Expanded Decision Tree (EDT) Peer Review

Reporting Period: Phase 1 and 2

Final Report

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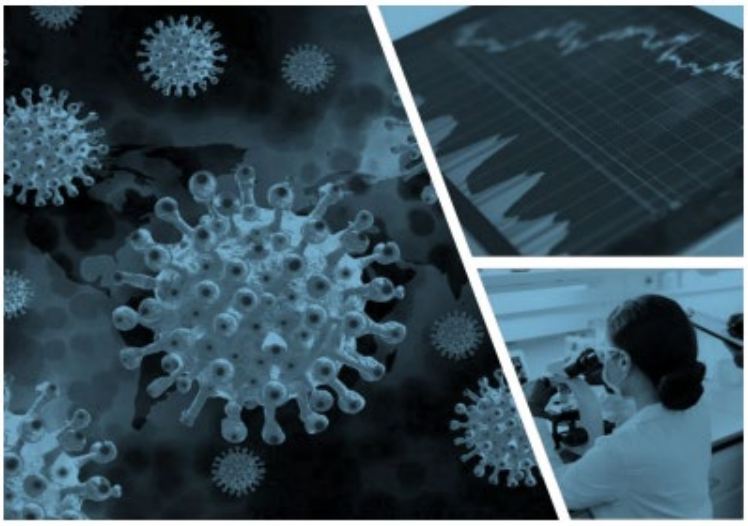

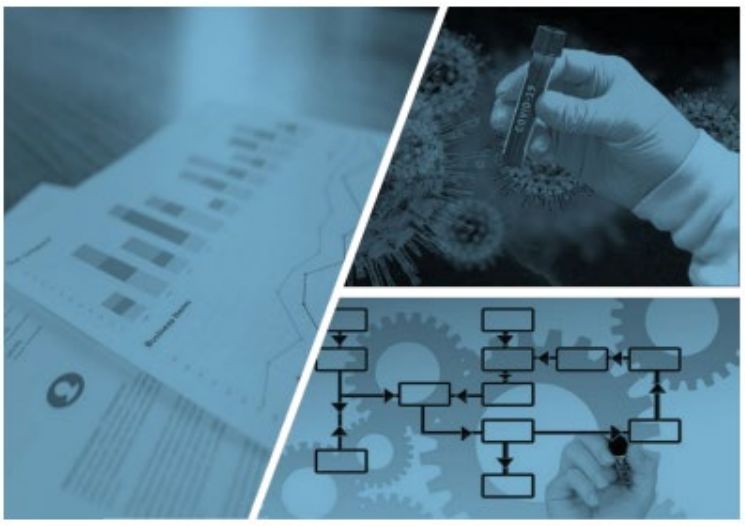

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PEER REVIEW CHARGE REPORT

1.0 SUBJECT MATTER EXPER SUBJECT MATTER EXPERT (SME) — Dr. Mark Cronin

Phase I:

Questions for section 1 (The Expanded Decision Tree (EDT)):

Question 1: Has FDA clearly explained, with adequate examples, all guidelines and definitions for use with the EDT? If not, please provide suggestions for alternate text and/or additional examples.
Response: I am basing this response on my interpretation of Section 1.5 (please clarify if this is incorrect). FDA has done an excellent job in describing the chemistry basis of the EDT in many aspects. I found the description of chemistry in Section 1.5 to be clear and very logical. This is appropriate for use in a toxicological risk assessment tool such as the EDT. The description inevitably requires the reader to have some background in organic chemistry, without which the reader will not have the full understanding of the subtlety and sophistication of the scheme. I do not see that this is a problem, if the user of the scheme required detailed interpretation and they did not have that level of chemistry, then they would need to seek it from a more qualified person. Taking my own experience as an example, I can understand the chemistry, but I would not be able to comment on whether the chemistry is correct or appropriately set out (I have no reason to believe that it is not).
To be critical of Section 1.5, it may be useful to include structures for classes A-F (Aliphatic – Aromatic ring). This may be especially helpful if and when the scheme is coded computationally. I also wonder whether this information may be easier to comprehend in a table, for instance with headings such as (although this may not be possible or practical):

Class	Title	Description	Detail	Relating to EDT Question

Section 1.5 contains a variety of different aspects of chemistry, going from fundamental classes of compounds such as being aliphatic or aromatic to different types of functional groups (see Section H) including very specific functional groups including reactive moieties etc. It might be more logical to organize this section into major structural features, different types of scaffolds (e.g., bridged compounds, zigzag etc.) and then organize the functional groups in a logical manner.
Another aspect that I found lacking from the whole EDT is a section describing the applicability domain of the scheme (I comment on this elsewhere below). It may not be appropriate to evaluate this with regard to Section 1.5, but it is a key definition of the scheme. For instance, is there a molecular weight cut off?
The term “guidelines” is used in Section 1.5, I do not believe there are real guidelines provided here but these are definitions. For the avoidance of doubt, it may be appropriate to remove the term “guidelines” or clearly denote what it means.
Overall, this Section 1.5 is very comprehensive which could be made clearer with organization of the information into a table and different levels of structural definition. It would also benefit from a statement at the beginning of the section that defines clearly what is the purpose of this section and how it is set out, with the expectations but the reader would gain from it - for instance it could be stated that there is a requirement for a knowledge of organic chemistry to interpret information. What we do not want is put off non-chemists from using the EDT as they may feel it is a purely chemistry-based tool with no basis in toxicology (this is clearly not the case!!).
Section 1.5, “I. Reactive moiety” It would be helpful to define what is meant by reactive here e.g., electrophilic, nucleophilic etc.

Question 2: Are all EDT questions clear as to which structural features they are describing? If no, please identify the question by its number, explain why you find the question ambiguous or confusing, and suggest alternative text to ensure that it is clear what kind of structural features the question is aiming to capture.
Response: I found the EDT questions to be clear in terms of the structural features they intend to describe. What is described are structural alerts in their most simplistic form, in other words the molecular environment is not defined for these alerts, i.e., no substituents are defined. In the documentation for the EDT, it may be worth explaining this along with the rationale for doing so. There are advantages to this approach, namely that it is fundamental, and all identify any compound with a structural feature, there are also disadvantages as a structural feature may be buried within a molecule and may not be relevant for activity. The FDA appears to wish to maintain the ethos of the original Cramer decision tree (Cramer et al, 1978, *Food and Chemical Toxicology* 16: 255-276) which was based on chemical classes and groupings alone. This was quite revolutionary in so doing at that time, but there would be no problem in making the definition of chemical classes within the questions more sophisticated at the current time (I will make a comment related to this later).
With regard to a previous comment made in Question 1, in the application of the EDT, it may be worth having a general statement or guidance on what the applicability domain of this is in terms of physio-chemical properties such as molecular weight. It should also be noted that the EDT is for single chemical structures. Clear guidance should be given before entering the EDT on how to address issues such as salts, counterions etc., i.e., should be neutralized form of a molecule be utilized?
It would be helpful for each "question" to have a descriptive title to give the reader some insight into what it relates to.

Question 3: Most questions place compounds into one of six classes of toxic potential depending on their structural features. Does the EDT place the type of compounds that are captured at each question into the appropriate class of toxic potential? If not, please explain why and provide a recommendation for the appropriate class of toxic potential.
Response: I will use my response to this charge question to make some general comments that will hopefully address this question but also go on into other issues with regard to the EDT. The FDA may wish to accept or ignore these comments!
It seems a simple question to ask if the EDT places compounds into the appropriate class of toxic potential. This is actually a very difficult question to tease apart and answer using the information given. To answer this question FDA needs to provide clear and upfront description of the six classes and their justification. The classes are defined in Tables 2 and 3 in Section 3.4. It almost seems as if the definition of classes becomes a self-fulfilling prophecy, were the classes defined *a priori*, or on the basis of the data? It is my understanding of the Cramer scheme that it did not set out to develop the classes on anything more than fundamental toxicological principles, less so on chemical groupings and data (although there is, of course, evidence of structure-activity in Cramer et al (1978). Whilst Fig 2 in Cramer et al (1978) plots some NOAELs, it was only Munro et al (1996, *Food and Chemical Toxicology* 34: 829-867) who started to put any significant numbers on the Cramer classes. So, I can agree that the EDT sets out six classes that go from "less toxic" (Class 1) to substances of very high toxicity (Class 6). My interpretation of the scheme, as described is that compound classes are generally in the right place, i.e. less toxic compounds are class 1, most toxic class 5 and 6. My feeling is that FDA has done a very good job in allocating chemicals classes to appropriate EDT classes. There were none that I felt uneasy with, although whether they are 100% correct will probably come with experience of using the scheme. I have no doubt that some refinements will be required at some point in the future, but what is describing appears to be an excellent starting point.
To be honest, I found the technical description of the chemistry Section 1.7 really difficult to take in and comprehend. Whilst this is a criticism, it does not belittle or criticize the incredible job that has been done by FDA in organizing this. In terms of this review, this may come to life more in Phase II. With regard to

presentation of the questions, I found myself going back and forth in the questions to determine which chemistries were associated with a particular EDT class. To help get the chemistry and EDT class across, possibly putting this information in a table, with chemistry linked to a class would be helpful and clearer. To fully understand the six EDT classes, it would be helpful to have a direct comparison with the Cramer scheme, i.e. is the chemistry in Cramer class 1 analogous to EDT class 1 etc. There is no reason Cramer and EDT classes should be the same, but it would help me understand the new classes, and I would imagine it would be helpful to others.
Some other points:

- FDA need to be very clear what this scheme is to be used for. There are a growing number of TTC schemes (and variations thereof) e.g. for skin sensitization, inhalation toxicity, ecological effects. I assume this scheme and the associated six classes relate solely to repeated, chronic oral exposure.
- It would be helpful to define whether (assuming they do) the six classes replace the requirement to deal with DNA reactive compounds and the Cohort of Concern separately.
- There is no easy way to demonstrate and document such a complex and detailed chemical analysis – perhaps this should be summarized in a publication with full access to the information via a computational / software application. I am thinking of perhaps of how meta information / data are held for structural alerts e.g. in Derek Nexus or the OECD QSAR Toolbox.
- The allocation of classes in the questions should be stated along with the description of chemistry, as it is, for some questions I was required to go several pages forward to find the result. Also (this should be very easy for the software as noted above), it would be helpful to have the explanations provided in Appendix 1 with the questions – I ended up flicking back and forward to find / interpret this information.
- Ultimately it would be great to have mechanistic information linked to the questions, even relevant AOPs (accepting there will be gaps in AOP coverage). There has long been appreciation of the potential value of a mechanism / mode-based classification system – although I am not sure how possible that is in practical terms.

A final comment here is rather fundamental. The EDT is by its name a “decision tree”, however no tree is provided. I am thinking here how familiar we are with the CDT, in particular Figure 1 in Cramer et al (1978), I was expecting to see something similar in this report. This actually raises the question of whether a yes / no decision is appropriate. What happens if a molecule contains two “alerts”, the first is less toxic and the molecule is assigned to that class early in the decision tree? I fully understand the strength of the decision tree approach, but now we can do things differently – why not assess the molecule against all alerts and use the most conservative classification? In this way, the classification is most protective, and it would be transparent. I realize we are very familiar with the decision tree approach, but it does not mean that it should applied in the new scheme.

Question 4: Commonly, structurally related compounds (e.g., γ-diketones) can have common toxicological endpoint (in this case γ-diketone type neurotoxicity). Compounds that can either hydrolyze and/or metabolize to these compounds can exhibit the same type of toxicity. FDA aimed to capture hydrolytic and metabolic precursors of structurally related compounds with similar toxicities at numerous questions. Are there any questions where you can suggest any possible metabolic and/or hydrolytic precursors to the types of compounds addressed that are currently not mentioned/captured in the question?
Response: The FDA should be congratulated on the completeness of the coverage of the EDT, especially capturing hydrolytic and metabolic precursors. This is not a particular area of personal expertise for myself, so I am unable to comment other than to say there is nothing obvious to me that has been omitted.

Question 5: Are the example structures provided after each EDT question correct and adequate for understanding what type of compounds the question aims to capture? Are there different or additional example structures for any of the questions that would help increase the understandability of the question?
Response: I am very grateful to the FDA for including chemical structures alongside the questions. This is a great help to me (as chemically aware scientist, but not a chemist). I am sure it will be helpful to many others. I believe the compounds provided are correct (I did not see anything incorrect). It may be helpful to have chemical / compound classes (which may help define domains) and individual exemplar compounds (most / all of which would never be dealt with by TTC as they will have data).
Again, the chemical classes could be linked to mode / mechanism, but also maybe to HPVC classes and other means of classification and inventories.

