SPECIFIC PROTOCOL

20th European Proficiency Test on Pesticides in Food of Animal Origin and Commodities with High Fat Content

„Pesticides in liquid whole egg (EUPT-AO20)“

(Update Version 1.1 - 25 March 2025)

Changes: Additional information on fluralaner

➢ 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. 12[1]. The current proficiency test covers pesticides that can be determined by multi-residue methods. This EUPT is to be performed by all National Reference Laboratories for Pesticides in Food of Animal Origin and Commodities with High Fat Content (NRL-AO) as well as by all official EU laboratories (OfLs) responsible for official pesticide residue controls on food of animal origin, as far as their scope overlaps with that of the EUPT-AO20. The commodity chosen to prepare the test material is liquid whole egg and it is considered as a representative commodity for “Egg, Group 9” (see Annex A of document SANTE 11312/2021v2)[2].

➢ PT item (test material)

This proficiency test concerns the analysis of pesticide residues in liquid whole egg. The matrix contains only spiked pesticides, which will be used for evaluation. **Based on checks by EURL AO the blank material contains fluralaner. If the participating laboratories analyse for fluralaner, they can report the result on a voluntary basis. If enough results will be available, evaluation will be done and reported in a separate chapter of the final report.**

Since 2020, the EURLs for pesticide residues do not provide blank PT items. Each laboratory is asked to use “pesticide free” representative eggs 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.

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[1] https://www.eurl-pesticides.eu/userfiles/file/EurlALL/General_Protocol_V12_2025.pdf

[2] https://food.ec.europa.eu/system/files/2023-11/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf

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 ~ 90 g of liquid whole egg PT item (one bottle) containing spiked pesticides.

- **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.

In previous EUPTs a quite number of laboratories had problems to report results for spinosad and spinosyn A and D. Please notice the **new footnote for spinosad and spinosyn A and D**. 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. damaged can 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 **07 April 2025**.

PT items will be shipped in bottles in a thermal isolated box with 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 and DHL. 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 frozen (-18°C) to avoid any deterioration/spoilage and to minimise possible pesticide losses.

Bring the content of the PT item to room temperature 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 sample amount into analytical test portions and weigh them into the tubes used for extraction. All analytical test portions not used for 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 general safety requirements and operating instructions for handling hazardous substances and their disposal must be observed.

The homogeneity tests were conducted using 5 g of the PT item and automated EN1528 method (for GC-amenable pesticides) and 5 g of the PT item for QuEChERS-AO method (for LC-amenable pesticides). Further information on the methods are available on the EURL AO website. 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 EUPT-AO20), the date of receipt, the condition of the PT item, and its acceptance. The deadline for acceptance is **14 April 2025**. 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 **14 April 2025 at noon**, they must inform the organiser immediately by e-mail (eurl-pesticides@cvuafr.bwl.de).

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 **24 March to 04 April 2025**. 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 07 April 2025 onwards, it is possible to submit the results by logging into the EUPT Webtool. **The deadline for result submission is 12 May 2025 at 23:00 CEST.** Method information can be added within the next 7 days (until 19 May 2025). As fluralaner is not included in the target pesticides list, please use the field **"Lab test comment"** for result submission of this analyte.

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.

The expanded measurement uncertainty is queried purely on a voluntary basis for information purposes during result submission. The expanded measurement uncertainty is not intended to be published in reports, but is only used for internal plausibility checks.

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 EUPT-AO20). 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 **19 May 2025**).

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 EUPT-AO20) until 7 working days after result submission deadline (until **19 May 2025**).

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**

By sending the preliminary report to the participants, all participants are requested for a proof reading of the preliminary report. In particular the **NRLs and OFLs** with an individual **z-score ≥ 3.0** or **false positive/negative results** are requested to **give feedback to EURL-AO** on any actions undertaken to find out the reasons for poor performance. For reporting necessary corrections, please use the excel-sheet that will be attached to the email with the preliminary report.

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 EUPT AO20 can be found. Links to most of the documents are also available on the EUPT-AO20 website.

