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# Risk characterization for mercury, dichlorodiphenyltrichloroethane and polychlorinated biphenyls associated with fish consumption in Serbia

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ABSTRACT

The aim of this work was to assess the risk due to mercury (Hg), dichlorodiphenyltrichloroethane (DDT) and non-dioxin-like polychlorinated biphenyls (ndl PCBs) intake via fish consumption in Serbia. We have developed 24 scenarios using four concentration levels (mean, maximum, 50th and 95th percentile) of contaminants, determined in 521 samples of fish products available on Serbian market; two consumption levels (Food and Agriculture Organization/World Health Organization data and recommendation of American Heart Association); and three body weights (5th, 50th and 95th percentile). All the values concerning the intake of DDT are below the corresponding health based guidance value. Calculated weekly intake of Hg using maximal concentration, intake of 340 g/week and 5th percentile of body weight exceeded the provisional tolerable weekly intake (PTWI). When maximal and 95th percentile concentration of ndl PCBs was used, weekly intakes exceeded a "guidance value" with one exception *i.e.*, when 95th percentile of concentration along with 95th percentile of body weight were used. Concerning Hg and ndl PCBs, when extreme concentrations were used, HIs exceeded the value of 1, indicating that fish and fishery products may pose a threat to consumer's health.



## 1. Introduction

The potential health benefits related to fish consumption are due to the presence of proteins, minerals, vitamins, and unsaturated essential fatty acids, especially polyunsaturated fatty acids (PUFAs) like omega-3 PUFAs. Dietary omega-3 fatty acids have a variety of anti-inflammatory and immune-modulating effects that may be of relevance to atherosclerosis and its clinical manifestations of myocardial infarction, sudden death, and stroke (Simopoulos, 1991a,b; Rennie et al., 2003; Albert, 2004; Din et al., 2004; Ruxton et al., 2004; Nettleton and Katz, 2005; Antonijevic et al., 2007). A variety of biologic effects include effects on triglycerides, high-density lipoprotein cholesterol, platelet function, endothelial and vascular function, blood pressure, cardiac excitability, measures of oxidative stress, pro- and anti-inflammatory cytokines, and immune function. Essential fatty acids are structural components of all tissues and are indispensable for cell membrane synthesis. The brain, retina and other neural tissues are particularly rich in long-chain omega-3 PUFAs, which are a conditionally essential nutrient for adequate neurodevelopment in humans (Simopoulos, 1991b; Salem and Pawlosky, 1992; de Wilde et al., 2002; Sidhu, 2003). It has been also shown that omega-3 PUFAs may interfere and modulate the carcinogenic process (Terry et al., 2003; Ding et al. 2004; Larsson et al., 2004).

On the other hand, fish and fishery products are susceptible to contamination, with chemicals that have been recognized as ubiquitous environmental pollutants such as heavy metals and polychlorinated organic compounds (EFSA, 2005a; Storelli, 2008). Fish consumption could be therefore considered as one of the major sources of human exposure to all above-mentioned environmental contaminants of which, according to monitoring data from the National reference laboratory of the Institute of meat hygiene and technology in Serbia, the mostly abundant are mercury (Hg),

*Abbreviations:* AFSSA, French Agency for Food Safety; AHA, American Heart Association; ATSDR, Agency for Toxic Substances and Disease Registry; DDT, dichlorodiphenyltrichloroethane; EFSA, European Food Safety Authority; FAO/WHO, Food and Agriculture Organization/World Health Organization; HI, hazard index; JECFA, Joint FAO/WHO Expert Committee on Food Additives; JMPR, Joint FAO/WHO Meeting on Pesticide; LOD, limit of determination; LOQ, limit of quantification; Hg, mercury; MAC, maximum allowable concentration; MRL, maximum residue level; MetHg, methylmercury; NOAEL, No-observed adverse effect level; ndl PCBs, nondioxin-like PCBs; OCs, organochlorines; POPs, Persistent Organic Pollutants'; PCBs, polychlorinated biphenyls; PUFAs, polyunsaturated fatty acids; PTDI, provisional tolerable daily intake; PTWI, provisional tolerable weekly intake; 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TDI, tolerable daily intake; UNEP, United Nations of Environmental Programme; WI, weekly intake.

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dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) (Curcic et al., 2009; Jankovic et al., 2010).

