Update on CFSAN Toxicology Activities

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CFSAN/FDA
NCTR/SAB
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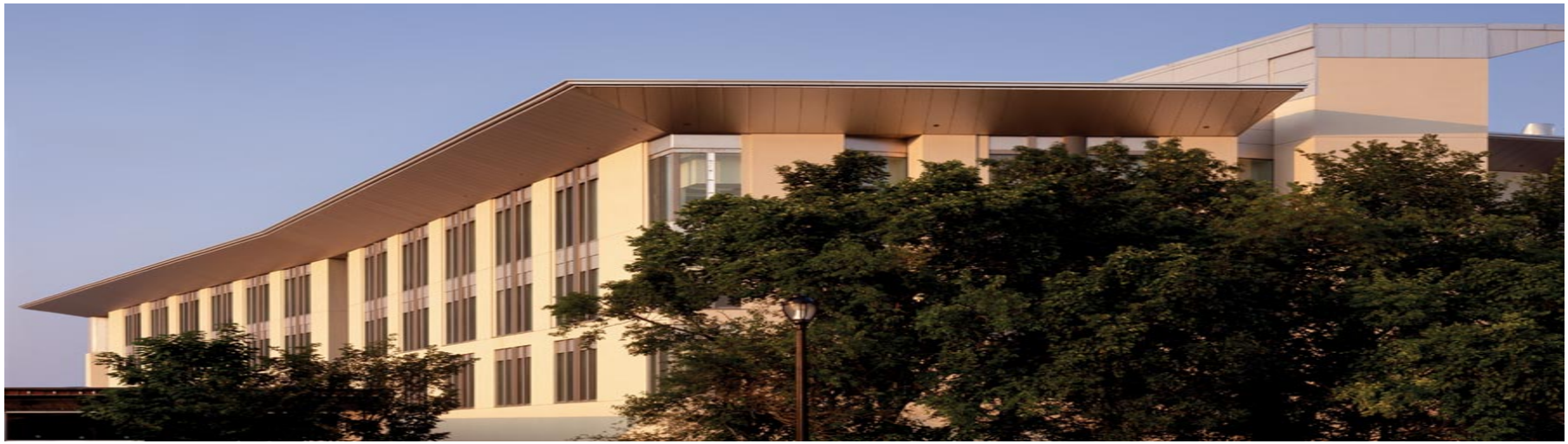
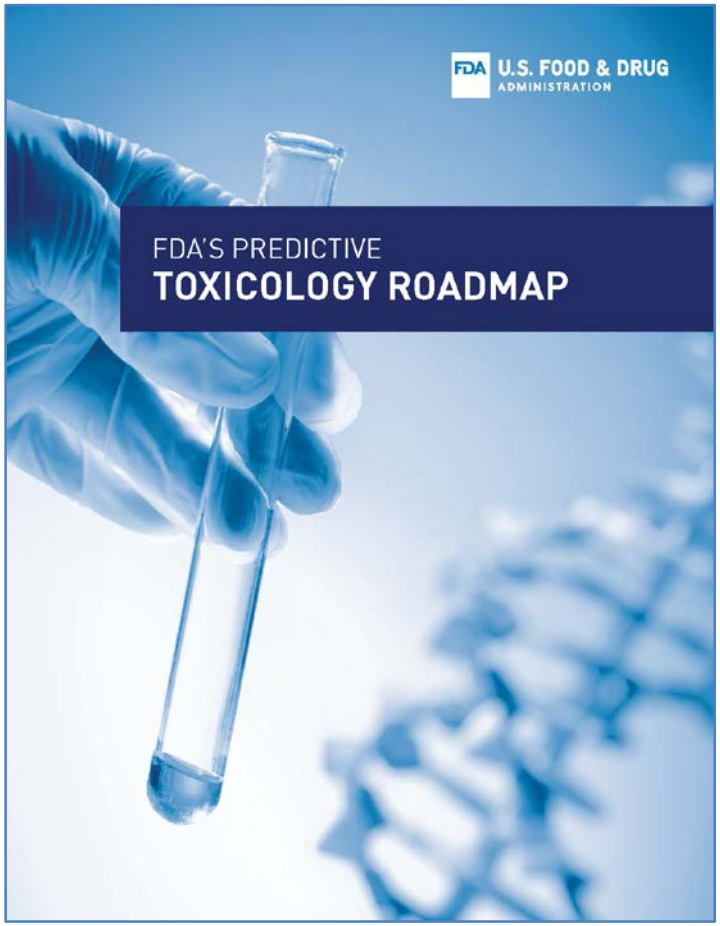


