ISO Fd Food ERA Chair

Safety Quality Traceability

# Speciacija organokositrovih spojin in polibromiranih difenil etrov v ribah in školjkah

Tea Zuliani, Petra Novak, Janez Ščančar, Radmila Milačič

Institut "Jožef Stefan" Ljubljana, Slovenija

# Pregled

- Organokositrove spojine (OKS)
- Polibromirani difenil etri (PBDE)
- Analizni postopek
- Prisotnost OKS in PBDE v ribah iz slovenskega tržišča

# Motilci delovanja endokrinega sistema

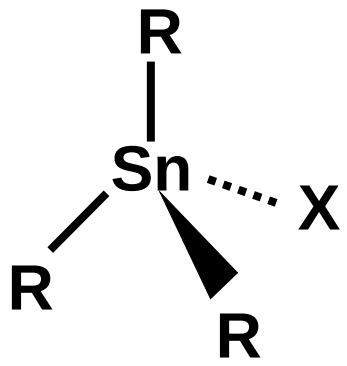
- snovi, ki spremenijo delovanje hormonskega sistema

- 87000 hormonskih motilcev

  - industrijske kemikalije (75500)
  - dodatki v kozmetiki in živilih (8000)
  - pomožne snovi v pesticidih (2500)
  - aktivne snovi v pesticidih (900)

