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**AMMONIUM PERFLUOROOCTANOATE:
CROSS-SECTIONAL SURVEILLANCE
IN A COMMUNITY SAMPLE
OF CLINICAL MEASURES OF GENERAL HEALTH STATUS
RELATED TO A SERUM BIOMARKER OF EXPOSURE**

A STUDY PROTOCOL

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TABLE OF CONTENTS

INTRODUCTION	1
BACKGROUND	1
A. Animal Toxicology	1
B. Human Epidemiology and Exposure Assessment	5
OBJECTIVES	7
STUDY OVERSIGHT, SPONSOR, AND TESTING FACILITIES	8
REGULATORY COMPLIANCE	9
STUDY DESIGN	9
A. Study Overview	9
B. Community Sample	11
C. Sample Size	11
D. Random Selection of the Community Sample	15
E. Random Digit Dialing Telephone Survey	16
MATERIALS AND METHODS	18
A. Test Substance/Exposure of Interest	18
B. Clinical Pathology Evaluation	18
DATA ANALYSES	20
HUMAN SUBJECTS PROTECTION	21
RECORDS AND SAMPLE STORAGE	22
PROPOSED STUDY DATES	22
REFERENCES	23
SIGNATURES	28

DRAFT

APPENDICES	29
APPENDIX A	COMPUTER-ASSISTED TELEPHONE
	INTERVIEWING (CATI) 30
APPENDIX B	CONSENT FORM 33
APPENDIX C	CLINICAL PATHOLOGY COLLECTION
	PARAMETERS 37
APPENDIX D	ELECTROCARDIOGRAPHY (EKG) 38
APPENDIX E	AUDIOMETRIC EXAMINATION 39
APPENDIX F	VISUAL ACUTTY AND TONOMETRY
	EXAMINATION 40
APPENDIX G	COMPREHENSIVE MEDICAL AND WORK HISTORY
	QUESTIONNAIRE 41
APPENDIX H	PHYSICAL EXAMINATION AND EVALUATION 42
APPENDIX I	EXYGEN STANDARD OPERATING PROCEDURE
	FOR PFOA ANALYSES 43

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INTRODUCTION

Ammonium perfluorooctanoate (APFO) is a fully fluorinated carboxylic acid used primarily as the ammonium salt to aid in the emulsion polymerization of fluoropolymers. APFO and its salts are soluble in water and readily dissociate to the carboxylate anion, perfluorooctanoate (PFOA). As a result of the presence and biopersistence of PFOA in the blood of humans, the potential health effects of PFOA have been examined in occupational cohorts. Background levels of serum PFOA have also been measured in some general population samples, but no data have been collected in these groups to measure general health status. Epidemiological investigations and medical surveillance of occupationally exposed workers have failed to find any association between PFOA exposure and adverse health effects. However, toxicological findings in animal studies and the slow elimination rate of PFOA observed in humans combine to indicate the desirability of additional surveillance in the general population.

This study is being undertaken to provide additional scientifically-credible data that can be used to understand whether or not exposure to PFOA in the community, as measured by serum PFOA levels, is related to adverse health outcomes as measured by standard health screening examinations.

DuPont has an external Epidemiology Review Board which will review this protocol, and DuPont will provide technical oversight for the duration of the study. The protocol will also be submitted for review and approval by the University of West Virginia Institutional Review Board (IRB).

BACKGROUND

The following sections describe studies on PFOA in animals and in humans.

A. Animal Toxicology

The liver is a primary target organ for both short-term and chronic effects of PFOA in rats (Griffith & Long, 1980; Olson & Anderssen, 1983; Kennedy, 1985; Pastoor *et al.*, 1987) and cynomolgus monkeys (Butenhoff *et al.*, 2002). The increased liver weight in both species does not appear to be a result of hepatocellular hyperplasia (no increase in nuclear DNA) and has been variously attributed to increases in peroxisomes, endoplasmic reticulum, and mitochondria (Ikeda *et al.*, 1985; Pastoor *et al.*, 1987; Butenhoff *et al.*, 2002; Berthiaume and Wallace, 2002; Biegel *et al.*, 2001). Higher doses lead to liver degeneration and necrosis and the appearance in the serum of enzymes reflecting liver damage.

PFOA and other perfluoroalkanoic acids are part of a widening group of substances including plasticizers (phthalate esters), hypolipidemic drugs (clofibrate, nafenopin) and some herbicides (phenoxyacetic acids), solvents (trichloroethylene), and naturally-occurring compounds such as long-chain fatty acids that are known as peroxisome proliferator-activated receptor alpha (PPAR α) agonists (Ikeda *et al.*, 1985; Just *et al.*, 1989; Pastoor *et al.*, 1987; Cook *et al.*, 1992, 1994; Biegel *et al.*, 1995, 2001; Cattley *et al.*, 1998). PFOA has been shown to activate the PPAR α receptor but not the PPAR γ receptor (Maloney and Waxman, 1999). As with all peroxisome proliferators (PPs), treatment of rodents with PFOA initiates a characteristic sequence of morphological and biochemical events in the liver and, to a lesser extent, the kidney. These events include: marked hepatocellular hypertrophy due to an increase in number and size of peroxisomes, and consequently, a large increase in peroxisomal fatty acid β -oxidation, an increased CYP450-mediated ω -hydroxylation of lauric acid, and a variety of changes in lipid metabolism (Ikeda *et al.*, 1985; Pastoor *et al.*, 1987; Berthiaume & Wallace, 2002). This response is initiated by the activation of the nuclear hormone receptor, PPAR α (Green, 1995; Ashby *et al.*, 1994; Lake, 1995).

There are, however, differences in the effects exerted by different groups of PPs. For example, PFOA did not cause hepatocellular hyperplasia like most other PPs (Pastoor *et al.*, 1987); although the study design did not include looking at early time points, such as 3-21 days. In rats, the hepatocellular response is primarily hypertrophic and is caused by an increase in the number of peroxisomes and proliferation of the smooth endoplasmic reticulum. In addition, there is evidence for proliferation of mitochondria that may, in part, account for increased liver mass (Butenhoff *et al.*, 2002; Berthiaume and Wallace, 2002).

Peroxisome proliferation induced by PFOA affects fatty acid metabolism and cholesterol synthesis in the liver (Haugom & Spydevold, 1992). Serum cholesterol levels were reduced 50-70% after only 24 hrs in rats fed diets containing 0.02% PFOA, and triglycerols were reduced to about 60% of controls after 7 days. Measurements of selected enzymes in hepatocytes from the PFOA-treated rats showed significant decreases in HMG-CoA reductase, the rate limiting step in cholesterol biosynthesis, and in acyl CoA cholesterol acyltransferase (ACAT), the enzyme responsible for the esterification of cholesterol. It was concluded that the hypolipidemic effect of PFOA is due, in part, to the reduced synthesis and esterification of cholesterol, combined with the enhanced oxidation of fatty acids in the liver (Haugom & Spydevold, 1992). Kudo *et al.* (1999) confirmed the earlier observation by Pastoor *et al.* (1987) that PFOA treatment increased the level of triglycerides in the liver and linked this with increases in two triglyceride synthesizing enzymes, glycerol 3-phosphotransferase and diacylglycerol acyltransferase. It also seems possible, however, that the increase in triglycerides in the liver as well as their decrease in the plasma following treatment with PFOA is associated with a decrease in triglyceride secretion from the liver. Clearly, PFOA has the ability to disrupt lipid metabolism by any of several mechanisms that

at the present time are not well understood. The importance of these, if any, in the toxicity of PFOA is not known.

A two-year chronic rat bioassay with PFOA indicates a significantly increased incidence of tumors (mainly adenomas) of the liver, testis (Leydig cell) and pancreas (acinar cell) (Biegel *et al.*, 2001). This is consistent with the fact that PFOA is a PP since, although most attention has been focused on PP-induced liver tumors, both Leydig cell tumors (Cook *et al.*, 1999), and pancreatic acinar cell tumors (Reddy & Rao, 1977; Svoboda and Azarnoff, 1979) are often observed following chronic exposure of rodents to other PPs. Ohmura *et al.* (1997) showed that the peroxisome proliferator, 4-chloro-6-(2,3-xylyldino)-2-pyrimidinylthio-(N-beta-hydroxyethyl)acetamide (DR931), a potent hepatocarcinogen in rats, was capable of inducing DNA synthesis in pancreatic acinar cells, yet had no effect on ductal or islet cells. Induction of liver, Leydig cell and pancreatic acinar cell tumors is a common finding for PPs (Cook *et al.*, 1999). In chronic bioassays in rats, Cook *et al.* (1999) reported that 7 out of 11 PPs induced all three tumor types (Cook *et al.*, 1999), and 10 of the 11 PPs produced liver and Leydig cell tumors (Cook *et al.*, 1999). The presence and established persistence of PFOA in human tissues, combined with the increased incidence of liver, Leydig cell and pancreatic acinar cell tumors in chronic studies with PFOA in rats, raises questions concerning the potential relevance of tumors observed in chronic studies in rats to potential human health risk.

It is generally agreed that liver tumors in rats produced by PPs are unlikely to be relevant to humans (Bentley *et al.*, 1993; Ashby *et al.*, 1994; Cattley *et al.*, 1998; Doull *et al.*, 1999). A large number of humans have been treated for relatively long periods of time with hypolipidemic drugs that are potent PPs in rodents. No significant changes in the peroxisome number or volume occur in humans taking substantial doses of these drugs for extended periods of time (up to 3 years) (Ashby *et al.*, 1994). Therefore, rodents appear to be poor models for human risk assessment with respect to liver effects observed with PPs. The reason for the non-responsiveness of humans to PPs is not yet fully understood; although, research shows differences in amount and expression of PPAR α between humans and rodents (Cattley *et al.*, 1998; Palmer *et al.*, 1998).

Experimental evidence for the mechanism of PFOA-induced Leydig cell tumor formation, while not conclusive, tends to support the hypothesis that a sustained increase in estradiol within the testes may be responsible for the increased incidence of Leydig cell tumors in male Sprague Dawley rats (Cook *et al.*, 1992; Biegel *et al.*, 1995; Liu *et al.*, 1996a, 1996b). It was initially thought that PP-induced Leydig cell tumors arose by a mechanism similar to that suggested for liver (Cook *et al.*, 1992). In the chronic PFOA study, however, there was no evidence that peroxisomes were induced in Leydig cells and no increase in β -oxidation activity above the normal baseline level (about 20 times less than that in liver) (Biegel *et al.*, 2001). Attention has subsequently focused on the role in Leydig cell hyperplasia and

neoplasia of a sustained increase in the level of serum estradiol observed in PFOA treated rats (Liu *et al.*, 1996a,b; Biegel *et al.*, 1995, 2001). It has been proposed that PPs, including PFOA, increase serum estradiol levels as well as levels in the testis (interstitial fluid) via induction of the enzyme aromatase, a CYP450-mediated enzyme in the liver (Liu *et al.*, 1996b; Biegel *et al.*, 1995, 2001; Upham *et al.*, 1998). It is then proposed that the increase in testicular estradiol will modulate growth factors, specifically TGF α , to stimulate cell proliferation in the Leydig cell as it is known to do in other (e.g., mammary) tissues (Liu *et al.*, 1987). The suggested role of elevated estradiol in Leydig cell neoplasia is still uncertain because estrogenic compounds do not induce Leydig cell tumors in rats. It is possible, however, that the failure of estrogenic compounds to cause Leydig cell tumors results from a depression of luteinizing hormone (LH) which has been demonstrated to be the chief driver of such tumors (Biegel *et al.*, 2001).

