

Reviewer's report

Title: Over-expression of AhR (aryl hydrocarbon receptor) induces neural differentiation of Neuro2a cells: Neurotoxicology study

Version: 1 **Date:** 24 April 2006

Reviewer: David DS Sherr

Reviewer's report:

General

This is an interesting and important paper that appears to demonstrate that AhR hyper-expression induces differentiation of neuro 2a neuronal cells. This would be an extremely important finding that would be of great interest to scientists working in the fields of CNS development or in AhR biology. However, there are a number of issues that significantly dampen enthusiasm for this paper. Because the writing is relatively weak, it is hard to tell if some of the problems are technical in nature or simple miscommunications.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The writing needs to be more clear.

Figure 2: It is unclear if the PCR primers are derived from the rat AhR sequence since the AhR gene itself was cloned from rat brain. If the primers were in fact derived from the rat, do they cross-react with the murine AhR? If not, then the negative amplification shown in figure 2A is misinterpreted.

Figure 4: Another interpretation of these data is that ectopic AhR expression induces cell death, thereby lowering the Hoechst signal. The authors should provide data demonstrating that AhR-transfected cells are as viable as cells transfected with the control plasmid. Similarly, a sentence in the "Conclusion" section on page 2 states that, "Activated AhR may disrupt the irregular differentiation occurring rather than cell death." No data are provided on cell viability.

The authors frequently refer to conditions as being "ligand-less". The authors do not consider the possibility that there is an endogenous ligand that is activating the AhR.

The statement, "AhR-mediated pathways are known to work essentially for the action of TCDD in the liver or reproductive organs" significantly understates the breadth of research on the AhR. For example, the AhR has been shown by many investigators to play a role in the development and function of the immune system. Similarly, several environmental AhR ligands are well known to be carcinogenic in many organs. In general, the literature is replete with examples of how the AhR and its environmental ligands influence development and function of many organ systems.

No description is given for what statistical tests were used. For data presented in Figures 2, 3, 4, 5, were statistics performed with replicates from 1 experiment? If so, each value is not an independent event and statistics cannot be performed. It is the experiment that must be replicated, not just the number of wells/samples/replicates in any one experiment. A minimum of 3 independent experiments must be performed before statistical analysis can be applied.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

At the end of paragraph 2 on page 9, what is meant by "formationText for this section"? I assume it is a typo.

There are many typos throughout the paper.

What is meant by "ontogenesis" on page 3.

What is meant by "is functional as ligand-binding AhR" on page 6?

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, and I have assessed the statistics in my report.