- **External supplier**

The following tasks were conducted by the assistance of external suppliers:

1. Generation of login credentials for EUPT webtool (EURL-CF, Lyngby, Denmark)
2. Programming and administration of EUPT-AO20 result submission website (EURL-CF, Lyngby, Denmark)

- **Time schedule**

Actor	Activity	Date
EURL	Preliminary announcement matrix liquid whole egg at EURL-NRL Workshop 2024 in Freiburg	08 - 09 October 2024
EURL	First information supplied to laboratories and call for participation (announcement)	08 January 2025
Participant	**Registration** via EUPT website	**17 December 2024 – 28 February 2025**
Participant	**Scope selection** via EUPT webtool	**24 March – 04 April 2025**
Participant	Proof of shipment address in EURL-Datapool	Ending 24 March 2025
EURL	**Dispatch of test material**	**07 April 2025**
Participant	Confirmation of test material receipt	08 - 14 April 2025
Participant	**Deadline for reporting of test results**	**12 May 2025***
Participant	Deadline for reporting of additional method information (no changes of reported results possible)	19 May 2025
EURL	Deadline for preliminary report	07 July 2025
Participant	Proof Reading of preliminary report	28 September 2025
EURL	Dispatch of the final report as pdf-file	Approx. end of 2025

*) 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.

- **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 eurl-pesticides@cvuafr.bwl.de before **01 April 2025**. Please make sure that the payment is made before the stipulated deadline stated on the invoice (**27 June 2025**). 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:

Björn Hardebusch

Head of EURL-AO (Food of Animal Origin and Commodities with High Fat Content)
CVUA Freiburg
Department of Residues and Contaminants
Bissierstrasse 5
D-79114 Freiburg

Phone: +49-761-8855-137
E-Mail: Bjoern.Hardebusch@cvuafr.bwl.de
eurl-pesticides@cvuafr.bwl.de

Organisation Team at the EURL AO Freiburg:

Björn Hardebusch	Head of EURL AO	CVUA Freiburg
Anja Muzyka	Food Chemist	CVUA Freiburg
Karin Tschiggfrei	Food Chemist	CVUA Freiburg
Sarah Obermeier	Food Chemist	CVUA Freiburg
Anna Büttner	Food Chemist	CVUA Freiburg

Quality Control Group:

Antonio Valverde	University of Almería, Almería, Spain
Paula Medina Pastor	EFSA, European Food Safety Authority, Parma, Italy

Advisory Group:

Amadeo R. Fernández-Alba	Head of EURL-FV, University of Almería, Spain
André de Kok	Wageningen Food Safety Research, Wageningen, The Netherlands
Carmen Ferrer Amate	EURL-FV, University of Almería, Spain
Finbarr O´Regan	Pesticide Control Laboratory, Department of Agriculture, Fisheries and Food, Kildare, Ireland
Hans Mol	Wageningen Food Safety Research, Wageningen, The Netherlands
Hermann Unterluggauer	Austrian Agency for Health and Food Safety (AGES), Innsbruck, Austria
Magnus Jezussek	Bavarian Health and Food Safety Authority, Erlangen, Germany
Marine Lambert	ANSES, Agence nationale de sécurité sanitaire de l'alimentation, de l'environement et du travail, Maisons-Alfort Cedex, France
Mette Erecius Poulsen	Head of EURL CF, DTU National Food Institute, Lyngby, Denmark
Michelangelo Anastassiades	Head of EURL SRM, CVUA Stuttgart, Fellbach, Germany
Patrizia Pelosi	Istituto Superiore di Sanità, Rome, Italy
Ralf Lippold	CVUA Freiburg, Freiburg, Germany
Stephan Radim	Czech Agriculture and Food Inspection Authority, Prague, Czech Republic
Tuija Pihlström	National Food Agency, Uppsala, Sweden

Annex 1

EUPT-AO20 Pesticide target list of mandatory analytes

Table A1: List of 79 **mandatory** analytes and minimum required reporting levels (MRRL) in EUPT-AO20. Results shall be rounded to three significant figures (e.g. 0.0581, 0.251 or 1.35).

