Bulgarian Chemical Communications, Volume 58, Issue 4 (pp. 504-514) 2026 *DOI: 10.34049/bcc.58.4.5757*

Advances in predictive toxicology for androgen receptor–mediated endocrine disruption

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Received: March 16, 2026; Revised: June 01, 2026

This article provides an overview of the achievements and limitations of predictive toxicology approaches for assessing endocrine-disrupting chemicals (EDCs). It focuses on the androgen receptor (AR) as an important target of EDCs. AR is a nuclear receptor involved in numerous physiological processes related to male development and health, including sexual differentiation, reproductive function, as well as non-reproductive processes such as development of bones, muscles, and nervous system. Due to its significance, AR dysregulation by EDCs is a subject of strong scientific and regulatory interest. Research on AR-mediated toxicity has led to the development of several non-animal testing alternatives to traditional safety assessment, including *in silico*, *in chemico*, and *in vitro* methodologies (referred to as New Approach Methodologies or NAMs). The review offers insights into the current understanding of the functioning and classification of AR ligands, as well as advancements in high-throughput screening assays for assessing their effects. The primary focus is on existing computational models, particularly those utilizing data from programs and projects of the U.S. Environmental Protection Agency and the Organization for Economic Co-operation and Development. The reviewed studies illustrate the effectiveness of multiple methodologies, with machine learning algorithms dominating current research and consensus modeling strategies having the best predictive performance. Overall, AR-related toxicity prediction demonstrates that NAMs hold significant potential in supporting chemical safety evaluation, prioritizing compounds for experimental testing, and lowering the overall cost and time required for chemical risk assessment. Additionally, the need for integrating predictive tools into regulatory risk assessment frameworks is emphasized.

Keywords: Predictive toxicology, Endocrine-disrupting chemicals, Androgen receptor, Computational models, New approach methodologies.

INTRODUCTION

Endocrine-disrupting chemicals (EDCs) are xenobiotic compounds that interfere with the human endocrine system thus causing adverse effects such as reproductive dysfunction, metabolic disorders (incl. diabetes type 2), neurodevelopmental impairments (Alzheimer's disease), and increased risk of cancer (e.g. prostate cancer) or skin disorders [1]. They act through multiple mechanisms, including direct interaction with receptors responsible for hormonal signaling, like the androgen receptor (AR), estrogen receptors ERα or ERβ, thyroid receptors, and modulation of hormone receptor expression [2, 3].

EDCs are present in a wide range of everyday products, including certain cosmetics, food and beverage packaging, toys, and pesticides. Some chemicals used as flame retardants may also function as endocrine disruptors. Human exposure to these substances can occur *via* air, diet, skin contact, and water. Consequently, endocrine disruption (ED) is explicitly addressed in major regulatory frameworks including the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH; No 1907/2006), the Biocides Regulation (BPR; Regulation (EU) No 528/2012), the Classification, Labelling and Packaging Regulation ([CLP; No 1272/2008) and the Endocrine Disruptor Screening Program (EDSP) of the United States Environmental Protection Agency (EPA; https://www.epa.gov/endocrine-disruption).

When assessing ED properties of chemicals, multiple types of information are integrated within a weight-of-evidence framework to support scientifically robust hazard identification. This process increasingly relies on predictive toxicology approaches that exploit fundamental knowledge of molecular structure, physicochemical properties, and chemical reactivity to anticipate biological effects. These methodologies provide the basis for non-animal testing strategies, now integral to both chemical safety assessment and regulatory decision-making.

Predictive toxicology integrates *in silico*, *in chemico*, and *in vitro* methodologies, collectively

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referred to as New Approach Methodologies [4]. These approaches enable systematic evaluation of structure–activity and structure–reactivity relationships, linking molecular features such as functional groups, stereochemistry, lipophilicity, electronic properties and other structural descriptors to endocrine-related toxicity. *In silico* methods - including quantitative structure–activity relationship (QSAR), molecular docking, and cheminformatics-based screening - allow rapid prioritization of chemicals and identification of potential interactions with endocrine system-related targets. Complementary *in chemico* assays provide insight into intrinsic chemical reactivity, while *in vitro* assays translate these properties into measurable biological responses.

Predictive toxicology approaches support safety evaluation and risk assessment for a broad range of substances, including industrial chemicals, pharmaceuticals, and environmental contaminants. They form the backbone of next-generation risk assessment, which emphasizes human-relevant mechanisms, scalability across chemical space, and reduced reliance on animal testing. Together, these approaches enable chemists to contribute directly to more efficient, mechanism-driven, and ethically responsible assessment of endocrine-disrupting chemicals.

This review aims to provide an overview of the most recent approaches used to predict ED effects of chemicals, discussing their achievements and current limitations. We particularly focus on the AR as an important target of EDCs to offer researchers exemplifying studies utilizing approaches of the advanced predictive toxicology.

