Tichler file 11/16/87

HASKELL LABORATORY FOR TOXICOLOGY
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October 29, 1987

AR226-1917

To: Charles Reinhardt, MD
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Two-Year Toxicity/Carcinogenicity Study of
Fluorochemical FC-143 in Rats

I have reviewed the pathology portions of the chronic rat study conducted by the 3M Company and have several comments regarding their findings.

I am in essential agreement with the interpretation of the non-neoplastic findings given in the report. Specifically, the increased incidence of hepatic "megalocytosis", vacuolation, "cystoid degeneration" and necrosis was clear evidence of hepatic metabolism and toxicity. The elevation of serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in male rats (30 and 300 ppm) correlated with these histological lesions. The absence of elevated hepatic enzymes in the females reflected the relatively less severe liver lesions in this sex.

There appears to have been some disagreement between the study pathologist (R. Geil) and the study director (L. Sibinski) with regards to the interpretation of some other non-neoplastic lesions. The pathology report described the slightly increased incidence of adrenal nodular hyperplasia (300 ppm males) and ovarian tubular hyperplasia (30 and 300 ppm females) as "secondarily compound related, probably through the FC-143 altered liver metabolism of endogenous steroids". While I tend to agree with this interpretation, the study director's characterization of these effects as "equivocal" was not unreasonable.

All of the hepatic neoplasms observed in this study were carcinomas and the incidence (males: 3,1,5; females: 0,0,1)* did not appear to be dose related. The incidence of nodular hyperplasia (males: 1,0,2; female: 0,0,3)* was slightly increased, though not statistically. As indicated in the report, this hyperplastic effect, if real, was a sequela of the liver degeneration rather than a pre-neoplastic change. The incidence of hepatocyte alterations (basophilic, eosinophilic and vacuolated) also did not suggest a pre-neoplastic effect.

* 0 ppm (Control), 30 ppm and 300 ppm, respectively

Although the study report concluded that "FC-143 is not considered to be carcinogenic in the rat" (page 25), it failed to adequately explain the following statements from the appended pathology report (page 123):

- "In female rats, there was a statistically significant increased incidence in mammary fibroadenomas at the 300 ppm level."
- "In male rats, there was a statistically significant, compound related occurrence of benign Leydig cell tumors of the testes at the 300 ppm level."

Although it was appropriate for historical incidence data to be applied to these tumors, the references cited in the study report were weak. In order to supplement the published literature, I have appended the control incidence data derived from Haskell Laboratory's most recent 13 chronic rat studies (Appendices A and B). This historical data includes 954 male and 947 female Crl:CD Sprague-Dawley rats which were at least 1 year of age but not terminally sacrificed until 2 years of age.

In the case of mammary tumors, it is more realistic to use the number of rats as the denominator, rather than the tissue number, since unexamined mammary gland usually indicates undeveloped or atrophied gland. The incidence of fibroadenomas (using 50 rats as the denominator) in the FC-143 study (20%, 38%, 42%)* certainly suggested a compound related effect. In its argument against such an effect, the study report states that "the incidence of fibroadenomas in the high-dose females ..... was similar to that reported for untreated aging rats (Prejean, et al. 1973)." The Prejean reference, however, reported an incidence of only 24% fibroadenomas! In any case, since the Prejean study included only 181 female rats, and they were terminally sacrificed at 18-months, it was an inappropriate historical reference. When the FC-143 mammary tumor data is compared to Haskell Laboratory's historical controls (Appendix C), there does not appear to be any compound related effect (42% vs. 37% [p=0.3]).