Analyte	MRRL (mg/kg)	Analyte	MRRL (mg/kg)
Aldrin	0.010	Epoxiconazole	0.010
Alpha-Cypermethrin (aka alphamethrin)	0.010	Etofenprox	0.010
Azinphos-methyl	0.010	Famoxadone	0.010
Bifenthrin	0.010	Fenpropidin	0.010
Bixafen	0.010	Fenpropimorph	0.010
Boscalid	0.010	Fenpyrazamine	0.010
Carbendazim	0.010	Fenthion-Oxon	0.010
Chlordane, cis-	0.005	Fenthion-Oxonsulfone	0.010
Chlordane, trans-	0.005	Fenthion-Oxonsulfoxide	0.010
Chlorfevinphos	0.010	Fenthion-Sulfone	0.010
Chlorpropham	0.010	Fenthion-Sulfoxide	0.010
Chlorpyrifos	0.010	Fenvalerate/Esfenvalerate (RR/SS/RS/SR)	0.010
Chlorpyrifos-Methyl	0.010	Fipronil	0.005
cis-Heptachlor epoxide	0.010	Fipronil-Sulfone	0.005
Cyfluthrin (sum)	0.010	Fluquinconazole	0.010
Cypermethrin (sum)	0.010	Flusilazole	0.010
Cyproconazole	0.010	HCH, alpha-	0.010
DDD, p,p-	0.010	HCH, beta-	0.010
DDE, p,p-	0.010	HCH, gamma-	0.010
DDT, o,p-	0.010	Heptachlor	0.010
DDT, p,p-	0.010	Hexachlorobenzene	0.005
Deltamethrin	0.010	Indoxacarb	0.010
Diazinon	0.010	Lambda-Cyhalothrin	0.010
Dieldrin	0.010	Malathion	0.010
Endosulfan, alpha-	0.010	Metaflumizone	0.010
Endosulfan, beta-	0.010	Methoxychlor	0.010
Endosulfansulfate	0.010	Nitrofen	0.010
Endrin	0.005	Oxychlordane	0.005

Analyte	MRRL (mg/kg)	Analyte	MRRL (mg/kg)
Parathion	0.010	Pyrazophos	0.010
Parathion-methyl	0.010	Quintozene	0.010
Pendimethalin	0.010	Resmethrin, (cis; trans)	0.010
Penflufen	0.010	Sulfoxaflor	0.010
Penthiopyrad	0.010	Tau-Fluvalinate	0.010
Permethrin (sum)	0.010	Tebuconazole	0.010
Phosmet	0.010	Tecnazene	0.010
Phoxim	0.005	Tetraconazole	0.010
Pirimiphos-Methyl	0.005	Thiacloprid	0.010
Prochloraz	0.010	trans-Heptachlor epoxide	0.010
Profenofos	0.010	Vinclozolin	0.010
Prothioconazole-Desthio	0.010		

Annex 2

EUPT-AO20 Pesticide target list of voluntary analytes

Table A2: List of 26 **voluntary** analytes and minimum required reporting levels (MRRL) in EUPT-AO20. Results shall be rounded to three significant figures (e.g. 0.0581, 0.251 or 1.35).

Analyte	MRRL (mg/kg)	Analyte	MRRL (mg/kg)
Benzalkonium chloride n-C12	0.010	Molinate	0.010
Benzalkonium chloride n-C14	0.010	Oxadiargyl	0.010
Benzovindiflupyr	0.010	Oxasulfuron	0.010
Bixafen, Desmethyl-	0.010	Oxyfluorfen	0.010
Boscalid-5-hydroxy (M510F01)	0.010	Picolinafen	0.010
BTS 44595	0.010	Propaquizafop	0.010
BTS 44596	0.010	Pyraclostrobin	0.010
Didecyldimethylammonium chloride n-C10	0.010	Quinoclamine	0.010
Fluopyram	0.010	Spinosad	0.010
Fluopyram-Benzamide (M25)	0.010	Spinosyn A	0.010
Hydroxy-Tebuconazole	0.010	Spinosyn D	0.010
Mefentrifluconazole	0.010	Spiroxamine	0.010
Metconazole	0.010	Thiophanate-Methyl	0.010

(1) Results for Spinosad shall always be reported, either if individual standards for Spinosyn A and D or a mixture of Spinosyn A and D are used for quantification.

(2) Results for Spinosyn A or D shall be reported, if the individual standards were used for quantification.