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MiniReview

Cytochrome P450 Induction and Xeno-Sensing Receptors Pregnane X Receptor, Constitutive Androstane Receptor, Aryl Hydrocarbon Receptor and Peroxisome Proliferator-Activated Receptor α at the Crossroads of Toxicokinetics and Toxicodynamics

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Abstract: Pregnane X receptor (PXR), constitutive androstane receptor (CAR), aryl hydrocarbon receptor (AHR) and peroxisome proliferator-activated receptor α (PPARα) are ligand-activated transcription factors that regulate expression of many xenobiotic-metabolizing enzymes including several cytochrome P450 (CYP) enzymes. Many xenobiotics induce CYP enzymes through these intracellular receptors and consequently affect toxicokinetics and possible metabolic activation of the receptor ligands and other xenobiotics utilizing similar metabolic pathways. However, it is now apparent that the xenobiotic receptors regulate also many endogenous functions and signalling pathways, and xenobiotic exposure thus may dysregulate an array of fundamental cell functions. This MiniReview surveys and discusses the multifaceted roles of xenobiotic receptors, for which CYP induction may serve as the first alert and possibly a biomarker for exposure to xenobiotics. With the current emergence of the adverse outcome pathway (AOP) concept, these receptors are being and will be assigned as molecular initiating events or key events in numerous discrete toxicity pathways.

The phenomenon 'induction of drug or xenobiotic metabolism'[1] was observed already more than 60 years ago as an increase in microsomal drug-metabolizing enzymes metabolizing various xenobiotics [1–4]. Soon afterwards, it was recognized that cytochrome P450s (CYPs) were the most important inducible enzymes in the endoplasmic reticulum of the liver and other tissues. Subsequently, the details of the mechanistic background of CYP induction via specific transcription factors were elucidated, firstly the aryl hydrocarbon receptor (AHR) and later the pregnane X receptor (PXR), the constitutive androstane receptor (CAR) and peroxisome proliferator-activated receptor α (PPARα) receptors. PXR, CAR, AHR and PPARα are often called 'nuclear' receptors, although AHR actually belongs to the family of basic helix/loop/helix (bHLH) receptors (bHLHe76) and only PXR, PPARα and CAR belong to the family of nuclear receptors (NR1I2 for PXR, NR1C1 for PPARα and NR1I3 for CAR), but because of the functional analogy and for purposes of this MiniReview, we shall include AHR as a nuclear receptor. Now these transcription factors are considered as the major molecular sensors for xenobiotic exposures. Traditionally, they are thought to be specialized for adjustment of the detoxication processes, but it has become increasingly apparent that they have much broader functions in various physiological processes. Consequently, their functions have to be assessed not merely in context with xenobiotic metabolism and toxicokinetics but also in the context of possible derangements of physiological functions.

An immediate incentive to write this MiniReview was based on the validation study of 'CYP induction *in vitro* method' co-ordinated by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM). In this project, the induction of four CYP-selective model enzyme reactions triggered by well-characterized inducers and non-inducers were assessed in primary human hepatocytes and HepaRG® cells. During this validation study, it became apparent that the induction phenomenon could be a potential marker

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[1]PubMed search (30 October 2017) for 'induction of cytochrome P450' resulted in about 14,500 hits and for 'cyp induction' about 2100 hits.

for physiological processes much wider than just xenobiotic metabolism. In this MiniReview, we aimed to:

1 Describe by which mechanisms receptor-controlled CYP induction can participate as an obligatory or modifying event in various chemical-initiated toxicities,
2 Survey the participation of xenobiotic receptors in regulating and potentially disturbing various physiological functions and
3 Briefly survey exogenous and endogenous compounds activating the xenobiotic receptors to illustrate the extent of various chemicals with potential to activate these nuclear receptors.

