Vanadium Pentoxide IRIS Assessment

Scientific Basis for the Application of the Stopping Rules

Vanadium Safety Readiness Program
Presentation to the EPA
December 15, 2014

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Agenda

Present the completed new science and demonstrate why it meets the Stopping Rules criteria.

- Describe the impact of the **new MOA data** on the **scientific credibility** of 2 of the key conclusions of the V2O5 IRIS assessment:
  - **#1 Cancer Classification**
  - **#2 Inhalation Unit Risk (IUR)**
- The studies are completed and in a publicly available form
  - **#3** Provide a few examples of **significant errors and omissions** that impact the **scientific credibility** of the assessment
- Discussion

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Stopping Rules after peer review

- "...the presumption shifts to not including new studies unless they have an impact on the credibility of an assessment's conclusions. "
- "Examples .... a strong new study that **might** change, in either direction, a major conclusion."
- "...such a study would likely have the ability to provide important mechanistic insights that would change the approach to dose-response assessment. "
- "Review the studies for pertinence, quality, and impact on the credibility of the assessment's conclusions. "
- "EPA will discuss its determination with the chair of the peer review panel."

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3

History: 2011 V2O5 Dataset

When the IRIS assessment was drafted by EPA, the NTP Inhalation Bioassay Study in rats and mice (2002) was for all practical purposes the only study that was useful for the determination of the Cancer Classification.

EPA concluded the MOA could not be identified due to a lack of information. This was confirmed by all peer reviewers

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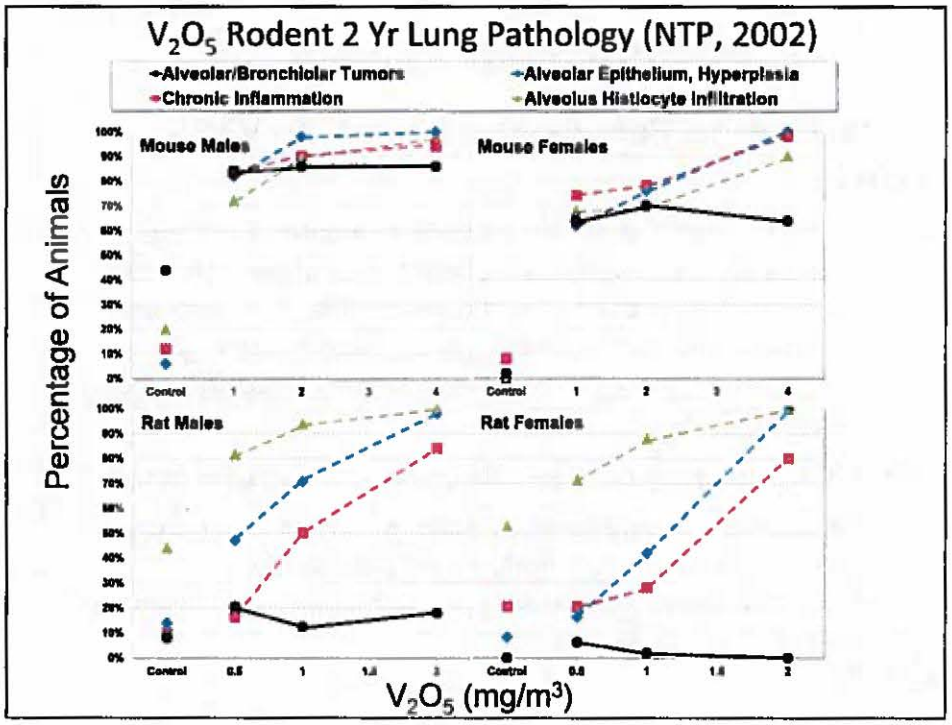
4



NTP Chronic V2O5 Inhalation Study in Rats and Mice

- marked inflammation in the lung for most rats and mice
- lung tumor response in mice is very different than the rat
- lung tumors in mice appear at maximal incidence over the narrow range tested
- the lung V burdens are proportional to the chamber concentration, not saturated
- no systemic pathology at any tissue site
- negative Ames, negative 13-week *in vivo mouse* RBC micronucleus
- more Kras mutations in some of the V_2O_5 mouse lung tumors compared to pooled historical controls

