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ACUTE INHALATION TOXICITY OF PYROTECHNICALLY DISSEMINATED TEREPHTHALIC ACID

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Groups of male, Fischer 344 rats were exposed by nose-only inhalation to 100, 200, and 400 mg/m³ of TPA for 30 min. Exposed rats and respective groups of air and fuse/fuel exposed controls were evaluated for physiological, bronchoalveolar lavage (BAL), and histopathological changes at 24-hr and 14-day post-exposures (PEs). There were no adverse changes in pulmonary function, BAL parameters, or histopathology. Inhalation of TPA resulted in a reversible, dose-related rhinorrhea; TPA is a mild irritant to mucous membranes. Gas-phase byproducts (CO, CO_2, NO_2, and SO_2) were below the threshold limit values for short-term exposures as established by the American Conference of Governmental Industrial Hygienists. The particulate products were nonmutagenic in the Ames mutagenicity assay. TPA appears to be an excellent candidate for a "safe" training smoke.

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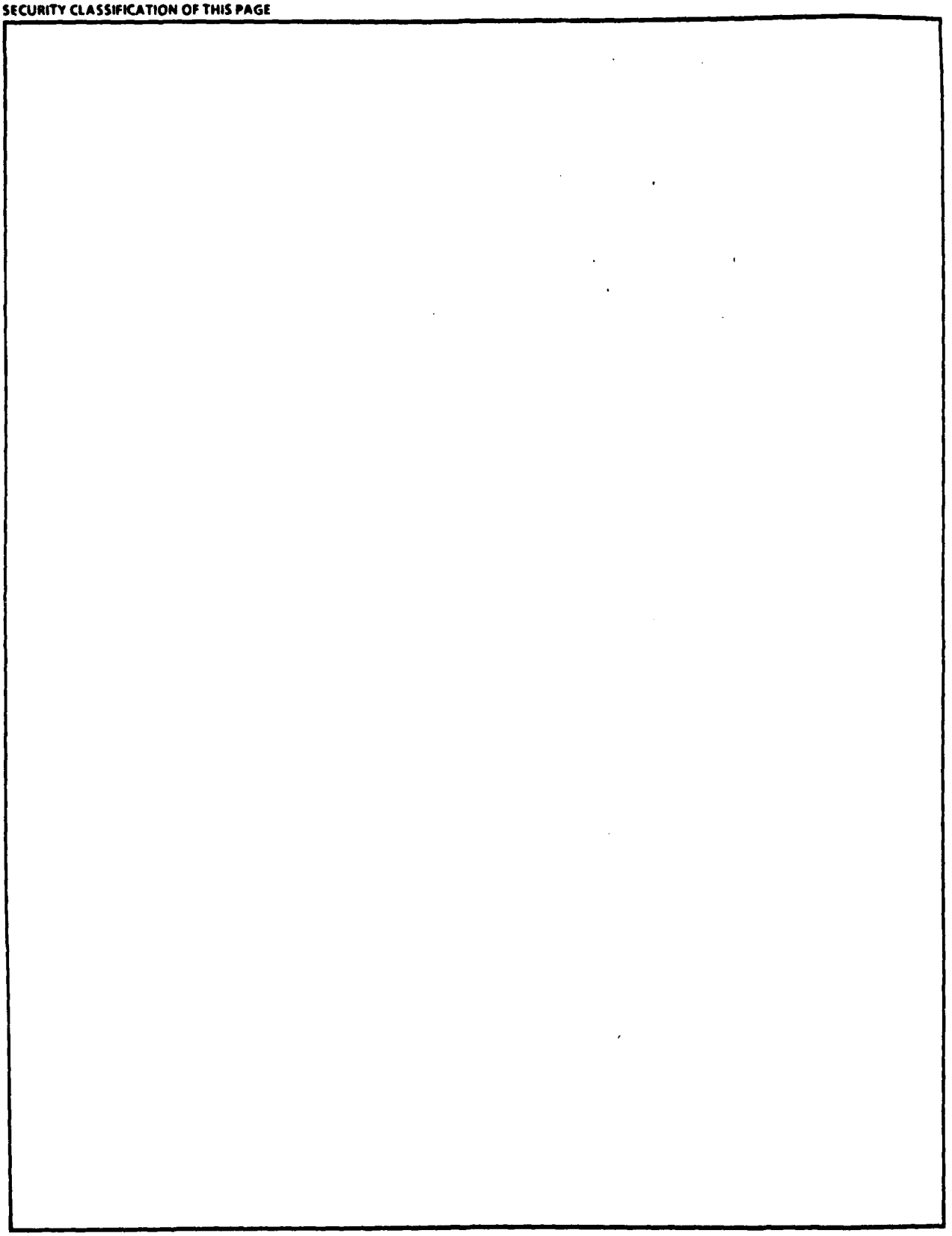

PREFACE

The work described in this report was authorized under Project No. 1L162622A552, Smoke and Obscurants. The work was started in February 1986 and completed in July 1986. The experimental data are contained in laboratory notebook nos. 82-0149, 81-0197, 85-0170, 86-0019, and 83-009.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Resources, National Research Council. This study was consistent with Good Laboratory Practice and was conducted in accordance with protocol #22086000A194 approved by the CRDEC Laboratory Animal Review Committee.

