



Editorial overview: The aryl hydrocarbon (Ah) receptor: From toxicology to human health

Michael S. Denison and Martin van den Berg

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Michael S. Denison

Dr. Michael S. Denison received a Ph.D. in Environmental Toxicology from Cornell University in 1983 and did postdoctoral research in Clinical Pharmacology at the Hospital For Sick Children in Toronto and in Molecular Pharmacology at Stanford University. In 1992, he joined the University of California, Davis, where he is a professor in the Department of Environmental Toxicology. Dr. Michael S. Denison's long-term research focus has been on the biochemical and molecular mechanisms by which the Ah receptor (AhR) mediates the biological/toxicological actions of dioxin-like chemicals and other classes of AhR ligands. He has been investigating the structural diversity of AhR ligands, the molecular interactions of these diverse ligands with residues within the AhR ligand binding domain that lead to activation/inhibition of the AhR and AhR signal transduction and other fun things. His laboratory developed a series of recombinant cell-based bioassays (the so-called CALUX bioassays) that are widely used for high-throughput screening analysis for AhR ligands and the detection and relative quantitation of dioxins and dioxin-like chemicals in extracts of diverse matrices.

Martin van den Berg

Martin van den Berg (1953, The Netherlands) is a professor of toxicology at the Utrecht University (The Netherlands). During the last decades his areas of research include: toxicokinetics, metabolism, reproductive and interactive effects of POPs, effects on steroid hormone synthesis and their relation with hormone dependent tumors, development of in vitro assays to detect endocrine disruptors. He has also been acting as an advisor or chair of several WHO, IARC, EU and US committees dealing with the (environmental) health effects of POPs and endocrine disruptors.

Early studies examining the biochemical and toxicological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related chemicals led to the identification of the aryl hydrocarbon receptor (AhR), a soluble cytosolic protein that binds TCDD specifically and with high affinity. A combination of biochemical, genetic and structure activity relationship studies subsequently provided strong support for a role of the AhR in mediating the diverse spectrum of effects produced by TCDD and related halogenated aromatic hydrocarbons. While the subsequent cloning and characterization of the AhR revealed that it was a ligand-dependent transcription factor and provided details of its detailed molecular mechanism(s) of action, it was the lack of biological and toxic effects of TCDD in AhR knockout mice that definitively demonstrated its role in mediating the action of these chemicals. Since that time, the number of publications on the AhR, AhR signal transduction mechanisms and the effects of AhR ligands have increased exponentially. Beyond new insights into the diversity of AhR ligands, the molecular mechanisms of AhR action, and species differences in AhR response, the AhR has been shown to play key role in a variety of developmental and physiological processes and human diseases. These advances have now led to the identification of the AhR as a target for the development of novel therapeutic agents for a variety of human diseases. In this special issue of *Current Opinion in Toxicology*, “The Aryl Hydrocarbon (Ah) Receptor: From Toxicology to Human Health”, world leading experts highlight the current state of knowledge of the AhR, AhR ligands and AhR signal transduction pathways, the role of the AhR in endogenous physiological processes, the adverse and beneficial effects of exogenous and endogenous AhR ligands and the more recent development of AhR-based therapeutic agents.

While understanding the mechanisms and effects of toxic chemicals is important from a potential preventative and therapeutic perspective, it can also reveal previously unknown biological or physiological pathways. Studies into the basis and mechanism of TCDD toxicity and what was responsible for the dramatic differences in TCDD sensitivity across a broad spectrum of species is one such example. How such studies led to the identification of the AhR and the elucidation of its diverse spectrum of biochemical and toxic mechanisms of action is presented in an article by Linda Birnbaum [[10.1016/j.cotox.2017.01.009](http://dx.doi.org/10.1016/j.cotox.2017.01.009)]. The ability of TCDD and other AhR ligands to produce biological and/or toxicological effects in a wide variety of species is not surprising given that phylogenetic analysis suggesting that the AhR is an ancient and evolutionary well conserved protein. The article by Mark Hahn *et al.* [[10.1016/j.cotox.2017.02.003](http://dx.doi.org/10.1016/j.cotox.2017.02.003)] describes the evolution of the AhR and suggests that an AhR homolog was

