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Pathways and Predications

AOP Report: An Upstream Network for Reduced Androgen Signaling Leading to Altered Gene Expression of Androgen Receptor–Responsive Genes in Target Tissues

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Abstract:

Adverse outcome pathways (AOPs) can aid with chemical risk assessment by providing plausible links between chemical activity at the molecular level and effect outcomes in intact organisms. Because AOPs can be used to infer causality between upstream and downstream events in toxicological pathways, the AOP framework can also facilitate increased uptake of alternative methods and new approach methodologies to help inform hazard identification. However, a prevailing challenge is the limited number of fully developed and endorsed AOPs, primarily due to the substantial amount of work required by AOP developers and reviewers. Consequently, a more pragmatic approach to AOP development has been proposed where smaller units of knowledge are developed and reviewed independent of full AOPs. In this context, we have developed an upstream network comprising key events (KEs) and KE relationships related to decreased androgen signaling, converging at a nodal KE that can branch out to numerous adverse outcomes (AOs) relevant to androgen-sensitive toxicological pathways. Androgen signaling represents an extensively studied pathway for endocrine disruption. It is linked to numerous disease outcomes and can be affected by many different endocrine-disrupting chemicals. Still, pathways related to disrupted androgen signaling remain underrepresented in the AOP-wiki, and endorsed AOPs are lacking. Given the pivotal role of androgen signaling in development and function across vertebrate taxa and life stages of both sexes, this upstream AOP network serves as a foundational element for developing numerous AOPs. By connecting the upstream network with various downstream AOs, encompassing different species, it can also facilitate cross-species extrapolations for hazard and risk assessment of chemicals. *Environ Toxicol Chem* 2024;00:1–9. © 2024 The Author(s). *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Adverse outcome pathway; Androgen signaling; Anti-androgens; Endocrine disruptors; Reproductive toxicology; Risk assessment

INTRODUCTION AND BACKGROUND

The adverse outcome pathway (AOP) framework has received great attention in recent years because of its potential utility to aid in chemical testing and regulation. However, despite a surge in attention and activities, a prevailing challenge with using AOPs for regulatory purposes is that there still are very few fully developed AOPs in the AOP-wiki (https://aopwiki.org/) that are endorsed by the Organisation for Economic Co-operation and Development (OECD), that is, citable AOPs. One major reason for this paucity is the large body of work that is required to build and review complete AOPs. To address some of these challenges and facilitate increased AOP development, a pragmatic approach was proposed, encouraging the development and

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peer review of smaller units of knowledge that can be used as building blocks for constructing larger, integrated AOP networks (Svingen et al., 2021).

The AOP framework can be used to assess all types of potential toxicological effects, including endocrine disruption. For 30 years, endocrine disruption has been an important focus area in the European Union, the OECD, the United States, and Japan. Tremendous resources have been set aside to develop test methods, explore exposure and effects, and build regulatory systems to minimize exposure to endocrine-disrupting chemicals (EDCs). In current chemical legislations within the European Union, specific scientific criteria for EDC identification have been implemented for biocidal products (European Commission, 2017), plant protection products (European Commission, 2018), and classification under the Classification, Labelling and Packaging (CLP) regulation (European Commission, 2023a). These criteria, and the identification of EDCs under the regulation concerning Registration, Evaluation, Authorisation, and Restriction of Chemicals (European Commission, 2006), builds on the well-established definition by the World Health Organization/International Programme on Chemical Safety (Damstra et al., 2002), which requires a biologically plausible link between endocrine activity and an adverse effect in an intact organism. Demonstration of the adverse effect typically relies on extensive animal studies, which contrasts with the overall political aim of reducing the use of animals in chemical toxicity testing. With the introduction of the new hazard classes in the CLP regulation, an opening has been introduced to use non-animal data for EDC identification if it "provides an equivalent predictive capacity as" human or animal data (European Commission, 2023b). Providing this "predictive equivalent capacity," however, can be challenging because animals are complex biological systems, and the interactions between organs, tissues, cells, and physiological processes are difficult to mimic outside of a living organism, especially for delayed effects. Further, in vivo testing allows for the observation of systemic effects, including metabolism, distribution, and excretion of substances, which are challenging to replicate in nonanimal methods. Because AOPs can establish the biological plausibility of the links between molecular initiating events (MIEs), key events (KEs), and adverse outcomes (AOs), the development of AOPs can potentially be a valuable tool to help in both current and future testing and assessment strategies in the European Union and beyond.

