Compilation of Preliminary Individual Comments from Members of the Chartered SAB and SAB Chemical Assessment Advisory Committee on New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

(As of June 24, 2020)

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Dr. Hugh Barton

New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

Hugh A. Barton

June 18, 2020

1. EPA-OPP is participating in the Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) with government, non-governmental organization, and industry stakeholders (Section 2). ReCAAP is developing a risk-based weight of evidence (WOE) approach for waiving chronic and carcinogenicity studies. This proposed approach is consistent with existing guidance and current practice for other types of toxicology studies.
    a. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.

The draft risk-based WOE approach lays out reporting for a variety of information to consider for determining whether to waive chronic/carcinogenicity studies. It is essentially a format to be followed for preparing such a request. What it does not provide is information about how such a decision would be made other than a sentence here or there. Section 4.4 on Evidence of Immune Suppression begins with the statement "In the absence of genotoxicity, hormonal effects, or liver enzyme induction, indications of immunosuppression could raise concern for potential tumor formation." This is useful information, but nothing similar is said in the sections on genotoxicity or hormonal effects. The statement in Section 4.5 Genetic Toxicity is completely confusing – "If the chemical is mutagenic, then the evaluation is complete and no further documentation is needed (the pesticide would not undergo a cancer bioassay if the chemical is mutagenic, so no need to write a full carcinogenicity waiver)." Mutagenic chemicals would generally be of great concern, so it is unclear what this sentence means.

    b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

The case study is useful but seems such a simple example that it provides limited guidance for making decisions when results are more complex. It is noted in the white paper that additional case studies are in development for trying out the WOE approach; this would be valuable.

2. EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to begin to replace the chronic/carcinogenicity testing in mammals. In addition, EPA-OPP and ORD are working together to collect quantitative gene expression

data from short-term *in vivo* rat studies for a selected set of pesticides that cause liver tumors in rodent with known modes of action. All of these efforts are in the early stages of development and would benefit from expert and public input.

a. Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.

The three collaborative projects appear to be valuable efforts to move the disciplines of toxicology and risk assessment as well as the organizations dependent upon them, such as EPA, towards new alternative approaches to assessing and protecting human health. These projects are useful and deserving of sufficient support to allow them to make a difference in the next few years.

3. EPA is working with HESI, NTP, and other government and industry stakeholders to accelerate the incorporation of kinetically-derived maximum doses (KMD) into repeat dosing studies like the chronic/carcinogenicity study as an alternative to the traditional maximum tolerated dose (MTD). The KMD approach is consistent with numerous guidance documents developed by EPA, OECD and other international organizations as a more humane and human relevant approach to dose selection. One KMD study has been provided to the SAB along with the description and agenda of an upcoming workshop and the scope/charge of a workgroup at HESI to develop additional case study and a best practices document.

   a. Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?

Choices of the doses to use in toxicity studies have long posed a challenge. A kinetically maximum dose has been proposed as an additional selection criteria. The general concern is that toxicities observed at very high doses compared to human exposure may not be as indicative of potential human health effects as toxicities observed at lower doses. The materials provided do not make a strong case for use of kinetics as a determinant, but rather text in the white paper and the provided example argue for the importance of toxicodynamics or mode of action in making determinations about dose selections.

As higher internal concentrations of chemicals or drugs are obtained in toxicity studies with higher administered doses, several things may occur. There may be changes in how the body handles a chemical (pharmacokinetics or toxicokinetics) and/or what the chemical does to the body (pharmacodynamics or toxicodynamics also referred to as mode of action). Changes in pharmacodynamic processes at higher doses may result in adverse effects that would not occur at lower doses, so these effects have little or no relevance for human health risk assessment with low enough exposures. Some changes in pharmacodynamics, such as depletion of the scavenger molecule glutathione, can also change pharmacokinetics impacting whether appropriate extrapolations to lower doses are feasible, as in the example provided. Saturation of pharmacokinetic clearance processes (e.g., metabolism, urinary excretion) result in changes in the relationship between the external administered dose and the internal concentration time course of parent compound and metabolites. This may effect appropriate extrapolation to lower doses and

make pharmacokinetic modeling desirable, but does not by itself determine the human relevance of the findings. Higher internal concentrations in the absence of changes in toxicodynamic processes can result in observable effects that are unobservable at lower doses given the number of animals in the study and the duration of exposure and observations. Detection of such effects would generally be considered advantageous, since the biological processes occurring are the same as those occurring at lower concentrations but would require much larger numbers of animals to be observable at lower concentrations.

One challenge for using saturation of pharmacokinetics as a characteristic to limit the highest dose in a toxicity study is that pharmacokinetics alone does not identify when observed toxicities are not predictive for humans. The example provided (EPA-HQ-OPP-2013-0154-0104) describes decisions about a KMD for dichloropropene. It argues for selection of a KMD based upon nonlinear kinetics assessed in a steady state study in mice (p33) and then argues that the benign lung adenomas arose only at doses exceeding the KMD. However, the key issue is that glutathione depletion is observed (section 5B p35). Glutathione depletion impacts the pharmacokinetics because the major route of metabolism is glutathione conjugation (section 5A p 35), but importantly glutathione depletion is a major protective pathway in the toxicodynamics. With depletion it is expected that toxicodynamic processes would occur that would be much less likely with adequate glutathione present leading to the lung adenomas. Glutathione depletion, like excessive body weight loss, should be considered a criterion that would limit use of such high doses in toxicity studies or, if the study were already done as is the case for dichloropropene, argue that the data from that dose are too confounded to be appropriate to extrapolate to lower doses or to humans. Thus, the changes in toxicokinetics are here being used as an indicator of when changes are occurring in toxicodynamics. It would be preferable to use the data on glutathione depletion directly.

Another issue with the proposals around the KMD is how the shift from linear pharmacokinetics to saturation tends to be described and then the statistical methods applied. Saturable processes are most frequently well described mathematically by a rectangular hyperbola, often referred to as a Michaelis-Menten equation, referring to a common formulation in enzymology. This is a smooth curve that at low concentrations, well below the mathematical parameter describing the concentration giving half-maximal activity (called Km), behaves essentially linearly within measurement error. As one goes to higher concentrations, the curve increasingly deviates from the low dose linear behavior, effectively bending over from a straight line to result in a near plateau at the maximal activity. There is no "inflection" point as would be created at the point where two straight lines with different slopes join (hockey stick model), but rather a steadily increasing difference between a straight line and a hyperbola that bends over.

Thus, the question is how much of a deviation from the low dose linearity is considered “too much” deviation so one should not use such a dose; this appears a matter of choice. A widely used rule of thumb for experimental designs to determine the parameters of the Michaelis-Menten equation or equivalently equations for specific binding or pharmacological effects (Emax equations), is that one needs data spanning a range from 10-fold above the half maximal parameter (i.e., Km, or Kd, or EC50) to 10-fold below. The 100-fold range centered on the half maximal parameter covers the approximately linear center portion of the sigmoid shaped curve observed when the x-axis is plotted on the log scale. The differences between the curve shapes on the normal and log scales for

the x-axis leads to confusion about what are approximately linear portions of the curve. Fitting sparse pharmacokinetic data to obtain an "inflection" point seems a questionable approach.

One avenue for consideration in the various ongoing KMD activities, is whether the issues might be better addressed by focusing on the activities moving towards use of NAMs or transcriptomic PODs. Concerns over how to select doses in a traditional toxicity study, in particular a chronic/cancer study, should hopefully become a lower priority as one moves towards newer methods.

Dr. Janice Chambers

Chronic/Carcinogenicity Waivers: Comments from Jan Chambers

Question 1:

a. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.

The attachment seems to cover most of the information that would need to be analyzed to approve a chronic/carcinogenicity test waiver, but the statements are quite brief and do not provide any guidance to EPA staff regarding their analysis of these data. A WOE approach is reasonable and could definitely be science-based, but could be subject to judgment calls if guidance and rationales are not presented

b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

The case study makes a well-documented WOE case for waiving the chronic/carcinogenicity tests based on knowledge of rodent physiology and toxicology with other compounds and read-across as possible.

4. Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.

The shorter-term gene expression approach to predicting carcinogenic potential has considerable merit, especially if some well documented commonalities are found among toxicants resulting in the common endpoint considered here, i.e., liver tumors. However there are many uncertainties involved in extrapolating from short term changes in gene expression (which may or may not be related to the mechanism leading to tumors) and considerable analysis and consideration of the physiology reflected by these changes in gene expression must be made before accepting short-term changes as a surrogate for long-term effects.

5. a. Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?

The case study is very interesting and well described. It makes a very good case for a KMD which assesses the biology behind the kinetic results and makes an effective case for the KMD instead of the MTD as the high dose in a study. This new approach has considerable potential for more science-based risk assessments on

carcinogenicity compared to the earlier approach that indicates the adverse results seen at excessive dose levels must be considered in the risk assessment.

General comment: There was much to consider on these new approaches and very little time to consider them. Because these would rewrite the long-standing guidance that EPA has been following for risk assessments, these new approaches should be considered in a fashion that is not rushed and allows the SAB to provide more thoughtful advice than is currently possible.

Dr. Samuel Cohen

New Approach Methods in reducing the Use of Laboratory Animals

for Chronic Carcinogenicity Testing.

1. The format outlined for the study waiver request presents the needed information in a clear and comprehensive manner. I strongly support the agency in its attempts to no longer require the two-year bioassay in mice and rats for carcinogenicity testing. Not only is this wasteful of resources, including the use of too many animals, it has little predictive value for human carcinogenic risk, particularly for chemicals that are nongenotoxic. I strongly support the concept of using reference doses for carcinogenicity assessment for nongenotoxic chemicals with appropriate safety factors, since the toxicities that occur prior to development of cancer are necessary for ultimate development of the cancer. Since these are precursor lesions and are noncancerous endpoints, they can be handled the same way as other noncancer toxicities. Focusing on genetic toxicity, immunosuppression and hormone perturbation are appropriate, especially for toxicity assessment. However, for cancer assessments, the only hormonal perturbation known to be predictive of human cancer risk is estrogenic activity related to breast, endometrial, and to a much lesser extent, liver and possibly ovarian cancer. Other hormonal perturbations, although of consideration regarding toxicity, do not predict carcinogenic activity in humans. Probably the best example of nonrelevance are the numerous chemicals involved in producing thyroid follicular tumors in rats, all of which ultimately are related to hypothyroid induction with increased TSH stimulation and thyroid follicular proliferation. There are innumerable epidemiology studies indicating that hypothyroidism is not a risk factor for human thyroid cancer. The focus on immunosuppression is particularly important, since many of the immunosuppressive agents used in clinical medicine are actually negative in the two-year bioassay. Immunosuppression of any kind, whether inherited, secondary to therapy for transplantation, cancer, or autoimmune diseases, or due to AIDS, all increase the risk of virally related cancers and a few others. A two-year bioassay for such agents is a waste of resources. A few specific details could also be included in the listing. Some mention should be made about the quality of studies, although for pesticide evaluations these will often be GLP. In evaluation of genotoxicity, it should be stated that appropriate negative *in vivo* studies will usually negate positive findings in the *in vitro* studies.

