

## ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

### TEST SUBSTANCE

**Identity:** A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-206CE. (1-Octanesulfonic acid) (CAS # 2795-39-3).

**Remarks:** The 3M production lot number was 502.

The test sample is FC-206CE. Current information indicates it is a mixture of 0.92% PFOS, 15% diethylene glycol butyl ether, 76.09% water, 4% urea, 1.28% Sultone foamer, 1.55% sodium octyl sulfate, 1.05% polyoxyethylene monooctylphenyl ether, 0.06% sodium lauryl sulfate, and 0.05% tolyltriazole.

***The following summary applies to a mixture with incompletely characterized concentrations of impurities. Data may not accurately reflect the toxicity of the fluorochemical component of the test sample.***

### METHOD

**Method:** OECD 209

**Test type:** Acute static

**GLP:** No

**Year Completed:** 1989

**Analytical monitoring:** Dissolved oxygen concentrations, pH, and temp.

**Statistical methods:** Results were determined by calculation of the % inhibition in respiration rate.

**Test organism source:** Activated sludge mixed liquor collected from the Metro Waste Treatment Plant, St. Paul, MN.

**Test condition:**

**Dilution water:** Millipore Milli-Q™ water

**Synthetic sewage:** per OECD method

**Reference and test solution preparation:** A stock solution of the reference substance, 3,5-dichlorophenol, was prepared by dissolving 500 mg in 10mL 1N NaOH, diluted to 30mL with Millipore Milli-Q™ water, then brought to the point of incipient precipitation with 1N H<sub>2</sub>SO<sub>4</sub> and diluted to 1L with Milli-Q™ water. The pH of the reference solution was measured to be 7.6. Test substance was added directly to the test vessels and was apparently miscible with water.

**Test vessels:** Not given.

**Number of concentrations:** five plus 1 reference substance and 2 blank controls

**Temperature:** 20 – 22 °C

**Total suspended solids and pH on day of testing:** 3.6 g/L (TSS), initial pH 7.3 and final pH 7.3

**Element Basis:** Respiration inhibition as determined by oxygen consumption.

**Remarks:** Values corrected to 20 °C for calculations.

## RESULTS

**Nominal concentrations:** 2 Blank controls, reference substance, 100, 180, 320, 560, and 1000 mg/L

**Element values:** 30-minute  $EC_{50}$  = >1000 mg/L  
3-hour  $EC_{50}$  = >1000 mg/L

Element values based on nominal concentrations.

**Remarks:** Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical proportion alone.

## CONCLUSIONS

The FC-206CE 3-hour  $EC_{50}$  for activated sludge respiration inhibition was determined to be >1000 mg/L.

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

## DATA QUALITY

**Reliability:** Klimisch ranking 2. Testing meets all criteria for quality testing. However, sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

## REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request number G1358-2, 1989.

## OTHER

**Last changed:** 6/26/00

LR G1358-2

Date 6-23-89

Analyst SMB

ENVIRONMENTAL LABORATORY  
ACTIVATED SLUDGE MIXED LIQUOR

Type Test O&D Activated Sludge Respiration Inhibition Test #209

Source Metro Waste Treatment Plant St Paul, MN

Date Obtained 6-23-89 Date Used 6-23-89

Mixed Liquor Suspended Solids (MLSS) 3.6 g/L Adjustment None g/L

pH Initial = 7.3 Final = 7.3

Comments Test run with LR G1750

Test Substance

Sample Description FC - 206 CE Lot 502

Sample pH Highest Conc. 1000mg/L = 7.2 Adjustment None

Comments \_\_\_\_\_

Reference Substance

Sample Description 3,5- Dichlorophenol

Sample pH 7.6 (stock soln.) Adjustment None

Comments \_\_\_\_\_

Exposure Conditions

Test Duration 3-Hours

Test Temperature 20-22 °C

Comments \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Lab Request No. G1358-2

## Environmental Lab — Work Sheet

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## Sample Description

FC-206CE

Lot 502

yellow liquid

OECD ACTIVATED SLUDGE  
RESPIRATION INHIBITION TEST #209Date: 6-23-89Analyst: Juan A. Beach

