Environmental Toxicology and Risk Assessment

Second Volume

Gorsuch/Dwyer/
Ingersoll/LaPoint
editors

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Environmental Toxicology and Risk Assessment: 2nd Volume

Joseph W. Gorsuch, F. James Dwyer, Christopher G. Ingersoll, and Thomas W. La Point, editors

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Overview

The Second Symposium on Environmental Toxicology and Risk Assessment: Aquatic, Plant, and Terrestrial, held in Pittsburgh, PA, during 26–29 April 1992 continues in what has been a series of 18 successful ASTM Environmental Symposia. The meeting was well attended with 203 participants, including individuals from Canada, Sweden, India, Venezuela, the Netherlands, and the United States. The format for the Second Symposium was sightly different from the first in that it integrated Subcommittee development of standards with symposium presentations. This new format provided greater opportunity for participation by individuals attending the meeting in the development of standards, which is why ASTM is still such a necessary and respected Society.

The Symposium began with a plenary session titled "Greening of the International Marketplace" presented by James Fava (Weston Company) and Frank Consoli (Scott Paper Products). Environmentally friendly manufacture, use, and disposal of products is such an important topic that the presenters were asked, and graciously provided, a brief summary of Product Life-Cycle Assessment, which follows the Overview.

During the following three days, ten platform sessions and a poster session covered a wide array of topics. Some covered more traditional topics including: aquatic toxicology, biomarkers, ecosystems, effluent toxicology, plant toxicology, and sediment toxicology. It is somewhat surprising to realize that topics such as effluent and sediment toxicology are now considered "traditional," especially since we can remember when these efforts were just getting started. Many of the papers and related posters presented in these sessions are included in this volume.

As was the case in the first symposium, presentations on Ecological Risk Assessment were well-attended. Two sessions were devoted to Ecological Risk Assessment. The first session on Ecological Risk Assessment focused on the multiple uses of quantitative structure activity relationships (QSARs) by the U.S. EPA Office of Pollution Prevention and Toxics (OPPT). All the papers presented within this session have been published in a separate section within this Special Technical Publication (STP). Hopefully, this provides readers with pertinent information regarding OPPT's use of QSARs. The second session covered such topics as: issues of scale and uncertainty, defining scientific procedural standards, and the presentation of ecological risk assessments for specific contaminants and ecosystems.

There were several "firsts" for ASTM at this meeting. Subcommittee E47.12 on Behavioral Toxicology met for the first time. To launch this Subcommittee's activities, a session dedicated to topics associated with Behavioral Toxicology was held. Papers focused on the status of fish behavioral toxicology and included: avoidance-attractance, behavioral activity, respiratory monitoring, and predator-prey interactions, while other papers focused on behavioral toxicology of invertebrates and birds.

This Symposium presented the first session for ASTM dedicated to environmental neurotoxicology. The Neurotoxicology session was also well represented with five of the papers presented at the session published in this STP.

This STP contains 45 papers covering the areas of environmental toxicology and risk assessment. There are eight groupings of papers within the STP; however, as is always the case, several papers span multiple topics. The papers in this STP present the development of new research techniques, synopses of available techniques, findings concerning various

environmental stressors, and the application of techniques and processes to environmental assessment.

We would like to thank the session chairs who recruited speakers and organized sessions: Plant Toxicology, Jerry Barker (Mantech Environmental Technology, Inc., Corvallis, OR) and Jim Hoberg (Springborn Laboratories, Inc., Wareham, MA); Sediment Toxicology, Marcia Nelson (U.S. Fish and Wildlife Service, Columbia, MO) and Beth McGee (U.S. Environmental Protection Agency, Washington, DC); SAR/QSAR in the Office of Pollution Prevention and Toxics, Maurice Zeeman (U.S. Environmental Protection Agency, Washington, DC); Biomarkers, Greg Linder (Mantech Environmental Technology, Inc., Corvallis, OR); Effluent Toxicity, Tom Abrahamsen (Eastman Chemical Co., Kingsport, TN) and Remi van Compernolle (Shell Development Co., Houston, TX); Environmental Neurotoxicology, Behrus Jahan-Parwar (State University of New York, Albany, NY); Aquatic Toxicity, Laverne Cleveland (U.S. Fish and Wildlife Service, Columbia, MO); Ecological Risk Assessment, Greg Biddinger (Exxon Biomedical Sciences, East Millstone, NJ); Behavioral Toxicology, Ed Little (U.S. Fish and Wildlife Service, Columbia, MO); Poster Session, Mark Hinman (Exxon Biomedical Sciences, East Millstone, NJ) and John Walker (U.S. Environmental Protection Agency, Washington, DC). Also, we would like to thank the ASTM staff that helped us with planning the Symposium and this STP: Dorothy Savini (Symposium Planning); Kathy Dernoga, Therese Pravitz, and Lynn Hanson (Acquisitions and Review); Ken St. John (Representative of Committee on Publications (COP)), University of Mississippi, MS); and Susan Canning (Committee E 47 Staff Manager).

