SPECIFIC PROTOCOL

18th European Proficiency Test on Pesticides in Food of Animal Origin and Commodities with High Fat Content

„Pesticides in honey (EUPT AO18)“

(last update: 20.03.2023)

➢ 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. 10[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 AO18. The commodity chosen to prepare the test material is honey and it is considered as a representative commodity for “high sugar and low water content, Group 3” (see Annex A of document SANTE 11312/2021)[2].

➢ Test item (test material)

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

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

The organisers will check the test 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 ~ 50 g of honey test item (one bottle) containing spiked pesticides.

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1 http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=821&LabID=100&Lang=EN

2 https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_11312_2021.pdf

- **Analytical parameters**

The test 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. **However, in case of amitraz, laboratories are asked to additionally report the value for amitraz according to the residue definition and the equation used for calculation of amitraz in the comments field.**

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 test item**

Dispatch of the test item is planned on **17 April 2023**.

Test items will be shipped in plastic bottles in a normal box with no further cooling. The organisers will aim to ensure that all participating laboratories will receive their test items as soon as possible. Test 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 test item handling**

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

Bring the content of the test item to room temperature before taking out any analytical test portion. **It is recommended to divide the whole test 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 test item, laboratories employing methods requiring large analytical portions are advised to scale them down.

The homogeneity tests were conducted using 5 g of the test item and SweEt-method[3] with GPC (Gel Permeation Chromatography) clean up (for GC-amenable pesticides) and 5 g of the test item using QuEChERS-AO method (for LC-amenable pesticides) as published on the EURL webpage. 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://guest.dtu.dk/Sites/GuestLogin/RetrievePassword.aspx using your email address or your username.

1. Test item receipt and acceptance

Once the laboratory has received the test item, it must report to the organiser, via the EUPT Webtool (Result Submission Website EUPT AO18), the date of receipt, the condition of the test item, and its acceptance. The deadline for acceptance is **24 April 2023**. If the laboratory does not respond by this

[3] Determination of pesticide residues by ethyl acetate extraction using GC- and LC-MS/MS (SweEt); German version CEN/TS 17743:1011

deadline the organiser will assume that the test item has been received and accepted. If participants have not received the test items by the **24 April 2023 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 **27 March to 14 April 2023**. 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 17 April 2023 onwards, it is possible to submit the results by logging into the EUPT Webtool. **The deadline for result submission is 22 May 2023 at 23.00 CET.** Method information can be added the next 7 working days (until 31 May 2023).

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 EUPT AO18). 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 **31 May 2023**).

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 AO18) until 7 working days after result submission deadline (until **31 May 2023**).

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 EUPT AO18 can be found. Links to most of the documents are also available on the EUPT AO 18 website.

- **Subcontracting**

The following tasks were subcontracted:

1. Generation of login credentials for EUPT webtool (EURL-CF, Lyngby, Denmark)
2. Programming and administration of EUPT AO18 result submission website (EURL-CF, Lyngby, Denmark)
3. Purchase of blank honey (FoodQS GmbH, Langenzenn, Germany)
4. Provision of equipment for preparation of the test items (FoodQS GmbH, Langenzenn, Germany)

- **Time schedule**

Actor	Activity	Date
EURL	Preliminary announcement matrix honey at EURL-NRL workshop	27 October 2022
EURL	First information supplied to laboratories and call for participation	Mid of January 2023
Participant	**Registration** via EUPT website	**23 January – 10 March 2023**
Participant	**Scope selection** via EUPT webtool	**27 March – 14 April 2023**
Participant	Proof of shipment address in EURL-Datapool	Ending 06 April 2023
EURL	**Dispatch of test material**	**17 April 2023**
Participant	Confirmation of test material receipt	18 – 24 April 2023
Participant	**Deadline for reporting of test results**	**22 May 2023***
Participant	Deadline for reporting of additional method information (no changes of reported results possible)	31 May 2023
EURL	Deadline for preliminary report	22 July 2023
EURL	Dispatch of the final report as pdf-file	Approx. end of 2023

*) 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 **14 April 2023**. Please make sure that the payment is made before the stipulated deadline stated on the invoice (**02 June 2023**). 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.

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Annex 1

EUPT AO18 Pesticide target list of mandatory analytes

(last update: 17.03.2023; changes are marked in red)

