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Submitting Organization	MILES INC
Contractor	MILES INC
Document Title	RESPIRATORY SENSITIZATION STUDY IN GUINEAPIGS: CONTROLS CHALLENGED WITH 2,4- OR 2,6-TOLUENE DIISOCYANATE WITH COVER LETTER DATED072593
Chemical Category	2,4-TOLUENE DIISOCYANATE (584-84-9)

Section 8d

MILES

Miles Inc.
Mobay Road
Pittsburgh, PA 15205-9741
Phone 412 777-2000

86930000342

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July 29, 1993

Document Processing Center TS-790
Office of Toxic Substances Room L-100
Environmental Protection Agency
401 M Street SW
Washington, DC 20460

Contains No CBI

Attention: 8(d) Health and Safety Reporting Rule
(Notification/Reporting)

Gentlemen:

Enclosed is a copy of a Health and Safety Study that was reported as initiated by Miles Inc. in our letter to you dated August 24, 1992 (see letter attached).

We are submitting this study on behalf of Miles Inc., Mobay Road, Pittsburgh, Pennsylvania 15205. We are filing this Health and Safety Study to comply with the regulations codified at 40 CFR, Part 716. This submission contains no Confidential Business Information (CBI).

The information required at 40 CFR 716.30 is given below.

Chemical Name:	Toluene Diisocyanate; 2,4- and 2,6-
CAS No:	584-84-9 and 91-08-7
Name of Study:	Respiratory Sensitization Study in Guinea Pigs: Controls Challenged with 2,4- or 2,6-Toluene Diisocyanate (TDI)
Submitting Official:	Francis J. Rattay
Title:	Manager, Regulatory Affairs
Address:	Mobay Road Pittsburgh, Pa 15205
Telephone No.:	(412) 777-7471

93 AUG -3 AM 11:35
OTS CBIC

Sincerely,

Francis J. Rattay
Manager, Regulatory Affairs
(412) 777-7471

Attachment
Certified Mail No.: P 213 126 285

ATTACHMENT

MILES

Miles Inc
Mobay Road
Pittsburgh, PA 15205-9741
Phone: 412 777-2000

August 24, 1992

Document Processing Center TS-790
Office of Toxic Substances Room L-100
Environmental Protection Agency
401 M Street SW
Washington, DC 20460

Attention: 8(d) Health and Safety Reporting Rule
(Notification/Reporting)

Contains No CBI

Gentlemen:

This letter is to notify EPA of a Health and Safety Study that has been initiated by Miles Inc., Mobay Road, Pittsburgh, Pennsylvania 15205. This study was initiated July 27, 1992 and the report should be available in the first quarter of 1993.

The information required by 40 CFR, Part 716 is given below.

Chemical Name:	Toluene Diisocyanate; 2,4- and 2,6-
CAS No:	584-84-9 and 91-08-7
Nature of Study:	To determine whether direct and/or cross respiratory sensitization occurs with 2,4- and 2,6-toluene diisocyanate
Submitting Official:	Francis J. Rattay
Title:	Manager, Regulatory Affairs
Address:	Mobay Road Pittsburgh, Pa 15205
Telephone No.:	(412) 777-7471
FAX No.:	(412) 777-7484

If you have any questions, please contact me.

Sincerely,

Francis J. Rattay
Manager, Regulatory Affairs
(412) 777-7471

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Attachment
Certified Mail No.: P 827 215 577

FILE
7

Study Title

Respiratory Sensitization Study in Guinea Pigs:
Controls Challenged with 2,4- or 2,6-Toluene Diisocyanate (TDI)

Author

D. L. Warren

Contains No CBI

Study Completion Date

July 13, 1993

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OTS CBIC

Test Facility

Miles Inc.
Agriculture Division
Toxicology
17745 South Metcalf
Stilwell, Kansas 66085-9104

Study Number

92-344-PP

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the TSCA Good Laboratory Practice Standards of 40 CFR Part 792 and the OECD Principles of Good Laboratory Practice, C(81)30 (Final) Annex 2 (Paris, May 1981).

SUBMITTER

MILES INC.

J. H. Thyssen: _____
Vice President, Toxicology

Date: 7-13-93

SPONSOR

AGRICULTURE DIVISION

J. H. Thyssen: _____
Vice President, Toxicology

Date: 7-13-93

STUDY DIRECTOR

D. L. Warren: _____

Date: 7/13/93.

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SPONSOR

INTERNATIONAL ISOCYANATE INSTITUTE, INC.
119 Cherry Hill Road
Parsippany, NJ 07054

TEST FACILITY

MILES INC.
Agriculture Division
Toxicology
17745 South Metcalf
Stilwell, Kansas 66085-9104

DATES

Experimental Start Date: July 27, 1992
Terminal Sacrifice Date: October 23, 1992

PERSONNEL AND RESPONSIBILITIES

Toxicology:	J. H. Thyssen	J. H. Thyssen
Toxicology Laboratory:	G. K. Sangha	G. K. Sangha
Experimental Toxicology:	R. N. Shiotsuka	R. N. Shiotsuka
Study Direction:	D. L. Warren	D. L. Warren
Study Conduct:	L. A. Berry	L. A. Berry
	J. S. Gladbach	J. S. Gladbach
	A. T. Halliburton	Art Halliburton
Pathology Services:	B. P. Stuart	Barry P. Stuart
Animal Care:	R. E. Mueller	Robert E. Mueller
Gross Pathology:	H. E. Hoss	Herbert E. Hoss
Analytical Chemistry:	K. D. Moore	for K. D. Moore
Quality Assurance:	C. A. Balder	C. A. Balder by D. M. Wallace

QUALITY ASSURANCE STATEMENT

Audit reports have been submitted to the Study Director and Laboratory Management documenting the status of compliance with applicable departmental standard operating procedures, the study protocol and Good Laboratory Practice regulations.

All phases of study conduct for this study type have been periodically inspected by the Quality Assurance Unit. Audit reports have been submitted to Laboratory Management and the Study Director, documenting the status of compliance with applicable departmental standard procedures, the study protocol and Good Laboratory Practices regulations.

AUDIT			REPORT TO STUDY DIRECTOR/ MANAGEMENT
Date	Phases	Auditor	
	Phase of Study		
07/29/92	Lab Tech Computer Operation/Data, Collection, Verification of Temperature-Humidity Probes, Recording of Temperature, Humidity, and Airflows, Balance Verification, Body Weights, Observations, Animal Inventory, Load Restrainers/Plethysmographs/Chamber.	J. Hagensen	07/29/92
09/03/92	Generator, Mixing Chamber and Plethysmographs, Airflows, Static Pressure Readings, Protein Conjugate Sampling.	J. Hagensen	09/03/92
09/23/92	Test Substance Sampling	J. Hagensen	09/23/92
02/10/93-03/04/93	Final Report Review	J. Hagensen	03/04/93
	Phase of Study Type		
02/28/92	Animal Identification	J. Hagensen	02/28/92
03/2-3/92	Use of Gilibrator, Verification of Airflows, Test Substance Weighing Before and Following Exposure, Presensitization Body Weights, Observations, Animal Inventory, Placement of Animals in Restrainers/Chamber, Acclimation, Onset of Exposure, Lab Tech Computer Operation and Data Collection, End Exposure, Removal of Animals From Chamber/Restrainers and Post-Exposure Observations.	J. Hagensen	03/04/92

QUALITY ASSURANCE STATEMENT (Continued)

AUDITS			REPORT TO STUDY DIRECTOR/ MANAGEMENT
Date	Phases	Auditor	
	Phase of Study Type (Continued)		
03/30/92	Balance Verification, Removal of Test Substance From Storage, Weighing of Test Substance, Return to Storage, Test Substance Preparation, Calibration of Generator Airflow, Generator Operation, Delivery Pump Operation, Chamber Atmosphere and Particle Size Sampling, and Wet Test Meter Calibration.	J. Hagensen	03/30/92
04/7-8/92	Balance Verification, Pre-challenge Body Weights, Observations, Placement of Animals in Plethysmographs, Acclimation, Calibration of Mixing Chamber, Test Substance Generator and Plethysmograph Airflows, Static Pressure (magnahelic) Readings, Calibration of Respiratory Rate Recorders, Weight of Test Substance Before and After Exposure, Pre-exposure Acclimation Start, Start of Pre-exposure Respiratory Measurements, Start and End of Challenge Exposure, Sampling of Mixing Chamber and Plethysmograph Atmospheres, Airflow and Static Pressure Readings, Post Challenge Recovery Period, Post-Exposure Body Weights and Observations.	J. Hagensen	04/08/92
04/16/92	Blood Collection, Anesthetization, Centrifuging of Blood and Necropsy.	J. Hagensen	04/16/92
	Clinical Laboratory Support Functions		
05/08/92	Hematology Parameters	J. Ranjbar	05/08/92
05/08/92	Differential Counts	J. Ranjbar	05/08/92

QUALITY ASSURANCE STATEMENT (Continued)

Date	AUDITS Phases	Auditor	REPORT TO STUDY DIRECTOR/ MANAGEMENT
	Analytical Support Functions		
05/06/92	Preparation For Analysis, Analytical Method, Submission of Samples, Determination of Test Substance Concentration, Standardization of Chromatographic Instruments and Analytical Standards.	S. Bailey	05/07/92
	Animal Care Support Functions		
01/31/92	Bedding, Rack Change and Feeding	C. A. Cox	02/03/92
02/04/92	Cleaning of Racks, Cages, Trays and Feeders	C. A. Cox	02/04/92
02/20/92	Filter Inspection/Change	C. A. Cox	02/20/92
02/25-26/92	Receiving, Shipment Examination, Randomization, Quarantine, Feeding and Observations	C. A. Cox	02/26/92
03/02/92	Release of Shipment for Study Use, Special Procedures For Guinea Pigs	C. A. Cox	03/02/92
03/09/92	Room Disinfection and Light Timer Check	C. A. Cox	03/09/92

In compliance with the Good Laboratory Practice regulations, this final report for study number 92-344-PP has been reviewed by the Quality Assurance Unit. The results presented in this report accurately describe the methods and standard procedures and reflect the raw data collected during the conduct of the study.

C.A. Halder by D.M. Wallace
C. A. Halder, Quality Assurance

3/30/93
Date

ABSTRACT

The potential for sham-sensitized guinea pigs to display apparent hypersensitivity reactions upon challenge with 2,4- or 2,6-Toluene Diisocyanate (TDI) were investigated. Twelve animals per group were sham-exposed during the sensitization phase (exposures for three hours/day for five consecutive days) to room air. All animals were then housed under standard conditions for two weeks. Animals were subsequently challenged three times (at approximately one-week intervals) to assess apparent hypersensitivity reactions, as shown below.

	Sensitization	Challenges
Group 1	Sham	Sham; Sham; GSA
Group 2	Sham	2,4-TDI; 2,4-TDI; 2,4-TDI-GSA
Group 3	Sham	2,6-TDI; 2,6-TDI; 2,6-TDI-GSA

GSA = Guinea pig serum albumin
TDI-GSA = GSA protein conjugate

Increases in respiratory rate were taken as a primary indicator for apparent hypersensitivity responses and the respiratory rate of each animal was monitored before, during, and after each challenge exposure. Sustained respiratory rate increases shortly after exposure represent immediate-type while increases at times long after the end of exposure indicate delayed-type responses. Animals were also monitored for clinical signs and alterations in the rate of body weight gain as well as for lesions at necropsy.

No clinical signs were observed from any animal during this experiment. Analysis of body weight data showed few differences between groups and no lesions were observed during gross necropsy from any animal.

All animals were evaluated for apparent immediate- and delayed-type hypersensitivity reactions during each of the three challenges. Apparent immediate-type responses were occasionally observed from groups 1 and 3, while apparent delayed-type responses were observed from all groups. In that recurrence of a particular response during successive episodes of challenge may be indicative of true hypersensitivity reactions, the incidence of this recurrence was assessed from each animal. Only one repeated immediate-type and one repeated delayed-type response were observed (from groups 1 and 2, respectively). Each group is considered to be indistinguishable with respect to apparent responses or recurrent responses. This low incidence of reaction during challenge represents a background level of response for this test system.

MATERIALS: 2,4-Isomer

I. The test substance was supplied by Miles Inc., Industrial Chemicals Division, Mobay Road, Pittsburgh, PA 15205-9741, with the following information:

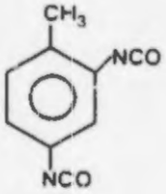
Test Substance:

Identification:	Mondur TDS Grade II
Physical Appearance:	Pale yellow liquid
Lot No.:	0074
Purity:	98.9%
Stability Under Storage Condition:	One year

Active Ingredient:

Common Name:	Toluene Diisocyanate
Chemical Name:	2,4-Toluene Diisocyanate

Structural Formula:



2,4 - TDI

CAS Registry No.:	584-84-9

II. During the study the test substance was stored and archived at room temperature.

MATERIALS: 2,6-Isomer

I. The test substance was supplied by Aldrich Chemical Co., Inc., with the following information:

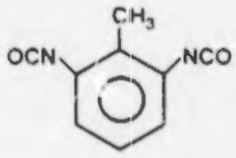
Test Substance:

Identification:	2,6-Toluene Diisocyanate
Physical Appearance:	Clear liquid
Cat. No.:	22386-7
Purity:	97%
Stability Under Storage Condition:	Not available*

Active Ingredient:

Common Name:	Toluene Diisocyanate
Chemical Name:	2,6-Toluene Diisocyanate

Structural Formula:



2,6 - TDI

CAS Registry No.:	91-08-7

II. During the study the test substance was stored under refrigerator conditions.

*Stability under storage is presumed to be similar to that of the 2,4- isomer.

PURPOSE

The purpose of this study is to evaluate the potential for sham-sensitized guinea pigs to display apparent hypersensitivity reactions upon challenge with either 2,4- or 2,6-TDI. The protocol for this experiment was based on test procedures described by Karol and co-workers [1-5]. This system involves exposure of guinea pigs during a "sensitization" phase and, at a later time, inhalation exposures during a "challenge" phase. For this experiment all animals were sham-sensitized with conditioned air. Hypersensitivity reactions are typified by an increase in the respiratory rate during or after challenge.

The approaches used in this report for the identification and characterization of both immediate- and delayed-type hypersensitivity reactions have previously been validated with subtilisin [5], ovalbumin [6], and purified tuberculin protein [2], as positive controls.

This report is intended to be a companion document to a report completed previously [7], in which animals received both sensitization exposures and challenge exposures with 2,4- and 2,6-TDI.

TEST GUIDELINES

There are no test guidelines for this study type.

METHODS

Animal Information

Source, Number, Weight and Age

Young adult male Hartley guinea pigs obtained from Sasco, Inc., Madison, Wisconsin, were used in this study. Guinea pigs are the species of choice for respiratory sensitization testing (Karol et al. [1,2,3]). Results from a total of 36 animals are reported. Body weights prior to the first sensitization exposure ranged from 241 to 296 grams. These animals were approximately three to four weeks of age at the start of exposure.

Examination and Acclimation

Guinea pigs were examined upon receipt and held for at least five or six days prior to study initiation. During the holding period each guinea pig was examined for changes in general appearance, behavior and gross external abnormalities. Animals with abnormalities were not used in this study.

Care and Housing

Prior to and following the exposure period, animals were housed individually in polycarbonate shoe-box type cages with ALPHA-dri bedding. Bedding was changed at least twice weekly and guinea pigs were transferred to clean cages with every bedding change. The environmental conditions were set for a room temperature range of 18.3 to 25.5°C, a relative humidity (RH) range of 40-70%, and a photoperiod with 12 hours of light alternating with 12 hours of darkness. Environmental conditions were continuously monitored. Potable municipal tap water and Agway Prolab Guinea Pig Chow were available for ad libitum consumption except during the exposure period.

Identification

Each animal was identified by earmark and cage card.

Randomization

Animals were assigned to sequentially numbered cages using a list of random numbers generated using SAS software [8]. Animals were selected, for each exposure group, in numerical order after randomization.

Experimental Design

Exposure Regimen

This study was conducted in three phases: sensitization, induction, and challenge, as described below, and each animal experienced each phase.

Three groups of 12 animals were used and each group of controls received different challenge exposures, as shown below.

Group	Exposure	
	Sensitization	Challenge
Group 1	Sham	Sham
Group 2	Sham	2,4-Isomer
Group 3	Sham	2,6-Isomer

The course of these experiments followed a six-week protocol:

Phase	Regimen	Description
Sensitization	Week 1	5 x 3 hours/day sham-exposed to conditioned room air
Induction	Weeks 2,3	Conventional housing
Challenge	Week 4	Challenge #1 to room air or TDI vapor
	Week 5	Challenge #2 to room air or TDI vapor
	Week 6	Challenge #3 to unconjugated protein or TDI protein conjugate

These phases are described more completely below.

Note: The day of the first exposure was denoted as study day 0.

Sensitization Phase

Sham exposures to room air were administered to each group for three hours per day on each of five consecutive days (days 0-4). These exposures were conducted at approximately the same time of day for each group.

Induction Phase

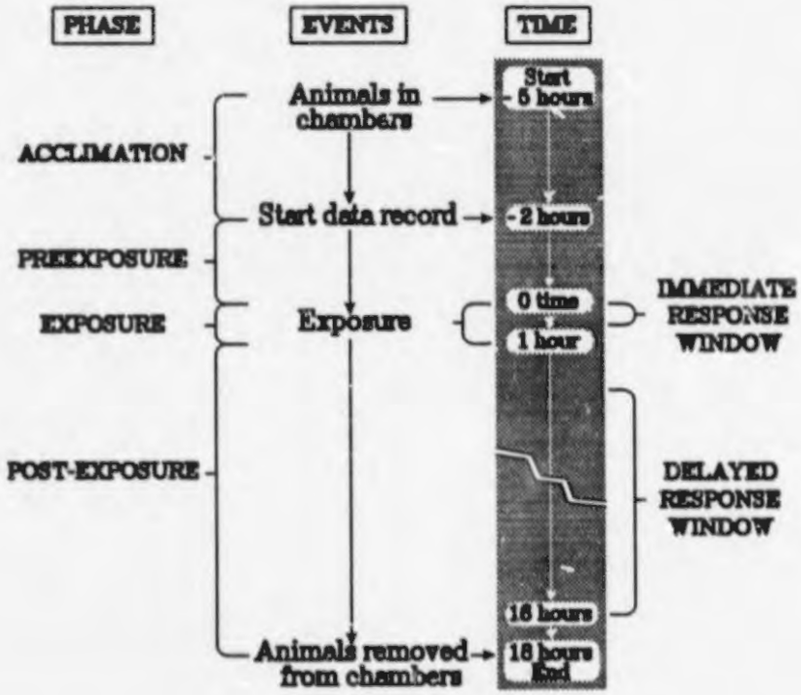
Each animal was conventionally housed for approximately two weeks (vide supra).

Challenge Phase

This phase was conducted identically for all three groups and animals were exposed to the same isomer of TDI during each challenge exposure for a given group. These exposures were started between 12:00 p.m. and 3:00 p.m. so as to minimize the potential for diurnal variations in response.

The events during challenge that are associated with respiratory rate data collection are shown schematically below.

EVENTS DURING CHALLENGE



Procedurally, subgroups of four animals were placed into whole-body plethysmographs on day 21. Subsequent subgroups (from the original group of 12 animals that were given sensitization exposures) were challenged on days 22, 23, or 24. Animals were allowed to acclimate for approximately three hours under quiet surroundings. Monitoring of the respiratory activity of each animal was then started and was taken for approximately the next two hours (preexposure period). Animals were then exposed for approximately 60 minutes (exposure) and respiratory rate monitoring continued for the ensuing 16 hours (post-exposure) while animals breathed conditioned room air. Thus for each animal the respiratory activity was monitored continuously for the preexposure, exposure, and post-exposure periods (approximately 20-24 hours of continuous measurement). Nappa Nectar (SE LAB Group, Cincinnati, OH) was provided as a water source (available ad libitum) after the end of exposure during the challenge phase. Approximately 24 hours after being placed in the plethysmographs animals were removed and returned to conventional housing conditions.

Animals were rechallenged to the same atmosphere approximately one week later (challenge week 2) and then challenged with either unconjugated GSA or the appropriate TDI-GSA conjugate during the following week (challenge week 3). The regimen for each challenge with respect to exposure duration and data collection was identical. After the third challenge, animals were sacrificed and necropsied.

Exposures: Generation and Monitoring

Exposure Chamber - Sensitization Phase

Animals were exposed in a nose-only inhalation exposure chamber which had a nominal volume of 75 L. These chambers are constructed of plexiglass, are cylindrical in design, and have attached specially designed guinea pig restrainers with nose cones so as to allow nose-only exposure. These chambers were operated in a dynamic mode (constant airflow) and the airflow was monitored continuously and recorded at five-minute intervals (Labtech software [9]). An exhaust airflow of approximately 23 - 29 Lpm (18 - 23 chamber-volume changes per hour) was used and a static pressure was maintained at approximately -0.02 inch of water.

Exposure Chamber - Challenge Phase

Animals were housed and exposed, unrestrained, in whole-body plethysmographs. These rectangular plethysmographs had a nominal volume of 2.0 liters and were constructed of opaque plexiglass, with an internal source of water (Nappa Nectar; SE LAB Group, Cincinnati, OH). Plethysmographs were operated in a dynamic mode (constant airflow of 1.7 Lpm and a static pressure of approximately -0.4 inch of water). Airflow was recorded twice during the one hour exposure. Each plethysmograph was connected to a mixing chamber (2.0 L) and atmospheres were generated into this mixing chamber for distribution.

Generation of Test Substance Atmosphere

2,4- and 2,6-TDI were generated as a vapor by a bubbler. This generator was maintained at a constant temperature of approximately 25°C during challenge exposures. Dried compressed air was supplied with a flow of approximately 0.25-0.41 Lpm during challenge. Unconjugated GSA and TDI-GSA conjugates were generated as a liquid aerosol in borate buffer (50 mM, pH=9.4, 1.0% w/v solution) using an air-blast type glass nebulizer. This protein solution was delivered by a peristaltic pump.

Generator output was mixed with conditioned room air at the mixing chamber and total airflows were approximately 4 - 18 Lpm for challenge. During sensitization exposures airflows were monitored continuously and were recorded at approximately five-minute intervals (Labtech software [9]). During the challenge phase, airflows were calibrated immediately before animals were placed in plethysmographs and were recorded twice during exposure.

Analytical Concentration

Analytical concentrations of chamber atmospheres were determined by sampling during the exposure period. Chamber concentrations of TDI vapor were quantified by reverse-phase high pressure liquid chromatographic analysis of atmosphere impinger samples after derivation with N-4-nitrobenzyl-N-n-propylamine [10]. Chamber concentrations of TDI-GSA conjugate were quantified gravimetrically after filter sampling (Millipore HAWG filters, pore size = 0.5 μm, sampling flow rate was approximately 1.7 Lpm).

Units of Concentration

All references to the concentration of the chamber atmosphere are expressed as ppb of TDI vapor or mg/m^3 of TDI-GSA conjugate.

Preparation and Characterization of the TDI-GSA Conjugate

Guinea pig serum albumin (GSA) conjugates of 2,4-TDI and 2,6-TDI were prepared by methods previously described [11]. After dialysis and lyophilization the product was characterized as to the percent substitution of TDI onto GSA molecules. This characterization was performed by a TNBS derivitization method [12], and the results consistently indicated a percent of substitution of approximately 85% for each conjugate.

Particle Size Distribution

The particle size character of the TDI-GSA atmospheres generated during this study have previously been assessed [7]. Under identical conditions to those used here, Mass Median Aerodynamic Diameter values for aerosols ranged from 0.87-0.94 μm and associated geometric standard deviation values ranged from 1.3 to 1.4. Thus, the TDI-GSA aerosols used during this study were easily respirable by guinea pigs.

Chamber Monitoring of Temperature and Relative Humidity

The desired ranges for temperature and relative humidity (RH) are, as in our animal housing facility, 18.3-25.5°C and 40-70%, respectively. During sensitization, temperatures and RH were taken by Solomat probes (Pt Chamber, 100/%RH) with transmitters [13]. During challenges, temperatures were taken by hand with a National Institute of Standards and Technology (NIST) certified thermometer twice during exposure. Relative humidity monitoring was not done during challenge exposures in that our Hygroscopic-Dielectric type sensors are incompatible with isocyanates. No suitable substitutes for these sensors are available.

Treatment of Chamber Exhaust

Exhaust air from the exposure chambers and plethysmographs was filtered through an activated-charcoal trap and a HEPA filter before it was vented externally.

Body Weights/Clinical Signs

Individual body weights and clinical observations were recorded just prior to exposure on days 0-4, on days 7 and 14, and before as well as after challenge in the whole-body plethysmographs. Additionally, clinical observations were taken just after exposure on days 0-4.

Gross Pathology

Animals were sacrificed by transecting the posterior vena cava after blood collection. A complete gross necropsy was performed on each guinea pig, which involved an examination of all organs, body cavities, cut surfaces, external orifices, and surfaces. All gross abnormalities were recorded and selected tissues were saved for possible future analysis.

Respiratory Measurements

Data Collection

Respiratory activity was measured during each challenge (from plethysmographs) using Validyne differential pressure transducers (Model Dp 45-16). The analog signal was amplified using a Gould 2400S system and was converted to a digital format using a BUXCO (BUXCO Electronics Inc.) system [14].

Respiratory activity was recorded by the BUXCO system every 0.1 minute from each animal during the preexposure and exposure periods. Generally during the post-exposure period (typically one-half hour after end of exposure) the recording rate was changed to one-minute intervals and this rate was maintained until the end of data collection.

Data Evaluation

The respiratory rate was statistically and graphically evaluated as it is the primary indicator for a respiratory sensitization response. Phases of the physiological response during challenge have been described as "immediate-onset," occurring shortly after commencement of exposure, and "delayed-onset," occurring some hours (typically 5-15) after exposure (see scheme for "events during challenge" vide supra). As animals are continuously monitored for approximately 16 hours after the start of exposure, detection of each phase of a potential response is possible.

Respiratory rate data are collected from the BUXCO system (dBase format [15]) and electronically transferred into a Lotus 1-2-3 [16] spreadsheet. In this format respiratory rate data taken during or after exposure from an individual animal may be compared to the mean respiratory rate for that animal taken before exposure (the preexposure period). These comparisons are consistent with the scheme for data analysis that has been adopted by Karol et al. [4,5]. Mean preexposure respiratory rate values are used to discern a **RESPONSE THRESHOLD** for a particular animal on an individual day of challenge. Exceeding this threshold value is taken as an indicator for a hypersensitivity response. Our specific criteria for identifying responses are based on the work of Karol et al. [1-5]. These are: 1) Immediate-type hypersensitivity reaction: The animal will maintain a respiratory rate greater than the response threshold for at least three consecutive minutes at some point during the first 0.5 hour of exposure. Moreover, the shape of the response curve is such that the respiratory rate will rise from a near normal level to a level that is above the threshold. 2) Delayed-type hypersensitivity reaction: The animal will sustain an increase in respiratory rate that is above the response threshold for at least 15 consecutive minutes. This increase will occur at some point that is from 2 to 16 hours after the start of exposure. Either an immediate-type or delayed-type response will be taken to suggest background levels of response to challenge with 2,4- or 2,6-TDI.

<u>Establishing a Response Threshold</u>: This is a unique value that was determined each time an animal was challenged. The threshold value is based on the average preexposure respiratory rate for each animal. During the preexposure period respiratory rate was collected (0.1 minute samples) for at least two hours. From this data set spurious values were removed and data was retained for a continuous period of time, generally at least one hour in duration, that appeared to represent a quiescent period. This was to establish a baseline for reference, and the mean and standard deviation of this continuous data set was calculated. The limits we have chosen to impose on the response threshold value are 140-160 breaths/minute (bpm); the threshold value will always be within this range. These limits are compared with the calculated values of the preexposure mean + 3 SD and 150% of the preexposure mean. If either of these calculated values is within the range of 140-160 BPM then it is taken as the response threshold. If both are within the range or are either greater than or lower than this range, then the value closer to the preexposure mean + 3 SD is selected. This threshold value is compared to all of the exposure and post-exposure data after spurious point values are removed. Our criteria for selecting a response threshold is consistent with that established previously [4] wherein analysis of data from a large pool of controls indicated that a 3 SD increase from the mean respiratory rate was equivalent to a 47% increase.

Graphical Representation of Data: Respiratory rates are graphically represented from the start of exposure until the end of data collection. In each case two graphs are included: 1) one that includes the data from the exposure period (one hour) plus approximately the first 0.5 hour after the end of exposure (all data collected at 0.1 minute intervals) and 2) one that presents the post-exposure period from approximately 1.5 hour after the start of exposure until the end of data collection (1.0 minute intervals for data collection). These two graphs are to facilitate the identification of immediate-type and delayed-type hypersensitivity reactions, respectively. To prepare the data for graphing, first spurious points are removed, then in sequence every pair of values is averaged together, thus reducing the data set by half, finally a moving average of groups of 15 values are sequentially calculated to generate a set of smoothed data for graphing (i.e., graphing data point #1 is the mean of data values 1-15, graphing point #2 is the mean of values 2-16, etc.). The numerical manipulations of data were performed on Lotus 1-2-3 [16], while graphs were prepared using a Mass-11 Draw package (Microsystems Engineering Corporation) after transferal of data from Lotus. This smoothing of data prior to graphing is essential so as to present interpretable figures. Each graph contains information such as the exposure concentration, the response threshold, and trends in respiratory rate as a function of time from the start of exposure.

Statistical Analysis

Respiratory parameters collected by the BUXCO System [14] were transferred to dBase IV [15] files to delete those parameters not relevant to this study. These dBase files were then transferred to a Lotus 1-2-3 [16] spreadsheet to compute means and descriptive statistics. Other descriptive statistics were generated by Lotus 1-2-3 [16] as needed.

Archival Procedures

The protocol, raw data, a sample of the test substance, and the final report will be archived at locations specified by Miles Inc., Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, Kansas 66085-9104.

RESULTS AND DISCUSSION

Animal Care and Housing

Continuous monitoring of the room housing guinea pigs during this study showed that there were no deviations from the desired range for temperature (18.3 to 25.5°C). On 14 separate days the room RH was 1 or 2% below the desired range (40-70%). These excursions ranged from 11 to 170 minutes in duration and did not affect the outcome of these experiments.

Chamber Parameters

Temperature and % RH

Weekly mean values for chamber temperature and RH during the sensitization phase (days 0-4) from all groups ranged from 20.4 to 21.5°C and 52 to 61% respectively. Weekly mean values for temperature and RH per group are:

Group 1: 21.1°C and 52% (range of daily means: 20.5-21.9°C and 48-60%, respectively)

Group 2: 21.6°C and 55% (range of daily means: 20.6-22.0°C and 43-64%, respectively)

Group 3: 20.4°C and 61% (range of daily means: 19.4-20.9°C and 59-62%, respectively)

During the challenge phase, plethysmograph temperature readings were taken approximately 3 hours or 20 hours after animals were enclosed. The range of temperature values recorded was 20-25°C (inclusive of all measurements, n=82). Plethysmograph temperatures were similar between groups; means of recorded values from each group were 23°C, 23°C and 24°C (groups 1, 2 and 3, respectively). No routine monitoring of RH was possible during the challenge phase.

Summarily, these data indicate that animals on this study were not subjected to physical stresses related to either temperature or relative humidity. All chamber temperature and % RH values recorded were within the desired range for our facility (18.3 to 25.5°C and 40 to 70% RH, respectively).

Exposure Concentrations

Challenge Phase

The choice of TDI concentration during challenge should be low enough so as to avoid a sensory irritation type response but high enough to provoke a hypersensitivity type response should animals be sufficiently predisposed. Concentrations of 2,4- or 2,6-TDI that were used during this study were consistent with those used previously [7,4,1].

Individual challenge concentrations for vaporous and GSA-conjugated TDI are presented in Appendix II. Weekly mean values were very consistent and ranged from 20 to 37 ppb for TDI vapor and from 30 to 38 mg/m³ for the TDI-GSA-conjugate.

Biologic Observations and Measurements

Clinical Observations

No clinical signs were observed from any animal during the course of this study.

Body Weights

A summary of the body weight data collected during this study is presented in Table 2, while individual values are presented in Appendix I. These data indicate that all groups showed weight gain at each time point through day 14. A few animals from group 3 lost weight between day 14 and week 1. This is due to alteration of drinking procedures and is not considered to be related to exposures. It is also noted that animals from all groups lost a substantial amount of weight during the course of each challenge.

Mortalities and Replacement Animals

There were no exposure-related mortalities and no animals were replaced during this study.

Gross Pathology

Each animal was gross necropsied after the final challenge and no lesions were observed from any animal.

Physiological Indications of Hypersensitivity

Response threshold values were not disparate between groups. Average response threshold values from each of the three groups (means of all animals from all three weeks of challenge) were 142, 148, and 142 bpm (groups 1-3, respectively). Individual response threshold values are presented in Appendix II. This information indicates that the criteria used to assess hypersensitivity reactions was not dissimilar between groups.

A summary of the incidence of apparent hypersensitivity responses from each group is presented in Table 3, while individual responses are identified in Appendix II. This table identifies both immediate- as well as delayed-type responses. Additionally, individual respiratory rate patterns during the exposure and post-exposure periods of each challenge conducted during this study are presented in Figures 1 - 108 (information is included on each figure concerning exposure concentration, animal number, response threshold, and etc.; see the "Guide to Figures" for more explanation).

As indicated in Table 3, apparent immediate-type hypersensitivity reactions were only sporadically observed. A total of five incidences were recorded, three from group 1 and two from group 3. No outstanding differences were observed in the incidence of apparent immediate-type hypersensitivity reactions between sham-challenges or those with TDI vapor and challenges with native or conjugated GSA.

A greater incidence of apparent delayed-type hypersensitivity reactions were observed from each group by comparison to apparent immediate-type responses. A total of 11 apparent delayed-type responses were noted from group 1, a total of eight apparent responses were noted from group 2, and a total of two apparent responses were noted from group 3. The week 1 challenges from group 1 showed by far the highest incidence of apparent delayed-type responses; 80%. This incidence is unreasonably high from animals that are sham-challenged and suggests that problems of a technologic or data-acquisition nature occurred during this week. Maintenance and reparative work was conducted on the air handling systems of our laboratories during this week and the impact of this disturbance cannot be assessed. Thus this data was excluded from further analysis.

All animals were sham sensitized, thus stimulus was not provided for the development of an authentic hypersensitivity response. With respect to findings of apparent delayed-type responses, it is improbable that each of these observations represent a true hypersensitivity response. This suggests that the criteria used for identification of an apparent delayed-type hypersensitivity response may be too sensitive, and thus may deserve further refinement. Authentic hypersensitivity responses would be expected to recur upon subsequent challenge. Thus assessment of response recurrence upon sequential challenge provides a means to distinguish between sporadic responses and those suspected to be associated with true hypersensitivity.

One indication that an apparent response may be the consequence of authentic hypersensitivity is a recurrence of a particular type of response during our regimen of three individual challenges. Table 4 presents the incidence of repeated immediate- as well as delayed-type apparent hypersensitivity responses from each of the three groups. To be included in this incidence table a particular animal must have shown the same type of apparent response on at least two out of the three challenges. This table shows a low incidence of repeated responses from each group. Only one repeated immediate-type and one repeated delayed-type response were observed (from groups 1 and 2, respectively). In that the incidence of apparent responses or recurrent responses did not substantially differ between groups, each of these groups is considered to be indistinguishable with respect to apparent hypersensitivity reactions during challenge.

Summarily, these data suggest a low but considerable incidence of apparent hypersensitivity responses from each group. In that each of these groups represent control animals, the incidence of response observed in this study is considered to be a background level for this test system.

SUMMARY AND CONCLUSIONS

Background levels of respiratory hypersensitivity-like reactions to either 2,4- or 2,6-TDI were evaluated in male guinea pigs. Three groups were sham-sensitized and subsequently were challenged three times (at one week intervals) with either isomer of TDI or were sham-challenged. In each case, the final challenge was to GSA; either the native protein or the appropriate TDI conjugate. No clinical signs, lesions at gross necropsy, or substantial differences in weight-gain between groups were noted. Sustained increases in respiratory rates were taken as indicators for apparent immediate-type and delayed-type responses. A similar incidence of apparent immediate-type responses was observed between groups, while fewer delayed-type responses were observed from the group challenged with 2,6-TDI than from other groups. Animals from each group appeared to demonstrate more delayed-type rather than immediate-type apparent responses. Few recurrences of a specific type of response were seen during the three weeks of challenge, thus, the background level of hypersensitivity to TDI in guinea pigs appears to be low.

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Table 1

Summary of Plethysmograph Concentrations During Challenge

Study Number 92-344-PP

Group	Challenge Atmosphere	Challenge Week	n=	Concentration (Mean + SD)
1	Sham	1	12	NA
	Sham	2	12	NA
	GSA	3	12	38 ± 9 mg/m³
2	2,4-TDI	1	12	20 ± 8 ppb
	2,4-TDI	2	9	27 ± 12 ppb
	2,4-TDI-GSA	3	12	32 ± 14 mg/m³
3	2,6-TDI	1	12	37 ± 18 ppb
	2,6-TDI	2	12	37 ± 18 ppb
	2,6-TDI-GSA	3	12	30 ± 6 mg/m³

SD = Standard Deviation
NA = Not Applicable

Table 2

Summary of Body Weights During a Respiratory Sensitization Study with 2,4- or 2,6-TDI

Study Number 92-344-PP

Group	Initial Body Weight Range		Analysis of Body Weights (g) Day of Study[1] 0	4	7	14	Mean Weight Change During Challenge (g) Week 1	Week 2	Week 3
1 (Sham)	243-275	Avg	258	287	307	361	-32	-33	-45
		SD	11	13	14	22			
		MC		29	20	54			
2 (2,4-TDI)	241-280	Avg	263	293	320	374	-43	-54	-56
		SD	13	18	22	31			
		MC		30	27	54			
3 (2,6-TDI)	255-296	Avg	272	293	319	357	-32	-42	-43
		SD	13	15	17	24			
		MC		21	26	38			

SD = Standard Deviation
MC = Mean Change
[1] = Day 0 denotes first day of exposure.
[2] = Averages are presented for all animals challenged. For individual data, see Appendix I.

Table 3

Incidence of Apparent Hypersensitivity Responses Observed During Challenge

Study Number 92-344-PP

	Incidence of Apparent Response*					
	Immediate			Delayed		
	Wk. 1	Wk. 2	Wk. 3	Wk. 1	Wk. 2	Wk. 3
Group 1 (Sham; GSA)**	2/12 (17%)	0/12 (0%)	1/12 (8%)	8/10 (80%)	3/9 (33%)	0/12 (0%)
Group 2 (2,4-TDI; 2,4-TDI-GSA)	0/12 (0%)	0/12 (0%)	0/12 (0%)	4/11 (36%)	2/12 (17%)	2/12 (17%)
Group 3 (2,6-TDI; 2,6-TDI-GSA)	0/12 (0%)	1/12 (8%)	1/12 (8%)	0/10 (0%)	0/11 (0%)	2/12 (17%)

* Responses during each week of challenge / group size.
** Material used for challenge exposures (weeks 1, 2 and 3); see text.

Table 4

Incidence of Repeated Apparent Responses Observed During Three Weeks of Challenge

Study Number 92-344-PP

Challenge Group[1]	Incidence[2]	
	Immediate	Delayed
Sham	1	0[3]
2,4-TDI	0	1
2,6-TDI	0	0

[1] Material used for exposures; see text.

[2] Number of animals showing the same type of apparent response during two out of three weeks of challenge.

[3] Apparent delayed-type responses from challenge week 1 were excluded from this analysis.

GUIDE TO FIGURES

One page of Figures is presented for each challenge of each animal. Included are separate graphs for approximately the first 1.5 hours after the start of exposure and a second graph for the remaining duration of data collection. These are presented separately so as to facilitate identification of suspected immediate-type and delayed-type responses, respectively. The response threshold is indicated on each graph and the actual exposure concentration for the indicated animal is shown within a shaded area that indicates the actual time of exposure. Also included on each Figure is information regarding the animal challenged and the atmosphere compositions for sensitization and challenge exposures.

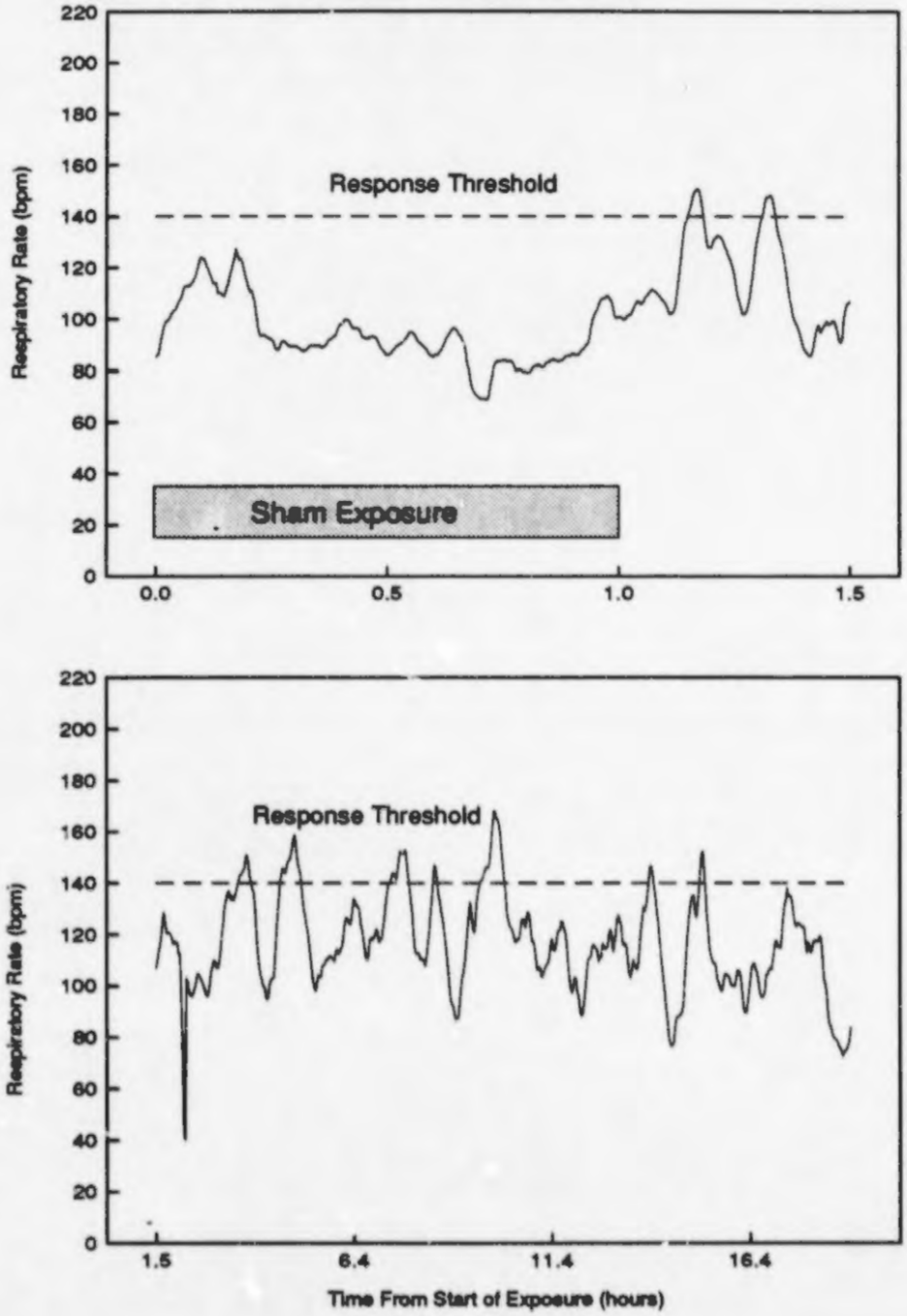
Each Figure is separately numbered from 1 to 108. They are presented in the order of groups 1 - 3, and, for each group data for the first week of challenges are presented in sequence and these are followed by data from the second and then finally the third week of challenge. The following table identifies figure numbers that are associated with each group.

