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THE *TETRAMITUS* ASSAY

The field of toxicology frequently uses short-term tests, known as bioassays, to determine the presence and relative concentrations of toxicants in various biological samples. One of the most well know and frequently used bioassays is the Ames Test. Others include the various Microtox™ tests, Mutatox™, AMAX II™, as well as the numerous invertebrate, fish and amphibian tests for aquatic toxicity. Despite their widespread use, many bioassays serve as imperfect data retrieval systems for the detection of toxic agents for several reasons: the tests are costly, time-consuming, poorly suited for field applications, yield imprecise results, and are scientifically complex. Moreover, most bioassays have had limited utility in ascertaining the toxicity of complex particulate samples because suspected toxicants must be extracted from contaminated sediments before testing can begin.

ETL's *Tetramitus* Assay measures the effects of toxic agents on the flagellate phenotype of *Tetramitus rostratus*, a protozoan organism. When a sample concentrate or particle suspension containing toxic agents is added to a *Tetramitus* culture, cell division is inhibited in direct proportion to the concentration of individual or multiple toxic agents. The *Tetramitus* Assay measures the dose-response relationship, thus indicating the presence and degree of toxicity in that sample. Because *Tetramitus* flagellates have a gullet and are particle feeders, the assay allows for the measurement of whole particle toxicity without prior extraction or solvent substitution procedures. This feature is a significant advancement in the field of toxicity testing and is but one of many that distinguishes the *Tetramitus* Assay from other bioassays.

ETL has focused its research on applying the *Tetramitus* Assay to water testing, where the assay has significant environmental implications. Standard monopollutant water testing measures only the levels of individual toxicants and compares this amount with levels deemed acceptable by various regulatory agencies. The *Tetramitus* Assay is particularly important because it measures the total toxicity of a sample, thereby reflecting the synergistic or antagonistic effects of multiple toxicants. The test provides a comprehensive screening for cytotoxicity, thereby minimizing the need for expensive tests for specific toxicants. The assay's whole particle analysis capability is a noteworthy breakthrough in the water testing field because particles contained in sediment contain

higher levels of toxicity than the dissolved components of the water sample itself. In addition to its application of analyzing the toxicity of water samples, ETL plans to develop the protocol for use of the assay in other biological (food, urine, etc.) and air samples.

The *Tetramitus* Assay has many important characteristics that will enable it to achieve a leadership position in the field of toxicity testing. The assay's whole particle testing capability is unique amongst bioassays. The *Tetramitus* Assay, which can detect toxicants at low concentrations, is sensitive to a broad range of mutagens, including aminobiphenyl (main carcinogen in cigarette smoke), 2-aminoflourene (a nitroaromatic similar to many dyes), benzo[A]pyrene (a combustion by-product), and MX (predominant mutagen found in chlorinated water). Dose-response curves are also demonstrated with compounds positive for Salmonella strains TA97, TA98, TA100 and TA102. In a test with 31 coded samples there were no false positives or negatives (on the basis of Ames Test values). Dose-response curves for three mutagens (aminobiphenyl, cadmium and para-phenylenediamine) measured three years apart, showed excellent agreement. Furthermore, repeat assays for 2-aminoflourene using populations of flagellates growing at two vastly different initial growth rates, were in close agreement. These results demonstrate the robustness of the *Tetramitus* Assay. Because of the *Tetramitus* Assay's positive response to diverse types of DNA damaging agents, there is strong circumstantial evidence that the *Tetramitus* Assay is, in fact, measuring genotoxicity. The technology behind the *Tetramitus* Assay is scientifically simple. Very little technical training is required to run the test and minimal scientific equipment is needed. Further, results are achieved rapidly, the test is cost-effective and environmentally friendly. ETL has developed software which facilitates the statistical analysis of the results of the *Tetramitus* Assay.

Most of the data collected to date on the *Tetramitus* Assay has been compared to Ames Test values. The Ames Test, still considered by many to be the gold standard of bioassays and toxicological testing, is slow, expensive and scientifically complex. While the *Tetramitus* Assay performs very well when compared to the Ames Test, the competitors of this technology are the several rapid, inexpensive, benchtop toxicology tests now on the market, such as those offered by Azur Environmental, Xenometrix Corporation and others. The *Tetramitus* Assay seems to enjoy many advantages over other test systems:

- The *Tetramitus* Assay is inexpensive to perform. It can be run for a fraction of the cost of other benchtop tests. The primary expense associated with the *Tetramitus* Assay is the cost of the labor necessary to conduct the test.
- The *Tetramitus* Assay requires no specialized preparations or test kits, only inexpensive commercially available reagents and media. Results are obtained within 4-24 hours. While this is competitive with Microtox™, most other bioassays take considerably longer. The

testing protocol is simple and can be mastered by a technician with only a few hours of training.

