Center for Regulatory Effectiveness

Suite 500
1601 Connecticut Avenue, N.W.
Washington, D.C. 20009
Tel: (202) 265-2383 Fax: (202) 939-6969
secretary1@mbsdc.com www.TheCRE.com

Information Quality Guidelines Staff (Mail Code 2811R) July 10, 2008
U.S. EPA
1200 Pennsylvania Ave., NW
Washington, DC 20460

Please find attached for filing a Request for Correction under the Information Quality Act. Please contact Scott Slaughter, 202/265-2383, Slaughter@mbsdc.com, with regard to this Request.

Sincerely,

Scott Slaughter

Attachment

INFORMATION QUALITY ACT ("IQA") REQUEST FOR CORRECTION ("RFC") OF ENVIRONMENTAL PROTECTION AGENCY'S ("EPA") INFORMATION DISSEMINATIONS REGARDING THE AMPHIBIAN METAMORPHOSIS ASSAY [*]

The Center for Regulatory Effectiveness ("CRE"), Jim J. Tozzi and Scott Slaughter request that EPA correct its many public statements that the Amphibian Metamorphosis Assay ("AMA") is reproducible and properly validated. The public disseminations which need correction include the following:

1) The following EPA statement about the AMA Peer Reviewers' conclusions : "In summary, the overall intra- and inter-laboratory reproducibility is considered to be demonstrated...." [1]

2) The following EPA statement summarizing the AMA Peer Reviewers comments: "Overall, it is concluded that that the amphibian metamorphosis assay is valid for its intended purpose."[2]

3) The following EPA statement to the March 25, 2008 Science Advisory Panel for the Endocrine Disruptor Screening Program ("EDSP"):

   "So in summary it's a two tier program, chemical assays for Tier 1 screening battery includes both in vitro and in vivo, mammalian and nonmammalian assays that have gone through validation process and peer review, EPA considers them to be validated and ready for use."[3]

These statements are inaccurate and misleading. EPA should correct them by stating:

1) In summary, external peer reviewers concluded that the AMA's overall intra- and inter-laboratory has not been demonstrated.

---

[*] ***Submitted on July 10, 2008, by mail to Information Quality Guidelines Staff (Mail Code 2811R), U.S. EPA, 1200 Pennsylvania Ave., NW, Washington, DC 20460; by Fax to (202) 565-2441; and by E-mail to*** quality@epa.gov

[1] EPA's Response to the Peer Review Results for the Amphibian Metamorphosis Assay (hereinafter "EPA Response"), page 5, available online at http://www.epa.gov/endo/pubs/ama_peer_review_response_final.pdf

[2] *Id.*, page 1, available online at http://www.epa.gov/endo/pubs/ama_peer_review_response_final.pdf .

[3] Page 32 of Science Advisory Panel ("SAP") transcript available online at (http://www.epa.gov/scipoly/sap/meetings/2008/march/transcript2008-03-25.pdf)

2) Overall, external peer reviewers concluded that that the amphibian metamorphosis assay is not valid for its intended purpose.

3) So in summary it's a two tier program, chemical assays for Tier 1 screening battery includes both in vitro and in vivo, mammalian and nonmammalian assays that have gone through validation process and peer review, and peer reviewers did not consider the AMA to be validated and ready for use.

All other EPA public statements that the AMA is reproducible and validated should also be corrected to say just the opposite.

THE AMA FAILED PEER REVIEW BECAUSE IT IS NOT REPRODUCIBLE

The AMA is one of the assays that EPA proposes to require companies to use during Tier 1 of the EDSP. EPA cannot use the AMA in the EDSP unless EPA demonstrates that the assay is validated for its intended endpoints.[4] In order to validate the AMA, EPA must demonstrate that the AMA is reproducible both within a single laboratory and among different laboratories." [5]

For Influential Scientific Information, such as the AMA and the EDSP, EPA's IQA Guidelines require that EPA "ensure reproducibility for disseminated original and supporting data according to commonly accepted scientific, financial, or statistical methods."[6]

EPA asked five experts from outside the Agency to peer review the AMA and state their opinions as to whether the AMA has been properly validated. These peer reviewers produced a report on their review of the AMA, and EPA responded to it. [7]

With regard to whether the AMA is reproducible, one peer reviewer stated in the Report : "This is a major flaw of the material provided ...."[8]

---

[4] 21 U.S.C. § 346a(p).

