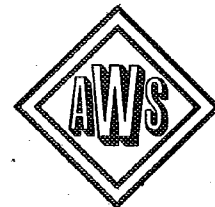
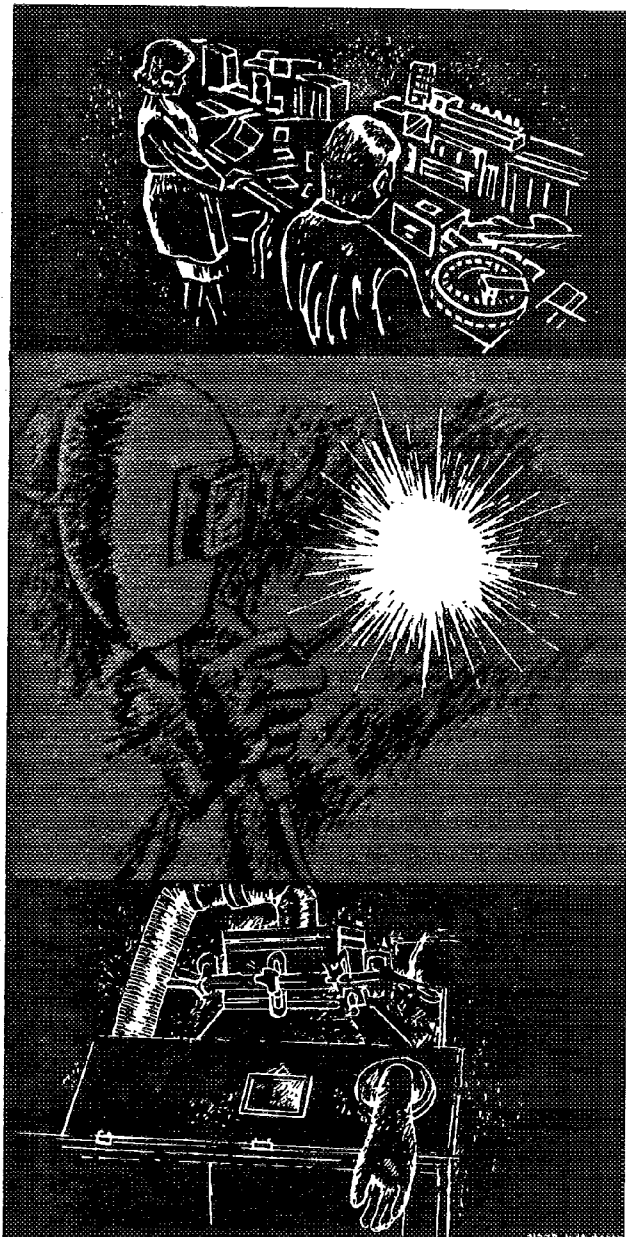


Toxicity of Welding Fume in Rats



AMERICAN WELDING SOCIETY

Key Words — welding fume,
toxicity, inhalation, research,
Hazleton Laboratories

Toxicity of Welding Fume in Rats (Complete Report)

Research performed by the Hazleton Laboratories America, Incorporated, Vienna, Virginia, under contract with the American Welding Society and supported by industry contributions.

Performed By:

William B. Coate, Ph.D.

August, 1985

Abstract

This report contains results of a study made on the toxicity of welding fumes in rats. Six welding fumes were administered to rats by inhalation to determine relative ranking of toxicity. Fumes used were generated from E6010, E7018, E70S-3, E70T-1, E308-16 and E5356 consumables. Results were evaluated in terms of acute inhalation toxicity, pulmonary pathologic and fibrogenic potential, *in vivo* cytogenetic potential and mutagenic potential of urinary excretions. Sampling in the chamber included measurement of particle concentration and size, ozone, carbon monoxide, and the oxides of nitrogen.

The Summary Report contains text of the study findings and selected tabular data. The Complete Report contains the same text, with the tabular data in its entirety.

Prepared for
SAFETY AND HEALTH COMMITTEE
AMERICAN WELDING SOCIETY
550 N.W. LeJeune Road, P.O. Box 351040
Miami, FL 33135

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Personnel

AWS Safety and Health Research Committee

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<i>W. S. Howes</i>	NEMA
<i>E. Mastromatteo*</i>	Consultant
<i>R. A. Parent*</i>	Consultox, Limited
<i>R. J. Simonton</i>	INCO Alloys International

*Advisors

Foreword

(This foreword is not part of the *Toxicity of Welding Fume in Rats* but is included for information purposes only.)

The health of workers in the welding environment is a major concern of the American Welding Society. As part of a continuing health effects research program, contract research projects have included studies of fumes and gases, ventilation, radiation and noise associated with various processes.

One area of concern has been the inadequate information reported in welding fume toxicity studies where laboratory animals are used. This report represents a contractual effort to rank relative toxicities of fume from six commonly used welding consumables. The results, while not definitely conclusive, indicate a need for more extensive studies of this kind.

Acknowledgments

Dr. Richard Parent, Consultox, Ltd., Baton Rouge, Louisiana, developed the test protocol and provided consultant services during the toxicity testing.

Funds for this project were provided by the American Welding Society.

The American Welding Society gratefully acknowledges the financial support of the program by industry contributions.

Supporting Organizations

Air Products and Chemicals, Incorporated
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Truck Trailer Manufacturers Association
Walker Stainless Equipment Company
Weld Tooling Corporation

Many other organizations have made contributions to support the ongoing program from May 1979 to the present.

AWS Safety and Health Research Committee Study Objective

The following study objective was prepared by the AWS Safety & Health Research Committee. It summarizes the study purpose and explains the technical problems encountered.

The objective of the study was to assess the relative toxicity to rats of six welding fumes in common usage. The toxicity potential was evaluated in terms of:

- Acute inhalation effects
- Lung fibrogenicity
- Cytogenetic effects
- Pulmonary pathology
- Mutagenic effects

In a study of acute toxicity, the relevance of the findings to a welder population with chronic fume exposure at much lower levels can be questioned. The issue is raised: should there be a chronic lifetime exposure study at more realistic fume levels? This might be the next logical step, taking into account cost and technical difficulties to be encountered. Also, a further investigation into the toxicity of E308-16 fume may be in order.

Future studies, if any, should be designed with more consideration given toward extrapolation to humans, as well as prospective epidemiological investigations of the general welding population.

Hazleton Laboratories
Office of Quality Assurance

Project Title: Toxicity Study of Welding Fumes in Rats

Project Number: 2236-100/101

Quality Assurance inspections of the study and review of the final report of the above referenced project were conducted according to the standard operating procedures of the Office of Quality Assurance and according to the general requirements of the appropriate Good Laboratory Practice Regulations. Findings from the inspections and final report review were reported to management and to the study director.

To the best of my knowledge, the phases of this study were conducted in compliance to the FDA-GLP Regulations and the report accurately reflects the data generated.

Frederick G. Snyder (signature on original)

Frederick G. Snyder Date
Director
Office of Quality Assurance

kms/2772

Subject: Toxicity Study of Welding Fumes in Rats
Project No. 2236-100/101
Hazleton Laboratories

We, the undersigned, hereby declare that the work was performed under our supervision, according to the procedures herein described.

Study Director:

William B. Coate (signature on original)
WILLIAM B. COATE, Ph.D.
Life Sciences Division

Laboratory Supervision:

Robert J. Hardy (signature on original)
ROBERT J. HARDY
Life Sciences Division

Pathology:

Thomas F. Hastings (signature on original)
THOMAS F. HASTINGS, D.V.M., Ph.D.
Diplomate, American College of
Veterinary Pathologists
Life Sciences Division

Study Coordinator:

Nancy McCarroll (signature on original)
NANCY McCARROLL, B.S.
Life Sciences Division

Study Coordinator:

Thomas Cortina (signature on original)
THOMAS CORTINA, B.S.
Life Sciences Division

Study Coordinator:

Marie Draus (signature on original)
MARIE DRAUS, M.S.
Life Sciences Division

Study Coordinator:

Julie A. Ralph (signature on original)
JULIE A. RALPH, A.A.S.
Life Sciences Division

tlh

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Toxicity of Welding Fume in Rats

Summary and Conclusions

Mutagenicity, fibrogenicity, cytotoxicity, and general histopathological manifestations were evaluated in a series of *in vivo* and *in vitro* tests in Sprague-Dawley rats subsequent to a single exposure to various welding fumes. Forty rats per sex per group were placed in a 1.0 m³ chamber which housed the welding unit used for the generation of the specific welding fumes. The fumes were exhausted into the animal exposure chamber at an airflow rate of approximately 200 liters per minute. Target concentration for each fume was 0.6 mg per liter, averaged over a single 6-hour exposure period. Homogeneity, total particulate/gravimetric concentration, particle size distribution, temperature, relative humidity, and the concentration of atmospheric gasses were monitored.

Histopathological evidence of exposure to the toxic welding fumes were noted primarily in the lungs as perivascular and intra-alveolar edema and a seropurulent or fibrinopurulent pneumonitis. Pigmented alveolar macrophages were indicative of fume/particle deposition in the airspaces with subsequent phagocytosis and clearance from the lung. Based on histopathological data from lungs exposed to these fumes, E308-16 demonstrated the most severe morphological changes as displayed by vascular damage and inflammatory cell infiltration. These findings were supported by analysis of lavage fluids obtained from another subgroup of rats exposed to E308-16. Lavagates appeared turbid and contained an appreciable number of red blood cells which rendered these samples inappropriate for macrophage assessment. Generally, animals exposed to the other fumes demonstrated a mild to moderate increase in alveolar macrophages, many of which were pigmented. Although the histopathology from these animals was fairly nonremarkable, other indications of pulmonary toxicity were present. This was especially true of cell recovery and percent macrophages obtained from bronchoalveolar lavage

fluids. In contrast to light microscopic evidence of a mild histiocytosis, lavage fluids from all males and females exposed to fumes E7018 and E70T-1 contained a significantly decreased number of macrophages. However, further characterization of the macrophages which were present indicated that macrophage viability was not influenced by the welding fumes. Although the phagocytic ability of the macrophages was apparently decreased following exposure to all fumes except E5356, the nature of the test makes it less sensitive than the exclusion of trypan blue in the viability test.

Comparisons between pulmonary histopathology and *in vitro* viability EC₅₀ data demonstrated good correlations in regard to the relative cytotoxicities. The viability EC₅₀ of the welding fumes showed that E7018 was only slightly more cytotoxic than E308-16 to alveolar macrophages and that E70T-1 and E6010 also possessed relatively low EC₅₀ viabilities. In contrast, the viability EC₅₀ of E5356 and E70S-3 were relatively high which indicated that the two fumes were virtually nontoxic. Histopathological assessment of lungs exposed to these welding fumes illustrated the cytotoxic effects *in vivo*. Pulmonary irritation, as displayed by edema and pneumonitis, was seen in animals exposed to E308-16, whereas clinical observations disclosed some obvious respiratory tract effects with exposure to E7018, E70T-1, and E6010. It should be noted however, that the *in vitro* tests evaluated the cytotoxicity of welding fumes on macrophages and their functional capabilities. The *in vivo* exposures resulted principally in edematous changes which could actually be an evaluation of the relative toxicities of the welding fumes on the vascular endothelium, which are perhaps less resistant to the fumes.

Therefore, in ranking the relative toxicological potentials of the welding fumes, histopathology and *in vitro* macrophage assessment permits classification into three categories: highly toxic, moderately toxic, and non-toxic. Welding fume E308-16, due to its ability to cause

irritation in the deep lung and its relatively low EC_{50} , may be regarded as highly toxic. E7018, E6010, and E70T-1 also possess the potential of causing respiratory tract irritation, albeit in the upper respiratory tract, and had relatively low EC_{50} viability values; therefore, these fumes may be considered moderately toxic. Based on histopathologic evaluations and EC_{50} data of animals exposed to the welding fumes designated E5356 and E70S-3, these may be regarded as nontoxic at the concentrations tested in this study.

Lastly, no mutagenic or cytogenic effects were found as a result of exposure to any of the fumes. In addition, the relative fibrogenic potential of these fumes could not be assessed.

Introduction

This project, sponsored by the American Welding Society, was designed to determine the relative ranking of fumes generated from six different welding electrodes (three different arc welding processes), in terms of their acute inhalation toxicity, pulmonary fibrogenic potential¹, *in vivo* cytogenetic² potential, pulmonary pathologic potential, and mutagenic³ potential of urinary excretions from exposed rats. To this end, a representative of each of six types of electrodes was used in manual or semiautomatic mode by skilled welders to provide comparable particulate concentrations (gravimetrically determined) of fresh fume over a six-hour period in an inhalation chamber containing 40 rats per fume. Fume was generated on one chamber and pulled through a fume scavenger by negative pressure into the inhalation chamber. In addition to sampling to determine fume particulate concentration, aerodynamic particle size distribution, ozone, nitrogen oxides, and carbon monoxide concentrations were measured in the inhalation chamber.

This project permits a direct ranking of the toxicity of the fumes to rats (at the same particulate concentrations) and a ranking of the potential hazards given knowledge of the total fume concentrations produced under standardized simulated welding conditions. This knowledge may be contained in AWS F1.2, *Laboratory Method for Measuring Fume Generation Rates and Total Emission for Welding and Allied Processes*, a publication of the American Welding Society.

1. Fibrogenic potential: ability for growth of white fibrous connective tissue in organ or part in excess of that naturally present.
2. Cytogenetic: structure, behavior, growth and reproduction of cells and their relation to genetics.
3. Mutagenic: causing abrupt change in the make up of an organism not resulting from recombination.

Welding Processes and Test Materials

Three welding processes with one or more representative electrodes were used to generate fume for testing as listed:

Process	Electrodes	Shielding Gas
Shielded Metal Arc/ Covered Electrodes	E308-16; E6010; E7018	—
Gas Metal Arc/ Solid Wire	E5356 E70S-3	Argon CO ₂
Flux Cored Arc	E70T-1	CO ₂

Test Animals

Sprague-Dawley descended rats, approximately six weeks of age, were obtained in seven separate shipments of 55 or 65 per sex from Charles River Breeding Laboratories, Kingston, New York. They were quarantined for 12 days during which time they were culled of those showing physical defects or signs of disease. Only those found in good health by the staff veterinarian and of normal body weight were accepted for study. Of those found acceptable, 39 or 48 per sex were selected and 26 or 30 per sex were randomly assigned to seven subgroups and the remainder for *in vitro* tests as indicated on the following page. Upon assignment to subgroups, the animals were permanently identified by monel metal ear tag numbers. The unused rats from each shipment were destroyed.

