Organisation for Economic Co-operation and Development

ENV/JM/MONO(2018)26

Unclassified

English - Or. English

19 September 2018

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) FOR ESTROGENICITY OF THE SUBSTITUTED PHENOLS

Series on Testing and Assessment
No. 290

JT03435852

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. 290

CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) FOR OESTROGENICITY OF THE SUBSTITUTED PHENOLS

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 2018

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 36 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in twelve different series: **Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides; Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; Safety of Manufactured Nanomaterials;** and **Adverse Outcome Pathways**. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/chemicalsafety/).

This publication is available electronically, at no charge.

Also published in the Series on testing and Assessment link

For this and many other Environment,
Health and Safety publications, consult the OECD's
World Wide Web site www.oecd.org/chemicalsafety/

or contact:

OECD Environment Directorate,
Environment, Health and Safety Division
2, rue André-Pascal
75775 Paris cedex 16
France
Fax : (33-1) 44 30 61 80
E-mail : ehscont@oecd.org

FOREWORD

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was developed by Canada and the United States for illustrating practical use of IATA and submitted to the 2017 review cycle of the IATA Case Studies Project. This case study was reviewed by the project team. The document was endorsed at the 2nd meeting of the Working Party on Hazard Assessment in June 2018.

The following three case studies were also reviewed in the project in 2017 and are published with this case study:

1. PRIORITISATION OF CHEMICALS USING THE INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA)-BASED ECOLOGICAL RISK CLASSIFICATION, ENV/JM/MONO(2018)27, Series on Testing & Assessment No. 291.
2. CASE STUDY ON GROUPING AND READ-ACROSS FOR NANOMATERIALS GENOTOXICITY OF NANO-TiO_2, ENV/JM/MONO(2018)28, Series on Testing & Assessment No. 292.
3. A CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR SUB-CHRONIC REPEATED-DOSE TOXICITY OF SIMPLE ARYL ALCOHOL ALKYL CARBOXYLIC ESTERS: READ-ACROSS, ENV/JM/MONO(2018)29, Series on Testing & Assessment No. 293.

These case studies are illustrative examples, and their publication as OECD monographs does not translate into direct acceptance of the methodologies for regulatory purposes across OECD countries. In addition, these cases studies should not be interpreted as official regulatory decisions made by the authoring member countries.

A considerations document summarizing the learnings and lessons of the review experience of the case studies is published with the case studies:

> REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) -Third Review Cycle (2017) - ENV/JM/MONO(2018)25, Series on Testing & Assessment No. 289.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

LIST OF ABBREVIATIONS

AC50	Concentration at 50% of maximum activity
ADE	Applied Dose Equivalent
AER	Activity Exposure Ratio
AO	Adverse Outcome
AOP	Adverse Outcome Pathway
BER	Bioactivity Exposure Ratio
CERAPP	Collaborative Estrogen Receptor Activity Prediction Project (U.S.)
CEPA	Canadian Environmental Protection Act
CMP	Chemicals Management Plan
DSL	Domestic Substances List
EDSP	Endocrine Disruptor Screening Program (U.S. EPA)
EPA	Environmental Protection Agency (U.S.)
ER	Estrogen Receptor
ERE	Estrogen Response Element
ESRAB	Existing Substances Risk Assessment Bureau (Health Canada)
GSM	Global Similarity Method
HPG	Hypothalamic-Pituitary-Gonadal
HTTK	High Throughput Toxicokinetics
HTS	High Throughput Screening
IATA	Integrated Approaches for Testing and Assessment
IPCS	International Programme on Chemical Safety
IVIVE	*In vitro* to *in vivo* extrapolation
KE	Key Event
KER	Key Event Relationship
LEL	Lowest Effect Level
LSM	Local Similarity Method
LUT	Look-up table
MIE	Molecular Initiating Event
MOA	Mode of Action
NAM	New Approach Methodology
NCCT	National Center for Computational Toxicology (U.S.)
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (U.S.)
NTP	National Toxicology Program (U.S.)
OECD	Organisation for Economic Cooperation and Development
QA	Quality assurance
QC	Quality control
QSAR	Quantitative Structure-Activity Relationship
VTG	Vitellogenin
WHO	World Health Organization

Disclaimer: This case study has been developed for illustrative purposes only and should not to be interpreted as a regulatory decision by Health Canada or the U.S. EPA. Further, it is not intended to inform the EPA Endocrine Disruption Screening Program (EDSP) or be interpreted as the hazard characterisation for the Health Canada risk assessment.

INTRODUCTION

This case study is intended to offer practical insights to inform the development of guidance for deriving and applying Integrated Approaches for Testing and Assessment' (IATA) in a regulatory context. The case study is the result of a collaborative effort by staff at both the Existing Substances Risk Assessment Bureau (ESRAB) at Health Canada and the National Center for Computational Toxicology (NCCT) at the U.S. Environmental Protection Agency (EPA). The case study focuses on a set of substituted phenols (hindered and non-hindered) and uses IATA to examine their estrogenic potential. The selected phenols used in this case study were of particular interest because they are listed on the Canadian Domestic Substances List (DSL)[1] and will be addressed under the third phase of Canada's Chemicals Management Plan (CMP)[2]. The final approach applied for the CMP screening risk assessment report currently under development by Health Canada is considered separate from this document and the approaches taken are subject to change. This document is not intended to provide complete characterisation of health effects for the CMP phenolic substances (target chemicals), nor does it provide information regarding their exposure for the general population of Canada.

The goal of this case study is to demonstrate that *in silico* and *in vitro* data can be used to screen for estrogenic potential of chemical substances, and that these data sources provide a good proxy for estimating the *in vivo* point of departure dose. The estrogenic potential of the three target chemicals was determined using an IATA that combines (Q)SAR approaches and data from *in vitro* and *in vivo* studies. (Q)SAR predictions were generated using selected publicly available and commercial models. The *in vitro* high throughput screening (HTS) data from multiple assays were combined into a consensus prediction of estrogenic potential. Extrapolation of HTS bioactivity to an estimated applied dose equivalent (ADE) was performed through the application of reverse dosimetry. For the target substance that showed estrogenic potential, the ADE was compared to effect levels from traditional *in vivo* animal studies to demonstrate the utility of these HTS data for use during prioritisation and assessment. The methods and application of the IATA illustrated here may broadly support priority setting for further evaluation as well as hazard characterisation for risk assessment.

1 The Domestic Substances List - http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=5f213fa8-1
2 Canada's Chemicals Management Plan - https://www.canada.ca/en/health-canada/services/chemical-substances/chemicals-management-plan.html

1. PURPOSE

1.1. Purpose of use of this IATA

This case study provides insights that will be helpful during the refinement of existing guidance on IATA and chemical grouping approaches. It is envisioned that the methods and application of this IATA may support priority setting, which could include prioritisation for further testing and/or information gathering and/or formal risk assessment. Further, it may also support hazard characterisation in the context of regulatory decision making (depending on specific program needs and requirements). The goal of this case study is to demonstrate that *in silico* and *in vitro* data can be used to screen for estrogenic potential of chemical substances, and that these data sources provide a good proxy for estimating the *in vivo* point of departure dose.

This IATA has been performed with a dual purpose. The first, to provide support for the hypothesis (described in Section B, below); the second, to build confidence in the use of data from alternative methods. To this end, a bi-directional approach is used. To provide support for the hypothesis, read-across between target substance and source analogues was conducted in the horizontal dimension (inter-chemical) during analysis of the data matrices. To build confidence for the use of alternative methodologies in risk assessment, data from many different streams (traditional and alternative) were tabulated, integrated and compared in the vertical dimension (intra-chemical).

1.2. Rationale for selection of target chemicals

The objective of this case study is to evaluate the potential for the select set of chemical substances to interact with the endocrine system; specifically, the female reproductive system. Endocrine disruption is defined as being caused by: *an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism or its progeny, or (sub)populations*[3]. Under the Canadian Environmental Protection Act (CEPA, 1999), "a "hormone disrupting substance" means a substance having the ability to disrupt the synthesis, secretion, transport, binding, action or elimination of natural hormones in an organism or its progeny, which are responsible for the maintenance of homeostasis, reproduction, development or behaviour of the organism. Environment and Climate Change Canada and Health Canada scientists consider "hormone disruption" or "endocrine disruption" to be attributable to numerous and diverse mechanisms within an organism, which may include those involving estrogen, androgen, progestrogen, glucocorticoid, mineral corticoid and a myriad of peptide hormone effects, among others[4].

There are chemicals in the environment that have structures that are similar to that of estrogen. Sometimes, these structurally similar chemicals are able to bind to the estrogen receptor (ER) and affect downstream changes that would typically be caused by estrogen. Because estrogen is a powerful hormone, both the quantity of estrogen and the timing of its release are under tight physiological control. Therefore, estrogenic

3 The World Health Organization (WHO) International Programme on Chemical Safety (IPCS)

4 Federal research on hormone disrupting substances as required under the Canadian Environmental Protection Act, 1999. http://www.oag-bvg.gc.ca/internet/English/pet_340_e_37607.html

chemicals can produce confusing signals in the cell that disrupt this control and can lead to adverse effects (including reproductive, developmental and carcinogenic effects). Estrogenic endocrine disruptors have been well studied and are reviewed elsewhere (Kiyama and Wada-Kiyama 2015). The rationale for selecting three substituted phenols for this case study is that substituted phenols are included among the substances with estrogenic potential, which is the endpoint of interest of this IATA. Further, these substances were of particular interest because they are listed on the Canadian DSL and will be addressed under the third phase of Canada's CMP.

1.3. Target chemicals: the substituted phenols

Three target chemicals were selected for this study: 4-tert-butylphenol, 2,4-di-tert-butylphenol and octabenzone (Table 1). These substances are phenols (i.e., they contain a benzene ring with a hydroxyl) that are substituted (i.e., there are additional 'R' groups around the benzene ring). When the substituents are beside the hydroxyl group, the phenol is considered to be hindered. For example, in Figure 1, a fully hindered phenol would have substituents at both R1 and R5, a partially-hindered phenol would have one substituent at either R1 or R5, and a non-hindered phenol would not have substituents at R1 or R5. Therefore, 4-tert-butylphenol is non-hindered, and 2,4-di-tert-butylphenol and octabenzone are partially-hindered.

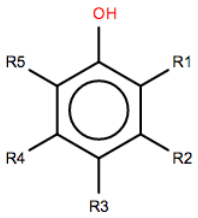
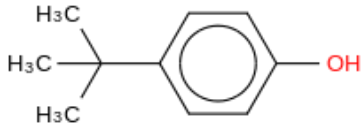
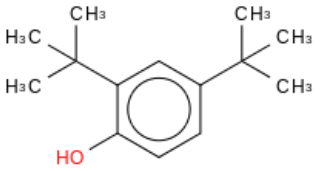
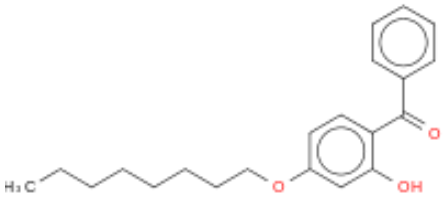


Figure 1. Phenol scaffold.

Table 1. Target chemicals.

Chemical Name	CAS RN	Chemical Structure
Non-hindered		
4-Tert-Butylphenol	98-54-4	
Partially-hindered		
2,4-Di-Tert-Butylphenol	96-76-4	
Octabenzone	1843-05-6	

1.4. Endpoints

The endpoint of interest for this IATA is disruption of the female reproductive system—i.e., the ability to act in the place of estrogen and thus interfere with an organism's normal biochemistry and physiology. Therefore, we selected data from assays that test for estrogen receptor binding and transactivation of the genomic estrogen response element. We also queried algorithms that search the published literature, and *in silico* models that use quantitative structure-activity relationships (QSAR) to make predictions regarding the estrogenic potential of the substance. Collectively, these types of assays and data can be thought of as 'lower tier', which is terminology that indicates that the data were generated using high-throughput screening tools (e.g., *in silico* models, cell-free or cell-based assays). By comparison, higher tier data would be used to identify and characterise specific adverse effects (e.g., an *in vivo*, multi-generational reproductive and developmental toxicity study). The lower tier data that are gathered herein include tests that would be similar to those found in levels 1-3 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters, or tier 1 of the EPA EDSP assessment. Briefly, the OECD framework considers existing data and *in silico* approaches (level 1), *in vitro* screening assays (level 2), and *in vivo* screening assays (level 3); and the EPA EDSP tier 1 battery has considerable overlap with the OECD levels 2-3 and comprises *in vitro* and *in vivo* screening assays. These tier/level designations and corresponding assays are described in greater detail elsewhere 5,6. The *in silico* models used herein include both publically available and proprietary models (summarised in Table 4). The *in vitro* tests used herein include assays that are aimed at detecting endpoints like receptor agonism/antagonism and transcriptional

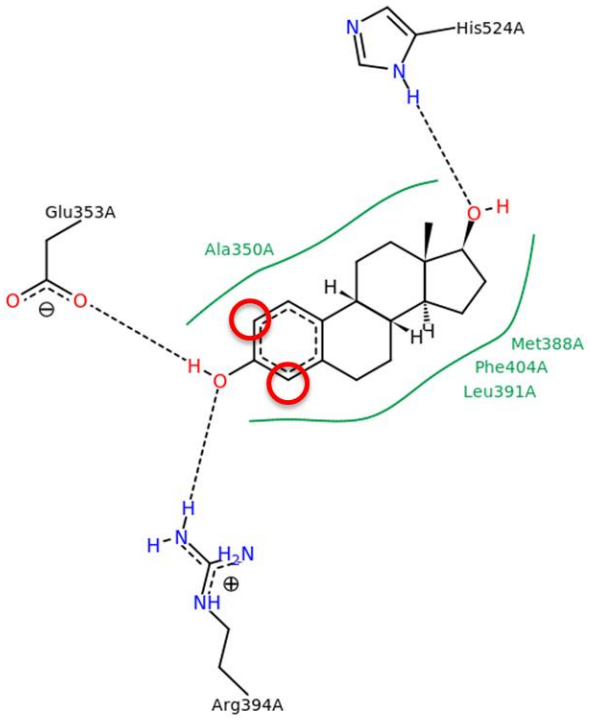
5 http://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm#CONCEPTUAL
6 https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-battery-assays

activation (i.e., ToxCast, CERAPP). The *in vivo* tests considered herein measure organ- and organism-level effects (i.e., the uterotrophic assay, which evaluates the ability of a chemicals to cause an increase in uterine weight, a change that is used a as an indicator of estrogenic potential). The specific data sources queried herein are tabulated in the appended data matrices and are described in greater detail in Section D.2.

Taken together, this case study examines lower tier data to provide support for a hypothesis regarding the estrogenic potential of a set of phenolic substances. These data are used to indicate if a chemical has the potential to alter endocrine function and estimate the *in vivo* dose at which this might occur.

2. HYPOTHESIS FOR PERFORMING IATA

(a) Estrogen, the endogenous ligand of the estrogen receptor (ER), is a non-hindered phenol



(b)

Figure 2). Certain phenols are known to mimic the activity of natural estrogen, which results in the possibility of endocrine disruption. Binding of phenols to ERs occurs due to the presence of the hydroxyl groups (Figure 2b). Therefore, it is expected that the availability of the phenolic hydroxyl group is an important factor in determining the degree and potency of estrogenicity of a substance.

Hypothesis: The estrogenic potential of substituted phenols is determined by the type, number and position of the substituent(s) relative to the phenolic hydroxyl group.

Prediction: Non-hindered phenols are expected to be estrogenic, whereas hindered phenols are expected not to be.

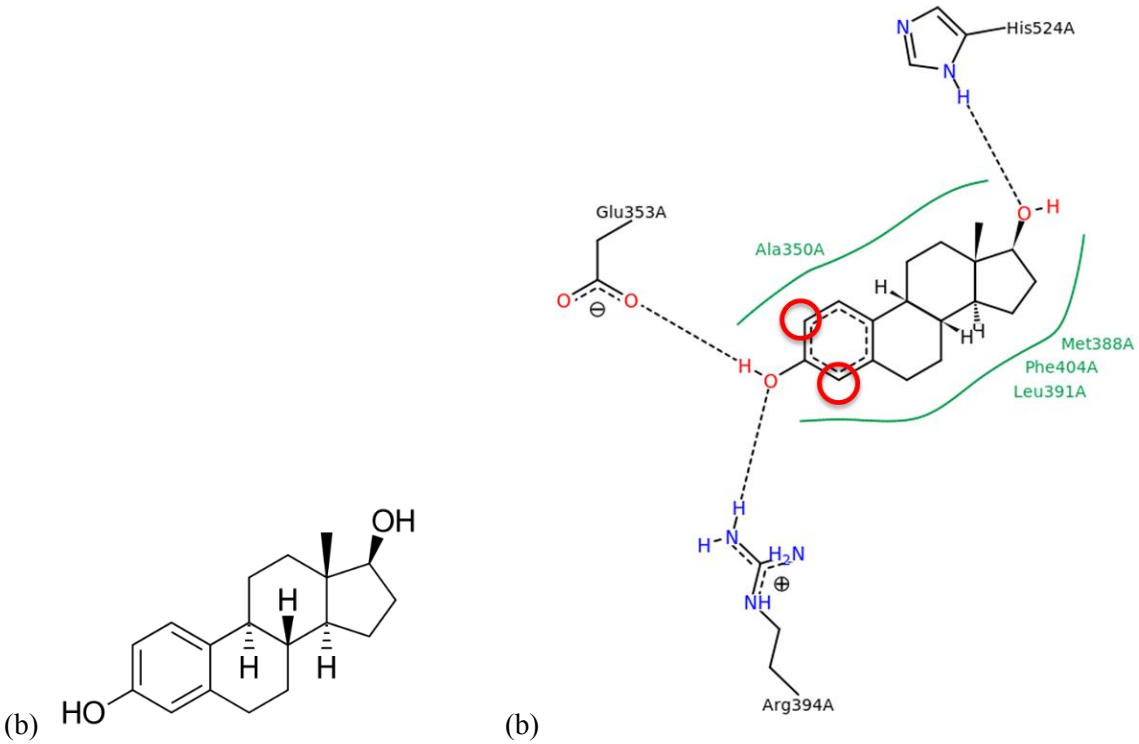


Figure 2. (a) Chemical structure of estradiol (estrogen). (b) The three key residues on the estrogen receptor (ER) for estrogen-ER binding. Red circles indicate substituent locations on hindered phenols.

3. APPROACHES

To provide support for the hypothesis, read-across target substance and source analogues was conducted in the horizontal dimension (inter-chemical) for the purpose of data-gap filling between. To build confidence for the use of alternative methodologies in risk assessment, data from many different streams (traditional and alternative) were tabulated, integrated and compared in the vertical dimension (intra-chemical). The resultant two-dimensional data matrices provide a weight of evidence that has been produced for three target chemicals and their source analogues, and is an integrated approach for making predictions regarding the estrogenicity of the substituted phenols. Aspects that were considered when formulating this approach were: the mode of action (MOA) of estrogen (Section C.1.), and the availability of MOA-relevant adverse outcome pathways (AOPs) and the OECD IATA guidance for the integration of IATA and AOPs (Section C.2., Appendix A). The description of the final IATA workflow for estrogenicity potential of phenols is described in Section C.3 and Figure 4.

3.1. Mode of Action (MOA)

The ER is a ligand-activated nuclear receptor, which means that it can translocate to the nucleus and modify the expression of a variety of genes when it is bound to estrogen. Since it is small and lipophilic, estrogen can enter the cell passively through the plasma membrane. Once inside the cell, estrogen binds to the ER in a specific binding pocket, thereby activating the receptor. Two activated ERs will then combine to form a dimer that will translocate into the cell's nucleus. Once in the nucleus, it will scan the DNA until it finds an estrogen response element (ERE; which is a specific sequence in the DNA to which the estrogen-bound ER dimer can bind). The ERE is associated with an estrogen-responsive gene, whose expression will be altered (typically increased) upon binding of the ER. These changes in gene expression will have a variety of effects on the immediate biology of the cell that can lead to effects in the organism as a whole (Kiyama and Wada-Kiyama 2015). The data sources queried herein target various stages of the MOA in order to establish if a substance of interest is able to interact with or disrupt estrogen signalling (Table 2).

Table 2. Assays used to measure steps in the estrogenic mode of action.

Mode of Action	**Description**	**Relevant Assay(s) and *in silico* models**
1. ER complex formation	Binding of estrogen (or estrogenic substance) to the ER	ToxCast cell-free protein binding assays, CERAPP, OASIS TIMES, ACD Percepta, OECD Toolbox
2. DNA binding and trans-activation of gene expression	Binding of the ER-estrogen complex to the ERE causing changes in estrogen-dependent gene expression	ToxCast ERE reporter gene assays, OASIS TIMES, ACD Percepta
3. Protein expression	Translation of mRNA to protein	Western blot; proteomics (not assessed herein)
4. Altered levels of circulating hormones	Abnormal levels of circulating estrogen and related hormones	ELISA (enzyme-linked immunosorbant assay (not assessed herein)
5. Organ-level changes	E.g., a change in uterine weight	Uterotrophic assay (TG 440); Derek Nexus Expert System
6. Organismal-level changes	E.g., reproductive or developmental effects	Two-generation reproductive toxicity assay (TG 416); Derek Nexus Expert System

3.2. Adverse Outcome Pathway (AOP)

During the development of this case study, the AOP-Wiki[7] was searched for AOPs that include estrogen and/or the ER; however, none of the currently available AOPs could be used to identify a putative adverse outcome for the group of phenols considered here. There were a handful of AOPs that are currently 'under development' and might be useful for this purpose once they are published (Appendix A). Therefore, it is proposed that future applications of this IATA might be informed by information from AOPs, once these relevant AOPs are fully developed and published.

3.3. IATA Workflow

A schematic of the workflow developed and used for this case study on the use of IATA for estrogenicity of the substituted phenols is outlined in Figure 3 and explained in more detail below.

7 AOP Wiki: https://aopwiki.org/

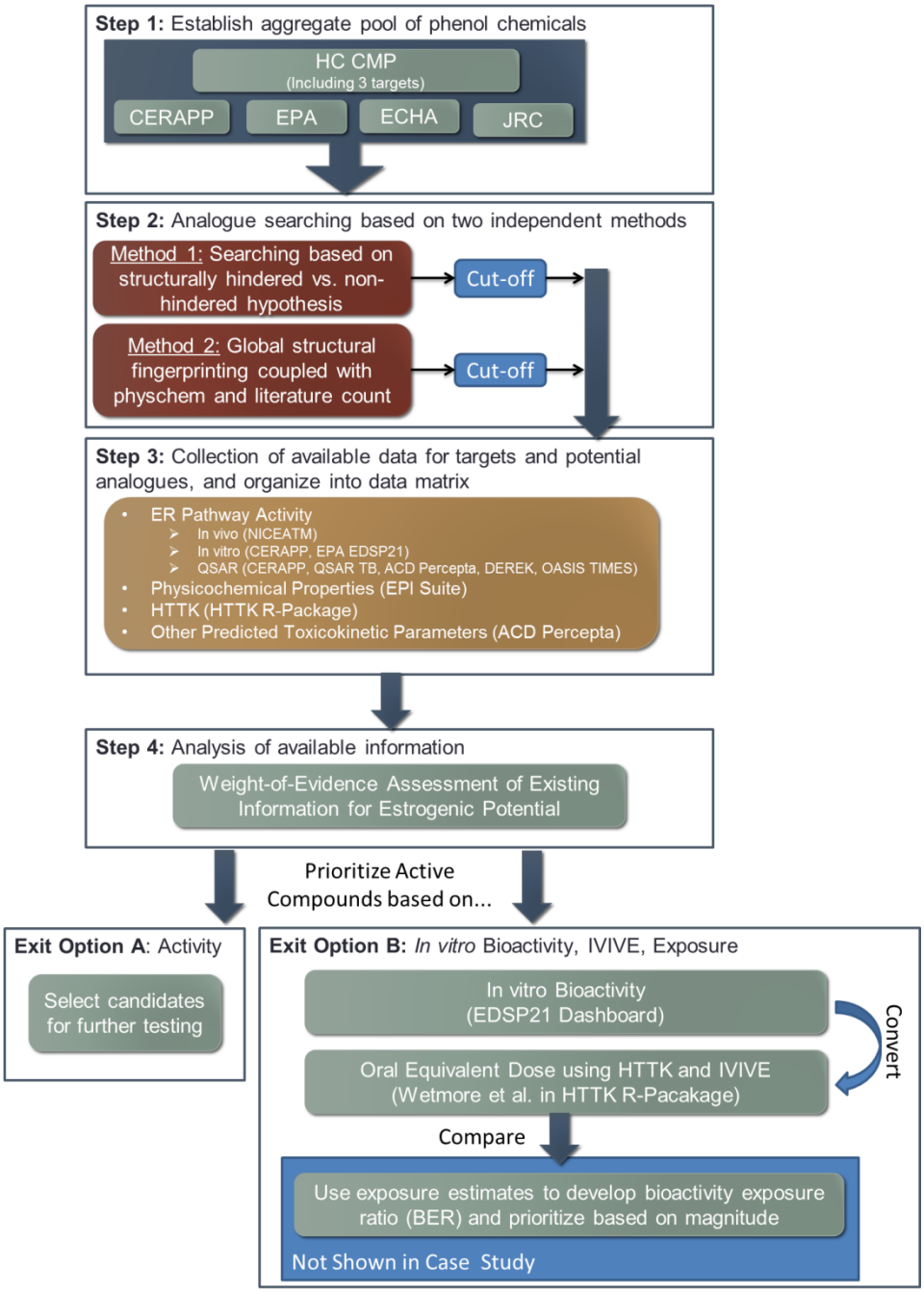


Figure 3. IATA workflow for estrogenicity potential of phenols.

