

**APPLICATION FOR THE APPROVAL OF TOUCHI EXTRACT  
UNDER REGULATION (EC) NO 258/97 OF THE EUROPEAN  
PARLIAMENT AND OF THE COUNCIL OF 27 JANUARY  
1997 CONCERNING NOVEL FOODS AND NOVEL FOOD  
INGREDIENTS**

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CBC Co., Ltd

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# APPLICATION FOR THE APPROVAL OF TOUCHI EXTRACT UNDER REGULATION (EC) NO 258/97 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 27 JANUARY 1997 CONCERNING NOVEL FOODS AND NOVEL FOOD INGREDIENTS

## Table of Contents

	Page
EXECUTIVE SUMMARY .....	i
ADMINISTRATIVE DETAILS .....	1
Name and Contact Details for Correspondence.....	1
INTRODUCTION.....	1
Confidential Information .....	2
I SPECIFICATION OF THE NOVEL FOOD .....	4
I.A Identity .....	5
I.A.1 Proposed Names .....	5
I.A.2 Trade Names .....	5
I.B Specification .....	5
I.C Analytical Information .....	6
I.C.1 Potentially Toxic Inherent Constituents and External Contaminants .....	6
I.C.2 Nutrients.....	8
I.D Representative Commercial Scale Batch Data .....	10
I.E Formulation Data .....	11
I.F Stability Data .....	11
I.F.1 Stability of the Bulk Powder .....	11
I.F.2 Stability Formulated Products .....	12
II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD .....	13
II.A Production Process .....	14
II.A.1 Production of Fermented Black Beans.....	14
II.A.2 Production of Touchi Extract .....	14
II.B History of the Production Process .....	15
II.C Identification of Potential Hazards .....	16
II.C.1 Toxicological Hazards.....	16
II.C.2 Nutritional Hazards.....	16
II.C.3 Microbiological Hazards.....	16
II.D Control of the Production Process.....	16
II.E Potential for Adverse Effect on Public Health or Micro-organism Contamination .....	17
III HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD .....	18
III.A Source of Touchi Extract .....	19
III.A.1 Identity of the Soybean .....	19
III.A.2 Cultivation of the Soybean .....	19

**Non-Confidential**

III.A.3	Fermentation of the Soybean.....	19
III.B	GM Status of Touchi Extract.....	20
III.C	Characterisation of the Small Soybean .....	20
III.D	Characterisation of <i>Aspergillus Oryzae</i> .....	20
III.E	Potential for Detrimental Effects on Human Health .....	21
III.E.1	Source Soybeans.....	21
III.E.2	<i>Aspergillus Oryzae</i> .....	21
IX	ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD .....	22
IX.A	Anticipated Food Uses and Maximum Use Level.....	23
IX.A.1	Intended Use and Use-Levels.....	23
IX.A.2	Consumer Awareness.....	24
IX.A.3	Exposure Estimates .....	24
X	INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE.....	27
X.A	Previous Human Exposure.....	28
X.B	Allergenicity of Touchi Extract .....	28
XI	NUTRITIONAL INFORMATION ON THE NOVEL FOOD .....	29
XI.A	Comparison of Touchi Extract to Traditional Counterparts.....	30
XI.A.1	Soy Proteins.....	30
XI.A.2	Soy Isoflavones.....	30
XI.A.3	Alpha-glucosidase Inhibitory Action .....	31
XI.B	Labelling of Touchi Extract .....	32
XII	MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD .....	33
XII.A	Information on Microorganisms and their Metabolites.....	34
XIII	TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD.....	35
XIII.A	Experimental Animal Data .....	36
XIII.A.1	Acute Toxicity.....	36
XIII.A.2	Subacute/Subchronic Toxicity.....	36
XIII.A.3	Reproductive/Developmental Toxicity.....	41
XIII.A.4	Mutagenicity/Genotoxicity .....	41
XIII.A.5	Chronic Toxicity.....	44
XIII.A.6	Other Preclinical Studies.....	44
XIII.B	Human Data .....	48
	OVERALL CONCLUSIONS .....	53
	REFERENCES.....	54
	GLOSSARY .....	58

**List of Appendices**

Appendix 1	Certificates of Analysis for Touchi Extract Lot 1
Appendix 2	Certificates of Analysis for Touchi Extract Lot 2
Appendix 3	Certificates of Analysis for Touchi Extract Lot 3
Appendix 4	Certification of Raw Material Soybean

**Non-Confidential**

Appendix 5	Determination of Touchi Extract alpha-Glucosidase Inhibitory Activity (Confidential)
Appendix 6	Manufacturing Controls for Touchi Extract
Appendix 7	Typical Examples of Food Products Containing Fermented Black Bean Ingredients

**List of Tables and Figures**

Table I.B-1	Proposed Specification for Touchi Extract.....	5
Table I.C.1.2-1	Heavy Metals Analysis for Touchi Extract .....	7
Table I.C.1.3-1	Dioxins and Dioxin-like PCBs Analysis for Touchi Extract.....	7
Table I.C.1.4-1	PAH Analysis for Touchi Extract.....	7
Table I.C.1.6-1	3-MCPD Analysis for Three Batches of Touchi Extract .....	8
Table I.C.2.1-1	Nutrient Profile for Three Batches of Touchi Extract .....	9
Table I.C.2.2-1	Nutrient Profile for Three Batches of Touchi Extract .....	9
Table I.C.2.3-1	Alpha-Glucosidase Inhibitory Action for Three Batches of Touchi Extract.....	9
Table I.C.2.3-2	Enzyme Inhibitory Action of Touchi Extract .....	10
Table I.D-1	Product Analysis for Three Batches of Touchi Extract .....	10
Table I.F.1-1	Stability of Touchi Extract at Room Temperature in Bulk Powder Form .....	11
Table I.F.1-2	Accelerated Stability Data for Touchi Extract in Bulk Powder Form .....	11
Table I.F.1-3	Stability Data for Touchi Extract in 10% Aqueous Solution .....	12
Table IX.A.3-1	Summary of the Estimated Daily Intake of Touchi Extract from All Proposed Food Categories when Consumed as Conventional Foods in the UK By Consumers Only (Concise Database Data).....	25
Table IX.A-1	Comparison of Nutrient Profiles for Fermented Black Beans and the Extract.....	30
Table XII.A-1	Microbiological Analysis for Touchi Extract .....	34
Table XIII.A.2-1	Body Weights in Rats Treated Orally with Touchi Extract for 28 Days.....	37
Table XIII.A.2-2	Haematology in Rats Treated Orally with Touchi Extract for 28 Days.....	38
Table XIII.A.2-3	Blood Chemistry in Rats Treated Orally with Touchi Extract for 28 Days.....	39
Table XIII.A.4.1-1	Results of Bacterial Reverse Mutation Test of Touchi Extract (-S9).....	42
Table XIII.A.4.1-2	Results of Bacterial Reverse Mutation Test of TE (+S9) .....	43
Table XIII.A.4.2-1	Results of Micronucleus Test.....	44
Table XIII.A.6-1	Preclinical Pharmacological Studies Conducted using Touchi Extract.....	46
Table XIII.B.1-1	Clinical Studies Conducted with Touchi Extract .....	49
Figure II.A.1-1	Overview of the Fermentation Process.....	14
Figure II.A.2-1	Overview of the Extraction Process.....	15
Figure IX.A.2-1	Example of a Food Supplement Product in Sachet Form.....	24
Figure XI.A.3-1	Digestion of Carbohydrate (Starch and Sucrose) by the Body .....	31

# **APPLICATION FOR THE APPROVAL OF TOUCHI EXTRACT UNDER REGULATION (EC) NO 258/97 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 27 JANUARY 1997 CONCERNING NOVEL FOODS AND NOVEL FOOD INGREDIENTS**

## **EXECUTIVE SUMMARY**

CBC Co., Ltd. wishes to market Touchi Extract (TE), a protein-rich product in powder form obtained by aqueous extraction of small soybeans that have been fermented using the fungus *Aspergillus Oryzae* (also known as “salted black beans”) for use in food supplement products in the European Union.

Fermented black beans have been widely used in China for the past 1000 years as a traditional seasoning and people are familiar with black beans as a protein source that can be preserved by drying without damage to the nutrient content. In Europe, fermented black beans have also been consumed for many years in Chinese dishes containing “black bean sauce” or involving “black bean paste” as an ingredient, and extensive discussions with the UK Novel Foods Competent Authority have indicated that the use of Touchi extract would not be considered novel for flavouring or seasoning. However, CBC Co., Ltd. also intend to market this product for use in food supplements in the European Union and for these purposes Touchi extract would be considered “novel” and fall under category (e) of Article 1(2) of Regulation (EC) No 258/97 (European Parliament and the Council of the European Union, 1997) (foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practises).

The application for placing Touchi extract on the market as a novel food or novel food ingredient is required to follow the European Commission’s Scientific Committee on Food (SCF) Recommendation 97/618/EC (Commission of the European Communities, 1997). Under Section 4 of this Recommendation, pertaining to the scientific classification of novel foods for the assessment of wholesomeness, Touchi extract would be considered as “Class 2 Complex Novel Food from non-GM sources: 2.1 the source of the novel food has a history of use in the Community”. Unlike the well-known large soybean, the small soybean has not been genetically modified and therefore, Touchi extract is derived from a non-GM source.

The tradename Touchi Extract (TE) will be used for marketing the food ingredient to the manufacturers. The proposed name for labelling purposes is “fermented black (soya) bean extract”, “Touchi extract” or “fermented black bean extract” (where \*made from soya may be used as a footnote for the latter 2). Analytical data has been provided to show that Touchi extract does not contain toxic inherent constituents or external contaminants that might be associated with soybean or oilseed type products. Specifically the results of analysis for pesticides, heavy metals, dioxins, dioxin-like PCBs, PAHs, mycotoxins and 3-MCPD have

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been presented. The nutrient profile of Touchi extract is well understood and the protein composition comparable to that of fermented black beans from which it is obtained. In addition, the results of analysis concerning soy isoflavones and alpha-glucosidase inhibitory activity are presented. The specification has been well defined and analysis for three non consecutive production batches provided to show that the product can be manufactured consistently in accordance with clearly defined chemical and physical parameters.

The production process involves the water extraction of fermented black beans followed by spray drying to yield Touchi extract in the powder form. No chemical modification of the fermented black beans occurs and conventional extraction procedures are employed. As an indication of scale, one tablespoon (15 g) of fermented black beans corresponds to 4.5 g of Touchi extract. The process is performed in accordance with the principles of Hazards Analysis and Critical Control Points (HACCP) and Good Manufacturing Practice (GMP). The inclusion of a pasteurisation stage and the maximum 7% limit on water content in the product specification both ensure that microbial growth is minimised.

The raw material used to prepare Touchi extract is the small soybean (*Glycine max.*) which has been extensively used in the Sichuan province of China for centuries where it is known as the small yellow bean. Fermentation is performed using the fungus *Aspergillus oryzae* which has been used for hundreds of years in the production of soy sauce, miso, and sake.

Touchi extract is intended to be consumed as a nutritional support by people who wish to slow the breakdown of carbohydrate following a meal. Such a “carbohydrate blocking” property may help people dieting to feel less hungry for longer after a meal.

In order to provide reassurance in relation to the use of such products by at risk groups, Touchi extract will only be marketed in food supplement form either as capsules or in sachet form (*e.g.*, tea formulation) at levels that would not exceed 4.5 g per daily serving/dose. It is anticipated that such products would always be consumed with a meal. Touchi extract will only be added to products as a single dose supplement formulation where presentation to the consumer is controlled by Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements and will not be added to general food products. Furthermore, risk management measures will be applied under the conditions of the Corrigendum to Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 (European Parliament and the Council of the European Union, 2007) on nutrition and health claims made on foods.

As stated above, the results of microbiological testing confirm the absence of pathogens.

The safety of Touchi extract is based principally on its equivalence to fermented black beans, which have been consumed since ancient times and are widely available in culinary dishes throughout the European Union. At the maximum consumption level of 4.5 g per day, Touchi extract is equivalent to approximately a 1 tablespoon (15 g) serving of fermented black beans. The solvent used is water and no selective extraction occurs with the result

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that no chemical modification is involved and the composition is comparable its traditional counterpart (fermented black beans). Touchi extract has an inhibitory effect on the enzyme alpha-glucosidase so delaying the digestion of carbohydrate in the small intestinal tract after a meal. Undigested carbohydrates or disaccharides are then excreted rather than being absorbed by the body thus providing possible assistance in weight control regimes. The approval of Touchi extract is requested only for specific food supplement products (in tablet or sachet form) that would be taken with meals at levels not exceeding 4.5 g/day and as such would have specific risk management and labelling clauses that would prevent the consumption by at risk groups such as diabetics. The safety of Touchi extract at these levels is supported by a range of toxicological safety studies, including a 28-day sub-chronic study in the rat, which established a no-observed-adverse-effect level (NOAEL) of 2500 mg/kg body weight/day (Fujita and Yamagami, 2007), equivalent to 150 g/day for a 60 kg adult, or equivalent to approximately a 33-fold safety factor compared to maximum daily consumption of 4.5 g. Numerous human clinical studies have been conducted in both healthy and diabetic subjects with Touchi extract at levels up to 10 g per daily serving and durations of up to 6 months. The absence of major adverse effects in subjects offers additional evidence for the safety of Touchi extract.

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## **INTRODUCTION**

CBC Co., Ltd. wishes to market Touchi extract (TE) or fermented black bean extract, a protein-rich product in powder form obtained by aqueous extraction of small soybeans that have been fermented using the fungus *Aspergillus Oryzae* (also known as "salted black beans") for use in certain specified food supplement products in the European Union.

