Key Characteristics of Female and Male Reproductive Toxicants

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Proposed Key Characteristics of Female Reproductive Toxicants as an Approach for Organizing and Evaluating Mechanistic Data in Hazard Assessment

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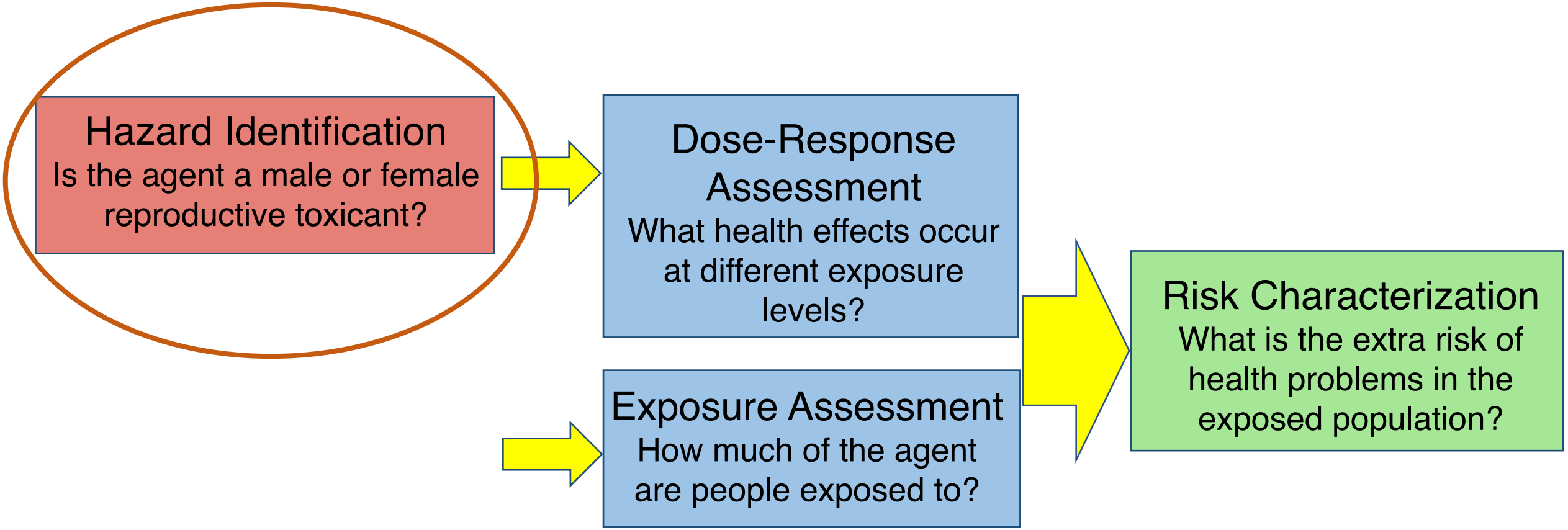
Chemical Risk Assessment



The Bottleneck in Chemical Hazard Assessment

- Tens of thousands of chemicals in commerce, but very few have been evaluated for female or male reproductive toxicity.
- Hazard assessment for human health risk assessment relies strongly on animal toxicology bioassays and human epidemiological studies.
- These are expensive and time-consuming.
- Less emphasis has been placed on mechanistic data.

The Key Characteristics Concept

- Pioneered for carcinogens by IARC working group in 2012
- Characteristics commonly exhibited by established carcinogens
- Provides a uniform approach for searching, organizing, and evaluating mechanistic evidence for carcinogen hazard identification.
- Utilized by IARC (Guyton et al, 2018, Carcinogenesis)
- 2017 NASEM report recommended approach be expanded to other endpoints, including reproductive toxicity

Working Group on KCs of Reproductive Toxicants and Endocrine Disruptors

Berkeley CA, March 7-8, 2018

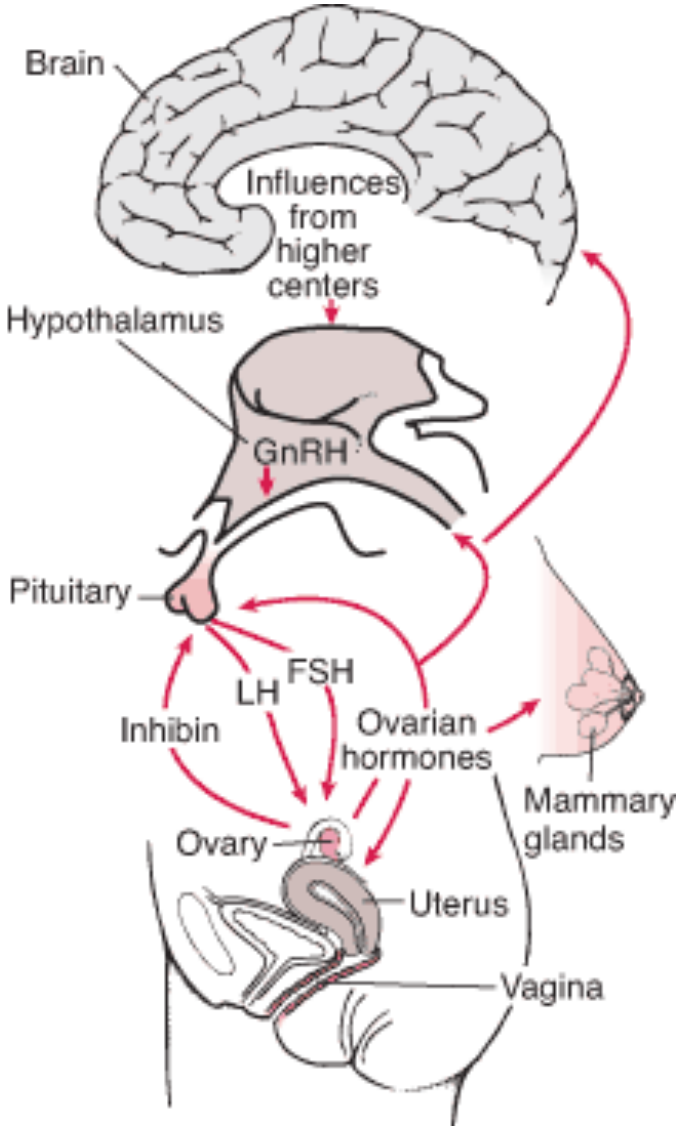
Application of KCs approach to reproductive toxicants