Androgen receptor, types of ligands, reference compounds

One of the main subjects of research in predictive toxicology is nuclear receptors, including the AR. The AR is a nuclear transcription factor of key importance for male health that regulates sexual differentiation, reproductive function, and spermatogenesis, as well as non-reproductive processes such as the development of bones, muscles, and the nervous system. The AR functions by binding androgen ligands (hormones). Upon ligand binding, a sequence of events is initiated, including conformational changes, dissociation from heat shock proteins (HSPs), and translocation from the cytoplasm to the nucleus (if not already localized there) (Figure 1). In the nucleus, the receptor interacts with specific DNA sequences known as androgen response elements, ultimately leading to the modulation of gene expression in target cells, including testicular cells. Representative AR target genes include *KLK3* (Prostate-Specific Antigen/PSA), *KLK2*, *FKBP5*, *NKX3-1*, *IGF1*, *MYC*, and *CCND1* (Cyclin D1). Activation of these and other involved genes promotes key biological responses such as cellular proliferation, differentiation, survival, metabolism, secretion, and tissue homeostasis, thereby reflecting the normal physiological functioning of AR signaling.

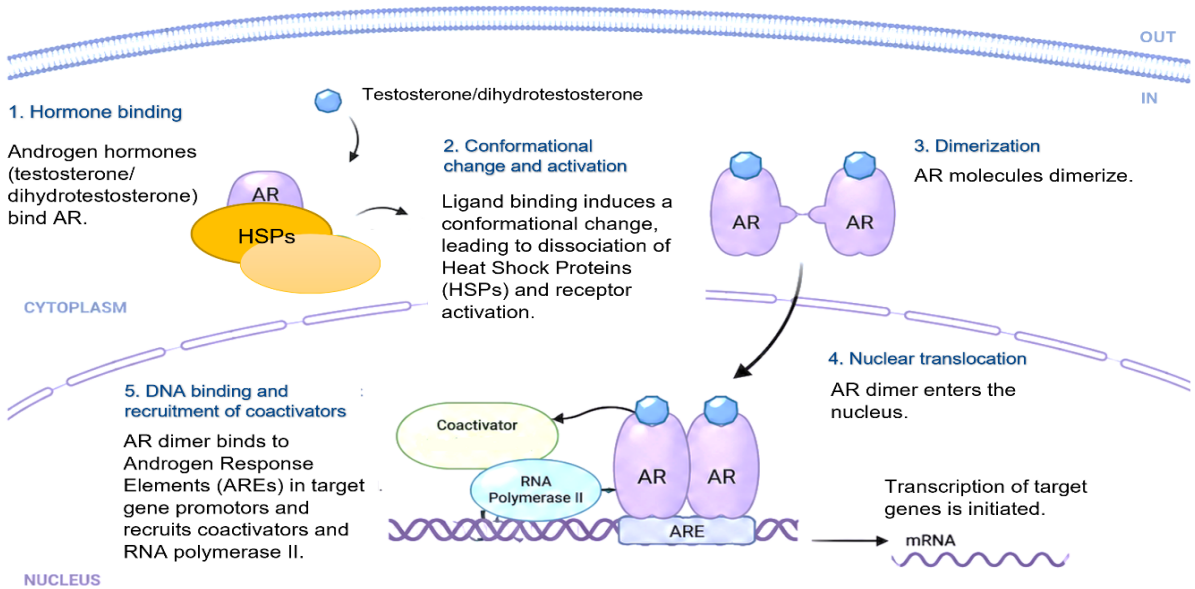
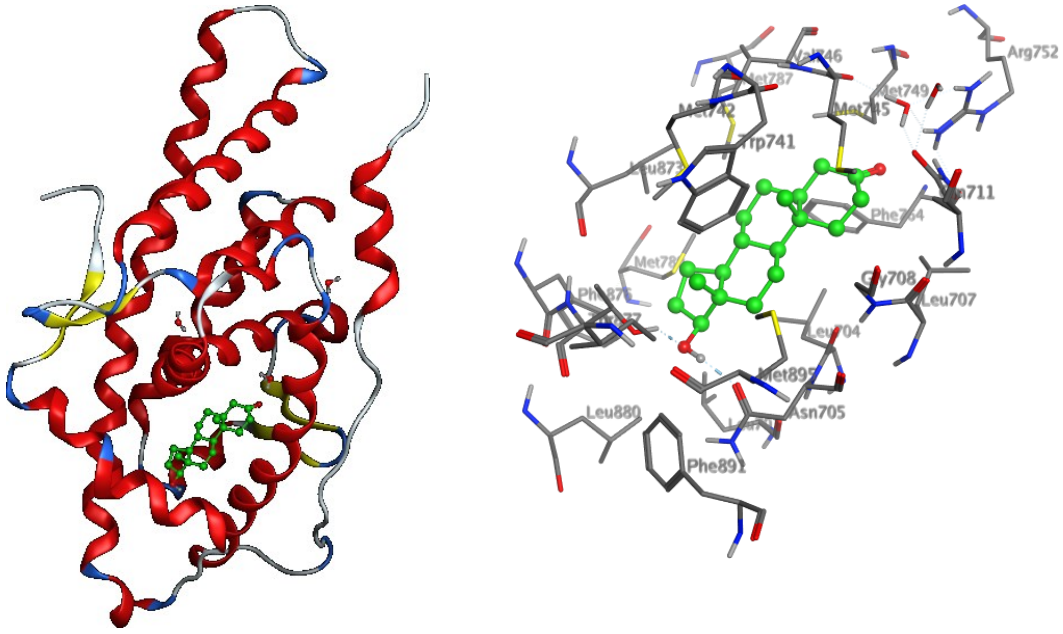