Fermented black beans have been widely used in China for the past 1000 years as a traditional seasoning and people are familiar with black beans as a protein source that can be preserved by drying without damage to the nutrient content. In Europe, fermented black beans have also been consumed for many years in Chinese dishes containing "black bean sauce" or involving "black bean paste" as an ingredient, and extensive discussions with the UK Novel Foods Competent Authority have indicated that the use of Touchi extract would not be considered novel for flavouring or seasoning. However, CBC Co., Ltd. also intend to market this product for use in food supplement products in the European Union and for these purposes Touchi extract would be considered "novel" and fall under category (e) of Article

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1(2) of Regulation (EC) No 258/97 (European Parliament and the Council of the European Union, 1997) (foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practises).

The application for placing Touchi extract on the market as a novel food or novel food ingredient is required follow to the European Commission's Scientific Committee on Food (SCF) Recommendation 97/618/EC (Commission of the European Communities, 1997). Under Section 4 of this Recommendation, pertaining to the scientific classification of novel foods for the assessment of wholesomeness, Touchi extract would be considered as "Class 2 Complex Novel Food from non-GM sources: 2.1 the source of the novel food has a history of use in the Community". Unlike the well-known large soybean, the small soybean has not been genetically modified and therefore, Touchi extract is derived from a non-GM source.

In accordance with this SCF Recommendation (97/618/EC) the following structured sections are required to establish the safety of a Class 2.1 novel food:

- I Specification of the novel food
- II Effect of the production process applied to the novel food
- III History of the organism used as the source of the novel food
- IX Anticipated intake/extent of use of the novel food
- X Information from previous human exposure to the novel food or its source
- XI Nutritional information on the novel food
- XII Microbiological information on the novel food
- XIII Toxicological information on the novel food

We present the application in this structured format. At the start of each section the information that must be addressed in that particular structured scheme is specified in more detail.

A glossary is provided at the end of this document to explain abbreviated terms referred to in the dossier.

## **Confidential Information**

CBC Co., Ltd requests that certain information contained within Sections I.E (Formulation Data), I.F (Stability Data) and II.A.2 (Production of Touchi Extract) be considered confidential information. The Sections I.E and I.F marked confidential provide detailed descriptions of representative formulations and their respective properties, and Section II.A.2 provides a detailed description of the manufacturing process to obtain Touchi Extract. These Sections

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therefore, include proprietary information of significant commercial value to the company. For public consideration a Non-Confidential version of these Sections has been provided herein which contains an overview of the relevant information. Notably, the Non-Confidential information provides detailed stability information on the bulk powder to reasonably allow for a general evaluation of the safety of the product. The stability of the material when incorporated into a formulation would have to be demonstrated for each individual product and the data presented herein should only be considered evidence that this can be achieved by the appropriate research and development programs.

## **I SPECIFICATION OF THE NOVEL FOOD**

Based on the Commission Recommendation 97/618/EC structured schemes the following information must be provided pertaining to the specification of the novel food (Commission of the European Communities, 1997):

- a. "...is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"
- b. "Is the information representative of the novel food when produced on a commercial scale?"
- c. "Is there an appropriate specification (including species, taxon *etc.* for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

These points are addressed in the section that follows.

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### I.A Identity

Touchi extract (TE) is a protein-rich powder containing an alpha-glucosidase inhibitor obtained on water extraction of small soybeans fermented with the fungus *Aspergillus Oryzae*.

#### I.A.1 Proposed Names

Fermented black bean (soya) extract”

“Touchi extract” (where “\*made from soya” may be used as a footnote)

“Fermented black bean extract” (where “\*made from soya” may be used as a footnote)

#### I.A.2 Trade Names

Fermented black bean extract, Touchi extract, fermented black bean concentrate

### I.B Specification

The proposed regulatory specification for Touchi extract is presented in Table I.B-1. The specification includes both nutrient and purity measures.

<b>Table I.B-1 Proposed Specification for Touchi Extract</b>		
<b>Parameter</b>	<b>Specification</b>	<b>Analytical Method</b>
<b>Characteristics</b>		
Appearance	Light brown powder	Visual
Taste	‘Pleasant’	Taste
Fat	Max. 1%	Soxhlet extraction
Protein	Min. 55%	Kjeldahl method, AOAC 981
Water	Max. 7%	AOAC 925.1
$\alpha$ -glucosidase inhibitor activity	IC <sub>50</sub> min. 0.025	Enzyme assay <sup>1</sup>
<b>Contaminants</b>		
Arsenic	Max. 10 $\mu$ g/kg	Atomic Absorption Spectroscopy
Aflatoxins	Max. 5 $\mu$ g/kg	HPLC
3-MCPD	Max. 50 $\mu$ g/kg	GCMS
Total heavy metals (expressed as lead)	Max. 20 $\mu$ g/kg	Na <sub>2</sub> S Colorimetric method
<b>Microbiological Requirements</b>		
Total bacteria count	$\leq$ 1000 cfu/g	USP23
Total mould and yeast count	$\leq$ 300 cfu/g	USP23
<i>Escherichia coli</i>	Negative /g	USP23

<sup>1</sup> Modified method of Miwa *et al.*, *Chem Pharm. Bull.*, 34:838, 1986

## **I.C Analytical Information**

Analytical information concerning potentially toxic inherent constituents, external contaminants and nutrients has been obtained for three non-consecutive batches of Touchi extract that are representative of the commercial product to be marketed in the European Union (EU). All analysis was conducted by independent laboratories with the appropriate country accreditation and the test methods comply with the relevant Commission (EC) Regulations and Directives laying down methods of sampling and analysis of potential contaminants (2002/63/EC, 1883/2006 and 333/2007; Commission of the European Communities, 2002, 2006a and 2007). The three non-consecutive batches of Touchi extract analysed and discussed herein are labelled as follows:

Lot 1 Touchi extract manufactured 17/02/07

Lot 2 Touchi extract manufactured 18/12/06

Lot 3 Touchi extract manufactured 16/11/06

The Certificates of Analyses for the data discussed below and the relevant testing laboratory accreditation details are provided as Appendices 1 to 3.

### **I.C.1 Potentially Toxic Inherent Constituents and External Contaminants**

#### *I.C.1.1 Pesticides*

Fermented black beans are derived from the small soybean grown in the Sichuan province. No pesticides are used at any stage of the production process or are used on other crops in regions in which the small soybean is grown. Pesticides use is regulated at both local and governmental level in the province and the appropriate certification has been issued by the Organic Food Development and Certification Centre (OFDC, 2007; accredited by the International Federation of Agriculture Movements, IFOAM) and is presented in Appendix 4. In addition, a pesticide screen has been conducted on the three production batches of Touchi extract and full details are provided in Appendices 1 to 3. No residues of pesticides were recorded above the limits of detection.

#### *I.C.1.2 Heavy Metals*

Heavy metals analysis for three batches of Touchi extract is presented in Table I.C.1.2-1. EU Regulation 1881/2006 (Commission of the European Communities, 2006b) concerning contaminants in foodstuffs specifies a 0.2 µg/kg limit for lead in cereals, legumes and pulses and for cadmium in soybeans. All results meet these regulatory requirements for lead and cadmium and either fall within acceptable ranges or are below detection limits for the other heavy metals tested.

<b>Table I.C.1.2-1 Heavy Metals Analysis for Touchi Extract</b>				
Parameter	Maximum Level <sup>1</sup> (mg/kg wet wt)	TE Lot Results (mg/kg)		
		1	2	3
Arsenic (As)	-	1.02	1.12	0.783
Lead (Pb)	0.2	0.11	0.11	0.08
Cadmium (Cd)	0.2	0.09	0.09	0.09
Mercury (Hg)	-	<0.004	<0.004	<0.004

<sup>1</sup>According to EU Regulation 1881/2006 (Commission of the European Communities, 2006b)

*I.C.1.3 Dioxins and Dioxin-like PCB's*

Dioxins and dioxin-like PCBs have been analysed for three batches of Touchi extract and the results are summarised in Table I.C.1.3-1. At the proposed intake of Touchi extract of up to 4.5 g/day (see Section IX) the levels of dioxins and dioxin-like PCBs are considered to fall within acceptable ranges for the three batches.

<b>Table I.C.1.3-1 Dioxins and Dioxin-like PCBs Analysis for Touchi Extract</b>			
Parameter	TE Lot Results (pg/g) <sup>1</sup>		
	1 <sup>2</sup>	2 <sup>3</sup>	3 <sup>4</sup>
Sum of dioxin-like PCBs only (WHO-PCB-TEQ)	28	29	28
Sum of dioxins and furans (WHO-PCDD/F-TEQ)	48	46	45
Sum of dioxins, furans and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)	76	75	73

<sup>1</sup>Upperbound concentrations, calculated on the assumption that all values of different congeners below the limit of quantification are equal to the limit of quantification; results refer to sample containing 12% moisture where dry matter of sample <sup>2</sup>95.8%, <sup>3</sup>95.2%, <sup>4</sup>96.3%, respectively.

*I.C.1.4 Polycyclic Aromatic Hydrocarbons (PAHs)*

Three batches of Touchi extract have been analysed for polycyclic aromatic hydrocarbon (PAH) contamination according EU Regulation 1881/2006 (Commission of the European Communities, 2006b). The full list of PAHs screened is provided in Appendices 1 to 3 and all results are below detection limits. As described in the Regulation, benzo(a)pyrene can be used as a marker for the occurrence of PAHs in food and the results for this contaminant are summarised in Table I.C.1.4-1.

<b>Table I.C.1.4-1 PAH Analysis for Touchi Extract</b>			
Parameter	TE Lot Results (µg/kg)		
	1	2	3
Benzo(a)pyrene	<0.5	<0.5	<0.5

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### **I.C.1.5      *Mycotoxins***

Aflatoxins are produced by certain species of *Aspergillus*, the fungus employed in the fermentation of the small soybeans to form Touchi extract. Three batches of Touchi extract were analysed for aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>. None of the batches were found to contain these mycotoxins above the limits of detection (<0.2 µg/kg). As far as we are aware *Aspergillus oryzae* is not a source of Ochratoxin A (Fennel, 1976; Stoloff *et al.*, 1977), an opinion supported by experts at the accredited independent testing laboratory.

### **I.C.1.6      *3-Monochloropropane-1,2-diol (3-MCPD)***

The presence of 3-monochloropropane-1,2-diol (3-MCPD) has been determined for three batches of Touchi extract according to EU Regulation 1881/2006 (Commission of the European Communities, 2006b). Under this Regulation the maximum levels of 3-MCPD permitted in hydrolysed vegetable proteins and soy sauce is 20 µg/kg for a liquid containing 40% dry matter, which corresponds to 50 µg/kg in the dry matter. The levels of 3-MCPD are <30 µg/kg for the three batches tested and therefore fall well below the regulatory requirements. In addition, the maximum intake level of up to 4.5 g per day proposed for Touchi extract (Section IX) ensures this product will not contribute significantly to the dietary intake of 3-MCPD. A 3-MCPD specification of 0 to 50 mg/kg has been introduced to the product specification (Table I.B-1) in order to provide further assurance of the safety of products containing Touchi extract.

<b>Table I.C.1.6-1      3-MCPD Analysis for Three Batches of Touchi Extract</b>				
<b>Parameter</b>	<b>Maximum Level<sup>1</sup> (µg/kg)</b>	<b>TE Lot Results (µg/kg)</b>		
		<b>1</b>	<b>2</b>	<b>3</b>
3-MCPD	50	<10	<30	<30

<sup>1</sup>Hydrolysed vegetable protein and soy sauce, EU Regulation 1881/2006 (Commission of the European Communities, 2006b) where level adjusted for dry matter; dry matter of samples tested was Lot 1(95.8%), Lot 2 (95.2%) and Lot 3 (96.5%).

## **I.C.2      **Nutrients****

### **I.C.2.1      *Nutrient Profile***

As discussed later in Section II, one portion of a dish using black bean sauce would generally contain 15 g of fermented black beans, which on extraction is equivalent to 4.5 g of Touchi extract. As a result, the proposed maximum daily intake of up to 4.5 g per day of Touchi extract is equivalent to one serving of a typical dish containing a traditional form of fermented black beans (e.g., sauce, paste). On extraction of fermented black beans the water soluble fraction is removed from the insoluble part to give a product that is consequently high in protein but low in fat. No chemical modification is involved in the extraction of the water-soluble fraction of fermented black beans and therefore, the nature of the nutrients remains identical to the traditional fermented black bean counterparts. A summary of the fat and protein content is provided in Table I.C.2.1-1 for 3 batches of Touchi extract. Soy proteins are a well-established source of all the essential amino acids required

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in the human diet, as detailed in Section XII. Essentially, Touchi extract is a concentrated form of the protein fraction of fermented black beans.

<b>Table I.C.2.1-1 Nutrient Profile for Three Batches of Touchi Extract</b>			
<b>Nutrient</b>	<b>TE Lot Results (% w/w total composition)</b>		
	<b>Lot 1</b>	<b>Lot 2</b>	<b>Lot 3</b>
Fat	0.2	0.1	0.2
Protein	63.1	66.3	65.0

*I.C.2.2 Isoflavone Aglycones Content*

The soy isoflavone aglycones content of three batches of Touchi extract is presented in Table I.C.2.2-1. The Certificates of Analysis and testing laboratory accreditation details are provided in Appendices 1-3.

