



LAS-Learning

Trainees Instructions

EU Module 5 - Recognition of pain, suffering and distress - Species specific: Zebrafish

Development of interactive e-learning modules on specific areas of the Education & Training framework facilitating implementation of DIR 2010/63/EU

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1. Module Description

1.1 Overview

This module - Recognition of pain, suffering and distress - Species-specific: Zebrafish - will equip you with the knowledge to identify critical indicators of distress and pain in zebrafish, enabling you to assess their welfare. Studies have shown that various laboratory procedures, such as netting, fin clipping, tagging, surgery, and heart damage, can cause distress and/or pain in zebrafish.

This module will cover five topics: Clinical evaluation, How to recognise pain and distress, Management of pain and distress: anaesthesia, analgesia and others, Humane endpoints and euthanasia, and Severity classification.

If you are visiting the site independently, you should consult the EC Training and Education framework [guidance document](#), which provides an overview of training requirements for individuals with different responsibilities under their relevant national legislation. Additional education and training may be necessary to meet national or institutional requirements.

This document offers suggestions for supporting your training. Each module aligns with the learning outcomes specified by the EU Education & Training framework for laboratory animal science. This module was developed by Lynne U. Sneddon and Ana Maria Valentim, two prominent researchers in animal welfare and pain perception in aquatic animals, mainly fish with extensive experience researching, publishing, and training.

Your collaboration and feedback are greatly appreciated, as the module is currently in the testing phase.

1.2 Topics Covered

- Clinical evaluation
- How to recognise pain and distress
- Management of pain and distress: anaesthesia and others
- Humane endpoints and euthanasia
- Severity classification

1.3 Learning Objectives

- Develop proficiency in assessing patients' health status through systematic clinical evaluations, including history-taking, physical examinations, and interpretation of diagnostic tests.
- Acquire the ability to identify signs of pain and distress in patients by understanding physiological and behavioral indicators, and apply appropriate assessment tools.



- Learn to implement effective strategies for managing pain and distress, including the use of anaesthesia, analgesia, and non-pharmacological interventions.
- Understand the principles of determining humane endpoints in clinical and research settings, and gain knowledge of ethical and effective euthanasia practices when necessary.
- Comprehend the classification systems for assessing the severity of pain, suffering, and distress, and apply these frameworks to guide clinical decisions and ensure ethical standards.

To support your ongoing learning, each module concludes with a curated list of recommended readings and cited references. Whenever possible, these references are linked to facilitate further exploration.



2. Prerequisites and Requirements

No specific prior knowledge is required. However, a basic understanding of searching bibliographic databases and a background in laboratory animal science and/or non-animal methods can be advantageous.

This module is designed to guide you step by step, eliminating the need for prior study.

2.1 Requirements

- Completion of lessons and understanding of learning objectives.
- Participation in knowledge checks and assessments to evaluate understanding.



3. Grading and Completion

Grading will be based on the successful completion of knowledge checks and assessments provided at the end of each module. Upon finishing all parts of the module, students will receive a certificate of completion.



4. Textbooks and Reading Materials

The **“References and Further Reading”** lesson provides most references and readings. They comprise scientific articles, sections of books, websites, and videos. Clicking on any link will open a new window to download or visualise the additional material. Several links to further resources can also be found in the module contents to better guide the reader.

The additional materials provide more information on specific topics, tools, and resources.



5. Course Program

The module is organised into seven chapters, with lessons and learning objectives as follows:

Chapter	Lesson	Learning objectives
Introduction	1-4	Principles of clinical evaluation Humane Endpoints Principles of pain management in zebrafish Assess your knowledge
Clinical evaluation	5-10	Get to know the learning objectives Clinical and behavioural signs to monitor Methodologies: Score sheets Methodologies: Analysis of Behaviour Other factors affecting behaviour Assess your knowledge
How to recognise pain and distress	11-15	Introduction - recognition of pain and distress Normal zebrafish behaviour Nociception and pain in zebrafish Distress in zebrafish Assess your knowledge
Management of pain and distress: anaesthesia, analgesia and others	17-21	Introduction - Anaesthesia and analgesia Anaesthetics Analgesics Management of pain and distress, examples Other strategies to manage pain and distress Assess your knowledge
Humane endpoints and euthanasia	22-25	Introduction Humane Endpoints Euthanasia and humane killing Assess your knowledge
Severity classification	26-27	Severity classification of laboratory procedures Assess your knowledge
Summary and knowledge check	28-30	Module summary List of references and further reading Assess your knowledge

Table 1 - Learning objectives per parts and lessons.

