# PCBs, Dioxins, and Furans in Fish Commonly Available on the Slovene Market

ESTER HEATH[1,2], TINA KOSJEK[1,2] , DAVID HEATH[1]

[1]Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia

[2]Jožef Stefan International Postgraduate School, Jamova cesta 39, 1000 Ljubljana, Slovenia

## Background

- By EU standards, Slovenia is not considered a large consumer of fish. The average Slovenian consumes approximately approx. 10.8 kg per capita per year of fish, which is less than half of the EU28 average (25.5 kg per capita per year) and has one of the lowest expenditures of fish per household in the EU28 [1].
- Consumption of fish per capita has been steadily increasing and the National Slovenian Institute of Public Health continues to promote fish as part of a healthy diet [2].
- Fish are an excellent source of omega-3 fatty acids (DHA & EPA) - 0.5-1.8 g of DHA + EPA per day significantly reduces deaths from heart disease and all causes.
- FAO dietary guidelines on eating healthy recommends eating 2-3 portions of fish per week [3].

## Problem Statement

- People need to balance nutritional benefits of eating fish and the potential exposure to harmful contaminants.
- FAO/WHO recommends that countries "develop, maintain and improve existing databases on specific contaminants, particularly methylmercury and dioxins and dioxin like compounds in fish consumed in their region".
- Few studies estimating the risk posed from dioxin and dioxin like compounds in fish readily available on the Slovene market exist such that data on exposure to these compounds is at best limited.

## Objectives

- Obtain information related to PCB, dioxin, and furan residues in commonly consumed wild, farmed, and frozen fish (and squid) available on the Slovenian market.

## Methods: Sampling

- In total, 121 samples were collected from various supermarkets and fish markets in Slovenia including:
  - Fish and squid caught in the Northern Adriatic;
  - Fish farmed in Slovenia;
  - Imported fish and squid species (10 samples).
- Fish were first eviscerate and fillets set aside for sampling (squid were analysed individually).
- Samples were transported on ice, snap frozen (liquid nitrogen) and homogenized.

## Method: Sample Analysis

1) Samples: (20 g) were Soxhlet extracted with hexane and acetone (9:1, v/v) for 16 h (EPA 1668B and 8082A)
2) Lipid removal: $H_2SO_4$ (96% p.a.) and residual water with $Na_2SO_4$
3) Purified extracts: Florisil column (60/100 mesh)
4) Analysis: GC (Agilent ) with ECD detection
5) Column: DB–XLB (30m x 0.25 mm ID., 0.25 µm film thickness). Oven: 100 °C, (1 min) to 300 °C at 10 °C/min, (7 min). Injection: 1 µl, splitless., carrier gas He (1 ml/min).
6) Confirmatory analysis: 450-GC coupled to a 240 IT-MS (Varian USA) in CI mode.
7) Dioxins and furans were analysed at ALS Group (CZ).

## Method: Validation

- LOD: 0.003-0.007 ng/g LOQ: 0.010-0.024 ng/g

| Congener | CRM IAEA 406 | | |
|---|---|---|---|
| | ng.g$^{-1}$ | C. V. | 95% C.I. |
| PCB 28 | 0.42 | 0.57 | 0.43 - 1.3 |
| PCB 31 | 0.67 | 0.29 | 0.23 - 0.47 |
| PCB 52 | 1.6 | 1.3 | 1 - 2.2 |
| PCB 101 | 2.7 | 3.1 | 2.2 - 3.4 |
| PCB 105 | 0.77 | 0.71 | 0.48 - 0.88 |
| PCB 138 | 4.8 | 4 | 2.5 - 6.3 |
| PCB 153 | 3.9 | 3.7 | 2.9 - 6.0 |
| PCB 156 | 0.27 | 0.27 | 0.21 -0.59 |
| PCB 180 | 1.1 | 1.2 | 1.0 - 1.2 |

CRM IAEA 406 (lyophilized homogenated fish)

