Association of South East Asian Nations (ASEAN)

ANNEX IV
ASEAN GUIDELINES FOR MINIMISING THE RISK OF TRANSMISSION OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES IN HEALTH SUPPLEMENTS

Disclaimer:

This document is provided for information purpose only and subject to changes, pending the finalisation of the [ASEAN Agreement on Regulatory Framework for Traditional Medicines]. Official references to this document can only be made once the said Agreement has been finalised.

Version 2.0

DOCUMENT INFORMATION

This version is adopted at the 27th ACCSQ TRADITIONAL MEDICINES AND HEALTH SUPPLEMENTS SCIENTIFIC COMMITTEE MEETING (ATSC) 1-2 June 2015, Kuala Lumpur, Malaysia and endorsed at the 23rd ACCSQ TRADITIONAL MEDICINES AND HEALTH SUPPLEMENTS PRODUCT WORKING GROUP (TMHSPWG) MEETING 12-16 October 2015, Makati City, Philippines.

Version No.	History of adoption and endorsement		Reasons for revision
	ATSC adoption date	TMHSPWG endorsement date	
1	3rd ATSC Meeting 3 Jun 2008	9th TMHSPWG Meeting 5-6 June 2008	-
2	XX ATSC Meeting XX Jun 2015	XX TMHSPWH Meeting XX June 2015	To limit the scope of document to Health Supplements (HS) To update the name of EMEA to EMA To update the content due to: • EMA's update of the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) in 2011 • WHO's update on Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies in 2010 • TGA's update of their approach to minimising the risk of exposure Transmissible Spongiform Encephalopathies

CONTENTS

Purpose 3

Background 3

Guiding principles 4

1. ASEAN approach to minimising transmissible spongiform encephalopathy risk in Health Supplements 4

2. ASEAN guidelines to manage the risk of TSE in bovine-derived materials 4

2.1. Scope of guidelines 4

2.2. Guidelines in managing raw materials 5

2.3. Regulatory Requirements 7

References 9

Appendices 11

Appendix 1 BSE-Risk Classification Systems 11

Appendix 2 Levels of tissue infectivity* 12

Appendix 3 Sample of TSE Declaration Form 20

Appendix 4 Sample of Checklist for Self-Assessment 21

PURPOSE

To use an ASEAN harmonized approach to minimise the risk of Transmissible Spongiform Encephalopathy (TSE) in Health Supplements (HS).

BACKGROUND

Transmissible Spongiform Encephalopathies (TSEs) are a group of chronic degenerative diseases that is characterised by the accumulation of pathologically misfolded 'Prion' protein (PrP) that accumulates in the central nervous systems of infected individuals.

PrP^{TSE}, which has been identified as the pathogenic agent responsible for TSEs, is highly resistant to protease and heat denaturation treatments. The occurrence of TSE spans across different species and some forms of TSE include:

- Bovine Spongiform Encephalopathy (BSE) in cattle
- Scrapie in sheep and goats
- Chronic Wasting Disease (CWD) in cervids (deers and elks)
- Different forms of Creutzfeld-Jakob Disease (CJD) in humans
- Gerstmann-Sträussler-Scheinker Syndrome in humans

The WHO had concluded a causal link between Variant CJD (vCJD)[1] and BSE through epidemiological, biochemical and transmission studies. Studies have shown that human exposure to BSE is mainly via BSE-contaminated food.

Although WHO epidemiological analysis does not indicate medicinal products, blood and blood derived products to have been sources of vCJD infection, it is prudent to introduce measures to minimise risk of TSE transmission to humans via the use of ruminant materials in HS products.

[1] Details on the terms TSE, PrP^{TSE} and vCJD can be found in references no. 1, 4 and 5 listed in the References of this set of Guidelines.

GUIDING PRINCIPLES

1. ASEAN APPROACH TO MINIMISING TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHY RISK IN HEALTH SUPPLEMENTS

ASEAN has adopted a risk management approach to minimise the transmission risk of TSE in HS that is consistent with the approach recommended by the World Health Organisation (WHO) and the European Medicines Agency (EMA).

The ASEAN Guidelines should be read in conjunction with the latest publications by the WHO, EMA and the World Organisation for Animal Health (Office International des Epizooties (OIE)) on TSE.