# Organokositrove spojine - OKS

## Zgradba

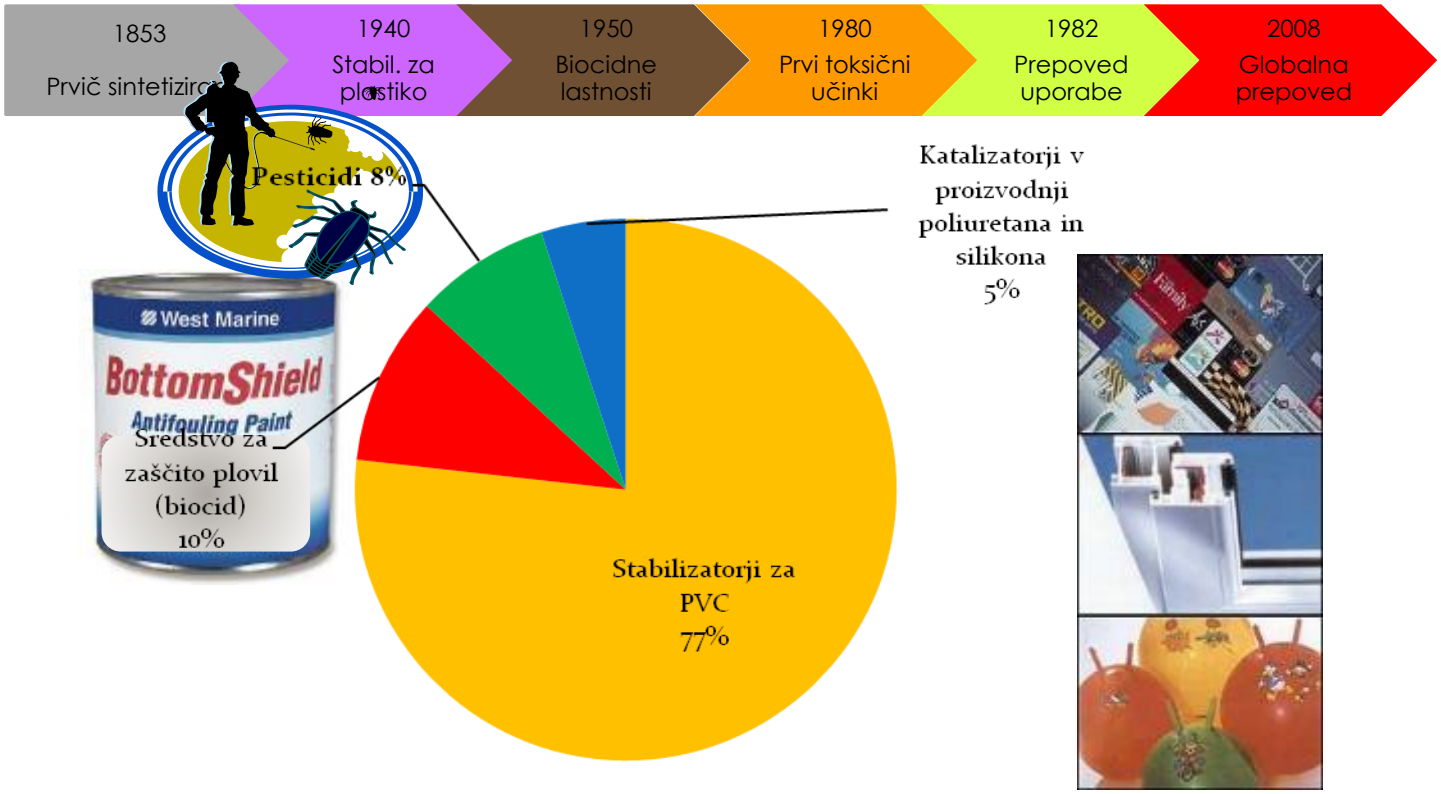



**R** :organski ligandi (metilna, etilna, butilna, oktilna, cikloheksilna skupina, ...)

**X** : anionski ligandi (halid, hidroksid, acetat, oksid, ...)

| *Kratica* | *Spojina* |
|---|---|
| TBT | tributil kositer |
| DBT | dibutil kositer |
| MBT | monobutil kositer |

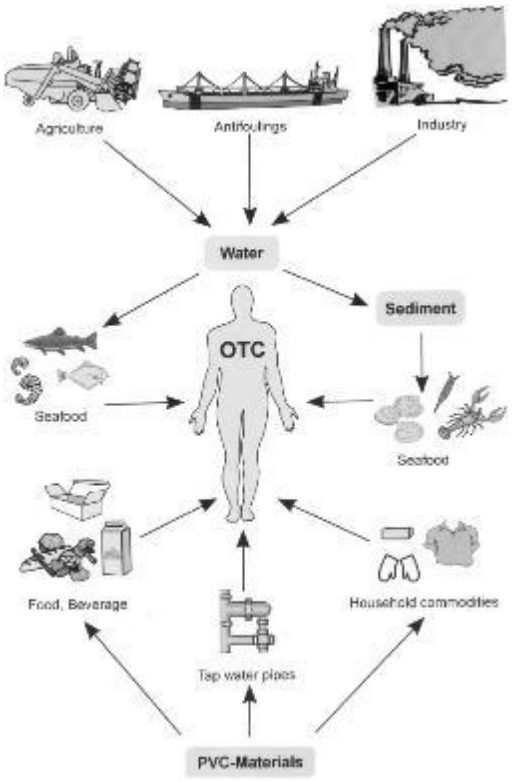
# OKS – zgodovina in področja uporabe



# OKS - strupenost

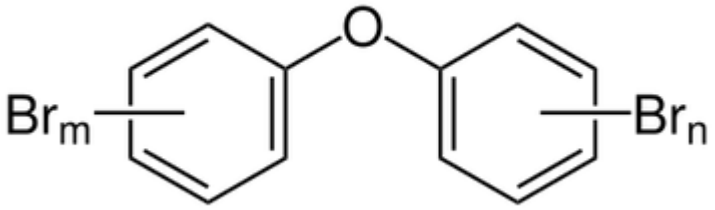
$R_4Sn \cong R_3Sn^+ > R_2Sn^{2+} > RSn^{3+} > Sn^{4+}$

- Izpostavljenost
  - intenzivnost, čas, pogostost and način
- Organizem
  - npr. sesalci: metil- ethil- >> fenil- > oktil-



