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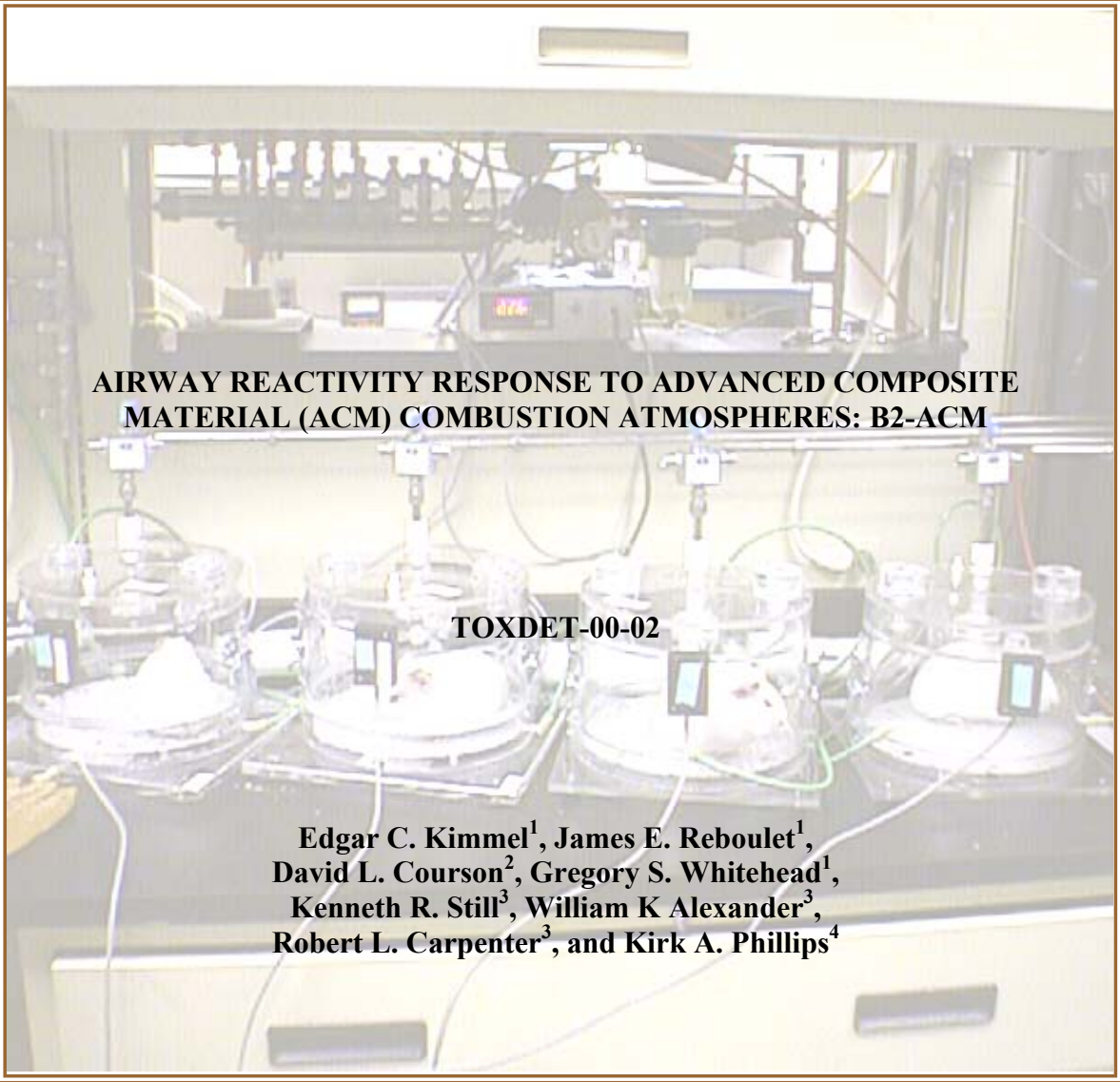

AIRWAY REACTIVITY RESPONSE TO ADVANCED COMPOSITE MATERIAL (ACM) COMBUSTION ATMOSPHERES: B2-ACM

TOXDET-00-02

Edgar C. Kimmel[1], James E. Reboulet[1], David L. Courson[2], Gregory S. Whitehead[1], Kenneth R. Still[3], William K Alexander[3], Robert L. Carpenter[3], and Kirk A. Phillips[4]

1. Geo-Centers Inc.
2. ManTech Environmental Sciences.
3. Naval Health Research Center Detachment (Toxicology) – NHRC/TD
   Bldg 433
   2612 5th St.
   Wright-Patterson AFB, OH 45433-7903
4. 509 ADOS/SGGB, Whiteman AFB, MO

Correspondence to Dr. E.C. Kimmel at NHRC/TD

PREFACE

This document contains the results of an investigation of the acute airway response in laboratory guinea pigs produced by acute exposure to smoke generated from combustion of advanced composite material (ACM). Various types of ACM are currently being used the construction and retrofit of numerous military systems and vehicles. The type of ACM under investigation was material commonly used in the construction of the B2 bomber (B2-ACM) and was provided by Northrop Grumman Corp. The report is intended to provide information pertinent to the evaluation of the potential health risks to humans exposed to B2-ACM smoke. This work was conducted at the Naval Health Research Center Detachment Toxicology (NHRC/TD) under the direction of CAPT Kenneth R. Still, MSC, USN, Officer-in-Charge NHRC/TD. The research was requested by Capt Kirk A. Phillips, BSC, USAF, Bioenvironmental Engineering Flight Commander 509 ADOS/SGGB. This work was sponsored by the 509 ADOS/SGGB and the Naval Medical Research and Development Command under Work Unit # 63706N-M00095.004.1714.

The opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of Defense, the Department of the Navy or the Naval Services at large.

Animal handling procedures used in this study were subject to review and approval by the Animal Care and Use Committee located at Wright-Patterson AFB and the Airforce Surgeon General. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Research, National Research Council, DHHS, National Institutes of Health Publication 85-23, 1985, and the Animal Welfare Act of 1966, as amended.

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EXECUTIVE SUMMARY

PROBLEM

Advanced composite materials (ACMs) are a family of man-made products that are currently being used to replace metallic components in a variety of military and civilian vehicles, primarily aircraft. ACMs consist of woven fiber mesh fabrics that are impregnated with and imbedded in a polymer matrix. There are a variety of ACMs depending upon the type of fibers and polymers used in their manufacture. The form examined in this investigation was a carbon-graphite fiber/epoxy ACM used in the construction of the B2 bomber (B2-ACM). The major disadvantage of the increased use of ACMs is the fact that this material is combustible; evolving chemically and physically complex atmospheres of gases, vapors, and particulate material in high concentrations when burned. The health risks associated with exposure to these atmospheres are largely unknown, yet the potential to expose large numbers of military as well as civilian personnel is great. There are numerous possible exposure scenarios associated with the use and combustion of ACMs. This investigation focussed on the exposure of unprotected personnel whose respiratory tract may be sensitive to the irritating effects of ACM smoke.

OBJECTIVE

The production of potentially toxic smoke from pyrolysis of ACM is highly likely in the event of aircraft mishap. Should the mishap occur in or near a population center, the smoke exposure risk is high for large numbers of unprotected individuals. Smoke can be immediately lethal (asphyxiation) or lead to acute lung injury and severe lung disease (acute respiratory distress syndrome). However, a significant fraction of sensitive individuals (estimated 10 to 20 %) may also be at increased risk of severe, possibly lethal, acute airway reactivity (AR) or related airway hyperreactivity (AHR) responses. These effects are produced by exposure to very low concentrations of smoke. Our objective was to examine this possibility using an animal model of AR and AHR responses and to identify, if possible, a concentration of smoke at which there was no observable effect. This information is intended to provide a basis for the establishment of guidelines for cordon of an aircraft mishap site.

RESULTS

Diluted smoke from the combustion of as little as 5 grams of B2-ACM was found to elicit AR responses after a brief exposure. Exposure to diluted smoke from burning a larger amount (100 grams) of B2-ACM elicited severe bronchospasm leading to convulsions. The minimal response from exposure to atmospheres produced by burning 2 grams of B2-ACM suggests that there may be a threshold level for this acute airway reaction to this smoke. Two pertinent factors must be considered, the smoke concentrations were sufficiently low as to not be visible and removal of particulate matter from the smoke did not significantly alter the response.

CONCLUSION

The inhalation of dilute smoke from pyrolysis of at least one type of ACM, B2-ACM, can produce an acute AR response similar to an asthmatic episode in sensitive individuals. Severe AR reactions if prolonged could be fatal. Furthermore, because of the relationship between AR and AHR responses there is a distinct possibility that sensitization of exposed individuals will greatly increase the probability of a non-specific AHR response upon subsequent exposure to any one of numerous upper respiratory irritants. Investigation of the latter phenomenon was not in the scope of the present investigation, however the results of this investigation indicate this is a probable health risk associated with ACM smoke exposure.

ABSTRACT

Exposure for 30 minutes to diluted smoke from pyrolysis of advanced composite material used in the construction of the B2 bomber (B2-ACM) caused an airway reactivity (AR) response in naïve guinea pigs reminiscent of a human asthmatic episode. Animals exposed to diluted smoke from pyrolysis of 5, 10 and 100 grams of B2-ACM showed changes in a number of parameters characterizing ventilation, breathing pattern, and breath structure. These changes are considered indicative of bronchoconstriction. The highest exposure concentration also elicited convulsions in the animals, which may or may not be related to the AR response. Upon treatment with fresh air there was recovery period in which breathing returned to normal. However the recovery was transient with respiratory parameters returning to abnormal levels; indicating a "rebound" constrictive event even in the presence of clean air. Animals exposed to diluted smoke from the pyrolysis of 2 grams of B2-ACM demonstrated minimal changes in only a few of the respiratory parameters, suggesting that there might be a threshold for B2-ACM smoke elicited AR response.

KEY WORDS AND PHRASES

Advanced composite material, Smoke, Airway reactivity response.

GLOSSARY OF TERMS AND ABBREVIATIONS

Note: Common chemical and measurement abbreviations are not included.

ACM – advanced composite material

AHR – airway hyperreactivity

AHS – airway hypersensitivity

AR – airway reactivity

B2-ACM – ACM used in construction of the B2 bomber

BC - bronchoconstrictor

f– breathing frequency

FDP – flow derived parameter

NIST – National Institutes of Standards and Technology

PI – pulmonary irritant

PEF – peak expiratory flow

Penh – enhanced pause

PEP - peak expiratory pressure

PIF - peak inspiratory flow

Rt – relaxation time

SI – sensory irritant

Te – expiratory time

Ti – inspiratory time

Ti/(Ti+Te) – respiratory duty cycle

Ve – minute ventilation

VS – vagal stimulant

Vt – tidal volume

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INTRODUCTION

Advanced composite materials (ACMs) are being used in increasing quantity as the material of choice for construction and retrofit of military and civilian vehicles, particularly aircraft. In some of these craft ACMs are the predominant structural material. The term ACMs refers to a variety of engineered materials that are composed of continuous fiber fabrics impregnated with and imbedded in a polymer matrix. Plys of ACM are bonded together in layers to form laminates, which are the basic structural material. Several materials are used in the manufacture of the ACM's fabric mesh; the most common being fibers of high strength glass, carbon/graphite, tungsten/boron, or aramid materials such as Kevlar ® (Dupont Chemical). Several polymer types are used in the manufacture of ACMs as well. These include thermosetting resins such as epoxy, cyanate esters, bismaleimdes, polyesters, and vinyl esters. Thermoplastic resins such as polyetherketones and polysulfones are used to make molded parts and to strengthen thermosetting resins for some applications. Epoxy resins are most commonly used in the manufacture of ACMs. All of these materials are flammable and have high heat release rates when burned. Consequently, unlike their metal counterparts, many structural ACM components are combustible. In 1999 there were several mishaps resulting in the destruction of 25 US Air Force aircraft (Gideon, 2000), many of which contained ACMs. This gives cause for heightened concern about potential health risks to military and civilian personnel associated with exposure to smoke generated from fires related to mishaps involving ACM laden aircraft. Of particular concern is the exposure of large numbers of people , should such a mishap occur in or near a population center.

Smoke is a complex mixture of gases, vapors, and particulate matter that varies in composition depending on the material being burned and specific combustion kinetics. Because of this complexity there are a variety of untoward responses that may be elicited by exposure to a given type of smoke. Given the varied composition of ACMs it is likely that their smoke composition also will vary widely. It follows that there also may be a variety of untoward responses that may be elicited from exposure to ACM smoke. We recently reported the results of an investigation of short term pulmonary injury produced in animals by exposure smoke generated by burning one type of ACM used in the

construction of the B2 bomber (Kimmel et al., 2000a). That investigation focussed on acute lethal toxicity from asphyxiation and examination of pathophysiologic effects associated with damage of lung tissue. However there are several respiratory responses that are associated with stimulation of sensory receptors distributed throughout the respiratory tract, which are not necessarily linked to tissue damage. These responses can be incapacitating and, if severe enough, lethal. An airway reactivity (AR) response associated with irritation of respiratory mucosa and airway constriction is one such respiratory effect. AR responses often are related to immediate and delayed onset airway hyperreactivity (AHR) responses. The difference being that AHR responses are, as a rule, immunologically mediated. Over the past several years there have been a few incidents reported (primarily in the popular press) in which emergency response personnel have suffered skin irritation and respiratory effects after responding mishaps involving aircraft with ACM components. Particularly alarming is the fact that many of these individuals were afforded respiratory protection during their exposure. In 1997, 22 Baltimore area firefighters required hospitalization for complaints of labored breathing, eye and skin irritation, nausea, and headache after responding to an USAF F-117A mishap. Ten years ago, workers recovering the engine from a RAF GR.5 Harrier downed in Denmark reported respiratory and dermal irritation. Again, these workers were equipped with industrial respiratory and eye protection equipment. Several anecdotal comments forthcoming from personnel handling combusted ACM suggest a "contact dermatitis" response to this material. Because of similar mechanisms of action at the molecular level between dermal irritation and AR responses, these reports imply that AR and AHR responses are a highly probable consequence ACM smoke exposure. However, the most telling evidence is the persistent diminished flow volume capacity and a heightened reactivity to histamine challenge in an individual exposed at the crash site of a Navy F-18 fighter in the late 1980's (Seibert, 1990). These reports and what is known of the chemical constituency of B2-ACM smoke (Kimmel et al., 2000a; Libscomb et al., 1997) prompted the present investigation of B2-ACM smoke induced AR response.

METHODS AND MATERIALS

Test Material

Single ply carbon/graphite/epoxy ACM coupons measuring 10 x 10 x 0.3 cm were provided by Northrop Grumman Corp. for this investigation (Ret # B01718, ASDL #146 – Mr. Dan Ellison – personal communication). To simulate the extensive damage to ACM that occurs upon high impact (Flight International, 1991) these coupons were ground in a laboratory cutting mill (Fritsch model LC-124, Gilson Col, Worthington, OH). A coarse 6 x 6 mm sieve was used in the mill and grinding produced splintered fragments of ACM that typically measured 2 to 3 mm in width and 20 to 40 mm in length. Some delamination of the fiber mesh and the epoxy did occur, however this did not appear the affect the total fraction of B2-ACM that was pyrolyzed under given conditions. Nor was the pyrolysis rate substantially affected by the increase in surface area to mass ratio caused by grinding.

Animals

40 male guinea pigs (Hartley, Outbred, Crl(HA)BR) ranging between 390 and 440 g were obtained from a commercial supplier for this investigation (Charles River Laboratories, Wilmington, MA). Newly arrived animals were examined by a veterinarian and were quarantined for 10 days prior to use. The animals were housed individually in plastic, shoebox cages over adsorbent bedding for the duration of the investigation. Food and water were provided *ad libitum* and the animals were maintained on a 12 hr diurnal cycle. Prior to exposure the animals maintained in a fully, AALAC accredited facility.

Experimental Design

Four animals were exposed to B2-ACM smoke in a 690-L (Kimmel et al, 1997) whole-body exposure chamber for 30 minutes in a pilot investigation of survivability. The remaining 36 animals were divided among 9 exposure groups. Exposures were conducted using 2, 5, 10 or 100 grams of milled B2-ACM. These weights refer to the amount of B2-ACM loaded into the combustion furnace and are **NOT** TO BE CONSTRUED AS SMOKE EXPOSURE CONCENTRATIONS. For convenience, the term load is used to distinguish between these quantities of uncombusted B2-ACM and their corresponding nominal and/or measured smoke concentrations. Also for convenience, the experimental

groups are designated by this load weight. The term nominal concentration refers to the concentration of smoke calculated by dividing the amount of B2-ACM burned in a given time by the volume of air that passed through the furnace during that time. Smoke exposures were 30 minutes in duration. Individual animals in a given exposure group were exposed simultaneously in 4 separate 4.5 L whole body plethysmographs (vide infra). Two exposures were conducted for each load. One exposure was to whole smoke and the other to a corresponding concentration of smoke from which particulate matter had been filtered.. Groups exposed to the filtered smoke have the designator F. The 9 experimental groups, consisting of 4 naïve, animals each are as follows:

- Control – room air exposure.
- 2 – 2 g B2-ACM load.
- 2F – 2 g B2-ACM load, filtered.
- 5 – 5 g B2-ACM load.
- 5F – 5 g B2-ACM load, filtered.
- 10 – 10 g B2-ACM load.
- 10F – 10 g B2-ACM load, filtered.
- 100 – 100 g B2-ACM load
- 100F – 100 g B2-ACM load, filtered.

Airway response to the smoke exposure was measured in each animal in real time during the 30-minute exposure period. Prior to delivery of smoke to the plethysmograph ventilation, breathing pattern and breath structure measurements were made in the animals for a 30-minute period to establish baseline parameters for normal breathing.

Exposure System and Smoke Characterization

The exposure system including the furnace, the combustion methods, and the smoke characterization methods used in this investigation are essentially those that have been described in detail previously (Kimmel et al., 2000a). For this investigation the 690-L whole-body, inhalation exposure chamber served as a reservoir from which smoke was drawn into each plethysmograph through a 1-m length of 1.25 cm i.d. stainless steel tubing. For the filtered smoke exposures provisions were made to insert an absolute, filter in the transport line (PALL, HEPA capsule # 12144, Gelman Laboratory, Ann Arbor, MI).

Smoke was transported from the reservoir at 9.2 L/min for 3 minutes. Based on Silver's criteria (Silver, 1946) and mixing efficiency calculations (Cholette and Cloutier, 1959) this allowed for filling of the plethysmographs with smoke to greater than 99.9% capacity while maintaining laminar flow (Reynolds number ≤ 2000) in the transport lines. The smoke was allowed to age in the reservoir for 30 minutes prior to delivery to the plethysmographs. Aging of the smoke was intended to simulate the dilute smoke exposure conditions that would occur at a site remote from the combustion source. In our previous investigation, we found that the particulate fraction of the B2-ACM combustion atmospheres lost approximately 30 % of its initial mass due to evaporation of volatile components (Kimmel et al., 2000a). This evaporative loss was complete within 30 minutes of formation of the smoke. In addition, there was a significant loss of aerosol particle mass in the smoke within the first 30 minutes of generation due to gravitational settling. Quantitative analysis demonstrated that initial concentrations of the aerosol, CO, CO_2, NO_2, SO_2 components of the smoke could account for approximately 82 % of nominal concentration of the smoke regardless of the load mass. The concentrations of these smoke constituents in the reservoir were determined immediately prior to delivery of the smoke to the plethysmographs. Aerosol concentration and size distribution also were determined at this time. After 30 minutes of exposure, each plethysmograph was flushed at a rate of 9 L/min for 3 minutes with fresh air to clear the smoke. Breathing parameters were recorded for an additional 30 minutes. A schematic diagram of the reservoir/plethysmograph exposure apparatus is shown in Figure 1.

Respiratory Physiology

Since restraint and anesthesia can effect AR responses, respiratory measurements were made in unrestrained and unanesthetized animals, employing barometric techniques pioneered by Chapin, (1954) and Drorbaugh and Fenn (1955). Ventilation, breathing pattern, and breath structure measurements were made in real-time using four commercially available, 4.5 L, bias-flow plethysmographs (PLY/BF-GP, Buxco Electronics, Sharon, CT – see Figure 2). Four plethysmographs were used so that the four animals in each experimental group could be exposed simultaneously to the same atmosphere. Generally measurement of changes in ventilation, breathing pattern, and

breath structure are considered indirect assessments of airway reactivity events such as bronchoconstriction (DiMaria et al., 1987; Briatico-Vangosa et al., 1994). However, several improvements of the basic barometric technique have been made that have increased the reliability of this indirect approach (Jaeger and Bouhuys, 1969, Johanson and Pierce, 1971; Epstein and Epstein, 1978; Pennock et al., 1979; Jacky, 1980; Bartlett and Tenney, 1980;Fleming et al., 1983; Silbaugh and Mauderly, 1984). Given these improvements and the freedom from the complications of restraint and anesthesia, barometric techniques remain viable approaches to initial evaluation of an inhalation threat for AH and AHR risk (Thorne and Karol, 1988; Blaikie et al., 1995). Barometric techniques are useful as screening tools in a multi-tiered approach toward evaluating potency of potential respiratory irritants and allergens (Sarlo and Clark, 1992).

Differential pressure across 2 screen mesh pneumotachographs located in the plethysmograph walls was measured with a differential pressure transducer (0 –5.2 mmHg, S0X1, Synsym Co., Malpitas, CA) to determine flow.. The flow signal was electronically integrated to obtain tidal volume (Vt). Prior to each use the pressure transducers were calibrated using a pressure standard with National Institute of Standards and Technology (NIST) traceable certification (Meri-Cal # LP200C, Meriam Instruments, Cleveland,OH). The pneumotachographs also were calibrated using a bubble flow meter standard with NIST traceable certification (Gilibrator, Gilian Instruments, Wayne, NJ). The transducer signal was amplified, processed and recorded using hardware and software designed for this purpose (MAXII, Biosystems XA, Buxco Electronics, Sharon, CT) and a personal computer. In addition to Vt, breathing frequency (*f*), minute ventilation (Ve), inspiratory time (Ti), expiratory time (Te), relaxation time (Rt), peak inspiratory flow (PIF), and peak expiratory flow (PEF) were measured for each breath. The average values of these parameters for 30 breaths were recorded each minute. Calculated parameters included respiratory duty cycle [Ti/(Ti+Te)], enhanced pause (penh = (PEP/PIP)[(Te-Rt)/Rt], Hamelmann et al., 1997), andflow derived parameter (FDP = PEF(Ti+Te)/Vt, Pauluhn and Eben, 1991). These calculated parameters also were recorded for each minute. Figure 3 is a diagram of a typical breath with several of these parameters depicted. Table 1 lists the parameters measured with appropriate units.

During the 30-minute period prior to exposure the plethysmographs were operated with a 1 L/min bias flow to remove animal waste gases. However, during the 30-minute exposure period this flow was stopped to prevent artificial depletion of the test atmosphere. As a result there was a build up of CO_2 in the plethysmograph which affected some of the parameters being measured. These effects were reflected in the control group results (vide infra) and were accounted for (Kimmel et al., 2000b). Because the pneumotachographs remained open, O_2 depletion and pressure build in the plethysmograph were not factors. After the flushing and during the post-exposure period bias flow through the plethysmographs was restored. Filling and flushing the plethysmographs required opening an inlet port, which invalidated plethysmograph calibration. Thus recordings made during the 3-minute fill and flush periods were ignored. To allow the animals to explore and acclimatize to the plethysmograph, recordings during the first 10 minutes of the pre-exposure baseline period likewise were ignored.

Data Analysis

The mean of the last 10 (last 10 minutes) values recorded for a parameter during the pre-exposure period was considered as the normal, baseline value of that parameter for that animal. Parameter values recorded or calculated for each minute were transformed by dividing them by the baseline value for that animal. Displayed data are the mean group value. Comparisons were made among control and experimental group values for any given minute into the test period. Comparisons of parameter values for given blocks of time within an experimental group also were made. Comparisons either between groups or between time periods within a group were made using Student's t tests, with $p \leq 0.05$ considered as significant. Because of the large quantity of data presented designators for significant differences are not displayed on the graphics. However error bars representing the standard error of the mean are periodically shown.

