Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification

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What are Endocrine Disrupting Chemicals?

Endocrine Disrupting Chemicals (EDCs) are defined by the Endocrine Society as:

"an exogenous [non-natural] chemical, or mixture of chemicals, that interferes with any aspect of hormone action."

Endocrinology, September 2012, 153(9):4097–4110

POSITION STATEMENT

Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society

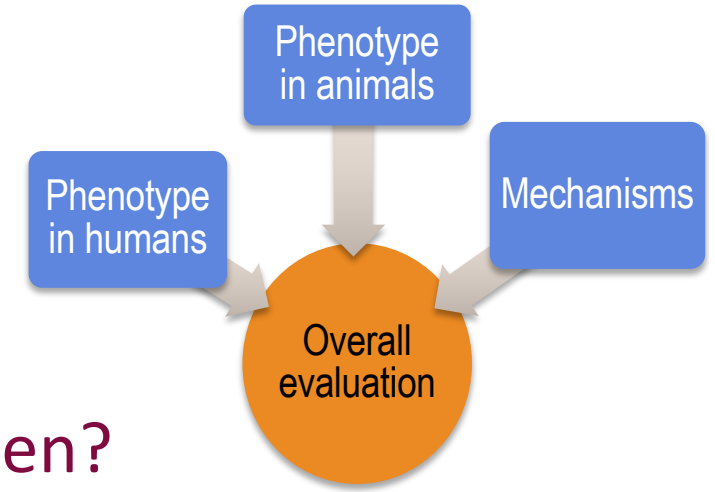
R. Thomas Zoeller, T. R. Brown, L. L. Doan, A. C. Gore, N. E. Skakkebaek, A. M. Soto, T. J. Woodruff, and F. S. Vom Saal

How can we identify EDCs?

- Hazard= an intrinsic property or characteristic that makes a substance dangerous

- A complex literature of mechanistic studies provides evidence on ED hazard, yet there is no widely-accepted, systematic method to integrate these data to help identify EDCs.

Let us learn history lessons



- Who decides if a chemical is a carcinogen?
  - **Many groups** (GHS, EU, USEPA, USNTP, CalEPA Prop 65) decide from Monographs of the **International Agency for Research on Cancer IARC**, part of the World Health Organization
- How does IARC identify carcinogens?

  ⬇ Epidemiology, rodent assays

  ⬆ Mechanistic, *in vitro* assays
- Key Characteristics of Carcinogens
  - A framework for organizing data related to the intrinsic properties of carcinogens
  - Incomplete 'mechanistic pathway' ≠ decision-making inaction
  - Help identify data gaps

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Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis

Martyn T. Smith,[1] Kathryn Z. Guyton,[2] Catherine F. Gibbons,[3] Jason M. Fritz,[3] Christopher J. Portier,[4] Ivan Rusyn,[5] David M. DeMarini,[3] Jane C. Caldwell,[3] Robert J. Kavlock,[3] Paul F. Lambert,[6] Stephen S. Hecht,[7] John R. Bucher,[8] Bernard W. Stewart,[9] Robert A. Baan,[2] Vincent J. Cogliano,[3] and Kurt Straif[2]*

Expert Meeting on Advancing the Key Characteristics Framework to Reproductive Toxicants and EDCs

- March 7-8th, 2018 in Berkeley CA
- Sponsored by: CalEPA
- Zoeller and La Merrill invited to lead the evaluation of whether developing KCs of EDCs was feasible

