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JOANNA KUC, ADAM GROCHOWALSKI, TOMASZ ŚWIERGOSZ*

CORRELATION OF HEXABROMOCYCLODODECANE AND CONTENT OF FAT IN FISH

BADANIE KORELACJI STĘŻENIA HEKSABROMOCYKLODODEKANU I ZAWARTOŚCI TŁUSZCZU W RYBACH

Abstract

A study on the correlation between the concentration of hexabromocyclododecane and the percentage of fat in meat tissues of selected marine fish species, both marine and farmed, has been carried out. The relationships between variables based on a determination of the coefficients of linear correlation r-Pearson were determined and graphs illustrating these dependencies were presented.

Keywords: hexabromocyclododecane, correlation analysis

Streszczenie

Przeprowadzono badanie korelacji pomiędzy stężeniem heksabromocyklododekanu i procentową zawartością tłuszczu w tkance mięsnej wybranych gatunków ryb morskich połowowych i hodowlanych. Określono zależności pomiędzy zmiennymi na podstawie wyznaczania współczynników korelacji liniowej r-Pearsona i przedstawiono wykresy obrazujące te zależności.

Słowa kluczowe: heksabromocyklododekanm, badanie korelacji

* Ph.D. Eng Joanna Kuc, Prof. Ph.D. D.Sc. Eng. Adam Grochowalski, Eng. Tomasz Świergosz, Department of Analytical Chemistry, Faculty of Chemical Engineering and Technology, Cracow University of Technlogy.

Abbreviations

BFR – Brominated Flame Retardant
EPS – Expanded Polystyrene
ESIS – European Chemical Substances Information System
HBCD – 1,2,5,6,9,10 – hexabromocyclododecane
HIPS – High Impact Polystyrene
HPV – High Production Volume
OECD – Organization for Economic Cooperation And Development
POP – Persistent Organic Pollutant
REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals
SD – Standard Deviation
XPS – Extruded Polystyrene

1. Introduction

Hexabromocyclododecane (CAS: 25637-99-4 for HBCD; 3194-55-6 for 1,2,5,6,9,10 – HBCD) is a brominated fire retardant added to many products made from plastic in order to ensure their incombustible properties [1–3]. According to information provided by the European Chemical Substances Information System (ESIS) [4], HBCD is on the list of HPV substances [5]. The HBCD production volume between 1995 and 1997 is estimated at 11,500 tons per year [6], while in 2001, production increased to 16700 tons of HBCD per year, of which most (9,500 tons) was in Europe [7]. Statistical data from 2002 indicate that the global production volume of HBCD from 2003 to 2007 also saw an increase [8], and as in the previous years, the largest consumer is Europe, especially Poland and Eastern European countries [6]. HBCD is mainly used as an additive to non-flammable expanded and extruded (EPS and XPS) polystyrene foams, high impact polystyrene (HIPS) and the textile industry. The segment production of building materials EPS and XPS consume the largest quantities of the total amount of HBCD production – more than 90% [9, 10]. EPS and XPS foams are known under the trade name ‘styrofoam’, which is mainly used as an insulating material of external and internal walls, foundations and at the attics, but also as insulation boards in transport vehicles and trucks as well as in child car seats, and as packaging materials [11, 12].

The physicochemical properties of polystyrene and especially the low density value and high porosity make it a highly desirable insulating material, and thus it is used in various products intended for the coastal aquaculture environment. The results of studies [13] confirm that buoys made with ESP contain significant levels of HBCD, which enters the water and bottom sediments, and consequently also the tissues of aquatic organisms. Furthermore, the containers used for storage and fish transport are also often made from EPS or XPS containing HBCD, resulting in a passage of this xenobiotic to the fish’s tissues [14].

There is no doubt that, in view of the risks to human health, the use of HBCD in EPS and XPS materials in products used in aquaculture coastal environments and on the market for the sale and breeding of fish seems to be pointless, especially as the danger of possible ignition of these materials is negligible. Fish are an important research material to the assessment of human exposure to the adverse effects of pollution, even though they are less consumed than meat and dairy products [15]. Another factor, which justifies the need to monitor the

concentrations of HBCD in fish, is frequently observed that the tissues of aquatic organisms, particularly fish with high fat content, is determined BFR at higher concentration levels than in the case of terrestrial organisms [16–18].