<b>Table I.C.2.2-1 Nutrient Profile for Three Batches of Touchi Extract</b>			
<b>Nutrient</b>	<b>TE Lot Results (g/100 g)</b>		
	<b>Lot 1</b>	<b>Lot 2</b>	<b>Lot 3</b>
Soy isoflavone aglycones (aglycone equivalent) <sup>1</sup>	0.11	0.13	0.13

<sup>1</sup> Based on "Test method for Soy Isoflavone Aglycones (Aglycone equivalent) In Foods" taken from "Guidelines for Food for Specified Health Uses (FOSHU) etc., Including Soy Isoflavones", (Ministry of Health, Labour and Welfare Japan, 2006)

*I.C.2.3 Enzyme Inhibitory Activity*

It is well established that fermented black beans exhibit alpha-glucosidase inhibitory action and on extraction this activity is retained (see Section XII). The IC<sub>50</sub> value for ten production lots of Touchi extract is presented in Appendix 5 and shows activity is consistently in the range 0.025 to 0.059 mg/mL. The alpha glucosidase inhibitory action for three batches of Touchi extract is also provided in Table I.C.2.3-1. The Appendix also outlines the details of the assay used to determine alpha-glucosidase inhibitory activity. Touchi extract has also been screened for inhibition of other enzymes, (see Table I.C.2.3-2 for representative batch analysis) but only alpha-glucosidase inhibitory action has been observed.

<b>Table I.C.2.3-1 Alpha-Glucosidase Inhibitory Action for Three Batches of Touchi Extract</b>			
<b>Enzyme</b>	<b>TE Lot Results (IC<sub>50</sub> mg/mL)</b>		
	<b>Lot 1</b>	<b>Lot 2</b>	<b>Lot 3</b>
Alpha-glucosidase	0.047	0.055	0.54

<b>Table I.C.2.3-2 Enzyme Inhibitory Action of Touchi Extract</b>	
<b>Enzyme</b>	<b>Activity (mg/mL)</b>
α-Glucosidase (Sucrose)	0.1
α-Glucosidase (Maltose)	0.8
α-amylase	ND
Lactase	ND
Lipase	ND
Protease – Pepsin	ND
Protease – Trypsin	ND
Protease – Chymotrypsin	ND

ND = not detected

**I.D Representative Commercial Scale Batch Data**

Three non-consecutive batches of Touchi extract that are representative of production on a commercial scale were analysed according to the product specification (see Table I.B-1), and the results are provided in Table I.D-1.

<b>Table I.D-1 Product Analysis for Three Batches of Touchi Extract</b>				
<b>Parameter</b>	<b>Specification and Units</b>	<b>TE Lot Results (µg/kg)</b>		
		<b>1</b>	<b>2</b>	<b>3</b>
<b>Characteristics</b>				
Appearance	Light brown powder, Visual inspection	Light brown powder	Light brown powder	Light brown powder
Taste	Pleasant, taste inspection	Pleasant	Pleasant	Pleasant
Fat	Max. 1%	0.2	0.1	0.2
Protein	Min 55%	63.1	66.3	65.0
Water	Max. 7%	3.5	3.2	3.9
α-Glucosidase inhibitor activity	IC <sub>50</sub> min. 0.025	0.047	0.055	0.54
<b>Contaminants</b>				
Arsenic	Max. 10 µg/kg	1.0	1.1	0.8
Aflatoxins	Max. 5 µg/kg	<0.2	<0.2	<0.2
3-MCPD	Max. 50 µg/kg	<10	<30	<30
Total heavy metals (expressed as lead)	Max. 20 µg/kg	<20	<20	<20
<b>Microbiological Requirements</b>				
Total bacteria count	Less than 1000 cfu/g	<1000	<1000	<1000
Total mould and yeast count	Less than 300 cfu/g	<300	<300	<300
<i>Escherichia coli</i>	Negative /g	Negative	Negative	Negative

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**I.E Formulation Data**

It is anticipated that Touchi extract may be used as a bulk powder or incorporated into formulations for presentation to the consumer as a food supplement product. For example, formulations for both tablet and tea formulation have been approved as Foods for Specific Health Uses (FOSHU) in Japan.

**I.F Stability Data**

In order to confirm the stability of Touchi extract in the anticipated delivery forms, the product has been monitored over time as a both as a bulk powder and in typical tablet and green tea formulations.

**I.F.1 Stability of the Bulk Powder**

The stability of Touchi extract has been confirmed over 36 months at room temperature by measuring the taste, appearance, and alpha-glucosidase inhibitory activity at regular intervals. The results of these studies are summarised in Table I.F.1-1. In addition, accelerated tests (at elevated temperature) and tests in as a 10% (w/w) solution in water are provided in Table I.F.1-2 and I.F.1-3.

Parameter	Month						
	Initial	1	3	6	12	24	36
Appearance and Taste	-	NC	NC	NC	NC	NC	NC
$\alpha$ -Glucosidase activity (% of initial)	100.0	102.0	96.2	94.4	96.2	96.2	98.1
$\alpha$ -Glucosidase activity, IC <sub>50</sub> (mg/mL)	0.51	0.50	0.53	0.54	0.53	0.53	0.52

NC = no change

Parameter	Temperature/Month								
	37 °C			45 °C			55 °C		
	Initial	3	6	Initial	3	6	Initial	3	6
Appearance and Taste	-	NC	NC	-	NC	NC	-	NC	NC
$\alpha$ -Glucosidase activity (% of initial)	100.0	97.7	97.7	100.0	97.6	97.4	100.0	102.0	100.2
$\alpha$ -Glucosidase activity, IC <sub>50</sub> (mg/mL)	0.52	0.53	0.53	0.52	0.53	0.53	0.52	0.51	0.52

NC = no change

<b>Table I.F.1-3 Stability Data for Touchi Extract in 10% Aqueous Solution</b>										
<b>Parameter</b>	<b>Conditions/Time Period</b>									
	<b>Autoclave (121 °C, 20 min)</b>						<b>Stored at 4 °C</b>		<b>Stored at 40 °C</b>	
	<b>pH 4</b>		<b>pH 7</b>		<b>pH 9</b>		<b>Initial</b>	<b>Wk 4</b>	<b>Initial</b>	<b>Wk 4</b>
	<b>Before</b>	<b>After</b>	<b>Before</b>	<b>After</b>	<b>Before</b>	<b>After</b>				
α-Glucosidase activity (% of initial)	100.0	98.1	100.0	102.0	100.0	96.3	100.0	98.1	100.0	102.0
α-Glucosidase activity, IC <sub>50</sub> (mg/mL)	0.52	0.52	0.52	0.51	0.52	0.54	0.52	0.53	0.52	0.51

**I.F.2 Stability Formulated Products**

The stability of Touchi extract has also been monitored over time in typical formulations (e.g., as tablet and tea products) both at relative time (RT) and at elevated temperature (accelerated studies).

## **II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD**

Based on Commission Recommendation 97/618/EC decision trees the following questions must answered pertaining to the production process of the novel food (Commission of the European Communities, 1997):

- a. "Does the novel food undergo a production process?"
- b. "Is there a history of use of the production process for the food?" If no, "does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?"
- c. "Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?"
- d. "Are the means identified for controlling the process to ensure that the novel food complies with its specification?"
- e. "Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?"
- f. "After processing is the novel food likely to contain microorganisms of adverse public health significance?"

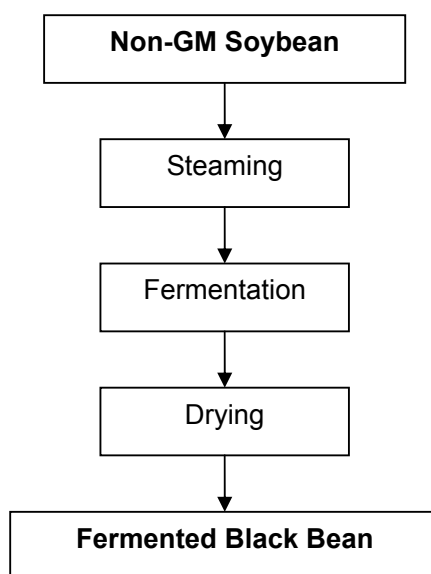
These points are addressed in the section that follows.

## **II.A Production Process**

### **II.A.1 Production of Fermented Black Beans**

The process involved in the formation of fermented black beans is outlined in Figure II.A.1-1. The starting material, small soybeans are prepared for fermentation by washing and screening for foreign material. The soybeans are then steamed and fermented in an aerobic environment using the fungus, *Aspergillus Oryzae*. The fermentation process follows conventional methods well established in the industry. The resulting fermented black bean product is then washed, dried, screened for any foreign material once again and packaged under vacuum for use in the proceeding extraction process (Section II.A.2). Notably, *Aspergillus oryzae* use will be regulated in the future under pending regulation in the EU establishing a common authorisation procedure for food additives, food enzymes and food flavours (European Parliament and the Council of the European Union, 2006a,b).

**Figure II.A.1-1 Overview of the Fermentation Process**



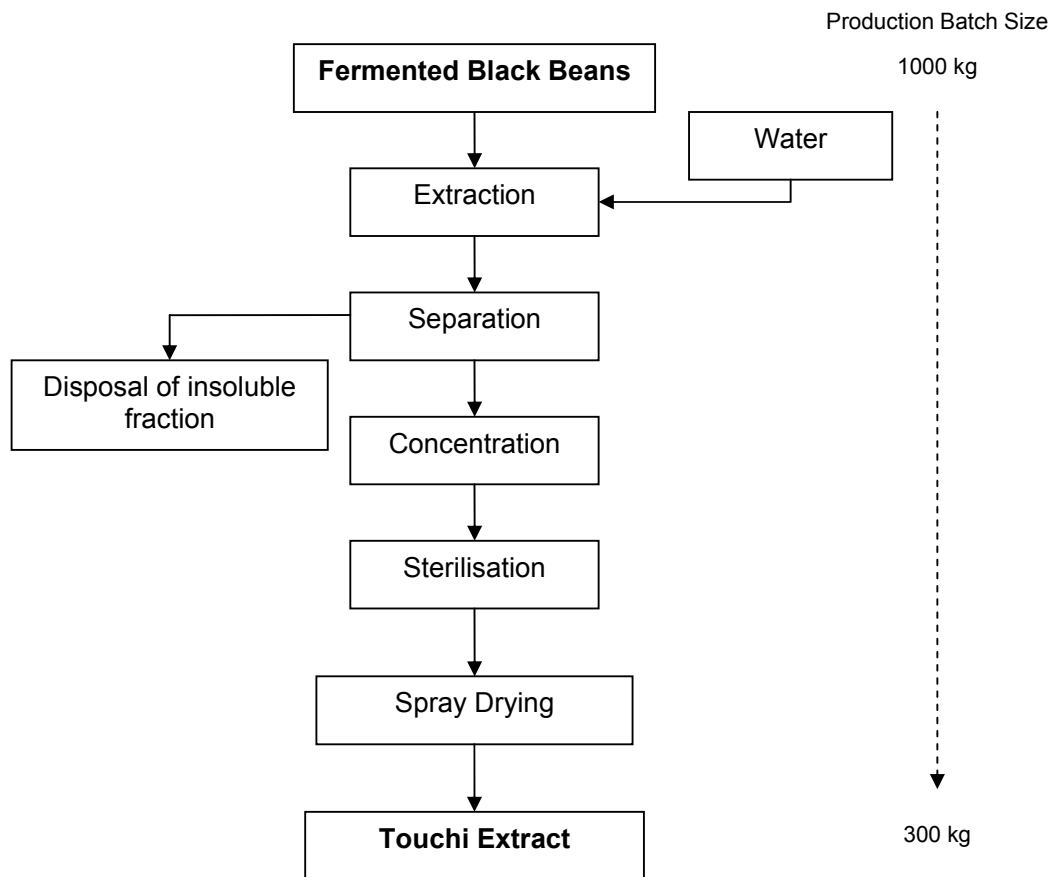
### **II.A.2 Production of Touchi Extract**

An overview of the extraction process to yield Touchi extract is provided in Figure II.A.2-1. The fermented black beans are essentially milled and then suspended in boiling water to achieve extraction into the aqueous phase. Following extraction, the aqueous phase is separated from the insoluble fraction following a series of purification steps before being concentrated and spray dried to yield the final product, Touchi extract, as a pale brown powder (water content less than 7%). No chemical modification of the fermented black beans is involved and the only solvent is water. The process is typical of the industry and

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follows conventional extraction procedures. To give an indication of scale, one tablespoon (15 g serving) of fermented black beans corresponds to 4.5 g of Touchi extract. The intake levels of up to 4.5 g of Touchi extract per day proposed is therefore equivalent to consumption of one serving of fermented black beans in traditional form as part of a Chinese dish.

**Figure II.A.2-1 Overview of the Extraction Process**



**II.B History of the Production Process**

Fermentation using the *Aspergillus Oryzae* fungus is a well established procedure employed in the production of soy sauce, sake, and miso (Wood, 1977). Over time strains of *Aspergillus oryzae* have been identified that are suitable for fermentation and it has been safely used in the food industry for several hundred years. In the production of soy sauce, *Aspergillus oryzae* is combined with soybeans and wheat (a combination known as koji) and for the formation of sake is combined with rice. There are a number of different types of miso, all of which are fermented using *Aspergillus oryzae* but involve different sources of carbohydrate including rice, barley or soybeans. The exact fermentation conditions that lead to a high quality product without the risk of toxic by-product formation have been established over years of industrial experience, thereby ensuring the safety of Touchi extract.

