Activation and Potentiation of Human GABA<sub>A</sub> Receptors by Non-Dioxin–Like PCBs Depends on Chlorination Pattern

Elsa C. Antunes Fernandes,*† Hester S. Hendriks,* Regina G. D. M. van Kleef,* Ad Reniers,* Patrik L. Andersson,† Martin van den Berg,* and Remco H. S. Westerink*

*Neurotoxicology Research Group, Toxicology Division, Institute for Risk Assessment Sciences, Utrecht University, NL-3508 TD Utrecht, The Netherlands; and †Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden

1To whom correspondence should be addressed at Neurotoxicology Research Group, Toxicology Division, Institute for Risk Assessment Sciences, Utrecht University, PO Box 80.177, NL-3508 TD Utrecht, The Netherlands. Fax: +31-30-2535077. E-mail: e.c.antunesfernandes@uu.nl.

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants, which have been used in numerous industrial and commercial applications. Worldwide, more than 1.5 million tons of PCBs were produced until their production, commercialization, and use was largely prohibited in the 1970s (Breivik et al., 2002). Mainly due to improper waste disposal, PCBs still enter the environment, and because of their lipophilicity and biopersistence, they tend to accumulate in biota (ATSDR_2000). Food is one of the major routes for human PCB exposure, although inhalation of (indoor) air and house dust can also provide significant exposure, mainly to lower chlorinated congeners (Broding et al., 2007; Peper et al., 2005). Regardless the route of exposure, levels of individual PCBs in human blood have been shown to be in the subnanomolar to lower nanomolar range. However, humans are not exposed to a single congener, and sum of the levels of the most common PCBs in blood can add up to higher nanomolar range (Gabrie et al., 2000; Petrik et al., 2006).

According to their chemical and toxicological properties, PCBs can be divided into two groups: non-dioxin-like polychlorinated biphenyls (NDL-PCBs) and dioxin-like polychlorinated biphenyls (DL-PCBs). Contrary to DL-PCBs, NDL-PCBs have one or more chlorines in the ortho-positions, resulting in nonplanar structures. Consequently, NDL-PCBs have little or no affinity to the aryl hydrocarbon receptor (AhR) and display a different toxicological profile. Several epidemiological studies have indicated that perinatal exposure to NDL-PCBs can result in neurodevelopmental and neurobehavioral effects in children (Faroon et al., 2001; Winneke et al., 1998). Moreover, in vivo studies have shown that animals dosed with NDL-PCBs displayed neurobehavioral effects, including changes in motor activity, learning, memory, and attention (Boix et al., 2010; Eriksson et al., 2010).

The neurotoxic potential of non-dioxin-like polychlorinated biphenyls (NDL-PCBs) is characterized by disruption of pre-synaptic processes, including calcium homeostasis and neurotransmitter transport. Recently, using a limited set of congeners, we demonstrated that PCB28 and PCB52 can potentiate postsynaptic GABA<sub>A</sub> receptors. In the present study, effects of 20 NDL-PCBs and 2 dioxin-like PCBs, selected based on their chemical variation and abundance in the environment, on human GABA<sub>A</sub> receptors were investigated. GABA<sub>A</sub> receptors were expressed in Xenopus oocytes, and NDL-PCB effects were determined using the two-electrode voltage-clamp technique. Results demonstrate that lower chlorinated PCB19, PCB28, PCB47, PCB51, PCB52, PCB95, and PCB100 act as a partial agonist (at low receptor occupancy), i.e., potentiating the receptor response during coapplication with GABA (at EC<sub>20</sub>). Importantly, PCB19, PCB47, PCB51, and PCB100 can also act as full agonist, i.e., activate the GABA<sub>A</sub> receptor in the absence of GABA. Potentiation and activation of the GABA<sub>A</sub> receptor is concentration dependent and limited to NDL-PCBs that have 3–5 chlorine atoms, 1–3 ortho-substitutions, an equal number (0–1) of meta-substitutions on both phenyl rings, and do not have an adjacent para- and meta-substitution on the same phenyl ring. Activation and potentiation of the GABA<sub>A</sub> receptor by PCB47, the most potent congener (lowest observed effect concentration of 10nM), is attenuated when coapplied with PCB19, PCB28, PCB153, or PCB180, indicative for competitive binding. Considering the importance of GABA-ergic signaling for brain development, motor coordination, learning, and memory, this mode of action can contribute to the previously observed NDL-PCB–induced neurobehavioral and neurodevelopmental effects and should be included in human risk assessment.

