Key characteristics of male reproductive toxicants: an approach for screening and sorting mechanistic evidence.

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Abstract

Since the introduction of ten key characteristics of carcinogens as a basis for organizing mechanistic data on carcinogenesis, the National Academy of Sciences has recommended that key characteristics approaches also be developed for noncancer hazards. The aim of this project was to identify a set of key characteristics that can be used for searching, screening, and sorting mechanistic evidence on chemical-induced toxicological responses in the male reproductive system. An expert workgroup was convened at the University of California-Berkeley in March 2018 to review the key characteristics approach and determine whether it can be applied to endocrine disruptors and male and female reproductive toxicants. For male reproductive toxicants, eight key characteristics were identified based on survey of established mechanisms, and include alterations in: 1) germ cell functions, 2) somatic cell functions, 3) reproductive hormone levels/production, 4) hormone receptors, 5) DNA damage, 6) epigenetic modifications, 7) oxidative stress, and 8) inflammation. As a proof of principle, this set of key characteristics was used to organize mechanistic evidence from in vivo and in vitro studies on the PCB mixture Aroclor 1254 and effects in the male reproductive system. The proposed key characteristics of male reproductive toxicants facilitates the systematic screening and categorization of mechanistic data from diverse research methods, models, and endpoints, as well as from a variety of known pathways for chemical-induced toxicity that can support hazard characterization. Disclaimer: *The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.*

Background – Introduction

Hazard identification as part of a human health risk assessment consists of an analysis of the available evidence on chemical-induced adverse health effects that are focused on cancer and non-cancer outcomes such as male reproductive toxicity. Evaluation of epidemiological, toxicological, and mechanistic studies for direct evidence of effects after chemical exposures plays a critical role in the hazard identification process. Analysis of mechanistic events that are precursors to apical endpoints seen in animals and humans support evidence of a hazard, identify potential susceptible populations and lifestages, and inform the human relevance of effects observed in animals, and identify data gaps.

Identification of the eight key characteristics of male reproductive toxicants.

- The ten key characteristics (KCs) of human carcinogens were introduced as the basis of a uniform approach for identifying, organizing, and evaluating mechanistic evidence to support cancer hazard identification (Smith et al. 2016, Guyton et al. 2018).
- In its 2017 report on “Using 21st Century Science to Improve Risk-Related Evaluations”, the National Academy of Sciences, Engineering and Medicine (NASEM) opined that the KCs approach “avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence”
- The 2017 NASEM report also recommended that the KCs approach be expanded to other endpoints, including reproductive effects, endocrine disruption and cardiovascular disease.
- Here, we have attempted to develop a set of key characteristics of male reproductive toxicants based on our current knowledge of the mechanisms by which chemicals cause reproductive toxicity.
  - A workgroup was convened at the University of California-Berkeley on March 2018 to address this topic. The objective was to review the key characteristics approach and determine whether it can also be applied to male reproductive toxicants.
  - Additionally, the review literature was evaluated to identify environmental contaminants known to target the male reproductive system and established cellular/molecular pathways linked to adverse outcomes.

- Each key characteristic is described in the context of mechanisms/pathways by which exposure to male reproductive toxicants can lead to adverse health effects.
- Finally, there is a shift away from whole animal testing for apical endpoints towards high-throughput in vitro testing and toxicogenomic profiling due to cost, timelines, ethical considerations, regulatory constraints on animal testing, and the large volume of chemicals needing evaluation.

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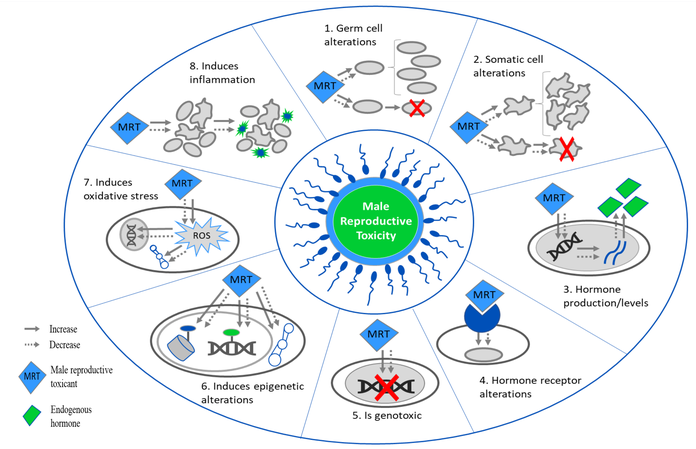
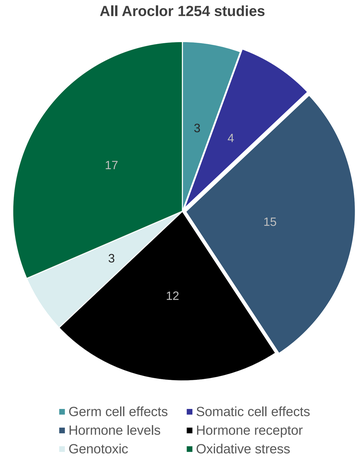
Key characteristics of male reproductive toxicants

Characteristic	Examples of relevant evidence
1. Alters germ cell development, functions, or death	Increases in germ cell apoptosis; alterations in sperm acrosome reaction and motility
2. Alters somatic cell development, functions, or death	Increased Sertoli cell apoptosis; alterations in Sertoli cell functions, cytoskeleton, and interactions with germ cells; alterations in Leydig cell development.
3. Alters production and levels of reproductive hormones	Decreased Leydig cell steroidogenic functions; increased hepatic metabolism and excretion of sex hormones.
4. Alters hormone receptor levels/functions	Androgen receptor antagonism, estrogen receptor activation, decreased LH-Receptor expression
5. Is genotoxic	DNA damage, chromosome fragmentation, altered sperm cell chromosome numbers.
6. Induces epigenetic alterations	Altered sperm microRNAs, germ cell DNA methylation patterns, and histone retention sites
7. Induces oxidative stress	Reduced tissue antioxidant levels.
8. Induces inflammation	Increased testicular expression of pro-inflammatory markers and prostaglandin levels
Abbreviations: LH, Luteinizing hormone	