https://doi.org/10.1038/s41574-019-0273-8

CONSENSUS STATEMENT

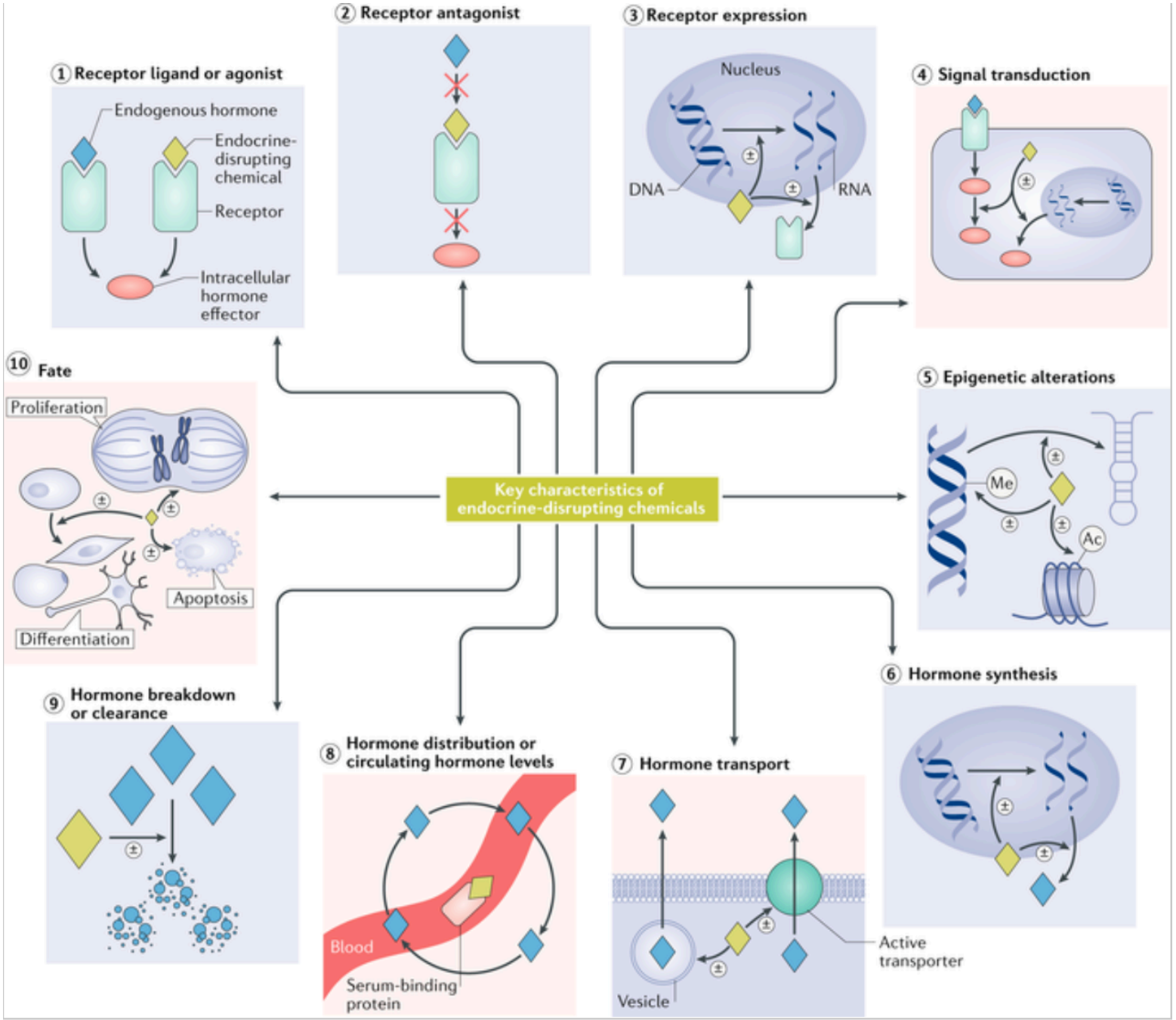
NATURE REVIEWS | ENDOCRINOLOGY

OPEN

Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification

Michele A. La Merrill[1], Laura N. Vandenberg[2], Martyn T. Smith[3], William Goodson[4], Patience Browne[5], Heather B. Patisaul[6], Kathryn Z. Guyton[7], Andreas Kortenkamp[8], Vincent J. Cogliano[9], Tracey J. Woodruff[10], Linda Rieswijk[3,11], Hideko Sone[12], Kenneth S. Korach[13], Andrea C. Gore[14], Lauren Zeise[15] and R. Thomas Zoeller[16]*

Universal EDC Characteristics Are



Recommended uses of the KCs of EDCs in searching and organizing the relevant literature.

- Systematically search the scientific literature for mechanistic data, by using appropriate combinations of keyword terms (i.e. controlled ontologies) to reproducibly identify endpoints relevant to the KCs.
- Organize and integrate the gathered evidence on endocrine disruption across data streams. Such data may arise from molecular epidemiology studies, in vivo and in vitro tests in experimental models, high-throughput tests and in silico modeling. The latter data sources may be germane when the former mechanistic data sources are sparse.

A hazard of the 'relevant literature' is that it may be sparse due to poor coverage of the KCs by standardized tests for regulatory decision-making.

Table 1 | **Key characteristics of EDCs and representative standardized tests that address them**