The examples that were provided are quite good, particularly the bladder and calculus example for cancer assessment and the use of KMD in addition to MTD for assessment of dose. My only comment regarding the bladder cancer example is that there actually are examples of carcinogenicity testing of sulfonamides, dating back to the 1960s. Although these were not GLP studies, they clearly showed an increased incidence of bladder tumors in mice. The chemical was 4-ethylsulphonylnaphthalene-1-sulphonamide (ENS) (Br. J. Urol., 26 :26-34, 1964), and was the example for which David Clayson ultimately hypothesized that the tumors induced by chemicals related to calculi were due to the calculi and not to the chemical itself, this dating back to 1974 (Clayson, JNCI, 52: 1685-1689, 1974). The conclusion is that this is quantitatively not relevant to humans given the differences in exposure. However, one could also argue that qualitatively this is not relevant to human exposures either (Cohen, Toxicol. Res., 7: 565-575, 2018). For EPA and its focus on risk-based assessment, this distinction is not important. However, for agencies, such as the European Chemical Agency, which is hazard based, this can become a significant issue for classification.

2. The efforts of the EPA's collaborations with NTP and HESI to consider NAM-based approaches is strongly supported. For now, a combination of short-term *in vivo* studies with appropriate *in vitro* studies can readily accomplish a screening for carcinogenicity. I would recommend that the studies in mice for carcinogenicity be completely eliminated since they are of no predictive value for humans beyond that which is provided from studies in rats. The EPA might reexamine the basis for the non-use of mice in European pharmaceutical evaluations in the 1980s and 1990s, prior to ICH. They only incorporated the mouse into the overall evaluation when the international harmonization efforts were undertaken due to the requirements by both the United States and Japan for two species. Some of the significant issues that still need to be addressed in these collaborative efforts is the focus on screening for tissues other than the liver. Most of the studies so far have investigated predictive values of genomic and other methods utilizing liver carcinogens. However, this will not screen for carcinogens for a number of other tissues, keeping in mind that there are very few chemicals actually associated with human liver cancer (ethanol, aflatoxin, estrogen). How does the agency propose to screen for carcinogens with target organs beside the liver? Rodent assays will be of little value since the major cancers in humans frequently are not affected in the rodent bioassay, or the rodent model is not predictive of changes in humans. For example, colon, stomach, and pancreas ductal adenocarcinomas are rarely produced in the rodent bioassay with any chemicals, particularly not with nongenotoxic chemicals. Likewise, the prostate in the rodent is not similar to the human prostate either morphologically or endocrinologically. The list could go on extensively. I would encourage the collaborations to develop human cell-based assays that would be more predictive of human risk. However, for nongenotoxic chemicals, it is the toxicity in short-term assays, whether in human cells or in animals, that could provide the basis for a risk assessment since protecting for the short-term toxicities will also protect for the ultimate development of cancers. (For further details, see Cohen, Toxicol. Res., 7: 565-575, 2018; Toxicol. Sci., 80: 225-229, 2004; Toxicol. Pathol., 38: 487-501, 2010; Cohen et al., Reg. Toxicol. Pharmacol., 103: 100-105, 2019)

3. I strongly support the efforts by the EPA and its collaborators in further developing the KMD-related evaluations. The example that was provided for dichloropropene highlights the advantages of such an approach to assess overall risk. Clearly, as long as exposures in humans will be below the KMD, studies performed at doses higher than the KMD are irrelevant to human risk. Combining this with the efforts in reducing the number of animals, a short-term evaluation for KMD in addition to MTD dose determinations could be used in establishing appropriate doses for the short term studies, and completely eliminating the two-year bioassay as described above.

Samuel M. Cohen, MD, PhD
Professor, Department of Pathology and Microbiology
University of Nebraska Medical Center

Dr. Tony Cox

Preliminary comments in response to the charge questions for the SAB consultation on New Approach Methods and Reducing the use of Laboratory Animals for Chronic and Carcinogenicity Testing

1a. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.
1b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

The proposed WoE approach is mostly clearly explained, and the motivation and rationale provided seem to me to be compelling and admirable. However, the proposal is not fully clear and complete in the following areas:

- Section 4 (Toxicity).
    - Attachment 1 says that "Indications of immunosuppression could raise concern for potential tumor formation." How should it be determined whether such concern is warranted by the evidence? Does a feeling of concern have relevance for risk assessment, or is the risk assessment to be driven by data independent of feelings of concern that it might not or might not engender?
    - Some immune response-related endpoints may be extremely sensitive to even low levels of exposure to some chemicals, and yet be irrelevant for risk assessment. (For example, some peripheral blood lymphocytes may respond to very low concentrations of benzene, and yet have no relevance for risk of acute myeloid leukemia.) How should the relevance of such sensitive responses for risk assessment be determined?
    - A change that "could raise concerns for potential tumor formation" in a non-specialist might not do so in a specialist who understands why the change is irrelevant for tumor formation. In such cases, is the concern itself to be used as a basis for decision-making, or should the possibility of concern instead be discussed by the registrant, and reasons for sharing or rejecting the possible concern be explained?

- Section 5 (Evidence of Chronic Toxicity from Related Chemicals). Attachment 1 says "As outlined above, providing a rationale for why the indicator molecule(s) were chosen as the best comparators to the candidate molecule is an important element of this section." How is "best" defined here? Might the "best comparators" still not be very good? Are there objective tests or principles that should be applied to select indicator molecule(s) as comparators?

- Section 6 (Proposed Points of Departure and Prospective Risk Assessments). Attachment 1 says "Calculate estimates for cancer risk (the Margin of Exposure) – by

linear or non-linear cancer risk assessment methods as appropriate for the molecule." How should the registrant (and EPA) determine which methods are "appropriate for the molecule"? Might several different methods be appropriate? If there is uncertainty about which methods are most appropriate, how should that uncertainty be addressed?

- Section 7 (Conclusion). How is a conclusion supposed to be derived (and supported) from the data considered? The template in Attachment 1 says "**Based on a WOE approach, the registrant requests that the chronic/carcinogenicity toxicity studies [be/not be (*as appropriate*)] required at this time for [Chemical X].** This approach considered all of the available hazard and exposure information for [Chemical X], including: [provide a summary of why studies should not be required]." This leaves unclear exactly how the registrant (and the EPA) should get from "all of the available hazard and exposure information" to a specific request.
    - Might the same body of information lead to different requests by different parties?
    - On what basis should it be decided whether a request should be granted based on the data provided? In other words, this final step, of getting from data considered to the action requested, needs to be explained further.

2. *EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to begin to replace the chronic/carcinogenicity testing in mammals. In addition, EPA-OPP and ORD are working together to collect quantitative gene expression data from short-term in vivo rat studies for a selected set of pesticides that cause liver tumors in rodent with known modes of action. All of these efforts are in the early stages of development and would benefit from expert and public input.*

    a. *Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.*

- Characterizing the predictive validity (rather than just the descriptive validity) of NAM-based approaches using gene expression data is critically important for assessing the practical value of this approach. What are its false-positive and false-negative rates (and how should these be defined and estimated from realistically limited data)?

- How does the value of information (VoI) provided by the NAM-based approaches compare to the VoI from traditional approaches? (Animal testing at relatively high concentrations has, arguably, not been very informative about human responses at realistic concentrations, although the decades-old debate on this point continues. Are NAM-based approaches demonstrably better, or at least not demonstrably worse?)

- Is it worth considering that some chemicals might be carcinogenic at toxic concentrations but not at relevant environmental concentrations?

3. *EPA is working with HESI, NTP, and other government and industry stakeholders to accelerate the incorporation of kinetically-derived maximum doses (KMD) into repeat dosing studies like the chronic/carcinogenicity study as an alternative to the traditional maximum tolerated dose (MTD).*

    a. *Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?*

- Great idea! This is important work and appears to be well thought-out. An additional activity to consider might be to carefully examine the effects of *timings* of repeated exposures (as well as their concentrations), as they relate to non-proportional responses. For example, dose fractionation and stop-exposure experiments on various chemicals have shown that the same cumulative exposure per unit time (e.g., per week) can have large or small toxic effects, depending on how it is distributed over time. This may require considering pharmacodynamics and well as pharmacokinetics. (See e.g., Figures 3 and 4 of "Implications of nonlinearity, confounding, and interactions for estimating exposure concentration-response functions in quantitative risk analysis www.sciencedirect.com/science/article/pii/S0013935120305314.)

Dr. Thomas Parkerton

General Comments

EPA correctly acknowledges some of the shortcomings with the present approach used for determining the need for chronic toxicity and carcinogenic data and should be commended for developing alternative approaches. The Office of Pesticide Programs has led efforts to provide a more flexible and fit for purpose approach in these assessments given the activities, actions and projects described. However, it is encouraged this initiative includes outreach and engagement across EPA. In addition to the Office of Research & Development, the Office of Pollution Prevention and Toxics (OPPT) should be viewed as an essential partner in actions that are planned consistent with the direction identified in the update of TSCA. Regrettably, OPPT does not appear involved in the Rethinking Carcinogenicity Assessment for Agrochemicals (ReCAAP) Project which would help ensure alignment on the development of a risk-based weight of evidence (WOE) approach for waiving chronic and carcinogenicity studies. Further, OPPT scientists should contribute to the HESI eSTAR program to ensure perspectives and concerns are addressed if they are not already active participants. As this initiative is progressed, efforts should be made to ensure consultation and alignment for implementation with other relevant EPA program offices such as the Office of Superfund Remediation and Technology Innovation, Office of Water, Office of Air and Radiation, and the National Center for Environmental Assessment.

Response to Charge Questions

1. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.

   RESPONSE: The draft WOE template appears like a positive step forward. Two questions for further consideration:
   How can this document be extended beyond agricultural chemicals?
   Read across is mentioned in step 5 in relation to carcinogenicity assessment. Can read across principles be extended to other toxicity endpoints if confirmative short terms assays indicate similar biological activity (specifically, subchronic and mode of action studies) ?

2. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

3. Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.

4. Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?

   RESPONSE: It is recommended that kinetically-derived maximum dose (KMD) concept for determining dose ranges proposed for carcinogenicity testing be extended to all repeat dose

toxicity testing. OPP is using KMD for pesticides and OPPT should be encouraged to apply this approach for industrial chemicals. Further, as this approach is further adopted and more broadly implemented across the EPA, will there a process or effort to reassess prior decisions and evaluation with regard to carcinogenic hazard/risk? More specifically, how will prior substance evaluations for which hazard thresholds were derived above the KMD and for which NAM data, MOAs, human relevance were not available or considered be reevaluated in light of updated assessment paradigms and new data.

Dr. Mara Seeley

Charge Questions: NAMS/Reducing Use of Laboratory Animals for Chronic and Carcinogenicity Testing

1a. Risk-based WOE approach for waiving chronic/carcinogenicity studies

- The WOE approach seems reasonable and well-thought out, as a framework
- EPA should consider incorporating evidence of pathways or findings potentially associated with an increased likelihood of tumor formation, such as suppression of programmed cell death, inflammation/regenerative cell proliferation

1b. Draft case study

- The draft case study is comprehensive and clearly presented.
- The case study should include reference to testing protocols or studies relied on for the toxicity testing

2. Direction and scope of collaborative NAMs projects

The direction and scope of the NAMs projects should advance the science of risk assessment away from reliance predominantly on traditional apical endpoints from animal bioassays.

3. Current KMD-related activities

The KMD seems like a preferable alternative to the MTD, and thus developing best practices for KMD analysis would be a worthwhile effort for EPA.

Dr. Kimberly White

Charge questions for the SAB consultation on new approach methods and reducing the use of laboratory animals for chronic and carcinogenicity testing.

1. Question: Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.

Answer: The "Draft Risk-Based Weight of Evidence Framework for Chronic/Carcinogenicity Studies with Agrochemicals" is clear regarding what information would be required of a registrant submitting a waiver request. However, it does not provide information on the criteria that the agency may use to review the information or support granting the waiver (i.e. is there a base level set of information that would be required for the waiver to be considered complete). Perhaps the approach could include an additional section between I and II that addresses this component. Additionally, section II.4 Toxicity, requests the registrant to "summarize how available studies can be used to inform chronic outcomes." This section should also consider including whether this information is specific to the chemical being assessed or is data also permitted for a surrogate chemical that is anticipated to act similarly to the chemical under review.

While there is a separate section II.5 which discussed read across information it was unclear if this information also applied to section II.4. In several sections of the draft risk-based WOE approach it requests information on mode of action data and associated key events. The document should consider highlighting specific mode of action frameworks that have already been accepted by the agency. As noted in the draft whitepaper provided to the SAB, the "CARC has evaluated tumor mode of action data for > 60 pesticides and has accepted the proposed MOA and conducted non-linear dose-response assessment for > 50 pesticides." Additionally, it is unclear how or when information about study quality should be presented throughout section II or if full study reports or manuscripts would also be requested as supporting information.

2. Question: Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

Answer: Some additional areas for clarity in the draft case study include the following: In Section 1. Use and Exposure Profile – additional information regarding other potential exposure routes (e.g. inhalation, dermal). The current draft notes "All exposure scenarios, including dietary (food and water), residential, aggregate, and occupation" but doesn't identify what those exposure routes are. In Section 4. Toxicity – Acute toxicity information is summarized in paragraph form and then a summary table in appendix B. Does this information need to include more specific study data and associated references?

Another potential consideration for the case study is whether it should include a summary table of the weight-of-evidence in the Section 7, Conclusion, that includes the lines of evidence available, relevancy of that data to the studies being requested to waive, the strength of that

evidence and the reliability/uncertainty associated with this evidence

3. Question: Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.

Answer: The direction and scope of the three collaborative projects to help support efforts to replace the use of animals in chronic/carcinogenicity testing appears reasonable. Some thoughts are provided below for consideration:

- DNTP Efforts to Improve Carcinogenic Assessment of Environmental Substances

– An important component of this project as noted in focus area 1 will be "Developing a translational toxicology pipeline (TTP) of capacities to characterize the potential for environmental substances to cause or contribute to the development of cancer." Having an understanding of what translational changes and at what level they represent an adverse impact will be important. Additionally, understanding the role of reversibility of any identified change and impacts to understanding the development or progression of cancer will also be important.

- Health and Environmental Sciences Institute (HESI) Point of Departure Program Overview

– Case study examples demonstrating how the PODs can be established will be important, perhaps this will be included in the 2021 manuscript. As well, addressing the issue of non-correlation of adverse effects with mode of action information and if that impacts the confidence of the established POD.

- Gene Expression Evaluation of Pesticides with Established Liver Tumor Modes of Action

– The agency should provide more details about the specific deliverables of this project.

4. Question: Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?

Answer: The project description should be updated to include specific deliverables, outcomes and overall timeline for the effort. For example, will the case studies that will be identified result in a publication or whitepaper; will the planned workshop result in a publication; are there communication or education aspects of this project that should be included; are there any specific challenges to implementation that have been identified?

Comments from SAB Chemical Assessment Advisory Committee Members

Dr. Richard Belzer

Preliminary Comments on SAB/CAAC Review of New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

June 22, 2020

1. **EPA-OPP is participating in the Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) with government, non-governmental organization, and industry stakeholders (Section 2). ReCAAP is developing a risk-based weight of evidence (WOE) approach for waiving chronic and carcinogenicity studies. This proposed approach is consistent with existing guidance and current practice for other types of toxicology studies.**

    a. **Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.**

The draft weight-of-evidence (WoE) framework in Attachment 1 ("Draft Risk-Based Weight of Evidence Framework for Chronic/ Carcinogenicity Studies with Agrochemicals") provides a well-organized list of categories of information that a registrant would be expected to provide. I see a few potential problems, however:

§ II is ambiguous with respect to informational requirements.

How would a registrant know that a petition is adequately supported? This section includes references to *potential* dietary and non-dietary exposure (§ II.1), bioaccumulation (§ II.2), chronic exposure (§ II.1), chronic toxicity (§ II.3), tumor formation (§ IV.4), molecules in the same chemical class (§ IV.5), and human exposure (§ IV.7). Outside of physics, *potential* has no scientific meaning. Thus, § II appears to be less about weight-of-*evidence* (WoE) than weight-of-*worry* (WoW).

§ II.6 concerns *safety* assessment, not *risk* assessment, and as such it cannot produce outputs compatible with regulatory benefit-cost analysis.

Each of the items requested here is part of safety assessment. There is no scientific definition of *safe*, so transparency requires that this be properly characterized as risk *management.* I realize that safety assessment is the dominant form of what federal and state governments do under the rubric of *risk* assessment. Nonetheless, the outputs of safety assessment are not refutable, inherently subjective, and hence nonscientific. They cannot meet established principles for information quality, including substantive and

presentational objectivity.[1] And they cannot be used as inputs to regulatory benefit-cost analysis. § II.7 reinforces the inference that the registrant would be asked to propose a risk *management* decision with respect to whether the default requirement to conduct additional studies should be waived.

For these reasons, the second half of the title of § II.6 should be labeled as Proposed/Prospective Risk *Management* Decision. If USEPA does not want to invite the registrant to opine on risk management, then this subsection should be revised accordingly.

This WoE framework is missing a coherent model and the weights that would be used to run it.

Is USEPA inviting registrants to propose which (WoE? WoW?) model the Agency should use and what weights it should apply? (To be clear, this is a question for clarification and not a criticism. Registrants may be better positioned to propose a choice of model and weights, and document these proposed choices in a way that is transparent and reproducible.)

The problem remains that the choice of WoE/WoW model and weights may be (correctly) criticized as subjective and perhaps self-serving. How to solve this? USEPA should give serious consideration to adopting a WoE framework focused solely on information, one that explicitly compares the societal burden of acquiring information and compares that to the practical utility of this information for producing human health benefits.[2]

While it seems logical to me that value-of-information principles should guide decisions to acquire information, these principles seem nowhere in evidence. A rational justification for waiving default informational requirements is that the burden of obtaining the information exceeds its practical utility given available scientific knowledge. This may be hard to quantify, but even a qualitative or semi-quantitative case could be highly illuminating. Clearly, the case for waiver is strongest when burden vastly exceeds practical utility and is weakest when burden exceeds practical utility by only a small amount.[3] Information of this type would inform the balancing test necessary to support rational choice. It also would help USEPA document the basis for its decisions.

As an alternative, a registrant could offer to describe what results these default studies would have to yield to alter the default decision set forth in § II.6. It may be

[1] Office of Management and Budget (2002); U.S. Environmental Protection Agency (2002).
[2] "Societal" burden is the value of scarce resources expended to obtain knowledge, which is never free. Leaving aside technical details not important here, societal burden is indifferent to whether the burden of knowledge discovery is borne by the private or public sector. Existing USEPA regulations and guidance give virtually zero weight to burdens borne by the private sector.
[3] Where practical utility demonstrably exceeds burden, there would be no justification for waiving the requirement.

reasonable for the registrant to describe how extreme the results of the studies proposed for waiver would have to be, and how likely it is that such extreme results would be obtained given existing scientific knowledge. The case for waiver is strongest if results must be extraordinarily extreme and unlikely to materially change the Agency's decision. Of course, registrants might need guidance from the Agency concerning specifically what results equate to what the hypothetical implementation of the WoW framework in Attachment 2 calls a "potential for concern" (there's that word, again). Still, this approach would use credible value-of-information principles to inform the decision to waive default information collection burdens, As a result, Agency decisions could be substantially more reproducible, and more easily defended if challenged.

b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

i. The case study in Attachment 2 ("Rethinking Carcinogenicity Assessment for Agrochemicals Project [ReCAAP]") invents, but does not define, new terms of art that are not in Attachment 1 ("Draft Risk-Based Weight of Evidence Framework for Chronic/ Carcinogenicity Studies with Agrochemicals").

At the top of this list is "potential for concern" in § I ("Purpose of the Analysis"), a phrase that is ambiguous in both nouns. The case study's elaboration on this purpose is unhelpful. The analysis is supposed to be informed by "the *potential* for long-term exposure from dietary sources" when "*possible* total chronic exposure is very low" (emphasis added). Is *potential* different from *possible*? If so, how? If not, why are different terms used? This ambiguity is partially relieved by text that seems to invoke value-of-information principles, but the lack of any concrete expression of those principles raises doubt as to whether that inference is correct (or whether a registrant interpreting the text this way might later be blindsided).

