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With new technologies we can detect even more pesticide residues and other unwanted contaminants in our food. Photo: Ragnar Våga Pedersen (NIBIO)

Pesticide residues in food from Asia on the Norwegian market and the importance of efficient screening methods for pesticides

Plant protection products (PPPs) are sprayed to strengthen crop plant health and protect crops against microbial diseases, insect damage and weed infestations. Residues of the pesticide active ingredients from the PPPs in the produce, however, are examples of unwanted chemical pollution. Pesticide residues may pose a risk to human health if the maximum residue levels (MRLs) in the produce are exceeded. The MRL describes the maximum allowed pesticide residue concentration in a food product that is still considered safe for human consumption (FAO, 2020). A PPP used in accordance with the instruction on the product label, should not result in pesticide residues above the MRL in the produce.

However, if spraying of the crop is done too close to the harvest date, with higher pesticide dosages than allowed, or with banned pesticides, the MRLs can be exceeded in the crop. Food safety legislation, national monitoring programs and efficient pesticide residue analysis methods are important tools in ensuring that our food is safe to eat.

MORE EFFICIENT FOOD MONITORING ANALYSIS USING LC-HRMS SCREENING METHOD

Each year, NIBIO analyses approximately 1300 food samples for the content of pesticides in the official monitoring programme, commissioned by the Norwegian Food Safety Authority. 70 % of the samples are food imported to the Norwegian market, whereas 30 % are domestic food products. All samples are analysed with two targeted multi-methods using LC-MS/MS (NIBIO method no. M86) and GC-MS/MS (NIBIO method no. M93) technology, respectively. A selection of samples also undergoes analysis with a set of single-residue methods (SRMs) targeting more analytically challenging pesticides. The multi-methods and most of the SRMs are accredited (NS-EN ISO/IEC 17025:2017). The two multi-methods cover in total 379 pesticides and selected pesticide metabolites. Our targeted analysis methods are in line with the requirements of current EU/EEA regulations and are annually updated to ensure the mandatory scope of pesticides and metabolites.

At NIBIO, we have recently established a screening method which expands our pesticide scope to over 800 pesticides and metabolites, including all LC-pesticides in method M86 and 53 out of 107 GC-pesticides in method M93. For this we utilize high resolution mass spectrometry (LC-HRMS Thermo QExactive, NIBIO method no. M121 (qualitative screening) and M119 (quantitative screening)). Under current regulations there is an option to apply qualitative screening methods on 15 % of the samples included in the EU monitoring programme (Commission Implementing Regulation (EU) 2021/601). Screening methods are specifically useful because they enable a view of all contaminants in a sample, including emerging pesticide contaminants and metabolites that are not in the scope of the targeted methods. Furthermore, the stored instrument raw files from the screening analysis allows for a retrospective analysis when novel questions and food safety challenges arise.

NIBIOs new screening method was proven to report the correct pesticide results in annual proficiency tests in onion, eggplant and tomato arranged by the European reference laboratory for pesticides in fruit and vegetables in the period 2020-2022. In most cases, the reported pesticide concentration from the LC-HRMS screening method was within +/- 20 % of the concentration reported by NIBIOs targeted methods (LC- and GC-MS/MS) (Figure 1). These tests also showed the advantage of the screening method through the detection of pesticides not previously included in our targeted methods, e.g. alachlor, diuron, fluacrypyrim, fonofos, metazachlor, monolinuron, orthosulfamuron, tetrachlorvinphos and valifenalate. These pesticides were subsequently added to our quantitative screening method.

