

# SPECIFIC PROTOCOL

## 1<sup>st</sup> Interlaboratory Study on Pesticides in Food of Animal Origin and Commodities with High Fat Content

### „Pesticides in Fish (ILS F-01)“

(last update: 22.04.2024)

#### ➤ Introduction

This protocol is complementary to the valid version of the General Protocol for EU Proficiency Tests for Pesticide Residues in Food and Feed, Ed. 11<sup>1</sup>. The current interlaboratory study covers pesticides mainly be determined by multi-residue methods and some specific SRM compounds that may occur in that matrix. This ILS is voluntary for all laboratories. The commodity chosen to prepare the test material is fish and it is considered as a representative commodity for “meat (muscle) and seafood, Group 7” (see Annex A of document SANTE 11312/2021v2)<sup>2</sup>.

#### ➤ PT item (test material)

This proficiency test concerns the analysis of pesticide residues in fish. The matrix contains only spiked pesticides.

Since 2020 the EURLs for pesticide residues do not provide blank PT items. Each laboratory is asked to use “pesticide free” representative fish for recovery experiments as well as for the preparation of matrix-matched or procedural calibration standards.

The organisers will check the PT items for sufficient homogeneity and for stability using conditions that reproduce sample shipment and storage for the duration of the proficiency test.

All these tests will be conducted by the EURL AO which is accredited according to ISO 17043 for organising proficiency tests.

The participants will receive ~ 100 g of fish PT item (one can) containing spiked pesticides.

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<sup>1</sup> <http://www.eurl-pesticides.eu/docs/public/tmpl Article.asp?CntID=821&LabID=100&Lang=EN>

<sup>2</sup> [https://food.ec.europa.eu/system/files/2023-11/pesticides\\_mrl\\_guidelines\\_wrkdoc\\_2021-11312.pdf](https://food.ec.europa.eu/system/files/2023-11/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf)

➤ **Analytical parameters**

The PT item contains several pesticides from the target pesticide lists given in Annex 1 and 2 of this document.

**It is mandatory to analyse all pesticides included in Annex 1. Pesticides included in Annex 2 can be analysed on voluntary basis.**

Laboratories should carefully read the target pesticide lists, where important information about reporting of results, as well as the Minimum Required Reporting Levels (MRRLs), are given. The target pesticide lists contain only individual compounds and results should only be reported for individual compounds, no matter how the residue definitions are set.

During EUPT AO 12 - 16 a quite number of laboratories had problems to report spinosyn A and D individually. Therefore, a possibility for reporting the result of spinosad (sum of spinosyn A and D) was added. Please, report results for spinosyn A and D only, if you use the individual standards for calibration.

The MRRL values will be used to identify false positive and false negative results and for the calculation of z-scores for false negatives.

Please, report important observations during analysis in the EUPT Webtool in the special field for comments (e.g. broken bottle or losses, additional pesticides not listed in the target lists). Please consider therefore the EUPT Webtool Guideline.

➤ **Shipment of PT item**

Dispatch of the PT item is planned on **06 May 2024**.

PT items will be shipped in cans in a normal box with no further cooling. The organisers will aim to ensure that all participating laboratories will receive their PT items as soon as possible. PT items will be shipped with TNT/FedEx. Prior to shipment an e-mail will be sent to the participating laboratories from the shipper.

Laboratories must make their own arrangements for the receipt of the package. They should inform the organiser of any public holidays in their country/city during the week of the shipment, and must make all necessary arrangements to receive the shipment, even if the laboratory is closed.

➤ **Instructions on PT item handling**

Once received, the PT item shall be stored cooled (4°C) to avoid any deterioration/spoilage and to minimise possible pesticide losses.

**Bring the content of the PT item to room temperature before taking out any analytical test portion and homogenise the whole sample thoroughly before analysis, including any liquid that may be present due to the technical processing.**

It is recommended to divide the whole PT item into analytical test portions and weigh them into the tubes used for the extraction of the analytical test portions. All analytical test portions not used for the analysis should be stored chilled. This procedure helps to avoid possible losses caused by several thawing steps of the test material.

All participants should use their own routine standard operating procedures for extraction, clean-up and analytical measurement and their own reference standards for identification and quantification purposes. Considering the available amount of PT item, laboratories employing methods requiring large analytical portions are advised to scale them down.