Step 1: Establish aggregate pool of phenol chemicals

An aggregate pool of phenolic chemicals was constructed to facilitate searches aimed at identifying and evaluating potential source analogues for each of the three target chemicals. Phenols were extracted from various international inventories and chemical lists as described in Table 3. The chemical structures used in the workflow were available in the EPA Distributed Structure-Searchable Toxicity (DSSTox) Database (DSSTox). Any potential source analogue needed to have a CAS RN and a discrete

structure in DSSTox to be considered for inclusion into the aggregate pool. An initial pool of approximately 3800 unique phenols was established. There was considerable substance overlap within the selected inventories with the chemical set from the Collective Estrogen Receptor Activity Prediction Project (CERAPP). The motivation for selecting the inventories used was based on the availability of associated, case-study-relevant data. In particular, data sources were chosen based on whether they contained or were specifically for endocrine disrupting chemicals. All phenols on the phase three work plan of Canada's Chemicals Management Plan (CMP) were part of the phenol pool. However, only three CMP target chemicals were selected to demonstrate the practical utility of the IATA workflow. The workflow could be used more broadly to inform the assessment of other phenols.

Table 3. Inventories utilised for establishing the aggregate pool of phenol chemicals.

Inventory	# of Phenols	# of Phenols not in CERAPP	Source
Canadian Chemicals Management Plan Phase 3 List (including 3 targets substances)	128	1	https://www.canada.ca/en/health-canada/services/chemical-substances/third-phase-chemicals-management-plan.html#s3
Collective Estrogen Receptor Activity Prediction Project (CERAPP)	3711	0	https://www.epa.gov/chemical-research/cerapp-collaborative-estrogen-receptor-activity-prediction-project-0
EPA Endocrine Disruptor Screening Program Data Availability List	672	6	Personal communication
National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods (NICEATM) Uterotrophic DB	54	0	(Kleinstreuer, et al. 2016a)
European Chemicals Agency (ECHA) Community rolling action plan (CoRAP)	24	0	https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table
ECHA Public Activities Coordination Tool (PACT)	25	2	https://echa.europa.eu/addressing-chemicals-of-concern/substances-of-potential-concern/pact
ECHA Registrations Dossiers	441	64	https://echa.europa.eu/information-on-chemicals/registered-substances
European Commission Endocrine Active Substances Information System (EASIS)	97	17	https://easis.jrc.ec.europa.eu/veil/

Step 2: Analogue searching based on two different methods

Identifying analogues and evaluating their suitability forms a critical component in read-across prediction and the subsequent weight-of-evidence assessment performed for the

three target substances. Analogues were identified using two different methods in an effort to ensure a comprehensive approach. Both methods are described in greater detail in Section D1. The first method (Local Similarity Method; LSM) was developed around the hypothesis that the type of substituent(s) and their position(s) relative to the hydroxyl group influences the estrogenic potential and potency of a given phenol. Thus, analogues were identified based on their local structural similarities, taking into account the positions on either side of the phenolic hydroxyl group only. Alternatively, the second method (Global Similarity Method; GSM) identified potential analogues based on their global structural similarity, taking into account the entire molecule. GSM Analogues were then filtered based on physicochemical properties considered to be relevant in driving ER activity. The two methods were considered complementary as they permitted a search for analogues based on postulated receptor interactions while at the same time investigating potentially unknown structural features that may influence activity. Analogues identified from both methods were carried forward to the next step in the IATA workflow.

Step 3: Collection of available data for targets and potential analogues, and organise into data matrix

Lower tier data related to estrogenic activity (similar to tier 1of the EDSP [8] and levels 1-3 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters [9]) were collected from various sources. These data included *in vivo* and *in vitro* data as well as *in silico* predictions. The NICEATM Uterotrophic database, CERAPP, and High-throughput toxicokinetic (HTTK) data were collected for *in vitro* to *in vivo* extrapolation (IVIVE) [represented as "Exit Option B" of the workflow (Figure 3)]. Data extraction from many of the data sources was facilitated by developing scripts to collect the data and organise them in the OECD data matrix template. The data sources, models and methods for this step are described in more detail in Section D.2.

The data collection exploited comprehensive, publicly available collections (databases) of curated data; their use allowed for both accurate and efficient data collection. It is recognised that the data collection was not exhaustive and there may be additional data sources that could also inform an assessment of estrogenic activity. Nonetheless, the approach leverages the significant efforts made by others on compiling data, as well as assuring its quality [i.e., quality assurance and quality control (QA/QC) measures].

Step 4: Analysis of available information

A prescribed data interpretation procedure across all lines of evidence (*in vivo, in vitro, in silico*) is not described herein; therefore, this approach is not considered to be an OECD 'defined approach' (OECD 2016). Rather, an expert judgement weight of evidence assessment of the estrogenic activity of a given target chemical was made using data for the target (where available) and its respective source analogues.

8 EPA EDSP Tier 1: https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-battery-assays

9 OECD Conceptual Framework for Endocrine Disrupting Chemicals, Levels 1-3: http://www.oecd.org/env/ehs/testing/oecdworkrelatedtoendocrinedisrupters.htm

Exit Options: Prioritizing Chemicals for Testing or Further Assessment

- Exit Option A: Depending on the focus of the regulatory program the activity call alone could be used to prioritise chemicals for further tier 2 testing (e.g., *in vivo* testing); or,
- Exit Option B: Prioritisation using *in vitro* bioactivity. HTTK-based IVIVE is used together with exposure estimates to develop a margin of exposure (MOE)-like metric called a Bioactivity Exposure Ratio (BER)[10].

Exit Option B is based on a data-driven framework for tiered toxicity testing that was published by the Hamner Institutes for Health Sciences (Thomas, et al. 2013), which can be found in Appendix B. The first tier of this framework bins chemicals according to 'selective' or 'non-selective' MOAs based on data from high-throughput *in vitro* assays (for the current case study, the phenols fall under 'selective' MOA). *In vitro* bioactivity is converted to an applied dose (i.e., an applied dose equivalent, ADE) by IVIVE. The next step, which was not conducted in the current case study, is to use high-throughput exposure models to estimate human exposure levels. The BER is derived by comparing the ADE to the exposure estimate. If the BER is above a certain cut-off, no further testing is required (cut-off values tend to be purpose- or program-specific). However, if the BER is less than the cut-off, further testing and/or refinements are undertaken. The concept of the BER as a metric for risk based prioritisation has been of recent international interest, including within the Existing Substances Program at Health Canada (Canada 2016). Further, the 'data driven framework' described above continues to evolve to include 'new thinking'; for example, by including a 'tier 0' that screens chemicals using high-throughput transcriptomics (the 2013 framework is reproduced in Appendix B).

10 NB: In order to distinguish this type of bioactivity-based calculation from traditional MOE calculations, it is now referred to as the (bio)activity-to-exposure ratio (AER or BER). However, the original publication refers to an 'MOE' (Thomas et al. 2013).

4. DATA/INFORMATION GATHERING

4.1. Analogue chemicals

Two methods for analogue identification (LSM and GSM) were explored in this case study. The LSM and GSM are described in detail below; however, first we provide a high-level overview of each approach. The LSM identifies potential analogues based on their local position(s) relative to the hydroxyl group; whereas, the GSM identifies potential analogues based on the global structural similarity of the entire molecule. Therefore, these two methods tackle the same problem using very different inputs, and, as a result, also produce different outputs. The local approach of the LSM is informed by the mechanistic knowledge that the hydroxyl group is essential for the ability of the substance to bind to the ER. Thus, the model focuses locally on the R1 and R5 substituents (recall, Fig. 1) and is based on the hypothesis that these are the most important for facilitating or interfering with ER binding. Alternatively, the GSM considers the molecules on a global scale and therefore does not consider the exact placement of substituents relative to the hydroxyl. The GSM then filters analogues based on physicochemical properties considered to be relevant in driving ER activity and availability of information for each analogue (applying a minimum threshold of at least four studies for inclusion in the final list). In addition to using these two differing approaches for analogue identification (local versus global), the two methods also use two unique 'Similarity Score' metrics. Importantly, these two metrics cannot be directly compared (the reason for this is explained in greater detail at the end of this section). Taken together, these approaches can be considered to be distinct, but complementary. For the purpose of this case study we've included results and analysis for each; however, when considering future applications of the IATA, whether to use the LSM, GSM or both would be at the author's discretion.

Local Similarity Method (LSM)

The intention of the LSM was to select phenols that were similar to the target chemicals, both structurally (i.e., with respect to the substitution relative to the phenolic hydroxyl) and chemically (i.e., functionally). A similarity metric that included substituent position and chemical identity was developed and applied to analogue selection. These phenols were then used as source analogues for read-across. The LSM is described below, first via a technical description, and then using an illustrative example.

Technical description of the LSM: Using a phenol scaffold, molecules were decomposed into *R*-positions about the aryl ring and set of fragments at each position. For each *R*-position: (i) the unique set of fragments was determined; (ii) the fragments were fingerprinted; (iii) pair-wise similarity look-up tables (LUTs) were calculated; (iv) the full similarity matrices from the LUTs, including a similarity penalty for molecule pairs having different substitution patterns were filled; (v) the global similarity matrix was calculated; and, (vi) analogues were selected by filtering on global similarity. To perform these steps, a program was written in Python[11] that made use of the Indigo[12]

11 Python - https://www.python.org/
12 Indigo - http://lifescience.opensource.epam.com/indigo

cheminformatics library for decomposition, fingerprinting, and fragment similarity calculation, and the Pandas[13] library for handling matrices. The method is depicted schematically in Figure 4.

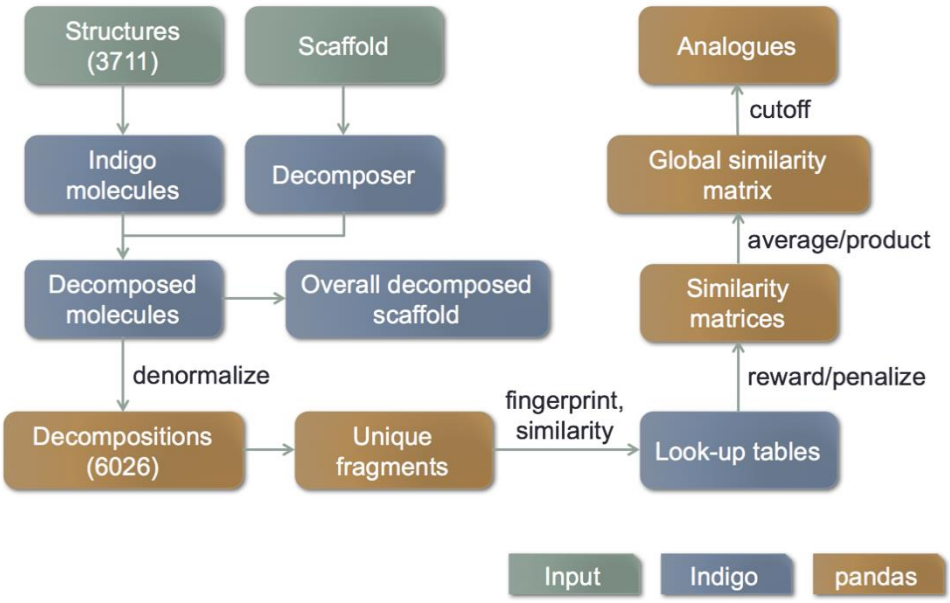


Figure 4. Analogue selection workflow for the LSM.

The phenol scaffold was defined using SMARTS [14] notation as c1ccccc1[OX2H1]. An example of decomposition is shown in Figure 5. The molecule (left) is decomposed into a scaffold with substitution positions R_2 and R_5, and the fragments corresponding to those positions, $CH(OH)CH_2NH_2$ and -OH.

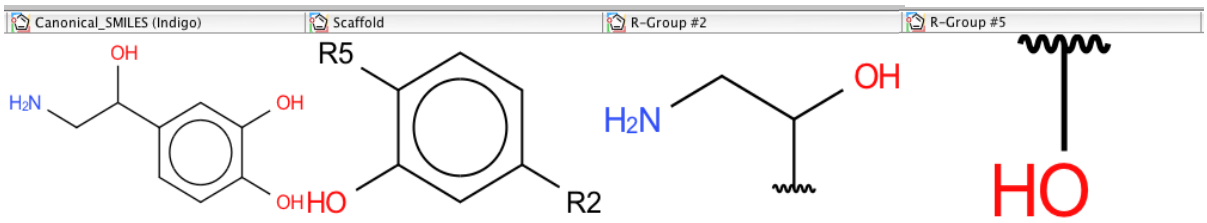


Figure 5. Example decomposition.

In this example, the phenol scaffold appears more than once, which leads to multiple decompositions. Not all the decompositions are unique however – they may be identical by symmetry. The approximately 3800 phenols considered resulted in about 6000 decompositions. Overall, 18 unique R-positions were identified that include conjoined and bridging rings (Figure 6).

13 Pandas - http://pandas.pydata.org/
14 SMARTS: http://www.daylight.com/dayhtml/doc/theory/theory.smarts.html

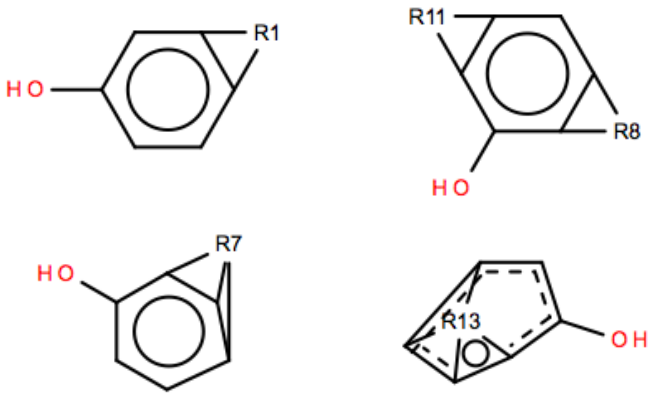


Figure 6. Examples of conjoined ring substitution patterns.

For the fragments, the Indigo similarity fingerprint was utilised, and the Tanimoto index for the pair-wise similarity of each fragment at each *R*-position was calculated (Tanimoto 1957). For each *R*-position, the pair-wise similarities were collected in a similarity look-up table (LUT). This was done for efficiency with regards to both computation time and memory usage. For example, the R_1 position was found to have 340 unique fragments, resulting in a 340 by 340 look-up table (roughly 116000 calculations). In contrast, the full similarity matrix for R_1 would have required 6000 x 6000 = 36 million calculations. Thus the full similarity matrix S_{R_k} for position R_k can be rapidly populated from its corresponding LUT. Additional efficiencies could be gained by recognizing that S_{R_k} is symmetric and the diagonal elements are 1. Therefore, only the upper triangle U_{R_k}, needs to be filled and the remainder of the matrix can be completed using:

$$S_{R_k} = U_{R_k} + I_N + U_{R_k}^T$$

where, I_N is the identity matrix, N is the number of unique decompositions, and $U_{R_k}^T$ is the transpose of the upper triangle. A reward or penalty to the similarity based on substitution pattern was applied at this point. If both molecules i and j were unsubstituted at position R_k, then they were rewarded with a similarity $s_{ij,R_k} = 1$. On the other hand, if one molecule was substituted at position R_k and the other was not, the pair was penalised with a similarity $s_{ij,R_k} = 0$. This is shown with the pseudo-code in Figure 7. This process was repeated for all 18 unique *R*-positions.

if ($fragment_{i,R_k}$ is not null) and ($fragment_{j,R_k}$ is not null) then:

$$s_{ij,R_k} = similarity\left(fp\left(fragment_{i,R_k}\right), fp\left(fragment_{j,R_k}\right)\right)$$ LUT

else if ($fragment_{i,R_k}$ is null) and ($fragment_{j,R_k}$ is null) then:

$$s_{ij,R_k} = 1.0$$ Reward

else:

$$s_{ij,R_k} = 0.0$$ Penalize

Figure 7. Pseudo-code for similarity calculation, where "similarity" is the Tanimoto index function and "fp" is the fingerprinting function.

Next, the *R*-position similarity matrices were combined into a global similarity matrix by taking the element-wise product. Thus, for two molecules *i* and *j*, their global similarity (considering all *R*-positions *k*) is

$$S_{ij} = \prod_k s_{ij,R_k}$$

This applied a strict substitution pattern requirement for analogue selection – if any $s_{ij,R_k} = 0$ then $S_{ij} = 0$. Like the Tanimoto index, the global similarity is limited to the range $0 \leq S_{ij} \leq 1$. Finally, for each target, the 10 most similar analogues with associated *in vivo* or *in vitro* data were selected and carried forward to step 4 of the IATA workflow.

Illustrative example of the LSM: The LSM is best illustrated with an example. Consider three molecules A, B, C, where A is the target molecule and B, C are potential analogues. A and B are substituted at the same position but have different functional groups. A and C are substituted at different positions but have the same functional groups.

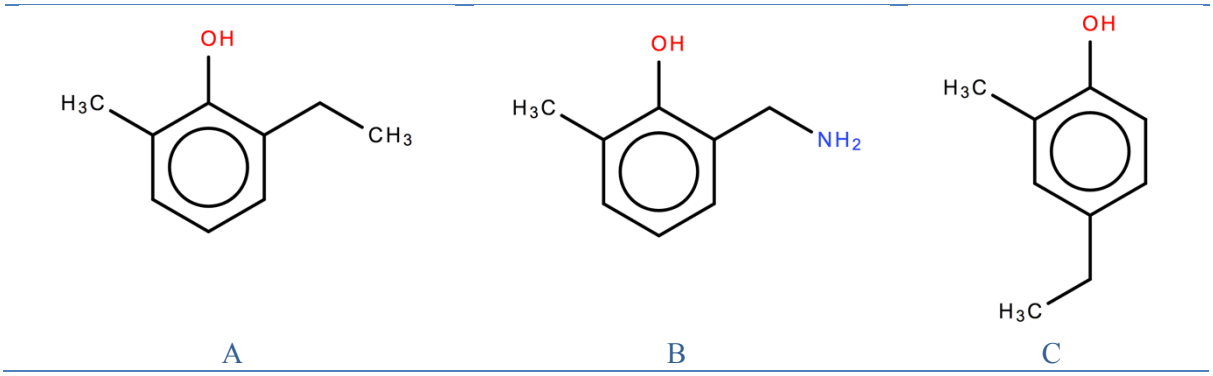


Figure 8. Hypothetical target A and potential analogues B and C.

The molecules are decomposed by their R-positions using the phenol scaffold (refer to Figure 1) in Table 3, below.

Table 4. Fragments resulting from decomposition by R-position for molecules A, B, C.

Molecule	R_1	R_2	R_3	R_4	R_5
A	$-CH_3$	-H	-H	-H	$-CH_2CH_3$
B	$-CH_3$	-H	-H	-H	$-CH_2NH_2$
C	$-CH_3$	-H	$-CH_2CH_3$	-H	-H

In this case, the similarity matrix for a particular R-position (denoted here as position 'k') can be written as:

$$S_{R_k} = \begin{bmatrix} s_{AA,R_k} & s_{AB,R_k} & s_{AC,R_k} \\ s_{BA,R_k} & s_{BB,R_k} & s_{BC,R_k} \\ s_{CA,R_k} & s_{CB,R_k} & s_{CC,R_k} \end{bmatrix}$$

Notice that along the diagonal the same fragment from the same molecule is being compared with itself, which will always result in a similarity of 1. Also, the order in which molecules are compared does not affect their similarity. In other words, $s_{AB,R_k} = s_{BA,R_k}$. This gives rise to the symmetry observed in the similarity matrices.

For molecules A, B, C, the fragment similarities for the R-positions may be expressed by the matrices

$$S_{R_1} = S_{R_2} = S_{R_4} = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix} \qquad S_{R_3} = \begin{bmatrix} 1 & 1 & 0 \\ 1 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \qquad S_{R_5} = \begin{bmatrix} 1 & 0.25 & 0 \\ 0.25 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

The strict substitution pattern requirement can be seen in e.g., $s_{AC,R_3} = 0$ where A is unsubstituted and C is substituted by $-CH_2CH_3$.

The global similarity matrix is calculated as the element-wise product of the R-position matrices, and results in:

$$S = \prod_k S_{R_k} = \begin{bmatrix} 1 & 0.25 & 0 \\ 0.25 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

Therefore, with a score of 0.25, molecule B may be chosen as an analogue to A (S_{AB} = 0.25); whereas, with a score of 0, C would not (S_{AC} = 0). Importantly, this is in contrast to Method 2 below where, on the basis of structure alone, C would be the best analogue.

In summary, this method culminated in the selection of source analogues that were structurally and functionally similar, possessed identical substitution patterns with respect to their targets, and were associated with relevant empirical data.

Global Similarity Method (GSM)

The Global Similarity Method (GSM) for analogue selection was based on the global filtering protocol developed as part of a parallel read-across case study (Pradeep, et al. 2017). Pradeep et al., (2017) describes the method in much more detail; however, a brief

summary is provided below. Figure 9 Overall steps for the read-across analysis workflow adapted from Pradeep et al., 2017. Figure 9 provides a schematic of the GSM workflow.