present in organisms approximately 600 million years ago. At least five different classes of AhR-like proteins have evolved in vertebrates and a given species can contain multiple AhRs or AhR-like proteins that exhibit individual differences in ligand binding, ligand specificity and functional activity. While understanding the similarities and differences in the functional activity of these proteins will likely provide further insights into the diversity of AhR signal transduction, the ability of the AhR and AhR-like proteins to modulate gene expression via distinctly different mechanism has also been observed. The article by Eric Wright *et al.* [[10.1016/j.cotox.2017.01.001](#)] describes the current state of knowledge regarding the canonical and non-canonical AhR mechanisms of gene regulation. It also highlights the functional activity of the newly identified AhR:KLF6 heterodimer. In addition to multiple signalling mechanisms, the ability of very structurally diverse ligands to bind and activate an AhR response is notable. Furthermore, the broad range ligand- and species-selective differences suggest additional levels of regulation that can contribute to the diversity in AhR response. The article by Michael S. Denison and Samantha Faber [[10.1016/j.cotox.2017.01.006](#)] clearly highlights the diversity in AhR ligands and ligand-dependent AhR responses and it also discusses multiple mechanisms that can contribute to ligand-specific differences in AhR response. While the lack of experimentally determined structures of functional domains of the AhR has hampered detailed analysis of the molecular mechanisms of AhR activation, molecular modelling approaches have provided new insights. The current state-of-the-art of molecular modelling of functional domains of the AhR, the analysis of ligand binding by docking approaches and more recent structural information of AhR related proteins is providing approaches to study ligand-dependent AhR transformation and DNA binding, which is discussed in the article by Laura Bonati *et al.* [[10.1016/j.cotox.2017.01.011](#)]. Such experimental approaches will certainly help to elucidate key structural differences that will contribute to our understanding of species differences in AhR ligand selectivity and ligand specific responses.

The ability of TCDD and related TCDD-like chemicals to bind to and activate the AhR and produce AhR-dependent toxicity is well established. This led to the development of toxic equivalency factors (TEFs), values that are used internationally for human risk assessment to predict the toxicity of mixtures of TCDD-like compounds. However, the majority of the TEFs are based on *in vivo* rodent studies. Considering documented differences in ligand specificity and relative effect potencies (REPs) between rodent and human AhRs, there are significant uncertainties in the predictive nature of rodent TEFs for effects in humans. Focusing on dioxin-like polychlorinated biphenyls (DL-PCBs), the article by van Majorie Duursen *et al.* [[10.](#)

[1016/j.cotox.2017.01.005](#)] reviews the TEF approach, its application and limitations with respect to human health effects for these compounds. Moreover, it discusses the impact that ligand- and species-specific differences in human and rodent AhRs and other pathways have on overall estimation of the toxic potency of these chemicals to humans. While a significant amount of information is known about the role that the AhR plays in regulation of gene expression, understanding of the mechanisms by which the AhR mediates the toxicity of TCDD and TCDD-like chemicals lags far behind. Recent advances in our understanding of AhR-dependent hepatotoxicity mechanisms have come from analysis of TCDD-mediated differential gene expression networks and phenotypic analysis. These aspects are discussed further in the article by Kelly Fader and Timothy Zacharewski [[10.1016/j.cotox.2017.01.010](#)]. TCDD-dependent, AhR-mediated toxicity is proposed to result from a collective response to cumulative changes in metabolic reprogramming. This involves multiple pathways and variations in effects and is suggested to contribute to documented species and tissues differences in response. Although the ability of the AhR to bind and be activated by structurally diverse ligands, in mammals, AhR-dependent toxicity is observed only with metabolically persistent ligands (e.g. TCDD and TCDD-like chemicals). The reasons for this differential response have remained an open question for many years. The article by Jason Matthews [[10.1016/j.cotox.2017.01.013](#)] discusses a new negative feedback loop involving an AhR induced gene product, the TCDD-inducible poly-ADP-ribose polymerase (TIPARP). This protein not only appears to negatively regulate ligand activated AhR activity, but TIPARP is also linked to AhR-dependent toxicity. It was found that the loss of TIPARP results in enhanced ligand-inducible and AhR-dependent toxicity. Based on these observations, the further analysis of the molecular mechanism of TIPARP action may provide a new and novel avenue in which key regulatory events in the molecular mechanisms of AhR-dependent toxicity should be investigated.