5.1 Progress Tracking

Once you begin working through a module, your progress is tracked, and you can break off and resume your studies at any point. Once the module is completed, the trainee can access any section to refresh their understanding of a topic.



5.2 Module Structure

The module is divided into several parts, and although they were designed to be followed sequentially, they can also be taken iteratively. Please note that a certificate of completion is only issued to learners who complete all module parts.

5.3 In-Depth Explanation Lesson by Lesson

Lesson	Title	LO	Explanation
1	Principles of pain management in zebrafish		Pain management in zebrafish represented in 1 photo, 1 quote and 1 list. “Pain - Modulation and Consequences” represented with 2 lists and 1 quiz. “Treatment of pain - the drug classes” represented with 1 list, 1 collapsible, 1 diagram and 1 table. “Analgesia - Pre-emptive and multimodal analgesic regimes” represented with 1 collapsible. “Non-pharmacological management of pain” represented with 1 interactive figure.
2	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
3	Introduction		Short introduction
4	Normal zebrafish behaviour	5.1	Description of what is normal for a zebrafish represented with 1 video.
5	Clinical and behavioural signs to monitor	5.3	Body condition with 3 photos in a slack of 3 flip-cards and 1 figure. External morphology represented with 1 photo and 6 flip-cards. Eyes represented in 4 flip-cards. Gills represented in 2 flip-cards. Fins represented in 2 flip-cards. Behaviour represented in 1 interactive labelled figure.
6	Methodologies : Score sheets	5.3	Score sheets represented with a 3-step process, 1-list and 1 1-timeline.
7	Methodologies : Analysis of Behaviour	5.3	Ethogram description. Decide on the sampling approach description with 1 list and 1 figure. Choose a recording technique represented in 1 list.
8	Other factors affecting behaviour	5.3	Factors that can affect behaviour are represented with 1 list.



9	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
10	Introduction - recognition of pain and distress	5.1 5.2	Recognising and alleviating pain with 1 photo and 1 gif.
11	Nociception and pain in zebrafish	5.2	Recognising pain in zebrafish description. Criteria described for animals that feel pain, represented with a 1 list. Pain Assessment description. General Indicators represented in 1 table. Physiological Indicators description with 1 figure. Behavioural Indicators description with 1 interactive labelled figure and 1 figure.
12	Distress in zebrafish	5.2	Good working practice to avoid distress represented with 1 list and 12 flip-cards.
13	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
14	Introduction - Anaesthesia and analgesia	5.2 5.6	Welfare in zebrafish description.
15	Anaesthetics	5.2 5.6	Anaesthesia description with 1 video and 1 figure in a 3 window tab, and a table. types of anaesthetics used in fish, represented with a slack of 5 flip-cards and 1 table.
16	Analgesics	5.2 5.6	Managing pain description with 1 photo, 1 slack of 3 flip-cards and 2 tables. Drug administration represented in 1 interactive labelled figure.
17	Management of pain and distress, examples	5.2 5.6	Examples of management of pain and distress represented in a 6-step process and 5 figures.
18	Other strategies to manage pain and distress	5.2 5.6	Environmental enrichment represented with 1 photo and 1 slack of 3 flip-cards with 2 photos.
19	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
20	Introduction	5.4	Introduction text on humane endpoints and euthanasia.
21	Humane Endpoints	5.4	Ending experiments to reduce or prevent suffering descriptions with 1 photo and a 1 tab with 2 windows. When a humane endpoint is reached, represented in 1 list.
22	Euthanasia and humane killing	5.4	Euthanasia represented with 2 lists. “When is euthanasia needed?” represented with 1 list.



			<p>Considerations of euthanasia methods represented with 1 list and 1 process with 3 steps and 1 figure.</p> <p>Rapid cooling method represented with 1 interactive labelled figure.</p> <p>Euthanasia of larval stages represented with 1 list and 1 image.</p> <p>Confirmation of death represented with 1 list and 1 interactive labelled figure.</p>
23	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
24	Severity classification of laboratory procedures	5.5	<p>“What a procedure is” represented with 2 quotes, 6 flip-cards and 1 table.</p> <p>Cumulative severity represented with a 1 list.</p>
25	Knowledge-check		Assesses progress and knowledge acquired during the chapter.
26	Summary		Module summary
27	References and Further Reading		References for additional materials
28	Knowledge-check		Assesses progress and knowledge acquired during the module

Table 2 - Explanation lesson by lesson.



6. Target Audience

This module is intended for (bio)medical researchers, participants in laboratory animal science courses, university students, biology/medical teachers, animal welfare body members, regulators, and anyone interested in learning more about animal research ethics and the principles of Replacement, Reduction, and Refinement of animal use for scientific and educational purposes.