RESULTS

Combustion Conditions

Combustion conditions for this investigation were identical to those described previously (Kimmel et al., 2000a). Despite the presence of a spark source in the furnace B2-ACM combustion did not enter the flaming mode. There was no notable depletion of O_2 in the air stream through the furnace. Within seconds of the start, the temperature in the furnace reached 630 ^oC and rose rapidly to as high as 740 ^oC as the B2-ACM pyrolyzed, demonstrating that B2-ACM combustion was exothermic. The average ratio of CO_2 to CO production was 2.2 to 1. Under these conditions, the fires were given an ISO/TC92/SC3 classification of non-flaming-1b. An average of 26.5 % of the B2-ACM loaded into the furnace pyrolyzed at these temperatures (Table 2) compared to slightly over 20% in previous experiments. This overall increase in burn efficiency was attributed to the fact smaller B2-ACM loads were burned in the present investigation. These data are in agreement with those of other investigators. Courson and colleagues (1996) found that approximately 30% of carbon/bismaleimide ACM pyrolyzed at 650 to 700 ^oC. Soranthia and colleagues (1992) found an range of 6 to 28 % mass loss from ACMs with either carbon or glass fibers and a variety of different polymer matrices, that were irradiated at 50-kW/m^2.

Smoke Concentration and Chemistry

Nominal concentrations of B2-ACM smoke ranged from 0.77 to 28.70 g/m^3 (Table 2). Concentration of particulates in the exposure atmospheres ranged from 0.15 to 2.2 g/m^3 (Table 3), for an approximately 15-fold difference between the lowest and highest aerosol concentrations among the exposure atmospheres. However, the particle size distributions were nearly identical with mass median aerodynamic diameters (MMADs) and geometric standard deviations (σgs) ranging from 1.3 to 1.6 μm and 1.7 to 1.8, respectively. The concentrations of common gas phase constituents in the exposure atmospheres differed over a wider range, with the high and low concentrations of CO_2, CO, NO_2, and SO_2 differing by factors of approximately 22, 28, 38, and 45 respectively. However, evolution

of these gases appeared to be a linear function of the mass of B2-ACM that pyrolized (Figure 4).

Overt Response to Exposure

Compared to the pre-exposure period, there was an increase in activity and exploratory behavior in the animals, which became evident almost immediately upon onset of exposure, regardless of smoke concentration. This behavioral change also was present, but not as marked, in the control animals. This increase in activity subsided in the control, 2, 2F, 5, and 5F group animals within a few minutes of the start of the exposure period. A second increase in animal activity was observed immediately after the flushing of the plethysmographs. at the end of exposure. Animals in the 10 and 10F groups also showed a marked increase in activity, as well as signs of labored breathing which persisted throughout the exposure period and in some animals persisted into the post-exposure period. Animals in the 100 and 100F exposure groups demonstrated similar behavior and signs of respiratory distress. Between 15 and 20 minutes into exposure 7 of the 8 animals in these two exposure groups became ataxic and developed spasms and seizures which persisted throughout the remainder of the exposure period. We speculate that these seizures would have been lethal had the exposure protocol been extended for a few more minutes Seizure activity subsided upon flushing of the plethysmographs and was replaced by a shivering behavior that persisted for 10 to 15 minutes.

Ventilation, Breathing Pattern and Breath Structure

Normal ventilation and breath structure parameters for the baseline period are listed in Table 4. Exposure to all concentrations of B2-ACM smoke, both whole and filtered, caused breathing frequency to become erratic, in many cases more than doubling the variability of *f* during both the exposure and post-exposure periods. Breathing frequency in animals in the 2 and 2F exposure groups did not vary significantly from control group animals (Figure 5). Group 5 and 5F animals had a significantly decreased *f* during much of the exposure and post-exposure periods (Figure 6). (Depression of f was more pronounced in both the 10 and 10F group animals, however there were signs of recovery in the post-exposure period (Figure 7). Animals exposed to the 100 and 100F smoke demonstrated a

pronounced depression f starting immediately upon smoke exposure Unlike the other exposure groups there was a compensatory elevation of *f* during the latter part of the exposure period that persisted for 10 to 20 minutes into the post-exposure period. There was a substantial difference in response to the filtered versus unfiltered smoke at this load. The compensatory elevation of *f* was more pronounced in animals exposed to unfiltered smoke, where f reached significantly higher levels than corresponding controls (Figure 8). In control, 2 and 2F animals there was a steady, slight increase in Vt over the course of the exposure period, which immediately returned to normal levels on plethysmograph flushing (Figure 9). As a result there was a slight corresponding increase in Ve (Figure 10). . A significant elevation of Vt was observed during the exposure period in group 5 and 5F animals. However in these animals the return to normal Vt immediately after flushing of the plethysmographs was temporary. In both groups Vt again rose to significantly higher levels than controls over the course of the post-exposure period (Figure 11). Since *f* in these animals was depressed the elevation of Vt was not reflected by a remarkable elevation of Ve during the exposure period. However, in the final few minutes of the post-exposure period Ve declined to levels significantly lower than controls (Figure 12).. Vt in 10 and 10F animals was significantly elevated from onset of exposure and remained elevated during the post-exposure period. This elevation of Vt was offset by the depression of *f* in these animals, therefore Ve remained normal or slightly lower than normal throughout (Figures 13 & 14). There was a significant elevation of Vt in group 100 and 100F animals during the exposure period, which immediately returned to normal during the post-exposure period (Figure 15).As a result of these changes in tidal volume and corresponding changes in breathing frequency, Ve was initially depressed on exposure but elevated rapidly over the course of exposure in both groups. This substantial elevation of Ve persisted in both groups into the first few minutes of the post-exposure period (Figure 16).

Neither PIF nor Ti were effected in group 2 and 2F animals by exposure to smoke (Figures 17 & 18). In group 5 and 5F animals PIF was slightly depressed during the exposure period and continued to diminish over the course of the post-exposure period (Figure 19). These changes in PIF were reflected by a corresponding elevation of Ti in these animals (Figure 20). A similar but more pronounced pattern of changes in PIF and Ti was observed in group 10 and 10F animals (Figures 21 & 22). PIF in group 100 and 100F

animals was depressed at he start of exposure. In a pattern similar to that of Vt, PIF increased over the course of exposure. By the end of the exposure period PIF was substantially elevated in these animals. In group 100 animals PIF rapidly returned to control levels at onset of the post-exposure period. In group 100F animals PIF fell below control values (Figure 23). In this instance, changes in Ti did not mirror changes in PIF. In both the 100 and 100F animals Ti was significantly elevated during exposure. In a pattern corresponding to changes in breathing frequency, Ti was lowered in these animals by purging the plethysmographs of smoke. Despite this reduction, Ti in group 100F animals remained elevated during the entire post-exposure period; whereas, Ti in group 100 animals returned to normal levels initially and then gradually increased (Figure 24). Expiratory time control, 2, and 2F animals was not significantly different (Figure 25). A elevation of Te in 5 and 5F that occurred in group 5 and 5F animals during the exposure period continued to increase in the post-exposure period (Figure 26). Elevation of Te during the exposure period was more pronounced in group 10 and 10F animals. During the post-exposure period Te remained elevated but at lower levels than during the exposures (Figure 27). Changes of Te in groups 100 and 100F reflected the pattern of changes of Ve observed in these animals. There was an immediate increase in Te upon exposure that gradually diminished to levels lower than controls over the course of the exposure. The decreased Te persisted into the post-exposure, initially, then gradually increased to control values by the end of this period (Figure 28).

Respiratory duty cycle [Ti/(Ti+Te)] remained constant, with little change in variability, during the exposure and post-exposure periods in all but group 100 and 100F animals (Figures 29-31).Duty cycle in these animals increased during the first 10 minutes of exposure and remained elevated throughout the remainder of the experiment (Figure 32). Exposure to 2 and 2F levels of B2-ACM smoke did not effect penh (Figure 33). However in group 5 and 5F animals there was an increase in the fluctuation of penh accompanied by gradual increase to significantly elevated levels during the last half of the post-exposure period (Figure 34). A similar pattern of increased fluctuation in penh was observed in group 10 and 10F animals. However, a significant increase in penh also was observed in group 10 animals immediately on exposure. Although reduced in magnitude penh remained elevated during the post-exposure period in these animals. Group 10F animals did

not exhibit a consistently elevated penh during exposure. It was not until latter half of the post exposure period that penh was significantly elevated in these animals (Figure 35). Penh increased immediately upon exposure in groups 100 and 100F animals and remained elevated throughout the post-exposure period in both groups (Figure 36). There was no apparent effect of smoke exposure on FDP in 2, 2F 5, and 5F animals (Figures 37 & 38). FDP increased significantly in group 10 animals but not in group 10F animals (Figure 39). There was an immediate, significant elevation of FDP upon exposure in group 100 and 100F animals that eventually returned to normal levels by the end of the post-exposure period (Figure 40).

DISCUSSION

AR response refers to changes in breathing pattern and breath structure that occur in naïve subjects when exposed to substances that stimulate one or more of a variety of sensory receptors and nerve fibers distributed throughout the respiratory tract (Paintal, 1981). AR responses are distinguishable from AHR or airway hypersensitivity (AHS) responses because latter imply a heightened sensitivity or degree of response resulting from an immunologically or pathologically mediated predisposition. AHS is thought to be immunologically mediated by specific antigens (Karol and Thorne, 1988); whereas AHR may be non-specific and not necessarily immunologically mediated (Costa et al., 1992). Furthermore, it has been suggested that AHS responses can be either immunogenic or neurogenic in origin (Pauluhn, 1997). All these airway reactivity responses may be related to one another and to other respiratory conditions such as chronic obstructive lung disease by complex and poorly understood mechanisms (O Connor et al, 1989, Thorne and Karol, 1988; Pauwels, 1990). Our data indicate that exposure of naïve animals to B2-ACM smoke elicits "asthma like" changes in breathing pattern and breath structure that can be incapacitating and possibly fatal. Furthermore, the possibility that exposure to B2-ACM smoke can lead to increased risk for an airway reactive disorder or event can not be discounted. Based on a synopsis of the control of breathing by Boggs (1992), Vijayaraghavan and colleagues (1993) developed a rationale attributing characteristic changes in breathing pattern and breath structure to the stimulation of specific efferent nerve endings located in different portions of the respiratory tract. . They postulated that stimulation of trigeminal and laryngeal nerve endings in the extrathoracic respiratory tract caused closure of the glottis resulting in an increase in laryngeal resistance. Agents eliciting this action were termed sensory irritants (SI). The result was slowing of the initial stage of expiration leading to an increase in Te and a reduction of *f*.. In their nomenclature agents eliciting constriction of the conducting airways were termed bronchoconstrictors (BC). Characteristic responses attributed to BCs were an increase in Te due to increased airway resistance as well as an increase in Ti. The result of which was a decrease in *f*. Direct stimulation of vagal C-fibers (J receptors) located in the alveolar region of the lung, produced characteristic breathing pattern changes that differed as a function of dose

(exposure concentration). Agents that elicited immediate, rapid responses and for which recovery was rapid once the agent was cleared from the lung were termed vagal stimulants (VS). Characteristic changes brought on by low doses of VS included a shortening of both Ti and Te leading to a decrease in Vt and an increase in *f*. These responses are a hallmark of rapid shallow breathing. VS at high doses also shortens Ti and Te thus decreasing Vt. However, there is an increase in pause between breaths leading to a net decrease in *f*. Similar dose dependant characteristic changes in breathing pattern of were attributed to pulmonary irritants (PI). The difference being that stimulation and recovery from stimulation of C-efferents by PI are slow acting. It was thought that PI effects were mediated through an inflammatory response including edema and congestion. The time required for the development as well as resolution of this inflammatory stimulation of the receptors was deemed responsible for the slower actions of PI. It is not clear as whether slower acting PI responses can be attributed to either release of inflammatory neuropeptides or to physical stimulation of the receptors. However both mechanisms appear feasible. .

The responses to B2-ACM smoke exposure observed in the present investigation did not readily fit in the scheme developed by Vijayaraghavan and colleagues. The depression of *f* observed in group 5, 5F,10, and 10F animals and the elevation of *f* observed in group 100 and 100F animals indicate that this not a VS response. This pattern as well as the rate at which f changes, with exception of 100 and 100F animals, also preclude a PI response. The pattern of change of Vt observed support these conclusions. Sasaki and colleagues (1987) also noted that vagal stimulation decreased Vt. In addition, Chanda and colleagues (1984) demonstrated that methacholine, a well-known bronchoconstrictor, had little effect on Vt. However, stimulation of ventilation by CO_2 may explain Ve elevation. In our laboratory, Ve elevation has characteristically started with an elevation of Vt at lower concentrations of CO_2 unaccompanied by significant change in *f* (Kimmel et al., 1999, 2000b). Thus, CO_2 effects on Vt confound the interpretation of these data. Other investigators have shown a similar pattern of CO_2 stimulated ventilation in which increases in f accompanied increases in Vt as concentration of CO_2 increased (Shams 1985; Tepper et al, 1988; Lai et al., 1978). Thus, the elevation of Vt in the present investigation could be attributed to CO_2 in the smoke as well as build up of expired CO_2 in the plethysmographs.

Nevertheless, continued elevation of Vt in group 5, 5F, 10 and 10F animals during the post-exposure period when fresh air flow was returned to the plethysmograph, suggests that some other mechanism also contributed to the increase in Vt. Tidal volume in animals exposed to 100 and 100F smoke returned to baseline levels during the post-exposure period. Given that this was not the case in animals in which Vt was elevated by exposure to lower concentrations of smoke it is difficult to attribute this to recovery of normal breathing pattern. Consequently the return of Vt to control levels in these animals can be considered to be the result of counteracting receptor stimulation in a manner that normally lowers Vt. However, depression of Vt is characteristic of both VS and PI stimulation. The transient elevation in Ve observed in the 100 and 100F group animals corresponded to the period when both f and Vt were elevated in these animals. This period of hyperventilation also corresponded with the time in which these animals demonstrated clear signs of ataxia and convulsions, suggesting a possible link between the two. Sadoul and colleagues (1985) demonstrated a similar link between diaphragmatic and intercostal neuromuscular stimulation/fatigue and change in ventilation in sheep.

The reduction of PIF brought on by exposure to smoke in all but group 2, 2F, and 5 animals was characteristic of bronchoconstriction. The response difference between the 5 and 5F animals indicated that filtration of the smoke may have moderated this effect. The same is suggested by comparison between group 10 and 10F animals, however at this smoke concentration the PIF in both filtered and unfiltered smoke differed from controls. When Vt is relatively constant then reduced a PIF would be expected to prolong Ti, as was observed in animals exposed to lower concentrations of smoke. In group 100 and 100F animals Ti remained elevated throughout the exposure despite an elevation of PIF from a significantly lower to a significantly higher flow than controls. We attributed this to a substantial elevation of Vt possibly caused by CO_2 stimulation of ventilation. The Te elevation observed in group 5, 5F (in the post exposure period), 10and 10F animals could be attributed to either SI or BC stimulation according to the scheme of Vijayaraghavan and co-workers. However, simultaneous elevation of Ti in these animals suggests BC stimulation. In group 100 and 100F animals the initial Te elevation might also be attributed to BC stimulation. In contrast to Vijayaraghavan and colleagues, Zuperku and Hopp (1985) found that vagal stimulation prolonged Te in a canine model. The Te

depression observed in group 100 and 100F animals late in the exposure and the early in post-exposure was characteristic of a VS or PI stimulation. However, characteristic changes in breathing pattern for these types of stimulants is accompanied by a depression of Vt, (Sasaki et al., 1987), which was not the case in this instance. In addition, Noble and colleagues (1987) noted a transient Te shortening in CO_2 exposed infants and a similar observation was made by Haddad and colleagues (1982) in dogs with sleep-related hypoxia. The effect of shortened Te was reflected by an increased respiratory duty cycle in these animals. However, respiratory duty cycle was not altered by smoke exposure at any other concentration. Respiratory duty cycle elevation also has been attributed to neuromuscular fatigue (Sadoul et al., 1985). Chanda and colleagues (1984) found that respiratory duty cycle was not modified in volunteer subjects challenged with methacholine.

Hamelmann and colleagues (1997) demonstrated penh to be a valid, noninvasive indicator of bronchoconstriction induced by methacholine in mice. Although an indirect measure of AR, penh correlated well with intraplueral pressure and direct measures of pulmonary resistance. Furthermore, penh was shown to be independent of changes in *f*, and a limited proportional dependence of penh upon Vt was demonstrated. A 2.5 fold increase in Vt produced a less than 2 fold increase in penh, leading to the conclusion that much larger increases (up to 11 fold, Hamelmann et al., 1997, Goldsmith et al., 1999) in penh induced by methacholine could not be accounted for by change in Vt. The moderate elevations of penh we observed are of the magnitude that could be attributed to CO_2 induced Vt elevation . However, the onset penh elevation in group 5, 5F, and 10F animals during the post-exposure period, when CO_2 was removed from the plethysmograph, suggested a different mechanism for the elevation of penh. Likewise, elevated penh persisted into the post-exposure period in group 10, 100 and 100F animals. Differences in penh between group 10 and 10F animals, particularly during exposure, indicate that bronchoconstrictive action could be attributed to constituents in the particulate fraction of the smoke. However the responses observed in group 100 and 100F animals show that at higher concentrations the ability of B2-ACM smoke to elicit a BC response does not reside solely in the particulate fraction. Using a nose-only, flow plethysmograph Pauluhn (1996) demonstrated that when compared to changes in f and Vt, elevation of the dimensionless

construct FDP was more sensitive to non-specific AR produced by acetylcholine challenge in guinea pigs. In addition, change in FDP was shown to be more conspicuous in animals which had been previously sensitized to BC type responses by phenyl(mono)isocyanate. In this and subsequent work (Pauluhn, 1997) suggested FDP was less responsive to breathing pattern changes characteristic of SI stimulation and could used to distinguish between SI and BC stimulation, particularly those that are immunologically mediated. In the present investigation, FDP was remarkably different from control values in group 10, 100, and 100F animals during and shortly after the exposure period; whereas many of the other parameters examined were significantly different from control values at lower concentrations.

Collectively, our data point toward B2-ACM smoke eliciting a BC response. However, the data also suggest that other types of responses cannot be ruled out. It is evident that stimulation of ventilation by CO_2 in the smoke was a confounding factor. Given the chemical complexity of the smoke it is likely that different types of AR responses were elicited simultaneously by different constituents of the smoke. This might explain the increase in the variability of given measurements. The erratic, minute by minute, fluctuations observed in respiratory pattern were, in part, due to conflicting responses elicited by simultaneous stimulation of various types of receptors. The responses observed that were characteristic of bronchoconstriction were markedly lower than those elicited by well known BC agents. Although this might suggest that B2-ACM smoke is not a powerful AR stimulant it could well be that a counteracting effects of stimulation of other receptors produced a dampening effect. A dampening of specific responses would explain the rather moderate changes in breathing pattern that accompanied the convulsions in some animals. The findings also suggest that filtration of the particulate material from B2-ACM smoke moderates but does not eliminate AR responses, suggesting that constituents capable if eliciting AR responses reside in both the particulate and gas phases of the smoke. Despite the complex and often confounding attributes the AR responses elicited by B2-ACM smoke, it is apparent that a dose response relationship exists between B2-ACM smoke and AR. Furthermore, the data indicate that there is a threshold below which AR responses are not elicited by smoke exposure. However, this threshold effect may be limited to smoke exposures of 30 minutes or less.

CONCLUSIONS

Short exposure to dilute B2-ACM smoke causes stimulation of various receptors located throughout the respiratory tract resulting in asthma-like changes of ventilation. The breathing pattern and breath structure changes we observed can be incapacitating and can be lethal if of sufficient severity and duration. These responses can be induced by B2-ACM smoke concentrations that are not visible, hence victims may not have adequate warning of exposure. Furthermore, AR responses have been shown to occur at smoke concentrations far below those that produce pulmonary dysfunction that persists after cessation of exposures. The ability to elicit AR responses is not limited to the smoke particulate fraction although smoke filtration may moderate some types of AR response. Thus, personal protective equipment (PPE) that eliminates only particles from hazardous atmospheres may not provide adequate protection for emergency response personnel. Personnel without PPE of any type are at greater risk.

Although the mechanisms by which AR, AHR, and AHS responses are related are not well understood it is clear that relationships among these responses exist. In this investigation B2-ACM was clearly shown to elicit an AR response, hence the possibility that it also may elicit AHR and AHS responses must be considered. It is possible that B2-ACM smoke exposure, even if it does not elicit an immediate response, can lead to heightened susceptibility and increased severity of response from subsequent exposure to smoke or other airborne contaminants capable of eliciting airway reactive disorders. This possibility points to another category of health risk associated with exposure to B2-ACM smoke that has not yet been verified experimentally.

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TABLE 1. GLOSSARY OF BREATHING PARAMETERS AND UNITS

TERM	DEFINITION	UNITS
f	breathing frequency	breaths/min.
Vt	tidal volume	mL
Ve	minute ventilation (*f* x Vt)	mL/min.
Rt	relaxation time	sec.
Ti	inspiratory time	sec.
Te	expiratory time	sec.
[Ti/(Ti+Te)]	Respiratory duty cycle	fractional
PIF	peak inspiratory flow	mL/sec.
PEF	peak expiratory flow	mL/sec.
penh	enhanced pause (Te/Rt –1)(PEP/PIP)	non dimensional
FDP	flow derived parameter (PEF)[(Te + Ti)/Vt]	non dimensional

TABLE 2. COMBUSTION EFFICIENCY AND NOMINAL CONCENTRATION

Mass loaded (g)	Mass burned (g)	Duration (min.)	Pyrolysis rate (g/min)	% burned	CO2:CO mass ratio	Flow * volume m^3	Nominal Concentration (g/m^3)
2.03	0.55	2.53	0.19	24.1	2.8 : 1	0.257	0.80
2.01	0.53	2.23	0.24	26.4	2.5 : 1	0.262	0.77
5.00	1.70	2.73	0.62	34.0	1.8: 1	0.252	2.46
5.02	1.69	2.76	0.61	33.6	2.0 : 1	0.257	2.45
10.01	2.65	2.78	0.95	26.5	1.9 : 1	0.254	3.84
9.98	2.61	2.92	0.89	26.1	2.1: 1	0.274	3.78
100.02	20.84	7.73	2.70	20.8	2.0 : 1	0.741	28.12
100.00	20.61	8.03	2.57	20.6	2.3 : 1	0.718	28.70

* If the volume of the flow through the furnace was less than the reservoir volume, nominal concentration was adjusted by dilution to reservoir volume (0.69 m^3).

