



The Human-Relevant Potency Threshold: Reliable, Replicated, and Consistent with 100 years of Mechanistic Pharmacology

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Disclosure

Over the past 20 years, Christopher J. Borgert has periodically received funding from the following entities for developing methods to evaluate endocrine disruption and pharmacological and toxicology potency:

Endocrine Policy Forum (EPF)

American Chemistry Council Bisphenol A Panel

American Chemistry Council Phthalates Panel

American Chemistry Council Silicones Environment, Health and Safety Center

BASF Corporation

CropLife International

Styrene Information and Research Center

LyondellBasell Corporation

Monsanto Corporation

Definition: “Endocrine Disruptor”

- A chemical that produces an adverse effect(s) as a consequence of an endocrine mode of action (MoA).
 - Chemical produces an adverse effect;
 - **Chemical operates via an endocrine MoA that causes the adverse effect.**

Potency Thresholds for Endocrine Disruption

- Potency for the Adverse Effect [Solecki et al., 2017. *Arch Toxicol*, 91(2), 1001-1006.]
 - **Potency via the Endocrine MoA (*Mechanistic Potency Threshold*)**
 - the “*limit*” or minimum strength by which the chemical must interact with components of an endocrine pathway “*below which no reaction occurs.*”
- [Borgert CJ, Matthews JC, Baker SP (2018) *Archives of Toxicology*, 92: 1685-1702.]

Fundamentals of
**Receptor, Enzyme,
and
Transport
Kinetics**

John C. Matthews

1993

- *Affinity*: the strength of attachment between a small molecular and it's bio-macromolecular target.
 - K_d : dissociation constant
- *Intrinsic activity / efficacy*: the relative ability of a bound ligand to elicit the full macromolecular response.
- *Potency*: proportional to *Affinity* and *Intrinsic Activity*
- *Law of Mass Action*: rates of chemical reactions are directly proportional to the product of the activities and concentrations of the reactants.

Hill Equation

- Hill AV, 1910

Michaelis-Menten Equation

- Michaelis & Menten, 1913

The receptor concept: pharmacology's big idea

***H.P. Rang**

Emeritus Professor of Pharmacology, University College London

BIOCHEMICAL PHARMACOLOGY 71 (2006) 1663–1670



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Commentary

Some implications of receptor theory for in vivo assessment of agonists, antagonists and inverse agonists

S. Stevens Negus*

Alcohol and Drug Abuse Research Center, 115 Mill Street, McLean Hospital, Harvard Medical School, Belmont, MA, United States



Review

TRENDS in Pharmacological Sciences Vol.27 No.3 March 2006

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The quantitative analysis of drug–receptor interactions: a short history

David Colquhoun

Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, UK

Regulatory Toxicology and Pharmacology 67 (2013) 83–88

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Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph



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Potency matters: Thresholds govern endocrine activity[☆]

Christopher J. Borgert^{a,*}, Stephen P. Baker^b, John C. Matthews^c

Hypothesis 1

Borgert CJ, Matthews JC, Baker SP (2018) *Archives of Toxicology*, 92: 1685-1702.

There exists a threshold mechanistic potency below which a chemical will not produce a physiological effect by that mode of action (MoA).

- We tested the hypothesis for the Estrogen-Receptor alpha (ER α) agonist MoA by comparing chemicals that vary widely in their mechanistic potencies via ER α with their ability to produce clinically observable ER α -mediated effects in humans.
- We used chemical potencies in the ERTA assay relative to 17 β -estradiol (E2) as a measure of mechanistic potency and compared those values with relative potencies in the rat uterotrophic assay (RUA) – which is relatively specific for the ER α MoA – to assess potential confounding by pharmacokinetics.
 - Positive Controls: endogenous steroidal (e.g., E2) and pharmaceutical estrogens (e.g., EE2)
 - Negative Controls: endogenous steroidal androgens and synthetic anabolic steroids.

Borgert CJ, Matthews JC, & Baker SP. (2018).
Human-relevant potency threshold (HRPT)
for ERα agonism.
Arch Toxicol, 92(5), 1685-1702.

