

# **FINAL REPORT**

## **REDUCED MIST GENERATION WITH MICROPCCM MACHINING COOLANTS**

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**June 29, 1999**

## LIST OF ABBREVIATIONS

<b>MicroPCM</b>	<b>Microencapsulated Phase Change Material</b>
<b>M</b>	<b>Dimensionless Mist Generation Number</b>
<b>Q<sub>m</sub></b>	<b>Metal Removal Rate</b>
<b>Q<sub>f</sub></b>	<b>Fluid Flow Rate</b>
<b>D</b>	<b>Rod Diameter</b>
<b>F</b>	<b>Cut Width or Tool Feed Rate</b>
<b><math>\omega</math></b>	<b>Rotational Speed</b>

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## **SIGNIFICANT FINDINGS**

This Phase I program demonstrated that microPCMs in an machining oil coolant could reduce the amount of mist generated at the highest mist generation numbers on the 2.5 hp bench-top lathe at UNC with aluminum by 31-33%. In cases where particularly deep cuts are rapidly made with relatively little coolant, machining numbers obtained in industry will be significantly greater than those generated here. In an earlier SBIR program for NSF at NCSU, we found that the quantity of mist generated was significantly higher during turning experiments with steel or titanium rods on an 8 hp engine lathe. In such cases, the benefit of microPCMs for reducing mist generation should increase well beyond those determined here. In cytological testing, microPCMs used in coolants were shown to be of minimal toxicity and produced no mutagenesis. A U.S. patent has already been awarded to TRDC for the use of microPCM machining coolants, and several potential Phase III sponsors, including General Motors, have indicated their interest in follow-on Phase II program support.

## **USEFULNESS OF FINDINGS**

Human safety is a concern in a working environment where machinists are sometimes covered with lubricating fluids and machining coolants. As industrial speeds increase, so does the tool interface temperatures that cause higher evaporation of the machine coolants and cutting fluids. The cost of replacing cutting and machining fluids also increases with the speed of machining. The costs, however, of environmental pollution, OSHA regulations, expensive air-handling equipment and associated workplace energy losses, along with continued exposure of machine operators is likely to be much higher.

MicroPCM coolants have demonstrated their ability to provide from 10X to 40X more cooling and heat transport than conventional fluids. Earlier programs for NSF also demonstrated the potential of these new coolants to reduce surface temperatures, reduce tool wear, and improve surface finishes.

The development of microPCM machining coolants, that have the potential for reducing mist generation and machining emissions by up to 90% during high speed operations with hard materials, could be of significance to worker safety and health.

## ABSTRACT

An earlier SBIR Phase I and II program for the National Science Foundation suggested that machine coolants containing microencapsulated phase change materials (microPCMs) may significantly reduce the quantity of particulates generated during high speed machining operations. To this end, an investigation has been performed in conjunction with the School of Public Health at the University of North Carolina in Chapel Hill, NC to determine whether the addition of microencapsulated phase change materials (microPCMs) to mineral oil could significantly reduce the size distribution and concentration of mist generated during a metal machining operation. The test coolant was formulated by adding 25% microencapsulated n-eicosane particles to a low viscosity mineral oil commonly used for such studies at UNC.

Mist was generated during the application of coolant while turning a three-inch diameter aluminum rod on a small 2.5 hp lathe. Different experimental conditions were examined by varying the depth of cut and the rotational speed of the lathe as well as the coolant flow. An Aerosizer LD (Amherst Process Instruments, Inc., Amherst, MA) was used to measure the mist size distribution and number concentration. Mist mass concentrations were measured gravimetrically using polytetrafluoroethylene (PTFE) filters.

The results showed that addition of the microPCMs to the mineral oil did not affect the size distribution of the mist generated, but did decrease the mass of mist generated by 31% at the highest mist generation numbers. These conditions represented the experimental variables with the fastest rotational speed and deepest cut on the lathe, and the lowest coolant flow rate. The mist generation number is the ratio of the metal removal rate divided by the coolant flow rate.

In addition, both toxicity and mutagenesis studies were also conducted by SITEK Laboratories in Silver Spring, MD with the microPCM coolant. These investigations determined that there was neither significant toxicity nor mutagenesis associated with the microPCM particles in the machining coolant.

The extensive use of microPCMs in apparel was licensed by TRDC to two companies: Frisby Technologies in Freeport, NY and Clemmons, NC as well as Outlast Technologies in Boulder, CO. MicroPCM-enhanced apparel products are currently being produced by over 30 U.S. manufacturers. For the past three years, investigators at General Motors Research Center in Warren, Michigan have also worked with TRDC to study how microPCM coolants could be utilized in grinding machining operations.

## Introduction

Metalworking fluids (coolants or lubricants) are used to increase production during machining operations. Production rates increase because the metalworking fluid cools the tool used to machine the workpiece and lubricates the tool-workpiece interface. Metalworking fluids also cool the tool by removing the metal chips formed during the cutting process. Approximately 80% of the heat generated by the cutting action is absorbed by these chips. By flushing the chips and thus the excess heat away from the tool with a metalworking fluid coolant, faster cutting speeds are possible and production rates can be increased. Metalworking fluids also reduce the friction between the tool and workpiece, thus increasing the life of the tool and resulting in a better surface finish on the workpiece.

Materials used in cutting and forming operations include: metals, alloys, plastics, ceramics and composites. The removal processes involved include: turning, milling, broaching, drilling, tapping, cutoff, grinding, polishing and lapping. These processes apply a tool or an abrasive at speeds with sufficient force to remove a given quantity of material. Material forming processes such as forging, rolling, extrusion, rod and wire drawing, tube drawing, deep drawing, swaging, and roll forming rely on plastic deformation. In material removal processes, the rate of production as well as the life of the tool is greatly influenced by whether or not a cutting fluid is used. Ineffective cooling can lead to thermal distortion of the workpiece that subsequently produces a loss in dimensional tolerances. The development of superior cutting or machining fluids or coolants, that facilitate the enhanced cooling of a cutting tool and workpiece or that may also improve lubricity, could be of great value to industrial manufacturing.

The two main functions of cutting fluids or machine coolants are lubrication at relatively low cutting speeds and cooling at relatively high cutting speeds. At high cutting speeds, there is normally not sufficient time for the fluid coolant to reach the chip-tool interface and significantly influence its lubrication. Machine coolants are usually either water- or oil-based. A variety of methods are utilized to apply them; including, dripping, flooding, high pressure jet, spray misting and manual brushing. Older methods generally flood the interface area from the top down, but for efficient high-speed machining, a jet directed under and about the chip by high pressure spraying has been found to be the most effective.

Water-based coolants have a higher heat capacity than those which are oil-based, and can sustain increased heat loads during high-speed machining. Water-based coolants, however, can promote corrosion in some materials where oil-based coolants will not. Oil-based fluids have approximately 1/4 to 1/3 the heat capacity of water-based coolants, and thus often require higher flow rates. Additives to some coolants also limit their usage with certain materials; e.g., lubricating fluids for iron and steel are normally not used with aluminum.

Some materials are also harder to machine than others. One particularly important property is thermal conductance or the ability to conduct the heat generated away from the contacted parts. The amount of heat generated during a machining operation depends upon the material and the rate of machining. High-speed machines commonly approach speeds of 1,000 ft/min, and at these very high speeds, cutting fluids or machine coolants become increasingly ineffective and important. The role of the machine coolant or cutting fluid is particularly important in high speed machining of materials with limited thermal conductance. Hot-rolled steels and many composites can cause problems for high speed machining, and improved lubricants or machine

coolants can facilitate their fabrication. Metals such as hot-rolled steel can dull even the best tungsten carbide tools in a short time. Ceramics have greater compressive properties and lower tensile strength, but can withstand higher temperatures than metals. In the case of some plastics and composite materials, the heat generated during high speed machining is sufficient to plasticize the work piece.

Straight oil, also known as insoluble oil, is derived from animal, vegetable, or mineral (petroleum) sources, and is not mixed with water. Straight oil is mainly comprised of alkanes, but may also contain other organic compounds such as polycyclic aromatic hydrocarbons. Supplements such as sulfur, chlorine, and phosphorous are frequently added to improve the lubrication and heat dissipation capabilities of a straight oil.

Another category of an oil-based metalworking fluid is a soluble oil, which is also called an emulsified oil because it forms an oil-water emulsion. Soluble oils contain small concentrations (5%-20%) of a straight oil mixed with water and an emulsifier, usually a sulfonate. The presence of water in a soluble oil often requires still more chemical additives to prevent bacterial growth and inhibit corrosion.

Finally, synthetic and semi-synthetic fluids are the newest type of oil-based metalworking fluids or coolants. Synthetic fluids are mixtures of organic or inorganic salts and water. Semi-synthetic fluids are similar, but can contain trace amounts (<1%) of straight oil. Both synthetic and semi-synthetic fluids can offer good microbial control, lubrication properties, and corrosion control. Wetting agents are typically added to increase the lubrication properties of the fluid for use under extreme pressure conditions.

Human safety is also a concern in a working environment where machinists sometimes become covered with lubricating fluids and machining coolants. One threat is the bacteria that can grow in water-based fluids; another is the generation of potentially hazardous vapors or mists during high speed machining. As machining speeds increase, so do the tool interface temperatures that cause higher evaporation of the machine coolants and cutting fluids. The cost of replacing evaporated fluids also increases with the speed of machining. However, the costs of environmental pollution, OSHA regulations, expensive air-handling equipment, associated energy losses and the continued exposure of machine operators to increased risk is undoubtedly much higher.

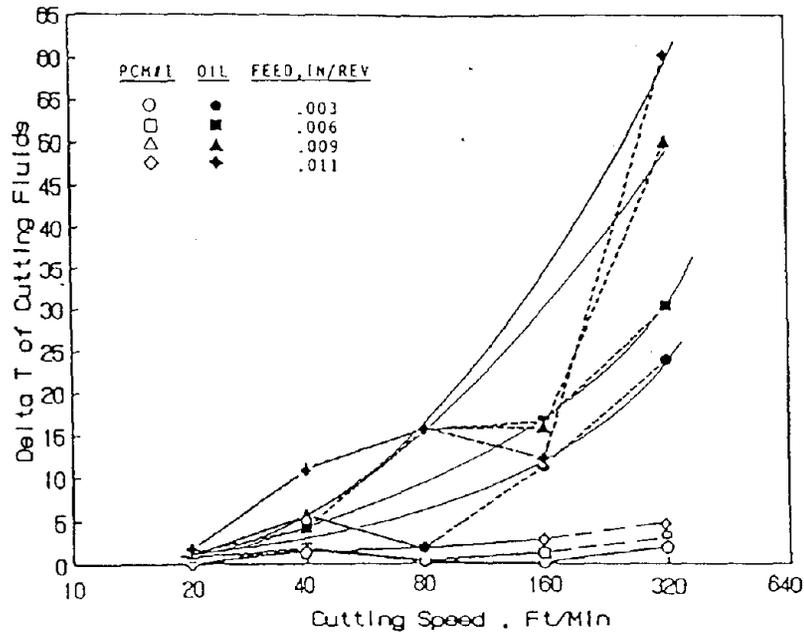
## **Background**

Over the past 15 years, researchers at Triangle Research and Development Corporation (TRDC) have pioneered the development and application of microencapsulated phase change materials (microPCMs) to a variety of materials in order to significantly enhance the thermal capacitance of liquid coolants, textile fibers, apparel foams, aerospace and agricultural coatings, and selected composites [Bryant, Colvin and Mulligan 2-15]. Since 1984 and through 25 Phase I and II SBIR programs for NASA, NSF, USAF, NAVY and USDA, TRDC investigators have demonstrated repeatedly how microPCMs could be added to conventional materials to enhance their thermal capacitance from 10 to 40 times (1,000 - 4,000%) and the heat transport coefficient (Nusselt Number) by as much as 300%. These microPCMs range from approximately 3 to 50 microns in diameter and consist of a paraffin core with a plastic shell that is normally less than 1 micron in thickness.

In Phase I and II SBIR programs for USAF, NASA, NAVY and NSF, TRDC investigators have developed an entirely new series of textile materials that contain microPCMs for enhanced thermal storage. These materials have already been licensed and sub-licensed to over 30 U.S. manufactures and several European and Japanese companies to produce lightweight, non-bulky apparel that affords up to 10X the thermal capacitance of conventional materials. In continuing programs for NSF, TRDC researchers are developing new microPCM capsule wall materials that permit them to reach temperatures over 300 C so they can be incorporated into melt-spun fibers such as nylons and polypropylenes. In a current Phase II program for DOD, TRDC investigators are developing new and larger encapsulated PCM materials for passive cooling garments.

In other Phase I and II SBIR programs for NSF and USDA, TRDC investigators are also developing microPCM spray coatings that can significantly enhance the effectiveness of mycoherbicides, fungicides, and insecticides. In Phase I programs, they have already demonstrated their capability to provide significant thermal protection to plants from both cold stress (frost and freeze) as well as heat stress (sterilization of corn tassels).

During earlier Phase I and Phase II programs for the National Science Foundation in 1994-96, investigators showed how microPCM machining coolants could (1) significantly enhance the cooling performance compared to the same material without microPCMs, (2) reduce the surface temperatures during machining operations by 15%, (3) reduce tool wear by 25-75% depending upon the machining speeds, and (4) generate a significantly decreased amount of both generated mist and evaporated coolant. They attributed the latter effect to the enhanced thermal capacitance of the microPCM coolant; however, this Phase I effort is the first to explore this phenomena. Figure 1 illustrates a comparison of cutting fluid differential temperatures across a heat exchanger placed in the return circulating coolant loop during turning experiments using a straight oil and an oil containing microPCMs. Note how cool the microPCMs held the coolant.



**Figure 1. Comparison of Cutting Fluid Differential Temperature Across the Heat Exchanger in a Machining Coolant Loop for Straight Oil and the Same Oil with MicroPCMs**

Reducing the amount of generated coolant mist is a commendable goal as epidemiological studies have associated coolant mist exposures with adverse health effects. In response to the potential health effects, last year NIOSH recommended setting the permissible exposure limit for metalworking fluid mist at  $0.5 \text{ mg/m}^3$  for total particulate and  $0.4 \text{ mg/m}^3$  for thoracic particles. However, given current control practices, it may be difficult and expensive to achieve the proposed standard; hence, a more basic means to minimize mist concentrations by preventing mist formation is worthwhile.

In 1989, over 1.8 million metal cutting machines operating in the United States exposed over 1.2 million operators to metalworking fluid mists [1]. To protect workers from the health damaging effects of oil mists, the ACGIH recommended a TLV of  $5 \text{ mg/m}^3$  of "respirable particles" in 1966 and OSHA adopted the TLV as the PEL in 1970. Since then, the installation of machine enclosures, local exhaust ventilation, and mist collectors has decreased the ambient concentrations of oil mists in the workplace. Due to the large number of workers exposed, many epidemiological studies have been conducted in an attempt to understand the exposure routes, and potential carcinogenic and non-carcinogenic health effects of metalworking fluids to humans.

Dermal contact is one exposure route for metalworking fluid mists [20]. Dermal exposure to metalworking fluid mists is associated with both benign and malignant skin conditions. Contact dermatitis of the hands and forearms due to exposure to soluble and synthetic metalworking fluids is the most prevalent health problem affecting metal machining workers [21,22,23]. In addition, excessive incidences of skin and scrotal cancer were discovered in workers who were exposed to straight oil mists, but cases are becoming less common as the carcinogens in straight oil are being removed by improving the mineral oil refining processes [24,25,26,27].

The other exposure route is inhalation. Inhaled oil mist droplets smaller than 10 microns are associated with non-malignant respiratory effects and a variety of cancers, but not lung cancer [28,29]. All three types of metalworking fluids are capable of inducing asthma, chronic bronchitis, and an acute cross-shift decrease in lung function for exposed workers at exposure concentrations less than  $5 \text{ mg/m}^3$  [30,31,32,33]. Several more recent studies have also demonstrated an increased incidence of many gastrointestinal cancers in metal machining workers after prolonged exposure to mists of all three types of metalworking fluids. For instance, Park *et al.* and Silverstein *et al.* suggested a positive association between synthetic or soluble oil used in a grinding operation and stomach cancer [34,35,36]. Another study, conducted by Silverman *et al.*, also indicated an increased risk of bladder cancer in metal machining workers, but did not specify the type of metalworking fluid associated with the cancer [37]. Furthermore, rectal and laryngeal cancers have been attributed to straight oils, and synthetic oils are also linked to pancreatic cancer [36,38,29,40].

Previous research has mainly focused on determining the sources and size distribution of the mist in industrial settings, and evaluating techniques to control the mist. Woskie *et al.* discovered that the size characteristics of the mist depend upon the type of machining process, the type of fluid being applied, and an interaction between the type of machine and type of fluid [41]. In a similar study, Chan *et al.* discovered that the largest particles ( $\sim 8 \mu\text{m}$ ) resulted from the application of the metalworking fluid, and that the smallest ( $< 1 \mu\text{m}$ ) were generated by the machining operation [42]. To collect the mist generated, industrial mist collectors may be used, but the removal efficiency of sub-micron droplets decreases over time [Leith].

Only one study has investigated altering metalworking fluid formulations to decrease worker exposure to machine mists. Smolenski *et al.* indicated that increasing the elongational viscosity of straight oil by adding a higher molecular weight polymer increased the mass median diameter of the atomized oil droplets from 20% to 200% [43]. Larger mist droplets decreased the exposure concentrations by improving the mist collector's capture efficiency, thus increasing droplet removal due to gravitational settling. Larger droplets will also not penetrate as deeply into worker's lungs.

Researchers at the University of North Carolina (UNC) in Chapel Hill have spent the last several years studying the generation, measurement, and control of mists from metalworking fluids [44-56]. Various aspects of engineering controls used to contain mists of metalworking fluids have been investigated both in the laboratory and at automotive plants. Recently, more fundamental research has focused on determining the mechanisms by which mist droplets are generated. For this study, an apparatus was built to determine the generation rate and size distribution of mist produced when an aluminum rod was turned on a lathe. This work characterized the mist formation process by quantifying the mist generated by impaction, centrifugal force, and evaporation/condensation and identifying the parameters affecting each mechanism.

By teaming with TRDC, researchers at UNC have continued to investigate new means to minimize mist formation and hence, mist concentration. Using previously established methods for generating mist from a metal machining process and for measuring the resultant mist concentration and size distribution, UNC researchers have investigated the potential of a machine coolant with microPCMs to reduce the quantity of mist generated during high speed machining operations. Thus, the effectiveness of microPCMs at preventing the formation of metalworking mists could be determined.

## **Phase I Objectives**

The objectives of this Phase I effort were to:

Task 1. Conduct a coordination meeting with relevant participants in the Phase I effort;

Task 2. Identify, design and test a selected microPCM machining coolant;

Task 3. Formulate the microPCM machining coolant into a control oil;

Task 4. Coordinate and conduct a series of experiments at UNC School of Public Health with measurement of effluent mists from the machining operations;

Task 5. Analyze the experimental results and compare them to the control oil without microPCMs;

Task 6. Conduct an initial investigation into the potential toxicity of the selected microPCM coolant materials;

Task 7. Coordination with potential Phase III sponsors;

Task 8. Prepare a final report describing the activities, results and conclusions of the Phase I effort.

## **Results of the Phase I Program**

The overall results of the Phase I effort will be discussed on a task by task basis as outlined in the objectives above.

### **Task 1. Coordination of the Phase I Activities**

Since this Phase I effort involved the timely participation of multiple organizations, it was important to coordinate the program goals and objectives to the participants and outline how their activities could impact the results of the projected six-month investigation. Dr. Colvin visited with the participants at UNC-School of Public Health in Chapel Hill, NC to outline the program goals and develop a working strategy. He also had conference calls with the president and project officer at SITEK Research Laboratories in Rockville, MD to discuss their role and procedures to determine the potential toxicity of the microPCM materials.

## Task 2. Identification, Design and Testing of a Selected MicroPCM Machining Coolant

Since 1984, TRDC investigators have developed numerous microPCM particles and coolants to support 25 SBIR programs for NASA, USAF, NAVY, NSF, and USDA as well as follow-on Phase III programs with the USAF, Lockheed Advanced Development Company (Skunk Works), McDonnell-Douglas Aerospace, Outlast Technologies, Frisby Technologies and General Motors Corporation. Most of the microPCMs that TRDC has developed consist of a paraffinic core that is 3-50 microns in diameter with a polymer shell as shown in Figure 2. Figure 3 illustrates a scanning electron photomicrograph of microPCM capsules enlarged 2,000X. The shell of the flexible microPCM capsules is usually less than 1 micron thickness to permit the necessary 12% volumetric expansion/contraction upon solid/liquid phase change. Two-component fluid slurries are made up of 20-30% solids concentration of microPCMs in a liquid media, either water or an oil. For the earlier SBIR program for NSF, TRDC investigators used microencapsulated n-eicosane particles to provide the enhanced thermal storage and transport of the metalworking coolant. When a microPCM coolant is circulated so that practically all of the heat generated by the machining process is carried away by the latent phase change of the particles instead of sensible temperature change of the fluid coolant, we term this condition "tuned"; i.e., the system is operated at its optimal level as shown in Figure 4. This procedure is so unique that it has already been granted a U.S. patent and its use for machining operations is also patented by Triangle Research and Development Corporation (TRDC) [15].

For this task as in the earlier program for NSF, TRDC investigators selected n-eicosane to be microencapsulated. This paraffin melts around 36 C, thus it will be fully solidified in the microcapsules at room temperature. TRDC utilized its Perkin-Elmer DSC-7 Differential Scanning Calorimeter to determine not only the temperature range for phase change, but also the quantity of latent heat that can be stored in the material. As expected, these values change somewhat when the paraffin is microencapsulated; i.e., the phase change shifts to a lower temperature. Additionally, the DSC for melting occurs at a significantly higher temperature than that for its refreezing. Figures 5a and 5b illustrate the DSCs for melting and freezing of the selected eicosane microPCM that was used for this Phase I investigation. Figure 6a and 6b are DSCs for the eicosane microPCMs in a 25% concentration of the mineral oil control. Since the microparticles would be recirculated within a closed loop, it was also necessary to determine the rate of leakage through the capsule walls using our Perkin-Elmer Thermogravimetric Analyzer or TGA under repetitive cycling. These tests indicated that the selected microPCM capsules were indeed quite hardy and did not exhibit any appreciable leakage during repetitive recycling across the phase change zone of melting and freezing.

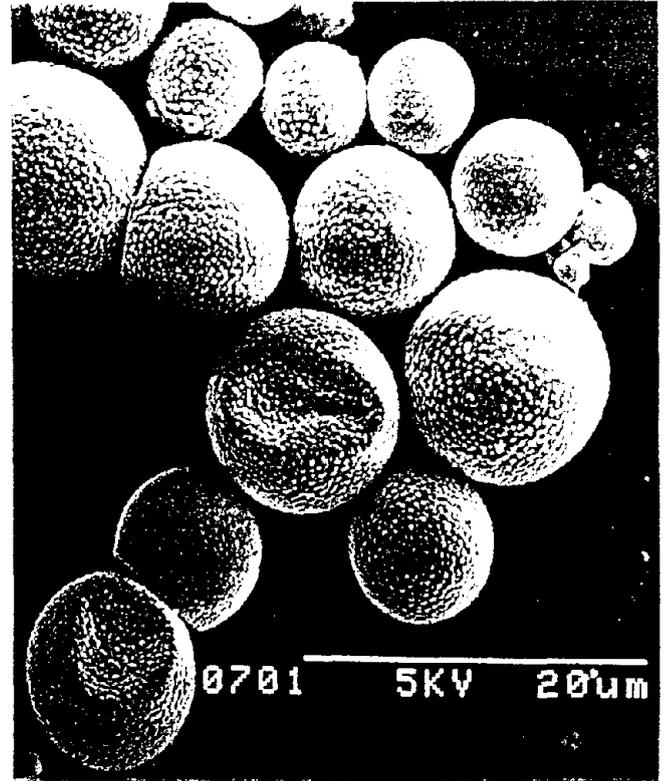
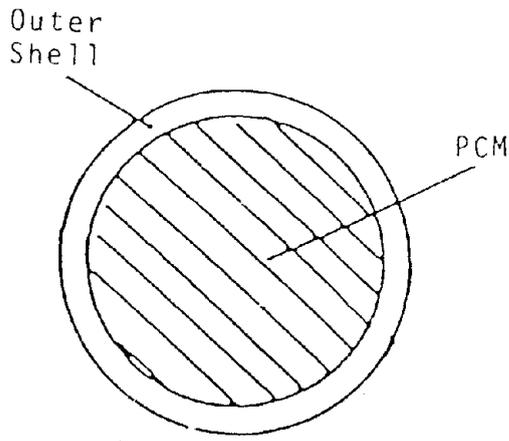


Figure 2. Cross-section of a microPCM capsule

Figure 3. Scanning electron photomicrograph of PCM microcapsules, 3-12 microns (2,000X)

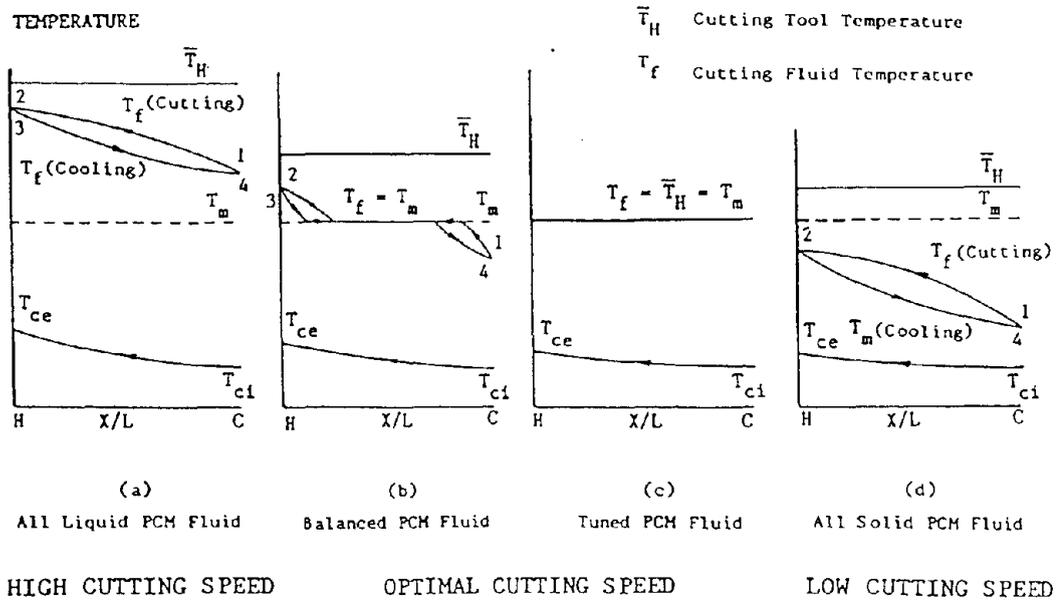
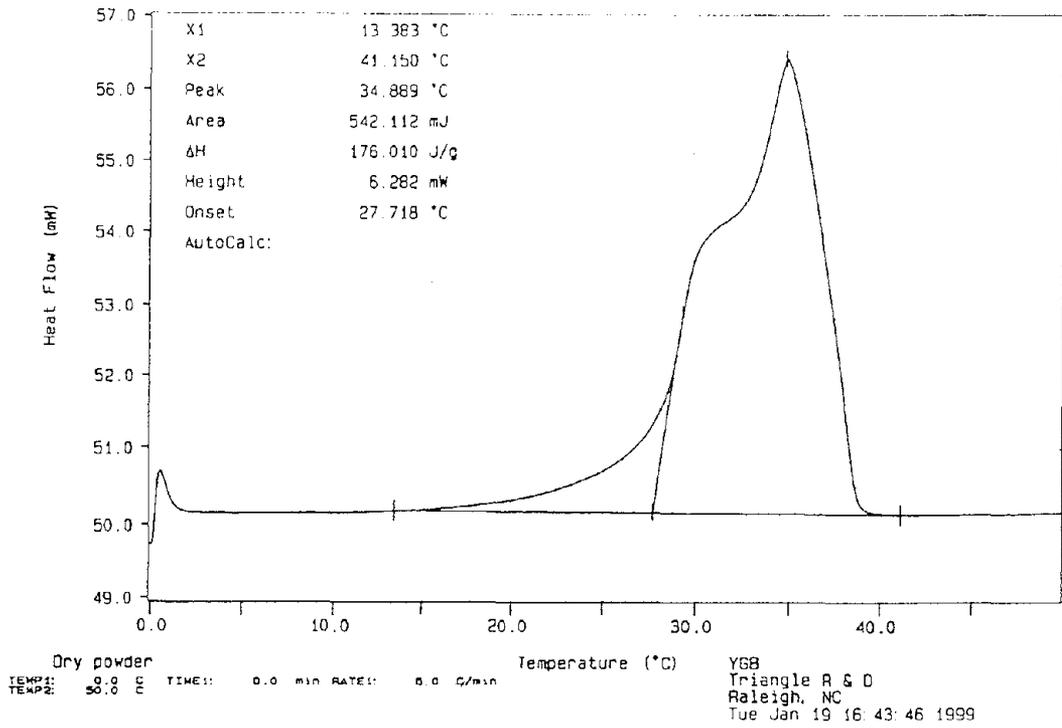


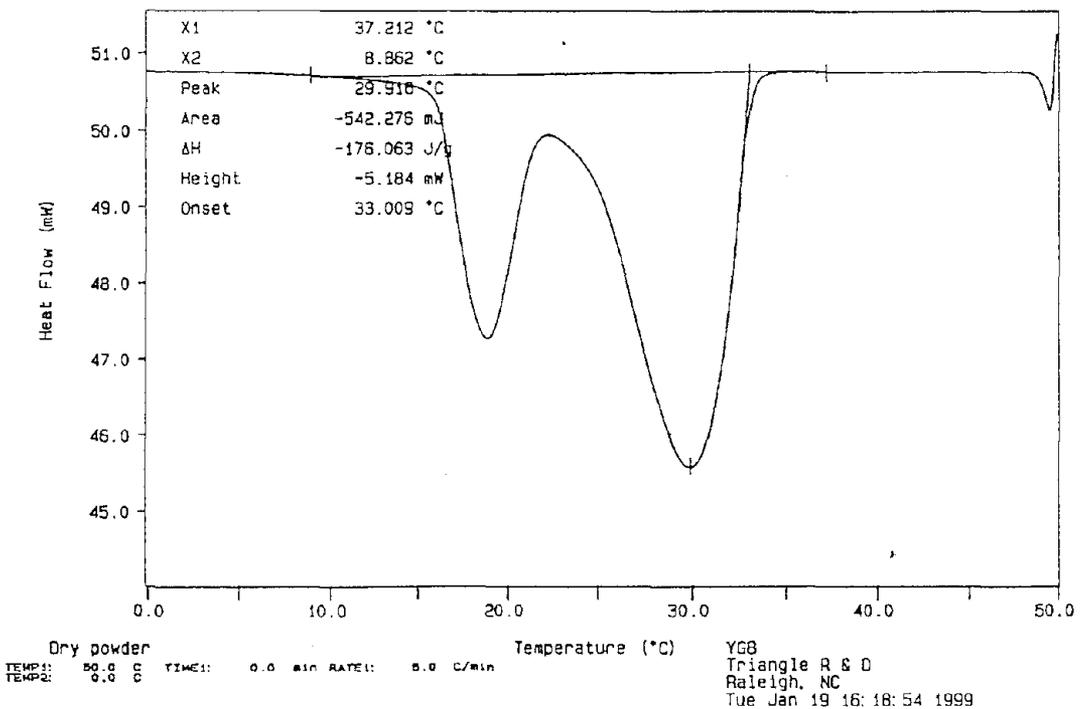
Figure 4. Optimal or "Tuned" Operation of a microPCM Coolant

Curve 1: DSC  
 File info: Machin001 Tue Jan 19 16:40:53 1999  
 Sample Weight: 3.080 mg  
 Eico(42-02), TG, 10-40 microns H1



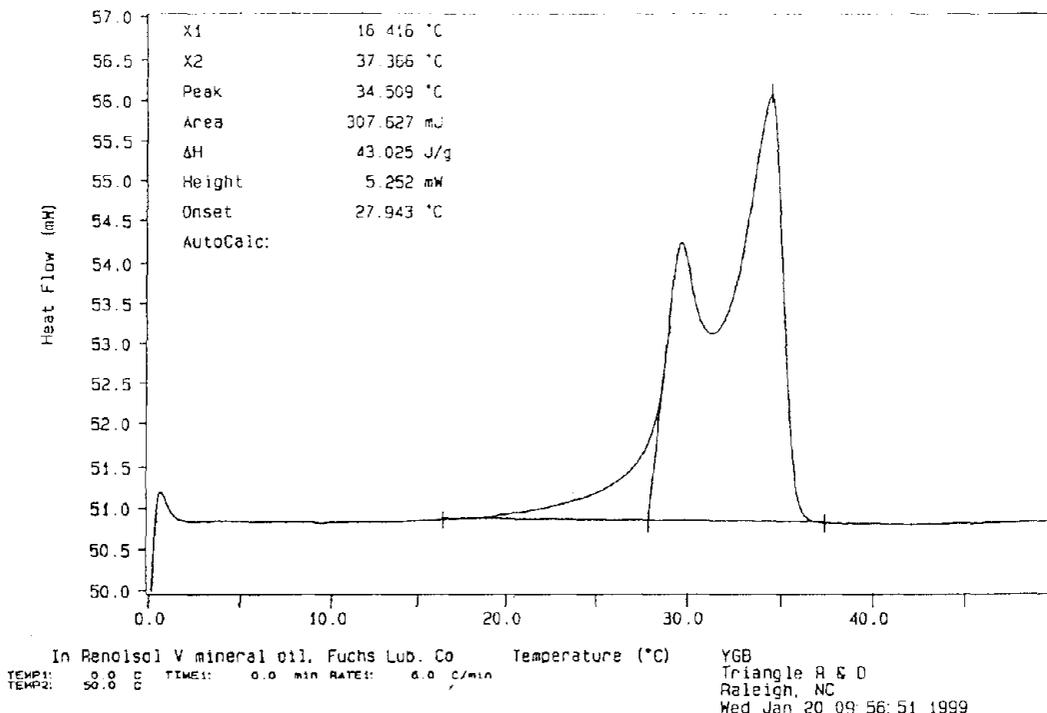
**Figure 5a. Differential Scanning Calorimetry (DSC) for Heating or Melting of the Eicosane MicroPCM**

Curve 1: DSC  
 File info: Machin000 Tue Jan 19 16:17:55 1999  
 Sample Weight: 3.080 mg  
 Eico(42-02), TG, 10-40 micron C1



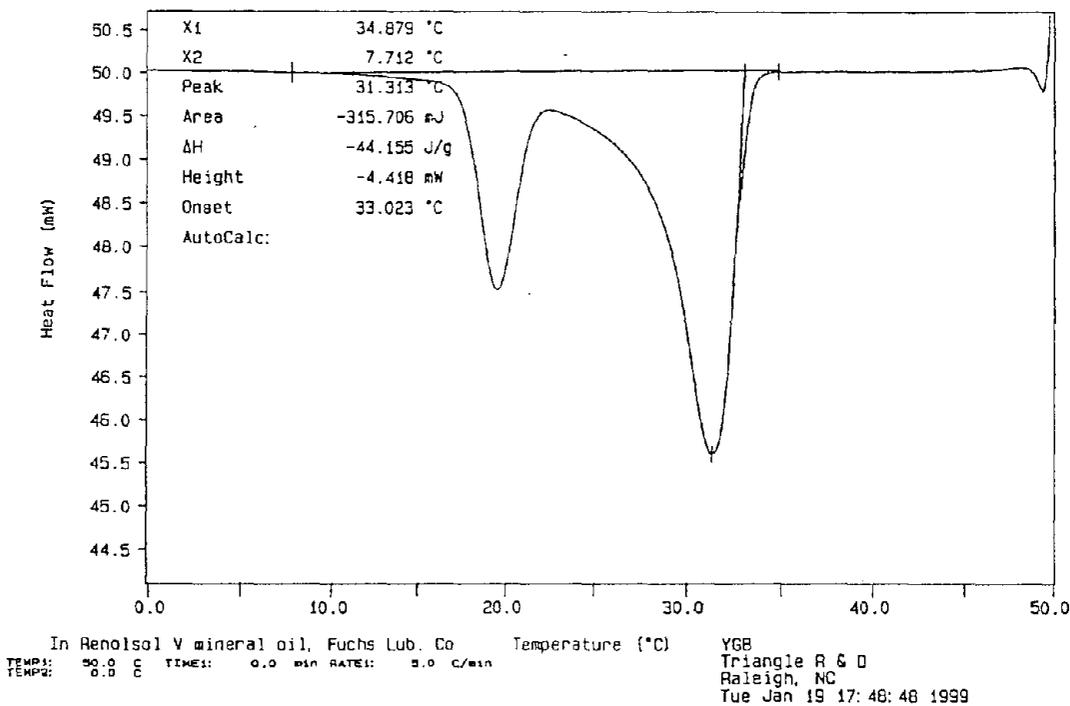
**Figure 5b. DSC for Cooling or Freezing of the Eicosane MicroPCM**

Curve 1: DSC  
 File info: Machin007 Tue Jan 19 17:59:28 1999  
 Sample Weight: 7.150 mg  
 Eico(42-02), TG, 25% (w/w) slurry H2



**Figure 6a. DSC for Heating or Melting of Eicosane MicroPCM at 25% Concentration in Machining Oil Coolant**

Curve 1: DSC  
 File info: Machin006 Tue Jan 19 17:48:21 1999  
 Sample Weight: 7.150 mg  
 Eico(42-02), TG, 25% (w/w) slurry C2



**Figure 6b. DSC for Cooling or Freezing of Eicosane MicroPCM at 25% Concentration in Machining Oil Coolant**

### **Task 3. Formulate a MicroPCM Coolant Using a Control Oil**

It is recognized that soluble and synthetic oils are increasing in popularity. However, in earlier experiments at UNC, it was found that a straight mineral oil would generate the most mist and therefore provide us with the greater opportunity for mist reduction. Therefore, it was believed that using mineral oil with microPCMs would likely benefit the most from the anticipated mist reduction. In addition, if the microPCM capsules should rupture during machining, their suspension in an oil would result in less deposits or contamination of the circulating coolant. To this end a control mineral oil was provided TRDC by UNC researchers. Compatible microPCMs were then formulated into the test coolant for experiments at Chapel Hill and approximately 5 gallons of the microPCM machining coolant was delivered by TRDC to UNC for their experiments.

### **Task 4. Conduct Machining Experiment with MicroPCM Coolants at UNC**

The objectives of these Phase I tasks were: (1) to measure the concentration and size distribution of the mist generated from the lathe operation under various operating conditions using both plain mineral oil and using mineral oil with microPCMs, and (2) to determine if the addition of microPCMs to the mineral oil caused a reduction in mist formation.

## **EXPERIMENTAL PROCEDURE**

### **Test Set-up**

A bench-top lathe (Sears-Roebuck, Chicago, IL) equipped with a carbide steel tool (MSC Industrial Supply, Plainview, NY) was used to investigate mist generation from straight oil and straight oil with microPCMs. As shown in Figure 7, mist was generated by turning aluminum rods three inches in diameter on a lathe powered by a 2.5 horsepower motor. The generated mist was confined to the area within a transparent, acrylic box placed around the lathe. A hinged lid on the acrylic enclosure allowed access to the carbide steel tool bit and the aluminum rods. Rubber gaskets were placed along the edges of the lid opening to seal the box. Cutting characteristics of the lathe were varied to the desired depth of cut and rotational speed.

Air was supplied to the acrylic box via a small gap between the rubber gaskets. Metal working fluid was applied directly onto the cutting surface; fluid flow rate was regulated by a calibrated rotameter. The applied metal working fluid was collected in a pan below the lathe, passed through a filter to remove the chips, drained into a sump, and re-used. The ambient relative humidity and temperature within the acrylic housing were monitored with a thermo-hygrometer (Fisher Scientific, Pittsburgh, PA).

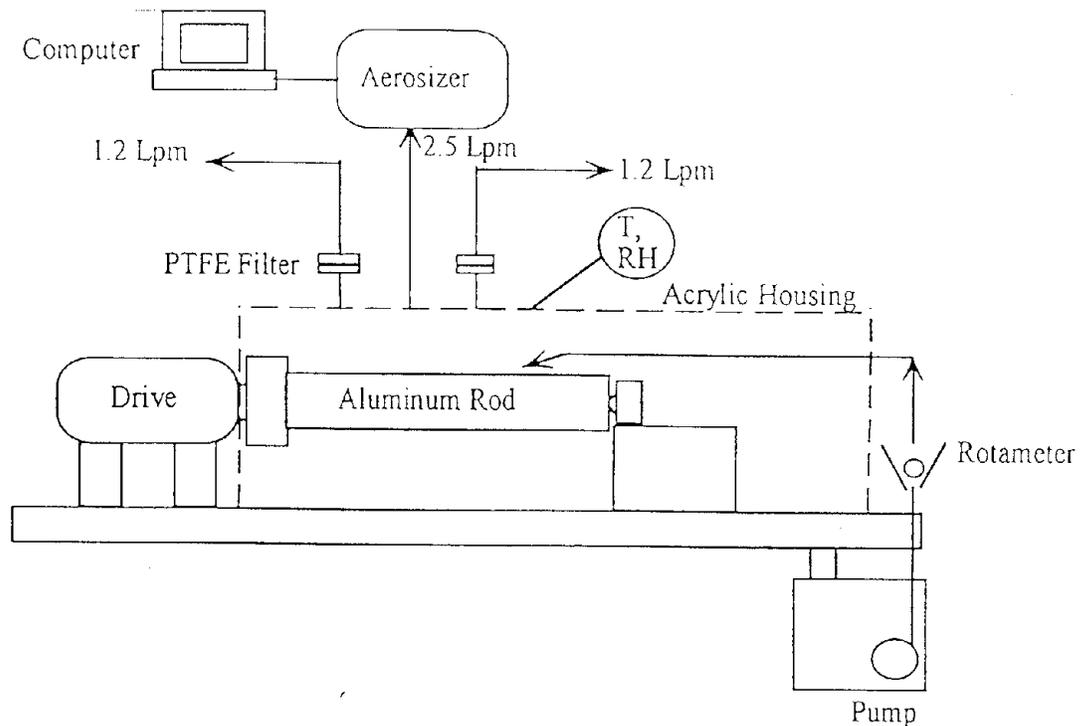


Figure 7. Bench-top lathe and sampling equipment used during experiments.

Mist was drawn at 2.5 Lpm through a sampling port in the center of the rear side of the acrylic box into an Aerosizer LD (Amherst Process Instruments, Inc., Amherst, MA). The Aerosizer uses time of flight and particle acceleration principles to measure the number concentration size and distribution of the mist. In addition, mist mass concentrations were measured gravimetrically. Samples of the mist were withdrawn at 1.2 Lpm from each of two sampling ports onto polytetrafluoroethylene (PTFE) filters (Omega Specialty Instruments, Chelmsford, MA). These filters were weighed on a Mettler MT-5 microbalance (Mettler-Toledo, Greifensee, Switzerland) to determine the mass collected. The standard operating procedures for these tests are given in Appendices A and B.

The amperage consumed by the lathe was monitored with an ammeter (A.W. Sperry Instruments Inc., Hauppauge, NY). The amperage applied to the tool, or cutting amperage, was calculated by subtracting the amperage necessary to operate the lathe without machining from the total amperage measured during a machining experiment. The cutting power was calculated by multiplying the cutting amperage by the voltage supplied to the lathe. In addition, TRDC supplied a variable-speed, centrifugal pump and controller system that had been used earlier and that had demonstrated its ability to circulate the microPCM coolant without consequential damage to the particles. A differential temperature measuring system was also provided to permit the circulating system to be operated in a "tuned" condition; i.e., with minimal sensible heat transport.

## Test Coolants

As stated earlier, the microPCM test coolant was formulated at TRDC by adding 25% microencapsulated n-eicosane particles to a low viscosity mineral oil, Fuchs 7911(Fuchs Lubricants Co., Harvey, IL). The unfilled Fuchs 7911 mineral oil served as the control coolant. The density of the coolant with the microPCMs was  $0.81\text{g/cm}^3$ ; the density of the control, the mineral oil alone, was also  $0.81\text{g/cm}^3$ .

## Experimental Conditions

Twenty-four experiments involving the machining of aluminum rods on a lathe were performed in random order. Table 1 lists the four experimental conditions investigated. Tests were conducted using straight oil with and without the added microPCMs. Each condition was repeated three times for both oils; the three replicates allowed the estimation of experimental error.

**Table 1. Experimental Conditions**

Rotational speed (RPM)	Depth of cut (inches)	Coolant flow (ml/s)	Mist generation Number, M
500	0.005	3.0	0.014
805	0.015	3.0	0.065
1270	0.014	2.5	0.115
1270	0.020	2.5	0.165

Rotational speed, cut depth, and coolant flow can be combined into a dimensionless mist generation number,  $M$ , defined as the metal removal rate,  $Q_m$ , divided by the fluid flow,  $Q_f$ .

$$M = \frac{Q_m}{Q_f} \quad \text{Eq. 1}$$

In Eq. 1, the metal removal rate equals the product of the surface area of metal removed per second and the cut depth,  $C$ :

$$Q_m = \pi D F \omega C \quad \text{Eq. 2}$$

where  $D$  is the rod diameter,  $F$  is the cut width (tool feed rate), and  $\omega$  is the rotational speed. Preliminary work at University of North Carolina indicated that the mist generation rate was highly correlated with the metal removal rate. Therefore, the rotational speed of the lathe and the depth of cut were the only machining parameters varied. The metal hardness (aluminum 6061), the tool rake angle of  $15^\circ$ , and the tool feed rate of 0.0062 inches per revolution were held constant throughout the tests. Tool rake angle refers to the angle between the tool and the aluminum workpiece. Tool feed rate refers to the rate the tool advances down the aluminum rod. The maximum rate the coolant pump was able to move the mineral oil with the microPCMs was 3.5 ml/sec. The difference between low and high mist generation number in the test design was maximized by changing the rotational speed and cut depth.

## Task 5. Data Analysis

Mist concentration was calculated from the mass collected on each PTFE filter divided by the sampling flow rate and time. The general linear models procedure in SAS (SAS Institute, Cary, NC) was used to analyze the data. Tukey multiple comparison tests were used to check for differences in the average mist generation rate and mass median diameter of the mist at each experimental condition. In addition, analysis of variance was used to check for unwanted correlations between the parameters of interest and confounding variables such as ambient temperature or relative humidity. All statistical tests were performed at the 95% confidence level.

Each mist generation rate determined in this work was taken as the mass of material collected on a sampling filter divided by the sampling time. Generation rates determined this way are properly comparable from experiment to experiment because all experiments were conducted in exactly the same way. Thus, these values are correctly interpreted as *relative* mist generation rates. These values are not meant to represent *absolute* mist generation rates as determination of absolute rates would require knowledge of sampling efficiency, a factor that is not easily determined.

## MACHINING TEST RESULTS

The SAS model showed that mist generation rate was not significantly affected by ambient temperature, relative humidity, or run order. In addition, the mass median diameter (MMD) was not influenced by the addition of the microPCMs to the mineral oil except perhaps at the lowest mist generation number,  $M = 0.014$ , ( $p = 0.04$ .) Addition of the microPCMs had no effect on the geometric standard deviation for the mist at any mist generation numbers.