[5] *E.g., NICEATM/ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods*, page 13, available online at http://iccvam.niehs.nih.gov/SuppDocs/SubGuidelines/SD_subg034508.pdf.

[6] EPA IQA Guidelines, p. 47, available online at http://www.epa.gov/quality/informationguidelines/documents/EPA_InfoQualityGuidelines.pdf

[7] The Peer Reviewers' Report is entitled *Peer Review Results for the Amphibian Metamorphosis Assay* ("Peer Review Report"). It is available online at http://www.epa.gov/endo/pubs/ama_peer_review_121907.pdf. EPA's Response to the Peer Review Report is entitled *EPA Response to Peer Review Results for the Amphibian Metamorphosis Assay ("*EPA Response*").* It is available online at http://www.epa.gov/endo/pubs/ama_peer_review_response_final.pdf

[8] EPA Response, page 5.

He also explained that the inter-laboratory inconsistencies obvious in just one table of the AMA validation study "would convince any reviewer for a reputable scientific journal to recommend rejection" of the validation study. [9]

He further stated "that the conclusions regarding inter-laboratory variability are not warranted and that it [the AMA test protocol] fails as a method for accomplishing the stated goal of the assay to be part of the Endocrine Disruptor Screening program (EDSP)."[10]

He advised EPA that "[b]efore the AMA can be used as a screening tool that is open to contract laboratories, the issues raised above should be addressed. The bottom line is that the AMA is not suitable as a screening tool for endocrine disrupting compounds." [11]

A second peer reviewer concluded in the Report:

> "One of the major concerns about the assay is the degree of inter-laboratory consistency.... while overall trends are observed (ie T4 accelerates, perchlorate and IOP delay), there is surprising inconsistency among the laboratories....Based on these observations, the consistency of findings across laboratories remains a major concern for the future viability of the assay system."[12]

A third peer reviewer was more positive, but even she concluded that "there was some variation and testing may need to be conducted independently in at least two separate labs."[13]

A fourth peer reviewer concluded that

> "Concerning was that not all aspects were always controlled for. Moreover, when conducting the inter-laboratory study using weak thyroid modulators, it seems that the consistency was lost."[14]

This peer reviewer also commented on the inconsistency of test result interpretation among laboratories performing the AMA:

> "A much stronger guideline for data interpretation within the AMA Test Method Documents is necessary.... *In summary, this phase trial demonstrated that data interpretation across the validation studies needs to be consistent, and guidelines need to be carefully developed to facilitate this interpretation.* In fact, in the AMA Test Method, there is no section on data interpretation, and in the overall ISR [Integrated Summary Report] ,

---

9 Peer Review Report, page 3-7.
10 Peer Review Report, page 2-1.
11 Peer Review Report, page 3-17.
12 EPA Response, page 5
13 EPA Response, page 5
14 EPA Response, at page 7.

> there are no clear guidelines for how many parameters need to be significantly different from controls before a compound is to be interpreted as thyroid disrupting. Such guidelines are essential and should be provided clearly in the final AMA Test Method Protocol, along with appropriate summary tables."[15]

The fifth and final peer reviewer concluded

> "One of my greatest concerns in the AMA documentation is the high variance in reproducibility of the results obtained from the various labs during the various test phases. I am disquieted by the little attention given to the variance between the labs, when their protocols were (supposedly) identical. Most of the chemicals used in these studies were well known inhibitors or accelerators of metamorphosis. The fact that inhibition and acceleration were seen in the test results is, of course, exactly what one expected. I did not expect, however, the variance in the reports between the different labs. It is bothersome that more effort was not made to explain the inter-laboratory variance."[16]

This reviewer also explained:

> "My greatest concerns about the AMA center on the document "Draft Method for the AMA." Various laboratories should be able to follow the methodology of this essential document and achieve identical results. **There is simply not enough detail in this methodology to be confident that the assays can be executed with adequate amounts of reproducibility."**[17]

These are just some examples of the Peer Reviewer's concern with the reproducibility of the AMA tests, and with the reproducibility of AMA test result interpretation. There are many other examples in the Peer Review Report.[18]

Consequently, the Peer Review Report does not support the following EPA summary of the peer reviewers' conclusions: "In summary, the AMA's overall intra- and inter-laboratory reproducibility is considered to be demonstrated.**"** [19]