## Results

| Sample | Atlantic salmon (L) | Rainbow trout (SM)[1,2] | Rainbow trout (K2) | Rainbow trout (K3) | Rainbow trout (K4) | Rainbow trout (K1) | Rainbow trout (K16) | European seabass (P) | European seabass (V) | European seabass (D) | European anchovy (K5) | European pilchard (K6) | Gilt-head (sea) bream (K7) | Gilt-head (sea) bream (K8) | Whiting (K9) | European squid (K10) | European squid (K11) | Patagonian Squid (K12) | Pangas (K13) | Pangas (K14) | Pangas (K15) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | *Salmo Salar* | *Oncorhynchus mykiss* | | | | | | *Dicentrarchus labrax* | | | *Engraulis encrasicolus* | *Clupea pilchardus* | *Sparus aurata* | | *Merlangius merlangus* | *Loligo vulgaris* | | *Loligo sp.* | *Pangasius sp.* | | |
| Location | Norway | *Slovenia* | *Slovenia* | *Slovenia* | *Bosnia-Herzegovina* | *Italy* | *Turkey* | Slovenia | Slovenia N. Adriatic | Greece | Croatia N. Adriatic | Croatia N. Adriatic | Croatia | Slovenia N. Adriatic | Slovenia N. Adriatic | Slovenia N. Adriatic | Croatia N. Adriatic | Patagonia | Vietnam | Vietnam | Vietnam |
| | farmed | farmed | farmed | farmed | farmed | farmed | farmed | farmed | wild | farmed | wild | wild | farmed | wild | wild | wild | wild | wild | wild (fresh) | wild (frozen) | wild (frozen) |
| Congener | ng g-1 ww | | | | | | | | | | | | | | | | | | | | |
| I-PCBs (di-ortho) | | | | | | | | | | | | | | | | | | | | | |
| PCB 28 | 0.048 | 0.64 | 0.48 | 0.37 | 0.66 | 0.30 | 0.063 | 0.088 | 0.38 | 0.27 | 0.18 | 0.38 | 0.31 | 0.39 | 0.046 | 0.078 | 0.053 | 0.038 | 0.056 | 0.020 | 0.073 |
| PCB 52 | 0.60 | 1.3 | 0.45 | 0.28 | 0.25 | 0.18 | 0.21 | 0.33 | 1.7 | 0.79 | 0.26 | 0.35 | 0.16 | 0.60 | 0.042 | 0.11 | 0.017 | 0.028 | 0.029 | 0.025 | 0.076 |
| PCB 101 | 0.63 | 2.0 | 0.72 | 0.82 | 0.51 | 1.7 | 0.38 | 1.2 | 5.6 | 2.0 | 0.99 | 0.73 | 0.67 | 1.3 | 0.45 | 0.59 | 0.078 | 0.069 | 0.15 | 0.12 | 0.30 |
| PCB 138 | 1.4 | 2.7 | 0.85 | 0.86 | 0.58 | 0.69 | 0.49 | 1.8 | 14 | 5.4 | 3.5 | 4.9 | 0.92 | 8.4 | 0.77 | 1.4 | 0.10 | 0.13 | 0.066 | 0.061 | 0.068 |
| PCB 153 | 0.53 | 2.2 | 0.76 | 0.72 | 0.51 | 0.68 | 0.36 | 1.6 | 11 | 4.0 | 2.3 | 3.2 | 0.74 | 4.8 | 0.58 | 1.1 | 0.065 | 0.10 | 0.056 | 0.10 | 0.065 |
| PCB 180 | 0.24 | 0.54 | 0.23 | 0.26 | 0.17 | 0.18 | 0.11 | 0.42 | 3.8 | 1.3 | 1.1 | 1.4 | 0.31 | 2.1 | 0.21 | 0.31 | 0.033 | 0.031 | 0.029 | 0.034 | 0.027 |
| DL-PCBs (non-ortho) | | | | | | | | | | | | | | | | | | | | | |
| PCB 77 | 0.077 | 0.34 | 0.089 | 0.029 | 0.080 | 0.043 | 0.026 | 0.088 | 0.69 | 0.15 | 0.33 | 0.19 | 0.046 | 0.32 | 0.040 | 0.12 | 0.026 | 0.038 | 0.025 | 0.046 | 0.031 |
| PCB 81 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| PCB 126 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| PCB 169 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| DL-PCBs (mono-ortho) | | | | | | | | | | | | | | | | | | | | | |
| PCB 105 | 0.19 | 0.28 | 0.18 | 0.14 | 0.12 | 0.067 | 0.062 | 0.28 | 2.2 | 0.83 | 0.43 | 0.31 | 0.16 | 0.60 | 0.13 | 0.21 | 0.044 | 0.048 | 0.027 | 0.039 | 0.038 |
| PCB 114 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| PCB 118 | 0.20 | 1.0 | 0.43 | 0.36 | 0.27 | 0.28 | 0.33 | 0.56 | 6.0 | 2.5 | 1.0 | 1.4 | 0.36 | 3.3 | 0.27 | 0.56 | 0.055 | 0.028 | 0.019 | 0.032 | 0.039 |
| PCB 123 | <LOD | <LOD | 0.059 | 0.0040 | 0.052 | 0.039 | 0.032 | 0.11 | 0.58 | <LOD | 0.10 | 0.15 | 0.011 | 0.34 | 0.057 | 0.017 | <LOD | 0.023 | <LOD | <LOD | <LOD |
| PCB 156 | 0.051 | 0.17 | 0.081 | 0.087 | 0.068 | 0.072 | 0.043 | 0.12 | 0.60 | 0.17 | 0.18 | 0.24 | 0.0040 | 0.38 | 0.083 | 0.030 | 0.023 | 0.007 | 0.024 | 0.006 | 0.033 |
| PCB 157 | 0.029 | 0.13 | 0.027 | 0.033 | 0.031 | 0.028 | 0.009 | 0.048 | 0.17 | 0.057 | 0.028 | 0.068 | <LOD | 0.069 | 0.021 | 0.010 | 0.011 | <LOD | <LOD | 0.006 | 0.022 |
| PCB 167 | <LOD | <LOD | 0.11 | 0.091 | <LOD | <LOD | 0.071 | <LOD | <LOD | <LOD | <LOD | <LOD | 0.068 | 0.49 | 0.10 | 0.094 | 0.044 | 0.067 | 0.050 | 0.072 | 0.030 |
| PCB 189 | <LOD | 0.044 | <LOD | <LOD | <LOD | <LOD | <LOD | 0.030 | 0.13 | <LOD | <LOD | 0.026 | <LOD | 0.045 | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD |
| ∑I-PCB | 3.4 | 9.4 | 3.5 | 3.3 | 2.7 | 3.8 | 1.6 | 5.4 | 37 | 14 | 8.3 | 11 | 3.1 | 18 | 2.1 | 3.5 | 0.34 | 0.40 | 0.39 | 0.35 | 0.61 |
| ∑DL-PCB | 0.61 | 2.0 | 1.0 | 0.79 | 0.68 | 0.58 | 0.62 | 1.3 | 10 | 3.7 | 2.1 | 2.4 | 0.70 | 5.6 | 0.73 | 1.1 | 0.26 | 0.26 | 0.20 | 0.25 | 0.25 |
| ∑PCB | 4.0 | 11 | 4.5 | 4.1 | 3.4 | 4.4 | 2.2 | 6.7 | 47 | 17 | 10 | 13 | 3.8 | 23 | 2.8 | 4.6 | 0.60 | 0.65 | 0.59 | 0.61 | 0.85 |