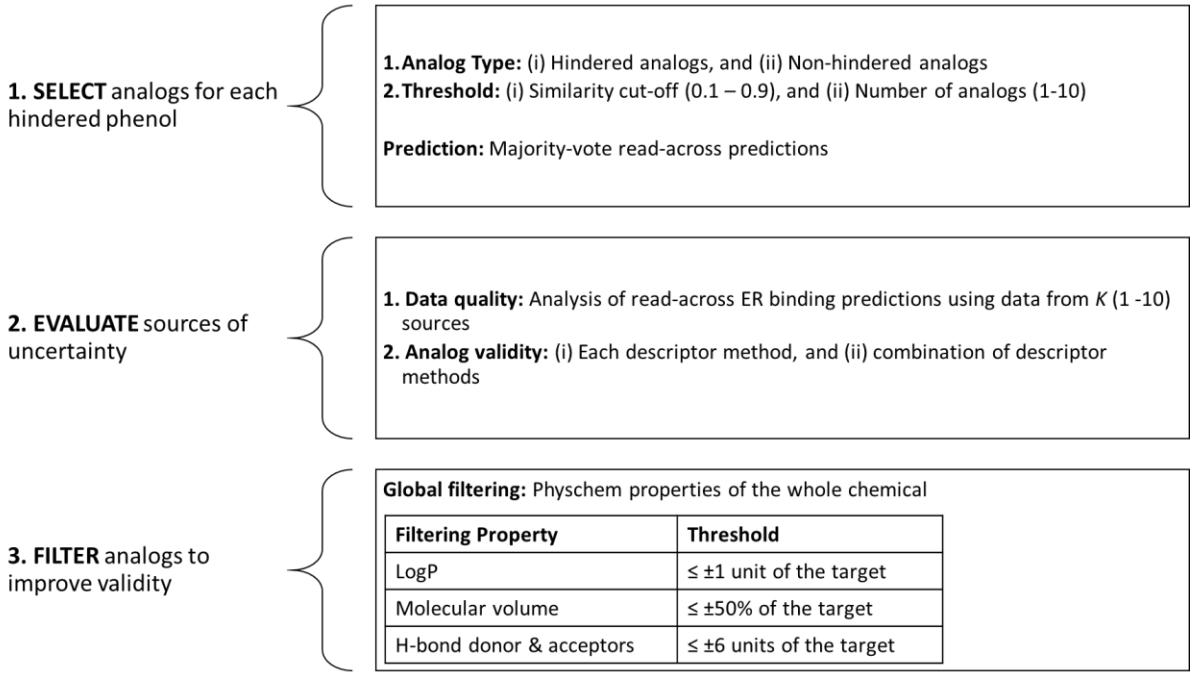


Figure 9 Overall steps for the read-across analysis workflow adapted from Pradeep et al., 2017.

PubChem fingerprints, a type of chemical structural fingerprints, were calculated for the target substances and the entire source analogue pool (Step 1 in the IATA workflow, Figure 3). PubChem fingerprints comprise 881 bits, where each bit represents the presence or absence of a specific substructure. The types of substructures represented within the PubChem fingerprint include hierarchical element counts, rings in a canonical extended smallest set of smallest rings, ring set, simple atom pairs, simple atom nearest neighbours, detailed atom neighbourhoods, simple SMARTS patterns, and complex SMARTS patterns. PubChem fingerprints were generated within the KNIME analytics platform (version 2.11.3).

The Jaccard distance/Tanimoto index (Tanimoto 1957) was then used to calculate pairwise similarity indices for all the chemicals as characterised by their PubChem fingerprints. The similarity index ranges from 0-1, where 0 indicates least similar (dissimilar) and 1 indicates most similar (mostly chemical similarity by itself). For the three target chemicals, a similarity index of 0.7 was taken as a cut off for the purposes of identifying a pragmatic starting number of structurally similar source analogues. The threshold of 0.7 was selected based on the concordance analysis performed in the read-across case study (Pradeep, et al. 2017), where concordance is defined as the ratio of number of analogues with the same experimental outcome as the target and the total number of target-analogue pairs within each similarity threshold. In general, a marked increase in concordance (> 80%) was observed at cut-offs above 0.7 along with a decrease in coverage (number of chemicals for which an analogue could be selected at the given similarity threshold).

Literature reports on *in vitro* ER activity of each compound had been compiled as part of the overall CERAPP project (Mansouri, et al. 2016). These data included an activity outcome (active / inactive) as well as a potency value for active compounds. Each phenol in the CERAPP dataset was associated with a literature data count (how many independent reports of activity were found), which was used as a surrogate for data quality (reproducibility). The expectation was that increasing numbers of consistent literature reports of ER activity, should give rise to more reliable estimates of true activity. Figure 9 shows the trend of maximum read-across prediction accuracy and associated balanced accuracy using the PubChem fingerprints as a function of the number of literature sources *k* (1-10). Structural similarity based read-across predictions improved with the confidence in experimental data, but there was a threshold for optimal performance (as discussed in Pradeep et al., 2017). Given the impact that data quality had on analogue suitability in the case study, source analogues identified using the PubChem fingerprints were filtered to remove any that had fewer than 4 literature data sources.

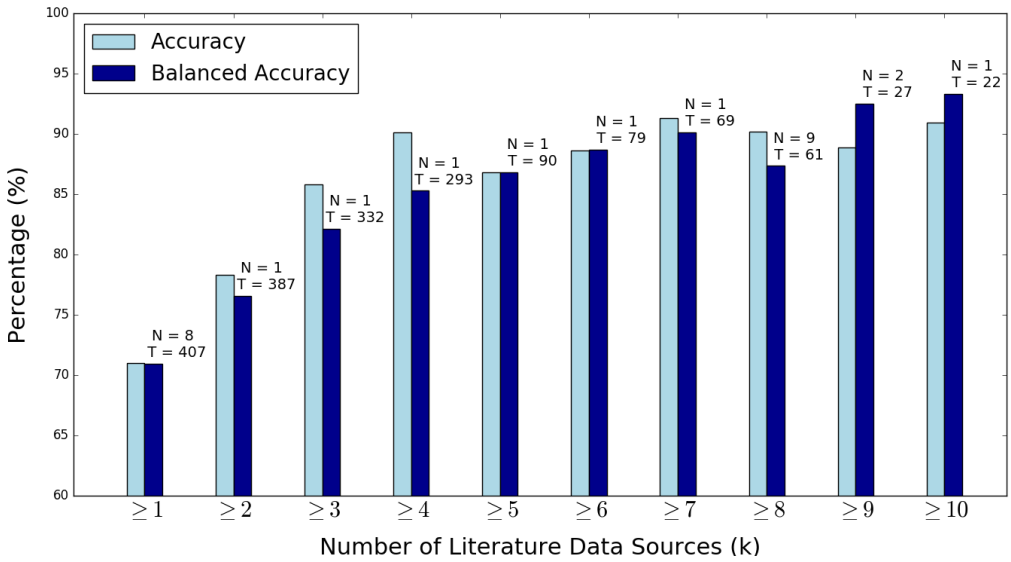


Figure 10: Literature data source analysis to observe the effect of data confidence on read-across predictions. N: number of analogues resulting in the best prediction, T: number of hindered phenols predicted from the restricted dataset. The x-axis corresponds to the threshold in number of data sources (measure of data confidence) and the y-axis corresponds to the maximum accuracy/balanced accuracy of prediction for the dataset. The text on top of each bar plot indicates the number of analogues resulting in the best prediction (N) and the number of hindered phenols that had at least *N* analogues (i.e. were predicted) from the restricted dataset (T). (Figure from Pradeep et al., 2017).

In Pradeep et al., (2017), read-across predictivity was evaluated on the basis of using hindered phenols, non-hindered phenols or both as a pool of source analogues for hindered phenols. Using both hindered and non-hindered phenols as analogues resulted in significantly greater coverage without a significant loss in performance, as compared to using one or the either chemical subgroups. Given the lack of difference in the concordance across the three sets of chemicals and the effect on coverage, the source

analogues were not evaluated separately as hindered or non-hindered phenols, but solely on the basis of the similarity index and number of analogues.

Source analogues were also evaluated for their suitability using a selection of physicochemical properties that were expected to be relevant for ER binding as evidenced by other published QSAR studies [ref: https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/doc/EUR_24522_EN.pdf]. The evaluation was performed using a global filtering approach which relied upon several whole molecule properties characterising physicochemical, steric and electronic features (e.g., molecular volume, LogP, energy of the highest occupied molecular orbital (eHOMO), energy of the lowest unoccupied molecular orbital (eLUMO) etc.). Molecular properties were computed in MOE software (version 2015.10) using canonical SMILES strings. A Student's t-test was performed to test which properties were significantly different for the phenols that bind to the ER as compared to the non-binders. On the basis of the t-test p-values, three properties were identified as significant: LogP, molecular volume and number of hydrogen bond donors and acceptors. The distribution of each of the selected properties was used to identify what a reasonable threshold for each property range should be in order to filter the source analogues.

The preliminary source analogues identified on the basis of PubChem fingerprints were filtered if:

1. they did not have 4 or more literature data sources;
2. molecular volume was beyond ±100% of the molecular volume of the target chemical, if logP was beyond ±1 unit, and if the total number of hydrogen bond donors and acceptors were beyond ± 6 units.

If an analogue did not meet the above criteria, it was discarded until *N* closest analogues were selected for each target substance within the 0.7 similarity threshold. If none of the analogues satisfied the acceptance thresholds, then the target was considered out of domain for analogue selection and any subsequent read-across predictions. The analogues selected were carried forward into step 4 of the IATA workflow (Figure 3).

Comparison of similarity values calculated using the LSM and GSM

The numerical similarity values of from the LSM and GSM can only be compared within methods and cannot be compared between methods. For a number of reasons (see the illustrative example of the LSM, above), the LSM will always give a lower similarity value than the GSM for the same target-analogue pair. Firstly, the LSM excludes the phenol scaffold from the similarity calculation while the GSM does not, resulting in values that will be lower than those from the GSM. Secondly, the multiplicative nature of the global similarity S_{ij} defined in the LSM results in lower values. S_{ij} is a product of fragment similarities, where each fragment similarity is bound between zero and one. The product of any two values less than one and greater than zero is a value that is less than the two initial values. The product of fragment similarities over each *R*-position drives S_{ij} towards zero. Finally, small substituents (e.g. 3 atoms or less, excluding H) can result in a low similarity in the LSM. For example, the LSM similarity for 4-methylphenol and 4-ethylphenol is 0.5, while for 4-butylphenol and 4-pentylphenol it is 0.8.

4.2. Data Sources

Several databases of aggregated test data related to estrogenicity of substances were queried and relevant data extracted and arrayed in the accompanying data matrix files to use in the approach. The information sources covered *in vivo* assays, *in vitro* assays and (Q)SAR predictive tools. Each data source is described below.

In vivo

The primary *in vivo* assay that can be used to detect if a chemical has the potential to interact with the estrogen hormone system is the uterotrophic assay. The uterotrophic assay is a short-term screening test that is based on the increases in uterine weight. It is mainly used to evaluate a chemicals ability to elicit biological activities (agonists) similar to endogenous estrogens (e.g. 17ß-estradiol). EPA and OECD Guidelines are available that describe the assay in more detail (EPA 2009, OECD 2007). A significant recent effort at the U.S National Toxioclogy Program (NTP) Interagency Center for the Evaluation of Alternative Toxiological Methods (NICEATM) performed a search of the peer-reviewed literature for uterotrophic assay data on the library of ToxCast compounds. A database of guideline-like studies was curated (GL-UTDB) from this extensive literature search. The GL-UTDB contains information from 400+ guideline-like studies extracted from 90+ publications, and encompasses data on 118 chemicals (Kleinstreuer, et al. 2016b). This high quality database has been made freely available on the NTP website[15]. Data on the target chemicals and potential analogues was extracted from this database (where available) and captured in the associated target chemical data matrices.

In vitro

High Throughput Screening (HTS)

High-throughput screening (HTS) *in vitro* assays are used to rapidly generate concentration response curves for a range of biological activity for large numbers of compounds. An example of such an initiative to generate this type of data is the U.S. EPA ToxCast program. To date, the combined efforts of ToxCast and the Tox21 consortium have tested over 8000 chemicals (Judson, et al. 2010).

The most developed approaches for applying the high-throughput *in vitro* assays and computational tools to inform a regulatory program are their recent development for use in the US EPA Endocrine Disruptor Screening Program (EDSP). Indeed, this approach has been approved as alternatives for the traditional *in vivo* assays for estrogenicity (Federal Register 9928-69 [16]). Briefly, the results from the 18 assays that aim to measure mechanistic events related to the ER Pathway (i.e. ER binding, dimerisation, chromatin binding, transcriptional activation and ER-dependent cell proliferation) are integrated into a computational model that can discriminate bioactivity from assay specific interference and responses related to cytotoxicity (Browne, et al. 2015). The model provides a score relative to estradiol ranging from 0 (inactive substances) to 1

15 Curated Database of Rodent Uterotrophic Bioactivity - https://ntp.niehs.nih.gov/pubhealth/evalatm/tox21-support/endocrine-disruptors/edhts.html

16 Federal Register 9928-69: https://www.federalregister.gov/documents/2015/06/19/2015-15182/use-of-high-throughput-assays-and-computational-tools-endocrine-disruptor-screening-program-notice

(bioactivity for 17β-estradiol). A score of 0, 0 to 0.1 and ≥ 0.1, are considered "inactive", "inconclusive" and "active", respectively. The score is characterised by the area under the curve (AUC) of the concentration-response profile which is a consensus of the curves for the individual assays. The results of the individual ER related assays and the ER bioactivity model scores for tested chemicals are available online [17]. The model was evaluated against a set of reference chemicals for which guideline type studies were available for the *in vivo* uterotrophic assay (mentioned above) and it was shown to be highly predictive (Judson, et al. 2015, Kleinstreuer, et al. 2017). The model has been adopted for regulatory acceptance and EPA intends to accept the ER Model Results as an alternative for select Tier 1 assays (ER binding, ERTA, and Uterotrophic Assays) [18]. The model is also listed in the Joint Research Centre (JRC) Tracking System for Alternative Methods towards Regulatory Acceptance (TSAR)[19]. The details of the 18 assays have not yet been summarised in the OECD template for alternative methods however an effort is underway to do so. In the meantime, details on the assays are available on the EDSP21 Dashboard online (see https://actor.epa.gov/edsp21/).

An automated script was written in order to search the online EDSP 21 dashboard and extract the results of the 18 ToxCast and Tox21 assays as well as the ER Model AUC scores for the target substances and potential analogues. All the data were added to the corresponding target chemical data matrices.

Literature collected under CERAPP

Literature reports on *in vitro* ER activity of each compound were also compiled as part of the Collective Estrogen Receptor Activity Prediction Project (CERAPP) project. These data included an activity outcome (active / inactive) as well as a potency value for active compounds.

In vitro studies collected under CERAPP:

The CERAPP was an international collaborative effort to predict estrogenic potential for tens of thousands of man-made chemicals found in the environment (Mansouri, et al. 2016). As part of the project information on the estrogenic activity of chemicals was mined and curated to serve as a validation set for predictions of the different models (see below). Experimental data were collected from the following sources:

- HTS data from Tox21 project
- U.S. FDA Estrogenic Activity Database (EADB),
- METI (Ministry of Economy, Trade and Industry, Japan) database
- ChEMBL database

Data are available for download on over 7000 substances on the U.S. EPA ToxCast™ Data website[20]. All data entries were categorised into three assay classes: (*a*) binding, (*b*) reporter gene/transactivation, or (*c*) cell proliferation and potency levels for binding,

17 EPA EDSP 21 Dashboard - https://actor.epa.gov/edsp21/

18 US EPA 2015. https://www.epa.gov/endocrine-disruption/use-high-throughput-assays-and-computational-tools-endocrine-disruptor#screening

19 JRC Tracking System for Alternative Methods Towards Regulatory Acceptance (TSAR) - http://tsar.jrc.ec.europa.eu/

20 CERAPP Experimental Data Evaluation Set- https://www3.epa.gov/research/COMPTOX/CERAPP_files.html

agonist and antagonist activity were assigned based on the results (see Mansouri et al. 2016 for details). The assigned potency level and literature count from the CERAPP dataset were extracted and added to the corresponding target substance data matrices.

In silico

Scores from the EPA ER *in vitro* consensus model described in the *in vitro* HTS section formed the basis of the training set for ER QSAR model development as part of the CERAPP project. 48 individual ER QSAR models were derived from which a final consensus prediction was generated. These consensus predictions were then forward validated against the literature-derived ER activity data set described above. Overall CERAPP predictions were made on ~32,000 unique chemical structures and the predictions for the target compounds and associated analogues were extracted from the EDSP21 Dashboard and added to the corresponding target chemical data matrices.

Predictions were generated with respect to ER binding potential from selected publicly available or commercial software tools outlined in Table 5.

Table 5. *In silico* tools used to support the IATA workflow.

Model Name (version)	Description	Available QMRF	Reference
Advanced Chemistry Development (ACD) Percepta (v2015) – Estrogen Receptor Binding module	Statistical model for classifying ER binding affinities.	No	ACD/ Percepta 2013 [21]
Estrogen Receptor Binding Profiler – (in OECD QSAR Toolbox v3.4)	Scheme is based on structural and parametric rules extracted from literature sources and supported by experimental data. The ER-binding profiler classifies chemicals as non- binders or binders depending on molecular weight (MW) and structural characteristics of the chemicals.	No	OECD 2016 [22]
US EPA rtER Expert System – (in OECD QSAR Toolbox v3.4)	Profiler consists of molecular definitions that mimic the structural criteria of chemical classes potential estrogen receptor-binders covered by US EPA Estrogen Receptor Expert System (ERES) The ERES profiler is an effects-based automated system used to predict estrogen receptor binding affinity.	No	OECD 2016 [11]
Lhasa, Derek Nexus (v5.0.2) Oestrogenicity	Expert system used to profile category members for alerts related to estrogenicity.	No	Derek Nexus 2016 [23]

21 ACD/Percepta 2013: http://perceptahelp.acdlabs.com/help_v2014/index.php/Endocrine_System_Disruption
22 OECD Toolbox: http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
23 Derek Nexus: https://www.lhasalimited.org/products/derek-nexus.htm

OASIS TIMES (v2.27.19.13) Estrogen Binding Affinity S9 activated (v4.04)	QSAR with metabolic simulator for prediction of relative binding affinity for ER	Yes	TIMES 2016 [24]

High throughput toxicokinetic data (HTTK) and in vitro to in vivo extrapolation (IVIVE)

The methods selected to calculate oral equivalent doses from the *in vitro* bioactivity for this case study are described in detail in (Wetmore, et al. 2012). Briefly, plasma protein binding and metabolic clearance are measured using *in vitro* assays (HTTK). These *in vitro* data are used to parameterise a simple pharmacokinetic model to estimate expected steady state blood concentrations at a given dose (assuming 100% oral bioavailability). Monte Carlo simulation provides estimates of the variability of the steady-state blood concentration (Css) in a population of healthy individuals. Using reverse dosimetry, oral doses can then be estimated that would result in a steady-state blood concentration equivalent to the AC50 from the ToxCast assay. HTTK data has been generated for many of the ToxCast chemicals and the data along with the Wetmore et al. IVIVE modelling values have been made available in a publicly accessible R package to facilitate the conversion of *in vitro* bioactivity concentrations to oral equivalent doses[25]. The R package was used for this case study and the ADE required to achieve the upper 95th percentile Css values equivalent to the AC50 value from the lowest active ER related assay were estimated and added to the target substance data matrices where applicable. See Section E2 for discussion.

24 LMC Oasis: http://oasis-lmc.org/products/models/human-health-endpoints/estrogen-binding-affinity.aspx

25 Wambaugh et al. 2017. https://cran.r-project.org/web/packages/httk/index.html

5. APPLICATION OF IATA

5.1. Summary of Data

This IATA was applied using three target chemicals, 4-tert-butylphenol (CAS 98-54-4), 2,4-di-tert-butylphenol (CAS 96-76-4) and octabenzone (CAS 1843-05-6). The results of the complementary analogue identification methods are integrated below. Briefly summarised, the results for each substance are as follows:

- 4-tert-butylphenol is a non-hindered phenol that is expected to be estrogenic. The applied dose equivalent (ADE) derived for this substance is more protective than the dose predicted using read-across. Further, the 2 methods used for identification of analogues produced similar lists of analogues that were predictive of the target's activity.
- 2,4-di-tert-butylphenol is a partially hindered phenol that is not expected to be estrogenic. For this substance, the 2 methods used for identification of analogues produced dissimilar lists of analogues. The LSM analogues had substituents in the R1 and R3 position (like the target), whereas the GSM analogues had substituents in a variety of positions around the aryl ring. The LSM analogues were more predictive of the target's activity than the GSM analogues.
- Octabenzone is also a partially hindered phenol that is not expected to be estrogenic. It is the most data-poor of the three target chemicals tested herein. Similar to the results for 2,4-di-tert-butylphenol, analogues with substituents in the same position as those on the target were most predictive of the target's activity.

The hypothesis that non-hindered phenols are expected to be estrogenic was substantiated by read-across. However, robust predictions for the partially-hindered phenols proved more challenging owing to the conflicting results from the source analogues identified. Notably, analogues with substituents in the same position on the aryl ring as those on the target substance were most predictive of the target's activity, particularly their position relative to the phenolic hydroxyl group. Further, data from *in vivo* and *in vitro* sources was more consistent across source analogues and hence made more compelling predictions for read-across compared to the predictions made using the *in silico* tools.

Target 1: 4-tert-butylphenol , CAS RN 98-54-4

The data that are discussed in the following section are summarised in the IATA data matrix for 4-tert-butylphenol (CAS 98-54-4). Here, the trends across the queried data sources for the source analogues are summarised.

As a non-hindered phenol, 4-tert-butylphenol is expected to be potentially estrogenic. It is a data-rich substance that was found to be active in the *in vivo* uterotrophic assay, as well as the *in vitro* ToxCast battery for ER agonism. The ToxCast ER agonist AUC model score for this chemical is 0.161, which is above the threshold to be considered active. A variety of *in silico* (QSAR) approaches have also identified it as likely to be active for ER binding, ER agonism and ER antagonism. This IATA also considered read-across using source analogues identified by 2 different methods. The LSM

identified seven analogues with a variety of 3-5 carbon substituents at the R3 position and one analogue with a 10 carbon substituent at the R3 position. The GSM identified nine analogues, four of which were also identified by the LSM. Of the GSM analogues, seven had 3-6 carbon substituents at the R3 position, one had two substituents (1 or 4 carbons at the R3 and R1 positions, respectively) and one had a methyl substituent at both the R2 and R3 positions. In general, the analogues that were identified by either method were similar to the target (Table 6).

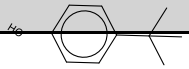
While most of the analogues had *in vitro* data associated with them, only one had *in vivo* data. 4-(Methylbutan-2-yl)phenol (CAS 80-46-6, identified by both methods) was found to be active in the uterotrophic assay (LEL=200 mg/kg-bw/day) (Yamasaki, et al. 2003).

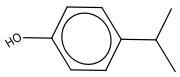
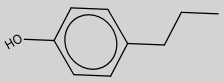
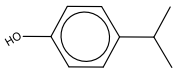
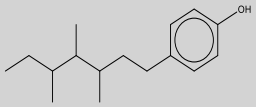
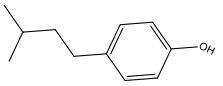
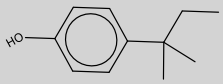
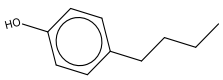
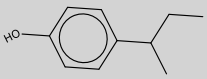
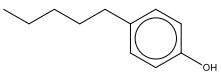
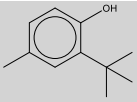
Five analogues have undergone *in vitro* testing in ToxCast. Three analogues that were identified by both methods were found to be active as ER agonists (AUC=0.111-0.282) and two analogues (LSM only) were predicted not to be (AUC=0.021-0.0275); none were found to be ER antagonists (AUC=0-0.0002). Taken together, the ToxCast data provided mixed support for ER agonism. Had there been no ToxCast data for the target, these results could have been approached in one of two ways: (1) the most conservative approach, which would be to air on the side of caution and assume ER agonism; or, (2) the nearest neighbour approach, which would be to choose the most structurally similar analogue to read-across from. However, the nearest neighbour approach is interesting in this case as the results from the two closest analogues are mixed. CAS 99-89-8 is not active (AUC=0.021) and CAS 99-71-8 is active (AUC=0.163); the true activity of the target is AUC=0.161, which is very close to the active call from CAS 99-71-8.

