ANNEX 3

EU POSITION

ON THE PROPOSED CHANGES TO OIE MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS

Presented in March 2012 for adoption in May 2012

SUMMARY AND GENERAL COMMENTS

The EU thanks the OIE for the considerable work undertaken to revise the disease specific chapters of the OIE Aquatic Manual and thanks the OIE for taking on board the majority of comments provided by the EU.

As regards the chapter on General recommendations and the chapters concerning crustacean diseases, the EU supports the adoption of those chapters.

As regards the chapters concerning fish and mollusc diseases, except the chapter on ISA, the EU supports the adoption of those chapters, but for some of the chapters the EU has some comments that it would request the Aquatic Animals Commission to consider in the future revisions of the chapters.

The EU cannot support the adoption of the chapter on ISA, see the rationale for this below.

Diseases of fish

2.3.0. General information

EU position
The EU supports the adoption of this modified chapter.

2.3.1. Epizootic haematopoetic necrosis

EU position
The EU supports the adoption of this modified chapter, but has some comments that it would invite the OIE to consider for further revisions of the text.

Specific comments

LINES 416-417: The EU would not agree with the following statement: "ELISA is useful for both diagnosis and certification."

ELISA for detection of virus directly in tissue material is only useful for diagnosis of clinical cases as the sensitivity is too low compared to cell culture techniques. It is therefore less suitable for surveillance in clinical healthy fish for demonstrating freedom of disease.

LINE 784: In the view of the EU the designations in table 5.1 would benefit from a reassessment, in particular as regards ELISA, see above comment. In the opinion of the EU PCR should be regarded as a useful method for surveillance, and thus be given the designation "a" or "b" rather than "d".

LINE 787 onwards: Following a reassessment of table 5.1, chapter 6 should also be revised.

LINE 794: The EU would invite the OIE to reassess the statement "For practical purposes, EHNV can only be detected in fish that are clinically affected of that have died with the infection." In experimental trials it has been possible to re-isolate the virus from clinically healthy fish.

2.3.2. Epizootic ulcerative syndrome

EU position
The EU supports the adoption of this modified chapter, but has some comments that it would invite the OIE to consider for future revisions of the text.

Specific Comments

LINES 54-56: The EU would propose that the text is amended as follows:

"It appears from observation of natural outbreaks that some fish, such as common carp (*Cyprinus capio*), Nile tilapia (*Oreochromis niloticus*) and milk fish (*Chanos chanos*), ~~have been~~ do not exhibit clinical signs and thus may be considered ~~naturally~~ resistant to EUS (though they may be subclinically infected) (Lilley *et al.*, 1998)."

LINES 195-198: The EU would propose deleting this text. Firstly, because it is a repetition of what is described later in the chapter. Secondly, the EU would not agree with histopathology as a main confirmatory method.

LINE 417: In table 5.1, the EU would propose that histopathology is given the rating of 'b' or 'c' rather than 'a' for confirmatory diagnosis, to ensure consistency with Point 7.2, which does not include histology as a confirmatory diagnosis. Histology is likely to produce both false negative and false positive results.

2.3.3. Gyrodactylosis (*Gyrodactylus salaris*)

EU position
The EU supports the adoption of this modified chapter, but has one comment that it would invite the OIE to consider for future revisions of the text.

Specific comments

LINES 6-7: The EU would propose adding the words "and other salmonids" after "(*salmo salar*)", as Atlantic salmon is not the only salmonid to be susceptible to GS according to the definition of susceptible species of the OIE.

2.3.4. Infectious haematopoetic necrosis

EU position
The EU supports the adoption of this modified chapter.

2.3.5. Infectious salmon anaemia

EU position
The EU cannot support the adoption of the modified chapter as it stands.

In the view of the EU the adoption of this modified chapter should be postponed until the discussion on how to deal with the different strains of ISAV has been finalised. To ensure consistency between the OIE Manual and Code as regards ISA, the respective chapters should be adopted simultaneously.

From the March report from the Aquatic Animal Health Standards Commission, it appears that the revised Code chapter on ISA will not be presented for adoption in May 2012.

2.3.6. Koi herpesvirus disease

EU position
The EU supports the adoption of this modified chapter.

2.3.7. Red sea bream iridoviral disease

EU position
The EU supports the adoption of this modified chapter.

2.3.8. Spring viraemia of carp

EU position
The EU supports the adoption of this modified chapter.

Specific comment

LINES 140: The EU would encourage the OIE to include the specific references as has been done elsewhere in the chapter.

2.3.9. Viral haemorrhagic septicaemia

EU position
The EU supports the adoption of this modified chapter.

2.3.10. *Oncorhynchus masau* virus disease

EU position
The EU supports the adoption of this modified chapter.

2.3.11. Viral encephalopathy and retinopathy

EU position
The EU supports the adoption of this modified chapter.

Diseases of molluscs

2.4.0. General information

EU position

The EU supports the adoption of this modified chapter, but has some comments that it would invite the OIE to consider for future revisions of the text.

Specific comments

LINE 63: The word "plaps" should be replaced by the word "palps".

LINES 282 and 284: The word "gluteraldehyde" should be replaced by the word "glutaraldehyde".

LINE 376: Section 2.6.2. should be re-named "Nucleic acid extraction" as this would fit better with the content of the section.

