Attached Sheet

Original: Japanese
Provisional translation

Fundamental Concepts in the Safety Assessment of Foods Containing Soy Isoflavones for the purpose of Specified Health Use

May 2006
Food Safety Commission
Novel Foods Expert Committee

Note 1: In this report “Soy isoflavones,” unless specified otherwise, indicates total isoflavone, including glycoside/glycosides and aglycone/aglycones, and where expressed as “soy isoflavone glycoside” or “soy isoflavone aglycone” indicates soy isoflavone glycoside or soy isoflavone aglycone respectively.

Note 2: In this report, when converting soy isoflavone glycoside to soy isoflavone aglycone, in the event that the composition ratio is not clearly defined, the ratio of the molecular weight of genistein (270.24) to that of its glycoside, genistin (432.38), i.e. (0.625), is used as the coefficient. The reason for using the value of genistein is that it has the greatest binding capacity to estrogen receptor of the soy isoflavone aglycones.

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Progress of evaluation

January 19, 2004	Request from the Minister of Health, Labour and Welfare for a food impact assessment relating to the application for approval as a food for specified health uses (Two food products containing soy isoflavone aglycone as ingredient exhibiting health functions (Isoflavone Miso, Oral Health Tablets - Calcium & Isoflavone))
January 22, 2004	29th Meeting of the Food Safety Commission (explanation of requested items)
March 9, 2004	7th Meeting of the Novel Foods Expert Committee
May 11, 2004	10th Meeting of the Novel Foods Expert Committee
May 28, 2004	Request from the Minister of Health, Labour and Welfare for a food impact assessment relating to the application for approval as a food for specified health use (A food product containing soy isoflavones as ingredient exhibiting health functions (Soy Isoflavones 40))
June 3, 2004	47th Meeting of the Food Safety Commission (explanation of requested items)
June 21, 2004	12th Meeting of the Novel Foods Expert Committee
August 30, 2004	15th Meeting of the Novel Foods Expert Committee
September 27, 2004	16th Meeting of the Novel Foods Expert Committee
October 18, 2004	17th Meeting of the Novel Foods Expert Committee
December 13, 2004	18th Meeting of the Novel Foods Expert Committee
March 16, 2005	21st Meeting of the Novel Foods Expert Committee
April 18, 2005	22nd Meeting of the Novel Foods Expert Committee
April 28, 2005	92nd Meeting of the Food Safety Commission
April 28 to May 25, 2005	Public Comments
June 14, 2005	24th Meeting of the Novel Foods Expert Committee
July 8, 2005	25th Meeting of the Novel Foods Expert Committee
November 7, 2005	29th Meeting of the Novel Foods Expert Committee
December 12, 2005	30th Meeting of the Novel Foods Expert Committee
January 31, 2006	32nd Meeting of the Novel Foods Expert Committee
February 20, 2006	33rd Meeting of the Novel Foods Expert Committee
March 9, 2006	134th Meeting of the Food Safety Commission
March 9 to April 5, 2006	Public Comments
May 8, 2006	36th Meeting of the Novel Foods Expert Committee
May 9, 2006	Report to Chairperson of the Food Safety Commission from the Chairperson of the Novel Foods Expert Committee
May 11, 2006	Notification of the result from safety assessment to the Minister of Health, Labour and Welfare

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1 Introduction

Soy isoflavones are types of flavonoids derived from soybeans and are particularly prevalent in soy germ. Up until now, foods for specified health use containg soy isoflavone glycoside as an ingredient responsible for health functions in the form of a soft drink (40 mg/day as soy isoflavone glycoside, 25 mg/day aglycone equivalent) to assist in maintaining bone calcium, have already been approved and are commercially available.

In this context a safety assessment was conducted with regard to the following products as food for specified health use based on a request from the Minister of Health, Labour and Welfare for a safety assessment relating to the application for approval as a food for specified health use, by the Food Safety Commission Novel Foods Expert Committee.

Product name	Ingredient responsible for health functions	Indicated volume	Product outline
Oral Health Tablet Calcium & Isoflavone	Soy isoflavone aglycone	9 mg/day	Food product in tablet form containing soy isoflavone aglycone & calcium
Isoflavone Miso	Soy isoflavone (aglycone and glycoside)	53 mg/day (48 mg/day aglycone equivalent [a)])	Miso paste with soy isoflavone aglycone added
Soy Isoflavone 40	Soy isoflavone glycoside	40 mg (26 mg/day aglycone equivalent [a)])	Food product in tablet form containing soy isoflavone glycoside

These three foods differ from the foods already approved, that are commercially available as foods for specified health use, in respect of ingredient for health functions (glycoside or aglycone), amount of ingredient, and type of food.

Japan, one of the foremost long average life span countries in the world, has a particularly low incidence of breast cancer and prostate cancer etc. and thus the diet of Japanese people has been believed to one of the associated factors for this. That diet includes soy products (tofu, natto, miso etc.) which are low in fat, and rich in plant protein, calcium etc, and has been considered to be a factor in the healthy diet of Japanese people.

In Japan, various soy products containing soy isoflavones have been consumed daily. In other words, Japanese people have ample experiencs of eating soy isoflavones as components/incradients of soy products. No specific concern has been raised on the dietary intake of soy products up until now.

People, however,-have no experience to eat foods concentrated in soy isoflavone alone, or foods surmounted by soy isoflavone. It is thus these foods differ in their ratios of soy isoflavone to other ingredients (protein, calcium etc.) from food of natural origins. In addition, there are debates on the use of soy isoflavone intake as a practical index for measurement of dietary intake of soy products, and on the use of high amounts for health

[a] Equivalent value based on materials submitted by the applicant

enhancement. Currently, efficacy of the intake of soy products is discussed as a source of protein as well as isoflavone in the Japanese. In another word, there are queries/ambiguities on whether soy isoflavones alone is effective to take, or whether the entire soy product is necessary to be consumed. It is also uncertain whether the overall Japanese dietary custom consuming relatively high amounts of soy products as the source of protein is effective in its entirety. In recent epidemiological studies on Japanese, the risk of breast cancer is shown to decrease from the small intake group to the high intake group, when divided based on dietary intake of soy products into four separate groups [94].

In different with protein and calcium, soy isoflavone has, however, not been regarded as essential nutrient.

Soy isoflavone is a group of phytoestrogens. It has a chemical structure similar to that of estrogen and thus binds to estrogen receptors. Soy isoflavone is shown to have the synagistic and antagonistic effects *in vitro* and in experimental animal tests. Soy isoflavone is reported to induce apoptosis in human cancer cells[111], It is also known on inhibition of proliferation[112] and of cell invasion[113], also the enhancement of carcinogenesis[137], and in *in vivo* there are reports of an anti-carcinogenetic effect[114][115] and an enhancement for carcinogenesis in internal organs[116][117]. It is inferred that these results could be demonstrated in humans too. For example, in spite of the preventative effect for osteoporosis, breast cancer and prostate cancer may be expected. There is also a possibility of the increased risks of breast cancer development or relapse. However, a great deal of research is still being conducted, and discussion on the efficacy and safety of soy isoflavone in humans has not yet established.

The Food Safety Commission Novel Foods Expert Committee has expressed the health concern on the safety of long term dietary intake from the viewpoint of insufficient experience for the eating of foods concentrated or enhanced with soy isoflavone aglycone alone, for uncertainty on the efficacy and safety of soy isoflavone aglycone, and also for the amount of isoflavone included. One of the three foods that are under application is expected to contain the isoflavone amount greater than the approved previously (about 1.9 times (aglycone equivalent)).

This report is investigating the fundamental concepts in the safety assessment of Foods for specified health use containing soy isoflavones, and investigations will proceed on the premise that;

(1) The intake characteristics (large and continuous intake) of foods containing soy isoflavone, based on the dietary intake of soy products in Japan, must be taken into consideration as Foods for specified health use.
(2) Both the benefits and hazards of soy isoflavone are investigated based on soy isoflavone data on humans, if those are available.
(3) Epidemiological data based on human endocrinological characteristics, which take into consider the age groups, gender and so on, are desirerable, but are not currently available as published reports. Thus the tests with experimental animals are included as auxiliary data.
(4) The soy isoflavone-mediated action throughestrogen receptor shall be used as the main indicator.

Note, in the results of animal experiments stated in (3) on aspects of safety, as the function of estrogen system is maintained/conserved throughout mammals including humans with high commonalities, results obtained in animal experiments may be reproduced in a certain situation in humans also (biological plausibility[b]). There are, however, differences in sensitivity among species and also within one species (subpopulations, it is thus uncertain whether the same phenomenon would be developed at the identical doses for humans and animals.

Following the above basic principles, the investigation on the fundamental concepts in the safety assessment of foods for specified health use containing soy isoflavones, was organized as follows.

- Intake of foods for specified health use was regarded as either additional intake over normal eating habits, or as a replacement.
- Different from pharmaceuticals where dosage and administration is managed under the instructions of medical personals, foods are consumed by a whole population including healthy, candidates for life-style related disease, young/old, male/female, for which foods for specified health use are no exception.
- In the National Dietary Survey, there have been no significant changes for the past 30 years in the dietary intake of soy products.
- In the past, people have experience of eating soy products, however have insufficient experience eating foods concentrated in or enhanced by soy isoflavone.

Based on these points, in the setting of the upper limit of the daily dietary intake of soy isoflavone as ingredients exhibiting health functions of the foods for specified health use, the amount of soy isoflavone consumed from soy products was determined as the basis for dietary experience. And the upper limit of daily dietary intake was also consideration. Assessment was done with the idea that the range for the upper limit is desirable to address a total amount of soy isoflavone taken both as various foods and foods for specified health use. In different from therapeutic medicines, foods for specified health use are not prescribed by doctors after diagnosis of individual symptoms, assessment was undertaken with the concept that the dietary intake level must ensure the sufficient safety.

Specifically, this report sets the intake amount of soy isoflavone as a food for foods for specified health use from the reports summarized from 2 to 4, and validates that amount for each age group.

[b] Biological plausibility: The cause and effect relationship at issue (or the relationship between two matters), has a consistency with already existing medical/biological knowledge. Here, with regard to the mechanism of action of estrogen, which is basic and universally inherent in mammals, we have made comparisons to the medical and biological knowledge that we have access to, for example, even if there is no direct confirmation in humans, it is indicated that there is sufficient consistency with confirmed information including animal test results, and an equivalent phenomenon can be expected in humans.

2 Overview of soy isoflavone

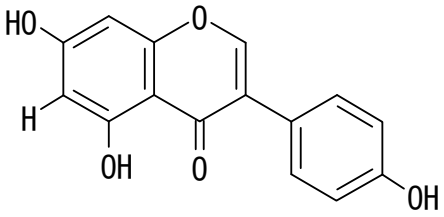
2.1 Properties of soy isoflavone

Soy isoflavone is a type of flavonoids contained in soybeans and is particularly prevalent in soy germ, there are three types of aglycones; genistein, daidzein, glycitein (isoflavone aglycone). These also exist as their respective glycosides (genistin, daizin, glycitin); glycoside acetyl forms and malonyl forms.

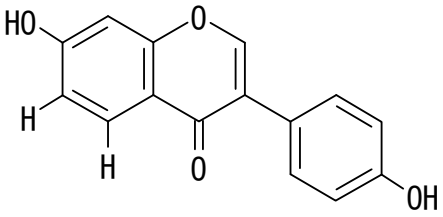
Soybean fermented foods such as miso and natto are rich in soy isoflavones, in these prepations, soy isoflavone glycosides share the major portions normally.

The chemical properties of the main aglycones (genistein, daidzein) are shown as follows.

[Structural formula]



Genistein



Daidzein

[Property]

Genistein

Molecular formula: $C_{15}H_{10}O_5$ (molecular weight 270) colorless long needle crystals with a melting point of 296-298℃. Poorly soluble in glacial acetic acid/cold ethanol, highly soluble in ether, hot ethanol. Becomes yellow after dissolving in alkali, and turned dark-red in the ethanolic iron chloride (III) solution.

Daidzein

Molecular formula: $C_{15}H_{10}O_4$ (molecular weight 254) colorless columnar crystals with a melting point of 315-320℃. Insoluble in water, and soluble in methanol, ethanol and acetone. Becomes yellow after dissolving in alkali, and detected by the fluorescence by UV irradiation. Decomposed to form formic acid, resorcin, and *p*-hydroxybenzoate with alkali.

2.2 Internal metabolism of soy isoflavones (kinetics)

Soy isoflavone glycosides are hydrolyzed by enzymes in saliva[133)] and small intestinal mucosa[134)], and also by ß-glucosidase contained in intestinal bacterium, whereby the aglycones daidzein, genistein are produced[1)]. The aglycones and metabolites are absorbed at the intestinal tract and then transported to the liver via the portal vein. The glucuronic acid and sulfate conjugates are excreted to the bile. Parts of the metabolites are deconjugated at the intestine mainly by ß-glucuronidase., contained in intestinal bacterium and subjected to enterohepatic circulation through the reabsorption. Therefore, the majorities of ingested levels of flavonoids are excreted in the urine[2)3)].

The secondary metabolites of soy isoflavone consist of equol and o-desmethylangolensin (*O*-DMA), which are respectively formed from daidzein. In addition, dihydro-genistein is produced from genistein.

A schematic profile of this soy isoflavone metabolism is shown in Appendix 1 as the pharmacokinetics of soy isoflavone.

2.3 Currently known biological effects of soy isoflavones

2.3.1 Biological effects via estrogen receptor

Soy isoflavones are structurally distinct from steroids, but share some extents of the similarity with estrogens, and thus bind to estrogen receptor (ER: ER-α, ER-ß), and demonstrate the biological effects.

ER-α is abundantly found in the female reproductive organs (uterus, vagina, ovary), and is also detected in the mammary glands, hypothalamus, endothelial cells and vascular smooth muscle. Another form, ER-ß is abundant in the prostate gland and ovary and also present in the lungs, brain, blood vessels and bones[7].

The following phenomena are observed on soy isoflavones' binding affinity to ER.

- Genistein rather than daizein shows a stronger affinity for binding activity to the ER. Equol, a metabolite of daidzein, has the slightly stronger affinity than does genistein[8].
- For the binding affinity of soy isoflavone to ER-α, genistein was estimated as 4/100 of estradiol (E2) and daidzein was 1/1000 in the solid-phase binding assay. According to solubilized receptor-ligand binding assay, genistein was calculated as 7/1000 (equivalent), and daidzein was 2/1000[9]. In another study, the binding affinity of genistein to ER-α was reported to 4/1000 of estradiol. Also, the binding affinity of ethynyl estradiol (EE) was assumed to be 1.9 times higher than that of E2[10].
- For the binding affinity of soy isoflavones to ER-ß, genistein was estimated as 87/100 of E2 and daidzein was 5/1000 in the solid-phase binding assay. According to solubilized receptor-ligand binding assay, genistein was calculated as 13/100(equivalent), and daidzein was 1/100[9].
- 10-100 nM genistein possess the activity quivalent to physiological levels of estrogens. Thus, the estrogenic potency of genistein at concentrations of 1000 nM was greater than that of physiological levels of E2[9].

Individual differences in the biological effects of soy isoflavones may come from alterations of absorption and metabolism. The difference in affinity of equol production, a soy isoflavone metabolite, has also been considered. Although yet to be sufficiently clarified scientifically, it is thought that certain types of intestinal micro flora may contribute to the production of equol[8]. Also, the frequency of people that produce equol, although it depends on race and sex, ranges between 20-60%[25].

2.3.2 Topoisomerase inhibition effect

Flavonoids such as soy isoflavones and quercetin inhibit topoisomerase II which works to maintain normal DNA structure, and these have the ability to cause abnormalities (mutations such as translocation/rearrangement) of *MLL* (myeloid-lymphoid leukemia) genes.

Rearrangement of *MLL* genes can also be induced by anti-cancer agents VP16 and

doxorubicin (topoisomerase II inhibitor), acute myeloid leukemia and acute lymphoid leukemia have been known to develop after treatment with these drugs.

MLL gene abnormalities are implicated in 65% of acute myeloid leukemia and 85% of acute lymphoid leukemia in infants, however this drops to only 5% for those diagnosed aged 1 or older, so it is thought that *MLL* gene associated leukemia in infants occurs inside the womb. These reports imply the concerns of the impact of exposure of a fetus to substances with topoisomerase II inhibiting properties during pregnancy.

The following report shows comparing soy isoflavone to chemotherapeutics in *MLL* gene cleavage due to topoisomerase II inhibition.

- In an experiment using hematopoietic system cultured cells, *MLL* gene cleavage caused by the anticancer agent VP16 (25 μM) was equivalent to those induced by 50 μM genistein, 100 μM genistin and 200 μM daidzein. The *in vitro* experiment that used purified human topoisomerase II and DNA showed that this effect was caused by topoisomerase II inhibition. It is reported that genistein produces inhibition level equivalent to VP16, and genistin and daidzein possess 50% of that affinity[118].

2.3.3 Other

Several reports resulted from animal and human tests are available on the effect on thyroid function, and in iodine deficient state, a thyroid peroxidase (TPO) inhibitory effect of soy isoflavone is detected. For this reason, some countries continue to supplement iodine to soy-based infant formula since 60's.