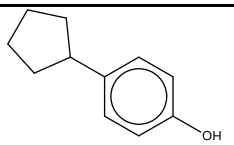
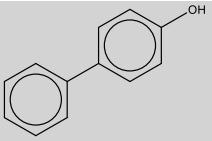
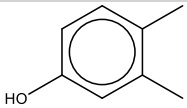
Finally, the IATA also made use of predictions for both the target and source analogues using both the CERAPP QSAR consensus model as well as selected *in silico* tools, namely: OASIS TIMES, the OECD Toolbox, Derek Nexus and ACD Percepta. Briefly, the CERAPP consensus QSAR model consistently predicted weak activity for 'consensus binding' and 'consensus agonism.' Alternatively, the calls for 'consensus antagonism' predicted moderate to strong activity (strong activity was associated with analogues that have larger substituents with five or more carbons). The ER binding model with OASIS TIMES gave predictions of 'weak' or 'low' activity for the target substance and its source analogues. The OECD Toolbox (ER profiler and US EPA rtER expert system) also predicted weak binding across most of the substances. Similarly, ACD Percepta predicted weak or no binding to the ER-alpha. Finally, Derek Nexus generated 'no alerts' for nine of the substances, including the target, and an alert of 'Mammal Plausible, Alkylphenol or precursor' for five of the analogues. Taken together, the *in silico* tool predictions were less compelling than the other data sources. However, because the overall call from the combined QSAR sources is 'active', these data are useful for early prioritisation and screening of chemicals.

4-tert-butylphenol is data rich; therefore, it provided a pragmatic proof-of-concept example to demonstrate both the value of incorporating *in vivo, in vitro* and *in silico* data as well as an analysis of the level of consistency of responses between the target substance and its source analogues.

Table 6. Analogues identified for 4-tert-butylphenol , CAS RN 98-54-4.

Method	Name	CAS RN	Structure
Target	4-tert-butylphenol	98-54-4	

LSM	(1-Methylethyl)-phenol	25168-06-3	
LSM	4-Propylphenol	645-56-7	
LSM	4-Isopropylphenol	99-89-8	
LSM	Phenol, dodecyl-, branched	121158-58-5	
LSM, GSM	p-Isoamylphenol	1805-61-4	
LSM, GSM	4-(2-Methylbutan-2-yl)phenol	80-46-6	
LSM, GSM	4-Butylphenol	1638-22-8	
LSM, GSM	4-(Butan-2-yl)phenol	99-71-8	
GSM	4-Pentylphenol	14938-35-3	
GSM	2-tert-Butyl-4-methylphenol	2409-55-4	

GSM	4-CYCLOPENTYLPHENOL	1518-83-8	
GSM	4-Phenylphenol	92-69-3	
GSM	3,4-Dimethylphenol	95-65-8	

Target 2: 2,4-di-tert-butylphenol , CAS RN 96-76-4

The data that are discussed in the following section are summarised in the IATA data matrix for 2,4-di-tert-butylphenol (CAS 96-76-4). Here, the trends across the queried data sources are summarised.

As a hindered phenol, 2,4-di-tert-butylphenol is not expected to be estrogenic. It has been tested and found to be inactive in the *in vivo* uterotrophic assay (maximum dose >1000 mg/kg-bw/day), as well as the *in vitro* ToxCast battery for ER agonism (AUC=0) and ER antagonism (AUC=0). A variety of *in silico* (QSAR) approaches have also identified it as likely to be inactive for ER binding, ER agonism and ER antagonism. No ADE was derived for this substance.

Between the two analogue identification methods, 19 analogues were identified for 2,4-di-tert-butylphenol, including one analogue that was identified by both methods (2,4-Diisopropylphenol). These analogues are listed in Table 7. The target has tert-butyl substituents at both the R1 and R3 positions. Similarly, the LSM identified 10 analogues with substituents at the R1 and R3 positions: five analogues have 1-5 carbon substituents, one analogue has two 9 carbon substituents, two analogues have substituents with more complex structures including an additional aromatic ring at R3, and two analogues have R3 methyl ether. An additional nine analogues were identified by the GSM. Unlike the LSM, the substituents of the GSM analogues are distributed at a variety of positions: two analogues have substituents at R1 and R3 (like the target), one analogue has a single substituent at R1 (partially-hindered), two have substituents at R1 and R5 (fully-hindered), and five analogues have a single substituent at R3 (unhindered). Taken together, the analogues produced by the LSM were all structurally similar to the target in terms of their substituent positions, whereas the analogues produced by the GSM were more structurally diverse (even though their pairwise Tanimoto indices were high).

Three analogues had *in vivo* data associated with them: 4-cumylphenol, 4-nonylphenol and 4-octylphenol. Each of these phenols is unhindered (single, large substituents are located at R3) and was identified by the GSM. Because they are unhindered, it was not anticipated that they would adequately predict the activity of the partially-hindered target (based upon what is known about the biochemistry of ER binding, described above). Indeed, 4-cumylphenol was found to be active in the uterotrophic assay (LEL= 20 and 200 mg/kg-be/day); 4-nonylphenol had mixed results based on nine iterations of

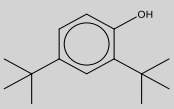
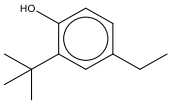
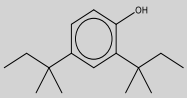
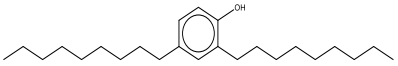
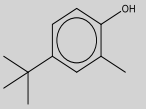
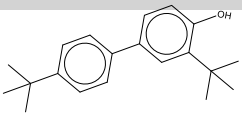
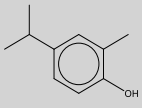
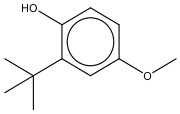
the assay (Active: LEL=75, 90, 100 and 200 mg/kg-bw/day; Not Active: Maximum dose= 2 and 200 mg/kg-bw/day); and, 4-octylphenol also had mixed results (Active: LEL= 100 mg/kg-bw/ day; Not Active: LEL=200 mg/kg-bw/day). These discordant outcomes are a known issue of the uterotrophic assay have been described elsewhere (Kleinstreuer, et al. 2016b). The target itself is not active in the uterotrophic assay (maximum dose=1000mg/kg-bw/day). The finding that none of these analogues could adequately predict the activity of the target demonstrates the importance of considering the position of the substituents on the phenolic ring during analogue selection.

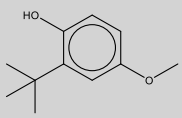
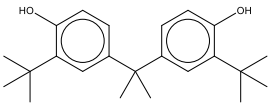
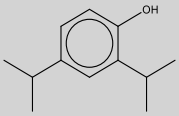
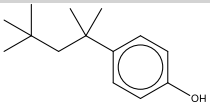
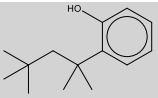
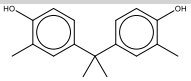
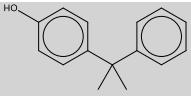
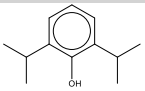
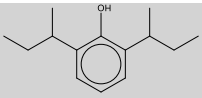
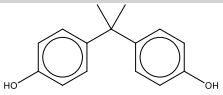
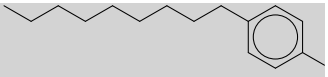
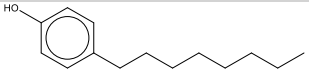
Ten analogues have undergone *in vitro* testing in ToxCast. One analogue (identified by both methods) was found to be not active as an ER agonist or antagonist (AUC=0); the four remaining LSM analogues were also found to be inactive for ER agonism and antagonism (AUC=0-0.0193). Therefore, the LSM analogues are good predictors of the activity of the target chemical. Alternatively, the six remaining GSM analogues had mixed results: four were active for ER agonism (AUC=0.118-0.45), two were not active (AUC=0-0.088), and all were not active for ER antagonism (AUC=0). The four GSM analogues that were found to be active were all unhindered phenols (4-(1,1,3,3-Tetramethylbutyl)phenol , 4-cumylphenol, bisphenol A and 4-octylphenol). Of the two that were found to be not active, one was fully hindered (propofol) and one was unhindered (4-nonylphenol). The target is a partially-hindered phenol that is not active for ER agonism or antagonism. Therefore, the GSM analogues were less able to accurately predict the activity of the target. Taken together, these data are consistent with the *in vivo* results, which also show the importance of considering the position of the substituents on the phenolic ring during analogue selection.

Finally, the IATA also considered predictions from the CERAPP consensus QSAR models and other selected *in silico* tools for the target and their analogues, including: OASIS TIMES, the OECD Toolbox, Derek Nexus and ACD Percepta. The target was ‘inactive’ across all of these models. For the analogues, the CERAPP consensus QSAR models made predictions of ‘inactive’ or ‘activity’ (ranging from very weak to moderate) for ‘consensus binding’ and ‘consensus agonism’ across all 19 analogues. The calls for ‘consensus antagonism’ ranged from ‘inactive’ to ‘strong activity’; and, the two substances predicted to be strongly active (4-cumylphenol and bisphenol A, identified by the GSM) also had the highest AUC scores for ER agonism (0.376 and 0.45), and the former was also active in the *in vivo* uterotrophic assay. For the LSM analogues, OASIS TIMES gave predictions of ‘not active’ or ‘weak activity’ for ER binding. For the GSM analogues, OASIS TIMES gave predictions of ‘not active’ or ‘weak/low/moderate activity’ for ER binding. Similarly, ACD Percepta gave predictions of ‘no binding’ or ‘weak binding’ for the LSM analogues; and, ‘weak’ or ‘strong’ binding for the GSM analogues. Finally, Derek Nexus generated ‘no alerts’ for the LSM analogues and the target. Alerts for the GSM substances included: ‘no alert’, ‘Mammal Plausible, Alkylphenol or precursor’ and ‘Mammal Plausible, bisphenol or precursor.’ Taken together, the *in silico* predictions for the LSM analogues were in better agreement with the predictions of the target substance than those for the GSM analogues.

The target, 2,4-di-tert-butylphenol, is a data rich substance that is not expected to be estrogenic due to partial hindrance of the hydroxyl by a substituent at the R1 position. It was observed that analogues that shared this structural feature were better at predicting the activity of the target, and would therefore be better for read-across had the target been data-poor. This result is consistent with the hypothesis that non-hindered phenols are expected to be potentially estrogenic, whereas hindered phenols are not.

Table 7. Analogues identified for 2,4 -di-tert-butylphenol, CAS RN 96-76-4.

Method	Name	CAS RN	Structure
Target	2,4-Di-tert-butylphenol	96-76-4	
LSM	2-tert-Butyl-4-ethylphenol	96-70-8	
LSM	2,4-Bis(2-methylbutan-2-yl)phenol	120-95-6	
LSM	2,4-Dinonylphenol	137-99-5	
LSM	4-(T-Butyl)-2-cresol	98-27-1	
LSM	3,4'-bis(1,1-dimethylethyl)[1,1'-biphenyl]-4-ol	42479-88-9	
LSM	2-Methyl-4-isopropylphenol	1740-97-2	
LSM	2-tert-Butyl-4-methoxyphenol	121-00-6	

LSM	Butylated hydroxyanisole	25013-16-5	
LSM	4,4'-Propane-2,2-diylbis(2-tert-butylphenol)	79-96-9	
LSM, GSM	2,4-Diisopropylphenol	2934-05-6	
GSM	4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	
GSM	Phenol, 2-(1,1,3,3-tetramethylbutyl)-	3884-95-5	
GSM	3,3'-Dimethylbisphenol A	79-97-0	
GSM	4-Cumylphenol	599-64-4	
GSM	Propofol	2078-54-8	
GSM	2,6-Di(butan-2-yl)phenol	5510-99-6	
GSM	Bisphenol A	80-05-7	
GSM	4-Nonylphenol	104-40-5	
GSM	4-Octylphenol	1806-26-4	

Target 3: Octabenzone, CAS RN 1843-05-6

The data that are discussed in the following section are summarised in the IATA data matrix for octabenzone (CAS 1843-05-6). Here, we summarise the trends across the queried data sources. As a partially-hindered phenol, octabenzone is not expected to be estrogenic. It has not been tested in the *in vivo* uterotrophic assay, and is negative in the ToxCast batteries for ER agonism and antagonism. No ADE was derived for this substance.

Eleven analogues were identified for this target (Table 8). The LSM identified four analogues with large substituents at the R1 and R4 positions. The R1 substituent is always a ketone linkage to an aryl ring (like the target). Three analogues have an R4 substituent that is an ether linkage to an alkyl group, and the fourth analogue has a nitrogen-containing compound at the R4 position. The GSM identified 7 analogues for the target, none of which overlapped with those identified by the LSM. The GSM analogues were also large and often complex; however, their location on the phenolic ring was not limited to the R1 and R4 positions. Therefore, with respect to substituent position, the analogues produced by the LSM were all structurally similar to the target, whereas the analogues produced by the GSM are more structurally diverse.

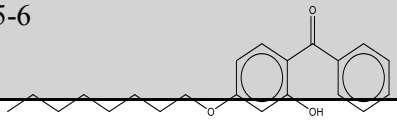
Two analogues have *in vivo* data associated with them: 2-hydroxy-4-methoxybenzophenone (identified by the LSM) and tiratricol (identified by the GSM). The former is inactive in the uterotrophic assay (maximum dose >1000 mg/kg-bw/day, n=2); whereas the latter is active (LEL=1000 mg/kg-bw/day, n=1). Of the two substances, 2-hydroxy-4-methoxybenzophenone is the nearest neighbor to the target; therefore, it would be considered to be the more appropriate choice for read-across.

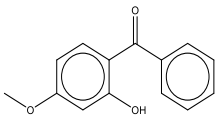
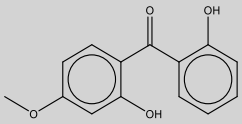
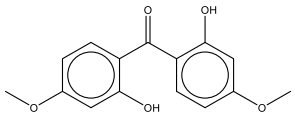
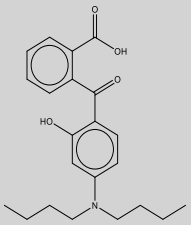
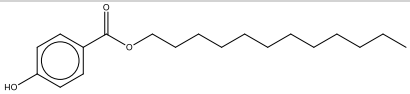
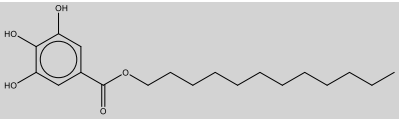
Three analogues have undergone *in vitro* testing in ToxCast. 2-Hydroxy-4-methoxybenzophenone (LSM) and dodecyl gallate (GSM) are inactive for ER agonism, whereas tiratricol (GSM) is active (AUC=0.179). All three are inactive for ER antagonism.

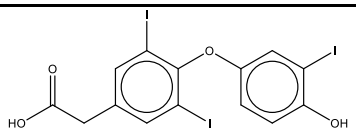
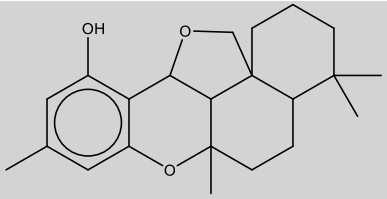
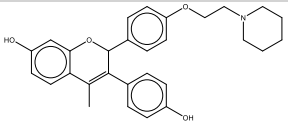
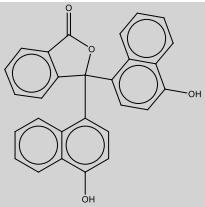
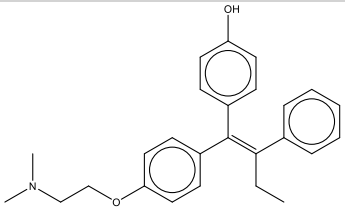
Finally, the IATA considered predictions from the CERAPP consensus QSAR models as well as selected *in silico* tools for the target and analogues, including: OASIS TIMES, the OECD Toolbox, Derek Nexus and ACD Percepta. Across these platforms, the LSM analogues typically had calls of inactivity or weak activity; whereas the GSM analogues had calls of inactivity, weak activity or moderate activity.

Taken together, octabenzone is a substance that does not have any *in vivo* data, but has been tested in ToxCast. Based on the additional data from the LSM and GSM analogues, a nearest neighbour approach is the best fit for this substance. The results for octabenzone are consistent with the hypothesis that hindered phenols are unlikely to be estrogenic. Taken together, source analogues with substituents in the same position as the target are more predictive of the target's activity, which is consistent with the results reported for 2,4-di-tert-butylphenol (the target compound discussed in the preceding section).

Table 8. Analogues identified for octabenzone, CAS RN 1843-05-6.

Method	Name	CAS RN	Structure
Target	Octabenzone	1843-05-6	

LSM	2-Hydroxy-4-methoxybenzophenone	131-57-7	
LSM	2,2'-Dihydroxy-4-methoxybenzophenone	131-53-3	
LSM	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone	131-54-4	
LSM	2-[4-(Dibutylamino)-2-hydroxybenzoyl]benzoic acid	54574-82-2	
GSM	Dodecylparaben	2664-60-0	
GSM	Dodecyl gallate	1166-52-5	

GSM	Tiratricol	51-24-1	
GSM	Siccanin	22733-60-4	
GSM	ACOLBIFENE	182167-02-8	
GSM	a-Naphtholphthalein	596-01-0	
GSM	(Z)-4-Hydroxytamoxifen	68047-06-3	

5.2. Uncertainty

This case study focussed on select phenolic chemicals has reinforced the established insights on what sources of uncertainties exist in read-across, namely the uncertainty associated with the similarity rationale to identify and evaluate analogues and the uncertainty associated with the underlying data supporting those analogues (Patlewicz, et al. 2014). In this case study, the uncertainty associated with the analogue identification and evaluation is brought into sharper focus in the application of two distinct yet complementary approaches used to identify source analogues for 3 target chemicals. The LSM and GSM result in different sets of analogues for each of the target chemicals with some overlaps. For the GSM, using global chemical fingerprints in conjunction with whole molecular properties and number of literature sources was not sufficient to identify the most predictive analogues for targets 2,4-di-tert-butylphenol (96-76-4) and octabenzone (1843-05-6), although it was found to be fit for purpose for 4-tert-butylphenol (98-54-4) where there was the greatest overlap in the analogues identified by the 2 methods. This highlights how challenging analogue identification can be and that the scaffolding of the target ought to be considered during analogue selection (especially when it dictates receptor binding dynamics).

While both methodologies seem appropriate, the distinction in the set of analogues highlights the difficulty in benchmarking one technique for analogue selection as a general guideline for analogue selection. Furthermore, the impact of underlying experimental data quality, use of hindered versus non-hindered phenols as analogues, and the effect of analogue filtering by endpoint relevant physicochemical properties as evaluated in the read-across case study (Pradeep, et al. 2017), illustrates that addressing the key sources of uncertainty in read-across can lead to improved read-across predictions.

Each of the models employed in this IATA case study has a degree of uncertainty surrounding it, which is related to the domain of applicability of the model. The domain of applicability can depend on a number of parameters and is typically established on a case-by-case basis. Therefore, while it is difficult to make broad statements regarding a model's domain of applicability, it is nevertheless an important consideration when employing a model in this (or any) context.

5.3. Strategy and integrated conclusion

This IATA was performed with a dual purpose. The first was to provide support for the hypothesis regarding estrogenicity of three substituted phenols. Using horizontal or inter-chemical read-across, the overall activity call for each target was found to be active for 4-tert-butylphenol (98-54-4) and inactive for 2,4-di-tert-butylphenol (96-76-4) and octabenzone (1843-05-6). These outcomes are consistent with the proposed hypothesis that non-hindered phenols are expected to be potentially estrogenic, whereas hindered phenols are not (Table 9). The second purpose was to build confidence in the use of data from alternative methods. Using a vertical or intra-chemical comparison of data tabulated from both traditional and alternative sources, confidence was built for the use of alternative methodologies in risk assessment.

Table 9. Summary of data for the three target substances from the sources queried.

	4-tert-butylphenol (98-54-4)	**2,4-di-tert-butylphenol (96-76-4)**	**Octabenzone (1843-05-6)**
In vivo			
Uterotrophic assay	**Active**	Inactive	No data
In vitro			
ToxCast	ER Agonism: **Active** ER Antagatonism: Inactive	ER Agonism: Inactive ER Antagatonism: Inactive	ER Agonism: Inactive ER Antagatonism: Inactive
CERAPP (literature sources)	ER binding: **Active (vw)** (15) ER Agonism: no data ER Antagonism: Inactive (6)	ER binding: **Active (vw)** (6) ER Agonism: Inactive (4) ER Antagonism: no data	ER binding: Inactive (12) ER Agonism: Inactive (6) ER Antagonism: Inactive (6)
QSAR			
CERAPP	ER binding: **Active (w)** ER Agonism: **Active (w)** ER Antagonism: **Active (s)**	ER binding: Inactive ER Agonism: Inactive ER Antagonism: Inactive	ER binding: **Active (vw)** ER Agonism: **Active (vw)** ER Antagonism: Inactive
OASIS TIMES	Binding (P): **Active (w)** Binding (M): **Active (low)**	Binding (P): Inactive Binding (M): Inactive	Binding (P): Inactive Binding (M): **Active (s)**
OECD Toolbox	**Binder (w)**	**Binder (s)**	**Binder (s)**
Derek Nexus Expert System	No alert	No alert	No alert
ACD Percepta	**Weak Binder ERα**	Inactive	**Weak Binder ERα**
Overall call based on LSM analogues	**Active**	Inactive	Inactive
Overall call based on GSM analogues	**Active**	**Active** (with some discordant results)	Inactive (with some discordant results)
Acronyms: ER=estrogen receptor; M=metabolite; P=parent; s=strong; vw=very weak; w=weak.			

Using in vitro bioactivity and HTTK based IVIVE for prioritisation

The HTS bioactivity data for 4-tert-butylphenol were used to estimate an administered dose equivalent (ADE) through the application of reverse dosimetry (Table 10). Available *in vivo* data for 4-tert-butylphenol are presented in Table 11. The lower limit of the ER-related ADE for 4-tert-butylphenol (11.22 mg/kg-bw/day) is more protective than the effect levels derived from *in vivo* studies found in levels 5 and 4 in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters supporting its utility for prioritisation purposes. In a two-generation reproductive toxicity study (OECD 416) the NOAEL and LOAEL were determined to be 70 and 200 mg/kg-bw/day, respectively. In a combined repeated dose and reproduction/developmental toxicity study (OECD 422) the NOAEL and LOAEL were determined to be 60 and 200 mg/kg-bw/day, respectively. This ADE is also lower than the lowest effect level (LEL=100 mg/kg-bw/day) from the uterotrophic assay (although it is acknowledged that the uterotrophic assay is not intended to be used to generate MOEs). Taken together, these data demonstrate consistency between *in vitro* and *in vivo* point of departure doses, with the BER for 4-tert-butylphenol being within an order of magnitude of the *in vivo* points of departure.

If one were to carry this assessment further, the ADE could be compared against an exposure estimate to derive a Bioactivity Exposure Ratio (BER) (an MOE-like metric). The magnitude of the BER is an indication of relative risk, which can be helpful during screening, priority setting, establishing category boundaries, read-across and/or hazard characterisation. Use for prioritisation is particularly supportable, with the potential for using the BER approach in streamlined or screening level risk assessments. Further, it can be used as a high-throughput risk approximation/classification tool, or as a supplemental line of evidence to support decision making in risk assessment. These applications are especially promising for jurisdictions with flexible regulatory frameworks (e.g., CEPA). However, they should be corroborated through further case studies of additional chemicals.

Table 10. HTS ER related AC50 values converted to oral equivalent dose for 4-tert-butylphenol (CAS RN 98-54-4).

	AC50 (µM)	**ADE (mg/kg bw/day)**
Lowest Activity for ER Pathway Assay	1.63[1]	11.22
Range of Activity for ER Pathway Assays	1.63 – 58.05	11.22 – 291.01

[1] Based on ER pathway assay with lowest AC50 (ATG_ERa_Trans_up)

Table 11. *In vivo* data available for 4-tert-butylphenol (CAS RN 98-54-4).