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6

History: Peer Review

- Final External Peer Review Report on V2O5 (2012):
  - Chair Dr. Mitch Cohen "Post-Meeting Update: The panel members appreciated receiving hard copies of the definitions used by the EPA to define test agents as "likely", "suggestive," "inadequate" etc carcinogens. It was clear there was no common view among the panel as to which categorization best applied to V2O5."
- EPA's Response to Peer Reviewers comments:
  - "The available tumorigenic evidence on vanadium pentoxide could be considered a borderline case between two descriptors: likely to be carcinogenic to humans and suggestive evidence of carcinogenicity. "

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7

History: Scientific Community's Response

- $1 Million reprogrammed by DoD's VSR program to address data gaps identified in EPA's draft IRIS assessment.
- Additional support from FDA's National Center for Toxicological the Research (NCTR), leveraged NIH NCBI databases, & Vanadium Producers and Reclaimers Association, VPRA
- Research implemented by well-respected experienced scientists, using state-of-art technologies

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8

Issue #1: Cancer Classification & MOA

- Basis:
  - –Causes lung tumors in rodents following inhalation exposure to concentrations causing significant chronic inflammation.
  - –No other toxicity or oncogenicity at any other tissue site. Site of contact effect.
- Cancer Classification and MOA:
  - –EPA: "Likely Human Carcinogen" MOA unknown
  - –Our position: the totality of the current science, including the new studies, supports a classification of "Suggestive". New data have ruled out a direct mutagenic MOA.

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9

New Scientific Studies 2011-2014

1) IN VIVO DNA DAMAGE (COMET ASSAY) and MOA Schuler et al. 2011, conducted at Harlan Switzerland and 3 collaborating laboratories

Species: B6C3F1 mice (lungs)

Exposure: nose only inhalation 6 hr/d for 16 consecutive days, (up to NTP range)

In vivo repeat exposure study in relevant species, strain, relevant route, and target organ (lung)

Comet assay in both lung and BAL cells NEGATIVE

Omitted from the draft IRIS assessment in 2011

Now has minimal mention and omitted from the table of Genetic Toxicology studies E-1

Status: Publication accepted 5-1-2011

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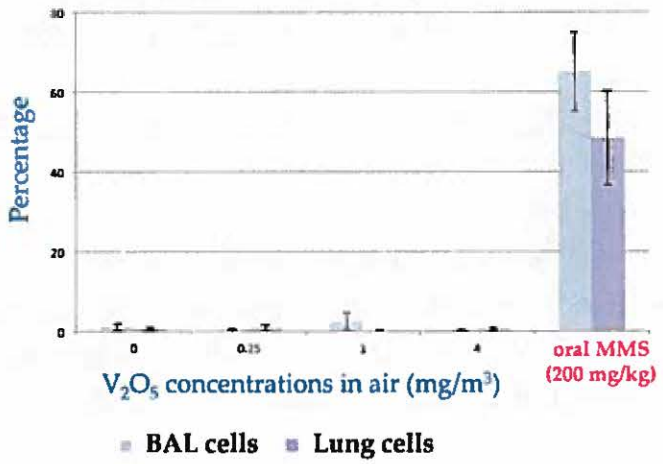
10

Comet Assay Tail Intensities in BAL and Lung Cells



(Schuler et al., 2011)

In Vivo Gene Mutation

2) TRANSGENIC CII ASSAY IN LUNG TISSUE, In life: IITRI; transgenic: Drs. Moore/Manjanatha FDA's NCTR Laboratory
Species: Big Blue B6C3F1 Mouse (lung tissue)
Exposure: inhalation, 6hr/d, 5d/wk, 4 and 8 weeks, 0.1 and 1 mg/m3

In vivo OECD guideline study in relevant species and strain, route, exposure level, and target organ

Transgenic gene mutation assay NEGATIVE

Does not support a mutagenic MOA

Status: IITRI Final In-life report dated 5-5-2014, CII Gene Mutation abstract and presentation at an International Vanadium Symposium 6-30-2014, SOT abstract accepted, final report dated 12-1-2014

Average lung weights and lung *cII* MFs in male BB mice exposed to 0, 0.1, and 1 mg/m^3 V_2O_5 for up to 8 weeks.

