MONITORING PROGRAMME FOR VETERINARY CONTROL ON SEAFOOD PRODUCTS IMPORTED TO NORWAY FROM THIRD COUNTRIES – RESULTS FROM 2020

In accordance with Commission Regulation (EC) No 136/2004, Annex II, Part 1

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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 2020. Samples were collected by personnel at the Norwegian Border Inspection Posts (BIP) and 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 very good cooperation during the conduct of this monitoring programme . A risk assessment for different groups of imported products, made the basis for the sampling plans and the selection of analytical activities. The current trend of hazards, as reported in The Rapid Alert System for Food and Feed (RASFF) notification system, the compositional nature of the products and the annual import quantity of relevant products, formed an up-to-date basis for this risk assessment.

A total of 91 samples from the NFSA, collected at the BIPs, were examined by a selection of analytical methods and assays for microorganisms and undesirable chemical substances. Eight of the samples examined contained undesirable microorganisms or had trace elements or persistent organic pollutants (POP's) exceeding respective maximum levels.

Selected microbiological analyses were performed on 74 samples. *Listeria monocytogenes* was detected qualitatively in four samples, with quantitative measurements below the criteria given by EU of 100 cfu/g at the day of expired shelf-life . Undesirable trace elements were analysed for in 86 samples with two samples (one fish and one squid) exceeding the respective maximum levels for cadmium and one fish species exceeding the maximum levels for mercury. Out of 30 samples examined for POP's, one was identified as non-compliant with its maximum levels for PCDD/Fs and DLPCBs.

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.

According to Commission Regulation (EC) No 136/2004 (EU 2004, FOR-2015-11-30-1347) the monitoring plans must be based upon the nature of the products and the potential risks associated with different product categories, considering all relevant factors such as frequency and number of incoming consignments and results from previous monitoring. The selection of parameters was based on previous findings in this program, as well as information provided in the RASFF, “Rapid Alert System for Food and Feed” system of the European commission.

The spectrum of products examined by NFSA at veterinary border inspection points is large and varied . Thus, the methods used to examine the products are also diverse.

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 and transport. The microbes found in fresh seafood reflects the environment from where it originated and the microorganisms present there. Further, viruses, bacteria, moulds and yeasts can contaminate the product during various steps during processing such as gutting, filleting, breading, etc., and for some of these microorganisms growth in the product may occur if storage conditions are poor, typically at high temperature or during long storage time.

High numbers of fecal bacteria of human or animal origin indicates unsatisfactory sanitary conditions during production or handling of the product. Several types of fecal bacteria can if present in unacceptable numbers also cause foodborne gastroenteritis. To evaluate possible fecal contamination, analyses for common indicator organisms were conducted including assays for coliform bacteria, bacteria in the Enterobacteriaceae family, enterococci and sulphate reducing clostridia.

Staphyloccocci are commonly found on the skin and mucosal linings of humans and animals. Contamination of food usually happens during processing and handling, and high numbers indicates poor hygiene during production. Coagulase positive staphylococci are of particular interest regarding food safety, as they can produce enterotoxins and are a common source of food-borne illnesses. The bacteria die during cooking, but the enterotoxins they produce are heat-stable and remain active after normal cooking procedures.

Furthermore, samples were analysed for specific pathogens relevant for food safety, including bacteria in the genera *Salmonella*, *Listeria* and *Vibrio*. Vibrios are marine and estuarine bacteria that are common in seawater and are therefore naturally present in many seafood products. Some *Vibrio* species can cause severe infections even among previously healthy people, and several more are opportunistic pathogens able to give infection in people with underlaying predisposing health conditions. The prevalence of pathogenic Vibrios are strongly linked to increased water temperature. *Salmonella* spp. and *Listeria monocytogenes* are globally among the most common food-borne pathogens that cause disease and mortality in humans. *Salmonella* are fecal bacteria and contamination usually occurs during production and handling, including during aquaculture production in contaminated water. *L. monocytogenes* originate from soil or the faeces of farmed animals such as sheep, thrive in factory environments and are commonly introduced as a contaminant during food processing. As *L. monocytogenes* are able to multiply at refrigeration temperatures, growth to unacceptable concentrations may occur during chilled storage for extended periods. 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.