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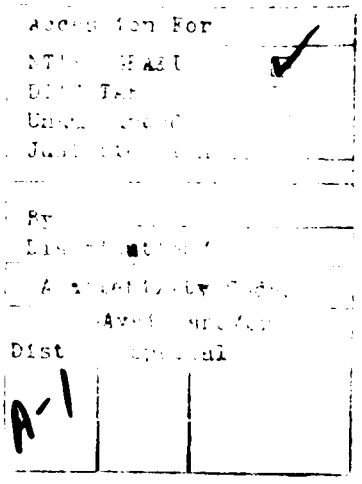

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ACUTE INHALATION TOXICITY OF PYROTECHNICALLY DISSEMINATED TEREPHTHALIC ACID

1. INTRODUCTION

Terephthalic Acid [(TPA) CAS# 100-21-0] is a component in several smoke grenades and pots and is used widely in the chemical industry for the production of polyesters. Because exposure of troops could occur from the pyrotechnic dissemination of TPA, the Munitions Directorate (Smoke Division) of the U.S. Army Chemical Research, Development and Engineering Center (CRDEC) requested that the Research Directorate (Toxicology Division) of CRDEC conduct an acute inhalation toxicological evaluation of TPA from thermally disseminated devices to mimic field exposure.

An Environmental Protection Agency (EPA) preliminary chemical hazard information profile on TPA found it to be relatively nontoxic although bladder stones were induced in rats following oral administration of TPA at 2-5% in the diet.[1] Hyperplasia of the bladder was observed in those animals with calculi; the irritation from these may result in neoplasms. It appears that critical saturating urinary concentrations of TPA and calcium were necessary for stones to develop from TPA exposure. The authors conclude that "the possibility that normal human exposure to these chemicals in the workplace or elsewhere could result in calculus formation appears to be remote."[2] The absence of bladder and kidney toxicity in rats and guinea pigs exposed to inhaled TPA has been reported by Lewis and co-workers.[3] In these studies, exposure to nuisance dust levels (10 mg/m^3) of TPA for 6 hr/day 5 days/week for 6 mo did not result in any adverse changes in body weight gain, organ/body weight ratios, clinical, chemistry, or histopathology.

Pharmacokinetic studies following intravenous and oral administration of carbon-14 TPA to Fischer 344 rats indicate that TPA was excreted unchanged in the urine within 24 hr.[1] TPA administered intraperitoneally to rabbits was rapidly absorbed by the plasma, reaching a maximum level within 1 hr. Moffit et al. also found that carbon-14 TPA was rapidly absorbed and excreted in rats after single or repeated oral and intratracheal administration.[4] In addition, there was no evidence of skin irritation or absorption after dermal application of 80 mg of carbon-14 TPA. Furthermore, there was no significant absorption of carbon-14 TPA when applied to the conjunctival sacs of the eyes of eight rabbits. TPA was rapidly absorbed, excreted, and not retained in rabbit and rat tissues. It appears that exposure to TPA in low concentrations does not result in deleterious changes. The purpose of this study was to determine if short-term inhalation exposure to high concentrations of TPA would have adverse effects on Fischer 344 rats.

2. MATERIALS AND METHODS

2.1 Experimental Design.

Groups of male rats were exposed by nose-only inhalation to 100, 200, and 400 mg/m^3 of TPA for 30 min. Only male rats were used in this study because literature review indicated that female rats were less susceptible to urinary calculi formation from TPA.[1] Exposed rats and respective groups of air and fuse/fuel exposed controls were evaluated for physiological, bronchoalveolar lavage (BAL), and histopathological changes at 24-hr and 14-day postexposure (PE). Previous studies with these pyrotechnic devices have shown a significant amount of particulate and vapor phase contribution from the fuse/fuel components.[5] This necessitated an extra fuse/fuel control group of rats.

The TPA used in this study was manufactured by Cape Industries (Wilmington, NC) and was a highly purified grade of TPA with negligible amounts of contaminants (Table 1).

Table 1. Impurities in Terephthalic Acid

Substance	ppm
4-carboxybenzaaldehyde	20.0
Monomethyl terephthalate	370.0
Dimethyl terephthlate	20.0-60.0
p-Toluic acid	Not detected
Benzoic acid	Not detected
Acetic acid	Not detected
Ash, sulfate	2.0
Iron	0.15
Nickel	0.25
Water	600.0

2.2 Chamber Operation and Sample Collection.

The rats were exposed in a 250-L, nose-only chamber connected to a 20,000-L chamber. TPA was disseminated from antidim cans, small end-burning test munitions. These pyrotechnic devices were filled with 54% TPA, 15% sugar, 26% potassium chlorate, 3% magnesium chlorate, and 2% nitrocellulose along with a small amount of starter mix for quick-match ignition as described by Gerber.[6] Gene Tracy, project engineer from CRDEC Munitions Directorate, prepared each antidim can to yield a concentration of approximately 200 mg/m^3 in the large chamber. The concentration in the nose-only chamber was maintained by varying the flow through the orifice valve on the exhaust filter. Doubling the concentration from 100-200 mg/m^3

was accomplished by igniting two antidim cans; four antidim cans produced a concentration of 400 mg/m^3. Four antidim cans (minus the TPA load) were ignited to determine the "worse case" fuse/fuel component of the generated aerosol. Sampling for concentration, particle size, oxygen, carbon dioxide, carbon monoxide, and oxides of nitrogen (the expected combustion products) was conducted at intervals during the 30-min exposure periods. Filter samples were analyzed for TPA by high performance liquid chromatography (HPLC) analysis because gravimetric analysis of the filter samples contained the mass of the combustion byproducts.

2.3 TPA Collection and Analysis.

During chamber exposure, airborne TPA was collected by drawing chamber air through 47-mm glass fiber filter pads (Gelman). The volume of chamber air drawn was measured with a calibrated rotameter. Sampling was conducted at 5-, 15-, and 25-min intervals per exposure. Samples consisted of a mixture of TPA, grenade components, and combustion products.

After sample collection, TPA was extracted from the filter pads by sonicating the pads in aqueous base. The filter pads were first transferred to a 250-mL polycarbonate flask (Corning). A stream of water was then directed onto the filter pad to loosen the sample. An appropriate amount of water and aqueous base were added to the flask to solubolize TPA. The amount of base added was computed based on the filter pad sample weight consisting of approximately 70% of TPA and on the addition of 2 moles of base per mole of TPA. Typically, 3 to 5 mg of TPA sample was dissolved into 100-150 mL of water containing 3 to 4 mL of 0.01 N NaOH. The resulting sample pH generally ranged from 7 to 8; otherwise, pH adjustments were made to achieve this range by adding dilute HNO_3 or NaOH. Solutions with a pH greater than 8 would damage the HPLC column.