- Working group of experts in female and male reproductive toxicity and endocrine disruption convened at UC Berkeley on March 7-8, 2018
  - Reviewed approach and agreed it could be applied to reproductive toxicants
  - Formulated initial draft lists of key characteristics
  - Three working groups were formed to continue working together
- Iterative processes in which the initial lists of key characteristics were refined based on discussions of the entire group and work of subgroups focused on developing and refining specific KCs and example chemicals

Key Characteristics are not apical endpoints

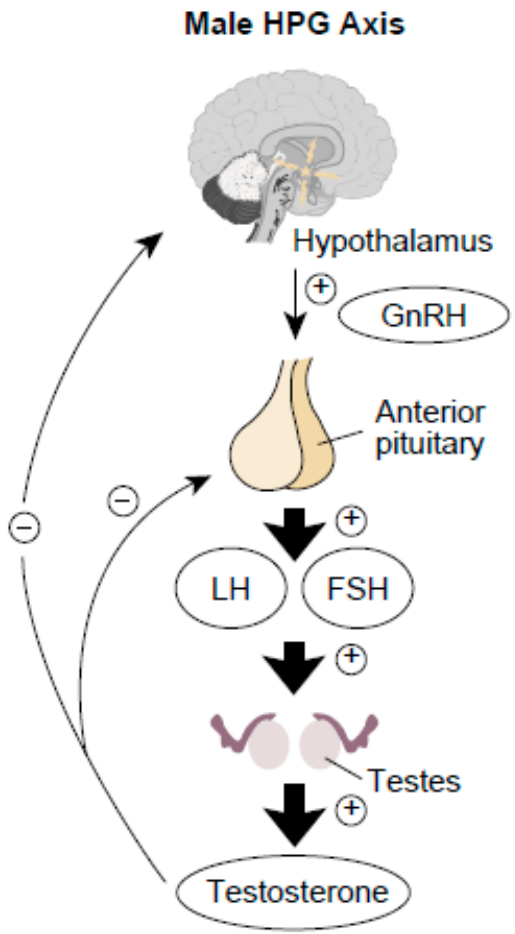
Female-specific apical endpoints of reproductive toxicity

Reproductive Process or Endpoint	Assay
Estrous cycling	Vaginal cytology
Reproductive organ size	Weights of ovaries, uterus (with oviducts and cervix), pituitary
Reproductive organ structure	Macroscopic and histopathological examination of ovaries, uterus, oviducts, cervix, vagina, pituitary, mammary gland. Enumeration of ovarian primordial follicles
Development	Puberty (vaginal opening, first vaginal estrus in rodents), anogenital distance, structure of external genitalia
Pregnancy	Pregnancy rate, number of implantation sites, preimplantation mortality, birth rate, number and sex of live and dead pups at birth, fetal/neonatal body weights