Key characteristics	Examples of relevant streams of mechanistic evidence	Guideline description (species) [agency and guideline number][a]
KC1. Interacts with or activates hormone receptors	Binding or agonism of hormone receptors	Androgen Receptor Binding (rat) [US EPA 890.1150]; Estrogen Receptor Binding (rat) [US EPA 890.1250, OECD TG 493]; Estrogen Receptor Transcriptional Activation (human stable transfection) [US EPA 890.1300, OECD TG 455]; Androgen Receptor Binding (rat) [US EPA 890.1150]; Androgen Receptor Transcriptional Activation (human stable transfection) [OECD TG 458]; Uterotrophic (rat) [US EPA 890.1600, OECD TG 440]; Hershberger [US EPA 890.1400, OECD TG 441]
KC2. Antagonizes hormone receptors	Antagonism of nuclear or cell surface hormone receptors	Estrogen Receptor Transcriptional Activation (human) [OECD TG 455]; Androgen Receptor Transcriptional Activation (human) [OECD TG 458]; Hershberger [US EPA 890.1400, OECD TG 441]
KC3. Alters hormone receptor expression	Abundance, distribution and degradation of hormone receptors	None
KC4. Alters signal transduction in hormone-responsive cells	Abundance of post-translational modifications, cofactors, transcription factors and transcripts, and activity of associated enzymes	None
KC5. Induces epigenetic modifications in hormone-producing or hormone-responsive cells	Chromatin modifications, DNA methylation and non-coding RNA expression	None
KC6. Alters hormone synthesis	Expression or activity of enzymes or substrates in hormone synthesis	Aromatase (human) [US EPA 890.1200]; Steroidogenesis (human) [US EPA 890.1550, OECD TG 456]
KC7. Alters hormone transport across cell membranes	Intracellular transport, vesicle dynamics or cellular secretion	None
KC8. Alters hormone distribution or circulating hormone levels	Blood protein expression and binding capacity, blood levels of pro-hormones and hormones	None
KC9. Alters hormone metabolism or clearance	Inactivation, breakdown, recycling, clearance, excretion or elimination of hormones	None
KC10. Alters fate of hormone-producing or hormone-responsive cells	Atrophy, hyperplasia, hypertrophy, differentiation, migration, proliferation or apoptosis	None

EDC, endocrine-disrupting chemical; OECD, Organisation for Economic Co-operation and Development; TG, test guideline; US EPA, US Environmental Protection Agency. [a]Only assays that serve as the basis of regulatory decisions of the OECD and US EPA are provided.

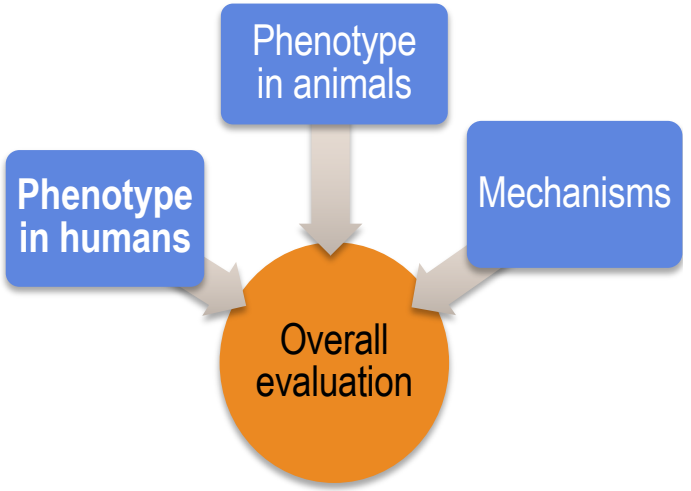
Recommended uses of the KCs of EDCs in searching, organizing and evaluating the relevant literature.

- Emancipate the investigator from describing an entire, specific molecular pathway. In combination with phenotypic data from epidemiology and animal studies, this strategy represents an important and practical addition to hazard identification.

Case Study: Bisphenol A, short-lived ubiquitous exposure

- Plastics
- Thermal paper receipts

KCs in Data integration: BPA phenotype in humans



There are now more than 100 epidemiology studies showing associations between BPA and adverse outcomes such as obesity, diabetes mellitus, female infertility, male sexual dysfunction, lower birth weight, and atypical neurobehaviors in children, among others. Although many of these studies are cross-sectional, others are longitudinal, providing stronger evidence for causal relationships between exposures and effects.

Bisphenol A is associated with increased diabetes in humans

Rancière et al. Environmental Health (2015) 14:46
DOI 10.1186/s12940-015-0036-5

ENVIRONMENTAL HEALTH

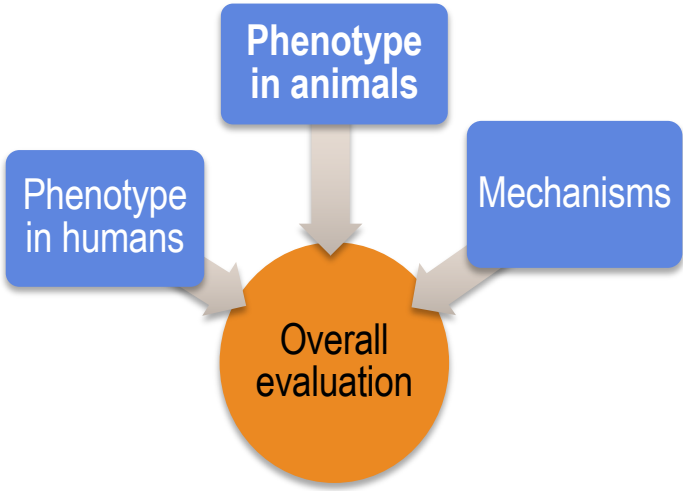
REVIEW Open Access

Bisphenol A and the risk of cardiometabolic disorders: a systematic review with meta-analysis of the epidemiological evidence

