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In vitro effects of PCBs and OH-PCBs on the basal and dexamethasone-modified thyroid hormone metabolism in chicken liver

Kinga Kowalik, Dorota Katarzyńska-Banasik, Karolina Frydrych, and Andrzej Sechman

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Original article

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To assess the *in vitro* effect of polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) on the metabolism of thyroid hormones (THs) in chicken liver, explants of liver tissue were incubated in a medium supplemented with dexamethasone (DEX) (100 nM), PCB118 (dioxin-like PCB), PCB153 (non-dioxin-like PCB), 4-OH-PCB107 and 3-OH-PCB153 (0.5×10^{-8} M), and with DEX together with each of the PCBs and OH-PCBs to determine the triiodothyronine (T_3) secretion, thyroxine (T_4) to T_3 conversion, mRNA expression and protein concentration of the iodothyronine deiodinases (DIO1, DIO2, DIO3), TH transporters (*OATP1C1, MCT8, MCT10, LAT1*) and TH receptors (*THRA*, *THRB)*. The results obtained revealed that the tested PCBs and OH-PCBs interacted with and/or abolished the inhibitory effects of DEX on T_3 secretion and T_4 to T_3 conversion. The tested dl- and ndl-PCBs and their hydroxylated metabolites affected the basal and DEX-modified mRNA expression and the protein concentration of all three deiodinases. The PCBs and OH-PCBs did not change the *MCT8* gene expression; however, PCB118 and 4-OH-PCB107 reduced the *MCT10* mRNA levels with a concomitant increase in the basal and DEX-stimulated *LAT1* mRNA expression. PCB153 and 3-OH-PCB153 did not influence the *MCT10* expression, but they elevated the basal and reduced DEX-stimulated *LAT1* mRNA levels. Among the four tested PCBs, only 4-OH-PCB decreased the *TRβ0* mRNA expression. In conclusion, to our knowledge, these results revealed for the first time that both dl-PCB and ndl-PCB and their OH-PCBs affect T_3 secretion and T_4 to T_3 conversion, as well as the expression of iodothyronine deiodinases and TH transporters in chicken liver. These results indicate that not only the parental PCBs, but also their hydroxylated derivatives may influence iodothyronine metabolism in a chicken's liver, resulting in changes in T_3 availability in the organism.

Key words: polychlorinated biphenyls, hydroxylated polychlorinated biphenyls, iodothyronine deiodinases, hen.

Kinga Kowalik✉, Dorota Katarzyńska-Banasik, Karolina Frydrych, Andrzej Sechman, Department of Animal Physiology and Endocrinology, University of Agriculture in Kraków, Poland.
E-mail: kinga.kowalik@urk.edu.pl

Thyroid hormones (THs) play an essential role in organisms. They exert a profound influence on avian development (both differentiation and growth), as well as being the main regulators of the basal metabolic rate, and are crucial for maintaining a constant body temperature. Because the chicken thyroid gland produces mostly 3,3′,5,5′-tetraiodothyronine (thyroxine; T_4), the availability of receptor-active 3,3′,5-triiodothyronine (T_3) must be regulated, primarily in the extrathyroidal tissues by a deiodination process. This process is catalysed by three enzymes called: type 1 (DIO1), type 2 (DIO2), and type 3 (DIO3) iodothyronine deiodinases (Darras *et al*. 2006). DIO1 and DIO2 both have outer ring deiodination (ORD) activity, so they participate in the production of T_3 as a result of the conversion of T_4, as well as and in the degradation of 3,3′,5′-triiodothyronine (rT_3) to 3,3′-diiodothyronine (3,3′-T_2).

On the other hand, DIO3 has inner ring deiodination (IRD) activity and degrades T_4 to rT_3, as well as T_3 to 3,3′-T_2 (Fig. 1). Thus, DIO1 and DIO2 are mostly engaged in the synthesis of T_3, while DIO3 is responsible for TH degradation and inactivation (Van Der Spek *et al.* 2017). The deiodinases are tissue-specific enzymes: DIO1 is present mostly in the liver, kidneys and muscles; DIO2 occurs in the brain and the liver; while DIO3 has been identified in almost all tissues (Köhrle 2000; Darras & Van Herck 2012; Orozco *et al*. 2012; Darras *et al.* 2015).

To fulfil their physiological role as regulators of gene transcription in the target cells, THs must cross the plasma membrane, and this process is mediated by specific TH transmembrane transporters that belong to the families of the monocarboxylate transporters (MCTs), Na-independent organic anion transporting polypeptides (OATPs) and the L-type amino acid transporters (LATs) (Van Der Deure *et al.* 2010). They are involved not only in the cellular TH uptake, but also in their efflux from the cell (Darras *et al*. 2015; Bourgeois *et al.* 2016). T_4 is mainly transported by the monocarboxylate transporter 8 (MCT8) and the organic anion transporting polypeptide 1C1 (OATP1C1), while T_3 is transported by MCT8 and the monocarboxylate transporter 10 (MCT10), and the L-type amino acid transporter 1 (LAT1) is predominantly involved in 3,3′-T_2 transport (Cosmo *et al.* 2010).

THs act on cells in the target tissues mainly through thyroid hormone receptors (TRs), which belong to the nuclear receptor superfamily. In birds, TRs are the product of two different genes, called THRA (TRα) and THRB (TRβ), which generate several isoforms (among them TRα1, TRβ0 and TRβ2, which preferentially bind T_3 (Decuypere *et al.* 2005; Vella & Hollenberg 2017).

One consequence of rapid technological and industrial development is the pollution of the environment with toxic substances. Some of these chemicals affect the activity of the endocrine system by disrupting the endogenous hormone signalling pathways, and are therefore called ‘endocrine disruptors’ (EDs) (Swedenborg *et al.* 2009). Polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) belong to the group of EDs. Due to their similar chemical structure, PCB and hydroxylated PCB may mimic or interfere with the action of the endogenous hormones, so these compounds may disturb the functions of the endocrine system. So far, there are known to be at least two different classes of PCBs: dioxin-like PCBs (dl-PCBs, e.g. 2,3′,4,4′,5-pentachlorobiphenyl; PCB118) and non-dioxin-like PCBs (ndl-PCBs, e.g. 2,2′4,4′,5,5′-hexachlorobiphenyl; PCB153). Both dl-PCB and ndl-PCB congeners have been detected in food items such as dairy products, meat, eggs, fish or vegetables, as well as in fruits (Mihtas *et al.* 2015; Güzel *et al.* 2020). Moreover, PCB118 and PCB153 have been detected in human plasma (Schettgen *et al.* 2012), while the presence of their hydroxylated metabolites has been found in urine (Quinete *et al.* 2016).

The experimental evidence for the effect of PCBs on DIO activity is incomplete and sometimes inconsistent. The effect of these compounds depends on the species and age of the animal, as well as

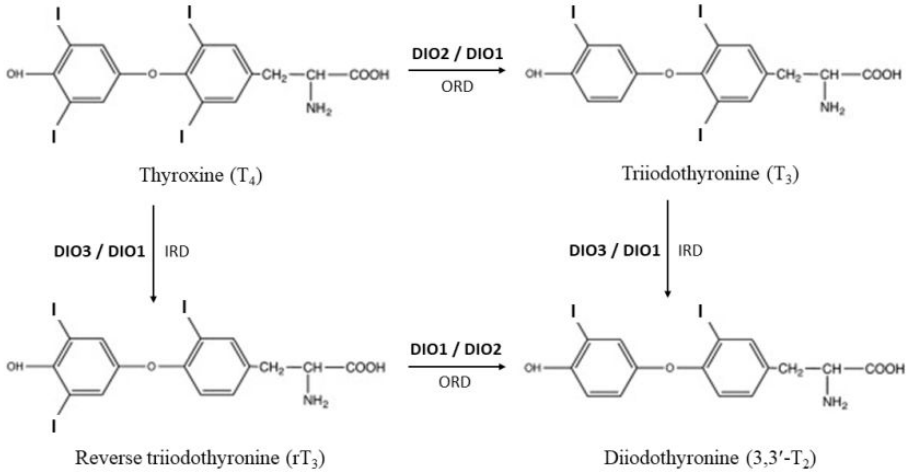

Fig.1. The main pathways of thyroxine deiodination (description in the text, diagram by the authors). DIO1 – deiodinase type I; DIO2 – deiodinase type 2; DIO3 – deiodinase type 3; ORD – Outer Ring Deiodination (5’-monodeiodination); IRD – Inner Ring Deiodination (5-monodeiodination).

the type of compound being tested and the route of administration. For example, experiments carried out on chicken embryos revealed that dl CB77 (3,3′,4,4′-tetrachlorobiphenyl) injected on Day 4 of incubation decreased the plasma TH concentration during the last stages of the chickens' development; however, its influence on DIO1 and DIO3 activity in the liver was limited. In turn, in the cerebellum and telencephalon, a compensatory increase in DIO2 activity with a concomitant decrease in DIO3 activity was observed (Beck *et al.* 2006). On the other hand, a previous study showed that both mixtures of PCBs, Aroclor 1242 and Aroclor 1254 (mixtures of PCBs containing 12 carbon atoms in the phenyl rings with an average chlorine content of 42 and 54%, respectively), reduced the hepatic DIO1 activity in chicken embryos (Gould *et al.* 1999). The literature also includes studies on other species, such as fish and mammals, which have confirmed the diversity of the effects of PCBs on TH metabolism. In the Nile tilapia, Aroclor 1254 increased DIO3 activity in the brain, but decreased DIO1 and DIO2 activity in the liver and brain, respectively (Coimbra *et al.* 2005). An experiment conducted by Kato *et al.* (2004) on Wistar and Gunn rats showed that PCB99 (2,2′,4,4′,5-pentachlorobiphenyl) decreased the hepatic DIO1 activity, but only in Gunn rats. These observed differences in the effects of PCBs and OH-PCBs on TH metabolism suggest the need for further studies focused on different species, which will allow for looking at the similarities and differences in the regulatory mechanisms in distant taxonomic groups of animals.

