

# **Critical Analysis of the Key Characteristics Approach Conceptual, Methodological, and Practical Limitations for Hazard Identification**

Carr J. Smith, Ph.D.

Scientific Integrity Webinar Series  
Endocrine Policy Forum, ISRTP, SOT ELFSI  
Specialty Section, SETAC Special Interest Group

# Purpose of this presentation

- KC approaches are widely promoted and already in regulatory use
- They have not been subjected to rigorous validation
- This talk synthesizes the first two webinars by topic
- **Focus:** conceptual failures, methodological pitfalls (KCC and KC-EDC), and examples of misapplications
- **Question:** Is the KC approach fit for purpose as a standalone hazard tool?

# What is the KC approach?

Premise: *Chemicals that cause a given adverse effect share “key characteristics” useful for hazard identification*

- Formulated by preselected like-minded experts meeting in workshops.
- Conclusions often labeled as “consensus.”
- Checklists of broad effects absent mechanistic considerations.
- First set: 10 key characteristics of carcinogens (KCCs; Smith MT, et al., 2016).
- Extended to endocrine disruptors (KC-EDCs; La Merrill et al., 2020) and many other endpoints.
- KC checklists are being proposed for additional types of toxicity.

## The two lists we focus on:

- KCCs: electrophilicity, genotoxicity, altered DNA repair, **epigenetic** change, **oxidative stress**, chronic inflammation, immunosuppression, receptor modulation, immortalization, altered **proliferation**/cell death.
- KC-EDCs: interacts with/activates hormone **receptors**, antagonizes **receptors**, alters **receptor** expression, alters signal transduction, **epigenetic** change in hormone cells; alters synthesis, transport, distribution, metabolism/clearance, or cell fate.
- Overlap (oxidative stress, receptor effects, proliferation, epigenetics) was later used to build umbrella KCs for other toxicities.
- No list specifies how many hits are required. Some papers say one “strong” hit can suffice, or that the full list need not be applied.

# Advantages Claimed vs. Advantages Realized

## Claimed:

- Based on mechanistic understanding ?
- Obviates the need to know the complete mode-of-action ?
- Superior to weight-of-evidence evaluations because simpler, faster, less expensive ?

## Question for the series:

Do KCs better protect and streamline public-health protections? Or does the KC checklist approach generate false-positives do to overly broad categorizations?

# Conceptual failure 1: no path to a negative

## “There is no there there.” *Gertrude Stein*

*A method that classifies toxicants must be able to evaluate some chemicals as not possessing the toxicity under consideration.*

A minimum number of characteristics are not required

Subjectively classified “Strong Evidence” for a single characteristic can be treated as sufficient

Absence of evidence is not exonerating so negative results are tentative

Transparent rules for assessing data quality or for resolving conflicts are lacking



**Result:** confirmatory method; incapable of falsifying premise of suspected toxicity

## Conceptual failure 2: Mechanism and “No Mechanism”

**“A thing cannot be and  
not be at the same time.”  
*Aristotle***

KC papers claim a foundation in known mechanisms and tout the advantage of not needing to know the mechanism.

This contradiction is incoherent for endocrine disruption.

- EDC definitions require an adverse effect *as a consequence* of an endocrine MoA . . . not merely an apical effect and a presumed MoA.

KCCs suffer similarly. Checklist hits are treated as mechanistic evidence. But events relevant to carcinogenesis are absent.

# Conceptual failure 3: “intrinsic” toxicity

Intrinsic properties belong to every molecule (molecular weight, composition).

- Toxicity is extrinsic: It depends on dose, species, life-stage, exposure route, duration, and kinetics.
- “Intrinsic hazard” elevates high-dose or saturating-condition effects to properties of the molecule.

Non-genotoxic carcinogens are a clear counter-example:

- Below the threshold for the key event, there is no carcinogenicity.

The same occurs for endocrine disruption:

- “*Potency Matters; Thresholds Govern Endocrine Activity.*”
  - *Borgert, Baker et al., 2013*

# Logical failure: induction and the absurd conclusion

**“For every complex problem there is an answer that is clear, simple, and wrong.” *H.L. Mencken***

KCAs attempt to superimpose results from agents already classified as toxic onto untested chemicals.

This invokes the Uniformity Principle and is circular reasoning (Hume; Popper).

- *Deductive reductio*: If multiple KC hits imply a carcinogen (or EDC), and essential molecules (ATP,  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ , water, linoleic acid) hit multiple KCs;
  - then essential molecules are carcinogens and EDCs. . .
- OR . . . Carcinogens and Endocrine Disruptors are essential for life.
  - Linoleic acid is required for membranes, yet it satisfies KCC and KC-EDC criteria at high concentrations.
  - A method that cannot exclude these molecules is nondiscriminatory and is not useful for hazard identification.