# Polibromirani difenil etri - PBDE

## Zgradba



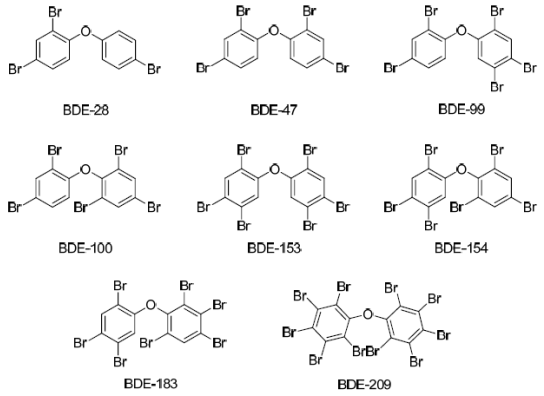

m = 1 – 5

n = 0 – 5

Σ 209

**Table 1:** Homologues, number of isomers for each homologue group and nomenclature for PBDEs.

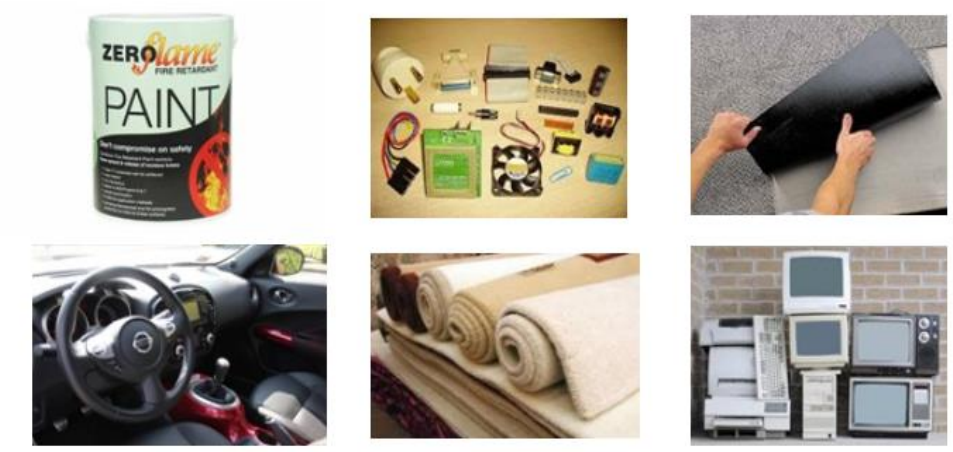
| Homologues | Chemical formula (Molecular mass) | Number of isomeric congeners | Congeners |
|---|---|---|---|
| monoBDEs | $C_{12}H_9Br_1O$ (MW: 249.1) | 3 | BDE-1 to BDE-3 |
| diBDEs | $C_{12}H_8Br_2O$ (MW: 328.0) | 12 | BDE-4 to BDE-15 |
| triBDEs | $C_{12}H_7Br_3O$ (MW: 406.9) | 24 | BDE-16 to BDE-39 |
| tetraBDEs | $C_{12}H_6Br_4O$ (MW: 485.8) | 42 | BDE-40 to BDE-81 |
| pentaBDEs | $C_{12}H_5Br_5O$ (MW: 564.7) | 46 | BDE-82 to BDE-127 |
| hexaBDEs | $C_{12}H_4Br_6O$ (MW: 643.6) | 42 | BDE-128 to BDE-169 |
| heptaBDEs | $C_{12}H_3Br_7O$ (MW: 722.5) | 24 | BDE-170 to BDE-193 |
| octaBDEs | $C_{12}H_2Br_8O$ (MW: 801.4) | 12 | BDE-194 to BDE-205 |
| nonaBDEs | $C_{12}H_1Br_9O$ (MW: 880.3) | 3 | BDE-206 to BDE-208 |
| decaBDE | $C_{12}Br_{10}O$ (MW: 959.2) | 1 | BDE-209 |




Vir: EFSA, 2011

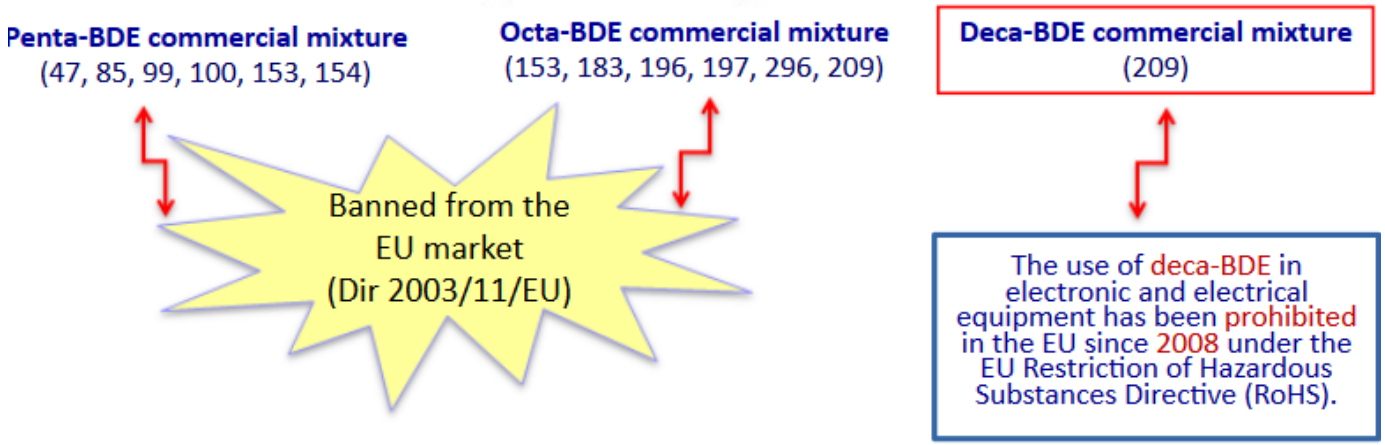
# PBDE – zgodovina in področja uporabe

- Zaviralci gorenja
- Začetek proizvodnje 1976
- Uporaba v električnih in elektronskih izdelkih, pohištvu, tekstilnih izdelkih, plastiki…

