INVESTIGATING DNA ADDUCT FORMATION BY FLAVOR CHEMICALS AND TOBACCO BYPRODUCT IN E-CIGARETTES USING IN SILICO APPROACHES

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Disclaimer: This is not a formal dissemination of information by FDA and does not represent Agency position or policy.

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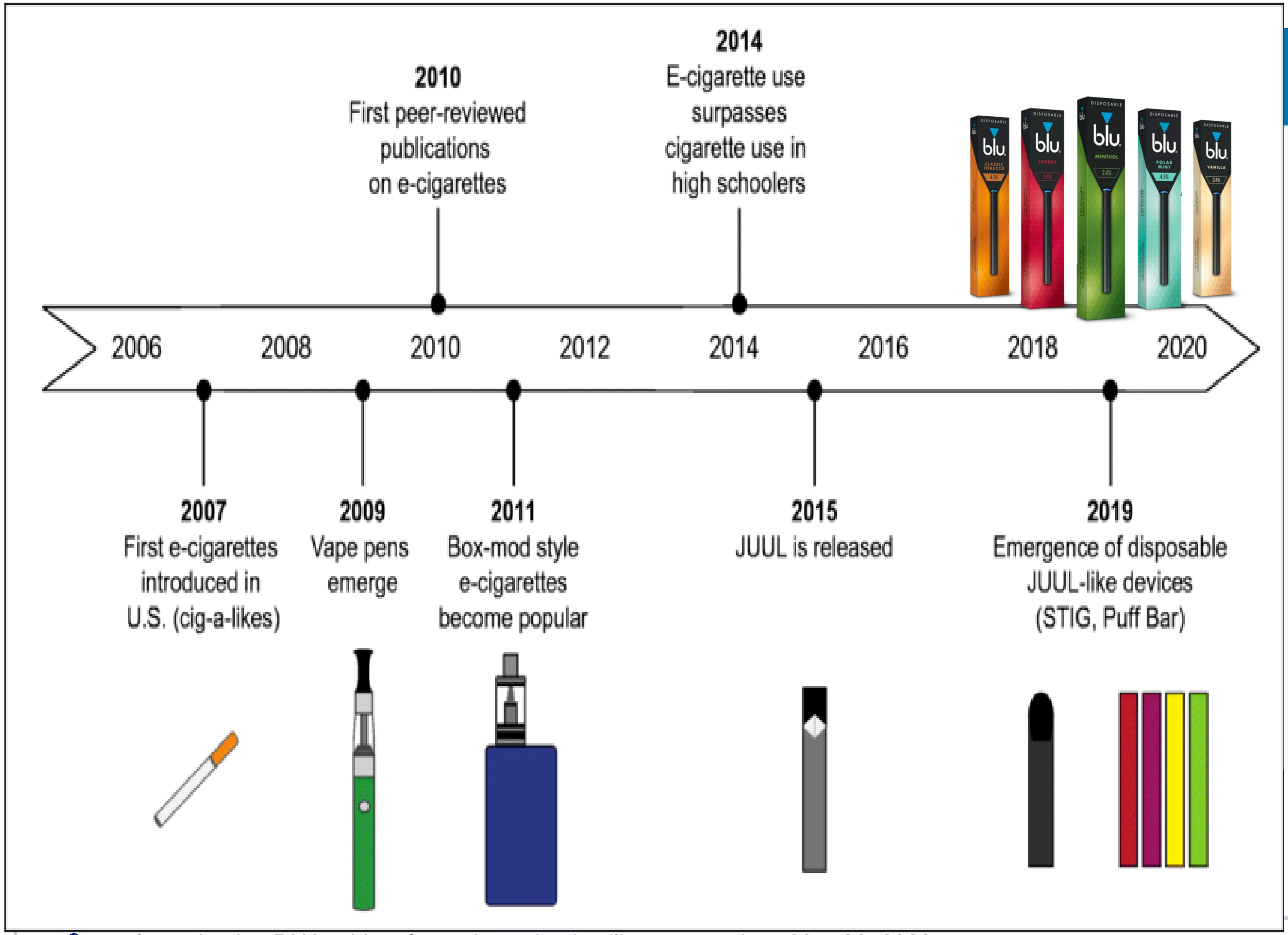
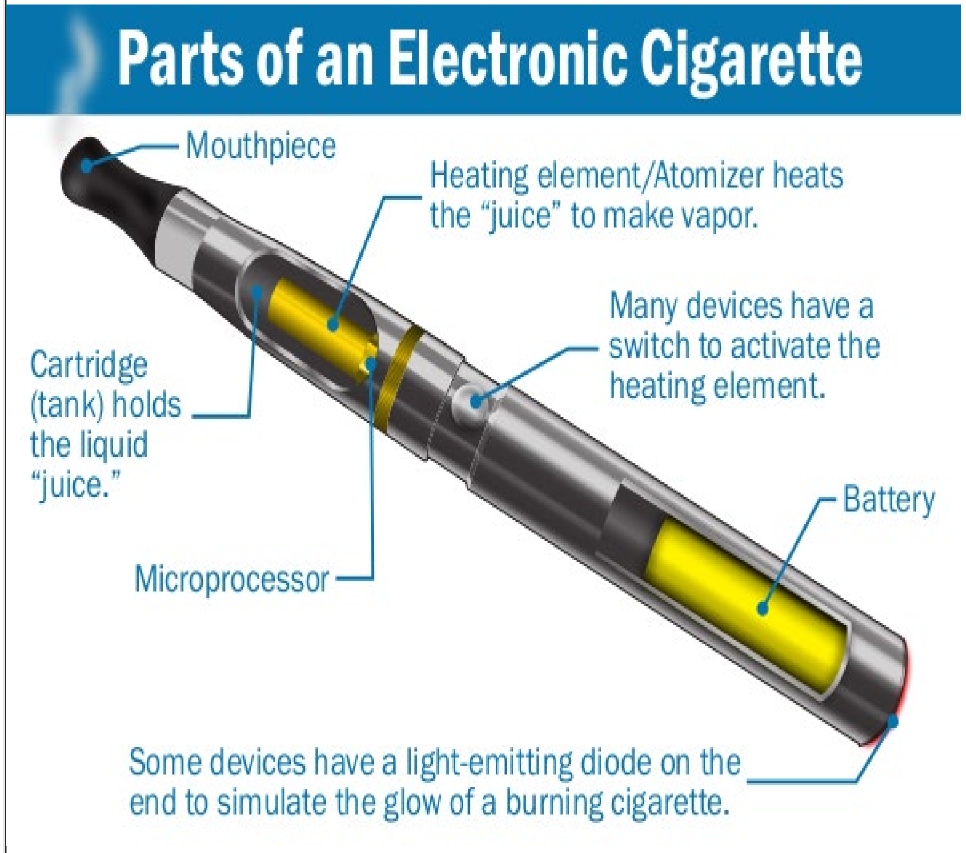
DISCLAIMERS

This presentation is not a formal dissemination of information by Center for Tobacco Products (CTP), FDA, and does not represent Agency position or policy

ANATOMY OF E-CIGARETTES





BACKGROUND

- E-cigarettes surging popularity – e.g., sleek designs, marketing, social media, and ***Flavors*** (80% youth e-cig users, National Youth Tobacco Survey, 2020)
- Flavors banned in refillable e-cigs in January 2020, but available in disposable e-cigs.
- E-liquid can react during mixing and storage at room temp. (Erythropel et al., 2018).
- Efficient transfer of flavor chemicals (e.g., cinnamaldehyde) from e-liquid into aerosol (Noel et al., 2018)
- Thermal degradation of e-liquid: propylene glycol (PG), vegetable glycerin (VG), and flavors are shown to generate acrolein, methylglyoxal, etc.