Study Type	**Effect Levels (mg/kg bw/day)**
Uterotrophic Assay (NICEATM DB) Immature Rat (Kleinstreuer et al. 2016a)	LEL - 100 Result: 1.3 fold increase; s.c. over 3 days; Crj:CD(SD) Rat

Sprague-Dawley rats; 2-generation study (OECD 416); oral via diet; 0, 800, 2500 and 7500 ppm corresponding to 0, 70, 200 and 600 mg/kg bw/day (EU 2008)	NOAEL/LOAEL: 70/200 Increased vaginal epithelium atrophy and reduced relative weight of ovaries and adrenal glands in females at 200 mg/kg bw/day
Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test (OECD TG 422) (REACH, 1996). Sprague-Dawley rats (male and female; n=13); oral gavage; 20, 60, 200 mg/kg body weight	NOAEL/LOAEL: 60 / 200 Abnormal respiratory sounds in a females in and decreased plasma albumin in males. Note: Ovary weights were not recorded in this study. Microscopic examination of the ovary tissue did not reveal any abnormalities.

6. REFERENCES

Al Jishi, T., Sergi, C., 2017. Current perspective of diethylstilbestrol (DES) exposure in mothers and offspring. Reprod. Toxicol. 71, 71-77.

Browne, P., Judson, R.S., Casey, W.M., Kleinstreuer, N.C., Thomas, R.S., 2015. Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model. Environ. Sci. Technol. 49, 8804-8814.

Canada, 2016. Health Canada Chemicals Management Plan (CMP) Science Committee Objectives Paper. Integrating New Approach Methodologies within the CMP: Identifying Priorities for Risk Assessment, Existing Substances Risk Assessment Program. <http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=172614CE-1>. Meeting no. 5.

EPA, 2009. Endocrine disruptor screening program test guidelines OPPTS 890-1600: Uterotrophic Assay. 740-C-09-0010.

EU, 2008. European Union Risk Assessment Report: P-TERT-BUTYLPHENOL, CAS No: 98-54-4 . EINECS No: 202-679-0.

Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen, H.M., Reif, D.M., Rotroff, D.M., Shah, I., Richard, A.M., Dix, D.J., 2010. *In vitro* screening of environmental chemicals for targeted testing prioritization: the ToxCast project. Environ. Health Perspect. 118, 485-492.

Judson, R.S., Magpantay, F.M., Chickarmane, V., Haskell, C., Tania, N., Taylor, J., Xia, M., Huang, R., Rotroff, D.M., Filer, D.L., Houck, K.A., Martin, M.T., Sipes, N., Richard, A.M., Mansouri, K., Setzer, R.W., Knudsen, T.B., Crofton, K.M., Thomas, R.S., 2015. Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 *In vitro* High-Throughput Screening Assays for the Estrogen Receptor. Toxicol. Sci. 148, 137-154.

Kiyama, R., Wada-Kiyama, Y., 2015. Estrogenic endocrine disruptors: Molecular mechanisms of action. Environ. Int. 83, 11-40.

Kleinstreuer, N.C., Ceger, P., Watt, E.D., Martin, M., Houck, K., Browne, P., Thomas, R.S., Casey, W.M., Dix, D.J., Allen, D., Sakamuru, S., Xia, M., Huang, R., Judson, R., 2017. Development and Validation of a Computational Model for Androgen Receptor Activity. Chem. Res. Toxicol. 30, 946-964.

Kleinstreuer, N.C., Ceger, P.C., Allen, D.G., Strickland, J., Chang, X., Hamm, J.T., Casey, W.M., 2016a. A Curated Database of Rodent Uterotrophic Bioactivity. Environ. Health Perspect. 124, 556-562.

Kleinstreuer, N.C., Ceger, P.C., Allen, D.G., Strickland, J., Chang, X., Hamm, J.T., Casey, W.M., 2016b. A Curated Database of Rodent Uterotrophic Bioactivity. Environ. Health Perspect. 124, 556-562.

Liang, J., Shang, Y., 2013. Estrogen and cancer. Annu. Rev. Physiol. 75, 225-240.

Mansouri, K., Abdelaziz, A., Rybacka, A., Roncaglioni, A., Tropsha, A., Varnek, A., Zakharov, A., Worth, A., Richard, A.M., Grulke, C.M., Trisciuzzi, D., Fourches, D., Horvath, D., Benfenati, E., Muratov, E., Wedebye, E.B., Grisoni, F., Mangiatordi, G.F., Incisivo, G.M., Hong, H., Ng, H.W., Tetko, I.V., Balabin, I., Kancherla, J., Shen, J., Burton, J., Nicklaus, M., Cassotti, M., Nikolov, N.G., Nicolotti, O., Andersson, P.L., Zang, Q., Politi, R., Beger, R.D., Todeschini, R., Huang, R., Farag, S., Rosenberg, S.A., Slavov, S., Hu, X., Judson, R.S., 2016. CERAPP: Collaborative Estrogen Receptor Activity Prediction Project. Environ. Health Perspect. 124, 1023-1033.

OECD, 2016. Guidance document on the reporting of defined approaches to be used within integrated apporaches to testing and assessment. No, 255.

OECD, 2007. Test no. 440: Uterotrophic Bioassay in Rodents. Organisation for Economic Co-operation and Development, Geneva, Switzerland.

OECD (2014), Guidance Document for Describing Non-Guideline *In vitro* Test Methods, Series on Testing and Assessment No. 211, ENV/JM/MONO(2014)35, OECD, Paris.

OECD (2016a), Guidance Document on the Reporting of Defined Approaches to Be Used within Integrated Approaches to Testing and Assessment No. 255, ENV/JM/MONO(2016)28, OECD, Paris.

OECD (2016b), Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to Be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation, Series on Testing and Assessment No. 256, ENV/JM/MONO(2016)29, OECD, Paris.

OECD (2016c), "Users' Handbook supplement to the Guidance Document for developing and accessing Adverse Outcome Pathways", OECD Series on Adverse Outcome Pathways, No. 1, OECD Publishing, Paris.

Patlewicz, G., Ball, N., Becker, R.A., Booth, E.D., Cronin, M.T., Kroese, D., Steup, D., van Ravenzwaay, B., Hartung, T., 2014. Read-across approaches--misconceptions, promises and challenges ahead. ALTEX 31, 387-396.

Pradeep, P., Mansouri, K., Patlewicz, G., Judson, R., 2017. A systematic evaluation of analogs and automated read-across prediction of estrogenicity: A case study using hindered phenols. Comput. Toxicol. 4, 22-30.

Tanimoto, T., 1957. An Elementary Mathematical theory of Classification and Prediction. Internal IBM Technical Report.

Thomas, R.S., Philbert, M.A., Auerbach, S.S., Wetmore, B.A., Devito, M.J., Cote, I., Rowlands, J.C., Whelan, M.P., Hays, S.M., Andersen, M.E., Meek, M.E., Reiter, L.W., Lambert, J.C., Clewell, H.J.,3rd, Stephens, M.L., Zhao, Q.J., Wesselkamper, S.C., Flowers, L., Carney, E.W., Pastoor, T.P., Petersen, D.D., Yauk, C.L., Nong, A., 2013. Incorporating new technologies into toxicity testing and risk assessment: moving from 21st century vision to a data-driven framework. Toxicol. Sci. 136, 4-18.

Wetmore, B.A., Wambaugh, J.F., Ferguson, S.S., Sochaski, M.A., Rotroff, D.M., Freeman, K., Clewell, H.J.,3rd, Dix, D.J., Andersen, M.E., Houck, K.A., Allen, B., Judson, R.S., Singh, R., Kavlock, R.J., Richard, A.M., Thomas, R.S., 2012. Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicol. Sci. 125, 157-174.

Yamasaki, K., Takeyoshi, M., Sawaki, M., Imatanaka, N., Shinoda, K., Takatsuki, M., 2003. Immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals. Toxicology 183(1-3):93-115.

APPENDIX A

AOPs are chemically agnostic, linear sequences of events that proceed through all levels of biological organisation. They are analytical constructs that are used to capture and understand the sequence of events that link the point at which a chemical interacts with a biological system (the molecular initiating event, MIE) to the associated disease phenotype (the adverse outcome). An MIE is connected to an adverse outcome by intervening key events (KE), which are connected to one-another by key event relationships. In its simplest form, estrogen signalling[26] can be described according to three main steps: (MIE) binding of a substance to the ER, (KE) binding of the activated ER complex to genomic DNA at an ERE, (KE) alteration of the expression of estrogen-dependent genes. On its own, this sequence of events is not necessarily adverse. However, it could trigger an adverse outcome (AO) if the exposure was of sufficient magnitude, frequency and/or duration. In this case, the possible AOs include a wide range of health disorders, including reproductive and developmental toxicity, and/or an increase of certain tumour types [e.g., (Al Jishi and Sergi 2017, Liang and Shang 2013)]. Alternatively, an absence of estrogenic activity provides some confidence to rule out toxicity produced by this specific AOP.

The OECD recently published a guidance document for the use of AOPs in developing IATA. It describes a framework for how an AOP can be applied to inform and structure IATA for regulatory purposes (Figure A) .A handful of AOPs that are currently 'under development' and might be useful in the context of this IATA once they are published are listed in Table A.

26 KEGG Estrogen Signaling Pathway: http://www.genome.jp/kegg-bin/show_pathway?hsa04915

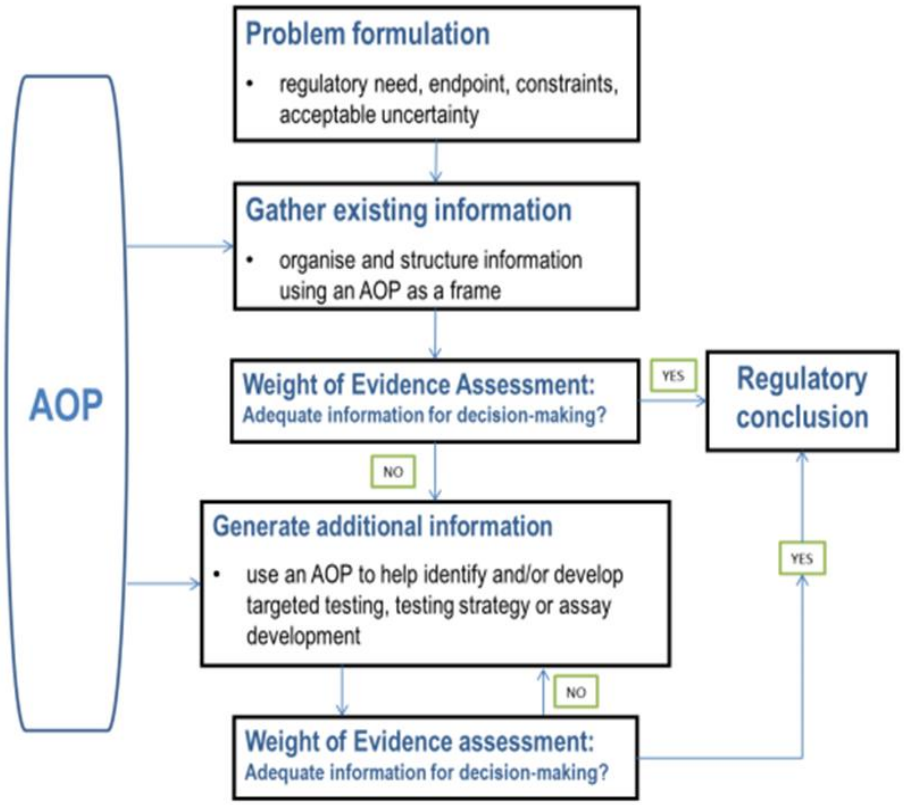


Figure A. OECD Framework for how an AOP can be applied to inform and structure IATA in a decision-making context (reproduced from OECD, 2016).

Table A. Adverse Outcome Pathways that involve Estrogen and/or the ER as identified through a search of the AOP-Wiki.

AOP #	AOP Title	MIE	AO	Relevant to Case Study?
AOPs endorsed by the TFHA/WNT				
25	Aromatase inhibition leading to reproductive dysfunction	Aromatase inhibition	Reproductive dysfunction	**No.** Aromatase is the enzyme that converts testosterone to estrogen. AOP-25, describes how aromatase inhibition leads to reduced levels of estrogen, which leads to reduced levels of VTG. Therefore, AOP-25 is not relevant for the same reasons described for AOPs 30 and 7.
AOPs approved by EAGMST				
23	Androgen receptor agonism leading to reproductive dysfunction	Agonism, Androgen receptor	Reproductive dysfunction	**No.** This AOP describes how reduced androgen synthesis leads to reduced estrogen levels, which leads to reduced VTG levels. Therefore, AOP-23 is not relevant for the same reasons described for AOPs 30 and 7.

AOPs under review by EAGMST				
30	ER antagonism leading to reproductive dysfunction	Antagonism of the ER	Reproductive dysfunction	**No**. The majority of the key events describe the effect of ER antagonism on vitellogenin (VTG) levels. VTG is an important protein in oviparous animals (i.e., animals that lay eggs); however, it is not relevant to mammalian reproduction.
7	Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female	PPAR-gamma activation	Impaired fertility	**No**. This AOP describes how activation of PPAR-gamma leads to a reduction in aromatase levels, which leads to a reduction in estrogen levels, which leads to irregular ovarian cycles and impaired fertility. Alternatively, estrogenic compounds are expected to simulate an increase in estrogen levels.
'under development'				
167	Early-life estrogen receptor activity leading to endometrial carcinoma in the mouse.			
146	Estrogen receptor activation and female precocious puberty			
200	Estrogen receptor activation leading to breast cancer			
29	Estrogen receptor agonism leading to reproductive dysfunction			
165	Antiestrogen activity leading to ovarian adenomas and granular cell tumours in the mouse			

Notes: EAGMST = Extended Advisory Group on Molecular Screening and Toxicogenomics which oversees the OECD AOP development programme; TFHA = Task Force on Hazard Assessment, now renamed the Working Party on Hazard Assessment (WPHA); WNT = Working Group of the National Coordinators for Test Guidelines Programme

APPENDIX B

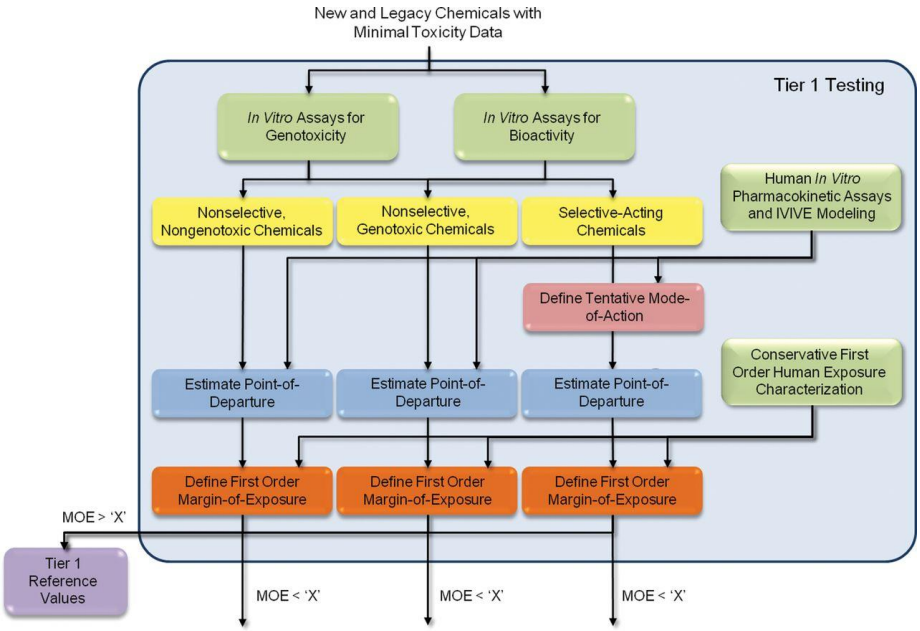


Figure B: Tier 1 of the proposed data-driven framework for tiered toxicity testing (Thomas, et al. 2013).

The green boxes illustrate the data package that includes experimental data and computational modelling results, which serve as inputs into the framework.

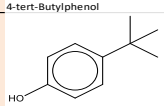
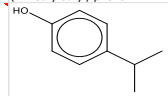
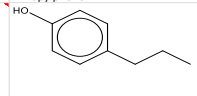
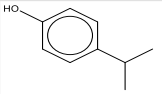
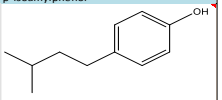
The yellow boxes are separate chemical categories that are determined by the *in vitro* genotoxicity and the high-throughput *in vitro* screening assays.

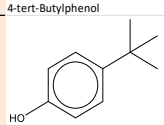
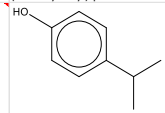
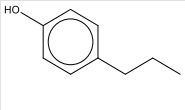
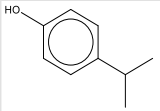
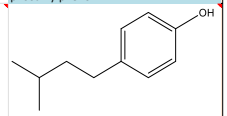
The red box (for selective chemicals) represents the determination of the MOA, which is based on what high-throughput *in vitro* assays were activated/inhibited.

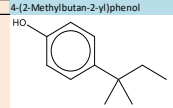
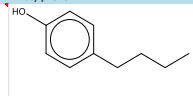
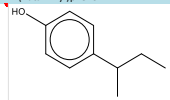
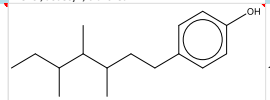
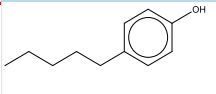
The blue and orange boxes represent the estimation of the point of departure and MOE (i.e., BER) using additional pharmacokinetic and exposure information, respectively. For those chemicals with a MOE (i.e., BER) greater than a defined cut-off, no further testing is performed. Chemicals with a MOE (i.e., BER) less than the cut-off are advanced to Tier 2.

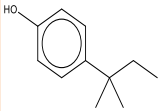
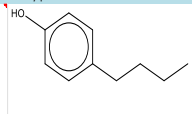
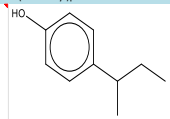
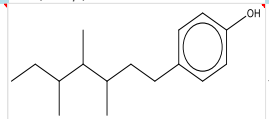
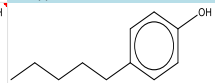
Note: The data-driven framework for tiered toxicity testing proposed by Thomas et al (2013) has been evolving and the present proposal includes a 'Tier 0' screening using toxicogenomics assays. This was presented at the ECHA Topical Scientific Workshop on New Approach Methodologies, April 20, 2016 see: https://echa.europa.eu/documents/10162/22816069/plenary2004_thomas_en.pdf/5494958f-72a7-43aa-9d31-da2429c22c90).

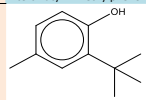
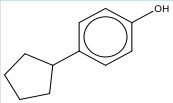
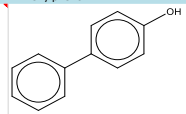
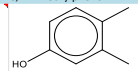
APPENDIX - IATA DTA MATRIC FOR 4-TERT-BUTYLPHENOL (98-54-4)

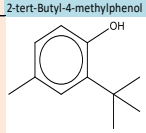
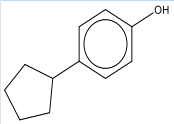
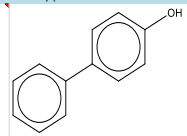
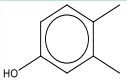
Chemical ID						
		Target	Source 3	Source 4	Source 5	Source 6
CAS RN		98-54-4	25168-06-3	645-56-7	99-89-8	1805-61-4
Name		4-tert-Butylphenol	(1-Methylethyl)-phenol	4-Propylphenol	4-Isopropylphenol	p-Isoamylphenol
Structure						
		$C_{10}H_{14}O$	$C_9H_{12}O$	$C_9H_{12}O$	$C_9H_{12}O$	$C_{11}H_{16}O$
SMILES		CC(C)(C)c1ccc(O)cc1	CC(C)c1ccc(O)cc1	CCCc1ccc(O)cc1	CC(C)c1ccc(O)cc1	CC(C)CCC1=CC=C(O)C=C1
Analogue Search Local Similarity Method (LSM) (Similarity Score)		1	0.75	0.75	0.75	0.666666667
Analogue Search Global Similarity Method (GSM) (Similarity Score)		N/A	N/A	N/A	N/A	0.921348333
Integrated Conclusion for Estrogenicity (For Target)						
Summary in vivo data						
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)		1.Active LEL - 100 mg/kg/day Result: 1.3 fold increase s.c. over 3.0 days Crj:CD(SD) rat (PND 20) 2. Active LEL - 99.24936976 mg/kg/day Result: ND Reported as log lowest effective dose (2.82) s.c. over 3.0 days Crj:CD(SD)IGS rat (PND 20)	ND	ND	ND	ND
Pubertal female		ND	ND	ND	ND	ND
Supporting data related to the target endpoint(s)						
In vitro	ER Binding (CERAPP Literature)	Active (VeryWeak) Literature Sources: 15	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0
	ER Agonist - Reporter Gene / Transcriptional Acitivation (CERAPP Literature)	ND (ND) Literature Sources: 0	Inactive (Inactive) Literature Sources: 2	ND (ND) Literature Sources: 0	Inactive (Inactive) Literature Sources: 2	ND (ND) Literature Sources: 0
	ER Antagonist - Reporter Gene / Transcriptional Acitivation (CERAPP Literature)	Inactive (Inactive) Literature Sources: 6	Inactive (Inactive) Literature Sources: 2	Inactive (Inactive) Literature Sources: 2	Inactive (Inactive) Literature Sources: 2	ND (ND) Literature Sources: 0
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score	0.161		0.0275	0.021	
	ToxCast ER Antagonist AUC Score	0		0	0	
	ACEA_T47D_80 h_Positive	6.60413032601		30.4060260774	47.2997452789	
	ATG_ERE_CIS_up	4.48461562405		31.7227444556	25.5873725701	
	ATG_ERa_TRANS_up	1.6322048577		13.6629120046	19.8164288039	
	NVS_NR_bER	Inactive		Inactive	Inactive	
	NVS_NR_hER	Inactive		Inactive	13.8529904419	
	NVS_NR_mERa	27.0942322233		Inactive	Inactive	
	OT_ER_ERaERa_0480	57.0456988263		69.072680903	35.6121886802	
	OT_ER_ERaERa_1440	58.5040399712		Inactive	Inactive	
	OT_ER_ERaERb_0480	32.0989034179		45.2105238003	51.0442659531	
	OT_ER_ERaERb_1440	24.2300990309		56.6617657244	48.9229407558	
	OT_ER_ERbERb_0480	36.0987016926		39.7786050586	63.7079935013	
	OT_ER_ERbERb_1440	21.4774617912		57.036511678	55.3415379846	
	OT_ERa_EREGFP_0120	17.0226996272		Inactive	Inactive	
	OT_ERa_EREGFP_0480	10.8565314558		Inactive	Inactive	
	Tox21_ERa_BLA_Agonist_ratio	Inactive		Inactive	Inactive	
	Tox21_ERa_BLA_Antagonist_ratio	Inactive		Inactive	Inactive	
	Tox21_ERa_LUC_BG1_Agonist	Inactive		Inactive	Inactive	
	Tox21_ERa_LUC_BG1_Antagonist	Inactive		Inactive	Inactive	
	Cytotoxicity Lower Limit (all 'burst assays')	161.9273999		161.9273999	161.9273999	