Animal Husbandry

Upon receipt and throughout their laboratory stay, the rats were individually caged in stainless steel mesh cages with Purina #5001 Laboratory Chow® and with tap water, both provided *ad libitum* except during exposure. They were housed in environmentally controlled chambers provided with 12 or more air changes per hour and a timer-controlled 12-hour light/dark cycle. Temperature and relative humidity were maintained between 66–78°F and 32 and 86 percent, respectively.

Generation of Test Atmospheres

Each of the welding fumes tested was generated in a cubical 1-m³ glass and stainless steel open-front chamber by a skilled welder using manual or semiautomatic technique as appropriate to the electrode. The work piece of appropriate parent metal plate was sited under an 18 in. L × 15 in. W galvanized fume scavenger

with stainless steel skirt on three sides. The front opening of the skirt was 11 inches high above a grate floor on which the work piece rested at a 15 degree angle. The fume scavenger was connected to the turret top of a second 1-m³ glass and stainless steel exposure chamber. The exposure chamber was fitted with a perforated stainless steel diffusion ceiling, held 40 individual stainless steel mesh restraining cages for rats on a single tier, and was exhausted from the bottom at 200 liters per minute under negative pressure. Thus, the fume laden air captured by the fume scavenger was swept into and out of the exposure chamber at the calibrated rate of 200 liters

per minute. Unscavenged fume was exhausted from the top of the first (welding) chamber or escaped into the well-ventilated room. Figure 1 gives a schematic view of the experimental setup.

The welder, seated in front of the workpiece, was surrounded on three sides by a welder's screen and on the fourth by the welding chamber. The exposure chamber was covered with black plastic tape on the two sides nearest the welding chamber in order to shield the rats from indirect radiation from the arc. Additional cooling of the exposure chamber (as needed to maintain its internal temperature below 80°F) was provided by a

Electrode	Subgroup							<i>In Vitro</i> Test	Date of Test Exposure
	A	B	C	D	CCA	CCB	CCD		
E70S-3	5/5	5/5	5/5	5/5	2/2	2/2	2/2	13/13	10/17/83
E308-16	5/5	5/5	5/5	5/5	2/2	2/2	2/2	13/13	10/24/83
E7018	5/5	5/5	5/5	5/5	2/2	2/2	2/2	13/13	10/31/83
E6010	5/5	5/5	5/5	5/5	2/2	2/2	2/2	13/13	11/07/83
E70T-1	5/5	5/5	5/5	5/5	2/2	2/2	2/2	13/13	11/14/83
E5356	5/5	5/5	5/5	5/5	2/2	2/2	2/2	13/13	11/28/83
E308-16 ^a	5/5	5/5	5/5	5/5	5/5	5/5	5/5	13/13	1/09/84

^a. Additional test at concentration selected for minimal lethality.

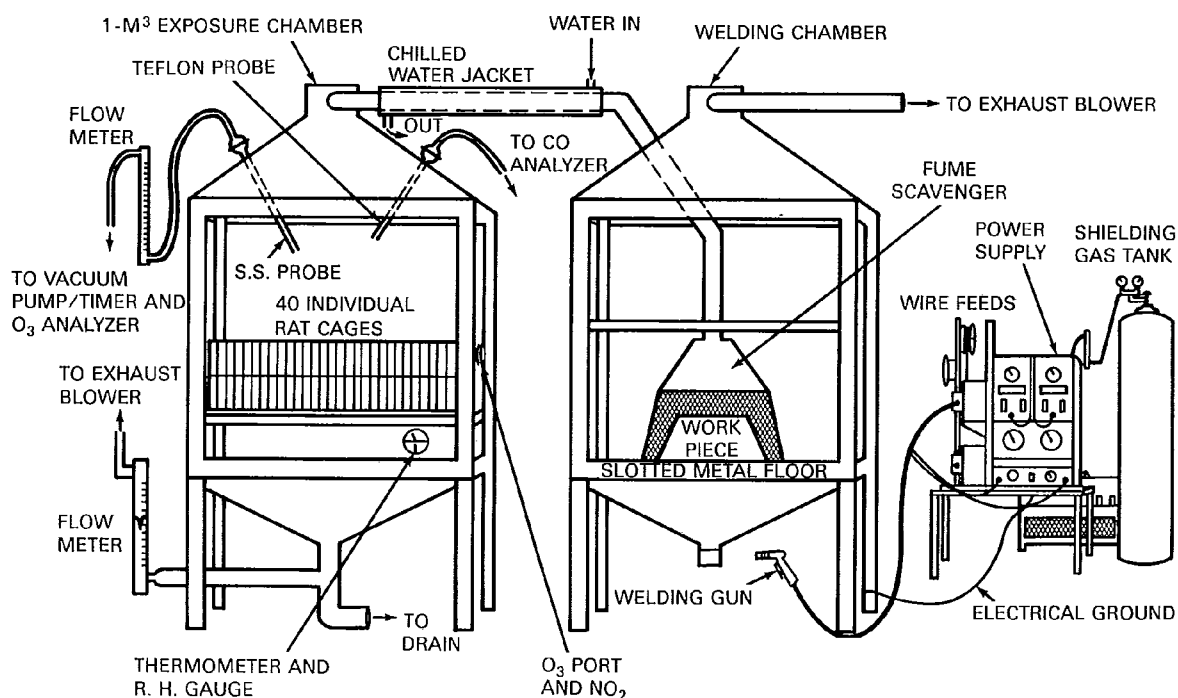


Figure 1 — Schematic View of the Arrangement for Exposing Rats to Welding Fumes

plastic bag filled with ice cubes on the chamber roof and a cold-water jacket on the copper pipe connecting the fume scavenger to the exposure chamber. A third 1-m³ glass and stainless steel chamber with a 200-liter per minute HEPA-filtered airflow from a clean air source was used to "expose" control animals concurrently.

Welding fume was generated for six hours using continuous 10-minute duty cycles⁴. Two skilled welders, spelling each other and assisting each other in the replacement of work pieces as needed, welded beads on the plates. The maximum welding period was nine minutes out of every 10 minutes. During each welding period, bead was laid continuously except for momentary breaks for workpiece exchange and restarting of the arc. A Hobart MEGA-FLEX 650 RVS power supply was used for all welding.

Preliminary runs were made with each electrode to determine the arc current and the on/off periods during each duty cycle needed to achieve mean six-hour fume (particulate) concentrations in the exposure chamber as near to 0.6 mg per liter as possible. This target concentration was selected based on a preliminary six-hour exposure with the stainless steel electrode (E308-16) fume which resulted in the deaths of 40/40 rats at a mean concentration of 1.78 mg per liter.

Ten closed-face filter (Gelman 47-mm GN-6) samples (gravimetric) of the fume in the exposure chamber were obtained at predetermined times during each six-hour run. Each sample was pulled via a stainless steel [0.5 in. (12.7 mm) I.D.] standard probe extended near the breathing zone of the animals. The sampling rate was 5.0 liters per minute for two minutes. Flow rate was measured with a Fischer-Porter 36-541-21 flowmeter calibrated with a wet test meter and remained constant throughout each sampling period. Samples were obtained during random portions of the duty cycles and each electrode fume was sampled similarly.

In addition, 47 mm open-face Nuclepore filter (0.2 μ pore size) samples of the fumes in the exposure chamber and at the welding site were obtained and shipped to Dr. Margaret Nasta, Mellon Institute, Carnegie Mellon University for particle sizing by automated electron beam analysis with SEM and chemical characterization by energy-dispersive X-ray analysis. A separate report on these analyses has been submitted by Dr. Nasta.

Table 1 lists the various parameters used with each electrode during the six-hour welding fume exposures. Arc current and voltage were recorded continuously on Moseley millivolt recorders. Arc length and drag angle

were maintained for optimal welds by the welder. Shielding gas flow rates were monitored with calibrated flow-raters appropriate to the gases. Wire-feed rates were controlled by Hobart 27 and Dualmatic CVS arc welding systems. Electrode materials were preweighed and postweighed at the end of each welding session to obtain M.O. rates.

Exposure Atmosphere Characterization

Homogeneity of Fume

Prior to the first test exposure, the homogeneity of particulate fume in the exposure chamber at the plane of the animal cages was determined using the E70S-3 electrode on steel plate. Four open-face filter samples were obtained simultaneously at four locations on top of the cages plus a closed-face sample via the standard probe. The gravimetric concentration was obtained from each sample. The results indicated a reasonably homogeneous (± 10 -18%) atmosphere in the breathing zone with a $\approx 25\%$ underestimation by the standard probe. Data are shown below.

E70S-3 Preliminary Gravimetric Concentrations (mg/L)					
Open-Faced Filter				Closed-Face Filter	
Sample:	1	2	3	4	1
	0.84	0.76	1.08	1.02	0.70

Total Particulates/Gravimetric Concentration

During each exposure, total particulate samples were obtained on 47 mm closed-face Gelman GN-6 membrane filters, via the standard probe, during 10 nearly evenly spaced periods within each six-hour fume generation period, starting 30 minutes after initiation of welding cycles. Each sample was collected at 5.0 liters per minute for 2.0 minutes. These samples were immediately weighed on an analytical balance (Mettler Model H 54AR) reading to 0.1 mg and then stored in glass vials within Teflon®-lined caps under nitrogen for possible future metal analysis. On the basis of sample weights obtained, the gravimetric concentration of particulate fume during each two-minute period was calculated. The mean and standard deviation of the 10 samples was taken to characterize the particulate concentration during the exposure period. (During the third hour,

4. Except for E5356, in which continuous two-minute duty cycles were employed.

Table 1
Fume Generation Parameters

Electrode	Diameter (inches)	Volts	Amps	Current	Shielding Gas	Gas Flow CFM	Workpiece	Continuous On/Off Periods	Melt Off Rate lb/hour
								Ten Minute Duty Cycles	
E70S-3	0.045	28	180	DC+	CO ²	40	1/2 in. steel	8 ft/2 ft	5.63
E308-16	1/4	24	210	DC+	—	—	1/2 in. steel	8 ft/2 ft	5.10
E7018	3/16	24	195	DC+	—	—	3/4 in. steel	8 ft/2 ft	4.66
E6010	3/16	29	125	DC+	—	—	1 in. steel	9 ft/1 ft	1.54
E70T-1	0.93	25	350	DC+	CO ²	45	1 in. steel	{ 8 ft/2 ft (3.5 hr) 6 ft/4 ft (2.5 hr)	9.20
E5356	3/64	26	135	DC+	Argon	45	1 in. aluminum	1 ft 54 in./6 in. ^a	0.14
E308-16	1/4	23	160	DC+	—	—	1 in. steel	5 ft/5 ft	2.84

a. E5356 electrode was melted during continuous two-minute duty cycles versus standard ten-minute duty cycle.

additional such filter samples were obtained to provide 100 mg of particulate to be used in *in vitro* macrophage studies.)

Particle Size Distribution and Characterization

Twice during each exposure, samples were obtained using Andersen 1-CFM eight-stage cascade impactors with backup filters. The stage plates and filters were pre and postweighed on the above analytical balance. The resulting distributions of total particulate mass collected were used to calculate the mass median aerodynamic diameter (MMAD) and σ_g for each sample of each fume.

Oxygen (O₂) Concentration

The O₂ percentage in the exposure chamber was monitored hourly with a Fyrite oxygen gauge (1 percent accuracy) with a protective particulate filter in the sampling line. Provision for supplementary O₂ was available in case the O₂ percentage fell below 19 percent, which it never did.

Ozone (O₃) Concentration

The O₃ concentration (in ppm) in the exposure chamber was monitored hourly using an AID Model 560 Ozone Analyzer (chemiluminescence principle) calibrated in 0.1 ppm increments up to 1.0 ppm with an AID Model 565 Ozone Generator (UV source) and with O₃-free room air. Samples were obtained via 1/4 inch Teflon® and stainless steel tubing with a Gelman GN-6 protective particulate filter in line.

Nitrogen Oxides (NO₂ and NO + NO₂) Concentrations

NO₂ (0.5 to 10 ppm) and NO + NO₂ (2 to 50 ppm) concentrations were separately monitored at hourly intervals with Draeger CH31001 and CH30001 tubes, sampling via a port in the chamber wall. In addition, an attempt was made to measure nitric oxide (NO) concentration directly, using a Miran 1A Infrared Vapor (IR) Analyzer calibrated with pure NO (99 percent) injected via a septum from a gas-tight syringe into a closed loop (5.61 liters) on the instrument cell. An analytical wavelength of 5.35 microns and a 20.25-meter pathlength was used. At this wavelength, NO₂ showed no absorbance. When compared to the results obtained by subtracting the Draeger NO₂ reading from the Draeger NO + NO₂ reading, no correlation between the obtained IR and the derived Draeger NO values could be established. It was subsequently found that the IR Analyzer was responding to some waste product diffusing from the feces of the exposed rats (but not of control rats), an emission which seemed to vary from electrode to electrode test. Thus, the NO readings obtained with the IR Analyzer were uninterpretable and could not be used to measure NO concentrations in the exposed chamber.

Carbon Monoxide (CO) Concentration

CO concentration was measured hourly in ppm using a second Miran 1A Infrared Vapor Analyzer sensitive to 0.2 ppm calibrated with pure CO injected via a septum from a gas-tight syringe into a closed loop (5.61 liters) on the instrument cell. An analytical wavelength of 4.61

microns and a 20.25-meter pathlength were used and the cell was maintained at a constant temperature in excess of 100° F. Samples were pulled at 5.0 liters per minute for five to six minutes (in excess of T_{99} for complete cell flushing) and the instrument response recorded on a stripchart. The peak response during each sampling period was logged as the CO concentration for that interval. Samples were pulled via a Teflon® line from the middle of the chamber through a protective in-line Gelman A/E glass fiber filter.

Temperature and Relative Humidity

Temperature and relative humidity were monitored in both exposure and control chambers with Abbeon Certified Temperature and Relative Humidity Gauges located at the level of the animals. The readings were logged from each exposure chamber every 15 minutes during chamber occupancy.

Experimental Design

The animals selected for each electrode test and concurrent common controls were subdivided randomly into Subgroups A, B, C, D, CCA, CCB, and CCD, with an additional 13 per sex to be used as donors of pulmonary macrophages for *in vitro* tests. The subgroup designations are defined in the following subsections, in terms of the use to which the animals were put:

(A) Subgroup A animals (five per sex per electrode) were used for two donations of urine (excreted 0–24 and 72–96 hours postexposure) to be pooled (by sex) and used in bacterial plate assays after concentration by lyophilization, and for gross and histopathology studies following a 14-day recovery period.