Question 6: Are there any congeneric groups that the EDT does not adequately address, but for which enough safety data exist that could serve as the basis of additional EDT questions to address these groups? If yes, please identify and provide all toxicological data for the congeneric group(s) that may form the basis of one or more additional questions. If possible, please propose the wording for such additional EDT questions. (Substances within a congeneric group are structurally and metabolically similar.)
Response: I do not believe that there are any obvious congeneric groups with data that have been omitted from the EDT.
A couple of thoughts:

- If not attempted (it may be in Phase II), it would be a useful exercise to map the EDT questions to available data e.g. take the compounds in a database and determine which question and EDT class they relate to.
- Mapping the EDT classes will probably help with the definition of applicability domain e.g. which types of chemistry the EDT could be applied to.
- Experience shows that many *in silico* models are used well beyond their intended domain and purpose. Therefore, should the EDT aim to help with e.g.
    - Sugars, amino acids – especially those considered GRAS
    - Polymer components
    - Botanicals – expand the knowledge to more botanical classes
    - Pharmaceuticals – there are many data and knowledge of mechanisms etc.
    - Nanomaterials
    - Biocides – consideration of pesticide data and mechanisms
    - Natural toxins e.g. mycocystins

  The final two examples (biocides / natural toxins) may be particularly relevant to food.

The need for animal data to extend the EDT further will be restrictive, thus there may be good reason to extend on a mechanistic basis, e.g., using omics, NAM etc. data

Question 7: Should any questions be further subdivided to ensure a more refined grouping of related substances? If yes, please suggest wording for the refined question(s) and provide the data justifying the suggestion.
Response: The FDA has done an incredible job in defining and dividing the questions, resulting in very fine granularity. This shows very great expertise and thought. There were no refinements to the classes that seemed obvious to me at this time.

Question 8: Are there any terms used in the EDT questions that should be added to the guidelines and definitions section to help users of the EDT? If yes, what additional terms should we define?
Response: The guidelines and definition focus on chemistry and defining that. The chemistry does not need any more definition in my opinion.
Here I summarize some thoughts expressed above, as well as others:

- The guidelines could give an overall statement of the use of the EDT and the information it can provided i.e. TTC for oral repeat dose / chronic exposure etc.
- An applicability domain for the EDT could be given.
- I would have preferred to see a description of the six EDT classes – or at least an overview / brief explanation – before reading the questions. Information is given in Section 1.8 and I only read that after reading the questions, so I was trying to interpret the questions without knowing what the EDT classes meant!

I realize that answering this question is really a moot point. Most users will simply go to a usable piece of software and never consider the original description in the paper.
Other comments that do not fit elsewhere:

- Sections 1.1 and 1.2. The start of these sections would benefit from shorter sentences, more punctuation etc.
- Section 1.2, paragraph 3. It could be stated that there have been updates of the ToxTree implementation of CDT, as well as other implementations e.g. VEGA, COSMOS NG, OECD QSAR Toolbox etc.

Questions for section 2 (The Expanded Decision Tree Chemistry, Toxicity, and Metabolism Database (EDT DB)):

Question 9: Has FDA clearly explained where the toxicological data found in the EDT DB were collected from? If not, what additional information should we provide?
Response: FDA should be congratulated for the data collection exercise and attempting to record the data as clearly as possible. It is vital that all data are transparently collected. It is well known that this was a significant problem in the original Munro data set i.e. the source of data, especially the selection of the NO(A)EL was sometimes not clear.
I strongly encourage FDA to include a transparent and fully documented and curated database of compounds, experimental details and the source of data (as, for instance, with COSMOS DB, see Yang et al (2017, https://doi.org/10.1016/j.fct.2017.08.043; 2021, https://doi.org/10.1016/j.comtox.2021.100175). This could even be linked to the questions / decisions i.e. which data relate to which alert etc. There is reference to the "EDT DB" at the end of Section 2.1, paragraph 3, and this must be made available and future-proofed, i.e. adequately described such that it could be updated by other workers not involved in this project.

Question 10: Has FDA clearly explained the study selection criteria and provided adequate information and/or data to support its opinion that these criteria are appropriate for data inclusion in the DB? If not, what additional information should we provide?
Response: It is essential that the study selection criteria are reported and explained. This appears to be done and must be recorded in the database. I am not aware of any other information that should be provided as long as it is clear how a decision on e.g. the selection of a NO(A)EL, LO(A)EL etc. has been made. This should be more obvious when the data are recorded in the database, and I can attempt to follow how a decision has been made.
The study selection criteria would be clearer if the list of criteria, e.g., in Section 2 were put in a table, this could include how many data were taken from each source, the source etc.
Whilst it may appear trivial to FDA, something that could be reported is a "worked example" of how the value has been determined and the decisions made. It should be remembered that most TTC users do not appreciate the analysis of the original data. This could also allow for the easier updating of information if more data / knowledge become available and the criteria are updated.

Question 11: FDA used various factors based on study duration to derive duration adjusted no-effect-levels (NELs) to estimate chronic NELs. Has FDA provided adequate information and/or data to support its opinion that these duration adjustment factors are adequate to derive chronic NELs? If you generally agree, are there any exceptions in which these factors might be problematic to the derivation of duration adjusted NELs?
Response: As previous two responses, it is vital that the adjustment factors are recorded transparently, as well as the process of applying them (this could be included in the aforementioned "worked example". The FDA has done a thorough and rigorous job of defining and applying the factors to derive the NEL. As before, it would be clearer if it could be summarized within a table, but this is a minor issue.
Other comments:

- Section 2: reference to the "Original" EDT... I found the use of the adjective "original" in the title to Section 2 quite confusing. If there will be different versions of the EDT then I would recommend referring to them as ver. 1.0, 1.1. 2.0 etc. rather than "original".

Questions for section 3 (The Preliminary (Pre-validation) Threshold of Toxicological Concern Levels):

Question 12: Based on Figure 2 and all other information provided, in your opinion, does the EDT better resolve the differing toxic potentials of chemicals with broad structural variation compared to the CDT? Please explain why or why not.
Response: Unfortunately, I do not feel I can give a definitive answer to this question! To do this would require a greater understanding of the data, illustration of the data distribution etc., this has not been provided. It is also difficult to give a definitive answer as the functional groups etc. will be associated with a range of potencies – I agree that some are intrinsically more toxic than others e.g. replacing a primary alcohol by a nitroso group will make the molecule more toxic. However, the data distribution is dependent on the compounds that have been tested.
Whilst I cannot give a definitive response, I do believe the EDT provides a much improved decision tree to support TTC analysis. The classes have been updated with contemporary knowledge and understanding, as well as newer data. This, in itself, gives me greater confidence in the use of the EDT.
Comments on the data in Table 2:

- The difference between the total number of substances in each class and that used for the TTC calculation should be carefully explained and the compounds omitted also included in the data (with reasons for exclusion).
- Class VI: I have no doubt FDA will be criticized that this class has a low number of substances (just as Cramer Class II in Munro has been). The reason for the low number is obvious i.e., these are unique and identifiably highly toxic compounds so not many will be tested. Whilst there may well be criticism, I would like to offer my support for this class, despite the low number of substances.
- Could further analytics be added to Table 2 e.g., range of molecular weights (this should be part of the applicability domain) and even range of NELs (along with 5^{th} / 95^{th} percentiles to make it more realistic)?
- It might be helpful to explain why molar units are used rather than mg, and how does this affect the distribution (remember since Munro et al 1996 we have traditionally used mg values)

On a note of presentation. Fig 2 in the FDA review document (Overlap of NELs...). My preference would for the x-axis (log NEL) to increase in value from left to right, this is more logical when thinking of dose data and consistent with Fig 2 in Munro et al (1996). It may also be possible to indicate the 5^{th} percentile such that the derivation of the TTC value would be apparent.

Questions for section 4 (The Validation of the Expanded decision Tree):

Question 13: Has FDA clearly explained the source of the validation DB and how the data was verified pre-validation? If not, what additional information should we provide?
Response: The FDA indicate clearly that the validation DB was harvested from the US Environmental Protection Agency (EPA) ToxVal DB. It would be good practice to give the URL / reference for the ToxVal DB (obviously it can be found with an internet search engine, but that is not the point). Also, the version number of ToxVal DB, dates of data collection etc. should be given. The purpose of such detail is that any updates in ToxVal DB can be accounted for.
The verification of data is described in Section 4.3.4. There is a really interesting and important statement here that the original study report often did not agree with ToxVal DB (as I understand what is stated). As an aside, this should be reported and the reasons for it established. Section 4.3.4 states that "our own judgement" as used to settle on the NOAEL. This is a potential area of ambiguity, I am assuming that the process is as described in Section 4.3.5, although that is not made clear. Regardless, FDA should ensure that this process is described fully (Section 4.3.5 is probably adequate) and all decisions are recorded in the database. As with my response to Question 10, this may be a place where a "worked example" would help others understand how this has been done.

Question 14: Has FDA clearly laid out how the validation DB received from EPA was processed to enable its use for the external validation of the EDT? If not, please explain why not.
Response: The FDA has clearly listed how the validation DB was processed.
Some comments:

- Was an IUPAC name recorded (where available)?
- Which SMILES code was used? There are multiple types of SMILES, plus many ways of recording the same (e.g. toluene could be Cc1ccccc1 vs c1cc(C)ccc1 vs CC1=CC=CC=C1 etc). I would recommend a single approach is used e.g. canonical SMILES. The other option is the use of InChI / InChIKeys, which would be a good standard identifier anyway. This point is vital to identify duplicates both within the Validation DB and the EDT DB.
- Section 4.3.2. Applicability domain is mentioned here but much less so for the EDT DB. There is an opportunity to properly investigate the chemical space of EDT DB and whether the Validation DB meets this. For instance, this could be achieved with Principal Component Analysis of descriptors / fingerprints, or many of the widely used machine learning techniques.
- Sectio 4.3.3. Read-across data. The FDA is correct not to use read-across data. Did the data for the "source compound" of the read-across enter the same evaluation procedure as the ToxVal DB data? Data from ECHA's DB are notoriously difficult to use, as well as having the problems of commercial sensitivity.
- Typos
    - Section 4.3.1. Paragraph 1, line 4 "exists" should be "exist"
    - Section 4.3.1. Paragraph 2, line 1 delete space in "counter ion"

Question 15: Has FDA provided adequate information and/or data to show that the validation DB was processed appropriately for its intended use? If not, what additional information should we provide?
Response: This question is also difficult to answer without the results, presumably which will be released as part of Phase II.
It would have been helpful to clearly define what the meant by and required from the term "validation". Validation means different things to different people. Was the purpose here to allow for regulatory acceptance? I assume not... I believe the validation process was to evaluate the performance, whether it is fit for purpose etc. As such, I think Section 4.1 should describe more fully what is intended here.
I agree with and support the process of evaluation described in Section 4.1. However, it would be useful to define properly what is meant by the EDT TTCs being "protective", I assume this means the TTC is significant below the NEL (how far below?). Section 4.1 ii) states the validation will "accurately predict

the chronic oral toxicity....". Surely the purpose of TTC is not to predict toxicity, that is more like QSAR / Read-across. I appreciate these comments may be addressed by Phase II.
So, I believe adequate information is provided to show the data were processed appropriately, but the problem formulation could be improved.

Phase II:

Questions for section 4 (The Validation of the Expanded decision Tree):

Question 16: Some of the pre-validation EDT questions were updated, and some new sub- and sub-sub-questions were created based on the validation results. Has FDA provided adequate information to justify all updates? If not, which changes/updates were not fully justified and what information should we provide to justify them?
Response: I found that the updates to the pre-validation EDT questions were well explained and justified. They gave me sufficient detail to understand why the update had been made in a clear and unambiguous manner. It is certainly impressive how much additional work, post-validation, that has been performed and how much knowledge was extracted from the whole validation procedure.