Androgens are essential for reproductive development across animal taxa and play essential roles throughout life (Schuppe et al., 2020). For example, mouse knockouts have established the crucial roles for the androgen receptor (AR) in brain, bone, muscle, and adipose tissue (Rana et al., 2014). And besides the recognized roles of androgen signaling in male fertility (Davey & Grossmann, 2016; Walters et al., 2010; Welsh et al., 2007), AR is also important for female fertility where, among other things, it regulates folliculogenesis (Panagiotou et al., 2022; Prizant et al., 2014; Walters et al., 2010). Likewise in fish, androgens are important for the development of sexual characteristics and behavioral traits (Ogino et al., 2014, 2023). Androgen signaling has been shown to be disrupted by EDCs, which causes reproductive effects in humans and other animals (Bergman et al., 2013; Gaudriault et al., 2017; Schwartz, Christiansen, et al., 2019; Skakkebaek et al., 2016). Many EDCs can perturb androgen signaling by various mechanisms, including binding and antagonizing the AR or disrupting the synthesis of androgens such as testosterone (T) and dihydrotestosterone (DHT; Wilson et al., 2008).

Given the crucial role for androgen signaling in vertebrate development and function in both sexes, across animal species, and at different life stages, numerous AOPs could be developed that share common upstream MIEs, KEs, and KE relationships (KERs). To provide a solid building block for developing an array of AOPs relating to disrupted androgen signaling and to facilitate future AOP network-building, we have developed an upstream AOP network for reduced androgen signaling. It describes the KEs and KERs that lead to reduced AR activation and altered AR gene transcription in more complex biological systems, including in vivo.

UPSTREAM AOP NETWORK DESCRIPTION

Androgen signaling is essential for many developmental processes as well as functions in adult life. The two main androgens acting as AR ligands in mammals are T and DHT, with DHT synthesized from T by the enzyme 5α-reductase in target tissues (Davey & Grossmann, 2016; Marks, 2004; Robitaille & Langlois, 2020). Notably, in other vertebrates such as fishes, 11-ketotestosterone is the second main ligand (Schuppe et al., 2020). Nevertheless, the main androgen ligands bind to, and activate, the AR. Subsequently, the ligand–receptor complex homodimerizes and translocates to the nucleus, where it binds to androgen response elements on the DNA leading to transcriptional regulation of target genes (Davey & Grossmann, 2016). Although a few cases of ligand-independent AR activation have been shown (Bennesch & Picard, 2015), androgen signaling in mammals is mainly achieved by AR activation by binding of T or DHT, with DHT being the more potent ligand (Grino et al., 1990).

There are several steps from androgen synthesis (steroidogenesis) to AR activation in target tissues that are essential (Gerald & Raj, 2022; Naamneh Elzenaty et al., 2022; Nef & Parada, 2000; Swerdloff et al., 2017) and that can be targeted by EDCs (Wilson et al., 2008). We have developed an upstream network including various KEs and KERs (see also individual KE/KER descriptions in the Supporting Information or at aopwiki.org) that all merge at a nodal KE describing altered gene transcription in vivo (KE 286 "Altered, Transcription of genes by AR"). This upstream AOP network (Figure 1) is applicable to many AOs relevant for both sexes and across animal taxa. In contrast, we envision downstream events to be more specific to animal taxa, life stage, and sex. For example, downstream of KE 286 several AOs are already established for mammalian male reproductive effect outcomes with regulatory relevance, such as shorter anogenital distance (AOPs 305, 306, 307 [currently under development]), nipple retention (AOP 344 [currently under development]), and hypospadias (AOP 477 [currently under