# Pseudoscientific aspects of the Key Characteristics Approach

Pseudoscientific methods lack falsifiability, even though they may sometimes yield correct results (Moberger, 2020).

## KCAs:

- rely on confirmation rather than severe attempts at falsification.
- lack internal procedures that would allow a negative result.
- treat non-specific cellular/molecular events as diagnostic.
- were not validated against negative examples, or even all positives.

Correct classifications reached by incorrect means do not rehabilitate the flawed methodology.

# KCC derivation and specificity

KCCs were excerpted from IARC Group 1 carcinogen data sets. Negative examples were not part of the derivation set.

Consequences (CJ Smith et al. 2021; related analyses):

- Many IARC-classified carcinogens possess none of the KCCs.
- Many EPA non-carcinogens possess several KCCs.

Guyton et al. (2018) have argued that a single KCC can sometimes indicate carcinogenic potential.

- This maximalist view of sufficiency converts the list into a checkbox.

# Empirical test: high-throughput data

## “Fate shuffles the cards and we play.” *Schopenhauer*

Becker et al. (2017):

- EPA-classified carcinogens and non-carcinogens.
- ToxCast/Tox21 assays mapped to 7 KCCs.
- Adjusted for cytotoxicity burst.
- Machine-learning evaluation of predictiveness.

Result: no better than chance.

- A tool that cannot beat a coin flip is not useful for prospective hazard identification.

# Cancer Treatment Decisions are Driven by Individual Patient Tumor Mutation Profiles – *Specificity not Generality*

| Cancer type                | Tumor Mutation Profile                                        |
|----------------------------|---------------------------------------------------------------|
| Non-small cell lung cancer | EGFR, ALK and ROS1 rearrangements, BRAF, MET, RET, KRAS, HER2 |
| Breast cancer              | HER2 amplification, PIK3CA, BRCA1, BRCA2                      |
| Colorectal cancer          | KRAS, NRAS, BRAF (V600E), MSI-H/dMMR                          |
| Prostate cancer            | BRCA1, BRCA2, ATM, AR-V7 variant                              |
| Melanoma                   | BRAF, KIT                                                     |
| Blood cancers              | BCR-ABL fusion gene, FLT3, IDH1, IDH2                         |
| Thyroid cancers            | BRAF, RET, NTRK                                               |
| Pancreatic cancer          | BRCA1, BRCA2, RAS                                             |
| Ovarian cancer             | BRCA1, BRCA2, homologous recombination deficiency (HRD)       |

**Biomarker relevance varies by tumor subtype, assay, and clinical context.**

# Oxidative stress is the weakest “Key” Characteristic

- Not cancer-specific; a key event in many non-cancer pathways
- Produced by essential nutrients at high concentration
- Often participatory or secondary, not causal
- Paraquat and diquat: strong redox cyclers, not carcinogens
- Many cited assays [certain Comet protocols, antibody 8-Oxo-2'-deoxyguanosine (8-oxodG)] fail community quality control criteria

*Counting papers reporting oxidative-stress does not support a determination of carcinogenic potential.*

# KC-EDC construction

## Endocrine pharmacology requires:

|                                                    |                                                     |                             |                       |               |                                                      |
|----------------------------------------------------|-----------------------------------------------------|-----------------------------|-----------------------|---------------|------------------------------------------------------|
| potency<br>relative to the<br>endogenous<br>ligand | receptor<br>occupancy and<br>laws of mass<br>action | feedback and<br>homeostasis | life-stage<br>windows | reversibility | distinction<br>between<br>activity and<br>disruption |
|----------------------------------------------------|-----------------------------------------------------|-----------------------------|-----------------------|---------------|------------------------------------------------------|



High-concentration *in vitro* binding, transcriptional activation, or cytotoxicity-confounded responses do not establish endocrine disruption.

