

## 5.0 DATA ON *IN VITRO* ER BINDING ASSAYS

### 5.1 Introduction

Methods and ER binding data were collected from 72 publications reporting studies in which the competitive binding of a substance to the ER was measured and RBA values were included or could be calculated. When provided, the specific information extracted for each substance included its name, source, purity, methodological details, relevant binding data ( $K_i$ ,  $IC_{50}$ , and/or RBA values for positive studies, highest dose tested [HDT] for negative studies), and the citation. For studies in which chemical structures only were provided, every effort was made to identify the name of each substance tested. No attempt was made to identify the source and purity of a substance if the investigators did not provide such information. If available, a Chemical Abstract Service Registry Number (CASRN) was entered for each substance. This identifier was obtained from various sources, including the publication, the National Library of Medicine's ChemID database, and *The Merck Index*. Chemical name synonyms were entered for substances that were identified in the literature by more than one name, and for substances where the literature name may have been different from the generic name. All substances with the same CASRN were listed under the same name, regardless of the name that was used in the original publication. **Appendix C** provides information on the names, synonyms, CASRN, and chemical/product class, where available, for each substance, while **Appendix D** contains the *in vitro* ER binding data sorted alphabetically by substance name.

### 5.2 Availability of Detailed *In Vitro* ER Binding Protocols

The scientific methods presented in the publications containing data from competitive *in vitro* ER binding studies provided various levels of detail. To the extent possible, the most important method parameters were extracted from each publication and summarized in **Appendix A**. Details about the following method parameters are included in the Appendix to the extent this information was available:

- Preparation of the receptor (e.g., species or cell line, buffer used for preparation of cytosol, protein concentration of cytosol);
- Competitive binding assay (e.g., concentration of radiolabeled estrogen, solvent used to dissolve the test substance, concentration range of the test substance, number of replicates within an assay, number of times assay was repeated);

- Separation of ligand (e.g., type of slurry used, incubation time, temperature); and
- Data calculations (e.g., method used for calculating data, data format).

### 5.3 Availability of *In Vitro* ER Binding Data

ER binding data were collected for a total of 638 substances tested in competitive binding studies with ER obtained from the following sources:

1. Rat uterine cytosol (RUC);
2. Mouse uterine cytosol (MUC);
3. Rabbit uterine cytosol (RBC);
4. Cytosol from human adenocarcinoma MCF-7 cells (MCF-7 cytosol);
5. Intact MCF-7 cells (MCF-7 cells);
6. Semi-purified human ER protein (hER<sub>α</sub>);
7. Semi-purified human ER protein (hER<sub>β</sub>);
8. Semi-purified rat ER protein (rER<sub>α</sub>);
9. Semi-purified human ER as measured by FP (hER<sub>α</sub>-FP);
10. Glutathione-S-transferase fusion proteins consisting of the def domains of the human ER receptor (GST-hERdef);
11. Glutathione-S-transferase fusion proteins consisting of the def domains of the mouse ER receptor (GST-mERdef);
12. Glutathione-S-transferase fusion proteins consisting of the def domains of the lizard (anole) (GST-aERdef);
13. Glutathione-S-transferase fusion proteins consisting of the def domains of the chicken (GST-cERdef); and
14. Glutathione-S-transferase fusion proteins consisting of the def domains of the rainbow trout (GST-rtERdef).

In all studies, competitive binding was measured by the displacement of radiolabeled ( $[^3\text{H}]$  or  $[^{125}\text{I}]$ ) 17 $\beta$ -estradiol from the ER-estrogen complex or by the change in anisotropy of the fluorescent ER-estrogen complex by the test substance. **Appendix D** presents the extracted and compiled data sorted first by substance name and then by assay. In those cases in which the RBA value was not provided in the citation, this value was calculated, when possible, from

provided IC<sub>50</sub> values. Not all of these values were reported in all publications. In some publications, neither the IC<sub>50</sub> nor the RBA values were presented. In many of these cases, the binding of the test substance to the ER over a range of concentrations was presented graphically, so that the IC<sub>50</sub> values of 17 -estradiol and the test substance could be estimated. These estimated IC<sub>50</sub> values and corresponding calculated RBA values are italicized in **Appendix D**. For substances that did not bind sufficiently well to the ER to displace the reference estrogen (i.e., an IC<sub>50</sub> value could not be calculated), the only parameter that could be entered into the database was the HDT.

#### **5.4 *In Vitro* ER Binding Assay Results for Individual Substances**

The number of *in vitro* ER binding assays in which each substance was tested is provided in **Appendix E**. These data, shown in **Table 5-1**, are summarized by assay and ranked according to the number of substances tested. Of the 638 substances tested in the 14 different *in vitro* ER binding assays, the majority of substances (376 or 59%) had been tested in the RUC assay. Only 133 (21%) of these substances had been tested in the next most frequently used assay, hER . For five of the 14 assays (hER -FP, RBC, rER , GST-mER def, GST-cERdef), published data on less than 50 substances for each assay were located.

As presented in **Table 5-2**, only 14 (excluding the reference compound 17 -estradiol) of the 638 substances (2.4%) had been tested in 10 or more assays, and of these, only three substances (0.47%) had been tested in all 14 assays. As stated in **Section 3**, 94% (600) of the substances in the database had been tested in one to five assays, with 63% (403) tested in one assay only.

#### **5.5 Use of Coded Chemicals and Compliance with Good Laboratory Practice (GLP) Guidelines**

Based on the available information in the scientific literature, it appears that the published *in vitro* ER binding assay studies neither used coded chemicals nor were they conducted in compliance with GLP guidelines (see **Section 8**).

**Table 5-1** Number of Substances Tested in Various *In Vitro* ER Binding Assays (638 Substances)\*

Assay	Number of Substances Tested	% of Total Substances Tested
RUC	376	59%
hER	133	21%
hER	101	16%
GST-hER def	99	16%
MCF-7 cytosol	94	15%
GST-rtERdef	86	13%
GST-aERdef	85	13%
MUC	75	12%
MCF-7 cells	66	10%
hER -FP	48	8%
RBC	45	7%
rER	37	6%
GST-mER def	34	5%
GST-cERdef	34	5%

\*Assays sorted according to the number of substances tested.

**Table 5-2** Substances Tested in Ten or More *In Vitro* ER Binding Assays\*

Substance	Number of Assays	Number of Publications
17 -Estradiol	14	72
Diethylstilbestrol	14	30
Bisphenol A	14	22
Tamoxifen	14	13
Estrone	13	12
<i>p,p'</i> -Methoxychlor	13	11
4-Hydroxytamoxifen	13	10
<i>o,p'</i> -DDT	12	9
Estriol	12	9
Genistein	11	9
Coumestrol	11	7
Kepone	10	7
2,2-Bis( <i>p</i> -hydroxyphenyl)-1,1,1-trichloroethane (HPTE)	10	7
5 -Dihydrotestosterone	10	6
Zearalenone	10	5

\*Substances sorted by the number of assays tested and then by the number of publications.