Some text in Attachment 2 is a source of potential … worry.

In my comments on the proposed WoE framework in response to Question 1(a), I said that text looked more like a WoW framework. Attachment 2 reinforces this concern. *Potential*, an adjective that is ambiguous without bound, appears 18 times in the case study. *Possible* appears another six times. In none of these uses, however, is the adjective defined to reveal, for example, an associated probability – particularly the probability sufficient to trigger enough worry to deny a waiver request.

The stated facts in this case study make the waiver decision easy, so it's superfluous *in this case* to unpack the meaning of *potential.* But that does not illuminate how the framework would be implemented if the facts were less one-sided.

Where simple declarative scientific statements are made, they are sometimes undermined by ambiguity.

§ II.1 makes a factual statement that contains substantial probative information establishing a strong case for waiver given the precautionary character of HED's *levels of concern*:

> All exposure scenarios, including dietary (food and water), residential, aggregate, and occupation, are reported to be below the level of concern for EPA's Health Effects Division (HED).

If all exposure scenarios are below HED's worry threshold, that ought to be sufficient. Why, then, does the text follow with a second sentence that undermines the first?

> While there is the potential for long-term exposure from dietary sources, the possible total chronic exposure is very low.

Text in §§ II.2, II.3, II.4.5, and II.5 provide better models. § II.2 explains, in just two sentences, everything that is needed to infer that Herbicide1 should not trigger concern (worry?) about bioaccumulation. § II.3 makes the same point in a few short paragraphs, all with the same message. The paragraph on excretion includes a profoundly powerful scientific inference: " "*no* bioaccumulation *potential*" (emphasis added). § II.4.5 clearly states, "All [genotoxicity] studies were negative, *clearly eliminating* any concern for tumor formation via genotoxic mechanisms" (emphasis added). § II.5 reports that Herbicide 1 "does not contain" elements "demonstrated to be associated [causally associated?] with sensitization reactions" and "showed *no effects* on the thyroid" (emphasis added).[4] Scientifically correct declarative statements that cut off a risk pathway should be encouraged.

Taking these texts at face value, the burden of collecting additional information based on what is scientifically known almost certainly exceeds the practical utility of the information which the studies proposed for waiver would supply. It would be helpful if USEPA invited registrants to characterize the available science in such value-of-information terms.

WoE/WoW inferences are not obvious from the evidence presented.

§ II.4.2 says continuous administration of Herbicide1 leads to calculi-induced hyperplasia in dogs at specified (high) doses. These effects are said to be reversible. Why, then, does this evidence "strongly support that the threshold of concern for toxicity for Herbicide1 is the exposure that leads to calculi formation regardless of the duration of the exposure"? A rational basis for this inference seems to be missing. Does reversibility not

[4] Qualifying such statements with dubious probability statements is unhelpful. After declaring the absence of thyroid effects, stating that "Herbicide1 is *highly unlikely* to result in thyroid tumor formation" (emphasis added) creates contradictory ambiguity.

matter? Is there truly no difference between exposure for a lifetime versus exposure for a single day? Absent a rational basis, WoE and WoW inferences are indistinguishable.

WoE/WoW inferences cannot be interpreted in welfare economic terms.

The benefits of avoiding a risk depend on willingness-to-pay (WTP). An economist asked to estimate WTP to avoid reversible calculi-induced hyperplasia would be stymied. This biological effect of is not easily understood by nonexperts, and valuation requires that a risk be well-defined. Moreover, any WTP is severely attenuated precisely because the effects are reversible. Given the information provided, the best estimate of the value of avoiding this presumptively adverse effect is zero. If that is so, the proposed WoE framework clearly has strayed into WoW territory. The authors need to make a stronger case why reversible calculi-induced hyperplasia is meaningful to actual people, particularly if doses are below the threshold whereby calculi formation can act as urinary bladder carcinogens. Similar concerns arise for hormone perturbation as a presumptively adverse effect. It is likely that perturbation within normal boundaries would not be construed by actual people as an adverse effect. And actual people might interpret some hormone perturbations (or even permanent changes) as beneficial. A biological effect, whose interpretative meaning to actual people is unclear in both magnitude and sign, cannot be presumed to be adverse just because toxicologists can find it in laboratory experiments.

The case study description of chronic urinary tract toxicity suggests that information essential for valuation is missing (or perhaps was not reported). The summary inference – *with sufficient exposure*, crystalluria and the accompanying toxicity might be expected for Herbicide1" (emphasis added) begs the question what exposure is *sufficient*. If that exposure level materially exceeds environmental concentrations, then WTP to avoid it is likely to be zero and the social benefit from denying the waiver would be negligible at best. Only if the level of exposure determined to be *sufficient* is environmentally relevant to human would there be any practical utility in acquiring the information.

The achievement of safety-assessment type dose indicators (e.g., NOAELs) also cannot be valued, which makes them dubious health-based targets. Setting aside for the moment the fact that purported adverse effects understood only by experts are not well-defined goods, a monetary value in principle can be estimated for the avoidance of any response in a causal dose-response relationship. But dose indicators that are not located on a causal dose-response relationship are much more challenging to value. They consist of two parts: (1) WTP to avoid the nearest dose causally located, and (2) WTP for the additional "safety" achieved by reducing exposure below this causally located dose, though without any information concerning the magnitude of additional risk avoided. The second part is purely speculative, and the greater the implied safety factor the more speculative it is. WTP for the latter part is likely to be zero, as would be the WTP for any noncausal dose-response.

The value of waiving default studies on value-of-information grounds is diminished when risk management adjustments are made without their burdens being accounted for

in the analysis. Despite the strong case given for why chronic toxicity in humans is impossible at environmentally relevant doses, the case study proposes to add an additional 10x factor for database uncertainty (see Table 7). The proper question is the net practical utility of a waiver under both scenarios. Only if net practical utility is higher under the scenario in which the 10x database uncertainty factor is added is it possible to justify adding that factor on value-of-information grounds. Otherwise, it is a just a risk management concession.

2. **EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to begin to replace the chronic/carcinogenicity testing in mammals. In addition, EPA-OPP and ORD are working together to collect quantitative gene expression data from short-term *in vivo* rat studies for a selected set of pesticides that cause liver tumors in rodent with known modes of action. All of these efforts are in the early stages of development and would benefit from expert and public input.**

The description of the NAM project does not explicitly say so, but it appears to be grounded on the realization that traditional rodent bioassays, which formerly were universally characterized as the "gold standard" in toxicology, have significant information quality deficiencies, most notably "imperfect translational relevance to human health." See the list on p. 2. In addition, rodent bioassays have been assailed for lacking practical utility sufficient to justify their burden. Practical utility limitations are self-evident from this acknowledged information quality deficiency, and burden includes both cost, delay, and low throughput. A relatively recent addition to these burdens is rising salience of ethical concerns about animal sacrifice.

I am concerned that the "3R principles" summarized in § 1.2 of the Cancer NAMs White Paper may lack a proper respect for value-of-information principles. There are optimal levels of reduction, replacement, and refinement, each of which depends on the result of value-of-information analysis.[5] But I cannot find any discussion in the White Paper that properly addresses tradeoffs. Tradeoffs are hinted at by noting "there will not be a 'one-size fits all' solution" (see p. 4), but nowhere are tradeoffs transparently discussed and analyzed.[6] Further, the "risk-based weight of evidence analysis framework" does not transparently include value-of-information principles or recognize tradeoffs. If it is true that "NAMs are expected to improve the scientific foundation of risk assessments by providing more human-relevant information that is more efficient and less costly," (pp. 4-

[5] If the concern about animal sacrifice were truly a matter of ethics, such experimental would be simply banned instead of reduced, replaced, and/or refined.

[6] Even this limited acknowledgement of tradeoffs is subsequently discarded in favor of on-size fits all approaches to KMD: "There is an immediate need to *standardize* these approaches for broad regulatory use and facilitate *global harmonization*" (p. 5). "Standardization" is the *sine qua non* of a one-size-fits-all solution; "global harmonization" makes clear that "one-size" is not an approximation.

5), then why has the technology of toxicology been mired for decades in tools that generate low-value information in a manner that it inefficient and expensive?[7]

Conventional safety assessment wages war against scientific risk assessment and regulatory benefit-cost analysis, and at least one of the proposed reforms in "Replacement" would continue this war in a new guise. Kudos to HESI for convening "a multi-sector and multi-disciplinary working group," but doing so to "build and implement [yet another] framework" for "health protective point[s] of departure" (p. 5, bullet 2), that are by definition *not* part of risk assessment and are fundamentally incompatible with information quality principles and regulatory benefit-cost analysis, is not a productive step forward.

a. Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.

I am concerned that a key attribute of these three projects is the preservation of certain institutional controls over the path of reform, and that these controls will operate in ways that are incompatible with objective risk assessment and regulatory benefit-cost analysis.[8]

An early sign of trouble can be found in the simultaneous adoption of the "fit for purpose" concept and the goals of "characteriz[ing] the *potential* for environmental exposures to cause *or contribute to* the development of cancer in humans…" (emphasis added, White Paper, p. 8). These goals are incompatible with regulatory benefit-cost analysis, a key purpose of risk assessment, in which *potential* exposures (probability unknown) that *contribute to* risk (in some amorphous way) actually are risk management thumbs on the risk assessment scale. The replacement of one institutionalized incompatible toxicology technology with another is not reform in any meaningful sense. Perhaps the development of a "translational toxicology pipeline" would be useful, but it won't be if it substitutes "carcinogenic *potential*" for risk.[9] Adverse Outcome Pathways do not necessarily bring us closer to objectively estimating cancer *risk*. Rather, they may be used to justify the substitution of ever more arcane non- or pre-cancer endpoints – endpoints that actual people cannot understand or value.[10]

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[7] Similarly, if a kinetically-derived maximum dose approach "not only lessens or avoids unnecessary pain and distress in animals, but also generates data that are relevant and more predictive of human health risks" (p. 5), why have conventional methods that callously imposed pain and distress without producing useful information been used for so long?

[8] It was NTP that institutionalized the 2-year rodent bioassay and for decades protected it from criticism, and the short description in § 3.1 suggests that it seeks to retain this role.

[9] That the plan would retain the 2-year rodent bioassay and "an option" is similarly worrisome. These bioassays should be justified only by rigorous application of value-of-information analysis, never treated as acceptable defaults in which the burden of proof rests with alternative technologies.