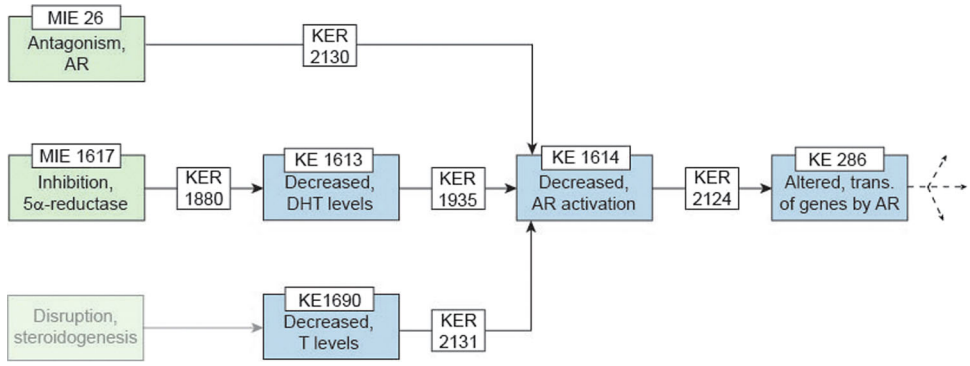

FIGURE 1: Upstream network for reduced androgen signaling. Upstream network of key events (KEs) and KE relationships (KERs) that lead to decreased androgen receptor (AR) activation and consequently altered AR-mediated gene transcription. Disruption of steroidogenesis (faded box) is included as a placeholder molecular initiating event (MIE), representing upstream events in steroidogenesis currently not included in the network. The dashed arrows from KE 286 (altered, transcription of genes by AR) indicate that the network may connect to many different downstream pathways and adverse outcomes. DHT = dihydrotestosterone; T = testosterone; trans. = transcription.

development]), as well as decreased fertility (AOP 345 [currently under development]) in mammalian females. But the network is also relevant for AOs suggested for reproductive effects in fish, for example, 5α-reductase inhibition leading to impaired fecundity in female fish (AOP 289 [currently under development]) downstream of KE 1613 (decreased DHT levels).

As depicted in Figure 1, the upstream network currently includes two MIEs: antagonism of the AR (MIE 26; Pedersen et al., 2022) and inhibition of 5α-reductase (MIE 1617). Disruption of steroidogenesis is currently functioning as a placeholder MIE in our network because this includes various mechanisms. This is because disrupted steroidogenesis has many potential outcomes regarding steroid hormone levels, with "decreased T" being only one of many. A separate steroidogenesis pathway is planned for development, allowing the direct linkage to the androgen network but also to other steroid hormone pathways such as estrogen signaling. For the current network, the immediate downstream KE from disrupted upstream steroidogenesis is decreased T levels (KE 1690). By different causal KERs, these events (MIE 26, KEs 1617 and 1690) all result in decreased AR activation (KE 1614) and ultimately altered AR gene transcription (KE 286; Panagiotou et al., 2022).

In many tissues, especially during development, activation of AR requires the presence of the more potent ligand DHT instead of T, as is the case in, for example, development of male external genitalia and perineal tissue in mammals (Robitaille & Langlois, 2020; Schwartz, Christiansen, et al., 2019). Endocrine-disrupting chemicals inhibiting 5α-reductase activity, and thereby reducing DHT levels (KER 1880), would be potent antiandrogens (Bowman et al., 2003; Schwartz, Vinggaard, et al., 2019; Yamana et al., 2010). The causal link represented by KER 1880 is well established and a central step in the upstream network.

Direct antagonism of the AR (MIE 26) will cause a reduction in AR activation (KE 1614), as described with KER 2130. It should be noted that KE 1614 (decreased AR activation) refers to changes in vivo without appropriate methods currently available but may be assessed indirectly with methods used to measure upstream MIEs/KEs or directly in, for example, fish (OECD, 2022; Sébillot et al., 2014). The rationale is that KE 1614 represents the activation of AR in tissues (e.g., in the living organism) with all the complexity it entails, for instance, that the reduced AR activation can result from several different upstream events, such as reduced testosterone levels or AR antagonism. One might initially think that this KE could be measured by in vitro AR reporter gene assays, but this carries the risk of missing certain mechanisms/MIEs. An example is inhibition of 5α-reductase (MIE 1617), where a reduction in DHT will lead to a decreased AR activation, an effect which is not measurable in a traditional cell-based AR reporter gene assay. This is especially important to keep in mind if the AOP network is used for chemical risk assessment and this nodal KE is used to predict AOs. In the future, new approach methodologies that can better capture the complexity of the in vivo situation may become available, such as tissue organoids and microfluidics/organs-on-a-chip (Leung et al., 2022).

