Safe commodity assessments for WOAH listed aquatic animal diseases

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Background

The Safe commodity assessments for OIE listed aquatic animal diseases publication (2016) included the aquatic animal product assessments that were conducted against the criteria in Article 5.4.1. by the WOAH *ad hoc* Group between 2009-2016 to support the products listed in Article X.X.3. of the disease-specific chapters of the *Aquatic Code*.

The scientific literature on thermal stability for WOAH listed diseases had not been reviewed since the *ad hoc* Group had completed its work.

In 2020, the Aquatic Animal Health Standards Commission agreed to amend the approach used in Article X.X.3. of the disease-specific chapters of the *Aquatic Code* to include the specific time/temperatures required to inactivate the specific pathogenic agents rather than the previous approach of listing specific products and the industry standard time/temperatures used during processing. The Commission agreed to maintain the use of equivalent time/temperature combinations to ensure the inactivation of the pathogenic agents and flexibility for Members.

The Commission agreed that the assessments conducted against the criteria in Article 5.4.1. for the aquatic animal products and the time/temperatures for inactivation currently listed in Articles X.X.3. should be reviewed given that the scientific evidence on thermal stability for these assessments had not been reviewed since the completion of the initial assessments. In addition, the Commission agreed to provide more guidance regarding equivalent time/temperature combinations that could be used by Members (e.g. longer times at lower temperatures or shorter times at higher temperatures).

Between October and December 2022, a consultant undertook new or revised assessments for WOAH listed aquatic animal diseases using all available scientific evidence on thermal stability. A summary of the time/temperatures for inactivation recommended for each WOAH listed disease is provided in Table 1 and the complete assessments are presented in the following pages.

Table 1. Summary of inactivation time/temperatures for heat-treated and mechanically dried products for WOAH listed aquatic animal diseases.

Listed disease	Product	Temperature	Time
Diseases of amphibians listed by WOAH (Article 1.3.4.)			
Infection with *Batrachochytrium dendrobatidis*	Heat treated amphibian products	60°C	5 minutes
	Mechanically dried amphibian products	60°C	5 minutes
Infection with *Batrachochytrium salamandrivorans*	Heat treated amphibian products	60°C	5 minutes
	Mechanically dried amphibian products	60°C	5 minutes
Infection with Ranavirus species	Heat treated amphibian products	60°C	30 minutes

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Listed disease	Product	Temperature	Time
	Mechanically dried amphibian products	60°C	30 minute
Diseases of crustaceans listed by WOAH (Article 1.3.3.)			
Acute hepatopancreatic necrosis disease	Heat treated crustacean products	100°C	1 minute
Infection with *Aphanomyces astaci* (Crayfish plague)	Heat treated crustacean products	100°C	1 minute
Infection with *Hepatobacter penaei* (Necrotising hepatopancreatitis)	Heat treated crustacean products	95°C	5 minutes
Infection with infectious hypodermal and haematopoietic necrosis virus	Heat treated crustacean products	100°C	2 minutes
Infection with infectious myonecrosis virus	Heat treated crustacean products	75°C	5 minutes
Infection with *Macrobrachium rosenbergii* nodavirus (White tail disease)	Heat treated crustacean products	50°C	5 minutes
Infection with taura syndrome virus	Heat treated crustacean products	70°C	108 minutes
Infection with white spot syndrome virus	Heat treated crustacean products	60°C	1 minute
Infection with yellow head virus genotype 1	Heat treated crustacean products	60°C	15 minutes
Infection with decapod iridescent virus 1	Heat treated crustacean products	80°C	30 minutes
Diseases of fish listed by WOAH (Article 1.3.1.)			
Infection with epizootic haematopoietic necrosis virus	Heat treated fish products	60°C	15 minutes
	Mechanically dried eviscerated fish	60°C	15 minutes
Infection with *Aphanomyces invadans* (Epizootic ulcerative syndrome)	Heat treated fish products	100°C	1 minute
	Mechanically dried eviscerated fish	100°C	1 minute
Infection with *Gyrodactylus salaris*	Heat treated fish products	40°C	1 minute
	Mechanically dried fish products	40°C	1 minute
Infection with infectious salmon anaemia virus	Heat treated fish products	56°C	5 minutes
	Mechanically dried eviscerated fish	56°C	5 minutes
Infection with salmonid alphavirus	Heat treated fish products	60°C	60 minutes
	Mechanically dried eviscerated fish	60°C	60 minutes
Infection with infectious haematopoietic necrosis virus	Heat treated fish products	90°C	30 seconds
	Mechanically dried eviscerated fish	90°C	30 seconds
Infection with koi herpesvirus	Heat treated fish products	50°C	1 minute
	Mechanically dried fish products	50°C	1 minute
Infection with red sea bream iridovirus	Heat treated fish products	56°C	30 minutes
	Mechanically dried eviscerated fish	56°C	30 minutes

Listed disease	Product	Temperature	Time
Infection with spring viraemia of carp virus	Heat treated fish products	60°C	60 minutes
	Mechanically dried eviscerated fish	60°C	60 minutes
Infection with viral haemorrhagic septicaemia virus	Heat treated fish products	60°C	60 minutes
	Mechanically dried eviscerated fish	60°C	60 minutes
Infection with tilapia lake virus	Heat treated fish products	60°C	120 minutes
	Mechanically dried eviscerated fish	60°C	120 minutes
Diseases of molluscs listed by WOAH (Article 1.3.2.)			
Infection with abalone herpesvirus	Heat treated abalone products	50°C	5 minutes
	Mechanically dried abalone products	50°C	5 minutes
Infection with *Bonamia exitiosa*	Heat treated mollusc products	100°C	15 minutes
Infection with *Bonamia ostreae*	Heat treated mollusc products	100°C	15 minutes
Infection with *Marteilia refringens*	Heat treated mollusc products	121°C	3 minutes and 36 seconds
Infection with *Perkinsus marinus*	Heat treated mollusc products	60°C	60 minutes
Infection with *Perkinsus olseni*	Heat treated mollusc products	60°C	60 minutes
	Mechanically dried abalone products	60°C	60 minutes
Infection with *Xenohaliotis californiensis*	Heat treated abalone products	95°C	5 minutes
	Mechanically dried abalone products	95°C	5 minutes

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1. Assessments for WOAH listed diseases of amphibians using criteria in Article 5.4.1. (conducted in December 2022)

1.1. Aquatic animal product assessments for infection with *Batrachochytrium dendrobatidis*

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated amphibian products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 5 minutes, or a time/temperature equivalent) (refer to Table 3 for the assessment).

   ii. Mechanically dried amphibian products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 5 minutes or a time/temperature equivalent) (refer to Table 4 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 3 and 4 has been incorporated into Table 2 to support Members in determining time/temperature equivalence:

Table 2. Time required for >99.9% inactivation of *Batrachochytrium dendrobatidis*

Batrachochytrium dendrobatidis	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	47	30			
	50				
	60		5		
	70				
	80				
	90				
	100			1	
	110				
	121				

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for *Batrachochytrium dendrobatidis*.

Table 3. Heat treated amphibian products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Infection with *B. dendrobatidis* is confined to the superficial keratin-rich layers of the epidermis in adult amphibians (Berger *et al*., 2005; Voyles *et al.*, 2007). Canned product is likely to consist of meat only.	Yes
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable water is used to process canned product. There is evidence that *B. dendrobatidis* will survive for up to three weeks in potable tap water (Johnson & Speare, 2003). However, water potentially contaminating the product will be subject to the same temperature/time treatment as the product and the final product is sealed.	No
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	*B. dendrobatidis* is inactivated by heating to 100°C for 1 min, 60°C for 5 min or 47°C for 30 min (Johnson *et al.*, 2003).	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION ***Batrachochytrium dendrobatidis* will be inactivated by heat treatment. Therefore, heat treated amphibian products (i.e. heat treatment of at least 60°C for at least 5 minutes or a time/temperature equivalent) are eligible for inclusion in Article 8.1.3.**		

Table 4. Mechanically dried amphibian products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Infection with *B. dendrobatidis* is confined to the superficial keratin-rich layers of the epidermis in adult amphibians (Berger *et al*., 2005; Voyles *et al.*, 2007). Mechanically dried product is likely to be mainly meat, but there is no guarantee that skin would not also be present.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Water is used in the processing but the product undergoes a drying process.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	*B. dendrobatidis* is inactivated by heating to 100°C for 1 min, 60°C for 5 min or 47°C for 30 min (Johnson *et al*., 2003).	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)	–	–
AND/OR		
c) Biological (e.g. fermentation)	–	–
CONCLUSION ***Batrachochytrium dendrobatidis* will be inactivated by mechanical drying. Therefore, mechanically dried amphibian products (i.e. heat treatment of at least 60°C for at least 5 minutes or a time/temperature equivalent) are eligible for inclusion in Article 8.1.3.**		

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JOHNSON, M.L. & SPEARE, R. (2003). – Survival of *Batrachochytrium dendrobatidis* in water: Quarantine and disease control implications. *Emerging Infectious Diseases*, **9** (8), 922–925. doi:10.3201/eid0908.030145

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1.2. Aquatic animal product assessments for infection with *Batrachochytrium salamandrivorans*

1. Assessed and met the criteria in Article 5.4.1. and will be presented in Article 8.2.3. for:

   i. Heat treated amphibian products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 5 minutes, or a time/temperature equivalent) (refer to Table 6 for the assessment).

   ii. Mechanically dried amphibian products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 5 minutes or a time/temperature equivalent) (refer to Table 7 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 6 and 7 has been incorporated into Table 5 to support Members in determining time/temperature equivalence:

Table 5. Time required for >99.9% inactivation of *Batrachochytrium salamandrivorans*

Batrachochytrium dendrobatidis	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	47	30			
	50				
	60		5		
	70				
	80				
	90				
	100			1	
	110				
	121				

Use of surrogate:: Because of a lack of relevant scientific information for the heat inactivation for *Batrachochytrium salamandrivorans*, *Batrachochytrium dendrobatidis* was used as a surrogate pathogenic agent.

Batrachochytrium dendrobatidis was chosen as it was determined to be the closest related pathogenic agent (Martel *et al*., 2013) for which there was available information for heat treatment inactivation.