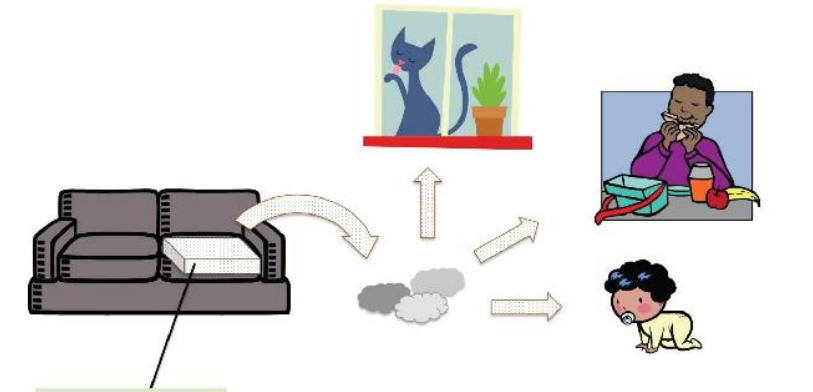


# PBDE – zgodovina in področja uporabe

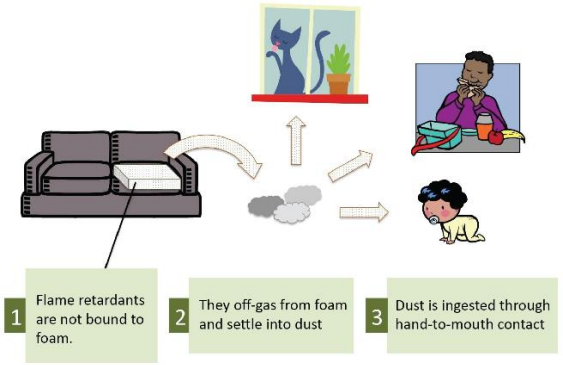
- Zaviralci gorenja
- Začetek proizvodnje 1976
- Uporaba v električnih in elektronskih izdelkih, pohištvu, tekstilnih izdelkih, plastiki…

