MONITORING PROGRAMME FOR VETERINARY CONTROL ON SEAFOOD PRODUCTS IMPORTED TO NORWAY FROM THIRD COUNTRIES - RESULTS FROM 2022

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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 2022. Eight samples of products imported in 2021 but analysed in 2022 are also included.

Samples were collected by personnel 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 114 seafood samples, were examined by a selection of analytical methods and assays for microorganisms and undesirable chemical substances.

Selected microbiological analyses were performed on 99 of the samples, undesirable trace elements were measured in 114 samples and persistent organic pollutants (POPs) were measured in 31 samples. The chemical spoilage indicator histamine and traces of drug residues and dyes were examined in a selection of 20 relevant samples.

Sammendrag (engelsk):

Denne rapporten oppsummerer resultater fra det pågående overvåkingsprogrammet for veterinær grensekontroll av sjømatprodukter importert til Norge fra land utenfor EU og EØS i 2022. Åtte prøver av produkter importert i 2021, men analysert i 2022 er også inkludert. Prøvene ble samlet inn av 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) og produktenes sammensetning og opprinnelse dannet et oppdatert grunnlag for risikovurderingen.

Totalt 114 sjømatprøver ble undersøkt med et utvalg analysemetoder for mikroorganismer og uønskede kjemiske stoffer. Utvalgte mikrobiologiske analyser ble utført på 99 av prøvene, uønskede sporstoffer ble målt i 114 prøver og persistente organiske miljøgifter (POP-er) ble målt i 31 prøver. Histamin og spor av legemiddelrester og fargestoffer ble undersøkt i 20 relevante prøver.

Innhold

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 evaluate possible faecal contamination, analyses for common indicator organisms were conducted, including assays for coliforms, 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, samples were analysed for specific pathogens relevant for food safety, including norovirus, hepatitis A virus, and bacteria in the genera *Salmonella*, *Listeria* and *Vibrio*. The EU microbiological criteria for *Salmonella* spp. and *Listeria monocytogenes* [1], implemented by Norway has through the EEA agreement, formed a basis for the evaluation.

1.2 Drug residues and dyes

Farmed seafood products were analysed for several 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 [2]. Samples were also analysed for the dyes; malachite green, crystal violet and brilliant green.

1.3 Chemical spoilage indicators

The survey also included the biogenic amine histamine, following Commission Regulation (EU) No 1019/2013 [3] of 23 October 2013 amending Annex I to Regulation (EC) No 2073/2005 [3] as regards histamine in fishery products.

1.4 Carbon monoxide

Carbon monoxide (CO) has been illegally used on fresh fish fillet and especially tuna to retain a fresh, red appearance for a longer storage period. It reacts with the oxy-myoglobin to form a cherry red carboxy-myoglobin complex. No direct health implications from eating CO-treated fish are known. However, the practice of food cosmetics is problematic, since the customer is misled regarding the product freshness. Potentially, CO could mask spoilage, as the CO-complex can be stable beyond the fish-fillet shelf life. As no official maximum level is provided, a sample was judged as CO treated if the analysed level was above 200 µg/kg, as described in Marrone et al. (2015) [4].

1.5 Undesirable trace elements

Undesirable trace elements relevant for seafood safety occur naturally in the environment, with large geographical variations. The analysed levels reflect the geological presence, as well as anthropogenic sources. 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 (as listed in 2022/2388 (EC)) [5] was evaluated. Arsenic (As), was also included, although there is no maximum level in seafood, in contrast to the maximum limits in terrestrial foods.

1.6 Persistent organic pollutants – POPs' (dioxin, PCB, PBDE)

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 yet been established for the BDEs. The EU recommends a monitoring of the BDE compounds in food [6]. Seafood is considered a potential contributor to BDE-99 exposure, which is the BDE compound considered most relevant to food-safety [7].

1.7 Polycyclic aromatic hydrocarbons (PAH)

Polycyclic aromatic hydrocarbons (PAHs) are formed from incomplete combustion of organic matter. PAHs have been found to exhibit food safety issues, and maximum levels are established for smoked fishery products. The maximum levels are set for Benzo(a)pyrene (BaP) alone, as well as for the lower bound sum of four PAH compounds (PAH4): BaP, Benzo(a) anthracene, Benzo(b)fluoranthene and chrysene.

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 to prevent contamination. The samples were then further 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 species 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 [8], unless otherwise specified.

The evaluations of the analytical data in the report were based on the EU maximum levels and recommendations [1,3,5]. 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 114 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 samples from different 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.