Our recent study (Kowalik & Sechman 2022) revealed that PCBs and OH-PCBs may affect TH synthesis and secretion, as well as iodothyronine metabolism in the chicken thyroid gland, by the changing *DIO1* and *DIO3* mRNA expression and the protein abundances of each DIO. Moreover, even though OH-PCBs are more water soluble than their corresponding parent PCBs, they still retain a high hydrophobicity, which may potentially lead to an accumulation in the liver and adipose tissues. Despite the extensive knowledge concerning the effects of PCBs on many biological processes, there is still scarce data regarding the impact of OH-PCBs, whose concentrations in the environment are currently increasing. The molecular mechanism of action of these compounds and their effect on iodothyronine metabolic processes in the avian liver, which is the main organ of iodothyronine metabolism, has not been fully explored. Therefore, in this study, we tested the hypothesis that PCBs and their OH-PCBs would affect the metabolism of iodothyronines in chicken liver. To verify this hypothesis, *in vitro* experiments were carried out to examine the effects of PCB118 and PCB153 and their hydroxylated metabolites (4-OH-PCB107 and 3-OH-PCB153, respectively) on: (i) T_3 secretion and T_4 to T_3 conversion; (ii) DIO1, DIO2 and DIO3 concentration; and (iii) the expression of the genes encoding a) these three deiodinases, b) the transmembrane transporters of T_4 and T_3, and c) the TH receptors in control and dexamethasone (DEX)-treated chicken liver explants. DEX is a well-known synthetic glucocorticoid analogue that affects the deiodinase mRNA expression and activity in embryonic chicken livers (Van Der Geyten *et al*. 1999; Van Der Geyten *et al*. 2001; Verhoelst *et al*. 2004; Reyns *et al*. 2005). In addition, PCBs and OH-PCBs, due to their similar chemical structure to thyroid hormones, may also affect deiodination. Therefore, it was decided to investigate the influence of PCBs and OH-PCBs on the iodothyronine metabolisms in both basic and DEX-modified conditions. Moreover, due to the fact that the effect of DEX on the deiodination process has been well described in the literature, this compound served as an internal control to confirm that the technical conditions did not interfere with the course of the experiments.

Material and Methods

Animals and experimental procedure

The experiments and animal procedures were accepted by the Second Local Animal Ethics Committee in Krakow, Poland (Resolution No. 20/2022). Hy-Line Brown hens (n = 30; at the age of 24-28 weeks), purchased from a commercial farm, were kept in individual cages with free access to tap water and feed (commercial compound feed for egg laying hens – DJ, from 'Barbara SP. Z O.O., Turza, Poland) in a 14L:10D light regime. The studies were divided into 2 stages: (1) the preliminary stage (the experiments were carried out on 8 hens), which was aimed at selecting an appropriate dose of tested PCBs and OH-PCBs; and (2) the real stage (the experiments were performed on 22 hens), in which the effects of the PCBs and OH-PCBs, alone and in combination with DEX, on: (i) T_3 secretion to the medium and T_4 to T_3 conversion (Exp. 1, n=8 hens); (ii) deiodinase concentrations (Exp. 2, n=8 hens); and (iii) the mRNA expression of deiodinases, TH transporters and receptors (Exp. 3, n=6 hens) in the hen livers were investigated. In both stages, the birds were decapitated in the morning (between 8 and 10 a.m.) to rule out the influence of physical factors on the daily changes

in TH secretion and metabolism. From each hen, the liver was isolated, immediately placed on ice, and divided into small and equal explants. In the first part of the *in vitro* experiments, in order to show the dose-dependent PCB and OH-PCB action on the conversion of T_4 to T_3, the liver explants were incubated for 24 h in 1 ml Eagle's medium [from the Laboratory of Sera and Vaccines (Lublin, Poland)], which was supplemented with a 0.05% bovine serum albumin (BSA) and 2 µl/ml antibiotic-antimycotic solution (10,000 units penicillin, 10 mg streptomycin and 25 µg amphotericin B/ml) from Sigma-Aldrich (St. Louis, MO, USA) as a control medium, or with the addition of PCB118 (CAS No. 31508-00-6) and PCB153 (CAS No. 35065-27-1) from AccuStandard (New Haven, USA), as well as 4-OH-PCB107 (4-OH-2,3,3',4',5-pentachlorobiphenyl, CAS No. 152969-11-4) or 3-OH-PCB153 (3-hydroxy-2,2',4,4',5,5'-hexachlorobiphenyl, CAS No. 54284-55-8) from Wellington Laboratories (Guelph, Canada) at concentrations ranging from 10^{-9} M to 10^{-5} M (n=8 in each group). In the second stage of the studies, in three consecutive experiments (Exp. 1: n=8; Exp. 2: n=8; and Exp. 3: n=6), the hen liver explants were incubated for 6 h in the following groups: control, in a medium without PCBs or OH-PCBs; and experimental, where the medium was supplemented with: DEX (Cat No. D1756 from Sigma-Aldrich, St. Louis, MO, USA) at a dose 100 nM (10^{-7} M, the dose chosen according to Mörk *et al.* 2016); PCB118, PCB153, 4-OH-PCB107 or 3-OH-PCB153, at a dose 0.5×10^{-8} M; and combinations of DEX with the above PCBs and OH-PCBs. The doses of the PCBs and OH-PCBs were chosen on the basis of the literature data that showed their levels in avian tissues (Jaspers *et al.* 2006; Luzardo *et al.* 2014), as well as the toxicity equivalent factor (TEF) in birds described by Van Den Berg *et al.* (1998) and the dose-response data obtained from our preliminary studies (see Table 3).

In each experiment, incubation was carried out at 39°C in an atmosphere of 95% air and 5% CO_2. After the incubation, the liver explants and media were collected and frozen at -80ºC and -20ºC, respe ctively, until T_3 determination in the medium (by the RIA method), protein determination by Bradford's method (Bradford 1976), T_4 to T_3 conversion (according to the method described by Darras *et al.* 1991), iodothyronine deiodinases (DIO1, DIO2 and DIO3 by the ELISA method) and the mRNA expression (Real-time qPCR method) in the liver tissue of the following genes: i) enzymes involved in the metabolism of T_4 and T_3: DIO1 (*DIO1*), DIO2 (*DIO2*) and DIO3 (*DIO3)*; ii) proteins involved in transmembrane TH transport: OATP1C1 (*SLCO1C1)*, MCT8 (*SLC16A2)*, MCT10 (*SLC16A10)* and LAT1 *(SLC7A5)*; and iii) TH receptors involved in the target gene expression: TRα (*THRA)* and TRβ0 (*THRB)*.

T_3 measurement in the medium

The T_3 concentrations in the collected media were determined using T_3-RIA-CT kits (Cat No. RIA-4534) from DIAsource (Belgium), according to the manufacturer's instructions. To exclude the influence of the incubation medium on the concentration of the measured hormones, the standard curve and controls were reconstituted in the medium. The quality control data of the RIA kit was presented in the previous publication (Kowalik & Sechman 2022).

T_4 to T_3 conversion in liver homogenate

The T_4 to T_3 conversion in the liver homogenates was determined according to the method described by Darras *et al.* (1991). Briefly, the liver explants were homogenised in an ice-cold sodium phosphate buffer (0.15 M, pH = 6.5) and centrifuged at 2400 g for 10 min at 4°C. To avoid freeze/thaw cycles, immediately after protein isolation, the protein concentration was determined by Bradford's method using a Pierce Detergent Compatible Bradford Assay Kit from Thermo Scientific (Rockford, IL, USA) with the BSA from Sigma-Aldrich (St. Louis, MO, USA) as the standard. The reaction mixture contained 50 µl of protein supernatant, 200 µl of phosphate buffer with DTT [1,4-dithiothreitol, from Sigma-Aldrich (St. Louis, MO, USA)] and 10 µl T_4 [Sigma-Aldrich (St. Louis, MO, USA)]. The final concentrations of DTT and T_4 in the incubation volume were 2.4 mM and 0.13 µM, respectively. The blank was prepared without the addition of a protein homogenate. After incubation for 1 h at 37°C, the reaction was stopped with 1 ml of ice-cold 0.63% Brij 35 (polyoxyethylene mono-lauryl ether from Serva Electrophoresis GmbH, Germany). The T_3 was measured using T_3-RIA-CT kits with the following modification: the standards and controls were dissolved in Brij 35 to avoid the influence of this compound on the T_3 determination. The T_4 to T_3 conversion (minus the blank) was expressed per milligram of protein.