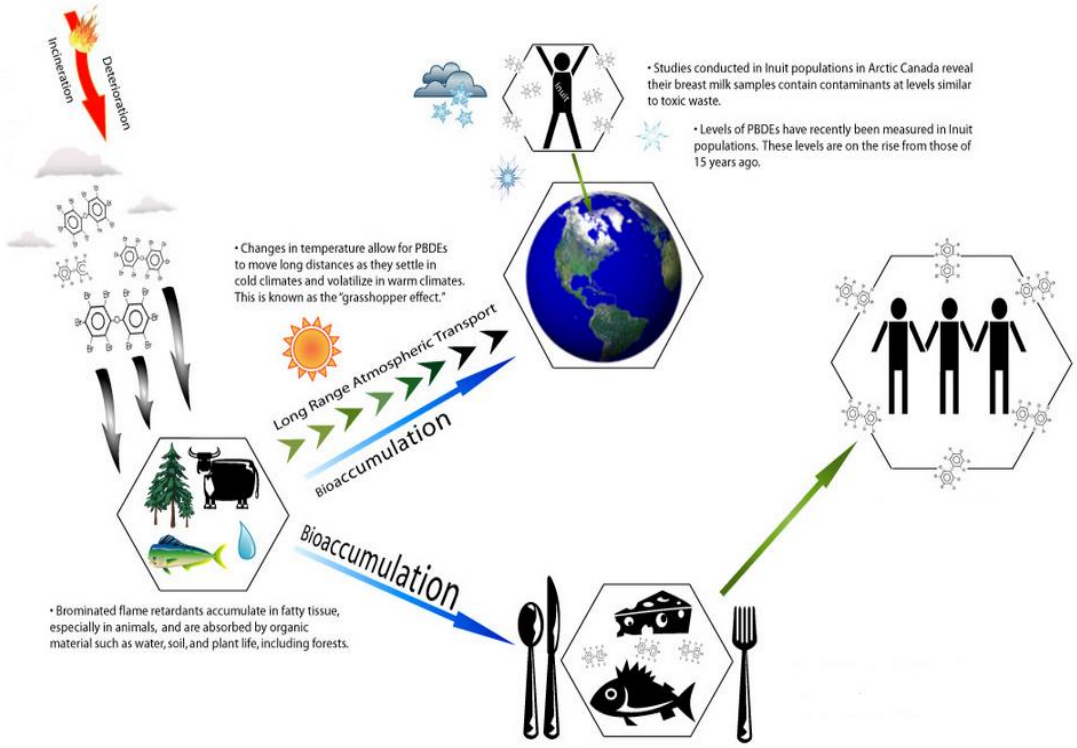


# PBDE – izpostavljenost



# PBDE – izpostavljenost



# Prisotnost PBDE in OKS v bioti

| Location | Sampling year | Sample type | ∑PBDEs | References |
|---|---|---|---|---|
| | | Fish | ng $g^{-1}$ ww | |
| Finland Sea | 2007-2008 | farmed fish | 0.9 - 1.7 | (Souminen, et al., 2011) |
| Baltic Sea | 2004-2006 | herring, salmon | 1.2 - 2.5 | (Szlinder-Richert et al. 2010) |
| Belgium Sea | 2003 | plaice, whiting | 0.06 - 6.1 | (Voorspoels, et al., 2003) |
| German rivers | 2013 | bream | 0.14 – 18 | (Lohmann, et al., 2015) |
| England rivers | 2007-2011 | roach | 2 – 44 | (Jürgens, et al., 2013) |
| Czech Rep. Elbe river | 2002-2003 | chub, bream, trout | 2 – 18 | (Pulkrabova, et al., 2007) |
| Norway lakes | 1998-2004 | brown trout | 0.3 – 23 | (Mariussen, et al., 2008) |
| Italy | 2011-2012 | halibut, blue fish, trout | 0.03 - 0.5 | (Martellini, et al., 2016) |
| | | Mussels | ng $g^{-1}$ ww | |
| Greece | 2005-2007 | mussel | 0.07-1.51 | (Dosis, et al., 2016) |
| Portugal | 2002-2004 | mussel | 0.18-0.86 | (Gama, et al., 2016) |
| Italy | 2008 | mussel | 0.17-1.04 | (Giandomenico, et al., 2013) |
| Belgium | 2002 | mussel | 0.15-1.8 | (Covaci, et al., 2005) |
| Norway | 2002 | mussel | 0.2 | (Knutsen, et al., 2008) |
| U.K. | 2006 | mussel | 0.2-3 | (Fernandes, et al., 2008) |
| | | Birds | ng $g^{-1}$ lipid | |
| Island | 2002-2004 | eider | 44 | (Jörundsdóttir, et al., 2013) |
| Island | 2002-2004 | great skua | 2400 | (Jörundsdóttir, et al., 2013) |
| Belgium | 2001-2003 | buzzard | 17-4400 | (Voorspoels, et al., 2005) |
| Belgium | 2001-2003 | sparrow hawk | 79-18000 | (Voorspoels, et al., 2005) |
| | | Mammals | ng $g^{-1}$ lipid | |
| Belgium | 2004 | red fox | 1-44 | (Voorspoels ,et al., 2006) |
| Norway | 2001 | polar bear | 13-70 | (Wolkers, et al., 2004) |
| Norway | 1999 | ringed seal | 14.1-23.7 | (Wolkers, et al., 2004) |
| Greenland | 2006 | dolphin | 249-308 | (Rotander, et al., 2012) |
| Island | 2003-2006 | Minke whale | 64-111 | (Rotander, et al., 2012) |
| Norway | 2006 | ringed seal | 21-33 | (Rotander, et al., 2012) |

| Species | Origin | Organotin compound (ng Sn $g^{-1}$ ww) | | | References |
|---|---|---|---|---|---|
| | | MBT | DBT | TBT | |
| Sardine (Sardina pilchardus) | PT | <0.3 – 3.1 | 1.2 – 5.1 | 13.2 – 29.6 | MM Santos et al., 2009 |
| | FR | 1.2 | 1.7 | 1.5 | T Guerin et al., 2007 |
| | GR | n.d. | n.d. | 0.10 – 19.50 | AP Louppis et al., 2010 |
| | SP | n.d. | n.d. | 7.40 – 14.3 | OT SAFE 2004 |
| Anchovy | FR | 1.3 | 0.6 | 1.5 | T Guerin et al., 2007 |
| | GR | n.d.<br>n.d. | n.d.<br>n.d. | 0.20 – 4.35<br>12.7 – 20.8 | AP Louppis et al., 2010 |
| | SP | n.d. | n.d. | 1.60 – 12.3 | OT SAFE 2004 |
| | IT | n.d. | n.d. | 3 dw | T Fortibuoni et al., 2013 |
| Whiting | SP | n.d. | n.d. | 0.40 – 9.0 | OT SAFE 2004 |
| | NL | n.d. | n.d. | 0.80 – 1.60 | |
| | IT | n.d. | n.d. | < LOD* | T Fortibuoni et al., 2013 |
| Salmon | BE | n.d. | n.d. | 0.80 | OT SAFE 2004 |
| | DE | n.d. | n.d. | 0.40 – 3.30 | |
| | FR | n.d.<br>1.80 | n.d.<br>1.90 | 2.45 – 2.85<br>0.70 | T Guerin et al., 2007 |
| | SP | n.d. | n.d. | 0.80 – 4.50 | OT SAFE 2004 |
| Seabream | GR | n.d. | n.d. | 5.30 | T Guerin et al., 2007 |
| | FR | 1.1 | 1.0 | 1.0 | |
| | IT | n.d. | n.d. | 7 dw | T Fortibuoni et al., 2013 |
| Seabass | GR | n.d. | n.d. | 0.40 – 2.80 | OT SAFE 2004 |
| | FR | 1.1 | 1.4 | 4.3 | T Guerin et al., 2007 |
| | IT | n.d. | n.d. | 4 - 50 dw | T Fortibuoni et al., 2013 |
| Squid | SP | n.d. | n.d. | 1.60 – 9.80 | OT SAFE 2004 |
| | GR | n.d. | n.d. | 0.40 – 1.60 | |
| | FR | 0.9 | 1.4 | 10.0 | T Guerin et al., 2007 |
| Mussel | ES | n.d. | n.d. | 11 | OT SAFE 2004 |
| | FR | n.d. | n.d. | 2 - 26 | OT SAFE 2004 |
| | | 0.8 | 0.5 | 1.1 | T Guerin et al., 2007 |
| | GR | n.d. | n.d. | 3 - 12 | OT SAFE 2004 |
| | IT | n.d. | n.d. | 132 - 307 | |
| | NL | n.d. | n.d. | 2 - 8 | |
| | PT | 3.8 - 9.1 | 29.1 - 32.2 | 70 - 100 | MM Santos et al., 2009 |
| | UK | n.d. | n.d. | 2.50 – 21.0 | OT SAFE 2004 |