(B) Subgroup B animals (five per sex per electrode) were used to donate pulmonary macrophages by lung lavage for determination of macrophage count (recovery) and cell viability, as well as for ATP, lysozyme, and acid phosphatase concentrations, and phagocytosis activity as possible indicators of fibrogenicity potential.

(C) Subgroup C animals (five per sex per electrode) were used to donate bone (femur) marrow cells 24 hours postexposure for cytogenetic analysis.

(D) Subgroup D animals (five per sex per electrode) were similarly used to donate bone marrow cells 72 hours postexposure for cytogenetic analysis.

(CCA) Subgroup CCA animals (12 per sex) were used to donate urine and to be necropsied/histopathology as common controls for all A subgroups.

(CCB) Subgroup CCB animals (12 per sex) were used to donate pulmonary macrophages as common controls for all B subgroups.

(CCD) Subgroup CCD animals (12 per sex) were used to donate bone (femur) marrow as common controls for both Subgroup C and Subgroup D.

In Vitro Tests

The additional 13 unexposed animals per sex selected for each electrode fume test were used to donate untreated pulmonary macrophages for *in vitro* tests of the relative toxicity and fibrogenicity potential of the particulate phase of each fume collected from the exposure chamber.

Methodologies employed with each of these tests are described below under the respective subgroup designations and *in vitro* macrophage tests.

Supplemental Test

The initial test exposure to E308-16 fume at 0.601 mg/liter resulted in the deaths of 19 males and 20 females during or within 24 hours after the exposure; therefore, no further use was made of these animals (except to necropsy Subgroup A). A subsequent test exposure of 20 per sex was made at a particulate concentration expected to be the maximum acute nonlethal concentration of this fume, based on a series of "dose-ranging" exposures at different concentrations.⁵ This test exposure took place approximately six weeks after completion of the other electrode exposures, and was conducted as a separate experiment with its own control group (10 per sex).

Specimen, Raw Data, and Final Report Storage

All specimens, raw data, and the final report are stored in the archives of Hazleton Laboratories America, Incorporated.

Assay Methods

Subgroups A and CCA

Observations and Records

All of the Subgroup A rats were observed for toxic signs prior to exposure, at least every half hour during exposure, after removal from the exposure chamber, and

5. Exposure data, clinical signs, and mortality ratios for the "dose ranging" exposures are presented in Appendices 14A-14E (complete report).

twice daily thereafter for 14 consecutive days.⁶ Individual body weights were recorded initially at pre and post-exposure, on Days 7 and 14, and at termination.

Sacrifice and Gross Pathology

At termination, all surviving rats were sacrificed by exsanguination under sodium pentobarbital anesthesia. Terminal body weights were recorded prior to necropsy. Necropsies were also performed on animals that were found dead.

Organ Weights

Only the brains, lungs,⁷ livers, hearts, and kidneys of terminally sacrificed rats were weighed and organ/body weight ratios determined.

Tissue Preservation

The following tissues from all rats were preserved in 10 percent neutral buffered formalin:

Brain	Jejunum
Thyroid	Ileum
Trachea	Cecum
Heart	Colon
Liver	Rectum
Spleen	Urinary bladder
Kidneys	Testis with epididymis
Adrenals	Prostate
Stomach	Ovaries
Pancreas	Uterus
Duodenum	Nasal turbinates

The lungs with bronchi were preserved in a formalin-glutaraldehyde fixative.

Histopathology

The lungs of these animals were embedded in Paraplast®, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Statistical Analyses

Absolute body weights and organ weight data of the common control group were compared statistically against the data of the fume-exposed groups of the same sex. Absolute body weights were evaluated at initiation, on Day 7, and at termination (Day 14).

6. The control animals were sacrificed after the morning observation on Day 13 because of scheduling necessity.

7. Lungs from the Subgroups C and D, as well as from CCD animals, were similarly weighed upon removal and trimmed for wet weight, and then were air-dried for 24 hours, further dried for 48 hours in a desiccator, and then reweighed for dry weight. The lung wet/dry weight ratio for each animal was calculated and presented by subgroup.

Analyses of the above data were performed in the following order. Levene's test for homogeneity of variances (Levene, 1960; Draper and Hunter, 1969) was performed, and if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA) (Winer, 1971). If the variances proved to be heterogeneous, a series of transformations was performed until variance homogeneity was achieved. These transformations were $\log_{10}$, square (X^2), square root ($X^{1/2}$), reciprocal ($1/X$), angular (arcsine $X^{1/2}$), and rank, respectively. If rank transformation was ineffective in removing variance heterogeneity, ANOVA of ranked data was completed. If ANOVA of untransformed or transformed data was significant, Dunnett's t-test (Dunnett, 1955 and 1964) was used for control versus compound-treated group mean comparisons. If ANOVA was not significant, the analysis was complete.

Data transformations are designated throughout this report as presented below:

A = Data analyzed following $\log_{10}$ transformation

B = Data analyzed following square (X^2) transformation

C = Data analyzed following square root ($X^{1/2}$) transformation

D = Data analyzed following reciprocal ($1/X$) transformation

E = Data analyzed following angular (arcsine $X^{1/2}$) transformation

F = Data analyzed following rank transformation
For mean tables, the above indications followed by the letters M, F, or B indicate the transformation occurred for either the Male, Female, or Both male and female groups.

Tests for homogeneity of variances and ANOVA were evaluated at the 5.0 percent one-tailed probability level. Control versus compound-treated group mean comparisons of the above data were evaluated at the 5.0 percent two-tailed probability level.

Statistical references are appended to this report, and statistically significant differences, as indicated by the aforementioned tests, are designated throughout this report by the term *significant* and/or as follows:

S+ = Significantly higher than the control value

S- = Significantly lower than the control value

Subgroup A — Body Fluid Mutagenicity Assay

Materials

Control Substances. The control substances listed on the following page were used in this study and were considered stable under the conditions of this assay.

Chemical	Manufacturer	Lot Number	Purity
Corn Oil (Mazola)	Best Foods	Not Applicable	100%
2-Aminoanthracene (2-AA)	Sigma	11 of 0600	Practical Grade
Deionized Glass Distilled Water (DGDH ₂ O)	Collected from AG-11 Automatic Collection System	Not Applicable	Assume 100%
Dimethylsulfoxide (DMSO)	Fisher	730913	Certified ACS
Methylnitronitroso-guanidine (MNNG) ^a	Aldrich	010247	97%
9-Aminoacridine (9-AA) ^a	Sigma	23F-0382	Grade II
2-Nitrofluorene (2-NF) ^a	Aldrich	261OPE	98%

a. Positive control agents used directly in the mutational assay.

Body Fluid Collection Procedure

Test Materials

1. Urine Collection. Urine from each animal was collected in individual Roth metabolism jars over a 24-hour period immediately following the conclusion of welding fume exposure (or air control exposure) and an additional 24-hour collection was performed starting 72 hours postexposure. The urine samples from test and control animals were collected into flasks held at 0–4°C, filter-sterilized and held frozen at approximately –20°C. The frozen samples were later lyophilized and used for analysis in the *Salmonella*/Mammalian Microsome Mutagenicity Assay.

2. Positive Control Group. Prior to initiation of the study, three male Sprague-Dawley descended rats were administered a single intraperitoneal injection of 2-Aminoanthracene (2-AA), 80 mg/kg dosing volume = 1 ml/100 gm. Pooled urine, collected from these animals for a 24-hour period following treatment, was held frozen and later concentrated as described below until performance of the mutational assay.

Urine was also collected and concentrated from five untreated male rats and served as the negative control for this group.

3. Concentration. Individual urine samples from each fume exposure and the accompanying control series, which were collected at the same time as the appropriate fume exposure urine, were pooled as follows:

Test Groups (Fume Exposure)	Negative Control Group
24 Hour Male Urine	24 Hour Male Urine
24 Hour Female Urine	24 Hour Female Urine
72 Hour Male Urine	72 Hour Male Urine
72 Hour Female Urine	72 Hour Female Urine

Pooled urine samples from each test fume and control exposure were concentrated by lyophilization. The residual dried material for each group was weighed and held frozen at approximately –20°C until performance of the mutational assay.

To expedite sample analysis in the mutational assay, the negative control urines collected from two animals per sex per fume for fume exposures E5356, E70S-3, E70T-1, E6010 and E7018 were separately pooled, concentrated, and used as the specific negative control for the appropriate test fume exposure. For fume exposure E308-16, the control urines collected from 10 animals per sex were treated in a similar manner and constituted the negative control for this test.

Mutagenicity Assay

1. Preparation of the Test Material. Sufficient weight of each residual material from each pooled urine sample was weighed and dissolved in deionized glass distilled water (DGDH₂O) to yield a 100 mg/ml solution. It should be noted that reconstitution of all residues resulted in cloudy, homogenous suspensions. The test suspensions for fume exposure groups, prepared as

described, were further diluted 1:10 in DGDH₂O; therefore, two concentrations of the residue collected from urine pooled by sex and postexposure time for each test fume exposure were evaluated in the *Salmonella*/Mammalian Microsome Mutational Assay. All control residues were prepared in DGDH₂O to contain 100 mg/ml, and were tested at a single concentration.

2. Mutational Assay. Exposure of the auxotrophic *S. typhimurium* strains to the concentrated urine samples was accomplished by the plate incorporation assay. For induction of reverse mutations, the reconstituted urine samples, prepared as described, were tested against five bacterial strains: TA1535, TA1537, TA1538, TA98 and TA100, with and without S-9 metabolic activation. Pooled urine samples from test animals and controls were plated in triplicate.

To each 2.5 ml of complete top agar the following were added: 0.1 ml of an overnight broth culture of each tester strain and 0.1 ml of the appropriate dilution of reconstituted test urine or control urine. For the activated phase of testing, 0.5 ml of the S-9 mix and 0.1 ml of a Beta-glucuronidase preparation were also added. The contents of the tube were mixed thoroughly and poured onto VBE minimal agar plates. Concentrated urine collected from animals administered the positive control were treated in a similar manner.

Plates were gently rotated and tilted to assure uniform distribution of the top agar, allowed to harden on an even surface for one hour, inverted and put into a dark 37°C ± 0.5°C incubator. After two days, the colonies in both test plates and controls were counted using an electronic colony counter.

3. Positive Control Compounds. In addition to the positive urine control, the following known mutagens were included in the experiment:

Strain	Minus S-9	Plus S-9
TA1535	MNNG (5 µg/plate)	2-AA (5 µg/plate)
TA1537	9-AA (75 µg/plate)	2-AA (5 µg/plate)
TA1538	2-NF (50 µg/plate)	2-AA (5 µg/plate)
TA98	2-NF (50 µg/plate)	2-AA (5 µg/plate)
TA100	MNNG (5 µg/plate)	2-AA (5 µg/plate)

4. Metabolic Activation. The mammalian microsome fraction used for metabolic activation was prepared from male Sprague-Dawley rats. The liver homogenate (S-9) was prepared in advance and stored frozen until used.

A. Preparation of Liver Homogenate (S-9). Enzymes were induced in male Sprague-Dawley rats by a single intraperitoneal injection of a polychlorinated biphenyl mixture (200 mg Monsanto Aroclor 1254 in

corn oil) at a dosage of 500 mg/kg five days before sacrifice. The animals were deprived of food and water prior to sacrifice.

The rats were sacrificed by decapitation, the livers removed, placed in sterile ice-cold beakers, and washed in an equal volume of 0.15 M KCl. All livers were minced with sterile scissors in three volumes of the sterile KCl rinsing solution and homogenized. The homogenates were centrifuged at 9000Xg in a refrigerated centrifuge and the supernate (S-9 fraction) was retained. The S-9 fraction was distributed in aliquots quickly frozen on dry ice, and stored in a freezer. As needed, sufficient aliquots of the S-9 fraction were thawed and kept on ice until incorporated into the S-9 mix. The unused portion was discarded at the end of the day.

B. Preparation of S-9 Mix. The S-9 mix contained per ml:

(1) S-9	0.05 ml ⁸
(2) MgCl ₂ - 0.4 M	0.02 ml
(3) KCl - 1.65 M	0.02 ml
(4) NADP - 0.05 M	0.08 ml
(5) glucose-6-phosphate - 0.1 M	0.05 ml
(6) sodium phosphate pH 7.4 - 0.2 M	0.50 ml
(7) H ₂ O	0.28 ml
	1.00 ml

The S-9 mix was freshly prepared on the day of the experiment. Ingredients (1), (2), (3), (6) and (7) were mixed and passed through a Millipore prefilter, a 0.8 µ membrane and a 0.45 µ sterile membrane. Ingredients (4) and (5) were aseptically added following filtration. The S-9 mix was kept on ice; the unused portion was discarded at the end of the day. Stock solutions of MgCl₂, KCl and NaH₂PO₄ were stored in the refrigerator; stocks of reagents G-6-PO₄ and NADP were held in a freezer.

C. Preparation of Beta-glucuronidase. Beta-glucuronidase was prepared at a concentration of 2.5 mg/ml in phosphate buffer, filter-sterilized, and kept on ice; the unused portion was discarded at the end of the day.

Subgroup B — *In Vivo* Rat Alveolar Macrophage Assay

Procedure

Media and Reagents. All media and reagents, including serum, required for this program were prepared in bulk

8. The appropriate amount of S-9 to be added was determined on the basis of standardization experiments using the promutagens: 2-acetylaminofluorene and 2-AA.

prior to the start of this program. Storage was appropriate, so that no degradation of media or reagents occurred.

Test System Information. The respiratory system has effective mucociliary transport and phagocytic cells that prevent the accumulation of inhaled particles in the lung and clear them away from the respiratory membrane.

Alveolar macrophages are phagocytes located on the surface of the small airways and in the alveoli of the lungs. These cells actively engulf bacteria and foreign particles that enter the lung and they detoxify these foreign substances. Since alveolar macrophages play a vital role in maintaining normal lung function, it is of considerable importance to determine the effects of potential environmental pollutants in these cells.

This work was designed to study the effects of six welding fumes on the alveolar macrophage cells lavaged from the lungs. Viability was assessed by trypan blue exclusions. Ability of macrophages to utilize oxidative phosphorylation was determined by measuring ATP levels. Phagocytic activity of the cells was assessed using an *in vitro* challenge to latex particles. In order to examine the impact of phagosomal welding fumes on lysosomal enzyme content, the cellular activities of lysozyme and acid phosphatase were determined.