A second mechanism/pathway which has been proposed to be also involved in the formation of PFOA induced Leydig cell tumors is the inhibition of testosterone biosynthesis, which disrupts the hypothalamic-pituitary-thyroid (HPT) axis. In *ex vivo* studies with PFOA, exposure to PFOA resulted in an inhibition of enzymes critical to the testosterone biosynthetic pathway, which, if occurring *in vivo*, would subsequently lead to a decrease in circulating testosterone (Biegel *et al.*, 1995). In *in vitro* studies, 13 PPAR α agonists were demonstrated to inhibit testosterone biosynthesis (Liu *et al.*, 1996a). The decrease in testosterone levels results in compensatory increases in LH, increased binding of LH to the LH receptor on Leydig cells, thus resulting in increases in Leydig cell proliferation (Clegg *et al.*, 1997). In a two-year mechanistic bioassay with PFOA and another PPAR α agonist, LH levels were not consistently increased, yet estradiol levels were increased at several time points (Biegel *et al.*, 2001). This is consistent with PFOA inducing circulating estradiol levels, which would attenuate the elevation of LH caused by the inhibition of testosterone biosynthesis.

There is, however, evidence indicating a substantial difference in the susceptibility of rats and humans to Leydig cell tumorigenesis. There are numerous pharmaceuticals and chemicals that have been documented to produce Leydig cell tumors in rats and other laboratory animals, but not in humans. These include androgen-receptor antagonists, dopamine agonists, estrogen agonists/antagonists, other PPAR agonists (clofibrate, gemfibrozil), sugars (lactose, lactitol), and nicotine (Clegg *et al.*, 1997). Studies have also indicated that there does not appear to be a difference in the morphology of Leydig cell tumors, whether spontaneous or chemically induced.

Since PFOA and other PPs do not increase peroxisome levels in the testis, current ideas regarding the mechanism of PP-induced Leydig cell hyperplasia and neoplasia suggest the involvement of hormone-mediated (estradiol) induction of testicular growth factors (Biegel *et al.*, 2001). The increased levels of estradiol are thought to result from the induction of a

hepatic cytochrome-P450 (CYP450) mediated aromatase. If this is not part of a pleiotropic PPAR-mediated response, Leydig cell tumorigenicity could involve a distinct hormone-mediated mechanism that might be of some relevance to humans. There is, however, evidence indicating a substantial difference in the susceptibility of rats and humans to Leydig cell tumorigenesis. Thus, while the spontaneous incidence of Leydig cell adenomas in ageing Crl:CD⁶ BR rats ranges from approximately 0-12% and can approach 100% in F344 rats, the rate in humans is reported to be only about 0.4 per million (0.00004%) (Schottenfeld, 1996). It seems highly unlikely that PFOA exposures represent any significant human risk with respect to Leydig cell cancer. There was no evidence of any increase in serum estradiol or testicular cancer in 3M plant workers exposed to PFOA (Olsen, 1998) and in the 6-month PFOA study with cynomolgus monkeys, estradiol levels were not increased (Butenhoff *et al.*, 2002). Furthermore, there was no increased incidence of testicular or other cancers in humans ingesting high daily doses of fibrates and other rodent PPs for hyperlipidemia (Ashby *et al.*, 1994).

The mechanism by which PFOA and some other PPs induce pancreatic acinar cell tumors is not well understood. It has been hypothesized that it might involve release of cholecystikinin (CCK) in the gut with subsequent stimulation of the acinar cells in the pancreas to secrete pancreatic enzymes into the gut (Biegel *et al.*, 2001; Herrington & Adrian, 1995). However, it must be concluded that, at the present time, this is a speculative mechanism that is not supported by experimental evidence for PFOA (Biegel *et al.*, 2001; Butenhoff *et al.*, 2002). Even if this is found to be the mechanism operating in rats, its applicability to humans is highly uncertain (Gavin *et al.*, 1996, 1997; Cattley *et al.*, 1998; Pandol, 1998). To assess the hypothesis in production workers at 3M, plasma CCK-33 measurements were made by radioimmunoassay during the 1997 medical surveillance of 75 male production workers (Olsen *et al.*, 2002). The results showed a weak negative association between serum PFOA and plasma CCK levels and serum liver enzyme tests showed no indication of cholestasis. There was no increased mortality from pancreatic cancer in these workers (Alexander *et al.* 2002).

B. Human Epidemiology and Exposure Assessment

The presence of organic, covalently bound fluorine in human blood was reported by Taves (1968a,b), and Taves *et al.* (1976) tentatively identified a component of the organic fluorine as PFOA. Following that finding, 3M began monitoring its fluorochemical production workers (Ubel, 1980). Workplace monitoring was originally based on determination of total organofluorine in serum. Speciation and quantitation of PFOA rather than total organofluorine was introduced in 3M's medical monitoring program in the 1990s. 3M has also surveyed sera from the general (non-occupational) population. Using high performance liquid chromatography-electrospray tandem mass spectrometry Hansen *et al.* (2001), Olsen *et al.* (2002a; 2002b; 2002c) have shown in three U.S. general population

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studies serum concentrations of PFOA, regardless of age, averaging approximately 5 parts per billion (ppb) with an upper bound of the 95th percent tolerance limit approximating 10 to 15 ppb. In another study, Olsen *et al.* (2003a) examined a total of 30 human donor livers for the presence of PFOA. Almost all donor livers were below the lower limit of quantitation which ranged between 5.4 to 35.9 ng/g. Of the two donor livers measured above the lower limit of quantitation, one liver had a PFOA concentration of 2.5 ng/g and the other had a mean analysis of 46.9 ng/g.

The first published report of serum concentrations in an ammonium perfluorooctanoate (APFO) production workforce (Ubel *et al.* 1980) indicated a total organic fluorine content of 1 to 71 ppm in 3M employees at an electrochemical fluorination plant in Cottage Grove, MN. The highest serum concentrations were observed in employees with the longest production work history and among a subset of workers whose jobs involved drying and packaging operations. Using gas chromatographic techniques, Ubel *et al.* reported that 90% of the organic fluorine was PFOA.

Additional analyses of total organic fluorine or serum PFOA measurements have been conducted since Ubel *et al.* in conjunction with medical surveillance of these Cottage Grove production workers (Gilliland, 1992; Gilliland and Mandel 1996; Olsen *et al.* 1998; 2000; 2003b) and other production workers (Olsen *et al.* 2003c). Serum PFOA concentrations during the 1990's were comparable to those initially estimated by Ubel *et al.* (1980) with means (range in parentheses) of 5.0 ppm (0-80 ppm), 6.8 ppm (0.0-114) and 6.4 ppm (0.1-81 ppm) in 1993, 1995 and 1997, respectively (Olsen *et al.* 2000).

Medical surveillance of these Cottage Grove production workers has included a medical questionnaire; measurement of height and weight; pulmonary function testing; and standard biochemical and hematology tests (Ubel *et al.* 1980; Gilliland *et al.* 1996; Olsen *et al.* 2000; 2003b). Hepatic-and lipid-related blood tests have included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma glutamyl transferase (GGT), total and direct bilirubin, total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides. Ubel *et al.* (1980) initially reported that no health problems were associated with exposure to fluorochemicals in this workforce although aggregate analyses were not presented. Others have subsequently not shown abnormal associations between clinical chemistry or hematology findings with either total organic fluorine levels (Gilliland and Mandel 1996) or serum PFOA concentrations (Olsen *et al.* 2000; 2003b) in this workforce. Although Gilliland and Mandel (1996) suggested that serum total organic fluorine levels might modulate hepatic responses to obesity and alcohol, these interactions were not observed in three subsequent surveillance examinations that analyzed specifically for serum PFOA concentrations (Olsen *et al.* 2000).

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In response to toxicological findings that suggested PFOA might modulate endocrine activity in the rat (Biegel *et al.* 1995, 2001), serum levels of several hormones (estradiol, free and bound testosterone, FSH, LH, prolactin and TSH) were incorporated in the medical surveillance program of these Cottage Grove workers starting in 1990. Other hormones (dehydroepiandrosterone sulfate, 17 hydroxyprogesterone and sex hormone binding globulin) were added in 1993 and 1995. Gilliland (1992) reported a positive nonlinear association with estradiol and a negative nonlinear association with free or bound testosterone in relation to serum total organic fluorine concentrations. These associations were not confirmed with analyses specific to serum PFOA concentrations although mean estradiol levels were 10 percent greater among employees with the highest serum PFOA concentrations (30 ppm) (Olsen *et al.* 1998). Possible explanations for the disparity between findings may involve the use of different biomonitoring and hormonal assay methods and possible misclassification of confounding variables, most importantly, body mass index (Olsen *et al.* 1998). Based on their data, Olsen *et al.* (1998) concluded there was reasonable assurance of no significant hormonal changes associated with PFOA at the serum levels measured among these male production employees.

Contrary to the relatively rapid rates of elimination reported in laboratory animals (DuPont, 1982; Johnson and Ober, 1980; Butenhoff *et al.*, 2002), PFOA appears to be slowly eliminated in humans (Ubel *et al.* 1980; Burris *et al.* 2002). In an interim report, Burris *et al.* (2002) reported the serum half-life of elimination for PFOA was 4.4 years (S.D. 3.5) in nine retired fluorochemical production employees who had serum concentrations measured over an 18 month time period following cessation of exposure. Initial concentrations ranged from 0.1 to 1.8 parts per million (ppm). Although non-occupational sources of PFOA exposure during the follow-up time period were not accounted for and the half-life study is still in progress, the slow elimination rate observed suggests that humans have the least ability to eliminate PFOA of any species studied. This slow elimination does, however, allow the use of a single measurement to significantly reflect current, recent, past, and chronic/subchronic exposure.

This observation, combined with the effects seen in animal studies, warrant further evaluation of the potential health consequences that might result from exposure to PFOA

OBJECTIVES

The overall objective of this community study is to generate scientifically sound data to further the understanding by DuPont, regulatory agencies, and others of the nature and potential effect of human exposures resulting from PFOA emissions from Washington Works.

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The first objective for this study will be to develop a statistical model describing the variation in the exposure biomarker (serum PFOA), if any, in relation to environmental exposure to PFOA considering traditional epidemiology descriptors, such as age, sex, race, Body Mass Index (BMI), alcohol use, prescription medications, and cigarette smoking status.

A second objective will be to model health outcome in terms of the adjusted serum PFOA values. For both the first and second objectives, standard regression methodology will be used to determine which of these potential explanatory variables should be included in the final models.

The third objective will be to identify appropriate clinical markers, if any, related to changes in PFOA concentrations in serum to allow proper follow-up through continued surveillance.

STUDY OVERSIGHT, SPONSOR, AND TESTING FACILITIES

This community survey will be conducted for DuPont by *The Sapphire Group, Inc.* The following entities have key roles in this endeavor.

