Appendix G:

Amendments and Deviations

(Copies)

Study Plan Deviation

Study No.: 01 G 00 0 02

Date: 8.5.00

Deviation No.: 1

Subject : Fabric conditioning

Page: 8 Paragraph: 5.3.2

Change: In addition to the description in the study plan (paragraph 5.3.2, page 8) the fabrics were pre-conditioned with deionisized water (spray application).

Reason: Improvement of ability to take either saline (groups A, C, E, G, I) or liquid test article (groups B,D,F.H) into the fabrics.

Signature Study Director: i.V. B. Sokol

date 8.5.00

Head, QAU: M.B. Ketkar

date 08.05.2000.

Original: study director
copy: QUA

Study Plan Deviation

Study No.: 01 G 00 0 02

Date: 10.5.00

Deviation No.: 2

Subject : group description and labelling

Page: 8 Paragraph: 5.3, last section

Change: Test article dosage will be as follows: for the dried cotton gauze approxi mately 1 square inch will be applied daily (**groups C, E, G and I**); for the liquid 0.1 ml will be applied daily (**groups B, D, F and H**); for controls (group A) 0.1 ml of saline will be applied daily.

Reason: Typing error

Signature Study Director: i.V. B. Sörl
date 10.5.00

Head, QAU: M.B. Ketkar
date 10.05.2000

Original: study director
copy: QUA

Study Plan Amendment

Date: November 26, 2001

No.: 1

Subject: Blood serum analysis for PFOS, PFHS and M570 on 52 blood samples at CENTRE, State College, PA, U.S.A.

Page: 10
Paragraph: 5.10 Line: 4,5

Change: Since PFOS was found in the liver samples, PFOS, PFHS and M570 will be analyzed in 52 blood samples according to the attached tabulation
at CENTRE Analytical Laboratories, Inc.
(attn. Dr John Flaherts & Mrs. Kim Doolittle),
Research Drive
State College, PA 16801, U.S.A.

Reason: Bottle-neck in the bio-analytical department at Fraunhofer ITA

Signature Approval:

Fraunhofer ITA
Study Director

26.11.2001 C. Dasenbrock
Date C. Dasenbrock

Monitor of Sponsor

John L. Butenhoff 1/10/02
Date J.L. Butenhoff

cc:
Fraunhofer ITA QAU

26.11.2001 M.B. Ketkar
Date M.B. Ketkar

Animal and Serum Sample Identification

Fraunhofer ITA Study N°: **01 G 00 0 02**

Day of shipment on dry ice: November 26, 2001.
Each plastic bag containing the requested sample groups (2M/2F) is marked in its 1st line with "01 G 00 002 Serum", in its 2nd and 3rd line with the group number / consecutive animal number within the group and the day of sample taking, e.g. "Gr. 15/03,04 D14" for males and "Gr. 16/03,04 D14" for females. The same information is given on each of the tubes containing the rat blood serum.

Repeat-Dose Dermal Toxicity/Absorption Study of Several Fabric Protectors in Rats
Serum Analysis of Collected Samples

The following serum samples will be analyzed in duplicate for PFOS, PFHS and M570 with one matrix spike per group at Day 28:

Group ID	Sample	D4 = Day 4	D14 = Day 14	D28 = Day 28	D42 = Rec. Day 14	Totals
A 01 A 02	Control	None	None	2M=**Gr. 01/ 05, 06** 2F=**Gr. 02/ 05, 06**	2M=**Gr. 01/ 07, 08** 2F=**Gr. 02/ 07, 08**	**8**
C 05 C 06	USA Fabric Protector Cloth (Red Can) T-7338	None	None	2M=**Gr. 05/ 05, 06** 2F=**Gr. 06/ 05, 06**		**4**
E 09 E 10	OUS Fabric Protector Cloth (Red Can) T-7339	None	None	2M=**Gr. 09/ 05, 06** 2F=**Gr.10/ 05, 06**	None	**4**
G 13 G 14	Carpet Protector Cloth (Mill Applied) T-7341	None	None	2M=**Gr. 13/ 05, 06** 2F=**Gr. 14/ 05, 06**	None	**4**
H 15 H 16	Carpet & Uphol. Protector Liquid (K-Salt) T-7342	2M=**Gr. 15/ 01, 02** 2F=**Gr. 16/ 01, 02**	2M=**Gr. 15/ 03, 04** 2F=**Gr. 16/ 03, 04**	2M=**Gr. 15/ 05, 06** 2F=**Gr. 16/ 05, 06**	2M=**Gr. 15/ 07, 08** 2F=**Gr. 16/ 07, 08**	**16**
I 17 I 18	Carpet & Uphol. Protector Cloth (K-Salt) T-7343	2M=**Gr. 17/ 01, 02** 2F=**Gr. 18/ 01, 02**	2M=**Gr. 17/ 03, 04** 2F=**Gr. 18/ 03, 04**	2M=**Gr. 17/ 05, 06** 2F=**Gr. 18/ 05, 06**	2M=**Gr. 17/ 07, 08** 2F=**Gr. 18/ 07, 08**	**16**
	Grand Total Number of Samples					**52**

