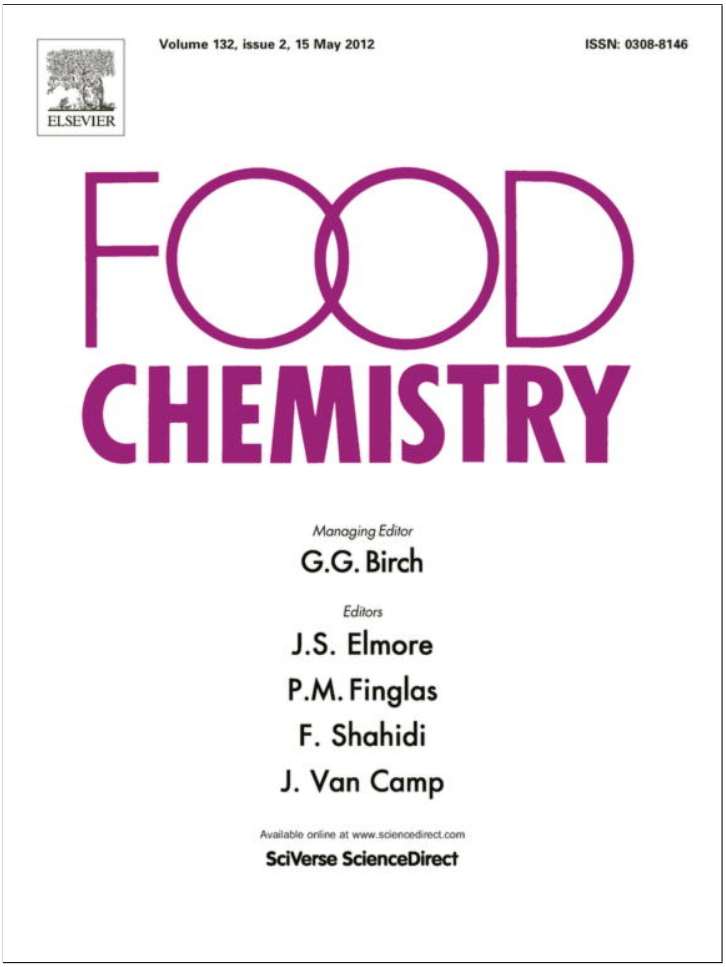

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The assessment of organic mercury in Baltic fish by use of an *in vitro* digestion model

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ABSTRACT

The organic mercury content of five commercially valuable fish species (cod, flounder, turbot, perch and herring) was determined by use of an *in vitro* digestion model in order to assess health risk of fish caught within the Polish Exclusive Economic Zone of the Baltic Sea. Concentrations of total mercury and organic mercury were measured in the muscle tissue of fish and in the products of two-stage gastrointestinal digestion, using atomic absorption spectroscopy (AMA 254). The highest concentrations of organic mercury were found in the muscles of predatory fish that dwell in near-bottom waters. Based on a bioaccessibility estimate obtained from the *in vitro* digestion model, it was found that only 26–62% of organic mercury, depending on the species of fish, was released into the intestinal lumen during the digestion of muscle. Therefore, to postulate the potential toxicity of fish, based on the organic mercury content of the muscle tissue ingested by consumers, is unfounded. The risk assessment should be carried out on the basis of another parameter – the bioaccessibility of organic mercury.

1. Introduction

Mercury (Hg) occurs in the natural environment in a number of forms which differ from each other with regard to physical and chemical properties. Toxicity, distribution and accumulation of mercury in specific environmental compartments depend on these properties. Seafood is a source of valuable dietary components, such as protein, unsaturated fatty acids, vitamins A and D, phospholipids and minerals, but it can also contain organic mercury (Hg_{org}) which is present in marine organisms mainly in the form of methylmercury (MeHg). In societies which consume fish and shellfish, exposure to methylmercury may often occur and this is particularly dangerous during the prenatal period of pregnancy (Burger & Gochfeld, 2007; Carbonell, Bravo, Fernández, & Tarazona, 2009).

The maximum admissible dose of mercury tolerable to a healthy human being is 5.0 μg kg^{-1} of body weight, which includes 1.6 μg of methylmercury per one kilogramme of body weight. According to the World Health Organisation (WHO, 1990), mercury concentration in the muscles of fish intended for human consumption should not exceed 0.5 μg Hg g^{-1} wet mass. Regulation (EC) No. 466/2001 and regulation (EC) No. 221/2002, which are constituents of European Union Legislation on food hygiene, establish the maximum limit for mercury in a whole fish at 0.5 μg g^{-1} w.w. An exception is made for predatory fish which are allowed to have a higher mercury concentration of 1.0 μg g^{-1} w.w. Although the period of exposure is necessarily important in assessing the risk associated with the consumption of fish by a healthy adult, especially if it is prolonged, in pregnant women and small children, a single meal may pose a danger, particularly when it consists of predatory fish (Burger & Gochfeld, 2007).