Question 17: Was the validation adequate to show that the EDT is suitable for the classification of compounds in its applicability domain according to their toxic potentials? If not, describe what type of validation would be needed.
Response: As there is no advice to the contrary, I take the term "applicability domain" to be as it is used with regard to a QSAR. That is the applicability domain is a representation of the chemical and structural property space, including metabolism and, where possible toxicology and mechanism of action. Therefore, I take this question to be asking whether the domains for the six classes have been assessed with the validation exercise.
My personal opinion is that the validation exercise was an extremely useful and valuable activity, but it has shown little about the applicability domains within the EDT. By this I mean that the applicability domains within the EDT were not defined as I would like and as a user would find helpful. For instance, it would be helpful for each of the six classes to be summarised in a figure with the types of chemistry that are associated with them. This is obviously cross referenced against the questions. Further the applicability domain could give an indication of the range of physicochemical properties, for instance log P, molecular weight etc for the compounds associated with them in the supporting database. Hopefully the software that is being developed could have this type of functionality, so that a user would be able to know, at least in broad terms, whether their compound falls within the domain of the EDT.
In terms of the validation exercise that could be undertaken, the prevalidation database could be defined as described above, with regard to structural features and properties, and the validation database mapped onto those properties and features for each of the six classes.

Question for section 5 (Conclusions):

Question 18: Has FDA provided adequate information and/or data to support the conclusions found in this section? If not, what additional information should we provide?
Response: A number of broad conclusions are given in Section 5. I believe the report in its entirety has provided adequate information to support the conclusions made in Section 5, with the possible exception of read across, as noted below.

- I agree on the hypothesis that structurally or metabolically similar chemicals can be expected to be toxicologically similar. I agree that the FDA has made a laudable effort and great improvement in the capability of grouping chemicals, establishing trends and using the TTC approach in chemical safety assessment. I also agree that the groupings are based on a large and reliable database of publicly available toxicology data. However, I find it more difficult to support the conclusion that there were many ADME data considered (see, for instance the middle of the first paragraph of the conclusions).

- At the end of the first paragraph reference is made to performing read-across. I accept that the knowledge within the EDT, particularly the structural groupings, will be of great benefit to performing read across and I am excited by this prospect. However, this is a topic that is not developed in the report so I cannot agree that FDA has provided adequate information to support this particular conclusion - whilst I do accept it is valid.
- I can accept in the second paragraph the statement about the level of data available and that the EDT shown that further data can be added in to improve and expand the analysis. I fully support the conclusion that periodic review will help reaffirm and strengthen the scientific basis of the EDT, and I hope that this will be possible and undertaken in the future by FDA or others.
- I also agree with the third paragraph that the EDT will be an invaluable tool for application with TTC and I anticipate that this will become the industry standard and then their future.
- Finally, I can support the conclusion that the EDT, as part of a tiered strategy or an initiative such as Next Generation Risk Assessment, will be a valued part of reducing animal use in, and the cost of, chemical safety assessment.

Appendix 1 aims at providing a brief explanation of each EDT question. By no means are these explanations meant to be. With that in mind, please respond to the following questions.

Question 19: Are all explanations clear and concise? If not, please identify the explanation by question number and elaborate as to how we can more clearly explain the question.
Response: I am basing my response to Questions 19 and 20 with regard to Appendix 1 that was provided in the first document for review (Phase I). It is noted that this has not been updated post validation. I apologise if I have this incorrect.
I found the explanations in Appendix 1 clear and correct. I believe that in combination with the details on the questions, these will be very valuable for users of the EDT.

Question 20: Should FDA add anything to these explanations to improve the reader's understanding of each question's rationale? If yes, please identify the explanation by question number and explain how we should revise. Please note that these explanations were designed to be concise and not all-encompassing.
Response: I found Appendix 1 to be clear and will aid the reader's understanding. They are written at the correct level, i.e. a toxicologist (or risk assessor) with a knowledge of physiology, biochemistry and chemistry. They are well referenced and with adequate evidence.
As noted in response to a previous question, I hope that it will be possible to combine the information in Appendix 1 with the questions in some way, hopefully in the software, such that the user can see both together. My only comment with regard to making Appendix 1 clearer for the reader would be to add a title to each question, summarizing and describing the individual EDT questions. Referring to the previous question on applicability domain – aspects of this could be summarized here, as well as exemplar compounds with data. It would also be good to have an indication of how many compounds in the database were classified according to the EDT question.

Appendix 2 contains the combined, finalized EDT Chemistry, Toxicology and Metabolism DB on which the finalized TTCs were based.

Question 21: Are the set of chemicals in the database sufficient to cover the chemical domain of applicability described in the document? If not, please explain.
Response: This is a very difficult question to answer. The FDA has assembled a very large database of toxicological information, having carefully curated the values. This, in itself, is a great achievement. I am assuming from the question that the "chemical domain of applicability" described in the document relates to the EDT questions. In order to address this question, I would need to see a breakdown of how many compounds were associated with each question and each assignment of a class (many EDT questions have multiple classes).

In order to understand the applicability domain of the classes, I refer to Table 8 (pages 131-132) in the Phase II document. With the exception of Class VI, there are a significant number of data points in each class. Without analytics, e.g. structural features, ranges of properties etc., it is not possible to give a definitive answer with regard to Classes I to V. Class VI is quite unique and, as noted in the report, it has limited numbers of compounds due to the high toxicity associated with this class. I have no doubt of the need of Class VI and its validity. Despite the small number of data, I am satisfied Class VI is relevant and significant.

Overall question:

Question 22: Do you have any other comments or suggestions?
Response: I fully acknowledge the work that has been undertaken to create the EDT, its validation and the preparation of the database. I understand that it has been many years in the making, and the complexity and level of detail as well as coverage across chemical classes is testament to the hard work of the FDA. I look forward to the publication(s) that will arise from the EDT and encourage FDA to include as much detail as possible. This is particularly the case because we know from the original Cramer scheme there was much ambiguity. This is, of course, not a criticism of the original scheme which was quite revolutionary in its time. I also hope that FDA will promote the scheme, e.g., at conferences, webinars, social media etc. There will also be a need for training in the new scheme and this in itself is an opportunity for dissemination and to increase understanding in the whole concept of the EDT and TTC. The "make or break" for the EDT will be the software platform on which it is based. I really look forward to seeing this and I hope there is an opportunity to start with a clean slate, rather than seeing it as an update of the implementations of the Cramer scheme. I hope the informatics implementation will be robust and representative of the chemistry as described in the EDT. Anyone who has used the implementations of the Cramer scheme well understand the limitations and ambiguities. I would also encourage the FDA to link the EDT to the data in the database, as well as provide a full description of the classes and questions, investigating property and chemical structure space - there is an opportunity here to implement some analyses with, for instance, the ToxPrint fingerprints and assessment of chemical / property space. I realise, however, that a wish list for the software is going beyond the remit of the charge questions!
One further comment: Phase II report. Page 17. Section 4.4.3.20. I assume the reference to Appendix 3 should be Appendix 2?

2.0 SUBJECT MATTER EXPER SUBJECT MATTER EXPERT (SME) — Dr. Kim Li

Phase I:

Questions for section 1 (The Expanded Decision Tree (EDT)):

Question 1: Has FDA clearly explained, with adequate examples, all guidelines and definitions for use with the EDT? If not, please provide suggestions for alternate text and/or additional examples.
Response: Overall, the guidelines and definitions for use with the EDT are clearly explained. However, it is noted that some definitions are composed specifically for the EDT questions and therefore do not have the same meaning as in the general literature, which created some challenges:

- 1.5.J: EDT evaluates the organic salts and metal ion salts as their neutral forms. While this simplifies the decision process and may not impact the final classification, it contradicts the general understanding that valence states often determine the reactivity and therefore toxicity outcome.
- 1.5.K. EDT defines "hydrolysis" as addition of element(s) of water to a molecule leading to either a different molecule or multiple molecules. EDT does not define "reduction" but refers to hydrolysis or reduction of functional groups in Figure 1. It would be useful to define both terms to improve illustration in Figure 1.

- 1.5.N. "Corresponding" hydrolysis products are described and illustrated for primary and secondary alcohols. The structures have R1 to R5 substitutions but only R3, R4 and R5 are described.
- 1.5.R. The text refers Bridgehead atoms to definition AA which may be a typo.
- 1.5.X. The structures illustrate PHAs with the solo, duo, trio and quartet bonds which form bay and/or fjord regions. Please describe the difference between the bay and fjord regions.

Question 2: Are all EDT questions clear as to which structural features they are describing? If no, please identify the question by its number, explain why you find the question ambiguous or confusing, and suggest alternative text to ensure that it is clear what kind of structural features the question is aiming to capture.
Response: Almost all the EDT questions are clear on the structural features and those with definitions are bolded for ease of reference. Colors are sometimes used to call out the structural features, and this is particularly helpful. However, Question 2 is extremely difficult to follow. In this question, the "=" can be a double bond or may mean "equal to".

Question 3: Most questions place compounds into one of six classes of toxic potential depending on their structural features. Does the EDT place the type of compounds that are captured at each question into the appropriate class of toxic potential? If not, please explain why and provide a recommendation for the appropriate class of toxic potential.
Response: Section 1.8 provides the rationale for the EDT classification based on structural features and predictions for metabolic activation/detoxification/biological reactivity. The 47 questions in the pre-validation EDT illustrate how appropriate EDT Classes are determined. Appendix 1 provides the short explanation for each of the 47 question and sub-questions. Without Appendix 1, rationale behind each question can be easily lost.
Section 1.8 also defines Classes I through VI with high level examples for each class. The significant toxicity endpoints are addressed in the Charge Questions, with the exception of skin sensitization and systemic hypersensitivity. If no consensus is reached whether or not EDT should address these endpoints, it would be good to highlight these for future research efforts.
While most questions are challenging for a non-chemist, Question 28 is particularly difficult. This question is a terminal question that covers a large number of biologically reactive moieties with increased potential for toxicity and assigns them to Classes III, IV or V. Appendix 1 gives a detailed description of the compounds and the associated mechanisms of action and toxicity endpoints (e.g. cardiovascular toxicity, neurotoxicity, carcinogenicity, oxidative stress). It would be helpful to provide the basis for sub-questions i), ii), iii), iv) and v) in ranking these toxicity endpoints into Classes III, IV or V.

Question 4: Commonly, structurally related compounds (e.g., γ-diketones) can have common toxicological endpoint (in this case γ-diketone type neurotoxicity). Compounds that can either hydrolyze and/or metabolize to these compounds can exhibit the same type of toxicity. FDA aimed to capture hydrolytic and metabolic precursors of structurally related compounds with similar toxicities at numerous questions. Are there any questions where you can suggest any possible metabolic and/or hydrolytic precursors to the types of compounds addressed that are currently not mentioned/captured in the question?
Response: The proximate carcinogens may help with this question. Perhaps they can be captured in the questions with PAHs.

Question 5: Are the example structures provided after each EDT question correct and adequate for understanding what type of compounds the question aims to capture? Are there different or additional example structures for any of the questions that would help increase the understandability of the question?

Response: The example structures are challenging for non-chemists. In compounds with substitutions, it would be helpful to label the carbon positions. An example is the structure in Question 32 d), 7-oxocyclohepa-1,3-dien-1-yl propionate.

Question 6: Are there any congeneric groups that the EDT does not adequately address, but for which enough safety data exist that could serve as the basis of additional EDT questions to address these groups? If yes, please identify and provide all toxicological data for the congeneric group(s) that may form the basis of one or more additional questions. If possible, please propose the wording for such additional EDT questions. (Substances within a congeneric group are structurally and metabolically similar.)
Response: Siloxanes are often detected as extractables/leachables from food packaging materials. There are linear and cyclic structures with a wide molecular weight range. There is a wealth of toxicology information that can be leveraged for EDT questions.

Question 7: Should any questions be further subdivided to ensure a more refined grouping of related substances? If yes, please suggest wording for the refined question(s) and provide the data justifying the suggestion.
Response: The groupings appear adequate at this point. When the EDT database is shared in Phase II, we can revisit this question.

Question 8: Are there any terms used in the EDT questions that should be added to the guidelines and definitions section to help users of the EDT? If yes, what additional terms should we define?
Response: Section 1.5 provides the guidelines and definitions which are very helpful for the EDT questions. Many endogenous and exogenous compounds may exist as stereoisomers and enantiomers, e.g flavonoids in Question 15. It may be helpful to add definitions for the common isomeric forms.

Questions for section 2 (The Expanded Decision Tree Chemistry, Toxicity, and Metabolism Database (EDT DB)):

Question 9: Has FDA clearly explained where the toxicological data found in the EDT DB were collected? If not, what additional information should we provide?
Response: Section 2.2 provides criteria for data collection. However, the source of the toxicological data is not explained until Section 4.2 Creation of the External Validation Database.