Figure 1. Schematic representation of AR signaling under normal conditions.



A B

Figure 2. A. 3D crystal structure (PDB ID 1T7T) of the androgen receptor ligand binding domain (the protein backbone rendered in ribbon) in complex with the agonist 5-alpha dihydrotestosterone (in green); B. Closer view of the active site with the amino acid residues forming the receptor binding pocket.

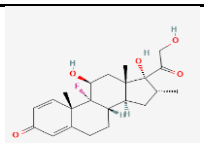
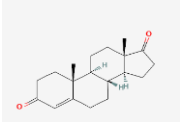
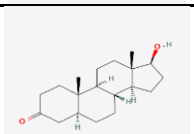
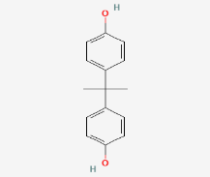
Disruptions in the AR function can result in severe reproductive disorders, ranging from complete androgen insensitivity syndrome to milder forms of impaired fertility. Due to its central role in androgen-dependent pathways that control male development and physiology, the AR is often described as a "master regulator" of these processes [5]. These factors also explain the strong scientific interest in the AR as a target of predictive toxicology research, alongside the estrogen receptors and other nuclear receptors involved in the regulation of essential physiological processes in the living organisms. The official symbol for the androgen receptor according to the HGNC (HUGO Gene Nomenclature Committee, https://www.genenames.org/) nomenclature is AR. Other symbols reported in the scientific literature include NR3C4 (nuclear receptor subfamily 3, group C, member 4), KD, AIS, AR8, TFM, DHTR, SBMA, HYSP1, SMAX1, HUMARA. Compounds that interact with AR are referred to as AR ligands and may be either endogenous (hormones) or exogenous (xenobiotics). AR is most commonly localized in the cytoplasm, where it forms a complex with heat-shock proteins. Activation of AR is initiated by binding to androgen hormones. Androgen-activated AR dissociates from heat-shock proteins and subsequently moves to the nucleus, where it mediates AR pathways [6]. Dihydrotestosterone is the most potent known endogenous ligand of the receptor. It binds to human AR with a dissociation constant (Kd) of 0.25–0.5 nM, which corresponds to an approximately 2–3-fold higher affinity than that of testosterone (Kd = 0.4–1.0 nM) and a 15–30-fold higher affinity than that of adrenal androgens. Fig. 1 illustrates the X-ray crystal structure of AR in complex with dihydrotestosterone retrieved from the Protein Data Bank (PDB; https://www.rcsb.org/). EDCs can mimic the interaction of the AR with its natural hormones, thereby interfering with its normal functions. The ability of EDCs to bind to AR and their specific mode of interaction represent the key molecular initiating event underlying the associated toxic effects. AR-targeting EDCs can disrupt the tightly regulated transcriptional processes controlled by AR signaling (Figure 2), leading to altered gene expression, epigenetic dysregulation, impaired reproductive development, endocrine dysfunction, and increased susceptibility to hormone-dependent diseases, including prostate cancer and infertility. Assessing these effects requires distinguishing between EDCs that act as agonists (activators) or antagonists (inhibitors) based on their ability to activate or inhibit transcription of AR target genes [7]. For example, bisphenol A is reported to antagonize AR signaling, alter the expression of *KLK3 (PSA)* and *NKX3-1* genes and is associated with reduced sperm quality, impaired male fertility, altered prostate development, endocrine dysfunction, and increased susceptibility to

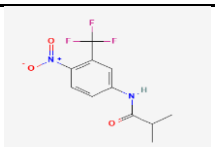
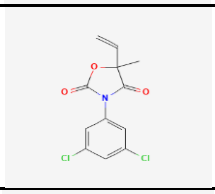
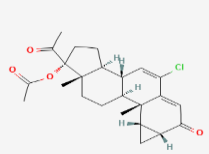
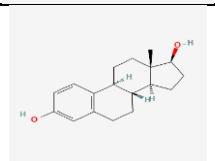
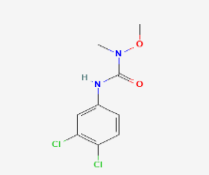
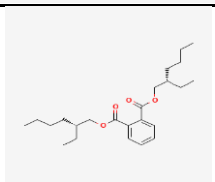
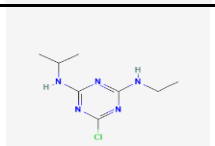
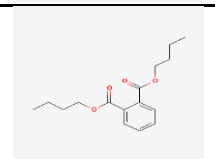
hormone-dependent cancers [8]. Similarly, vinclozolin metabolites function as AR antagonists, blocking androgen-dependent transcription and reducing expression of genes involved in male reproductive differentiation and steroidogenesis. Physiological consequences include cryptorchidism, hypospadias and impaired spermatogenesis [9].