LINES 456-457: The reference to Douglas et al (2010) should be deleted as it is a duplication with the reference in line 444-447 (Courbeil *et al* 2010).

2.4.1. Infection with abalone herpes-like virus

EU position

The EU supports the adoption of this modified chapter.

2.4.2. Infection with *Bonamia exitiosa*

EU position

The EU supports the adoption of this modified chapter, but has one comment that it would invite the OIE to consider for future revisions of the text.

Specific comments

LINES 129-130 The EU would propose that the last sentence is amended to read as follows:

"For polymerase chain reaction (PCR) assays, samples ~~must~~ should be preserved in 95–100% ethanol and not denatured alcohol, or in a suitable DNA storage solution or can be frozen."

2.4.3. Infection with *Bonamia ostreae*

EU position

The EU supports the adoption of this modified chapter, but has one comment that it would invite the OIE to consider for future revisions of the text.

Specific comments

LINES 131-132: The EU would propose that the last sentence is amended to read as follows:

"For polymerase chain reaction (PCR) assays, samples ~~must~~ should be preserved in 95–100% ethanol and not denatured alcohol, or in a suitable DNA storage solution or can be frozen."

2.4.4. Infection with *Marteilia refringens*

EU position

The EU supports the adoption of this modified chapter, but has one comment that it would invite the OIE to consider for future revisions of the text.

Specific comments

LINES 141-142: The EU would propose that the last sentence is amended to read as follows:
For polymerase chain reaction (PCR) assays, samples ~~must~~ should be preserved in 95–100% ethanol and not denatured alcohol, or in a suitable DNA storage solution or can be frozen."

2.4.5. Infection with *Perkinsus marinus*

The EU supports the adoption of this modified chapter.

2.4.6. Infection with *Perkinsus olseni*

The EU supports the adoption of this modified chapter.

2.4.7. Infection with *Xenohaliotis californiensis*

The EU supports the adoption of this modified chapter.

2.4.8. Infection with *Mikrocytos mackini*

The EU supports the adoption of this modified chapter.

2.4.9. Infection with ostreid herpesvirus-1

EU position

The EU can support the adoption of this chapter, but would strongly encourage the OIE to further develop this chapter.

The EU welcomes that the Aquatic Animals Commission in its March 2012 report recognises the different significance of the different variants of OsHV-1, in particular OsHV-1 µvar. However, in the opinion of the EU the OIE Manual chapter on OsHV-1 should be subject to further revision to include more specific information on the specificities of OsHV-1 µvar, in particular as regards diagnostic methods.

The EU also notes that the Commission highlights that the case definition is specifically designed so that Member Countries need only to report outbreaks with increased mortality. However, the proposed definition of a confirmed case in the OIE Aquatic Manual does not include such a criterion. In the view of the EU, an inclusion of such a criterion would be contrary to the general definition of disease in the OIE Aquatic Code. Furthermore, if only detection with mortality is reported, the epidemiological value of that information will be reduced considerably.

Specific comments

LINES 62/204: The EU suggests that it is indicated which species should preferentially be sampled.

LINES 62-65 (section 2.2.1) and 69-73 (section 2.2.3): The EU would request that it is specified which species are susceptible to the different variants of OsHV-1.

LINES 102-104 (section 2.2.7): Since it is only DNA that has been detected and not viable virus, a scenario might also be that these species are neither susceptible, resistant nor vectors to the disease. The EU would therefore propose that the paragraph is amended to read:

"OsHV-1 µvar DNA has been recently detected in France in blue mussel, *Mytilus edulis,* and in *Donax trunculus* (Renault, comm. pers.). However, in these cases, it remains unknown if these bivalve species play a role in the transmission of the virus, including whether they are susceptible, resistant or may act as vector species."

LINE 216: Please add the words "and digestive gland tissues" after the words "Gonad tissues".

LINE 321 onwards: The text of Section 4.3.1.2.3on molecular techniques has improved, but in the view of the EU the text is still incomplete. The EU would in particular highlight the following:

1. *Choice of PCR method:* The text should be drafted in a manner to provide advice on which PCR methods to use, not only contain a mere description of the methods used. When drafting such advice the

following should be taken into consideration:Single round conventional assays are appropriate to confirm suspected clinical cases of OsHV1 during an outbreak. However, their lack of sensitivity makes them less suited suitable for use in targeted surveillance (ie detection of virus in clinically normal oysters). The use of C9/C10 or DPFor /DPRev primers in a real time Sybr®Green is recommended as an assay with proven high sensitivity and suitable for use in targeted surveillance. The DPFor /DPRev primer pair might be useful because it targets the DNA polymerase catalytic subunit which is supposed to present less polymorphism than other part of the viral genome especially the C region. Other assays such as the real time TaqMan assay (Martenot *et al*. 2010) and two round amplification of a conventional PCR (nested assay) may also achieve a similar level of sensitivity and be suitable for use in targeted surveillance.

2. *Identification of OsHV1 µvar:* A description of the appropriate methods to use for the identification of OsHV-1µvar should be included. In the view of the EU, the use of conventional PCR using C2C6 or the CFCR primer sets and sequencing analysis is needed to identify the new variant.

LINES 556-557: The EU would propose the following wording for point ii) of Chapter 7.2.:

ii) In other cases, a confirmed case is positive results of PCR or real-time PCR targeting different virus genome areas confirmed by

(a) sequencing of several PCR products, or,

(b) ISH.