- The *Tetramitus* Assay measures the toxicity of whole particles by flagellate ingestion via the gullet. Most other bioassays require the extraction of toxicants, frequently using solvents, before testing of a particle sample can begin. The efficacy of this extraction process, as well as any solvent's impact on the accuracy of test results, are unclear. This aspect of the *Tetramitus* Assay, the ability to perform true and reproducible whole particle testing, is a significant competitive advantage over other systems.
- The *Tetramitus* Assay does not require the addition of an exogenous activation mixture (Rat Liver S9) necessary for many other toxicity screens, because *Tetramitus* has its own activating enzymes. Accordingly, the *Tetramitus* Assay does not require the use of laboratory animals. This aspect of the *Tetramitus* Assay gives it significant ethical advantages over those bioassays and test systems that still use animals and animal products.
- The *Tetramitus* Assay is sensitive to a broad range of toxicants. Many other test systems still require the use of multiple strains of the indicator organisms or the use of different test kits for the detection of different classes of compounds.
- The *Tetramitus* Assay is not affected by dyes or other coloration in water samples, unlike other test systems.

The market for this technology is expansive. The *Tetramitus* Assay makes toxicity screening quick and inexpensive for water utilities, state and federal regulatory agencies, watershed projects, environmental testing companies, individual homeowners and any industry required to monitor effluent discharge. This technology addresses many goals in new state and federal clean water legislation, as it provides an inexpensive means of self-regulation and monitoring for industry. Moreover, the EPA recently announced a policy shift in favor of *in vitro* toxicity testing, and away from *in vivo* toxicity testing. The market for environmental technology, which is expanding rapidly, is but one small area of the *Tetramitus* Assay's application. The potential use of this technology in the pharmaceutical, chemical, cosmetic and hazardous waste industries may well expand beyond its extensive environmental testing applications.

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Islamabad and Washington.

Pakistani authorities had already made several arrests in connection with Mr. Pearl's abduction, including that of Ahmed Omar Sheikh, a Muslim militant who has publicly claimed credit for the

of officials who maintained a relationship with the Taliban in Afghanistan. The killing of Danny Pearl reinforces the urgency of General Musharraf's effort to turn Pakistan away from radical Islamic fundamentalism.

Breathing the Air Downtown

Recent hearings and analyses of air quality near the site of the World Trade Center collapse have produced some encouraging findings. The most reasonable judgment at this point is that the outside air in Lower Manhattan is safe to breathe and that indoor spaces that have been properly cleaned are fine as well. The main concern is a few hot spots outdoors and whether some apartments or offices need a better cleaning than they have previously received.

It is distressing that, despite a generally heroic response to the terrorist attack, no city, state or federal agency took responsibility for ensuring that indoor spaces were appropriately cleaned before reoccupation. Only after prodding from such elected officials as Senator Hillary Rodham Clinton and Representative Jerrold Nadler has the federal Environmental Protection Agency agreed to set up a task force to deal with indoor air problems.

The judgment that the outdoor air is safe comes from readings at air monitors around ground zero. By one recent count, of some 5,300 air samples analyzed for asbestos by the E.P.A. in Lower Manhattan, only 15 exceeded the pertinent safety standard and only 4 of those occurred after Sept. 30. Surveying the whole range of data available, the Natural Resources Defense Council, a respected environmental organization, concluded in a preliminary report last week that outdoor air quality was now "approaching or similar to" levels in the area before the buildings collapsed.

Even so, at least 10,000 New Yorkers suffered short-term health effects, the environmental group estimates. They include rescue workers who toiled in the rubble for weeks, often without wearing

respirators, and survivors of the attack who were engulfed in the billowing dust clouds as they fled. Most alarmingly, say doctors who treat the victims, many office workers and residents who returned to the area after the attack have also come down with respiratory problems. Some cases are mild and go away with time, but for susceptible individuals, the symptoms can persist.

