Chloroorganic insecticides in the fat of different assortment of rainbow trout (*Oncorhynchus mykiss*) meat

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Received: April 9, 2014  Accepted: September 30, 2014

Abstract

The aim of the study was to determine the content of chlorinated hydrocarbon residues in fat of different assortment of rainbow trout (*Oncorhynchus mykiss*). The research material consisted of 108 fish from six different producers. Thirty-six fish were analysed as fresh, 36 fish as frozen after six months storage, and 36 fish as traditionally smoked. Chromatographic determination of γ-HCH, DDT, DDD, and DDE was performed with an Agilent Technologies 6890N. The presence of the compounds was detected in all tested fat samples. The content of γ-HCH was five-fold higher in frozen fish (average 2.11 ng/g of fat) than in fresh and smoked fish (0.42 ng/g of fat and 0.43 ng/g of fat, respectively). Total DDT (ΣDDT) content was found higher after refrigerated storage but did not exceed acceptable levels, indicating that the fat in the meat of rainbow trout is an attractive nutritional compound with a low concentration of tested chemical pollutants.

Keywords: *Oncorhynchus mykiss*, fat, DDT, γ-HCH.

Introduction

In Poland, a diet rich in fish and fish preserves is currently being promoted and recommended for almost everyone. The average consumption of fish, fish preserves, and seafood is approximately 13 kg (of live weight) per Polish citizen and the consumption of trout and its preserves constitutes 0.35 kg (16). Fish and fish preserves have a beneficial impact on human health and support the optimal physical and mental condition. Fish are also a very good source of proteins, rich in essential amino acids, fats, macro- and trace elements, and fat-soluble vitamins. Fish contain saturated, monoenoic and valuable polienoic (LC n-3 PEFA) fatty acids (18) that, among other benefits, have a hypocholesterolic effect (anti-arteriosclerosis) (8).

However, this valuable fat may also carry harmful substances, which reduce the health properties of fish meat. Substances such as pesticides (*e.g.* DDT, HCH) were introduced by the man into the natural environment in order to eliminate disease-carrying (mainly malaria) insects and as plant protection compounds. Nowadays, these substances are thought to pose a risk to health due to well-documented chronic toxicity, and the fact that human exposure to these pollutants results mainly from food consumption (1, 7, 21). Chloroorganic insecticides are dangerous for humans and animals, though it has not been explicitly documented that these compounds, when accumulated in fat tissue, cause teratogenic, mutagenic, or cancerogenic effects. DDT may cause a potential effect at a molecular level and disrupt the intercellular connections, which may result in a loss of control over growth and differentiation of cells. It has been found that DDT has an impact on the bone marrow, blood cells, and the immune and nervous systems. The mechanism of neurotoxic action is probably associated with hormonal regulation. It may modify the electrophysiological and enzymatic properties of cell membranes and nerve cells by destroying the oestrogen receptors in the human hormonal system (17, 20). High concentrations of these compounds and their metabolites detected in humans’ and animals’ tissues worldwide (even in places where they had not been used), and documented harmful effects on human body contributed to the legal ban on DDT use, and established guidelines on the content of these compounds in food (4).

These pesticides constitute a group of persistent organic pollutants (POPs) found in surface and ground
waters. The highest concentrations of these compounds have been detected during agrochemical procedures as well as during snow-melt season and water runoff. However, a lack of pesticides in water does not necessarily indicate the purity of a given reservoir since pesticides accumulate mainly in bottom sediments and aquatic organisms. Fish, in particular, ingest residues of chloroorganic pesticides during respiration and with feed (5, 14). Especially predatory species, are most vulnerable to intensive accumulation of chloroorganic insecticides in their bodies due to their position in the food chain (7). Therefore, it has become important to evaluate production (i.e. culture conditions in this case) and processing (freezing and smoking) procedures, because improper technology may introduce harmful substances to food products.

The aim of the study was to determine the content of selected chloroorganic insecticides in the fat of fresh, frozen, and smoked rainbow trout meat and the impact of culture conditions and processing procedures on the concentration of these insecticides.

Material and Methods

The research material consisted of 108 rainbow trout from six different producers. Thirty-six fish were analysed as fresh, 36 fish as frozen after six months of storage, and 36 fish as traditionally smoked. Fresh fish were purchased directly from the breeders. Fish farms were situated in the province of Warmia and Mazury and were under veterinary supervision. At these farms, the production cycle differed in the type of feed, water, and processing (freezing and smoking) procedures, because recovery analyses for DDT, DDE, DDD, and lindane were 85%, 92%, 90%, and 86% respectively.