Just the opposite is true. Peer review concluded that the AMA's overall intra- and inter-laboratory reproducibility has not been demonstrated. As one peer reviewer noted:

> "This section [of EPA's AMA validation study under review] proclaims 'The reproducibility of the [A]MA, for screening purposes, has been well-demonstrated using

---

15 Peer Review Report, page 3-31.

16 EPA Response, page 7.

17 Peer Review Report, page 2-33 (emphasis in the original).

18 *E.g.*, Peer Review Report, pages 2-8 to 2-11, 2-14 to 2-15, 2-21 to 2-24, 2-25 to 2-26, 2-27, 2-67 to 2-70, 3-1, 3-7, 3-8, 3-17, 3-25, 3-26, 3-27, 3-31, 3-44, 3-56, to 3-58, 3-59, 3-66, 3-67, 3-69, 3-70, 3-72, 3-80.

19 EPA Response, page 1, available online at http://www.epa.gov/endo/pubs/ama_peer_review_response_final.pdf

> several representative thyroid-active chemicals across geographically diverse laboratories.' However, if the variation between the labs cannot be explained, then one cannot feel as confident about this proclamation as the author of the review."[20]

In light of the negative peer review results, EPA's public statements that reproducibility is demonstrated through peer review--and that the AMA is reproducible and validated--are inaccurate and misleading. Consequently, these statements violate EPA's IQA Guidelines because the IQA's Objectivity Standard requires that EPA ensure that information the Agency disseminates is reproducible and "accurate, reliable, and unbiased."[21]

Because they are inaccurate and reliable, these EPA statements also violate the IQA's utility requirement. Inaccurate, unreliable statements are not useful.

Because the AMA does not meet the IQA standards, EPA cannot use, rely on, or otherwise disseminate any information generated by the AMA.

EPA'S PUBLIC STATEMENTS THAT THE AMA IS VALIDATED ARE INACCURATE AND VIOLATE THE IQA FOR OTHER REASONS

EPA publicly states that the AMA has been properly validated and is ready for regulatory use: *e.g.*,

> "Overall, it is concluded that that the amphibian metamorphosis assay is valid for its intended purpose" [22]; and

> "So in summary it's a two tier program, chemical assays for Tier 1 screening battery includes both in vitro and in vivo, mammalian and nonmammalian assays that have gone through validation process and peer review, EPA considers them to be validated and ready for use."[23]

As demonstrated above, these statements are inaccurate because reproducibility is required for test validation, and the AMA has never been demonstrated to be reproducible.

There are still other reasons why the AMA is not validated.

---

[20] Peer Review Report, page 2-27.

[21] EPA IQA Guidelines, pages 15 and 22.

[22] Page 1 at of EPA's Response to the Peer Review Results for the Amphibian Metamorphosis Assay (emphasis in the original), available online at http://www.epa.gov/endo/pubs/ama_peer_review_response_final.pdf (hereinafter "EPA Response").

[23] Page 32 of SAP transcript available online at (http://www.epa.gov/scipoly/sap/meetings/2008/march/transcript2008-03-25.pdf). The referenced Tier 1 screening battery include the AMA.

For example, the governing validation criteria include the following criterion: "Ideally all data supporting the validity of a test method should have been obtained in accordance with the principles of GLP [Good Laboratory Practices]."[24]

The lab data used by EPA to develop and assess the AMA were not developed in accordance with GLP. [25] Consequently, EPA's statements that the AMA is validated are inaccurate and misleading and violate the IQA.

The American Chemistry Council ("ACC") submitted comments to EPA which demonstrated that the AMA was not validated for still other reasons. Some of ACC's comments are set forth below:

> " The amphibian metamorphosis assay results indicate that a purported "negative control" test article actually yielded a positive response and a "positive control" test article actually yielded a negative response."
>
> ***
>
> "The amphibian metamorphosis assay, which has been proposed by the Agency for inclusion into the Tier 1 Battery as a screen for thyroid agents, has not met the scientific standard of a valid assay because it has not been shown to be reliable and specific. Data re-analysis indicates a purported "negative control" test article actually yielded a positive response and a "positive control" test article actually yielded a negative response, which is contrary to the analyses EPA circulated to the Agency's peer reviewers (see attached detailed comments). Therefore, at a minimum, the correct statistical analyses must be sent to the peer review panelists and they should be asked to re-evaluate their previous comments in light of new information, and if warranted, adjust their review comments accordingly." [26]