Some of the existing websites are provided below:

- WHO: <http://www.who.int/zoonoses/diseases/bse/en/>
- EMA: <http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003700.pdf>
- OIE: <http://www.oie.int/eng/normes/mcode/en_sommaire.htm>

Although the recommendations of EMA are primarily for medicinal products, the approach is applicable to health products meant for human consumption, which would include HS.

The ASEAN Guidelines are intended to provide guidance to dealers (including importers and manufacturers) based on the principles mentioned in preceding sections, with the aim to reduce the transmission risk of TSE in the HS that are made available in ASEAN. This document will be subject to regular update and review as the development in the scientific knowledge of the diseases and the effectiveness of the recommended measures evolve with time.

2. ASEAN GUIDELINES TO MANAGE THE RISK OF TSE IN BOVINE-DERIVED MATERIALS

2.1. SCOPE OF GUIDELINES

2.1.1. This set of guidelines covers all substances of ruminant origin that are used in HS.

2.1.2. The use of ruminant as raw materials, in particular bovine and ovine materials, is common in HS. This would present a risk of TSE transmission to humans via the use of such products.

2.1.3. There is no known cure for TSE in humans but the risk of infection could be minimised through the use of certain precautionary measures on the use of ruminant derived materials in HS.

2.1.4. The range of materials covered in this set of guidelines would include the raw materials and all the substances in the product as well as those that it may be exposed to during the stages of processing. This would include the active substances, excipients and adjuvants, raw and starting materials, as well as reagents used in production.

2.2. GUIDELINES IN MANAGING RAW MATERIALS

2.2.1. Minimising transmission risk of TSE, particularly BSE, is based upon considerations of 3 closely related parameters.

2.2.1.1. Source animals and their geographical origin (section 2.2.2)

- Materials from countries of high BSE risk[2] would not be acceptable unless justified[3].
- Most satisfactory source is from countries where the risk of BSE in cattle is absent or low.

2.2.1.2. Nature of animal material used (section 2.2.3)

- Animal-derived materials of high infectivity risk would not be allowed unless justified;
- Use animal-derived materials of lowest risk

2.2.1.3. Production process(es) (section 2.2.4)

- Use of a quality assurance system at manufacturing facilities to monitor the production process so as to ensure consistency and traceability;
- Considerations to minimise the risk of cross-contamination should be taken in manufacturing process, especially for raw materials; and
- Validation should be done when production processes are claimed to contribute significantly to the safety of a product.

2.2.2. Source animals and their geographical origin

[2] Countries of high BSE risk would include, but are not limited to those as identified by WHO to be high risk.
[3] A product from high or undetermined BSE risk countries would be allowed only if a valid European Directorate for the Quality of Medicines (EDQM) Certificate for that product is available.

2.2.2.1. Controlled sourcing is the most important criterion to enhance the safety of a product and to minimise the risk of TSE transmission.

2.2.2.2. The most satisfactory source of ruminant raw materials is from countries where BSE risk in cattle is absent or low.

2.2.2.3. Materials sourced from countries where BSE risk is high or undetermined would not be acceptable unless justified.

2.2.2.4. Currently, the BSE risk status ascribed to countries [4] is based on the classification systems of the OIE (Appendix 1). Some factors that were considered in the risk assessment include:

- Reports of BSE cases in the country
- Length of time of which a country has remained BSE free from the last occurrence of BSE in that country
- Procedures/Methods taken to reduce BSE transmission within the country etc.

2.2.3. Nature of animal material used

2.2.3.1. The tables in Appendix 2 depict the levels of infectivity in different organs and secretions of BSE-infected animals or in infected humans[5].

2.2.3.2. The WHO and EMA categorise tissues and body parts into 3 categories of infectivity based on research done.

- High Infectivity (Category IA)
- Lower infectivity (Category IB)
- Tissues with no detectable infectivity or PrP^{TSE} (Category IC)

2.2.3.3. Some specific Ruminant Derived Material (RDM) may be considered to be of negligible infectivity when processed appropriately. Such RDM include, but are not limited to:

2.2.3.3.1. Gelatin / Collagen

- From skin – Acid or alkaline treatment is acceptable
- From bones - Bones should be taken from BSE-free countries or countries with low BSE-prevalence. In general, alkaline treatment is preferred over acid treatment alone.
- Skull and vertebral columns should not be used.

