APPENDIX AVAILABLE ON THE HEI WEB SITE

Research Report 166

Advanced Collaborative Emissions Study (ACES) Subchronic Exposure Results: Biologic Responses in Rats and Mice and Assessment of Genotoxicity

Part 1. Biologic Responses in Rats and Mice to Subchronic Inhalation of Diesel Exhaust from U.S. 2007-Compliant Engines: Report on 1-, 3-, and 12-Month Exposures in the ACES Bioassay

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Appendix G. Memorandum on Rat Strain Decision

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This document was reviewed by HEI's ACES Review Panel but did not undergo the HEI scientific editing and production process.

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APPENDIX G

Memorandum

To: File
From: Annemoon van Erp and Maria Costantini
Date: July 15, 2010
Re: Decision on rat strain for ACES

This memorandum documents the decision process regarding the choice of rat strain to be used in the chronic inhalation bioassay of ACES Phase 3B.

Initial Steps (2006 / 2007)

Initial discussions regarding the rat strain for ACES were held during the study protocol development in 2005 and 2006. At the time, NTP was considering switching from F344 rats, which had a high incidence of leukemia, to another strain, possibly Wistar rats. The final version of the ACES Project Plan of May 2006, which was part of the ACES Request for Proposals 06-1, considered using F344 (which had been used in the previous HEI-funded bioassay at the Lovelace Respiratory Research Institute) or Wistar rats, which had been used in previous inhalation bioassays, including for diesel exhaust at the Fraunhofer Institute (and had found similar tumor response to the Lovelace work). At the time, as excerpted below, the Project Plan recommended Wistar rats rather than F344, and suggested the Wistar WU strain over the Wistar Han strain because adult male Wistar Han rats reach a higher bodyweight, which would require larger cages and would reduce the number of animals that could be exposed. A final decision on the rat strain (Wistar Han or Wistar WU) would be made later, with further input from experts and the investigator's team (to be selected).

> Selected text from the Project Plan, page 40:
> "*Rat strain.* Two strains of rats are being considered for use in the ACES bioassay: F344 and Wistar WU. Earlier diesel bioassays have for the most part used F344 rats (Ishinishi et al 1986; Iwai et al 1986; Mauderly et al 1987; Brightwell et al 1989; Mauderly et al 1994); some have used Wistar Han rats (Heinrich et al 1986; Heinrich et al 1995). Initially F344 rats were recommended for ACES to improve our ability to compare results with earlier chronic diesel bioassays and because it has been the strain of choice for chronic NTP bioassays. However, the F344 strain has poor survival and an increasing spontaneous incidence of leukemia, which could confound the analysis of lung lesions and shorten the life span of the animals. In addition, this strain has a high incidence of spontaneous testicular tumors (70-90%) making it insensitive for testicular effects. The NTP has been discussing alternatives to

using the F344 rats (NTP 2005), and is considering recommending Wistar rats for inhalation studies and F344 rats from the National Center for Toxicological Research at the Food and Drug Administration (which have better fecundity than F344 from NTP) for non-inhalation studies. Because of the problem with the F344 rats, two Wistar substrains have been considered for ACES. Wistar WU rats have also been used extensively in chronic bioassays and in inhalation studies in Europe and have comparable growth curves and final bodyweights to F344 rats. Their background tumor incidence of both leukemia and testicular tumors is relatively low compared to the F344 strain. The Wistar Han rats have been considered also, but their final bodyweight of around 650 g for males would limit the number of rats that could fit into the exposure chambers after 6 months of exposure. Therefore, the Wistar Han substrain should not be considered for ACES. With either F344 or Wistar WU, the total number of animals that could be housed in the inhalation chambers and analyzed at the various sacrifice times would be the same.

(..) The final decision about the rat and mouse strains will be made by the HEI ACES Oversight Committee after reviewing recent data on growth curves, survival rates, and background tumor incidence and other age-related diseases, and while considering insights that may be provided by the applicants to RFP 06-1 for the chronic bioassay and the HEI ACES Advisory Committee."

In December 2006, Dr. Gene McConnell of the HEI ACES Oversight Committee notified HEI that NTP had officially switched to the Wistar Han strain. At this time, HEI started looking into the feasibility of using Wistar WU or Han rats for ACES. The main issues weighing into the decision were: survival, tumor incidence in unexposed animals, bodyweight, and availability at suppliers in the U.S.

In March 2007, Dr. Joe Mauderly at LRRI, whose team was selected to conduct the bioassay, proposed a strategy to accommodate larger Wistar Han rats. This would include purchasing custom made cages for rats larger than 500 g, according to specifications set by the NIH Guide for the Care and Use of Laboratory Animals. In April 2007, HEI obtained information on survival curves of the Wistar WU and Han strains. Attempts to obtain background tumor data from studies conducted in Europe was only partially successful because some of the data were proprietary. However, available data indicated that there were no major health problems with these strains and that spontaneous tumor incidence was low. Dr. Mauderly located a supplier of Wistar Han rats in the U.S. (Han:Tac:WH strain from Taconic) that indicated that the bodyweight of male Wistar Han was only slightly larger than for Wistar WU. Based on this information, Drs. McConnell and Mauderly agreed that the Wistar Han strain was the best possible alternative to using the F344 rat strain. On April 13, 2007, Dr. Jane Warren communicated this recommendation to the ACES Oversight Committee. Based on the available information and a preference to purchase rats from a supplier based in the U.S., the Oversight Committee approved the choice of the Wistar Han rat strain.