## Test Solutions Preparation

| Test Conc.                       | mg added   | M.Q. | ml DI water | ml syn. sewage  | ml microbial        | Total volume |     |   |     |
|----------------------------------|------------|------|-------------|-----------------|---------------------|--------------|-----|---|-----|
| mg/L                             | per 500 ml |      | per 500 ml  | feed per 500 ml | inoculum per 500 ml | ml           |     |   |     |
| Blank <sup>(1)</sup><br>Controls | -          |      | 284         | +               | 16                  | +            | 200 | = | 500 |
| 100                              | 50         | +    | 284         | +               | 16                  | +            | 200 | = | 500 |
| 180                              | 90         | +    | 284         | +               | 16                  | +            | 200 | = | 500 |
| 320                              | 160        | +    | 284         | +               | 16                  | +            | 200 | = | 500 |
| 560                              | 280        | +    | 284         | +               | 16                  | +            | 200 | = | 500 |
| 1000 <sup>(2)</sup>              | 500        | +    | 284         | +               | 16                  | +            | 200 | = | 500 |

<sup>(1)</sup> Blank Control II shared with LRG 1750, as Blank Control I<sup>(2)</sup> Test substance apparently miscible with water.

Initial pH highest conc. tested, 1000 mg/L; = 7.2

Milli-Q water used: Prepared 6-19-89, aeration started 6-20-89

pH = 6.0

## ENVIRONMENTAL LABORATORY

## OECD ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

Pg 2 of 3

## RAW DATA

Test Substance: FC-206CE Lot 502Source of Activated Sludge Mixed Liquor: Metro Waste Treatment Plant St Paul, MNDate Received: 6-23-89Date Used: 6-23-89Analyst: Jusan A. Beach

## 30 - MINUTE EXPOSURE

| Test Substance<br>Concentration (mg/L) | DISSOLVED OXYGEN CONCENTRATIONS mg/L |     |     |     |     |     |     |     |                        |     |                       | TEMP<br>C° |
|----------------------------------------|--------------------------------------|-----|-----|-----|-----|-----|-----|-----|------------------------|-----|-----------------------|------------|
|                                        | TIME-MINUTES                         |     |     |     |     |     |     |     |                        |     |                       |            |
|                                        | 0.5                                  | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0                    | 9.0 | 10.0                  |            |
| CONTROL I                              | 7.4                                  | 7.2 | 6.6 | 6.1 | 5.6 | 5.1 | 4.5 | 4.0 | 3.5                    | 3.0 | 2.5                   | 22         |
| 100                                    | 8.1                                  | 7.8 | 7.3 | 6.8 | 6.3 | 5.8 | 5.3 | 4.8 | 4.3                    | 3.8 | 3.3                   | 21         |
| 180                                    | 7.8                                  | 7.5 | 7.0 | 6.4 | 5.9 | 5.3 | 4.8 | 4.2 | 3.7                    | 3.2 | 2.7                   | 20         |
| 320                                    | 7.7                                  | 7.4 | 6.8 | 6.2 | 5.6 | 5.0 | 4.4 | 3.8 | 3.2                    | 2.6 | 2.0                   | 21         |
| 560                                    | 7.4                                  | 7.1 | 6.5 | 5.8 | 5.1 | 4.5 | 3.8 | 3.1 | 2.5                    | 1.8 | 1.2                   | 21         |
| 1000                                   | 5.8                                  | 5.4 | 4.6 | 3.9 | 3.1 | 2.4 | 1.7 | 0.9 | <del>0.5</del><br>7.30 |     |                       | 21         |
|                                        |                                      |     |     |     |     |     |     |     |                        |     |                       |            |
| CONTROL II                             | 7.3                                  | 7.1 | 6.6 | 6.1 | 5.5 | 5.0 | 4.5 | 4.0 | 3.5                    | 3.0 | <del>2.5</del><br>3.2 | 22         |