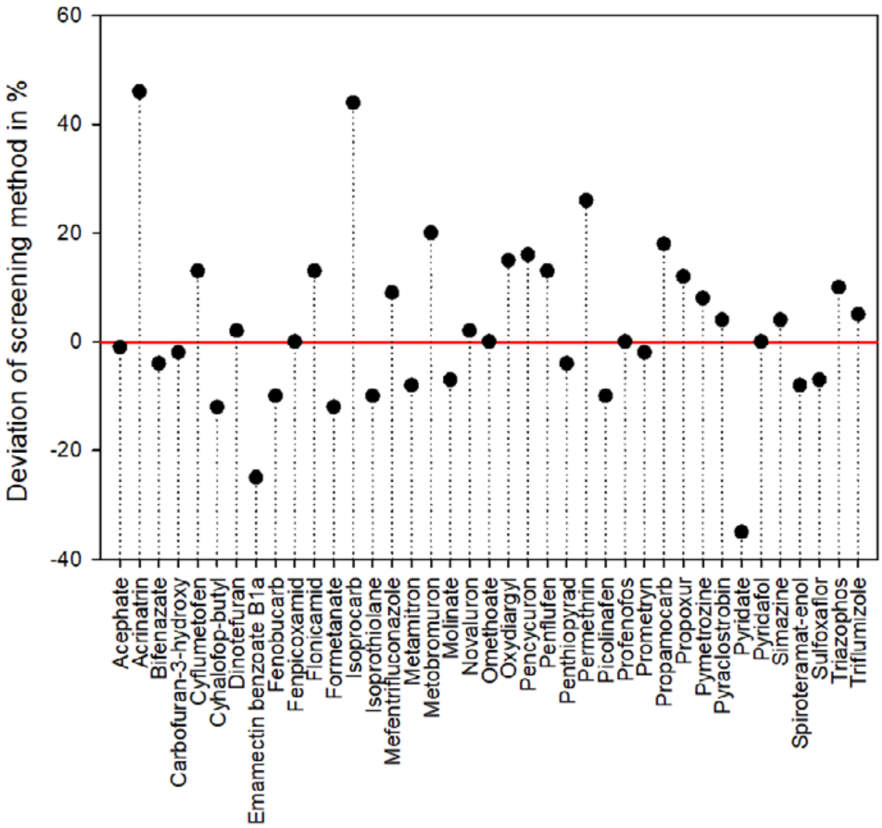

Figure 1: The figure shows the percentage difference in pesticide concentrations detected in annual proficiency tests (2020-2022) in onion, eggplant and tomato in a sample extract analyzed both with our LC-HRMS screening method and our targeted LC-MS/MS and GC-MS/MS methods. The red line represents the results from the targeted methods whereas the black dots are the percentage deviation of the pesticide concentration results by the screening method.

The screening method was further utilized to detect pesticides in food samples from the Norwegian monitoring programme in the year 2020. 52 extracts of various food samples from Asia (including Turkey) previously analysed for pesticides with the targeted NIBIO methods M86 and M93, were collected and stored frozen at -20°C and subsequently screened using LC-HRMS NIBIO method M121. The targeted methods reported 61 detections above the limit of quantification (LOQ of 0.01 mg/kg) in 22 of the samples, whereas the screening method reported 64 detections at or above LOQ in the same samples. The screening method furthermore detected pesticide contaminants outside the scope of the targeted methods, namely piperonyl butoxide (in black tea) and the pesticide metabolites acetamiprid-desmethyl (in chilipepper), imidacloprid-desnitro (in mint) and thiabendazole-5-hydroxy (in clementine). These findings did not represent illegal residues, since piperonyl butoxide is a synergist that lacks a defined maximum residue level, and the three other metabolites are not included in the residue definitions of their mother compounds and hence have no defined MRLs.

The 52 samples from Asia, including 7 rice samples, were also screened for the content of an additional 45 pesticides that were not previously included in our screening or targeted methods. The 45 pesticides were approved in rice in China (Table 1). The analysis was limited to full scan MS data only, and we did not purchase reference standards for them. None of the 45 pesticides were detected in the samples. 27 out of the 45 pesticides do not have any record in the EU Pesticides Database. Of the 18 pesticides with a record, 13 of them were not approved in EU. The pesticide benziothiazolinone is produced in China, and better known as benzisothiazolinone (CAS 2634-33-5) in Europe. The compound is used as a biocide and a pesticide co-formulant in Europe but is not approved as a pesticide in EU. In a recent study in China, benziothiazolinone was detected repeatedly in apples and in some of the samples exceeding the MRL level in EU; but did not exceed the MRL level set in China (Liu et al. 2023).

The production and use of pesticides for which there are yet no records or regulations in the European pesticide regulatory system might be a challenge for food safety. However, screening methods that can search for suspected pesticides in food samples will turn out very useful in this respect. We work continuously to expand the scope of our screening database to include more pesticides, but also co-formulants, veterinary drugs, natural toxins, biocides and other undesirable substances in food.

PESTICIDE RESIDUES IN FOODS FROM ASIA ON THE NORWEGIAN MARKET

Between 2015-2020 a total of 566 food samples of imported produce from 12 different Asian countries were collected and analysed under the Norwegian food safety monitoring programme for pesticides in food and feed. Most of the samples (80 %) were from Turkey, Thailand, China and India. Rice and tea were the most analysed commodities during this period.