Note: several disinfectants were less active against *Batrachochytrium salamandrivorans* than against *Batrachochytrium dendrobatidis*, therefore the use of *Batrachochytrium dendrobatidis* as a surrogate may not necessarily result in equivalent inactivation (VanRooij *et al*., 2017).

Table 6. Heat treated amphibian products

Article 5.4.1. Criteria		***Rationale***	***Assessment***
1.	***Absence of pathogenic agent in the traded commodity:***		
	a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Like *B. dendrobatidis*, *B. salamandrivorans* is confined to the superficial keratin-rich layers of the epidermis in adult amphibians (Martel *et al*., 2013). Canned product is likely to consist of meat only.	Yes
	AND		
	b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable water is used to process canned product. However, water potentially contaminating the product will be subject to the same temperature/time treatment as the product and the final product is sealed.	No
OR			
2.	***Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:***		
	a) Physical (e.g. temperature, drying, smoking)	Information is lacking regarding inactivation conditions for *B. salamandrivorans.* However, the closely related *B. dendrobatidis* is inactivated by heating to 100°C for 1 min, 60°C for 5 min or 47°C for 30 min (Johnson *et al.*, 2003).	Yes
	AND/OR		
	b) Chemical (e.g. iodine, pH, salt, smoke)	-	-
	AND/OR		
	c) Biological (e.g. fermentation)	-	-
CONCLUSION			
Based on the use of *Batrachochytrium dendrobatidis* as a surrogate, *Batrachochytrium salamandrivorans* will be inactivated by heat treatment. Therefore, heat treated amphibian products (i.e. heat treatment of at least 60°C for at least 5 minutes or any time/temperature equivalent) are eligible for inclusion in Article 8.2.3.			

Table 7. Mechanically dried amphibian products

Article 5.4.1. criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Like *B. dendrobatidis*, *B. salamandrivorans* is confined to the superficial keratin-rich layers of the epidermis in adult amphibian is confined to the superficial keratin-rich layers of the epidermis in adult amphibians (Martel *et al*., 2013). Mechanically dried product is likely to be mainly meat, but there is no guarantee that skin would not also be present.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Water is used in the processing but the product undergoes a drying process.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Information is lacking regarding inactivation conditions for *B. salamandrivorans*. However, the closely related *B. dendrobatidis* is inactivated by heating to 100°C for 1 min, 60°C for 5 min or 47°C for 30 min (Johnson *et al.*, 2003).	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)	–	–
AND/OR		
c) Biological (e.g. fermentation)	–	–
CONCLUSION **Based on the use of *Batrachochytrium dendrobatidis* as a surrogate*, Batrachochytrium salamandrivorans* will be inactivated by mechanical drying. Therefore, mechanically dried amphibian products (i.e. heat treatment of at least 60°C for at least 5 minutes or any time/temperature equivalent) are eligible for inclusion in Article 8.2.3.**		

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JOHNSON, M.L. & SPEARE, R. (2003). – Survival of *Batrachochytrium dendrobatidis* in water: Quarantine and disease control implications. *Emerging Infectious Diseases*, **9** (8), 922–925. Doi:10.3201/eid0908.030145

JOHNSON, M.L., BERGER, L., PHILIPS, L. & SPEARE, R. (2003). – Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms,* **57**, 255-260. Doi:10.3354/dao057255.

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VAN ROOIJ, P., PASMANS, F., COEN, Y. & MARTEL, A. (2017). – Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus *Batrachochytrium salamandrivorans*. *PloS ONE,* **12(10),** e0186269.

1.3. Aquatic animal product assessments for infection with *Ranavirus*

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated amphibian products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 30 minutes, or a time/temperature equivalent) (refer to Table 9 for the assessment).

   ii. Mechanically dried amphibian products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 30 minutes, or a time/temperature equivalent) (refer to Table 10 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 9 and 10 has been incorporated into Table 8 to support Members in determining time/temperature equivalence:

Table 8. Time required for >99.9% inactivation of *Ranavirus*

Ranavirus	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	56	120			
	58		60		
	60			30	
	65			30	
	70				
	80				
	90				
	100				
	110				
	121				3.6

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for infection with *Ranavirus*.

Table 9. Heat treated amphibian products

Article 5.4.1. Criteria		*Rational*	*Assessment*
1.	***Absence of pathogenic agent in the traded commodity:***		
	a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Amphibian *Ranavirus* is present in skin and a wide range of internal organs (Cunningham *et al.*, 2008). Available information indicates that amphibian *Ranavirus* is additionally present in skeletal muscle (Cunningham *et al.*, 1996; Gantress *et al.*, 2003; Miller *et al.*, 2008).	No
	AND		
	b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Water is used to process the product but the water potentially contaminating the product will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR			
2.	***Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:***		
	a) Physical (e.g. temperature, drying, smoking)	The amphibian *Ranavirus* type species, FV3, is inactivated by heating to 65°C for 30 min (Granoff *et al.*, 1965). TEV, a strain of FV3, is inactivated within 2 min at 56°C (Wolf *et al.*, 1968). Another amphibian *Ranavirus*, BIV was inactivated after 2 h at 56°C (Speare & Smith, 1992), 60 min at 58°C and 30 min at 60°C (LaFauce *et al*., 2012). Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *Ranavirus*.	Yes
	AND/OR		
	b) Chemical (e.g. iodine, pH, salt, smoke)		
	AND/OR		
	c) Biological (e.g. fermentation)		
CONCLUSION ***Ranavirus*** **will be inactivated by heat treatment. Therefore, heat treated amphibian products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 30 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 8.3.3.**			

Table 10. Mechanically dried amphibians

Article 5.4.1. criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Amphibian *Ranavirus* is present in skin and a wide range of internal organs (Cunningham *et al.*, 2008). Available information indicates that amphibian *Ranavirus* is additionally present in skeletal muscle (Cunningham *et al.*, 1996; Gantress *et al.*, 2003; Miller *et al.*, 2008).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Water is used in the processing but the product undergoes a drying process.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	The amphibian *Ranavirus* type species, FV3, is inactivated by heating to 65°C for 30 min (Granoff *et al.*, 1965). TEV, a strain of FV3, is inactivated within 2 min at 56°C (Wolf *et al.*, 1968). Another amphibian *Ranavirus*, BIV, was inactivated after 2 h at 56°C (Speare & Smith, 1992), 60 min at 58°C and 30 min at 60°C (LaFauce *et al*., 2012). Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *Ranavirus*.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		NA
AND/OR		
c) Biological (e.g. fermentation)		NA
CONCLUSION ***Ranavirus* will be inactivated by mechanical drying. Therefore, mechanically dried amphibians (i.e. heat treatment to attain a core temperature of at least 60°C for at least 30 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 8.3.3.**		

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2. Assessments for WOAH listed diseases of crustaceans using criteria in Article 5.4.1. (conducted in December 2022)

2.1. Aquatic animal product assessments for acute hepatopancreatic necrosis disease

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 1 minute, or a time/temperature equivalent) (refer to Table 12 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 12 has been incorporated into Table 11 to support Members in determining time/temperature equivalence:

Table 11. Time required for >99.9% inactivation of *Vibrio parahaemolyticus*

Vibrio parahaemolyticus	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	52	22+			
	60		5+		
	70			2+	
	80		15*		
	90			10*	
	100				1*
	110				
	121				

+ Denotes data for >99.9% (3 log) inactivation of *V. parahaemolyticus* outside shrimp tissue

* Denotes data for >99.9% (3 log) inactivation of *V. parahaemolyticus* inside shrimp tissue. Only 100°C for 1 min provides 100% inactivation of *V. parahaemolyticus* inside shrimp tissue.

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for *Vibrio parahaemolyticus*, including strains responsible for AHPND.

Table 12. Heat treated crustacean products

Article 5.4.1. criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	This commodity largely contains muscle tissue. The AHPND bacteria is present in gut-associated tissues (Tran *et al*., 2013; Kumar *et al*., 2020); examination of muscle tissue for presence of the bacterium has not been reported in the literature and there is possibility of contamination of muscle by gut associated tissue.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	*Vibrio parahaemolyticus* in oyster tissue is inactivated when heated to 52°C for 22 min (Andrews *et al*., 2003). Zhang *et al*., (2014) reported >99.9% inactivation of *Vibrio parahaemolyticus* in alkaline peptone water-salt broth after 5 min at 60°C, or 2 min at 70°C. *Vibrio* species in artificial seawater were also inactivated after 2 min at 70°C (Johnston & Brown 2002). However, none of these studies used shrimp or prawn tissue as the matrix for studying inactivation. There is evidence that the decimal reduction time (D-value) depends on the matrix homogenate used and that *V. parahaemolyticus* may be more heat resistant than other *Vibrio* species (Johnston & Brown 2002). Vanderzant & Nickelson (1972) reported that *Vibrio parahaemolyticus* were recoverable on direct plating and enrichment after 6 log10 reduction following heating shrimp tissue to 80°C for 15 min. Exposure of shrimp tissue to 90°C for 10 min is probably effective, and 1 min at 100°C is confirmed as 100% effective (Vanderzant & Nickelson, 1972).	Yes
AND/OR		

b) Chemical (e.g. iodine, pH, salt, smoke)

AND/OR

c) Biological (e.g. fermentation)

CONCLUSION

VpAHPND is highly likely to be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 9.1.3.

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2.2. Aquatic animal product assessments for infection with *Aphanomyces astaci* (crayfish plague)

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated crayfish products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 1 minute, or a time/temperature equivalent) (refer to Table 14 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 14 has been incorporated into Table 13 to support Members in determining time/temperature equivalence:

Table 13. Time required for >99.9% inactivation of *Aphanomyces astaci*

Aphanomyces astaci	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	37	720			
	40				
	50				
	60		5		
	70		5		
	80				
	90				
	100			1	
	110				
	121				

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for *Aphanomyces astaci*.