As we wrap up this second volume on Environmental Toxicology and Risk Assessment, the third symposium will have been held, new techniques and modification of old techniques will have been presented for environmental assessments, and Subcommittees will have been working towards consensus on standard methods. There are many other societies and organizations to which many of us belong. And while some of these groups may have highly visible roles in various aspects of the environmental sciences, ASTM provides a forum for consensus and standardization of methods necessary to evaluate and protect our fragile environment.

Joseph W. Gorsuch

Eastman Kodak Company, Rochester, NY; symposium chirman and editor.

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U.S. Fish and Wildlife Service, Columbia, MO; symposium chairman and editor.

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Synopsis of Plenary Session

Product Life-Cycle Assessment

The purpose of this material is to describe Life-Cycle Assessment (LCA) and to briefly discuss possible applications.

Product life-cycle assessment is a process used to evaluate the environmental burdens associated with a product, process, or activity. This data is then used to assess the impact of those energy and material uses and releases on the environment, and to evaluate and implement opportunities to achieve environmental improvements. LCA includes the entire life-cycle of the product, process or activity, encompassing, extracting, and processing of raw materials; manufacturing, transportation, and distribution; use, re-use, maintenance; recycling; and final disposal. In 1991, the Society of Environmental Toxicology and Chemistry developed a technical framework for LCA. That report provides a discussion on the state-of-the-practice of product LCA.

A complete LCA consists of separate but interrelated components: inventory, impact, and improvement analysis. It should be emphasized that most of the past efforts to develop or conduct life-cycle assessment have focused on the inventory component. These three components comprise an integrated approach that, when combined with other appropriate data, can provide the insight needed to achieve and maximize environmental improvements.

Environmental benefits can be realized at each step in the LCA process. For example, the inventory alone may be used to identify opportunities for reducing emissions, energy, and material use. The impact analysis and improvement analysis tools, however, help ensure that these potential reduction strategies are optimized and that improvement programs do not produce unanticipated impacts.

The LCA is a dynamic and iterative process of evaluation. For example, changes in the material input to a manufacturing process or changes in the process itself may trigger the need for an updated inventory. Likewise, new information pertaining to human or environmental exposure and toxicity may trigger the need to update the impact and improvement analyses.

Life-Cycle Inventory

The foundation, and most practiced component of an LCA is the inventory. It is in this component that a quantification of energy, raw materials, and environmental releases occur throughout the life cycle of the product. The major life-cycle inventory stages focus on (1) raw materials acquisition, (2) manufacturing, processing, and formulation; (3) distribution and transportation, (4) use/re-use/maintenance, (5) recycling, and (6) waste management. In general, each stage receives inputs of materials and energy and produces outputs of materials or energy that move to subsequent stages and wastes that are released into the environment.

An example illustrates how companies can use the application of product LCAs today to understand and make decisions concerning environmental and resource issues associated with their products, processes, or packaging. Scott Paper Company has decided to apply the concept of product LCA internally to gain insights in its operations. Scott has developed a strategy to consider resource and environmental issues along the entire life cycle of products

starting from natural resources, raw material, manufacture, product and packaging development, and product use and disposal. The ultimate endpoint for Scott's LCA work will be to incorporate life-cycle consideration into its product/process development system.

In conclusion, companies who have integrated life-cycle environmental, health, safety and resource considerations with their product design and development systems and total quality management practices will become preeminent among their peers. A proactive program provides a management tool to effectively and efficiently implement actions, on a product-by-product basis, to continually improve the environmental quality of products, packaging, processes, and activities. Overall, this results in the long-term sustained success of a company.