A classic approach for distinguishing AR agonists from antagonists is based on evaluating their ability to induce N/C interactions—interactions between the NH_2-terminal (N-terminal) and the COO-terminal (C-terminal) of the receptor [10]. Using this criterion high-affinity AR agonists (e.g., dihydrotestosterone, mibolerone, testosterone, and methyltrienolone) have concentrations between 0.1 and 1 nM and induce the N/C interaction more than 40-fold. Agonists with lower affinity require concentrations of 10–100 nM for up to 23-fold induction of the N/C interaction (oxandrolone, fluoxymesterone). No N/C interaction is recorded for antagonists at concentrations up to 1 μM (hydroxytamoxifen, cyproterone acetate or RU56187, estradiol, progesterone, medroxyprogesterone acetate); each of these steroids inhibits the dihydrotestosterone-induced N/C interaction at concentrations of 1–500 nM. Kemppainen *et al.* [10] concluded that all ligands exhibited concentration-dependent AR agonist activity that corresponded with induction of the N/C interaction, while antagonists and weaker agonists did not trigger this interaction. Their results suggest that the N/C interaction enhances agonist potency at low physiological ligand concentrations, as shown by transcription, dimerization/DNA binding, and stabilization assays. However, at sufficiently high ligand concentrations, the N/C interaction is not required for agonist activity, and its inhibition does not necessarily indicate antagonist behavior.

The current classification of AR-targeting EDCs used in predictive toxicology deals with four main classes of activity: agonists, antagonists, selective AR modulators (SARMs), and inactive compounds [11]. SARMs are defined as small molecules that can act as agonists or antagonists with tissue-selective activities, which are their key characteristics. Table 1 illustrates this classification using 12 reference compounds for AR, reported in [10] and based on reports from the ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) [12] and the OECD (Organization of Economic Cooperation and Development) [13].

Table 1. Representatives of main activity classes of reference chemicals; SARM - selective androgen receptor modulator (according to Kleinstreuer *et al.* [11]).

Substance (product class)	CAS ref. no.	Chemical structure	Chemical class	Activity class
Dexamethasone (pharmaceutical)	50-02-2		Steroid, nonphenolic	AR agonist
4-Androstenedione (hormone)	63-05-8		Steroid, nonphenolic	Strong AR agonist
5α-Dihydrotestosterone (hormone)	521-18-6		Steroid, nonphenolic	Strong AR agonist
Bisphenol A (chemical intermediate)	80-05-7		Diphenylalkane bisphenol; Phenol	AR antagonist

Flutamide (pharmaceutical)	13311-84-7		Amide; Anilide; Nitrobenzene	AR antagonist
Vinclozoline (pesticide)	50471-44-8		Organochlorine; Cyclic imide; Carbamate	AR antagonist
Cyproterone acetate (pharmaceutical)	427-51-0		Nitrile; Diphenyl ether; Organochlorine	SARM
17β-Estradiol (hormone)	50-28-2		Steroid, phenolic; Estrene	SARM
Linuron (pesticide)	330-55-2		Urea	SARM
Diethylhexyl phthalate (used in a variety of customer products)	117-81-7		Phthalate	Inactive
Atrazine (pesticide)	1912-24-9		Aromatic amine; Triazine; Arylamine	Inactive
Di-*n*-butyl phthalate (plasticizer)	84-74-2		Phthalate	Inactive

ICCVAM published a list of 78 substances recommended for use in future validation studies of AR endocrine disruptors [12] with a summary of the available quantitative data on AR binding *in vitro* for each substance. Relative binding affinity (RBA) data are available for 33 (42%) of these 78 recommended substances.

Development and validation of new approach methodologies based on relevant toxicological data

- *The EPA's ToxCast and Tox21 programs for assessing the effects of chemicals on AR pathways (AR pathway model).* EPA has developed and maintains the Toxicity Forecaster (ToxCast) program, which was subsequently expanded under the Tox21 initiative (https://www.epa.gov/comptox-tools/exploring-toxcast-data). This program has generated data using *in vitro* high-throughput screening (HTS) assays measuring activity of chemicals at multiple points along the AR activity pathway (AR pathway model). The primary objective of ToxCast/Tox21 is to use these data to predict AR toxicity potential and prioritize testing of chemicals that pose the greatest risk to human health and the environment [14]. In practice, ToxCast/Tox21 provides the largest data for

evaluating the effects of chemicals on AR pathways. The data are collected from various sources, and a diverse range of chemical collections has been studied. A number of tests are used to assess AR activity, with a combination of HTS assays most often used to provide a more comprehensive evaluation of the potential toxic effects of the studied compounds.

