MONITORING PROGRAMME FOR PHARMACEUTICALS, ILLEGAL SUBSTANCES, CONTAMINANTS AND MICROBIOLOGY IN AQUATIC PRODUCTS IMPORTED TO NORWAY FROM THIRD COUNTRIES

Annual report for 2024

RAPPORT FRA HAVFORSKNINGEN NR. 2025-36

Title (English and Norwegian):

Monitoring programme for pharmaceuticals, illegal substances, contaminants and microbiology in aquatic products imported to Norway from third countries

Overvåkingsprogram for legemidler, ulovlige stoffer, miljøgifter og mikrobiologi i akvatiske produkter importert til Norge fra tredjeland

Subtitle (English and Norwegian):

Annual report for 2024

Årlig rapport for 2024

Report series:	Year - No.:	Date:
Rapport fra havforskningen	2025-36	25.06.2025

ISSN:1893-4536

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Distribution:

Open

Project No.:

15220, 15949

On request by:

The Norwegian Food Safety Authority (Mattilsynet)

Program:

Trygg og sunn sjømat

Research group(s):

Fremmed- og smittestoff (FRES)
Sjømat og ernæring

Number of pages:

15

Summary (English):

This report summarises results from the ongoing monitoring programme for veterinary border control on seafood products imported to Norway from countries outside the EU and the European Economic Area in 2024.

Samples were collected by personnel from the Norwegian Food Safety Authorities at the Norwegian Border Inspection Posts (BIP). The Institute of Marine Research (IMR) carried out the analytical work on behalf of the Norwegian Food Safety Authority (NFSA). We want to thank NFSA for good cooperation during the conduct of this monitoring programme. A risk assessment for different groups of imported products formed the basis for the selection of analytical activities, where current trend of hazards, as reported in The Rapid Alert System for Food and Feed (RASFF) notification system and the compositional nature of the products and origin formed an up-to-date basis for the risk assessment.

A total of 96 seafood samples, were examined by a selection of analytical methods and assays for microorganisms and undesirable chemical substances.

Selected microbiological analyses were performed on 96 of the samples, contaminants were measured in 26 samples, analyses of residues of authorised veterinary drugs were performed in six samples and analysis of unauthorised and prohibited substances were performed in 19 samples. H istamine was examined in a selection of eight relevant samples, and two samples were analysed for the presence of anisakid nematodes.

Summary (Norwegian):

Denne rapporten oppsummerer resultater fra det pågående overvåkingsprogrammet for veterinær grensekontroll av sjømatprodukter importert til Norge fra land utenfor EU og EØS i 2024. Prøvene ble samlet inn av Mattilsynets personell ved de norske grensekontrollstasjonene (BIP), og Havforskningsinstituttet utførte analysearbeidet på oppdrag fra Mattilsynet. Vi takker Mattilsynet for godt samarbeid under gjennomføringen av dette overvåkingsprogrammet.

En risikovurdering for ulike grupper av importerte produkter dannet grunnlaget for valg av analyseaktiviteter, der nåværende trend av farer, som rapportert i meldingssystemet Rapid Alert System for Food and Feed (RASFF), samt produktenes sammensetning og opprinnelse dannet et oppdatert grunnlag for risikovurderingen.

Totalt 96 sjømatprøver ble undersøkt med et utvalg analysemetoder for mikroorganismer og uønskede kjemiske stoffer. Utvalgte mikrobiologiske analyser ble utført på alle de 96 prøvene. Miljøgifter ble analysert i 26 prøver, rester av lovlige veterinære legemidler ble undersøkt i 6 prøver og uautoriserte og ulovlige stoffer ble undersøkt i 19 prøver. Histamin ble undersøkt i åtte relevante prøver, og to prøver ble analysert for tilstedeværelsen av anisakid nematoder.

