# Non-coplanar polychlorinated biphenyls (PCBs) are direct agonists for the human pregnane-X receptor and constitutive androstane receptor, and activate target gene expression in a tissue-specific manner

Fadheela Al-Salman and Nick Plant

Centre for Toxicology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH

**Corresponding Author:**

Dr Nick Plant

Centre for Toxicology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH

Tel: +44 (0)1483 686412

Fax: +44 (0)1483 686401

Email: N.Plant@Surrey.ac.uk

**Abbreviations:** AhR, Aryl hydrocarbon receptor; CAR, constitutive androstane receptor; PCB, polychlorinated biphenyls; PXR, pregnane X-receptor

**Abstract**

The polychlorinated biphenyl group of possess high environmental persistence, leading to bioaccumulation and a number of adverse effects in mammals. Whilst coplanar PCBs elicit their toxic effects through agonism of the aryl hydrocarbon receptor; however, non-coplanar PCBs are not ligands for AhR, but may be ligands for members of the nuclear receptor family of proteins. To better understand the biological actions of non-coplanar PCBs, we have undertaken a systematic analysis of their ability to activate PXR and CAR-mediated effects

Cells were exposed to a range of non-coplanar PCBs (99, 138, 153, 180 and 194), or the coplanar PCB77: Direct activation of PXR and CAR was measured using a mammalian receptor activation assay in human liver cells, with rifampicin and CITCO used as positive controls ligands for PXR and CAR, respectively; activation of target gene expression was examined using reporter gene plasmids for CYP3A4 and MDR1 transfected into liver, intestine and lung cell lines.

Several of the non-coplanar PCBs directly activated PXR and CAR, whilst the coplanar PCB77 did not. Non-coplanar PCBs were also able to activate PXR/CAR target gene expression in a substitution- and tissue-specific manner.

Non-coplanar PCBs act as direct activators for the nuclear receptors PXR and CAR, and are able to elicit transcriptional activation of target genes in a substitution- and tissue-dependent manner. Chronic activation of PXR/CAR is linked to adverse effects and must be included in any risk assessment of PCBs.

**Keywords:**

Nuclear Receptor; Gene Expression; Drug Metabolism; PXR; CAR

## Introduction

Polychlorinated biphenyls (PCBs) were historically used in a large number of industrial applications, including coolants, plasticisers, lubricants and insulators (Safe, 1984; Safe, 2001). Their high chemical stability has resulted in environmental persistence, and coupled with their highly lipophilic nature raises the potential for bioaccumulation in higher mammals, including humans.

There are 209 potential PCB congeners, of which 36 congeners are considered to be environmentally threatening due to environmental prevalence, bioaccumulation in animal tissues and known toxic effects (McFarland and Clarke, 1989). These congeners can be divided into non-*ortho* PCBs that have a coplanar configuration, and *ortho*-substituted PCBs with a non-coplanar configuration. PCB congeners with zero or one *ortho*-chlorine substituent (e.g. PCB77) exhibit toxic effects similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and these are mediated through the aryl hydrocarbon receptor (AhR); thus, these PCBs are referred to as dioxin-like PCBs. These adverse effects include hepatotoxicity, elevated blood lipids, immune suppression, reproductive and developmental toxicity and carcinogenicity (Safe, 1984; Safe, 2001). Due to these highly undesirable properties, PCB manufacture was banned in the late 1970s and their usage severely restricted. However, due to the highly persistent nature of these chemicals, it is estimated that there remains over 750,000 tonnes of PCBs in the biosphere, and hence they still represent an important potential toxic contaminant.

Whilst the majority of risk assessment interest has focussed on the coplanar dioxin-like PCBs, non-coplanar PCBs are also highly environmentally prevalent, with PCB138, 153 and 180 being widely present in the environment and thus must also be included in any hazard/risk assessment (Safe, 1994). Non-coplanar PCBs (e.g. PCB153) possess *ortho* chlorine substituents on the biphenyl ring that twists the structure away from a single plane. This significantly reduces the affinity for AhR, and indeed these compounds are likely to act as inhibitors of AhR-mediated activation (Suh *et al.*, 2003). Non-coplanar PCBs are more likely to act as ligands for members of the nuclear receptor family of transcription factors (Wu *et al.*, 2009). This activity may be important considering that not all of the adverse effects associated with PCB exposure are mediated via AhR. For example, the hydroxylated metabolites of PCBs are weak oestrogen receptor agonists, as well as inhibitors of oestrogen sulphotransferase; as such, PCBs may have an endocrine disrupting effect, which may

underlie their reproductive toxicity (Kester *et al.*, 2000). Thus it is important to assess the hazard of non-coplanar PCBs to humans to inform a full risk assessment

It has been suggested that non-coplanar PCBs may act as ligands for the constitutive andorstane receptor (CAR) and/or the pregnane X receptor (PXR) (Schuetz *et al.*, 1998; Jacobs *et al.*, 2005), and may thus activate CAR/PXR target genes expression. Due to this activity, this group of PCBs is often referred to as 'Phenobarbital-like PCBs'.

There is thus a need to examine the non-AhR mediated effects of PCBs, and specifically the non-coplanar phenobarbital-like PBCs, for comprehensive risk assessments to be undertaken. As such, we have examined the ability of the non-coplanar PCBs PCB99, 138, 153, 180 and 194 to directly activate CAR and PXR, as well as their ability to activate target gene expression in liver, lung and intestinal cells *in vitro*.

## Materials and Methods

**Reagents:** Fugene-6 transfection reagent was purchased from Roche Diagnostics, Lewes, UK. Unless otherwise stated all other chemicals were of molecular biology grade and obtained from Sigma-Aldrich. (Poole, UK). The Huh7 human hepatocellular carcinoma cell line (Nakabayashi *et al.*, 1982) was a kind gift from Dr Steve Hood (GSK, Ware, UK). Human intestinal (Caco2) and lung (A549) cell lines were purchased from ECACC (Porton Down, UK). All cells were routinely cultured in minimal essential medium with Earle's salts supplemented with 1 % non-essential amino acids, 2 mM L-glutamine, 100U/ml penicillin, 100 μg/ml streptomycin and 10 % foetal bovine serum. All cell culture medium and supplements were purchased from Invitrogen (Paisley, UK). In order to maintain phenotypic consistency, Huh7 cells were only used for three weeks (approximately 6 passages) following recovery from liquid nitrogen. The expression plasmid for PXR was kindly provided by Prof. S. Kliewer (University of Texas, Dallas, USA).