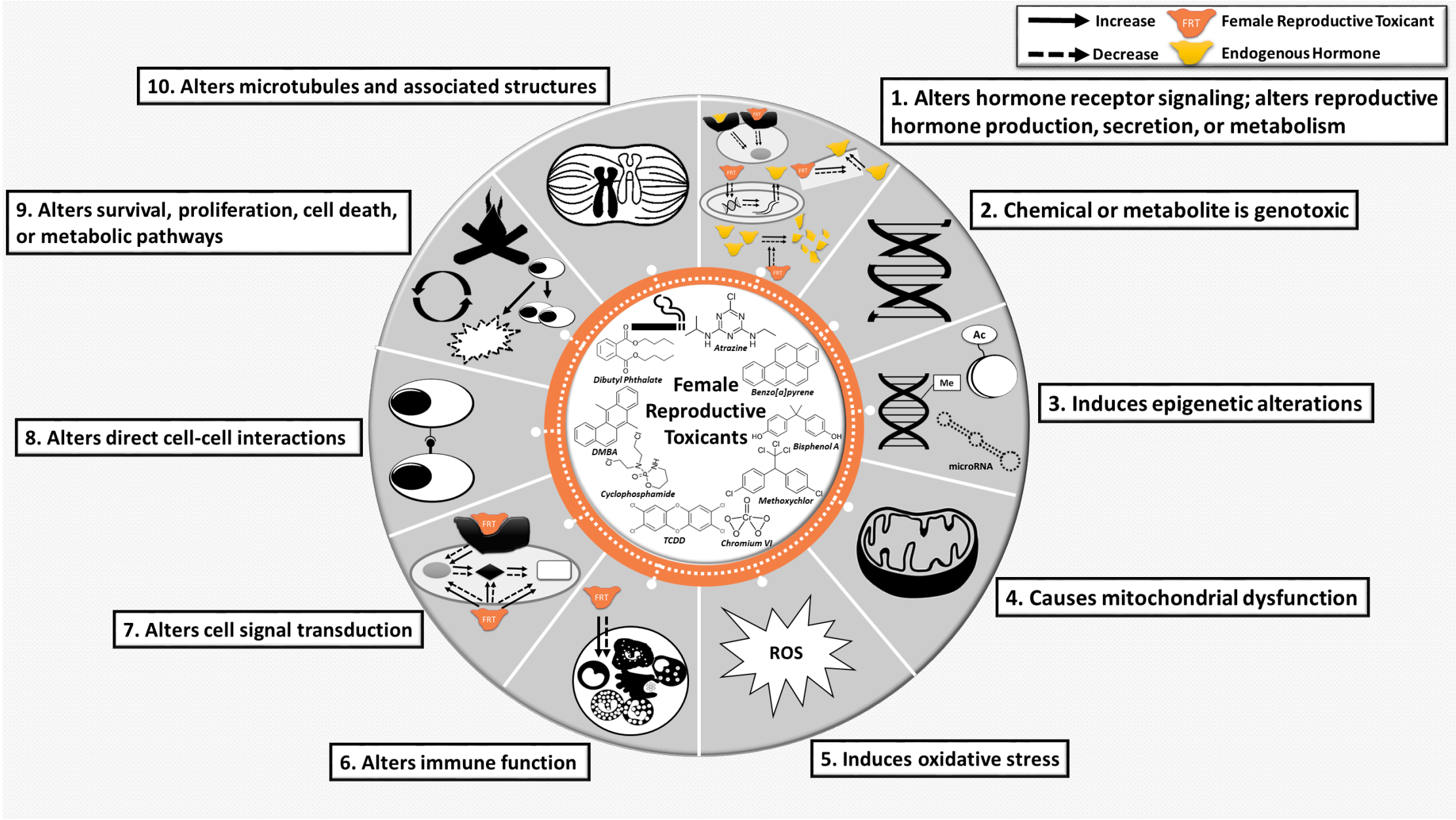
Male-specific apical endpoints of reproductive toxicity

Reproductive Process or Endpoint	Assay
Reproductive organ size	Weights of testes, epididymides, seminal vesicles, prostate, pituitary
Reproductive organ structure	Macroscopic and histopathological examination of testes, epididymides, seminal vesicles, prostate, pituitary
Sperm evaluation	Sperm number (count) and quality (morphology, motility)
Development	Testis descent, puberty (preputial separation),sperm production, anogenital distance, structure of external genitalia
Sexual behavior	Mounts, intromissions, ejaculations



https://en.wikipedia.org/wiki/File:Hypothalamic%E2%80%93pituitary%E2%80%93gonadal_axis_in_males.png

Key Characteristics of Female Reproductive Toxicants

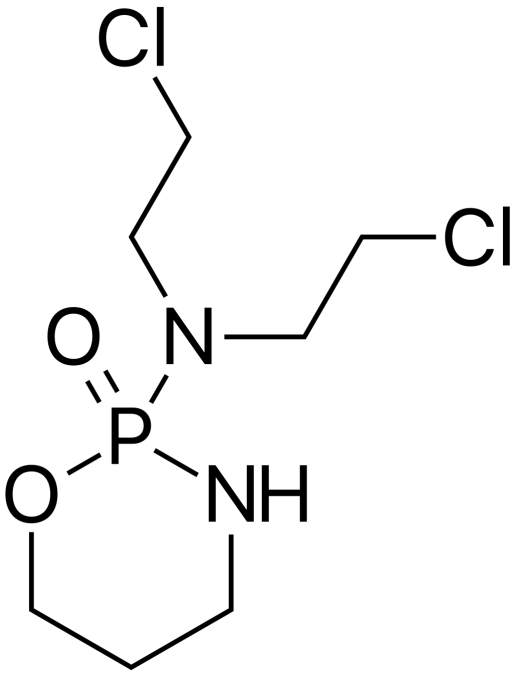


Application of KCs to known female reproductive toxicants

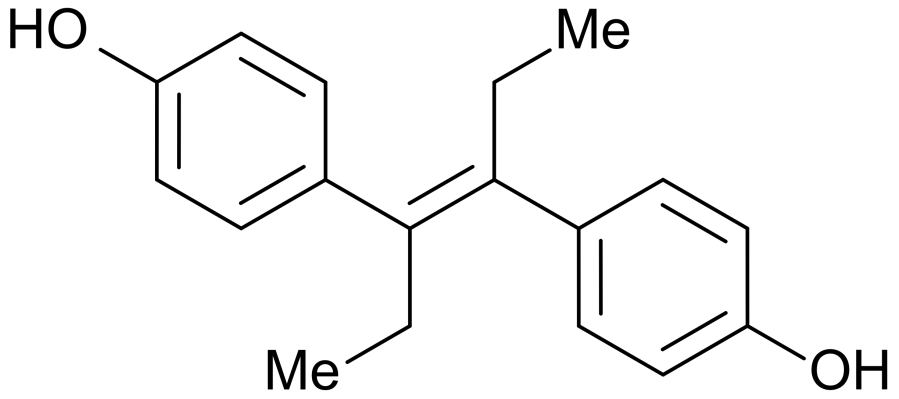
- Example toxicants for which abundant epidemiological and/or *in vivo* toxicology data illustrate female reproductive toxicity
- Explicitly chose toxicants with differing modes of action:
  - Diethylstilbestrol
  - Cyclophosphamide
  - TCDD