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The subsequent extraction of the fermented black beans is a conventional method, which only uses hot water. No other solvents or processes are involved and following extraction only the soluble fraction is spray dried to form the finished product. The extraction procedure does not change the composition of Touchi extract compared to the traditional counterpart fermented black beans, but offers advantages in terms of application. Fermented black beans and fermented black bean paste are common ingredients of Chinese dishes and have been consumed in Europe for many years. However, fermented black beans are not completely soluble in water and can often give rise to an unsightly precipitate in dishes such as consommé soups or to an uneven flavour distribution during the cooking process. In addition, the powdered nature of Touchi extract makes it much easier to incorporate into other products as a seasoning requiring less mixing than fermented black beans or the paste. Although soy sauce is most commonly used in the liquid form it is also available for incorporation into products such as 'instant' foods (e.g., noodles) in the powder form. In many ways Touchi extract is analogous to powdered soy sauce differing only in flavour.

### **II.C Identification of Potential Hazards**

It is not anticipated that any potential hazards are associated with the processes employed in the production of Touchi extract. Although there has been concern regarding toxin formation including aflatoxins during fermentation with different fungi, it has been established that no hazardous materials are formed under proper, regulated manufacturing conditions using *Aspergillus oryzae* (U.S. EPA, 1997). In terms of composition, there are no changes in the nature of the nutritional components compared to its starting material and traditional food counterpart, fermented black beans; essentially Touchi extract can be considered a concentrated form of the protein fraction.

#### **II.C.1 Toxicological Hazards**

See Section I.C.1.

#### **II.C.2 Nutritional Hazards**

See Section I.C.2.

#### **II.C.3 Microbiological Hazards**

See Section II.E and XII.

### **II.D Control of the Production Process**

The procedures involved in the manufacture of Touchi extract follow the principles of HACCP and GMP. Details of these controls are provided in Appendix 6. The process and intermediate products are monitored routinely to ensure that the Touchi extract meets specification. If a final product batch is found not to meet the required specification it is discarded. The nature of the manufacturing process (water extraction of fermented black

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beans) is such that it is not currently feasible to reprocess final material if it fails to meet the specification.

**II.E Potential for Adverse Effect on Public Health or Micro-organism Contamination**

The manufacture of Touchi extract complies with principles laid out in Directive 93/43/EEC on the hygiene of foodstuffs (Council of the European Communities, 1993) at all stages of the process. Generally, foods formed by fermentation have a higher risk of containing micro-organisms that may be a concern to safety and as a result a pasteurisation step is included in the final stages of product manufacture. Whereas the pasteurisation process can be problematic to perform on fermented soybeans in the solid form or as a paste, the hot extraction of fermented black beans allows sterilisation and filtration to be performed more effectively so greatly increasing the safety of the product compared to its traditional counterparts. In addition, preparation of the product in the powder form lowers the amount of moisture to <15% thereby reducing the potential for microbial growth and increasing the shelf-life of the product. Limits on microbial growth are included in the product specification and analytical data is provided in Section XII.

### **III HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD**

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to the history of the source organism (Commission of the European Communities, 1997):

- a. "Is the novel food obtained from a biological source, *i.e.*, a plant, animal, or microorganism?"
- b. "Has the organism used as the source of the novel food been derived using GM?"
- c. "Is the source organism characterised?"
- d. "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

These points are addressed in the section that follows.

### **III.A Source of Touchi Extract**

#### **III.A.1 Identity of the Soybean**

The raw material used to prepare fermented black bean is the small soybean (*Glycine max.*) which has been extensively used in the Sichuan province of China for centuries where it is known as the small yellow bean. The species has been cultivated for many years to generate a species that is resistant to insect and environmental damage. The soybean is a species of legumes that grows annually and is native to East Asia. The height, growth, and environment in which it is found can vary. Growing prostrate to around 20 cm or erect to 2 m, the pods, stems, and leaves are characterised by a brown or grey pubescence, and the leaves are trifoliate having up to five leaflets which are 6 to 15 cm in length and 2 to 7 cm in width. The plant exhibits small pink, purple or white flowers growing in the axil of the leaf and hairy pod-type fruit 3 to 8 cm in length that grow in clusters of 3 to 5 where each pod typically contains 2 to 4 soybeans (*i.e.*, seeds). Notably the leaves of the plant are lost before the seeds mature. The hull of the soybean is hard and water resistance so protecting the seed from damage.

#### **III.A.2 Cultivation of the Soybean**

To generate the small soybean for production of Touchi extract, seed is distributed to the contract farmers in the Sichuan province and a local agricultural specialist advises on the crop growth and harvesting procedures. The farms are managed in a sustainable manner, where the nutritive quality of the soil is adjusted naturally for cultivation by planting wheat, rice, and corn in rotation and by addition of an approved fermented organic fertiliser. No agro-chemicals are used on the crop and this is confirmed at government, agricultural society and local level (see Appendix 4). In addition, there are no potential sources of pollution in the vicinity of the contract farms. When harvesting the crop, countermeasures are employed to reduce possible contamination with foreign materials (*e.g.*, stones, chips, and husk) and the storage facilities are maintained to suitable hygiene standards. Records are maintained at all stages of the process to ensure full traceability of the small soybean.

#### **III.A.3 Fermentation of the Soybean**

The fermentation of the small soybeans is performed using the fungus, *Aspergillus oryzae*, which has been used for hundreds of years in the production of soy sauce, miso, and sake. The fungus is a member of the *Aspergillus* genus, a group that are thought to reproduce asexually (Fungi Imperfecti) and are characterised by spore formation on large black or brown conidia in phialides found on the vesicle. The genus is separated into groups based on morphological characteristics of the individual species and *Aspergillus oryzae* is a member of the *Aspergillus flavus* group. *Aspergillus oryzae* has undergone extensive selection over the years in order to produce the current strains that adapted for safe and suitable use in fermentation processes.

### **III.B GM Status of Touchi Extract**

Unlike the well-known large soybean, the small soybean has not been genetically modified and therefore, Touchi extract is derived from a non-GM source. The large soybean has been extensively developed in the Northern and East Coast of China and crops grown in these areas have a high risk contamination with genetically modified organisms. However, the small soybean has a low efficiency of production making it unsuitable for large-scale production and as a result has not been the focus of genetic modification. The seed of the small soybean used for cultivation has been analysed by PCR at the “Technical Centre of Sichuan Entry-Exit Inspection and Quarantine Bureau of People’s Republic of China” and GMO-free certification issued.

### **III.C Characterisation of the Small Soybean**

The small soybean plant is a species of legume and the soybeans obtained are considered oilseeds.

Classification of the small soybean is as follows:

Kingdom	Plantae
Subdivision	Tracheobionta (Vascular plants)
Superdivision	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicotyledons)
Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Genus	<i>Glycine</i>
Species	<i>Glycine max</i>

### **III.D Characterisation of *Aspergillus Oryzae***

Characterisation of *Aspergillus Oryzae* is as follows:

Kingdom	Fungi
Phylum	Ascomycota
Subphylum	Pezizomycotina
Class	Eurotimomycetes
Order	Eurotiales
Family	Trichocomaceae
Genus	<i>Aspergillus</i>
Species	<i>oryzae</i>

### **III.E Potential for Detrimental Effects on Human Health**

There are two key components of the product to consider in terms of potential detrimental health effects on humans, the source soybean, and the fungus, *Aspergillus oryzae*.

#### **III.E.1 Source Soybeans**

The main feature of the soybean that has led to its extensive use in foodstuffs is that it contains high levels of protein, which can be preserved by drying and then re-hydrated without any loss in nutrient value. In addition, there is a large market for soybean oil, which contains considerable amounts of linoleic acid (C18:2, n-6, approximately 50%).

A dried soybean consists of 40% protein, 20% oil, 35% carbohydrate, and 5% ash, approximately by weight. The majority of the soybean protein is a relatively heat-resistant protein suitable for the preparation of products which undergo high temperatures such as soy flour (vegetable protein) or milk and tofu. Fermented black beans, from which the protein-rich extract is obtained, have a long history of safe use in China and more recently as a component of many Chinese dishes consumed in Europe.

#### **III.E.2 *Aspergillus Oryzae***

*Aspergillus Oryzae* has a long history of safe use in food production spanning several hundred years. In addition to being used in many fermentation processes that are analogous to the one described herein for fermented black beans, it is also used in the production of livestock feed supplements.

## **IX ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD**

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to the intake/extent of use of the novel food (Commission of the European Communities, 1997).

- a. "Is there information on the anticipated uses of the novel food based on its properties?"
- b. "Is there information to show anticipated intakes for groups predicted to be at risk?"
- c. "Will introduction of the novel food be restricted geographically?"
- d. "Will the novel food replace other foods in the diet?"

These points are addressed in the section that follows.

## **IX.A Anticipated Food Uses and Maximum Use Level**

### **IX.A.1 Intended Use and Use-Levels**

It is anticipated that Touchi extract is consumed as a nutritional support during a meal in order to hinder the digestion of carbohydrates in the small intestinal tract in an analogous way to food ingredients such as resistant starch or “starch blockers” which are currently on the market in the EU. Such a property may help people dieting feel less hungry for longer after a meal, similar in principle to the purpose of so-called “low glycaemic” foods.

In order to provide reassurance in relation to the use of such products by at risk groups, Touchi extract will only be available in food supplement products at levels that would not exceed 4.5 g per daily serving/dose. The form of the supplement may vary but will be clearly marked as providing a dose of Touchi extract and labelled to indicate that the product should only be consumed with food. For example, Touchi extract may be delivered in tablet form or alternatively in a tea or soup-style formulation where the flavour of the ingredient will also be appreciated by the consumer.

Food Supplements as regulated by Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements (European Parliament and The Council of the European Union, 2002), for which Touchi extract would be considered an “other substance”, clearly states in Article 6:

*3. Without prejudice to Directive 2000/13/EC, the labelling shall bear the following particulars:*

- (a) the names of the categories of nutrients or substances that characterise the product or an indication of the nature of those nutrients or substances;*
- (b) the portion of the product recommended for daily consumption;*
- (c) a warning not to exceed the stated recommended daily dose;*
- (d) a statement to the effect that food supplements should not be used as a substitute for a varied diet;*
- (e) a statement to the effect that the products should be stored out of the reach of young children.*

As a result of Touchi extract only being available in clearly marked food supplement products for which there are already risk management procedures in place throughout the EU, presentation to the consumer will be controlled. Capsules/tablets or sachets (e.g., tea or soup type formulations) would be clearly marked as providing a dose of Touchi extract where levels would not exceed 4.5 g per serving/dose per day. Further risk management measures will be applied under the conditions of the Corrigendum to Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods (European Parliament and the Council of the European Union, 2007).

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**IX.A.2 Consumer Awareness**

Although traditionally foods supplements are delivered in tablet or capsule form there are a number of examples of these products being provided in sachet form for delivery as a stand-alone drink or for addition to other beverages/foodstuffs. An example of this type of product available from popular UK “high street stores” and the accompanying labelling is presented in Figure IX.A.2-1. It is therefore envisaged that consumers will be aware of this type of food supplement form and follow the appropriate directions for use.

**Figure IX.A.2-1 Example of a Food Supplement Product in Sachet Form**



**Benefiber Soluble Fibre Food Supplement Trial Pack**

*“Benefiber can help maintain your digestive health system by increasing your fibre intake as part of a healthy, varied and well balanced diet and active lifestyle”*

*Now its easier than ever to add fibre to your diet. You can mix Benefiber sachets with almost anything, and because Benefiber is taste free and non-thickening it won't alter the taste or texture of your foods or beverages.*

*Suitable for adults and children 12 years and over.*

*Suggested daily intake: One sachet of Benefiber (mixed with food or drink) twice a day. Ensure that you drink plenty of water, experts recommend 8 glasses a day.*

*Do not exceed the recommended daily intake.*

[Boots Pharmacy, 2008 -

[http://www.boots.com/shop/product\\_details.jsp?productid=1087633&classificationid=1046313](http://www.boots.com/shop/product_details.jsp?productid=1087633&classificationid=1046313)]

**IX.A.3 Exposure Estimates**

As a result of Touchi extract only being available in clearly marked food supplement products for which there are already risk management procedures in place throughout the EU, presentation to the consumer will be controlled. Capsules/tablets or sachets (e.g., tea or soup type formulations) would be clearly marked as providing a dose of Touchi extract where levels would not exceed 4.5 g per serving/dose per day.

On the basis that Touchi extract is to be marketed as a food supplement product, detailed exposure estimates using food consumption databases for conventional foods are not relevant. For the purposes of this safety assessment however, an exposure estimate has been performed based on the worst case scenario whereby consumers of teas and soups incorporate these types of products as part of their conventional food intake. Data from the United Kingdom provided as part of the “Concise European Food Consumption Database” has been used to determine the mean (g/day) and 97.5<sup>th</sup> percentile (g/day) intakes of “vegetable soups” and “coffee, tea, cocoa” for consumers only for the adult population (16 to

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64 years) (EFSA, 2008a). Typical UK food portion sizes for a “mug of tea” of 240 g/serving and “cup-a-soup” type products of 215 g/serving have been used equate the intakes in g/day to servings/day (FSA, 2002) which are then translated into Touchi extract consumption based on the maximum proposed use-level of 4.5 g/serving. The estimated intakes of Touchi extract based on this “worst case scenario” are summarised in Table IX.A.3-1.

<b>Table IX.A.3-1 Summary of the Estimated Daily Intake of Touchi Extract from All Proposed Food Categories when Consumed as Conventional Foods in the UK By Consumers Only (Concise Database Data)</b>							
Food Category <sup>1</sup>	Exposure Assessment						
	% Consumers	Mean (g/day)	97.5 <sup>th</sup> Percentile (g/day)	Approx. No. of Servings <sup>2</sup>		Total Touchi Extract Intakes (g/day) <sup>3</sup>	
				Mean	97.5 <sup>th</sup>	Mean	97.5 <sup>th</sup>
Vegetable Soups	33.0	74	247	<1	1	<4.5	4.5
Coffee, Tea, Cocoa (expressed as liquid)	97.0	752	1848	3	8	13.5	36

<sup>1</sup> Based on consumption of Touchi-extract containing products only within the broad food category; <sup>2</sup> Food portion sizes: 215 g/serving cup-a-soup style vegetable soups and 240 g/serving “coffee, tea, cocoa as liquid” (FSA, 2002); <sup>3</sup> assumes maximum use-level of 4.5 g/serving.