Samples were analyzed for TPA by HPLC. A 10-mL sample aliquot was first withdrawn from each flask and filtered through a 0.45-μ filter (Millipore). The filtered samples were then injected onto the HPLC column for TPA separation and quantitation. Peak areas of the extracted samples were compared to the peak areas generated from a standard curve to determine TPA concentration in solution. The concentration of TPA in the chamber was then calculated by the following equation:

$$\text{TPA (mg/m}^3\text{)} = \frac{\mu\text{g/mL TPA (std. curve)} \times \text{(dilution vol. - mL)}}{\text{Liters Air Sampled}}$$

Instrumental and solvent conditions for the HPLC are listed in Appendix A. A paired-ion reagent (0.01 M TBA - tetrabutyl ammonium phosphate) was used as one of the eluting solvents to more effectively partition the TPA from the column.

According to Cape Industries, the purity of the TPA standards was >99.9%. The standards were extracted and analyzed in the same manner as the samples. A four point calibration curve with concentrations bracketting the sample concentrations was used for quantitation.

2.4 Principle of Operation for Gas Monitoring.

Pyrotechnic dissemination of TPA smoke resulted in the concurrent release of CO and CO_2 gasses. These gasses were continuously monitored in a 200-L chamber with a Miran 80 Infrared Analyzer. Air samples were drawn from the chamber through appropriate gas sample tubes (Kitagawa) as an alternate method to determine CO and CO_2, as well as SO_2 and NO_2 concentrations.

2.4.1 Miran Calibration.

The Miran was set up to conduct real-time continuous analysis for CO and CO_2 levels in the chamber. A 3-ft air sampling hose with a particulate filter was connected from one of the chamber sampling ports to the Miran. This sample line was situated within the breathing zone of the exposed animals at approximately the mid-height level of the chamber. The Miran's built-in pump was then used to draw sample air from the chamber, circulate it through the Miran, and exhaust it back into the chamber.

To establish a baseline, dry air was purged through the Miran prior to calibration. The instrument was then zeroed for absorbance at the select wavelength for each gas, CO (4.75 μ and 4.85 μ), CO_2 (4.13 μ), and reference (7.499 μ). These wavelengths were determined by scanning the IR region for each monitored gas injected into the IR and isolating the wavelength of maximum absorbance. Instrumental settings for the Miran are listed in Appendix A.

Calibration for CO was conducted by using Validyne mass flow controllers. A controlled ratio of the CO gas standards (300 ppm and 3000 ppm Matheson) and dry air were metered into the Miran to determine the concentration versus absorbance for each proportion of CO gas. A calibration curve was then established ranging from 103.0 to 150 ppm CO (Figure 1).[10]

Calibration for CO_2 was conducted by injecting known quantities of gas (CO_2 Government supply - 99.9% purity) into the Miran's closed loop calibration system. A calibration curve was established above the ambient CO_2 level and ranged from 15 to 500 ppm CO_2 (Figure 2).

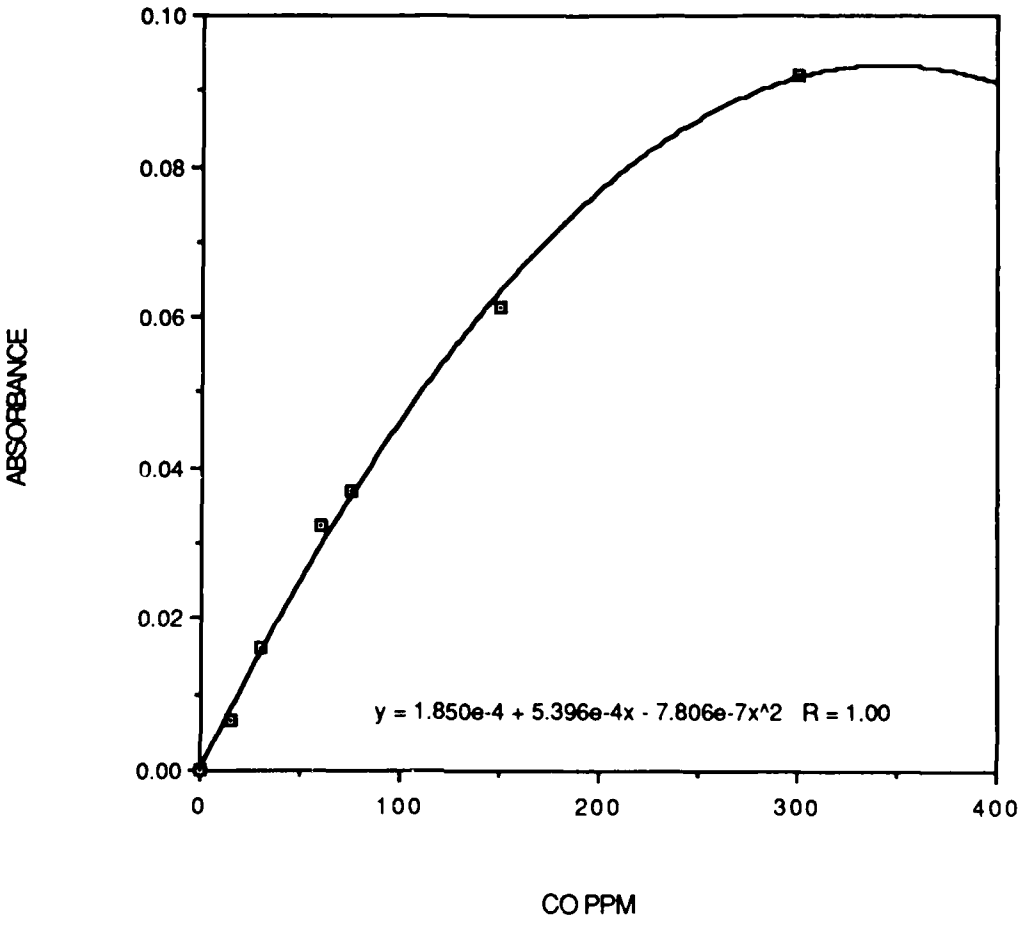

Figure 1. Carbon Monoxide Calibration Curve.