Key Words: non-dioxin-like PCB; human GABA<sub>A</sub> receptor; receptor activation and potentiation; binary mixture; structure-activity relationship; human risk assessment.
In vitro studies identified regulation of the intracellular calcium concentration ([Ca\(^{2+}\)], which is essential for neural activity and cell viability, as one of the critical parameters affected by NDL-PCBs (Kim et al., 2009; Shafer et al., 1996; Tilson and Kodavanti, 1998). Additional in vitro studies revealed that NDL-PCBs can also affect other presynaptic processes essential for proper neurotransmission, such as inhibition of the uptake of dopamine, serotonin, glutamate, and GABA in synaptosomes and synaptic vesicles isolated from rat brain (Mariussen and Fonnum, 2001; Mariussen et al., 2006).

With respect to postsynaptic processes, acute, direct effects of NDL-PCBs on postsynaptic GABA\(_A\) receptors have been described only recently (Antunes Fernandes et al., 2010). GABA\(_A\) is a pentameric receptor in which \(\alpha_2\beta_2\gamma_2\) is the most common subunit composition in central nervous system (CNS) (for review, see Mohler, 2007). GABA binds to the receptor via its two specific binding sites (between the \(\alpha\)- and \(\beta\)-subunits), resulting in an opening of the receptor and a subsequent Cl\(^-\) current across the membrane. There are, however, several other binding sites that are capable of modulating GABA\(_A\) receptor function, and depending on the binding site involved, the GABA-induced Cl\(^-\) current can be potentiated or inhibited. Modulation of the GABA\(_A\) receptor is of particular relevance as GABA is the main inhibitory neurotransmitter in the adult mammalian CNS and provides the main inhibitory feedback for learning and memory as well as motor activity (for review, see Mohler, 2007).

Based on six abundant NDL-PCBs, it was shown that lower chlorinated congeners are more effective in potentiating the GABA\(_A\) receptor than higher chlorinated NDL-PCBs (Antunes Fernandes et al., 2010). However, only a limited set of NDL congeners was studied, hampering identification of a structure-activity relation (SAR) for NDL-PCBs. Furthermore, studying six congeners, it was unclear whether NDL-PCBs are also able to modulate the GABA\(_A\) receptor in the absence of GABA, i.e., act as full agonist on the human GABA\(_A\) receptor.

Based on their physical-chemical properties and abundance in food and human samples, Stenberg and Andersson (2008) have described a selection of 20 NDL-PCBs. In the present study, this set of NDL-PCBs and two DL-PCBs was used to measure acute effects of NDL-PCBs on GABA\(_A\) receptor functioning and to elucidate a SAR. Furthermore, as human exposure is not restricted to single congeners, we also investigated the effects of binary mixtures of NDL-PCBs on GABA\(_A\) receptor function.

**EXPERIMENTAL SECTION**

**Animals.** All experiments were in accordance with Dutch law and approved by the Utrecht University Ethical Committee for Animal Experiments. Adult female specimens of *Xenopus laevis* frogs (provided by Dr Wim Scheenen, Radboud University, Nijmegen, The Netherlands) were kept in copper-free tap water (pH 6.5, 23°C) in standard aquaria (0.5 × 0.4 × 1 m; 1–15 per aquarium) with a 12-h light/dark cycle. The animals were fed earthworms (Hagen's, Nijkerkerveen, The Netherlands) three times a week.

**Chemicals.** GABA, gabazine, neomycin solution (10 mg neomycin/ml in 0.9% NaCl), collagenase type I, NaCl, and 3-amino-benzoic acid ethyl ester (MS-222) were obtained from Sigma Chemical (St Louis, MO). CaCl\(_2\) (1M solution), MgCl\(_2\) (1M solution), MgSO\(_4\), NaHCO\(_3\), NaOH, Ca(NO\(_3\))\(_2\), KCl, and HEPES were purchased from Merck (Darmstadt, Germany). Complementary DNA (cDNA) of human GABA\(_A\) subunits was synthesized and provided by Paul J. Whiting (Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Harlow, Essex, UK).