The mean estimated total intakes of Touchi extract consumed as a conventional “cup-a-soup” type product or as a tea (assuming all consumption in these category is in the form of tea) are less than 4.5 g and 13.5 g, respectively (equivalent to <1 and approximately 3 servings, respectively). The highest level estimated consumption (97.5<sup>th</sup> percentile of users) of Touchi extract is 4.5 g and 36 g, respectively (equivalent to 1 or 8 servings approximately). As recommended by the EFSA in their “Guidance Document for the Use of the Concise European Food Consumption Database in Exposure Assessment” (EFSA, 2008b) one approach to estimate the total exposure from all food sources is to assume that an individual might be a high level consumer of two food categories and an average consumer of others. Since there are only 2 food categories involved in this intake assessment it is likely to be a gross over estimation to assume that consumers will be high-level consumers (97.5<sup>th</sup> percentile intake) of both “vegetable soups” and “tea, coffee and cocoa” and thus in this assessment it is assumed that the highest level consumers would be high consumers of one category and only average consumers for another. The 2 scenarios whereby a consumer is considered high-level for “vegetable soups” but average for “tea, coffee and cocoa” and *vice versa* result in estimated total exposures to Touchi extract of 18 g and 40.5 g, respectively. Notably, an NOAEL of 2,500 mg/kg body weight/day has been determined for Touchi extract (see Section XI of the dossier) which equates to 150 g/day of Touchi extract for a typical 60 kg adult. The average intakes of approximately 3 and less than half a serving for “tea, coffee and cocoa” and “vegetable soups”, respectively combined lead to a maximum intake of less than 4 servings or less than 18 g/day of Touchi extract and an 8 fold safety factor. The highest level consumption of an average amount of “vegetable soup” and high levels of “tea, coffee and cocoa” of 1 and approximately 8

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servings, respectively combined give rise to a maximum intake of less than 9 servings or less than 40.5 g/day and a 3- to 4-fold safety factor. It is important to note, however, that the Touchi extract is not proposed for conventional use and this quick method of screening the average exposure to the food ingredient largely over estimates intakes and is not a realistic model. Moreover, Touchi extract is only intended for consumption with food and this will further limit the users of products (typically to 3 meals per day) and in practice eliminate casual consumption of tea or soup between meals. Overall, from the data provided it is reasonable to conclude that Touchi extract-containing products will not pose a safety concern based on the proposed food-uses and food-levels and there is no evidence at this time to warrant a more detailed exposure assessment being performed.

## **X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE**

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to previous human exposure to the novel food:

- a. “Is there information from previous direct, indirect, intended, or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?”
- b. “Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?”

These points are addressed in the section that follows.

## **X.A Previous Human Exposure**

Fermented black beans have been widely used in China for the past 1000 years as a traditional seasoning and people are familiar with black beans as a protein source that can be preserved by drying without damage to the nutrient content. In Europe, fermented black beans have also been consumed for many years in Chinese dishes containing “black bean sauce” or involving “black bean paste” (prepared by crushing fermented black beans) as a seasoning, typically at levels of around 15 g per serving. Typical examples of popular dishes containing fermented black beans or products of fermented black beans as seasoning are provided in Appendix 7. Note that a serving of 15 g of fermented black beans in a traditional Chinese dish is equivalent to the maximum intake level of 4.5 g per day proposed for Touchi extract (see Section II.A.2).

## **X.B Allergenicity of Touchi Extract**

Soybeans are a common source of food allergenicity and are included in Annex IIIa of EU Directive 2003/89/EC regarding the indication of ingredients present in foodstuffs (European Parliament and the Council, 2003), which states that all products containing soybeans and products thereof as an ingredient must be clearly labelled. In accordance with this legislation, all products containing Touchi extract will be clearly labelled as made from soya or soybeans. Touchi extract is only requested for use in food supplements and the presence and origin of the ingredient will therefore be clearly apparent to the consumer.

Food allergies to soy are thought to mainly occur in children (often with eczema) and to be less common in adults. Proteins in the soybean, particularly the ‘storage proteins’ vicilin and legumin, involved in new plant growth, are thought to be responsible for the allergic response of certain individuals to soya-containing products (InformAll, 2007). Although the storage proteins are stable to heat, during the fermentation process, degradation may occur to form fragments that do not exhibit the same allergenic response in susceptible individuals. Notably, none of the subjects in the 6-month clinical trial performed by Fujita *et al.* (2003) complained of any allergy-related effects.

Touchi extract is currently approved as a Food for Specific Health Use (FOSHU) in Japan. CBC Co., Ltd. has not received any consumer complaints to date regarding adverse effects to the consumption of its fermented black bean products.

## **XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD**

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to nutritional information available on the novel food (Commission of the European Communities, 1997):

- a. "Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"

This point is addressed in the section that follows.

## **XI.A Comparison of Touchi Extract to Traditional Counterparts**

From a nutritional safety perspective Touchi extract can be considered substantially equivalent to fermented black beans and fermented black bean paste which have been on the market in the EU for many years.

One portion of a dish using black bean sauce would generally contain 15 g of fermented black beans, and on extraction as outlined in the schematic of the production process in Section II.A.2, 15 g of fermented black beans corresponds to 4.5 g of Touchi extract. For comparison, the nutrient profiles for 15 g of fermented black beans and 4.5 g of Touchi extract are provided in Table XI.A-1. The relative amount of each nutrient changes during processing because the water-insoluble fraction is removed (*e.g.*, the fat content), however no chemical modification is involved, therefore, the nature of the nutrients remains the same for the extract and the fermented black beans.

<b>Constituent</b>	<b>Composition (% w/w total)</b>	
	<b>Fermented Black Beans (Data from Japanese Food Index)</b>	<b>TE (Lot Manufactured 17/3/05)</b>
Protein	18.6	61.2
Fat	8.1	0.2
Carbohydrate	31.5	27.3
Water	24.4	3.5
Ash	17.4	7.8
Total	100	100

Touchi extract and its traditional counterparts are a valuable source of protein. Soybeans can be effectively dried and rehydrated without loss in the nutrient values of the protein content and is therefore considered a valuable source of protein that can be preserved.

### **XI.A.1 Soy Proteins**

Isolated soybean proteins, even following cooking or other forms of denaturing, typically have a Protein Digestibility Corrected Amino Acid Score (PDCAA) approaching one, similar to that of milk proteins and egg white (FAO, 1991). As a result, soy proteins are generally regarded as “complete proteins” providing all of the essential amino acids in sufficient quantities to meet human nutritional needs. Studies have also shown that comparable scores (>0.9) are achieved by soybean protein isolate or concentrate and it is logical that Touchi extract has the same nutritional value.

### **XI.A.2 Soy Isoflavones**

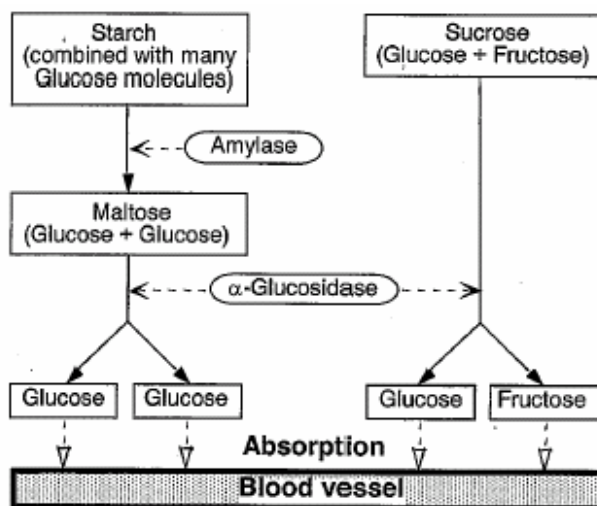
Soybeans contain a number of isoflavones which are flavonoids with structural similarities to oestrogen. The whole soybean contains approximately 200 mg of isoflavones per 100 g and

significant amounts are present in the majority of soy based products (USDA database). Other good sources of isoflavones and isoflavonols (related polyphenolic compounds) include kale (45 mg/100 g), onions (30 mg/kg) and broccoli (10 mg/kg) (Gunstone, 2003).

### **XI.A.3 Alpha-glucosidase Inhibitory Action**

Touchi extract can inhibit alpha-glucosidase action, thus aiding the body's self-regulation of this enzyme. As a consequence, consumption of Touchi extract as a food supplement alongside a meal can delay carbohydrate digestion in the small intestinal tract. The digestion of carbohydrate in the body is outlined in Figure XI.A.3-1. Briefly, starch is first digested by the action of amylase in saliva and in the small intestinal juices to form maltose. Both the disaccharides maltose, formed by the breakdown of starch, and sucrose are converted to their monosaccharide components by alpha-glucosidase at the mucosa in the small intestine. Touchi extract has the ability to inhibit the activity of the alpha-glucosidase enzyme so limiting the breakdown of carbohydrates and the subsequent formation of glucose and fructose (monosaccharides) (Clissold and Edwards, 1988; Toeller, 1994; Fujita *et al.*, 2005). Undigested carbohydrates or disaccharides are then excreted rather than being absorbed by the body thus potentially offering assistance in weight control regimes.

**Figure XI.A.3-1 Digestion of Carbohydrate (Starch and Sucrose) by the Body**



The clinical data supporting the ability of Touchi extract to delay the digestion of carbohydrate when consumed in conjunction with food is summarised in Section XIII.B. Fujita *et al.* (2001a) report that the minimum dose at which Touchi extract demonstrates alpha-glucosidase inhibitory activity is 0.3 g and the recommended daily dose of up to 4.5 g/day (Section IX) clearly delivers sufficient for the desired properties to be achieved.

The inhibition of alpha-glucosidase is found only in beans fermented with *Aspergillus* sp.; it is not observed in the raw or boiled bean, in bean whey or in beans fermented with *Bacillus subtilis natto* (*i.e.*, "natto" a traditional Japanese appetiser).

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The effect of Touchi extract consumption on carbohydrate digestion is analogous to that observed in a variety of other foods including indigestible dextrin, resistant starch, and alpha-amylase inhibitors. For example, extracts of white kidney bean (*Phaseolus vulagaris*) have also been shown to hinder the digestion of complex carbohydrates and play a role in weight control. In contrast to Touchi extract, white bean extract inhibits the amylase enzyme rather than alpha-glucosidase (see Figure XI.A.3-1 above). These extracts are currently available as supplements in the EU where they are marketed as “starch blockers”.

**XI.B            Labelling of Touchi Extract**

For nutritional labelling purposes, Touchi extract can be considered as providing approximately 60 g protein (minimum 55 g), no more than 1 g fat and 25 to 30 g of carbohydrate per 100 g subject to measurements on a batch to batch basis.

Further details on the requirements for labelling of food supplement products in relation to Touchi extract are discussed in Section IX.A.

## **XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD**

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to microbiological information available for the novel food (Commission of the European Communities, 1997):

- a. “Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?”

These points are addressed in the section that follows.

**XII.A Information on Microorganisms and their Metabolites**

The production process to Touchi extract involves sterilisation and filtration of the aqueous extract in order to minimise the risk of microbial contamination. In addition, the moisture content of the product in the powder form is <7% so limiting the potential for microbial growth. The results on an independent microbiological screen on the same three production batches of Touchi extract discussed in Section I are presented in Table XII.A and the Certificates of Analyses provided in Appendices 1-3, respectively. No contamination was detected in any of the batches tested.

<b>Table XII.A-1 Microbiological Analysis for Touchi Extract</b>			
<b>Contaminant</b>	<b>Microbiological Growth Results</b>		
	<b>Lot 1</b>	<b>Lot 2</b>	<b>Lot 3</b>
Total bacteria count	Less than 1000 cfu/g	<1000	<1000
Total mould and yeast count	Less than 300 cfu/g	<300	<300
<i>Escherichia coli</i>	Negative /g	Negative	Negative

The manufacturing site has a certified HACCP system in place and all procedures comply with GMP as further assurance of the quality of the Touchi extract (Appendix 6).

### **XIII TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD**

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to toxicological information available on the novel food (Commission of the European Communities, 1997):

- a. "Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"
- b. "Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?"

OR

- c. "Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?"
- d. "Is there information which suggests that the novel food might pose an allergenic risk to humans?"

These points are addressed in the section that follows.

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**XIII.A Experimental Animal Data**

**XIII.A.1 Acute Toxicity**

No studies that addressed the acute toxicity of Touchi extract specifically were found in the published scientific literature.

Touchi extract was evaluated for acute oral toxicity in mice in an unpublished study in accordance with OECD Guidelines for the Testing of Chemicals 401 (Japan Food Research Laboratories 2000a). Oral administration of 5000 mg/kg body weight/day of Touchi extract to male and female ICR mice caused no deaths during the experimental period. No clinical abnormalities or significant body weight changes were observed. No remarkable changes were found in any organ at necropsy. The LD<sub>50</sub> was thus considered to be >5000 mg/kg body weight.

**XIII.A.2 Subacute/Subchronic Toxicity**

A 28-day toxicity study with Touchi extract was recently submitted to the International Journal of Toxicology for publication by Fujita and Yamagami (2007). Male and female SPF rats of CrI:CD(SD) strain were obtained from Charles River Japan, Inc. at the age of 4 weeks. Animals were quarantined for 3 days and subsequently acclimated for 5 days (male) or 6 days (female). Ten healthy animals of each sex were randomly assigned to 4 groups in an attempt to equalise mean group body weights by a computerised randomisation procedure. The rats were given doses of 250, 1000, and 2500 mg/kg body weight Touchi extract per day.