**Nuclear Receptor Activation Assay.** Nuclear reporter activation assays were undertaken using a modification of the Checkmate mammalian two-hybrid system (Promega, UK), as described previously (Howe *et al.*, 2011). Briefly, Huh7 cells were seeded into 96-well plates (Nunc International, Leicestershire, UK) at a concentration of 20,000 cells/well and incubated at 37 °C for 24 hrs. FuGENE 6-mediated DNA co-transfections, using 25ng/well luciferase reporter plasmid and 12.5ng/well NR-Gal4 fusion plasmid were undertaken, using serum-free

medium for the six-hour transfection period; this was then replaced with fresh, complete medium, containing charcoal-stripped serum, for the remaining culture period. The NR-Gal4 construct encodes a fusion protein of the GAL4-DBD and an extended ligand binding region for human PXR or CAR, representing amino acids 141-434 and 162-348 respectively.

Transfections were allowed to proceed for 24 hours, in the presence of PCB, positive control chemical or vehicle control (0.1% DMSO) as indicated, and then luciferase activity determined using the Dual-Luciferase reporter system (Promega, UK) according to the manufacturer's protocol. Luminescent output was measured using a LumiCount automated plate reader (Canberra Packard, UK).

**Transcript level measurement:** Specific primers and TAMRA/FAM dual labelled probe sets were designed against CYP1A1 and 18s using the Primer Express software (Applied Biosystems, Warrington, UK) and were purchased from Eurofins MWG (Wolverhampton, UK).

Huh7, A549 or Caco2 cells were seeded into 96-well plates (Nunc International, Leicestershire, UK) at a concentration of 10,000 cells/well and incubated at 37 °C for 24 hrs in a humidified container for attachment. Cells were exposed to PCB, positive control chemical or vehicle control as indicated for 48 hours. Following compound exposure, total RNA was extracted using the RNAeasy system (Qiagen, Crawley, UK) and treated with RNase-free DNase (Promega, Southampton, UK) to remove genomic contamination. Reverse transcription was primed with random hexamers and carried out by Superscript II (Invitrogen) as per the manufacturer's instructions.

cDNA was amplified using TaqMan Universal PCR Master Mix with 400 nM primers and 200 nM fluorogenic probe in a total reaction volume of 25µl: cDNA generated from 50ng input total RNA was used per reaction to measure CYP1A1, whilst cDNA derived from 50pg input total RNA was used for 18s RNA quantitation. Quantitative polymerase chain reaction (Q-PCR) reactions were run on the ABI7000 SDS instrument and quantitation was carried out using the ABI proprietary software against a standard curve generated from human genomic DNA (Promega), and normalised against 18s rRNA expression levels.

**Reporter gene assay:** Reporter gene assays were undertaken as described in Aouabdi et al (Aouabdi *et al.*, 2006). Briefly, cells were seeded into 96-well plates (Nunc International, Leicestershire, UK) at a concentration of 10,000 cells/well and incubated at 37 °C for 24 hrs

in a humidified container for attachment. FuGENE 6-mediated DNA co-transfections, with 75ng/well reporter gene constructs and 25ng/well over-expression plasmid as appropriate, were undertaken in serum-free medium for the six-hour transfection period; this was then replaced with fresh, complete medium, containing charcoal-stripped serum, for the remaining culture period.

Transfections were allowed to proceed for 48 hours, in the presence of PCB, positive control chemical or vehicle control as indicated, and then secretory alkaline phosphatase (SEAP) activity determined. Endogenous alkaline phosphatase activity was deactivated by heat-treatment of the medium at 65 °C for 30 minutes, and SEAP activity was assayed using the AURORA system (ICN, Thame, UK), according to the manufacturer's protocol. Chemiluminescent output was measured using a LumiCount automated plate reader (Canberra Packard, UK), and fold induction relative to vehicle control calculated as described previously (Plant *et al.*, 2000).

Data was fitted to a non-linear regression model using GraphPad Prism v5.04 (La Jolla, CA, USA). The model utilised a four parameter variable slope to fit log(agonist) versus response, as given by the equation: Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope)).

**Results**

A range of PCB congeners were selected for study that encompassed the coplanar PCB77, and the non-coplanar PCB99, 138, 153, 180 and 194 (Figure 1). Initially, we confirmed that only the coplanar PCB77 was able to activate AhR-mediated gene expression. Human liver, intestine and lung cell lines were exposed to each of the PCBs (10μM) for 48 hours and CYP1A1 transcript levels measured by Q-PCR. PCB77 elicited a significant increase in CYP1A1 transcript levels in all three cell lines; Caco2 cells were the most responsive, with Huh7 and A549 being equally responsive, demonstrating 64±6, 3.8±0.4 and 4.2±0.6 fold inductions versus vehicle control (0.1% DMSO), respectively (Figure 2). In contrast, none of the non-coplanar PCBs were able to significantly increase the level of CYP1A1.

To examine the relationship between each PCB and the nuclear receptors PXR and CAR, we utilised a mammalian receptor activation assay, which contained extended ligand-binding domains for PXR and CAR respectively, to directly examine interactions. When transfected into Huh7 liver cells, the PXR activation reporter construct was activated by the positive control ligand rifampicin in a dose-dependent manner (maximum 7.6-fold versus vehicle control; Figure 3A), but was not activated by the coplanar PCB77. Within the non-coplanar

PCBs examined, the PXR activation assay was activated in a dose dependent manner by PCB138, 153, 180 and 194. PCB153 elicited the greatest effect, being approximately equipotent compared to rifampicin (maximum 9-fold versus vehicle control). The CAR activation assay was activated by the human-specific positive control agonist CITCO in a dose dependent manner (maximum 5.8-fold versus vehicle control; Figure 3B It was also activated by PCB99, 153, 180 and 194, although all were less potent than CITCO, producing maximal activations of 2.5-, 3.3-, 3.8- and 2.7-fold versus vehicle control, respectively.

Having characterised the ability of selected non-coplanar PCBs to directly activate the nuclear receptors PXR and CAR, we next examined their ability to activate target gene expression for these nuclear receptors. We used reporter gene constructs for CYP3A4 and MDR1, which are classical target genes for PXR (Geick *et al.*, 2001; Bombail *et al.*, 2004; Plant, 2007), and can also be activated by CAR (Xie *et al.*, 2000). Cells were exposed to the classical PXR agonist rifampicin (10μM), the mixed CAR/PXR agonist phenobarbital (500μM), or each PCB (10μM).

In the human liver cell line Huh7, both rifampicin and phenobarbital activated expression of the CYP3A4 reporter gene (approximately 10- and 6-fold compared to vehicle control, respectively); no effect was observed on MDR1 reporter gene expression for either the upstream or downstream regulatory regions. Within the tested PCBs, only PCB153 had any significant impact on PXR/CAR-mediated gene expression, significantly increasing CYP3A reporter gene expression by approximately 7.6-fold compared to vehicle control (Figure 4A).

