To the EPA and Its Science Advisory Board

October 27 – 29, 2010 Meeting

Continued Review of EPA's Reanalysis of Key Issues Related to Dioxin toxicity and Response to NAS Comments

Prepared by Robert A. Budinsky, Ph.D.
Senior Toxicologist at the Dow Chemical Company

These comments accompany the slide presentation reviewed by Dr. Robert Budinsky on the "Dose-Response Approaches for Nuclear Receptor-Mediated Modes of Action" workshop held on September 27-29, 2010 at the National Institutes of Environmental Health Sciences (NIEHS). This workshop aimed to initiate a robust dialogue and consensus on the biological modeling and modes-of-action (MOA) for chemical hepatic carcinogens that act via nuclear receptors. The workshop was developed and conducted by regulatory, academic, industry and consulting scientists and was sponsored by many different organizations including SOT and EPA (more information is available on the TERA website: http://www.tera.org/peer/nuclearreceptor/). The conference was organized by a steering committee co-chaired by Drs. Mel Andersen (Hamner Institute) and Julian Preston (EPA). Logistical and technical assistance was managed by Toxicological Excellence in Risk Assessment (TERA), which is a non profit organization specializing in risk assessment issues.

The workshop's intent was to examine and/or develop a MOA case study for three nuclear receptors, CAR/PXR, AHR and PPARα). The case study evaluations relied upon the MOA framework originally developed by the International Programme on Chemical Safety that was later adopted in the U.S. EPA's 2005 Cancer Guidelines. Each nuclear receptor case study was co-chaired by two scientists who led an expert panel comprised of experts in the area of a specific nuclear receptor, including nuclear receptor biologists, toxicologists, regulatory scientists, and risk assessors. Following plenary session lectures on nuclear receptor biology, dose-response modeling of nuclear receptors, rodent liver carcinogenicity, and the Human Relevance Framework, each case study panel was responsible for presenting and critiquing the mode of action and its underlying key events during a day and a half period. Charge questions guided the discussion in order to derive conclusions regarding the MOA, Key Events, and dose-response relationships regarding rodent liver tumors in a systematic, weight-of-the-evidence approach.

The AHR receptor expert panel, co-chaired by Drs. Dieter Schrenk and Bob Budinsky, was comprised of scientific experts familiar with the AHR's biology, toxicology and risk assessment issues (Table 1).

Table 1: AHR Case Study Expert Panel Members

Dr. Nigel Walker (NIEHS) Dr. Amy Brix (EPL) Dr. Gary Perdew (Penn State Univ.) Dr. Tom Gasiewicz (Univ. of Rochester) Dr. Martin van den Berg (Uetrecht Univ.) Dr. Dieter Schrenk (Kaiserlautern Univ.) Dr. Norbert Kaminski (Michigan State Univ).	Dr. Thomas Starr (TBS, inc.) Dr. Lesa Aylward (Summitt Toxicology) Dr. Jay Silkworth (General Electric) Dr. Bruce Allen (Allen Consulting) Dr. Bob Budinsky (Dow Chemical) Dr. Ted Simon (Ted Simon, L.L.C.)

Dr. Rusty Thomas (Hamner Institute) and Dr. Craig Rowlands (Dow) provided background data on genomics. Dr. Mel Andersen (Hamner Institute) reviewed the zonal activation-dependency of the AHR. Dr. Andy Maier of TERA acted as rapporteur and panel facilitator. Finally, the panel benefitted from the input from a number of "roving" experts, including Julian Preston of EPA, Dr. Mike Honeycutt of the Texas Commission of Environmental Quality, Dr. Stoney Simons of the NIH, and Dr. Mike Dourson of TERA.

After careful review of MOA and Key Events data, the panel came to agreement that a basic understanding of the MOA, Key Events, and dose-response relationships was supported by the available data. Overall, the supporting data could be modeled to generate No-Observable Effect Levels. These conclusions were reviewed with the larger conference attendees on day 3 of the workshop.

The MOA and Key Events developed by the AHR expert panel consisted of pathways that led to either hepatocellular tumors or to bile duct tumors. Central to both types of tumors was sustained AHR activation and all the potential Associative Events that could be modeled for this Key Event, e.g, CYP1A induction, gene expression changes.

- For hepatic tumors, sustained AHR activation occurring in pre-existing, initiated hepatocytes inhibits apoptosis resulting in increased proliferation and clonal expansion of altered hepatic foci.
- Accumulation of tissue TCDD levels over time causes a pathological outcome that has been labeled as hepatopathy by extensive Pathology Working Group input.
- This hepatopathy and its associative events results in subsequent regeneration and mitogenic stimulation of the pre-existing hepatocytes within the altered hepatic foci.
- Hepatopathy, along with the additional inhibition of apoptosis, eventually causes the pre-existing initiated hepatocytes to escape growth control and transform into cancer cells and tumors.
- For the hepatocellular tumors, multinucleated hepatocytes were observed as part of the overall hepatopathy and are concluded to be an important associative event (biomarker). However, many other Key Event, Associative Events, and

Modulatory Factors can serve as important dose-response relationships for characterizing the threshold nature of dioxin-induced hepatocellular tumors.

- For biliary tumors, a similar set of Key Events occurs within pre-existing initiated biliary cells, or stem cells, followed by TCDD-induced cholangiofibrosis that leads to regenerative repair and indirect mitogenic stimulation of the pre-existing initiated biliary cells or oval cells (stem cells) with the final result of biliary tumor formation. Although more limited when compared to the studies and data available for evaluating Key Events, Associative Events, and Modulatory Factors for hepatocellular cancer, biliary tumor development is still highly dependent upon sustained AHR activation.
- For the bile duct tumors, oval cell hyperplasia was thought to be a significant Key Event.

In summary, an expert panel, during a workshop dedicated to examining the MOA, Key Events, and dose-response relationships for important nuclear receptors, was able to derive a MOA for dioxin-promoted rodent liver tumors. The AHR MOA supported the 2006 NAS panel's recommendation for using a threshold model for describing dioxin's carcinogenic risk in rodents. In contrast, the EPA after many years of examining the data and having the benefit of multiple science advisory panels, an NAS review panel, and their own expert contractors, claimed to have not found sufficient information for supporting a MOA. A likely reason for EPA's failure to identify a MOA is their failure to follow a systematic, weight-of-the-evidence process known as the MOA framework as specified by their own 2005 Cancer Guidelines. .