Study oversight, coordination, and data evaluation:	<u><i>The Sapphire Group, Inc.</i></u> Bethesda, MD Carol Gevecker Graves, Ph.D., Principal Investigator
Physical and clinical examinations and laboratory analyses:	<u>Examinetics</u> Kansas City, MO Mary H. Dimitri, Senior Sales Executive (Subcontract to Pacific Toxicology, Los Angeles, CA, for standard laboratory analyses)
Serum PFOA level analyses:	<u>Exygen Research</u> State College, PA John Flaherty, Vice President of Operations
Cohort interviews and enrollment:	<u>Aspen Systems</u> Rockville, MD Marie Pogozelski, Manager, Survey Operations

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Random sample
selection:

Genesys Sampling
Fort Washington, PA

Sponsor:

Haskell Laboratory for Health and Environmental Sciences
E.I. Du Pont De Nemours and Company
Newark, DE
Robin C. Leonard, Ph.D., M.A.C.E.

REGULATORY COMPLIANCE

This study will be conducted according to guidance provided by the American College of Epidemiology Ethics and Standards of Practice Committee which can be accessed at <http://www/acepidemiology2.org/policystmts/EthicsGuide.asp>.

The laboratory analyses provided by the contract laboratories, Examintetics, Pacific Toxicology, and Exygen, will be conducted in accordance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice (GLP) Standards (1989). Appropriate chain of custody procedures for samples and results will be followed (Exygen Research, Standard Operating Procedure). See Appendix I. Additionally, the final laboratory analyses report will be reviewed and audited by experienced GLP auditors.

STUDY DESIGN

This study will consist of a cross-sectional sample of community residents randomly selected from five water districts to determine the range of values for serum PFOA and various health screening endpoints for people potentially environmentally exposed to PFOA.

Each study participant will complete a self-administered health status questionnaire using a questionnaire similar to that used in a study of workers at DuPont's Washington Works facility. Each study participant will receive a physical examination similar to that received by Washington Works employees who participate in the worker study. The examination will be administered in the principal community of each water district.

A. Study Overview

This cross-sectional survey will use standard epidemiologic and statistical analyses of several types of clinical data and a biomarker of exposure (serum PFOA) to determine the relationships, if any, between exposure to PFOA and any early clinical signs of adverse effect in humans. To this end, the study will incorporate data from clinical chemistry and

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hematology analyses, as well as clinical parameters customarily utilized in traditional health surveillance examinations, such as chest X-ray, pulmonary function tests, and hearing and vision tests.

A broad panel of testing will be conducted that will provide a reasonably extensive snapshot of general health and well-being. These include Chem Screen 25/CBD/Urinalysis, chest X-ray, pulmonary function test, EKG, audiometry, and visual acuity tests. Additional specific clinical pathology tests are included that may have more relevance to the overall objectives of the study, based on findings in animal studies and occupational surveillance. These include special tests for lipid profiles, hormone measurements, C-reactive protein, and prostate-specific antigen (Appendix C).

Results from the cross-sectional survey will note the prevalence of test values for several health endpoints in community participants. The prevalence of these test values will also be compared across stratification of serum PFOA levels into quartiles.

Briefly, the procedures for the community study include these major tasks:

- Identify service boundaries of the five water districts with detectable PFOA.

- Select a random sample of telephone numbers of the targeted number of community members in each water district. Preliminary assessment of the sample size needed suggests that approximately 800 residents exposed through drinking water will be a sample size adequate to make meaningful statistical analyses.

- Communicate via telephone with the randomly selected community residents to inform them of the study and to invite their participation in the study.

- Coordinate between those selecting the sample and inviting participation and the field team to schedule appointments for physical examination for each participant.

- Track the review and signing of consent forms and the completion of self-administered questionnaires.

- Perform the physical examination, medical tests, and blood drawing for each selected community participant at a convenient location in each water district.

- Each participant will be examined once and have blood drawn once for this study. In the event of laboratory accidental loss of specimens, the participant will be notified and asked if he/she is willing to provide an additional specimen.

DRAFT

Following the blood draw, a small bagged snack will be provided to each participant to mitigate the effects of the fast before the participant leaves the examination site.

Transfer data electronically to *The Sapphire Group* for analysis.

Analyze data, prepare reports, and communicate study results to participants. Each study participant will receive a printout of his/her individual results.

B. Community Sample

The five water districts in which PFOA has been detected (and hence which are members of the class) are

Lubeck, WV
Belpre, OH
Little Hocking, OH
Tuppers Plains, OH
Village of Pomeroy, OH

The sample for the community study will be approximately 800 persons chosen randomly from the five water districts in the class. In addition, the study will include some persons who obtain their water from private wells.

Adults, defined as persons 18 years of age and older, will be invited to participate.

C. Sample Size

A power study was conducted to determine the sample sizes needed to have reasonable assurance of finding biologically relevant levels of serum PFOA (J. Green, personal communication). Statistical power considerations are relevant to the proposed statistical analyses since the decision to include or exclude a potential explanatory variable in the proposed regression analyses and the interpretation of the magnitude of a regression coefficient can only be done by taking into account the variance of the estimated coefficient. That variance is in turn related to the power of the analysis.

The power study consisted of a Monte Carlo computer simulation study and was based on the variance-covariance structure of the 3M study (Olsen *et al.* 2000, 2003b). While the variability in the community data may differ from that observed in the 3M study, that study constitutes the most reliable information available on variation in serum PFOA levels.

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The following table and figures illustrate the results of the power study. They indicate how many subjects are required in any given cohort to detect a difference of a specified percent change based on the population studied by 3M. The sample size required depends on several factors:

Size effect one wants to be able to detect

Level of confidence one wants to have in detecting that size effect

Variability in serum PFOA levels in the population cohort being studied

The variability in the proposed study population is presumed to be the same as in the 3M population. Should the variability in the study population be higher, the required sample sizes will increase. As a rule, the objective is to have high confidence (85-95 %) of detecting the size effect deemed important.

The results below are divided into gender and age groups, as statistically significant differences were observed in the variation within these populations. For example, the coefficient of variation (CV) in adult males was 0.40, while in adult females the CV was 0.45. The CV observed among elders of either sex was 0.35.

Table 1 presents the results of the power study. Figure 1 illustrates the sample sizes required for various power levels (0.75, 0.85, and 0.95) for a CV of 0.25. Figure 2 is a similar illustration for a CV of 0.35, and Figure 3 illustrates sample sizes for a CV of 0.45. An example from the table and figures shows that it is possible to have 95% confidence of detecting a 20% change in serum PFOA levels of adult males or females with 66 subjects. The same size effect can be detected in elders of either gender with only 40 subjects.

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Table 1. Sample Size (N) Based on Proportion Change To Be Detected, Power Desired, and Coefficient of Variation in the Cohort Being Sampled

Proportion change	Power	CV = 0.25	CV = 0.35	CV = 0.45
		N	N	N
0.1	0.75	38	73	120
	0.85	50	97	161
	0.95	75	146	242
0.2	0.75	11	20	33
	0.85	14	27	44
	0.95	21	40	66
0.3	0.75	5	10	16
	0.85	7	13	22
	0.95	10	20	32
0.4	0.75	3	6	10
	0.85	4	8	13
	0.95	6	12	20
0.5	0.75	3	5	7
	0.85	3	6	9
	0.95	5	9	14

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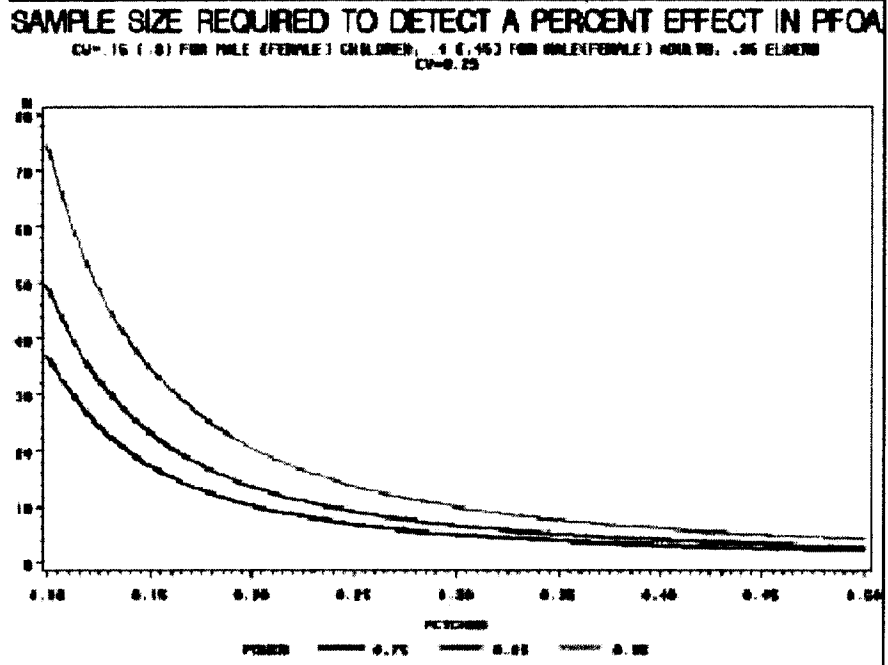