TABLE 3. EXPOSURE CONCENTRATIONS

Exposure Group	Aerosol (g/m^3)	CO_2 (g/m^3)	CO (g/m^3)	NO_2 (g/m^3)	SO_2 (g/m^3)	Total* (g/m^3)
Control	0	0	0	0	0	0
2	0.15	0.42	0.15	0.01	0.02	0.75
2F	0	0.45	0.18	0.01	0.03	0.67
5	0.26	1.05	0.57	0.06	0.06	2.00
5F	0	1.18	0.60	0.04	0.08	1.90
10	0.48	1.96	1.03	0.09	0.12	3.59
10F	0	2.03	0.96	0.10	0.12	3.21
100	2.2	9.23	4.73	0.43	1.16	17.75
100F	0	10.13	4.47	0.38	1.10	16.08

TABLE 4. BASELINE VENTILATORY PARAMETERS

Group Parameter (n=4)	Control	2g	2g-F	5g	5g-F	10g	10g-F	100g	100g-F
BW	439 (8.2)	435 (10.7)	437 (21.0)	436 (11.1)	410 (17.4)	410 g (10.7)	422 g (9.6)	430 (8.2)	420 (8.9)
f	117.6 (6.23)	113.0 (9.62)	103.2 (10.84)	114.3 (20.66)	116.6 (19.54)	114.2 (10.42)	124.8 (7.64)	116.7 (11.51)	128.8 (5.23)
Vt	1.8 (0.14)	2.0 (0.14)	2.1 (0.15)	2.2 (0.20)	1.9 (0.18)	1.8 (0.09)	1.7 (0.08)	1.9 (0.09)	1.7 (0.04)
Ve	196.8 (17.51)	208.2 (9.21)	198.3 (5.68)	214.5 (13.05)	213.4 (14.22)	181.2 (10.04)	212.3 (20.99)	208.0 (16.24)	208.5 (4.07)
Ti	0.206 (0.0106)	0.225 (0.0163)	0.237 (0.0185)	0.233 (0.0299)	0.227 (0.0292)	0.227 (0.0283)	0.197 (0.0145)	0.219 (0.0216)	0.19 (0.0044)
Te	0.346 (.0014)	0.352 (0.0280)	0.400 (0.0385)	0.398 (0.0576)	0.376 (0.0386)	0.381 (0.0293)	0.299 (0.0108)	0.342 (0.0289)	0.306 (0.0110)
PIF	13.7 (1.05)	13.7 (0.67)	14.0 (0.60)	16.6 (0.84)	17.2 (1.07)	12.9 (1.22)	15.4 (1.71)	14.0 1.08)	14.1 (0.14)
PEF	7.7 (0.83)	8.2 (0.33)	7.7 (0.30)	9.6 (0.48)	9.9 (0.46)	7.1 (0.25)	8.8 (0.78)	8.2 (1.97)	7.9 (0.22)
Rt	0.236 (0.0083)	0.236 (0.0153)	0.267 (0.0289)	0.258 (0.0284)	0.249 (0.02489)	0.256 (0.0200)	0.202 (0.0082)	0.232 (0.0168)	0.215 (0.0072)
penh	0.271 (0.0155)	0.309 (0.0172)	0.292 (0.2252)	.318 (0.0382)	0.304 (0.0159)	0.282 (0.0310)	0.264 (0.0042)	0.284 (0.0216)	0.243 (0.0039)
FDP	2.34 (0.062)	2.38 (0.035)	2.34 (0.053)	2.43 (0.036)	2.38 (0.034)	2.41 (0.059)	2.40 (0.015)	2.34 (0.046)	2.27 (0.025)

Baseline respiratory parameters were determined as mean of all breaths recorded for a 20-minute period immediately preceding exposure in each of 4 animals per exposure group. Values are mean and (standard error). See TABLE 1 for parameter definitions and units.

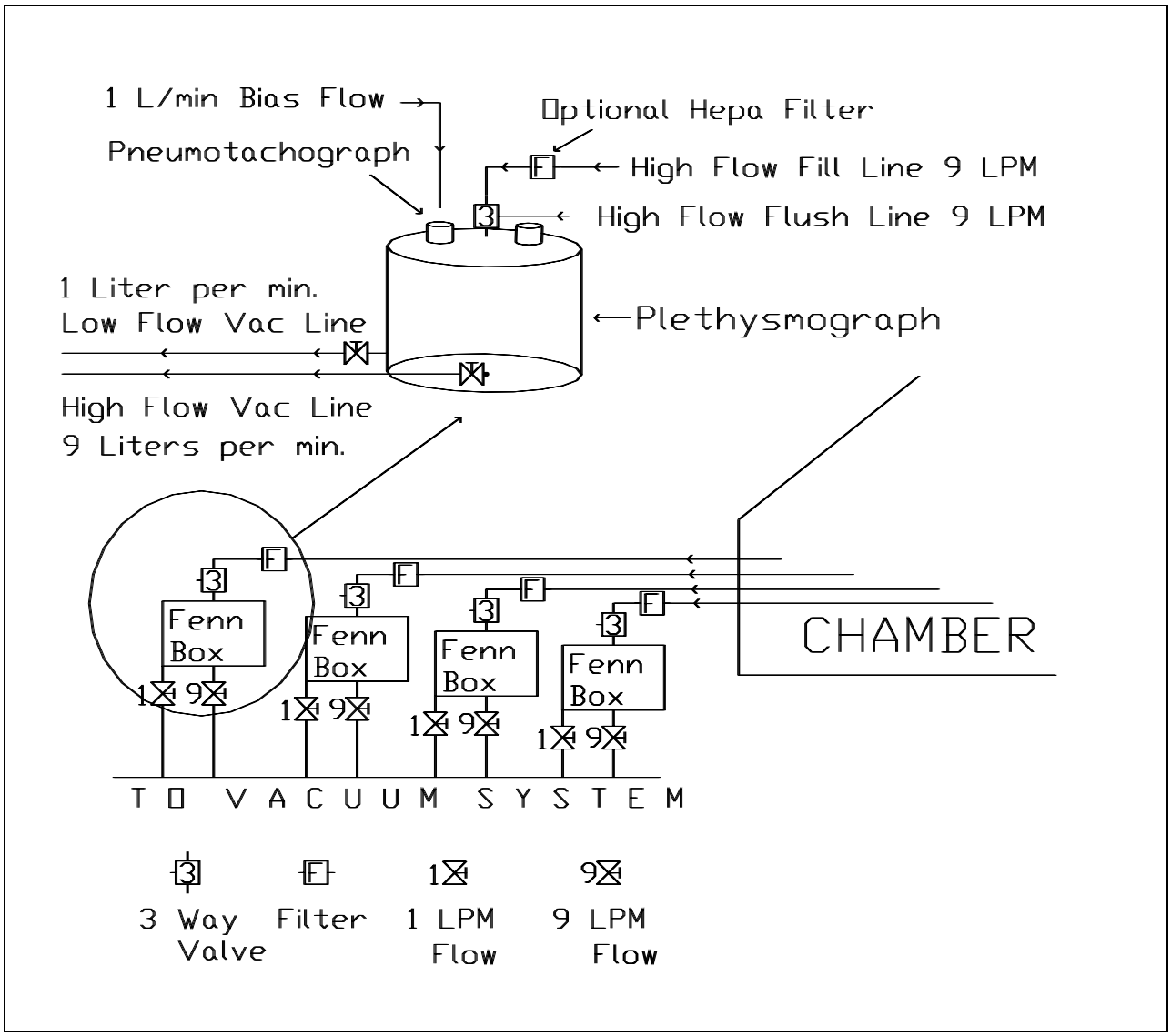


Figure 1. Schematic of the plethysmograph(s)/exposure system.

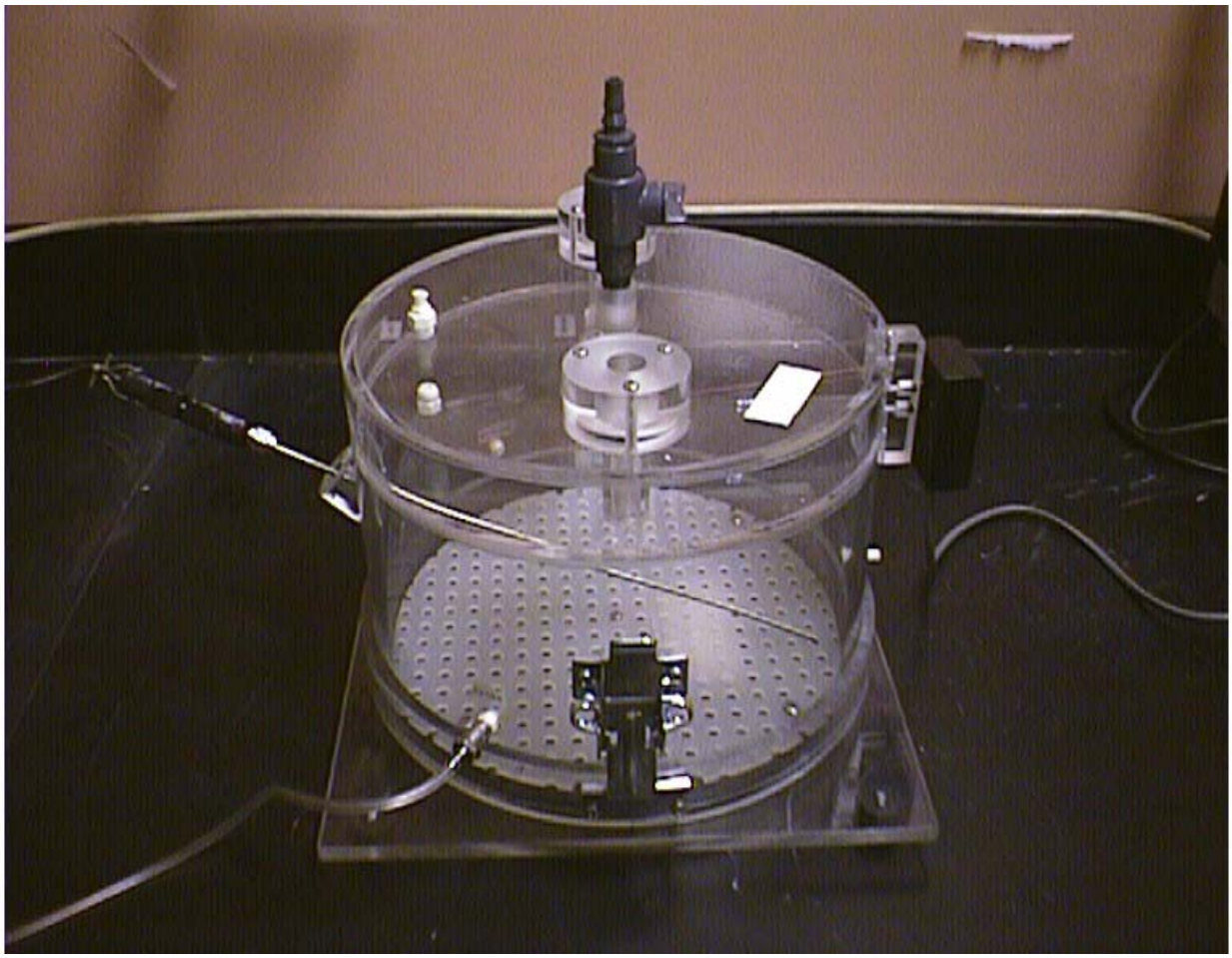


Figure 2. Photograph of a bias flow, barometric plethysmograph

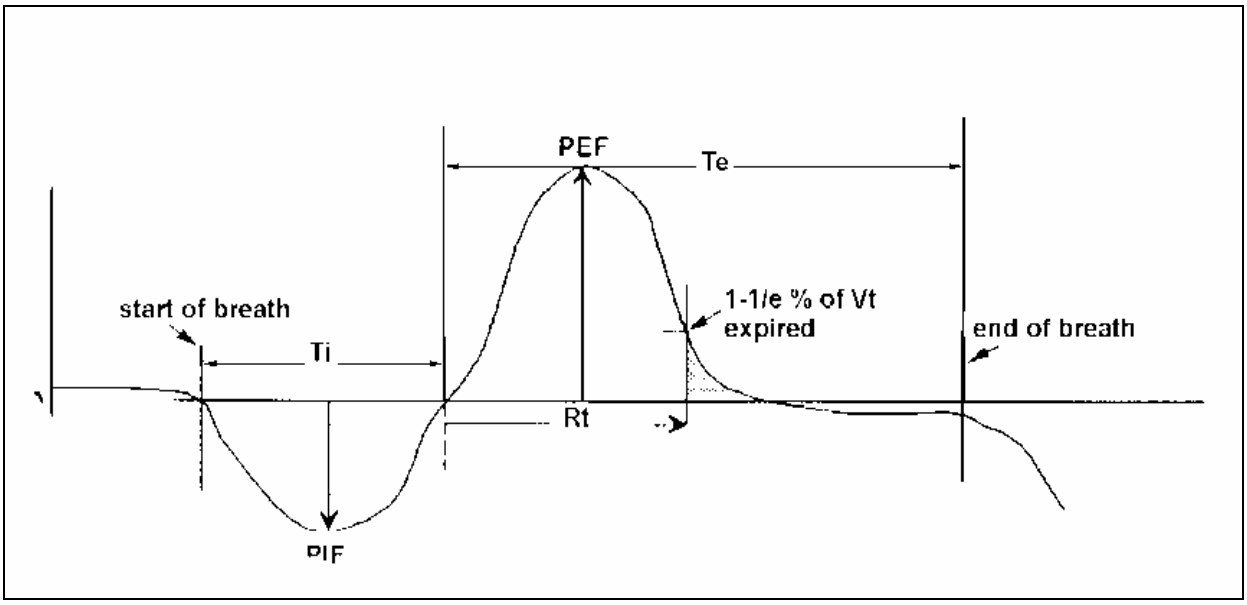


Figure 3. Diagram of a typical breath.

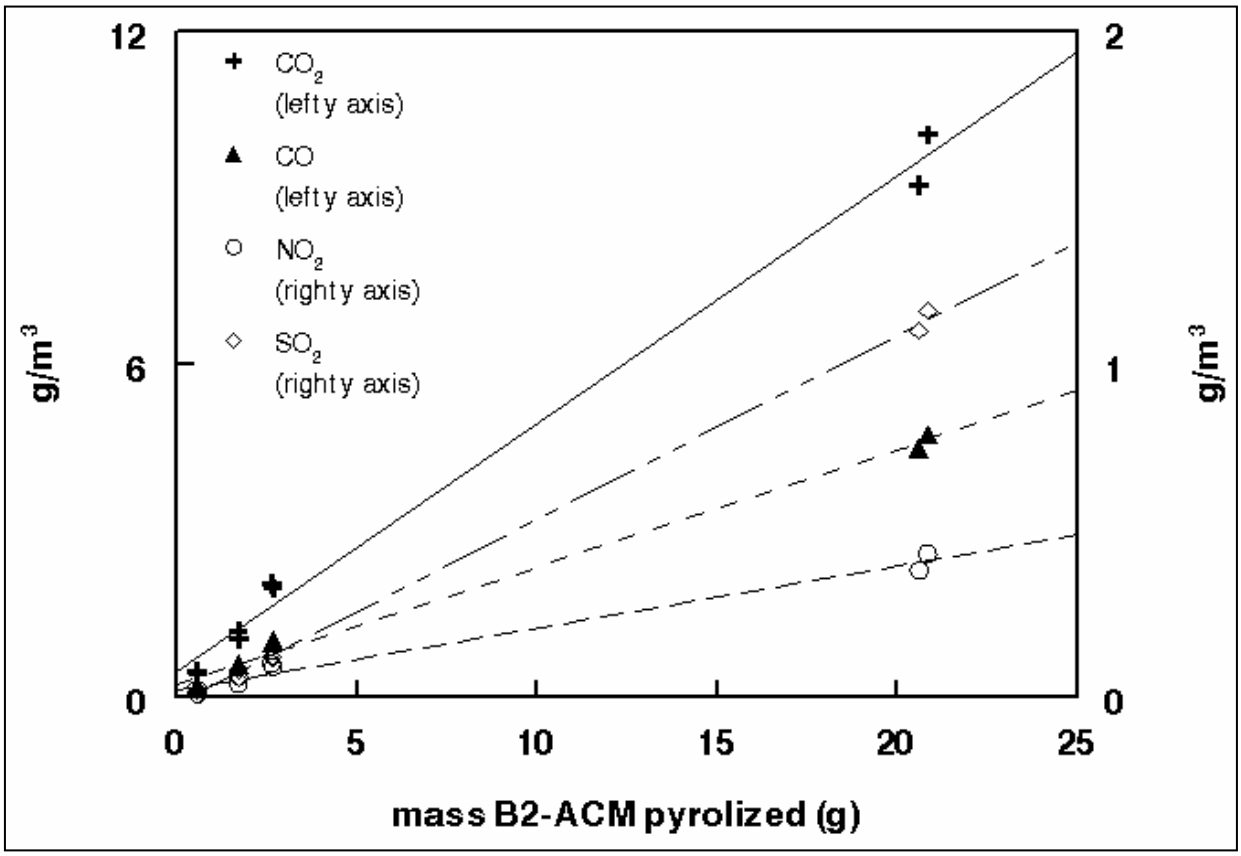


Figure 4. Concentrations of CO_2, CO, NO_2, and SO_2 as a function of the mass of B2-ACM pyrolized.

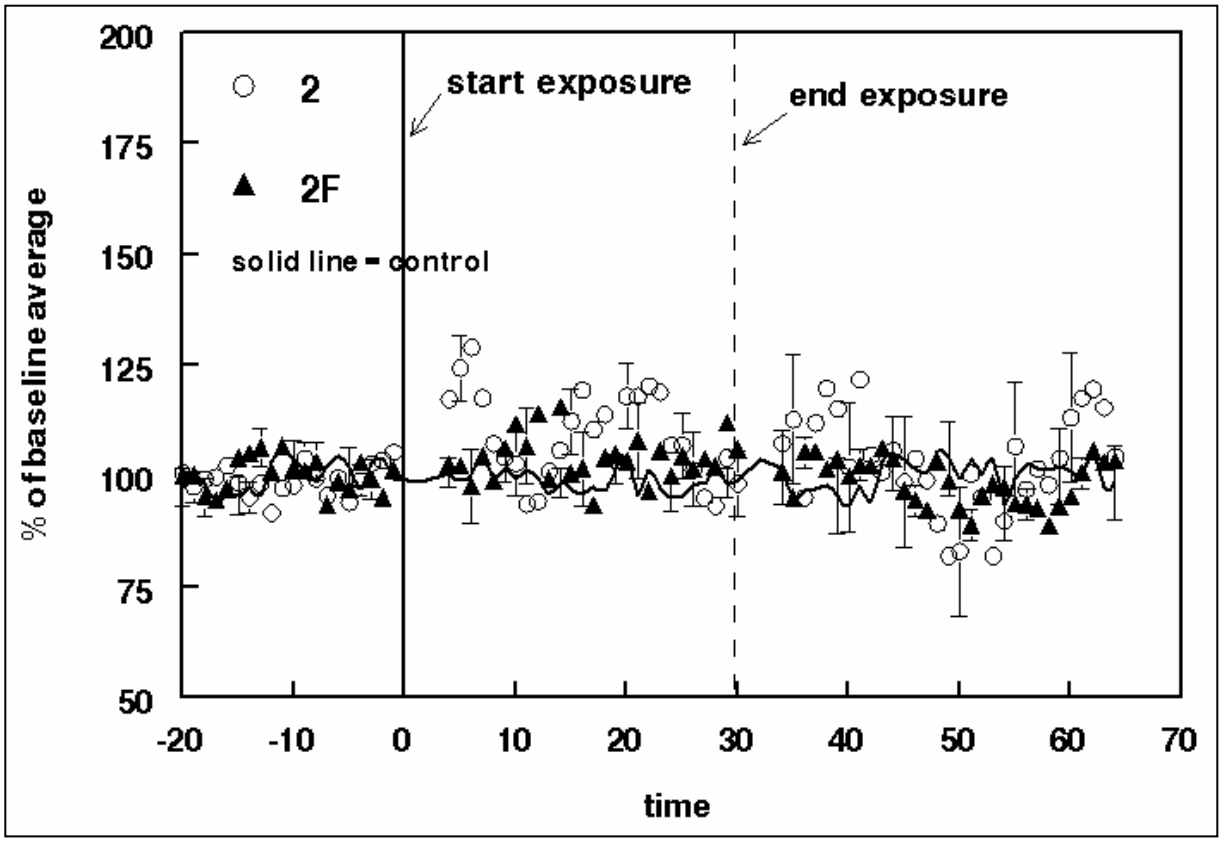


Figure 5. Breathing frequency, exposure groups 2 and 2F.

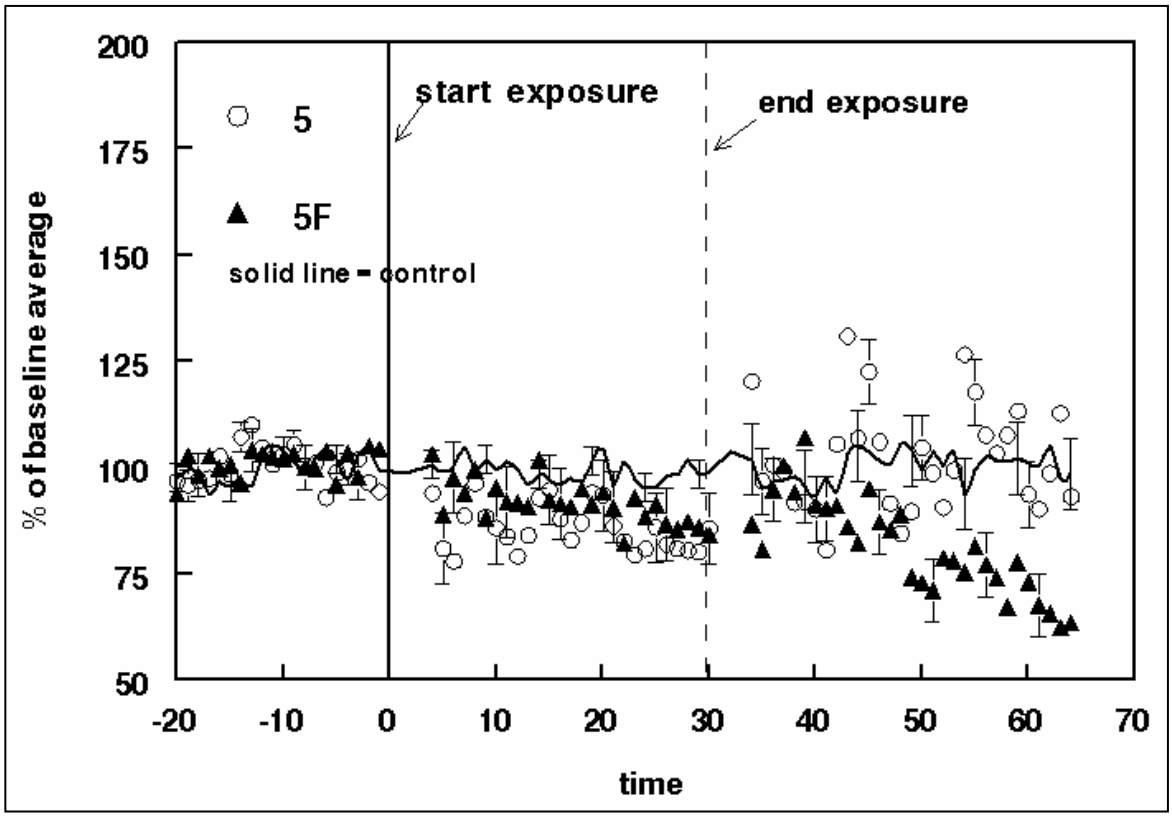


Figure 6. Breathing frequency, exposure groups 5 and 5F.