Cyclophosphamide

- On Prop 65 list of known female reproductive toxicants.
- Causes temporary or permanent amenorrhea and early menopause due to ovarian follicle depletion.
- KC2: is metabolically activated to genotoxic metabolites; ↑ dsDNA breaks in cultured neonatal ovaries
- KC5: ↑ ROS and oxidative DNA damage in cultured granulosa cells; ↑ ROS in oocytes
- KC7: *in* vivo treatment ↑ ovarian phosphorylation of AKT and its target FOXO3
- KC9: ↑ apoptosis in cultured granulosa cells and in granulosa cells of ovarian follicles with *in vivo* dosing
- KC10: disrupted microtubules in meiotic spindles of oocytes



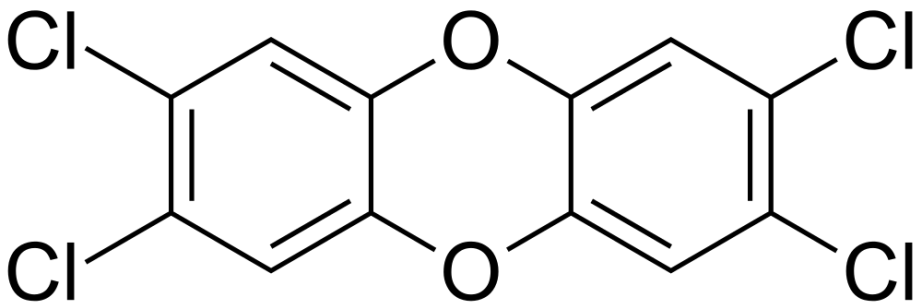
Diethylstilbestrol (DES)



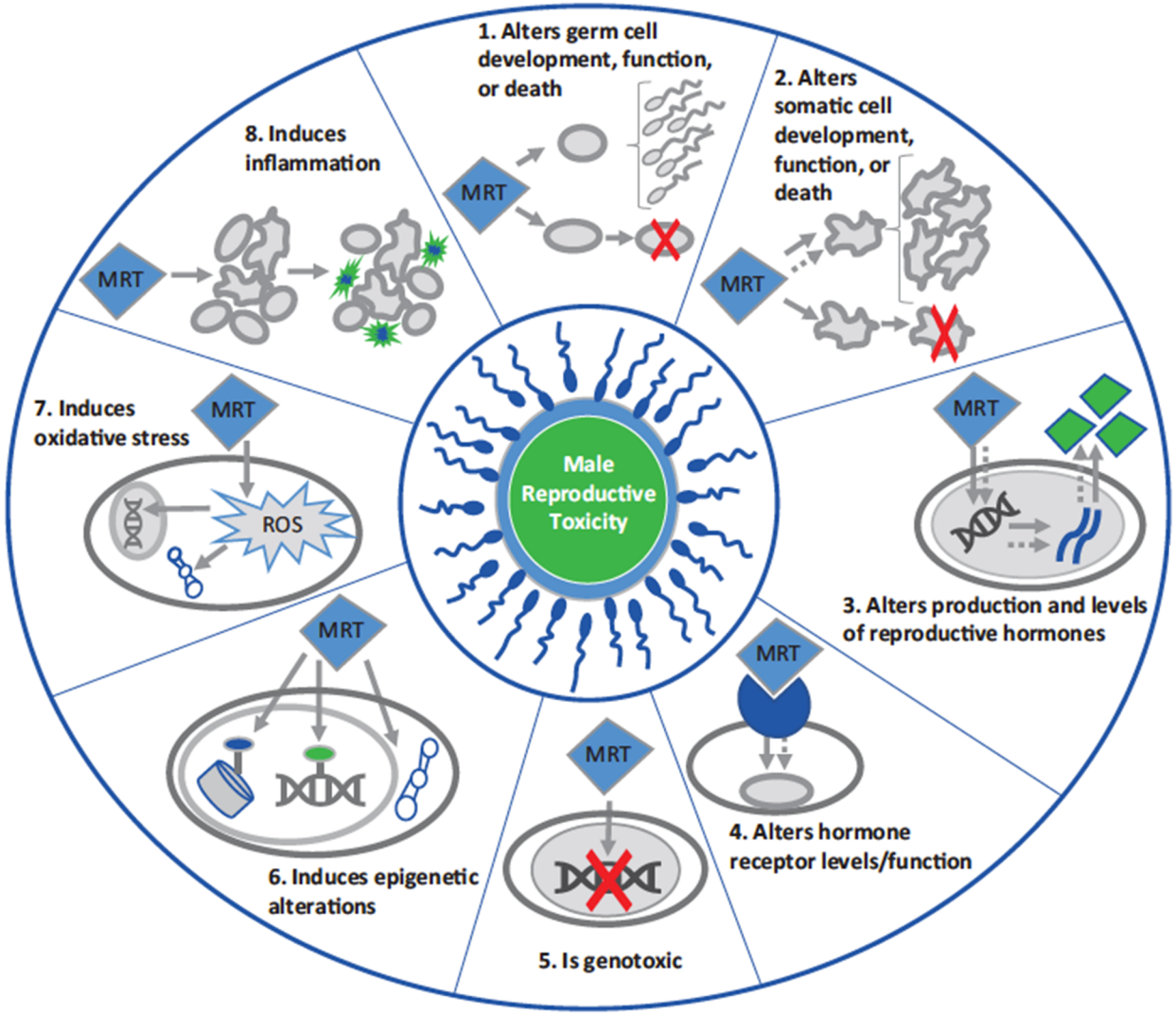
- Daughters of mothers treated during pregnancy with DES developed vaginal adenocarcinoma and malformations of the uterus, cervix, and vagina
- KC1: potent synthetic estrogen; binds and activates ERα
- KC7: altered *Hoxa10* gene expression in cultured endometrial cells and in the uterus of developmentally exposed mice
- KC3: altered DNA methylation, histone acetylation and methylation in the developing uterus