Hg enters the environment by both, natural sources (such as volcanic activity, erosions, and weathering what contribute to the presence of Hg in the water, soil, and the atmosphere) and human activities – mining, fossil fuel combustion, industrial emissions, direct application of fertilizers and fungicides as well as disposal of solid waste. Conversion of inorganic Hg to the methylated form is of critical importance in terms of Hg in food. The methylation of inorganic Hg into methylmercury (MetHg) occurs primarily as a result of microbial activity (Merritt and Amirbahman, 2009; Ersoy and Çelik, 2010; Saei-Dehkordi et al., 2010). MetHg exposure can cause neurological symptoms such as paresthesia, ataxia, and has also been associated with developmental delays in children whose mothers were exposed during pregnancy (WHO, 1990). Much of the information about the effects of MetHg comes from high level poisoning episodes in Iraq (Bakir et al., 1973) and in Minamata and Nigata, Japan (Irukayama et al., 1977). However, there is also concern that MetHg can cause developmental and other neurological effects at lower level of exposure more consistent with the usual patterns of fish consumption (Davidson et al., 1995; Grandjean et al., 1997).

Over the past few decades, the occurrence of organochlorines (OCs) in the environment is of great concern due to their persistent and long-range transportable nature as well as toxic biological effects (Tanabe et al., 1994; Wania and Mackay, 1996; Bourdon et al., 2010). In May 2001, the 'Stockholm Convention on Persistent Organic Pollutants' (POPs) adopted by the United Nations of Environmental Programme (UNEP) highlighted the need to control the global contamination by toxic environmental chemicals. The treaty promotes the global regulations on the production and usage of persistent OCs (Mariscal-Arcas et al., 2010) such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) (Moon et al., 2009). Even these contaminants are present in the environment at low levels, they could be taken up by aquatic organisms and bioconcentrate and bioaccumulate to the progressively higher levels in the food chain, particularly in longer-living and predatory fish. Toxic effects of DDT were widely recognized in the 1960s and 1970s in relation to avian eggshell thinning, and other adverse reproductive effects in birds have been reported since that time (ATSDR, 2002). These adverse effects may include neurological deficits, liver dysfunction, reproductive anomalies, behavioral abnormalities, immune dysfunction, endocrine disruption and carcinogenesis (ATSDR, 1999, 2000, 2002). The developing fetus and neonate are particularly vulnerable to exposure due to transplacental and lactational transfer of maternal burdens at critical periods of development and growth.

The potential toxicity of PCBs was first recognized following incidents in Japan in 1968 and Taiwan in 1979 when rice oil used for cooking was contaminated by heat-degraded PCBs during the manufacturing process (Chen et al., 1985; Hsu et al., 1985; Masuda et al., 1985; Rogan et al., 1986; Yen et al., 1989). These incidents in addition to both human occupational exposure studies (Taylor et al., 1984, 1989) and other animal studies (Allen et al., 1974, 1979, 1980; Allen and Barsotti, 1976; Barsotti et al., 1976; Brezner et al., 1984; Peterson et al., 1993; Brouwer et al., 2001) have led to PCBs being implicated as reproductive toxic substances.

Surveys carried out in a number of countries on the daily exposure to these contaminants in humans have shown that over 90% of exposure occurs through the diet, with foods of animal origin usually being the predominant source (Smith and Gangolli, 2002; Lorán et al., 2010; Radosevic et al., 2010). Because of the fact that freshwater and marine fish may be highly contaminated, to the regulatory/advisory bodies consumption of this type of food and corresponding products became a major concern. Numerous national and international regulatory bodies have established fish consumption guidelines, particularly for those fish known to accumulate a variety of chemicals, as well as recommendations of alternative lifestyle or diets.

In the past years, fish consumption has increased in Serbia primarily due to public campaign on health benefits through popular media (Lekic-Arandjelovic et al., 2008). In general, health benefits and fish consumption are informed through different organizations but rarely there is an insight into the potential health risk (Antonijevic et al., 2007; Pieniak et al., 2010; Ruxton, 2011). In order to make contribution to the issue the aim of this study was to assess the risk due to Hg, DDT, and non-dioxin-like PCBs (ndl PCBintake associated with fish consumption by integration of empirical contaminant concentrations measured in fish and fish canned products available on Serbian market and data on fish consumption.