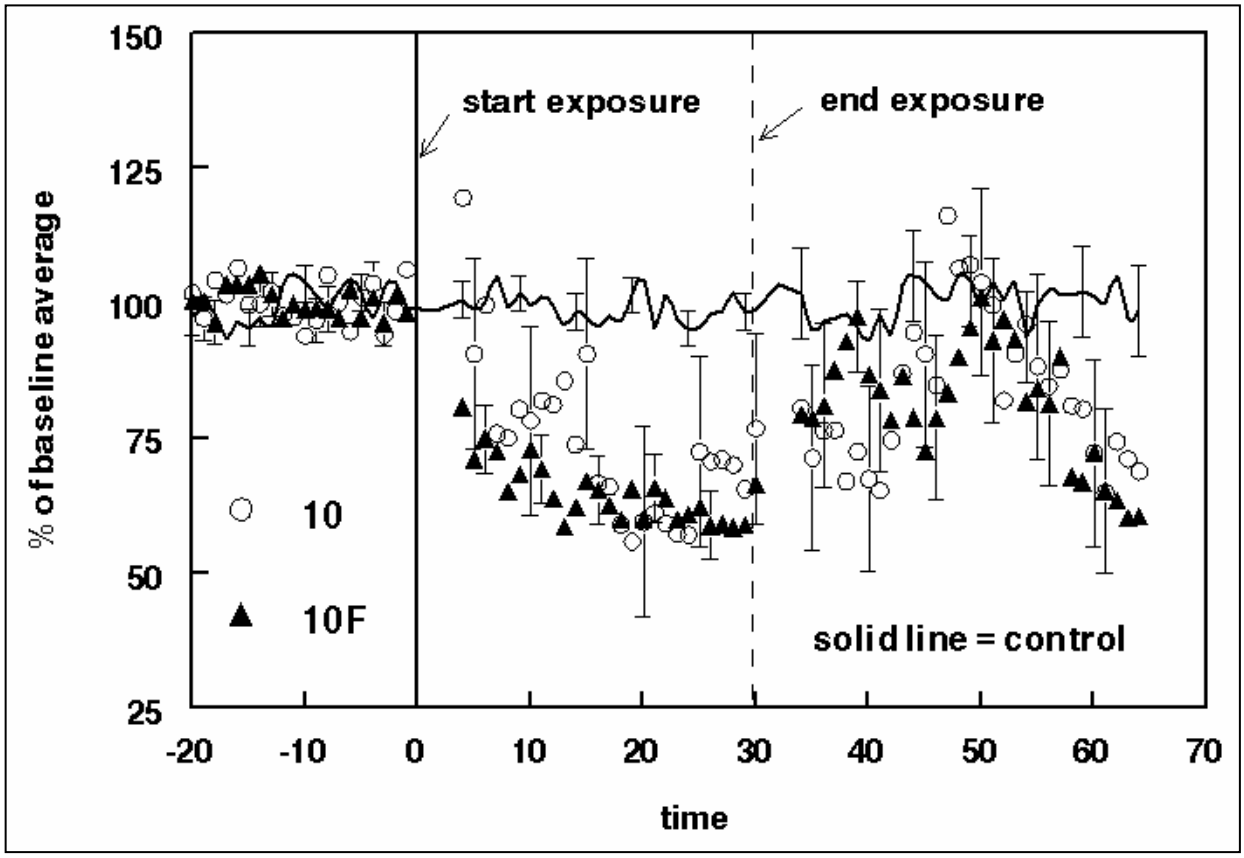


Figure 7. Breathing frequency, exposure groups 10 and 10F.

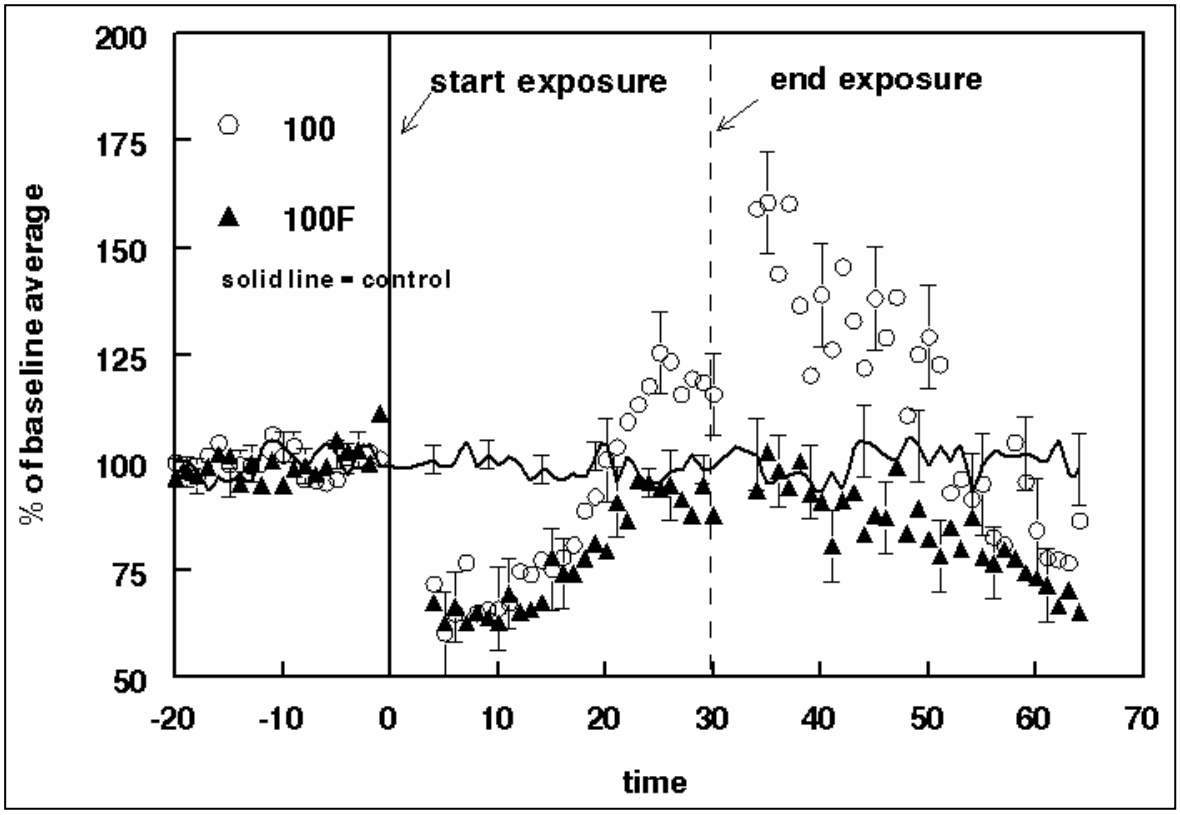


Figure 8. Breathing frequency, exposure groups 100 and 100F.

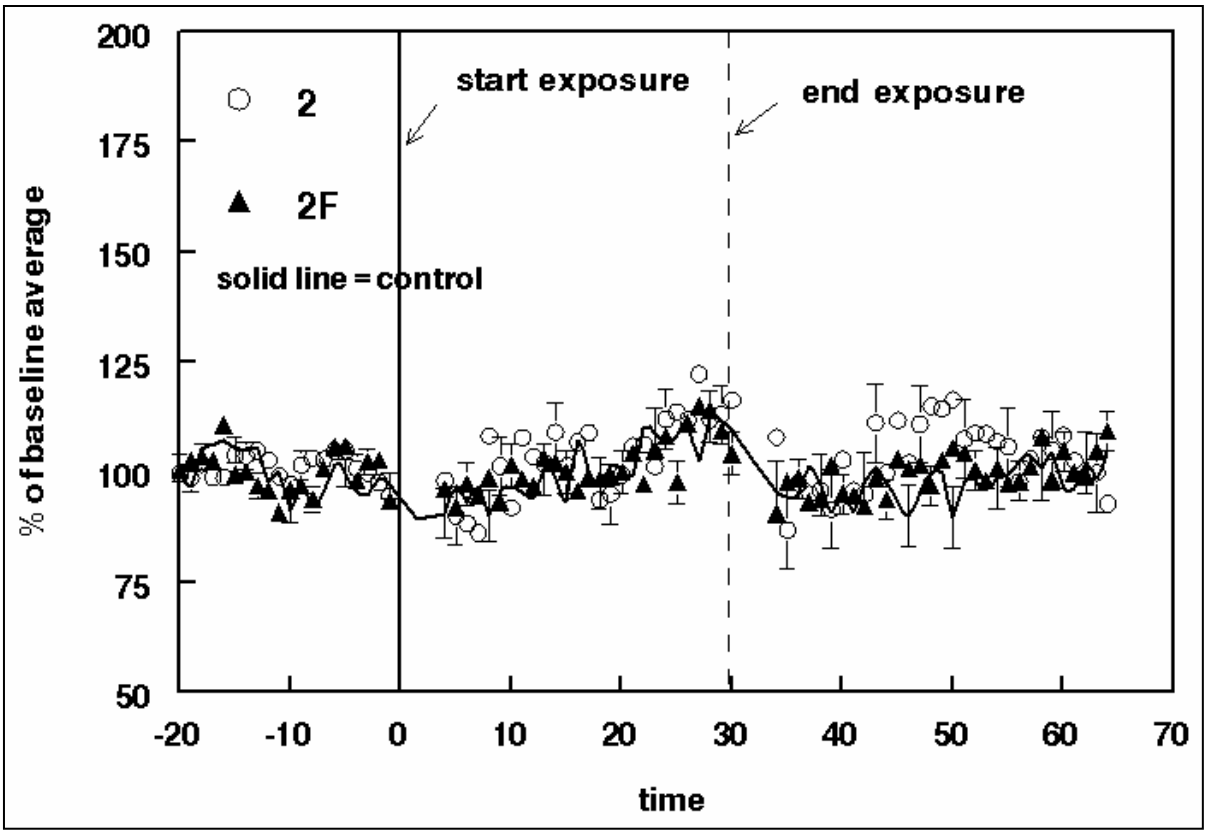


Figure 9. Tidal volume, exposure groups 2 and 2F.

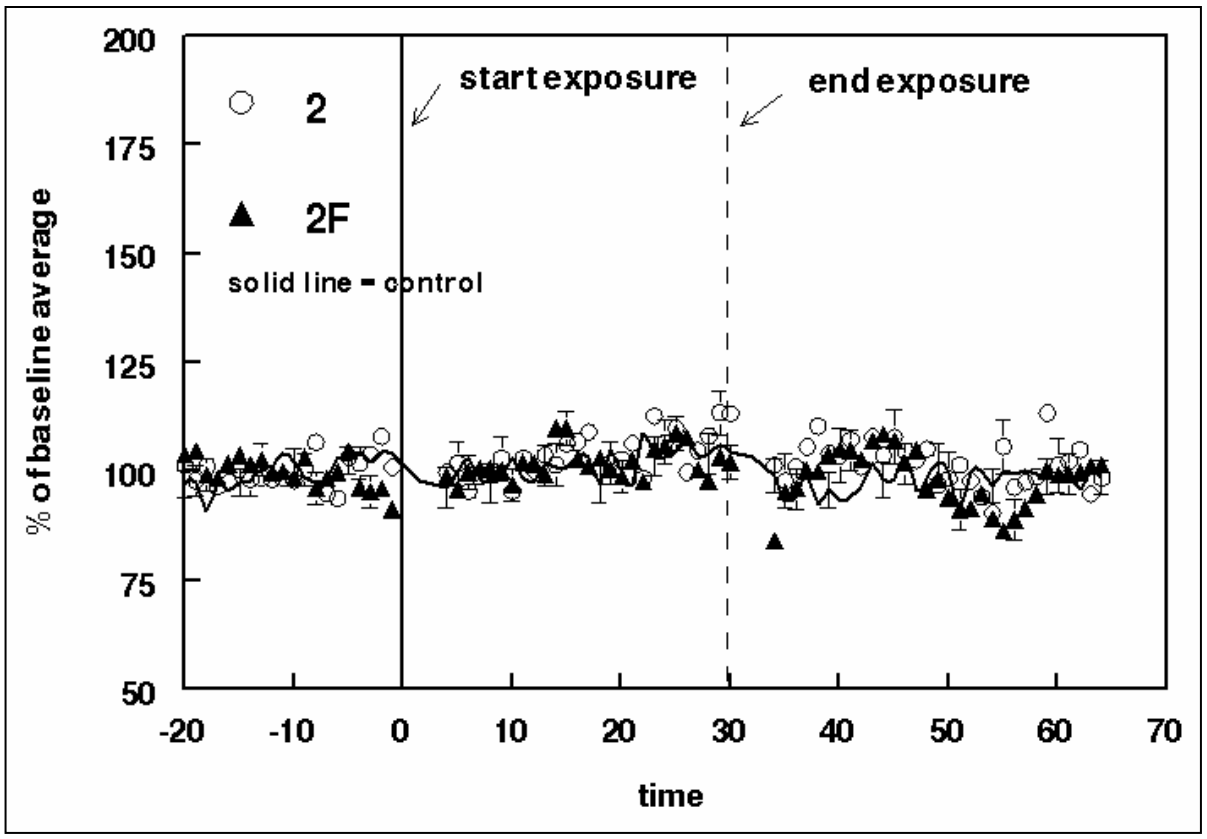


Figure 10. Minute ventilation, exposure groups 2 and 2F.

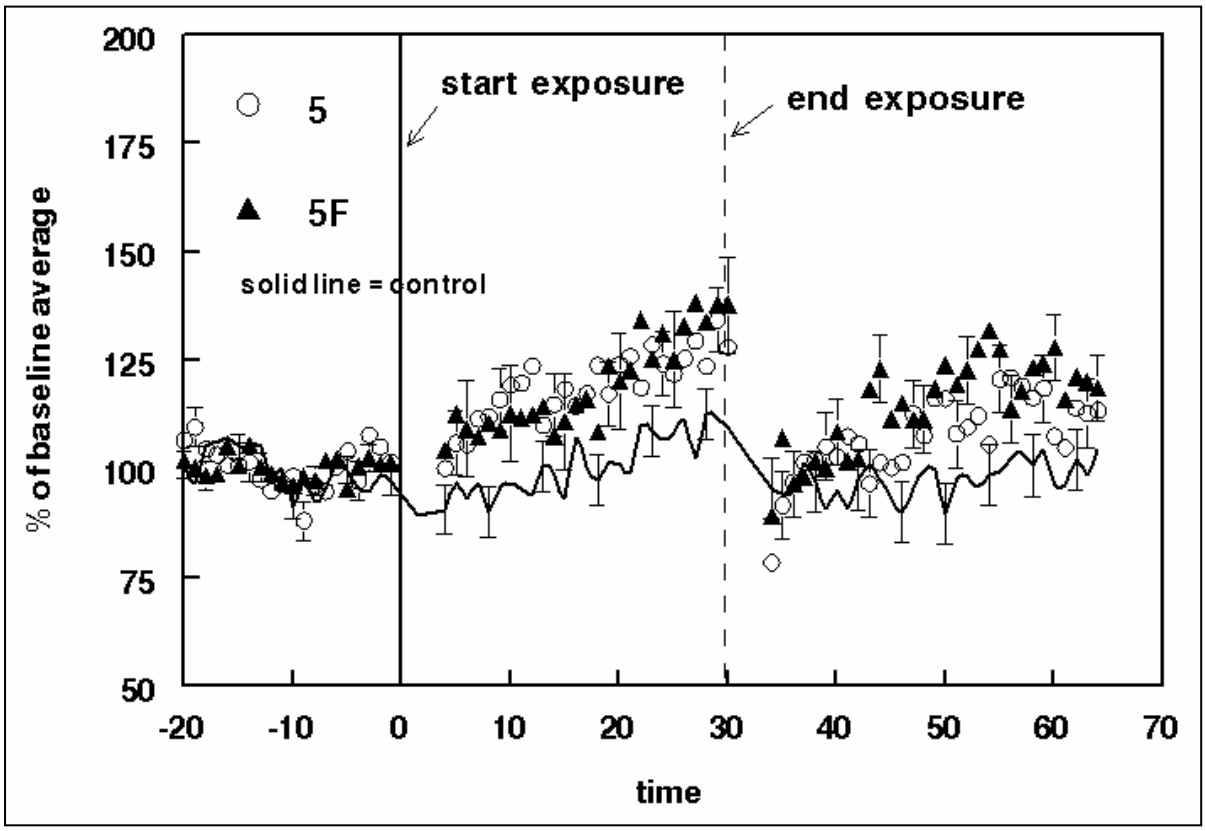


Figure 11. Tidal volume, exposure groups 5 and 5F.

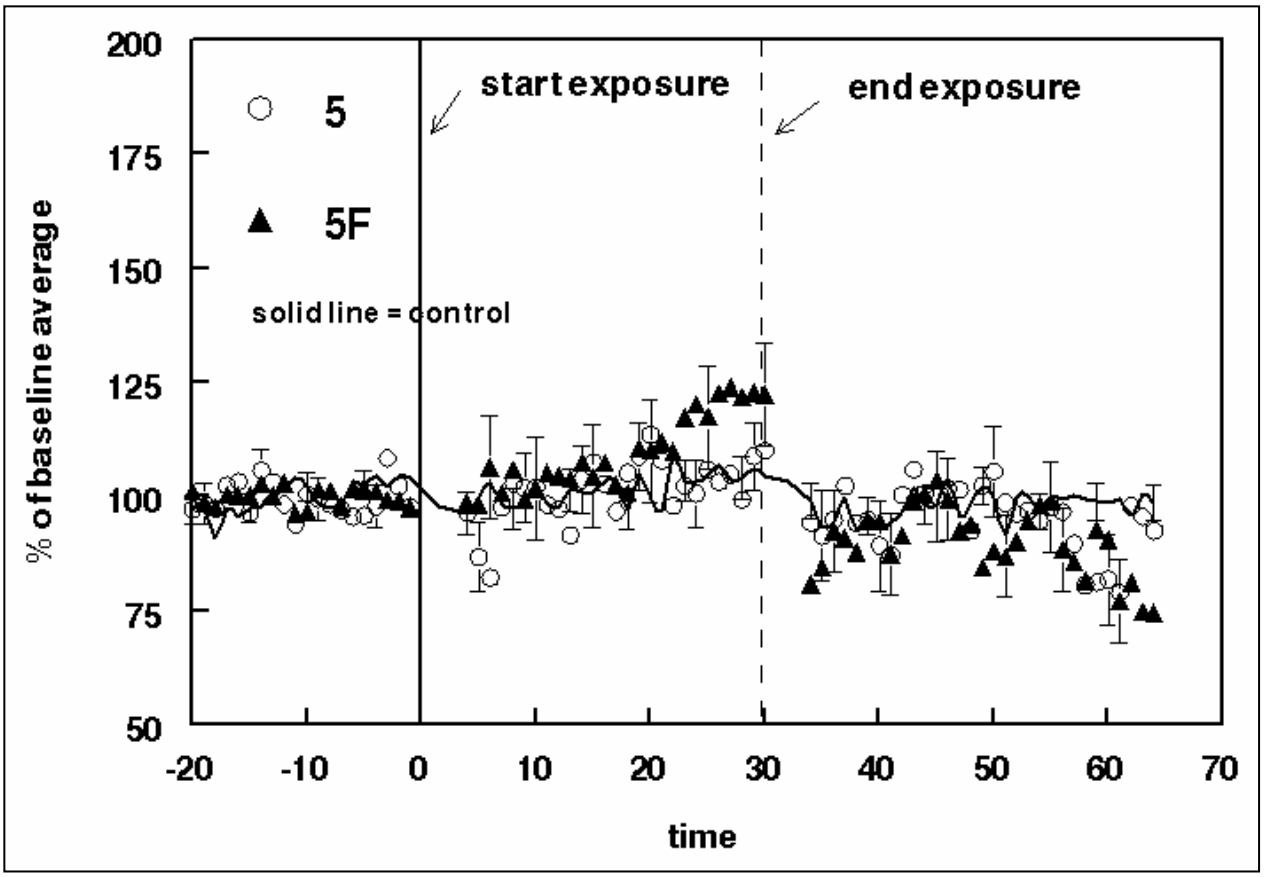


Figure 12. Minute ventilation, exposure groups 5 and 5F.

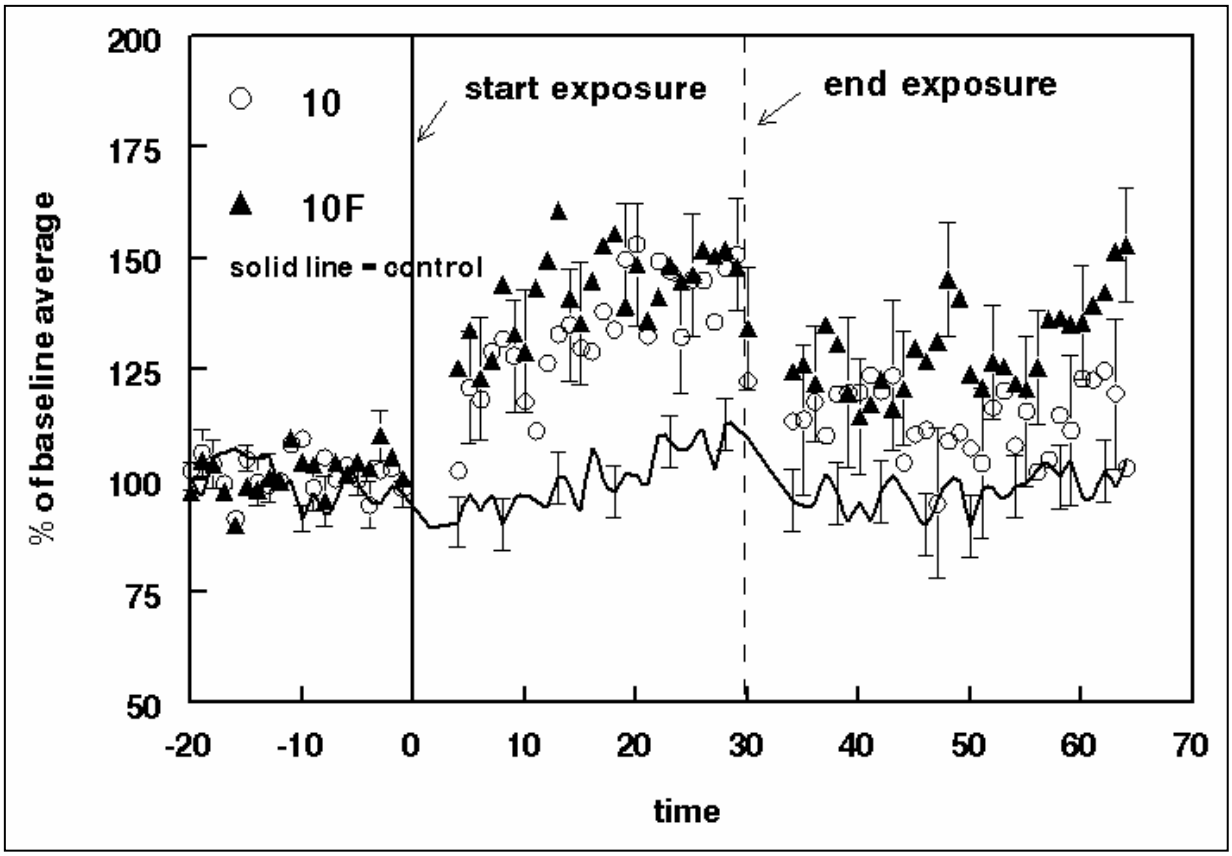


Figure 13. Tidal volume, exposure groups 10 and 10F.

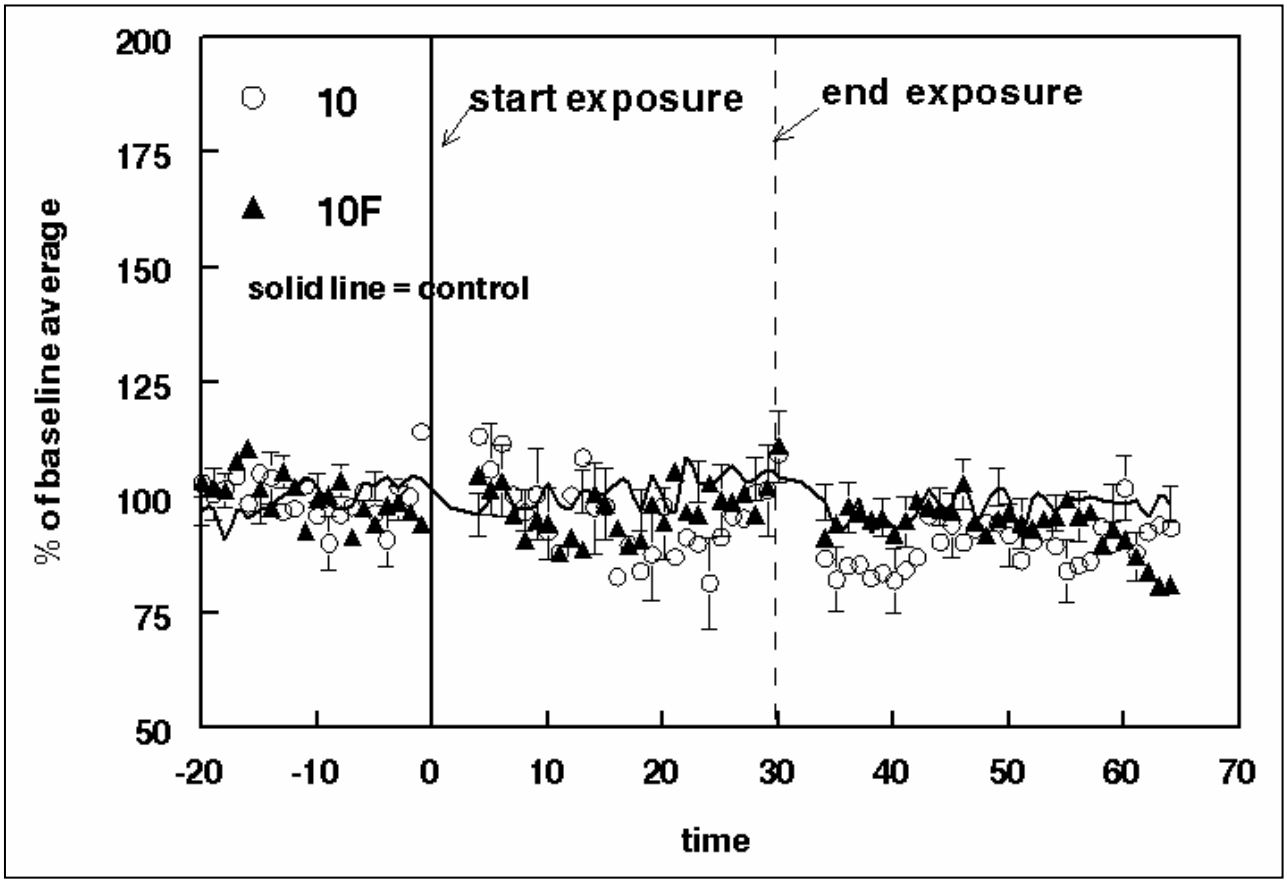


Figure 14. Minute ventilation, exposure groups 10 and 10F.

