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Letter

Repeated 28-day and 13-week dose toxicity studies of oils prepared from the internal organs of the Japanese giant scallop (*Patinopecten yessoensis*) in rats

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ABSTRACT — The discarded internal organs of the Japanese giant scallop (*Patinopecten yessoensis*) are abundant resources rich in n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid and docosahexaenoic acid. However, they have not been utilized due to contamination with toxic substances such as cadmium (Cd) and the occurrence of diarrhetic shellfish toxins (DST). We have successfully prepared a high-quality scallop oil (SCO) from its internal organs with negligible contamination with Cd and DST. The scallop internal organs were obtained from two different scallop processing areas, Mutsu and Uchiura bays, Japan, and referred to as SCO-M and SCO-U, respectively. To evaluate the safeties of SCO-M and SCO-U as food ingredients and n-3 PUFA supplements, repeated 28-day and 13-week dose oral toxicity studies in rats were conducted. Rats were fed diets containing 1% and 5% of SCO-M and SCO-U, respectively, in the repeated 28-day dose oral toxicity study and 5% SCO-M or SCO-U in the repeated 13-week dose oral toxicity study (limit test). No adverse toxicological effects were observed when rats were fed diets containing SCO-M and/or SCO-U at up to 5% for 28 days and 13 weeks. These results suggest that SCO-M and SCO-U are safe products in terms of subacute toxicity under these experimental conditions.

Key words: n-3 Polyunsaturated fatty acids, Eicosapentaenoic acid, Docosahexaenoic acid, Scallop internal organs, Safety study

INTRODUCTION

The health-promoting functions of n-3 polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been well documented. For example, the intake of EPA and DHA has been reported to reduce the risk of hyperlipidemia (AbuMweis *et al.*, 2018), cardiovascular diseases (Manuelli *et al.,* 2017), infant allergies (Warstedt *et al.*, 2016), and Alzheimer's disease (Freund-Levi *et al.*, 2006). In response to this, various organizations are recommending the intake of EPA and DHA. For example, Food and Drug Administration (FDA)/World Health Organization, the American Dietetic Association, and

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American Heart Association now recommend a total n-3 PUFA dietary intake of 1.4 to 2.5 g/day, with EPA and DHA ranging from 140 to 600 mg/day, depending on the authority issuing the guidelines. In addition, the FDA has recommended that consumers should not exceed a total of 3 g/day intake of EPA and DHA, with no more than 2 g/day from a dietary supplement because excess consumption of EPA and DHA can lead to a slower clotting time. Due to the growing demand of EPA and DHA as supplements and pharmaceutical products worldwide, there are concerns about the exhaustion of EPA and DHA supply sources. Therefore, a search for new EPA and DHA sources is underway.

Well-known sources of n-3 PUFA are seafood by-products that include belly flaps, heads, liver, roe, skin, viscera, and meat adhered to the bones (Vazquez *et al.*, 2019). Coasts of Hokkaido island and Aomori prefecture in northern Japan are one of the largest habitats of scallops (*Patinopecten yessoensis*). Approximately 500,000 tons/year of scallops are landed at Hokkaido island and Aomori prefecture, but the edible part (adductor muscle) constitutes only about 15% (w/w) and other parts, including the outer shells and internal organs, are waste. The internal organs of scallops such as the hepatopancreas, gonads, mantles, and gills are obtained during scallop processing in a quantity of approximately 32,000 and 6,000 tons/year in Hokkaido island and Aomori prefecture, respectively. A total of 2,200 thousand tons of *P. yessoensis* were landed in 2016 worldwide, and the hepatopancreas is considered to have been discarded (FAO, 2020). Previous studies showed that the hepatopancreas of scallop has an extremely high n-3 PUFA content, especially EPA (Hayashi, 1986, 1988). Therefore, an attempt to use oil prepared from the internal organs of scallop as a functional food material was undertaken. However, they failed to remove toxic metals such as cadmium (Cd) and diarrheic shellfish poison (DST) from oil because of the high levels of these components in the hepatopancreas of scallops (Kruzynski, 2004; Matsushima *et al.*, 2018). For this reason, the internal organs of scallops have not been used as an n-3 PUFA source. Recently, we successfully prepared a high-quality scallop oil (SCO) from its internal organs that satisfies the specifications for its use as a food product, by removing the Cd and DST (Okuyama *et al.*, 2019). In addition, we prepared SCO using the method by Okuyama *et al.* (2019) from the internal organs of *P. yessoensis*, obtained from two different processing areas and referred to them as SCO-M (SCO from Mutsu bay, Aomori, Japan) and SCO-U (SCO from Uchiura bay, Hokkaido, Japan). Therefore, SCO-M and SCO-U could be a new source for the n-3 PUFA supply, thus the safety of SCO needs to be ensured. Our previous study showed that SCO-M and SCO-U did not have a genotoxicity under *in vitro* (Ames test) and *in vivo* (Micronucleus test) experiments (Sugimoto *et al.*, 2019). Further safety evaluations on SCO-M and SCO-U are necessary for the production as a food ingredient and supplement. In this study, the safeties of SCO-M and SCO-U were evaluated in Wistar rats by carrying out repeated 28-day and 13-week dose oral toxicity studies. The effects of n-3 PUFA were also compared to the ones of tuna oil, which is already available in the market.

MATERIALS AND METHODS

Materials

The internal organs of the Japanese giant scallop (*P. yessoensis*) from Mutsu and Uchiura bays were provided by SATO CHIKURO Co. (Aomori, Japan) and Yakumo fishery cooperative (Hokkaido, Japan), respectively. The giant scallop's internal organs obtained from Mutsu bay consisted only of the hepatopancreas and were obtained from October to November 2017. In contrast, the giant scallop's internal organs obtained from Uchiura bay contained not only hepatopancreas, but also gonads, gills, and mantles, and were obtained from August to September 2017. SCO-M and SCO-U were prepared according to the method described previously (Sugimoto *et al.*, 2019). Soybean oil was obtained from Merck KGaA (Darmstadt, Germany). Tuna (*Thunnus orientalis*) oil was provided by Yashima Shoji Co., Ltd. (Shizuoka, Japan). The components of the experimental diet were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan) and Fujifilm Wako Pure Chemical Co. (Osaka, Japan). All other chemicals were of reagent grade and were obtained from Merck KGaA and Nacalai Tesque, Inc. (Kyoto, Japan).

Lipid compositions of oil from the Japanese giant scallop's internal organs

The fatty acid compositions of SCO-M and SCO-U were determined using a gas chromatographic (GC) system (GC-2014; Shimadzu Co., Kyoto, Japan) equipped with an Omegawax® capillary column (Merck KGaA) as described previously (Fukunaga *et al.*, 2016). The phospholipid (PL) contents of SCO-M and SCO-U were determined using phosphorus analyses as described previously (Rouser *et al.,* 1970). The cholesterol contents of SCO-M and SCO-U were measured by GC (GC-2014; Shimadzu Co.) equipped with a DB-5 capillary column (Agilent Technologies Japan Ltd., Tokyo, Japan) with an internal standard of 5α-cholestane (Kaneda *et al.*, 1980). The α-tocopherol

(α-Toc) contents of SCO-M and SCO-U were measured by using a high-performance liquid chromatographic (HPLC) system of Model Prominence Series (Shimadzu Co.) equipped with a reversed phase column (Inertsil® ODS column, 250 × 4.6 mm I. D., GL Sciences Inc., Tokyo, Japan) (Ueda *et al.,* 1993). The peroxide values and acid values of SCO-M and SCO-U were determined as described in the JOCS Standard Methods for the Analysis of Fats, Oils and Related Materials (Japan Oil Chemists' Society, 2013). In addition, Cd, arsenic, mercury, dioxin, pesticide residues, polychlorobiphenyl, and DST in SCO-M and SCO-U were analyzed using the official analytical methods performed by a commercial service (Japan Food Research Laboratories, Tokyo, Japan).