In the colon-derived cell line Caco2, both rifampicin and phenobarbital increased CYP3A4 reporter gene expression (9 and 4-fold compared to control, respectively). In addition, they also significantly activated the MDR1 upstream regulatory region, by 2.5- and 3.5-fold versus vehicle control, respectively. PCB153 was again able to increase CYP3A4 reporter gene activity (5.6-fold compared to vehicle control), and also increased MDR1 upstream regulatory region activity (3.8-fold compared to vehicle control), but had no effect on the MDR1 downstream regulatory region. PCB194 elicited a significant 2.6-fold increase in CYP3A4 reporter gene expression, but had no impact on either MDR1 reporter genes. All other PCBs tested had no effect on any of the reporter genes examined (Figure 4B)

In general, effects within the lung cell line A549 were of a smaller magnitude compared to those observed in either the Huh7 or Caco2 cells (Figure 4C). Rifampicin did not increase CYP3A4 reporter gene expression, but did increase MDR1 upstream reporter gene expression

by 3-fold compared to vehicle control. In comparison, phenobarbital had no effect on MDR1 reporter gene expression, but did significantly increase CYP3A4 reporter gene expression by 4.3-fold compared to control. PCB138 increased CYP3A4 reporter gene expression, as did PCB153 (3.2- and 4-fold compared to vehicle control). PCB153 and PCB180 both increased expression of MDR1 reporter genes, although PCB153 increased the upstream regulatory region reporter (3.5-fold compared to vehicle control), whilst PCB180 increased the downstream regulatory region reporter (3-fold compared to vehicle control).

PCB153 was selected for further analysis due to its direct and potent activation of both PXR and CAR, as well as its ability to activate PXR/CAR target gene expression, and its high environmental prevalence. Using the CYP3A4 and MDR1 reporter gene constructs we produced full concentration response curves in each of the three cell lines. As can be seen in figure 5A, in the Huh7 liver cell line, PCB153 is equipotent with rifampicin at activating the CYP3A4 reporter gene (EC50=5.5±1.3 and 6.8±1.4, respectively), consistent with its equipotent activation of PXR in the ligand binding assay (Figure 3). Both PCB153 and rifampicin have no significant effect on MDR1 reporter gene constructs in Huh7 cells, consistent with previous data (Figure 4).

In contrast to the equipotent effect observed in the liver cell line, PCB153 was a more potent activator than rifampicin of the CYP3A4 reporter gene in Caco2 cells (EC50=1.5μM±2.3 and 10μM±4.6, respectively; Figure 5B): However, the maximum induction observed was greater for rifampicin than PCB 153 (13.3±1.2 versus 6.1±0.4 cold compared to vehicle control, respectively). In addition, both rifampicin and PCB153 caused a dose-dependent increase in the MDR1 upstream regulatory region reporter gene: PCB153 was more potent that rifampicin (EC50= 1.3μM ±4.3 and 8.6μM±1.2, respectively), with the maximal induction observed for rifampicin (5.2-fold±0.3 compared to vehicle control) being greater than that observed with PCB153 (2.7-fold±0.3 compared to vehicle control).

Both rifampicin and PCB153 were poor activators of CYP3A4 in the A549 lung cell line (figure 5C). Both compounds also failed to elicit statistically significant, dose-dependent, activation of the MDR1 upstream regulatory region, although rifampicin did cause an approximate 8-fold increase in MDR1 downstream regulatory region-mediated reporter gene activity at high concentrations.

**Discussion**

Previous data has provided indirect evidence for the interaction of non-coplanar PCBs with the nuclear receptors PCR and CAR. Using a reporter gene system, Tabb et al were able to demonstrate that highly chlorinated, non-coplanar, PCBs were able to impact on PXR-mediated transcription (Tabb *et al.*, 2004). Interestingly, they demonstrated that these highly chlorinated PCBs were able to activate reporter gene expression of target genes via mouse and rat PXR, but inhibited human PXR-mediated transcriptional events. Both PCB153 and PCB 180 were demonstrated to exhibit Ki values in the low micromolar range, with PCB153 being the more potent inhibitor. In addition, PCB153 was demonstrated to bind to human PXR via a scintillation proximity assay; it is important to note that this assay is merely a binding assay and does not distinguish between agonist and antagonist behaviour. Herein, we demonstrate the ability of non-coplanar PCBs to both activate the nuclear receptors PXR and CAR in vitro, and also link this to tissue-specific activation of target gene expression. PCB153 has been shown to also be an agonist for the PXR paralogue CAR (Sakai *et al.*, 2006), although again this was only evident at relatively high concentrations, with significant activations occurring above 20ppm. Whilst our current data may initially appear contradictory to the suggestion that non-coplanar PCBs act as inhibitors of human PXR-mediated transcriptional events. However, these data can be reconciled when one considers that inhibition of CYP3A reporter gene expression was observed by Tabb et al. at low micromolar concentrations, whilst we demonstrate activation of reporter gene expression at concentrations of 10μM; in addition, we demonstrate no direct activation of PXR or CAR by non-coplanar PCBs at 0.1μM, which is not inconsistent with the observations of Tabb et al. Taken together, such data may suggest that the mode-of-action for non-coplanar PCBs may be as mixed antagonist-agonists, with agonist activity only occurring at higher concentrations. Such a hypothesis is further supported by the work of Kopec et al, who studied the effect of non-coplanar PCBs on the murine transcriptome. In experiments where mice were exposed to 1-300mg/kg PCB153 for 24-168 hours, they observed an activation of "PXR/CAR target genes" that was not evident in mice exposed to the coplanar TCDD (Kopec *et al.*, 2010). It should be noted that no attempt was made to delineate between PXR-specific and CAR-specific target genes, with this target gene set being treated as a single unit. Subsequently, Kopic et al also demonstrated that TCDD and PCB153 activate unique target gene sets even when given as a mixture, further supporting their alternate modes of action via the AhR and PXR/CAR, respectively (Kopec *et al.*, 2011). The doses used in these studies achieved hepatic PCB153 concentration in the micromolar range, consistent with the in vitro doses used in our current work.