Fanny Rancière[1,2,3†], Jasmine G. Lyons[3†], Venurs H. Y. Loh[3], Jérémie Botton[1,2,4], Tamara Galloway[5], Tiange Wang[6], Jonathan E. Shaw[3] and Dianna J. Magliano[3*]

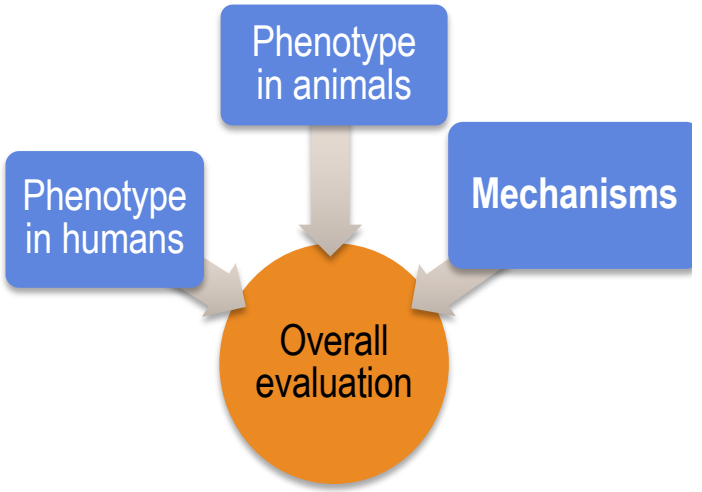
Eight cross-sectional studies reported on the relation between uBPA and diabetes [32, 37, 39, 40, 42, 46, 51, 56], and seven supported a positive association (Table 2).

KCs in Data integration: BPA phenotype in animals



In rodents, hundreds of studies demonstrate that even low doses of BPA can disrupt development of the brain, male and female reproductive tracts, mammary gland, and metabolic tissues under endocrine control, among others. BPA can also induce precancerous and cancerous lesions of the mammary gland and prostate.

KCs in Data integration: BPA mechanistic data



The expertise of the team provided the opportunity to conduct a thought exercise examining how the mechanistic evidence for BPA mapped to the KCs of EDCs.

This BPA example was not performed as a systematic review, hence some mechanistic data was likely missed.