Content

1 - Introduction

As a member of the European Economic Area (EEA), Norway is obliged to monitor the conformity of food and feed products imported to the EEA area. Included in this activity is analytical examinations of seafood with respect to microorganisms or the presence of undesirable substances. The Norwegian Food Safety Authority (NFSA) is the competent authority regarding veterinary border control in Norway. On behalf of NFSA, IMR carried out the analytical examination of the seafood samples in this monitoring programme and elaborated this report.

1.1 - Microbial parameters

A selection of microbiological parameters was used to evaluate the safety and quality of seafood products and whether proper hygienic measures were applied during production or transport. To examine for possible faecal contamination, analyses for commonly applied indicator organisms were conducted, including assays for coliforms, thermotolerant coliform bacteria, bacteria in the Enterobacteriaceae family, *Escherichia coli* and enterococci. In addition, examination for coagulase positive staphylococci and sulphite reducing clostridia were conducted on a selection of samples, either heat treated or under vacuum. Furthermore, a selection of samples was examined for specific pathogens relevant for food safety, including norovirus, hepatitis A virus, *Vibrio* spp., bacteria in the genus *Salmonella* and *Listeria monocytogenes* . Vibrios are naturally occurring in marine and estuarine environments, with some species being potential human pathogens. There are currently no regulations on vibrios in food, however their presence in seafood is monitored as they are expected to become an increasing problem with increasing seawater temperatures. *Salmonella* are faecal bacteria commonly found in the gastrointestinal tract of warm-blooded animals and may contaminate a range of food and feed items. *Salmonella* is one of the leading causes of food borne infections in the world and can cause serious disease in humans depending on serotype involved and the susceptibility of the infected person. *L. monocytogenes* (*Lm*) can be found among animals and humans , as well as in the environment. *Lm* can establish in food processing facilities due to their biofilm-forming abilities and natural resistance to commonly used decontaminants, from which they can contaminate food processed in those facilities. *Lm* can cause serious infections, particularly in young children, the elderly and people with a compromised immune system. It is an increasing food-safety issue that is mainly relevant for lightly preserved foods with an extended shelf-life and that are not intended for heat-treatment prior to consumption, as exemplified by cold-smoked non frozen fish products. The EU microbiological criteria for *Salmonella* and *Lm* [1] , implemented by Norway has through the EEA agreement, formed a basis for the evaluation.

1.2 - Parasitic nematodes

Fillets of fish were analysed for the presence of anisakid nematodes using the UV-press method (ISO 23036-1:2021) [2] . European regulations states that fisheries products that are obviously contaminated with parasites must not be placed on the marked for human consumption [3] .

1.3 - Unauthorised and prohibited substances

Farmed seafood products were analysed for several unauthorised and prohibited veterinary medicinal products. Chloramphenicol is an antibiotic agent that exhibit activity against a broad spectrum of microorganisms. Due to a rare but serious dose-independent adverse effect (aplastic anaemia), this agent is not authorized in the treatment of food-producing animals, including fish. Nitrofurans were previously widely used in veterinary medicine as an antimicrobial agent. They were banned by the European Union (EU) in 1995 due to concerns

about the carcinogenicity of possible residues in the edible tissue [4] . Samples were also analysed for the dyes; malachite green, crystal violet and brilliant green; metronidazole and s teroids .

1.4 - Authorised veterinary drugs

Farmed seafood samples were analysed for residues of veterinary drugs that are authorised for use in food producing animals and checked for compliance according to the Maximum Residue Limits (MRLs) as listed in EU 2010/37 [11] . The samples were analysed for residues of antibiotic agents, drugs used against intestinal parasites and anti sea lice agents.

1.5 - Histamine

The survey also included the biogenic amine histamine, following Commission Regulation (EU) No 1019/2013 [5] of 23 October 2013 amending Annex I to Regulation (EC) No 2073/2005 [1] as regards histamine in fishery products .