[4] A reference list of countries classified based on the OIE system can be found at <http://www.oie.int/animal-health-in-the-world/official-disease-status/bse/list-of-bse-risk-status/>

[5] Source from the guidance note published by WHO: "WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies" (updated 2010) <http://www.who.int/bloodproducts/tse/en/

2.2.3.3.2. Tallow and tallow derivatives

- WHO and EMA consider such substances unlikely to be infectious because production processes are rigorous.
- May be allowed without restrictions when prepared by extraction and purification processes at high temperatures and GMP is controlled[6].

2.2.3.3.3. Milk and milk derivatives

- WHO and EMA considered such substances safe, provided:
  - Milk is sourced from healthy ruminants fit for human consumption;
  - Potentially infectious ruminant-derived materials are not used in manufacturing process.

2.2.3.3.4. Measures to prevent and/or reduce cross contamination of materials in Categories IA, IB and IC should be taken into consideration when raw materials are obtained.

2.2.4. Production Process(es)

2.2.4.1. Production processes should be designed to take into considerations all available information on processes that could reduce or inactivate infectivity or remove infectivity from starting materials, as this would augment safety provided by sourcing.

2.2.4.2. WHO and EMA recommend for quality assurance systems be in place to monitor the production process in the processing of RDM e.g. in form of GMP.

2.2.4.3. If claims to increase safety of product through manufacturing process are made, relevant information should be submitted to the Authority for validation when required.

2.2.5. European Directorate for the Quality of Medicines (EDQM) Certificate

2.2.5.1. The EDQM Certificate is useful as a source of reference for evidence of compliance, and therefore should be obtained if possible. However, national regulators can request for more information if required.

2.3. REGULATORY REQUIREMENTS

2.3.1. National regulators may require a declaration by the person placing the product in the market or the manufacturer, on compliance with the recommendations in this set of Guidelines. Such documents to prove compliance should be held by the person who shall make these readily available to the regulatory agencies when required to do so.

[6] Refer to European Pharmacopoeia for manufacturing conditions

2.3.2. A sample copy of the Declaration and a suggested checklist for self-assessment are shown in Appendices 3 and 4. (The checklist may form part of the requirements for submission to the regulatory agencies during pre-marketing approvals).

REFERENCES

1. *WHO Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products*. World Health Organization. Geneva: World Health Organization. 2003.

2. *WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies.* World Health Organization. Geneva: World Health Organization. 2006.

3. *WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies.* World Health Organization. Geneva: World Health Organization. 2010.

4. *Report of a WHO Consultation on medicinal and other products in relation to human and animal Transmissible Spongiform Encephalopathies, with participation with the Office international des Epizooties (OIE)*. World Health Organization. Geneva: World Health Organization. 24-26 March 1997.

5. *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01 Rev.2-Oct 03) adopted by the Committee for Proprietary Medicinal Products (CPMP) and by the Committee for Veterinary Medicinal products (CVMP)*. Official Journal of the European Union. 2004; C 24, 28.1.2004: 6.

6. *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products ((EMA/410/01 rev.3) adopted by the Committee for Proprietary Medicinal Products (CPMP) and by the Committee for Veterinary Medicinal products (CVMP)*. Official Journal of the European Union. 5.3.2011; C 73/1.

7. *Guidance for Industry: The sourcing and processing of gelatin to reduce the potential risk posed by Bovine Spongiform Encephalopathy (BSE) in FDA-Regulated products for human use*. U.S. Department of Health and Human Services: Food and Drug Administration. 1997.

8. *TGA approach to minimising the risk of exposure Transmissible Spongiform Encephalopathies (TSE)*. Australian Government Department of Health: Therapeutic Goods Administration. April 2014.

9. *Minimising the risk of transmitting TSE via medicinal products*. European Pharmacopoeia 5.0; Sec 5.2.8.

APPENDICES

APPENDIX 1 BSE-RISK CLASSIFICATION SYSTEMS

A. World Organisation for Animal Health (Office International des Epizooties (OIE)) Classification System

Categories of BSE-risk of country:

1. Undetermined BSE risk
2. Controlled BSE risk
3. Negligible BSE risk

Reference website:
Terrestrial Animal Health Chapter 11.4. Code Bovine spongiform encephalopathy <http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_bse.htm>
Status of BSE risk <http://www.oie.int/animal-health-in-the-world/official-disease-status/bse/list-of-bse-risk-status/>

APPENDIX 2 LEVELS OF TISSUE INFECTIVITY*

The information in the tables is based exclusively upon observations of naturally occurring disease, or primary experimental infection by the oral route (in ruminants), and does not include data on models using strains of TSE that have been adapted to experimental animals, because passaged strain phenotypes can differ significantly and unpredictably from those of naturally occurring disease.

