**Corporate Health Physics**
**Corporate Occupational Medicine**
**Corporate Product Responsibility**
**Corporate Toxicology**
**3M Medical Department**

3M Center, 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 1110

**3M**

## Serum Fluorochemical trends of out-of-county residents in CLUE I (1974) and CLUE II (1989) epidemiologic investigations.

This project is a joint collaboration between 3M and Johns Hopkins University School of Public Health researchers. The objective of this study is to analyze 358 adult sera samples that were collected in 1974 and 1989 as part of the CLUE I and CLUE II studies conducted in Washington County, Maryland. The sera samples represent out-of-county residents who participated in the study. A total of 59 samples are from individuals who donated in both 1974 and 1989. An additional total of 120 samples will be analyzed in each year of different individuals. Fluorochemicals to be measured include perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexanesulfonate, N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA), N-methyl perfluorooctanesulfonamidoacetate (M570), perfluorooctanesulfonamidoacetate (M556), and perfluorooctanesulfonamide (PFOSA). The timeline for study completion, estimated at January 1, 2001, is entirely dependent upon a validated analytical method to analyze 1 ml of human serum for these fluorochemicals with a lower limit of quantitation of $\leq$ 10 ppb, based on high performance liquid chromatography electrospray tandem mass spectrometry methods.

## TITLE OF RESEARCH PROPOSAL

Serum fluorochemical trends of out-of-county residents in CLUE I (1974) and CLUE II (1989) epidemiologic investigations

1. **RESEARCH QUESTION:**

Are fluorochemicals detectable in the serum samples of individuals who donated blood to a community-based specimen bank in 1974 and 1989 and to what extent do levels change over time?

2. **RATIONALE/BACKGROUND:**

The 3M Company (sponsor of this proposal) manufactures widely-used products that contain fluorochemicals. Fluorochemicals transform metabolically to perfluorooctane sulfonate (PFOS) as an endstage metabolite. PFOS is a surfactant and used in industrial and commercial processes. PFOS concentrates in the liver and undergoes enterohepatic circulation. Subchronic studies in rats and primates suggest a cumulative toxicity resulting in lowered serum total cholesterol levels and metabolic wasting. These effects may be due to effects on fatty acid transport, metabolism, membrane functions, and peroxisome proliferation.

Exposure rates to these chemicals among the general population are unknown. Occupationally exposed individuals have been monitored for the presence of fluorochemicals (Olsen et al). Among male production employees the mean levels of perfluorooctane sulfonate (PFOS) in 1995 and 1997 were 2.19 ppm and 1.75 ppm, respectively. No substantial changes in serum hepatic enzymes, cholesterol, or lipoprotein levels were noted among individuals with levels less than 6 ppm. Only 7 individuals in 1995 and 5 in 1997 had levels in excess of 6 ppm; no marked changes in lipid or hepatic enzymes were noted. It is unknown to what extent these levels can be detected in the general population. This proposal is an exploratory study to determine for the first time whether serum concentrations of fluorochemicals among a non-occupationally exposed population increase over time and whether concentrations vary by gender or age.

3. **METHODS**

From August through November, 1974, the Campaign against Cancer and Stroke was conducted in Washington County, MD. Referred to as CLUE I (from the slogan, "Give us a Clue to Cancer"), a total of 25,802 persons donated blood, of whom 20,305 were county residents. The brief history form was completed at the time of blood collection (Appendix 1). The Campaign against Cancer and Heart Disease (CLUE II) was similar to CLUE I in most respects. It was conducted from May through October, 1989. Brief histories (Appendix 1) and blood pressures were taken, and 20 ml of blood were drawn. Participants were given a food frequency questionnaire (Appendix 1) to complete at home and were asked to return it with a toenail clipping for trace metal assays. A total of 32,898 persons participated (table 1).

For this project we will use specimens from participants who are not Washington County residents and who, therefore, are not in the analytic cohort. These individuals are referred to as the "out-of-county" group. A total of 358 samples will be selected for assays (see table 1). Fifty-nine individuals in the "out-of-county" group gave blood samples in 1974 and 1989. The age distribution of these 59 individuals in 1974 was as follows: age 25-44 years - 28; age 45-64 years - 20; age 65+ years - 11. We will analyze all of this group for serum fluorochemical levels to determine change in concentrations overtime within individuals. An additional sample of 120 individuals from CLUE I and CLUE II will be selected stratified by gender and age.

**Table 1. Demographic Distribution of Total Number (N = 358) of Serum Samples to be Analyzed**

| | Number of Subjects | |
|---|---|---|
| | 1974 | 1989 |
| **Same individuals both years (n = 59/yr)** | | |
| Females* | 32 | 32 |
| Males* | 27 | 27 |
| **Different individuals each year (1974, 1989)** | | |
| Females | | |
| <40 yrs | 20 | 20 |
| 40-60 yrs | 20 | 20 |
| >60 yrs | 20 | 20 |
| Males | | |
| <40 yrs | 20 | 20 |
| 40-60 yrs | 20 | 20 |
| >60 yrs | 20 | 20 |

*Age in 1989 is provided below (1974 data and distribution by age by gender was not known at the time this proposal was written)

Assuming the correlation between the two measurements obtained in 1974 and 1989 is 0.8, the sample size of 59 provides a statistical power of 85% to detect a 10% difference between the two time periods.

