

MONITORING PROGRAMME FOR VETERINARY CONTROL ON SEAFOOD PRODUCTS IMPORTED TO NORWAY FROM THIRD COUNTRIES - RESULTS FROM 2021

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Sammendrag (norsk):

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 2021.

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

Selected microbiological analyses were performed on 84 of the samples, undesirable trace elements were measured in all 100 samples and persistent organic pollutants (POPs) were measured in 30 samples. The chemical spoilage indicator histamine was examined in a selection of 20 relevant samples. All values were below the maximum permitted level, when established.

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 100 sjømatprøver ble undersøkt med et utvalg analysemetoder for mikroorganismer og uønskede kjemiske stoffer. Utvalgte mikrobiologiske analyser ble utført på 84 av prøvene, uønskede sporstoffer ble målt i 100 prøver og persistente organiske miljøgifter (POP-er) ble målt i 30 prøver. Histamin og spor av legemiddelrester og fargestoffer ble undersøkt i 20 relevante prøver. Alle målte verdier var under de respektive grenseverdiene.

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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, (Commission Regulation (EC) 2019/1871). 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* (Commission Regulation 2073/2005), implemented by Norway has through the EEA agreement, formed a basis for the evaluation.

1.2 Antibiotic resistance

Antimicrobial resistance is a prevalent challenge to the global public health. Carbapenemase-producing and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are priority pathogens for which research and urgent development of new antibiotics is needed (WHO 2017). Despite findings of carbapenem resistant Enterobacteriaceae in seafood imported from Southeast Asia (Janecko, Martz et al. 2016), there are currently no regulations in place for the screening for antibiotic resistant pathogens in imported seafood. As a preliminary survey with the goal of establishing a pipeline for the detection of antibiotic resistant pathogens, farmed fish and shellfish imported from Asia and South America were selected for analyses targeting carbapenem and/or third generation cephalosporin resistant Enterobacteriaceae.

1.3 Prohibited substances

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.

1.4 Chemical spoilage indicators

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

1.5 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).

1.6 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 1881/2006 (EC)) was evaluated. Arsenic (As), was also included, although there is no maximum level in seafood, in contrast to the maximum limits in terrestrial foods.

1.7 Persistent organic pollutants POPs' (PCDD/F, 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 (Commission Regulation (EC) 1259/2011) was evaluated for these contaminants: dioxins, furans, dioxin-like 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 (EU 2014). Seafood is considered a potential contributor to BDE-99 exposure, which is the BDE compound considered most relevant to food-safety (EFSA CONTAM Panel 2011).

1.8 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, unless otherwise specified (see Annex 1, Table 2 for details).

The evaluations of the analytical data in the report were based on the EU maximum levels and recommendations (Commission Regulation (EU) No. 2006/1881, Commission Regulation (EU) No. 2073/2005, 37/2010 and 1019/2013) and EU recommendations. 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 100 samples from the NFSA at Norwegian BIPs, were examined by a selection of methods for microorganisms and undesirable chemical species (see Annex 1 for details on methods) as shown in Table 1. All samples were found to be compliant with EU regulations. Samples with detected microorganism levels or undesirable chemical species above detection limits are listed in Annex 2.

Table 1: Analyses performed on samples from different seafood categories. The "other" category includes all processed food items such as roe, crabsticks, fishcakes, battered, steamed, dried and salted, and marinated and canned food items.

Samples and assays included in the Norwegian veterinary border control of seafood 2021									
	Fish	Crustaceans	Cephalopods	Bivalves	Feed/Flour	Marine Oils	Other	Total number	
Microbiology	33	16	8	3		6	18	84	
Antibiotic resistance	7	12						19	
Drug residues and dyes	6	8		1			6	21	
Chemical spoilage indicators	6					2	12	20	
Carbon monoxide	3							3	
Undesirable trace elements	36	15	7	3		6	33	100	
POPs' (PCDD/F, PCB, PBDE)	7		2			6	15	30	
PAH				3			1	4	

3.1 Microbial parameters

Eighty-four samples were analysed for the presence of potential human pathogenic bacteria, spoilage bacteria and moulds/yeasts. All samples were compliant regarding the regulations. Details about samples exceeding the detection limit can be found in Annex II, Table 3.