1: Triploid, 2: Mix of composite samples taken in May and March

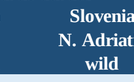
3: Van den Berg, M., Birnbaum et al., (2006). The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicological Sciences : An Official Journal of the Society of Toxicology, 93(2), 223–241.

| Sample | European anchovy (K5) | European pilchard (K6) | Gilt-head (sea) bream (K8) | Rainbow trout (SM) | European seabass (V) | European seabass (D) | WHO-TEFs |
|---|---|---|---|---|---|---|---|
| Species | *Engraulis encrasicolus* | *Clupea pilchardus* | *Sparus aurata* | *Oncorhynchus mykiss* | *Dicentrarchus labrax* | *Diceutrarchus labrax* | |
| Location | Croatia Northern Adriatic | Croatia Northern Adriatic | Slovenia Northern Adriatic | Slovenia | Slovenia Northern Adriatic | Greece | |
| | wild | wild | wild | farmed | wild | farmed | |
| | ng g$^{-1}$ ww | | | | | | |
| 2,3,7,8-TCDD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 1 |
| 1,2,3,7,8-PeCDD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 1 |
| 1,2,3,4,7,8-HxCDD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.1 |
| 1,2,3,6,7,8-HxCDD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.1 |
| 1,2,3,7,8,9-HxCDD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.1 |
| 1,2,3,4,6,7,8-HpCDI | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.01 |
| OCDD | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.0003 |
| 2,3,7,8-TCDF | <0.49 | 1.7 | 2.4 | <1.3 | 3.2 | 3.9 | 0.1 |
| 1,2,3,7,8-PeCDF | <LOD | <0.17 | 0.38 | <LOD | 0.52 | <0.14 | 0.03 |
| 2,3,4,7,8-PeCDF | <0.20 | 0.42 | 0.76 | <LOD | 0.72 | 0.44 | 0.3 |
| 1,2,3,4,7,8-HxCDF | <LOD | <LOD | <LOD | <LOD | <LOD | <0.19 | 0.1 |
| 1,2,3,6,7,8-HxCDF | <LOD | <LOD | <0.27 | <LOD | <LOD | <LOD | 0.1 |
| 1,2,3,7,8,9-HxCDF | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.1 |
| 2,3,4,6,7,8-HxCDF | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.1 |
| 1,2,3,4,6,7,8-HpCDI | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.25 |
| 1,2,3,4,7,8,9-HpCDI | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.25 |
| OCDF | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | 0.65 |
| ∑PCDDs | <LOD | <LOD | <LOD | <LOD | <LOD | <LOD | |
| ∑PCDFs | <LOD | 3.1 | 4.6 | <1.3 | 6.5 | 6.8 | |