The Member States of the Organization for Economic Cooperation and Development (OECD) on the 24th meeting in 2007 agreed that HBCD has a high potential for bioaccumulation and biomagnification at different levels of the trophic pyramid and the properties indicating a threat to the environment including human health [19]. Bioaccumulation is a process resulting in the increase of concentration of the chemical substances in the living body as compared to the concentration observed in the environment in which the organism exists, taking into account all routes of exposure or absorption via the respiratory tract, skin and in food [20]. The HBCD bioaccumulation potential is linked to the lipophilic properties of the compound [21]. The biomagnification process can be considered as a special case of bioaccumulation, which relates to the increase in concentration of chemical substances in the living body compared to the concentration of the substance in the diet [20] and it is often the relationship between predator and prey thereof [22].

Tomy G. et al. [23, 24] confirm the strong positive linear correlation between concentrations of HBCD and trophic levels in the food web of Lake Ontario and the waters of the Arctic areas, which demonstrates the ability of HBCD to bioaccumulate and biomagnification in the chain of trophic relationships. Similar findings also indicate Law et al. based on the study of aquatic life in Lake Winnipeg (Canada) [25] and Morris et al. leader of the study samples from the North Sea (Europe) [26].

The procedure for the determination of HBCD is similar to other BFR or POP, and is usually multi-step and time consuming. Methods of extracting and purifying the samples have been well-developed over the past few/several years and they are commonly used as a reference in the analysis of environmental and biological samples as well as in food [27, 28]. HBCD is normally secreted from the sample in the extraction process, the extract is then purified, analytes are isolated from the sample matrix and separated from the interfering components. Choosing the appropriate techniques at different stages of the analytical methodology mainly depends on the sample matrix and the purpose of research. Various analytical techniques are applicable to samples, which are in various states of aggregation. Keeping quality control at every stage of the analytical methodology is a necessary element. In order to ensure the integrity of research, it is important to care about the representativeness of the taken samples, to ensure the uniformity and the immutability of the composition of samples from the moment of its collection to the final determination, validation of individual stages and the whole procedure, analytical and statistical evaluation of measurement results using various chemometric methods as a tool dedicated to analytical chemistry [29]. According to chemometric measurements, tools for the visualisation of the relationships in the data collection and classification of objects are useful as well as the relation between variables, which is implemented on the basis of determination of the coefficients of linear correlation (r) – the most common r-Pearson, together with graphs showing these dependencies [30].

2. Materials

The samples of food constitutes meat tissues from farmed, marine and freshwater fish, such as the Atlantic salmon (Norwegian), rainbow trout, pangasius and tilapia and marine fish species: Alaska pollack, cod, hoki, hake, Atlantic herring, Atlantic halibut, Atlantic mackerel and sole. The study was selected from among the most common species of fish consumed in Poland, according to the information provided in reports of the Statistical Institute of Agricultural and Food Economics [15] and in [31]. In a few cases, two fish of the same species were investigated. All of the tested fish were purchased from the fish shop, to which the products are delivered directly from suppliers in fisheries or farming, according to information provided by the owner.

The general scheme of the analytical procedure with respect to the research is presented in Fig. 1. Details describing the individual steps of the experiment and the parameters of validation methods have been presented in previous studies [32].

