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Role of sample processing strategies at the European Union National Reference Laboratories (EU-NRLs) concerning the analysis of pesticide residue control

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ABSTRACT

The guidance document SANTE 11945/2015 recommends that cereal samples be milled to a particle size preferably smaller than 1 mm and that extensive heating of the samples should be avoided. The aim of the present study was therefore to investigate the differences in milling procedures, obtained particle size distributions and the resulting pesticide residue recovery when cereal samples were milled at the European Union National Reference Laboratories (EU-NRLs) with their routine milling procedures. A total of 23 NRLs participated in the study. The oat and rye samples milled by each NRL were sent to the European Union Reference Laboratory on Cereals and Feedingstuff (EURL-CF) for the determination of the particle size distribution and pesticide residue recovery. The results showed that the NRLs used several different brands and types of mills. Large variations in the particle size distributions and pesticides recoveries were observed even between samples milled by the same type of mill.

KEYWORDS: Pesticides, QuEChERS, extraction efficiency, cereals, milling, particle size, SANTE 11945/2015

INTRODUCTION

Several factors can influence the extractability of pesticides from cereals, such as the physicochemical properties of the pesticide, its accumulation in the grain's inner or outer parts, the type of pesticide application, and the cereal type, among other factors [1]. Incurred pesticide residues are not always easily extracted because they might be enclosed in cells, starch, or fat particles or undergo strong interactions with matrix. To facilitate the extractability of analytes, it is common to homogenize/grind or mill the sample either by a standard procedure or to produce the smallest particles possible with the available equipment. This strategy is based on the assumption that as the particle size decreases, the efficiency of the extraction increases because the surface area accessible to the solvent increases with decreasing particle size. Therefore, thorough sample processing to obtain small and homogeneous particle size is of great importance in pesticide residue analysis and all other fields of quantitative analysis. Nevertheless, this parameter is not evaluated when laboratories participate in proficiency tests regarding pesticide residue analysis or when they perform recovery studies on spiked samples. Proficiency test materials are processed by the provider of the test. Our previous study (unpublished data) showed that the particle size of cereal flour affects the extraction efficiency of incurred pesticides. A better extraction efficiency was achieved with smaller particle size. The guidance document SANTE 11945/2015 [2] recommends that laboratories mill to a particle size preferably less than 1 mm. However, the milling procedures used in pesticide laboratories vary, and the equipment for studying particle size distribution is often not available. We have therefore investigated the differences in milling procedures and determined the particle size distributions in cereal samples milled by the European Union National Reference Laboratories (EU-NRLs) using their routine milling procedures as well as the extractability of pesticide residues from these

samples. By this study, we expected to shed light on the variation in the sample processing strategies employed at the EU-NRLs performing pesticide residue control analysis as well as to obtain further data on the relationship between the particle size and extraction efficiency.

EXPERIMENTAL

Chemicals

Pesticide standards (all with a purity > 96%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile (HPLC Grade S) was purchased from Rathburn Chemicals (Walkerburn, UK) and acetic acid and ammonium acetate were purchased from Merck (Darmstadt, Germany). Magnesium sulfate was purchased from J.T. Baker, Aventor Performance Materials B.V (Center Valley, PA 18034, USA), sodium chloride was from Merck & Co. (Whitehouse Station, NJ, USA), sodium citrate dehydrate and sodium citrate sesquihydrate were from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany) and the clean-up sorbent PSA was from Agilent Technologies (Santa Clara, CA 95051, USA). The pesticide standard stock solutions of 1 mg/ml were prepared in toluene and stored at −18 ºC in ampoules under an argon atmosphere. The standard mixtures of 10 µg/ml in acetonitrile were prepared from these stock solutions. The working solutions were prepared by the dilution of the standard mixtures with acetonitrile and finally matching them 1:1 with an extract of blank flour (not containing pesticide residues) to obtain a concentration range of 0.0003–0.333 µg/ml. The extracts of cereal samples with no pesticide content obtained by the in-house standard extraction and clean-up procedure, described in Section 2.2, were used for matrix matching.

Sample and Analytical Methods

The oat and rye used in this study were grains grown and sprayed in the field produced in connection with the production of proficiency test material for the two EUPTs; EUPT-C3 (oat) and -C4 (rye). The levels of the incurred pesticides in the oat and rye test material, determined with our in-house method involving milling with a 1 mm sieve size, extraction by the in-house QuEChERS method (see section 0) and analysis by LC-MS/MS and GC-MS/MS, are presented in Table 1. One-hundred grams of both oat and rye grain were sent to 23 NRLs located in different EU regions. The samples were milled by these NRLs, using their routine sample processing procedure for pesticide analysis in cereals, and sent back to us for further studies.

