Larry R. Zobel, MD, MPH
Staff Vice President
and Medical Director

3M Medical Department

3M Center, Building 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 5181 Office
651 733 5152 Facsimile

3M

April 20, 2000

FEDERAL EXPRESS 929 6711 024
CUSTOMER PACKAGE TRACKING NUMBER — PULL UP PURPLE TAB

Dr. Oscar Hernandez
US EPA/OPPT/CCD (7405)
401 M Street, SW
Room E615B
Washington, DC 20460

Dear Dr. Hernandez:

I am writing to clarify information contained in the two-generation reproduction study on potassium perfluorooctanesulfonate. A report of this study was submitted on February 15, 2000 as a supplement to the TSCA Section 8(e) Notice of 9/14/98. That notice had been filed midway through the study when we became aware of F1 pup mortality at the highest two doses in the study (1.6 and 3.2 mg/kg/d). The study was conducted with perfluorooctanesulfonate given by oral gavage at four doses, 0.1, 0.4, 1.6 and 3.2 mg/kg/d. Following the pup mortality seen in the 1.6 and 3.2 mg/kg/day dose groups, only the 0.1 and 0.4 mg/kg/day doses groups were continued into the second generation.

Language in the report indicated that F2 pup viability was affected in the 0.4 mg/kg/day dose group. This is not the case. Attached is an amended report from the laboratory performing the study, Primedica, Argus Division. The amended language clearly states that there were no toxicologically important effects on pup survival or growth at the highest dosage tested 0.4 mg/kg/day. I have attached table E20 for your reference. It shows no significant differences between the vehicle control group and the 0.4 mg/kg/day dose group for number of stillborn (0.3+/- 0.6 and 0.5 +/- 0.9), pup viability (97.1 % and 96.2%) or number of pups surviving per litter, preculling, on day 4 (13.7 +/- 3.3 and 13.8 +/- 2.4).

Dr. Marv Case, Director of Toxicology at 3M and Dr. Mildred Christian and Dr. Raymond York of Primedica Argus prepared a paper for publication that reflects this interpretation. This paper is still under internal review. Dr. John (Jack) Moore, formerly of EPA and now a consultant in Toxicology, has also reviewed the full report and agrees with this interpretation.

I request that you treat the attached report as superceding the earlier report. You will note that the study report submitted as follow up to the 8e had only summary data tables. The enclosed report includes all of the data tables, individual as well as summary.

Exhibit
1667
State of Minnesota v. 3M Co.,
Court File No. 27-CV-10-28862

Also enclosed is an analytic report that gives the perfluorooctanesulfonate concentrations in liver and serum samples collected from selected animals in the two-generation reproduction study. The serum and liver analytical data included in the report offer a limited understanding, because of the timing and limited number of samples, of the pharmacokinetics of perfluorooctanesulfonate.

Two additional studies have been undertaken to develop an understanding of the disposition of perfluorooctanesulfonate during gestation and lactation. In the first of these studies, potassium perfluorooctanesulfonate was administered by gavage up to the point of mating, and disposition of the body burden in dams to pups was followed during gestation. In the second study, dosing continued through gestation and lactation, and disposition was followed through lactation. The in-life aspects of these studies are complete, and reports are enclosed. Protocols for the analytical work associated with these two studies is enclosed. Final analytical reports should be available within three months.

A complete cross fostering study was also done. This study was done with only two groups, a control and a group dosed at 1.6 mg/kg/day. This dose level had minimal effect on the dams but produced neonatal mortality in the original two generation study. The cross-fostering study shows that neonatal pup mortality is related to exposure of pups *in utero* during gestation, although there appears to be a contribution to the mortality from the treated dams if pups were also exposed *in utero*. A copy of the final report is enclosed.

Two ancillary reports are associated with the cross-fostering study. In the first, serum perfluorooctanesulfonate levels in dams and pups were monitored at lactation days 14 and 21 or 22. This report demonstrates that pups are exposed *in utero* and can be exposed via milk. Litters with exposure *in utero* and via milk from treated foster-dams have serum values approximating foster-dam serum values on days 21 or 22 of lactation. Litters which were born of untreated dams but cross-fostered to treated dams have serum concentrations roughly one-third of foster-dam values at the end of lactation. Litters born of treated dams but cross-fostered to untreated dams have serum values of approximately half their birth dams at the end of lactation. The untreated foster dams are found to acquire single-digit ppm levels of perfluorooctanesulfonate in their serum, presumably from exposure to pup urine and feces.

The second ancillary report to the cross-fostering study relates to electron microscopy conducted on lung and liver tissue of pups culled shortly after birth. Lung tissue was examined for possible effects of perfluorooctanesulfonate on pulmonary surfactant production. There was no difference between control and exposed pups with regard to lung architecture and the presence of lamellar bodies in Type II pneumocytes. Surfactant production appeared normal. Since perfluorooctanesulfonate causes peroxisome proliferation in rodents, liver tissue was examined for the presence of peroxisomes. Pups exposed *in utero* were found to have a two to three fold increase in peroxisomes. Copies of both reports are enclosed.