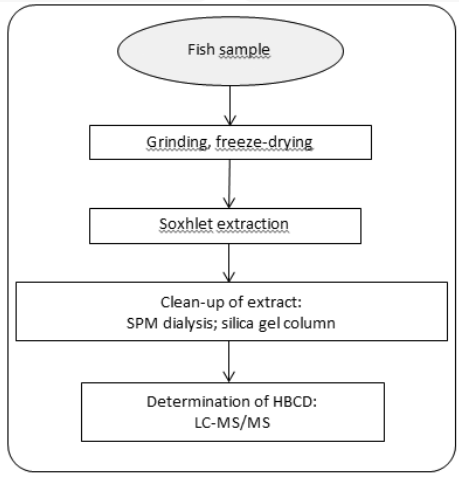

Fig. 1. Scheme of analytical procedure to prepare fish samples for LC MS/MS analysis

3. Results and discussion

For the determination of lipophilic compounds in food samples, such as HBCD, an important step is to determine the fat content [33, 34]. Hence, before cleaning extract/ isolation of HBCD from fat matrix, fat determinations were performed for each sample. Using a rotary evaporator, the solvent was evaporated and the residual fat fraction was weighed and it determined the weight of raw fat, based on the weight of the sample to be analysed. The results of the fat content of the test samples of fish are summarised together with the result indications of HBCD in Table 1.

The lipophilic nature of HBCD causes that this compound accumulates in the adipose tissue of living organisms. Therefore, a study for the assessment of the concentration of the sum of isomers of HBCD on the fat content in the test samples has been conducted. In order

to test the correlation, all of the obtained results and studied groups were considered, and they were limited to the following factors:

a) type of sample, all samples and one group consisted of farmed fish (No.1–6) and the other consisted of marine fish (No. 7–16),
b) fat content: divided into two groups of samples – the first comprised the fish that the fat content was less than 7%; the second group of those containing more than 7% fat. The value of 7% was taken as the limit based on the distribution of fish, taking into account the fat content in fish muscle PN-A-86770: 1999 [35]. Thus, the first group accounted for an average of fatty fish and lean fish, while the second group of oily fish.

The results are shown below.

Table 1

Fat and HBCD content of the test samples of fish

No.	Fish	Fat content %	Concentration of HBCD [pg/g fresh weight] ± SD
1	Atlantic salmon (Norwegian)	13	434 ± 44
2	Atlantic salmon (Norwegian)	14	152 ± 16
3	Rainbow trout	6.5	78 ± 7.1
4	Rainbow trout	6.0	101 ± 12
5	Pangasius	12	5.4 ± 0.65
6	Tilapia	12	2.4 ± 0.17
7	Pollack	4	6.4 ± 0.58
8	Cod	1	376 ± 36
9	Hoki	2	23 ± 2.1
10	Hake	1	14 ± 1.4
11	Atlantic herring	16	404 ± 33
12	Atlantic halibut	11	154 ± 14
13	Atlantic halibut	10	366 ± 26
14	Atlantic Mackerel	15	906 ± 75
15	Atlantic Mackerel	18	650 ± 55
16	Sole	12	18 ± 2.4

Based on a survey of correlation between the concentration of HBCD and fat content in the samples, it was found that there is a strong positive linear relationship ($r = 0.6105$) only in the case of samples with a fat content of more than 7%. The correlation coefficient for marine fish catch is also positive, and it is a highly linear relationship ($r = 0.6377$), which may result from the fact that, in the present group, most fish species are oily fish.

It can therefore be argued that the potential accumulation of HBCD will increase with increasing fat content in the tissue of the test fish, due to the lipophilic properties of the compound. The statistical significance of the correlation coefficients was confirmed by the *t*-test ($|t| \geq t_{\alpha = 0,05,\ n-2}$).

Test results of HBCD in the tissues of fish confirm the ability of this compound to bioaccumulate, while the results of the correlation between the analyte concentration and fat content of the samples confirm the lipophilic character of the compound. In the study on the category of "Fish meat" [36], the results indicate a significant relationship between the fat content and the concentration of polybrominated diphenyl ethers (PBDEs), which are also in the group of additive flame retardants.

References

[1] Heeb N.V., Schweizer W.B., Kohler M., Gerecke A.C. *Structure elucidation of hexabromocyclododecanes – a class of compounds with a complex stereochemistry.* Chemosphere, 61, 2005, 65–67.