V_2O_5 Concentration (mg / m^3)	4 Week		8 Week	
	Lung Weight (mg)	*cII* MF x 10^{-6}	Lung Weight (mg)	*cII* MF x 10^{-6}
0	101.7 ± 3	29.5 ± 4.2	111 ± 9.2	29.2 ± 3.4
0.1	111.6 ± 4.7	38.5 ± 7.9	116 ± 10.3	47.8 ± 14.3
1	**138.3 ± 3.0***	24.3 ± 4.4	**142.7 ± 8.3***	17 ± 2.8

* Significantly different from corresponding control ($P \leq 0.05$)

13

In Vivo Kras Mutation Study

3) MUTATIONS AT THE Kras LOCUS IN LUNG TISSUE. In life: IITRI; transgenic: Dr. Barbara Parsons, FDA's NCTR Laboratory

Species: Big Blue B6C3F1 Mouse (measured mutants at the Kras loci in lung tissue)

Exposure: inhalation, 6hr/d, 5d/wk, 4 and 8 weeks, 0.1 and 1 mg/m3

In vivo repeat exposure study in relevant species and strain, route, exposure level, and target organ

Kras mutations not increased in the lung up to 8 weeks of treatment

Kras mutations NOT an early event in lung tumor formation

Does not support a mutagenic MOA

Status: IITRI Final in-life report dated 5-5-2014, SOT abstract accepted, final report dated 11-17-2014.

14

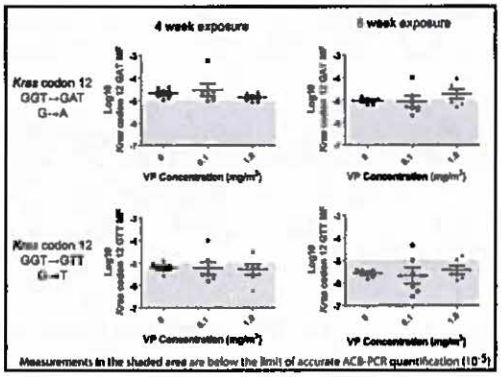
Quantification of *Kras* Mutant Fraction in the Lung DNA of Mice Exposed to Aerosolized Particulate Vanadium Pentoxide by inhalation

Rationale: a) cancer driver mutations are important targets for cancer risk assessment; b) In an NTP study, *Kras* mutation was detected in a larger percentage of VP-induced lung tumors than in spontaneous, historical control tumors; c) ACB-PCR is a sensitive mutation detection method, which has detected the induction of cancer driver mutations after short in vivo exposures to model mutagenic carcinogens.

Alternate Hypotheses/Potential Supporting Results:
Hypothesis #1 - VP induces *Kras* mutation via oxidative DNA damage/expect to observe selective increase in the codon 12 GTT mutation
Hypothesis #2 - VP causes early amplification of spontaneous *Kras* mutation/expect early increases in *Kras* codon 12 GAT & GTT mutations
Hypothesis #3 - The increase in *Kras* mutation is a late event in VP-induced lung carcinogenesis/expect no effect on either *Kras* mutation

Conclusions

- Inhalation of aerosols of particulate VP for 4 or 8 weeks did not result in significant changes in levels of *Kras* codon 12 GAT or GTT mutation.
- Spontaneous *Kras* mutation (GAT>GTT) is present in lung tissue of control mice.
- Accumulation of additional *Kras* mutants is not an early event, and/or the proliferative advantage of *Kras* mutant clones requires either longer expression times or larger cumulative VP exposures.