# What KC-EDCs omit, ... but Endocrine pharmacology requires:

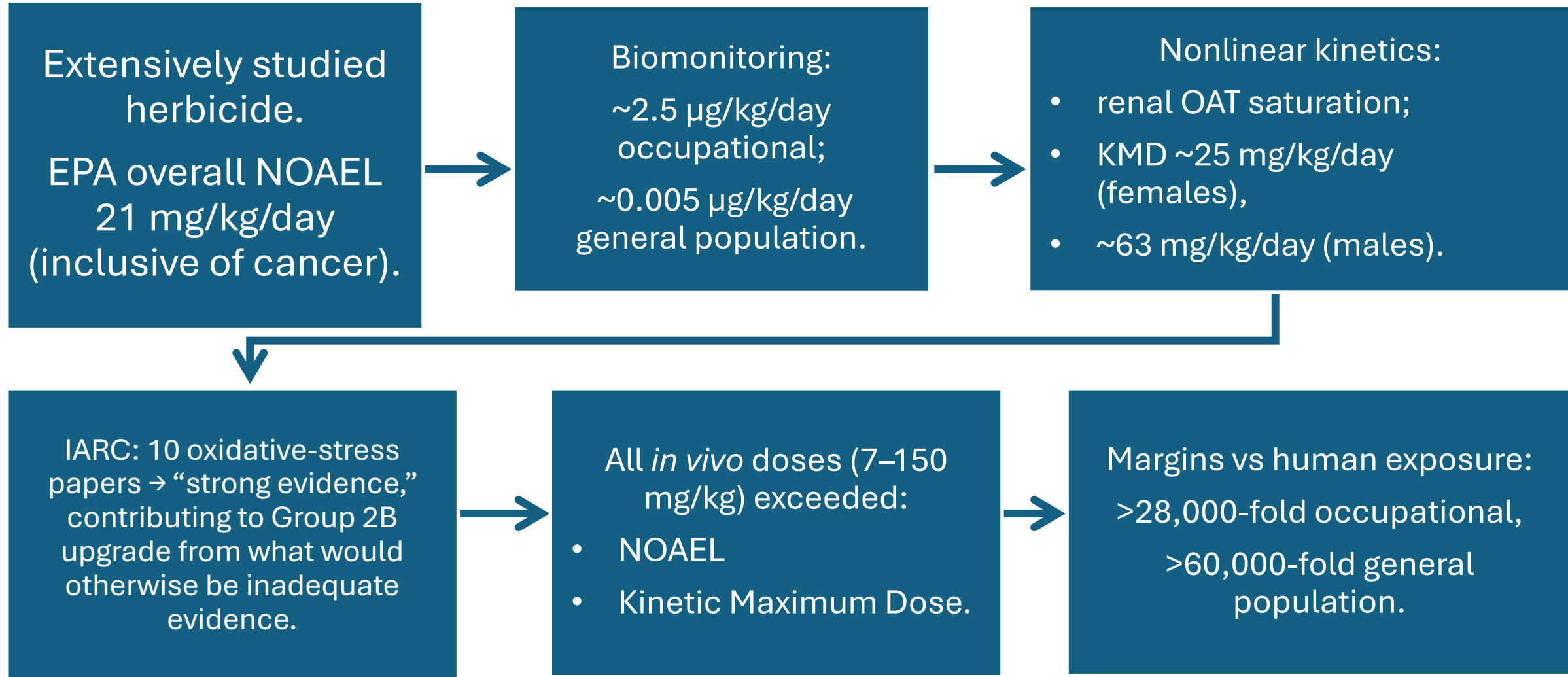
- Potency relative to the endogenous ligand
- Receptor occupancy and laws of mass action
- Feedback and homeostasis

*High-concentration in vitro binding, transcriptional activation, or cytotoxicity-confounded responses do not establish endocrine disruption.*

- Life-stage windows
- Reversibility
- Distinction between activity and disruption
- Must link adverse effect to endocrine mechanism

*KC-EDCs cannot separate endocrine-mediated from non-endocrine mechanisms (proliferation, epigenetics, oxidative stress).*

# Case: 2,4-D and the oxidative-stress bin



## 2,4-D: what data integration would have shown

- 3 of 7 *in vivo* papers used a Tunisian formulation later identified as the 2-butoxyethyl ester.
- Liberated 2-butoxyacetic acid causes rodent RBC hemolysis; human RBCs are not susceptible.
- Hemolysis is not an apical effect of 2,4-D acid.
- *In vitro* concentrations (200–500 µg/mL) exceed saturated-kinetic plasma levels.
- One yeast study at low pH promotes ion trapping.

**Smith MT, et al. (2016):**

**KC bins should be used to form hypotheses; hypotheses should be tested for dose–relevance and species–relevance.**

**KC approach omitted this critical step. Paper counting replaced integration.**

# Case: glyphosate genotoxicity and oxidative stress

IARC Monograph 112: “strong evidence” of genotoxicity and oxidative stress, used in a 2A classification.

Genotoxicity claim relied on public literature, often formulations, often single high doses.

- A cited human micronucleus study: authors stated that causality could not be assigned, response lacked strength and dose concordance, recovery was inconsistent, and sample/exposure data precluded conclusions.

ECHA (2016, 2022): GLP OECD studies “reliable without restriction” and negative; public literature lower tier.

- Conclusion: not genotoxic, not carcinogenic.