Table 14. Heat treated crayfish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Aphanomyces astaci* is mainly present in exoskeleton (the cuticle) of crayfish but may also invade other tissues (Oidtmann *et al.*, 1997, 2006). All these tissues may be used in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Oidtmann *et al.*, (2002) found that *Aphanomyces astaci* mycelium in crayfish is inactivated by heating to 37°C for at least 12 h. However, some strains of *A. astaci* may survive this treatment, hence use of temperatures greater than 50°C are recommended (K. Söderhall, pers. comm., cited in DAWE (2019). CEFAS (2000) reported that *A. astaci* spores and mycelium are inactivated after 5 min exposure to 60°C or 70°C. Oidtmann *et al.*, (2002) found that all stages of *Aphanomyces astaci* in crayfish tissues are 100% inactivated by boiling at 100°C for 1 min.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION ***Aphanomyces astaci* will be inactivated by heat treatment. Therefore, heat treated crayfish products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 9.2.3.**		

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2.3. Aquatic animal product assessments for Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 95°C for at least 5 minutes, or a time/temperature equivalent) (refer to Table 16 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 16 has been incorporated into Table 15 to support Members in determining time/temperature equivalence:

Table 15. Time required for >99.9% inactivation of *Hepatobacter penaei*

Hepatobacter penaei	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60				
	65	45			
	70				
	80				
	90				
	95		10	5	
	100				
	110				
	121				3.6

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for *Hepatobacter penaei*, the bacterium *Anaplasma phagocytophilium* was used as a surrogate pathogenic agent.

Anaplasma phagocytophilium was chosen as it was determined to be the closest related pathogenic agent (Nunan *et al*., 2013) for which there was available information for heat treatment inactivation.

Table 16. Heat treated crustacean products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	This commodity contains muscle tissue. The NHP bacterium is present in the hepatopancreas (Frelier *et al.*, 1992). However, examination of muscle tissue for presence of the bacterium has not been reported in the literature.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	There is no specific information about inactivation of *Hepatobacter penaei* with heat. *Anaplasma phagocytophilium* (a closely related obligate intracellular bacterium, see Nunan *et al.*, 2013), is inactivated after exposure to 65°C for 45 min (Pedra *et al.*, 2008), or 95°C for 5-10 min (Borjesson *et al.*, 2005). Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *H. penaei*.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of *Anaplasma phagocytophilium* as a surrogate, *Hepatobacter penaei* is highly likely to be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 95°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 9.3.3.**		

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2.4. Aquatic animal product assessments for infection with infectious hypodermal and haematopoietic necrosis virus

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 2 minutes, or a time/temperature equivalent) (refer to Table 18 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 18 has been incorporated into Table 17 to support Members in determining time/temperature equivalence:

Table 17. Time required for >99.9% inactivation of Infectious hypodermal and haematopoietic necrosis virus (IHHNV)

Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	55	720			
	60				
	70		20		
	80			420	
	90			20	
	100				2
	110				
	121				

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for infectious hypodermal and haematopoietic necrosis virus, other densoviruses in the Family *Parvoviridae* were used as surrogate pathogenic agents.

These were chosen as they were determined to be the closest related pathogenic agents (Bonami *et al*., 1990; Shike *et al*., 2000) for which there was available information for heat treatment inactivation.

Table 18. Heat treated crustacean products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Tissue tropism of IHHNV is reported to include mesodermal and ectodermal tissues including: lymphoid organs, connective tissue, cuticular epithelium, antennal gland, heart, nerves, ganglia, gonads and skeletal muscle (Owens *et al.*, 1992; Sithigorngul *et al.*, 2009). Infection may therefore be present in muscle tissue used in the manufacture of these products.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	There is no specific information about inactivation of IHHNV, however it has been classified as a densovirus in the Family *Parvoviridae* (see Bonami *et al.*, 1990; Shike *et al.*, 2000) so other parvoviruses can be used as surrogates. Porcine parvovirus has been reported to be inactivated after 11 h to 12 h at 55°C (Lund *et al.*, 1996). An insect densovirus was completely inactivated after exposure to 70°C for 20 min (Seki, 1986). Canine parvovirus is inactivated after exposure to 80°C for over 7 h, and at 100°C after 2 min (McGavin, 1987). Bovine parvovirus was completely inactivated within 1 min at 100°C (Rehman 1987), but at least 20 min was required at 90°C to achieve the same result (Mahnel & Von Brodorotti 1981).	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION		

Based on the use of other densoviruses in the Family Parvoviridae as surrogates, infectious hypodermal and haematopoietic necrosis virus is highly likely to be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 2 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 9.5.3.

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2.5. Aquatic animal product assessments for infection with infectious myonecrosis virus

1. Assessed and met the criteria in Article 5.4.1for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 75°C for at least 5 minutes, or a time/temperature equivalent) (refer to Table 20 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 20 has been incorporated into Table 19 to support Members in determining time/temperature equivalence:

Table 19. Time required for >99.9% inactivation of infectious myonecrosis virus (IMNV)

Infectious myonecrosis virus (IMNV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60	60			
	70				
	75		5		
	80				
	90				
	100				
	110				
	121			3.6	

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for IMNV, *Giardia lamblia* virus (GLV) and betanodaviruses were used as surrogate pathogenic agents.

Giardia lamblia virus was chosen as it was determined to be the closest related pathogenic agent (Poulos *et al*., 2006) for which there was available information for heat treatment inactivation, while betanodaviruses are an aquatic virus generally similar in size and genomic construction to IMNV.

Table 20. Heat treated crustacean products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	The principal target tissues for IMNV include the striated muscles (skeletal and less often cardiac), connective tissues, haemocytes, and the lymphoid organ parenchymal cells (Lightner *et al.*, 2004; Tang *et al.*, 2005; Poulos *et al.*, 2006). This commodity contains muscle tissue.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	There is no specific information about inactivation of IMNV with heat. Infectious myonecrosis virus is a small, non-enveloped dsRNA virus approximately 40-45 nm in diameter (Poulos *et al.*, 2006; Tang *et al*., 2008) that is most closely related to *Giardia lamblia* virus (GLV) (Poulos *et al*., 2006). Janssen *et al*., (2015) found that purified GLV virions were more thermoresistant than other closely related viruses, but were inactivated after 5 min exposure to 75°C. Betanodaviruses are similar sized, non-enveloped ssRNA viral particles, and Frerichs *et al*., (2000), found that sea bass nodavirus, is inactivated within 60 min at 60°C. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of IMNV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION		
Based on the use of *Giardia lamblia* virus as a surrogate, infectious myonecrosis virus is highly likely to be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 75°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 9.4.3.		

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2.6. Aquatic animal product assessments for infection with *Macrobrachium rosenbergii* nodavirus (White tail disease)

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 50°C for at least 5 minutes, or a time/temperature equivalent) (refer to Table 22 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 22 has been incorporated into Table 21 to support Members in determining time/temperature equivalence:

Table 21. Time required for >99.9% inactivation of *Macrobrachium rosenbergii* nodavirus (MrNV)

Macrobrachium rosenbergii nodavirus (MrNV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50	5			
	60	5			
	70				
	80				
	90				
	100				
	110				
	121		3.6		

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for *Macrobrachium rosenbergii* nodavirus.

Table 22. Heat treated crustacean products

Article 5.4.1. Criteria		*Rationale*	*Assessment*
1.	***Absence of pathogenic agent in the traded commodity:***		
	a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	MrNV is present in muscle tissues (Arcier *et al.*, 1999; Sahul Hameed *et al.*, 2004; Tung *et al.*, 1999) and these may be used in the commodity.	No
	AND		
	b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR			
2.	***Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:***		
	a) Physical (e.g. temperature, drying, smoking)	Ravi & Sahul Hameed (2016) found that MrNV in tissue suspensions was inactivated after exposure to 50°C or 60°C for at least 5 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of MrNV.	Yes
	AND/OR		
	b) Chemical (e.g. iodine, pH, salt, smoke)		
	AND/OR		
	C) Biological (e.g. fermentation)		
CONCLUSION ***Macrobrachium rosenbergii* nodavirus will be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 50°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 9.6.3.**			

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2.7. Aquatic animal product assessments for infection with taura syndrome virus

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 70°C for at least 108 minutes or a time/temperature equivalent) (refer to Table 24 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 24 has been incorporated into Table 23 to support Members in determining time/temperature equivalence:

Table 23. Time required for >99.9% inactivation of taura syndrome virus (TSV)

Taura syndrome virus (TSV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	41	>360			
	50				
	60				
	65		360		
	70			108	
	80				
	90				
	100				
	121				3.6

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for TSV, Israeli acute paralysis virus (IAPV), cricket paralysis virus (CrPV), and other similar viruses in the Family *Dicistroviridae* were used as surrogate pathogenic agents.

IAPV, CrPV and other similar viruses in the Family *Dicistroviridae* which infect insects were chosen as they were determined to be the closest related pathogenic agents (Mari *et al*., 2002; Valles *et al*., 2017) for which there was available information for heat treatment inactivation.

Table 24. Heat treated crustacean products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	TSV is present in muscle tissues (Nunan *et al*., 2004) and these may be used in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	TSV remains viable after passage through the gut of seagulls and chickens, which equates to survival at least 6 h at 41°C (Vanpatten *et al*., 2004). Although there is no specific information for inactivation of TSV, closely related viruses in the Family *Dicistroviridae* which infect insects are likely to be useful surrogates. Tomkies *et al*., (2015) found that Israeli acute paralysis virus (IAPV) was inactivated in honey after exposure to 65°C for 6 h or 70°C for 108 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of TSV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of Israeli acute paralysis virus (IAPV) and other viruses in the Family *Dicistroviridae* as surrogates, Taura syndrome virus is highly likely to be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment of at least 70°C for at least 108 minutes or a time/temperature equivalent) are eligible for inclusion in Article 9.7.3.**		

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2.8. Aquatic animal product assessments for infection with white spot syndrome virus

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 1 minute, or a time/temperature equivalent) (refer to Table 26 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 26 has been incorporated into Table 25 to support Members in determining time/temperature equivalence:

Table 25. Time required for >99.9% inactivation of white spot syndrome virus (WSSV)

White spot syndrome virus (WSSV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50	60			
	60		1		
	70			0.2	
	80				
	90				
	97				1
	100				
	110				
	121				

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for WSSV.