James A. Fava

Roy F. Weston, Inc.,
West Chester, PA.

Frank J. Consoli

Scott Paper Company,
Philadelphia, PA.

Contents

Overview—J. W. GORSUCH, F. J. DWYER, C. G. INGERSOLL, AND T. W. LA POINT ix

Summary of Plenary Session: Product Life-Cycle Assessment—J. A. FAVA AND F. J. CONSOLI xi

AQUATIC TOXICOLOGY AND USE OF EXPERIMENTAL ECOSYSTEMS

The Use of Stimulable Bioluminescence from Marine Dinoflagellates as a Means of Detecting Toxicity in the Marine Environment—D. LAPOTA, G. J. MOSKOWITZ, D. E. ROSENBERGER, AND J. G. GROVHOUG 3

Effect of Varying Environmental Conditions on the Toxicity of Copper to Salmon—J. E. MUDGE, T. E. NORTHSTROM, G. S. JEANE, W. DAVIS, AND J. L. HICKAM 19

Quality Assurance in Programs Regulated by the U.S. Environmental Protection Agency—P. D. ROYAL AND K. M. JOP 34

Invertebrate Behavior as an Indicator of Contaminated Water and Sediments—E. H. SMITH AND D. T. LOGAN 48

Ameliorating Effect of Sodium Chloride on the Tolerance of *Cheumatopsyche pettiti* (Trichoptera: Hydropsychidae) to Water Acidification—J. A. CAMARGO AND J. V. WARD 62

Avoidance-Preference Testing in Aquatic Toxicology: Towards a Standardized Methodology—C. W. STEELE, D. H. TAYLOR, AND S. STRICKLER-SHAW 73

Community Change and Ecosystem Functional Complexity: A Microcosm Study of Copper Toxicity—J. R. PRATT AND J. L. ROSENBERGER 88

Utility of Laboratory Microcosms for Predicting the Environmental Fate of Chemicals: A Comparison of Two Microcosm Designs with Butyl Benzyl Phthalate—W. J. ADAMS AND V. W. SAEGER 103

BIOMARKERS

Chlorophyll Fluorescence: Its Status and Future as a Rapid Assay of Plant Stress—L. A. KAPUSTKA 123

Allozyme Frequency Analysis of Aquatic Populations as an Indicator of Contaminant-Induced Impacts—R. B. GILLESPIE AND S. I. GUTTMAN 134

Correlation with Fetax of a Cellular Bioassay—Cell Health Assay of Water Quality—CHAWQ—J. T. BLANKEMEYER, B. K. STRINGER, J. A. BANTLE, AND M. FRIEDMAN 146

A Short-Term Test for Dioxin Teratogenicity Using Chicken Embryos—D. S. HENSHEL, B. M. HEHN, M. T. VO, AND J. D. STEEVES 159

Biomarkers in Avian Field Studies—B. A. WILLIAMS 175

Effects of a Single Dose Exposure to Aroclor 1254 on Mouse Hepatic Cytochromes P450—L. E. BEEBE, L. W. FORNWALD, S. D. FOX, H. J. ISSAQ, AND L. M. ANDERSON 184

EFFLUENT TOXICITY AND SIMULATED TREATMENT

Comparison of Acute and Short-Term Chronic Test Methods for Effluent Toxicity Monitoring at Sewage Treatment Facilities—L. E. DUNBAR, T. T. HAZE, AND A. A. IACOBUCCI, JR. 197

Characterization, Identification and Confirmation of Total Dissolved Solids as Effluent Toxicants—W. L. McCULLOCH, W. L. GOODFELLOW, JR., AND J. A. BLACK 213

Important Considerations in Choosing a Synthetic Feed for Laboratory-Scale Wastewater Treatment Systems—H. M. WATSON 228

Toxicity Characterization of Chronically Toxic Industrial Wastewaters—A. A. KHAN, D. J. KENT, J. F. BARBIERI, F. P. SWEENEY, AND S. A. KHAN 240

ENVIRONMENTAL NEUROTOXICOLOGY

A Receptor Binding Assay Applied to Monitoring the Neurotoxicity of Parathion to *Peromyscus* After Oral Exposure—D. A. JETT, A. T. ELDEFRAWI, AND M. E. ELDERFRAWI 253