The homogeneity tests were conducted using 10 g of the PT item and automated EN1528 method<sup>3</sup> (for GC-amenable pesticides) and 5 g of the PT item using QuEChERS-AO method<sup>4</sup> (for LC-amenable pesticides). As sub-sampling variability increases with decreasing analytical portion size, sufficient homogeneity can only be guaranteed where participants employ analytical portions that are equal or larger than those stipulated in the previous sentence.

#### ➤ EUPT Webtool and Deadlines

Sample receipt acknowledgement, scope selection, analytical results and method information are to be submitted via the [EUPT Webtool](#) (open in incognito or private window). Please consider the guideline on how to use the EUPT Webtool: [EUPT Webtool Guideline](#). To access the EUPT Webtool participants must use their unique login data (username and password), which was sent to you from the colleagues from DTU Denmark. The password can be retrieved via <https://quest.dtu.dk/Sites/GuestLogin/RetrievePassword.aspx> using your email address or your username.

#### 1. PT item receipt and acceptance

Once the laboratory has received the PT item, it must report to the organiser, via the EUPT Webtool (Result Submission Website ILS F-01), the date of receipt, the condition of the PT item, and its acceptance. The deadline for acceptance is **13 May 2024**. If the laboratory does not respond by this deadline the organiser will assume that the PT item has been received and accepted. If participants have not received the PT items by the **13 May 2024 at noon**, they must inform the organiser immediately by e-mail ([eurl-pesticides@cvuafr.bwl.de](mailto:eurl-pesticides@cvuafr.bwl.de)).

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<sup>3</sup> [EURL AO Validation report 2021-06 GC-Orbitrap GC-MS MS quantification fish offals](#)

<sup>4</sup> [EURL AO Validation report 2021-07 LC-MSMS quantification offal and fish](#)

## 2. Scope selection

The analytical scope must be selected prior to the shipment of the samples. This is done via the EUPT Webtool. The scope selection subpage will be opened from **22 April to 03 May 2024**. As default all mandatory pesticides are preselected. Results can only be reported for analytes that have been selected during the scope selection procedure.

**Important:** If you did not select your scope in time, all analytes of the mandatory pesticide list (see Annex 1) will be selected for your scope!

## 3. Results and method submission

After shipment of the samples, from 06 May 2024 onwards, it is possible to submit the results by logging into the EUPT Webtool. **The deadline for result submission is 05 July 2024 at 23:00 CEST.** Method information can be added the next 7 working days (until 16 July 2024).

**Important:** After the final submission it will NOT be possible to edit the results. The website will not be accessible after this date and any results reported after the deadline will not be included in the statistical treatment, or in the final report. Participants will receive an email confirming the submission of their results. Attached to the email will be an excel file with all their submitted data and a pdf of the pesticide and concentration submitted.

## 4. Reporting of qualitative and quantitative results

Results shall NOT be reported where a pesticide

- a) was not detected,
- b) was detected below the RL (Reporting Limit) of the laboratory or
- c) was detected below the MRRL.

Results reported as “< RL” will be considered as „not detected“.

Significant Figures:

Residue levels shall be expressed to three significant figures, e.g. 0.0581, 0.251 or 1.35 mg/kg.

## 5. Reporting information on analytical methodology

All laboratories are requested to provide information on the analytical method(s) they have used via EUPT Webtool (Result Submission Website ILS F-01). The laboratories are requested to fill-in this important information in order to minimise the administrative burden of collecting this information at a later date. Submission of method information is only possible until 7 working days after result submission deadline (until **16 July 2024**).

## 6. Reporting of supplementary information in case of false negative results

In case of false negative results, the affected laboratories will be asked via e-mail to provide details of the methodology used after the deadline for results submission. This can be done by accessing the

EUPT Webtool (Result Submission Website ILS F-01) until 7 working days after result submission deadline (until **16 July 2024**).

**Important:** If no sufficient information on the methodology used is provided, the organiser reserves the right not to accept the analytical results reported by the participant.

➤ **Follow-up actions**

According to article 94 2c of Regulation (EU) No 2017/625, underperformance of any NRL AO in comparative testing will be followed by EURL AO.

➤ **Documents**

In the EURL-document repository (CIRCA BC) all documents relating to ILS F-01 can be found. Links to most of the documents are also available on the [ILS F-01 website](#).