Size Distribution Analysis

The sample size distribution for each of the samples milled by the 23 NRLs was determined after arrival at the EURL using a vibratory sieve shaker (Retsch AS 200, Haan, Germany). The sieving parameters (sample size, sieve time and amplitude) were optimized using different sample sizes (50 and 100 g), amplitudes (2-3 mm) and sieving times (10-20 min). The optimal sieving conditions, which produced the most efficient separation of the flour particles, were obtained when using a sample size of 50 g of flour, 20 min of sieving time and an amplitude of 2.5 mm. The flour samples were separated into fractions according to sieve mesh sizes of 50 µm to 4.8 mm (see number and sieve mesh seizes in Table 3).

To ease the comparison between particle size distributions, the relative particle size (RPS) of each milled sample was calculated using the following formula:

$$RPS = \sum_{i=1}^{n} (AS * SA)$$

RPS: relative particle size

AS: average sieve mesh size (calculated as average between sieve n and sieve n-1)

SA: sample amount in sieve n (g)

Pesticide Extraction and Clean-Up Procedure

The samples were extracted using our standard procedure for cereals (QuEChERS) [3]. In brief, 5.0 g of milled cereal was added to 10 ml of cold water and immediately extracted with 10 ml of acetonitrile by shaking the tube for 1 min by hand. To optimize the extraction, a ceramic homogenizer was used. Afterward, 4.0 g magnesium sulfate, 1.0 g sodium chloride, 1.0 g sodium citrate dihydrate and 0.5 g sodium citrate sesquihydrate were added. After 1 min of shaking by hand and centrifugation for 10 min at 4300 *g*, 8 ml of the supernatant was transferred to a clean tube and stored at −80 ºC for a minimum of 1 h. The extract was then thawed and when still very cold, it was centrifuged at 4300 *g* for 5 min. Thereafter, 6 ml of cold extract was mixed with 150 mg PSA and 900 mg magnesium sulfate. After shaking for 30 s and centrifugation for 5 min at 4300 *g*, 4 ml of the extract was withdrawn and mixed with 40 µl of 5% formic acid solution. To determine the effect of shaking on the extraction efficiency, a sample of rye test material milled with a centrifugal mill with a sieve size of 1 mm was extracted by shaking for 1 min and 5 min using a mechanical shaker

(2010 Geno/Grinder, SPEX SamplePrep, Stanmore, UK) and compared with manual shaking for 1 min. This final extract was analyzed by GC-MS/MS and LC-MS/MS.

Chromatographic Separation and Detection

GC-MS/MS analysis was performed on a Quattro Micro Tandem GC–MS/MS (Waters, USA). The system consisted of a PAL-GC autosampler, an Agilent GC 6890N and a Quattro Micro Tandem mass spectrometer. The GC was equipped with a Gerstel PTV injector for large volumes, and 4 µl was injected. The injector program started with an initial temperature of 30 ºC for 0.8 min followed by a ramp of 480 ºC/min to 290 ºC, which was held for 2 min. Finally, the temperature was raised 720 ºC/min to 330 ºC to clean the injector. The GC oven program started with an initial temperature of 60 ºC held for 3 min, followed by a ramp of 30 ºC/min to 180 ºC. This temperature was held for 0.8 min and following by a ramp of 5 ºC/min to 280 ºC, which was held for 3 min. To clean the column, the temperature was raised 40 ºC/min to 300 ºC for 10 min and 120 ºC/min to 310 ºC for 1 min. Chromatographic separation was performed on a RESTEK, Rxi®-5 ms, 30 m, 0.25 mm ID, 0.25 µm df column with a constant flow of 1.3 ml/min of helium as the carrier gas. The temperatures of the transfer line and ion source were set at 250 ºC and 180 ºC, respectively. The mass spectrometer was operated in the electronic ionization mode (EI, 70 eV).

LC-MS/MS analysis was performed on a HP1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) connected to a Micromass Quattro Ultima Triple Quadrupole Instrument. The injection volume was 10 µl. The chromatographic separation was performed on a Genesis C18 column, 100 mm × 3 mm, 4 m pore size, (Gracevydac, Hengoed, UK). In front of the separation column was a Phenomenex SecurityGuard column, C18 ODS, 4 mm × 2 mm (Cheshire, UK).

Solvent A was ammonium acetate/acetic acid solution at a concentration of 20 mM each. Solvent B was 95% methanol and 20 mM of both ammonium acetate and acetic acid. The total flow rate of eluents A and B was 0.3 ml/min. The initial gradient was 100% A, which was decreased to 50% A after 2 min and 0% A after 20 min. Solvent A was held at 0% until 24 min. The total run time was 30 min. Ionization was performed by electrospray ionization in positive ion mode (ESI+). The capillary voltage was set to 1.0 kV. The source temperature was 120 ºC and the desolvation temperature was 350 ºC. Nitrogen was used as the desolvation gas (flow 550 l/h) and cone gas (flow 50 l/h), and argon was used as the collision gas at a pressure of 1.7×10^{-3} mbar.