BACKGROUND – DNA ADDUCTS

- Some flavor chemicals and tobacco byproducts are found to induce DNA adducts: estragole, eugenol, cinnamaldehyde, methylglyoxal, etc. *(Zhou et al., 2007; Sakano et al., 2004; Kiwamoto et al., 2016; Frischmann et al., 2005)*
- Formed from electrophilic chemicals or chemicals that can be metabolic activated to become electrophiles, which then covalently bind to DNA or/and proteins
- DNA adducts → DNA damage → Carcinogenesis?
- DNA adducts levels are associated with cancer risk (*Poirier, 2016; Hemminki et al., 2000*)

IARC MONOGRAPH
TEN KEY CHARACTERISTICS OF CARCINOGENS

FDA

A Section 508–conformant HTML version of this article
is available at http://dx.doi.org/10.1289/ehp.1509912.

Review

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

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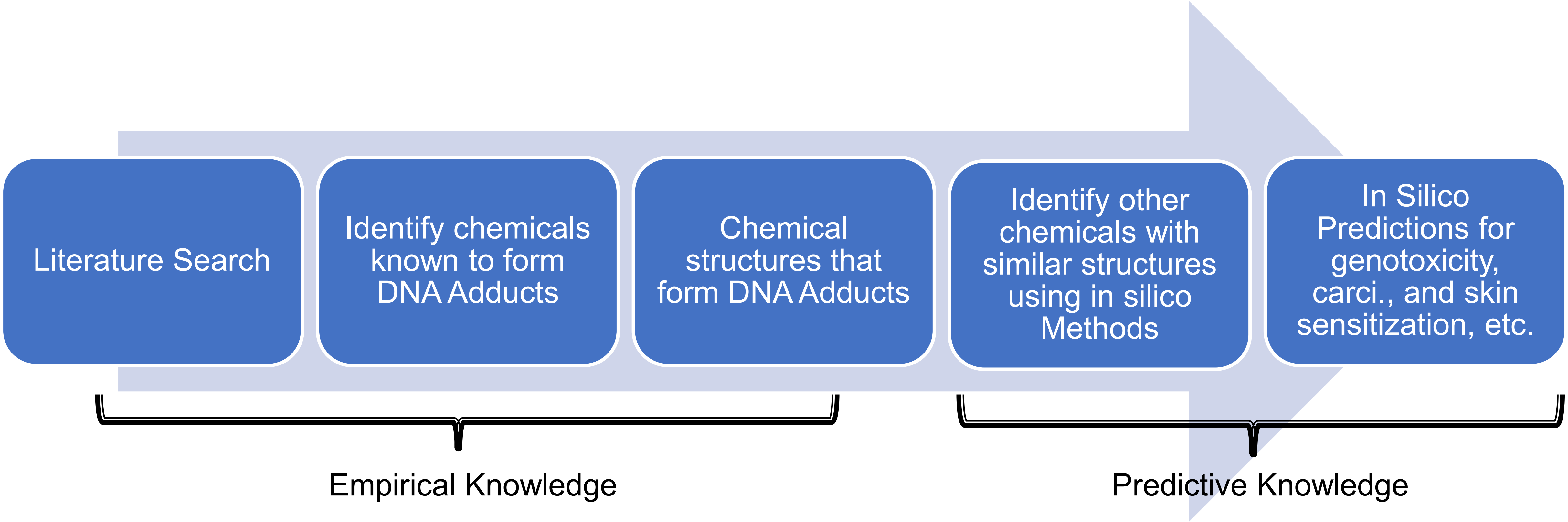
Key characteristics of carcinogens.

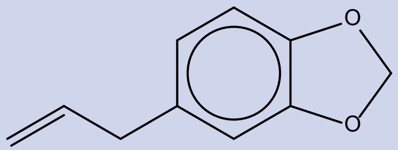
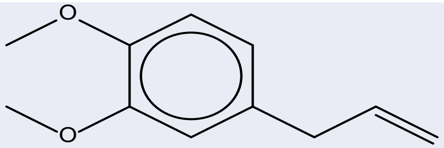
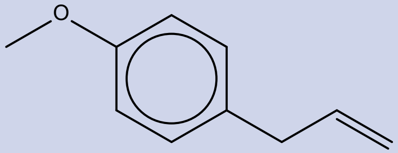
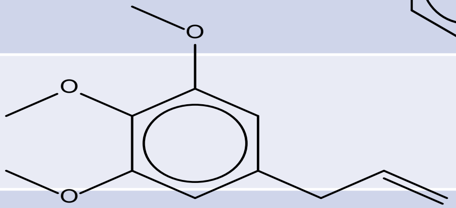
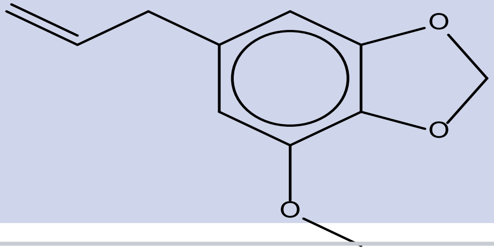
Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)

METHODS


Literature Search
Identify chemicals known to form DNA Adducts
Chemical structures that form DNA Adducts
Identify other chemicals with similar structures using in silico Methods
In Silico Predictions for genotoxicity, carci., and skin sensitization, etc.
Empirical Knowledge
Predictive Knowledge

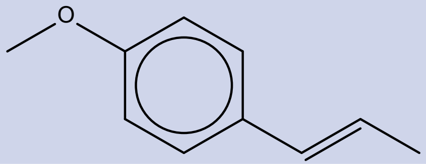
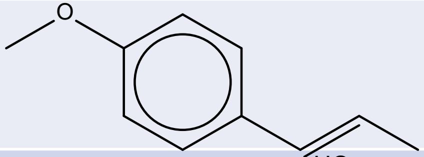
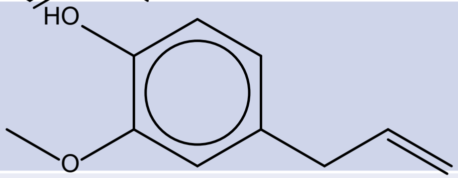
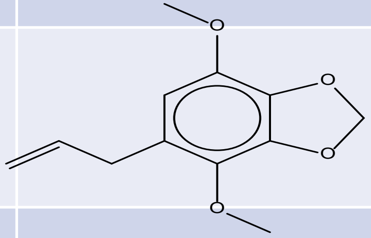
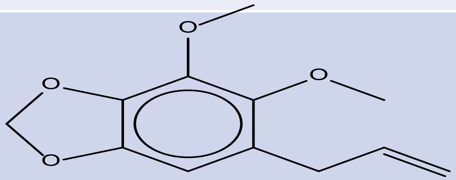
ALKENYLBENZENES

Kang & Valerio, 2020

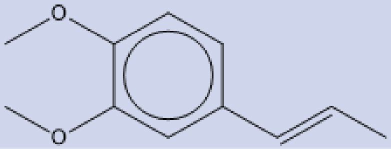
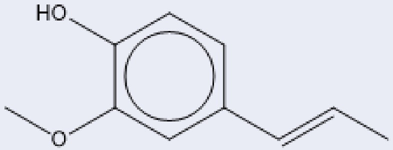
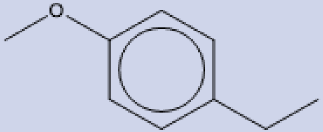
Chemical Name	CASRN	DNA Adduct Citations	Structures	Similarity Metric to Safrole
Safrole	**94-59-7**	**Munerato et al., 2005; Zhou et al., 2007; Kobet et al., 2016**		**1.0000**
Methyleugenol	**93-15-2**	**Kobet et al., 2016; Tremmel 2017; Kobet et al., 2018**		**0.9067**
Estragole	**140-67-0**	**Zhou et al., 2007; Kobet et al., 2016; Schulte-Hubbert et al., 2019**		**0.7886**
Elemicin	**487-11-6**	**Phillips et al., 1984; Kobet et al., 2016**		**0.7809**
Myristicin	**607-91-0**	**Phillips et al., 1984; Randerath et al., 1993; Zhou et al., 2007; Kobet et al., 2016**		**0.8316**