FDA Predictive Toxicology Roadmap

- https://blogs.fda.gov/fdavoice/index.php/2017/12/fda-launches-predictive-toxicology-roadmap-to-enable-advances-in-toxicity-testing/



Roadmap-Responsive Activities

- Each center developed its own plan to meet roadmap goals.
- Two goals for updating our toxicology toolboxes:
  - Revaluate Existing Tools For Regulatory Use
    - Use of Dog for Chronic Testing
    - Use of Chronic Rodent Bioassays
  - Evaluate New Tools for regulatory Use
    - *C.Elegans*
    - Organs on Chips
    - Read Across

Considering Traditional Tests

- Toxicity Studies in Dogs
  - *Use of Dog Studies in FDA's Safety Assessments for Food Additives and Color Additives*, Anyangwe et al., abstract submitted to the 2019 SOT Annual Meeting.
- Rodent Bioassays
  - SOT-FDA Colloquium Series, February 20, 2019.

Redesigning the Rodent Bioassay for the 21st Century- Beginning the Discussion….

- FDA and SOT held a colloquia February 20, 2019
- Follow-up at both Winter Tox Forum and Eurotox 2020
- The following presentations are available on the SOT website.
  - **The Chronic Cancer Bioassay Is Frequently Conducted for Pesticides When It Is Not Always Needed to Protect Human Health** Doug Wolf, Syngenta
  - **Threshold-based Risk Assessment is the Same for Cancer and Non-cancer Endpoints for Non-DNA Reactive Carcinogens-**Samuel Monroe Cohen, University of Nebraska Medical Center,
  - **Is the Two-Year Rodent Bioassay Needed to Address Carcinogenic Risk for Human Pharmaceuticals?** Frank D. Sistare, Merck & Co Inc., West Point, PA
  - W**eight of Evidence Approach to Cancer Assessment-**Alan R. Boobis, Imperial College,

Reassessment of the Dog for Food and Color Additive Safety Assessments

- Goal of the project
  - To determine the impact of studies conducted in dogs on decisions regarding the safe use of food and color additives that have been the subject of petitions submitted to OFAS.
  - To apply the knowledge obtained from these findings to update toxicology testing recommendations.
- Reviewed162 food and color additive petitions containing one or more dog studies were submitted to FDA from 1950-2018.
- Since 2000, very few dog studies have been submitted.
- There were no unique toxicity that were seen only in the dog.
- Concluded that rodent studies combined with ADME data are could be sufficient to evaluate the safe use of food and color additives.
- Next Step- looking at modeling.

Read-Across

- According to FDA's Predictive Toxicology Roadmap read across is a methodology that "uses data from a data-rich substance for a data-poor substance that is considered similar enough to use the same data as a basis for assessing safety."
- Agreement with Underwriter's labs
- The Expanded Decision Uses Read-Across.

Goals of the Research Collaborative Agreement Plan between Underwriter's labs and FDA/CFSAN

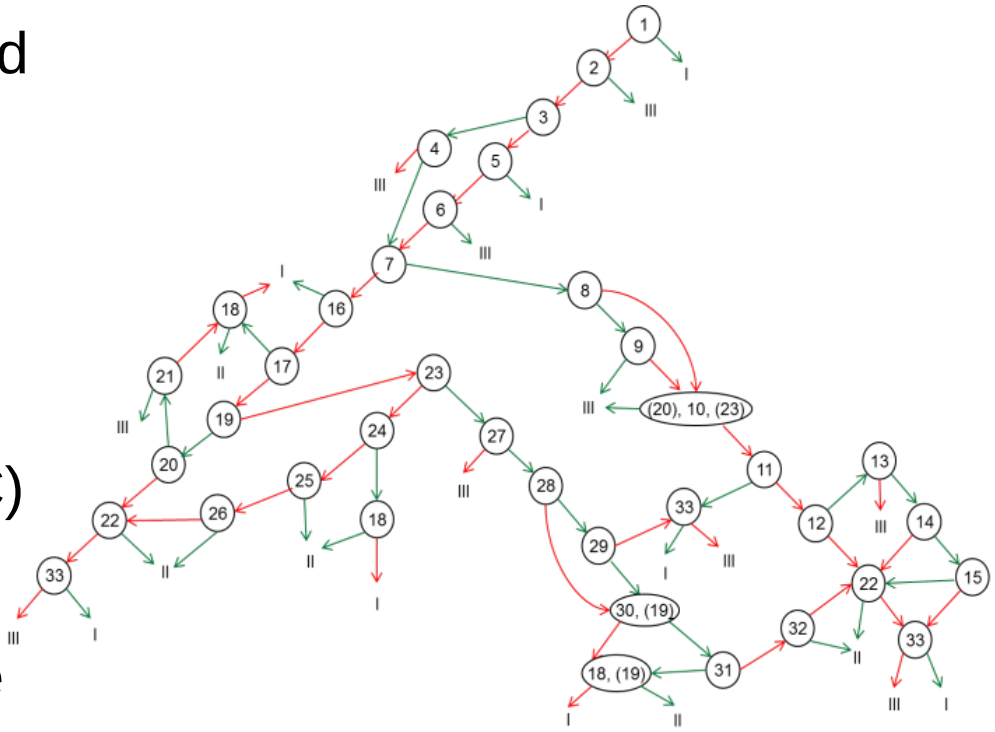
- To provide CFSAN employees the option to use UL Cheminformatics Tool Kit.
- To provide UL with feedback on how well UL Cheminformatics Tool Kit performs in predicating specific toxicities of chemicals found in CFSAN-regulated products.
- To evaluate the usefulness of Read Across as a Regulatory Tool for use in safety/risk assessment.
- To share the results of our finding with members of the FDA Toxicology Working Group.

