Utility of the Laser Particle Counter for Determination of Drinking-Water Toxicity

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Particle toxicity in various water samples depends on the mixture of toxicants in each sample. Toxicity values for different samples can be expressed using the new metric: Particle Toxicity.

Use of the Spectrex PC-2000 was the instrument of choice in enumerating the particle concentrations for the calculation of the particle toxicity of drinking-water and reference toxicant suspensions. Generation of Particle Size Distribution Graphics with the PC-2000 also provided information which characterized the particle concentration, surface area %, and volume % for specified particle-size subpopulations (1-2③m, 2-4③m, 4-8③m, 8-16③m, etc.). The parsing of surface area % into specific particle-size subpopulations is especially useful because the surface area of the particles is the parameter which determines the efficiency of toxicant adsorption for a given particle-size subpopulation. Further identification of the specific toxic agents observed in particles from each sample can be determined using EPA TIE Analyses[2]; the fractionation of toxicities into different chemical classes can be monitored using the *Tetramitus* Assay. Thus, the PC-2000 is an invaluable tool for whole particle toxicity studies of drinking-water and the identification of contaminants of concern.

Current regulations for water quality assessment express maximal allowable levels of single contaminants as a weight per unit volume metric; i.e., mg/liter. There is no provision for addressing the issue of toxicity of mixtures. Furthermore, there are no regulations which deal with particle toxicity. White, *et.al*,[3] have demonstrated that for 50 industrial effluents, the particle toxicity, as measured by the *E.coli* SOS assay, is four orders of magnitude (10,000 X) greater than the soluble toxicity. Thus, the use of a particle toxicity metric offers a more relevant standard for the assessment of contaminants in drinking- and source-water.

In the White, *et.al*, study[4], the toxic agents adsorbed onto the particles required solvent extraction and solvent substitution procedures for determination of the toxicity in the SOS system. We have developed a rapid and cost-effective method for measuring whole particle toxicity: The *Tetramitus* Growth Inhibition Assay[5]. Whole particle toxicity tests obviate the need for solvent extraction of particles and eliminate solvent extraction bias[6].

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[2] http://www.epa.gov/nheerl/publications/files/Sediment TIE Guidance Document.pdf

[3] White, P.A, Rasmussen, J.B, and C. Blaise, (1996) Comparing the Presence, Potency and Potential Hazard of Genotoxins Extracted From a Broad Range of Industrial Effluents, *Env .and Molec. Mutagenesis*, 27: 116-139.

[4] Ibid.

[5] Jaffe, R.L., Rapid Assay of Cytotoxicity Using Tetramitus Flagellates. *Toxicology and Industrial Health* 11: 543-558, 1995.

[6] Jaffe, R.L, The Tetramitus Assay, in "Biomonitors and Biomarkers as Indicators of Environmental Change", 2000, Ed: Butterworth, F.M., A.Gunatilaka, and M.E. Gonsebatt pp.391- 425.

In addition, determination of whole particle toxicity also would address the issue of the toxicity of mixtures.

Determination of sample toxicity is achieved by measuring the growth inhibition of *Tetramitus* flagellates which are exposed to increasing concentrations of toxic agents present in a given sample. *Tetramitus* is a single cell organism which can exist in three forms; ameba, flagellate and cyst. It has lived on this planet for over 1 billion years.[7] *Tetramitus* flagellates (Figure 1) have been grown in the laboratory as stable populations for the last 25 years. Flagellate growth inhibition is the basis for the study of toxic agents found in the environment (Figure 2).[8, 9,]

Figure 1. Scanning Electron micrograph of a *Tetramitus* flagellate.

In order to produce toxicity dose response graphics the Slope Ratio (Growth Inhibition) is plotted against the number of particles (x 10^3) per ml.

The rate of growth, is measured by counting the concentration of flagellates (cells per ml) over time; the numerical value of the growth rate is the **slope**, expressed as concentration of cells per unit time (hours).Toxicity is expressed as the ratio of the slope of the exposed culture to the slope of the control culture: the **Slope Ratio**. Thus a slope ratio of 0.8 ($SR._{80}$) represents *Tetramitus* flagellates which grow at a 20% reduced growth rate.

Kluwar Academic/Plenum Publishers (New York)

[7] Sagan, L. ,"On the Origin of Mitosing Cells", *J. Theoret. Biol*, 14: 224 274, (1967).

[8] Jaffe, R.L., "Rapid A*ssay* of Cytotoxicity Using Tetramitus Flagellates*", Toxicology and Industrial Health* 11: 543-558, 1995.