Decision revisited (2009)

In January 2009, Dr. McConnell contacted HEI indicating the NTP had changed towards using the Harlan Sprague Dawley (SD) rat strain. This decision appeared to be driven by the desire to use the same strain for different types if studies, including reproductive and developmental studies. Apparently the Wistar Han (from Taconic) were not producing large enough litters to accommodate those other types of studies. However, this issue did not affect the ACES study. Dr. McConnell also mentioned that a recent

chronic study at the Hamner Institute (formerly CIIT) had observed a so-called "bumblefoot" (pododermatitis) problem in Wistar Han rats, which would be of concern. It was unclear if a similar problem existed at other suppliers. He mentioned that Harlan had become a supplier for the European Wistar Han strain (based on breeding pairs from RCC Switzerland, where many of the European carcinogenicity studies had been conducted), which provided an additional choice of suppliers.

The issue was brought before the Oversight Committee for their input. At this time a thorough comparison was made of the F344, SD, Wistar Han, and Wistar WU rat stains with regards to (1) the existence of a historical database, (2) whether the NTP had used or would be using them, (3) any known health issues, (4) survival curves, and (5) bodyweight. Further research into the bumblefoot problem indicated that this could be related to housing conditions. During a conference call on February 6, 2009, Oversight Committee members decided to stay with the original recommendation to use Wistar Han rats. As before, the F344 strain was not considered suitable because of the leukemia incidence. The SD strain was not considered suitable due to the large bodyweight (900 g), a lack of a historical data (especially for inhalation studies), and a high incidence (>50%) of mammary tumors in females, which in many cases requires euthanasia.

The final recommendation was to obtain Wistar rats of European origin. Because the Wistar Han/RCC strain was now available in the U.S. through Harlan (which had bought RCC in the meantime) this was a preferable option over shipping Wistar WU rats from Europe (due to higher cost). Other Wistar Han strains (available from Taconic or Charles River) were considered less desirable because they were not recently derived from the stock at RCC.

Additional discussions (2010)

In January 2010, HEI was contacted by Dr. John Budroe of the HEI ACES Advisory Committee. He indicated that OEHHA was concerned with the choice of the Wistar Han strain because of a mutation in the aryl hydrocarbon (Ah) receptor. He mentioned a recent paper[1] that stated "For example, the Wistar Han rat would not be used in studies evaluating dioxins or other selected aromatic hydrocarbons. Most Wistar Han rats are homologous for a mutant aryl hydrocarbon (Ah) receptor (Pohjanvirta et al. 1998)[2]. This mutation makes the Wistar Han resistant to the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and certain other "dioxin-like" chemicals; therefore, this model would not be the appropriate choice when studying these types of compounds."

HEI brought up the issue with members of the Oversight Committee. Although the Committee acknowledged that this issue could potentially be important, the levels of dioxin already measured in the 2007 engine exhaust were two orders of magnitude below previous models and unlikely to be a significant contributor of effects in the

[1] King-Herbert AP, Sills RC, Bucher JR. 2010. Commentary: update on animal models for NTP studies. Toxicol Pathol. 38(1):180-181

[2] Pohjanvirta R, Wong JM, Li W, Harper PA, Tuomisto J, Okey AB. 1998. Point mutation in intron sequence causes altered carboxyl-terminal structure in the aryl hydrocarbon receptor of the most 2,3,7,8-tetrachlorodibenzo-p-dioxin-resistant rat strain. Mol Pharmacol. 54(1):86-93.

animal. It also felt that this was only one of many factors playing into the rat strain decision. In response to Dr. Budroe, HEI noted the following:

> "The choice of which rat strain to use for ACES was based on a number factors, which included general long-term health, susceptibility to specific kinds of tumors, longevity, body weight, and availability. Based on these factors, the Wistar Han emerged as the best choice for our purposes.
>
> (1) Longevity was a major decision factor. We thought it was important to have sufficient survival after 24 months because we would like to extend the ACES study to 30 months, if possible, to provide the maximum opportunity for potential changes to occur. F344 and Sprague Dawley do not have as good survival curves as Wistar Han do.
>
> (2) In terms of long-term health, we did not see major problems with Wistar Han. You mentioned possible sensitivity to dioxin and similar compounds that act via the aryl hydrocarbon receptor. This is possible, but given the very low levels of dioxin with the 2007 heavy-duty engines (about 100x lower than previous models - see CRC ACES Phase 1 report; we also discussed this with appropriate people within EPA) and uncertainty about the possible involvement of the Ah receptor mechanism in non-cancer endpoints we do not think this is a concern that is major enough to warrant revisiting the decision to use Wistar Han.
>
> (3) Body weight was also considered important due to the limited size of the inhalation chambers. By choosing a medium-size rat we could maximize the number of animals exposed in this study and thus increase the power of the study. Sprague Dawley are bigger than Wistar Han which poses a logistical problem for ACES in terms of their size.
>
> (4) Wistar Han rats have been used before in diesel exhaust inhalation studies, and there is a historical database of cancer endpoints in this strain from other exposures[3]. The paper by King-Hebert indicates that NTP is currently constructing a reference database for Sprague Dawley because it doesn't yet exist.
>
> (5) One of the deciding factors for NTP to start using Sprague Dawley rats rather than Wistar Han was reproductive success (as mentioned in the King-Hebert paper). This is of course not a concern for ACES.
>
> All of the above gives us confidence that the Wistar Han is the optimal choice for ACES and believe it will provide a full and fair opportunity to test whether there are effects from these long-term exposures."

HEI also noted that, if ACES stakeholders remain concerned about this issue, a possibility remains to conduct additional short-term studies with different rodent models after the chronic bioassay has been completed (originally part of the ACES Project Plan as Phase 3C – if funding is available). At that time a more informed decision can be made, based on results from the chronic bioassay and any other information that may have become available in the meantime.

[3] Additional information on background tumor incidence has become available at the Harlan website: https://webapps.harlan.com/wistarhannover/. (*Note that people need to create a user account to access the website. They will then receive a call from a Harlan representative.)*