Question 10: Has FDA clearly explained the study selection criteria and provided adequate information and/or data to support its opinion that these criteria are appropriate for data inclusion in the DB? If not, what additional information should we provide?
Response: Section 2.2 explains the criteria for study selection in the original EDT database. Table 1 shows the distribution of studies in the original EDT DB based on the exposure route. While there are few subcutaneous, intravenous, and intraperitoneal studies individually, please consider combining these routes and analyze them collectively for parenteral route.
Section 4.3.5 gives a brief description of the selection criteria for the studies in the external validation database. It is not surprising that there are conflicting data that require best judgement by the EDT panel of experts. The example provided for Tolclofos-methyl is very helpful. It would be good to understand the EDT panel peer review process.

Question 11: FDA used various factors based on study duration to derive duration adjusted no-effect-levels (NELs) to estimate chronic NELs. Has FDA provided adequate information and/or data to support its opinion that these duration adjustment factors are adequate to derive chronic NELs? If you generally agree, are there any exceptions in which these factors might be problematic to the derivation of duration adjusted NELs?

Response: The derivation of the duration adjustment factors is discussed in Sections 2.2 and 3.3. A duration factor of 1 is used for studies > 98 days, 3 for studies 84-98 days, and 10 for studies < 84 days (Section 2.2, p. 80). The 3 distinct durations are non-conventional, but the reason is explained later (Section 3.3, p. 85). Further, the factors used by ICH Q3D, ICH Q3C(R6), ECETOC, ECHA and REACH are reviewed. The final decision for EDT to use the middle ground values and to continue with the Munro et al (1996) approach to establish the Cramer Decision Tree classes was also explained. While the EDT factors reflect a pragmatic approach, they will likely raise questions when compared to science-based principles as presented in ICH Q3C(R6) Appendix 3. Of important note is that ICH accounts for species-specific lifespan and life-stages.

Questions for section 3 (The Preliminary (Pre-validation) Threshold of Toxicological Concern Levels):

Question 12: Based on Figure 2 and all other information provided, in your opinion, does the EDT better resolve the differing toxic potentials of chemicals with broad structural variation compared to the CDT? Please explain why or why not.
Response: Figure 2 appears to give better resolution of the 6 EDT classes than the 3 CDT classes. EDT proposes to use mmol/kg bw/day for NEL. The rationale and literature support are explained in Section 2, p. 80. Although the large MW compounds such as proteins and polymers are out of scope, the MW range for the EDT database may still be quite wide. In addition, a single molecule may have multiple alert features and/or reactive sites on the same molecule. It would be good to compare how the proposed unit works for the more complicated molecules that exhibit multiple toxicity endpoints.
Table 3 compares the pre-validation EDT TTCs with and without use of the median MW. It would be helpful to include the MW ranges in the table. High MW compounds may have been excluded for various reasons. Is there a cutoff for MW before a large complex organic compound is excluded?

Questions for section 4 (The Validation of the Expanded decision Tree):

Question 13: Has FDA clearly explained the source of the validation DB and how the data was verified pre-validation? If not, what additional information should we provide?
Response: Section 4.2 describes the EPA ToxVal DB as the original source. By applying filters, a small subset was created with defined structures with sub-chronic and chronic oral studies and derived NELs. The processing and verification for pre-validation appears robust (Section 4.3).

Question 14: Has FDA clearly laid out how the validation DB received from EPA was processed to enable its use for the external validation of the EDT? If not, please explain why not.
Response: Section 4.3 provides details on the processing and verification of the data received from EPA with respect to elimination of duplicate substances, applicability domain, read-across data, selection of NEL, selection of best representative study, as well as dose and purity adjustment. The details are clear and well laid out.

Question 15: Has FDA provided adequate information and/or data to show that the validation DB was processed appropriately for its intended use? If not, what additional information should we provide?
Response: Please see comments to Questions 13 and 14. The FDA has provided adequate information and/or data for the pre-validation DB. Section 4.3.7 points to the additional processing of data. This additional information will be useful in Phase 2.

Phase II:

Questions for section 4 (The Validation of the Expanded decision Tree):

Question 16: Some of the pre-validation EDT questions were updated, and some new sub- and sub-sub-questions were created based on the validation results. Has FDA provided adequate information to justify all updates? If not, which changes/updates were not fully justified and what information should we provide to justify them?

Response: The updates to the pre-validation EDT questions are very helpful, based on the validation results. FDA shows great care to address the comments from the chemists and provide thoughtful resolutions for toxicological evaluations. Below are general comments for consideration, much of which is probably already work-in-progress for the next phase of EDT development. Further insights on the ongoing updates would be much appreciated. Below are general, high-level comments with cross-reference to other charge questions as relevant and appropriate.

- Appendix 1: The updates are explained in Section 4.4 and corresponding edits are tracked (red) in Section 4.5.4 for the post-validation, finalized EDT questions. Appendix 1 provides short explanations for the pre-validation EDT questions. Now that the EDT questions are finalized, it would be very helpful to integrate all the changes and update Appendix 1. [Please also see also comments to Appendix 1, Charge Questions 19 and 20]
- Read-across: In Phase II, read-across is introduced as a very important scientific tool to fill data gaps for data-poor compounds in congeneric groups. Read-across has helped to greatly expand the number of compounds in the final EDT database. I am in total agreement to integrate read-across into EDT. While there is no consensus standard in the scientific or regulatory communities on read-across methodologies, please consider elaborating on the principles, key criteria and literature references (Escher et al., 2019; European Chemical Agency, 2017; Firman et al., 2021; Patlewicz et al., 2024; Patlewicz & Shah, 2023; Punt et al., 2020). With the recent advances in computational (Q)SAR modeling, it would be helpful to compare available software programs such as EPAs GenRA and ECHA's OECD Toolbox and provide transparency on the EDT read-across approach. [Please also see comment to Charge Questions 17 and 18]
- Bioavailability: The EDT DB collects data on toxicokinetic and metabolic fate of substances to evaluate the influence (or lack) of absorption and metabolism on the toxicity of the substances, rather than gathering comprehensive ADME datasets (Section 2.1). It is true that absorption is among the key determinants of the bioavailability of substances. However, oral absorption does not truly represent systemic (internal) absorption. One scenario is that substances that are poorly absorbed may be rapidly eliminated to a large extent. Such substances may be biologically inert and eliminated from the body with no adverse effects. Then there is another scenario where reactive substances may be sequestered/bound in body organs/tissues, resulting in low distribution and low elimination. Therefore toxicological risk assessments would benefit from use of broader ADME understanding to predict systemic (internal) rather than oral (external) exposure dose (Partosch et al., 2015). Commercially available software programs, e.g. ACD Percepta (http://www.acdlabs.com/products/percepta/), may be useful for this purpose. [Please also see updated comment to Question 12]

Question 17: Was the validation adequate to show that the EDT is suitable for the classification of compounds in its applicability domain according to their toxic potentials? If not, describe what type of validation would be needed.

Response: The finalized external validation DB contains 1,242 substances bringing the Combined EDT DB to 3142 substances (Appendix 2). In order to evaluate whether they are suitable for their classification in the applicability domain, it would be important to clearly define the EDT DB domain and describe the criteria and validation for QSAR modeling purposes. In Section 4.3.2, there is only mention of compounds eliminated when not in the applicability domain: "unhyrolyzable polymers, proteins, inorganic substances, and substances with undefined structures in addition to most mixtures". In Section 4.6.1 and Section 4.6.2, there is intention to broaden the applicability domain but without explicitly defining the domain. A recent publication by Mora et al (2024) describing applicability domain analysis in QSAR models may be helpful.

For the EDT DB domain, it would be helpful to identify the compounds by their intended uses, e.g. as direct/indirect food additives, monomers, plasticizers. In addition, many of the substances are identified as naturally occurring, e.g. plant flavonoids (Appendix 1 explanation on Charge Question 15).

Identifying naturally occurring substances would help assess the applicability domain of the EDT DB for the dietary intake of the general population as well as the special populations at risk. The suggestion is not to burden the EDT DB with naturally occurring substances of low order of toxicity. Rather it is to understand whether the EDT DB includes naturally occurring substances with known significant bioactivity and potential toxicity.
ClassyFire is a freely accessible program (http://classyfire.wishartlab.com/) that classifies chemicals into structure-based taxonomy (Djoumbou Feunang et al., 2016). It may expedite classification of the substances in final EDT DB into chemical families and/or congeneric groups and provide further support for applicability domain, as well as read-across approach.

Question for section 5 (Conclusions):

Question 18: Has FDA provided adequate information and/or data to support the conclusions found in this section? If not, what additional information should we provide?
Response: The EDT project is a monumental undertaking and reaching Phase 2 is a significant milestone. The conclusion acknowledges that as additional information and data become available, the EDT can be further refined. The current conclusions are based on the premise that substances of similar structures are expected to have similar metabolic fate and to lead to similar toxicological outcome. However there is desire for deeper understanding and interpretation of more comprehensive ADME datasets, particularly how oral absorption translates to systemic bioavailability. Given that the oral studies make up about 96% of all the studies in the EDT DB, it would be of great value to the scientific community if EDT DB can relate to other exposure routes by modeling bioavailability and can provide guidance on route-to-route extrapolation factors.
FDA considers EDT as a tool for toxicologists to predict toxic potentials of compounds and provide safe intake levels (TTC). The sequence of chemical structure-based yes/no questions leads to the assignment of the substance to one of the 6 classes of TTC. These questions may be intuitive to a chemist but they are difficult for a toxicologist to follow. However, we are encouraged that the EDT software is currently in development to automate read-across. We look forward to the next phase of collaboration between the chemists and the toxicologists for a user-friendly automated computer program.
As the applicability domain grows with additional external data, the EDT DB will be periodically reviewed, assessed and refined. The scientific community would certainly welcome broadening of the domain and the transparency to the ongoing data collection and nomination process.

Appendix 1 aims at providing a brief explanation of each EDT question. By no means are these explanations meant to be comprehensive. With that in mind, please respond to the following questions.

Response: The updates for Phase 2 are explained in Section 4.4 and corresponding edits are tracked (red) in Section 4.5.4 for the post-validation, finalized EDT question. Appendix 1 provides short explanations for the pre-validation EDT questions. Now that the EDT questions are finalized, it would be very helpful to update Appendix 1 to facilitate review of the finalized questions.

Question 19: Are all explanations clear and concise? If not, please identify the explanation by question number and elaborate as to how we can more clearly explain the question.
Response: Appendix 1 gives a detailed description of the compounds and the associated mechanisms of action and toxicity endpoints (e.g. cardiovascular toxicity, neurotoxicity, carcinogenicity, oxidative stress). The terminal question, Question 28, was updated but it is still confusing (to a toxicologist). It would be helpful to provide the basis for this terminal question, sub-questions i), ii), iii), iv) and v) in ranking these toxicity endpoints into Classes III, IV or V. For example, is weighting applied to the endpoints to account for severity leading to different Class assignments?

Question 20: Should FDA add anything to these explanations to improve the reader's understanding of each question's rationale? If yes, please identify the explanation by question number and explain how we should revise. Please note that these explanations were designed to be concise and not all-encompassing.
Response: Integrating the updates in Section 4.4 and corresponding edits (Section 4.5.4) into Appendix 1 would be crucial for fuller understanding and feedback to the explanations. Question 28 is the critical terminal decision question that classifies substances into EDT Classes III, IV or V. Please consider additional details on the weighting and ranking of the toxicity endpoints that differentiate these higher classes

Appendix 2 contains the combined, finalized EDT Chemistry, Toxicology and Metabolism DB on which the finalized TTCs were based.

Question 21: Are the set of chemicals in the database sufficient to cover the chemical domain of applicability described in the document? If not, please explain.
Response: Question 21 (for Appendix 2) and Question 17 (for Section 4) both relate to suitability/adequacy of the chemical and applicability domain of EDT DB. Specifically for Appendix 2 (spreadsheet), please consider:

- Adding MW unit to Column E (i.e. µg/kg bw/day or mmol/kg bw/day)
- Add footnote or text to explain Column E on how molecular weight is adjusted based on # of subunits. It is not clear if subunits refer to repeating units as in polymers and/or copolymers.
- Adding column for intended/approved use of the compound (e.g. direct food additive, indirect food additive, polymer production, monomer, plasticizer, catalyst, cross-linking agent, curing agent) (Sheftel, 2000)
- Adding column for Chemical congeneric group/class/family for each compound
- Adding column for critical toxicity endpoint(s) on which the EDT class is based

Question 21 does not specifically ask the reviewers to critique the critical studies selected for the derivation of NELs or LELs. Perhaps this is planned when new and significant studies are published.