The 22 PCBs used in this study were PCB19, PCB28, PCB47, PCB51, PCB52, PCB53, PCB74, PCB77, PCB95, PCB100, PCB101, PCB104, PCB118, PCB122, PCB126, PCB128, PCB136, PCB138, PCB153, PCB170, PCB180, and PCB190 (see Supplementary table 1 for full names and number of ortho-chloro-substitution of each congener). PCBs were purchased from Neosync Inc., and possible impurities, e.g., polychlorinated dibenzo-p-dioxins/ polychlorinated dibenzofurans (PCDD/Fs) and DL-PCBs, were removed by applying the PCBs dissolved in n-hexane on an active carbon column and collecting them after elution with n-hexane as described by Danielsson et al. (2008). The highly purified PCBs were dissolved in purity-checked dimethyl sulphoxide (DMSO). PCB stock solutions of 25mM were further diluted to obtain a final experimental concentration ranging from 1nM to 10µM. DMSO at concentrations up to 0.5% (vol/vol) had no effect on the GABA\(_A\) receptor-mediated Cl\(^-\) currents. In the present experiments, the final concentration of DMSO in PCB-containing saline was always kept below 0.1% (vol/vol).

**Expression of \(\alpha_2\beta_2\gamma_2\) GABA\(_A\) receptors in *Xenopus laevis* oocytes.** Oocytes from *X. laevis*, injected with foreign cDNA of the receptor of choice, are a commonly used tool in studying direct effects of plasma membrane receptors (for review, see Sigel and Minier, 2005). Oocyte preparation and injection was as described previously (Antunes Fernandes et al., 2010). Briefly, female *X. laevis* were anaesthetized by submersion in 0.1% MS-222, and ovarian lobes were surgically removed. Oocytes were treated with collagenase type I (1.5 mg/ml Ca\(^{2+}\)-free Barth’s solution) for 90 min at room temperature before manual defolliculation. cDNA coding for the human \(\alpha_1\), \(\beta_2\), and \(\gamma_2\) subunits of human GABA\(_A\) receptors, dissolved in distilled water at a 1:1:1M ratio, was injected into the nuclei of stage V or VI oocytes using a Nanoject Automatic Oocyte Injector (Drummond, Broomall, PA). The injected volume was 23 nl per oocyte (~1 ng of each subunit). Sham-injected oocytes were injected only with 23 nl of distilled water, i.e., without cDNA. Following injection of the cDNA, or only demineralized water, oocytes were incubated at 21°C in modified Barth’s solution containing (in mM) 88 NaCl, 1 KCl, 2.4 NaHCO\(_3\), 0.3 Ca(NO\(_3\))\(_2\), 0.41 CaCl\(_2\), 0.82 MgSO\(_4\), 15 HEPES, and 10 µg/ml neomycin (pH 7.6 with NaOH). Experiments were performed on oocytes after 2–5 days of incubation, i.e., when receptor expression is maximal. Each experiment was repeated with oocytes obtained from at least two different animals.

**Electrophysiological recording.** Ion currents associated with GABA\(_A\) receptor activity were measured with the two-electrode voltage-clamp technique using a Gene Clamp 500B amplifier (Axon Instruments) with high-voltage output stage as described previously (Antunes Fernandes et al., 2010). Recording microelectrodes (0.5–2.5 M\(\Omega\)) were filled with 3M KCl. Oocytes, placed in a Teflon recording tube, were voltage clamped at −60 mV and continuously superfused (~30 ml/min) with saline solution, containing (in mM): 115 NaCl, 2.5 KCl, 1 CaCl\(_2\), and 10 HEPES (pH 7.2 with NaOH). Aliquots of freshly thawed stock solutions of GABA (1M) in demi-water and of the different PCBs in DMSO were added to the saline immediately before the
experiments. Oocytes were exposed to compounds by switching the perfusate from saline to PCB- and/or GABA-containing saline using a servomotor-operated valve. The maximum PCB concentration tested was 10μM as the solubility of PCBs is limited and higher concentrations lack toxicological relevance. Consequently, it was not possible to establish complete concentration-response curves and to calculate the corresponding EC_{50}s for all PCBs. For specific experiments, oocytes were exposed to a binary mixture of PCBs (1μM PCB47 + 10μM PCB19; 1μM PCB47 + 10μM PCB28; or 1μM PCB47 + 10μM PCB180) or to a mixture of GABA (at EC_{20} concentration) and PCBs (0.01μM PCB47 + 10μM PCB153). In order to minimize absorption of PCBs to the perfusion system, glass reservoirs and Teflon tubes (PTFE; 4 × 6 mm; Rubber, Hilversum, The Netherlands) were used.