Group No.	Figure No.
1	1-36
2	37-72
3	73-108

Study Number 92-344-PP

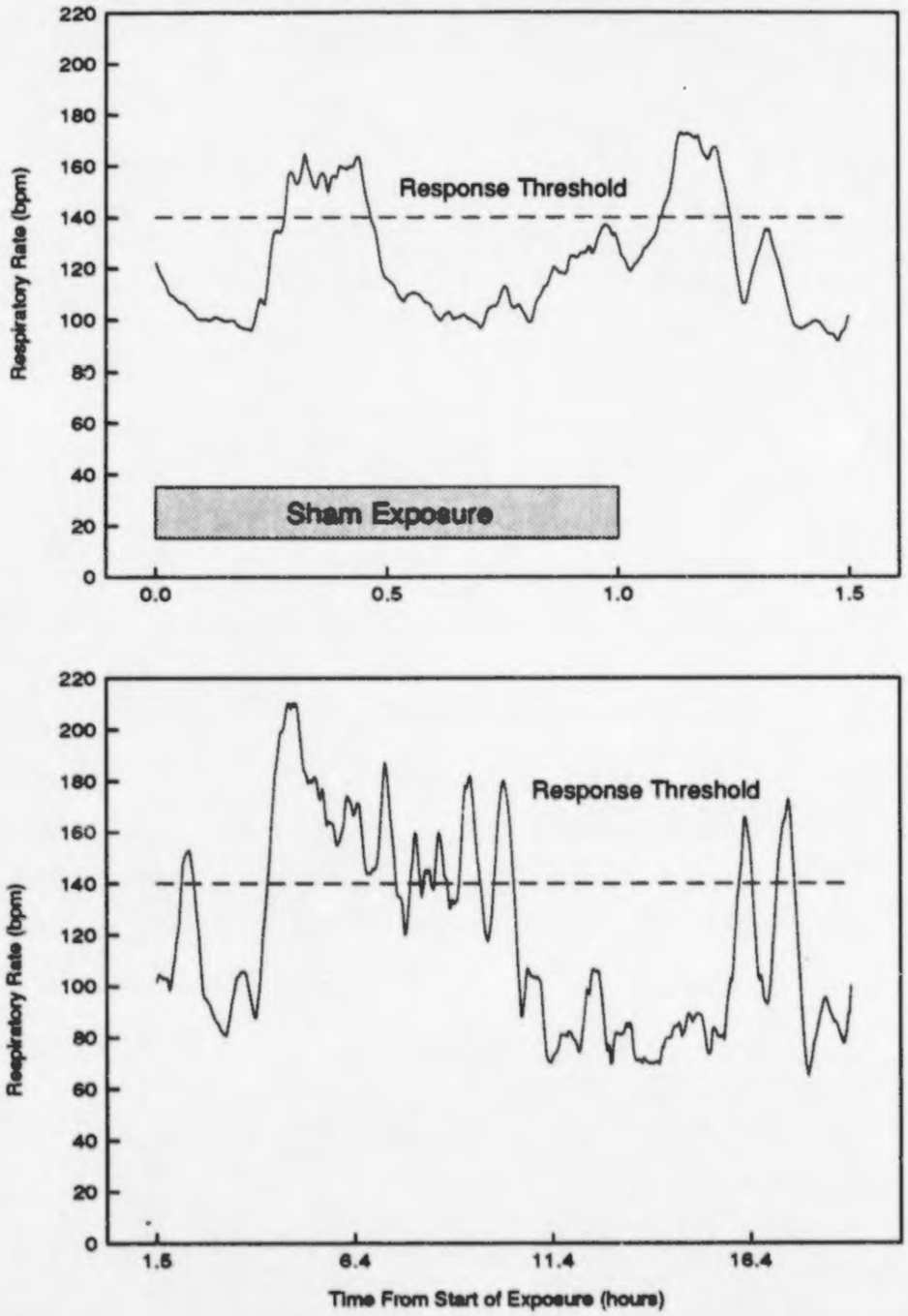
Animal # 193: Sham Sensitization

Figure: 1 Sham Challenge Week 1

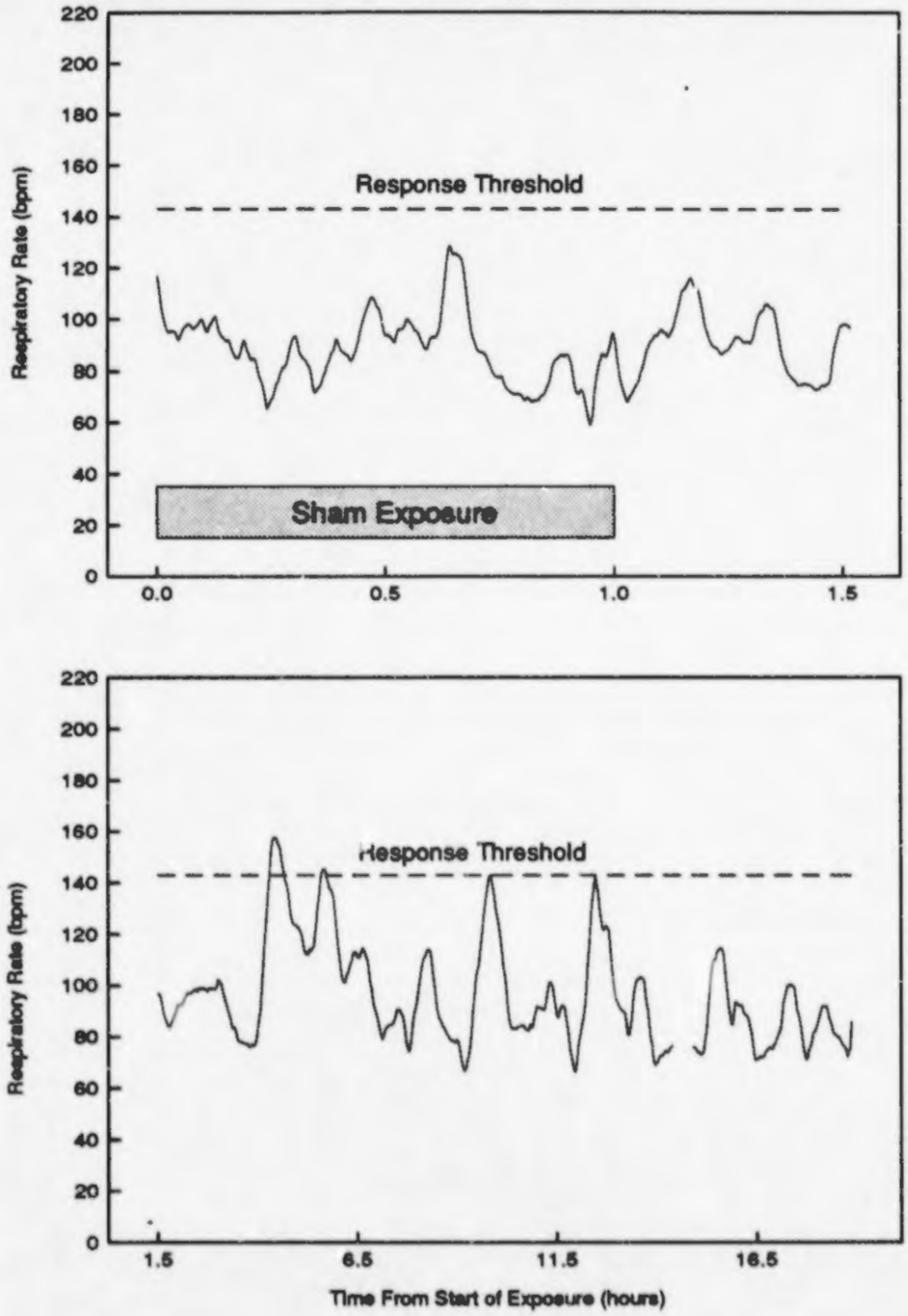


Study Number 92-344-PP
Animal # 194: Sham Sensitization
Sham Challenge Week 1

Figure: 2

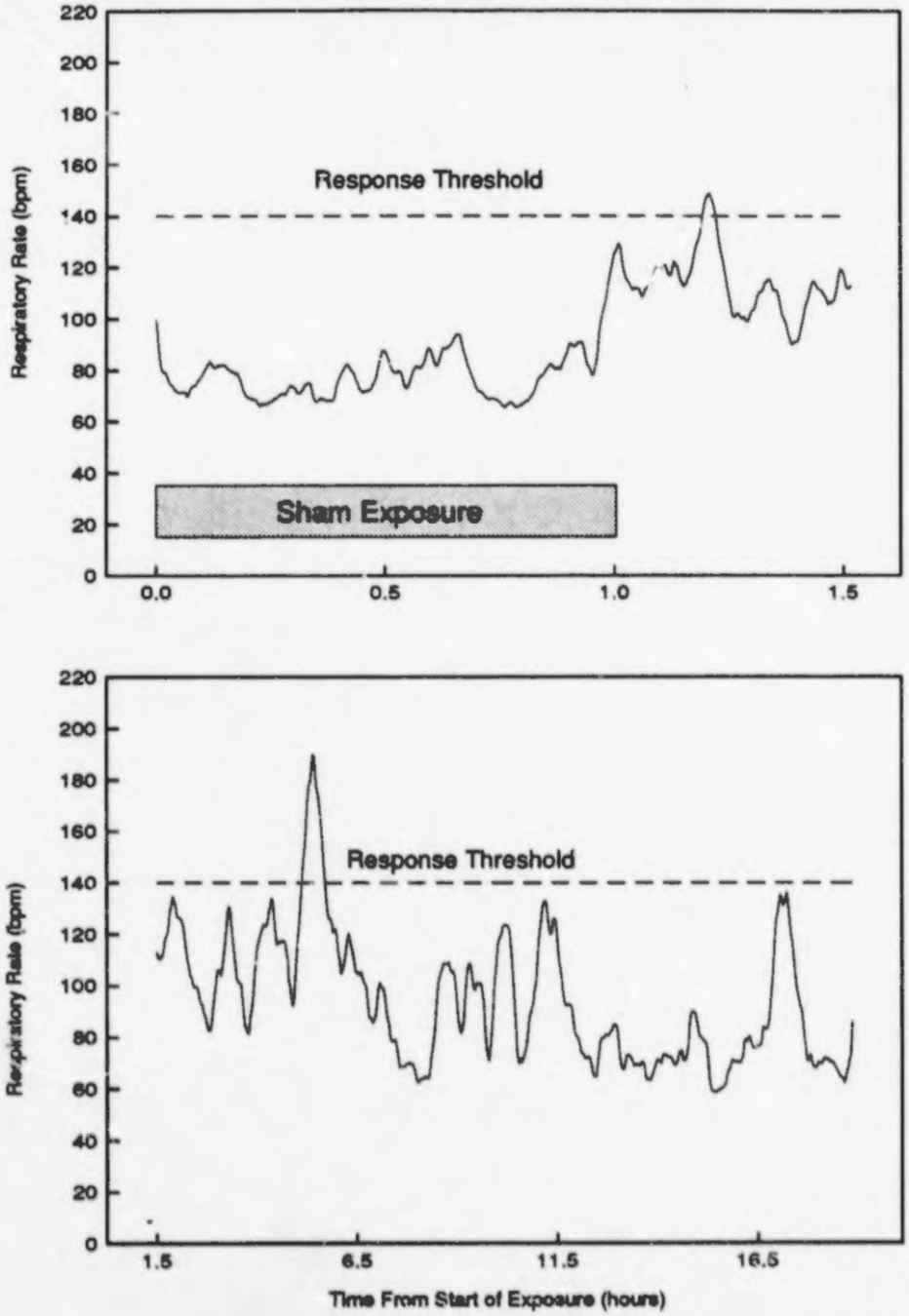


Study Number 92-344-PP
Animal # 195: Sham Sensitization
Figure: 3 Sham Challenge Week 1



Study Number 92-344-PP
Animal # 196: Sham Sensitization
Sham Challenge Week 1

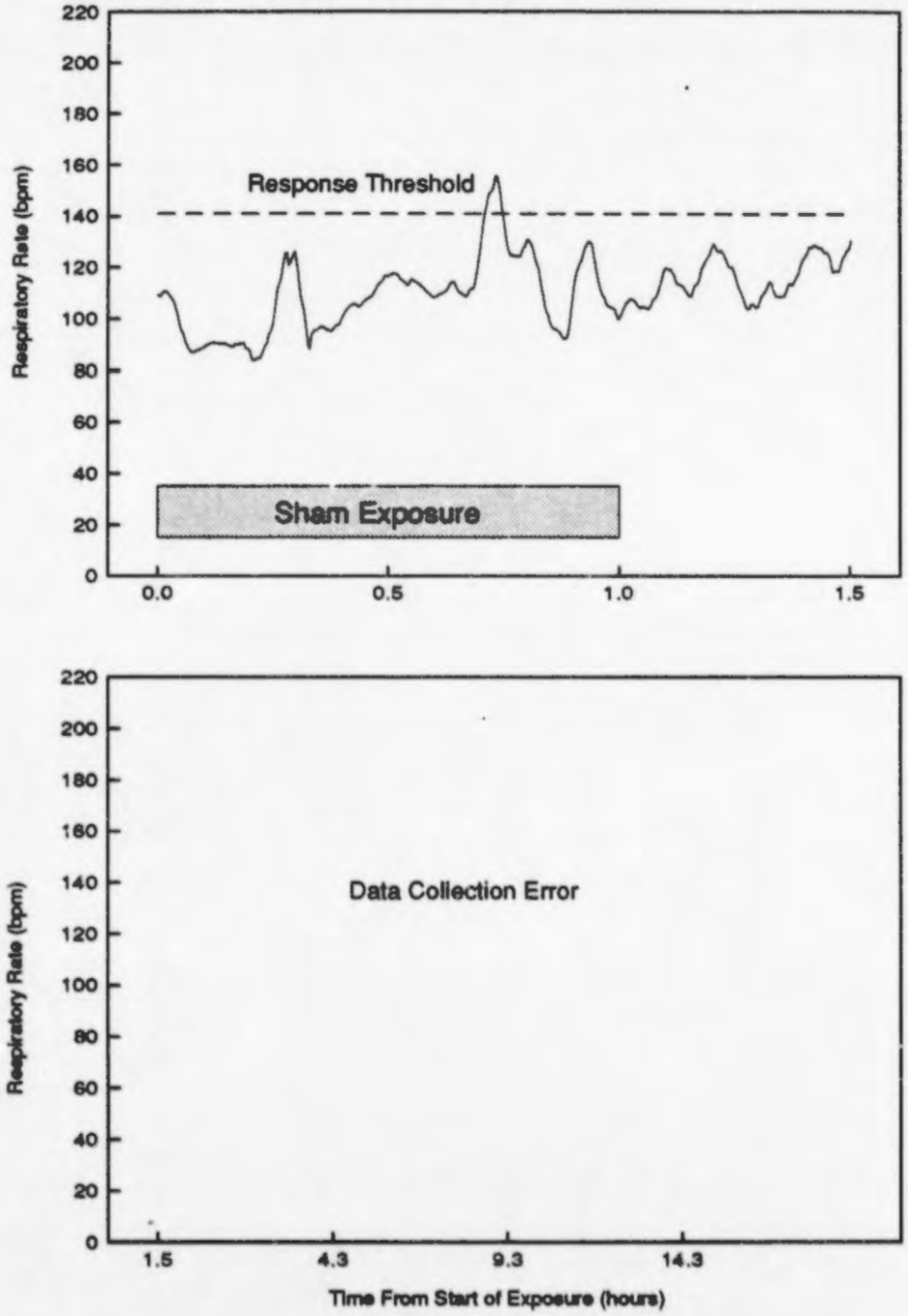
Figure: 4



Study Number 92-344-PP

Animal # 197: Sham Sensitization

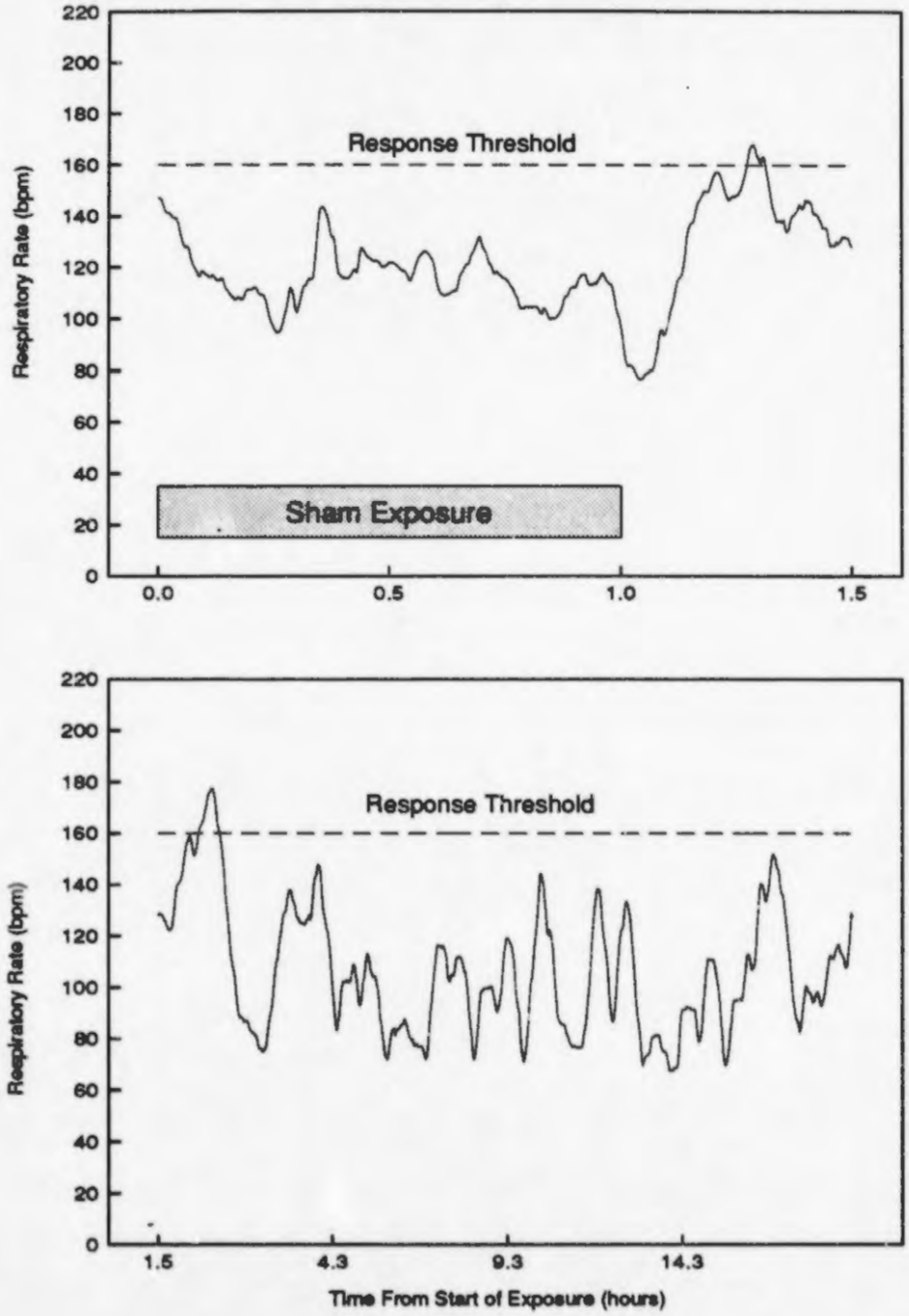
Figure: 5 Sham Challenge Week 1



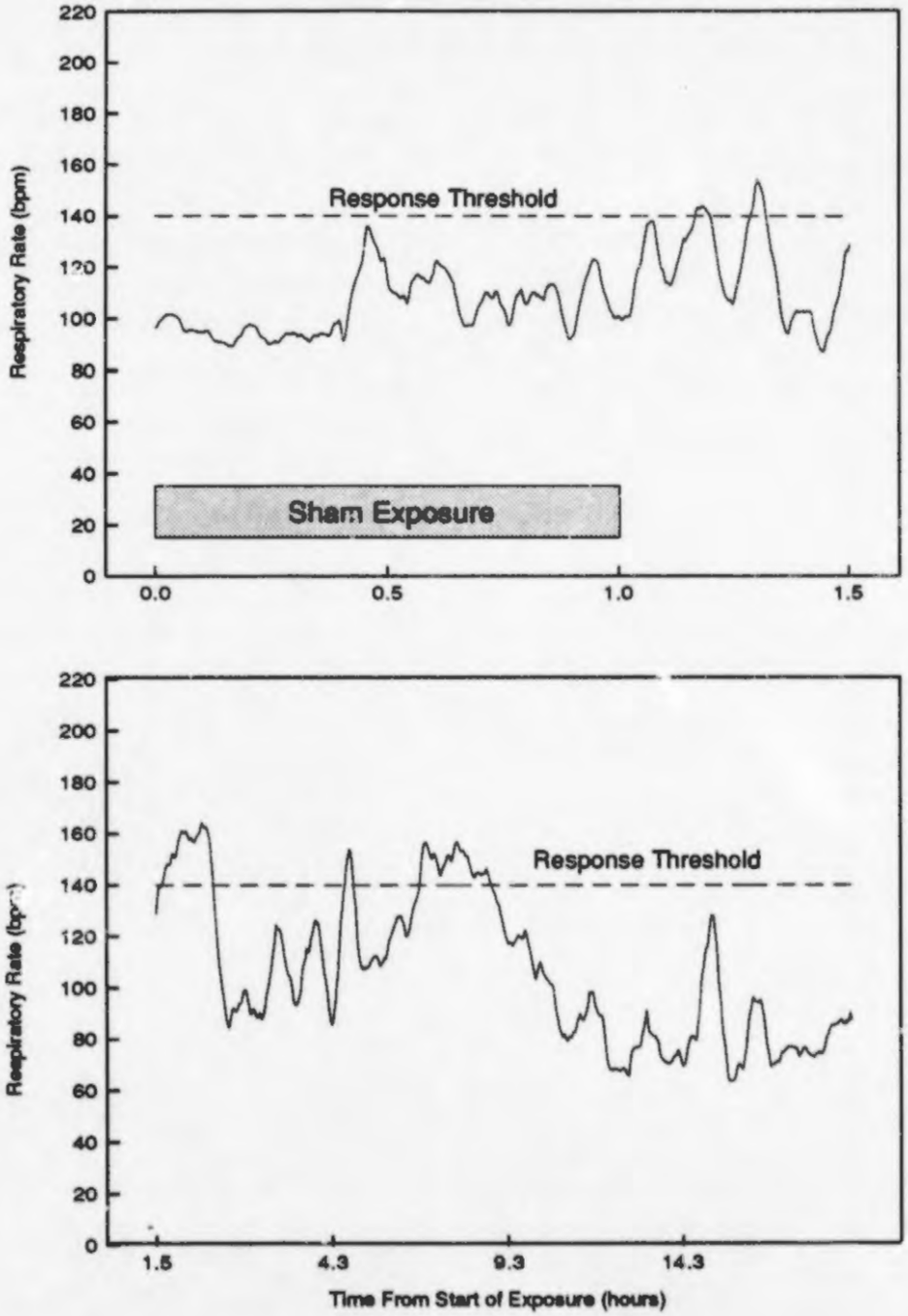
Study Number 92-344-PP

Animal # 198: Sham Sensitization

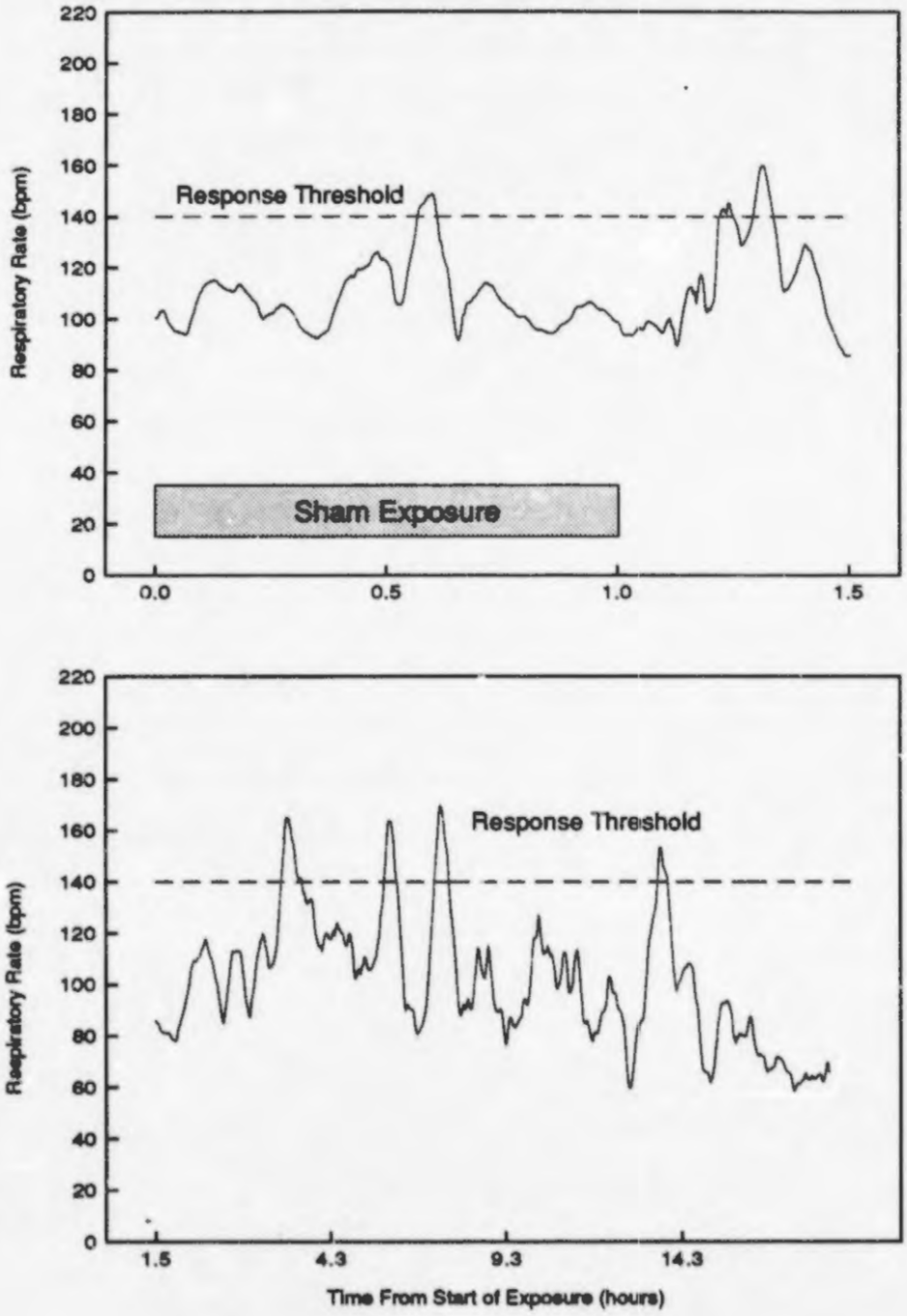
Figure: 6 Sham Challenge Week 1



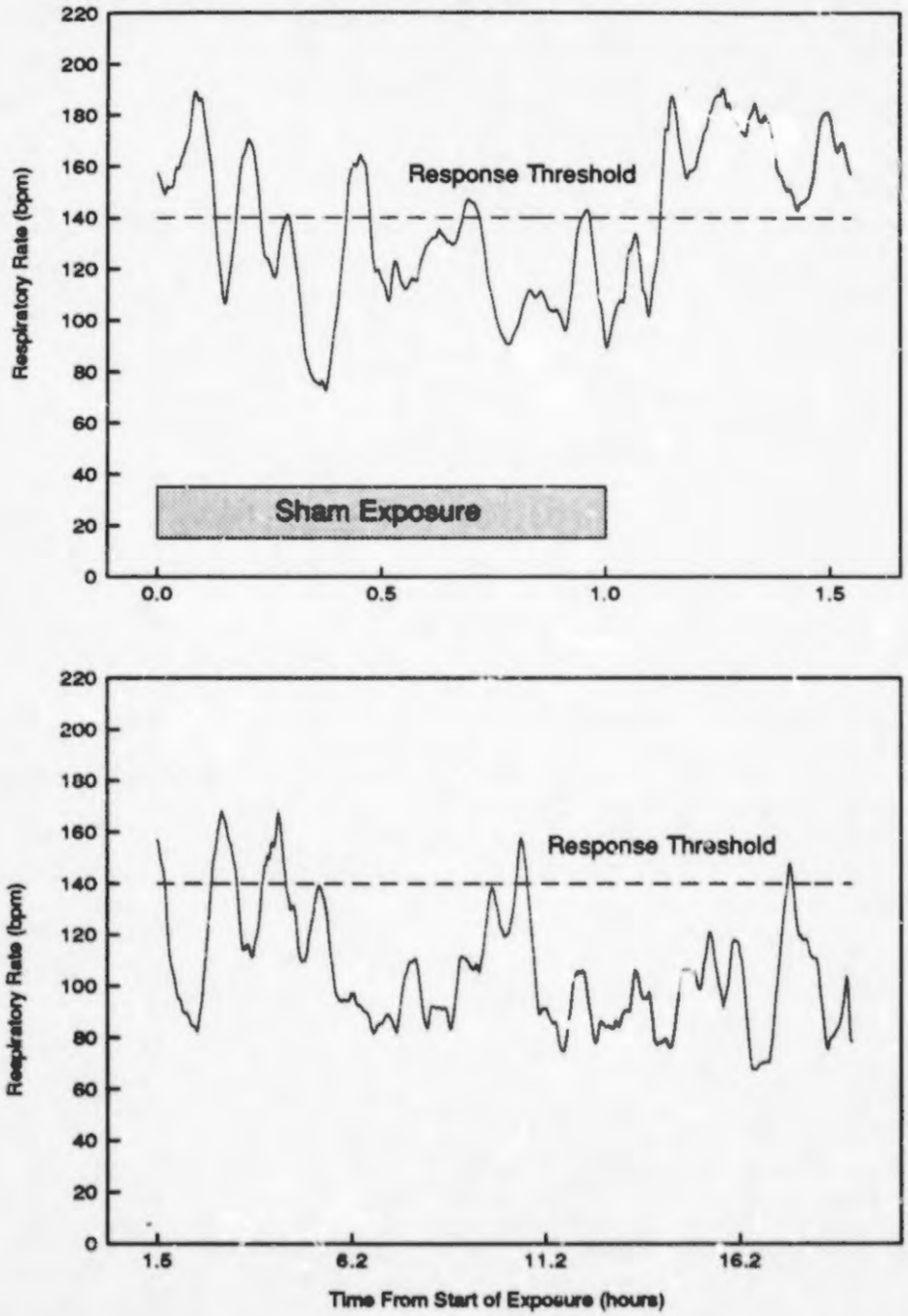
Study Number 92-344-PP
Animal # 199: Sham Sensitization
Figure: 7 Sham Challenge Week 1



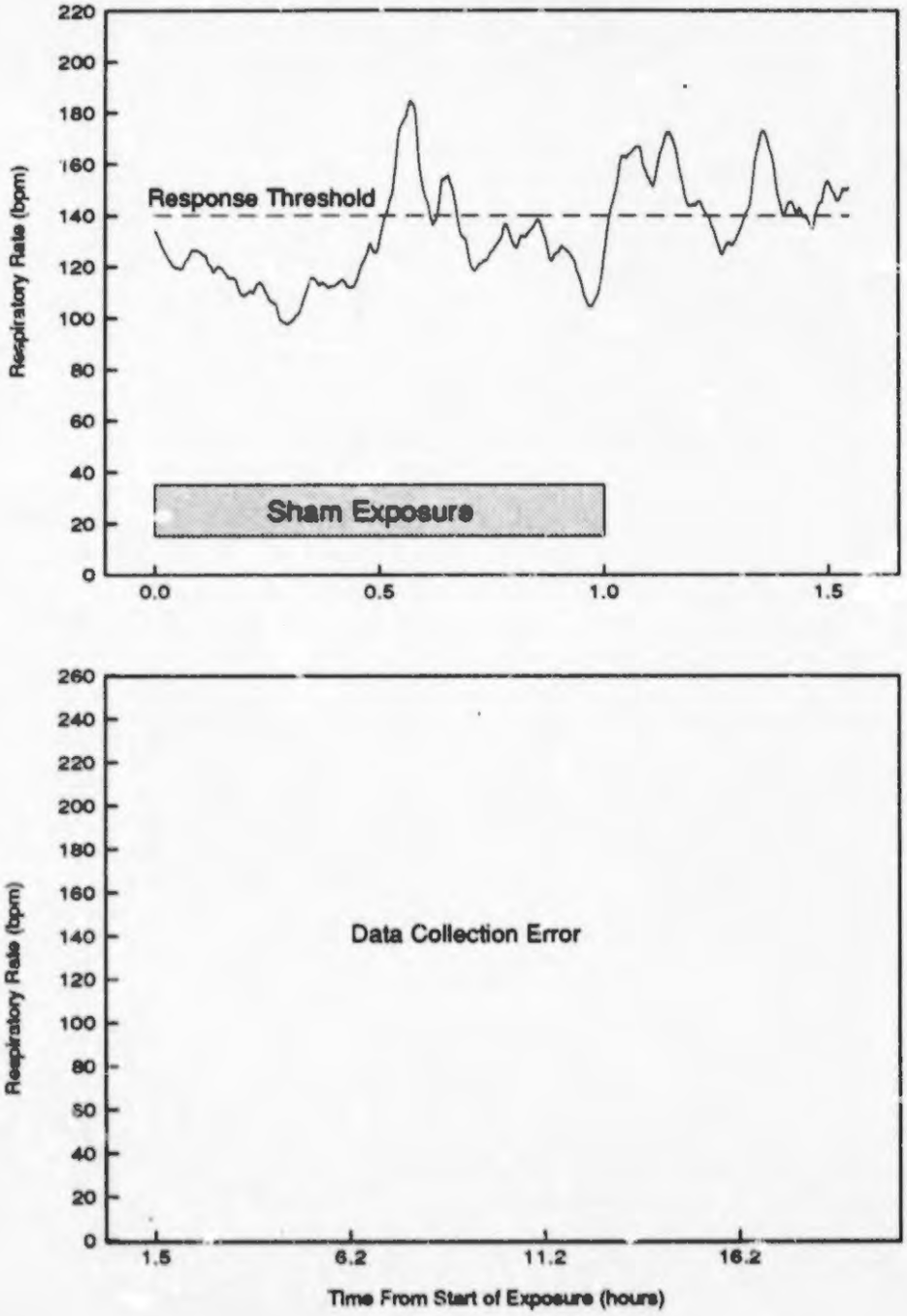
Study Number 92-344-PP
Animal # 200: Sham Sensitization
Figure: 8 Sham Challenge Week 1



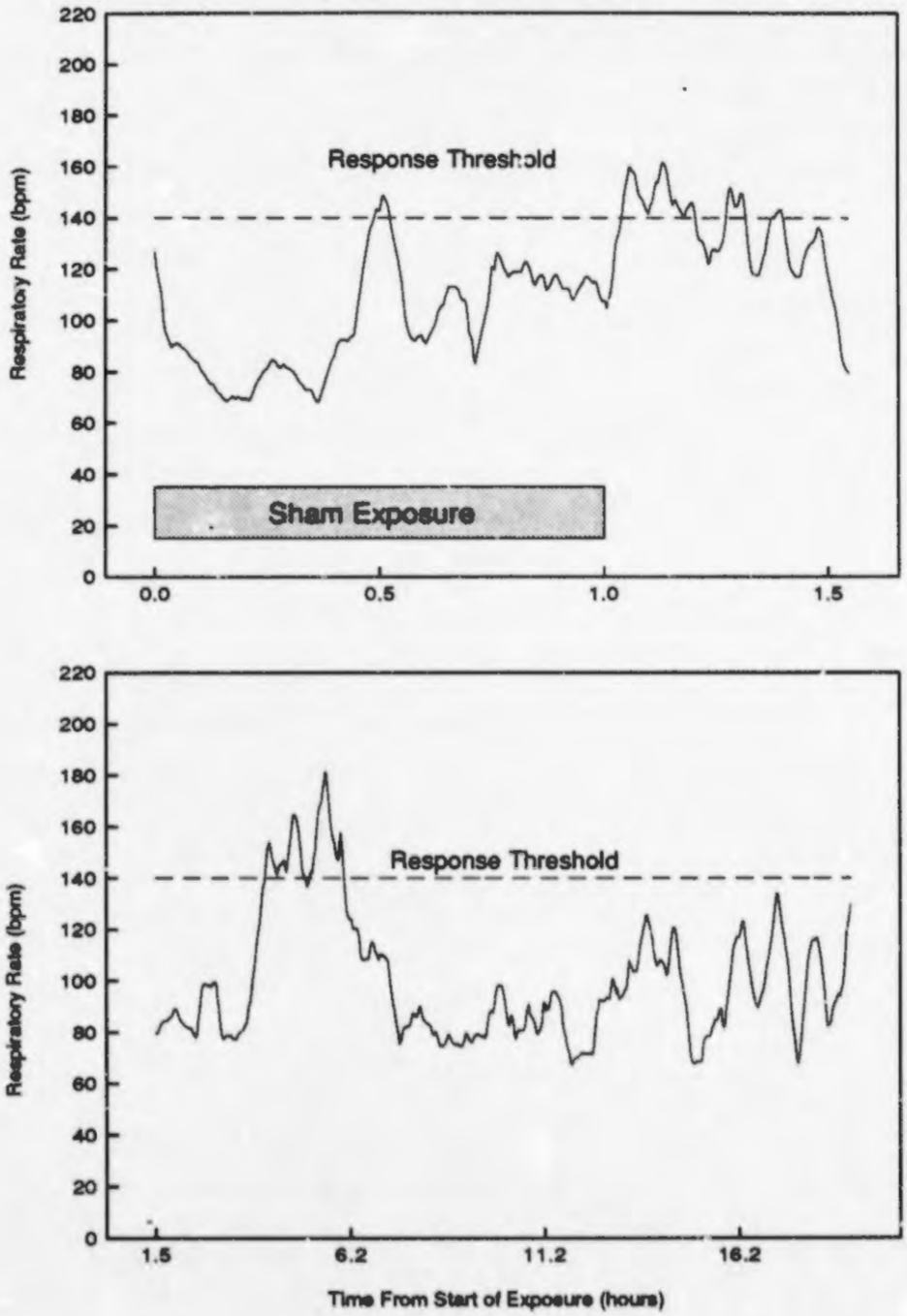
Study Number 92-344-PP
Animal # 201: Sham Sensitization
Figure: 9 Sham Challenge Week 1



Study Number 92-344-PP
Animal # 202: Sham Sensitization
Figure: 10 Sham Challenge Week 1



Study Number 92-344-PP
Animal # 203: Sham Sensitization
Figure: 11 Sham Challenge Week 1

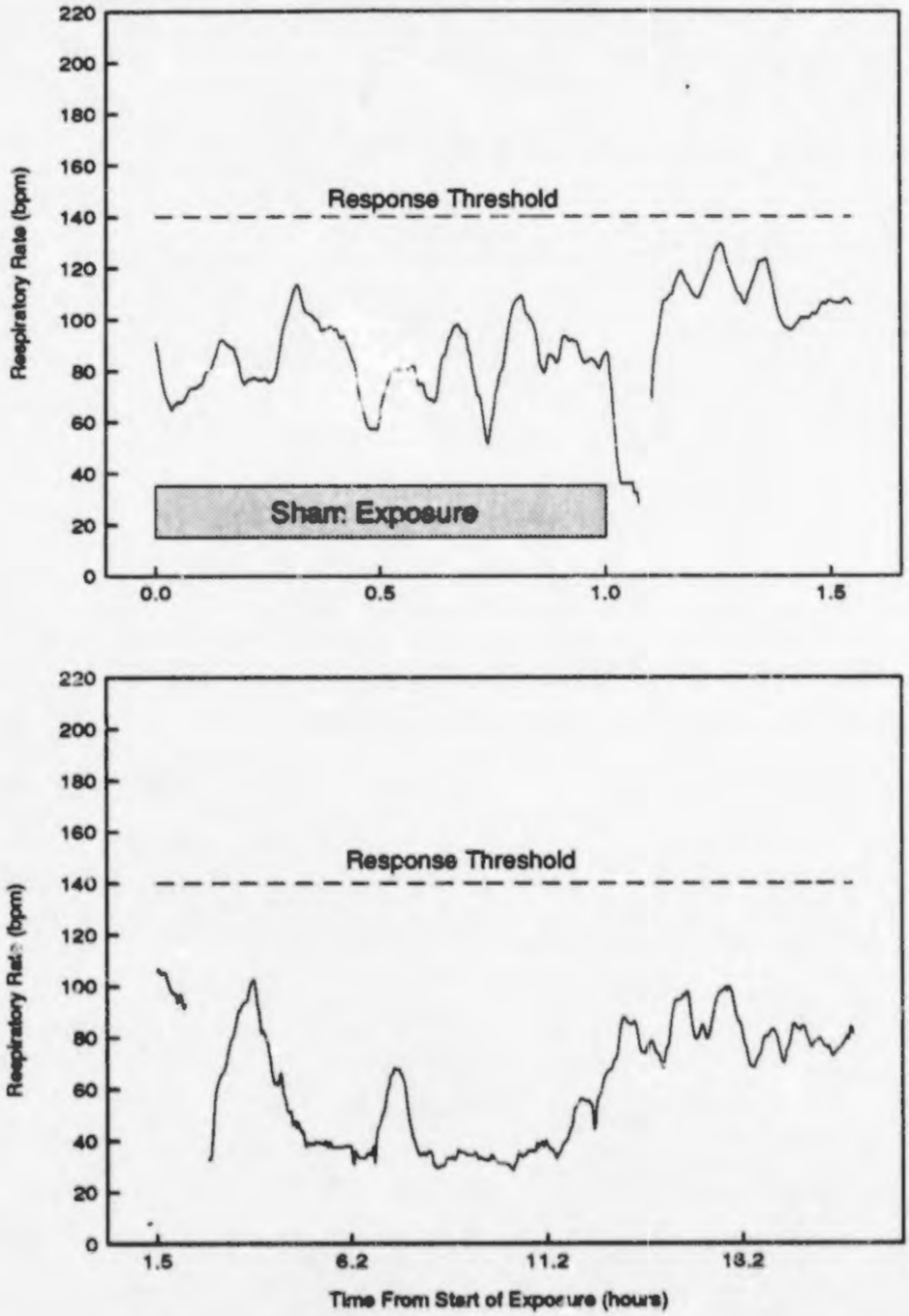


0 0 4 3

Study Number 92-344-PP

Animal # 204: Sham Sensitization

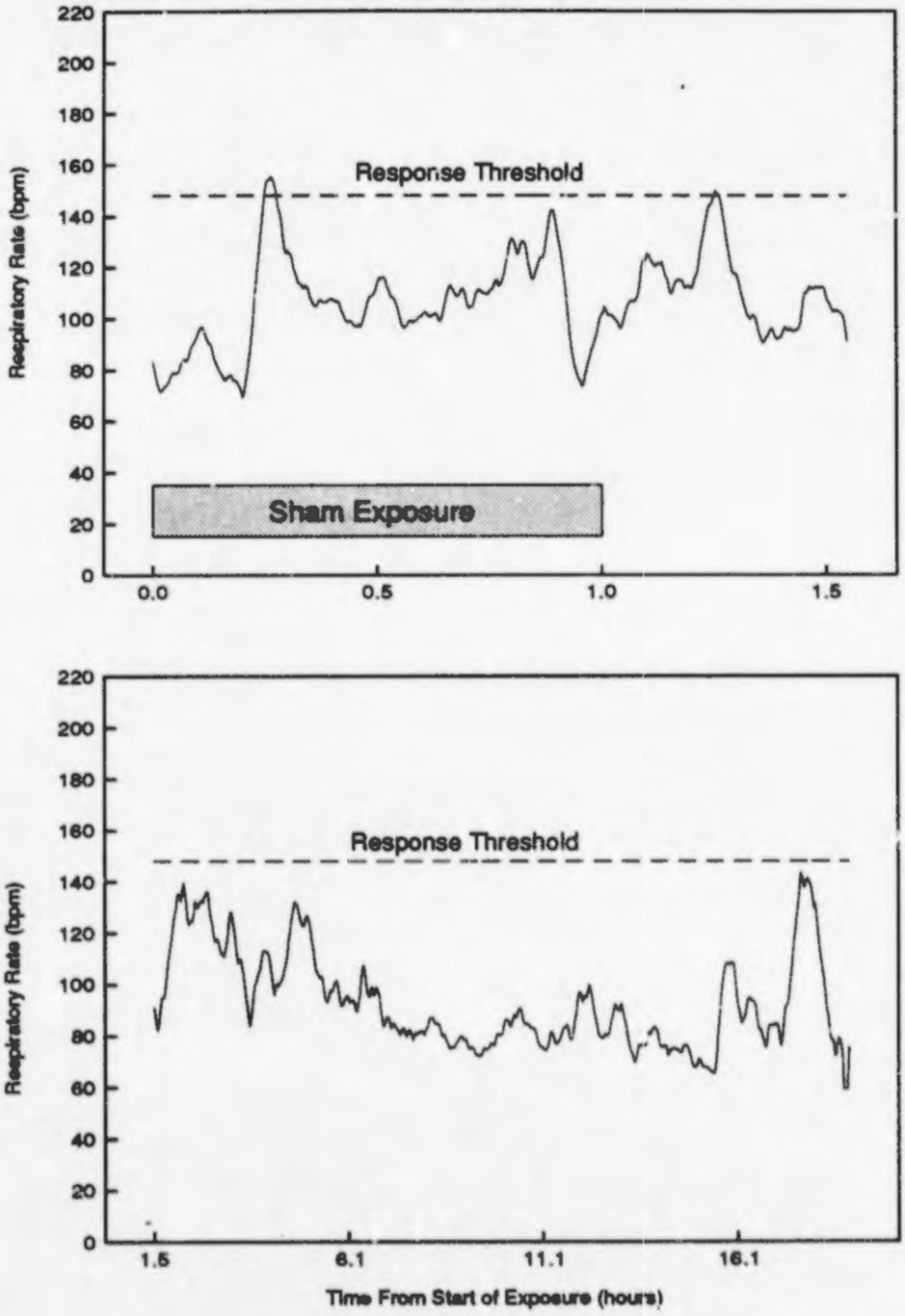
Figure: 12 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 193: Sham Sensitization