Both the GC-MS/MS and the LC-MS/MS were operated in MRM mode to perform mass spectrometric quantifications of the pesticides. The employed MRM transitions, retention times and collision energies are listed in Table 2. Quantification was based on bracketing calibration curves of five matrix matched standard solutions covering the relevant concentration range. Oat and rye blank material milled with a sieve size of 1 mm was used for the preparation of matrix matched calibration solutions.

RESULTS AND DISCUSSION

In a previous study, we studied the effect of particle size on the extraction efficiency of pesticides (unpublished data). It was confirmed that there is a relationship between decreasing particle size and increasing efficiency with which incurred pesticide residues are extracted from different cereals (barley, oat, rye and wheat). To obtain an overview of the situation in EU laboratories, a survey on the milling equipment, procedures employed and the particle size distributions obtained was performed. A total of 23 NRLs participated in the survey. Various types of mills including centrifugal

mills (6), knife mills (10), hammer mills (3) and universal horizontal mills (4) were used by the different NRLs.

Several NRLs participating in the study used common coffee grinders (knife mill) to mill the cereal grains and several reported that heat was produced during milling. The determination of the particle size distribution of the oat and rye samples milled by NRLs revealed large variations in the particle size distribution and thereby also the RPS (Tables 3 and 4). The RPS ranged from 24-129 for oat and 16-155 for rye (Tables 5 and 6). Generally, samples milled by hammer mills and knife mills were inhomogeneous, with more than 35% of the sample accounted for as particles larger than 0.7 mm (Figure 1 a and b), whereas samples milled by centrifugal mills (at 0.5 or 1.0 mm) were more homogenous and mainly consisted of particles smaller than 0.7 mm. The smallest relative particle size (RPS) values of 16 and 24 for rye and oat, respectively, were observed for two samples that were milled frozen by a centrifugal mill. The largest RPS values of 129 and 155 were produced by a knife mill and a hammer mill for oat and rye, respectively (Tables 5 and 6). However, large variations were observed in the RPS for samples milled using the same type of mill, especially for rye samples milled by universal horizontal mills. These variations may be due to differences in size, quality, and the brand of the mill as well as the milling procedure employed. As observed in Tables 5 and 6, knife mills may result in either quite efficient milling with an RPS of 30 or relatively insufficient milling with an RPS of 141. When comparing the particle size distribution for oat and rye obtained when ground with the same mill, a bigger fraction of larger particles was observed for rye than for oat. This may be due to differences in the texture, fat and moisture content of the oat and rye grains (Figure 1 a and b).

Determination of the extractability/recovery of the incurred pesticide from the samples milled by the NRLs showed that recovery was generally higher in samples with

smaller RPS (Table 5 and 6). Consequently, a higher recovery was generally observed for samples milled by centrifuge mills with a sieve size of 0.5 or 1.0 mm than for samples milled by knife mills and hammer mills. The recovery ranged from 71 to 108% for oat and from 75 to 119% for rye. The low extraction efficiency found for some samples, in spite of relatively low RPS, may be related to factors other than particle size, e.g., the degradation of the pesticides due to unfavorable handling, storage and/or transportation conditions. However, the NRLs were instructed to store grain and flour in a freezer, and stability tests of the corresponding EUPT test materials did not show degradation of the pesticides during 4 weeks of storage at room temperature.

The results for oat samples showed a clear relationship between decreasing particle size and increasing extraction efficiency of pesticides (Figure 2a). The recovery obtained for the samples with the smallest particles was up to 40% higher than the recovery for the largest particles. Significant differences (t-test, $p<0.05$) were observed between the seven oat samples with the smallest RPS and the seven samples with the largest RPS in terms of the recovery of carbendazim, chlorpyrifos, cyprodinil, fenpropimorph, fenvalerate, flusilazole, lambda-cyhalothrin and pyraclostrobin. For rye, the relationship was less clear. However, significant differences in recovery were also observed for rye samples between those with the smallest and largest RPS, i.e., carbendazim, chlorpyrifos-methyl, fenpropimorph, flutriafol, kresoxim-methyl and spiroxamine (Figure 3b). The results indicate that if the laboratories change their milling procedure to obtain an RPS of 20 instead of 120/150, it may increase the overall extraction efficiency by more than 35%. As mentioned, efficient milling resulting in a low RPS may be obtained with several types of mill; however, by using a centrifugal mill with a specific sieve size, it is possible to

have the process under control and obtain a reproducible result without relying on the visual judgment of the operator.

In accordance with our previous study (unpublished data), the present study shows that that smaller particle sizes increase extraction efficiency. In most routine laboratories, the homogeneity of a sample is probably based on a visual and personal evaluation unless a mill equipped with a specific sieve size is used. Thus, most pesticide residue laboratories do not know which particle size distribution they obtain by their standard homogenization procedure. Differences in the milling procedures may therefore contribute significantly to the variability in the results of the samples of grain analyzed by the pesticide laboratories as part of the common EU as well as their national pesticide control programs.