## 2. Materials and methods

### 2.1. Contamination data

Concentrations of Hg, DDT, and ndl PCBs were measured in fish and fish canned products available on Serbian market. The term DDT actually comprises DDT and related metabolites: dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD). Totally 521 samples were analysed: 252 samples of marine fish (hake-*Merluccius merluccius*, mackerel-*Scomber scombrus*, sprat-*Sprattus sprattus*, scorpanea-*Scorpaena scrofa*, gilthead-*Sparus aurata*, anchovy-*Engraulidae*, salmon-*Salmo salar*), 52 samples of freshwater fish (trout-*Salmo irideus* and carp-*Cyprinus carpio*) and 217 samples of canned fish products (tuna and sardines). These data were collected during 2010 year. The origin of fish analysed for the purpose of this study differs depending on fish species: salmon – SAD and Bulgaria, gilthead – Greece, hake – Argentina, China and Norway, sprat – Poland and Ireland, anchovy – Greece and Spain, mackerel – Spain and Ireland, scorpanea – Island; carp – Serbia and Bosnia and Herzegovina, trout – Serbia and Bosnia and Herzegovina; canned fish – Tailand, Filipines, Maroco, Spain, and Croatia. Measurements were done in National reference laboratory of the Institute for meat hygiene and technology, in compliance with ISO standard 17 025. Quantitatively and qualitatively, analysed samples could be accepted as representative on the national level.

Fish specimens were kept frozen at −20 °C before analysis. Edible parts were chopped into 2–3 cm thick portions and homogenized. Samples for Hg analysis were prepared by microwave digestion (ETHOS Milestone). Analyses were carried out on atomic absorption spectrometer Varian "SpectrAA 220" with VGA 77 hydride system. Cold vapor technique for Hg was applied. The limit of quantification (LOQ) for Hg was 5 ng/g. Analytical quality control was achieved by using certified reference material BCR 186. Replicate analyses were in the range of certified values. DDT and ndl PCBs were extracted and separated by elution from fat in small glass columns filled with partially deactivated alumina. The eluate was evaporated to an appropriate volume. An aliquot of 1 μl was injected into a gas chromatograph coupled with electron capture detector. GC Varian Model 3800 equipped with a $^{63}$Ni electron capture detector and Varian VF 5-ms column (30 m × 0.25 mm i.d. and 0.25 μm film thickness) were used for analysis of PCBs (congeners 28, 52, 101, 118, 138, 153, and 180) and DDT. Operating conditions were the following: injector 250 °C; detector 300 °C; column oven program: initial 50 °C raised to 200 °C at 50 °C/min, hold for 2 min then raised to 215 °C at 2.5 °C/min, hold for 5 min and finally raised to 230 °C at 2 °C/min, hold for 9.5 min. The highly purified nitrogen carrier gas flow was 1 ml/min. Data acquisition was performed by Varian Star software. Analysis of sample blank showed no interference peaks with the individual PCB congener and DDT analysis. The limit of determination (LOD) for each compound was determined as the mean of 10 times background noise from five reagent blank samples. Methods LOQs, which depended on congener type, were in the range 0.2–0.5 ng/g. Analytical quality control was achieved by using certified reference material ERM-BB446. Accuracy and intermediate precision were fulfilled according to the specific requirements for determination of ndl PCBs (State Institute for Chemical and Veterinary Analysis of Food, 2008). Values below the LOD were assigned one half of LOD.

### 2.2. Intake assessment

Total diet study has not been undertaken in Serbia, so far. Instead of such comprehensive data base, for the purpose of intake assessment, we used the only available data taken from the GEMS/Food Consumption Cluster Diets database (FAO/WHO, 2006). According to this data source estimated average weekly consumption of total fish related to adults in Serbia is 154 g/week of which 106.4 g belongs to marine fish, 29.4 g to freshwater fish and 18.2 g to fish canned products.The following formula was used for calculation of intake assessment expressed as weekly intake (WI) in μg/kg b.w.:

$$\mathrm{WI} = \frac{\text{Weekly consumption}(g) \times \text{Concentration of contaminant}(\mu g/g)}{\text{Body weight}(kg)}$$

Having into consideration differences in dietary habits a "composite fish dish" – a theoretical mixture of relevant marine, freshwater species and canned fish products has been developed on the basis of the existing fish consumption profile in the baseline study (FAO/WHO, 2006). The relative contribution for every single "fish"' component in the "composite fish dish" was thereby calculated on the basis of the observed 7-day average intake for all tree consumed fish items (Table 1).