In Vivo Gene Expression Analysis Using Mouse Whole Genome Arrays

4) DATA MINING OF NIH DATABASE OF ACC Study of 26 CHEMICALS (including V2O5) Hamner Institutes, Drs M Black and Mel Andersen

Species: mice (14 mouse lung tumorigens and 12 non tumorigens)

Exposures: 90 days for each compound, tumorigenic levels

Repeat exposure study in relevant species, strain, route and target tissue (lung)

No evidence of a mutagenic MOA

Effects on lipid metabolism detected, well-established in the pharmacology literature

Status: Final report (4-29-2014), Presented at V9 International Symposium (6-30-2014)

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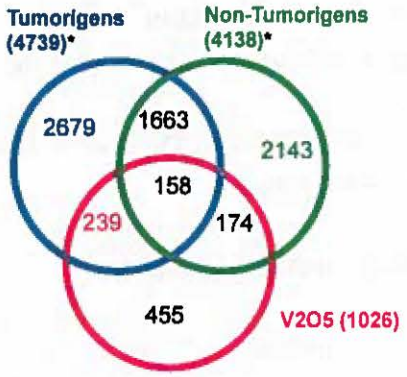
16

Significant Genes by ANOVA with orthogonal linear contrasts
Significance by [FDR < 0.05 *AND* (FC > +1.5 *OR* FC < -1.5)]



*Significant if significance threshold met by any chemical in category

4) Summary of Gene Expression Analysis Using Mouse Whole Genome Arrays

- The 239 genes in common with V2O5 and at least one other tumorigen did not yield any significantly enriched pathways.
  - Interpretation: little commonality between V_2O_5 and other lung tumorigens
- No evidence for changes in cell cycle/proliferation, DNA-damage or oxidative stress related pathways with the genes differentially expressed by V_2O_5
  - Interpretation: no evidence in support of a mutagenic mode of action in lungs of mice exposed to V_2O_5 for 90 days

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18

In Vivo Biomarker and Pathology Evaluation

5) *In* Vivo Biomarker and Pathology Evaluation, Drs. Jim Klaunig and Z. Wang, Indiana U.

Species: Wild type B6C3F1 mice (lung tissue)

Exposures: 6 hr/d, 5d/wk, 4 or 8 weeks to 0.1 and 1 mg/m3

Repeat exposure *in vivo* study in relevant species, strain, route and target tissue (lung)

Data supports an inflammatory MOA

Oxidative stress MOA not supported

Status: Final report for the in-life portion 5-5-2014; Phase 1 biomarker work presented at V International Symposium 6-30-2014, Phase 2 underway

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19

Biomarker Study: Summary of results: oxidative stress, inflammation and proliferation markers in the lungs of B6C3F1 mice exposed to V_2O_5 for 4 and 8 weeks

Groups	8-OHdG (pg/ml/mg)	IFNγ (ng/ml/mg)	IL-1α (pg/ml/mg)	IL-6 (pg/ml/mg)	Ki-67 (ng/ml/mg)
4 week					
Sham	318±41	19.7±1.7	432±57	539±225	120±49
0.1 mg/m³	299±30	19.3±2.3	418±71	466±106	97±15
1.0 mg/m³	275±39*	17.2±1.6*	438±106	349±30*	79±11*
8 week					
Sham	260±52	17.4±1.7	434±67	386±107	90±17
0.1 mg/m³	262±44	18±2.6	458±81	455±170	120±55
1.0 mg/m³	273±38	17.3±1.4	531±112*	384.1±65.5	91±18

*P < 0.05 in comparison with respective controls (Sham) by one way ANOVA followed by Dunnett's test. Values represent Mean ± SD of 10 samples in each group.

Male Rat Lung Tumor Response Analyses

6) Starr et al. 2012 constructed enlarged historical control dataset for rats fed only with the NTP2000 diet. **Included studies conducted after** NTP (2002). Utilized non-parametric K-S tests to assess before vs. after heterogeneity of HCs without having to make any distributional assumptions.