C. Darnbrook

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PROTOCOL AMENDMENT

Amendment Number: 2
Effective Date: 1/11/02

Exygen Study Number: 023-068 Protocol Number: 01G 00 0 02

DESCRIPTION OF AMENDED SECTION

Section 5.10 Blood and Liver Samples

AMENDED TO

The rat serum samples will be analyzed according to the method entitled "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Serum For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-4.1) with the modifications listed on the attached pages.

RATIONALE

To clarify the analytical method to be used for the analysis of the rat serum samples.

IMPACT ON STUDY

No negative impact.

Emily R Decker — Principal Investigator Signature — Date 4/15/02

C. Darrin Woods (Fraunhofer ITH) — Study Director Signature — Date 25-April 2002

John 2. Butenhoff — Sponsor Representative Signature — Date 3-May 2002

John M Flaherty — Management Signature — Date 5/7/02

Exygen QAU Review — Date 3/4/02 4/4/02

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3058 Research Drive
State College, PA 16801, USA
T: 800.281.3219
F: 814.272.1019
exygen.com

Protocol No. 01G 00 0 02
Amendment #2

ANALYTICAL METHOD MODIFICATIONS

1. There will be no surrogate standard used in the extraction procedure. The stock, fortification, and calibration standards will be prepared as follows:

Stock Solution
Prepare a stock solution of PFOS at 100 µg/mL by weighing out 10.0 mg of analytical standard (corrected for percent salt and percent purity). Adjust final volume to 100 mL with methanol in a 100-mL volumetric flask. Store this stock solution (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

Fortification Solutions

a. 1.0 µg/mL Fortification Solution – Pipette 1.0 mL of the 100 µg/mL stock solution into a 100 mL volumetric flask. Bring up to volume with methanol.

b. 0.1 µg/mL Fortification Solution – Pipette 10.0 mL of the 1.0 µg/ml fortification solution into a 100-mL volumetric flask and bring up to volume with methanol.

c. 0.01 µg/mL Fortification Solution – Pipette 10.0 mL of the 0.1 µg/mL fortification solution into a 100-mL volumetric flask and bring up to volume with methanol

Store all fortification standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

Calibration Standards
Prepare six LC/MS/MS calibration standards in methanol via dilution of the 0.1 µg/mL and 0.01 µg/mL fortification solutions.

This is a typical example; additional concentrations may be prepared as needed.

Initial Conc. (µg/mL)	Volume (mL)	Diluted to (mL)	Final Conc. (µg/mL)
0.1	5	100	0.005
0.1	2	100	0.002
0.1	1	100	0.001
0.01	5	100	0.0005
0.01	2	100	0.0002
0.01	1	100	0.0001

Store all calibration standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

3. The sample volume for the red blood cells samples will be 100 µL. The amount of 0.5 M TBA added to the sample will be 0.5 mL and the amount of 0.25 M sodium carbonate/sodium bicarbonate buffer added will be 1.0 mL. The samples will only be filtered with 0.2 µm nylon mesh filter if necessary.

4. The samples will be analyzed under the following conditions:

LC/MS/MS System and Operating Conditions (TurboIonspray)

Mass Spec: PE SCIEX API 3000 Biomolecular Mass Analyzer

Interface: SCIEX TurboIon Spray Liquid Introduction Interface
Harvard infusion pump

Computer: Dell UltraScan P1110

Software: PE SciexAnalyst 1.1
Windows NT

HPLC: Hewlett Packard (HP) Series 1100
HP Quat Pump
HP Vacuum Degasser
HP Autosampler
HP Column Oven

HPLC Column:Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temp.: 35° C
Injection Vol.: 10 µL
Mobile Phase (A): 2 mM Ammonium Acetate in ASTM type I water
Mobile Phase (B): Methanol
Flow Rate: 0.3 mL/min.

Time	% A	% B
0	60	40
1.0	0	100
7.0	0	100
7.5	60	40
11.0	60	40

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g., 2.1 mm x 30 mm) and columns from different manufacturers (Keystone

Betasil C_{18} etc.) can be used, provided equivalent chromatography is obtained.

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time (min)
PFOS	negative	499 → 99	4.40

On a day-to-day basis, the retention times may vary slightly depending on the batch of mobile phase, etc.

Example Tune File Parameters

The following values are provided as an example. Actual values may vary from instrument to instrument. Also, these values may be changed from time to time in order to optimize for greatest sensitivity.