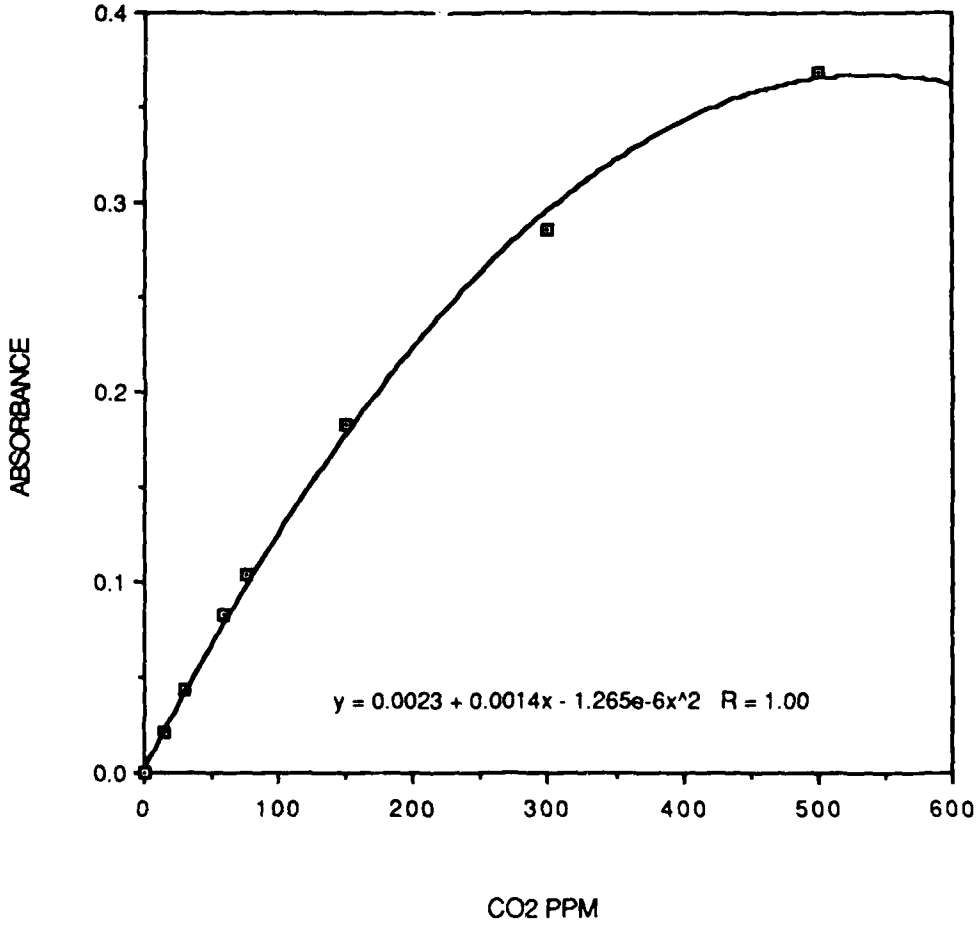

Figure 2. Carbon Dioxide Calibration Curve.

2.4.2 Monitoring Procedure (Miran and Gas Sample Tubes).

Prior to chamber analysis, ambient air was purged into the Miran, and the monitoring wavelengths were zeroed. Once TPA exposure began, samples of chamber air were continuously circulated through the Miran. Absorbance values for CO and CO_2 were measured approximately every minute and sent to a Tetronix computer for data storage and computer graphics.

Because it was zeroed at ambient air, the Miran monitored only the CO_2 generated from the TPA smoke. Total CO_2 in the chamber was determined by drawing chamber air into gas sampling tubes (Kitagawa). Quantitation of CO_2 by this method involved a direct reading from the tube that corresponded to a colorimetric change of the gas sample tube reagent. In addition, the total amount of CO, SO_2, and NO_2 was also measured once per exposure level using the gas sampling tube method.

2.5 Particle Size Analysis.

Using a Sierra Instruments Cascade Impactor (Model 2210-k, 10 stage), the aerodynamic particle size of the generated TPA aerosol was determined. The test material was collected on lightly greased stainless steel substrates that were weighed prior to and following sampling to determine mass collected in each size range. Particle size sample data was analyzed by log normal regression (least squares method) of particle size versus cumulative relative mass. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (θ_g) of each sample were determined for the fuse/fuel component and the 100-, 10-, and 1-mg/m^3 concentrations.

2.6 Animal Observations.

The rats were housed in temperature (75 + 5 °F) and humidity (40-60%) controlled Bioclean units in a separate room adjacent to the inhalation chambers. Individual, stainless steel cages were used for housing. Cage trays were changed three times/week. Certified Purina rodent chow and water was available ad lib. The animals were randomized, tattooed, and weighed before exposure and at weekly intervals during the experimental periods. During exposure, the rats were held in nose-only exposure cages. After each exposure period, the animals were removed from the exposure chamber and returned to their holding cages. Before and after exposure, the rats were examined for signs of toxicity.