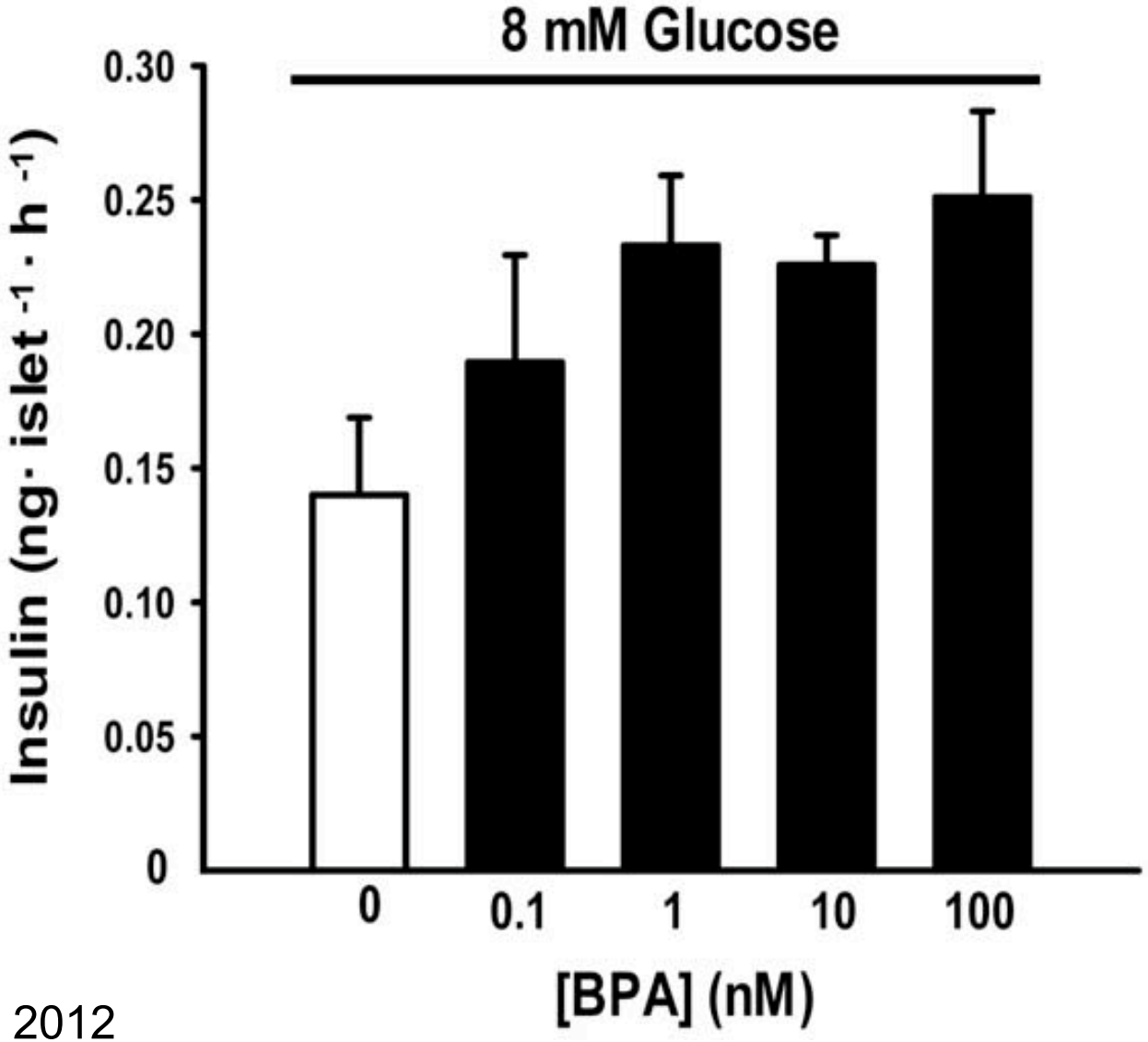
Case Study of BPA Characteristics

EDC Characteristic	Mechanistic evidence for BPA
1. Interacts with or activates hormone receptors	BPA activates nuclear ERs, membrane ER and GPR30 in a variety of species.
2. Antagonizes hormone receptors	BPA antagonizes androgen receptor.
3. Alters hormone receptor expression	BPA increases the expression of ER mRNA, and its location, in specific regions of the brain in mice exposed during gestation.
4. Alters signal transduction in hormone- responsive cells	**BPA represses pancreatic beta cell calcium signaling.** BPA induces the expression and recruitment of SRC to ERα, ERβ, and THRβ. BPA induces ERK phosphorylation in Sertoli TM4 cells. In a human testicular seminoma cell line (JKT-1). BPA activates cAMP- and cGMP-dependent protein kinase pathways to phosphorylate CREB.
5. Induces epigenetic modifications in hormone- producing or responsive cells	BPA affects promoter specific- methylation in brain, prostate, and human breast cancer cells. The ER binding region of the long-non coding RNA HOTAIR promoter is enriched by trimethylation on H3K4 and by H3K4-specific methyl-transferases in human breast cancer cells. In mouse prostate, neonatal BPA activates the histone methyltransferase MLL1 to persistently increase H3K4 trimethylation at genes associated with prostate cancer.

Case Study of BPA Characteristics

EDC Characteristic	Mechanistic evidence for BPA
6. Alters hormone synthesis	BPA inhibits steroidogenesis in the rat testis. BPA reduces cytochrome p450 aromatase levels and the expression of other steroidogenic regulatory proteins.
7. Alters hormone transport across cell membranes	**Low BPA doses increase insulin secretion from vesicles.**
8. Alters hormone distribution or circulating hormone levels	In men, BPA exposure is associated with increased SHBG levels, as well as decreased circulating levels of androstenedione and free testosterone.
9. Alters hormone metabolism or clearance	No evidence identified.
10. Alters fate of hormone-producing or responsive cells	Developmental exposures to BPA alters the differentiation of mammary epithelial cells, and increased the number of alveolar buds (structures that eventually produce milk in lactating females) in the mammary gland. **BPA also increases the proliferation index in the pancreas,** mammary gland, and uterine endothelial cells, among others.