[2] Heeb N.V., Schweizer W.B., Mattrel P., Haag R., Gerecke A.C., Kohler M., Schmid P., Zennegg M., Wolfensberger M., *Solidstate conformations and absolute configurations of (+) and ()alpha-, beta-, and gamma-hexabromocyclododecanes (HBCDs)*, Chemosphere 68, 2007, 940–950.

[3] Law R.J., Kohler M., Heeb N.V., Gerecke A.C., Schmid P., Voorspols S., Covaci A., Becher G., Janak K., Thomsen C., *Hexabromocyclododecane challenges scientists and regulators*. Environmental Science & Technology, 39, 2005, 281A–287A.

[4] Data Sheet: *Result for EC#: 247-148-4 HBCD* http://esis.jrc.ec.europa.eu/index.php?PGM=hpv (access january 2015).

[5] *HPV data summary and test plan for hexabromocyclododecane (HBCD) CAS No. 3194556.* American Chemistry Council Brominated Flame Retardant Industry Panel (BFRIP) 1300 Wilson Blvd Arlington, Virginia 2001.

[6] *Risk Assessment Hexabromocyclododecane CAS-No.: 25637-99-4 EINECS-No.: 247-148-4* Final report May 2008. Final approved version, European Union Risk Assessment Report. 2008. Office for Official Publications of the European Communities. R044_0805_env_hh_final_ECB.doc

[7] Janák K., Covaci A., Voorspoels S., Becher G., *Hexabromocyclododecane in marine species from the Western Scheldt Estuary: diastereoisomer- and enantiomer-specific accumulation*. Environmental Science & Technology, 39, 2005, 1987–1994.

[8] Arctic Monitoring and Assessment Programme (AMAP) Report 2007:6 SFT Report TA–2440/2008. Final Report of Phase I of the ACAP Project on Brominated Flame Retardants (BFRs). Phase I: *Inventory of sources and identification of BFR alternatives and management strategies*, Oslo 2007.

[9] *Data on manufacture, import, export, uses and releases of HBCDD as well as information on potential alternatives to its use*. The technical work on this report has been led by IOM Consulting, supported by BRE, PFA and Entec under framework contract ECHA/2008/02/SR4/ECA.226.

[10] Exploration of managements options for Hexabromocyclododecane. *Report to the 8th meeting of the UNECE Task Force on Persistent Organic Pollutants*, Montreal 2010.
[11] U.S. Environmental Protection Agency (2012). *Significant New Use Rule for Hexabromocyclododecane and 1,2,5,6,9,10-Hexabromocyclododecane*, 77 FR 17386-17394.
[12] NICNAS, *National Industrial Chemicals Notification and Assessment Scheme. Priority Existing Chemical Assessment* Report No.34. Hexabromocyclododecane. Sydney 2012.
[13] Al-Odaini N. A., Shim W. J., Han G. M., Jang M., Hong S. H., *Enrichment of hexabromocyclododecanes in coastal sediments near aquaculture areas and a wastewater treatment plant in a semi-enclosed bay in South Korea*. Science of the Total Environment, 505, 2015, 290–298.
[14] Rani M., Shim W. J., Han G. M., Jang M., Song Y. K., Hong S. H., *Hexabromocyclododecane in polystyrene based consumer products: An evidence of unregulated use*, Chemosphere, 110, 2014, 111–119.
[15] Pieńkowska B.. *Spożycie ryb*, IERiGŻ: *Rynek ryb: Stan perspektywy. Analizy rynkowe*, Wyd IERiGŻ, Warszawa 2008.
[16] Darnerud P. O., *Toxic effects of brominated flame retardants in man and in wildlife.* Environment International, 29, 2003, 841–53.
[17] Thomann R. V.., *Modeling organic chemical fate in aquatic systems: significance of bioaccumulation and relevant time-space scales,* Environmental Health Perspectives, 103, 1995, 53–57.
[18] Elskus A., Collier T. K., Monossone E.Ch., 4: *Interactions between lipids and persistent organic pollutants in fish,* Biochemistry and Molecular Biology of Fishes, 6, 2005.
[19] Organization for Economic Cooperation And Development (OECD). SIDS Initial Assessment , *Profile for Cas. No. 25637-99-4, 3194-55-6, Hexabromocyclododecane (HBCDD).* 2007. http://webnet.oecd.org/Hpv/UI/handler.axd?id=ea58ac11-e090-4b24-b281-200ae351686c (access: january 2015).
[20] Mackay D., Fraser A., *Bioaccumulation of persistent organic chemicals: mechanisms and models,* Environ. Pollut., 110, 2000, 375–391.
[21] *CHL report for hexabromocyclododecane. Proposal for Harmonised Classification and Labelling.* Based on the CLP Regulation (EC) No 1272/2008, Annex VI, Part 2 Substance Name: Hexabromocyclododecane, 2009.
[22] Newman M. C.Ch., 5: *Bioaccumulation from Food and Trophic Transfer w Fundamentals of Ecotoxicology: The Science of Pollution*, CRC Press, Taylor & Francis Group.
[23] Tomy G. T., Budakowski W., Halldorson T., Whittle D. M., Keir M. J., Marvin C., MacInnis G., Alaee M., *Biomagnification of α- and γ-hexabromocyclododecane isomers in a Lake Ontario food web*, Environmental Science & Technology, 38, 2004, 2298–2303.
[24] Tomy G., Pleskach K., Oswald T., Halldorson T., Helm P. A., MacInnis G., Marvin C. H.., *Enantioselective bioaccumulation of hexabromocyclododecane and congener-specific accumulation of brominated diphenyl ethers in an eastern Canadian Arctic marine food we,.* Environmental Science & Technology, 42, 2008, 3634–3639.
[25] Law K., Halldorson T., Danell R., Stern G., Gewurtz S., Alaee M., Marvin C., Whittle M., Tomy G., *Bioaccumulation and trophic transfer of some brominated flame retardants in a Lake Winnipeg (Canada) food web*, Environmental Toxicology and Chemistry, 25, 2006, 2177–2186.