Adverse Outcome Pathway (AOP) Concept

During recent years, there have been efforts to develop integrated schemes to describe toxicity processes and effects from molecules to populations. The adverse outcome pathway (AOP) concept has emerged as a programme run by The Organisation for Economic Co-operation and Development (OECD), which aims to dissect toxicity pathways into distinct steps: the molecular initiating event (MIE), other intermediary key events (KE) and key event relationships (KER). AOP is built by organizing and weighing the evidence coming from *in silico* and *in vitro* models to ecotoxicological and human epidemiological studies. By assessing different building elements according to modified Bradford Hill criteria, including biological plausibility, dose and temporal concordance and strength of association, several AOPs were described [5–7]. An example of AOPs is provided in fig 1B. A dedicated Internet site AOPwiki already contains more than 200 AOPs, although most of them are under development and only a fraction of them have been extensively scrutinized and endorsed (http://aopkb.org/ and https://aopwiki.org/). Consequently, we have made explicit reference only to those AOPs that are published in scientific literature. In this MiniReview, we describe potential roles of nuclear receptors in various physiological/toxicological effects and reflect on the use of the AOP concept as a potential platform for linking nuclear receptor activation with toxicological effects. It is also assumed that nuclear receptor activation might be a key event in a toxicity pathway without the necessary CYP involvement.

The Central Role of Xenobiotic Receptors and CYP Induction in Toxicokinetics and Toxicodynamics

Cytochrome P450 induction was recognized for the first time while investigating carcinogenesis by various chemicals, including polycyclic aromatic hydrocarbons (PAH) [8] and drug tolerance of barbiturates and other analogous compounds [9]. Describing these observations in modern AOP terms, CYP induction, or more exactly the binding of a ligand to a nuclear receptor, could be characterized in different schemes either as a molecular initiating event (MIE) or as a modifying factor of an AOP. Ligand binding to NR is a MIE if it leads via downstream KEs to adverse outcome (AO) and this possibility will be dealt with in connection of physiological roles of NRs. In other scenarios, ligand binding to NR is not a MIE, or an intermediary KE, if it is not a part of a linear pathway. In such a case, receptor activation could be envisaged as a modifying biological event that contributes to an AOP without being a part of the AOP itself. As an example, it is amply demonstrated, as in the case of PAH carcinogenesis, that the formation of a reactive carcinogen metabolite by a CYP enzyme and the binding of a metabolite to DNA are key initial events leading to manifest cancer. In this scenario, the DNA binding of a DNA-reactive carcinogen metabolite and the ensuing DNA damage may be envisaged as a MIE for the linear AOP scheme. The induction of carcinogen-activating CYP enzymes by NRs modifies this KE/MIE in the carcinogenic process, and thus, the induction could be regarded as a modifying factor. In a case of barbiturate tolerance, the induction of metabolizing enzymes affects the concentration of a drug and consequently its binding (activation) to the $GABA_A$ receptor with a subsequent central nervous system (CNS) depression in an animal (or in human being). In these scenarios, CYP induction as such may not be regarded as a key event (KE), because it is not a KE in the sequential process leading from MIE to AO. These scenarios are presented schematically in fig. 1. On the other hand, an alternative way to interpret the above examples is to regard the activation of a nuclear receptor as a MIE and the change (increase or decrease) in the concentration of the active molecule, either DNA-reactive metabolite or receptor-activating parent drug, as a downstream KE leading ultimately to AO. Difficulties and ambiguities of incorporating metabolic processes including the formation of reactive metabolites, which usually are regarded as part of toxicokinetics and ADME, into the AOP concept have been described recently [7].

CYP3A4 is probably the most important CYP enzyme for metabolism of chemicals, and for this reason, the induction and variability of CYP3A4 are important modifying factors in a large number of chemical-induced toxicities, especially regarding pharmaceuticals [10,11]. For example, anticancer therapy-related adverse effects have been associated with CYP3A4 variability due to induction and genetic polymorphisms in breast, prostate, gynaecological and colorectal cancers [12–14], and consequently, the interest of using nuclear receptor activation and target gene induction as biomarkers for revealing potential risks and for the translation into successful treatments of diseases has grown during the recent years [15].

Endogenous compounds.