Non-Invasive Measures of Neurotoxicity in Terrestrial and Aquatic Oligochaetes—C. D. DREWES, A. LINGAMNENI, AND R. W. ROGGE 263

A Non-Invasive Neurotoxicity Assay Using Larval *Medaka*—D. FEATHERSTONE, C. D. DREWES, J. R. COATS, AND S. P. BRADBURY 275

Effects of PCB Exposure on Dopamine Levels in the Nervous System of a Mollusc—R. C. SOKOL AND B. JAHAN-PARWAR 289

Effect of Embryonic PCB Exposure on Hatching Success, Survival, Growth and Developmental Behavior in Landlocked Atlantic Salmon, *Salmo salar*—J. P. FISHER, J. M. SPITSBERGEN, B. BUSH, AND B. JAHAN-PARWAR 298

PLANTS FOR TOXICITY ASSESSMENTS

A Review of Terrestrial Plants as Biomonitors—T. G. PFLEEGER, H. C. RATSCH, AND R. A. SHIMABUKU 317

The Allium Test—A Potential Standard for the Assessment of Environmental Toxicology—G. FISKSESJÖ 331

Activation and Detoxification of Nitro-Aromatic Compounds by Plant Tissue Culture Cells—B. S. SHANE, C. R. MANY, AND D. J. LONGSTRETH 346

Phytotoxicity: Recommendations for Measuring and Reporting Dosage—C. McFARLANE, J. S. FLETCHER, S. BRESLER, M. MATTHIES, AND J. BARKER 362

Quantitative Structure Activity Relationships for the Photoinduced Toxicity of Polycyclic Aromatic Hydrocarbons to Duckweed—A Preliminary Model—B. M. GREENBERG, X.-D. HUANG, D. G. DIXON, L. REN, B. J. McCONKEY, AND C. L. DUXBURY 369

RISK ASSESSMENT

Ecological Risk Assessment of a Novel Marine Antifoulant—W. D. SHADE, S. S. HURT, A. H. JACOBSON, AND K. H. REINERT 381

Experimental Versus Empirical Approaches to Setting Water Quality Objectives—M. GILBERTSON 409

A Proposed Approach to Quantitatively Assess Potential Ecological Impacts to Terrestrial Receptors from Chemical Exposure—G. E. WATKIN AND M. E. STELLJES 422

Defining Scientific Procedural Standards for Ecological Risk Assessment—D. A. BELLUCK, R. N. HULL, S. L. BENJAMIN, R. D. FRENCH, AND R. M. O'CONNELL 440

The TSCA Interagency Testing Committee, 1977 to 1992: Creation, Structure, Functions, and Contributions—J. D. WALKER 451

Metal Accumulation in Blood and Milk of Dairy Cows Grazed or Fed by Fodder Grown on a Sewage Water Disposal Site—K. VARADARAJAN, K. PALIWAL, AND C. RAJAMANICKAM 510

SAR QSAR IN THE OFFICE OF POLLUTION PREVENTION AND TOXICS

The Development of SAR/QSAR for Use Under EPA's Toxic Substances Control Act (TSCA): An Introduction—M. G. ZEEMAN, J. V. NABHOLZ, AND R. G. CLEMENTS 523

Structure/Activity Relationships for Evaluation of Biodegradability in the EPA's Office of Pollution Prevention and Toxics—R. S. BOETHLING 540

The Use of Quantitative Structure-Activity Relationships (QSAR's) as Screening Tools in Environmental Assessment—R. G. CLEMENTS, J. V. NABHOLZ, D. E. JOHNSON, AND M. G. ZEEMAN 555

Validation of Structure Activity Relationships Used By the USEPA's Office of Pollution Prevention and Toxics for the Environmental Hazard Assessment of Industrial Chemicals—J. V. NABHOLZ, R. G. CLEMENTS, M. G. ZEEMAN, K. C. OSBORN, AND R. WEDGE 571

Quantitative Structure-Activity Predictions for Amine Toxicity to Algae and Daphnids—L. D. NEWSOME, D. E. JOHNSON, AND J. V. NABHOLZ 591

Baseline Toxicity QSAR Models: A Means to Assess Mechanism of Toxicity for Aquatic Organisms and Mammals—R. L. LIPNICK 610

SEDIMENT TOXICOLOGY

Developing Chronic Sublethal Sediment Bioassays: A Challenge to the Scientific Community—T. M. DILLON 623