No clinical signs or changes in body weight (see Table XIII.A.2-1) or food consumption related to the administration of Touchi extract were observed. No abnormal changes were observed in any urinalysis parameter in treated animals as compared with the control animals.

		Dose (mg/kg bw)			
		Control (Water) (n = 10)	TE		
			250 (n = 10)	1000 (n = 10)	2500 (n = 10)
Male	Day 1	199 ± 7	199 ± 4	199 ± 8	198 ± 5
	Day 8	249 ± 10	252 ± 12	250 ± 14	254 ± 8
	Day 15	302 ± 18	301 ± 23	304 ± 22	310 ± 12
	Day 22	341 ± 25	340 ± 31	345 ± 30	349 ± 15
	Day 28	371 ± 28	370 ± 39	378 ± 37	381 ± 16
	Necropsy	341 ± 25	339 ± 34	348 ± 33	351 ± 15
Female	Day 1	158 ± 7	157 ± 6	156 ± 7	156 ± 6
	Day 8	177 ± 7	181 ± 9	175 ± 14	177 ± 9
	Day 15	196 ± 11	202 ± 9	197 ± 19	197 ± 13
	Day 22	213 ± 14	219 ± 13	210 ± 22	215 ± 16
	Day 28	228 ± 16	235 ± 16	223 ± 23	229 ± 18
	Necropsy	212 ± 14	217 ± 13	205 ± 21	211 ± 17

Values shown as the Mean ± Standard Deviations  
 No significant difference from control was noted

As shown in Table XIII.A.2-2, a statistically significant decrease was seen in mean corpuscular haemoglobin and mean corpuscular volume for males in the 1000 mg/kg group, while mean corpuscular haemoglobin concentration was statistically increased in these animals. However, these changes were considered to be unrelated to the test substance, because no dose-dependent effects were noted, or changes were within the range of background data. No other significant changes were seen in haematological parameters. Similarly, a statistically lower chloride value for males in the 1000 and 2500 mg/kg groups, and a lower  $\gamma$ -GTP value for females in the 250 mg/kg group were observed (see Table XIII.A.2-3). These changes were likewise judged to be unrelated to the test substance, because they were unrelated to dose or considered a mild change within the range of background data. No other significant effects on clinical chemistry parameters were noted.

		Dose (mg/kg bw)			
		Control (Water) (n = 10)	TE		
			250 (n = 10)	1000 (n = 10)	2500 (n = 10)
Male	RBC (10 <sup>4</sup> /μL)	832 ± 27	846 ± 32	853 ± 22	818 ± 31
	WBC (10 <sup>2</sup> /μL)	88.0 ± 21.6	87.4 ± 23.2	87.4 ± 21.2	100.5 ± 24.7
	Ht(%)	45.4 ± 1.6	45.1 ± 0.8	44.2 ± 0.8	44.0 ± 1.6
	Hb (g/dL)	16.5 ± 0.5	16.5 ± 0.4	16.2 ± 0.3	16.0 ± 0.6
	MCH (pg)	19.8 ± 0.5	19.5 ± 0.6	19.0 ± 0.5*	19.6 ± 0.5
	MCV (fL)	54.6 ± 1.6	53.4 ± 2.0	51.9 ± 2.0*	53.8 ± 1.4
	MCHC (g/dL)	36.3 ± 0.4	36.5 ± 0.6	36.7 ± 0.2**	36.4 ± 0.2
	Ret (%)	3.65 ± 0.47	3.68 ± 0.44	3.40 ± 0.31	3.73 ± 0.26
	Ret (10 <sup>4</sup> /μL)	30.36 ± 3.89	31.06 ± 3.09	28.97 ± 2.94	30.53 ± 2.96
	Plt (10 <sup>4</sup> /μL)	127.0 ± 14.2	128.9 ± 16.5	134.6 ± 12.9	120.3 ± 14.5
	Differential leukocyte counts				
	Baso (%)	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
	Eosi (%)	1.0 ± 0.4	0.8 ± 0.3	0.8 ± 0.2	1.0 ± 0.5
	Neut (%)	18.9 ± 7.3	17.6 ± 5.4	21.4 ± 4.8	19.9 ± 4.4
	Lym (%)	78.3 ± 7.0	79.7 ± 6.0	75.6 ± 5.3	77.1 ± 4.6
	Mono (%)	1.7 ± 0.8	1.9 ± 0.8	2.1 ± 0.8	2.0 ± 0.8
	Baso (10 <sup>2</sup> /μL)	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
	Eosi (10 <sup>2</sup> /μL)	0.9 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.9 ± 0.3
	Neut (10 <sup>2</sup> /μL)	16.0 ± 6.2	14.6 ± 3.2	18.6 ± 6.1	19.8 ± 6.4
	Lym (10 <sup>2</sup> /μL)	69.5 ± 21.1	70.5 ± 22.4	66.2 ± 17.7	77.7 ± 20.6
Mono (10 <sup>2</sup> /μL)	1.5 ± 0.8	1.6 ± 0.8	1.8 ± 0.8	2.0 ± 0.9	
Female	RBC (10 <sup>4</sup> /μL)	824 ± 31	816 ± 26	819 ± 42	818 ± 41
	WBC (10 <sup>2</sup> /μL)	76.1 ± 38.0	59.7 ± 16.9	65.8 ± 22.2	58.0 ± 14.9
	Ht(%)	42.5 ± 1.5	43.0 ± 1.6	42.3 ± 1.5	43.4 ± 2.4
	Hb (g/dL)	15.8 ± 0.5	15.8 ± 0.4	15.6 ± 0.5	15.9 ± 0.8
	MCH (pg)	19.2 ± 0.5	19.4 ± 0.3	19.1 ± 0.6	19.5 ± 0.6
	MCV (fL)	51.6 ± 1.4	52.7 ± 0.8	51.8 ± 1.9	53.0 ± 1.6
	MCHC (g/dL)	37.1 ± 0.6	36.7 ± 0.6	36.9 ± 0.4	36.8 ± 0.3
	Ret (%)	3.27 ± 0.78	3.02 ± 0.44	3.13 ± 0.58	3.25 ± 0.42
	Ret (10 <sup>4</sup> /μL)	26.85 ± 6.19	24.66 ± 3.76	25.51 ± 4.21	26.52 ± 3.50
	Plt (10 <sup>4</sup> /μL)	135.3 ± 8.3	131.8 ± 12.3	121.8 ± 12.6	124.4 ± 15.1
	Differential leukocyte counts				
	Baso (%)	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
	Eosi (%)	1.0 ± 0.3	1.0 ± 0.4	1.1 ± 0.4	1.3 ± 0.6
	Neut (%)	18.7 ± 6.4	16.9 ± 4.8	18.0 ± 4.7	21.1 ± 9.1
	Lym (%)	79.2 ± 6.8	81.2 ± 4.8	79.9 ± 4.8	76.5 ± 9.2
Mono (%)	1.1 ± 0.4	0.8 ± 0.5	1.1 ± 0.6	1.0 ± 0.3	

<b>Table XIII.A.2-2 Haematology in Rats Treated Orally with Touchi Extract for 28 Days</b>		<b>Dose (mg/kg bw)</b>			
		<b>Control (Water) (n = 10)</b>	<b>TE</b>		
			<b>250 (n = 10)</b>	<b>1000 (n = 10)</b>	<b>2500 (n = 10)</b>
	Baso (10 <sup>2</sup> /μL)	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
	Eosi (10 <sup>2</sup> /μL)	0.8 ± 0.6	0.6 ± 0.2	0.7 ± 0.4	0.8 ± 0.4
	Neut (10 <sup>2</sup> /μL)	14.3 ± 11.3	9.8 ± 3.2	11.2 ± 2.9	11.9 ± 5.6
	Lym (10 <sup>2</sup> /μL)	60.1 ± 29.5	48.8 ± 15.0	53.2 ± 20.0	44.7 ± 14.3
	Mono (10 <sup>2</sup> /μL)	0.9 ± 0.8	0.5 ± 0.4	0.7 ± 0.4	0.6 ± 0.3

Values shown as the Mean ± Standard Deviations

\*p < 0.01: Significantly different from control by Dunnett's test

\*\*p < 0.05: Significantly different from control by Steel's test

<b>Table XIII.A.2-3 Blood Chemistry in Rats Treated Orally with Touchi Extract for 28 Days</b>		<b>Dose (mg/kg bw)</b>			
		<b>Control (Water) (n = 10)</b>	<b>TE</b>		
			<b>250 (n = 10)</b>	<b>1000 (n = 10)</b>	<b>2500 (n = 10)</b>
Male	AST (IU/L)	66.1 ± 8.6	70.1 ± 8.2	66.0 ± 6.4	67.5 ± 9.2
	ALT (IU/L)	27.3 ± 4.9	27.6 ± 4.5	25.3 ± 3.9	25.8 ± 5.6
	ALP (IU/L)	175.6 ± 32.5	187.2 ± 37.7	176.8 ± 30.5	175.6 ± 22.0
	LDH (IU/L)	195.9 ± 39.1	192.1 ± 37.3	170.7 ± 24.3	168.7 ± 33.2
	γ-GTP (IU/L)	1.84 ± 0.50	1.80 ± 0.72	1.62 ± 0.63	1.61 ± 0.57
	Glu. (mg/dL)	131 ± 17	122 ± 15	130 ± 12	132 ± 15
	T.Cho. (mg/dL)	28.5 ± 3.3	30.6 ± 4.9	27.5 ± 5.2	30.2 ± 3.0
	TG (mg/dL)	44.0 ± 9.0	50.6 ± 21.9	54.5 ± 17.8	48.9 ± 31.0
	PL (mg/dL)	89 ± 13	95 ± 11	92 ± 9	97 ± 7
	TP (g/dL)	6.0 ± 0.3	6.1 ± 0.2	6.1 ± 0.3	6.0 ± 0.3
	Alb. (g/dL)	2.4 ± 0.2	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
	A/G	0.66 ± 0.04	0.64 ± 0.03	0.65 ± 0.04	0.66 ± 0.04
	BUN (mg/dL)	17.4 ± 2.6	16.5 ± 1.5	16.8 ± 1.5	17.2 ± 2.5
	Crea. (mg/dL)	0.49 ± 0.07	0.51 ± 0.11	0.46 ± 0.06	0.51 ± 0.08
	T.Bil (mg/dL)	0.39 ± 0.14	0.42 ± 0.17	0.39 ± 0.11	0.44 ± 0.08
	Na (mEq/L)	142 ± 1	142 ± 1	143 ± 1	143 ± 1
	K (mEq/L)	4.5 ± 0.3	4.5 ± 0.3	4.7 ± 0.2	4.7 ± 0.4
	CL (mEq/L)	112 ± 2	112 ± 1	110 ± 1*	110 ± 2*
	P (mg/dL)	8.2 ± 0.8	7.7 ± 0.6	8.1 ± 0.5	7.9 ± 0.5
Ca (mg/dL)	9.4 ± 0.3	9.4 ± 0.3	9.5 ± 0.2	9.3 ± 0.2	

		<b>Dose (mg/kg bw)</b>			
		<b>Control (Water) (n = 10)</b>	<b>TE</b>		
			<b>250 (n = 10)</b>	<b>1000 (n = 10)</b>	<b>2500 (n = 10)</b>
Female	AST (IU/L)	69.3 ± 6.1	64.6 ± 4.7	69.1 ± 10.9	70.5 ± 7.7
	ALT (IU/L)	22.4 ± 3.6	20.2 ± 2.6	21.1 ± 2.3	20.3 ± 2.7
	ALP (IU/L)	114.7 ± 53.7	119.3 ± 32.6	104.0 ± 19.3	105.5 ± 29.8
	LDH (IU/L)	175.8 ± 55.1	165.5 ± 39.8	174.3 ± 35.5	166.1 ± 33.7
	γ-GTP (IU/L)	2.47 ± 0.49	1.88 ± 0.45*	2.48 ± 0.39	1.96 ± 0.64
	Glu. (mg/dL)	123 ± 24	120 ± 14	120 ± 17	123 ± 15
	T.Cho. (mg/dL)	31.8 ± 7.0	31.7 ± 4.6	35.9 ± 8.3	31.1 ± 6.9
	TG (mg/dL)	22.3 ± 11.3	17.7 ± 8.2	19.4 ± 9.5	22.0 ± 14.2
	PL (mg/dL)	119 ± 21	112 ± 8	124 ± 17	111 ± 17
	TP (g/dL)	6.4 ± 0.4	6.3 ± 0.2	6.3 ± 0.3	6.2 ± 0.3
	Alb. (g/dL)	2.7 ± 0.2	2.6 ± 0.1	2.6 ± 0.2	2.6 ± 0.2
	A/G	0.72 ± 0.03	0.68 ± 0.03	0.70 ± 0.07	0.71 ± 0.05
	BUN (mg/dL)	21.6 ± 2.3	19.0 ± 2.8	20.0 ± 2.1	19.5 ± 2.4
	Crea. (mg/dL)	0.53 ± 0.08	0.55 ± 0.09	0.58 ± 0.08	0.59 ± 0.10
	T.Bil (mg/dL)	0.36 ± 0.12	0.40 ± 0.17	0.41 ± 0.09	0.41 ± 0.09
	Na (mEq/L)	143 ± 2	142 ± 2	143 ± 2	143 ± 2
	K (mEq/L)	4.3 ± 0.3	4.5 ± 0.2	4.3 ± 0.3	4.3 ± 0.4
	CL (mEq/L)	113 ± 2	113 ± 2	112 ± 2	112 ± 2
P (mg/dL)	6.7 ± 0.8	6.6 ± 0.8	6.7 ± 1.0	6.7 ± 1.3	
Ca (mg/dL)	9.4 ± 0.2	9.4 ± 0.1	9.5 ± 0.2	9.3 ± 0.3	

Values shown as the Mean ± Standard Deviations  
 \*p < 0.05: Significantly different from control by Dunnett's test

Although a statistically lower value was seen in the relative thymus weight for males in the 1000 mg/kg group (123 ± 24 vs. 156 ± 33 mg%; p<0.05 by Dunnett's test), this change was not considered to be related to the administration of Touchi extract since it was a mild change unrelated to dose and was within the range of background data.