2.3.3.1 Animal testing

- It has been reported that soybean feeding and iodine deficiency synergistically induce thyroid follicle cell hyperplasia, increases in serum thyroid stimulating hormone (TSH) concentration and thyroid weight, and also induce ultra structural changes to the thyroid and pituitary glands in female rats[91].
- It has also been reported that administration of soy isoflavone in female rats under the conditions of iodine deficiency, did not influence thyroid weight or thyroid tissue histo-pathology[92].
- In the experiment with rats fed soy isoflavones with soy-free feed, TPO activity is decreased dependent on the concentration, but it has no impact on thyroid weight or hormone concentration[93].

2.3.3.2 Human tests etc.

- In the 1950s and 1960s, 12 cases were reported on changes in thyroid function in infants fed soy-based infant formula, The most cases were assumed to be caused by goiter despite the fact there were no symptoms of decreased thyroid function due to iodine deficiency[11)37)].
- It has been reported that, under iodine-deficient dietary conditions, genistein and daidzein inhibit TPO activity, an enzyme involved in iodothyronine hormone synthesis, and iodine supplementation to soy-based infant formula was implemented in the 1960s[11].
- There is a clinical report, a male infant receiving soy-based infant formula was diagnosed with congenital hypothyroidism, and treated using thyroxine (T4) commencing 11 days

after the birth, however the his hypothyroidism continued until the diet was changed to cow's milk[11].

In Japan, there are high levels of dietary intake of iodine from foods such as seaweed. No clitical impact on thyroid function for adults is thus considered. However, infants intake low amounts of seaweed as compared to adults, and so issues wthe level of soy isoflavone intake cannot be ruled out.

2.4 Diagnostic markers used in soy isoflavones safety assessment

Of the biological effects enumerated in 2.3, this report, in principle, is conducting investigations using ER mediated effects as the indicator of soy flavonoid action from the following reasons.

Soy isoflavones are assumed to contribute to human health, principally, ER-mediated actions. Hence, both the “efficacy” and “harmful effects” are possible to appear.

While topoisomerase inhibition is clear to appear at a comparatively high concentration, the ER mediated effects are observed at low concentration ranges.

Also, at present no reliable reports are available on the benefits of soy isoflavonoid action on topoisomerase inhibition.

We set the diagnostic marker as the lowest dose of ER-mediated effects, regarding the relationship between dietary intake (amount) of soy isoflavone and human health effects, using data obtainable/scientific findings at this point in time. Therefore individual investigations were also undertaken in accordance with conditions of subjects.

As the ER mediated effects of soy isoflavones are being used as the indicator, considering receptivity to estrogen as the basis, subjects are divided into premenopausal females, postmenopausal females, and males. Pregnant women, fetuses, infants and small children are to be investigated separately.

3 Dietary intake of soy isoflavone from soy food products

A provisional calculation of the dietary intake of soy isoflavone in Japan was made after sub-dividided by gender and age using soy isoflavone contents in various soy products, which are based on the daily consumption of soy products such as tofu, miso and and the results of the 2002 National Nutrition Survey[26].

3.1 Content of soy isoflavones in various soy food products

Based on the reported values of soy isoflavones determined in various soy products, the soy isoflavone aglycones (equivalent value) in soy products are summarized in Table 1[27].

The values are described as soy isoflavone aglycones analyzed with acid hydrolysis.

Table 1　Soy isoflavone aglycone content in various soy products (equivalent value)

(soy isoflavone aglycone mg/100 g)

Name of food (sample No.)	Content	Average content
Soybean (11 samples)	88.3-207.7	140.4
Cooked soybeans (3 samples)	69.0-74.7	72.1
Roasted soybeans (1 sample)	200.7	200.7
Soy flour (2 samples)	211.1-321.4	266.2
Tofu (4 samples)	17.1-24.3	20.3
Frozen tofu (1 sample)	88.5	88.5
Tofu refuse (1 sample)	10.5	10.5
Kinzanji miso (1 sample)	12.8	12.8
Fried (tofu) (3 samples)	28.8-53.4	39.2
Natto (2 samples)	65.6-81.3	73.5
Miso (8 samples)	14.3-81.4	49.7
Soy sauce (8 samples)	0.7-1.2	0.9
Soymilk (3 samples)	6.9-53.8	24.8

3.2 Dietary intake of soy isoflavones from soy food products based on 2002 National Nutrition Survey (provisional calculation)

In order to grasp the soy isoflavone dietary intake of Japanese people (aged 15 and over), a provisional calculation of the daily intake of soy isoflavone aglycone (equivalent value) was conducted based on the 2002 National Nutrition Survey[26].

[About the National Nutrition Survey]

The 2002 National Nutrition Survey calculated the dietary records of the survey participants (11,491 subjects of 4,246 ramdomly selected households) for one specified day in November 2002, to express nutrient intake for one person for one day nationwide.

Although the average dietary intake is considered to express the average daily intake for one person nationwide, it is considered dietary intake distribution and variance do not indicate the percentages of people who consume a lot of a nutrient daily and those that consume none at all. This means that it is unclear from the results of the National Nutrition Survey whether a person who consumed 90g of a specific food on the specific day of the National Nutrition Survey continued to consume that amount from the next day onwards.

In the provisional calculation, using the national average for dietary intake of soybean/its products, and miso/soy sauce from the results of the 2002 National Nutrition Survey, consumers of those foods 15 years old and over were classified as specified in 2.4 into premenopausal females (15-59 y.o.), postmenopausal females (50 y.o. and over) and males, and the dietary intake of soy products calculated for each group. The values were then multiplied by the average soy isoflavone content in the various soy products shown in Table 1, and the daily intakes of soy isoflavone aglycone (equivalent value) were determined. The dietary intake distribution for each group is shown in Appendix 2. Note, because there are individual differences in the age of menopause, 50-59 year old females were classified as both premenopausal and postmenopausal.

As a result, the median soy isoflavone intake and 95 percentile value[c)] were 16 mg/day, 64 mg/day respectively for premenopausal females, 22 mg/day, 74 mg/day for postmenopausal females and 18 mg/day, 76 mg/day respectively for males (Table 2, Appendix 2).

Note, according to the results of National Dietary Surveys from 1975 to 2002, the daily dietary intake of soy products has moved between the ranges of 63.2-70.2 g, so it is considered that there hasn't been considerable change in soy isoflavone dietary intake from soy products.

Table 2 Daily intakes of soy isoflavone aglycone (equivalent)[d)] provisionally calculated from the results of the 2002 National Nutrition Survey

(Soy isoflavone aglycone dietary intake (equivalent) mg/day/person)

	Median (50 percentile value)	95 percentile value
Premenopausal females (15-59 y.o.)	16	64
Postmenopausal females (50 y.o. and older)	22	74
Males (15 y.o. and older)	18	76
Total	18	70

3.3 Others

There are some written reports with regard to daily intakes of soy isoflavone from soy products consumed in the dietary habits of Japanese people (adults).

- According to a one day dietary records in Japan (Tokai region) targeting 1232 people (886 males: 54.4±7.7 years, 346 females: 57.8±4.8 years), and a dietary records totaling 16 days targeting 88people (46 males: 52.5±4.5 years, 42 females: 49.8±8.6 years), the median of total dietary intake of genistein and daidzein was 31.7 mg, 24.4 mg/day respectively and 75 percentile value was 51.4 mg/day, 31.6 mg/day respectively[28)].
- In Japan (Tokai region), the soy isoflavone average dietary intake calculated using soy isoflavone content analyzed from meal samples manufactured using the market basket method was 22.2 mg/day (soy isoflavone aglycone equivalent)[29)].
- According to a dietary survey that targeted 1528 female farmers in 5 regions of Japan, the 90 percentile value for total dietary intake for daidzein and genistein was calculated to be 75.8 mg/day and the 95 percentile value was 91.3 mg/day[30)].

  According to a 3 day dietary records that targeted 50 females (aged 32-68) in Japan (Tohoku region), the average total dietary intake of daidzein and genistein was 39.5 mg/day (range 7.8-87.7 mg/day)[31)].

- In a 3 day dietary records targeting 115 females mostly employed in farming (29-78 y.o) in northern Japan, the average total dietary intake of daidzein and genistein was 47.2 mg/day (12.0-118.9 mg/day). Also, 6 people (5.2%) of this group exceeded 100 mg/day[32)].

[c] Percentile value: When the measurement values are placed in ascending order, the measurement value in the position of the arbitrary position for the number of measurement values. e.g.) The 10 percentile value in 1,000 sample measurement values indicates the measurement value in the 10% position (No100) of the measurement values in ascending order.

[d] Total soy isoflavone aglycone (equivalent) contained in soybean/processed food (soybean (whole)/ processed food, tofu, fried tofu, natto, other soybean processed food), and as seasonings, miso and soy sauce, in the table of National Dietary Survey by food groups.

Soybeans and soy products are considered as one of the sources for plant protein during infanthood and school age in Japan. Meanwhile it has also been reported that soy-based infant formula has been consumed for more than 50 years in the US.

4 Test reports on soy isoflavones

Of the soy isoflavone dietary intake values within the study reports etc., that are marked with an asterisk, it is unclear whether it is glycoside or aglycone, otherwise unless stated, it refers to the amount of aglycone.

4.1 Reports on pharmacokinetics

4.1.1 Animal testing

Some tests using rats, have noted the kinetics of soy isoflavone metabolites, and that glucuronic acid conjugate fraction presents to a maximum of 90%, is biologically inactive, and free and sulphate conjugate fractions are normally biologically active[11].

Also, it has been reported that the source of the soy isoflavone affects on the bioavailability of metabolites into the blood and also on the influence with female estrous cycle.

- On the level of daidzein, genistein and specific metabolites equol and 4-ethylphenol in the blood, urine and feces after single oral administration of soy isoflavone glycoside to rats, plasma daidzein concentration showed a peak about 2 hours after the intake, with the concentration approximately 2 times higher than that of genistein. The concentration, however, decreased afterwards, and no difference was detected in both concentrations after 15 hours. Urinary excretion of daidzein was estimated as approximately 17% of the dose 48 hours after intake and 12% for genistein. Also, approximately 5% of daidzein was excreted as equol, and approximately 42% of genistein was excreted as 4-ethylphenol. Excretion in feces 48 hours after intake was 2.3% daidzein and 3.4% genistein, and thus the bioavailability of soy isoflavone in rats was assumed to be higher for daidzein than for genistein[12].
- A 16 weeks term study was done on the influence to growth with post weaned rats fed soy isoflavones. Soy-based infant formula or soy extract was used as the source of the soy isoflavones. Plasma concentrations of soy isoflavones increased dependent on the dose, however the rate of metabolism of the soy-based infant formula was lower. The length of estrous cycle was increased in a dose-responsive manner; however the influence was not striking for the soy-based infant formula. These results suggest that there is a possibility that the effects of soy isoflavones differ slightly depending on the source[13].
- Pharmacokinetics of genistein was compared with male and female mice after the subcutaneous–administration–on postnatal days 1-5. The average fraction presents as active aglycone was similar to those in fetal and neonatal rats from placental and lactational exposures. Also, the elimination half-time was 3-7 times longer than that of rats and 2-3 times that of humans. These results suggest the low conjugation capacityas the significant factor on the estrogen effect in neonates[121].
- Daidzein glucoside is hydrolysed by lactase phlorizin hydrolase in the alimentary canal and then absorbed. This is thought to be one reason for the rapid absorption in rats after

intake of conjugates[134].

4.1.2 Human tests and observational studies etc.

There are several reports on the pharmacokinetics of soy isoflavone in healthy Japanese adult male and females, pre and postmenopausal females and also on other ethnics including Americans.

In the human pharmacokinetics of soy isoflavone, differences in the form of isoflavone intake (form of food) and individual differences in amounts of soy isoflavone excreted in the urine varied several times to several dozen times, In addition a striking individual differences was observed on the excretion of the daidzein metabolites equol and *O*-DMA, In literature reporting individual differences in amounts of equol excreted,the diffedrence in the urine during peak period reached more than 1500 times[25], suggesting the possibility of racial differences in the extents of individual differences.

- Infants soy isoflavone absorption and excretion may differ from adults[11], because of limited capacity for hydrolysis of soy isoflavone glycosides in developing intestinal microflora in infants.
- A studty on the relationship between GTT (Gut transit time) and the bioavailability of soy isoflavone was done with 68 females (18-43 y.o., 35 Asian females, and 33 Caucasian females) given soymilk powder.the GTT was 40±8 hours with of the Asian female subjects that excreted less genistein in the feces, and the genistein excretion ratio in the urine was 11±2.7%. While the GTT was much longer (63±5 hours), with a lower excretion ratio in the urine (4.0±1.7%) with the Asian female subjects that excreted more genistein in the feces. The GTT of the Caucasian females (84±5 hours) was longer than Asian females (56±6 hours), and the genistein excretion ratio in the urine was similar to the Asian female subjects that excreted more genistein in the feces. From these results, it was considered that subjects with a rapid GTT and lower isoflavone excretion in the feces had a higher bioavailability of soy isoflavone (migration rate to circulatory blood flow)[15].
- Soy isoflavone-containing tablets (0.11 mmol: glycoside 50 mg/day or aglycone 30 mg/day) were given to 4 males and 4 females (Japanese, 31-58 y.o.). The blood levels of daidzein and genistein reached their maximal at 2 hours after aglycone intake and 4 hours after glycoside intake, and the levels were more than twice higher after aglycone intake than glycoside intake. The higher blood levels of genistein than of daidzein were observed in both aglycone and glycoside intake. Soy isoflavone elimination from the blood was faster after aglycone intake[16].
- Soy isoflavone-containing tablets were given to 4 males and 4 females (Japanese, 38-57 y.o.), which contain soy isoflavones (1.7 mmol: glycoside 760 mg/day and aglycone 450 mg/day., The blood levels of daidzein and genistein reached their maximal concentrations at 4 hour after aglycone intake, and also at 4 hours after for daidzein and 6 hours after for genistein of glycoside intake. Isoflavone concentration was more than 5 times higher after aglycone intake than after glycoside intake. Soy isoflavone was eliminated quickly from the blood after aglycone intake as compared to glycoside intake, but no differences are detected on their lconcentrations at 24 hours after intake[16].
- Soy isoflavone-containing tablets, containing soy isoflavone aglycones (30 mg/day), were given to 7 males and 6 females (Japanese, 30.9±4.2 y.o.) for 2 weeks. The blood

concentrations of daidzein and genistein were significantly raised during the ingestion period as compared with before intake, but reversed to the level observed prior to intake at 1 week after the ingestion period[17].

- Judging from the data of a dietary survey in Japan (Tohoku district), the average total dietary intake of daidzein and genistein is estimated as approximately 0.18 mmol/day (47 mg/day), with the maximum value 0.45 mmol/day (119 mg/day). The average soy isoflavone concentration in the blood was calculated as daidzein 0.11 μmol/L, genistein 0.31 μmol/L, and the maximum value 1.77 μmol/L and 2.46 μmol/L respectively[18]
- In a double blind crossover experiment with single administration,–miso fortified either soy isoflavone aglycone or glycoside (containing equivalent amounts) was given to 10 females (5 premenopausal females, 5 post menopausal females), on the soy isoflavones in the blood up to 24 hours after intake, the blood concentration after the agycone intake did not exceed the blood concentration observed after the glycoside intake[19].
- Tablets containing soy isoflavone (1.3 mmol/day: glycoside 130 mg/day and aglycone 80 mg/day) were given 8 males (Japanese, 38-55 y.o.) for 4 weeks each. The concentration of daidzein and genistein in the blood was more than 2 times higher during the aglycone intake period than that during the glycoside intake period, and maintain a certain level throughout the experimental period[16].
- Tablets containing soy isoflavone glycoside (aglycone equivalent 47 mg/day) were given to 33 postmenopausal females (Japanese 53.8±2.9 y.o.) for a 6 month period, and the daidzein, genistein and glycitein concentrations in the blood after 6 months were determined to 0.89±0.84 μmol/L, 0.34±0.30 μmol/L and 0.17±0.156 μmol/L respectively, and the daidzein and glycitein concentrations were significantly higher than the start of the experiment[122].
- Roasted soybeans (15, 30, 60 mg/day), were given to 5 premenopausal females (29-48 y.o.) and 5 postmenopausal females (49-56 y.o.) (of unknown race, however experiment conducted in US) daidzein, genistein and equol in the blood and urine were determined after intake. No difference in the pharmacokinetics of soy isoflavones were detected between pre and postmenopausal females. The concentration of daidzein and genistein in the urine increased in line with an increase in soy isoflavone intake, while the excretion ratio as a percentage of administered doses decreased. Also, it was confirmed that excretion of equol in the urine was slower than daidzein and genistein[20].
- Pharmacokinetics of daidzein, genistein and equol were investigated after administration of tablets containing soy isoflavone (about 0.11 mmol/L: glycoside 48 mg and aglycone 30 mg), 15 females (excluding 1 dropout, American, 46±6 y.o.). Mean Cmax, tmax, and AUC values for genistein were not significantly different after ingestion of aglycone or glucoside. However, Cmax and AUC values, but not tmax were significantly higher for daidzein after aglycone ingestion. The AUC for equol was significantly higher after ingestion of the glucoside tablets[21].
- On measurement of daidzein and genistein in the blood and urine of 6 males (5 Caucasians, 1 Asian, 21-48 y.o.) given a drink containing dissolved soybean powder (6.3 μmol/kg BW: about 1.6 mg/kg BW), The blood concentration rose slowly, and reached to a maximum values 3.14±0.36 μmol/L at 7.42±0.74 hours for daidzein, and 4.09±0.94 μmol/L at 8.42±0.69 hours for genistein. The amount of daidzein excreted in the urine was greater than genistein, however the AUC ratios were similar to as observed with a test meal, suggesting the similar capacity for bioavailabilities of both daidzein and genistein[22].