Overall question:

Question 22: Do you have any other comments or suggestions?
Response: It is quite a humbling experience for a toxicologist to read through the rationale, the thought processes and actual structure-based decision steps that help transform CDT to EDT. Toxtree, the software based on CDT, has been the pragmatic solution to predict and screen toxicity potential of data-poor compounds and to classify them into Cramer Classes I-III to estimate the corresponding Cramer TTCs. The desire for EDT to broaden the chemical domain and to advance the structure-based framework is admirable. In Section 4.6.3, it is stated "Going forward, the finalized EDT TTCs will be used." The process to achieve consensus with the scientific and regulatory communities is of utmost importance to share the EDT framework, scientific rationale, the derivation of the EDT Classes and TTCs through workshops and conferences on the ongoing transformation efforts.

3.0 SUBJECT MATTER EXPER SUBJECT MATTER EXPERT (SME) — Dr. Mark Nelms

Phase I:

Questions for section 1 (The Expanded Decision Tree (EDT)):

Question 1: Has FDA clearly explained, with adequate examples, all guidelines and definitions for use with the EDT? If not, please provide suggestions for alternate text and/or additional examples.
Response: For the most part all guidelines and definitions are clearly explained with adequate examples. Below are the areas where I think there could be some updates to make things a little clearer.

- Section 1.5

- I think it might be best to rearrange the first 6 bullets to be ordered like this: Aliphatic, Acyclic, Alicyclic, Aromatic, Heterocyclic, Heteroaromatic.
- Alicyclic – maybe change to say: "means the presence of at least one ring composed of only carbon atoms with our without the presence of ring alkene(s) (i.e., C=C) that do not form an aromatic ring."
- Heterocyclic – maybe change to say: "means the presence of a ring with at least one ring atom that is not carbon…"
- Heteroaromatic – maybe change to say "means that the substance contains at least one ring, which is composed of at least one ring heteroatom…"
- Pseudo-aromatic – maybe rearrange to say "…by incorporating the election pair of a functional group into an enolic double bond…"
- Oxygenated functional group – maybe it'd be good to reference Figure 1 for users to refer to to see example structures.
- Corresponding – should "Corresponding" be in bold? It's a little confusing that the word being defined is being used in the definition, maybe you could use "equivalent" instead?
- Related – should "Related" be in bold?
- Bridged – this is very minor, but it might be helpful to show the examples in the order they are defined (i.e., Bridged, Fused, Spiro, Singly bonded).
- Electron pair donors – it might be useful to have a figure illustrating some of the SMARTs patterns outlined (e.g., ester, carboxylate, etc.)
- Solo, duo, trio, quartet – I'm unsure what this definition is trying to say. Is it that each of the carbons must be bonded to an atom other than an atom in the aromatic ring, rather than "can be"? That is, the carbons have to be on the outside edge of the structure.
    - Should there also be a definition of what the Bay and Fjord regions are?

Question 2: Are all EDT questions clear as to which structural features they are describing? If no, please identify the question by its number, explain why you find the question ambiguous or confusing, and suggest alternative text to ensure that it is clear what kind of structural features the question is aiming to capture.
Response: I think for the most part, the EDT questions are relatively clear which structural features they are describing. Below are some areas where I have some suggestions that may help to make things clearer:

- Throughout – it'd be useful to have the sub-sub-bullets [e.g., i), ii), etc.] on a new line [like is done for the exceptions in Q1a)]. I think this would help a user more easily identify the different types of structures discussed in the question.
- Throughout – double check that all terms defined in Section 1.5 are bold [e.g., "corresponding" in Q1a), Q1b), Q1c), etc.; "α" in Q1g), Q1h), Q3a) etc.].
- Throughout – I think it'd be useful to have a sub-heading saying under each question identifying where the assignment answers are [e.g., If yes assign to Class X, if no proceed to QX].
- Throughout – When examples in Figures contain substructures and/or functional groups present in the question, I think it'd be helpful to highlight the matching substructure.
- In Q1 questions and Q3f) -, it'd be useful to underline the "and"/"or" before the sub-sub-bullets indicators [e.g., ii)]. This is used in Q2 and makes it easier to discern where the break is.
- Q1e) – Should these be "A monosaccharide, or hydrolysable oligosaccharide or polysaccharide…" The way I read the current version seems to suggest the substance needs to contain all substituents to be considered a "Yes".
- Q3g)ii) – azide – should the SMARTS pattern here be "$-N^{-}-N^{+}\equiv N$"?
- Q4 – for the albuterol sulfate example, I think it'd be helpful to include an illustration of the sulphate group with a note that it is to be disregarded.

- Q6 b)ii) – even though there is no "i)" sub-bullet in Q6, it may be more easily understood if this was rewritten as "the compound does not have any of the skeletal structures listed in b)i)" so it's not confused for i).
- Q6 c) – Is it the additional ring system that is to have the ≥2 oxygenated functional groups or the macrocyclic ring?
- Q7 g)iv) – Should this be "a halogen"?
- Q8 d) – Should the last line read "…ortho, meta, and para positions each (does not have to be on the same ring), and each ring must be substituted…"?
- Q12 e) I would recommend switching the "4≤" to "≤4".
- Q18 b) – Can any of the skeletons mentioned (up to the aromatic ring) be with or without the moieties mentioned (i.e., from the primary alcohols) or only the aromatic ring? This needs to be made clearer
- Q18 assignments – ii) is missing "If yes"
- Q22 – The wording of this is a little confusing, but I'm not sure how to reword it.
- Q30 a) – It's not too clear what is being described here. Is this question saying the aliphatic chain can have ≥1 of the functional groups or only the alicyclic ring(s)?
- Q45 – Can the substituents mentioned after alcohol be attached to the aromatic substituent mentioned just before (e.g., alicyclic ring, methylenedioxy, etc.), directly to the aromatic ring, or either?
- Q47 f) – Final sentence, reword to "In addition, other than the tetrahydropyran ring that is fully substituted, all rings should have…"

Question 3: Most questions place compounds into one of six classes of toxic potential depending on their structural features. Does the EDT place the type of compounds that are captured at each question into the appropriate class of toxic potential? If not, please explain why and provide a recommendation for the appropriate class of toxic potential.
Response: I think this question is probably best answered after I have received Appendix 2. That way I can look at some of the chemicals and go through the questions to assign a subset of the chemicals and compare what I get to the assignments in the Appendix.

Question 4: Commonly, structurally related compounds (e.g., γ-diketones) can have common toxicological endpoint (in this case γ-diketone type neurotoxicity). Compounds that can either hydrolyze and/or metabolize to these compounds can exhibit the same type of toxicity. FDA aimed to capture hydrolytic and metabolic precursors of structurally related compounds with similar toxicities at numerous questions. Are there any questions where you can suggest any possible metabolic and/or hydrolytic precursors to the types of compounds addressed that are currently not mentioned/captured in the question?
Response: There are no questions where I can suggest other metabolic and/or hydrolytic precursors that aren't currently captured.

Question 5: Are the example structures provided after each EDT question correct and adequate for understanding what type of compounds the question aims to capture? Are there different or additional example structures for any of the questions that would help increase the understandability of the question?
Response: The example structures appear to be correct and adequate for understanding the types of compounds covered by the questions.

- As mentioned above for Question 2 – I think something that could help increase the understandability of the question, especially where several substructures are listed [e.g., Q2b)], would be to highlight the substructure in the example chemical. I think this would help to identify the substructures much more easily.

- Q12 e) - In the example here, I think it would be useful to also use a substance with an ellagic acid backbone as an example, because it was mentioned in Q12 a) that these substances are dealt with here.
- Q47 g) – Should the example structures here be in Kekule form to match the other aromatic rings?

Question 6: Are there any congeneric groups that the EDT does not adequately address, but for which enough safety data exist that could serve as the basis of additional EDT questions to address these groups? If yes, please identify and provide all toxicological data for the congeneric group(s) that may form the basis of one or more additional questions. If possible, please propose the wording for such additional EDT questions. (Substances within a congeneric group are structurally and metabolically similar.)
Response: I'm not sure whether there are any other congeneric groups that have enough safety data where a question could be written that isn't covered by the EDT.

Question 7: Should any questions be further subdivided to ensure a more refined grouping of related substances? If yes, please suggest wording for the refined question(s) and provide the data justifying the suggestion.
Response: I'm not sure. This may be something I can better answer after looking at the chemicals in Appendix 2.

Question 8: Are there any terms used in the EDT questions that should be added to the guidelines and definitions section to help users of the EDT? If yes, what additional terms should we define?
Response: Maybe it would be useful to define the terms:

- Conjugated.
- Dimer
- Organyl
- Connector

Questions for section 2 (The Expanded Decision Tree Chemistry, Toxicity, and Metabolism Database (EDT DB)):

Question 9: Has FDA clearly explained where the toxicological data found in the EDT DB were collected? If not, what additional information should we provide?
Response:

- Section 2.1:
    - When discussing searching the literature, please mention what search terms were used and what was used to search (i.e., PubMed, Google). For example, what types of information/studies were searched for, just subchronic and chronic?
    - Where was information on food contact substances gathered from?
    - When mentioning study details included, did the substance need to have all this information to be included in the EDT DB?

Question 10: Has FDA clearly explained the study selection criteria and provided adequate information and/or data to support its opinion that these criteria are appropriate for data inclusion in the DB? If not, what additional information should we provide?
Response:

- Section 2.2:
    - I think this section would be easier to follow if there were headings relating to each criterion and they were discussed in turn, e.g., what species were allowed? What durations?
    - If an NEL wasn't identified by a study, did you convert the LEL to an NEL?
        - This is mentioned in Section 3.3 (i.e., no LELs were converted to NELs), but it would be good to mention that here too
    - In Section 4.3.6 it says that NELs in the EDT DB were adjusted based on the purity of the substance and dosing schedule, but that's not mentioned in this Section, please add that.
    - What species were allowed for inclusion in the EDT DB?
    - Page 80. line 22 – What number of animals are considered adequate to ensure statistical significance?
        - Did you go back and re-evaluate the statistical significance?
    - Page 80, line 24 – Did you re-evaluate if the reported effects were adverse or not?
    - Page 80, line 27 – "Limited reporting" on what? The number of animals, whether the effects were adverse, or something else? Please expand.
    - Page 80, line 33 – is the NEL for the shorter duration study the original NEL or the adjusted NEL?
    - Page 80, line 35-39 – What was the shortest duration allowed in the EDT DB? (Shortest in Munro was subchronic)
    - Page 81, line 32-36 – This example is a little confusing as written, suggest to swap around as:
        - For example, for aliphatic, alicyclic, or aromatic ketones or hydrocarbons of sufficient molecular weight and lipophilicity that cause α_{2u}-globulin-type nephropathy, an endpoint not relevant to humans, and observed exclusively in male rats, we used the toxicological data (e.g., NEL and LEL values) for female rats only for inclusion in the EDT DB.
    - Page 81, line 47-Page 82, line 4 – Both can't be true, either the offspring NEL was chosen if it was equal to the parent or the parent NEL was chosen.
- Section 2.3
    - Why did you decide not to have a separate class to distinguish (non-)genotoxic compounds?
        - The carcinogenic TTC identified by Kroes et al (2004) is roughly x10 lower than the Class III TTC.

Otherwise, the reasoning behind the selection criteria is clearly explained.

Question 11: FDA used various factors based on study duration to derive duration adjusted no-effect-levels (NELs) to estimate chronic NELs. Has FDA provided adequate information and/or data to support its opinion that these duration adjustment factors are adequate to derive chronic NELs? If you generally agree, are there any exceptions in which these factors might be problematic to the derivation of duration adjusted NELs?
Response: I think there is adequate information provided to support the use of the adjustment factors FDA chose when adjusting the NELs for a chronic duration, especially when converting the different lengths of oral studies. Unfortunately, there didn't seem to be any information about what conversion factors were used for the non-oral studies.

- Page 82, line 5 – What conversion factors (if any) were used for the non-oral studies? Please expand why a conversion factor was (not) used.