Unilateral pelvic dilation was observed in the right kidney of one male at the 2500 mg/kg dose group at necropsy. Upon histopathological examination, slight interstitial mononuclear cell infiltration and unilateral dilatation of pelvis in the kidney were observed. These changes were observed in only one animal and thus considered to be of spontaneous origin. No other changes were seen upon pathological or histopathological examination.

Because no toxic changes were observed at 2500 mg/kg body weight/day in both sexes, the NOAEL for Touchi extract was considered to be more than 2500 mg/kg in males and

## ***Non-Confidential***

females. These results are consistent with fermented black bean's status as a traditional Chinese food derived from fermented soybeans and its purported long history of use.

### **XIII.A.3 Reproductive/Developmental Toxicity**

No studies that addressed the reproductive and developmental toxicity of Touchi extract were found in the published scientific literature. However, given the origin of Touchi extract and the long safe history of use of fermented black beans no problems are anticipated.

### **XIII.A.4 Mutagenicity/Genotoxicity**

No studies that addressed the mutagenic or genotoxic potential of Touchi extract specifically were found in the published scientific literature. A reverse mutation assay and an *in vivo* micronucleus test have been submitted for publication to the International Journal of Toxicology by Fujita and Yamagami (2007). These studies are summarised in Sections XIII.A.4.1 and XIII.A.4.2

#### *XIII.A.4.1 Reverse Mutation Assay*

Touchi extract was tested in *Salmonella typhimurium* strains TA98, TA1537, TA100, TA1535, and *Escherichia coli* WP2uvrA (Fujita and Yamagami, 2007). Based on the results of a concentration-range finding test (data not shown), Touchi extract was dissolved in water and repeatedly tested at five dose levels of 5000, 2500, 1250, 625 and 313 µg/plate both with and without metabolic activation. The positive control substances were AF2, 9-aminoacridine, 2-aminoanthracene, and sodium azide; a vehicle control (water) was also included. Tests were performed by the pre-incubation method, and 3 plates per dose were used.

After the 48-hour incubation period, revertant colony counts were measured manually. A colony analyzer (CA-11D, System Science Inc.) was used for the counting of about 100 or more colonies (TA100 strain and positive control group). The precipitation of the test article on the plate was checked grossly, and the state of growth inhibition (background lawn) was examined under a stereoscope. The revertant colony counts, the mean and standard deviation for test article groups (each dose), negative control group, and positive control group were tabulated for each cell strain. Concentration-response curves were drawn for the test article groups. The test was repeated to determine reproducibility of the results.

The mutagenicity test was conducted at five concentrations (313 to 5000 µg/plate), with a common ratio of 2. As shown in Tables XIII.A.4.1-1 and XIII.A.4.1-2, the number of revertants did not increase to more than twice that of the negative control in any strain used, with or without S9 mix. No microbial contamination was detected in the test solution at the highest concentration, or S9 mix in any of the tests. The mutagenicity of the positive control substances was confirmed, and the numbers of revertants in positive and negative controls were within the range (mean  $\pm$  3 x S.D.) of historical control values (data not shown). No precipitation of the test article or growth inhibition was observed in any cell strains. The data of the test were judged as acceptable since (1) the negative control values were appropriate

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in comparison with the background values; (2) the positive control value was more than twice the negative control value and appropriate in comparison with the background values; and (3) there were no abnormalities in the sterility test.

	TE concentration (µg/plate)	Mean number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2 <i>uvrA</i>	TA98	TA1537
S9 mix (-)	0 (negative control) <sup>1</sup>	133 ± 16 128 ± 25	9 ± 4 9 ± 2	29 ± 8 27 ± 2	17 ± 8 20 ± 1	4 ± 2 7 ± 3
	313	129 ± 17 118 ± 13	13 ± 4 10 ± 1	22 ± 5 27 ± 7	14 ± 3 21 ± 4	3 ± 2 7 ± 1
	625	129 ± 5 118 ± 10	9 ± 2 10 ± 4	21 ± 5 33 ± 5	15 ± 4 17 ± 3	4 ± 1 5 ± 2
	1,250	137 ± 16 134 ± 10	11 ± 3 7 ± 4	26 ± 4 28 ± 6	16 ± 3 20 ± 5	5 ± 2 7 ± 1
	2500	136 ± 12 134 ± 13	9 ± 4 8 ± 3	25 ± 5 27 ± 2	22 ± 2 27 ± 7	4 ± 2 8 ± 2
	5000	150 ± 8 132 ± 11	10 ± 5 12 ± 2	20 ± 6 29 ± 7	16 ± 2 27 ± 6	4 ± 2 8 ± 2
Positive control S9 mix (-)	Chemical	AF-2	SA	AF2	AF2	9AA
	Concentration (µg/plate)	0.01	0.5	0.01	0.1	80
	Mean number of colonies/plate	504 ± 3 404 ± 14	655 ± 43 678 ± 49	106 ± 11 132 ± 12	575 ± 42 550 ± 15	315 ± 116 96 ± 20

1: Water for injection  
 AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide  
 SA: Sodium azide  
 9AA: 9-Aminoacridine

<b>Table XIII.A.4.1-2 Results of Bacterial Reverse Mutation Test of TE (+S9)</b>						
	TE concentration (µg/plate)	Mean number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2 <i>uvrA</i>	TA98	TA1537
S9 mix (+)	0 (negative control) <sup>1</sup>	154 ± 6 130 ± 7	9 ± 2 9 ± 3	19 ± 7 32 ± 4	25 ± 1 26 ± 3	12 ± 3 12 ± 1
	313	135 ± 21 138 ± 19	9 ± 1 9 ± 3	24 ± 4 33 ± 3	21 ± 3 29 ± 3	8 ± 1 8 ± 2
	625	150 ± 14 140 ± 8	10 ± 2 10 ± 1	28 ± 10 28 ± 10	21 ± 4 24 ± 1	7 ± 1 8 ± 2
	1,250	140 ± 14 152 ± 21	9 ± 2 9 ± 5	25 ± 2 33 ± 2	22 ± 5 23 ± 3	7 ± 3 9 ± 3
	2500	160 ± 13 157 ± 27	9 ± 3 8 ± 2	25 ± 2 32 ± 3	22 ± 5 23 ± 3	7 ± 3 9 ± 3
	5000	185 ± 13 172 ± 15	11 ± 3 11 ± 3	22 ± 3 31 ± 6	30 ± 4 38 ± 9	8 ± 3 9 ± 2
	Positive control S9 mix (+)	Chemical	2-AA	2-AA	2-AA	2-AA
Concentration (µg/plate)		1.0	2.0	10	0.5	2.0
Mean number of colonies/plate		779 ± 16 701 ± 49	225 ± 21 178 ± 13	685 ± 22 740 ± 28	270 ± 17 246 ± 24	139 ± 32 178 ± 13

1: Water for injection  
2-AA: 2-Aminoanthracene

The *in vitro* mutagenic potential of Touchi extract (313 to 5000 µg/plate) was also evaluated in an unpublished reverse mutation assay with *Salmonella typhimurium* strains TA100, TA98, TA1535, and TA1537 and *Escherichia coli* WP2 *uvrA* with and without rat-liver metabolic activation using the pre-incubation method. No evidence of mutagenic effects was observed with Touchi extract in any strain with or without metabolic activation (Japan Food Research Laboratories, 2000b).

#### XIII.A.4.2 *In vivo* Micronucleus Assay

Touchi extract was also evaluated in an *in vivo* micronucleus test in rats (Fujita and Yamagami, 2007). The toxicity of the test article was assumed to be very low because Touchi extract has been eaten as a fermented food for many years and therefore, the high-dose level for this study was determined to be 2000 mg/kg/day. The middle and low dose levels were selected to be 1000 and 500 mg/kg/day using a common ratio of 2, respectively.

Male SPF rats of Crl:CD(SD) strain were obtained from Charles River Japan, Inc. at the age of 7 weeks. In accordance with the Guideline for Genetic Toxicity Studies, rats were administered Touchi extract by oral gavage on 2 successive days. The dosing volume was 10 mL/kg. Control animals received water at the same volume. A single 0.4 mg/mL dose of mitomycin C (MMC) was administered intraperitoneally (dosing volume 10 mL/kg) as the positive control substance.

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The incidence of micronuclei was examined for 2000 polychromatic erythrocytes (PCEs) per rat, or 1000 per slide of 1 animal (2 slides) using 1000-fold microscopy. Polychromatic erythrocytes were counted similarly for 1000 erythrocytes per animal, or 500 erythrocytes were counted per slide of 1 animal (2 slides). The frequency (%) of polychromatic erythrocytes to the total erythrocytes was calculated for each bone marrow specimen.

The results of the study are shown in Table XIII.A.4.2-1. The mean incidence of polychromatic erythrocytes with micronuclei in the Touchi extract-treated groups was equivalent or less than in the negative control group (0.14, 0.09, and 0.06% in the 500, 1000, and 2000 mg/kg groups, respectively, and 0.13% in the negative control group). Thus, no dose-dependent increase was observed in the incidence of polychromatic erythrocytes with micronuclei among Touchi extract-treated groups, whereas a significant increase in the incidence of polychromatic erythrocytes with micronuclei (4.92%) was observed in the positive control group treated with MMC.

The mean frequency of polychromatic erythrocytes to the total erythrocytes was 48.3, 49.2, and 47.4% in the 500, 1000, and 2000 mg/kg groups, respectively, and 47.8% in the negative control group. In contrast, a significant decrease in the frequency of polychromatic erythrocytes was observed in the MMC dosing group (41.8).

<b>Test substance</b>	<b>Dose (mg/kg)</b>	<b>Number of Animals</b>	<b>Mn-PCE/2000 PCE Mean <math>\pm</math> S.D. (%)</b>	<b>PCE/1000 RBC Mean <math>\pm</math> S.D. (%)</b>
Negative control (water for injection)	0	6	0.13 $\pm$ 0.06	47.8 $\pm$ 1.5
Touchi extract	500	6	0.014 $\pm$ 0.07	48.3 $\pm$ 2.4
	1000	6	0.09 $\pm$ 0.07	49.2 $\pm$ 0.9
	2000	6	0.06 $\pm$ 0.05	47.4 $\pm$ 1.7
Positive control (MMC)	4	6	4.92 $\pm$ 0.83 <sup>1</sup>	41.8 $\pm$ 8.0 <sup>2</sup>

Frequency of micronucleated polychromatic erythrocytes (PCE) per 2000 PCE.

Frequency of PCE per 1000 RBC.

<sup>1</sup>Significantly different from the control group by Kastenbaum and Bowman method ( $p < 0.01$ ).

<sup>2</sup>Significantly different from the control group by Dunnett's test ( $p < 0.05$ ).

### **XIII.A.5 Chronic Toxicity**

No studies that addressed the chronic toxicity of Touchi extract specifically were found in the published scientific literature.

### **XIII.A.6 Other Preclinical Studies**

The activity of Touchi extract was evaluated in tissue, animal, and anti-infective *in vitro* assays as part of a pharmacological screen (PharmaScreen<sup>®</sup>, MDS Panlabs Pharmacology Services, USA)

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Touchi extract did not produce autonomic signs or effects on the central nervous system, cardiovascular system, and gastrointestinal system. No metabolic effects were seen, nor were indications of allergy or inflammation observed. No significant activity was observed at dose levels and concentrations tested. No microbiological pathogens were detected (MDS Panlabs, 2000).

A limited number of pharmacological studies in laboratory animals were identified. No adverse effects associated with the administration of Touchi extract were reported in rats and mice in these studies. The absence of major adverse effects in these studies offer support for the safety of Touchi extract. A summary of studies examining the pharmacological effects of Touchi extract is provided in Table XIII.A.6-1.