Van den Berg, M., Birnbaum et al., (2006). The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicological Sciences: An Official Journal of the Society of Toxicology, 93(2), 223–241.

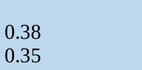
Rainbow Trout *Oncorhynchus mykiss*

Atlantic Salmon *Salmo salar*

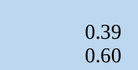


European pilchard *Sardina pilchardus*

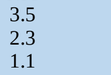
European Anchovy *Engraulis encrasicolus*



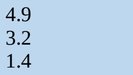
European Seabass *Dicentrarchus labrax*



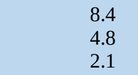
Pangas catfish *Pangasius*



Gilt-head (sea) bream *Sparus aurata*



Whiting *Merlangius merlangus*



European squid *Loligo vulgaris*

| Sample | | FDA tolerance limit* | WHO daily intake limit** | EU tolerance limit*** | | |
|---|---|---|---|---|---|---|
| | | ∑ PCB | 20 ng of typical PCB mixture/kg BW/day | ∑PCDD/F-TEQ | ∑DL-PCB and PCDD/F-TEQTEQ | ∑I-PCB |
| | | 2µg.g$^{-1}$ | | 3.5 pg g$^{-1}$ ww | 6.5 pg g$^{-1}$ ww | 75 ng g$^{-1}$ ww |
| *Engraulis encrasicolus* | (K5) | 0.010 | 1.6 | 0.49 | 2.0 | 8.3 |
| *Clupea pilchardus* | (K6) | 0.013 | 2.0 | 0.68 | 2.2 | 11 |
| *Sparus aurata* | (K8) | 0.023 | 3.5 | 0.81 | 2.5 | 18 |
| *Oncorhynchus mykiss* | (SM) | 0.037 | 5.5 | 0.47 | 2.0 | 9.4 |
| *Dicentrarchus labrax* | (V) | 0.047 | 7.2 | 0.95 | 2.8 | 37 |
| *Diceutrarchus labrax* | (D) | 0.017 | 2.6 | 1.1 | 2.7 | 14 |

*CFR - Code of Federal Regulations Title 21, volume 2, (2016)

**WHO (2003), Polychlorinated Biphenyls. Human Health effects. Concise International Assessment document, Vol 55. Geneva.

***COMMISSION REGULATION (EU) No 1259/2011 (Dec., 2011)

Uncertainty of each 2,3,7,8-PCDD/F congener is 30% and total WHO-TEQ is 20% expressed as doule (k=2) relative standard deviation (RSD%) and corresponds to 95% interval of reliability

## Conclusion

Analysed samples did not exceed recommended tolerance limit values (FDA and EU) and daily intake values (WHO).

## References

1) https://www.eumofa.eu/consumptionmon
2) FAO/WHO (2011). Consultation on the Risks and Benefits of Fish Consumption. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization, 50 pp.
3) http://www.fao.org/nutrition/education/food-dietary-guidelines

## Funding Source: EU

Slovene National Project CRP V4 1120 "Quality of fish on Slovene market"
ISO-FOOD received funding from the European 7 Framework Programme under grant agreement no 621329 (2014-2019)
www.isofood.eu