A separate group of 10 clinically healthy Sprague-Dawley descended rats (five males and five females) aged eight to nine weeks were used for each fume tested. In addition, a common control group of 12 males and 12 females was evaluated. Only cell preparations free of mucous and blood were used for the assay.

Methodology. Alveolar macrophages from each exposed animal were cultured separately. Concurrent CCB control animals (two per sex) were included for each fume and the results pooled. A separate control group of five per sex was used for the E308-16 exposure test.

Lung Lavage. Approximately 20 hours following the six-hour exposure, the animals were euthanized by i.p. injection of sodium pentobarbital just before lung lavage via tracheostomy. The site for tracheal incision was washed thoroughly with alcohol, and the tracheostomy performed using aseptic techniques. Sterile saline (0.85 NaCl solution) containing penicillin (100 units/ml) (Gibco), streptomycin (100 µg/ml) (Gibco) and Kanamycin (100 µg/ml) (Gibco) was warmed to 37°C and used to lavage the lungs *in situ*. The first instillation was allowed to remain in the lungs for 15 minutes, and five subsequent instillations were removed immediately.

Cells from the lavage fluid were then pelleted by centrifugation at 1200 rpm for 10 minutes. The supernatant was removed and the cells resuspended in growth medium. An aliquot of this suspension was mixed with

0.4 percent trypan blue (Gibco) and counted in a hemocytometer. Total cells, percent live cells (unstained), and percent dead cells were recorded. The remaining cells were pelleted again and resuspended in prewarmed Medium 199 (Gibco) with Hank's balanced salt solution supplemented with 20 percent heat-inactivated, fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 100 µg/ml Kanamycin.

A cell count was performed and the suspension adjusted to approximately 1×10^6 cells/ml.

Design. Cells from each animal were established as individual cultures. Depending on the number of cells obtained from each animal, one to three cultures were established per animal. Initial viabilities were also measured. Only preparations having at least 95 percent viability were used in culture. The cell cultures were incubated at $37^\circ\text{C} \pm 0.5^\circ\text{C}$ in a humidified atmosphere with 5 percent CO_2 in air for 20 hours.

At the end of incubation, the medium containing unattached cells was decanted into a centrifuge tube. The attached cells were removed by trypsinization using trypsin—EDTA (0.25%) (Gibco). For each sample, the original medium and trypsinized cells were combined and separate aliquots of cells were removed for viability, ATP, lysozyme, acid phosphatase, and phagocytosis determinations.

Viability Determination. The cell viability was determined by counting the number of cells that excluded trypan blue dye (live), the number of cells that retained the dye (dead), and summing for total cells. Percentages of viable cells and a viability index were calculated for each culture.

Cellular ATP Determination. An aliquot of cells was removed from each starting cell suspension which contained viable and nonviable cells, and an equal volume of ATP-releasing reagent (Turner) and Hepes buffer (Turner) was added to the cells. The sample was placed into a photometer (Turner Instruments), and a luciferin-luciferase reaction mixture (Turner) was dispensed into the sample. The ATP in each sample of cells was calculated by comparison to an internal ATP standard (Turner).

Lysozyme Determination. For each culture, a sample of sonicated cell extract, prepared from each starting cell suspension, was added to an equal volume of *Micrococcus luteus* and incubated for 30 seconds at room temperature. Results were determined by reading the change in percent light transmission of the suspension at 645 nm. Enzyme determination was calculated by comparison to an internal crystalline lysozyme standard.

Acid Phosphatase Determination. For each culture, a sample of sonicated cell extract, prepared from each starting cell suspension, was placed on ice and 16 nM disodium p-nitrophenyl phosphate (Sigma) was added. The reaction tubes were incubated at 37°C. After 30 minutes, the incubation tubes were returned to the ice bath and 0.25N NaOH was added to stop the reaction. Each tube was vortexed and absorbance at 410 nm was read for each sample. Enzyme determination was calculated by comparison to an enzyme standard.

Phagocytosis Determination. Freshly isolated rat alveolar macrophage cells were placed in six-well dishes containing coverslips, and polystyrene latex particles (1.0 μ m diameter) were added to each culture at approximately 20 latex particles per macrophage. Cultures were placed at 37°C. After 30 minutes incubation, each culture was fixed, and stained with Giemsa, destained with 10 percent rosin, and rinsed in ethanol. The slides were processed in 100 percent xylene for three hours to remove extracellular particles.

The phagocytic index was determined for each culture by microscopically counting 200 macrophages per culture. Macrophages were classified according to numbers of latex spheres phagocytized: 0, 1–3, 4–6, 7–9, or >10. Results of cultures exposed to welding fumes were compared with control cultures and statistical differences noted, if present. Macrophages containing at least one particle were considered to be functionally active. Macrophages containing 10 particles or more were not considered to be functionally active due to the inability to distinguish particles from debris.

Statistical Analyses. Viability, cellular ATP, lysozyme, acid phosphatase, and phagocytosis determinations were analyzed by a Student's t-test (Snedecor and Cochran, 1980). All analyses were evaluated at the 5.0 percent one-tailed probability level.

Subgroups C, D, and CCD — *In Vivo* Bone Marrow Cytogenetic Analysis

Procedure

Administration of Colchicine. Twenty hours after removal from the exposure chamber, five animals per sex (Subgroup C) per fume, and 70 hours after removal from the chamber another five animals per sex (Subgroup D) per fume, and 10 common control animals per sex (Subgroup CCD, plus 10 additional control animals per sex for the E308-16 fume only), were administered colchicine by intraperitoneal injection at 2.0 mg/kg body weight (dosing factor = 5.0 ml/kg) to inhibit mitosis and arrest cells in metaphase. The colchicine was dissolved in Hank's Balanced Salt Solution (HBSS).

Sacrifice of Animals. Approximately two hours after injection of colchicine, the animals were sacrificed by injection of sodium pentobarbital.

Cytogenetic Techniques

1. Preparation of Slides. The bone marrow cells were processed according to the modified techniques described by Evans (1976) and Killian, et al. (1977). Immediately following sacrifice, bone marrow cells were collected from both femurs of each animal by aspiration into 5 ml of HBSS, prewarmed to 37°C. The aspirate was centrifuged for five minutes at approximately 1100 rpm. The supernate was decanted and 7.0 ml of prewarmed (37°C) 0.075M KCl were added to each tube. After 25 minutes, five drops of freshly prepared fixative (methanol:acetic acid, 3:1) were added to each tube. The tubes were immediately capped, gently inverted to mix the contents and centrifuged for five minutes at 1100 rpm. The supernate was decanted, and 5.0 ml of fixative was added slowly down the sides of each tube. The cells were resuspended and recentrifuged for five minutes. The supernate was discarded, and a new addition of 5.0 ml of fresh fixative was added to each tube. The cells were washed once more with fixative, then refrigerated. The chilled preparations were centrifuged at ~1100 rpm for five minutes, the supernate was decanted and the cells suspended in 0.5–2.0 ml fresh fixative. Several drops of the final cell suspension were dispersed onto precleaned glass microscope slides and air-dried. Cell density was checked using a phase-contrast microscope. Two slides were made for each animal and were marked with the animal's identification number.

2. Stain Preparation. Fresh stain was prepared by mixing 10 ml of Giemsa stain (Harleco) with 240 ml of glass distilled deionized water. The cells were stained in this solution for 10 minutes. Slides were rinsed twice in glass distilled water, air-dried, and subsequently mounted with glass cover slips in CoverBond® mounting media.

Cytogenetic Analysis

1. Chromosome Evaluation. After all slides were prepared, stained, and cover-slipped, a code number was temporarily assigned to each animal by a person not involved in the scoring of the slides. The slides were not decoded until all had been analyzed.

An attempt was made to examine at least 50 cells in metaphase from each group. In some instances, it was not possible to locate 50 spreads, so as many spreads as could be found were analyzed. The slides were scanned with a low power objective (10X or 25X) to find cells in the metaphase stage, and the chromosomes were analyzed with high power oil immersion lens (100X). Only those cells in the metaphase stage of mitosis were

analyzed for the presence of cytogenetic abnormalities. The following items were recorded for each animal: numbers and types of chromosomal aberrations, mitotic index, modal number for each metaphase, and the vernier location of each metaphase containing damage. The mitotic index was recorded as the number of cells undergoing mitosis per 500 cells counted and was represented as a percentage.

2. Classification of Cytogenetic Aberrations. Chromosomal aberrations were characterized as follows:

- (1) chromatid breaks—including fragments and deletions
- (2) chromosome breaks—including acentric fragments, deletions, and minutes
- (3) chromatid and chromosome gaps
- (4) exchanges—rings, dicentrics, translocations, quadriradials and triradials
- (5) cells with ≥ 10 aberrations
- (6) pulverized cells

Chromatid aberrations involve a break in only one arm of the chromosome; chromosomal aberrations involve both arms at identical sites. A break is defined as any separation that exceeds the width of a chromatid arm or a discontinuity accompanied by disturbance of the axial integrity. A break may or may not be associated with an identifiable piece of displaced chromatid material; i.e., fragment.

Chromatid and chromosome gaps are paucities in the stain involving one or both chromatid arms, and are less than the width of the arm(s). The origin of this type of damage is still in question and, although recorded, was not included in the statistical analysis.

Aberrant configurations which alter the structural appearance of an entire chromosome or involve the joining together of more than one chromosome were categorized as exchanges. Exchanges consist of dicentrics, rings, translocations, triradials, and quadriradials. Those cells with 10 or more scorable aberrations were classified separately. Thus, when determining the total number of aberrations in the cells analyzed for each individual animal, cells with ≥ 10 aberrations were counted as 10 aberrations, as suggested by Killian, et al. (1977).

3. Statistical Analyses. Upon completion of all scoring, the slides were decoded and the data was collated into the appropriate groups for statistical analyses.

The mean mitotic indices, mean modal numbers, percent aberrant cells, and the mean number of aberrations per cell for each group were statistically compared using the Kruskal-Wallis nonparametric analysis of variance and nonparametric pairwise group comparisons (Kruskal and Wallis, 1952; 1966). All tests were one-tailed at the 95 percent confidence interval ($p \leq 0.05$).

Subgroup B—*In Vitro* Rat Alveolar Macrophage Assay

Procedure

Test Materials

1. Collection of Fumes. For each of the six fumes generated, approximately a 100 mg gravimetric sample was taken on Gelman GN-6 membrane filters during the third hour of exposure. Immediately after removal from the chamber, each sample was weighed and stored in a glass vial under nitrogen for no more than 30 minutes before use in the *in vitro* assay.

2. Control Articles. Cell culture medium was used as the negative control. Cab-O-Sil at 50 $\mu\text{g}/\text{ml}$ in saline solution (see *Lung Lavage*) was used as the positive control.

3. Media and Reagents. All media and reagents, including serum, required for this program were prepared in bulk prior to the start of this program. Storage was appropriate, so that no degradation of media or reagents occurred.

Test System Information. A separate group of 26 clinically healthy animals (13 males and 13 females) aged eight to nine weeks, and breathing only ambient laboratory air, was used to obtain macrophages for each fume tested. Only individual lung lavage preparations free of mucous and blood were used. From each group of animals, a pooled sample of the lavage fluid was stained with Giemsa and checked for cell type. Preparations were pooled and all had at least 90 percent macrophages and initial macrophage viabilities of at least 95 percent.

Methodology. Each fume was tested at eight half-log concentrations of fume particulate in triplicate, beginning at 10,000 $\mu\text{g}/\text{ml}$. Appropriate negative and positive controls were included in each assay.

Lung Lavage. On the day of sacrifice, the animals were euthanized by i.p. injection of sodium pentobarbital just before lung lavage via tracheostomy. The site for tracheal incision was washed thoroughly with alcohol, and the tracheostomy performed using aseptic techniques. Sterile saline (0.85% NaCl solution) containing penicillin (100 units/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and Kanamycin (100 $\mu\text{g}/\text{ml}$) was warmed to 37°C and used to lavage the lungs *in situ*. The first instillation was allowed to remain in the lungs for 15 minutes, and five subsequent instillations were removed immediately.

Cells from the lavage fluid were then pelleted by centrifugation at 1200 rpm for 10 minutes. The supernatant was removed and the cells resuspended in growth medium. A portion of this suspension was mixed with

0.4 percent trypan blue and counted on a hemocytometer. Total cells, percent live cells (unstained), and percent viable cells and viability index were recorded. The remaining cells were pelleted again and resuspended in prewarmed Medium 199 with Hank's balanced salt solution supplemented with 20 percent heat-inactivated fetal bovine serum, 100 units/ml of penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin and 100 $\mu\text{g}/\text{ml}$ Kanamycin.

A cell count was performed and the suspension adjusted to approximately 1×10^6 cells/ml. The prepared cell suspension was added to each of three cell culture wells just prior to treatment with the fume sample dilutions.

Design and Treatments. For each of the six fumes generated, a special gravimetric sample was collected on three or more Gelman GN-6 membrane filters during the third hour of exposure. After removal from the chamber, this sample was weighed immediately and stored in a glass vial under nitrogen for not more than 30 minutes. Approximately 100 mg of each fume was collected in this manner.

Each filter was then extracted with an appropriate volume of medium and sonicated to remove any remaining particulates from the filter. Stock suspensions of particulate sample were prepared to contain the fume particulate at approximately 2000 $\mu\text{g}/\text{ml}$ on a weight per volume basis. This was $\approx 2X$ concentration of the highest test concentration.

The collected test fume suspension was then added to the media in eight half-log concentration levels, starting at 10,000 $\mu\text{g}/\text{ml}$. Each assay (for each concentration) was performed in triplicate. The suspensions were then incubated at $37^\circ \pm 0.5^\circ \text{C}$ in a humidified atmosphere of 5 percent CO_2 in air for a period of 20 hours.

Viability Determinations. At the end of the incubation, the medium containing unattached cells was decanted into a centrifuge tube. The attached cells were removed by trypsinization, using trypsin-EDTA (0.25%). For each sample, the original medium and the trypsinized cells were combined and an aliquot of cells removed for each of the following determinations: viability, ATP, lysozyme, acid phosphatase, and phagocytosis.

Cell Viability Determination. The cell viability was determined by recording total cells counted, percent cells excluding trypan blue (live), and percent cells retaining the dye (dead). In addition, the concentration of collected fume that reduced cell viability by 50 percent (the EC_{50}) was determined. All six fumes were evaluated in this manner and compared with both positive and negative control groups and with each other.