[26] Morris S., Allchin C.R., Zegers B. N., Haftka J.J.H., Boon J. P., Belpaire C., Leonards P. E.G., van Leeuwen S., de Boer J., *Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs*, Environmental Science & Technology, 38, 2004, 5497–5504.

[27] Morris S., Bersuder P., Allchin C. R., Zegers B., Boon J. P., Leonards P. E. G., de Boer J., *Determination of the brominated flame retardant, hexabromocyclodocane, in sediments and biota by liquid chromatography-electrospray ionisation mass spectrometry*, Trends in Analytical Chemistry, 25, 2006, 343–349.

[28] Xu W., Wang X., Cai Z., *Analytical chemistry of the persistent organic pollutants identified in the Stockholm Convention: A review*, Analitica Chimica Acta, 790, 2013, 1–13.

[29] Zuba D., Parczewski A., *Chemometria w nauce i praktyce*, IES Kraków 2009.

[30] Konieczka P., Namieśnik J., *Ocena i kontrola jakości wyników pomiarów analitycznych*, Wydawnictwo Naukowo Techniczne, Warszawa 2007.

[31] Pieniak Z., Kołodziejczyk M., Kowrygo B., Verbeke W., *Consumption patterns and labelling of fish and fishery products in Poland after the EU accession*, Food Control, 22, 2011, 843–850.

[32] Kuc J., Grochowalski A., Mach S., Placha D., *Level of hexabromocyclododecane isomers in the tissue of selected commonly consumed fish in Central European countries*, Acta Chromatographica, 26, 2014, 575–585.

[33] Gómara B., Lebrón-Aguilar R., Quintanilla-Lópezb J.E., José M., *Development of a new method for the enantiomer specific determination of HBCD using an ion trap mass spectrometer*, Analitica Chimica Acta, 605, 2007, 53–60.

[34] Covaci A., Voorspoels S., Ramos L., Neels H., Blust R., *Recent developments in the analysis of brominated flame retardants and brominated natural compounds*,Journal of Chromatography A, 1153, 2007, 145–171.

[35] PN-A-86770:1999, Ryby i przetwory rybne. Terminologia.

[36] Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain, EFSA Journal 9, 2011.