1.6 - Undesirable trace elements

Undesirable trace elements relevant for seafood safety occur naturally in the environment, with large variations. The analysed levels are influenced by different factors including the geological presence, anthropogenic sources and environmental conditions. These compounds may accumulate in food chains and thus find their way into seafood. Farmed seafood can be affected via contaminated feed. The elements cadmium (Cd), mercury (Hg), and lead (Pb) were measured and the compliance of the values with the EU maximum levels (ML) (as listed in EU 2023/915) [6] was evaluated. Arsenic (As), was also included, although there is currently no maximum level in seafood.

1.7 - Persistent organic pollutants - POPs' (dioxin, PCB, PBDE, PFAS)

Persistent organic pollutants (POP's) form a diverse group of substances with a range of chemical and toxicological characteristics. POPs are persistent in the environment and accumulate in food chains. Some classes of POPs are considered a human health dietary risk. The compliance of selected samples with established maximum levels for food stuffs [6] was evaluated for these contaminants: sum of dioxins (polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)), sum of dioxins and dioxins like PCBs (dl-PCBs), and the EU selected "non-dioxin like-PCBs". In addition, flame-retardant compounds in the polybrominated diphenyl ethers family (PBDEs) were measured. However, maximum levels in food have not been established for the BDEs. The EU recommends a monitoring of the BDE compounds in food [7] . Seafood is considered a potential contributor to BDE-99 exposure, which is the BDE compound considered most relevant to food-safety [8] . In 2023, maximum levels were established for perfluorinated compounds. The levels were set for perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA): perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS), individually and for their sum[5]. For the sum, lower bound concentration is calculated on the assumption that all the values below the limit of quantification are zero[6] .

2 - Material and Methods

Sampling was carried out by NFSA at the Norwegian Border Inspection Posts (BIPs) while analytical examinations and the writing of this report was carried out by IMR. The sampling targeted hazards associated with different imported products, and took into account import volumes, compositional nature of the products, results from previous monitoring, geographical origin of samples, and information available in the RASFF (Rapid Alert System for Food and Feed).

Fresh samples were shipped without delay to IMR whereas frozen samples were stored frozen in the BIPs until shipment in the frozen state to IMR for analysis. Upon arrival, samples were registered at the IMR sample reception unit, each sample was photographed, and relevant information registered in a Laboratory Information Management System (LIMS). Microbiological assays were done prior to all other sample handling procedures to prevent contamination. The samples were subsequently prepared for analyses and split in sub-samples (aliquots) for the different assays and analytical methods.

In general, the edible part was selected for analyses according to a manual specified for each type of sample. For undesirable chemical compounds where a legal maximum level is established, the tissue specified in the regulation was selected. The analytical methods and procedures used were quality assured and accredited according to the ISO 17025:2005 standard [9] , unless otherwise specified.

The evaluations of the analytical data in the report were based on the EU maximum levels and recommendations [1,5,6] . The maximum levels provide a legal framework for trade. For undesirables with no established maximum level, interpretation of the analytical values was based on scientific expert opinions when available.

3 - Results and Discussion

A total of 96 samples from the NFSA at Norwegian BIPs, were examined by a selection of methods for microorganisms, and undesirable chemical species as shown in Table 1.

Table 1: Analyses performed on 96 samples categorized in six seafood groups. The "other" category includes all processed food items such as roe, crabsticks, fishcakes, battered, steamed, boiled, dried and salted, and marinated and canned food items. Most of the samples were analysed for multiple parameters.

Samples and assays included in the Norwegian veterinary border control of seafood 2024							
	Fish	**Crustaceans**	**Cephalopods**	**Bivalves**	**Marine Oils**	**Other**	**Total number**
Microbiology	32	22	7	4	8	23	**96**
Parasites	2						**2**
Unauthorised and prohibited substances	5	13		1			**19**
Authorised veterinary drugs		6					**6**
Histamine - Chemical spoilage indicators	4					4	**8**
Undesirable trace elements	9	9	2	1	5		**26**
POPs' (dioxin, PCB, PBDE)	4	2	2		4		**12**
PFAS	4	6	2				**12**

3.1 - Microbial parameters

Ninety-six samples were analysed for the presence of potential human pathogenic bacteria and spoilage bacteria. All samples were compliant with the regulations.