Figure: 13 Sham Challenge Week 2



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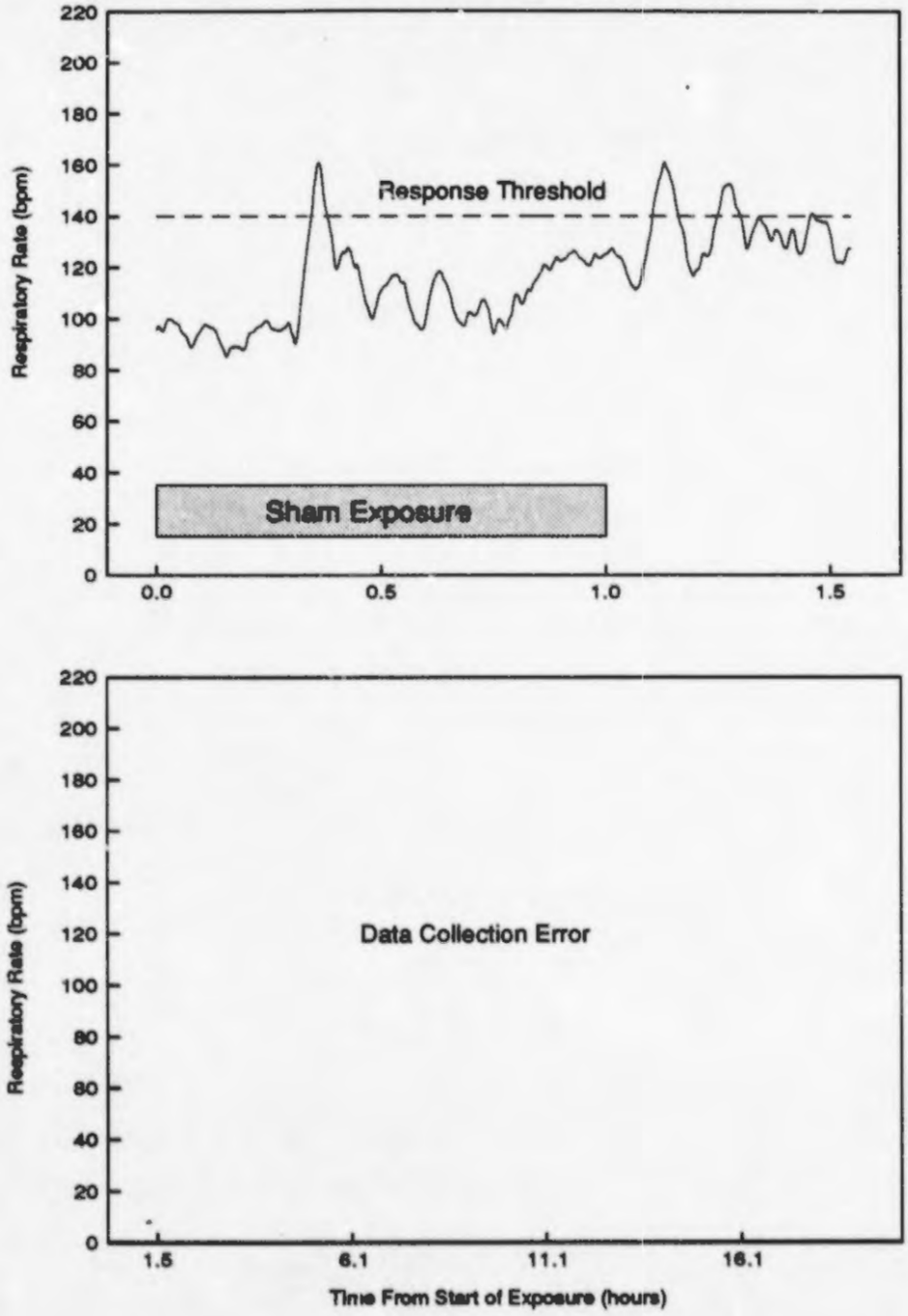
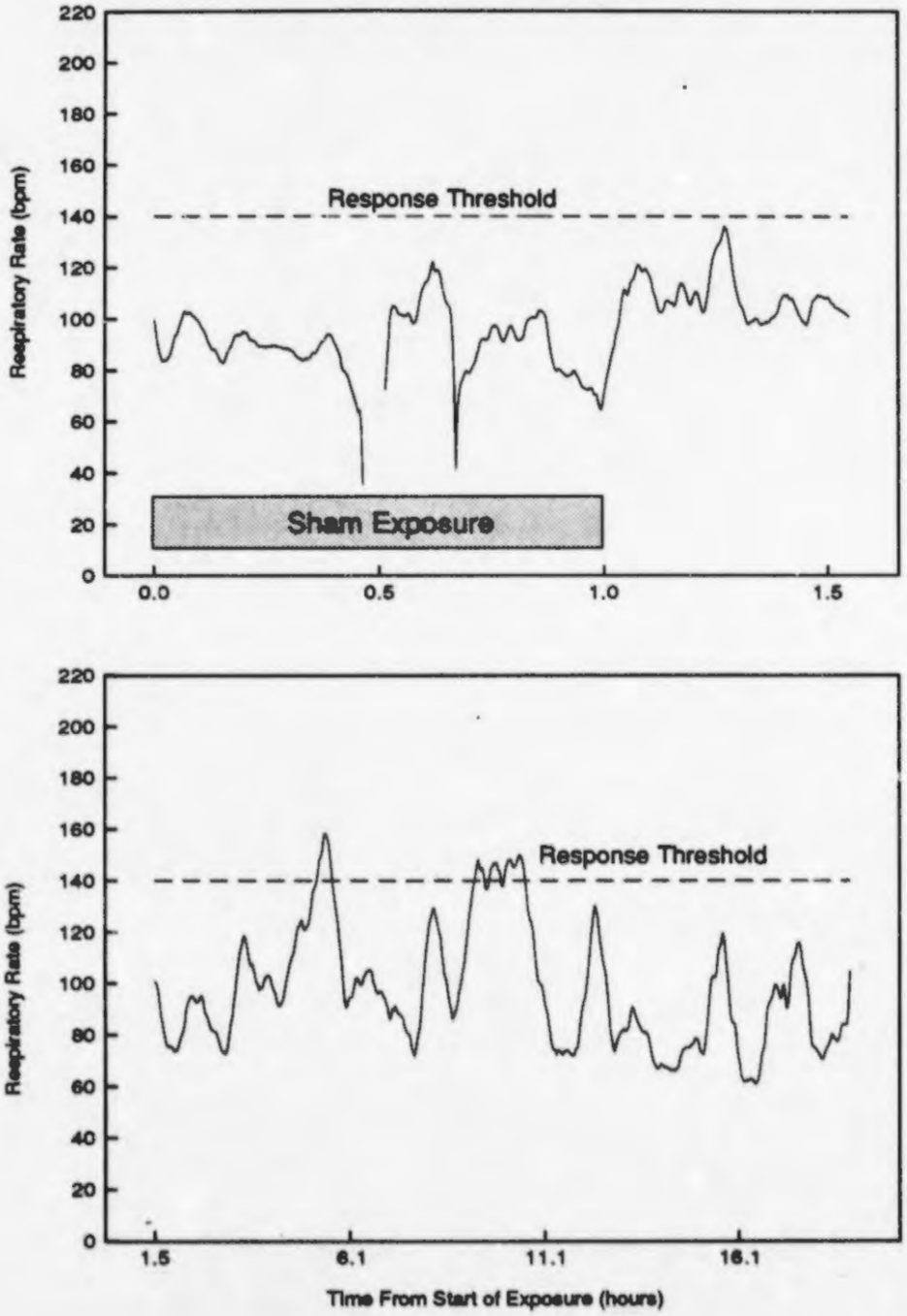
Study Number 92-344-PP
Animal # 194: Sham Sensitization
Figure: 14 Sham Challenge Week 2



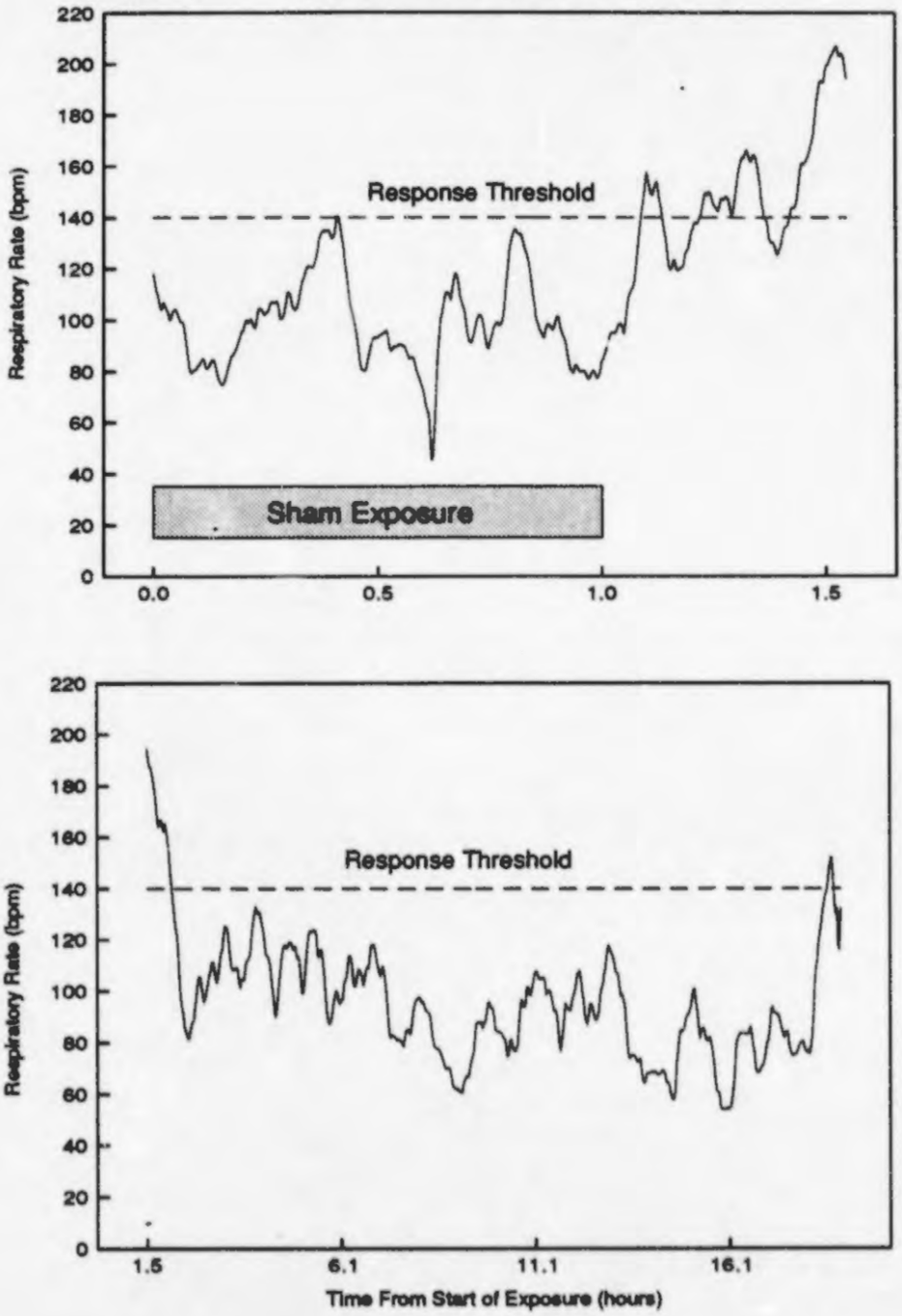
Study Number 92-344-PP
Animal # 195: Sham Sensitization
Figure: 15 Sham Challenge Week 2



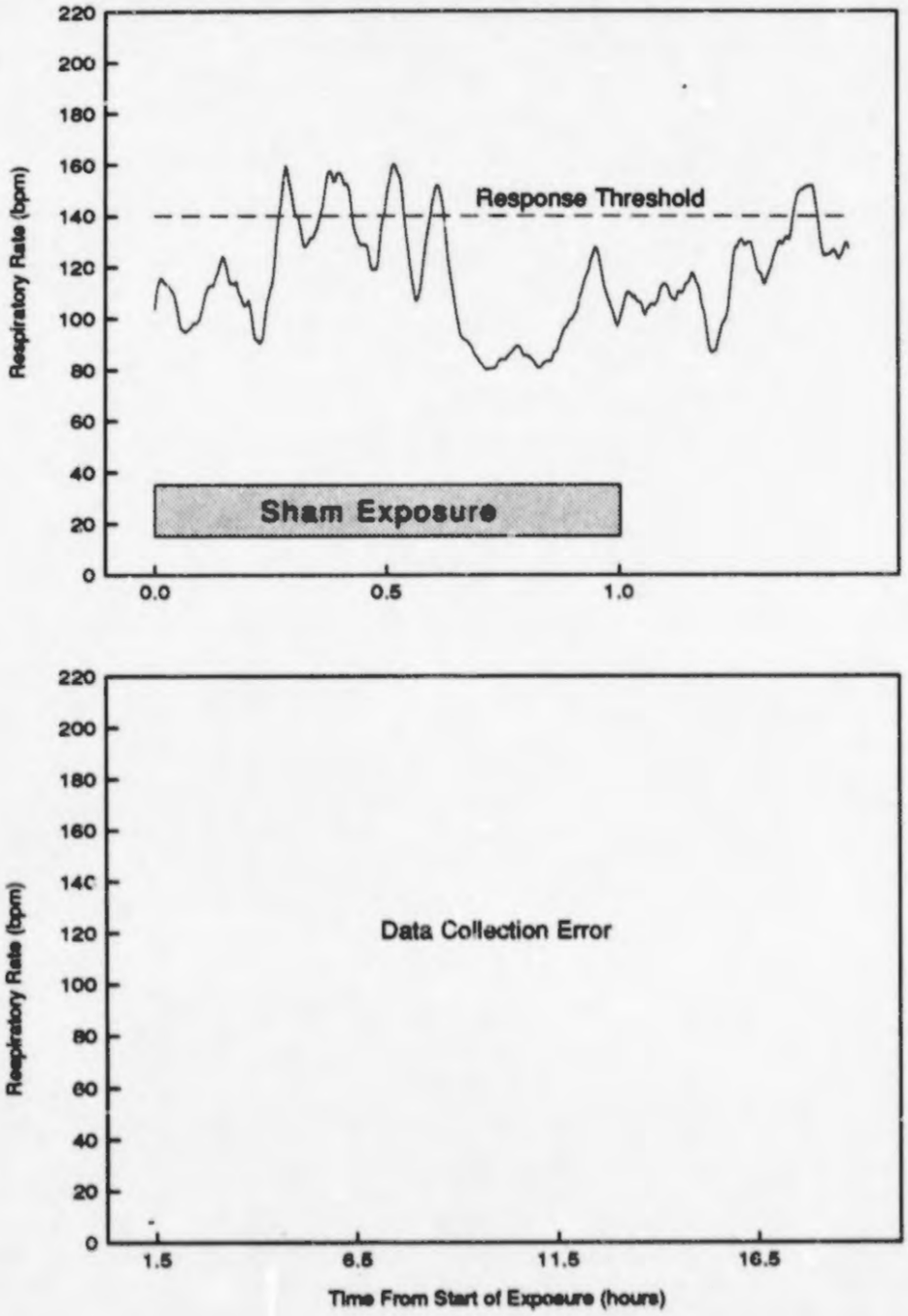
Study Number 92-344-PP

Animal # 196: Sham Sensitization

Figure: 16 Sham Challenge Week 2

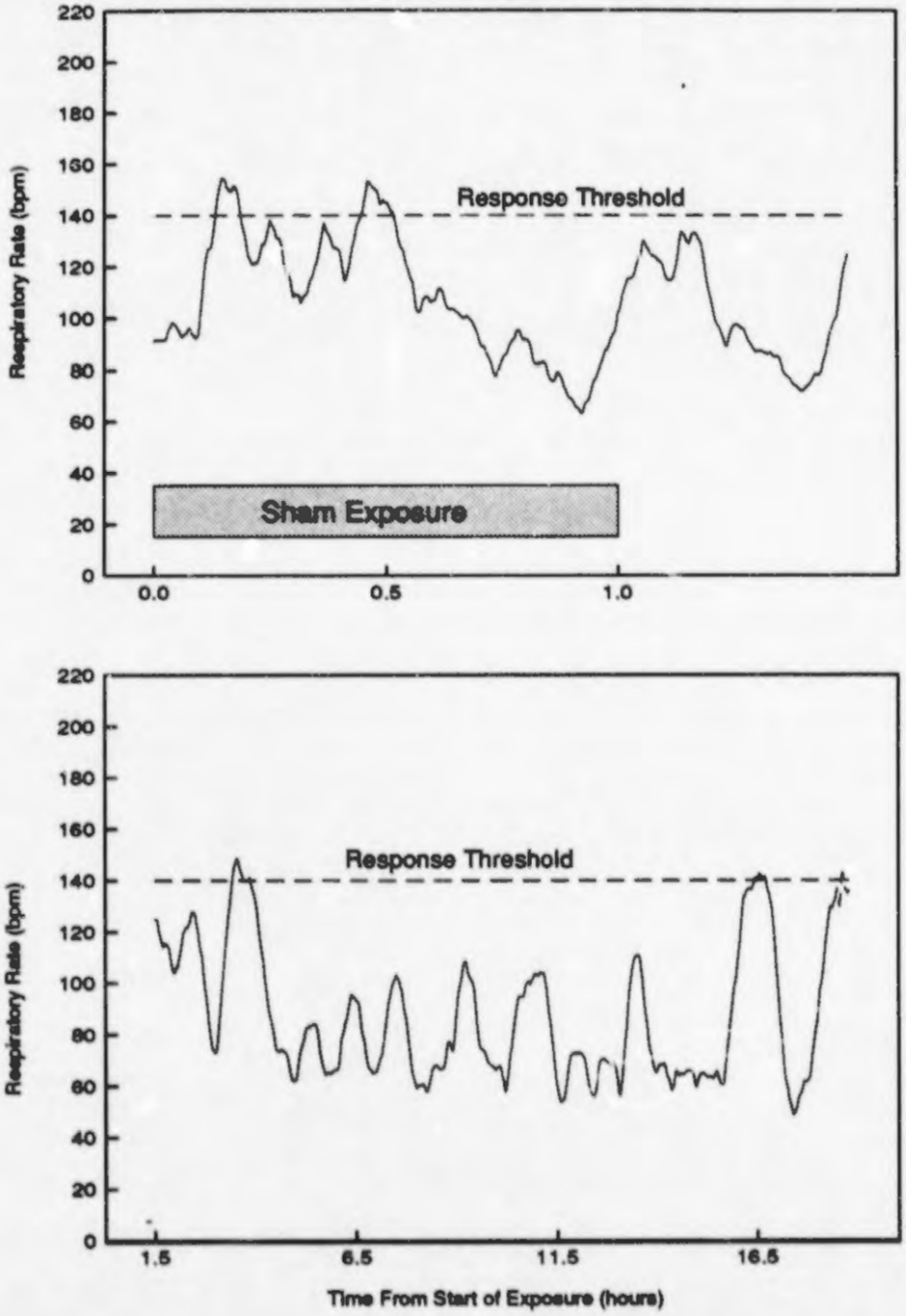


Study Number 92-344-PP
Animal # 197: Sham Sensitization
Figure: 17 Sham Challenge Week 2

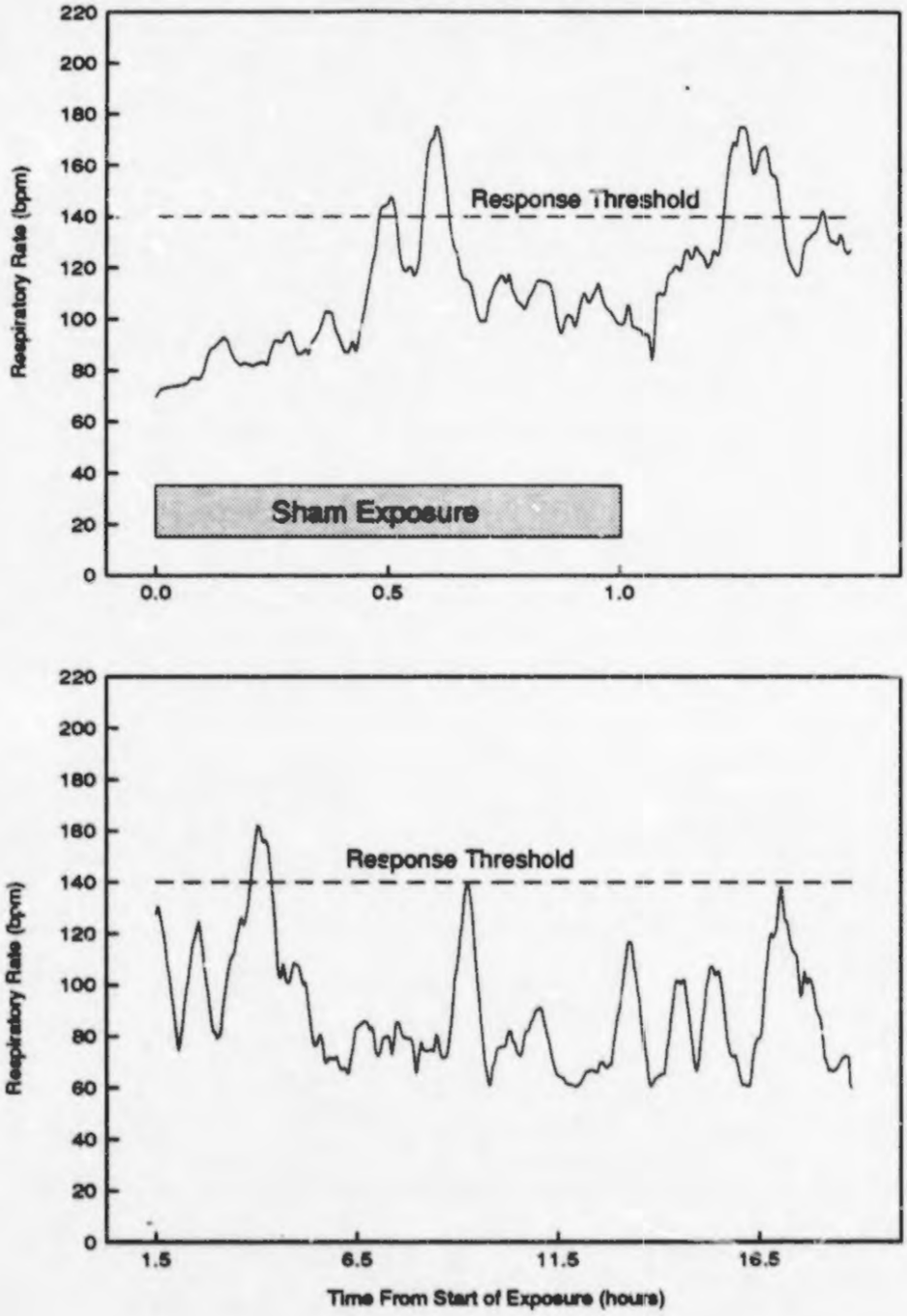


0 0 4 9

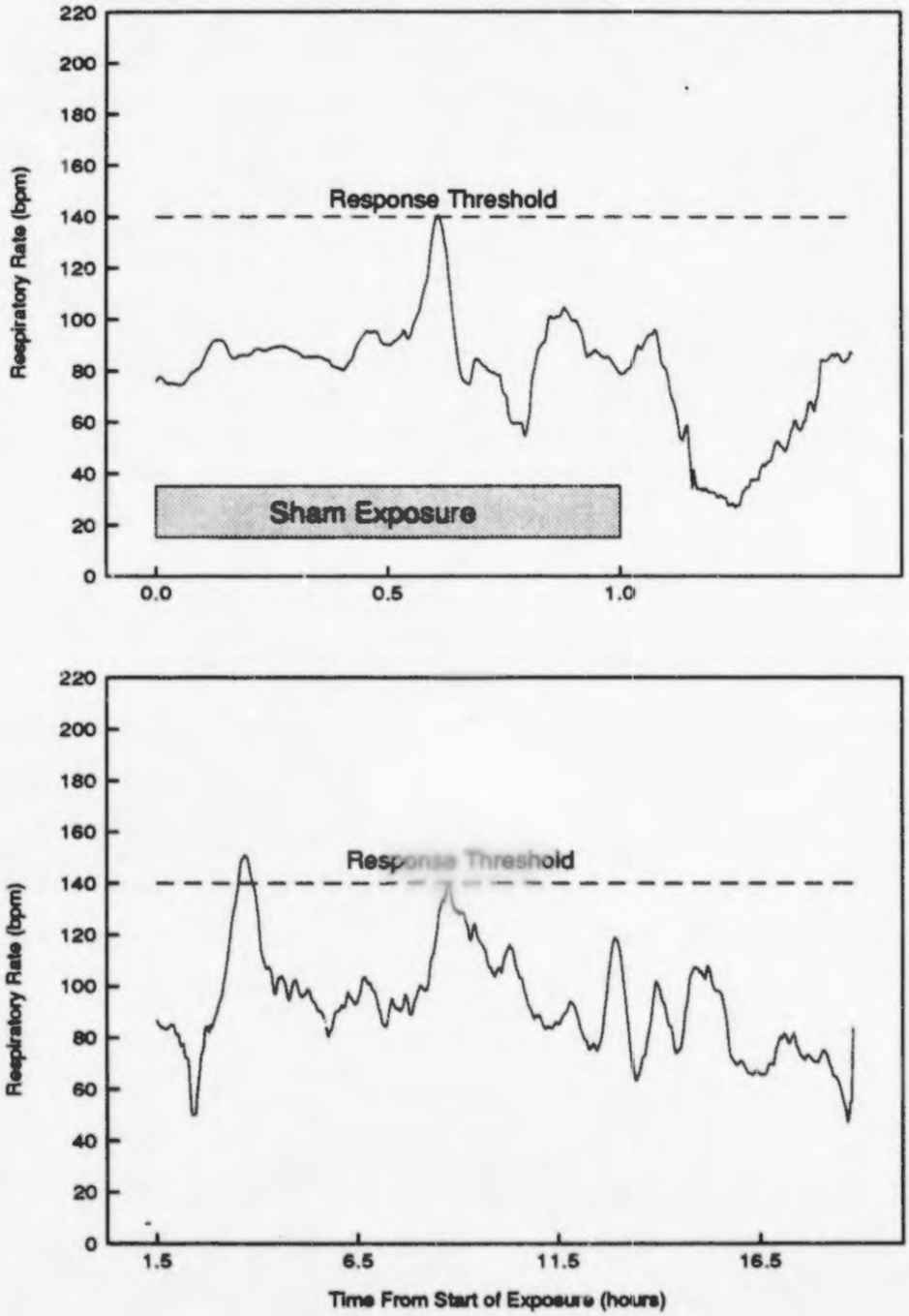
Study Number 92-344-PP
Animal # 198: Sham Sensitization
Figure: 18 Sham Challenge Week 2



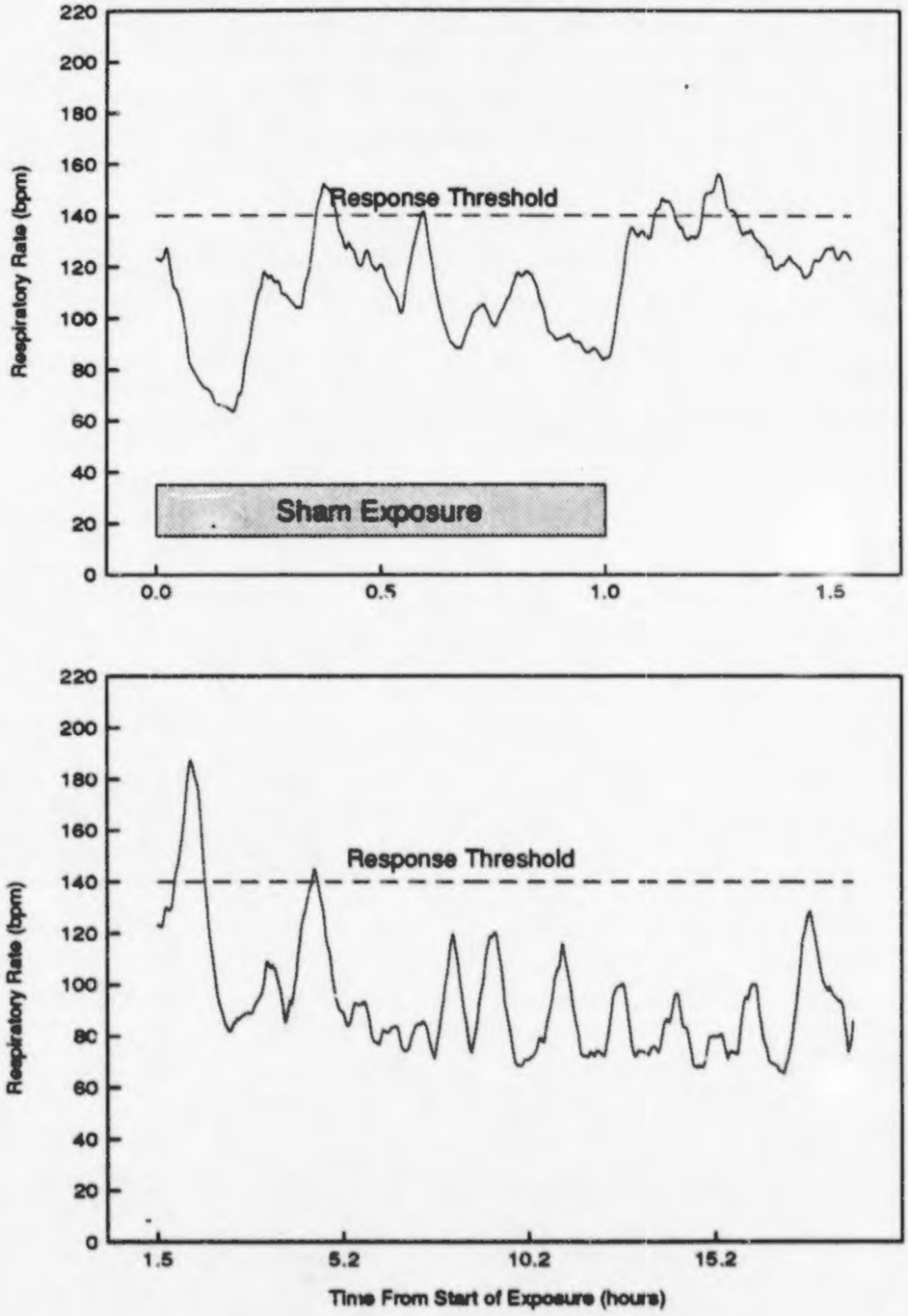
Study Number 92-344-PP
Animal # 199: Sham Sensitization
Figure: 19 Sham Challenge Week 2



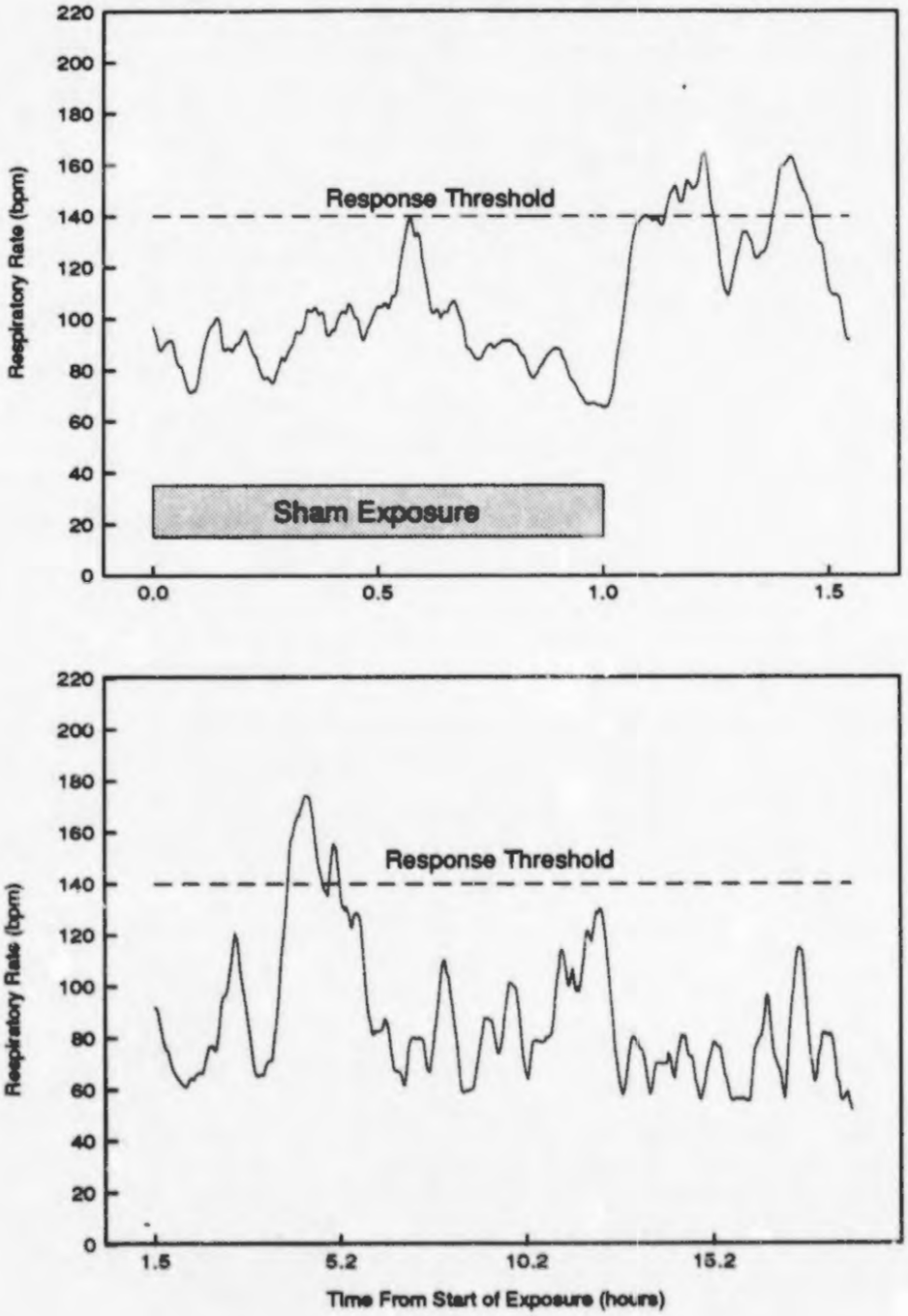
Study Number 92-344-PP
Animal # 200: Sham Sensitization
Figure: 20 Sham Challenge Week 2



Study Number 92-344-PP
Animal # 201: Sham Sensitization
Figure: 21 Sham Challenge Week 2

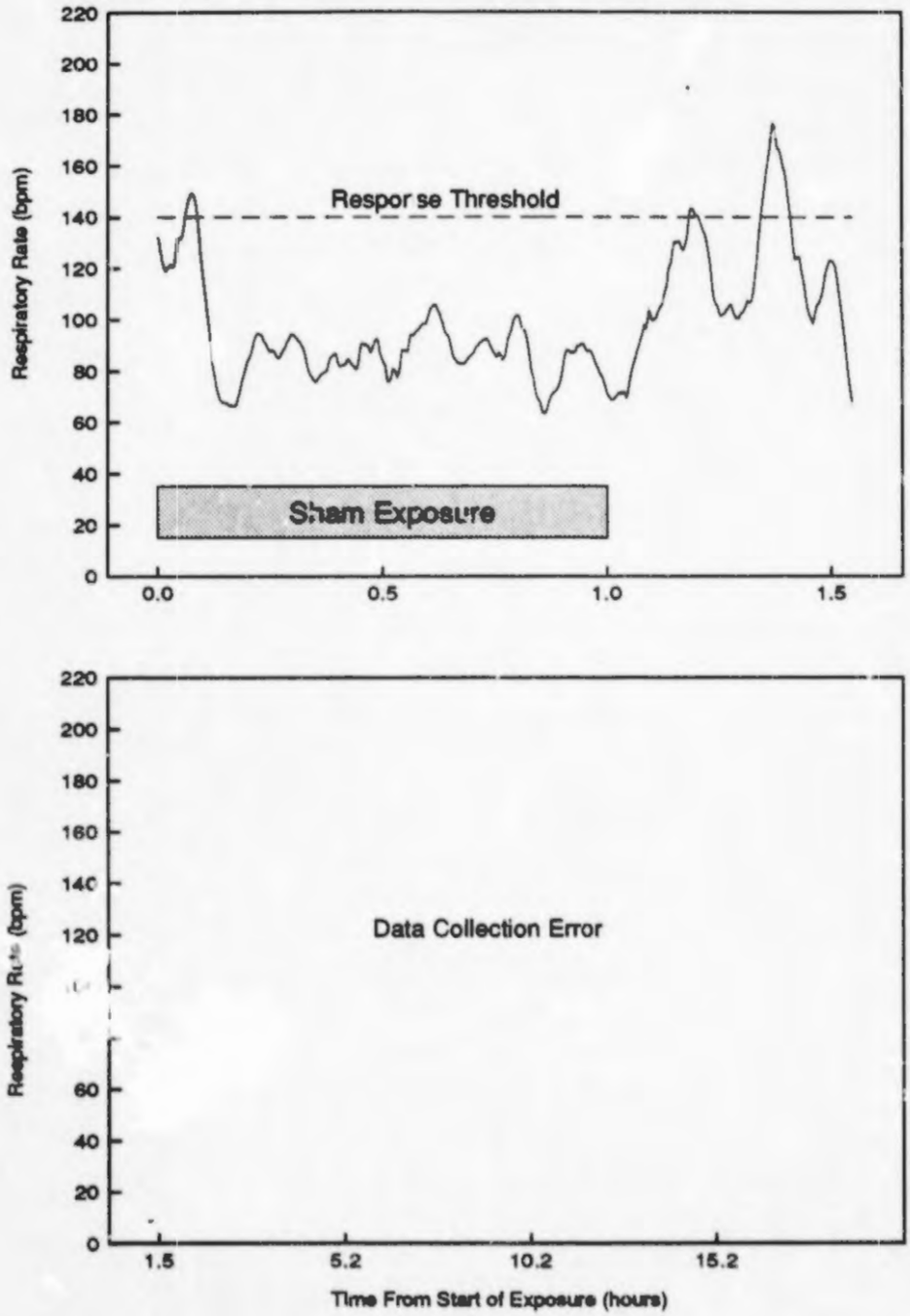


Study Number 92-344-PP
Animal # 202: Sham Sensitization
Figure: 22 Sham Challenge Week 2