Endpoints Used in the Evaluation of UL Cheminformatics Tool Kit for performance

- The endpoints that are looked at are:
  - Acute oral toxicity
  - Acute inhalation toxicity
  - Acute eye irritation
  - Acute dermal irritation
  - Acute dermal toxicity
  - Skin sensitization
  - Mutagenicity
  - Acute aquatic toxicity
  - chronic aquatic toxicity
  - DART* (under development)

Brief Decision Tree (DT) History

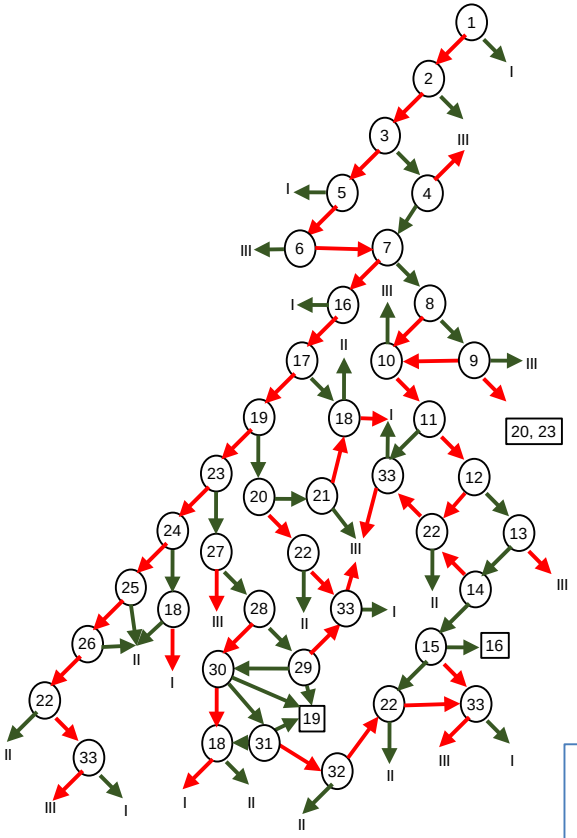
- 1978 FEMA designs sequence of structure-based questions (DT) that assigns chemicals to one of three toxic classes (Cramer et al. DT).
- 1996 Munro assigns 5th% NOEL each class and proposes Threshold of Toxicological Concern (TTC) for each class.
- JECFA develops a flavor safety evaluation procedure using DT and TTC concept.