Ethics and animals

The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai University (approval No. 1704) and followed the "Guide for the Care and Use of Experimental Animals" issued by the Prime Minister's Office of Japan.

Four-week-old male and female Wistar rats were obtained from Japan SLC, Inc. (Shizuoka, Japan), and used after acclimatization for 7 days. During acclimatization and the experimental period, rats were kept in an air-conditioned room (temperature: 21-23°C; humidity: 50-70%; illuminated in the 08:00-20:00 hr period). Rats were provided with the experimental diets based on the American Institute of Nutrition (AIN) 93G (Reeves *et al.*, 1993) and tap water *ad libitum*.

Repeated 28-day oral dose toxicity study

Seven groups of 8 male and female rats were fed diets containing 7% (w/w) experimental oils for a period of 28 days. The control diet was the AIN93G formula (Reeves *et al.*, 1993) containing 7% of soybean oil. The experimental diets contained 6% of soybean oil and 1% of either SCO-M and SCO-U (SCO-M 1% and SCO-U 1%, respectively), and 2% of soybean oil with 5% of either SCO-M and SCO-U (SCO-M 5% and SCO-U 5%, respectively). In addition, the tuna oil diets contained 6% of soybean oil and 1% of tuna oil (TO 1%), and 2% of soybean oil with 5% of tuna oil (TO 5%). Gross appearances of rats and food and water intakes were evaluated daily. The experimental diets were stored at -35°C, thawed, and fed to the rats on a daily discard and top-up routine. After 28 days and an overnight fasting, the rats were euthanized under isoflurane anesthesia, then macroscopic observation was conducted. Next, their blood was collected from the inferior vena cava without the use of anti-coagulants, and serum was obtained through centrifugation (2,000 × *g*, for 15 min). The abdominal organs were quickly removed, weighed, rinsed with cold saline, and frozen in liquid nitrogen. Aliquots of liver, spleen, kidney, and gonadal tissues were fixed in 10% neutral buffered formalin. The fixed tissues were then dehydrated by using an ethanol series and embedded in paraffin. Sections of 5 μm of thickness were sliced, then stained with hematoxylin and eosin, and evaluated by a pathologist.

Repeated 13-week oral dose toxicity study

Four groups of 8 male and female rats were used for repeated oral dose toxicity study for 13 weeks (limit test). The control diet was the AIN93G formula (Reeves *et al.*, 1993). The experimental diets contained 2% of soybean oil with 5% SCO-M, SCO-U, or tuna oil (SCO-M 5%, SCO-U 5%, and TO 5%, respectively). Other experimental and anatomical conditions were performed in the same manner as the 28-day repeated oral dose toxicity study described above, but no histological examination was performed.

Analysis of biochemical, electrolytical, and hematology parameters

Total protein, albumin, albumin/globulin (A/G), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), serum urea nitrogen (SUN), total lipid, PL, triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), inorganic phosphorus (IP), magnesium (Mg), red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured by a commercial service (Japan Medical Laboratory, Osaka, Japan). Additionally, in the repeated 13-week oral dose toxicity study, alkaline phosphatase (ALP) and creatinine were also measured.

Analysis of thiobarbituric acid reactive substances (TBARS)

Levels of TBARS in serum and liver were measured spectrophotometrically at 532 nm based on the color obtained after the reaction of thiobarbituric acid with malondialdehyde (Ohkawa *et al.,* 1979).

Statistical analyses

Data showed the mean values and standard errors of the mean (SEM). The differences between multiple groups were evaluated using analysis of variance (ANOVA) and Tukey's multiple comparison test. Statistical signifi-

cance was set at $p < 0.05$. The analyses were performed using GraphPad Prism software version 7.0d (GraphPad Software, CA, USA).

RESULTS AND DISCUSSION

Lipid compositions of the experimental oils

Table 1 shows the lipid compositions of experimental oils including soybean oil, SCO-M, SCO-U, and tuna oil. Compared to tuna oil, SCO-M and SCO-U showed high EPA (31.8 and 27.7 wt%, respectively) and PL (51 and 269 mg/g, respectively) contents, but a low DHA (6.5 and 5.2 wt%, respectively) content. In addition, SCO-M and SCO-U contained very little amounts of Cd (< 0.4 mg/kg), arsenic (< 2 ppm), mercury (< 0.4 ppm), dioxin (< 4 pg-TEQ/kg), pesticide residues (< 0.01 ppm, respectively), polychlorobiphenyl (< 3 ppm), and DST (< 0.16 mg okadaic acid Eq/kg), and demonstrated that the specifications for their use as food products are satisfied.

Repeated 28-day oral dose toxicity study

No mortality and no abnormalities in the gross appearance of the rats were observed during the experimental period. Table 2 shows growth parameters. In the male and female rats, there were no significant differences in the initial BW, final BW, BW gain, food intake, food efficiency, and water intake among the groups.

Macroscopic observation showed no abnormalities in the abdominal organs of all the SCO-M and SCO-U groups. Table 3 shows relative organ weights. In the male and female rats, there were no significant differences in the relative organ weights including the liver, kidney, spleen, heart, lung, gonadal organs (testis or ovary and uterus), brain, stomach, small intestine, cecum, large intestine, mesentery WAT, perirenal WAT, and epididymal WAT (male only) among the group. In addition, histological evaluations indicated no abnormalities in the liver, kidney, spleen and gonadal organs (testis and ovary) in all the SCO-M and SCO-U groups compared with the control and TO groups (data not shown), thus indicating that SCO-M and SCO-U have no adverse effects on these organs.

Table 4 shows the biochemical, electrolytical, and hematological parameters. In the male rats, the serum lipid parameters including PL, total-cholesterol, and HDL-C levels in the SCO-M 5% and SCO-U 5% groups were significantly lower than those of the control group. On the other hand, in the female rats, the SCO-U 5% group showed a significantly lower serum total-cholesterol level than that of the control group. Previous studies suggested that the dietary fish oil containing EPA and DHA decreased the serum lipid levels (Guo *et al.*, 2018; Nestel, 1990). In addition, the lowering of serum lipid parameters in male rats was also observed in the TO 5% group, when compared with the control group. Since fish oil has the effect of lowering the serum lipid concentration and the same phenomenon has been observed in the TO 5% group, the lowering of serum lipid parameters by dietary SCO-M and SCO-U does not seem to have an adverse effect. No significant differences were found in the other parameters between the control group and the SCO-M or SCO-U groups.

EPA and DHA are more easily oxidized than linoleic acid and alpha-linolenic acid because they are rich in active methylene groups (Miyashita, 2019). Therefore, dietary SCO, which are high in EPA, may promote lipid peroxidation in serum and in the liver. To examine the effect of lipid peroxidation, TBARS levels in serum and liver were analyzed. Table 5 shows the TBARS levels in serum and liver of male and female rats. No significant differences were observed in TBARS levels in the serum

Table 1. Lipid composition of the experimental oils.