The true toxicity of xenobiotics, including MeHg, depends on their bioaccessibility, meaning the percentage of the chemical compound released from food into the liquid present in the digestive tract during digestion and which is therefore available for absorption via intestinal mucosa for use by the organism (Shim, Ferruzzi, Kim, Janle, & Santerre, 2009). The assimilability of mercury released during digestion mainly depends on the form in which this toxin is introduced into an organism. This is because the chemical form determines the effective propensity for reacting with biological ligands which enter the area of absorption, mainly duodenum, in order to cross the intestinal wall.

The total mercury concentration in the tissue of consumed fish does not supply information on the true risk connected with fish consumption. Measurement of mercury concentration in the liquid part of digested food, which contains the bioaccessible fraction, allows estimation of safe intake levels of the consumed fish, based on the recommended standards. *In vivo* studies are expensive and labour-intensive and, for this reason, *in vitro* experiments, which are simple and relatively inexpensive, provide good alternatives for research conducted on animals. Despite their undeniable advantages, *in vitro* studies on xenobiotic bioaccessibility in food products remain rare (Hur, Lim, Decker, & McClements, 2011).

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The aim of the presented project was to answer the two following questions: first, what percentage of MeHg present in the muscle tissue of fish is released during the digestive process, and second, what is the influence of matrix composition on the bioaccessibility of this toxin, considering that this is the most often ingested form of fish muscle tissue by humans.

2. Materials and methods

2.1. General

Five commercially valuable fish species were studied: cod (*Gadus morrhua*; $n = 31$), flounder (*Platichthys flesus*; $n = 22$), turbot (*Psetta maxima*; $n = 16$), perch (*Perca fluviatilis*; $n = 20$); herring (*Clupea harengus*; $n = 22$). All fish were caught between January and December, 2009, in the Polish Exclusive Economic Zone (EEZ) of the southern Baltic Sea and all fish measurements and dissections, as well as subsequent chemical analyses, were conducted in the Mercury Laboratory, belonging to the Institute of Oceanography, University of Gdansk. The sampled fish were transported to the laboratory, rinsed with deionised water and measured to precision levels of 1 mm and 1 g for total length and total weight, respectively. The collected muscle samples were mechanically homogenised, placed in polyethylene bags and stored at a temperature of −20 °C prior to analysis. Samples were lyophilised and powdered prior to further analysis.

All reagents used during analysis were of analytical grade: L-cysteine hydrochloride, pepsin, pancreatin, bile salts, lipase, α-amylase, hydrochloric acid, sodium bicarbonate, toluene, trisodium citrate dehydrate and nitric acid (V). Before analysis, all laboratory glassware was soaked in 5 M HNO_3 for 24 h, followed by triple rinsing in deionised water and drying at 80 °C for 12 h.

2.2. Determination of total mercury (Hg_T)

Determination of total mercury concentrations in the tissue samples of the studied fish was performed by the atomic absorption spectroscopy method, with the use of an AMA-254 mercury analyzer.

Lyophilised tissue samples, weighing from 50 to 100 g, were placed in pre-heated nickel sample vessels and automatically introduced into the oven. The tissue samples were dried at 120 °C for 5 min, and ashed at 800 °C over 3 min. The combustion products were transported via a stream of oxygen gas and absorbed on a gold trap. After desorption, the rate of absorption was measured at a wavelength of 253.65 nm during a 1 min measurement cycle. The method precision was expressed by a repeatability variance value equal to ca. 10%. The total mercury concentration values were expressed in ng g^{-1} dry mass. Method precision and accuracy were verified by the analysis of mercury concentration in accordance with certified biological standards, namely QTM057BT and QTM055BT (Quasimene Laboratory Performance Studies Trace Metals in biota), for which the obtained margin of error was 4%. The determined detection limit for mercury was 0.005 ng g^{-1}.

2.3. Determination of organic mercury (Hg_{org})

Selective extraction of mercury from the muscle tissue of the investigated fish was conducted in accordance with the procedure described by Carbonell et al. (2009) and Barska and Skrzyński (2003) and duly modified. Samples of lyophilised fish muscle, weighing from 0.5 to 1.0 g (accuracy: 0.001 g), were placed in 50 cm^3 vials with ground glass stoppers. After adding 5 cm^3 of 6 M HCl and 5 cm^3 of toluene, the vials were incubated in an ultrasonic bath for 30 min, followed by centrifugation at 3500 rpm for 30 min at a temperature of 2 °C. The top layer of toluene was poured into 15 cm^3 borosilicate vials with screw caps; a volume of 5 ml of toluene was added to each remaining pellet and the vials were then shaken and spun. The toluene layer was again decanted into 15 cm^3 vials and, in this way, the toluene extract of organic mercury (ca. 10 cm^3) was obtained. The extract was treated with 1 cm^3 of a solution consisting of 1% cysteine hydrochloride in 20% v/v sodium citrate, and later shaken for 10 min and centrifuged. A volume of 0.1 cm^3 of the aqueous layer was placed in pre-heated nickel sample vessels and analysed for organic mercury content by means of an AMA-254 mercury analyzer. An appropriate programme, with a drying time of 1 min 10 s and an ashing period of 2 min 30 s, was chosen beforehand.

