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NEBRASKA'S HEALTH SCIENCE CENTER

PATHOLOGY AND MICROBIOLOGY

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Dear John,

I have reviewed the two-volume document entitled "Perfluorooctanesulfonyl Fluoride (POSF; T-7661.4), Toxicity Study by Inhalation Administration to CD Rats for 13 Weeks Followed by a 4 Week Recovery Period (Vol. 1 & 2). You asked me to specifically review the changes regarding the urinary tract, and especially the scanning electron microscopic findings reported in Vol. 2.

Overall, it appears that the major tissue treatment-related effect is in the liver. From what I understand, you have additional information regarding the effects of this compound on the liver, indicating that it is quite possibly related to peroxisome proliferation. I will not comment further regarding the liver changes.

With respect to the urinary tract, they list sporadic changes in the kidney, which I have described in the review of the slides in the paragraphs above. Again, I do not see anything here that suggests treatment-related effects on the kidney.

With respect to the urinary bladder, by light microscopy they are all listed as normal. By scanning electron microscopy, it is my impression that these also are normal. Again, there is no indication as to which of the photomicrographs belong to which treatment groups, but I do not see anything in any of the electron micrographs of the urothelium to suggest any abnormalities in any of the animals. Many of the animals show separation of the urothelial cells of the superficial layer, which is usually indicative of overdistention of the bladder with fixative at the time of preservation. In addition, there are some slight changes of occasional cell necrosis of superficial cells, but again, this is a normal finding as long as it is not extensive and is seen in only one or two cells at a time, rather than large areas. I do not see any such large areas in any of the sections portrayed. Based on the classification system that we devised for examination of subtle changes of rat urinary bladder (Scanning Microscopy, 4:135-142, 1990), I would classify all of these bladders as a 1 or a 2, which are considered normal.

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It is a bit unclear as to methodology of the preparation of these samples. Were the animals under anesthesia at the time of fixation of the bladder, or were the animals killed and the bladders removed and then placed in fixative? If the latter method, some of these slight changes we see in some of these bladders could well be due to autolysis, which occurs within 60 seconds of the time that an animal dies (J. Natl. Cancer Inst., 90: 19-25, 1998). Regardless of the specific methodology, I would classify all of these bladders as normal.

Also, the scanning electron micrographs of the urinary sediment I consider all normal. There are mostly illustrated examples of single or aggregates of magnesium ammonium phosphate crystals, which are a normal constituent of rat urine (as well as most other mammals). I see no abnormal urinary solids in any of these photographs.

The PCNA staining for cell proliferation showed no differences between the treated and controls, again supporting the conclusion that there was no effect on the urinary bladder urothelium. With the negative observations by light microscopy, scanning electron microscopy, and PCNA labeling index, it can strongly be concluded that there is no effect on the urothelium in this study. Scanning electron microscopy and labeling index are particularly sensitive in detecting early urothelial changes, and these were completely negative in this study.

Examination of urinary parameters also showed no differences secondary to treatment, although it is a bit difficult to ascertain the exact methodology used for collection and examination of these specimens. The fact that overnight urines were collected is not the best way, but is certainly an adequate way for examining specimens. Also, not providing food and water during collection of the urine is not optimum for identifying changes, but the fact that no changes were observed in urine specimens and no changes were observed in the urothelium of any of the animals, I can only conclude that there were no significant treatment-related effects on urinary composition, urinary sediment, or the urothelium.

In summary, I do not see any evidence of a treatment related effect on the urinary tract, including kidneys and urinary bladder, in response to treatment with POSF by inhalation.

Sincerely yours,

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