Several factors may influence the extraction efficiency of a pesticide, e.g., the heat sensitivity or polarity of the compound [4–8]. Most of the NRLs, especially those employing knife mills or hammer mills, noticed heat produced during milling. Only one of the NRLs milled frozen grains. We would expect that the degree of milling would have an influence on the extraction efficiency of pesticides if these pesticides are evenly distributed in the grain, whereas it would be of less importance if the residues primarily occurred on the surface or outer layers of the grain. A significant physical difference between oat and rye is that oat kernels are firmly covered by the hulls, while rye is not covered to the same extent [9]. Thus, the oat grain is protected from any direct application of pesticides. Further, when the grains mature, i.e., are allowed to dry in the field, the evaporation of water will occur in the oat on the surface of the hulls, whereas in rye, it will occur on the surface of the grain itself. Consequently, the rye grain is exposed to direct application, and when water evaporates from the surface of the rye grain, pesticide residue may precipitate. Thus, if the pesticide residue present in rye grain is primarily associated with the outer parts, i.e., the bran

of the grains, it could be a reason for the observed weaker correlation between a higher extraction efficiency and lower particle sizes for rye. As oat is protected by the hulls and the evaporation of water during maturation will occur on the hulls, the pesticide residue in oat grain may therefore be more evenly distributed, and the degree of milling will consequently be of more importance.

Thus, the present study showed good correlation between increased extraction efficiency and decreasing particle size. It may be assumed that this correlation is observed because the extraction itself is not sufficient and that small particles then compensate for insufficient extraction time. Hepperle et al.[1] found up to a 20% higher recovery of pesticides from pineapple by increasing the extraction time from 1 to 15 minutes. We consequently tested if using a mechanical shaker and/or extending the extraction time could overcome the differences in extraction efficiency due to particle size. No difference was observed when the mechanical shaker was used for 1 min or 5 min compared to 1 min with hand shaking using rye test material with incurred pesticides milled with a centrifugal mill with a sieve size of 1 mm. Thus, the manual extraction used for this study was shown to be sufficient. We also studied whether poor recovery as a result of poor milling (large particles) could be compensated for by increasing the extraction time. The recovery from rye test material milled using a sieve size of 5 mm was not affected by increasing the extraction time from 1 to 5 minutes using the mechanical shaker. Thus, the present study shows that, using mechanical extraction, even for a longer time, will not compensate for the poor milling of cereal. This phenomenon, of course, is only valid if the manual shaking is performed effectively. Furthermore, ceramic homogenizers are important to obtain optimal extraction.

NRLs use different types of mill/homogenization equipment for the sample processing of cereal grains, which result in very different particle size distributions.

Generally, the lowest RPS was obtained when the sample had been milled by a centrifugal mill at a specific mesh size (0.5 mm). By using this equipment, the procedure was also under control and reproducible. Common knife mills in most cases produced very inhomogeneous samples with high RPS. A clear relationship between an increasing extraction efficiency of incurred pesticides and decreasing RPS was found for all pesticides in oat and for most pesticides in rye. It is expected that the difference between oat and rye is due to more evenly distributed pesticides in the oat kernel because it is protected against direct exposure to pesticides by its hull. It was also shown that using mechanical extraction, even for a longer time, will not compensate for the poor milling of cereal.

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Figure captions

Figure 1. Average particle size of a) oat and b) rye samples milled by NRLs using different types of mills.

Figure 2. Average relative particle size (RPS) and average pesticide recovery (%) for the seven samples with the smallest, middle and largest RPS, a) oat and b) rye. Differences are significant (t-test, $P<0.05$) for pesticides marked with *.

Table 1. Levels of incurred pesticides (mg/kg) in the oat and rye test materials.

	Reference value (mg/kg)	
Compound	**Oat**	**Rye**
Azoxystrobin	0.18	0.31
Carbendazim	0.44	1.12
Chlorpyrifos	1.05	-
Chlorpyrifos-methyl	-	0.09
Cyproconazole	1.01	-
Cyprodinil	0.07	-
Deltamethrin	-	0.06
Fenbuconazole	0.55	-
Fenpropimorph	0.16	2.06
Fenvalerate	0.10	-
Fludioxonil	0.11	-
Fluquinconazole	-	0.63
Flusilazole	0.81	-
Flutriafol	-	2.81
Kresoxim-methyl	-	0.42
Lambda-cyhalothrin	0.03	0.04
Malathion	-	0.09
Metconazole	0.56	-
Pirimiphos-methyl	-	0.05
Pyraclostrobin	0.82	-
Spiroxamine	-	1.15
Tebuconazole	1.79	-
Triadimenol	-	1.83

Table 2: Quantifier ions, qualifier transitions and collision energies for the GC-MS/MS and LC-MS/MS amenable pesticides included in the oat and rye test materials.