Reference material from the European Community Bureau of Reference relating to the presence of BRC-463 mercury and methylmercury in tuna fish ($[MeHg^+] = 3.04 \pm 0.16$ mg kg^{-1}) was used to check the accuracy and precision of organic mercury (methylmercury) extraction from the muscle tissue of the studied fish. This reference material was analysed according to the aforementioned methodology and the results ($n = 7$) are presented in Table 1. The average rate of recovery from this reference material was 96.2% (range: 90.3–102%), while the standard deviation was equal to 3.95. The coefficient of variation for three replicates returned a value lower than 10% and replicate analysis on organic mercury extracts from fish muscle tissue was performed for 94% of samples. The mean value of the blank sample, based on 10 measurements, was 0.379 Hg_{org} ng cm^{-3} of extract.

2.4. Calibration

Calibration was conducted by dissolving 125.2 mg of methylmercury chloride (CH_3HgCl) in 3 cm^3 of acetone in a 100 cm^3 measuring flask, and filling the flask with 0.1 M HCl to obtain a stock solution of 1000 μg cm^{-3}. A solution of 10 μg cm^{-3} was prepared from the stock to be used for further dilutions. Volumes of 0.2, 0.3, 0.4 and 0.6 cm^3 of the diluted stock solution were transferred into 100 cm^3 measuring flasks and 0.1 M HCl was added to create a final volume of 100 cm^3 in each flask. The prepared diluted solutions were then used to calibrate the analyzer.

2.5. In vitro digestion procedure for fish muscle tissue

A 5 g sample of mechanically homogenised muscle tissue was collected per fish and digested *in vitro* according to the procedure described by Shim et al. (2009) and Cabañero, Madrid, and Cámara (2004). Raw muscle was dried at 40 °C for 2 days. Samples were placed in 100 cm^3 conical flasks and 15 cm^3 of digestive juices (10% w/v pepsin in 0.1 M HCl) were added. Samples were incubated in a thermostatic water bath with periodic shaking at a temperature of 37 °C for 2 h. After 1 h of stomach digestion simulation, pH was checked and immediately lowered to pH 2 with 6 M HCl. Upon completion of the gastric digestion, pH was raised to 6.8 with

Table 1
Concentrations of $MeHg^+$ [mg kg^{-1}] in reference material BCR-463.

No.	$MeHg^+$
1	2.853
2	2.885
3	3.038
4	2.988
5	2.873
6	3.093
7	2.746

a saturated solution of sodium bicarbonate. Ten cubic centimetre of intestinal juices were then added (1.5% w/v pancreatin, 0.5% w/v α-amylase, 0.5% w/v lipase and 2.5% w/v bile salts in 0.1 M $NaHCO_3$) and intense shaking was conducted for 1 min. Following this, samples were placed in a thermostatic water bath with periodic shaking at a temperature of 37 °C for 2.5 h. After finishing the digestion, 10 cm^3 aliquots were transferred into glass vials and centrifuged at 3500 rpm for 1 h. The supernatant was then filtered through a 0.45 μm Millipore filter and analysed for organic mercury content.

2.6. Analytical procedure for determining organic mercury in supernatant after a two-stage in vitro gastrointestinal digestion

A volume of 2.5 cm^3 of toluene was added to 2.5 cm^3 of post-ultrafiltration supernatant (10 kDa), the solution was shaken for 10 min in an ultrasonic bath and then centrifuged at 3000 rpm for 30 min at an approximate temperature of 2 °C. The top layer of toluene was decanted into a 15 cm^3 vial, while the remaining volume was treated with 2.5 cm^3 of toluene, shaken and spun as described previously. The top layer was again poured off into a 15 cm^3 vial to leave a toluene extract containing around 5 cm^3 of organic mercury. One cubic centimetre of 1% cysteine hydrochloride in 20% sodium citrate solution was added to the extract, which was then shaken and centrifuged as described above. The concentration of organic mercury was measured in 10^{-3} cm^3 of aqueous layer using an AMA-254 mercury analyzer.

2.7. Statistical analysis

Data were analysed with the use of the StatSoft STAISTICA 9 statistical package. A median value was used during the analysis because most data displayed nonparametric distribution. The Shapiro–Wilk test was used to evaluate the fitting of variables to the normal curve, while the Spearman's test was applied to obtain rank correlations. The ANOVA test, the Kruskal–Wallis ranking and the median test were applied to estimate the significance of differences between multiple independent samples. All hypotheses were tested at a significance level of $p < 0.05$. Bioaccessibility (%) of organic mercury was calculated using the following equation:

$$\%\mathrm{bioHg_{org}} = \frac{[\mathrm{Hg_{org}}] \cdot Vs}{[\mathrm{Hg_{org}}]_{\mathrm{tm}} \cdot M_{\mathrm{tm}}} 100\% \quad (1)$$

where, $\%\mathrm{bioHg_{org}}$ – bioaccessibility (%), $[\mathrm{Hg_{org}}]$ – organic mercury concentration in supernatant after ultrafiltration (10 kDa) (ng g^{-1}), $[\mathrm{Hg_{org}}]_{\mathrm{tm}}$ – organic mercury concentration in muscle tissue (ng g^{-1}), M_{tm} – mass of muscle tissue (g), Vs – solution volume (cm^3).