The experimental data for the size distribution and gravimetric tests of the two oils for the four test conditions are summarized in Table 2 below, and included in Appendix C. Figure 8 presents the average mist generation rate for each test condition. Each bar on this graph represents the average of six mass samples taken on PTFE filters. Error bars on each bar represent one standard deviation in the measurements that made up the average generation rates plotted. Analysis of variance found a significant difference between the mist generation rates for oils with and without the microPCMs at the two highest mist generation numbers. Addition of microPCMs to the mineral oil decreased the mass of mist generated at  $M = 0.115$  by 31% and at  $M = 0.165$  by 33%. These results were significant at the  $p = 0.02$  and  $p = 0.006$  levels, respectively.

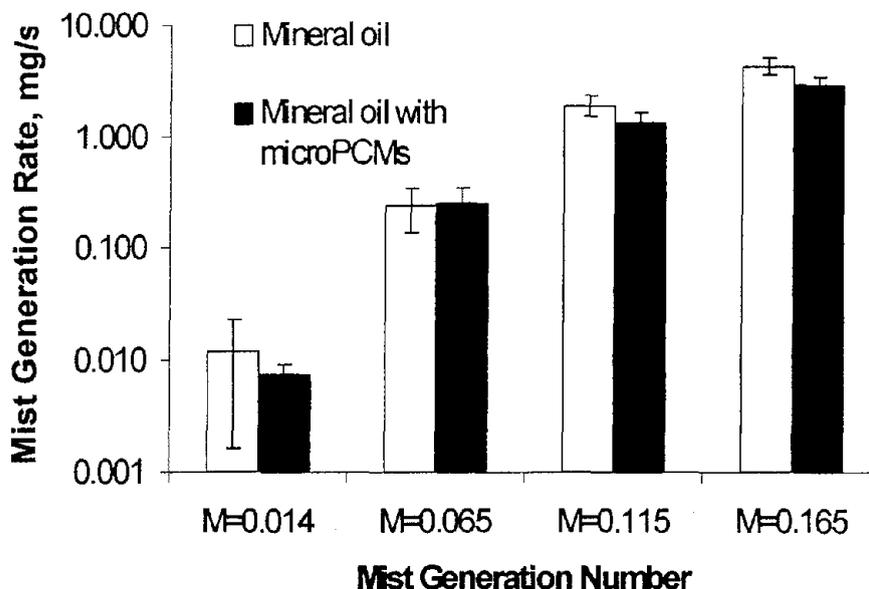


Figure 8. Average mist generation rate (mg/s) for the two oils at the four test conditions

#### Task 6. Investigation into the Potential Toxicity of a MicroPCM Machining Coolant

During an earlier SBIR program for NASA/MSFC to investigate the potential use of a microPCM coolant in a space station, researchers found no evidence that neither the microPCM particles nor the aqueous two-component coolants were toxic. When microPCMs were added to various oil-based coolants during an earlier SBIR program for NSF as well as follow-on Phase III programs at General Motors, no evidence was found that the microPCM capsules were potentially toxic. Paraffinic microPCMs are currently being used in numerous articles of apparel by both of our Phase III apparel sponsors: Outlast Technologies In Boulder, CO and Frisby Technologies in Long Island, NY. They have also conducted numerous toxicity investigations, and in no case has significant toxicity been found. However, we had determined earlier that mist generation and potential hazards from evaporation for respiration warranted additional examination. To this end, samples of microPCMs were forwarded to SITEK Research Laboratories in Rockville, MD to perform an objective third-party investigation.

SITEK Research Laboratories performs a variety of toxicity testing including in-vitro testing as alternatives to animal testing, in-vivo testing, animal acute toxicity testing with rodents, rabbits, pigs and dogs, as well as primary dermal irritation testing and intracutaneous reactivity tests. They also can perform various analytical chemistry, formulation and pharmacokinetics studies and testing for a wide range of materials as well as biomonitoring for human and animal exposures. A standardized test protocol that is routinely used for U.S. FDA submissions was to be used for these initial safety evaluations.

M #	Test #	Mist Generation Rate, mg/s			MMD, um	Temp, C	% RH	M #	Test #	Mist Generation Rate, mg/s			MMD, um	Temp, C	%RH
		Sampling Position Left	Sampling Position Right	Average						Sampling Position Left	Sampling Position Right	Average			
0.014	8	0.005	0.006	0.012	4.450	26.9	41.0	0.014	15	0.004	0.009	0.007	2.565	25.0	5.0
	10	0.020	0.029		4.553	27.2	38.0		17	0.006	0.007		2.935	23.6	6.0
	12	0.006	0.005		6.952	27.5	31.0		22	0.008	0.009		2.968	24.2	19.5
0.065	5	0.255	0.307	0.238	2.169	25.6	16.0	0.065	13	0.262	0.346	0.248	2.020	25.8	11.0
	11	0.296	0.339		2.652	27.8	33.0		19	0.118	0.146		1.824	24.4	15.0
0.115	7	0.105	0.124	1.930	1.703	26.1	43.0		23	0.303	0.311	1.330	2.272	23.9	20.0
	3	1.636	2.038		1.896	26.1	15.0	0.115	16	1.183	1.511		1.731	24.7	6.0
0.165	4	1.392	1.673		1.891	25.6	16.0		20	1.627	1.650		1.865	24.4	16.0
	9	2.374	2.466	4.432	2.777	27.2	39.5		21	0.937	1.074	2.960	1.798	24.4	22.0
	1	**	4.372		1.667	24.2	16.0	0.165	14	2.171	2.629		1.721	25.0	5.0
	2	3.506	3.925		1.670	25.0	14.5		18	2.648	3.276		1.911	23.3	6.0
	6	4.687	5.670		**	25.3	16.5		24	3.569	3.468		2.824	23.9	20.0

\*\* Data lost

Table 2. Experimental Data for Size Distribution and Gravimetric Testing of Oils

Samples of eicosane dry powder were forwarded to SITEK for examination using two different test procedures. One procedure examined the eicosane dry powder for its potential cytotoxicity, and the other tested its potential to cause mutations at the histidine operon of *Salmonella typhimurium* strains. In the first test, a decrease in the relative cloning efficiency (RCE) of cultured BALB/c-3T3 cells was examined at a ratio of 1 gm of test powder /10 ml of culture media at 37 C for 72 hours in a shaker incubator with mild agitation and 5% CO<sub>2</sub> in air. Two reference polymers were used as the positive control which was also incubated under the same conditions. Two hundred cells/plate were seeded in media and tested in triplicate. After incubation for 9 days, the cells were counted and the RCE determined to plot the IC/50 values. In the second test to study mutagenesis, eicosane dry powder was tested with four *Salmonella typhimurium* tester strains and with *E-Coli* strain WP2uvrA with both positive and negative controls. Details of the two tests at SITEK are provided in the attached Appendices D and E.

In addition to these two studies, TRDC had also requested that its licensee, Frisby Technologies in Clemmons, NC as well as a current subcontractor, Southern Research Institute in Birmingham, AL also conduct toxicity studies since they are assisting us with the development of many of these new products. Frisby Technologies currently obtains its microPCM particles under an exclusive contract with 3M. The microPCMs used in this Phase I study were obtained from another source: one that supplied them to us earlier for the NSF cutting fluid study. As mentioned earlier, Frisby Technologies has focussed on the development of new apparel, including foams that contain microPCM particles. Frisby had already contracted with NamSA, a world leader in testing services for the medical device industry to examine the cytotoxicity of the 3M microPCMs for Comfortemp foams with the USP agar diffusion method. An in-vitro biocompatibility study, based upon USP guidelines, was conducted on the Comfortemp foam containing the microPCMs. A portion of the test article, the negative control and the positive control were placed on duplicate agarose surfaces directly overlaying confluent monolayers of L-929 mouse fibroblast cells. After incubation at 37 C for 24-27 hours, the cell cultures were examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis, if any. The cultures were then examined microscopically at 100X to verify any decolorized zones and to determine cell morphology in proximity to and beneath the test articles. Under the conditions of this study, the microPCM foam test article showed no evidence of causing cell lysis or toxicity greater than a grade 2 (mild activity), which met the requirements of the USP.

In still another Phase II SBIR program for the U.S. Marines, TRDC is developing a macroPCM cooling garment to be worn beneath NBC (Nuclear-Biological-Chemical) protective clothing. This garment will contain approximately 4-5 lbs. of a PCM macroencapsulated within particles 2-3mm in diameter. The passive USMC PECS protective vest is expected to provide sufficient cooling to fighting Marines for 1-2 hours in both jungle and desert environments without batteries or circulating coolants. Southern Research Institute (SRI) in Birmingham, AL is a principal sub-contractor to TRDC for the development of a pilot process to fabricate the macroPCM particles for later inclusion in the PECS garments by Delta Thermal Systems, Inc. - subsidiary company of TRDC. To this end, SRI has initiated an acute toxicity study by PTRL East, Inc. in Richmond, KY using subcutaneous implantation of the encapsulated PCMs in rats and mice. While the results of this particular SRI study were not available before the completion of this report, they should be available by preparation of a Phase II proposal later this year.

## **Task 7. Coordination with Potential Phase III Sponsors**

TRDC has already applied for and received U.S. Patent protection for this technology in August 1992, patent no. 5,141,079 for the use of microPCM coolants in machining operations [15]. This technology has already been licensed to Frisby Technologies located in both Freeport, NY and Clemmons, NC. Frisby Airborne Hydraulics, the parent company of Frisby Technologies, machines and fabricates numerous hydraulic components (flap actuators and control surface actuators) that are used in most of the airliners currently flying. In addition, Frisby Technologies in an IPO in the Spring of 1998, announced their intentions to develop numerous products that would include microPCMs as thermal enhancers. These include: foams, composites, coatings and coolants. To this end, TRDC is already working with them to further develop this technology. In 1994, Dr. Colvin presented the results of the Phase I-II SBIR program for NSF at a conference at M.I.T [10]. Dr. C.H. Shen, the director of machining research at General Motors, also indicated that GM would like to become a Phase III sponsor and conduct additional machining experiments with the new coolants. Surprisingly, they have found that microPCM coolants are useful for heat intensive grinding operations, which produce the highest tool wear. With the new environmental standards, particularly in California, it is anticipated that other companies will also become interested in a safer workplace for their workers; especially when these coolants provide superior fabrication and significant reductions in tool wear - the most costly factor in manufacturing today for most materials.

## **Phase I Results and Discussion**

As anticipated, machining aluminum with the 2.5 hp bench-top lathe at UNC generated minimal heat and therefore resulted in minimal generation of machining mists for analysis. Nevertheless, the addition of the microPCMs to the mineral oil still significantly reduced the amount of mist generated at the two highest machining rates by 31-33%. This finding is consistent with the design attribute of the microPCMs, which was to increase the thermal capacitance of the oil. Thus, the addition of microPCMs should have its greatest effect at the highest mist generation numbers - particularly with steels and harder metals at much higher speeds. High mist generation rates with the 2.5 hp lathe occurred with a combination of factors including the fastest rotational speed, the slowest coolant rate, and the deepest depth of cut. The microPCMs effected no substantial or significant change in the mass median diameter or geometric standard deviation of the generated mist. The earlier results for NSF with a 8 hp engine lathe at North Carolina State University generated significantly more oil mist with steel and titanium rods; therefore, it will be necessary to utilize a more powerful lathe and higher machining speeds in a follow-on Phase II program to produce results more representative of industrial machining operations.

Toxicology testing at SITEK and NamSA indicated minimal or no cytotoxicity and no mutagenesis with the microencapsulated phase change materials currently being produced. The use of dry powder for the cytotoxicity testing should, however, be considered a "worst case" because in use, the powder will only constitute 25% of the solids concentration in the oil coolant. It should also be pointed out that TRDC works with several suppliers of microPCMs and that the processes for producing microPCMs is an evolving one that is very likely to result in lower levels of toxicity than those currently being demonstrated. The 99% pure paraffins, which constitute most of the microPCM structure and absorb the heat of evaporation, are inherently

non-toxic. The enveloping polymers are the suspect materials, and these are undergoing further change and washing as the Phase III commercialization process develops. General Motors has found no reason to become alarmed even though they have utilized microPCM coolants for turning, milling and grinding operations, which produces much higher coolant evaporation or mist generation. Dr. C.H. Shen, director of machining research at GM, will present a paper this Fall at the ASME International Congress in Nashville describing the performance of these new microPCM coolants during grinding operations.

### **Phase I Conclusions**

The Phase I program showed that microPCMs in an oil coolant on the 2.5 hp benchtop lathe at UNC reduced the amount of mist generated at the highest mist generation numbers evaluated in these studies by 31-33%. In many industrial operations coolants flood the workpiece; in these cases mist generation numbers may be lower than those tested here. In cases where particularly deep cuts are rapidly made and if relatively little coolant is used, machining numbers achieved in industry will be significantly greater than those generated here as was found in the earlier tests with the 8 hp lathe at NCSU. In such cases, the benefit of microPCMs at reducing mist generation should increase well beyond those determined here. In cytotoxicology testing, microPCMs to be used in coolants have also been shown to be of minimal toxicity, and non-mutagenic. A U.S. patent has already been awarded to TRDC for the use of microPCM machining coolants, and several potential Phase III sponsors have indicated their interest in follow-on Phase II support.

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## Appendix A. Standard Operating Procedures for the Coolant Experiments

1. Set Aerosizer settings to the following:

System set up: Control-S

Dual sensitivity	Off
Noise threshold	6
PMT	1150
Clock	40 mhz
Sample pressure	Pulse-jet
Baseline	0.1
Channels	55

Select proper sample density: F4

Autocombine ON, Sample rate 1.5 Lpm

Select proper directory: F9

Program measurements: Manual, F10

2. Turn on dilution system
  - Set Variac for fan at 30%
  - Set vacuum pump suction for 4 to 6 cfh greater than outlet
3. Turn on fluid pump for warm-up
4. Set up lathe as follows:
  - Measure rod diameter and re-set spindle
  - Set rotational speed
  - Set cut depth (one mark equals 0.001 inch)
  - Move tool carriage to starting point
5. Pre-test conditions
  - Record temperature and relative humidity
  - Prepare filters
6. Turn on MWF flow
  - Loosen clamp on tubing to get desired flow
  - Set rotameter to ~8 for 3 ml/s, ~7.5 for 2.5 ml/s
  - Check flow with graduated cylinder and stopwatch
7. Collect MWF sample for temperature measurement; record temperature
8. Connect filters to apparatus

9. Start lathe
  - Turn switch to “on” position; make sure aluminum rod spinning forwards
  - On right side of tool carriage, flip lever down to move carriage
10. Start Aerosizer – F7
11. Monitor filter sample and MWF flows
12. Stop lathe
  - On right side of tool carriage, flip lever up
  - Turn switch to off position
13. Stop Aerosizer – F11
14. Disconnect filters
15. Measure final fluid flow and temperature
16. Stop MWF flow
17. Post test conditions
  - Measure temperature and relative humidity
  - Re-weigh filters
18. Record miscellaneous information
  - Measure final rod diameter, calculate actual cut depth
  - Record average filter sample flows
  - Record filter sample time (Aerosizer time plus ten seconds)

## APPENDIX B. BAITY LAB STANDARD OPERATING PROCEDURE FOR WEIGHING FILTERS ON METTLER MT5 MICROBALANCE

Note: This protocol should be used unless modifications have been approved by PI.

1. Remove sampling filter and blank from package using tweezers. If using PVC filters, discharge first on static strip for approximately 30 seconds.
2. Place filter and blank in plastic petri dish.
3. Desiccate filters for minimum of 24 hours.
4. Remove filter/petri dish from desiccator and place in equilibration chamber. The equilibration chamber consists of a clean Tupperware box with several ports open to the atmosphere through glass fiber filter partitions.
5. Equilibrate filter and blank for minimum of 24 hours at room conditions. Keep lab door closed.
6. Calibrate and zero microbalance.
7. Weigh a calibration weight that has approximately the same weight as the filters to be weighed. Weigh blank filter(s) using protocol of steps 8 – 11 below.
8. Using tweezers, remove filter from petri dish and place on Polonium strip for minimum of 30 seconds.
9. Place filter on center of weighing pan and wait until stability indicator disappears.
10. Record weight when reading remains stable for at least 15 seconds.
11. Remove filter, close balance door, and make sure balance returns to zero. If not, re-zero balance and repeat steps 9 – 11.
12. Repeat steps 8 – 11. If two weights differ by more than 3  $\mu\text{g}$ , repeat steps 8 – 11 until the disagreement is resolved.
13. Place filter, rough side up, in cassette and cap both ends.
14. Repeat step 7. If weights differ by more than a significant amount (e.g. 5  $\mu\text{g}$ ), resolve disagreement and reweigh all filters.
15. Record room temperature, relative humidity, and barometric pressure.
16. After sampling, repeat steps 1 - 15

## Modifications to Baity Lab SOP Weighing Procedures for Mist Generation with MicroPCM Machining Coolants

Modifications to the Baity Lab SOP for Weighing Filters on the Mettler MT5 Microbalance were necessary due to the volatility of the mineral oil and potential for the collected sample to evaporate off the PTFE filter. To determine if sample evaporation was occurring during desiccation and equilibration of the filters, all the filters and blanks were weighed immediately after sampling, after desiccation, and after room equilibration. Steps #1-15 of the Baity Lab SOP for Weighing Filters on the Mettler MT5 Microbalance were followed. The modifications are listed below.

16. Immediately after sampling, re-weigh filters and blanks following steps #6 - 12, and 14.
17. Desiccate filters and blanks according to steps #2 - 3. Re-weigh filters and blanks after desiccation following steps #6 - 12 and 14.
18. Equilibrate filters and blanks according to step #5. Re-weigh filters and blanks according to steps #6 - 12 and 14.

The additional weighings showed that sample evaporation was occurring. Therefore, the filter and blank weights obtained in modified step #16 were used as the post-sampling weights in the analyses.

Test 1 Mineral Oil, 1270 rpm, 02", 2.5 ml/s

	Run 1	% Under	Run 1	Lower SIZE	Upper SIZE	Run 1 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	actual	Best log-normal fit dg	
Mineral Oil		5%	0.63	0.1	0.12	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264			rg	1.425
1/17/99 14:12		10%	0.68	0.12	0.14	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.966113			r2	0.992
AERODYNAMIC No. DISTRIBUTION		15%	0.72	0.14	0.16	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.832581				
Material:		20%	0.77	0.16	0.18	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.714798				
Density (g/cc):	0.81	25%	0.82	0.18	0.22	0	0	0.2	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.514128				
Correlating Factor:	1	30%	0.87	0.22	0.25	0	0	0.235	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.386294				
Run Length (sec):	64.55	35%	0.92	0.25	0.29	0	0	0.27	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.237874				
PMT Voltage (Volts):	1150	40%	0.96	0.29	0.34	0	0	0.315	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.078810				
Laser Current (mA):	42.06	45%	1.01	0.34	0.4	0	0	0.37	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-0.916291				
Clock Freq (MHz):	40	50%	1.06	0.4	0.46	0	0	0.43	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-0.776529				
Sum of channels:	1889189	55%	1.11	0.46	0.54	7.62E+03	0	0.5	4.03E-03	4.04E-10	4.04E-07	2.06E-04	-3.532041	-0.616186	0.477161	0.54		
Lower Size Limit:	0.1	60%	1.17	0.54	0.63	8.13E+04	7.62E+03	0.585	4.71E-02	6.90E-09	6.90E-06	3.73E-03	-2.675297	-0.462035	0.646349	0.63		
Upper Size Limit:	199.6	65%	1.23	0.63	0.74	2.30E+05	8.89E+04	0.683	1.69E-01	3.13E-08	3.13E-05	1.97E-02	-2.059169	-0.301105	0.803993	0.74		
Mean Size:	1.08	70%	1.3	0.74	0.86	2.27E+05	3.19E+05	0.8	2.89E-01	4.93E-08	4.93E-05	4.49E-02	-1.695971	-0.150823	0.914380	0.86		
Standard Deviation:	1.43	75%	1.38	0.86	1	2.82E+05	5.46E+05	0.93	4.38E-01	9.62E-08	9.62E-05	9.41E-02	-1.315832	0	1.046183	1.00		
D(4.3):	32.29	80%	1.48	1	1.2	3.59E+05	8.28E+05	1.1	6.28E-01	2.02E-07	2.02E-04	1.98E-01	-0.850227	0.182322	1.233776	1.20		
D(3.2):	2.1	85%	1.6	1.2	1.4	2.51E+05	1.19E+06	1.3	7.61E-01	2.33E-07	2.33E-04	3.17E-01	-0.476242	0.336472	1.408544	1.40		
Spec surf area (m <sup>2</sup> /g):	3.53	90%	1.76	1.4	1.6	1.72E+05	1.44E+06	1.5	8.52E-01	2.45E-07	2.45E-04	4.42E-01	-0.144930	0.470004	1.583932	1.60		
Mode (Linear scale):	0.7	95%	2.04	1.6	1.8	1.09E+05	1.61E+06	1.7	9.10E-01	2.28E-07	2.28E-04	5.59E-01	0.147768	0.587787	1.756986	1.80		
Mode Lower Bound:	0.67			1.8	2.2	1.08E+05	1.72E+06	2	9.67E-01	3.67E-07	3.67E-04	7.46E-01	0.62910	0.788457	2.108715	2.20		
Mode Upper Bound:	0.72			2.2	2.5	3.36E+04	1.83E+06	2.35	9.85E-01	1.85E-07	1.85E-04	8.41E-01	0.998084	0.916291	2.374550	2.50		
Lower combine size:	5.67			2.5	2.9	1.97E+04	1.86E+06	2.7	9.95E-01	1.64E-07	1.64E-04	9.25E-01	1.437652	1.064711	2.774627	2.90		
Upper combine size:	5.75			2.9	3.4	7.44E+03	1.88E+06	3.15	9.99E-01	9.85E-08	9.85E-05	9.75E-01	1.961744	1.223775	3.340651	3.40		
Dispenser				3.4	4	1.91E+03	1.89E+06	3.7	1.00E+00	4.09E-08	4.09E-05	9.96E-01	2.653178	1.386294	4.267776	4.00		
Flow : 0.0				4	4.6	0	1.89E+06	4.3	1.00E+00	0.00E+00	0.00E+00	9.96E-01						
Flow Inc. : 0.0				4.6	5.4	0	1.89E+06	5	1.00E+00	0.00E+00	0.00E+00	9.96E-01						
Pulse : 0.0				5.4	6.3	2.76E+01	1.89E+06	5.85	1.00E+00	2.34E-09	2.34E-06	9.97E-01						
Pulse Inc. : 0.0				6.3	7.4	3.21E+01	1.89E+06	6.85	1.00E+00	4.37E-09	4.37E-06	9.99E-01						
Nebulizer : 0.				7.4	8.6	2.44E+00	1.89E+06	8	1.00E+00	5.29E-10	5.29E-07	1.00E+00						
Neb. Inc. : 0.0				8.6	10	1.62E+00	1.89E+06	9.3	1.00E+00	5.52E-10	5.52E-07	1.00E+00						
Low Limit : 4				10	12	0	1.89E+06	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
High Limit: 80				12	14	0	1.89E+06	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Baseline Offset	0.1			14	16	0	1.89E+06	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Noise Filter (Sigmas)	6			16	18	0	1.89E+06	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Nozzle Type				18	22	0	1.89E+06	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Flow Rate Range (l/min):	2.51			22	25	0	1.89E+06	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
lo:	2.51			25	29	0	1.89E+06	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Mass Loading (mg/m <sup>3</sup> ):	1.55			29	34	0	1.89E+06	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Concentration (#/m <sup>3</sup> ):	7.00E+08			34	40	0	1.89E+06	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Counting efficiency:	1			40	46	0	1.89E+06	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				46	54	0	1.89E+06	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				54	63	0	1.89E+06	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				63	74	0	1.89E+06	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				74	86	0	1.89E+06	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				86	100	0	1.89E+06	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				100	120	0.00E+00	1.89E+06	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				120	140	0	1.89E+06	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				140	160	0	1.89E+06	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				160	180	0	1.89E+06	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				180	220	0	1.89E+06	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				1889217						1.96E-06	1.96E-03							

## Test 1 (Cont.) Mineral Oil, 1270 rpm, .02", 2.5 ml/s

### Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	222.056	222.069	0.012	2.850	137.000	3.903	0.000	0.005
Filter R	214.183	224.888	10.704	2.750	137.000	3608.301	0.078	4.372
Blank	149.84	149.841	0.001					

### Actual Machining Conditions

Flow	2.540
Depth	0.018
RPM	1270.000
Base Amp	na
Cut Amp	na
Diameter	7.620

M# = 0.146

Mact = 0.146

Test 2 Mineral Oil, 1270 rpm, .02", 2.5 ml/s

	Run 3	% Under	Run 3	Lower SIZE	Upper SIZE	Run 3 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	actual	Best log-normal fit
Mineral Oil	0.66	5%	0.66	0.12	0.12	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-2.12026	-2.12026			1.405
1/1799 15:04	0.72	10%	0.72	0.12	0.14	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.96611	-1.96611			0.987
AERODYNAMIC NO. DISTRIB.	0.77	15%	0.77	0.14	0.16	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.83258	-1.83258			
F-7211	0.83	20%	0.83	0.16	0.18	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.7148	-1.7148			
Density (g/cc):	0.88	25%	0.88	0.18	0.22	0	0	0.2	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.51413	-1.51413			
Correlating Factor:	0.93	30%	0.93	0.22	0.25	0	0	0.235	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.38629	-1.38629			
Run Length (sec):	0.98	35%	0.98	0.25	0.29	0	0	0.27	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.23787	-1.23787			
PMT Voltage (Volts):	1.02	40%	1.02	0.29	0.34	0	0	0.315	0.00E+00	0.00E+00	0.00E+00	0.00E+00					
Laser Current (mA):	1.07	45%	1.07	0.34	0.4	0	0	0.37	0.00E+00	0.00E+00	0.00E+00	0.00E+00					
Clock Freq (MHz):	1.12	50%	1.12	0.4	0.46	0	0	0.43	0.00E+00	0.00E+00	0.00E+00	0.00E+00					
Sum of channels:	1.17	55%	1.17	0.46	0.54	0	0	0.5	0.00E+00	0.00E+00	0.00E+00	0.00E+00					
Lower Size Limit:	1.23	60%	1.23	0.54	0.63	3.60E+04	0	0.585	2.34E-02	3.06E-09	3.06E-06	1.71E-03	-2.926827	-0.46204	0.61677	0.63	
Upper Size Limit:	1.29	65%	1.29	0.63	0.74	1.54E+05	3.60E+04	0.685	1.24E-01	2.10E-08	2.10E-05	1.35E-02	-2.211855	-0.30111	0.78666	0.74	
Mean Size:	1.36	70%	1.36	0.74	0.86	1.62E+05	1.90E+05	0.8	2.29E-01	3.52E-08	3.52E-05	3.32E-02	-1.836115	-0.15082	0.89396	0.86	
Standard Deviation:	1.42	75%	1.42	0.86	1	2.25E+05	3.52E+05	0.93	3.75E-01	7.65E-08	7.65E-05	7.60E-02	-1.432368	0	1.02563	1	
D(4.3):	1.54	80%	1.54	1	1.2	3.09E+05	5.77E+05	1.1	5.75E-01	1.74E-07	1.74E-04	1.74E-01	0.18232	0.18232	1.21272	1.2	
D(3.2):	1.66	85%	1.66	1.2	1.4	2.26E+05	8.86E+05	1.3	7.22E-01	2.10E-07	2.10E-04	2.91E-01	0.33647	0.33647	1.38493	1.4	
Spec surf area (m <sup>2</sup> /g):	1.83	90%	1.83	1.4	1.6	1.58E+05	1.11E+06	1.5	8.24E-01	2.26E-07	2.26E-04	4.18E-01	-0.208247	0.47	1.5564	1.6	
Mode (Linear scale):	1.88	95%	1.88	1.6	1.8	1.05E+05	1.27E+06	1.7	8.92E-01	2.18E-07	2.18E-04	5.40E-01	0.099371	0.58779	1.72732	1.8	
Mode Lower Bound:	1.82		1.82	1.8	2.2	1.05E+05	1.37E+06	2	9.61E-01	3.57E-07	3.57E-04	7.39E-01	0.641132	0.78846	2.07702	2.2	
Mode Upper Bound:	2.5		2.5	2.2	2.5	3.30E+04	1.48E+06	2.35	9.82E-01	1.82E-07	1.82E-04	8.41E-01	0.998571	0.91629	2.34567	2.5	
Lower combine size:	6.61		6.61	2.9	3.4	7.80E+03	1.53E+06	3.15	9.99E-01	1.58E-07	1.58E-04	9.30E-01	1.473159	1.06471	2.75681	2.9	
Upper combine size:	6.71		6.71	2.9	3.4	8.39E+02	1.54E+06	3.7	1.00E+00	1.80E-08	1.80E-05	9.98E-01	2.241522	1.22378	3.58069	3.4	
Dispenser	0.0		0.0	4	4.6	0	1.54E+06	4.3	1.00E+00	0.00E+00	0.00E+00	9.98E-01	2.818924	1.38629	4.35815	4	
Flow Inc.: 0.0	0.0		0.0	4.6	5.4	0	1.54E+06	5	1.00E+00	0.00E+00	0.00E+00	9.98E-01	2.818924	1.6864	4.35815	5.4	
Pulse : 0.0	0.0		0.0	5.4	6.3	0	1.54E+06	5.85	1.00E+00	0.00E+00	0.00E+00	9.98E-01	2.818924	1.84055	4.35815	6.3	
Pulse Inc.: 0.0	0.0		0.0	6.3	7.4	3.16E+01	1.54E+06	6.85	1.00E+00	4.30E-09	4.30E-06	1.00E+00					
Nebulizer: 0.0	0.0		0.0	7.4	8.6	0	1.54E+06	8	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Neb. Inc.: 0.0	0.0		0.0	8.6	10	0	1.54E+06	9.3	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Low Limit: 40	40		40	10	12	0	1.54E+06	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
High Limit: 80	80		80	12	14	0	1.54E+06	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Baseline Offset	0.1		0.1	14	16	0	1.54E+06	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Noise Filter (Sigmas)	6		6	16	18	0	1.54E+06	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nozzle Type	200um		200um	18	22	0	1.54E+06	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):	2.51		2.51	22	25	0	1.54E+06	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
to:	2.51		2.51	25	29	0	1.54E+06	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):	1.34		1.34	29	34	0	1.54E+06	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (#/m <sup>3</sup> ):	7.87E+08		7.87E+08	34	40	0	1.54E+06	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:	1		1	40	46	0	1.54E+06	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				46	54	0	1.54E+06	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				54	63	0	1.54E+06	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				63	74	0	1.54E+06	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				74	86	0	1.54E+06	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				86	100	0	1.54E+06	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				100	120	0	1.54E+06	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				120	140	0	1.54E+06	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				140	160	0	1.54E+06	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				160	180	0	1.54E+06	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				180	220	0	1.54E+06	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				1539821.79						1.79E-06	1.79E-03						

Test 2 (cont.) Mineral Oil, 1270 rpm, .02", 2.5 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rat mg/sec	Gen. Rate mg/sec
Filter L	226.763	235.022	8.258	2.850	132.000	2787.959	0.063	3.506
Filter R	219.38	228.399	9.018	2.780	132.000	3121.028	0.068	3.925
Blank	149.84	149.841	0.001					

Actual Machining Conditions

Flow	2.580
Depth	0.022
RPM	1270.000
Base Amp	na
Cut Amp	na
Diameter	7.530

Actual M# = 0.176

Mact = 0.173

Test 3 Mineral Oil, 1270 rpm, .014", 2.5 ml/s

	Run 5	% Under	Run 5	Lower SIZE	Upper SIZE	Run 5 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit	
Taken on:	1/17/99 15:58	5%	0.63	0.1	0.12	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-2.120264			dg 1.486	
ABRODYNAMIC No. DISTRIB.		10%	0.68	0.12	0.14	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.9661113			r2 0.996	
Material:	F-7211	15%	0.73	0.14	0.16	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.832381				
Density (g/cc):	0.81	20%	0.78	0.16	0.18	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.714798				
Correlating Factor:	1	25%	0.84	0.18	0.22	0	0	0.2	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.514128				
Run Length (sec):	121.03	30%	0.89	0.22	0.25	0	0	0.235	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.386294				
PMT Voltage (Volts):	1150	35%	0.94	0.25	0.29	0	0	0.27	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.237874				
Laser Current (mA):	42.54	40%	0.99	0.29	0.34	0	0	0.315	0.00E+00	0.00E+00	0.00E+00	0.00E+00					
Upper Size Limit:	40	45%	1.05	0.34	0.4	0	0	0.37	0.00E+00	0.00E+00	0.00E+00	0.00E+00					
Lower Size Limit:	0.1	50%	1.11	0.4	0.46	0	0	0.43	0.00E+00	0.00E+00	0.00E+00	0.00E+00				0.34	
Sum of channels:	3427641	55%	1.17	0.46	0.54	2.22E+04	0	0.5	6.47E-03	1.18E-09	1.18E-06	2.54E-04	-3.476162	0.478548	0.54		
Lower Size Limit:	0.1	60%	1.23	0.54	0.63	1.53E+05	2.22E+04	0.585	5.17E-02	1.32E-08	1.32E-05	3.10E-03	-2.736524	0.641435	0.63		
Upper Size Limit:	199.6	65%	1.31	0.63	0.74	3.85E+05	1.77E+05	0.685	1.64E-01	5.24E-08	5.24E-05	1.45E-02	-2.184752	0.798113	0.74		
Mean size:	1.13	70%	1.39	0.74	0.86	3.75E+05	5.62E+05	0.8	2.73E-01	8.13E-08	8.13E-05	3.20E-02	-1.851517	0.910720	0.86		
Standard Deviation:	1.49	75%	1.49	0.86	1	4.50E+05	9.37E+05	0.93	4.03E-01	1.54E-07	1.54E-04	6.53E-02	-1.512008	1.041801	1		
DX(4,3):	2.14	80%	1.6	1	1.2	5.89E+05	1.39E+06	1.1	5.70E-01	3.32E-07	3.32E-04	1.37E-01	-1.093292	1.229727	1.2		
DX(3,2):	1.74	85%	1.74	1.2	1.4	4.41E+05	1.98E+06	1.3	7.03E-01	4.10E-07	4.10E-04	2.26E-01	-0.752382	1.407504	1.4		
Spec surf area (m <sup>2</sup> /g):	4.25	90%	1.93	1.4	1.6	3.28E+05	2.42E+06	1.5	8.01E-01	4.69E-07	4.69E-04	3.27E-01	-0.447109	1.588402	1.6		
Mode (Linear scale):	0.7	95%	2.27	1.6	1.8	2.30E+05	2.74E+06	1.7	8.67E-01	4.78E-07	4.78E-04	4.31E-01	-0.174143	1.769738	1.8		
Mode Lower Bound:	0.66		1.8	2.2	2.57E+05	2.97E+06	2	9.43E-01	9.43E-01	8.72E-07	8.72E-04	6.20E-01	0.304310	0.788457	2.2		
Mode Upper Bound:	0.72		2.2	2.5	9.33E+04	3.23E+06	2.35	9.70E-01	9.70E-01	5.13E-07	5.13E-04	7.31E-01	0.614571	2.139014	2.5		
Lower combine size:	7.36		2.5	2.9	5.99E+04	3.32E+06	2.7	9.87E-01	9.87E-01	5.00E-07	5.00E-04	8.39E-01	0.989203	1.064711	2.9		
Upper combine size:	7.47		2.9	3.4	2.89E+04	3.38E+06	3.15	9.96E-01	9.96E-01	3.82E-07	3.82E-04	9.21E-01	1.414808	2.320725	3.4		
Dispenser			3.4	4	1.19E+04	3.41E+06	3.7	9.99E-01	9.99E-01	2.55E-07	2.55E-04	9.77E-01	1.988810	1.386294	4.168391	4	
Flow Inc. : 0.0			4	4.6	3.20E+03	3.42E+06	4.3	1.00E+00	1.00E+00	1.08E-07	1.08E-04	1.00E+00					
Pulse Inc. : 0.0			4.6	5.4	0	3.43E+06	5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Pulse Inc. : 0.0			5.4	6.3	0	3.43E+06	5.85	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nebulizer : 0.0			6.3	7.4	0	3.43E+06	6.85	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Neb. Inc : 0.0			7.4	8.6	0	3.43E+06	8	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Low Limit : 40			8.6	10	0	3.43E+06	9.3	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
High Limit: 80			10	12	0	3.43E+06	11	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Baseline Offset			12	14	0	3.43E+06	13	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Noise Filter (Sigmas)			14	16	0	3.43E+06	15	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nozzle Type			16	18	0	3.43E+06	17	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):			18	22	0	3.43E+06	20	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
to:			22	25	0	3.43E+06	23.5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):			25	29	0	3.43E+06	27	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (#/m <sup>3</sup> ):			29	34	0	3.43E+06	31.5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:			34	40	0	3.43E+06	37	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			40	46	0	3.43E+06	43	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			46	54	0	3.43E+06	50	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			54	63	0	3.43E+06	58.5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			63	74	0	3.43E+06	68.5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			74	86	0	3.43E+06	80	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			86	100	0	3.43E+06	93	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			100	120	0	3.43E+06	110	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			120	140	0	3.43E+06	130	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			140	160	0	3.43E+06	150	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			160	180	0	3.43E+06	170	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			180	220	0	3.43E+06	200	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
						342763				4.62E-06	4.62E-03						

**Test 3 (Cont.) Mineral Oil, 1270 rpm, .014", 2.5 ml/s**

**Generation Rate Calculations**

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	211.97	215.1515	3.180	2.800	121.000	1192.291	0.026	1.636
Filter R	223.1335	226.9565	3.822	2.700	121.000	1485.839	0.032	2.038
Blank	149.84	149.841	0.001					

**Actual Machining Conditions**

Flow	2.470	<b>M# = 0.100</b>
Depth	0.012	
RPM	1270.000	
Base Amp	na	
Cut Amp	na	
Diameter	7.420	<b>Mact = 0.097</b>

Test 4 Mineral Oil, 1270 rpm, 014", 2.5 ml/s

	Run 7	% Under	Run 7	Lower SIZE	Upper SIZE	Run 7 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	actual	Best log-normal fit
Mineral Oil		5%	0.62	0.1	0.12	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264			1.891
1/17/99 16:45		10%	0.67	0.12	0.14	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.966113			1.469
AERODYNAMIC No.DISTRIB.		15%	0.72	0.14	0.16	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.832581			0.988
Material:		20%	0.77	0.16	0.18	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.714798			
Density (g/cc):	0.81	25%	0.83	0.18	0.22	0	0	0.2	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.514128			
Correlating Factor:	1	30%	0.88	0.22	0.25	0	0	0.235	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.386294			
Run Length (sec):	122.69	35%	0.93	0.25	0.29	2.97E+01	0	0.27	1.09E-05	2.48E-13	2.48E-10	6.80E-08	-4.377216	-1.078810	0.351513	0.34	
PMT Voltage (Volts):	1150	40%	0.99	0.29	0.34	1.66E+03	2.97E+01	0.315	6.23E-04	2.20E-11	2.20E-08	6.12E-06	-4.079193	-0.916291	0.394184	0.4	
Laser Current (mA):	42.54	45%	1.04	0.34	0.4	2.77E+03	1.69E+03	0.37	1.64E-03	5.96E-11	5.96E-08	2.25E-05	-4.051253	-0.776529	0.398441	0.46	
Clock Freq (MHz):	40	50%	1.1	0.4	0.46	2.97E+02	4.47E+03	0.43	1.75E-03	1.00E-11	1.00E-08	2.52E-05	-2.707857	-0.462035	0.514683	0.54	
Sum of channels:	2718741	55%	1.16	0.46	0.54	2.27E+04	4.76E+03	0.5	1.01E-02	1.20E-09	1.20E-06	3.55E-04	-2.172601	-0.301105	0.667812	0.63	
Lower Size Limit:	199.6	60%	1.22	0.54	0.63	1.30E+05	2.75E+04	0.585	5.80E-02	1.10E-08	1.10E-05	3.39E-03	-1.842845	-0.150823	0.931267	0.74	
Upper Size Limit:	1.12	70%	1.38	0.74	0.86	2.98E+05	4.66E+05	0.8	2.81E-01	6.47E-08	6.47E-05	3.27E-02	-1.090477	0.182322	1.243621	1.2	
Standard Deviation:	1.5	75%	1.48	0.86	1	3.56E+05	7.64E+05	0.93	4.12E-01	1.21E-07	1.21E-04	6.60E-02	-1.506378	0.000000	1.059864	1	
DX(3):	20.59	80%	1.59	1	1.2	4.63E+05	1.12E+06	1.1	5.82E-01	2.61E-07	2.61E-04	1.38E-01	-0.751504	0.336472	1.416714	1.4	
DX(2):	2.27	85%	1.73	1.2	1.4	3.46E+05	1.58E+06	1.3	7.10E-01	3.22E-07	3.22E-04	2.26E-01	-0.448157	0.470004	1.591946	1.6	
Spec surf area (m <sup>2</sup> /g):	3.26	90%	1.93	1.4	1.6	2.57E+05	1.93E+06	1.5	8.04E-01	3.67E-07	3.67E-04	3.27E-01	-0.178330	0.587787	1.765948	1.8	
Mode (Linear scale):	0.68	95%	2.26	1.6	1.8	1.79E+05	2.19E+06	1.7	8.70E-01	3.72E-07	3.72E-04	4.29E-01	0.293423	0.788457	2.117098	2.2	
Mode Lower Bound:	0.66			1.8	2.2	2.00E+05	2.37E+06	2	9.43E-01	3.98E-07	3.98E-04	7.25E-01	0.597081	0.916291	2.379243	2.5	
Mode Upper Bound:	0.72			2.2	2.5	7.24E+04	2.57E+06	2.35	9.70E-01	3.98E-07	3.98E-04	7.25E-01	0.956557	1.064711	2.731845	2.9	
Lower combine size:	7.24			2.5	2.9	4.62E+04	2.64E+06	2.7	9.87E-01	3.86E-07	3.86E-04	8.31E-01	1.364624	1.223775	3.195847	3.4	
Upper combine size:	7.36			2.9	3.4	2.29E+04	2.68E+06	3.15	9.95E-01	3.03E-07	3.03E-04	9.14E-01	1.847220	1.386294	3.847328	4	
Dispenser				3.4	4	9.13E+03	2.71E+06	3.7	9.99E-01	1.96E-07	1.96E-04	9.68E-01	2.569323	1.526056	5.078316	4.6	
Flow : 0.0				4	4.6	2.95E+03	2.72E+06	4.3	1.00E+00	9.93E-08	9.93E-05	9.95E-01					
Flow Inc. : 0.0				4.6	5.4	3.50E+02	2.72E+06	5	1.00E+00	1.86E-08	1.86E-05	1.00E+00					
Pulse : 0.0				5.4	6.3	0	2.72E+06	5.85	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Pulse Inc. : 0.0				6.3	7.4	0	2.72E+06	6.85	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nebulizer : 0.0				7.4	8.6	0	2.72E+06	8	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Neb. Inc. : 0.0				8.6	10	0	2.72E+06	9.3	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Low Limit : 40				10	12	0	2.72E+06	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
High Limit: 80				12	14	0	2.72E+06	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Baseline Offset	0.1			14	16	0.00E+00	2.72E+06	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Noise Filter (Sigma)	6			16	18	0	2.72E+06	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nozzle Type	200um			18	22	0	2.72E+06	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):	1.49			22	25	0	2.72E+06	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
to:	1.53			25	29	0	2.72E+06	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):	1.98			29	34	0	2.72E+06	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (l/m <sup>3</sup> ):	8.81E+08			34	40	0	2.72E+06	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:	1			40	46	0.00E+00	2.72E+06	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				46	54	0	2.72E+06	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				54	63	0	2.72E+06	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				63	74	0	2.72E+06	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				74	86	0.00E+00	2.72E+06	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				86	100	0.00E+00	2.72E+06	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				100	120	0	2.72E+06	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				120	140	0	2.72E+06	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				140	160	0	2.72E+06	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				160	180	0	2.72E+06	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				180	220	0	2.72E+06	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
				2718614.07						3.64E-06	3.64E-03						

Test 4 (Cont.) Mineral Oil, 1270 rpm, .014", 2.5 ml/s

Generation Rate Calculations										
	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec		
Filter L	206.5185	209.9055	3.386	2.900	133.000	1114.981	0.025	1.392		
Filter R	191.8695	195.661	3.791	2.700	133.000	1340.637	0.029	1.673		
Blank	149.84	149.841	0.001							

Actual Machining Conditions	
Flow	2.570
Depth	0.014
RPM	1270.000
Base Amp	na
Cut Amp	na
Diameter	7.360

M# = 0.112

Mact = 0.108

Test 5 Mineral Oil, 805 rpm, .015", 3 ml/s

	Run 9	% Under	Run 9	Lower SIZE	Upper SIZE	Run 9 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit actual	dg	2.169
Mineral Oil	1/17/99 17:25	5%	0.62	0.1	0.12	6.56E+01	0	0.11	5.31E-05	3.70E-14	3.70E-11	1.89E-08		-2.120264			rg	1.547
Taken on:		10%	0.68	0.12	0.14	5.91E+01	6.23E+01	0.13	1.01E-04	5.51E-11	5.51E-11	4.69E-08		-1.966113			r2	0.997
AERODYNAMIC No. DISTRIB.		15%	0.73	0.14	0.16	7.31E+01	1.21E+02	0.15	1.60E-04	1.05E-13	1.05E-10	1.00E-07		-1.832581				
Material:	E-7211	20%	0.8	0.16	0.18	6.30E+01	1.94E+02	0.17	2.11E-04	1.31E-13	1.31E-10	1.67E-07		-1.714798				
Density (g/cc):	0.81	25%	0.85	0.18	0.22	8.09E+01	2.57E+02	0.2	2.76E-04	2.74E-13	2.74E-10	3.07E-07		-1.514128				
Correlating Factor:	1	30%	0.91	0.22	0.25	1.63E+02	3.38E+02	0.235	4.08E-04	8.98E-13	8.98E-10	7.64E-07		-1.386294				
Run Length (sec):	192.67	35%	0.97	0.25	0.29	4.07E+02	5.02E+02	0.27	7.37E-04	3.39E-12	3.39E-09	2.49E-06		-1.237874				
PMT Voltage (Volts):	1150	40%	1.02	0.29	0.34	1.35E+03	9.09E+02	0.315	1.83E-03	1.79E-11	1.79E-08	1.16E-05	-4.228204	-1.078810	0.342466	0.34		
Laser Current (mA):	42.54	45%	1.08	0.34	0.4	2.11E+03	2.26E+03	0.37	3.53E-03	4.52E-11	4.52E-08	3.46E-05	-3.979076	-0.916291	0.381813	0.4		
Clock Freq (MHz):	40	50%	1.14	0.4	0.46	1.13E+03	4.36E+03	0.43	4.44E-03	3.79E-11	3.79E-08	5.40E-05	-3.871974	-0.776529	0.400088	0.46		
Sum of channels:	1236448	55%	1.2	0.46	0.54	1.04E+04	5.49E+03	0.5	1.29E-02	5.52E-10	5.52E-07	3.35E-04	-3.401656	-0.616186	0.491274	0.54		
Lower Size Limit:	0.1	60%	1.28	0.54	0.63	5.25E+04	1.59E+04	0.585	5.33E-02	4.45E-09	4.45E-06	2.60E-03	-2.794040	-0.462035	0.640504	0.63		
Upper Size Limit:	199.6	65%	1.35	0.63	0.74	1.23E+05	6.84E+04	0.685	1.55E-01	1.67E-08	1.67E-05	1.11E-02	-2.286033	-0.301105	0.795530	0.74		
Mean Size:	1.16	70%	1.44	0.74	0.86	1.25E+05	1.91E+05	0.8	2.53E-01	2.70E-08	2.70E-05	2.49E-02	-1.961653	-0.150823	0.921159	0.86		
Standard Deviation:	1.52	75%	1.54	0.86	1	1.53E+05	3.16E+05	0.93	3.79E-01	5.22E-08	5.22E-05	5.15E-02	-1.630542	0.000000	1.064412	1		
D(4.3):	2.34	80%	1.65	1	1.2	2.07E+05	4.69E+05	1.1	5.47E-01	1.17E-07	1.17E-04	1.11E-01	-1.221142	0.182322	1.272706	1.2		
D(3.2):	1.88	85%	1.8	1.2	1.4	1.61E+05	6.76E+05	1.3	6.77E-01	1.50E-07	1.50E-04	1.88E-01	-0.886826	0.336472	1.472691	1.4		
Spec surf area (m <sup>2</sup> /g):	3.93	90%	2.01	1.4	1.6	1.24E+05	8.37E+05	1.5	7.78E-01	1.78E-07	1.78E-04	2.78E-01	-0.588055	0.470004	1.677859	1.6		
Mode (Linear scale):	0.68	95%	2.36	1.6	1.8	8.81E+04	9.62E+05	1.7	8.49E-01	1.84E-07	1.84E-04	3.72E-01	-0.327209	0.587787	1.880224	1.8		
Mode Lower Bound:	0.66			1.8	2.2	1.02E+05	1.05E+06	2	9.31E-01	3.45E-07	3.45E-04	5.47E-01	0.118762	0.788457	2.284343	2.2		
Mode Upper Bound:	0.71			2.2	2.5	3.81E+04	1.15E+06	2.35	9.62E-01	2.09E-07	2.09E-04	6.54E-01	0.396021	0.916291	2.578262	2.5		
Lower combine size:	4.64			2.5	2.9	2.49E+04	1.12E+06	2.7	9.82E-01	2.08E-07	2.08E-04	7.60E-01	0.706027	1.064711	2.951898	2.9		
Upper combine size:	4.71			2.9	3.4	1.24E+04	1.22E+06	3.15	9.92E-01	1.64E-07	1.64E-04	8.43E-01	1.008543	1.223775	3.368647	3.4		
Dispenser				3.4	4	5.80E+03	1.23E+06	3.7	9.97E-01	1.25E-07	1.25E-04	9.07E-01	1.321496	1.386294	3.861787	4		
Flow : 0.0				4	4.6	2.05E+03	1.23E+06	4.3	9.99E-01	6.92E-08	6.92E-05	9.42E-01	1.572316	1.526056	4.308653	4.6		
Flow Inc. : 0.0				4.6	5.4	1.13E+03	1.24E+06	5	1.00E+00	6.00E-08	6.00E-05	9.73E-01	1.920716	1.686399	5.016431	5.4		
Pulse : 0.0				5.4	6.3	3.96E+02	1.24E+06	5.85	1.00E+00	3.36E-08	3.36E-05	9.90E-01	2.317029	1.840550	5.963922	6.3		
Pulse Inc. : 0.0				6.3	7.4	1.48E+02	1.24E+06	6.85	1.00E+00	2.01E-08	2.01E-05	1.00E+00						
Nebulizer : 0.0				7.4	8.6	0	1.24E+06	8	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Neb. Inc. : 0.0				8.6	10	0	1.24E+06	9.3	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Low Limit : 40				10	12	0	1.24E+06	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
High Limit: 80				12	14	0	1.24E+06	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Baseline Offset	0.1			14	16	0	1.24E+06	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Noise Filter (Sigmas)	6			16	18	0	1.24E+06	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Nozzle Type	200um			18	22	0	1.24E+06	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Flow Rate Range (l/min):	1.49			22	25	0	1.24E+06	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
to:	1.53			25	29	0	1.24E+06	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Mass Loading (mg/m <sup>3</sup> ):	0.59			29	34	0	1.24E+06	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Concentration (l/m <sup>3</sup> ):	2.55E+08			34	40	0	1.24E+06	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
Counting efficiency:	1			40	46	0	1.24E+06	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				46	54	0	1.24E+06	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				54	63	0	1.24E+06	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				63	74	0	1.24E+06	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				74	86	0	1.24E+06	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				86	100	0	1.24E+06	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				100	120	0	1.24E+06	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				120	140	0	1.24E+06	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				140	160	0	1.24E+06	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				160	180	0	1.24E+06	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				180	220	0	1.24E+06	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00						
				180	220	1236398.76				1.96E-06	1.96E-03							

Test 5 (Cont.) Mineral Oil, 805 rpm, .015", 3 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	211.3255	212.749	1.422	2.850	203.000	312.278	0.007	0.255
Filter R	212.6125	214.236	1.622	2.700	203.000	375.972	0.008	0.307
Blank	149.84	149.841	0.001					

Actual Machining Conditions

Flow	3.000
Depth	0.016
RPM	805.000
Base Amp	na
Cut Amp	na
Diameter	7.290

M# = 0.070

Mact = 0.067

Test 6 Mineral Oil, 1270 rpm, .02", 2.5 ml/s

Power surge turned off all equipment. Lost size distribution data.