Questions for section 3 (The Preliminary (Pre-validation) Threshold of Toxicological Concern Levels):

Question 12: Based on Figure 2 and all other information provided, in your opinion, does the EDT better resolve the differing toxic potentials of chemicals with broad structural variation compared to the CDT? Please explain why or why not.

Response: Based on the information provided it does seem like the EDT better resolves the differing toxic potentials of chemicals compared to the CDT. This is because 1) the EDT Classes cover a wider range of NEL values than the three Munro classes and 2) the fact there are more EDT Classes means that the TTCs can be associated with a finer selection of chemicals with a more similar toxicity. However, there are some questions/suggestions I have that could be used to make this clearer and improve the comparison against the CDT:

- How many orders of magnitude does the CDT DB data span? This comparison would be useful to highlight the extra NELs the EDT covers.
- How were the number of EDT Classes chosen?
- If you have the CDT DB data and you're able to recalculate the TTCs in ug/kg bw/day, it could be good to convert the NELs in the CDT DB to mmol/kg bw/day and calculate the CDT TTC values using the median MW (similar to what you did for the EDT TTC). This would give you another way to compare the results for the CDT DB to those for the EDT TTC.
- Figure 2 is a nice graphic to illustrate the additional coverage provided by the extra EDT Class and what the distribution of NELs looks like. But it is a little difficult to distinguish where the lower NELs values are, so it might not be the best to show the decreased overlap in NELs. Maybe it'd be good to use a box and whisker plot or violin plot to show that?
- It might also be worth performing separate pairwise comparisons of the distributions for the CDT and EDT Classes to show whether, for example, the CDT Class I is statistically different from the EDT Class I/II. Or whether the EDT Class I is statistically different from the EDT Class II, etc.
    - This could be done using a metric like the Kolmogorov-Smirnov test.
- What CDT Class are the chemicals in the example on Page 86, line 23 in? Do these chemicals fall into the same CDT Class?
- This may be something for an earlier Section, but how was the default EDT Class chosen?

Questions for section 4 (The Validation of the Expanded decision Tree):

Question 13: Has FDA clearly explained the source of the validation DB and how the data was verified pre-validation? If not, what additional information should we provide?

Response:

- Page 88, line 4 - What version of the ToxVal DB was used?
- Page 88, line 6 - What was the minimum number of days a study had to be conducted for in order to be used in the validation DB?

Question 14: Has FDA clearly laid out how the validation DB received from EPA was processed to enable its use for the external validation of the EDT? If not, please explain why not.

Response:

- Page 88, line 20 - How was the cross-referencing performed to remove chemicals already in the EDT DB?
    - What was used as the comparator, the CASRN? SMILES?
- Page 88, line 34-Page 89, line 2 - Do you have a list of the counter ions that were removed up-front from the chemicals in the validation DB or were chemicals with different salt forms run through the EDT workflow and only removed after having an EDT Class assigned?

- Page 89, line 12 – Were the SMILES from the EDT DB and external validation DB canonicalized using the same software before comparing them?
- Page 90, line 40 – What did you do when different agencies had different ADIs/RfDs?
    - Did you choose the lowest or use judgement to identify which you thought was more appropriate? If it was the latter, what factors did you consider?
    - Page 91, lines 7-15 – What were the reasons behind this study being deemed more appropriate? The length of study, the adverse effects, it having a lower NEL?
        - Trying to get a sense of the types of criteria that were used when choosing a NEL from outside of ToxVal DB

Question 15: Has FDA provided adequate information and/or data to show that the validation DB was processed appropriately for its intended use? If not, what additional information should we provide?
Response: Yes, I believe FDA has provided adequate information to show that the validation DB was appropriately processed.

Phase II:

Questions for section 4 (The Validation of the Expanded decision Tree):

Question 16: Some of the pre-validation EDT questions were updated, and some new sub- and sub-sub-questions were created based on the validation results. Has FDA provided adequate information to justify all updates? If not, which changes/updates were not fully justified and what information should we provide to justify them?
Response: I think, for the most part, FDA has provided adequate information to justify the updates based upon the validation results and feedback from the validation chemists. However, I have some minor clarifying questions:

- Q6a – While the clarification on page 24, lines 38-44 make sense, did you use data from the EDT DB or references to back up this change?
- Q7 – There are references to Q6b(i) and Q6b(ii) on page 27, I am assuming these are typos, but wanted to include them.
- Q7g – Could you add an explanation for the clarifications made to Q7g.
- Q7 assignments – Could you add an explanation for the reassignment of chemicals answering yes at Q7g(iv).
- Q14 assignments – Could you add an explanation for the update for moving chemicals where "…the epoxides are substituted by or fused to a polyaromatic ring…" if yes to Q14b(ii).
    - The pre-validation EDT assigned polyepoxides to Class V, rather than moving them onto Q33. In Appendix 2, the results for the 4 chemicals answering yes to Q14b(ii) also seem to suggest they were not moved onto Q33.

Question 17: Was the validation adequate to show that the EDT is suitable for the classification of compounds in its applicability domain according to their toxic potentials? If not, describe what type of validation would be needed.
Response: Yes, I think the validation is adequate to show the suitability of the EDT. The use of the pre-validation EDT questions to classify the validation chemicals by the validation chemists helped to identify areas of the workflow that were not covered by the chemicals in the pre-validation DB. I believe combining this with the evaluation of the EDT Classes after the assignment of the validation chemicals was a necessary step in the validation of the EDT to ensure that chemicals were not being erroneously assigned to Classes that were too under/overprotective of the chemicals toxic potential. Together these aspects of the validation allowed for clarification and/or updating the EDT questions to cover the (slightly) broader range of chemicals present in the combined EDT DB.
There are a couple of analyses that I think may help compare the pre- and post-validation EDTs:

- Figure 4 compares the NEL distributions for the combined EDT DB chemicals, what do the distributions of the NELs in the pre- and post-validation EDT look like for the subset of chemicals in the original EDT DB?
    - This would provide a good "apples-to-apples" comparison between the pre- and post-validation EDTs.
- Even though the NEL distributions look different visually, did you use any statistical method(s) (e.g., Kolmogorov-Smirnov [K-S] test) to verify that the distributions between the post-validation EDT Classes are different?
    - This could also be performed to compare the pre- and post-validation EDT Classes
- Even though the pre- and post-validation EDT TTCs are very similar, you could use a bootstrapping approach to compare the 5th percentile values for the pre- and post-validation EDT to show they are not statistically different.

Question for section 5 (Conclusions):

Question 18: Has FDA provided adequate information and/or data to support the conclusions found in this section? If not, what additional information should we provide?
Response: The basis on which the EDT was created is scientifically sound (i.e., structurally similar chemicals are [expected to be] toxicological/metabolically similar). The EDT questions and the workflow as a whole are structured in a way that enables the chemical groupings to be of use for read-across. The validation effort that was undertaken also illustrates that when additional data are available the EDT can be refined to classify chemicals into a more appropriate class.

Appendix 1 aims at providing a brief explanation of each EDT question. By no means are these explanations meant to be comprehensive. With that in mind, please respond to the following questions.

Question 19: Are all explanations clear and concise? If not, please identify the explanation by question number and elaborate as to how we can more clearly explain the question.
Response: Given the complexity of what some of the questions are trying to identify and group, I think the explanations are clear and concise. One possible way to make the explanations more easily understood may be to have them in the same order they appear in the questions. However, this could make them less concise because it may involve some repetition; for example, Q7 would involve splitting the explanation of the bioactivation of chemicals via GHS [captured by Q7g(i)] from the explanation of detoxification of chemicals via GHS [captured by Q7b(iii)].

Question 20: Should FDA add anything to these explanations to improve the reader's understanding of each question's rationale? If yes, please identify the explanation by question number and explain how we should revise. Please note that these explanations were designed to be concise and not all-encompassing.
Response: I think where there is some explanation of the reaction mechanism by which groups of chemicals may become (de)toxified [e.g., Qs 7b(ii), 19b, 19d, 32a, 33, and 38] to include an example illustration of what the reaction mechanism looks like.

Appendix 2 contains the combined, finalized EDT Chemistry, Toxicology and Metabolism DB on which the finalized TTCs were based.

Question 21: Are the set of chemicals in the database sufficient to cover the chemical domain of applicability described in the document? If not, please explain.
Response: Yes, I believe the chemicals in the combined EDT DB are sufficient to cover the chemical domain of applicability described.

Overall question:

Question 22: Do you have any other comments or suggestions?
Response: Along with the updates suggested above I have some clarifying questions on the validated EDT questions/comments:

- Throughout – I think the questions would be easier to read if the additional context in parentheses were included using square brackets, saving the parentheses for the question numbers and SMARTS patterns.
    - For example, Q3g(viii)) is an example where it is especially unclear what is (not) meant to be in parentheses.
- Throughout – I think the questions would be clearer if each sub-sub question were on its own line
- Section 4.4.3.56 – page 38, line 20, should "alicyclic block" be "acyclic block"
    - It appears that Q24a and Q24b are for acyclic chemicals
- Section 4.4.3.57 – page 39, line 14, should this be Q33c rather than Q43c?
- Section 4.4.3.58 – page 40, lines 9, 11, and 14; should this be Q34a(i))/Q34a(iii)) rather than Q43a(i))/Q43a(iii))?
- Definition Q – Page 51, line 12, "see definition AA" should be updated to definition Z.
- Q1c – Is the exemption of ≥8 contiguous conjugated double bonds [Q1a(iii)] only applicable to Q1c(i) or all of Q1c?
    - Page 6, line 5 seems to suggest it is all of Q1c, but the question itself reads as it is only Q1c(i).
- Q2e – Can the -OH group(s) on the alkyl chain be directly attached to the P?
- Q2e – Can both -OH and the ester/N be on the same alkyl chain?
- Q2 assignments – Should iv) also check if yes to Q2a)?
    - Should it read "If yes to a) and e(i)) or e(ii)) assign to Class II?
- Q2 assignments – Should v) also check for e(i)) and e(ii))?
- Q3c(ii)) – It would be good to use the statement "…(but these cannot be a part of a heterocyclic ring itself)…" here to be consistent with Q3c(i)).
- Q3d – Does the definition of "connector" on Page 9, line 28-29 need to be added here or with the other definitions?
- Q3f(iii)) – Does it matter if the N+ is directly or indirectly substituted?
- Q3f(vi) – There are some typos here "…a maximum or 2"; comma between "one or more" and "ether, alcohol, ester…", and; "single heteroaromatic rings"
- Q3f(vii)) – The term "…only one ring with a single ring N+…" may be easier to understand as "…one ring with a single N+ as a ring atom…" to keep consistent with how it is in other questions
- Q3g(ii)) – Page 69, line 1: May be good to add "[i.e., completely contained within a ring]" after "derivatives can be fully cyclic".
- Q3g(viii)) – I think the closing parenthesis may be missing.
- Q5 assignments – The "Examples for no" heading either needs to be reworded or new examples should be included. The current examples (methylmercury and darinaparsin) are now examples of chemicals that contain atoms in points (i) and (ii) rather than examples for no.
- Q6e(iii) – Should the "Examples for Q3e(iii)" be "Examples for Q6e(iii)"?
- Q6h(iii) examples – It would be clearer if the example ring structures started on a new line
    - I think "cains" is a typo and should be "chains"
- Q18b examples – I am assuming the substituents of interest are currently in blue and the rest of the structure is in red because it is an addition to the post-validation EDT. I would recommend having the substituted associated with "yes" assignment in red in the final EDT documentation.

- Q28n(ii)) – The text in parentheses "(other than in the previous sub-sub-question)", may be better written as "(other than those captured in sub-sub Q28n(i)))" so that it is more inline with similar text in Q18a(ii)).
- Q36c – Was "linear aliphatic chain of ≤6" a typo in the pre-validation EDT? Is that why it was updated in the post-validation EDT.

4.0 SUBJECT MATTER EXPER SUBJECT MATTER EXPERT (SME) — Dr. George Kass

Phase I (revised):

Questions for section 1 (The Expanded Decision Tree (EDT)):

Question 1: Has FDA clearly explained, with adequate examples, all guidelines and definitions for use with the EDT? If not, please provide suggestions for alternate text and/or additional examples.
Response: A fundamental revision of the TTC databases and threshold definitions is very welcomed. To introduce the new concept of EDT, a very extensive and complete document was presented.