# Prisotnost PBDE in OKS v bioti

- Priporočilo EU 2014/118/EU o monitoringu prisotnosti bromiranih zaviralcev gorenja v živilih
- zahtevana meja detekcije < **10 pg $g^{-1}$**
- monitoring izveden v času 2014-2015
- 3971 vzorcev živil
- Belgija, Češka, Nemčija, Estonija, Španija, Finska, Francija, Irska, Nizozemska, Norveška, Anglija
- izmed 19 kongenerjev najbolj pozorni na BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 in BDE-209
- rezultat: - najpogosteje določena BDE-47 in BDE-209
  - koncentracije nizke, ki naj ne bi ogrožale ljudi

# Analizni postopek

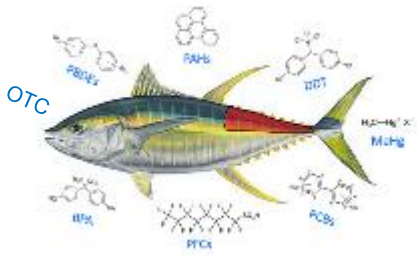
## OKS

Predpriprava vzorca
(homogeniziranje, liofilizacija, …)

Ekstrakcija

Derivatizacija / Ekstrakcija

Ločba in detekcija
(GC-PFPD, GC-MS, GC- ICP-MS)




## PBDE

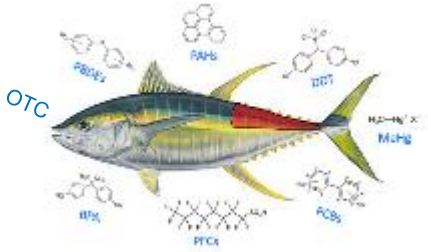
Predpriprava vzorca
(homogeniziranje, liofilizacija, …)

Ekstrakcija

Čiščenje ekstrakta / Koncentriranje

Ločba in detekcija
(GC- ECD, GC-MS, GC-MS-MS,
GC- ICP-MS)

# Analizni postopek




## OKS

Biološki vzorci
+
Interni standard / izotopsko obogaten standard

↓

Ekstrakcija
(0,1 mol $L^{-1}$ HCl v metanolu, mehansko stresanje 2h, ultrazvok 1h)

↓

Derivatizacija/Ekstrakcija
(2 % $NaBEt_4$, izo-oktan, 45 min stresanja)

↓

Ločba in detekcija
GC-ICP-MS

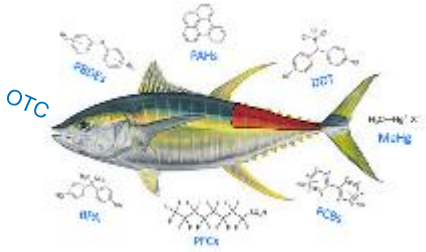
+ ekstrakt vzorca
+ acetatni pufer (pH 4.8-5)
+ $NaBEt_4$ (2%)

+ izooktan

45 min stresanja

organska faza (2µL)

# Analizni postopek




## OKS

Biološki vzorci
+
Interni standard / izotopsko obogaten standard

↓

Ekstrakcija
(0,1 mol $L^{-1}$ HCl v metanolu, mehansko stresanje 2h, ultrazvok 1h)