Chemical ID						
		Target	Source 3	Source 4	Source 5	Source 6
CAS RN		98-54-4	25168-06-3	645-56-7	99-89-8	1805-61-4
Name		4-tert-Butylphenol	(1-Methylethyl)-phenol	4-Propylphenol	4-Isopropylphenol	p-Isoamylphenol
Structure						
		$C_{10}H_{14}O$	$C_9H_{12}O$	$C_9H_{12}O$	$C_9H_{12}O$	$C_{11}H_{16}O$
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Active (Weak)	Active (VeryWeak)	Active (VeryWeak)	Active (VeryWeak)	ND (ND)
	CERAPP Consensus Agonist (Potency Level)	Active (VeryWeak)	Active (VeryWeak)	Active (VeryWeak)	Active (VeryWeak)	ND (ND)
	CERAPP Consensus Antagonist (Potency Level)	Active (Strong)	Active (Moderate)	Active (Moderate)	Active (Moderate)	ND (ND)
	OASIS TIMES ER Binding QSAR - Parent	0.001<RBA<0.1 Weak Active	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active	0.001<RBA<0.1 Weak Active
	OASIS TIMES ER Binding QSAR - Metabolites	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active
	EPA rtER Expert System v1 - Profiler (OECD Toolbox v3.4)	Alkylphenol	Alkylphenol	Alkylphenol	Alkylphenol	Alkylphenol
	EPA rtER Expert System v1 - QSAR (OECD Toolbox v3.4)	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%
	Estrogen Receptor Binding -Profiler (OECD Toolbox v3.4)	Weak Binder, OH group	Weak Binder, OH group	Weak Binder, OH group	Weak Binder, OH group	Weak Binder, OH group
	Derek Nexus Expert System (Oestrogenicity Mammal)	No Alert	No Alert	No Alert	No Alert	Mammal Plausible Alkylphenol or precursor
	ACD Percepta (Estrogen Receptor Binding)	Weak Binder ERα	No Binding to ERα	No Binding to ERα	No Binding to ERα	Weak Binder ERα
Physical-chemical data						
Molecular Weight (Da)		150.2176	136	136.191	136.191	164.2441
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		36.91	13.8	38.3	27.49	49.36
Boiling point (°C)(EPI Suite 4.1 - MPBPVP v1.43)		229.66	218	229.65	218.32	254.63
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		0.596	2.01	4.12	2.013	0.685
LogPow (EPI Suite 4.1 - WSKOW v1.42)		3.42	2.97	3.04	2.97	3.95
Kinetics Parameters						
in vitro	95% Quantile Css @ 1mg/kg/day intrinsic clearance assay @ 1µM (Wetmore et al.) (mg/L)	0.0218126				
	95% Quantile Css @ 1mg/kg/day intrinsic clearance assay @ 10µM (Wetmore et al.) (mg/L)	0.03019832				
QSAR ACD Percepta 2015 - PK Explorer Single Dose	Lipinski rule violations	0	0	0	0	0
	Oral bioavailability (%F) (1 mg/kg)	99.3	99.4	99.4	99.4	99.3
	Cmax (ug/ml) (1 mg/kg)	0.43	0.47	0.32	0.47	0.4
	Tmax (min) (1 mg/kg)	69	61	61	61	78
	AUC0-inf (ug.h/ml) (1 mg/kg)	5.94	6.09	4.17	6.09	5.5
Exposure Estimates(s)						
EPA ExpoCast (mg/kg BW/day) [median]		0.0000608		0.000000092	0.000000136	
EPA ExpoCast (mg/kg BW/day) [95%]		0.00416		0.00000552	0.00000819	
In Vitro Bioactivity Oral Equivalent Dose (OED)						
OED - ER related lower bound (HTS) (mg/kg/day)		11.22556				

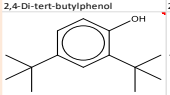
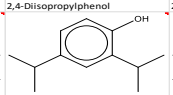
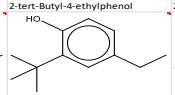
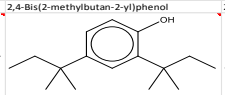
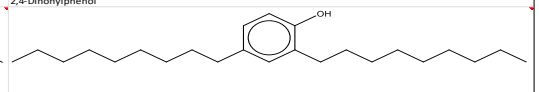
Chemical ID						
		Source 9	Source 10	Source 11	Source 12	Source 13
CAS RN		80-46-6	1638-22-8	99-71-8	121158-58-5	14938-35-3
Name		4-(2-Methylbutan-2-yl)phenol	4-Butylphenol	4-(Butan-2-yl)phenol	Phenol, dodecyl-, branched	4-Pentylphenol
Structure		 $C_{11}H_{16}O$	 $C_{10}H_{14}O$	 $C_{10}H_{14}O$	 $C_{16}H_{26}O$	 $C_{10}H_{14}O$
SMILES		CC(C)(CC)c1ccc(O)cc1	CCCCc1ccc(O)cc1	CCC(C)c1ccc(O)cc1	CCC(C)C(C)C(C)CCc1ccc(O)cc1	CCCCCc1ccc(O)cc1
Analogue Search Local Similarity Method (LSM) (Similarity Score)		0.666666687	0.600000024	0.600000024	0.571428597	N/A
Analogue Search Global Similarity Method (GSM) (Similarity Score)		0.977272749	0.931034505	0.954545438	N/A	0.931818187
Integrated Conclusion for Estrogenicity (For Target)						
Summary in vivo data						
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)		1. Active LEL - 200 mg/kg/day Result: 2.6 fold increase s.c. over 3.0 days Crj:CD(SD) rat (PND 19) 2. Active LEL - 200 mg/kg/day Result: 2.6 fold increase s.c. over 3.0 days Crj:CD(SD) rat (PND 19) 3. Active LEL - 202.0667245 mg/kg/day Result: ND Reported as log lowest effective dose (3.09) s.c. over 3.0 days Crj:CD(SD)IGS rat (PND 20) 4. Active LEL - 200 mg/kg/day Result: 2.3 fold increase s.c. over 3.0 days ND rat (PND 19)	ND	ND	ND	ND
Pubertal female		ND	ND	ND	ND	ND
Supporting data related to the target endpoint(s)						
In vitro	ER Binding (CERAPP Literature)	Active (Weak) Literature Sources: 14	Active (ND) Literature Sources: 4	Active (ND) Literature Sources: 9	ND (ND) Literature Sources: 0	Active (Weak) Literature Sources: 6
	ER Agonist - Reporter Gene / Transcriptional Activi	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	Active (Weak) Literature Sources: 3
	ER Antagonist - Reporter Gene / Transcriptional A	Inactive (Inactive) Literature Sources: 6	Inactive (Inactive) Literature Sources: 2	Inactive (Inactive) Literature Sources: 4	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score	0.282	0.111	0.163		
	ToxCast ER Antagonist AUC Score	0.000192	0	0		
	ACEA_T47D_80 h_Positive	3.35719456352	7.19630215626	9.86734571118		
	ATG_ERE_CIS_up	2.02825215562	8.42657725054	2.97526502644		
	ATG_ERa_TRANS_up	2.9221089108	4.81201649738	3.15066786985		
	NVS_NR_bER	Inactive	Inactive	Inactive		
	NVS_NR_hER	Inactive	Inactive	Inactive		
	NVS_NR_mERa	22.075548259	Inactive	Inactive		
	OT_ER_ERaERa_0480	10.8157999282	19.2218323651	43.541629371		
	OT_ER_ERaERa_1440	16.1237250261	21.80778883	70.5022599701		
	OT_ER_ERaERb_0480	7.00274765843	12.1410247036	50.6837181161		
	OT_ER_ERaERb_1440	10.3861472186	17.1026984034	40.8829837769		
	OT_ER_ERbERb_0480	5.41868015127	14.918125846	19.5502707995		
	OT_ER_ERbERb_1440	7.54253818572	17.6954549005	35.7279049448		
	OT_ERa_EREGFP_0120	0.633585555129	19.6353502725	11.4916199575		
	OT_ERa_EREGFP_0480	1.32529279851	Inactive	20.4331356255		
	Tox21_ERa_BLA_Agonist_ratio	Inactive	Inactive	Inactive		
	Tox21_ERa_BLA_Antagonist_ratio	Inactive	Inactive	Inactive		
	Tox21_ERa_LUC_BG1_Agonist	12.577655956	63.7244175956	Inactive		
	Tox21_ERa_LUC_BG1_Antagonist	Inactive	Inactive	Inactive		
	Cytotoxicity Lower Limit (all 'burst assays')	19.30762015	161.9273999	161.9273999		

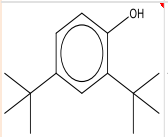
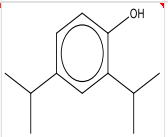
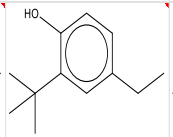
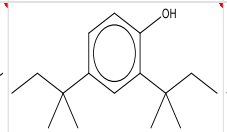
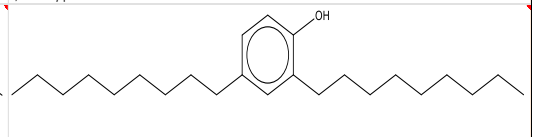
Chemical ID						
		Source 9	Source 10	Source 11	Source 12	Source 13
CAS RN		80-46-6	1638-22-8	99-71-8	121158-58-5	14938-35-3
Name		4-(2-Methylbutan-2-yl)phenol	4-Butylphenol	4-(Butan-2-yl)phenol	Phenol, dodecyl-, branched	4-Pentylphenol
Structure		 $C_{11}H_{16}O$	 $C_{10}H_{14}O$	 $C_{10}H_{14}O$	 $C_{16}H_{26}O$	 $C_{10}H_{14}O$
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Active (Weak)	Active (VeryWeak)	Active (Weak)	Active (Weak)	Active (VeryWeak)
	CERAPP Consensus Agonist (Potency Level)	Active (Weak)	Active (VeryWeak)	Active (Weak)	Active (Weak)	Active (VeryWeak)
	CERAPP Consensus Antagonist (Potency Level)	Active (Strong)	Active (Strong)	Active (Strong)	Active (Strong)	Active (Strong)
	OASIS TIMES ER Binding QSAR - Parent	0.001<RBA<0.1 Weak Active	0.001<RBA<0.1 Weak Active	0.001<RBA<0.1 Weak Active	Not Active	0.001<RBA<0.1 Weak Active
	OASIS TIMES ER Binding QSAR - Metabolites	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active	Not Active	0<RBA<0.001 Low Active
	EPA rtER Expert System v1 - Profiler (OECD Toolbox v3.4)	Alkylphenol	Alkylphenol	Alkylphenol	Alkylphenol	Alkylphenol
	EPA rtER Expert System v1 - QSAR (OECD Toolbox v3.4)	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%
	Estrogen Receptor Binding -Profiler (OECD Toolbox v3.4)	Weak Binder, OH group	Weak Binder, OH group	Weak Binder, OH group	Strong Binder, OH group	Weak Binder, OH group
	Derek Nexus Expert System (Oestrogenicity Mam	No Alert	Mammal Plausible Alkylphenol or precursor	No Alert	Mammal Plausible Alkylphenol or precursor	Mammal Plausible Alkylphenol or precursor
	ACD Percepta (Estrogen Receptor Binding)	Weak Binder ERα	No Binding to ERα	No Binding to ERα	Strong Binder ERα	Weak Binder ERα
Physical-chemical data						
Molecular Weight (Da)		164.2441	150.2176	150.2176	234.38	164.24
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		47.7	49.21	38.56	79.75	59.85
Boiling point (°C)(EPI Suite 4.1 - MPBPVP v1.43)		247.72	247.71	236.93	313.59	264.87
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		0.1547	1.707	1.117	0.012	1.48
LogPow (EPI Suite 4.1 - WSKOW v1.42)		3.91	3.53	3.46	6.26	4.02
Kinetics Parameters						
in vitro	95% Quantile Css @ 1mg/kg/day intrinsic clearance assay @ 1µM (Wetmore et al.) (mg/L)	0.4504448		0.05048554		
	95% Quantile Css @ 1mg/kg/day intrinsic clearance assay @ 10µM (Wetmore et al.) (mg/L)	0.3468825		0.04297087		
QSAR ACD Percepta 2015 - PK Explorer Single Dose	Lipinski rule violations	0	0	0	1 (LogP ≥ 5)	0
	Oral bioavailability (%F) (1 mg/kg)	99.3	99.3	99.3	61.9	98.93
	Cmax (ug/ml) (1 mg/kg)	0.39	0.42	0.43	0.12	0.35
	Tmax (min) (1 mg/kg)	81	69	68	316	154
	AUC0-inf (ug.h/ml) (1 mg/kg)	5.39	5.74	5.95	1.03	4.28
Exposure Estimates(s)						
EPA ExpoCast (mg/kg BW/day) [median]		0.0000374	1.71E-08	0.00000029		
EPA ExpoCast (mg/kg BW/day) [95%]		0.00188	0.00000217	0.0000218		
In Vitro Bioactivity Oral Equivalent Dose (OED)						
OED - ER related lower bound (HTS) (mg/kg/day)		0.2309851		8.852918		

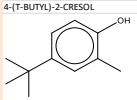
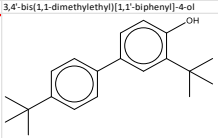
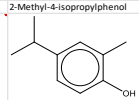
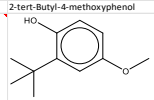
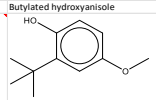
Chemical ID					
		Source 14	Source 15	Source 16	Source 17
CAS RN		2409-55-4	1518-83-8	92-69-3	95-65-8
Name		2-tert-Butyl-4-methylphenol	4-CYCLOPENTYLPHENOL	4-Phenylphenol	3,4-Dimethylphenol
Structure					
SMILES		Cc1ccc(O)c(c1)C(C)(C)C	Oc1ccc(cc1)C1CCCC1	Oc1ccc(cc1)-c1ccccc1	Cc1cc(O)ccc1C
Analogue Search Local Similarity Method (LSM) (Similarity Score)		N/A	N/A	N/A	N/A
Analogue Search Global Similarity Method (GSM) (Similarity Score)		0.923913062	0.922222197	0.922222197	0.922222197
Integrated Conclusion for Estrogenicity (For Target)					
Summary in vivo data					
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)		ND	ND	ND	ND
Pubertal female		ND	ND	ND	ND
Supporting data related to the target endpoint(s)					
In vitro	ER Binding (CERAPP Literature)	Inactive (Inactive) Literature Sources: 8	Active (Weak) Literature Sources: 5	Active (Weak) Literature Sources: 21	Inactive (Inactive) Literature Sources: 8
	ER Agonist - Reporter Gene / Transcriptional Acitivation (CEI	Inactive (Inactive) Literature Sources: 4	Active (Weak) Literature Sources: 2	Active (Weak) Literature Sources: 9	Inactive (Inactive) Literature Sources: 4
	ER Antagonist - Reporter Gene / Transcriptional Acitivation (	Inactive (Inactive) Literature Sources: 4	ND (ND) Literature Sources: 0	Inactive (Inactive) Literature Sources: 4	Inactive (Inactive) Literature Sources: 4
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score				
	ToxCast ER Antagonist AUC Score				
	ACEA_T47D_80 h_Positive				
	ATG_ERE_CIS_up				
	ATG_ERa_TRANS_up				
	NVS_NR_bER				
	NVS_NR_hER				
	NVS_NR_mERa				
	OT_ER_ERaERa_0480				
	OT_ER_ERaERa_1440				
	OT_ER_ERaERb_0480				
	OT_ER_ERaERb_1440				
	OT_ER_ERbERb_0480				
	OT_ER_ERbERb_1440				
	OT_ERa_EREGFP_0120				
	OT_ERa_EREGFP_0480				
	Tox21_ERa_BLA_Agonist_ratio				
	Tox21_ERa_BLA_Antagonist_ratio				
	Tox21_ERa_LUC_BG1_Agonist				
	Tox21_ERa_LUC_BG1_Antagonist				
	Cytotoxicity Lower Limit (all 'burst assays')				

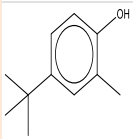
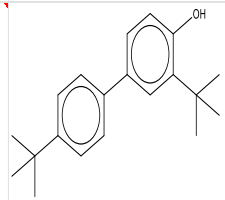
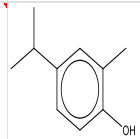
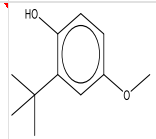
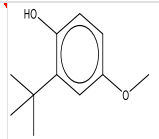
Chemical ID					
		Source 14	Source 15	Source 16	Source 17
CAS RN		2409-55-4	1518-83-8	92-69-3	95-65-8
Name		2-tert-Butyl-4-methylphenol	4-CYCLOPENTYLPHENOL	4-Phenylphenol	3,4-Dimethylphenol
Structure					
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Inactive (Suspicious)	Active (VeryWeak)	Active (Weak)	Inactive (Suspicious)
	CERAPP Consensus Agonist (Potency Level)	Inactive (Suspicious)	Active (VeryWeak)	Active (VeryWeak)	Inactive (Suspicious)
	CERAPP Consensus Antagonist (Potency Level)	Inactive (Suspicious)	Active (Weak)	Active (Weak)	Inactive (Suspicious)
	OASIS TIMES ER Binding QSAR - Parent	Not Active	0.001<RBA<0.1 Weak Active	0.001<RBA<0.1 Weak Active	0<RBA<0.001 Low Active
	OASIS TIMES ER Binding QSAR - Metabolites	Not Active	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active	0<RBA<0.001 Low Active
	EPA rtER Expert System v1 - Profiler (OECD Toolbox v3.4)	Alkylphenol	No Alert	Phenylphenols	Alkylphenol
	EPA rtER Expert System v1 - QSAR (OECD Toolbox v3.4)	RBA>0.00001%	Out of Domain	RBA>0.00001%	RBA>0.00001%
	Estrogen Receptor Binding -Profiler (OECD Toolbox v3.4)	Weak Binder, OH group	Weak Binder, OH group	Moderate Binder, OH group	Weak Binder, OH group
	Derek Nexus Expert System (Oestrogenicity Mammal)	No Alert	Mammal Plausible Alkylphenol or precursor	No Alert	No Alert
	ACD Percepta (Estrogen Receptor Binding)	Weak Binder ERα	No Binding to ERα	Weak Binder ERα	No Binding to ERα
Physical-chemical data					
Molecular Weight (Da)		164.2441	162.2283	170.2072	122.1644
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		51.87	59.48	86.56	25.37
Boiling point (°C)(EPI Suite 4.1 - MPBPVP v1.43)		247.72	270.72	317.36	210.67
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		1.74	0.381	0.00231	2.45
LogPow (EPI Suite 4.1 - WSKOW v1.42)		3.97	3.84	3.28	2.61
Kinetics Parameters					
in vitro	95% Quantile Css @ 1mg/kg/day intrinsic clearance assay @ 1µM (Wetmore et al.) (mg/L)				
	95% Quantile Css @ 1mg/kg/day intrinsic clearance assay @ 10µM (Wetmore et al.) (mg/L)				
QSAR ACD Percepta 2015 - PK Explorer Single Dose	Lipinski rule violations	0	0	0	0
	Oral bioavailability (%F) (1 mg/kg)	99.32	99.22	98.98	99.42
	Cmax (ug/ml) (1 mg/kg)	0.38	0.4	0.44	0.52
	Tmax (min) (1 mg/kg)	73.67	111.33	144	56.33
	AUC0-inf (ug.h/ml) (1 mg/kg)	5.22	5.65	8.08	6.24
Exposure Estimates(s)					
EPA ExpoCast (mg/kg BW/day) [median]					
EPA ExpoCast (mg/kg BW/day) [95%]					
In Vitro Bioactivity Oral Equivalent Dose (OED)					
OED - ER related lower bound (HTS) (mg/kg/day)					

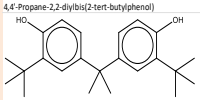
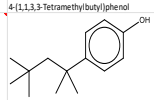
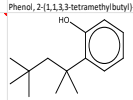
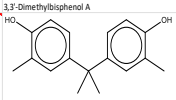
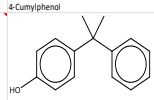
APPENDIX - IATA DATA MATRIX FOR 2,4-DI-TERT-BUTYLPHENOL (96-76-4)

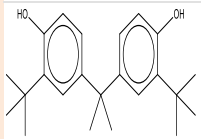
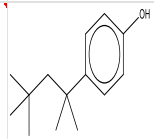
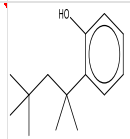
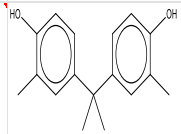
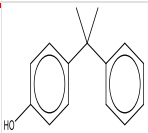
Chemical ID						
		Target	Source 1	Source 2	Source 3	Source 4
CAS RN		96-76-4	2934-05-6	96-70-8	120-95-6	137-99-5
Name		2,4-Di-tert-butylphenol	2,4-Diisopropylphenol	2-tert-Butyl-4-ethylphenol	2,4-Bis(2-methylbutan-2-yl)phenol	2,4-Dinonylphenol
Structure						
SMILES		CC(C)(C)c1cc(ccc1O)C(C)(C)C	CC(C)c1cc(ccc1O)C(C)C	CCc1cc(c(O)cc1)C(C)(C)C	CC(C)(CC)c1cc(ccc1O)C(C)(C)CC	CCCCCCCCCc1cc(CCCCCCCCC)c(O)cc1
Analogue Search Local Similarity Method (LSM) (Similarity Score)		1	0.5625	0.5	0.444444471	0.25
Analogue Search Global Similarity Method (GSM) (Similarity Score)		N/A	0.967741907	N/A	N/A	N/A
Integrated Conclusion for Estrogenicity						
Summary in vivo data						
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)		Inactive Max dose - 1000.0 mg/kg/day s.c. over 3.0 days Crj:CD(SD)IGS rat (PND 20)	ND	ND	ND	ND
Supporting data related to the target endpoint(s)						
In vitro		Active (VeryWeak)	Inactive (Inactive)	Active (Weak)	ND (ND)	ND (ND)
	ER Binding (CERAPP Literature)	Literature Sources: 6	Literature Sources: 4	Literature Sources: 1	Literature Sources: 0	Literature Sources: 0
		Inactive (Inactive)	Inactive (Inactive)	ND (ND)	Inactive (Inactive)	ND (ND)
	ER Agonist - Reporter Gene / Transcriptional	Literature Sources: 4	Literature Sources: 2	Literature Sources: 0	Literature Sources: 4	Literature Sources: 0
		ND (ND)	Inactive (Inactive)	Inactive (Inactive)	ND (ND)	ND (ND)
	ER Antagonist - Reporter Gene / Transcriptio	Literature Sources: 0	Literature Sources: 2	Literature Sources: 2	Literature Sources: 0	Literature Sources: 0
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score	0	0	0.0193	0.00528	
	ToxCast ER Antagonist AUC Score	0	0	0	0.00113	
	ACEA_T47D_80 h_Positive	Inactive	Inactive	Inactive	Inactive	
	ATG_ERE_CIS_up	Inactive	36.753033701	Inactive	Inactive	
	ATG_ERa_TRANS_up	Inactive	27.3376098151	22.2914793418	33.3751796776	
	NVS_NR_bER		Inactive	Inactive		
	NVS_NR_hER		0.0571877772638	Inactive	Inactive	
	NVS_NR_mERa		Inactive	7.78788147455		
	OT_ER_ERaERa_0480	Inactive	Inactive	Inactive	Inactive	
	OT_ER_ERaERa_1440	Inactive	Inactive	Inactive	Inactive	
	OT_ER_ERaERb_0480	Inactive	Inactive	Inactive	Inactive	
	OT_ER_ERaERb_1440	Inactive	Inactive	Inactive	Inactive	
	OT_ER_ERbERb_0480	Inactive	14.6078070448	21.326093121	4.73492094704	
	OT_ER_ERbERb_1440	Inactive	Inactive	Inactive	Inactive	
	OT_ERa_EREGFP_0120	Inactive	Inactive	Inactive	Inactive	
	OT_ERa_EREGFP_0480	Inactive	Inactive	Inactive	Inactive	
	Tox21_ERa_BLA_Agonist_ratio	Inactive	Inactive	Inactive	Inactive	
	Tox21_ERa_BLA_Antagonist_ratio	Inactive	Inactive	Inactive	Inactive	
	Tox21_ERa_LUC_BG1_Agonist	Inactive	Inactive	19.8069646734	Inactive	
	Tox21_ERa_LUC_BG1_Antagonist	44.4612393484	Inactive	Inactive	55.7366351929	
	Cytotoxicity Lower Limit (all 'burst assays')	4.305684796	0	6.001668521	3.30245152	