- Soy milk (soy isoflavone aglycone 158 mg/day) and a control meal ("isoflavone free" soy milk) were given to 8 premenopausal females (6 Caucasians, 2 African Americans, 33±6.1 y.o.) for a menstrual cycle in a cross-over study, and the excretion in the urine of the metabolite 17ß-estradiol was quantified. There was a 47% (P=0.03) increase in 2-hydroxyesterone in the urine for a group of the test meal intake as compared to the intake of the control. On the other hand no difference in the amount of 16α-hydroxyesterone was observed. These results suggest the enhanced metabolism of endogenous estrogen during intakes of soy milk[23].
- In a randomized double blind cross over study with 6 postmenopausal females (European, 55.5±5 y.o.). Drinks containing soy isoflavone aglycone and glycoside (each consisting of 1 mg/kg body weight/day as soy isoflavone aglycone) were given. No observed difference in the soy isoflavone pharmacokinetics in plasma or urine, suggests no apparent difference in the bioavailability of isoflavones[24].
- In a randomized cross over study where 21 premenopausal females (18-53 y.o), 17 postmenopausal females (48-69 y.o.), 21 males (18-55 y.o.) are involved, soy milk, soy extract protein, or Tempe (each corresponding to 0.44 mg/kg weight) were given. Serum isoflavone concentrations increased after intake of each test food, and genistein concentrations exceeded daidzein concentrations. Also, females had a higher maximum blood concentration of daidzein. In the intake of Tempe, considered to be rich in aglycone, there was a higher soy isoflavone maximum concentration shift compared to that of soy extract protein. Soy isoflavone is metabolized faster and reaches a maximum blood concentration earlier for soy milk intake, suggesting a difference in the kinetics of soy isoflavone depending on the form of food taken[135].
- Regarding the proportion of soy isoflavone in the serum at 3-4.5 hours after intake of soy isoflavone, it was reported that genistein derived components/metabolites exists as 50-90% glucuronic acid conjugate, 5-20% sulphate conjugate and 10-25% aglycone. Daidzein derived components exists as 30-60% glucuronic acid conjugate, 25% sulphate conjugate and close to 20% aglycone[4)5)6)].

As a result of survey of these test reports, there are no obvious differences in the pharmacokinetics of soy isoflavone between pre and postmenopausal females, and in neonates, conjugation capacity is low and excretion half-life is long. When comparing intake of soy isoflavone from food and the intake of glycoside in tablet form (aglycone equivalence), although there is no difference in blood concentration of genistein, the latter gives higher concentrations of the equol precursor daidzein.

From the above, it is considered that both orally taken soy isoflavone glycoside and aglycone are comparatively efficiently absorbed by the body. However, deconjugation capacity can be expected to differ between races.

4.2 Test reports regarding safety

4.2.1 Animal tests

Oral and subcutaneous administration experiments using mouse, rat, and monkey, have been conducted mainly on genistein, which has high estrogen activity, in order to investigate it (1) as an analyte amongst various chemicals measured in bioassays to measure estrogen activity (uterine hypertrophy etc.) or (2) its effect on pregnant animals and fetuses through

experiments administering to pregnant animals or effect of administering to neonates etc. In these experiments, in (1) it caused an increase in uterine weight and was interpreted as estrogen effect positive, and in (2) abnormal etc reproductive function was observed in not only the mother animal but in the offspring as well.

Regarding the results of tests, we investigated extrapolative potential, and possibility of effect on humans from administration period (animal age in weeks)/ administration dosage, and observed symptoms and no-observed-effect level.

- Subcutaneous administration (50 mg/kg/day) of genistein to mouse neonates induced a significant gain in uterine weights 5 days after birth, and 18 months later in all cases, there was aberrant growth of fallopian tube epithelium and absence of corpora lutea. Also, complicated endometrial hyperplasia (47%), atypical endometrial hyperplasia (5%), squamous metaplasia (64%) and uterine gland cancer (35%) were observed in the uterus. While in the control group, uterine adenomyosis (6%) and complicated endometrial hyperplasia (19%) were observed. The same level of ovarian cysts was observed in both groups[34].
- No significant difference was observed in the day of vaginal opening using subcutaneous administration (0.5, 5, 50 mg/kg/day) of genistein from 1-5 days after birth of mouse neonates compared with the control group, however there was a lengthening of abnormal estrous cycles accompanying an increase in severity of symptoms corresponding to dosage and age in weeks. Also the number of surviving female mouse infants at periods of 2, 4 and 6 months decreased with increases in genistein dosage and with the passing of time. The number of live pups rate at 6 months, where the control groups was 100%, was 60%, 40%, 0% for mice administered 0.5, 5, 50 mg/kg/day of genistein. At 2 months of age, more than 60% of the 50 mg/kg/day administration group were fertile as determined by uterine implantation sites, but pregnancy was not maintained[123].
- On investigation of the effect on ovarian differentiation using subcutaneous administration (50 mg/kg/day) of genistein 1-5 days after birth of mouse neonates, mice treated with genistein had fewer single oocytes and a higher percentage of oocytes not enclosed in follicles. Despite the intercellular bridges disappearing 2 days after birth in the control group, it remained even 4 days after birth in the test group. Also, there was also an increase in the number of oocyte that survived during the nest breakdown period, and there were fewer oocytes that undergoing apoptosis. This information suggests genistein exposure during development alters ovarian differentiation by inhibiting oocyte nest breakdown and attenuating oocyte cell death[124].
- On investigation of the effect on pregnant rat and offspring rat using dosed feeding of genistein (5, 25, 100, 250, 625, 1,250 ppm), the weight and intake amount of the pregnant rat prior to birth and the weight of the offspring rat 50 days after birth were significantly reduced compared to the control group (1,250 ppm). In the offspring rat, decreased weight of the male ventral prostate and an increased relative weight of the pituitary gland in males and females were observed (1,250 ppm). Also, in pathohistological testing, ductal/alveolar hyperplasia of the mammary glands (female 250-1,250 ppm, males greater than 25 ppm), abnormal cellular maturation in the vagina (female 625, 1,250 ppm) and abnormal ovarian antral follicles (female 1,250 ppm) and aberrant or delayed sperm

atogenesis in the seminiferous tubules (males 1,250 ppm) were observed. Although a decrease in sperm was observed in the epididymis (male 625, 1,250 ppm), there was no significant difference in testicular spermatid head counts and epididymal spermatozoa counts compared to the control group. Also, an increase in the incidence of renal tubal mineralization was observed (male and females 250 ppm and above). These symptoms are considered to correspond to effects from estrogenic activity[125].

- Pregnant rats were given genistein (5-300 mg/kg/day) throughout gestation and lactation, a decrease in testosterone in male offspring, late testes development and abnormal reproductive function were observed[35].
- Pregnant Rhesus monkeys were given genistein (8 mg/kg/day) for 7 weeks, and compared with the control group, no difference was observed in the maternal weight gained during pregnancy, or in fetal weight or placental weight at delivery. The E2 concentration in the maternal blood, the fetus, the uterine vein/ovarian vein was significantly higher compared to the control group, however there was no effect on progesterone concentration[36].
- In Co-twin male marmosets were given soy-based infant formula (1.6-3.5 mg/kg/day*) or milk for the period 4-45 days after birth, at the completion of intake, there was no difference in testes weight. The testosterone surge was suppressed in the soy formula fed animals, and plasma testosterone concentration decreased. Also, although a consistent difference in sertoli or germ cell numbers were not observed, Leydig cell numbers increased by an average of 74%[37].
- An increase in dry weight of uterus of rat neonates (postnatal days (PND) 1-5) was reported after subcutaneous administration of equol (1000 μg/day) on PND5. Compared with a control group there was a significant decrease in uterine weights at PND 20 and 25 after administration of equol 100 μg/day on PND 1-5, and in uterine weights at PND 15 after administration of equol 100 μg/day on PND 1-10, however there was no effect on the ER level. Also, on administration of equol 10, 100, 1000 μg/day on PND 10-14, there was a decrease in uterine glands that wasn't accompanied by an increase in uterine weight or abnormal development[38].
- Uterine hypertrophy was confirmed in 3 day gavage administration of genistein 20-500 mg/kg/day to immature female rat (18-20 days after birth) and 60-300 mg/kg/day to mature rat (8-10 weeks) which had an ovariectomy at 6-8 weeks of age. Also, in the increased uterine weight observed after 3 days subcutaneous administration 35 mg/kg/day to immature rat and mature rat after ovariectomy, the increase was especially pronounced in the immature rat[10][39][40].
- On 90 day oral administration of fermented soybean extract (about 8, 56, 400 mg/kg/day) to male and female rats, a decrease in body weight was observed in the male 56, 400 mg/kg and the female 400 mg/kg group. Also, in pathohistological findings, kidney deposit of calcium was observed in the male 56, 400 mg/kg group, and in 400 mg/kg group of male ventral prostate, epithelium hyperplasia, cyst, depletion of secretory fluid and hypersecretion of epithelium cell were observed[42].
- In a 2 week subcutaneous administration experiment of genistein (0.7-5 mg/day) to osteoporotic model mice with an ovariectomy, the genistein ED 50 (50% effect dose) for causing uterine hypertrophy and osteopenia inhibition was 3 mg/day and 0.29 mg/day respectively, and it was considered that sensitivity differences between the uterus and bones for isoflavone was 10 times[14].

Regarding the results of animal tests, as a result of summarizing and investigating administration periods (animal age in weeks)/ dosages etc and symptoms observed, and no-observed-effect level etc, the tests using pregnant animals suggest the possibility of a health effect (risk) to mother animals and fetuses from high exposure. Also, an impact was observed in both male and female offspring and mature animals.

4.2.2 Observational studies in human

Regarding tests on human subjects and observational findings etc, we organized the scientific findings related to safety assessment concerning the biological effects by intake of soy isoflavone in each group classified by gender/age.

4.2.2.1 Premenopausal females

Regarding the intake of soy isoflavone by premenopausal females, as shown below, there are a large number of reports on the extension of the menstrual cycle length and changes in serum hormone concentrations.

In general, premenopausal females have a large fluctuation in serum hormone levels in accordance with the menstrual cycle, and the biological effect by fluctuations in the menstrual cycle and hormone levels observed in the reports is yet to be sufficiently clarified.

- When giving premenopausal females (20-29 y.o., of unknown race, however experiment conducted in US) soy protein (14.4 mg genistein/day: 6 females, 28.1 mg/day: 6 females), miso (25 mg/day: 3 females, excluding 3 subject dropouts), and isoflavone free soy products (5 females) for one menstrual cycle, in the case of taking 28.1 mg/day soy protein, the follicular phase was significantly lengthened ($P<0.01$) and E2 concentration during the follicular phase was significantly raised ($P<0.05$) compared with before the experiment. Also, LH (luteinizing hormone) and FSH (follicle-stimulating hormone) significantly decreased during the ovulation phase ($P<0.05$, $P<0.01$ respectively). Although there was a lengthening of menstrual cycle days and the follicular phase period due to intake of miso, the subject number was small and a significant difference was not acknowledged. However, a delay in the peak of progesterone was observed. No significant fluctuations were observed in any other sexual hormone levels before or after intake in each experiment[44].
- Premenopausal females (of unknown age, however experiment conducted on students, Japanese), were divided into two groups, on group given tablets containing soy isoflavone (20, 40 mg/day: 19 subjects, 50 mg/day: 3 subjects) for 1 menstrual cycle in addition to their normal eating habits including soy products (approximately 10 mg/day), and a control group (20 females, continued their normal eating habits) The menstrual cycle was lengthened in half of the subjects in the 20 mg/day group and in 70-75% of subjects in the 40 mg/day group compared with that before the experiment. In the 50 mg/day intake group, the menstrual cycle shortened in 1 subject and was lengthened in the other two subjects[45].
- On giving 20 premenopausal females (21-44 y.o., 10 non Asians, 10 Asians) 3 types of soy products (non Asian: 28 mg/day, Asian: 36 mg/day) for 3 menstrual cycles, E2 concentration for Asian females during luteal phase significantly decreased ($P=0.005$)

compared with before the experiment. A significant increase in SHBG (sex hormone binding globulin) concentrations in both the follicular phase and the luteal phase (P=0.009, P=0.05 respectively) were observed in non-Asian females. On the other hand, significant fluctuations in the menstrual cycle or progesterone concentration were not observed[46].

- On sampling and analysis of Nipple Aspiration Fluid (NAF) of 14 premenopausal females (29-50 y.o., Caucasian) given soy protein (genistein 37.4 mg/day*) for 6 menstrual cycles, an increase in NAF volumes were observed during the test period compared with the non soy protein intake period, and the mean concentrations of GCDFP-15 (Gross Cystic Disease Fluid Protein-15: specific tumor marker for breast cancer) in the NAF decreased, however that concentration clearly increased in 2 subjects. Despite hyperplastic cells in the NAF cytological diagnosis detected in only 1 subject prior to the experiment (7.1%), they were detected in 4 subjects (28.6%) during the experiment and in 3 months after soy consumption periods[48].
- In a randomized cross over experiment (of unknown race, however experiment conducted in US) where 16 premenopausal females (29.7±6.4 y.o., excluding 4 subject dropouts) and 20 premenopausal females taking oral contraceptive for longer than 3 months (22.8±2.9 y.o.) were given a drink of skim milk with added soy protein (38 mg/day) and a control meal (skim milk) for 2 menstrual cycles each, intake of the test food did not effect the serum E1 (estrone), E2, SHBG, prolactin, progesterone or menstrual cycle length[49].
- 47 premenopausal females with either benign or malignant breast disease (excluding 1 subject dropout, of unknown race, however experiment conducted in UK), were divided at random into a group (18 subjects, excluding 1 subject dropout, 30.6±8.0 y.o.) given bread rolls containing soy protein (45 mg/day*) for 2 weeks, and a control group (29 subjects, 33.6±8.1 y.o., continued normal eating habits), and on collection and thymidine labeling of breast epithelial cells two weeks later, there was an increase in the rate of breast epithelial cell proliferation and a significant increase in PR expression[50].
- 81 premenopausal females with benign breast disease (excluding 3 subject dropouts, of unknown race, however experiment conducted in UK) were divided at random into a group of 28 subjects (31.6±7.3 y.o.) given bread rolls containing soy protein (45 mg/day*) for 2 weeks and a control group of 53 subjects (of which mammary gland tissues of 33 subjects were obtained from a tissue bank and used, 34.9±8.8 y.o., excluding 3 subject dropouts, continued normal eating habits). The comprehensive estrogenic effects on the breasts by the test diet was investigated by analysis of serum and nipple aspirate, thymidine and Ki67 labeling of breast epithelial cells and using ER, PR and Bcl-2 as indicators. In the intake group, there was a significant decrease in concentration of apolipoprotein D in nipple aspirate (P=0.002), an indicator of estrogen effect, and an increase in pS2 concentration (P<0.001). However there was no increase in breast epithelial cells, or effect on ER and PR expression etc[51].
- 189 premenopausal females (excluding 31 subject dropouts, Asian, indigenous Hawaiian, Caucasian etc) were divided at random into a group (92 subjects, excluding 17 subject dropouts, 43.2±2.7 y.o. at time of starting experiment) given various soy products etc (59 mg/day level*) for 2 years, and a control group (97 subjects, excluding 14 subject dropouts, 42.8±2.9 y.o. at time of starting experiment, 97 subjects at completion of experiment, continued normal eating habits), and the effect on serum sexual hormone concentrations

and menstrual cycle investigated. As a result, mean E1, E2 and free E2 concentrations increased during the luteal phase for the intake group in the first 3 months, however no difference was observed in either group from 6 months to 12 months, and no significant difference was observed between the groups either. The menstrual cycles shortened for both groups, however no significant difference was observed between the groups[52].