↓

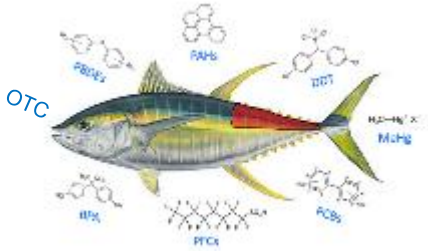
Derivatizacija/Ekstrakcija
(2 % $NaBEt_4$, izo-oktan, 45 min stresanja)

↓

Ločba in detekcija
GC-ICP-MS

| OKS | Ribe | | Školjke | | Mehkužci | |
|---|---|---|---|---|---|---|
| (µg Sn $kg^{-1}$) | LOD | LOQ | LOD | LOQ | LOD | LOQ |
| MBT | 0.036 | 0.117 | 0.047 | 0.155 | 0.061 | 0.203 |
| DBT | 0.011 | 0.035 | 0.026 | 0.085 | 0.039 | 0.130 |
| TBT | 0.014 | 0.050 | 0.018 | 0.060 | 0.059 | 0.195 |

# Analizni postopek




## PBDE

0.25 g freeze dried tissue or 1.5 g fresh tissue

+ BDE 77

+ $^{81}$Br enriched PBDEs

10 mL 25 % TMAH in $H_2O$
Ultrasound assisted extraction, 30 min

50 mL Tris-citrate buffer (pH 6) + 2 mL iso-octane
Mechanical shaking, 2 h (300 rpm)

Organic phase collected + 0.5 mL 25% KOH in MeOH
Centrifugation, 5 min 5000 x *g*

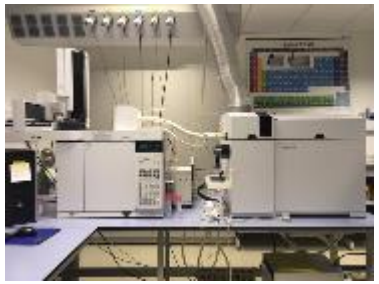
Extract (iso-octane) passed through a Florisil column, evaporated under $N_2$ to 30 µL

GC-ICP-MS

ID-GC-ICP-MS

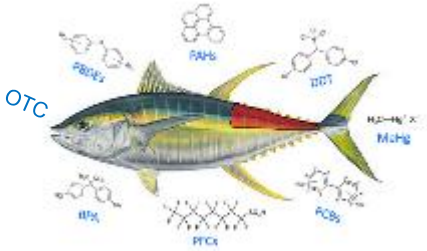
organska faza

stresanja

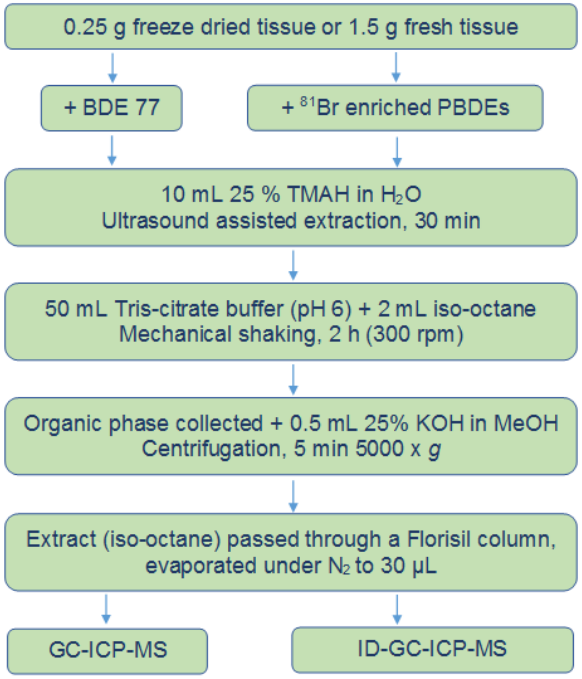


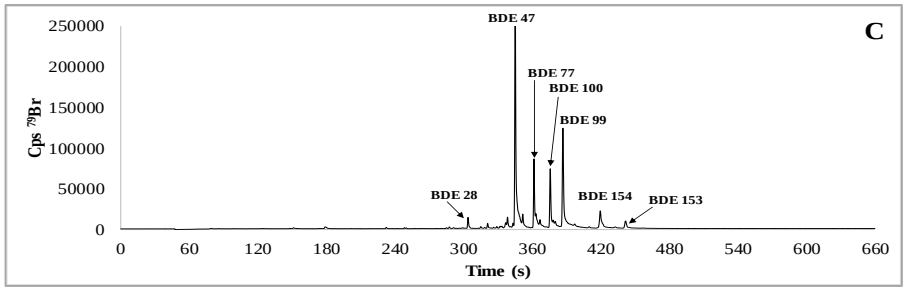
Vir: Novak P. in sod., Journal of Chromatography A, 2017