Table 26. Heat treated crustacean products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	The major targets of WSSV infection are tissues of ectodermal and mesodermal embryonic origin, especially the cuticular epithelium and subcuticular connective tissues (Lightner, 1996; Momoyama *et al.*, 1994; Wongteerasupaya *et al.*, 1995), but WSSV is also found in muscle tissue (Durand *et al.*, 2003). This commodity contains muscle tissue.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	WSSV is inactivated in less than 60 min at 50°C, less than 1 min at 60°C, and in 0.2 min at 70°C (Nakano *et al.*, 1998). The results of Reddy *et al.* (2011) which alleged WSSV survived cooking at 100°C for 30 min were not able to be replicated by Aranguren Caro *et al.*, (2020), who found cooking shrimp carcasses for 1 min at 97°C resulted in 100% inactivation of WSSV. Aranguren Caro *et al.*, (2020) found that uncooked shrimp stored on ice with an initial carcass temperature of 8.9°C reached 60°C in 37 seconds and 70°C in 48 seconds when placed in fresh water boiling at 97°C, using a shrimp:water ratio of 1:20 w/v (100 g of shrimp into 2 L of water).	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION
White spot syndrome virus will be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 9.8.3.

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2.9. Aquatic animal product assessments for infection with yellow head virus genotype 1

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent) (refer to Table 28 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 28 has been incorporated into Table 27 to support Members in determining time/temperature equivalence:

Table 27. Time required for >99.9% inactivation of yellow head virus genotype 1 (YHV-1)

Yellow head virus genotype 1 (YHV-1)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60	15			
	70				
	80				
	90				
	100				
	110				
	121		3.6		

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for YHV-1.

Table 28. Heat treated crustacean products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	YHV targets tissues of ectodermal and mesodermal origin including lymphoid organs, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia (Chantanachookin *et al.*, 1993; Lightner, 1996, Ma *et al*., 2009). All or some of these tissues can make up the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	YHV has been reported to be inactivated by heat at 60°C for 15 min (Flegel *et al.,* 1995). Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of YHV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Yellow head virus will be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 9.9.3.**		

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2.10. Aquatic animal product assessments for infection with decapod iridescent virus 1

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 80°C for at least 30 minutes, or a time/temperature equivalent) (refer to Table 30 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 30 has been incorporated into Table 29 to support Members in determining time/temperature equivalence:

Table 29. Time required for >99.9% inactivation of decapod iridescent virus 1 (DIV 1)

Decapod iridescent virus 1 (DIV 1)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60				
	70	60			
	80		30		
	90				
	100				
	110				
	121			3.6	

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for decapod iridescent virus 1 (which is the only member of the genus *Decapodiridovirus* within the Family *Iridoviridae*, see Li *et al*., 2017; Qiu *et al*., 2017), invertebrate iridescent virus 6 (IIV-6) was used as a surrogate pathogenic agent.

IIV-6 was chosen as it was determined to be the closest related pathogenic agent (Li *et al*., 2017; Qiu *et al*., 2017; Liao *et al*., 2022) for which there was available information for heat treatment inactivation.

Table 30. Heat treated crustacean products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	The principal target tissues for DIV-1 infection include hematopoietic tissue, haemocytes, antennal gland, pleopods, uropods, gills, lymphoid organ, hepatopancreas and muscle (Qiu *et al*., 2017, 2019; Sanguanrut *et al*., 2020; Liao *et al*., 2022), with hematopoietic tissue containing the highest viral load (Qiu *et al*., 2019). This commodity contains muscle tissue.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Like other iridoviruses, DIV-1 is likely to be thermolabile and sensitive to thermal inactivation (Marina *et al*., 2000; Guo *et al*., 2022). Although there is no specific information for inactivation of DIV-1, closely related viruses such as Invertebrate iridescent virus 6 (IIV-6) are likely to be useful surrogates. Martinez *et al*., (2003) found that IIV-6 was 100% inactivated after exposure to 70°C for 60 min or 80°C for 30 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of DIV-1.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of invertebrate iridescent virus 3 and 6 (IIV-3, IIV-6) as surrogates, decapod iridescent virus 1 is highly likely to be inactivated by heat treatment. Therefore, heat treated crustacean products (i.e. heat treatment to attain a core temperature of at least 80°C for at least 30 minutes or a time/temperature equivalent) are eligible for inclusion in Article 9.10.3.**		

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3. Assessments for WOAH listed diseases of fish using criteria in Article 5.4.1. (conducted in December 2022)

3.1. Aquatic animal product assessments for infection with epizootic haematopoietic necrosis virus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent) (refer to Table 32 for the assessment).

   ii. Mechanically dried eviscerated fish (i.e. a heat treatment to attain a core temperature of at least 60°C for at least 15 minutes or a time/temperature equivalent) (refer to Table 33 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 32 and 33 has been incorporated into Table 31 to support Members in determining time/temperature equivalence:

Table 31. Time required for >99.9% inactivation of epizootic haematopoietic necrosis virus (EHNV)

Epizootic haematopoietic necrosis virus (EHNV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40	1440			
	50				
	60		15		
	70				
	80				
	90				
	100				
	110				
	121			3.6	

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for EHNV.

Table 32. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	EHNV is present in most internal organs as well as skin and muscle (Langdon *et al*., 1988; Ariel *et al.*, 2009).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Langdon (1989) incubated EHNV infected tissue culture medium at various temperatures and found the virus was inactivated after 15 min at 60°C or 24 h at 40°C. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of EHNV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Epizootic haematopoietic necrosis virus will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.1.3.**		

Table 33. Mechanically dried eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	EHNV is present in most internal organs as well as skin and muscle (Langdon *et al*., 1988; Ariel *et al.*, 2009).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Langdon (1989) incubated EHNV infected tissue culture medium at various temperatures and found the virus was inactivated after 15 min at 60°C or 24 h at 40°C. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of EHNV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Epizootic haematopoietic necrosis virus will be inactivated by mechanical drying. Therefore, mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 15 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.1.3.**		

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3.2. Aquatic animal product assessments for infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. a heat treatment to attain a core temperature of at least 100°C for at least 1 minute, or a time/temperature equivalent) (refer to Table 35 for the assessment).

   ii. Mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 100°C for at least 1 minute, or a time/temperature equivalent) (refer to Table 36 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 35 and 36 has been incorporated into Table 34 to support Members in determining time/temperature equivalence:

Table 34. Time required for >99.9% inactivation of *Aphanomyces invadans*

Aphanomyces invadans	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	37	720			
	40				
	50				
	60		5		
	70		5		
	80				
	90				
	100			1	
	110				
	121				

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for *Aphanomyces invadans*, *Aphanomyces astaci* was used as a surrogate pathogenic agent.

Aphanomyces astaci was chosen as it was determined to be the closest related pathogenic agent (Makkonen *et al*., 2016) for which there was available information for heat treatment inactivation.

Table 35. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Aphanomyces invadans* is present in muscle and other edible tissues (Ahmed *et al.,* 1999; Callinan *et al.*, 1989; Chinabut *et al.*, 1995; Chinabut & Roberts, 1999; Das & Mukherjee, 1998; Miyazaki & Egusa, 1972, 1973; Noga *et al.*, 1988).	No.
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Although there is no specific information about thermal inactivation of *A. invadans*, it is known that the closely related *Aphanomyces astaci* is inactivated by heat. Oidtmann *et al.,* (2002) found that *A. astaci* mycelium in crayfish is inactivated by heating to 37°C for at least 12 h, whilst CEFAS (2000) reported that *A. astaci* spores and mycelium are inactivated after 5 min exposure to 60°C or 70°C. Oidtmann *et al*., (2002) found that all stages of *A. astaci* in crayfish tissues are 100% inactivated by boiling at 100°C for 1 min. Based on these data, *A. invadans* is also unlikely to survive this process.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of *Aphanomyces astaci* as a surrogate, *Aphanomyces invadans* is highly likely to be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 10.2.3.**		

Table 36. Mechanically dried eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
c) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Aphanomyces invadans* is present in muscle and other edible tissues (Ahmed *et al.,* 1999; Callinan *et al.*, 1989; Chinabut *et al.*, 1995; Chinabut & Roberts, 1999; Das & Mukherjee, 1998; Miyazaki & Egusa, 1972, 1973; Noga *et al.*, 1988).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Although there is no specific information about thermal inactivation of *A. invadans*, it is known that the closely related *Aphanomyces astaci* is inactivated by heat. Oidtmann *et al.,* (2002) found that *A. astaci* mycelium in crayfish is inactivated by heating to 37°C for at least 12 h, whilst CEFAS (2000) reported that *A. astaci* spores and mycelium are inactivated after 5 min exposure to 60°C or 70°C. Oidtmann *et al*., (2002) found that all stages of *A. astaci* in crayfish tissues are 100% inactivated by boiling at 100°C for 1 min. Based on these data, *A. invadans* is also unlikely to survive this process.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of *Aphanomyces astaci* as a surrogate, *Aphanomyces invadans* is highly likely to be inactivated by mechanical drying. Therefore, mechanically dried eviscerated products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 10.2.3.**		

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3.3. Aquatic animal product assessments for infection with *Gyrodactylus salaris*

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 40°C for at least 1 minute or a time/ temperature equivalent) (refer to Table 38 for the assessment).

   ii. Mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 40°C for at least 1 minute, or a time/temperature equivalent) (refer to Table 39 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 38 and 39 has been incorporated into Table 37 to support Members in determining time/temperature equivalence:

Table 37. Time required for >99.9% inactivation of *Gyrodactylus salaris*

Gyrodactylus salaris	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	35	2			
	40		1		
	50				
	60				
	70				
	80				
	90				
	100				
	110				
	121				

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for *Gyrodactylus salaris*.