[10] It is far from clear that toxicologists can consistently rank alternative points along an AOP. If they cannot, the purported AOP lacks sufficient scientific content to be used for estimating risk or benefits to human health.

3. **EPA is working with HESI, NTP, and other government and industry stakeholders to accelerate the incorporation of kinetically-derived maximum doses (KMD) into repeat dosing studies like the chronic/carcinogenicity study as an alternative to the traditional maximum tolerated dose (MTD). The KMD approach is consistent with numerous guidance documents developed by EPA, OECD and other international organizations as a more humane and human relevant approach to dose selection. One KMD study has been provided to the SAB along with the description and agenda of an upcoming workshop and the scope/charge of a workgroup at HESI to develop additional case study and a best practices document.**

    a. **Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?**

The observed historical difference in how KMD has been used in pharmacology and chemical safety assessments[11] deserves more thoughtful explanation (though some is presented in Attachment 3). If this difference is attributable primarily to science, a concerted application of science may be useful. But it is primarily attributable to institutional factors, the public interest argues against any governmental entity (or group of entities) preserving institutional control over reform.[12] Some support for the former is supplied early in Attachment 3, but unfortunately the structure of the project, and thus constraints imposed upon it, support the latter. To oversimplify, the principle values of KMD appear to be obtaining information at dose ranges closer to human exposure and determining where and why the decades-long assumption of linearity breaks down. Why, then, is the proposed project so focused on "best practices"?

"Best practices" are, of course, generally preferred; who, after all, is against what's "best"? Problems can and do arise when, under the guise of "best practices," stakeholders (including government agencies) seek to establish their preferred models and/or outcomes as "best," to the exclusion of their scientific or institutional competitors. Especially at this stage, therefore, it may be premature to establish the goal of seeking a consensus on "best practices," as the HESI project, summarized in Attachment 4, appears trying to do. A better

[11] "While KMD is routinely considered in preclinical tests to provide perspective on the relevance of study results to human safety assessment for drugs, KMD is rarely used in chemical safety assessment" (Cancer NAM White Paper, p. 13).

[12] By "institutional factors" I am, of course, alluding to Public Choice theory. See, e.g., Buchanan and Tullock (1962) and Downs (1967). For a historical perspective on today's moment of reform in toxicology, see Downs (1972). The second stage in Down's "issue-attention cycle" ("alarmed discovery and euphoric enthusiasm") is best captured by the publication of National Research Council (2007). It's a matter for debate whether reform is now in stage 3, 4 or 5. Evidence for each can be found in the White Paper.

approach may be to seek reasonable minimum performance standards that would exclude only demonstrably inferior approaches.[13]

The most salient concern to me is that this workshop emphasis the need for outputs that are compatible with objective risk assessment and regulatory benefit-cost analysis. Outputs that, like the longstanding toxicological tools that would be replaced, are inconsistent with these purposes are not helpful. Clarity that objective risk assessment is intended would substantially ameliorate this concern.

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[13] The stated objectives include both exclusivity ("Selecting *the* appropriate PK parameter to examine dose proportionality," emphasis added) and inclusivity ("Defining a *minimum* dataset considered necessary to set a KMD," emphasis added).

Dr. Tiffany Bredfeldt

Charge Questions for June, 2020 Science Advisory Board

New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

In accordance with the September, 2019 directive from EPA Administrator Andrew Wheeler, EPA's Office of Pesticide Programs (OPP) and Office of Pollution Prevention and Toxics (OPPT) are working to reduce the number of laboratory animal studies requested or required for pesticides and industrial chemicals. Beyond the ethical issues associated with animal use, new approach methods (NAMs) are expected to improve the scientific foundation of risk assessments by providing human-relevant information that is more efficient and less costly. In collaboration with the Office of Research and Development and multiple stakeholders, EPA-OCSPP has developed a draft white paper highlighting three projects that are improving the science used in risk assessment for chronic/carcinogenicity testing. These activities are organized by the 3Rs principles for laboratory animal testing-- *reduce, replace, refine* as originally proposed by Russell and Burch (1). Because of the complexities in biology and toxicology, there will not be a "one-size-fits-all" solution to improving chronic/carcinogenicity testing. As such, EPA and its collaborators are taking a multifaceted approach that advances several areas simultaneously. The agency requests the SAB provide comment on the following charge questions.

1. EPA-OPP is participating in the Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) with government, non-governmental organization, and industry stakeholders (Section 2). ReCAAP is developing a risk-based weight of evidence (WOE) approach for waiving chronic and carcinogenicity studies. This proposed approach is consistent with existing guidance[14] and current practice[15] for other types of toxicology studies.
    a. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.

        i. Under Section II item 1, the USEPA should consider adding a section to the framework where study waiver applicants can report any known biomoritoring data or any form of historical exposure data if/when available.
        ii. Under Section II item 2 of the framework, the applicants are requested to report what is known about metabolism in mammals and which metabolites are formed in the environment. Metabolites

[14] https://www.epa.gov/sites/production/files/2015-04/documents/data-require-guide-principle.pdf ; https://www.epa.gov/sites/production/files/2014-02/documents/part158-tox-data-requirement.pdf

[15] Craig et al (2019) Reducing the Need for Animal Testing While Increasing Efficiency in a Pesticide Regulatory Setting: Lessons From the EPA Office of Pesticide Programs' Hazard and Science Policy Council. Regul Toxicol Pharmacol, 108, 104481 Nov 2019. PMID: 31546018

formed in the environment would be better placed in a section describing environmental transport and fete of the chemical in questions to avoid confusion. This section could be referred to as needed throughout the document.

iii. In item 2 of Section II, the framework document asks applicants to describe results from available toxicokinetic studies. The second bullet describing repeated dose evaluations is worded in a confusing manner. Please consider breaking these instructions into more sentences or rewording for clarity.

iv. A MOA section should be included as a stand-alone section. Then, following MOA section, more emphasis could be placed on specific MOA where it is important to the exposure duration in each section. This just seems like MOA is being included as too much of an afterthought in subsections when it should be critical in the event chronic studies are being waived.

v. Overall, the framework for this waiver are clear and well-written. However, they read like instructions. What is not clear is what WOE will be specifically required to waive long-term cancer bioassays. Though, the WOE is likely considered on a case-by-case basis, the guidelines need to consider in their scope required WOE (or do so in additional case studies). The framework could be more widely used and should be written in a manner that is more comprehensive when it comes to required WOE along with examples.

vi. It is clear that the central MOAs for carcinogenicity, i.e., genotoxicity, endocrine disruption, and immunosuppression, are being addressed in the framework. Additional data to be considered should more clearly requested, e.g., receptor-mediated assays, instead of adding such data into a vague "additional" data section, which is where this data was placed in the example provided in Attachment 2.

vii. The human-relevance of the proposed MOA should be discussed in the MOA section(s) or as a separate section. This evidence is a critical point of discussion that may be best emphasized in a MOA section rather than scattered throughout the document.

b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

i. For a variety of places in the document, figures (e.g., metabolism and MOA) would greatly improve clarity and ease of reading. It is valuable to encourage applicants to produce figures in these documents for ease of reading and transparency.

ii. The EPA provided a very thorough WOE analysis for Herbicide1. The overall report is clear and convincing. The available data support the choice to not require cancer bioassays for this chemical based upon

what is known of the MOA and the available toxicity database. However, it would be nice to have another example where the chemical-specific database is less data rich. Such an example may provide clearer guidance for when EPA can apply this waiver and when they cannot. The criteria for waiving or not waiving data is not clear from the available framework. To have consistent application of the waiver, it may be necessary to discuss required WOE a bit more. This might require the production of more prescriptive guidelines or additional WOE case studies, which are apparently underway, to be made available.

2. EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to begin to replace the chronic/carcinogenicity testing in mammals. In addition, EPA-OPP and ORD are working together to collect quantitative gene expression data from short-term *in vivo* rat studies for a selected set of pesticides that cause liver tumors in rodent with known modes of action. All of these efforts are in the early stages of development and would benefit from expert and public input.

    a. Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.

        1. The scope of the three focus areas for the TTP is appropriate and timely. The strong focus on AOP or MOA framework early in the process appears to be a wise choice for this collaborative project.
        2. The scope and direction of the future goals for 2020 are appropriate. While initial work seems focused on collecting methods or models, there is significant emphasis on the mutational comparison between rodents and humans. Other cancer MOA should also be included in these early stages, so that they may matriculate in tandem. I would consider comparison of rodent versus human endocrine disruptors and immunosuppressors also important. I have concern that too much focus on comparing rodent to human data may simply be an act of comparative biology if contextualization is not well integrated throughout these early stages.
        3. The structure and key elements of the TTP will be critical for its success. At this time, it is unclear what that structure will be of the TTP. A figure or scheme of that structure would benefit the clarity and make component stages of the pipeline more transparent in regard to their use and scope. It would seem that the obvious and most simple stages that make up the TTP

are a development and growth stage for establishing assays, a stage for increased use in the scientific and risk assessment communities for developing and shaping application of assays or methods within these communities. Finally, the mature, characterized assays in final stages should be made available for broad and required use in the regulated community.

4. In addition to clarifying TTP stages, it will be important for inclusion and exclusion criteria to be developed within the context of an AOP/MOA framework for the application of new tools developed or refined in the TTP. Importantly, human relevance should be integrated into throughout the framework to enable it to be truly fit for purpose.
5. The Health and Environmental Sciences Institute (HESI) Point of Departure Program Overview project is of high value and importance. It is critical that we learn to derive POD from evolving methods in vivo and in vitro. Some impressive progress has been made on these fronts.
6. The EPA and collaborators are right to begin their POD finding studies in rodent cell lines for comparison against in vivo studies. However, the challenge of human relevance is not necessarily met in this approach because studies in human cell lines are not being referred to in a manner that indicates they will be a critical part of POD finding studies. The EPA should consider the pathway forward that moves away from animals and animal cell lines and how to conduct risk assessments in human cell lines. It is apparent that EPA has considered these endpoints per the studies they have published in the past. However, it is unclear how EPA will incorporate human cells for comparison to rodent model systems in the described research projects.
7. In the Gene Expression Evaluation of Pesticides with Established Liver Tumor Modes of Action Project, the EPA is fortunate to have a collection of guideline studies and mechanistic information for many pesticides and I agree that these studies and provide a logical bridge between traditional studies and NAMS. These studies are critical for the risk assessment community to build confidence in NAMs.
8. The EPA chose 6 MIEs that are logical for the investigation of liver cancer in rodents. They at least establish a foundation upon which to build. The EPA indicates that the methods were derived using Affymetrix microarray data of rats exposed to pharmaceutical compounds. The following objective is a bit unclear. Does the EPA intend to generate a novel or new profiling platform or a custom one? More information is

needed in this section to provide clarity upon what the EPA intends to do.