Determination of deiodinase concentrations

The deiodinase concentrations in the liver homogenates were determined as described previously (Kowalik & Sechman 2022). Briefly, the tissues were homogenised in liquid nitrogen and suspended in the RIPA Lysis and Extraction Buffer from Applied Biosystems/Thermo Fisher Scientific (Foster City, USA) containing 10 µl/ml of protease inhibitor cocktail from Sigma-Aldrich (St. Louis, MO,

USA), then sonicated and centrifuged at 12,000 rpm for 20 min at 4°C. The protein was determined by Bradford´s method as described above. The isolated protein was used to measure the concentration of iodothyronine deiodinases in the liver tissue by using chicken DIO1 (Cat No. E12262c), DIO2 (Cat No. 0578c) and DIO3 (Cat No. E12263c) ELISA kits (Wuhan EIAab Science Co. Ltd., China), according to the manufacturer's instructions. The quality control data of the ELISA kits was shown in the previous publication (Kowalik & Sechman 2022).

Gene expression analysis

The total RNA from the liver explants was isolated according to the modified method of Chomczynski & Sacchi (2006) using the TRI reagent from Thermo Scientific (Rockford, IL, USA). The A260/A280 ratios and an electrophoretic separation were performed to evaluate the quality of the extracted RNA. The reverse transcription (RT) and real-time PCR were carried out according to our recent (Kowalik & Sechman 2022) and previous work Katarzyńska-Banasik *et al.* (2017). Briefly, the RT reaction was performed on one µg of total RNA using the RevertAid RT Transcription Kit (from Thermo Scientific) in the Mastercycler personal thermocycler (Eppendorf, Germany) with the following thermal profile: 5 min at 25°C, 60 min at 42°C and 5 min at 70°C. Next, 10-fold diluted cDNA was used in a real-time polymerase chain reaction (qPCR) for the tested and reference genes in a 96-well thermocycler (StepOne Plus, Applied Biosystems, Foster City, CA, USA), according to the following cycling programme: 2 min at 50°C, 10 min at 95°C and 40 cycles of denaturation for 15 s at 95°C, followed by 1 min of annealing at 60°C. TaqMan probes for the investigated and reference genes were designed and provided by Applied Biosystems (Table 1 and Table 2, respectively). Before proceeding with the determination of the mRNA expression of the tested genes, six reference genes – glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), hypoxanthine-guanine phosphoribosyltransferase (*HPRT1*), 60S ribosomal protein L13 (*RPL13*), succinate dehydrogenase complex flavoprotein subunit A (*SDHA*), TATA-binding protein (*TBP*) and vimentin (*VIM*) – were tested to select the most appropriate one. According to the statistical analysis, which was performed with the NormFinder programme, the most stable gene was SDHA (stability value 0.028); while the stability value for the best combination of two genes, *VIM* and *SDHA*, was 0.032. For this reason, the *SDHA* gene was used as a reference gene. Because the reaction efficiency for the tested genes was in the range of 90-110%, the normalised relative quantities (NRQ) of the genes were calculated according to the Pfaffl (2001) method.

Statistical analysis

The data was statistically analysed by a one-way ANOVA, followed by a Tukey's multiple range test (for independent trials) performed using Sigma Stat 2.03 (Systat Software GmbH, Germany). In order to standardise the statistical analysis of the results obtained, and due to the fact that parametric tests are considered stronger compared to non-parametric tests, log transformations were made as needed to maintain the normal distribution of the data (a loga-

Table 1

The summary of TaqMan Gene Expression Assays characteristic for investigated genes

Gene symbol	Description	Assay ID	Context sequence	Amplicon size (bp)
DIO1	Deiodinase, iodothyronine type I	Gg03361636_m1	CTTCAGTTTCATGCGAGATAACCGA	69
DIO2	Deiodinase, iodothyronine type II	Gg03362313_m1	ACAAGCAGGTCAAACTTGGAG-GAGA	76
DIO3	Deiodinase, iodothyronine type III	Gg03363066_s1	GGCTACAAGATCTCGGAGCTGCGGA	93
SLCO1C1 (OATP1C1)	Solute carrier organic anion transporter family member 1C1	Gg03357846_m1	ATCTTACCATGGGACCAAGCAGAAG	62
SLC16A1 (MCT8)	Solute carrier family 16 member 1	Gg03369202_m1	TGGGGGTCGTAGGGGGCCTTGGACT	68
SLC16A10 (MCT10)	Solute carrier family 16 member 10	Gg03316338_m1	TTCTTTTGTAAGCACCATCGAACCT	84
SLC7A5 (LAT1)	Solute carrier family 7 member 5	Gg03350324_m1	CGGTGGCTGTGGACTTTGGGAATCA	67
THRA	thyroid hormone receptor, alpha 27	Gg03371711_m1	CCCATGTTCTCGGAGCTGCCGTGCG	86
THRB	thyroid hormone receptor, beta 2	Gg03371488_m1	CAACAGATTTGGTGTTGGATGACAG	84

Table 2

The summary of TaqMan Gene Expression Assays characteristic for reference genes

Gene symbol	Description	Assay ID	Context sequence	Amplicon size (bp)
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	Gg03346982_m1	TCGTCAAGCTTGTTTCCTGGTATGA	107
HPRT1	Hypoxanthine-guanine phosphoribosyl-transferase	Gg03338899_m1	GATATCGGCCAGACTTTGTTGGATT	89
RPL13	60S ribosomal protein L13	Gg03348054_m1	TTATGCCGATCAGGAACGTTTTCAA	66
SDHA	Succinate dehydrogenase complex flavo-protein subunit A	Gg03330765_m1	GCAGAAGACAATGCAAAGCCATGCT	103
TBP	TATA-binding protein	Gg03366488_m1	CAGGAGCAAAAAGCGAGGAACAGTC	82
VIM	Vimentin	Gg03360311_m1	GGAAACTAGAGATGGACAGGTTATT	85

Table 3

Effect of PCB118 and PCB153 and their hydroxylated metabolites (4-OH-PCB107 and 3-OH-PCB153) at doses from 10^{-9} M to 10^{-5} M on T_4 to T_3 conversion in the chicken liver after 24 h of incubation. The T_4 to T_3 conversion was determined according to the method described by Darras *et al*. 1991. Values are expressed as mean ± SEM (n = 8 per each group) in pg T_3/mg protein. ** $p < 0.01$, * $p < 0.05$ vs control group; one-way ANOVA followed by Tukey's multiple range test

Dose (M)	Tested compound			
	PCB118	4-OH-PCB107	PCB153	3-OH-PCB153
0 (Control)	152.38 ± 6.27	155.04 ± 5.08	154.64 ± 13.56	156.53 ± 8.85
10^{-9}	169.52 ± 8.44	227.25 ± 8.14 **	254.09 ± 12.42 **	234.86 ± 11.12 **
10^{-8}	186.54 ± 7.44 *	224.45 ± 14.55 **	216.24 ± 11.65 **	219.12 ± 12.31 **
10^{-7}	183.62 ± 3.32 *	209.18 ± 7.45 **	179.17 ± 15.47	205.51 ± 9.66 *
10^{-6}	160.85 ± 4.66	175.43 ± 14.51	161.31 ± 11.03	216.62 ± 15.37 **
10^{-5}	164.73 ± 7.64	180.72 ± 8.21	196.13 ± 6.37	298.05 ± 10.05 **

rithmic transformation of the data was used only for hypothesis testing). Values are shown as the mean ± SEM (presented on the original scale) and are considered to be significantly different at $p < 0.05$. The figures were prepared using Grapher 17 (Golden Software Inc., USA).

Results

Selection of the dose of the tested PCBs and OH-PCBs

In the control groups, the conversion of T_4 to T_3 in the liver explants ranged from 152.38 ± 6.27 to 156.53 ± 8.85 pg T_3/mg protein (Table 3). Depending on the applied dose, all the tested compounds increased the T_4 to T_3 conversion. PCB118 (at doses of 10^{-8} and 10^{-7} M) and 4-OH-PCB107 (at doses from 10^{-9} to 10^{-7} M) elevated the T_4 to T_3 conversion by an average of 21% ($p < 0.05$) and 46% ($p < 0.01$), respectively. PCB153 at a concentration of 10^{-9} and 10^{-8} M augmented the measured conversion by an average of 52% ($p < 0.01$). All the applied doses of 3-OH-PCB increased the T_4 to T_3 conversion by an average of 50% ($p < 0.05$-0.01).