	Soybean oil	SCO[1]		Tuna oil
		SCO-M[2]	SCO-U[3]	
Fatty acid composition (wt%)				
C14:0	-	7.7	6.6	3.2
C14:1	-	1.6	0.8	-
C16:0	11.2	15.4	18.2	20.8
C16:1	0.1	12.4	12.9	4.9
C16:2	-	1.2	1.0	1.0
C16:4	-	1.4	0.5	-
C18:0	3.7	5.4	4.5	4.4
C18:1n-9	22.6	4.6	8.2	19.6
C18:2n-6	54.7	0.7	1.8	1.2
C18:3n-6	-	1.1	1.0	-
C18:3n-3	2.5	-	-	-
C20:1	-	2.8	2.0	2.6
C20:4n-6	-	2.3	2.8	0.7
C20:5n-3	-	31.8	27.7	8.3
C22:6n-3	-	6.5	5.2	26.6
Phospholipid (mg/g)	N.D.	51	269	1
Cholesterol (mg/g)	N.D.	4.7	7.7	1.2
α-Tocopherol (μg/g)	0.26	4.03	4.04	0.64
PV(meq/kg)	2.43	7.39	3.72	6.90
AV(mg/g)	0.1	2.7	17.4	0.3

[1] The contents of cadmium (< 0.4 mg/kg), arsenic (< 2 ppm), mercury (< 0.4 ppm), dioxin (< 4 pg-TEQ/kg), pesticide residues (< 0.01 ppm) respectively, polychlorobiphenyl (< 3 ppm), and diarrheic shellfish poison (< 0.16 mg okadaic acid Eq/kg) in SCO-M and SCO-U were determined using official analytical methods. The results confirmed that the specifications for their use as food products, have been satisfied.

[2] Oil prepared from the internal organs of *P. yessoensis*, by-produced in Mutsu bay area (Aomori, Japan).

[3] Oil prepared from the internal organs of *P. yessoensis*, by-produced in Uchiura bay area (Hokkaido, Japan).

AV, acid value; N.D., not detected; PV, peroxide value; SCO, scallop oil.

Table 2. Growth parameters in rats administered with oil prepared from the Japanese giant scallop (*P. yessoensis*) internal organs for 28 days.

	Control	SCO-M[1]		SCO-U[2]		TO	
		1%	5%	1%	5%	1%	5%
Male							
Initial BW (g)	115.8 ± 1.7	116.2 ± 2.1	116.7 ± 2.7	115.0 ± 2.4	116.0 ± 2.2	115.3 ± 2.3	116.1 ± 3.1
Final BW (g)	292.1 ± 4.0	309.9 ± 8.6	310.8 ± 5.9	295.9 ± 6.8	312.6 ± 6.3	308.0 ± 3.4	301.3 ± 7.7
BW gain (g/day)	6.30 ± 0.11	6.91 ± 0.35	6.93 ± 0.18	6.46 ± 0.20	7.02 ± 0.27	6.88 ± 0.12	6.61 ± 0.26
Food intake (g/day)	15.1 ± 0.8	14.8 ± 0.4	16.0 ± 0.5	14.6 ± 0.4	16.1 ± 0.5	15.7 ± 0.4	15.1 ± 0.5
Food efficiency (g/g)[3]	0.434 ± 0.007	0.486 ± 0.027	0.451 ± 0.012	0.456 ± 0.014	0.450 ± 0.018	0.452 ± 0.008	0.455 ± 0.018
Water intake (mL/day)	40.3 ± 2.2	40.4 ± 2.4	45.8 ± 3.3	43.0 ± 2.8	41.8 ± 2.5	41.4 ± 2.5	43.6 ± 2.4
Female							
Initial BW (g)	94.7 ± 1.9	94.2 ± 2.6	95.0 ± 2.3	95.0 ± 2.1	94.8 ± 1.2	95.1 ± 2.0	94.8 ± 2.4
Final BW (g)	212.0 ± 2.9	215.2 ± 6.4	213.7 ± 5.6	208.7 ± 5.0	210.0 ± 6.2	215.7 ± 3.4	224.6 ± 3.6
BW gain (g/day)	4.05 ± 0.14	4.17 ± 0.19	4.09 ± 0.16	3.92 ± 0.17	3.97 ± 0.19	4.16 ± 0.11	4.48 ± 0.11
Food intake (g/day)	11.6 ± 0.6	10.9 ± 0.4	11.0 ± 0.4	11.0 ± 0.2	11.0 ± 0.2	11.4 ± 0.3	11.6 ± 0.5
Food efficiency (g/g)	0.364 ± 0.012	0.399 ± 0.018	0.385 ± 0.015	0.369 ± 0.013	0.373 ± 0.018	0.379 ± 0.009	0.400 ± 0.010
Water intake (mL/day)	30.3 ± 0.8	29.8 ± 1.1	27.1 ± 0.6	27.7 ± 0.5	28.3 ± 0.6	28.5 ± 0.7	30.4 ± 0.8

Data show means ± SEM (n = 8).

[1] Oil prepared from the internal organs of *P. yessoensis*, by-produced in Mutsu bay area (Aomori, Japan).

[2] Oil prepared from the internal organs of *P. yessoensis*, by-produced in Uchiura bay area (Hokkaido, Japan).

[3] Food efficiency (g/g) = BW gain (g/day) / food intake (g/day).

BW, body weight; SCO, scallop oil; TO, tuna oil.

and the liver among the groups both in male and female rats. Therefore, the intakes of SCO-M and SCO-U at concentrations of 1% and 5% (w/w) in the diets for 28 days did not affect TBARS levels in the serum and the liver.

A decrease in serum lipid parameters was observed in the SCO-M 5% and SCO-U 5% groups, which is considered to be due to the effects of EPA and DHA contained in SCO-M and SCO-U, because serum lipid parameters were similarly decreased in the TO 5% group. Other parameters in all the SCO-M and SCO-U groups were not significantly different from the control group and were considered within the reference interval from the view point of normal biological and laboratory limits. These results demonstrated that SCO-M and SCO-U are safe components under these experimental conditions. In addition, the minimum daily intakes of SCO-M and SCO-U during the experimental period were 3,321 and 3,384 mg/kg BW in male rats and 3,321 and 3,384 mg/kg BW in female rats, respectively. These results indicated that the dietary administration of SCO-M and SCO-U in diets at a 5% percentage, did not show any toxic effects in the repeated 28-day oral dose toxicity study using rats.

Repeated 13-week oral dose toxicity study

As mentioned above, rats fed diets containing 5% SCO-M and SCO-U did not show any toxic effects in the repeated 28-day oral dose toxicity study. Therefore, the only diets containing 5% of the test substances were used for repeated 13-week oral dose toxicity study using rats (limit test). The toxicities of SCO-M and SCO-U were determined for the repeated oral dose toxicity study for 13 weeks. No mortality and no abnormalities in the gross appearance of rats fed diets containing SCO-M and SCO-U were observed during the experimental period. Table 6 shows the growth parameters. There were no significant differences in the initial BW, final BW, BW gain, food intake, food efficiency, and water intake among the groups in male and female rats.

Table 7 shows the relative organ weights. Macroscopic observation showed no abnormalities in the abdominal organs of the SCO-M and SCO-U groups. No significant differences in the relative organ weights including the liver, kidney, spleen, heart, lung, gonadal organs (testis or ovary and uterus), brain, stomach, small intestine, cecum, large intestine, mesentery WAT, perirenal WAT, and epididymal WAT (male only) were observed among the groups. On the other hand, macroscopic observation and relative organs weights did not reveal any abnormalities, thus no histological evaluations were performed.

Table 8 shows the biochemical, electrolytical, and hematology parameters. No significant differences were found between the control group and the SCO-M or SCO-U groups in male and female rats. Serum lipid parameters in male rats including total lipid, PL, total-cholesterol and HDL-C levels in the SCO-M and SCO-U

Table 3. Weight of the internal organs of the rats administered with oil prepared from Japanese giant scallop (*P. yessoensis*) internal organs for 28 days.