Norovirus and Hepatitis A are globally the two most common food-borne viruses associated with food-poisoning from seafood. They reproduce in the colon of humans and can contaminate bivalves and other filter feeding organisms that grow in contaminated water. Norovirus in particular is extremely contagious, and even a very low concentration can cause disease.

Moulds and yeasts both cause spoilage of food products, and moulds can produce allergens and potent mycotoxins. Contaminated food can thus lead to severe allergic reactions or food poisoning. Several types of mycotoxins are also considered carcinogenic, and high concentrations of moulds and yeasts should therefore be avoided in food. Moulds and yeasts are not commonly associated with the marine environment but are widely distributed in terrestrial environments such as soil, in the air, on human skin and several stages across food production lines such as fishing vessels and factory environments with unsatisfactory sanitary conditions. High numbers of both moulds and yeasts present in a food product indicates contamination during production or poor storage conditions.

Antimicrobial resistance is a prevalent challenge to global public health. Carbapenemase-producing Enterobacteriaceae and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are priority pathogens for which research and urgent development of new antibiotics is needed (WHO 2017). Although carbapenem resistant Enterobacteriaceae have been detected in seafood imported from South East Asia (Janecko, Martz et al. 2016), there are currently no regulations on 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, we analysed farmed fish and shellfish imported from Asia and South America for the presence of ESBL-producing Enterobacteriaceae and carbapenem resistant Enterobacteriaceae.

According to current EU legislation (Directive 96/23), certain drugs are illegal in feed used for animals intended for food production. Thus, farmed seafood products were analysed for a selection of seafood-relevant pharmaceuticals: 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.

The survey also included the chemical spoilage indicator histamine. Histamine or “scombroid” poisoning gives symptoms similar to an allergic reaction and is associated with products from fish species that naturally contain high levels of the amino acid histidine. Histidine can over time be turned into histamine by bacteria present in the product, and high levels indicate mishandling (e.g. storage at high temperatures), or that the product is past its shelf-life.

Carbon monoxide (CO) is used to treat fresh fish fillet and especially tuna to retain a fresh, red appearance for a longer storage period. No direct health implications from eating CO-treated fish is known. However, the practice of food cosmetics is problematic, and considered fraudulent. CO reacts with the oxy-myoglobin to form a cherry red carboxy-myoglobin complex. As this CO-complex can be stable beyond the fish-fillet shelf life it can mislead the customer regarding the product quality and mask spoilage, potentially exposing the consumer to high levels of microbes, microbial toxins or histamine. 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).

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. As implemented in EC 1881/2006 (FOR-2015-07-03-870) , the elements cadmium (Cd), mercury (Hg), and lead (Pb), were measured and the compliance of the values with the EU maximum levels was evaluated. Arsenic (As), was also included, although there is no maximum level in seafood . Marine Arsenic primarily exists as molecular species with moderate to low toxicity.

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 dietary human health risk. The compliance of selected samples with established maximum levels for food stuffs (EC1881/2006) was evaluated for these contaminants: polychlorinated dibenzo(p)dioxin and furans (PCDD/Fs), dioxin-like polychlorinated biphenyls (PCBs), and the EU selected “non-dioxin like-PCBs” (ICES-6, which comprise the Sum of PCB 28, 52, 101, 138, 153 and 180) . In addition, flame-retardant compounds in the polybrominated diphenyl ethers family (PBDEs) were measured. The EU recommends a monitoring of the BDE compound s in food (EU 2014). However, maximum levels in food have not yet been established for the BDEs. 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).

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 conducted by IMR. The sampling targeted hazards associated with each kind of imported products, and considered import volumes, compositional nature of the products, results from previous monitoring, geographical origin of samples, and information available in the Rapid Alert System for Food and Feed (RASFF). This report concerns samples imported to Norway in 2020. Due to the Covid-19 situation a decrease in seafood imports was ob served in 2020, and this is reflected in a reduced number of samples in this program.

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 photographed, and relevant information registered in a Laboratory Information Management System (LIMS). The microbiological assay was carried out prior to all other sample handling to avoid contamination. The sample was 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 contaminants where a legal maximum level was established, the tissue specified in the regulation was selected. The analytical methods and procedures used were accredited according to the ISO 17025 standard, unless otherwise specified.