BPA increases insulin secretion in mice



Soriano et al 2012

BPA causes ER beta dependent insulin secretion

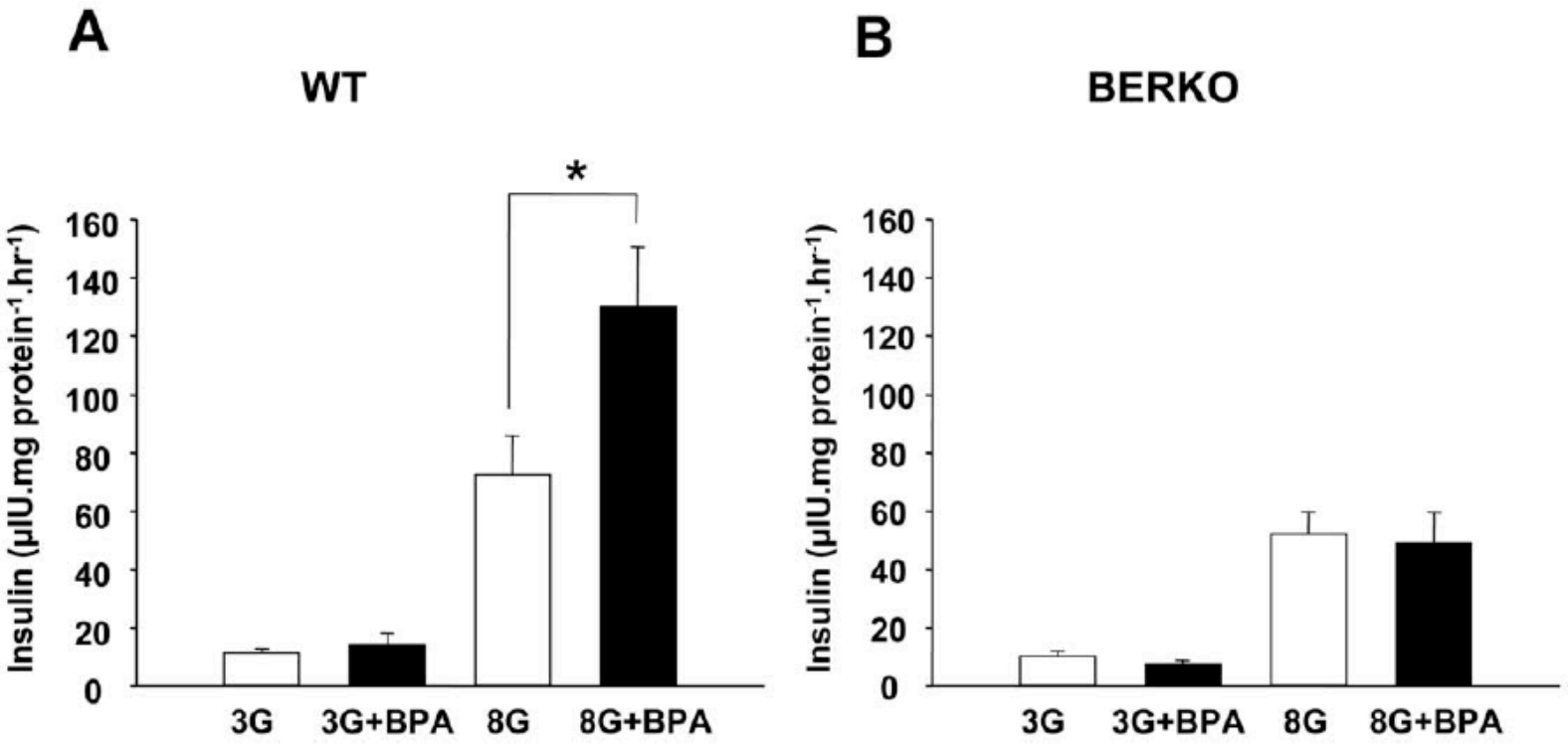


Figure 5. BPA effect on glucose-induced insulin secretion from isolated islets from WT and ERβ−/− mice. A, 1 nM BPA induced insulin secretion from WT islets exposed to 3 mM glucose and 8 mM glucose for 1 h. Note that 1 nM BPA action was significant only when 8 mM glucose was used. B, Same experiment as performed in A, but using ERβ−/− islets. Note that 1 nM BPA effect was abolished at 8 mM glucose (n = 5). *, $P<0.05$ Student's t-test comparing 8 mM glucose with 8 mM glucose in presence of 1 nM BPA.

