3.0 CHARACTERIZATION OF SUBSTANCES TESTED IN *IN VITRO* ER TA ASSAYS

3.1 Introduction

In vitro ER TA data were collected for a total of 698 substances that had been evaluated for their ability to activate or inhibit transcription of estrogen-inducible genes. The data were obtained from 86 peer-reviewed, scientific journal articles and two submitted reports containing unpublished data. Relevant information on the substances tested (i.e., chemical name, Chemical Abstract Service Registry Number [CASRN], chemical supplier or source, and purity) was extracted from the publications and reports and entered into a database. While the two unpublished reports included all of this information, many of the publications did not. For publications in which only chemical structures were provided, every effort was made to identify chemical names and CASRNs. The CASRNs were obtained from various sources, including the National Library of Medicine's ChemID database and *The Merck Index*. However, no attempt was made to determine the source or purity of test substances if this information was not provided. Different studies often used a unique chemical name for the same substance. When this occurred, the most commonly used chemical name was chosen and assigned to the substance, regardless of the name used in a particular publication or report, and the unique chemical nomenclature was entered into the database as a synonym (**Appendix C**).

3.2 Rationale for Selection of Substances/Products Tested in *In Vitro* ER TA Assays

The majority of the *in vitro* ER TA studies described in this BRD were conducted between 1995 and 2001. In these studies, various substances were tested for their potential to act as ER agonists or antagonists or used to study characteristics of specific ER-sensitive cell models. Most of the substances tested were industrial chemicals, pesticides, phytoestrogens, and environmental contaminants. Typically, these studies also reported on the utility of a particular *in vitro* ER TA assay as a screen for endocrine disruptor activity.

Some of the tested substances, particularly the natural estrogens (e.g., estrone, estriol) and synthetic anti-estrogens (e.g., ICI 182780, ICI 164384, hydroxytamoxifen), were studied to obtain a better understanding of their different potencies and biological activities. Some of the synthetic anti-estrogens were investigated in *in vitro* ER TA studies to evaluate their mechanisms

of action as therapeutic agents, and to determine why some of these substances have both agonist and antagonist activities.

A number of the 17β-estradiol analogs (e.g., estratriene-1,17β-diol, 1-aminoestratriene-17β-ol) were investigated to determine SAR for the development of quantitative SAR (QSAR) models. Certain pesticide and polycyclic aromatic hydrocarbon metabolites (e.g., *p,p'*-DDE, *p,p'*-DDD, 1-hydroxy-benzo[a]pyrene, 3-hydroxy-benzo[a]pyrene, 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane) were tested to determine which metabolite enhanced or inhibited ER-induced TA and/or to provide information relating to SAR.

A few of the substances tested in *in vitro* ER TA assays were selected to address basic research questions regarding the nature of ER binding and TA processes. With the discovery of a second subtype of the ER (i.e., ERβ) in 1996, some of these substances were tested to determine whether they interacted preferentially with ERα or ERβ. These types of studies investigated the steps involved in ER activation or inhibition of target genes, and used both naturally-occurring ER-binding substances (i.e., steroids and phytoestrogens) and synthetic ER agonists and antagonists.

3.3 Chemical and Product Classes Tested

Chemical and product class information for the substances tested in *in vitro* ER TA assays is provided in **Appendix C**. Substances were assigned to chemical classes based on available information from standardized references (e.g., *The Merck Index*) and from an assessment of their chemical structures. As shown in **Table 3-1**, the chemical classes that have been tested most extensively are polychlorinated biphenyls, organochlorines, polycyclic aromatic hydrocarbons, phenolic steroids, nonphenolic steroids, phthalates, phenols, and alkylphenols. Of the 698 substances included in this BRD, 682 could be assigned to at least one chemical class, while 76 could be assigned to two chemical classes, and two could be assigned to three chemical classes.

Product classes were assigned based on information from *The Merck Index* and the National Library of Medicine's ChemFinder. A wide range of product classes is represented, as shown in **Table 3-2**. The most common product classes tested in *in vitro* ER TA assays are pesticides

Table 3-1 Major Chemical Classes Tested in *In Vitro* ER TA Assays*

Chemical Class	Number of Substances
Alkylphenol	30
Alkylphenyl ether	6
Aromatic hydrocarbon	6
Benzophenone	10
Biphenyl	12
Bisphenol	19
Chalcone	5
Chlorinated cyclodiene	7
Diphenylalkane	5
Flavone	10
Hydroxybenzoic acid	4
Imidazole	4
Isoflavone	19
Nitrile	4
Organochlorine	71
Organophosphate	4
Organothiophosphate	6
Paraben	6
Phenol	39
Phthalate	37
Polybrominated diphenyl ether	17
Polychlorinated biphenyl	82
Polycyclic aromatic hydrocarbon	69
Pyrethrin	6
Pyrethroid ester	5
Resorcyclic acid lactone	6
Salicylic acid	9
Steroid, nonphenolic	47
Steroid, phenolic	55
Stilbene	18
Thiophene	4
Triazine	8
Triphenylethylene	4
Urea	4

*Includes only those chemical classes for which at least four substances had been tested in *in vitro* ER TA assays.

(including their metabolites and degradation products), pharmaceuticals, chemical intermediates, dielectric fluid components, natural products (including several phytoestrogens), and plasticizers. Of the substances included in this BRD, 266 had no known commercial use, so were not classified within a product class.

Table 3-2 Major Product Classes Tested in *In Vitro* ER TA Assays*

Product Class	Number of Substances
Antimicrobial agent or Disinfectant	9
Antioxidant	11
Chemical intermediate	49
Cosmetics	3
Dielectric fluid	41
Dye, Pigment, or Stain	4
Flame retardant/Flame retardant additive	18
Flavor	7
Food or Food additive	5
Fragrance, Fragrance ingredient, or Perfume	8
Monomer	3
Natural product (e.g., phytoestrogens)	30
Pesticide (including metabolites and degradation products)	132
Pharmaceutical/Pharmaceutical additive	111
Plant growth regulator	3
Plasticizer	23
Preservative	15
Reagent (analytical, colorimetric, sulfhydryl)	3
Solvent	12
Surfactant	5
UV light absorber	9
Not classified	266

*Includes only those product classes for which at least three substances had been tested in *in vitro* ER TA assays.