Table A1: List of **mandatory** analytes and minimum required reporting levels (MRRL) in EUPT AO18 (test item honey). 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)
2,4-Dimethylphenylformamid (amitraz metabolite)(1)	0.010	Hexachlorcylcohexane (HCH), alpha-isomer	0.010
4,4´-Methoxychlor	0.010	Hexachlorcylcohexane (HCH), beta-isomer	0.010
Acetamiprid	0.010	Hexachlorcylcohexane (HCH), gamma-isomer (Lindane)	0.010
Aldrin	0.010	Hexachlorobenzene (HCB)	0.010
Azinphos-methyl	0.010	Hexythiazox	0.010
Azoxystrobin	0.010	Imazalil	0.010
Bifenthrin	0.010	Imidacloprid	0.010
Bixafen (parent only)	0.010	Indoxacarb (sum of isomers)	0.010
Boscalid (parent only)	0.010	Iprodione	0.010
Bromopropylate	0.010	Malaoxon	0.010
BTS 44595 (M201-04) (prochloraz metabolite)	0.010	Malathion	0.010
BTS 44596 (M201-03) (prochloraz metabolite)	0.010	Metaflumizone (sum of isomers)	0.010
Buprofezin	0.010	Methidathion	0.010
Carbendazim (carbendazim only)	0.010	Methiocarb	0.010
Chlordane, Cis-	0.010	Methiocarb-sulfone	0.010
Chlordane, Trans-	0.010	Methiocarb-sulfoxide	0.010
Chlorfenvinphos	0.010	Myclobutanil (parent only)	0.010
Chlorobenzilate	0.010	N-2,4-Dimethylphenyl-N-methylformamidin (amitraz metabolite) (1)	0.010
Chlorpropham (parent only)	0.010	o,p´-DDT	0.010
Chlorpyrifos	0.010	Oxychlordane	0.010
Chlorpyrifos-methyl	0.010	p,p´-DDD	0.010
Clothianidin	0.010	p,p´-DDE	0.010
Coumaphos	0.010	p,p´-DDT	0.010
Cyfluthrin (sum of isomers)	0.010	Paraoxon-methyl	0.010
Cyhalothrin, Lambda- (sum of isomers)	0.010	Parathion	0.010
Cypermethrin (sum of isomers)	0.010	Parathion-methyl (parent only)	0.010
Cyproconazole	0.010	Pendimethalin	0.010
Cyprodinil	0.010	Permethrin (sum of isomers)	0.010
Deltamethrin	0.010	Phosalone	0.010
Diazinon	0.010	Pirimicarb	0.010

Dieldrin	0.010	Pirimicarb, Desmethyl-	0.010
Diethyl-m-toluamid, N,N-, (DEET)	0.010	Pirimiphos-ethyl	0.010
Difenoconazole	0.010	Pirimiphos-methyl	0.010
Dimethoate	0.010	Prochloraz	0.010
Dimethomorph	0.010	Profenofos	0.010
Dimoxystrobin	0.010	Propargite	0.010
Endosulfan, Alpha-	0.010	Prothioconazole-desthio (sum of isomers)	0.010
Endosulfan, Beta-	0.010	Pyraclostrobin	0.010
Endosulfan-sulfate	0.010	Pyrazophos	0.010
Endrin	0.010	Pyrimethanil	0.010
Epoxiconazole	0.010	Resmethrin (sum of isomers)	0.010
Ethoprophos	0.010	Spinosad(2)	0.010
Etofenprox	0.010	Spinosyn A(3)	0.010
Famoxadone	0.010	Spinosyn D(3)	0.010
Fenhexamid	0.010	Spiroxamine	0.010
Fenitrothion	0.010	tau-Fluvalinate	0.010
Fenpropidin (parent only)	0.010	Tebuconazole	0.010
Fenpropimorph (sum of isomers) (parent only)	0.010	Tebufenozide	0.010
Fenvalerate/Esfenvalerate (sum of RR, SS, RS and SR isomers)	0.010	Terbuthylazine	0.010
Fipronil	0.005	Tetraconazole	0.010
Fipronil sulfone (MB46136)	0.005	Tetramethrin	0.010
Fluquinconazole	0.010	Thiacloprid	0.010
Flusilazole (parent only)	0.010	Thiamethoxam	0.010
Flutriafol	0.010	Thiophanate-methyl	0.010
Heptachlor	0.010	Triazophos	0.010
Heptachlorepoxid, Cis-	0.010	Trichlorfon	0.010
Heptachlorepoxid, Trans-	0.010	Trifloxystrobin (parent only)	0.010
Heptenophos	0.010	Vinclozolin	0.010

(1) The residue definition for amitraz is: amitraz including the metabolites containing the 2,4-dimethylaniline moiety expressed as amitraz. If an amitraz metabolite was detected, please report additionally the value for amitraz according to the residue definition and the equation used for calculation of amitraz in the comments field.

(2) 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.

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

Annex 2

EUPT AO18 Pesticide target list of voluntary analytes

Table A2: List of **voluntary** analytes and minimum required reporting levels (MRRL) in EUPT AO18 (test item honey). Results shall be rounded to three significant figures (e.g. 0.0581, 0.251 or 1.35)

Analyte	MRRL (mg/kg)
Ametoctradin (parent only)	0.01
Benzovindiflupyr	0.01
Fenpyrazamine (parent only)	0.01
Fenthion	0.01
Fenthionoxon	0.01
Fenthionoxonsulfone	0.01
Fenthionoxonsulfoxide	0.01
Fenthionsulfone	0.01
Fenthionsulfoxide	0.01
Flonicamid (parent only)	0.01
Orthophenylphenol (2-phenylphenol)	0.01
Picoxystrobin	0.01
Spirotetramat	0.01
Spirotetramat-enol	0.01