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REVIEW OF PROLIFERATIVE LESIONS OF THE EXOCRINE PANCREAS IN TWO CHRONIC FEEDING STUDIES IN RATS WITH AMMONIUM PERFLUOROOCTANOATE

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1.0 INTRODUCTION AND METHODS

As part of study investigating the relationship between peroxisome-proliferating compounds and tumorigenesis in the rodent liver, testis, and pancreas, male CD rats were exposed to ammonium perfluorooctanoate (APFO) for 2-years at dietary concentrations of 300 ppm (Biegel et al., 2001). This study was conducted at DuPont's Haskell Laboratory during 1991 and 1992. Among the findings in that study were increased incidences of pancreatic acinar cell hyperplasia and adenoma in APFO-treated rats. These results were in apparent contrast to a previous chronic feeding study with APFO in which the same rat strain was exposed to the same dietary concentration of 300 ppm (3M, 1987). In that study, no compound-related proliferative lesions of the exocrine pancreas were identified. This latter study was sponsored by the 3M company and was conducted between 1981 and 1983.

In an effort to address the apparent discrepancies between the two studies, the following activities were undertaken sequentially:

- Slides of the pancreas from the 3M study were reviewed by Dr. Steven R. Frame, a veterinary pathologist at DuPont (the study pathologist for the DuPont Study). This review focused on slides from male rats in the control and 300 ppm (high dose) groups of the 3M study.

- At the request of 3M, pancreas slides (control and treated) from the DuPont study were reviewed by Dr. Ernest E. McConnell, a consulting veterinary pathologist.

- Dr. McConnell then reviewed all pancreas slides from the 3M study.

- Drs. Frame and McConnell jointly reviewed selected pancreas slides from both the 3M and DuPont studies. This review included pancreas slides from the DuPont study stained with 5-bromo-2'-deoxyuridine (BrdU) to detect increased cell proliferation.

The slide reviews outlined above were conducted to help clarify the findings relative to pancreatic acinar cell lesions in the two chronic feeding studies with APFO. These reviews were not, however, designed as exhaustive pathology peer reviews. Thus, not all slides from all groups were examined but only those deemed necessary to assess and compare the effects on the exocrine pancreas in the two studies. The reviewing pathologists were in agreement regarding the general conclusions of these reviews (see Results and Discussion below).

2.0 RESULTS AND DISCUSSION

Based on the review of Dr. McConnell, the conclusions of the DuPont study were confirmed. Specifically, chronic exposure to 300 ppm APFO was associated with increased incidences of pancreatic acinar cell hyperplasia and adenoma of the pancreas in male rats. Dr. McConnell confirmed all acinar cell lesions diagnosed as acinar cell neoplasia (adenoma or carcinoma). He also confirmed an increased incidence of hyperplasia in APFO-exposed rats.

Based on the joint review by Drs. McConnell and Frame, exposure of male rats to 300 ppm APFO in the 3M study was associated with a slight increase in acinar cell hyperplasia but not adenoma or carcinoma. Thus, APFO was not a pancreatic tumorigen in the 3M study.

The reviews of both the 3M and DuPont studies indicate that the results of the two studies are more similar than dissimilar when considered in the context of the pathogenesis and diagnostic criteria established for proliferative lesions of the exocrine pancreas of the rat. Proliferative lesions of the acinar pancreas are thought to exist along a continuum of hyperplasia, adenoma and carcinoma. However, the likelihood of progression or regression along that continuum is unknown in most cases, and typically there are no cytological features that clearly distinguish hyperplasia from adenoma (Hansen et al., 1995; Eustis et al., 1990). Indeed, some authors have used terms such as hyperplasia and adenomatous hyperplasia to describe nonmalignant acinar cell lesions (Greaves and Faccini, 1992). To achieve some uniformity in diagnoses across laboratories, diagnostic criteria have been established to differentiate acinar cell hyperplasia from adenoma, and the criterion most commonly employed is the somewhat arbitrary feature of two-dimensional size of the lesion (Hansen et al., 1995; Eustis and Boorman, 1990). Thus, for many acinar cell proliferations, the difference between the diagnoses of hyperplasia and adenoma reflects a difference in the two-dimensional size of a random section through the lesion rather than known differences in biological potential.