Effect of PCBs and OH-PCBs on T_3 secretion and T_4 to T_3 conversion

In the control conditions, the explants of the chicken liver secreted 1.24 ± 0.06 (Fig. 2A) and 1.18 ± 0.08 ng of T_3/mg protein (Fig. 2B). In both trials, the DEX inhibited T_3 secretion from the liver explants by 27% and 24%, respectively ($p < 0.05$; Figs 2A, 2B). PCB118, as well as PCB153, decreased the T_3 concentration in the medium by 28% ($p < 0.05$; Fig. 2A) and 40% ($p < 0.01$; Fig. 2B), respectively. On the other hand, 3-OH-PCB153 increased the T_3 secre-

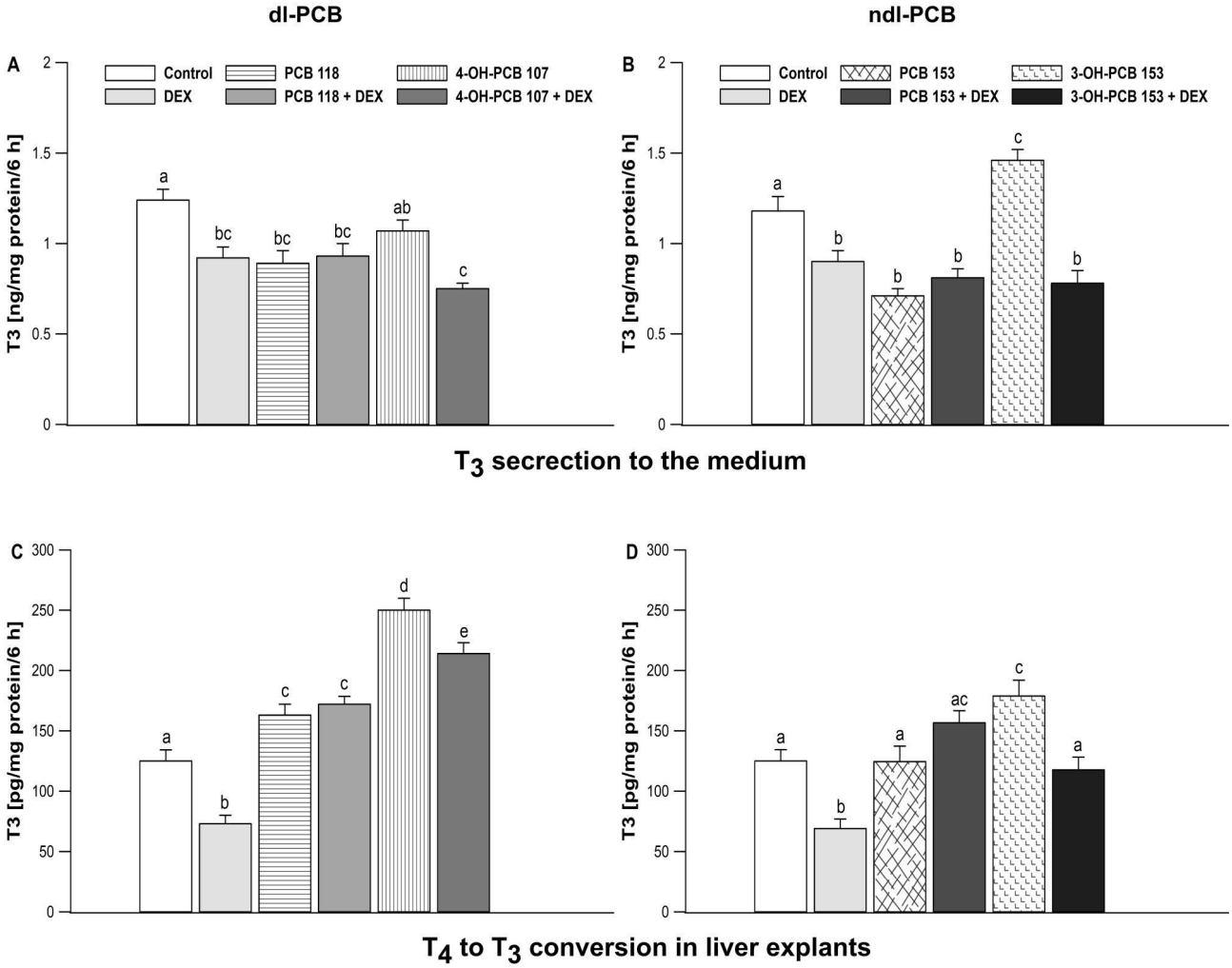

Fig. 2. Effect of dl-PCB (PCB118 and 4-OH-PCB107; A, C) and ndl-PCB (PCB153 and 3-OH-PCB153; B, D) on the basal and DEX--affected T_3 secretion (A, B) and T_4 to T_3 conversion (C, D) in the chicken livers. T_3 concentrations in the collected media and T_4 to T_3 conversion in the liver homogenates were determined by radioimmunoassay (RIA) and according to the method described by Darras *et al*. 1991, respectively. Values are expressed as the mean ± SEM (n = 8 per each group). Different letters denote statistically significant differences ($p < 0.05$).

tion by 24% ($p < 0.05$; Fig. 2B). Neither PCB nor OH-PCB influenced the DEX-inhibited T_3 secretion from the liver explants ($p < 0.05$; Figs 2A, 2B). However, this steroid reversed the stimulatory effect of 3-OH-PCB153; as a result, the T_3 secretion in this group was 47% lower in comparison with the control group ($p < 0.01$; Fig. 2B).

The effects of dl- and ndl-PCB and their OH-PCB congeners on the T_4 to T_3 conversion rate in the liver explants are shown in Fig. 2C and Fig. 2D. In the two control groups, the concentration T_3 (representing the T_4 to T_3 conversion in the liver homogenates) was 125.24 ± 9.22 and 122.14 ± 9.39 pg T_3/mg protein, respectively (Figss 2C, 2D). In both trials, DEX inhibited the T_4 to T_3 deiodination by 44% and 48%, respectively ($p < 0.01$; Figs. 2C, 2D). In comparison to the control group, PCB118 and 4-OH-PCB107 increased the T_4 to T_3 conversion by 36% and 100%, respectively ($p < 0.01$; Fig. 2C). PCB153 did not affect the T_4 to T_3 conversion; however, 3-OH-PCB153 elevated this conversion by 43% ($p < 0.01$; Fig. 2D). Furthermore, all the tested PCBs and OH-PCBs counteracted the inhibitory effect of DEX on T_4 to T_3 deiodination in the chicken liver homogenates ($p < 0.01$ in comparison to DEX; Figs 2C, 2D). On the other hand, the stimulatory effects of 4-OH-PCB107 and 3-OH-PCB153 on the T_4 to T_3 conversion were reduced by the DEX by 22% ($p < 0.05$; Fig. 2C) and 34%, respectively ($p < 0.01$; Fig. 2D).

Effect of PCBs and OH-PCBs on the mRNA expression of iodothyronine deiodinases

In comparison with the control group, DEX decreased the *DIO1* mRNA level in the chicken liv-

er explants by 20% ($p < 0.05$; Fig. 3A) and 21% ($p < 0.05$; Fig. 3B), but did not influence the expression of the *DIO2* mRNA (Figs. 3C, 3D) and reduced the *DIO3* mRNA expression by 19% ($p < 0.05$; Fig. 3E) and 24% ($p < 0.05$; Fig. 3F).

PCB118 and 4-OH-PCB107 increased the basal levels of *DIO1* mRNA by 32% and 25%, respectively ($p < 0.01$; Fig. 3A). However, DEX reduced these stimulatory effects by 60% and 30%, respectively ($p < 0.01$; Fig. 3A). Moreover, in comparison with the DEX-treated group, in the PCB118 + DEX-treated group, the *DIO1* mRNA level was lower by 21% ($p < 0.05$; Fig. 3A). There were no significant alterations between the treated groups with respect to ndl-PCBs (Fig. 3B).

In comparison with the control group, only 3-OH-PCB153 increased the basal expression of *DIO2* mRNA by 26% ($p < 0.05$; Fig. 3D). The addition of DEX to the medium elevated the *DIO2* mRNA expression in the PCB118 + DEX and 4-OH-PCB107