Sediment Toxicity Testing: Comparison of Methods and Evaluation of Influencing Factors—P. V. WINGER AND P. J. LASIER 640

Density, Diversity, and Incidence of Deformities of Benthic Invertebrates Near a Vinyl Chloride Discharge Point Source in the Niagara River Watershed—M. D. DICKMAN AND G. RYGIEL 663

The Importance of Biological Testing in the Assessment of Metal Contamination and Site Remediation: A Case Study—C. R. LEE, J. W. SIMMERS, D. L. BRANDON, AND B. L. FOLSOM, JR. 681

The TSCA Interagency Testing Committee's Role in Facilitating Development of Test Methods: Toxicity and Bioconcentration Testing of Chemicals Added to Sediments—J. D. WALKER 688

Author Index 723

Subject Index 725

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Aquatic Toxicology and Use of Environmental Ecosystems

David Lapota,[1] Gwendolyn J. Moskowitz,[2] Dena E. Rosenberger[2], and Joseph G. Grovhoug[3]

THE USE OF STIMULABLE BIOLUMINESCENCE FROM MARINE DINOFLAGELLATES AS A MEANS OF DETECTING TOXICITY IN THE MARINE ENVIRONMENT.

REFERENCE: Lapota, D., Moskowitz, G. J., Rosenberger, D. E., and Grovhoug, J. G., **"The Use of Stimulable Bioluminescence from Marine Dinoflagellates as a Means of Detecting Toxicity in the Marine Environment,"** Environmental Toxicology and Risk Assessment: 2nd Volume, STP 1216, J. W. Gorsuch, F. J. Dwyer, C. G. Ingersoll, and T. W. La Point, Eds., American Society for Testing and Materials, Philadelphia, 1993.

ABSTRACT: Phytoplankton bioassays have been used as biological tools in assessing environmental impact from contaminants. Series of experiments were designed to measure the acute and sublethal effects of heavy metals (tributyltin, copper, and zinc) and storm drain effluent on the light output from marine bioluminescent dinoflagellates (*Pyrocystis lunula* in earlier experiments and *Gonyaulax polyedra* in later experiments). Cultured cells were exposed to various concentrations of a metal or storm drain effluent from hours up to 11 days. Measurable differences in light output have been observed in as little as 3 h when compared to control cells.

KEYWORDS: bioluminescence, toxicity testing, dinoflagellates, metals, storm drain effluent

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Phytoplankton bioassays have been used as biological tools in assessing environmental contamination. The use of these organisms in bioassays is justified by the ecological role they play as primary producers with respect to other trophic levels, and their inherent sensitivity to toxic chemicals (Salazar 1985; Walsh et al. 1985). Also, phytoplankton bioassays tend to be simple, rapid, and inexpensive when compared to more complicated and involved assays using fish and invertebrate species.

Historically, phytoplankton bioassays have involved the enumeration of phytoplankton cells to determine stress in algal populations when exposed to a single toxicant or chemical mixture (Lapota et al. 1989). These assays have been successful, but can be labor intensive. Therefore, other toxicological endpoints for the measurement of toxicity in phytoplankton are being investigated. In our laboratory, a bioassay has been developed which measures the light output from bioluminescent dinoflagellates for assessment of toxic effects when exposed to a single toxicant or mixture. Bioluminescence is a visible blue light produced either intermittently or continuously by numerous terrestrial and aquatic organisms. Many of the marine dinoflagellate species are able to produce bioluminescence as part of their daily physiological processes. Similarly, some marine bacteria also emit this light. Early observations indicated that the presence of various toxicants reduced the light intensity of bioluminescent bacterial cultures (Beijerinck 1889). The utility of bioluminescent bacteria as a test organism has since been investigated in testing air quality (Sie and Thanos 1966), herbicides (Tchan and Chiou 1977), and other toxic chemicals (Johnson et al. 1942). A bacterial bioluminescent assay (using freeze-dried cultures of a cold water bacterium) has been developed for the detection of toxic compounds (Bulich 1979).