### Test 6 (Cont.) Mineral Oil, 1270 rpm, .02", 2.5 ml/s

#### Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	215.1825	221.04	5.856	2.800	97.000	2738.663	0.060	4.687
Filter R	207.705	214.412	6.706	2.650	97.000	3313.418	0.069	5.670
Blank	149.84	149.841	0.001					

#### Actual Machining Conditions

Flow	2.560	M# =	0.169
Depth	0.021		
RPM	1270.000		
Base Amp	na		
Cut Amp	na		
Diameter	7.210	Mact =	0.160

Test 7 Mineral Oil, 805 rpm, .015", 3 ml/s

	Run 11	% Under	Run 11	Lower	Upper	Run 11	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
	Mineral Oil	3%	0.59	0.1	0.12	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-2.120264		dg	1.703
Taken on:	1/18/99 11:13	10%	0.64	0.12	0.14	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.966113		r2	0.973
AERODYNAMIC No. DISTRIB.		15%	0.68	0.14	0.16	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.832581			
Material:	F-7211	20%	0.72	0.16	0.18	1.84E+00	0	0.17	7.88E-06	3.83E-15	3.83E-12	1.78E-08	-1.714798			
Density (g/cc):	0.81	25%	0.77	0.18	0.22	5.05E+00	1.84E+00	0.2	2.96E-05	1.71E-14	1.71E-11	9.73E-08	-1.514128			
Correlating Factor:	1	30%	0.82	0.22	0.25	3.04E+01	6.89E+00	0.235	1.60E-04	1.67E-13	1.67E-10	8.74E-07	-1.386294			
Run Length (sec):	121.17	35%	0.86	0.25	0.29	1.29E+02	3.73E+01	0.27	7.12E-04	1.07E-12	1.07E-09	5.86E-06	-1.237874			
PMT Voltage (Volts):	1150	40%	0.91	0.29	0.34	4.21E+02	1.66E+02	0.315	2.52E-03	5.58E-12	5.58E-09	3.18E-05	-1.078810	0.348911	0.34	
Laser Current (mA):	42.54	45%	0.96	0.34	0.4	5.43E+02	5.87E+02	0.37	4.83E-03	1.17E-11	1.17E-08	8.59E-05	-0.916291	0.384056	0.4	
Clock Freq (MHz):	40	50%	1.01	0.4	0.46	2.94E+02	1.13E+03	0.43	6.11E-03	9.91E-12	9.91E-09	3.21E-04	-3.648456	0.401079	0.46	
Sum of channels:	233193	55%	1.06	0.46	0.54	3.40E+03	1.42E+03	0.5	2.07E-02	1.80E-10	1.80E-07	9.69E-04	-3.099558	0.498553	0.54	
Lower Size Limit:	0.1	60%	1.11	0.54	0.63	1.51E+04	4.83E+03	0.585	8.54E-02	1.28E-09	1.28E-06	6.91E-03	-2.461911	0.641902	0.63	
Upper Size Limit:	199.6	65%	1.17	0.63	0.74	3.17E+04	1.99E+04	0.685	2.21E-01	4.32E-09	4.32E-06	2.69E-02	-1.921674	0.793279	0.74	
Mean Size:	1.02	70%	1.24	0.74	0.86	2.98E+04	5.16E+04	0.8	3.49E-01	6.47E-09	6.47E-06	3.70E-02	-1.580534	0.910288	0.86	
Standard Deviation:	1.46	75%	1.32	0.86	1	3.40E+04	8.14E+04	0.93	4.95E-01	1.16E-08	1.16E-05	1.11E-01	-1.222238	0.000000	1	
DX(3):	1.78	80%	1.42	1	1.2	4.14E+04	1.15E+05	1.1	6.73E-01	2.34E-08	2.34E-05	2.19E-01	-0.774482	0.182322	1.2	
DX(5):	1.51	85%	1.53	1.2	1.4	2.79E+04	1.57E+05	1.3	7.97E-01	2.60E-08	2.60E-05	3.40E-01	-0.412381	0.336472	1.4	
Spec surf area (m <sup>2</sup> /g):	4.91	90%	1.69	1.6	1.6	1.90E+04	1.83E+05	1.5	8.74E-01	2.71E-08	2.71E-05	4.66E-01	-0.085448	0.470004	1.6	
Mode (Linear scale):	0.68	95%	1.96	1.6	1.8	1.17E+04	2.04E+05	1.7	9.24E-01	2.44E-08	2.44E-05	5.79E-01	0.199510	0.587787	1.8	
Mode Lower Bound:	0.65		1.8	1.8	2.2	1.13E+04	2.13E+05	2	9.72E-01	3.83E-08	3.83E-05	7.57E-01	0.696057	0.788457	2.2	
Mode Upper Bound:	0.7		2.2	2.5	2.5	3.44E+03	2.27E+05	2.35	9.87E-01	1.89E-08	1.89E-05	8.45E-01	0.104205	0.916291	2.5	
Lower combine size:	4.79		2.5	2.9	3.4	2.01E+03	2.30E+05	2.7	9.96E-01	1.67E-08	1.67E-05	9.72E-01	1.421522	1.064711	2.9	
Dispenser			2.9	3.4	4	7.97E+02	2.32E+05	3.15	9.99E-01	1.06E-08	1.06E-05	9.71E-01	1.902645	1.223775	3.4	
Flow Inc : 0.0			3.4	4	4	2.17E+02	2.33E+05	3.7	1.00E+00	4.66E-09	4.66E-06	9.93E-01	2.462257	1.386294	4	
Pulse Inc : 0.0			4.6	5.4	6.3	1.11E+01	2.33E+05	5.85	1.00E+00	1.35E-10	1.35E-07	9.94E-01	2.496090	1.686399	5.4	
Nebulizer : 0.0			6.3	7.4	8.6	3.00E+00	2.33E+05	6.85	1.00E+00	9.44E-10	9.44E-07	9.98E-01	2.894740	1.840550	6.3	
Neb. Inc : 0.0			7.4	8.6	10	0	2.33E+05	8	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Low Limit : 600			8.6	10	12	0	2.33E+05	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
High Limit : 800			10	12	14	0	2.33E+05	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Baseline Offset			12	14	16	0	2.33E+05	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Noise Filter (Sigmas)			14	16	18	0	2.33E+05	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Nozzle Type			16	18	22	0	2.33E+05	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Flow Rate Range (l/min):			18	22	25	0	2.33E+05	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
to:			22	25	29	0	2.33E+05	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Mass Loading (mg/m <sup>3</sup> ):			25	29	34	0	2.33E+05	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Concentration (#/m <sup>3</sup> ):			29	34	40	0	2.33E+05	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Counting efficiency:			34	40	46	0	2.33E+05	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			40	46	54	0	2.33E+05	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			46	54	63	0	2.33E+05	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			54	63	74	0	2.33E+05	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			63	74	86	0	2.33E+05	83	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			74	86	100	0	2.33E+05	90	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			86	100	120	0	2.33E+05	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			100	120	140	0	2.33E+05	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			120	140	160	0	2.33E+05	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			140	160	180	0	2.33E+05	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			160	180	220	0	2.33E+05	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			180	220	233187283	0	2.33E+05	200	1.00E+00	2.15E-07	2.15E-04	1.00E+00				

Test 7 (Cont.) Mineral Oil, 805 rpm, .015", 3 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	222.613	222.8555	0.248	2.850	132.000	83.558	0.002	0.105
Filter R	209.29	209.5675	0.283	2.750	132.000	98.842	0.002	0.124
Blank	149.842	149.837	-0.005					

Actual Machining Conditions

Flow	3.150
Depth	0.017
RPM	805.000
Base Amp	na
Cut Amp	na
Diameter	7.105

M# = 0.070

Mact = 0.066

Test 8 Mineral Oil, 500 rpm, .005", 3 ml/s

Run 13	% Under	Run 13	Upper SIZE	Lower SIZE	Run 13 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
Mineral Oil	5%	0.35	0.12	0.12	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264		dg
1/18/99 11:56	10%	0.51	0.12	0.14	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.966113		rg
AERODYNAMIC No. DISTRIB.	15%	0.59	0.14	0.16	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.832581		r2
Material:	20%	0.64	0.16	0.18	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.714798		
Density (g/cc):	25%	0.69	0.18	0.22	0	0	0.2	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.514128		
Correlating Factor:	30%	0.76	0.22	0.25	1.13E+01	0	0.235	4.56E-03	6.22E-14	6.22E-11	3.44E-06		-1.386294		
Run Length (sec):	35%	0.84	0.25	0.29	3.50E+01	1.13E+01	0.27	1.87E-02	2.92E-13	2.92E-10	1.96E-05		-1.237874		
PMT Voltage (Volts):	40%	0.92	0.29	0.34	6.83E+01	4.63E+01	0.315	4.62E-02	9.05E-13	9.05E-10	6.97E-05		-1.078810	0.427630	0.34
Laser Current (mA):	45%	1.01	0.34	0.4	6.19E+01	1.15E+02	0.37	7.12E-02	1.33E-12	1.33E-09	1.43E-04		-0.916291	0.478158	0.4
Clock Freq (MHz):	50%	1.09	0.4	0.46	2.83E+01	1.77E+02	0.43	8.27E-02	9.54E-13	9.54E-10	1.96E-04		-0.776529	0.502911	0.46
Sum of channels:	55%	1.18	0.46	0.54	8.01E+01	2.05E+02	0.5	1.15E-01	4.25E-12	4.25E-09	4.31E-04		-0.616186	0.573412	0.54
Lower Size Limit:	60%	1.31	0.54	0.63	1.79E+02	2.85E+02	0.585	1.87E-01	1.52E-11	1.52E-08	1.27E-03		-0.462035	0.695626	0.63
Upper Size Limit:	65%	1.46	0.63	0.74	2.46E+02	4.64E+02	0.685	2.87E-01	3.35E-11	3.35E-08	3.13E-03		-0.301105	0.828288	0.74
Mean Size:	70%	1.63	0.74	0.86	1.76E+02	7.10E+02	0.8	3.58E-01	3.82E-11	3.82E-08	5.24E-03		-0.150823	0.922249	0.86
Standard Deviation:	75%	1.88	0.86	1	2.19E+02	8.86E+02	0.93	4.46E-01	7.45E-11	7.45E-08	9.37E-03		0.000000	1.048434	1
DX(4.3):	80%	2.4	1	1.2	2.75E+02	1.11E+03	1.1	5.57E-01	1.55E-10	1.55E-07	1.80E-02		-2.097977	1.224817	1.2
DX(3.2):	85%	3.66	1.2	1.4	1.95E+02	1.38E+03	1.3	6.36E-01	1.82E-10	1.82E-07	2.80E-02		-1.910930	1.374127	1.4
Spec surf area (m <sup>2</sup> /g):	90%	4.12	1.4	1.6	1.34E+02	1.58E+03	1.5	6.90E-01	1.91E-10	1.91E-07	3.86E-02		-1.767376	1.500952	1.6
Mode (Linear scale):	95%	4.95	1.6	1.8	1.16E+02	1.71E+03	1.7	7.36E-01	2.42E-10	2.42E-07	5.20E-02		-1.626131	1.637156	1.8
Mode Lower Bound:	0.65		1.8	2.2	1.22E+02	1.83E+03	2	7.86E-01	4.14E-10	4.14E-07	7.49E-02		-1.440444	1.835196	2.2
Mode Upper Bound:	0.66		2.2	2.5	5.20E+01	1.95E+03	2.35	8.07E-01	1.82E-10	2.86E-07	9.07E-02		-1.336484	1.956355	2.5
Lower combine size:	2.63		2.5	2.9	3.72E+01	2.00E+03	2.7	8.22E-01	3.10E-10	3.10E-07	1.08E-01		-1.237916	2.078610	2.9
Upper combine size:	2.67		2.9	3.4	3.58E+00	2.04E+03	3.15	8.23E-01	4.74E-11	4.74E-08	1.10E-01		-1.223898	1.223775	3.4
Dispenser			3.4	4	1.39E+02	2.04E+03	3.7	8.79E-01	2.99E-09	2.99E-06	2.76E-01		-0.595159	1.386294	4
Flow Inc. : 0.0			4	4.6	1.73E+02	2.18E+03	4.3	9.49E-01	5.84E-09	5.84E-06	5.99E-01		0.250366	1.526056	4.6
Pulse Inc. : 0.0			4.6	5.4	1.08E+02	2.35E+03	5	9.93E-01	5.72E-09	5.72E-06	9.15E-01		1.375292	10.368090	5.4
Nebulizer : 0.0			5.4	6.3	1.80E+01	2.46E+03	5.85	1.00E+00	1.53E-09	1.53E-06	1.00E+00				
Low Limit : 40			6.3	7.4	0	2.48E+03	6.85	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
High Limit : 80			7.4	8.6	0	2.48E+03	8	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Baseline Offset			8.6	10	0	2.48E+03	9.3	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Noise Filter (Sigmas)			10	12	0	2.48E+03	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Nozzle Type			12	14	0	2.48E+03	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Flow Rate Range (l/min):			14	16	0	2.48E+03	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
to:			16	18	0	2.48E+03	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Mass Loading (mg/m <sup>3</sup> ):			18	22	0	2.48E+03	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Concentration (#/m <sup>3</sup> ):			22	25	0	2.48E+03	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Counting efficiency:			25	29	0	2.48E+03	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			29	34	0	2.48E+03	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			34	40	0	2.48E+03	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			40	46	0	2.48E+03	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			46	54	0	2.48E+03	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			54	63	0	2.48E+03	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			63	74	0	2.48E+03	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			74	86	0	2.48E+03	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			86	100	0	2.48E+03	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			100	120	0	2.48E+03	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			120	140	0	2.48E+03	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			140	160	0	2.48E+03	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			160	180	0	2.48E+03	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			180	220	0	2.48E+03	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			2478.116						1.81E-08	1.81E-05					

Test 8 (Cont.) Mineral Oil, 500 rpm, .005", 3 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	221.396	221.442	0.051	2.800	283.000	8.174	0.000	0.005
Filter R	232.859	232.9115	0.058	2.600	283.000	9.925	0.000	0.006
Blank	149.842	149.837	-0.005					

Actual Machining Conditions

Flow	2.970
Depth	0.008
RPM	500.000
Base Amp	na
Cut Amp	na
Diameter	7.300

M# = 0.022

Mact = 0.021



Test 9 (Cont.) Mineral Oil, 1270 rpm, .014", 2.5 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	219.898	224.433	4.540	2.800	120.000	1716.117	0.038	2.374
Filter R	206.3535	210.8965	4.548	2.700	120.000	1782.813	0.038	2.466
Blank	149.842	149.837	-0.005					

Actual Machining Conditions

Flow	2.410
Depth	0.011
RPM	1270.000
Base Amp	na
Cut Amp	na
Diameter	7.255

M# = 0.094

Mact = 0.089

Test 10 Mineral Oil, 500 rpm, 005" 3 ml/s

	Run 17	% Under	Run 17	Lower	Upper	Run 17	# In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
	Mineral Oil	5%	0.57	0.1	0.12	2.51E+01	0	0	0.11	1.29E-04	1.41E-14	1.41E-11	1.72E-08		-2.120264		dg
Taken on:	1/18/99 13:58	10%	0.66	0.12	0.14	2.36E+01	2.37E+01	2.37E+01	0.13	2.51E-04	2.20E-14	2.20E-11	4.39E-08		-1.966113		rg
AERODYNAMIC No. DISTIB.		15%	0.74	0.14	0.16	2.03E+01	4.73E+01	4.73E+01	0.15	3.56E-04	2.90E-14	2.90E-11	7.91E-08		-1.832581		
Material:	F-7211	20%	0.82	0.16	0.18	1.14E+01	6.76E+01	6.76E+01	0.17	4.15E-04	2.38E-14	2.38E-11	1.08E-07		-1.714798		
Density (g/cc):	0.81	25%	0.9	0.18	0.22	6.92E+01	7.90E+01	7.90E+01	0.2	7.72E-04	2.35E-13	2.35E-10	3.93E-07		-1.514128		
Correcting Factor:	1	30%	0.98	0.22	0.25	2.04E+02	1.48E+02	1.48E+02	0.235	1.82E-03	1.12E-12	1.12E-09	1.76E-06		-1.386294		
Run Length (sec):	267.75	35%	1.06	0.25	0.29	7.66E+02	3.52E+02	3.52E+02	0.27	5.82E-03	6.47E-12	6.47E-09	9.61E-06		-1.237874		
PMT Voltage (Volts):	43.02	40%	1.14	0.29	0.34	1.85E+03	1.13E+03	1.13E+03	0.315	1.54E-02	2.45E-08	3.94E-05	3.94E-05	-3.948808	-1.078810	0.304881	0.34
Laser Current (mA):	40	50%	1.22	0.34	0.4	1.70E+03	2.98E+03	2.98E+03	0.37	2.41E-02	3.64E-11	3.64E-08	8.36E-05	-3.764872	-0.916291	0.345799	0.4
Sum of channels:	193897	55%	1.42	0.46	0.54	2.53E+03	5.41E+03	5.41E+03	0.5	4.09E-02	1.34E-10	1.34E-07	2.76E-04	-3.454043	-0.616186	0.427809	0.54
Lower Size Limit:	0.1	60%	1.53	0.54	0.63	7.33E+03	7.94E+03	7.94E+03	0.585	7.88E-02	6.22E-10	6.22E-07	1.03E-03	-3.080931	-0.462035	0.552326	0.63
Upper Size Limit:	199.6	65%	1.65	0.63	0.74	1.42E+04	1.53E+04	1.53E+04	0.685	1.52E-01	1.93E-09	1.93E-06	3.37E-03	-2.708912	-0.301105	0.712552	0.74
Mean Size:	1.33	70%	1.79	0.74	0.86	1.39E+04	2.94E+04	2.94E+04	0.8	2.24E-01	3.02E-09	3.02E-06	7.04E-03	-2.455236	-0.150823	0.847711	0.86
Standard Deviation:	1.76	75%	1.96	0.86	1	1.73E+04	4.34E+04	4.34E+04	0.93	3.13E-01	5.90E-09	5.90E-06	1.42E-02	-2.191846	0.000000	1.015238	1
DX(4.3):	5.52	80%	2.16	1	1.2	2.40E+04	6.06E+04	6.06E+04	1.1	4.36E-01	1.35E-08	1.35E-05	3.06E-02	-1.871695	0.182322	1.264054	1.2
Spec surf area (m <sup>2</sup> /g):	2.47	90%	2.77	1.2	1.4	1.74E+04	1.05E+05	1.05E+05	1.5	6.29E-01	2.49E-08	2.49E-05	8.35E-02	-1.614494	0.336472	1.507457	1.4
Mode (Linear scale):	0.68	95%	3.36	1.6	1.8	1.40E+04	1.22E+05	1.22E+05	1.7	7.02E-01	2.92E-08	2.92E-05	1.19E-01	-1.180408	0.470004	1.767514	1.6
Mode Lower Bound:	0.65		1.8	2.2	2.06E+04	1.36E+05	1.36E+05	2	8.08E-01	6.98E-08	6.98E-05	2.04E-01	-0.828720	0.788457	2.581649	2.2	
Mode Upper Bound:	0.71		2.2	2.5	1.06E+04	1.57E+05	1.57E+05	2.35	8.63E-01	8.31E-08	8.31E-05	2.75E-01	-0.598720	0.916291	3.021957	2.5	
Upper combine size:	5.09		2.5	2.9	9.97E+03	1.67E+05	1.67E+05	2.7	9.14E-01	8.31E-05	8.31E-05	3.76E-01	-0.317058	1.064711	3.664726	2.9	
Lower combine size:	5.17		2.9	3.4	7.41E+03	1.77E+05	1.77E+05	3.15	9.52E-01	9.82E-08	9.82E-05	4.95E-01	-0.013156	1.223775	4.512401	3.4	
Dispenser			3.4	4	4.75E+03	1.85E+05	1.85E+05	3.7	9.77E-01	1.02E-07	1.02E-04	6.18E-01	0.301534	1.386294	5.597339	4	
Flow Inc.: 0.0			4	4.6	2.26E+03	1.89E+05	1.89E+05	4.3	9.88E-01	7.61E-08	7.61E-05	7.11E-01	0.555923	1.526056	6.662312	4.6	
Pulse : 0.0			4.6	5.4	1.41E+03	1.92E+05	1.92E+05	5	9.96E-01	7.47E-08	7.47E-05	8.01E-01	0.846953	1.686399	8.131369	5.4	
Pulse Inc.: 0.0			5.4	6.3	5.98E+02	1.93E+05	1.93E+05	5.85	9.99E-01	5.07E-08	5.07E-05	8.63E-01	1.094213	1.840550	9.631349	6.3	
Nebulizer : 0.0			6.3	7.4	1.72E+02	1.94E+05	1.94E+05	6.85	1.00E+00	2.34E-08	2.34E-05	8.91E-01	1.234444	2.001480	10.601933	7.4	
Neb. Inc. : 0.0			7.4	8.6	1.11E+01	1.94E+05	1.94E+05	8	1.00E+00	2.40E-09	2.40E-06	8.94E-01	1.250244	2.151762	10.717248	8.6	
Low Limit : 40			8.6	10	1.77E+01	1.94E+05	1.94E+05	9.3	1.00E+00	6.03E-09	6.03E-06	9.02E-01	1.291392	2.302585	11.023480	10	
High Limit: 80			10	12	8.93E+00	1.94E+05	1.94E+05	11	1.00E+00	5.04E-09	5.04E-06	9.08E-01	1.327533	2.484907	11.299660	12	
Baseline Offset			12	14	0.518	1.94E+05	1.94E+05	13	1.00E+00	4.82E-10	4.82E-07	9.08E-01	1.331084	2.639057	11.327170	14	
Noise Filter (Sigma)			14	16	2.20E+00	1.94E+05	1.94E+05	15	1.00E+00	3.14E-09	3.14E-06	9.12E-01	1.354622	2.772589	11.511196	16	
Nozzle Type			16	18	0	1.94E+05	1.94E+05	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.354622	2.890372	11.511196	18	
Flow Rate Range (l/min):	1.53		18	22	1.79E+00	1.94E+05	1.94E+05	20	1.00E+00	6.07E-09	6.07E-06	9.20E-01	1.402379	3.091042	11.893818	22	
to:	1.53		22	25	9.86E+00	1.94E+05	1.94E+05	23.5	1.00E+00	5.42E-08	5.42E-05	9.85E-01	2.181832	3.218876	20.281213	25	
Mass Loading (mg/m <sup>3</sup> ):	0.2		25	29	0.465	1.94E+05	1.94E+05	27	1.00E+00	3.88E-09	3.88E-06	9.90E-01	2.331944	3.367296	22.476579	29	
Concentration (#/m <sup>3</sup> ):	2.84E+07		34	40	0.378	1.94E+05	1.94E+05	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00	2.331944	3.526361	22.476579	34	
Counting efficiency:	1		40	46	0	1.94E+05	1.94E+05	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			46	54	0	1.94E+05	1.94E+05	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			54	63	0	1.94E+05	1.94E+05	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			63	74	0	1.94E+05	1.94E+05	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			74	86	0	1.94E+05	1.94E+05	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			86	100	0	1.94E+05	1.94E+05	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			100	120	0	1.94E+05	1.94E+05	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			120	140	0	1.94E+05	1.94E+05	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			140	160	0	1.94E+05	1.94E+05	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			160	180	0	1.94E+05	1.94E+05	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			180	220	0	1.94E+05	1.94E+05	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			193892.089								8.24E-07	8.24E-04					

## Test 10 (Cont.) Mineral Oil, 500 rpm, .005", 3 ml/s

### Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	202.258	202.4625	0.210	2.800	278.000	34.183	0.001	0.020
Filter R	209.383	209.6675	0.290	2.700	278.000	48.986	0.001	0.029
Blank	149.842	149.837	-0.005					

### Actual Machining Conditions

Flow	2.960
Depth	0.006
RPM	500.000
Base Amp	na
Cut Amp	na
Diameter	7.200

M# = 0.016

Mact = 0.016

Test 11 Mineral Oil, 805 rpm, .015", 3 ml/s

	Run 21	% Under	Run 21	Upper SIZE	Run 21	# In	Run 21	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(night)	predicted log normal	Best log-normal fit
			SIZE	SIZE													dg
Mineral Oil		5%	0.64	0.12	5.64E+01	0	0.11	7.03E-05	3.18E-14	3.18E-11	1.23E-08			-2.120264			1.558
1/18/99 14:42		10%	0.72	0.12	1.53E+01	5.32E+01	0.13	8.94E-05	1.43E-14	1.43E-11	1.79E-08			-1.966113			1.558
AERODYNAMIC No. DISTRIB.		15%	0.8	0.14	2.04E+01	6.86E+01	0.15	1.15E-04	2.91E-14	2.91E-11	2.92E-08			-1.832581			1.558
F-7211		20%	0.87	0.16	1.96E+01	8.89E+01	0.17	1.39E-04	4.08E-14	4.08E-11	4.50E-08			-1.714798			1.558
Density (g/cc):	0.81	25%	0.95	0.18	1.54E+01	1.09E+02	0.2	1.58E-04	5.24E-14	5.24E-11	6.53E-08			-1.514128			1.558
Correlating Factor:	1	30%	1.02	0.22	6.80E+01	1.24E+02	0.235	2.43E-04	3.74E-13	3.74E-10	2.10E-07			-1.386294			1.558
Run Length (sec):	163.98	35%	1.09	0.25	2.47E+02	1.92E+02	0.27	5.52E-04	2.06E-12	2.06E-09	1.01E-06			-1.237874			1.558
PMT Voltage (Volts):	1150	40%	1.16	0.29	6.23E+02	4.39E+02	0.315	1.33E-03	8.25E-12	8.25E-09	4.21E-06			-1.078810	0.368444	0.34	1.558
Laser Current (mA):	43.5	45%	1.24	0.34	8.75E+02	1.06E+03	0.37	2.42E-03	1.88E-11	1.88E-08	1.15E-05			-0.916291	0.406830	0.4	1.558
Clock Freq (MHz):	40	50%	1.32	0.4	6.69E+02	1.94E+03	0.43	3.25E-03	2.26E-11	2.26E-08	2.02E-05			-0.776529	0.429266	0.46	1.558
Sum of channels:	802260	55%	1.41	0.46	5.63E+03	2.61E+03	0.5	1.03E-02	2.98E-10	2.98E-07	1.36E-04			-0.616186	0.527712	0.54	1.558
Lower Size Limit:	0.1	60%	1.5	0.54	2.60E+04	8.23E+03	0.585	4.27E-02	2.21E-09	2.21E-06	9.92E-04			-0.462035	0.673126	0.63	1.558
Upper Size Limit:	199.6	65%	1.61	0.63	7.84E+04	3.43E+04	0.685	1.16E-01	7.96E-09	7.96E-06	4.08E-03			-0.301105	0.820651	0.74	1.558
Mean Size:	1.35	70%	1.73	0.74	6.07E+04	9.27E+04	0.8	1.91E-01	1.32E-08	1.32E-05	9.19E-03			-0.150823	0.932297	0.86	1.558
Standard Deviation:	1.62	75%	1.87	0.86	1.77E+04	1.53E+05	0.93	2.88E-01	2.65E-08	2.65E-05	1.95E-02			0.000000	1.061604	1	1.558
D(4.3):	3.14	80%	2.04	1	1.11E+05	2.31E+05	1.1	4.27E-01	6.29E-08	6.29E-05	4.38E-02			0.182332	1.243752	1.2	1.558
D(3.2):	2.51	85%	2.26	1.2	9.44E+04	3.43E+05	1.3	5.45E-01	8.79E-08	8.79E-05	7.79E-02			0.336472	1.413498	1.4	1.558
Spec surf area (m <sup>2</sup> /g):	2.96	90%	2.57	1.4	8.11E+04	4.37E+05	1.5	6.46E-01	1.16E-07	1.16E-04	1.23E-01			-1.160872	0.470004	1.6	1.558
Mode (Linear scale):	1.01	95%	3.09	1.6	6.42E+04	5.18E+05	1.7	7.26E-01	1.34E-07	1.34E-04	1.75E-01			0.87787	1.751526	1.8	1.558
Mode Lower Bound:	0.83			1.8	2.2	8.94E+04	5.82E+05	2	8.37E-01	3.03E-07	3.03E-04			-0.546831	0.788457	2.2	1.558
Mode Upper Bound:	1.17			2.2	2.5	4.31E+04	6.72E+05	2.35	8.91E-01	2.37E-07	2.37E-04			-0.294330	0.916291	2.5	1.558
Lower combine size:	5.58			2.5	2.9	3.63E+04	7.15E+05	2.7	9.36E-01	3.02E-07	3.02E-04			0.003779	1.064711	2.9	1.558
Upper combine size:	5.67			2.9	3.4	2.44E+04	7.51E+05	3.15	9.66E-01	3.23E-07	3.23E-04			0.232622	1.223775	3.4	1.558
Dispenser				3.4	4	1.42E+04	7.84E+05	3.7	9.84E-01	3.04E-07	3.04E-04			0.658339	1.386294	3.4	1.558
Flow Inc. : 0.0				4	4.6	6.50E+03	7.90E+05	4.3	9.92E-01	2.19E-07	2.19E-04			0.953401	1.526056	4.6	1.558
Flow Inc. : 0.0				4.6	5.4	3.84E+03	7.96E+05	5	9.97E-01	2.04E-07	2.04E-04			1.333092	1.686399	5.4	1.558
Pulse Inc. : 0.0				5.4	6.3	1.79E+03	8.00E+05	5.85	9.99E-01	1.52E-07	1.52E-04			1.847193	1.840550	6.3	1.558
Pulse Inc. : 0.0				6.3	7.4	6.01E+02	8.02E+05	6.85	1.00E+00	8.19E-08	8.19E-05			3.234600	2.001480	7.4	1.558
Nebulizer : 0.0				7.4	8.6	7.24E+00	8.02E+05	8	1.00E+00	1.57E-09	1.57E-06						1.558
Neb. Inc. : 0.0				8.6	10	0	8.02E+05	9.3	1.00E+00	0.00E+00	0.00E+00						1.558
Low Limit : 40				10	12	0	8.02E+05	11	1.00E+00	0.00E+00	0.00E+00						1.558
High Limit: 80				12	14	0	8.02E+05	13	1.00E+00	0.00E+00	0.00E+00						1.558
				14	16	0	8.02E+05	15	1.00E+00	0.00E+00	0.00E+00						1.558
				16	18	0	8.02E+05	17	1.00E+00	0.00E+00	0.00E+00						1.558
Baseline Offset				18	22	0	8.02E+05	20	1.00E+00	0.00E+00	0.00E+00						1.558
Noise Filter (Sigmas)				22	25	0	8.02E+05	23.5	1.00E+00	0.00E+00	0.00E+00						1.558
Nozzle Type				25	29	0	8.02E+05	27	1.00E+00	0.00E+00	0.00E+00						1.558
Flow Rate Range (l/min):				29	34	0	8.02E+05	31.5	1.00E+00	0.00E+00	0.00E+00						1.558
Mass Loading (mg/m <sup>3</sup> ):				34	40	0	8.02E+05	37	1.00E+00	0.00E+00	0.00E+00						1.558
Concentration (#/m <sup>3</sup> ):				40	46	0	8.02E+05	43	1.00E+00	0.00E+00	0.00E+00						1.558
Counting efficiency:				46	54	0	8.02E+05	50	1.00E+00	0.00E+00	0.00E+00						1.558
				54	63	0	8.02E+05	58.5	1.00E+00	0.00E+00	0.00E+00						1.558
				63	74	0	8.02E+05	68.5	1.00E+00	0.00E+00	0.00E+00						1.558
				74	86	0	8.02E+05	80	1.00E+00	0.00E+00	0.00E+00						1.558
				86	100	0	8.02E+05	93	1.00E+00	0.00E+00	0.00E+00						1.558
				100	120	0	8.02E+05	110	1.00E+00	0.00E+00	0.00E+00						1.558
				120	140	0	8.02E+05	130	1.00E+00	0.00E+00	0.00E+00						1.558
				140	160	0	8.02E+05	150	1.00E+00	0.00E+00	0.00E+00						1.558
				160	180	0	8.02E+05	170	1.00E+00	0.00E+00	0.00E+00						1.558
				180	220	0	8.02E+05	200	1.00E+00	0.00E+00	0.00E+00						1.558
				220	802269.802						2.58E-06	2.58E-03					1.558

Test 11 (Cont.) Mineral Oil, 805 rpm, .015", 3 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	212.732	213.958	1.231	2.900	174.000	309.843	0.007	0.296
Filter R	207.2445	208.6275	1.388	2.850	174.000	355.489	0.008	0.339
Blank	149.842	149.837	-0.005					

Actual Machining Conditions

Flow	3.000
Depth	0.018
RPM	805.000
Base Amp	na
Cut Amp	na
Diameter	7.170

M# = 0.078

Mact = 0.074

Test 12 Mineral Oil, 500 rpm, .005", 3 ml/s

	Run 23	% Under	Run 23	Lower SIZR	Upper SIZR	Run 23 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit actual	dg
Mineral Oil	1/18/99 15:25	5%	0.3	0.12	0.12	699E+00	0	0.11	3.98E-04	3.94E-15	3.94E-12	4.20E-08		-2.120264			fg
Run on:		10%	0.35	0.12	0.14	6.60E+00	6.99E+00	0.13	6.58E-04	6.15E-15	6.15E-12	1.07E-07		-1.966113			fg
AERODYNAMIC No. DISTRIB.		15%	0.43	0.14	0.16	8.58E+00	1.36E+01	0.15	1.07E-04	1.23E-14	1.23E-11	2.38E-07		-1.832381			r2
Material:	F-7211	20%	0.56	0.16	0.18	1.14E+01	2.22E+01	0.17	1.62E-03	2.37E-14	2.37E-11	4.91E-07		-1.714798			
Density (g/cc):	0.81	25%	0.62	0.18	0.22	6.03E+01	3.36E+01	0.2	4.54E-03	2.04E-13	2.04E-10	2.67E-06		-1.514128			
Correlating Factor:	1	30%	0.66	0.22	0.25	1.46E+02	9.38E+01	0.235	1.16E-02	8.01E-13	8.01E-10	1.12E-05		-1.386294			
Run Length (sec):	301.96	35%	0.7	0.25	0.29	5.06E+02	2.39E+02	0.27	3.61E-02	4.22E-12	4.22E-09	5.61E-05		-1.237874			
PMT Voltage (Volts):	1150	40%	0.76	0.29	0.34	1.10E+03	7.45E+02	0.315	8.94E-02	1.46E-11	1.46E-08	2.12E-04	-3.525056	-1.078810	0.313693	0.34	
Laser Current (mA):	43.02	45%	0.82	0.34	0.4	1.04E+03	1.85E+03	0.37	1.40E-01	2.24E-11	2.24E-08	4.50E-04	-3.320165	-0.916291	0.375592	0.4	
Clock Freq (MHz):	40	50%	0.89	0.4	0.46	3.47E+02	2.89E+03	0.43	1.57E-01	1.17E-11	1.17E-08	5.75E-04	-3.251189	-0.776529	0.399067	0.46	
Sum of channels:	20655	55%	0.96	0.46	0.54	6.77E+02	3.24E+03	0.5	1.89E-01	3.59E-11	3.59E-08	9.57E-04	-3.103341	-0.616186	0.454445	0.54	
Lower Size Limit:	0.1	60%	1.04	0.54	0.63	1.54E+03	3.91E+03	0.585	2.64E-01	1.31E-10	1.31E-07	2.35E-03	-2.826782	-0.462035	0.579495	0.63	
Upper Size Limit:	199.6	65%	1.12	0.63	0.74	2.50E+03	5.45E+03	0.685	3.85E-01	3.40E-10	3.40E-07	5.97E-03	-2.513807	-0.301105	0.762990	0.74	
Mean Size:	0.9	70%	1.21	0.74	0.86	1.93E+03	7.95E+03	0.8	4.78E-01	4.18E-10	4.18E-07	1.04E-02	-2.310608	-0.150823	0.912187	0.86	
Standard Deviation:	1.93	75%	1.33	0.86	1	2.00E+03	9.88E+03	0.93	5.75E-01	6.82E-10	6.82E-07	1.77E-02	-2.104034	0.000000	1.093798	1	
DX(4.3):	8.88	80%	1.48	1	1.2	2.44E+03	1.19E+04	1.1	6.93E-01	1.38E-09	1.38E-06	3.24E-02	-1.846956	0.182322	1.371099	1.2	
DX(3.2):	4.47	85%	1.67	1.2	1.4	1.72E+03	1.43E+04	1.3	7.76E-01	1.60E-09	1.60E-06	4.94E-02	-1.650401	0.336472	1.629664	1.4	
Spec surf area (m <sup>2</sup> /g):	1.66	90%	1.98	1.4	1.6	1.19E+03	1.60E+04	1.5	8.34E-01	1.70E-09	1.70E-06	6.76E-02	-1.494168	0.470004	1.869542	1.6	
Mode (Linear scale):	0.34	95%	2.64	1.6	1.8	8.51E+02	1.72E+04	1.7	8.75E-01	1.77E-09	1.77E-06	8.64E-02	-1.362989	0.587787	2.098016	1.8	
Mode Lower Bound:	0.33		1.8	2.2	2.2	9.91E+02	1.81E+04	2	9.23E-01	3.96E-09	3.96E-06	1.22E-01	-1.163885	0.788457	2.499256	2.2	
Mode Upper Bound:	0.35		2.2	2.5	2.5	4.29E+02	1.91E+04	2.35	9.44E-01	2.36E-09	2.36E-06	1.47E-01	-1.047781	0.916291	2.767770	2.5	
Lower combine size:	3.8		2.5	2.9	2.9	2.92E+02	1.95E+04	2.7	9.58E-01	2.44E-09	2.44E-06	1.73E-01	-0.941013	1.064711	3.040082	2.9	
Upper combine size:	3.86		2.9	3.4	3.4	2.07E+02	1.98E+04	3.15	9.68E-01	2.75E-09	2.75E-06	2.03E-01	-0.832295	1.223775	3.344918	3.4	
Dispenser			3.4	4	4	1.38E+02	2.00E+04	3.7	9.75E-01	2.97E-09	2.97E-06	2.34E-01	-0.724834	1.386294	3.676251	4	
Flow Inc.: 0.0			4	4.6	4.6	1.75E+02	2.01E+04	4.3	9.83E-01	5.90E-09	5.90E-06	2.97E-01	-0.532746	1.526056	4.352404	4.6	
Pulse Inc.: 0.0			5.4	6.3	6.3	4.67E+01	2.04E+04	5	9.89E-01	6.72E-09	6.72E-06	3.69E-01	-0.335211	1.686399	5.177641	5.4	
Nebulizer Inc.: 0.0			6.3	7.4	7.4	9.27E+01	2.05E+04	6.85	9.92E-01	3.97E-09	3.97E-06	4.11E-01	-0.225031	1.840550	5.704139	6.3	
Neb. Inc.: 0.0			7.4	8.6	8.6	5.08E+01	2.06E+04	8	9.96E-01	1.26E-08	1.26E-05	5.45E-01	0.114244	2.001480	7.685957	7.4	
Low Limit: 40			8.6	10	10	1.63E+01	2.06E+04	9.3	9.99E-01	5.56E-09	5.56E-06	7.22E-01	0.589355	2.302585	11.669633	10	
High Limit: 80			10	12	12	1.07E+00	2.06E+04	11	1.00E+00	6.06E-10	6.06E-07	7.29E-01	0.608715	2.484907	11.869904	12	
Baseline Offset			12	14	14	0	2.07E+04	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00	0.608715	2.639057	11.869904	14	
Noise Filter (Sigmas)			14	16	16	0.714	2.07E+04	15	1.00E+00	1.02E-09	1.02E-06	7.40E-01	0.641880	2.772589	12.221015	16	
Nozzle Type			16	18	18	4.06E+00	2.07E+04	17	1.00E+00	8.45E-09	8.45E-06	8.30E-01	0.952305	2.890372	16.054705	18	
Flow Rate Range (l/min):			18	22	22	4.72E+00	2.07E+04	20	1.00E+00	1.60E-08	1.60E-05	1.00E+00					
to:			22	25	25	0	2.07E+04	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):			25	29	29	0	2.07E+04	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (#/m <sup>3</sup> ):			29	34	34	0	2.07E+04	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:			34	40	40	0	2.07E+04	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			40	46	46	0	2.07E+04	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			46	54	54	0	2.07E+04	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			54	63	63	0	2.07E+04	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			63	74	74	0	2.07E+04	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			74	86	86	0	2.07E+04	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			86	100	100	0	2.07E+04	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			100	120	120	0	2.07E+04	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			120	140	140	0	2.07E+04	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			140	160	160	0	2.07E+04	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			160	180	180	0	2.07E+04	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			180	220	220	0	2.07E+04	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
								20656.101		9.39E-08	9.39E-05						

### Test 12 (Cont.) Mineral Oil, 500 rpm, .005", 3 ml/s

#### Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	223.98	224.0495	0.075	2.850	312.000	10.641	0.000	0.006
Filter R	202.744	202.81	0.071	2.800	312.000	10.322	0.000	0.005
Blank	149.842	149.837	-0.005					

#### Actual Machining Conditions

Flow	2.970	M# =	0.022
Depth	0.008		
RPM	500.000		
Base Amp	na		
Cut Amp	na		
Diameter	7.075	Mact =	0.020