Potential human pathogenic bacteria

Of the 84 samples, 64 were examined for the presence of coliform bacteria. Four samples had levels above or at the detection limit (10 cfu/g). Further, eighty-one samples were analysed for the presence of thermotolerant coliform bacteria, and two had levels above the method detection limit (10 cfu/g). Three samples were analysed for Enterobacteriaceae and all were below the detection limit of 10 cfu/g.

For enterococci, four of 68 samples examined were found above the detection limit of 100 cfu/g.

All 84 sample were analysed for the presence of *Salmonella* spp. and found negative. *Listeria monocytogenes* was not found in any of the 55 samples analysed.

Fourteen samples were analysed for the presence of coagulase positive staphylococci, and 16 samples were analysed for the presence of sulphite reducing clostridia. None of these samples had levels above the detection limit (100 cfu/g).

Twenty-five samples were analysed for the presence of potentially human pathogenic *Vibrio* spp., and three samples of whiteleg shrimp and scampi, all from Vietnam were positive. The isolated strains were identified using MALDI-TOF

MS, and two were found to be V. parahaemolyticus and one was identified as V. lutrae.

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). One sample of Pacific oysters imported from South Korea was positive for the presence of Norovirus type II. Three samples were examined for the presence of Escherichia coli, and both had numbers below detection limit.

Mould and yeast

The presence of mould and yeast was examined in three samples, all were below the detection limit of 100 cfu/g.

3.2 Antibiotic resistance

A total of 19 samples were analyzed for the presence of carbapenemase-producing and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Five of the 19 samples tested showed presence of the members of the family Enterobacteriaceae. All five positive samples were whiteleg shrimps. Three of the samples carried Enterobacter spp and two of the samples carried Klebsiella pneumoniae. Enterobacter spp. carry AmpC betalactamase that confers resistance to cephalosporin, hence further analysis is needed to confirm the presence of ESBLs in these isolates (Jacoby, 2009). Klebsiella pneumoniae is an emerging threat to the human health and is known for causing several nosocomial infections, ESBL producing Klebsiella pneumoniae is a clinical problem in Norway (Fostervold et al, 2022). Thus, detection of these isolates in imported seafood is of concern from seafood safety point of view.

3.3 Prohibited substances

A selection of 21 samples of fish, crustaceans, processed seafood, and bivalves were examined with respect to antimicrobial agents and/or dyes (Table 1). None of these samples contained detectable residues of malachite green, leuco-malachite green, brilliant green, crystal violet or leuco-crystal violet. Furthermore, none of the samples contained chloramphenicol or any of the nitrofuran metabolites.

3.4 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. 20 relevant samples were selected for analysis, and all measured values were below the maximum permitted levels.

3.5 Carbon monoxide

Three samples of Yellowfin tuna were analysed for the presence of added carbon monoxide, and all showed levels below 200 μ g/kg, which is the internationally accepted level for physiological CO content in muscle tissue (Marrone et al., 2015). The highest observed value was 171 μ g/kg in a fillet of Kawakawa from Vietnam.

3.6 Undesirable trace elements

100 samples were analysed for undesirable trace elements. One sample of Pacific saury from Taiwan had cadmium values at 0.22 mg/kg, which is above the legal limit of 0.05 mg/kg for fish. However, this product was not meant for human consumption. Two samples of squid cut-offs including viscera from Argentina, not meant for human consumption, also contained high levels of cadmium with 43 and 33 mg/kg respectively. No further action was taken with these three samples. One sample of crushed lobster carapaces from Canada had a cadmium content of 1.4 mg/kg, which is above the legal limit of muscle from crustaceans of 0.5 mg/kg. However, there are no legal limits for carapaces, no further action was taken.

None of the other undesirable trace elements were measured at levels above legal limits.

3.7 Persistent organic pollutants - POP's (PCDD/F, PCB, PBDE)

Thirty 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 PCDD/Fs, the PCBs and the PBDEs. All analysed samples were compliant. Four samples were analysed for the presence of PAHs, and all were compliant according to the regulations.

3.8 Polycyclic aromatic hydrocarbons (PAH)

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

4 - Conclusion

A total of 100 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 2021.

Selected microbiological analyses were performed on 84 samples, undesirable trace elements were measured in 100 samples and POPs' were measured in 30 seafood samples. All samples were compliant with the current legislation. The chemical spoilage indicator histamine was examined in 20 relevant samples, all values were below the maximum permitted level.

One sample of Pacific saury from Taiwan and two samples of squid cut-offs were found with cadmium values above the legal limit. However, these products were not intended for human consumption. One sample of crushed lobster carapaces from Canada also had a high Cd value, but was deemed compliant as it was intended as a flavour enhancer, and not to be eaten whole.