Relative Potency
Range

3.5E-02 – 1.0E+00	Hormones (11)
2.6E-02 – 2.0E+00	Drugs (19)
3.3E-05 to 7.0E-02	Botanicals (109)
2.5E-06 to 7.1E-06	Androgens (5)



Human-relevant potency threshold (HRPT) for ERα agonism

Christopher J. Borgert¹ · John C. Matthews² · Stephen P. Baker³

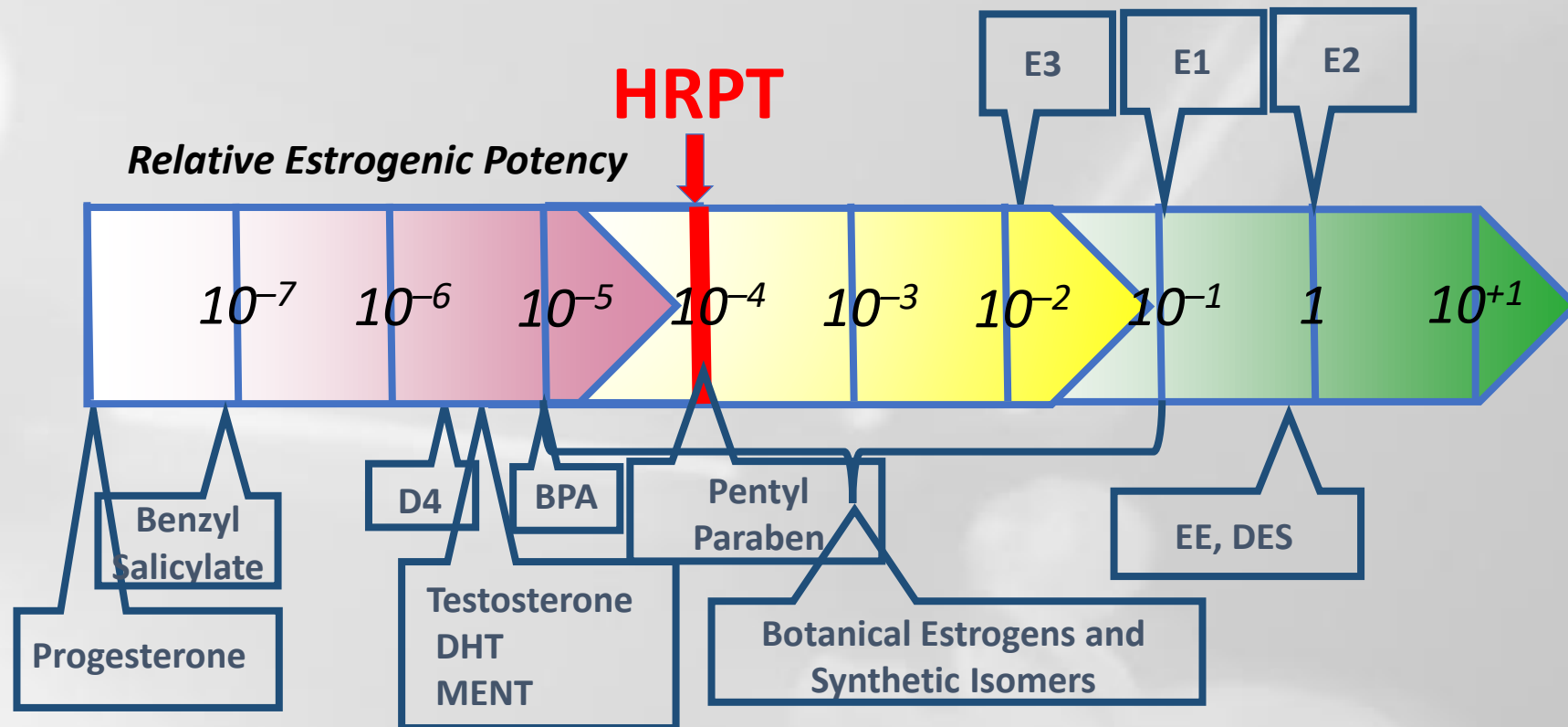
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Abstract

