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Styrene Information and Research Center Briefing Paper: Styrene's Mode of Action

Background

Chemical testing of mice and rats is used extensively by health scientists to determine the likelihood that humans exposed to the same chemical may be affected in a similar manner. These tests underpin most of the current government regulations on chemicals today.

Is a mouse's reaction to a test chemical a good predictor of human health? Health scientists today would answer this question: "Usually, but there are instances where the unique characteristics of the test animal cause it to react to the same chemical differently from humans." For this reason, the U.S. Environmental Protection Agency's (EPA's) *Guidelines for Carcinogen Risk Assessment* emphasize that assessors need to understand as best they can the "**Mode of Action**" by which a substance acts biologically within and upon an organism. In many cases, this understanding will help confirm that humans are likely to react the same way as the test animal. But in some cases, this same examination will show that what happened in the test animal is unlikely to happen in humans because of the different species' genetic makeup.

Introduction

Extensive testing of styrene over the years generally has shown little cause for concern. In the mid-1990's, however, published studies commissioned by the Styrene Information and Research Center (SIRC) raised questions about styrene's cancer-causing potential. Specifically, the studies found lung tumors in exposed mice, but no tumors in rats, even though the rats were exposed to much higher levels.

Because the data on workers do not indicate any increase in cancer -- lung or otherwise -- from styrene exposure (see "Eminent Scientists Find No Causal Relationship to Human Cancer" in this paper), scientists were left with the key question of why mice get cancer, but rats and humans apparently do not. Here is where the investigation into styrene's Mode of Action -- how it acts biologically within and upon an organism -- comes into play.

As soon as the mouse lung tumors were found, the styrene industry launched additional research to study styrene's Mode of Action -- MoA for short -- for two key reasons:

- If the results in mice prove to be relevant to humans, then the industry would have a stewardship responsibility to its workers

and to the public to protect them from these effects.

- If the mice would appear to be unique in some way that makes their reaction to styrene not relevant to human health, then government agencies charged with regulating chemicals would need additional information about the MoA by which these effects occurred in mice as a way of assuring themselves and the public that the results in mice would not be relevant to human health.

Since the mouse lung tumors were found, the styrene industry through SIRC in the United States and its European counterpart, the Styrenics Steering Committee of Cefic, have sponsored some \$5 million worth of research to better define the process through which styrene forms tumors in the mouse (styrene's MoA) and examine why styrene does not seem to produce the same response in rats and humans. These further studies have expanded our knowledge greatly of styrene's MoA in mice and increasingly reveal that lung tumors in mice exposed to styrene are not relevant for human risk assessment.

Much of the newest SIRC-commissioned MoA research uses genetically modified mice -- as described further along in this paper; it relies

on techniques that won the Nobel Prize in Medicine¹ in 2007.

Styrene MoA Research – The Early Years

From 1996 through 2001, peer-reviewed scientific journals published 15 papers on styrene MoA-related research commissioned by the styrene industry. In 2002, a report² in the peer-reviewed *Journal of Regulatory Toxicology and Pharmacology* summarized the results of these first several years of SIRC/Cefic-sponsored research.

This early research showed that the lung tumors in the mice most likely were not the result of damage to mouse DNA (the most common way that cancerous tumors develop – a “genotoxic” MoA), but rather resulted from cell toxicity (“cytotoxicity”) that led in turn to a non-tumorous increase in the number of cells in the lung (hyperplasia). This finding was consistent with results from rat studies, where there were no lung tumors seen and where there was no evidence of cytotoxicity.

These early studies also concluded “that styrene respiratory tract toxicity in mice and rats, including mouse lung tumors, is mediated by...metabolites³. The (applicable) model predicts that humans do not generate sufficient levels of these metabolites in the terminal bronchioles to reach a toxic level. Therefore, the postulated mode of action for these effects indicates that respiratory tract effects (of styrene) in rodents are not relevant for human risk assessment.”

The essential finding from this early research was that mouse lungs exposed to styrene produce sufficient quantities of certain metabolites to develop tumors, but rat lungs

do not. The question remained: What were the metabolites responsible for these phenomena? With this information, one could examine whether these same metabolites are of concern for humans.

Identifying Metabolites that Mediate Tumor Formation

From 2002 through 2009, peer-reviewed journals carried 11 more papers based on SIRC-commissioned research to identify the responsible metabolites and, accordingly, answer the fundamental question of why humans and rats exposed to styrene do not develop tumors, while mice do.

In 2009, the peer-reviewed *Journal of Regulatory Toxicology and Pharmacology* carried a report⁴ summarizing the body of MoA mouse lung tumor research to date. The underlying research involved styrene and several related chemicals.

These studies found that styrene is catalyzed in the mouse lung by the CYP2F⁵-type enzyme. The metabolic products of this enzyme in the mouse cause severe damage to a specific type of cells called Clara cells, which are found in small airways of the lungs. The mouse lung removes these damaged cells and replaces them with new Clara cells that do not make as much of a protein called CC10, which is a normal Clara-cell product that has tumor-preventing properties.