During its review of the EDSP Tier 1 Battery, the SAP expressed concern about false negative/false positive problems with the assays. For example Dr. Delicos, one member of the SAP, stated:

> "I just have one question. I guess about a legal definition. I may be the only person confused here, but representing assays as validated...and some of the public commentors are saying these assays were not validated...for instance, if you did not have a...a demonstration of a chemical which you would expect to be negative and it's not demonstrated to be negative in these pubertal assays,

[24] Page 8 of EPA Response
[25] Id.
[26] ACC EDSP Comments dated March 20, 2008, page 3 of transmittal letter and page 3 of attached comments, available online at http://www.regulations.gov/search/search_results.jsp?No=0&sid=11A556147894&Ne=2+8+11+8053+8054+8098+8074+8066+8084+8055&Ntt=EPA-HQ-OPP-2008-0012&Ntk=All&Ntx=mode+matchall&N=0&css=0

could you go forward with that program in August as you, as you plan,
or do you have to stop and...and do that?
Is that a legal requirement for the validation?"

"DR. TIMM [from EPA]: I think it's...it's clearly necessary to show that....
Some people would like to...and we would like to, actually...have had a clear negative.
We...we...we didn't choose well.
I don't think that that means there isn't one out there.
It just means we...we didn't make a very good choice." [27]

CORRECTIONS REQUESTED

For the reasons stated above, the following EPA statements are inaccurate and violate the IQA:

1. "In summary, the overall intra- and inter-laboratory reproducibility is considered to be demonstrated...." [28]

2. "Overall, it is concluded that that the amphibian metamorphosis assay is valid for its intended purpose. [29]

3. "So in summary it's a two tier program, chemical assays for Tier 1 screening battery includes both in vitro and in vivo, mammalian and nonmammalian assays that have gone through validation process and peer review, EPA considers them to be validated and ready for use."[30]

EPA should correct these inaccurate and misleading statements by deleting them from public dissemination and substituting the following language for them:

1) In summary, external peer reviewers concluded that the AMA's overall intra- and inter-laboratory has not been demonstrated.

---

[27] Pages 43-44 at http://www.epa.gov/scipoly/sap/meetings/2008/march/transcript2008-03-26.pdf

[28] EPA's Response to the Peer Review Results for the Amphibian Metamorphosis Assay, available online at http://www.epa.gov/endo/pubs/ama_peer_review_response_final.pdf (hereinafter "EPA Response"), page 5, available online at http://www.epa.gov/endo/pubs/ama_peer_review_response_final.pdf

[29] Page 1 at of EPA's Response (emphasis in the original), available online at http://www.epa.gov/endo/pubs/ama_peer_review_response_final.pdf (hereinafter "EPA PR Response")

[30] Page 32 of SAP transcript available online at http://www.epa.gov/scipoly/sap/meetings/2008/march/transcript2008-03-25.pdf

2) Overall, external peer reviewers concluded that that the amphibian metamorphosis assay is not valid for its intended purpose.

3) So in summary it's a two tier program, chemical assays for Tier 1 screening battery includes both in vitro and in vivo, mammalian and nonmammalian assays that have gone through validation process and peer review, and peer reviewers did not consider the AMA to be validated and ready for use.

All other EPA information disseminations that the AMA is reproducible and validated should also be corrected to say just the opposite.

HOW REQUESTERS ARE AFFECTED

The undersigned and Jim J. Tozzi use substances on the EPA's proposed list of substances to be tested by the Tier 1 battery of assays. Accurate and reliable tests are necessary to reduce and protect against any risks to them from those assays.

CRE itself is an entity dedicated to ensuring effective regulation, including the proper validation of tests used for regulation. CRE has been a leading and active proponent of the Information Quality Act since before the Act was passed. CRE commented on the EDSP to EPA.

CONTACT PERSON

Please contact Scott Slaughter, The Center for Regulatory Effectiveness, Suite 500, 1601 Connecticut Ave. NW 20009, 202/265-2383, slaughter@mbsdc.com, with regard to this RFC.

_____

Scott Slaughter

Submitted July 10, 2008