Because the detection of misfolded prion protein (PrP^{TSE}) broadly parallels infectivity titers in various tissues, PrP^{TSE} testing results are presented in parallel with bioassay data. Although a given tissue may be positive or negative in different varieties of TSE, the expert group considered a tissue to be potentially infectious even if a positive result occurred in only a single disease. The categorical assignment of tissues will almost certainly undergo further revision as new data accumulate from increasingly sensitive tests.

Category IA: High infectivity tissues: CNS tissues that attain a high titre of infectivity in the later stages of all TSEs, and certain tissues that are anatomically associated with the CNS.

Category IB: Lower-infectivity tissues: peripheral tissues that have tested positive for infectivity and/or PrP^{TSE} in at least one form of TSE.

Category IC: Tissues with no detectable infectivity or PrP^{TSE}: tissues that have been examined for infectivity and/or PrP^{TSE} with negative results.

Data entries are shown as follows:

+ Presence of infectivity or PrP^{TSE}
- Absence of detectable infectivity or PrP^{TSE}
NT Not tested
NA Not applicable
? Controversial or uncertain results
() Data limited to one or two tested specimens (human tissues)
[ ] Infectivity or PrP^{TSE} data based exclusively on bioassays in transgenic (Tg)mice over-expressing the PrP-encoding gene or PrP^{TSE} amplification methods.

A word of caution is offered about tissues in Table of Category IB for which positive results are so far limited to either detection of PrP^{TSE} using amplification techniques (PMCA), or infectivity bioassays in Tg mice that over-express PrP. The amounts of pathological protein

or infectious agent detected by these exquisitely sensitive assays may well fall below the threshold of transmissibility for normal animals and humans.

A good example is illustrated in the studies of urine and feces from deer infected with CWD: bioassays using normal deer as recipient subjects were negative; subsequent bioassays performed in Tg mice were positive. A similar discordance was observed for BSE muscle inoculated into cattle and Tg mice. Until more evidence is compiled showing that positive results in experimental PMCA and Tg mouse assays equate to a risk of transmitting disease under natural conditions, it cannot be assumed that such results imply the existence of a substantial risk to the health of animals or humans.

Considering the succession of updated Tables of Infectivity of the past few years, and the fact that inflammation has been shown to result in PrP^{TSE} deposition in tissues that are not normally involved in TSE pathogenesis, it is evident that as testing continues, more tissues will find their way from Table of Category IC into Table of Category IB (but probably not from either Table of Category IC or IB into Table of Category IA). It is also evident that the data generated to date are far from complete, and that a great deal more work needs to be done if conclusions about the tissue distribution and significance of infectivity in a given TSE are to be based on direct measurements rather than by analogy to other forms of the disease.

* Appendix 2 was referenced from the "WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies" (updated 2010) < http://www.who.int/bloodproducts/tse/en/>

Category IA: High infectivity tissues

Tissues	Humans				Cattle		Sheep & goats		Elk & deer	
	vCJD		Other TSEs		BSE		Scrapie		CWD	
	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}
Brain	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	NT	+
Retina	NT	+	+	+	+	NT	NT	+	NT	+
Optic nerve[2]	NT	+	NT	+	+	NT	NT	+	NT	+
Spinal ganglia	+	+	NT	+	+	+	+	+	NT	+
Trigeminal ganglia	+	+	NT	+	+	+	NT	+	NT	-
Pituitary gland[3]	NT	+	+	+	-	NT	+	+	NT	+
Dura mater[3]	NT	(+)	+	-	NT	NT	NT	NT	NT	NT