Table 38. Heat treated products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
1) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Skin may be present in the commodity. *Gyrodactylus salaris* is present on the skin, fins and gills of fish living in fresh water (Jensen & Johnsen, 1992). Infected fish transferred from fresh water to seawater of 7.5 ppt or higher become free of the parasite by 56 days after transfer (Soleng & Bakke, 1997).	No (fresh-water fish) Yes (marine fish in seawater >7.5 ppt for >56 days)
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Thermal tolerance of *G. salaris* was examined by Koski *et al*., (2016), who found that parasites exposed to heated water in petri dishes survived < 2 min at 35°C and <1 min at 40°C.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION ***Gyrodactylus salaris* will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 40°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 10.3.3.**		

Table 39. Mechanically dried, eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Skin, fins and gills are part of the commodity. *Gyrodactylus salaris* is present on the skin, fins and gills of fish living in fresh water (Jensen & Johnsen, 1992). Infected fish transferred from fresh water to seawater of 7.5 ppt or higher become free of the parasite by 56 days after transfer (Soleng & Bakke, 1997).	No (fresh-water fish) Yes (marine fish in seawater >7.5 ppt for >56 days)
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Thermal tolerance of *G. salaris* was examined by Koski *et al*., (2016), who found that parasites exposed to heated water in petri dishes survived < 2 min at 35°C and <1 min at 40°C.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **_Gyrodactylus salaris_ will be inactivated by mechanical drying. Therefore, mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 40°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 10.3.3.**		

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3.4. Aquatic animal product assessments for infection with infectious salmon anaemia virus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 56°C for at least 5 minutes, or a time/temperature equivalent) (refer to Table 41 for the assessment).

   ii. Mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 56°C for at least 5 minutes, or a time/temperature equivalent) (refer to Table 42 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 41 and 42 has been incorporated into Table 40 to support Members in determining time/temperature equivalence:

Table 40. Time required for >99.9% inactivation of infectious salmon anaemia virus (ISAV)

Infectious salmon anaemia virus (ISAV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	56	5			
	60		1		
	70				
	80				
	90				
	100				
	110				
	121				

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for ISAV.

Table 41. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, skin, fins and bones may be present in the commodity. ISAV infects endocardial cells, endothelial cells, leucocytes and erythrocytes, and therefore the virus will be present in many tissues (Hovland *et al.,* 1994; Nylund *et al.,* 1996; Byrne *et al.,* 1998).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	ISAV is heat-sensitive under experimental conditions. Falk *et al.,* (1997) found that free ISAV in viral suspension was inactivated within 5 min at 56°C. ISAV was also inactivated within 1 min at 60°C (Torgersen, 1998).	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Infectious salmon anaemia virus will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 56°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.4.3.**		

Table 42. Mechanically dried, eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, bones, head, gills, skin, and fins may be part of the commodity. ISAV infects endocardial cells, endothelial cells, leucocytes and erythrocytes, and therefore the virus will be present in many tissues (Hovland *et al.,* 1994; Nylund *et al.,* 1996; Byrne *et al.,* 1998).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	ISAV is heat-sensitive under experimental conditions. Falk *et al.,* (1997) found that free ISAV in viral suspension was inactivated within 5 min at 56°C. ISAV was also inactivated within 1 min at 60°C (Torgersen, 1998).	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Infectious salmon anaemia virus will be inactivated by mechanical drying. Therefore, mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 56°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.4.3.**		

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3.5. Aquatic animal product assessments for infection with salmonid alphavirus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 44 for the assessment).

   ii. Mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 45 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 44 and 45 has been incorporated into Table 43 to support Members in determining time/temperature equivalence:

Table 43. Time required for >99.9% inactivation of salmonid alphavirus (SAV)

Salmonid alphavirus (SAV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50	30[+]			
	60		60*		
	70				
	80				
	90				
	100				
	110				
	121			3.6	

[+] Denotes data for purified virus.

* Denotes data for purified virus in the presence of organic matter, which is more representative of treatment of SAV within fish tissues.

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for SAV.

Table 44. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. ***Absence of pathogenic agent in the traded commodity:***		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, skin and fins may be present in the commodity. Infection with SAV may induce a viraemia during the acute phase. SAV has been detected by RT-PCR or virus isolation in the following tissues: blood, brain, gill, and heart (Graham *et al.*, 2006; Graham *et al.*, 2007a; Jansen, *et al.*, 2010) as well as in mucus and faeces (Graham *et al.*, 2012). Demonstration of presence or absence of viable virus in skin, fins, skeletal muscle and other tissues has not been systematically studied in fish undergoing slaughter/destruction although all ages of finfish can experience infection with SAV.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. ***Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:***		
a) Physical (e.g. temperature, drying, smoking)	Purified SAV is inactivated within 30 min at 50°C (Nelson *et al.*, 1995), while SAV in the presence of organic matter is inactivated within 1 h at 60°C (Graham *et al.*, 2007b). The latter result is considered more representative of treatment of SAV within fish tissues. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of SAV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Salmonid alphavirus will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.5.3.

Table 45. Mechanically dried, eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, bones, head, gills, skin, and fins may be part of the commodity. Infection with SAV may induce a viraemia during the acute phase. SAV has been detected by RT-PCR or virus isolation in the following tissues: blood, brain, gill, and heart (Graham *et al.*, 2006; Graham *et al.*, 2007a; Jansen, *et al.*, 2010) as well as in mucus and faeces (Graham *et al.*, 2012). Demonstration of presence or absence of viable virus in skin, fins, skeletal muscle and other tissues has not been systematically studied in fish undergoing slaughter/destruction although all ages of finfish can experience infection with SAV.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Purified SAV is inactivated within 30 min at 50°C (Nelson *et al.*, 1995), while SAV in the presence of organic matter is inactivated within 1 h at 60°C (Graham *et al.*, 2007b). The latter result is considered more representative of treatment of SAV within fish tissues. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of SAV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Salmonid alphavirus will be inactivated by mechanical drying. Therefore, mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.5.3.**		

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3.6. Aquatic animal product assessments for infection with infectious hematopoietic necrosis virus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent) (refer to Table 47 for the assessment).

   ii. Mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent) (refer to Table 48 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 47 and 48 has been incorporated into Table 46 to support Members in determining time/temperature equivalence:

Table 46. Time required for >99.9% inactivation of infectious hematopoietic necrosis virus (IHNV)

Infectious hematopoietic necrosis virus (IHNV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	38	140			
	40	20[+]			
	45		10[+]		
	50	30		2[+]	
	55				1[+]
	60		15		
	70				
	80				
	90			0.5	
	100				10*
	110				
	121				

[+] Denotes data from Whipple & Rohvec, (1994) using purified virus. These data have not been replicated by other researchers, and may not be representative of treatment of IHNV within fish tissues.

* Denotes a one off study by Amend *et al.,* (1969) which is likely to represent a thermal overdose.

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for IHNV.

Table 47. Heat treated fish product

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, skin and fins may be present in the commodity. IHNV is present in muscle, heart, brain, gill, skin, skin mucus, fins, pyloric caecae, intestines, kidney, spleen, liver and stomach (Brudeseth *et al.,* 2002; Engelking & Kaufman, 1994; La Patra *et al.*, 1989, 1995; Yamamoto & Clermont, 1990; Yamamoto *et al.,* 1990).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	IHNV is thermolabile as shown by Whipple & Rohvec, (1994), who used purified virus and found IHNV was inactivated after 20 min at 40°C, 10 min at 45°C and 1-2 min at 50-55°C. However, these data have not been replicated by other researchers, and hence they may not be representative of treatment of IHNV within fish tissues. For example, Gosting & Gould (1981) found IHNV was inactivated after 140 min at 38°C, while Anderson *et al*., (2008) found IHNV was inactivated after 30 min at 50°C. Spickler (2007) stated that IHNV was inactivated after 15 min at 60°C, and Traxler & Richard, (2004) found IHNV was inactivated within 30 seconds at 90°C. The original description of IHNV by Amend *et al.,* (1969) included data showing inactivation of the virus after 10 min at 100°C. However, in light of the more recent data now available, the one-off treatment used by Amend *et al.,* (1969) is likely to represent a large thermal overdose.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION		

Infectious hematopoietic necrosis virus will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent) are eligible for inclusion in Article 10.6.3.

Table 48. Mechanically dried, eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, bones, head, gills, skin, and fins may be part of the commodity. IHNV is present in muscle, heart, brain, gill, skin, skin mucus, fins, pyloric caecae, intestines, kidney, spleen, liver and stomach (Brudeseth *et al.,* 2002; Engelking & Kaufman, 1994; La Patra *et al.*, 1989, 1995; Yamamoto & Clermont, 1990; Yamamoto *et al.,* 1990).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	IHNV is thermolabile as shown by Whipple and Rohvec, (1994), who used purified virus and found IHNV was inactivated after 20 min at 40°C, 10 min at 45°C and 1-2 min at 50-55°C. However, these data have not been replicated by other researchers, and hence they may not be representative of treatment of IHNV within fish tissues. For example, Gosting and Gould (1981) found IHNV was inactivated after 140 min at 38°C, while Anderson *et al.*, (2008) found IHNV was inactivated after 30 min at 50°C. Spickler, (2007) stated that IHNV was inactivated after 15 min at 60°C, and Traxler & Richard, (2004) found IHNV was inactivated within 30 seconds at 90°C. The original description of IHNV by Amend *et al.,* (1969) included data showing inactivation of the virus after 10 min at 100°C. However, in light of the more recent data now available, the one-off treatment used	Yes

by Amend *et al.,* (1969) is likely to represent a large thermal overdose.

AND/OR

b) *Chemical (e.g. iodine, pH, salt, smoke)*

AND/OR

c) *Biological (e.g. fermentation)*

CONCLUSION

Infectious hematopoietic necrosis virus will be inactivated by mechanical drying. Therefore, mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent) are eligible for inclusion in Article 10.6.3.

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3.7. Aquatic animal product assessments for infection with koi herpesvirus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 50°C for at least 1 minute, or a time/temperature equivalent) (refer to Table 50 for the assessment).

   ii. Mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 50°C for at least 1 minute, or a time/temperature equivalent) (refer to Table 51 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 50 and 51 has been incorporated into Table 49 to support Members in determining time/temperature equivalence:

Table 49. Time required for >99.9% inactivation of koi herpesvirus (KHV)

Koi herpesvirus (KHV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50	1			
	60		0.5		
	70			0.5	
	80				
	90				
	100				
	110				
	121				

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for KHV.