- All definitions relevant to the chemistry contained in the different questions for the EDT are clearly and concisely presented.
- The definitions (and guidelines) for the toxicological endpoints are less clear. In part this is due to the nature, but a clear position would have been helpful. For example, the concepts of NOAEL and NEL can be quite different. When was adversity taken into account and when where effects not necessarily linked to adversity considered? There is only reference to small increases in relative liver weight from metabolic loading, and this was excluded. I agree that the earlier literature used by Munro for their TTC was not always very clear on this point but still now there are discussions on when an effect is considered adverse and when it is considered adaptive. Perhaps the text could be clarified on this point, where an effect is judged as adverse.
- Another point where the guidelines are not clear for the pre-validated EDT is on how the doses were calculated. The creation of the External Validation Database goes into much detail when administration of the chemical was not every day or when the compound had a lower purity. However, it is not stated whether the same approach was used to create the pre-validated EDT. More details here would be important.
- A similar comment can be made regarding the chemicals used to pre-validated EDT. The issue of inaccurate and multiple CAS numbers, incomplete description of the chemical, etc. is described in the External Validation Database but not for the pre-validated EDT.
- The examples provided give a good illustration of the information sought after in the questions.
- A general comment on the presentation of the first document is that the individual questions could be better highlighted. It is easy to get lost in the document as the questions are only indicated by a number, and navigation because of the need to refer to other questions throughout the text is not easy. Would a flowchart be possible?

Question 2: Are all EDT questions clear as to which structural features they are describing? If no, please identify the question by its number, explain why you find the question ambiguous or confusing, and suggest alternative text to ensure that it is clear what kind of structural features the question is aiming to capture.
Response: The EDT questions are a mix of:

- purely chemically defined questions (general structural features, elements contained in the structure, functional groups, etc.) (e.g., Q1,3-5, 12-33)
- Structures associated families of known toxicants (e.g., Q2 for OP compounds, Q34 for phthalates)
- Known biochemicals (e.g. Q1i)
- Pharmacologically (toxicologically) active compounds of natural or man-made origin (e.g., Q6)

From what I can judge, the purely chemically based questions are quite complete, whereas the questions dealing with naturally occurring compounds are limited to a few groups of pharmacologically (toxicologically) active compounds. This is also acknowledged in the document (p100). Will the incompleteness be compensated by the purely chemically based questions? The concern is that the most toxic compounds known are naturally occurring ones rather than man-made.
It is difficult to reconstruct how the different questions are interlinked. Therefore, the fact that two different questions can address the same chemical and come to different class assignments may or may not occur when using the EDT. For example, TCDD can be answered in Q8(b) (assigned to Class VI) but also in Q18(a) (assigned to Class V).
I tried to identify bisphenol through the questions. Q36, takes you to 41 then 42, then 43, then???

Question 3: Most questions place compounds into one of six classes of toxic potential depending on their structural features. Does the EDT place the type of compounds that are captured at each question into the appropriate class of toxic potential? If not, please explain why and provide a recommendation for the appropriate class of toxic potential.
Response: This question is difficult to answer. The document does not provide many examples with clear class assignments. Where it is the case, classification falls in line with their previous one under the extended Cramer one. Particularly helpful would have been a more complete list, and perhaps more importantly the chemicals falling outside the 95th percentile.
There is some unclarity here regarding some endogenous phosphorylated compounds that are formed from precursor macromolecules (phospholipids) or further phosphorylated in the process. Some are captured by Q1i, others by Q2c. Examples: IP3, IP4, IP6. In the former case, they are classified as I. In the latter case, this leads to class III, and this an overclassification. An example would be IP6 (phytic acid).
Q18 puts TCDD and congeners into class V which will not be sufficiently protective (0.031 ug/kg bw/day vs 0.25 pg/kg bw/day). Subsequent check with the EDT spreadsheet has TCDD listed with a NEL of 1 pg/kg bw.

Question 4: Commonly, structurally related compounds (e.g., γ-diketones) can have common toxicological endpoint (in this case γ-diketone type neurotoxicity). Compounds that can either hydrolyze and/or metabolize to these compounds can exhibit the same type of toxicity. FDA aimed to capture hydrolytic and metabolic precursors of structurally related compounds with similar toxicities at numerous questions. Are there any questions where you can suggest any possible metabolic and/or hydrolytic precursors to the types of compounds addressed that are currently not mentioned/captured in the question?
Response: The EDT does a very good job in assigning appropriate classes to reactive intermediates. The above mentioned 2,5-hexanedione example is assigned to class IV. Both Toxtree and the QSAR Toolbox also assign it to Cramer class III. Here the EDT shows its strength as it picks up the precursor and correctly assigns it to class IV whereas both Toxtree and QSAR Toolbox put it into Cramer Class I. The intermediate metabolite 2-hexanol is put in Cramer class II and I, respectively. EDT assigns it Class III (Q24 – if I am correct). For the further metabolite, 2-hexanone, the QSAR toolbox assigns it to Class I, Toxtree to Class II, and EDT to Class IV. The related 1,2-diethylbenzene that manifests the same type of neurotoxicity is in Cramer Class I but EDT Class IV.
The neurotoxicity of hexane is well documented. My understanding is that long term exposure to high concentrations is necessary to elicit human toxicity. Would a Cramer Class I still be protective. The US National Institute for Occupational Safety and Health (NIOSH) has set a recommended exposure limit (REL) for n-hexane of 50 ppm (180 mg/m^3 over an 8-hour workday. Cramer Class I would be 2 orders of magnitude lower (let's ignore routes of exposure for the moment). Therefore, is the need to assign all hexanes to EDT class IV justified?
The problem often encountered in practice is that exposure will not be directly to the reactive intermediate but to appropriate precursor molecules. The reactive molecules described in the different questions may

be too unstable to be of relevance from an external exposure point of view. This issue of metabolism is addressed for some families of compounds (e.g. epoxide formation from PAHs) where the pathway is well-established but may miss many other parent compounds. Would there be room to consider linking the EDT questions to third-party predictors of metabolism? Prediction of metabolism would facilitate the inclusion of a number of substances in the questions dealing with reactive chemicals if it could be predicted that they would be formed as part of their metabolism.

Question 5: Are the example structures provided after each EDT question correct and adequate for understanding what type of compounds the question aims to capture? Are there different or additional example structures for any of the questions that would help increase the understandability of the question?
Response: Generally, yes but examples for where the answer to the question is negative can be difficult to reconstruct as it can lead to multiple questions with negative answers (e.g. dapsone).

Question 6: Are there any congeneric groups that the EDT does not adequately address, but for which enough safety data exist that could serve as the basis of additional EDT questions to address these groups? If yes, please identify and provide all toxicological data for the congeneric group(s) that may form the basis of one or more additional questions. If possible, please propose the wording for such additional EDT questions. (Substances within a congeneric group are structurally and metabolically similar.)
Response: For metals and organometals, additional questions on their solubility and pKa values under physiological conditions to predict their ADME properties would allow to refine the grouping. This would allow to address the considerable differences in the toxicity of some metal salts, e.g. Ba salts.

Question 7: Should any questions be further subdivided to ensure a more refined grouping of related substances? If yes, please suggest wording for the refined question(s) and provide the data justifying the suggestion.
Response: See answer for Q6.

Question 8: Are there any terms used in the EDT questions that should be added to the guidelines and definitions section to help users of the EDT? If yes, what additional terms should we define?
Response: The explanations to the different questions given under Appendix 1 is very useful but should be incorporated into the questions themselves.

Under Appendix 1 a number of explanatory terms such as receptors, pharmacological activity are used. As for the bullet point above, it would be good to have these concepts included in the questions themselves as they represent the rationale for several of the questions.

Questions for section 2 (The Expanded Decision Tree Chemistry, Toxicity, and Metabolism Database (EDT DB)):

Question 9: Has FDA clearly explained where the toxicological data found in the EDT DB were collected? If not, what additional information should we provide?
Response: The description of the sources of data used for the pre-validated EDT. Some additional information would have been useful such as number of chemicals from the different sources, curation to get unique substances would have been useful. A table would help.
The issue of inaccurate and multiple CAS numbers, incomplete description of the chemical, etc. is described in the External Validation Database but not for the pre-validated EDT.
Missing is also the extensive discussion found in the External Validation Database but not for the pre-validated EDT on the approach used when administration of the chemical was not every day or when the compound had a lower purity.

Limited information on inclusion and exclusion criteria is provided. The TTC approach comes with exclusion criteria regarding some groups of chemicals where the chemical space represented by the substances in the database was considered outside the domain of applicability. Examples are organosilicon substances, substances with a potential for bioaccumulation, endocrine disrupting chemicals, inorganic substances, nanomaterials, etc. Some of these groups have been incorporated in the EDT which is seen as positive but some more information on the underlying criteria would be helpful. This would also be very useful for those compounds that interact with nuclear receptors linked to endocrine activity. Some are addressed from a structural point of view in Q6 and Q34 (and Q33 for AhR ligands). While the TTC (Cramer class III) is found protective for the majority of man-made EDs, there are a number of exceptions. For example, among pesticides recently shown to display ED activity, the TTC would not be protective for chlorpyrifos (CAS # 2921-88-2), clodinafop (CAS #105512-06-9), flutamide (CAS # 13311-84-7).

Question 10: Has FDA clearly explained the study selection criteria and provided adequate information and/or data to support its opinion that these criteria are appropriate for data inclusion in the DB? If not, what additional information should we provide?
Response: The issue of inaccurate and multiple CAS numbers, incomplete description of the chemical, etc. is described in the External Validation Database but not for the pre-validated EDT.
Missing is also the extensive discussion found in the External Validation Database but not for the pre-validated EDT on the approach used when administration of the chemical was not every day or when the compound had a lower purity.
For the External Validation Database there is a description of the issue of conflicting interpretations between organizations (e.g. conflicting NELs). Such issues would also have occurred but are not mentioned in the pre-validated EDT, given the number of databases used where the same chemicals are likely to have their evaluation reported.
Naturally occurring substances, some endogenously produced by the body, are covered to some extent but the coverage is not complete, and the limitation of the approach is acknowledged (see answer to Q2).
Unfortunately, only limited information of the selection criteria for data inclusion or exclusion in the DB is provided. Given that similar compounds can give rise to a wide range of different toxicological activities, it is also not very clear what the inclusion or exclusion criteria as to toxicological properties were.
The definitions (and guidelines) for the toxicological endpoints are less clear. In part this is due to the nature, but a clear position would have been helpful. For example, the concepts of NOAEL and NOEL can be quite different. When was adversity taken into account and when where effects not necessarily linked to adversity considered? There is only reference to small increases in relative liver weight from metabolic loading, and this was excluded. Finally, there is no mention of PODs using the BMD approach. Weren't there any cases where BMDL values were used? What was the decision if both BMDL and NO(A)EL values were available?
Reference is made to 'toxic effects in the context of enzyme catalyzed and uncatalyzed metabolism' (p 81). What is exactly meant with uncatalyzed metabolism? Spontaneous degradation? GSH conjugation without GST? Redox cycling?

Question 11: FDA used various factors based on study duration to derive duration adjusted no-effect-levels (NELs) to estimate chronic NELs. Has FDA provided adequate information and/or data to support its opinion that these duration adjustment factors are adequate to derive chronic NELs? If you generally agree, are there any exceptions in which these factors might be problematic to the derivation of duration adjusted NELs?
Response: The External Validation Database but not for the pre-validated EDT has an extensive section on the approach used when administration of the chemical was not every day or when the compound had

a lower purity or when administration was of shorter duration. The same level of description should be used to describe how the EDT was created.
How was the situation handled where a NEL for the shorter duration study was lower than that for the chronic study?
In the situation where substances were tested only in single-dose studies, it is stated that they were included if the NEL 'was within an order of magnitude as that of other members of the congeneric group in multiple dose level sub chronic or chronic studies (e.g., dimethyl disulfide)'. Would this not lead to some form of confirmatory bias?
For developmental, reproductive, or combined reproductive/developmental studies, it is stated that 'parental NEL were used in cases where the maternal or paternal NEL was less than or equal to the NEL for the offspring. Duration factors (3 or 10) were employed to adjust for the study duration for dams and males.' I could see a justification for this duration factor in the absence of any additional repeated dose study. However, if the toxicity is only manifested as a result of pregnancy than the entire duration of the pregnancy should be seen as complete, i.e. without the need for an additional duration factor.
The analysis of the impact of non-oral studies is welcomed.
The decision not to generate additional NELs from LELs is welcomed.
It is described on p85 that 'to calculate the Class VI TTC, we decided to also use the 11 NELs from studies with a duration of less than 84 days (but no one-day studies). We used a factor of 10 to adjust for the short duration to calculate chronic DNELs, as described earlier.' While the argument to ensure maximum protection can be defended, it would be helpful to see an analysis the impact of the factor 10.