Of the samples analysed, 11.8 % contained pesticide residues above the MRL, 31 % had residues below the maximum residue level whereas 57 % had no detectable pesticide residues (Figure 2). A closer look at the samples from China exclusively, showed that 3 % of samples from China contained pesticide residues above the MRL, 30 % had residues below the MRL, whereas 67 % of the samples had no detectable residues (Figure 2). The domestic samples from Norway (in 2020), had less pesticide residues, with 0.6 % of samples having pesticide residues above the MRL, 31 % with detectable residues below MRL, whereas 68 % of the samples had no pesticide residues (Mattilsynet, 2021).

There was a higher share of samples from Asia and China that had no detectable pesticide residues (57 and 67 %, respectively) as compared to all samples imported to Norway from outside EU/EEA (40%) in the same period (2015-2020, 2326 samples). On the other hand, the Asia samples – but not the China samples - had a higher share of samples with pesticide residues exceeding MRL (11.8%) than the samples from outside EU/EEA (5 %). The selection of food commodities from Asia for the annual monitoring campaigns is to some extent focused on foods that are empirically known to contain high levels of pesticide residues, which could explain the higher percentage of MRL exceedances in Asia samples. The most MRL exceedances per commodity were found in spring onion (71 % of all spring onion samples), coriander (45 %) and beans with pods (41 %) - but few samples were in total taken of these commodities. Rice, tea and grapes were sampled at high rates and constituted 30 % of all samples, but had less exceedances, e.g. 14 % of rice samples exceeded MRL whereas 70% of them had no residues. The high percentage of no residues in the Asia samples on the other hand, is partly due to arbitrary sampling of commodities from Asia containing no residues in this period, e.g. dry beans and hazelnut, and sampling of certain

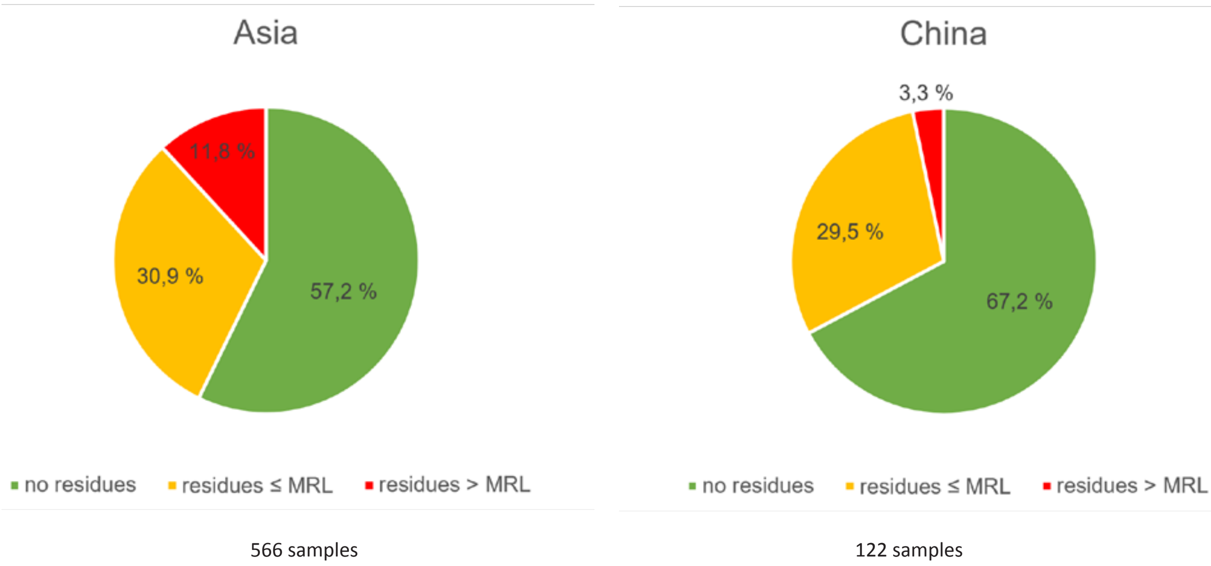

Figure 2: Distribution of pesticide residues in food from Asia (all countries; left) and China (right) analysed under the Norwegian food safety monitoring programme during the period 2015-2020.

commodities at a high rate, e.g. rice, with 70 % of samples having no pesticide residues.

Pesticide residue detections above MRL were most frequently encountered in produce from Thailand, Laos, India and Turkey. A sample of coriander from Thailand showed the largest exceedance, with a measured concentration 330 times higher than the MRL for the pesticide propiconazole (MRL = 0.02 mg/kg). A pesticide concentration above the MRL does not necessarily mean a health threat, but the Food Safety Authority always assesses the need for action; like removing the product from the market. MRL exceedances are published by the national Food Safety Authorities (for Norway: Mattilsynet) and food recall notices in the EU are published via the Rapid Alert System for Food and Feed portal (RASFF).