Chemical ID						
		Target	Source 1	Source 2	Source 3	Source 4
CAS RN		96-76-4	2934-05-6	96-70-8	120-95-6	137-99-5
Name		2,4-Di-tert-butylphenol	2,4-Diisopropylphenol	2-tert-Butyl-4-ethylphenol	2,4-Bis(2-methylbutan-2-yl)phenol	2,4-Dinonylphenol
Structure						
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Inactive (Suspicious)	Inactive (Suspicious)	Inactive (Suspicious)	Active (VeryWeak)	Active (Weak)
	CERAPP Consensus Agonist (Potency Level)	Inactive (Suspicious)	Inactive (Suspicious)	Inactive (Suspicious)	Active (VeryWeak)	Active (Weak)
	CERAPP Consensus Antagonist (Potency Lev	Inactive (Suspicious)	Inactive (Suspicious)	Inactive (Suspicious)	Inactive (Suspicious)	Active (Moderate)
	OASIS TIMES ER Binding QSAR - Parent	Not Active	Not Active	Not Active	Not Active	Not Active
	OASIS TIMES ER Binding QSAR - Metabolites	Not Active	Not Active	Not Active	Weak Active (0.001<RBA<0.1)	Not Active
	EPA rtER Expert System v1 - Profiler (OECD To	Alkylphenols	Alkylphenols	Alkylphenols	Alkylphenols	Alkylphenols
	EPA rtER Expert System v1 - QSAR (OECD Tool	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%
	Estrogen Receptor Binding - Profiler (OECD Ti	Strong binder, OH group	Moderate binder, OH grooup	Moderate binder, OH grooup	Strong binder, OH group	Strong binder, OH group
	Derek Nexus Expert System (Oestrogenicity	No Alert	No Alert	No Alert	No Alert	No Alert
	ACD Percepta (Estrogen Receptor Binding)	No Binding to Erα	Weak Binding to Erα	Strong Binding to Erα	No Binding to Erα	No Binding to Erα
Physical-chemical data						
Molecular Weight (Da)		206.3239	178.2707	178.2707	234.377	346.5897
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		76.96	55.74	62.31	89.03	162.58
Boiling point (°C)(EPI Suite 4.1 - MPBPVP v1.43)		281.15	261.41	264.88	310.95	430.19
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		0.356	0.4066	0.2893	0.04626	2.013E-07
LogPow (EPI Suite 4.1 - WSKOW v1.42)		5.33	4.42	4.46	6.31	10.47
Kinetics Parameters						
in vitro	95% Quantile Css @ 1mg/kg/day (intrinsic clearance assay @ 1µM (Wetmore et al.) (mg/L)					
	95% Quantile Css @ 1mg/kg/day (intrinsic clearance assay @ 10µM (Wetmore et al.) (mg/L)					
QSAR						
ACD Percepta 2015 - PK Explorer	Lipinski rule violations	0	0	0	1 (Log P)	1 (Log P)
	Oral bioavailability (%F) (1 mg/kg)	98.19	99.27	99.25	89.86	0.04
	Cmax (ug/ml) (1 mg/kg)	0.29	0.37	0.34	0.18	0
	Tmax (min) (1 mg/kg)	181.67	82.67	92	259.67	559.83
	AUC0-inf (ug.h/ml) (1 mg/kg)	5.93	5.47	5.18	3.64	0
Oral Equivalent Dose						
OED - ER related lower bound (mg/kg/day)						
OED - ER related lower bound (HTS) (mg/kg/day)						

		Chemical ID				
		Source 5	Source 6	Source 7	Source 8	Source 9
CAS RN		98-27-1	42479-88-9	1740-97-2	121-00-6	25013-16-5
Name		4-(T-BUTYL)-2-CRESOL	3,4'-bis(1,1-dimethylethyl)[1,1'-biphenyl]-4-ol	2-Methyl-4-isopropylphenol	2-tert-Butyl-4-methoxyphenol	Butylated hydroxyanisole
Structure						
SMILES		Cc1cc(ccc1O)C(C)(C)C	CC(C)(C)c1ccc(cc1)-c1cc(c(O)cc1)C(C)(C)C	Cc1cc(ccc1O)C(C)C	COc1cc(c(O)cc1)C(C)(C)C	COc1cc(c(O)cc1)C(C)(C)C
Analogue Search Local Similarity Method (LSM) (Similarity Score)		0.25	0.222222224	0.1875	0.166666672	0.166666672
Analogue Search Global Similarity Method (GSM) (Similarity Score)		N/A	N/A	N/A	N/A	N/A
Integrated Conclusion for Estrogenicity						
		Summary in vivo data				
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)		ND	ND	ND	ND	ND
Supporting data related to the target endpoint(s)						
In vitro	ER Binding (CERAPP Literature)	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0
	ER Agonist - Reporter Gene / Transcriptional Acitivation (CE	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0
	ER Antagonist - Reporter Gene / Transcriptional Acitivation (	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	Inactive (Inactive) Literature Sources: 4	Inactive (Inactive) Literature Sources: 4
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score				0.0139	
	ToxCast ER Antagonist AUC Score				0	
	ACEA_T47D_80 h_Positive				5.18536491976	
	ATG_ERE_CIS_up				Inactive	
	ATG_ERa_TRANS_up				23.8325525748	
	NVS_NR_bER					
	NVS_NR_hER				Inactive	
	NVS_NR_mERa					
	OT_ER_ERaERa_0480				Inactive	
	OT_ER_ERaERa_1440				Inactive	
	OT_ER_ERaERb_0480				62.3932430395	
	OT_ER_ERaERb_1440				53.0811678218	
	OT_ER_ERbERb_0480				32.3952065019	
	OT_ER_ERbERb_1440				59.3778356853	
	OT_ERa_EREGFP_0120				Inactive	
	OT_ERa_EREGFP_0480				Inactive	
	Tox21_ERa_BLA_Agonist_ratio				Inactive	
	Tox21_ERa_BLA_Antagonist_ratio				Inactive	
	Tox21_ERa_LUC_BG1_Agonist				Inactive	
	Tox21_ERa_LUC_BG1_Antagonist				Inactive	
	Cytotoxicity Lower Limit (all 'burst assays')				8.090873264	

Chemical ID						
		Source 5	Source 6	Source 7	Source 8	Source 9
CAS RN		98-27-1	42479-88-9	1740-97-2	121-00-6	25013-16-5
Name		4-(T-BUTYL)-2-CRESOL	3,4'-bis(1,1-dimethylethyl)[1,1'-biphenyl]-4-ol	2-Methyl-4-isopropylphenol	2-tert-Butyl-4-methoxyphenol	Butylated hydroxyanisole
Structure						
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Active (VeryWeak)	Active (Moderate)	Active (VeryWeak)	Inactive (Suspicious)	Inactive (Suspicious)
	CERAPP Consensus Agonist (Potency Level)	Active (VeryWeak)	Active (Weak)	Inactive (Suspicious)	Inactive (Suspicious)	Inactive (Suspicious)
	CERAPP Consensus Antagonist (Potency Level)	Active (VeryWeak)	Active (Weak)	Inactive (Suspicious)	Inactive (Suspicious)	Inactive (Suspicious)
	OASIS TIMES ER Binding QSAR - Parent	Not Active	Not Active	Weak Active (0.001<RBA<0.1)	Low Active (0<RBA<0.001)	Low Active (0<RBA<0.001)
	OASIS TIMES ER Binding QSAR - Metabolites	Not Active	Not Active	Low Active (0<RBA<0.001)	Not Active	Not Active
	EPA rtER Expert System v1 - Profiler (OECD Toolbox v3.3)	Alkylphenols	Phenylphenols	Alkylphenols	Alkoxyphenols	Alkoxyphenols
	EPA rtER Expert System v1 - QSAR (OECD Toolbox v3.3)	RBA>0.00001%	Unknown Binding Potential	RBA>0.00001%	RBA>0.00001%	RBA>0.00001%
	Estrogen Receptor Binding -Profiler (OECD Toolbox v3.3)	Weak binder, OH group	Strong binder, OH group	Weak binder, OH group	Moderate binder, OH grooup	Moderate binder, OH grooup
	Derek Nexus Expert System (Oestrogenicity Mammal)	No Alert	No Alert	No Alert	No Alert	No Alert
	ACD Percepta (Estrogen Receptor Binding)	Weak Binding to Erα	Weak Binding to Erα	No Binding to Erα	No Binding to Erα	No Binding to Erα
Physical-chemical data						
Molecular Weight (Da)		164.2441	282.4199	150.2176	180.2435	180.25
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		51.87	144.31	38.1	66.21	66.21
Boiling point (°C)(EPI Suite 4.1 - MPBPVP v1.43)		247.72	389.2	236.92	265.53	265.53
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		2.9	2.78E-05	4.78	0.312	0.312
LogPow (EPI Suite 4.1 - WSKOW v1.42)		3.97	7.1	3.52	3.5	3.5
Kinetics Parameters						
in vitro	95% Quantile Css @ 1mg/kg/day (intrinsic clearance assay @ 1µM (Wetmore et al.) (mg/L)				0.1444037	
	95% Quantile Css @ 1mg/kg/day (intrinsic clearance assay @ 10µM (Wetmore et al.) (mg/L)				0.1988537	
QSAR						
ACD Percepta 2015 - PK Explorer	Lipinski rule violations	0 1 (Log P)		0	0	0
	Oral bioavailability (%F) (1 mg/kg)	99.32	50.88	99.34	99.36	99.36
	Cmax (ug/ml) (1 mg/kg)	0.39	0.09	0.43	0.42	0.42
	Tmax (min) (1 mg/kg)	73.67	372.33	64.33	72.67	72.67
	AUC0-inf (ug.h/ml) (1 mg/kg)	5.33	2.27	5.87	5.38	5.38
Oral Equivalent Dose						
OED - ER related lower bound (mg/kg/day)						
OED - ER related lower bound (HTS) (mg/kg/day)						

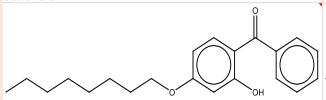
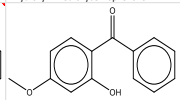
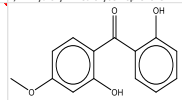
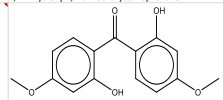
Chemical ID						
		Source 10	Source 11	Source 12	Source 13	Source 14
CAS RN		79-96-9	140-66-9	3884-95-5	79-97-0	599-64-4
Name		4,4'-Propane-2,2-diylbis(2-tert-butylphenol)	4-(1,1,3,3-Tetramethylbutyl)phenol	Phenol, 2-(1,1,3,3-tetramethylbutyl)-	3,3'-Dimethylbisphenol A	4-Cumylphenol
Structure						
SMILES		CC(C)(c1cc(c(O)cc1)C(C)(C)C)c1cc(c(O)cc1)C(C)(C)C	CC(C)(C)CC(C)(C)c1ccc(O)cc1	CC(C)(C)CC(C)(C)c1ccccc1O	CC(C)(c1cc(C)c(O)cc1)c1cc(C)c(O)cc1	CC(C)(c1ccccc1)c1ccc(O)cc1
Analogue Search Local Similarity Method (LSM) (Similarity Score)		0.137931034	N/A	N/A	N/A	N/A
Analogue Search Global Similarity Method (GSM) (Similarity Score)			0.956521749	0.956521749	0.948453605	0.926315784
Integrated Conclusion for Estrogenicity						
Summary in vivo data						
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)		ND	Active LEL - 200 mg/kg/day Result: 3 fold increase s.c. over 3.0 days ND rat (PND 19)	ND	ND	Active LEL - 200 mg/kg/day Result: 273 % of control s.c. over 3.0 days Crj:CD(SD) rat (PND 20 - 22) Active LEL - 20 mg/kg/day Result: 1.3 fold increase s.c. over 3.0 days ND rat (PND 19)
Supporting data related to the target endpoint(s)						
In vitro	ER Binding (CERAPP Literature)	ND (ND) Literature Sources: 0	Active (Weak) Literature Sources: 30	Active (Weak) Literature Sources: 4	Active (Weak) Literature Sources: 11	Active (Weak) Literature Sources: 13
	ER Agonist - Reporter Gene / Transcriptional Acitivation (CERAPP Literat	ND (ND) Literature Sources: 0	Active (Moderate) Literature Sources: 11	Active (Weak) Literature Sources: 2	Active (Weak) Literature Sources: 5	Active (Weak) Literature Sources: 6
	ER Antagonist - Reporter Gene / Transcriptional Acitivation (CERAPP Lit	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	Active (VeryWeak) Literature Sources: 2	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score		0.393			0.376
	ToxCast ER Antagonist AUC Score		0			0
	ACEA_T47D_80 h_Positive		0.5744			1.7078
	ATG_ERE_CIS_up		1.8243			0.235
	ATG_ERa_TRANS_up		3.3894			0.1662
	NVS_NR_bER		12.8405			9.17533
	NVS_NR_hER		3.185			0.8511
	NVS_NR_mERa		10.4576			Inactive
	OT_ER_ERaERa_0480		3.2452			9.2261
	OT_ER_ERaERa_1440		4.7083			9.5923
	OT_ER_ERaERb_0480		2.2473			2.397
	OT_ER_ERaERb_1440		2.817			7.8894
	OT_ER_ERbERb_0480		0.7209			2.5649
	OT_ER_ERbERb_1440		1.2884			2.2544
	OT_ERa_EREGFP_0120		0.3145			0.6344
	OT_ERa_EREGFP_0480		0.5296			0.7552
	Tox21_ERa_BLA_Agonist_ratio		4.6374			19.8519
	Tox21_ERa_BLA_Antagonist_ratio		51.0184			46.8361
	Tox21_ERa_LUC_BG1_Agonist		1.4714			0.7134
	Tox21_ERa_LUC_BG1_Antagonist		100.5862			Inactive
	Cytotoxicity Lower Limit (all 'burst assays')		4.50753			4.552236

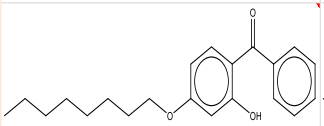
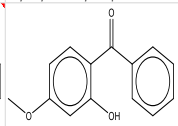
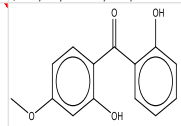
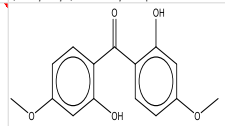
Chemical ID						
		Source 10	Source 11	Source 12	Source 13	Source 14
CAS RN		79-96-9	140-66-9	3884-95-5	79-97-0	599-64-4
Name		4,4'-Propane-2,2-diylbis(2-tert-butylphenol)	4-(1,1,3,3-Tetramethylbutyl)phenol	Phenol, 2-(1,1,3,3-tetramethylbutyl)-	3,3'-Dimethylbisphenol A	4-Cumylphenol
Structure						
			CC(C)(C)CC(C)(C)c1ccc(O)cc1	CC(C)(C)CC(C)(C)c1ccccc1O	CC(C)(c1ccc(O)cc1)c1ccc(O)cc1	CC(C)(c1ccccc1)c1ccc(O)cc1
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Active (VeryWeak)	Active (Weak)	Active (VeryWeak)	Active (VeryWeak)	Active (Weak)
	CERAPP Consensus Agonist (Potency Level)	Active (VeryWeak)	Active (Weak)	Active (VeryWeak)	Active (VeryWeak)	Active (Weak)
	CERAPP Consensus Antagonist (Potency Level)	Active (VeryWeak)	Active (Strong)	Active (Weak)	Active (Moderate)	Active (Strong)
	OASIS TIMES ER Binding QSAR - Parent	Can't Classify	Moderate Active (0.1<RBA<10)	Not Active	Moderate Active (0.1<RBA<10)	Weak Active (0.001<RBA<0.1)
	OASIS TIMES ER Binding QSAR - Metabolites	Can't Classify	No Active	Not Active	Moderate Active (0.1<RBA<10)	Low Active (0<RBA<0.001)
	EPA rtER Expert System v1 - Profiler(OECD Toolbox v3.3)	No alert found	Alkoxyphenols	Alkoxyphenols	No alert found	Phenylphenols
	EPA rtER Expert System v1 - QSAR(OECD Toolbox v3.3)	Unknown Binding Potential	RBA>0.00001%	RBA>0.00001%	Out of Domain	RBA>0.00001%
	Estrogen Receptor Binding -Profiler (OECD Toolbox v3.3)	Very strong binder, OH group	Strong binder, OH group	Strong binder, OH group	Very strong binder, OH group	Strong binder, OH group
	Derek Nexus Expert System (Oestrogenicity Mammal)	No Alert	Mammal Plausible Alkyl phenol or precursor	No Alert	Mammal Plausible Bisphenol or precursor	No Alert
	ACD Percepta (Estrogen Receptor Binding)	Weak Binding to Erα	Weak Binding to Erα	Weak Binding to Erα	Weak Binding to Erα	Weak Binding to Erα
Physical-chemical data						
Molecular Weight (Da)		340.499	206.3239	206.3239	256.3395	212.2869
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		181.38	72.79	72.79	152.11	102.69
Boiling point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		433.17	281.15	281.15	386.74	327.76
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		8.786E-07	0.0691	0.0907	1.01E-05	0.00391
LogPow (EPI Suite 4.1 - WSKOW v1.42)		7.46	5.28	5.28	4.74	4.12
Kinetics Parameters						
in vitro	95% Quantile Css @ 1mg/kg/day (Intrinsic clearance assay @ 1µM (Wetmore et al.) (mg/L)		0.0525223			
	95% Quantile Css @ 1mg/kg/day (Intrinsic clearance assay @ 10µM (Wetmore et al.) (mg/L)		0.1186207			
QSAR						
ACD Percepta 2015 - PK Explorer	Lipinski rule violations	1 (Log P)	0	0	0	0
	Oral bioavailability (%F) (1 mg/kg)	64.49	95.52	95.52	98.36	98.32
	Cmax (ug/ml) (1 mg/kg)	0.09	0.28	0.27	0.28	0.31
	Tmax (min) (1 mg/kg)	361.83	219.67	219.67	173.33	177.33
	AUC0-inf (ug.h/ml) (1 mg/kg)	3.79	4.51	4.29	5.4	6.12
Oral Equivalent Dose						
OED - ER related lower bound (mg/kg/day)						
OED - ER related lower bound (HTS) (mg/kg/day)						

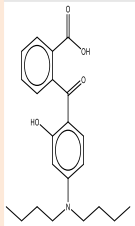
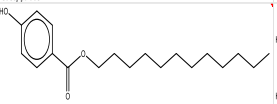
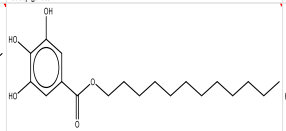
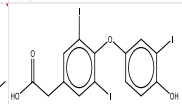
Chemical ID						
		Source 15	Source 16	Source 17	Source 18	Source 19
CAS RN		2078-54-8	5510-99-6	80-05-7	104-40-5	1806-26-4
Name		Propofol	2,6-Di(butan-2-yl)phenol	Bisphenol A	4-Nonylphenol	4-Octylphenol
Structure		CC(C)c1cccc(C(C)C)c1O	CC(CC)c1cccc(C(C)CC)c1O	CC(C)(c1ccc(O)cc1)c1ccc(O)cc1	CCCCCCCCCc1ccc(O)cc1	CCCCCCCCc1ccc(O)cc1
SMILES		CC(C)c1cccc(C(C)C)c1O	CC(CC)c1cccc(C(C)CC)c1O	CC(C)(c1ccc(O)cc1)c1ccc(O)cc1	CCCCCCCCCc1ccc(O)cc1	CCCCCCCCc1ccc(O)cc1
Analogue Search Local Similarity Method (LSM) (Similarity Score)		N/A	N/A	N/A	N/A	N/A
Analogue Search Global Similarity Method (GSM) (Similarity Score)		0.924731195	0.924731195	0.916666687	0.913043499	0.913043499
Integrated Conclusion for Estrogenicity						
Summary in vivo data						
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)		ND	ND		Active LEL - 75 mg/kg/day Result: 140.5228758 Calculated from table 2. p.o. over 3.0 days Alpk:AP rat (PND 21 - 22) Inactive Max dose - 2.0 mg/kg/day s.c. over 3.0 days SD rat (PND 21) Active LEL - 200 mg/kg/day Result: 1.5 fold increase over control s.c. over 3.0 days SD rat (PND 21) Inactive Max dose - 200.0 mg/kg/day s.c. over 3.0 days SD rat (PND 63) Inactive Max dose - 200.0 mg/kg/day s.c. over 3.0 days Crj:CD(SD) rat (PND 20 - 22)	"Inactive Max dose - 200.0 mg/kg/day s.c. over 3.0 days Crj:CD(SD) rat (PND 20 - 22)" "Active LEL - 100 mg/kg/day Result: 1.5 fold increase p.o. over 4.0 days Wistar rat (PND 22)"
Supporting data related to the target endpoint(s)						
In vitro	ER Binding (CERAPP Literature)	Inactive (Inactive) Literature Sources: 12	Inactive (Inactive) Literature Sources: 4	Active (Weak) Literature Sources: 41	Active (Weak) Literature Sources: 42	Active (Weak) Literature Sources: 20
	ER Agonist - Reporter Gene / Transcriptional	Inactive (Inactive) Literature Sources: 6	Inactive (Inactive) Literature Sources: 2	Active (Weak) Literature Sources: 13	Active (Weak) Literature Sources: 20	Active (VeryWeak) Literature Sources: 8
	ER Antagonist - Reporter Gene / Transcriptic	Inactive (Inactive) Literature Sources: 6	Inactive (Inactive) Literature Sources: 2	ND (ND) Literature Sources: 0	Inactive (Inactive) Literature Sources: 4	Inactive (Inactive) Literature Sources: 2
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score	0		0.45	0.088	0.118
	ToxCast ER Antagonist AUC Score	0		0	0	0
	ACEA_T47D_80 h_Positive	Inactive		0.385957796198	5.4911	3.4382
	ATG_ERE_CIS_up	Inactive		0.0981414036581	2.1848	2.2393
	ATG_ERa_TRANS_up	Inactive		0.11946298153	3.9075	2.6021
	NVS_NR_bER			0.420986099013	Inactive	Inactive
	NVS_NR_hER			0.607594540954	Inactive	Inactive
	NVS_NR_mERa			0.87048206385	Inactive	
	OT_ER_ERaERa_0480	Inactive		5.32337807316	23.1188	15.077
	OT_ER_ERaERa_1440	Inactive		3.94159854444	13.3433	19.5724
	OT_ER_ERaERb_0480	Inactive		1.05046402257	13.7329	10.9206
	OT_ER_ERaERb_1440	Inactive		1.88278300276	19.2942	15.1127
	OT_ER_ERbERb_0480	Inactive		0.514622233352	10.2212	10.2043
	OT_ER_ERbERb_1440	Inactive		0.518436511368	12.0261	7.3196
	OT_ERa_EREGFP_0120	Inactive		0.73797176627	Inactive	Inactive
	OT_ERa_EREGFP_0480	Inactive		0.821759154102	Inactive	Inactive
	Tox21_ERa_BLA_Agonist_ratio	Inactive		1.36800785552	Inactive	Inactive
	Tox21_ERa_BLA_Antagonist_ratio	Inactive		84.6649852913	Inactive	Inactive
	Tox21_ERa_LUC_BG1_Agonist	Inactive		0.40817219083	29.0104	30.9436
	Tox21_ERa_LUC_BG1_Antagonist	Inactive		Inactive	Inactive	Inactive
	Cytotoxicity Lower Limit (all 'burst assays')	161.9273999		3.639546744	2.473653468	4.32403008