The European Commission has recently proposed draft criteria for the identification of endocrine disrupting chemicals (EDCs) that pose a significant hazard to humans or the environment. Identifying and characterizing toxic hazards based on the manner by which adverse effects are produced rather than on the nature of those adverse effects departs from traditional practice and requires a proper interpretation of the evidence regarding the chemical’s ability to produce physiological effect(s) via a specific mode of action (MoA). The ability of any chemical to produce a physiological effect depends on its pharmacokinetics and the potency by which it acts via the various MoAs that can lead to the particular effect. A chemical’s potency for a specific MoA—its mechanistic potency—is determined by two properties: (1) its affinity for the functional components that comprise the MoA, i.e., its specific receptors, enzymes, transporters, transcriptional elements, etc., and (2) its ability to alter the functional state of those components (activity). Using the agonist MoA via estrogen receptor alpha, we illustrate an empirical method for determining a human-relevant potency threshold (HRPT), defined as the minimum level of mechanistic potency necessary for a chemical to be able to act via a particular MoA in humans. One important use for an HRPT is to distinguish between chemicals that may be capable of, versus those likely to be incapable of, producing adverse effects in humans via the specified MoA. The method involves comparing chemicals that have different ERα agonist potencies with the ability of those chemicals to produce ERα-mediated agonist responses in human clinical trials. Based on this approach, we propose an HRPT for ERα agonism of 1E-04 relative to the potency of the endogenous estrogenic hormone 17β-estradiol or the pharmaceutical estrogen, 17α-ethinylestradiol. This approach provides a practical way to address Hazard Identification according to the draft criteria for identification of EDCs recently proposed by the European Commission.

Keywords Endocrine disruptor/disruption · Hazard identification · Potency · HRPT · European Commission · Estrogen system

Human-Relevant Potency-Threshold (HRPT) for the ER α -Agonist MoA adapted from Borgert et al. (2018) with data added for benzyl salicylate and BPA from Natsch et al. (2021) and for parabens from Fayyaz et al. (2021).



Borgert CJ, Matthews JC, & Baker SP. (2018). *Arch Toxicol*, 92(5), 1685-1702.

Hypothesis 2

Borgert CJ, Burgoon LD, Matthews JC. 2024.
Archives of Toxicology, 98(6), 1795-1807

There is a physiological and biochemical basis for the HRPT below which a chemical will not produce a physiological effect by an endocrine mode of action (MoA).

- Data were obtained from the literature for circulating levels and ER α affinities for endogenous estrogens.
- Established formulae were used to calculate fractional ER α occupancies for mixtures of these ER α ligands and theoretical exogenous ligands.
- Physiological relevance of receptor occupancies was interpreted in terms of normal fluctuations in levels of the endogenous estrogens.

Hormones and EDCs *Do Not* Compete in a Vacuum

Borgert CJ, Burgoon LD, Matthews JC. 2024. *Archives of Toxicology*, accepted February 2024.

DHEA, DHEA-sulfate, androstenedione, androstenediol (Adiol), estrone and estriol are endogenous, naturally occurring products of human metabolism that all exhibit measurable affinity and efficacy for both ER α and ER β .

- Range in potency 1/100 (Adiol, estrone, estriol) to 1/10,000 (DHEA) relative to estradiol.
- Are present in blood at relatively high concentrations: picomolar to 10 micromolar.
- DHEA and Adiol are considered physiological estrogens and produce observable effects on many estrogen-responsive tissues.
- Receptor occupancy by these metabolites alone is considerable and precludes significant binding by low-affinity exogenous chemicals.