Test I3 Mineral Oil/PCM Slurry, 805 rpm, .015", 3 ml/s

	Run 25	% Under	Run 25	Upper SIZE	Run 25 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit	
Min. Oil/PCM	0.62	0.1	0.12	0	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264		rg	2.020
1/19/99 14:13	0.67	0.12	0.14	2.69E+01	0	0	0.13	1.80E-05	2.51E-14	2.51E-11	1.35E-08		-1.966113		r2	0.990
AERODYNAMIC No.DISTRIB.	0.72	0.14	0.16	5.99E+01	2.69E+01	0.15	0.15	5.82E-05	8.57E-14	8.57E-11	5.97E-08		-1.832581			
Material:	0.77	0.16	0.18	2.69E+01	8.68E+01	0.17	0.17	7.62E-05	5.61E-14	5.61E-11	9.00E-08		-1.714798			
Density (g/cc):	0.81	0.25	0.22	5.44E+02	1.14E+02	0.2	0.2	1.14E-04	1.84E-13	1.84E-10	1.89E-07		-1.514128			
Correlating Factor:	1	0.87	0.22	2.25	1.64E+02	0.235	0.235	2.23E-04	9.04E-13	9.04E-10	6.77E-07		-1.386294			
Run Length (sec):	189.74	0.92	0.25	5.15E+02	3.33E+02	0.27	0.27	5.68E-04	4.30E-12	4.30E-09	3.00E-06		-1.237874			
PMT Voltage (Volts):	1150	0.98	0.29	0.34	1.66E+03	8.47E+02	0.315	1.68E-03	2.20E-11	2.20E-08	1.49E-05	-4.172325	-1.078810	0.332490	0.34	
Laser Current (mA):	42.54	1.03	0.34	0.4	2.58E+03	2.51E+03	0.37	3.41E-03	5.54E-11	5.54E-08	4.48E-05	-3.916211	-0.916291	0.371435	0.4	
Sum of channels:	1491563	1.14	0.46	0.54	1.36E+04	6.42E+03	0.5	1.34E-02	7.23E-10	7.23E-07	4.59E-04	-3.314635	-0.616186	0.481807	0.54	
Lower Size Limit:	0.1	60%	1.2	0.54	0.63	7.11E+04	0.585	6.11E-02	6.03E-09	6.03E-06	3.71E-03	-2.677189	-0.462035	0.634747	0.63	
Upper Size Limit:	199.6	65%	1.28	0.63	0.74	1.70E+05	0.685	1.75E-01	2.32E-08	2.32E-05	1.62E-02	-2.139232	-0.301105	0.801016	0.74	
Mean Size:	1.1	70%	1.35	0.74	0.86	1.67E+05	0.8	2.87E-01	3.63E-08	3.63E-05	3.58E-02	-1.801827	-0.150823	0.926859	0.86	
Standard Deviation:	1.48	75%	1.45	0.86	1	2.00E+05	0.93	4.21E-01	6.83E-08	6.83E-05	7.26E-02	-1.456460	0.000000	1.076172	1	
D(4:3):	2.09	80%	1.55	1	1.2	2.62E+05	1.1	5.97E-01	1.48E-07	1.48E-04	1.52E-01	-1.026644	0.182322	1.296019	1.2	
D(3:2):	1.7	85%	1.67	1.2	1.4	1.94E+05	1.3	7.27E-01	1.81E-07	1.81E-04	2.50E-01	-0.674739	0.336472	1.509062	1.4	
Spec surf area (m <sup>2</sup> /g):	4.35	90%	1.86	1.4	1.6	1.41E+05	1.5	8.22E-01	2.02E-07	2.02E-04	3.59E-01	-0.360968	0.470004	1.728386	1.6	
Mode (Linear scale):	0.7	95%	2.16	1.6	1.8	9.68E+04	1.7	8.87E-01	2.02E-07	2.02E-04	4.68E-01	-0.080736	0.587787	1.951079	1.8	
Mode Lower Bound:	0.66		1.8	2.2	1.02E+05	1.32E+06	2	9.55E-01	3.46E-07	3.46E-04	6.54E-01	0.396853	0.788457	2.398708	2.2	
Mode Upper Bound:	0.72		2.2	2.5	3.35E+04	1.42E+06	2.35	9.77E-01	1.84E-07	1.84E-04	7.54E-01	0.686057	0.916291	2.718294	2.5	
Lower combine size:	4.37		2.9	3.4	8.50E+03	1.48E+06	2.7	9.91E-01	1.63E-07	1.63E-04	8.43E-01	1.005621	1.064711	3.121176	2.9	
Upper combine size:	4.43		2.9	3.4	8.50E+03	1.48E+06	3.15	9.96E-01	1.13E-07	1.13E-04	9.03E-01	1.301441	1.223775	3.547154	3.4	
Dispenser			3.4	4	3.32E+03	1.49E+06	3.7	9.99E-01	7.12E-08	7.12E-05	9.42E-01	1.570779	1.386294	3.985364	4	
Flow Inc. : 0.0			4	4.6	1.08E+03	1.49E+06	4.3	9.99E-01	3.64E-08	3.64E-05	9.62E-01	1.768831	1.526056	4.341769	4.6	
Pulse : 0.0			4.6	5.4	5.38E+02	1.49E+06	5	1.00E+00	2.85E-08	2.85E-05	9.77E-01	1.993803	1.686399	4.785439	5.4	
Pulse Inc. : 0.0			5.4	6.3	2.38E+02	1.49E+06	5.85	1.00E+00	2.02E-08	2.02E-05	9.88E-01	2.251109	1.840550	5.348718	6.3	
Nebulizer : 0.0			6.3	7.4	1.33E+02	1.49E+06	6.85	1.00E+00	1.82E-08	1.82E-05	9.98E-01	2.821398	2.001480	6.844842	7.4	
Neb. Inc. : 0.0			7.4	8.6	1.61E+01	1.49E+06	8	1.00E+00	3.49E-09	3.49E-06	9.99E-01	3.284658	2.151762	8.363239	8.6	
Low Limit : 40			8.6	10	2.77E+00	1.49E+06	9.3	1.00E+00	9.46E-10	9.46E-07	1.00E+00					
High Limit: 80			12	14	0	1.49E+06	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Baseline Offset			14	16	0	1.49E+06	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Noise Filter (Sigmas)			16	18	0	1.49E+06	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nozzle Type			18	22	0	1.49E+06	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):			22	25	0	1.49E+06	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
to:			25	29	0	1.49E+06	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):			29	34	0	1.49E+06	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (#/m <sup>3</sup> ):			34	40	0	1.49E+06	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:			40	46	0	1.49E+06	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			46	54	0	1.49E+06	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			54	63	0	1.49E+06	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			63	74	0	1.49E+06	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			74	86	0	1.49E+06	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			86	100	0	1.49E+06	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			100	120	0	1.49E+06	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			120	140	0	1.49E+06	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			140	160	0	1.49E+06	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			160	180	0	1.49E+06	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			180	220	0	1.49E+06	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			220		0	1.49E+06	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
					1491800.314				1.85E-06	1.85E-03						

Test 13 (Cont.) Mineral Oil/PCM Slurry, 805 rpm, .015", 3 ml/s

Generation Rate Calculations									
	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec	
Filter L	197.325	198.719	1.393	2.800	200.000	315.932	0.007	0.262	
Filter R	199.316	201.124	1.807	2.750	200.000	417.278	0.009	0.346	
Blank	149.84	149.841	0.001						

Actual Machining Conditions	
Flow	3.030
Depth	0.012
RPM	805.000
Base Amp	7.600
Cut Amp	8.750
Diameter	7.620
M# =	0.052
Amp =	1.150
Mact =	0.052

Test 14 Mineral Oil/PCM Slurry, 1270 rpm, 02", 2.5 ml/s

	Run 27	% Under	Run 27	Lower SIZE	Upper SIZE	Run 27	# In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
Mineral Oil/PCM	5%	0.63	0.1	0.12	0	0	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-2.120264		FB	1.435
Taken on:	1/19/99	15:08	0.68	0.12	0.14	0	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.966113		F2	0.988
AERODYNAMIC No. DISTRIB	15%	0.72	0.14	0.16	0	0	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.832581			
Material:	20%	0.77	0.16	0.18	0	0	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.714798			
Density (g/cc):	0.81	25%	0.82	0.18	0.22	0	0	0	0.2	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.514128			
Correlating Factor:	1	30%	0.87	0.22	0.25	0	0	0	0.235	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.386294			
Run Length (sec):	119.34	35%	0.92	0.25	0.29	0	0	0	0.27	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.237874			
PMT Voltage (Volts):	1150	40%	0.97	0.29	0.34	0	0	0	0.315	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.078810		0.34	
Laser Current (mA):	41.58	45%	1.02	0.34	0.4	0	0	0	0.37	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-0.916291		0.4	
Clock Freq (MHz):	40	50%	1.07	0.4	0.46	0	0	0	0.43	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-0.776529		0.46	
Sum of channels:	4127131	55%	1.13	0.46	0.54	1.58E+04	0	0	0.5	3.84E-03	8.39E-10	8.39E-07	1.83E-04	-3.564055	0.474544	0.54	
Lower Size Limit:	0.1	60%	1.19	0.54	0.63	1.77E+05	1.58E+04	0.585	0.585	4.68E-02	1.50E-08	1.50E-05	3.45E-03	-2.701199	0.648257	0.63	
Upper Size Limit:	199.6	65%	1.25	0.63	0.74	5.02E+05	1.93E+05	0.685	0.685	1.68E-01	6.83E-08	6.83E-05	1.83E-02	-2.089691	0.808639	0.74	
Mean Size:	1.1	70%	1.33	0.74	0.86	4.91E+05	6.95E+05	0.8	0.8	2.87E-01	1.06E-07	1.06E-04	4.13E-02	-1.733579	0.919740	0.86	
Standard Deviation:	1.45	75%	1.41	0.86	1	5.87E+05	1.19E+06	0.93	0.93	4.29E-01	2.00E-07	2.00E-04	8.50E-02	-1.371995	1.048177	1	
DX(3):	1.86	80%	1.51	1	1.2	7.49E+05	1.77E+06	1.1	1.1	6.11E-01	4.23E-07	4.23E-04	1.77E-01	-0.976912	1.231157	1.2	
DX(4):	1.6	85%	1.64	1.2	1.4	5.47E+05	2.52E+06	1.3	1.3	7.43E-01	5.09E-07	5.09E-04	2.88E-01	-0.560021	1.405775	1.4	
Spec surf area (m <sup>2</sup> /g):	4.63	90%	1.81	1.4	1.6	3.79E+05	3.07E+06	1.5	1.5	8.33E-01	5.43E-07	5.43E-04	4.06E-01	-0.238257	1.579187	1.6	
Mode (Linear scale):	0.7	95%	2.09	1.6	1.8	2.58E+05	3.45E+06	1.7	1.7	8.98E-01	5.38E-07	5.38E-04	5.23E-01	0.057303	1.757264	1.8	
Mode Lower Bound:	0.67		1.8	2.2	2.5	2.63E+05	3.71E+06	2	2	9.61E-01	8.91E-07	8.91E-04	7.17E-01	0.573052	2.117438	2.2	
Mode Upper Bound:	0.74		2.2	2.5	2.5	8.36E+04	3.97E+06	2.35	2.35	9.82E-01	4.60E-07	4.60E-04	8.17E-01	0.902985	2.385672	2.5	
Lower combine size:	7.59		2.5	2.9	3.4	4.73E+04	4.05E+06	2.7	2.7	9.93E-01	3.95E-07	3.95E-04	9.62E-01	1.296762	2.750645	2.9	
Upper combine size:	7.7		2.9	3.4	2.07E+04	4.10E+06	3.15	3.15	9.98E-01	2.74E-07	2.74E-04	9.62E-01	1.776875	1.223775	3.271999	3.4	
Dispenser			3.4	4	4	6.96E+03	4.12E+06	3.7	3.7	1.00E+00	1.49E-07	1.49E-04	9.95E-01	2.557790	4.339287	4	
Pulse-let			4	4.6	4.6	7.18E+02	4.13E+06	4.3	4.3	1.00E+00	2.42E-08	2.42E-05	1.00E+00				
Flow Inc. : 0.0			4.6	5.4	5.4	0	4.13E+06	5	5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Flow Inc. : 0.0			5.4	6.3	6.3	0	4.13E+06	5.85	5.85	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Pulse : 0.0			6.3	7.4	7.4	0	4.13E+06	6.85	6.85	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Pulse Inc.: 0.0			7.4	8.6	8.6	0	4.13E+06	8	8	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Nebulizer : 0.0			8.6	10	10	0	4.13E+06	9.3	9.3	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Neb. Inc. : 0.0			10	12	12	0	4.13E+06	11	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Low Limit : 8000			12	14	14	0	4.13E+06	13	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
High Limit: 8000			14	16	16	0	4.13E+06	15	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Baseline Offset			16	18	18	0	4.13E+06	17	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Noise Filter (Sigmas)			18	22	22	0	4.13E+06	20	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Nozzle Type			22	25	25	0	4.13E+06	23.5	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Flow Rate Range (l/min):			25	29	29	0	4.13E+06	27	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
to:			29	34	34	0	4.13E+06	31.5	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Mass Loading (mg/m <sup>3</sup> ):			34	40	40	0	4.13E+06	37	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Concentration (#/m <sup>3</sup> ):			40	46	46	0	4.13E+06	43	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Counting efficiency:			46	54	54	0	4.13E+06	50	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			54	63	63	0	4.13E+06	58.5	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			63	74	74	0	4.13E+06	68.5	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			74	86	86	0	4.13E+06	80	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			86	100	100	0	4.13E+06	93	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			100	120	120	0	4.13E+06	110	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			120	140	140	0	4.13E+06	130	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			140	160	160	0	4.13E+06	150	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			160	180	180	0	4.13E+06	170	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			180	220	220	0	4.13E+06	200	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
							4127070.2				4.60E-06	4.60E-03					

**Test 14 (Cont.) Mineral Oil/PCM Slurry, 1270 rpm, .02", 2.5 ml/s**

**Generation Rate Calculations**

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	213.311	218.109	4.797	2.800	129.000	1686.756	0.037	2.171
Filter R	232.399	238.002	5.602	2.700	129.000	2042.772	0.043	2.629
Blank	149.84	149.841	0.001					

**Actual Machining Conditions**

Flow	2.340	M# =	0.176
Depth	0.020		
RPM	1270.000		
Base Amp	7.750	Amp =	3.000
Cut Amp	10.750		
Diameter	7.550	Mact =	0.174

Test 15 Mineral Oil/PCM Slurry, 500 rpm, 005", 3 ml/s

	Run, 29	% Under	Run, 29	Lower	Upper	Run, 29	# In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
	Min. Oil/PCM	5%	Run, 29	SIZE	SIZE	Run, 29	# In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
Taken on:	1/19/99 15:54	10%	0.55	0.1	0.12	0	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264		lg 1.678
AERODYNAMIC No. DISTRIB		15%	0.64	0.12	0.14	0	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.9666113		r2 0.980
Material:		20%	0.69	0.14	0.16	0	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.832581		
Density (g/cc):	0.81	25%	0.74	0.16	0.18	0.917	0	0	0.17	1.90E-05	1.91E-15	1.91E-12	3.54E-07		-1.714798		
Correlating Factor:	1	30%	0.8	0.18	0.22	1.01E+01	0.917	0	0.2	2.28E-04	3.42E-14	3.42E-11	3.54E-07		-1.514128		
Run Length (sec):	301.17	35%	0.86	0.22	0.25	4.87E+01	1.10E+01	0.235	0.235	1.24E-03	2.68E-13	2.68E-10	2.98E-06		-1.386294		
PMT Voltage (Volts):	1150	40%	0.92	0.25	0.29	1.65E+02	5.97E+01	0.27	0.27	4.67E-03	1.38E-12	1.38E-09	1.65E-05		-1.237874		
Laser Current (mA):	41.58	45%	0.99	0.29	0.34	5.38E+02	2.25E+02	0.315	0.315	1.58E-02	7.13E-12	7.13E-09	8.64E-05		-1.078810	0.367330	0.34
Clock Freq (MHz):	40	50%	1.05	0.34	0.4	6.80E+02	7.63E+02	0.37	0.37	3.00E-02	1.46E-11	1.46E-08	2.30E-04		-0.916291	0.418501	0.4
Sum of channels:	48167	55%	1.13	0.4	0.46	2.93E+02	1.44E+03	0.43	0.43	3.61E-02	9.87E-12	9.87E-09	3.26E-04		-0.776529	0.439559	0.46
Lower Size Limit:	0.1	60%	1.2	0.46	0.54	4.99E+02	1.74E+03	0.5	0.5	4.64E-02	2.64E-11	2.64E-08	5.85E-04		-0.616186	0.478165	0.54
Upper Size Limit:	199.6	65%	1.29	0.54	0.63	2.26E+03	2.24E+03	0.585	0.585	9.32E-02	1.91E-10	1.91E-07	2.46E-03		-0.462035	0.598519	0.63
Mean Size:	1.14	70%	1.38	0.63	0.74	5.19E+03	4.49E+03	0.685	0.685	2.01E-01	7.07E-10	7.07E-07	9.39E-03		-0.301105	0.760277	0.74
Standard Deviation:	1.67	75%	1.62	0.86	1	5.34E+03	1.44E+04	0.93	0.93	3.00E-01	1.03E-09	1.03E-06	1.95E-02		-2.064207	0.881343	0.86
D(4,3):	2.88	80%	1.77	1	1.2	6.68E+03	1.98E+04	1.1	1.1	5.49E-01	3.77E-09	3.77E-06	7.43E-02		-1.444537	1.214507	1.2
Spec surf area (m <sup>2</sup> /g):	3.35	85%	1.97	1.2	1.4	5.29E+03	2.65E+04	1.3	1.3	6.59E-01	4.92E-09	4.92E-06	1.23E-01		-1.162325	1.403464	1.4
Mode (Linear scale):	0.68	90%	2.24	1.4	1.6	4.05E+03	3.17E+04	1.5	1.5	7.43E-01	5.80E-09	5.80E-06	1.79E-01		-0.917832	0.470004	1.6
Mode Lower Bound:	0.65	95%	2.67	1.6	1.8	3.09E+03	3.58E+04	1.7	1.7	8.07E-01	6.44E-09	6.44E-06	2.42E-01		-0.698461	0.587787	1.8
Mode Upper Bound:	0.72		1.8	2.2	4.17E+03	3.89E+04	2	2	8.94E-01	1.41E-08	1.41E-05	3.81E-01		-0.303013	0.788457	2.192444	2.2
Lower combine size:	4.43		2.2	2.5	1.90E+03	4.31E+04	2.35	2.35	9.33E-01	1.05E-08	1.05E-05	4.83E-01		-0.041665	0.916291	2.509919	2.5
Upper combine size:	4.5		2.9	3.4	9.36E+02	4.65E+04	3.15	3.15	9.84E-01	1.24E-08	1.24E-05	7.29E-01		0.273062	1.064711	2.953838	2.9
Dispenser			3.4	4	4.49E+02	4.74E+04	3.7	3.7	9.94E-01	9.64E-09	9.64E-06	8.24E-01		0.610098	1.223775	3.516632	3.4
Flow Inc. : 0.0			4	4.6	1.48E+02	4.79E+04	4.3	4.3	9.97E-01	5.00E-09	5.00E-06	8.73E-01		0.928969	1.386294	4.147488	4
Pulse : 0.0			4.6	5.4	9.73E+01	4.80E+04	5	5	9.99E-01	5.15E-09	5.15E-06	9.23E-01		1.138606	1.526056	4.622698	4.6
Pulse Inc. : 0.0			5.4	6.3	3.67E+01	4.81E+04	5.85	5.85	9.99E-01	3.11E-09	3.11E-06	9.54E-01		1.426029	1.686399	5.563973	5.4
Nebulizer : 0.0			6.3	7.4	2.70E+01	4.81E+04	6.85	6.85	1.00E+00	3.68E-09	3.68E-06	9.90E-01		1.680382	1.840550	6.119150	6.3
Netb. Inc. : 0.0			7.4	8.6	0	4.82E+04	8	8	1.00E+00	0.00E+00	0.00E+00	9.90E-01		2.313755	2.001480	8.491422	7.4
Low Limit : 40			8.6	10	0	4.82E+04	9.3	9.3	1.00E+00	0.00E+00	0.00E+00	9.90E-01		2.313755	2.151762	8.491422	8.6
High Limit: 80			10	12	1.87E+00	4.82E+04	11	11	1.00E+00	1.06E-09	1.06E-06	1.00E+00		2.313755	2.302585	8.491422	10
Baseline Offset			12	14	0	4.82E+04	13	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Noise Filter (Sigmas)			14	16	0	4.82E+04	15	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nozzle Type			16	18	0	4.82E+04	17	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):			18	22	0	4.82E+04	20	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
to:			22	25	0	4.82E+04	23.5	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):			25	29	0	4.82E+04	27	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (#/m <sup>3</sup> ):			29	34	0	4.82E+04	31.5	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:			34	40	0	4.82E+04	37	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			40	46	0	4.82E+04	43	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			46	54	0	4.82E+04	50	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			54	63	0	4.82E+04	58.5	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			63	74	0	4.82E+04	68.5	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			74	86	0	4.82E+04	80	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			86	100	0	4.82E+04	93	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			100	120	0	4.82E+04	110	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			120	140	0	4.82E+04	130	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			140	160	0	4.82E+04	150	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			160	180	0	4.82E+04	170	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			180	220	0	4.82E+04	200	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			481.66.027														

Test 15 (Cont.) Mineral Oil/PCM Slurry, 500 rpm, .005", 3 ml/s

Generation Rate Calculations										
	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec		
Filter L	212.684	212.741	0.056	2.750	311.000	8.316	0.000	0.004		
Filter R	228.668	228.784	0.115	2.750	311.000	17.078	0.000	0.009		
Blank	149.84	149.841	0.001							

Actual Machining Conditions	
Flow	2.770
Depth	0.005
RPM	500.000
Base Amp	7.500
Cut Amp	7.800
Diameter	7.450
	M# = 0.015
	Amp = 0.300
	Mact = 0.014

Test 16 Mineral Oil/PCM Slurry, 1270 rpm, 0.14", 2.5 ml/s

	Run 31	Run 31	Upper	Run 31	Run 31	Upper	Run 31	Run 31	# Under	Mid-Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
	Min. Oil/PCM	% Under	SIZE	Lower	Upper	SIZE	# In	# Under	Mid-Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit	
Taken on:	1/19/99 16:46	5%	0.1	0.12	0	0	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264		rg	
AERODYNAMIC No.DISTRIB.		10%	0.66	0.12	0.14	0	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.9661113		r2	
Material:	F-7211	15%	0.71	0.14	0.16	0	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.832581			
Density (g/cc):	0.81	20%	0.75	0.16	0.18	0	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.714798			
Correlating Factor:	1	25%	0.79	0.18	0.22	0	0	0	0.23	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.514128			
Run Length (sec):	115.13	30%	0.84	0.22	0.25	0	0	0	0.27	1.00E-06	2.41E-14	2.41E-11	8.21E-09		-1.237874			
PMT Voltage (Volts):	41.58	40%	0.94	0.29	0.34	1.42E+03	2.89E+00	0	0.315	5.19E-04	1.88E-11	1.88E-08	6.41E-06	-4.358590	-1.078810	0.345564	0.34	
Laser Current (mA):	40	50%	1.04	0.4	0.46	4.28E+03	7.48E+02	1.42E+03	0.37	2.08E-03	9.18E-11	9.18E-08	3.77E-05	-3.958121	-0.916291	0.400697	0.4	
Clock Freq (MHz):	2736280	60%	1.16	0.54	0.63	2.10E+04	6.45E+03	5.70E+03	0.43	2.36E-03	2.52E-11	2.52E-08	4.63E-05	-3.909227	-0.776529	0.408005	0.46	
Lower Size Limit:	0.1	70%	1.22	0.63	0.74	3.43E+05	1.69E+05	2.74E+04	0.585	1.00E-02	1.11E-09	1.11E-06	4.24E-04	-3.316463	-0.616186	0.504210	0.54	
Upper Size Limit:	1.07	80%	1.38	0.86	1	3.88E+05	5.27E+05	8.70E+05	0.8	3.18E-01	7.43E-08	7.43E-05	4.65E-02	-1.680141	-0.150823	0.930042	0.86	
Standard Deviation:	1.46	85%	1.49	1	1.2	4.77E+05	1.26E+06	1.1	1.1	6.34E-01	2.69E-07	2.69E-04	1.83E-01	-0.903372	0.182322	1.239363	1.2	
D(4.3):	1.72	90%	1.62	1.2	1.4	3.43E+05	1.73E+06	1.3	1.3	7.59E-01	3.19E-07	3.19E-04	2.92E-01	-0.547799	0.336472	1.413445	1.4	
D(3.2):	4.31	95%	2.08	1.6	1.8	1.59E+05	2.31E+06	1.7	1.7	8.44E-01	3.33E-07	3.33E-04	4.05E-01	-0.239224	0.470004	1.584217	1.6	
Spec surf area (m <sup>2</sup> /g):	0.66		1.8	2.2	2.2	1.63E+05	2.47E+06	2	2	9.62E-01	5.53E-07	5.53E-04	7.06E-01	0.542744	0.788457	2.115169	2.2	
Mode Lower Bound:	0.74		2.2	2.5	2.9	5.39E+04	2.63E+06	2.35	2.35	9.82E-01	2.96E-07	2.96E-04	8.07E-01	0.867983	0.916291	2.385371	2.5	
Mode Upper Bound:	7.36		2.9	3.4	3.4	1.34E+04	2.72E+06	3.15	3.15	9.98E-01	2.66E-07	2.66E-04	8.98E-01	1.269559	1.064711	2.767078	2.9	
Upper combine size:	7.47		3.4	4	4	4.58E+03	2.73E+06	3.7	3.7	1.00E+00	9.84E-08	9.84E-05	9.92E-01	1.731928	1.223775	3.282812	3.4	
Dispenser			4	4.6	7.08E+02	2.74E+06	4.3	4.3	4.3	1.00E+00	2.39E-08	2.39E-05	1.00E+00	2.403049	1.386294	4.207089	4	
Flow Inc. : 0.0			4.6	5.4	0	2.74E+06	5	5	5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nozzle Type			5.4	6.3	0	2.74E+06	5.85	5.85	5.85	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):			6.3	7.4	0	2.74E+06	6.85	6.85	6.85	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
lo:			7.4	8.6	0	2.74E+06	8	8	8	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):			8.6	10	0	2.74E+06	9.3	9.3	9.3	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (#/m <sup>3</sup> ):			10	12	0	2.74E+06	11	11	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:			12	14	0	2.74E+06	13	13	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			14	16	0	2.74E+06	15	15	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			16	18	0	2.74E+06	17	17	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			18	22	0	2.74E+06	20	20	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			22	25	0	2.74E+06	23.5	23.5	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			25	29	0	2.74E+06	27	27	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			29	34	0	2.74E+06	31.5	31.5	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			34	40	0	2.74E+06	37	37	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			40	46	0	2.74E+06	43	43	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			46	54	0	2.74E+06	50	50	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			54	63	0	2.74E+06	58.5	58.5	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			63	74	0	2.74E+06	68.5	68.5	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			74	86	0.00E+00	2.74E+06	80	80	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			86	100	0	2.74E+06	93	93	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			100	120	0	2.74E+06	110	110	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			120	140	0	2.74E+06	130	130	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			140	160	0	2.74E+06	150	150	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			160	180	0	2.74E+06	170	170	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			180	220	0	2.74E+06	200	200	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
							2736225.19					2.94E-06	2.94E-03					

Test 16 (Cont.) Mineral Oil/PCM Slurry, 1270 rpm, .014", 2.5 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	215.771	218.14	2.368	2.700	125.000	891.124	0.019	1.183
Filter R	210.255	213.279	3.023	2.700	125.000	1137.613	0.024	1.511
Blank	149.84	149.841	0.001					

Actual Machining Conditions

Flow	2.650	M# =	0.109
Depth	0.014		
RPM	1270.000		
Base Amp	7.750	Amp =	2.250
Cut Amp	10.000		
Diameter	7.425	Mact =	0.106

Test 17 Mineral Oil/PCM Slurry, 500 rpm, .005", 3 ml/s

	Run 33	% Under	Run 33	Upper SIZE	Run 33	# In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	In(high)	predicted log normal	Best log-normal fit
Min. Oil/PCM	5%	0.39	0.12	0	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264		dg
1/1999 17:28	10%	0.59	0.12	0	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.966113		rg
AERODYNAMIC No. DISTRIB.	15%	0.65	0.14	0.16	2.69E+00	0	0.15	8.39E-05	3.84E-12	5.54E-08	5.54E-08	5.54E-08		-1.832581		r2
F-7211	20%	0.69	0.16	0.18	5.72E+00	2.69E+00	0.17	2.62E-04	1.19E-14	1.19E-11	2.27E-07	2.27E-07		-1.714798		
Density (g/cc):	25%	0.74	0.22	0.25	1.44E+01	8.40E+00	0.2	7.11E-04	4.88E-14	4.88E-11	9.29E-07	9.29E-07		-1.514128		
Correlating Factor:	30%	0.79	0.22	0.25	4.53E+01	2.28E+01	0.235	2.13E-03	2.49E-13	2.49E-10	4.52E-06	4.52E-06		-1.386294		
Run Length (sec):	35%	0.85	0.25	0.29	2.08E+02	6.81E+01	0.27	8.62E-03	1.74E-12	1.74E-09	2.95E-05	2.95E-05		-1.237874		
PMT Voltage (Volts):	40%	0.91	0.29	0.34	6.36E+02	2.76E+02	0.315	2.85E-02	8.42E-12	8.42E-09	1.51E-04	1.51E-04	-3.613532	-1.078810	0.351596	0.34
Laser Current (mA):	45%	0.97	0.34	0.4	8.11E+02	9.12E+02	0.37	5.38E-02	1.74E-11	1.74E-08	4.02E-04	4.02E-04	-3.351597	-0.916291	0.410055	0.4
Clock Freq (MHz):	50%	1.03	0.4	0.46	3.50E+02	1.72E+03	0.43	6.47E-02	1.18E-11	1.18E-08	5.72E-04	5.72E-04	-3.252644	-0.776529	0.434588	0.46
Sum of channels:	55%	1.11	0.46	0.54	4.20E+02	2.07E+03	0.5	7.78E-02	2.22E-11	2.22E-07	8.92E-04	8.92E-04	-3.124005	-0.616186	0.468687	0.54
Lower Size Limit:	60%	1.18	0.54	0.63	1.74E+03	2.49E+03	0.585	1.32E-01	1.48E-10	1.48E-07	3.02E-03	3.02E-03	-2.745437	-0.462035	0.585365	0.63
Upper Size Limit:	65%	1.27	0.63	0.74	3.77E+03	4.24E+03	0.685	2.50E-01	5.13E-10	5.13E-07	1.04E-02	1.04E-02	-2.310953	-0.301105	0.755491	0.74
Mean Size:	70%	1.37	0.74	0.86	3.47E+03	8.00E+03	0.8	3.58E-01	1.26E-09	1.26E-06	3.94E-02	3.94E-02	-1.757144	0.000000	1.045834	1
Standard Deviation:	75%	1.49	0.86	1	3.70E+03	1.15E+04	0.93	4.74E-01	2.47E-09	2.47E-06	7.50E-02	7.50E-02	-1.439780	0.182322	1.260078	1.2
DX(3):	80%	1.65	1	1.2	4.37E+03	1.52E+04	1.1	6.10E-01	3.08E-09	3.08E-06	1.19E-01	1.19E-01	-1.178673	0.316472	1.468877	1.4
DX(2):	85%	1.86	1.2	1.4	3.30E+03	1.95E+04	1.3	7.13E-01	3.27E-09	3.27E-06	1.66E-01	1.66E-01	-0.988582	0.470004	1.661740	1.6
Spec surf area (m <sup>2</sup> /g):	90%	2.15	1.4	1.6	2.29E+03	2.29E+04	1.5	7.85E-01	3.52E-09	3.52E-06	2.17E-01	2.17E-01	-0.782302	0.587787	1.853825	1.8
Mode (Linear scale):	95%	2.68	1.6	1.8	1.69E+03	2.51E+04	1.7	8.37E-01	7.55E-09	7.55E-06	3.26E-01	3.26E-01	-0.451510	0.788457	2.251272	2.2
Mode Lower Bound:		0.65	1.8	2.2	2.23E+03	2.68E+04	2	9.07E-01	5.51E-09	5.51E-06	4.05E-01	4.05E-01	-0.240066	0.916291	2.548888	2.5
Mode Upper Bound:		0.75	2.2	2.5	1.00E+03	2.91E+04	2.35	9.38E-01	6.62E-09	6.62E-06	5.00E-01	5.00E-01	0.001226	1.064711	2.936874	2.9
Lower combine size:		4.37	2.5	2.9	7.93E+02	3.01E+04	2.7	9.63E-01	6.98E-09	6.98E-06	6.01E-01	6.01E-01	0.255980	1.223775	3.410775	3.4
Upper combine size:		4.43	2.9	3.4	5.27E+02	3.08E+04	3.15	9.79E-01	7.00E-09	7.00E-06	7.02E-01	7.02E-01	0.529644	1.386294	4.005379	4
Dispenser			3.4	4	3.26E+02	3.14E+04	3.7	9.90E-01	4.85E-09	4.85E-06	7.72E-01	7.72E-01	0.744569	1.526056	4.544165	4.6
Flow Inc. : 0.0			4	4.6	1.44E+02	3.17E+04	4.3	9.94E-01	6.07E-09	6.07E-06	8.59E-01	8.59E-01	1.076771	1.686399	5.522973	5.4
Pulse Inc. : 0.0			5.4	6.3	4.70E+01	3.20E+04	5.85	9.99E-01	3.99E-06	3.99E-06	9.17E-01	9.17E-01	1.383319	1.840550	6.612249	6.3
Pulse Inc. : 0.0			6.3	7.4	2.75E+01	3.20E+04	6.85	1.00E+00	3.75E-09	3.75E-06	9.71E-01	9.71E-01	1.891349	2.001480	8.910620	7.4
Nebulizer : 0.0			7.4	8.6	0	3.20E+04	8	1.00E+00	0.00E+00	0.00E+00	9.71E-01	9.71E-01	1.891349	2.151762	8.910620	8.6
Neb. Inc. : 0.0			8.6	10	0	3.20E+04	9.3	1.00E+00	0.00E+00	0.00E+00	9.71E-01	9.71E-01	1.891349	2.302585	8.910620	10
Low Limit : 40			10	12	0	3.20E+04	11	1.00E+00	0.00E+00	0.00E+00	9.71E-01	9.71E-01	1.891349	2.484907	8.910620	12
High Limit : 80			12	14	1.92E+00	3.20E+04	13	1.00E+00	1.79E-09	1.79E-06	9.96E-01	9.96E-01	2.696506	2.639057	14.296859	14
Base line Offset			14	16	0.17	3.20E+04	15	1.00E+00	2.43E-10	2.43E-07	1.00E+00	1.00E+00				
Noise Filter (Sigmas)			16	18	0	3.20E+04	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
Nozzle Type			18	22	0	3.20E+04	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
Flow Rate Range (l/min):			22	25	0	3.20E+04	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
to:			25	29	0	3.20E+04	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
Mass Loading (mg/m <sup>3</sup> ):			29	34	0	3.20E+04	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
Concentration (#/m <sup>3</sup> ):			34	40	0	3.20E+04	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
Counting efficiency:			40	46	0	3.20E+04	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			46	54	0	3.20E+04	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			54	63	0	3.20E+04	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			63	74	0	3.20E+04	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			74	86	0	3.20E+04	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			86	100	0	3.20E+04	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			100	120	0	3.20E+04	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			120	140	0	3.20E+04	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			140	160	0	3.20E+04	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			160	180	0	3.20E+04	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			180	220	0	3.20E+04	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00	1.00E+00				
			220			3.20E+04	200	1.00E+00	6.94E-08	6.94E-08	6.94E-05	6.94E-05				
							32029.005									

Test 17 (Cont.) Mineral Oil/PCM Slurry, 500 rpm, .005", 3 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	208.589	208.661	0.071	2.700	304.000	10.986	0.000	0.006
Filter R	204.103	204.19	0.086	2.700	304.000	13.307	0.000	0.007
Blank	149.84	149.841	0.001					

Actual Machining Conditions

Flow	3.050	M# =	0.013
Depth	0.005		
RPM	500.000		
Base Amp	7.500	Amp =	0.400
Cut Amp	7.900		
Diameter	7.355	Mact =	0.013

Test 18 Mineral Oil/PCM Slurry, 1270 rpm, .02", 2.5 ml/s

	Run 37	% Under	Run 37	Lower	Upper	Run 37	# In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	actual	Best log-normal fit
	Min. OUP/PCM	5%	SIZE	SIZE	SIZE	Run 37	Run 37	Run 37	Run 37	Run 37	Run 37	Run 37	Run 37	Run 37	Run 37	Run 37	Run 37	Run 37
Taken on:	1/19/99 18:13	10%	0.12	0.14	0	0	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-2.120264				1.911
AERODYNAMIC No. DISTRIB.		15%	0.14	0.16	0	0	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.966113				1.440
Material:	F-7211	20%	0.16	0.18	0	0	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.832381				0.951
Density (g/cc)	0.81	25%	0.18	0.22	0	0	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.714798				
Correlating Factor:	1	30%	0.22	0.25	0	0	0	0	0.235	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.386794				
Run Length (sec)	119.65	35%	0.25	0.29	0	0	0	0	0.27	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.237874				
PMT Voltage (Volts)	1150	40%	0.29	0.34	0	0	0	0	0.315	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-1.078810				0.34
Laser Current (mA)	41.11	45%	0.34	0.4	0	0	0	0	0.37	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-0.916291				0.4
Clock Freq (MHz)	40	50%	0.4	0.46	0	0	0	0	0.43	0.00E+00	0.00E+00	0.00E+00	0.00E+00	-0.776529				0.46
Sum of channels:	4263005	55%	0.54	0.63	0	0	0	0	0.5	2.09E+03	4.71E+10	4.71E+07	7.16E-05	-3.799796	0.478726			0.54
Lower Size Limit:	0.1	60%	0.63	0.74	0.63	1.49E+05	8.90E+03	0	0.585	3.71E-02	1.77E-08	1.77E-05	2.02E-03	-2.874804	0.670562			0.63
Upper Size Limit:	199.6	65%	0.74	0.86	0.74	4.66E+05	1.58E+05	0.685	0.685	1.46E-01	6.33E-08	6.33E-05	1.18E-02	-2.263387	0.837811			0.74
Mean Size:	1.17	70%	0.86	1	0.86	4.68E+05	6.24E+05	0.8	0.8	2.56E-01	1.02E-07	1.02E-04	2.75E-02	-1.919625	0.946659			0.86
Standard Deviation:	1.5	75%	1	1.2	1	5.57E+05	1.09E+06	0.93	0.93	3.87E-01	1.90E-07	1.90E-04	5.67E-02	-1.583221	1.073479			1
DX(2):	2.15	80%	1.2	1.4	1.2	7.13E+05	1.63E+06	1.1	1.1	5.53E-01	4.04E-07	4.04E-04	1.19E-01	-1.180765	1.242997			1.2
DX(3):	1.82	85%	1.4	1.6	1.4	5.46E+05	2.36E+06	1.3	1.3	6.83E-01	5.08E-07	5.08E-04	1.97E-01	-0.832069	1.401123			1.4
Specific surface area (m <sup>2</sup> /g):	4.06	90%	1.6	1.8	1.6	3.96E+05	2.91E+06	1.5	1.5	7.76E-01	5.67E-07	5.67E-04	2.84E-01	-0.569739	1.552911			1.6
Mode (Linear scale):	0.7	95%	1.8	2.2	1.8	2.94E+05	3.31E+06	1.7	1.7	8.44E-01	6.12E-07	6.12E-04	3.79E-01	-0.309003	1.707655			1.8
Mode Lower Bound:	0.67		2.2	2.5	2.2	3.50E+05	3.60E+06	2	2	9.27E-01	1.19E-06	1.19E-03	5.62E-01	0.155052	2.021194			2.2
Mode Upper Bound:	0.74		2.5	2.9	2.5	1.41E+05	3.93E+06	2.35	2.35	9.60E-01	7.73E-07	7.73E-04	6.81E-01	0.469508	2.267651			2.5
Lower combine size:	7.36		3	3.4	3	9.63E+04	4.09E+06	2.7	2.7	9.87E-01	8.03E-07	8.03E-04	8.03E-01	0.838383	2.612787			2.9
Upper combine size:	7.47		3.4	4	3.4	5.03E+04	4.19E+06	3.15	3.15	9.94E-01	6.67E-07	6.67E-04	9.07E-01	1.324779	3.096886			3.4
Dispenser			4	4.6	4	2.07E+04	4.24E+06	3.7	3.7	9.99E-01	4.44E-07	4.44E-04	9.76E-01	1.974331	3.923460			4
Flow Inc.: 0.0			4.6	5.4	4.6	4.38E+03	4.26E+06	4.3	4.3	1.00E+00	1.48E-07	1.48E-04	9.99E-01	1.386794	5.670596			
Pulse Inc.: 0.0			5.4	6.3	5.4	1.74E+02	4.26E+06	5	5	1.00E+00	9.20E-09	9.20E-06	1.00E+00	2.985325				
Nebulizer: 0.0			6.3	7.4	6.3	0	4.26E+06	5.85	5.85	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Neb. Inc.: 0.0			7.4	8.6	7.4	0	4.26E+06	8	8	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Low Limit: 400			8.6	10	8.6	0	4.26E+06	9.3	9.3	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
High Limit: 800			10	12	10	0	4.26E+06	11	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Baseline Offset	0.1		12	14	12	0	4.26E+06	13	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Noise Filter (Sigmas)	6		14	16	14	0	4.26E+06	15	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nozzle Type	200um		16	18	16	0	4.26E+06	17	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):	1.49		18	22	18	0	4.26E+06	20	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):	1.53		22	25	22	0	4.26E+06	23.5	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):	3.15		25	29	25	0	4.26E+06	27	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (l/m <sup>3</sup> ):	1.42E+09		29	34	29	0	4.26E+06	31.5	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:	1		34	40	34	0	4.26E+06	37	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			40	46	40	0	4.26E+06	43	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			46	54	46	0	4.26E+06	50	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			54	63	54	0	4.26E+06	58.5	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			63	74	63	0	4.26E+06	68.5	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			74	86	74	0	4.26E+06	80	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			86	100	86	0	4.26E+06	93	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			100	120	100	0	4.26E+06	110	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			120	140	120	0	4.26E+06	130	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			140	160	140	0	4.26E+06	150	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			160	180	160	0	4.26E+06	170	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			180	220	180	0	4.26E+06	200	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			4263006.6								6.49E-06							

Test 18 (Cont.) Mineral Oil/PCM Slurry, 1270 rpm, .02", 2.5 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	216.114	221.845	5.730	2.700	130.000	2073.374	0.044	2.648
Filter R	245.1	252.059	6.958	2.650	130.000	2565.225	0.054	3.276
Blank	149.84	149.841	0.001					

Actual Machining Conditions

Flow	2.700
Depth	0.020
RPM	1270.000
Base Amp	7.900
Cut Amp	11.750
Diameter	7.330

M# = 0.152

Amp = 3.850

Mact = 0.147



Test 19 (Cont.) Mineral Oil/PCM Slurry, 805 rpm, .015", 3.0 ml/s

Generation Rate Calculations									
	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec	
Filter L	215.306	215.871	0.565	2.750	192.000	135.908	0.003	0.118	
Filter R	228.128	228.845	0.717	2.800	192.000	169.391	0.004	0.146	
Blank	149.834	149.834	0.000						

Actual Machining Conditions	
Flow	3.090
Depth	0.018
RPM	805.000
Base Amp	7.600
Cut Amp	8.750
Diameter	7.230
M# =	0.076
Amp =	1.150
Mact =	0.072



Test 20 (Cont.) Mineral Oil/PCM Slurry, 1270 rpm, .014", 2.5 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	247.991	251.361	3.370	2.750	126.000	1235.256	0.027	1.627
Filter R	222.952	226.492	3.540	2.850	126.000	1252.040	0.028	1.650
Blank	149.834	149.834	0.000					

Actual Machining Conditions

Flow	2.540
Depth	0.014
RPM	1270.000
Base Amp	7.750
Cut Amp	9.750
Diameter	7.140

M# = 0.113

Amp = 2.000

Mact = 0.106

Test 21 Mineral Oil/PCM Slurry, 1270 rpm, .014", 2.5 ml/s

	Run 43	% Under	Run 43	Upper	Run 43	# In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
	Min. Oil/PCM	5%	0.61	SIZE	0	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.170264		dg
Taken on:	1/20/99 16:25	10%	0.66	0.12	0	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.966113		rg
AERODYNAMIC No.DISTRIB.		15%	0.7	0.14	0	0	0	0.15	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.832581		r2
Material:	F-7211	20%	0.75	0.16	0	0	0	0.17	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.714798		
Density (g/cc):	0.81	25%	0.8	0.18	0	0	0	0.2	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.514128		
Correlating Factor:	1	30%	0.85	0.22	0	0	0	0.235	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.386294		
Run Length (sec):	117.43	35%	0.89	0.25	0	0	0	0.27	1.55E-04	2.84E-12	2.84E-09	1.20E-06		-1.237874		
PMT Voltage (Volts):	11.50	40%	0.94	0.29	0.34	2.04E+03	3.40E+02	0.315	1.09E-03	2.71E-11	2.71E-08	1.26E-05	-4.209578	-1.078810	0.342662	0.34
Laser Current (mA):	41.58	45%	1	0.34	0.4	4.27E+03	2.38E+03	0.37	3.04E-03	9.18E-11	9.18E-08	5.13E-05	-3.883615	-0.916291	0.389591	0.4
Clock Freq (MHz):	40	50%	1.05	0.4	0.46	1.32E+03	6.66E+03	0.43	3.64E-03	4.44E-11	4.44E-08	7.00E-05	-3.809109	-0.776529	0.401190	0.46
Sum of channels:	2190702	55%	1.11	0.46	0.54	2.01E+04	7.97E+03	0.5	1.28E-02	1.07E-09	1.07E-06	5.19E-04	-3.280293	-0.616186	0.494063	0.54
Upper Size Limit:	199.6	65%	1.23	0.63	0.74	2.80E+05	1.44E+05	0.685	1.94E-01	3.82E-08	3.82E-05	2.07E-02	-2.038560	-0.301105	0.805620	0.74
Lower Size Limit:	1.07	70%	1.31	0.74	0.86	2.66E+05	4.24E+05	0.8	3.15E-01	5.78E-08	5.78E-05	4.51E-02	-1.694220	-0.150823	0.922604	0.86
Standard Deviation:	1.47	75%	1.39	0.86	1	3.03E+05	6.90E+05	0.93	4.53E-01	1.04E-07	1.04E-04	8.90E-02	-1.346982	0.000000	1.057781	1
DX(4.3):	1.89	80%	1.49	1	1.2	3.82E+05	9.96E+05	1.1	6.29E-01	2.15E-07	2.15E-04	1.80E-01	-0.916150	0.182322	1.253349	1.2
DX(3.2):	1.6	85%	1.62	1.2	1.4	2.73E+05	1.38E+06	1.3	7.53E-01	2.56E-07	2.56E-04	2.88E-01	-0.559598	0.336472	1.442667	1.4
Spec surf area (m <sup>2</sup> /g):	4.62	90%	1.79	1.4	1.6	1.92E+05	1.65E+06	1.5	8.42E-01	2.74E-07	2.74E-04	4.03E-01	-0.244427	0.470004	1.632837	1.6
Mode (Linear scale):	0.66	95%	2.08	1.6	1.8	1.31E+05	1.85E+06	1.7	9.02E-01	2.72E-07	2.72E-04	5.18E-01	0.045745	0.587787	1.830479	1.8
Mode Lower Bound:	0.66			1.8	2.2	1.33E+05	1.98E+06	2	9.63E-01	4.52E-07	4.52E-04	7.09E-01	0.550119	0.788457	2.232633	2.2
Mode Upper Bound:	0.72			2.2	2.5	4.31E+04	2.11E+06	2.35	9.82E-01	2.37E-07	2.37E-04	8.09E-01	0.873138	0.916291	2.53457	2.5
Lower combine size:	2			2.5	2.9	2.44E+04	2.15E+06	2.7	9.93E-01	2.04E-07	2.04E-04	8.93E-01	1.251578	1.064711	2.942883	2.9
Upper combine size:	17.07			2.9	3.4	9.90E+03	2.18E+06	3.15	9.98E-01	1.31E-07	1.31E-04	9.50E-01	1.644012	1.223775	3.434651	3.4
Dispenser				3.4	4	3.54E+03	2.19E+06	3.7	1.00E+00	7.61E-08	7.61E-05	9.82E-01	2.096276	1.386294	4.104156	4
Flow : 0.0				4	4.6	5.53E+02	2.19E+06	4.3	1.00E+00	1.87E-08	1.87E-05	9.90E-01	2.320794	1.526056	4.483513	4.6
Flow Inc. : 0.0				4.6	5.4	2.66E+02	2.19E+06	5	1.00E+00	1.41E-08	1.41E-05	9.98E-01	2.634442	1.686399	5.072886	5.4
Pulse : 0.0				5.4	6.3	7.31E+01	2.19E+06	5.85	1.00E+00	6.20E-09	6.20E-06	9.98E-01	2.947781	1.840550	5.739036	6.3
Pulse Inc. : 0.0				6.3	7.4	2.64E+01	2.19E+06	6.85	1.00E+00	3.59E-09	3.59E-06	1.00E+00				
Nebulizer :				7.4	8.6	0.953	2.19E+06	8	1.00E+00	2.07E-10	2.07E-07	1.00E+00				
Neb. Inc :				8.6	10	0	2.19E+06	9.3	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Low Limit :	40			10	12	0	2.19E+06	11	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
High Limit:	80			12	14	0	2.19E+06	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Baseline Offset	0.1			14	16	0	2.19E+06	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Noise Filter (Sigmas)	6			16	18	0	2.19E+06	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Nozzle Type	200um			18	22	0	2.19E+06	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Flow Rate Range (l/min):	1.49			22	25	0	2.19E+06	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Mass Loading (mg/m <sup>3</sup> ):	1.17			25	29	0	2.19E+06	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Concentration (#/m <sup>3</sup> ):	7.41E+08			29	34	0	2.19E+06	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Counting efficiency:	1			34	40	0	2.19E+06	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				40	46	0	2.19E+06	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				46	54	0	2.19E+06	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				54	63	0	2.19E+06	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				63	74	0	2.19E+06	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				74	86	0	2.19E+06	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				86	100	0	2.19E+06	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				100	120	0	2.19E+06	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				120	140	0	2.19E+06	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				140	160	0	2.19E+06	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				160	180	0	2.19E+06	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				180	220	0	2.19E+06	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
				2190838.183						2.37E-06	2.37E-03					

