



Memorandum

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Date October 1, 2002

From Division of Food Contact Substance Notification Review (DFCSNR)

Subject Worst-case estimate of the unit cancer risk for ammonium perfluorooctanoic acid (APFO)

To Regulatory Group 1-DFCSNR  
Attn.: Vivian Gilliam

Through David G. Hattan, Ph.D. David G. Hattan  
Toxicology Review and CAC/QRAC Coordinator

**FOOD CONTACT NOTIFICATION No. 260**

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**RELATED PETITIONS/NOTIFICATIONS**

FMF 000336 Ammonium perfluoroalkylcarboxylate (Fluorochemical FC-143)

**INTRODUCTION**

This memorandum calculates the worst-case unit cancer risk for ammonium perfluorooctanoic acid (APFO, CAS No 3825-26-1), (b)(4) using the most potent estimate derived from the review of two bioassays on APFO. The first assay, "Two year oral (diet) toxicity/carcinogenicity study of fluorochemical FC-143 in rats" was previously submitted in FMF 000336 as well as FCN 260. The "incomplete" review of this study by PML Siu, Ph.D. is located in FMF 000336 on pages 001237 - 001253 (Siu/Biddle, 03/18/1988, RE FMF 336). No conclusions were contained in the review, which was awaiting additional data prior to submission to the Cancer Assessment Committee (CAC). Interpretations of the results of this study have been aided by P. Dua, D.V.M., Ph.D (Attachment: Dua/Twaroski, 08/29/2002, RE Fluorochemical FC-143 Study in Rats - Comments).

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## MUTAGENICITY

APFO was negative for mutagenic activity in an Ames assay using *Salmonella typhimurium* and *Saccharomyces cerevisiae*<sup>1</sup>, in an *in vitro* chromosome aberration assay using Chinese hamster ovary (CHO) cells<sup>2</sup>, and in an *in vivo* micronucleus assay in mice<sup>3</sup>. Dyneon LLC also submitted additional mutagenicity studies on a similar compound, sodium perfluorooctanoate (SPFO, CAS No. 335-95-5). SPFO was negative for mutagenic activity in an Ames assay using *S. typhimurium* and *Escherichia coli*<sup>4</sup>, a chromosome aberration assay using human peripheral lymphocytes<sup>5</sup>, and in a mouse bone marrow micronucleus assay<sup>6</sup>. SPFO induced chromosome aberrations in CHO cells treated for short durations with  $\geq 3730 \mu\text{g/ml}$  with and without metabolic activation (S9)<sup>7</sup>.

## CARCINOGENICITY

Two rat oral bioassays on APFO were submitted in FCN 260:

- "Two year oral (diet) toxicity/carcinogenicity study of fluorochemical FC-143 in rats", Riker Laboratories, Inc. St. Paul, Minnesota, Study No. 0281CR0012, August 1987<sup>8</sup>
- Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC. (2001) Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol Sci.* 60(1):44-55.<sup>9</sup>

### Riker Study

In the Riker study, Sprague-Dawley rats [CrI:COBSr CD(SD)BR] (50/sex/treatment) were administered FC-143 (APFO) in the diet at doses of 0, 30 and 300 ppm for 2 years. Male rats treated with 300 ppm had an increase in food consumption with a concomitant decrease in body weight, indicating that the maximum tolerated dose (MTD) was likely reached. Although females treated with 300 ppm demonstrated decreased body weights, the body weights were inconsistent and they also ate less than control animals. Mortality was slightly or significantly decreased in 300 ppm treated females or males, respectively. The study authors concluded that the compound was "not considered to be carcinogenic in the rat". The significant neoplastic findings are tabulated below.

Table 1a. Riker Study: Summary of Neoplasia Incidence - Males

Lesion	CONTROL		30 PPM		300 PPM	
	Incidence	%	Incidence	%	Incidence	%
Leydig cell adenoma	0/49	0	2/50	4	7/50	14*
Hepatocarcinoma/hyperplastic nodule <sup>1</sup> combined	3/49	6%	1/50	2%	8/50	16% <sup>a</sup>

\*Statistically different from controls,  $p \leq 0.05$

<sup>1</sup>The study authors did not address the occurrence of hepatocellular adenomas. At the time the study was conducted (1980s) hepatocellular adenoma was not a common term, but hyperplastic nodule was. Hyperplastic nodules are usually classified as either foci of cellular alteration or hepatocellular adenomas. Accordingly, they have been combined to address the neoplastic findings of the liver (Dua/Twaroski)

<sup>a</sup>Significant based on "histopathological findings in the liver, it can be stated that there is an increase in proliferative hepatocellular lesions in the high dose males compared to the control group suggesting a treatment related effect. The increased incidence of non-neoplastic findings in the liver is further evidence that this is a target organ" (Dua/Twaroski).