9. The primary mention of dose in section 3.3 seems to indicate that studies are being conducted with tumorigenic doses. It is unclear if the EPA intends to conduct studies with a range of doses for liver tumorigenic agents. It would seem logical for the EPA to not only include tumorigenic doses but lower doses where tumorigenesis may not be expected to determine the behavior of genes responding early in the process or whether there are transitions in the toxicity in or among AOP. It is unclear how the EPA intends to conduct these studies and clarity is needed.

3. EPA is working with HESI, NTP, and other government and industry stakeholders to accelerate the incorporation of kinetically-derived maximum doses (KMD) into repeat dosing studies like the chronic/carcinogenicity study as an alternative to the traditional maximum tolerated dose (MTD). The KMD approach is consistent with numerous guidance documents developed by EPA, OECD and other international organizations as a more humane and human relevant approach to dose selection. One KMD study has been provided to the SAB along with the description and agenda of an upcoming workshop and the scope/charge of a workgroup at HESI to develop additional case study and a best practices document.

    a. Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?

        i. The EPA's interest in reducing animal research is commendable. However, it is unclear how the KMD-related activities will reduce animal use given that kinetic studies must be conducted to find the KMD.
        ii. "KMD refers to the highest dose at, or slightly above the point of departure from dose proportionality, or PK linearity". The KMD approach is interesting, but it appears applicability will be highly chemical-dependent. There are a variety of reasons non-linear kinetics may occur: saturation in metabolism, saturation of absorption, and saturation of excretion. These cases may give rise to different toxicities depending on what step and chemical/metabolite is driving toxicity. With that in mind, the concept of KMD is complex. In cases where the KMD is achieved by saturation that results in accumulation of a less toxic form of a given chemical the saturation kinetics may not drive a maximumly tolerated dose or a meaningful maximal dose. Clearly, MOA must inform the decision to utilize KMD. Such a policy may make the application of KMD variable and, as such,

inconsistent, an issue that EPA is aware of as mentioned in the Cancer NAMs White Paper. Further, too low dose selection during toxicity testing may give rise to misleading interpretations of toxicity data. It seems that KMD doses are higher than human exposure. Thus, if a KMD is to be used, it will be important to define human exposure when that data is available to contextualize the use of the KMD.

iii. The concept of inflection point where dose proportionality is lost may be flawed when one considers that saturation, by whatever mechanism, is generally not a rapid change. Thus, it may be a gradual event since saturation may not appear as a simple threshold or inflection point on the dose response curve. If we assume this could happen, at least in some instances, the KMD concept will be flawed. It is unclear how EPA may deal with this issue.

iv. The EPA should consider benchmark dose approach (BMD) as an alternative to the KMD approach. In this approach, it may be possible to use more dose groups to characterize the dose-response curve. In cases where animal suffering appears significant, high dose studies could be halted.

v. Please see:

1. Harringa MB, Cnubben NHP, Slob W, Pronk MEJ, Muller A, Woutersen M, Hakkert BC. 2020. Use of the kinetically-derived maximum dose concept in selection of top doses for toxicity studies hampers proper hazard assessment and risk management. Regulatory Toxicology and Pharmacology. 114:104659. https://doi.org/10.101106/j.yrtph.2020.104659
2. Woutersen M, Muller A, Pronk MEJ, Cnubben NHP, Hakkert BC. 2020. Regulating human safety: how dose selection in toxicity studies impacts human health hazard assessment and subsequent risk management options. Regulatory Toxicology and Pharmacology. 114:104660. https://doi.org/10.1016/j.yrtph.2020.104660
3. Kalantari F, Ringblom J, Sand S, Oberg M. 2017. Influence of distribution of animals between dose groups on estimated benchmark dose and animal distress for quantal responses. Risk Analysis:37:1716-1728. https://doi.org/10.1111/risa.12741

Dr. Karen Chou

Charge Questions for June, 2020 Science Advisory Board

New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

In accordance with the September, 2019 directive from EPA Administrator Andrew Wheeler, EPA's Office of Pesticide Programs (OPP) and Office of Pollution Prevention and Toxics (OPPT) are working to reduce the number of laboratory animal studies requested or required for pesticides and industrial chemicals. Beyond the ethical issues associated with animal use, new approach methods (NAMs) are expected to improve the scientific foundation of risk assessments by providing human-relevant information that is more efficient and less costly. In collaboration with the Office of Research and Development and multiple stakeholders, EPA-OCSPP has developed a draft white paper highlighting three projects that are improving the science used in risk assessment for chronic/carcinogenicity testing. These activities are organized by the 3Rs principles for laboratory animal testing-- *reduce, replace, refine* as originally proposed by Russell and Burch (1). Because of the complexities in biology and toxicology, there will not be a "one-size-fits-all" solution to improving chronic/carcinogenicity testing. As such, EPA and its collaborators are taking a multifaceted approach that advances several areas simultaneously. The agency requests the SAB provide comment on the following charge questions.

1. EPA-OPP is participating in the Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) with government, non-governmental organization, and industry stakeholders (Section 2). ReCAAP is developing a risk-based weight of evidence (WOE) approach for waiving chronic and carcinogenicity studies. This proposed approach is consistent with existing guidance[16] and current practice[17] for other types of toxicology studies.
    a. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.

    The outline is well done.

    1) Under section II. 1. Use pattern & exposure scenarios: It may be helpful to state that the statements about exposure profile should include information on both environmental exposure pathways and the routes of exposure of the test substance in humans.

[16] https://www.epa.gov/sites/production/files/2015-04/documents/data-require-guide-principle.pdf ; https://www.epa.gov/sites/production/files/2014-02/documents/part158-tox-data-requirement.pdf

[17] Craig et al (2019) Reducing the Need for Animal Testing While Increasing Efficiency in a Pesticide Regulatory Setting: Lessons From the EPA Office of Pesticide Programs' Hazard and Science Policy Council. Regul Toxicol Pharmacol, 108, 104481 Nov 2019. PMID: 31546018

2) Many factors influence the difference between short-term toxicity and chronic toxicity. Log (Kow) is one of them. Among the other factors that are necessary to maintain an organism's health are the continuous availability of hydrophilic endogenous molecules required in Phase II biotransformation. Manifestation of short-term toxicity of a test substance may be prevented by Phase II reactions, while continuous and long-term exposure may deplete one or more of the endogenous molecules, when the rate of synthesis exceeds the rate of expenditure. Frequent and long-term depletion of these molecules (e.g., glycine, taurine, glutamine, sulfate, glutathione, glucuronide, or donors of acetyl group and methyl group), can result in adverse effects that could not be observed in short-term studies. Impairment of an organism's capacity to compensate for additional stress is a criterion in evaluating potential adverse effect, based on EPA's definition of "adverse effect". The reviewer, therefore, suggests that potential depletion of these endogenous molecules to be reported, considered in the WoE assessment, and reviewed for the waiver decision.

b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

The draft case study is clear written. It provides appropriate information and is outlined as that is shown in Attachment 1. The WoE framework, as evident in Appendix G, delivers a reasonable approach for the retrospective analysis. In the case of Herbicide 1, the WoE analysis has provided sufficient certainty for the waiver decision.

Additional comments:

1) Section II.1, Paragraph Exposure.: The exposure profile should include statements for all possible environmental exposure pathways and the routes of exposure in humans. In the example presented in Attachment 2, the routes of dermal and inhalation exposure are not mentioned. If some of the routes of exposure are unlikely based on the proposed use of Herbicide 1, it is worthy to be stated so to provide clarity.
2) P. 4, Absorption: The absorption of all potential routes of exposure to Herbicide 1 should be reported. If data are not available for certain routes, it should be stated so to provide clarity. The information provide for the route of oral exposure is clear, condensed, and informative.
3) P. 5, first paragraph: Explain/define first label and second label. Otherwise, the paragraph is well done.
4) Section 3. The ranges of test doses in the studies reported under the subsections of Absorption, Distributing, Metabolism, and Excretion need to be reported, for comparing with the amounts of likely exposure of normal use, comparing with the results from other studies, and evaluating potential saturation of ADME pathways.
5) P. 7, "The NOAEL in rat was 58 mg/kg/day and 70 mg/kg/day for males and females respectively": Based on the data presented in Table 4, the reviewer finds the selection of NOAEL values questionable.

6) The WoE framework, as evident in Appendix G, provides a reasonable approach for the retrospective analysis. In the case of Herbicide 1, the WoE analysis provides sufficient certainty for the waiver decision.

2. EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to begin to replace the chronic/carcinogenicity testing in mammals. In addition, EPA-OPP and ORD are working together to collect quantitative gene expression data from short-term *in vivo* rat studies for a selected set of pesticides that cause liver tumors in rodent with known modes of action. All of these efforts are in the early stages of development and would benefit from expert and public input.
   a. Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.

The three projects will likely to provide supporting information for threshold dose-response relationship for liver cancer, justification for using data generated from short-term study for the assessment of potenital long-term effects, including carcinogenicity, and potentially eliminating some of the required toxicity testing studies for human health risk assessment. Together, they will provide information, tools, and justification for the harmonization of guidelines for cancer and noncancer effects, including the assumption of threshold dose-response relationship.

Specifically, in project 1, the US National Toxicology Program (DNTP) will populate the translational toxicology pipeline (TTP) with existing technologies and database and implementing AOP and IATA approaches, as a part of the effort to expand the use of alternative methods in assessing chemical carcinogenicity. A TPP framework will be developed to link mechanisms to pathways for site-specific cancers. Carci HEI could enhance the communication among federal agencies and develop a communication strategy to deliver clear and actionable information to all stakeholders.

In the second project, led by HESI, alternative approaches will be applied to evaluate omic-technology and non-apical effect-based POD, such as transcriptomic POD, for human health risk assessment. Results from previous studies, although limited, have demonstrated the possibility that short-term non-apical PODs may be used to predict chronic apical PODs.

The third project is designed to provide evidence to support the replacement of the long-term carcinogenicity testing study with short-term studies, by using (a) the liver-tumor database evaluated by CARC of EPA-OPP, (b) the discovery that liver-cancer biomarkers observed after short-term exposure can accurately identify chemical-dose combinations that cause liver tumors at 2-years post exposure, and (3) the observed threshold in the dose-response relationships of the six biomarkers.