- In a randomized cross over experiment where 14 premenopausal females (26.5±4.7 y.o., of unknown race, however test conducted in US) were given soy protein powder (control 10, low dose 64, high dose 128 mg/day) for 3 menstrual cycles + 9 days each time, there was a significant increase($P<0.05$) in E1 concentration during the mid follicular phase for the low dose compared with the control period, however there was a significant decrease during high dosage ($P<0.05$), and a significant difference between both the high and low dosages($P=0.02$) was observed. There was a significant decrease in LH and FSH concentrations during the ovulation phase for the low dose compared with the control period ($P=0.009$, $P=0.04$ respectively), a significant decrease in the concentrations for the high dose ($P<0.05$ for each), and the low dose had the significantly lower concentrations compared with the high dosage ($P<0.05$). Also, concentration of free T3 during the early follicular phase significantly decreased ($P=0.02$) for the high dosage compared with the control and the low dosage, and DHEA sulphate significantly decreased for the high dosage compared with low dosage ($P=0.02$). No significant fluctuation of menstrual cycles was observed during the test period and no effect from the test diet was observed from the endometrial biopsies[53].
- 10 premenopausal females (excluding 16 subject dropouts, 23-42 y.o., 7 Caucasians, 3 African Americans) were given soymilk for 1 menstrual cycle, and there was a 25% decrease ($P \leqq 0.05$) in E2 concentration throughout the menstrual cycle and a significant decrease in progesterone in the luteal phase ($P=0.002$). However, no significant difference in LH, FSH and the menstrual cycle length were observed[54].
- On investigation of effect on serum sexual hormone values, where 60 premenopausal females (Japanese) were divided at random into a group (31 subjects, 26.1±7.9 y.o.) given soymilk supplemented to normal eating habits, including soy products (total daily intake 72.8 mg/day) for 2 menstrual cycles and a control group (29 subjects, 26.9±6.8 y.o., continued normal eating habits), there was a significant decrease ($P=0.02$) in serum E1 concentration during the follicular phase for the intake group compared with at the start of the experiment. E2 concentration decreased by 27%, however it wasn't a significant fluctuation. Within the intake group, for the 21 subjects for which serum collection before and after the experiment was conducted in days 9-12 of the menstrual cycle (total daily intake 75.7 mg/day) there was a significant reduction in E1 concentration by 30% ($P=0.005$), and a significant trend compared with the control group ($P=0.07$). E2 concentration decreased by 33% ($P=0.10$). Also, mean menstrual cycle length was increased[55].
- On investigation of effect on mammographic densities before and after experiment, 30 premenopausal females (excluding 4 subject dropouts, 16 Caucasians, 10 Japanese and Chinese, 4 Hawaiians) were divided at random using the double blind method into a group (15 subjects, 41.1±3.1 y.o.) given tablets containing soy isoflavone (76 mg/day) for 1 year and a control group (15 subjects, 43.3±1.7 y.o.), there was no significant changes either in the size of dense areas or in the percent densities in the intake group compared

to the control group[56].

- 6 premenopausal females (22-29 y.o., 4 Caucasians, 1 African American, 1 Hispanic) were given soy milk (147 mg/day) for 1 menstrual cycle, and there was a significant decrease (P=0.03) in E2 concentration throughout the menstrual cycle and that decreasing tendency continued for 2-3 cycles after the experiment. There was a significant decrease in the progesterone concentration in the luteal phase (P=0.002), and DHEA sulphate gradually decreased during the experiment (P=0.03). Also, a lengthening of the average number of menstrual cycle days was observed (P=0.06)[57].

As a result of investigating soy isoflavone intake amount and the effect on endocrine function from these test reports targeting premenopausal females, two points that are suggested as biological effects of soy isoflavone are; the fluctuation of endogenous estrogen such as estradiol and progesterone in the blood, and the lengthening of the menstrual cycle.

4.2.2.2 Postmenopausal females

Regarding soy isoflavone intake by females during the period (about 5 years) to reach full menopause and the postmenopausal period after that, as shown below, there are few reports suggesting an effect on the level of hormones in the blood, however endometrial effects with soy isoflavone has been observed in reports of long term clinical studies.

- In a double blind experiment where 73 postmenopausal females with hypercholesterolemia for which the last menstrual period was one or more years ago (excluding 8 subject dropouts, of unknown race, however experiment conducted in US) were divided into 3 groups of those given soy protein (43 mg/day: 24 subjects, average 59.3 y.o., 72 mg/day: 23 subjects, average 61.9 y.o.) and a control diet (casein 26 subjects, average 61.0 y.o.) for 6 months, a significant increase in thyroxine (T4) and free T4 in the 43 mg/day intake group (P=0.02, P=0.03 respectively), and a significant increase in thyroid stimulating hormone (TSH) and triiodothyronine (T3) (P=0.01, P=0.04 respectively) was observed compared with the control group. However, no significant difference was observed in serum E2, E1 sulphate, cortisol, DHEA sulphate, insulin, glucagon, or FSH[58].
- 63 postmenopausal females (excluding 7 subject dropouts, of race unknown, however experiment conducted in Italy) were divided at random into a group given tablets containing genistein (36 mg/day, 32 subjects, excluding 3 subject dropouts, 51.9±1.85 y.o., the mean postmenopausal period of 17.6±3.1 months) and a group given control tablets (calcium, 31 subjects, excluding 4 subject dropouts, 51.6±1.75 y.o., the mean postmenopausal period of 17.0±3.4 months) for 48 weeks, and in the intake group there was a significant reduction in the menopausal syndrome index (KI) (P<0.05) compared with at baseline and the control group. At the end of the experiment, increase (6.8-7.0 mm) of endometrial thickness was observed in 1 subject of intake group and 2 subjects of control group, however endometrial thickness in other subjects during the observational period were 5 mm or less, and no difference was observed between the groups[59].
- In a randomized crossover experiment where 18 postmenopausal females (56.9±5.8 y.o., excluding 5 subject dropouts, of unknown race, however experiment conducted in US, the mean postmenopausal period of 7.6±4.7 years) were given soy protein powder (control 7, low dosage 65, high dosage 132 mg/day) for 93 days for each period, there was a significant

decrease in estrone sulphate (E1-S) during high dosage intake compared with during the control dosage intake and low dosage intake, and an E2 and E1 decreased and SHBG increased significantly compared with during the control period. There was no significant effect due to dosage in vaginal cytology or endometrial biopsy results[60].

- In a double blind experiment where 39 postmenopausal females (of unknown race, however experiment conducted in Italy), were divided at random into 2 groups and one group given soy extract tablets (50 mg/day*, 20 subjects, 54±7.1 y.o., the mean postmenopausal period of 6.2±4 years) and the other control tablets (19 subjects, 53±3.9 y.o. the mean postmenopausal period of 5±4.3 years) for 6 weeks, and then given in combination with conjugated equine estrogens for 4 weeks, and then with medroxyprogesterone acetate added for 2 weeks, frequency of hot flushes significantly decreased in the single intake experiment compared with before the experiment (approx 45% decrease, $P<0.01$). No effect to vaginal cytology, endometrial thickness or serum sexual hormone values were acknowledged. No difference in the frequency of hot flushes between the test substance and the control in the concomitant intake with hormones was observed, with both groups showing the same action[61].
- In a randomized double blind test where 128 postmenopausal females (45-60 y.o., excluding 8 subject dropouts, Japanese, the mean postmenopausal period of 2-5 years) were divided into 4 groups and one group was given soy isoflavone glycoside tablets (47 mg/day) for 6 months (33 subjects, excluding 1 subject dropout), one group combined with walking (31 subjects, excluding 3 subject dropouts), a control group (33 subjects, excluding 1 subject dropout), and an exercise group (31 subjects, excluding 3 subject dropouts), a significant effect was not observed on the blood estradiol concentration in the test diet intake[122].
- In a randomized double blind crossover experiment where 13 menopausal females and 45 postmenopausal females (58±7 y.o., Japanese) were given tablets containing soy isoflavone while continuing normal eating habits (42.2 mg/day, total intake 51.1 mg/day) and a control tablet (total intake 13.7 mg/day) for 4 weeks each, E2 concentration decreased by 52% in menopausal females compared with baseline values, however it was not statistical significance. While there was a significant increase in E2 concentrations in postmenopausal females compared to baseline values and control ($P<0.05$ each), and there was a significant increase in progesterone concentration compared to baseline values. Also hot flushes decreased significantly ($P<0.05$) and a significant decrease in deoxypyridinoline in the urine was observed (time since end of menopause unknown)[126].
- In a double blind experiment where 47 postmenopausal females (excluding 11 subject dropouts, of unknown race, however experiment conducted in Australia) were divided at random into 2 groups, one group given soy flour (approximately 100 mg/day level, 23 subjects, excluding 5 subject dropouts, 53.8±1.1 y.o., the mean postmenopausal period of 4.7±1.1 years) and the other taken a control diet (wheat flour, 24 subjects, excluding 6 subject dropouts, 56.0±1.0 y.o., the mean postmenopausal period of 6.7±1.3 years) for 12 weeks, the frequency of hot flushes decreased by 40% in the intake group compared with baseline values and 25% in the control group ($P<0.001$ for each), however there was no significant difference between the groups. There was no significant effect on vaginal cell maturity, plasma lipids, or urinary calcium[62].
- In a double blind experiment where 79 postmenopausal females (excluding 25 subject

dropouts, of unknown race, however experiment conducted in Italy) were divided at random into 2 groups, one group given soy protein (76 mg/day, 40 subjects, excluding 11 subject dropouts, 48-61 y.o., the mean postmenopausal period of 46.8±7.2 months) and the other taken a control diet (casein, 39 subjects, excluding 14 subject dropouts, 45-62 y.o., the mean postmenopausal period of 46.2±5.8 months) for 12 weeks, there was a significant reduction in the frequency of hot flushes per day in the intake group compared with the control group ($P<0.01$). There was no difference between both groups in harmful effects such as nausea, vomiting, bloating and constipation[63].

- In a double blind experiment where 90 postmenopausal females (47-57 y.o., of unknown race, however experiment conducted in Italy) were divided at random into 3 groups, one group given tablets containing genistein (54 mg/day, 30 subjects, 52±0.6 y.o., the mean postmenopausal period of 7±1.1 years), one group given a control tablet (30 subjects, 51±0.7 y.o., the mean postmenopausal period of 6±0.9 years) and one group treated estrogen-progesterone therapy (30 subjects, 52±0.9 y.o., the mean postmenopausal period of 7±0.6 years) for one year, there was a significant reduction in the frequency of hot flushes in the test intake group compared with the control group (24% decrease, $P<0.01$) No significant effect on endometrial thickness due to intake of the test diet was observed. Also after completion of the experiment, an endometrial biopsy was performed on 3 women in both the intake group and control group and 2 women in the estrogen-progesterone therapy group who showed an endometrial thickness grater than 5mm, however no abnormalities were observed[64].
- In a randomized double blind experiment where 49 postmenopausal females (excluding 13 subject dropouts, 45-60 y.o., of unknown race, however experiment conducted in Italy) were given tablets containing soy isoflavone (61 mg/day, 22 subjects, excluding 6 subject dropouts, the mean postmenopausal period of 2.4±1.2 years) and control tablets (27 subjects, excluding 7 subject dropouts, the mean postmenopausal period of 2.4±1.5 years) for 6 months, frequency of hot flushes decreased in both groups, however no difference was observed between the groups. No effect was acknowledged for endometrial thickness or vasoconstriction in the uterus or brain[65].
- 20 postmenopausal females (54.2±5.7 y.o., of unknown race, however experiment conducted in Chile) were given dried soy milk powder (69 mg/day) for 10 weeks, and there was a significant increase in SHBG compared with before the test ($P<0.05$), and a correlation between blood isoflavone concentration and rate of SHBG change were observed. (time since end of menopause unknown)[66].
- In a randomized double blind experiment where 38 postmenopausal females (excluding 4 subject dropouts, 64-83 y.o., 37 Caucasians, 1 unknown) were taken food containing soy isoflavone with iodine supplementation (90 mg/days, 22 subjects, excluding 3 subject dropouts) and a control diet (16 subjects, excluding 1 subject dropout) for 6 months, there was no significant change in the values for TSH, T3, or T4 between both groups (time since end of menopause unknown)[67].
- 91 females, at least 2 years past last menses (excluding 6 subject dropouts, 45-65 y.o., 97% Caucasians) were divided at random into 2 groups, one group given various soy products (165 mg/day) for 4 weeks (66 subjects) and the other continued normal eating habits as the control group (25 subjects), no significant difference was observed in the values for FSH, LH, SHBG before and after the experiment or between the groups. An increase in

percentage of vaginal superficial cells in 12 subjects (19%) of the intake group was observed, however there was a decrease in 8 subjects (13%). There was an increase in 2 subjects (8%) in the control group, and there was a decrease in 5 subjects (21%)[68].

- In a randomized double blind cross over experiment where 28 females not observed menstrual bleeding in the past 12 months (excluding 14 subject dropouts, 54.9±1.0 y.o., 26 Caucasians, 1 African American, 1 Ethiopian) were given soy protein (107 mg/day), soy protein without isoflavone (2 mg/day) and a control diet (milk protein) for 6 weeks each, postocclusion peak flow velosity of the brachial artery was significantly lower during administration of soy protein containing soy isoflavone (P=0.03). There ware no significant changes in biochemical cardiovascular disease risk markers[69].
- In a randomized double blind experiment where 53 postmenopausal females (excluding 3 subject dropouts, 86% Caucasians) were given soy extract as tablets (110 mg/day*, 27 subjects, excluding 1 subject dropout, 59.9±4.0 y.o., menopausal age 48.4±6.8 y.o.) and control tablets (26 subjects, excluding 2 subject drop outs, 61.5±6.3 y.o., menopausal age 51.2±4.3 y.o.) for 6 months, congnitive function improved in the intake group compared with the control group (P=0.02)[70].
- In a randomized double blind experiment where 50 postmenopausal females (of unknown race, however experiment conducted in Australia) were given soy protein (118 mg/day*, 30 subjects, 61±1 y.o.) and a control diet (casein, 20 subjects, 62±1 y.o.) for 3 months, there was a significant increase in serum C reactive protein (CRP) in both groups, and SHBG and thyroid hormone binding globulin (TBG) decreased significantly, however there were no significant differences between the groups. FSH, LH, and DHEA sulphate did not change significantly in either group. (time since end of menopause unknown)[71].
- On NAF collection and analysis of 10 postmenopausal females (45-57 y.o., Caucasian) given genistein in the form of soy protein (37.4 mg/day*) for 6 menstrual cycles, a small increase in NAF volume was observed, however it was not a significant change. However, a clear increase in NAF volumes was observed in 4 subjects carrying out estrogen replacement therapy. There was almost no change between subjects in the mean concentration of GCDFP-15 in NAF, however a clear increase was observed in 3 subjects. Also, hyperplastic cells were detected in 3 subjects in the NAF cytology (time since end of menopause unknown)[48].
- In a double blind experiment to investigate the effect on endometrium in postmenopausal females, 30 postmenopausal females (about 55 y.o., excluding 9 subject dropouts, of unknown race, however experiment conducted in US) were divided at random into 4 groups (E2 0.5 mg/day or 1.0 mg/day added to soy protein powder 120 mg/day or control diet). Soy protein powder did not protect the endometrium from E2-induced hyperplasia in postmenopausal women. (time since end of menopause unknown)[72].
- In a randomized double blind experiment where 319 postmenopausal females (excluding 57 subject dropouts, of unknown race, however experiment conducted in Italy) were given soy isoflavone tablets 150 mg/day (154 subjects, excluding 25 subject dropouts, 49±4.3 y.o. at time of starting experiment, the mean postmenopausal period of 5.6±4.3 years) and control tablets (165 subjects, excluding 32 subject dropouts, 50±3.9 y.o. at time of starting experiment, the mean postmenopausal period of 5.8±4.5 years) for 5 years, endometrium was sampled at baseline, 30th month and at the end of the study. As a result, no subjects were diagnosed with endometrial hyperplasia or malignancy at 30th month. 5th year after

the beginning of the experiment, 3.8% of the intake group (6 subjects) were diagnosed with endometrial hyperplasia (5 were simple and 1 was complex hyperplasia). On the other hand none were acknowledged in the control group. There were no subjects diagnosed with atypical endometrial hyperplasia or endometrial carcinoma in either group. Also, proliferative phase cells observed in 1.1% (2 subjects) of the intake group and 1.5% (3 subjects) of the control group at baseline, they were not observed in either group at 30th month., but were observed in 3.2% (5 subjects) of the intake group at the end of the experiment[96].

As a result of investigating these test reports targeting postmenopausal females, the focus of the comparatively short term and low dose tests using soy isoflavone in postmenopausal females corresponds mainly to efficacy as hormone replacement therapy, and it was judged that information concerning safety was mostly unobtainable, however in the test report where 150 mg/day soy isoflavone was taken for 5th year, there were significantly more subjects diagnosed with endometrial hyperplasia in the intake group, which is thought to indicate the possibility of an adverse reaction in large amount and long term continuous use of soy isoflavone.

4.2.2.3 Males

As shown below, there are several reports on the intake of soy isoflavone in males, suggesting changes in some serum hormone values, however the majority of reports suggest that these changes were not clinically significant.