The greatest concern is the quality of air inside buildings, where measurements and cleanup tasks were largely left to building managers and residents. Critics believe that residual contamination in many buildings could pose a long-term threat if not removed. A survey of 59 apartments in 30 buildings by the Centers for Disease Control and the city Health Department found no elevated levels of asbestos in the air but low levels of asbestos in some dust samples and fiberglass in other samples. No one knows how many indoor spaces might be contaminated. The E.P.A. and the city's own environmental agency should determine the scope of the problem and ensure a proper cleanup.

The Trade center attack was such an unprecedented event that it left many questions unanswered. Nobody is sure what the health effects of very tiny fibers pulverized in the collapse might be or how the hundreds of toxic materials in the dust clouds might interact to affect health. The greatest risks may well be faced by immigrant day laborers hired to clean many of the buildings, often without proper instruction or gear. As for the wider public, concerned doctors offer this advice: Make sure your building has been cleaned properly — and stay alert for any symptoms of respiratory distress that may indicate exposure to toxic substances.

Editorial Observer/VERLYN KLINKENBORG

All of New York on the Same Page

It seems like such a simple, worthwhile idea. Invite all the residents of a city, young and old alike, to read the same book during the same week, then use that common literary ground to strike up a lively conversa-

What began as a good idea looks more and more like a bad one.

this citywide event will have to get the taste of cod-liver oil out of their mouths before they can actually sav-
Mr. Lee's remarkable prose.

The message behind all of this, of course, is that reading is good for

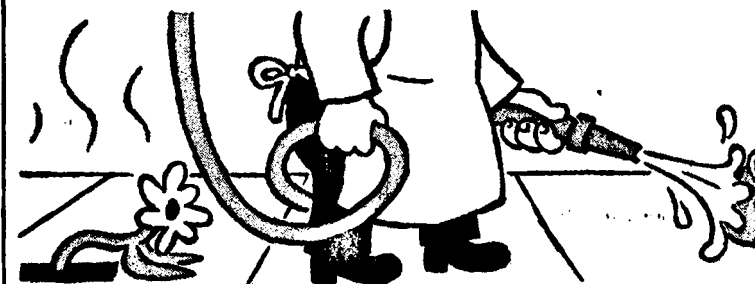
learn from this tragedy, these crimes will be committed again.

JAMES T. SUGRUE
Yonkers, Feb. 20, 2002

To the Editor:
Re "Facing a Sin of the Fathers"

ing numbers of Catholics. Their interest in preserving "an outmoded hierarchical system is more important to them than serving the people of God."
JAMES C. BARRING
Austin, Tex., Feb. 18,

Note to the Super: Psst! There's a Drought



To the Editor:

Re "Drought on East Coast Raises Worries of Water: Rationing" (front page, Feb. 20): Millions of gallons of water are wasted by building superintendents washing down sidewalks. My super says he just received a note from the managing agent saying sidewalks could be washed before 7 a.m. I have asked him not to have the staff do it at all.

Where are the brooms? Bring on the brooms; they're already here!
ANDREW F. M. HOK JR.
New York, Feb. 20, 2002

To the Editor:

Re "Drought on East Coast Raises Worries of Water Rationing" (front page, Feb. 20):

Daily, we hear about the severe drought on the East Coast. It seems prudent, therefore, to take steps today, before we reach the crisis level.

Why not ask all restaurants to get out from diners if they want to before filling and refilling glasses would be such an easy "first step"?
DORIS F.
Floral Park, Queens, Feb. 20,

Who Should Take the Risks for Mideast Peace

To the Editor:

Re "Will Israel Take a Chance?" (Op-Ed, Feb. 21): I ask Henry Siegman how many more chances Israel must take. The Saudi response should be explored, but I wonder where the Saudi leaders were five and 10 years ago.

Further, what other chances must I take when I fear walking the streets of Jerusalem? My children fear riding the buses, and other children I know have developed serious anxiety disorders to confront the anxiety of returning home from school.

When will the Palestinians take a real chance and say that no matter what, they want peace? There seem to be no voices advocating a true settlement at this time. In the past 1

on defending its pre-1967 borders.
CARL MAJ
Newtonville, Mass., Feb. 21

To the Editor:

Thomas L. Friedman asserts full Israeli withdrawal to the 1967 lines is "in accord with Resolution 242" (column, Feb. 20). Resolution 242, drafted by the UN after painstaking diplomacy, specifically stated that Israel would draw "from territories" to "secure and recognized boundaries." The efforts to put the word "all" in the word "territories" were rejected.

The intent of this language is crystal clear to the British foreign secretary, George Brown, who years later: "The proposal sa-