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To: Dr. Angela King-Herbert

Subj: Rodent Cancer Bioassay Workshop

Dear Dr. King-Herbert,

I am writing concerning the NTP workshop regarding selection of rodent strains for chronic bioassays to be held 16-17 May 2005. First, I want to congratulate you for conducting this Workshop. I'm certain that it will provide important information and be helpful in guiding the NTP in the future. I am interested in attending and making a short presentation (see below). I have been working in this area for over 3 decades and would like to present the results of some of my experience to the members of your workshop. Specifically, I would like to talk about the "best" strain of rat to use in chronic inhalation studies. Some of my concerns with the F344N rat are as follows:

Background: First, in my view there is *no perfect strain* of rat, i.e. "one strain doesn't fit all". One should choose the most appropriate strain to answer the most questions of concern and have the least disadvantages with the particular xenobiotic being studied. The confounding results of many studies could have been precluded by the use of a different strain. In my opinion, the best way to approach the selection of the most appropriate strain is to have a clear understanding of the potential toxicity of a given chemical, including potential target organs, and then select the strain that best meets these needs. At a minimum this would require ADME and basic PK data. One might also try to anticipate the "down-stream" use of the data by the appropriate regulatory agency and their particular concerns.

F344N rat: As you know, this strain of rat has been the primary strain used by the NTP since its' inception. It has provided a wealth of data on the potential toxic and carcinogenic potential of a wide variety of chemicals. In addition, it has become the strain of choice in the USA, in no small part because it is the one used by the NTP and the NTP is considered the "Gold Standard". However, in my experience some of the data derived from this strain has been compromised as to its usefulness for regulatory needs (the public's' use of the data), which in my view should be the basic reason for conducting these studies. Some of the concerns with the F344N rat are as follows:

- 1) As you know, the F344N rat has a high incidence of leukemia. Unfortunately, this disease often affects the lung causing the capillaries to be packed with the leukemic cells. While this does not preclude the diagnosis of overt neoplasms, it can and does mask

inflammation and preneoplastic lesions, e.g. bronchoalveolar hyperplasia, and to some degree subtle visceral pleural lesions. This is particularly important when evaluating the effects of inhaled particulates. The leukemic response in the liver also confounds the diagnosis of hepatocellular proliferative lesions and interferes with the evaluation of chronic renal lesions.

So, what is the best alternative? In my view, the Wistar rat (Han strain) is superior. It does not have this problem, is of a size that works nicely with nose-only protocols, and has a large historical data base, although in Europe. However, I don't think the lack of a USA data base should preclude its' use. This Fischer rat type of leukemia is rarely found in the Wistar rat, therefore, chronic lung, liver and kidney lesions are much more easily ascertained. Also, the Wistar has a low incidence of lymphatic tumors, so if that is an endpoint of interest, it is still valuable because it isn't refractory to neoplastic lesions of the lymphoreticular and hematopoietic systems. The Sprague-Dawley rat would be my second choice for inhalation studies, but it has the problem of size and a high spontaneous incidence of large mammary tumors in females, both of which prevent them from being used in standard nose-only tubes.

- 2) The F344N rat also has a high incidence of Leydig cell tumors of the testes which essentially prevents it from being of any value for this endpoint.

Solution? Again, in my view the Wistar strain is superior because of its relatively low incidence of this lesion. The Sprague-Dawley is also suitable for this endpoint, but again, has the problems listed above, in particular for inhalation studies.

- 3) The F344N rat also has the problem of developing thyroid follicular cell tumors with hepatotoxic compounds. There is convincing data that such thyroid induced neoplasms have no bearing on the risk of thyroid cancer in humans. While I do not know if other strains of rats have this same problem, the question should be answerable by reviewing the available data.

Using the above as examples, I would like to offer a suggestion for how to address the overall issue of strain selection. First, I would not allow the issue of "We have used the F344N strain for so long that we can't abandon it because of the large data base" to drive or even significantly influence the selection of the strain. If statistical concern is an issue with the selection of a new strain of rat, this can be sufficiently addressed by increasing the number of rats in the control group or using two control groups. Second, I think that it would be useful to evaluate and compare the most "popular" strains of rats with regard to their advantages and disadvantages, e.g. background incidence of a given neoplasm, complications from the use of that strain (see above leukemia discussion), and which strain best mimics what is known about ADME and PK in humans. This would probably mean that a given strain might be used for one chemical and another for a second one, etc. Such an approach would make the results of the chronic bioassay more scientifically credible and much more useful for the regulators.

Thank you for allowing me to respond to your invitation for “public comments”. If I am allowed to comment, I would restrict my comments to the above. Also, if you allow me to comment, it would help me to know which day would be appropriate because of my time constraints.

Thank you for your consideration in this matter.

Sincerely,

Gene McConnell