A large number of endogenous compounds, for example steroid hormones, cholesterol derivatives and bile acids, are substrates for xenobiotic-metabolizing enzymes, but it should be remembered that the majority of CYPs associated with the synthesis of steroids and many other endogenous substances are distinct from 'xenobiotic CYPs' [16]. Consequently, nuclear receptor-mediated regulation of metabolism of endogenous substrates has received increased attention as a potential mechanism affecting homoeostasis. Severe derangements of the homoeostasis may lead to adverse effects. CYP enzymes

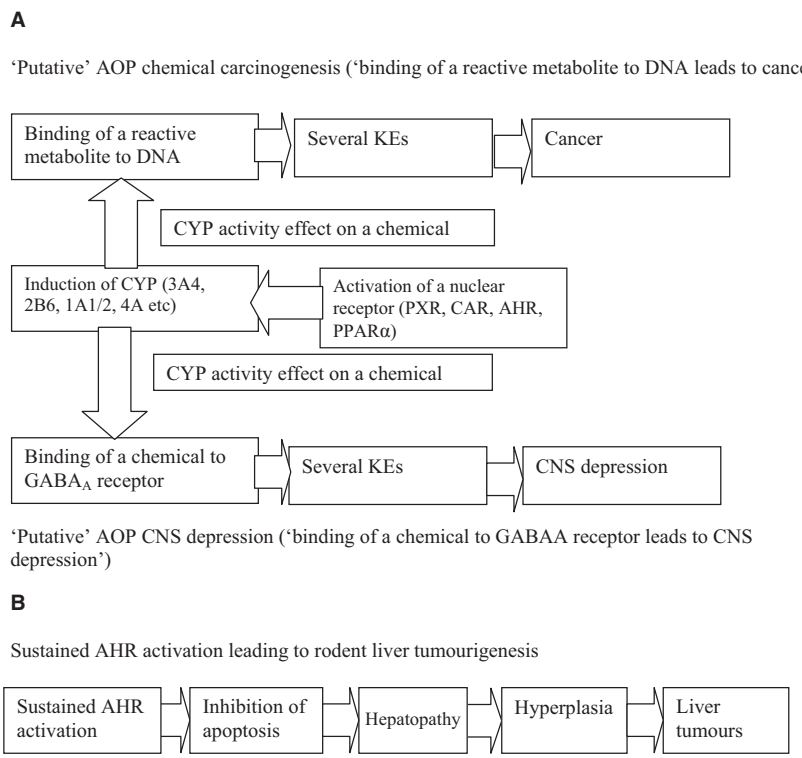

Fig. 1. Examples of CYP induction as a significant factor in chemical adversities. (A) Depicts putative adverse outcome pathways (AOP) for chemical carcinogenesis [1,2] and barbiturate tolerance [3,4]. In these cases, CYP induction leads to an increase or decrease in the concentration of a chemical bound to the receptor. (B) Depicts a published AOP 'sustained AHR activation leading to rodent liver tumourigenesis' [54]. In this AOP, 'sustained AHR activation' is MIE and 'rodent liver tumourigenesis' is AO, and the intermediary steps are KEs.

have a key role in the metabolism of several endogenous hormones including steroid hormones (e.g. oestradiol, progesterone, testosterone), and their synthetic derivatives and endocrine-disrupting chemicals are capable of inducing CYP enzymes perturbing the normal steroid metabolism [17–19]. It is obvious that severe disturbances in physiological hormone levels could lead to variable adverse effects. Another example is related to cholestatic liver injury: PXR binds lithocholic acid, a secondary bile acid which is rather toxic to the liver, and causes induction, which triggers the protective mechanism towards the toxicity of lithocholic acid and its metabolite [20,21].

Thyroid disruption.

Together with other endocrine disruptors, compounds affecting the thyroid axis ('thyroid disrupters') have recently received increased attention. In EU, the EURL ECVAM has launched a validation study to assess *in vitro* methods for the detection of chemicals which disrupt thyroid function. Based on the OECD detailed review article on 57 thyroid hormone disruption assays [22], EURL ECVAM has identified 17 *in vitro* methods as candidates for this validation study in collaboration with EU-NETVAL. The well-performing methods may be selected for further assessment with a view to their eventual use in a regulatory context. There are a number of potential targets leading to thyroid disruption, for example those associated with thyreotropin and thyroid gland processes and those involved in thyroid hormone synthesis, catabolism, kinetics and actions. Regarding the topic of this MiniReview, it is amply demonstrated that the levels of principal thyroid hormones, thyroxine (T4) and triiodothyronine (T3) are decreased by the PXR-, CAR-, AHR- and PPARα-associated induction of some phase II (especially sulphotransferases (SULT) and UDP-glucuronosyltransferases (UGT)) enzymes in the liver [17,23,24], resulting in relative tissue and/or circulating T3/T4 deficiency and its possible adverse sequel. As an example, triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether) decreases rat serum thyroxine via nuclear receptor interaction(s) and subsequent induction of hepatic thyroxin clearance. *In vitro* studies indicate that triclosan interacts with multiple nuclear receptors, including human PXR and human CAR, in addition to rat CAR [25]. An *in vitro* method measuring CYP induction as part of the mechanism of action (MoA) battery of chemicals that interfere with the thyroid signalling pathway could provide a warning signal about the possibility of liver–thyroid axis disruption. Appropriate AOPs to capture essential processes are under development (see AOP Knowledge Base: http://aopkb.org/ and https://aopwiki.org/).