- In a randomized crossover experiment conducted on 42 males (35-62 y.o., excluding three subject dropouts, Caucasians) given tofu (74 mg/day) and lean meat as the source of protein in the normal diet for four weeks each, no significant change was acknowledged in the serum sexual hormone values for either intake period[73].
- Eleven males (18-35 y.o., excluding four subject dropouts, of unknown race, test conducted in the UK) were given tablets containing soy isoflavone (40 mg/day) for two months, and no significant change was acknowledged in the serum sexual hormone values, volume and concentration of semen, or the number and motility of sperm[74].
- In a double blind experiment where 81 males (excluding 31 subject dropouts, 90% Caucasians) were divided at random into two groups and one group was given a soy protein drink (83 mg/day, 34 subjects, excluding 17 subject dropouts, 64.9±7.7 y.o.) and the other was given a soy protein drink without soy isoflavone (47 subjects, excluding 14 subject dropouts, 63.9±7.2 y.o.) for 1 year, serum PSA concentration increased in both groups, but no significant difference was acknowledged in the values between the groups, before or after the test[75].
- Twenty males with prostate cancer (stage B, C or D) (excluding three subject dropouts, 53-82 y.o., 19 Caucasians, one African American) were given soy isoflavone capsules (398 mg/day: 28 days, 796 mg/day: 56 days) for a total of 84 days, and breast change and increase of frequency of hot flushes were observed. Also, on investigation of DNA damage to peripheral lymphocytes taken from subjects using a comet assay, the comet tail moment on day 28 of intake was significantly lower compared to that at the start of the experiment. However, test diet induced rearrangements of *MLL* genes were not observed[77][78].

- According to an epidemiological survey targeting 69 males (60.5±10.7 y.o., Japanese) in Japan (Chubu district), there was a significant negative correlation between serum estradiol concentration and intake of soy products (r=-0.32, P=0.009)[79].
- Thirty-four males (excluding one subject dropout, Japanese) were divided at random into two groups and one group was given soymilk added to their normal diet (48 mg/day, total intake amount 58 mg/day, 17 subjects, excluding one subject dropout, 32.0±8.4 y.o.) for eight weeks, and the other continued their normal diet (11 mg/day, 17 subjects, 32.8±8.3 y.o.), and there was a significant decrease in estrone concentration in the intake group compared with the control group. No effect was observed on estradiol, testosterone or SHBG[80].
- Thirty males (40-69 y.o., 23 Caucasians, five Blacks, two Asians) were given a single administration of a soy isoflavone capsule (as genistein 1, 2, 4, 8, 16 mg/kg body weight/day*) and conceivable effects due to the test diet, loss of appetite (4, 8 mg/kg/day), pedal edema (4 mg/kg/day), and abdominal tenderness (8 mg/kg/day) were observed. Also, there was a rise in lipase (2, 8 mg/kg body weight/day), a rise in amylase (2 mg/kg body weight/day), and leukopenia (16 mg/kg body weight/day) and hypophosphatemia (4, 8 mg/kg body weight/day) were observed[76].

As a result of investigating these test reports, it was clear the majority of tests on males were comparatively short, and no obvious adverse events were observed in the experiment measuring blood hormone values with up to 74 mg/day soy isoflavone intake. However, although males have a homeostatic mechanism, because they have a small amount of endogenous estrogen, it easily surpasses the modulating amount, and as reported in the expression of breast change in the experiment where soy isoflavone was taken for a total of 84 days (398 mg/day: 28 days, 796 mg/day: 56 days), it is possible that this may cause an estrogen agonist effect. On that point, it is thought that it is a possible that sensitivity to exogenous estrogen from soy isoflavone etc does not differ greatly from that of pre and postmenopausal females.

4.2.2.4 Pregnant females (including potentially pregnant), fetuses, infants and small children

There are various reports, as shown below, concerning the effects of soy isoflavone on pregnant females (including potentially pregnant), fetuses, infants and small children, with particular emphasis on fetuses and infants, including the possible of effect on fetuses in the first stages of pregnancy.

Infants have been given soy-modified milk for many years, so there are several findings concerning the intake of soy-modified milk.

- According to a survey on the relationship between meals during pregnancy and hypospadias in newborn males (UK), a significant correlation was observed between a vegetarian diet during pregnancy and the occurrence of hypospadias in newborn males (odds ratio 3.88, 95% confidence interval 1.69-8.92, compared with non-vegetarians)[81].
- In a retrospective cohort study that investigated the relativity between soy-formula intake or cow milk during infanthood, and health status and reproductive function in later years for young adults (20-34 y.o.), no effect on general health or reproductive outcomes was seen

with the intake of soy-formula, although a significant increase in discomfort with menstruation was acknowledged in females (P=0.04). However no difference was acknowledged in the level of menstrual pain. Also, a slightly longer duration of menstrual bleeding without an increase in menstrual flow, was acknowledged (P=0.02)[82].

- Soy isoflavone concentrations in serum, cord plasma and amniotic fluid at time of birth were similar in seven pregnant females (20-30 y.o., Japanese), and it was assumed that soy isoflavone is transported to the new born from the mother's body[83][84].
- The plasma concentrations of genistein and daidzein in four month-old infants (seven subjects, of unknown race, in an experiment conducted in the US) given 900-1000 ml/day of soy-based infant formula (28-47 mg/day* (about 4.5-8 mg/kg/day)) were significantly higher compared to infants given cow-milk or human breast milk (P<0.05). Also, the soy isoflavone concentration in human breast milk from mothers who consume soy products was elevated compared to the non consuming soy period, but it was lower than concentrations from soy-based infant formula[85].
- On analysis of plasma soy isoflavone in 2.5-5 months-old infants (four subjects, of unknown race, in an experiment conducted in Switzerland) given soy-based infant formula (43-48 mg/day*), no aglycone was detected. From these findings it was considered that soy isoflavone in infants was metabolized to a sulphate conjugate and that a glucuronic acid conjugate and these metabolites were rapidly excreted[86].
- According to a report which examined the effect of soy isoflavone contained in soy-based infant formula on endocrine and other functions, there was insufficient data to make a judgment regarding the safety of intake by infants, and it was concluded that more study was necessary in the future[119].
- In a matched pair case control study using 120 pairs of girls (of unknown race, in an experiment conducted in Puerto Rico), a weak relationship between thelarche observed before two years of age and soy-based infant formula intake was acknowledged[37].
- On an ethical drug package insert for hormonal agents (estrogen), careful administration to children before puberty with the possibility of causing premature epiphyseal closure and premature sexual maturation are listed.[87]
- In the treatment of short stature in young children, because sexual hormone may cause epiphysis closure, consideration of starting age for the treatment is required[88].

As a result of investigating these reports, it is clear that genistein etc is transferred to the blood of the fetus via the cord, although experts are still discussing the level of exposure to the fetus and whether there is a threshold for the soy isoflavone effect or not.

There is clearly insufficient test data regarding pregnant females (including potentially pregnant), fetuses, infants and small children, and as a result, it was not possible to judge the potential health effects, either beneficial and harmful.

4.2.2.5 Other human tests

- A total 123 male and female subjects (85% males, excluding 27 subject dropouts, of unknown race, in an experiment conducted in the US) were divided at random into two groups and one way given a soy protein drink (83 mg/day, 58 subjects, excluding 16 subject dropouts, 64.7±7.9 y.o.), and one way given a soy protein drink without soy isoflavone (65 subjects, excluding 11 subject dropouts, 65.1±7.9 y.o.) for one year, and there was no

difference in changes in serum insulin-like growth factor (IGF) in either groups. Also, no significant difference was observed in values before and after the experiment[89].

Also, the following is a report regarding soy isoflavone intake in subjects with disease.

- In a randomized crossover experiment, a total 31 male and female subjects with hypolipidemic (19 males, 12 postmenopausal females, 56.5±9.0 y.o., of unknown race, in an experiment conducted in Canada) were given a low fat test diet containing soy protein (53 mg/day) and a control diet (low-fat diet) for one month each. The test diet contained twice as much dietary fiber as the control diet. As a result, a significant decrease in total cholesterol, LDL cholesterol and oxidized LDL was observed ($P<0.001$) during the test diet intake period. There was no significant difference in the excretion of urinary sexual hormone metabolites[90].

4.2.3 Others

4.2.3.1 Reports on risk of cancer etc.

Regarding phytoestrogens such as soy isoflavone, there is concern regarding the risk of cancers with higher sensitivity to estrogen such as breast cancer.

Of note, there have been reports demonstrating the anti estrogenic effects of soy isoflavone intake with regard to development of breast cancer *in vivo*.

4.2.3.2 Relativity to cancer with high sensitivity to estrogen

As shown below, there are several reports regarding soy isoflavone and the risk of cancers with higher sensitivity to estrogen such as breast cancer.

Of note, currently in the obtainable findings based on human tests, there are no reports directly associating soy isoflavone intake with an increase in incidence of breast cancer in females.

4.2.3.2.1 Animal testing

- In female rats given an ovariectomy after confirmation of appearance of MNU (Methyl nitrosourea) induced mammary tumora, oral administration of genistein (750 ppm, 495 mg/kg feed/day equivalent[h]) was started, and on intraperitoneal injection of bromodeoxyuridine (BrdU) on the 90th day, were examined postmortem. As a result, compared to the control group, there was a significant increase in ER positive adenocarcinoma weight, a significant increase in BrdU labeling rate of tumor cells and a significant increase in uterine weight in the test group[41].
- In ovariectomized athymic mice implanted with human breast cancer cells (MCF-7), on investigation of the effect on growth of the MCF-7 by genistein (mixed feed 125-1000 μg/g), it was found that genistein promoted tumor growth, cell proliferation and pS2 expression in a dose-responsive manner[129].
- In ovariectomized athymic mice implanted with human breast cancer cells (MCF-7), on investigation of the interactions between genistein (mixed feed 1000 ppm) and tamoxifen (anti-estrogen drug used in treatment of breast cancer), genistein negated the inhibitory

[h] Calculated on the assumption that body weight of 20 week old SD rat is 300g, and daily food intake is 20g/day.

effect of tamoxifen, and had an expression inhibition effect for estrogen response genes (pS2, PR, cyclin D1). In fact, genistein produced increased tumor growth and increased expression of response genes[130].

4.2.3.2.2 Human tests and observational studies etc.

- In the "Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases (JPHC study)" (10 year prospective tracking survey targeting 40-59 y.o. females) conducted by the research group of the Ministry of Health, Labour and Welfare, analysis was conducted on the intake of soybean foods. It was reported that there was a relationship between intake of miso soup and soy isoflavone, and a reduction in risk of breast cancer[94], and compared to the intake group at around 7 mg genistein, the intake group at a level of 13 mg and above had a reduced risk of breast cancer.
- In a follow up study of breast cancer patients (1,459 subjects) conducted in China, it was reported that no relationship was observed between soybean food intake prior to breast cancer diagnosis, and the period until relapse[131].
- The American Cancer Society has published a guide relating to daily eating habits aimed at patients receiving cancer therapy. It states that soy products and soy isoflavone have both estrogenic and anti estrogenic effects, and as no conclusion has been reached regarding the beneficial or harmful effects at this point in time, it warns breast cancer survivors not to take tablets containing concentrated soy, or supplements with soy isoflavone extract or concentrate[132].
- The American Heart Association, on assessment of recent test reports concerning soy protein and soy isoflavone gives the following scientific recommendation to professionals. Soy isoflavone does not have a reducing effect on vasomotion symptoms in menopause (dizziness, hot flushes etc), and results are mixed with regard to the slowing of postmenopausal bone loss. Also, as the efficacy and safety regarding prevention and treatment of breast cancer, endometrial cancer and prostate cancer is yet to be established, and with scarce evidence from clinical reports and warnings regarding the possibility of adverse reactions, it has been concluded that it is not recommended to take soy isoflavone supplements in food or tablets. In contrast, many soy products such as tofu or soy burgers contain high amounts of polyunsaturated fatty acids, dietary fiber, vitamins and minerals, and have a low content of saturated fatty acids, so the replacement of animal protein containing considerable amounts of saturated fatty acids and cholesterol with soy products is useful for cardiovascular disease and general health[136].

4.2.3.3 Points of concern regarding intake of hormonal agents (pharmaceuticals) etc.

As soy isoflavone has estrogen-like effects, the following are points of concern regarding the harmful effects referred to on the package insert etc of hormones (pharmaceuticals) for oral intake.

- Regarding follicular hormone and luteinizing hormone mixed hormone replacement therapy for postmenopausal women, the following information is contained on the pharmaceutical package insert.
  - Regarding hormone replacement therapy (HRT) and the risk of breast cancer, as a result of randomized testing targeting postmenopausal females in the US, it was

reported that the combination administration group of conjugated estrogen preparation and luteinizing hormone had a significantly higher risk of breast cancer than the control group (hazard ratio: 1.24)[97)98)].

- Also, regarding HRT and the risk of coronary heart disease[99)], HRT and risk of cerebral stroke[100)], HRT and the risk of dementia[101)], it was reported that there was a significantly higher risk compared with the control group for each.

- Information warning regarding the increased risk of ovarian cancer from long term doses of follicular hormone for postmenopausal females is contained on pharmaceutical package insert[102)103)].
- Development of thrombosis, cardiac failure and angina pectoris are listed as important adverse reactions contained in the pharmaceutical package insert for estrogen used in the treatment for prostate cancer[104)].

5 Situation of investigations in other countries

5.1 Situation of Food Standard Agency (FSA) investigations

The FSA, in the assessment concerning phytoestrogen substances (Phytoestrogens and Health (May, 2003))[37)] conducted an investigation of the health effects from diet derived phytoestrogen intake.

In the report, it was pointed out that in the bioactivity of phytoestrogens, the mechanism that enables inducement of the estrogen receptor mediated agonist/antagonist effect is complex and there are differences in types between animals and humans, differences in pharmacokinetics and sexual development and differences in effect depending on race and the amount of past exposure to phytoestrogens. Also, the majority of animal experiments have used administration of high phytoestrogen doses, and as these experiment conditions are not equivalent to exposure in the human diet, interpretation is difficult.

Furthermore, regarding population that could be estimated to exceed the average phytoestrogen intake (vegetarians and vegans, Asians, consumers of dietary supplements containing soy products or phytoestrogens), such people have large individual differences in metabolism and biological activity, in particular, differences in intestinal flora which contribute to the production of equol. However, the effect from transport of phytoestrogens from the body of the mother to the fetus, and the effect on thyroid function in these people are yet to be clarified.

In addition, many of the studies were short-term intervention studies in adults that did not address the possibility that exposure to phytoestrogens at an earlier age may influence the risk of disease later in life, while it is mentioned that there is an effect on menopausal symptoms, osteoporosis, cardiovascular disease and cancer.

The FSA has listed the following research as recommended research to enable future risk assessment:

- research concerning population groups consuming large amounts of phytoestrogens including soy isoflavone, and thr health implications
- clarification of the use of soy-based infant formula in the UK

- concerning the possibility of phytoestrogen effect on infants given soy-based infant formula
- concerning the potential interaction of phytoestrogens with hypothyroidism
- clarification of the phytoestrogen effect mechanism
- long term prospective cohort study (Analysis of relativity between phytoestrogen intake and breast cancer, prostate cancer and osteoporosis) and short term interventional study (assessment of effect of phytoestrogen intake on menopausal symptoms and disease risk markers such as osteoporosis and cancer) considering the role of phytoestrogen metabolic products such as equol

In the same way, the following research has been listed concerning the field of supplements:

- as the health implications of *in utero* exposure to phytoestrogens are unclear, regarding the continuous health effects in the fetal and infant stages due to maternal exposure of phytoestrogens
- concerning the drug interaction between pharmaceuticals possessing hormonal effects and intake of dietary supplements containing phytoestrogens
- concerning the difference in phytoestrogenic metabolism between races

In addition, in the FSA annual report (2004), an analysis using a large-scale prospective cohort study on the associated risks between phytoestrogen intake and development of breast cancer and prostate cancer, a double blind crossover experiment using soy isoflavone targeting patients with hypothyroidism, and analysis related to phytoestrogen intake in postmenopausal females diagnosed with breast cancer, were scheduled[105].

5.2 Situation of Food and Drug Administration (FDA) and Agency for Healthcare Research and Quality (AHRQ) investigations

The FDA, based on clinical trials where a decrease in plasma LDL (low-density lipoprotein) was observed follwing soy protein intake, in 1999 authorized the health claim that soy protein may reduce the risk of coronary heart disease[106].

Regarding the health effects of soybeans and soy isoflavone, the AHRQ released a report (2005) based on human tests from the perspective of cardiovascular effects, efficacy for menopausal symptoms, effects on endocrine function, proliferative effect on cancer cells and effect on bones etc[120].

It is concluded that the evidence does not support the positive effect of soy products on endocrine function, menstrual length, or bone health, although evidence was often limited and of poor quality.

According to the AHRQ, the following points need to be addressed in future research:

- Fully report the components of soy products being tested
- Compare different doses, soy products, and populations
- More closely evaluate the effects of different soy components, non-protein, non-isoflavone components

- Fully consider the types of foods bring replaced by soy products and the controls being used
- Use the CONSORT statement as a guide to designing and reporting studies

5.3 Situation of Agence Française de Sécurité Sanitaire des Aliments (AFSSA) investigations

In the report "Présentation du rapport sur "Sécurité et bénéfices des phyto-estrogènes apportés par l'alimentation -Recommandations", concerning phytoestrogens (March 2005)[107], the AFSSA considered estrogen like effects of phytoestrogen substances containing soy isoflavones from various viewpoints.