ALKENYLBENZENES

Kang & Valerio, 2020

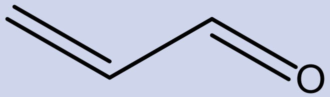
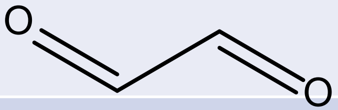
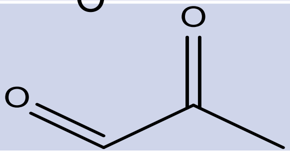
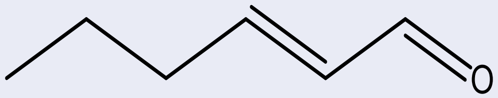
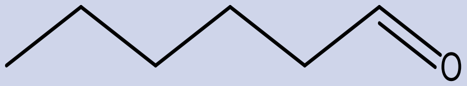
Chemical Name	CASRN	DNA Adduct Citations	Structures	Similarity Metric to Safrole
trans-Anethole (Anise camphor)	4180-23-8	Kobet et al., 2016; Fuhbrueck et al., 2018		0.6614
cis-Anethole	25679-28-1	Fuhbrueck et al., 2018		0.6614
Eugenol	97-53-0	Sakano et al., 2004; Munerato et al., 2005		0.8790
Apiol	523-80-8	Phillips et al., 1984; Zhou et al., 2007		0.7185
Dillapiole	8025-95-4 (484-31-1)	Phillips et al., 1984; Zhou et al., 2007		0.7610

FLAVOR COMPOUNDS WITH SIMILAR STRUCTURES TO SAFROLE [1]

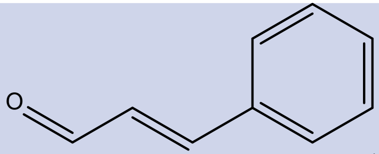
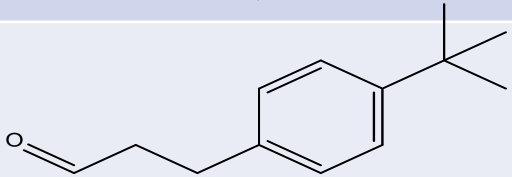
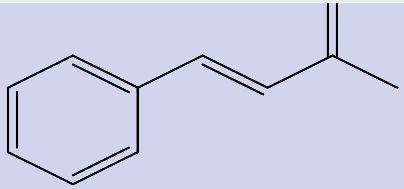
Chemical Name	CASRN	DNA Adduct Citations	Structures	Similarity Metric to Safrole
Methyl-isoeugenol	93-16-3	NA		0.789505
Isoeugenol	97-54-1	NA		0.759281
4-ethylanisole (1-ethyl-4-methoxybenzene)	1515-95-3	NA		0.720409

[1] Identified by Vitic V3.0 and ChemTunes V1.2

TOBACCO OR/AND FLAVOR-RELATED ALDEHYDES

Chemical Name	CASRN	DNA Adduct Citations	Structures	Similarity Matrix to Acrolein
Acrolein (2-propenal)	107-02-8	Lee et al., 2015; Kiwamoto et al., 2015		1.0000
Glyoxal	107-22-2	Vilanova et al. 2017		0.9786
Methylglyoxal; Pyruvaldehyde	78-98-8	Frischmann et al., 2005		0.9120
trans-2-hexenal (HEX); Hex-2(trans)-enal	6728-26-3	Schuler and Eder 1999; Stout et al., 2008		0.9050
Hexanal (Caproic aldehyde)	66-25-1	Gölzer et al., 1996;		0.8757

TOBACCO OR/AND FLAVOR RELATED ALDEHYDES

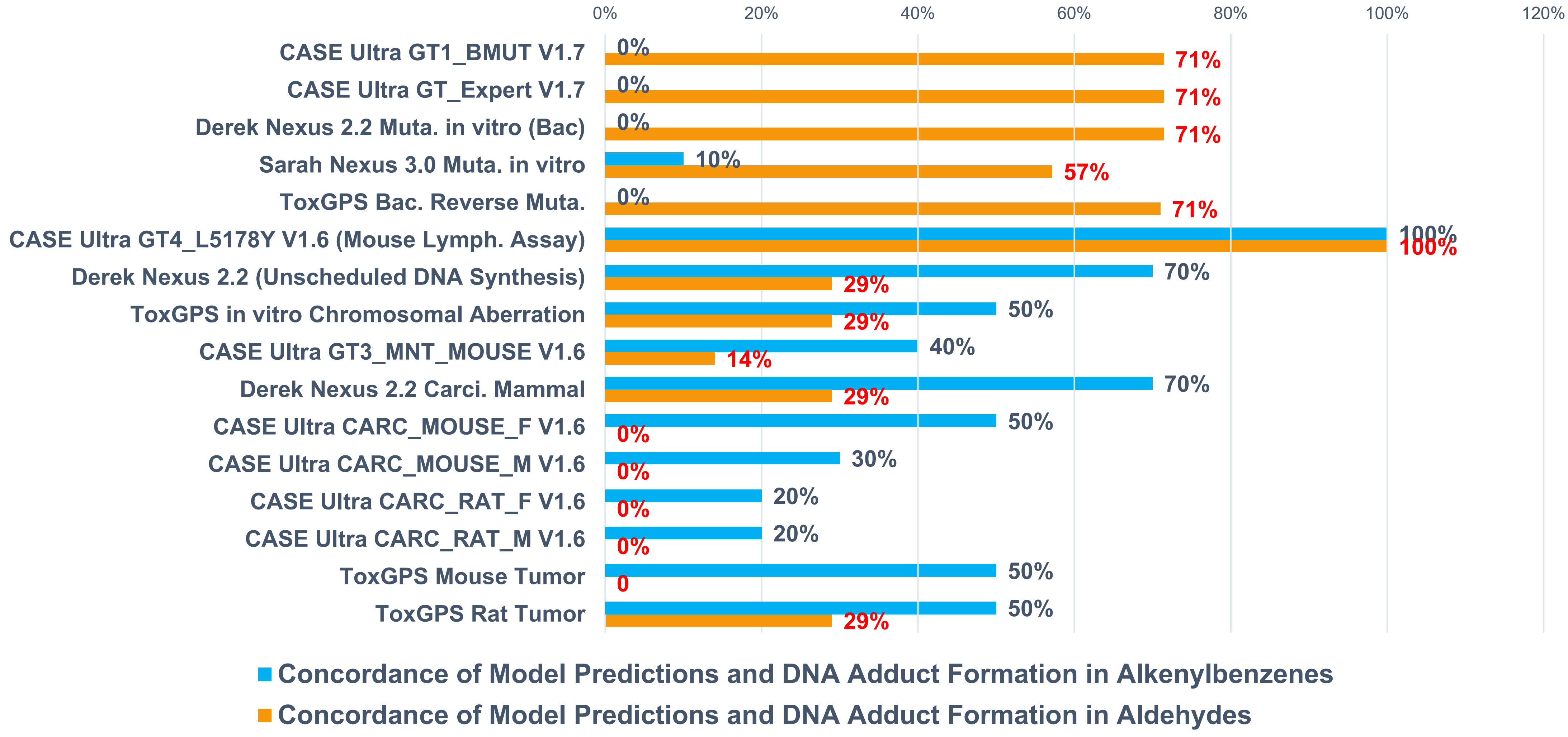
Chemical Name	CASRN	DNA Adduct Citations	Structures	Similarity Matrix to Acrolein
trans-Cinnamaldehyde; (2E)-3-phenylprop-2-enal	14371-10-9	Kiwamoto et al., 2016		0.8235
Bourgeonal; 3-(4-tert-butylphenyl) propanal (BDHCA)	18127-01-0	Kobet et al., 2018		0.7099
Flavor Compounds with Similar Structures to Acrolein				
4-methylcinnamaldehyde				
Benzalacetone (Benzylideneacetone)	122-57-6	N/A		0.6948