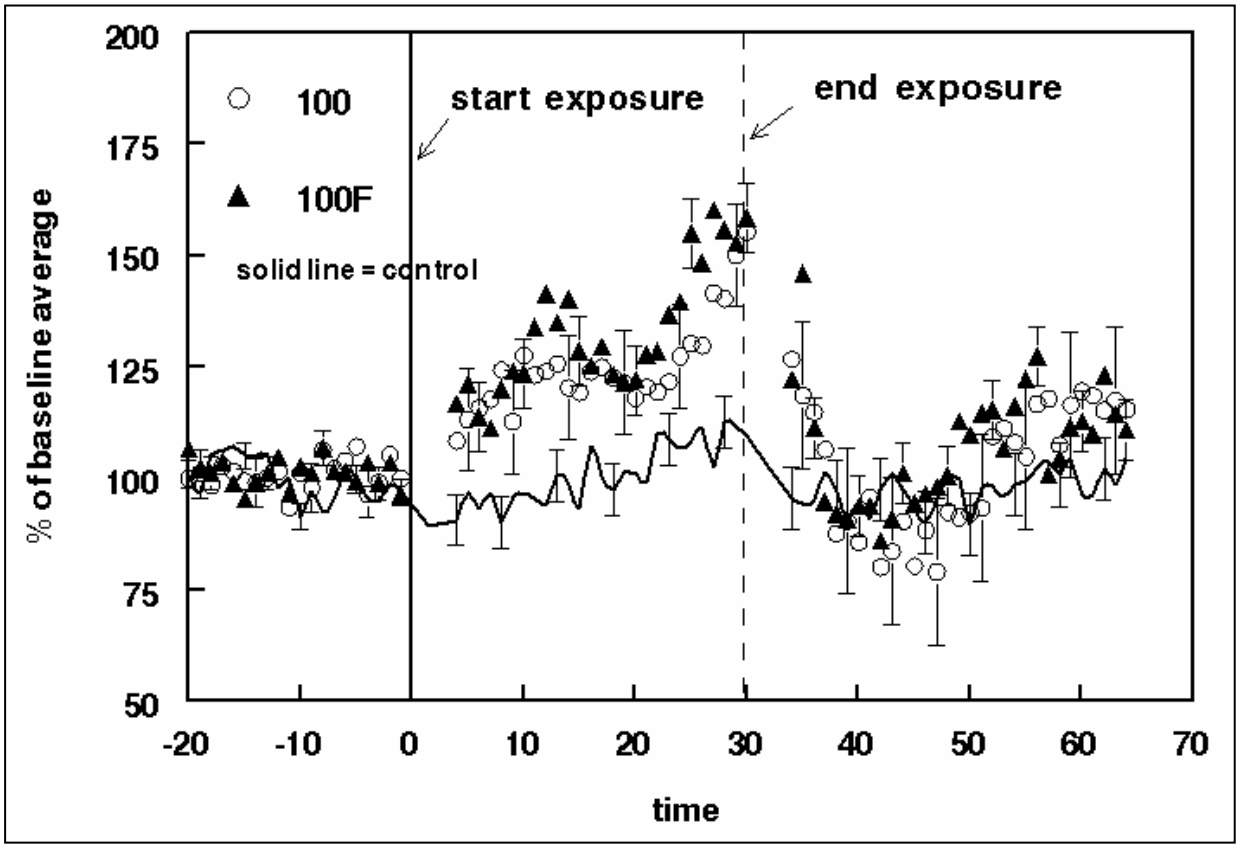


Figure 15. Tidal volume, exposure groups 100 and 100F.

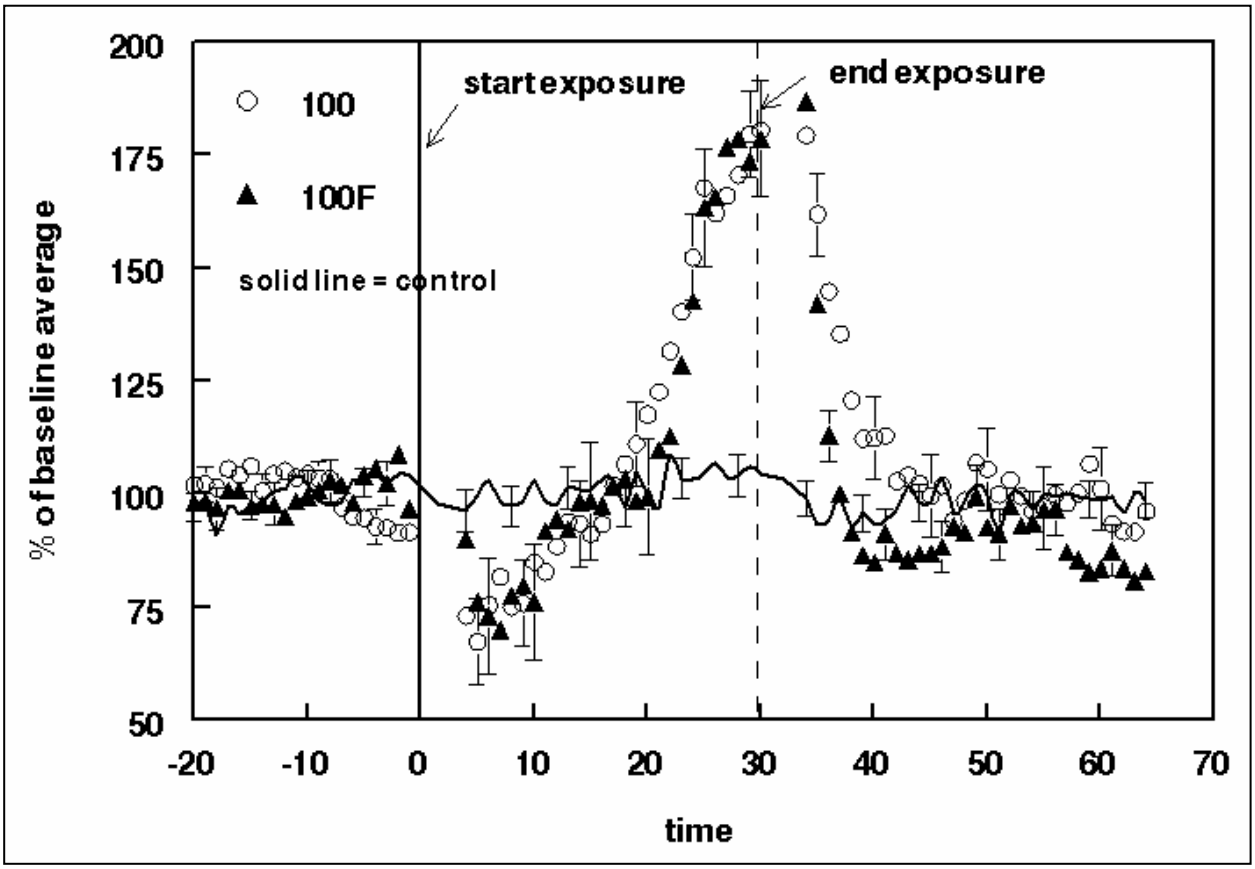


Figure 16. Minute ventilation, exposure groups 100 and 100F.

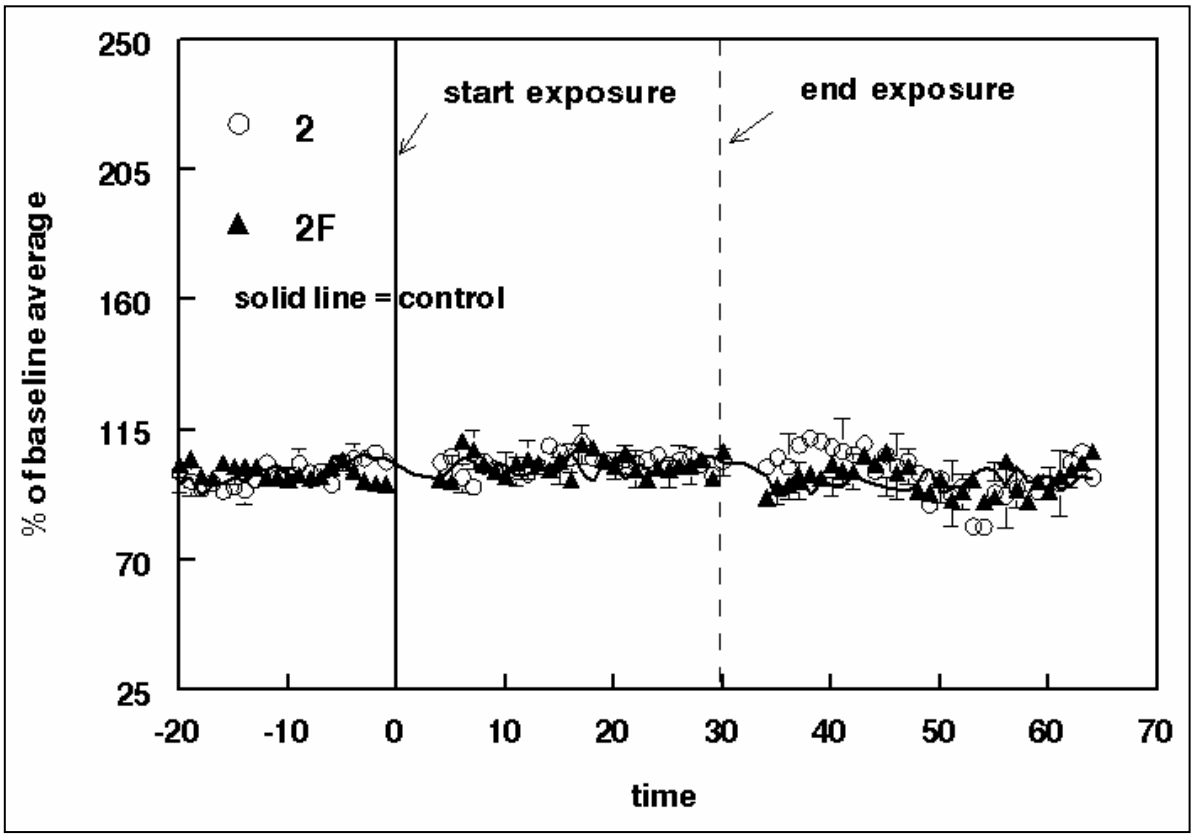


Figure 17. Peak inspiratory flow, exposure groups 2 and 2F.

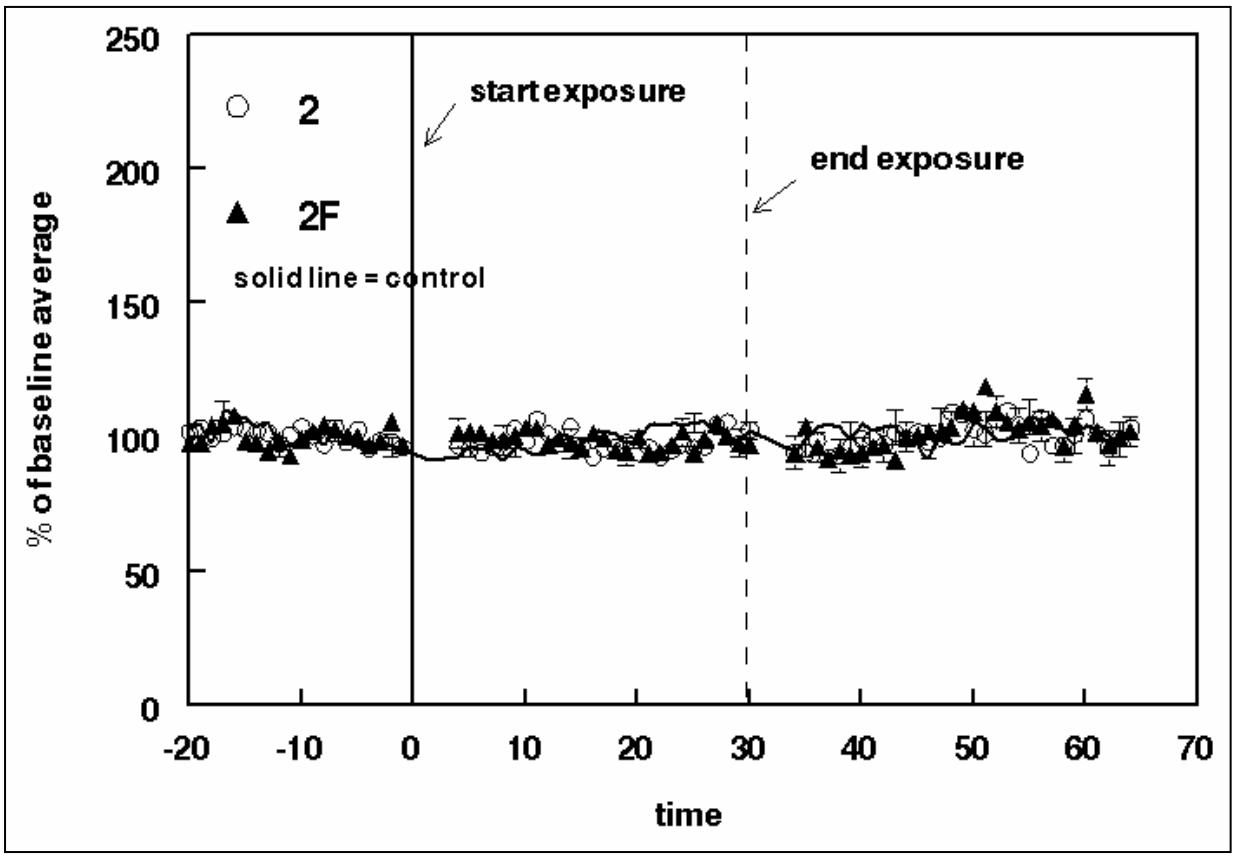


Figure 18. Inspiratory time, exposure groups 2 and 2F.

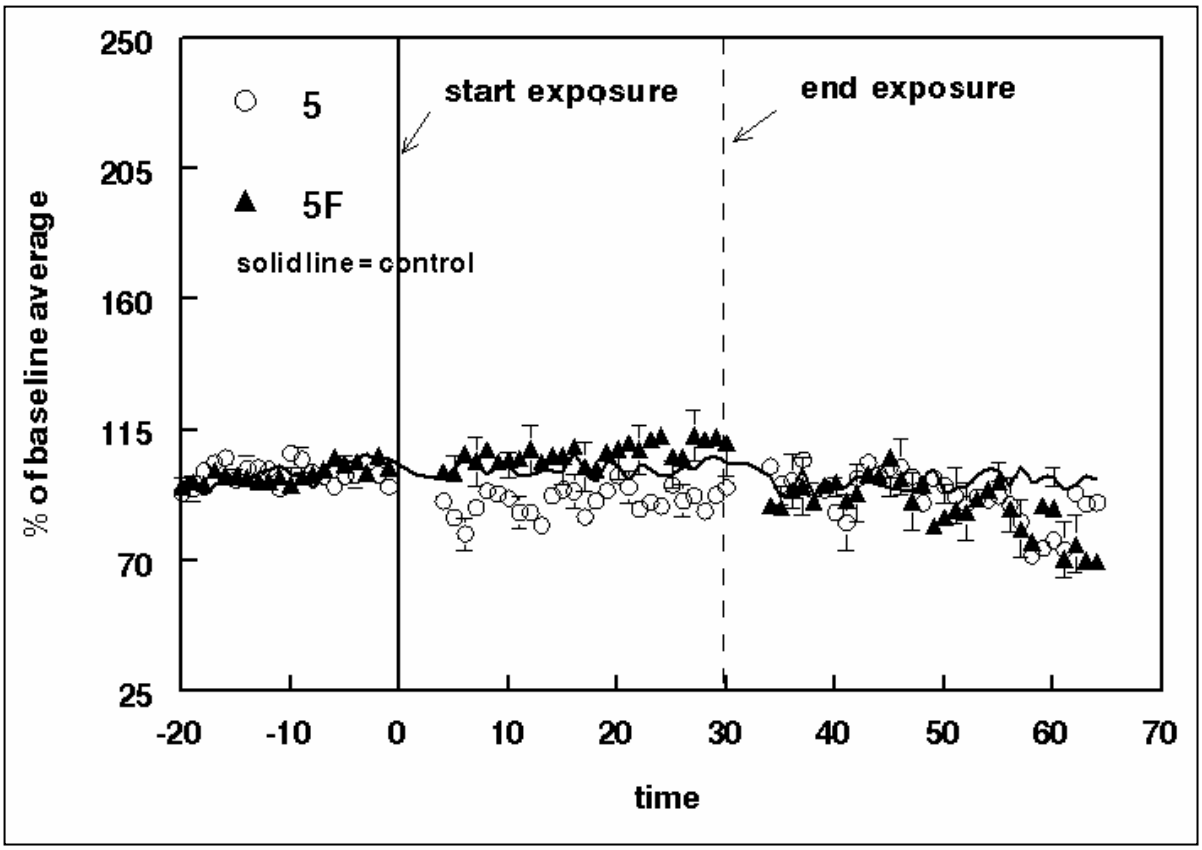
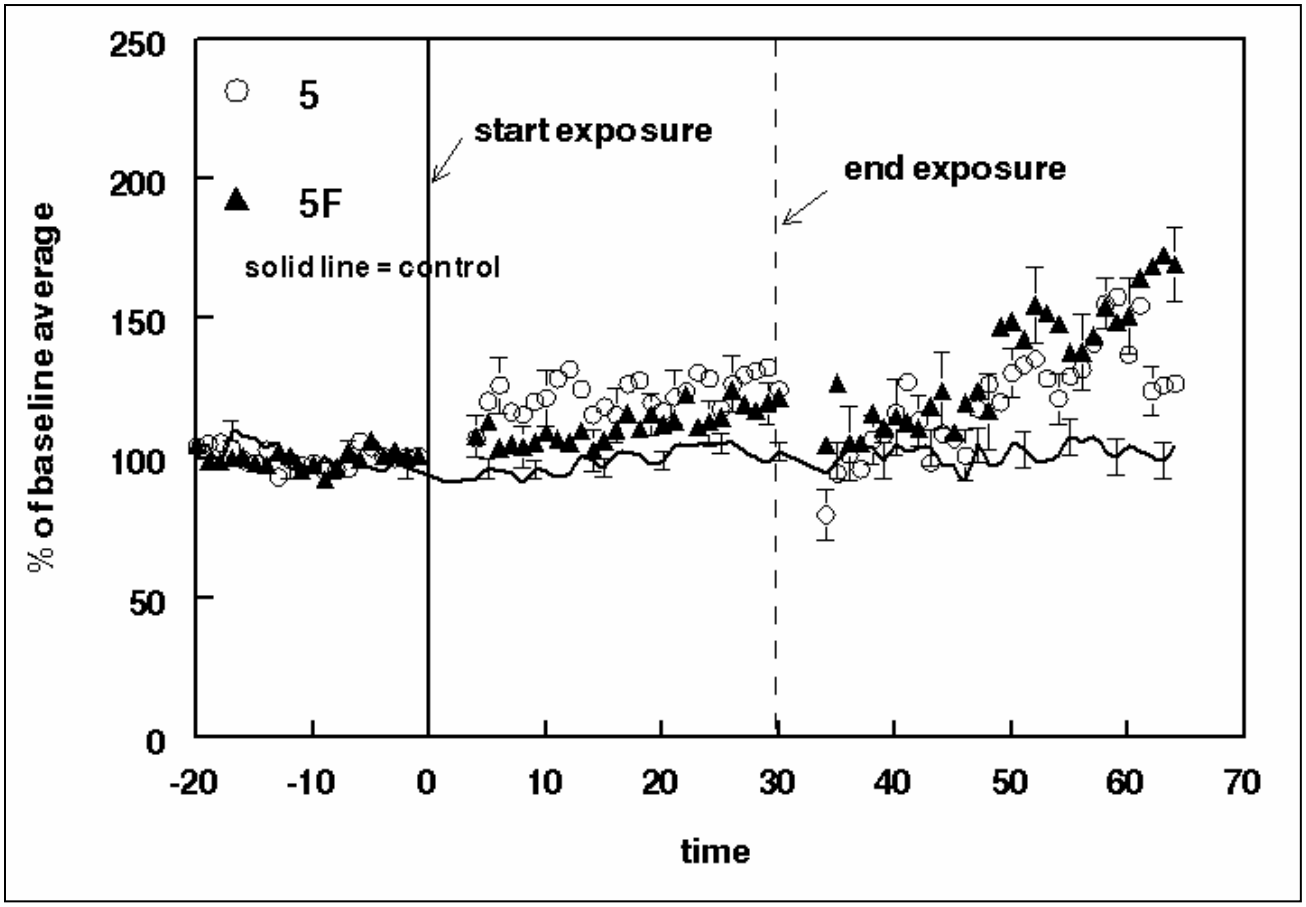


Figure 19. Peak inspiratory flow, exposure groups 5 and 5F.



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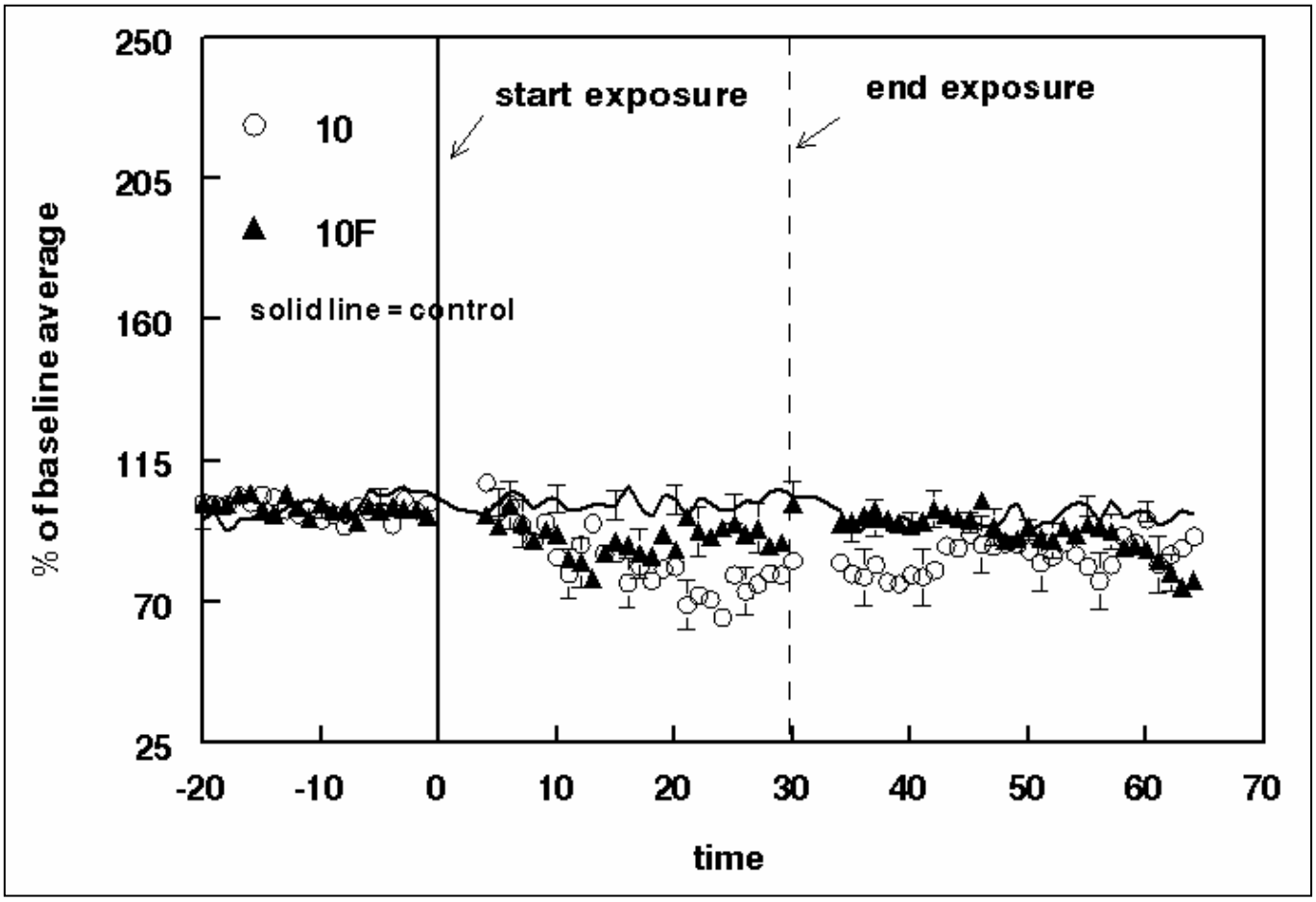


Figure 21. Peak inspiratory flow, exposure groups 10 and 10F.