2.7 Physiological Evaluations.

Lung lavage and pulmonary physiological testing were performed on the same animal to enable correlation of biochemical with functional changes. At the aforementioned PE times, the rats were anesthetized intraperitoneally with sodium pentabarbital (40 mg/kg), and a tracheal catheter was surgically inserted. Before the lavage procedure was performed, the tracheal catheter was connected to a Fleisch pneumo-tachometer for the measurement of respiratory flow. An air-filled esophageal catheter was inserted into the esophagus approximately to the level of the thoracic inlet and was connected to Statham (PM131TC + 2.5) differential pressure transducer to measure esophageal pressure. Both flow and pressure signals were processed on a Pulmonary Function Computer (Buxco Electronics, Incorporated, Sharon, CT). Flow, tidal volume, transpulmonary pressure, compliance, and resistance were recorded on a Buxco Data Logger. Compliance, measured by the ratio of the volume change in a tidal breath to the pressure change between end expiration and end inspiration, is a standard physiological method of assessing the overall elasticity or distensibility of the lungs and thorax. Restrictive pulmonary diseases (e.g., fibrosis, silicosis) result in decreases in compliance due to a stiffening effect that increases the work of breathing. Resistance is a measure of the pressure difference-

required for a unit flow change. Inhalation of dust may lead to an increase in airway resistance. Both compliance and resistance were measured as indicators of functional impairment.

2.8 Bronchoalveolar Lavage.

Immediately following the pulmonary physiology measurements, the esophageal catheter was removed, and the lavage procedure started. The lung washing technique consisted of instilling a calculated volume of normal saline (0.015 mL/g body weight) into the lung and immediately withdrawing the saline until a slight pressure was felt on the syringe plunger. Two lavage washes were done in quick succession. The recovered lavage fluid from both washes was pooled and centrifuged at 300 g for 10 min at 4 °C.

Following centrifugation, the fluid was separated into supernatant and pellet fractions. The pellet was resuspended in 1-mL of 50% bovine serum albumin, and total cell counts were taken on a ZBI Coulter Counter. A differential cell count was made using a modified Wright's staining method. The supernatant lavage fluid was assayed for total protein with the Bio Rad Protein Assay and for enzymatic activity of B-Glucuronidase (B-Glu), lactate dehydrogenase (LDH), and alkaline phosphate (ALKP). LDH and ALKP activities were determined on an Abbott VP Series II using an Abbott analysis kit, and B-Glu was assayed using a Sigma Chemical Company kit.

2.9 Pathological Evaluation.

At the end of the experimental periods (i.e., 24-hr PE, 14-day PE), all scheduled rats were euthanized with carbon dioxide gas and complete necropsies were performed by Pathology Associates Incorporated (Ijamsville, MD) in accordance with contract #DAAA15-85-D-0002. All tissues were fixed in 10% neutral buffered formalin, trimmed, dehydrated, embedded in paraffin, sectioned at 6 mm, and stained with hematoxylin and eosin. Representative sections were examined for all dose levels and controls.

2.10 Data Analysis.

As described by Gad and Weil, data analysis was conducted according to a statistical "decision tree."[7] First, Bartlett's test for homogenicity of variance followed by analysis of variances (ANOVA) was used. Finally, Duncan's Multiple Range Test was used to determine which group (or groups) differed significantly from which other group (or groups). The ANOVA1PR, based on Sokal and Rolf's Biometry Programs for Apple II, was the program used for the statistical analyses.

2.11 Ames Mutagenicity Assay.

Additional pyrotechnically disseminated TPA samples were collected on fiberglass filters, extracted with dichloromethane, and evaluated for mutagenicity in the Ames Salmonella assay. The results indicated that in this test system, the extract contained no substances that were mutagenic.[8]

3. RESULTS

3.1 Chamber Analyses.

Figure 3 shows the disseminated TPA being released from a single antidim can into the large 20,000-L chamber. The generated aerosol produced a dense white cloud in the large chamber. The experimental setup (Figure 4) includes a 250-L nose-only chamber connected to a large 20,000-L generation chamber. The results of the chamber analyses for particulate and vapor phase components are summarized in Table 2. Insignificant amounts of nitrogen and sulfur oxides were measured in the test atmosphere. The carbon monoxide and dioxide generated were well below the threshold limit value (TLV) established by the American Conference of Governmental Industrial Hygienists for short-term exposure defined as a 15-min time-weighted average not to be exceeded during a work day. The majority of the carbon monoxide appears to be contributed from the fuse/fuel moiety of the test munition. The contribution of the fuse/fuel mass is approximately one third of the total particulate mass that is in agreement with Deskin et al.[5] The HPLC analysis of the filter samples for TPA represented the other two thirds of the total particulate mass. The MMAD of the particulate phase is within the respirable region of the lung.

3.2 Toxic Observations.

Rats exposed to 30 min of pyrotechnically disseminated TPA exhibited minor adverse toxic effects; the only toxic sign observed was rhinorrhea that was dose responsive. The low-concentration exposed rats had slight rhinorrhea 15 min into the exposure; the medium concentration rats had slight rhinorrhea 7 min into the exposure while the high concentration rats had moderate rhinorrhea after 2.5 min of exposure. All exposed rats recovered within 1 hr of the exposure. The fuse/fuel exposed rats did not exhibit rhinorrhea; therefore, it was presumed that exposure to TPA was the causative factor. There were no other compound-related toxic reactions to exposure to TPA. All of the exposed rats gained weight at the same rate as the controls.