The Role of Xenobiotic Receptors as Key Events in Signalling Pathways with Potentially Adverse Consequences

As stated above, CYP induction *per se* is just an increase in CYP enzyme activity, which then leads to an increase in

metabolism of a compound. While this in general enhances detoxication, in certain situations, the induction may ultimately lead to enhanced toxicity (metabolic activation). A well-known textbook example is paracetamol and liver injury [26]. These toxicokinetic consequences of CYP induction are fully integrated into drug development and into some areas of clinical drug treatment, for example prevention of potential drug interactions based on CYP induction [27,28]. Another scenario is the participation of nuclear receptors in physiological regulatory processes, for example proliferation and differentiation (AHR), glucose tolerance and energy balance (PXR) and gluconeogenesis, lipid metabolism (PPARα), bile acids homoeostasis and hormonal regulation (CAR). In context of these fundamental cellular pathways, nuclear receptor activation is a genuine MIE, which initiates a process potentially leading to adverse outcome. In these cases, the induction of CYPs may be observed (if it is at all measured), but here it represents just a biomarker for nuclear receptor activation triggering a pathway leading to potentially adverse consequences.

It is of importance to remember that nuclear receptors do not cause their effects in mechanistic isolation. Downstream processes initiated by nuclear receptor activation are triggered by multicomponent protein complexes [29]. There are large numbers of co-effectors, co-activators and co-repressors participating in the regulatory process, which ultimately results in an up-regulation (or down-regulation, for that matter) of target gene transcription. Cell- or tissue-specific differences in the regulatory machinery may be of importance in the final outcome, for example when studying induction responses in various cell lines even if they are derived from the same tissue or cell type [29].

The Role of PXR in Potential Adverse Effects

As stated above, PXR is a key regulator of xenobiotic metabolism including many CYPs and phase II enzymes (UGTs, SULTs, etc.) and consequently of utmost importance for instituting efficacious drug treatment and preventing chemical toxicities. In addition, PXR binding with exogenous and endogenous molecules has been shown to up- or down-regulate several metabolic and signalling pathways associated with energy homoeostasis, bile acid metabolism, cell cycle, proliferation and inflammation [30–33]. Some examples that are more extensively researched are shown in table 1. It has been stressed that in many cases, detailed mechanisms and pathways have not been adequately elucidated and further research will show the significance of these observations in drug treatment or adverse reactions. Also, many of these findings have been interpreted preferably from the point of view of novel therapeutic options and drug development. Potential toxicological implications have remained less well developed. However, PXR activation and ensuing induction of CYPs and transporters have been incorporated into AOPs dealing with liver diseases, including cholestasis, steatosis and fibrosis [34–37]. For example, in cholestatic liver diseases or drug-induced injuries, it is implied that the role of PXR is more linked with changes in concentrations of endogenous molecules such as specific bile acids [35].