In order to calculate Hg, ndl PCBs, and DDT intake, we have developed 24 different scenarios, using $4 \times 2 \times 3$ design. Namely, four concentration levels of certain contaminants were chosen: mean, maximal, 50th and 95th percentile; two fish consumption levels were included: the first one, obtained from Food and Agriculture Organization/World Health Organization (FAO/WHO) data base (FAO/WHO, 2006) – 154 g total fish/week and the second one obtained from recommendation of American Heart Association (AHA) – 340 g total fish/week (AHANC, 2006). Finally, three body mass values were used: a body mass of 51 kg that represents 5th percentile, 70 kg that represents 50th percentile, and a body mass of 98 kg that represents 95th percentile of a total population of 808 adult healthy volunteers (395 females and 413 males), included in national survey from the Department of Endocrinology of Clinical Center Vojvodina, Novi Sad and Faculty of Medicine, University of Novi Sad (Srdic, 2002).For each of twelve scenarios hazard index (HI) was calculated based on formulas given below:

$$\mathrm{HI} = \frac{\text{calculated weekly intake for certain contaminat}}{\text{provisional tolerable weekly intake}}$$

$$\mathrm{HI}_{\mathrm{TOTAL}} = \mathrm{HI}_{\mathrm{Hg}} + \mathrm{HI}_{\mathrm{DDT}} + \mathrm{HI}_{\mathrm{PCB}}$$

## 3. Results and discussion

Analytical method for Hg determination in fish was linear in the range of 1–75 ng/g ($r$ = 0.9993). Repeatability was confirmed by corresponding coefficient of variation of 2.46%, while limit of quantification (LOQ) was 5 ng/g. Analytical quality control was achieved by using certified reference material BCR 186, with certified value for mercury of 1.97 ± 0.04 μg/g. Results for our replicate analysis – 1.99 ± 0.05 μg/g, were in the range of certified values. Linearity of the analytical method for DDT and ndl PCBs determination in fish samples was in the range of 1–300 ng/g ($r$ = 0.9977 and $r$ = 0.9980, respectively), while repeatability was 5.04% and 6.57%, respectively. Calculated LOQ value for DDT was 0.5 ng/g and for congeners of ndl PCBs it varied in the range 0.2–0.5 ng/g, depending on congener type. Analytical quality control was achieved by using certified reference material ERM-BB446, with certified value for ndl PCBs of 207 ± 11 ng/g. Our results for replicate analysis (198 ± 13 ng/g) were in the range of certified values. Accuracy and intermediate precision were fulfilled according to the specific requirements for determination of ndl PCBs (State Institute for Chemical and Veterinary Analysis of Food, 2008).

The data in Table 1 were presented using minimal, maximal, and mean values as well as using quartiles to describe better concentration distributions. In the structure of "composite fish dish" the major part belongs to the marine fish followed by freshwater fish and canned products. All the values have indicated that Hg is predominant contaminant particularly of canned products (Table 1).

To protect public health, maximum levels of Hg in fishery products are laid down by the Commission Regulation (EC) No 1881/2006 of 19 December 2006 (EC 1881/2006, 2006). The levels should be as low as reasonably achievable, taking into account that for physiological reasons certain species concentrate Hg more easily in their tissues than others. Hg limit for fishery products in general is 500 ng/g fresh weight. It is 1000 ng/g fresh weight for anglerfish, Atlantic catfish, bass, blue ling, bonito, eel, halibut little tuna, marlin, pike, plain bonito, Portuguese dogfish, rays, redfish, sail fish, scabbard fish, shark, snake, mackerel, sturgeon, swordfish, and tuna. According to the Serbian Rulebook on quantities of pesticides, metals and metalloids and other toxic substances, chemotherapeutics, anabolics, and other substances that can be found in foodstuffs (Official Journal of FRY 5/92, 1992) maximum

**Table 1**
Structure and contents of Hg, DDT, and ndl PCBs in a composite fish dish.

| Fish item | Composite fish (g/week) | Composite fish (%) | Hg (ng/g) | | | | | | | DDT (ng/g) | | | | | | | ndl PCBs (ng/g) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | min | max | mean | 25th | 50th | 75th | 95th | min | max | mean | 25th | 50th | 75th | 95th | min | max | mean | 25th | 50th | 75th | 95th |
| Marine fish | 106.4 | 69.09 | n.d. | 286 | 34 | 16 | 29 | 41 | 78 | n.d. | 118 | 12 | n.d. | n.d. | 12 | 61 | n.d. | 66 | 7 | n.d. | n.d. | 2 | 34 |
| Freshwater fish | 29.4 | 19.09 | 5 | 61 | 23 | 14 | 17 | 26 | 61 | 1 | 54 | 8 | 1 | 4 | 4 | 54 | n.d. | 45 | 5 | n.d. | n.d. | n.d. | 45 |
| Canned products | 18.2 | 11.82 | n.d. | 502 | 58 | 26 | 40 | 70 | 131 | n.d. | 231 | 10 | n.d. | n.d. | n.d. | 51 | n.d. | 94 | 5 | n.d. | n.d. | n.d. | 31 |
| | | | Composite fish dish | | | | | | | | | | | | | | | | | | | | |
| Σ | 154.0 | 100 | 1.4 | 268.6 | 34.7 | 16.8 | 28.0 | 41.6 | 81.0 | 0.6 | 119.1 | 11 | 0.6 | 1.2 | 9.2 | 58.5 | 0.5 | 63.2 | 6.4 | 0.5 | 0.5 | 1.5 | 35.7 |

n.d. – not detected.