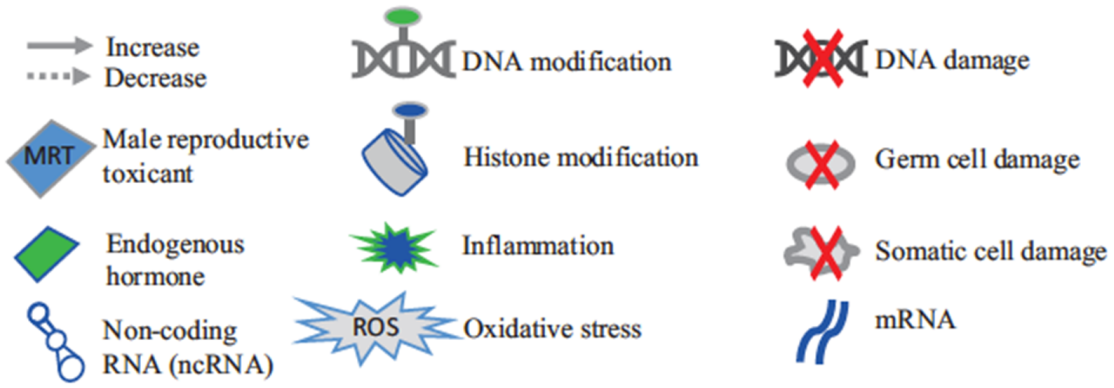
2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

- *In utero* exposure in rodents adversely affects female reproductive system development and function
- KC1: altered estrogen receptor signaling in cultured breast cancer cells; decreased E production in luteinized granulosa cells; developmental exposure decreased uterine PR expression and uterine responses to P
- KC3: developmental exposure resulted in hypermethylation of uterine progesterone receptor promoter in F1 and F3 females
- KC6: altered endometrial immune function, promoting growth of endometrial implants in mouse endometriosis model
- KC7: disrupted multiple cell-signaling pathways in cultured endocervical cells and luteinized granulosa cells
- KC9: altered protein levels of cell cycle regulators in endocervical cells from monkeys treated with TCDD