Samples and assays included in the Norwegian veterinary border control of seafood 2022								
	Fish	Crustaceans	Cephalopods	Bivalves	Feed/Flour	Marine Oils	Other	Total number
Microbiology	44	13	10*	4		8	20	99
Drug residues and dyes	3	68		1			8	20
Chemical spoilage indicators	6*						13	19
Carbon monoxide	3							3
Undesirable trace elements	45	13	10	4		8	34	114
POPs’ (dioxin, PCB, PBDE)	9*		4			6	12	31
PAH				2			1	3

*Includes products not intended for humans, “non-human consumption” (NHC) products

3.1 Microbial parameters

Ninety-nine samples were analysed for the presence of potential human pathogenic bacteria and spoilage bacteria. Seven of these samples were imported in 2021 and sent to IMR and analysed in 2022. All samples were compliant regarding the regulations.

Of the 99 samples, 78 were examined for the presence of coliform bacteria. Five samples had levels above or at the detection limit (10 cfu/g), where the highest value found was 40 cfu/g. Further, 94 samples were analysed for the presence of thermotolerant coliform bacteria, and one had levels above the method detection limit (10 cfu/g) with 20 cfu/g found.

For enterococci, three of 58 samples examined contained 100 cfu/g. The detection limit for the method is 100 cfu/g.

All 99 sample were analysed for the presence of *Salmonella* spp. and found negative. *Listeria monocytogenes* was not found in any of the 32 samples analysed.

Fourteen samples were analysed for the presence of coagulase positive staphylococci. None of these samples had levels above the detection limit (100 cfu/g). Seventeen samples were analysed for the presence of sulphite reducing clostridia, and one sample consisting of boiled, frozen apple snails from Viet Nam had 2800 cfu/g, which is above the detection limit of 100 cfu/g.

Nineteen samples were analysed for the presence of potentially human pathogenic *Vibrio* spp., and two samples were positive. One sample of whiteleg shrimp from Vietnam was found to contain *V. parahaemolyticus* and one sample of pacific oysters from South Korea contained *V. alginolyticus*. The vibrio strains isolated from these two samples were were identified using MALDI-TOF MS.

Three samples were examined for the presence of Norovirus type I and II, 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). None of the samples were positive. Three samples were examined for the presence of Escherichia coli, and all had numbers below detection limit.

3.2 Drug residues and dyes

A selection of 20 samples of fish, crustaceans, processed seafood, and bivalves were examined with respect to unauthorised antimicrobial agents and/or dyes (Table 1), and two samples contained unauthorised antimicrobial agents or dyes (Table 2). One sample of Pangasius from Vietnam contained malachite green (0.35 ng/g ww) and leuco-malachite green (35 ng/g ww), the decision limit (CCa) of both malachite green and leucomalachite green is 0.15 ng/g ww, the reference point for action (RPA) is 0.5 ng/g for the sum of malachite green and leucomalachite green [9] . None of the other samples contained detectable residues of malachite green, leuco-malachite green, brilliant green, crystal violet or leuco-crystal violet. The nitrofuran metabolite 3-Amino-2-oxazolidinone (AOZ) was detected in one sample of whiteleg shrimp from Vietnam. The measured level was 17 ng/g ww, after washing the sample according to procedure the measured level was 13 ng/g ww The RPA for AOZ is 0.5 ng/g. Chloramphenicol was not detected in any of the samples.

3.3 Chemical spoilage indicators

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. 19 relevant samples were selected for analysis, and all measured values were below the maximum permitted levels.

3.4 Carbon monoxide

Three samples of Yellowfin tuna from Vietnam were analysed for the presence of added carbon monoxide, and all showed levels below 200 µg/kg, which is the internationally accepted level for physiological CO content in muscle tissue [4], with the highest level observed being 38 µg/kg.

3.5 Undesirable trace elements

114 samples were analysed for undesirable trace elements.

For cadmium, there were three samples exceeding the maximum level (Table 2): One sample of boiled American lobster imported from China had a cadmium concentration of 0.58 mg/kg wet weight, which is above the maximum level of 0.5 mg/k wet weight for white muscle meat. It has been shown earlier that cadmium can leak from hepatopancreas to muscle meat during boiling and thawing of crustaceans [10], which also seems to be a plausible explanation in the current case.

One sample of sardines in sunflower oil imported from Philippines containing the species Sardinella lemuro was measured to contain 0.084 mg/kg wet weight which is over the maximum level of 0.05 mg/kg wet weight (Table 2). While a higher maximum level of 0.25 mg /kg wet weight has been set for the sardine species Sardina pilchardus this does not apply to the here measured Sardinella lemuro. A sample of muscle Northern shortfin squid, Illex illecebrosus, imported from Canada had a cadmium concentration of 1.38 mg/kg wet weight, exceeding the legal limit of 1.0 mg/kg wet weight (Table 2).

For mercury, one sample of European lobster imported from United Kingdom had a mercury concentration just on the maximum level of 0.5 mg/kg wet weight in the white muscle meat (Table 2).