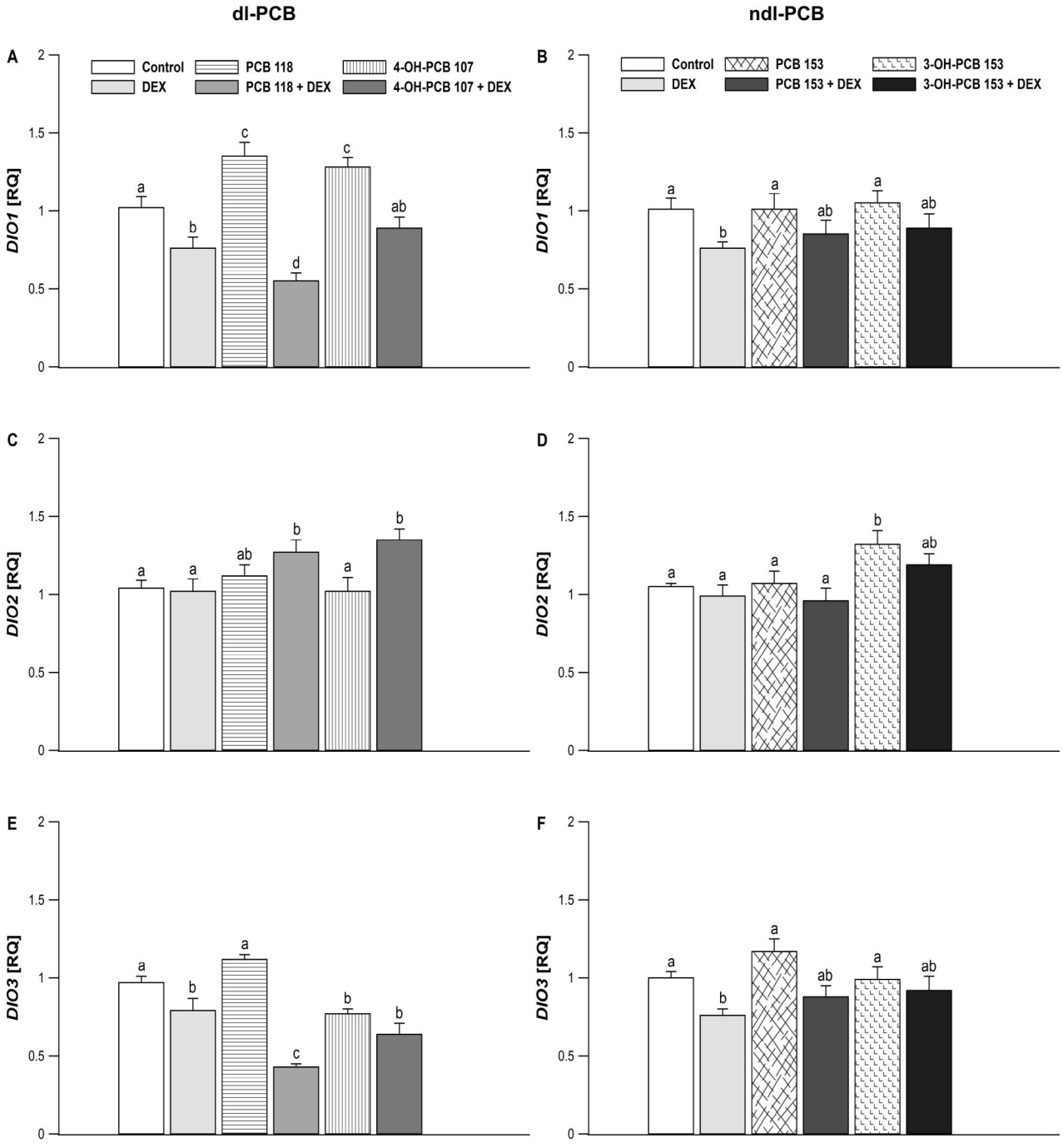

Fig. 3. Effect of dl-PCB (PCB118 and 4-OH-PCB107; A, C, E) and ndl-PCB (PCB153 and 3-OH-PCB153; B, D, F) on the basal and DEX-affected mRNA expression, evaluated by a real-time qPCR of: *DIO1* (A, B), *DIO2* (C, D) and *DIO3* (E, F) genes in the chicken livers. Each value represents the mean of the relative quantity (RQ) ± SEM (n = 6 per group), normalised to *SDHA* as a reference gene and standardised to the abundance in the control group. The values marked with different letters differ at $p < 0.05$.

+ DEX treated groups by 25% ($p < 0.05$; Fig. 3C) and 32% ($p < 0.01$; Fig. 3C), respectively.

4-OH-PCB107 decreased the *DIO3* mRNA level by 21% ($p < 0.05$; Fig. 3E). There were no significant changes in the *DIO3* mRNA levels in the other experimental groups; however, in comparison with the DEX-inhibited group, the *DIO3* mRNA level was lower by 46% in the PCB118 + DEX-treated group ($p < 0.01$; Fig. 3E).

Effect of PCBs and OH-PCBs on iodothyronine deiodinase concentrations

In the control liver explants, the concentrations of DIO1 were 21.14 ± 0.08 (Fig. 4A) and 21.24 ± 0.72 ng/mg protein (Fig. 4B), the DIO2 concentrations were 8.23 ± 0.42 (Fig. 4C) and 8.9 ± 0.53 ng/mg protein (Fig. 4D), and the DIO3 concentrations were 18.71 ± 0.52 (Fig. 4E) and 19.06 ± 0.75 ng/mg protein (Fig. 4F).

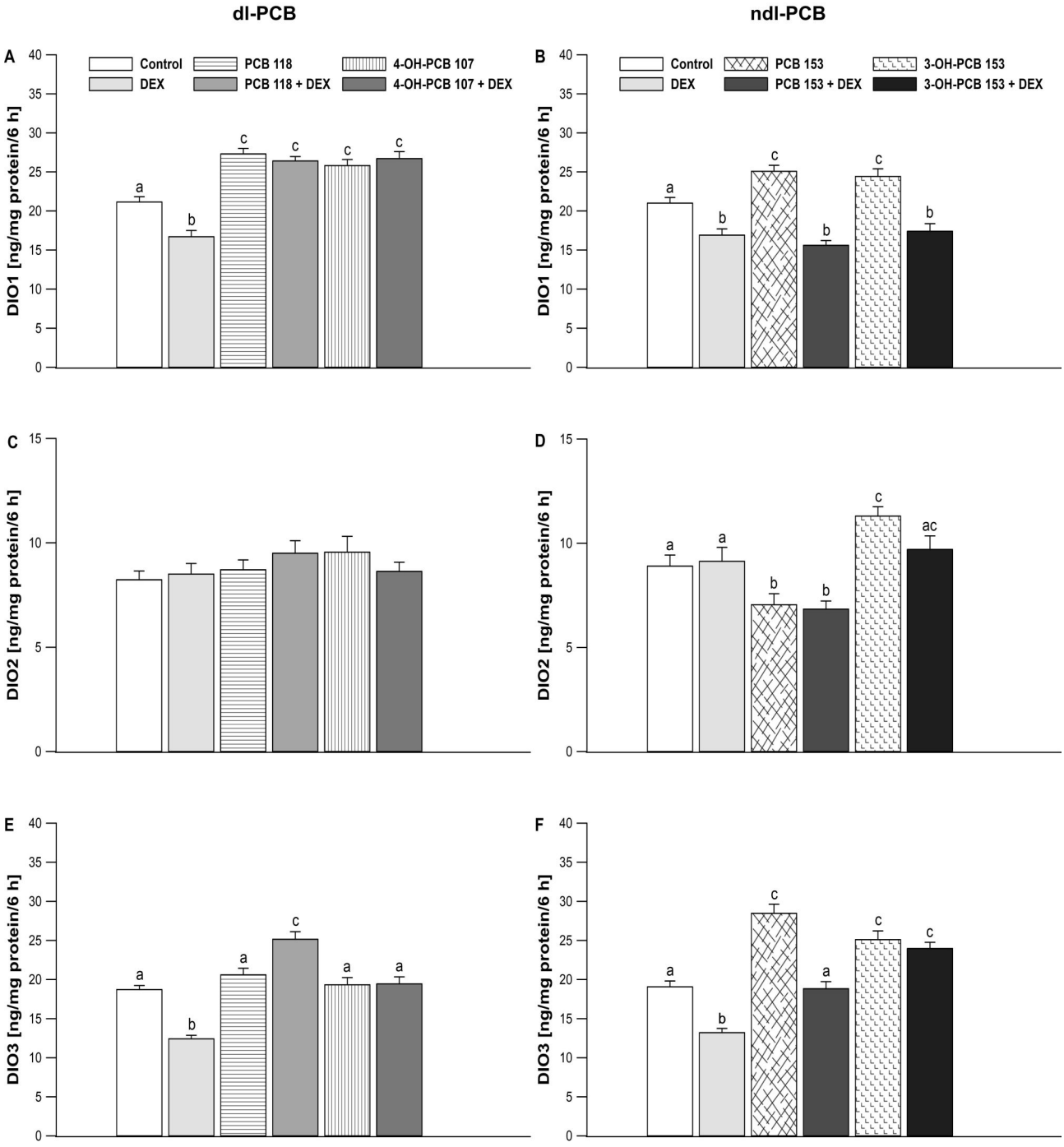

Fig. 4. Effect of dl-PCB (PCB118 and 4-OH-PCB107; A, C, E) and ndl-PCB (PCB153 and 3-OH-PCB153; B, D, F) on the basal and DEX-affected DIO1 (A, B), DIO2 (C, D) and DIO3 (E, F) concentrations, as determined by ELISA, in the chicken livers. For further explanations, see Fig. 2.

In the two trials, DEX reduced the DIO1 concentrations in the chicken liver by 30% ($p < 0.01$; Fig. 4A) and 28% ($p < 0.05$; Fig. 4B), respectively, and did not influence the DIO2 concentrations (Fig. 4C, Fig. 4D). Furthermore, it decreased the DIO3 levels by 34% ($p < 0.01$; Fig. 4E) and 31% ($p < 0.01$; Fig. 4F), respectively.

In comparison with the control group, PCB118 and 4-OH-PCB107 enlarged the basal DIO1 levels by 19% and 52%, respectively ($p < 0.05$-0.01; Fig. 4A). Moreover, these compounds abolished the DEX inhibitory effect in such a way that there were no differences between the dl-PCBs and dl-PCBs + DEX-treated groups (Fig. 4A). PCB153 and 3-OH-PCB153 increased the DIO1 concentrations by 18% and 16%, respectively ($p < 0.05$), but did not affect the DEX-inhibited DIO1 concentrations (Fig. 4B).

The tested dl-PCB did not change the concentrations of DIO2 in the chicken liver (Fig. 4C). PCB153 decreased while 3-OH-PCB153 increased the DIO2 concentration by 20% and 31%, respectively ($p < 0.05$-0.01; Fig. 4D). Furthermore, in combination with DEX, PCB153 diminished the DIO2 concentration by 21% ($p < 0.05$; Fig. 4D).