Given the suggested mixed agonist/antagonist of non-coplanar PCBs against human PXR/CAR, it is pertinent to consider what the likely human exposures are. Measurement of PCB levels in humans has been undertaken in a number of countries, with blood concentrations for PCB153 in the range 5-550 ng/g lipid having been reported (Petersen *et al.*, 2007; Rudge *et al.*, 2012). In addition, similar levels have been reported in fatty tissues such as breast (170±364 ng/g lipid (Petreas *et al.*, 2011)), demonstrating the rapid distribution of these lipophilic compounds. Given the rapid and complete uptake of PCBs into human liver cells (Ghosh *et al.*, 2010), it is likely that similar concentrations are found in the liver. As the lipid content of the liver is approximately 5% w/w (Garbow *et al.*, 2004), it can be estimated that human liver concentrations are in the range 0.25-25ng/g total liver: This would equate to PCB concentrations in the low micromolar region, which is where effects were observed in the current study. Studies looking at the PCB burden in other tissues shows that there is, generally, only a small degree of variance between median tissue levels (expressed as ng/g lipid) throughout the body; hence, other tissue burdens can be expected to be within 5-fold of the liver, meaning that these too likely to be within the micromolar range (Brandt and Bergman, 1987; Zhao *et al.*, 2009). These estimates of PCB body burden, plus the activation data presented herein are therefore consistent with non-coplanar PCBs being able to activate PXR/CAR-dependent pathways at know human exposure levels. Indeed, Petersen et al. demonstrated that CYP3A activity was increased in Faroese adults with increased PCB exposure, which is supportive of PXR/CAR-mediated effects of PCBs occurring at physiologically relevant concentrations (Petersen *et al.*, 2007).

The differential effect of PCBs in human liver, lung and intestinal cell lines is of interest, and may be relevant to future risk assessments. The differential activation of target genes, is most probably reflective of the differential coregulator pool present in each tissue, and reflects the most efficient response to chemical challenge. For example, previous experiments demonstrated that CYP3A was induced in both rat liver and intestine following exposure to the PXR ligands dexamethasone and L742694 but that MDR1 was only increased in the intestine (Hartley *et al.*, 2004); this most probably reflects the relative importance of metabolism and transport in chemical handling within the liver and intestine. The data presented herein is consistent with such a tissue-specific induction profile, and this may be important given that oral exposure is likely to be the most common route of non-occupational PCB exposure. Exposure to PCBs via inhalation is likely to be only a minimal non-occupation exposure route, and herein we demonstrate that lung cells are relatively

insensitive to the PXR/CAR-mediated effects of non-coplanar PCBs. It should be noted that some caution must be applied when interpreting this data, as the in vitro experiments undertaken herein utilised transformed cell lines, which may not be fully representative of the in vivo tissue. However, we have selected those cell lines previously reported to be amongst the closest models of the relevant primary tissue (Lieber *et al.*, 1976; Thelen and Dressman, 2009; Lin *et al.*, 2012), which may mitigate this potential confounder to some degree.

Given the diverse functions that nuclear receptors undertake within the body in general (Bookout *et al.*, 2006), and the liver specifically (Plant and Aouabdi, 2009), it is likely that agonism of PXR and/or CAR by non-coplanar PCBs may have impacts on these systems. In particular, nuclear receptors are associated with the regulation of metabolic homeostasis, through both the action of endosensing receptors such as LXR, FXR and PPAR (Barbier *et al.*, 2002; Mohan and Heyman, 2003), and xenosensing receptors such as PXR and CAR (Bachmann *et al.*, 2004; Pascussi *et al.*, 2008; Wada *et al.*, 2009). Thus, exposure to non-coplanar PCBs may lead to chronic activation of PXR/CAR and potential dysfunction of these metabolic processes. Such effects have been reported for chronic exposure to other PXR/CAR ligands, supporting this hypothesis (Francis *et al.*, 2003; Wada *et al.*, 2009). Coupled with the reported association of coplanar PCB exposure and metabolic syndrome (Uemura *et al.*, 2009), this highlights an important area for future risk assessment.

In summary, we demonstrate that non-coplanar PCBs may act as direct activators of the nuclear receptors PXR and CAR, and that they are able to activate target gene expression in a tissue- and substituent-dependent manner. Considering that these effects occur at concentrations that have been measured in humans, and that chronic activation of PXR/CAR has been linked to metabolic dysfunction and disease, it is important that these effects are included in risk assessments.

## Conflict of Interests

None

## References


Aouabdi, S., Gibson, G. G., and Plant, N. (2006). Transcriptional regulation of PXR: Identification of a PPRE within the proximal promoter responsible for fibrate-mediated transcriptional activation of PXR. *Drug Metab. Dispos.* **34**, 138-144.

Bachmann, K., Patel, H., Batayneh, Z., Slama, J., White, D., Posey, J., Ekins, S., Gold, D., and Sambucetti, L. (2004). PXR and the regulation of apoA1 and HDL-cholesterol in rodents. *Pharmacological Research* **50**, 237-246.

Barbier, O., Torra, I. P., Duguay, Y., Blanquart, C., Fruchart, J. C., Glineur, C., and Staels, B. (2002). Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. *Arteriosclerosis Thrombosis and Vascular Biology* **22**, 717-726.

Bombail, V., Taylor, K., Gibson, G., and Plant, N. (2004). Role of Sp1, C/EBPa, HNF3 and PXR in the basal and xenobiotic-mediated regulation of the CYP3A4 gene. *Drug Metab. Dispos.* **32**, 525-535.

Bookout, A. L., Jeong, Y., Downes, M., Yu, R. T., Evans, R. M., and Mangelsdorf, D. J. (2006). Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. *Cell* **126**, 789-799.

Brandt, I., and Bergman, A. (1987). PCB methul sulfones and related-compounds - Identification of target cells and tissues in different species. *Chemosphere* **16**, 1671-1676.

Francis, G. A., Fayard, E., Picard, F., and Auwerx, J. (2003). Nuclear receptors and the control of metabolism. *Annual Review of Physiology* **65**, 261-311.

Garbow, J. R., Lin, X., Sakata, N., Chen, Z., Koh, D., and Schonfeld, G. (2004). In vivo MRS measurement of liver lipid levels in mice. *Journal of Lipid Research* **45**, 1364-1371.

Geick, A., Eichelbaum, M., and Burk, O. (2001). Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *Journal of Biological Chemistry* **276**, 14581-14587.

Ghosh, S., De, S., Chen, Y., Sutton, D. C., Ayorinde, F. O., and Dutta, S. K. (2010). Polychlorinated biphenyls (PCB-153) and (PCB-77) absorption in human liver (HepG2) and kidney (HK2) cells in vitro: PCB levels and cell death. *Environment international* **36**, 893-900.