The increased incidence of Leydig cell adenomas (0%, 4%, 14%)* was also suggestive of a compound effect. The study report cited two references in its contention that 14% was in line with the normal background incidence of Leydig cell tumors in Sprague-Dawley rats. The first reference (Anver, et al.) was of little value considering the small number of rats (96), the multiple sacrifices, and the duration of the study (38 months of age). The use of the older rats (24-38 months of age) for comparison with the FC-143 study rats (13-25 months of age) is questionable, especially since no Leydig tumors were observed in the 12-23 month old rats (0/28). The second reference (Hazleton Laboratories) was stated as

* 0 ppm (Control), 30 ppm and 300 ppm, respectively

documentation of a Leydig cell tumor incidence of 28.7%. This latter figure is dramatically different from our own experience and warrants some skepticism. In fact, Sid Jones, chief of the Hazleton Pathology Department, says that their 1984 incidence data showed a Leydig cell tumor incidence of 10% (30/198), with a range of 2-24% in individual studies. Our own historical data (Appendix B), demonstrates an incidence of 6.1% (58/954) with a range of 1-12%. Comparing this to the FC-143 Leydig cell tumor incidence (Appendix C) shows a statistically significant increase ($p=0.04$) in the high-dose group (300 ppm). Combining the Haskell and Hazleton data (88/1152) gives an incidence of 7.6%, which is not statistically different from the FC-143 incidence of 14% ($p=0.09$).

In conclusion, it is my opinion that the FC-143 chronic rat study report failed to adequately address the possibility of either mammary gland or Leydig cell carcinogenesis. The pathology report did not include a conclusion where the pathologist clearly stated his interpretation of the biological significance of his findings. In addition, the author of the study text did not apply appropriate historical data to refute the implications of the pathology report. It should be mentioned that reliable historical data for chronic rodent studies has generally not been published and that the Haskell Laboratory data was not available to Riker Laboratory.

Based on our experience at Haskell Laboratory, there is no evidence that FC-143 resulted in an increase in any type of rat mammary tumor.

The effect of a chronic oral exposure to 300 ppm on the incidence of Leydig cell tumors is still unclear and might be appropriately termed equivocal. Compared to both the study controls and the Haskell historical control data, a 14% incidence is suspicious, particularly in light of the possible steroid-related non-neoplastic effects noted previously. Although I personally believe that the Leydig cell tumors are probably compound related, the evidence is not great enough to be definitive. I would appreciate the opportunity to review the testes on this study.

Jones, S (Hazleton Labs.). Telephone conversation with C. Keenan (Haskell Labs.). October 20, 1987.

Prejean JD, Peckham JC, Casey AE, Griswold DP, Weisburger EK and Weisburger JH. Spontaneous Tumors in Sprague-Dawley Rats and Swiss Mice. Cancer Research 33:2768-2773 (1973).

Anver MR, Cohen BJ, Lattuada CP and Foster SJ. Age-Associated Lesions in Barrier-Reared Male Sprague-Dawley Rats. Experimental Aging Research 8:3-24 (1982).

APPENDIX A
FEMALE RATS - MAMMARY GLAND TUMORS

HISTORICAL INCIDENCE DATA - HASKELL LABORATORY
Charles River Laboratories : Sprague-Dawley Rats (Crl:CDBR(SD) RATS)

STUDY (2-YEAR Crl:CDBR(SD) RAT) :	A	B	C	D	E	F	G	H	I	J	K	L	M	=	13
# CONTROL RATS (1-2 YRS ON TEST):	98	87	85	77	69	66	59	77	64	78	68	60	59	=	947
Mammary Gland Adenocarcinoma :	24	6	20	5	7	10	7	5	7	16	6	9	12	=	134
Malignant Mixed Mammary Tumor :	1	-	1	-	-	-	-	-	-	-	-	-	-	=	2
Squamous Cell Carcinoma :	-	-	-	-	-	-	-	-	-	-	-	-	-	=	-
Fibrosarcoma :	-	-	-	-	-	-	-	-	-	1	-	-	-	=	1
TOTAL MALGNANT TUMORS -->	25	6	21	5	7	10	7	5	7	17	6	9	12	=	137
Mammary Gland Adenoma :	-	2	1	2	-	6	1	2	-	-	2	2	-	=	18
Mammary Gland Fibroadenoma :	42	47	23	37	27	16	18	37	26	22	24	16	19	=	354
Mammary Gland Fibroma :	-	-	1	-	-	1	-	-	-	-	-	-	-	=	2
TOTAL BENIGN TUMORS -->	42	49	25	39	27	23	19	39	26	22	26	18	19	=	374
TOTAL MAMMARY GLAND TUMORS -->	67	55	46	44	34	33	26	44	33	39	32	27	31	=	511