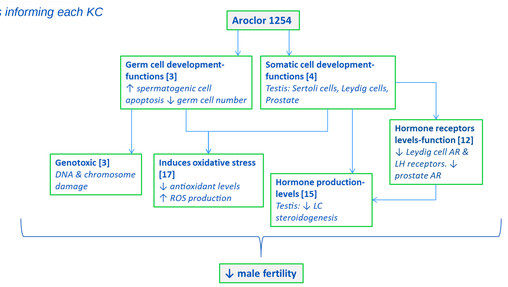
Preliminary evaluation of mechanistic effects caused by the PCB mixture Aroclor 1254



Aroclor 1254 Studies reporting on oxidative stress					
Study & HERO ID	**Age at exposure & exposure duration**	**Exposure route and duration**	**Doses - concentration**	**Species and train**	**Exposure outcomes**
Murugesan et al 2008; 614690	Not reported	Cell culture; 6 or 12 hrs	0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M	Rat – Wistar	Isolated Leydig cells: ↑ lipid peroxidation, hydrogen peroxide, and hydroxy radical production; ↓ superoxide dismutase, catalase, GPX, GR, GST, and gamma-GT; ↓ cellular levels of Vit C and Vit E
Krishnamoorthy et al 2005; 626034	90 days old	Cell culture; 6, 12 or 24 hrs	0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M		Isolated Sertoli cells: ↓ activity SOD, CAT, GPx, gamma-GT activities. ↑ lipid peroxidation.
Murugesan et al 2007; 1297571	90 days old	Cell culture; 24 hrs	0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M		Isolated Leydig cells: ↑ lipid peroxidation, hydrogen peroxide, and hydroxy radical production; ↓ superoxide dismutase, catalase, GPX, GR, GST, and gamma-GT; ↓ cellular levels of Vit C and Vit E (with and without and LH).
Aly 2013; 2149875	Not reported	Cell culture; 3 hrs	0, 10^{-9}, 10^{-8}, 10^{-7} M		↑ reactive oxygen species production and lipid peroxidation in testis mitochondrial fraction. ↓ testicular mitochondria activities of SOD, CAT, GPx and GR, and levels of glutathione and Vit C.
Ateşşahin et al 2010; 1403617	8 weeks old	Injection; 8 weeks	0, 2 mg/kg-day	Rat – Spregue Dawley	↓ SOD activity. No effect on TBARs, GSH, GSH-Px, CAT activities, or testosterone levels.
Krishnamoorthy et al 2007; 626036	90 days old	30 days	0, 2 mg/kg-day	Rat – Wistar	Epididymal sperm: ↑ levels of reactive oxygen species (HO-, H2O2, & lipid peroxides). ↓ levels of alpha tocopherol, GSH, and ascorbic acid. ↓ activity of SOD, CAT, GPx, GR, and GST in epididymal sperm
Selvakumar et al 2011; 759831					In prostate: ↑ lipid peroxidation & levels of H2O2. Effects were ameliorated by co-treatment with Vitamin E.
Murugesan et al 2005; 1416186					↑ Leydig cell lipid peroxidation, hydrogen peroxide, and hydroxyradical levels. ↓ Leydig cell superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase, glutathione reductase, gamma-glutamyl transpeptidase.
Murugesan et al 2005; 1417568					↑ testicular lipid peroxidation, hydrogen peroxide production, hydroxyl radical production. ↓ testicular Vit E and Vit C levels. ↓ testicular superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase, and glutathione reductase.
Murugesan et al 2005; 1417737					Isolated Leydig cells: ↑ lipid peroxidation, hydrogen peroxide and hydroxy radical production; ↓ superoxide dismutase, catalase, GPX, GR, GST.
Venkataraman et al 2004; 1418753					↓ prostate superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase. ↑ prostate lipid peroxidation, hydrogen peroxide production. No effect on prostatic acid phosphatase.
Senthil kuma et al 2004; 1418887					↑ Sertoli cell lipid peroxidation, hydrogen peroxide, and hydroxyradical levels. ↓ Sertoli cell lactate levels. ↓ Sertoli cell superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase, glutathione reductase, gamma-glutamyl transpeptidase.
Sridhar et al 2004; 2162224					↓ prostate superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase, prostatic acid phosphatase. ↑ prostate lipid peroxidation, hydrogen peroxide production.
Raj et al 2014; 2149829	Not reported	Injection; 24 hrs			↑ epididymis H2O2 production, lipid peroxidation (caput, corpus, cauda).

Proposed overview for Aroclor 1254 – induction of six key characteristics of male reproductive toxicants

[ ] number of studies informing each KC



Concluding remarks

- Approaches which facilitate the systematic evaluation of toxicological and mechanistic evidence can improve the transparency and strength of evidence analyses performed as part of a risk assessment.
- The key characteristics of male reproductive toxicants can provide a structure for systematically searching and organizing the relevant literature on mechanistic information in support of an evaluation of a chemical for reproductive toxicity.
- In combination with lifestage and causality applied to human and animal evidence the key characteristics of male reproductive toxicants can be evaluated to identify pathways/networks that are conserved across species and therefore relevant to human health.

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