Figure 1. Sample size graph for CV = 0.25

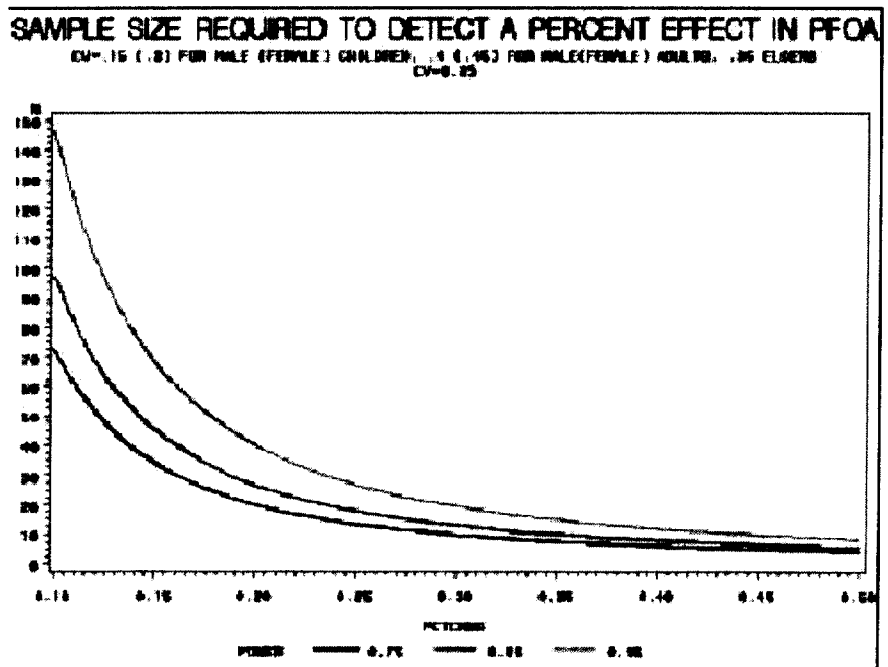


Figure 2. Sample size graph for CV = 0.35

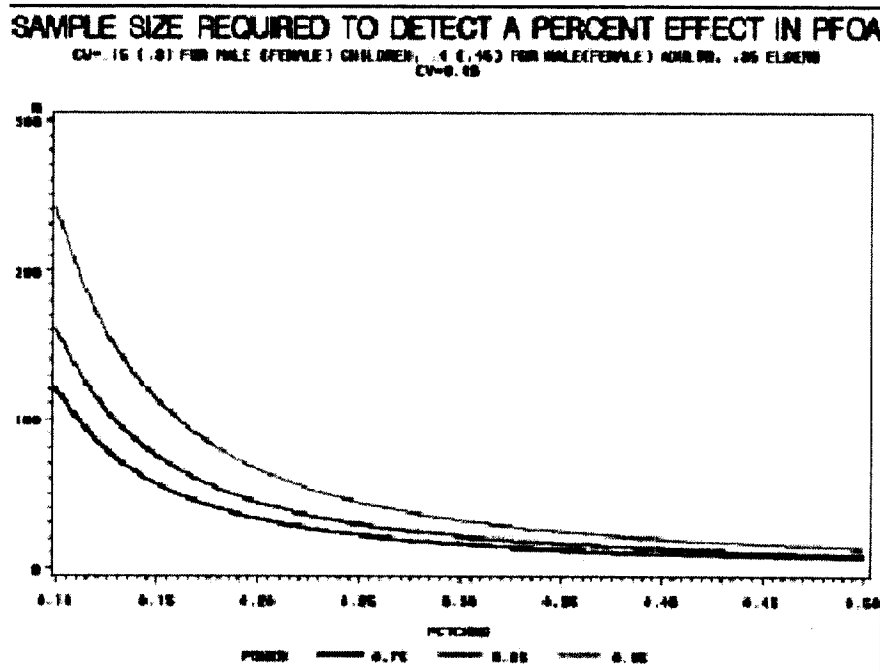


Figure 3. Sample size graph for CV = 0.45

In setting the sample size for the community study, a percent change of 10% (*i.e.*, a proportion change of 0.1) was selected along with a power of 85% (0.85). From the table, it can be seen that a sample size of 161 is required to observe this percent change in serum PFOA in adult males or females who exhibit a CV of 0.45. Because there are five water districts (*i.e.*, five sub-cohorts), a sample size of approximately 800 is required (*i.e.*, 160 per water district x 5 water districts = 800).

D. Random Selection of the Community Sample

Two methods were considered as the basis for sample selection random selection from water district customer lists or random digit dialing based on Census blocks served by a water district. The telephone survey using random digit dialing was deemed preferable to a random selection based on water district customer lists for several reasons. A name on the utility list may be the owner, but not the occupier, of the residence using the water. The utility lists may not readily identify commercial and other non-residential users. Because negotiations for customer lists may be protracted, obtaining the lists from all five utilities may cause a time burden delaying the initiation of the study. Finally, since it may not be possible to obtain lists from all water districts, the alternate method would have to be used in some water districts. Less bias would be introduced if the same selection method were used in all water districts.

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Random digit dialing (RDD) will be used to select study participants from each water district (Waksberg, 1978; Groves and Kahn, 1980). Assuming a participation ratio of 10-to-1, RDD will be used to screen an initial list of 8,000 eligible residents aged 18 and older, with a residential listing, and living in the five water districts based on Census blocks. This approach will yield an estimated sample of approximately 800 participants balanced by age group and gender.

An RDD sample of residential phone numbers will be obtained from Genesys Sampling Systems, a product of the Marketing System Group (MSG) of Fort Washington, PA. Genesys is recognized as one of the top ten systems for producing RDD samples. Genesys will be provided with the Census blocks for each water district. They will provide RDD blocks of 100 residential phones excluding all businesses, hospitals, schools, prisons, nursing homes, etc. They will provide blocks totaling 8,000 households distributed among the five water districts. Assuming that only one in every 10 persons contacted will agree to participate in the study, this method will generate the desired sample size of approximately 800.

Within each water district, the stratified random sample of approximately 160 will be balanced by gender and age groups. Males and females will be sampled approximately equally reflecting the distribution of genders in the community.

Within each gender, age groups will be balanced in three broad age categories: 18-44 years, 45-64 years, and 65+ years. The computer-assisted telephone interviewing (CATI) system software will provide daily/weekly counts of sampling quotas incorporating the stratification criteria to ensure balanced recruitment of all three age groups for each gender. (See Appendix A.)

E. Random Digit Dialing Telephone Survey

Recruitment of the community sample will be subcontracted to Aspen System Corporation's Survey Operations Center. They will employ RDD and CATI to accomplish the following tasks:

A recruitment call will establish study enrollment eligibility. The phone call will include a brief introduction about the purpose and value of the study to the community and to the individual (*i.e.*, free health assessments conducted in a location convenient to their home). Questions will be asked concerning place of occupation and source of water (*e.g.*, public water supply or private well, name of water utility). DuPont employees will be excluded from the community study, as will pregnant women and people on chemotherapy. Telephone calls will be placed between 5 and 9 P.M. EST.

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The recruitment screening questionnaire will be programmed for CATI administration offering full telephone interviewing automation and real-time monitoring of study progress as data are collected. The range of capabilities of the software promote ease of telephone interviewing by simplifying questionnaire programming, call management, quota cell control (*i.e.*, grouping of respondents based on demographic characteristics such as gender and age), calling results, interviewer monitoring, and data reports and delivery. The CATI system offers daily electronic reporting of results to monitor total calls made, quota cell reports of completed interviews by gender and age group, and instantaneous quality control.

A pilot study of 100 telephone numbers will be conducted to test the telephone screening questions and participation rates. Based on the results of the pilot test, the recruitment script (introduction and/or questions) may be modified and the recruitment survey retested, as necessary, for clarity. Any persons who agree to participate in the study during this pilot test will be included in the study.

Up to four telephone attempts will be made to reach and recruit a randomly selected adult in a household.

Upon reaching an adult and after introductory remarks, the telephone interviewer will determine the number of adults living in the household. If there are more than two adults, an adult respondent will be selected randomly based on the nearest birthday strategy (*i.e.*, the adult whose birthday is closest to the date of the interview will be selected for study participation).

If the selected adult is eligible after responding to the screening questions (exclude individuals who ever worked for DuPont and verify that their gender and age group quotas remain open for enrollment), their participation in the study will be requested and their home address will be recorded. It is anticipated that for every 10 calls made one person will agree to participate in the community study on the average.

Level one of study participation will be based on verbal agreement attained in the telephone recruitment interview. Nightly summaries of eligible respondents who agree to participate will be calculated by the telephone survey vendor to revise the age and gender enrollment quotas within each of the water districts for the next evening's survey.

Physical examination appointments will be made at this time and confirmed in writing.

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A follow-up letter confirming participation and the date of the examination, the consent form (Appendix B), the self-administered questionnaire (Appendix G), and a pre-paid return envelope will be distributed to each study participant via Federal Express or USPS priority mail.

Respondents who do not return the questionnaire, do not make an appointment, and/or do not show up for their physical exam appointment will be telephoned up to three times and urged to participate. A sample in excess of 800 will be recruited to have approximately 800 participants remaining after no-shows are accounted.

Level two of assessing the final study participation rates will be calculated weekly, using results from completed self-administered questionnaires and completed physical exams. Study participant rates by age group and gender within each of the water districts will be calculated weekly to adjust subsequent balanced enrollment.

MATERIALS AND METHODS

A. Test Substance/Exposure of Interest

Test Substance:	Ammonium perfluorooctanoate (APFO), assimilated through environmental exposure
Analytical Standard:	Exygen (for serum PFOA analyses) and Pacific Toxicology (for all other standard analyses) will provide standard operating procedures that describe their quality control/quality assurance practices.
Additional Information:	Community residents may have exposure to chemicals other than PFOA. This potential will be addressed in the questionnaire by inquiring about occupation.

B. Clinical Pathology Evaluation

Approximately 30 mL of blood will be drawn into several vacutainer tubes with the appropriate anticoagulants, barriers, preservatives, and other additives as required for the list of analyses listed in Appendix C. This will require the participants to fast for at least 12 hours before the blood collection. In addition to a standard ChemScreen 25/CBD, other serum markers will be measured.

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1. Collection Sites and Samples

Sample Volumes: All sample volumes are approximate. (See Appendix C)

Collection Parameters: See Appendix C

2. Electrocardiography (EKG) Evaluation

Frequency: Once

Scope: See Appendix D

3. Chest X-Ray Evaluation

Frequency: Once

Scope: 14 x 17 PA Chest (full chest x-ray, one view)

4. Audiometric Examination

Frequency: Once

Scope: See Appendix E

5. Pulmonary Function Test Evaluation

Pulmonary function testing (PFT), or lung function testing, is a method of determining how well the lungs and airways are working. The most common PFT is called spirometry. Each participant will take in as deep a breath as possible, blowing out all of the air as fast and as hard as possible into a tube through which the volume of air is measured. Three attempts are needed, and the best effort is recorded. The results will be recorded as FEV1, FVC, FEV1/FVC%, FEV1% predicted, and FVC%.

Frequency: Once

Scope: Forced Expiratory Volume 1 (FEV1), Forced Vital Capacity (FVC), FEV1/FVC%, Forced Expiratory Flow (FEF25- 75), Peak Expiratory Flow Rate (PEFR)

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6. Visual Acuity

Frequency: Once

Scope: See Appendix F

7. Questionnaire

Frequency: Once

Scope: See Appendix G

8. Physical Examination

Frequency: Once

Scope: See Appendix H

DATA ANALYSES

The first objective for this study will be to develop a statistical model describing the variation in the exposure biomarker (serum PFOA), if any, in relation to environmental exposure to PFOA considering traditional epidemiology descriptors, such as age, sex, race, Body Mass Index (BMI)¹, alcohol use, prescription medications, and cigarette smoking status.

A second objective is to model health outcome in terms of the adjusted serum PFOA values. For both the first and second objectives, standard regression methodology will be used to determine which of these potential explanatory variables should be included in the final models.

The third objective will be to identify appropriate clinical markers, if any, related to changes in PFOA concentrations in serum to allow proper follow-up through continued surveillance.

In the analyses, each explanatory variable will be broken into a small number of categories. To avoid over-parameterization, a variable that does not contribute significantly to a model will be discarded.

¹ BMI will be calculated as $\text{weight} / (\text{height}^2) \times 100$ using metric units.

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Missing values for the age, sex, race, relative body weight or height variables will be entered into the unknown subgroup for that particular variable rather than excluding the subject from the analysis. Assessments of model assumptions will be conducted to ensure that the appropriate statistical procedures/models are used. The data will be displayed with box plots of the distributions and variation for clinical endpoints. It may be necessary to use normalizing, variance stabilizing transformations for these responses.

For continuous variables, such as blood pressure, analyses will be based on group means, adjusted for any potentially confounding variables. For discrete outcome variables, such as the prevalence of liver disease, analyses will be based on logistic or Poisson regression. In addition, analyses will be performed on the proportion of a group's measurements that fall in the highest or lowest decile. For disease prevalence rates based on sparse data, exact logistic regression may be used.

HUMAN SUBJECTS PROTECTION

Specific procedures that will be put in place for human subjects protection during this study include, but are not limited to, the following:

- Detailed consent form from each participant that identifies study purpose, requirements, voluntary nature of participation, right of withdrawal, possible risks, and potential benefits (Appendix B).

- Coded identification to link biological specimens, exam results, and completed questionnaires.

- Scheduling of exam appointments to ensure that waiting areas are not crowded.

- Use of mobile office/laboratory units with sufficient capacity to ensure that only one participant is occupying a test area.

- Establishment of coded folders or envelopes that will contain each participant's results for archiving.

- Analytic data files that identify participants only through study subject identification code.

- Contact information for study personnel so that any problems or questions that should arise for participants can be addressed as quickly as possible.