Oocytes were repeatedly exposed to different GABA- and/or PCB-containing solutions. To correct for desensitization or run-up, oocytes were superfused with GABA-containing saline to evoke a control response (EC_{max} or EC_{20}) before and after each PCB exposure, so each oocyte could serve as its own control. Furthermore, between each application, a washout period of 2–5 min was introduced to allow receptors to recover from possible desensitization. The lipophilic nature of the PCBs apparently did not affect the observed rapidly reversible potentiation of the GABA-evoked response, as repeated applications of PCBs did not change the observed effects (data not shown). Membrane currents were low-pass filtered (8-pole Bessel; 3 dB at 0.3 kHz), digitized (12 bits; 1024 samples per record), and stored on disk for computer analysis.

**Data analysis and statistics.** Peak amplitudes of GABA-induced or PCB-induced ion currents were measured and normalized to the maximum amplitude of GABA-induced responses (1μM) to adjust for differences in receptor expression levels among oocytes and for small variations in response amplitude over time. The percentage of the PCB receptor activation was calculated as a quotient of the maximum amplitude of PCB and GABA (at 1μM). The percentage of PCB-induced potentiation of the GABA-induced ion current was calculated from the quotient of the maximum amplitude of the GABA-PCB coapplication response and the maximum amplitude of the control response (GABA at EC_{20} concentration). As such, the effects of the PCB exposure are always expressed as a % of control, with each oocyte serving as its own control, corrected for changes over time. Data are expressed as mean ± SEM of number oocytes. Statistical differences (p < 0.05) were calculated using paired and unpaired two-tailed Student’s t-test where appropriate.

**RESULTS**

**Effects of NDL-PCBs on GABA_{A} Receptor Activation**

Oocytes, voltage-clamped at −60 mV, were exposed to saline containing 0.3μM–3mM GABA, and GABA-induced inward Cl\(^{-}\) currents were normalized to the maximum GABA-induced current (1μM) to obtain a GABA concentration-response curve (data not shown). This curve was used to determine the EC_{20} and EC_{50}, i.e., concentrations producing 20 and 80% of maximal response, which amounted to 22 and 193μM, respectively (n = 10; Antunes Fernandes et al., 2010).

First, 20 NDL-PCBs and 2 DL-PCBs (Supplementary table 1) were screened for possible agonistic effects on the human GABA_{A} receptor by exposing oocytes to PCB-containing (1 or 10μM) saline. It was previously shown that PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180 were unable to activate the GABA_{A} receptor (Antunes Fernandes et al., 2010). In the present study, the majority of the tested PCBs were also unable to act as full agonist at the GABA_{A} receptor (see Table 1).

However, PCB19, PCB47, PCB51, and PCB100 were able to activate the GABA_{A} receptor. Subsequently, these four congeners were tested at lower concentrations (30nM–10μM) to determine the lowest observed effect concentration (LOEC) and, if possible, the EC_{50} for activation of the GABA_{A} receptor (Fig. 1, Table 1, and Supplementary fig. 1). At 1μM, PCB47 clearly induced GABA_{A} receptor activation, whereas at 0.1 and 0.3μM, this induction was very modest and not concentration dependent. Still, PCB47 was the most potent tested congener, with an LOEC for GABA_{A} receptor activation of 0.1μM (n = 3), an EC_{50} of 0.67μM, and maximum activation of the GABA_{A} receptor amounting to 42.5 ± 4.5% at 3μM (n = 3). PCB19 and PCB100 had the highest activation of the GABA_{A} receptor, 18.3 ± 7.0% (PCB19, n = 4) and 12.1 ± 3.6% (PCB100, n = 8).

These results thus demonstrate that some lower chlorinated NDL-PCBs can act as full agonist on the human GABA_{A} receptor and that activation of the GABA_{A} receptor is at least partly dependent on the chlorination pattern of the congener.