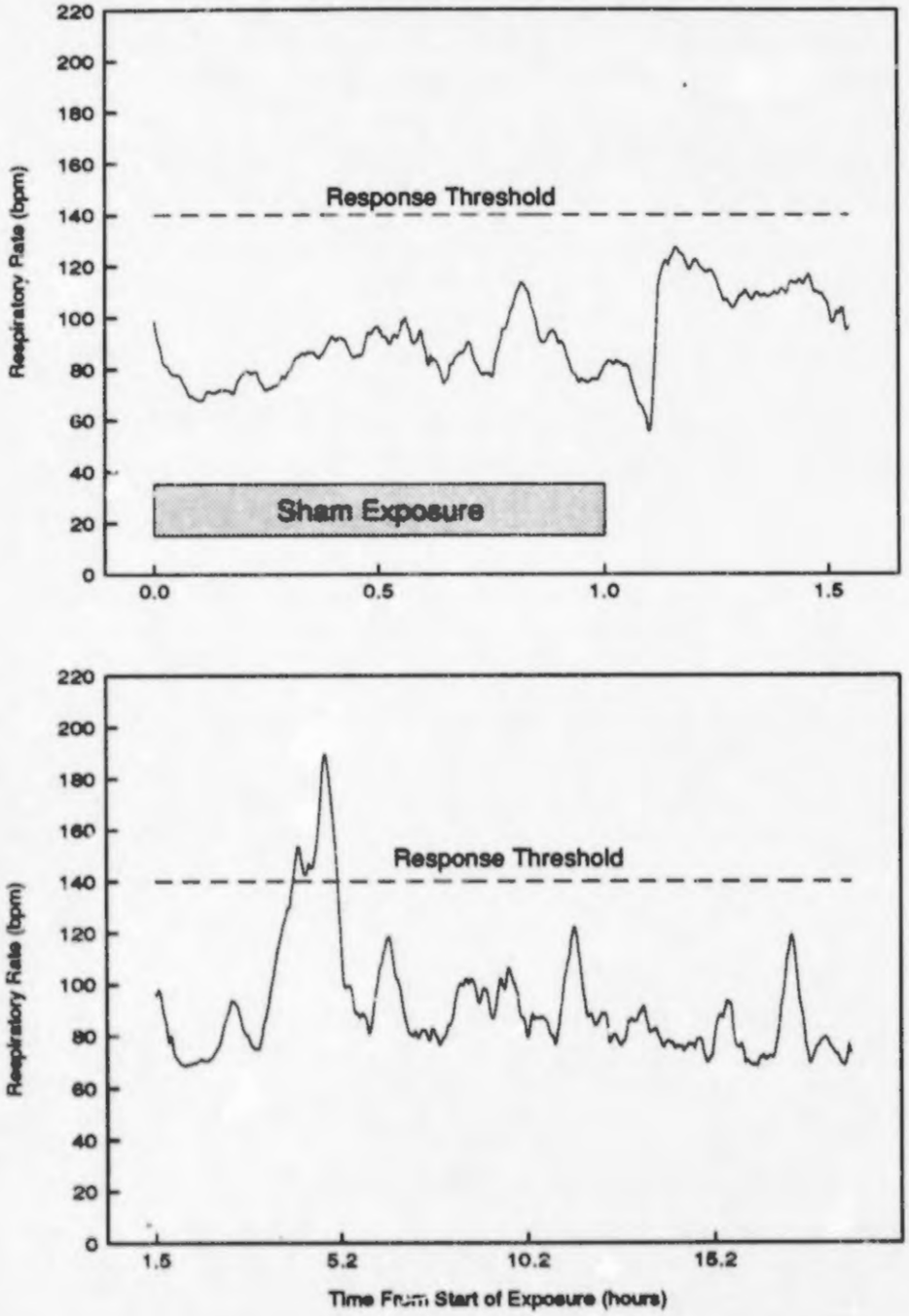


Study Number 92-344-PP
Animal # 203: Sham Sensitization
Sham Challenge Week 2

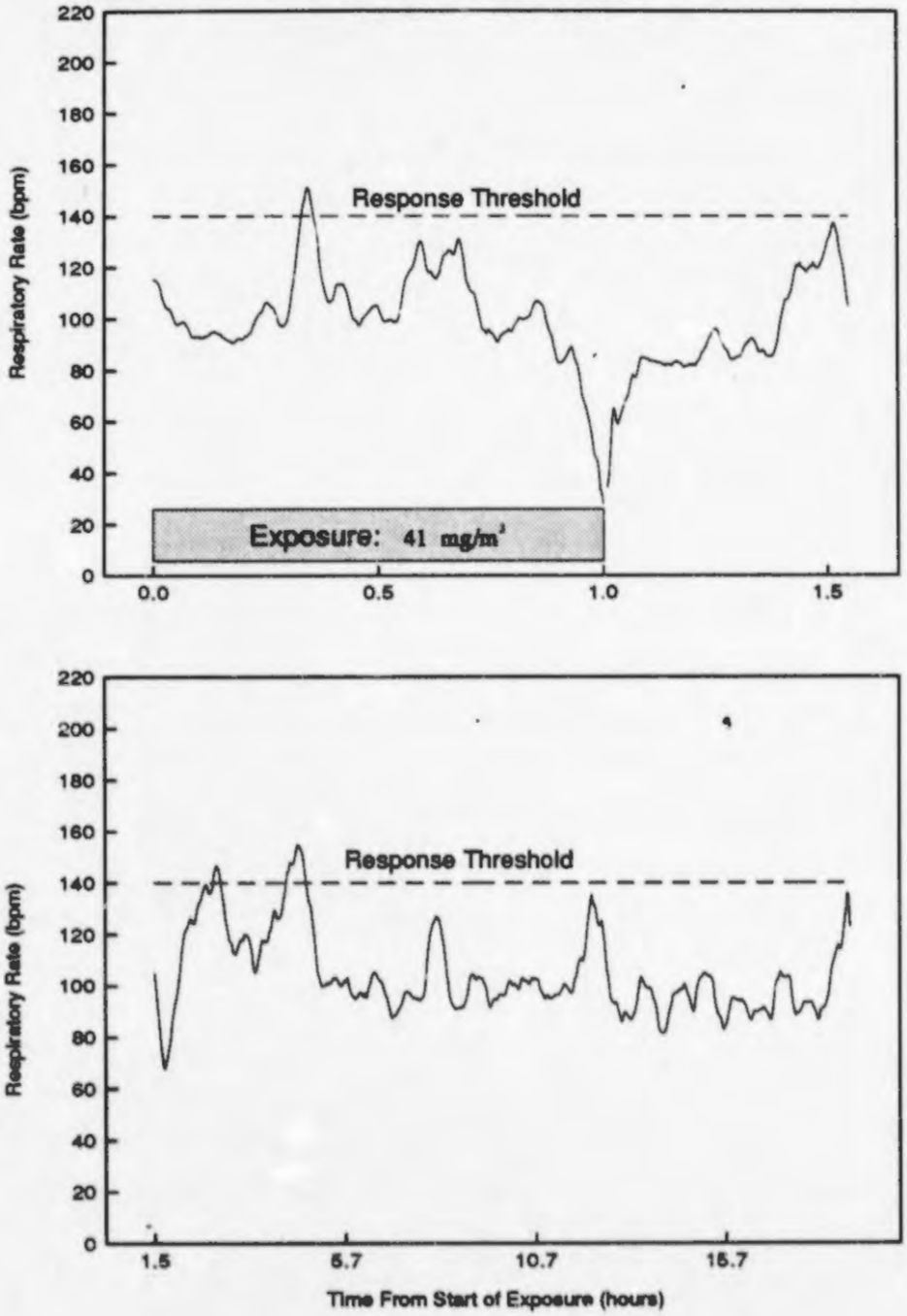
Figure: 23



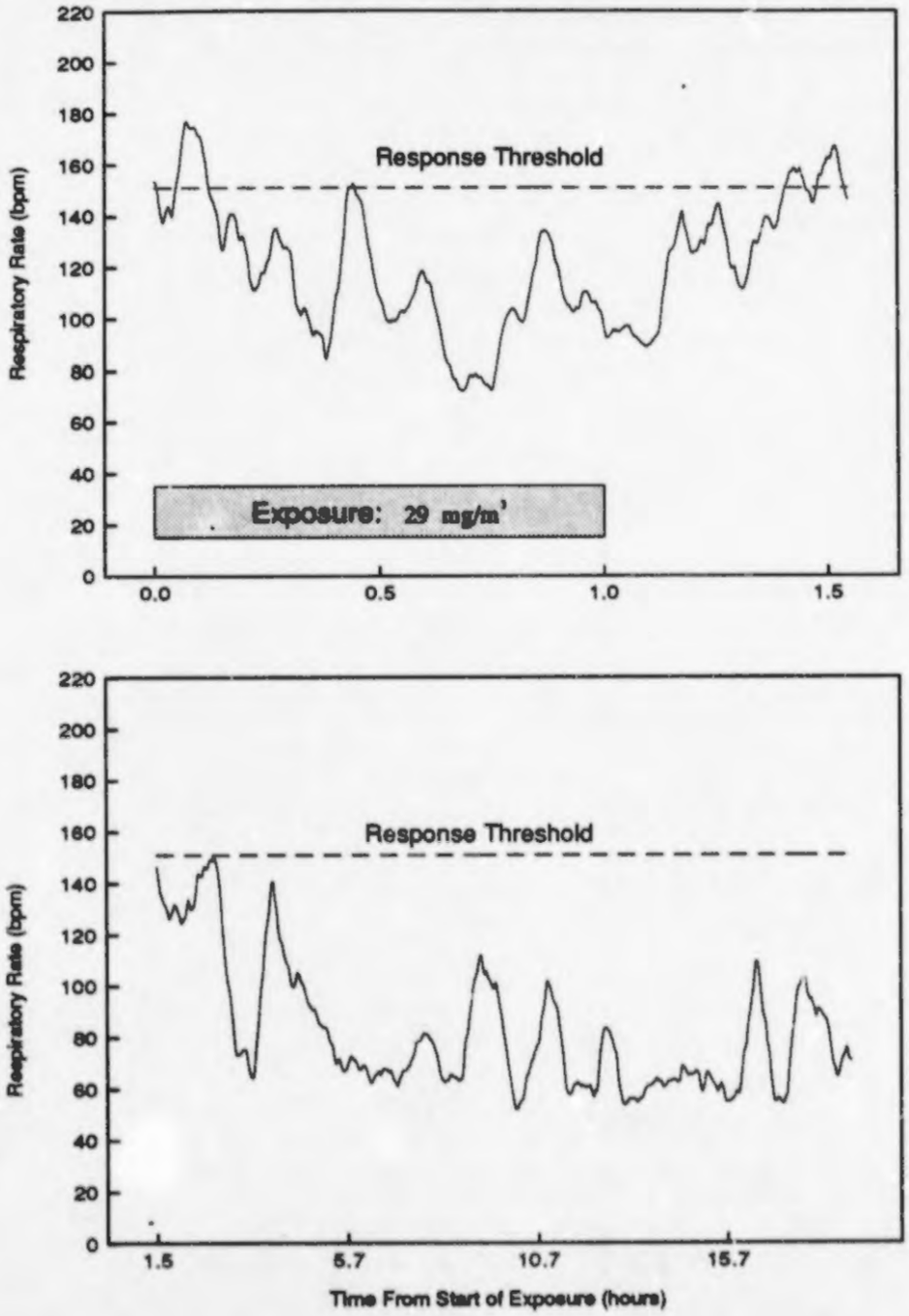
Study Number 92-344-PP
Animal # 204: Sham Sensitization
Figure: 24 Sham Challenge Week 2



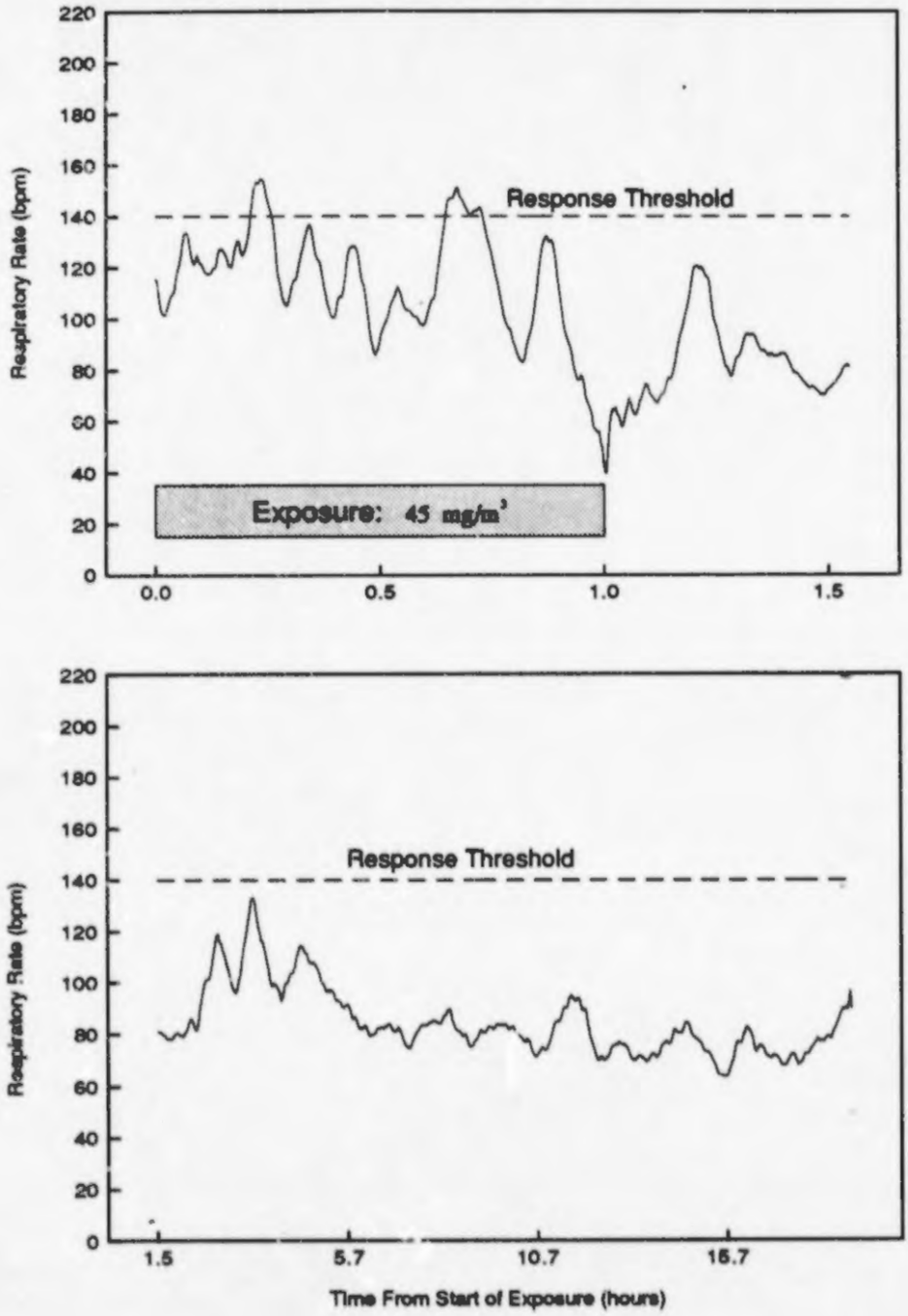
Study Number 92-344-PP
Animal # 193: Sham Sensitization
Figure: 25 GSA Challenge Week 3



Study Number 92-344-PP
Animal # 194: Sham Sensitization
Figure: 26 GSA Challenge Week 3



Study Number 92-344-PP
Animal # 195: Sham Sensitization
Figure: 27 GSA Challenge Week 3



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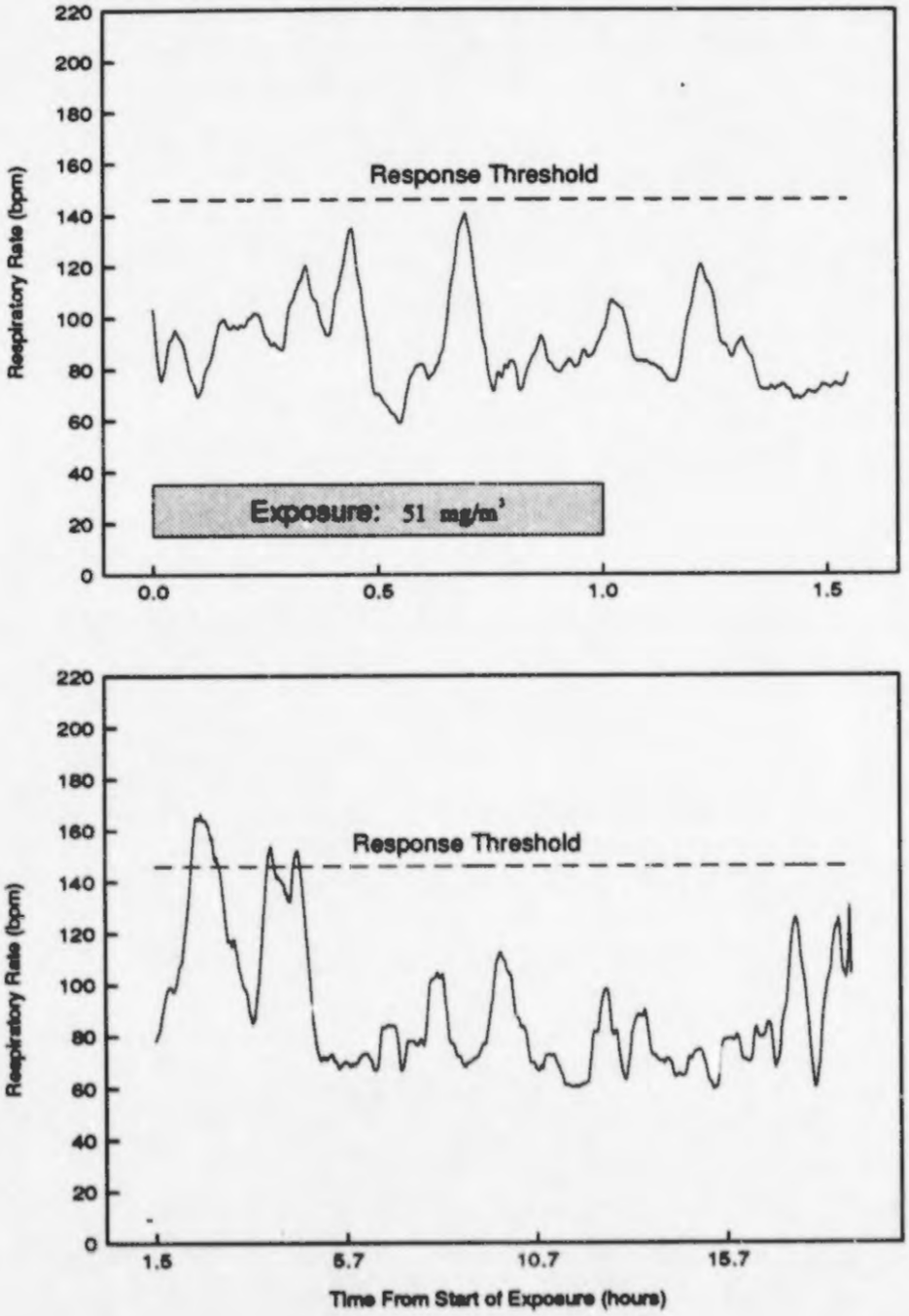
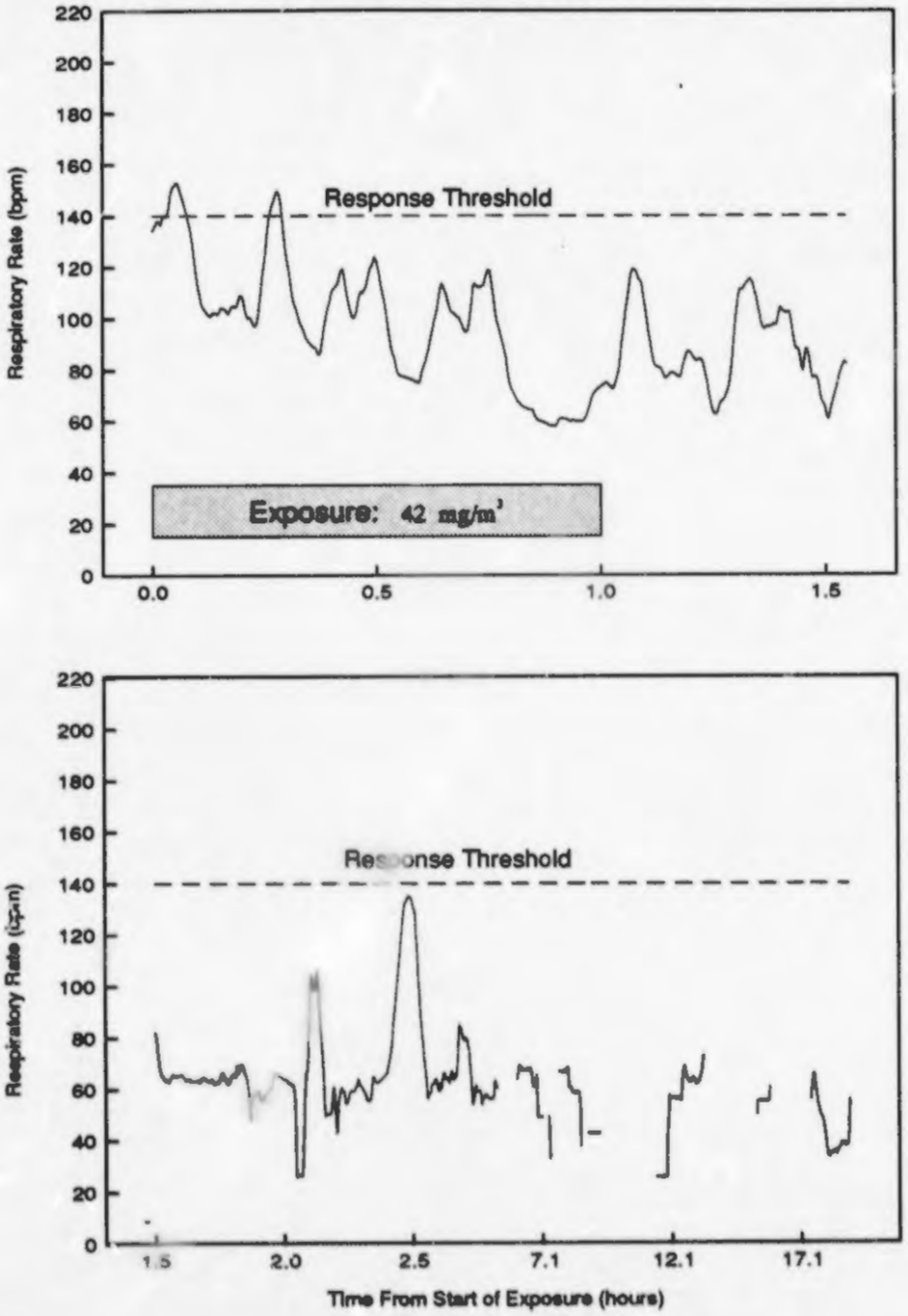
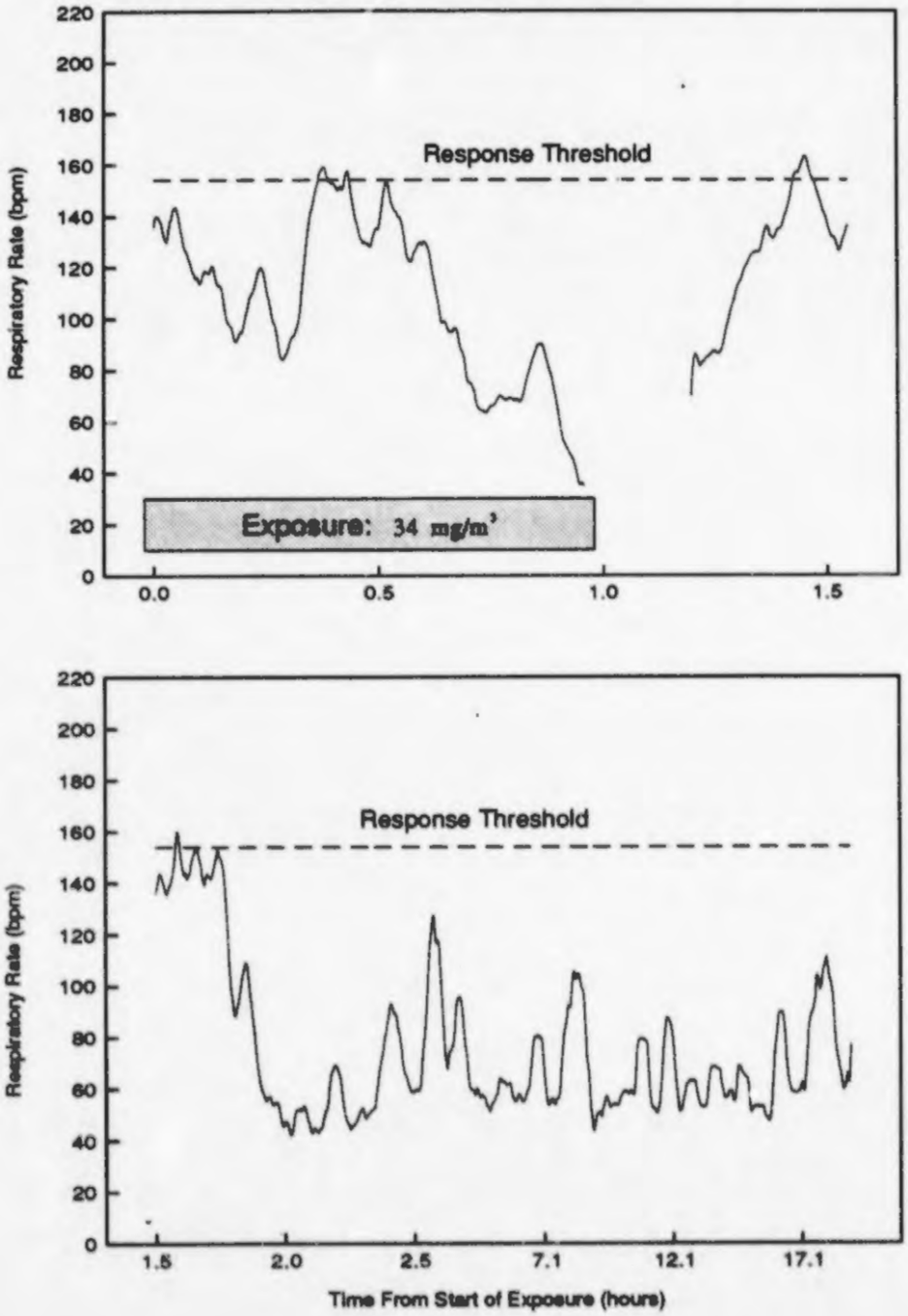
Study Number 92-344-PP
Animal # 196: Sham Sensitization
Figure: 28 GSA Challenge Week 3



Study Number 92-344-PP
Animal # 197: Sham Sensitization
Figure: 29 GSA Challenge Week 3



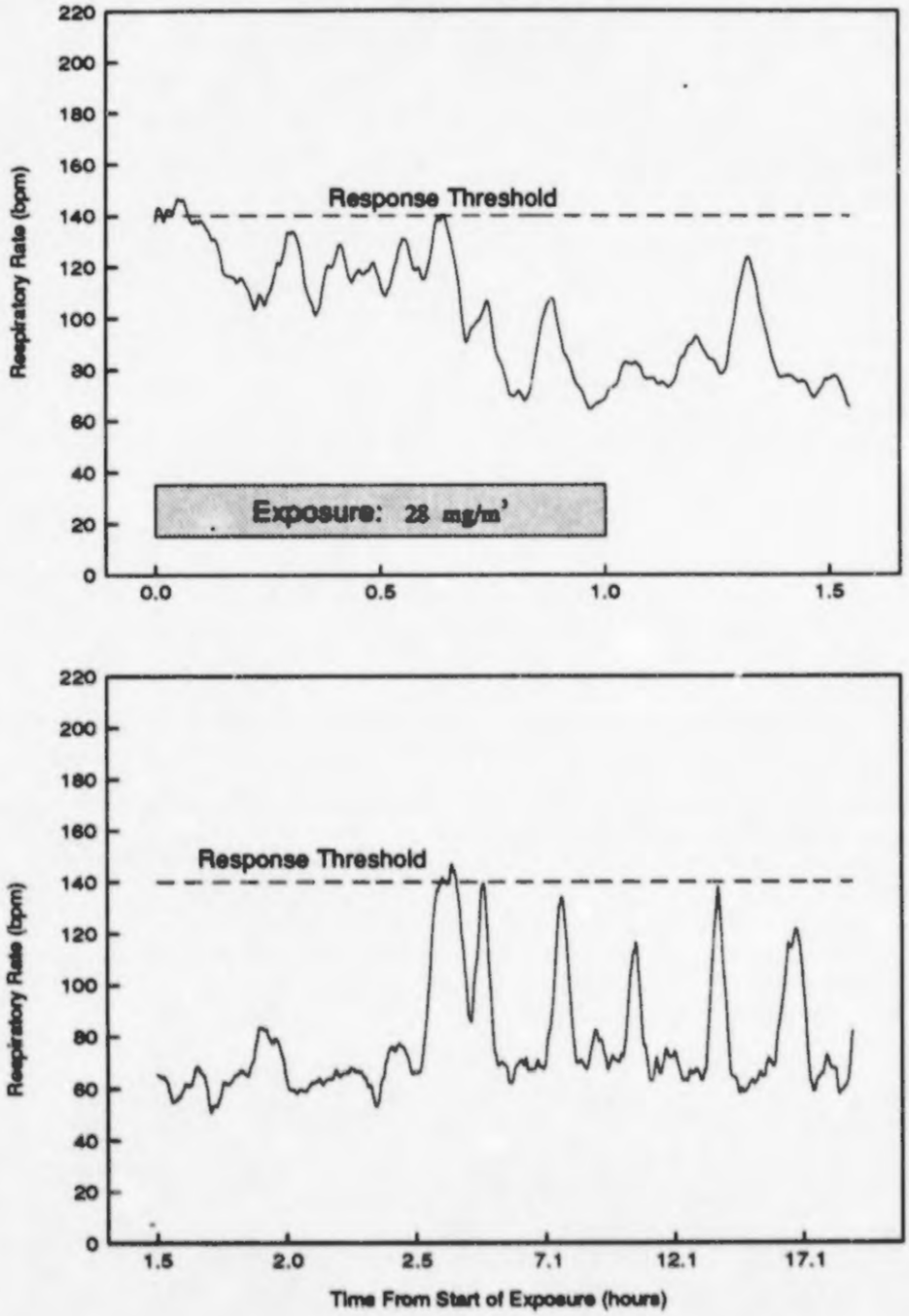
Study Number 92-344-PP
Animal # 198: Sham Sensitization
Figure: 30 GSA Challenge Week 3



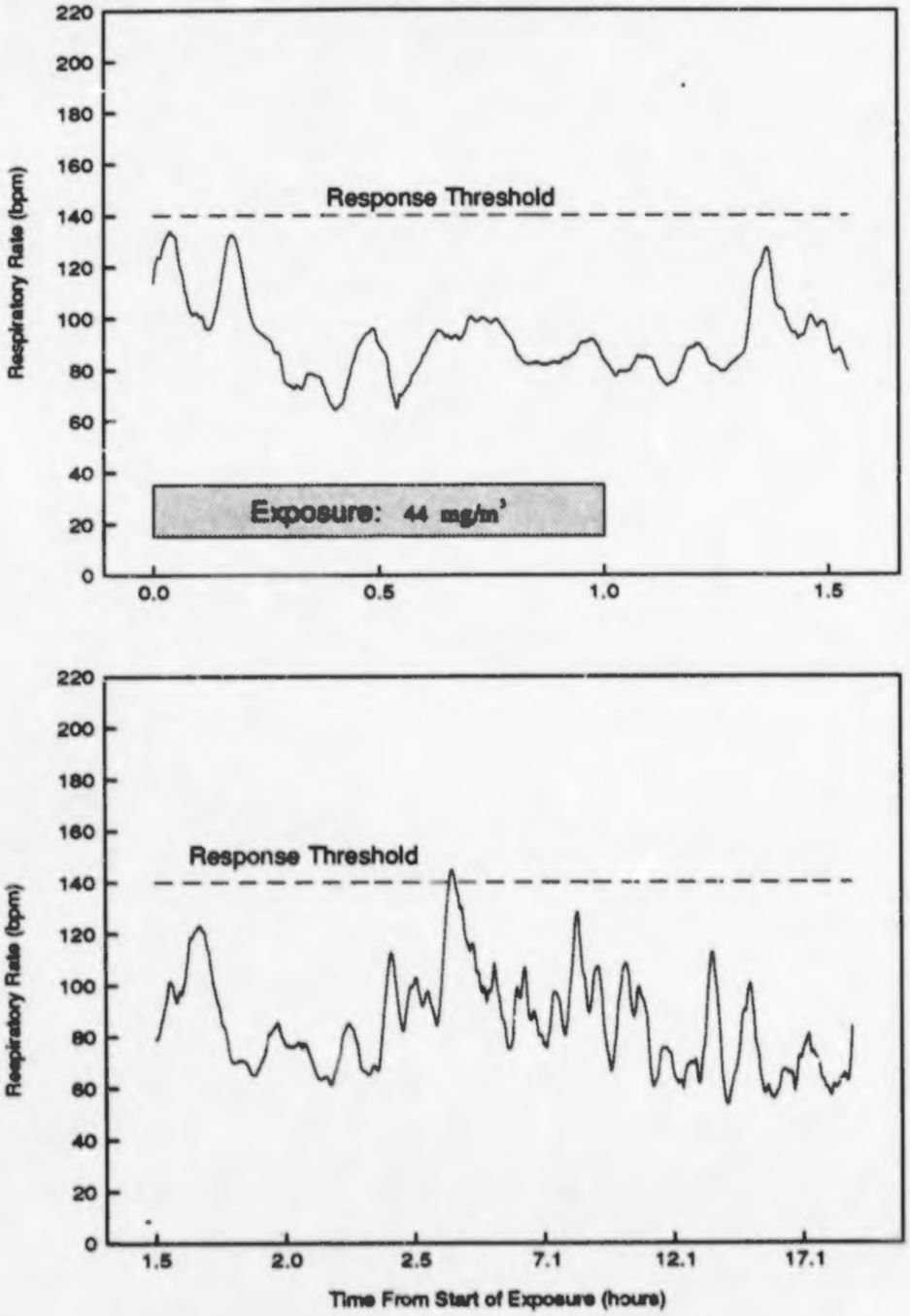
Study Number 92-344-PP

Animal # 199: Sham Sensitization

Figure: 31 GSA Challenge Week 3



Study Number 92-344-PP
Animal # 200: Sham Sensitization
Figure: 32 GSA Challenge Week 3



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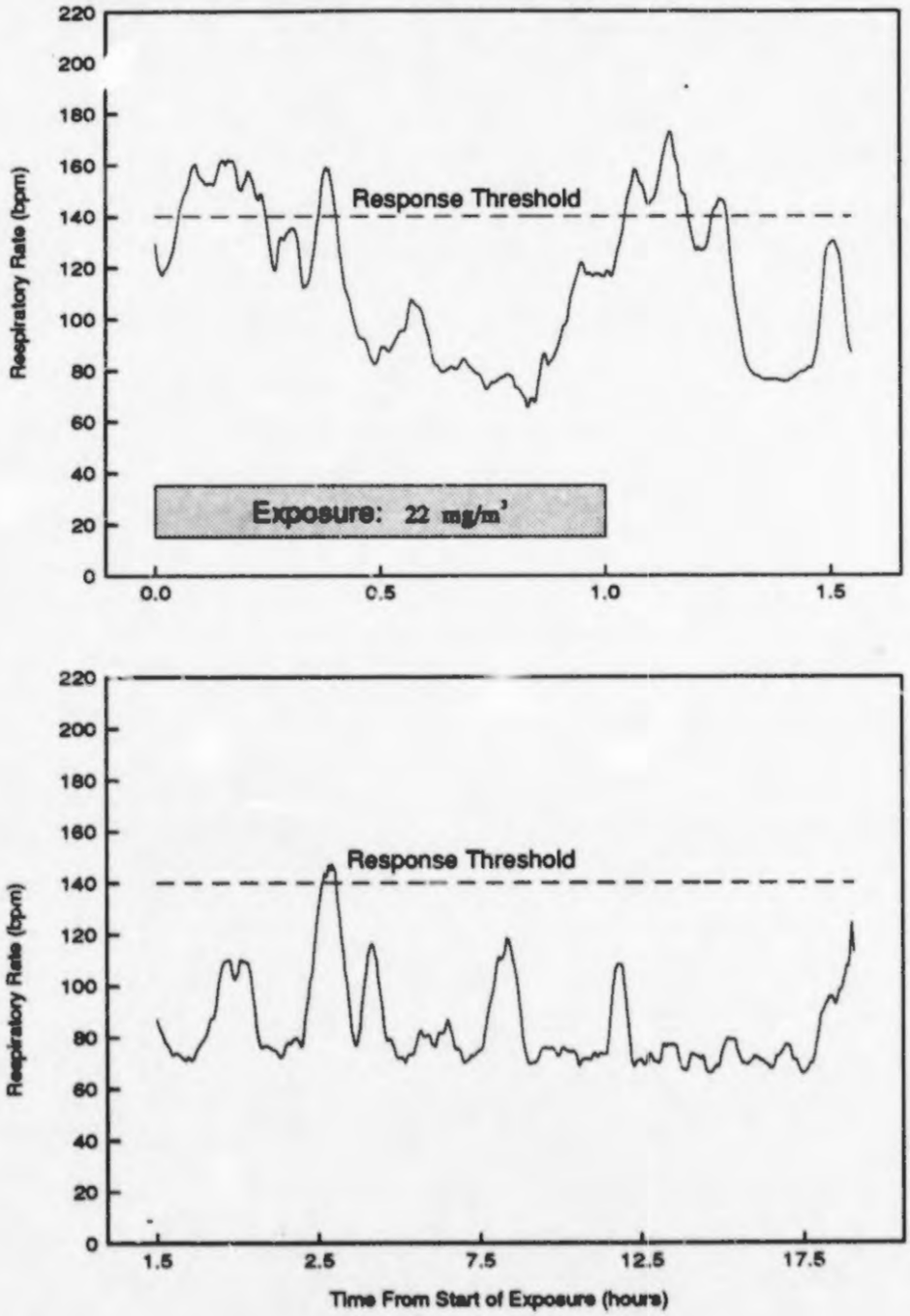
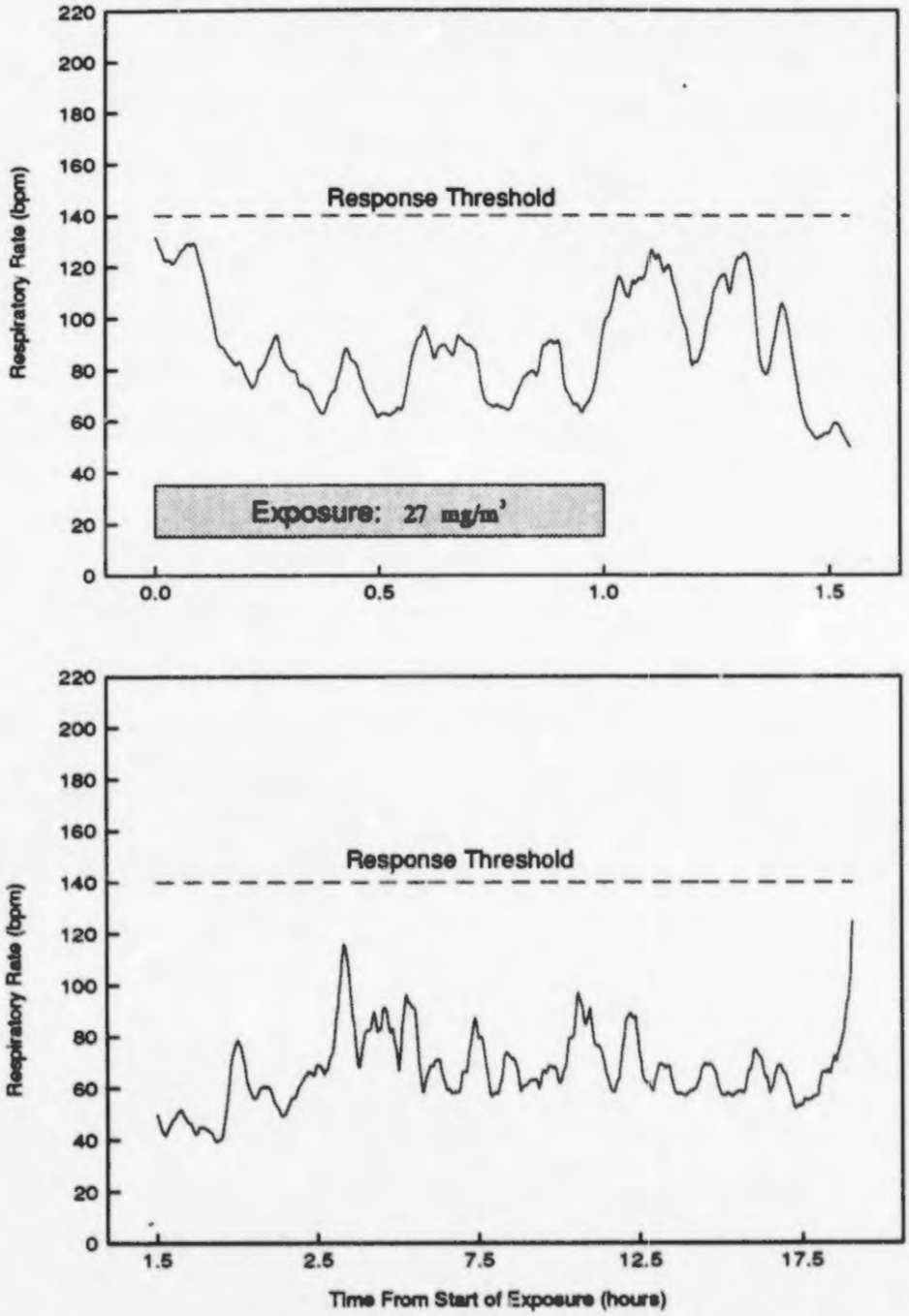
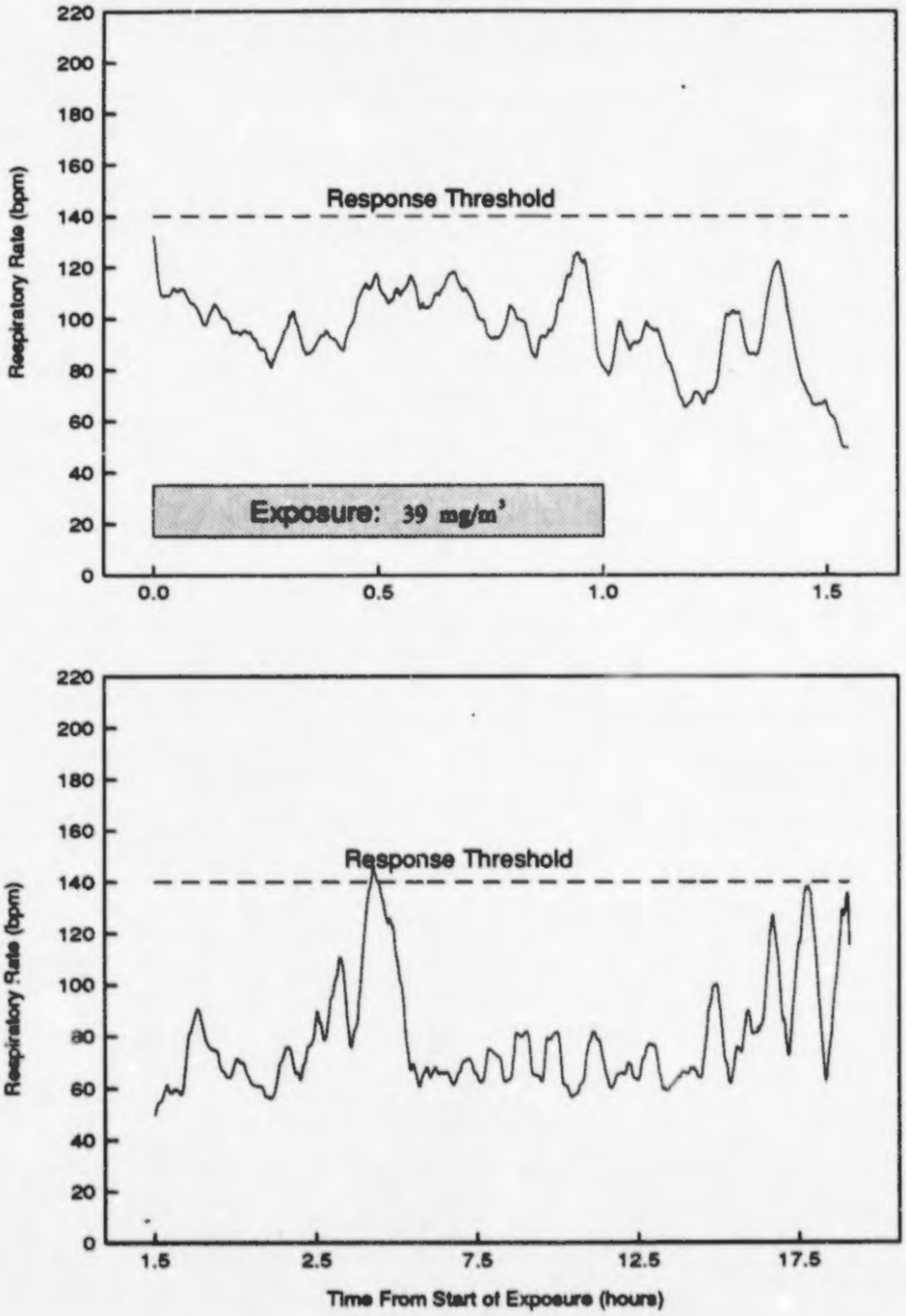
Study Number 92-344-PP
Animal # 201: Sham Sensitization
Figure: 33 GSA Challenge Week 3