In conclusion, the report indicated 1 mg/kg body weight/day of isoflavone aglycone as the amount for which health effect (risk) from phytoestrogen intake could not be considered.

Also, for infants given prepared foods with soy protein as the main component, the amount of phytoestrogen contained in that food should be limited to 1 mg/L. In addition, for breast cancer patients or people with a history of breast cancer in the family, intake should be restricted in consideration of increased risk of tumor growth.

In particular, with regard to foods in supplement form or foods prepared for infants, information on soy isoflavone content should be provided to the consumer. Therefore, it is recommended that foods with soy as the main ingredient such as tofu, miso and soy-milk carry the labels "Contains X mg soy isoflavone", "Take in moderation" and "Not recommended for children under 3".

Furthermore, it is recommended that regarding foods in supplement form or enriched food, in addition to carrying the labels for the content of soy isoflavone, also display "Must not exceed 1 mg/kg body weight per day", "Not recommended for women with a history themselves or within the family of breast cancer" and "Consult your physician".

5.4 Situation of investigations in other countries

In Israel, there is information regarding the recommendation that consumption of soy products by small children is restricted, and if allowed, it is recommended not to be given to infants. Note, it is also advised that intake of soy products by adults, until completion of test reports in future, should not exceed a moderate amount.

In Italy, a recommendation was given in July 2002 that daily intake from foods supplemented with phytoestrogen or soy isoflavones should not exceed 80 mg/day.

In paragraphs 6 and 7, references to soy isoflavone related to dietary intake indicate soy isoflavone aglycone.

6 Concept regarding safety assessment

This paragraph, expresses the concept of safety assessment related to the intake of foods for specified health use with soy isoflavone as the ingredients exhibiting health functions, based on the basic policy outlined in "1 Introduction".

Specifically, in determining the daily dietary intake upper limit for foods for specified health use with soy isoflavone as the ingredient exhibiting health functions, investigation was conducted from the perspective of safety based on as many test reports etc (test reports summarized from 2 to 4 in this report) that could be obtained on soy isoflavone, and although investigation was based around the results of human tests, where sufficient findings on humans could not be obtained, proceeded using the results of animal testing. Also, the soy isoflavone intake amount as foods for specified health use established from these reports were examined for each age group.

6.1 Establishing a safe upper limit for standard intakes of soy isoflavone

6.1.1 Establishing an upper limit for standard daily intakes of soy isoflavone based on dietary experience

In Japan, there has been no examination of high consumption of soy products as an indicator of health damage, however in findings up to the present, there have been no reports of clear health damage from consumption of soy isoflavone at a level contained in soy products of the normal eating habits of Japanese people.

Therefore, in this safety assessment, the 95 percentile value 64-76 mg/day (premenopausal females: 64 mg/day, postmenopausal females: 74 mg/day, males: 76 mg/day) made clear by the soy isoflavone intake amount (provisional calculation) shown in 3.2 of this report based on the 2002 National Nutrition Survey, including the amount consumed as foods for specified health use, was determined as the upper limit for standard daily intakes of soy isoflavone regarded as safe at this time based on dietary experience.

6.1.2 Establishing an upper limit for standard daily intakes of soy isoflavone based on human clinical research

In the soy isoflavone tablet (150 mg/day) 5 year long term intake experiment targeting postmenopausal females[96], endometrial hyperplasia was not observed in the intake group at 30th month (2 years and 6 months) compared with the control group, however development of endometrial hyperplasia was significantly higher at 60th month (5 years). According to this report, it is considered that soy isoflavone intake of 150 mg/day is the “effect level” for which there is concern for development of damage to human health. As sustained and excessive stimulation of estrogen is pointed out as a development factor in endometrial hyperplasia, in the case of long term continuous intake of soy isoflavone, it is considered that similar effects may develop due to the estrogen effect.

In the report examined in 4.2.2.1, decrease in serum E2 concentration and lengthening of menstrual cycles were reported as an effect from 150 mg/day soy isoflavone in premenopausal females, and were also reported at the lower soy isoflavone intake of around 60 mg/day.

It has been pointed out that this kind of effect on the internal estrogen environment, in postmenopausal females considered to have a lowered homeostatic mechanism, may be expressed at an even lower soy isoflavone intake amount, i.e. sensitivity to exogenous estrogen is higher than premenopausal females.

In the report on males examined in 4.2.2.3, expression of gynecomasty was reported as an effect on males taking several hundred mg/day of soy isoflavone.

It is possible that these kinds of biological effects, in males who possess a homeostatic mechanism however have naturally low levels of endogenous estrogen, are due to the level of exogenous estrogen easily surpassing the adjustable amount and causing an estrogen agonist effect. On that point, it is thought that there is not a large difference between estrogen sensitivity with premenopausal and postmenopausal females.

Dosage and administration of foods for specified health use, as opposed to pharmaceuticals, are not strictly determined, and once accustomed to taking, it is recommended to continue taking for a long time to obtain the expected health enhancement effect, and assuming intake by people in various age groups, healthy people, sick people, and candidates for life-style related disease, it is necessary to sufficiently consider individual differences.

Due to equol production capacity, absorption efficiency of soy isoflavone into the blood from the alimentary canal and metabolic efficiency in the liver etc varying greatly between people, even when the same amount of soy isoflavone is taken there are large differences in the effect.

Therefore, individual differences, and the various issues surrounding the biological effect of female hormones, are a factor that must be taken into account when establishing an upper limit for standard intakes of soy isoflavone.

From the above, in consideration of the soy isoflavone tablet (150 mg/day) 5 year long term intake experiment targeting postmenopausal females[96], and individual differences, 75 mg/day, half of the 150 mg/day considered as the "effect level" for which there is concern for development of damage to human health in that experiment, is determined as the safe upper limit for standard intakes of soy isoflavone for humans (premenopausal females, postmenopausal females, males) at this time, based on clinical trials.

6.1.3 Establishing the upper limit for daily extra dietary intake of soy isoflavone as a food for specified health use based on human clinical research

As a result of examining clinical research reports on humans, as stated in 4.2.2.1 Premenopausal females (Test reports regarding safety), it is considered that the two biological effects from soy isoflavone in premenopausal females is the variation in the endogenous estrogen concentrations such as blood estradiole and an effect on the menstrual cycle length.

Of the 13 clinical research reports on intake of soy isoflavones in addition to normal eating habits in premenopausal females, 4 reports (5 experiments) with sufficient data on subject numbers and presence of analysis of hormone values were selected (Appendix 3). Based on these 4 reports (5 experiments), using change in blood E2 concentration and effect on menstrual cycle length as indicators, examination of the daily extra dietary intake standard for soy isoflavone as a food for specified health use was conducted.

(1) Effect of soy isoflavone on serum E2 concentration (effect on serum endogenous estrogen)

In the 4 reports selected (5 experiments), increase or decrease of serum E2 concentration before and after intake of soy isoflavone were compared to the soy isoflavone added intake amount.

In the experiment with a soy isoflavone added intake amount of 28.1 mg/day, serum E2

concentrations significantly increased (40%) due to soy isoflavone intake, however in the experiment with a soy isoflavone added intake amount of 57.3 mg/day, a decreasing (33%) tendency in serum E2 concentration and a significant decrease (30%) in serum E1 concentration were observed, and in the experiment with a soy isoflavone added intake amount of 147.0 mg/day, serum E2 concentration significantly decreased by 81% due to soy isoflavone intake. In the experiment with a soy isoflavone added intake amount of 14.4 mg/day and 38.0 mg/day, there was a decrease in the average serum E2 concentration, however it wasn't a significant difference.

(2) Effect of soy isoflavone on menstrual cycle length of premenopausal females

In the 4 reports selected (5 experiments), lengthening or shortening of the menstrual cycle before and after intake of soy isoflavone were compared to the soy isoflavone added intake amount.

In the experiment with a soy isoflavone added intake amount of 147.0 mg/day, the menstrual cycle showed a lengthening (12%) tendency. In other experiments with a soy isoflavone added intake amount, there was almost no change in menstrual cycle in a 38.0 mg/day experiment, menstrual cycle shortened due to soy isoflavone intake in a 14.4 mg/day experiment, and menstrual cycle lengthened due to soy isoflavone intake in a 28.1 mg/day and 57.3 mg/day experiment, however none of these were significant changes.

It is thought that soy isoflavone works as a partial agonist or as a partial antagonist. It is thought at low dosage it acts inhibitively as a partial antagonist to the hypothalamo-pituitary, promoting E2 secretion from the ovaries. However, in this case, as the E2 secreted from the ovaries stimulates the hypothalamo-pituitary, the rise in E2 can be considered transitory (clinically cases are known where anti estrogen drugs transitorily raise blood E2 concentration). On the other hand, for high doses exceeding a certain amount, agonist activity becomes most prominent, and there is a sustained decrease in serum E2 secreted from the ovaries via stimulative action to the hypothalamo-pituitary (negative feedback), and it is thought that as an effect on the adjustment function of the estrous cycle a lengthening of the menstrual cycle is brought about as a result. In (1), the effect on serum E2 concentration caused by soy isoflavone intake is a significant increase at low dosage (28.1 mg/day) and a significant decrease or an observed decreasing tendency at high dosage (57.3 mg/day and 147.0 mg/day). In (2), the menstrual cycle lengthened at high dosage (147.0 mg/day). These factors can be considered the grounds for these effects.

Whether the rise in serum E2 concentration at a low dosage can be taken as an adverse event or not requires further debate. Meaning that it is assumed that the fluctuation band and directionality of the variation differs easily depending on the period of administration and the timing of the measurement and uniform handling is difficult. With regard to this, the decrease in serum E2 concentration at high dosage can be assumed to be comparatively continuous and dependent on dosage, effecting control of ovarian function by gonadotrophic hormones, and it is considered that a hypothetical extremely high exposure may have the biological effect of amenorrhea. In this report, decreased serum E2 concentration and lengthening of the menstrual cycle (result of the negative feedback mechanism working) from added intake of soy isoflavone was judged to be directly related to adverse events.

Note, in the event that increased serum E2 concentration in low doses is acknowledged as

a harmful effect, this will be reexamined.

The lack of test reports providing reliable measurement data, the differences in the test design for each of those tests (subject numbers, form of administered matter, intake period, blood collection period etc), the differences in subjects (subjects who consumed soy isoflavone from foods in their normal eating habits, and subjects who didn't) were all factors that must be considered when establishing the daily extra dietary intake of soy isoflavone as a food for specified health use.

Moreover, other factors to be considered are: the difficulty of uniform consideration of the effect of increasing serum E2 concentration at low dosage and decreasing at high dosage, and the difficulty of assuming the no-observed-effect level from the dose-response curve.

From the above, as shown in (1), data for the soy isoflavone dietary intake of 57.3 mg/day which showed a significant decrease in serum E1 concentration and a decreasing tendency for serum E2 concentration (P=0.1), can be considered the minimum effect level for daily extra dietary intake of soy isoflavone within normal eating habits.

In consideration of the test numbers and differences in test design as well as the difficulty in establishing the no-observed-effect dose, the daily extra dietary intake of soy isoflavone as a food for specified health use for premenopausal females is calculated as half of that 57.3 mg/day, to be 28.7 mg/day, or roughly 30 mg/day.

6.1.4 Summary of establishment of upper limit for standard intakes of soy isoflavone

6.1.4.1 Upper limit for standard daily intakes of soy isoflavone

In consideration of 6.1.1 (the safe upper limit for standard daily intakes of soy isoflavone for humans at this point in time based on dietary experience of 64-76 mg/day) and 6.1.2 (the safe upper limit for standard daily intakes of soy isoflavone at this point in time based on human clinical research of 75 mg/day), it was determined to set the safe upper limit for standard daily intakes of soy isoflavone in this health impact assessment at 70-75 mg/day.

Note, this upper limit, due to lack of data, does not include investigation of fetuses, infants, small children or pregnant females (including potentially pregnant). For infants, it is thought that because their homeostatic mechanism is not fully developed, sensitivity to exogenous estrogen is possibly higher than that of premenopausal females. This possibility is suggested from animal experiments.

It is considered that exceeding the dietary intake of 70-75 mg/day of isoflavone will not give rise to health effects of immediate concern, however the daily dietary intake standard upper limit (premenopausal females, postmenopausal females, males) was established, from the fact that the hormone effect of this substance is realized by a life mechanism (estrogen hormone system) which has high commonality between mammals, and while taking into consideration biological plausibility against a background of biological effects (including harmful effects) observed in laboratory animals and medical knowledge and based on findings concerning humans at this point of time, to what is considered safe.

Through the progress of future research concerning soy isoflavone, it is expected that an

even more appropriate upper limit shall be established in future based on scientific findings.

6.1.4.2 Upper limit for daily extra dietary intake of soy isoflavone as a food for specified health use

As observed in 6.1.3, from the perspective of the effect on endocrine function of premenopausal females of a decrease in the serum E2 concentration and a lengthening of the menstrual cycle due to intake of soy isoflavone, the upper limit for daily extra dietary intake of soy isoflavone as a food for specified health use in this safety assessment was determined to be 30 mg/day.

Unlike premenopausal females, there are no reports showing an effect on endocrine function in postmenopausal females and males. However, as discussed in 6.1.2, as sensitivity of postmenopausal females is not low compared to premenopausal females, and as there appears to be no certainty that male sensitivity differs greatly from that of females, the extra intake amount of premenopausal females was extrapolated, giving 30 mg/day.

Currently, the amount of soy isoflavone approved by the Ministry of Health, Labour and Welfare as food for specified health use as having efficacy in being "useful in maintaining bone calcium" is 40 mg/day, which is 25 mg/day soy isoflavone aglycone equivalent.

The upper limit for the safe daily extra dietary intake of soy isoflavone as a food for specified health use of 30 mg/day (soy isoflavone aglycone (equivalent value)) is considered to be the value that satisfies both suggested efficacy and safety at this point in time.

6.2 Validation of established safe dietary intake standards for soy isoflavone

6.2.1 Premenopausal females, postmenopausal females, and males

The following was confirmed based on the daily soy isoflavone dietary intake from everyday soy products clarified from soy isoflavone dietary intake (provisional calculation) based on the 2002 National Nutrition Survey discussed in 3.2 (Table 2 median value: 16-22 mg/day, hereafter normal dietary intake.), the soy isoflavone daily dietary intake standard upper limit obtained from the establishment of a safe upper limit dietary intake standard for soy isoflavone in 6.1, and the upper limit for the safe daily extra dietary intake of soy isoflavone as a food for specified health use.

Premenopausal females
16 mg/day + 30 mg/day = 46 mg/day < 70-75 mg/day
Postmenopausal females
22 mg/day + 30 mg/day = 52 mg/day < 70-75 mg/day
Males
18 mg/day + 30 mg/day = 48 mg/day < 70-75 mg/day

From this, for those premenopausal females, postmenopausal females and males alike, who consume the normal dietary intake (16-22 mg/day) of soy isoflavone from soy products every day, even when consuming 30 mg/day soy isoflavone as food for specified health use, will not exceed the 70-75 mg/day safe upper limit for standard daily intakes of soy isoflavone obtained from 6.1.4.1 of this report.

Also, for those premenopausal females, postmenopausal females and males alike, who

consume 40-45 mg/day (Overall: 80-85 percentile value equivalent, premenopausal females: 83-87 percentile value equivalent, postmenopausal females: 73-80 percentile value equivalent, males: 78-83 percentile value equivalent) of soy isoflavone from soy products every day, it is considered that even when consuming 30 mg/day soy isoflavone as food for specified health use, they will not exceed the 70-75 mg/day safe upper limit for standard daily intakes of soy isoflavone.

6.2.2 Pregnant females (including potentially pregnant), fetuses, infants and small children

For pregnant females (including potentially pregnant), fetuses, infants and small children, as there was insufficient human test data, animal testing results were also taken into consideration when assuming possible health effects.

6.2.2.1 Pregnant females (including potentially pregnant) and fetuses

From data that is currently obtainable, in experiments using pregnant animals, there is concern for potential health effects (risk) from exposure to high concentrations. Also, from the literature, it was clear that genistein etc passes through to the fetus blood via the umbilical cord. Experts are still currently debating whether there is a presence of a threshold for these effects.

Levels of exposure to human fetuses, and what potential health effects may occur in the fetus as a result, have not been clarified scientifically at this point in time so consideration was made based on animal testing etc.

As discussed in 4.2.1 Animal testing (Test reports regarding safety) in this report, there are reports suggesting an effect on reproductive function of fetuses through exposure of high concentrations of soy isoflavone to pregnant animal mothers.

Also, as discussed in 2.3.2 of this report, it has been reported that because flavonoids including soy isoflavone possess a topoisomerase II inhibition effect, they may potentially cause *MLL* gene abnormality.