**Effects of NDL-PCBs on GABA_{A} Receptor Potentiation at Low Receptor Occupancy**

As previously reported (Antunes Fernandes et al., 2010), at low receptor occupancy (EC_{20}), the lower chlorinated PCB28...
TABLE 1
Summary of the Effects of NDL-PCBs on Human GABA<sub>A</sub> Receptor Function

<table>
<thead>
<tr>
<th>Congener</th>
<th>LOEC (µM)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Maximum potentiation</th>
<th>LOEC (µM)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Maximum activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB19</td>
<td>0.3</td>
<td>2.4</td>
<td>119.5 ± 15.6% (10µM)</td>
<td>3</td>
<td>N.D.</td>
<td>18.3 ± 7.0% (10µM)</td>
</tr>
<tr>
<td>PCB28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3</td>
<td>N.D.</td>
<td>98.2 ± 12.5% (10µM)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PCB47</td>
<td>0.01</td>
<td>0.17</td>
<td>223.4 ± 34.2% (3µM)</td>
<td>0.1</td>
<td>0.67</td>
<td>42.5 ± 4.5% (3µM)</td>
</tr>
<tr>
<td>PCB52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3</td>
<td>N.D.</td>
<td>24.5 ± 1.4% (10µM)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PCB51</td>
<td>0.03</td>
<td>1.03</td>
<td>168.0±39.6% (3µM)</td>
<td>0.3</td>
<td>1.1</td>
<td>6.9 ± 1.6% (3µM)</td>
</tr>
<tr>
<td>PCB95</td>
<td>0.3</td>
<td>0.34</td>
<td>20.0 ± 3.5% (1µM)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PCB100</td>
<td>0.03</td>
<td>0.15</td>
<td>122.1 ± 17.6% (3µM)</td>
<td>10</td>
<td>N.D.</td>
<td>12.1 ± 3.6% (10µM)</td>
</tr>
</tbody>
</table>

Note. Effects can be classified as partial (left) and full (right) agonistic, i.e., potentiation and activation of the receptor, respectively. Values for maximum activation are presented as mean ± SEM (n = 3–19). N.D., not determined, indicating that EC<sub>50</sub> could not be determined as the maximum potentiating effect was not yet reached at the highest concentration tested (10µM).

<sup>a</sup>Results described previously (Antunes Fernandes et al., 2010).

and PCB52 are able to potentiate the GABA-induced current in a concentration-dependent manner. However, this potentiation effect is not seen at high receptor occupancy (EC<sub>50</sub>). In the present study, none of the 20 NDL-PCBs and 2 DL-PCBs tested (1 or 10µM) showed any agonistic or antagonistic properties when coapplied with GABA at EC<sub>50</sub> concentration (data not shown). However, when coapplied with GABA at EC<sub>20</sub>, PCB19, PCB47, PCB51, PCB95, and PCB100 were able to potentiate the GABA-evoked current (Fig. 2, Table 1, and Supplementary fig. 2.). These PCBs were further tested at a concentration ranging from 1nM to 10µM.

Again, PCB47 was the most potent NDL-PCB with an LOEC for potentiation of the GABA<sub>A</sub> receptor of 0.01µM (n = 9) and an EC<sub>50</sub> of 0.17µM. Maximum potentiation was reached at 3µM and amounted to 223.4 ± 34.2% (n = 5). Although not statistically significant, the potentiating effect attenuated at 10µM (198.3 ± 13.2%; n = 3). Potentiation of the GABA<sub>A</sub> receptor by PCB51 and PCB100 was comparable, although less potent than PCB47. Both PCBs had an LOEC of 0.03µM and a maximum potentiation of the GABA<sub>A</sub> receptor of ~120 to 160% at 3µM. However, the EC<sub>50</sub> of PCB51 (1.03µM) was approximately one order of magnitude higher than that of PCB100 (0.15µM). Comparable with PCB28 and PCB52 (Antunes Fernandes et al., 2010), PCB19 and PCB95 have an LOEC for potentiation of the GABA<sub>A</sub> receptor of ~0.3µM. However, at higher concentrations (>1µM), PCB19 and PCB28 were more effective in potentiating the GABA<sub>A</sub> receptor than PCB52 and PCB95 (see Table 1 for details). These results thus demonstrate that several lower chlorinated NDL-PCBs can act as partial agonist on the human GABA<sub>A</sub> receptor when coapplied with GABA at EC<sub>50</sub> and that this action depends at least on the chlorination pattern of the congener.