Determination of fat content. The determination of fat content was performed with Soxhlet's extraction method with ethyl ether as a solvent. The fat was dried to a fixed mass in an electric dryer at 105°C (12).

Extraction of fat from samples. The extraction of fat was conducted with the Schmid-Ratzlaff method, which consisted in hydrolysing meat with concentrated hydrochloric acid and triple extraction of fat with a mixture of ethyl and petroleum ether (at 1:1 ratio). After separation of the ether layer, it was distilled and dried to a fixed mass at 105°C, providing pure fat for further analyses (13).

Analytic procedure. The extraction of chloroorganic insecticides from fat was conducted according to Ludwicki et al. (9). The method was based on dissolving fat in n-hexane and purifying it with sulphuric acid until a layer of acid becomes transparent. The hexane layer was then dried with anhydrous sodium sulphate.

Chromatographic analysis. Chromatographic separation of γ-HCH, DDT, DDD, and DDE was performed with an Agilent Technologies 6890N gas chromatograph with electron capture detector (ECD) with a capillary column, which was 25 m in length and 0.32 mm in diameter. The liquid phase was PAS-1701 with a 0.25 µm thick film. The temperatures of separation were 250°C for the dispenser, 200°C for the column, and 280°C for the detector. Helium was the carrier gas with a flow velocity of 2.5 mL/min (the dispenser with 10:1 scale). The compounds were identified by comparing the peak retention times of the samples and standards, and the quantitative analysis was performed based on the surface of peaks calculated with Chemstation software. The detection limit was 0.05 ng/g for γ-HCH, DDT, and DDE, and 0.01 ng/g for DDD. Recovery analyses for DDT, DDE, DDD, and lindane were 85%, 92%, 90%, and 86% respectively.

Statistical analysis. The analysis of variance was applied (Fisher-Snedecor test) for one-factor and two-factor experiments. The origin of fish and technological processing (fresh, frozen, and smoked fish) were assumed as the grouping factors. A Newman-Keuls test was used to collect information on the diversification of individual means and groups of homogenous means (i.e. that did not differ statistically). A MS Excel sheet and Statistica PL9 software were used for statistical calculations.

Results

Fresh fish. The average content of fat in fresh fish was 6.14% and ranged from 4.97% to 7.75%. It should be emphasised that the determined fat content in fresh fish did not differ from the concentration of lipids in fresh rainbow trout, which ranges from 6% to 8% (2, 3). The average concentration of γ-HCH in the tested samples was 0.42 ng/g of fat (Table 1).

The average content of DDT and its metabolites in fresh fish was: DDE – 16.47 ng/g of fat, DDD – 4.83 ng/g of fat and DDT – 6.69 ng/g of fat. The average content of total DDT (DDT+DDE+DDD, ΣDDT) in fresh fish was 28 ng/g of fat (Table 1).

The percentage of insecticide content in the fat of trout from different producers is presented in Fig. 1. It
should be noted that the DDE metabolite constitutes the highest fraction (from 51.32% to 62.49%) of total DDT in the fat of fresh fish. In the fat, average content of DDE, DDD, and DDT in ΣDDT was 58.12%, 17.44%, and 24.44% respectively.

**Frozen fish.** The average fat content in frozen fish was 4.87% (from 3.2% to 6.27%) and was lower than in fresh fish, which might have resulted from the leakage of some fat during technological processes. The average content of γ-HCH was 2.11 ng/g of fat. The highest, statistically significant, average content of lindane was detected in the fat of fish from the producer 4 (6.55 ng/g of fat). The fat of fish, originating from this producer, also contained the highest average content of ΣDDT (92.93 ng/g of fat) and its metabolites.

The average content of DDT and its metabolites in the fat of frozen fish was 23.99 ng/g of fat for DDE, 12.94 ng/g of fat for DDT, and 9.49 ng/g of fat for DDD. The average content of ΣDDT was 46.42 ng/g of fat. The content of individual compounds in frozen fish is presented in Table 1.

The percentage content of individual metabolites ranged from 40.81% to 63.39% for DDE, from 17.01% to 41.74% for DDT, and from 15.01% to 26.69% for DDT in ΣDDT. The percentages of the individual insecticides in the fat of trout originating from different producers are presented in Fig. 2.