Hartley, D. P., Dai, X., He, Y. D., Carlini, E. J., Wang, B., Huskey, S. E., Ulrich, R. G., Rushmore, T. H., Evers, R., and Evans, D. C. (2004). Activators of the rat pregnane X

receptor differentially modulate hepatic and intestinal gene expression. *Mol. Pharmacol.* **65**, 1159-1171.

Howe, K., Sanat, F., Thumser, A. E., Coleman, T., and Plant, N. (2011). The statin class of HMG-CoA reductase inhibitors demonstrate differential activation of the nuclear receptors PXR, CAR and FXR, as well as their downstream target genes. *Xenobiotica* **41**, 519-529.

Jacobs, M. N., Nolan, G. T., and Hood, S. R. (2005). Lignans, bacteriocides and organochlorine compounds activate the human pregnane X receptor (PXR). *Toxicol. Appl. Pharmacol.* **209**, 123-133.

Kester, M. H. A., Bulduk, S., Tibboel, D., Meinl, W., Glatt, H., Falany, C. N., Coughtrie, M. W. H., Bergman, A., Safe, S. H., Kuiper, G., Schuur, A. G., Brouwer, A., and Visser, T. J. (2000). Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: A novel pathway explaining the estrogenic activity of PCBs. *Endocrinology* **141**, 1897-1900.

Kopec, A. K., Burgoon, L. D., Ibrahim-Aibo, D., Mets, B. D., Tashiro, C., Potter, D., Sharratt, B., Harkema, J. R., and Zacharewski, T. R. (2010). PCB153-elicited hepatic responses in the immature, ovariectomized C57BL/6 mice: Comparative toxicogenomic effects of dioxin and non-dioxin-like ligands. *Toxicol. Appl. Pharmacol.* **243**, 359-371.

Kopec, A. K., D'Souza, M. L., Mets, B. D., Burgoon, L. D., Reese, S. E., Archer, K. J., Potter, D., Tashiro, C., Sharratt, B., Harkema, J. R., and Zacharewski, T. R. (2011). Non-additive hepatic gene expression elicited by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,2 ',4,4 ',5,5 '-hexachlorobiphenyl (PCB153) co-treatment in C57BL/6 mice. *Toxicol. Appl. Pharmacol.* **256**, 154-167.

Lieber, M., Smith, B., Szakal, A., Nelsonrees, W., and Todaro, G. (1976). Continuous tumor-cell line from a human lung carcinoma with properties of type-II alveolar epithelial cells. *International Journal of Cancer* **17**, 62-70.

Lin, J., Schyschka, L., Muhl-Benninghaus, R., Neumann, J., Hao, L., Nussler, N., Dooley, S., Liu, L., Stockle, U., Nussler, A. K., and Ehnert, S. (2012). Comparative analysis of Phase I and II enzyme activities in 5 hepatic cell lines identifies Huh-7 and HCC-T cells with the highest potential to study drug metabolism. *Archives of Toxicology* **86**, 87-95.

McFarland, V. A., and Clarke, J. U. (1989). Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. *Environ. Health Perspect.* **81**, 225-239.

Mohan, R., and Heyman, R. A. (2003). Orphan nuclear receptor modulators. *Current Topics in Medicinal Chemistry* **3**, 1637-1647.

Nakabayashi, H., Taketa, K., Miyano, K., Yamane, T., and Sato, J. (1982). Growth of human hepatoma cell lines with differentiated functions in chemically defined medium. *Cancer Research* **42**, 3858-3863.

Pascussi, J. M., Gerbal-Chaloin, S., Duret, C., Daujat-Chavanieu, M., Vilarem, M. J., and Maurel, P. (2008). The tangle of nuclear receptors that controls xenobiotic metabolism and transport: Crosstalk and consequences. *Annual Review of Pharmacology and Toxicology* **48**, 1-32.

Petersen, M. S., Halling, J., Damkier, P., Nielsen, F., Grandjean, P., Weihe, P., and Brosen, K. (2007). Polychlorinated biphenyl (PCB) induction of CYP3A4 enzyme activity in healthy Faroese adults. *Toxicol. Appl. Pharmacol.* **224**, 202-206.

Petreas, M., Nelson, D., Brown, F. R., Goldberg, D., Hurley, S., and Reynolds, P. (2011). High concentrations of polybrominated diphenylethers (PBDEs) in breast adipose tissue of California women. *Environment international* **37**, 190-197.

Plant, N. (2007). The human cytochrome P450 3A sub-family: transcriptional regulation, inter-individual variation and interaction networks. *Biochimica et Biophysica Acta* **1770**, 478-488. .

Plant, N., and Aouabdi, S. (2009). Nuclear receptors: the controlling force in drug metabolism of the liver? *Xenobiotica* **39**, 597-605.

Plant, N., Ogg, M., Crowder, M., and Gibson, G. (2000). Control and statistical analysis of in vitro reporter gene assays. *Analytical Biochemistry* **278**, 170-174.

Rudge, C. V. C., Sandanger, T., Rollin, H. B., Calderon, I. M. P., Volpato, G., Silva, J. L. P., Duarte, G., Neto, C. M., Sass, N., Nakamura, M. U., Odland, J. O., and Rudge, M. V. C. (2012). Levels of selected persistent organic pollutants in blood from delivering women in seven selected areas of Sao Paulo State, Brazil. *Environment international* **40**, 162-169.

Safe, S. (1984). Polychlorinated-biphenyls (PCBs) and polybrominated biphenyls (PBBs) - biochemistry, toxicology, and mechanism of action. *Crc Critical Reviews in Toxicology* **13**, 319-395.

Safe, S. (2001). Molecular biology of the Ah receptor and its role in carcinogenesis. *Toxicol. Lett.* **120**, 1-7.

Safe, S. H. (1994). Polychlorinated-biphenyls (PCBs) - Environmental-impact, biochemical and toxic responses, and implications for risk assessment. *Critical Reviews in Toxicology* **24**, 87-149.

Sakai, H., Iwata, H., Kim, E. Y., Tsydenova, O., Miyazaki, N., Petrov, E. A., Batoev, V. B., and Tanabe, S. (2006). Constitutive androstane receptor (CAR) as a potential sensing biomarker of persistent organic pollutants (POPs) in aquatic mammal: Molecular characterization, expression level, and ligand profiling in Baikal seal (Pusa sibirica). *Toxicological Sciences* **94**, 57-70.

Schuetz, E. G., Brimer, C., and Schuetz, J. D. (1998). Environmental xenobiotics and the antihormones cyproterone acetate and spironolactone use the nuclear hormone pregnenolone X receptor to activate the CYP3A23 hormone response element. *Mol. Pharmacol.* **54**, 1113-1117.