3. EPA is working with HESI, NTP, and other government and industry stakeholders to accelerate the incorporation of kinetically-derived maximum doses (KMD) into repeat dosing studies like the chronic/carcinogenicity study as an alternative to the traditional maximum tolerated dose (MTD). The KMD approach is consistent with numerous guidance documents developed by EPA, OECD and other international organizations as a more humane and human relevant approach to dose selection. One KMD study has been provided to the SAB along with the description and agenda of an upcoming workshop and the scope/charge of a workgroup at HESI to develop additional case study and a best practices document.

    a. Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?

    The assumption that an observed inflection point reflects kinetic saturation is possible and likely to be demonstrable using certain datasets generated from toxicity testing studies, but unlikely to be universal. Studies have shown that some fitted toxicokinetic models provide no inflection points (Heringa et al. 2020). In addition, toxicity testing studies are designed for observations that are the outcome of changes in both types, toxicokinetic and toxicodynamic pathways. Selectively excluding test doses based on toxicokinetics alone could severely undermine the scope of toxicological observations, such as observations for mechanistic insights.

    As for the application of the KMD approach in the case of 1,3-dichloropropene, which is cited in the White Paper as an example for the KMD approach, observations from the test dose of 60 ppm was dismissed from the toxicity assessment. The decision was made based on the inflection point of 40 ppm, without deliberating potential human exposure. Furthermore, the magnitude of the difference between the two doses, 40 ppm and 60 ppm, is not considered in the judgment of dismissal.

    Recommendation for additional topics to be discussed at the KMD Workshop:

    1. Define validation requirements before the KMD concept can be implemented in testing guidelines; the examples provide in the White Paper provide only limited information.
    2. Defining unacceptable top doses when KMD approach is applied in toxicity testing studies. The magnitude of unacceptable exceedance, when top dose exceeds KMD, needs to be carefully considered. The proposed risk assessment or testing guidelines involving the use of KMD should make an effort to minimize the situations where results from high dose exposure are excluded from the toxicity assessment after looking at the toxicity effects.

Dr. Harvey Clewell

Charge to the SAB on *New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing*

Discussion/Charge Questions

1. EPA-OPP is participating in the Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) with government, non-governmental organization, and industry stakeholders (Section 2). ReCAAP is developing a risk-based weight of evidence (WOE) approach for waiving chronic and carcinogenicity studies. This proposed approach is consistent with existing guidance and current practice for other types of toxicology studies.

    a. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.

I found the draft framework to be clearly written and comprehensive. I'm sure it will require modification/expansion as additional case studies are evaluated.

    b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

The case study provides an excellent example of the potential application of the framework for a chemical that causes renal toxicity from calculi formation, which is a fairly straightforward case. I would suggest that additional case studies are needed to illustrate the application of the guideline for other cases such as liver toxicity associated with enzyme induction, metabolism-related thyroid toxicity, etc. It would also be useful to included cases where the mutagenicity data is equivocal but read across suggests a non-mutagenic mode of action.

2. EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to begin to replace the chronic/carcinogenicity testing in mammals. In addition, EPA-OPP and ORD are working together to collect quantitative gene expression data from short-term *in vivo* rat studies for a selected set of pesticides that cause liver tumors in rodent with known modes of action. All of these efforts are in the early stages of development and would benefit from expert and public input.

    a. Please comment on the direction and scope of the three collaborative projects described in Section 3 of the draft white paper.

The OPP efforts described in Section 3, including the collaborative projects with NIEHS and HESI, are important for moving methods of carcinogenic assessment into the future, increasing the capability for rapidly identifying carcinogenic potential while reducing animal testing requirements, and should be continued.

3. EPA is working with HESI, NTP, and other government and industry stakeholders to accelerate the incorporation of kinetically-derived maximum doses (KMD) into repeat dosing studies like the chronic/carcinogenicity study as an alternative to the traditional maximum tolerated dose (MTD). The KMD approach is consistent with numerous guidance documents developed by EPA, OECD and other international organizations as a more humane and human relevant approach to dose selection. One KMD study has been provided to the SAB along with the description and agenda of an upcoming workshop and the scope/charge of a workgroup at HESI to develop additional case study and a best practices document.

    a. Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?

I applaud EPA-OPP for taking a proactive role in developing approaches for evaluating submissions that propose the consideration of a KMD to reduce (or interpret) animal testing. The HESI activities will provide an opportunity to document multiple case studies that illustrate both the value and the limitations of the KMD approach.

The 1,3-DCP case study provides an example of a well-constructed argument for the application of a KMD to interpret animal toxicity study results (Bartels et al 2020). The argument included evidence of a kinetic nonlinearity together with evidence of significant depletion of glutathione in the target tissue at exposures about the KMD.

However, in the EPA's evaluation of the carcinogenic potential of 1,3-DCP (EPA-HQ-OPP-2013-0154-0104) the determination to accept the proposed use of a KMD to eliminate consideration of tumor outcomes at high concentrations/doses was supported solely by the observations of kinetic nonlinearities. The crucial evidence of GSH depletion above the KMD is mentioned in the report, but it is not cited as weighing into the decision to apply the KMD. This illustrates the importance of the HESI effort to develop principles for documenting and evaluating KMD decisions.

Dr. Wayne Landis

EPA reviews June 23-24 2020 SAB Committee meetings
Wayne G. Landis

My reviews are focused on the Consolidated Human Toxicity Assessment Guideline and the New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing document. These reviews match my expertise in toxicology and risk assessment. During my career I have also been the Chair of my university's Institutional Animal Care and Use Committee (IACUC). Reduce, refine and replace has been an important consideration for several decades and is discussed in the current textbooks in the field.

I applaud the consideration of probabilistic risk assessment in decision making. By definition, it is not risk assessment unless it is probabilistic. However, there are a number places where the legacy of non-probabilistic approaches exist. One is the continued use of NOAELs and similar measures based on the outcomes of hypothesis testing. The issues with such point estimates can easily be found with a google search. The same can be said of taking a point estimate, even the lower confidence interval, from a regression model. Often, we are attempting to extrapolate to the effects at very low doses because the standard experimental designs are not asking the questions appropriate for risk assessment. Experimental designs need to be altered to answer the key questions in risk assessment, not risk assessment being compromised attempting to accommodate outdated methods.

I have long been a proponent of the use of exposure-response curve fitting instead of hypothesis testing to describe toxicity. I have co-authored several papers on the topic. For a decade I have also worked to integrate causality into a risk analysis and have become increasing skeptical of many approaches claiming to be weight of evidence. I downloaded the EPA guidance document for exposure-response to evaluate its application as a Module.

During my long stint on several SAB subcommittees from the mid 2000s to the early 2010s and with several administrators. I was used to extensive documentation and having time to conduct our own analysis when necessary to answer the charge questions. In comparison this process resembles a rapid screening review than a careful consideration and analysis. I also discourage EPA from referencing documents published behind paywalls in journals in their documentation. Such an example is " (Cumulative risk assessment lessons learned: A review of case studies and issues)". My understanding is that work produced by a U. S. agency cannot be copyrighted by a third party. I also examined several other documents that were available to document different modules.

New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

1. EPA-OPP is participating in the Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) with government, non-governmental organization, and industry stakeholders (Section 2). ReCAAP is developing a risk-based weight of evidence (WOE) approach for waiving chronic and carcinogenicity studies. This proposed approach is consistent with existing guidance[1] and current practice[2] for other types of toxicology studies.

    a. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.

I do not have much to say in support of qualitative WOE approaches. I have seen them misused in arguments with such topics as atrazine effects and others. There are quantitative approaches to WOE published by EPA authors. Below are two citations.

Carriger et al. 2016. Bayesian networks improve causal environmental assessments for evidence-based policy. Environ. Sci. Technol. 2016, 50, 13195−13205. DOI: 10.1021/acs.est.6b03220

Carriger JF, Barron MG. 2016. A practical probabilistic graphical modeling tool for weighing ecological risk-based evidence. Soil and Sediment Contamination: An International Journal, 25:4, 476-487, DOI: 10.1080/15320383.2016.1171293

The approaches do require specific kinds of information to be collected during the WoE process.

    b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

Seems too simple and not probabilistic. If the goal is to provide information for risk assessment then how can an approach that does address uncertainty with an attempt to be quantitative be the best approach. It is not clear to me how this approach would fit into the more quantitative approach as described by Carriger et al (see citations above).

2. EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to begin to replace the chronic/carcinogenicity testing in mammals. In addition, EPA-OPP and ORD are working together to collect quantitative gene expression data from short-term *in vivo* rat studies for a selected set of pesticides that cause liver tumors in rodent with known modes of action. All of these efforts are in the early stages of development and would benefit from expert and public input.

As a former chair of the WWU IACUC and a proponent of the 3Rs I have some very specific questions

What are the goals for accuracy and precision? Are they similar in predictive ability as conventional methods and where are the data?

How do NAM approaches compare to conventional animal testing for accuracy and prediction of human toxicity? If there are deficits, are they acceptable given the goal of reducing the number of and pain to the animals.

How are the extrapolations going to be made from both the NAM and gene-expression studies to human carcinogenicity? Are there better data extrapolation-modeling tools than have been conventional used and will the approach lead to the 3 Rs being realized?

Dr. Dennis Paustenbach

New Approach Methods and Reducing The Use of Laboratory Animals for Chronic and Carcinogenicity Testing

My Overview on The Document: I have been interested, as a toxicologist, in reducing the number of animals involved in testing new chemicals and I have encouraged the profession to move away from the more painful ones for the animals. My post-doc in 1986 focused on how to use physiologically-based pharmacokinetic approaches to select doses for chronic bioassays. Interestingly, it has taken about 30 years for those thoughts to take hold.

Interestingly, even though I have lobbied to significantly reduce the number of animals tested, I still believe that given our current level of understanding of the chemicals, it is premature for the agency to arbitrary say that there were will no more or very limited testing by 2035. We currently don't have a sufficient scientific bases for doing that and I believe it is implausible that we can rely on in-vitro and in-silico tests alone for safety evaluations.

I wholeheartedly support using kinetics to select the maximum dose in animal testing. MTD has existed for far too long and its use has often led us down the wrong paths.

Using computers, zebra fish, in-vitro assays, gene arrays, and subtle perturbations to guide us to understand the safety in humans seems like a worthy management objective that deals with the concerns of many stakeholders but it is unlikely that these, alone, will allow us to safely bring chemicals to the marketplace.