IN SILICO TOXICOLOGY SOFTWARE/MODELS AND ENDPOINTS

FDA

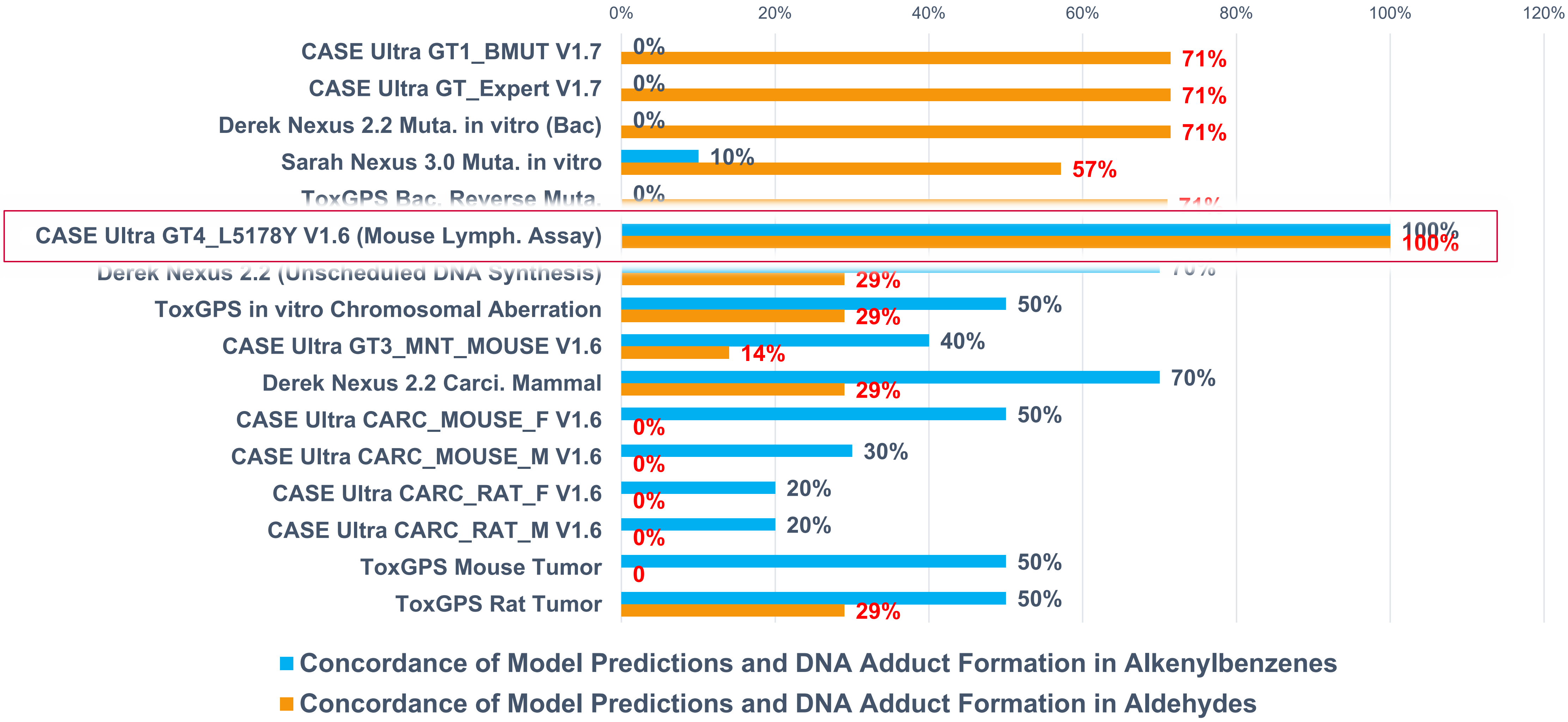
Toxicity Endpoints	In silico Software
Bacterial mutagenicity (AMES)	CaseUltra, Sarah Nexus, Derek Nexus, ChemTunes-ToxGPS
Mammalian mutagenicity	CaseUltra
Chromosome damage (mammal)	Derek Nexus, CaseUltra
Micronuclei formation	CaseUltra, Chem-Tune - ToxGPS
Unscheduled DNA synthesis in vitro (mammal)	Derek Nexus
Rodent carcinogenicity	CaseUltra, Derek Nexus, ToxGPS
Skin Sensitization	CaseUltra, Derek Nexus, ToxGPS