Not only the exceedance of the MRL, but also the number of different pesticides detected in one food sample can give an indication on how safe a food is. Our monitoring data showed that the highest numbers of different pesticides in the same sample were detected in raisins imported to Norway. Three raisin samples from Turkey showed 16, 15 and 12 different pesticides, respectively, and a raisin sample from China contained residues of 14 different pesticides. Both a chili pepper sample from Laos and a mandarin sample from Turkey showed residues of 12 different pesticides. According to the present EU legislation, samples are compliant with legislation if none of the pesticide concentrations are above the MRL after subtraction of the measurement uncertainty. The sum concentration of pesticides in a sample is not considered. However, there were several samples where multiple pesticides exceeded the MRL, including a jambolana sample from Thailand that contained 8 pesticides (etofenprox, chlorantraniliprole, cypermethrin, fenpyroximate, omethoate, deltamethrin, dimethoate and acetamiprid) all at levels exceeding the MRLs.

NATIONAL PESTICIDE AND FOOD SAFETY MRL REGULATIONS IN A GLOBAL MARKET

With increasing international trade, agricultural produce from all over the world are available. Variation among regions and countries, in terms of climate, agricultural practices, crops and food safety regulations can result in differences in pesticide use and in pesticide residue levels. Many countries are developing strategies to reduce the use and dependency of pesticides. China have implemented policies aimed to reduce pesticide use and the occurrence of residues in food with the Pollution-Free Food action plan and the Action Plan for Zero Growth in Pesticide use by 2020 (Liu et al., 2020). The *European Green Deal* is part of the European Commission's priorities for 2019-2024, and includes a Farm-to-Fork Strategy which aims to reduce pesticide use in general, and specifically to reduce the use of hazardous pesticides by 50 % by 2030 (EC, 2020).

Importing countries are often high-income countries with stricter food safety regulations compared to exporting developing or emerging countries (FAO, 2020). Meeting these regulations can result in higher

Extracts of fruit and vegetables at NIBIO. Photo: Marit Almvik, (NIBIO)

New screening technology bringing pesticide residue analysis to a new level. Photo: Erling Fløistad (NIBIO)

costs for the exporting country, which can be problematic for emerging and developing countries. Harmonized standards can help to facilitate trade and ensure food safety. MRLs are part of the Codex Alimentarius, a collection of international standards, practices, and guidelines regarding food safety and fairness in international trade (FAO/WHO, 2018). The Codex MRLs are however not mandatory. This means, that countries or regions still need their own legislation on pesticide residues, and not all are in alignment with the Codex MRLs. Some countries adopt Codex MRLs directly, while other countries might choose to implement MRLs that are either less or more strict than the MRLs suggested by Codex (FAO, 2020). The FAO compared the MRLs set for rice in different regions and countries and found that less than half of the countries had an MRL corresponding to the Codex MRL in rice in their national legislation (FAO, 2020). Also, the number of MRLs for pesticides in rice varied greatly between countries, the largest difference being between the EU with 486 MRLs and Cambodia with 11 MRLs. The number of MRLs in rice as defined by Codex is 82.

Figure 3 gives an overview of the MRLs in rice for the countries/regions included in the Norwegian monitoring programme that were part of the study by FAO (FAO, 2020). Among samples analysed under the Norwegian monitoring programme during 2015-2020 which showed pesticide residues exceeding the MRL in the EU/EEA, 30 % originated from Thailand. The picture of MRLs in rice is illustrative for the general situation with few MRLs set in Thailand as compared to the EU. However, a great number of the EU-MRLs for rice are set at the limit of quantification because the pesticide active ingredient is not registered in Europe or has no approved use on rice in Europe.

The mandatory analysis scope for pesticides in the EU coordinated monitoring program follows from this comprehensive framework for MRLs. The use of targeted multimethods complemented by high resolution accurate mass (HRMS) screening methods for real broad scope screening with the option to include detection of known chemicals and toxins also outside the scope of current regulations, are important means to achieve food safety.