<sup>1</sup> Griffieth, F.D. and Long, J.E. (1980) Animal toxicity studies with ammonium perfluorooctanoate, *Am Ind. Hyg Assoc J* 41, 576-583 (submitted in FCN 260)

<sup>2</sup> Corning Hazelton Inc. study number 17388-0-437, reviewed by ICF for the FDA under contract number 223-00-2450, work assignment number 2002-29 (study and review included in FCN 260).

<sup>3</sup> Corning Hazelton Inc. study number 17388-0-455, reviewed by ICF for the FDA under contract number 223-00-2450, work assignment number 2002-29 (study and review included in FCN 260)

<sup>4</sup> Corning Hazelton Inc. study number 17073-0-409, reviewed by ICF for the FDA under contract number 223-00-2450, work assignment number 2002-29 (study and review included in FCN 260)

<sup>5</sup> Corning Hazelton Inc. study number 17073-0-449CO, reviewed by ICF for the FDA under contract number 223-00-2450, work assignment number 2002-29 (study and review included in FCN 260)

<sup>6</sup> Corning Hazelton Inc. study number 17073-0-455, reviewed by ICF for the FDA under contract number 223-00-2450, work assignment number 2002-29 (study and review included in FCN 260)

<sup>7</sup> Corning Hazelton Inc. study number 17073-0-437CO, reviewed by ICF for the FDA under contract number 223-00-2450, work assignment number 2002-29 (study and review included in FCN 260).

<sup>8</sup> Reviewed by ICF for the FDA under contract number 223-00-2450, work assignment number 2002-29 (study and review included in FCN 260)

<sup>9</sup> Submitted in FCN 260

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Table 1b: Riker Study: Summary of Neoplasia Incidence - Females

Mammary Gland Lesion	CONTROL		30 PPM		300 PPM	
	Incidence	%	Incidence	%	Incidence	%
Adenocarcinoma	7/46	15	14/45	31	5/44	11
Fibroadenoma	10/46	22	19/45	42	21/44	48*
Carcinoma	1/46	2	0/45	0	0/44	0
Adenoma	3/46	6	0/45	0	0/45	0
Fibroadenomas and adenomas	13/46	28	19/45	42	21/44	48
Fibroadenomas and adenocarcinomas <sup>1</sup>	16/46	35	29/45	64	22/44	50

\*Statistically different from controls,  $p \leq 0.05$

<sup>1</sup>"It is appropriate to evaluate the combined incidence of benign and malignant tumors" (Dua/Twaroski)

The study authors indicated that there was a statistically significant increase ( $p \leq 0.05$ ) in mammary gland fibroadenomas. However, analysis of the data in combination with other mammary tumor findings revealed that the combined incidence of benign and malignant tumors "does not indicate a dose relationship of mammary tumors to treatment" (Dua/Twaroski). These data, combined with the lack of lobular hyperplastic lesion findings and considering the background occurrence of mammary tumors in SD rats, lead to the conclusion that "mammary tumors in this study does not appear to be treatment related" (Dua/Twaroski). Accordingly, although the mammary fibroadenoma lesion finding was statistically significant, this data will not be used to calculate the unit cancer risk for this study.

In the absence of scientific data that suggests a more appropriate approach, the following assumptions have been made in order to calculate a unit cancer risk (UCR) for APFO based on the Riker study in rats: 1) the UCR is defined as the slope of the dose-response curve drawn from the lowest apparent effect dose of APFO to zero, 2) that tumors arising at multiple sites or from different tissues at the same site are independent of each other and are additive in calculating the UCR; 3) the lowest dose at which the incidence of neoplastic effects was significant is used to calculate the UCR, and 4) the 300 ppm APFO delivered in feed equates to a dose of 14.2 mg/kg/day and 16.1 mg/kg/day for males and females, respectively.

$$\begin{aligned} \text{Unit Cancer Risk}_{\text{Males}} &= ((7/50 - 0/49)/14.2) + ((8/50 - 3/49)/14.2) \\ &= 0.017 \text{ (mg/kg bw/day)}^{-1} \end{aligned}$$