Table 1: Dissociation constants and plasma concentrations used for receptor occupancy calculations

Ligand Label	Endogenous Steroid or Exogenous Ligand	Kd (M)	Affinity Relative to E2	Mid-Point Plasma Concentration (M)	Minimal Plasma Concentration (M)
A	17β-Estradiol (E2)	1.0E-10	1.00E+00	1.84E-09	7.34E-12
B	Androstenediol	4.5E-09	2.22E-02	1.8E-09	1.1E-09
C	DHEA	1.1E-06	9.09E-05	1.9E-08	7.0E-09
D	DHEA-Sulfate	1.1E-04	9.09E-07	7.8E-06	5.4E-07
E	Estrone (E1)	2.2E-09	4.55E-02	1.5E-07	2.7E-11
F	Estriol (E3)	2.2E-09	4.55E-02	1.5E-07	2.7E-11
G	HRPT Ligand	1.0E-06	1.00E-04	1.0E-06	1.0E-09
H	Supra-HRPT Ligand	1.0E-07	1.00E-03	1.0E-06	1.0E-09
I-a	Supra-HRPT Ligand 10X	1.0E-08	1.00E-02	1.0E-06	1.0E-09
I-b	Supra-HRPT Ligand 10X	1.0E-07	1.00E-03	1.0E-05	1.0E-08

Borgert CJ, Burgoon LD, Matthews JC. 2024. *Archives of Toxicology*, 98(6), 1795-1807

Table 2: Fractional Receptor Occupancy of Sub-HRPT Chemical in the Presence of Normal Plasma Concentrations of Endogenous Steroidal ERα Ligands

Ligand Components	Percent ER Occupied at Mid-Point Plasma Concentration (<u>M</u>)	Percent ER Occupied at Minimal Plasma Concentration (<u>M</u>)
Fraction occupied by E2 alone	94.8347	6.8381
Fraction occupied by E2 in presence of B – F	11.7533	5.4223
Fraction occupied by E2 in presence of B – F, G	11.6785	5.4183
Fraction occupied by E2 in presence of B – F, H	11.0461	5.3826
Fraction of ER occupied by A-F	99.3598	26.1263
Fraction of ER occupied by A – F, G	99.3639	26.1809
Fraction of ER occupied by A – F, H	99.3984	26.6681
Difference Due to G	0.0041	0.0545
Difference Due to H	0.0385	0.5417
Difference Due to I-a or I-b	0.2498	5.0819

Borgert CJ, Burgoon LD, Matthews JC. 2024. *Archives of Toxicology*, 98(6), 1795-1807

Conclusions

Borgert CJ, Burgoon LD, Matthews JC. 2024. *Archives of Toxicology*, accepted February 2024.

- The physiologically relevant alteration of receptor occupancy is defined by normal fluctuations in endogenous steroid levels, including but not only, 17 β -estradiol.
- **The endogenous metabolic milieu** of hormones, their precursors, metabolites, and other small molecules **forms the physiological and biochemical basis for the HRPT.**
- The *Potency Threshold* for ER α is in the range of 1E–04 to 1E–03 relative to the potency of 17 β -estradiol (E2) and applies to both agonist and antagonist modes of action.
- *Potency Thresholds* exist for *all* mechanisms that involve interactions of small molecules with biological macromolecules: receptors, enzymes, transporters, DNA response modifiers, etc.

Pande P, Fleck SC, Twaddle NC, Churchwell MI, Doerge DR, & Teeguarden JG. (2019). *Food Chem Toxicol*, 125, 341-353.

Comparison of Affinity Values and Normal Plasma Concentrations of Endogenous Steroidal ER α Ligands: Human Versus Rat

Ligand	K _d (M) Human ER α	K _d (M) Rat ER α	Human Plasma Concentration	Rat Plasma Concentration
17 β -estradiol (E2)	1.00E – 10	2.00E – 10	1.84E – 09	1.00E – 11
Androstenediol (Adiol)	4.50E – 09	6.00E – 09	1.80E – 09	2.00E – 10
DHEA	1.10E – 06	2.20E – 06	1.90E – 08	9.00E – 09
DHEA-Sulfate	1.10E – 04	2.20E – 04	7.80E – 06	5.40E – 07
Estrone (E1)	2.20E – 09	4.55E – 09	1.50E – 07	5.00E – 10
Estriol (E3)	2.20E – 09	4.55E – 09	1.50E – 07	5.00E – 11

▽ Rat Endpoint

○ Human DES Cohorts

○ A1**

○ A2*