Of the 96 samples, 75 were examined for the presence of coliform bacteria. Eleven samples had levels above or at the detection limit (10 colony forming units, cfu/g), where the highest value found was 4300 cfu/g in a sample of "popcorn shrimps" (category "other"/"processed seafood"). The remaining samples with values above the detection limit contained 10-40 cfu/g, and consisted of frozen fish fillets or shrimp imported from Viet Nam, China, The Philippines or India. Further, 93 samples were analysed for the presence of thermotolerant coliform bacteria, where nine samples had levels above the method detection limit (10 cfu/g). Of these, the highest measured value was 3100 cfu/g in the aforementioned sample of "popcorn shrimp", whereas values between 10-70 cfu/g were measured in other processed seafood products, fish fillets and fresh oysters imported from Viet Nam, Canada, China or the Philippines.

For enterococci, ten of 61 samples examined contained values at or above the detection limit of 100 cfu/g. The highest values detected were 1100, 2100 and 3100 cfu/g in three separate samples of frozen whiteleg shrimp or giant tiger prawns imported from Viet Nam or India, and 11 400 cfu/g in the previously mentioned sample of "popcorn shrimp", imported from Viet Nam (Table 2). The remaining six positive samples contained between 100 to 700 cfu/g, and consisted of frozen whiteleg shrimp or processed seafood imported from Viet Nam (Table 2).

High numbers of intestinal bacteria such as faecal coliforms and enterococci indicates faecal contamination of the product, either from the environment or during production, and may convey gastrointestinal diseases to the consumer. However, sufficient h eat-treatment will kill such bacteria if present. In any case, good kitchen hygiene should be practised when handling these products to avoid cross-contaminating surfaces and other food-items.

Five samples, where four consisted of fresh or frozen oysters imported from Canada or Korea, and one consisted of “seafood cocktail” (Category Processed seafood), were examined for the presence of *Escherichia coli* , and the numbers were below detection limit in all samples.

Ninety-three sample were analysed for the presence of *Salmonella* and found negative. *Listeria monocytogenes* was detected qualitatively in four of the 50 samples analysed, and the subsequent quantitative analysis found the number to below the detection limit of 10 cfu/g (Table 2). The positive samples consisted of two trout fillets imported from Peru, one sample of Pangasius fillets imported from Viet Nam, and one sample of Humboldt squid fins imported from Chile (Table 2). As these products were not intended to be consumed without further heat treatment, the products were compliant with EU regulations.

Thirteen samples were analysed for the presence of coagulase positive staphylococci. None of these samples had levels above the detection limit (100 cfu/g). Twenty samples were analysed for the presence of sulphite reducing clostridia. Two samples were of fish oil imported from Peru both had 100 cfu/g (Table 2). The detection limit of the method is 100 cfu/g.

Twenty samples were analysed for the presence of potentially human pathogenic *Vibrio* spp., where four samples were found positive. One sample of Eastern oysters imported from Canada contained *Vibrio alginolyticus* , one sample of frozen whiteleg shrimp imported from Viet Nam contained *V. parahaemolyticus* , another sample of frozen whiteleg shrimp also from Viet Nam contained *V. cholera* , and finally, one sample of Giant tiger prawns from India contained *V. parahaemolyticus* and *V. cholerae* (Table 2) . *Vibrio*- strains isolated from these samples were identified using both MALDI-TOF MS and biochemical tests (API 20E). The *V. cholerae* isolated from whiteleg shrimp was analysed for the presence of genes associated with toxin production by the Norwegian Institute of Public Health, and was found to not be toxin-producing. Non-toxin producing *V. cholerae* does not cause cholera, however, they may still cause milder gastrointestinal symptoms, including diarrhoea. *V. parahaemolyticus* may also cause gastrointestinal infections depending on the presence of toxin genes. *V. algionolyticus* on the other hand, is usually associated with waterborne wound- or ear-infections, and not with foodborne gastrointestinal infections. Heat-treatment will kill vibrios if present in the products, but good kitchen hygiene should be practised when handling these products to avoid cross-contaminating surfaces and other food-items.