The data from the HTS of ToxCast/Tox21 consist of approximately 9,500 substances that have been tested across roughly 1,400 assays. The ToxCast invitroDB v3.3 database (published in September 2020) is available at the following link: https://www.epa.gov/chemical-research/exploring-toxcastdata-downloadable-data [last accessed 11 March 2026]. The dataset includes AC_{50} values (the concentration at which 50% of the observed effect is achieved), binary codes representing activity (1) or inactivity (0) for each chemical–assay pair, and chemical-specific cytotoxicity scores.

Many aspects of AR activity have been investigated using a variety of assays. Most assess binding affinity together with agonistic and antagonistic activity, while some focus specifically on either antagonism or agonism. Transcriptional activation assays are also used to evaluate the ability of chemicals to stimulate AR-regulated gene transcription. These assays employ cell lines containing reporter genes under the control of AR-dependent promoters. Lynch and co-authors [15] screened the Tox21 library to identify AR activators using two reporter gene cell lines: AR β-lactamase (AR-bla) and AR luciferase (AR-luc). Seventy-five compounds identified in the primary assay were subsequently characterized as potential agonists or inactive compounds through confirmatory screening and secondary assays. Biochemical binding assays and AR nuclear translocation assays further confirmed the direct binding to and activation of AR by these compounds. Multiple AR activators were identified, including well-known AR agonists such as testosterone, as well as novel or less well-characterized compounds such as prulifloxacin [15].

One of the first comprehensive test collections includes a panel of nine high-throughput screening assays (five from ToxCast and four from Tox21). These tests evaluate receptor binding, cofactor recruitment, gene transcription, and protein production across multiple cell types, including eight human and one animal (chimpanzee) cell types [11]. The developed computational model assumes that the observed effect value (efficacy, A) at a given chemical concentration is a linear sum of contributions from the receptors being measured. It is based on the calculation of area under the curve (AUC) [11]. The model was initially trained using a set of 28 reference compounds, and subsequently tested on 1,846 ToxCast chemicals: 1,549 chemicals were inactive in the model; 115 chemicals were predicted to have strong effects as AR binders, either as agonists or antagonists; the remaining 182 chemicals showed results in an intermediate range.

In a subsequent study, Kleinstreuer *et al.* [16] expanded the panel from nine to eleven high-throughput assays by adding a rat AR binding assay (radioligand cell-free measurement). The list of unique reference compounds was also expanded from 28 to 44 to improve the evaluation and validation of the computational model. In one of the most recent studies conducted under the EPA's EDSP for endocrine disruptor screening, the assessment panel was further expanded to 14 high-throughput assays (Table 2) [17].

Table 2. List of assays used in models for assessing the effects of chemicals on AR pathways (14 assays) [17].

ToxCast name of the assay	Description of the assay (pathway)
NVS_NR_hAR	Receptor binding assay with human AR (antagonist and agonist pathway)
NVS_NR_cAR	Receptor binding assay with chimpanzee AR (antagonist and agonist pathway)
NVS_NR_rAR	Receptor binding assay with rat AR (antagonist and agonist pathway)
OT_AR_ARSRC1_ 0480	Coregulator recruitment assay at 8 h (antagonist and agonist pathway)
OT_AR_ARSRC1_ 0960	Coregulator recruitment assay at 16 h (antagonist and agonist pathway)
UPITT_HCI_U2OS_ AR_TIF2_ Nucleoli_Agonist	Nuclear translocation assay (antagonist and agonist pathway)
ATG_AR_TRANS_ up	Reporter gene assay (agonist pathway)
OT_AR_ARELUC_ AG_1440	Reporter gene assay (agonist pathway)
TOX21_AR_BLA_ Agonist_ratio	Reporter gene assay (agonist pathway)
TOX21_AR_LUC_ MDAKB2_Agonist	Reporter gene assay (agonist pathway)
ACEA_AR_ Agonist_80h	Real time impedance assay (agonist pathway)

UPITT_HCI_ U2Os	Nuclear translocation assay (antagonist pathway)
TOX21_AR_BLA_ Antagonist_ratio	Reporter gene assay (antagonist pathway)
TOX21_AR_LUC_ MDAKB2_ Antagonist_ 0.5nM_R1881	Reporter gene assay (antagonist pathway)

The aim of the study by Bever *et al.* [17] is to demonstrate a method for processing data to identify optimal minimal assay panels derived from a larger, comprehensive model, to provide a unified framework for evaluating the performance of future minimal panels, and to incorporate chemical cluster analysis into this evaluation. Although several of the assays used in the previously described AR pathway models are no longer available from the original provider, such an approach remains valuable for future evaluations of minimal assay models intended for prioritization and screening purposes. The authors compare two previously published models and report that the expanded 14-assay model shows higher sensitivity for antagonists, whereas the original 11-assay model demonstrates slightly higher sensitivity for agonists. They further analyze subsets of assays within the original AR pathway model to optimize overall testing strategies that reduce costs while maintaining sensitivity across a broad chemical space.