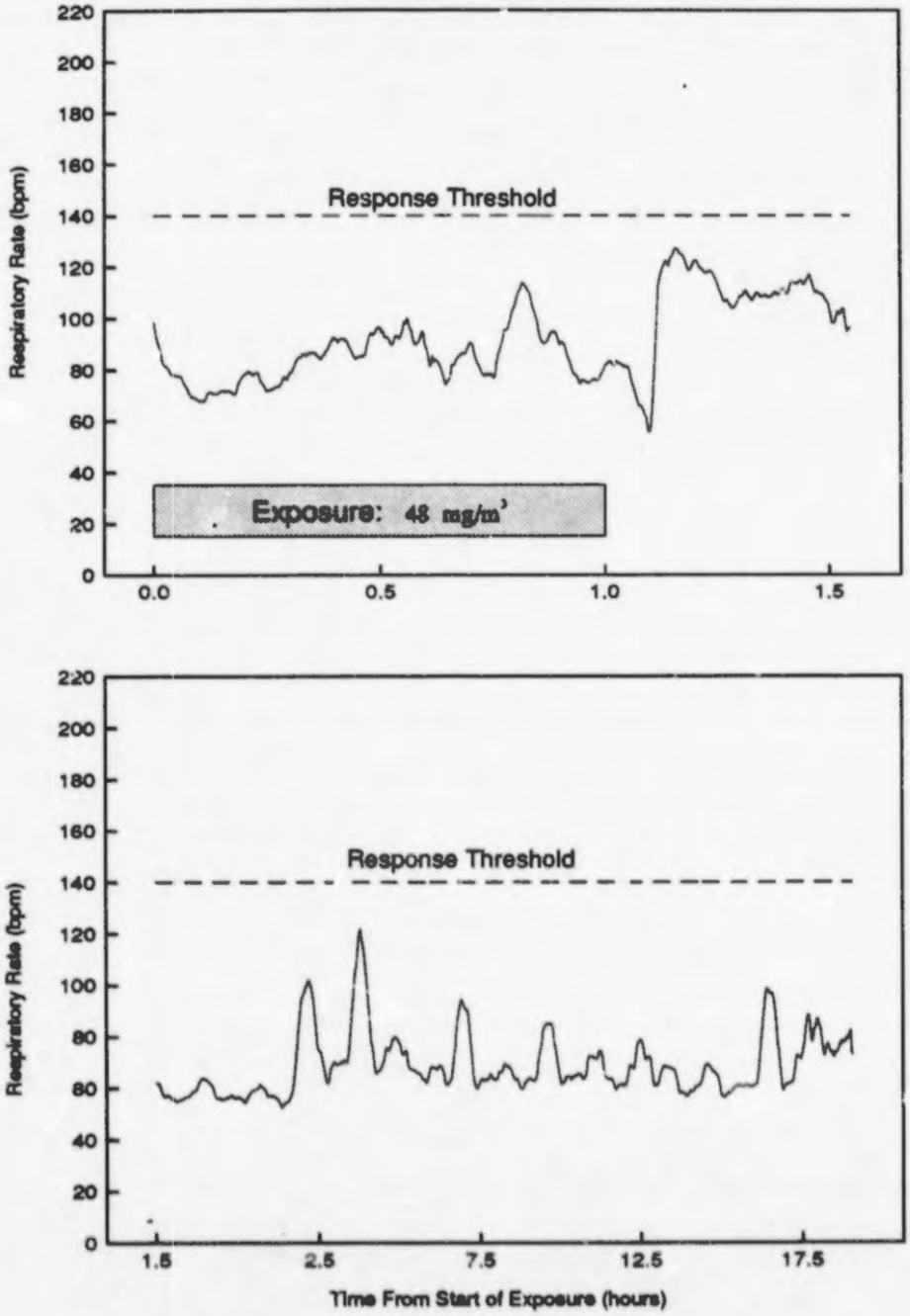
Study Number 92-344-PP
Animal # 202: Sham Sensitization
Figure: 34 GSA Challenge Week 3



Study Number 92-344-PP
Animal # 203: Sham Sensitization
Figure: 35 GSA Challenge Week 3



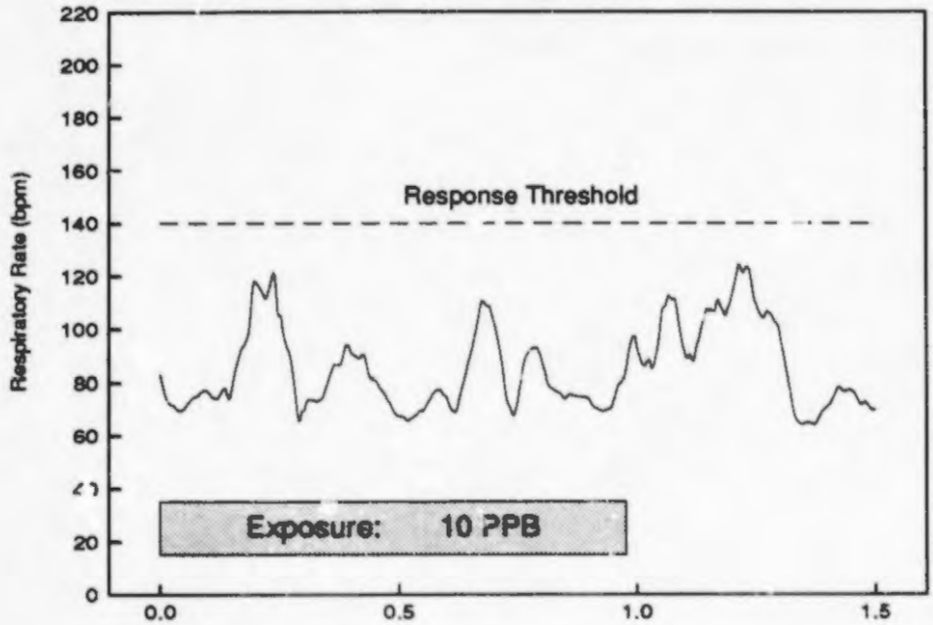
Study Number 92-344-PP
Animal # 204: Sham Sensitization
Figure: 36 GSA Challenge Week 3

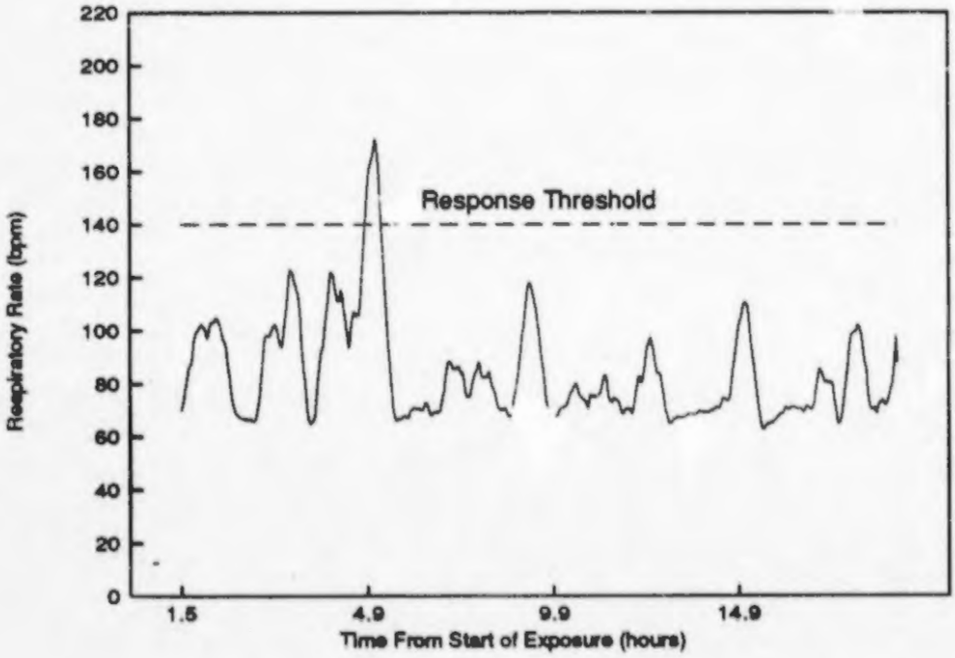


Study Number 92-344-PP

Animal # 222: Sham Sensitization

Figure: 37 2,4-TDI Challenge Week 1

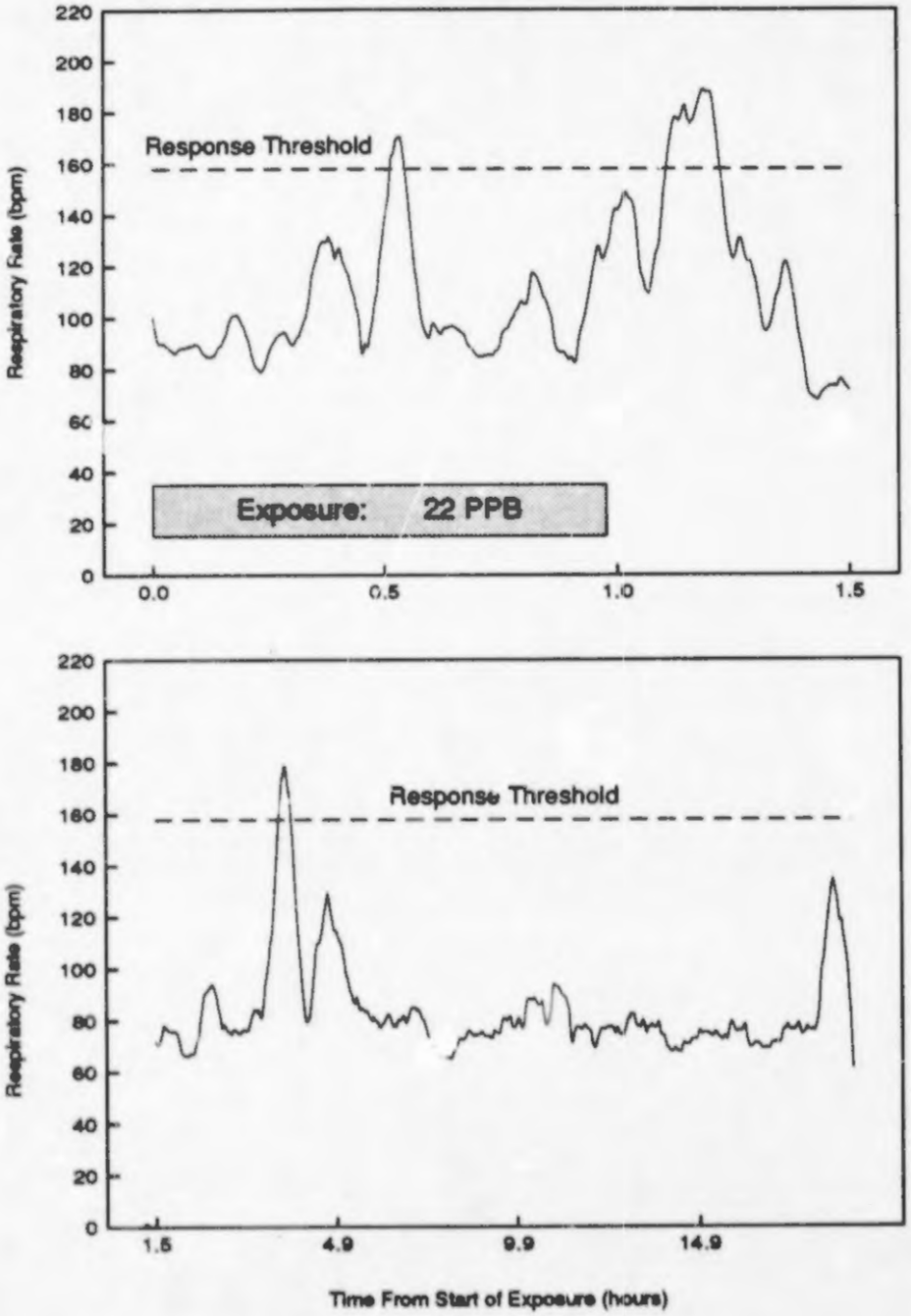




Study Number 92-344-PP

Animal # 223: Sham Sensitization

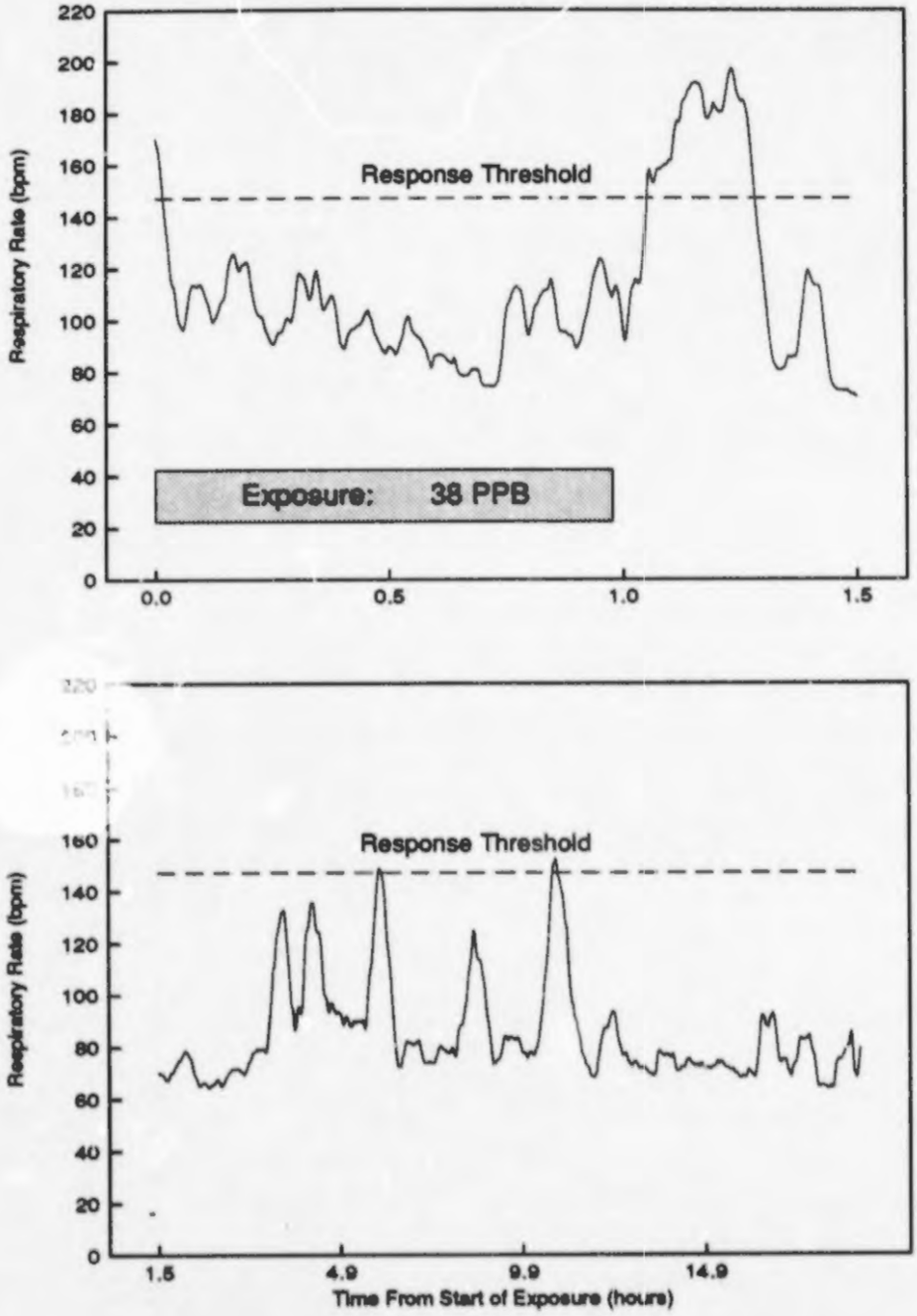
Figure: 38 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 224: Sham Sensitization

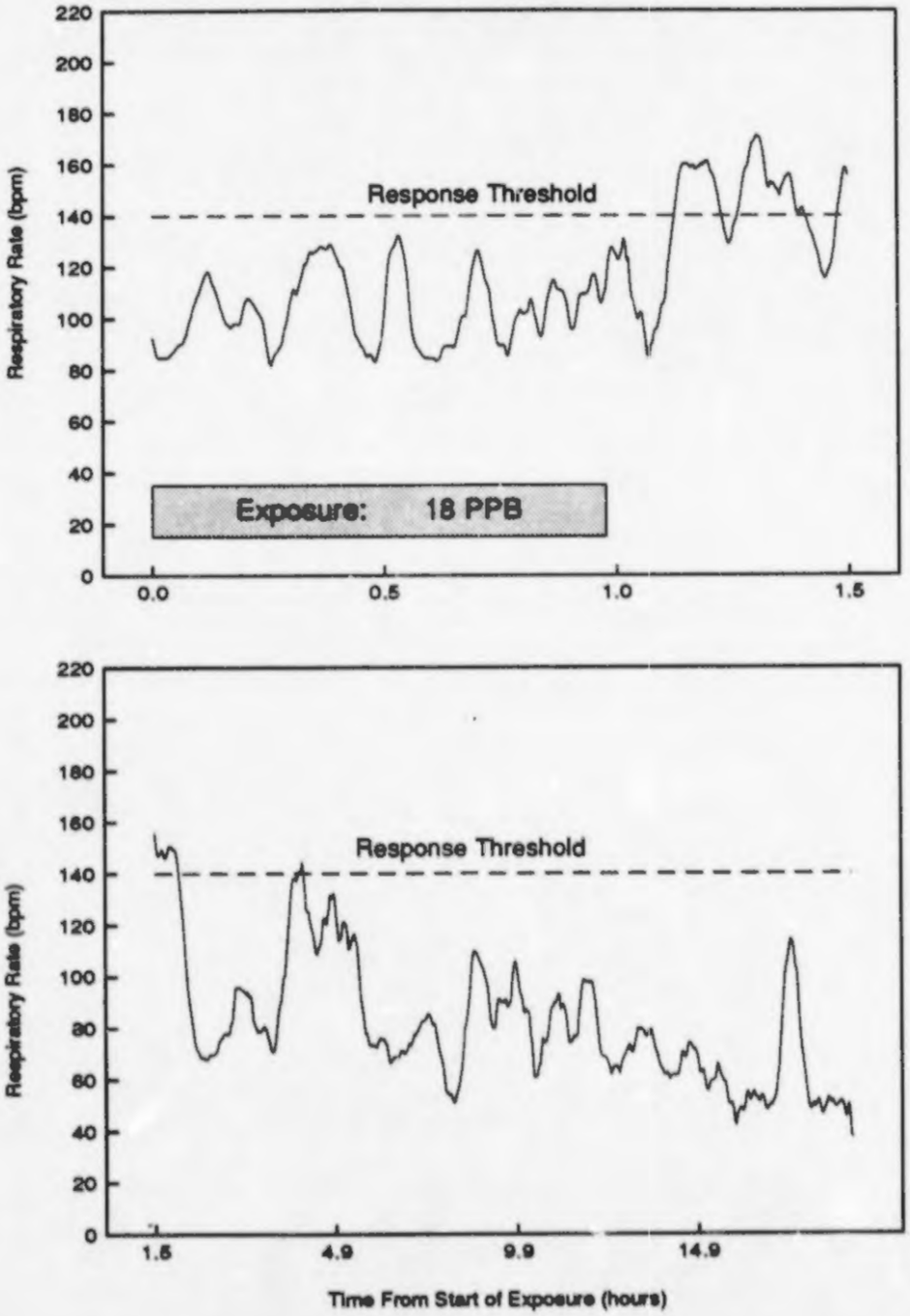
Figure: 39 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 225: Sham Sensitization

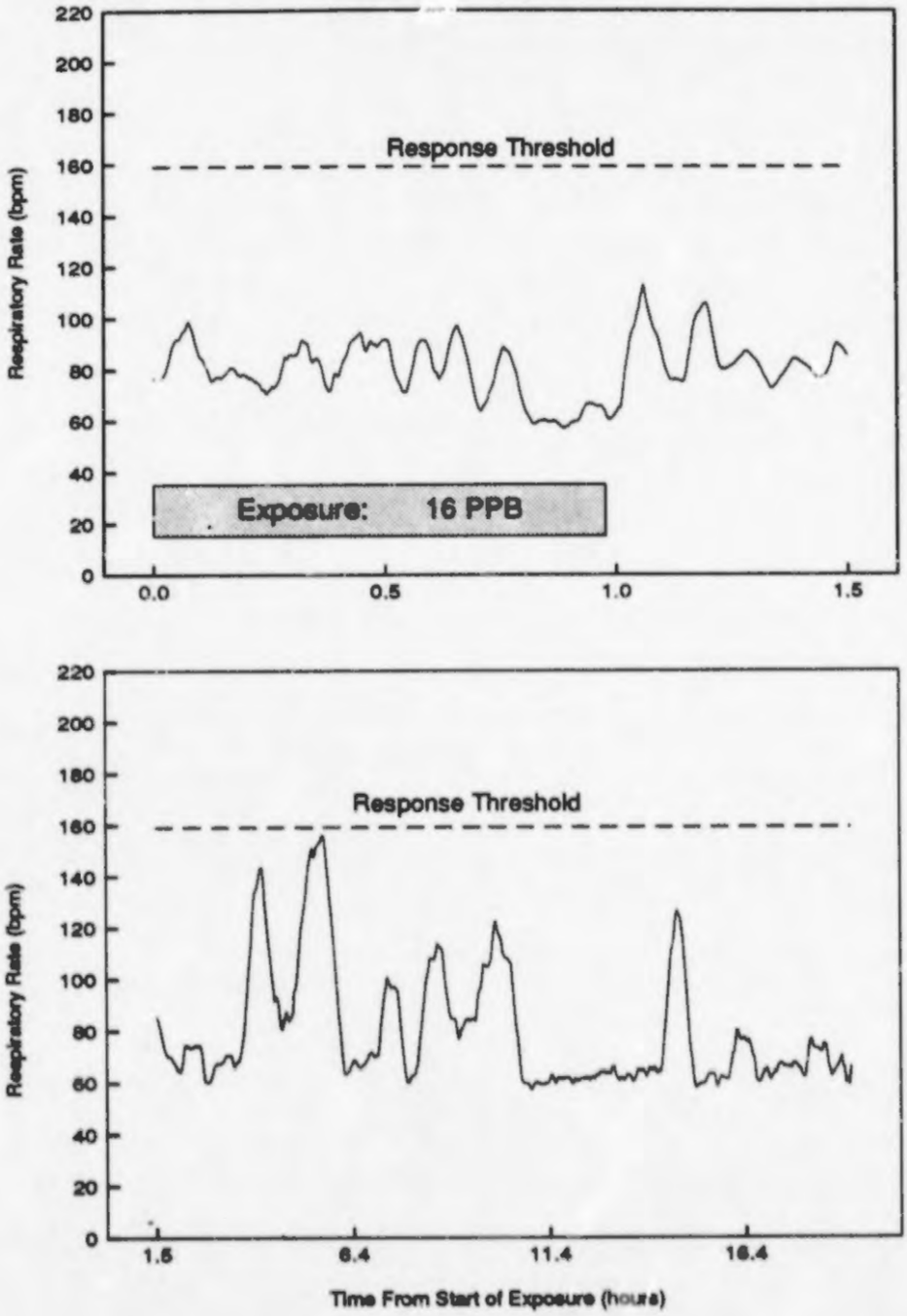
Figure: 40 2,4-TDi Challenge Week 1



Study Number 92-344-PP

Animal # 226: Sham Sensitization

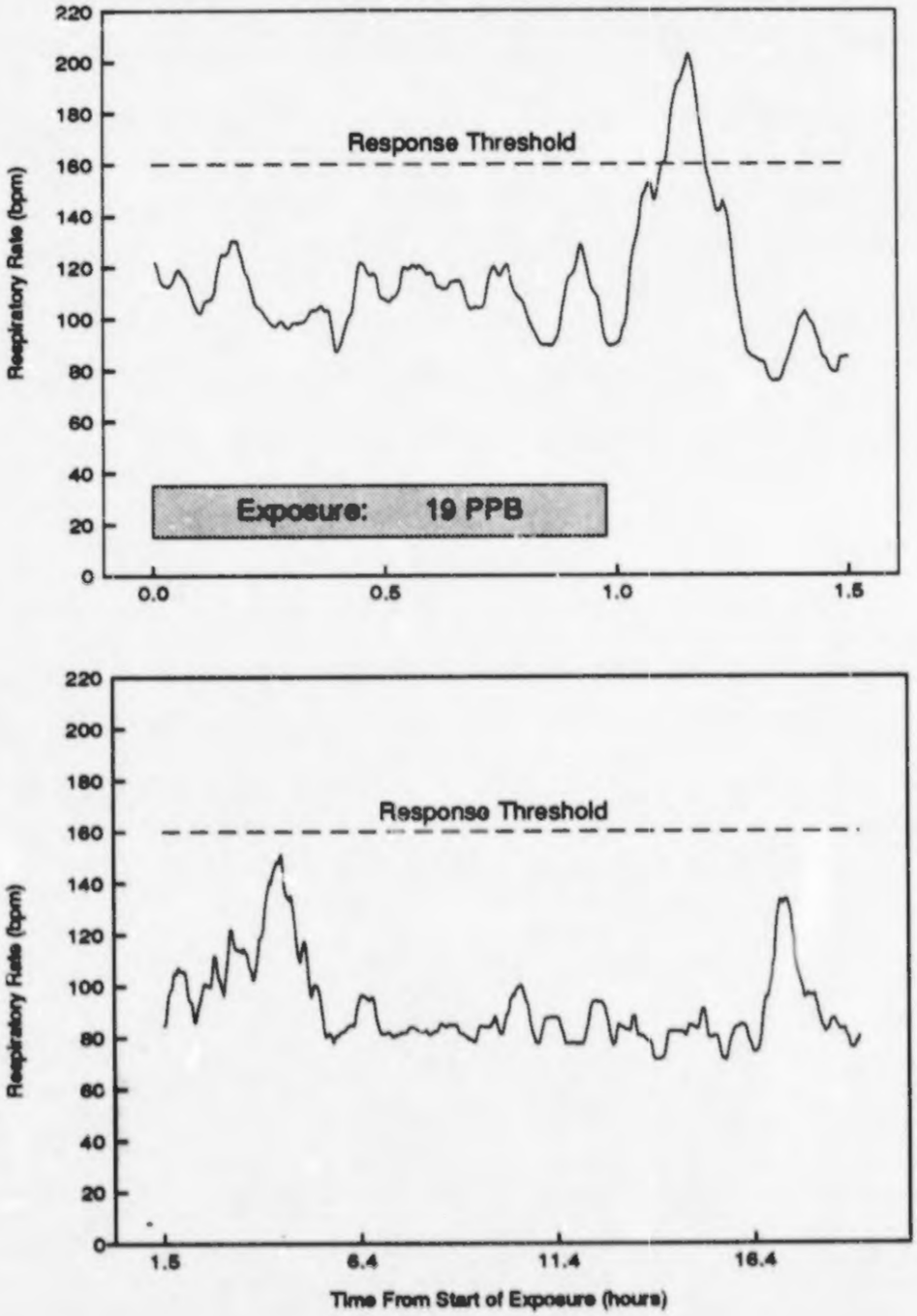
Figure: 41 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 227: Sham-Sensitization

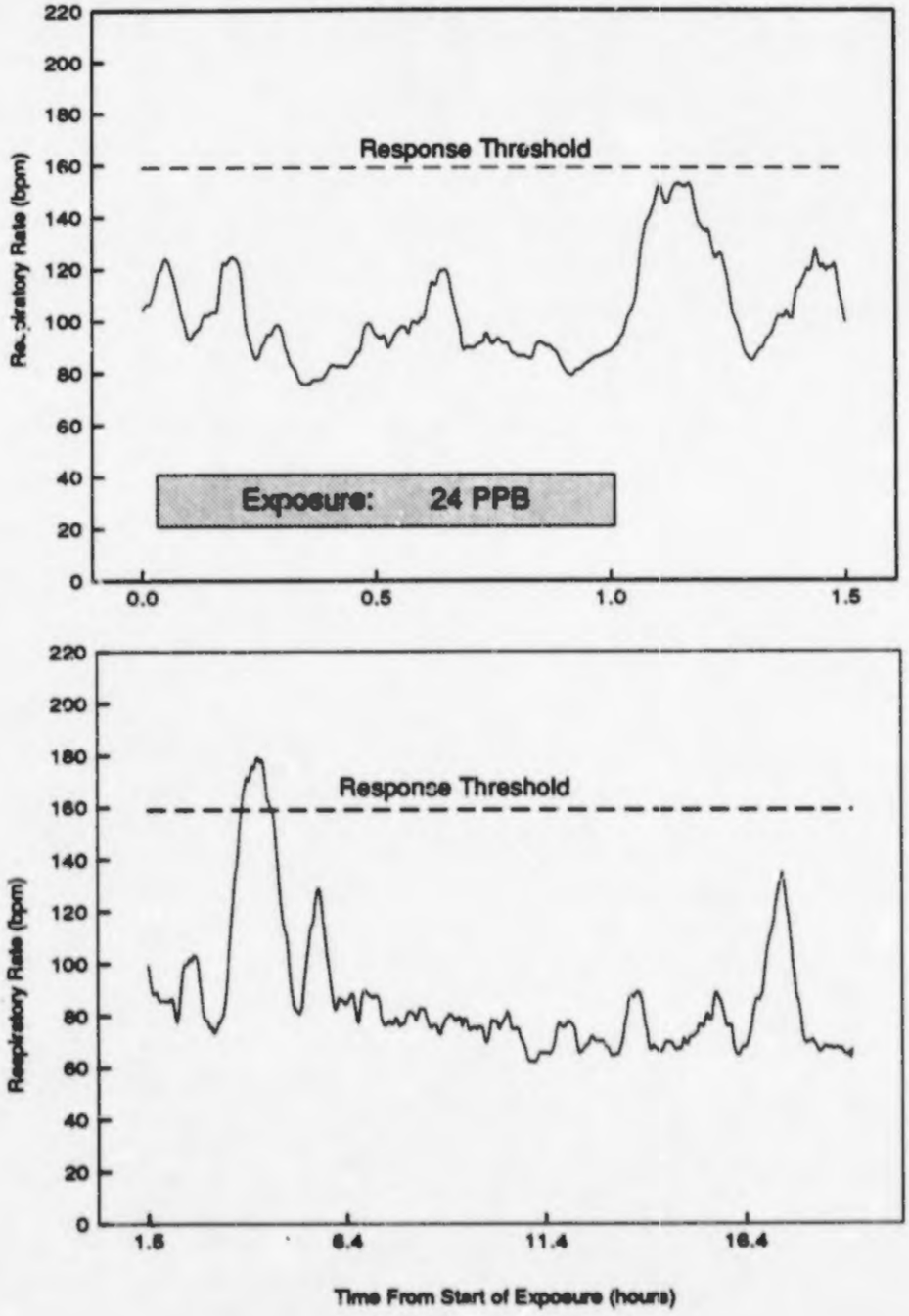
Figure: 42 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 228: Sham Sensitization

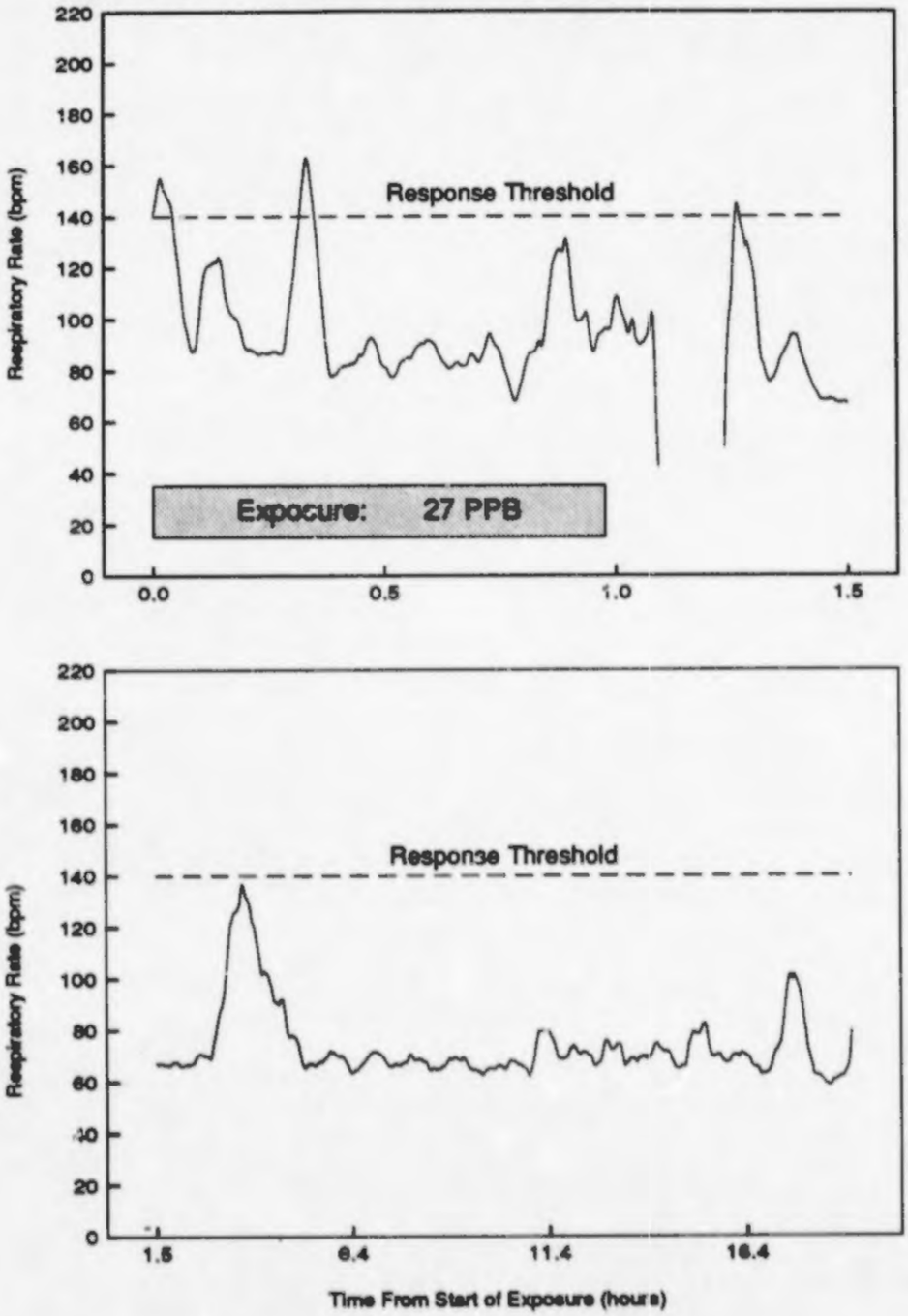
Figure: 43 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 229: Sham Sensitization

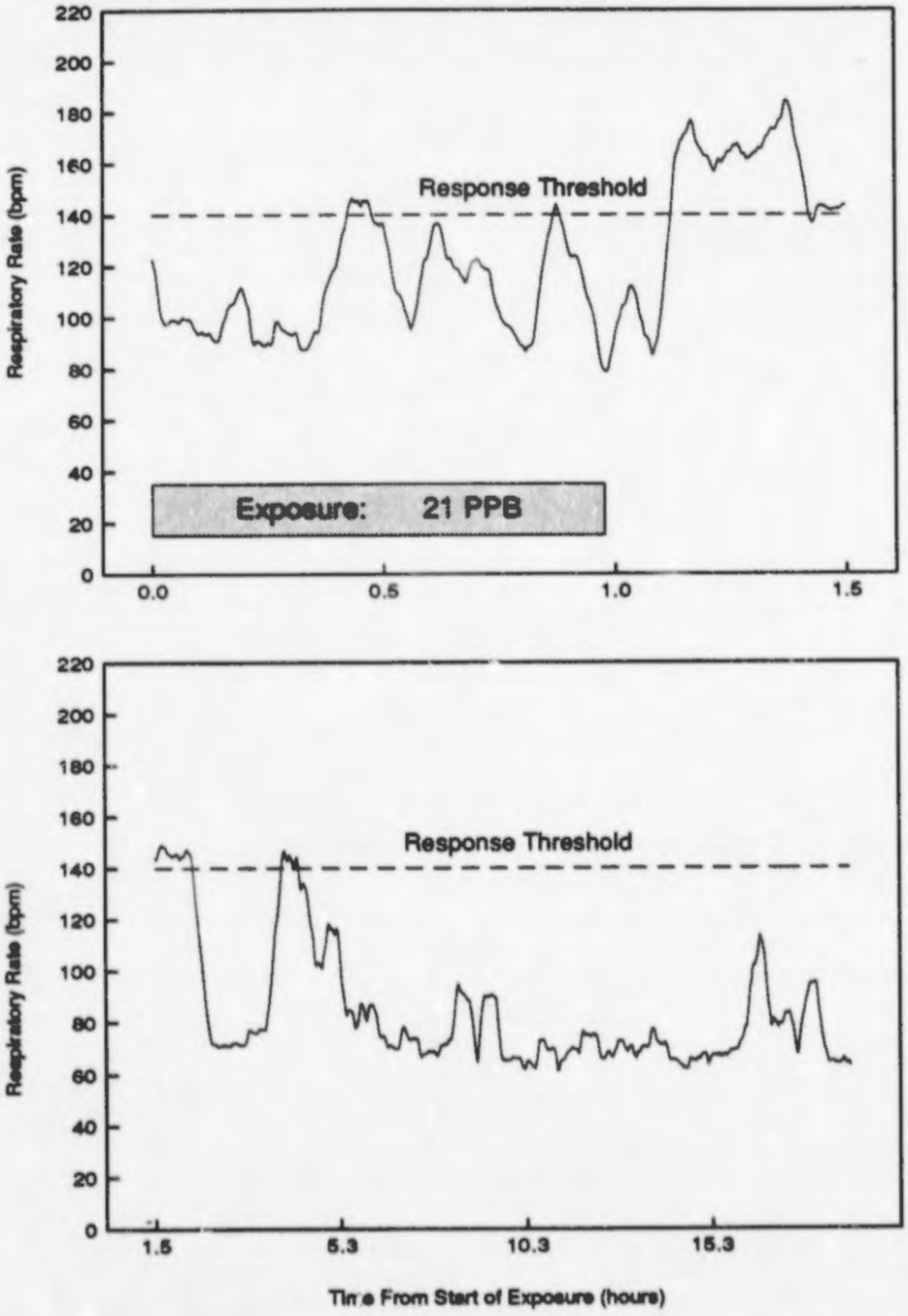
Figure: 44 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 230: Sham Sensitization