Category IB: Lower infectivity tissues

Tissues	Humans				Cattle		Sheep & goats		Elk & deer	
	vCJD		Other TSEs		BSE		Scrapie		CWD	
	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}
Peripheral Nervous system										
Peripheral nerves	+	+	(-)	+	[+]	+	+	+	NT	+
Autonomic ganglia[4]	NT	+	NT	(-)	NT	+	NT	+	NT	+
Lymphoreticular tissues										
Spleen	+	+	+	+	-	-	+	+	NT	+
Lymph nodes	+	+	+	-	-	-	+	+	NT	+
Tonsil	+	+	NT	-	+	-	+	+	NT	+
Nictitating membrane	NA	NA	NA	NA	+	-	[+]	+	NT	+
Thymus	NT	+	NT	-	-	NT	+	+	NT	-
Alimentary tract[5]										
Esophagus	NT	-	NT	-	-	NT	[+]	+	NT	+
Fore-stomach[6] (ruminants only)	NA	NA	NA	NA	-	NT	[+]	+	NT	+
Stomach/ abomasum	NT	-	NT	-	-	NT	[+]	+	NT	+
Duodenum	NT	-	NT	-	-	-	[+]	+	NT	+
Jejunum[7]	NT	+	NT	-	-	+	[+]	+	NT	NT
Ileum[7]	NT	+	NT	-	+	+	+	+	NT	+
Appendix	(-)	+	NT	-	NA	NA	NA	NA	NA	NA
Colon/caecum[7]	NT	+	NT	-	-	-	+	+	NT	+
Rectum	[+]	+	NT	NT	NT	NT	NT	+	NT	+
Reproductive tissues										
Placenta[8]	NT	-	(+)	-	-	NT	+	+	NT	-
Ovary[3]	NT	- (+)	NT	-	-	NT	-	-	NT	-
Uterus[3]	NT	- (+)	NT	-	-	NT	-	-	NT	-
Other tissues										
Mammary gland/udder[9]	NT	-	NT	-	-	NT	-	+	NT	NT
Skin[3],[10]	NT	- (+)	NT	-	-	NT	-	+	[+]	[+]
Adipose tissue	NT	-	(-)	-	-	NT	NT	NT	[+]	NT
Heart/pericardium	NT	-	-	-	-	NT	-	NT	NT	+
Lung	NT	-	+	-	-	NT	-	-	NT	+
Liver[3]	NT	- (+)	+	-	-	NT	+	-	NT	-
Kidney[3,11]	NT	- (+)	+	-	-	-	[+]	+	NT	+
Adrenal	NT	+	-	-	[+]	+	+	-	NT	+
Pancreas[3]	NT	- (+)	NT	-	-	NT	+	NT	NT	+
Bone marrow[12]	-	-	(-)	-	(+)	NT	+	NT	NT	-
Skeletal muscle[13]	NT	+	(-)	+	[+]	NT	[+]	+	[+]	-
Tongue[14]	NT	-	NT	-	-	NT	[+]	+	NT	-
Blood vessels	NT	+	NT	+	-	NT	NT	+	NT	-
Nasal mucosa[15]	NT	NT	NT	+	-	NT	+	+	NT	+
Salivary gland	NT	-	NT	-	-	NT	+	NT	-	-
Cornea[16]	NT	-	+	-	NT	NT	NT	NT	NT	NT
Body fluids, secretions and excretions										
CSF	-	-	+	-	-	NT	+	-	NT	NT
Blood[17]	+	?	-	?	-	?	+	?	+	?
Saliva	NT	-	-	NT	NT	NT	-	NT	+	[-]
Milk[18]	NT	NT	(-)	NT	-	-	+	[+]	NT	NT
Urine[19]	NT	-	-	-	-	NT	-	-	- [+]	[+]
Feces[19]	NT	NT	-	NT	-	NT	-	NT	- [+]	NT

Category IC: Tissues with no detected infectivity or PrP^{TSE}

Tissues	Humans				Cattle		Sheep & goats		Elk & deer	
	vCJD		Other TSEs		BSE		Scrapie		CWD	
	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}	Infectivity[1]	PrP^{TSE}
Reproductive tissues										
Testis	NT	-	(-)	-	-	NT	-	-	NT	-
Prostate/Epididymis/ Seminal vesicle	NT	-	(-)	-	-	NT	-	-	NT	-
Semen	NT	-	(-)	-	-	NT	-	-	NT	NT
Placenta fluids	NT	NT	(-)	(-)	-	NT	NT	NT	NT	NT
Fetus[20]	NT	NT	NT	NT	-	NT	-	-	NT	(-)
Embryos[20]	NT	NT	NT	NT	-	NT	?	NT	NT	NT
Musculo-skeletal tissues										
Bone	NT	-	NT	-	-	NT	NT	NT	NT	NT
Tendon	NT	-	NT	-	-	NT	NT	NT	NT	NT
Other tissues										
Gingival tissue	NT	-	-	-	NT	NT	NT	NT	NT	NT
Dental pulp	NT	-	NT	-	NT	NT	NT	NT	NT	NT
Trachea	NT	-	NT	-	-	NT	NT	NT	NT	-
Thyroid gland	NT	-	(-)	-	NT	NT	-	NT	NT	-
Body fluids, secretions and excretions										
Colostrum[21]	NT	NT	(-)	NT	(-)	-	(?)	NT	NT	NT
Cord blood[21]	NT	NT	(-)	NT	-	NT	NT	NT	NT	NT
Sweat	NT	NT	-	NT	NT	NT	NT	NT	NT	NT
Tears	NT	NT	-	NT	NT	NT	NT	NT	NT	NT
Nasal mucus	NT	-	-	NT	NT	NT	NT	NT	NT	NT
Bile	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