[9] Jaffe, R.L, "The Tetramitus Assay", in Biomonitors and Biomarkers as Indicators of Environmental Change, Ed: Butterworth, F.M., A.Gunatilaka, and M.E. Gonsebatt, Pp.391- 425, Kluwar Academic/Plenum Publishers (New York), 2000.

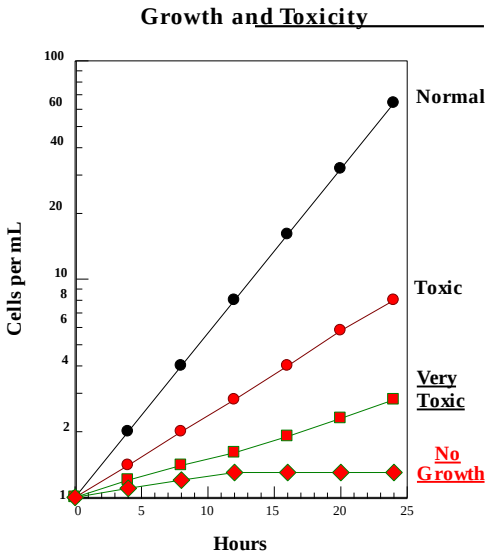

Figure 2 Schematic representation of growth of *Tetramitus* Flagellates. Both normal growth and growth of cells exposed to different concentrations of toxic agents are illustrated.

Tetramitus dose response relationships were observed when flagellates were exposed to the reference toxicants used in the EPA WTC mouse inhalation studies.[10] Particle concentrations for these suspensions were obtained using the Spectrex PC2000 Laser Particle Counter (Figure 3). The linearity of the dose-response regression curves produced high r^2 values (correlation coefficients); this attribute allows for single dose screening of a larger number of water samples prior to performing a 5-dose assay with particle enumeration.

[10] Jaffe, R.L. (2004) Utility of the Tetramitus Assay for the Assessment of Air Quality following Terrorist Attacks. Report to the U.S. Environmental Protection Agency Pulmonary Toxicology Branch, Experimental Toxicology Division, NHEERL, Revised Report October 11, 2004 (http://envirotoxlab.purehost.com/studies/wtcreferencesamples.pdf)

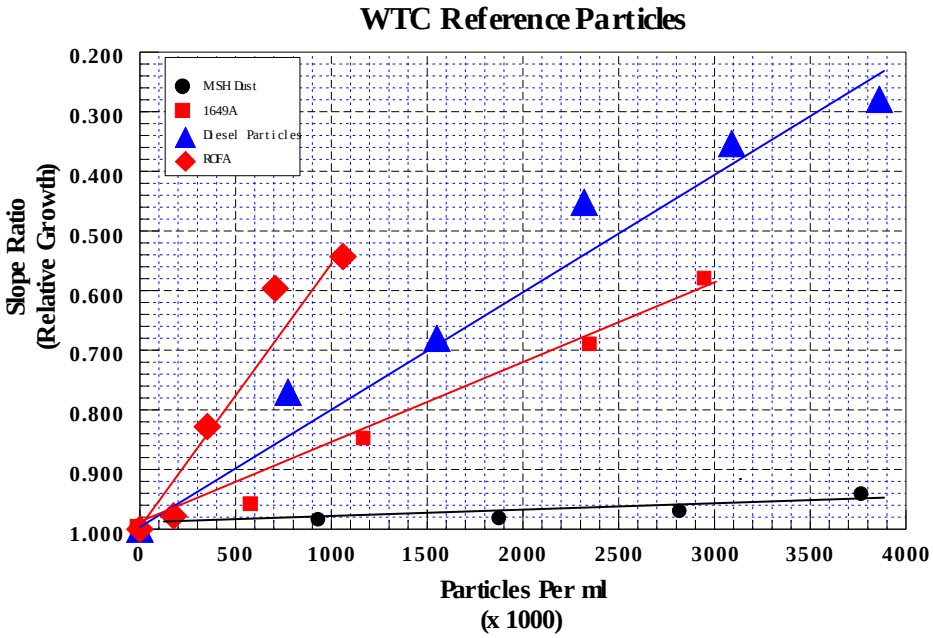

Figure 3. Dose Response of *Tetramitus* flagellates exposed to different concentrations of EPA Reference Samples, used in the WTC Mouse Toxicity Study and diesel particulate matter. At ROFA doses above 1.6 x 10^6 particles per ml, flagellate growth was completely inhibited after 25 hours of exposure. (ROFA – Residual Oil Fly Ash, 1649A – NIST Composite Air Sample taken in Washington, D.C., MSH – Mount St. Helen's Dust, Diesel Particles – Diesel Particulate Matter, NIST SRM # 2975)

In 2006, a toxic water sample was obtained in from a well in Windham, NY (Figure 4). Note that the toxicity of these particles was similar to diesel particulates (Table 1). There is limited evidence that diesel exhaust is a human carcinogen.[11] Thus, further study using EPA TIE methods is indicated in order to evaluate the chemical identity of the toxic agents found in these particles.