Table 50. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, skin and fins may be present in the commodity. KHV has been detected in skin mucus, gill, liver, gut, kidney, spleen and brain (Gilad *et al.*, 2004).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Kasai *et al.,* (2005) used purified virus and found KHV was not inactivated after 5 min at 40°C, but was completely inactivated after exposure to 50°C for 1, 3 and 5 min, 60°C for 0.5 min and at 70°C for 0.5 min.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Koi herpesvirus will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 50°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 10.7.3.

Table 51. Mechanically dried eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, bones, head, gills, skin and fins may be part of the commodity. KHV has been detected in skin mucus, gill, liver, gut, kidney, spleen and brain (Gilad *et al.*, 2004).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Kasai *et al.,* (2005) used purified virus and found KHV was not inactivated after 5 min at 40°C, but was completely inactivated after exposure to 50°C for 1, 3 and 5 min, 60°C for 0.5 min and at 70°C for 0.5 min.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Koi herpesvirus will be inactivated by mechanical drying. Therefore, mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 50°C for at least 1 minute, or a time/temperature equivalent) are eligible for inclusion in Article 10.7.3.

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3.8. Aquatic animal product assessments for infection with red sea bream iridovirus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent) (refer to Table 53 for the assessment).

   ii. Mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent) (refer to Table 54 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 53 and 54 has been incorporated into Table 52 to support Members in determining time/temperature equivalence:

Table 52. Time required for >99.9% inactivation of red sea bream iridovirus (RSIV)

Red sea bream iridovirus (RSIV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	56	30			
	60				
	65		20		
	70				
	80				
	90				
	100				
	110				
	121			3.6	

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for RSIV. However, some data for heat inactivation of the closely related Infectious spleen and kidney necrosis virus (ISKNV) was also used (Fusianto *et al*., 2019). ISKNV was chosen to add additional information for determining time/temperature equivalency for heat treatment inactivation.

The close taxonomic relatedness between ISKNV, and other megalocytiviruses including turbot reddish body iridovirus (TRBIV) (see Go *et al*., 2016) suggests the heat inactivation data for RSIV and ISKNV can also be used for Megalocytivirus (excluding Scale drop disease virus), including TRBIV.

Table 53. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Red sea bream iridovirus (RSIV) occurs in multiple tissues in infected fish. RSIV DNA has been detected in muscle tissue (Choi *et al.*, 2006) and characteristic basophilic inclusion bodies reported in muscle (Jung *et al.*, 1997).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Nakajima & Sorimachi, (1994) studied purified virus and found RSIV was heat labile and inactivated after exposure to 56°C for 30 min. Fusianto *et al*., (2019) examined the closely related ISKNV and found virus in homogenised tissue was not inactivated after exposure to 40°C for 20 min, or 60°C for 5 min, but was completely inactivated after exposure to 65°C for 20 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of RSIV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Red sea bream iridovirus will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.8.3.

Table 54. Mechanically dried eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Red sea bream iridovirus (RSIV) occurs in multiple tissues in infected fish. RSIV DNA has been detected in muscle tissue (Choi *et al.*, 2006) and characteristic basophilic inclusion bodies reported in muscle (Jung *et al.*, 1997).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Nakajima & Sorimachi, (1994) studied purified virus and found RSIV was heat labile and inactivated after exposure to 56°C for 30 min. Fusianto *et al.*, (2019) examined the closely related ISKNV and found virus in homogenised tissue was not inactivated after exposure to 40°C for 20 min, or 60°C for 5 min, but was completely inactivated after exposure to 65°C for 20 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of RSIV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Red sea bream iridovirus will be inactivated by mechanical drying. Therefore, mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.8.3.

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3.9. Aquatic animal product assessment for infection with spring viraemia of carp virus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 56 for the assessment).

   ii. Mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 57 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 56 and 57 has been incorporated into Table 55 to support Members in determining time/temperature equivalence:

Table 55. Time required for >99.9% inactivation of spring viraemia of carp virus (SVCV)

Spring viraemia of carp virus (SVCV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	38	140			
	40				
	50				
	60		60		
	70				
	80				
	90			1	
	100				
	110				
	121				

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for SVCV. However, some data for heat inactivation of other closely related rhabdoviruses including viral haemorrhagic septicaemia virus (VHSV) and infectious hypodermal and haematopoietic necrosis virus (IHNV) was also used (Dixon *et al*., 2012). These were chosen to add additional information for determining time/ temperature equivalency for heat treatment inactivation.

Table 56. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	High titres of spring viraemia of carp virus (SVCV) occur in the liver and kidney of infected fish, but much lower titres occur in the spleen, gills and brain (Faisal & Ahne, 1984; Fijan *et al.,* 1971).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Dixon *et al*., (2012) demonstrated that SVCV is thermolabile and was inactivated after exposure to 60°C for 60 min. Other rhabdoviruses such as VHSV and IHNV have also been demonstrated to be heat sensitive under experimental conditions. For example, Gosting and Gould (1981) found IHNV was inactivated after 140 min at 38°C, while Traxler & Richard, (2004) found VHSV is inactivated within 1 min at 90°C, while IHNV was inactivated within 30 seconds at 90°C. Dixon *et al*., (2012) also found that VHSV was inactivated after exposure to 60°C for 60 min.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Spring viraemia of carp will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.9.3.

Table 57. Mechanically dried eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	High titres of spring viraemia of carp virus (SVCV) occur in the liver and kidney of infected fish, but much lower titres occur in the spleen, gills and brain (Faisal & Ahne, 1984; Fijan *et al.,* 1971).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Dixon *et al*., (2012) demonstrated that SVCV is thermolabile and was inactivated after exposure to 60°C for 60 min. Other rhabdoviruses such as VHSV and IHNV have also been demonstrated to be heat sensitive under experimental conditions. For example, Gosting & Gould (1981) found IHNV was inactivated after 140 min at 38°C, while Traxler & Richard, (2004) found VHSV is inactivated within 1 min at 90°C, while IHNV was inactivated within 30 seconds at 90°C. Dixon *et al*., (2012) also found that VHSV was inactivated after exposure to 60°C for 60 min.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Spring viraemia of carp virus will be inactivated by mechanical drying. Therefore, mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.9.3.**		

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3.10. Aquatic animal product assessments for infection with viral haemorrhagic septicaemia virus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 59 for the assessment).

   ii. Mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 60 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 59 and 60 has been incorporated into Table 58 to support Members in determining time/temperature equivalence:

Table 58. Time required for >99.9% inactivation of viral haemorrhagic septicaemia virus (VHSV)

Viral haemorrhagic septicaemia virus (VHSV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60	60			
	70				
	80				
	90		1		
	100				
	110				
	121				

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for VHSV.

Table 59. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, skin and fins may be present in the commodity. VHSV is present in multiple tissues of finfish including muscle, heart, spleen, kidney, blood, intestine, testes, ovaries, eye, skin and brain (Castric & de Kinkelin, 1980; Enzmann, 1981; Hedrick *et al.*, 2003; Iida *et al.*, 2003; Neukirch, 1986; Nishizawa *et al.*, 2006; Wizigmann & Hoffmann, 1982).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	VHSV is heat-sensitive under experimental conditions. Dixon *et al.*, (2012) found that VHSV was inactivated after exposure to 60°C for 60 min, while Traxler & Richard, (2004) found VHSV is inactivated within 1 min at 90°C.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Viral haemorrhagic septicaemia virus will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.10.3.

Table 60. Mechanically dried, eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, bones, head, gills, skin, and fins may be part of the commodity. VHSV is present in multiple tissues of finfish including muscle, heart, spleen, kidney, blood, intestine, testes, ovaries, eye, skin and brain (Castric & de Kinkelin, 1980; Enzmann, 1981; Wizigmann & Hoffmann, 1982; Neukirch, 1986; Hedrick *et al.*, 2003; Iida *et al.*, 2003; Nishizawa *et al.*, 2006).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	VHSV is heat-sensitive under experimental conditions. Dixon *et al*., (2012) found that VHSV was inactivated after exposure to 60°C for 60 min, while Traxler & Richard, (2004) found VHSV is inactivated within 1 min at 90°C.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Viral haemorrhagic septicaemia virus will be inactivated by mechanical drying. Therefore, mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.10.3.

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3.11. Aquatic animal product assessments for infection with tilapia lake virus

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 120 minutes or a time/temperature equivalent) (refer to Table 62 for the assessment).

   ii. Mechanically dried eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 120 minutes, or a time/temperature equivalent) (refer to Table 63 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 62 and 63 has been incorporated into Table 61 to support Members in determining time/temperature equivalence:

Table 61. Time required for >99.9% inactivation of tilapia lake virus (TiLV)

Tilapia lake virus (TiLV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60	120			
	70				
	80				
	90				
	100				
	110				
	121		3.6		

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for TiLV. Although TiLV has been described as an orthomyxo-like virus, most of its segmented negative-sense RNA genome has little homology to any other known viruses (Bacharach *et al*., 2016), leading to its placement in a new Family *Amnoonviridae* within the Order Articulavirales (see Turnbull *et al*., 2020). Due to an apparent lack of closely related viruses, it is not clear at this time whether inactivation data from orthomyxoviruses can be reliably used to provide additional information for determining time/temperature equivalency for heat treatment inactivation of TiLV.

Table 62. Heat treated fish products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, bones, head, gills, skin, and fins may be part of the commodity. TiLV is present in multiple tissues of finfish including liver, brain, spleen, kidney, blood, intestine, testes, ovaries, eye and skin (Eyngor *et al*., 2014; Chiamkunakorn *et al*., 2019; Surachetpong *et al*., 2020; Tang *et al*., 2021).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009). Furthermore, the water will be subject to the same temperature/ time treatment as the product and the final product is sealed.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Mai *et al*., (2021) demonstrated that TiLV is thermolabile when they found cultured free virus remained viable after 120 min at 25°C, but was inactivated after exposure to at least 60°C for 120 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of TiLV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		

CONCLUSION

Tilapia lake virus will be inactivated by heat treatment. Therefore, heat treated fish products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 120 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.11.3.