Found no significant heterogeneity, and widened HC incidence ranges. Also, the concurrent control group appeared to be a near-outlier relative to the enlarged HC database. This could invalidate all comparisons involving HCs, forcing reliance on the most relevant comparison group, the concurrent controls.

Concluded that V_2O_5 is not carcinogenic in rats based on both the updated HC ranges and previous concurrent control comparisons.

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21

Male Rat Lung Tumor Response Analyses

EPA 2014 draft asserts **positive** response using different HC data **BUT:**

- CC data were included inappropriately with the HC data
- HC groups were pooled despite finding significant heterogeneity
- Adjustments for heterogeneity are inadequately documented
- No accounting for survival differences with poly-3 adjustments for intercurrent mortality. This could invalidate all comparisons that make use of historical controls (Elmore and Peddada 2009)
- The EPA conclusion runs counter to current best practices: **"The concurrent control group is the most relevant comparator for determining treatment-related effects"** (Keenan et al. 2009)
- Unbalanced discussion defends new EPA analyses without noting limitations or shortcomings; rejects previous analyses outright
- Not peer-reviewed; not published; not publicly available

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22

Issue #2: Inhalation Unit Risk

- The lung tumor response in mice is constant across the narrow range of the 3 concentrations NTP tested.
  - EPA nevertheless derived an inhalation unit risk
  - Our position: No IUR can be derived scientifically from these data
  - Significant dose-response across the **exposed** groups is needed to do this
- Peer reviewer Dr. Max Costa: "I agree with most of the conclusions..., except the use of the NTP data to extrapolate to lower levels. How can this be done when all the doses give the same cancer incidence?"

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23

No Scientific Basis for Deriving an Inhalation Unit Risk

7) Starr and MacGregor (2014) provided to EPA in May 2014, but the findings are not discussed or cited in the new draft

- **We found no significant dose-related trends in lung tumor incidence among exposed male or female mice** either with or without poly-3 adjustments for intercurrent mortality
- Saturated high-dose response is problematic for dose-response modeling: **It provides no information on shape of the response at lower doses.** EPA's 2012 BMD Guidance recognizes this problem, and it does not recommend developing a unit risk. It states that the ideal solution is to have more data at lower doses. **Without additional data at lower doses, there is no scientific basis to support IUR-based extrapolations below 1 mg/m^3**

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24

Issue #3: Serious Process /Science Deficiencies

Ex. The NTP *in vivo* RBC micronucleus test following 3 month inhalation exposure in mice is reported in table E-1 as **positive** whereas it is clearly **negative** (NTP #507). This may have adversely affected the public & peer review process.

The error was reported to the EPA during the comment period in 2011, but it has not been corrected.

Ex. While this IRIS assessment is stated to be only on vanadium pentoxide, in multiple places data/studies on other V compounds are still included. EPA received critical comments on this issue but it has only partially been addressed. Ex. appendix E remains that describes vanadium levels in ambient air without relating them to V2O5. Studies with V exposures are also included.

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25

Serious Process /Science Deficiencies

Ex. The Schuler *et al* publication (2011) which was negative in an *in vivo* comet assay in the target tissue (lung) has been given little mention (2 lines), and **omitted** from the table of genetic toxicology studies (E-1) although it was the **most relevant** genetic toxicology study that had been published. EPA discounts this genetox study as "less useful" because it is only short term and not chronic (see p. A-23), however guidelines call for genetox studies to be short-term.

Ex. The EPA conducted additional modeling to derive the RfC as suggested by several Peer reviewers however it has not used it. The RfC is now based on dividing the low effect level by a larger (3000 fold) uncertainty factor, an approach which has not received either public or peer review.

Ex. EPA has conducted a new historical control analysis of rat lung tumor data that has not been seen by the public or been peer reviewed.