➤ **Subcontracting**

The following tasks were subcontracted:

1. Generation of login credentials for EUPT webtool (EURL-CF, Lyngby, Denmark)
2. Programming and administration of ILS F-01 result submission website (EURL-CF, Lyngby, Denmark)
3. Purchase of blank fish (Gertrud-Luckner-Gewerbeschule, Freiburg, Germany)
4. Provision of equipment for preparation of the PT items (Gertrud-Luckner-Gewerbeschule, Freiburg, Germany)

➤ **Time schedule**

Actor	Activity	Date
EURL	Preliminary announcement Interlaboratory Study Fish at Joint Workshop in Stuttgart	20 October 2023
EURL	First information supplied to laboratories and call for participation	Beginning of February 2024
Participant	<b>Registration</b> via EUPT website	<b>15 December 2023 – 01 April 2024</b>
Participant	<b>Scope selection</b> via EUPT webtool	<b>22 April – 03 May 2024</b>
Participant	Proof of shipment address in EURL-Datapool	Ending 26 April 2024
EURL	<b>Dispatch of test material</b>	<b>06 May 2024</b>
Participant	Confirmation of test material receipt	07 – 13 May 2024
Participant	<b>Deadline for reporting of test results</b>	<b>05 July 2024*</b>
Participant	Deadline for reporting of additional method information (no changes of reported results possible)	16 July 2024
EURL	Deadline for preliminary report	06 September 2024
EURL	Dispatch of the final report as pdf-file	Approx. end of 2024

\*) Please make sure to report your results on time as there will be no extension of the deadline.

➤ **Participation fee**

There is a **fee of EUR 200.00** for shipping and handling to participants within the European Union and EFTA countries (**including NRLs**). Fees for participants from **other countries** are **EUR 400.00**. An invoice will be sent as pdf-file via e-mail. If you participate in other studies, please refer to the announcement for the fees. In this case you will receive an individual invoice!

➤ **Delays in Payment**

The participants will receive an **invoice as pdf-file via e-mail** to the corresponding e-mail address given during registration. Laboratories wishing to additionally receive an invoice in paper form should write the request to [eur-pesticides@cvuafr.bwl.de](mailto:eur-pesticides@cvuafr.bwl.de) before **26 April 2024**. Please make sure that the payment is made before the stipulated deadline stated on the invoice (**28 June 2024**). If the invoice is not paid within the stipulated time, reminders will be sent within a four week period.

**From the second reminder onwards an administration fee of 25 € will be charged per reminder.** Based on Reg. (EC) 625/2017, OfLs not paying the EUPT sample delivery fee will be initially warned that their participation in subsequent EUPTs could be denied. In case of a repetitive non-payment, the EUPT organisers will inform the corresponding NRL or the competent authority to take action.

**EUPT-Coordinator:**

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**Organisation Team at the EURL AO Freiburg:**

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## Annex 1

 ILS F-01 Pesticide target list of **mandatory** analytes

**Table A1:** List of 53 **mandatory** analytes and minimum required reporting levels (MRRL) in ILS F-01 (PT item fish). Results shall be rounded to three significant figures (e.g. 0.0581, 0.251 or 1.35). Version 1.1, changes are marked in **red**