	Pesticide	Retention time	Precursor ion-1	Product ion-1	Collisions energy 1 (V)	Precursor ion-2	Product ion-2	Collision energy 2 (V)
GC-MS/MS	Azoxystrobin	15.83	344	329	15	388	345	15
	Chlorpyrifos	14.12	197	169	10	314	258	12
	Chlorpyrifos-methyl	12.98	286	93	20	125	79	5
	Cyprodinil	20.11	226	225	15	223	208	15
	Deltamethrin-cis	29.29	181	152	10	253	174	10
	Fenbuconazole	25.70	198	129	10	129	102	15
	Fenpropimorph	14.27	303	128	5	117	115	10
	Fenvalerate	14.07	167	125	10	125	99	10
	Kresoxim-methyl	19.17	206	116	4	206	131	10
	Lambda-cyhalothrin	22.26	197	141	10	208	181	10
	Malathion	14.00	173	99	10	173	127	5
	Metconazole	21.85	125	89	10	127	89	10
	Pirimiphos-methyl	20.47	305	290	10	290	233	10
	Tebuconazole	20.08	250	125	15	125	89	10
	Triadimenol	15.50	168	70	5	128	100	10
LC-MS/MS	Carbendazim	10.03	192	160	20	192	30	29
	Cyprodinil	20.11	226	93	33	226	77	40
	Flusilazole	18.93	316.17	247	17	316.17	165	20
	Kresoxim-methyl	19.26	314	116	30	314	131	20
	Pirimiphos-methyl	20.51	306	164	20	306	108	20
	Pyraclostrobin	20.23	388.15	194	11	388.15	163	25
	Spiroxamine	18.00	298.26	144	20	298.26	100	30

Table 3. Particle size distribution for oat samples milled by NRLs using different mills, presented by the number of grams collected in the individual sieves.

Sieve size (mm)	0.05-0.1	0.1-0.15	0.15-02	0.2-0.25	0.25-0.35	0.35-0.5	0.5-0.71	0.71-2.8	>2.8
Centrifugal	3.3	16.5	13.5	16.2	26.4	15.6	8.4	0.7	0.0
Centrifugal	0.3	9.5	8.5	19.2	10.2	9.6	14.3	29.4	0.0
Centrifugal	0.4	5.1	5.0	10.0	20.7	13.4	22.3	23.1	0.0
Centrifugal	1.2	15.5	20.7	23.5	30.1	7.9	0.7	0.4	0.0
Centrifugal	0.3	6.6	17.3	18.0	14.7	12.2	20.2	10.8	0.0
Centrifugal	0.4	6.0	6.5	17.2	13.0	13.0	19.5	24.6	0.0
Knife	0.5	4.3	7.8	18.4	8.0	9.3	14.3	38.1	0.0
Knife	0.0	1.8	9.4	22.7	8.6	7.2	12.7	37.3	0.0
Knife	1.2	13.5	11.2	11.9	9.0	10.7	19.1	23.2	0.0
Knife	0.0	3.9	6.1	11.4	18.0	7.6	13.0	39.7	2.8
Knife	0.0	3.5	5.5	13.8	28.7	16.7	22.3	9.4	0.0
Knife	0.4	3.1	3.6	14.0	31.9	13.7	20.0	12.9	0.0
Knife	0.5	5.2	4.2	11.7	25.8	11.8	15.5	26.9	0.0
Knife	1.1	4.4	3.0	4.9	6.5	8.5	11.7	59.1	2.8
Knife	0.0	0.0	10.4	6.0	14.0	10.4	10.6	47.8	2.0
Knife	0.3	6.0	7.4	17.6	15.6	11.4	17.9	23.6	0.0
Hammer	0.4	3.8	4.0	12.2	8.3	7.1	9.6	54.4	1.4
Hammer	0.3	4.0	11.4	5.1	7.5	5.7	7.1	58.9	1.5
Horizontal	0.0	4.5	17.1	23.9	14.6	19.6	20.0	0.5	0.0
Horizontal	0.2	4.6	4.4	15.4	30.6	15.0	14.0	15.6	0.0
Horizontal	0.4	6.2	11.0	17.9	11.9	13.8	18.3	21.1	0.0
Horizontal	0.2	2.8	3.3	5.0	18.0	7.4	11.4	52.3	0.0
Unknown	0.3	2.7	3.0	12.9	20.2	10.4	14.8	35.5	0.0

Table 4. Particle size distribution for rye samples milled by NRLs using different mills, presented by the number of grams collected in the individual sieves.