The monthly consumption limits (MLC) of the investigated fish were calculated according to the method used by the US EPA (US Environmental Protection Agency). (1997).

$$\mathrm{MLC} = \frac{DF \cdot MB}{[\mathrm{Hg_{org}}]} \cdot \frac{30.44\ \mathrm{day} \cdot \mathrm{month}^{-1}}{MC} \quad (2)$$

where, DF – reference dose (10^{-4} mg kg^{-1} body weight day^{-1}), MB – body mass (70 kg), $[\mathrm{Hg_{org}}]$ – organic mercury (methylmercury) concentration in muscles (mg kg^{-1}), MC – meal mass (0.227 kg $meal^{-1}$).

3. Results

The concentrations of total and organic mercury in the muscle tissue of the fish caught in the southern Baltic were characterised by a considerably wide range within each species, in which concentrations tended to increase with the length and weight of the specimens (Table 2). Research carried out in other bodies of water confirms the directly proportionate relationship between mercury concentration in the muscles of a fish and the length of the fish (Amlund, Lundebye, & Berntssen, 2007; Polak-Juszczak, 2009) and a statistically relevant correlation between these two parameters has been proven ($r = 0.74$, $p < 0.0001$) (Burger & Gochfeld, 2007). In addition to this, Staveland, Marthinsen, Norheim, and Julshamn (1993) also noted a statistically relevant relationship between mercury concentration and the age of the fish ($r = 0.57$, $p < 0.02$).

In cod, flounder and turbot, the determined levels of total mercury and organic mercury in muscle tissue and organic mercury, in liquid containing the bioaccessible fraction of this metal, were distribution-free (nonparametric). The corresponding values for perch and herring were distributed parametrically. The highest concentrations of total mercury and organic mercury were found in the muscles of turbot, perch, cod and flounder (Table 2) and the highest percentage shares of organic mercury were measured in the same species. Mercury concentrations in herring muscle were significantly lower than those in the other species and the share of bioaccessible organic mercury fraction was also smaller (<80%) ($p < 0.05$).

After a two-stage gastrointestinal *in vitro* digestion of fish muscle tissue, solutions were obtained that contained the bioaccessible organic mercury fraction. The highest amount of organic mercury per 1 g of raw muscle was released during the digestion of perch, turbot and flounder tissue. In the cases of cod and herring, the concentrations of bioaccessible organic mercury fraction in the solutions obtained after digestion of 1 g of dry muscle tissue were lower. However, the highest levels of organic mercury bioaccessibility (%) were measured in the muscles of herring and perch. Flounder and turbot muscles were characterised by lower mercury bioaccessibility, while those of cod returned the lowest value for this parameter (Table 2).

4. Discussion

Fish muscles, which are the most commonly consumed parts of a fish by humans, are the main points of accumulation for methylmercury (Wiener, Krabbenhoft, Heinz, & Scheuhammer, 2003) and this is due to interaction between MeHg and cysteine-rich myogens in the sarcoplasm (Kannan et al., 1998). Owing to the high capacity of MeHg to bioaccumulate in the trophic chain in the muscles of predatory fish, this compound constitutes over 90% of total mercury (Kasper et al., 2009).

Ingestion is the primary route of entry for MeHg into the bodies of fish; it is characterised by a high coefficient of intestinal wall permeability, ranging from 56% to 95%, in some cases reaching up to 100%, while the intestinal permeability, coefficient for inorganic mercury ranges from 10% to 27% (Kasper et al., 2009; Wiener et al., 2003). It is then transported via the blood, mainly in erythrocytes, into the cells of the organism and it has been suggested that muscle tissue, where it is the only organic form of this element to be found, acts as a reservoir (Cabañero, Madrid, & Cámara, 2007; Kasper et al., 2009; Wiener et al., 2003). Therefore, muscles are the primary points of accumulation in fish originating from areas with low level contamination (Havelková, Dušek, Némethová, Poleszczuk, & Svobodová, 2008). Of the fish studied as part of this investigation, only herring displayed organic mercury concentrations that were statistically different in comparison with the other species ($p < 0.05$) (Fig. 1).

It has been proven that organic mercury concentration in fish muscle increases with an increased share of animal origin food in the fish's diet (Kasper et al., 2009). This explains why fish from lower trophic levels, such as herring, exhibit a lower share of or-

Table 2
Statistical description of biometric parameters, total mercury concentrations [Hg_T (ng g^{-1})], organic mercury concentrations [Hg_{org} (ng g^{-1})], bioaccessible fraction of organic mercury [$bioHg_{org}$ (ng g^{-1})] and bioaccessibility of organic mercury (%$bioHg_{org}$) in the muscle tissue of fish. Symbols: x – mean value, SD – standard deviation, min – minimal value, max – maximal value, M – median, d.m. – dry mass, w.m. – wet mass.