1. Infectivity bioassays of human tissues have been conducted in either primates or mice (or both); bioassays of cattle tissues have been conducted in either cattle or mice (or both); and most bioassays of sheep and/or goat tissues have been conducted only in mice. In regard to sheep and goats not all results are consistent for both species; for example, two goats (but no sheep) have contracted BSE naturally [Eurosurveillance, 2005, Jeffrey et al., 2006]. Similarly, most of the results described for CWD were derived from studies in deer, and findings may not be identical in elk or other cervids.
2. In experimental models of TSE, the optic nerve has been shown to be a route of neuroinvasion, and contains high titers of infectivity.
3. No experimental data about infectivity in pituitary gland or dura mater in humans with all forms of human TSE have been reported, but cadaveric dura mater patches, and growth hormone derived from cadaveric pituitaries have transmitted disease to hundreds of people and therefore must be included in the category of high-risk tissues. PrP^{TSE} was detected by immunoblot in the dura mater of a vCJD patient who died in the US after an unusually long incubation period (see also Table IB for other positive tissues: skin, kidney, liver, pancreas, ovary and uterus) [Notari et al., 2010]. It must be mentioned that earlier studies of numerous cases examined in the UK reported all of these tissues to be negative [Ironside et al., 2002; Head et al., 2004].
4. In cattle, PrP^{TSE} is reported to be inconsistently present in the enteric plexus in the distal ileum, but immunohistochemical examination of tissues from a single 'fallen stock' case of BSE in Japan suggested (albeit equivocally) involvement of myenteric plexuses throughout the small and large intestine [Kimura and Haritani, 2008].

5. In vCJD, PrP^{TSE} is limited to gut-associated lymphoid and nervous tissue (mucosa, muscle, and serosa are negative).
6. Ruminant forestomachs (reticulum, rumen, and omasum) are widely consumed, as is the true stomach (abomasum). The abomasum of cattle (and sometimes sheep) is also a source of rennet.
7. When a large BSE oral dose was used to infect cattle experimentally, infectivity was detected in the jejunum and the ileo-caecum junction in Tg mice overexpressing PrP [courtesy of Dr. M Groschup]. PrP^{TSE} was detected at low incidence in lymphoid tissue of ileum [Terry et al., 2003] and has been detected at an even lower frequency in jejunal lymphoid tissue of cattle similarly infected by the oral route [EFSA, 2009].
8. A single report of transmission of sporadic CJD infectivity from human placenta has never been confirmed and is considered improbable.
9. PrP^{TSE} has been detected in scrapie-infected sheep with chronic mastitis, but not from infected sheep without mastitis [Ligios et al., 2005].
10. Studies in hamsters orally infected with scrapie revealed that PrP^{TSE} deposition in skin was primarily located within small nerve fibers. Also, apical skin 'velvet' from the antlers of CWD-infected deer are reported to contain PrP^{TSE} and infectivity [Angers et al., 2009].
11. PrP^{TSE} detected by immunocytochemistry in the renal pelvis of scrapie-infected sheep [Siso et al., 2006]; and in lymphoid follicles within connective tissue adjacent to the renal pelvis in CWD-infected mule deer [Fox et al., 2006].
12. A single positive marrow in multiple transmission attempts from cattle orally dosed with BSE-infected brain [Wells et al., 1999; Wells et al., 2005; Sohn et al., 2009].
13. Muscle homogenates have not transmitted disease to primates from humans with sporadic CJD, or to cattle from cattle with BSE. However, intracerebral inoculation of a semitendinosus muscle homogenate (including nervous and lymphatic elements) from a single cow with clinical BSE has transmitted disease to transgenic mice that overexpress PrP at a rate indicative of trace levels of infectivity [Buschmann and Groschup, 2005]. Also, recent published and unpublished studies have reported the presence of PrP^{TSE} in skeletal muscle in experimental rodent models of scrapie and vCJD [Beekes et al., 2005], in experimental and natural scrapie infections of sheep and goats [Andreoletti et al., 2004], in sheep orally dosed with BSE [Andreoletti, unpublished data], and in humans with sporadic, iatrogenic, and variant forms of CJD [Glatzel et al., 2003; Kovacs et al., 2004; Peden et al., 2006]. Bioassays of muscle in transgenic mice expressing cervid PrP have documented infectivity in CWD-infected mule deer [Angers et al, 2006], and experiments are underway to determine whether detectable PrP^{TSE} in other forms of TSE is also associated with infectivity.
14. In cattle, bioassay of infectivity in the tongue was negative, but the presence of infectivity in palatine tonsil has raised concern about possible infectivity in lingual tonsillar tissue at the base of the tongue that may not be removed at slaughter [Wells et al., 2005; EFSA, 2008]. In sheep naturally infected with scrapie, 7 of 10 animals had detectable PrP^{TSE} in the tongue [Casalone et al., 2005; Corona et al., 2006].