The potential role of PXR activation in toxicological effects associated with energy metabolism has attracted significant attention during the last few years and PXR has been suggested as a potential mediator of endocrine-disrupting (or more specifically metabolism-disrupting) effects of chemicals [38,39]. Several studies have observed changes in lipid and glucose metabolism after treatment with PXR ligands, and also, the PXR knockout has metabolic effects [33]. However, many controversies remain in the field and the studies have often produced conflicting results in different models. Obviously, *ex vivo* and cell culture models often do not continue to express 'physiologically normal' levels of these nuclear receptors and/or their targets. Moreover, a majority of studies have been carried out in rodents and much less is known of the metabolic effects of PXR activation in human beings. Recently, Spruiell *et al.* [40,41] demonstrated that human PXR gene promotes glucose intolerance in a transgenic PXR-humanized mouse model fed with high-fat diet; however, the effect on obesity was gender-specific. Treatment of human volunteers with rifampicin for one week impaired glucose tolerance as observed in oral glucose tolerance test, suggesting prodiabetogenic effect [42]. Also a clinical study using another PXR activator, St. John's wort, observed impaired glucose tolerance after 21 days treatment with the herbal product [43]. Thus, in human beings, PXR activation appears detrimental for metabolic health. Several potential PXR target genes relevant for glucose metabolism and lipid synthesis have been identified and may be regarded as potential KEs, although the causality for the AOPs may not be fully confirmed.

It is worth of mentioning that thus far, only a few examples of the role of PXR in AOPs can be found in the AOP Knowledge Base (http://aopkb.org/ and https://aopwiki.org/). One of the reasons may be that toxic effects based on the PXR-regulated xenobiotic metabolism – for example those based on drug–drug interactions or formation of reactive intermediates – are extensively researched and widely recognized especially regarding pharmaceuticals and clinical medicine. As described above, some other potentially significant effects on physiological processes such as energy homoeostasis or cell cycle and proliferation have been found more recently, and it could be just a matter of time when appropriate tentative AOPs will emerge.

The Role of CAR in Potential Adverse Effects

The targets and ensuing regulatory functions of CAR resemble in many ways those of PXR, although conspicuous differences exist. A potentially significant difference between CAR and PXR is related to the constitutive activity of CAR without a ligand; thus, potential ligands could be agonists or inverse agonists as well as antagonists. CAR activation is further complicated by indirect activators, that is compounds such as phenobarbital which do not bind to the receptor itself, but still activate it via other mechanisms [29].

Table 1.
The role of PXR ligand interactions in physiological functions and signalling pathways.

Pathway/biological process	Examples of target gene	Mechanism	Function/adverse effect	References[1]
Energy homoeostasis				
Gluconeogenesis	*G6PC* *PCK1*	Repression of FOXO1 and CREB	Gluconeogenesis ↓	Kodama, Koike *et al.* (2004), Kodama, Moore *et al.* (2007)
Hepatic glucose transport	*SLC2A2 (GLUT2)*	Repression	Glucose uptake ↓	Rysä, Buler *et al.* (2013)
Lipogenesis	*ELOVL6* *CD36* *LPIN1* PPARG	Direct induction	Hepatic steatosis ↑	Zhou, Zhai *et al.* (2006), He, Gao *et al.* (2013), Zhou, Febbraio *et al.* (2008)
β-oxidation	*CPT1A*	Repression of FOXA2	Hepatic steatosis ↑	He, Gao *et al.* (2013)
Ketogenesis	*HMGCS2*	Repression of FOXA2	Hepatic steatosis ↑	Nakamura, Moore *et al.* (2007)
Bile acid metabolism				
Bile acid synthesis	*CYP7A1* *CYP8B1*	Interaction with PGC1α	Bile acid synthesis ↓	Bhalla, Ozalp *et al.* (2004)
Bile acid catabolism and transport	*CYP2B* *CYP3A* *ABCC3*	Direct induction	Bile acid catabolism and transport ↑	Wagner, Halilbasic *et al.* (2005)
Cell cycle and proliferation				
Proliferation	*CDKN1A (p21)* *CDKN1B*	Repression of FOXO3	Proliferation or antiproliferation depending on the context	Shizu, Abe *et al.* (2016), Ouyang, Ke *et al.* (2010)
Cell migration	*GADD45B* *IGFBP1*	Direct induction Repression of HNF4α	Changes in cell morphology and migration	Kodama, Negishi (2011), Kodama, Yamazaki *et al.* (2015)
Apoptosis	*BCL2* *TP53 (p53)*	Induction Reduced p53 transactivation	Anti-apotosis, promotion of malignant phenotype	Zucchini, de Sousa *et al.* (2005), Robbins, Cherian *et al.* (2016)
Inflammation				
	NFKB1 (NF-κB) *TLR4*	Interaction with NF-κB Repression of TLR4 pathway	Anti-inflammation, regulation of innate immunity	Qiu, Cervantes *et al.* (2016), Zhou, Tabb *et al.* (2006), Gu, Ke *et al.* (2006)

[1]Reference list in the Appendix S1.