Analyses of model subsets derived from the 14-assay model identified three critical assays for predicting antagonism and two critical assays for predicting agonism. At least nine assays are required to predict both agonism and antagonism with high sensitivity (95%). The authors further demonstrate that testing workflows guided by chemical structure–based clustering can reduce the average number of assays required per chemical. In this approach, assay selection is informed by the probability that a chemical acts as an AR agonist based on its structural features. The results indicate that a multi-stage testing workflow can achieve 95% sensitivity while using only 48% of the resources needed to perform all assays included in the original full models. Resource use can be further reduced by incorporating *in silico* activity predictions. The main conclusion of the study is that integrating chemical clustering and the simultaneous evaluation of both antagonistic and agonistic mechanisms provides efficient approach to chemical screening. This strategy can minimize the total number of assays required to predict AR activity, thereby maximizing the number of chemicals that can be tested and supporting data-driven prioritization for further screening under the EDSP Universe of Chemicals list. The Universe of Chemicals list includes 10,272 compounds identified for potential screening (available at: https://www.epa.gov/sites/default/files/2015-07/documents/edsp_chemical_universe_list_11_12.pdf; last accessed 11 March 2026).

In their analysis of the 14-assay panel, Judson *et al.* [18] emphasize the importance of including AR homodimerization assays. However, they also note that not all AR assays are currently available from their original sources. Therefore, to apply subset models effectively, it may be necessary to identify existing assays or develop new ones that exhibit behavior similar to those selected for the optimal subsets. The authors further highlight that one of the major challenges in using *in vitro* data to predict *in vivo* outcomes is the limited representation of xenobiotic metabolism in *in vitro* systems.

To address the metabolic limitations of *in vitro* systems, the latest ToxCast strategic plan proposes the transfection of chemically modified mRNAs encoding human cytochrome P450 (CYP) enzymes into cells to induce intracellular CYP expression and functional metabolic activity [19, 20]. The study evaluated 2,165 ToxCast chemicals using an AR homodimerization assay conducted in parallel with 10 human liver CYP enzymes, with the aim of identifying antiandrogenic parent compounds and their metabolites. The authors conclude that the AR dimerization assay, when combined with metabolism testing, can detect chemicals that are either activated or deactivated by CYP-mediated metabolism. AR dimerization and CYP450 data are available for 131 agonists, 260 antagonists, and 128 tested compounds.

In 2023 OECD published a Test Guideline that established methods for identifying chemicals with androgenic properties. It exclusively addresses transcriptional activation and inhibition of an androgen-regulated reporter gene (Androgen Receptor TransActivation (ARTA) assays) and formulates the minimum standards for the performance of experimental controls and reference standards [21].

The *in vitro* assays developed under the ToxCast / Tox21 initiatives, and OECD guideline protocols, are designed to measure key components of the AR pathway. This pathway is activated through a series of well-characterized key events, beginning with receptor binding (molecular initiating event) and dimerization with cofactors, which subsequently modulate transcriptional activity, leading to the production of functional proteins and either stimulation or inhibition of downstream receptor responses. Studies have demonstrated that the

integration of multiple assays can quantitatively discriminate between AR-active and inactive substances [22]. The regulatory application of AR pathway–based methods is currently under consideration in several regulatory programs, for instance, EDSP.

- *Computational models used to predict the effects of chemical compounds on AR.* The overview of models presented below highlights recent *in silico* models to reflect current trends in *in silico* prediction of AR toxicity. The majority of these models are based on ToxCast/Tox21 data.

One of the earliest models utilizing ToxCast AR *in vitro* data are the OASIS QSAR models proposed by Bhhatarai *et al.* [23]. The authors rely on the OASIS methodology proposed by Mekenyan *et al.* [24] for the estrogen receptor by integrating molecular structure, metabolism, and mechanisms of action in QSAR models. OASIS QSAR AR models show high sensitivity (92–100%) and specificity (70–81%) for predicting AR binding. The authors recommended to apply a tiered screening approach for integrative testing and risk assessment.