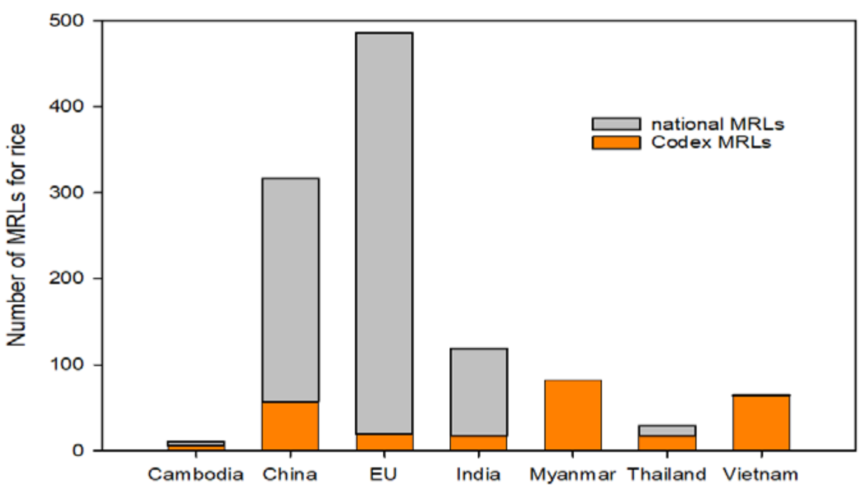

Figure 3: Number of MRLs for pesticides in rice per country with grey representing national MRLs and orange the MRLs set by Codex. The Codex MRLs presented here are in the country's legislation with the same value as the Codex. For example, Cambodia has 11 MRLs in rice of which 5 correspond to Codex MRLs and 6 are national. Graph made after data from FAO (FAO, 2020).

Table 1. 45 Pesticides approved in rice in China that were not originally included in NIBIOs screening or targeted analysis methods. Food samples from Asia were screened for the content of these pesticides based on match to their ionized exact mass (m/z of [M+H]+).

Pesticide approved in rice in China	Formula	m/z [M+H]
Benziothiazolinone	C7H5NOS	152.01646
Bisultap	C5H13NS4O6	311.96985
Bronopol	C3H6BrNO4	199.95530
Butachlor (Machette)	C17H26ClNO2	312.17248
Carbosulfan	C20H32N2O3S	381.22064
Cartap	C7H15N3O2S2	238.06784
Chlorobromoisocyanuric acid	C3HO3N3ClBr	241.89626
Chlorothalonil	C8Cl4N2	264.88884
Chromafenozide	C24H30N2O3	395.23292
Clotrimazole	C22H17ClN2	345.11530
Cyantraniliprole	C19H14BrClN6O2	473.01229
Cyhalofop-butyl	C20H20FNO4	358.14491
Dimetachlone	C10H7Cl2NO2	243.99266
Enestroburin	C22H22ClNO4	400.13101
Ethoxysulfuron	C15H18N4O7S	399.09690
Florpyrauxifen-benzyl	C20H14Cl2F2N2O3	439.04223
Flucetosulfuron	C18H22FN5O8S	488.12459
Flufiprole	C16H10Cl2F6N4OS	490.99293
Imidaclothiz	C7H8ClN5O2S	262.01600
Isotianil	C11H5Cl2N3OS	297.96031
Metaldehyde	C8H16O4	177.11214
Metamifop	C23H18ClFN2O4	441.10119
Metazosulfuron	C15H18ClN7O7S	476.07497
Methazine	C19H16ClNO4	358.08406
Monosultap	C5H11NS4O6	309.95420
Moroxydine hydrochloride	C6H13N5O*HCl	172.11929
Octylamine, acetate	C8H19N	130.15903
Oxadiargyl	C15H14Cl2N2O3	341.04542
Oxaziclomefone	C20H19Cl2NO2	376.08656
Paichongding	C17H23ClN4O3	367.15314
Pentoxazone	C17H17ClFNO4	354.09029
Phenamacril	C12H12N2O2	217.09715
Polyxin A	C23H32N6O14	617.20493
Propyrisulfuron	C16H18ClN7O5S	456.08514
Pyribenzoxim	C32H27N5O8	610.19324
Pyridapenthion	C14H17N2O4PS	341.07194
Pyriminobac-methyl	C17H19N3O6	362.13466
RH-5849	C18H20N2O2	297.15975
Simetryne	C8H15N5S	214.11209
Tetrachlorantraniliprole	C17H10BrCl4N5O2	535.88447
Thifluzamide	C13H6Br2F2N2O2S	526.84937
Thiocyclam	C5H11NS3	182.01264
Thiodiazole-copper	C2H2N3S2	132.97629
Triafamone	C14H13F3N4O5S	407.06315
Triflumezopyrim	C20H13F3N4O2	399.10634

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