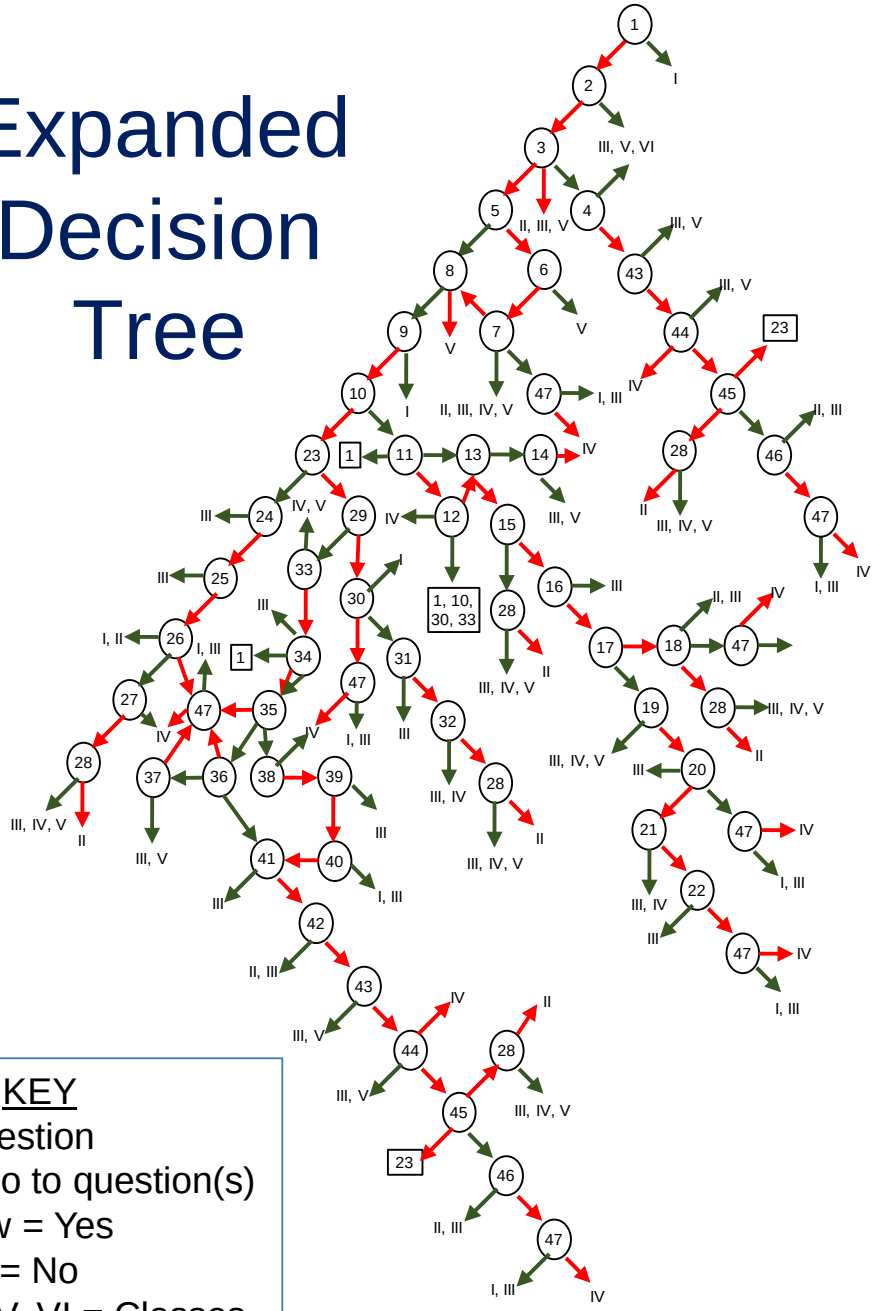
Expanded Decision Tree (EDT)

- Comprehensive revision of the DT to update scientific data underpinning Cramer et al. DT questions
- Remove non-structure-based questions in old DT
- Increase scope of EDT to address majority of substances in food (six vs. three classes)
  - Increase elements and functional moieties
- EDT incorporates “mode of action”/species differences
  - Address alpha-2u-globulin, peroxisome proliferation, progressive renal nephropathy, etc.
- Increase scope and size of EDT database (~2000)

