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**Pathology Review of Reported Tumorigenesis
in a Two Year Study of FM-3924 in Rats**

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BY

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Pathology Review of Tumors Reported in Rats Given FM-3924

Introduction: This report presents an independent assessment of tumorigenesis data obtained from a study to determine the chronic toxicity and carcinogenic potential of the fluorochemical FM-3924 in rats. The study, entitled "Two Year (Diet) Toxicity / Carcinogenicity Study of Fluorochemical FM-3924 in Rats," was sponsored by the 3M Company, St. Paul, Minnesota. Biophase procedures were conducted at Riker Laboratories, Inc., between April, 1981 and May, 1983 and were in compliance with FDA Good Laboratory Practice (GLP) guidelines. At Riker Laboratories, Leonard J. Sibinski, BA, served as the Study Director. Following in-life procedures, all major organs and tumors were processed into microslides and examined by Robert G. Geil, DVM, DACVP. Dr. Geil's findings and interpretations were incorporated into the final report for the study.

For the tumorigenesis review, the complete study report, including all relevant pathology data was forwarded to Pathology Associates International (PAI), West Chester, Ohio for examination by Richard H. Bruner, DVM, DACVP. The sponsor (3M) regarded original pathology interpretations by Dr. Geil as adequate, and examination of microscopic tissue sections was not included in the review process. Specific objectives of the review were to evaluate tumor data and to provide an opinion relative to the potential relationship of reported neoplasms with the test material based upon: 1. The incidence and morphology of observed tumors and associated proliferative and non-proliferative lesions. 2. A literature review to examine the biologic behavior and carcinogenetic potential of similar fluorochemicals, 3. Contemporary knowledge of tumor mechanistic data, especially with respect to possible epigenetic pathways, 4. Consideration of chronic toxicity, immunosuppression, hormonal modulation or ancillary biochemical interactions which may serve as modulating factors in the development of tumors in this study, and 5. Personal experience in evaluating rodent carcinogenesis bioassays.

Review Procedures and Findings

1. Reported tumorigenesis and ancillary pathologic changes for FM-3924: Based upon reported pathology findings, the review pathologist concurred that treatment-related changes were present at both the 1-Year (interim) and 2-Year (terminal) sacrifices as well as in some unscheduled deaths. At the 1-Year interim sacrifice, treatment-related changes were generally restricted to the liver and were characterized by a dose-dependent increase in hepatocellular cytomegaly, vacuolation and necrosis along with slightly increased inflammatory cell infiltrates. Cytomegaly

and vacuolation were generally consistent with treatment-induced perturbation of liver cell metabolism resulting in hypertrophy via proliferation of peroxisomes and/or smooth endoplasmic reticulum (P-450 enzyme induction). Hepatocellular necrosis was largely attributed to chronic liver cell swelling with compromised metabolism and/or reduced blood perfusion (hypoxia).

Treatment-related microscopic findings in unscheduled deaths and animals continued until the terminal (2-Year) sacrifice included persistent hepatocellular cytomegaly and vacuolation in both sexes. Additionally, cystoid degeneration ("spongiosis hepatis") of the liver was significantly increased in high dose males. Most notably, hyperplastic nodules were increased in the liver of both sexes and hepatocellular adenomas and carcinomas were increased in females.

Based upon reported pathology data, dietary levels of 100 ppm FM-3924 for two years resulted in unequivocal hepatocellular cytomegaly, vacuolation and cystoid degeneration as well as increased hyperplastic nodules in both sexes and increased adenomas and carcinomas in high dose females. Although increased hepatocellular neoplasms were not reported in males, it is likely that some of the "hyperplastic nodules" (in both sexes) would be regarded as hepatocellular adenomas if contemporary diagnostic criteria were applied (1). It is noteworthy, also, that hepatic cytomegaly was observed in males given 10 and 30 ppm of the test material suggesting that a NOEL for hepatocytomegaly was not achieved for males assigned to this investigation

2. Literature review of the biologic behavior and carcinogenic potential of fluorochemicals

The reviewer regarded the test material (N-ethylperfluorooctanesulfonamido ethanol) as a unique xenobiotic with unknown structure-activity relationships. A limited literature review was conducted to determine if the biological behavior, including tumorigenesis, of similar fluorochemicals had been reported. The literature review included a survey of rodent carcinogenesis bioassays completed by the National Toxicology Program (NTP) and select scientific journals and biological extracts. Although no carcinogenesis bioassays with "complex" fluorochemicals were discovered, several relevant reports concerning subchronic investigations were located. Additionally, several reports, including the NTP 2-year bioassay of sodium fluoride were available to provide perspectives on the biological effects of long-term fluoride exposure. Results of these studies are briefly summarized as follows:

1. Subchronic toxicity studies in rats with "complex" fluorochemicals have identified treatment-related hepatocellular hypertrophy (cytomegaly) similar to findings with FM-3924. In studies conducted by Van Rafelghem et al, a single intraperitoneal injection of perfluoro-*n*-decanoic acid (PFDA) in several rodent

species resulted in persistent hepatocellular swelling which, ultrastructurally, was characterized by peroxisomal proliferation (12).