CONCORDANCE BETWEEN DNA ADDUCT FORMATION AND VARIOUS IN SILICO TOX PREDICTIONS



CONCORDANCE BETWEEN DNA ADDUCT FORMATION AND VARIOUS IN SILICO TOX PREDICTIONS

FDA

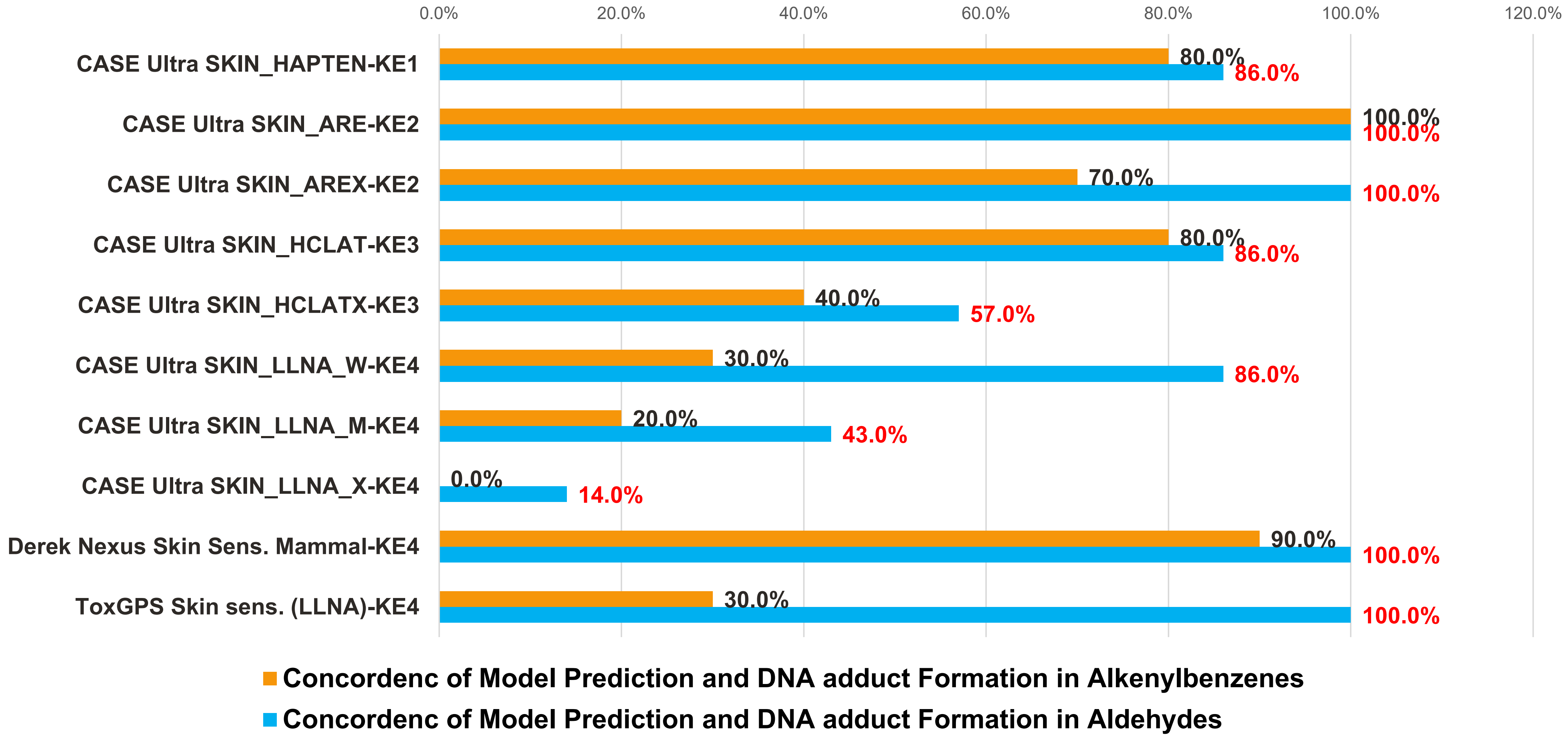


MAJOR SIMILARITIES – HIGHLY CONCORDANT MODELS

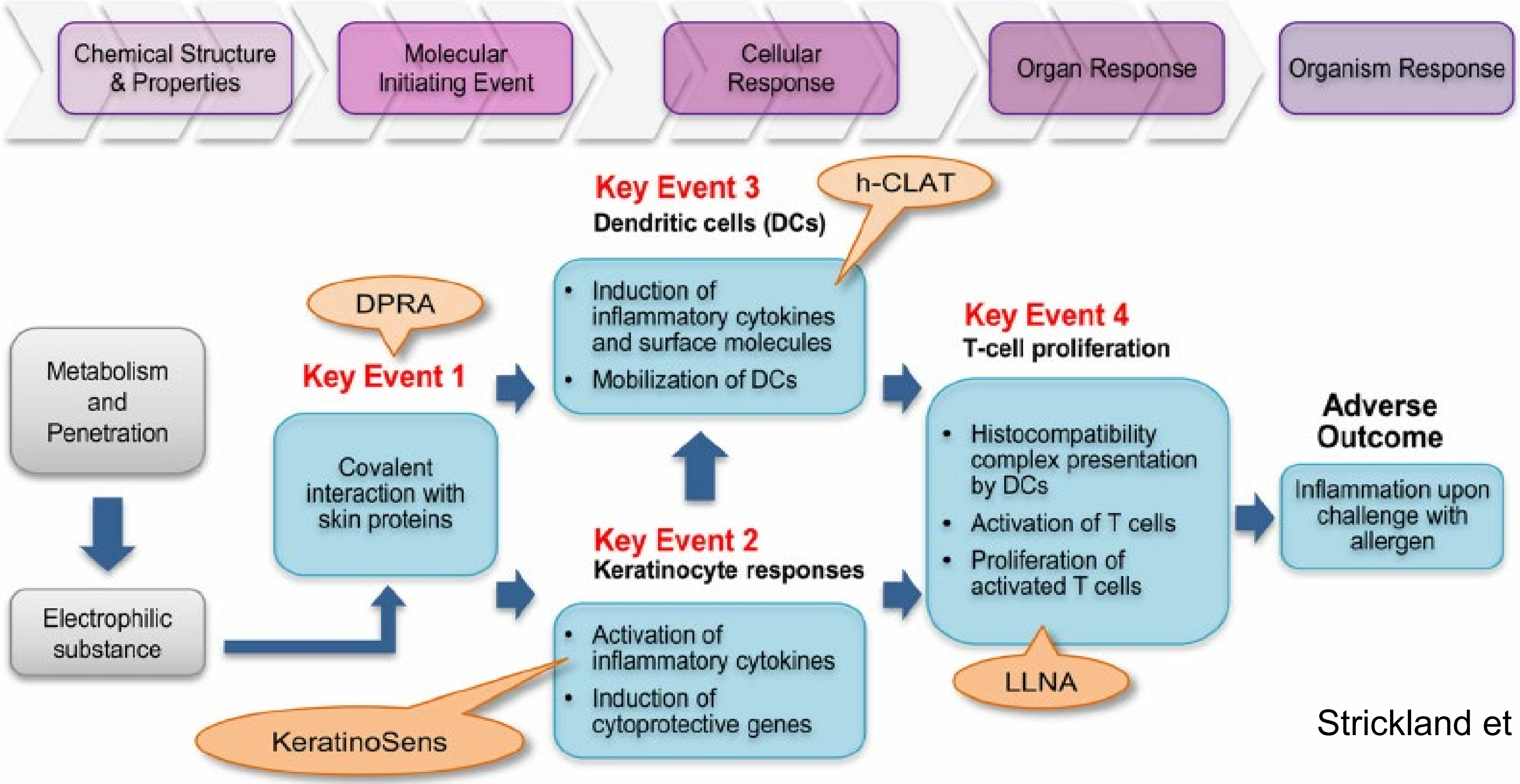
L5178Y (in vitro mouse lymphoma assays, CASEUltra)

- 100% concordance with DNA adduct formation in both chemical classes
- L5178Y – widely used for regulatory genotoxicity test
  - ICH S2B guidelines (Muller et al., 1999; ICH, 1997)
  - OECD – for testing of chemical. TG 490 (OECD, 2016)
  - Detects thymidine kinase (tk) locus of L5178Y cells (tk +/- → tk -/-) including point mutation, deletion, chromosomal rearrangements, and translocation, etc.
  - Signal events that are resulted from DNA damage caused by DNA adduct formation