Cramer et al. Decision Tree



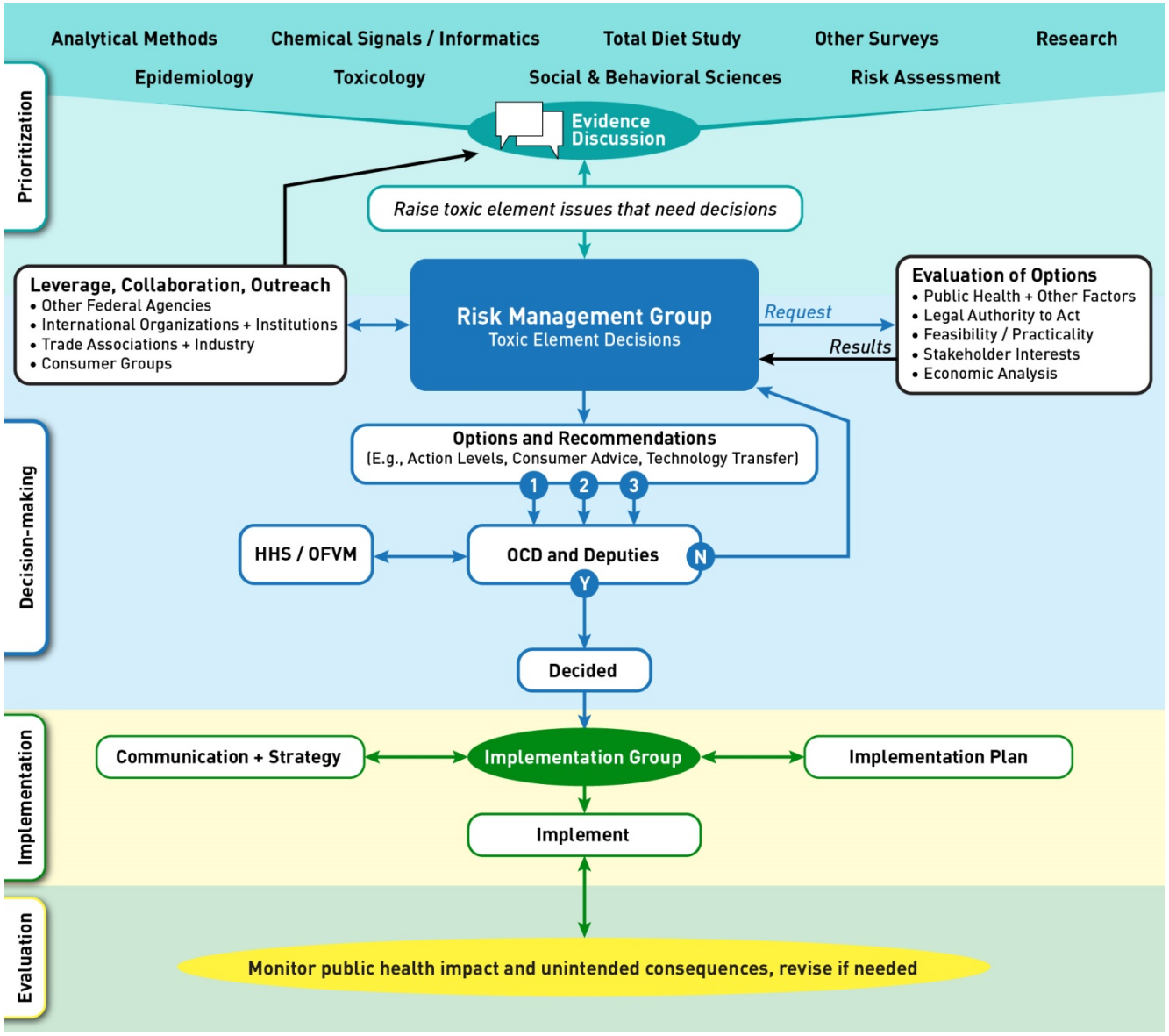
Expanded Decision Tree



KEY

Circle = question
Square = go to question(s)
Black arrow = Yes
Red arrow = No
I, II, III, IV, V, VI = Classes

Toxic Element Strategic Framework



Key Features

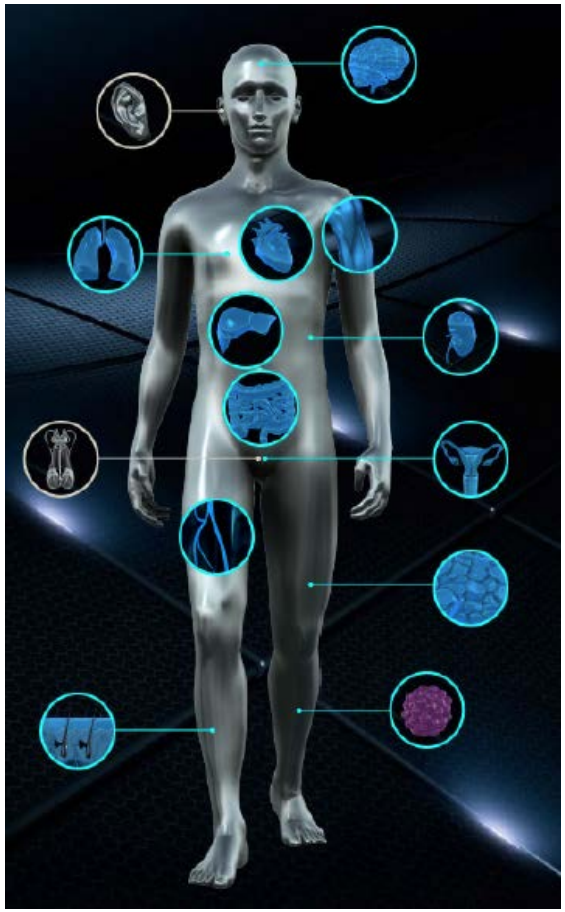
- Four phases: prioritization, decision-making, implementation, & evaluation
- New groups to address science & policy
  - Evidence Discussion Group, Risk Management Group
- Systems and/or practices to ensure documentation
  - Project manager and archival system
- Systematic approach to identify, target, and prioritize efforts
- Ensure a participatory process