In subchronic studies sponsored by the 3M Company, Griffith and Long administered ammonium perfluorooctanoate to rats, mice and monkeys via oral routes (3). At doses of 30 ppm or greater, rats displayed hepatocellular hypertrophy. This change was more prevalent and severe in males. Noteworthy was the observation that all mice given doses of 1000 ppm *ad libidum* and all monkeys given 100 mg/kg/day (gavage) died preterminally. Additionally, all monkeys given 30 mg/kg/day displayed anorexia, emesis, black stools, facial pallor, and prostration; and one monkey given 10 mg/kg/day displayed anorexia, black stools and facial pallor. Liver effects, however, were not observed in monkeys assigned to these studies. Furthermore, microbial assays using five *Salmonella* stains and one *Saccharomyces* strain, with and without metabolic activation, did not reveal mutagenic activity for the test material.

2. Review of studies relating to the chronic toxicity of fluoride and fluoride-containing compounds was limited to the NTP 2-year carcinogenesis bioassay of sodium fluoride and select environmental studies of fluoride in cattle. In the NTP study, treatment-related pathologic changes were not observed in the liver of rats given dietary concentrations of up to 175 ppm sodium fluoride in the drinking water for two years (11). Furthermore, in an extensive survey of cattle exposed to high environmental fluoride concentrations for lifetime periods, increased liver disease or neoplasia was not reported although many animals exhibited dental and skeletal changes typical of advanced fluorosis (9).

3. Mechanisms resulting in increased hepatocellular nodular hyperplasia and neoplasia in rats given FM-3924:

Results of possible genotoxicity studies with FM-3924 were not provided to the review pathologist. It was reasoned that this complex fluorochemical probably was not strongly mutagenic and that most biologic effects were due to perturbation of liver cell metabolism, with peroxisomal proliferation and general disruption of multiple metabolic pathways. Accordingly, it is likely that the development of liver cell tumors (and nodular hyperplasia) was associated with epigenetic mechanisms, possibly including peroxisomal proliferation and genetic damage (ploidy) associated with oxidative stress and/or altered regulation of the cell cycle. Following publication of the final report for this study (1988), numerous journal articles have been published which identify carcinogenesis in rodents following exposure to non-genotoxic test materials. Subsets of these chemicals which induce liver cell tumors in rodents are characterized by antecedent hepatocytomegaly and peroxisomal proliferation (2,5,6,8).

4. Effects of ancillary biochemical interactions which may have served as tumor promoters:

Comments relative to all metabolic aberrations which may have influenced liver cell tumorigenesis in this study would be largely speculative. It should be noted, however, that hepatic toxicity was unequivocally linked with ingestion of FM-3924, and at high dose levels liver cell alterations had resulted in necrosis. Correspondingly, accelerated proliferation of hepatocytes would be expected to repair damaged tissue, and may have served as a tumor promoter (10). Although it should be emphasized that increased liver cell proliferation which may occur in rodents following exposure to many toxic materials does not invariably result in increased tumor formation, most pathologists agree that cell damage which promotes in increased mitotic activity may contribute to tumor formation (4,10,13).

5. Personal opinion relative to a relationship between proliferative lesions and FM-3924 observed in this study:

It is my opinion that distinct increases in hepatocellular neoplasms in high dose females, combined with increased hyperplastic nodules in both sexes are clear indicators that the test material should be regarded as a liver carcinogen in Sprague Dawley rats. Other proliferative lesions and neoplasms were considered to be spontaneous alterations or secondary to the systemic effects of altered hepatocellular metabolism (7). Based upon histomorphologic changes observed in this study, it is likely that epigenetic mechanisms (especially peroxisomal proliferation, oxidative stress and other factors which deregulate the cell cycle) were key factors in the development of hepatocellular proliferative lesions. Ancillary data which would support epigenetic pathways for tumorigenesis in this study would provide a rationale for selection of exposure thresholds in humans. Based upon reference literature available for the preparation of this review, liver damage, including carcinogenesis, has not been reported in humans exposed to test materials that promote hepatocytomegaly and neoplasia in rodents via peroxisomal proliferation and associated metabolic perturbations

**Additional Information which might Contribute to the Safety
Assessment of FM-3924**

1. Mutagenesis assays or ancillary procedures to establish the genotoxic potential of the test material.
2. Cell proliferation studies to provide an index treatment-related increases in the cell cycle.
3. Ultrastructural analyses or contemporary analytical procedures to confirm the possible peroxisomal proliferation and ancillary metabolic perturbations.
4. Recovery studies to establish the persistence of liver cell effects following subchronic exposures to FM-3924.

Conclusions: Based upon review of the information available in the study report, it is my opinion that dietary FM-3924 for 2 years resulted in chronic liver changes (megalocytosis) in males at all dose levels (10, 30 and 100 ppm) and for females at the high dose concentration (100 ppm). At the high dose level, hyperplastic nodules were increased in both sexes and hepatocellular tumors (adenomas and carcinomas) were increased in females. Incidence values for liver proliferative lesions indicated that FM-3924 should be regarded as a liver carcinogen for Sprague Dawley rats under the conditions of this study. The presence of persistent, dose-dependent liver cell cytomegaly suggested that epigenetic mechanisms were causative for tumorigenesis in this study, and that safe exposure thresholds may be established providing that toxicokinetic and ancillary data do not indicate additional adverse effects such as reduced excretion and bioaccumulation.



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Date

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