CONCORDANCE BETWEEN DNA ADDUCT FORMATION AND SKIN SENSITIZATION PREDICTIONS



ADVERSE OUTCOME PATHWAY (AOP) SKIN SENSITIZATION



Strickland et al., 2019

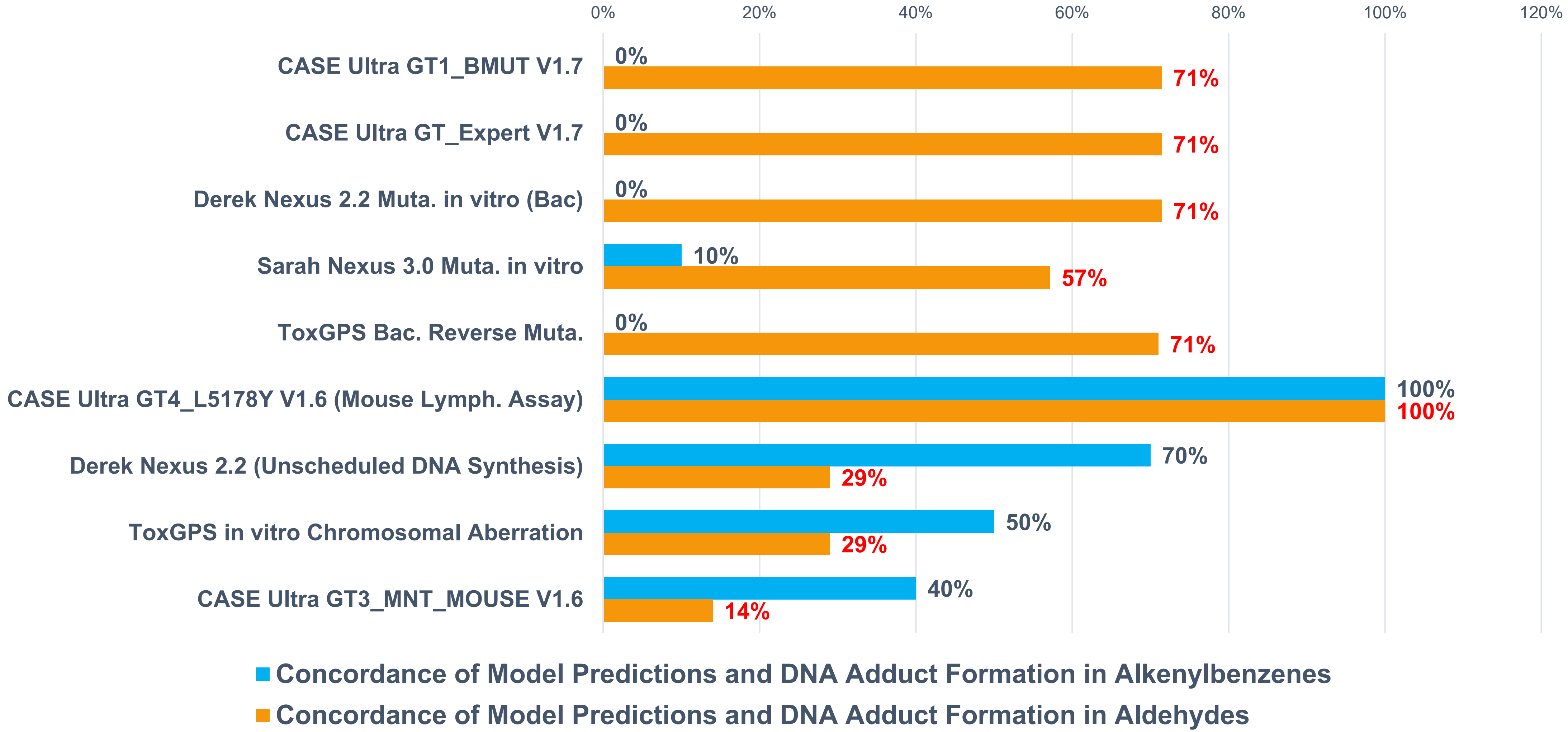
MAJOR SIMILARITIES – HIGHLY CONCORDANT MODELS

FDA

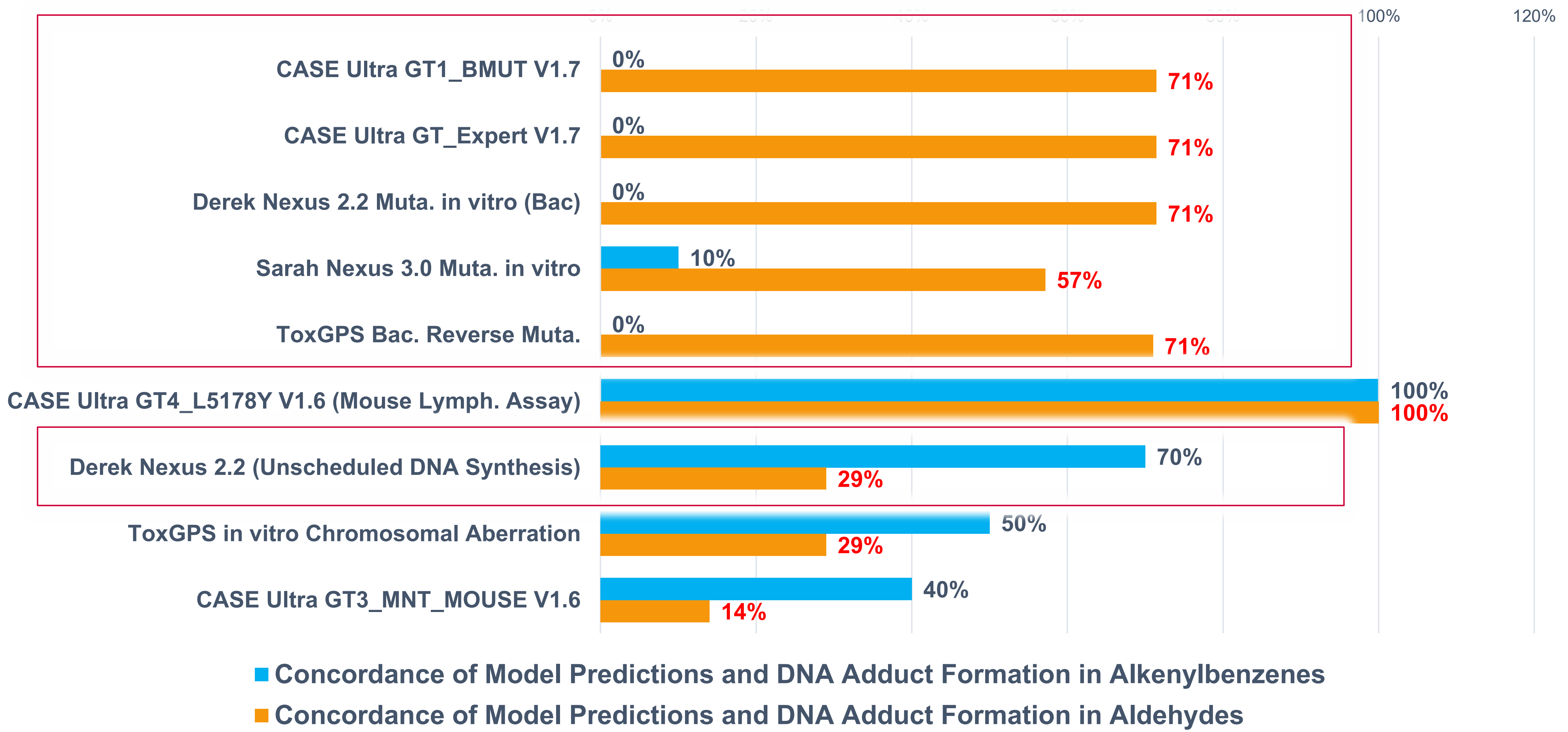
Skin Sensitization

- Both chemical classes produce high concordance with predictions for skin sensitization, with 90-100% in several models
- Corresponding to 4 key events (KEs) in skin sensitization Adverse Outcome Pathways (AOP)
- Aldehydes are known skin sensitizers, e.g., cinnamaldehyde
- Very few alkenylbenzenes are confirmed skin sensitizers, e.g., isoeugenol
- MOA – ***Electrophilic Reactive intermediates*** that can form ***covalent bonds*** with proteins (skin sensitization) and DNA (DNA adducts)

MAJOR DIFFERENCES IN PREDICTION ENDPOINTS



MAJOR DIFFERENCES IN PREDICTION ENDPOINTS



MAJOR DIFFERENCES BETWEEN TWO CHEMICAL CLASSES

Bacterial Mutagenicity

- CaseUltra, ToxGPS, Derek and Sarah Nexus
- **Alkenylbenzenes**
  - Mostly negative
  - ***Require bioactivation*** to form electrophilic intermediates
  - Bacterial assays (AMES) may not contain necessary enzymes for biotransformation
- **Aldehydes**
  - High rates of positive results
  - ***Direct acting*** – do not require bioactivation

MAJOR DIFFERENCES BETWEEN TWO CHEMICAL CLASSES

Non-specific genotoxicity - Derek Nexus

- ***Unscheduled DNA Synthesis (UDS)***
  - Alkenylbenzenes (70%) vs. Aldehydes (29%)

Aldehydes

- Inhibit DNA repairs, including nucleotide excision repair (NER), base excision repair (BER), and mismatch repair proteins
- Bind to DNA repair protein and lead to protein degradation
- Multi-faceted reactions of aldehyde toward both DNA and proteins lead to increased chemical insults to normal cellular functions