Sieve size (mm)	<0.05	0.05-0.1	0.1-0.15	0.15-02	0.2-0.25	0.25-0.35	0.35-0.5	0.5-0.71	0.71-2.8	>2.8
Centrifugal	0.9	9.4	13.8	8.8	8.7	14.6	17.6	18.9	8.3	0.0
Centrifugal	3.5	10.4	13.5	8.1	6.9	12.5	14.6	17.0	13.6	0.0
Centrifugal	0.7	7.0	10.3	5.2	5.7	10.1	13.8	23.2	23.8	0.0
Centrifugal	10.3	25.7	21.9	12.8	10.9	12.4	6.4	0.1	0.0	0.0
Centrifugal	0.4	7.9	10.6	10.4	11.0	15.4	18.7	18.7	6.6	0.0
Centrifugal	0.4	4.4	7.3	5.5	6.9	10.8	15.9	30.0	19.6	0.0
Knife	0.5	2.7	8.2	4.6	5.3	6.9	8.3	11.6	52.7	2.1
Knife	0.3	8.8	19.1	10.4	11.2	13.4	14.8	15.1	7.4	0.0
Knife	1.0	7.0	8.9	6.2	6.3	8.4	10.2	15.9	36.7	1.2
Knife	0.3	5.3	10.7	8.4	12.1	9.5	8.4	10.2	34.9	0.0
Knife	0.4	4.1	12.9	19.4	19.9	19.1	13.6	8.9	2.0	0.0
Knife	0.9	6.0	4.8	4.1	3.8	8.4	11.2	17.9	42.4	0.0
Knife	0.4	4.5	14.9	12.2	9.4	12.0	12.4	13.9	20.7	0.0
Knife	0.3	2.5	2.5	1.6	2.0	3.5	4.4	7.3	75.5	0.0
Knife	0.5	1.9	3.4	3.4	3.7	5.8	7.9	12.1	61.0	3.5
Hammer	6.5	23.2	26.8	10.6	14.5	10.5	6.2	0.7	1.2	0.0
Hammer	0.0	0.8	1.5	1.2	1.7	3.6	4.3	7.3	79.2	2.1
Hammer	0.3	2.8	8.3	2.8	4.5	6.8	8.2	13.9	52.4	0.0
Horizontal	0.5	5.1	11.5	13.7	12.0	10.3	10.2	13.2	24.3	0.0
Horizontal	0.0	6.4	9.3	6.4	8.1	14.1	21.1	24.9	8.8	0.0
Horizontal	0.9	4.7	5.5	3.7	4.2	7.7	11.9	22.9	39.1	0.0
Horizontal	0.6	2.1	3.6	2.8	2.9	5.2	7.2	9.9	66.5	0.0
Unknown	0.3	2.1	9.9	9.2	6.8	8.0	9.2	13.4	41.4	0.0

Table 5. Relative particle size and pesticide recovery (%) obtained for oat samples milled by NRLs using different mills.

Mill type	Rel. particle size	Azoxystrobin	Carbendazim	Chlorpyrifos	Cyprodinil	Fenbuconazole	Fenpropimorph	Fenvalerate	Flusilazole	Lambda-cyhalothrin	Metconazole	Pyraclostrobin	Mean recovery
Centrifugal	24.5	97	118	121	126	98	109	106	108	91	120	99	108
Centrifugal	29.4	110	126	110	108	105	92	118	116	87	131	100	109
Horizontal	34.8	106	107	120	116	103	106	118	103	96	117	99	108
Centrifugal	48.8	98	119	89	106	98	80	100	108	85	108	96	99
Knife	50.4	106	110	125	116	99	117	114	102	87	112	93	107
Knife	54.5	98	114	96	93	96	72	102	105	86	113	93	97
Horizontal	56.4	97	116	121	108	87	150	97	92	78	103	88	103
Horizontal	64.4	97	123	102	111	91	102	99	108	83	117	88	102
Knife	65.9	96	108	97	91	96	86	102	99	90	110	89	97
Knife	67.9	103	117	88	86	105	90	104	104	89	112	92	99
Centrifugal	69.8	103	112	99	103	101	96	105	105	85	112	92	101
Centrifugal	70.3	81	110	63	63	81	50	82	97	65	103	85	80
Knife	73.4	96	110	92	98	98	86	105	100	86	105	94	97
Centrifugal	74.6	101	129	79	78	103	62	112	118	89	124	104	100
Unknown	85.7	84	108	81	78	83	66	83	100	65	107	78	85
Knife	85.8	106	108	94	101	106	85	117	102	106	111	93	103
Knife	87.9	96	110	71	70	92	64	96	99	79	104	88	88
Knife	100.9	110	107	88	86	105	66	108	97	92	105	101	97
Horizontal	109.3	90	106	80	86	85	66	87	93	66	102	82	86
Knife	109.8	69	101	65	68	63	60	59	87	65	98	62	72
Hammer	116.2	84	116	64	60	84	53	105	105	61	108	83	84
Hammer	121.5	101	105	79	75	101	70	95	101	80	109	94	92
Knife	129.2	83	105	57	64	80	53	79	93	79	97	84	79

Table 6. Relative particle size and pesticide recovery (%) obtained for rye samples milled by NRLs using different mills.