This report is an assessment concerning soy isoflavone ER mediated effect, however, no benefit from soy isoflavone has been found with regard to fetuses, and under the recognition that the harmful effects must be given priority, it was judged that the effect (*MLL* gene associated leukemia) on the fetus through intrauterine exposure to these substances must be taken into consideration.

6.2.2.2 Infants and small children

Soy-based infant formula targeted at infants with lactose intolerance etc, has a long dietary history in Japan as well as overseas, and there have been studies conducted concerning damaging effects based on that. They include a report suggesting a slight hormone effect, and a hypothesis has been suggested that infants, as a specific trait, possibly have a high soy isoflavone conjugation ratio or high excretion capacity. However, it is thought that administration of soy modified milk to infants with lactose intolerance is being conducted on the instructions of a physician and monitored appropriately.

As discussed in 4.2.1 Animal testing (Test reports regarding safety) in this report, there is

a report suggesting an effect on reproductive function of neonate animals and immature animals through exposure to high concentrations of soy isoflavone.

In data that is currently available, there are findings of epiphyseal closing and premature puberty concerning the use of hormones (estrogen) as pharmaceuticals in small children.

As a summary of the above, it was not possible to scientifically judge a safe standard amount regarding biological effect of soy isoflavone intake in infants and small children.

6.2.3 Regarding equol production capacity

There are considerable individual differences regarding equol production after intake of soy isoflavone, with reports that it isn't detected in 60-70% of humans.

Data concerning persons producing equol is limited, and as it wasn't possible to take into consideration the distinction between persons capable of producing equol in the alimentary canal in this report, it is considered that this result was obtained under the conditions that persons not producing equol accounted for a certain percentage of the examined population.

Regarding equol, it has been reported[138] that in a human breast cancer cell graft experiment, development was not promoted, however as discussed in 2.3.1, in consideration of the high conjugation capacity of equol to ER, presumptively, in the event of investigating only persons producing equol, it is thought that an equivalent effect may be obtained from a lower intake of soy isoflavone. Therefore, it is considered irrefutable that continuous high doses by these persons increases the possible risk factors compared to non equol producing persons.

7 Results of safety assessment

7.1 Premenopausal females, postmenopausal females, and males

Regarding premenopausal females, postmenopausal females, and males consuming a normal dietary intake of soy isoflavone (16-22 mg/day) from soy products every day in daily eating habits, it is believed that there are no problems from the aspect of safety if the dietary intake of soy isoflavone from foods for specified health use on top of normal daily eating habits can be suitably controlled to within the range of 30 mg/day as soy isoflavone aglycone.

Also, regarding premenopausal females, postmenopausal females, and males consuming a dietary intake of soy isoflavone from soy products in excess of the 80-85 percentile value equivalent amount (40-45 mg/day) according to the results of the 2002 National Nutrition Survey, every day in daily eating habits, although consumption of 30 mg/day equivalent soy isoflavone as a food for specified health use will exceed the 70-75 mg/day safe upper limit for standard daily intake of soy isoflavone, it is thought that there are no grounds that persons already consuming soy isoflavone from soy products in excess of the 80-85 percentile equivalent amount, should be consuming 30 mg/day equivalent soy isoflavone as foods for specified health use.

7.2 Pregnant females (including potentially pregnant), fetuses, infants and small children

7.2.1 Pregnant females (including potentially pregnant) and fetuses

As fetuses can't control amount of intake themselves, pregnant females (including potentially pregnant) are covered.

As shown in 6.2.2.1, in animal testing, there have been reports suggesting exposure to high concentrations of soy isoflavone to pregnant animals effects the reproduction function of fetuses, however from data on humans, it is not possible to judge what concentration of soy isoflavone intake there would be a problem with safety, and it was not possible to scientifically judge safety in the case of additional intake as a food for specified health use.

However, in consideration of the fact that there are no known benefits concerning additional intake of soy isoflavone by pregnant females (including potentially pregnant) as well as the topoisomerase II inhibition effect of flavonoids including soy isoflavone, intake of soy isoflavone as a food for specified health use by pregnant females (including potentially pregnant) in addition to normal eating habits cannot be recommended.

7.2.2 Infants and small children

As shown in 6.2.2.2, in animal testing, there are reports suggesting an effect on reproductive function of neonate animals and immature animals through exposure to high concentrations of soy isoflavone, however from data on humans, it was not possible to scientifically judge what concentration of soy isoflavone intake there would be a problem with safety.

However, in consideration of reports suggesting an effect on reproductive function of neonate animals and immature animals through exposure to high concentrations of soy isoflavone and the ER mediated effect of soy isoflavone, it is considered that as long as the safety of adding intake of soy isoflavone to normal eating habits as a food for specified health use for the infants and small children with undeveloped sexual reproduction function cannot be clarified, it cannot be recommended.

8 Summary

8.1 Premenopausal females, postmenopausal females, and males

8.1.1 Expected upper limit for safe standard daily intakes of soy isoflavone

(1) Establishment based on dietary experience

As there have been no clear reports of health damage from the dietary intake of soy isoflavone from soy products consumed by Japanese over many years, that amount is roughly thought to be safe. And based on dietary experience, the 95 percentile soy isoflavone dietary intake value of 70 mg/day (64-76 mg/day: aglycone equivalent value) provisionally calculated from the 2002 National Nutrition Survey, was determined as the safe upper limit for standard daily intake for humans at this point in time.

(2) Establishment based on human clinical research

In an experiment targeting postmenopausal females who continued to take soy isoflavone tablets 150 mg/day for 5 years overseas (Italy), as the development of endometrial hyperplasia was significantly higher in the intake group, it can be considered that 150 mg/day soy isoflavone is the “effect level” for which there is concern for

development of damage to human health. In consideration that the intake subjects were all postmenopausal females as well as individual differences, 75 mg/day as soy isoflavone aglycone, half of the 150 mg/day, was determined as the safe upper limit for standard daily intake for humans based on human clinical studies at this point in time.

From (1) and (2) above, 70-75 mg/day as soy isoflavone aglycone was determined as the upper limit for the safe standard daily intakes at this point in time.

8.1.2 Expected upper limit for safe daily extra dietary intake of soy isoflavone as a food for specified health use

4 reports (5 experiments) with sufficient data were selected from clinical tests (13 reports) targeting premenopausal females given soy isoflavone (soy milk, soy protein, tablets etc), and using the effect on serum E2 concentration and menstrual cycle length observed from intake of soy isoflavone as an indicator, the relationship with soy isoflavone was examined. As a result, as a decrease in serum E2 concentration and lengthening of the menstrual cycle were observed together in an experiment where 57.3 mg/day and 147 mg/day soy isoflavone were given to subjects, 57.3 mg/day soy isoflavone, the lesser intake amount, was determined as the minimum effect level in the case of added intake. In consideration of differences in test design and individual differences, half of the 57.3 mg/day, roughly 30 mg/day soy isoflavone aglycone was established as the upper limit for safe daily extra dietary intake of soy isoflavone as a food for specified health use for premenopausal females.

Although there are no reports that can be investigated concerning the upper limit for extra dietary intake on top of normal eating habits for postmenopausal females and males, sensitivity of postmenopausal females is not low compared to premenopausal females, and as there appears to be no certainty that male sensitivity differs greatly from that of females, the upper limit was determined to be 30 mg/day as soy isoflavone aglycone on extrapolation of the result of premenopausal females.

8.2 Regarding pregnant females, fetuses, infants and small children

It was not possible to establish a safe dietary intake for pregnant females, fetuses (covered by pregnant females), infants and small children from human test and animal test data, however in consideration of harmful effects reported from animal experiments for pregnant females and fetuses, and the topoisomerase II inhibition effect of soy isoflavone, it was determined that additional intake to normal eating habits as a food for specified health use could not be recommended.

In consideration that sexual reproduction function in infants and small children is undeveloped, it was determined that additional intake to normal eating habits as a food for specified health use could not be recommended.

9 Conclusions

Soybeans are a food rich in nutrients such as plant protein and calcium, and the Japanese diet type that takes protein from soybeans is low in fat and calorie intake and is considered healthy compared with Western diet types, which mainly use livestock products as the

source of protein.

However it is to be emphasized that the upper limit for standard daily intakes of soy isoflavone of 70-75 mg/day, is the upper limit for the average value when taking this amount every single day over many years, and that exceeding this upper limit in dietary intake from soy products will not immediately lead to health damage.

This safety assessment of foods for specified health use containing soy isoflavone is not an investigation of safety concerns of soy or soy products present for many years in the Japanese diet, but an investigation of the safety of taking soy isoflavone in addition to normal eating habits as a food for specified health use.

Also, regarding the expected upper limit for standard daily intakes and daily extra dietary intake as a food for specified health use, this is the upper limit for the average value when taking this amount every single day over many years, and is the value at this point in time based on test reports collected and examined up until now, and due to the fact the following information was not obtainable, it is necessary to note this is a cautious value with emphasis placed on safety.

- surveys enabling establishment of intake standards based on intake groups of large amounts of soy isoflavone enriched foods
- long term epidemiological survey concerning soy isoflavone intake from a safety perspective
- survey considering high risk groups (fetuses, pregnant females, infants, small children and cancer patients etc)

Currently there is debate on the convenient use of soy isoflavone dietary intake as an index for simple measurement of dietary intake of soy products, and the health enhancements of that amount. However, discussion on the efficacy of the intake of soy products as a source of protein in the Japanese diet cannot be limited to soy isoflavone alone. There are debates on the use of soy isoflavone intake as a practical index for measurement of dietary intake of soy products, and on the use of high amounts for health enhancement. Currently, efficacy of the intake of soy products is discussed as a source of protein as well as isoflavone in the Japanese. In another word, there are queries/ambiguities on whether soy isoflavones alone is effective to take, or whether the entire soy product is necessary to be consumed. It is also uncertain whether the overall Japanese dietary custom consuming relatively high amounts of soy products as the source of protein is effective in its entirety. In recent epidemiological studies on Japanese, the risk of breast cancer is shown to decrease from the small intake group to the high intake group, when divided based on dietary intake of soy products into four separate groups[94].

The field of study on the biological effect of soy isoflavone phytoestrogen is a field where research is progressing. As it is considered new information will be obtained in future, while continuing to regard this new information, if new findings are obtained, it will be necessary to conduct the assessment once again and accurately clarify the range from the perspective of efficacy and safety.

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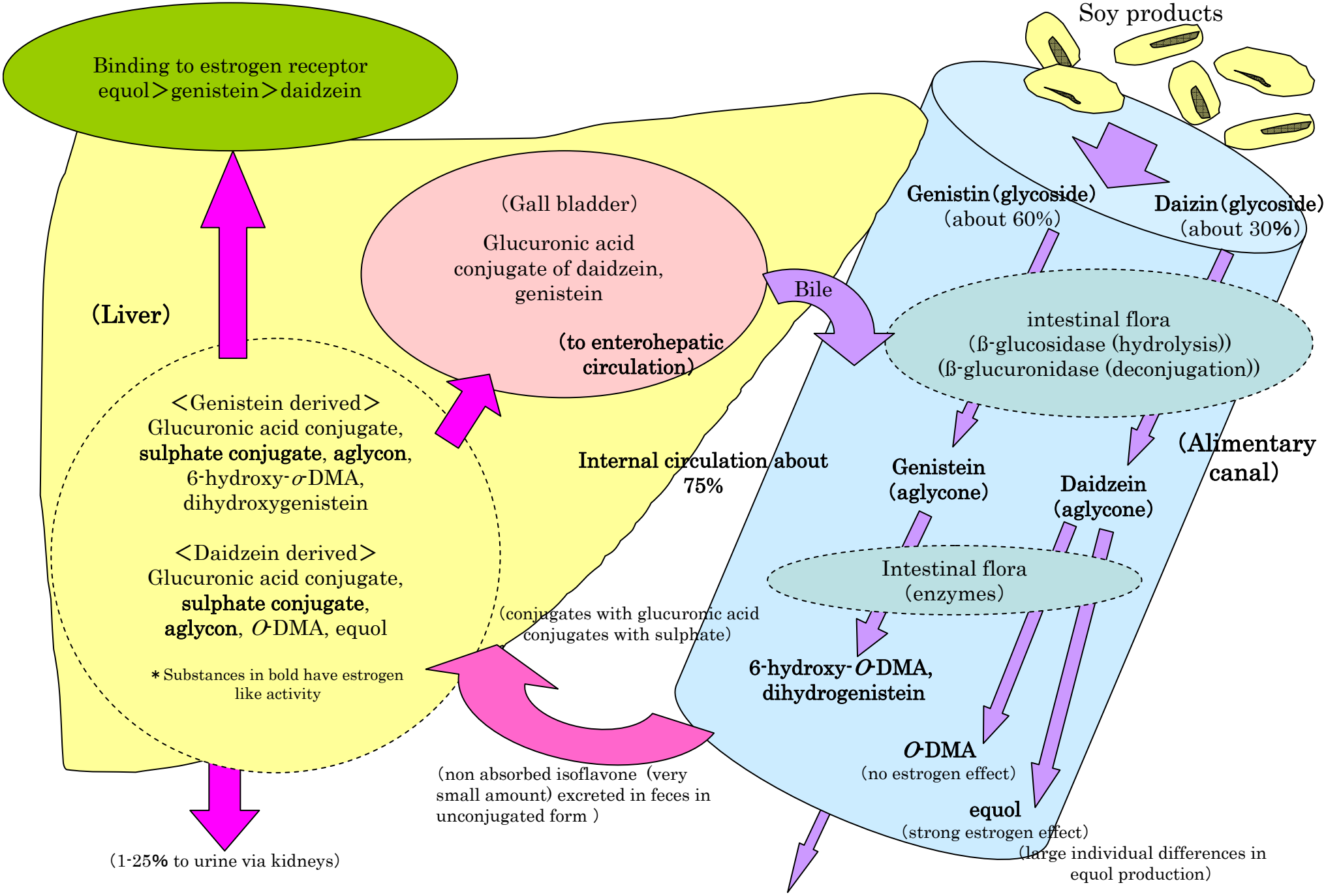
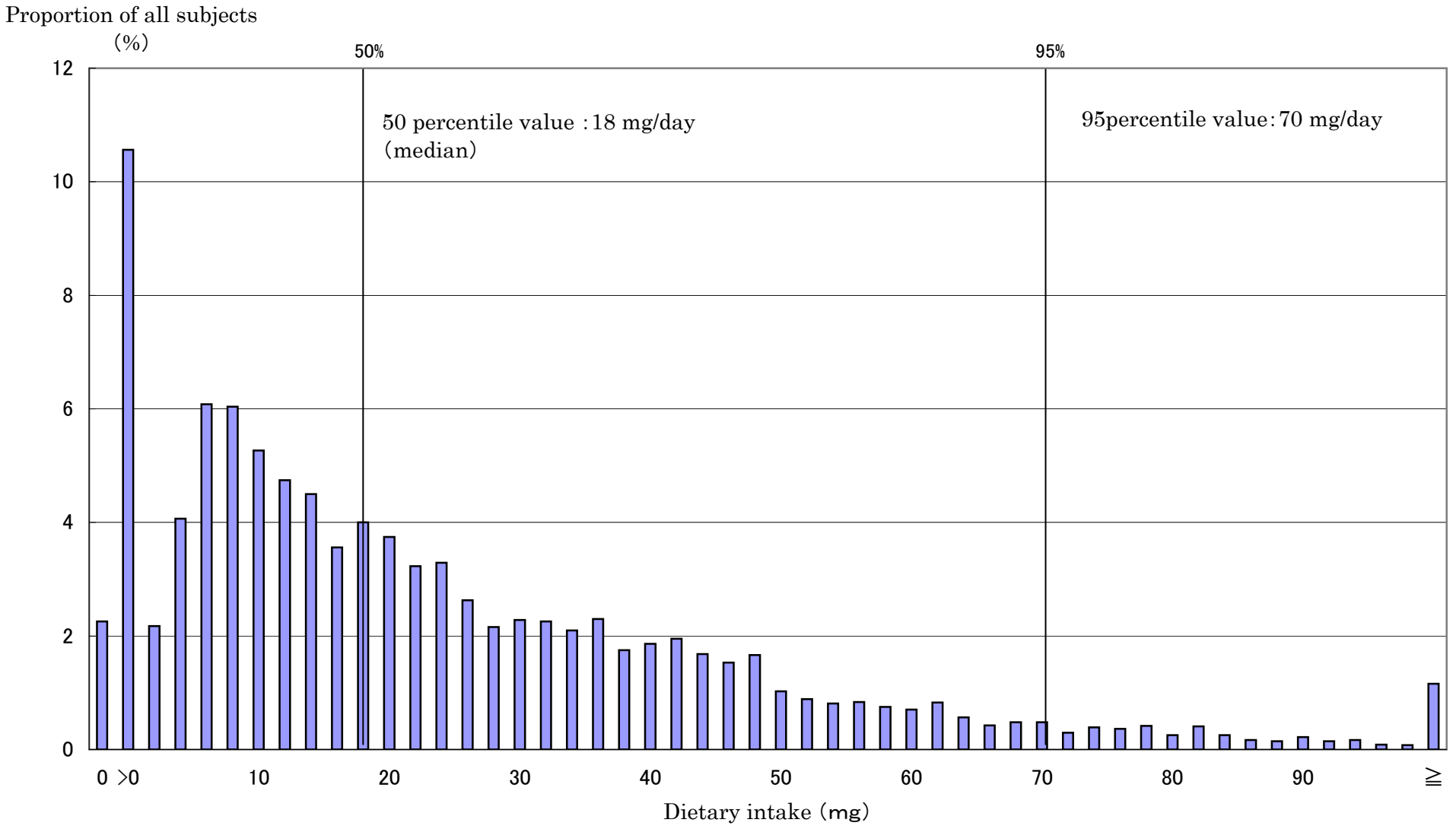
Pharmacokinetic flow chart of soy isoflavones