Cellular ATP Determination. An aliquot of cell suspension was removed from the culture, and an equal volume of ATP-releasing reagent and Hepes buffer was added to the cells. The sample was placed into a photometer (Turner Instruments) and a luciferin-luciferase reaction mixture was dispensed into the sample. The ATP in each sample of cells was calculated by comparison to a concurrent ATP standard.

Lysozyme Determination. For each culture, a sample of sonicated cell extract was added to an equal volume of *Micrococcus luteus* and incubated for 30 seconds at room temperature. Results were determined by reading the change in percent light transmission of the suspension at 645 nm. Enzyme determination was calculated by comparison to a crystalline lysozyme standard.

Acid Phosphatase Determination. For each culture, a sample of sonicated cell extract was placed on ice, and 16 nM disodium-p-nitrophenyl phosphate was added. The reaction tubes were incubated at 37°C . After 30 minutes, the incubation tubes were returned to the ice bath, and 0.25N NaOH was added to stop the reaction. Each tube was vortexed and absorbance at 410 nm was read for each sample. Enzyme determination was calculated by comparison to an enzyme standard.

Phagocytosis Determination. Freshly isolated rat alveolar macrophage cells were placed in six-well dishes containing coverslips, and polystyrene latex particles (1.0 μm diameter) were added to each culture at approximately 20 latex particles per macrophage. Cultures were placed at 37°C . After 30 minutes incubation, each culture was fixed, and stained with Giemsa, destained with 10 percent rosin, and rinsed in ethanol. The slides were processed in 100 percent xylene for three hours to remove extracellular particles.

The phagocytic index was determined for each culture by microscopically counting 200 macrophages per culture. Macrophages were classified according to numbers of latex spheres phagocytized: 0, 1-3, 4-6, 7-9, or 10. Results of cultures exposed to welding fumes were compared with control cultures and statistical differences noted, if present. Macrophages containing at least one particle were considered to be functionally active. Macrophages containing 10 particles or more were not considered to be functionally active due to the inability to distinguish particles from debris.

Statistical Analyses. Viability, cellular ATP, lysozyme, acid phosphatase, and phagocytosis determinations were analyzed by a Student's t-test (Snedecor and Cochran, 1980). All analyses were evaluated at the 5.0 percent one-tailed probability level.

Results

Gravimetric Concentrations

The mean ($\pm$ S.D.) particulate concentration for each exposure is presented in Table 2, along with the mean data for the other chamber parameters monitored during exposures.

Individual and mean gravimetric concentration values for each exposure are presented in Appendix 1.

Particle Size Analyses

Individual mass median aerodynamic diameters (MMAD's) and geometric standard deviations (σ_g 's) are presented in Appendix 2.

Test Atmospheric Gas Concentrations

Hourly test concentrations of oxygen, ozone, nitrogen oxides (NO_2 and $\text{NO} + \text{NO}_2$), and carbon monoxide are presented in Appendix 3.

Temperature and Relative Humidity

Hourly temperature and relative humidity readings in test and control chambers are presented in Appendix 4.

Subgroups A and CCA

Body Weights and Clinical Observations

Individual and mean body weights are presented in Appendices 5A and 5B. Group mean body weights are presented in Tables 1A and 1B (Complete Report). Clinical observations and mortality (by group) are summarized in Tables 2A and 2B (Complete Report).

No treatment-related trends were noted in the mean body weights although mean body weights of the E70S-3

males were significantly lower than CCA males at Day 7. This was not considered to be biologically significant because this group had a somewhat lower mean at Day 1 (pre-exposure) when compared to the CCA control group. All remaining postexposure body weight data were comparable to the CCA control group.

Clinical signs noted among the E308-16 Subgroup A (initial test) rats included rhinorrhea, salivation, and urine stains around the anus as well as dyspnea during the exposure. All these animals were found dead by Day 3. Signs noted among rats exposed to E7018, E6010, and E70T-1 fumes, as well as to fume on the second E308-16 exposure, included dyspnea, polypnea, wheezing, and bloody crust on the muzzle as well as brown dust or stain on the fur. One E308-16 second exposure female died during Day 1 postexposure. All E5356 and E70S-3 animals appeared normal except for brown dust on the fur in the latter group. All animals surviving for 14 days appeared normal by Day 2 postexposure.

Gross Pathology

Individual gross pathology findings are presented in Appendix 6 (Complete Report). Summarized findings are presented in Table 3 (Complete Report).

The majority of the gross pathology found was comparable to that in the CCA control group or incidental in nature. Gross pathology findings noted at a greater incidence in the fume-exposed groups were noted in the lung and kidneys. Findings in the lungs of exposed rats included failure to collapse, mottled, dark, firm, and pale focus(i)/area(s) in the E70S-3, E308-16, and E7018 animals, and findings in the kidneys included dark focus(i)/area(s), fluid under the capsule, or fluid-filled, dilated pelvis(es), and granular material in the E70T-1 animals. Dilated pelvis(es) were also noted in some control animals, rendering this finding ambiguous.

Table 2
Measured Mean Fume Characteristics

Electrode	Particulate Concentration mg/liter	Particle Size		O_2 %	O_3 ppm	NO_x ppm	NO_2 ppm	CO ppm	Temp. °F	R.H. %
		MMAD μ	$\pm \sigma_g$							
E70S-3	0.672 $\pm$ 0.138	0.765	2.24	19.7	0	2.5	<0.5	797	78	51
E308-16	0.601 $\pm$ 0.098	1.08	1.54	19.4	0	9.6	0	47.6	79	61
E7018	0.641 $\pm$ 0.132	0.890	1.78	19.3	0	16.8	<0.5	69.2	76	44
E6010	0.551 $\pm$ 0.084	0.860	2.06	19.7	0	11.3	0	41.2	78	45
E70T-1	0.890 $\pm$ 0.187	0.865	2.16	19.6	0	4.3	0	931	78	41
E5356	0.559 $\pm$ 0.066	0.845	2.11	19.6	0	20.0	0	2.5	77	51
E308-16	0.268 $\pm$ 0.117	1.02	2.13	19.5	0	9.5	0	37.0	77	33

Terminal Body Weights, Organ Weights, and Organ/Body Weight Ratios

Individual terminal body weights, organ weights, and organ/body weight ratios are presented in Appendix 7 (Complete Report). Mean values are presented in Table 4 (Complete Report).

Terminal body weights were comparable between the CCA control group and all fume-exposed groups.

Several occurrences of statistically significant organ weight differences were noted between the CCA control group and the fume-exposed groups as indicated in the chart below.

Lung Wet/Dry Weight Ratios

Lung wet/dry weights and wet/dry ratios are presented in Appendix 8 (Complete Report) and the means are presented in Table 5 (Complete Report).

Analysis of wet and dry lung weights revealed significant increases in the E7018 wet lung weights of males and females and the E70T-1 wet and dry lung weights of females. No significance in wet/dry lung ratios was observed.

Histopathology

Individual histopathology findings are presented in Appendix 9 (Complete Report). Findings are summarized in Table 6 (Complete Report).

E70S-3 — Pigmented macrophages were present in alveoli of all treated rats. One female had a minimal focal acute pneumonitis.

E7018 — Minimal to slight increases in pigmented macrophages were noted in alveoli in all but one treated rat. Occasional increases in normal alveolar macrophages were noted also.

E6010 — Minimal to moderate increases in pigmented macrophages were noted in all treated rats.

Minimal multifocal perivascular edema was noted in two males.

E70T-1 — Slight to moderate increases in pigmented macrophages were noted in all treated rats.

E5356 — Minimal increases in pigmented macrophages were noted in two male and one female treated rat. Minimal to slight increases in nonpigmented alveolar macrophages were noted in two animals of each sex. Five animals were completely unremarkable.

E308-16 (Initial Test) — All treated rats died prior to the scheduled sacrifice (see second test results below).

E308-16 (Second Test) — Lungs from treated animals of both sexes had variable numbers of pigmented macrophages, largely in the alveoli. In addition, two treated female rats had perivascular edema with one of those (died) having an acute pneumonitis (interstitial pneumonia). No other changes were noted. The presence of pigmented macrophages indicated that the test material was reaching the level of the alveoli and was being phagocytized and removed in a normal manner. The edema and pneumonia in two females indicated a moderate irritation with breakdown of vascular integrity. The change in the rat with pneumonitis was considered to be the cause of death (the animal died prior to 14 days postexposure). Welding fume generated from electrode E308-16 was noted to cause a persisting mild to moderate irritation and edema in the lungs of some female rats exposed to 0.27 mg per liter by inhalation for six hours. Male rats and three of five female rats showed only normal phagocytic response 14 days after the exposure.

Subgroup A — Body Fluid Mutagenicity Assay

The mean number of revertant colonies in the five *S. typhimurium* strains tested with pooled, concentrated, and reconstituted urine samples collected from male and

Males				
Parameter	E6010	E5356	E70S-3	E308-16 E7018 (second test)
Lung weight	S+	S+		
Lung/body weight ratio	S+			S+
Heart weight			S-	S-
Females				
Parameter	E70T-1	E5356	E7018	E308-16 (second test)
Lung weight	S+	S+	S+	S+
Lung/body weight ratio	S+	S+	S+	

female rats following exposure to the fumes of the six electrodes are shown in Tables 7–12 (Complete Report). The results indicated that none of the six fumes produced detectable levels of mutagenic urinary contents or metabolites, as evidenced by the lack of a two-fold or greater increase in revertant colonies at either of the test concentrations as compared to the appropriate control series. These findings were not altered by the inclusion of metabolic activation and Beta-glucuronidase into the test system.

Diagnostic positive controls with an agent (2-AA) that produces known urinary mutagenic metabolites along with standard controls for this assay are included with each table and confirm the sensitivity of the test system to detect mutagenic activity.

Subgroup B—*In Vivo* Rat Alveolar Macrophage Assay

Test System Information

Body weights of animals used in the study appear in Appendices 10A through 10F (Complete Report). No statistical differences were observed in mean body weights of animals exposed to the different fumes as compared to controls. The cell recovery and percentage of macrophage from each lavage fluid was determined. These data also appear in Appendices 10A through 10F (Complete Report).

As shown in Tables 13 and 14 (Complete Report), statistically significant decreases in cell recovery and in percentage macrophages were observed in all exposed male groups. Statistically significant decreases in cell recovery and in percentage macrophages were also observed in females exposed to Fumes E7018 and E70T-1. There were not enough cells to evaluate Fume E6010.

No cultures were set up for animals exposed to Fume E308-16 because all lavages yielded bloody preparations.

Viability Determinations

Initial viabilities were determined for each lavage fluid. Macrophage viability was again measured at 20 hours after culture initiation. No statistical differences in viabilities were observed for animals exposed to any fume. These results appear in Appendices 11A through 11F (Complete Report), and are summarized in Table 15A (Complete Report).

Phagocytosis

The effect of each fume on phagocytic ability of macrophages from exposed animals was determined. These results appear in Appendices 11A through 11F (Complete Report), and are summarized in Table 15B (Complete Report). Apparent decreases in phagocytic

ability were observed in males and females for all fumes studied except Fume E5356. Preparations from Fume E70S-3 male and Fumes E7018 and E6010 females, had too few samples to evaluate statistically. No cultures were set up for animals exposed to Fume E3018-16. This decrease may be due to the inability to distinguish between particle ingestion and debris when counting greater than 10 particles, and may not be physiological due to this limitation of the test system.

ATP Determinations

The effect of each fume on ATP levels in macrophages from exposed animals was determined. These results appear in Appendices 12A through 12F (Complete Report). Significant differences in ATP levels (Table 16) were observed for male rats exposed to Fumes E70S-3, E6010, E70T-1, and E5356, and for female rats exposed to Fumes E70S-3, E70T-1, and E5356. No results were obtained from males exposed to Fumes E308-16 and E7018, and females exposed to Fumes E308-16, E7018, and E6010 due to bloody preparations or too few cells recovered.

Acid Phosphatase Determination

The effect of each fume on acid phosphatase in macrophages from exposed animals was determined. These results appear in Appendices 12A through 12F (Complete Report). Statistically significant increases in acid phosphatase (Table 17) levels were observed for Fume E70S-3 male and females. This fume was the only fume of the six fumes evaluated statistically for this parameter. No data were available for evaluation of the other fumes due to bloody preparations or insufficient cells recovered as noted in Table 17 (Complete Report).

Lysozyme Determination

The effect of each fume on lysozyme in macrophages from exposed animals was determined. These results appear in Appendices 12A through 12F (Complete Report), and are summarized in Table 18 (Complete Report). As noted in Table 18, no statistical data is available comparing lysozyme levels of exposed animals with control levels.

Subgroups C, D, and CCD—*In Vivo* Bone Marrow Cytogenetic Analysis

Cytogenetic Analysis

A summary of the chromosomal aberration data is presented in Table 20 (Complete Report). Individual animal data are presented in Appendix 13 (Complete Report).

Results from the analysis of data showed no statistically significant increase in the frequency of chromosomal aberrations compared to the control value in any of the six groups exposed to welding fumes.

Modal Number

The mean modal number and standard deviation for each group are presented in Table 21 (Complete Report).

The average number of chromosomes in the examined metaphases was determined for each animal, and all test groups were statistically compared to the appropriate control group. Rats have a normal diploid chromosome number of 42. Statistical analysis revealed a small but significant increase in the E7018 test animals versus their control animals at 20 hours postexposure ($p = 0.0117$) but not at 70 hours.

Mitotic Index

The number of cells undergoing mitosis per 500 cells counted (mitotic index) was determined for each animal. The mean mitotic index and standard deviation for each group appear in Table 22 (Complete Report).

No statistically significant differences between the mean mitotic indices of the test groups and the controls were seen.

Subgroup B — *In Vitro* Rat Alveolar Macrophage Assay

Viability Determinations

Macrophage viabilities and viability indices were determined for each of the six fumes, and the results appear in Tables 23A through 23F (Complete Report). From these results, the concentration of fume particulates that reduced viability by 50 percent (EC_{50}) was calculated by graphing the percent viable for each fume. Relative toxicities of the six fumes were determined by comparing the EC_{50} values as shown in Table 24 (Complete Report).