The CoMPARA (Collaborative Modeling Project for Androgen Receptor Activity) project [25] brings together scientists specializing in computational toxicology from 25 research groups around the world. Using a variety of machine learning methods—Artificial Neural Networks (ANN), Support Vector Machines (SVM), Random Forest (RF), k-Nearest Neighbor (KNN), Decision Trees (DT), structural alerts, and others—the project enhances the statistical performance of the developed models. More than 90 predictive models have been developed, along with consensus models for predicting the binding, agonistic, and antagonistic activity of chemicals. In total 1,746 chemicals composed of a combined dataset based on the HTS panel of 11 *in vitro* assays from ToxCast™/Tox21 were included as a common training set. The predictive accuracy of the consensus models (on the evaluation set) is approximately 80%. The models have been implemented in the free, open-source OPERA application, enabling the screening of new chemicals with a defined applicability domain and accuracy assessment (https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera; last assessed 11 March 2026). This implementation has been used to screen the entire EPA DSSTox database of approximately 875,000 chemicals. The predicted AR activities are available on the EPA CompTox Chemicals Dashboard and in the National Toxicology Program's Integrated Chemical Environment [26].

Maran *et al.* published classification QSAR models [27] based on the same dataset, used in CoMPARA project. The models were based on two datasets by the EPA's National Center for Computational Toxicology. The first dataset contains 1,688 compounds and was used to construct the training sets for model development. The second dataset, consisting of 5,273 compounds collected from multiple EPA sources, was used to evaluate the actual predictive accuracy of the models (test set). The developed classification models categorize compounds using the RF method into three activity groups: androgen-binding (binders), agonistic, and antagonistic activity. In addition, a multi-class approach was developed that simultaneously distinguishes between inactive compounds, agonists, and antagonists. For the test set, the classification models predict agonists with an accuracy of 80%, while the corresponding values for antagonists and binders are 72% and 78%, respectively. Combining agonists, antagonists, and inactive compounds in the multi-class approach results in a prediction accuracy of 64% for the evaluation set. Considering the size of the training datasets and their imbalance, the authors regard the achieved prediction accuracy as very good. The final classification models are available for study and prediction in the QsarDB repository [28].

The fingerprint model developed by Zron and co-workers [29] is based on invitroDBv3.1 summary files (EPA, U. EPA ToxCast & Tox21 Summary Files from invitrodb_v3.1. https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data (April 16, 2019). From the initial dataset of 9214 substances 8645 substances were removed after curation (substances without structures, water samples, mixtures, incorrectly defined, polymers). "Coding" was performed using identifiers from two central sources, one for AC50 data from ToxCast/Tox21 and the other for AUC data (see section 2.1 above). The machine learning models use fingerprints with extended connectivity with a maximum diameter of 6, generated by the Chemistry Development Kit. Predictions are evaluated using a probability threshold, where a prediction of 0.5 or higher defines the chemical as active. Sets of nine agonist pathway analyses or seven antagonist AR pathway analyses were used for predictions: if more than half of the models in the group (i.e., at least five models for agonist pathway analysis or four models for antagonist pathway analysis) predict the compound as active, it is considered active. A limitation of this modelling is the consistent prediction of steroid chemicals as active, regardless of the test group, but this is a common limitation of

models that use fingerprints as molecular descriptors and may be overcome by analyzing other descriptors in future studies.

The model by Allen *et al.* uses an automated search for the maximum common substructure to generate 2D structural alerts from AR data from ToxCast. The model makes predictions based on 87 structural alerts for two receptors—AR and the glucocorticoid receptor (Glucocorticoid Receptor) — again using ToxCast data [30].

The models presented above were developed primarily using machine learning approaches, mainly quantitative structure–activity relationship (QSAR) classification methods. In the terminology of drug design and computational toxicology, these approaches are referred to as ligand-based methods [31]. However, models have also been developed using structure-based methods, primarily involving molecular docking of ligands in the active site of the receptor. The most significant of these models are described below.

The models of Trisciuzzi *et al.* [32] use a strategy based on the three-dimensional structure of the AR, selected from structures currently available in the Protein Data Bank (PDB; see Section 2.3 below for more information on the PDB dataset). From the nine initially selected structures, the one based on the complex with PDB ID 2PNU was identified as the best-performing structure-based classification model. The models were trained on a dataset of 1,689 curated compounds provided by the CoMPARA consortium and integrated with a two-stage applicability domain in order to reduce the number of false negatives. Three groups, each comprising 2,590 compounds extracted from the DUD-E collection (https://dude.docking.org/; last accessed 11 March 2026), were used to evaluate the model and the effectiveness of integrating the applicability domain. The model based on the 2PNU structure was subsequently applied to screen and prioritize two test groups of chemicals: A) a small set of 12 representative androgenic compounds that were accurately classified based on in-depth molecular-level analysis; and B) a large external set of 55,450 chemicals with potential for human exposure. In practice, this represents the largest external test set used in AR modeling studies.