The mouse lung also continues making extra cells. This increased cell production leads to a greater risk of the increased presence of DNA-damaged cells and less of the protective agent (CC 10) in the mouse lung, and therefore to an increased incidence of lung tumors in mice. Rats have much smaller amounts of CYP2F-type enzyme in their lungs and do not produce sufficient metabolites to cause this cellular toxicity; accordingly, there is no increase in lung tumors. And humans have very little CYP2F-type enzyme, leading

¹ Mario R. Capecchi, Sir Martin J. Evans, Oliver Smithies “for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells.”

² “Styrene Respiratory Tract Toxicity and Mouse Lung Tumors are Mediated by CYP2F-Generated Metabolites,” *Journal of Regulatory Toxicology and Pharmacology*. 35, 308-319 (2002)

³ Foreign substances typically are removed from organisms through a “metabolic” (chemical) process that results in the formation of “metabolites” (products of chemical process) that are either removed from the organism or, in turn, are subject to further metabolic processes.

⁴ “Mouse Specific Lung Tumors from CYP2F2-Mediated Cytotoxic Metabolism: An Endpoint/Toxic Response Where Data from Multiple Chemicals Converge to Support a Mode of Action,” *Regulatory Toxicology and Pharmacology*, 55:205-218 (2009).

⁵ CY2PF is a particular gene.

to the conclusion that styrene would not cause human lung tumors.

The SIRC-supported MoA research summarized above is described in more detail in the 2009 summary report cited above⁴. Readers are encouraged to explore this subject more fully by reading this report, copies of which are available upon request to SIRC.

Further Validating Styrene's Lack of Human Carcinogenicity

As part of its continuing research, SIRC commissioned work that uses mice with a CYP2F-type gene removed ("knock-out mice") in an effort to make them unsusceptible to styrene toxicity and, accordingly, to demonstrate that limiting CYP2F-type metabolism within the mouse greatly reduces styrene lung toxicity.

A small colony of "knock-out mice" for this important research was established and the work commenced in August 2010. Preliminary findings clearly support a conclusion that styrene is not a human carcinogen⁶. This continuing research should help further illuminate the essential role of CYP2F in mouse-lung tumor formation, and should help identify which specific styrene metabolites are responsible for the tumors observed in prior research. Results are expected in 2011, and will be offered for publication in a peer-reviewed journal.

SIRC is developing a program of additional MoA research that also would use the genetically modified mice. The key CYP2F enzyme thought to be responsible for toxicity and tumors in mouse lung will be replaced by the related human enzyme in the "knock-in" mice. Exposure of these mice to styrene will help determine if CYP2F MoA is relevant to the assessment of human risk from styrene exposure.

⁶ "Initial CYP2F2 Knock-Out Mouse Exposure Study Findings" abstract available upon request; additional information may be found in the SIRC briefing paper "New Research Findings Show Mouse Tumors Are Not Relevant for Human Risk Assessment; Styrene Does not Belong in NTP *Report on Carcinogens*."

Eminent Scientists Find No Causal Relationship to Human Cancer

In 2009, the peer-reviewed *Journal of Occupational and Environmental Medicine* published a SIRC-commissioned review showing that the "available evidence does not support a causal relationship between styrene exposure and any type of human cancer." The paper, "Epidemiologic Studies on Styrene and Cancer: A Review of the Literature," represents an independent report by a "blue ribbon" panel⁷ of epidemiologists.

SIRC asked the panel to review all of the epidemiologic⁸ literature and produce an authoritative report on styrene's human cancer-causing potential for consideration in regulatory and worker-safety decision-making.

Also, SIRC has commissioned an extension of one of the largest studies of styrene-exposed workers ever conducted. "A cohort mortality study of workers exposed to styrene in the manufacture of glass-reinforced plastics" was last updated in 1994. That new work, which is to be completed in 2011, will add 15 years of follow-up data, increasing the years-at-risk to 500,000 from 350,000.

The Path Forward

All of the research that the styrene industry underwrites is provided to federal and state regulators and other policy makers to inform their decision making on styrene. Currently, the U.S. National Toxicology Program, U.S. EPA and California Environmental Protection Agency are evaluating styrene's potential human carcinogenicity. SIRC will continue to underwrite independent, cutting-edge research and to make the findings available to these groups to ensure that they have access to the latest scientific findings that are relevant to their deliberations on styrene.

⁷Dr. Paolo Boffetta of the International Prevention Research Institute, Lyon, France, led the panel, which included Drs. Hans Olov Adami and Dimitrios Trichopoulos of the Harvard School of Public Health, Boston; Dr. Philip Cole of the University of Alabama-Birmingham, and Dr. Jack S. Mandel of the University of Toronto.

⁸ Epidemiology is the study of disease in human populations. The epidemiological literature on styrene comprises primarily studies on the long-term health of manufacturing workers exposed to styrene.