Assessment of Mixtures of Metals

- FDA designed a novel worm Development and Activity Test (wDAT) that maps the timing of *C. elegans* developmental milestone acquisition as well as stage-specific activity levels.
- FDA is using this tool to look at mixtures of metals in children's food.
- A planned 20-compound, blinded qualification study will clarify the utility of the wDAT for human-predicative developmental neurotoxicity testing.
- Follow-up with using zebra fish testing on mixtures- possibly in conjunction with Cornell University.
- Corelates with NCTR Rodent Study on effects of arsenic and developmental neurotoxicity.

Organ on a Chip Technology

- FDA is exploring organ-on-a-chip technology
- We're helping to advance this technology with FDA grants
- We expect that this technology will be developed enough in the future to support regulatory decision-making about safety



NATURE | NEWS

Miniature liver on a chip could boost US food safety

- CFSAN Researchers will be evaluating the effectiveness of this technology to better understand the effects of medicines, disease-causing bacteria in foods, chemicals, and other potentially harmful materials on the human body

Evaluation of the utility of ToxCast HTS and high-throughput toxicokinetic data for food chemical safety risk assessment via comparison with *in vivo* animal data.

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Abstract

New approach methodologies (NAMs) are currently being developed and evaluated for use in chemical safety risk assessment, including chemicals used in food. NAMs include in vitro high-throughput screening (HTS) assays, such as the ToxCast and Tox21 assays. The ToxCast/Tox21 assays have been run for thousands of compounds, including hundreds of compounds used in food. However, the relationship of these NAM data with traditional in vivo animal data, and the utility of NAMs for risk assessment, remain under evaluation. The goal of the present study is to evaluate the utility of ToxCast/Tox21 HTS data in food safety risk assessment. To do this, bioactive concentrations of a subset of food-use compounds in ToxCast were converted to oral equivalent doses (OEDs) via in-vitro to in-vivo extrapolation (IVIVE) using either in vitro or in silico-based toxicokinetic parameters for a subset of food-use compounds. These OEDs were then compared to doses demonstrated to cause effects in in vivo animal tests (using data compiled by EPA and FDA). Initial comparisons demonstrated great variability in the correlation between ToxCast and in vivo data, so steps are being taken to further refine the toxicokinetic information, chemical groups, and in vivo endpoints in an effort to identify additional information and conditions necessary to utilize HTS data for preliminary food safety assessment. This work does not reflect the official policy of the US EPA or the US FDA.

Introduction

- The development and implementation of NAMs in food and chemical risk assessment is an ongoing goal in toxicology.
- High-throughput screening data have been generated for a large number of compounds through the ToxCast/Tox21 project, including several food-use chemicals.
- Use of these HTS data in food chemical safety risk assessment remains under evaluation.
- Ongoing work is being done to relate concentrations in HTS assays to doses given orally in animal studies by *in vitro* to *in vivo* extrapolation (IVIVE).
- Work done by Friedman *et. al.* (2019) determined administered equivalent doses (AEDs) for 448 ToxCast compounds using the high-throughput toxicokinetics (HTTK) package for the IVIVE, and did a screening level comparison to *in vivo* animal data[1].
- The present study builds on these data, with the goal of evaluating the utility of ToxCast/Tox21 HTS data in food safety risk assessment.

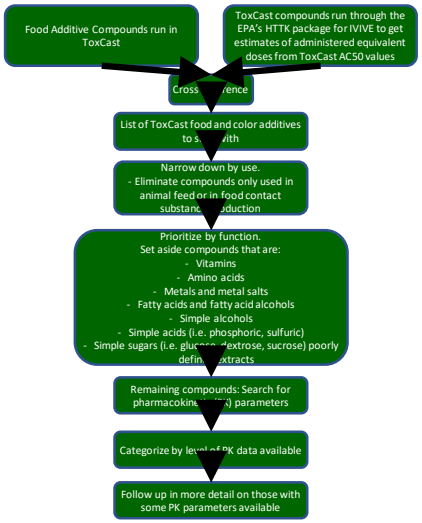
Acknowledgements

The authors would like to thank Patra Volarath for her helpful discussion on this project.