**Effects of Binary NDL-PCB Mixtures on GABA<sub>A</sub> Receptor Activity**

Humans are generally not exposed to one single PCB congener. Moreover, it was previously suggested that higher chlorinated NDL-PCBs (PCB153) can competitively bind to the GABA<sub>A</sub> receptor, thereby attenuating the potentiating effects of PCB28 and PCB52 (Antunes Fernandes et al., 2010). In order to further investigate if the composition of a PCB mixture determines its net effect on GABA<sub>A</sub> receptor activation or potentiation, several binary mixtures of NDL-PCBs have been tested.

In the present study, PCB47 is the most potent congener with respect to potentiation of the GABA<sub>A</sub> receptor at low receptor occupancy (EC<sub>20</sub>). Conversely, PCB153 (up to 10µM) was not able to potentiate the GABA-evoked current. When oocytes
were coexposed to GABA (at EC20) and a binary mixture of PCB47 (0.01μM) and PCB153 (10μM), the degree of receptor potentiation evoked by PCB47 alone decreased from 11.7 ± 1.4 to 3.5 ± 1.1% (p < 0.05; Fig. 3A). In line with previous research (Antunes Fernandes et al., 2010), this suggests that the higher chlorinated PCB153 competently binds to the GABA\(_\text{A}\) receptor, without being able to potentiate it.

To investigate the possibility of competitive binding by other NDL-PCBs with respect to GABA\(_\text{A}\) receptor activation (instead of potentiation), oocytes were first exposed to 1μM of the full agonist PCB47. The PCB47-induced ion current was set at 100%, and oocytes were subsequently exposed to binary mixtures of PCB47 (1μM) with another but less potent full agonist (PCB19; 10μM), with a partial agonist (PCB28; 10μM) or with a higher chlorinated NDL-PCB (PCB180; 10μM). All binary mixtures reduced the PCB47-evoked ion current to, respectively, 73.3 ± 2.4% (n = 3; p < 0.01), 40.4 ± 1.7% (n = 3; p < 0.001), and 17.6 ± 0.7% (n = 3; p < 0.001; Fig. 3B). In a comparable set of experiments, oocytes were first exposed to 10μM of the full (but weak) agonist PCB19 (PCB19-evoked current set at 100%) and subsequently to a binary mixture of PCB19 (10μM) and PCB47 (1μM). In this case, the binary mixture increased the inward current evoked by PCB19 to 883.6 ± 26.7% (n = 3; p < 0.01; Fig. 3C). These data thus suggest competitive binding to the GABA\(_\text{A}\) receptor and that the presence of less potent or inactive PCBs in the mixture attenuates the effect of more potent PCBs.

**DISCUSSION**

Previously, Antunes Fernandes et al. (2010) identified potentiation of the GABA\(_\text{A}\) receptor by the lower chlorinated NDL-PCBs 28 and 52 as a novel mode of action. The present study shows that also PCB19, PCB47, PCB51, PCB95, and PCB100 have comparable effects (Fig. 2 and Supplementary fig. 2). This potentiating effect is observed only when receptor occupancy is low, i.e., when coapplied with GABA at EC\(_{20}\).

Interestingly, PCB19, PCB47, PCB51, and PCB100 are also able to concentration dependently activate the GABA\(_\text{A}\) receptor, i.e., producing an inward Cl\(^{-}\) current in the absence of the endogenous agonist GABA (Fig. 1 and Supplementary fig. 1). Some NDL-PCBs thus mimic the effect of natural agonist GABA, possibly resulting in an indirect interaction of other modulators of the GABA\(_\text{A}\) receptor. This may be translated into an exacerbation of the effect of partial agonists of the GABA\(_\text{A}\) receptor, such as neurosteroids or ethanol.