	Control	SCO-M[1]		SCO-U[2]		TO	
		1%	5%	1%	5%	1%	5%
				g/100 g BW			
Male							
Liver	3.66 ± 0.17	3.86 ± 0.16	3.54 ± 0.04	3.78 ± 0.17	4.03 ± 0.04	3.94 ± 0.07	4.02 ± 0.06
Kidney	0.67 ± 0.01	0.68 ± 0.01	0.65 ± 0.01	0.61 ± 0.05	0.61 ± 0.03	0.65 ± 0.01	0.65 ± 0.05
Spleen	0.20 ± 0.01	0.22 ± 0.02	0.18 ± 0.01	0.18 ± 0.01	0.21 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
Heart	0.35 ± 0.01	0.34 ± 0.01	0.32 ± 0.01	0.35 ± 0.02	0.37 ± 0.02	0.37 ± 0.03	0.37 ± 0.01
Lung	0.46 ± 0.01	0.46 ± 0.01	0.45 ± 0.01	0.47 ± 0.02	0.47 ± 0.01	0.44 ± 0.01	0.45 ± 0.02
Testis	0.93 ± 0.02	0.97 ± 0.04	0.91 ± 0.03	0.93 ± 0.04	0.90 ± 0.02	0.90 ± 0.02	0.87 ± 0.05
Brain	0.69 ± 0.02	0.73 ± 0.04	0.64 ± 0.01	0.68 ± 0.03	0.64 ± 0.01	0.59 ± 0.04	0.63 ± 0.03
Stomach	1.59 ± 0.13	1.66 ± 0.27	1.25 ± 0.10	1.60 ± 0.11	1.28 ± 0.10	1.56 ± 0.13	1.52 ± 0.06
Small intestine	2.58 ± 0.05	2.50 ± 0.07	2.56 ± 0.08	2.37 ± 0.15	2.45 ± 0.06	2.55 ± 0.13	2.69 ± 0.10
Cecum	1.15 ± 0.12	1.09 ± 0.08	1.08 ± 0.07	1.05 ± 0.11	0.89 ± 0.08	1.12 ± 0.10	1.19 ± 0.08
Large intestine	0.45 ± 0.03	0.42 ± 0.01	0.44 ± 0.04	0.48 ± 0.03	0.42 ± 0.02	0.45 ± 0.03	0.45 ± 0.02
Mesentery WAT	1.30 ± 0.04	1.26 ± 0.05	1.42 ± 0.05	1.25 ± 0.06	1.23 ± 0.04	1.37 ± 0.04	1.29 ± 0.06
Perirenal WAT	1.52 ± 0.15	1.61 ± 0.15	1.79 ± 0.14	1.49 ± 0.12	1.66 ± 0.13	1.71 ± 0.15	1.38 ± 0.07
Epididymal WAT	1.42 ± 0.08	1.33 ± 0.20	1.44 ± 0.05	1.41 ± 0.09	1.29 ± 0.04	1.54 ± 0.05	1.21 ± 0.04
Female							
Liver	3.55 ± 0.08	3.66 ± 0.09	3.87 ± 0.11	3.80 ± 0.10	3.88 ± 0.08	3.72 ± 0.12	3.82 ± 0.07
Kidney	0.68 ± 0.01	0.72 ± 0.03	0.68 ± 0.02	0.70 ± 0.01	0.68 ± 0.01	0.68 ± 0.01	0.70 ± 0.00
Spleen	0.22 ± 0.00	0.21 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.23 ± 0.00	0.21 ± 0.01	0.22 ± 0.01
Heart	0.37 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.35 ± 0.01	0.36 ± 0.01	0.41 ± 0.03
Lung	0.51 ± 0.01	0.51 ± 0.01	0.54 ± 0.01	0.52 ± 0.01	0.51 ± 0.01	0.55 ± 0.04	0.52 ± 0.01
Ovary	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Uterus	0.25 ± 0.02	0.32 ± 0.04	0.30 ± 0.02	0.25 ± 0.03	0.29 ± 0.02	0.23 ± 0.01	0.24 ± 0.03
Brain	0.92 ± 0.01	0.92 ± 0.02	0.92 ± 0.02	0.83 ± 0.06	0.89 ± 0.02	0.90 ± 0.02	0.81 ± 0.04
Stomach	2.10 ± 0.24	1.78 ± 0.25	2.01 ± 0.20	1.97 ± 0.16	2.04 ± 0.21	2.04 ± 0.25	1.72 ± 0.14
Small intestine	3.01 ± 0.20	3.09 ± 0.26	2.87 ± 0.10	2.83 ± 0.14	3.01 ± 0.06	3.09 ± 0.06	2.86 ± 0.06
Cecum	1.09 ± 0.11	1.03 ± 0.12	1.15 ± 0.06	1.16 ± 0.09	1.04 ± 0.10	1.36 ± 0.09	1.17 ± 0.07
Large intestine	0.54 ± 0.02	0.57 ± 0.05	0.51 ± 0.04	0.52 ± 0.04	0.59 ± 0.03	0.56 ± 0.05	0.57 ± 0.03
Mesentery WAT	1.34 ± 0.06	1.35 ± 0.06	1.28 ± 0.09	1.37 ± 0.04	1.37 ± 0.06	1.36 ± 0.07	1.25 ± 0.08
Perirenal WAT	1.23 ± 0.11	1.39 ± 0.19	1.23 ± 0.11	1.23 ± 0.12	1.23 ± 0.16	1.25 ± 0.10	1.28 ± 0.06

Data show means ± SEM (n = 8). Values in the same row not sharing a common superscript are significantly different at $p < 0.05$ using Tukey's multiple comparison test.

[1] Oil prepared from internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).
[2] Oil prepared from internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).
BW, body weight; SCO, scallop oil; TO, tuna oil; WAT, white adipose tissue.

Table 4. The biochemical, electrolytical, and hematology parameters of rats administered with oil prepared from Japanese giant scallop (*P. yessoensis*) internal organs for 28 days.

	Control	SCO-M[1]		SCO-U[2]		TO	
		1%	5%	1%	5%	1%	5%
Male							
Total protein (g/dL)	5.75 ± 0.06	5.83 ± 0.08	5.66 ± 0.05	5.64 ± 0.06	5.63 ± 0.06	5.66 ± 0.05	5.71 ± 0.05
Albumin (g/dL)	3.68 ± 0.04	3.75 ± 0.03	3.71 ± 0.03	3.69 ± 0.04	3.66 ± 0.04	3.78 ± 0.05	3.79 ± 0.03
A/G	1.78 ± 0.02	1.81 ± 0.04	1.90 ± 0.02	1.89 ± 0.04	1.88 ± 0.06	1.94 ± 0.03	1.94 ± 0.01
AST (IU/L)	80.6 ± 2.3	80.3 ± 1.3	87.5 ± 3.5	84.0 ± 2.8	86.5 ± 1.9	80.0 ± 1.1	85.3 ± 1.8
ALT (IU/L)	45.0 ± 2.7	48.8 ± 2.3	47.1 ± 1.5	44.9 ± 1.7	46.0 ± 2.4	46.5 ± 1.0	48.1 ± 2.6
LDH (IU/L)	346 ± 38	294 ± 38	310 ± 40	361 ± 40	294 ± 29	276 ± 41	204 ± 26
SUN (mg/dL)	16.3 ± 0.9	15.3 ± 0.5	14.5 ± 0.4	13.6 ± 1.4	14.4 ± 0.6	15.1 ± 0.6	14.8 ± 0.8
Total lipid (mg/dL)	284 ± 30 [a]	285 ± 19 [a]	214 ± 17 [ab]	244 ± 12 [ab]	161 ± 7 [c]	280 ± 22 [ab]	203 ± 11 [bc]
PL (mg/dL)	141 ± 6 [a]	143 ± 4 [a]	105 ± 4 [c]	126 ± 4 [ab]	93 ± 4 [c]	138 ± 3 [ac]	113 ± 3 [bc]
TG (mg/dL)	77.6 ± 15.9	76.0 ± 9.8	60.9 ± 8.7	67.6 ± 7.6	38.4 ± 4.3	81.3 ± 13.6	53.5 ± 5.9

Table 4. (Continued).