allowable concentration (MAC) has been fixed at 500 ng/g fresh weight. In the present study these limits were not exceeded. Obtained ndl PCBs concentrations are below the MAC of 3000 ng/g fresh weight, established by Serbian Rulebook from 1992 (Official Journal of FRY 5/92, 1992), which refers to total PCBs, i.e. including dl PCBs, and are also below the limit of 2000 ng/g proposed by Food and Drug Administration, USA (US FDA, 2001). For the sake of completeness, it should be mentioned that no maximum levels for ndl PCB in feed and food have been set in the European Union, so far. According to Commission Regulation (EC) No. 199/2006 (199/2006 EC, 2006), maximal levels based on cumulative risk assessment and relative toxicity regarding 2,3,7,8-TCDD, have been given only for dl PCBs. The maximum level of 100 ng/g fresh weight for the sum of six ndl PCBs in fish has been proposed by the European Commission (EC) draft regulation (AFFSA, 2007). It can be seen from the Table 1 that mean concentration of ndl PCBs is far from European draft maximum level (Jankovic et al. 2008, 2010).

New EU legislation, i.e. Regulation (EC) No 178/2006 (EC 178/2006, 2006) and Regulation (EC) No 149/2008 (EC 149/2008, 2008) did not set maximum residue level (MRL) for DDT in fish, but only in meat, preparations of meat, offals, blood, animal fats fresh chilled or frozen, salted, in brine, dried or smoked or processed as flours or meals; for other processed products such as sausages and food preparations based on these products with fat content less than 10% maximum level is 0.1 mg/kg. Limit for DDT given by actual national sub law (Official Journal of RS 25/2010, 2010) is equal to EU MRL and represents cut off value for meat and meat products. All the values measured for DDT were several folds below the specified limits in EU and Serbia.

Weekly intake of Hg, DDT, and ndl PCBs through fish consumption has been calculated by deterministic model using fixed mean, maximum, 50th or 95th percentile of contaminants concentrations. The calculated weekly intakes of contaminants by adults of 51 kg (5th percentile), 70 kg (50th percentile), and 98 kg (95th percentile) body weights are summarized in Table 2.

For the purpose of evaluating the health risk the estimated dietary exposures were compared to the corresponding health based guidance values. In the period 2004–2007, several opinions concerning human dietary exposure to Hg were issued (EFSA 2004, 2005a; UK-COT 2004, 2007; Japan FSC, 2005; Canada BCS, 2007). All these documents indicate that fish (marine and freshwater) and seafood are the major source of Hg intake in humans. Depending on species, MetHg accounts for 70–100% total Hg in fish (EFSA, 2005a). However, for conservative assessment purposes, it is generally assumed that 100% of the Hg found in fish and shellfish is MetHg. According to European Food Safety Authority (EFSA), the range of average fish consumption is from 10 to 80 g per day for six European countries, corresponding to Hg weekly intake from 1.3 to 92 μg, per person (EFSA, 2004), that is similar to the intake calculated in our study (4.3–91.3 μg, per person). Markedly higher values were reported for Faroe Islands (average 252 μg/week), while in the Seychelles the daily Hg intake was estimated to be 103 μg, assuming a per capita consumption of fish of 75 kg per year (205 g per day) (Robinson and Shroff, 2004). The estimated average weekly intake of Hg by the French population is 68 μg for adults aged 15 years or more (corresponding to 1.1 μg/kg b.w. per week for a 60 kg person) and 55 μg for children aged 3–4 years (Leblanc et al., 2005). Estimated weekly intake of total Hg in the population from Catalonia (Bocio et al., 2005) is 148 μg, corresponding to 2.1 μg/kg b.w. per week, and is due principally to the high consumption of fish in this region. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) of 1.6 μg MetHg/kg b.w. (i.e. 0.228 μg/kg b.w./day) based on epidemiological studies that investigated the relationship between maternal exposure to Hg and impaired neurodevelopment in their children (FAO/WHO, 2003).

The Joint FAO/WHO Meeting on Pesticide (JMPR) (FAO/WHO, 2001) derived for DDT a provisional tolerable daily intake (PTDI) of 0.01 mg/kg b.w. on the basis of the lowest No-observed adverse effect level (NOAEL) of 1 mg/kg b.w. per day for numerous developmental effects in rats as summarized by Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 1999).