Suh, J. H., Kang, J. S., Yang, K. H., and Kaminski, N. E. (2003). Antagonism of aryl hydrocarbon receptor-dependent induction of CYP1A1 and inhibition of IgM expression by di-ortho-substituted polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* **187**, 11-21.

Tabb, M. M., Kholodovych, V., Grun, F., Zhou, C. C., Welsh, W. J., and Blumberg, B. (2004). Highly chlorinated PCBs inhibit the human xenobiotic response mediated by the steroid and xenobiotic receptor (SXR). *Environ. Health Perspect.* **112**, 163-169.

Thelen, K., and Dressman, J. B. (2009). Cytochrome P450-mediated metabolism in the human gut wall. *J. Pharm. Pharmacol.* **61**, 541-558.

Uemura, H., Arisawa, K., Hiyoshi, M., Kitayama, A., Takami, H., Sawachika, F., Dakeshita, S., Nii, K., Satoh, H., Sumiyoshi, Y., Morinaga, K., Kodama, K., Suzuki, T., Nagai, M., and Suzuki, T. (2009). Prevalence of Metabolic Syndrome Associated with Body Burden Levels of Dioxin and Related Compounds among Japan's General Population. *Environ. Health Perspect.* **117**, 568-573.

Wada, T., Gao, J., and Xie, W. (2009). PXR and CAR in energy metabolism. *Trends in Endocrinology and Metabolism* **20**, 273-279.

Wu, B., Zhang, Y., Kong, J., Zhang, X. X., and Cheng, S. P. (2009). In silico predication of nuclear hormone receptors for organic pollutants by homology modeling and molecular docking. *Toxicol. Lett.* **191**, 69-73.

Xie, W., Barwick, J. L., Simmon, C. M., Pierce, A. M., Safe, S., Blumberg, B., Guzelian, P. S., and Evans, R. M. (2000). Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes and Development* **14**, 3014-3023.

Zhao, G., Wang, Z., Zhou, H., and Zhao, Q. (2009). Burdens of PBBs, PBDEs, and PCBs in tissues of the cancer patients in the e-waste disassembly sites in Zhejiang, China. *Science of the Total Environment* **407**, 4831-4837.