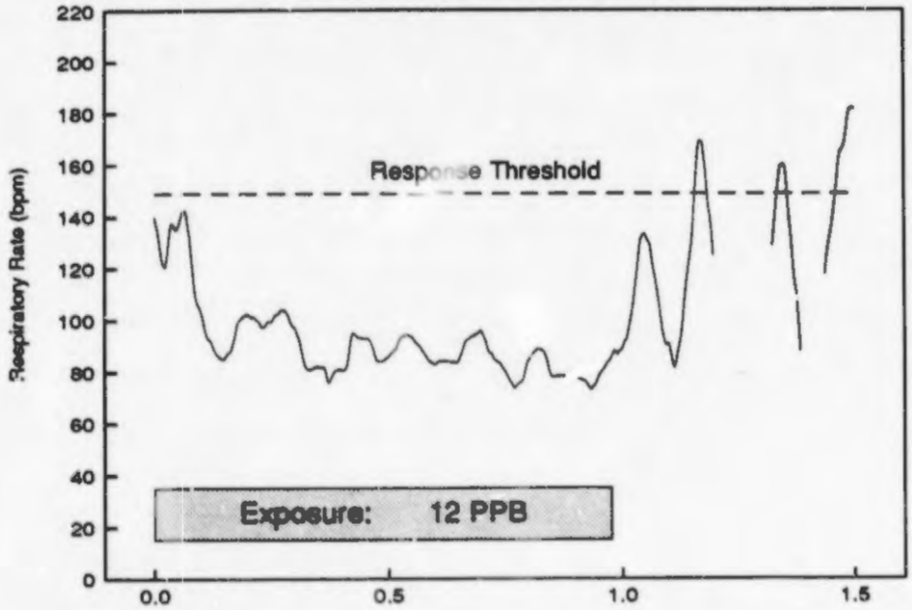
Figure: 45 2,4-TDI Challenge Week 1

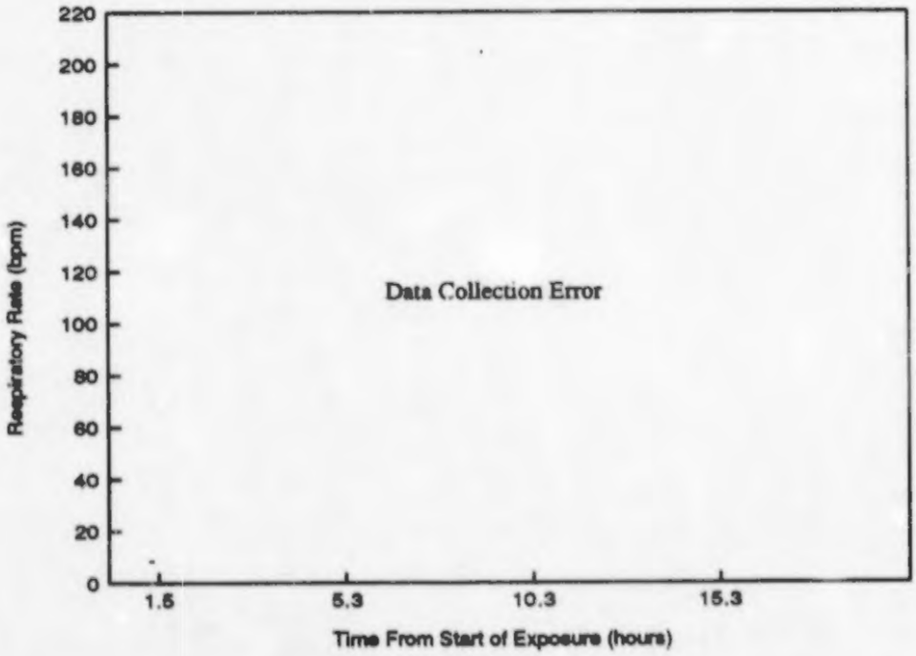


Study Number 92-344-PP

Animal # 231: Sham Sensitization

Figure: 46 2,4-TDI Challenge Week 1

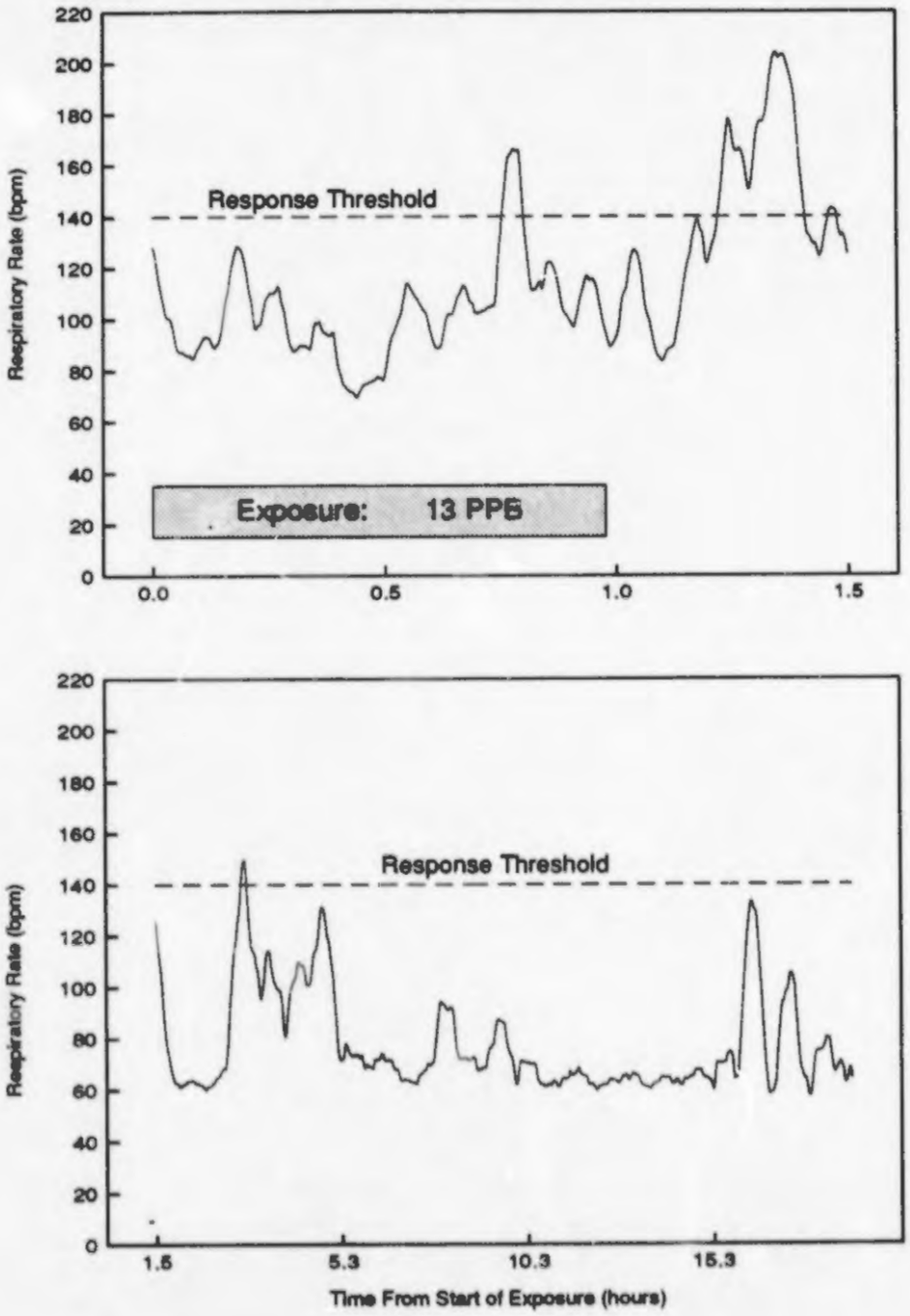




Study Number 92-344-PP

Animal # 232: Sham Sensitization

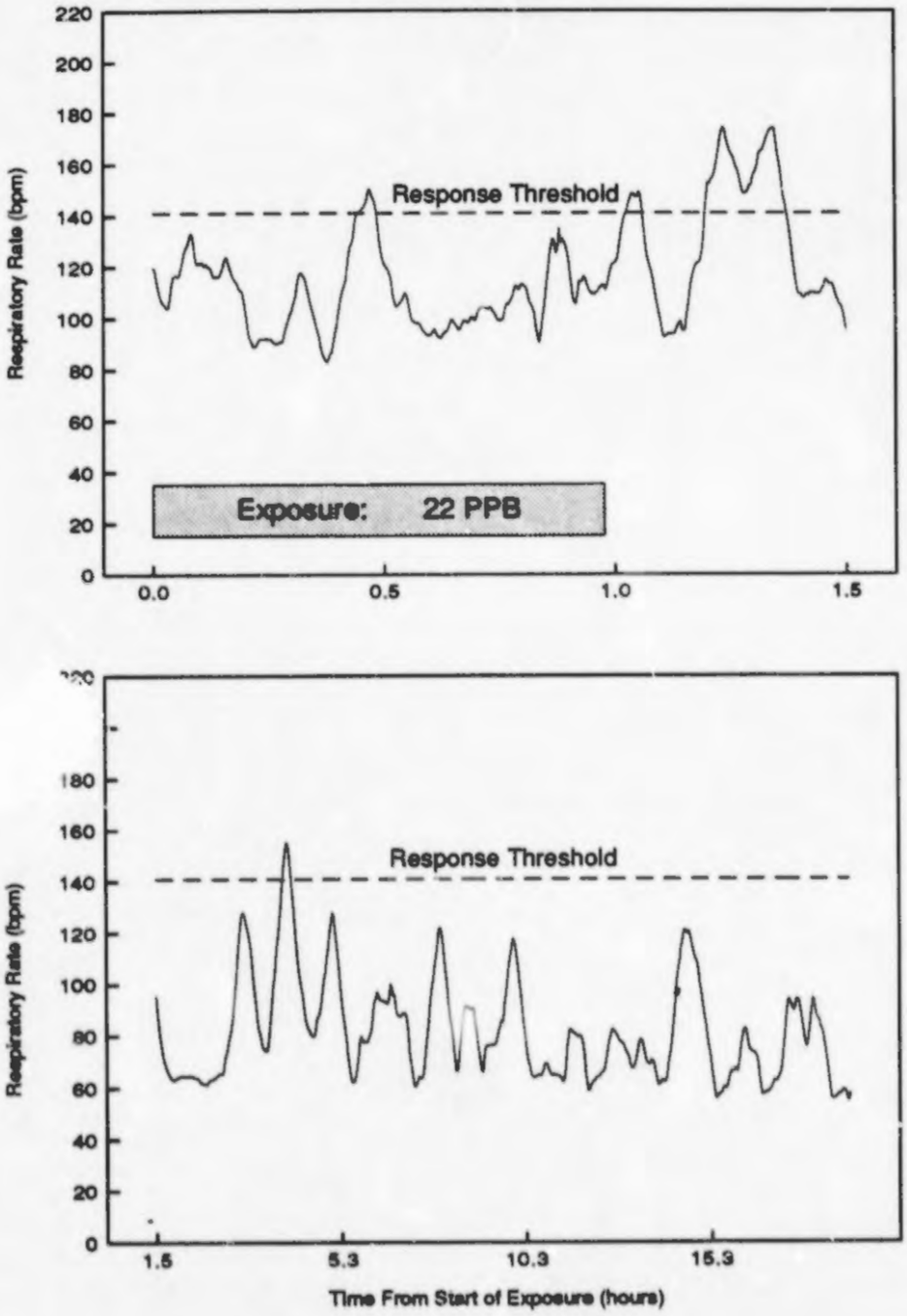
Figure: 47 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 233: Sham Sensitization

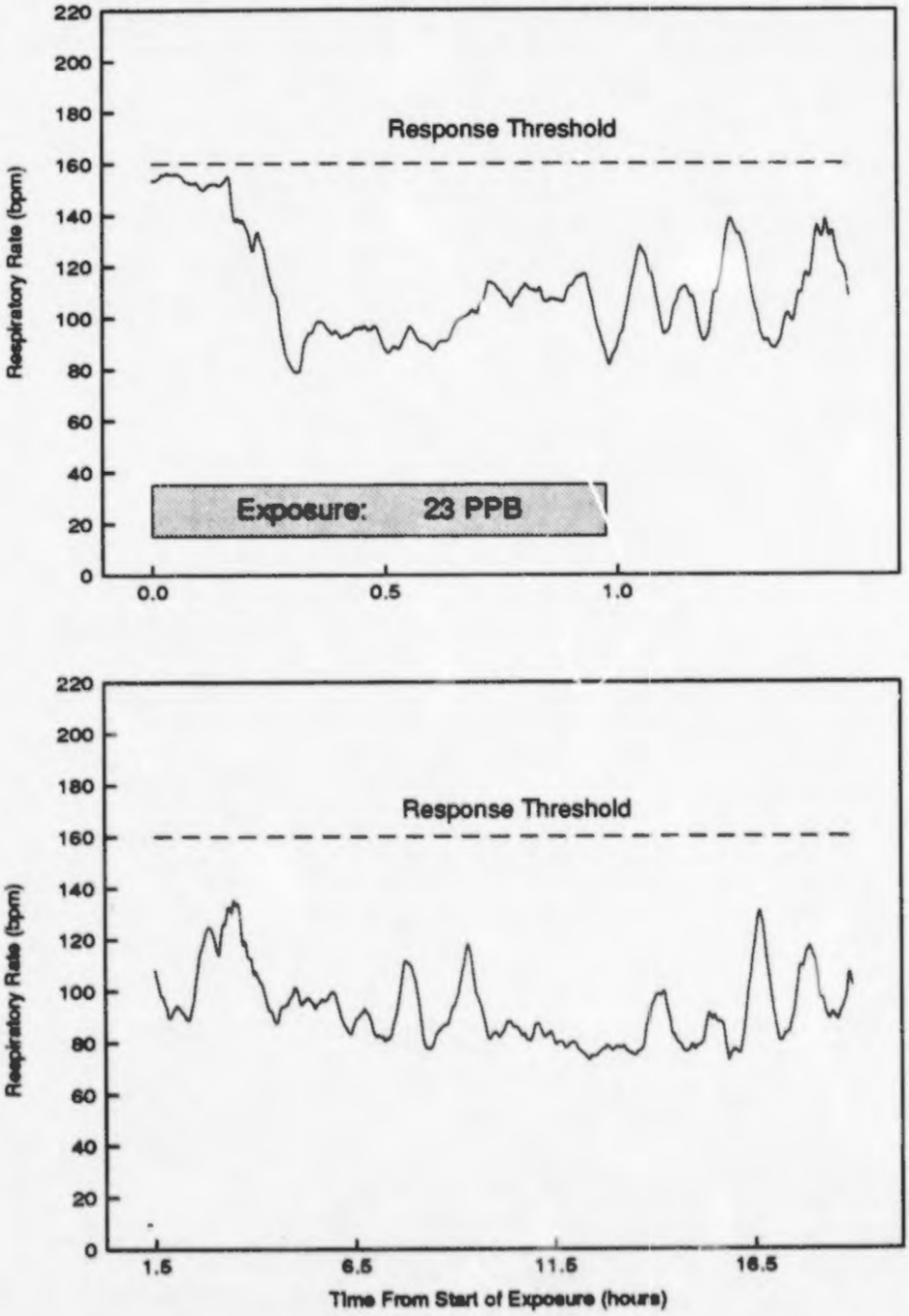
Figure: 48 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 222: Sham Sensitization

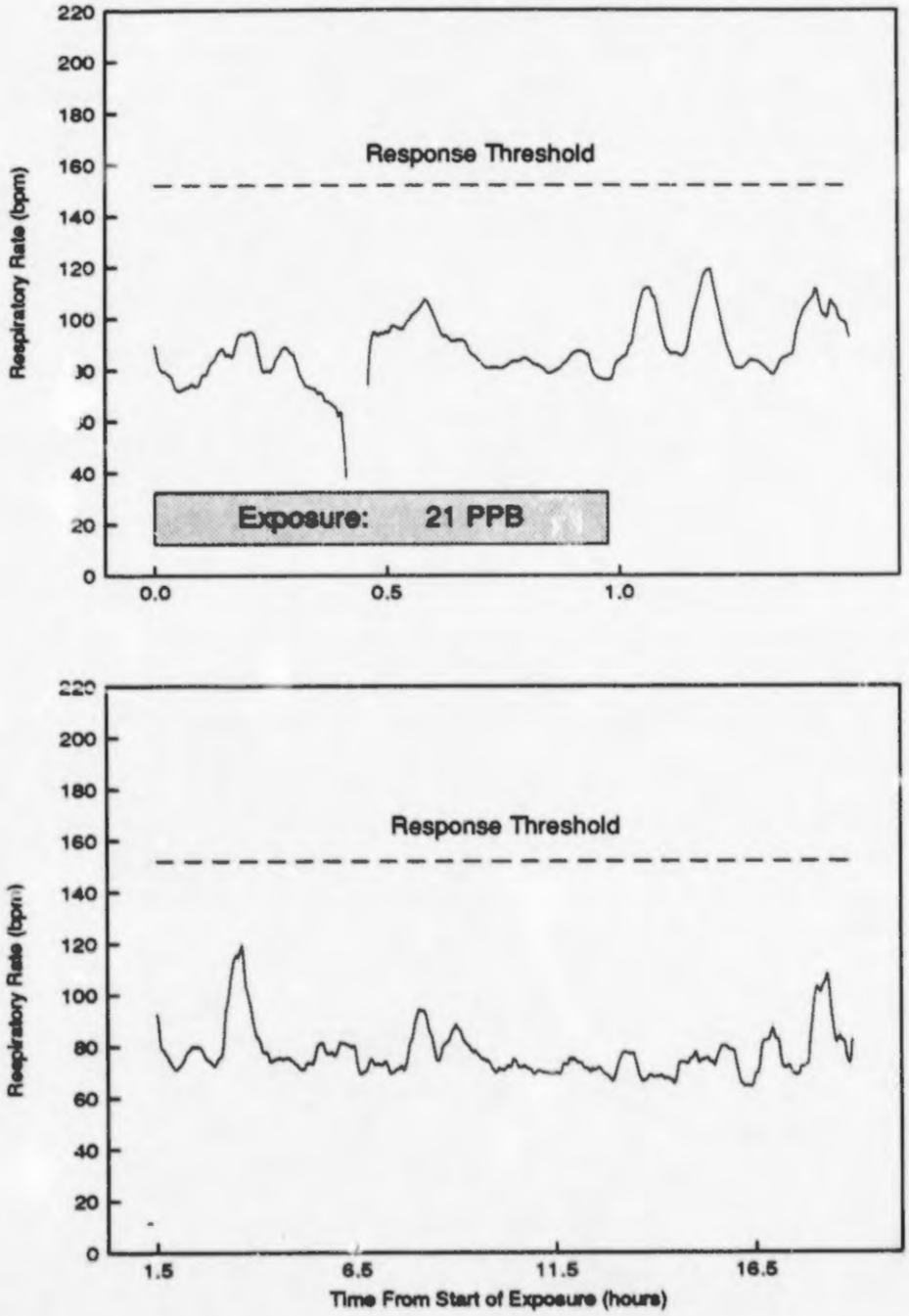
Figure: 49 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 223: Sham Sensitization

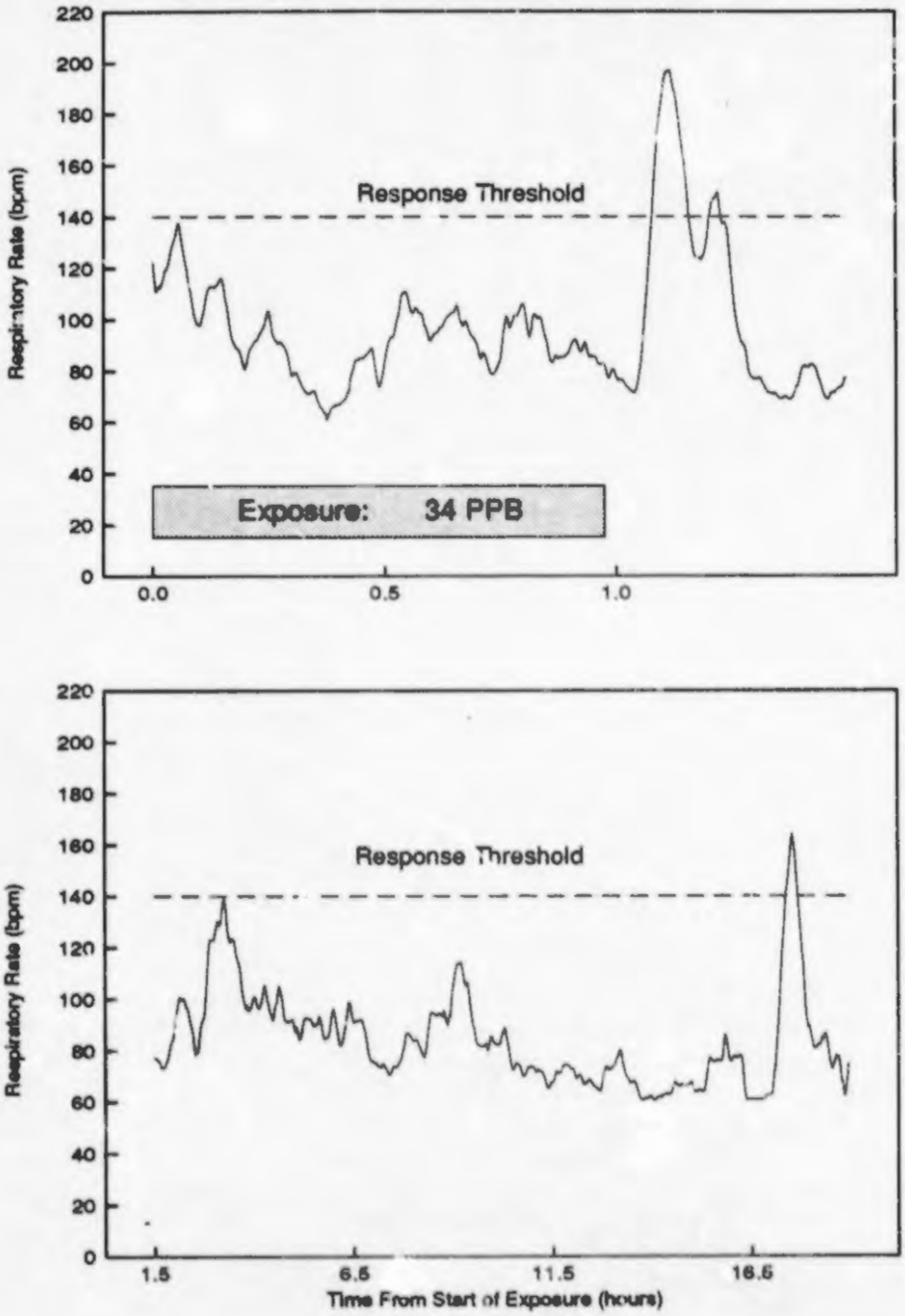
Figure: 50 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 224: Sham Sensitization

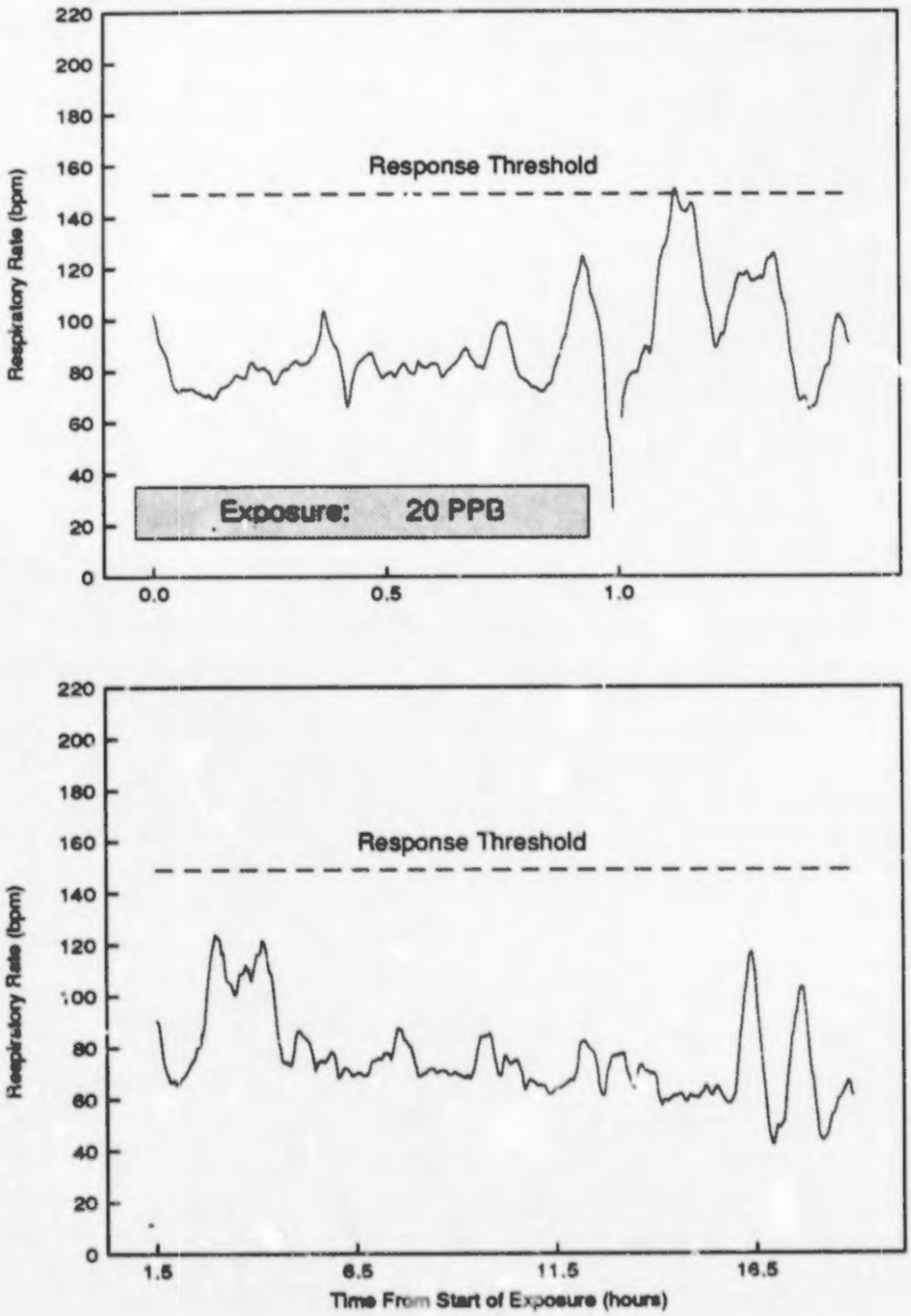
Figure: 51 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 225: Sham Sensitization

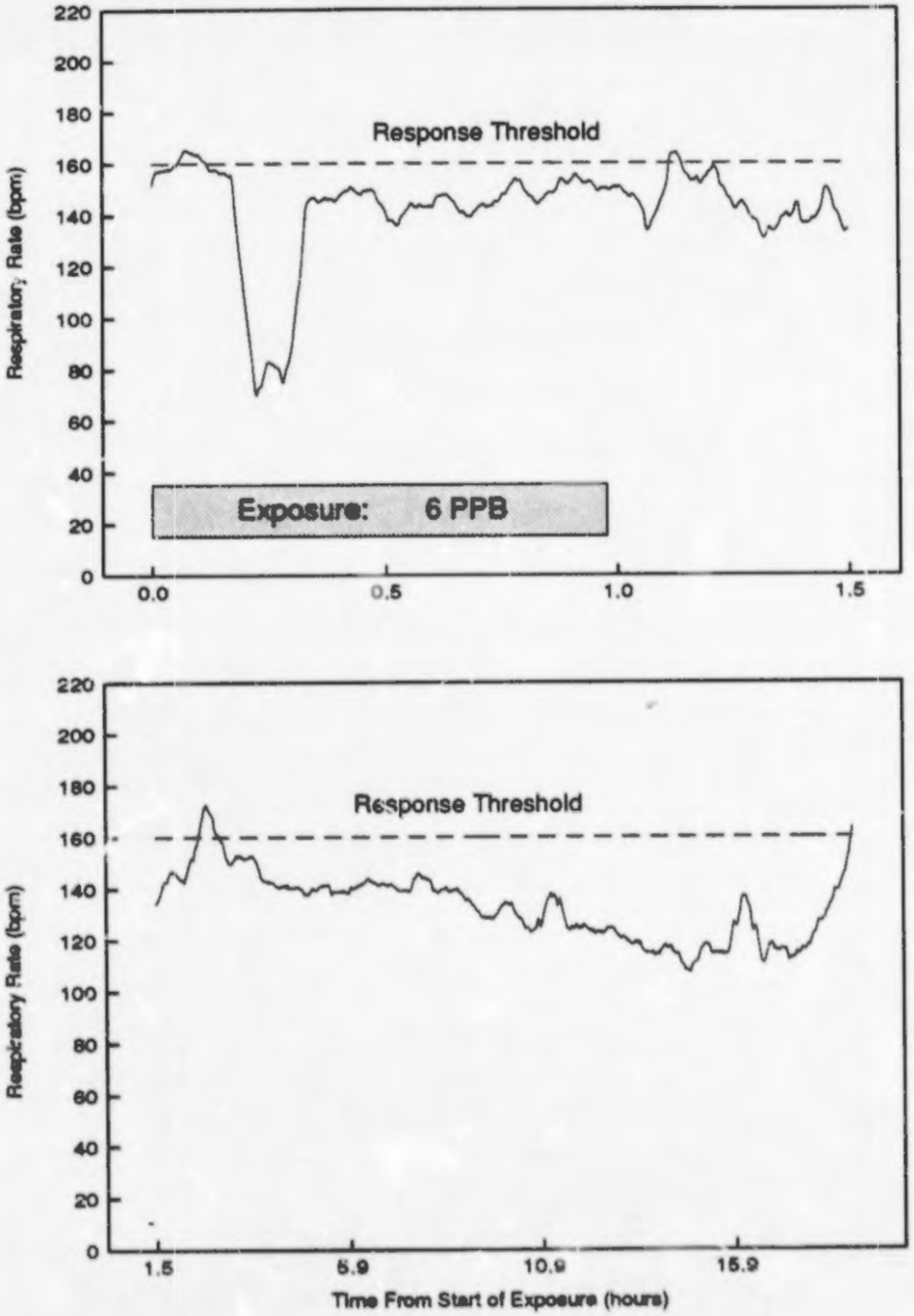
Figure: 52 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 226: Sham Sensitization

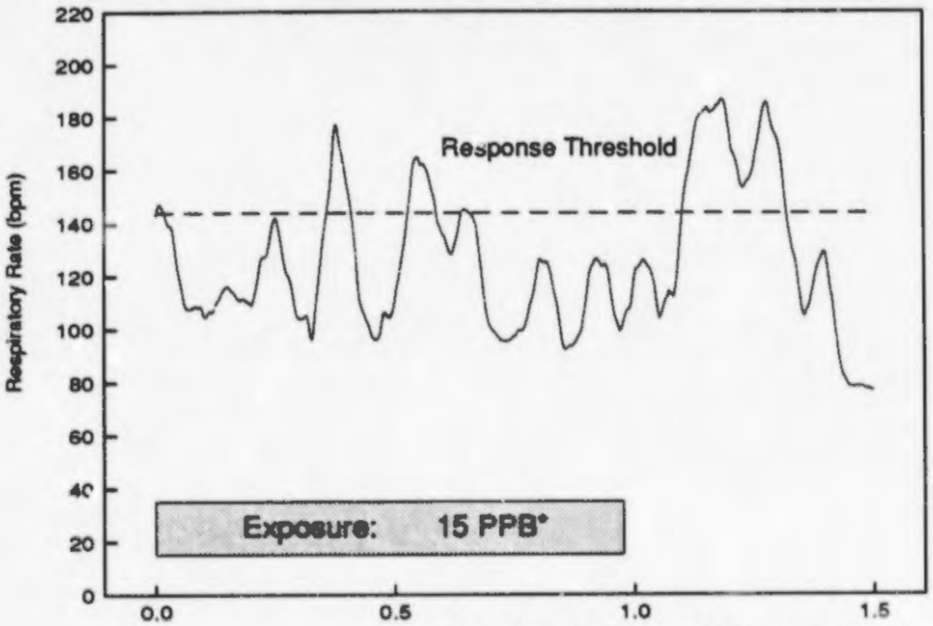
Figure: 53 2,4-TDI Challenge Week 2



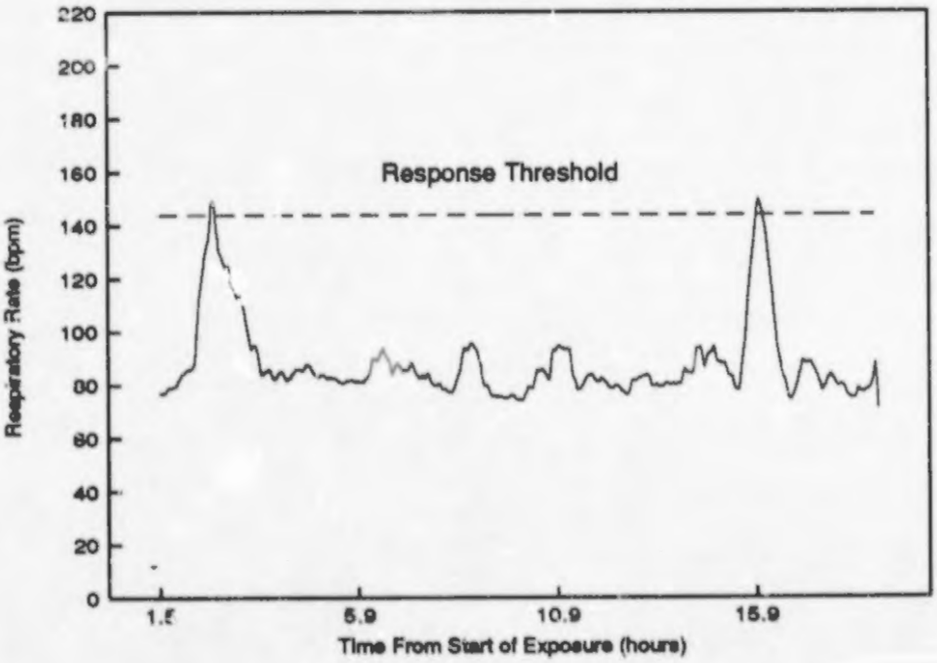
Study Number 92-344-PP

Animal # 227: Sham Sensitization

Figure: 54 2,4-TDI Challenge Week 2



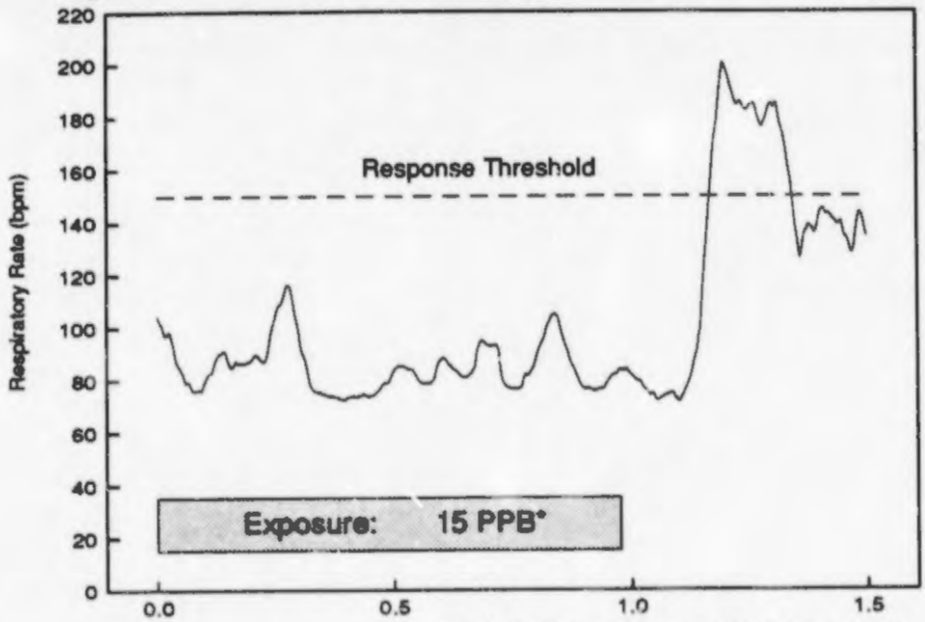
* Data Collection Error - Daily Average Used To Represent Individual Concentration



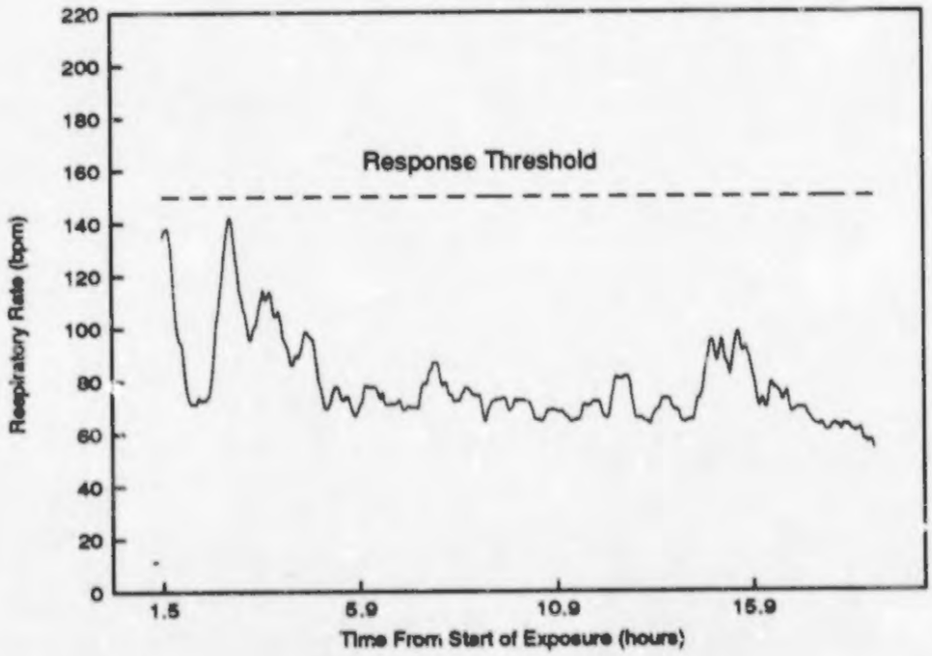
Study Number 92-344-PP

Animal # 228: Sham Sensitization

Figure: 55 2,4-TDI Challenge Week 2



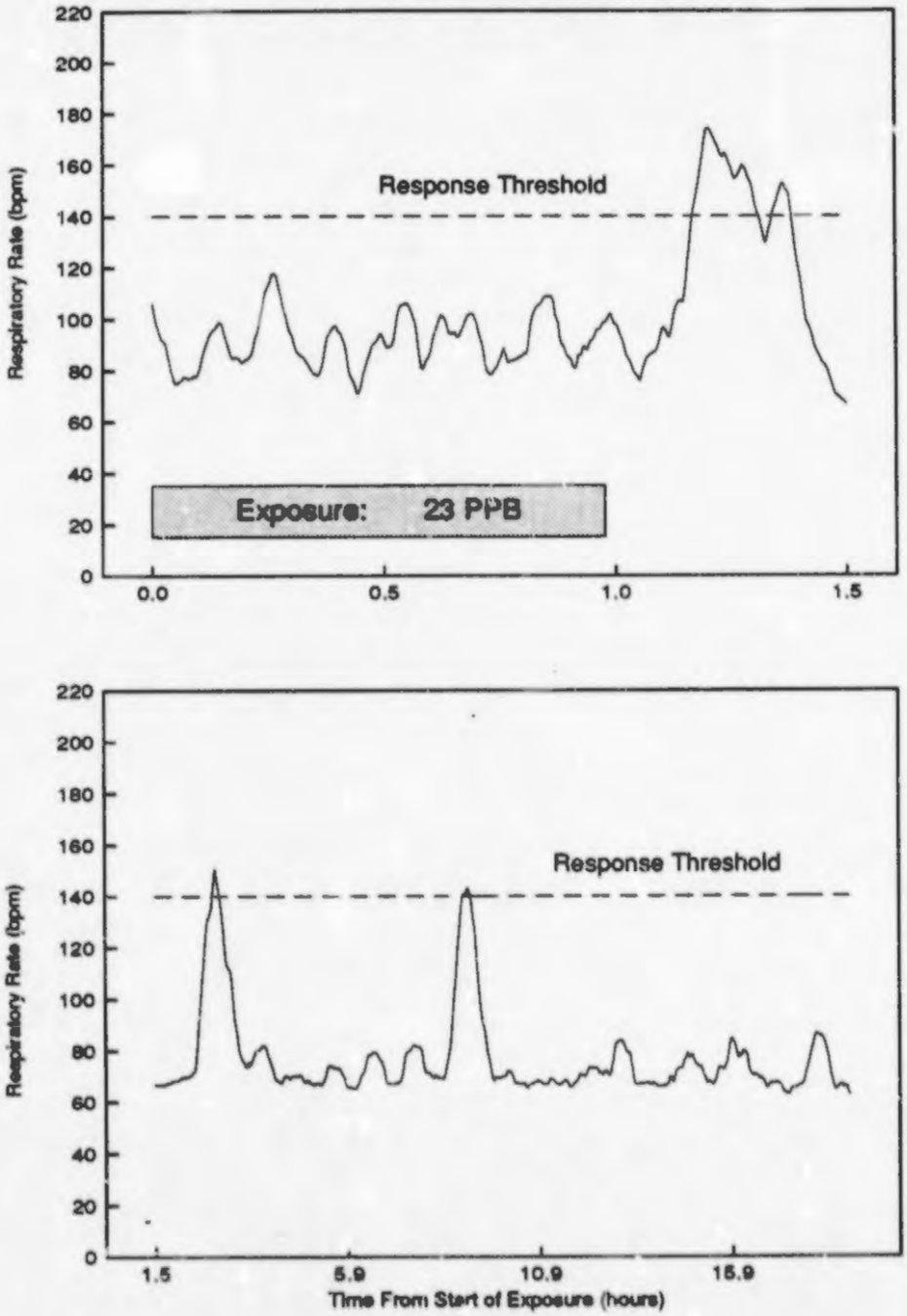
* Data Collection Error - Daily Average Used To Represent Individual Concentration



Study Number 92-344-PP

Animal # 229: Sham Sensitization

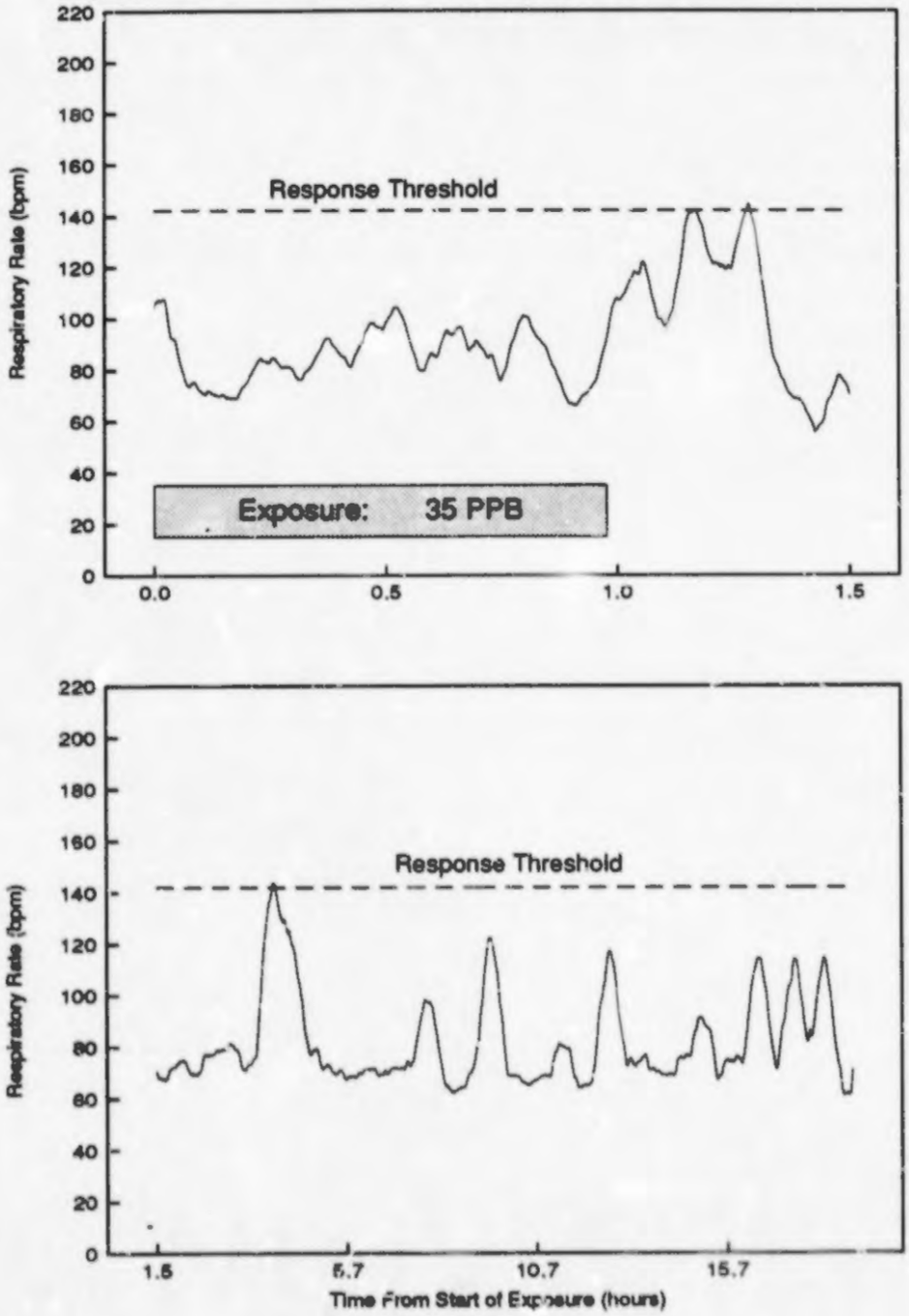
Figure: 56 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 230: Sham Sensitization