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RECORDS AND SAMPLE STORAGE

During the execution of the study and the analysis of data, raw data will be analyzed by *The Sapphire Group*. These data will be stored in locked file cabinets in a locked office area. Electronic data will be kept on secure servers accessible only by passwords. Access to data with personal identifiers will be limited to select study personnel.

Laboratory-specific or site-specific raw data, such as personnel files and equipment records, and specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, DE, or at Iron Mountain Records Management, Wilmington, DE.

PROPOSED STUDY DATES

Study dates are subject to date of protocol approval and sign-offs.

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SIGNATURES

Approved by:

Robert W. Rickard, Ph.D.
Scientific Director

Carol Gevecker Graves, Ph.D.
Principal Investigator

**FINAL PROTOCOL SUBJECT TO
EPIDEMIOLOGY REVIEW BOARD AND
INSTITUTIONAL REVIEW BOARD APPROVAL**

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APPENDICES

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APPENDIX A

COMPUTER-ASSISTED TELEPHONE INTERVIEWING (CATI)

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Aspen Systems Corporation's Survey Operations Center offers the latest in CATI technology. Aspen's boutique telephone center consists of 16 interviewer workstations and a supervisor/monitoring station located within Aspen's corporate headquarters in Rockville, Maryland.

Aspen uses CATI software that offers full telephone interviewing automation and real-time monitoring of studies while the data are being collected. The software's range of capabilities promote the ease of telephone interviewing by simplifying questionnaire programming, call management, quota cell (grouping of respondents based on demographic characteristics) control, calling results, interviewer monitoring, and data reports and delivery, including:

CATI System. The Survey Operations Center uses the Windows-based CATI software WinCati 4.2. The software promotes the efficiency of sample management by controlling the time and day when calls are made based on time zones and/or the results of the previous call. It also controls the number of times a number is attempted, works available sample evenly, and allows for the adjustment and scheduling of callbacks. RapidDial (WinCati's autodialing system) further enhances the automation of the interviewing process by increasing interviewer productivity and lowering dialing errors.

Interviewer Training. Telephone interviewers are provided project-specific instruction and training prior to placing calls. As part of their training, interviewers are given question-by-question instruction manuals as well as background information pertinent to the survey and must actively participate in scripted telephone role-playing. Additional project-specific job aids (e.g., square footage conversion charts, acronym lists, etc.) for collecting data are provided as needed.

Quality Control and Supervision of Interviewers. Quality control is an integral part of the data collection process at Aspen. It begins with interviewer training and continues throughout the data collection period until the final data file is delivered. From the supervisor/monitoring station, WinCati allows for instantaneous on-screen monitoring of telephone interviews in progress via SuperView. SuperView, coupled with our audio monitoring capabilities, allows supervisors both to see and hear the interviewers work and spot potential problems more readily. Supervisors can then rate interviewers based on their performance. The Remote Monitoring feature allows our clients to listen to calls from their offices.

CATI Reports. The WinCati software offers built-in reporting capabilities that permit the overall progress of the data collection effort to be monitored on a continual basis. Standard reports showing the current status of the sample

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database such as the distribution of records by number of call attempts, by time zone, by quota cells, by callback time, and by most recent disposition can be generated at any time. Quota cell reports, which show the number of completed interviews in relation to pre-set goals for respondents with specified characteristics, can also be run. The reporting system even monitors interviewer productivity by reporting interviewer log-on hours and completed interviews per hour. In addition to printing the reports, they can be downloaded as Excel spreadsheets and sent electronically to the client's desktop for viewing.

Data Delivery. At the completion of the telephone data collection period, a clean survey data file in ASCII, Excel, Access, SPSS, or SAS can be created on a read-only CD and/or delivered via a secured Internet account established for Aspen clients only. Additional features of WinCati allow the coding of answers to open-ended questions and the automatic generation of documentation for certain types of output files.

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APPENDIX B
CONSENT FORM

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CONSENT TO PARTICIPATE IN PFOA EXPOSURE AND HEALTH RESEARCH PROGRAM

Cross-Sectional Surveillance in a Community Sample of Clinical Measures of General Health Status Related to a Serum Biomarker of Exposure

Introduction: You are being asked to participate in a health surveillance and research program. Your participation is voluntary.

This health surveillance is being conducted in community members and parallels a health surveillance being conducted in workers at DuPont's Washington Works. Please ask questions of the site study coordinator if there is anything you do not understand.

What is the purpose of this study?

The purpose of the study is to learn whether there is any relationship between general health status and the blood level of PFOA, also known as C-8. General health status will be measured by a physical examination, blood work, pulmonary function tests, EKG, and urinalysis. These tests are similar to the general health exam that is performed by your personal physician.

What does this study involve?

Your participation in this study requires one physical exam that includes drawing of blood (about 3 tablespoons) for serum level of PFOA, clinical chemistry, and hematology (red and white blood cell counts, hemoglobin, and types of white blood cells). We will also provide urinalysis, pulmonary function tests, chest X-ray, and EKG. A physician will conduct a physical examination. In addition, you will be asked to complete a confidential questionnaire about tobacco and alcohol use and medical and work history.

Will the study be affected if you do not participate?

This research study design was chosen carefully to ensure good statistical analysis with good representation of community members across the five water districts making up the class in a class action suit against DuPont. Random sampling of the community is essential so that we have a good cross-section of people using the water in these water districts. The study will be much stronger if the people selected for sampling by the random process all participate.

What are the risks involved with being enrolled in this study?

The only risk of participating in this study would be the risk of a bruise (hematoma) where the blood is drawn and possible ordinary discomfort associated with drawing blood. Some people feel light-headed and dizzy when doing lung function tests. Rarely, a person will

faint. There will be a chair nearby where you could sit down if necessary, and the technicians are trained to monitor you for any feeling of dizziness.

Are there any benefits from participating in this study?

The benefit of participating in this study is that you may gain important knowledge about your general health with regard to, for example, your cholesterol, blood pressure, and the level of important enzymes that assist with digestion and liver and kidney function. We also hope to learn more about how exposure to PFOA may vary depending on where in the community people live.

Other important items you should know:

Withdrawal from the study: You may choose to stop your participation in this study at any time.

New information: To the best of our ability, any significant new findings during this research survey will be made known to you. The clinical services provider for this study, Examinetics, will mail a complete report of your results to your home address, or to any other address that you may provide. Your PFOA blood level will be communicated to you by letter from *The Sapphire Group*.

Confidentiality: Every effort will be taken to protect the names of the participants in this study. All analyses will be conducted on files that have had names removed, and the results will be reported as grouped data. No individuals will be identified as such in any of the published results. All data are retained in locked cabinets in offices of the study overseers, *The Sapphire Group, Inc.*, Bethesda, MD. On completion of the study, data will be transferred to locked cabinets in the Epidemiology Group, DuPont Haskell Laboratory, Newark, DE. Electronic data are kept on secure servers in databases that can only be accessed by *The Sapphire Group* or DuPont Epidemiology staff. However, you need to know that in litigation pending in the Circuit Court of Wood County, West Virginia, styled *Jack W. Leach et al.*, plaintiffs, vs. E.I. Du Pont De Nemours Co., defendant, civil action No. 01-C-608, attorneys for the parties will have access to the information generated by this study. However, access to such medical information is governed by the terms of a protective order entered by the court in the litigation.

Funding: Funding for this study has been provided by E.I. Du Pont De Nemours Co.

Number of participants: We expect about 800 participants to be enrolled in this community study.

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Who should you call with questions about this study?

Questions about this study may be directed to the principal investigator, Dr. Carol Graves, at *The Sapphire Group* (301-657-8008, ext. 205) during normal business hours.

Will you be paid to participate in this study?

No. We estimate the monetary value of the physical exam and blood work to be about \$500. You may find your personal results helpful as you consider your own health promotion activities.

CONSENT

I have read the above information about the comparison of health outcomes in the community potentially exposed to PFOA, and I have been given an opportunity to ask questions. I agree to participate in this study, and I have been given a copy of this consent document for my own records.

Your name:

Your signature:

Date:

Witness:

Date:

DRAFT

APPENDIX C

CLINICAL PATHOLOGY COLLECTION PARAMETERS

This appendix will be identical to that in the protocol for the worker study.

DRAFT

APPENDIX D
ELECTROCARDIOGRAPHY (EKG)

This appendix will be identical to that in the protocol for the worker study.

DRAFT

APPENDIX E
AUDIOMETRIC EXAMINATION

This appendix will be identical to that in the protocol for the worker study.

DRAFT

APPENDIX F

VISUAL ACUTY AND TONOMETRY EXAMINATION

This appendix will be identical to that in the protocol for the worker study.

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APPENDIX G

COMPREHENSIVE MEDICAL AND WORK HISTORY QUESTIONNAIRE

[Examinetics questionnaire in separate files.]

DRAFT

APPENDIX H

PHYSICAL EXAMINATION AND EVALUATION

[Examinetics physical examination form will be inserted here.]

DRAFT

APPENDIX I

EXYGEN STANDARD OPERATING PROCEDURE FOR PFOA ANALYSES

[Exygen SOP in separate file.]



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HEALTH EXAMINETICS

An Exemplar International Company

Confidential General Health Questionnaire

INSTRUCTIONS

PLEASE READ AND FOLLOW THESE INSTRUCTIONS CAREFULLY BEFORE ARRIVING FOR YOUR APPOINTMENT

1. Allow at least 30 minutes to fill out this health questionnaire at home, before your appointment. If you have difficulty with English or understanding the questions, have someone help you. Bring this completed questionnaire with you to your appointment.
2. Record your name and other information on page 2. Unless instructed otherwise, print your physician's or health professional's complete name, address, zip code and telephone number in the appropriate spaces on page 2.
3. Read each question carefully. Mark your answer in the oval next to the questions using an ink pen (or a very dark pencil). Completely fill in the appropriate oval. (see example below).

USE AN INK PEN (OR A VERY DARK PENCIL)

Please fill in answer choices completely



Right



Wrong



Wrong



Wrong

4. If you will be having blood drawn, consume no food or liquid (except plain water, black coffee, tea [no sweeteners/creamers] and necessary medications) for 12 hours before your appointment. This includes alcohol, soft drinks, chewing gum, candy and breath mints. **DIABETICS OMIT THIS INSTRUCTION.**
5. Please drink plenty of water so that laboratory specimens (blood and/or urine) may be obtained while you are on the unit. Fluid replacement is also important for your kidneys if you are fasting.
6. **Do not smoke** for at least 4 hours before your appointment.
7. If you wear prescription eyeglasses, bring them with you.
8. If you wear contact lenses (hard, soft, or gas permeable) please plan to remove them for the glaucoma (tonometry) test while you are on the unit. Be sure to bring a storage case and whatever solutions you may need.
9. Do not photocopy, bend, fold, staple or otherwise mutilate this questionnaire.

For _____

Location _____

Date _____

Time _____



7883

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Please fill in answer choices completely

Right Wrong Wrong Wrong

A PERSONAL AND BIOGRAPHICAL

Compared with other people your own age, how do you rate your health?

- Excellent ☐
- Very good ☐
- Good ☐
- Fair ☐
- Poor ☐

How is your health compared to last year?