Three samples contained potentially human pathogenic *Vibrio* spp., and five of the analysed samples carried cefotaxime resistant Enterobacteriaceae isolates. 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 or had trace elements, POPs' of PAH exceeding the respective maximum levels.

5 - Acknowledgements

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Five laboratories at IMR contributed with analyses in 2021: The Sample Reception and Vessel Laboratory, the Molecular Biology Laboratory, the Inorganic Chemistry Laboratory, the Nutrients Laboratory and the Chemistry and Undesirables Laboratory.

We wish to thank all laboratory leaders and everyone who contributed to this project for a good collaboration in 2021.

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7 - Annex I

Table 2: Methods used for detection and quantification of microbial and chemical parameters.

Parameter	Method of detection/quantification	Accreditation/Validation
Microbial parameters		
Salmonella	ISO 6579-1:2017	Accredited
Listeria monocytogenes	ISO 11290-1:2017	Accredited
Coliform bacteria	3M petrifilm CC*	Accredited
Thermotolerant coliform bacteria	3M petrifilm CC*	Accredited
Enterobacteriaceae	3M petrifilm Enterobacteaceae*	Accredited
Enterococci	ISO 7899-2:2000	Accredited
Coagulase positive staphylococci	3M petrifilm StaphExpress*	Accredited
Sulphite reducing clostridia	NMKL method nr.56 (2015)	Validated
Vibrio spp.	NMKL method nr.156 (1997)	Accredited
Norovirus and Hepatitis A	ISO 15216-1:2017	Validated
Mould and Yeast	NMKL method nr.98 (2005)	Accredited
Antibiotic resistance		
Carbapenemase-producing and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae	Internal method using selective growth media containing antibiotics	Currently under internal validation

Malachite green	LCMS/MS (QQQ)	Accredited
Leuco-malachite green	LCMS/MS (QQQ)	Accredited
Brilliant green	LCMS/MS (QQQ)	Accredited
Crystal violet	LCMS/MS (QQQ)	Accredited
Leuco-crystal violet	LCMS/MS (QQQ)	Accredited
Chloramphenicol	LCMS/MS (QQQ)	Accredited
Nitrofuran metabolites	LCMS/MS (QQQ)	Accredited
Chemical spoilage indicators		
Histamin	HPLC	Accredited
Carbon monoxide		
Carbon monoxide	GC-FID	Validated
Heavy metals		
Cadmium (Cd)	Quantitative ICP-MS, (NMKL method nr.186, 2007)	Accredited after NMKL prosedure nr. 4 (2005)
Mercury (Hg)	Quantitative ICP-MS, (NMKL method nr.186, 2007)	Accredited after NMKL prosedure nr. 4 (2005)
Lead (Pb)	Quantitative ICP-MS, (NMKL method nr.186, 2007)	Accredited after NMKL prosedure nr. 4 (2005)
Arsenic (As)	Quantitative ICP-MS, (NMKL method nr.186, 2007)	Accredited after NMKL prosedure nr. 4 (2005)
Persistent organic pollutants		
POPs	HRGC/HRMS, GC-MSMS or GC-MS (NCI)	Accredited
РАН	GC-MS/MS	Accredited

*AFNOR validated after ISO 16140

8 - Annex II

Table 3: Samples with measured values above detection limit. All samples were compliant according to current regulations.

	IMD	Samples above LOD/LOQ				Compliant/non
Parameter	IMR sample No.	Product	Country of origin	Measured value	Legal limit	compliant
Microbial parameters						
Salmonella	-	-		-	0 cfu/g	Compliant
Listeria monocytogenes	-	-		-	100 cfu/g	Compliant
Coliform bacteria	2021- 1094	Argentine shortfin squid	Argentina	90 cfu/g	No limit in seafood	Compliant
	2021- 1497	Whiteleg shrimp	Vietnam	60 cfu/g		Compliant
	2021- 1067	Scampi	Vietnam	80 cfu/g		Compliant
	2021- 1088	Marinated milkfish	Phillipines	10 cfu/g		Compliant
Thermotolerant coliform bacteria	2021- 1094	Argentine shortfin squid	Argentina	30 cfu/g	No limit in seafood	Compliant
	2021- 1088	Marinated milkfish	Phillipines	30 cfu/g		Compliant
Enterobacteriaceae	-	-	-	-	No limit in seafood	
Enterococci		Argentine shortfin squid	Argentina	100 cfu/g	No limit in seafood	Compliant