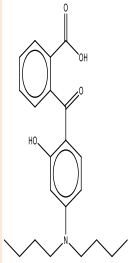
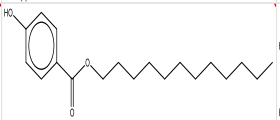
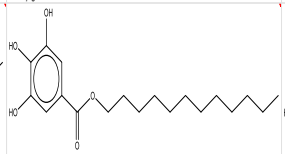
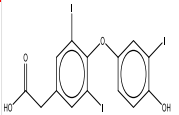
Chemical ID						
		Source 15	Source 16	Source 17	Source 18	Source 19
CAS RN		2078-54-8	5510-99-6	80-05-7	104-40-5	1806-26-4
Name		Propofol	2,6-Di(butan-2-yl)phenol	Bisphenol A	4-Nonylphenol	4-Octylphenol
Structure		CC(C)c1cccc(C(C)C)c1O	CC(CC)c1cccc(C(C)CC)c1O	CC(C)(c1ccc(O)cc1)c1ccc(O)cc1	CCCCCCCCCc1ccc(O)cc1	CCCCCCCCc1ccc(O)cc1
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Inactive (Suspicious)	Active (VeryWeak)	Active (Weak)	Active (VeryWeak)	Active (VeryWeak)
	CERAPP Consensus Agonist (Potency Level)	Inactive (Suspicious)	Active (VeryWeak)	Active (Weak)	Active (VeryWeak)	Active (VeryWeak)
	CERAPP Consensus Antagonist (Potency Lev	Inactive (Suspicious)	Active (VeryWeak)	Active (Strong)	Active (Strong)	Active (Moderate)
	OASIS TIMES ER Binding QSAR - Parent	Not Active	Not Active	Moderate Active (0.1<RBA<10)	Moderate Active (0.1<RBA<10)	Moderate Active (0.1<RBA<10)
	OASIS TIMES ER Binding QSAR - Metabolites	Not Active	Not Active	Weak Active (0.001<RBA<0.1)	Not Active	Not Active
	EPA rtER Expert System v1 - Profiler (OECD To	No alert found	No alert found	No alert found	Alkylphenols	Alkylphenols
	EPA rtER Expert System v1 - QSAR (OECD Tool	RBA<0.00001%	RBA<0.00001%	Out of Domain	RBA>0.00001%	RBA>0.00001%
	Estrogen Receptor Binding - Profiler (OECD T	Non binder, impaired OH	Non binder, impaired OH	Very strong binder, OH group	Strong binder, OH group	Strong binder, OH group
	Derek Nexus Expert System (Oestrogenicity	No Alert	No Alert	Mammal Plausible Bisphenol or precursor	Mammal Plausible Alkyl phenol or precursor	Mammal Plausible Alkyl phenol or precursor
	ACD Percepta (Estrogen Receptor Binding)	Weak Binding to Erα	Strong binding to Erα	Weak Binding to Erα	Weak Binding to Erα	Weak Binding to Erα
Physical-chemical data						
Molecular Weight (Da)		178.2707	206.3239	228.2863	220.3505	206.3239
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		55.74	76.34	131.76	91.8	82.77
Boiling point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		261.41	293.39	363.54	324.47	310.93
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		1.073	0.9839	0.00003026	0.0914	0.013
LogPow (EPI Suite 4.1 - WSKOW v1.42)		3.57	4.56	3.64	5.99	5.5
Kinetics Parameters						
in vitro	95% Quantile Css @ 1mg/kg/day (Intrinsic clearance assay @ 1µM (Wetmore et al.) (mg/L)			0.394328		0.2910886
	95% Quantile Css @ 1mg/kg/day (Intrinsic clearance assay @ 10µM (Wetmore et al.) (mg/L)			0.3078826		0.3182475
QSAR						
ACD Percepta 2015 - PK Explorer	Lipinski rule violations	0	0	0	1 (LogP >5)	
	Oral bioavailability (%F) (1 mg/kg)	68.1	53.13	99.12	52.21	57.32
	Cmax (ug/ml) (1 mg/kg)	0.19	0.12	0.36	0.11	0.15
	Tmax (min) (1 mg/kg)	301.33	331	121	355.33	344.67
	AUC0-inf (ug.h/ml) (1 mg/kg)	2.69	1.86	5.53	2.23	3.11
Oral Equivalent Dose						
OED - ER related lower bound (mg/kg/day)						
OED - ER related lower bound (HTS) (mg/kg/day)						

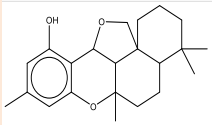
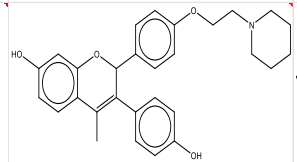
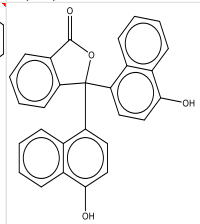
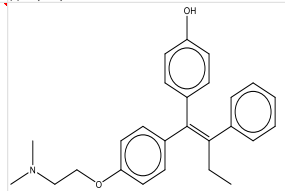
APPENDIX - IATA DATA MATRIX FOR OCTABENZONE (1843-05-6)

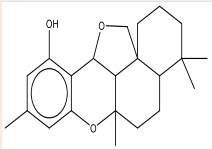
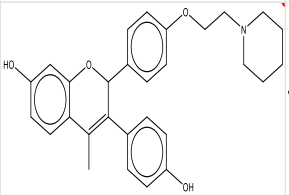
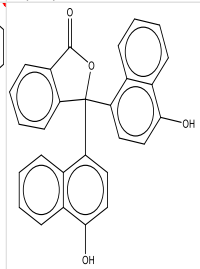
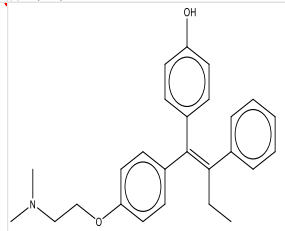
Chemical ID					
		Target	Source 1	Source 2	Source 3
CAS RN		1843-05-6	131-57-7	131-53-3	131-54-4
Name		Octabenzone	2-Hydroxy-4-methoxybenzophenone	2,2'-Dihydroxy-4-methoxybenzophenone	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone
Structure					
SMILES		CCCCCCCCOc1cc(O)c(cc1)C(=O)c1ccccc1	COc1cc(O)c(cc1)C(=O)c1ccccc1	COc1cc(O)c(cc1)C(=O)c1ccccc1O	COc1cc(O)c(cc1)C(=O)c1ccc(cc1O)OC
Analogue Search Local Similarity Method (LSM) (Similarity Score)		1	0.333333343	0.209876544	0.177083339
Analogue Search Global Similarity Method (GSM) (Similarity Score)					
Integrated Conclusion for Estrogenicity					
Summary in vivo data					
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)			1. Inactive Max dose - 1000.0 mg/kg/day p.o. over 7.0 days C57BL/6J mouse (PND 56) 2. Inactive Max dose - 1000.0 mg/kg/day s.c. over 7.0 days C57BL/6J mouse (PND 56)		
Supporting data related to the target endpoint(s)					
		Inactive (Inactive)	ND (ND)	Inactive (ND)	ND (ND)
In vitro	ER Binding (CERAPP Literature)	Literature Sources: 12	Literature Sources: 0	Literature Sources: 3	Literature Sources: 0
		Inactive (Inactive)	ND (ND)	Inactive (Inactive)	Active (Weak)
	ER Agonist - Reporter Gene / Transcriptional Acitivatio	Literature Sources: 6	Literature Sources: 0	Literature Sources: 4	Literature Sources: 2
		Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)
	ER Antagonist - Reporter Gene / Transcriptional Activa	Literature Sources: 6	Literature Sources: 6	Literature Sources: 4	Literature Sources: 2
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score	0	0.0645		
	ToxCast ER Antagonist AUC Score	0	0		
	ACEA_T47D_80 h_Positive	Inactive	0.0433060107721		
	ATG_ERE_CIS_up	Inactive	8.28307105129		
	ATG_ERa_TRANS_up	Inactive	6.90406281684		
	NVS_NR_bER				
	NVS_NR_hER				
	NVS_NR_mERa				
	OT_ER_ERaERa_0480	Inactive	61.4427465689		
	OT_ER_ERaERa_1440	Inactive	Inactive		
	OT_ER_ERaERb_0480	Inactive	31.0342437236		
	OT_ER_ERaERb_1440	Inactive	19.7136808933		
	OT_ER_ERbERb_0480	Inactive	63.3489766115		
	OT_ER_ERbERb_1440	Inactive	Inactive		
	OT_ERa_EREGFP_0120	Inactive	Inactive		
	OT_ERa_EREGFP_0480	Inactive	Inactive		
	Tox21_ERa_BLA_Agonist_ratio	Inactive	Inactive		
	Tox21_ERa_BLA_Antagonist_ratio	Inactive	Inactive		
	Tox21_ERa_LUC_BG1_Agonist	Inactive	26.0389468454		
	Tox21_ERa_LUC_BG1_Antagonist	Inactive	Inactive		
	Cytotoxicity Lower Limit (all 'burst assays')	2.34948008	161.9273999		

Chemical ID					
		Target	Source 1	Source 2	Source 3
CAS RN		1843-05-6	131-57-7	131-53-3	131-54-4
Name		Octabenzone	2-Hydroxy-4-methoxybenzophenone	2,2'-Dihydroxy-4-methoxybenzophenone	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone
Structure					
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Active (VeryWeak)	Active (VeryWeak)	Active (VeryWeak)	Active (VeryWeak)
	CERAPP Consensus Agonist (Potency Level)	Active (VeryWeak)	Active (VeryWeak)	Active (VeryWeak)	Active (VeryWeak)
	CERAPP Consensus Antagonist (Potency Level)	Inactive (Suspicious)	Active (Weak)	Active (Weak)	Active (Weak)
	OASIS TIMES ER Binding QSAR - Parent	Not active	Weak Active 0.001<RBA<0.1	Moderate Active 0.1<RBA<10	Moderate Active 0.1<RBA<10
	OASIS TIMES ER Binding QSAR - Metabolites	Strong active RBA>10	Weak Active 0.001<RBA<0.1	Moderate Active 0.1<RBA<10	Moderate Active 0.1<RBA<10
	EPA rtER Expert System v1 - Profiler (OECD Toolbox v3.3	No alert found	No alert found	No alert found	No alert found
	EPA rtER Expert System v1 - QSAR (OECD Toolbox v3.3)	RBA<0.00001%	Out of Domain	Out of Domain	Out of Domain
	Estrogen Receptor Binding - Profiler (OECD Toolbox v3.	Strong binder, OH group	Strong binder, OH group	Very strong binder, OH group	Very strong binder, OH group
	Derek Nexus Expert System (Oestrogenicity Mammal)	No alert	No alert	No alert	No alert
	ACD Percepta (Estrogen Receptor Binding)	Weak binding to Erα	No binding to Erα	No binding to Erα	No binding to Erα
Physical-chemical data					
Molecular Weight (Da)		326.4294	228.2433	244.2427	274.2687
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		177.27	129.8	162.17	176.11
Boiling point (°C)(EPI Suite 4.1 - MPBPVP v1.43)		444.63	363.41	398.24	421.9
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		0.000009226	0.0008826	0.00002586	9.826E-07
LogPow (EPI Suite 4.1 - WSKOW v1.42)		6.96	3.52	3.82	3.9
Kinetics Parameters					
in vitro	95% Quantile Css @ 1mg/kg/day (intrinsic clearance ass	N/A			
	95% Quantile Css @ 1mg/kg/day (intrinsic clearance ass	0.2122555			
QSAR		1			
ACD Percepta 2015 - PK Explorer	Lipinski rule violations	(LogP > 5)	0	0	0
	Oral bioavailability (%F) (1 mg/kg)	12.81	98.92	99.17	98.88
	Cmax (ug/ml) (1 mg/kg)	0.02	0.47	0.49	0.47
	Tmax (min) (1 mg/kg)	439.83	143	105.67	145.33
	AUC0-inf (ug.h/ml) (1 mg/kg)	0.54	9.13	7.95	7.93
Oral Equivalent Dose					
OED - ER related lower bound (mg/kg/day)					
OED - ER related lower bound (HTS) (mg/kg/day)					

Chemical ID					
		Source 4	Source 5	Source 6	Source 7
CAS RN		54574-82-2	2664-60-0	1166-52-5	51-24-1
Name		2-[4-(Dibutylamino)-2-hydroxybenzoyl]benzoic acid	Dodecylparaben	Dodecyl gallate	Tiratricol
Structure					
SMILES		CCCCN(CCCC)c1cc(O)c(c1)C(=O)c1ccccc1C(O)=O	CCCCCCCCCCCOC(=O)c1ccc(O)cc1	CCCCCCCCCCCOC(=O)c1cc(O)c(O)c(O)c1	Oc1ccc(cc1I)Oc1c(I)cc(CC(O)=O)cc1I
Analogue Search Local Similarity Method (LSM) (Similarity Score)		0.139917693			
Analogue Search Global Similarity Method (GSM) (Similarity Score)			0.820899493	0.780821919	0.75
Integrated Conclusion for Estrogenicity					
Summary in vivo data					
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)					Active LEL - 1000 mg/kg/day Result: ND See supplemental info for paper s.c. over 7.0 days C57BL/6J mouse (PND 56)
Supporting data related to the target endpoint(s)					
In vitro	ER Binding (CERAPP Literature)	Active (VeryWeak) Literature Sources: 1	Active (Moderate) Literature Sources: 7	Active (Weak) Literature Sources: 5	Active (Weak) Literature Sources: 6
	ER Agonist - Reporter Gene / Transcriptional Activation (CERAPP Literature)	Inactive (Inactive) Literature Sources: 2	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0
	ER Antagonist - Reporter Gene / Transcriptional Activation (CERAPP Literature)	ND (ND) Literature Sources: 0	Active (Weak) Literature Sources: 2	Active (Weak) Literature Sources: 4	ND (ND) Literature Sources: 0
In vitro HTS (ToxCast/IC/AC50 µM)	ToxCast ER Agonist AUC Score			0.0000527	0.179
	ToxCast ER Antagonist AUC Score			0.0773	0
	ACEA_T47D_80 h_Positive			Inactive	10.7314
	ATG_ERE_CIS_up			Inactive	5.8586
	ATG_ERa_TRANS_up			10.0416	3.6316
	NVS_NR_bER			Inactive	Inactive
	NVS_NR_hER			Inactive	1.0031
	NVS_NR_mERa			11.0217	Inactive
	OT_ER_ERaERa_0480			Inactive	37.6755
	OT_ER_ERaERa_1440			Inactive	45.2472
	OT_ER_ERaERb_0480			15.4569	35.1356
	OT_ER_ERaERb_1440			Inactive	39.0926
	OT_ER_ERbERb_0480			Inactive	Inactive
	OT_ER_ERbERb_1440			Inactive	Inactive
	OT_ERa_EREGFP_0120			72.3056	Inactive
	OT_ERa_EREGFP_0480			Inactive	Inactive
	Tox21_ERa_BLA_Agonist_ratio			63.218	Inactive
	Tox21_ERa_BLA_Antagonist_ratio			25.7526	5.1745
	Tox21_ERa_LUC_BG1_Agonist			Inactive	15.3034
	Tox21_ERa_LUC_BG1_Antagonist			39.4955	Inactive
	Cytotoxicity Lower Limit (all 'burst assays')			6.67	2.9170092

Chemical ID					
		Source 4	Source 5	Source 6	Source 7
CAS RN		54574-82-2	2664-63-0	1166-52-5	51-24-1
Name		2-[4-(Dibutylamino)-2-hydroxybenzoyl]benzoic acid	Dodecylparaben	Dodecyl gallate	Tiratricol
Structure					
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Inactive (Suspicious)	Active (Weak)	Active (VeryWeak)	Active (VeryWeak)
	CERAPP Consensus Agonist (Potency Level)	Inactive (Suspicious)	Active (Weak)	Active (VeryWeak)	Active (VeryWeak)
	CERAPP Consensus Antagonist (Potency Level)	Inactive (Suspicious)	Active (Moderate)	Inactive (Suspicious)	Active (Weak)
	OASIS TIMES ER Binding QSAR - Parent	Not Active	Out of Domain	Not Active	Out of Domain
	OASIS TIMES ER Binding QSAR - Metabolites	Not Active	Not Active	Not Active	Not Active
	EPA rtER Expert System v1 - Profiler(OECD Toolbox v3.3)	No alert found	Parabens	Gallates	No alert found
	EPA rtER Expert System v1 - QSAR(OECD Toolbox v3.3)	RBA<0.00001%	RBA>0.00001%	RBA>0.00001%	RBA<0.00001%
	Estrogen Receptor Binding -Profiler(OECD Toolbox v3.3)	Strong binder, OH group	Strong binder, OH group	Strong binder, OH group	Non binder, MW>500
	Derek Nexus Expert System (Oestrogenicity Mammal)	No alert	No alert	No alert	No alert
	ACD Percepta (Estrogen Receptor Binding)	Weak binding to Era	Weak binding to Era	Weak binding to Era	Weak binding to Era
Physical-chemical data					
Molecular Weight (Da)		369.4541	306.45	338.45	621.94
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		224.88	142	197.66	239.88
Boiling point (°C)(EPI Suite 4.1 - MPBPVP v1.43)		526.29	398.35	468.02	558.41
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		6.46E-10	1.70E-05	1.36E-07	2.68E-10
LogPow (EPI Suite 4.1 - WSKOW v1.42)		5.76	7.4	6.21	5.67
Kinetics Parameters					
In vitro	95% Quantile Css @ 1mg/kg/day (Intrinsic clearance assay @ 1µM)(Wetmore et al.) (mg/L)				
	95% Quantile Css @ 1mg/kg/day (Intrinsic clearance assay @ 10µM)(Wetmore et al.) (mg/L)				
QSAR		1	1	1	2
ACD Percepta 2015 - PK Explorer	Lipinski rule violations	(LogP > 5)	(LogP > 5)	(LogP > 5)	(LogP > 5; MW>500)
	Oral bioavailability (%F) (1 mg/kg)	87.42	7.81	31.36	87.55
	Cmax (ug/ml) (1 mg/kg)	1.72	0.02	0.11	3.73
	Tmax (min) (1 mg/kg)	255.33	390.33	227	258.67
	AUCO-inf (ug.h/ml) (1 mg/kg)	22.77	0.28	0.65	54.86
Oral Equivalent Dose					
OED - ER related lower bound (mg/kg/day)					
OED - ER related lower bound (HTS) (mg/kg/day)					

Chemical ID					
		Source 8	Source 9	Source 10	Source 11
CAS RN		22733-60-4	182167-02-8	596-01-0	68047-06-3
Name		Siccanin	ACOLBIFENE	a-Naphtholphthalein	(Z)-4-Hydroxytamoxifen
Structure					
SMILES		CC1=CC2=C(C3C4C(O2)(CCC5C4(CCCC5(C)C)CO3)C)C(=C1)O	CC1=C(C(OC2=C1C=CC(=C2)O)C3=CC=C(C=C3)OCCN4CCCCC4)C5=CC=C(C=C	Oc1ccc(c2ccccc12)C1(OC(=O)c2ccccc12)c1ccc(O)c2ccccc21	CCC(=C(c1ccc(cc1)OCCN(C)C)c1ccc(O)cc1)c1ccccc1
Analogue Search Local Similarity Method (LSM) (Similarity Score)					
Analogue Search Global Similarity Method (GSM) (Similarity Score)		0.721854329	0.717948735	0.711538434	0.703448296
Integrated Conclusion for Estrogenicity					
Summary in vivo data					
Uterotrophic Assay (NICEATM UT Database Guideline Studies) (Kleinstreuer et al. 2015)					
Supporting data related to the target endpoint(s)					
In vitro	ER Binding (CERAPP Literature)	Inactive (Inactive) Literature Sources: 4	Active (ND) Literature Sources: 8	Active (Weak) Literature Sources: 5	Active (Strong) Literature Sources: 5
	ER Agonist - Reporter Gene / Transcriptional Acitivation (CERAPP Literat	Inactive (Inactive) Literature Sources: 2	ND (ND) Literature Sources: 0	Active (Weak) Literature Sources: 2	ND (ND) Literature Sources: 0
	ER Antagonist - Reporter Gene / Transcriptional Acitivation (CERAPP Lit	Inactive (Inactive) Literature Sources: 2	ND (ND) Literature Sources: 0	ND (ND) Literature Sources: 0	Active (Strong) Literature Sources: 4
In vitro HTS (ToxCast)(AC50 µM)	ToxCast ER Agonist AUC Score				
	ToxCast ER Antagonist AUC Score				
	ACEA_T47D_80 h_Positive				
	ATG_ERE_CIS_up				
	ATG_ERa_TRANS_up				
	NVS_NR_bER				
	NVS_NR_hER				
	NVS_NR_mERa				
	OT_ER_ERaERa_0480				
	OT_ER_ERaERa_1440				
	OT_ER_ERaERb_0480				
	OT_ER_ERaERb_1440				
	OT_ER_ERbERb_0480				
	OT_ER_ERbERb_1440				
	OT_ERa_EREGFP_0120				
	OT_ERa_EREGFP_0480				
	Tox21_ERa_BLA_Agonist_ratio				
	Tox21_ERa_BLA_Antagonist_ratio				
	Tox21_ERa_LUC_BG1_Agonist				
	Tox21_ERa_LUC_BG1_Antagonist				
	Cytotoxicity Lower Limit (all 'burst assays')				

Chemical ID					
		Source 8	Source 9	Source 10	Source 11
CAS RN		22733-60-4	182167-02-8	596-01-0	68047-06-3
Name		Siccanin	ACOLBIFENE	a-Naphtholphthalein	(Z)-4-Hydroxytamoxifen
Structure					
(Q)SAR	CERAPP Consensus Binding (Potency Level)	Active (VeryWeak)	Active (Weak)	Active (Moderate)	Active (Moderate)
	CERAPP Consensus Agonist (Potency Level)	Active (VeryWeak)	Active (VeryWeak)	Active (Weak)	Inactive (Suspicious)
	CERAPP Consensus Antagonist (Potency Level)	Active (Moderate)	Active (Weak)	Active (Weak)	Active (Moderate)
	OASIS TIMES ER Binding QSAR - Parent	Out of Domain	Out of Domain	Out of Domain	Strong active RBA>10
	OASIS TIMES ER Binding QSAR - Metabolites	Not Active	Strong Active RBA>10	Moderate Active 0.1<RBA<10	Strong active RBA>10
	EPA rtER Expert System v1 - Profiler (OECD Toolbox v3.3)	No alert found	No alert found	No alert found	Tamoxifen-Like compounds
	EPA rtER Expert System v1 - QSAR (OECD Toolbox v3.3)	Out of Domain	Out of Domain	Out of Domain	Out of Domain
	Estrogen Receptor Binding -Profiler (OECD Toolbox v3.3)	Strong binder, OH group	Very strong binder, OH group	Very strong binder, OH group	Strong binder, OH group
	Derek Nexus Expert System (Oestrogenicity Mammal)	No alert	Plausible (mammal) 4,4'-Dihydroxydiphenyl-ethane or -ethene	No alert	No alert
	ACD Percepta (Estrogen Receptor Binding)	Weak binding to Erα	Strong binding to Erα	Weak binding to Erα	Strong binding to Erα
Physical-chemical data					
Molecular Weight (Da)		342.48	457.57	418.45	387.53
Melting point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		175.88	260.97	284.64	211.01
Boiling point (°C) (EPI Suite 4.1 - MPBPVP v1.43)		421.38	603.56	654.23	503.04
Vapour Pressure (Pa) (EPI Suite 4.1 - MPBPVP v1.43)		1.73E-06	1.65E-13	5.69E-15	4.14E-09
LogPow (EPI Suite 4.1 - WSKOW v1.42)		4.46	6.91	5.41	5.82
Kinetics Parameters					
in vitro	95% Quantile Css @ 1mg/kg/day (intrinsic clearance assay @ 1µM (Wetmore et al.) (mg/L)				
	95% Quantile Css @ 1mg/kg/day (intrinsic clearance assay @ 10µM (Wetmore et al.) (mg/L)				
QSAR ACD Percepta 2015 - PK Explorer	Lipinski rule violations	1 (LogP > 5)	1 (LogP > 5)	1 (LogP > 5)	1 (LogP > 5)
	Oral bioavailability (%F) (1 mg/kg)	59.81	89.82	15.21	61.72
	Cmax (ug/ml) (1 mg/kg)	0.12	0.06	0.03	0.05
	Tmax (min) (1 mg/kg)	346	277.67	390.33	394.83
	AUC0-inf (ug.h/ml) (1 mg/kg)	2.63	2.67	0.39	4.75
Oral Equivalent Dose					
OED - ER related lower bound (mg/kg/day)					
OED - ER related lower bound (HTS) (mg/kg/day)					