Questions for section 3 (The Preliminary (Pre-validation) Threshold of Toxicological Concern Levels):

Question 12: Based on Figure 2 and all other information
provided, in your opinion, does the EDT better resolve the differing toxic potentials of chemicals with broad structural variation compared to the CDT? Please explain why or why not.
Response: The steepness of the curve supports the argument for a better differentiation. However, it would have been interesting to see the EDT curves against the more recent improvements to the TTC by having a total of five groups (the three Cramer ones, the OP-carbamate one and the DNA-reactivity one). The differentiation is also helped by the fact that the EDT range is much wider than the TTC one, primarily helped by the fact that the thresholds of the two lower classes are higher than in the original TTC.

Questions for section 4 (The Validation of the Expanded decision Tree):

Question 13: Has FDA clearly explained the source of the validation DB and how the data was verified pre-validation? If not, what additional information should we provide?
Response: Contrary to the pre-validated EDT, the information on the validation DB (External Validation Database) is well described. What is missing is a clear overview of the databases used for the EDT and the External Validation Database, the number of chemicals extracted from each database and any overlap. Some tables and figures would be helpful here to guide the reader.

Question 14: Has FDA clearly laid out how the validation DB received from EPA was processed to enable its use for the external validation of the EDT? If not, please explain why not.
Response: The answer here would be similar to the one provided for Q13. See also answer to Q9.

Question 15: Has FDA provided adequate information and/or data to show that the validation DB was processed appropriately for its intended use? If not, what additional information should we provide?
Response: In addition to my previous answers, it would have been useful to have an overview of the types of compounds that were used to populate the EDT and the External Validation Database. By type I am referring to the principal use of the chemicals, i.e. pesticide, food flavouring, food colour, endocrine active substances, pharmacologically active substances, etc. This information would be important in the

evaluation of the intended use of the EDT in the different sectors and to see that there is sufficient confidence on the applicability domains. It is also not clear how well persistent chemicals are represented and how robust the EDT is for dealing with this group of chemicals.

Phase II:

1. General Impressions

Response: The idea of using thresholds of exposure to support regulatory decision making was first formulated in the 1960s and further developed into the concepts of virtual safe doses and the Threshold of Regulation (for exposure to potential carcinogens) and into Munro's TTC approach in the 1990s (for non-cancer endpoints). The original TTC was derived from a database of 613 chemicals that were allocated into one of three so-called Cramer classes based on structural considerations. Over the subsequent decades, the TTC approach was further refined, primarily by the inclusion of additional chemicals, adjustments of some of the Cramer questions, the reassignment of some groups of chemicals, the definition of exclusion criteria, and perhaps the most impactful of all, the definition of additional threshold values for organophosphates and carbamates, and for compounds with structural alerts for genotoxicity. However, it is fair to say that the impact of extending the chemical database has been seen mainly as confirmatory for the approach, and that in practice the three Cramer class thresholds have remained unchanged. Therefore, while the TTC approach has been widely acknowledged as a useful screening and prioritization tool for the risk assessment of chemicals when hazard data are incomplete, concerns always remained as to its coverage of the chemical space because of its reliance on just over 600 chemicals. There was also concern over the small number of chemicals used to derive the Cramer Class II threshold value. For these reasons, the initiative of FDA to engage in a complete re-assessment of the Cramer et al. Decision Tree (CDT) approach using not only a much-extended chemical database (DB) of 3142 different chemicals but also by developing from scratch a set of questions for the decision tree approach is very welcome.
The project sent out for the reviewing process was divided into two phases. The first phase consisted in the review of the original Expanded Decision Tree (EDT) followed by its external validation. This phase included the charge questions. Phase II focused on the validation of the EDT and presentation of the outcome of the finalised post-validation. In the finalised post-validated EDT, many of the EDT questions were refined or expanded by introducing sub-questions to avoid misclassifications (too conservative or insufficiently protective, dead-ends or oversight of groups of chemicals or structures. Reviewing EDT Phase II was facilitated by the provision of the final EDT DB which allowed this reviewer to test the EDT questions and compare with the class assignments reported in the DB. The lack of access to the final DB rendered some aspects of the review of Phase I challenging.

2. Detailed Comments

Response: The EDT enables the assignment of chemicals into one of six EDT classes. A clear advantage over the original Munro TTC is the much-reduced overlap between classes as compared with the Cramer classes plus a wider range between the Class I and the Class VI thresholds. It is, however, difficult to compare the distribution of the EDT classes with the distribution of the organophosphate-carbamate and the DNA-reactive classes of chemicals. Of the 3142 chemicals found distributed in EDT DB, 2057 were used to derive the six threshold values. From the description provided, it is not very clear what inclusion/exclusion criteria were used to determine which compounds contributed to calculate the TTC values and which did not. It would have been useful and more transparent to show in the Appendix 2 spreadsheet (EDT DB) which compounds contributed to the TTC calculation. Also, useful would have been an indication in the spreadsheet which question (or group of questions) within the EDT were used to assign the class for each compound. Some of the questions regarding the choice of endpoint and extrapolations used to define the point of departure values were raised in the answers to the charge questions.

The final EDT contains 47 main questions, most of which lead to several sub-questions. These appear to have gone through a rigorous assessment to ensure that any permutation in chemical structure can be either assigned to an EDT class or be forwarded to another question. The EDT is also aimed at supporting read-across. Here, future guidance will be needed to see how the EDT can be incorporated in a read-across framework, but the intention is welcome. Overall, the number of chemicals per EDT class appears sufficiently large to support the six classes. However, EDT Class VI is limited 68 unique chemicals, corresponding to only 3% of the total number of substances.

Where the approach used to define the EDT questions becomes more problematic is when the questions focus on chemicals that have pharmacological activity and where this pharmacological activity is responsible for the toxicity of the compound or group of compounds. The EDT addresses well the poly-halogenated compounds that fall in the dioxin group and that operate through the Ah receptor. This is a well-characterised group of compounds, and the existing TEFs have helped to distribute the members of the dioxin-like chemicals into their appropriate classes. For the phthalates, the evidence for lower potency to induce developmental effects in the shorter phthalates is perhaps not sufficiently strong to have them in Class III as compared to Class IV (e.g. DEP, a suspected ED). Sometimes, it is not clear why some closely related compounds are assigned to different classes while their points of departure would not support this. For instance, MEHP is Class III although its LEL is lower than that of its parent DEHP in Class IV.

Question 6 addresses, among others, structures known to act through oestrogen receptors (e.g. diethylstilbestrol and tamoxifen). Among these, there will be structural analogues devoid of or with much reduced pharmacological activity, and where a read-across will lead to an over-protective classification. Information on such analogues may not necessarily be available in the public domain. In theory, the opposite may also occur where future structural analogues may show much enhanced potency and where the EDT class may not be sufficiently protective. In the modification of the original TTC approach introduced by Kroes et al., many of these compounds were excluded, and while this reviewer welcomes the effort to minimise the exclusion categories from the EDT, for those chemicals that are toxic by virtue of their pharmacological properties, the level of uncertainty from using simple structural criteria and chemical read-across may be quite high. However, since the class assignment is generally based on the most potent members, the EDT approach would be expected to be sufficiently protective for the group of interest.

The threshold for Class VI is approximately 5-fold lower than Kroes' threshold for potential DNA-reactive mutagens and/or carcinogens. Unlike for the latter, the EDT DB does not exclude the so-called cohort of concern (COC) for which it was concluded that the TTC of 0.0025 µg per kg bw per day would not be sufficiently protective. The question is whether Class VI would be sufficiently protective for the members of the COC. It is also noted that in the EDT DB, the nitrosamine DEN, a member of the COC, is classified as Class V which has a TTC value that is higher than Kroes' threshold for potential DNA-reactive mutagens and/or carcinogens. Another chemical that was excluded from the TTC approach by Kroes et al. is TCDD. The EDT places TCDD into Class VI yet both the TWI established by EFSA in 2017 and the PTMI established by the JECFSA in 2002 are much lower than the equivalent Class VI TTC value. Based on these conclusions, Class VI may not be sufficiently protective for dioxins and dioxin-like compounds. Alternatively, this group of compounds could be removed from the EDT TTC as members of an exclusion category.

In Question 1i, the instructions are to disregard "the following commonly encountered and relatively nontoxic or of low toxicity i) metal counterions: sodium, potassium, calcium, magnesium, barium, aluminum, titanium, zinc, manganese, copper, iron, and bismuth". The relatively high EDT Class I TTC value may become problematic for counterions like aluminium, manganese and copper as their health-based guidance values could be exceeded. In addition, the formation of complexes with chemicals falling in Class I may lead to enhanced bioavailability of the metal counterion.

Question 1 also deals with nucleotides, nucleosides, phospholipids, monophosphates of amino acids, and their hydrolysis products, to place them into Class I. A number of such compounds may not have been

captured by Question 1. For example, the inositol derivative phytic acid is classified as III. It has very low bioavailability in non-ruminants and needs to be synthesised by our bodies. It could be considered as a hydrolysis product of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) (after further phosphorylation of IP4). Similarly, CDP-choline is in Class I but choline Cl (its hydrolysis product) is in Class II, and phosphatidyl ethanolamine is not listed but ethanolamine is in Class II.
Question 6d aims at capturing naturally occurring toxins, and structural criteria are provided. However, quite a number of such toxins are left out. Given their structural complexity it is difficult to see where they would end up if run through the EDT questions. Just some examples from the marine biotoxin groups: Okadaic acid and dynophysistoxins, TTX and saxitoxins, palytoxins. Another challenge is that for many naturally occurring toxins there are often multiple variants. For instance, there are 12 known ciguatoxins found in Caribbean and tropical Atlantic waters and 29 reported ciguatoxins in Pacific waters but likely to be with different potencies, i.e. different TEFs. It is also difficult to see whether the main driver for Question 6d is chemistry or the pharmacology responsible for the toxicity of the compounds. For instance, the highly potent brevetoxins and ciguatoxins are given as example and classification under cat VI is justified. However, for the structurally very similar yessetoxins (MoA currently unknown but not acting through Navs), which are much less potent biotoxins, Class VI would be overprotective.
The chemical bisphenol S would be redirected from Q 36 to Q 41. No clear indication of further redirection is given and under Q 41, BPS would be assigned to Class III. In the EDT DB it is assigned to Class II.

3. Conclusions

In conclusion, the developers of the EDT TTC are to be congratulated. The EDT TTC is a major improvement over the original Munro TTC and over its subsequent improvements. A major achievement is that the EDT TTC is based on an extensive DB that covers most existing regulatory assessments that are available and that it extends considerably the chemical space on which the TTC is based. This should have a positive impact on the confidence in the TTC approach for regulatory decision making. Several issues remain that would need addressing. The inclusion of questions aimed at identifying pharmacological properties or at grouping of substances based on such properties is welcome but comes with its own challenges. However, it is clear that an EDT based exclusively on chemical determinants would have been limiting and would rapidly face similar problems as read-across based exclusively on chemical similarity, and for which now an important focus is on incorporating metabolism and biological similarity. The EDT does not use exclusion categories. It is assumed that the EDT will not be applicable to nanomaterials and radioisotopes but among the organic chemicals addressed, some remain problematic, and it is not clear whether the Classes V and VI are sufficiently protective for potent genotoxic carcinogens such as aflatoxins and nitrosamines or non-genotoxic Ah receptor agonists such as TCDD. With some of the charged compounds that carry a metal counterion, the counterion may be more problematic than the organic molecule at the class threshold. Inclusion of naturally occurring toxins such as mycotoxins and marine biotoxins is welcome but the toxin space covered is currently somewhat incomplete.
Finally, one of the strengths of the Munro TTC is that it is supported by software platforms that help the risk assessor to assign a chemical to its appropriate Cramer Class. The development of a software platform to support the EDT is mentioned in the document, and the availability of such software will be extremely important, not only to support risk assessors to help them navigate through the complexity of the EDT questions but also to help the acceptance of the EDT TTC by the global community of risk assessors.