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<b>Table XIII.A.6-1 Preclinical Pharmacological Studies Conducted using Touchi Extract</b>			
<b>Reference</b>	<b>Objective</b>	<b>Study Design</b>	<b>Result and Evaluation of Safety Parameters</b>
Fujita and Yamagami, 2001	To evaluate the long-term antiglycaemic effects of TE in mice	Eight-week-old male genetically modified diabetic model KKA <sup>y</sup> mice were fed chow containing 0.08% or 0.4% TE for 60 days. Daily intakes of mice were 133 and 655 mg/kg/day in the 0.08 and 0.04% groups, respectively. After 60 days, a sucrose tolerance test was performed.	Food intake and body weights changes in TE-treated mice were similar to controls. Fasting blood glucose levels were significantly ( $p < 0.05$ ) decreased in the high-dose group after the 60-day ingestion period. Postprandial levels were also significantly reduced ( $p < 0.01$ ) after only 30 days. After oral loading with sucrose (2 g/kg), postprandial increases in the blood glucose level at 30 ( $p < 0.05$ ) and 60 ( $p < 0.01$ ) minutes were significantly attenuated in the 0.4% TE-treated animals compared with controls. In the 0.08% group, a similar significant decreasing tendency in post-loading blood glucose levels was observed at 60 minutes. Total cholesterol and triglyceride levels were dose-dependently and significantly decreased after TE ingestion. Glutamic-oxalacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), and gamma-glutamic transaminase (GGT), indices of hepatic function, were suppressed in the TE-treated animals compared to controls. Organ weights of the heart, kidney, jejunum, liver, and spleen increased in control KKA <sup>y</sup> mice due to hyperinsulenemia, but the respective organ weights and jejunum length were significantly reduced in the TE -extract treated groups. Authors suggested the latter was possibly due to attenuation of the glucagons-like peptide 2 (GLP-2), through decreases in the serum insulin level.
Fujita <i>et al.</i> , 2001a	To evaluate the antiglycaemic effects of TE in rats after a single oral treatment	Rats were administered 2 g/kg sucrose with or without TE at doses of 100 and 500 mg/kg.	TE significantly depressed the postprandial rise in blood glucose after oral sucrose loading in a dose-dependent manner.  At 500 mg/kg, TE significantly ( $p < 0.01$ ) decreased the postprandial rise in blood glucose compared with the control group at 15-60 minutes after sucrose loading. Similarly, the lower dose (100 mg/kg) significantly ( $p < 0.05$ ) depressed the postprandial rise in blood glucose compared to controls at 30 minutes after sucrose loading. The TE-extract treated animals had significantly lower postprandial rises in blood glucose levels compared with the control group when the area under the curve values were compared.

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<b>Table XIII.A.6-1 Preclinical Pharmacological Studies Conducted using Touchi Extract</b>			
<b>Reference</b>	<b>Objective</b>	<b>Study Design</b>	<b>Result and Evaluation of Safety Parameters</b>
Fujita <i>et al.</i> , 2005	To evaluate the efficacy and safety of TE ingestion on lipid metabolism	Sprague-Dawley rats (5 rats/group) were fed TE at doses of 0.08%, 0.5%, and 2% (wt/wt, final concentrations) for 3 weeks. Final TE intakes were calculated to be 54, 275, and 1350 mg/kg bw/day, in the .08%, 0.5%, and 2% dose groups, respectively. Food intakes and body weights were monitored. GOT, GPT, total cholesterol, high-density lipoprotein (HDL), triglyceride (TG), and blood glucose levels, and heart, liver, kidney, and epididymal and renal adipose tissue weights were measured.	Serum and liver triglycerides were dose-dependently and significantly decreased. Treated rats showed decreased adipose tissue weights, with the most significant decrease in renal adipose tissue weight seen in the 2% TE group. No changes in other organ weights were noted, and the levels of clinical markers such as cholesterol, HDL, TG, GPT, and GOT were unchanged.

### **XIII.B Human Data**

Human studies of Touchi extract were identified in the published literature. These clinical studies examined the effects of Touchi extract on carbohydrate digestion following a meal in healthy, hyperlipidemic, and diabetic subjects. Additionally, patients were monitored for changes in various haemological and biochemical parameters, body weights, and subjective side effects. The absence of major adverse effects in subjects does offer support for the safety of Touchi extract. A summary of the clinical trials with Touchi extract is provided in Table XIII.B.1-1.

Note that no gastrointestinal effects were seen in clinical studies with Touchi extract. Similarly, although soybeans can inhibit gastrointestinal proteases and thus induce diarrhoea or abdominal pain, Touchi extract did not demonstrate protease inhibition (see Section I, Table I.C.2-1) nor cause this effect (Matsuo *et al.*, 1992).

<b>Table XIII.B.1-1 Clinical Studies Conducted with Touchi Extract</b>					
<b>Reference</b>	<b>Study Design</b>	<b>Subject Population</b>	<b>Dosing Regimen</b>	<b>Endpoints Measured</b>	<b>Results/ Evaluation of Safety Parameters</b>
<b>Safety Data on Healthy Subjects</b>					
Nippon Supplement Inc. Research and Development, 1999a	Safety	3 healthy male subjects	Single bolus dose of 10 g TE	Haemological, biochemical, and urinary parameters, objective and subjective symptoms	No notable abnormalities were observed in any test.
Nippon Supplement Inc. Research and Development, 1999b	Safety	10 healthy male subjects	1 g TE before each meal (3 g/day) for 12 weeks	Haemological, biochemical, and urinary parameters, objective and subjective symptoms	No notable abnormalities were observed in any test.
Hiroyuki <i>et al.</i> , 2001	Confirmation of safety	9 healthy males (26-56 years of age)	1 g TE 3 times daily before meals for 12 weeks	Haemological and biochemical parameters, body weight, and body mass index, subjective symptoms	No remarkable changes in haemological, relevant biochemical data, body weights, or body mass indices were observed. Fasting blood glucose and glycated haemoglobin were not altered and there were no reports of gastrointestinal symptoms or any other adverse effects related to ingestion of TE.
<b>Data on Diabetic Subjects</b>					
Hiroyuki <i>et al.</i> , 2001	Non-comparative study	18 subjects (male and female) with non-insulin dependent diabetes mellitus	0.3 g TE 3 times daily before each meal for 6 months	Haemological and biochemical parameters, body weights and body mass indices, plasma lipids, and subjective symptoms	Fasting blood glucose and glycated haemoglobin levels were significantly reduced after 6 months of TE ingestion. The extract effectively attenuated the fasting blood glucose levels in 14 subjects (77.8%) and glycated haemoglobin levels in 11 patients (61.1%). A moderate decrease in total cholesterol levels was observed while high-density lipoprotein levels were reportedly moderately increased. Triglyceride levels were significantly decreased after 3 and 6 months of treatment. There were no complaints of gastrointestinal side effects and no remarkable changes in haemological, relevant biochemical parameters, body weights, or body mass indices were observed.

<b>Table XIII.B.1-1 Clinical Studies Conducted with Touchi Extract</b>					
<b>Reference</b>	<b>Study Design</b>	<b>Subject Population</b>	<b>Dosing Regimen</b>	<b>Endpoints Measured</b>	<b>Results/ Evaluation of Safety Parameters</b>
Fujita <i>et al.</i> , 2001a	Effects of Touchi on blood glucose levels after sucrose-loading	8 borderline diabetic subjects  4 diabetic subjects	0.1-10.0 g TE before sucrose loading (75 g)  0.3 g TE before eating 200 g of cooked rice	Blood glucose and insulin levels, haemological and biochemical parameters, subjective side effects	<p>Dose-dependent decreases the glycaemic response were observed. Significant suppression of postprandial blood glucose levels was seen at 60 and 90 minutes after sucrose loading. Area under the curve showed significant antiglycaemic effects at a minimum effective dose of 0.3 g.</p> <p>The postprandial increases in both blood glucose and mean insulin levels observed with TE administration were significantly depressed at 60 and 120 minutes after ingestion compared with levels when no TE was administered.</p> <p>Neither borderline nor diabetic patients complained of any side effects such as abdominal pain, diarrhoea, retching, or flatulence after the ingestion of TE. No abnormalities in haemological or biochemical parameters were observed.</p>
Fujita <i>et al.</i> , 2001b	Double-blind, placebo-controlled study	36 subjects (15 males, 21 females) with borderline and mild type-2 diabetes	Houji-tea with steamed soybean powder (placebo) or 0.3 g of TE before meals, 3 times per day for 3 months	Haemological and biochemical parameters, subjective symptoms	<p>Glycated haemoglobin (HbA1c) and fasting blood glucose levels were significantly reduced after 2 and 3 months, respectively, with TE supplementation. Triglyceride concentrations also tended to decrease at 2 months post-TE ingestion. No such effects were observed in the placebo group. No other significant changes on haemological or biochemical parameters or body weights and body mass indices were observed, and there were no reports of gastrointestinal side effects or other adverse events. There was no deterioration as assessed by fasting blood glucose and glycated haemoglobin levels after withdrawal of TE.</p>

<b>Table XIII.B.1-1 Clinical Studies Conducted with Touchi Extract</b>					
<b>Reference</b>	<b>Study Design</b>	<b>Subject Population</b>	<b>Dosing Regimen</b>	<b>Endpoints Measured</b>	<b>Results/ Evaluation of Safety Parameters</b>
Fujita <i>et al.</i> , 2003	Randomised, double-blind, placebo-controlled	47 borderline and mild type-2 diabetic subjects	0.3 g TE before meals, 3 times daily for 6 months	Blood glucose and insulin levels, haemological and biochemical parameters, subjective side effects	<p>Fasting blood glucose, glycated haemoglobin levels, and triglyceride levels were significantly decreased in the TE-treated group. Total cholesterol levels tended to decrease in subjects consuming TE, however this decrease did not reach statistical significance. The HDL content was unchanged in both the placebo and TE-treated groups.</p> <p>No adverse effects on haemological or biochemical parameters were observed. Body weight and BMI values at the completion of the study were comparable to pre-study values. No subjective side effects were reported.</p>
Fujita <i>et al.</i> , 2005	Randomised, double-blind, placebo-controlled study	50 nondiabetic, mild and borderline hypertriglyceridemic subjects	0.3 g of TE in tablet form before meals 3 times daily for 6 months	Haemological and biochemical parameters, subjective symptoms	<p>Four subjects were excluded from the study for reasons unrelated to their health and the administration of TE. Of the 46 subjects that completed the study, 25 received the TE tablets while 21 ingested placebo tablets. After 1 month, the triglyceride level in treated subjects was lower than the initial baseline value. This trend continued as the study progressed, and significant reductions were observed at the 2-, 4-, and 6-month time points compared to baseline. In contrast, the TG level of the placebo group did not change significantly. Significant reductions in mean TG levels were seen in treated subjects compared to placebo after 2, 4, and 6 months.</p> <p>Results were evaluated again by subdividing both the placebo and treatment groups into 2 subgroups each based on initial TG levels (borderline vs. mild hypertriglyceridemia). Both borderline and mild hypertriglyceridemics experienced significant reductions in TG levels compared to their respective baseline values after treatment with TE. In subjects with borderline hypertriglyceridemia, significant decreases in TG levels were observed at 2 and 4 months compared to borderline hypertriglyceridemic subjects in the placebo group. Significant decreases in TG levels were seen at 4 and 6 months in treated mild hypertriglyceridemics compared to placebo subjects with mild hypertriglyceridemia.</p>

<b>Table XIII.B.1-1 Clinical Studies Conducted with Touchi Extract</b>					
<b>Reference</b>	<b>Study Design</b>	<b>Subject Population</b>	<b>Dosing Regimen</b>	<b>Endpoints Measured</b>	<b>Results/ Evaluation of Safety Parameters</b>
					<p>After 1 month, the total fasting blood glucose level in the TE-fed subjects (both mild and borderline hypertriglyceridemic) had decreased, with significant decreases seen at 4 and 6 months. In contrast, the total cholesterol levels of treated and placebo groups did not change markedly, and there were no changes in total HDL levels in treated or placebo groups over the study period.</p> <p>Total body weight and BMI of the treatment and placebo groups did not differ significantly over the study period. The subjects reported no side effects such as abdominal distention, abdominal pain, diarrhoea, retching, increased flatulence, or allergic symptoms. No adverse effects were seen on haemological and biochemical parameters.</p>

## **OVERALL CONCLUSIONS**

The safety of Touchi extract is based principally on its equivalence to fermented black beans, which have been consumed since ancient times and are widely available in culinary dishes throughout the European Union. At the maximum consumption level of 4.5 g per day, Touchi extract is equivalent to approximately a 1 tablespoon (15 g) serving of fermented black beans. The solvent used is water and no selective extraction occurs with the result that no chemical modification occurs and the composition is comparable to the traditional counterpart, fermented black beans. Touchi extract has the ability to inhibit the activity of the alpha-glucosidase enzyme so delaying the digestion of carbohydrate in the small intestinal tract following consumption of food. Undigested carbohydrates or disaccharides are then excreted rather than being absorbed by the body thus providing possible assistance in weight control regimes. The approval of Touchi extract is requested only for specific food supplement products (in tablet or sachet form) that would be taken with meals at levels not exceeding 4.5 g/day and as such would have specific risk management and labelling clauses that would prevent the consumption by at risk groups such as diabetics. The safety of Touchi extract at these levels is further supported by a range of toxicological safety studies, including a 28-day sub-chronic study in the rat, which established a NOAEL of 2500 mg/kg body weight/day, equivalent to 150 g/day for a 60 kg adult or to approximately a 33-fold safety factor compared to maximum daily consumption of 4.5 g. Numerous human clinical studies have been conducted in both healthy and diabetic subjects with Touchi extract at levels up to 10 g per daily serving and durations of up to 6 months. The absence of major adverse effects in subjects offers additional evidence for the safety of Touchi extract.

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## **GLOSSARY**

3-MCPD	3-Monochloropropane-1,2-diol
FAO	Food and Agricultural Organisation
FOSHU	Foods for Specific Health Use
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HACCP	Hazards Analysis and Critical Control Points
HDL	High-density lipoprotein
IFOAM	International Federation of Organic Agriculture Movements
NOAEL	No-Observed-Adverse-Effect Level
OFDC	Organic Food Development and Certification Centre
PAH	Polycyclic aromatic hydrocarbon
PARNUTS	Foods for Particular Nutritional Purposes
PCB	Polychlorinated biphenyl
PCEs	Polychromatic Erythrocytes
PCR	Polymerase Chain Reaction
PDCAA	Protein Digestibility Corrected Amino Acid Score
RT	Relative Time
SCF	Scientific Committee on Food
TE	Touchi Extract
U.S. EPA	U.S. Environmental Protection Agency
WHO	World Health Organisation