Only recently has work been initiated using bioluminescent dinoflagellates as bioassay organisms. It was observed that exposure to toxic compounds quenched the bioluminescence from cultures of the dinoflagellate *Pyrocystis lunula* by as much as 40 percent following a 2-h exposure (Hannan et al. 1986). This species has also been used in acute assays to detect toxicity from drilling muds (Stiffey, pers. comm.). In our laboratory, successful use of this type of bioassay has provided data on the acute response and has demonstrated the chronic effects, from hours up to 11 days, on dinoflagellate cells of *Pyrocystis lunula* and *Gonyaulax polyedra* upon exposure to several metals and storm drain effluent. In this paper, the methods and preliminary results of these tests using stimulable bioluminescence as an endpoint for the detection of toxicity will be discussed.

MATERIAL AND METHODS

Materials

Materials used were as follows: reagent grade copper(II)sulfate pentahydrate and zinc sulfate heptahydrate (Aldrich Chemical Co.); tributyltin chloride (Aldrich Chemical Co.); American Society for Testing and Materials sea-salts (Lake Products Co.).

Culture and Bioassay Containers

Optical grade spectrophotometric cuvettes and polycarbonate flasks were used. All cuvettes and polycarbonate flasks were seawater aged for several days prior to first time use. Containers used in experiments were routinely soaked in RBS (critical cleaner) for 24 h and then soaked in 4N nitric acid for 4 h.

Bioassay Organisms

Two species of marine bioluminescent dinoflagellates were used in the assays. *Pyrocystis lunula* was obtained from the North East Pacific Culture collection at the University of British Columbia, Canada. The other species, *Gonyalaux polyedra*, was isolated from San Diego Bay. Both species were maintained in enriched seawater medium (ESM) according to American Society for Testing and Materials Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae (ASTM 1990). Tetrasodium ethylenediaminetetraacetic acid (EDTA), a chelator, was removed from preparation of the ESM during assays (Davey et al. 1970). Cultures were maintained in 250-mL polycarbonate flasks under a light regime of 12:12 h (light:dark) at approximately 100 fc from cool white bulbs. The cells' day-night cycle was reversed to accommodate daytime testing while the cells were in their night phase and most stimulable for light production. Cultures of *P. lunula* were maintained at a room temperature of 22°C prior to and during the assay; where as, *G. polyedra* was maintained at 18°C. Typically, *P. lunula* was cultured at approximately 3 to 4×10^3 cells/mL with medium changes at 1 month intervals. *G. polyedra* was cultured at twice this density with medium changes weekly due to their higher cell division rates.

Experimental Design

Dinoflagellate cells were exposed to various concentrations of tributyltin chloride (TBTCl), copper (II) sulfate (Cu_2SO_4), zinc sulfate ($ZnSO_4$) or storm drain

effluent. P. lunula was used in earlier experiments with TBTCl, storm drain effluent, and Cu_2SO_4; whereas, G. polyedra was tested in later experiments with Cu_2SO_4 and $ZnSO_4$. P. lunula was eliminated from further testing because it is an open ocean species and therefore may not be representative of species found in polluted bay and coastal waters. Consequently, G. polyedra was adopted for use in the assays because of its wide distribution in bay and coastal waters of most continents.

The emphasis of this study also shifted to the use of metals recommended by the Environmental Protection Agency for the standardization of bioassays. The range of metal concentrations tested was primarily determined based on the known sensitivities of other species.

A working solution of test article (test metal or storm drain effluent) or deionized water (for controls), seawater medium, and dinoflagellate cells was prepared for each experimental concentration and control. Aliquots of the working solution were delivered to cuvettes (test chambers) and light output measured. Assays ranged in duration from 3 to 11 days.

Three hundred cells per cuvette were used in all experiments. This number was determined from previous experiments in which light output vs. cell number in P. lunula was measured. The results indicated that as light output increased with cell number the coefficient of variation (C.V.) decreased (Fig. 1). A C.V. of approximately 10% was obtained using 300 cells; therefore, this cell number was adopted for use in the assay.

Determination of stock culture density

At the beginning of each assay, the stock culture density was determined by pipetting 1 mL of stock culture into 25 mL of medium (stock cultures were diluted for ease of enumeration). Subsamples of the dilute stock solution, in 1-mL aliquots, were pipetted into settling chambers which contained a 5% buffered formalin solution. The subsamples were enumerated in the settling chambers with an Olympus IMT-2 inverted microscope at 40x magnification. A total of 4 subsamples were enumerated and the mean calculated. The mean cell number was then multiplied by the dilution factor for determination of stock density.

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