Phagocytosis

The effect of each fume on the phagocytic ability of rat alveolar macrophages was determined. These results are presented in Tables 25A through 25F (Complete Report). Fume E6010 depressed phagocytic activity of rat alveolar macrophage cells at 1089 $\mu\text{g}/\text{ml}$; at higher concentrations, no intact cells were observed. Fume E308-16 depressed phagocytic activity at 13 and 39 $\mu\text{g}/\text{ml}$; at higher concentrations, no intact cells were observed. Fume E7018 depressed activity at 39 $\mu\text{g}/\text{ml}$ and Fume

E70T-1 at 118 $\mu\text{g}/\text{ml}$; at higher concentrations, no intact cells were observed. Fume E5356 was depressed at 359 $\mu\text{g}/\text{ml}$, but cells remained up to 72 percent viable at 3300 $\mu\text{g}/\text{ml}$. Fume E70S-3 showed close to normal phagocytic activity and viability at all concentrations tested.

ATP Determinations

The effect of each fume on cellular ATP levels was determined. These results appear in Tables 26A through 26F (Complete Report). ATP values were significantly different than controls for Fume E70S-3 at 359, 1089, 3300, and 10,000 $\mu\text{g}/\text{ml}$; for Fume E308-16 at all concentrations; for E7018 at all concentrations; for E6010 beginning at 359 $\mu\text{g}/\text{ml}$; for E70T-1 at 359, 1089, and 10,000 $\mu\text{g}/\text{ml}$; and for Fume E5356 at 1089 $\mu\text{g}/\text{ml}$ through 10,000 $\mu\text{g}/\text{ml}$. ATP values were significantly lower than controls for Fume E5356 at 1089, 3300, and 10,000 $\mu\text{g}/\text{ml}$; for Fume E70S-3 at 359, 1089, 3300, and 10,000 $\mu\text{g}/\text{ml}$; for Fume E70T-1 at 359, 1089, 3300, and 10,000 $\mu\text{g}/\text{ml}$; for Fume E6010 at 359, 1089, 3300, and 10,000 $\mu\text{g}/\text{ml}$; and for Fumes E7018 and E308-16 at all concentrations.

Acid Phosphatase Determinations

The effect of each fume on cellular acid phosphatase levels was determined. These results appear in Tables 27A through 27F (Complete Report). Acid phosphatase values were significantly lower than controls for Fume E70S-3 beginning at 4 $\mu\text{g}/\text{ml}$, but higher at 39 and 110 $\mu\text{g}/\text{ml}$. At 359 $\mu\text{g}/\text{ml}$, the samples were too turbid to read. There was no effect on acid phosphatase levels following exposure to up to 1089 $\mu\text{g}/\text{ml}$ of Fume E308-16; concentrations greater than 1089 $\mu\text{g}/\text{ml}$ were too turbid to read. Values were also significantly lower for Fume E7018 at 13, 39, and 359 $\mu\text{g}/\text{ml}$, and at 4, 13, 39, and 359 $\mu\text{g}/\text{ml}$ for Fume E6010. At the higher concentrations, the samples were too turbid to read. Acid phosphatase levels were significantly higher at 4, 39, and 118 $\mu\text{g}/\text{ml}$ of Fume E70T-1, and too turbid to read at the higher concentrations. Fume E5356 was significantly lower only at 13 $\mu\text{g}/\text{ml}$ and too turbid to read beginning at concentrations of 359 $\mu\text{g}/\text{ml}$.

Lysozyme Determinations

The effect of each fume on cellular lysozyme was determined. These results are presented in Tables 28A through 28F (Complete Report). No significant differences were observed between control values and any of the six fumes. It is important to note here the wide range in values among triplicate samples.

Table 1A
Mean Body Weights and Standard Deviations (grams)
Toxicity Study of Welding Fumes in Rats

2235-100/101

Group		Pre-Exposure	Day Postexposure				Found Dead
		Day 1	Day 1	Day 7	Day 14	Day 15	
MALES							
Control Group CCA	Mean	287.3	273.7	309.2	328.8	298.3	
	S.D.	18.68	18.42	17.49	30.95	23.36	
E5356—Group A	Mean	286.4	276.0	319.0	353.4	320.6	
	S.D.	31.55	28.59	20.54	15.14	16.01	
E70S-3—Group A	Mean	263.4	249.8	279.4 ^{S-}	303.8	281.8	
	S.D.	9.61	8.14	11.17	11.48	8.76	
E70T-1—Group A	Mean	284.8	270.8	303.2	346.2	310.8	
	S.D.	23.58	18.61	28.67	34.56	32.44	
E308-16—Group A ^a	Mean	287.8	263.6				239.0 14.16
	S.D.	5.54	4.28				
E6010—Group A	Mean	279.2	263.6	302.0	341.6	308.0	
	S.D.	10.35	9.37	5.79	4.51	5.74	
E7018—Group A	Mean	289.2	273.8	309.8	338.0	305.8	
	S.D.	30.71	27.62	23.64	26.28	23.03	
E308-16—Group A (Repeat)	Mean	261.4	249.2	277.0	326.8	289.6	
	S.D.	9.21	8.87	18.87	19.28	20.23	
Control Group CCA (Repeat)	Mean	255.8	245.0	288.8	317.6	286.8	
	S.D.	17.34	14.20	11.82	16.01	15.66	
FEMALES							
Control Group CCA	Mean	196.8	188.3	205.5	221.7	199.0	
	S.D.	6.55	6.54	11.04	13.15	11.95	
E5356—Group A	Mean	205.4	194.6	211.0	227.0	203.0	
	S.D.	9.63	10.45	10.49	13.10	11.79	
E70S-3—Group A	Mean	190.8	181.4	204.2	213.0	194.8	
	S.D.	5.54	5.55	8.23	8.19	6.76	
E70T-1—Group A	Mean	209.6 ^{S+}	198.8	215.8	227.5	205.0	
	S.D.	3.36	2.28	3.19	3.49	3.08	
E308-16—Group A ^a	Mean	189.2	179.4				156.6 22.51
	S.D.	10.99	11.13				
E6010—Group A	Mean	194.4	182.6	205.6	219.2	196.2	
	S.D.	5.27	6.43	9.29	8.04	7.60	
E7018—Group A	Mean	197.2	185.4	202.2	216.8	195.6	
	S.D.	9.20	7.06	8.23	12.13	9.56	
E308-16—Group A (Repeat)	Mean	193.2	180.8	200.0	222.5	195.0	
	S.D.	4.15	6.02	1.83	5.07	5.89	
Control Group CCA (Repeat)	Mean	184.6	178.0	203.8	216.2	192.0	
	S.D.	14.22	11.34	4.44	3.03	3.54	

a. All animals were found dead prior to the Day 7 observation.

2236-100/101

Table 1B
Mean Initial Body Weights and Standard Deviations (grams)
Toxicity Study of Welding Fumes in Rats

Group		Day 1	
		Pre-Exposure	Postexposure
MALES			
Control Group CCB	Mean	282.0	266.2
	S.D.	17.75	16.36
Control Group CCD	Mean	286.5	270.8
	S.D.	21.67	22.29
E5356—Group B	Mean	301.2	287.6
	S.D.	11.17	9.81
—Group C	Mean	304.2	289.6
	S.D.	11.08	10.85
—Group D	Mean	305.6	290.2
	S.D.	12.26	10.73
E70S-3—Group B	Mean	270.4	254.2
	S.D.	14.29	11.43
—Group C	Mean	266.0	253.0
	S.D.	8.83	8.31
—Group D	Mean	266.4	253.0
	S.D.	17.18	15.57
E70T-1—Group B	Mean	286.8	270.6
	S.D.	6.69	6.07
—Group C	Mean	276.4	261.4
	S.D.	9.63	8.71
—Group D	Mean	281.8	265.6
	S.D.	17.54	15.29
E308-16—Group B	Mean	282.0	259.2
	S.D.	8.86	7.53
—Group C	Mean	278.0	253.2
	S.D.	4.80	7.46
—Group D	Mean	284.0	261.4
	S.D.	6.28	10.67
E6010—Group B	Mean	285.8	268.6
	S.D.	12.11	9.89
—Group C	Mean	273.4	259.6
	S.D.	14.48	13.74
—Group D	Mean	275.2	259.8
	S.D.	15.21	13.97
E7018—Group B	Mean	296.8	273.2
	S.D.	8.81	9.58
—Group C	Mean	289.6	272.0
	S.D.	10.90	12.71
—Group D	Mean	306.4	277.0
	S.D.	4.04	18.79

Table 1B (Continued)

2236-100/101

Group		Day 1	
		Pre-Exposure	Postexposure
MALES			
E308-16—Group B (Repeat)	Mean	264.6	245.6
	S.D.	8.96	6.19
—Group C	Mean	254.8	246.0
	S.D.	14.17	5.15
—Group D	Mean	268.8	255.2
	S.D.	7.09	7.46
E308-16—Group CCB (Repeat)	Mean	262.8	248.8
	S.D.	6.50	5.81
—Group CCD	Mean	263.8	249.0
	S.D.	8.20	7.35
FEMALES			
Control Group CCB	Mean	196.3	184.3
	S.D.	11.71	10.60
Control Group CCD	Mean	195.8	184.7
	S.D.	9.33	9.87
E5356—Group B	Mean	200.0	190.6
	S.D.	8.69	7.64
—Group C	Mean	196.0	184.2
	S.D.	7.78	5.40
—Group D	Mean	190.4	179.6
	S.D.	12.46	12.93
E70S-3—Group B	Mean	193.4	184.6
	S.D.	3.05	2.97
—Group C	Mean	189.6	181.6
	S.D.	8.50	6.88
—Group D	Mean	193.4	182.6
	S.D.	15.27	14.48
E70T-1—Group B	Mean	205.8	193.6
	S.D.	8.17	9.21
—Group C	Mean	203.6	194.2
	S.D.	10.55	9.68
—Group D	Mean	203.8	194.0
	S.D.	4.15	6.08
E308-16—Group B	Mean	193.8	177.4
	S.D.	10.96	10.67
—Group C	Mean	189.2	171.2
	S.D.	9.34	11.23
—Group D	Mean	190.4	177.6
	S.D.	12.20	12.22

(Continued)

Table 1B (Continued)

2236-100/101

Group		Day 1	
		Pre-Exposure	Postexposure
FEMALES			
E6010—Group B	Mean	192.0	177.6
	S.D.	7.52	6.58
—Group C	Mean	193.2	181.6
	S.D.	5.63	4.98
—Group D	Mean	196.0	186.2
	S.D.	10.56	9.20
E7018—Group B	Mean	202.2	187.8
	S.D.	9.26	9.88
—Group C	Mean	193.2	180.4
	S.D.	2.68	3.51
—Group D	Mean	199.8	187.2
	S.D.	6.76	6.30
E308-16—Group B (Repeat)	Mean	193.0	181.0
	S.D.	5.29	5.96
—Group C	Mean	195.4	182.8
	S.D.	9.13	8.04
—Group D	Mean	194.2	183.0
	S.D.	5.93	4.42
E308-16—Group CCB (Repeat)	Mean	189.4	180.0
	S.D.	8.88	6.08
—Group CCD	Mean	190.2	179.0
	S.D.	4.82	5.92

Key to Table 2A

NOTE: Due to the poor visibility in the chamber during exposure, the exact number of animals exhibiting clinical signs could not be determined at certain intervals. The following approximations were made at these intervals and are indicated in the Table as follows:

Table 2A
Summary of Clinical Observations
Toxicity Study of Welding Fumes in Rats
Exposure Period—Subgroup A Males and Females

2236-100/101

Observations	Time During Exposure (Hour)											
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
<u>E5356</u>												
Number of animals observed	10	10	10	10	10	10	10	10	10	10	10	10
Appeared normal	10	10	10	10	10	10	10	10	10	10	10	10
<u>E70S-3</u>												
Number of animals observed	10	10	10	10	10	10	10	10	10	10	10	10
Appeared normal	10	10	10	10	10	10	10	10	0	0	0	0
Brown dust on fur	0	0	0	0	0	0	0	0	10	10	10	10
<u>E308-16</u>												
Number of animals observed	10	10	10	10	10	10						
Appeared normal	10	10	10	10	10	10						
Dyspnea (gasping, labored respiration)	0	0	0	0	0	0	c	a	a	a	a	a
Rhinorrhea (slight dark fluid, dark or red bloody discharge)	0	0	0	0	0	0	c	c	c	c	c	c
<u>E7018</u>												
Number of animals observed	10										10	10
Appeared normal	10	b	b	b	b	b	b	b	b	b	10	10
Dyspnea	0				c	c	c				0	0
Polypnea	0	c	c	c	c	c	c	c	c	c	0	0
<u>E6010</u>												
Number of animals observed	10	10	10					10	10	10	10	10
Appeared normal	10	10	10	a				10	10	10	10	10
Polypnea				b	b	b	b	0	0	0	0	0
<u>E70T-1</u>												
Number of animals observed												
Appeared normal	a	a	a									
Brown stain on nose	b	b	b	b	b	b	b	b	b	b	b	b
Polypnea			b									
Dyspnea				c	b	b	b	c	c	c	c	c
Brown dust on fur							a		c	c	c	c
<u>Control Group CCA</u>												
Number of animals observed	12	12	12	12	12	12	12	12	12	12	12	12
Appeared normal	12	12	12	12	12	12	12	12	12	12	12	12
<u>E308-16—Group A (Repeat)</u>												
Number of animals observed	10	10	10	10	10	10	10	10	10	10	10	10
Appeared normal	10	10	10	10	10	10	10	10	10	10	10	10

- a. Indicates several of the animals that were visible showed the designated clinical sign.
b. Indicates most of the animals that were visible showed the designated clinical sign.
c. Indicates that remaining animals that were visible appeared normal.