- Health is better (improved) in the past year ☐
- Health is about the same (no change) ☐
- Health is worse ☐

Have you ever been rated as high risk or rejected for life insurance? ☐

What is your primary race or ethnic origin? (Response Optional)

- Native American ☐
- Asian/Asian American ☐
- Black/African American ☐
- Hispanic/Latin American ☐
- White (Caucasian) ☐
- Other ☐

B OCCUPATIONALWhat type of work do you currently do? (mark one response only)

- Manufacturing/production ☐
- Transportation equipment operator ☐
- Other equipment operator ☐
- Craft/trade ☐
- Clerical ☐
- Managerial/administrative ☐
- Sales/marketing ☐
- Professional/technical ☐
- Service ☐
- Other type of work ☐
- Retired or not working ☐

In the past year, did you breathe, at least weekly, irritating or toxic gases, vapors, dusts, fumes, or mists at work? (mark one response only)

- No, or less than once per week ☐
- Yes, 1 to 2 days per week ☐
- Yes, 3 to 4 days per week ☐
- Yes, almost constantly ☐

If you were exposed to dust, was the dust exposure:

- Not exposed ☐
- Mild ☐
- Moderate ☐
- Severe ☐

B OCCUPATIONAL (continued)

If you were exposed to irritating or toxic gases, was the exposure:

- Not exposed ☐
- Mild ☐
- Moderate ☐
- Severe ☐

In your work, have you performed task(s) that have caused pain, soreness or stiffness that interfered with your job, hobbies, and/or personal life in any of the following places? (mark all that apply)

- Fingers ☐
- Hands ☐
- Wrists ☐
- Arms ☐
- Elbows ☐
- Shoulders ☐
- Neck ☐
- Back ☐
- Hips ☐
- Legs ☐
- Knees ☐
- Ankles ☐
- Feet ☐

Have you ever had daily or weekly exposure (on your skin or by breathing) to any of the following at work? (mark all that apply)

- Arsenic ☐
- Asbestos ☐
- Cadmium ☐
- Coal dust ☐
- Dust or smoke ☐
- Isocyanates ☐
- Lead ☐
- Mercury ☐
- Nickel or chromium ☐
- Polychlorinated biphenyls (PCBs) ☐
- Radiation or X-rays ☐
- RCF (refractory ceramic fibers) ☐
- Silica (foundry or sandblasting) ☐
- Solvents or degreasers ☐
- Talc ☐
- Urethane foams ☐
- Welding fumes or soldering agents ☐
- Wood dust ☐
- None of the above ☐

5567



7883

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Please fill in answer choices completely



Right



Wrong



Wrong



Wrong

C HOBBIES

Do you have any hobbies or "side jobs" that require you to regularly use chemicals, such as solvents, lead, mercury, furniture stripper, paint, insecticides, herbicides, or chemical foams? ☐

Do you have any hobbies or "side jobs" that cause you to be exposed to a lot of dust (woodworking, autobody work, construction, sandblasting, demolition work, auto mechanics, etc.)? ☐

D FAMILY HISTORY

Have any of your blood relatives had any of the following?
(mark all that apply)

	Mother and/or Father	Sister, Brother, Son or Daughter
Asthma	☐	☐
Birth defects	☐	☐
Cancer of the breast	☐	☐
Cancer of the colon (large intestine or rectum)	☐	☐
Cancer of the lung	☐	☐
Cancer of the prostate	☐	☐
Cancer of uterus, cervix or ovaries	☐	☐
Depression	☐	☐
Drinking problem/alcoholism	☐	☐
Glaucoma	☐	☐
Hearing loss or deafness before age 50	☐	☐
Heart attack before age 50	☐	☐
High blood pressure	☐	☐
High cholesterol level	☐	☐
Intestinal polyps (growths)	☐	☐
Lymphoma or leukemia	☐	☐
Osteoporosis (thinning of the bones)	☐	☐
Peripheral vascular disease (very poor circulation)	☐	☐
Psychological or mental disorder	☐	☐
Severe bleeding tendency (e.g. hemophilia, "bleeder")	☐	☐
Sickle cell anemia	☐	☐
Stroke	☐	☐
Sugar diabetes	☐	☐
Tuberculosis (TB)	☐	☐
Other cancer (malignancy)	☐	☐
Other major lung problem(s)	☐	☐

E CANCER

Have you ever been diagnosed as having cancer or other tumor in any of the following? (mark all that apply)

Bladder	☐
Bone	☐
Brain	☐
Breast	☐
Colon (large intestine or rectum)	☐
Gallbladder	☐
Kidney	☐
Leukemia	☐
Liver	☐
Lung:	
In the past year	☐
More than a year ago	☐
Lymphoma	☐
Mesothelioma	☐
Prostate	☐
Skin	☐
Stomach	☐
Testicular	☐
Uterus, cervix or ovaries	☐
Other cancer or tumor not listed above	☐

F INFECTIOUS DISEASES

Have you had a diphtheria/tetanus (lockjaw) booster vaccination in the past ten years? ☐

Have you been vaccinated against hepatitis? ☐

Have you had any of the following conditions?

Encephalitis	☐
Hepatitis B or C	☐
Mumps	☐
Measles	☐
Meningitis	☐
Rheumatic fever:	
In the past year	☐
More than a year ago	☐
Tuberculosis:	
In the past year	☐
More than a year ago	☐

5567

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7883

G HOSPITALIZATION AND SURGICAL

Have you been in the hospital as a patient in the past year? ☐

Have you ever had a surgical operation on any of the following?
(mark all that apply)

- Skin ☐
- Brain ☐
- Eye ☐
- Nose ☐
- Ear ☐
- Throat ☐
- Esophagus ☐
- Voice box (larynx) ☐
- Chest:
 - In the past year ☐
 - More than a year ago ☐
- Breast removal (mastectomy) ☐
- Lung ☐
- Heart ☐
- Artery, coronary bypass, or angioplasty (to correct blocked artery or aneurysm):
 - In the past year ☐
 - More than a year ago ☐
- Stomach ☐
- Intestine or colon ☐
- Appendix ☐
- Gallbladder ☐
- Liver ☐
- Kidney ☐
- Hernia (rupture) ☐
- "C" Section (Caesarean section) ☐
- Both ovaries removed:
 - At age 44 or younger ☐
 - At age 45 or older ☐
- Hysterectomy ☐
- Prostate ☐
- Testicles ☐
- Rectum (hemorrhoids or piles) ☐
- Neck ☐
- Back ☐
- Varicose veins ☐
- Other surgery (please specify) ☐
- Never had surgery ☐

Please fill in answer choices completely

☐ Right
 ☐ Wrong
 ☒ Wrong
 ☒ Wrong

H MEDICATIONS

Are you currently taking prescription medication for any condition?

- Yes, and I usually take the medication as prescribed ☐
- Yes, and I sometimes take the medication as prescribed ☐
- Yes, and I seldom take the medication as prescribed ☐
- No ☐

Have you taken any of the following medications for more than 10 days? (mark all that apply)

	Currently Taking	In the Past Year
Antacids or stomach medicine	☐	☐
Antibiotics (penicillin, amoxycillin, etc.)	☐	☐
Anticoagulants (blood thinners)	☐	☐
Anticonvulsant, seizure medicine (dilantin, phenobarbital, etc.)	☐	☐
Antidepressants / mood elevators	☐	☐
Asthma, breathing or lung medicine	☐	☐
Birth control pills	☐	☐
Cholesterol lowering agents	☐	☐
Cortisone or steroids	☐	☐
Diuretic (water) pills	☐	☐
Fay fever medicines	☐	☐
Heart medicine (such as digitalis, nitroglycerine, lisdal/propranolol, isordil/isosorbide dinitrate, etc.)	☐	☐
Herbal remedies	☐	☐
High blood pressure pills	☐	☐
Hormone replacement therapy	☐	☐
Iron	☐	☐
Insulin shots for diabetes	☐	☐
Non-prescription pain killers more than twice weekly (e.g., Anacin, Bufferin, APC's, Tylenol, Advil, Motrin, Aleve, aspirin, ibuprofen, acetaminophen, etc.)	☐	☐
Pills for diabetes	☐	☐
Prescription pain killers (codeine, morphine, demerol, Darvocet, Percodan, etc.)	☐	☐
Sedatives or sleeping pills (barbiturates, nembutal, etc.)	☐	☐
Stimulants (benzadrine, amphetamines, etc.)	☐	☐
Thyroid medicine	☐	☐
Tranquilizers	☐	☐
Weight control pills	☐	☐
Other hormones	☐	☐
Other prescription medications	☐	☐

5567



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7883

I ALCOHOL USE

How many drinks do you consume on average? (Include bottles or cans of beer, cocktails or shots of hard liquor, or glasses of wine) (mark one response only)

- None ☐
- 1 to 3 per week ☐
- 4 to 7 per week ☐
- 1 - 2 per day ☐
- 3 - 4 per day ☐
- 5 - 6 per day ☐
- More than 6 per day ☐

When you drink alcohol, what is the maximum number of drinks you consume in one hour?

- 1 or fewer per hour ☐
- 2 per hour ☐
- 3 per hour ☐
- 4 or more per hour ☐

In the past 6 months, have you felt you should cut down on your drinking? ☐

In the past 6 months, have people annoyed you by criticizing your drinking? ☐

In the past 6 months, have you felt badly or guilty about your drinking? ☐

In the past 6 months, have you taken a drink first thing upon awakening to get rid of a hangover or to steady your nerves (eye opener)? ☐

J MOTOR VEHICLE SAFETY

How many times in the past year did you drive after having three or more alcohol-containing drinks within 2 hours, or ride with a driver who had this much to drink?

- Never ☐
- 1 to 3 times ☐
- 4 to 11 times ☐
- Monthly ☐
- Weekly ☐
- Daily ☐

On average, how close to the speed limit do you usually drive?

- Below or within 5 mph over the limit ☐
- 6 to 10 mph over the limit ☐
- 11 to 15 mph over the limit ☐
- More than 15 mph over the limit ☐

Please fill in answer choices completely

Right Wrong Wrong Wrong

J MOTOR VEHICLE SAFETY (continued)

What percentage of the time do you wear a seatbelt or shoulder harness while driving or riding in a car, truck, or van?

- Always (100%) ☐
- Usually (51 to 99%) ☐
- Occasionally (20 to 50%) ☐
- Rarely (less than 20%) ☐

K TOBACCO USE

Do you now or did you ever smoke cigarettes? (mark one response only)

- No, I never have ☐
- Yes, I still smoke ☐
- I quit 10 or more years ago ☐
- I quit 5 to 9 years ago ☐
- I quit 1 to 4 years ago ☐
- I quit less than a year ago, but not in the past month ☐
- I quit in the past month ☐

Mark the number of packs of cigarettes you smoked per day in the past two years or the last two years before quitting: (1 pack = 20 or 25 cigarettes)

- Less than 1/2 pack per day ☐
- 1/2 pack per day ☐
- 1 pack per day ☐
- 2 or more packs per day ☐

Do you currently use any of the following tobacco products at least once a day?

- Pipes ☐
- Cigars ☐
- Smokeless (snuff, chewing tobacco) ☐

5567



7883

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Please fill in answer choices completely



Right



Wrong



Wrong



Wrong

L PHYSICAL ACTIVITY

Compared with other people your age, how would you describe the amount of exercise you get, counting work and leisure activities?

- Much more active ☐
- Somewhat more active ☐
- About average ☐
- Somewhat less active ☐
- Much less active ☐
- Medical condition limits my activity ☐

In an average week, how many times do you engage in vigorous aerobic physical activity? (Aerobic means work that lasts at least 20 minutes without stopping and that is hard enough to make you breathe heavier and your heart beat faster.)