Key Characteristics of Male Reproductive Toxicants

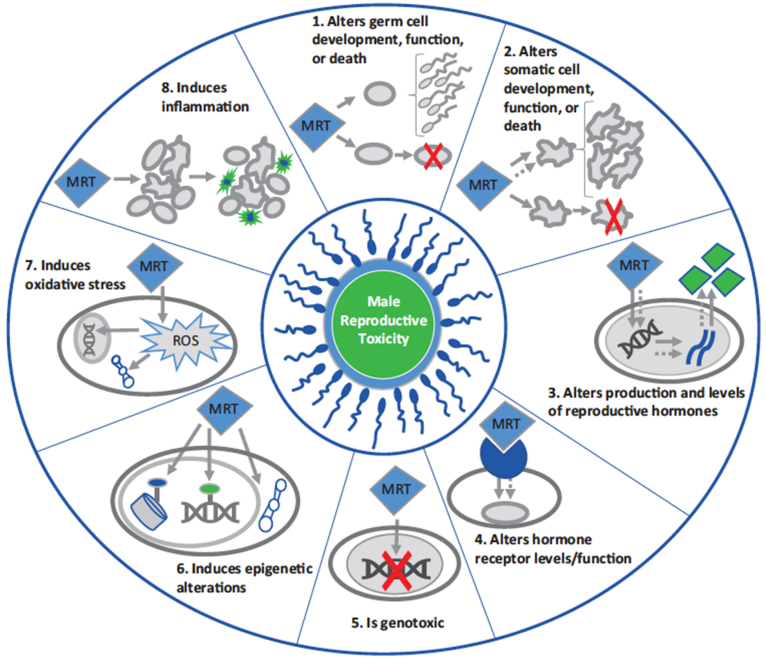




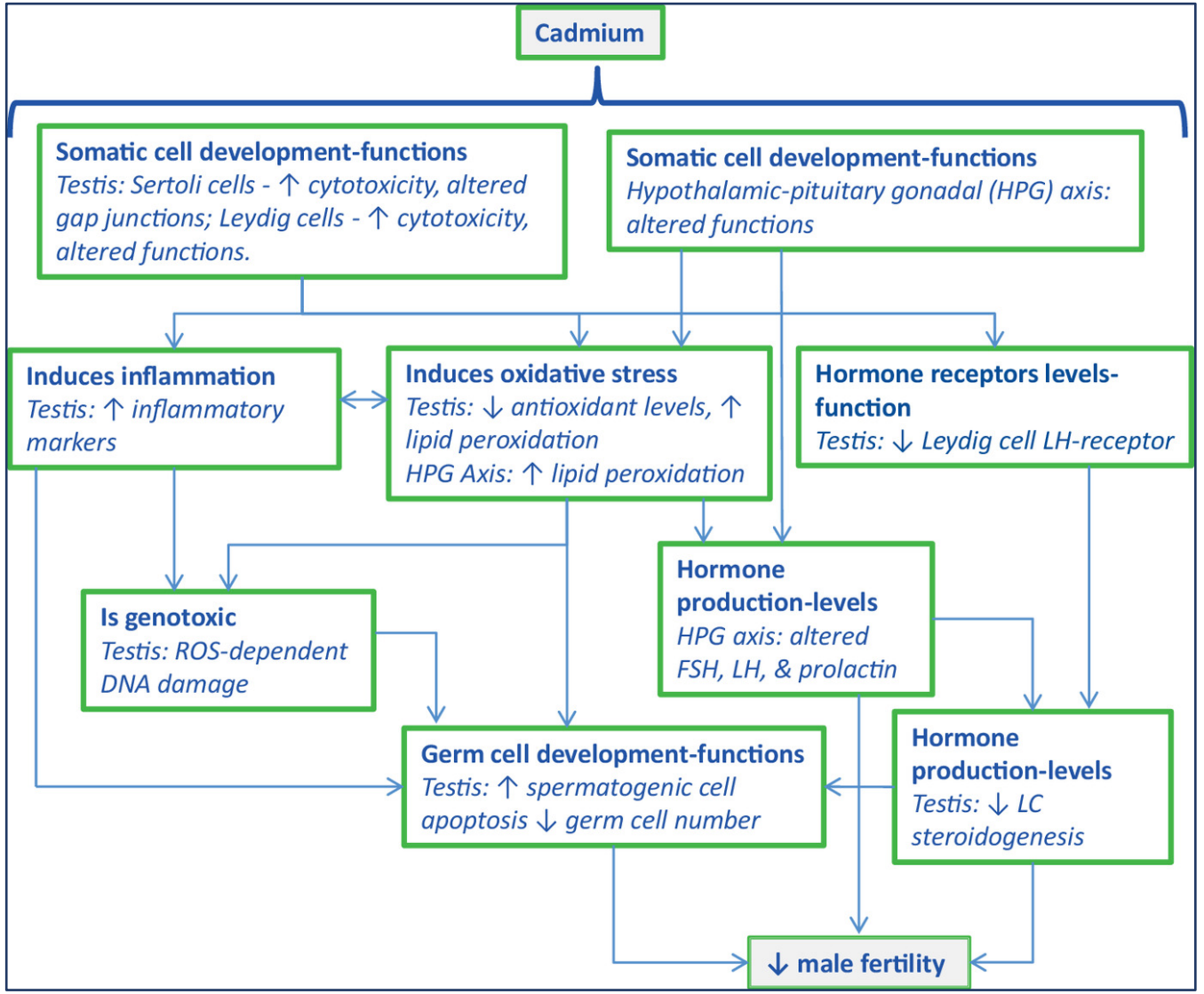
Cadmium: ♂ reproductive toxicant

- *Known* to cause temporary or permanent male infertility due to direct testicular effects, altered sperm motility and function, hormone changes and other factors.
- KC1: ↑ sperm cell death, ↓ sperm count/motility
- KC2: alters Sertoli cell gap junctions;

  Leydig cell cytotoxicity
- KC3: ↓ pituitary LH, FSH, PRL levels → decreased T
- KC4: ↓ Leydig cell LH receptors
- KC5 & 7: Testes; ROS-dependent DNA damage
- KC8: ↑ testicular inflammation