There is an undertone in toxicology today, and it is reflected in this document, that these in-silico and isolated cell perturbation assays will be effective at performing the hazard identification step in the risk assessment. This is possible and I would support it. However, for these to be used to give us insight about the dose-response relationship in mammals is probably asking for too much. The number of compensatory mechanisms and feed-back looks that can occur in-vivo vs the information gleaned from cellular assays (which don't have compensatory mechanisms) are many. Thus, I would urge that EPA "not" continue to say that they will achieve their goal by a particular date.

If the data are truly convincing and there are not too many exceptions as to when you can extrapolate, then it can be decided what a modern era test program can look like. In my experience, you can learn a vast amount by conducting whole body, in-vivo, assays in a much more limited number of animals; but we need animals.

My impression is that the Agency recognizes that if you declare NOELs in in-vitro assays as the Point of Departure, you will end up with remarkably low regulatory guidelines which, by definition, will be protective of the public. We have seen these inferences made in many

dozens of in-vitro and in-silico papers of the past 15 years. However, if we just want to choose very low numbers, using any approach possible, I would dare say that you won't need many toxicologists to do that and the nation will surely decrease the number of new chemicals which can be used in the coming years.

I don't think it should be the role of EPA to stifle technological advancement by setting limits on chemicals that are unnecessarily low just because it can be done. Whenever we have done that, there have been unintentional consequences that prevent significant advancement. With the technological age at its infancy, especially with respect to microdevices and in developing vaccines and antibiotics, EPA would want to be nervous about unintentional "holding back" some important discoveries.

So, as having watched this initiative "get legs" over the past 25 years, and having served as chairman of one of the BOSC committees to look over this work, I would just add a word of caution that EPA's first objective is protect the safety of our citizens and that this should not be compromised by an elected official or an appointed bureaucrat who is well intended (and wanting to be popular among certain stakeholders). The Agency needs to be driven by the facts and it could take many years to really understand how far these modern tests can take us.

Part B

I did not see charge questions so have no comments on that front. I did read the **Rethinking Carcinogenicity Assessment for Agrochemicals** and have some comments.

a) As an engineer, and then a toxicologist, I am very fond of example problems for illustrating the strengths and weaknesses of an approach. So, I thought this exercise was valuable.
b) The objective was to allow the reader to decide if a 2 year bioassay was needed for this new chemical.
c) It was wise to start with the exposure assessment.
d) It was also wise to introduce more toxicologists to the important role of chemical and physical properties. This topic is nearly always understated with respect to its importance.
e) It is unclear how the ADME info was incorporated into the decision making other than to know it did not accumulate in tissues with repeated exposure.
f) The example suffers having a control group of limited size (see subchronic testing). In my experience, in recent years, it is wise to have twice as many controls as you use in the treatment groups.
g) Moving to the conclusions. It is interesting that the risk assessment is driven by data from the in-vivo studies and that were was virtually no reliance on gene arrays or subtle in-vitro tests for a myriad of effects.

h) I am not sure a great argument was made for NOT conducting a 2 year bioassay but, perhaps, that is the direction we are headed.

Overall, this is an interesting analysis but very few of the NAM methods were applied.

Dr. Ted Simon

Charge Questions on White Paper "New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

1a. Risk-based WOE approach for waiving chronic carcinogenicity studies.

Comment 1: An exposure-based triage is missing from this first section. For many chemicals, exposure estimates may be available from far-field studies [2–4]. Any extant exposure estimates should be used and if unavailable, the methods used to develop them could be used within a read-across type inter-chemical extrapolation for exposure [5–10].

These exposure estimates could then be used as a prioritization/triage scheme in the development of PODs (#6, Proposed Points of Departure).

Comment 2: I am in favor of the use of MOE to address cancer risk, which appears to be the statement in the 7th bullet in section 6. The qualification of "…by linear or nonlinear cancer risk assessment methods as appropriate …" is also unclear. How would the choice of methods be determined?

I would propose the use of a WOE for this determination as proposed by several authors in the scientific literature [11; 12]

1b. Case Study with "Herbicide1"

Comment 1: A table of the exposures for all the scenarios would be helpful in "1. Use and Exposure Profile."

Comment 2: Essentially, this section used the exposure estimate for infants as the highest. Hence, consistent with my comment above, what's missing from the first section is this infant dose.

2a. Direction and Scope of Section 3 in the white paper

Comment 1: I disagree with the use of the key characteristics of cancer (KCCs). The KCCs have no better predictive ability than random chance, as demonstrated with a set of chemicals identified by EPA-OPP CARC as carcinogenic or not [13].

3a. Use of the KMD

Comment 1: I agree with proposals outlined in attachments 3 and 4.

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Dr. Eric Smith

Comments from EP Smith - New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing

1. EPA-OPP is participating in the Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP) with government, non-governmental organization, and industry stakeholders (Section 2). ReCAAP is developing a risk-based weight of evidence (WOE) approach for waiving chronic and carcinogenicity studies. This proposed approach is consistent with existing guidance[18] and current practice[19] for other types of toxicology studies.
    a. Please comment on the draft risk-based WOE approach for waiving chronic/ carcinogenicity studies (Attachment 1). Please include in your comments a discussion of the clarity and completeness of the proposal.
    b. Please comment on the draft case study provided (Attachment 2). Please include in your comments a discussion of the clarity and completeness of the proposal.

Comments on: Attachment 2 June 2020 SAB ReCAAP Waiver_Draft Final
Information in the tables related to statistical summarization or testing is lacking. I would suggest requiring that information. In particular

1. When there is an interval what is the confidence level, or are the values mean plus or minus standard deviation or standard error?
2. When p-values are reported, what is being tested? It is not always clear.
3. Samples sizes should be included. Typically, these studied are balanced so the sample sizes should be the same for each dose. If not the same, there should be a comment as to why not.

2. EPA is collaborating with Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS) and HESI to consider NAM-based approaches to begin to replace the chronic/carcinogenicity testing in mammals. In addition, EPA-OPP and ORD are working together to collect quantitative gene expression data from short-term *in vivo* rat studies for a selected set of pesticides that cause liver tumors in rodent with known modes of action. All of these efforts are in the early stages of development and would benefit from expert and public input.
    a. Please comment on the direction and scope of the three collaborative projects described in **Section 3 of the draft white paper**.

[18] https://www.epa.gov/sites/production/files/2015-04/documents/data-require-guide-principle.pdf ; https://www.epa.gov/sites/production/files/2014-02/documents/part158-tox-data-requirement.pdf
[19] Craig et al (2019) Reducing the Need for Animal Testing While Increasing Efficiency in a Pesticide Regulatory Setting: Lessons From the EPA Office of Pesticide Programs' Hazard and Science Policy Council. Regul Toxicol Pharmacol, 108, 104481 Nov 2019. PMID: 31546018

3. EPA is working with HESI, NTP, and other government and industry stakeholders to accelerate the incorporation of kinetically-derived maximum doses (KMD) into repeat dosing studies like the chronic/carcinogenicity study as an alternative to the traditional maximum tolerated dose (MTD). The KMD approach is consistent with numerous guidance documents developed by EPA, OECD and other international organizations as a more humane and human relevant approach to dose selection. One KMD study has been provided to the SAB along with the description and agenda of an upcoming workshop and the scope/charge of a workgroup at HESI to develop additional case study and a best practices document.

    a. Please comment on EPA's current KMD-related activities as described in Section 4 and Attachments 3 and 4. Does the SAB have additional activities that EPA could consider?

Comments regarding: Agenda and description of the upcoming workshop the on kinetically-derived maximum dose (Attachment 3 and 4)

One of the objectives is Conducting statistical analyses to determine a KMD for interpreting dose-response data which is good. Would there be an opportunity for designing experiments, especially computer aided experiments be useful? For example, what is a good design for determination of dose proportionality?
Should the potential effect of interactions be considered?

Case study 7: Statistical tests to determine KMD from sparse data points – again it may be useful to consider different experimental designs, especially adaptive designs for estimation of KMD.

There is some literature on the problem that should be considered in the workshop:

L.G. McFadden, M.J. Bartels, D.L. Rick, P.S. Price, D.D. Fontaine, S.A. Saghir Statistical methodology to determine kinetically derived maximum tolerated dose in repeat dose toxicity studies Regul. Toxicol. Pharmacol., 63 (2012), pp. 344-351.

They argue that an appropriate method is to fit linear then add a quadratic term and evaluate if it is statistically significant. Design comes important here as dose spacing and number of replicates can affect significance. Guidance on design could be valuable.

The paper below seems relevant.
Minne B. Heringa, Nicole H.P. Cnubben, Wout Slob, Marja E.J. Pronk, Andre Muller, Marjolijn Woutersen, Betty C. Hakkert, Use of the kinetically-derived maximum dose concept in selection of top doses for toxicity studies hampers proper hazard assessment and risk management, Regulatory Toxicology and Pharmacology,Volume 114, 2020, 104659,

https://doi.org/10.1016/j.yrtph.2020.104659.

The authors argue that KMD is ill-advised for top-dose estimation. The following is indicated in their paper

"KMD concept aims at having top doses in toxicity tests below non-existing inflection point. The KMD leads to lower test doses, resulting in less informative or inconclusive data. Testing at too low doses does not meet 3R principle and has regulatory consequences."

Note also the recent paper

Marjolijn Woutersen, Andre Muller, Marja E.J. Pronk, Nicole H.P. Cnubben, Betty C. Hakkert, Regulating human safety: How dose selection in toxicity studies impacts human health hazard assessment and subsequent risk management options, Regulatory Toxicology and Pharmacology, Volume 114, 2020, 104660,https://doi.org/10.1016/j.yrtph.2020.104660.

Both of these papers indicate the need to have the design and analysis of studies, as well as the interpretation, have an important role in the workshop and guidance documents. Perhaps a part of the workshop and document would include discussion of criticisms of the approach.

Comments on: EPA-HQ-OPP-2013-0154-0104: 1,3-Dichloropropene: Report of the Cancer Assessment Review Committee

There are a considerable number of statements of the form: statistically significant ($p>0.05$) that are not supported with additional information. There has been considerable pushback in the statistical and scientific community about the use of p-values and the reliance of statistical significance as a surrogate for scientific significance (Ioannidis 2005, 2019). The American Statistical Association has published two editorials on the topic with some recommendations (Wasserstein and Lazar, 2016, Wasserstein, Schirm, and Lazar, 2019). In particular, the p-value by itself gives little information. When sample sizes ar small, a large effect is needed for statistical significance. If sample sizes are large then a small effect would give statistical significance. Hence without sample size or effect size, there is difficulty in interpreting the p-value. Consider implementing some of the recommendations perhaps requiring effect size, sample sizes and/or confidence intervals rather than just p-values.