No other exceedances of the maximum levels for undesirable trace elements were found.

3.6 Persistent organic pollutants – POPs' (dioxin, PCB, PBDE)

Thirty-one samples, selected based on risk assessment-criteria, were analysed for three classes of organo-halogen compounds considered undesirable and relevant for seafood. The selected POPs' classes were: The dioxin, the PCB and the PBDE.

Two samples of oil were found to contain concentrations of sum dioxin and sum dioxin and dl-PCB exceeding the maximum levels (Table 2): One sample of squid oil imported from South Korea had a sum dioxin of 4.5 pg(WHO2005-TEQ)/g wet weight and sum dioxin and dl-PCB of 13.9 pg(WHO2005-TEQ)/g wet weight. The second oil was produced from fish and imported from Turkey, containing sum dioxin of 2.99 pg(WHO2005-TEQ)/g wet weight and sum dioxin and dl-PCB of 9.45 pg(WHO2005-TEQ)/g wet weight. Thereby both are exceeding the maximum levels given for oil of 1.75 and 6.0 pg(WHO2005-TEQ)/g for sum dioxin and sum dioxin and dl-PCB, respectively.

3.7 Polycyclic aromatic hydrocarbons (PAH)

Three samples were analysed for the presence of PAH-compounds. None contained levels above legal limits.

Table 2: Samples with measured values (microbial or chemical) above maximum levels. Measurement uncertainty (MU) is indicated in parentheses. Microbiological measurements are presented as colony forming units (cfu) per ml of sample, undesirable trace elements are measured as mg per kg sample of wet weight (ww), and concentrations of POPs are given as (pg TEQ/g wet weight) sum dioxin and sum dioxin and dl-PCB

IMR journal number	Product	Country of origin	Variable above acceptable value	Measured value	Acceptable maximum value of variable
Drug residues and dyes					
2022-2601/1	Whitefoot shrimp	Vietnam	Nitrofuran	13 ng/g	0.5 ng/g
2022-805	Pangasius	Vietnam	Malachite green	0.83 ng/g	0.15 ng/g
			Leuco-malachite green	35 ng/g	0.15 ng/g
Undesirable trace elements					
2022-2607	Lobster	China	Cadmium	0.58 mg/kg ww	0.50 mg/kg ww
2022-965	Sardines in sunflower oil	Philippines	Cadmium	0.084 mg/kg ww	0.05 mg/kg ww
2022-728	Lobster	United Kingdom	Mercury	0.5 mg/kg ww	0.5 mg/kg ww
2022-926	Squid	Canada	Cadmium	1.38 mg/kg ww	1.0 mg/kg ww
POPs					
2022-2593	Squid oil	South Korea	Sum dioxin	4.51 pg(WHO2005-TEQ)/g	1.75 pg(WHO2005-TEQ)/g
			Sum dioxin and dl-PCB	13.9 pg(WHO2005-TEQ)/g	6.0 pg(WHO2005-TEQ)/g
2022-1215	Fish oil	Turkey	Sum dioxin	2.99 pg(WHO2005-TEQ)//g	1.75 pg(WHO2005-TEQ)//g
			Sum dioxin and dl-PCB	9.45 pg(WHO2005-TEQ)//g	6.0 pg(WHO2005-TEQ)//g

4. Conclusions

A total of 114 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 2022.

Selected microbiological analyses were performed on 99 samples. Two samples contained potentially human pathogenic Vibrio spp. However, there are currently no regulations or limits regarding the presence of these bacteria in food items and they were deemed compliant.

None of the other samples examined were identified with undesirable microorganisms.

Undesirable trace elements were measured in 114 samples, POPs' were measured in 31 seafood samples and PAHs were measured in three samples. Three samples contained cadmium concentrations above their respective maximum level. American lobster imported from China, one sample of sardines in sunflower oil imported from the Philippines and one sample of squid from Canada. For mercury, one sample of European lobster imported from United Kingdom had a mercury concentration just on the maximum level. Two oil samples, one made of squid imported from South Korea and one made of fish imported from Turkey exceeded the maximum levels for sum dioxin and sum dioxin and dl-PCB in oils. None of the other samples examined had trace elements, POPs' or PAH exceeding the respective maximum levels.

Drugs prohibited for use in animals intended for food; chloramphenicol, nitrofurans, and dyes, were analysed in 20 samples. Malachite green and leuco-malachite green were detected in one sample of Pangasius from Vietnam. The nitrofuran metabolite AOZ was detected in one sample of whiteleg shrimp from Vietnam. Chloramphenicol was not detected in any of the samples.

The chemical spoilage indicator histamine was examined in 19 relevant samples, all values were below the maximum permitted level.

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