The evaluations of the analytical data in the report is based on the EU maximum levels (Commission Regulation (EU) No. 2006/1881, of which a summary is presented in Annex 1 of this report; Commission Regulation (EU) No. 2073/2005, 37/2010 and 1019/2013) and EU recommendations. The maximum levels provide a legal framework for trade. For measured variables with no established maximum level, interpretation of the analytical values was based on scientific expert opinions (when available), or on the analytical range commonly observed for this undesirable in seafood from pristine or semi-pristine waters.

3 - Results and Discussion

A total of 91 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. Samples with measured values (microbial or chemical) above acceptable levels are listed in Table 2. A summary of EU maximum levels for certain contaminants in foodstuffs are listed in Annex 1.

Table 1: *Analyses performed on different food groups. The "other" category includes all processed food items such as crabsticks, fishcakes, battered, steamed, dried and salted, and marinated and canned food items. The numbers reflect a reduction in the quantity of seafood imports to Norway in 2020 compared to previous years.*

Samples and assays included in the Norwegian veterinary border control of seafood in 2020								
	Fish	Crustaceans	Cephalopods	Bivalves	Feed/Flour	Marine Oils	Other	Total number
Microbiology	40	7	3	2		6	16	74
Antibiotic resistance	3	5						8
Drug residues and dyes	5	7					2	14
Chemical spoilage indicators	20							20
Carbon monoxide	4							4
Undesirable trace elements	42	11	3	2		5	23	86
POP's (PCDD/F, PCB, PBDE)	30							30

3.1 – Microbiology

Seventy-four samples were analysed for the presence of potential human pathogenic bacteria, spoilage bacteria, viruses and mould and yeast.

Sixty samples were examined for the presence of coliform bacteria. Three samples had levels above detection limit (10 cfu/g). These were one sample of *Dissostrichus* sp. (Toothfish) fillet (230 cfu/g) imported from Uruguay, one sample of Yellowfin tuna steak (10 cfu/g), and one sample of *Pangasius* (40 cfu/g), both imported from Vietnam. Further, 72 samples were analysed for the presence of thermotolerant coliform bacteria, and the same sample of Pangasius imported from Vietnam had levels above detection limit (20 cfu/g), which is 10 cfu/g. Two samples analysed for Enterobacteriaceae were both below the detection limit of 10 cfu/g.

For enterococci, two of 62 samples examined were above the detection limit of 100 cfu/g. These consisted of one sample of *Dissostrichus* sp. (Toothfish) fillet (200 cfu/g) imported from Uruguay, and one sample of peeled, frozen Whiteleg shrimp (100 cfu/g) imported from Vietnam.

Listeria monocytogenes was found qualitatively in four out of 57 samples analysed. These consisted of two samples of *Pangasius* sp. fillets from Vietnam, one sample of Rainbow trout fillets from Peru, and one sample of imitation shrimp balls made of toothfish (*Nemipterus* sp.) from Thailand. Further quantitative analyses found that all four samples had <10 cfu/g, which is below the detection limit for the quantitative method.

Eleven samples were analysed for the presence of coagulase positive staphylococci. Of these, two samples had levels above detection limit (100 cfu/g), one sample of Alaskan pollock (1300 cfu/g), and one sample of Greater forkbear (1200 cfu/g). Both samples were imported from China and consisted of dried and salted fillets.

***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 gram 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 dioxins and furans (sum PCDD/F) and sum dioxins, furans and dioxin-like PCBs (sum PCDD/F+DLPCB).*

I MR journal number	Product	Country of origin	Variable above acceptable value	Measured value	Acceptable maximum value of variable
Microbiology					
2020-2287*	Surimi of *Nemipterus* sp.	Thailand	*L. monocytogenes*	Found qualitatively	
				<10 cfu/g	100 cfu/g
2020-1067*	Trout, fillet	Peru	*L. monocytogenes*	Found qualitatively	
				<10 cfu/g	100 cfu/g
2020-1086*	*Pangasius* sp. fillet	Vietnam	*L. monocytogenes*	Found qualitatively	
				<10 cfu/g	100 cfu/g
2020-1088*	*Pangasius* sp. fillet	Vietnam	*L. monocytogenes*	Found qualitatively	
				<10 cfu/g	100 cfu/g
Metals					
2020-2457	*Stolephorus* sp. dried	Vietnam	Cadmium (Cd)	0.48 mg/kg ww	0.05 mg/kg (MU: 20%)
2020-1113	Squid, muscle	New Zealand	Cadmium (Cd)	1.2 mg/kg ww	1.0 mg/kg (MU: 20%)
2020-1950	*Dissostichus* sp. fillet	Uruguay	Mercury (Hg)	0.88 mg/kg ww	0.50 mg/kg (MU: 20%)
Dioxins and PCBs					
2020-1155	Squid oil	South Korea	sum PCDD/F (TEQ pg/g)	3.3	1.75 (MU 25%)
			sum PCDD/F + DLPCB (TEQ pg/g)	10.3	4 (MU 20%)