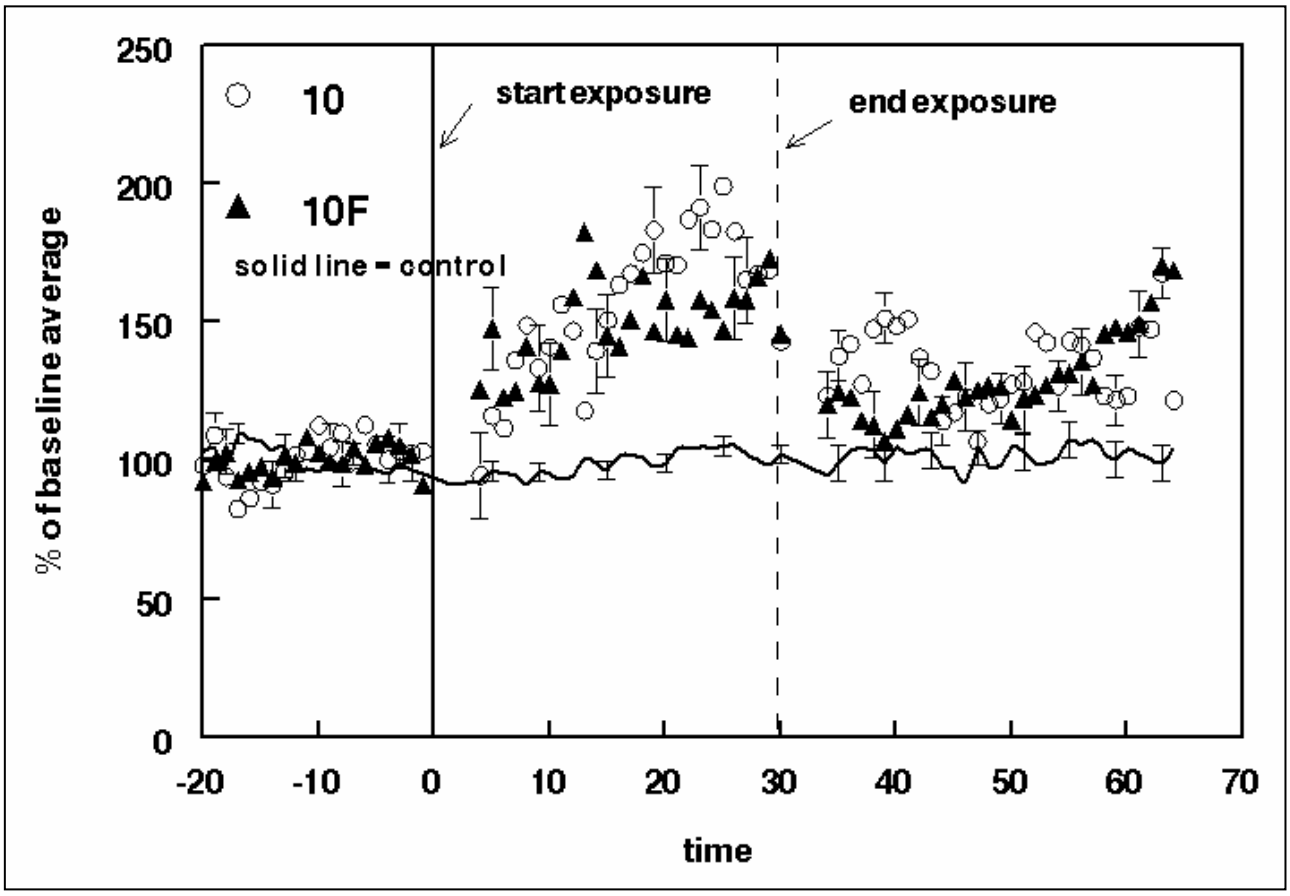


Figure 22. Inspiratory time, exposure groups 10 and 10F.

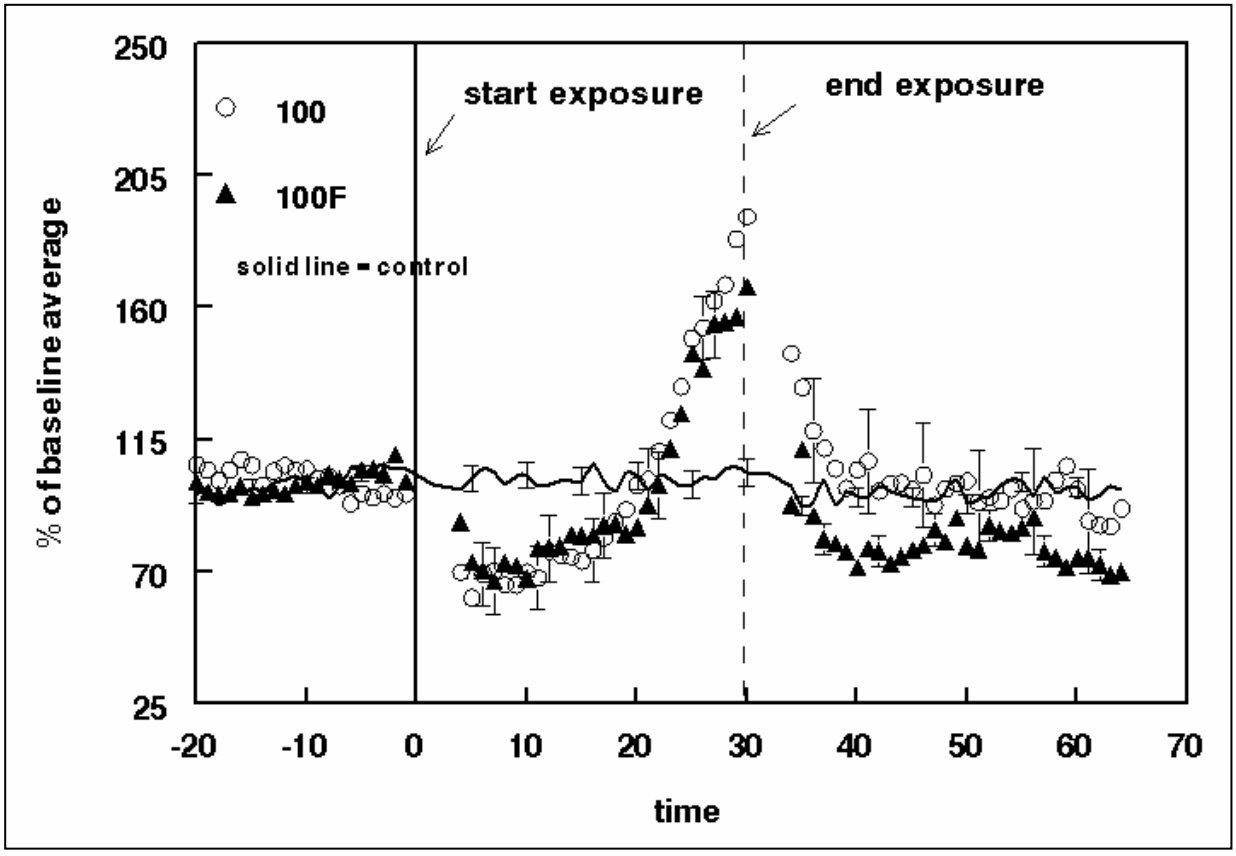


Figure 23. Peak inspiratory flow, groups 100 and 100F.

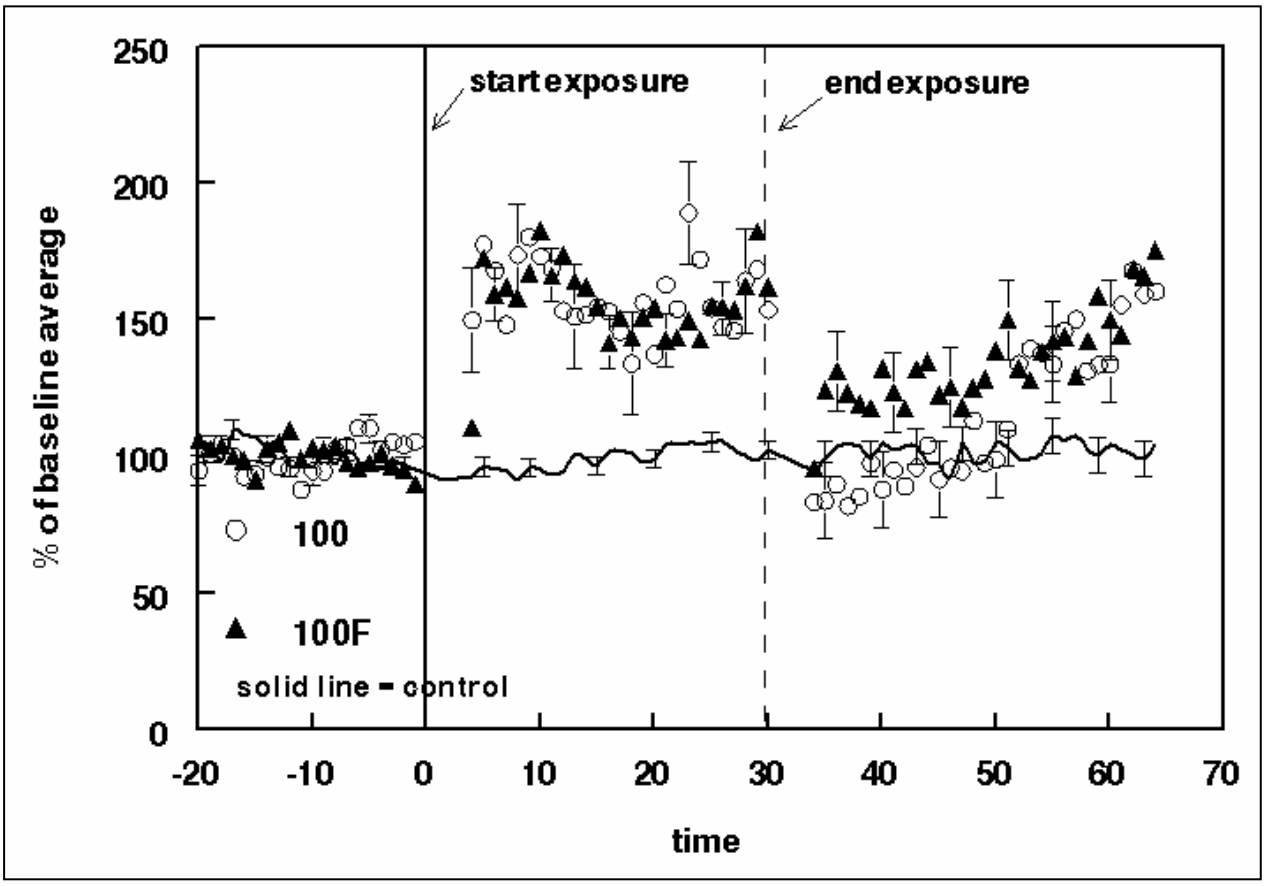


Figure 24. Inspiratory time, exposure groups 100 and 100F.

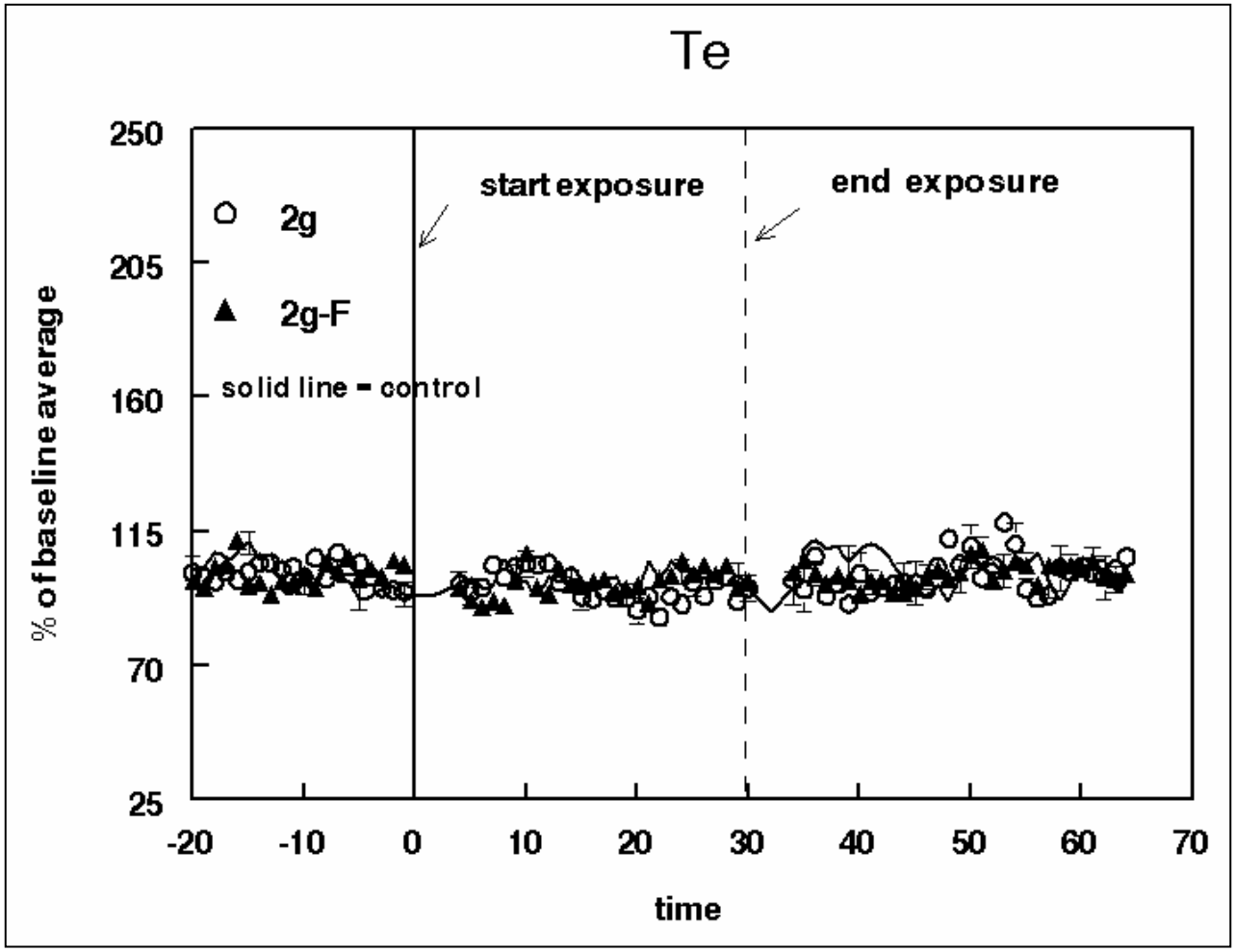


Figure 25. Expiratory time, exposure groups 2 and 2F.

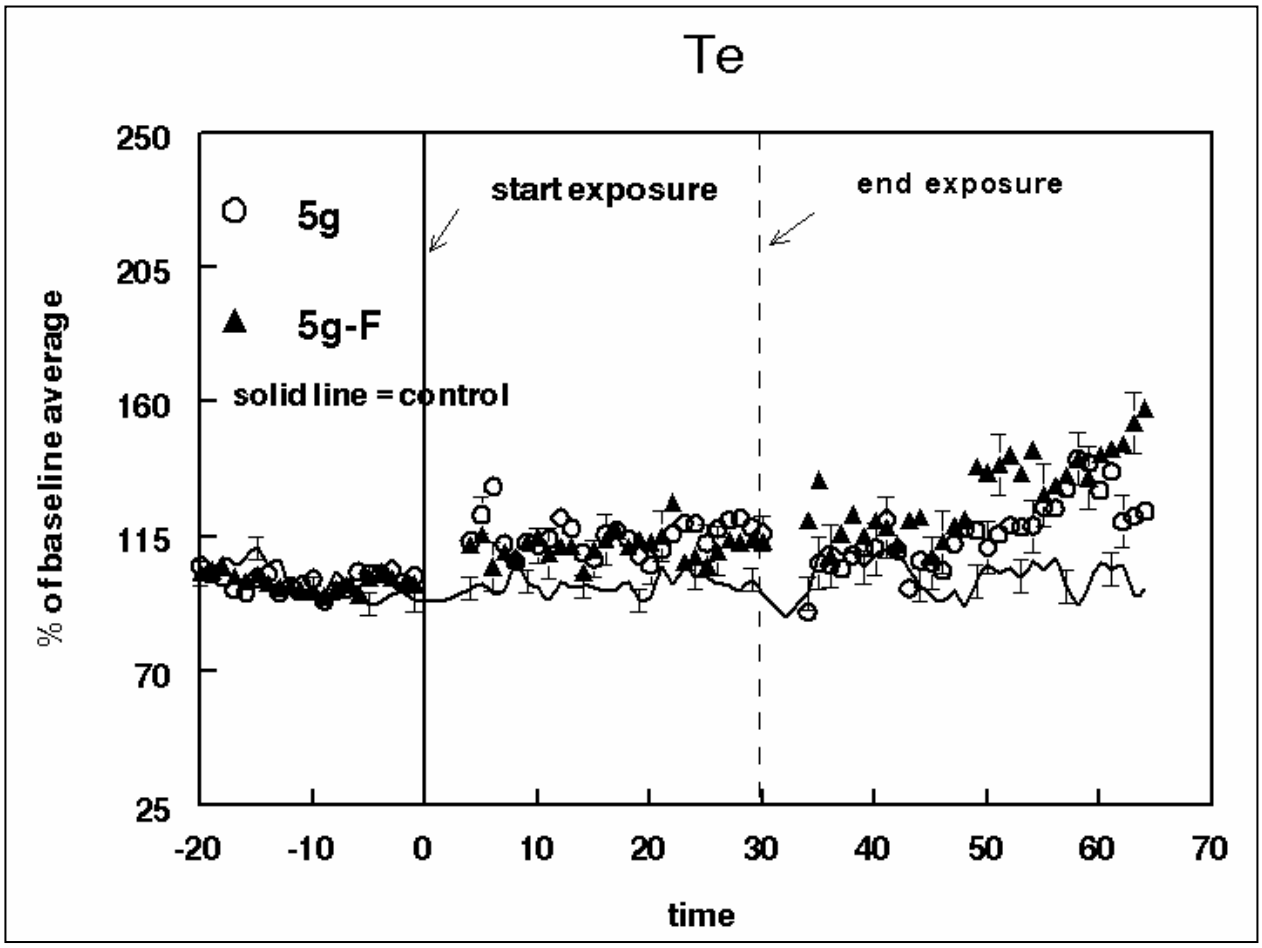
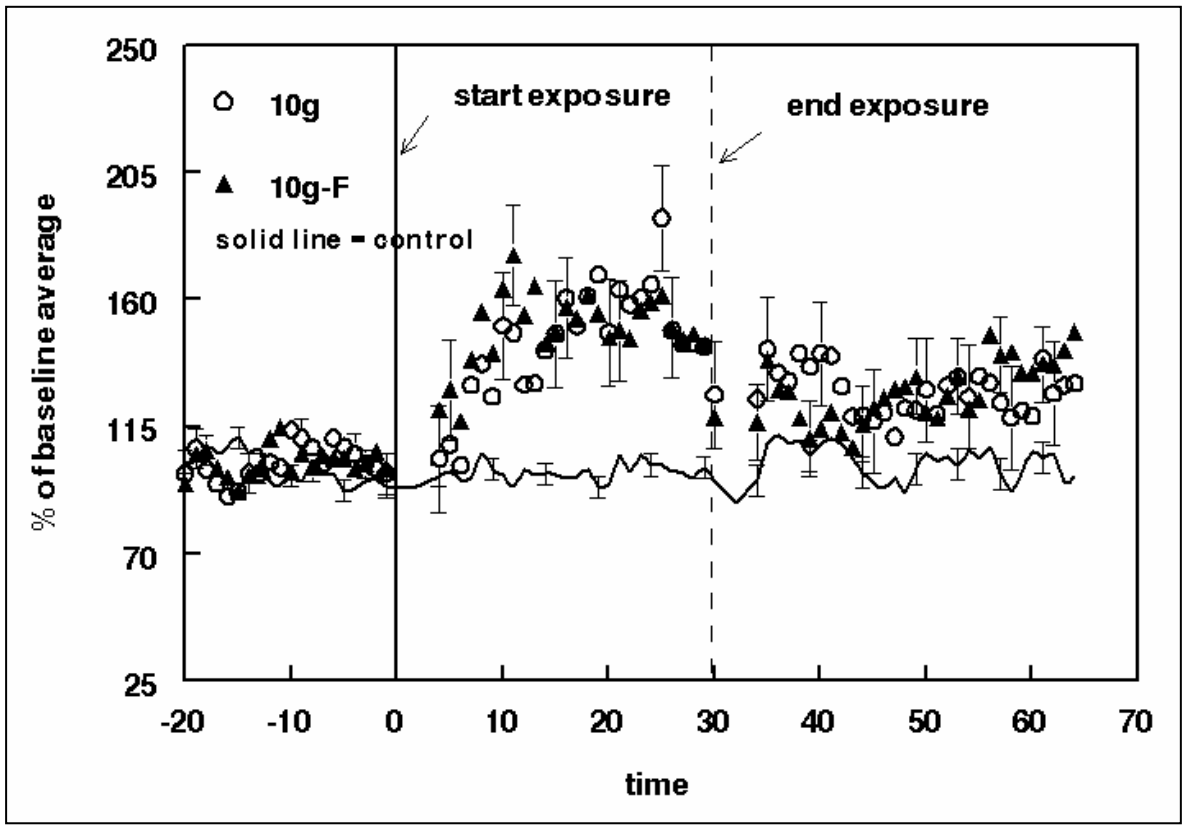
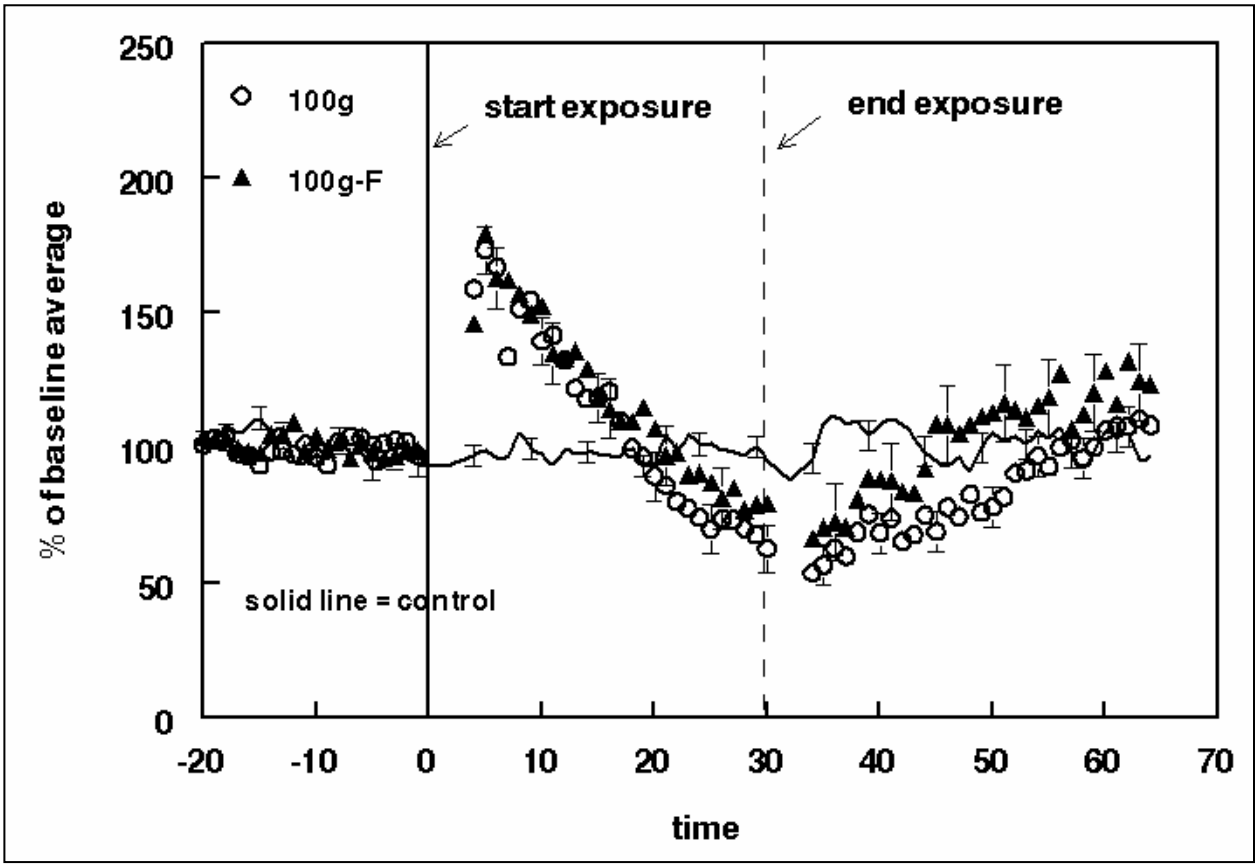


Figure 26. Expiratory time, exposure groups 5 and 5F.