	Control	SCO-M[1]		SCO-U[2]		TO	
		1%	5%	1%	5%	1%	5%
Total cholesterol (mg/dL)	74.0 ± 4.2 [a]	75.1 ± 3.9 [a]	54.4 ± 2. 5 [b]	62.8 ± 2.9 [ab]	45.3 ± 1.0 [c]	70.1 ± 3.5 [a]	53.9 ± 2.2 [b]
HDL-C (mg/dL)	49.4 ± 2.7 [a]	52.1 ± 3.2 [a]	36.5 ± 1.8 [c]	42.9 ± 2.4 [abc]	33.5 ± 0.9 [c]	49.9 ± 2.5 [ab]	41.8 ± 1.3 [bc]
Na (mEq/L)	141 ± 0	140 ± 0	141 ± 0	141 ± 0	141 ± 0	141 ± 0	141 ± 0
K (mEq/L)	4.85 ± 0.19	4.80 ± 0.18	4.54 ± 0.11	4.68 ± 0.06	4.53 ± 0.08	4.66 ± 0.13	4.58 ± 0.10
Cl (mEq/L)	101 ± 1	99 ± 1	99 ± 1	101 ± 0	101 ± 1	101 ± 0	100 ± 1
Ca (mEq/L)	10.9 ± 0.2	10.9 ± 0.1	10.8 ± 0.1	10.8 ± 0.1	10.7 ± 0.1	10.8 ± 0.1	10.8 ± 0.1
IP (mEq/L)	7.86 ± 0.34	7.83 ± 0.17	8.16 ± 0.26	7.23 ± 0.20	7.75 ± 0.14	7.49 ± 0.20	7.75 ± 0.22
Mg (mEq/L)	2.00 ± 0.06	1.90 ± 0.05	1.90 ± 0.06	1.89 ± 0.05	1.93 ± 0.05	1.94 ± 0.05	1.88 ± 0.03
RBC (10^4 cell/μL)	740 ± 9	759 ± 11	738 ± 9	747 ± 11	766 ± 5	725 ± 12	751 ± 7
WBC (cell/μL)	9000 ± 539	9963 ± 775	10550 ± 131	9900 ± 268	9588 ± 573	8975 ± 551	9650 ± 539
PLT (10^4 cell/μL)	68.9 ± 2.6	67.7 ± 2.3	63.6 ± 2.6	67.3 ± 3.4	61.2 ± 1.6	63.2 ± 2.8	60.7 ± 0.9
Hb (g/dL)	14.5 ± 0.1	14.8 ± 0.2	14.6 ± 0.3	14.5 ± 0.2	14.6 ± 0.1	14.8 ± 0.4	14.6 ± 0.1
Ht (%)	43.8 ± 0.6	45.1 ± 0.6	44.1 ± 0.8	43.9 ± 0.6	44.2 ± 0.4	43.8 ± 0.6	44.4 ± 0.3
MCV (fL)	59.3 ± 0.3 [ab]	59.5 ± 0.5 [ab]	59.9 ± 0.9 [a]	58.9 ± 0.2 [ab]	57.6 ± 0.4 [b]	60.5 ± 0.4 [ab]	59.1 ± 0.5 [ab]
MCH (pg)	19.6 ± 0.1	19.5 ± 0.1	19.9 ± 0.5	19.4 ± 0.1	19.1 ± 0.1	20.4 ± 0.7	19.4 ± 0.2
MCHC (g/dL)	33.1 ± 0.2	32.9 ± 0.2	33.2 ± 0.8	33.0 ± 0.1	33.1 ± 0.2	33.7 ± 1.0	32.8 ± 0.1
Female							
Total protein (g/dL)	5.66 ± 0.07	5.78 ± 0.08	5.84 ± 0.08	5.79 ± 0.12	5.69 ± 0.07	5.78 ± 0.08	5.68 ± 0.09
Albumin (g/dL)	3.79 ± 0.05	3.81 ± 0.06	3.88 ± 0.04	3.90 ± 0.07	3.79 ± 0.06	3.85 ± 0.06	3.80 ± 0.05
A/G	2.03 ± 0.06	1.94 ± 0.03	1.98 ± 0.03	2.07 ± 0.04	1.99 ± 0.04	2.00 ± 0.05	2.03 ± 0.05
AST (IU/L)	86.1 ± 3.0	82.9 ± 3.9	91.9 ± 4.9	87.9 ± 2.8	93.1 ± 2.7	95.6 ± 6.6	86.0 ± 3.0
ALT (IU/L)	38.5 ± 1.6	40.8 ± 2.9	48.3 ± 3.6	40.3 ± 3.0	47.8 ± 2.0	47.9 ± 2.3	50.3 ± 2.5
LDH (IU/L)	287 ± 47	392 ± 98	356 ± 72	383 ± 88	382 ± 56	430 ± 85	320 ± 48
SUN (mg/dL)	21.5 ± 1.6	22.3 ± 1.7	23.6 ± 2.6	19.3 ± 1.5	22.0 ± 1.9	23.8 ± 1.9	21.9 ± 2.5
Total lipid (mg/dL)	254 ± 13	265 ± 16	246 ± 14	255 ± 17	228 ± 12	257 ± 7	220 ± 6
PL (mg/dL)	139 ± 4 [ab]	138 ± 5 [ab]	129 ± 5 [abc]	140 ± 5 [ab]	113 ± 4 [c]	147 ± 4 [a]	124 ± 3 [bc]
TG (mg/dL)	55.0 ± 8.6	64.3 ± 9.4	51.9 ± 7.4	56.9 ± 9.6	60.4 ± 7.5	46.3 ± 6.4	33.4 ± 3.3
Total cholesterol (mg/dL)	75 ± 2	74 ± 4	73 ± 4	74 ± 4	61 ± 3	81 ± 2	73 ± 3
HDL-C (mg/dL)	50.9 ± 1.1 [ab]	51.6 ± 3.5 [ab]	50.1 ± 3.4 [ab]	53.5 ± 2.6 [ab]	44.3 ± 2.5 [b]	57.5 ± 1.8 [a]	53.8 ± 2.2 [ab]
Na (mEq/L)	139 ± 1	139 ± 1	139 ± 0	140 ± 1	140 ± 0	139 ± 0	139 ± 0
K (mEq/L)	4.18 ± 0.08	4.29 ± 0.08	4.24 ± 0.08	4.36 ± 0.09	4.13 ± 0.08	4.39 ± 0.17	4.21 ± 0.11
Cl (mEq/L)	102 ± 1	101 ± 1	101 ± 1	102 ± 1	102 ± 0	100 ± 1	102 ± 1
Ca (mEq/L)	10.8 ± 0.2	10.8 ± 0.1	10.8 ± 0.1	10.6 ± 0.1	10.5 ± 0.1	10.8 ± 0.1	10.7 ± 0.1
IP (mEq/L)	6.30 ± 0.23	6.38 ± 0.18	6.30 ± 0.32	6.04 ± 0.26	6.56 ± 0.06	6.80 ± 0.34	6.64 ± 0.30
Mg (mEq/L)	2.01 ± 0.09	1.94 ± 0.05	1.95 ± 0.05	1.98 ± 0.06	1.91 ± 0.04	2.03 ± 0.05	1.98 ± 0.06
RBC (10^4cell/μL)	779 ± 11	774 ± 12	772 ± 10	776 ± 11	788 ± 12	785 ± 9	760 ± 13
WBC (cell/μL)	9350 ± 642	9450 ± 714	10438 ± 875	8043 ± 569	9763 ± 477	9000 ± 570	8988 ± 268
PLT (10^4 cell/μL)	51.8 ± 4.2	61.3 ± 2.1	60.6 ± 3.2	60.4 ± 3.5	62.6 ± 1.6	63.8 ± 1.6	58.5 ± 2.8
Hb (g/dL)	15.2 ± 0.2	15.1 ± 0.2	15.2 ± 0.2	15.2 ± 0.2	15.3 ± 0.2	15.4 ± 0.2	14.9 ± 0.2
Ht (%)	45.8 ± 0.5	45.6 ± 0.8	46.0 ± 0.6	45.5 ± 0.6	45.9 ± 0.6	46.5 ± 0.5	45.5 ± 0.6
MCV (fL)	59.0 ± 0.3	59.1 ± 1.0	59.4 ± 0.5	58.6 ± 0.2	58.4 ± 0.6	59.5 ± 0.5	59.9 ± 0.6
MCH (pg)	19.5 ± 0.3	19.5 ± 0.2	19.6 ± 0.1	19.6 ± 0.1	19.5 ± 0.2	19.7 ± 0.1	19.7 ± 0.2
MCHC (g/dL)	33.1 ± 0.3	33.1 ± 0.2	33.0 ± 0.2	33.4 ± 0.2	33.4 ± 0.2	33.2 ± 0.2	32.8 ± 0.1