Mill type	Rel. particle size	Azoxystrobin	Carbendazim	Chlorpyrifos-methyl	Deltamethrin	Fenpropimorph	Flutriafol	Fluquinconazole	Kresoxim-methyl	Lambda-cyhalothrin	Malathion	Pirimiphos-methyl	Spiroxamine	Triadimenol	Mean recovery
Centrifugal	16.2	106	95	112	88	120	77	106	103	103	112	105	112	104	103
Hammer	18.8	95	96	110	91	116	75	93	101	81	100	116	117	102	99
Knife	30.4	93	93	93	79	97	76	99	96	99	102	113	106	99	96
Knife	39.8	108	99	107	102	113	81	106	97	91	118	115	111	106	104
Centrifugal	41.8	108	97	110	101	119	79	105	103	93	114	109	102	106	104
Centrifugal	43.9	98	101	91	87	98	81	98	97	88	108	94	97	50	91
Horizontal	48.4	116	98	89	105	94	78	117	97	103	123	79	76	93	98
Centrifugal	49.7	76	87	85	63	92	66	78	80	67	80	86	92	88	80
Knife	60.1	108	98	104	109	121	78	106	102	99	114	105	100	102	104
Horizontal	65.0	113	122	104	105	131	94	115	127	96	123	158	142	121	119
Centrifugal	66.5	91	94	108	89	112	78	101	97	93	114	132	108	95	101
Centrifugal	68.8	111	97	108	110	115	83	113	95	109	117	124	97	100	106
Knife	79.8	118	96	106	102	117	85	120	100	109	114	94	93	99	104
Knife	89.7	125	97	138	122	152	88	123	110	120	144	101	96	113	118
Unknown	91.6	100	96	95	89	98	79	103	93	95	100	101	106	86	95
Horizontal	92.5	91	87	91	90	97	75	90	94	84	104	86	82	99	90
Knife	95.3	109	90	95	91	103	79	108	92	105	112	86	85	101	97
Hammer	108.7	92	99	68	82	91	69	94	94	92	85	109	89	88	89
Knife	116.2	88	89	80	75	76	64	87	81	77	93	79	80	82	81
Horizontal	129.2	112	91	85	78	90	70	112	98	96	114	139	77	89	96
Knife	134.6	82	82	59	44	95	61	78	73	67	76	86	94	74	75
Knife	141.0	101	95	102	91	109	79	104	95	98	108	128	102	105	101
Hammer	155.2	98	95	91	115	94	77	104	91	112	100	90	92	86	96

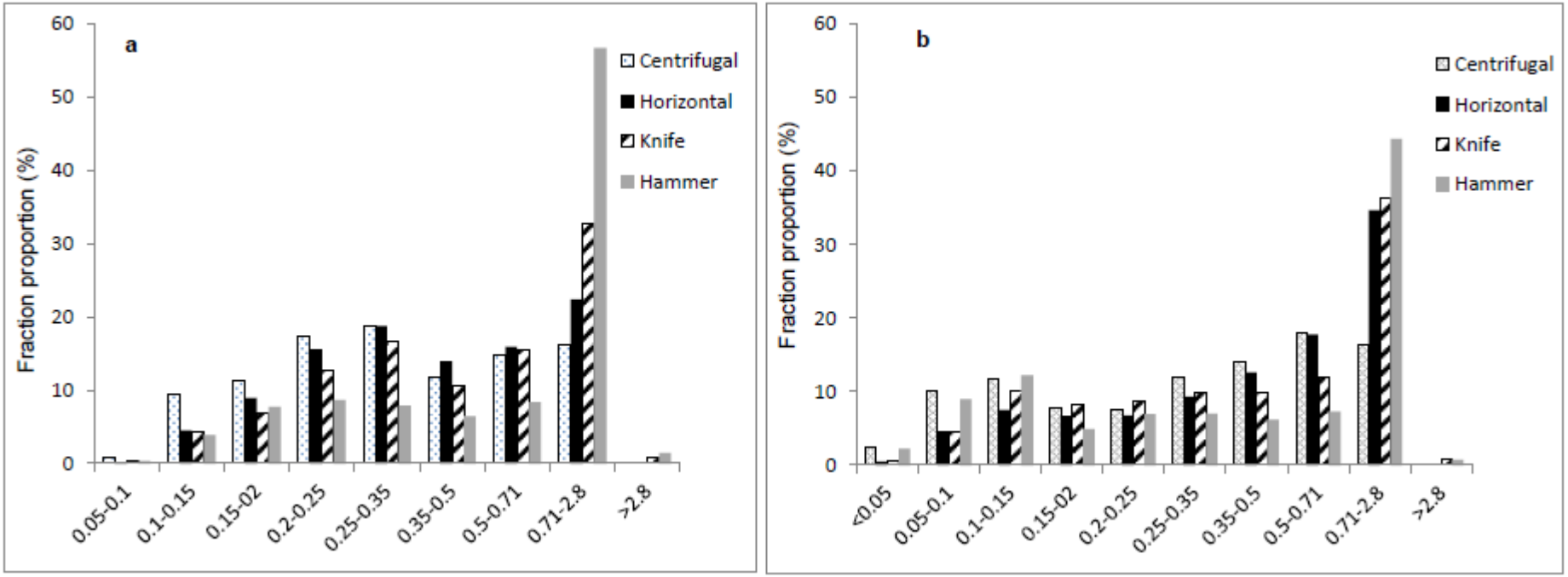
a
b
Fraction proportion (%)
Centrifugal
Horizontal
Knife
Hammer
0
10
20
30
40
50
60
<0.05
0.05-0.1
0.1-0.15
0.15-02
0.2-0.25
0.25-0.35
0.35-0.5
0.5-0.71
0.71-2.8
>2.8