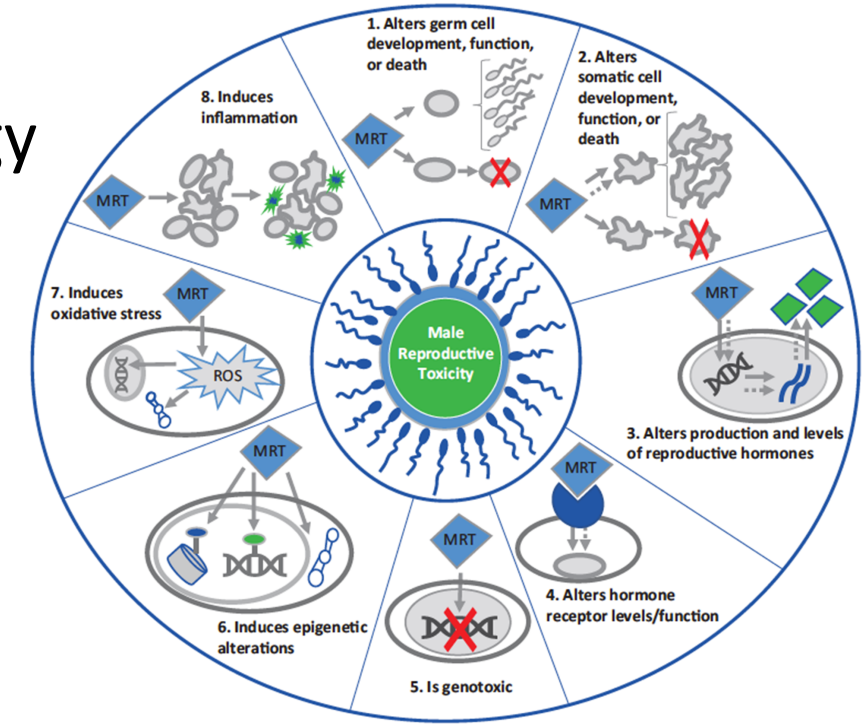
Cadmium: ♂ reproductive toxicant



KCs guide a *mechanistic network*

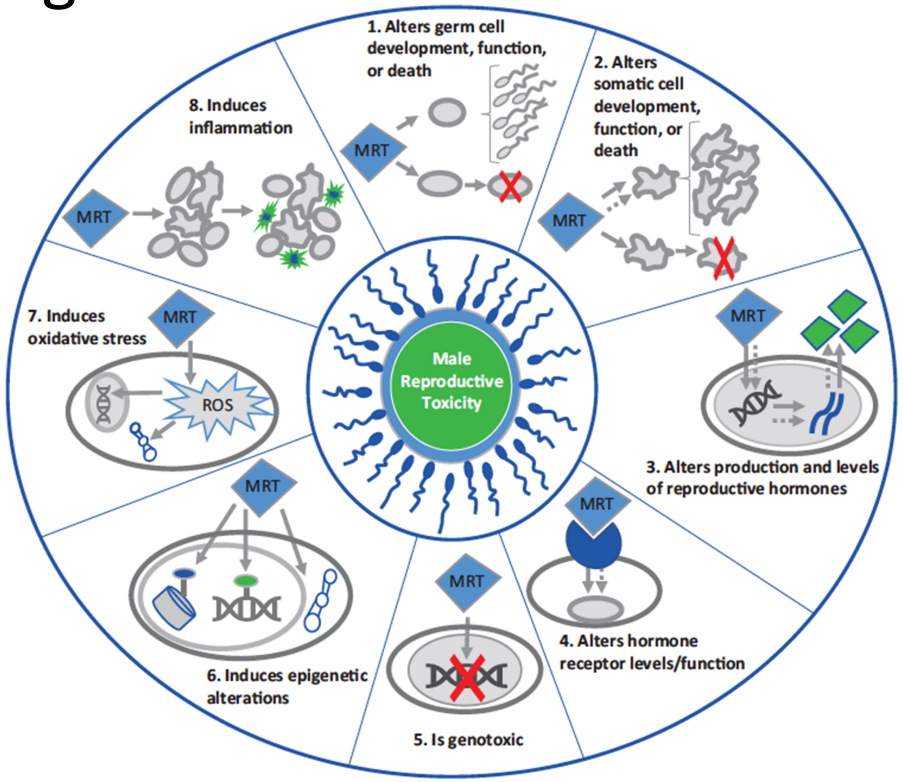
Phthalates: plasticizers

- Evidence in humans for ↑time to pregnancy due to ↓ male fertility.
  Strongest evidence for DEHP and DBP.
  Effects shown with *fetal* exposures and in *adult* men.
- KC1: Germ cell degeneration, apoptosis
  ↓ sperm counts, motility; poorer morphology
- KC2: Altered Sertoli-germ cell interactions
  ↑ Sertoli cell apoptosis
  ↓ ano-genital distance in newborn males
  ↑ hypospadias/cryptorchidism
- KC3: ↓ testosterone levels in adult men
- KC6: Alters sperm ncRNAs in mice



4-Methylbenzylidenecamphor (4-MBC)

- EDC, UV filter used in sunscreens .
- KC1: ↑ sperm hyperactivation motility by altering CatSper channel (Ca++ mobilization) – humans
- KC4: alters or activates steroid receptors
  - ERβin vertebrates, EcR in aquatic species
- KC6: ↓ hatching; transgenerational effects in crustaceans → epigenetic changes ?
- KC7: induces oxidative stress



Why Key Characteristics?

- Provides a *starting point* for identification, organization, analysis of mechanistic data that inform whether a chemical *can cause adverse reproductive effects*.
- Focuses on known organs and systems that impact reproductive functions.
- Development of targeted literature search strategies for a chemical using combinations of KC terms for endpoints.
- Development of literature inventories and KC networks for a chemical.
- Guide prioritization of data-poor chemicals for further evaluation.
- Identification of data gaps → research needs
- Development of new assays for toxicants
- KCs can inform development of adverse outcomes pathways (AOP)

Current Classification/Organizational Systems

- MoA = Mode of Action Classification
  - Describes a functional or anatomical change, resulting from the exposure of a living organism to a substance.
- AOP = Adverse Outcomes Pathways
  - Links in a *linear way* existing knowledge along one or more series of causally connected **key events** (**KE**) between two points — a **molecular initiating event** (**MIE**) and an **adverse outcome** (**AO**) that occur at a level of biological organization