**Smoked fish.** The study was conducted on traditionally (directly) smoked fresh fish, originating from different producers.

The average content of γ-HCH in the tested samples was 0.43 ng/g of fat.

The average content of DDT and its metabolites was 15.50 ng/g of fat for DDE, 7.21 ng/g of fat for DDD, and 8.92 ng/g of fat for DDT, whereas the average content of ΣDDT was 31.65 ng/g of fat. The detailed results of the analyses are presented in Table 1.

**Table 1.** Average content of lindane, DDD, DDE, DDT, and ΣDDT (DDT+DDE+DDD) in fat of fresh, frozen, and smoked fish obtained from six producers (ng/g of fat)

<table>
<thead>
<tr>
<th>Material</th>
<th>Producer number</th>
<th>Breeding conditions</th>
<th>Fat content (%)</th>
<th>γ-HCH</th>
<th>DDE</th>
<th>DDD</th>
<th>DDT</th>
<th>ΣDDT</th>
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<tr>
<td>Fresh fish</td>
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<td>x ± s</td>
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<tr>
<td></td>
<td>A A A</td>
<td>5.64 ± 1.43</td>
<td>0.42 ± 0.22a</td>
<td>12.02 ± 3.13a</td>
<td>3.88 ± 0.97a</td>
<td>7.53 ± 1.33a</td>
<td>23.43 ± 4.84ab</td>
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<tr>
<td></td>
<td>B A B</td>
<td>7.29 ± 1.22</td>
<td>0.49 ± 0.19a</td>
<td>11.21 ± 2.9a</td>
<td>4.28 ± 0.75ab</td>
<td>5.81 ± 2.96a</td>
<td>21.3 ± 6.27a</td>
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<tr>
<td></td>
<td>C B C</td>
<td>5.05 ± 2.11</td>
<td>0.47 ± 0.37a</td>
<td>20.87 ± 5.61b</td>
<td>5.72 ± 0.85bc</td>
<td>6.80 ± 2.61a</td>
<td>33.39 ± 7.08b</td>
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<td>D B D</td>
<td>4.97 ± 0.83</td>
<td>0.30 ± 0.14a</td>
<td>21.16 ± 6.75bc</td>
<td>6.16 ± 1.18c</td>
<td>7.04 ± 3.2a</td>
<td>34.37 ± 9.45b</td>
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<tr>
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<td>E B E</td>
<td>6.14 ± 0.92</td>
<td>0.39 ± 0.22a</td>
<td>19.66 ± 4.58b</td>
<td>4.61 ± 1.16ab</td>
<td>7.82 ± 1.11a</td>
<td>32.08 ± 4.95b</td>
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<td>F B F</td>
<td>7.75 ± 1.54</td>
<td>0.44 ± 0.25a</td>
<td>13.94 ± 3.62ac</td>
<td>4.36 ± 0.84ab</td>
<td>5.17 ± 1.94a</td>
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<tr>
<td>Average</td>
<td></td>
<td>6.14 ± 1.16</td>
<td>0.42 ± 0.24a</td>
<td>16.47 ± 6.06</td>
<td>4.83 ± 1.22</td>
<td>6.69 ± 2.35</td>
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<tr>
<td>Smoked fish</td>
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<td></td>
<td>F = 0.26;</td>
<td>F = 4.67;</td>
<td>F = 1.90;</td>
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<td>P = 0.002</td>
<td>P = 0.12;</td>
<td>P = 0.005</td>
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</table>

± - standard deviation, n/t - not tested, a,b,c - significance of difference between the samples; P = 0.01, A-D - breeding conditions – factors indicated with the same letters are identical within columns.

Fig. 1. The average percentage of DDE, DDD, and DDT contents in $\Sigma$DDT in fat of fresh fish obtained from different producers and in the fresh fish in total.

Fig. 2. The average percentage content of DDE, DDD, DDT in $\Sigma$DDT in the fat of frozen fish obtained from different producers and in the frozen fish in total.

Fig. 3. The average percentage content of DDE, DDD, DDT in $\Sigma$DDT in the fat of smoked fish obtained from different producers and in the smoked fish in total.
**Discussion**

The analysis of lindane content did not reveal any statistically significant differences between the fat sampled from fresh fish from different producers. It is important that none of the samples exceeded the highest concentration of γ-HCH allowed by European law, *i.e.* 20 ng/g of fat. The determined values are several times lower than this threshold (6).