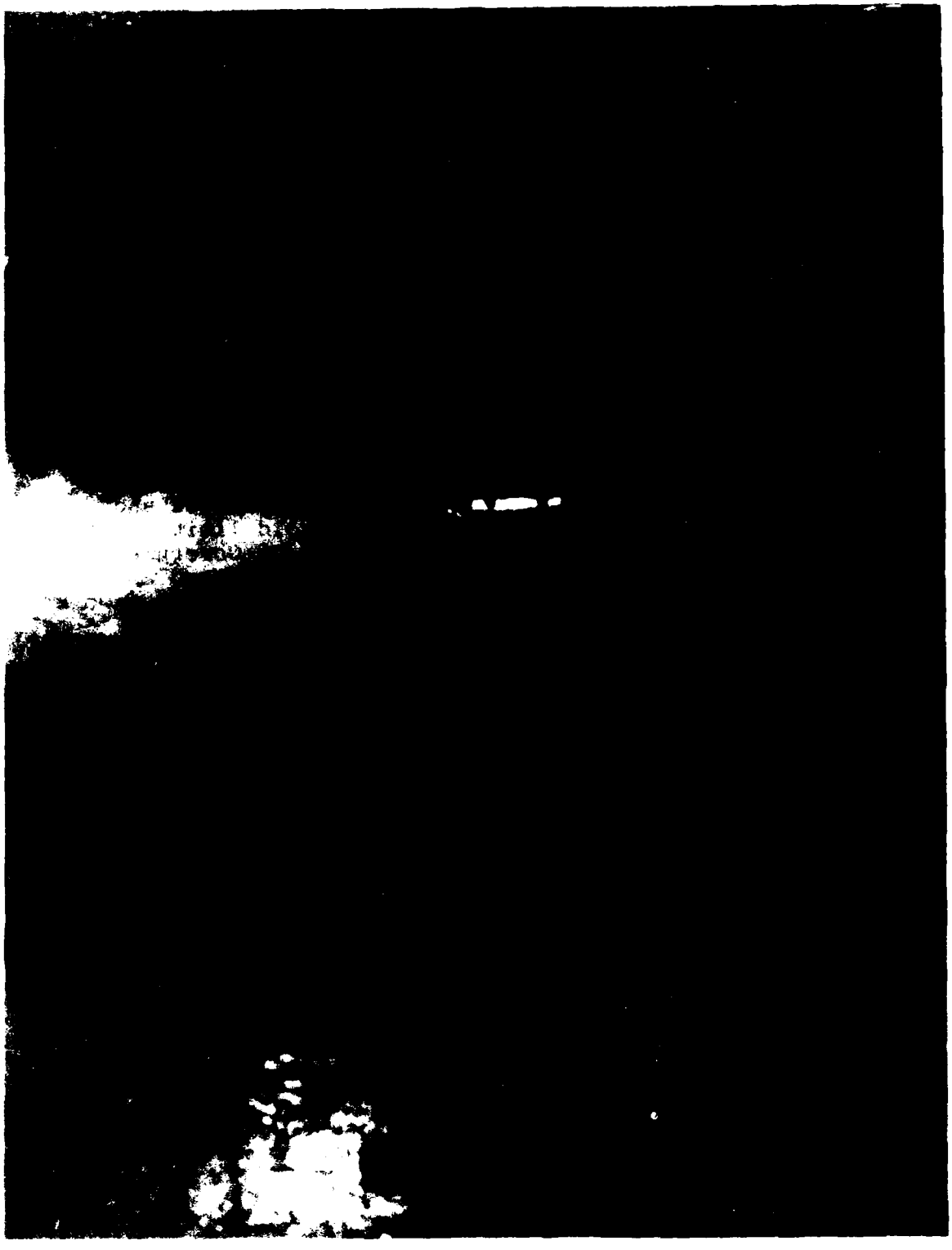

Figure 3. Dissemination of TPA.

Figure 4. Generation and Exposure Chambers.

3.3 Physiological and BAL Response.

The results of the physiological and BAL evaluations are presented in Tables 2-5. There were no statistically significant differences in physiological or BAL parameters between exposed and control rats.

3.4 Pathological Evaluations.

One rat from each of the control, low, and high concentration groups died during exposure. Necropsy of the spontaneous deaths found peritracheal and axillary hemorrhage, suggesting trauma from confinement in the nose-only holder. Subsequent adjustment of the cone on the holders and modification in the animal loading technique eliminated the spontaneous deaths. There was no compound-related mortalities. Results of the pathological evaluations are contained in Table 6.

Table 2. Chamber Concentrations and Particle Size Distribution for TPA Acute Inhalation Exposures (30 min).

	Total Mass[a] mg/m^3	TPA[a] HPLC Analysis (mg/ m^3)	CO[b] (CO_2)[c] (ppm)	CO_2[b] (CO_2)[c] (ppm)	NO_2[c] (ppm)	SO_2[c] (ppm)	MMAD[d] (θg) (μm)
Control	0	---	10 (1)	580(475)	0	0	----------
Fuse/Fuel	112.0	---	70 (55)	670(650)	1	1	0.40(3.23)
100 mg/m^3	78.2	55.0	15 (10)	625(600)	1	1	0.65(2.57)
200/mg/m^3	155.0	109.0	28 (25)	700(600)	1	1	0.69(2.58)
400 mg/m^3	340.0	235.0	50 (48)	760(625)	1	1	0.87(2.70)
TLV-TWA[e]	---	---	50	5,000	3	2	----------
TLV-STEL[f]	---	---	400	30,000	5	5	----------

[a]Average of six samples from two runs.
[b]Measured with a Miran Infrared Analyzer.
[c]Measured with Kitagawa gas sample tubes (average two samples).
[d]MMAD is mass median aerodynamic diameter; Og is geometric standard deviation.
[e]TLV-TWA threshold limit value - time weighted average established by American Conference of Governmental Industrial Hygienists (ACGIH).
[f]TLV-STEL threshold limit value - short-term exposure limit (ACGIH).