Figure 27. Expiratory time, exposure groups 10 and 10F.





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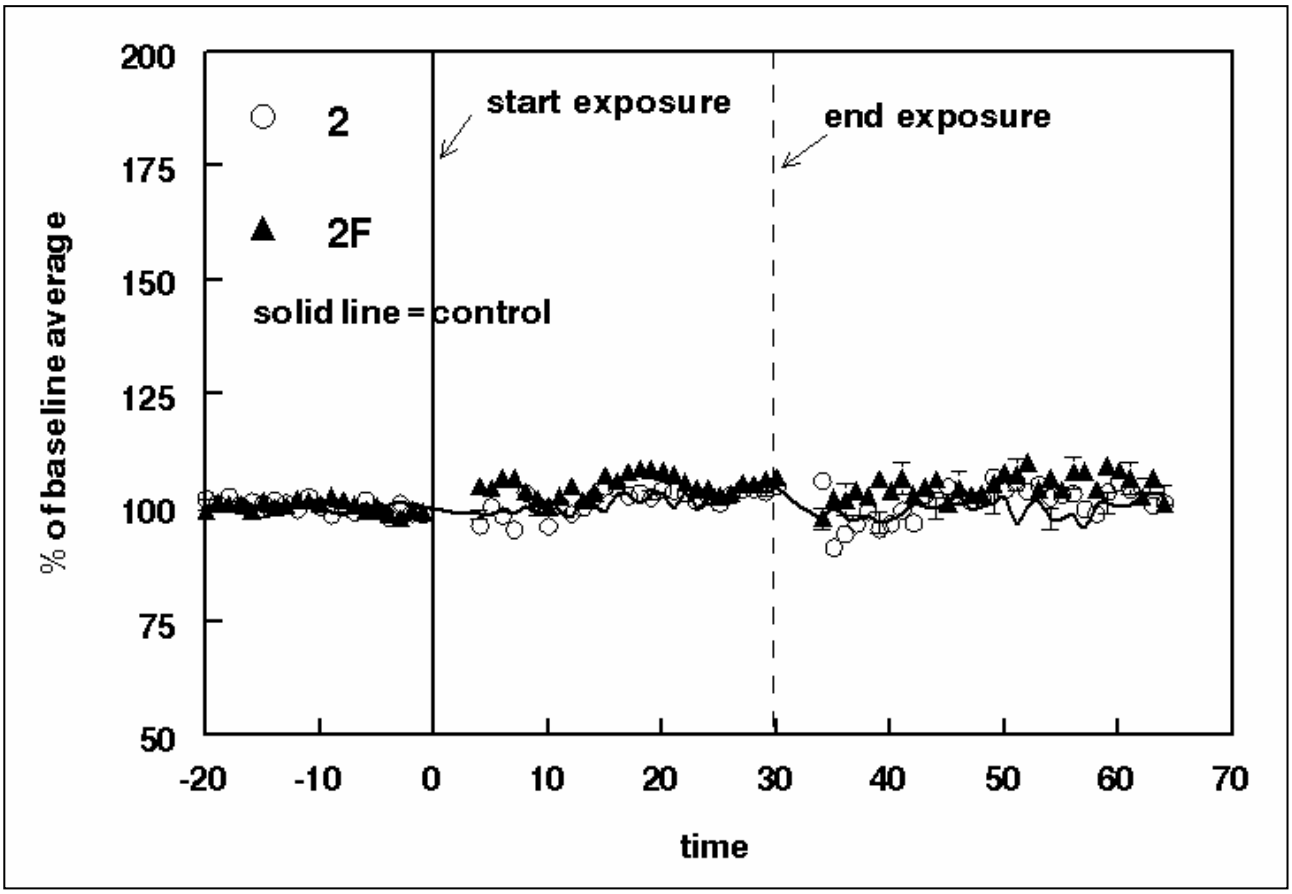


Figure 29. Respiratory duty cycle, exposure groups 2 and 2F.

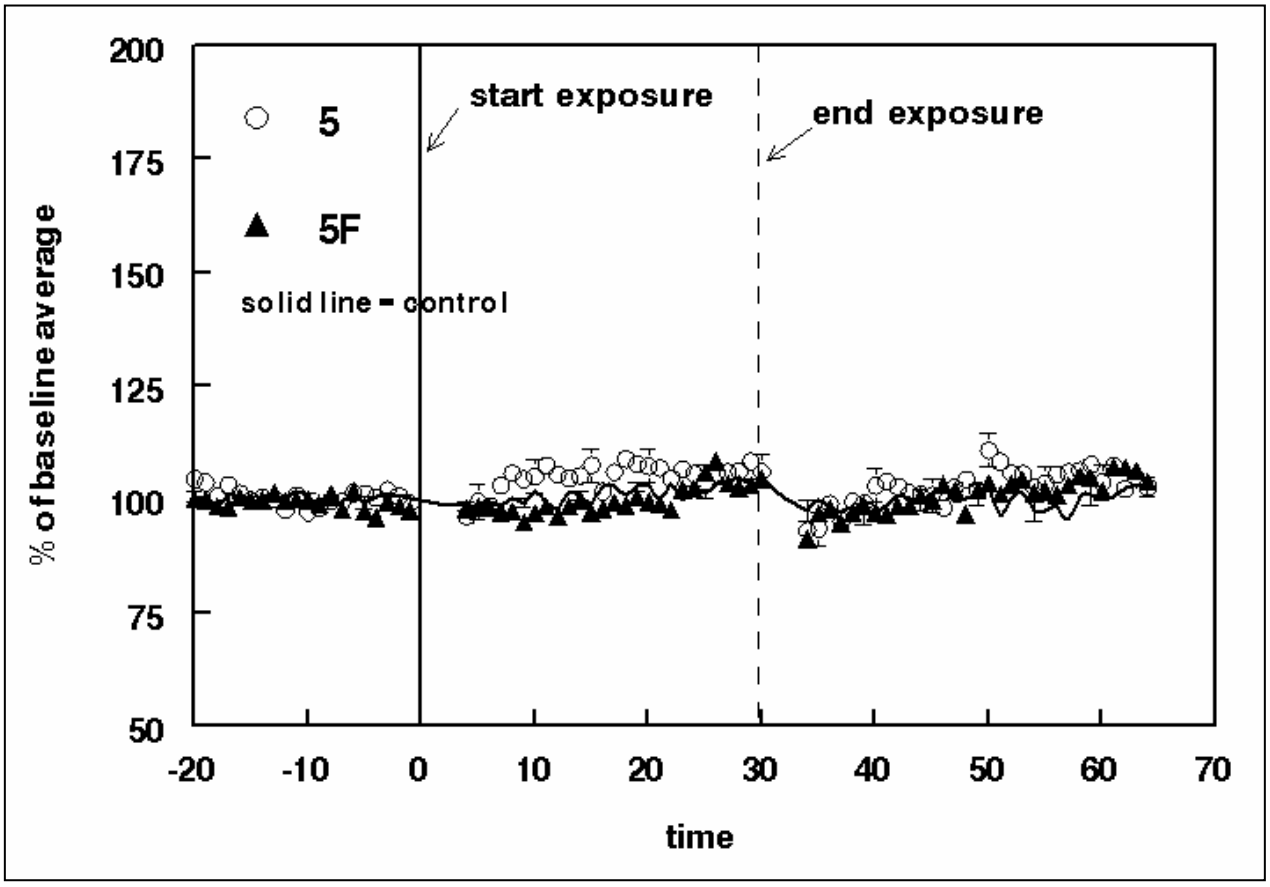


Figure 30. Respiratory duty cycle, exposure groups 5 and 5F.

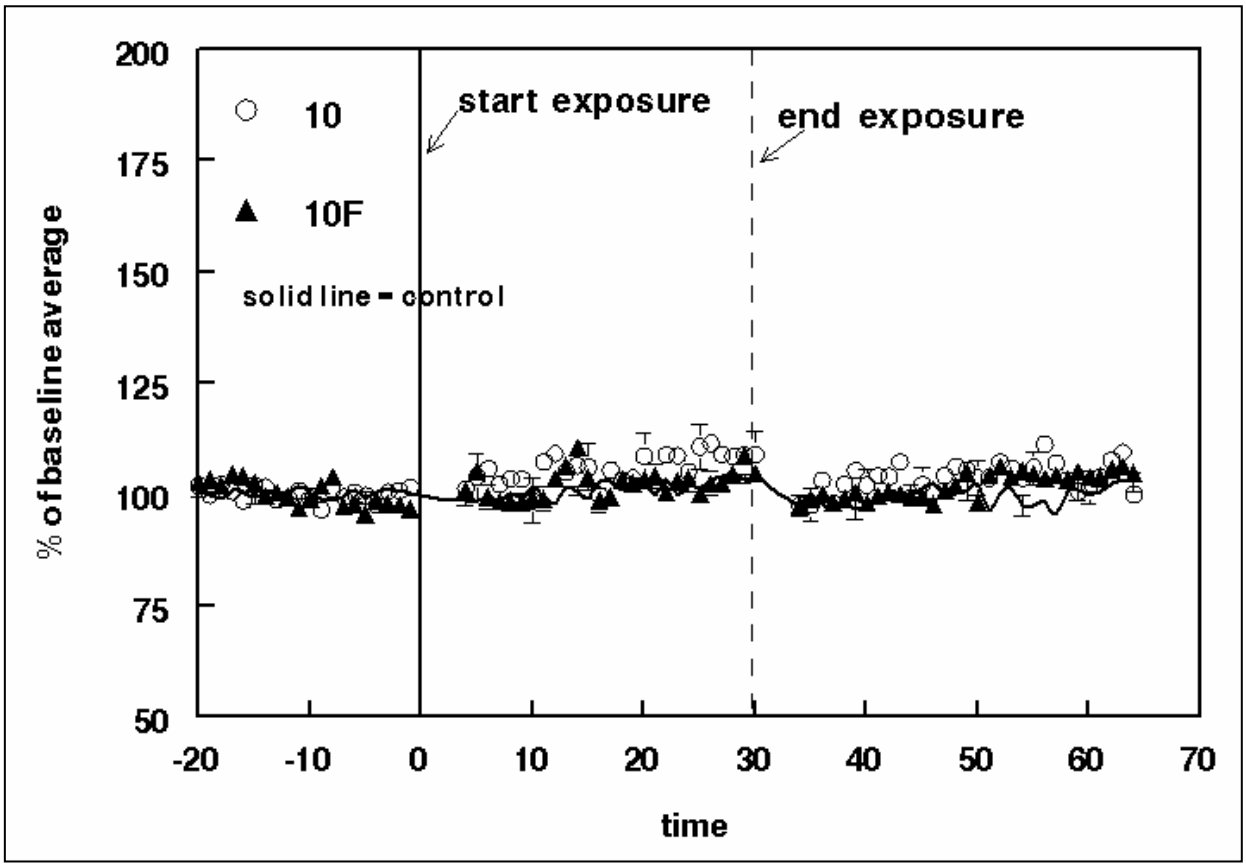


Figure 31. Respiratory duty cycle, exposure groups 10 and 10F.

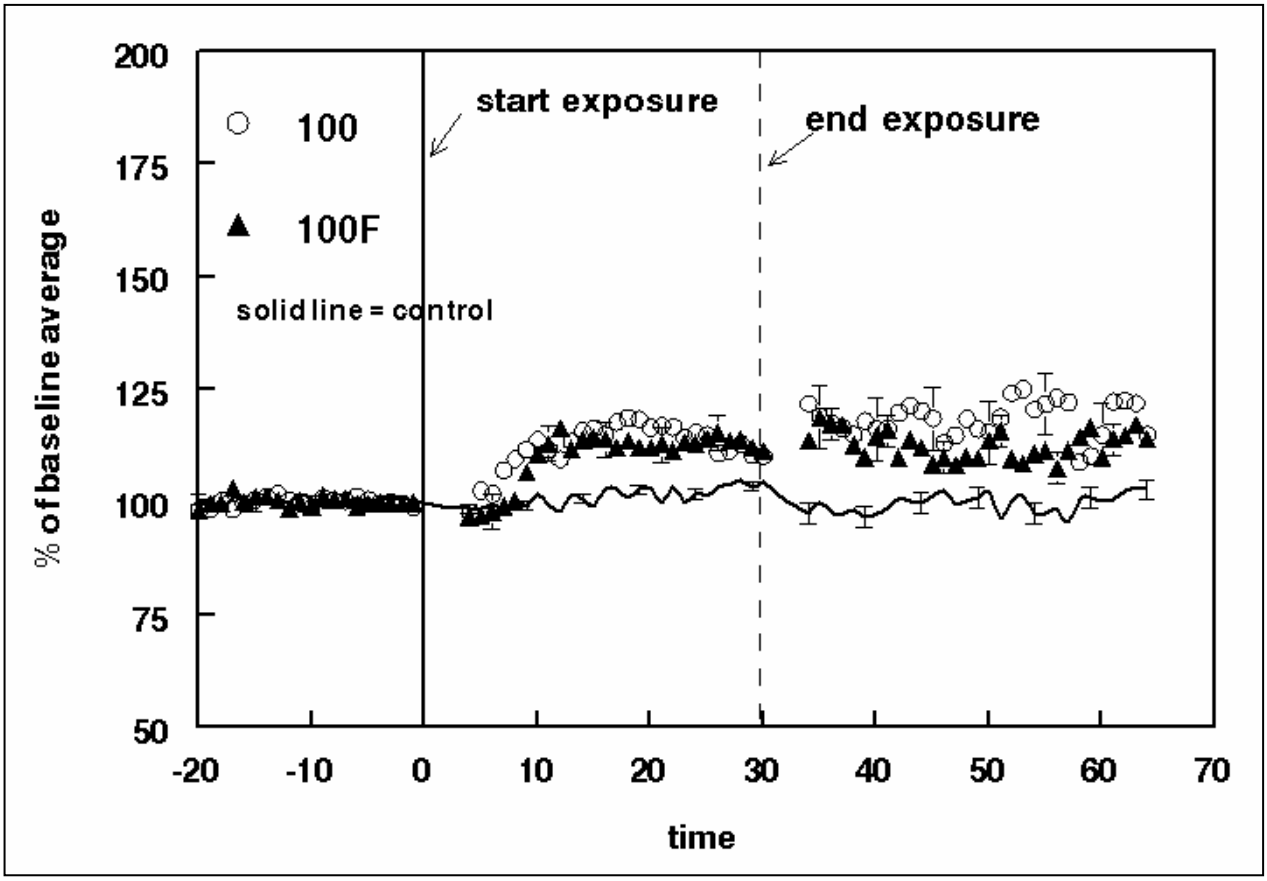


Figure 32. Respiratory duty cycle, exposure groups 100 and 100F.

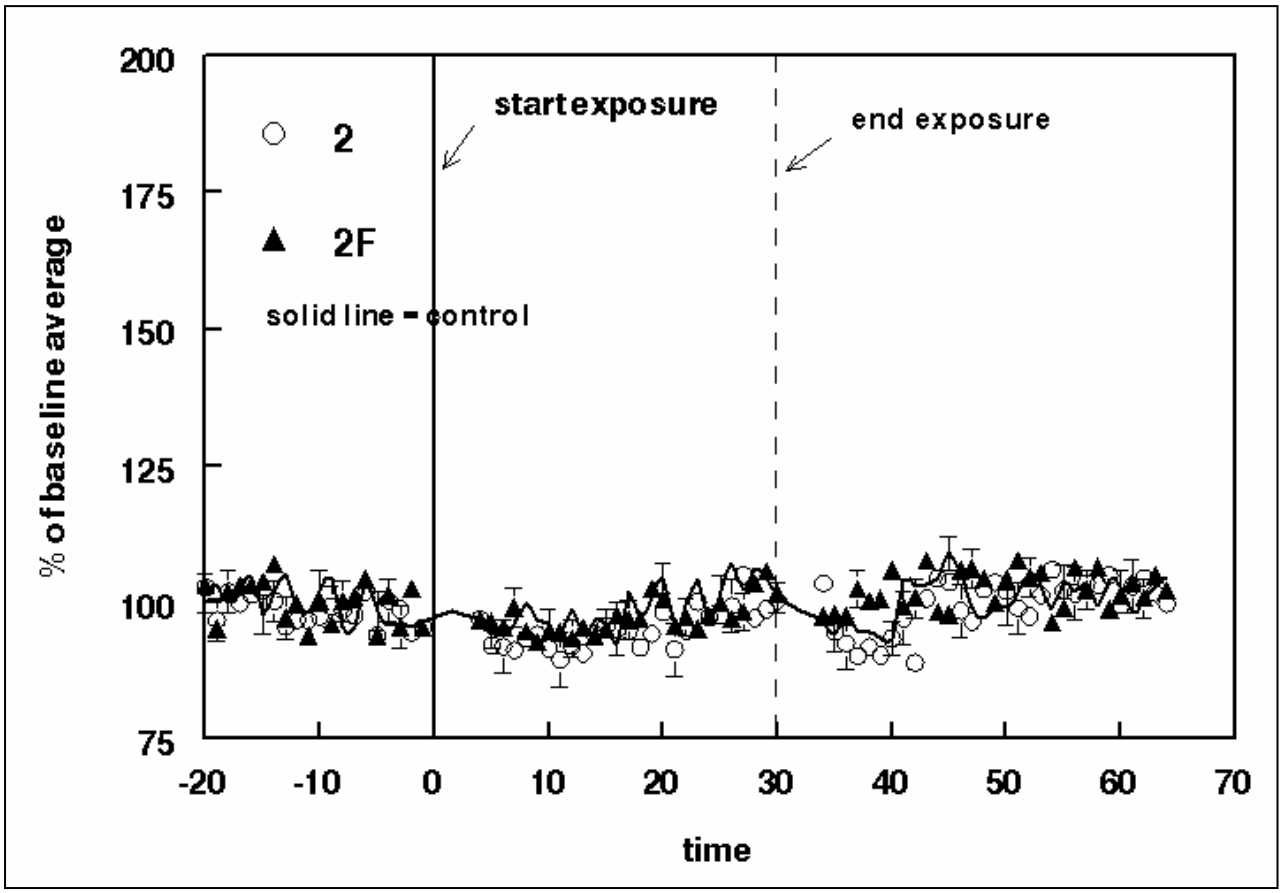


Figure 33. Enhanced pause, exposure groups 2 and 2F.

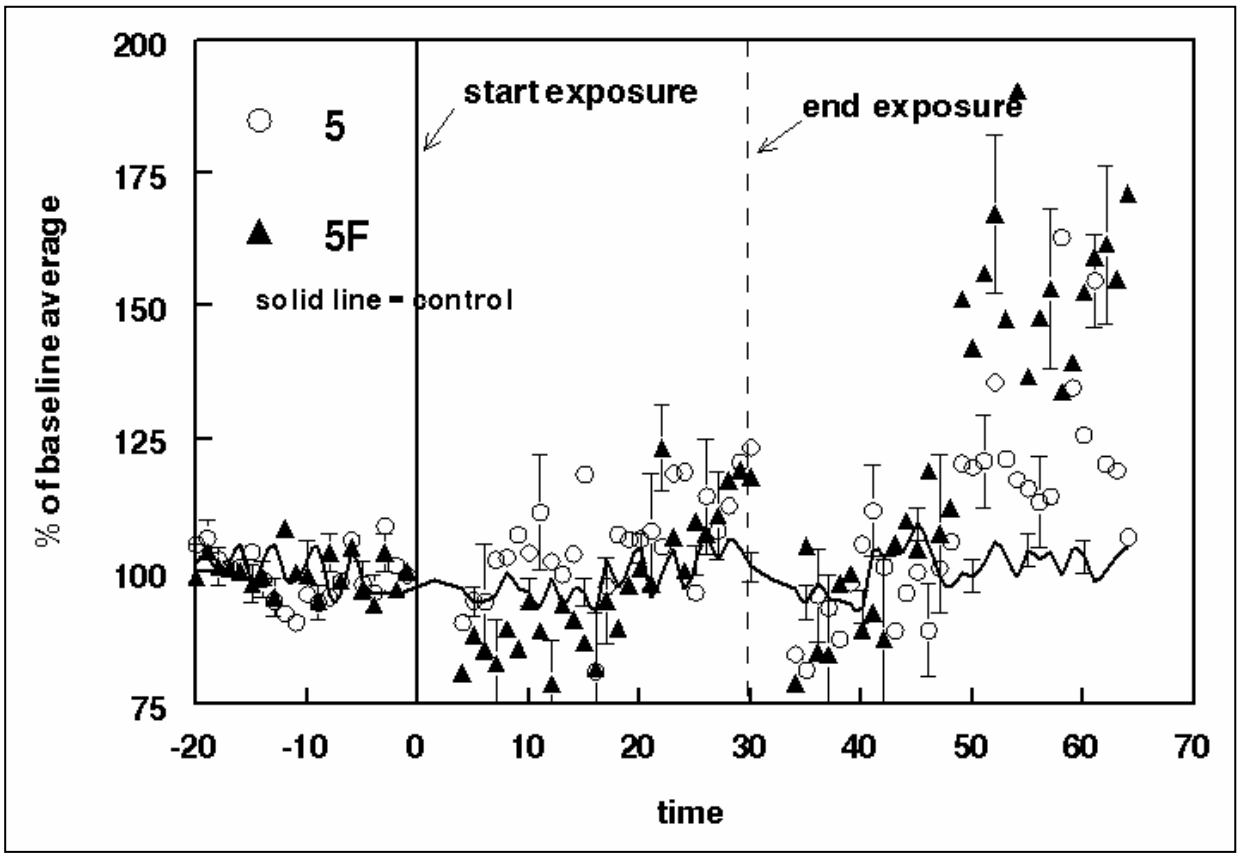


Figure 34. Enhanced pause, exposure groups 5 and 5F.