The statistical analysis of ΣDDT content in the fat of fresh fish meat from the producers 1, 2, and 6 revealed that there were no significant differences in this group of producers similarly to that in fat of fish meat from the producers 3, 4, and 5. As the specificity of culture conditions in the individual producers was different (Table 1), the detected statistical differences may result from the type of feed administered to fish and water used for fish culture. The comparable content of ΣDDT may be related to the fact that producers 1 and 6 obtained water from the same river and at the same place which, together with feed, may lead to an impact on statistically homogenous average content of ΣDDT. Despite the fact that producer 2 obtained water from a different source, the content of ΣDDT in the fat of fish meat from his farm did not differ statistically from the concentration of ΣDDT in the fat of fish meat from the producers 1 and 6. Thus, it may be assumed that water did not influence the content of ΣDDT, but the feed had a significant impact, as it was identical in the case of these three producers. A similar correlation was observed in the fat of fish meat from producers 3, 4, and 5, who used the same feed, which differed from the feed used by producers 1, 2, and 6. Other studies also confirmed the influence of the type of the feed on the content of tested compounds (10).

In the studies conducted by the National Veterinary Institute in Pulawy in 2006, 66 samples of fish (carp and trout) were tested for pesticide residues (11). The average content of ΣDDT in these products was 0.12 mg/g of fat, which was six times higher than the values determined in our study. Szelinder-Richert *et al.* (15) examined rainbow trout from Polish farms and detected 4.6 µg/kg w.w of ΣDDT, which was a three times higher value than that recorded in our studies.

Statistical analysis of lindane content in frozen fish did not reveal any significant statistical differences among the producers; only the average content of γ-HCH in frozen fish from producer 4 was significantly (few times) higher, but it did not exceed the maximum acceptable level of lindane (20 ng/g of fat) (6). This analysis indicated a lack of significant differences in the content of ΣDDT in fish from producers 1, 2, 3, 5, and 6, whereas frozen fish from producer 4 contained significantly higher residues of pesticides.

The correlations of statistically significant differences did not combine them with any of the factors associated with fish culture. The changes were probably connected with irregular leakage of some fat during thawing, which resulted in accumulation or loss of insecticide residues in fish meat. In each of the tested frozen fish, the residues of DDE constituted the highest percentage in ΣDDT (except producer 5). Frozen fish contained several times less ΣDDT than allowed by the European law (1000 ng/g of fat) for chickens, geese, ducks, turkeys, guinea fowls, ostriches, and pigeons (6).

The analysis of lindane content in smoked fish did not reveal any statistical differences between the fat of fish from different producers. The recorded values did not exceed the highest content of γ-HCH, *i.e.* 20 ng/g of fat (for chickens, geese, ducks, turkeys, guinea fowls, ostriches, and pigeons), allowed by European law (6). The determined levels are several times lower than the approved thresholds, and are comparable with the concentrations detected in fresh fish.

The evaluation of DDT, DDD and DDE contents revealed a lack of statistical differences between the producers 1, 2, 3, 4, and 6; only the smoked fish from the producer 5 contained higher amounts of tested compounds, which were confirmed statistically.

The average content of fat in the smoked fish tested by Usydus *et al.* was 5.31% and was similar to the value of our studies. Those authors also demonstrated that the content of ΣDDT in smoked trout was 5.03 ng/g wet weight (19). This result is three times higher than the level determined in our experiment.

The analyses of fat in the meat of fresh rainbow trout, which originated from six different producers did not reveal any significant differences in lindane content, whereas significant differences in ΣDDT concentration indicates that the type of feed was a differentiating factor. It should be emphasised that fish meat is used to produce feeds which are administered to rainbow trout under intensive rearing conditions, and the residues of insecticides detected in fish meal accumulate in the fat of fish meat.

Insecticides were detected in all tested samples, but none of them exceeded acceptable concentration limits. The detected amounts of chloroorganic insecticides (DDT, lindane) were several times lower than the approved thresholds, which leads us to assume that the fat in the meat of rainbow trout is an attractive nutritional compound with a low concentration of tested chemical pollutants.

In Europe, the maximum residue level of ΣDDT in fat of animal origin is 1.0 mg/kg, whereas for lindane (γ-HCH) it is 0.02 mg/kg of fat. None of the tested samples were free from the residues of chloroorganic insecticides. It should be noted that DDE constituted the highest percentage in ΣDDT. The relatively high content of DDT may indicate secondary contamination of the environment, for instance, from leaking pesticide tombs.
References