OVERALL SUMMARY AND CONCLUSION

FDA

- Identify *in vitro mouse lymphoma assays (L5178Y) that may correlate with DNA adduct formation*
- Identify areas that have increased confidence by comparing multiple prediction software and endpoints to *in vitro* and *in vivo* data
  - Bacterial mutagenicity – sensitivity, specific, and consensus in multiple software, ToxGPS, Derek Nexus, CaseUltra
  - Skin sensitization – widely available and validated
- Proof of concept – in silico tox can be utilized in tobacco product ingredient research to increase knowledge of potential toxicity and assist in prioritizing additional analysis and testing

STUDY LIMITATION

- Small number of chemicals studied
- In silico limitations: model transparency, data quality is critical for generating good predictions
- Biological relevance and implications for DNA adduct formation?
  - Hard to capture downstream carcinogenicity effects
  - DNA repair, oxidative stress, chronic inflammation, etc. (Smith et al., 2016)
  - Polymorphism in many xenobiotic-metabolizing enzymes, DNA repair, etc.
- In silico software used in this study does not factor exposure or specific exposure regarding inhalation route

STUDY STRENGTH – OVERALL

- Moving toward 3 Rs – Reduce, Refine, and Replace animal testing
  - FDA Predictive Toxicology Roadmap (FDA 2017)
- Highlight and recapitulate mechanistic aspects of chemical safety assessment
- Highlight the importance of looking beyond bacterial mutagenicity assay, or other genotoxicity assays (Ames, Chromosomal aberration, Micronucleus, etc.)
  - Lack of human metabolic enzymes that are present in vitro (even with S9)

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- Nabanita Nag
- Sang Ki Park
- John Knoblach

Questions and Comments?

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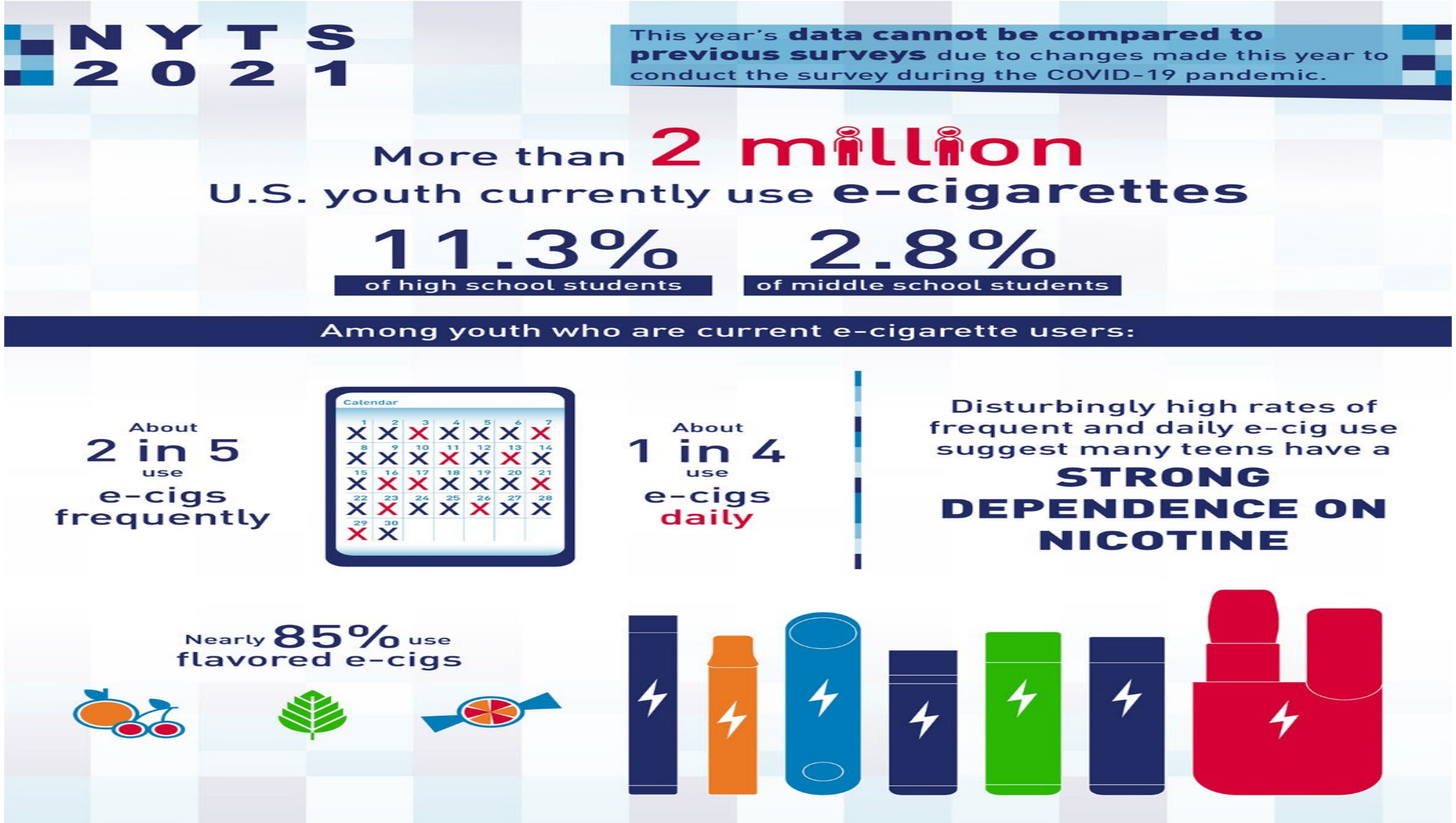
EXTRA SLIDES