Data show means ± SEM (n = 8). Values in the same row not sharing a common superscript are significantly different at $p < 0.05$ using Tukey's multiple comparison test.

[1] Oil prepared from the internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).

[2] Oil prepared from the internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; A/G, albumin/globulin; Ca, calcium; Cl, chlorine; Hb, hemoglobin; Ht, hematocrit; HDL-C, high-density lipoprotein cholesterol; IP, inorganic phosphorus; LDH, lactate dehydrogenase; K, potassium; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Mg, magnesium; Na, sodium; PL, phospholipid; PLT, platelet; RBC, red blood cell; SUN, serum urea nitrogen; SCO, scallop oil; TG, triglyceride; TO, tuna oil; WBC, white blood cells.

Table 5. Thiobarbituric acid reactive substances (TBARS) levels in the serum and liver of rats administered with oil prepared from Japanese giant scallop (*P. yessoensis*) internal organs for 28 days.

	Control	SCO-M[1]		SCO-U[2]		TO	
		1%	5%	1%	5%	1%	5%
Male							
Serum TBARS (nmol/mL)	5.83 ± 1.04	5.04 ± 1.05	4.95 ± 1.06	4.40 ± 0.62	5.17 ± 0.69	5.53 ± 0.92	5.27 ± 1.05
Liver TBARS (μmol/g)	86.2 ± 6.0	92.7 ± 8.5	93.1 ± 6.0	87.4 ± 7.7	98.8 ± 10.5	96.6 ± 4.7	99.2 ± 7.7
Female							
Serum TBARS (nmol/mL)	4.06 ± 0.38	4.23 ± 0.29	4.25 ± 0.48	4.17 ± 0.44	3.63 ± 0.36	4.50 ± 0.41	3.84 ± 0.38
Liver TBARS (μmol/g)	89.5 ± 7.2	86.9 ± 11.8	89.3 ± 7.3	97.5 ± 9.1	91.3 ± 6.9	97.5 ± 6.3	82.7 ± 7.6

Data show means ± SEM (n = 8). Values in the same row not sharing a common superscript are significantly different at $p < 0.05$ using Tukey's multiple comparison test.

[1] Oil prepared from internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).
[2] Oil prepared from internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).
SCO, scallop oil; TBARS, thiobarbituric acid reactive substances; TO, tuna oil.

Table 6. Growth parameters in rats administered with oil prepared from Japanese giant scallop (*P. yessoensis*) internal organs for 13 weeks.

	Control	SCO-M[1] 5%	SCO-U[2] 5%	TO 5%
Male				
Initial BW (g)	81.7 ± 1.0	83.9 ± 1.1	83.3 ± 0.5	81.5 ± 0.8
Final BW (g)	552.0 ± 13.1	534.0 ± 16.7	536.3 ± 12.9	516.0 ± 8.6
BW gain (g/day)	5.23 ± 0.15	5.00 ± 0.18	5.03 ± 0.14	4.88 ± 0.09
Food intake (g/day)	19.6 ± 1.3	19.4 ± 1.2	19.8 ± 1.2	19.5 ± 1.1
Food efficiency (g/g)[3]	0.267 ± 0.008	0.258 ± 0.009	0.254 ± 0.007	0.248 ± 0.005
Water intake (mL/day)	52.8 ± 3.4	44.7 ± 2.4	47.8 ± 2	43.5 ± 2.1
Female				
Initial BW (g)	72.8 ± 1.1	72.1 ± 1.1	71.0 ± 0.5	72.4 ± 0.8
Final BW (g)	301.3 ± 7.0	308.4 ± 4.8	322.8 ± 5.0	318.0 ± 6.4
BW gain (g/day)	2.55 ± 0.08	2.60 ± 0.05	2.77 ± 0.05	2.70 ± 0.06
Food intake (g/day)	13.1 ± 0.68	13.4 ± 0.71	14.1 ± 0.75	14.1 ± 0.66
Food efficiency (g/g) [3]	0.187 ± 0.006	0.194 ± 0.004	0.198 ± 0.004	0.191 ± 0.005
Water intake (mL/day)	27.5 ± 0.99	30.3 ± 1.05	28.9 ± 1.33	29.0 ± 1.25

Data show means ± SEM (n = 8). Values in the same row not sharing a common superscript are significantly different at $p < 0.05$ using Tukey's multiple comparison test.

[1] Oil prepared from the internal organs of *P. yessoensis*, by-produced in Mutsu bay area (Aomori, Japan).
[2] Oil prepared from the internal organs of *P. yessoensis*, by-produced in Uchiura bay area (Hokkaido, Japan).
[3] Food efficiency (g/g) = BW gain (g/day) / food intake (g/day).
BW, body weight; SCO, scallop oil; TO, tuna oil.

groups were significantly lower compared to those of the Control group. In addition, in female rats, the serum PL and total-cholesterol levels in the SCO-M and SCO-U groups were significantly lower than those of the control group. In addition, the decreased serum lipid parameters were also observed in the TO group in male rats. Therefore, the reduction in the serum lipid parameters due to SCO-M and SCO-U intake, as is the case with the repeated 28-day oral dose toxicity study, did not appear to be an adverse effect. No significant differences were found in the other parameters between the control group and the SCO-M or SCO-U groups.

Table 9 shows the TBARS levels in the serum and liver of male and female rats. In the female rats, serum TBARS level in the SCO-M 5% group was significantly higher than that in the control group. Despite fish oils reducing the risk of cardiovascular disease via mechanisms underlying atherosclerosis, thrombosis and inflammation, there have been concerns that these fatty acids may increase lipid peroxidation (Mori, 2004). A previous study reported that the long-term consumption of fish oils increases plasma lipid peroxide, particularly in older woman (Meydani *et al.*, 1991). Another paper showed that the consumption of EPA and DHA statistically and significantly increased plasma TBARS levels, but this change was so small that its clinical relevance is questionable (Wanderand Du, 2000). EPA and DHA appear to reduce the risk of cardiac arrhythmia, sudden cardiac death, and modestly reduce atherosclerotic plaque formation, even though TBARS are elevated (Wang *et al.*, 2006; Yokoyama *et al.*, 2007).