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26

Why the New V_2O_5 Science Meets the Stopping Rule Criteria

1) <u>Pertinence Criterion Met</u>:

- ✓Relevant compound and form (respirable V2O5)
- ✓ relevant route of administration (inhalation)
- ✓ relevant target tissue (lung)
- ✓ relevant species (mouse)
- ✓relevant strain (B6C3F1)
- ✓ relevant tests
- ✓ relevant data analysis

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27

Why the New V_2O_5 Science Meets the Stopping Rule Criteria

<u>2) Quality Criterion Met</u>:

- ✓Conducted by very experienced laboratories
- ✓Directed by well-respected senior scientists
- ✓Accepted study designs utilized
- ✓Full documentation available. Final reports or publications submitted to EPA

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28

Why the New V_2O_5 Science Meets the Stopping Rule Criteria

3) Impact Criterion Met:

- ✓ There is a significant increase in the science to assess the Cancer classification, MOA and IUR
- ✓ New studies are the state-of-the-art and cover the range of genotoxicity testing currently available
- ✓ Peer review group previously divided on the Cancer Classification and Inhalation Unit Risk
- ✓ Data supports an inflammatory MOA

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29

Why the New V_2O_5 Science Meets the Stopping Rule Criteria

3) Impact Criterion Met:

- ✓ EPA's Cancer Guidelines specifically use "DNA reactivity or effects on cell growth control" as criteria for the "likely classification". These effects have been ruled out
- ✓ A non mutagenic MOA does not support EPA's linear conservative extrapolation to derive an IUR
- ✓ The new MOA data are critical to informing the IRIS cancer classification and IUR evaluations

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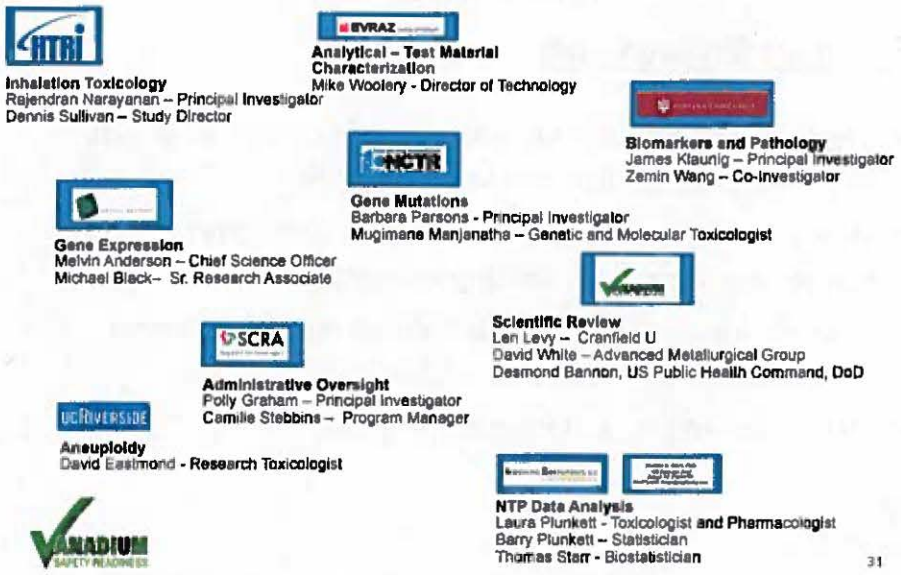
30

Mode of Action Collaborators



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Funding for some projects was also provided by the Vanadium Producers & Reclaimers Association.

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32

What Happens Next

- **Consult the Chair of the Peer Review Panel:**
  - We suggest providing the Chair with a copy of today's slides and the study abstracts.
  - Confirm with the Chair on the record that the stopping rule criteria are met.
- **Revise the IRIS assessment:**
  - Incorporate the new studies.
  - Correct the errors in the assessment.
  - Conduct a new explicit Evidence Integration
- **Submit the revised assessment for public comment and peer review by the CAAC.**
- **Publicly re-affirm NCEA's enhancement principles:**
  - IRIS driven by the need for both the best science and throughput
  - Stopping rules for post-peer review cases are strict but the criteria were met in this case.

33

Discussion

34