Further to the inclusion of a KE for both "AR activation in vivo" (KE 1614) and "altered AR-mediated gene transcription" (KE 286), rather than these two being combined, this is based on the rationale that nuclear receptor activation itself can be monitored independent of transcriptional profiling and that KE 286 will also serve as an umbrella KE for new KEs that are specific for downstream AOs. For example, it is reasonable to assume that the transcriptome of the prostate, and changes on exposure, will be different from that of, for example, the perineum. These will link to separate AOs such as prostate cancer or decreased anogenital distance, respectively, and may be caused by both shared and unique transcriptional changes. Future transcriptomics studies will likely shed light on these patterns of effects and may even reveal a common effect pattern at the transcriptional level that can be exploited to develop alternative test methods capable of capturing such changes in response to stressors.

OVERVIEW OF UPSTREAM AOP NETWORK DEVELOPMENT APPROACH

The KEs and KERs of the AOP upstream network are generally well characterized across vertebrate taxa and broadly

accepted to be canonical knowledge in the scientific literature. Hence, we did not perform extensive systematic literature reviews otherwise recommended for the development of non-canonical KERs (de Vries et al., 2021; Svingen et al., 2021). Instead, we made use of key review articles with support from selected primary literature, as described in the *AOP Developer's Handbook* (Villeneuve et al., 2023). Notably, all the included KEs and KERs were developed with a strong focus on mammalian species. It is, however, acknowledged that many of the events most likely have a much broader domain of applicability extending to nonmammalian vertebrates. Additional relevant knowledge to expand on the applicability to also include other vertebrates could be contributed by other AOP developers, ultimately improving on the utility of the upstream network.

The literature search strategies employed in the present study were decided on a case-by-case basis, and a narrative review methodology was applied. Primary research articles that were considered essential based on expert judgment for description of the scientific evidence supporting certain KERs were included. The objective was not to cover all existing information but rather to gather enough relevant and reliable information to sufficiently describe the supporting evidence. A prime focus during the development of the upstream network was on regulatory use, such as careful descriptions of appropriate methods for measuring KEs.

To ensure inclusion of information already available in the AOP-wiki, we initially performed a manual screening of the AOP-wiki pages for KEs and KERs relevant for the upstream network for reduced androgen signaling. We identified 19 AOPs (details can be found in Zilliacus et al., 2024) that were relevant for upstream antiandrogen signaling (AOPs 18, 19, 51, 64, 111, 120, 124, 476, and those included in Textbox 1). In these, there were nine redundant KEs and five redundant KERs that essentially described the same event or relationship, albeit most entries contained little more than a KE or KER title. Redundant KEs and KERs were merged for the purpose of the present study (merging was also proposed in the AOP wiki), to better support AOP network development.

SCIENTIFIC ASSESSMENT OF THE AOP UPSTREAM NETWORK

Adverse outcome pathway development guidelines (OECD, 2017) require a summary of scientific assessments of the evidence supporting the included KEs and KERs based on a weight-of-evidence analysis. This assessment includes considerations of biological plausibility and empirical evidence of KERs and essentiality of KEs in support of predicted outcomes and the applicability domain of the AOP. The assessment provides a summary of the confidence in the AOP described and exposes possible uncertainties, gaps, or weaknesses. We present an upstream AOP network which does not contain any AOs. Nevertheless, scientific assessments of the network have been performed because this will support its inclusion in future AOPs relevant for inhibition of androgen signaling (Table 1). These assessments are summarized in the following paragraphs.

TEXTBOX 1: Adverse outcome pathway (AOP) upstream network identification

- Formal AOP title: N/A
- AOP authors: M. K. Draskau, A. K. Rosenmai, H. K. L. Johansson, E. M. Panagiotou, N. Bouftas, M. L. Holmer, E. Elmelund, E. B. Pedersen, P. Damdimopoulou, J. Zilliacus, S. Christiansen, T. Svingen
- AOP number: N/A
- OECD WP project: part of 1.90
- List of key events (KEs):

ID	Title	Linked AOPs
26	Antagonism, AR	306, 344, 345, 372, 477
1617	Inhibition, 5α-reductase	289, 305
1613	Decreased, DHT levels	288, 289, 305, 307
1690	Decreased, testosterone levels	307
1614	Decreased, AR activation	288, 305, 306, 307, 344, 372, 477
286	Altered, transcription of genes by AR	305, 307, 344, 345, 495

OECD = Organsiation for Economic Co-operation and Development; AR = androgen receptor; DHT = dihydrotestosterone.