Once the instrument is tuned, the optimized parameters are saved as a "tune file". This tune file is then used during routine analysis. The tuning procedure may be repeated as necessary to ensure optimal sensitivity.

Tune File Parameters

Controls	Set
IS-Iospray	-4200.0
DP-Declustering Potential	-51.0
FP-Focusing Potential	-230.0
EP-Entrance Potential	10.0
CE-Collision Energy	-70
CXP-Collision Cell Exit Potential	-5
DF-Deflector	300.0
CEM-Channel Electron Multiplier	2800.0

Gas Flows	Set
Nebulizer Gas	12
Curtain Gas	13
Collision Gas	4
TIS Temperature	350°C

Calibration Procedures

a. Inject the same volume (between 10 to 20 µL) of each calibration standard (prepared in MeOH) into the LC/MS/MS.

b. Use linear, 1/x weighted standard curves for quantification. Linear standard curves are generated for each set by linear regression using the appropriate software system. Any calibration standards falling outside ± 30%, based on its calculated concentration, may

be excluded from the calibration curve. However, the total number of calibration standards that may be excluded must not exceed 20% of the total number of standards injected.

c. The correlation coefficient (r) for calibration curves generated must be ≥0.9925 (r^2≥0.985). If calibration results fall outside these limits, then appropriate steps should be taken to adjust instrument operation, and the relevant set of samples must be reanalyzed.

Sample Analysis

Inject the same volume used for the calibration standards (between 10 to 20 µL) of each sample, fortification, control, etc. into the LC/MS/MS. Each sample must be analyzed in duplicate.

a. Standards corresponding to at least six concentrations must be included in an analytical set.

b. Inject an entire set of standards (six) at the beginning of the run and inject standards interspersed about every 5-10 samples. All sample injections must be bracketed by standard injections.

c. Each set of samples analyzed (not to exceed 25) must include at least one reagent control (extraction solvents only), at least one matrix control, and two matrix control samples fortified at known concentrations and carried through the procedure to verify recovery. Each set must also include at least one methanol blank and one ASTM Type I H_2O blank.

d. The concentration of each sample, fortification, control, etc. is determined from the standard curve based on the peak area of the analyte in all standards injected during a set. The standard responses must bracket responses of the residue found in the sample set. If necessary, dilute the samples and re-analyze to give a response within the standard curve range.

e. Fortification recoveries within 80 to 120% are acceptable for fortifications at the LOQ (10 ng/mL) and at levels greater than the LOQ. Failure to meet these criteria requires an investigation of cause and a full reanalysis of the affected samples.

f. Samples in which no peaks are detected at the corresponding analyte retention times will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention times but are less than the response of the lowest standard (0.0001 µg/mL) will be reported as NQ (not quantifiable).

g. Background levels of analyte found in control/blank samples that correspond to values below the LOQ, but are still quantifiable, will be used to correct fortification recoveries.

h. If samples are not loaded on the instrument to be analyzed the day they are extracted, samples must be stored refrigerated at approximately 2°C to 6°C until analysis and analyzed preferably within a week.

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PROTOCOL DEVIATION

Deviation Number: 1
Date of Occurrence: 1/15-16,17/02

Exygen Study Number: 023-068 Protocol Number: 01G 00 0 02

DESCRIPTION OF DEVIATION

Protocol Amendment 2 - Sample Analysis.e-Accepted recovery of 79% for PFOS for 0113831 Spk A in Set 011502A. For 0113831 Spk A and B and their duplicate injections in Sets 011502AD, 011602A, 011602B, accepted recoveries for PFHS of 53%, 56%, 56%, 60%, 70%, 70%, 69%, 70%, 67%, 68%, 68%, and 68%. In Set 011702A for 0113831 Spk B and its dup. inj., accepted recoveries for PFHS of 73% and 74%. In Sets 011602A and 011602B, accepted recoveries for PFHS of 72% (0114158 Spk C and dup. inj.), 78% and 77% (0114162 Spk D and dup. inj.), 76% (0114166 Spk E and dup. inj.), 74% (0114170 Spk C and dup. inj.), and 78% and 77% (0114174 Spk D and dup. inj.)

ACTIONS TAKEN
for example - deviation issued, SOP revision, etc.

Protocol deviation issued.

Recorded By: Date:

IMPACT ON STUDY

No negative impact on study.

Emily R Decker — Principal Investigator Signature — Date 4/15/02

C. Daus Wood — Study Director Signature (Fraunhofer ITEM) — Date 25-April 2002

Sponsor Representative Signature — Date

Management Signature — Date

Exygen QAU Review

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3058 Research Drive
State College, PA 16801, USA
T: 800.281.3219
F: 814.272.1019
exygen.com