Test 21 (Cont.) Mineral Oil/PCM Slurry, 1270 rpm, .014", 2.5 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m3)	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	225.217	227.26	2.043	2.850	127.000	716.886	0.016	0.937
Filter R	209.8	212.1	2.300	2.800	127.000	821.479	0.018	1.074
Blank	149.834	149.834	0.000					

Actual Machining Conditions

Flow	2.650
Depth	0.014
RPM	1270.000
Base Amp	7.750
Cut Amp	9.750
Diameter	7.070

M# = 0.109

Amp = 2.000

Mact = 0.101

Test 22 Mineral Oil/PCM Slurry, 500 rpm, .005", 3.0 ml/s

	Run 45	% Under	Run 45	Lower SIZE	Upper SIZE	Run 45 # In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted log normal	Best log-normal fit
Min Oil/PCM	1/20/99	17:10	0.12	0.14	0	0	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264		dg 2.968
Taken on:			0.61	0.12	0.14	0	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.966113		fg 1.784
AERODYNAMIC No.DISTRIB.			0.67	0.14	0.16	2.69E+00	0	0.15	3.63E-05	3.85E-15	3.85E-12	2.23E-08		-1.832581		r2 0.988
Material:	F-7211		0.72	0.16	0.18	6.03E+00	2.69E+00	0.17	1.18E-04	1.26E-14	1.26E-11	9.60E-08		-1.714798		
Density (g/cc):			0.78	0.18	0.22	1.95E+01	8.72E+00	0.2	3.81E-04	6.63E-14	6.63E-11	4.84E-07		-1.514128		
Correlating Factor:			0.84	0.22	0.25	6.62E+01	2.83E+01	0.235	1.27E-03	3.64E-13	3.64E-10	2.61E-06		-1.386294		
Run Length (sec):	264.03		0.91	0.29	0.34	3.12E+02	9.45E+01	0.27	5.48E-03	2.60E-12	2.60E-09	1.78E-05		-1.237874		
PMT Voltage (Volts):	1150		0.97	0.29	0.34	8.72E+02	4.06E+02	0.315	1.72E-02	1.16E-11	1.16E-08	8.54E-05		-1.078810	0.337105	0.34
Laser Current (mA):	41.58	45%	1.04	0.34	0.4	1.22E+03	1.28E+03	0.37	3.37E-02	2.62E-11	2.62E-08	2.39E-04		-0.916291	0.392956	0.4
Clock Freq (MHz):	74225	55%	1.18	0.46	0.54	8.43E+02	2.50E+03	0.43	4.50E-02	2.84E-11	2.84E-08	4.03E-04		-0.776529	0.426986	0.46
Sum of channels:			1.27	0.54	0.63	3.70E+03	3.34E+03	0.5	6.52E-02	7.94E-11	7.94E-08	8.69E-04		-0.616186	0.484409	0.54
Lower Size Limit:			1.36	0.63	0.74	7.49E+03	8.54E+03	0.685	2.16E-01	1.02E-09	1.02E-06	8.67E-03		-0.462035	0.593210	0.63
Upper Size Limit:			1.48	0.74	0.86	7.14E+03	1.60E+04	0.8	3.12E-01	1.55E-09	1.55E-06	1.77E-02		-0.301105	0.748705	0.74
Mean Size:			1.7	0.86	1	8.14E+03	2.32E+04	0.93	4.22E-01	2.77E-09	2.77E-06	3.40E-02		-0.150823	0.878557	0.86
Standard Deviation:			2.433	1.75	1	1.02E+04	3.13E+04	1.1	5.60E-01	5.78E-09	5.78E-06	6.78E-02		0.182322	1.250823	1.2
D(4.3):			4.25	1.95	1.2	1.4	7.99E+03	4.15E+04	1.3	6.67E-01	7.44E-09	1.11E-01		-1.219637	1.465026	1.4
D(3.2):			2.23	1.4	1.6	6.10E+03	4.95E+04	1.5	7.50E-01	8.73E-09	8.73E-06	1.62E-01		-0.984789	1.678351	1.6
Spec surf area (m <sup>2</sup> /g):			2.7	1.6	1.8	4.82E+03	5.56E+04	1.7	8.14E-01	1.00E-08	1.00E-05	2.21E-01		-0.768634	1.902046	1.8
Mode (Linear scale):			0.65	1.8	2.2	6.04E+03	6.05E+04	2	8.96E-01	2.05E-08	2.05E-05	3.41E-01		-0.410325	2.340425	2.2
Mode Lower Bound:			0.74	2.2	2.5	2.77E+03	6.65E+04	2.35	9.33E-01	1.52E-08	1.52E-05	4.30E-01		-0.176667	2.679373	2.5
Mode Upper Bound:			2.9	2.5	2.9	2.22E+03	6.93E+04	2.7	9.63E-01	1.85E-08	1.85E-05	5.38E-01		0.095479	3.136510	2.9
Lower combine size:			3.4	3.4	4	7.68E+02	7.28E+04	3.7	9.92E-01	1.65E-08	1.65E-05	7.40E-01		0.641842	4.303230	4
Upper combine size:			4.6	4.6	5.4	2.03E+02	7.36E+04	4.3	9.95E-01	9.50E-09	9.50E-06	7.95E-01		0.874261	4.782471	4.6
Dispenser			5.4	6.3	6.29E+01	7.41E+04	5.85	9.99E-01	1.07E-08	1.07E-05	1.07E-02	8.89E-01		1.221565	5.515991	5.4
Pulse Jet			6.3	7.4	3.15E+01	7.41E+04	6.85	9.99E-01	4.29E-09	4.29E-06	4.29E-03	9.14E-01		1.366927	6.547429	7.4
Flow Inc.: 0.0			7.4	8.6	1.90E+01	7.42E+04	8	1.00E+00	4.12E-09	4.12E-06	4.12E-03	9.38E-01		1.540625	7.239955	8.6
Flow Inc.: 0.0			8.6	10	1.92E+01	7.42E+04	9.3	1.00E+00	1.00E+00	6.56E-09	6.56E-06	9.77E-01		1.988974	9.385220	10
Nebulizer: 40			10	12	7.08E+00	7.42E+04	11	1.00E+00	3.99E-09	3.99E-06	3.99E-03	1.00E+00				
Low Limit: 40			12	14	0	7.42E+04	13	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
High Limit: 80			14	16	0	7.42E+04	15	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Baseline Offset			16	18	0	7.42E+04	17	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Noise Filter (Sigmas)			18	22	0	7.42E+04	20	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
2000um			22	25	0	7.42E+04	23.5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Flow Rate Range (l/min):			25	29	0	7.42E+04	27	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
to:			29	34	0	7.42E+04	31.5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Mass Loading (mg/m <sup>3</sup> ):			34	40	0	7.42E+04	37	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Concentration (#/m <sup>3</sup> ):			40	46	0	7.42E+04	43	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
Counting efficiency:			46	54	0.00E+00	7.42E+04	50	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			54	63	0	7.42E+04	58.5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			63	74	0	7.42E+04	68.5	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			74	86	0	7.42E+04	80	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			86	100	0	7.42E+04	93	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			100	120	0	7.42E+04	110	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			120	140	0	7.42E+04	130	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			140	160	0	7.42E+04	150	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			160	180	0	7.42E+04	170	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			180	220	0	7.42E+04	200	1.00E+00	1.00E+00	0.00E+00	0.00E+00	1.00E+00				
			74221.81							1.71E-07	1.71E-04	1.00E+00				

Test 22 (Cont.) Mineral Oil/PCM Slurry, 500 rpm, .005", 3.0 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	230.033	230.111	0.078	2.800	274.000	12.913	0.000	0.008
Filter R	252.532	252.623	0.091	2.800	274.000	15.065	0.000	0.009
Blank	149.834	149.834	0.000					

Actual Machining Conditions

Flow	3.000	M# =	0.019
Depth	0.007		
RPM	500.000		
Base Amp	7.600	Amp =	0.270
Cut Amp	7.870		
Diameter	7.300	Mact =	0.018

Test 23 Mineral Oil/PCM Slurry, 805 rpm, 0.15", 3.0 ml/s

	Run 47	Run 47	Upper	Run 47	Run 47	# In	# Under	Mid Size	Cum % Under	Mass (g)	Mass (mg)	Cum % Mass	Mass Probits	ln(high)	predicted	actual	Best log-normal fit
	Min Oil/PCM	% Under	SIZE	Lower	Upper	SIZE	SIZE	SIZE	SIZE	SIZE	SIZE	SIZE	SIZE	SIZE	log normal	log normal	dg
Taken on:	1/20/99 17:47	5%	0.1	0.12	0.1	0.12	0	0.11	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-2.120264			1.529
AERODYNAMIC No.DISTRIB.		10%	0.7	0.12	0.14	0.14	0	0.13	0.00E+00	0.00E+00	0.00E+00	0.00E+00		-1.966113			0.959
Material:	F-7211	15%	0.76	0.14	0.16	0.16	0	0.15	1.35E-06	1.93E-15	1.93E-12	9.09E-10		-1.832581			
Density (g/cc):	0.81	20%	0.82	0.16	0.18	0.18	1.35E+00	0.17	3.29E-06	4.07E-15	4.07E-12	2.82E-09		-1.714798			
Correlating Factor:	1	25%	0.89	0.18	0.22	0.22	1.59E+00	0.2	4.87E-06	5.38E-15	5.38E-12	3.53E-09		-1.514128			
Run Length (sec):	167.31	30%	0.95	0.22	0.25	0.25	2.98E+01	0.235	3.45E-05	1.64E-13	1.64E-10	8.23E-08		-1.386294			
PMT Voltage (Volts):	1150	35%	1.01	0.25	0.29	0.29	2.63E+02	0.27	2.97E-04	2.20E-12	2.20E-09	1.12E-06		-1.237874			
Laser Current (mA):	41.58	40%	1.07	0.29	0.34	0.34	1.01E+03	0.315	1.30E-03	1.34E-11	1.34E-08	7.41E-06		-1.078810	0.360195	0.34	
Clock Freq (MHz):	40	45%	1.14	0.34	0.4	0.4	1.87E+03	0.37	3.17E-03	4.02E-11	4.02E-08	2.63E-05		-0.916291	0.408762	0.4	
Sum of channels:	1003950	50%	1.21	0.4	0.46	0.46	1.12E+03	0.43	4.28E-03	3.77E-11	3.77E-08	4.40E-05		-0.776529	0.430316	0.46	
Lower Size Limit:	199.6	55%	1.29	0.46	0.54	0.54	6.65E+03	0.5	1.09E-02	3.52E-10	3.52E-07	2.10E-04		-0.616186	0.508529	0.54	
Upper Size Limit:	1.23	60%	1.36	0.63	0.74	0.74	3.60E+04	0.585	4.68E-02	3.06E-09	3.06E-06	1.65E-03		-0.462035	0.652769	0.63	
Standard Deviation:	1.56	65%	1.46	0.63	0.74	0.74	9.07E+04	0.685	1.37E-01	1.24E-08	1.24E-05	7.46E-03		-0.301105	0.808662	0.74	
D(4.3):	95.98	70%	1.55	0.74	0.86	0.86	9.23E+04	0.8	2.29E-01	2.00E-08	2.00E-05	1.69E-02		-0.150823	0.922898	0.86	
D(3.2):	6.72	75%	1.67	0.86	1	1	1.13E+05	0.93	3.41E-01	3.83E-08	3.83E-05	3.50E-02		0.000000	1.052937	1	
Spec surf area (m <sup>2</sup> /g):	1.1	80%	1.81	1	1.2	1.2	1.54E+05	1.1	4.95E-01	8.68E-08	8.68E-05	7.57E-02		0.182322	1.236290	1.2	
Mode (Linear scale):	0.67	85%	1.99	1.2	1.4	1.4	1.27E+05	1.3	6.21E-01	1.18E-07	1.18E-04	1.31E-01		0.336472	1.412056	1.4	
Mode Lower Bound:	0.74	90%	2.24	1.4	1.6	1.6	9.96E+04	1.5	7.20E-01	1.42E-07	1.42E-04	1.98E-01		0.470004	1.585285	1.6	
Mode Upper Bound:	7.7	95%	2.66	1.6	1.8	1.8	7.78E+04	1.7	7.97E-01	1.62E-07	1.62E-04	2.74E-01		-0.600003	1.761575	1.8	
Lower combine size:	0.67		1.8	2.2	2.2	2.2	9.72E+04	2	8.94E-01	3.30E-07	3.30E-04	4.29E-01		-0.178281	2.106853	2.2	
Upper combine size:	7.82		2.5	2.9	2.9	2.9	4.14E+04	2.35	9.35E-01	2.28E-07	2.28E-04	5.36E-01		0.091154	2.362097	2.5	
Dispenser			3.4	4	4	4	1.83E+04	3.15	9.84E-01	2.43E-07	2.43E-04	7.72E-01		0.406748	1.064711	2.9	
Flow Inc. : 0.0			4.6	5.4	5.4	5.4	1.83E+03	3.7	9.94E-01	2.02E-07	2.02E-04	8.67E-01		1.111366	1.386294	3.4	
Pulse Inc. : 0.0			5.4	6.3	6.3	6.3	6.87E+02	4.3	9.97E-01	1.26E-07	1.26E-04	9.26E-01		1.445510	1.526056	4	
Nebulizer : 0.0			6.3	7.4	7.4	7.4	6.40E+00	5	9.99E-01	9.69E-08	9.69E-05	9.71E-01		1.901399	1.686399	5.4	
Flow : 0.0			7.4	8.6	8.6	8.6	0	8	1.00E+00	0.00E+00	0.00E+00	9.99E-01		3.034220	1.840550	6.3	
Low Limit : 40			8.6	10	10	10	3.00E+00	9.3	1.00E+00	0.00E+00	0.00E+00	9.99E-01		3.157475	2.001480	7.4	
High Limit: 80			10	12	12	12	0	11	1.00E+00	1.69E-09	1.69E-06	1.00E+00		3.157475	2.302585	8.6	
Baseline Offset			12	14	14	14	0	13	1.00E+00	0.00E+00	0.00E+00	1.00E+00				10	
Noise Filter (Sigmas)			14	16	16	16	0	15	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Nozzle Type			16	18	18	18	0	17	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Flow Rate Range (l/min):			18	22	22	22	0	20	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
to:			22	25	25	25	0	23.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Mass Loading (mg/m <sup>3</sup> ):			25	29	29	29	0	27	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Concentration (#/m <sup>3</sup> ):			29	34	34	34	0	31.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
Counting efficiency:			34	40	40	40	0	37	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			40	46	46	46	0	43	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			46	54	54	54	0.00E+00	50	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			54	63	63	63	0	58.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			63	74	74	74	0	68.5	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			74	86	86	86	0	80	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			86	100	100	100	0	93	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			100	120	120	120	0	110	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			120	140	140	140	0.00E+00	130	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			140	160	160	160	0.00E+00	150	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			160	180	180	180	0	170	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
			180	220	220	220	0	200	1.00E+00	0.00E+00	0.00E+00	1.00E+00					
							1004024.764										

Test 23 (Cont.) Mineral Oil/PCM Slurry, 805 rpm, .015", 3.0 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cft)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	220.878	222.115	1.237	2.750	177.000	322.771	0.007	0.303
Filter R	231.142	232.412	1.270	2.750	177.000	331.381	0.007	0.311
Blank	149.834	149.834	0.000					

Actual Machining Conditions

Flow	2.890
Depth	0.016
RPM	805.000
Base Amp	7.750
Cut Amp	8.650
Diameter	7.265

M# = 0.072

Amp = 0.900

Mact = 0.069



Test 24 (Cont.) Mineral Oil/PCM Slurry, 1270 rpm, .02", 2.5 ml/s

Generation Rate Calculations

	Initial (mg)	Final (mg)	Mist Mass (mg)	Flow (cfh)	Time (sec)	Conc. (mg/m <sup>3</sup> )	Smpl Rate mg/sec	Gen. Rate mg/sec
Filter L	205.29	211.89	6.600	2.800	118.000	2537.080	0.056	3.569
Filter R	218.219	224.632	6.413	2.800	118.000	2465.196	0.054	3.468
Blank	149.834	149.834	0.000					

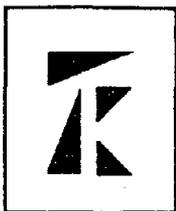
Actual Machining Conditions

Flow	2.560
Depth	0.020
RPM	1270.000
Base Amp	7.600
Cut Amp	10.750
Diameter	7.185

M# = 0.161

Amp = 3.150

Mact = 0.152



**FINAL REPORT**

Study Title

Mammalian Cell Colony Suppression Test With Cultured  
BALB/c-3T3 Cells

Test Article I.D.

EICOSANE, TG, DRY POWDER

Author

Kamala J. Pant, M.S.

Performing Laboratory

SITEK Research Laboratories  
15235 Shady Grove Road, Suite 303  
Rockville, Maryland 20850

Laboratory Project ID

SITEK Study No. 0538-7760

Study Initiation Date

March 24, 1999

Study Completion Date

June 9, 1999

Sponsor

Triangle Research and Development  
P.O. Box 12696  
Research Triangle Park, NC 27709-2696

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Study No. 0538-7760

Sponsor's Test Article I.D. EICOSANE, TG, DRY POWDER

The study described in this report was conducted in compliance with the following Good Laboratory Practice standard:

United States Food and Drug Administration,  
Title 21, Code of Federal Regulations Part 58,  
Revised April 1, 1996.

Signature Kamala Pant  
Kamala J. Pant, M.S.  
Study Director

6.9.99  
Date

QUALITY ASSURANCE UNIT'S STATEMENT

Study No. 0538-7760

Sponsor's Test Article I.D. EICOSANE, TG, DRY POWDER

The performance of this study was audited for adherence to the Good Laboratory Practice regulations for nonclinical laboratory studies by the Quality Assurance Unit of SITEK Research Laboratories. In this context, the facilities, equipment, personnel, methods, practices, controls, original data and reports have been inspected as per SITEK's Quality Assurance Unit's Standard Operating Procedures. The information contained within this report accurately reflects the raw data generated from this study.

Protocol Review Date: 04-16-99

The following phases were inspected for this study:

<u>Inspection Date</u>	<u>Phases Inspected</u>	<u>Date Findings Reported to Study Director</u>	<u>Date Findings Reported to Management</u>
<u>04-21-99</u>	<u>Treatment</u>	<u>04-21-99</u>	<u>04-21-99</u>
<u>05-19-99</u>	<u>Workbook Audit</u>	<u>05-20-99</u>	<u>05-24-99</u>
<u>05-20-99</u>	<u>Draft Report Audit</u>	<u>05-20-99</u>	<u>05-24-99</u>
<u>06-09-99</u>	<u>Final Report Audit</u>	<u>06-09-99</u>	<u>06-09-99</u>

Signature *Marcie A. Bauernschub* 6/9/99  
 Marcie A. Bauernschub, B.S. Date  
 Manager, Quality Assurance Unit

STUDY DIRECTOR'S SIGNATURE PAGE

This study was performed under the supervision of Kamala J. Pant, M.S., Study Director for the Colony Suppression Test, at SITEK Research Laboratories, 15235 Shady Grove Road, Suite 303, Rockville, Maryland 20850.

The Final Report on this study was written by the Study Director and released on June 9, 1999.

Signature

Kamala J. Pant, M.S. Kamala Pant Date 6.9.99  
Study Director

## ABSTRACT

The extract of test article, EICOSANE, TG, DRY POWDER, was evaluated for its potential to cause cytotoxicity as manifested by a decrease in the Relative Cloning Efficiency (RCE) of cultured BALB/c-3T3 cells. The procedures used in this study were similar to the ones described in Test Methods of Cell Toxicology (1).

The test article was extracted in the culture media at the ratio of 1 gram of test article per 10 mL of culture media at  $37 \pm 1^\circ\text{C}$  for 72 hours in a shaker incubator with mild agitation (60 rpm) and 5%  $\text{CO}_2$  in air. The extract concentrations evaluated were 0.063, 0.125, 0.25, 0.5 and 1.0 mL/mL in culture media.

Two reference polymers (SPRM A and SPRM B) were used as the positive control articles. Each polymer was extracted in culture media (3 cm<sup>2</sup> polymer/mL media) at  $37 \pm 1^\circ\text{C}$  for 72 hours with mild agitation (about 60 rpm). Positive control extracts were evaluated at concentrations of 0.001, 0.005, 0.01, 0.05, 0.1 and 0.25 mL/mL media (SPRM A) and 0.01, 0.025, 0.05, 0.1, 0.125 and 0.25 mL/mL media (SPRM B). The solvent control was also incubated under the same conditions prior to use in the assay.

Two hundred (200) cells/plate were seeded 18-24 hours before treatment. The culture media used consisted of Eagle's Minimum Essential Medium containing 2 mM L-glutamine, 10% heat inactivated fetal bovine serum, 50 units/mL penicillin and 50  $\mu\text{g}/\text{mL}$  streptomycin. Each concentration of the test article extract, positive control extract, and the solvent control were tested in triplicate.

After the treatment, the plates were incubated undisturbed for 9 days at  $37 \pm 1^\circ\text{C}$  in approximately 5%  $\text{CO}_2$  and 95% air. At the end of this period, the cells were washed with Dulbecco's Balanced Salt Solution (DPBS), fixed with methanol and stained with Giemsa stain. The colonies were counted, and the average number of colonies per plate was calculated. The RCE was determined using the following formula:

$$\text{RCE} = \frac{\text{Average No. of Colonies in Test Plates}}{\text{Average No. of Colonies in Solvent Control Plates}} \times 100$$

The Relative Cloning Efficiencies (RCEs) for the test article extract concentrations, ranging from 0.063 to 1.0 mL/mL, were from 0% to 32%.

The RCEs and the percent dilutions of the test article extracts were plotted on a semi-logarithmic graph to determine the  $IC_{50}$  value for the test article extract. The  $IC_{50}$  is the percent dilution of the extract that inhibits colony formation by 50% compared to that of the solvent control value. The  $IC_{50}$  value for the test article extract was found to be approximately 4.0%.

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## INTRODUCTION

This study was conducted by Kamala J. Pant, M.S., Lijuan Zhang, B.S., Jie Zhang, M.D., and Benjamin N. Tran, from April 21, 1999 to May 3, 1999, at SITEK Research Laboratories. The experimental procedures used to perform this study were essentially those described in Test Methods of Cell Toxicology (1).

The purpose of this study was to evaluate the test article, EICOSANE, TG, DRY POWDER, extracted in culture medium for its potential to cause cytotoxicity as manifested by a reduction in the Relative Cloning Efficiency (RCE) of cultured BALB/c-3T3 cells. Colony Suppression Assays using the BALB/c-3T3 and other mammalian cell lines have been used extensively and have been demonstrated to be effective in detecting the cytotoxicity of chemicals from a wide range of chemical classes (1-7).

**MATERIALS****TEST ARTICLE**

1. Identification:	<u>EICOSANE, TG, DRY POWDER</u>
2. Lot/Batch No.:	<u>42-02</u>
3. Description:	<u>White Powder</u>
4. Date Received:	<u>March 17, 1999</u>
5. Storage Conditions:	<u>Room Temperature</u>
6. Expiration Date:	<u>Not provided</u>

**CONTROL SUBSTANCES****Positive Control**

The following Standard Positive Reference Material (SPRM) extracts were tested as the positive controls.

Positive SPRM-A - Segmented polyetherurethane film containing 0.1% Zinc Diethyldithiocarbamate (ZDEC)

Source: Hatano Research Institute

Lot No.: 96010A

Storage conditions: Room Temperature

Expiration Date: 10-04-01

Positive SPRM-B - Segmented polyetherurethane film containing 0.25% Zinc Dibutyldithiocarbamate (ZDBC)

Source: Hatano Research Institute

Lot No.: 96008B

Storage conditions: Room Temperature

Expiration Date: 10-04-01

**Solvent Controls**

The test and positive control article polymers were extracted in culture medium, therefore, culture medium was used as the solvent control in this study.

Culture Medium - Eagle's Minimum Essential Medium with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 50 units/mL penicillin and 50  $\mu$ g/mL streptomycin.

Source: SITEK Research Laboratories

Batch No.: 18

Storage Conditions: 1-5° C

Expiration Date: October 22, 1999

## INDICATOR CELLS

### Source

The BALB/c-3T3 cell line (clone A31) used in this study was originally established by Dr. T. Kakunaga, National Institutes of Health, Bethesda, Maryland. A subpassage of this clone was obtained from Mobil Oil Corporation, Princeton, New Jersey. The cells were subcultured and cryopreserved in a large number of ampules for BALB/c-3T3 Colony Suppression Assays.

### Culture Conditions

The BALB/c-3T3 cells were routinely cultured in Eagle's Minimum Essential Medium containing 10% heat-inactivated fetal bovine serum and 2mM L-glutamine (MEM) in a humidified CO<sub>2</sub> incubator in an atmosphere of approximately 5% CO<sub>2</sub> and 95% air. The cultures were routinely subpassaged before reaching confluency.

### Stock Cultures

The BALB/c-3T3 cells were propagated in antibiotic-free medium to obtain a sufficient number of cells for freezing a large number of stock ampules. The cells were cryopreserved in MEM containing 8% dimethyl sulfoxide (DMSO) and stored in liquid nitrogen. Prior to using the stock cultures for the assay, representative ampules were tested for contaminating microorganisms, including mycoplasma. Stock ampules free of contaminating organisms were used to initiate the stock cultures for the assay. The cultures obtained from the stock ampules were maintained by subculturing for a maximum period of one month and used to initiate cultures for the Colony Suppression Assay.

## EXPERIMENTAL PROCEDURES

### DOCUMENTATION

The materials, experimental procedures used in the performance of the study, experimental results, and the methods used in the evaluation of the results were documented in the study workbook.

### TEST SYSTEM IDENTIFICATION

The 60 mm plates for each dose were placed inside of a 150 mm plate, and each 150 mm plate was labeled with SITEK's test article number, experiment number, and final concentration of the test article extract, positive control extracts or the solvent control. All dosing procedures were conducted under UV-filtered lights to avoid possible photoinactivation of the test and control article preparations.

### PREPARATION OF TEST ARTICLE EXTRACT

The test article extract was made on a weight to volume ratio of 1.0 gram of test article per 10 mL of culture media. The test article was weighed (3.5 gram) in a glass container and then 35 mL of culture media was added to the flask. The flask was gassed with 5% CO<sub>2</sub>, sealed with parafilm, and then placed in a shaker incubator set at 37 ± 1°C and 60 rpm for 72 hours. Approximately 100 mL of culture media, used as the solvent control, was placed in a second container, gassed with CO<sub>2</sub> and sealed. The solvent control was placed in the shaker incubator for the same length of time under the same conditions.

At the end of the extraction period, the extract media was decanted from the test article and filtered through a 0.45 µM filter. The test article extract and the solvent control flask were stored refrigerated until they were used in treatment (within 24 hours). Although the extraction was initiated with 35 mL of culture media, only 32.5 mL of extract could be decanted from the test article. Serial dilutions of the extract were made by varying the amount of extract delivered to the cultures.

The strength and stability of the test article extract under experimental conditions were not determined by SITEK Research Laboratories.

## PREPARATION OF POSITIVE CONTROL EXTRACTS

The extracts for the positive controls SPRM-A and SPRM-B were prepared at the ratio of 3 cm<sup>2</sup> surface area per 1.0 mL of culture medium. The positive controls were incubated in culture medium for 72 hours at 37 ± 1°C at 60 rpm and 5% CO<sub>2</sub> in air. At the end of this incubation period, the extracts were decanted and stored refrigerated until used in the experiment. Serial dilutions of the positive control extracts were made by varying the volumes of extract delivered to the plates.

The stability of the control extracts under experimental conditions were not determined by SITEK.

## PREPARATION OF TEST CULTURES

The BALB/c-3T3 stock cultures for the assay were grown in culture medium. Cultures growing in T-75 cm<sup>2</sup> tissue culture flasks and showing approximately 50-90% confluency were harvested and used to prepare the test cultures. The cells were washed with Dulbecco's Balanced Salt Solution (DPBS). The cells were then dissociated by adding 2.0 mL of a 0.25% trypsin EDTA solution [DPBS supplemented with EDTA (0.02%) and trypsin (0.25%) (trypsin-EDTA)] to each flask. The cells were rinsed with trypsin-EDTA, and the excess trypsin-EDTA was removed with a Pasteur pipet. The flasks were incubated at 37 ± 1°C until the cells dissociated. 5.0 mL of culture medium was added to each of the stock culture flasks, and the cell suspension was aspirated to obtain a single cell suspension. The cells from a number of stock culture flasks were pooled and centrifuged at 800 rpm for 5 minutes.

The supernatant was removed, and the cells were resuspended in culture medium. An aliquot of the cell suspension was diluted to the appropriate concentration and counted in a cell counter. Based on the cell counts, a separate cell suspension with 1x10<sup>5</sup> cells/mL was prepared to seed the culture plates. The cell suspension was further diluted to achieve 40 cells/mL. An appropriate number of 60 mm tissue culture plates was seeded with 5.0 mL of cell suspension to obtain test cultures with 200 cells/plate. The plates were incubated at 37 ± 1°C in a humidified incubator in an atmosphere of approximately 5% CO<sub>2</sub> and 95% air for 18-24 hours.

## COLONY SUPPRESSION ASSAY

In order to determine the test article and positive control article extract concentrations that would produce 0-100% cytotoxicity, several concentrations of each were evaluated.

**Test Article Extract Concentrations**

0.063, 0.125, 0.25, 0.5 and 1.0 mL/mL

**SPRM A Extract Concentrations**

0.001, 0.005, 0.01, 0.05, 0.1 and 0.25 mL/mL

**SPRM B Extract Concentrations**

0.01, 0.025, 0.05, 0.1, 0.125 and 0.25 mL/mL

**Negative Control - Culture Medium**

Each concentration of each treatment was evaluated in triplicate using 200 cells/plate. The cells were seeded 18-24 hours prior to treatment. The cytotoxicity of each treatment was assessed by determining the ability of the treated cells to form colonies. The cultures were exposed to the treatments for 9 days without any disturbance in a humidified incubator with approximately 5% CO<sub>2</sub> at 37 ± 1°C. After the exposure period, the cells were washed with DPBS, fixed with methanol, stained with Giemsa stain, and the colonies were counted. A cluster of more than 50 cells growing within a confined area was considered to be a colony. The average number of colonies per plate was calculated, and the Relative Cloning Efficiency (RCE) was determined using the following formula:

$$\text{RCE} = \frac{\text{Average No. of Colonies in Test or Positive Control Plates}}{\text{Average No. of Colonies in Solvent Control Plates}} \times 100$$

## EVALUATION OF TEST RESULTS

The Relative Cloning Efficiency versus treatment concentration was plotted on a semi-logarithmic graph for each treatment. The treatment concentration (expressed as % of undiluted treatment) that inhibited colony formation by 50% compared to the RCE obtained for the solvent control cultures ( $IC_{50}$ ) was calculated from the graph.

## CRITERIA FOR A VALID TEST

Since Standard Positive Reference Materials (SPRMs) were used, the  $IC_{50}$  value of the positive SPRMs should not exceed the following values: SPRM-A=7% and SPRM-B=70%.

## ARCHIVES

All of the raw data and the final report of the study are maintained at SITEK Research Laboratories' Archives at 15235 Shady Grove Road, Suite 303, Rockville, Maryland 20850, according to the terms and conditions of the study.

## RESULTS AND CONCLUSIONS

The results of the Colony Suppression Assay are presented as follows. The corresponding graphs (1-3) are also presented.

	<u>Table</u>	<u>Graph</u>
Test Article Extract	1	1
SPRM A Extract	2	2
SPRM B Extract	2	3

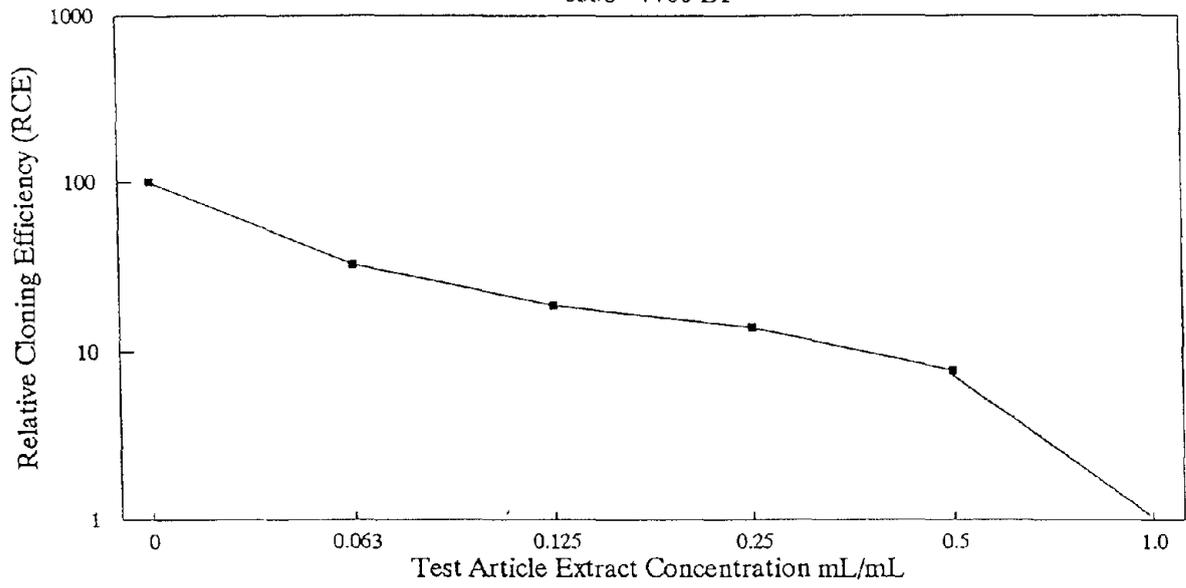
The corresponding solvent control results are included for comparison in Tables 1-2.

The results of the Colony Suppression Assay indicated that all of the test article extract concentrations were cytotoxic. The  $IC_{50}$  for the test article extract was calculated from the graph and found to be approximately 4.0%.

The positive controls, SPRM A and SPRM B, gave  $IC_{50}$  values of approximately 0.24% and 13.7% extract concentrations, respectively.

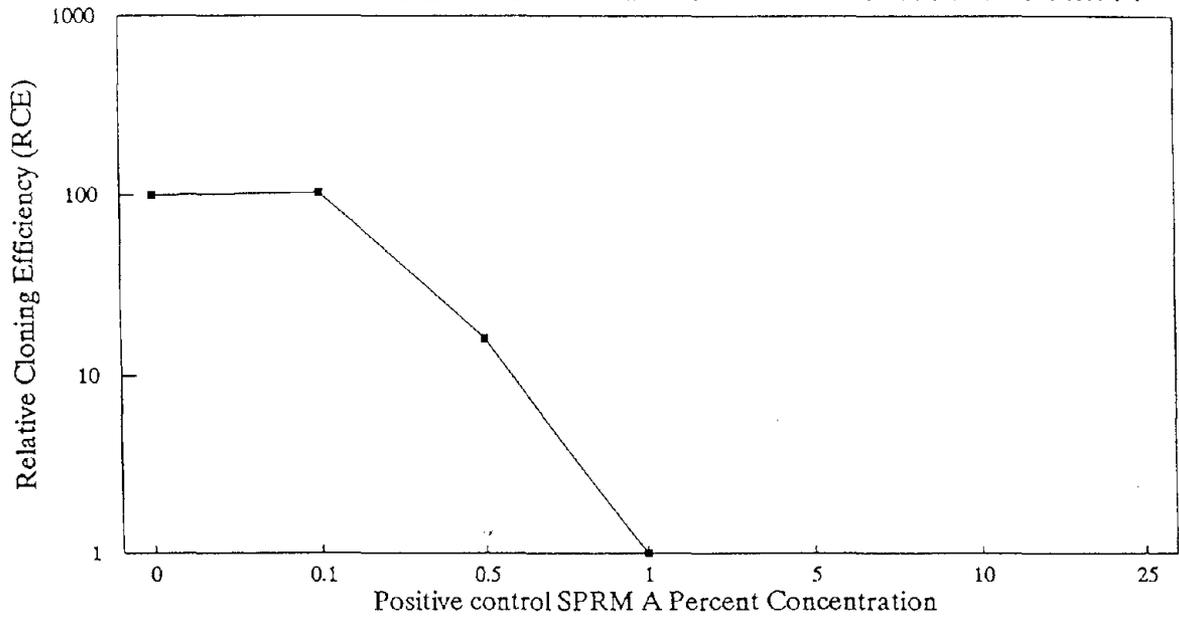


GRAPH 1 – RCE AND TEST ARTICLE EXTRACT CONCENTRATIONS mL/mL  
0538-7760 B1

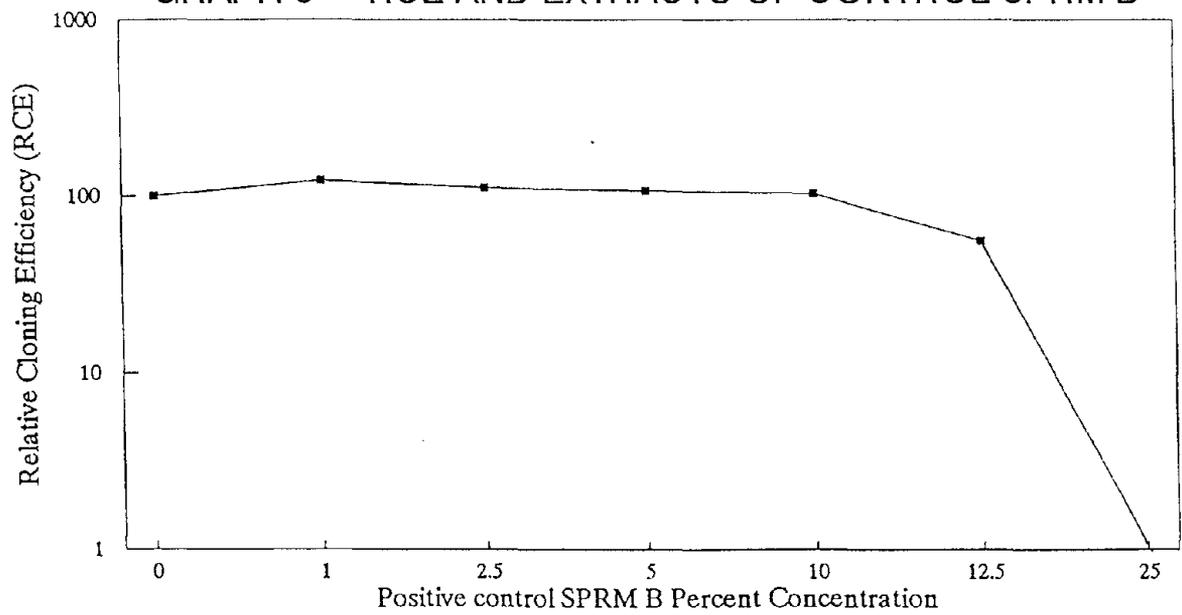




GRAPH 2 – RCE AND EXTRACTS OF CONTROL SPRM A



GRAPH 3 – RCE AND EXTRACTS OF CONTROL SPRM B



## REFERENCES

1. Test Methods of Cell Toxicology: Japanese Society of Cell Culture. Asakura Shoten (ed.), Tokyo, 1991.
2. Nakamura, A., Y. Ikarashi, T. Tsuchiya, M.-A. Kaniwa, M. Sato, K. Toyoda and M. Takahashi. Correlations among chemical constituents, cytotoxicities and tissue responses: In the cases of natural rubber latex materials. *Biomaterials*, II, *Biomat.*, 89:92-94, 1990.
3. Ikarashi, Y., K. Toyoda, N. Ohsawa, T. Uchima, T. Tsuchiya, M.-A. Kaniwa, M. Sato, M. Takahashi and A. Nakamura. Comparative studies by cell culture and in vivo implantation test on the toxicity of natural rubber latex materials. *J. Biomed. Mater. Res.*, 26:339-356, 1992.
4. Tsuchiya, T., Y. Ikarashi, H. Hata, K. Toyoda, M. Takahashi, T. Uchima, N. Tanaka, K. Sasaki and N. Nakamura. Comparative studies of the toxicity of standard reference materials in various cytotoxicity tests and in vivo implantation tests. *J. Appl. Biomaterials*, 4:153-156, 1993.
5. Tsuchiya, T., T. Arai, J. Ohhashi, K. Imai, H. Kojima, S. Miyamoto, H. Hata, Y. Ikarashi, K. Toyoda, M. Takahashi and A. Nakamura. Rabbit eye irritation caused by wearing toxic contact lenses and their cytotoxicities. In vivo/in vitro correlation study using standard reference materials. *J. Biomed. Mater. Res.*, 27:885-893, 1993.
6. Tsuchiya, T., Y. Ikarashi, T. Arai, J. Ohhashi, K. Isama and A. Nakamura. In vivo tissue/biomaterials toxic responses: Correlation with cytotoxic potential but not cell attachment. *Clinical Materials*, 16:1-8, 1994.
7. Tsuchiya, T. Studies on the standardization of cytotoxicity tests and new standard reference materials useful for evaluating the safety of biomaterials. *J. Biomaterials Appl.*, 2:138-157, 1994.

APPENDIX I

STUDY PROTOCOL, PROTOCOL ADDENDA,  
PROTOCOL AMENDMENT AND PROTOCOL DEVIATION



**MAMMALIAN CELL COLONY SUPPRESSION TEST  
WITH CULTURED BALB/c-3T3 CELLS**

This protocol is presented in two parts. Part One is designed to collect specific information pertaining to the test article and study. Part Two describes the study design in detail. Please complete all sections in Part One and sign section 8.0 to approve the protocol.

**PART ONE**

**1.0 SPONSOR**

- 1.1 Name: TRIANGLE RESEARCH AND DEVELOPMENT CORPORATION
- 1.2 Address: P.O. BOX 12696  
RESEARCH TRIANGLE PARK, NC 27709-2696
- 1.3 Sponsor's Study Coordinator: DAVID P. COLVIN, Ph.D.

**2.0 TESTING FACILITY**

- 2.1 Name: SITEK Research Laboratories
- 2.2 Address: 15235 Shady Grove Road, Suite 303  
Rockville, Maryland 20850
- 2.3 Study Director: Kamala J. Pant, M.S.

**3.0 STUDY NUMBERS**

- \* 3.1 Testing Facility's Study No.: 0538-7760
- 3.2 Sponsor's Study No.: \_\_\_\_\_

**4.0 TEST ARTICLE**

4.1 Identification

- Name: EICOSANE, TG, DRY POWDER
- Batch/Lot No.: 42-02

\* To be completed by the Testing Facility.



4.2 Description

Color: WHITE

Physical Form: POWDER

4.3 Analysis

Purity Information: \_\_\_\_\_

Does the Sponsor require the use of a correction factor to account for impurity?

Yes  No

If yes, what is the correction factor? \_\_\_\_\_

Determination of the test article characteristics as defined by Good Laboratory Practices will be the responsibility of the Sponsor. The specific GLP references for U.S. agencies are: FDA = 21 CFR, 58.105; EPA TSCA = 40 CFR, 792.105 and EPA FIFRA = 40 CFR 160.105.

4.4 Stability

Storage Conditions (check one):

Room Temperature  Refrigerated (1-5°C)

Frozen (-10 to -20°C)

Other (please specify): \_\_\_\_\_

Expiration Date: \_\_\_\_\_

4.5 Preferred Solvent (check one):

H<sub>2</sub>O  Culture Medium  DMSO  Acetone  Ethanol

Other (please specify): Culture medium

To be decided by the Testing Facility

4.6 Special Handling Instructions:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



**5.0 REGULATORY AGENCY SUBMISSION**

This study will be conducted in compliance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Parts 160 and 792, Revised July 1, 1995.

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Revised April 1, 1996.

Japanese Ministry of Agriculture, Forestry and Fisheries, 59 Nohsan, Notification No. 3850, Agriculture Production Bureau, August 10, 1984.

Japanese Ministry of Health and Welfare, Ordinance No. 21, April 1, 1997.

Japanese Ministry of International Trade and Industry, Ordinance 21, April 1, 1997.

Organisation for Economic Cooperation and Development, The OECD Principles of Good Laboratory Practice, Environment Monograph No. 45, Paris 1992.

Will this study be submitted to a regulatory agency?

Yes       No

If so, which agency(ies)? FDA

**6.0 DOSING SOLUTIONS**

The Sponsor will be responsible for determining the strength and stability of the dosing solutions. The U.S. requirements for analysis of dosing solutions are specified in: FDA = 21 CFR, 58.113; EPA TSCA = 40 CFR, 792.113 and EPA FIFRA = 40 CFR 160.113.

Does the Sponsor request samples of the dosing solutions?\*

Yes       No

Which concentration(s)? \_\_\_\_\_

What amount of each concentration? \_\_\_\_\_

\*\* Please note that there will be an additional charge for each shipment. See Special Services price schedule.