Essentiality

In the overall assessment of an AOP, the essentiality of each KE must be evaluated, meaning if an upstream KE is blocked or does not take place, then subsequent downstream KEs, or the AO, are prevented or modified. In other words, is the KE essential for progression of the pathway? But because this is an upstream network, essentiality assessment cannot be made in relation to specific AOs. Individual essentiality assessments can be made, such as that inhibiting enzymatic conversion of T to DHT by 5α-reductase (KE 1617) is essential for the downstream event of decreased DHT (KE 1613). However, with both T and DHT acting as ligands for AR in a spatiotemporally regulated manner, and there being different requirements for androgen signaling across animal taxa and sexes, it is our suggestion that essentiality assessments are best made when the network is connected to complete AOPs.

Biological plausibility

The upstream network for disrupted androgen signaling is considered highly plausible based on extensive scientific knowledge about the molecular actions of androgens and the role of AR signaling in mammalian development and function (Davey & Grossmann, 2016; Gao et al., 2005). As detailed in each of the KERs of the network (see Supporting Information

TABLE 1: Examples of assays associated with individual key events in the adverse outcome pathway network

KE ID	Title	Associated test assays
1617	Inhibition, 5α-reductase	Inhibition of 5α-reductase can be assessed using transfected cell lines, measuring substrate and product formation by using radiolabeled steroids, ELISA, or advanced analytical methods such as LC-MS/MS.
1613	Decreased, DHT levels	Conventional immunoassay methods (ELISA or RIA) and advanced analytical methods such as LC-MS/MS can be used to measure DHT concentrations.
1690	Decreased, T levels	Conventional immunoassay methods (ELISA or RIA) and advanced analytical methods such as LC-MS/MS can be used to measure T concentrations.
26	Antagonism, AR	Antagonism of AR can be measured in vitro in, for example, transactivation assays using cells stably or transiently transfected with reporter genes. This method is also used in OECD guideline Test No. 458 (OECD, 2020). Nuclear translocation of AR can be monitored by various assays (Campana et al., 2015), and AR dimerization can also be assessed in vitro (Lee et al., 2021), thereby providing an increased mechanistic understanding.
1614	Decreased, AR activation	This KE specifically focuses on decreased in vivo AR activation, but most methods used to measure AR activity are carried out in vitro, though the RADAR assay included in OECD Test No. 251 can detect decreased AR activation in fish embryos (OECD, 2022). In vitro AR activation assays, on the other hand, provide indirect information about the KE and are described in lower tier MIE/KEs (see, e.g., MIE 26, KE 1690 or 1613).
286	Altered, transcription of genes by AR	Either RT-qPCR or RNA sequencing methods can be used to measure the transcription level of AR target genes. Because this KE aims to capture patterns of effect at the transcriptome level, bioinformatics analyses will typically be required to identify AR-mediated effect patterns.

AR = androgen receptor; DHT = dihydrotestosterone; ELISA = enzyme-linked immunosorbent assay; KE = key event; LC-MS/MS = liquid chromatography–tandem mass spectrometry; OECD = Organsiation for Economic Co-operation and Development; RIA = radioimmunoassay; MIE = molecular initiating event; RADAR = Rapid androgen disruption activity reporter; RT-qPCR = reverse transcription quantitative polymerase chain reaction; T = testosterone.

or aopwiki.org), all components are considered canonical knowledge.

The biological plausibility is considered high for all KERs in this upstream antiandrogenic network. Two cardinal natural ligands for AR activation are T and its more potent metabolite DHT, and molecular events that either inhibit the synthesis or availability of these ligands—for example, disruption of steroidogenesis or inhibition of 5α-reductase—can reduce AR activity (Davey & Grossmann, 2016). Given the dependency of ligand binding for the activation of AR, any stressor that can inhibit AR ligand binding will reduce the overall AR activation. Once activated, AR primarily functions as a nuclear transcription factor that regulates gene expression together with transcriptional cofactors. Stressors affecting recruitment of cofactors to AR can thereby also result in decreased AR activity (Heinlein & Chang, 2002). A causal effect of reduced AR activation is thus an alteration in transcription of AR-regulated genes, which can differ significantly depending on tissues and life stages, as well as between organisms or sex of the organism (Davey & Grossmann, 2016; Eder et al., 2001).