Analyte	MRRL (mg/kg)	Analyte	MRRL (mg/kg)
<b>Aldrin</b>	<b>0.005</b>	<b>Heptachlor</b>	<b>0.005</b>
Azinphos-ethyl	0.010	<b>Heptachlorepoxyd, Cis-</b>	<b>0.005</b>
Azinphos-methyl	0.010	<b>Heptachlorepoxyd, trans-</b>	<b>0.005</b>
Bifenthrin (sum of isomers)	0.010	<b>Hexachlorcyclohexane (HCH), alpha-isomer</b>	<b>0.005</b>
<b>Chlordane, cis-</b>	<b>0.005</b>	<b>Hexachlorcyclohexane (HCH), beta-isomer</b>	<b>0.005</b>
<b>Chlordane, trans-</b>	<b>0.005</b>	<b>Hexachlorcyclohexane (HCH), gamma-isomer (Lindane)</b>	<b>0.005</b>
Chlorfenvinphos	0.010	Hexachlorobenzene (HCB)	0.005
Chlorpyrifos(-ethyl)	0.010	Indoxacarb (sum of isomers)	0.010
Chlorpyrifos-methyl	0.010	Malathion (parent only)	0.010
Cyfluthrin (sum of isomers)	0.010	Methidathion	0.010
Cyhalothrin, Lambda- (sum of isomers)	0.010	Methoxychlor, 4,4'-	0.010
Cypermethrin (sum of isomers)	0.010	<b>Nitrofen</b>	<b>0.005</b>
<b>DDD, p,p'- (TDE)</b>	<b>0.005</b>	<b>Oxychlordane</b>	<b>0.005</b>
<b>DDE, p,p'-</b>	<b>0.005</b>	Parathion(-ethyl)	0.010
<b>DDT, o,p'-</b>	<b>0.005</b>	Parathion-methyl (parent only)	0.010
<b>DDT, p,p'-</b>	<b>0.005</b>	Pendimethalin	0.010
Deltamethrin (cis-isomer)	0.010	Permethrin (sum of isomers)	0.010
Diazinon	0.010	Phosmet (parent only)	0.010
<b>Dieldrin</b>	<b>0.005</b>	Phoxim	0.010
<b>Endosulfan sulfate</b>	<b>0.005</b>	Pirimiphos-methyl	0.010
<b>Endosulfan, alpha-</b>	<b>0.005</b>	Profenofos	0.010
<b>Endosulfan, beta-</b>	<b>0.005</b>	Pyrazophos	0.010
<b>Endrin</b>	<b>0.005</b>	<b>Quintozene (parent only)</b>	<b>0.005</b>
Famoxadone (MACP mandatory)	0.010	Resmethrin (sum of isomers)	0.010
Fenvalerate/Esfenvalerate (sum of RR, SS, RS & SR isomers)	0.010	<b>Tecnazene</b>	<b>0.005</b>
Fipronil	0.005	<b>Vinclozolin (parent only)</b>	<b>0.005</b>
Fipronil sulfone	0.005		



## Annex 2

### ILS F-01 Pesticide target list of **voluntary** analytes

**Table A2:** List of 47 **voluntary** analytes and minimum required reporting levels (MRRL) in ILS F-01 (PT item fish). Results shall be rounded to three significant figures (e.g. 0.0581, 0.251 or 1.35). Version 1.1, changes are marked in **red**

Analyte	MRRL (mg/kg)	Analyte	MRRL (mg/kg)
Benzalkonium Chloride (C8,C10,C12,C14,C16,C18)	0.01	Hexaflumoron	0.01
Benzovindiflupyr	0.01	Imidacloprid	0.01
Bixafen (parent only)	0.01	Lufenuron	0.01
Bixafen-desmethyl	0.01	Mefentrifluconazole	0.01
Boscalid (parent only)	0.01	Metaflumizone (sum of isomers)	0.01
Carbendazim (Carbendazim only)	0.01	Penflufen (sum of isomers)	0.01
Chlorate	0.01	Penthiopyrad	0.01
Chlorpropham (parent only)	0.01	Prochloraz (parent only)	0.01
Cyproconazole	0.01	BTS 44595 (Prochloraz metabolite)	0.01
Didecyldimethylammoniumchlorid ( <b>DDAC-C10</b> )	0.01	BTS 44596 (Prochloraz metabolite)	0.01
Diflubenzuron (parent only)	0.01	Prothioconazole-desthio	0.01
Emamectin	0.01	Spinosad <sup>(1)</sup>	0.01
Epoxiconazole	0.01	Spinosyn A <sup>(2)</sup>	0.01
Ethoxyquin	0.01	Spinosyn D <sup>(2)</sup>	0.01
Ethoxyquin dimer, metabolite of ethoxyquin	0.01	Spiroxamine (parent only, sum of isomers)	0.01
Ethoxyquin quinone imine (EQI)	0.01	Sulfoxaflor (sum of isomers)	0.01
Etofenprox	0.01	tau-Fluvalinate (sum of isomers)	0.01
Fenpropidin (parent only)	0.01	Tebuconazole	0.01
Fenpropimorph (parent only)	0.01	Tebuconazole, Hydroxy- (free phenol only)	0.01
Fenpyrazamine	0.01	Teflubenzuron	0.01
Fluopyram	0.01	Tetraconazole	0.01
Fluopyram-benzamide	0.01	Thiacloprid	0.01
Fluquinconazole	0.01	Thiophanate methyl	
Flusilazole (parent only)			

<sup>(1)</sup> Results for Spinosad should be reported either if individual standards for Spinosyn A and D or a mixture of Spinosyn A and D are used for quantification.

<sup>(2)</sup> Results for Spinosyn A or D should be reported, if the individual standards were used for quantification.