SUMMARY:

	# of Tumors	% of 947 Control Female Rats
Mammary Gland Adenocarcinoma	134	14.1
TOTAL MALIGNANT TUMORS	137	14.5
Mammary Gland Fibroadenoma	354	37.4
TOTAL BENIGN TUMORS	374	39.5
TOTAL MAMMARY GLAND TUMORS	511	54

NOTES:

- Since a few animals were diagnosed as having more than one type of mammary tumor (eg. adenocarcinoma and fibroadenoma), the percent of female rats with mammary tumors is slightly less than 54 %.

APPENDIX B
MALE RATS - LEYDIG CELL TUMORS

HISTORICAL INCIDENCE DATA - HASKELL LABORATORY
Charles River Laboratories : Sprague-Dawley Rats (Crl:CDBR(SD) RATS)

STUDY (2-YEAR Crl:CDBR(SD) RAT) :	A	B	C	D	E	F	G	H	I	J	K	L	M	=	
# CONTROL RATS (1-2 YRS ON TEST):	98	89	89	79	69	66	54	79	68	75	65	62	61	=	954
Leydig Cell Adenomas :	12	2	1	6	5	3	2	6	8	9	2	2	-	=	58
Leydig Cell Adenocarcinomas :	-	-	-	-	-	-	-	-	-	-	-	-	-	=	0
TOTAL LEYDIG CELL TUMORS :	12	2	1	6	5	3	2	6	8	9	2	2	-	=	58

SUMMARY:

	# of Tumors	% of 954 Control Male Rats
Leydig Cell Adenocarcinoma	0	0
TOTAL MALIGNANT TUMORS	0	0
Leydig Cell Adenoma	58	6.1
TOTAL BENIGN TUMORS	58	6.1
TOTAL LEYDIG CELL TUMORS	58	6.1

APPENDIX C

COMPARISON OF THE HASKELL HISTORICAL DATA (Crl:CDBR(SD) RATS) WITH THE FC-143 CHRONIC RAT STUDY DATA

I. LEYDIG CELL TUMORS

SUMMARY:	HASKELL HISTORICAL DATA		FC-143: 300 ppm Group		FISHER EXACT TEST (FC-143, 300 ppm vs Haskell Hist. Data) p value =
	# of Tumors	% of 954 Control Male Rats	# of Tumors	% of 50 Test Male Rats	
Leydig Cell Adenocarcinoma	0	0	0	0	-
Leydig Cell Adenoma	58	6.1	7	14.0	0.037
TOTAL LEYDIG CELL TUMORS	58	6.1	7	14.0	0.037

II. MAMMARY GLAND TUMORS

SUMMARY:	HASKELL HISTORICAL DATA		FC-143: 300 ppm Group		FISHER EXACT TEST (FC-143, 300 ppm vs Haskell Hist. Data) p value =
	# of Tumors	% of 947 Control Female Rats	# of Tumors	% of 50 Test Female Rats	
Mammary Gland Adenocarcinoma	134	14.1	5	10	0.279
TOTAL MALIGNANT TUMORS	137	14.5	5	10	0.259
Mammary Gland Fibroadenoma	354	37.4	21	42	0.303
TOTAL BENIGN TUMORS	374	39.5	21	42	0.415
TOTAL MAMMARY GLAND TUMORS	511	54	26	52	0.449