Like PXR, CAR is an important regulator of xenobiotic metabolism and transport, and furthermore, it has a role in bile acid synthesis and catabolism, thyroid hormone homoeostasis, glucose and lipid metabolism as well as in apoptosis, cell cycle and proliferation (table 2). Although the target genes have been identified relatively convincingly, relationships of more distal cellular effects such as apoptosis or senescence with toxicologically significant adverse effects are less clear. Despite the significant similarities in the functions of PXR and CAR, there appears to be important differences in effects of the activation. For example, CAR activation ameliorates diabetes and fatty liver disease, while PXR activation has a detrimental effect [42,44,45]. CAR activation and ensuing induction of UGTs occurring in rats have been shown to lead to thyroid tumours as a consequence of prolonged liver–thyroid axis disturbance following increased T4 (and T3) elimination by the liver and subsequent thyroid stimulation by TSH [46]. This effect was not seen in mice *in vivo* and *in vitro* [23,47], highlighting species-specific differences. It has been reported that CAR (and PXR) activation leads to the up-regulation of hepatic uptake transporters Oatp (SLCO1A2) and Oatp2 (SLCO1B1) together with the up-regulation of CYPs and UGTs [48], accounting for increased metabolic clearance of thyroid hormones.

Thus far, only a couple of examples of the role of CAR in AOPs can be found in the AOP Knowledge Base (http://aopkb.org/ and https://aopwiki.org/).

The Role of AHR in Potential Adverse Effects

The specific role of AHR in the regulation of CYP1 and some other xenobiotic-metabolizing enzymes by polycyclic aromatic hydrocarbons became apparent in the 1970s and 1980s [49]. With a few exceptions [50], AHR seems to have a smaller role in regulating other CYPs, as compared especially with PXR, but regulates a number of important phase II enzymes such as specific UGT, SULT and aldo-keto reductase (AKR) enzymes [51]. In any case, AHR is an important mediator of various cancers in experimental animals and its role in human cancers presumably caused by polycyclic aromatic hydrocarbons and analogous compounds has been proven quite convincingly [48]. Furthermore, the toxicology of dioxins and polychlorinated biphenyls and related compounds has been largely linked with AHR. Additionally, AHR has been suggested as a target for potential anticancer therapies [52]. Since the 1990s, the participation of AHR signalling pathway has been demonstrated for several endogenous functions and processes, from reproduction to senescence [49], and some examples are shown in table 3.

Table 2.
The role of CAR in physiological functions and signalling pathways.

Pathway	Examples of target gene	Mechanism	Function/adverse effect	References[1]
Energy homoeostasis				
Gluconeogenesis	*G6PC*	Repression of	Gluconeogenesis ↓	Kodama, Koike *et al.* (2004),
	PCK1	FOXO1 and HNF4		Miao, Fang *et al.* (2006)
Lipogenesis	*INSIG1*	Induction	Hepatic steatosis ↓	Roth, Looser *et al.* (2008),
	SULT2B1	Induction	LXR activation ↓	Dong, Saha *et al.* (2009)
Cholesterol and bile acid metabolism				
Cholesterol synthesis	*HMGCR* and several other cholesterol synthesis genes	Induction	Hepatic cholesterol ↑	Kobayashi, Hashimoto *et al.* (2015)
Bile acid synthesis	*CYP7A1*	Induction	Changes in bile acid	Protection against bile
	CYP8B1	Repression	composition	acid-induced liver injury
Bile acid transport	*ABCC2*	Induction	Bile acid efflux ↑	
	ABCC4			
Cell cycle and proliferation				
Apoptosis	*GADD45B*	Induction, interaction with GADD45B	Apoptosis ↓	Yamamoto, Moore *et al.* (2010)
	MCL1	Induction	Apoptosis ↓, liver injury ↓	Baskin-Bey, Huang *et al.* (2006)
Proliferation	*FOXM1*	Induction	Proliferation ↑	Dong, Lee *et al.* (2015)
	MDM2	Induction	Cell senescence ↓	
Cell cycle	*MIR122 (miR-122)*	Repression of HNF4α	E2F1 ↑	Kazantseva, Yarushkin *et al.* (2015)

[1]Reference list in the Appendix S1.