According to EFSA document (EFSA, 2005b) more than 90% of the ndl PCBs exposure in the general population is via food. From the same document average daily dietary intakes of total ndl PCBs can be estimated to be in the range of 10–45 ng/kg b.w. per day. Results obtained in our study were in the range of 0.14–60.1 ng/kg b.w per day. Limited exposure data for young children, up to six years of age, indicates that the average intake (breastfeeding excluded) of total ndl PCBs is about 27–50 ng/kg b.w. per day (EFSA, 2005b). In general, children had exposure levels 2.5-fold higher than adults. In specific subpopulations with high dietary PCBs exposure such as Baltic Sea fishermen the daily intake from fish of the sum of the six ndl PCBs could be about 40 ng/kg b.w., corresponding to an intake of total ndl PCBs of 80 ng/kg b.w. per day before taking into account the rest of the diet. Breastfed infants are a group of high ndl PCBs intake which might be two orders of magnitude higher than adult exposure (EFSA, 2005b).

In the period 1995–2008 a detailed analysis of PCBs in food and feed samples collected from 18 EU Member States, Iceland, and Norway showed that the sum of the six ndl PCBs were on average close to five times higher than the sum of the 12 dl PCBs (EFSA, 2010). This relationship varied across food groups and is presumably related to the origin of samples and the contamination source.

Technical mixtures used in toxicity studies contain both ndl PCBs and dioxin-like compounds such as dl PCBs. These mixtures exert a variety of toxicological effects. However, these effects are not all specific for ndl PCBs but are also to be seen following exposure to polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dl PCBs. Although attempt has been made to establish health based guidance value for ndl PCBs, it was concluded that no such value could be established because simultaneous exposure to ndl PCBs and dioxin-like compounds hampers the interpretation of the results of the toxicological and epidemiological studies, and the database on effects of individual ndl PCB congeners is rather limited. The health based advice is, therefore, that the intake of ndl PCBs should be as low as possible (EFSA, 2010).

Considering the whole spectrum of data and the convergence of different types of toxicological studies for a similar reference value

**Table 2**
Weekly intakes of Hg, DDT, and ndl PCBs via fish consumption.

| Weekly intake (μg/kg b.w.) | | | | |
|---|---|---|---|---|
| Fish intake Concentration | FAO/WHO Mean/50th | AHA Mean/50th | FAO/WHO Max/95th | AHA Max/95th |
| *5th percentile of body weight* | | | | |
| Hg | 0.104/0.085 | 0.232/0.187 | 0.811/0.245 | 1.791/0.540 |
| DDT | 0.033/0.004 | 0.073/0.008 | 0.360/0.177 | 0.795/0.390 |
| ndl PCBs | 0.019/0.002 | 0.043/0.003 | **0.191**/**0.109** | **0.421**/**0.240** |
| *50th percentile of body weight* | | | | |
| Hg | 0.076/0.062 | 0.169/0.136 | 0.591/0.178 | 1.305/0.393 |
| DDT | 0.024/0.003 | 0.053/0.006 | 0.262/0.129 | 0.579/0.284 |
| ndl PCBs | 0.014/0.001 | 0.031/0.002 | **0.139**/**0.079** | **0.307**/**0.175** |
| *95th percentile of body weight* | | | | |
| Hg | 0.055/0.044 | 0.121/0.097 | 0.422/0.127 | 0.932/0.281 |
| DDT | 0.017/0.002 | 0.038/0.004 | 0.187/0.092 | 0.413/0.203 |
| ndl PCBs | 0.010/0.001 | 0.022/0.002 | **0.099**/0.057 | **0.219**/**0.125** |

FAO/WHO – intake of fish proposed by Food and Agriculture Organization/World Health Organization (154 g/week); AHA – intake of fish proposed by American Heart Association (154 g/week).
Provisional tolerable weekly intakes (PTWI): 1.6 μg/kg b.w./week for Hg, 70 μg/kg b.w./week for DDT and 0.07 μg/kg b.w./week for ndl PCBs.