The observed effects on the GABA\(_\text{A}\) receptor are specific and not due to activation of another natively expressed receptor as sham-injected oocytes exposed to the NDL-PCBs did not show any effect (data not shown). Moreover, the onset and washout of the PCB effect is very rapid (both within seconds), and the PCB-induced activation/potentiation of the GABA response remains stable over time as well as over repeated applications. It is thus unlikely that the observed effects on the GABA\(_\text{A}\) receptor are due to indirect PCB-induced effects, e.g., on intracellular calcium levels. Experiments with the partial agonist PCB28 and gabazine, which blocks the GABA-binding site, have shown that the potentiating effects of PCBs at least involve the GABA-binding site (Antunes Fernandes et al., 2010), likely in combination with a positive allosteric site, e.g., the benzodiazepine-binding site. However, the lack of truly specific agonists or antagonists for the different positive allosteric sites precludes more detailed assessment of the binding sites involved.

NDL-PCBs can thus act as (partial) agonist on the human GABA\(_\text{A}\) receptor. However, as full agonist, PCB47 and PCB51 show a clear reduction in the activation of the GABA\(_\text{A}\) receptor at higher concentrations (10μM). Similarly, as partial agonist, i.e., coapplied with GABA at EC\(_{20}\), PCB47, PCB57, and PCB100 show a clear attenuation of receptor potentiation at 10μM (Table 1, Fig. 2 and Supplementary fig. 2.). Whether PCB19, PCB28, and PCB95 show the same tendency is not clear because the highest tested concentration (10μM) was not enough to evoke a complete concentration-response curve and higher concentrations lack toxicological relevance and are

**FIG. 3.** GABA\(_\text{A}\) receptor potentiation and activation by binary NDL-PCB mixtures. (A) Potentiation of GABA-induced ion current (at EC\(_{20}\)) by PCB47 (0.01μM) is reduced when coapplied as binary mixture with PCB153 (10μM) (*p < 0.01). (B) Activation of the GABA\(_\text{A}\) receptor by PCB47 (1μM) was set as 100% to facilitate comparison with the effect of PCB47 in subsequent coapplication with PCB19 (10μM), PCB28 (10μM), or PCB180 (10μM) (**p < 0.01, ***p < 0.001). (C) Activation of the GABA\(_\text{A}\) receptor by PCB19 (10μM) was set as 100% to facilitate comparison with the effect of PCB19 in subsequent coapplication with PCB47 (1μM) (*p < 0.01). Bars represent mean ± SEM. (n = 3–4).
precluded due to the limited solubility of PCBs. Attenuation of receptor responses is observed for several GABA_A receptor agonists at higher concentrations and is possibly due to rapid receptor desensitization (Akk and Steinbach, 2000; Muroi et al., 2009; Pistis et al., 1997). Alternatively, it can be suggested that these NDL-PCBs bind to an additional (low-affinity) inhibitory binding site of the GABA_A receptor or exert their inhibitory effect indirectly, e.g., due to partitioning in the membrane (Akk et al., 2009; Hosie et al., 2003).

LOECs and EC_{50}s for activation and potentiation of the GABA_A receptor differ by more than one order of magnitude. Additionally, the maximum level of activation or potentiation differs considerably between the different NDL-PCBs (Table 1), making it difficult to establish a general rank-order potency. It is, however, evident that in this study, PCB47 is the most potent NDL-PCB, both as partial and as full agonist. Human exposure to PCBs is generally not exclusive to a single congener but to a mixture of PCBs. Our previous results suggested that the potentiating effects of PCB28 and PCB52 are apparently additive. Conversely, coapplication of PCB153 and PCB28 attenuated the potentiation of the GABA_A receptor by PCB28, suggesting competitive binding (Antunes Fernandes et al., 2010). In line with this, the present results demonstrate that the potentiating effect of PCB47 is decreased by coapplication with PCB153. Moreover, activation of the receptor by the most potent congener, PCB47, is decreased by coapplication with the less potent full agonist PCB19, partial agonist PCB28, or high-chlorinated nonactive PCB180. Furthermore, activation of GABA_A receptor by PCB19 is enhanced by subsequent application of PCB47. This supports the idea that competitive binding is involved, as observed previously for disruption of thyroid hormone receptor, estrogen receptor, and AhR binding (Chauhan et al., 2000; Gutleb et al., 2010; Petulis and Bunce, 2000).