Fish	Cod $n = 31$		Perch $n = 20$		Herring $n = 22$		Flounder $n = 22$		Turbot $n = 16$	
Estimator	$\bar{x} \pm$ SD min–max	M	$\bar{x} \pm$ SD min–max	M	$\bar{x} \pm$ SD min–max	M	$\bar{x} \pm$ SD min–max	M	$\bar{x} \pm$ SD min–max	M
Length [cm]	51 ± 21 30–102	42	32 ± 5 21–41	34	22 ± 2 18–26	22	36 ± 5 28–47	36	36 ± 8 27–57	34
Weight [g]	2068 ± 2858 381–11,140	771	679 ± 353 122–1400	638	153 ± 75 55–298	151	651 ± 207 219–967	672	1011 ± 606 572–2994	853
[Hg_T] w.m.	62 ± 74 8–335	37	40 ± 24 8–92	31	18 ± 9 6–39	17	66 ± 57 14–254	59	113 ± 81 18–336	80
[Hg_T] d.m.	420 ± 417 77–1944	322	383 ± 167 87–685	355	95 ± 46 20–208	91	303 ± 294 60–1373	258	617 ± 455 92–1871	428
[Hg_{org}] w.m.	53 ± 67 6–288	31	32 ± 18 9–70	26	14 ± 7 5–31	14	55 ± 49 14–231	45	91 ± 62 19–268	68
[Hg_{org}] d.m.	365 ± 380 63–1674	223	316 ± 128 89–579	305	75 ± 38 18–151	64	253 ± 259 59–1247	198	476 ± 364 83–1424	364
%Hg_{org}	85 ± 9 61–100	86	85 ± 9 74–100	81	80 ± 14 57–100	77	82 ± 10 65–98	81	82 ± 10 66–100	82
[$bioHg_{org}$] w.m.	12 ± 15 4–81	8	18 ± 16 2–60	13	9 ± 6 2–23	7	16 ± 13 3–51	12	25 ± 25 2–84	13
[$bioHg_{org}$] d.m.	74 ± 93 21–490	45	147 ± 84 42–332	120	42 ± 25 14–101	36	74 ± 66 15–285	57	101 ± 81 13–283	68
%$bioHg_{org}$	28 ± 19 2–62	21	48 ± 21 19–87	47	62 ± 25 16–97	61	38 ± 22 6–91	35	26 ± 19 5–65	23

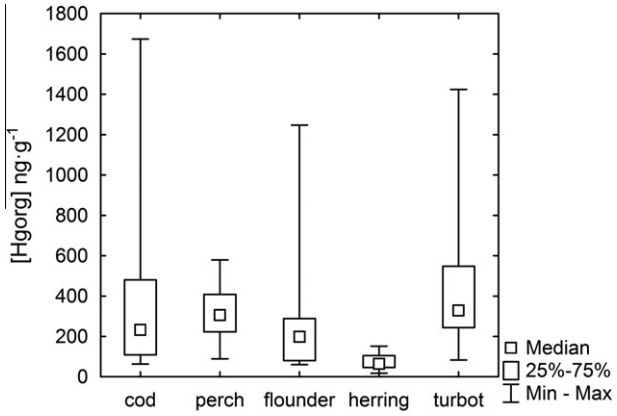

Fig. 1. Organic mercury concentrations in dry mass of skeletal muscle of five fish species.

ganic mercury in their muscle (ca. 80%) when compared with other species, a trait supported by the findings of Barska and Skrzyński (2003). Herring from the southern Baltic feed on planktonic crustaceans (*Pseudocalanus min. elongatus*) as a matter of preference (Falkowska, Kwaśniak, & Bełdowska, 2010), while those in shallow coastal waters tend to prey on larval fish from the *Gobiidae* family (Wyszyński, 1997), so the location of the feeding ground may also be regarded as a significant factor.

Organic mercury concentrations in pelagic fish are usually lower than those in benthic fish (Falkowska et al., 2010). A relationship between diet and sediment, the latter being the principle place of mercury storage and methylation in aquatic basins, results in higher concentrations of organic and inorganic forms of mercury in detritivores (Zhou & Wong, 2000). The highest determined concentrations of total mercury, with a large share of organic mercury, in the muscles of flounder, turbot and cod have confirmed this relationship. Balshaw, Edwards, Ross, and Daughtry (2008) and Nakao, Seoka, Tsukamasa, Kawasaki, and Ando (2007) have been able to conclude that the organic mercury content in fish muscle is inversely proportional to the fat content in tissue, and that it also decreases with a decreased protein fraction due to the reduction of

Table 3
The contents of protein (g.100 g^{-1} w.m.), fat (g.100 g^{-1} w.m.) and total and soluble selenium (μg g^{-1} w.m.) in fish from the North Sea and the Baltic.