Table 2B
Summary of Clinical Observations
Toxicity Study of Welding Fumes in Rats
Postexposure Period—Subgroup A Males and Females

2236-100/101

Observations	Exposure Off/ Removal from Chamber	Day Postexposure														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MALES																
E5356																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
E70S-3																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	2	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on body, nose and/or mouth	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E308-16																
Number of animals observed	5	5	2													
Appeared normal	0	0	0													
Found dead	0	3	2													
Dyspnea (gasping)	5	2	1													
Wheezing	5	0	1													
Bloody crust on nose, mouth and/or eyes	5	2	1													
Salivation	0	0	1													
Urine stains on anus	0	0	1													
E7018																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on fur	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chromadacryorrhea	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bloody crust on eye	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E6010																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on fur	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bloody crust on nose	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E70T-1																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	0	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on fur/body	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown stain on nose	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bloody crust on face	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control Group CCA																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
E308-16—Group A (Second Exposure)																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on fur	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(Continued)

Table 2B (Continued)

2236-100/101

Observations	Exposure Off/ Removal from Chamber	Day Postexposure														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FEMALES																
E5356																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
E70S-3																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	0	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on body and/or nose	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E308-16																
Number of animals observed	5	5	2	1												
Appeared normal	0	0	0	0												
Found dead	0	3	1	1												
Dyspnea (gasping)	5	2	0	0												
Wheezing	5	0	0	0												
Bloody crust on nose	5	0	1	1												
Salivation	1	0	0	0												
Languid	0	0	1	1												
E7018																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on fur	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E6010																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on fur	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bloody crust on nose	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E70T-1																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown stain on fur/body	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown stain on nose	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control Group CCA																
Number of animals observed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Appeared normal	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
E308-16—Group A (Second Exposure)																
Number of animals observed	5	5	5	4	4	4	4	4	4	4	4	4	4	4	4	4
Appeared normal	0	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Found dead	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown stain on fur	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wheezing	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key to Tables 3-6

223610A—Subgroup A

Group 1 = Common Control
Group 2 = E5356 Repeat
Group 3 = E70S-3
Group 4 = E308-16
Group 5 = E7018
Group 6 = E6010
Group 7 = E70T-1

223610C—Subgroups C and D

Group 1 = Common Control
Group 2 = E5356 Repeat
Group 3 = E70S-3
Group 4 = E308-16
Group 5 = E7018
Group 6 = E6010
Group 7 = E70T-1

223610G—Subgroup A

Group 1 = Common Control
Group 2 = E308-16 Repeat

Standard Key to Gross Pathology Incidence Summary

Locations of Tissue Masses Observed Grossly

DFL = Dorsal-Front-Left
DFR = Dorsal-Front-Right
DHL = Dorsal-Hind-Left
DHR = Dorsal-Hind-Right
DFM = Dorsal-Front-Mid
DHM = Dorsal-Hind-Mid
VFL = Ventral-Front-Left
VFR = Ventral-Front-Right
VHL = Ventral-Hind-Left
VHR = Ventral-Hind-Right
VFM = Ventral-Front-Mid
VHM = Ventral-Hind-Mid

The above locations are used in conjunction with the keywords MASS and SMALL MASS.

Other Symbols and Notations

H- = Finding noted during processing
of tissues in histology
(precedes keyword)

Table 3
Summary of Gross Pathology Findings
Toxicity Study of Welding Fumes in Rats

2236-100/101

Hazleton Laboratories America, Inc. Path/Tox System Output Printed: 21-May-84
 Department of Pathology Toxicity Study of Welding Fumes
 Vienna, Virginia GROSS PATHOLOGY INCIDENCE SUMMARY Study Number: 223610A

Organ and Keyword(s) or Phrase	Number:	Number of Animals Affected						
		MALE						
		1	2	3	4	5	6	7
Table Includes:								
Sex = All; Group = All; Weeks = All	Sex:							
Death = All; Subset = All	Group:							
Organ and Keyword(s) or Phrase	Number:	12	5	5	5	5	5	5
Top of List								
Brain (BR)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Adrenals (AD)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Thyroid (TY)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Trachea (TR)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Lung w/Bronchi (LU)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	1	0	5	3	4
Failure to Collapse		0	0	0	5	0	0	0
Dark		0	0	2	2	0	2	0
Pale Focus (I)/Area (S)		0	0	0	1	0	0	0
Mottled		0	0	2	3	0	0	0
Dark Focus (I)/Area (S)		0	0	0	0	0	0	1
Heart (HT)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	4	5	5	5	5
Pale Focus (I)/Area (S)		0	0	1	0	0	0	0
Spleen (SP)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	11	5	5	5	5	5	5
Pale		0	0	0	0	0	0	0
Pale Film on Capsule		1	0	0	0	0	0	0
Liver (LI)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Dark Focus (I)/Area (S)		0	0	0	0	0	0	0
Pale Focus (I)/Area (S)		0	0	0	2	0	0	0
Kidneys (KD)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	11	5	4	5	4	5	5
Fluid Filled		0	0	0	0	0	0	0
Dilated Pelvis(es)		1	0	0	0	1	0	0
Granular Material		0	0	0	0	0	0	0
Granular/Pitted/Rough		0	0	0	0	0	0	0
Stomach (ST)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	4	5	5	5
Pale Focus (I)/Area (S)		0	0	0	1	0	0	0
Dark Focus (I)/Area (S)		0	0	0	0	0	0	0

Table 3 (Continued)

2236-100/101

Hazleton Laboratories America, Inc.
Department of Pathology
Vienna, Virginia

Path/Tox System Output
Toxicity Study of Welding Fumes
GROSS PATHOLOGY INCIDENCE SUMMARY

Printed: 21-May-84
Study Number: 223610A

Table Includes: Sex = All; Group = All; Weeks = All Death = All; Subset = All		Number of Animals Affected							
		Sex:	MALE						
		Group:	1	2	3	4	5	6	7
Organ and Keyword(s) or Phrase	Number:		12	5	5	5	5	5	5
Duodenum (DU)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Jejunum (JE)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Ileum (IL)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Pancreas (PA)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Cecum (CE)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Dark Focus (I)/ Area (S)			0	0	0	0	0	0	0
Colon (CO)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Rectum (RE)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Testes w/Epidid (TP)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Prostate (PR)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Urinary Bladder (UB)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Ovaries (OV)	Number Examined:		0	0	0	0	0	0	0
	Not Remarkable:		0	0	0	0	0	0	0
Uterus (UT)	Number Examined:		0	0	0	0	0	0	0
	Not Remarkable:		0	0	0	0	0	0	0
Intraluminal Fluid			0	0	0	0	0	0	0
Nasal Turbinates (NT)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Mesenteric L.N. (MS)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Peritoneal Cavit (PC)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	5
Lymph Node, Other (LN)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	4	5	5
Dark			0	0	0	0	1	0	0
Thymus (TH)	Number Examined:		12	5	5	5	5	5	5
	Not Remarkable:		12	5	5	5	5	5	4

(Continued)

Table 3 (Continued)

2236-100/101

Hazleton Laboratories America, Inc.
Department of Pathology
Vienna, Virginia

Path/Tox System Output
Toxicity Study of Welding Fumes
GROSS PATHOLOGY INCIDENCE SUMMARY

Printed: 21-May-84
Study Number: 223610A

Table Includes: Sex = All; Group = All; Weeks = All Death = All; Subset = All	Sex:	Number of Animals Affected						
		MALE						
		1	2	3	4	5	6	7
Organ and Keyword(s) or Phrase	Number:	12	5	5	5	5	5	5
Dark Focus (I)/Area (S)		0	0	0	0	0	0	1
End of List								

	Sex:	Number of Animals Affected						
		FEMALE						
		1	2	3	4	5	6	7
Organ and Keyword(s) or Phrase	Number:	12	5	5	5	5	5	5
Top of List								
Brain (BR)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Adrenals (AD)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Thyroid (TY)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Trachea (TR)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Lung w/Bronchi (LU)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	0	5	4	4
Failure to Collapse		0	0	0	4	0	0	0
Dark		0	0	0	0	0	0	1
Pale Focus (I)/Area (S)		0	0	0	0	0	0	0
Mottled		0	0	0	5	0	0	0
Dark Focus (I)/Area (S)		0	0	0	0	0	1	0
Heart (HT)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Pale Focus (I)/Area (S)		0	0	0	0	0	0	0
Spleen (SP)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	4	5	5	5
Pale		0	0	0	1	0	0	0
Pale Film on Capsule		0	0	0	0	0	0	0
Liver (LI)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	11	5	5	4	5	3	3
Dark Focus (I)/Area (S)		0	0	0	0	0	1	0
Pale Focus (I)/Area (S)		1	0	0	1	0	1	2
Kidneys (KD)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	9	5	4	3	5	5	3

Table 3 (Continued)

2236-100/101

Hazleton Laboratories America, Inc.
Department of Pathology
Vienna, Virginia

Path/Tox System Output
Toxicity Study of Welding Fumes
GROSS PATHOLOGY INCIDENCE SUMMARY

Printed: 21-May-84

Study Number: 223610A

Table Includes:

Sex = All; Group = All; Weeks = All
Death = All; Subset = All

Organ and Keyword(s) or Phrase	Number:	Number of Animals Affected						
		FEMALE						
		1	2	3	4	5	6	7
	Sex:							
	Group:							
		12	5	5	5	5	5	5
Fluid Filled		0	0	0	1	0	0	0
Dilated Pelvis(es)		2	0	1	2	0	0	2
Granular Material		0	0	1	0	0	0	1
Granular/Pitted/Rough		1	0	0	0	0	0	0
Stomach (ST)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	11	5	5	4	5	5	4
Pale Focus (I)/Area (S)		0	0	0	1	0	0	0
Dark Focus (I)/Area (S)		1	0	0	0	0	0	1
Duodenum (DU)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Jejunum (JE)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Ileum (IL)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Pancreas (PA)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Cecum (CE)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	4	5	5	5	5	5
Dark Focus (I)/Area (S)		0	1	0	0	0	0	0
Colon (CO)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Rectum (RE)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Testes w/Epidid (TP)	Number Examined:	0	0	0	0	0	0	0
	Not Remarkable:	0	0	0	0	0	0	0
Prostate (PR)	Number Examined:	0	0	0	0	0	0	0
	Not Remarkable:	0	0	0	0	0	0	0
Urinary Bladder (UB)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Ovaries (OV)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Uterus (UT)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	10	3	5	5	4	3	4
Intraluminal Fluid		2	2	0	0	1	2	1
Nasal Turbinates (NT)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Mesenteric L.N. (MS)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5

(Continued)

Table 3 (Continued)

2236-100/101

Hazleton Laboratories America, Inc.
Department of Pathology
Vienna, Virginia

Path/Tox System Output
Toxicity Study of Welding Fumes
GROSS PATHOLOGY INCIDENCE SUMMARY

Printed: 21-May-84

Study Number: 223610A

Table Includes: Sex = All; Group = All; Weeks = All Death = All; Subset = All		Number of Animals Affected						
		FEMALE						
		1	2	3	4	5	6	7
Organ and Keyword(s) or Phrase	Number:	12	5	5	5	5	5	5
Peritoneal Cavit (PC)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Lymph Node, Other (LN)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	5
Dark		0	0	0	0	0	0	0
Thymus (TH)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	12	5	5	5	5	5	4
Dark Focus (I)/Area (S)		0	0	0	0	0	0	1
Dark		0	1	0	0	0	0	0

****End of List****

Hazleton Laboratories America, Inc. Path/Tox System Output Printed: 06-Apr-84
Department of Pathology Acute Inhalation Toxicity of Welding Fumes
Vienna, Virginia Project 2236-101
OBSERVATIONS INCIDENCE SUMMARY Study Number: 223610G

Table Includes: Sex = All; Group = All; Weeks = All Death = T; Subset = All		Number of Animals Affected			
		MALE		FEMALE	
		1	2	1	2
Organ and Keyword(s) or Phrase	Number:	5	5	5	4
Top of List					
Brain (BR)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	4	4
Indentation (Dorsal Surface)		0	0	1	0
Adrenals (AD)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Thyroid (TY)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Trachea (TR)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Lung (LU)	Number Examined:	5	5	5	4
	Not Remarkable:	5	1	3	2
Mottled		0	1	0	1
Dark Focus (I)/Area (S)		0	1	1	0
Dark		0	2	0	1
H-Collapsed		0	0	1	0
Heart (HT)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Spleen (SP)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4

Table 3 (Continued)

2236-100/101

Hazleton Laboratories America, Inc. Path/Tox System Output
 Department of Pathology Acute Inhalation Toxicity of Welding Fumes Printed: 06-Apr-84
 Vienna, Virginia Project 2236-101
 OBSERVATIONS INCIDENCE SUMMARY Study Number: 223610G

Table Includes:

Sex = All; Group = All; Weeks = All
 Death = T; Subset = All

Organ and Keyword(s) or Phrase	Sex:	Number of Animals Affected			
		MALE		FEMALE	
		1	2	1	2
Group:	Number:	5	5	5	4
Liver (LI)	Number Examined:	5	5	5	4
	Not Remarkable:	5	4	5	4
Pale Focus (I)/ Area (S)		0	1	0	0
Kidneys (KD)	Number Examined:	5	5	5	4
	Not Remarkable:	2	4	5	4
Dilated Pelvis(es)		3	1	0	0
Stomach (ST)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Duodenum (DU)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Jejunum (JE)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Ileum (IL)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Pancreas (PA)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Cecum (CE)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Colon (CO)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Rectum (RE)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Testes w/Epidid (TP)	Number Examined:	5	5	0	0
	Not Remarkable:	5	5	0	0
Prostate (PR)	Number Examined:	5	5	0	0
	Not Remarkable:	5	5	0	0
Urinary Bladder (UB)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Ovaries (OV)	Number Examined:	0	0	5	4
	Not Remarkable:	0	0	5	4
Uterus (UT)	Number Examined:	0	0	5	4
	Not Remarkable:	0	0	5	4
Nasal Turbinates (NT)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	5	4
Bone(s), Other (OB)	Number Examined:	5	5	5	4
	Not Remarkable:	5	5	4	4
Perforation(s)		0	0	1	0

** End of List **

Table 4
Mean Organ Weights and Organ/Body Weight Ratios with Standard Deviations
Toxicity Study of Welding Fumes in Rats

2236-100/101

Hazleton Laboratories America, Inc. Path/Tox System Output Printed: 27-Mar-84
 Department of Pathology Toxicity Study of Welding Fumes
 Vienna, Virginia MEAN ABSOLUTE ORGAN WEIGHTS (g) Study Number: 223610A

