Revisiting the TTC Approach for Cancer Assessment: Part of a Road Map of Computational Methods at FDA CFSAN OFAS

Jason Aungst[1], Kristi Muldoon-Jacobs[1], Diego Rua[2], Kirk Arvidson[1], Dimitar Hristozov[1], Benon Mugabe[1], Ed Matthews[1], Annette McCarthy, Chihae Yang[3], Mitchell A. Cheeseman[1]

1. U.S. FDA/CFSAN/OFAS College Park, MD; 2. U.S. FDA/CFSAN/OCAC College Park, MD; 3. Altamira, LLC, Columbus, OH

1.Abstract

Alternative methods in risk assessment workflows are attracting attention due to international legislation on providing safety data for large numbers of chemicals. The Threshold of Toxicological Concern (TTC) is one method of interest and was developed based on the Threshold of Regulation (TOR) policy of US FDA Center for Food Safety and Applied Nutrition (CFSAN). TTC offers a pragmatic solution for assessing the safety of low-exposure food-contact materials. The goal of this project is to aid regulatory risk assessment procedures by refining the TTC process. The first refinement is providing a cancer TTC dataset based on regulatory study review criteria. This process helps gain better regulatory acceptance and supports building a high quality training set for other computational methods. The Carcinogenicity Potency Database (CPDB) was integrated into the Chemical Evaluation and Risk Estimation System (CERES) database at CFSAN. Study details and TD_{50} values were selected for the cancer TTC dataset only from studies in the CDPB that were in accordance with defined regulatory criteria (e.g., test substance administration route, duration, sample size, dose levels, lesion classification). The second refinement is to incorporate known genotoxic mechanisms into the TTC decision tree. Positive and negative Ames assay data were curated from various databases. These data were then incorporated into and analyzed as part of the cancer TTC dataset. This analysis shows Ames positive carcinogens to be much more potent than Ames negative carcinogens. This analysis has two additional benefits. (1)The qualification of data through regulatory screening criteria increases the regulatory applicability and acceptance of TTC methods. (2)Our methodology provides a mechanism for incorporation of alternative and in vitro test methods into a regulatory risk assessment.

2. Introduction

The Code of Federal Regulations 170.39 provides exemption for food-contact substances (e.g., food-packaging or food-processing equipment) that migrates, or that may be expected to migrate, into food below the "threshold of regulation" (TOR), a level considered to be of negligible risk (<1 in 10^6). That threshold was set considering dose and effects in chronic and short-term animal feeding studies. Previous work attempted to incorporate TTC analyses (varying structure and endpoints) to expand the threshold to multiple levels (Cheeseman et al., 1999). That work examined an expanded CPBD database, structural alerts, and Ames assay data to correlate result with the initial dataset upon which TOR was based and to identify different potencies between structural classes and mutagenicity of chemicals. The current project attempts to expand on this work by building a training set based on regulatory criteria for bioassay data and expand the structural, physiochemical, and toxicological (mutagenicity) analyses to increase relevance for use by a regulatory agency.

3. Dataset and Regulatory Criteria

Preparation of Cancer TTC dataset: Publicly available CPDB database was downloaded from http://potency.berkeley.edu/. The lowest TD_{50} values from oral studies with significant tumorigenic effects (p≤0.01) were selected. The study inclusions also meet the following criteria in accordance with FDA Redbook 2000 recommendations.

- Positive tumorigenic effects with p <0.01. TBA (all tumor bearing animals), MXA, and MXB not included
- Tumors from one cell type (males or females per species).
- GLP studies or equivalent protocols: appropriate sample size (>40), excluded single dose studies, MTD achieved, and acceptable duration (18 months mouse, 24 months rats)
- Shorter exposures are included if results were statistically positive
- Negative studies with no significant tumor findings excluded
- Equivocal studies also excluded from this analysis
- Dataset available from http://www.altamira-llc.com

Ames mutagenicity data: Ames mutagenicity data were selected from the Altamira Ames mutagenicity training set implemented in CERES. This dataset includes the data from US FDA CFSAN, NTP, ISSCAN, Tokyo Eiken, and approved drugs (drugs@fda.gov and pharmapendium) as well as salmonella data from CPDB.

The following criteria in the order listed were applied to establish Ames calls.

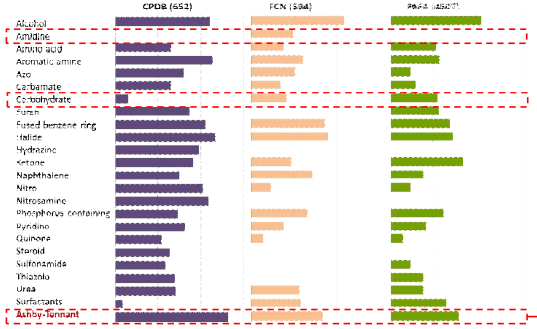
- US FDA regulatory calls preceded all others.
- NTP studies with calls made by Errol Zeiger (DSSTox NTPGTZ)
- Positive studies from TA 102 and 104 strains from Tokyo Eiken
- Used ISSCAN and CPDB salmonella calls when other information is available. ISSCAN call preceded CPDB salmonella call.