**Figure Legends**

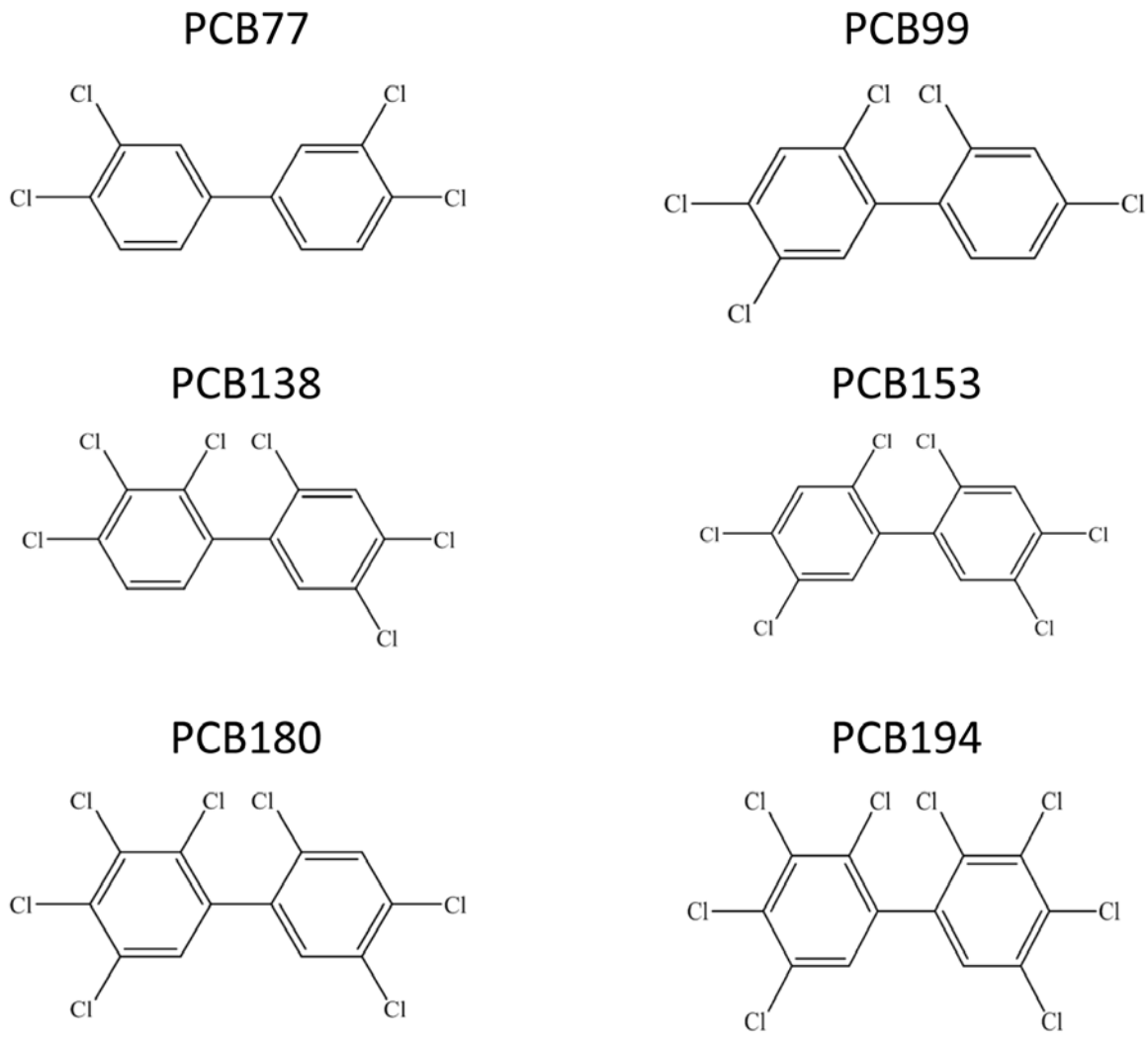


**Figure 1: Chemical structure of PCBs under study.**

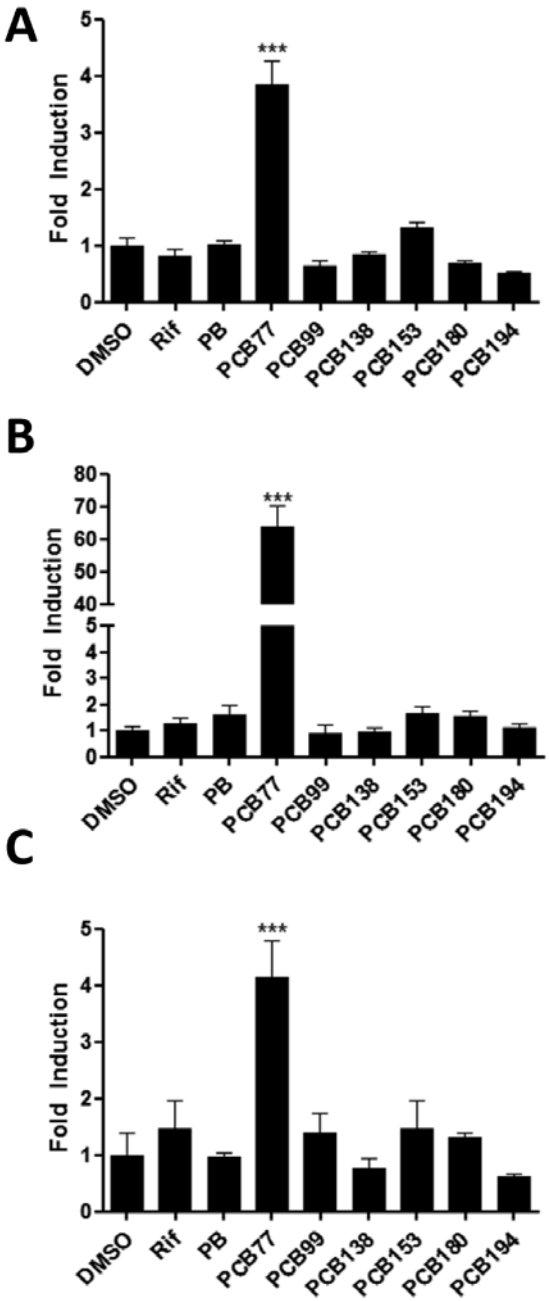


**Figure 2: Induction of CYP1A1 transcripts PCBs in liver, intestine and lung cell lines.** Huh7 (A), Caco2 (B) or A549 (C) cells were exposed to 10μM of the indicated chemical or vehicle control (0.1% DMSO) for 48 hours. Total RNA was extracted and then CYP1A1 transcript level measured by TaqMan and normalised to the 18S transcript level. Each data point represents n=6, and statistical analysis was via one-way ANOVA; ***=$p<0.001$ relative to vehicle control. Graph is representative of triplicate repeat experiments.

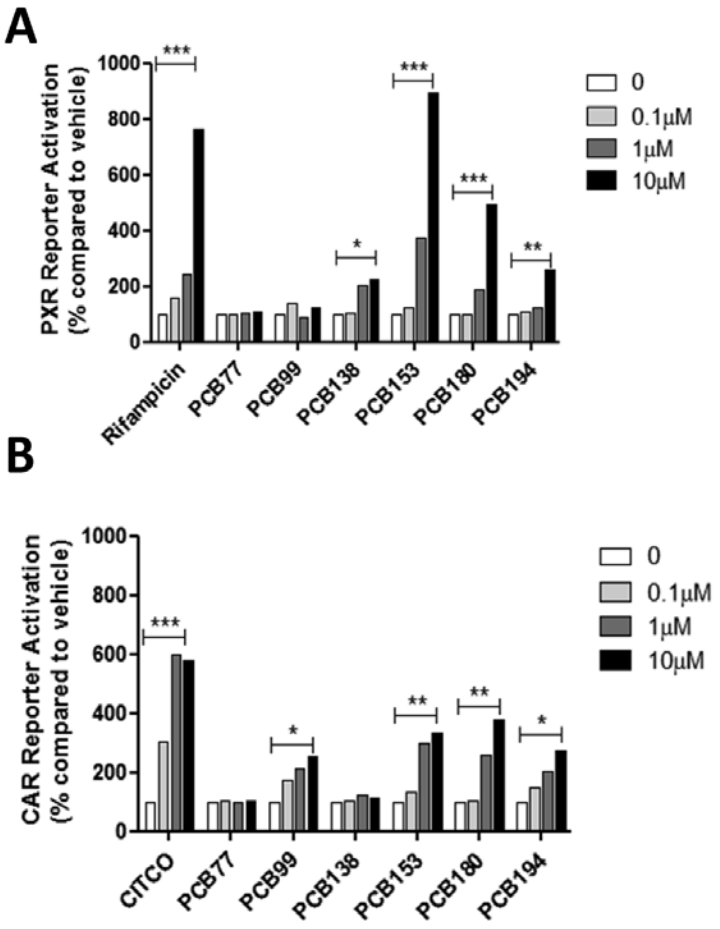


**Figure 3: Dose- and chemical-specific agonism of the nuclear receptors PXR and CAR by non-coplanar PCBs.** Huh7 cells were transiently transfected with an expression plasmid for PXR (A) or CAR (B) LBD-Gal4 DBD fusion construct, plus a luciferase reporter plasmid. Cells were then exposed to 0.1μM, 1μM or 10μM of the indicated chemical or vehicle control (0.1% DMSO) for 24 hours, and then luciferase measured. Each data point represents n=6, and statistical analysis was via one-way ANOVA; *=$p<0.01$, **=$p<0.05$ and ***=$p<0.001$ relative to vehicle control. Graph is representative of triplicate repeat experiments.