* *Listeria monocytogenes* was found in the samples using a qualitative method, but further quantitative analyses indicated that the number was below the detection limit of 10 cfu/g, which is below the criteria given by EU of 100 cfu/g at the day of expired shelf-life.

Seventeen samples were analysed for the presence of sulphite reducing clostridia, and one sample of dried baby-shrimp imported from Vietnam had concentrations above detection limit (100 cfu/g), of 400 cfu/g.

All 74 sample were analysed for the presence of *Salmonella* spp. and found negative. Twelve samples were analysed for the presence of potentially human pathogenic *Vibrio* spp., and all were negative. Two samples were examined for the presence of Norovirus type I and II and Hepatitis A by RT-PCR in accordance with ISO15216-1 (Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -Part 1: Method for quantification) . Both samples were negative for viruses. Two samples were examined for the presence of *Escherichia coli*, and both had numbers below detection limit.

Seven samples were examined for the presence of moulds and yeasts. Three samples were at the detection limit of 100 cfu/g for yeast; one sample of dried and salted Alaskan pollock imported from China, one sample of frozen baby-shrimp from Vietnam, and one sample of freeze-dried *Stolephorus* sp. powder imported from Thailand. Two samples were above the detection limit for mould. One sample of dried baby-shrimp powder from Vietnam (100 cfu/g), and one sample of freeze-dried *Stolephorus* sp. powder imported from Thailand.

3.2 – Antibiotic resistance

A total of eight samples were analyzed for the presence of carbapenemase-producing Enterobacteriaceae and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Although, other antibiotic resistant bacteria showed growth on the plates, none of the samples contained carbapenemase-producing Enterobacteriaceae or extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae.

3.3 – Drug residues and dyes

A selection of 14 samples were examined with respect to antimicrobial agents and/or dyes. None of the samples contained detectable residues of the dyes malachite green, leuc-omalachite green, brilliant green, crystal violet or luco-chrystal violet. Furthermore, no samples contained chloramphenicol or any of the nitrofuran metabolites. One sample of dried baby prawn imported from Vietnam, gave indications of residues of sulphonamide residues when examined by an inhibition bioassay. This sample was re-examined by more sensitive and specific chemical methods for a total of 84 drug residues, including 25 sulphonamides, without detection of any agents.

3.4 – Chemical spoilage indicators

Histamine is a toxic compound released if scombroid fish species are exposed to improper storage or transport conditions. Twenty relevant samples were selected for analysis. All measured values were below the maximum levels.

3.5 – Carbon monoxide

Four samples of Yellowfin tuna 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 (Marrone et al., 2015). The highest observed value was 139 µg/kg.

3.6 – Undesirable trace elements

Eighty-six samples were analysed for undesirable trace elements.

Two samples exceeded the maximum level for cadmium (Cd). The Cd concentration in a sample of dried anchovy, *Stolephorus* spp. imported from Vietnam was measured to be 0.48 mg/kg in the dried sample. Assuming the consumption of whole fish, the maximum limit of 0.05 mg/kg ww would be exceeded. A dry matter content of 25%, is often found in other anchovy species like *Engraulis* sp. The Cd concentration in a sample of squid muscle imported from New Zealand, was measured to be 1.2 mg/kg ww. exceeding the maximum level of 1.0 mg/kg ww. The measurement uncertainty for Cd is 20%.

One sample exceeded the maximum level for mercury (Hg). A fillet sample of *Dissostichus sp.* imported from Uruguay exceeded the maximum level of 0.5 mg/kg ww with a measured Hg content of 0.88 mg/kg ww and a measurement uncertainty of 20%.