FDA

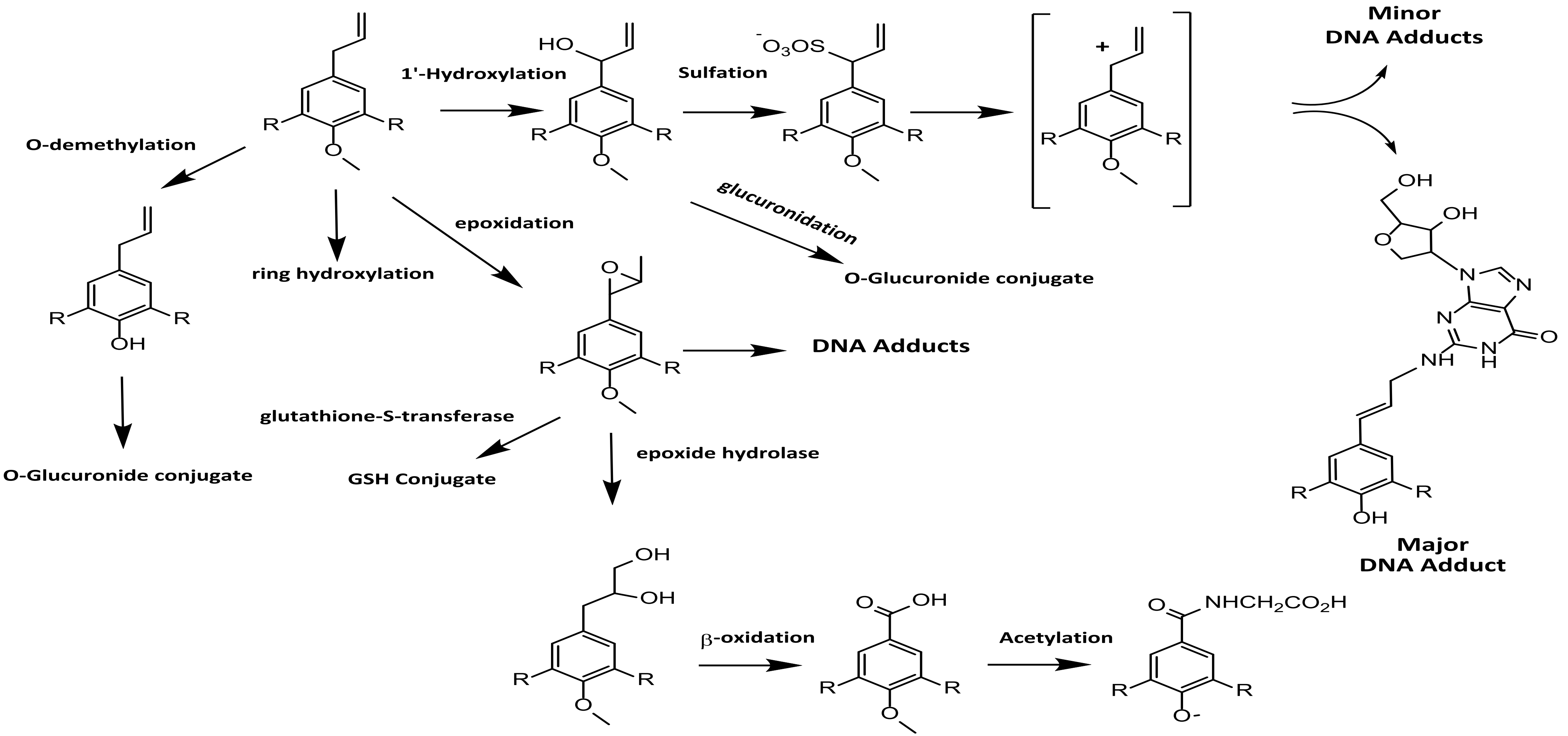
POSSIBLE FUTURE STUDIES

- Using L5178Y assays to screen chemicals??
- Screen tobacco ingredients (e.g., flavors) for DNA adduct formation
  - Structure similarity?
  - Metabolites?
  - Thermal degradation products?

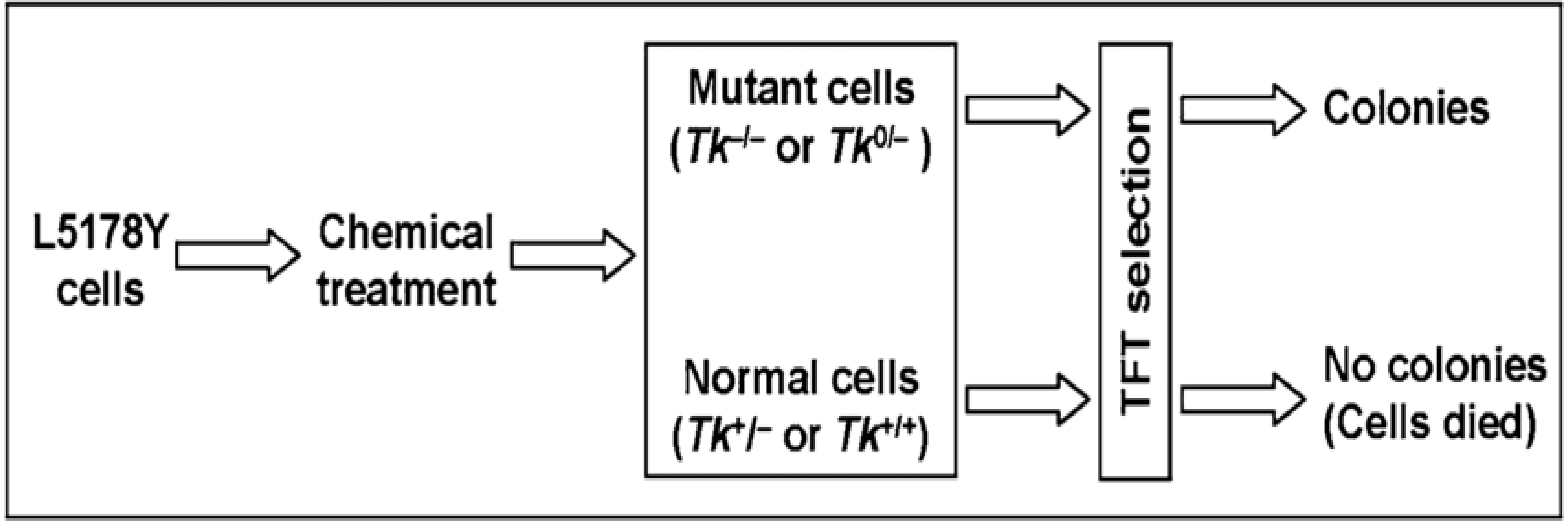
E-CIGARETTES



ALKENYLBENZENES METABOLISMS AND DNA ADDUCT FORMATION



L5179Y MOUSE LYMPHOMA ASSAY (MLA)

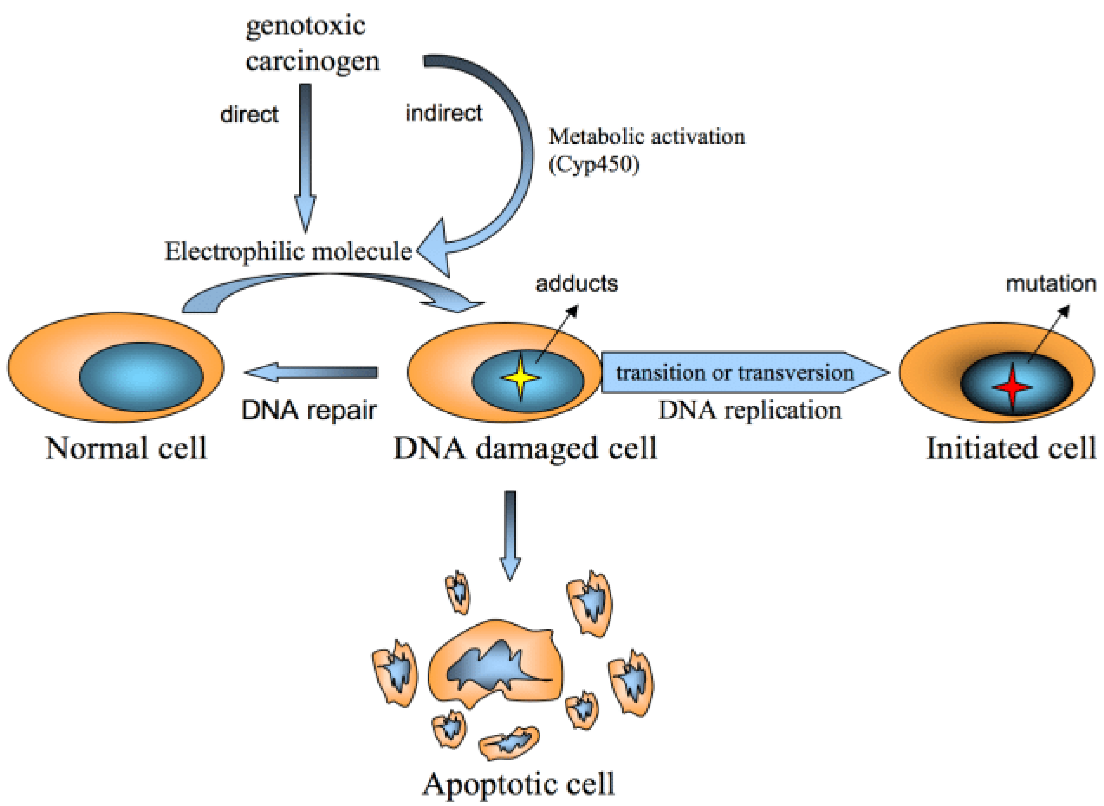


WHAT ARE THESE CHEMICALS

Alkenylbenzene

- Found in herb, e.g., basil, nutmeg, dills, parsley, etc.

DNA ADDUCT AND CARCINOGENESIS



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