15. Limited chiefly to regions involved in olfactory sensory reception.

16. Because only one case of iatrogenic CJD has been certainly attributed to a corneal transplant among hundreds of thousands of recipients (one additional case is considered probable, and another case only possible), cornea has been categorized as a lower-risk tissue; other anterior chamber tissues (lens, aqueous humor, iris, conjunctiva) have been tested with a negative result both in vCJD and other human TSEs, and there is no epidemiological evidence that they have been associated with iatrogenic disease transmission.

17. A wealth of data from studies of blood infectivity in experimental rodent models of TSE have been extended by recent studies documenting infectivity in the blood of sheep with naturally occurring scrapie and in sheep transfused with blood from BSE-infected cattle [Houston et al., 2008]; of deer with naturally occurring CWD [Mathiason et al., 2006]; and (from epidemiological observations) in the red cell fraction (which includes significant amounts of both plasma and leukocytes) of four blood donors in the pre-clinical phase of vCJD infections [reviewed in Brown, 2006; Hewitt et al., 2006]. Plasma Factor VIII administration has also been potentially implicated in a subclinical case of vCJD in a hemophilia patient [Peden et al., 2010]. Blood has not been shown to transmit disease from humans with any form of 'classical' TSE [Dorsey et al., 2009], or from cattle with BSE (including fetal calf blood). A number of laboratories using new, highly sensitive methods to detect PrP^{TSE} are reporting success in a variety of animal and human TSEs. However, several have experienced difficulty obtaining reproducible results in plasma, and it is not yet clear that positive results imply a potential for disease transmissibility, either because of false positives, or of 'true' positives that are due to sub-transmissible concentrations of PrP^{TSE}. Because of these considerations (and the fact that no data are yet available on blinded testing of specimens from naturally infected humans or animals) the expert group felt that it was still too early to evaluate the validity of these tests with sufficient confidence to permit either a negative or positive conclusion.

18. Evidence that infectivity is not present in milk from BSE-infected bovines includes temporo-spatial epidemiologic observations failing to detect maternal transmission to calves suckled for long periods; clinical observations of over a hundred calves suckled by infected cows that have not developed BSE; and experimental observations that milk from infected cows reared to an age exceeding the minimum incubation period has not transmitted disease when administered intracerebrally or orally to mice [Middleton and Barlow, 1993; Taylor et al., 1995]. Also, PrP^{TSE} has not been detected in milk from cattle incubating BSE following experimental oral challenge [SEAC, 2005]. However, low levels (µg to ng/L) of normal PrP have been detected in milk from both animals and humans [Franscini et al., 2006]. PrP^{TSE} has been detected in the mammary glands of scrapie-infected sheep with chronic mastitis [Ligios et al., 2005], and very recently it has been reported that milk (which in some cases also contained colostrum) from scrapie-infected sheep transmitted disease to healthy animals [Konold et al., 2008; Lacroux et. al., 2008].