Table 7. Organs weights in rats administered with oil prepared from Japanese giant scallop (*P. yessoensis*) internal organs for 13 weeks.

	Control	SCO-M[1] 5%	SCO-U[2] 5%	TO 5%
		g/100 g BW		
Male				
Liver	3.43 ± 0.20	3.15 ± 0.05	3.15 ± 0.08	3.06 ± 0.08
Kidney	0.62 ± 0.07	0.53 ± 0.02	0.53 ± 0.01	0.56 ± 0.01
Spleen	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.00
Heart	0.31 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.32 ± 0.01
Lung	0.42 ± 0.01	0.38 ± 0.01	0.38 ± 0.02	0.37 ± 0.01
Testis	0.69 ± 0.03	0.66 ± 0.03	0.67 ± 0.07	0.70 ± 0.02
Brain	0.45 ± 0.02	0.44 ± 0.01	0.43 ± 0.01	0.45 ± 0.01
Stomach	1.42 ± 0.12	1.11 ± 0.10	1.18 ± 0.08	1.53 ± 0.18
Small intestine	1.78 ± 0.15	1.56 ± 0.08	1.52 ± 0.06	1.60 ± 0.08
Cecum	0.83 ± 0.08	0.72 ± 0.10	0.64 ± 0.07	0.72 ± 0.11
Large intestine	0.35 ± 0.04	0.33 ± 0.03	0.37 ± 0.04	0.38 ± 0.06
Mesentery WAT	2.13 ± 0.23	1.88 ± 0.12	2.10 ± 0.11	2.04 ± 0.10
Perirenal WAT	2.21 ± 0.24	2.03 ± 0.16	2.44 ± 0.20	2.04 ± 0.12
Epididymal WAT	1.89 ± 0.11	1.56 ± 0.08	1.72 ± 0.11	1.69 ± 0.03
Female				
Liver	2.82 ± 0.10	3.11 ± 0.07	3.28 ± 0.09	2.98 ± 0.08
Kidney	0.60 ± 0.01	0.61 ± 0.01	0.58 ± 0.02	0.60 ± 0.02
Spleen	0.17 ± 0.00	0.20 ± 0.01	0.20 ± 0.00	0.18 ± 0.01
Heart	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01
Lung	0.46 ± 0.01	0.47 ± 0.01	0.42 ± 0.01	0.43 ± 0.01
Ovary	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.00
Uterus	0.17 ± 0.01	0.16 ± 0.01	0.14 ± 0.02	0.16 ± 0.01
Brain	0.72 ± 0.03	0.68 ± 0.01	0.67 ± 0.01	0.67 ± 0.01
Stomach	1.33 ± 0.23	1.53 ± 0.21	1.45 ± 0.14	1.17 ± 0.11
Small intestine	1.94 ± 0.13	2.01 ± 0.07	1.90 ± 0.10	1.91 ± 0.10
Cecum	0.81 ± 0.11	0.84 ± 0.07	0.66 ± 0.11	0.74 ± 0.11
Large intestine	0.44 ± 0.05	0.41 ± 0.03	0.46 ± 0.04	0.43 ± 0.05
Mesentery WAT	2.02 ± 0.18	2.05 ± 0.12	2.46 ± 0.15	2.33 ± 0.12
Perirenal WAT	3.11 ± 0.27	3.01 ± 0.30	3.59 ± 0.20	3.30 ± 0.20

Data show means ± SEM (n = 8). Values in the same row not sharing a common superscript are significantly different at $p < 0.05$ using Tukey's multiple comparison test.

[1] Oil prepared from the internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).
[2] Oil prepared from the internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).
BW, body weight; SCO, scallop oil; TO, tuna oil; WAT, white adipose tissue.

Table 8. The biochemical, electrolytical, and hematology parameters in rats administered with oil prepared from Japanese giant scallop (*P. yessoensis*) internal organs for 13 weeks.

	Control	SCO-M[1] 5%	SCO-U[2] 5%	TO 5%
Male				
Total protein (g/dL)	6.16 ± 0.13	6.28 ± 0.05	6.30 ± 0.07	6.01 ± 0.07
Albumin (g/dL)	3.66 ± 0.09	3.69 ± 0.05	3.83 ± 0.05	3.80 ± 0.05
A/G	1.46 ± 0.02 [bc]	1.36 ± 0.02 [c]	1.55 ± 0.03 [b]	1.72 ± 0.04 [a]
AST (IU/L)	85.3 ± 6.5	82.9 ± 3.7	83.9 ± 1.2	78.5 ± 2.7
ALT (IU/L)	47.4 ± 7.1	42.4 ± 1.4	49.1 ± 1.6	46.3 ± 1.9
LDH (IU/L)	359 ± 54	305 ± 61	261 ± 39	228 ± 28
ALP (IU/L)	1184 ± 369	1102 ± 49	988 ± 43	1172 ± 83
SUN (mg/dL)	20.5 ± 1.4	20.4 ± 1.3	19.4 ± 1.7	20.0 ± 0.8
Creatinine (mg/dL)	0.31 ± 0.01	0.34 ± 0.01	0.30 ± 0.01	0.31 ± 0.01
Total lipid (mg/dL)	347 ± 17 [a]	226 ± 16 [b]	232 ± 19 [b]	236 ± 22 [b]
PL (mg/dL)	170 ± 8 [a]	115 ± 5 [b]	114 ± 4 [b]	123 ± 5 [b]
TG (mg/dL)	74.5 ± 4.2 [a]	40.0 ± 5.5 [b]	49.1 ± 11.0 [ab]	45.9 ± 6.4 [b]

Table 8. (Continued).