Figure 1.

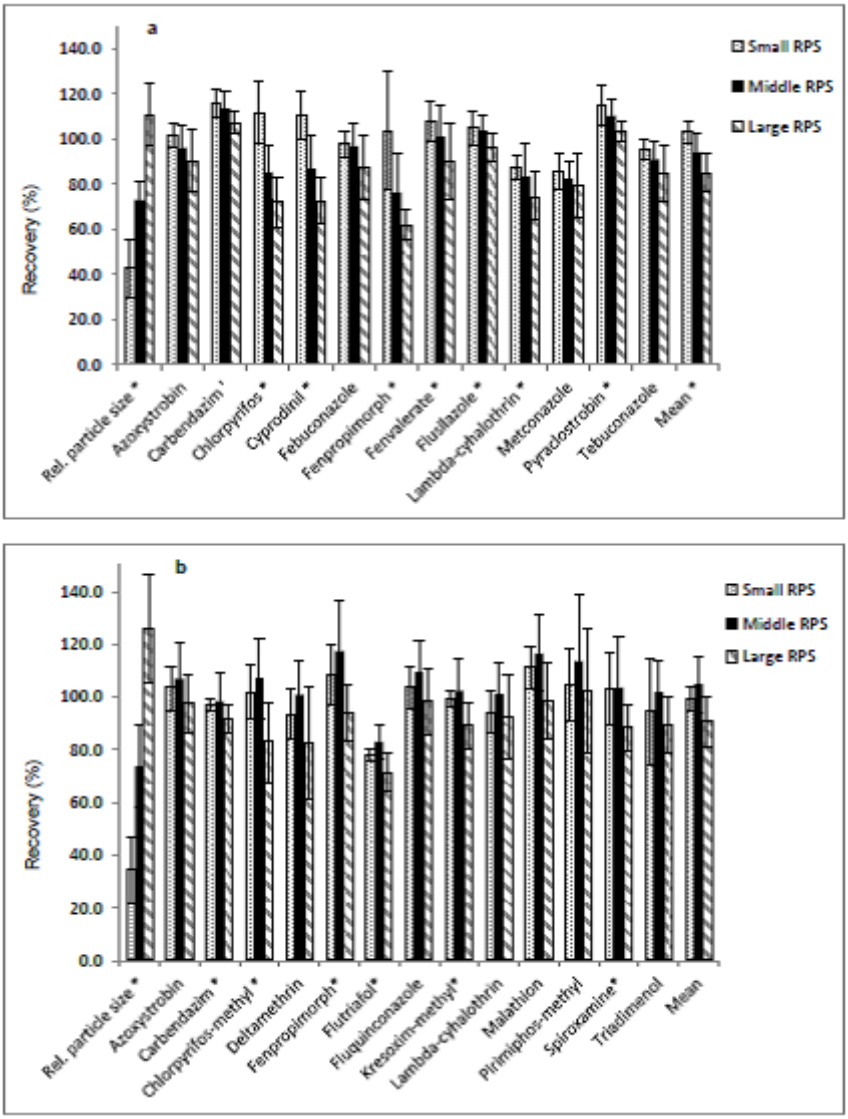
a
Small RPS
Middle RPS
Large RPS
Recovery (%)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Rel. particle size *
Azoxystrobin
Carbendazim '
Chlorpyrifos *
Cyprodinil *
Febuconazole
Fenpropimorph *
Fenvalerate *
Flusilazole *
Lambda-cyhalothrin *
Metconazole
Pyraclostrobin *
Tebuconazole
Mean *
b
Small RPS
Middle RPS
Large RPS
Recovery (%)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Rel. particle size *
Azoxystrobin
Carbendazim *
Chlorpyrifos-methyl *
Deltamethrin
Fenpropimorph*
Flutriafol*
Fluquinconazole
Kresoxim-methyl*
Lambda-cyhalothrin
Malathion
Pirimiphos-methyl
Spiroxamine*
Triadimenol
Mean

Figure 2.

TOC Graphic

Sent to EU-NRLs
Return to EURL-CF
Proficiency Test Materials
Milling
QuEChERS Extraction