Perhaps the most exciting recent development in AhR biology is the demonstration of its role in modulating/mediating a variety of endogenous physiological processes and signalling mechanisms. This may suggest that some of the adverse effects of TCDD and related AhR ligands may result from disruption of normal AhR functions. As such, increased understanding the role of the AhR in normal physiology is providing new avenues of study into how activation or inhibition of AhR can produce its diverse spectrum of biological and toxicological effects. The identification of a variety of indole-containing and/or tryptophan-derived AhR ligands that are produced endogenously as well as by enteric bacteria has further expanded the focus of studies into the endogenous functions of the AhR. These studies suggest that the AhR

may be a key regulator of host–microbiota interactions, modulating host immune responses and metabolism within the gastrointestinal (GI) tract but also impacting the microbiota. The article by Limin Zhang *et al.* [10.1016/j.cotox.2017.02.001] not only highlights how the combination of metabolomics and microbiota characterization has resulted in new insights into AhR function, but also the role that the AhR plays in modulating host–microbiota interactions. Iain Murray and Gary Perdew [10.1016/j.cotox.2017.01.003] discuss the spectrum of AhR active tryptophan metabolites that are produced endogenously or by enteric bacteria and the role that these chemicals play in modulating gut homeostasis and immune responses. The potential use of AhR ligands as therapeutic agents for inflammatory gastrointestinal disease or injury is suggested from a variety of studies. In addition to the GI tract, the AhR has also been shown to strengthen epithelial barrier function and immunity in the skin. Katja Merches *et al.* [10.1016/j.cotox.2017.02.002] discuss the role of the AhR and the ability of endogenous and exogenous AhR ligands to produce beneficial or adverse effects on skin integrity, inflammatory responses and health. Clearly, there is significant potential for the development of therapeutic AhR ligands, the ability of such chemicals to produce beneficial effects in one tissue and/or species and adverse effects in another. These observations indicate that mechanisms of ligand selectivity of AhR responses must be further understood in order to optimize the beneficial effects of an AhR targeted drug. The complexity in the development of selective therapeutic AhR agents extends beyond its documented ability to modulate immune responses in barrier tissues. The AhR has also been shown to play a role in other physiological and developmental processes. The article by Raimo Pohjanvirta [10.1016/j.cotox.2017.01.002] discusses the recent evidence for a novel role of the AhR in energy balance regulation and diet induced obesity. This was illustrated with inhibition or knockout of the AhR reducing obesity in mice fed a high fat diet and the activation of the AhR increasing obesity. Furthermore, the AhR has been recently shown to play a key role in pluripotency in embryonic stem cells. Chia-I Ko and Alvaro Puga [10.1016/j.cotox.2017.01.004] discuss evidence that supports a role for the AhR in modulation and maintenance of pluripotency with inhibition or knockout of the AhR promoting expansion and proliferation of pluripotent stem cells. In contrast, ligand-dependent activation of the AhR could interfere with normal AhR modulation of pluripotency and results in aberrations that perturb or disrupt normal embryonic development.