References

1. Friedman, K.P. Et. Al. Toxicol Sci. 2019 Sep 18

Materials and Methods



Results

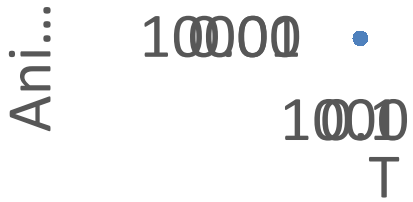


Figure 1. Comparison of ToxCast AEDs with in vivo animal data values for all initially identified compounds.
The active ToxCast assays for each compound were filtered based on curve-fitting caution flag and uncertainty information, and the AC50 values remaining were classified into percentiles for each compound. The 5th percentile AC50 value for each compound was converted to an administered equivalent dose (AED) using the HTTK package, and plotted against the lowest dose reported in *in vivo* animals studies in the ToxVal database. The black line delineates the 1:1 identity line.

Results and Discussion

Table 1. Criteria for Pharmacokinetic Data Classification

Determination	Criteria
No	Lack of data OR unsuitable for in vitro comparison (compound completely transformed before absorption in the GI tract)
Maybe	Some PK data, with potential issues
Yes	Some PK data available to use

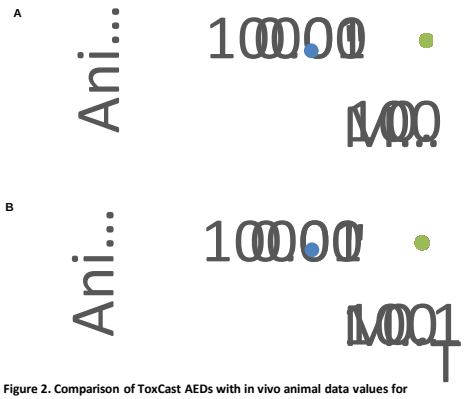


Figure 2. Comparison of ToxCast AEDs with in vivo animal data values for prioritized compounds.
The 5th percentile filtered AC50 values from ToxCast for each compound (as determined in Figure 1) were converted to administered equivalent doses using the HTTK package, and plotted against the lowest value reported for *in vivo* animals studies in the ToxVal database(A), or against the lowest low effect level in animals from the CompTox Dashboard in (B). Black line delineates the 1:1 identity line. Compounds are divided into those with no PK or are unsuitable for comparison ("no"), those that have potentially some PK data ('maybe"), and those with some level of PK data available for use ("yes")

Table 2. ToxCast data for 18 compounds selected for more detailed

Compound	CASRN	ToxCast Assays run	ToxCast assays active	ToxCast 5th % AC50 (μM)
Styrene	100-42-5	211	1	100.0000
Cyclohexylamine	108-91-8	639	6	0.0016
Butylated hydroxytoluene	128-37-0	401	61	7.8826
Sodium saccharin	128-44-9	211	1	69.8740
Estragole	140-67-0	427	5	21.2958
Butylated hydroxyanisole	25013-16-5	211	22	17.0508
Etidronic acid	2809-21-4	211	5	65.9696
Sodium benzoate	532-32-1	670	1	100.0000
Glycerol	56-81-5	669	17	0.0028
1,2-Propylene glycol	57-55-6	640	12	0.0334
Caffeine	58-08-2	676	53	1.4245
Sodium nitrate	7631-99-4	210	0	100.0000
Sodium nitrite	7632-00-0	638	4	2.5073
Potassium nitrate	7757-79-1	427	7	6.5230
Saccharin	81-07-2	428	4	1.22E-05
Propylparaben	94-13-3	719	99	7.4093
Eugenol	97-53-0	696	28	0.1320
Methylparaben	99-76-3	690	23	0.1215

Table 3. Initial values and pharmacokinetic data available for the 18 compounds selected for further analyses