At what temperature should the dosing solutions be stored?

\_\_\_ Room Temperature      \_\_\_ Frozen (-10 to -20°C)

\_\_\_ Refrigerated (1-5°C)

7.0 STUDY DATES

\* 7.1 Proposed Experimental Start Date: 4.8.99

Defined as the date the cells are first treated with the test article.

\* 7.2 Anticipated Experimental Completion Date: 4.29.99

Defined as the last date on which data are collected directly from the study.

8.0 PROTOCOL APPROVAL

\* Kamala Paut  
Study Director

3.24.99  
Date

[Signature]  
Sponsor's Study Coordinator

3-24-99  
Date

\* [Signature]  
Quality Assurance Manager

4/16/99  
Date

\* [Signature]  
Safety Officer

4/16/99  
Date

\* To be completed by the Testing Facility.



## STUDY DESIGN

### PART TWO

#### 9.0 PURPOSE

The purpose of this study is to evaluate the extract(s) of the test article(s) for its potential to cause cytotoxicity as manifested by the Relative Cloning Efficiency (RCE) of cultured BALB/c-3T3 cells.

#### 10.0 JUSTIFICATION FOR SELECTION OF TEST SYSTEM

Colony Suppression Assays using the BALB/c-3T3 and other mammalian cell lines have been used extensively and have been demonstrated to be effective in detecting the cytotoxicity of chemicals from a wide range of chemical classes (1-7).

#### 11.0 ABBREVIATIONS

- DPBS - Ca<sup>++</sup>-free and Mg<sup>++</sup>-free Dulbecco's balanced salt solution
- HIFBS - Heat-Inactivated Fetal Bovine Serum
- HDPS - High Density Polyethylene Sheet
- IC<sub>50</sub> - 50% of Control Value
- MEM - Eagle's Minimum Essential Medium containing 2mM L-glutamine and 10% HIFBS
- RCE - Relative Cloning Efficiency
- SNRM - Standard Negative Reference Material
- SPRM - Standard Positive Reference Material
- ZDEC - Zinc Diethyldithiocarbamate
- ZDBC - Zinc Dibutyldithiocarbamate

Trypsin-EDTA - Trypsin solution - DPBS supplemented with EDTA (0.02%) and trypsin (0.05%)

Culture Medium - Eagle's Minimum Essential Medium with 10% heated-inactivated fetal bovine serum, 2mM L-glutamine, 50 units/mL penicillin and 50 µg/mL streptomycin



## **12.0 INDICATOR CELLS**

### **12.1 Source**

The BALB/c-3T3 cell line (clone A31) to be used in this study was originally established by Dr. T. Kakunaga, National Institutes of Health, Bethesda, Maryland. A subpassage of this clone was obtained from Mobil Oil Corporation, Princeton, New Jersey. The cells were subcultured and cryopreserved in a large number of ampules for BALB/c-3T3 assays.

### **12.2 Culture Conditions**

The BALB/c-3T3 cells are routinely cultured in Eagle's Minimum Essential Medium containing 10% heat-inactivated fetal bovine serum (HIFBS) and 2mM L-glutamine (MEM) in a humidified CO<sub>2</sub> incubator in an atmosphere of approximately 5% CO<sub>2</sub> and 95% air. Cultures are routinely subpassaged before reaching confluency.

### **12.3 Stock Cultures**

The BALB/c-3T3 cells were propagated in antibiotic-free medium to obtain a sufficient number of cells for freezing a large number of stock ampules. The cells were cryopreserved in MEM containing 8% dimethyl sulfoxide and stored in liquid nitrogen. Prior to using the stock cultures for the assay, representative ampules will be tested for contaminating microorganisms, including mycoplasma. Stock ampules will also be tested for background and induced transformation frequencies. Stock ampules, free of contaminating organisms and showing acceptable levels of background and induced transformation frequencies, will be used to initiate the stock cultures for the assay. The cultures obtained from the stock ampules will be maintained by subculturing for a maximum period of 1 month and used to initiate cultures for the Colony Suppression Assay.

## **13.0 ROUTE OF ADMINISTRATION OF TEST ARTICLE**

The test article will be administered in vitro directly or as an extract. This is the only route of administration available in this test system.

## **14.0 TEST SYSTEM IDENTIFICATION**

All of the test cultures will be labeled using an indelible ink pen with a code system which clearly identifies the experiment number, test article, controls and concentrations.

## **15.0 CONTROL SUBSTANCES**

### **15.1 Negative Control**

In the event the test article is extracted in a solvent and the extract is tested in the assay, the solvent used for extraction will be used as the negative control.



If a Standard Negative Reference Material (SNRM) is required to be tested, high density polyethylene sheets (HDPSS) or the extract of HDPSSs will be used as the negative control.

#### 15.2 Positive Control

Zinc diethyldithiocarbamate (ZDEC) or zinc dibutyldithiocarbamate (ZDBC) will be used as the positive control at a concentration that causes approximately 50% reduction in the RCE.

If Standard Positive Reference Materials (SPRMs) are required to be tested, extracts from the following SPRMs will be used as the positive controls:

Positive SPRM-A - Segmented polyetherurethane film containing 0.1%  
ZDEC

Positive SPRM-B - Segmented polyetherurethane film containing 0.25%  
ZDBC

The extracts for the positive controls will be prepared in culture medium.

### 16.0 DOCUMENTATION

All of the procedures, results, significant observations, and methods used for analysis of the results will be documented in a study notebook. The study notebook will also include copies of the protocol, all protocol amendments and protocol deviations, study reports, and all relevant communications with the Sponsor.

### 17.0 EXPERIMENTAL PROCEDURE

#### 17.1 Preparation of Test Cultures

The BALB/c-3T3 stock cultures for the assay will be grown in culture medium. Cultures growing in T-75 cm<sup>2</sup> tissue culture flasks and showing approximately 50-90% confluency will be harvested and used to prepare the test cultures. The medium from the T-75 cm<sup>2</sup> flasks will be discarded, and the cells will be washed with Ca<sup>++</sup>-free and Mg<sup>++</sup>-free Dulbecco's balanced salt solution (DPBS). The cells will then be dissociated by adding 2.0 mL of 0.05% trypsin solution - DPBS supplemented with EDTA (0.02%) and trypsin (0.05%) (trypsin-EDTA) to each flask. The cells will be rinsed with trypsin-EDTA, and the excess trypsin-EDTA will then be removed with a Pasteur pipet. The flasks will be incubated at 37 ± 1.0°C until the cells dissociate. 5.0 mL of culture medium will then be added to each of the stock culture flasks, and the cell suspension will be aspirated to obtain a single cell suspension. The cells from a number of stock culture flasks will be pooled and centrifuged at 800 rpm for 5 minutes. The supernatant will be removed, and the cells will then be resuspended in culture medium. An aliquot of the cell suspension will be diluted to the appropriate concentration and counted in a cell counter. Based on the cell counts, a separate cell suspension with 1x10<sup>5</sup> cells/mL



will be prepared to seed the culture plates. An appropriate number of 60 cm<sup>2</sup> tissue culture plates will be seeded with 5.0 mL of cell suspension to obtain test cultures with 200 cells/plates. In the case of test articles which react with plastic, sterile glass culture flasks will be used instead of plastic culture plates. The flasks or plates will be incubated at 37 ± 1.0°C in a humidified incubator in an atmosphere of approximately 5% CO<sub>2</sub> and 95% air for 18-24 hours.

#### 17.2 Preparation of Test Article Extract(s)

The procedure to be used for the preparation of the test article extract(s) will be added to the protocol by a protocol addendum. The extract(s) will be tested in the Colony Suppression Assay.

#### 17.3 Colony Suppression Assay

In order to determine the test article extract concentration that will produce 0-100% cytotoxicity, the test article extract will be measured and a serial dilution will be prepared. If there is no prior knowledge of cytotoxicity or the Sponsor specifies differently, the treatment concentrations will be undiluted and 50%, 25%, 12.5% and 6.25% of the test article extract.

The test cultures seeded approximately 18-24 hours earlier will be used in the Colony Suppression Assay. Triplicate cultures will be used at each test article, positive control and negative control level.

The cytotoxicity of the test article will be assessed by determining the ability of the treated cells to form colonies. The cultures will be treated with various concentrations of the test article extract for 9-11 days without any disturbance in a humidified incubator with approximately 5% CO<sub>2</sub> at 37 ± 1°C. After the exposure period, the cells will be washed with Ca<sup>++</sup>-free and Mg<sup>++</sup>-free DPBS, fixed with methanol, and stained with Giemsa stain, and the colonies will be counted. A cluster of more than 50 cells growing within a confined area will be considered a colony. The average number of colonies per plate will be calculated, and the RCE will be determined by the following formula:

$$\text{RCE} = \frac{\text{Average No. of Colonies in Test Plates}}{\text{Average No. of Colonies in Culture Medium Plates}} \times 100$$

#### 17.4 Evaluation of Test Results

The RCEs and the percent dilutions of the extracts will be plotted on a semilogarithmic graph, and the percent dilution that inhibits colony formation to 50% of the control value (IC<sub>50</sub>) will be calculated from the graph.

To obtain the value of IC<sub>50</sub>, statistical computer calculations may be applied.

#### 17.5 Criteria for a Valid Test

1. A statistically significant inhibition of colony formation should not be observed in the negative control if an SNRM is used.



2. A statistically significant inhibition of colony formation should not be found in the solvent control when a solvent other than medium is used for extraction.

3. In cases where SPRMs are used, the  $IC_{50}$  value of the positive SPRM should not exceed the following values: SPRM-A = 7% and SPRM-B = 70%.

## 18.0 PROTOCOL AMENDMENTS AND DEVIATIONS

If changes in the approved protocol are necessary, such changes will be documented in the form of protocol amendments and protocol deviations. Protocol amendments will be generated when changes in the protocol are made prior to performing a study or part of a study affected by the changes. In such cases, a verbal agreement to make such changes will be made between the Study Director and the Sponsor. These changes and the reasons for them will be documented and attached to the protocol as an addendum. Protocol deviations will be generated when the procedures used to perform the study do not conform to the approved protocol. The Sponsor will be informed of these deviations, and as soon as practical, such changes, along with their reasons or explanations, will be documented and kept in the study notebook.

## 19.0 REPORT OF RESULTS

### 19.1 Content

The results of the study will be submitted to the Sponsor in the form of a final report. The report will include, but not be limited to, the following:

1. Name and address of the facility performing the study, and the dates on which the study was initiated and completed.
2. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
3. Statistical methods employed for analyzing the data.
4. The test and control articles identified by name, chemical abstracts number or code number, strength, purity and composition, or other appropriate characteristics.
5. A description of the methods used.
6. A description of the test system used. Where applicable, the final report shall include the number of cells used and the name and source of the cell line used.
7. A description of the treatment procedures, vehicle used for treatment and duration of treatment.
8. A description of all circumstances that may have affected the quality or integrity of the data.
9. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel involved in the study.



10. A description of the transformations, calculations or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.

11. The signed and dated reports of each of the individual scientists or other professionals involved in the study.

12. The locations where the raw data and final report are to be stored.

13. A statement from the Quality Assurance Unit.

#### 19.2 Changes and Corrections to the Final Report

All changes to the final report will be in the form of report amendments which will include the reason(s) for the change, and these amendments will be added to the final report as an addendum.

### 20.0 ARCHIVES

The raw data, documentation, protocol, electronic file containing the data tables, and the final report of the study will be maintained in the SITEK Research Laboratories Archives, 15235 Shady Grove Road, Suite 303, Rockville, Maryland, according to the terms and conditions of the study.

### 21.0 REFERENCES

1. Test Methods of Cell Toxicology: Japanese Society of Cell Culture. Asakura Shoten (ed.), Tokyo, 1991.

2. Nakamura, A., Y. Ikarashi, T. Tsuchiya, M.-A. Kaniwa, M. Sato, K. Toyoda and M. Takahashi. Correlations among chemical constituents, cytotoxicities and tissue responses: In the cases of natural rubber latex materials. *Biomaterials*, II, *Biomat.*, 89:92-94, 1990.

3. Ikarashi, Y., K. Toyoda, N. Ohsawa, T. Uchima, T. Tsuchiya, M.-A. Kaniwa, M. Sato, M. Takahashi and A. Nakamura. Comparative studies by cell culture and in vivo implantation test on the toxicity of natural rubber latex materials. *J. Biomed. Mater. Res.*, 26:339-356, 1992.

4. Tsuchiya, T., Y. Ikarashi, H. Hata, K. Toyoda, M. Takahashi, T. Uchima, N. Tanaka, K. Sasaki and N. Nakamura. Comparative studies of the toxicity of standard reference materials in various cytotoxicity tests and in vivo implantation tests. *J. Appl. Biomaterials*, 4:153-156, 1993.

5. Tsuchiya, T., T. Arai, J. Ohhashi, K. Imai, H. Kojima, S. Miyamoto, H. Hata, Y. Ikarashi, K. Toyoda, M. Takahashi and A. Nakamura. Rabbit eye irritation caused by wearing toxic contact lenses and their cytotoxicities. In vivo/in vitro correlation study using standard reference materials. *J. Biomed. Mater. Res.*, 27:885-893, 1993.



6. Tsuchiya, T., Y. Ikarashi, T. Arai, J. Ohhashi, K. Isama and A. Nakamura. In vivo tissue/biomaterials toxic responses: Correlation with cytotoxic potential but not cell attachment. *Clinical Materials*, 16:1-8, 1994.

7. Tsuchiya, T. Studies on the standardization of cytotoxicity tests and new standard reference materials useful for evaluating the safety of biomaterials. *J. Biomaterials Appl.*, 9:138-157, 1994.

## PROTOCOL ADDENDUM I

### Preparation of Test Article Extracts

The test article extracts were prepared on a weight to volume ratio of 1.0 gram per 10 mL of extraction fluid. Therefore, 3.5 gram of the material was weighed in a glass flask and 35 mL of culture media was added to the flask. The flask was gassed with 5% CO<sub>2</sub> in air, capped, sealed with parafilm and placed in a shaker incubator set at 37 ± 1°C and 60 rpm for 72 hours. The culture medium from the flask was decanted, filtered through 0.45 μM filter and stored refrigerated until it was used in treatment (within 24 hours).

## PROTOCOL ADDENDUM II

### Positive Control Extract Preparation

The following positive SPRM A and SPRM B extracts were used as positive controls in the Colony Suppression Assay:

SPRM A Lot No.: 96010A Source: Hatano Research Inc.,  
Expiration Date: 10-04-01

SPRM B Lot No.: 96010B Source: Hatano Research Inc.,  
Expiration Date: 10-04-01

Strips of 3x5 cm<sup>2</sup> size were cut from each of the films. The total surface area was 60 cm<sup>2</sup> (2x3x5). The strips were cut into smaller pieces and placed in glass flasks. The flasks were steam sterilized at 15 lbs pressure for 15 minutes. After sterilization, 10 mL (at the ratio of 6 cm<sup>2</sup> surface area per 2.0 mL of culture medium) of culture medium was added to each flask and the flasks were placed at 37 ± 1°C for 72 hours at 60 rpm. At the end of this 72 hours incubation period, the culture medium from each flask was decanted and stored refrigerated until it was used in treatment (within 24 hours).

PROTOCOL AMENDMENT

Amendment No.: 1

Sponsor: Triangle Research and Development  
PO Box 12696  
Research Triangle Park, NC 27709-2696

Testing Facility: SITEK Research Laboratories  
15235 Shady Grove Road, Suite 303  
Rockville, Maryland 20850

SITEK's Study No.: 0538-7760

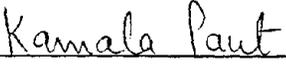
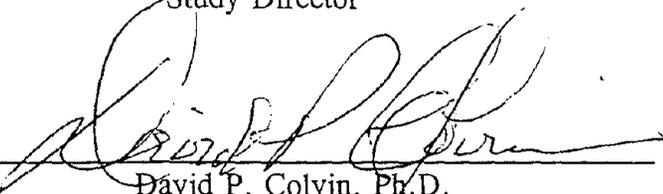
Test Article I.D.: EICOSANE, TG, DRY POWDER

Protocol Title: Mammalian Cell Colony Suppression Test With  
Cultured BALB/c-3T3 Cells

Amendment No. 1: Protocol Page 8, Section 17.4, Evaluation of Test Results - In the first sentence replace the word "valve" with "value".

Reason for Amendment No. 1: Typographical error.

APPROVAL:

 _____ Kamala J. Pant, M.S. Study Director	<u>5.26.99</u> Date
 _____ David P. Colvin, Ph.D. Sponsor's Study Coordinator	<u>6-9-99</u> Date

PROTOCOL DEVIATION

Amendment No.: 1

Sponsor: Triangle Research and Development  
PO Box 12696  
Research Triangle Park, NC 27709-2696

Testing Facility: SITEK Research Laboratories  
15235 Shady Grove Road, Suite 303  
Rockville, Maryland 20850

SITEK's Study No.: 0538-7760

Test Article I.D.: EICOSANE, TG, DRY POWDER

Protocol Title: Mammalian Cell Colony Suppression Test With  
Cultured BALB/c-3T3 Cells

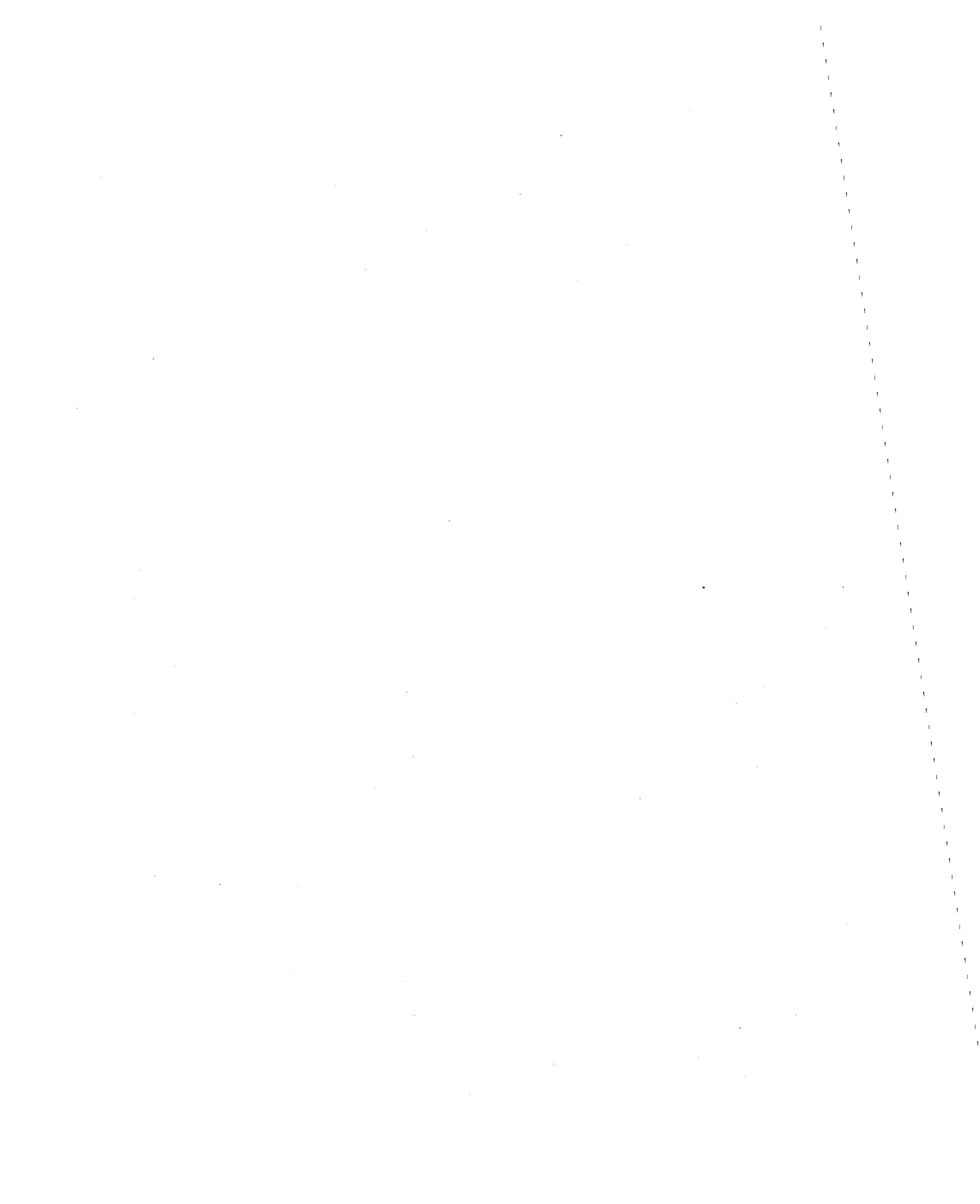
Amendment No. 1: Protocol Page 5, Section 11.0, Abbreviations and Protocol Page 7, Section 17.1, Preparation of Test Cultures 0.25% trypsin EDTA was used for trypsinization instead of 0.5%.

Reason for Amendment No. 1: 0.25% trypsin EDTA dissociates cells more easily. This change in the trypsinization procedure did not have any effect on the study outcome.

APPROVAL:

Kamala Pant  
Kamala J. Pant, M.S.  
Study Director

5-21-99.  
Date





# SITEK RESEARCH LABORATORIES

15235 Shady Grove Road, Suite 303, Rockville, Maryland 20850 • 301/926-4900 FAX 301/926-8891

## FINAL REPORT

### Study Title

Evaluation of a Test Article in the *Salmonella typhimurium*/  
*Escherichia coli* Plate Incorporation Mutation Assay  
in the Presence and Absence of Induced Rat Liver S-9

### Test Article I.D.

Eicosane, TG, Dry Powder

### Author

Kamala J. Pant, M.S.

### Performing Laboratory

SITEK Research Laboratories  
15235 Shady Grove Road, Suite 303  
Rockville, Maryland 20850

### Laboratory Project ID

SITEK Study No. 0538-2140

### Study Initiation Date

March 24, 1999

### Study Completion Date

June 9, 1999

### Sponsor

Triangle Research and Development Corporation  
P. O. Box 12696  
Research Triangle Park, NC 27709-2696

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Study No.: 0538-2140

Sponsor's Test Article I.D.: Eicosane, TG, Dry Powder

The study described in this report was conducted in compliance with the following Good Laboratory Practice standard:

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Revised April 1, 1998.

Signature: Kamala Pant 6.9.99  
Kamala J. Pant, M.S. Date  
Study Director

QUALITY ASSURANCE UNIT'S STATEMENT

Study No.: 0538-2140

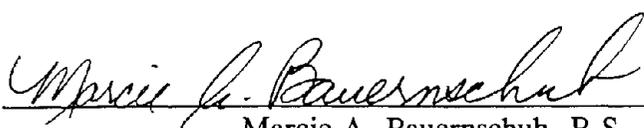
Sponsor's Test Article I.D.: Eicosane, TG, Dry Powder

The performance of this study was audited for adherence to the Good Laboratory Practice regulations for nonclinical laboratory studies by the Quality Assurance Unit of SITEK Research Laboratories. In this context, the facilities, equipment, personnel, methods, practices, controls, original data and reports have been inspected as per SITEK's Quality Assurance Unit's Standard Operating Procedures. The information contained within this report accurately reflects the raw data generated from this study.

Protocol Review Date: 03-26-99

The following phases were inspected for this study:

<u>Inspection Date</u>	<u>Phases Inspected</u>	<u>Date Findings Reported to Study Director</u>	<u>Date Findings Reported to Management</u>
<u>04-27-99</u>	<u>Workbook Audit</u>	<u>04-27-99</u>	<u>04-29-99</u>
<u>04-27-99</u>	<u>Draft Report Audit</u>	<u>04-27-99</u>	<u>04-29-99</u>
<u>06-09-99</u>	<u>Final Report Audit</u>	<u>06-09-99</u>	<u>06-09-99</u>

Signature:  6/9/99  
 Marcie A. Bauernschub, B.S. Date  
 Manager, Quality Assurance Unit

\*Although an in-life critical phase was inadvertently not monitored during the experimental portion of this study, it had no effect on the study's outcome. An SOP Deviation was generated.

**STUDY DIRECTOR SIGNATURE PAGE**

This study was performed under the supervision of Kamala J. Pant, M.S., Study Director for *Salmonella typhimurium* and *Escherichia coli* Gene Mutation Assays, at SITEK Research Laboratories, Suite 303, 15235 Shady Grove Road, Rockville, Maryland 20850.

The Final Report on this study was written by the Study Director and released on June 9, 1999.

Signature

Kamala J. Pant, M.S.  
Study Director

Kamala Pant

6.9.99  
Date

ABSTRACT

The test article, Eicosane, TG, Dry Powder, was tested for its potential to cause mutation at the histidine operon of *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, and at the tryptophan operon of *Escherichia coli* strain WP2uvrA. The test article, mixed in 0.9% saline, was tested for toxicity to strains TA100 and WP2uvrA in a Range Finding Test (RFT) at test article concentrations ranging from 5.0-5000  $\mu\text{g}/\text{plate}$ . It should be noted that 5000  $\mu\text{g}/\text{plate}$  is the highest recommended test article concentration for this assay. The tester strains were exposed to the test article in the absence of exogenous activation and in the presence of induced rat liver S-9 plus cofactors. The toxicity was evaluated based on: 1) reversion frequency, 2) viability, and 3) integrity of the background lawn. In the first Range Finding Test, the S-9 activated portion was lost due to contamination and was repeated in a second experiment.

The Mutation Assay, using the plate incorporation method, was performed with the four *Salmonella typhimurium* tester strains and with *Escherichia coli* strain WP2uvrA. Based on the results of the Range Finding Tests, the test article was tested at the following concentrations in the Mutation Assay:

***Salmonella* Strains and *E. coli* With and Without Activation:**

313, 625, 1250, 2500 and 5000  $\mu\text{g}/\text{plate}$

Both negative and positive controls fulfilled the requirements of the test.

The results of the Mutation Assay indicated that the test article did not induce any significant increase in the number of revertant colonies for any of the tester strains in the presence or absence of induced rat liver S-9.

Under the conditions of this study, Eicosane, TG, Dry Powder was negative in the *Salmonella typhimurium/Escherichia coli* Plate Incorporation Mutation Assay.

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## INTRODUCTION

The purpose of this study was to evaluate the test article, Eicosane, TG, Dry Powder, for its potential to cause mutations in the histidine operon of *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and the tryptophan operon of *Escherichia coli* strain WP2uvrA. The *Salmonella typhimurium/Escherichia coli* Plate Incorporation Mutation Assay has been used extensively and has been demonstrated to be effective in detecting mutations caused by compounds from a wide range of chemical classes.

This study was conducted by Kamala J. Pant, M.S., Lijuan Zhang, B.S., Jie Zhang, M.D., and Laverane Wilson, from April 1, 1999 to April 19, 1999, at SITEK Research Laboratories. The experimental procedures used to perform this study were essentially those of B. N. Ames, et al. (1), D. Maron and B. N. Ames (2), M. H. L. Green and W. J. Muriel (3), and S. Venitt and J. M. Parry (eds.) (4).

## MATERIALS

## TEST ARTICLE

1. Name:	<u>Eicosane, TG, Dry powder</u>
2. Batch/Lot No.:	<u>42-02</u>
3. Physical Appearance:	<u>White powder</u>
4. Date Received:	<u>March 17, 1999</u>
5. Storage Conditions:	<u>Room Temperature</u>
6. Purity Information:	<u>Not provided</u>
7. Expiration Date:	<u>Not provided</u>

## CONTROL SUBSTANCES

Positive Controls

The positive control chemicals used for the tester strains in the presence and absence of exogenous metabolic activation are presented below:

Strain	S-9	Chemical	Concentration ( $\mu\text{g}/\text{plate}$ )
TA98	-	2-NF (2-Nitrofluorene)	5.0
TA98	+	2-AA (2-Aminoanthracene)	1.25
TA100	-	NaAz (Sodium Azide)	1.0
TA100	+	2-AA (2-Aminoanthracene)	1.25
TA1535	-	NaAz (Sodium Azide)	1.0
TA1535	+	2-AA (2-Aminoanthracene)	1.25
TA1537	-	9-AA (9-Aminoacridine)	50
TA1537	+	2-AA (2-Aminoanthracene)	1.25
WP2uvrA	-	MMS (Methyl Methanesulfonate)	4000
WP2uvrA	+	2-AA (2-Aminoanthracene)	10



## INDICATOR CELLS

### Source

The *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 were originally obtained from Dr. Bruce N. Ames, University of California, Berkeley. The *Escherichia coli* strain WP2uvrA was obtained from Dr. Elena C. McCoy, Case Western University, Cleveland, Ohio.

## CULTURE CONDITIONS

The cells were grown in Oxoid Nutrient Broth No. 2 in a shaker incubator rotating at approximately 120 rpm and maintained at a temperature of  $37 \pm 1^\circ\text{C}$ . Stock cultures of the tester strains were cryopreserved at SITEK Research Laboratories. Scrapes from the cryopreserved stock were used to initiate the overnight cultures for the test.

## S-9 METABOLIC ACTIVATION SYSTEM

For the activated portion of the Range Finding Tests or the Mutation Assay, the cells were exposed to the test article in conjunction with an exogenous metabolic activation system consisting of Aroclor 1254 or phenobarbital and  $\beta$ -naphthoflavone-induced rat liver S-9 in 0.15M KCl plus cofactors (S-9 mix). The components of the standard S-9 mix were 8mM  $\text{MgCl}_2$ , 33mM KCl, 5mM glucose-6-phosphate, 4mM NADP, 100mM sodium phosphate buffer (pH 7.4), and 10% rat liver homogenate prepared from Aroclor 1254 or phenobarbital-and  $\beta$ -naphthoflavone induced, Sprague-Dawley rats. The S-9 batches used in this study were also evaluated for their ability to metabolically activate a promutagen in the *Salmonella typhimurium* Plate Incorporation Mutation Assay (Ames Test) in tester strain TA100 using a single dose of 2-AA. The following is the information pertaining to the S-9 batches used in this study:

**SITEK Study No. 0538-2140**

<u>Source:</u>	Molecular Toxicology, Inc.	SITEK Research, Labs.
<u>Inducing Agent:</u>	Aroclor 1254	Phenobarbital and $\beta$ -naphthoflavone
<u>S-9 Batch No.:</u>	825	083198
<u>Protein Content:</u>	35.3 mg/mL,	34.2 mg/mL
<u>Storage Conditions:</u>	$\leq -70^{\circ}\text{C}$	$\leq -70^{\circ}\text{C}$
<u>Preparation Date:</u>	03-24-98	08-31-98
<u>Expiration Date:</u>	03-24-00	08-31-01

## EXPERIMENTAL PROCEDURES

### DOCUMENTATION

The materials, experimental procedures used in the performance of the study, experimental results, and the analytical methods used in the evaluation of the results were documented in the study workbook.

### TEST SYSTEM IDENTIFICATION

#### Labeling Plates for the Range Finding Test and Mutation Assays

A sufficient number of Vogel-Bonner agar plates were removed from refrigerated storage and allowed to warm to room temperature. Each plate was then labeled with the following information: SITEK's test article number, experiment phase, strain code, dose level code, and presence or absence of rat liver S-9 mixture. The following strain and dose level codes were used:

#### Strain Codes:

1 = TA98            3 = TA1535        5 = WP2uvrA  
2 = TA100          4 = TA1537

#### Dose Level Codes:

0 = Solvent for the Test Article  
1 = 1st or highest Test Article dose level  
2 = 2nd Test Article dose level  
3 = 3rd Test Article dose level  
4 = 4th Test Article dose level  
5 = 5th or lowest Test Article dose level for the  
    Mutation Assays  
6 = 6th Test Article dose level  
7 = 7th or lowest Test Article dose level for the Range  
    Finding Test

In addition to the above, the Range Finding Test and Mutation Assay viability plates that contained 10X histidine-biotin or 10X tryptophan were designated with the prefix "T".

### Labeling Positive Control Plates

Vogel-Bonner agar plates were removed from refrigerated storage and allowed to warm to room temperature. Triplicate sets were labeled with the test article number, identity and dose of the particular positive control, experimental phase, strain code, and the presence or absence of rat exogenous metabolic activation.

### Labeling Tester Strain Titer Plates

Each tester strain titer plate was labeled with the following information: SITEK test article number, tester strain identity, and experimental phase and the prefix T.

### Labeling Tester Strain Characterization Plates

#### Histidine Requirement

A single histidine-biotin plate was divided into four zones by drawing horizontal lines on the bottom of the plate with a marking pen and labeling each zone with a different *Salmonella* tester strain. A biotin-only control plate was labeled in a similar manner.

#### rfa Mutation

Nutrient agar plates were labeled with the *Salmonella* tester strain identification and "CV" (crystal violet).

#### R-Factor

A single ampicillin agar plate was labeled in a similar manner as the histidine-biotin plate.

#### Tryptophan Requirement

A tryptophan plate and a Vogel-Bonner agar control plate were labeled with the code for strain WP2uvrA and used for confirmation of the tryptophan requirement.

## SOLUBILITY TEST

Since the Sponsor had recommended saline as the suspending media a solubility test was not performed. The test article formed a homogeneous suspension in saline. Based on this information, it was decided to use 0.9% saline as the solvent.

## PREPARATION OF TEST CULTURES

The methods used for the cryopreservation and cultivation of the tester strains are modifications of the procedures used by B. N. Ames, et al. (1) and D. Maron and B. N. Ames (2).

### Inoculation Procedures

Frozen ampules of strains TA100 and WP2uvrA for the Range Finding Test or strains TA98, TA100, TA1535, TA1537 and WP2uvrA for the Mutation Assays were removed from liquid nitrogen and placed into crushed dry ice to prevent thawing. Scrapes were made using the tip of a sterile pipet, and these scrapes were transferred to a shaker flask containing approximately 50 mL of sterile Oxoid Nutrient Broth No. 2. The flasks were placed in a shaker incubator, and a timer was set to start the unit at a time which allowed the strains to incubate at approximately 120 rpm and  $37 \pm 1^\circ\text{C}$  for a period of 8-12 hours for the *Salmonella* strains and 4-6 hours for the *E. coli* strain before being harvested.

### Harvesting Overnight Cultures

Before starting the experiment, the cultures were sampled and their Percent Transmittance (%T) was determined using a spectrophotometer set to a wavelength of 650 nm.

When the desired cell density of  $5 \times 10^8$  to  $1 \times 10^9$  cells/mL (represented by a %T of between 25% and 10%, Optical Density of 0.6-1.0) was achieved, the cultures were placed on wet ice or kept at 1-5°C until needed.

## PREPARATION OF S-9 METABOLIC ACTIVATION SYSTEM

The S-9 cofactor mix was prepared as follows: For each mL of S-9 cofactor mix required, 0.335 mL of sterile, deionized, distilled water was combined with 0.5 mL of 0.2M sodium phosphate buffer (pH 7.4), 0.04 mL of a 0.1M NADP solution, 5.0  $\mu$ L of 1M glucose-6-phosphate, and 0.02 mL of a 0.4M  $MgCl_2$ /1.65M KCl salt solution. This mixture was maintained on ice until just prior to use, whereupon 0.10 mL of S-9 in 0.15N KCl was added to the mixture.

## PREPARATION OF TEST ARTICLE DOSING SOLUTIONS

The test article was suspended and diluted in 0.9% saline to prepare the dosing solutions. The test article formed homogeneous suspension in 0.9% saline, it was not completely soluble in 0.9% saline. All of the test article and control substance treatments were done under UV-filtered lights to avoid possible problems of photoinactivation.

The strength and stability of the test article and all dosing solutions used in this assay were not determined by SITEK Research Laboratories.

## RANGE FINDING TESTS (A-1 and A-2)

In order to determine the toxicity and to select the appropriate test article concentrations for the Mutation Assays, a Range Finding Test was performed using strains TA100 and WP2uvrA. Seven doses of the test article, ranging from 5.0-5000  $\mu$ g/plate, were evaluated with and without induced rat liver S-9, using one plate per dose. The S-9 activated portion of the first Range Finding Test (A-1) was lost due to contamination. This portion was repeated in a second Range Finding Test (A-2).

### Spontaneous Reversion Frequency

Treatment was performed by adding either 500  $\mu$ L of sterile, deionized, distilled water or 500  $\mu$ L of S-9 cofactor mix to tubes containing 2.0 mL of top agar supplemented with 1X histidine-biotin or 1X tryptophan solution. Immediately thereafter, 100  $\mu$ L of TA100 or WP2uvrA was added, followed by 100  $\mu$ L of the appropriate test article dose or solvent. Although the test article was in suspension in saline it did not form a visible

precipitate in the treatment media at the time of treatment. Each tube was vortexed for 2-3 seconds, and the contents were evenly distributed over a Vogel-Bonner bottom agar plate. Each plate was placed on a level surface until the top agar solidified. The plates were inverted and incubated at  $37 \pm 1^\circ\text{C}$  for approximately 65-70 hours.

### Viability Count Determination

Treatment and incubation were performed as described in the preceding paragraphs, except that approximately 250-500 cells of TA100 or WP2uvrA were added to top agar supplemented with 10X histidine-biotin or 10X tryptophan solution.

After the incubation period was completed, the plates, starting with the highest test article concentration, were observed for the presence of precipitate. Since only slight precipitate was seen at the top test article concentration (5000  $\mu\text{g}/\text{plate}$ ), the plates were counted using an automatic colony counter. The precipitate did not interfere with the counting of the colonies.

Automatic colony counting was performed by making three counts per plate, rotating the plate approximately  $120^\circ$  between each count, and recording the median count. An ARTEK Counter, Model 880, was used for this purpose.

The background lawn was also evaluated. The following notations were used for the precipitate and background lawn evaluation:

#### Chemical Precipitate:

- |    |   |   |
|----|---|---|
| NP | = | No precipitate present.   |
| SP | = | Slight precipitate - Noticeable compound on the plate; however, no influence on automated plate counting. |
| MP | = | Moderate precipitate - Marked precipitate requiring hand counting for colony enumeration.                 |
| HP | = | Heavy precipitate - Large amount of compound on the plate rendering hand counting difficult.              |

**Background Lawn Evaluation:**

- NL = Normal, healthy microcolony lawn.
- SR = A noticeable reduction of the microcolony lawn compared to that of the solvent control plates.
- MR = Marked reduction of the microcolony lawn and an increase in the size of the microcolonies compared to the solvent control plates.
- ER = Extreme reduction of the microcolony lawn and a large increase in the size of the microcolonies compared to the solvent control plates.
- AB = Absence of any microcolony bacterial lawn.
- OP = Obscured by precipitate.

**Determination of Relative Cloning Efficiency**

The corrected viability counts from each dose with and without activation in *Salmonella* strain TA100 and in *Escherichia coli* strain WP2uvrA were compared with the respective solvent control viability counts. The ratio was converted into a percentage, and the data were included in the Range Finding Test results.

**MUTATION ASSAY (B-1)**

Doses for the Mutation Assay were selected based on the results of the Range Finding Tests. The Mutation Assay was performed with the four *Salmonella typhimurium* tester strains and *Escherichia coli* strain WP2uvrA using the plate incorporation method of treatment. The test article was tested with the following concentrations in the Mutation Assay:

***Salmonella* Strains and *E. coli* With and Without Activation:**

313, 625, 1250, 2500 and 5000  $\mu\text{g}/\text{plate}$

Treatment was performed by adding either 500  $\mu\text{L}$  of deionized, distilled water or 500  $\mu\text{L}$  of rat S-9 cofactor mix to tubes containing 2.0 mL of top agar supplemented with 1X histidine-biotin or 1X tryptophan solution. Immediately thereafter, 100  $\mu\text{L}$  of strains

TA98, TA100, TA1535, TA1537 or WP2uvrA were added, followed by 100  $\mu\text{L}$  of the appropriate test article dose or solvent. The positive controls were treated with 100  $\mu\text{L}$  of the appropriate stock solutions. Each tube was vortexed for 2-3 seconds, and the contents were evenly distributed over a Vogel-Bonner bottom agar plate. Each plate was placed on a level surface until the top agar solidified. The plates then were inverted and incubated at  $37 \pm 1^\circ\text{C}$  for 66 hours.

#### **Tester Strain Titer Determination**

Each tester strain was diluted to determine the approximate number of viable cells delivered to the assay plates. Therefore, approximately 250-500 cells were added to top agar supplemented with 10X histidine-biotin or 10X tryptophan solution. Each tube was vortexed for 2-3 seconds, and the contents were evenly distributed on bottom agar plates. The plates were incubated at  $37 \pm 1^\circ\text{C}$  for approximately 66 hours.

#### **Tester Strain Characterization**

All of the *Salmonella typhimurium* strains used in the assay were confirmed for the histidine requirement and the rfa mutation. In addition, strains TA98 and TA100 were tested for the presence of the pKM101 plasmid. *Escherichia coli* strain WP2uvrA was confirmed for the tryptophan requirement.

#### **Histidine or Tryptophan Requirement**

A streak of each tester strain was made by dipping a flamed wire loop into the appropriate undiluted tester strain suspension and drawing it across the surface in the appropriate region of a labeled histidine-biotin or tryptophan plate, as well as control plates. The plates were incubated at  $37 \pm 1^\circ\text{C}$  for approximately 18 hours.

#### **rfa Mutation**

For each of the *Salmonella* tester strains, a 100  $\mu\text{L}$  aliquot of the undiluted culture was added to a tube containing 2.0 mL of 1X histidine-biotin solution top agar. Each tube was vortexed for 2-3 seconds, and the contents were poured onto an appropriately labeled nutrient agar plate. After allowing the plate to solidify, a sterile disc was aseptically placed in the center of the agar overlay. 10  $\mu\text{L}$  of a 1.0 mg/mL crystal violet solution was then added to the disc. The plates were incubated at  $37 \pm 1^\circ\text{C}$  for approximately 18 hours.

### R-Factor Plasmid

A streak of each of the *Salmonella* tester strains was made by dipping a flamed wire loop into the appropriate suspension and drawing it across the surface in the appropriate region of an ampicillin plate. The plates were incubated at  $37 \pm 1^\circ\text{C}$  for approximately 18 hours.

### Evaluation of Assay Results

After the incubation period was completed, the plates, starting with the highest test article concentration, were observed for the presence of precipitate. Plates having no interfering precipitate were counted using an automatic colony counter (ARTEK Counter, Model 880). Plates with precipitate that interfered with automatic counting were counted by hand. The method of counting was the same as that used in the Range Finding Test.

The background lawn was also evaluated. The notations for the precipitate and background lawn evaluation were the same as for the Range Finding Test.

### Evaluation of Tester Strain Characterization

The requirement for histidine or tryptophan was demonstrated by the growth of the tester strains on plates supplemented with histidine or tryptophan and the lack of growth on the control plates.

The presence of the *rfa* mutation was evaluated by measuring the zone of inhibition around the crystal violet disc. A zone about 14-15 mm in diameter was evidence of appropriate inhibition.

The presence of the pKM101 plasmid was demonstrated by the growth of strains TA98 and TA100 and the lack of growth of strains TA1535 and TA1537 streaked on ampicillin plates.

Tabulation of Colony Counts

The colony counts provided by the automatic colony counter were raw counts and were not corrected to reflect actual counts. Correction of the counts was performed by computer. The correction factor was determined by comparing a wide range of manual and automatic counts, as described in SITEK's SOP No. 21.0. The relationship was linear, and the counts were corrected by using the following formula:

$$\text{Corrected Count} = \text{Raw Counts} (1.0571607) + 3.09496$$

## CRITERIA FOR A VALID ASSAY

The following criteria were used as guidelines in evaluating the acceptability of the Mutation Assay. Since it is impossible to formulate criteria that would apply to every configuration of data generated by the assay, the Study Director was responsible for the ultimate decision regarding the acceptability of the results.

### Solvent Control Cultures

The mean reversion frequency of the test article solvent control plates for each tester strain should have fallen within the following ranges:

TA98	20 ± 15	WP2uvrA	15 ± 10
TA100	100 ± 70		
TA1535	20 ± 15		
TA1537	15 ± 12		

### Positive Controls

The results for the positive control cultures were considered acceptable if the treated strains had a mean reversion frequency that was three times or more greater than the mean reversion frequency of the solvent control plates.

### Tester Strain Characterization

All of the *Salmonella typhimurium* strains were confirmed positive for histidine dependence. *Escherichia coli* strain WP2uvrA was confirmed positive for tryptophan dependence.

All of the *Salmonella typhimurium* strains were confirmed positive for the *rfa* mutation as evidenced by sensitivity to crystal violet.

The R-factor strains, TA98 and TA100, were confirmed positive for the pKM101 plasmid as evidenced by ampicillin resistance.

The titer of the stock cultures for each strain indicated that the stock cultures contained approximately between  $5.0 \times 10^8$  and  $1.0 \times 10^9$  bacteria per mL.

## EVALUATION OF TEST RESULTS

The following criteria were used as guidelines in evaluating the results of the Mutation Assay for a negative, positive or equivocal response. Since it is impossible to write criteria that would apply to every configuration of data generated by the assay, the Study Director was responsible for the ultimate decision concerning the results.

### Criteria for a Negative Response

A response was considered to be negative if all of the strains treated with the test article had mean reversion frequencies that were no greater than twice that of the mean reversion frequencies of the corresponding solvent control plates in TA98 and TA100 and no greater than three times in TA1535, TA1537 and WP2uvrA, and there was no evidence of a dose-dependent response.

### Criteria for a Positive Response

A response was considered to be positive if either strain TA98 or TA100 exhibited a mean reversion frequency that was at least double the mean reversion frequency of the corresponding solvent control in at least one dose, or if either strain TA1535, TA1537 or WP2uvrA exhibited a threefold increase in the mean reversion frequency compared to the solvent control in at least one dose. In addition, the response must have been dose dependent or increasing concentrations of the test article must have showed increasing mean reversion frequencies. In evaluating the results, consideration was given to the degree of toxicity exhibited by the dose causing the twofold/threefold or greater increase in reversion frequency and the magnitude of the increase in reversion frequency.

### Criteria for an Equivocal Response

A response was considered equivocal if it did not fulfill the criteria of either a negative or a positive response and/or the Study Director did not consider the response to be either positive or negative.

**ARCHIVES**

All of the raw data, documentation, protocol, electronic file containing the data tables and draft report of the study are maintained at SITEK Research Laboratories' Archives at 15235 Shady Grove Road, Suite 303, Rockville, Maryland 20850, according to the terms and conditions of the study.

## RESULTS

### RANGE FINDING TESTS (A-1 and A-2)

Summaries of the results of the Range Finding Tests are presented in Tables 1-4 and included in Appendix I. The individual plate counts and background lawn evaluation are presented in Appendix II. Since the first Range Finding Test (A-1) using activation was lost due to contamination, a second Range Finding Test (A-2) with activation only, was performed. The results of both Range Finding Tests are summarized below.

#### TA100 Without Activation:

There were essentially no signs of toxicity related to test article treatment. There was no reduction in the microcolony lawn, and no reduction in the number of revertants either. The Relative Cloning Efficiencies (RCE) for the test article treated plates were 74% and 97% for the test article concentrations of 10 and 500  $\mu\text{g}/\text{plate}$ , respectively. All the other concentrations had RCEs greater than 100%.

#### TA100 With Activation:

There were essentially no signs of toxicity related to test article treatment. There was no reduction in the microcolony lawn, and no reduction in the number of revertants either. The Relative Cloning Efficiencies (RCE) for all of the test article concentrations were more than 100%.

