

Comparative Analysis of Substances Recommended for Validation of *In Vitro* AR Binding and *In Vitro* AR Transcriptional Activation (TA) Assays

In both the *in vitro* AR binding and the *in vitro* AR TA assay BRDs, a list of recommended substances for future validation efforts were proposed (**Section 12**). Each list included substances covering a range of activities (from highly active to non-active) in the respective assays. Ideally, to allow for a direct comparison of performance and reliability between binding and TA assays, the same set of substances should be tested in both types of assays. To help in the selection of a coordinated list, the binding and TA responses for substances included in each list are provided in **Table 1**. The data are organized alphabetically by substance.

For each substance, the nature of the response (positive or negative) and the corresponding number of assays in which it was tested is (shown in parenthesis) are provided. In cases where discordant data were obtained across assays, two values are provided in parenthesis. The first value indicates the number of assays in which the substance was positive, while the second value indicates the number of assays in which the substance was tested. In determining the call for each assay, a positive response was assigned if at least one positive test was reported. This approach was used to be consistent with the approach used in the BRD and was based on the observation that negative test data (in situations where discordant test results within an assay were reported) appeared to be more often the result of inadequate dose selection rather than a lack of intrinsic assay sensitivity. The number of plus signs (for positive substances) indicates the relative potency of the substance in the assay considered. In the AR binding assay, three pluses indicate that the median RBA value across assays was greater than 0.1, two pluses indicate the median RBA value ranged between 0.0001 and 0.1, and one plus indicates that the RBA value was less than 0.0001. Since there was little quantitative data for the TA assays, it was not possible in most cases to assign an indication of potency. In the absence of EC₅₀ data (for agonism assays), a single plus was used to identify a positive response. In cases where a quantitative measure of activity was available, an additional plus was assigned if the median EC₅₀ value was greater than 0.001 μM and two pluses if the EC₅₀ value was lower than 0.001 μM . For antagonism TA assays, an IC₅₀ value lower than 1 μM was assigned three pluses ; above 1 μM two pluses and one plus if positive but no IC₅₀ value was reported.

Substances that were suggested for validation in each of the assays are indicated with an asterisk. Currently, 22 substances are proposed in common for testing in *in vitro* AR binding and TA assays.

Table 1 Comparative Activity of Recommended Validation Substances in *In Vitro* AR Binding and *In Vitro* AR TA Assays

Substance	CASRN	AR Binding Assays [†]	AR TA Agonism Assays ^{††}	AR TA Antagonism Assays ^{†††}
4-Androstenedione	63-05-8	+++ (2)*	+++ (3)*	- (1)*
Atrazine	1912-24-9	++ (1)*	- (1)*	- (1)*
Bicalutamide	90357-06-5	+++ (2)	+ (2)*	+++ (4)*
Chlordecone	143-50-0	+ (2)*	- (2)*	++ (1/2)*
Corticosterone	50-22-6	+ (1)*	- (1)*	not tested
Cortisol	50-23-7	- (2)*	++ (3/5)*	not tested
Cyanoketone	4248-66-2	- (1)*	not tested	not tested
Cyproterone acetate	427-51-0	+++ (5)*	+ (7)*	+++ (5)*
<i>o,p'</i> -DDT	789-02-6	+ (2)*	- (2)*	+ (2)*
<i>p,p'</i> -DDE	72-55-9	++ (2)*	+ (3/4)	++ (5/6)*
<i>p,p'</i> -DDT	50-29-3	++ (2)*	- (1)*	+ (2)*
Dexamethasone	50-02-2	- (2)*	+ (2/4)	not tested
Diethylstilbestrol	56-53-1	++ (3/4)*	- (3)*	+++ (2)*
5a-Dihydrotestosterone	521-18-6	+++ (7)*	++ (6)	not tested
17 -Ethinyl estradiol	57-63-6	+++ (2)*	+ (1/2)	not tested
17 -Estradiol	50-28-2	+++ (8)*	++ (11/12)*	++ (4)*
Estrone	53-16-7	+++ (1)*	++ (2)*	not tested
Fenitrothion	122-14-5	not tested	+ (2)*	+ (2)*
Fluoxymestron	76-43-7	+++ (1)*	+ (1)*	- (1)*
Flutamide	13311-84-7	++ (3/5)	- (7)*	++ (5)*
4-Hydroxyandrostenedione	566-48-3	+++ (1)*	not tested	not tested
Hydroxyflutamide	52806-53-8	+++ (6)	++ (6/7)*	+++ (9)*
17 -Hydroxyprogesterone	68-96-2	++ (2)*	not tested	not tested
Levonorgestrel	797-63-7	+++ (1)*	+++ (3)*	not tested
Lindane	58-89-9	+ (1)	- (1)	- (2)*
Linuron	330-55-2	++ (4)*	+ (1)*	++ (2)*
Medroxyprogesterone acetate	71-58-9	+++ (4)*	+ (4)*	- (1)*
Melengestrol acetate	2919-66-6	+++ (1)*	not tested	not tested
Methoxychlor	72-43-5	+ (2)*	- (2)*	+ (2)*
Methyltestosterone	58-18-4	+++ (2)	+++ (2)*	not tested
Mifepristone	84371-65-3	+++ (1)	++ (4/5)*	+++ (2)*
Nilutamide	63612-50-0	+++ (2)	+ (1)*	+++ (2)*
Pregnenolone	145-13-1	+ (1/2)*	not tested	+ (1)
Procymidone	32809-16-8	+ (1)*	- (1)*	++ (2)*
Progesterone	57-83-0	+++ (8)*	++ (7/10)*	+ (2/4)*

Substance	CASRN	AR Binding Assays [†]	AR TA Agonism Assays ^{††}	AR TA Antagonism Assays ^{†††}
Spironolactone	52-01-7	+++ (1)*	+ (2)*	+++ (2/3)*
Testosterone	58-22-0	+++ (8)*	+++ (12)*	+ (1)*
Trenbolone	10161-33-8	+++ (1)*	not tested	not tested
Vinclozolin	50471-44-8	+ (3)*	- (1)*	+++ (3)*

A single number in parenthesis indicates the number of assays in which the substance was tested. Two numbers in parenthesis indicate that the outcomes in the assays for that substance were discordant; the first number indicates the number of positive responses and the second the number of assays in which the substance was tested.

- indicates that the substance was negative in the assay.

+ indicates that the substance was positive in the assay.

[†]+++ indicates an RBA value greater than 0.1; ++ indicates an RBA value between 0.1 and 0.0001; + indicates an RBA value below 0.0001.

^{††}+++ indicates an EC₅₀ value that is smaller than 0.001; ++ indicates an EC₅₀ value that is higher than 0.001; + indicates a positive response but where an EC₅₀ value was not reported.

^{†††}+++ indicates an IC₅₀ that is lower than 1 µM, ++ indicates an IC₅₀ that is greater than 1 µM; indicates that the substance is an antagonist but no IC₅₀ value was reported.

* Substances suggested for validation in the designated assay.

For many substances tested, there was concordance between the response in the binding and TA assays. Three substances (atrazine, corticosterone and lindane) were positive in binding assays but negative in TA assays for agonism or antagonism. Two substances (cortisol, dexamethasone) were negative for binding but positive for TA agonism. Eight substances were positive for binding, negative for TA in the agonism assay, but positive for TA in the antagonism assays. These substances were chlordecone, *o,p'*-DDT, *p,p'*-DDT, diethylstilbestrol, flutamide, methoxychlor, procymidone, and vinclozolin. It is interesting to note that many of this last group of substances are chlorinated hydrocarbons.