Species	Protein	Fat	Total selenium	Soluble selenium
Herring	16.3[a]	10.7[a]	0.347[b]	0.068[b]
Perch	18.4[a]	0.8[a]	–	–
Cod	17.7[a]	0.7[a]	0.305[c]	–
Flounder	16.5[a]	1.8[a]	0.371[b]	0.101[b]
Turbot	17.2[b]	–	0.473[b]	0.093[b]

[a] Kunachowicz, Nadolna, and Przygoda (2005).
[b] Onning (2000).
[c] Miklavcic et al. (2011).

binding sites. These findings explain the highest percentage organic mercury content, which was found in the low-fat muscles of cod, as compared to that which was determined in herring muscle (Table 3).

Organic mercury, released into the intestinal lumen during digestion of consumed fish muscle tissue, poses a true risk to human health. Based on conducted research it may be concluded that, in the cases of flounder, turbot, perch and herring, the concentration of the bioaccessible organic mercury fraction is highly correlated with organic mercury concentration in the muscle tissue. The linear correlation coefficients, confirmed by Pearson's test and representing the relationship between organic mercury concentration in muscle tissue and bioaccessible organic mercury concentration, were 0.59 and 0.60 for herring and perch, respectively, while those for flounder, turbot and cod (Spearman's test) were 0.65, 0.52 and 0.30, respectively. Therefore, as the concentration of organic mercury in the muscle tissue of the studied fish increases so does the concentration of bioaccessible organic mercury fraction in the products of tissue digestion (Fig. 2).

Despite the proportionality relationships indicated above, bioaccessibility, calculated as the percentage of organic mercury released during digestion in relation to the organic mercury content in muscles, is inversely proportional to the concentration of organic mercury in the muscle tissue of the investigated fish. This is demonstrated by the negative values of linear correlation

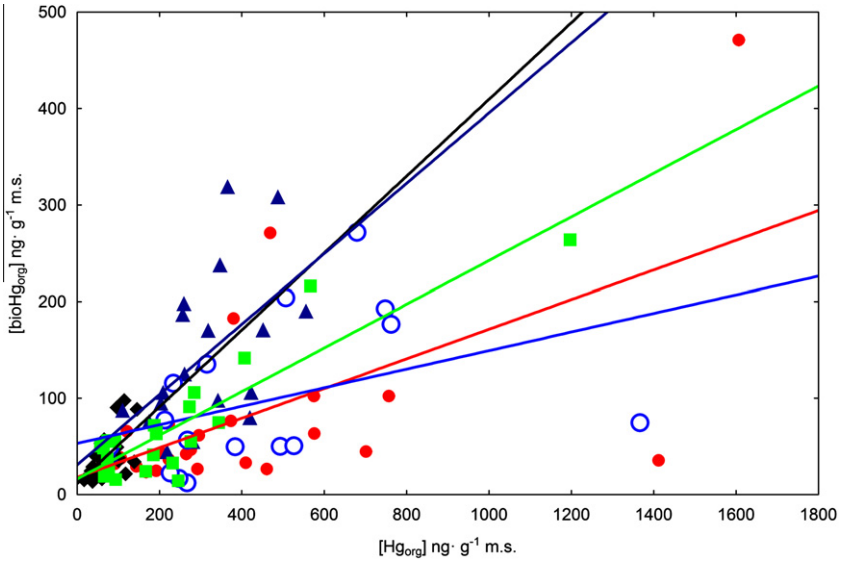

Fig. 2. Dependence of bioaccessible organic mercury fraction concentration [bioHg$_{org}$] on organic mercury concentration [Hg$_{org}$] in muscle tissue ■ flounder: $y = 0.26199x + 16.2898$ $R = 0.89$, ● cod: $y = 0.153517x + 17.9048$ $R = 0.68$, ◆ herring: $y = 0.391830x + 11.4696$ $R = 0.60$, ▲ perch: $y = 0.364566x + 30.8168$ $R = 0.56$, ○ turbot: $y = 0.096174x + 53.1842$ $R = 0.39$.

coefficient for the relationship between organic mercury bioaccessibility and organic mercury concentration in the muscle tissue of herring (−0.33), perch (−0.22), flounder (−0.42), turbot (−0.23) and cod (−0.66); the coefficient values for herring and perch were confirmed with Pearson's test, while those for flounder, turbot and cod were verified with Spearman's test. The differences in bioaccessibility of organic mercury obtained from the muscle tissue of the studied fish were not statistically significant ($p > 0.05$) (Fig. 3).

The mean bioaccessibility of organic mercury for the digested muscles of the investigated Baltic fish was ca. 37%. This value is comparable to that reported by Laird, Shade, Gantner, Chan, and Siciliano (2009) for the traditional food consumed and digested by the native inhabitants of northern Canada (35%), consisting mainly of fish and the innards of marine mammals. The bioaccessibility rates calculated by Cabañero et al. (2004, 2007) for the *in vitro* digested muscle tissue of tuna (*Thunnus* spp.), European pilchard (*Sardina pilchardus*) and black scabbardfish (*Aphanopus carbo*) were equal to 9%, 13% and 17%, respectively. It is notable that these values were considerably lower than the mean bioaccessibility rates determined for the Baltic cod (21%), perch (47%), herring (61%), flounder (35%) and turbot (23%) (Fig. 3).