Assuming the mean PFOS levels in the age group <40, 40-60 and >60 are 25, 30, and 36 ppb, respectively, and the standard deviation is 12 ppb for each group, we estimate 120 samples (40 in each age group) will provide a statistical power of more than 95% to detect a 20% difference among age groups. This sample size will also allow for a statistical power of 78% to detect a 20% difference between two groups (eg., men and women).

**Assay Measurements**

Northwest Bioanalytical will analyze for up to 8 serum organofluorochemicals (including PFOS) using high pressure liquid chromatography//electrospray tandem mass spectrometry (HPLC/ESMSMS) and evaluate versus an extracted curve. Because general population serum samples range in the area of the lower limit of quantitation (LLOQ) for PFOS that is currently

used by Northwest Bioanalytical, samples from this study will not be analyzed until the LLOQ for PFOS (31 ppb) at Northwest Bioanalytical can be reduced by approximately 1 order of magnitude. This validation effort is currently underway.

4. **RISKS/BENEFITS**

For the assessment of the proposed biomarkers, data and blood were collected previously in 1974 and/or in 1989 when the subjects volunteered to participate in a campaign to collect blood for a serum bank and signed an informed consent. We cannot conceive of any potential risk at this time except for possible breaches of confidentiality. The proposed substance to be measured have not been associated with adverse health outcomes in humans. This study is the first step to determine if exposure (as measured by internal dose) has even occurred among non-occupationally exposed individuals. No information will be given to individuals. Sera will be assayed and analyzed without individual identifiers. Statistical data will be presented by groups, without individual identifiers. At this point, there is no individual benefit and virtually no individual risk.

Protection against unauthorized access will be obtained by keeping the forms and computer files in rooms that are locked when not in use, and in a wing of the Health Department that is also locked when not in use. Access to these records are authorized only for students, employees of the Training Center and collaborators of approved studies who have signed a confidentiality pledge.

5. **DISCLOSURE/CONSENT**

Persons were recruited in 1974 and in 1989. At the times of blood donation, participants signed a general consent form, allowing their blood and data to be used for research purposes. For each of these campaigns, the consent form and history form were approved by the School's Committee on Human Research at its January, 2000 meeting.

6. **CONFIDENTIALITY/INSURANCES**

All persons who are permitted to have access to our records must have a legitimate purpose and must sign a pledge of confidentiality. Records for this study that contain personal identifiers are kept in rooms that are locked when not in use and in a wing of the Health Department that is also kept locked when not in use. This wing is rarely used by anyone other than employees of the Training Center or Health Department. Personal information that might be considered sensitive such as date of last menstrual period, and use of oral contraceptives or hormones is coded in a way that cannot be interpreted without a key. The key is kept in a different room from the files. When data are to be used outside of the Training Center, names and addresses are removed. In more than 36 years of the existence of the Training Center, we know of no breach of confidentiality.

The responsible persons are Dr. George W. Comstock, Director of the Training Center for Public Health Research, Ms. Sandra C. Hoffman, Asst. Director, (301-791-3230) and Dr. Kathy J. Helzlsouer, Director of the Specimen Banks, 410-955-9727.

7. **COLLABORATIVE AGREEMENTS:**

This proposal is not binding until a signed agreement is executed between the 3M Company and Johns Hopkins University.

## 8. OTHER IRB APPROVALS

N/A

## 9. SINGLE PROJECT ASSURANCE

N/A

## 10. PUBLICATIONS

It is 3M's intent to co-author information with Johns Hopkins University researchers derived from this study. The details of this will be outlined in the project research contract. Under this agreement Johns Hopkins University would retain the right to publish information from this study solely for its own teaching and research purposes.

## 11. PROPRIETARY DATA

Proprietary information will be addressed in the final agreement signed between the 3M Company and Johns Hopkins University.

## 12. BUDGET

The budget for the work contribution of the investigators from Johns Hopkins University is attached.

**Personnel (On-campus)**

| Name | Role on Project | Rate/hr | Total* |
|---|---|---|---|
| Kathy Helzlsouer | Principal investigator | $1,000 | $30,000 |
| Han-Yao Huang | Co-investigator | 500 | 45,000 |
| Gloria Zepp | Secretary | 200 | 18,000 |
| Total On-Campus Personnel | | | **$93,000** |

**Personnel (Off-campus)**

| | | | |
|---|---|---|---|
| Sandra Hoffman | Project coordinator | $ 400 | $ 4,000 |
| Clara Krumpe | Laboratory Technician | 300 | 18,000 |
| Judith Bolton | Data manager | 250 | 5,000 |
| Total Off-Campus Personnel | | | **$ 27,000** |

**Serum Bank Maintenance Costs**
(Maintenance, electricity, replacements)

| | | |
|---|---|---|
| Cost Per Participant Sample | $225.00 x 300 samples = | $ 67,500 |

**Other Expenses**

| | | |
|---|---|---|
| Laboratory supplies (specimen tubes, pipettes) | $225.00 | |
| Computer Supplies | 200.00 | |
| Shipping costs (FedEx, dry ice shipping containers) | 150.00 | |
| Total Other Expenses | | $ 575 |

**Total Project Costs (Johns Hopkins University)** **$188,075**

* Total is an estimate of time to complete work.

Travel costs by Johns Hopkins University investigators will be billed directly to 3M. All other research study costs (e.g., analytical costs by Northwest Bioanalytical) will be paid by 3M.