- Less than 1 time per week ☐
- 1 to 2 times per week ☐
- 3 or more times per week ☐

Do you do muscle-strengthening exercises such as vigorous manual labor, push-ups, sit-ups, or weight training three or more times per week? ☐

Do you do stretching and/or warm-up exercises for muscle and joint flexibility three or more times per week? ☐

M SKIN

Have you had any of the following in the past year?

- Noticed a mole, wart, or growth that is getting larger, darker or bleeding ☐
- Sore or swelling that has not healed in the past 2 months ☐
- Eczema or recurrent rash ☐
- Severe skin rash or dermatitis ☐
- Any type of skin rash you feel was related to your work ☐
- Itching, dryness, cracking, or peeling and scaling of the hands ☐
- Burning sensation in the hands, or reddening of the skin ☐
- Cracking or bleeding of the skin on your hands ☐
- Cracking or bleeding of the skin on your feet ☐
- Other skin problems ☐

N URINARY TRACT

Have you ever had any of the following?

- Kidney disease or inflammation/nephritis ☐
- Renal dialysis ☐

Have you had any of the following in the past year?

- Blood in your urine ☐
- Bladder disease ☐

O METABOLISM

Have you ever had any of the following?

- Thyroid problem or disorder ☐
- Elevated blood sugar or diabetes ☐
- Diabetes only during pregnancy ☐
- Childhood (before age 30) onset diabetes ☐
- Adult (at age 30 or older) onset diabetes ☐
- Other metabolic problems ☐

Have you had any of the following in the past year?

- Unintentional gain of 10 or more pounds ☐
- Unintentional loss of 10 or more pounds ☐

P EYES AND VISION

Have you ever had any of the following?

- Cataracts ☐
- Glaucoma ☐
- Left eye: blindness or lost vision ☐
- Right eye: blindness or lost vision ☐

Have you had any of the following in the past year?

- Corrective lens prescription has changed more than once ☐
- Sudden, temporary loss of vision ☐
- Eyesight has become worse and you have not seen a health professional concerning this ☐
- Vision is blurred or cloudy ☐
- Frequently irritated, red or bloodshot eyes ☐
- Burning, tearing or redness of eyes that occurs only while at work ☐
- Have experienced double vision ☐
- Bright colored rings (halos) seen around light bulbs ☐
- Any other eye or vision problem ☐
- None of the above ☐

Do you wear corrective lenses (eyeglasses, bifocals, or trifocals)? ☐

Do you wear contact lenses? ☐

How many hours do you spend daily looking at or working steadily with a computer monitor (VDT, Video Tube, CRT, LCD, etc.)?

- None ☐
- Less than 1 hour per day ☐
- 1 to 2 hours per day ☐
- 3 to 4 hours per day ☐
- 5 to 6 hours per day ☐
- 7 or more hours per day ☐

Do you have any difficulty distinguishing between colors?

- Yes, and this affects my ability to perform my job ☐
- Yes, but this does not affect my work ☐
- No ☐

5567



7883

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Please fill in answer choices completely

Right Wrong Wrong Wrong

Q EARS AND HEARINGHas your hearing been tested by someone other than your current employer? ☐Have you been to an ear specialist in the past year? ☐Have you ever had any of the following?Perforated (ruptured) eardrum ☐Frequent (more than 1 per year) ear infections after age 6 ☐Ear drainage ☐Ear surgery recommended but not performed ☐Noticeable hearing loss in left ear ☐Noticeable hearing loss in right ear ☐Hearing that comes and goes (fluctuating hearing loss) ☐Sudden or rapid loss of hearing ☐Severe persistent ringing, buzzing or roaring in the ears ☐Family or friends tell you that you have a hearing problem ☐Difficulty understanding speech in a crowded (noisy) room ☐Listen to loud music at a club, at home or with a "boom box", using a "walkman", or ear phones at high volume levels for 3 or more hours per week ☐Hobbies or home activities where you are exposed to loud noise (gunfire, lawn mowers, chain saw, gas or electric motors) ☐Have you had any of the following in one or both ears in the past year?Ear pain or discomfort ☐A feeling of fullness ☐Ear drainage ☐Imbalance ☐Dizziness (room spinning) ☐Severe persistent ringing, buzzing or roaring in the ears ☐Other ear disease, symptom, or injury ☐

Do you try to protect your hearing by wearing earplugs or earmuffs when you are exposed to loud noise?

Not exposed to loud noise ☐Always (100%) ☐Usually (51 to 99%) ☐Occasionally (21 to 50%) ☐Rarely (1 to 20%) ☐Never (0%) ☐**R RESPIRATORY**Have you had any of the following in the past year?More than 2 or 3 sinus infections (sinusitis) ☐Increased hoarseness or change in your voice ☐Noticed that certain chemicals make you cough ☐Usually have a cough (including smoker's cough) ☐Cough up phlegm (sputum) from the lungs (chest) daily for 3 or more months ☐If yes, is it more than 1/4 cupful of phlegm (from the lungs daily) ☐Other lung problems, disease, symptoms or injury in the past year ☐

Have you had any of the following?

Asthma ☐Abnormal chest X-ray ☐Asbestosis ☐Chronic bronchitis or emphysema ☐Pneumonia ☐Coughed up blood ☐Problems with wheezing (whistling) in the chest ☐None of the above ☐**S HEART AND BLOOD VESSELS**Have you ever had any of the following?Enlarged heart ☐Heart murmur ☐Angina ☐Myocardial infarction (heart attack) ☐Heart failure ☐Mitral Valve Prolapse ☐Abnormal ECG (electrocardiogram) ☐High blood pressure ☐Peripheral vascular disease (very poor circulation) ☐Fingers become cold, white or painful when hit or exposed to cold or vibration (e.g., Raynaud's disease) ☐Stroke ☐High cholesterol level (200 or higher) ☐Pain or cramps in legs when walking ☐Swelling in your legs or feet (not caused by standing or walking) ☐Palpitations (rapid or irregular pounding of the heart lasting seconds or minutes) more than once per month ☐Difficulty sleeping without being propped-up (for example: using 3 or more pillows) ☐Phlebitis (inflammation of the veins) in the legs ☐Other heart disease, problem or disorder ☐Other cardiovascular symptoms ☐

5567



7883

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Please fill in answer choices completely



Right



Wrong



Wrong



Wrong

T BLOODHave you ever had any of the following?

- Hemophilia (bleeding problem) ☐
- Sickle cell anemia ☐
- Other anemia ☐
- Tendency to bruise easily or bleed excessively ☐
- Have recently noticed enlarged "lymph" glands or nodes ☐
- Abnormal hemoglobin or red blood cells ☐
- Been refused as a blood donor in the past 5 years ☐
- Received a blood transfusion (another person's blood) in the past 10 years ☐
- Other blood disease, symptom or injury ☐

U BONES, MUSCLES AND JOINTSHave you ever had a back strain or injury?

- In the past year ☐
- More than a year ago ☐

Have you had any of the following in the past year?

- Back pains that prevented you from working (at home or at a job) more than once ☐
- Bursitis ☐
- Carpal Tunnel Syndrome ☐
- Degenerative disc disease ☐
- Herniated disc or sciatica ☐
- Elbows or shoulders often swollen and tender ☐
- Finger joints often swollen and tender ☐
- Gout ☐
- Hips, knees or ankles often swollen and tender ☐
- Osteoarthritis ☐
- Pain in your hand(s) at night ☐
- Rheumatoid arthritis ☐
- Tendonitis ☐
- Wake up in morning often with stiffness or aching in joints or muscles ☐
- Other bone or joint disease, symptom, injury or surgery ☐

V GASTROINTESTINAL TRACT**STOMACH, INTESTINES, BOWELS**

Have you had any of the following?

- Stomach, peptic or duodenal ulcers ☐
- Current non-repaired hernia (rupture) ☐
- Noticed blood in stools in the past year ☐
- Recent definite change in bowel movement, appearance and/or frequency ☐

V GASTROINTESTINAL TRACT (continued)**LIVER**

Have you had any of the following?

- Cirrhosis ☐
- Jaundice (yellow eyes or skin) ☐
- Liver disease, trouble or dysfunction ☐

BOWELS

If you are over the age of 40:

Do you have your stool checked for hidden (occult) blood at regular intervals (every 2 years)?

- Yes ☐
- No ☐
- I don't know ☐

Do you have regular (every 2 years) rectal examinations (gloved finger) performed by a health professional?

- Yes ☐
- No ☐
- I don't know ☐

Have you had a rectal examination using a sigmoidoscope or colonoscope in the past five years? ☐**W NUTRITION**How many servings of fruits and vegetables do you eat on a typical day? (mark one response only)

- | | |
|--|--|
| Zero servings ☐ | Four servings ☐ |
| One serving ☐ | Five servings ☐ |
| Two servings ☐ | Six servings ☐ |
| Three servings ☐ | Seven or more servings ☐ |

How many servings of whole-grain cereals, whole-grain breads, brown rice, beans, or bran do you eat on a typical day? (mark one response only)

- | | |
|--|--|
| Zero servings ☐ | Four servings ☐ |
| One serving ☐ | Five servings ☐ |
| Two servings ☐ | Six servings ☐ |
| Three servings ☐ | Seven or more servings ☐ |

How many servings of high-fat foods, such as those listed below, do you eat on a typical day? (red meat, processed meats [bologna, hot dogs, sausage, etc.] fried foods, gravy, cream or butter sauces, mayonnaise, salad dressing, whole-milk dairy products, etc.) (mark one response only)

- | | |
|--|--|
| Zero servings ☐ | Four servings ☐ |
| One serving ☐ | Five servings ☐ |
| Two servings ☐ | Six servings ☐ |
| Three servings ☐ | Seven or more servings ☐ |

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Please fill in answer choices completely

☐ Right ☐ Wrong ☒ Wrong ☒ Wrong

X NERVOUS SYSTEMS

Have you had any of the following?

- Psychological or mental disorder ☐
- Severe mood swings (e.g., feeling up and down) within the past 6 months ☐
- Depression or frequently feeling down, unable to enjoy life ☐
- Frequent episodes of nervousness or stress ☐
- Irritability and difficulty controlling temper ☐
- Chronic Fatigue Syndrome (CFS) or Chronic Fatigue Immune Deficiency Syndrome (CFIDS) ☐
- Neuritis (pinched or inflamed nerves) ☐
- Difficulty falling asleep, staying asleep or awakening early ☐
- Tend to have trouble concentrating (e.g. when calculating and doing arithmetic) ☐
- Tend to have trouble remembering (e.g. difficulty with short term memory) ☐
- Head injury that caused you to black out (concussion) in the past year ☐
- Fainted or loss of consciousness in the past year ☐
- Feel dizzy, light-headed, excessive drowsiness (like you have been drugged) ☐
- Severe headaches more than twice per year that were not relieved by non-prescription headache medications ☐
- A tremor (uncontrolled shaking) in the hands or legs ☐
- Narcolepsy ☐
- Numbness or tingling in arms (occurs every month) ☐
- Numbness or tingling in hands (occurs every month) ☐
- Numbness or tingling in feet (occurs every month) ☐
- Loss of feeling or sensation in arms, hands, legs or feet (occurs every month) ☐
- Felt tired, worn out, sad or blue in the last 6 months ☐
- Thoughts about suicide in the last 6 months ☐
- Other neurological or psychological disease, symptom, or injury ☐
- None of the above ☐

Frequent (at least once per week) problem with faintness or lightheadedness that occurs when:

- Standing or getting out of bed ☐
- Walking or running ☐
- Climbing stairs ☐

During the past month, were you under or did you feel you were under strain, stress, or pressure?