Table 3.
Examples of the participation of AHR signalling pathway in various physiological processes [49,66].

Pathway	Examples of target genes	Mechanism	Function/adverse effect	References[1]
Energy homoeostasis				
	FGF21	Transcriptional regulation, effect context specific	Insulin resistance, liver steatosis	Cheng, Vispute *et al.* (2014), Lu, Yan *et al.* (2015), Girer, Murray *et al.* (2016)
	TIPARP	Induction	Suppression of gluconeogenesis, NAD^+ level ↓	Diani-Moore, Ram *et al.* (2010)
Skin functions				
Melanocyte proliferation	*KITLG*	Not known	Tanning	Jux, Kadow *et al.* (2011)
Sebaceous gland differentiation	*PRDM1 (BLIMP1)*	Induction	Chloracne	Ikuta, Ohba *et al.* (2010), Bock (2016)
Cell cycle and proliferation				
Cell cycle	RB1 (pRB)	Interaction	Cell cycle arrest	Ge, Elferink (1998)
Differentiation and tissue regeneration	CTNNB1 (β-catenin)	Promotion of proteasomal degradation	Control of cell renewal/carcinogenesis	Kawajiri, Kobayashi *et al.* (2009)
Immunity				
T cell differentiation	*FOXP3*	Induction	Treg differentiation	Quintana, Basso *et al.* (2008)
	STAT1	Interaction	T_H17 cell development, IL22 production	Quintana, Basso *et al.* (2008), Veldhoen, Hirota *et al.* (2008), Kimura, Naka *et al.* (2008)
	MAF (c-Maf)	Interaction	Tr1 cell differentiation	Apetoh, Quintana *et al.* (2010)
Oxidative stress defence and hypoxia signalling				
	NFE2L2 (NRF2)	Induction	Antioxidative response	Miao, Hu *et al.* (2005)
	HIF1A	Competition for ARNT?	Disturbance of hypoxia signalling	Vorrink, Domann (2014)

[1]Reference list in the Appendix S1.

Recently, a central role of AHR activation in various toxicity effects has been envisaged, and a potential of AOP concept in analysing the significance of these effects has been suggested [53]. For example, there is strong evidence that sustained AHR activation lasting weeks or months is the MIE for rodent liver tumour promotion and the ensuing liver tumours

Table 4.
Examples of the participation of PPAR α signalling pathway in various physiological processes.

Pathway	Examples of target genes	Mechanism	Function/adverse effect	References[1]
Energy homoeostasis				
	SREBF1 (SREBP-1c) *JUN (c-Jun)*	Transcriptional regulation	Liver steatosis and hypertriglycemia	Yan *et al.* (2014), Lu *et al.* (2014), Mandard *et al.* (2004)
Cell cycle and proliferation				
Cell cycle	MKI67 (Ki-67)	Interaction	Hepatocellular hyperplasia	Maronpot *et al.* (2010), Zhang *et al.* (2014)
Apoptosis	*CASP3* *CASP7*	Inhibition	Hepatocellular hyperplasia, hepatocarcinoma	Misra *et al.* (2013), Mandard *et al.* (2004)
Inflammation	*IL6* *TNF (TNF-α)* *NFKB1 (NF-κB)*	Transcriptional regulation	Tumour growth	Misra *et al.* (2013), Zhang *et al.* (2014)
Oxidative stress				
	ACOX1 β-oxidation system	Induction	Peroxisome proliferation Antioxidative response Cell damage hepatomegaly	Misra *et al.* (2013), Shelby and Klaassen (2006), Guyton *et al.* (2009), Reddy (2004), Mandard *et al.* (2004)

[1]Reference list in the Appendix S1.

[54]. It is not known whether CYP induction and the following metabolic changes are required for this adverse outcome. Because of large interspecies differences, the significance and applicability of this AOP in human situation may be doubtful. Recent attempts to address the issue of species differences include development of humanized animal models. For example, a humanized mouse strain in which 31 mouse CYPs of families 2C, 2D and 3A were replaced by the major corresponding human counterparts, and PXR and CAR were humanized as well, may represent an interesting solution for the species differences [55].

