



ENVIRONMENTAL TOXICOLOGY LABORATORY, LLC

(Short Version)

Toxicity of Deutsche Bank Dust Samples⁽¹⁾

(Interim EPA Progress Report-I)

Environmental Toxicology Laboratory has developed 2 new **biomonitoring** tests, using single cell organisms to measure toxicity. These tests are capable of measuring the **synergistic effects** of pollutant **mixtures**, as contrasted to the chemical analyses of individual toxic agents. *Glaucoma chattoni* is a free-swimming ciliate (surface area covered with hair-like structures-cilia-which beat synchronously and propel the organism through liquid media). *Tetramitus rostratus* is a flagellate with four longer structures called flagella. The flagella act much the same as a boat propeller and their rapid beating causes forward movement.

The initial Swimming Behavior and Growth Inhibition Assays, describing the responses of *Glaucoma* and *Tetramitus* to exposure to toxic Deutsche Bank dust samples, provide a framework for the development of **rapid and cost-effective methods for the assessment of WTC contaminant exposure**. Because these protists ingest particles, the need for costly and labor-intensive particle extraction procedures is eliminated. Thus, **whole particle toxicity** measurement is now feasible. These assays could substantially increase the number of samples which could be evaluated in the proposed **Buildings Survey** of Lower Manhattan and Downtown Brooklyn and the **Deutsche Bank Deconstruction**.

The Deutsche Bank Dust Samples were sent to ETL by Drs. Nancy Adams and Jacky Rosati of the EPA Department of Homeland Security, Consequences and Decontamination Division. EPA has agreed to share the chemical analysis data of these dust samples with ETL in order to evaluate the toxicity test results.

The **rapid swimming behavior changes (10 minutes)** of *Glaucoma* ciliates (in response to Deutsche Bank dust exposures) could provide rapid assessment of filters recovered from **personal air monitors** of individual workers involved in the upcoming Deutsche Bank Deconstruction. Higher levels (hot spots) of contamination, which were not detected in general area air sample monitoring, may occur in “local” areas of work. **Worker exposures** could be continuously monitored and demolition procedures and work practices could be modified where appropriate.

Results

1. **Swimming Behavior abnormalities** were observed in the ciliate *Glaucoma chattoni*, which were exposed to Deutsche Bank particle suspensions. **(Changes were observed in less than 10 minutes.** See Figure 1)

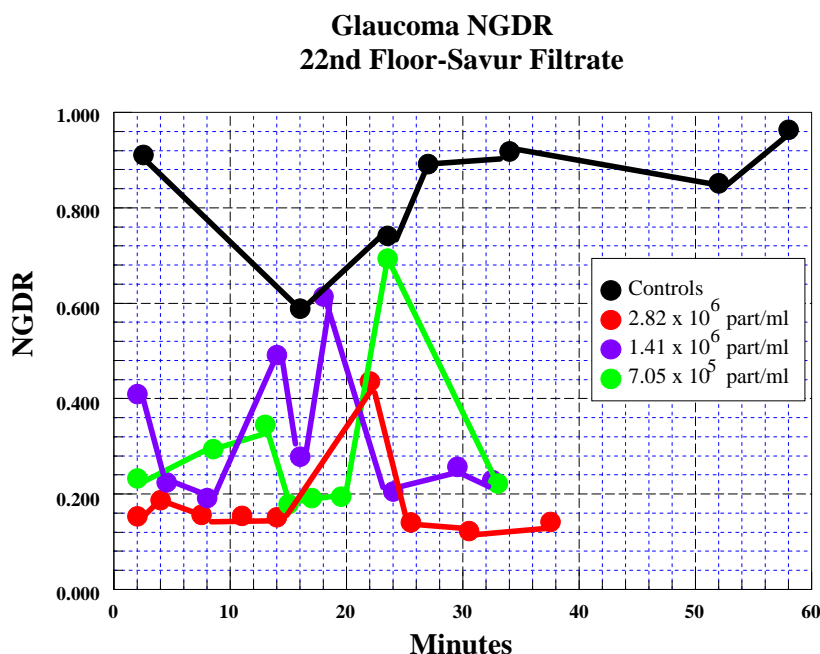


Figure 1. Swimming Behavior Response of *Glaucoma chattoni* ciliates which were exposed to a Deutsche Bank Dust Sample. The neat particle suspension was filtered through a Savur filter Assembly (pore size = 25 μ m) in order to remove larger particles.

The **NGDR** is the **net to gross displacement ratio**. This statistic is a measure of the relative straight distance traveled by the ciliates. *Glaucoma* ciliates normally swim in straight paths. Ciliates which are exposed to toxic agents swim in a circular path because some of the surface cilia are damaged. The normal beating synchrony of the cilia is disrupted.

A NGDR ratio of 1 would indicate a perfect straight line, while a ratio near zero would indicate a circular path. Multiple time points are sampled in order to evaluate statistical variations. Similar results also were obtained for 5th, 7th, and 41st floor dust samples

The NGDR parameter was developed by Dr. Scott Gallager of the Woods Hole Oceanographic Institution (WHOI). WHOI is the recipient of a DoD grant, with ETL as a sub-contractor, for the project "Terrorist Poisoning of Drinking Water".