**Table 3**
Hazard indexes for Hg, DDT, and ndl PCBs due to fish consumption among adult population in Serbia.

| Scenario | | | HI | | | |
|---|---|---|---|---|---|---|
| Fish intake | Body weight | Concentration | Hg | DDT | ndl PCBs | Total |
| FAO/WHO 5th | | Mean | 0.0652 | 0.0004 | 0.2745 | 0.3401 |
| | | 50th | 0.0531 | 0.0001 | 0.0286 | 0.0818 |
| AHA 5th | | Mean | 0.1449 | 0.0011 | 0.6079 | 0.7539 |
| | | 50th | 0.1169 | 0.0001 | 0.0429 | 0.1599 |
| FAO/WHO 50th | | Mean | 0.0475 | 0.0003 | 0.2000 | 0.2478 |
| | | 50th | 0.0388 | 0.0000 | 0.0143 | 0.0531 |
| AHA 50th | | Mean | 0.1056 | 0.0008 | 0.4429 | 0.5493 |
| | | 50th | 0.0850 | 0.0001 | 0.0286 | 0.1137 |
| FAO/WHO 95th | | Mean | 0.0344 | 0.0002 | 0.1429 | 0.1775 |
| | | 50th | 0.0275 | 0.0000 | 0.0143 | 0.0418 |
| AHA 95th | | Mean | 0.0756 | 0.0005 | 0.3143 | 0.3904 |
| | | 50th | 0.0606 | 0.0001 | 0.0286 | 0.0893 |
| FAO/WHO 5th | | Max | 0.5070 | 0.0051 | **2.7241** | **3.2362** |
| | | 95th | 0.1531 | 0.0025 | **1.5571** | **1.7127** |
| AHA 5th | | Max | **1.1186** | 0.0114 | **6.0181** | **7.1481** |
| | | 95th | 0.3375 | 0.0056 | **3.4286** | **3.7717** |
| FAO/WHO 50th | | Max | 0.3694 | 0.0037 | **1.9847** | **2.3578** |
| | | 95th | 0.1113 | 0.0018 | **1.1286** | **1.2417** |
| AHA 50th | | Max | 0.8150 | 0.0083 | **4.3846** | **5.2079** |
| | | 95th | 0.2456 | 0.0041 | **2.5000** | **2.7497** |
| FAO/WHO 95th | | Max | 0.2638 | 0.0027 | **1.4140** | **1.6805** |
| | | 95th | 0.0794 | 0.0013 | 0.8143 | 0.8950 |
| AHA 95th | | Max | 0.5825 | 0.0059 | **3.1328** | **3.7212** |
| | | 95th | 0.1756 | 0.0029 | **1.7857** | **1.9642** |

FAO/WHO- intake of fish proposed by Food and Agriculture Organization/World Health Organization (154 g/week); AHA – intake of fish proposed by American Heart Association (340 g/week). HI > 1, unacceptable risk.

(Tryphonas et al., 1989, 1991a,b; Tilson et al., 1990; Arnold et al., 1993a,b, 1995, 1999; Rice and Hayward, 1997, 1999), a reference dose of 20 ng/kg b.w./d for all 209 PCB congeners was proposed by WHO (IPCS/WHO CICAD, 2003). This reference dose, expressed as a tolerable daily intake (TDI) was adopted by the French Agency for Food Safety (AFSSA) in 2003 and 2007 (AFSSA, 2007; Baars et al., 2004). In addition, since the sum of six PCB congeners most commonly found in food matrices (PCB-28, 52, 101, 138, 153, and 180) accounts for approximately 50% of total ndl PCBs, a "guidance value" of 10 ng/kg b.w./day could be proposed for this group of six congeners (Arnich et al., 2009).

In our study, calculated weekly intake for Hg (right end column at 5th percentile) given in Table 2 exceeded the value of PTWI of 1.6 μg MetHg/kg b.w./day Also, in all cases where maximal and 95th percentile concentration of ndl PCBs was used, weekly intakes exceeded a "guidance value" of 10 ng/kg b.w./day (70 ng/kg b.w./week) with one exception *i.e.*, when 95th percentile of concentration along with 95th percentile of body weight were used.

Calculated HIs (Table 3), have shown that in anticipated scenarios there is no risk of exposure to DDT. HI derived from scenarios where maximal concentration of Hg was used was higher than the critical value of 1 (Max-AHA-5th). In the case of ndl PCBs, only when extreme, maximal concentration was used for calculation, HIs exceeded the value of 1. Similar results were obtained when 95th percentile of ndl PCBs, *i.e.* all but one HI exceeded the value of 1.