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## 3 - HOUR EXPOSURE

| Test Substance<br>Concentration (mg/L) | DISSOLVED OXYGEN CONCENTRATIONS mg/L |     |     |     |     |     |     |     |     |     |      | TEMP<br>C° |
|----------------------------------------|--------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------------|
|                                        | TIME-MINUTES                         |     |     |     |     |     |     |     |     |     |      |            |
|                                        | 0.5                                  | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 |            |
| CONTROL I                              | 7.5                                  | 7.2 | 6.7 | 6.2 | 5.6 | 5.1 | 4.6 | 4.1 | 3.5 | 3.0 | 2.5  | 22         |
| 100                                    | 8.0                                  | 7.9 | 7.5 | 7.1 | 6.7 | 6.2 | 5.8 | 5.4 | 5.0 | 4.6 | 4.1  | 20         |
| 180                                    | 8.3                                  | 8.1 | 7.7 | 7.4 | 7.0 | 6.6 | 6.2 | 5.8 | 5.5 | 5.1 | 4.7  | 20         |
| 320                                    | 8.3                                  | 8.1 | 7.7 | 7.3 | 6.9 | 6.5 | 6.1 | 5.7 | 5.3 | 4.9 | 4.5  | 20         |
| 560                                    | 8.3                                  | 8.1 | 7.8 | 7.5 | 7.1 | 6.8 | 6.4 | 6.0 | 5.7 | 5.3 | 4.9  | 20         |
| 1000                                   | 8.0                                  | 7.8 | 7.5 | 7.1 | 6.7 | 6.3 | 5.9 | 5.5 | 5.1 | 4.7 | 4.3  | 20         |
|                                        |                                      |     |     |     |     |     |     |     |     |     |      |            |
| CONTROL II                             | 7.3                                  | 7.1 | 6.5 | 6.0 | 5.5 | 5.0 | 4.4 | 3.9 | 3.3 | 2.9 | 2.3  | 22         |

pH of Activated Sludge Mixed Liquor: Initial 7.3

Final 7.3

pH of highest concentration tested: Initial 7.5

Final 7.5

Test Temperature: 20-22°C

# ENVIRONMENTAL LABORATORY

## ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209 CALCULATION OF RESULTS

LR G1358-2  
Date 6-28-89  
Analyst SJR

Test Substance FC-206CE Lot 502

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### 30 - MINUTE EXPOSURE

| Test Substance Concentration (mg/L) | Oxygen Uptake Rate R | R Corrected <sup>(1)</sup> to 20°C | % Inhibition in Respiration Rate <sup>(2)</sup> |
|-------------------------------------|----------------------|------------------------------------|-------------------------------------------------|
| CONTROL I                           | 0.5206               | 0.4749                             | -                                               |
| 100                                 | 0.5000               | 0.4776                             | -                                               |
| 180                                 | 0.5388               | 0.5388                             | -                                               |
| 320                                 | 0.6000               | 0.5731                             | -                                               |
| 560                                 | 0.6618               | 0.6321                             | -                                               |
| 1000                                | 0.7462               | 0.7127                             | -                                               |
| CONTROL II                          | 0.5127               | 0.4677                             | -                                               |

Percent Difference in Controls<sup>(3)</sup> 1.5%

EC<sub>50</sub> (95% Confidence Limits) mg/L > 1000 mg/L

### 3 - HOUR EXPOSURE

| Test Substance Concentration (mg/L) | Oxygen Uptake Rate R | R Corrected <sup>(1)</sup> to 20°C | % Inhibition in Respiration Rate <sup>(2)</sup> |
|-------------------------------------|----------------------|------------------------------------|-------------------------------------------------|
| CONTROL I                           | 0.5255               | 0.4794                             | -                                               |
| 100                                 | 0.4200               | 0.4200                             | 13                                              |
| 180                                 | 0.3776               | 0.3776                             | 22                                              |
| 320                                 | 0.4000               | 0.4000                             | 17                                              |
| 560                                 | 0.3576               | 0.3576                             | 26                                              |
| 1000                                | 0.3945               | 0.3945                             | 18                                              |
| CONTROL II                          | 0.5291               | 0.4827                             |                                                 |

Percent Difference in Controls<sup>(3)</sup> 0.7%

EC<sub>50</sub> (95% Confidence Limits) mg/L > 1000 mg/L

(1) Temp. Correction =  $\frac{R_{T,obs}}{\theta^x}$  (mg O<sub>2</sub>/L-min.)

(3) % Difference =  $\frac{|RC_1 - RC_2|}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

(2) % Inhibition =  $\frac{\left(\frac{RC_1 + RC_2}{2}\right) - R_S}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

The two control respiration rates should be within 15% of each other

See pg 1 for test soln.  
Prep.

Where:

R = Oxygen uptake rate (mg O<sub>2</sub>/L-min.)

