SUMMARY OF THE EFFECTS OF PFC's ON MITOCHONDRIAL BIOENERGETICS *IN VITRO*

The attached diagram describes the metabolic relationship for the various PFC's that were selected for inclusion in this study. The metabolic chart is adapted from the original as provided by Dr. Steve Gordon. Included in the diagram is a summary description of the biological activity of each metabolite, the details of which are described in the appended report and supplement. The identities of all test compounds were withheld from the person who performed the bioenergetic analyses (Dr. Anatoli Starkov) and the following legend reveals the code to the test compounds as referred to in the research report.

Code	3M identification
PF10	FC10
PF10H	the acetic acid of FC10
PF12L	FX12-linear
PF12M	FX12-mixed linear and branched
PF95	FC95
PF95M	the sulfonamide of FC95 (N-de-ethylated FX12)
PF143	FC143
SAL	salicylic acid included as a positive control

To briefly summarize, we found that FC95 and FC143 were weak inhibitors of mitochondrial bioenergetics *in vitro*. They would have to bioaccumulate to substantial levels to pose a significant concern *in vivo*. On the other hand, FX12 (both linear and mixed) and FC10 and its carboxylic acid metabolite (PF10H) were far more potent. The IC50 for the protonophoric uncoupling of mitochondrial respiration by FX12 (ca., 6 μM) compares to that of the classic uncoupler 2,4-dinitrophenol (DNP). Whether safety concerns are warranted depends on if these metabolites are stable and if they bioaccumulate to sufficient concentrations during the course of chronic exposures.

Our greatest concern is for the sulfonamide (PF95M), which is 5-fold more potent than DNP as an uncoupler of mitochondrial oxidative phosphorylation. The IC50 for the sulfonamide is approximately 1.5 μM, which compares with that of the most potent mitochondrial uncouplers known. It is highly reasonable to suspect that exposures to the sulfonamide, or its generation *in vivo* from an N-substituted precursor metabolite, may be associated with demonstrable symptoms attributable to the uncoupling of mitochondrial respiration. This concern is compounded by the rapid N-de-ethylation of FX12 *in vivo* and bioconcentration of the sulfonamide in liver[12]. Since this metabolite has been detected in sera from both rats and humans[3], an aggressive kinetic characterization seems warranted. We suggest that future research focus on the sulfonamide.

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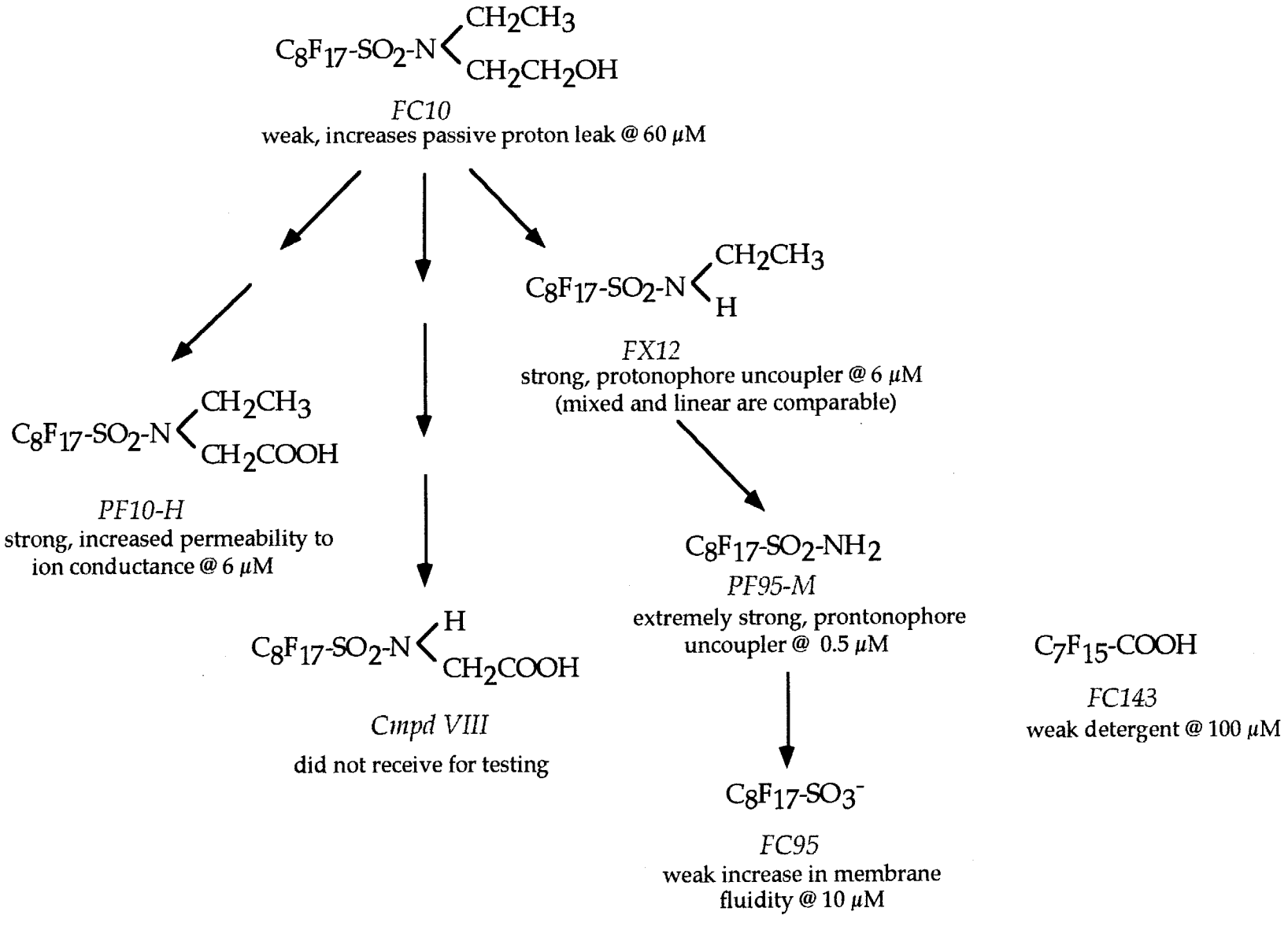
[1]Johnson, J.D. and Ober, R.D. (1979) Study No. 137-093, International Research and Development Corporation, Mattawan, MI

[2]Manning, R.O., Bruckner, J.V., Mispagel, M.E., and Bowen, J.M. (1990) Drug Metab. Dispos. 19: 205-211.

[3]Anon (1979). Tech. Report No., 723Q, 3M Central Analytical Laboratory, St. Paul, MN.


C_8F_{17}-SO_2-N(CH_2CH_3, CH_2CH_2OH)
FC10
weak, increases passive proton leak @ 60 μM
C_8F_{17}-SO_2-N(CH_2CH_3, H)
FX12
strong, protonophore uncoupler @ 6 μM
(mixed and linear are comparable)
C_8F_{17}-SO_2-N(CH_2CH_3, CH_2COOH)
PF10-H
strong, increased permeability to
ion conductance @ 6 μM
C_8F_{17}-SO_2-N(H, CH_2COOH)
Cmpd VIII
did not receive for testing
C_8F_{17}-SO_2-NH_2
PF95-M
extremely strong, prontonophore
uncoupler @ 0.5 μM
C_8F_{17}-SO_3^-
FC95
weak increase in membrane
fluidity @ 10 μM
C_7F_{15}-COOH
FC143
weak detergent @ 100 μM