In general, human health risk assessment of individual contaminants can be relatively simple procedure due to establishment of various health based guidance values determined by national and international authorities. However, humans are exposed to a mixture of chemicals at any one time. There is, for example, a positive relationship between Hg and PCB levels in fish, fish consumption by pregnant women, and deficits in neurobehavioral development in children (Schantz, 1990; IOM, 1991; Sparks and Shepherd, 1994; Jacobson and Jacobson, 1996; Lonky et al., 1996; NRC, 2000; Schantz et al., 2003; Stern et al., 2004; Roegge et al., 2004). A significant interaction between cord blood PCBs and maternal hair MetHg was found, such that negative associations between prenatal MetHg exposure and performance test were found in subjects with higher levels of prenatal PCB exposure (Stewart et al., 2003). Heath et al. (1972) have found that feeding Aroclor 1254 and DDE in the diet to 14-day-old Japanese quail gave additive results, but there was no evidence of mutual potentiation or antagonism. As some PCBs and DDT can stimulate microsomal enzyme activity, they can influence the action of other chemicals that undergo microsomal metabolism (ATSDR, 2000, 2002). Accordingly, it can be expected that they may potentiate the action of other chemicals that undergo microsomal activation, and antagonize the action of those that are detoxified.

Taking all this into account, after calculation of particular HI values, the intention was to summarize HIs originated from the intake of individual contaminants (Hg, DDT, and PCBs) (Table 3) and to calculate a total HI (Table 3). Expectedly, in all eleven cases where extreme concentration of ndl PCBs was used, total HI was higher than the limit value of 1. And HIs derived from scenarios where 95th percentile concentration was used were higher than the critical value of 1 only for ndl PCBs (95th-FAO/WHO-5th; 95th-AHA-5th; 95th-FAO/WHO-50th; 95th-AHA-50th and 95th-AHA-95th). In general, when mean or median concentrations have been taken into calculation obtained total HIs demonstrate that risk due to exposure to a combination of Hg, DDT, and ndl PCBs is likely to be insignificant. Based on different literature sources including monitoring data from the National reference laboratory of the Institute of meat hygiene and technology in Serbia, it could be assumed that Hg, DDT, and PCBs represent the most abundant fish contaminants. Besides, in 1999 UNEP/UNHCS reported the data on measurements of PCBs in the sediment of the Danube (Serbia), revealing the concentrations from 80 to 1600 ng/g (UNEP/UNCHS 1999). Our previously published results have shown that the sum of six PCBs congeners reaches the level of around 100 ng/g fresh weight of fish cought from the Danube whereas Hg and DDT levels were up to 50 and 120 ng/g, respectively (Spiric et al., 2001; Jankovic et al., 2008, 2009, 2010). However, it should be mentioned that other chemicals such as cadmium, lead, organochlorine insecticides (other than DDT), PCDDs, PCDFs etc. may be also present in fish. It is, however, difficult to predict human health impact of exposure to all these toxic compounds, particularly in view of the other exposure patterns. Global fish consumption varies significantly from one country to another depending primarily on geographic position, tradition, economic development, dietary habits, etc. Average annually fish consumption in Serbia is about 5 kg per capita that is significantly lower compared to the global consumption (16.4 kg) or EU average of 11 kg (in Austria) up to 57 kg (in Portugal) (Lekic-Arandjelovic et al., 2008). This means that food groups other than fish and fishery products i.e. meat, milk, eggs, and related products contribute much more to the body burden of humans, probably with exception of high fish consumers. Taking into account limitations of the study related to potential contribution of other contaminants as well as the other food items, total HI calculated in this study seems to be even lower than real one. Evaluation of human health impact of concomitant exposure to several chemicals also implies uncertainties due to lack of complete understanding of underlying mechanisms of their interactions, and interpretation in a dose-related manner.

## 4. Conclusion

Contaminant levels, particularly Hg, DDT, and PCBs may be sufficiently high in some fish species to cause adverse human health effects in people consuming large quantities. HIs derived from scenarios where maximal concentration was used were higher than the critical value of 1 for Hg (Max-AHA-5th) and ndl PCBs (Max-FAO/WHO-5th; Max-AHA-5th; Max-FAO/WHO-50th; Max-AHA-50th; Max-FAO/WHO-95th; Max-AHA-95th). Also HIs higher than the critical value were obtained from scenarios where 95th percentile of ndl PCBs concentration was used (95th-FAO/WHO-5th; 95th-AHA-5th; 95th-FAO/WHO-50th; 95th-AHA-50th and 95th-AHA-95th). Unlike the worst case scenario, when mean or median concentrations have been taken into calculation obtained total HIs demonstrate that risk due to exposure to a combination of Hg, DDT, and ndl PCBs associated with fish consumption is likely to be insignificant. In general, even calculated values of HIs related to fish consumption are below a critical value of 1, still one may not be sure that Serbian population is at the safe level unless result on complete dietary exposure is known.

## Conflict of Interest

The authors declare that there are no conflict of interest.


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