Soriano et al 2012

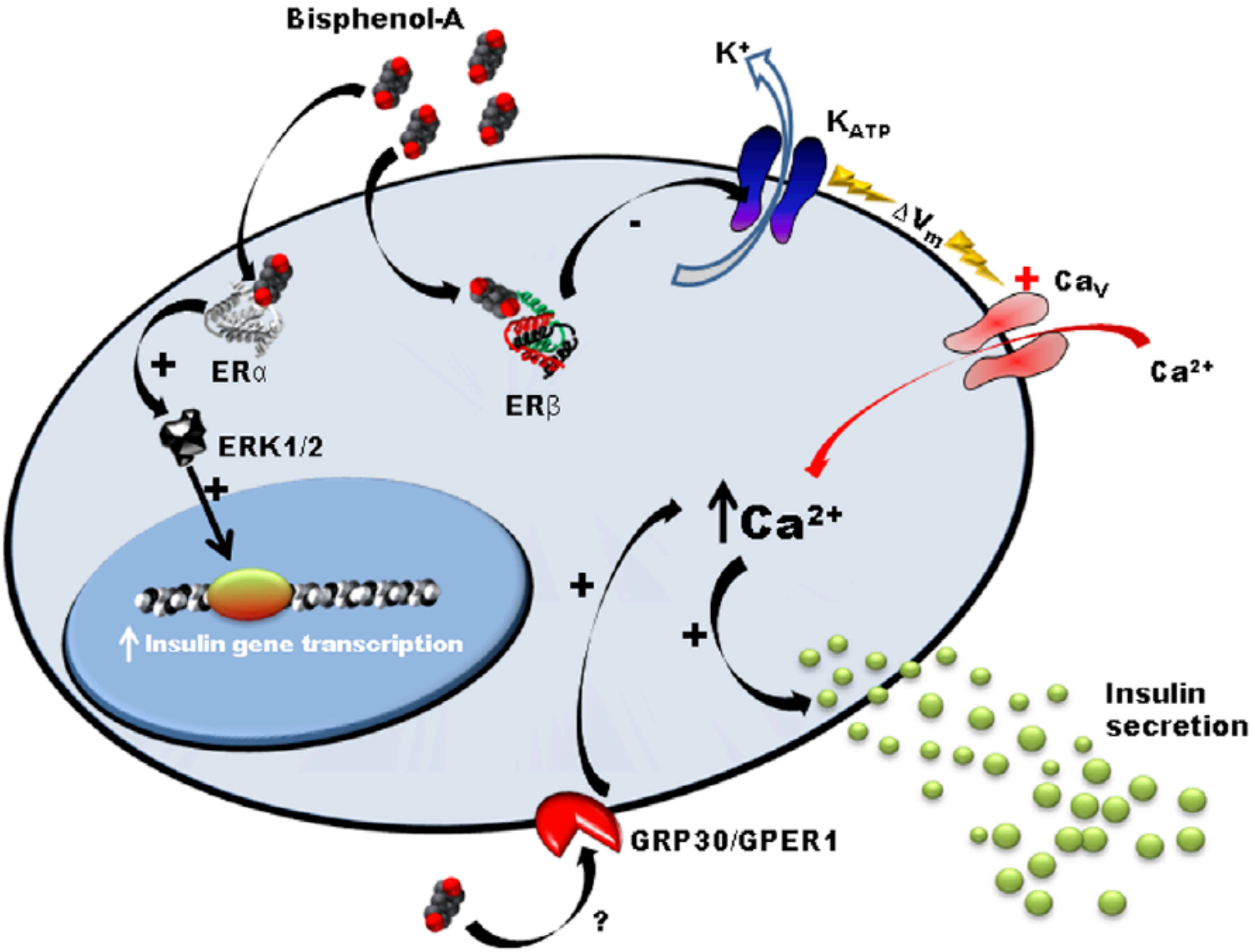
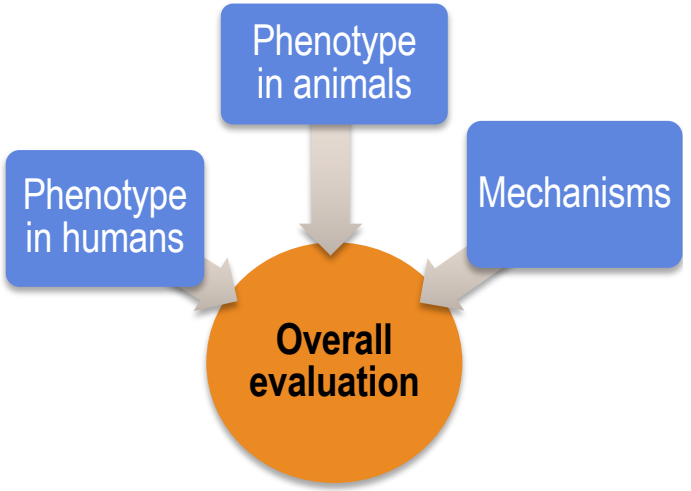


Figure 7. Model of BPA action on pancreatic β-cells. In the presence of stimulatory glucose concentrations, low concentrations of BPA rapidly decrease K_{ATP} channel activity through ERβ, enhancing glucose-induced $[Ca^{2+}]_i$ signals and insulin release. ERα is involved in the regulation of pancreatic insulin biosynthesis in response to BPA. In addition to ERβ, GPR30/GPER1 or another yet unidentified non-classical membrane estrogen receptor may participate in the insulinotropic effect of BPA on pancreatic β-cells. At the moment, this model applies to rodent beta cells. In humans, the receptors involved in the BPA regulation of K_{ATP} channel activity and insulin release are still undetermined.