2. Tetramitus Growth Inhibition

Tetramitus Growth Curves 2-5 um Particle Suspension

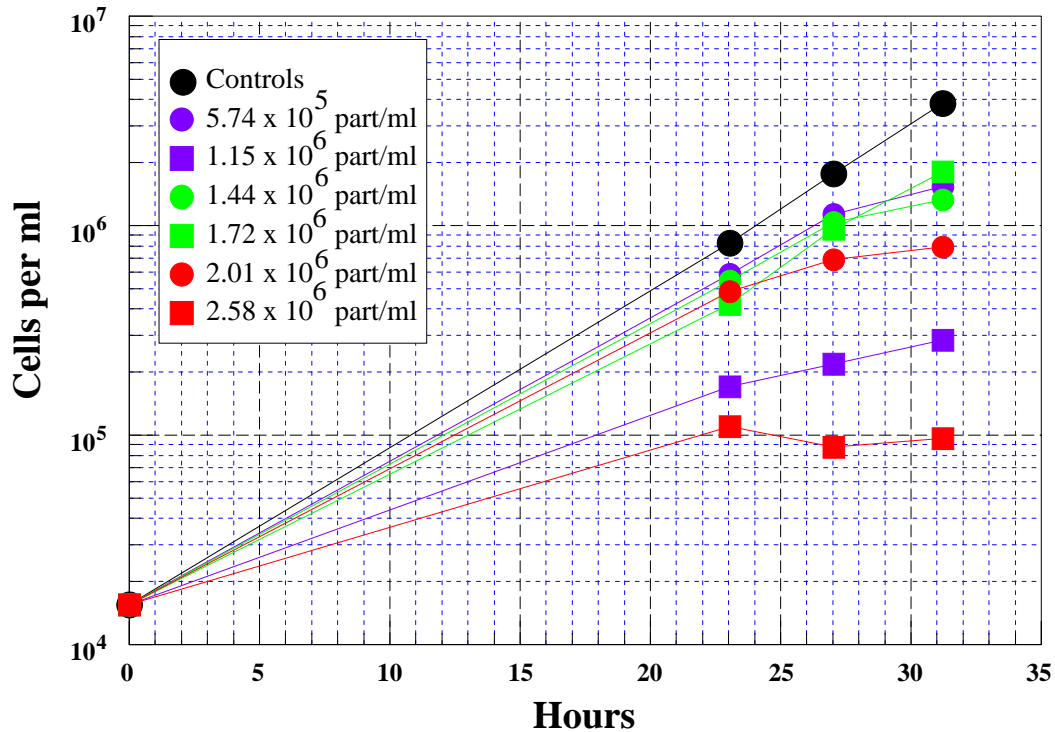


Figure 2 Growth of *Tetramitus* flagellates exposed to different concentrations of a 2-5 μm particle suspension prepared from a Deutsche Bank 41st Floor dust sample. Growth of flagellates was **completely inhibited** after 23 hours of incubation in cultures containing 2.58×10^6 particles/ml. This growth inhibition pattern is consistent (not proof positive) with **DNA damage** to the exposed flagellates at this concentration.

The ability of *Tetramitus* flagellates to ingest particles allows for the assessment of **whole particle toxicity**. Previous *Tetramitus* studies with known DNA damaging agents produced positive toxicity responses in 26/27 of the agents tested. A linear dose response also was observed in flagellates exposed to increasing concentrations of diesel particulates. (National Institute of Standards and Technology Standard Reference Material # 2975).

EPA has recently classified diesel particle exhaust as a human carcinogen.

Direct proof of DNA damage could be achieved by measuring the changes in the DNA (DNA adduct formation) extracted from flagellates exposed to different concentrations of a reference DNA-damaging agent such as benzo [A]pyrene; and subsequently to WTC particles. ETL has arranged for collaboration with Dr. David Mitchell of the U.T. M.D. Anderson Cancer Center for this DNA damage study. Dose-dependent DNA damage in *Tetramitus* flagellates exposed to WTC particles then could be calibrated to dose-dependent levels of flagellate growth inhibition. Thus, measurement of growth inhibition now would serve to indicate dose-dependent DNA damage to flagellates exposed to various dust samples. *Tetramitus* DNA then could serve as a surrogate for human DNA damage. Human DNA damage would indicate potential **long-term adverse health effects.** (DNA damage is the first step in the conversion of normal cells to cancer cells. DNA damage also impairs the immune system)

Conclusion:

The use of two protist organisms to assess the toxicity of WTC contaminants allows for the rapid and cost-effective **evaluation of mixtures** of contaminants and **whole particle toxicity.** The scope of the upcoming Buildings Survey can be substantially increased to cover a larger geographic area. Furthermore, worker exposure monitoring in the Deutsche Bank Deconstruction is now feasible, as well as increased frequency of air monitoring.

Proposals for further study are listed in the complete report (Appendix-III)

1. 2005, Robert L. Jaffe, Marianela Trichoche Camacaro, and Michael R. Jaffe Toxicity of Deutsche Bank Dust Samples, Report Submitted to the EPA Safe Buildings/Homeland Security Group, February 21, 2005.
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