One sample of Pacific oysters from Korea was examined for the presence of Norovirus type I and II, as well as Hepatitis A by RT-PCR in accordance with ISO 15216-1:2017 (Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -Part 1: Method for quantification) , and was found negative for all three parameters . The sample was analysed by the Norwegian University of Life Sciences (NMBU), who is the Norwegian NRL for Norovirus and Hepatitis A in food and water.

Table 2: Overview of the 27 samples with microbiological findings. Note that all samples were compliant with current regulations.

				CFU/g					
Country of origin	**Species**	**Latin name**	**Product description**	**Thermotolerant bacteria**	**Coliform bacteria**	**Enterococci**	**Sulph.red. Clostridia**	**Vibrio/20g sample**	**speci**
North America									
Canada	Eastern oyster	*Crassostrea virginica*	Bivalves, Fresh oysters					confirmed	*V. a*
Canada	Atlantic cod	*Gadus morhua*	Fish, frozen fillets	10					

Canada	Eastern oyster	*Crassostrea virginica*	Bivalves, Fresh oysters	10					
South America									
Peru	Trout	*Salmo trutta*	Fish, frozen fillets						
Peru	Trout	*Salmo trutta*	Fish, frozen fillets						
Peru	Anchovie	*Engraulis ringens*	Marine oil, fresh				100		
Chile	Humboldt squid	*Dosidicus gigas*	Cephalopods, frozen finns						
Asia									
China	Alaska pollock	*Theragra chalcogramma*	Fish, frozen fillets		40				
China	Alaska pollock	*Theragra chalcogramma*	Fish, frozen fillets	10	10				
China	blue grenadier	*Macruronus novaezelandiae*	Fish, frozen fillets		20				
India	Indian oil sardine	*Sardinella Longiceps*	Fish, frozen fillets		10				
India	Giant tiger prawns	*Penaeus monodon*	Crustaceans, frozen shrimp			700		confirmed	*V. c parah*
India	Whiteleg shrimp	*Penaeus vannamei* Boone	Crustaceans, frozen shrimp			1100			
Philippines	Milkfish	Chanos chanos	Fish, frozen fillets		40				
Philippines	Milkfish	Chanos chanos	Fish, frozen fillets	10	20				
Vietnam	Whiteleg shrimp	*Penaeus vannamei*	Processed products, dumplings with shrimp	70		700			
Vietnam	Striped catfish	*Pangasius hypophthalmus*	Fish, frozen fillets	10	10				
Vietnam	Whiteleg shrimp	*Penaeus vannamei* Boone	Crustaceans, frozen shrimp		10	3100			
Vietnam	Whiteleg shrimp	*Penaeus vannamei* Boone	Crustaceans, frozen shrimp			200		confirmed	*V.*
Vietnam	Whiteleg shrimp	*Penaeus vannamei* Boone	Crustaceans, frozen shrimp					confirmed	*parah*
Vietnam	Whiteleg shrimp	*Penaeus vannamei* Boone	Crustaceans, frozen shrimp			2100			

Vietnam	Whiteleg shrimp	*Penaeus vannamei* Boone	Crustaceans, frozen shrimp		10				
Vietnam	Whiteleg shrimp	*Penaeus vannamei* Boone	Crustaceans, frozen shrimp			100			
Vietnam	Yellowfin tuna	*Thunnus albacares*	Fish, frozen fillets	30	40				
Vietnam	Whiteleg shrimp	*Penaeus vannamei* Boone	Crustaceans, frozen shrimp			100			
Vietnam	Mix of species	*Loligo jaconica, Parapenaeopsis hardwickii, Sepiella japonica, Octopus vulgaris, Paphia undulata*	Processed products, frozen mix of squid, cuttlefish, octopus, shrimp and clams	10		200			
Vietnam	Argentine red shrimp	*Pleoticus muelleri*	Processed products, breaded ("popcorn") shrimp	3100	4300	11400			

3.2 - Parasitic nematodes

Fillets of frozen fish from two samples were analysed for the presence of anisakid nematodes. A sample consisting of three bullet mackerel imported from Viet Nam contained no parasites, whereas one sample of shortfin scad contained two parasites in the muscle tissue.