Soriano et al 2012

KCs in evidence integration:



- 1000s of mechanistic scientific papers on BPA that provide substantial evidence for 9 of the 10 KCs
- BPA (KC1) binds and (KC2) activates ERα and ERβ
- BPA then causes the (KC5) enrichment of H3K4 trimethylation and H3K4-specific methyl-transferases at the ER binding region of the HOTAIR promoter
- Activation of ER by BPA has multiple (KC4) signal transduction effects
  - (KC6) BPA activation of ERα mediated insulin transcription
  - (KC7) BPA activation of ERβ mediated ion flux underlying enhanced insulin secretion from pancreatic islets
- Consistent with the known mechanisms underlying the diverse adverse effects of BPA in humans and other animals
  - e.g. type II diabetes

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EDC group participants

Patience Brown (OECD)
Vincent Cogliano (US EPA)
Bill Goodson (SF, USA)
Kate Guyton (IARC)
Ken Korach (NIEHS, USA)
Andreas Kortenkamp (Brunel, UK)
Linda Rieswijk (UCB, USA)
Martyn Smith (UCB, USA)
Hideko Sone (NIES, Japan)
Laura Vandenberg (UMass, USA)
Tracey Woodruff (UCSF, USA)
Lauren Zeise (CalEPA)
Tom Zoeller (UMass, USA)

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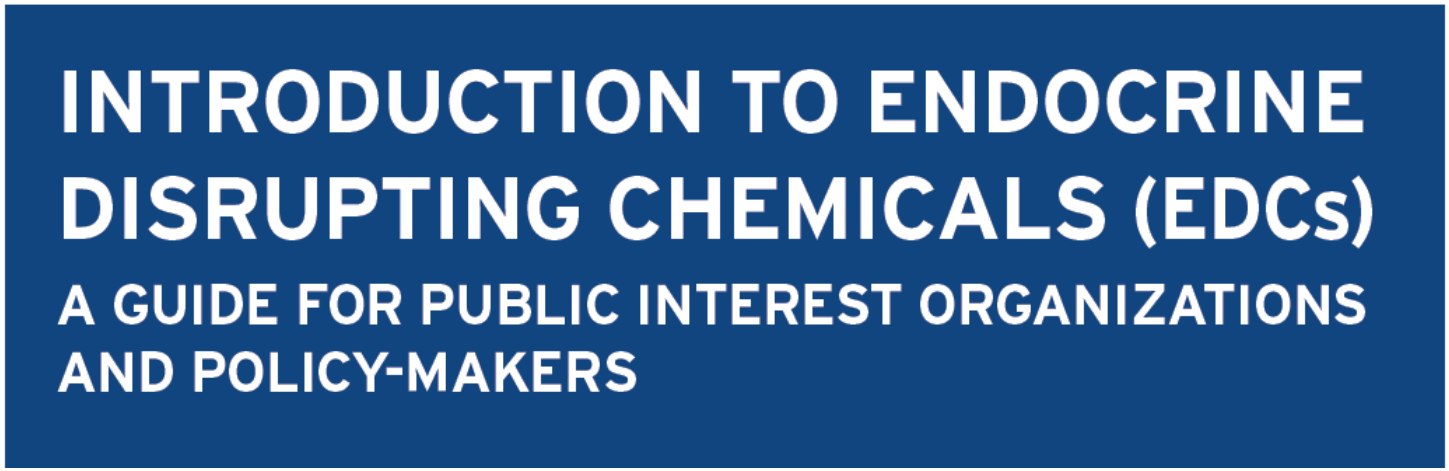
- **Characterize** the mechanistic evidence for an EDC as “strong”, “limited” or “inadequate” to reflect the wide variance in the extent and **quality of evidence** for any given KC, and **following the approach of IARC**.
- Emancipate the investigator from describing an entire, specific molecular pathway. In **combination with phenotypic data from epidemiology and animal studies**, this strategy represents an important and practical addition to hazard identification.

How you can be involved

- Educate your local organizations and policy-makers about
  - the importance of EDCs and
  - the need to take action
- Guide is available in six languages
  - English, Spanish, French, Russian, Arabic and Portuguese
  - Get it here: https://www.endocrine.org/topics/edc/introduction-to-edcs



Strategic **A**pproach to **I**nternational **C**hemicals **M**anagement (**SAICM**)

- **SAICM** is a policy framework to promote chemical safety around the world
- hosted by the United Nations Environment Programme
- Endocrine Society collaborated with non-profit organization IPEN to
  - educate conference attendees about EDCs,
    - Give out copies of the **Guide** to educate representatives about the importance of EDCs and the need to take action
  - draft and revise the text, and
  - build support for the resolution.
    - Over 120 governments

https://endocrinenews.endocrine.org/endocrine-society-influences-edc-policy-around-the-world/

SAICM Resolution key points

- Evidence in humans, laboratory animals and wildlife shows that exposure to endocrine-disrupting chemicals can result in adverse effects;
- The most critical window of exposure is during development;
- Exposure during early life stages can result in adult-onset disease, and an important focus should be on reducing exposure;
- There is a “cost of inaction” associated with EDCs exposure;
- Continued actions on endocrine-disrupting chemicals by all stakeholders will be needed in order to attain the objectives of the Strategic Approach.

The link to the guide again:
https://www.endocrine.org/topics/edc/introduction-to-edcs