PCB118 and 4-OH-PCB107 did not influence the DIO3 concentration in the chicken liver but eliminated the inhibitory effect of DEX; in comparison with the DEX-treated groups, the DIO3 levels were higher by 102% and 56%, respectively ($p < 0.01$; Fig. 4E). PCB153 and 3-OH-PCB153 increased the DIO3 concentrations by 49% and 32%, respectively ($p < 0.01$; Fig. 4F). Furthermore, these compounds abolished the DEX-inhibited effects on the DIO3 concentrations by 42% and 81%, respectively ($p < 0.01$; Fig. 4F).

Effect of PCBs and OH-PCBs on the mRNA expression of transmembrane TH transporters

In comparison with the control group, DEX did not affect the mRNA expression of the *MCT8* (Figs 5A, 5B) and *MCT10* (Figs 5C, 5D) transporters in the chicken explants, but increased the *LAT1* mRNA levels by an average of 42% ($p < 0.01$; Figs 5E, 5F).

All the tested PCBs and OH-PCBs, alone and in combination with DEX, did not influence the expression of *MCT8* mRNA (Figs 5A, 5B). The basal *MCT10* mRNA expression was inhibited in the groups incubated in a medium supplemented with PCB118 and 4-OH-PCB107 by 19% and 32%, respectively ($p < 0.05$; Fig. 5C). However, the addition of DEX to the incubation medium eliminated these inhibitory effects (Fig. 5C). PCB153 and 3-OH-PCB153 alone had no influence on the *MCT10* mRNA levels, but in combination with DEX increased the expression of this gene by 28% and 34%, respectively ($p < 0.05$-0.01; Fig. 5D).

An increase in the *LAT1* mRNA expression was observed in all the experimental groups treated both with dl- and ndl-PCB ($p < 0.01$; Figs 5E, 5F). The highest increase (by 138%; $p < 0.01$) was found in the 4-OH-PCB107-treated group (Fig. 5E). DEX augmented the PCB118-stimulated *LAT1* mRNA expression by 64% ($p < 0.01$; Fig. 5E). On the other hand, DEX diminished the PCB153- and 3-OH-PCB153-stimulated *LAT1* mRNA expression by 57% and 54%, respectively ($p < 0.01$; Fig. 5F).

The mRNA expression of the *OATP1C1* gene was not detected in the livers of the domestic hens.

Effect of PCBs and OH-PCBs on the mRNA expression of the receptors involved in target gene expression

The effects of the tested PCBs and OH-PCBs on the *TRα* and *TRβ0* mRNA expression are shown in Fig. 6. Only 4-OH-PCB107 decreased the mRNA expression of *TRβ0* by 22% ($p < 0.05$; Fig. 6C). In the other groups, no significant changes in the mRNA expression of *TRα* and *TRβ0* were observed (Fig. 6).

Discussion

Although the production of PCBs was banned in the year 2001 by the Stockholm Convention, PCBs and OH-PCBs, like other xenobiotics, are still present in the environment and therefore pose a threat to the health of living organisms (Borja *et al.* 2005; Pocar *et al.* 2006; Darras 2008). PCBs and OH-PCBs are detected not only in the environment (Borja *et al.* 2005; El-Rahman *et al.* 2019; Fang *et al.* 2020; Aziza *et al.* 2021) and in food products (Arnich *et al.* 2009; Mihats *et al.* 2015), but also in the blood plasma and urine of humans and animals (Schettgen *et al.* 2012; Koh *et al.* 2016; Quinete *et al.* 2016). Furthermore, the OH-PCBs produced by the oxidation of parental PCBs through a variety of mechanisms (such as metabolic transformations in living organisms or abiotic reactions with hydroxyl radicals) are still highly toxic, and probably play an important role in the resistance of PCBs to biodegradation (Kawano *et al.* 2005; Tehrani & Van Aken 2014). Both PCBs and their more water-soluble hydroxyl metabolites can easily accumulate in the adipose tissue and liver, or bind to the plasma proteins, because they retain a high hydrophobicity (Tampal *et al.* 2002; Tehrani & Van Aken 2014).

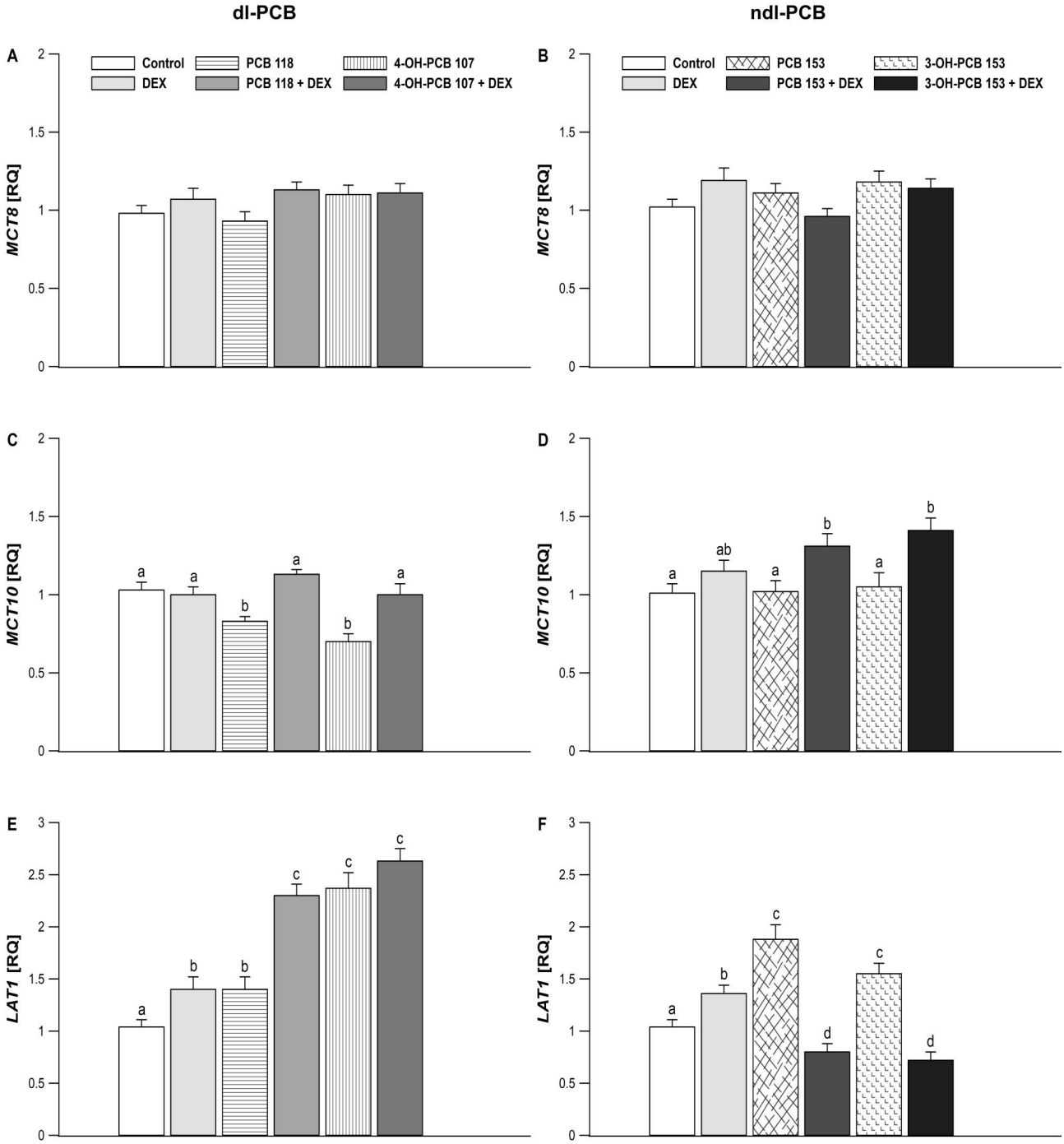

Fig. 5. Effect of dl-PCB (PCB118 and 4-OH-PCB107; A, C, E) and ndl-PCB (PCB153 and 3-OH-PCB153; B, D, F) on the basal and DEX-affected mRNA expression, evaluated by a real-time qPCR, of the thyroid hormone transporters: *MCT8* (A, B), *MCT10* (C, D) and *LAT1* (E, F) in the chicken livers. For further explanations, see Fig. 3.

Even though there is data in the literature on the influence of PCBs on the THs metabolism, it is difficult to clearly define the direction of the influence of these compounds. Moreover, their molecular mechanism of action in the cell, as well as the influence of OH-PCB on the metabolism and transport of THs in chicken liver, have not been fully explained. Therefore, this study was undertaken to investigate the effects of two different PCB classes, dl-PCBs and ndl-PCBs, represented by PCB118 and PCB153, respectively, and of their hydroxylated metabolites (4-OH-PCB107 and 3-OH-PCB153) on the T_3 secretion from liver explants, T_4 to T_3 conversion, iodothyronine deiodinase concentration, and the mRNA expression of the main genes involved in the metabolism, transport and THs receptors in chicken livers.