T<sub>obs</sub> = Sample temp., °C

θ = 1.047, x = T<sub>obs</sub> - 20°C

C = Control

Lab Request No. 61358-2  
G1750

## Environmental Lab — Work Sheet

Page 1 of 3Sample Description  
3,5-Dichlorophenol  
Reference Substance  
Hidrich Red Label, LotOECD ACTIVATED SLUDGE  
RESPIRATION INHIBITION TEST #209Date: 6-23-89Analyst: STBPurple crystals

## Stock and Test Solutions Preparation

STOCK SOLUTION: 500 mg 3,5-DCP/L Each 1.0 ml = 0.5 mg

- Dissolved 500mg 3,5-DCP in 10ml 1.0N NaOH
- Diluted to 30 ml with Milli-Q water
- Added, under stirring, 1.0N H<sub>2</sub>SO<sub>4</sub> dropwise until incipient ppt.
- Diluted to one liter with Milli-Q water.

Stock soln. pH = 7.6

| Test Conc.<br>mg/L    | ml stock soln<br>per 500 ml | ml DI water<br>per 500 ml | ml syn. sewage<br>feed per 500 ml | ml microbial<br>inoculum per 500 ml | Total volume<br>ml |
|-----------------------|-----------------------------|---------------------------|-----------------------------------|-------------------------------------|--------------------|
| Blank (1)<br>Controls | —                           | 284 +                     | 16 +                              | 200                                 | = 500              |
| 10                    | 10                          | 274 +                     | 16 +                              | 200                                 | = 500              |

(1) Controls shared with LR G1750

Milli-Q water used: Prepared 6-19-89, aeration started 6-20-89  
pH = 6.0

Pg 2 of 3

Date Used: 6-23-89

Final 7.5  
Final 7.9

# ENVIRONMENTAL LABORATORY

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST #209

CALCULATION OF RESULTS

61358-2  
LR 61750  
Date 6-23-89  
Analyst SIB

Test Substance 3,5-Dichlorophenol Reference Substance

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30 - MINUTE EXPOSURE

| Test Substance Concentration (mg/L) | Oxygen Uptake Rate R | R Corrected <sup>(1)</sup> to 20°C | % Inhibition in Respiration Rate <sup>(2)</sup> |
|-------------------------------------|----------------------|------------------------------------|-------------------------------------------------|
| CONTROL I                           | 0.5127               | 0.4677                             | -                                               |
| 10                                  | 0.3000               | 0.2737                             | 43                                              |
|                                     |                      |                                    |                                                 |
|                                     |                      |                                    |                                                 |
|                                     |                      |                                    |                                                 |
|                                     |                      |                                    |                                                 |
|                                     |                      |                                    |                                                 |
| CONTROL II                          | 0.5424               | 0.4948                             | -                                               |

Percent Difference in Controls<sup>(3)</sup> 5.6%

EC<sub>50</sub> (95% Confidence Limits) mg/L 10 mg/L induced 43% inhibition

3 - HOUR EXPOSURE

| Test Substance Concentration (mg/L) | Oxygen Uptake Rate R | R Corrected <sup>(1)</sup> to 20°C | % Inhibition in Respiration Rate <sup>(2)</sup> |
|-------------------------------------|----------------------|------------------------------------|-------------------------------------------------|
| CONTROL I                           | 0.5291               | 0.4827                             | -                                               |
| 10                                  | 0.2152               | 0.2055                             | 57                                              |
|                                     |                      |                                    |                                                 |
|                                     |                      |                                    |                                                 |
|                                     |                      |                                    |                                                 |
|                                     |                      |                                    |                                                 |
| CONTROL II                          | 0.5255               | 0.4794                             | -                                               |

Percent Difference in Controls<sup>(3)</sup> 0.7%

EC<sub>50</sub> (95% Confidence Limits) mg/L 10 mg/L induced 57% inhibition

(1) Temp. Correction =  $\frac{R_{T,obs}}{\theta^x}$  (mg O<sub>2</sub>/L-min.)

(3) % Difference =  $\frac{|RC_1 - RC_2|}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

(2) % Inhibition =  $\frac{\left(\frac{RC_1 + RC_2}{2}\right) - R_s}{\left(\frac{RC_1 + RC_2}{2}\right)} \times 100$

The two control respiration rates should be within 15% of each other

See pg 1 for stock + test soln. prep

Where:

R = Oxygen uptake rate (mg O<sub>2</sub>/L-min.)

T<sub>obs</sub> = Sample temp., °C

θ = 1.047, x = T<sub>obs</sub> - 20°C

C = Control