Based on these considerations it is apparent that the results of the DuPont and 3M studies are qualitatively similar, as APFO produced increased incidences of proliferative acinar cell lesions of the pancreas in both studies. The differences observed were quantitative, that is, more and larger focal proliferative acinar cell lesions were produced in the DuPont study compared to the 3M study. The reason for this quantitative discordance despite use of the same rat strain and dietary concentration of APFO (300 ppm) in the two studies is not known. However, there are a number of variables that could contribute to the differences observed. These studies were conducted approximately10 years apart and in different laboratories. It is expected that these temporal and locational variables were associated with some differences in animal husbandry, including diet, as well as potential genetic drifts within the rat strain. Of all possible factors, a difference in the diets is the most likely factor influencing the different incidences of

proliferative acinar cell lesions. Various forms of dietary manipulation, including feeding raw soy diets or diets deficient in choline, are known to moderate the incidences of acinar cell hyperplasia and neoplasia in the pancreas of rodents (Longnecker and Millar, 1990; McGuinness et al., 1980). The incidences of acinar cell hyperplasia and adenoma were increased five fold in control male F344 given corn oil by gavage compared to rats not gavaged (Eustis and Boorman, 1985).

In considering the human relevance of acinar cell hyperplasia and neoplasia in the rodent, it is noteworthy that the histological type of tumor seen in the rodent is distinctly different from tumors of the exocrine pancreas most commonly observed in humans. While rodent tumors are typically derived from acinar cells, the majority of human pancreatic neoplasms are of the ductular type (Longnecker and Millar, 1990). True ductular neoplasms of the pancreas are rare in rats (Greaves and Faccini, 1992). In addition, pancreatic acinar cell hyperplasia and adenoma have been produced in rats by a number of compounds which, like APFO, produce peroxisome proliferation in the liver. These include pharmaceuticals widely used in humans, such as the hypolipidemic agent, clofibrate. There is no known association between these compounds and tumors of the exocrine pancreas in humans. Thus, while the precise mechanism of formation and significance of pancreatic acinar cell proliferations in rats are not known, they probably do not indicate a cancer hazard for humans (Greaves, 2000).

3.0 CONCLUSIONS

Microscopic lesion observed in the exocrine pancreas in two chronic feeding studies in rats, conducted by 3M and DuPont respectively, were reviewed by two veterinary pathologists. These reviews were conducted to clarify apparent differences between the two studies relative to compound-related lesions of the exocrine pancreas; however, a formal pathology peer review of the pancreas from all study animals was not conducted. The results of the two studies were similar in that APFO produced proliferative lesions of pancreatic acini in both studies at dietary concentrations of 300 ppm. In the 3M study, treatment-related changes were limited to slight increases in acinar cell hyperplasia. In the DuPont study, increased incidences of hyperplasia and adenoma were observed. Thus, these studies produced qualitatively similar responses in the pancreas, with the differences noted being quantitative–higher overall incidences of proliferative lesions and greater tendency for progression of lesions to adenoma were observed in the DuPont study compared to the 3M study. Since the difference between pancreatic acinar hyperplasia and adenoma in the rat is often a reflection of arbitrary diagnostic criteria (size) and not necessarily biological potential, the results of the two studies are in reasonable concordance given the experimental variables that exist between them. Although the basis for the quantitative difference observed is not known, a number of variables, especially diet, are known to modulate the proliferative response of the acinar pancreas in rats and are likely operative in the differences in degree of response seen in the two chronic studies with APFO. Despite these differences, target organ effects on the pancreas, characterized by focal proliferative lesions of pancreatic acini, were common to both the 3M and DuPont studies.

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