Cabañero et al. (2004, 2007) and Laird et al. (2009) suggested that the differences in mercury bioaccessibility are influenced by

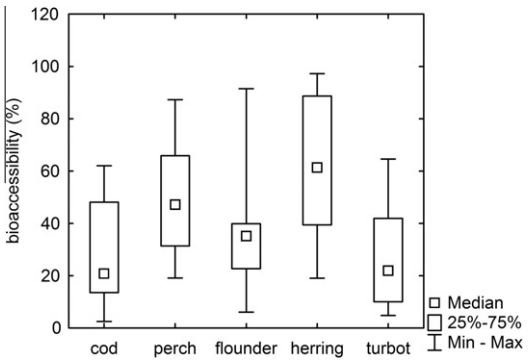

Fig. 3. Characteristic of bioaccessibility of organic mercury released during *in vitro* digestion from the muscle tissue of cod, perch, herring, flounder and turbot caught in the southern Baltic in 2009.

the species of fish, in particular by protein and fat contents, lower propensity of enzymes to free trace elements and the lack of intestinal villi enzymes during *in vitro* digestion. In addition to this, Cabañero et al. (2004), Cabañero et al. (2007) pointed to the antagonistic action of selenium, a natural component present in fish muscle, in relation to mercury toxicity. It is likely that the selenium forms complexes with the mercury, resulting in a low percentage of bioaccessible mercury in the products of digestion. Based on selenium concentrations measured in pelagic and near-bottom dwelling fish species (Table 3), there is a high probability that the low concentration of soluble selenium in herring muscles can explain the high bioaccessibility rate of organic mercury for this fish species. Laird et al. (2009) also underlined that the operational parameters of an *in vitro* model, such as enzyme concentrations, the numbers and durations of digestion stages and the presence of gastrointestinal microbial organisms, were also significant.

It has been observed that, in near-bottom dwelling fish (namely, turbot, flounder and cod), organic mercury concentration in muscles increases with the increasing age of the animal, while the compound's bioaccessibility decreases (Fig. 4). In the cases of perch and herring, an opposite relationship was observed and organic mercury bioaccessibility increased with the increasing concentration of the compound in the muscles and the length of fish. However, the linear correlation coefficients were lower than 0.5 and further research is needed on a sample with a higher number of fish.

Besides work-related exposure, fish are considered to be the main source of mercury for humans (Cabañero et al., 2007). The toxic effect of mercury in a human organism does not depend so much on the total concentration of the element in food products, but rather on the form in which it is present and its level of bioaccessibility. Using the values of organic mercury concentration in muscle tissue and the compound's bioaccessibility, the monthly limit of meals containing the studied Baltic fish species has been calculated with Eq. (2) (Table 4).

Based on the obtained calculations, it can be concluded that herring and cod are the safest sources of fish-based nutrition for consumers. This is due to the low organic mercury concentrations found in the products of human digestion for both species. It should be noted that data on the concentration of the bioaccessible organic mercury fraction present in the breakdown products of fish