Parameter		Samples a	bove LOD/I	_OQ		Compliant/non	
	IMR sample No.	Product	Country of origin	Measured value	Legal limit	compliant	
		Yellowfin tuna	Vietnam	200 cfu/g		Compliant	
	2021- 1497	Whiteleg shrimp	Vietnam	100 cfu/g		Compliant	
	2021- 1498	Whiteleg shrimp	Vietnam	800 cfu/g		Compliant	
Coagulase positive staphylococci		-	-	-	No limit in seafood	Compliant	
Sulphite reducing clostridia					No limit in seafood	Compliant	
Vibrio spp.		Whiteleg shrimp	Vietnam	Detected	No limit in seafood	Compliant	
		Whiteleg shrimp	Vietnam	Detected		Compliant	
		Scampi	Vietnam	Detected		Compliant	
Norovirus and Hepatitis A virus		Pacific oyster	South Korea	Norovirus detected	No limit in seafood	Compliant	
Mould and Yeast		-	-	-	No limit in seafood	Compliant	
Antibiotic resistance							
Multiresistant Klebsiella pneumoniae	2021- 484	Battered Whiteleg Shrimp	Vietnam	Detected	No limit in seafood	Compliant	
Resistant Enterobacter spp.	2021- 559	Peeled Whiteleg Shrimp	Vietnam	Detected		Compliant	

	IMR	Samples a	bove LOD/I	LOQ	Legal limit	Compliant/non
Parameter	sample No.	Product	Country of origin	Measured value		compliant
Resistant Enterobacter spp.	2021- 1071	Battered Whiteleg Shrimp	Vietnam	Detected		Compliant
Multiresistent Klebsiella pneumoniae	2021- 1081	Peeled Whiteleg Shrimp	Vietnam	Detected		Compliant
Resistant Enterobacter spp.	2021- 1497	Whiteleg Shrimp	Vietnam	Detected		Compliant
Prohibited substances						
Malachite green		-	-	-	Not allowed	Compliant
Leuco-malachite green		-	-	-	Not allowed	Compliant
Brilliant green		-	-	-	Not allowed	Compliant
Crystal violet		-	-	-	Not allowed	Compliant
Leuco-crystal violet		-	-	-	Not allowed	Compliant
Chloramphenicol		-	-	-	Not allowed	Compliant
Nitrofuran metabolites		-	-	-	Not allowed	Compliant
Chemical spoilage indicators						
Histamin	2021- 685	Tinned mackerel	United Kingdom	5.9 mg/kg ww	100-200 mg/kg ww	Compliant

	IMR	Samples at	oove LOD/L	_OQ		Compliant/non
Parameter	sample No.	Product	Country of origin	Measured value	Legal limit	compliant
	2021- 686	Tinned, chilifried mackerel	Thailand	9.3 mg/kg ww		Compliant
Carbon monoxide						
Carbon monoxide		Kawakawa fillet	Vietnam	171 μg/kg	No maximum limit in seafood, values >200µg/kg indicate potential addition of CO to product	Compliant
Heavy metals						
Cadmium (Cd)*	2021- 2502	Pacific saury	Taiwan	0.22 mg/kg*	0.1-1.0 mg/kg ww depending on product	Compliant
	2021- 1569	Squid cut- off	Argentina	43 mg/kg*		Compliant
	2021- 1568	Squid-cut- off	Argentina	33 mg/kg*		Compliant
	2021- 2934	Lobster Carapaces	Canada	1.4 mg/kg*		Compliant
Mercury (Hg)#			-	-	0.5-1.0 mg/kg ww depending on product	Compliant
Lead (Pb) #	-	-	-	-	0.1-1.5 mg/kg ww depending on product	Compliant
Arsenic (As) #	-	-	-	-	No limit in seafood	Compliant
Persistent organic pollutants						Compliant
POPs [#]	-	-	-	-	Varies with matrix and analyte	Compliant

Parameter	IMR sample No.	Product	Country of origin	Measured value	Legal limit	Compliant/non compliant
PAH [#]	-	-	-	-	Varies with matrix and analyte	Compliant

^{*} Not for Human Consumption

 $^{^{\#}}$ Most samples over LOD as the method is very sensitive, but all results were compliant with regulations



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