3.7 – Persistent organic pollutants (POPs)

Thirty samples, selected from risk assessment-criteria, were analysed for three classes of organo-halogen compounds considered undesirable and relevant in seafood. The selected POPs' classes were: The PCDD/Fs, the PCBs and the PBDEs. One sample was identified as non-compliant with its corresponding maximum limits for PCDD/Fs and DLPCBs. The sample was taken at the border inspection post in Ålesund harbour, from a lot of marine oil imported from South-Korea. All other analysed samples were measured with POPs values in the range commonly found in seafood from pristine or semi-pristine waters. The non-compliant sample, after re-analysis, was confirmed to have a sum PCDD/Fs of 3.3 pg/g (TEQ) (ww) and a sum of PCDD/DF an NDLPCBs of 11 pg/g (TEQ) (ww) (WHO-2005 TEF values).

4 - Conclusion

A total of 91 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 2020 .

Nine of the samples examined contained undesirable microorganisms or had trace elements or POPs' exceeding the respective maximum levels. Selected microbiological analyses were performed on 74 samples, and *Listeria monocytogenes* was detected qualitatively in four samples, with quantitative measurements below the criteria given by EU of 100 cfu/g at the day of expired shelf-life . Undesirable trace elements were measured in 86 samples with two samples (one fish and/one squid) exceeding the respective maximum limit for Cd and one fish species exceeding the maximum limit for Hg. POPs were measured in 30 seafood samples and only one sample was identified as non-compliant with regard to its corresponding maximum levels for PCDD/Fs and DLPCBs. The chemical spoilage indicator histamine was examined in 20 relevant samples, all values were below the maximum level.

Due to the Covid-19 pandemic a decrease in seafood imports was observed in 2020, and this was reflected in a reduced number of samples in this program compared to previous years.

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ANNEX 1: Regulatory maximum levels

Table A1: A selection of regulatory maximum levels for contaminants in seafood from the EU Commission regulation no 1881/2006

Selection of regulatory maximum levels for contaminants in seafood from the EU Commission regulation no 1881/2006											
Element or pollutant	Unit of measure-ment	Marin Fish Fillet [1]	Some fish species Fillet [1]	Wild caught Eel Fillet [1]	Fresh water Fish Fillet [1]	Smoked seafood products	Fish liver	Crustaceans: White meat	Bivalves and smoked bivalves [2]	Cephalo-pods [3]	Marine Oils HC [4]
Arsenic (As)	mg/kg ww [6]	-		-	-	-	-	-	-	-	-
Cadmium (Cd)		0.05	0.1-0.25 [8]	0.05	0.05	0.05-0.25 [6,8]	-	0.5	1.0 [6]	1.0	-
Mercury (Hg)		0.5	1.0	1.0	0.5	0.5 [6,8]	0.5	0.5	0.5 [6]	0.5	-
Lead (Pb)		0.3	0.3	0.3	0.3	0.3 [6,8]	-	0.5	1.5 [6]	0.3	-
Sum of dioxins and furans [5]	pg/g TEQ ww [6]	3.5	3.5	3.5	3.5	3.5 [6,8]	-	3.5	3.5 [6]	3.5	1.75
Sum of dioxin like PCBs [5]		-	-	-	-	-	-	-	-	-	-
Sum of dioxins. furans and dioxin like PCBs [5]		6.5	6.5	10	6.5	6.5 [6,8]	20	6.5	6.5 [6]	6.5	6
Sum of six NDLPCBs [5]	ng/g ww [6]	75	75	300	125	75 [6,8]	200	75	75 [6]	75	200
PAH Benzo[a]pyrene	µg/kg ww [6]	-	-	-	-	2-5 [2,6,8]	-	-	5 (6) [2]	-	2
PAH_4 , sum of 4 PAH compounds [7]	µg/kg ww [6]	-	-	-	-	12-30 [2,6,8]	-	-	30 (35) [2]	-	10
Based on Commission regulation 1881/2006, Commission Regulation 1259/2011 amending Regulation 1881/2006 and Commission regulation (EU) 835/2011 amending Regulation 1881/2006.	1) When fish is intended to be eaten whole, the level should be applied to the whole product. 2) Value in brackets concerns smoked bivalves. 3) Without viscera. 4) HC = Human consumption pg/g fat 5) Upper bound sum calculation is assumed. 6) Wet weight (w.w.); the concentration in a naturally moist sample. Values for dried or otherwise processed food should be transformed to w.w. 7) Benzo(a)pyrene, Benzo(a)anthracene, Benzo(b)fluoranthene and chrysene, assuming a lower bound sum calculation. 8) Value change with different biological species										

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