3.3 - Unauthorised and prohibited substances

Residues of malachite green and leuco malachite green were detected at a total concentration of 1.4 µg/kg in a sample of milkfish (*Chanos chanos*) from the Philippines. Leuco malachite green was also detected, 0.17 µg/kg, in a sample of barramundi (*Lates calcarifer*) from Vietnam. Residues of other dyes, crystal violet and brilliant green, was not detected in the samples. Residues of metronidazole was detected, 0.96 µg/kg, in a sample of whiteleg shrimp (*Penaeus vannamei Boone*) from Vietnam. No residues of nitrofurans or steroids were found in the samples.

3.4 - Authorised veterinary drugs

No residue of antibiotic agents was detected in the six samples analysed for antibiotic agents. One sample was analysed for drugs used against internal and external parasites; no residues were detected. C ypermethrin and deltametrin has been used as both anti sea lice agent and as a pesticide (Bernhard 2023) [12] . For 2024, cypermethrin and deltametrin were below the LOQ for the sample analysed.

3.5 - Histamine

Histamine is a biogenic amine produced by bacterial degradation of the amino acid histidine, if scombroid fish species are exposed to improper storage or transport conditions. A total of eight relevant samples were selected for analysis, and all measured values were below the maximum permitted levels.

3.6 - Undesirable trace elements

A total of 26 samples were analysed for undesirable trace elements. None of the samples exceeded the maximum levels established for mercury, cadmium or lead.

3.7 - Persistent organic pollutants – POPs' (dioxin, PCB, PBDE, PFAS)

Analyses of dioxin, dl-PCB, PCB6 and PBDE were performed in 12 samples, none of the results were above the established maximum levels for dioxin, dioxin and dl-PCB, PCB6. Analyses of PFAS were performed in 12 samples, also for this group of compounds, none of the results were above the established maximum levels.

4 - Conclusion

A total of 96 samples collected by the official staff at the Norwegian Border Inspection Posts of the Norwegian Food Safety Authority were examined for selected chemical and microbiological undesirables in 2024 .

Selected microbiological analyses were performed on 96 samples. Four samples contained *L. monocytogenes* , but as the samples were not from ready-to-eat products, they were considered compliant according to current regulations.

Four samples harboured potentially human pathogenic *Vibrio* spp. However, there are currently no regulations or limits regarding the presence of these bacteria in food items.

None of the other samples examined were identified with undesirable or unacceptable microorganisms according to EU regulations, though some contained high values of intestinal microorganisms. Most vibrios are marine bacteria that are naturally occurring in the environment, including in animals such as bivalves and crustaceans. Fecal coliforms and enterococci, however, are intestinal bacteria found in humans or animals, and may indicate poor hygienic conditions either during farming of animals (aquaculture) or during processing of the products (aquaculture and wild caught).

Undesirable trace elements were measures in twenty-six samples of seafood. Further, twelve samples were measured for dioxin, dl-PCB, PCB6, PBDE, and PFAS were measured in twelve samples of seafood. None of the samples had levels exceeding the respective maximum levels.

Substances prohibited for use in animals intended for food were analysed in nineteen samples. Residues of malachite green and leuco malachite green were detected in milkfish (*Chanos chanos*) from the Philippines. Leuco malachite green was also detected in barramundi (*Lates calcarifer*) from Vietnam. Residues of metronidazole was detected in whiteleg shrimp (*Penaeus vannamei* Boone) from Vietnam.

The chemical spoilage indicator histamine was examined in eight relevant samples, and all values were below the maximum permitted level.

5 - References

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