Table 63. Mechanically dried, eviscerated fish

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	Muscle, bones, head, gills, skin, and fins may be part of the commodity. TiLV is present in multiple tissues of finfish including liver, brain, spleen, kidney, blood, intestine, testes, ovaries, eye and skin (Eyngor *et al*., 2014; Chiamkunakorn *et al*., 2019; Surachetpong *et al*., 2020; Tang *et al*., 2021).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Potable fresh water is used to process the product (WHO/FAO, 2009), however the product undergoes a drying process and is sealed during transport.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Mai *et al*., (2021) demonstrated that TiLV is thermolabile when they found cultured free virus remained viable after 120 min at 25°C, but was inactivated after exposure to at least 60°C for 120 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of TiLV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Tilapia lake virus will be inactivated by mechanical drying. Therefore, mechanically dried, eviscerated fish (i.e. heat treatment to attain a core temperature of at least 60°C for at least 120 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 10.11.3.**		

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4. Assessments for WOAH listed diseases of molluscs using criteria in Article 5.4.1. (conducted in December 2022)

4.1. Aquatic animal product assessments for infection with abalone herpesvirus

1. Assessed and met the criteria in Article 5.4.1. for :

   i. Heat treated abalone products (i.e. heat treatment to attain a core temperature of at least 50°C for at least 5 minutes or any time/temperature equivalent) (Table 65).

   ii. Mechanically dried abalone products (i.e. heat treatment to attain a core temperature of at least 50°C for at least 5 minutes or any time/temperature equivalent) (Table 66).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 65 and 66 has been incorporated into Table 64 to support Members in determining time/temperature equivalence:

Table 64. Time required for >99.9% inactivation of abalone herpesvirus (AbHV)

Abalone herpesvirus (AbHV)	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50	5			
	60				
	70				
	80				
	90				
	100				
	110				
	121		3.6		

3. Because of a lack of relevant scientific information for the heat inactivation for AbHV, OsHV-1 microvariant was used as a surrogate pathogenic agent.

OsHV-1 microvariant was chosen as it was determined to be the closest related pathogenic agent (as another member of the Family *Malacoherpesviridae* in the Order Herpesvirales, see Savin *et al*., 2010, Adams *et al*., 2013), for which there was available information for heat treatment inactivation.

Table 65. Heat treated abalone products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	AbHV is found primarily in association with the ganglia and nerves (Chang *et al.*, 2005; Hooper *et al.*, 2007) and so could be present in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Clean seawater or potable water is used to process the product (WHO/FAO, 2009). The water used may therefore be contaminated, but any water used in the early stages of processing would then be subject to time/temperature treatments.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Warne (1988) recommends that abalone are blanched at 70°C for 5 min, followed by 35 min at 121.1°C or 93 min at 110°C. Aquatic herpesviruses are known to be heat labile (Wolf, 1988). Information is lacking regarding inactivation conditions for AbHV. However, there is some information for the closely related OsHV-1 microvariant. Hick et al. (2016) found that OsHV-1 microvariant remained viable after heating to 42°C for 5 min, but was inactivated by heating to 50°C for 5 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of AbHV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of OsHV-1 microvariant as a surrogate, AbHV will be inactivated by heat treatment. Therefore, heat treated abalone products (i.e. heat treatment to attain a core temperature of at least 50°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.1.3.**		

Table 66. Mechanically dried abalone products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	AbHV is found primarily in association with the ganglia and nerves (Chang *et al*., 2005; Hooper *et al*., 2007) and so could be present in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Water is used in the processing but the product undergoes a drying process.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Warne (1988) recommends that abalone are blanched at 70°C for 5 min, followed by 35 min at 121.1°C or 93 min at 110°C. Aquatic herpesviruses are known to be heat labile (Wolf, 1988). Information is lacking regarding inactivation conditions for AbHV. However, there is some information for the closely related OsHV-1 microvariant. Hick et al. (2016) found that OsHV-1 microvariant remained viable after heating to 42°C for 5 min, but was inactivated by heating to 50°C for 5 min. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of AbHV.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of OsHV-1 microvariant as a surrogate, AbHV will be inactivated by mechanical drying. Therefore, mechanically dried abalone products (i.e. heat treatment to attain a core temperature of at least 50°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.1.3.**		

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4.2. Aquatic animal product assessments for infection with *Bonamia exitiosa*

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated mollusc products (i.e. a heat treatment to attain a core temperature of at least 100°C for at least 15 minutes, or a time/temperature equivalent) (refer to Table 68 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 68 has been incorporated into Table 67 to support Members in determining time/temperature equivalence:

Table 67. Time required for >99.9% inactivation of *Bonamia exitiosa*

Bonamia exitiosa	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60				
	70				
	80				
	90				
	100	15			
	110				
	121		3.6		

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for *Bonamia exitiosa*, *Bonamia ostreae* was used as a surrogate pathogenic agent.

Bonamia ostreae was chosen as it was determined to be the closest related pathogenic agent in the phylum Haplosporidia (see Hine *et al*., 2001; Engelsma *et al*., 2014) for which there was available information for heat treatment inactivation.

Table 68. Heat treated mollusc products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Bonamia exitiosa* infects the haemocytes and connective tissue of host oysters (Hine *et al.*, 2001), and so is likely to be present in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Information is lacking regarding heat inactivation conditions for *Bonamia exitiosa*. Morga *et al*., (2009) reported that the closely related *Bonamia ostreae* was inactivated after exposure to 100°C for 15 min. In contrast, Gervais et al., (2015) used flow cytometry to record a mean parasite mortality rate of only 80.6% (± 19.02) after exposure of purified *B. ostreae* to 100°C for 15 minutes. Arzul *et al*., (2009) used dead purified *B. ostreae* exposed to boiling at 100°C for 15 min to calibrate the same flow cytometry method using propidium iodide dye for nucleic acid staining. They found that at least 98.1% of the boiled cells took up the dye and were presumed inactivated, although no bioassays were undertaken to test whether the remaining 1.9% of cells which did not take up the dye were infective. Taking these various sources of information into consideration, these data suggest that 100°C for 15 min is highly likely to be effective for inactivating *B. exitiosa*. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *B. exitiosa.*	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION		

Based on the use of *Bonamia ostreae* as a surrogate, *Bonamia exitiosa* will be inactivated by heat treatment. Therefore, heat treated mollusc products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 15 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.2.3.

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4.3. Aquatic animal product assessments for infection with *Bonamia ostreae*

1. Assessed and met the criteria in Article 5.4.1. for :

   i. Heat treated mollusc products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 15 minutes, or a time/temperature equivalent) (refer to Table 70 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 70 has been incorporated into Table 69 support Members in determining time/temperature equivalence:

Table 69. Time required for >99.9% inactivation of *Bonamia ostreae*

Bonamia ostreae	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60				
	70				
	80				
	90				
	100	15			
	110				
	121		3.6		

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for *Bonamia ostreae*.

Table 70. Heat treated mollusc products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Bonamia ostreae* infects the haemocytes and connective tissue of host oysters (Comps *et al*., 1980; Elston *et al*., 1986), and so is likely to be present in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Morga *et al*., (2009) reported that *Bonamia ostreae* was inactivated after exposure to 100°C for 15 min. In contrast, Gervais *et al*., (2015) used flow cytometry to record a mean parasite mortality rate of only 80.6% (± 19.02) after exposure of purified *B. ostreae* to 100°C for 15 minutes. Arzul *et al*., (2009) used dead purified *B. ostreae* exposed to boiling at 100°C for 15 min to calibrate the same flow cytometry method using propidium iodide dye for nucleic acid staining. They found that at least 98.1% of the boiled cells took up the dye and were presumed inactivated, although no bioassays were undertaken to test whether the remaining 1.9% of cells which did not take up the dye were infective. Taking these various sources of information into consideration, these data suggest that 100°C for 15 min is highly likely to be effective for inactivating *B. ostreae*. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *B. ostreae.*	Yes
AND/OR		

b) *Chemical (e.g. iodine, pH, salt, smoke)*

AND/OR

c) *Biological (e.g. fermentation)*

CONCLUSION

***Bonamia ostreae* will be inactivated by heat treatment. Therefore, heat treated mollusc products (i.e. heat treatment to attain a core temperature of at least 100°C for at least 15 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.3.3.**

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4.4. Aquatic animal product assessments for infection with *Marteilia refringens*

1. Assessed and met the criteria in Article 5.4.1. for:

    i. Heat treated mollusc products (i.e. a heat treatment to attain a core temperature of at least 121°C for at least 3.6 minutes, or a time/temperature equivalent) (refer to Table 72 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 72 has been incorporated into Table 71 to support Members in determining time/temperature equivalence:

Table 71. Time required for >99.9% inactivation of *Marteilia refringens*

Marteilia refringens	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60	1440			
	70				
	80				
	90				
	100				
	110				
	121		3.6		

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for *Marteilia refringens*, *Marteilia sydneyi* was used as a surrogate pathogenic agent.

Marteilia sydneyi was chosen as it was determined to be the closest related pathogenic agent in the phylum Paramyxida, (Perkins and Wolf 1976; Wolf 1979; Berthe *et al*., 2004) for which there was available information for heat treatment inactivation.

Table 72. Heat treated mollusc products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Marteilia refringens* is likely to be present in the commodity as it infects the digestive tract. Young plasmodia are found mainly in the epithelium of labial palps and the stomach (Grizel *et al.*, 1974). Sporulation takes place in the digestive gland tubules and ducts. Spores and presporogenic stages are released into the lumen of the digestive tract (Audemard *et al.*, 2002).	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	There is no specific information about heat inactivation of *M. refringens*. However, Wesche *et al.*, (1999) examined the viability of spores of the closely related *Marteilia sydneyi* and found they were 100% inactivated after exposure to 60°C for 24 h. As 24 h was the first time period after which spores were examined by Wesche *et al.*, (1999), this is highly likely to represent a thermal overdose. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *M. refringens.*	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of *Marteilia sydneyi* as a surrogate, *Marteilia refringens* will be inactivated by heat treatment. Therefore, heat treated mollusc products (i.e. heat treatment to attain a core temperature of at least 121°C for at least 3.6 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.4.3.**		

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4.5. Aquatic animal product assessments for infection with *Perkinsus marinus*

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated mollusc products (i.e. a heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 74 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Table 74 has been incorporated into Table 73 to support Members in determining time/temperature equivalence:

Table 73. Time required for >99.9% inactivation of *Perkinsus marinus*

Perkinsus marinus	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50	>1080			
	60		60		
	70				
	80				
	90				
	100				
	110				
	121			3.6	

Use of surrogate: The use of a surrogate pathogenic agent was not necessary for *Perkinsus marinus*.