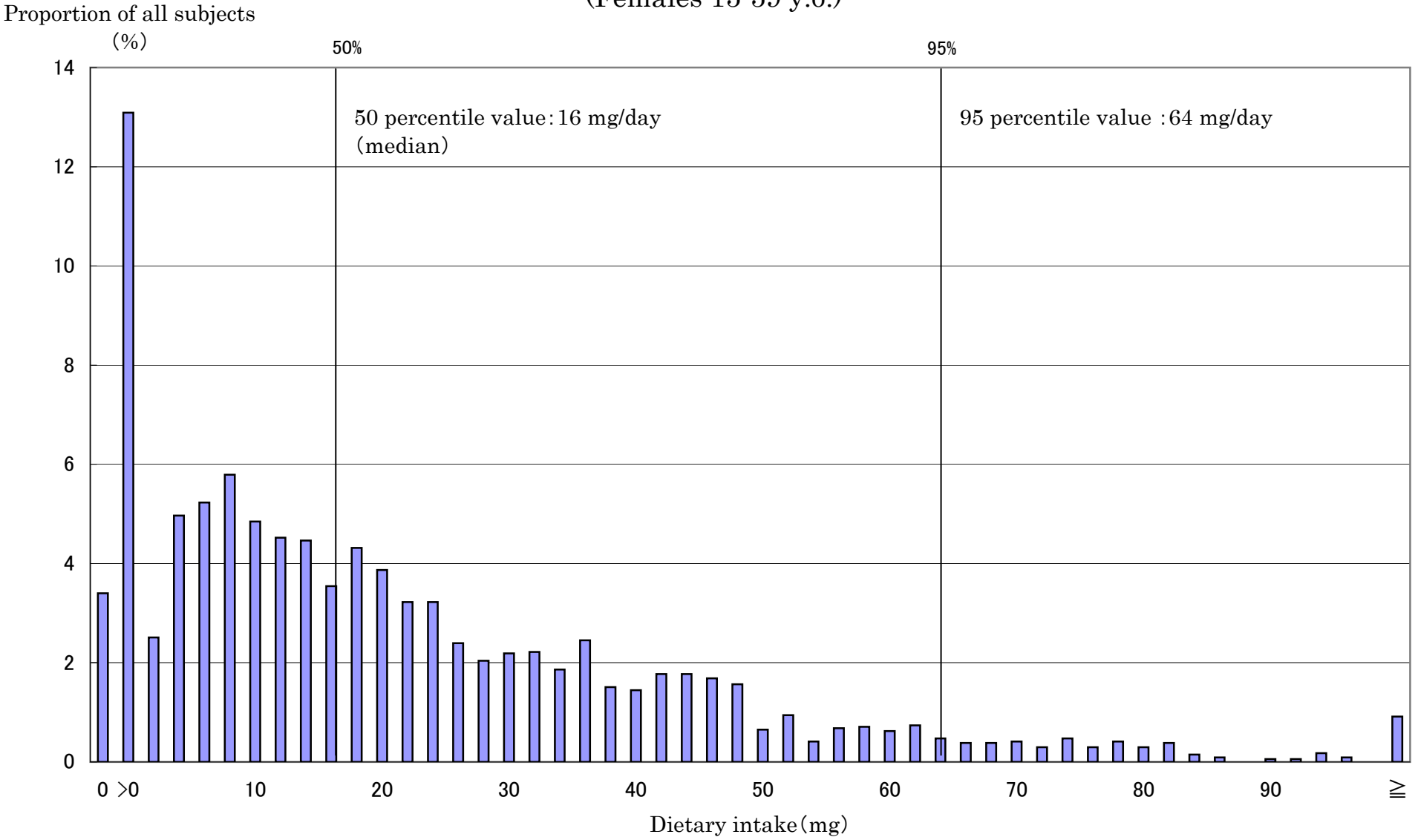
Appendix 1



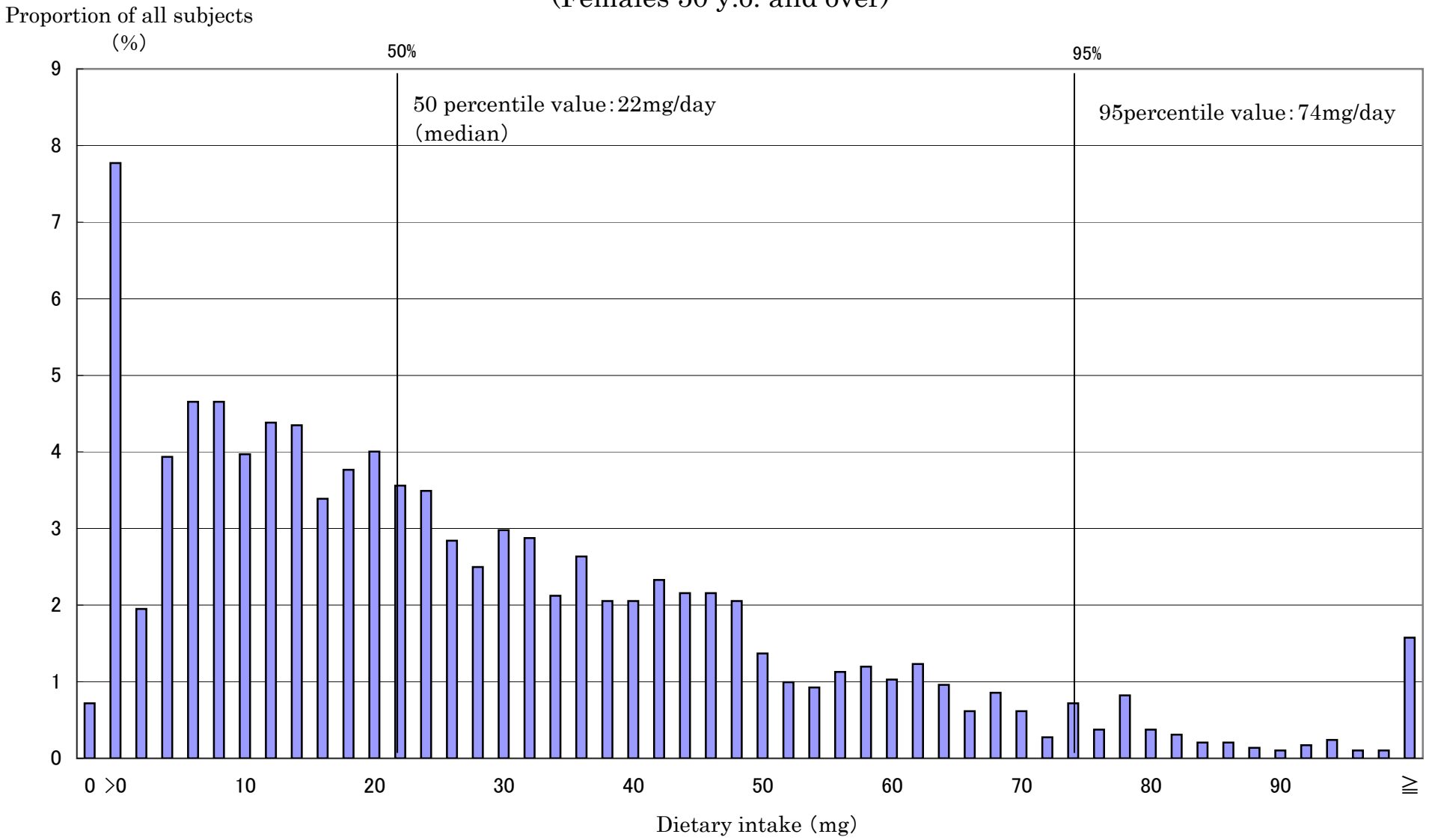
Distribution of soy isoflavone dietary intake from soy food products based on 2002 National Dietary Survey (Total)



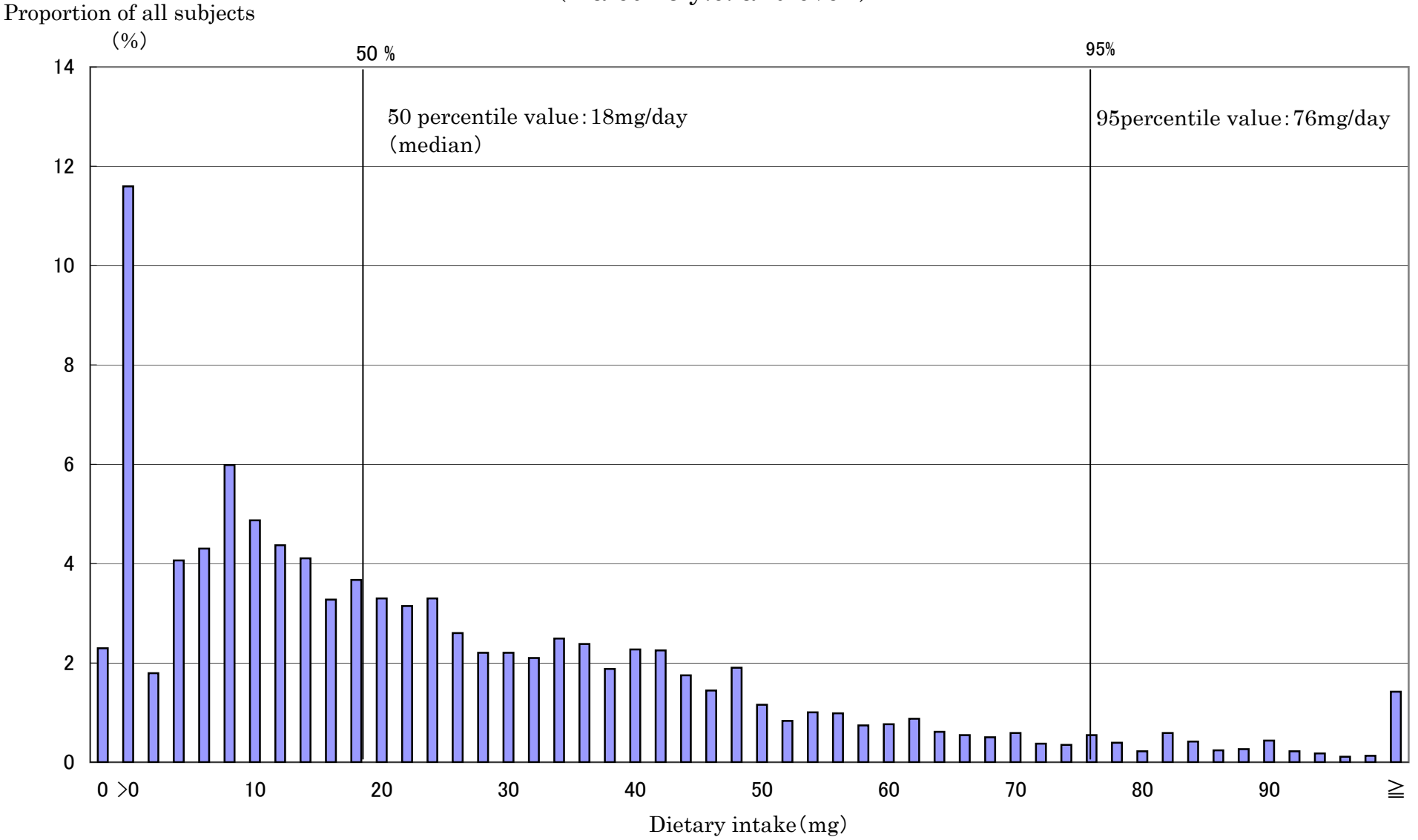
Distribution of soy isoflavone dietary intake from soy food products based on 2002 National Dietary Survey
(Females 15-59 y.o.)



Distribution of soy isoflavone dietary intake from soy food products based on 2002 National Dietary Survey
(Females 50 y.o. and over)



Distribution of soy isoflavone dietary intake from soy food products based on 2002 National Dietary Survey
(Males 15 y.o. and over)



List of test reports on safety (premenopausal females) Appendix 3-1

Reference No.	Soy isoflavone intake				Test content information		
	Normal dietary intake (mg/day)	Extra dietary intake (mg/day)	Total dietary intake (mg/day)	Reason for choosing test data	Subject number, age	Race (If unknown, location of test)	Intake period(Displayed as multiples of menstrual cycles)
44-1	-	28.1	28.1	E2 change P<0.05	6 subjects (20-29 y.o.)	Unknown (USA)	1
44-2	-	25.0	25.0	Only 3 subjects			
44-3	-	14.4	14.4		6 subjects (20-29 y.o.)	Unknown (USA)	1
45-1	10	20.0	30.0	No data start/end point (change only)			
45-2	10	40.0	50.0	No data start/end point (change only)			
45-3	10	50.0	60.0	Only 3 subjects			
46-1	-	36.2	36.2	No E2 data start/end point (% change only)			
46-2	-	27.7	27.7	No E2 data start/end point (% change only)			
49-1	-	38.0	38.0		16 subjects (29.7±6.4 y.o.)	Unknown (USA)	2
49-2	-	38.0	38.0	Subjects taking oral contraceptives			
48	-	37.4	37.4	No analysis data of hormone values according to menstrual cycle, no menstrual cycle data			
50	-	45.0	45.0	No analysis data of hormone values, no menstrual cycle data			
51	-	45.0	45.0	No analysis data of hormone values, no menstrual cycle data			
52	4	55.0	59.0	Analysis of hormone value is luteal stage			
53-1	-	10.0	10.0	Cross over test by dosage, No E2 data start point			
53-2	-	64.0	64.0	As above			
53-3	-	128.0	128.0	As above			
55-1	15.8	56.9	72.7	Overlap with 55-2 subjects			
55-2	18.4	57.3	75.7	Use data from 55-1 test where serum collection periods are all together, E2 change P=0.1	21 subjects	Japanese	2
54	-	154.0	154.0	E2 change P=0.01, no E2 data start/end point (%change only)			
56	1	75.0	76.0	No analysis data of hormone values			
57	-	147.0	147.0	E2 change P=0.03, Menstrual cycle change P=0.06	6 subjects (22-29 y.o.)	4 Caucasians, 1 African American, 1 Hispanic	1

List of test reports on safety (premenopausal females)

Appendix 3-2

Reference No.	Soy isoflavone intake			Follicular phase E2(pg/ml)				Changes to E2 before and after intake			Menstrual cycle				Changes to menstrual cycle before and after intake	
	Normal dietary intake (mg/day)	Extra dietary intake (mg/day)	Total dietary intake (mg/day)	Before intake	SE/SD*	After intake	SE/SD*	Change (Noted or change before and after intake)	Difference in E2 before and after intake (pg/ml)	Difference in E2 before and after intake (% change after intake compared to before intake)	Before intake (days)	SE/SD*	After intake (days)	SE/SD*	Change (Noted or change before and after intake)	Difference in mentstrual cycle before and after intake (% change after intake compared to before intake)
44-1	-	28.1	28.1	69.40	8.30	97.20	27.80	+27.80	+27.80	40.1%	27.5	2.4	29.0	2.0	+1.50	5.5%
44-2	-	25.0	25.0													
44-3	-	14.4	14.4	83.30	11.10	72.20	8.30	-11.10	-11.10	-13.3%	33.0	4.0	32.0	5.0	-1.00	-3.0%
45-1	10	20.0	30.0													
45-2	10	40.0	50.0													
45-3	10	50.0	60.0													
46-1	-	36.2	36.2													
46-2	-	27.7	27.7													
49-1	-	38.0	38.0	144.90	85.90	131.10	95.90	-13.80	-13.80	-9.5%	29.2	3.7	29.3	3.9	+0.10	0.3%
49-2	-	38.0	38.0													
48	-	37.4	37.4													
50	-	45.0	45.0													
51	-	45.0	45.0													
52	4	55.0	59.0													
53-1	-	10.0	10.0													
53-2	-	64.0	64.0													
53-3	-	128.0	128.0													
55-1	15.8	56.9	72.7													
55-2	18.4	57.3	75.7	98.00	85.00	65.40	51.70	-32.60	-32.60	-33.3%	29.0	4.2	32.4	8.7	+3.40	11.7%
54	-	154.0	154.0													
56	1	75.0	76.0													
57	-	147.0	147.0	186.90	99.30	35.50	range22 -57	-151.40	-151.40	-81.0%	28.3	1.9	31.8	5.1	+3.50	12.4%

*SE：standard error
SD：standard deviation