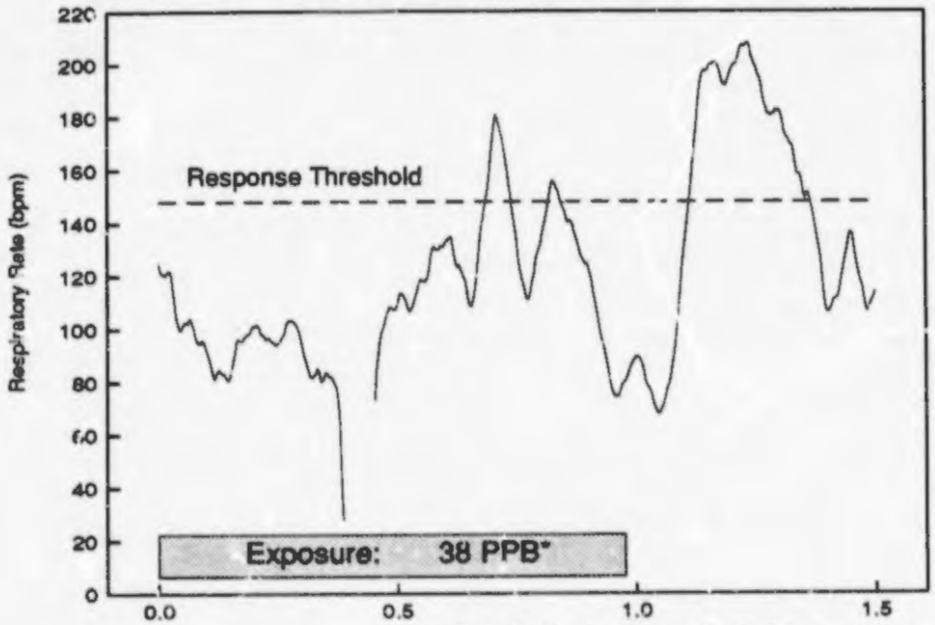
Figure: 57 2,4-TDI Challenge Week 2



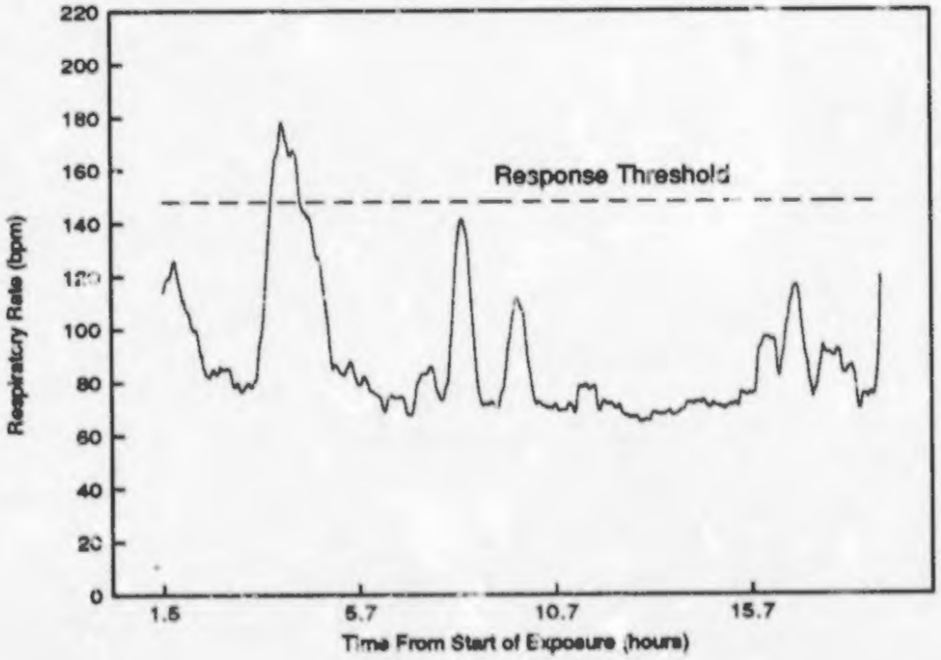
Study Number 92-344-PP

Animal # 231: Sham Sensitization

Figure: 58 2,4-TDI Challenge Week 2



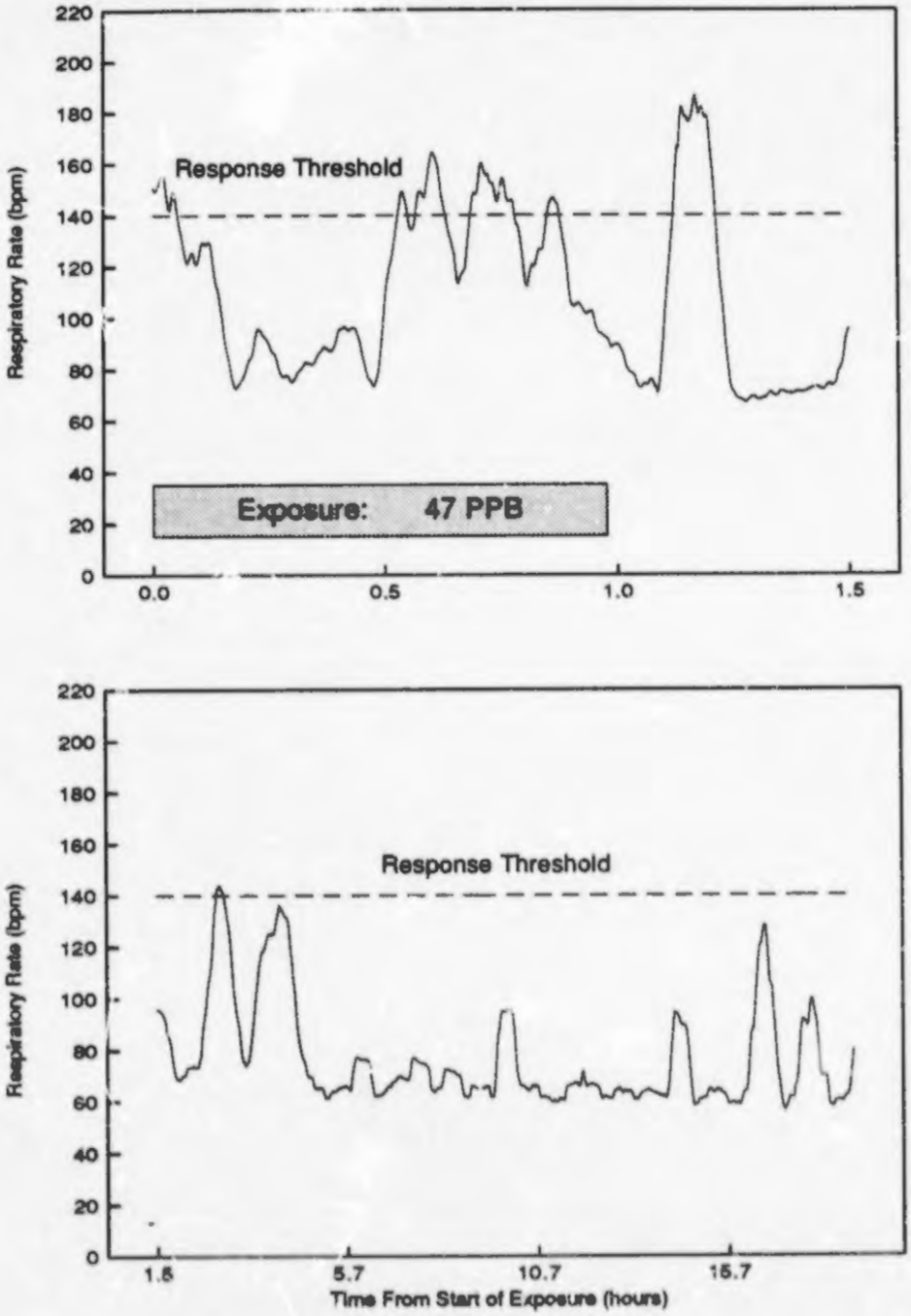
* Data Collection Error - Daily Average Used To Represent Individual Concentration



Study Number 92-344-PP

Animal # 232: Sham Sensitization

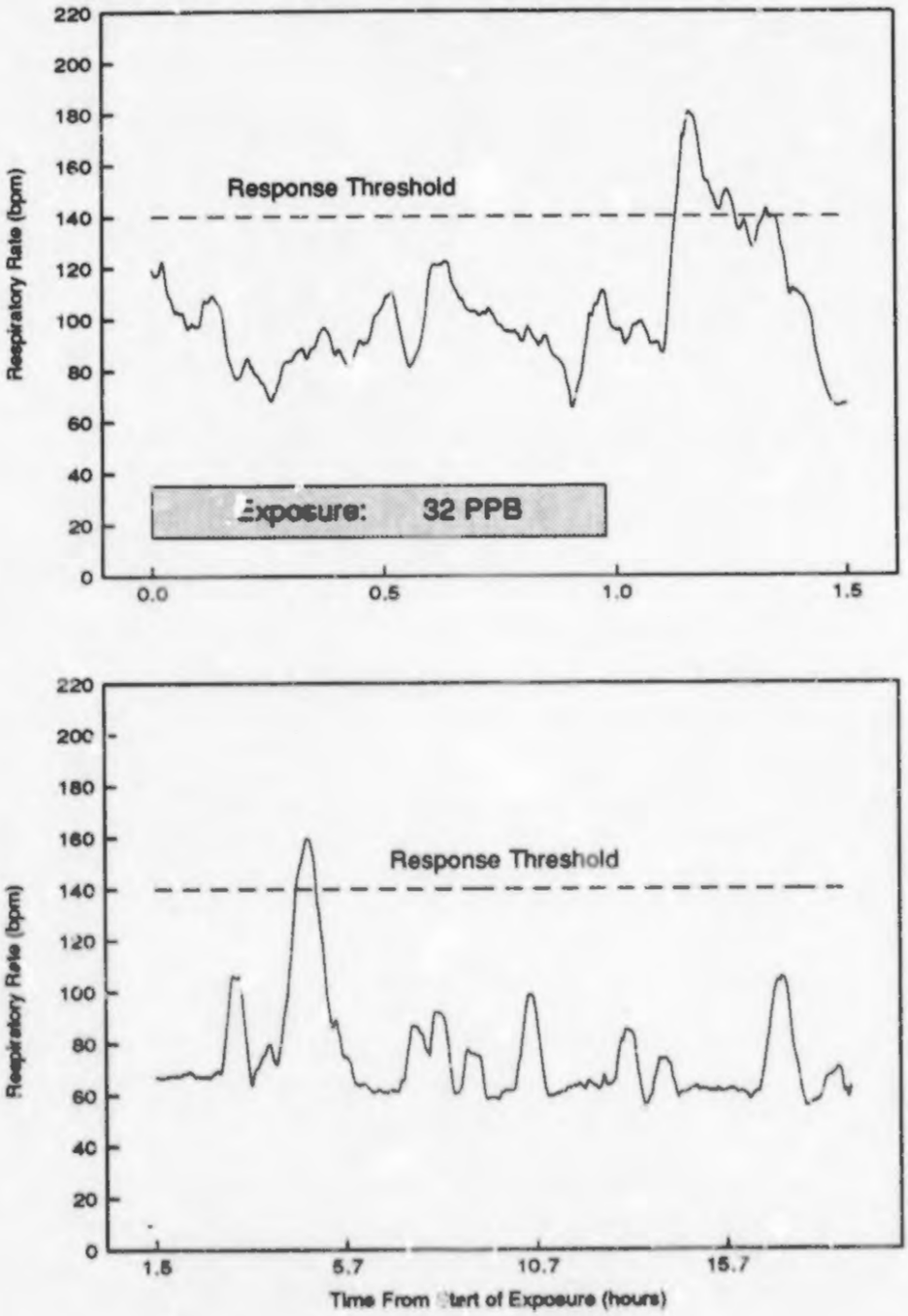
Figure: 59 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 233: Sham Sensitization

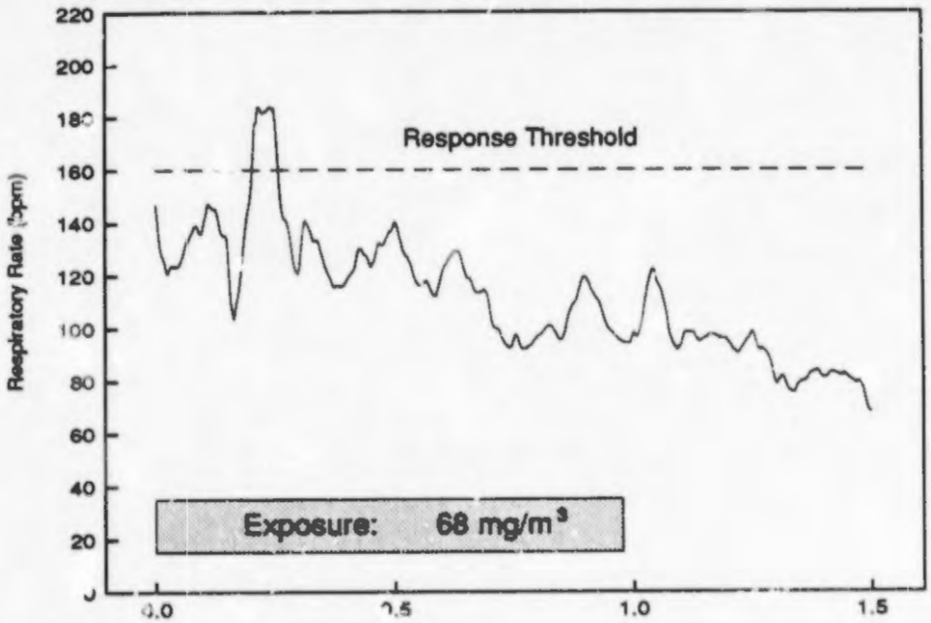
Figure: 60 2,4-TDI Challenge Week 2

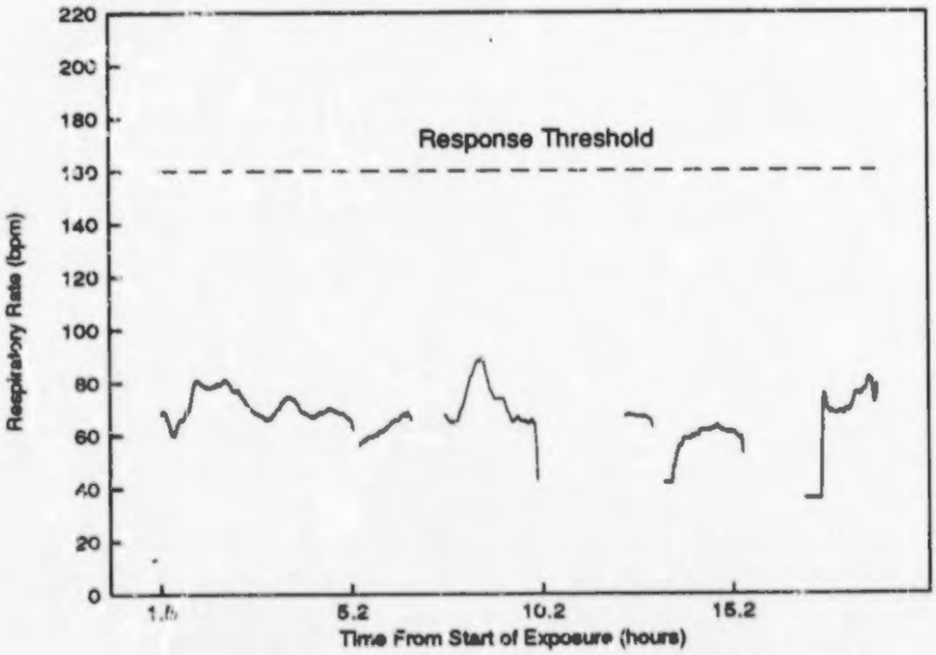


Study Number 92-344-PP

Animal # 222: Sham Sensitization

Figure: 61 2,4-TDI-GSA Challenge Week 3

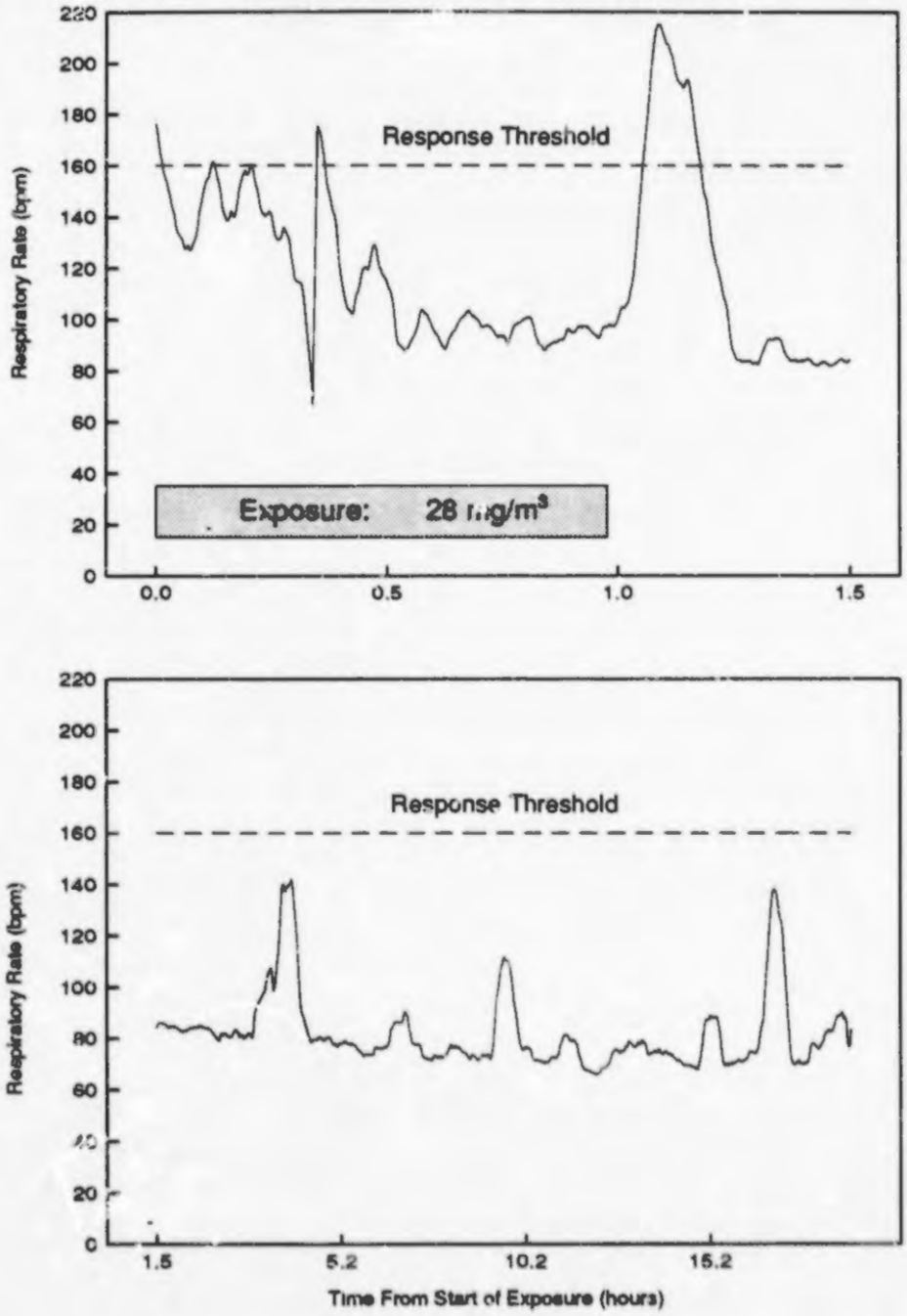




Study Number 92-344-PP

Animal # 223: Sham Sensitization

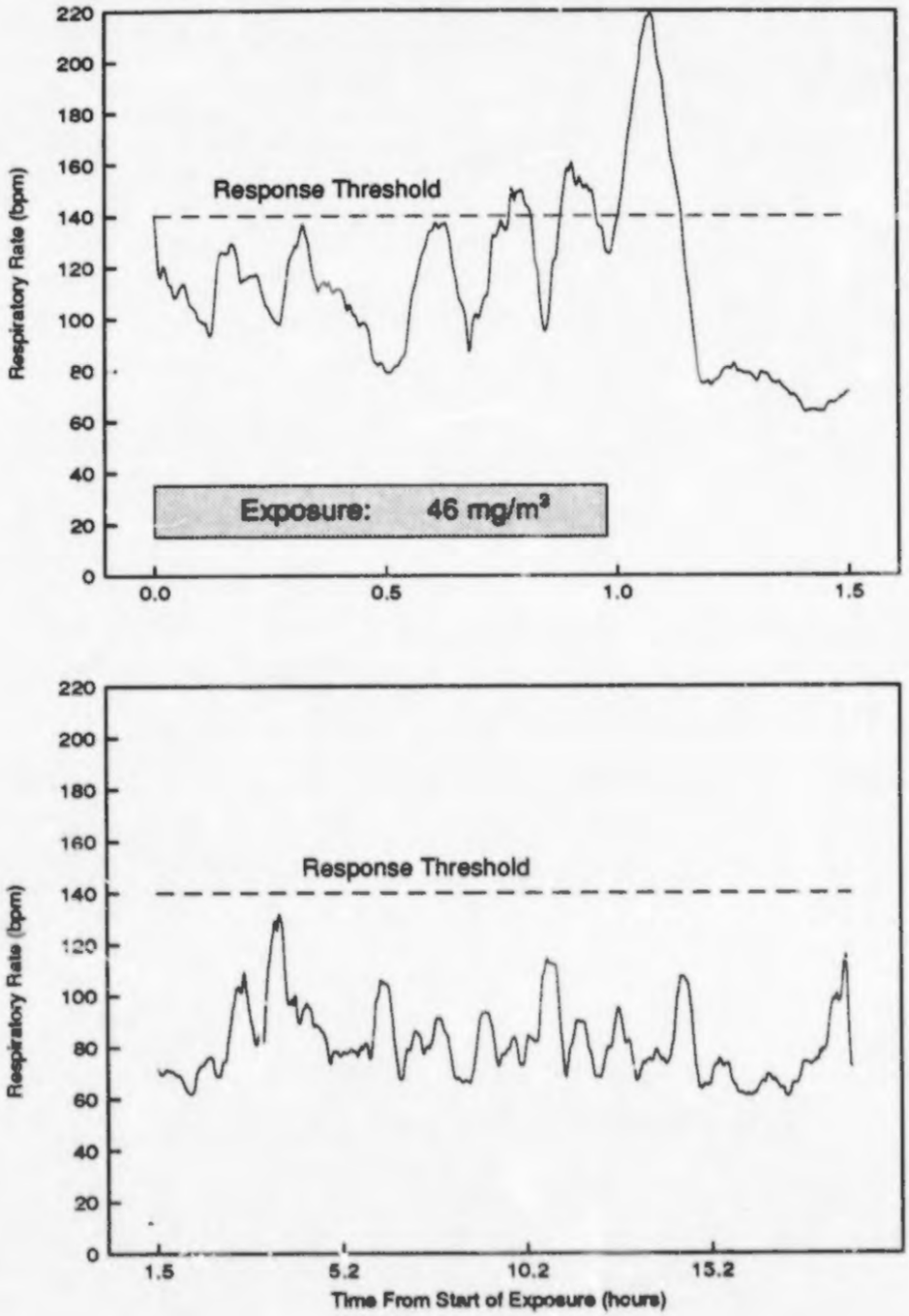
Figure: 62 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 224: Sham Sensitization

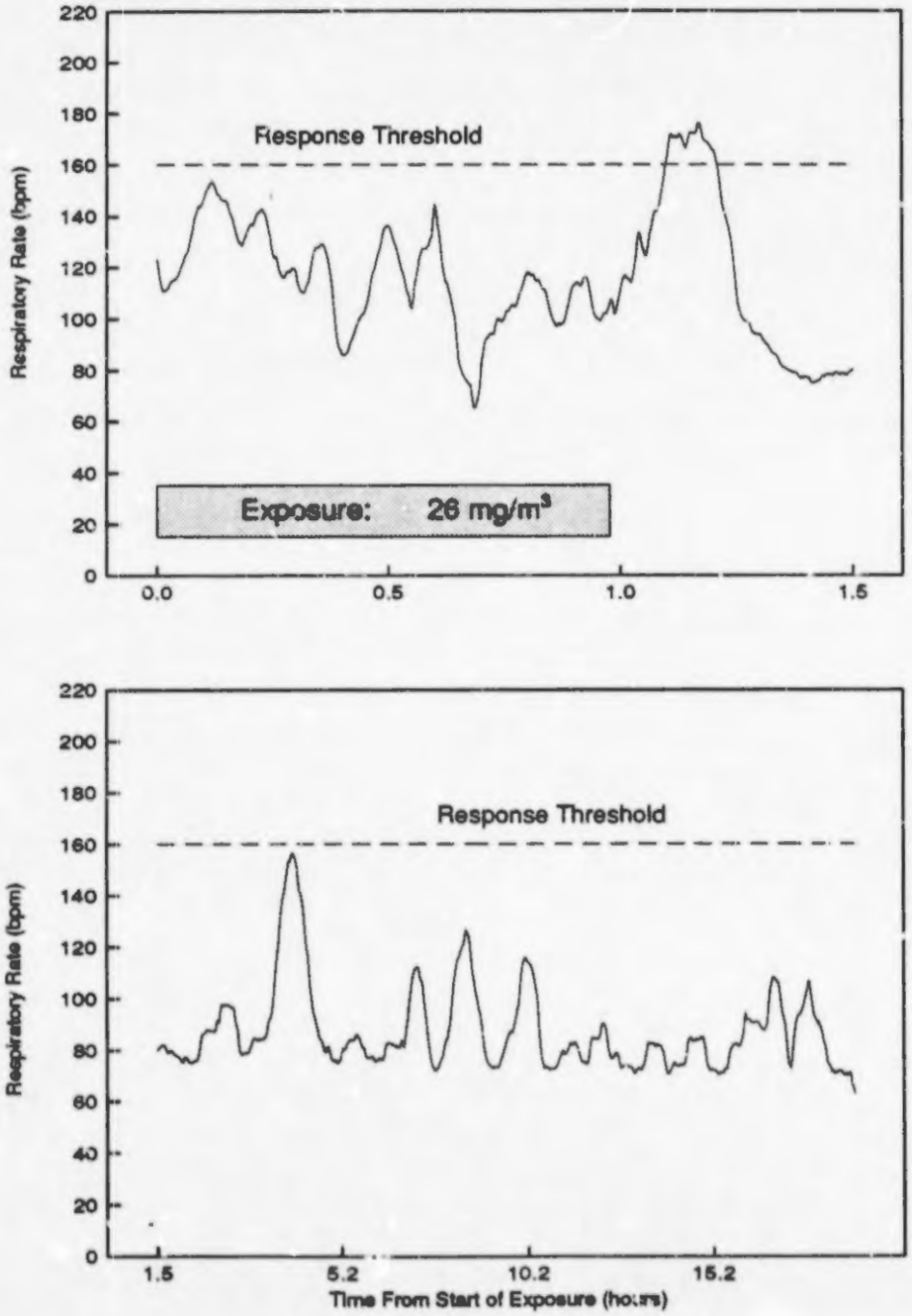
Figure: 63 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 225: Sham Sensitization

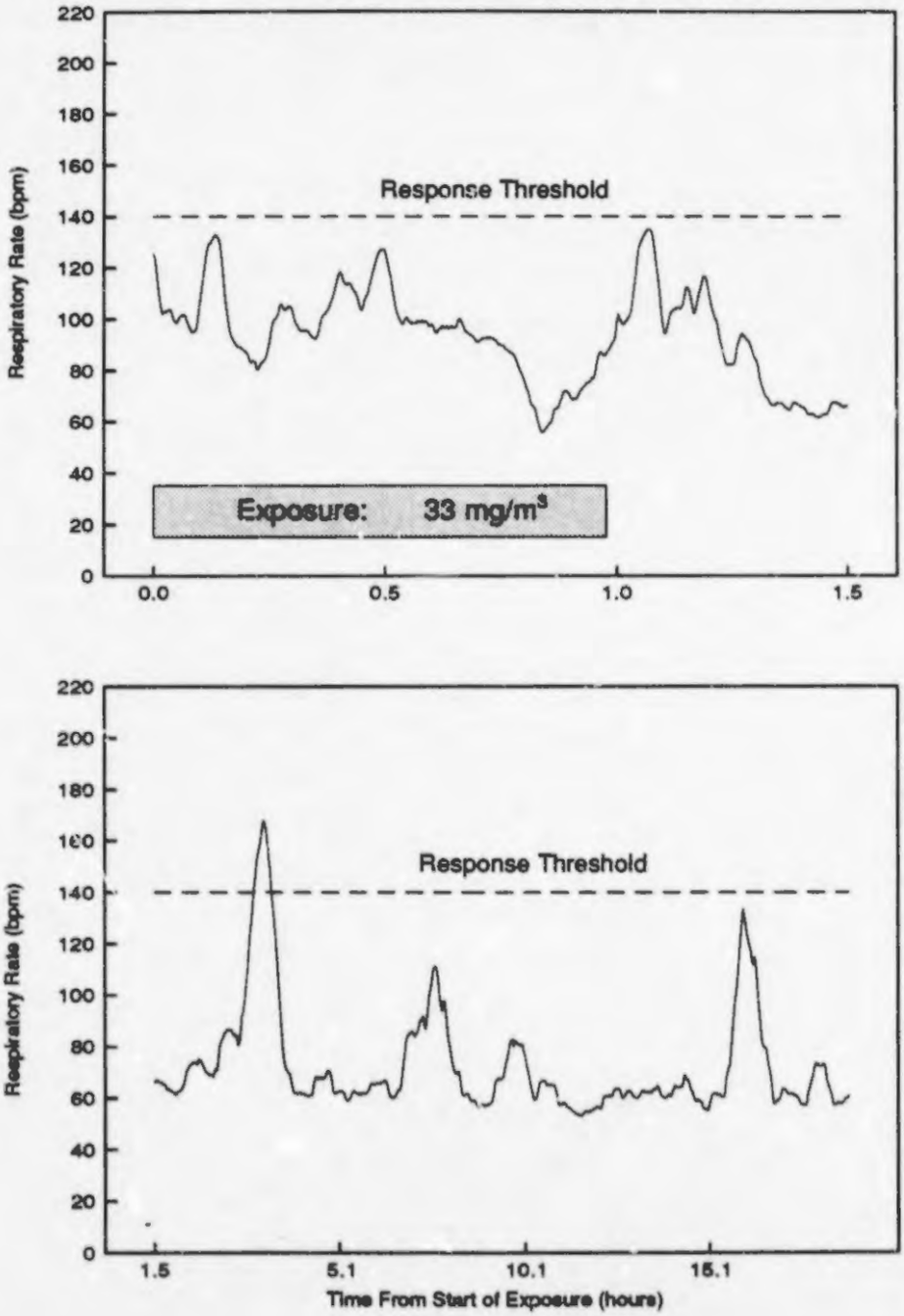
Figure: 64 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 226: Sham Sensitization

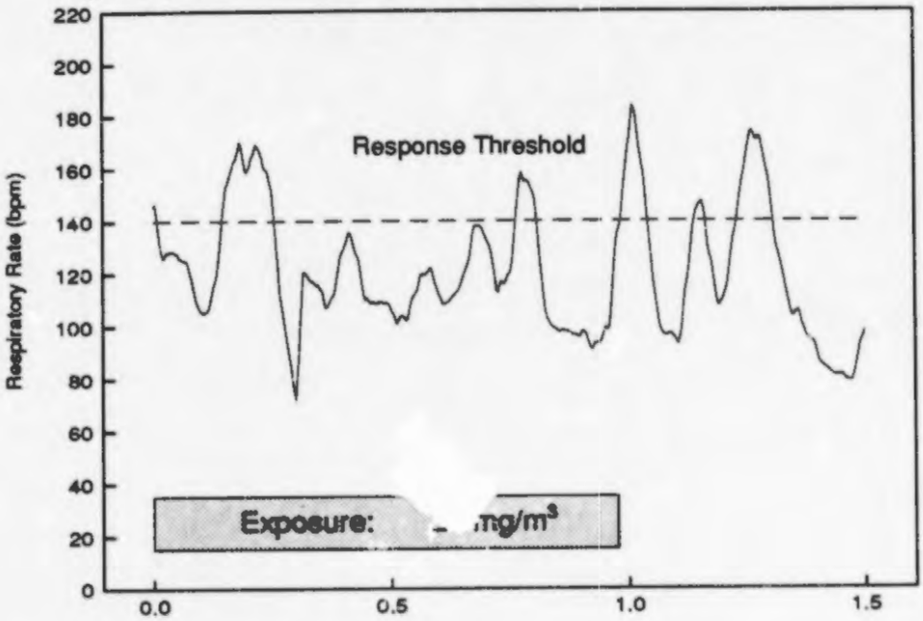
Figure: 65 2,4-TDI-GSA Challenge Week 3

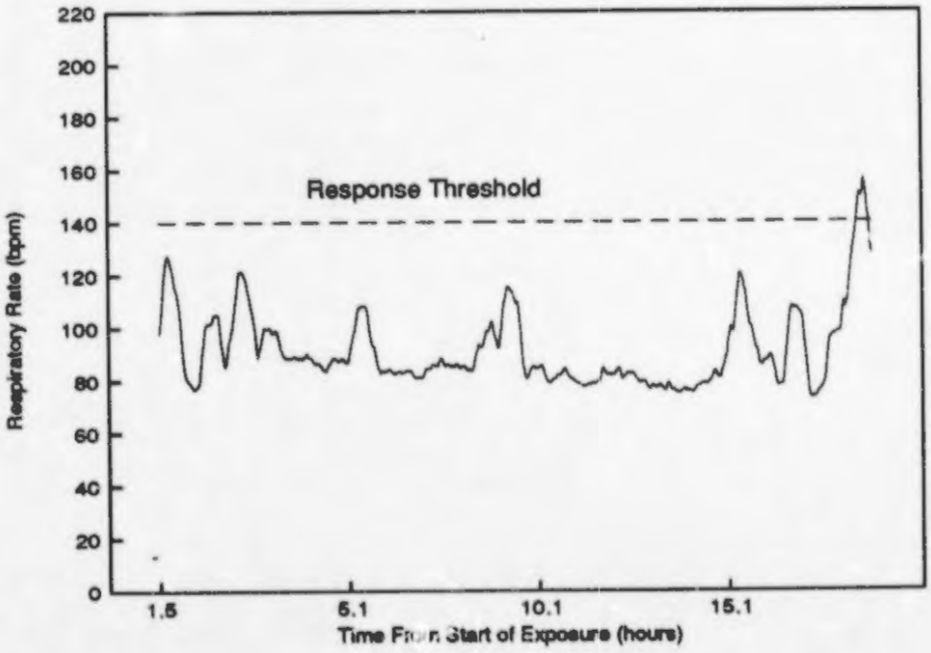


Study Number 92-344-PP

Animal # 227: Sham Sensitization

Figure: 66 2,4-TDI-GSA Challenge Week 3

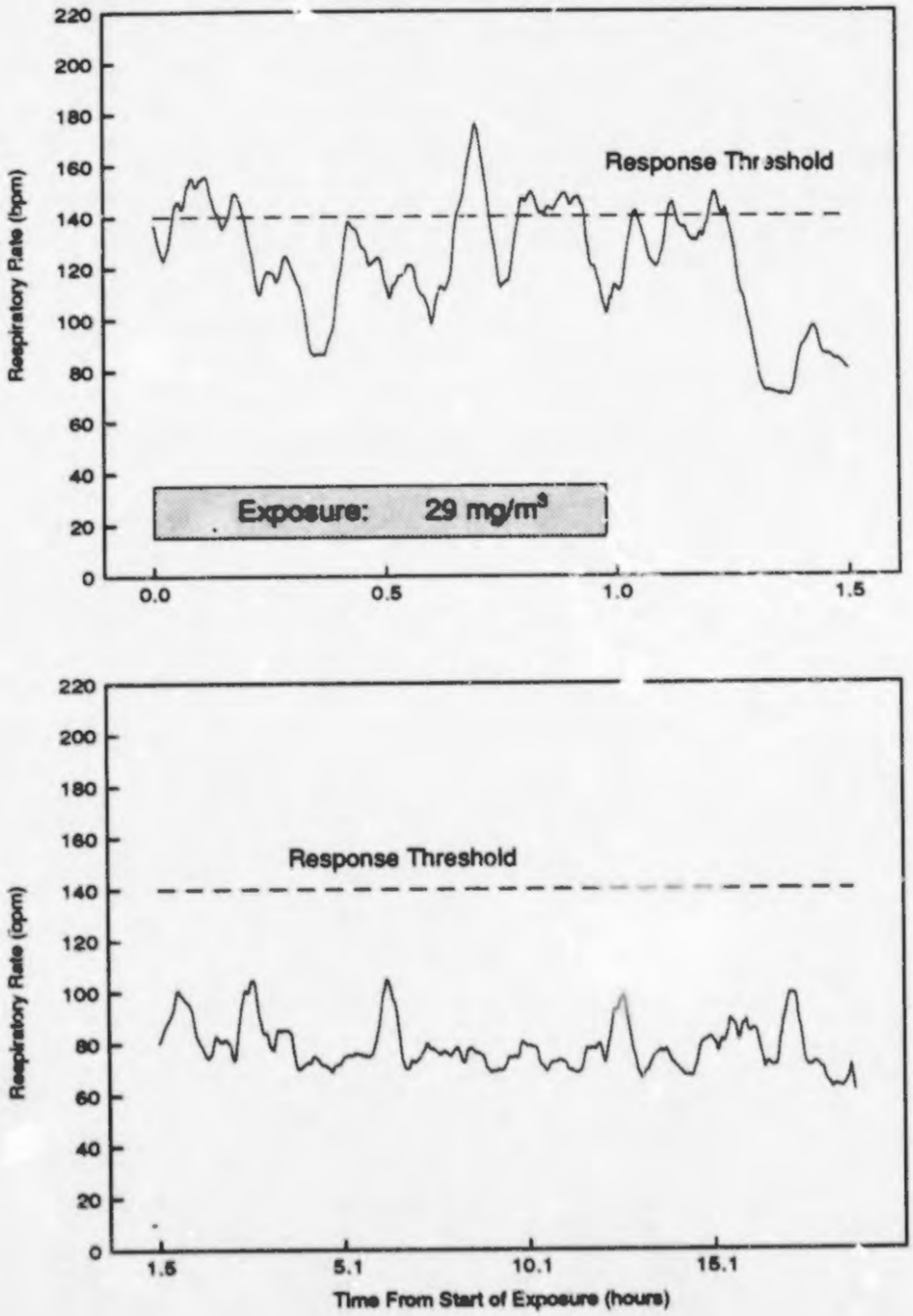




Study Number 92-344-PP

Animal # 228: Sham Sensitization

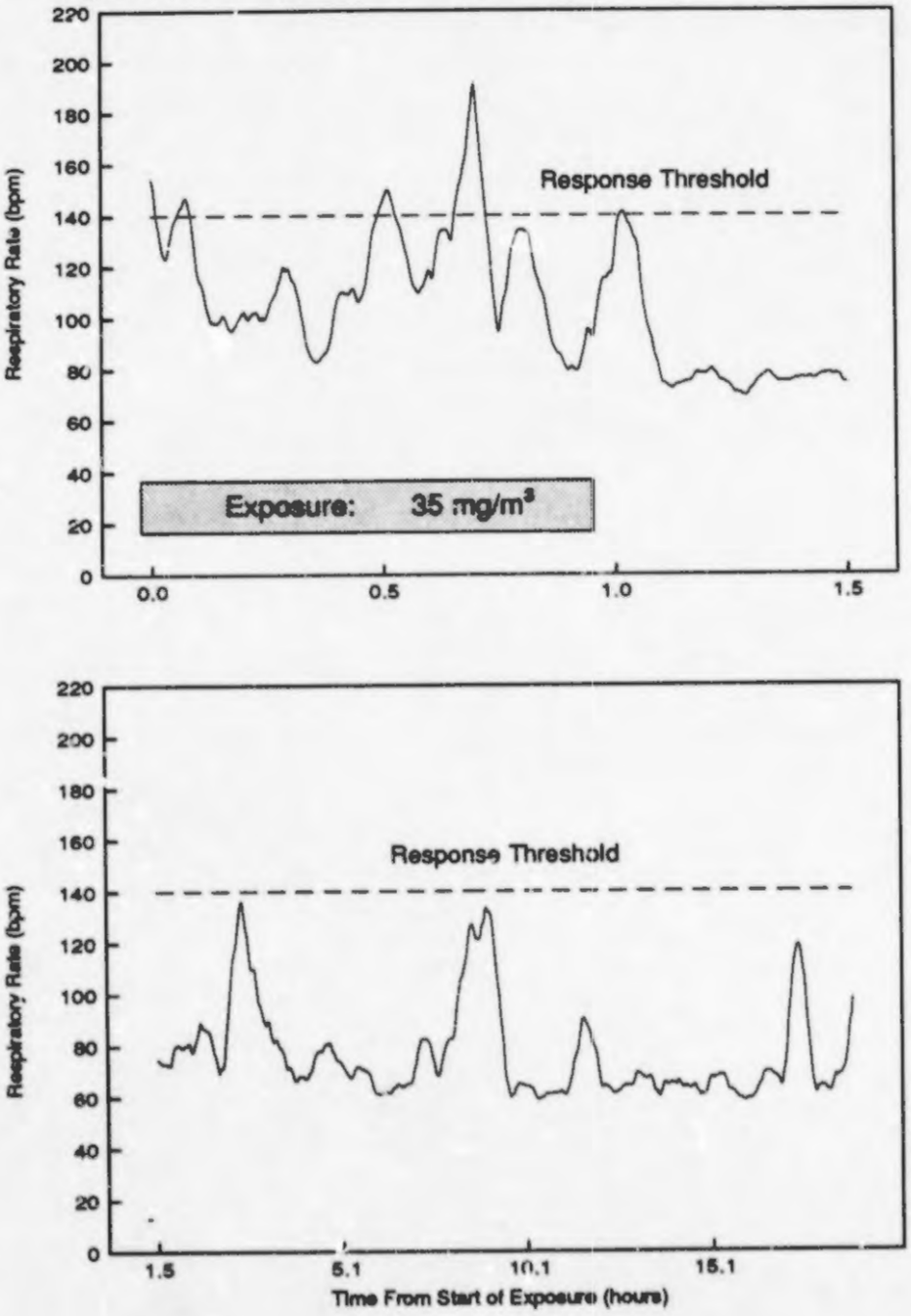
Figure: 67 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 229: Sham Sensitization

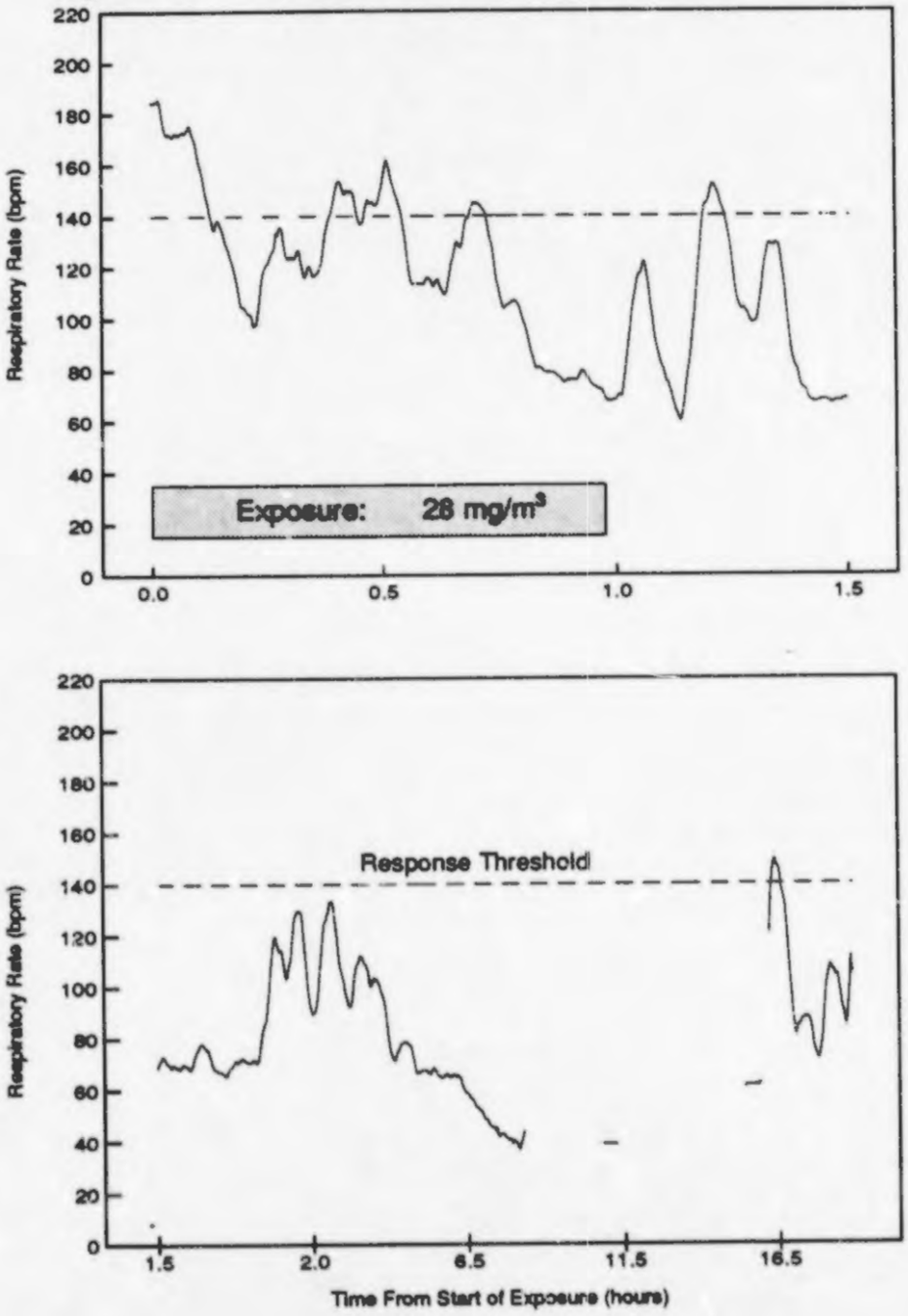
Figure: 68 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 230: Sham Sensitization