#### WP2uvrA Without Activation:

There were essentially no signs of toxicity related to test article treatment. There was no reduction in either the microcolony lawn or the number of revertants. The Relative Cloning Efficiencies (RCE) for the test article treated plates were greater than 100% for the concentrations ranging from 5.0 to 5000  $\mu\text{g}/\text{plate}$ , except for the 10 and 500  $\mu\text{g}/\text{plate}$  concentrations which had RCEs of 87% and 88%, respectively.

#### WP2uvrA With Activation:

There were essentially no signs of toxicity related to test article treatment. There was no reduction in either the microcolony lawn or the number of revertants. The Relative Cloning Efficiencies (RCE) for the test article treated plates ranged from 40% to 93% for the concentrations ranging from 5.0 to 5000  $\mu\text{g}/\text{plate}$ .

## MUTATION ASSAY (B-1)

Summaries of the results of the Mutation Assay are presented in Tables 5 and 6 included in Appendix I. The individual plate counts and background lawn evaluation are presented in Appendix II.

The Mutation Assay (using the plate incorporation method) was performed with the four *Salmonella* tester strains and with *E. coli* strain WP2uvrA. Based on the results of the Range Finding Tests, the following concentrations were tested in the Mutation Assay:

### ***Salmonella* Strains and *E. coli* With and Without Activation:**

313, 625, 1250, 2500 and 5000  $\mu\text{g}/\text{plate}$

All the test article concentrations were nontoxic. The test article did not induce any significant increase in the number of revertant colonies for any of the test strains with or without activation as compared to the solvent control. The average number of revertants per plate were within the acceptable range for the solvent controls. The positive controls also fulfilled the requirements for a valid assay.

## CONCLUSIONS

The test article, Eicosane, TG, Dry Powder, was tested in the *Salmonella typhimurium*/*Escherichia coli* Plate Incorporation Mutation Assay in the presence and absence of induced rat liver S-9.

The Mutation Assay, using the plate incorporation method, was performed with the four *Salmonella typhimurium* tester strains and *Escherichia coli* strain WP2uvrA.

The results of the Mutation Assay indicated that the test article did not induce any significant increase in the number of revertant colonies for any of the tester strains in the presence or absence of induced rat liver S-9. The positive and negative controls fulfilled the requirements of the test.

Under the conditions of this study, Eicosane, TG, Dry Powder was negative in the *Salmonella typhimurium*/*Escherichia coli* Plate Incorporation Mutation Assay.

REFERENCES

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4. Venitt, S., and J. M. Parry (eds.). *Mutagenicity Testing: A Practical Approach*. IRL Press, Oxford, England and Washington, D.C., 1984.

APPENDIX I

DATA TABLES

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TABLE 1

SITEK Study No. 0538-2140

SALMONELLA TYPHIMURIUM PLATE INCORPORATION MUTATION ASSAY  
RANGE FINDING TEST RESULTS

SPONSOR: Triangle Research & Development      SITEK STUDY NO.: 0538-2140  
EXPERIMENT NO.: A-1      SOLVENT: 0.9% Saline  
TEST ARTICLE: Eicosane, TG, Dry powder      STRAIN: TA100

WITHOUT ACTIVATION						WITH S-9 ACTIVATION					
Test Article Conc. µg/plate	No. of Rever-tants/ Plate	Chem. PPT. Eval.*	Back-ground Lawn Evalu-ation**	No. of Viable Colo-nies/ Plate	Rela-tive Cloning Effi-ciency (RCE)	Test Article Conc. µg/plate	No. of Rever-tants/ Plate	Chem. PPT. Eval.*	Back-ground Lawn Evalu-ation**	No. of Viable Colo-nies/ Plate	Rela-tive Cloning Effi-ciency (RCE)
5.0	74	NP	NL	1106	145%	This portion of the experiment was lost due to contamination. It was repeated in the second Range Finding Test (A-2)					
10	64	NP	NL	563	74%						
50	78	NP	NL	1267	166%						
100	70	NP	NL	887	117%						
500	86	NP	NL	741	97%						
1000	61	NP	NL	879	116%						
5000	94	SP	NL	785	103%						
SOLV. CONT.	78	NP	NL	761	100%						

$$RCE = \frac{\text{No. of Colonies in Test Plates}}{\text{No. of Colonies in Solvent Control Plates}} \times 100$$

## \* Chemical Precipitate Evaluation

NP = No precipitate

SP = Slight precipitate; noticeable precipitate on the plate, but no interference with automated plate counting

MP = Moderate precipitate; marked precipitate necessitating hand counting for colony enumeration

HP = Heavy precipitate; large amount of precipitate rendering hand counting difficult or impossible

## \*\* Background Lawn Evaluation

NL = Normal, healthy microcolony lawn

SR = Noticeable thinning of the microcolony lawn compared to control

MR = Marked thinning of the microcolony lawn and increase in size of microcolonies compared to control

ER = Extreme thinning of the microcolony lawn and large increase in size of microcolonies compared to control

AB = Absence of microcolonies

OP = Obscured by precipitate

TABLE 2

SITEK Study No. 0538-2140

ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY  
RANGE FINDING TEST RESULTS

SPONSOR: Triangle Research & Development      SITEK STUDY NO.: 0538-2140  
 EXPERIMENT NO.: A-1      SOLVENT: 0.9% Saline  
 TEST ARTICLE: Eicosane, TG, Dry powder      STRAIN: WP2uvrA

WITHOUT ACTIVATION						WITH S-9 ACTIVATION					
Test Article Conc. µg/plate	No. of Rever-Chem. tants/ PPT. Plate Eval.*	Back-ground Lawn Evalu-ation**	No. of Viable Colo-nies/ Plate	Rela-tive Cloning Effi-ciency (RCE)		Test Article Conc. µg/plate	No. of Rever-Chem. tants/ PPT. Plate Eval.*	Back-ground Lawn Evalu-ation**	No. of Viable Colo-nies/ Plate	Rela-tive Cloning Effi-ciency (RCE)	
5.0	14	NP	NL	1249	116%	This portion of the experiment was lost due to contamination. It was repeated in the second Range Finding Test (A-2)					
10	14	NP	NL	931	87%						
50	14	NP	NL	1933	180%						
100	12	NP	NL	1838	171%						
500	16	NP	NL	945	88%						
1000	16	NP	NL	1282	119%						
5000	12	NP	NL	1364	127%						
SOLV. CONT.	16	NP	NL	1075	100%						

$$RCE = \frac{\text{No. of Colonies in Test Plates}}{\text{No. of Colonies in Solvent Control Plates}} \times 100$$

## \* Chemical Precipitate Evaluation

NP = No precipitate

SP = Slight precipitate; noticeable precipitate on the plate, but no interference with automated plate counting

MP = Moderate precipitate; marked precipitate necessitating hand counting for colony enumeration

HP = Heavy precipitate; large amount of precipitate rendering hand counting difficult or impossible

## \*\* Background Lawn Evaluation

NL = Normal, healthy microcolony lawn

SR = Noticeable thinning of the microcolony lawn compared to control

MR = Marked thinning of the microcolony lawn and increase in size of microcolonies compared to control

ER = Extreme thinning of the microcolony lawn and large increase in size of microcolonies compared to control

AB = Absence of microcolonies

OP = Obscured by precipitate

TABLE 3

SITEK Study No. 0538-2140

SALMONELLA TYPHIMURIUM PLATE INCORPORATION MUTATION ASSAY  
RANGE FINDING TEST RESULTS

SPONSOR: Triangle Reseach & Development      SITEK STUDY NO.: 0538-2140  
 EXPERIMENT NO.: A-2      SOLVENT: 0.9% Saline  
 TEST ARTICLE: Eicosane, TG, Dry powder      STRAIN: TA100

WITHOUT ACTIVATION					WITH S-9 ACTIVATION				
Test Article Conc. µg/plate	No. of Rever-Chem. tants/ Plate	Back-ground Lawn Evalu-ation**	No. of Viable Colo-nies/ Plate	Rela-tive Cloning Effi-ciency (RCE)	Test Article Conc. µg/plate	No. of Rever-Chem. tants/ Plate	Back-ground Lawn Evalu-ation**	No. of Viable Colo-nies/ Plate	Rela-tive Cloning Effi-ciency (RCE)
This portion of the experiment was not repeated.	5.0	63	NP	NL	849	113%			
	10	71	NP	NL	842	112%			
	50	59	NP	NL	925	123%			
	100	53	NP	NL	939	125%			
	500	49	NP	NL	948	126%			
	1000	67	NP	NL	929	123%			
	5000	58	SP	NL	876	116%			
	SOLV. CONT.	68	NP	NL	754	100%			

$$RCE = \frac{\text{No. of Colonies in Test Plates}}{\text{No. of Colonies in Solvent Control Plates}} \times 100$$

## \* Chemical Precipitate Evaluation

NP = No precipitate

SP = Slight precipitate; noticeable precipitate on the plate, but no interference with automated plate counting

MP = Moderate precipitate; marked precipitate necessitating hand counting for colony enumeration

HP = Heavy precipitate; large amount of precipitate rendering hand counting difficult or impossible

## \*\* Background Lawn Evaluation

NL = Normal, healthy microcolony lawn

SR = Noticeable thinning of the microcolony lawn compared to control

MR = Marked thinning of the microcolony lawn and increase in size of microcolonies compared to control

ER = Extreme thinning of the microcolony lawn and large increase in size of microcolonies compared to control

AB = Absence of microcolonies

OP = Obscured by precipitate

TABLE 4

SITEK Study No. 0538-2140

ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY  
RANGE FINDING TEST RESULTS

SPONSOR: Triangle Reseach & Development      SITEK STUDY NO.: 0538-2140  
 EXPERIMENT NO.: A-2      SOLVENT: 0.9% Saline  
 TEST ARTICLE: Eicosane, TG, Dry powder      STRAIN: WP2uvrA

WITHOUT ACTIVATION					WITH S-9 ACTIVATION				
Test Article Conc. µg/plate	No. of Rever-Chem. tants/ PPT. Plate Eval.*	Back-ground Lawn Eval-u-ation**	No. of Viable Colo-nies/ Plate	Rela-tive Cloning Effi-ciency (RCE)	Test Article Conc. µg/plate	No. of Rever-Chem. tants/ PPT. Plate Eval.*	Back-ground Lawn Eval-u-ation**	No. of Viable Colo-nies/ Plate	Rela-tive Cloning Effi-ciency (RCE)
This portion of the experiment was not repeated.					5.0	17	NP	NL	1534 93%
					10	13	NP	NL	1005 61%
					50	10	NP	NL	919 55%
					100	14	NP	NL	778 47%
					500	14	NP	NL	664 40%
					1000	18	NP	NL	1279 77%
					5000	12	SP	NL	1290 78%
					SOLV. CONT.	10	NP	NL	1658 100%

$$\text{RCE} = \frac{\text{No. of Colonies in Test Plates}}{\text{No. of Colonies in Solvent Control Plates}} \times 100$$

## \* Chemical Precipitate Evaluation

NP = No precipitate

SP = Slight precipitate; noticeable precipitate on the plate, but no interference with automated plate counting

MP = Moderate precipitate; marked precipitate necessitating hand counting for colony enumeration

HP = Heavy precipitate; large amount of precipitate rendering hand counting difficult or impossible

## \*\* Background Lawn Evaluation

NL = Normal, healthy microcolony lawn

SR = Noticeable thinning of the microcolony lawn compared to control

MR = Marked thinning of the microcolony lawn and increase in size of microcolonies compared to control

ER = Extreme thinning of the microcolony lawn and large increase in size of microcolonies compared to control

AB = Absence of microcolonies

OP = Obscured by precipitate

SITEK Study No. 0538-2140

TABLE 5  
SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY  
 MUTATION ASSAY RESULTS - WITHOUT ACTIVATION

SPONSOR: Triangle Research&Development      SITEK STUDY NO.: 0538-2140  
 EXPERIMENT NO.: B-1      SOLVENT: 0.9% Saline  
 TEST ARTICLE: Eicosane, TG, Dry Powder      CONC. IN:  $\mu$ g/plate

<u>S. typhimurium</u>		Average No. of Revertants Per Plate						
		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: TA98 DATE PLATED: 4/14/99 CELLS SEEDED: $1.086 \times 10^8$	REVERTANTS	673	32	28	25	26	28	37
	STD. DEV.	104	1	5	3	5	9	18
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100 DATE PLATED: 4/14/99 CELLS SEEDED: $1.086 \times 10^8$	REVERTANTS	506	83	74	73	77	86	85
	STD. DEV.	19	7	6	13	5	12	20
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1535 DATE PLATED: 4/14/99 CELLS SEEDED: $1.740 \times 10^8$	REVERTANTS	441	17	10	9	13	15	16
	STD. DEV.	17	2	4	2	5	1	2
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537 DATE PLATED: 4/14/99 CELLS SEEDED: $0.862 \times 10^8$	REVERTANTS	220	11	10	7	9	11	7
	STD. DEV.	13	4	3	2	1	6	1
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

<u>E. coli</u>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: WP2uvrA DATE PLATED: 4/14/99 CELLS SEEDED: $1.432 \times 10^8$	REVERTANTS	648	25	23	22	23	23	24
	STD. DEV.	23	2	1	1	2	2	2
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn.

NP = No precipitate.

TABLE 6  
 SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY  
 MUTATION ASSAY RESULTS - WITH S-9 ACTIVATION

SPONSOR: Triangle Research&Development  
 EXPERIMENT NO.: B-1  
 TEST ARTICLE: Eicosane, TG, Dry Powder

SITEK STUDY NO.: 0538-2140  
 SOLVENT: 0.9% Saline  
 CONC. IN:  $\mu\text{g}/\text{plate}$

S. typhimurium		Average No. of Revertants Per Plate						
		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: TA98	REVERTANTS	1442	30	33	29	37	31	28
DATE PLATED: 4/14/99	STD. DEV.	172	4	2	5	5	4	6
CELLS SEEDED: $1.086 \times 10^8$	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100	REVERTANTS	1403	59	60	52	66	62	63
DATE PLATED: 4/14/99	STD. DEV.	129	14	3	10	18	2	6
CELLS SEEDED: $1.082 \times 10^8$	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1535	REVERTANTS	205	9	12	11	14	16	14
DATE PLATED: 4/14/99	STD. DEV.	19	1	3	3	5	5	5
CELLS SEEDED: $1.740 \times 10^8$	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537	REVERTANTS	103	5	8	5	5	7	6
DATE PLATED: 4/14/99	STD. DEV.	6	4	2	1	1	3	2
CELLS SEEDED: $0.862 \times 10^8$	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
E. coli		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: WP2uvrA	REVERTANTS	116	23	20	23	24	19	18
DATE PLATED: 4/14/99	STD. DEV.	9	2	2	2	1	2	2
CELLS SEEDED: $1.432 \times 10^8$	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn.

NP = No precipitate.

APPENDIX II  
DETAILED PLATE COUNTS AND  
BACKGROUND LAWN EVALUATION

	<u>Page No.</u>
First Range Finding Test (A-1)	37 - 38
First Range Finding Test (A-2)	39 - 40
Mutation Assay (B-1)	41 - 44









SITEK Study No. 0538-2140

SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY  
MUTATION ASSAY RAW COLONY COUNTS AND BACKGROUND LAWN EVALUATION

EXPERIMENT NO.: B-1  
TEST ARTICLE: Eicosane, TG, Dry Powder

SITEK STUDY NO.: 0538-2140  
SOLVENT: 0.9% Saline  
CONC. IN: µg/plate

WITHOUT ACTIVATION

<u>S. typhimurium</u>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: TA98	REVERTANTS	569	27	21	24	22	16	20
	PER	585	27	21	18	26	33	25
	PLATE	747	28	29	20	17	23	51
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDDED: 1.086 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100	REVERTANTS	495	76	62	63	66	91	79
	PER	474	82	73	79	69	68	95
	PLATE	460	70	65	55	75	76	58
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDDED: 1.082 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1535	REVERTANTS	419	13	6	7	5	10	12
	PER	427	11	11	4	13	12	13
	PLATE	397	14	4	6	11	12	10
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDDED: 1.740 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537	REVERTANTS	210	9	4	2	5	2	4
	PER	215	4	8	5	6	12	4
	PLATE	192	9	7	5	6	9	5
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDDED: 0.862 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

<u>E. coli</u>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: WP2uvrA	REVERTANTS	635	20	18	18	19	18	22
	PER	593	23	18	18	21	21	20
	PLATE	603	20	20	19	18	18	19
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDDED: 1.432 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn.

NP = No precipitate.

SITEK Study No. 0538-2140

SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY  
MUTATION ASSAY CORRECTED COLONY COUNTS AND BACKGROUND LAWN EVALUATION

EXPERIMENT NO.: B-1  
TEST ARTICLE: Eicosane, TG, Dry Powder

SITEK STUDY NO.: 0538-2140  
SOLVENT: 0.9% Saline  
CONC. IN: µg/plate

WITHOUT ACTIVATION

<i>S. typhimurium</i>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: TA98 DATE PLATED: 4/14/99 CELLS SEEDED: 1.086 x 10 <sup>8</sup>	REVERTANTS	605	32	25	28	26	20	24
	PER PLATE	622	32	25	22	31	38	30
		793	33	34	24	21	27	57
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100 DATE PLATED: 4/14/99 CELLS SEEDED: 1.082 x 10 <sup>8</sup>	REVERTANTS	526	83	69	70	73	99	87
	PER PLATE	504	90	80	87	76	75	104
		489	77	72	61	82	83	64
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1535 DATE PLATED: 4/14/99 CELLS SEEDED: 1.740 x 10 <sup>8</sup>	REVERTANTS	446	17	9	10	8	14	16
	PER PLATE	455	15	15	7	17	16	17
		423	18	7	9	15	16	14
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537 DATE PLATED: 4/14/99 CELLS SEEDED: 0.862 x 10 <sup>8</sup>	REVERTANTS	225	13	7	5	8	5	7
	PER PLATE	230	7	12	8	9	16	7
		206	13	10	8	9	13	8
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

<i>E. coli</i>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: WP2uvrA DATE PLATED: 4/14/99 CELLS SEEDED: 1.432 x 10 <sup>8</sup>	REVERTANTS	674	24	22	22	23	22	26
	PER PLATE	630	27	22	22	25	25	24
		641	24	24	23	22	22	23
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn.

NP = No precipitate.

SITEK Study No. 0538-2140

SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY  
MUTATION ASSAY RAW COLONY COUNTS AND BACKGROUND LAWN EVALUATION

EXPERIMENT NO.: B-1  
 TEST ARTICLE: Eicosane, TG, Dry Powder

SITEK STUDY NO.: 0538-2140  
 SOLVENT: 0.9% Saline  
 CONC. IN: µg/plate

WITH S-9 ACTIVATION

<i>S. typhimurium</i>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: TA98 DATE PLATED: 4/14/99 CELLS SEEDED: 1.086 x 10 <sup>8</sup>	REVERTANTS	1186	22	27	24	30	23	20
	PER PLATE	1390	25	26	30	28	26	29
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100 DATE PLATED: 4/14/99 CELLS SEEDED: 1.082 x 10 <sup>8</sup>	REVERTANTS	1227	42	55	54	58	56	63
	PER PLATE	1461	49	50	49	43	58	54
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1535 DATE PLATED: 4/14/99 CELLS SEEDED: 1.740 x 10 <sup>8</sup>	REVERTANTS	198	6	8	9	14	15	12
	PER PLATE	171	6	10	8	10	15	14
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537 DATE PLATED: 4/14/99 CELLS SEEDED: 0.862 x 10 <sup>8</sup>	REVERTANTS	98	0	6	3	2	7	2
	PER PLATE	96	4	3	2	1	4	1
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

<i>E. coli</i>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: WP2uvrA DATE PLATED: 4/14/99 CELLS SEEDED: 1.432 x 10 <sup>8</sup>	REVERTANTS	115	19	14	21	21	14	12
	PER PLATE	98	17	17	20	20	13	14
	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn.

NP = No precipitate.

SITEK Study No. 0538-2140

SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY  
 MUTATION ASSAY CORRECTED COLONY COUNTS AND BACKGROUND LAWN EVALUATION

EXPERIMENT NO.: B-1  
 TEST ARTICLE: Eicosane, TG, Dry Powder

SITEK STUDY NO.: 0538-2140  
 SOLVENT: 0.9% Saline  
 CONC. IN: µg/plate

WITH S-9 ACTIVATION

<u>S. typhimurium</u>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: TA98	REVERTANTS	1257	26	32	28	35	27	24
	PER	1473	30	31	35	33	31	34
	PLATE	1597	33	35	25	43	34	25
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 1.086 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100	REVERTANTS	1300	47	61	60	64	62	70
	PER	1548	55	56	55	49	64	60
	PLATE	1360	75	62	41	84	60	60
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 1.082 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1535	REVERTANTS	212	9	12	13	18	19	16
	PER	184	9	14	12	14	19	18
	PLATE	220	10	9	8	9	10	9
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 1.740 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537	REVERTANTS	107	0	9	6	5	10	5
	PER	105	7	6	5	4	7	4
	PLATE	96	7	9	4	6	5	8
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 0.862 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

<u>E. coli</u>		Positive Control	Solvent Control	Concentration per plate				
				313	625	1250	2500	5000
STRAIN: WP2uvrA	REVERTANTS	125	23	18	25	25	18	16
	PER	107	21	21	24	24	17	18
	PLATE	115	24	21	21	23	21	19
DATE PLATED: 4/14/99	LAWN	NL	NL	NL	NL	NL	NL	NL
CELLS SEEDED: 1.432 x 10 <sup>8</sup>	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn.

NP = No precipitate.

APPENDIX III

STUDY PROTOCOL AND PROTOCOL AMENDMENTS



EVALUATION OF A TEST ARTICLE IN THE SALMONELLA TYPHIMURIUM/  
ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY IN THE  
PRESENCE AND ABSENCE OF INDUCED RAT LIVER S-9

This protocol is presented in two parts. Part One is designed to collect specific information pertaining to the test article and study. Part Two describes the study design in detail. Please complete all sections in Part One and sign section 8.0 to approve the protocol.

PART ONE

1.0 SPONSOR

1.1 Name: TRIANGLE RESEARCH AND DEVELOPMENT CORPORATION

1.2 Address: P.O. BOX 12696  
RESEARCH TRIANGLE PARK, NC 27709-2696

1.3 Sponsor's Study Coordinator: DAVID P. COLVIN, Ph.D.

2.0 TESTING FACILITY

2.1 Name: SITEK Research Laboratories

2.2 Address: 15235 Shady Grove Road, Suite 303  
Rockville, Maryland 20850

2.3 Study Director: Kamala J. Pant, M.S.

3.0 STUDY NUMBERS

\*3.1 Testing Facility's Study No.: 0538  
~~0537~~-2140  
ee K<sup>2</sup>3/18/99

3.2 Sponsor's Study No.: \_\_\_\_\_

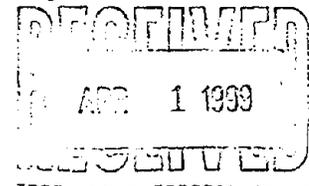
4.0 TEST ARTICLE

4.1 Identification

Name: Eicosane, TG, Dry powder

Batch/Lot No.: 42-02

\* To be completed by the Testing Facility.





4.2 Description

Color: white

Physical Form: Powder

4.3 Analysis

Purity Information: \_\_\_\_\_

Does the Sponsor require the use of a correction factor to account for impurity?

Yes  No

If yes, what is the correction factor? \_\_\_\_\_

Determination of the test article characteristics as defined by Good Laboratory Practices will be the responsibility of the Sponsor. The specific GLP references for U.S. agencies are: FDA =21 CFR, 58.105; EPA TSCA =40 CFR, 792.105 and EPA FIFRA =40 CFR 160.105.

4.4 Stability

Storage Conditions (check one):

Room Temperature  Refrigerated (1-5°C)

Frozen (-10 to -20°C)

Other (please specify): \_\_\_\_\_

Expiration Date: \_\_\_\_\_

4.5 Preferred Solvent (check one):

H<sub>2</sub>O  DMSO

Acetone  Ethanol

Other (please specify): 0.9% Saline

To be decided by the Testing Facility

4.6 Special Handling Instructions:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



### 5.0 REGULATORY AGENCY SUBMISSION

This study will be conducted in compliance with the following Good Laboratory Practice standards:

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Parts 160 and 792, Revised July 1, 1997.

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Revised April 1, 1998.

Japanese Ministry of Agriculture, Forestry and Fisheries, 59 Nohsan, Notification No. 3850, Agriculture Production Bureau, August 10, 1984.

Japanese Ministry of Health and Welfare, Ordinance No. 21, April 1, 1997.

Japanese Ministry of International Trade and Industry, Notification No. 85, Basic Industries Bureau, March 31, 1984.

Organisation for Economic Cooperation and Development, The OECD Principles of Good Laboratory Practice, Environment Monograph No. 45, Paris 1992.

Will this study be submitted to a regulatory agency?

Yes       No

If so, which agency(ies)? FDA

### 6.0 TEST ARTICLE/DOSING SOLUTIONS CHARACTERIZATION

The U.S. requirements for analysis of dosing solutions are specified in: FDA =21 CFR, 58.113; EPA TSCA =40 CFR, 792.113; and EPA FIFRA =40 CFR, 160.113.

Does the Sponsor want dosing solution analysis?

Yes\*\*       No

If yes, please complete the rest of this section.

If requested by the Sponsor, SITEK Research Laboratories will determine the strength and stability of the dosing solutions. The method of analysis may be provided by the Sponsor, or if requested by the Sponsor, SITEK Research Laboratories will develop the method of analysis.

\*\* Additional charges will apply. See Special Services price schedule.



Alternatively, the Sponsor will be responsible for determining the strength and stability of the dosing solutions.

Dosing solution analysis will be performed by:

SITEK Research Laboratories  Sponsor

What dosing solutions will be analyzed?

From the Range Finding Test?

Yes  No

From the Assay?

Yes  No

Which concentration(s)? \_\_\_\_\_

What amount of each concentration? \_\_\_\_\_

At what temperature should the dosing solutions be stored?

Room Temperature  Frozen (-10 to -20°C)

Refrigerated (1-5°C)

7.0 STUDY DATES

\*7.1 Proposed Experimental Start Date: 3.26.99

Defined as the first date the test article is applied to the test system.

\*7.2 Anticipated Experimental Completion Date: 4.26.99

Defined as the last date on which data are collected directly from the study.

\*To be completed by the Testing Facility.



8.0 PROTOCOL APPROVAL

\* Kamala Pant 3.24.99.  
Study Director Date

[Signature] 3-24-99  
Sponsor's Study Coordinator Date

\* [Signature] 3/26/99  
Quality Assurance Manager Date

\* [Signature] 3/26/99  
Safety Officer Date

\*To be completed by the Testing Facility.



## STUDY DESIGN

PART TWO

## 9.0 PURPOSE

The purpose of this study is to evaluate the test article for its potential to cause mutations in the histidine operon of Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 and the tryptophan operon of Escherichia coli strain WP2uvrA.

## 10.0 JUSTIFICATION FOR SELECTION OF TEST SYSTEM

The Salmonella typhimurium/Escherichia coli Plate Incorporation Mutation Assay has been used extensively and has been demonstrated to be effective in detecting the mutagenic activity of chemicals from a wide range of classes.

## 11.0 ABBREVIATIONS

2-AA	-	2-Aminoanthracene
2-NF	-	2-Nitrofluorene
9-AA	-	9-Aminoacridine
DMSO	-	Dimethyl Sulfoxide
MMS	-	Methyl Methanesulfonate
NaN <sub>3</sub>	-	Sodium Azide
NADP	-	Nicotinamide-adenine Dinucleotide Phosphate
O.D.	-	Optical Density
%T	-	Percent Transmittance
S-9	-	Induced Rat Liver Homogenate

## 12.0 INDICATOR CELLS

12.1 Source

The Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 were obtained from Dr. Bruce N. Ames, University of California, Berkeley, California. The Escherichia coli strain WP2uvrA was obtained from Dr. Elena C. McCoy, Case Western University, Cleveland, Ohio.



### 12.2 Culture Conditions

The Salmonella typhimurium and Escherichia coli strains are routinely grown in Oxoid Nutrient Broth No. 2 in a shaker incubator rotating at approximately 120 rpm and maintaining a temperature of  $37 \pm 1^\circ\text{C}$ .

### 12.3 Stock Cultures

The Salmonella typhimurium and Escherichia coli strains were propagated to obtain a sufficient number of cells for freezing a large number of stock ampules. The cells were cryopreserved in Oxoid Nutrient Broth No. 2 supplemented with 8-9% dimethyl sulfoxide (DMSO) and stored in liquid nitrogen vapor phase. Scrapes from stock ampules are used to initiate the stock cultures for the test.

## 13.0 METABOLIC ACTIVATION

The standard rat liver S-9 will be prepared by inducing male Sprague-Dawley rats with Aroclor-1254 or phenobarbital and/or -naphthoflavone. The livers will be aseptically removed, washed, minced, homogenized and centrifuged at 9000xg. The supernatant fraction will be pooled, dispensed in appropriate aliquots, and stored below  $-70^\circ\text{C}$  for up to 3 years. Prior to its use in this study, the S-9 will be evaluated for acceptable metabolic activity in a standard Salmonella typhimurium mutation assay using strain TA100 and a single dose of 2-AA.

## 14.0 ROUTE OF ADMINISTRATION OF TEST ARTICLE

The test article will be administered in vitro directly or through a solvent compatible with the test cultures. This is the only route of administration available in this test system.

## 15.0 TEST SYSTEM IDENTIFICATION

All test plates will be labeled using an indelible pen with a code system which clearly identifies the experiment number, the SITEK test article number, controls, doses, and whether or not the plate was treated in conjunction with an exogenous activation system.

The test article will be designated by the unique four-digit number assigned by SITEK when the test article is received (e.g., 0074). The experiment phase will be designated by the letter A (Range Finding Test) or B (Mutation Assay) followed by a number designating the trial number. This will be followed by the letter N (No Activation) or S (With S-9) which will be followed by the dose and strain identification numbers. The doses will be identified by the numbers 1, 2, 3, ... indicating the highest to the lowest dose. The strain identification numbers will be as follows:

<u>Salmonella typhimurium</u>	<u>Escherichia coli</u>
1 = TA98	5 = WP2uvrA
2 = TA100	
3 = TA1535	
4 = TA1537	



An example of a plate label follows:

0074B1-S-1-3

- 0074 = SITEK Test Article Number
- B1 = First Mutation Assay
- S = With S-9
- 1 = Highest Test Article Dose
- 3 = Strain TA1535

In addition to the above, the Range Finding Test and Mutation Assay viability plates that contain 10X histidine-biotin or 10X tryptophan will be designated with the prefix "T".

16.0 CONTROL SUBSTANCES

16.1 Positive Controls

The positive control chemicals that will be used for the tester strains in the presence and absence of exogenous metabolic activation are presented below. The abbreviations are defined in Section 11.0.

	<u>Strain</u>	<u>S-9</u>	<u>Chemical</u>	<u>Dose</u> <u>(µg/plate)</u>
<u>Salmonella</u>				
<u>typhimurium</u>				
	TA98	-	2-NF	2.5-7.5
	TA98	+	2-AA	1.25-5.0
	TA100	-	NaAz	0.5-2.0
	TA100	+	2-AA	1.25-5.0
	TA1535	-	NaAz	0.5-2.0
	TA1535	+	2-AA	1.25-5.0
	TA1537	-	9-AA	25-75
	TA1537	+	2-AA	1.25-5.0
<u>Escherichia</u>				
<u>coli</u>				
	WP2uvrA	-	MMS	2000-4000
	WP2uvrA	+	2-AA	10-20

If necessary, other appropriate positive controls can be used with the approval of the Sponsor.

DMSO will be used to solubilize the positive controls, except for NaAz and MMS, which will be dissolved in deionized, distilled H<sub>2</sub>O.



## 16.2 Solvent Control

The solvent used for dissolving the test article will be used as the solvent control. Deionized, distilled water, dimethyl sulfoxide (CAS #67-68-5), ethanol (CAS #64-17-5) and acetone (CAS #67-64-1) are some of the solvents which are compatible with this test system. If there is a need to use other solvents, the approval of the Sponsor will be obtained prior to their use.

## 17.0 DOCUMENTATION

All procedures, results, significant observations, and methods used for analysis of results will be documented in a study notebook. The study notebook will also include copies of the protocol, all protocol amendments and protocol deviations, study reports, and all relevant communications with the Sponsor.

## 18.0 EXPERIMENTAL PROCEDURE

### 18.1 Determination of Solubility/Miscibility

In order to determine the optimal vehicle for delivering the test article to the test system or to determine the maximum achievable concentration in the solvent requested by the Sponsor, a solubility/miscibility test will be performed. The solvents of choice for this system are water, DMSO, acetone and ethanol. If the test article is not sufficiently soluble in any of these solvents, additional solvents will be screened.

For solid and viscous test articles, the solubility test will consist of weighing out 20- to 100-mg aliquots of test article and adding solvent in 0.1 mL increments, with thorough mixing between additions, until the test article is dissolved as determined by visual inspection or until 5.0 mL of solvent has been added to the vessel. The volume of solvent required for complete dissolution and any additional observations will be recorded in the study notebook. Test articles that do not dissolve in 5.0 mL of solvent will be visually inspected and recorded as either "not soluble," "partially soluble forming a homogeneous suspension," or "partially soluble not forming a homogeneous suspension."

For liquid test articles, a miscibility test will be conducted. 0.5 mL of solvent will be added to 0.5 mL aliquots of the test article. The resulting solution will be thoroughly mixed and observed for miscibility. The test article will be rated by visual inspection as either "not miscible," "partially miscible," or "completely miscible" in each of the four preferred solvents. The miscibility rating and any additional observations will be recorded in the study notebook.

### 18.2 Preparation of Test Cultures

The strains of Salmonella typhimurium and Escherichia coli will be prepared from cultures that were started from scrapes placed in Oxoid Nutrient Broth No. 2. The cultures will be placed on the shaker, and a timer turns on the incubator approximately 8-12 or 4-6 hours for Salmonella typhimurium or Escherichia coli, respectively, prior to sampling the cultures for growth determination. The incubator will be set at 120 rpm and  $37 \pm 1^\circ\text{C}$ . Samples from each culture will be checked for Percent Transmittance (%T) at 650 nm.



Only cultures that have a %T of between 25% (O.D. 0.6) and 10% (O.D. 1.0) will be used. These cultures will have approximately between  $5.0 \times 10^8$  and  $1.0 \times 10^9$  cells per mL.

### 18.3 Preparation of S-9 Metabolic Activation Mix

For the portion of the Range Finding Test or the Mutation Assay in which the cells are exposed to the test article in conjunction with an exogenous metabolic activation system, induced rat liver S-9 plus cofactors (S-9 mix) will be used as the activation system. The components of the standard S-9 mix will be 8mM MgCl<sub>2</sub>, 33mM KCl, 5mM glucose-6-phosphate, 4mM NADP, 100mM sodium phosphate buffer (pH 7.4), and 10% rat liver S-9.

### 18.4 Preparation of Test Article

The desired amount of the test article as specified in the dilution scheme will be weighed or measured just prior to use in either the Range Finding Test or the Mutation Assay. The dosing solutions will be prepared by adding the appropriate volume of solvent to the test article and thoroughly mixing the resulting solution until the test article goes completely into solution or a homogeneous suspension is achieved. The remaining doses specified in the dilution scheme will be prepared by either performing a serial dilution or by varying the volume delivered from the stock concentration to the cultures. In all treatments the amount of solvent delivered to the target cultures will be limited to a level which has no cytotoxic effect on the cells. If necessary, the test article may be added directly to the top agar.

### 18.5 Range Finding Test

In order to determine the test article concentrations that will produce from 0-100% toxicity, a Range Finding Test will be performed with and without S-9 activation using tester strains TA100 and WP2uvrA only. The test article will be weighed or measured, and a serial dilution will be prepared. If there are no solubility/miscibility limitations, prior knowledge of cytotoxicity indicates differently, or the Sponsor specifies differently, the treatment concentrations for solid and viscous test articles will be 5000, 1000, 500, 100, 50, 10 and 5.0 µg/plate. If the results based on the dosing regimen indicate that the threshold level of complete toxicity is below 5.0 µg/plate an additional Range Finding Test will be performed.

#### 18.5.1 Treatment

2.0 mL aliquots of molten top agar, to which trace amounts of histidine and biotin have been added, will be dispensed to a series of culture tubes maintained at  $45 \pm 1^\circ\text{C}$ . Treatment will be performed by adding 0.5 mL of S-9 mix or 0.5 mL of sterile, distilled, deionized water, 0.1 mL of tester strain TA100 or WP2uvrA, and 0.1 mL of test article to the top agar. Appropriate solvent controls will also be prepared.

In addition, plates for determining viability will be prepared by plating the test article doses with a  $2.0 \times 10^3$  dilution of tester strain TA100 or WP2uvrA in top agar containing 10X histidine-biotin or 10X tryptophan, respectively.



The contents will be mixed by vortexing the tube, and then the contents will be poured onto a bottom agar plate and evenly distributed by gently tilting and rotating the plate. The plate will be placed on a flat, level surface until solidified. After all treatment is performed, the plates will be inverted and incubated at  $37 \pm 1^\circ\text{C}$  for 48-72 hours.

#### 18.5.2 Determination of Toxicity

After 48-72 hours of incubation, the plates will be removed from the incubator and evaluated or placed in cold storage ( $1-5^\circ\text{C}$ ) until evaluated.

Evaluation of test article toxicity on the tester strain will be based on three end points:

1. Viability of cells plated on minimal medium plates supplemented with excess histidine-biotin or tryptophan. Toxicity will be measured as a decrease in the number of colonies per plate with increasing test article concentration.
2. The number of revertant colonies on minimal medium plates supplemented with trace amounts of histidine-biotin or tryptophan. Toxicity will be measured as a reduction in the number of revertant colonies per plate with increasing test article concentration.
3. The integrity of the background microcolony lawn. Toxicity will be measured as a thinning or disappearance of the background lawn usually occurring with an increase in the size of the remaining microcolonies relative to the control plates.

The number of revertants per plate and the number of viable colonies per plate will be determined by counting them with an automatic colony counter or by hand as described in Sections 18.6.4.1 and 18.6.4.2.

The counts will be entered directly in the Lotus 123 computer program 2140A.WK3, and the calculations will be performed. The computer printouts will be included in the study notebook.

#### 18.6 Mutation Assay

The maximum concentration of nontoxic test articles that is tested will be 5 mg per plate, unless the Sponsor requests otherwise or precipitation of the test article on the plate warrants the use of a lower concentration. Test articles that produce a toxic effect will be tested at a maximum dose that significantly reduces the number of revertants per plate and/or causes thinning of the background lawn. Four lower doses will be selected that should not produce toxicity. Test articles that are insoluble at concentrations of 5 mg per plate or lower will be tested at a maximum dose that produces precipitate. A concentration that produces precipitate in the test system will be considered to be beyond the limits of solubility. The actual dose levels for the assay, once determined, will be added to the protocol in the form of an amendment. Each test article dose, the positive controls and solvent controls will be plated in triplicate.



#### 18.6.1 Test Culture Preparation and Exposure

Cultures of Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and Escherichia coli WP2uvrA for use in the Mutation Assay will be prepared as described in Section 18.2. The test article will be weighed or measured, and a serial dilution will be performed as previously described in Section 18.4. 2 mL aliquots of molten top agar to which histidine and biotin or tryptophan have been added will be dispensed to a series of culture tubes maintained at  $45 \pm 1^\circ\text{C}$ . Treatment will be performed by adding 0.5 mL of S-9 mix or 0.5 mL of sterile, distilled, deionized water, 0.1 mL of tester strain, and 0.1 mL of test article to the top agar. Appropriate solvent and positive controls will also be prepared. The contents will be mixed by vortexing the tube, and then the contents will be poured onto a bottom agar plate and evenly distributed by gently tilting and rotating the plate. The plate will be placed on a flat, level surface until solidified. After all treatment will be performed, the plates will be inverted and incubated at  $37 \pm 1^\circ\text{C}$  for 48-72 hours.

#### 18.6.2 Confirmation of Tester Strain Genotypes

On the same day as the plating of the Mutation Assay, the genotypes of the tester strains will be confirmed. All of the Salmonella typhimurium strains will be tested for histidine dependence and the rfa mutation. Each Salmonella typhimurium strain will be tested for the uvrB deletion after cryopreservation of the stock ampules. The tester strains TA98 and TA100 will also be tested for the pKM101 plasmid. The Escherichia coli WP2uvrA strain will be tested for tryptophan dependence.

#### 18.6.3 Tester Strain Viability Determination

After the Mutation Assay has been plated, a dilution of each tester strain will be prepared, and approximately 250-500 bacteria will be plated in top agar supplemented with 10X histidine-biotin or 10X tryptophan. These plates will be incubated for 48-72 hours, and then the total number of colonies that develop will be determined.

#### 18.6.4 Background Lawn Evaluation

The integrity of the background microcolony lawn will be evaluated by viewing each plate with the aid of a 2X to 4X microscope. The lawns will be rated as normal, slightly reduced, markedly reduced, extremely reduced or absent.

#### 18.6.5 Enumeration of Colonies

After 48-72 hours of incubation, the plates treated with the highest test article concentration will be observed for the presence of precipitate. If precipitate is absent, the entire assay will be counted using an automatic colony counter. If observation of the high dose plates reveals precipitate that interferes with accurate automatic counting, those plates will be counted by hand. The procedure will be repeated for each subsequent dose level or until no precipitate is evident.



#### 18.6.5.1 Automatic Colony Counting

Each plate will be placed on the stage, and three counts are made with the automatic counter. The plate will be rotated on the stage approximately  $120^\circ$  between each count, and the median count will be recorded.

#### 18.6.5.2 Hand Counting

Hand counting of colonies will be performed by marking a dot over each colony on the bottom of the plate while clicking off the counts on a digitometer. The hand count will be recorded for each plate.

The counts will be entered directly in the Lotus 123 computer program 2140B.WK3. The computer printouts will be included in the study notebook.

#### 18.7 Confirmatory Mutation Assay

If the first Mutation Assay gives negative or equivocal results, a confirmatory Mutation Assay will be performed. The test article treatment concentrations may be altered based on the results obtained in the first Mutation Assay. On the other hand, if the results of the first Mutation Assay are clearly positive, a confirmatory Mutation Assay may or may not be performed depending on the Sponsor's instructions.

#### 18.8 Criteria For a Valid Assay

The following criteria will be used as guidelines in determining the acceptability of the results. Since it is impossible to formulate criteria that would apply to every configuration of data generated by the Mutation Assay, the Study Director will be responsible for the ultimate decision regarding the acceptability of the results.

##### 18.8.1 Solvent Control Cultures

The mean reversion frequency of the test article solvent control plates for each strain must fall within the range presented below.

<u>Salmonella typhimurium</u>	<u>Escherichia coli</u>
TA98      20 ± 15	WP2uvrA  15 ± 10
TA100    100 ± 70	
TA1535   20 ± 15	
TA1537   15 ± 12	

##### 18.8.2 Positive Controls

The results for the positive control cultures will be considered acceptable if the treated strains have mean reversion frequencies that are three times or greater than the mean reversion frequencies of the test article solvent control plates.



### 18.8.3 Tester Strain Characterization

1. All of the Salmonella typhimurium strains will be confirmed positive for histidine dependence and the Escherichia coli strain for tryptophan dependence.
2. All of the Salmonella typhimurium strains will be confirmed positive for the rfa mutation as evidenced by sensitivity to crystal violet.
3. The R-factor strains, TA98 and TA100, will be confirmed positive for the pKM101 plasmid as evidenced by ampicillin resistance.
4. The titer of the stock cultures of each strain will indicate that the stock cultures contained approximately between  $5.0 \times 10^8$  and  $1.0 \times 10^9$  bacteria per mL.

### 18.9 Evaluation of Test Results

The following criteria will be used as guidelines in evaluating the results of the Mutation Assay for a negative, positive or equivocal response. Since it is impossible to write criteria that would apply to every configuration of data generated by the Mutation Assay, the Study Director will be responsible for the ultimate decision in the evaluation of the results. The factors considered in making the decision will be discussed in the report.

#### 18.9.1 Criteria for a Negative Response

A response will be considered negative if 1) strains TA98 and TA100 have mean reversion frequencies that are less than twice that of the mean reversion frequencies of the corresponding solvent control plates, 2) strains TA1535, TA1537 and WP2uvrA have mean reversion frequencies less than three times that of the corresponding solvent control plates, and 3) there is no evidence of a dose-dependent response.

#### 18.9.2 Criteria for a Positive Response

A response will be considered positive if either strain TA98 or TA100 has a dose that produces a mean reversion frequency that is greater than or equal to two times the mean reversion frequency of the corresponding solvent control plates or if either strain TA1535, TA1537 or WP2uvrA has a dose producing a three-fold or greater increase in the mean reversion frequency compared to the solvent control frequency. In addition, the response must be dose-dependent or increasing concentrations of the test article must show increasing mean reversion frequencies. In evaluating the results, consideration will be given to the degree of toxicity exhibited by the dose causing the two-fold/three-fold or greater increase in reversion frequency and the magnitude of the increase in reversion frequency.

#### 18.9.3 Criteria for an Equivocal Response

A response will be considered equivocal if it does not fulfill the criteria of either a negative or a positive response and/or the Study Director does not consider the response to be either positive or negative.



In addition, if either strain TA1535, TA1537 or WP2uvrA has a dose producing a twofold increase in mean reversion frequency compared to the solvent control frequency and there is a dose-dependent response at lower concentrations in this strain, then this results will be considered equivocal and the test may be repeated after consultation with the Sponsor.

#### 19.0 PROTOCOL AMENDMENTS AND DEVIATIONS

If changes in the approved protocol are necessary, such changes will be documented in the form of protocol amendments and protocol deviations. Protocol amendments will be generated when changes in the protocol are made prior to performing a study or part of a study affected by the changes. In such cases, a verbal agreement to make such changes will be made between the Study Director and the Sponsor. These changes and the reasons for them will be documented and attached to the protocol as an addendum. Protocol deviations will be generated when the procedures used to perform the study do not conform to the approved protocol. The Sponsor will be informed of these deviations, and as soon as practical, such changes along with their reasons or explanations will be documented and kept in the study notebook.

#### 20.0 REPORT OF RESULTS

##### 20.1 Content

The results of the study will be submitted to the Sponsor in the form of a final report. A draft report will be submitted before the final report is issued. The report will include, but not be limited to, the following:

1. Name and address of the testing facility and the dates on which the study was initiated and completed, terminated or discontinued.
2. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
3. Methods used to analyze the data.
4. The test and control substances.
5. Description of the methods used to perform the study.
6. The data, mean plate counts, +/-SD, and any observations regarding toxicity and precipitate.
7. The name and signature of the Study Director and the names of other technical personnel who participated in performing the study.
8. The location where the raw data and reports are to be stored.
9. A statement from the Quality Assurance Unit.



## 20.2 Changes and Corrections to the Final Report

All changes to the final report will be in the form of report amendments which will include the reason(s) for the change, and these amendments will be added to the final report as an addendum.

## 21.0 ARCHIVES

The raw data, electronic file containing the data tables, documentation, protocol and final report of the study will be maintained in the SITEK Research Laboratories Archives, 15235 Shady Grove Road, Suite 303, Rockville, Maryland, according to the terms and conditions of the study.