An additional follow-up study is being planned to explore the possible role of decreases in cholesterol at birth as a mechanism for reduced pup viability. It has been shown (Levin et. al., 1989) that rat pups undergo a burst of steroid synthesis activity prior to birth followed by a period of much reduced synthesis following birth (perhaps due to the presence of exogenous cholesterol during lactation). HMG CoA reductase and HMG CoA synthase mRNAs are highest during late fetal life and undergo a precipitous reduction immediately after parturition. Harb et. al. showed that fluvastatin, an HMG CoA reductase inhibitor marketed as Lescol for hypercholesterolemia, if administered prior to and during gestation, causes neonatal mortality. The mortality was prevented by co-administration of mevalonate, the product of the reaction catalyzed by HMG CoA reductase. Perfluorooctanesulfonate reduces serum cholesterol in laboratory animals and is reported to reduce activity of HMG CoA reductase (Haughom and Spydevold, 1992). Our planned study would test the hypothesis that the neonatal mortality observed in the high dose groups of the two-generation perfluorooctanesulfonate study is due to the reduction in cholesterol synthesis due to reduced HMG CoA reductase activity. This would be done by attempting mevalonate rescue, similar to the fluvastatin study. We will also attempt rescue with cholesterol.

Two additional objectives are being designed into the planned study. The first would be to more precisely define a NOEL and LOEL for pup mortality by testing doses between 0.4 and 1.6 mg/kg/day. The second is to obtain additional pharmacokinetic data.

Human infants do not undergo a similar precipitous drop in cholesterol synthesis at birth (Levin et. al., 1989). Establishing reduced cholesterol synthesis as a mechanism of action would have significant implications when assessing risk associated with the findings in the two-generation study. The planned study is currently under negotiation with a contract laboratory. We will share the protocol and expected completion date with you when they are available.

We are looking forward to discussions between our scientists and agency scientists about the information we have submitted and continue to submit on perfluorooctanesulfonate.

Sincerely,

LRZobel MD

Larry R. Zobel, MD MPH
Staff Vice President & Medical Director

c: Dr. Charles Auer, Director Chemical Control Division (no attachments)

References:

Harb, R.V., Hartman, H.A., and Cox, R.H. (1994), Prevention of fluvastatin induced toxicity, mortality, and cardiac myopathy in pregnant rats by mevalonic acid supplementation. *Teratology,* **50,** 19-26

Haughom, B. and Spydevold, O. (1992), The mechanism underlying the hyperlipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOS) and clofibric acid. *Biochimica et Biophysica Acta,* **1128**, 65-72.

Levin, M.S., Pitt, A.J.A., Schwartz, A.L., Edwards, P.A. and Gordon, J.I. (1989), Developmental changes in the expression of genes involved in cholesterol biosynthesis and lipid transport in human and rat fetal and neonatal livers. *Biochimica et Biophysica Acta,* **1003**, 293-300.

Attachments

Protocol 418-008 Combined oral (gavage) fertility, developmental and perinatal/postnatal reproduction toxicity study of PFOS in rats (Sponsor's study number: 6295.9), Table E20, 1-3.

Harb, R.V., Hartman, H.A., and Cox, R.H. (1994), Prevention of fluvastatin induced toxicity, mortality, and cardiac myopathy in pregnant rats by mevalonic acid supplementation. *Teratology*, **50**, 19-26

Haughom, B. and Spydevold, O. (1992), The mechanism underlying the hyperlipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOS) and clofibric acid. *Biochimica et Biophysica Acta*, **1128**, 65-72.

Levin, M.S., Pitt, A.J.A., Schwartz, A.L., Edwards, P.A. and Gordon, J.I. (1989), Developmental changes in the expression of genes involved in cholesterol biosynthesis and lipid transport in human and rat fetal and neonatal livers. *Biochimica et Biophysica Acta*, **1003**, 293-300.

Final Report Protocol 418-013 Oral (Gavage) Pharmacokinetic Study of PFOS in Rats (Sponsor's study number: T-6295.12), June 24, 1999

Protocol FACT-TOX-110 Oral (Gavage) Pharmacokinetic Study of PFOS in Rats, Protocol, June 8, 1999

Final Report Protocol 418-015 Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats (Sponsor's study number: T-6295.14), July 23, 1999

Protocol FACT-TOX-111 Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats, Protocol, June 8, 1999

Summary PFOS Rat Two-Generation Reproduction Study (Study numbers: 3M T-6295.9, Argus 418-008 [in-life], FACT-TOX-012 [analytical])

Report Amendment I, 13 April 2000, Protocol 418-008 (Sponsor's study number: 6295.9) Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats, Final Report

Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number: 2759-39-3) in the Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage, Laboratory Report No. <U2006>, Requestor Project No. <3M TOX 6295.9>, October 27, 1999

Interoffice Memo from Tom Kestner to Leo Gehlhoff, "Fluorochemical Isomer Distribution by ^{19}F-NMR Spectroscopy, December 1, 1997

Final Report Protocol 418-008 Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats (Sponsor's study number 6295.9, June 10, 1999

Summary PFOS Rat Cross-Fostering (Study numbers: 3M T-6295.13, Argus 418-014)

Final Report Protocol 418-014, Oral (Gavage) Cross-Fostering Study of PFOS in Rats (Sponsor's study number: T-6295.13), July 23, 1999

Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) (CAS Number: 2759-39-3) in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage, Laboratory Report No. U2779, Requestor Project No. 3M Tox 6295.13, June 10, 1999