[11] National Toxicology Program, Department of Health and Human Services, "Report on Carcinogens: Diesel Exhaust Particulates", Twelfth Edition, 2011, http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/DieselExhaustParticulates.pdf , (accessed September 17, 2011).

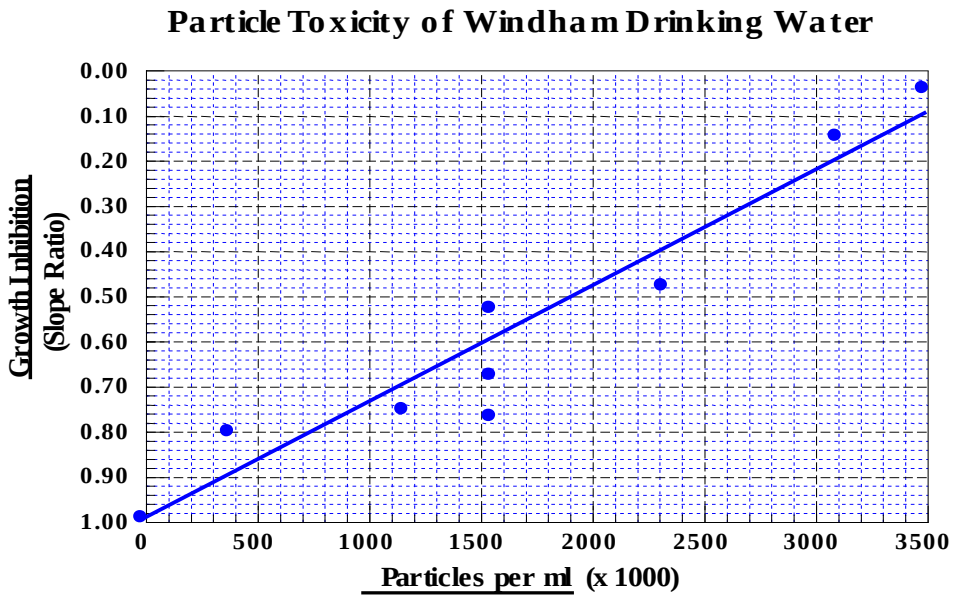

Figure 4. Toxicity of a concentrated particle suspension prepared from a neat drinking-water sample taken from a well in Windham, NY (8-May-2006). The dose response curve for the Windham particles was linear with a correlation coefficient (r^2) of 0.92.

Comparison of $SR_{.80}$ Particle Equivalents for Different Mixtures

We use the dose-intercept value of $SR_{.80}$ to compare the toxicities of different particle suspensions. Table 1 lists $SR_{.80}$ dose-intercept values for particle suspensions obtained from different sources. The $SR_{.80}$ dose-intercept value is that concentration of particles which produces a relative growth rate of 0.80 (20% growth inhibition), The $SR_{.80}$ dose-intercept value is obtained by using the Dependent Variable Intercept Calculator of the PSI-Plot, V7.5 software program (Poly Software International).

Table 1.

Toxic Mixture	$SR_{.80}$ dose-intercept (particles/ml x 1000)	r^2 (Regression)	Ratio to ROFA $SR_{.80}$
Mt. St. Helens Dust	13,838	.878	1/31.6
NIST 1649a	1,609	.964	1/3.67
Deutsche Bank Dust 41ST Floor (Unsonicated)[12]	1470	.980	1/3.36
Windham Drinking Water	927	.919	1/2.12
Diesel Particulates	844	.971	1/1.93
Deutsche Bank Dust 41ST Floor (Sonicated)[13]	450	.723	1/1.03
ROFA	438	.942	-----

[12] R.L.Jaffe, et.al., "Toxicity of Deutsche Bank Dust Samples", Report Submitted to the EPA Safe Buildings/Homeland Security Group, ETL Website, February 23, 2005, http://envirotoxlab.purehost.com/studies/toxicityofdeutschebankdustsamples.pdf

[13] Ibid.