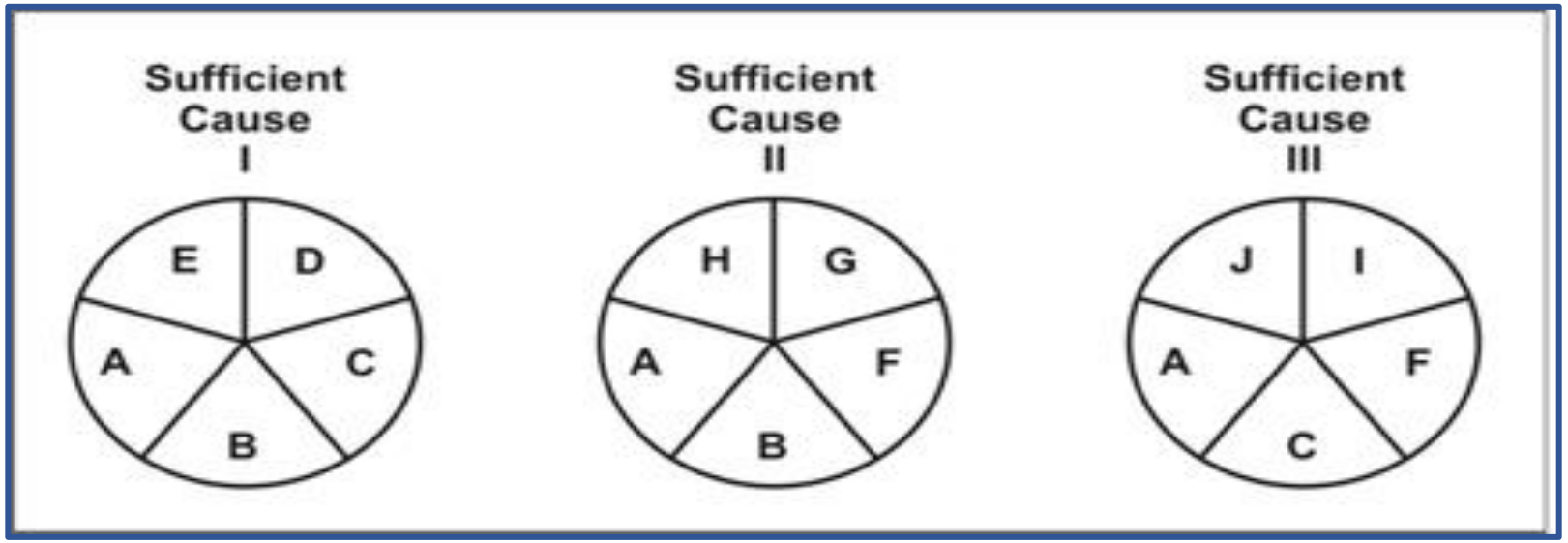
Why Key Characteristics? Limitations of MOA/AOP Approaches

MOA = Mode of Action
AOP = Adverse Outcomes Pathways

- Biology is not linear – influenced by feedback mechanisms, repair, background, susceptibilities…**Network of systems**
- Multiple ways to arrive at same conclusion – Does not fit with the *Causal Pie Concept*
- Limited by the current understanding of the disease process: recognized by Sir Bradford Hill, who noted that "*what is biologically plausible depends upon the biological knowledge of the day*"
- Key events are supposed to be quantifiable but in reality, they may be impossible to measure

Adapted from MT Smith

Rothman's Causal Pies:
Three causal pies each with various components.



- MOA/AOP approaches do not fit with Rothman's causal pies concept which envisages *multiple combinations of causes producing a disease*

Adapted from MT Smith

Why Key Characteristics?
Limitations of MOA/AOP Approaches

MOA = Mode of Action
AOP = Adverse Outcomes Pathways

- MOA/AOP may be incomplete or wrong
  - e.g. DEHP – Rusyn and Corton (2012)
- Focus on 'favorite' mechanism may introduce *bias*, especially on committees and public databases
- How many 'validated' AOPs needed for 100K chemicals producing 100s of adverse outcomes in different ways?

Adapted from MT Smith

Key Characteristics don't require risk assessor to guess the mechanism

- Mechanistic hypotheses in science *are beneficial* because if you test it and are wrong then you modify the hypothesis and get closer to the truth

- Mechanistic hypotheses in risk assessment *are problematic* because if you are wrong you may have made a bad risk decision that cannot easily be changed and may have caused medical or economic harm

Adapted from MT Smith

Summary and Conclusions

- **The Key Characteristics of *known* human reproductive toxicants provide a knowledge-based approach to organize and evaluate available data of other chemical(s) for evidence of reproductive harm.**

- Reproductive toxicants tend to act through multiple mechanisms producing the hallmarks of compromised human and animal fertility. As such, the KC approach can incorporate complexity, avoiding potential bias in MOA/AOP approaches.

- KC approach does not require *a priori* hypothesis of mode of action or causal linkage to an adverse outcome, which may be unavailable for 1000s of chemicals.

Summary and Conclusions

- Having one or several KCs does *not* conclusively identify a chemical as a repro toxicant! Rather, it aids risk assessors working with reproductive experts in prioritizing chemicals for additional toxicity testing.

- The KC approach can *identify data gaps* (e.g. mechanisms) and pinpoint areas requiring additional research (i.e. inform funding agencies).

- KCs may be useful in conjunction with a data science approach to *predict toxicity* and identify chemicals requiring further study.

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