# Analizni postopek




## PBDE

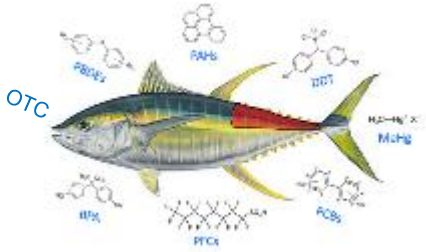



| BDE congener | LOD (ng $g^{-1}$ ww) | LOQ (ng $g^{-1}$ ww) |
|---|---|---|
| BDE 28 | 0.003 | 0.011 |
| BDE 47 | 0.006 | 0.019 |
| BDE 99 | 0.008 | 0.027 |
| BDE 100 | 0.004 | 0.013 |
| BDE 153 | 0.005 | 0.015 |
| BDE 154 | 0.009 | 0.030 |

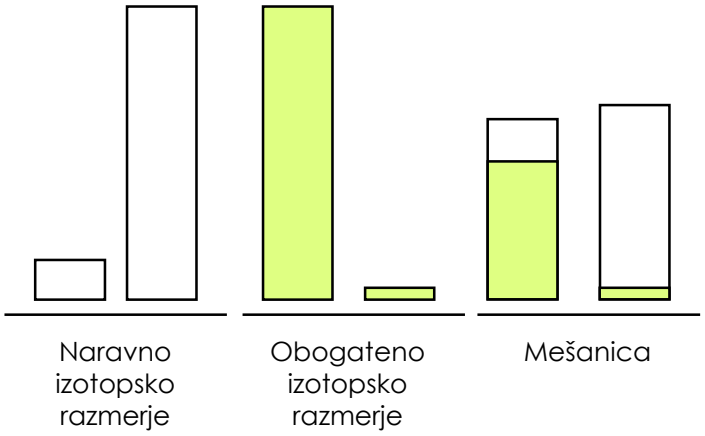
Vir: Novak P. in sod., Journal of Chromatography A, 2017

# Analizni postopek




Določanje koncentracije

- metoda standardnega dodatka
- metoda izotopskega redčenja




$$R_b^a = \frac{A^a}{A^b}$$

$$C_s = C_{sp}\,\frac{m_{sp}}{m_s}\,\frac{M_s}{M_{sp}}\,\frac{A_{sp}^b}{A_s^a}\left(\frac{R_{b,m}^a - R_{b,sp}^a}{1 - R_{b,m}^a R_{a,s}^b}\right)$$

$R^a{}_b$ - izotopsko razmerje med izotopoma a in b
$A_a$ - delež izotopa a v vzorcu
$A_b$ - delež izotopa b v vzorcu

$C_{s,\,sp}$ - koncentracija elementa v vzorcu oz. dodatku
$m_{s,sp}$ - masa vzorca oz. dodatka
$M_{s,\,sp}$ - atomska masa elementa v vzorcu oz. dodatku

# OKS in PBDE v ribah iz slovenskega tržišča

- OKS

| Vrsta | Poreklo | DBT (ng Sn $g^{-1}$ mt) | TBT (ng Sn $g^{-1}$ mt) | ΣBuT (ng Sn $g^{-1}$ mt) |
|---|---|---|---|---|
| Losos | NW | < 0.011 | 0.158 ± 0.010 | 0.158 |
| Brancin | SI | 0.0480 ± 0.0005 | 0.226 ± 0.025 | 0.274 |
| | GR | 0.0620 ± 0.0005 | 0.266 ± 0.024 | 0.328 |
| | Cr-wild | 0.333 ± 0.025 | 1.90 ± 0.15 | 2.233 |
| Orada | SI-wild | < 0.011 | 0.353 ± 0.025 | 0.353 |
| Mol | SI | < 0.011 | 0.162 ± 0.012 | 0.162 |
| Sardela | SI | < 0.011 | 1.35 ± 0.12 | 1.35 |
| Sardon | CR | < 0.011 | 3.82 ± 0. 25 | 3.82 |
| Sipa | SI | < 0.039 | 0.688 ± 0.053 | 0.688 |

# OKS in PBDE v ribah iz slovenskega tržišča

- PBDE

| | Location | Species | BDE (ng $g^{-1}$ mt) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | BDE 28 | BDE 47 | BDE 99 | BDE 100 | BDE 153 | BDE 154 |
| **The Northern Adriatic Sea** | Slovenia | sardele | 0.11 | 0.53 | 0.11 | 0.080 | < lod | < lod |
| | | brancin | 0.05 | 0.18 | 0.109 | 0.102 | 0.060 | 0.086 |
| | | mol | 0.07 | 0.14 | 0.069 | 0.068 | 0.034 | 0.065 |
| **Local market** | Slovenia | školjke | 0.03 | 0.09 | 0.05 | 0.07 | < lod | < lod |

# Hvala za pozornost!