## 22.0 REFERENCES

1. Ames, B. N., J. McCann and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/ mammalian-microsome mutagenicity test. *Mut. Res.*, 31:347-367, 1975.
2. Maron, D., and B. N. Ames. Revised methods for the Salmonella mutagenicity test. *Mut. Res.*, 113:173-215, 1983.
3. Green, M. H. L., and W. J. Muriel. Mutagen testing using trp+ reversion in Escherichia coli. In: B. J. Kilbey, et al. (eds.), *Handbook of Mutagenicity Test Procedures*, pp. 65-94, Elsevier North Holland Biomedical Press, Amsterdam, 1977.
4. Venitt, S., and J. M. Parry (eds.). *Mutagenicity testing: A practical approach*. IRL Press, Oxford, England and Washington, D.C., 1984.

PROTOCOL AMENDMENTS

Amendment Nos.: 1 and 2

Sponsor: Triangle Research and Development Corporation  
P.O. Box 12696  
Research Triangle Park, NC 27709-2696

Testing Facility: SITEK Research Laboratories  
15235 Shady Grove Road, Suite 303  
Rockville, Maryland 20850

SITEK's Study No.: 0538-2140

Test Article I.D.: Eicosane, TG, Dry powder

Protocol Title: Evaluation of a Test Article in the Salmonella typhimurium/Escherichia coli Plate Incorporation Mutation Assay in the Presence and Absence of Induced Rat Liver S-9

Amendment No. 1: Protocol Page 11, Section 18.6, Mutation Assay - The test article was tested with the following concentrations in the Mutation Assay:

**With and Without Activation:**

313, 625, 1250, 2500 and 5000  $\mu\text{g}/\text{plate}$

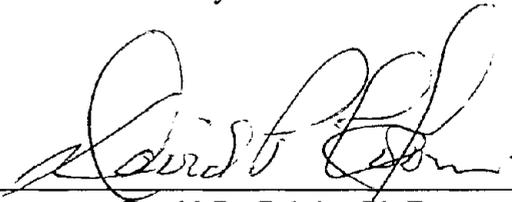
Reason for Amendment No. 1: As specified in the protocol.

Protocol Amendment Nos. 1 and 2  
SITEK Study No. 0538-2140  
Page 2

Amendment No. 2: Protocol Page 13, Section 18.7, Confirmatory Mutation Assay - A confirmatory Mutation Assay was not performed.

Reason for Amendment No.2: As per Sponsor's instructions.

APPROVAL:

 _____ Kamala J. Pant, M.S. Study Director	<u>4.29.99</u> Date
 _____ David P. Colvin, Ph.D. Sponsor's Study Coordinator	<u>5-6-99</u> Date





# United States Patent [19]

[11] Patent Number: **5,141,079**

Whitney et al.

[45] Date of Patent: **Aug. 25, 1992**

- [54] **TWO COMPONENT CUTTING/COOLING FLUIDS FOR HIGH SPEED MACHINING**
- [75] Inventors: **Raymond A. Whitney, Raleigh; Virginia S. Colvin; David P. Colvin, both of Apex; James C. Mulligan, Raleigh, all of N.C.**
- [73] Assignee: **Triangle Research and Development Corporation, Raleigh, N.C.**
- [21] Appl. No.: **736,388**
- [22] Filed: **Jul. 26, 1991**
- [51] Int. Cl.<sup>5</sup> ..... **F01M 5/00**
- [52] U.S. Cl. .... **184/6.14; 184/104.1; 165/104.17; 407/11**
- [58] Field of Search ..... **184/6.14, 104.1; 165/104.17; 407/11**

- 4,708,812 11/1987 Hatfield ..
- 4,747,240 5/1988 Voisinet et al. .
- 4,767,551 12/1989 Hunt et al. .
- 4,829,859 5/1989 Yankoff ..... 407/11
- 4,911,232 3/1990 Colvin et al. .... 165/104.17

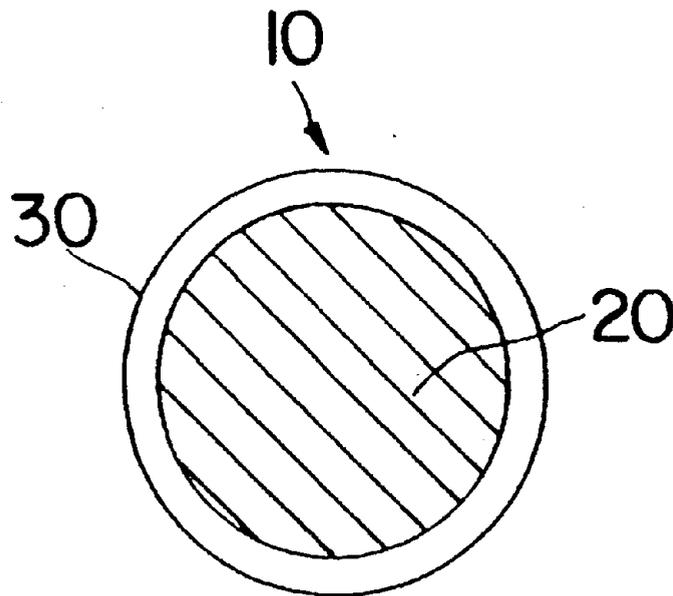
*Primary Examiner*—Albert W. Davis, Jr.  
*Attorney, Agent, or Firm*—Rosenthal & Putterman

### [57] ABSTRACT

A method of obtaining enhanced thermal energy between a material forming apparatus and a cooling fluid is disclosed. A two component heat transfer fluid of the type including a carrier fluid and a plurality of discrete particles that undergo a reversible latent energy transition upon the transfer of thermal energy thereto. The temperature of the particles is adjusted (heated or cooled as necessary) to the point of the beginning of latent energy transition of the particles. The fluid is then brought into contact with a heat source such as a metal forming apparatus and a workpiece, proximate the interface therebetween. The slurry may then be collected, adjusted to the point of the beginning of latent energy transition and re-circulated to the heat source.

- [56] **References Cited**
- U.S. PATENT DOCUMENTS**
- 3,605,551 9/1971 Steward ..... 407/11
- 3,729,064 4/1973 Wolf et al. .... 184/6.14
- 4,235,730 11/1980 Schlicht ..
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**8 Claims, 6 Drawing Sheets**



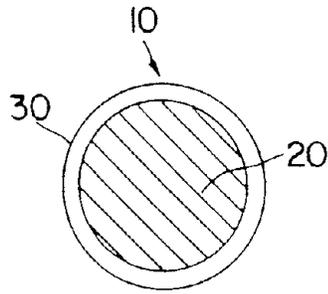


FIG. 1

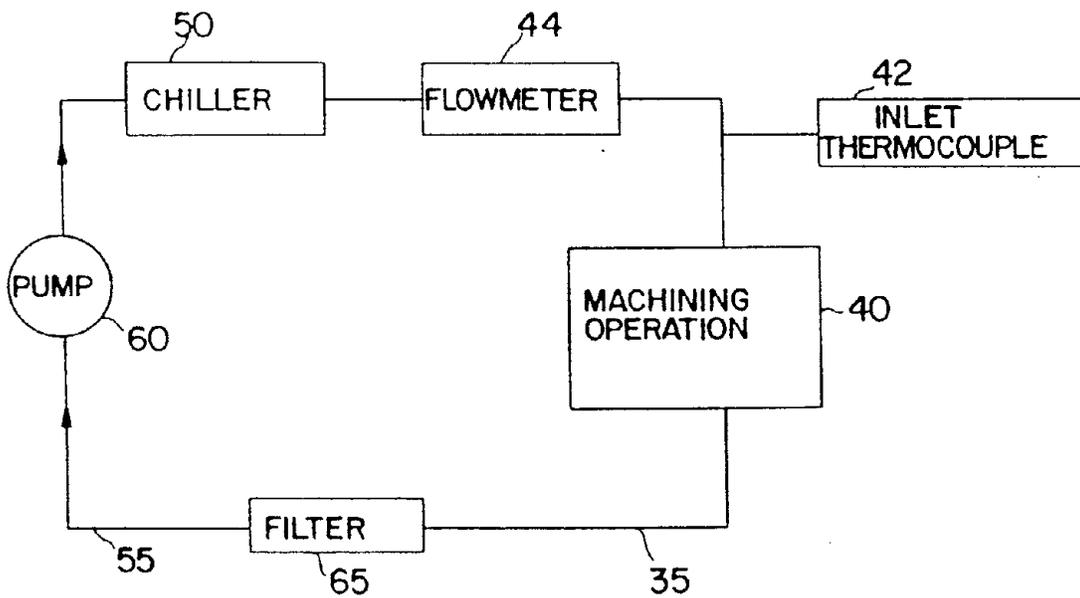


FIG. 2

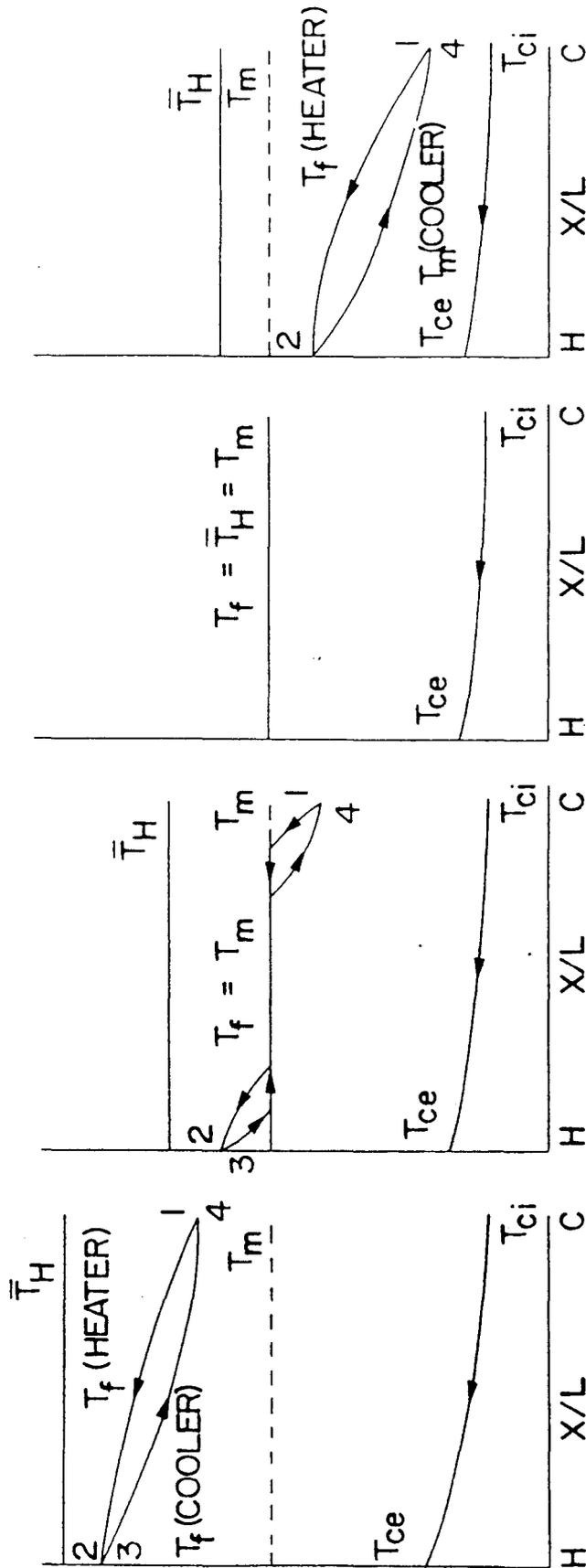


FIG. 3d

FIG. 3c

FIG. 3b

FIG. 3a

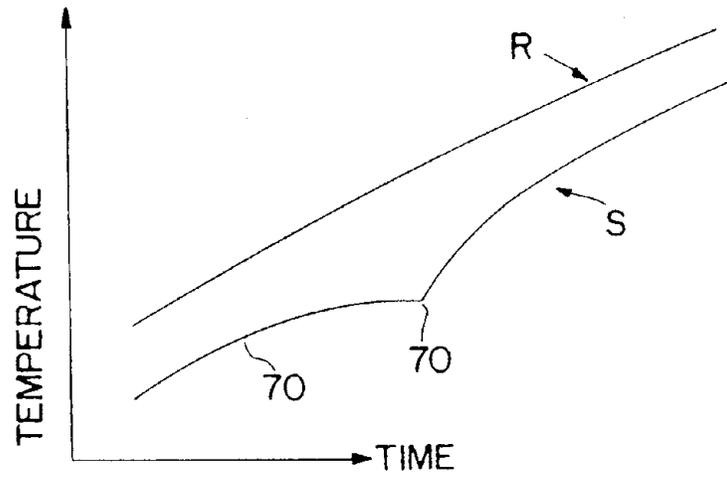


FIG.4a

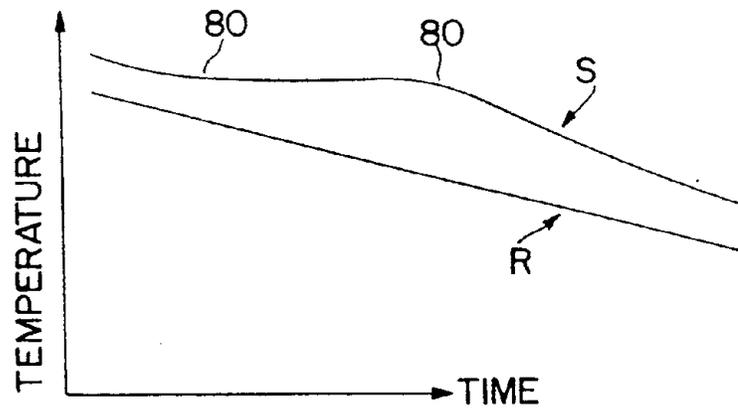


FIG.4b

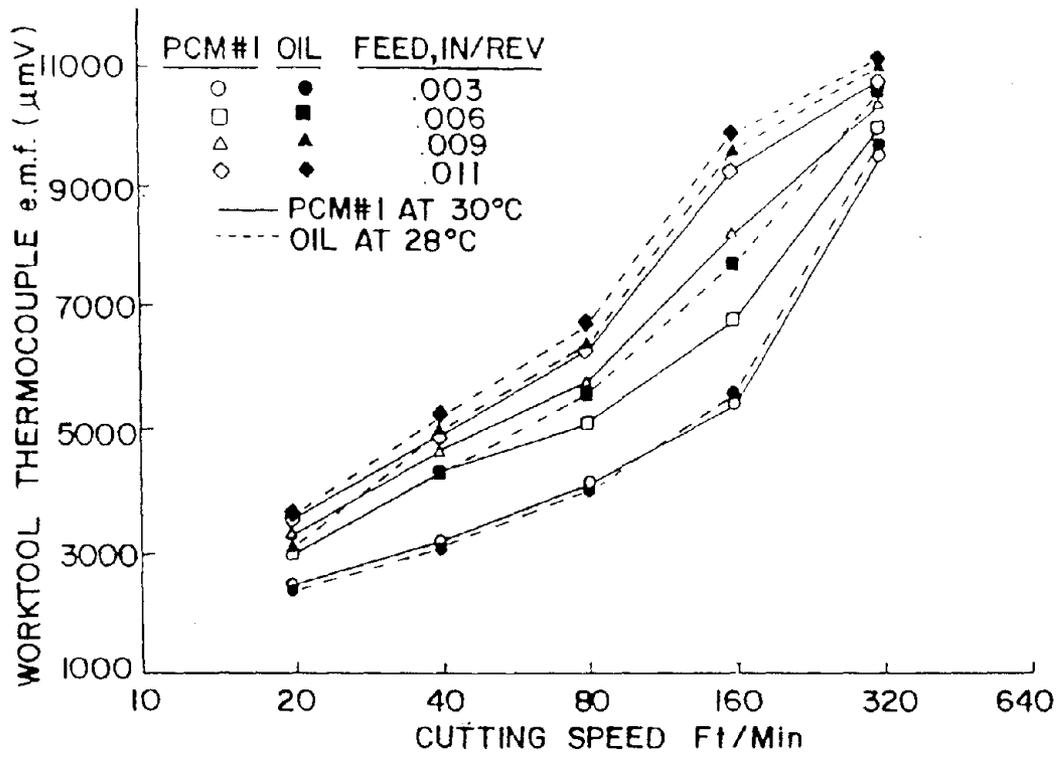


FIG. 5

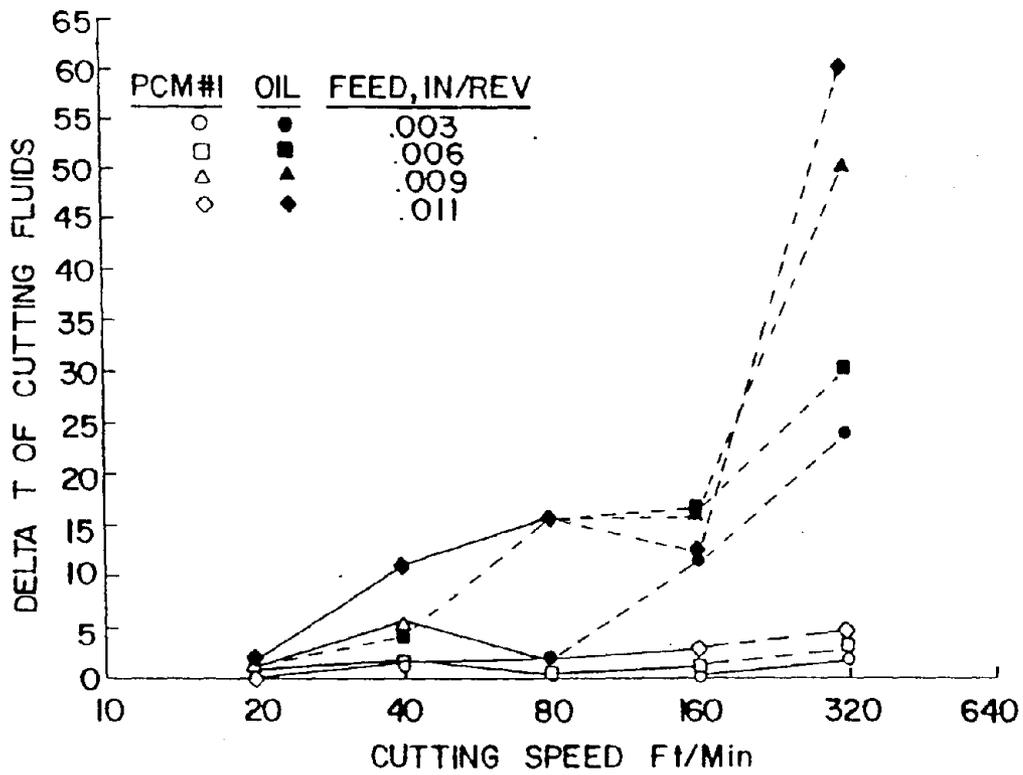


FIG. 6

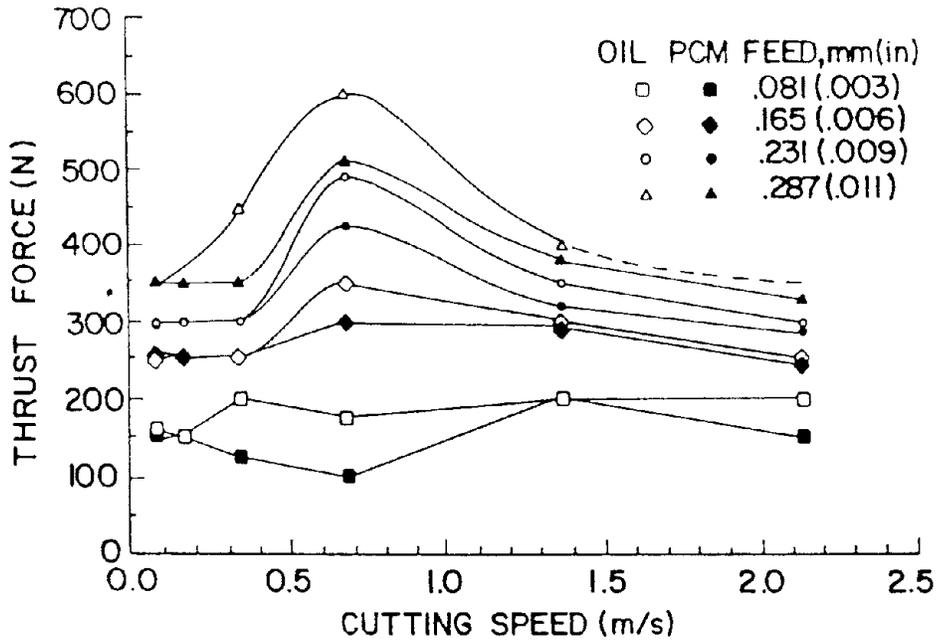


FIG.7

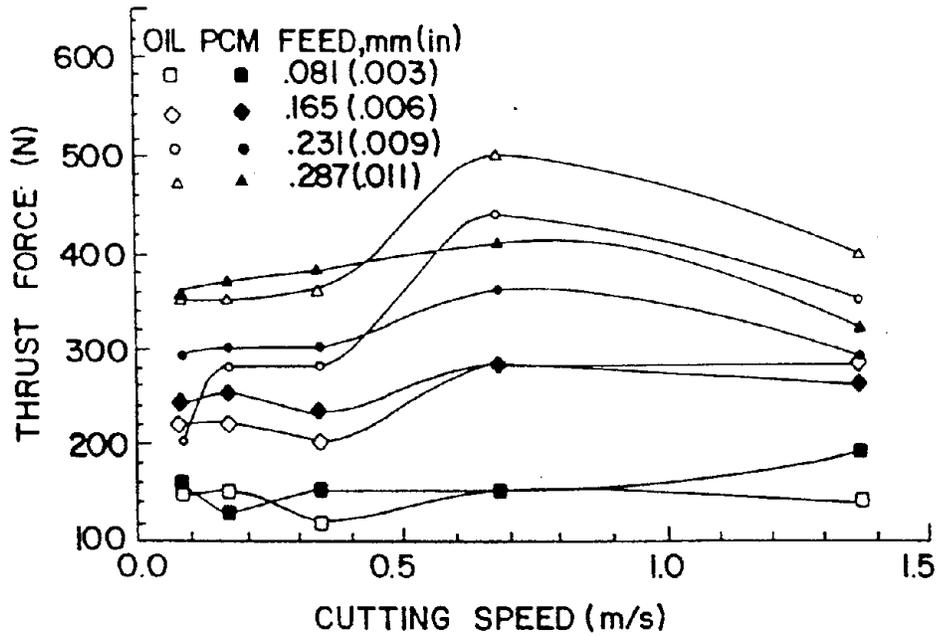


FIG.8

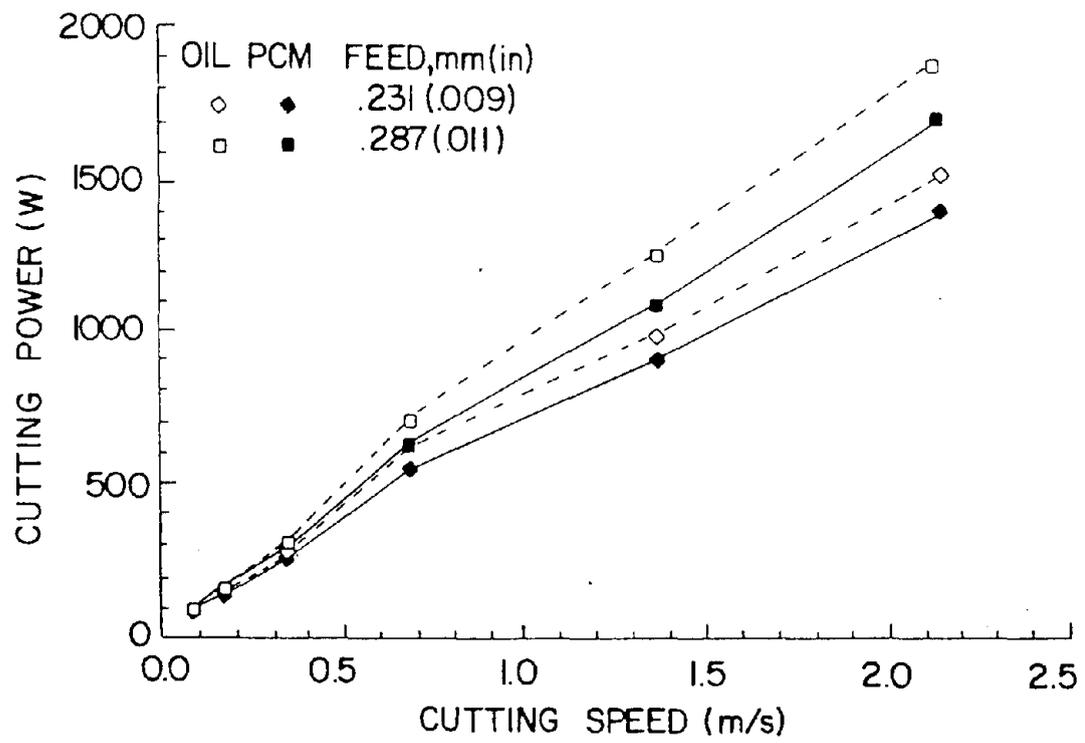


FIG.9

## TWO COMPONENT CUTTING/COOLING FLUIDS FOR HIGH SPEED MACHINING

### FIELD OF THE INVENTION

This invention relates generally to the field of cooling fluids and more specifically, to cooling fluids of the type used to cool cutting tools and drill bits used in high speed machining.

### BACKGROUND OF THE INVENTION

Approximately 60 million gallons of lubricants and cutting fluids are used each year in metal cutting and metal forming operations in the United States at a cost of more than \$350 million. This figure represents only a fraction of the cost associated with machines and their tools, bits, production materials, and labor. The selection of coolants or lubricants, however, is important for reasons other than cost. Particular cutting fluids are also selected for their performance: for the quality of the part produced, its accuracy and dimensional stability and finish, surface cleanliness, reduction in tool wear, corrosion protection, ease of machining, especially high-speed machining, and finally, for shop safety and environmental protection.

Materials that are processed into useful parts by cutting and forming operations include metals, alloys, plastics, ceramics, and composites. The removal processes include: turning, milling, broaching, drilling, tapping, cutoff, grinding, polishing, and lapping. These processes apply a tool or an abrasive at sufficient speed or force to remove a given quantity of material. Chip material-forming processes include: forging, rolling, extrusion, rod and wire drawing, tube drawing, deep drawing, swaging, and roll forming. These processes rely on plastic deformation of the material. In material removal processes, the rate of production as well as the life of the tool can be influenced by whether or not an effective cutting fluid is used. Ineffective cooling can lead to thermal distortion of the workpiece that subsequently produces a loss in dimensional tolerances.

The two main functions of cutting fluids are lubrication at relatively low cutting speeds and cooling at relatively high cutting speeds of the tool, chip, and workpiece. By serving these functions, cutting fluids (a) prevent tool, workpiece, and machine overheating, (b) increase tool life, (c) improve surface finish on the workpiece, and (d) help clear the swarf from the cutting area. Cutting fluids are usually either water or oil-based; the oil may be either natural or synthetic. Various methods are used to apply lubricants: dripping, flooding, high pressure jet, misting, and manual brushing. Older methods generally flood the interface area from the top down, but for efficient high-speed machining, a jet directed under the end clearance face and about the chip by high pressure spraying has been found to be very effective.

Water-based fluids have higher heat capacities than those with an oil-base and can sustain increased heat loads during high-speed machining. Water-based fluids, however, promote corrosion in some materials where oil coolants do not. Oil-based fluids normally have one-fourth to one-half the thermal capacity of water, often require higher flow rates, sometimes support bacteria growth, and may become toxic upon evaporation at high temperatures. Additives to some coolants also limit their use with certain materials; for example, lubricating fluids for iron and steel are normally not used with

aluminum. Similarly, cutting fluids with chlorine-bearing additives can not be used for machining titanium alloys. There is often concern for the cleanliness of the manufactured part because of the cutting fluids.

Some materials are also more difficult to machine than others. One particularly important quality is thermal conductance—the ability to conduct the heat generated during operation away from the chip formation zones. The heat generated depends on the material and the rate of machining. Thirty to forty years ago, machining speeds for steel were 40 to 70 feet/minute; common high speed machining today approaches speeds of 1,000 feet/minute. The effectiveness of cutting or cooling fluids is related to their contact or dwell time at the tool/workpiece interface. Since this contact time is reduced at high cutting speeds, the role of the cutting fluid is much more important in high-speed machining of materials with limited thermal conductance. High-strength steels and many composites are difficult to machine at high speeds, so improved lubricating or cooling fluids aid the fabrication with these materials. Metals such as high-strength steels and high temperature alloys dull even the best tungsten carbide tools in a short time. In some cases, ceramic tools can replace tungsten carbide tools because they have higher compressive strength and can withstand higher temperatures. However, they are brittle, have low tensile strength, and cannot be used with lubricants. With some plastics and composite materials, the heat generated during high-speed machining is sufficient to plasticize the chip and workpiece.

Operator safety is also a concern in an environment where machinists can become covered with lubricating fluids. One significant threat is the generation of potentially hazardous vapors during high-speed machining. As machine speeds increase, so do the chip-tool interface temperatures that cause evaporation of the cutting fluid. The cost of replacing evaporated fluids increases with the speed of cutting; however, the costs of environmental pollution, of observing OSHA regulations, air-handling equipment, associated energy loss, fluids disposal, and exposure of the operators to increased risk can be significantly higher. Thus, the development of superior cutting fluids that would facilitate enhanced cooling of the cutting tool and workpiece, would improve lubricity and workplace safety, and reduce environmental pollution, would be of great value to the manufacturing industries.

It is, accordingly, an object of the present invention to provide a cooling fluid and method that minimizes the risk of tool, workpiece and machine overheating.

It is another object of the present invention to provide a cooling fluid and method that extends useful tool life.

Yet another object of the present invention is to provide a cooling fluid and method that improves workpiece surface finish.

Still another object of the present invention is to provide a cooling fluid and method that enhances operator safety by reducing toxic fumes.

A still further object of the present invention is to provide a cooling fluid and method that reduces environmental hazards.

### SUMMARY OF THE INVENTION

To accomplish the foregoing objects, there is provided a method of obtaining enhanced thermal energy

transfer between a heat source such as a material forming apparatus and a cooling fluid. A two component heat transfer fluid of the type including a carrier fluid and a plurality of discrete particles (PCM) that undergo a reversible latent energy transition upon the transfer of thermal energy thereto is provided. The temperature of the particles is adjusted (i.e., heated or cooled as necessary) to the point of the beginning of latent energy transition of the particles. The two component heat transfer fluid (PCM slurry) is then brought into contact with a heat source such as material forming apparatus and a workpiece, proximate the interface therebetween.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Some of the features and advantages of the invention having briefly been described, others will appear from the detailed description which follows, when taken in connection with the accompanying drawings in which—

FIG. 1 is a cross-section of a microcapsule containing a phase change material as used in the present invention.

FIG. 2 is a schematic of a tool cooling system according to the present invention illustrating a typical heat source, a chiller/heater and a cooling or rejection heat exchanger.

FIG. 3a illustrates the temperature profiles for one thermal cycle in the system of FIG. 2. In this figure, the temperature of the PCM slurry always remains above the latent energy transition temperature and the energy transfer of the slurry is all sensible.

FIG. 3b illustrates the temperature profiles of FIG. 3a where the heating rate has been decreased so that the temperature swing of the PCM is centered on the latent energy transition temperature,  $T_M$ . The energy transfer of the PCM fluid is both sensible and latent.

FIG. 3c illustrates the temperature profiles of FIG. 3b where the PCM temperature is adjusted so that the temperature sensing of the PCM is centered on the latent energy transition temperature,  $T_M$ , but there is no excursion in temperature above or below the value  $T_M$ . The energy transfer of the PCM fluid in this case is completely latent, and represents optimal tuned system operation.

FIG. 3d illustrates the temperature profiles of the system of FIG. 2 where temperature of the PCM particles always remains below the latent energy transition temperature,  $T_M$ , and the energy transfer of the PCM fluid is again all sensible.

FIG. 4a and 4b illustrate the thermal characteristics of phase change material in heating (endotherm) and cooling (exotherm) cycles, illustrating the latent absorption (or release) of energy at a constant temperature in the plateau portions of the slurry temperature curves.

FIG. 5 illustrates the variation of work-tool thermocouple e.m.f. with cutting speed and feed rate for cutting oil containing PCM 1 with 25 weight percent solids, initially at 30 degrees centigrade and oil only at 28 degrees centigrade.

FIG. 6 illustrates the comparison of cutting fluid temperature (degrees centigrade) before and after the workpiece with cutting speed and feed rate for cutting oil with PCM 1 initially at 30 degrees centigrade and oil only at 28 degrees centigrade.

FIG. 7 illustrates the effect of cutting speed and feed on the thrust force component ( $F_x$ ) of the tool forces. The cutting oil and PCM lubricants are initially at seventeen degrees centigrade.

FIG. 8 illustrates the effect of cutting speed and feed on the thrust force component ( $F_x$ ) of the tool forces when using an oil and PCM lubricant, both starting at initial temperatures of 30 degrees centigrade.

FIG. 9 illustrates the variation of the cutting power with cutting speed and feed. Cutting is performed with the lubricants initially at 17 degrees centigrade.

#### DETAILED DESCRIPTION OF THE ILLUSTRATED EMBODIMENT

While the present invention will be described more fully hereinafter with reference to the accompanying drawings, in which a particular embodiment is shown, it is to be understood at the outset that persons skilled in the art may modify the invention herein described while still achieving the favorable results of this invention. Accordingly, the description which follows is to be understood as a broad teaching disclosure directed to persons of skill in the appropriate arts and not as limiting upon the present invention.

Referring now to the drawings and particularly to FIG. 2, a typical material forming process in the form of a machining operation in accordance with the present invention is schematically illustrated. Thermal energy is introduced on one side of the heat transport loop 35 in the machining operation as illustrated at 40 and is removed on the other side by a rejection heat exchanger such as a chiller 50. A two component slurry is circulated through the system by means of a pump 60.

The slurry 55 comprises a carrier fluid and a plurality of particles that undergo a reversible latent heat of fusion transition upon heating and cooling. More specifically, the carrier fluid can be almost any fluid depending upon the system requirements in which it must operate. Factors that influence the selection of a particular carrier fluid include temperature, toxicity, viscosity, pressure, etc. However, it will be noted that for the majority of applications, water or any one of a variety of lubricating oils well known to those skilled in the art could be employed. Lubricating oils are often used instead of water to prevent corrosion of the workpiece material in most cases.

The particles that actually absorb the thermal energy can take different forms depending on the temperature requirements of a given application. For relatively low temperature applications, a phase change material (PCM) is microencapsulated within a different material. For higher temperature applications and solid/solid transitions, one component particles can be employed that undergo solid/solid transformation. For still higher temperatures, microencapsulated metals may be used consisting of a metal shell coating over a metal phase change core material.

Microencapsulated phase change materials store thermal energy in the form of a physical change of state as the core material melts or freezes or undergoes solid/solid transition. The core material is isolated from the carrier fluid by a thin membrane or shell. FIG. 1 illustrates a microencapsulated particle generally indicated at 10 having a core 20 of a phase change material and a thin shell 30 of another material. Melted core material 20 is thus contained within the surrounding shell 30 and is restricted from mixing with this carrier fluid or from being deposited in undesired locations upon cooling. The technology of microencapsulating various materials, including those that undergo a phase change, is well known to those skilled in the art and further discussion thereof is not deemed necessary.

The one-component, unencapsulated particles may also take the form of a powder suspended within the carrier fluid. At a temperature below their melting point, certain materials may undergo a solid/solid energy transformation that also results in an enforced intake or release of heat at a specific temperature. Both sides of the material exhibit a specific endotherm and exotherm corresponding to the enhanced intake or release of heat. When the endotherm and exotherm are close enough together, the possibility exists for tuning a closed loop circuit or system to exhibit significantly enhanced heat transport. In this instance, the material remains solid during latent energy transition and a protective shell is not needed.

As stated above, the type of carrier fluid chosen depends largely on the steady state operating temperature of a given material forming operation. The following table illustrates exemplary carrier fluids and their respective operating temperature ranges.

Common Name	Approximate Temperature Range
Water	>0 C. to <100 C.
Water/glycol mixture	> -40 C. to <110 C.
Oils, silicone, hydrocarbons	> -50 C. to <250 C. depending on formulation
Liquid sodium	>100 C. to <900 C.
Liquid lithium	>180 C. to <1400 C.

Similarly, latent energy transition materials are chosen so that the melting point occurs at just below the steady state operating temperature of a given material forming operation. The following table illustrates exemplary latent energy transition materials and their approximate transition temperatures.

Common Name	Approximate Transition Temperature
Water	0 C.
Tetradecane	5-6 C.
Hexadecane	17-18 C.
Octadecane	24-27 C.
Methyl Palmitate	30-32 C.
Eicosane	35-39 C.
Sodium	98 C.
Lithium	181 C.
Pentaerythritol	184-186 C.
Neopentylglycol	184-186 C.
Tin	232 C.
Bismuth	271 C.
Zinc	420 C.
Barium	725 C.
Eutectic alloys containing Bismuth, Cadmium, Indium, Lead, Tin	47-138 C.

Exemplary of shell materials to encapsulate the latent energy transition materials are the following:

Polymers  
Polyamids  
Silver  
Gold  
Copper  
Nickel  
Cobalt

It will be noted with respect to the foregoing, that when paraffinic PCM's are microencapsulated, their endotherms and exotherms are usually altered from the pure material. Experience has shown that the crystallization of the microencapsulated PCM's is normally affected; i.e., the freezing occurs at lower temperature

(supercooling) than with pure paraffinic material. Thus, the difference in temperature between the endotherm and exotherm usually increases when the paraffins are microencapsulated.

According to the present invention, the effective thermal capacitance of the fluid slurry may be increased many times that of the carrier fluid alone (from slightly above one to well over ten times).

As illustrated in FIGS. 4a and 4b, the latent heat transition in both heating and cooling of the slurry S occurs in an isothermal plateau as compared with a referenced bath R which shows no such plateau. Thus, by carefully adjusting the system parameters, almost all the heat acceptance and heat rejection in the PCM slurry may be confined to the isothermal latent melting plateau 70 and cooling plateau 80 (heat plateaus) as illustrated in FIG. 4. The result is greatly enhanced heat transport, with an attendant decrease in temperature differential across the loop.

Experimental evidence has been obtained to demonstrate that the above described thermal fluid exhibits enhanced heat transport. Unlike two-phase heat transport using liquid to gas heat transitions with its attendant high pressures and large changes in volume, the two-component thermal fluid slurry operates at low pressures and with very small changes in system volume (approximately fifteen percent). Within the slurry, the microparticles store the majority of the thermal energy in the form of latent energy. The circulating loop is tuned to complete the endothermic energy capture just as the particles leave the tool/workpiece interface and then completes the exothermic energy release just as the particles exit the rejection heat exchanger or chiller 50. The heat to be transferred then can occur across a very narrow temperature gradient between the endothermic and exothermic levels. FIG. 4 illustrates the endothermic 70 and exothermic 80 plateaus or levels exhibited by a fluid slurry containing microencapsulated latent energy materials. If the temperature difference or thermal gradient between the thermal slurry endotherm and exotherm is small, it is possible to transport significantly more heat than sensible heat transport can provide under the same conditions of flow. It is this methodology that permits the enhanced nature for heat transport of the two-component thermal fluid slurry. Regardless of whether one uses microencapsulated phase change materials (solid/liquid PCM's) or unencapsulated powders of materials (solid/solid PCM's) that exhibit similar endothermic and exothermic temperature levels, the thermal fluid slurry can be tuned to produce enhanced heat transport characteristics.

FIG. 3 illustrates the dependence of heat transferred with system flow rate, heating rate, and cooling rate. The parameters contained therein are defined as follows:

$T_f$	= Fluid Temperature
$T_H$	= Average Temperature of Heater
$T_a$	= Temperature of Latent Energy Material Fluid
$T_m$	= Latent Energy Transition Temperature
$T_c$	= Coolant Temperature (i = inlet, e = exit)
X/L	= Cross Section Position in Thermal Loop Between Hot (H) and Cold (C)

At a particular combination of these variables, the system can be tuned to provide heat transport enhancement. The operating range for tunability is usually narrow, but its location can be determined by differential

scanning calorimetry or measurements of the fluid temperature at the exit of the chiller and at the machine/tool interface. Conditions for optimal heat transport will exist when the difference in the fluid temperature is minimized between these points. When this occurs, it indicates that most of the thermal energy is being transported in the form of latent phase or chemical bond energy rather than sensible thermal energy that normally is attributed to the difference in temperature between the aforementioned measurement locations. Testing has also revealed a 50 to 100% enhancement for the heat transfer coefficient  $h$ .

In general, the following three variables are available for adjustment in order to tune a PCM such as is described above:

1. Heat flux, or heat loaded into the PCM slurry.
2. Slurry flow rate.
3. Cooling capacity from the PCM slurry through the rejection heat exchanger.

A typical operating system may have either a fixed or variable heat load (flux) (generated by machining operation 40) to be dissipated, and a cooling system 50 utilizing chilled water or other coolant at a given inlet temperature (measured by thermocouple 42) and adjustable flow rate (controlled by flowmeter 44). The slurry 55 is then pumped (via pump 60) between the hot and cold sinks for heat transfer as illustrated in FIG. 2. A filter 65 is provided to remove workpiece chips, etc. prior to re-circulation to the tool-workpiece interface.

The temperature of the heat source must be higher than the melting temperature of the microencapsulated PCM, and the cooling temperature must be below the freezing temperature of the PCM.

The slurry flow rate must be adjustable through the laminar flow range or at least over the range of operation. The heat input and heat output heat exchanger in the chiller may be of the type generally suitable to fluid flow heat transfer applications.

It should be noted that the melting and freezing temperature points of a substance are not normally the same. The plateaus in the heating or cooling curves illustrate the latent energy that must be absorbed or released in order to cause a material to change its physical state from a solid to a liquid or visa versa. The closer the melting and freezing temperatures of the PCM are to each other, the greater is the tuning effect, and the greater the thermal enhancement.

FIG. 3 distinguishes between the untuned states above or below the melting temperature (FIG. 3a and 3d), the balanced state (FIG. 3b) and the optimally tuned state (FIG. 3c). As shown in FIG. 3b, the balanced system represents operation around the melting temperature, but FIG. 3c illustrates the practically isothermal condition wherein the temperature gradient  $dT = T_3 - T_1$  is minimized. Although the  $dT$  can be made to approach zero degrees centigrade, losses in the system sometimes require a temperature differential on the order of a fraction of a degree (0.1 degree centigrade). The following represent actual experimental data illustrating the differences between the tuned, balanced and untuned states for the system variables listed.

The discussion that follows compares the energy expended in a number process parameters when using a standard cutting oil such as Cling-Surface light thread cutting oil No. 26060 manufactured by Cling Surface Company, of Orchard Park, N.Y. and the same cutting oil with 25 weight percent of microencapsulated phase change material added thereto. The microcapsules in

the examples cited vary in diameter from 5-25 microns and contain eicosane as the core material. Microcapsules such as employed herein are well known to those skilled in the art and further discussion thereof is not deemed necessary.

FIG. 5 compares data for two coolants in the same machining system: one for an ordinary cutting oil and another containing microencapsulated PCMs. Both of the coolants were circulated at 28 to 30 degrees centigrade. Little improvement was evident at the lower machine speeds and feed rates due to the low deformation and frictional forces. However, at 160 ft/min and a feed rate of 0.006 in/rev where conditions approached tuned conditions, there was a significant reduction in the worktool thermocouple temperature for the microPCM coolant of 11.7%, while at a feed rate of 0.009 in/rev, the reduction was almost 15%.

FIG. 6 compares the temperature differential ( $\Delta T$ ) across the tool/workpiece of cutting fluids as a function of speed and feed rate. At a cutting speed of 160 ft/min, the change in temperature of the cutting oil was 10 to 15 degrees centigrade and at 320 ft/min, the  $\Delta T$  temperature gradient was from 20 to 60 degrees centigrade, depending upon the feed rate. For the same conditions of cutting speed and feed rate, the PCM cutting fluid was observed to undergo a  $\Delta T$  temperature gradient of less than 5 degrees centigrade—a reduction of over 90%. It is also important to note that for these cutting conditions, the temperature of the PCM cutting fluid bordered the melting and crystallization plateaus of the microparticle core materials. It is at this point; i.e., where the phase change of the particle cores and the full latent thermal capacitance of the PCM capsules is effectively utilized, that the PCM coolant can remove a greater quantity of heat than is possible using ordinary sensible thermal capacitance alone. At the same time, the smoke generated from the evaporating coolant and its environmental impact during high-speed machining operations were observed to be significantly reduced with the PCM coolant. With little evaporation and fluid loss, this could also mean reduced fluid replenishment and greater operator safety.

Thrust force between the tool and workpiece is necessary for machining operations and also affects the power consumption and tool life. In addition to cooling, cutting fluids also perform as lubricants to reduce these friction and power used in the cutting operation as well as increase the life of the cutting tool. FIG. 7 illustrates the thrust force versus the cutting speed for various feed rates for both a cutting oil and the PCM lubricant or coolant. Even with operation at 17 degrees centigrade which is well below the latent phase change temperature, there is a remarkable improvement in the thrust force for a cutting speed of 0.7 m/s of 20% at the higher feed rates to 75% for lower feed rates. In FIG. 8, however, when the threshold temperature is elevated to 30 degrees centigrade in order to produce a "tuned flow" condition for optimal latent heat absorption and cooling, the reduction in the thrust force is still approximately 25% at the higher feed rates. This reduction in thrust force should result in significantly reduced tool wear, which would translate to a direct economic advantage to the PCM coolants.

FIG. 9 also demonstrates a significant reduction (up to 10%) in the cutting power at the higher cutting speeds for the PCM lubricant/coolant when compared to the oil. Therefore, it has been demonstrated that a two-component cutting fluid consisting of microencap-

sulated phase change materials is a superior machine coolant as well as a better lubricant.

The foregoing embodiments and examples are to be considered illustrative, rather than restrictive of the invention, and those modifications which come within the meaning and range of equivalence of the claims are to be included therein.

That which is claimed is:

1. A method of obtaining enhanced thermal energy transfer between a material forming apparatus and a cooling fluid comprising the steps of:

(a) adjusting the temperature of a two component heat transfer fluid of the type including a carrier fluid and dispersed throughout the carrier fluid, a plurality of discrete particles that undergo a reversible latent energy transition upon the transfer of thermal energy to the fluid so that thermal energy is transferred thereto, to the point of the beginning of latent energy transition of the particles; and

(b) directing a flow of the two component heat transfer fluid into contact with the material forming apparatus and a workpiece, proximate the interface therebetween, whereby the ability of the heat transfer fluid to absorb and transfer thermal energy from the material forming apparatus is enhanced.

2. The method of claim 1 further including the steps of collecting the two component heat transfer fluid after it has been in contact with the material forming apparatus and the workpiece interface; and

readjusting the temperature of the plurality of discrete particles to the point of the beginning of their latent energy transition.

3. The method of claim 2 further including the step of re-directing the flow of the two component heat transfer fluid into contact with the material forming apparatus and workpiece proximate the interface therebetween.

4. The method according to claim 1 wherein the discrete particles comprise a microencapsulated phase change material.

5. The method according to claim 1 wherein the discrete particles comprise a material that undergoes a solid/solid phase transformation.

6. The method according to claim 1 wherein the carrier fluid is water.

7. The method according to claim 6 wherein the carrier fluid comprises a mixture of water and machine oil.

8. The method according to claim 1 wherein the carrier fluid is a machine oil.

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