		Sex:		MALE						
Table Includes:		Group:		1	2	3	4	5	6	7
Sex = All; Group = All; Weeks = All		Number:		12	5	5	5	5	5	5
Death = All; Subset = All										
Brain (BR)	Number in Group:			12	5	5	0	5	5	5
	Mean:			2.02	2.04	1.89		2.00	2.06	1.96
	Standard Deviation:			0.14	0.07	0.08		0.12	0.09	0.07
Lung (LU)	Number in Group:			12	5	5	0	5	4	5
	Mean:			1.37	1.56 ^{S+}	1.25		1.39	1.62 ^{S+}	1.47
	Standard Deviation:			0.11	0.14	0.10		0.15	0.08	0.18
Kidneys (KD)	Number in Group:			12	5	5	0	5	5	5
	Mean:			2.56	2.50	2.30		2.41	2.63	2.51
	Standard Deviation:			0.24	0.18	0.08		0.24	0.23	0.37
Heart (HT)	Number in Group:			12	5	5	0	5	5	5
	Mean:			0.98	0.95	0.87 ^{S+}		0.87 ^{S+}	0.96	0.94
	Standard Deviation:			0.09	0.10	0.06		0.07	0.03	0.09
Liver (LI)	Number in Group:			12	5	5	0	5	5	5
	Mean:			8.82	9.28	7.90		8.71	9.25	8.75
	Standard Deviation:			1.04	0.55	0.66		0.88	0.43	0.80
		Sex:		FEMALE						
		Group:		1	2	3	4	5	6	7
		Number:		12	5	5	5	5	5	5
Brain (BR)	Number in Group:			12	5	5	0	5	5	5
	Mean:			1.87	1.84	1.88		1.80	1.85	1.83
	Standard Deviation:			0.09	0.03	0.06		0.09	0.07	0.05
Lung (LU)	Number in Group:			12	5	5	0	5	5	5
	Mean:			1.09	1.31 ^{S+}	1.07		1.20 ^{S+}	1.16	1.23 ^{S+}
	Standard Deviation:			0.08	0.04	0.03		0.10	0.07	0.02
Kidneys (KD)	Number in Group:			12	5	5	0	5	5	5
	Mean:			1.71	1.68	1.68		1.70	1.57	1.65
	Standard Deviation:			0.12	0.09	0.14		0.14	0.12	0.19
Heart (HT)	Number in Group:			12	5	5	0	5	5	5
	Mean:			0.71	0.71	0.64		0.72	0.67	0.69
	Standard Deviation:			0.06	0.07	0.03		0.06	0.05	0.02
Liver (LI)	Number in Group:			12	5	5	0	5	5	5
	Mean:			6.40	6.21	6.02		6.15	5.96	6.05
	Standard Deviation:			0.58	0.62	0.71		0.47	0.24	0.28

Table 4 (Continued)

2236-100/101

Hazleton Laboratories America, Inc.		Path/Tox System Output				Printed: 06-Apr-84	
Department of Pathology		Acute Inhalation Toxicity of Welding Fumes					
Vienna, Virginia		Project 2236-101					
		ABSOLUTE ORGAN WEIGHT MEANS (g)				Study Number: 223610G	
Table Includes:		Sex:		MALE		FEMALE	
Sex = All; Group = All; Weeks = All		Group:		1	2	1	2
Death = T; Subset = All		Number:		5	5	5	4
Terminal Body Weight (g)	Number in Group:			5	5	5	4
	Mean:			286.8	289.6	192.0	195.0
	Standard Deviation:			15.7	20.2	3.5	5.9
Lung ^{BF} (LU)	Number in Group:			5	5	5	4
	Mean:			1.37	1.59	1.19	1.30 st
	Standard Deviation:			0.07	0.23	0.07	0.04
Heart (HT)	Number in Group:			5	5	5	4
	Mean:			0.91	0.97	0.71	0.71
	Standard Deviation:			0.03	0.08	0.07	0.02
Liver (LI)	Number in Group:			5	5	5	4
	Mean:			8.51	9.04	6.12	6.35
	Standard Deviation:			1.23	0.50	0.34	0.14
Kidneys (KD)	Number in Group:			5	5	5	4
	Mean:			2.45	2.61	1.57	1.58
	Standard Deviation:			0.21	0.33	0.10	0.06
Brain (BR)	Number in Group:			5	5	5	4
	Mean:			1.85	1.91	1.75	1.77
	Standard Deviation:			0.11	0.06	0.15	0.07

Hazleton Laboratories America, Inc.		Path/Tox System Output				Printed: 27-Mar-84			
Department of Pathology		Toxicity Study of Welding Fumes							
Vienna, Virginia		MEAN ORGAN/TERMINAL BODY WEIGHT RATIOS (%)				Study Number: 223610A			
Table Includes:		Sex:	MALE						
Sex = All; Group = All; Weeks = All	Group:	1	2	3	4	5	6	7	
Death = All; Subset = All	Number:	12	5	5	5	5	5	5	
Brain (BR)	Number in Group:	12	5	5	0	5	5	5	
	Mean:	0.683	0.637	0.670		0.658	0.668	0.634	
	Standard Deviation:	0.080	0.042	0.030		0.056	0.023	0.047	
Lung (LU)	Number in Group:	12	5	5	0	5	4	5	
	Mean:	0.459	0.485	0.445		0.456	0.524 ^{S+}	0.473	
	Standard Deviation:	0.022	0.028	0.031		0.025	0.029	0.029	
Kidneys (KD)	Number in Group:	12	5	5	0	5	5	5	
	Mean:	0.861	0.780	0.816		0.789	0.854	0.805	
	Standard Deviation:	0.074	0.028	0.048		0.046	0.077	0.058	
Heart (HT)	Number in Group:	12	5	5	0	5	5	5	
	Mean:	0.331	0.297	0.308		0.284	0.312	0.302	
	Standard Deviation:	0.042	0.021	0.021		0.021	0.014	0.005	
Liver (LI)	Number in Group:	12	5	5	0	5	5	5	
	Mean:	2.950	2.897	2.803		2.844	3.003	2.820	
	Standard Deviation:	0.190	0.134	0.175		0.122	0.083	0.098	

(Continued)

Table 4 (Continued)

2236-100/101

Hazleton Laboratories America, Inc.
Department of Pathology
Vienna, Virginia

Path/Tox System Output
Toxicity Study of Welding Fumes
MEAN ORGAN/TERMINAL BODY WEIGHT RATIOS (%)

Printed: 27-Mar-84

Study Number: 223610A

Table Includes: Sex = All; Group = All; Weeks = All Death = All; Subset = All		Sex:		FEMALE						
		Group:	Number:	1	2	3	4	5	6	7
				12	5	5	5	5	5	5
Brain (BR)	Number in Group:			12	5	5	0	5	5	5
	Mean:			0.942	0.907	0.968		0.921	0.943	0.892
	Standard Deviation:			0.063	0.057	0.029		0.041	0.053	0.024
Lung (LU)	Number in Group:			12	5	5	0	5	5	5
	Mean:			0.550	0.647 ^{S+}	0.548		0.614 ^{S+}	0.592	0.602 ^{S+}
	Standard Deviation:			0.036	0.023	0.021		0.051	0.036	0.015
Kidneys (KD)	Number in Group:			12	5	5	0	5	5	5
	Mean:			0.862	0.829	0.863		0.868	0.801	0.803
	Standard Deviation:			0.064	0.020	0.062		0.058	0.032	0.084
Heart (HT)	Number in Group:			12	5	5	0	5	5	5
	Mean:			0.357	0.349	0.331		0.367	0.340	0.334
	Standard Deviation:			0.022	0.016	0.010		0.020	0.021	0.015
Liver (LI)	Number in Group:			12	5	5	0	5	5	5
	Mean:			3.214	3.054	3.084		3.141	3.039	2.952
	Standard Deviation:			0.147	0.202	0.286		0.101	0.091	0.127

Hazleton Laboratories America, Inc. Path/Tox System Output Acute Inhalation Toxicity of Welding Fumes Printed: 06-Apr-84
Department of Pathology Project 2236-101
Vienna, Virginia ORGAN TO TERMINAL BODY WEIGHT RATIO MEANS (%) Study Number: 223610G

Table Includes: Sex = All; Group = All; Weeks = All Death = T; Subset = All		Sex:		MALE		FEMALE	
		Group:	Number:	1	2	1	2
				5	5	5	4
Lung ^{BF} (LU)	Number in Group:			5	5	5	4
	Mean:			0.480	0.547 ^{S+}	0.620	0.669 ^{S+}
	Standard Deviation:			0.027	0.043	0.042	0.027
Heart (HT)	Number in Group:			5	5	5	4
	Mean:			0.316	0.334	0.369	0.363
	Standard Deviation:			0.015	0.032	0.038	0.021
Liver (LI)	Number in Group:			5	5	5	4
	Mean:			2.960	3.131	3.188	3.256
	Standard Deviation:			0.337	0.211	0.186	0.065
Kidneys (KD)	Number in Group:			5	5	5	4
	Mean:			0.853	0.901	0.817	0.809
	Standard Deviation:			0.032	0.094	0.049	0.043
Brain (BR)	Number in Group:			5	5	5	4
	Mean:			0.646	0.662	0.912	0.908
	Standard Deviation:			0.049	0.032	0.070	0.055

Table 5
Mean Lung Wet/Dry Weights and Lung Wet/Dry Weight Ratios with Standard Deviations
Toxicity Study of Welding Fumes in Rats

2236-100/101

Hazleton Laboratories America, Inc.
 Department of Pathology
 Vienna, Virginia

Path/Tox System Output
 Toxicity Study of Welding Fumes
 MEAN ABSOLUTE ORGAN WEIGHTS (g)

Printed: 20-Feb-84

Study Number: 223610C

Table Includes:		Sex:	MALE						
Sex = All; Group = All; Weeks = All		Group:	1	2	3	4	5	6	7
Death = Sched; Subset = All		Number:	12	10	10	3	10	10	10
Lung - Wet (LU0)	Number in Group:		12	10	10	3	10	10	10
	Mean:		1.49	1.48	1.38	1.49	1.64 ^{S+}	1.50	1.48
	Standard Deviation:		0.15	0.15	0.08	0.06	0.14	0.09	0.13
Lung - Dry (LU1)	Number in Group:		12	10	10	3	10	10	10
	Mean:		0.31	0.31	0.30	0.33	0.34	0.33	0.30
	Standard Deviation:		0.02	0.03	0.02	0.01	0.03	0.03	0.02
		Sex:	FEMALE						
		Group:	1	2	3	4	5	6	7
		Number:	12	10	10	1	10	10	10
Lung - Wet (LU0)	Number in Group:		12	10	10	1	10	10	10
	Mean:		1.20	1.18	1.16	0.97	1.31 ^{S+}	1.22	1.31 ^{S+}
	Standard Deviation:		0.09	0.10	0.10		0.11	0.10	0.08
Lung - Dry (LU1)	Number in Group:		12	10	10	1	10	10	10
	Mean:		0.25	0.25	0.25	0.23	0.28	0.26	1.29 ^{S+}
	Standard Deviation:		0.02	0.02	0.03		0.02	0.02	0.03

Hazleton Laboratories America, Inc.
 Department of Pathology
 Vienna, Virginia

Path/Tox System Output
 Toxicity Study of Welding Fumes
 MEAN LUNG WET/DRY RATIOS (g)

Printed: 20-Feb-84

Study Number: 223610C

Table Includes:		Sex:	MALE						
Sex = All; Group = All; Weeks = All		Group:	1	2	3	4	5	6	7
Death = Sched; Subset = All		Number:	12	10	10	10	10	10	10
	Number in Group:		12	10	10	3	10	10	10
	Mean:		4.80	4.72	4.63	4.62	4.82	4.57	4.89
	Standard Deviation:		0.35	0.09	0.19	0.03	0.16	0.33	0.46
		Sex:	FEMALE						
		Group:	1	2	3	4	5	6	7
		Number:	12	10	10	1	10	10	10
	Number in Group:		12	10	10	1	10	10	10
	Mean:		4.73	4.70	4.61	4.22	4.78	4.65	4.60
	Standard Deviation:		0.18	0.11	0.16	—	0.08	0.22	0.36

Table 6
Summary of Histopathology Findings
Toxicity Study of Welding Fumes in Rats

2236-100/101

Hazleton Laboratories America, Inc. Path/Tox System Output Printed: 23-May-84
 Department of Pathology Toxicity Study of Welding Fumes
 Vienna, Virginia HISTOPATHOLOGY INCIDENCE SUMMARY Study Number: 223610A

Table Includes:
 Sex = All; Group = All; Screen = All; Weeks = All Sex:
 Death = All; Find = All; Subset = All Group:
 Organ and Finding Description Number:

		Number of Animals Affected						
		MALE						
		1	2	3	4	5	6	7
		12	5	5	5	5	5	5
Top of List								
Lung (LU)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	10	2	0	0	0	0	0
— Alveolar Macrophages		1	2	0	0	2	0	1
— Pigmented Macrophages		0	2	5	2	5	5	5
— Perivascular/Peribronchial Lymphoid Hyperplasia		0	0	0	0	0	0	0
— Pneumonitis		0	0	0	2	0	0	0
— Congestion		0	0	0	4	0	0	0
— Perivascular Edema		1	0	0	5	0	2	0
— Alveolar Edema		0	0	0	4	0	0	0
— Fibrinous Pneumonia		0	0	0	0	0	0	0
— Hemorrhage		0	0	0	1	0	0	0
— Seropurulent Pneumonia		0	0	0	2	0	0	0
End of List								

		Number of Animals Affected						
		FEMALE						
		1	2	3	4	5	6	7
		12	5	5	5	5	5	5
Top of List								
Lung (LU)	Number Examined:	12	5	5	5	5	5	5
	Not Remarkable:	9	3	0	0	1	0	0
— Alveolar Macrophages		2	2	1	0	0	0	0
— Pigmented Macrophages		0	1	5	2	4	5	5
— Perivascular/Peribronchial Lymphoid Hyperplasia		1	0	0	0	0	0	0
— Pneumonitis		0	0	1	4	0	0	0
— Congestion		0	0	0	5	0	0	0
— Perivascular Edema		0	0	0	5	0	0	0
— Alveolar Edema		0	0	0	5	0	0	0
— Fibrinous Pneumonia		0	0	0	1	0	0	0
— Hemorrhage		0	0	0	0	0	0	0
— Seropurulent Pneumonia		0	0	0	0	0	0	0
End of List								

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 Department of Pathology Acute Inhalation Toxicity of Welding Fumes
 Vienna, Virginia Project 2236-101
 HISTOPATHOLOGY INCIDENCE SUMMARY Study Number: 223610G

		Number of Animals Affected			
		MALE		FEMALE	
		1	2	1	2
		5	5	5	5
Top of List					
Lung (LU)	Number Examined:	5	5	5	5
— Perivascular/Peribronchial Lymphoid Hyperplasia		4	3	2	3
— Pigmented Macrophages		0	4	0	5
— Perivascular Edema		0	0	0	2
— Congestion		0	0	0	1
— Pneumonitis		0	0	0	1
End of List					