Biegel, LB, et al

In the Biegel, LB, et al. study, male CrI:CD@BR (CD) rats were feed *ab libitum*, pair fed, or fed 300 ppm C8 (APFO, rats were also fed 50 ppm Wy-14,643 as a positive control for peroxisome proliferation). Of the 156 rats/group, various numbers of animals were sacrificed at time intervals to determine hormonal measurements, cell proliferation, and for the evaluation of peroxisome proliferation. Animals were treated for 24-months, with those surviving to the end of the study (76-80/group) being euthanized and examined pathologically. The overall mean daily intake value for APFO was determined to be 13.6 mg/kg/day. The authors concluded that APFO, a peroxisome proliferator, induces a "tumor triad" increasing the incidence of tumors in the liver, Leydig cells, and pancreatic acinar cells. The tumor incidence data (Table 2 of the manuscript) and the calculated unit cancer risk derived from this data are detailed below.

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Table 2: Biegel, LB, *et al*: Summary of Neoplasia Incidence

Lesion	CONTROL		PAIR FED CONTROL		APFO	
	Incidence	%	Incidence	%	Incidence	%
Liver-Adenoma/carcinoma combined	2/80	3	3/79	4	10/76	13*
Testes - Leydig cell adenoma	0/80	0	2/78	3	8/76	11*
Pancreas –acinar cell adenoma/carcinoma combined	0/80	0	1/79	1	8/76	11*

\*Significantly different from the pair fed control group, p < 0.05.

In the absence of scientific data that suggests a more appropriate approach, the following assumptions have been made in order to calculate a unit cancer risk for APFO based on the Biegel, LB, *et al* study in rats: 1) the UCR is defined as the slope of the dose-response curve drawn from the lowest apparent effect dose of APFO to zero; 2) that tumors arising at multiple sites or from different tissues at the same site are independent of each other and are additive in calculating the UCR; 3) the lowest dose at which the incidence of neoplastic effects was significant is used to calculate the UCR; and 4) the 300 ppm APFO delivered in feed equates to a dose of 13.6 mg/kg/day.

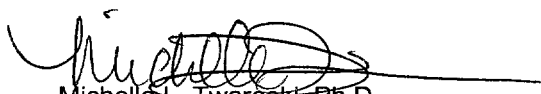
$$\begin{aligned} \text{Unit cancer risk} &= ((10/76-3/79)/13.6) + ((8/76-2/78)/13.6) + ((8/76-1/79)/13.6) \\ &= 0.0069 + 0.0058 + 0.0068 \\ &= 0.020 \text{ (mg/kg bw/day)}^{-1} \end{aligned}$$

For this risk assessment, the test substance (APFO) is assumed to be a carcinogen and the sex, species and study that results in the highest unit cancer risk for the test substance is used in future risk assessments for that chemical. Two bioassays have been reviewed and both show potentially positive tumor responses in the rat to APFO and are of suitable quality to use in a quantitative risk assessment. The unit cancer risk derived from the Riker data in male rats is 0.017 (mg/kg bw/day)<sup>-1</sup>. The unit cancer risk derived from male rats in the Biegel, LB, *et al* is 0.020 (mg/kg bw/day)<sup>-1</sup>. Therefore, the worst-case unit cancer risk for APFO is 0.020 (mg/kg bw/day)<sup>-1</sup>.

## CONCLUSION

This memorandum summarizes the neoplastic findings from two rat bioassays on APFO, (b)(4) being notified for in FCN 260, and the calculated unit cancer risks derived from these studies. The unit cancer risk derived from this analysis is based upon the conservative but unproven assumption that APFO is a carcinogen and that data derived from the rodent studies on APFO summarized herein can be used to estimate human cancer risk from exposure to APFO. This estimation of the unit cancer risk associated with APFO does not constitute a Center or Agency decision that the chemical is a carcinogen and data contained herein (with the exception of mutagenicity data) should be used for the sole purpose of estimating risk and not as supporting data for the development of policy or modeling of carcinogenic chemicals.

We ask your concurrence with the method used to calculate the unit cancer risk for APFO and the resulting conclusions

  
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HFS-275

Attachment: Memorandum Dua/Twaroski, 08/29/2002, RE: Fluorochemical FC-143 Study in Rats – Comments