The model of García-Sosa [33] applies deep neural networks (DNN) to molecular descriptors derived from docking (calculated protein–ligand binding), chemical fingerprint distances, and other statistical techniques, achieving good performance as indicated by AUC (0.87) and Matthews correlation coefficient (0.47). Another advantage of the model is the good interpretability of the descriptors and features used. The source code and data are publicly available on GitHub. In this publication, García-Sosa also provides a critical analysis of the training data used in the CoMPARA project, describing it as highly imbalanced, with a large number of non-binders (N = 1,468) compared with binders (N = 205). Such imbalances in datasets can strongly influence the performance of machine learning algorithms. Therefore, the author balances the training set in his model to ensure an equal number of compounds acting as binders and non-binders. The same author, together with Maran combined multivariate logistic regression, protein structural docking scores, extended connectivity fingerprints, and naïve Bayesians to predict compounds that bind to or disrupt the AR, improving model accuracy for toxicity prediction [34]. CoMPARA data set has been used also by Collins et al. [35] who applied RF method and developed models that predict androgen activity with high predictive capabilities (balanced accuracies of 93%, coverage of 89%).

Other models for predicting AR toxicity using different datasets and methodologies have also been described in the scientific literature. Wahl and Smiesko [36] used molecular dynamics to improve prediction of AR antagonist binding, but distinguishing between antagonists and agonists remained challenging. Gupta and Rana [37] developed a QSAR-based multilevel ensemble model to predict toxicity of small drug molecules binding to the AR, using a dataset of 10,273 molecules with feature selection and k-fold cross-validation. Banerjee *et al.* [38] utilized 2D-QSAR and chemical Read-Across models to predict AR binding affinity based on hydrophobicity, steroidal nucleus, bulkiness, and hydrogen bond donors and validated using stringent criteria and supported by pharmacophore mapping and molecular simulations. Ollitrault *et al.* [39] proposed QGexAR models to predict AR binding with a consensus balanced accuracy of 0.725 to 0.840 using gene expression profiles from LINCS (Library of Integrated Network-Based Cellular Signatures) database of NIH and machine learning algorithms.

The above analysis illustrates that the machine learning based approaches currently provide the most accurate predictions of chemical interactions with the AR. The CoMPARA project described above, and the consensus models generated within it using multiple machine learning algorithms, provide reliable predictions for thousands of compounds [40]. The integration of structure-based methods, such as molecular docking, further improves model performance [33, 41], particularly because structure-

based approaches offer easily interpretable models and represent a viable alternative for prediction in computational toxicology. Therefore, combining different computational approaches (ligand-based and structure-based) within a single framework significantly improves the predictive power of models and supports the development of consensus models for predicting AR toxicity.

CONCLUSIONS

Research on toxicity mediated through the AR highlights the biological and toxicological significance of this receptor. AR plays a key role in maintaining endocrine homeostasis and reproductive health, while also serving as a major molecular target through which numerous chemicals may exert adverse effects. Consequently, AR-mediated toxicity represents a well-established research field supported by extensive experimental and computational investigations.

A variety of computational approaches have been developed to predict AR-related toxicity, with consensus modeling strategies generally demonstrating the best predictive performance. The reviewed studies illustrate the effectiveness of multiple methodologies, with machine learning algorithms dominating current research. Approaches such as artificial neural networks, support vector machines, random forest models, k-nearest neighbor algorithms, and structural alert systems are widely applied to identify potential AR modulators. Among structure-based methods, molecular docking is commonly used to evaluate ligand–receptor interactions and predict binding affinity. In contrast, molecular dynamics simulations are applied less frequently due to their high computational cost, although they provide valuable mechanistic insight into receptor–ligand stability and conformational changes. Despite the availability of advanced computational techniques, the success of *in silico* modeling strongly depends on the availability of reliable and representative experimental data on AR-mediated toxicity. Current efforts focus on optimizing traditional *in vitro* high-throughput screening assays and complementing them with additional experimental approaches capable of capturing a broader range of biological responses across the signaling pathways associated with AR activity. The generation of cross-species toxicity data further contributes to improved understanding of endocrine mechanisms in both human and animal systems. Additionally, careful dataset curation and balancing remain essential to ensure adequate representation of chemical diversity and to improve model robustness.

However, despite the development of reliable predictive models for chemicals with potential ED activity, their regulatory implementation remains limited. By 2022, only a small number of pesticide active substances had been evaluated for endocrine-disrupting properties by EPA, and none had been formally classified as endocrine disruptors [41]. These findings emphasize the need for greater integration of predictive tools into regulatory risk assessment frameworks. In this context, new approach methodologies offer considerable potential to support chemical safety evaluation, prioritize compounds for experimental testing, reduce reliance on animal experiments, and lower the overall cost and time required for chemical risk assessment.

Acknowledgement: *The authors acknowledge the financial support by the project BG-RRP-2.017-0038-C02 "Web-based platform for in silico assessment of toxic effects of chemicals on human health and the environment" funded by the European Union through the NextGenerationEU instrument under the Recovery and Resilience Facility in implementation of investment C2I2: "Enhancing the innovation capacity of the Bulgarian Academy of Sciences in the field of green and digital technologies".*

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