Antiandrogenicity, as described in the upstream network, encompasses different mechanisms of action, ultimately blocking the action of androgen signaling. These are well established with prototypic drugs and effect outcomes. For instance, pharmaceutical AR antagonists such as megestrol acetate, spironolactone, flutamide, bicalutamide, enzalutamide, and so forth have various potentials to inhibit AR activation. Androgen synthesis inhibitors such as ketoconazole, seviteronel, abiraterone acetate, finasteride, alfatradiol, and so forth are well known to inhibit T or DHT biosynthesis. All of these antiandrogens can be used as medicines to treat androgen-sensitive conditions, for example, prostate cancer; and they have all been shown to affect various tissues or organs, attesting to the overall biological plausibility of the upstream network culminating in AR-regulated gene transcription.

Empirical evidence

Evidence from the open literature related to the events currently included in the upstream network for reduced androgen signaling is extensive and covers different species, sexes, and life stages. Based on an overall assessment, the empirical support for this upstream network is considered high. The assessment is primarily based on strong and consistent evidence of dose concordance for the KERs showing that $KE_{upstream}$ occurs at the same or lower doses of a chemical stressor than $KE_{downstream}$ (see individual KER descriptions in Supporting Information or aopwiki.org). Regarding temporal concordance, specific experiments demonstrating that $KE_{upstream}$ occurs at earlier time points than $KE_{downstream}$ are generally lacking for the individual KERs. Because the entire network acts on a timescale of minutes to hours, the $KE_{upstream}$ is often assessed at the same time point as the $KE_{downstream}$ (e.g., in animal toxicity studies). Empirical evidence for all KERs of the network is considered high.

Uncertainties and inconsistencies

Regarding the essentiality of certain KEs, other factors could alter the impact on a downstream KE. For example, DHT can be synthesized from, for example, androstanediol produced by other organs in some mammals, including humans, by the existence of an alternative ("backdoor") pathway (Renfree & Shaw, 2023). Also, it is not well characterized how much, or little, T must be present in tissues for sufficient DHT to be synthesized.

These factors must be carefully considered when performing essentiality assessments for complete AOPs.

Applicability domain

Androgen signaling is crucial for sexual development and reproductive function across vertebrates (Ogino et al., 2011). The current network is based on mammalian data; several units could be expanded to include other vertebrate species. In mammals, androgens are needed for correct development of male sexual characteristics and fertility. Androgens are also important for female reproduction and are critical for, for instance, ovary follicle development (Panagiotou et al., 2022; Prizant et al., 2014), with AR-ablated mice showing reduced fertility and disrupted estrous cycling (Prizant et al., 2014). With extensive knowledge and studies having established a central role for androgen signaling in vertebrates (also beyond reproduction), in both sexes and during development as well as in adult life, the upstream network for reduced androgen signaling has a broad applicability domain.

Known chemical stressors

Many industrial chemicals, plasticizers, pesticides, and pharmaceuticals have been shown to trigger the two MIEs of the upstream network, as well as the placeholder MIE "steroidogenesis" leading to reduced T levels (Figure 1). For KE 26, measured by, for instance, AR-reporter gene assays, examples of known stressors are azole fungicides (epoxiconazole, flusilazole, prochloraz, propiconazole, triticonazole, and tebuconazole) that can antagonize the AR at different potencies (Draskau & Svingen, 2022; Pedersen et al., 2022). But also other fungicides, such as procymidone, vinclozolin, or dimethomorph, as well as other types of pesticides including, for instance, linuron are AR antagonists (Orton et al., 2011; Sébillot et al., 2014). There are also several pharmaceuticals exploited for their AR antagonistic potency, such as flutamide, bicalutamide, and enzalutamide (Chen et al., 2022). For KE 1617, known inhibitors of 5α-reductase are pharmaceuticals designed to block the production of DHT, such as finasteride and dutasteride (Aggarwal et al., 2010). There is less evidence for environmentally relevant chemicals acting as strong inhibitors of 5α-reductase, but certain industrial chemicals and pesticides such as dibutyltin, tributyltin, triphenyltin, and prochloraz can interfere with 5α-reductase activity (Lo et al., 2007).

POTENTIAL APPLICATIONS OF THE UPSTREAM NETWORK

The upstream network for reduced androgen activity holds great utility in supporting the continued development of complete AOPs and AOP networks in that the early molecular events are shared between most AOs that originate from disrupted androgen signaling. Hence, when building causal pathways downstream of KE 286 ("altered [AR] gene regulation"), and the transcriptional effects have been characterized for specific tissues and specific life stages, it will be a great advantage that the upstream part of the AOPs is already developed (Figure 2).