4. Distribution of TD50 after data evaluation

	# chemical	Mean TD50 mg/kg_bw/d	Median mg/kg_bw/d	Lower 10% mg/kb_bw-d
Studies met criteria	652	375	16.5	0.33
Removed negative/equivocal studies	572	252	12.0	0.28
Having Ames studies	428	302	15.6	0.31

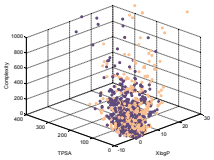
5. Comparison of chemical domain of revised TTC data set and food-contact substances

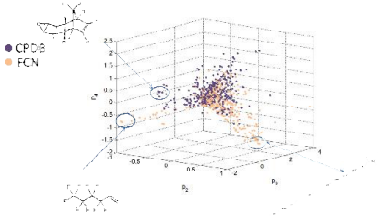
Only 21 chemical compounds are common to both CPDB (Cancer) TTC set and FCN list (as of Dec 2011).

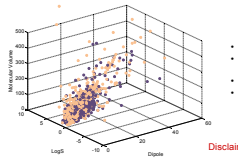


% in datasets (log scale)

6. Chemical space comparison of food-contact substances and TTC data sets

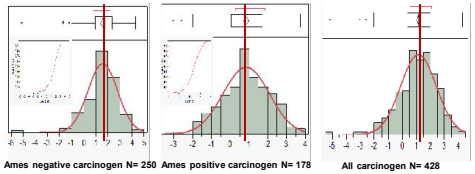






- Physicochemical space of Cancer TTC and FCN datasets seem rather similar.
- Structural feature space of Cancer TTC and FCN are very different. (Depicted in the PCA plots for the three principal components scores plot)
- 324 features from the FDA Redbook subgraphs from MOSES were used.
- Further investigation is required for the applicability of CPDB dataset for the assessment of FCS.

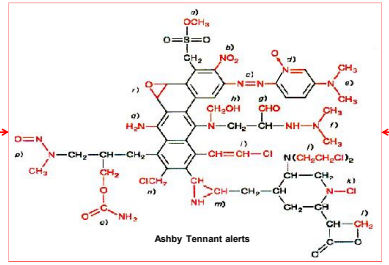
7. Distribution of TD50 values (log scale)



Ames negative carcinogen N= 250 Ames positive carcinogen N= 178 All carcinogen N= 428

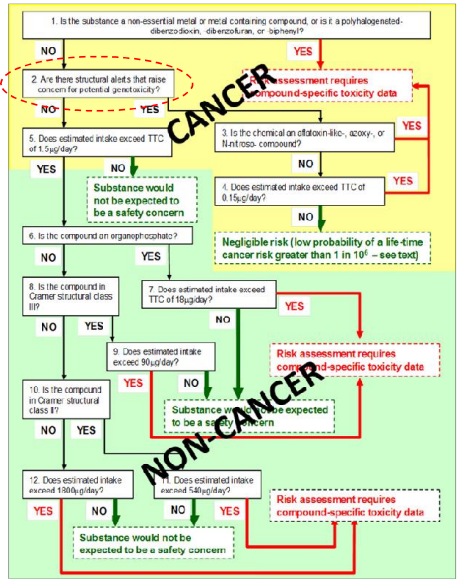
8. Separation of data into Ashby Tennant +/- and Ames +/-

	# chemical	Mean TD50 mg/kg_bw/d	Median TD50 mg/kg_bw/d
Total	428	302	15.6
Ames (-)	178	511	51.6
Ames (+)	250	153	6.4
AshbyTennant (+)	232	192	7.6
AshbyTennant (-)	193	436	40.6
AshbyTennant (+) & Ames (+)	184	98.2	5.5
AshbyTennant (+) & Ames (-)	48	552	41.7
AshbyTennant (-) & Ames (+)	64	315	17.2
AshbyTennant (-) & Ames (-)	129	497	51.5



Ashby Tennant alerts

9. TTC Decision Tree



Kroes, Renwick, Cheeseman, et. al. 2004, FCT, 42, 65-83

10.Future Directions

- Refine and expand data harvesting efforts within OFAS's submission workflow
- Continue development of QSAR models and alerts for endpoints other than carcinogenicity, developmental toxicity, *e.g.*, target organ toxicity, reproductive toxicity and endocrine disruptors, etc.
- Expand chemical space (applicability domain) of CERES to include: Additional CFSAN data including chemical, toxicity and exposure data Additional toxicity data through a collaborations
- Provide both structural and biological approaches to analog identification
- Bring biological analogs to reviewers through ToxCast and Tox21 projects

11. Acknowledgements

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Disclaimer References to Molecular Networks, CORINA and MOSES in this poster do not constitute an endorsement by the US FDA. This presentation is not an official US FDA guidance or policy statement.