Perhaps because of a longer history of AHR research, AHR activation as a MIE or KE can be found in several AOPs, which are currently under development or under review (http://aopkb.org/ and https://aopwiki.org/).

The Role of PPARα in Potential Adverse Effects

Peroxisome proliferator-activated receptor α is another member of the nuclear receptor family that has been implicated in the regulation of biotransformation enzymes [51,56]. PPARα induces expression of CYP4A in response to a heterogeneous group of peroxisome proliferators, agents such as hypolipidemic drugs, phthalates or herbicides [57]. Activation of PPARα has been demonstrated for a number of target genes participating in energy homoeostasis, cell cycle and proliferation and oxidative stress (table 4). Peroxisome proliferators produce a transient hepatocellular hyperplasia with subsequent hepatocellular hypertrophy. A combination of prolonged oxidative damage, due to peroxisome proliferation-related hydroperoxide formation and hepatocellular proliferation, is postulated to be responsible for liver tumour development [58]. However, liver tumours after PPARα ligand exposures are regarded specific to rodents and thus probably devoid of human relevance [59,60]. Additionally, prolonged activation of PPARα has been shown to induce disturbance of the liver–thyroid axis due to UGT induction and consequent thyroid hypertrophy, hyperplasia and tumour development [52,61]. As mentioned earlier, a humanized transgenic mouse model may be one of the tools to study such species differences [55], in addition to primary hepatocytes from human beings and rodents.

Currently, there are a few examples of the role of PPARα as a MIE or KE in AOPs under development in the AOP Knowledge Base (http://aopkb.org/ and https://aopwiki.org/).

Ligands for Nuclear Receptors – A Potential for Adversities

There are a huge number of chemicals identified as potential ligands for PXR. Shukla *et al.* [62] profiled 2816 clinically used pharmaceuticals for their ability to activate human PXR and induce human CYP3A4 at the cellular level and bind human PXR at the protein level. From 7 to 11% of drugs were identified as active across the three assays, which included assay-specific and pan-active compounds. A recent computational study [63] developed QSAR models based on *in vitro* PXR binding and activation, and CYP3A4 induction of pharmaceutical database and used these QSAR models to predict 'PXR binders' among the REACH database of >70 000 molecules. Approximately 5-15% of the chemicals were predicted to be 'PXR binders'. Naturally, only a small proportion of these compounds are high-affinity ligands [62] and the potential risk is dependent on real exposures.

It is possible that roughly similar numbers and shares of compounds with receptor affinity exist also for CAR [29,64], AHR [49] and PPARα [56], although the CAR ligand-binding pocket is smaller and less flexible than that of PXR accommodating perhaps a smaller number of chemicals [65]. However, even if large numbers of compounds are found in *in vitro* studies and cellular models, far less are found in *in vivo* animal experiments and there are only a handful of inducers confirmed in human beings. It is clear that *in vitro* model systems are not equivalent to, and cannot fully recapitulate, *in vivo* animal or human studies. Practically, all well-characterized human inducers are pharmaceuticals, for obvious reasons.

Thus, in the EURL ECVAM validation exercise, only drugs were tested as reference chemicals, because, for ethical reasons, it is difficult to obtain reliable human *in vivo* data on any other classes of chemicals.

Conclusions

With xenobiotic exposures, receptor activation controlling CYP induction is an adaptive response and, as such, CYP induction is an appropriate biomarker for informing about potential toxicokinetic and toxicodynamic alterations. In this context, CYP induction can be used in conjunction with other methods to show whether toxicity of a chemical is increased or decreased.

In physiological and potentially toxicological processes not directly involving induction of xenobiotic-metabolizing enzymes, CYP induction may serve as a biomarker of receptor activation, although in such a case the role of receptor activation is an initial factor in a specific signalling cascade for which the CYP induction as such has no role to play. In this case, further confirmation of the connection between a biomarker (CYP induction) and the receptor activation-mediated toxicity pathway is required. It is possible that different downstream processes with various target genes require different sets of cofactors. In the case of xenobiotic receptor-regulated target pathways, the role of CYP induction should be further elucidated.

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Disclosure

All authors state that they have nothing to disclose.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. List of references for tables 1–4.