Table 3. Pulmonary Function Summary for Rats Exposed to TPA for 30 Min.*

Groups	Time PE (days)	Weight (g)	Compliance (mL/cm H_2O)	Resistance (cm H_2O/L/s)	Tidal Volume (mL)	Rate (breaths/ min)	Minute Volume (mL/min)
Control	1	333±21	0.43±0.06	0.10±0.06	2.22±0.13	90.6±14.9	195±27
fuse/fuel	1	349± 7	0.53±0.11	0.08±0.05	2.23±0.15	91.1± 9.8	198±22
low dose	1	335±18	0.44±0.52	0.08±0.04	2.09±0.22	95.0±17.3	191±20
mid dose	1	322± 8	0.38±0.04	0.10±0.03	2.04±0.12	86.0±10.5	171±25
high dose	1	350±16	0.43±0.12	0.08±0.03	2.09±0.33	90.5±11.1	133±74
controls	14	350±14	0.50±0.11	0.08±0.05	2.42±0.22	93.3±14.4	209±37
fuse/fuel	14	367±11	0.46±0.04	0.07±0.03	2.34±0.23	90.4±16.2	199±42
low dose	14	369±31	0.44±0.07	0.10±0.04	2.29±0.21	90.2±10.6	195±22
mid dose	14	361± 8	0.49±0.07	0.07±0.01	2.45±0.31	77.6±22.7	179±51
high dose	14	352±18	0.45±0.09	0.08±0.02	2.37±0.23	92.8±11.2	207±32

*Each value represents mean ± SD (n=6), tested using Barlett's Test and ANOVA @ $P \leq 0.05$.

Table 4. Biochemical Analysis of Lavage Fluid from Rats Exposed to TPA.*

Groups	Time PE (days)	B Glu (sigma units/mL)	LDH U/L	ALKP μ/L	Protein μg/mL
Control	1	6.4±1.9	72±20	65±14	362± 78
fuse/fuel	1	5.7±0.5	62±16	66±11	323± 25
low dose	1	4.9±2.6	76±17	59±20	330± 35
mid dose	1	5.7±1.4	76±15	62±15	377± 80
high dose	1	5.0±1.8	80± 8	73±15	343± 99
controls	14	8.0±1.3	61±10	77± 8	367±128
fuse/fuel	14	8.0±2.2	59±14	76±10	292± 77
low dose	14	7.8±1.7 (n=5)	67±24	68±20	270± 73
mid dose	14	5.8±1.0	55±12	72±18	282± 67
high dose	14	7.8±1.3	77±31	84±11	420±125

*Except where indicated, each value represents mean ± SD (n=6), tested using Bartlett's Test and ANOVA @ $P \leq 0.05$.

Table 5. Cytological Analysis of Lavage Fluid from Rats Exposed to TPA.*

Groups	Time PE (days)	Total Nucleated Cells ($x10^3$)	Nucleated Cell Differential (%)		
			Macrophages	Lymphocytes	Polymorphonuclear Neutrophils
Control	1	1.35±0.30	91± 3	6±3	3±2
Fuse/Fuel	1	1.24±0.33	92± 4	7±4	1±1
Low Dose	1	2.30±2.08	94± 3	6±2	0±1
Mid Dose	1	1.18±0.25	96± 4	4±3	2±1
High Dose	1	1.55±0.34	94±10	4±3	2±1
Control	14	0.98±0.23	96± 3	4±3	0±1
Fuse/Fuel	14	1.00±0.30	93± 4	6±3	1±1
Low Dose	14	1.18±0.44	94± 3	6±2	0±0
Mid Dose	14	0.95±0.20	96± 2	4±1	0±1
High Dose	14	1.07±0.18	95± 3	4±2	1±1

*Each value represents mean ± SD (n=6).

Table 6. Histopathological Respiratory Lesions in Rats Exposed to TPA.

	Air	Fuse/Fuel	Low	Medium	High
24 Hr PE					
Trachea					
Lymphocytic infiltrate	0/6	0/6	3/5	2/6	3/6
Inflammation, acute	0/6	0/6	0/5	0/6	2/6
Larynx					
Lymphocytic infiltrate	0/6	2/6	2/5	3/6	0/6
Lung					
Inflamation, acute	0/6	0/6	0/5	1/6	2/6
Lymphocytic infiltrate, perivascular	0/6	1/6	1/5	3/6	0/6

Table 6. Histopathological Respiratory Lesions in Rats Exposed to TPA (Continued).

	Air	Fuse/Fuel	Low	Medium	High
14-Day PE					
Trachea					
Lymphocytic infiltrate	1/5	2/6	2/6	4/6	3/5
Larynx					
Lymphocytic infiltrate	1/5	4/6	1/6	2/6	3/5
Inflammation, chronic-active	0/5	0/6	0/6	1/6	0/5
Lung					
Lymphocytic infiltrate, perivascular	0/5	1/6	1/6	2/6	1/5
Inflammation, interstitial, chronic	2/5	0/6	2/6	1/6	0/5

Gross examination of the exposed rats at 24-hr PE revealed eight rats with urinary bladder plugs. These plugs were specifically recorded at necropsy because a previous study had associated ingested TPA with urinary bladder calculi (stones) in rats.[2] The plugs noted in this study were coagula, resulting from reflux of ejaculated fluids from the male secondary sex glands. The coagula and remaining gross lesions were considered incidental, insignificant findings and are listed in the gross to microscopic correlation tables contained in the files in the archives of CRDEC (Toxicology Division).

Three potentially compound-related lesions were noted in the rats exposed to TPA at the 24-hr PE period. These included minimal to mild (1 and 2 respectively on a scale of 4) focal, lymphocytic infiltrates of the trachea; focal, minimal, acute tracheal inflammation; and minimal to mild, focal, acute inflammation of the lung (Table 6). At the 14-day PE period, focal, lymphocytic infiltrates of the trachea were also noted in the air-only and fuse/fuel controls. Tracheal and lung inflammation showed no relationship to treatment at 14 days.