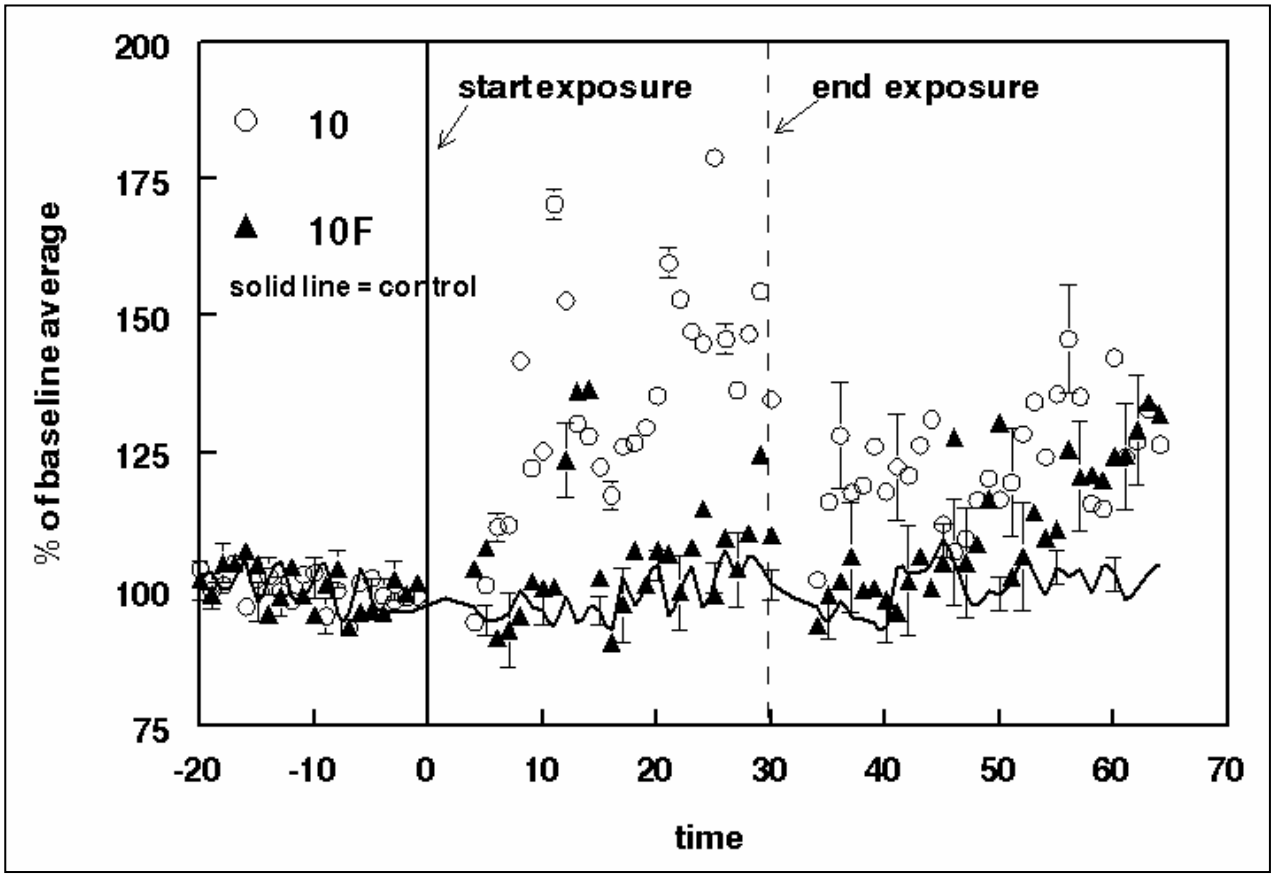


Figure 35. Enhanced pause, exposure groups 10 and 10F.

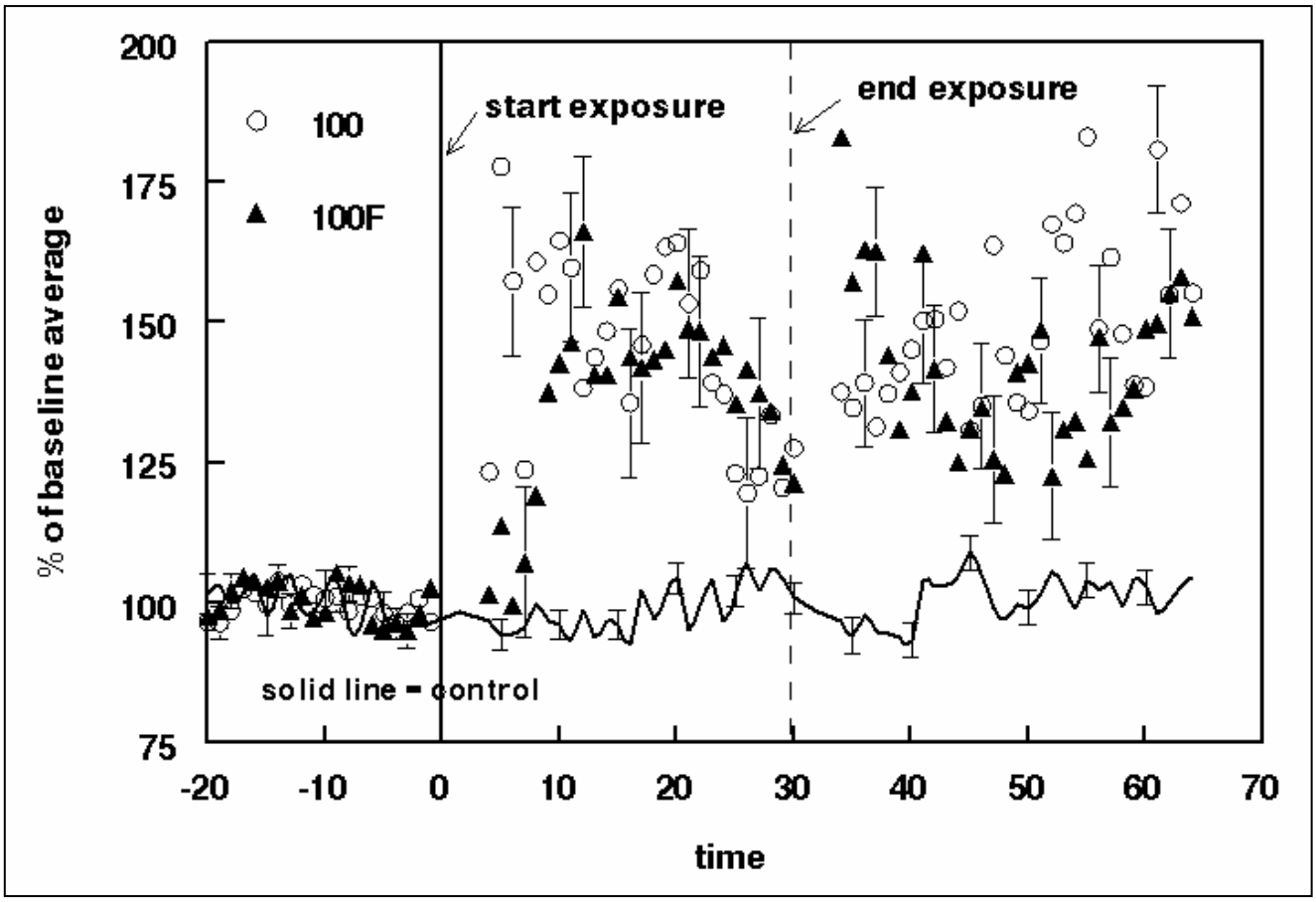
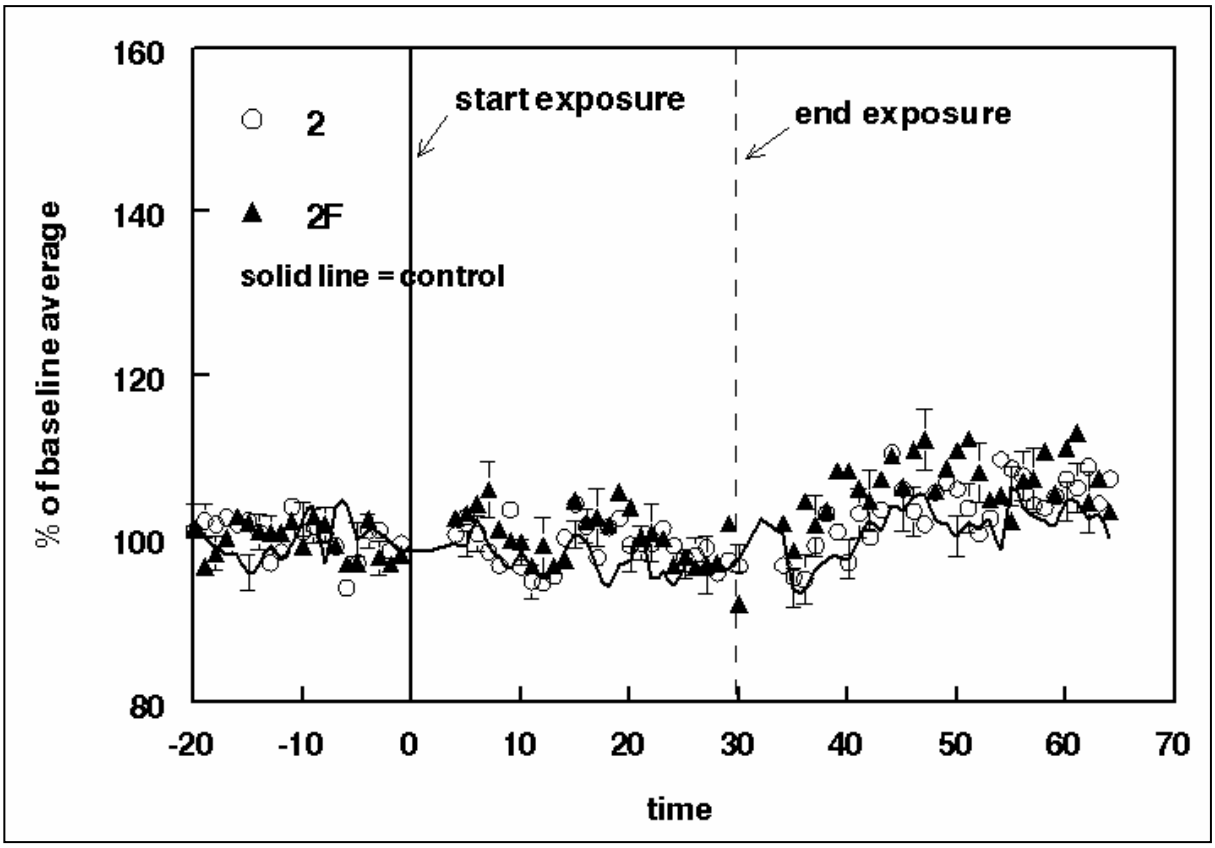


Figure 36. Enhanced pause, exposure groups 100 and 100F.

Figure 37. Flow derived parameter, exposure groups 2 and 2F.



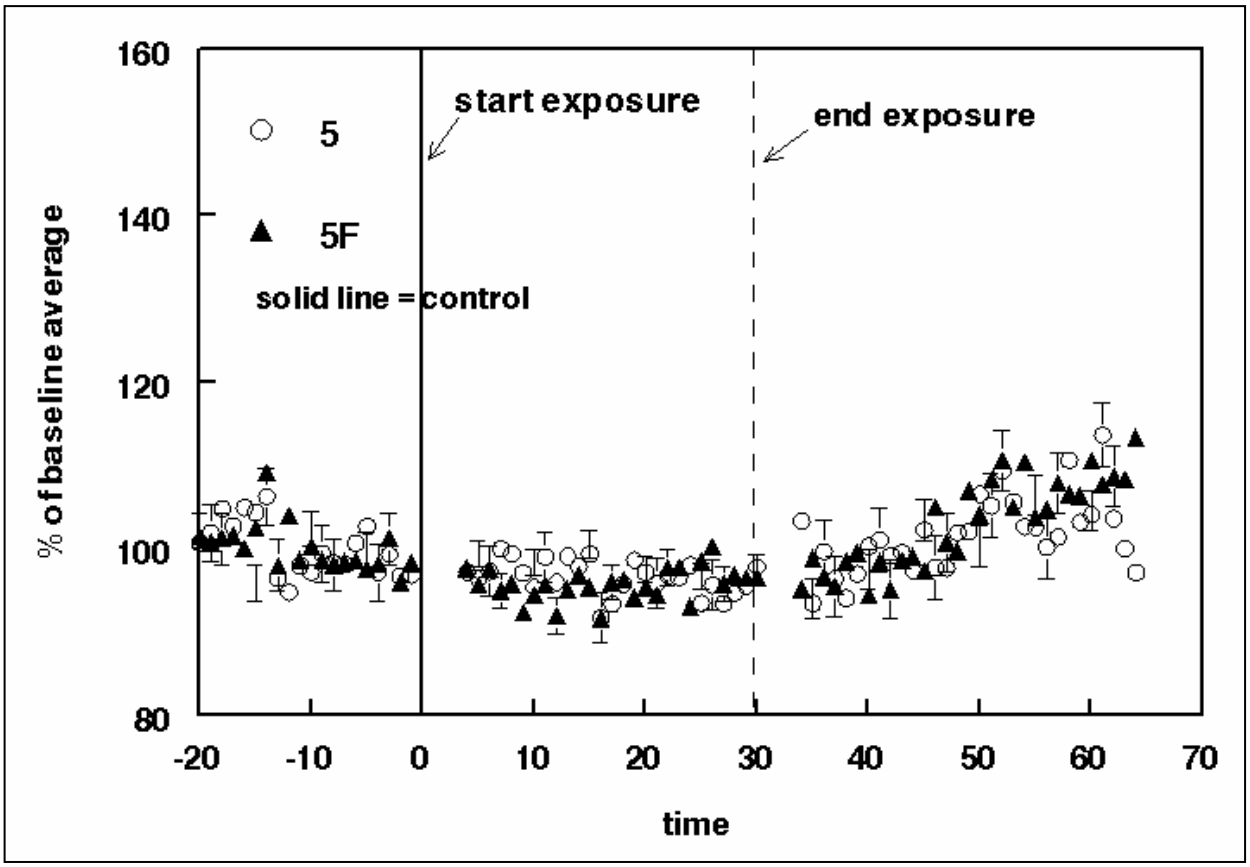


Figure 38. Flow derived parameter, exposure groups 5 and 5F.

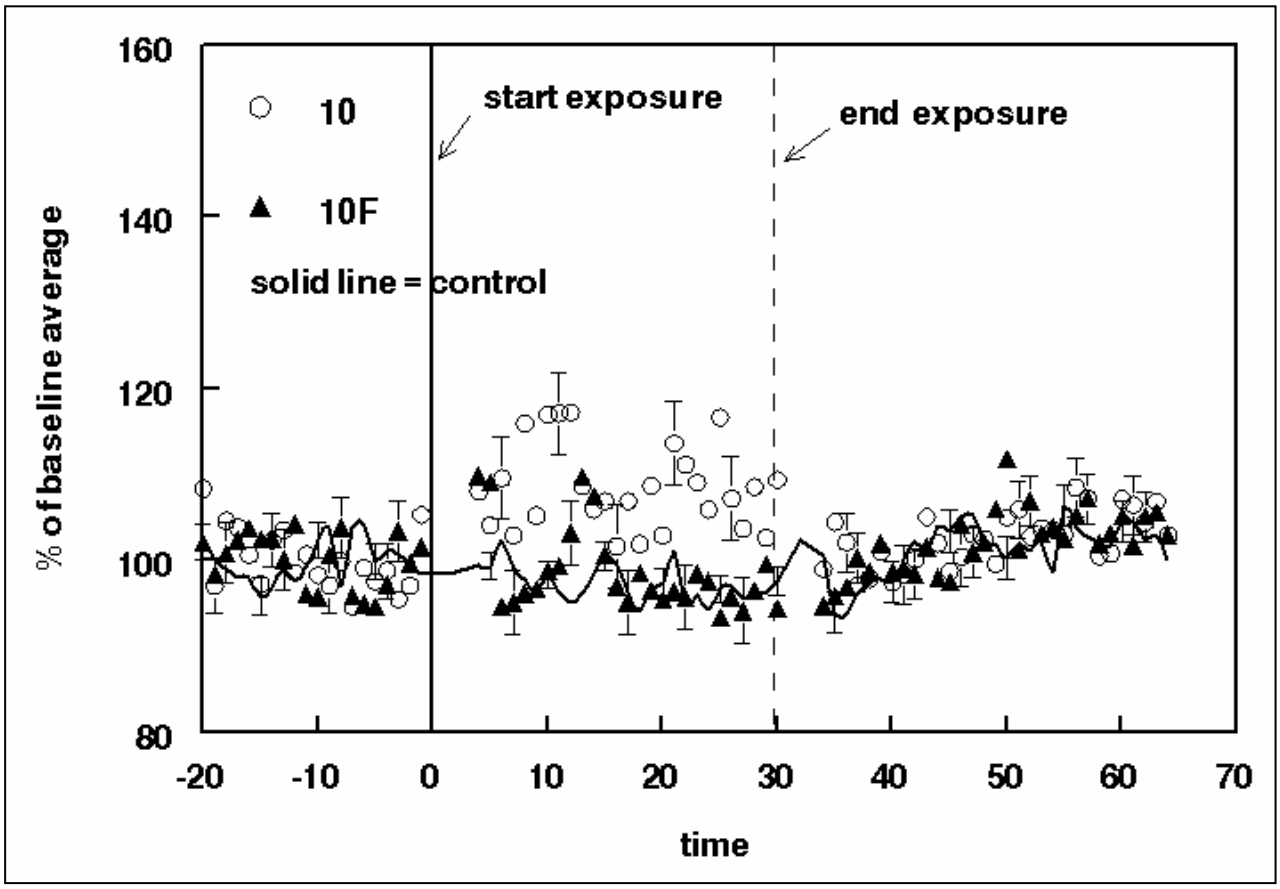


Figure 39. Flow derived parameter, exposure groups 10 and 10F.

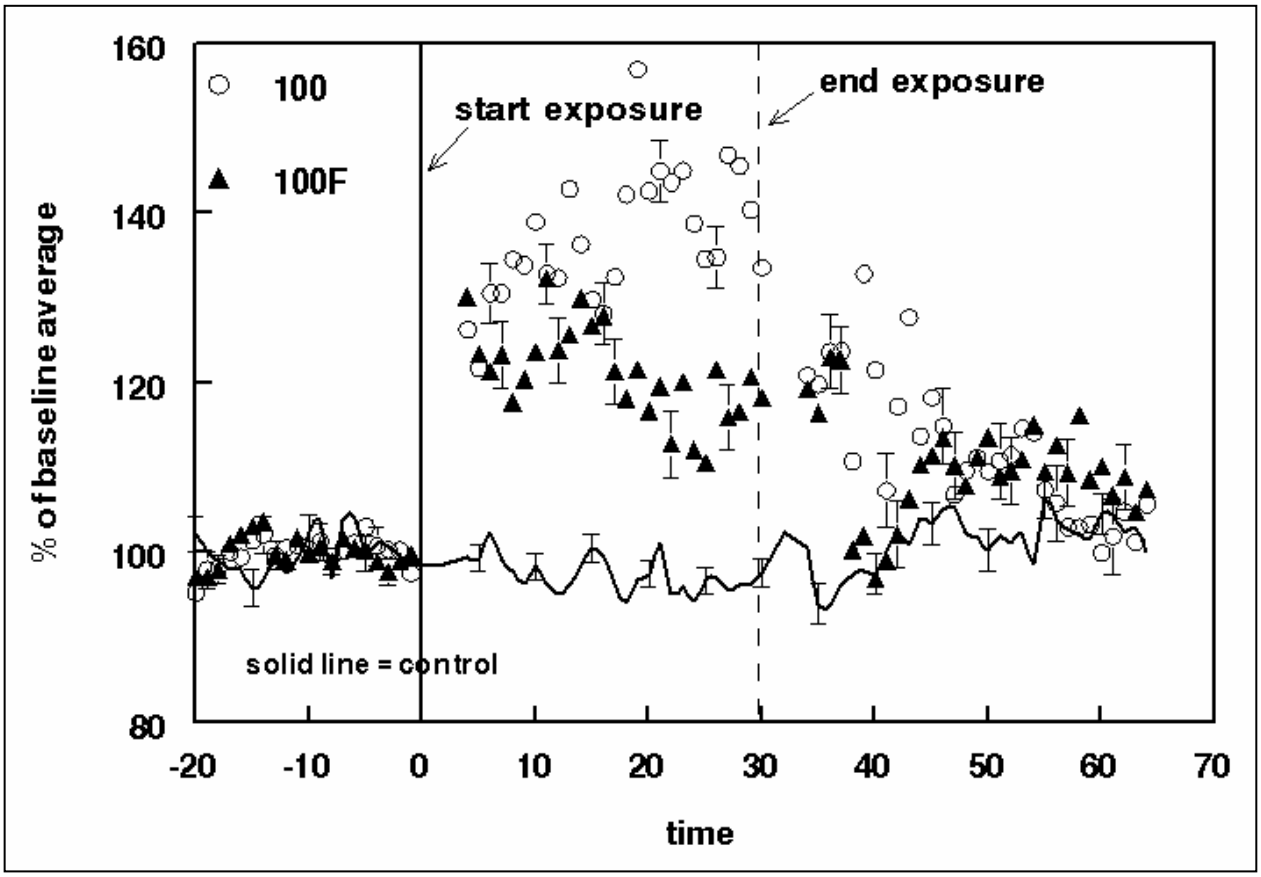


Figure 40. Flow derived parameter, exposure groups 100 and 100F.

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6. **AUTHOR(S)** Edgar C. Kimmel, James E. Reboulet, David L. Courson, Gregory S. Whitehead, Kenneth R. Still, William K Alexander, Robert L. Carpenter, and Kirk A. Phillips			
7. **PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)** Naval Health Research Center Detachment Toxicology NHRC/TD 2612 Fifth Street, Building 433 Area B Wright-Patterson AFB, OH 45433-7903			8. **PERFORMING ORGANIZATION REPORT NUMBER**
9. **SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)** Naval Health Research Center Detachment Toxicology NHRC/TD 2612 Fifth Street, Building 433 Area B Wright-Patterson AFB, OH 45433-7903			10. **SPONSORING/MONITORING AGENCY REPORT NUMBER** NHRC-99-XX
11. **SUPPLEMENTARY NOTES**			
12a. **DISTRIBUTION AVAILABILITY STATEMENT** Approved for public release; distribution is unlimited.			12b. **DISTRIBUTION CODE**
13. **ABSTRACT** *(Maximum 200 words)* Exposure for 30 minutes to diluted smoke from pyrolysis of advanced composite material used in the construction of the B2 bomber (B2-ACM) caused an airway reactivity (AR) response in naïve guinea pigs reminiscent of a human asthmatic episode. Animals exposed to diluted smoke from pyrolysis of 5, 10 and 100 grams of B2-ACM showed changes in a number of parameters characterizing ventilation, breathing pattern, and breath structure. These changes are considered indicative of bronchoconstriction. The highest exposure concentration also elicited convulsions in the animals, which may or may not be related to the AR response. Upon treatment with fresh air there was recovery period in which breathing returned to normal. However the recovery was transient with respiratory parameters returning to abnormal levels; indicating a "rebound" constrictive event even in the presence of clean air. Animals exposed to diluted smoke from the pyrolysis of 2 grams of B2-ACM demonstrated minimal changes in only a few of the respiratory parameters, suggesting that there might be a threshold for B2-ACM smoke elicited AR response.			
14. **SUBJECT TERMS** Advanced composite material, Smoke, Airway reactivity response.			15. **NUMBER OF PAGES** 78 16. **PRICE CODE**
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