19. A mixed inoculum of urine and feces from naturally infected CWD deer did not transmit disease during an 18 month observation period after inoculation of healthy deer with a heterozygous (96 G/S) *PRNP* genotype [Mathiason et al., 2006]. However,

recent bioassays in Tg mice have transmitted disease from both urine [Haley et al., 2009] and feces [Tamgüney et al., 2009]. In addition, mice with lymphocytic nephritis that were experimentally infected with scrapie shed both PrP^{TSE} and infectivity in urine, when bioassayed in Tg mice [Seeger et al., 2005]. Very low levels of infectivity have also been detected in the urine (and histologically normal kidneys) of hamsters experimentally infected with scrapie [Gregori and Rohwer, 2007; Gonzalez-Romero et al., 2008]. Finally, in an experimental scrapie-hamster model, oral dosing resulted in infectious feces when bioassayed in Tg mice over-expressing PrP [Safar et al., 2008].

20. Embryos from BSE-affected cattle have not transmitted disease to mice, but no infectivity measurements have been made on fetal calf tissues other than blood (negative mouse bioassay) [Fraser and Foster, 1994]. Calves born of dams that received embryos from BSE-affected cattle have survived for observations periods of up to seven years, and examination of the brains of both the unaffected dams and their offspring revealed no spongiform encephalopathy or PrP^{TSE} [Wrathall et al., 2002].

21. Early reports of transmission of sporadic CJD infectivity from human cord blood and colostrum have never been confirmed and are considered improbable. A bioassay from a cow with BSE in transgenic mice over-expressing bovine PrP gave a negative result [Buschmann and Groschup, 2005], and PrP^{TSE} has not been detected in colostrum from cattle incubating BSE following experimental oral challenge [SEAC, 2005].

APPENDIX 3 SAMPLE OF TSE DECLARATION FORM

TSE Submission Form
Brand & Product Name:
Kit Name:

Ingredient	Quantity	Animal Species	Tissue Used	Infectivity Category	Country of Origin	Reasons for Using

I hereby undertake that the above-mentioned product imported / manufactured (delete where appropriate) by my company complies with the ASEAN Guidelines for Minimising the Risk of Transmission of Transmissible Spongiform Encephalopathies in Health Supplements and I hold evidence to demonstrate that the product is prepared:

- from ruminant-derived materials without any risk of exposure to TSE, and the relevant authority in the country of origin has endorsed that the materials are sourced from TSE-free herds

-by manufacturing process with adequate measures taken to prevent cross-contamination between different tissues from different categories of infectivity

- by a manufacturing process that has shown experimentally to minimise the TSE transmissible agent, if the above product contains tallow and/or gelatin derived from ruminant-derived materials (including those for making capsule shells)

I shall retain all the necessary evidence at all times and would supply the evidence to the regulatory authority if required to do so. I shall report any changes in the TSE status of the ruminant-derived materials of the above product to the regulatory authority as soon as possible.

I hereby declare that the information on this form is current and correct.

I undertake the responsibility to check and ensure compliance to the latest ASEAN Guidelines for Minimising the Risk of Transmission of Transmissible Spongiform Encephalopathies in Health Supplements.

Name: …… Designation: ……

Company Name: ……

Tel: ……..…… Fax: ……..……..

Manufacturer Name: ……..Date: ……..……..

Company Stamp: …… Signature: ……..……

APPENDIX 4 SAMPLE OF CHECKLIST FOR SELF-ASSESSMENT

Check if available	Document	Enclosure number
	1. Source of Animal	
☐	Updated notification of BSE cases in country where each animal material is sourced, where applicable	
☐	Justification for using animal materials from BSE positive countries (if applicable)	
☐	Documentary proof issued by health authorities in source country to show that the raw materials used are sourced from TSE-free herds	
	2. Nature of animal tissue used in manufacturing	
☐	Detailed information on the nature and quantity of each animal-derived material: • Used in the manufacturing process (whether or not this is present in the final products) • Present in the final product formulation	
☐	Letter of confirmation or assurance from the raw materials supplier and/or manufacturer that: • Considerations to reduce cross contamination between different tissues have been taken when obtaining the raw materials	
	3. Manufacturing process(es)	
☐	Letter of confirmation or assurance from the manufacturer that: • Considerations to TSE inactivation / reduction methods have been taken when developing manufacturing methods • If claims have been made to inactivate TSE agents during manufacturing process, then the necessary documentary proof are held in possession and will be made available to the regulatory authority if needed • Quality assurance system is taken into consideration in developing and implementing the manufacturing processes	

	4. Other documentation	
☐	Company's assessment report for risk of TSE	
☐	Certificate of Suitability issued by European Directorate for the Quality of Medicines	