Lymphocytic infiltrates, although found only in TPA-exposed rats at 24 hr, are considered incidental for the following reasons. Lymphocytic infiltrates are rarely consistent with an inflammatory response of 24 hr or less duration; similar lesions were found in controls at 14 days indicating that the lesions were not likely TPA induced; and similar laryngeal lesions were found in the fuse/fuel controls at 24 hr. Moreover, perivascular lymphocytic infiltrates of the

lung (lesions often associated with various infectious agents) were found in one fuse/fuel control and several TPA-exposed rats at 24-hr PE. However, this lesion was absent in the high dosage rats at 24-hr PE. Therefore, the lymphocytic inflammatory lesions observed in TPA-exposed rats were not considered to be related to TPA exposure.

4. DISCUSSION

Groups of male, Fischer 344 rats were exposed to pyrotechnically disseminated TPA at target concentrations of 100, 200, and 400 mg/m^3 for 30 min. Additional control groups of rats were exposed to air or fuse/fuel components. At 24-hr and 14-day PE, the rats were evaluated for pulmonary function, BAL, and histopathological changes. There were no compound-related mortalities and no significant changes in BAL, pulmonary function, or histopathology. Cardiopulmonary effects from TPA have been demonstrated in earlier studies with dogs but at near lethal dosages. Grigas et al. found changes in cardiopulmonary parameters after the following levels were exceeded: at 100 mg/kg, respiratory minute volume increased; at 250 mg/kg, pulmonary resistance increased, pulmonary compliance decreased after 500 mg/kg; and aortic blood pressure decreased after 600 mg/kg.[9] The mean lethal dose was 767 mg/kg with respiratory arrest and an abrupt fall in aortic blood pressure. These dosages were administered at the rate of 2 mg/kg/min and are several orders of magnitude greater than the concentrations produced with airborne exposure.

The only adverse reaction observed in this study was a dose-related transient rhinorrhea that disappeared within 1-hr PE. Histopathological examination of the nasopharnygeal region revealed no compound-related changes from the rhinorrhea. As reported in literature, TPA appears to have mild irritating properties.[1]

Initially, there was some concern about the contribution of the combustion byproducts to the toxicity of pryrotechnically generated TPA. The disseminated TPA aerosol was sampled for particulate and vapor byproducts. The gases generated (CO, CO_2, NO_2, and SO_2) were all below their respective TLVs for short-term exposures (Table 2) and did not appear to contribute additional toxicity to the biological parameters examined in this study. The particulates were collected, extracted, and analyzed in the Ames mutagenicity assay with negative results.[8] These results agree with the study by Florin et al. in which TPA was tested for mutagenicity in four strains of Salmonella and was found to be nonmutagenic.[10] In another study, TPA inhibited spontaneous mammary tumorigenesis in mice when incorporated in the diet at the 0.5% level .[11] The authors postulated that TPA may have some homeostatic/modulating role as evidenced by its role in maintaining the activity of some metabolizing enzymes in rats.[12] Dimethyl TPA is hydrolyzed to TPA in the rat; therefore, it is relevant that a bioassay testing dimethyl TPA

in rats at doses of 2500 or 5000 ppm in the feed for 103 weeks produced no carcinogenicity.[13, 14]

5. CONCLUSIONS AND RECOMMENDATIONS

Exposing male, Fischer 344 rats to pyrotechnically disseminated TPA for 30 min resulted in no adverse changes in pulmonary function, BAL parameters, or histopathology. Inhalation of the TPA resulted in a reversible, dose-related rhinorrhea.

Gas-phase byproducts (CO, CO_2, NO_2, and SO_2) were below the TLVs for short-term exposures as established by the American Conference of Governmental Industrial Hygienists. The particulate products were nonmutagenic in the Ames mutagenicity assay.

TPA is widely used in the chemical industry, and the extensive existing data base on TPA shows it to be a mild irritant to skin and mucous membranes; it does not accumulate in tissues and is excreted unchanged; it is nonmutagenic; it is not carcinogenic; it does not bioaccumulate; and it is degraded by soil microorganisms. The major physiological effect of TPA reported in animal experiments is the derangement of urinary electrolyte excretion resulting in excess calcium concentrations in the bladder, followed by urolithiasis with irritation induced hyperplasia and neoplasms. However, these effects only occur at high dietary concentrations (2-5% TPA). Also, at these high levels, fetal effects were also shown with decreased survivability. Humans would not likely be exposed to the dosages required to produce these effects.

TPA would be an excellent candidate for a "safe" training smoke, but the following additional studies should be conducted to fill data gaps:

- Repeated and/or subchronic inhalation studies from pyrotechnically generated devices to determine the effects from long-term airborne exposure to TPA.

- Analysis of urinary electrolytes, pulmonary function, and reproductive studies should be conducted concurrently with the repeated and/or subchronic inhalation tests.

- No information was available to indicate the effect of TPA on planktonic organisms, aquatic vertebrates, or terrestrial plants. These ecological studies should also be conducted.

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APPENDIX

INSTRUMENT CONDITIONS

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APPENDIX

INSTRUMENT CONDITIONS

A. MIRAN 80

Pathlength:	9.75 m
Wavelength:	
Reference Air	7.5 μ
CO_2	4.13 μ
CO (primary)	4.75 μ
CO (secondary)	4.85 μ
Slit width:	1.0 mm
Mode:	Absorbance
Readout Parameters:	
Number of wavelengths	4
Number of components	3
Scanning Speed	2 (8 min)
Delay time	9 (1 min)
Read time	5 (7 sec)
Zero time	254 (2 ft, 50 in.)
Purge time	254 (2 ft, 50 in.)

B. HPLC

Model:	6000A (Waters)
Column:	C-18
Detector:	UV-254
Mode:	Absorbance (0.2)
Solvents:	• 0.01 M tetrabutylammonium phosphate (TBA) in water • 100% Methanol
Solvent Mixture:	50:50 mix of solvents A & B
Solvent Flow:	1.5 mL/min
Integrator:	**Model 3380A (Hewlett Packard)**