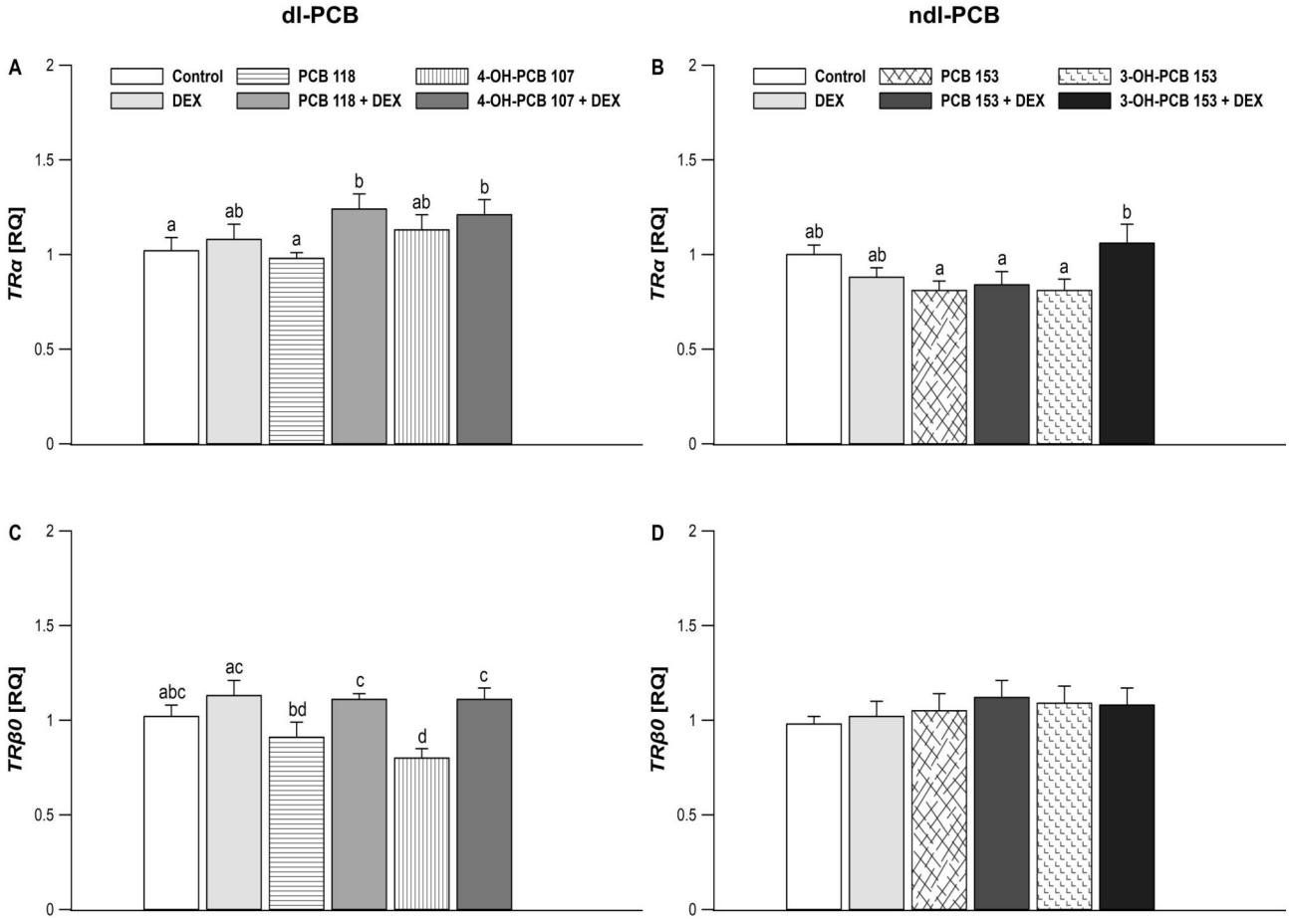

Fig. 6. Effect of dl-PCB (PCB118 and 4-OH-PCB107; A, C) and ndl-PCB (PCB153 and 3-OH-PCB153; B, D) on the basal and DEX--affected mRNA expression, evaluated by a real-time qPCR, of the thyroid hormone receptors: *TRα* (A, B) and *TRβ0* (C, D) in the chicken livers. For further explanations, see Fig. 3.

The first, preliminary stage of our research was to establish the concentrations of PCB118 and PCB153, as well as their hydroxylated metabolites, which would be used in further *in vitro* experiments. Jaspers *et al.* (2006) analysed the concentrations of several parental PCBs, including PCB118 and PCB153, in the livers of various aquatic and raptor birds. They showed that the median concentrations of PCB118 and PCB153 (ng/g fat mass) in this organ were respectively: 890 and 17,000 in the grey heron (*Ardea cinerea*), or 610 and 2,300 in the great crested grebe (*Podiceps cristatus*). In turn, in the representatives of raptors, the median concentrations of PCB118 and PCB153 (ng/g fat mass) were: 1,800 and 45,000 in the barn owl (*Tytoninae*), 71 and 2,000 in the common buzzard (*Buteo buteo*) or 6,000 and 31,000 in the sparrowhawk (*Accipiter nisus*). For the selection of the appropriate doses, values of the TEF coefficients of the applied PCBs evaluated for birds (Van Den Berg *et al.* 1998) and the results of our previous experiments (Katarzyńska *et al.* 2015; Sechman *et al.* 2016) were also taken into account. Based on the abovementioned literature data, for both PCB and OH-PCB, doses from 10^{-9} M to 10^{-5} M were selected and used in the preliminary experiment. The parental PCBs increased the T_4 to T_3 conversion in the groups incubated with the addition of PCB118 at concentrations of 10^{-8} M and 10^{-7} M, and PCB153 at concentrations of 10^{-9} M and 10^{-8} M. The same stimulating effect was observed in the groups incubated with 4-OH-PCB107 at concentrations ranging from 10^{-9} M to 10^{-7} M, as well as with 3-OH-PCB153 at all the tested concentrations. The observed effect depended on the concentration and form of the tested xenobiotics; while T_4 to T_3 deiodination was stimulated more by the OH-PCBs in comparison to the parental PCBs. Because changes were observed mainly in the groups incubated in a medium supplemented with the tested PCBs and OH-PCBs at concentrations of 10^{-8} M and 10^{-7} M, in order to standardise the plan and layout of the further experiments, an intermediate dose of 0.5 x 10^{-8} M was chosen and applied in this study.

The results of the *in vitro* experiments indicated that DEX has different effects on T_3 secretion and T_4 to T_3 conversion in the livers of adult chickens, in comparison with embryonic and post-hatch chickens. In our experiment, we found that DEX reduced both the T_3 secretion and T_4 to T_3 conversion in liver explants of the laying hen. Experiments carried out on 18-day-old chicken embryos clearly showed that during their embryonic development, DEX increases the concentration of T_3 in the blood plasma and liver (Darras *et al.* 1996; Van Der Geyten *et al.* 1999; Van Der Geyten *et al.* 2001; Reyns *et al.* 2005). On the other hand, in 8-day-old post-hatch chicks, DEX tended to lower the plasma T_3 (Darras *et al.* 1996). These results support the hypothesis that the effect of DEX on T_3 secretion from the chicken liver depends, to a large extent, on the age of the animal.

Our results revealed that the tested PCBs and OH-PCBs affected the basal and DEX-inhibited T_3 secretion from the chicken liver explants. PCB118 decreased the T_3 secretion; while its hydroxylated metabolite, i.e. 4-OH-PCB107, had no effect on the secretion of this hormone. PCB153 and 3-OH-PCB153 also changed the T_3 secretion from the liver explants, but in an opposite manner, i.e. the parental PCB decreased, while the hydroxylated metabolite increased the secretion of this iodothyronine. It is interesting that both PCB118 and PCB153, as well as their hydroxylated metabolites, did not change the DEX-inhibited T_3 secretion. To our knowledge, this is the first *in vitro* study to examine the T_3 secretion from liver explants. These results suggest that: (i) both parental PCBs, dl- and ndl-PCBs, inhibit T_3 secretion; (ii) the OH-PCB effects are OH-PCB congener dependent; and (iii) the tested PCBs did not influence the DEX-inhibited secretion of T_3 from the liver explants. One of the factors that could explain these changes in the T_3 secretion from the liver explants following PCBs and OH-PCBs treatment is T_4 to T_3 conversion. Our results showed that the investigated PCBs and OH-PCBs stimulated both basic and DEX-inhibited T_4 to T_3 conversion. Moreover, all the tested compounds abolished the inhibitory effect of DEX on the T_4 to T_3 conversion. The observed discrepancies between T_3 secretion and T_4 to T_3 conversion are difficult to explain; however, it cannot be excluded that PCBs and OH-PCBs may reduce the permeability of hepatocyte cell membranes and/or affect the functions of the TH transporters. Nonetheless, this hypothesis needs further investigation.