IARC had access to Tier 2 summaries of the guideline studies and set them aside.

# Case: glyphosate as an alleged EDC

EPA (2015) and EFSA (2017) weight-of-evidence, including validated EDSP Tier 1 and apical guideline studies: no convincing evidence of estrogen, androgen, or thyroid interaction.

Muñoz et al. (2020) using KC-EDCs: 8 of 10 characteristics met.

Problems in the supporting set:

- many studies used formulations, not glyphosate
- surfactants alone reproduce the cytotoxicity
- concentrations orders of magnitude above human blood levels
- internal contradictions (including concerns of estrogenic contamination)
- omission of the EPA/EFSA data sets
- no systematic quality or relevance filter

Later KCC re-evaluations that upgraded endocrine evidence cited this literature; some authors were plaintiff experts in litigations but no conflict of interest disclosed.

# Essential and beneficial molecules as false positives

Linoleic acid: oxidative stress, PPAR activation, ER-related proliferation signals, vaginal cornification — multiple KCC and KC-EDC hits — yet essential, and conjugated forms show protective effects.

Genistein: ER- $\alpha$  agonist, positive ERTA and uterotrophic assays; high-dose human menopausal study shows no proliferative endometrial effect; dietary staple for large populations.

Statins: 7 of 10 KCCs, carcinogenic in rodents at high doses, yet reduce cardiovascular death in millions of patients.

Phenobarbital (CAR) and PPAR- $\alpha$  agonists: multiple KCC hits; rodent liver tumors; human clinical and MOA data show no relevant cancer risk. Thresholds and reversibility are demonstrable and invisible to the checklist.

# Pattern across the misapplications

## Redundant errors include:

- Bin or count papers instead of testing a hypothesis
- Ignore dose, kinetics, and human exposure
- Treat formulations as if they were the active ingredient
- Omit higher-quality guideline and regulatory data
- Treat non-specific events (oxidative stress, proliferation, receptor binding) as diagnostic
- No rule that would have excluded a negative

When context is considered, the “strong evidence” claim is not supported.

# Why regulators still encounter KC outputs

IARC preamble evaluates hazard and does not integrate exposure.

- Hazard cannot be identified in the abstract; dose and conditions are part of the evidence
- Proposition 65 and similar statutes auto-adopt IARC classifications
- Delaney Clause still constrains some food-additive decisions
- Resource limits push agencies toward external lists
- Legislating a method ossifies outdated practices and protocols. Science changes; statutory methods do not.

# What limited use, if any, remains for KCAs?

Originally stated intent: organize mechanistic literature.

- Organizational objective fails if bins are counted rather than integrated conceptually.
- Following legitimate hypothesis construction, KCAs might assist broad literature searches and data gap identification.
- KCAs are not sufficient nor necessary for classification.
- Best employment is as optional key events inside an existing MOA / Adverse Outcome Pathway / Weight-of-Evidence analysis (IPCS, modified Bradford-Hill; Becker et al., 2025 archetypal MOA system).

# Requirements for any replacement

- Deductive, hypothesis-driven, aimed at potential for falsification
- Explicit data-quality and relevance criteria
- Ability to reach a negative conclusion
- Mandatory dose, toxicokinetic, and exposure context
- Temporal concordance and multi-stage biology
- Species and human relevance
- Prospective test against both known positives and known negatives

**Existing WoE and MOA frameworks  
already have these features.  
*KCAs do not.***

# Conclusions

1. KC approaches are not fit for purpose as standalone hazard tools.
2. They are confirmatory, non-specific, unvalidated against negatives, and prone to false positives when dose and kinetics are ignored.
3. 2,4-D, glyphosate, linoleic acid, genistein, statins, phenobarbital, and PPAR- $\alpha$  activators illustrate the same structural failure.
4. Scientific integrity requires reform under deductive standards or replacement by MOA and weight-of-evidence methods that can separate signal from noise at real-world exposures.

## Selected sources

- Becker et al., 2017, Regul Toxicol Pharmacol
- Borgert, 2023, Arch Toxicol
- La Merrill et al., 2020, Nat Rev Endocrinol
- Smith MT, et al., 2016, Environ Health Perspect
- Smith CJ, et al., 2021, Arch Toxicol
- McCarty, Borgert, Burgoon, 2020, Environ Toxicol Chem
- EPA and EFSA glyphosate endocrine WoE conclusions;
- ECHA RAC genotoxicity conclusions

# Discussion

## *Priority for challenging questions:*

- Where, if anywhere, is a KC checklist valid without MOA integration?
- What validation against negatives would be required before regulatory use?
- How should dose and exposure be mandated rather than optional?