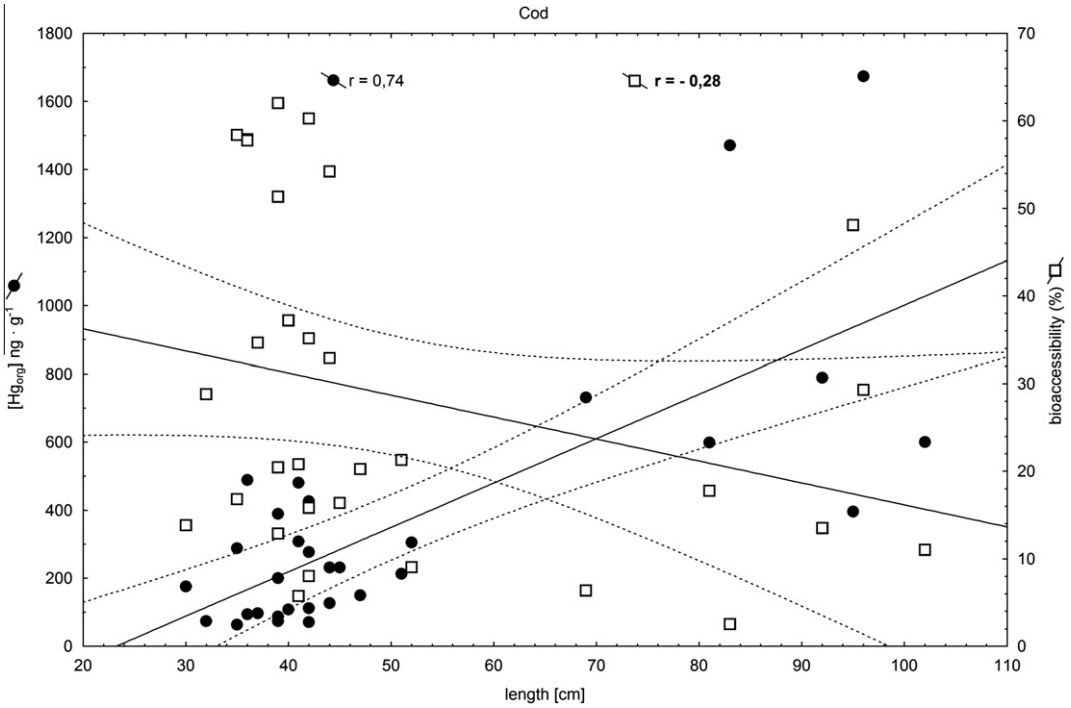

Fig. 4. Relationship between Hg_{org} concentration in muscles and bioaccessibility of Hg_{org}, and the length of near-bottom dwelling fish, for example cod.

Table 4
Number of risk-free meals per month based on organic mercury concentration [Hg_{org}] in muscles and the concentration of bioaccessible fraction released as a result of *in vitro* digestion [$bioHg_{org}$].

Fish species	Number of meals	
	[Hg_{org}]	[$bioHg_{org}$]
Cod (*Gadus morrhua*)	30	117
Perch (*Perca fluviatilis*)	36	72
Herring (*Clupea harengus*)	67	134
Flounder (*Platichthys flesus*)	21	78
Turbot (*Psetta maxima*)	14	72

digestion are not considered when the limits of risk-free consumption are being set. This is the case despite the fact that this parameter visibly indicates the lower bioaccessibility of organic mercury as compared to its expected value, a conclusion similarly reached by Cabañero et al. (2007). In order to exceed the admissible reference dose, and basing the estimate on organic mercury bioaccessibility, a consumer eating fish caught in the Polish Exclusive Economic Zone of the Baltic Sea would have to ingest one of the following amounts each month: ca. 30 kg of herring, 26 kg of cod, 18 kg of flounder, 16 kg of perch or 16 kg of turbot.

Furthermore, based on the study by Shim et al. (2009), it can be concluded that the environment of a digestive tract allows for important physiological interactions between mercury from fish tissue and the chemical components of concurrently digested food. It has been proven that naturally occurring chelators in food, such as catechins (green tea), theaflavins and thearubigins (black tea), soy protein and wheat bran, lower the bioaccessibility of mercury from *in vitro* digested fish tissue, probably due to the formation of insoluble complexes (Shim et al., 2009). Hojbjerg (1996) also reported that the high level of protein in food products decreases the absorption of organic and inorganic mercury forms after exposure via food. Therefore, the assessment of risk associated with fish consumption, based exclusively on total mercury concentration or methylmercury concentration in fish tissue, is unfounded. The presented results require a further comprehensive investigation based on much larger numbers of samples, as well as confirmation by *in vivo* studies.

5. Conclusions

The *in vitro* model of muscle tissue digestion was employed in order to establish the bioaccessibility of organic mercury and to assess the risk to which the consumers of Baltic fish are exposed. The presented research demonstrates that commercially valuable species of fish, namely cod, flounder, turbot, perch and herring, caught in the Polish Exclusive Economic Zone are a safe source of valuable nutritional components. The measured concentrations of organic mercury in the analysed muscle samples, despite the wide range of results obtained, were many-fold lower than the established reference doses that describe the admissible concentration of mercury in fish and other seafood. The highest total mercury concentrations and organic mercury concentrations were determined in the muscle tissue of flounder and turbot, while the lowest ones were found in herring muscle; the differences were statistically significant ($p < 0.05$). This finding indicates that the location of feeding ground, the food sources and the chemical composition of the muscle tissue all have influences on the accumulation of the toxin.

It was established that, along with the rise in concentration of organic mercury in the muscles of Baltic fish, there was an increase in the concentration of bioaccessible organic mercury present in their digestion products. An inversely proportional relationship was identified between the concentration of organic mercury in the fish muscle tissue and its level of bioaccessibility, calculated using Eq. (1). It was observed with bottom-dwelling species, however, such as turbot, flounder and cod, that, while the organic mercury concentrations in the muscles increased with the length of the fish, they were accompanied by a simultaneous decrease in bioaccessibility.

The average bioaccessibility rates for organic mercury from the digested muscles of Baltic fish species were as follows: cod 21%, turbot 23%, flounder 35%, perch 47%, herring 61%. The results of

the research carried out suggest that conclusions made about the potential toxicity of fish intended for human consumption, based only on the concentrations of total mercury or methylmercury in muscle tissue, are groundless. They also suggest that the bioaccessibility of organic mercury is a parameter of much greater relevance and one which should be considered during risk assessment. Future comprehensive investigations, by *in vivo* methods in this field of studies, should be continued.

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