Currently, the AOP-wiki only contains AOPs relevant for androgen signaling (mostly under development) that refer to reproductive outcomes in vertebrates (i.e., fish and mammals). However, the potential applications of inhibition of androgen signaling extend beyond reproduction, including other organs such as the liver, brain, bones, and skeletal muscle (Rana et al., 2014). By creating the upstream androgen signaling network, we aim to facilitate the downstream relationships of future AOPs and ease development of new AOPs with effects on species and organ systems not yet explored. We foresee our

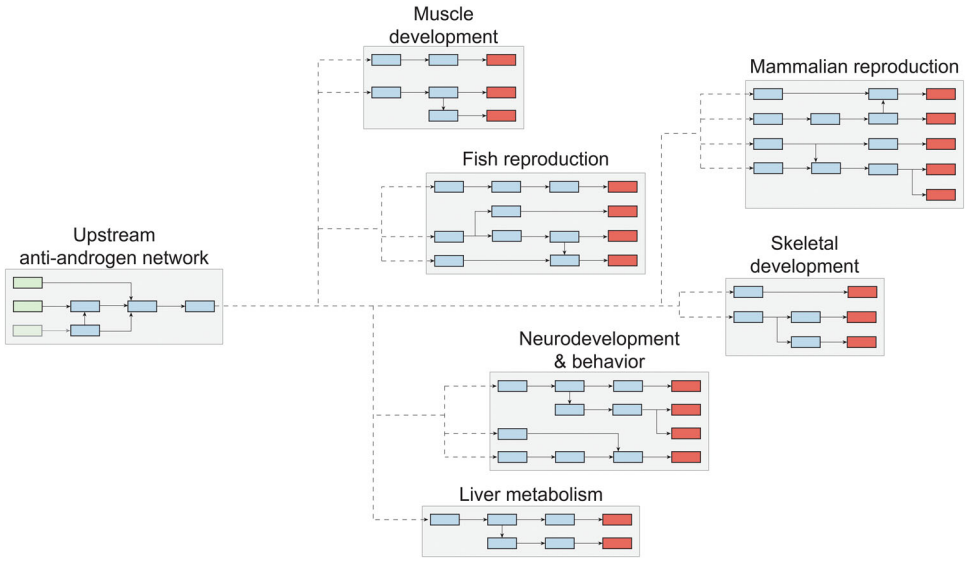

FIGURE 2: Potential applications of the upstream antiandrogen network. The upstream antiandrogen network may connect to many other biological networks within different taxa, tissues, and life stages, thus forming the foundation for the development of many different adverse outcome pathways starting from perturbed androgen signaling.

upstream androgen signaling network to be connected to various downstream AOPs and AOP networks leading to a range of AOs in different species. Such AOP networks may in the future be used to illustrate and facilitate cross-species extrapolations in hazard assessment of endocrine disruptors (Figure 2). The upstream network for reduced androgen signaling can in this way work as a core block of knowledge, accelerating the population of the AOP knowledge base and ultimately furthering chemical risk assessment and regulation.

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Author Contribution Statement—**Monica K. Draskau**: Data curation; Formal analysis; Investigation; Supervision; Validation; Visualization; Writing–original draft; Writing–review & editing. **Anna K. Rosenmai, Eleftheria M. Panagiotou, Marie L. Holmer**: Data curation; Formal analysis; Investigation; Validation; Writing–original draft; Writing–review & editing. **Nora Bouftas, Hanna K. L. Johansson**: Data curation; Formal analysis; Investigation; Writing–original draft; Writing–review & editing. **Emilie Elmelund**: Formal analysis; Investigation; Visualization; Writing–original draft; Writing–review & editing. **Johanna Zilliacus**: Formal analysis; Investigation; Methodology; Validation; Writing–review & editing. **Anna Beronius**: Funding acquisition; Methodology; Resources; Supervision; Validation; Writing–review & editing. **Pauliina Damdimopolou**: Funding acquisition; Resources; Supervision; Validation; Writing–review & editing. **Majorie van Duursen**: Formal analysis; Funding acquisition; Supervision; Validation; Writing–review & editing. **Terje Svingen**: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing–original draft; Writing–review & editing.

Data Availability Statement—Final PDF snapshots of all KE and KER descriptions are included as Supporting Information and can also be found in the AOP wiki (aopwiki.org) under the individual KE and KER pages.

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