- Yes, almost more than I could bear or stand ☐
- Yes, some; more than usual ☐
- Yes, some; about usual ☐
- Yes, a little ☐
- Not at all ☐

X NERVOUS SYSTEMS (continued)

During the past month, did you feel sad, hopeless, discouraged, or did you have so many problems that you wondered if anything was worthwhile?

- Extremely so (to the point that I have just about given up) ☐
- Very much ☐
- Quite a bit ☐
- Some; enough to bother me ☐
- A little bit ☐
- Not at all ☐

During the past month, how relaxed or tense were you?

- Very relaxed ☐
- Relaxed ☐
- Somewhat Relaxed ☐
- A little tense ☐
- Tense ☐
- Very tense ☐

During the past month, how depressed or cheerful were you?

- Very depressed ☐
- Depressed ☐
- A little depressed ☐
- Somewhat cheerful ☐
- Cheerful ☐
- Very cheerful ☐

Y OTHER ILLNESSESDo you have any other serious illness not mentioned throughout the questionnaire? ☐Are you currently being treated by a health professional for a condition or disease ☐Do you have a medical or personal problem you would like to discuss with a health professional? ☐**Z HEIGHT AND WEIGHT**

Please provide your correct height and weight (WITHOUT SHOES) by writing in the box and filling in the appropriate numbered bubbles.

	HEIGHT		HEIGHT		WEIGHT	
	Feet	Inches	Feet	Inches	pounds	
E						
X	5	07				
A	☐	☐	☐	☐	☐	☐
M	☐	☐	☐	☐	☐	☐
P	☐	☐	☐	☐	☐	☐
L	☐	☐	☐	☐	☐	☐
E	☐	☐	☐	☐	☐	☐

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Please fill in answer choices completely



Right



Wrong



Wrong



Wrong

AA FOR WOMEN ONLY

Health Examinetics understands that the following information is personal and confidential. These questions are necessary for assessing cancer risk.

How long has it been since your last mammogram (breast X-ray)?

- In the past year ☐
- 1 to 2 years ago ☐
- 3 or more years ago..... ☐
- Never ☐

How long has it been since you had your breasts examined by a physician or nurse?

- In the past year ☐
- 1 to 2 years ago ☐
- 3 or more years ago..... ☐
- Never ☐

Have you received instruction on how to properly examine your breasts?

- Yes, and I perform once per month ☐
- Yes, and I perform less than once per month ☐
- Yes, and I perform more than once per month ☐
- No or not sure ☐

How many biopsies or needle aspirations of the breast have you had?

- None or Do not know ☐
- One ☐
- Two or more ☐

How long has it been since you had your last pap smear?

- In the past year ☐
- 1 to 2 years ago ☐
- 3 or more years ago..... ☐
- Never ☐

How old were you when your menstrual periods started?

- 11 or younger ☐
- 12 to 13 ☐
- 14 or older ☐

How old were you when you had your first child?

- Have not had a child ☐
- 19 or younger ☐
- 20 to 24 ☐
- 25 to 29 ☐
- 30 or older ☐

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TITLE PAGE

Jack W. Leach, et al. v. E. I. du Pont de Nemours and Company
Circuit Court of Wood County, WV, Civil Action No. 01-C-608

Implementation Plan: May 1, 2003 Injunction Order
Appendix No. 2

Study-Specific Standard Operating Procedure :
Documentation, Accountability, and Sample Chain of Custody Requirements
for the Shipping of Serum Samples

IMPLEMENTATION DIRECTOR

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ANALYTICAL FACILITY MANAGEMENT

John Flaherty
Vice President of Operations
Exygen Research
3058 Research Drive
State College, PA 16801
(814) 272-1039

1. SCOPE AND APPLICABILITY

This SOP governs the documentation, accountability, and sample chain of custody requirements for the shipment of serum samples to the analytical facility.

2. REPONSIBILITIES

2.1 The Good Laboratory Practices (GLP) Expert and Analytical Facility Management are responsible for conducting the necessary training covering proper documentation, accountability, and sample chain of custody requirements. This training will, at minimum, be given to the Principal Contact from each of the three clinics.

2.2 The phlebotomist who performs the blood collection is responsible for ensuring that the Chain of Custody/Analysis Request Form (COC) (Figure 1) is completely and correctly filled out and signed by staff members who package and ship the serum samples.

2.3 The Principal Investigator at the analytical facility is responsible for ensuring that the chain of custody remains intact after the samples are received by the analytical facility.

3. DEFINITIONS

GLP Expert: Patricia D. Royal, M.S., President, Quality Systems Consultants, Inc., 80 Main Street, Plympton, MA 02367
(781) 585-9370

Analytical Facility Management: John Flaherty, Vice President of Operations, Exygen Research 3058 Research Drive, State College, PA 16801
(814) 272-1039

Principal Investigator: Emily Decker, Scientist
Exygen Research, 3058 Research Drive, State College, PA
16801 (814) 272-1039

4. MATERIALS AND EQUIPMENT

- 4.1 Chain of Custody/Analysis Request Forms, triplicate design.
- 4.2. A readily available source of dry ice pellets. Also, necessary personal protective equipment appropriate for handling dry ice.
- 4.3 Coolers numbered and coded with the clinic's unique identification assigned for this project. They must be purchased from Uline Shipping Supplies Specialists. The Uline part number for the required coolers is S-7888. (This part number corresponds to a package which includes one 19 x 12 x 12.5 cooler and one shipping box). These will be provided to each clinic.
- 4.4 Plastic bags for use as secondary sample packaging prior to shipping. These will also be obtained from Uline, part number S-1707 (3 x 5, 4 mil reclosable poly bags, 1000 per carton). These will be provided to each clinic.

5. PROCEDURE

- 5.1. Before collecting any blood, the completed and signed consent form must be verified. When each sample is collected the clinic will assign a unique alphanumeric identification code to each sample based on the clinic's identification code and patient code. This number will be entered into the Clinic Sample Identification column of the COC sheet. Likewise, the date and time of each blood sample collection will be recorded, and the date and time that the serum sample preparation is completed will be entered into the appropriate column on the COC sheet.
- 5.2. Coolers will be provided to each clinic (see section 4.3 above). Each cooler will be assigned a unique alphanumeric identification number, which will be written on the cooler. Each clinic will derive its own cooler numbering system, the only condition being that each number contains the clinic's identification code.
- 5.3. Before packaging, the primary serum sample containers must be placed individually into small plastic bags as secondary packaging (which will also be provided to each clinic; see section 4.4 above). The secondary packaging must have the unique Clinic Sample Identification sample code written on it in permanent black ink.
- 5.4. Before the samples are packed in dry ice, a person other than the packager will verify that the samples documented on the COC form match exactly with those being packed in the cooler. The verifier then initials and dates the Cooler Contents Verified prompt.

- 5.5. Samples are to be dispersed in a cooler full of dry ice pellets, arranged in such a way as to minimize the possibility of impact, and such that they are completely covered with the pellets. The cooler must be completely filled with dry ice pellets. No more than 20 samples are to be placed in one cooler. The cooler will be covered promptly after packing with dry ice pellets to minimize loss of dry ice to sublimation.
- 5.6. The time between the completion of serum preparation and packaging for shipment must be able to be accounted for on the COC page. **There must not be any unexplained gap in the chain of custody for the samples.**
- 5.7. After the serum samples are packaged, the Cooler ID# prompt will be completed. Section 2 (Personnel Involved in Shipment of Samples) of the COC form also will be completed at this time, if it has not been previously. The printed name, signature, initials and date of signature need to be recorded for each person who entered information anywhere on the form. The page number prompts also need to be filled in.
- 5.8. The person who packaged the samples then completes the first line in the Relinquished by section, and verifies that any unused prompts are lined out.
- 5.9. The COC form is then faxed to Exygen Research Sample Receiving using the fax number at the top of the form. After it is faxed, the pink copy is removed and becomes part of the clinic's records. The white original and yellow copies are to be placed in an envelope, which is then placed into the shipping carton with the cooler that contains the samples to which the COC form corresponds.
- 5.10. The serum samples are required to be shipped to Exygen Research Sample Receiving at the address given at the top of the COC form by **FedEx overnight** delivery. This is the only carrier and method of shipment that is acceptable.
- 5.11. Upon receipt of the samples by Exygen Research, the chain of custody procedure will follow Exygen SOP V401, Contract Research Sample Handling . After Exygen Sample Receiving completes the Received by section, a copy of the COC will be faxed back to the clinic, which will be kept with the clinic's records for this study.
- 5.12. Any problems with delivery or condition of samples upon arrival at Exygen will be documented. The Exygen Principal Investigator will notify the clinic and the Implementation Director of any problems immediately.

5.13. Correction and Clarification of Raw Data

5.13.1. As soon as any information has been entered on the COC form, it becomes raw data. At this point, this form is not to be discarded for any reason. If it becomes damaged, information may be transcribed onto another sheet, but the transcription process will be documented via footnote on the new page. The original damaged sheet must be attached to the transcribed page.

5.13.2. If errors are made in handwritten entries, or if clarifications are to be made, the errant information will be crossed out with a single line, so as not to obscure the original entry. Then, depending upon the nature of the error, an appropriate error code is chosen (see Figure 2), and the error code is initialed and dated. If the error is more complex than what is covered by the error code list, a detailed explanation will be provided by footnote on the COC page.

5.14. See Figure 3 for a properly filled out COC form, and for examples of the use of the error coding system.

6. REFERENCES

Exygen Research SOP V401, Contract Research Sample Handling

7. FIGURES

Figure 1. Chain of Custody/Analysis Request Form

Figure 2. List of Error Codes

Figure 3. Example COC Sheet and Use of Error Codes

8. SIGNATURES (SOP becomes effective on the date it is signed by the implementation director)

Robert L. Grob, Ph.D.
Implementation Director

Date

John M. Flaherty.
Analytical Facility Management

Date

Page 6 of 8

Figure 2. List of Error Codes

EXPLANATION OF ERROR CODING

Correction of raw data is made by drawing a single line through the erroneous data and/or write-over, and filling in the correct data. Care must be taken not to obscure the original entry. The correction is initialed and dated by the individual making the correction, and an explanation is given for the correction.

The following error codes shall be used to explain corrections:

- (wl) Inadvertently recorded in Wrong Location
- (cc) Changed for greater Clarity
- (wo) Write Over (Technician inadvertently wrote over the error instead of drawing a line through the error)
- (sp) Spelling error
- (ir) Inadvertently not Recorded at time of initial observation
- (ce) Calculation Error
- (re) Recording Error
- (ne) Numerical transcription Error
- (ee) Entry not initialed and dated at time of entry
- (te) Transcription Error
- (wd) Wrong Date entry
- (sm) Stray Marks appearing on data with no other feasible explanation
- (le) Late Entry
- (tl) Technical error

Codes, other than those defined above may be used, if explained or defined by footnote on the COC form. Errors that cannot be explained using an error code must be fully detailed at the time of correction.

Error codes are to be circled and written above or close to the correction. If this is not feasible, the error should be footnoted with an asterisk or other symbol, and explained at the bottom of the page.

Page 8 of 8