Several epidemiological and in vivo studies have described neurotoxic effects of NDL-PCBs following perinatal exposure. These effects can be at least partly explained by in vitro findings where NDL-PCBs were shown to alter neurotransmitter levels due to changes in neurotransmitter synthesis, metabolism, and transporters. Furthermore, exposure to NDL-PCBs has been reported to induce oxidative stress and disrupt Ca^{2+} homeostasis (for review, see Kodavanti, 2006; Pessah et al., 2010). As PCBs comprise a large number of congeners, previous studies have tried to classify their neurotoxic potential according to their chlorination pattern. It was shown that especially ortho-substituted congeners were able to inhibit vesicular and membrane neurotransmitter uptake (Mariussen and Fonnum, 2001; Mariussen et al., 2001). Disruption of Ca^{2+} homeostasis has been ascribed primarily to activation of intracellular ryanodine receptors, which appears to be specific for para-substituted NDL-PCBs (Kodavanti et al., 1996; Pessah et al., 2010). For activation or potentiation of GABA_A receptors, para-substitutions are not a prerequisite. Importantly, the observed effects are apparently dependent on the chlorination pattern of the PCBs, as DL-PCB77 and DL-PCB126 as well as NDL-PCBs with more than five chlorine atoms were unable to activate or potentiate the GABA_A receptor. Moreover, the number of ortho-substitutions, which to a large extent determines the planarity of the molecule, should be limited to 1–3. For example, the tri-ortho-substituted NDL-PCB51 and PCB100 are full agonists, whereas the structurally comparable but inactive PCB104 has four ortho-substitutions. Additionally, NDL-PCBs that have both a para- and a meta-substitution on the same phenyl ring are inactive. For example, PCB74, which has adjacent para- and meta-substitutions on one phenyl ring, is inactive, whereas PCB28, which is structurally similar except for the meta-substitution, acts as GABA_A receptor agonist. Similarly, PCB52 (partial agonist) and PCB101 (inactive) are structurally similar, except for an adjacent additional meta-substitution in PCB101. Finally, based on the current data set, it appears that both phenyl rings should have an equal number of meta-substitutions. For example, PCB52 and PCB95 have a single meta-substitution on each phenyl ring and act as partial agonist, whereas PCB53, which has a meta-substitution on only one ring, is inactive. Based on the present selection of 22 PCBs, it is suggested that to be (partial) GABA_A receptor agonist NDL-PCBs should (1) have no more than five chlorine atoms, (2) have 1–3 ortho-chlorinated positions, (3) not have an adjacentpara- and meta-substitution on the same phenyl ring, and (4) have an equal number (0–1) of meta-substitutions on both phenyl rings. Though this proposed SAR is based on 22 PCBs, future experiments will have to prove if this SAR is conclusive. Nonetheless, considering their LOECs and EC_{50}s, the most active NDL-PCBs (i.e., PCB47 and PCB51) appear to be di- and tri- ortho substituted, with one or two para-substitutions but no meta-substitutions.

Low–molecular weight NDL-PCBs are among the most abundant congeners in contaminated indoor air and dust samples in both public and private buildings (Harrad et al., 2010). Human plasma levels of individual lower chlorinated PCBs, including PCB28 and PCB47, amount on average to 0.03 and 0.02 nM, respectively, following exposure via contaminated indoor air. LOECs for activation of the GABA_A receptor derived from this study are between 0.1 and 10 μM. Though LOECs for receptor potentiation are one order of magnitude lower (0.01 μM for PCB47), these concentrations are well above the levels of individual lower chlorinated NDL-PCBs present in human plasma (in the low nanomolar range). Although at low concentrations additivity might occur, it should also be noted that the effect of the most potent PCB may be attenuated by the presence of less potent or inactive PCB due to competitive binding (Fig. 3).

Nonetheless, the effect concentrations reported here, as low as 10 nM for GABA_A receptor potentiation by PCB47, are in the same range or even well below the effect concentration previously reported for presynaptic adverse effects, including disruption of calcium homeostasis and neurotransmitter transport (Fonnum et al., 2006). Moreover, GABA is essential in early brain development (for review, see Ben-Ari et al., 2007; Owens and Kriegstein, 2002) and a key player in learning and memory.
as well as motor activity (for review, see Mohler, 2007). Consequently, the present data, demonstrating disruption of GABA_A receptor-mediated signaling, likely underlie at least part of the described neurobehavioral and neurodevelopmental effects following NDL-PCB exposure. As such, these findings justify a thorough exposure characterization and extensive human risk assessment for lower chlorinated NDL-PCBs.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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