To explain the observed effect of the investigated PCBs and OH-PCBs on T_3 secretion and T_4 deiodination, we tested the mRNA expression and protein level of iodothyronine deiodinases. This research confirmed the presence of all three types of iodothyronine deiodinases at the mRNA and protein levels in the chicken livers. The determination of these deiodinases by the chicken-specific ELISA method revealed that their concentrations in chicken liver are arranged in the following order: DIO1>DIO3>DIO2. A similar relationship was found in humans who were tested for the activity of these three types of deiodinases (Richard *et al.* 1998). In our research, DEX diminished *DIO1* and *DIO3*, and did not influence the *DIO2* mRNA expression and protein concentration in the liver explants. These results also confirm our earlier hypothesis (based on the obtained results and literature data; Darras *et al.* 1996; Van Der Geyten *et al.* 1999; Van Der Geyten *et al.* 2001; Reyns *et al.* 2005) that the effect of DEX on iodothyronine metabolism catalysed by deiodinase (T_3 concentrations, as well as the protein and mRNA level of deiodinase) depends on the age of the chicken. The data in the literature shows a different influence of DEX on the metabolism of THs and deiodinase activities in embryonic and post-hatch chickens: in 18-day-old embryos, a single injection of DEX did not affect DIO1 activity in the liver, but it rapidly (within just 2 h after the injection) decreased the DIO3 activity (Reyns *et al.* 2005). These changes were accompanied by a decrease in the *DIO3* mRNA expression, but without an influence on the *DIO1* mRNA levels following a DEX treatment (Van Der Geyten *et al.* 1999). Nevertheless, it has been found that in 18-day-old chicken embryos, the long-acting DEX may increase the DIO1 activity at 24 and 48 h after an injection (Darras *et al.* 1996). Alternatively, in 8-day-old chickens after hatching, this glucocorticoid decreases DIO1 but has no effect on the DIO3 activity (Darras *et al.* 1996). In the literature, there is no data on the effect of DEX on the *DIO2* mRNA levels in chicken liver; however, in all brain parts of 18-day-old embryonic chickens, DEX has been shown to affect the T_3 production capacity by an upregulation of DIO2 activity (Reyns *et al.* 2005).

Our experiment revealed that, in laying hens, PCB118 and 4-OH-PCB107 augmented the *DIO1* mRNA level. Moreover, 3-OH-PCB153 increased the *DIO2* while 4-OH-PCB107 decreased the *DIO3* mRNA expression. On the other hand, in the groups incubated in the medium with the addition of the tested dl-PCBs and their hydroxylated metabolites together with DEX, a greater effect of these compounds was observed than in the groups treated only with PCBs or OH-PCBs. In particular, it seems that dl-PCBs can interact with and/or counteract the effects induced by DEX on the deiodinase mRNA

levels. Compared to the mRNA expression analysis, the tested compounds showed a greater effect on the concentration of deiodinases in the chicken livers. Similarly to the mRNA expression, PCB118 and 4-OH-PCB107 augmented the DIO1 and did not change the DIO2 and DIO3 concentrations. Moreover, both of these compounds increased the DEX-inhibited level of DIO1 and DIO3. On the other hand, PCB153 and its hydroxylated metabolite influenced the concentrations of all three types of iodothyronine deiodinases, which suggests that ndl-PCB may disturb TH metabolism in the liver. Discrepancies observed between the mRNA expression and the protein levels of iodothyronine deiodinases in the liver explants may be related to differential transcriptional, posttranscriptional and/or translational regulation. The changes in the T_4 to T_3 conversion, as well as the deiodinase mRNA expression and concentrations demonstrated in our research are correlated. The enlarged T_4 deiodination after dl-PCB treatment may be a result of increased DIO1 mRNA and protein levels. It cannot be excluded that in the group incubated with PCB153, due to the increased concentration of DIO1 and DIO3, T_4 was converted to rT_3 to a greater extent than to T_3. This possibility is also confirmed by the decrease in the DIO2 level, which may suggest that DIO1 and DIO2 (responsible for the T_4 to T_3 conversion) can counteract each other, and as a result, the deiodination in this direction becomes less effective. Moreover, 3-OH-PCB153, by increasing the concentrations of DIO1, DIO2 and DIO3, may stimulate the deiodination process in each direction. The research on the influence of PCBs on deiodinases mainly concerns the activity of these enzymes. It has been shown that the hepatic activity of DIO1 remained unchanged after PCB77 treatment in chicken embryos (Beck *et al.* 2006). The same lack of effect was observed in chickens after hatching due to PCB54 (2,2′,6,6′-tetrachlorobiphenyl), PCB77 or PCB80 (3,3′,5,5′-tetrachlorobiphenyl; Gould *et al.* 1999; Beck *et al.* 2006). Experiments on rats showed that Aroclor 1254, a mixture of various PCB congeners, reduced DIO1 activity in the liver (Hood & Klaassen 2000). On the other hand, PCB77 and Aroclor 1254 exerted stimulatory effects on the DIO2 activity in the brains of chicken embryos (Beck *et al.* 2006) and in rat foetuses (Morse *et al.* 1996). Nevertheless, PCB77 did not affect the hepatic DIO3 activity in chicken embryos (Beck *et al.* 2006), while the 21-day administration of Arclor1254 increased DIO3 activity in the Nile tilapia (Coimbra *et al.* 2005).

The transport of THs across the cell membrane requires the presence of special carrier proteins (Bourgeois *et al.* 2016). Our studies showed a lack of *OATP1C1* mRNA expression in the chicken livers. Furthermore, the investigated PCBs, OH-PCBs and DEX did not influence the *MCT8* mRNA expression and had a slight effect on the *MCT10* mRNA expression, because only PCB118 and 4-OH-PCB107 diminished the mRNA level of this gene. These results suggest that the tested compounds hardly affect, or have little effect on T_4 and T_3 transport across the liver cell membrane by the MCT8 and MCT10 proteins. However, it cannot be ruled out that the observed reduction in T_3 secretion following the PCB118 and 4-OH-PCB107 treatment was associated with the decrease in the *MCT10* mRNA expression. On the other hand, a change in the *LAT1* mRNA level in all the experimental groups suggests that DEX, the tested PCBs and OH-PCBs, as well as the combination of these compounds, may mainly stimulate 3,3′-T_2 transport in hepatocytes. Therefore, the observed increase in the *LAT1* mRNA level seems to be related to the enlargement of the T_3 to 3,3′-T_2 deiodination catalysed by DIO1 and DIO3 to protect the cells from too high a concentration of T_3. Our results are consistent with the studies of Kato *et al.* (2017), who revealed that rats treated with KC500 (a mixture of seven different PCB congeners) increased the *LAT1* mRNA and *OATP2* expression in the liver, but these effects were observed at 48 and 96 h for *LAT1,* and at 96 h for *OATP2*.

Because PCBs and OH-PCBs have a similar chemical structure to TH, it seems likely that they may bind to TR. To explain the molecular mechanism of the PCB congener, as well as the action of OH-PCBs in the liver, we determined the mRNA expression of the genes encoding two isoforms of TRs, i.e. *TRα1* and *TRβ0*. Only 4-OH-PCB diminished the mRNA expression of *TRβ0*, which suggests that the tested PCB and OH-PCBs do not directly affect the TH-dependent gene expression in liver cells, but through changes in the secretion or deiodination of THs, they may change the functioning of hepatocytes. A similar effect was observed in rats where the authors investigated the effect of, inter alia, various PCB and OH-PCB congeners on the ability to bind to TRs in the hepatic nuclei, and the conclusion was that these compounds do not appear to bind to TRs in a competitive manner (Gauger *et al.* 2004). Cheek *et al.* (1999) showed that OH-PCBs have a relatively low affinity for the human thyroid receptor *in vitro*, and furthermore, none of the dl-PCB, PCB 77 and PCB 126 tested bound to TR. However, our results and those of other authors suggest that PCBs and OH-PCBs do not directly compete with TH for TR binding.

Although *in vitro* studies allow for more a precise control of the experimental conditions, as well as for examining the effects of compounds directly on the tissue, which allows researchers to exclude other mechanisms responsible for eliminating the effects of toxins in the body (e.g. it cannot be ruled out that the observed changes in the liver could be compensated by the xenobiotic metabolism processes taking place in other tissues), it would be very desirable to also conduct *in vivo* tests, in order to confirm the observed effects of PCBs and OH-PCBs on the iodothyronine metabolism in a chicken's liver.

Conclusions

In conclusion, this is the first *in vitro* study to describe the effects of PCBs and their hydroxylated metabolites on: basal and DEX-affected T_3 secretion; T_4 to T_3 conversion; iodothyronine deiodinase concentrations; and the mRNA expression of the genes involved in TH metabolism, membrane transport and TRs in chicken liver. Our results suggest that both dl-PCB and ndl-PCB and their hydroxylated metabolites mainly affect the T_4 to T_3 conversion and, to a lesser extent, the T_3 secretion from liver explants. Moreover, PCBs and OH-PCBs may influence the TH metabolism in chicken livers by a modification of the transcription and protein abundance of iodothyronine deiodinases. The changes we observed in the mRNA expression of the *MCT8* and *LAT1* indicated that PCBs and OH-PCBs may affect TH transport across the hepatocyte membrane. Our results suggest that not only parental PCBs, but also their hydroxylated metabolites may affect liver functions associated with the metabolism of iodothyronines. However, since *in vitro* studies take place in controlled experimental conditions, *in vivo* studies are recommended to confirm the observed effects of PCBs and OH-PCBs on iodothyronine metabolism in the liver of the chicken.

Author contributions

Research concept and design: K.K..; Collection and/or assembly of data: K.K., K.F.; Data analysis and interpretation: K.K., D.K.-B.; Writing the article: K.K., D.K.-B., A.S.; Critical revision of the article: A.S.; Final approval of article K.K., D.K.-B., A.S.:

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Conflicts of Interest

The authors declare no conflict of interest.

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