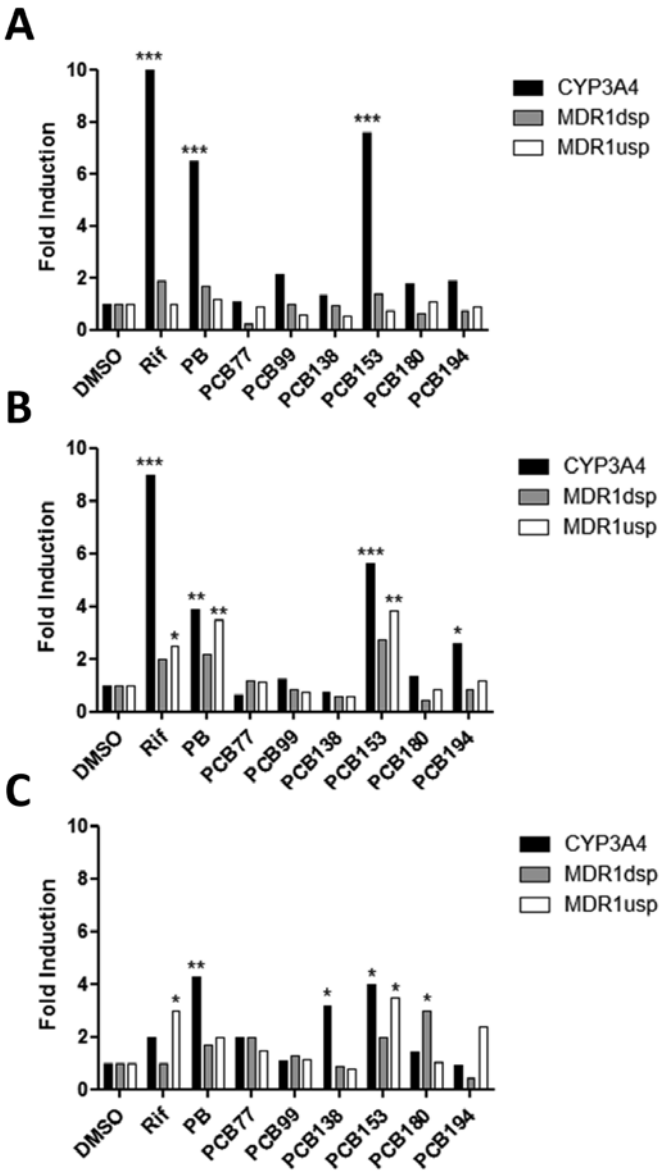


**Figure 4: Chemical-specific activation of CYP3A4 and MDR1 reporter gene expression by PCBs.** Huh7 (A), Caco2 (B) or A549 (C) cells were transiently transfected with a SEAP reporter gene under the control of the CYP3A4, MDR1 downstream (dsp) or upstream (usp) regulatory regions as indicated. Cells were then exposed to 10μM of the indicated chemical or vehicle control (0.1% DMSO) for 48 hours and SEAP activity measured, corrected for transfection efficiency, and presented as fold induction relative to vehicle control. Each data point represents n=6, and statistical analysis was via one-way ANOVA; *=$p<0.01$, **=$p<0.05$ and ***=$p<0.001$ relative to vehicle control. Graph is representative of at least triplicate repeat experiments.

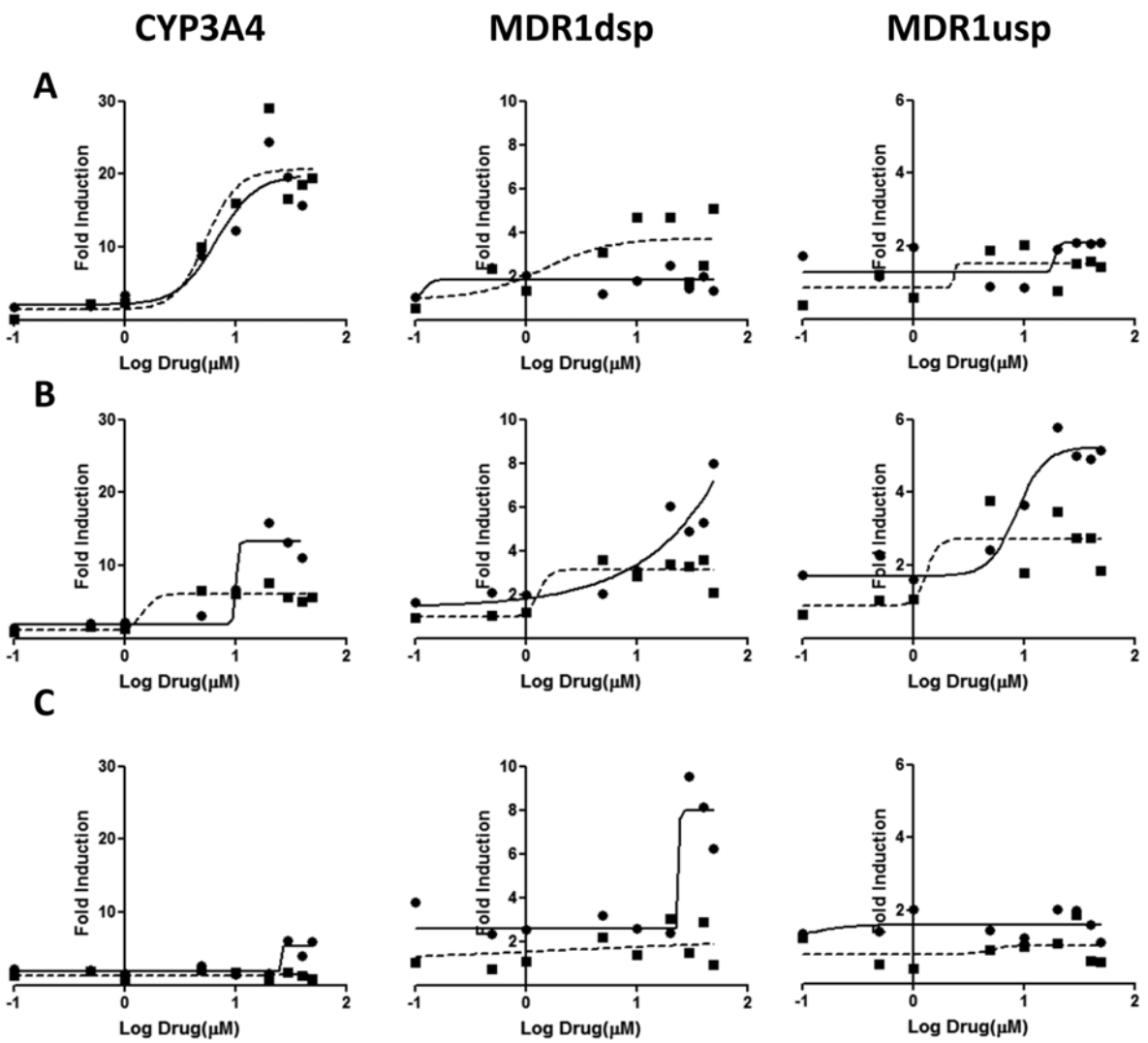


**Figure 5: Dose- and chemical-specific activation of CYP3A4 and MDR1 reporter gene expression by PCB153 and rifampicin occurs in a tissue-specificif manner.** Huh7 (A), Caco2 (B) or A549 (C) cells were transiently transfected with a SEAP reporter gene under the control of the CYP3A4, MDR1 downstream (dsp) or upstream (usp) regulatory regions as indicated. Cells were then exposed to indicated concentrations of PCB153 or rifampicin, or vehicle control (0.1% DMSO) for 48 hours and SEAP activity measured, corrected for transfection efficiency, and presented as fold induction relative to vehicle control. Each data point represents n=6, and statistical analysis was via one-way ANOVA; *=$p<0.01$, **=$p<0.05$ and ***=$p<0.001$ relative to vehicle control. Graph is representative of at least triplicate repeat experiments.