The role of the AhR in cancer has been previously linked to its ability to stimulate expression of cytochrome P450s that metabolically activate chemicals into their mutagenic form, which subsequently may lead to tumor formation. However, the AhR also appears to play other regulatory roles in cancer progression and tumor immunity. The article by Zhongyan Wang *et al.*

[10.1016/j.cotox.2017.01.008] discusses the evidence for the continuous activation of the AhR in tumor cells by endogenous AhR ligands produced. In this respect, the metabolism of tryptophan by indoleamine 2,3-dioxygenase (IDO) and tryptophan dioxygenase (TDO) is of particular interest because these can be induced by a ligand-activated AhR mechanism. The role of this “constitutively” activated AhR in promoting tumor formation, migration and its potential to regulate immune checkpoints in cancer are discussed further. Conversely, it is also shown that inhibition of AhR signalling in cancer cells can inhibit tumor migration and invasion. The AhR repressor (AHRH) is one such protein that plays a significant role in repressing AhR-dependent gene expression and tumor metastasis. AHRH is an AhR-like protein that is ligand independent. However, it can constitutively dimerize with ARNT and binds to the same DNA recognition site as ligand activated AhR:ARNT complexes, but is transcriptionally inactive. As far we know, it has been demonstrated only to function as a dominant negative form of the AhR. The article of Christoph Vogel and Thomas Haarmann-Stemmann [10.1016/j.cotox.2017.02.004] discusses the AHRH protein in more detail. Firstly, it reviews the functional activity of the AHRH as a feedback inhibitor of AhR signal transduction (its expression is induced by ligand activated AhR). Secondly, recent evidence is presented of the ability of AHRH to function as a regulator/repressor of inflammatory signalling pathways and as a tumor suppressor gene. Thus, while AhR antagonists can be developed as chemotherapeutic and immunomodulatory drugs, the use of AhR activators may be more problematic from a therapeutic point of view. Given that ligand-dependent activation of the AhR can produce both beneficial as well as toxic effects, a useful AhR-based therapeutic drug must be able to produce the desired therapeutic effect with no undesired AhR-dependent toxicity. Stephen Safe *et al.* [10.1016/j.cotox.2017.01.012] discusses the development of selective AhR modulators (SAhRMs) with tumor cell specific AhR agonist or antagonist activity. These SAhRMs could be used as cancer chemotherapeutic drugs and do not produce the spectrum of AhR-dependent toxic effects associated with TCDD or TCDD-like chemicals. Several currently available and extensively used pharmaceuticals with documented AhR agonist activity (e.g. omeprazole, flutamide, etc) could be repositioned from their current applications for use as AhR chemotherapeutics. One concern that has been raised about the development and use of AhR agonists as therapeutic agents is the fact that chronic exposure of humans to these chemicals will result in persistent activation of the AhR and AhR signal transduction. Such a mechanism has been proposed to explain the specific toxicity produced by TCDD and other metabolically stable TCDD-like chemicals. The article of Allison Ehrlich and Nancy Kerkvliet [10.1016/j.cotox.2017.01.007]

directly addresses the issue of whether chronic AhR activation produced by continual exposure to rapidly metabolized ligands (drugs) is safe for treatment of human diseases. The utility of AhR ligands as potentially safe therapeutic drugs is supported by the lack of any observed AhR-dependent toxic effects in humans that have been chronically exposed for long periods of time to a drug(s) that is also able to activate the AhR. Thus, the available evidence is supportive of the further development of AhR-based therapeutic agents that would lack AhR-dependent adverse effects (e.g. omeprazole, flutamide, laquinimod and others).

Overall, the diversity of opinion articles in this special issue highlights the range of our current understanding

of the AhR, its structure and function, and the key role that this regulatory factor plays in surprisingly wide variety in normal physiological processes and adverse health effects. Although the AhR was identified more than 40 years ago, the spectrum of biochemical and toxicological effects of AhR ligands and physiological processes and responses affected by the AhR continues to expand far beyond its original role of mediating the toxic and biological effects of TCDD. There is no doubt that the rapid expansion of documented roles and functions of the AhR will continue to increase in the near future and especially pharmacotherapeutical applications may offer significant perspectives in treatments of various diseases.