Compound	CASRN	Use	Initial ToxCast AED (mg/kg-bw/d)	Initial in vivo animal effect level (mg/kg-bw/d)	PK refinement
Styrene	100-42-5	Polymer production	4.2277	0	PBPK model
Cyclohexylamine	108-91-8	Boiler water additive	0.0001	15	Reported PK parameters in the literature
Butylated hydroxytoluene	128-37-0	Preservative	0.0118	25	Reported PK parameters in the literature
Sodium saccharin	128-44-9	Sweetner	2.4606	200	Reported PK parameters in the literature
Estragole	140-67-0	Flavor	1.9242	37	PBPK model, some human data
Butylated hydroxyanisole	25013-16-5	Preservative	1.1452	50	Reported PK parameters in the literature
Etidronic acid	2809-21-4	Boiler water additive, sanitizer	3.2042	30	Some reported PK parameters in the literature
Sodium benzoate	532-32-1	Preservative	1.3959	500	Some reported human PK parameters
Glycerol	56-81-5	Multiple	8.47E-05	1200	Reported PK parameters in the literature
1,2-Propylene glycol	57-55-6	Multiple (incl. antioxidant, flavor, stabilizer, solvent, humectant)	6.64E-04	1700	Reported PK parameters in the literature
Caffeine	58-08-2	Additive	0.1856	0	Reported PK parameters in the literature
Sodium nitrate	7631-99-4	Preservative	0.2244	500	PBPK model (based on nitrate ion)
Sodium nitrite	7632-00-0	Preservative	0.0220	25	PBPK model (based on nitrite ion)
Potassium nitrate	7757-79-1	Preservative	0.0164	290	PBPK model (based on nitrate ion)
Saccharin	81-07-2	Sweetner	3.96E-07	200	Reported PK parameters in the literature
Propylparaben	94-13-3	Preservative, antimicrobial, flavor	0.8345	12	Reported PK parameters in the literature
Eugenol	97-53-0	Flavor	0.1140	300	Reported PK parameters in the literature
Methylparaben	99-76-3	Antimicrobial, flavor	0.0437	100	Reported PK parameters in the literature

- On a first pass through the compounds, the ToxCast AED is often lower than the *in vivo* point of departure from animal studies, but not for all compounds.
- Many compounds run in the ToxCast assays are difficult to directly compare to *in vivo* animal data, for a variety of reasons, including things such metabolism or reactivity of the parent compound, compound volatility, and type of compound such that the compound is a vitamin, amino acid, or other component of normal metabolism in the body, among others.
- Results from these 18 prioritized chemicals can be used to help interpret the results of other chemicals in ToxCast.

Future Directions

- Use PK parameters identified in the literature to refine the IVIVE AEDs (and compare).
- Curate *in vivo* animal data to compare to studies used to make regulatory decisions.

This work does not reflect the official policy of the US EPA or the US FDA.

In Vitro Systems Working Group

- Consists of one representative from each FDA Center/Office.
- Chaired by CFSAN; Co-Chaired by NCTR
- Responsible for ensuring that IVSWG's goals are moving forward in a timely and transparent manner.
- Ensure FDA scientists are updated on new emerging *in vitro* methods and models.
- Inform FDA scientists on seminars, site visits, hands-on training, and other learning opportunities.
- Contact point for outside scientists wishing to present new technology to FDA.
- Develop proposals for potential public–private partnerships or applicable mechanisms to advance the development of *in vitro* technologies for regulatory science use.

IVSWG First Case Study

- Focus on coordinating, developing, and evaluating *in vitro* Microphysiological Systems (MPS) for regulatory use.

- This will be the first IVSWG case study on the viability of its implementation plan for *FDA's Predictive Toxicology Roadmap.*

- IVSWG program will be evaluated, and if needed, refined, after completion of its goals.

Some International Activities

- **International Liaison Group for Methods of Risk Assessment for Chemicals in Food (ILMERAC)-** Co-ordinated by EFSA. ILMERAC offers an opportunity for agencies with a regulatory risk assessment mandate to share and exchange experience with various partners on ongoing and planned activities aimed at developing and implementing methods for risk assessment of chemicals in food.
- **EU Tox Risk-** Under EU Horizon 2020 Program- FDA meets yearly and in discussions on TTC and Organs on a Chip for the follow-up EU Horizon grant.

Change Takes Time

Conclusions

- CFSAN is taking steps to advance the regulatory applicability and acceptance of novel methods, including TTC, read across, and organ-on-a-chip.
- CFSAN works collaboratively with FDA's five other product Centers and external stakeholders who are also actively taking steps to realize the goals of the Roadmap.
- In identifying critical priority activities for enhancing FDA engagement in new predictive toxicology methods, FDA will be better prepared to meet its mission today and in the future.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