Table 74. Heat treated mollusc products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Perkinsus marinus* can be found in gut epithelium, connective tissue of all organs and haemocytes (Mackin, 1951), and therefore is likely to be present in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	*Perkinsus marinus* can be inactivated by heat. Soudant *et al.*, (2005) used flow cytometry and staining with SYBR green and propidium iodide to determine that exposure to 60°C killed 96.6% of *P. marinus* meront cells within 30 min. Bushek *et al.*, (1997) reported that *P. marinus* in oyster tissues survived at least 18 h at 50°C, but were killed within 1 hr at 60°C. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *P. marinus.*	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION ***Perkinsus marinus* will be inactivated by heat treatment. Therefore, heat treated mollusc products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.5.3.**		

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4.6. Aquatic animal product assessments for infection with *Perkinsus olseni*

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated mollusc products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 76 for the assessment).

   ii. Mechanically dried abalone products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) (refer to Table 77 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 76 and 77 has been incorporated into Table 78 to support Members in determining time/temperature equivalence:

Table 75. Time required for >99.9% inactivation of *Perkinsus olseni*

Perkinsus olseni	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	60	60			
	70				
	80				
	90				
	100				
	110				
	121		3.6		

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for *Perkinsus olseni*, *Perkinsus marinus* was used as a surrogate pathogenic agent.

Perkinsus marinus was chosen as it was determined to be the closest related pathogenic agent in the Family *Perkinsidae* (see Goggin & Lester 1995; Villlalba *et al*., 2004) for which there was available information for heat treatment inactivation.

Table 76. Heat treated mollusc products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Perkinsus olseni* can be found in epithelia, connective tissue, muscle tissue and blood spaces (Lester & Davis, 1981; Googin & Lester 1995; Villlalba *et al.*, 2004), and therefore is likely to be present in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded		NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	There is limited information about heat inactivation of *Perkinsus olseni*. Goggin *et al*., (1990) found that 2% of *P. olseni* could survive in brine (120 ppt NaCl) heated to 50°C for up to 5 min, but none survived 10 min at the same temperature and salinity. Information for the closely related *Perkinsus marinus* suggests that exposure to 60°C for 30 min kills around 96% of parasites (Soudant *et al.,* 2005), while Bushek *et al*., (1997) reported that 100% of *P. marinus* are killed within 1 hr at 60°C. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *P. olseni*.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of *Perkinsus marinus* as a surrogate, *Perkinsus olseni* will be inactivated by heat treatment. Therefore, heat treated mollusc products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.6.3.**		

Table 77. Mechanically dried abalone products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Perkinsus olseni* can be found in epithelia, connective tissue, muscle tissue and blood spaces (Lester & Davis, 1981; Googin & Lester 1995; Villlalba *et al.*, 2004), and therefore is likely to be present in the commodity.	No
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Water is used in the processing but the product undergoes a drying process.	NA
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Warne (1988) indicates that abalone are blanched at 70°C for 5 min, followed by 35 min at 121.1°C or 93 min at 110°C. There is limited information about heat inactivation of *Perkinsus olseni*. Goggin *et al.*, (1990) found that 2% of *P. olseni* could survive in brine (120 ppt NaCl) heated to 50°C for up to 5 min, but none survived 10 min at the same temperature and salinity. Information for the closely related *Perkinsus marinus* suggests that exposure to 60°C for 30 min kills around 96% of parasites (Soudant *et al.,* 2005), while Bushek *et al.*, (1997) reported that 100% of *P. marinus* are killed within 1 hr at 60°C. Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *P. olseni*.	Yes
AND/OR		
b) Chemical (e.g. iodine, pH, salt, smoke)		
AND/OR		
c) Biological (e.g. fermentation)		
CONCLUSION **Based on the use of *Perkinsus marinus* as a surrogate, *Perkinsus olseni* will be inactivated by mechanical drying. Therefore, mechanically dried abalone products (i.e. heat treatment to attain a core temperature of at least 60°C for at least 60 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.6.3.**		

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4.7. Aquatic animal product assessments for infection with *Xenohaliotis californiensis*

1. Assessed and met the criteria in Article 5.4.1. for:

   i. Heat treated abalone products (i.e. a heat treatment to attain a core temperature of at least 95°C for at least 5 minutes, or a time/temperature equivalent) (refer to Table 79 for the assessment).

   ii. Mechanically dried abalone products (i.e. heat treatment to attain a core temperature of at least 95°C for at least 5 minutes or a time/temperature equivalent) (refer to Table 80 for the assessment).

2. Unless otherwise indicated, the specific time/temperature combination chosen for the pathogenic agent was selected to provide consistency of recommendations with previous WOAH documents, as well as consistency between disease agents of similar type.

3. Each time/temperature combination provided in Tables 79 and 80 has been incorporated into Table 78 to support Members in determining time/temperature equivalence:

Table 78. Time required for >99.9% inactivation of *Xenohaliotis californiensis*

Xenohaliotis californiensis	Temperature °C	Time (min)	Time (min)	Time (min)	Time (min)
	40				
	50				
	56	60			
	60				
	65		45		
	70				
	80				
	90				
	95			5	
	100				
	110				
	121				3.6

Use of surrogate: Because of a lack of relevant scientific information for the heat inactivation for *Xenohaliotis californiensis*, other marine rickettsial organisms which infect finfish (*Piscirickettsia salmonis*), and the closely related obligate intracellular bacterium *Anaplasma phagocytophilium* were used as surrogate pathogenic agents.

Piscirickettsia salmonis and *Anaplasma phagocytophilium* were chosen as they were determined to be the closest related pathogenic agents (Friedman *et al*., 2000; Cicala *et al*., 2017) for which there was available information for heat treatment inactivation.

Table 79. Heat treated abalone products

Article 5.4.1. Criteria	*Rationale*	*Assessment*
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Xenohaliotis californiensis* infects the epithelium of the oesophagus and intestine (Friedman *et al.*, 2000; Berthe 2003) and also occurs in faeces (Crosson *et al.*, 2020). Only foot muscle is edible, the viscera are waste tissues.	Yes
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Clean seawater or potable water is used to process the product (WHO/FAO, 2009). Transmission studies (Friedman *et al.*, 2002; Moore *et al.*, 2001) indicate that the organism can survive in seawater, and Crosson *et al.*, (2020) found that *X. californiensis* was inactivated after 48-72 h in seawater at 14-18°C. The water used may therefore be contaminated, but any water used in the early stages of processing would then be subject to time/temperature treatments.	No
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Warne (1988) indicates that abalone are blanched at 70°C for 5 min, followed by 35 min at 121.1°C or 93 min at 110°C. Information on inactivation conditions specifically for *X. californiensis* is limited. Similar rickettsial organisms in finfish are reported to be inactivated following exposure to 56°C for 1 h (Chen *et al.*, 2000). *Anaplasma phagocytophilium* (a closely related obligate intracellular bacterium, see Cicala *et al.*, 2017), is inactivated after exposure to 65°C for 45 min (Pedra *et al.*, 2008), or 95°C for 5-10 min (Borjesson *et al.*, 2005). Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely to result in complete inactivation of *X. californiensis*.	Yes

AND/OR

b) Chemical (e.g. iodine, pH, salt, smoke)

AND/OR

c) Biological (e.g. fermentation)

CONCLUSION

Based on the use of *Piscirickettsia salmonis* and *Anaplasma phagocytophilium* as surrogates, *Xenohaliotis californiensis* will be inactivated by heat treatment. Therefore, heat treated abalone products (i.e. heat treatment to attain a core temperature of at least 95°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.7.3.

Table 80. Mechanically dried abalone products

Article 5.4.1. Criteria	***Rationale***	***Assessment***
1. Absence of pathogenic agent in the traded commodity:		
a) There is strong evidence that the pathogenic agent is not present in the tissues from which the commodity is derived	*Xenohaliotis californiensis* infects the epithelium of the oesophagus and intestine (Friedman *et al.*, 2000; Berthe 2003) and also occurs in faeces (Crosson *et al*., 2020). Only foot muscle is edible, the viscera are waste tissues.	Yes
AND		
b) The water (including ice) used to process or transport the commodity is not contaminated with the pathogenic agent and the processing prevents cross contamination of the commodity to be traded	Water is used in the processing but the product undergoes a drying process.	No
OR		
2. Even if the pathogenic agent is present in, or contaminates, the tissues from which the commodity is derived, the treatment or processing to produce the commodity to be traded inactivates the pathogenic agent:		
a) Physical (e.g. temperature, drying, smoking)	Warne (1988) indicates that abalone are blanched at 70°C for 5 min, followed by 35 min at 121.1°C or 93 min at 110°C. Information on inactivation conditions specifically for *X. californiensis* is limited. Similar rickettsial organisms in finfish are reported to be inactivated following exposure to 56°C for 1 h (Chen *et al.*, 2000). *Anaplasma phagocytophilium* (a closely related obligate intracellular bacterium, see Cicala *et al*., 2017), is inactivated after exposure to 65°C for 45 min (Pedra *et al*., 2008), or 95°C for 5-10 min (Borjesson *et al*., 2005). Heat treatment at 121°C for 3.6 min (Ababouch, 1999, 2002) is also likely	Yes

	to result in complete inactivation of *X. californiensis*.
AND/OR	
b) *Chemical (e.g. iodine, pH, salt, smoke)*	
AND/OR	
c) *Biological (e.g. fermentation)*	
CONCLUSION **Based on the use of *Piscirickettsia salmonis* and *Anaplasma phagocytophilium* as surrogates, *Xenohaliotis californiensis* will be inactivated by mechanical drying. Therefore, mechanically dried abalone products (i.e. heat treatment to attain a core temperature of at least 95°C for at least 5 minutes, or a time/temperature equivalent) are eligible for inclusion in Article 11.7.3.**	

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