	Control	SCO-M[1] 5%	SCO-U[2] 5%	TO 5%
Total-cholesterol (mg/dL)	102 ± 5 a	72 ± 5 b	69 ± 3 b	72 ± 6 b
HDL-C (mg/dL)	68.8 ± 4.1 [a]	44.5 ± 3.3 [b]	51.0 ± 1.8 [b]	53.1 ± 2.9 [b]
Na (mEq/L)	138 ± 1	139 ± 0	140 ± 0	139 ± 0
K (mEq/L)	4.54 ± 0.09	4.59 ± 0.07	4.51 ± 0.11	4.70 ± 0.09
Cl (mEq/L)	99 ± 1	99 ± 0	99 ± 0	99 ± 0
Ca (mEq/L)	10.6 ± 0.1	10.7 ± 0.1	10.8 ± 0.1	10.7 ± 0.1
IP (mEq/L)	5.28 ± 0.18	5.59 ± 0.31	5.64 ± 0.20	5.43 ± 0.27
Mg (mEq/L)	2.13 ± 0.06	2.13 ± 0.04	2.04 ± 0.04	2.08 ± 0.04
RBC (10^4 cell/μL)	848 ± 9	829 ± 6	851 ± 12	846 ± 13
WBC (cell/μL)	11975 ± 959	12563 ± 522	12425 ± 710	12400 ± 739
PLT (10^4 cell/μL)	57.6 ± 2.0	53.7 ± 2.0	52.0 ± 1.4	52.0 ± 2.1
Hb (g/dL)	15.6 ± 0.2	15.2 ± 0.1	15.6 ± 0.2	15.2 ± 0.3
Ht (%)	44.5 ± 0.5	43.5 ± 0.3	44.3 ± 0.3	43.2 ± 0.6
MCV (fL)	52.5 ± 0.5	52.6 ± 0.4	52.3 ± 0.6	51.0 ± 0.3
MCH (pg)	18.4 ± 0.1 [a]	18.3 ± 0.1 [ab]	18.4 ± 0.2 [ab]	17.9 ± 0.1 [b]
MCHC (g/dL)	35.1 ± 0.2	34.9 ± 0.1	35.3 ± 0.2	35.1 ± 0.2
Female				
Total protein (g/dL)	6.04 ± 0.06	6.30 ± 0.9	6.28 ± 0.09	6.10 ± 0.07
Albumin (g/dL)	3.83 ± 0.05	3.96 ± 0.03	3.96 ± 0.07	3.91 ± 0.06
A/G	1.73 ± 0.03	1.66 ± 0.04	1.71 ± 0.05	1.78 ± 0.02
AST (IU/L)	69.6 ± 2.2	75.5 ± 2.1	76.8 ± 1.6	68.3 ± 1.7
ALT (IU/L)	37.2 ± 3.1	41.7 ± 1.9	49.5 ± 4.1	41.6 ± 1.9
LDH (IU/L)	175 ± 31	172 ± 35	209 ± 37	165 ± 29
ALP (IU/L)	865 ± 91	1009 ± 86	942 ± 56	1066 ± 64
SUN (mg/dL)	26.3 ± 2.7	22.3 ± 1.7	24.1 ± 1.8	22.5 ± 1.4
Creatinine (mg/dL)	0.34 ± 0.02	0.30 ± 0.02	0.28 ± 0.01	0.32 ± 0.01
Total lipid (mg/dL)	278 ± 11	246 ± 15	270 ± 14	253 ± 9
PL (mg/dL)	166 ± 4 [a]	137 ± 5 [b]	143 ± 5 [b]	149 ± 6 [ab]
TG (mg/dL)	45.7 ± 6.8	44.4 ± 6.6	54.2 ± 8.6	34.1 ± 2.7
Total-cholesterol (mg/dL)	94 ± 3 [a]	77 ± 3 [b]	76 ± 4 [b]	87 ± 3 [ab]
HDL-C (mg/dL)	69.1 ± 2.0 [a]	56.3 ± 2.2 [b]	61.3 ± 3.0 [ab]	68.3 ± 2.5 [a]
Na (mEq/L)	138 ± 0	138 ± 0	137 ± 0	138 ± 0
K (mEq/L)	4.16 ± 0.12	4.21 ± 0.04	4.16 ± 0.07	4.08 ± 0.13
Cl (mEq/L)	101 ± 1	101 ± 0	100 ± 1	101 ± 0
Ca (mEq/L)	10.2 ± 0.1	10.5 ± 0.1	10.6 ± 0.1	10.4 ± 0.1
IP (mEq/L)	3.76 ± 0.24	4.23 ± 0.22	4.56 ± 0.40	4.20 ± 0.37
Mg (mEq/L)	2.21 ± 0.05	2.21 ± 0.03	2.13 ± 0.02	2.13 ± 0.06
RBC (10^4 cell/μL)	809 ± 12	821 ± 18	807 ± 7	820 ± 7
WBC (cell/μL)	9800 ± 478	11388 ± 981	12371 ± 518	9800 ± 529
PLT (10^4 cell/μL)	58.1 ± 1.7	53.4 ± 1.3	55.4 ± 2.2	52.0 ± 2.4
Hb (g/dL)	15.7 ± 0.2	15.7 ± 0.3	15.5 ± 0.1	15.7 ± 0.1
Ht (%)	44.5 ± 0.4	44.6 ± 0.7	43.5 ± 0.3	44.5 ± 0.3
MCV (fL)	55.1 ± 0.5	54.5 ± 0.6	53.9 ± 0.1	54.3 ± 0.3
MCH (pg)	19.5 ± 0.1	19.1 ± 0.1	19.1 ± 0.1	19.1 ± 0.1
MCHC (g/dL)	35.4 ± 0.2	35.2 ± 0.2	35.5 ± 0.1	35.2 ± 0.1

Data show means ± SEM (n = 8). Values in the same row not sharing a common superscript are significantly different at $p < 0.05$ using Tukey's multiple comparison test.

[1] Oil prepared from the internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).

[2] Oil prepared from the internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; A/G, albumin/globulin; Ca, calcium; Cl, chlorine; Hb, hemoglobin, Ht, hematocrit; HDL-C, high-density lipoprotein cholesterol; IP, inorganic phosphorus; LDH, lactate dehydrogenase; K, potassium; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Mg, magnesium; Na, sodium; PL, phospholipid; PLT, platelet; RBC, red blood cell; SUN, serum urea nitrogen; SCO, scallop oil; TG, triglyceride; TO, tuna oil; Toc, tocopherol; WBC, white blood cell.

Table 9. Thiobarbituric acid reactive substances (TBARS) levels in the serum and liver of rats administered with oil prepared from Japanese giant scallop (*P. yessoensis*) internal organs for 13 weeks.

	Control	SCO-M[1] 5%	SCO-U[2] 5%	TO 5%
Male				
Serum TBARS (nmol/mL)	3.4 ± 0.4	3.5 ± 0.2	3.7 ± 0.1	4.2 ± 0.2
Liver TBARS (μmol/g)	139 ± 7	156 ± 9	157 ± 7	158 ± 6
Female				
Serum TBARS (nmol/mL)	3.0 ± 0.2 [b]	4.6 ± 0.6 [a]	3.9 ± 0.2 [ab]	4.3 ± 0.3 [a]
Liver TBARS (μmol/g)	150 ± 6	137 ± 13	152 ± 6	140 ± 15

Data show means ± SEM (n = 8). Values in the same row not sharing a common superscript are significantly different at $p < 0.05$ using Tukey's multiple comparison test.

[1] Oil prepared from the internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).
[2] Oil prepared from the internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).
SCO, scallop oil; TBARS, thiobarbituric acid reactive substances; TO, tuna oil.

In this study, the increased serum TBARS level was also observed in the TO 5% group compared with the control group in female rats. Thus, although the SCO-M group showed an increase in the serum TBARS level, this was not a serious toxicological problem because it was also observed in the intake of tuna oil.

The presence of SCO-M and SCO-U at a dose of 5% in the diets demonstrated a lack of toxicologically significant adverse effects in a repeated 13-week oral dose toxicity study. These results demonstrated that SCO-M and SCO-U are safe components under these experimental conditions. The minimum daily intakes of SCO-M and SCO-U were 2,087 and 2,052 mg/kg BW in male rats and 2,209 and 2,208 mg/kg BW in female rats, respectively. These results indicate that the dietary administration of SCO-M and SCO-U at a percentage of 5% in diets did not show any toxic effects in repeated a 13-week oral dose toxicity study using rats.

There is a limitation in the repeated 13-week oral dose toxicity study. Histological evaluation was not conducted in the repeated 13-week oral dose toxicity study because macroscopic observation and relative organ weights did not reveal any abnormalities. However, a toxicity study is often carried out along with a histological evaluation. Therefore, histological evaluation is required to assure the safety of the use of SCO-M and SCO-U on a repeated 13-week oral dose toxicity study.

In summary, in repeated oral doses toxicity studies, the intakes of SCO-M and SCO-U at concentrations of 5% (w/w) in the diets for 28 days and 13 weeks using rats did not show any significant toxicologic manifestations. SCO-M and SCO-U were well tolerated at these dietary levels as evidenced by the absence of significant changes in the general condition and appearance of the rats, growth, and hematology and clinical chemistry parameters. These results suggested that SCO-M and SCO-U demonstrated no adverse toxicological in-life and clinical chemistry effects at a dose level of 5% in diets for rats, when administered for 28 days and 13 weeks. In addition, SCO-M and SCO-U possessed a similar toxicity profile as that of other n-3 PUFA sources (Blum *et al.*, 2007; Robertson *et al.,* 2014; Schmitt *et al.*, 2012). These data support that SCO-M and SCO-U are safe sources of n-3 PUFA in terms of subacute toxicity under the present experimental conditions.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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