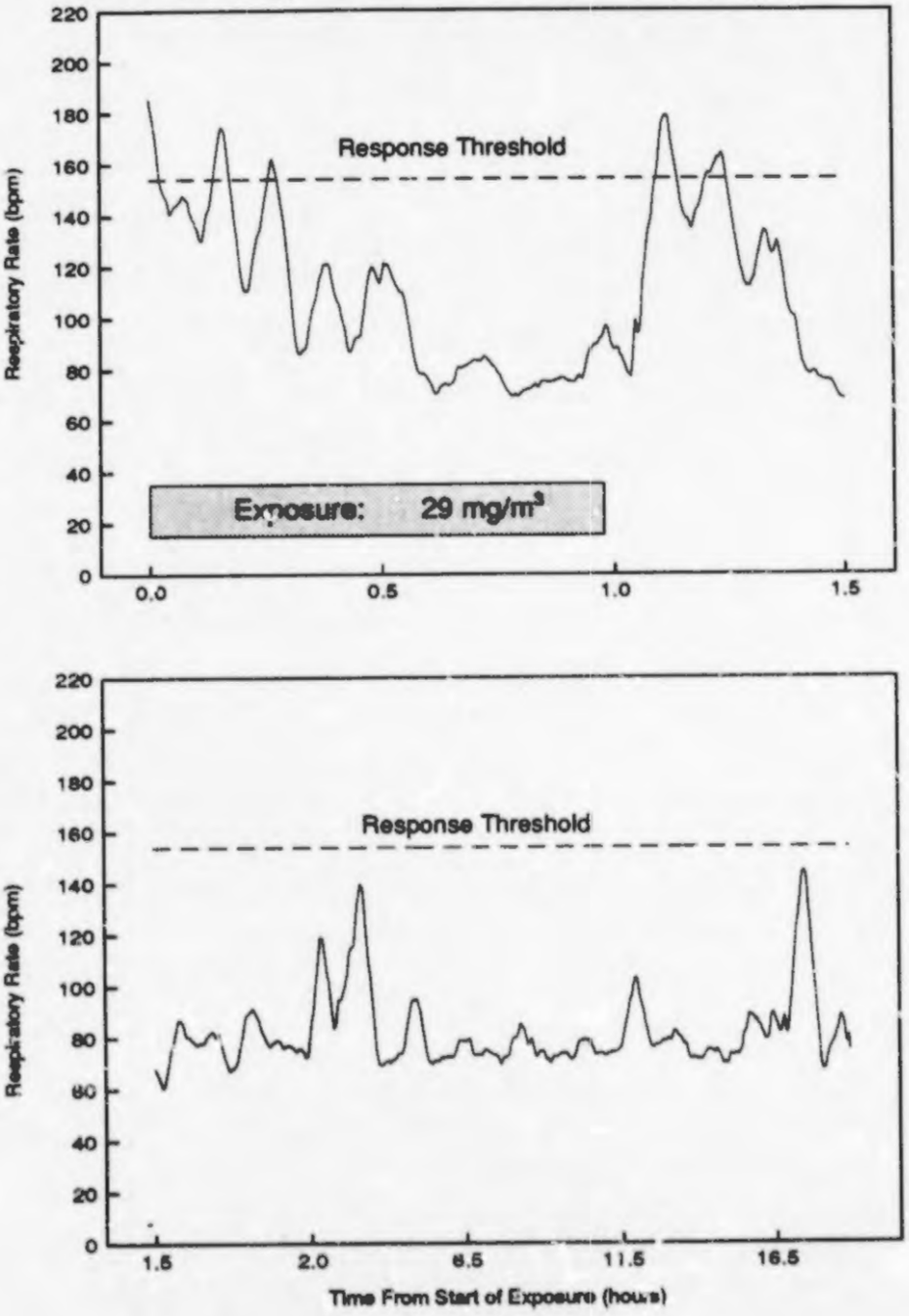
Figure: 69 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 231: Sham Sensitization

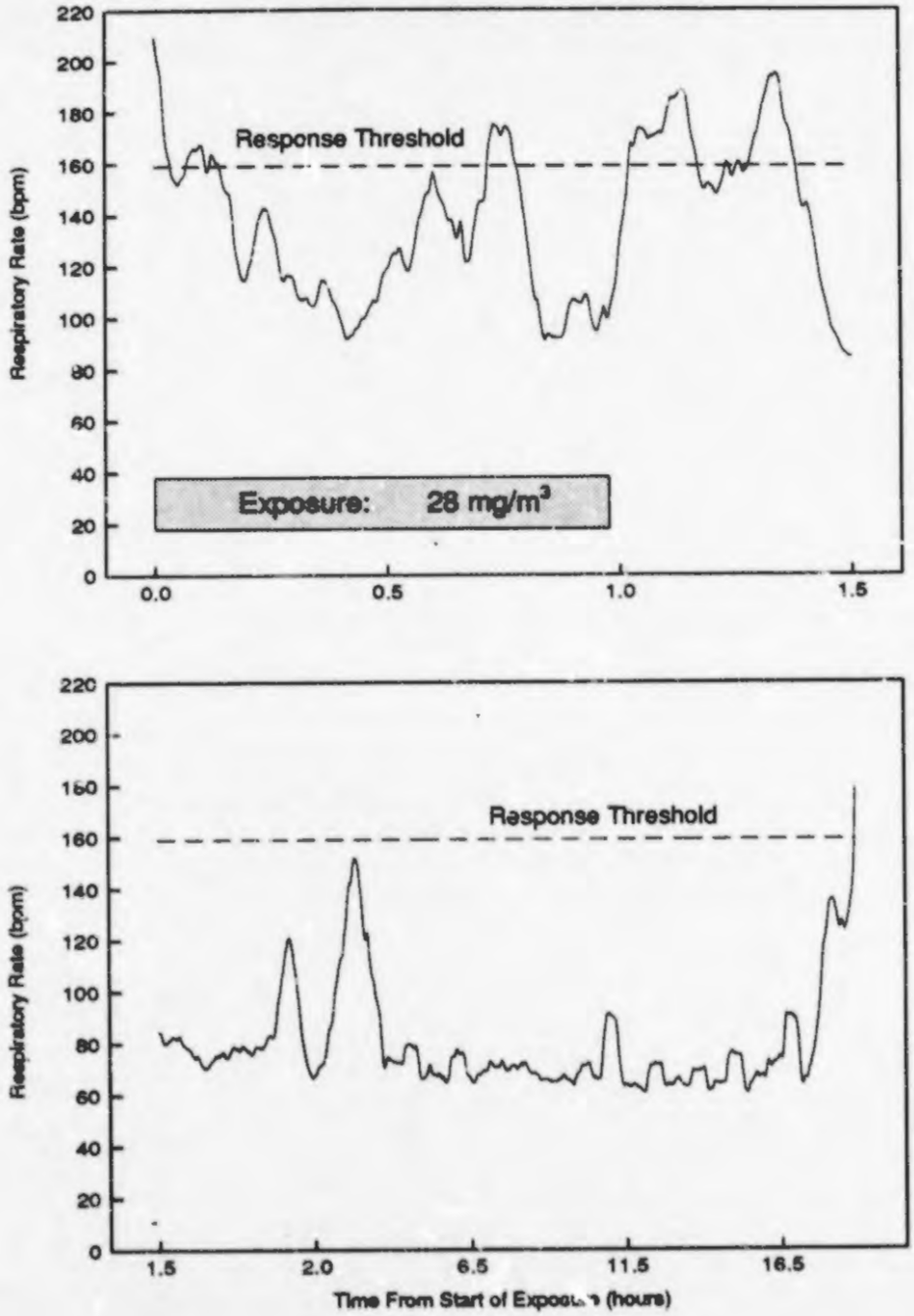
Figure: 70 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 232: Sham Sensitization

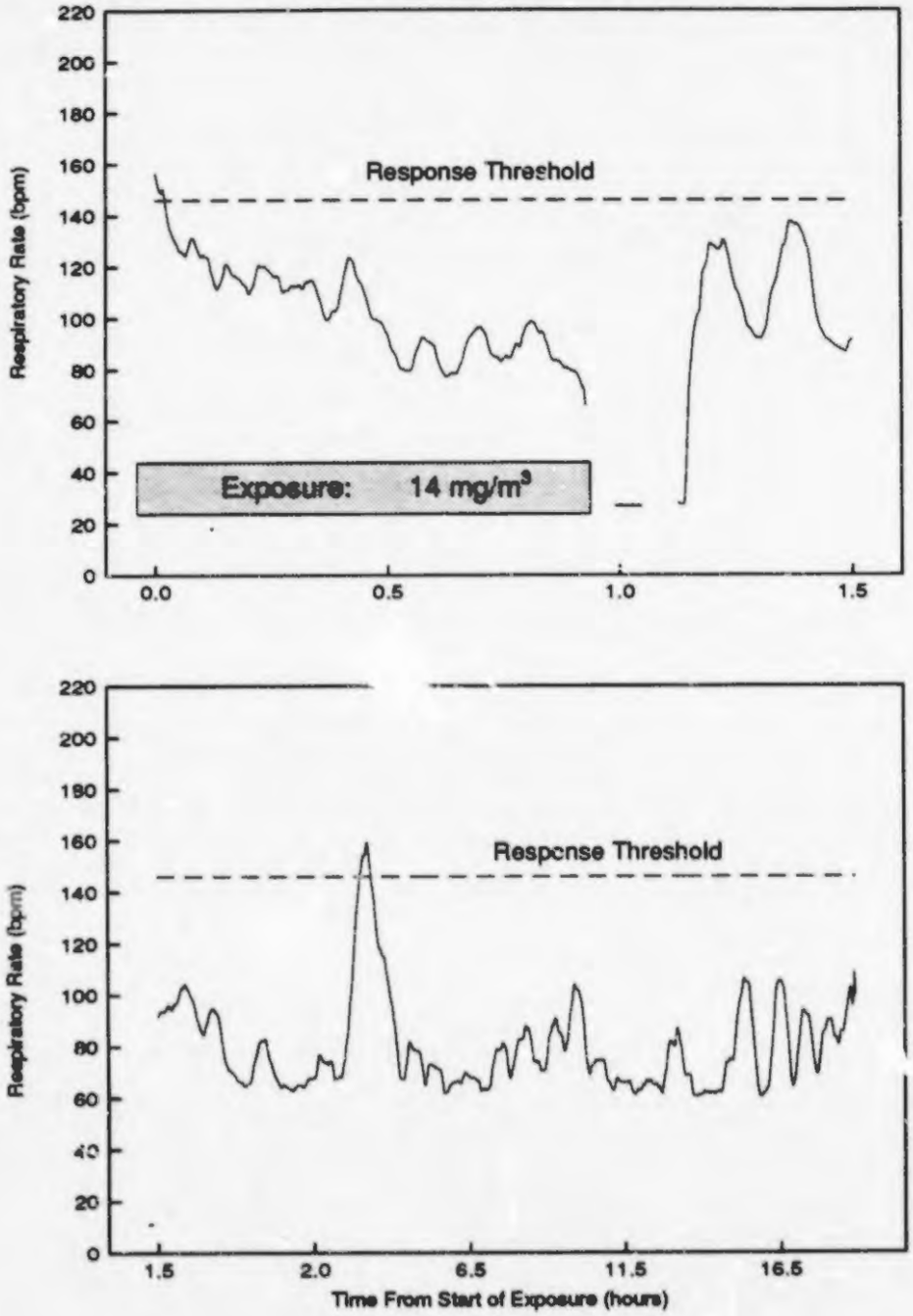
Figure: 71 2,4-TDI-GSA Challenge Week 3



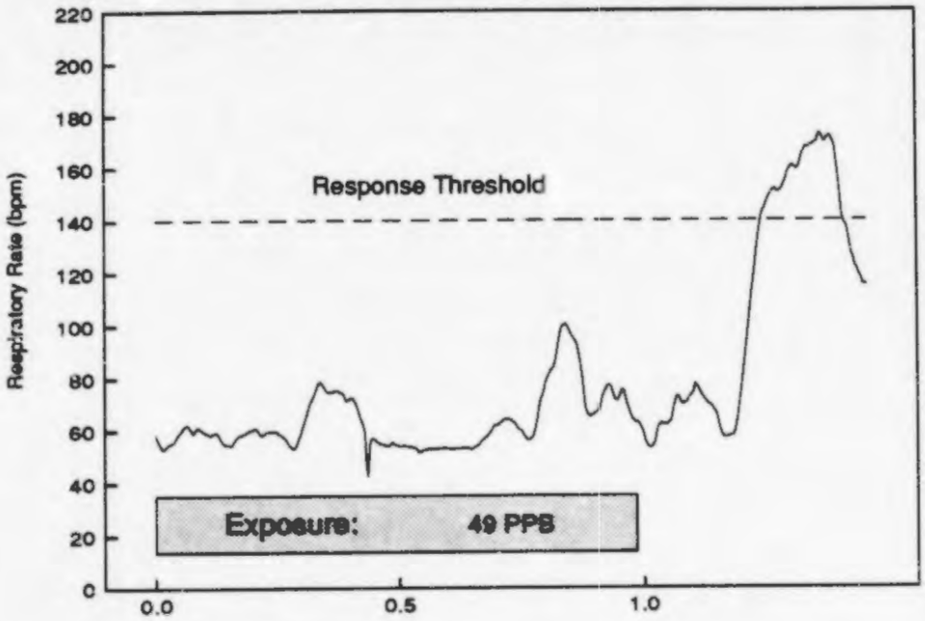
Study Number 92-344-PP

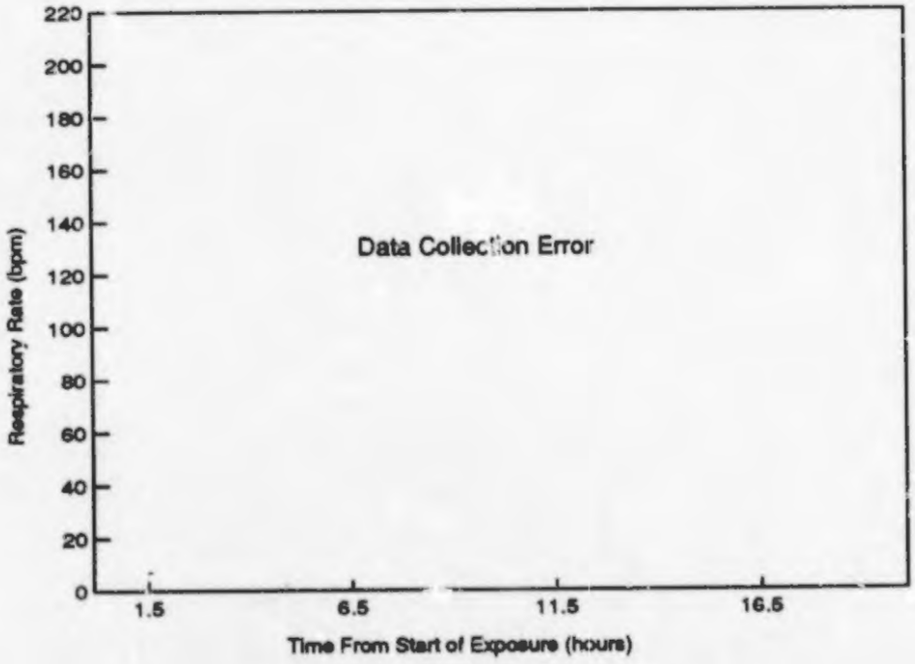
Animal # 233: Sham Sensitization

Figure: 72 2,4-TDI-GSA Challenge Week 3

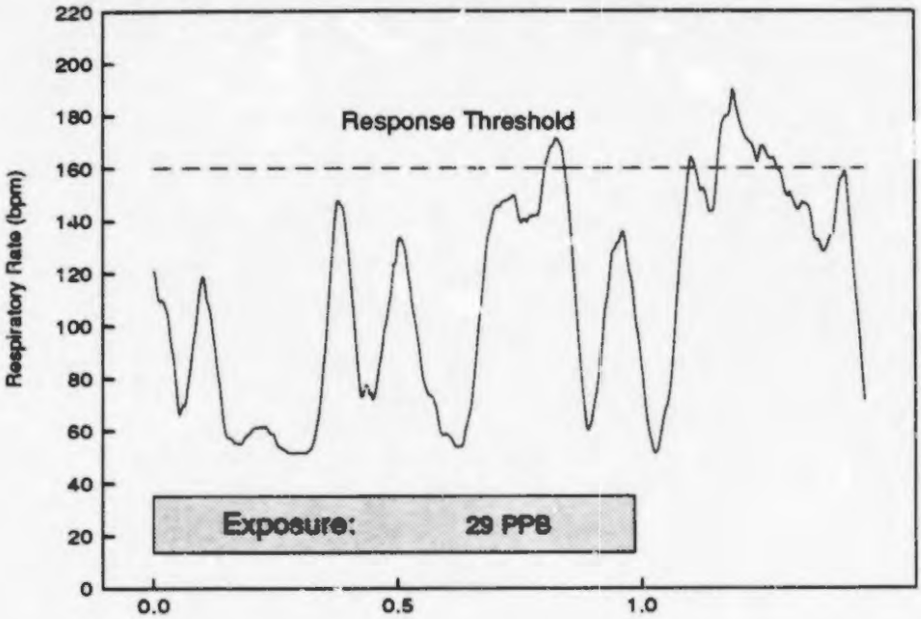


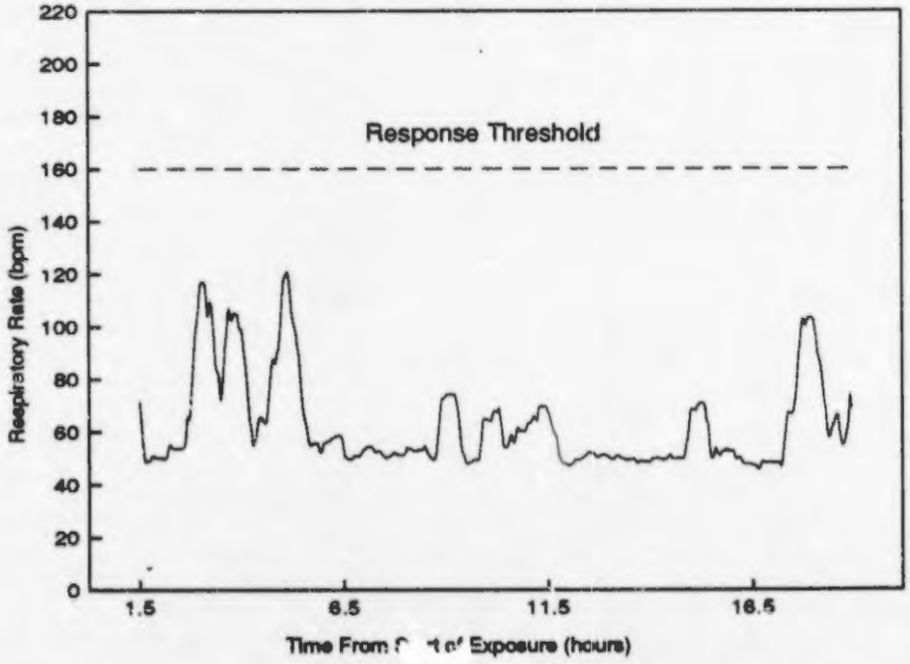
Study Number 92-344-PP
Animal # 207: Sham Sensitization
Figure: 73 2,6-TDI Challenge Week 1





Study Number 92-344-PP
Animal # 208: Sham Sensitization
Figure: 74 2,6-TDI Challenge Week 1

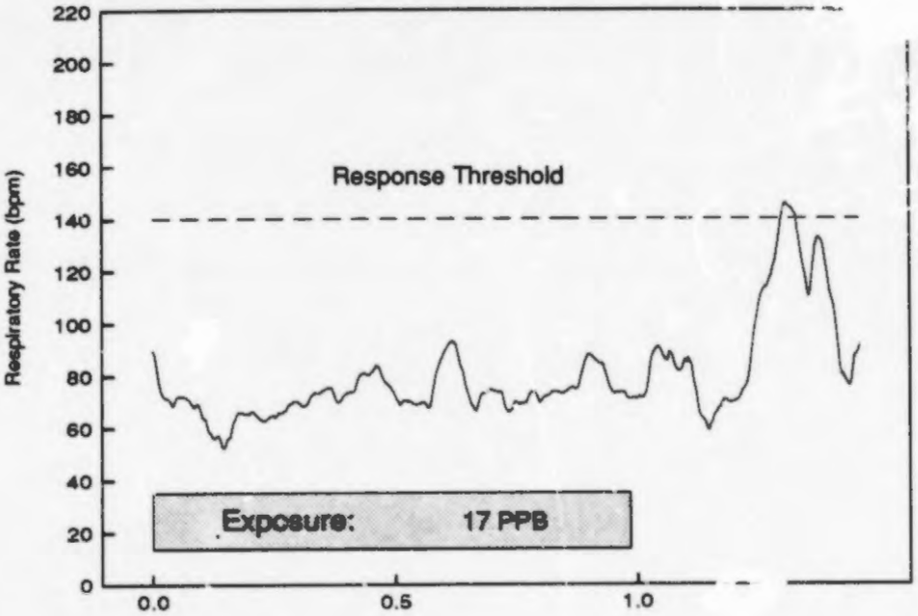


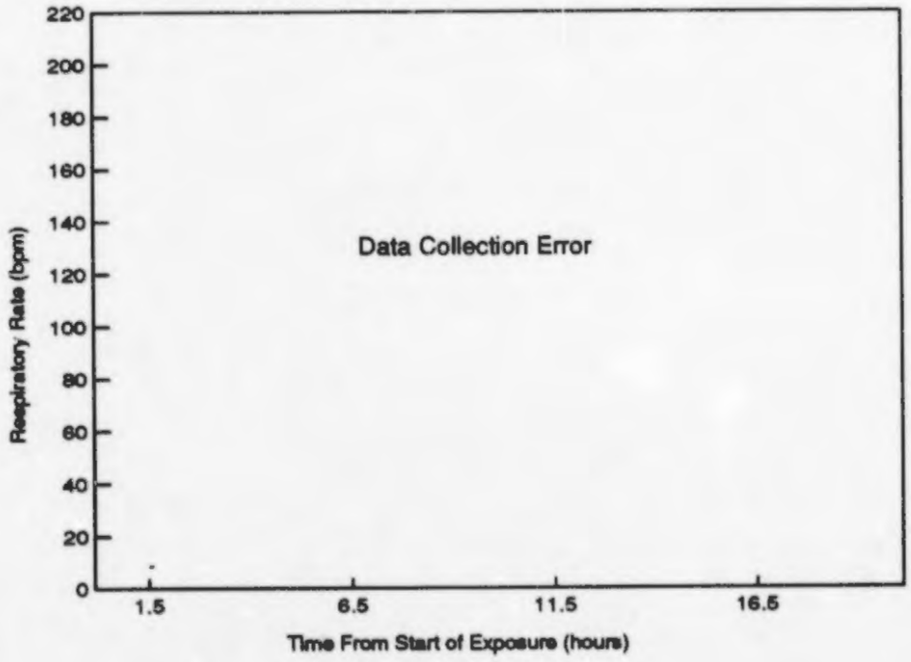


Study Number 92-344-PP

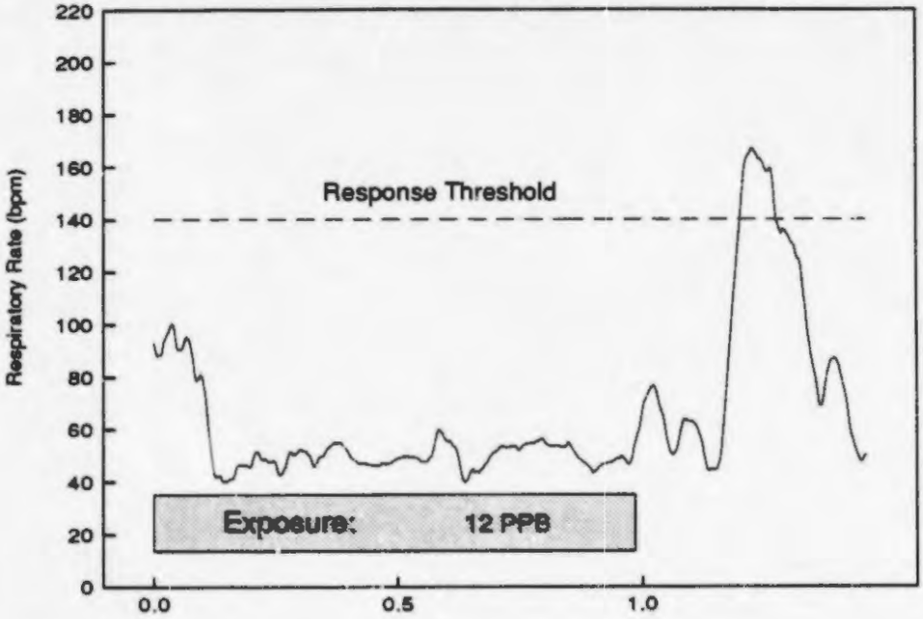
Animal # 209: Sham Sensitization

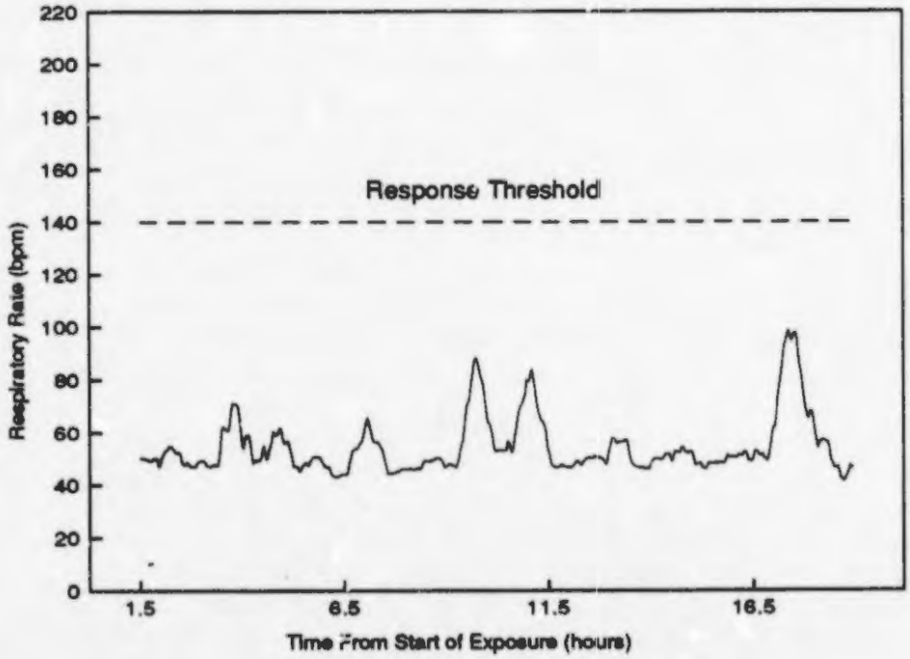
Figure: 75 2,6-TDI Challenge Week 1





Study Number 92-344-PP
Animal # 210: Sham Sensitization
Figure: 76 2,6-TDI Challenge Week 1

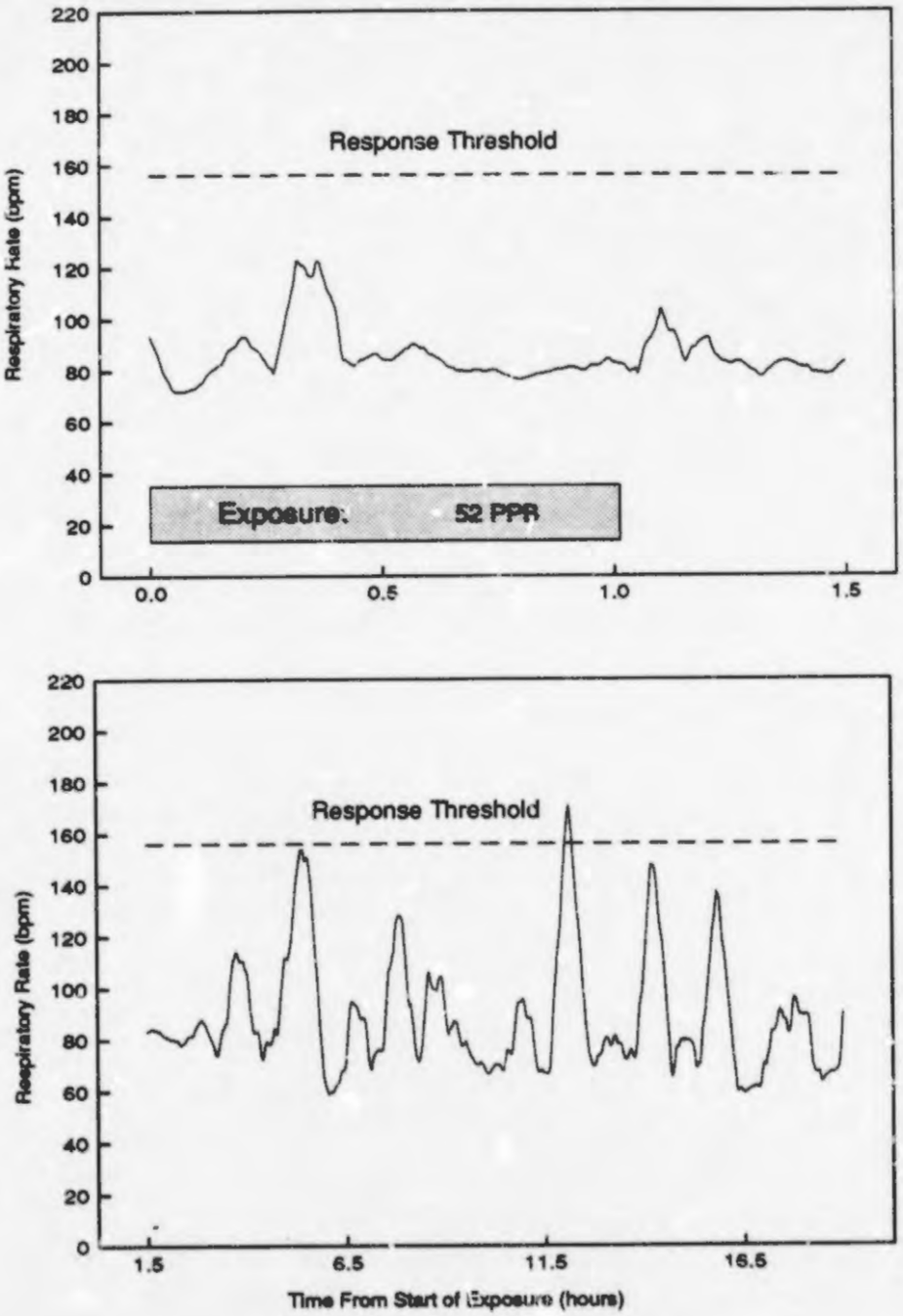




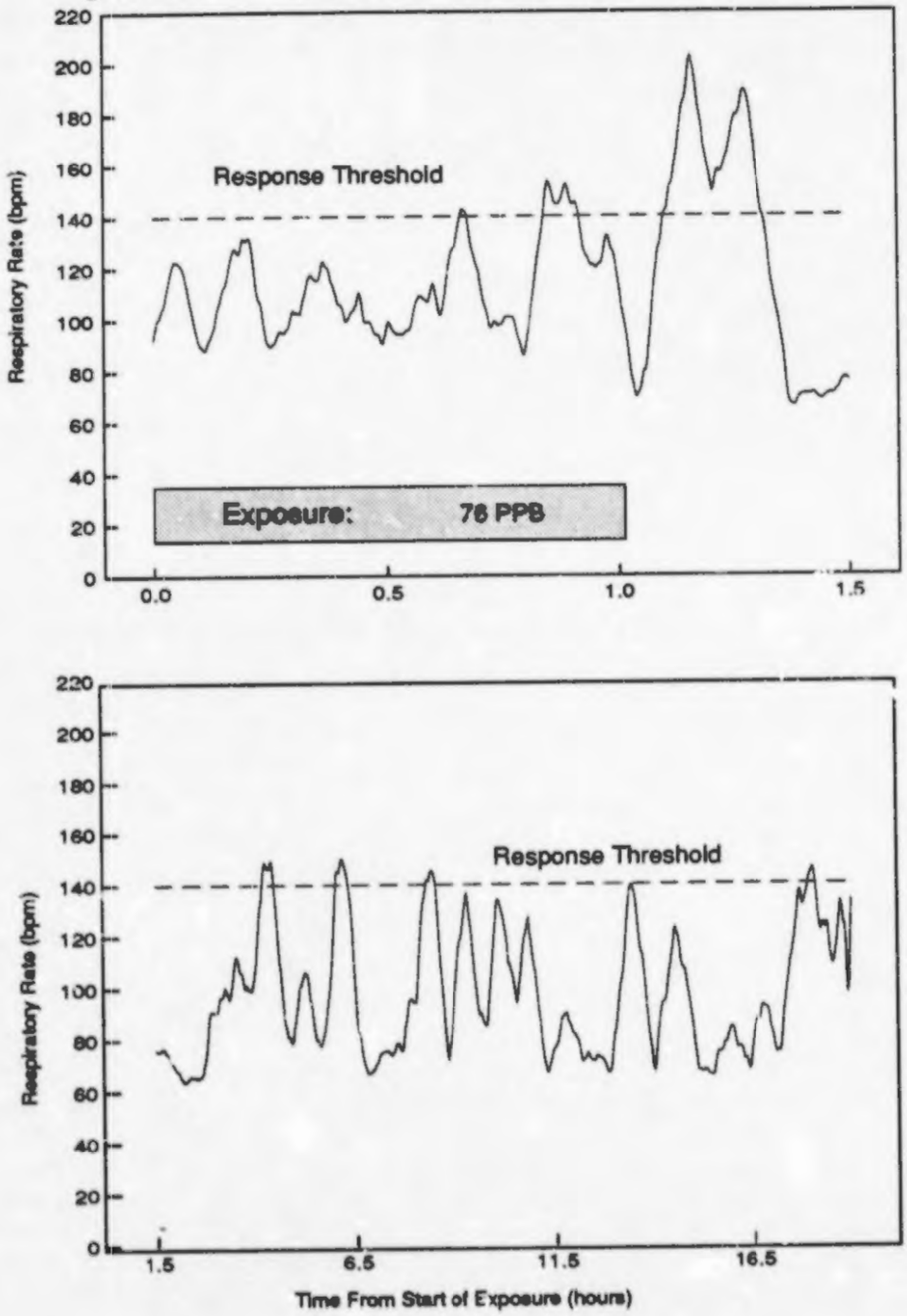
Study Number 92-344-PP

Animal # 211: Sham Sensitization

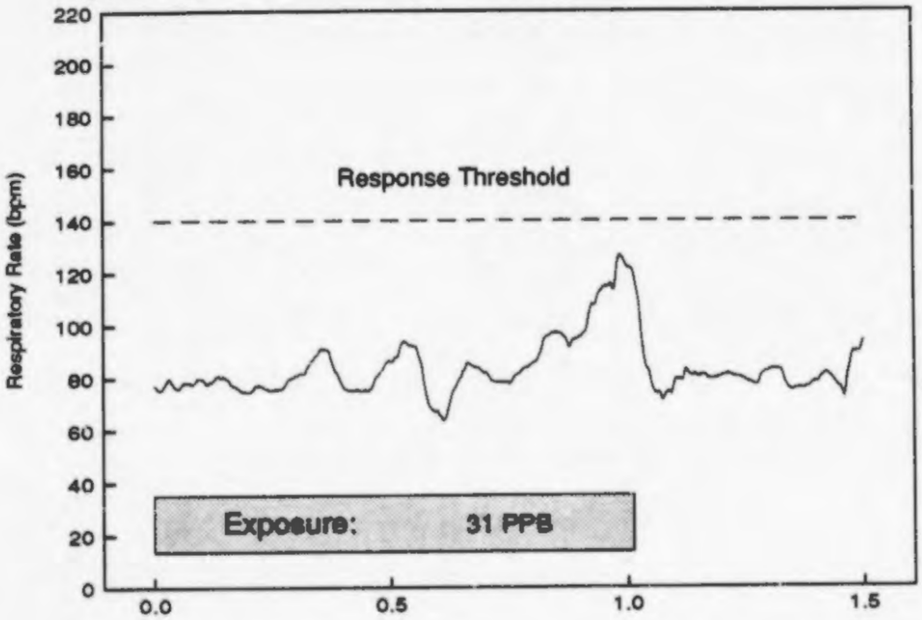
Figure: 77 2,6-TDI Challenge Week 1

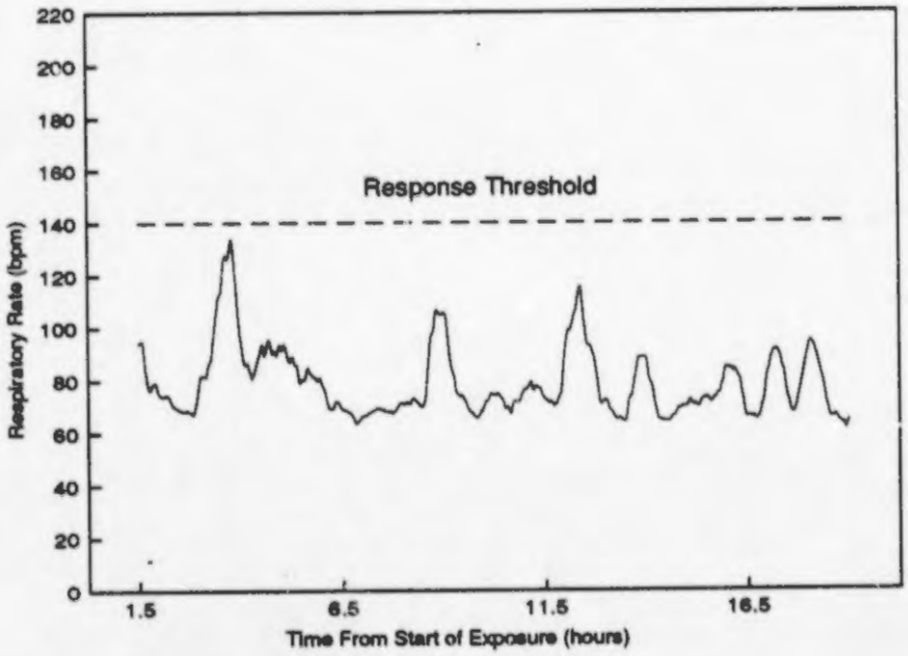


Study Number 92-344-PP
Animal # 212: Sham Sensitization
Figure: 78 2,6-TDI Challenge Week 1

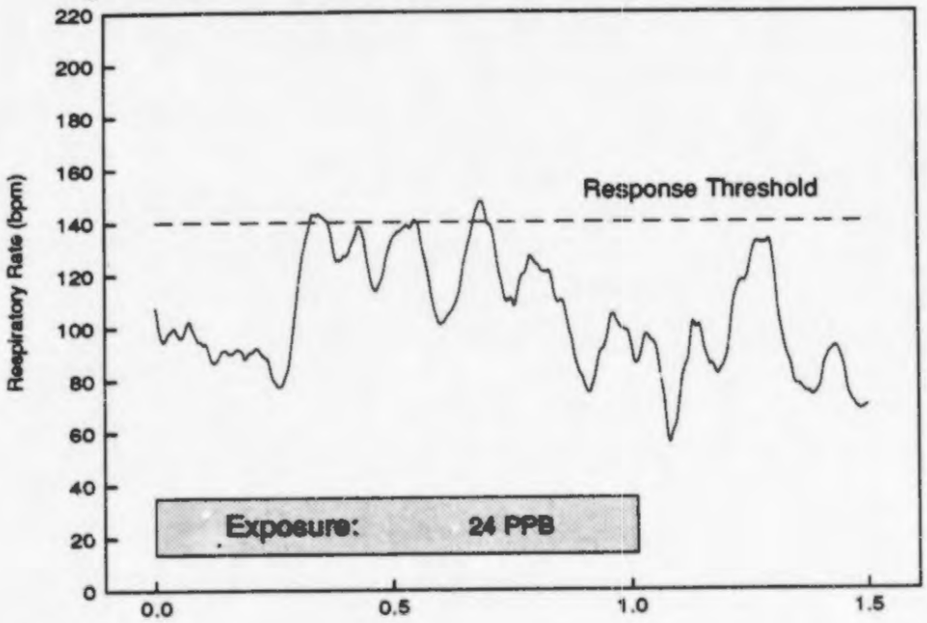


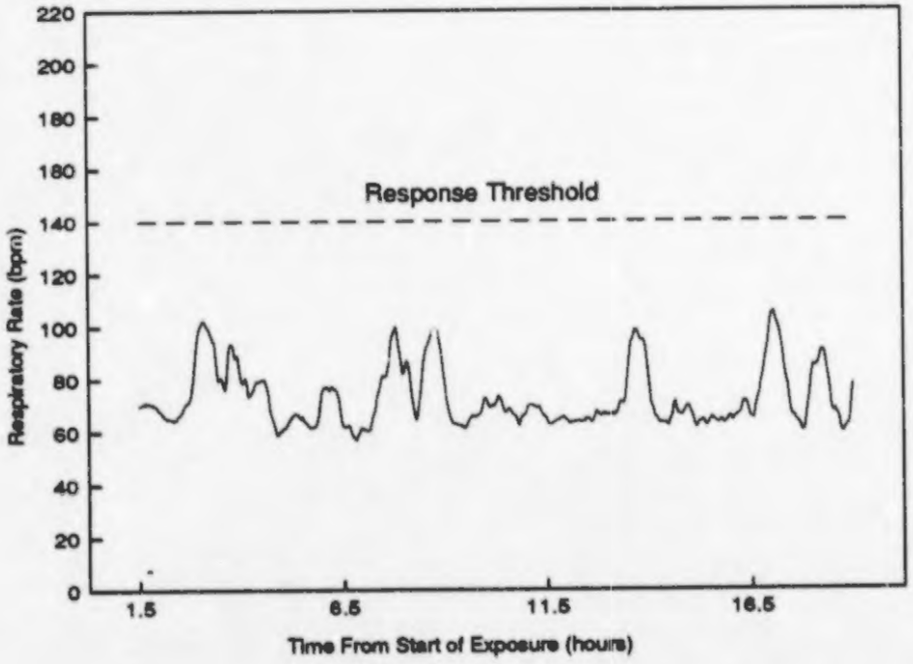
Study Number 92-344-PP
Animal # 213: Sham Sensitization
Figure: 79 2,6-TDI Challenge Week 1





Study Number 92-344-PP
Animal # 214: Sham Sensitization
Figure: 80 2,6-TDI Challenge Week 1

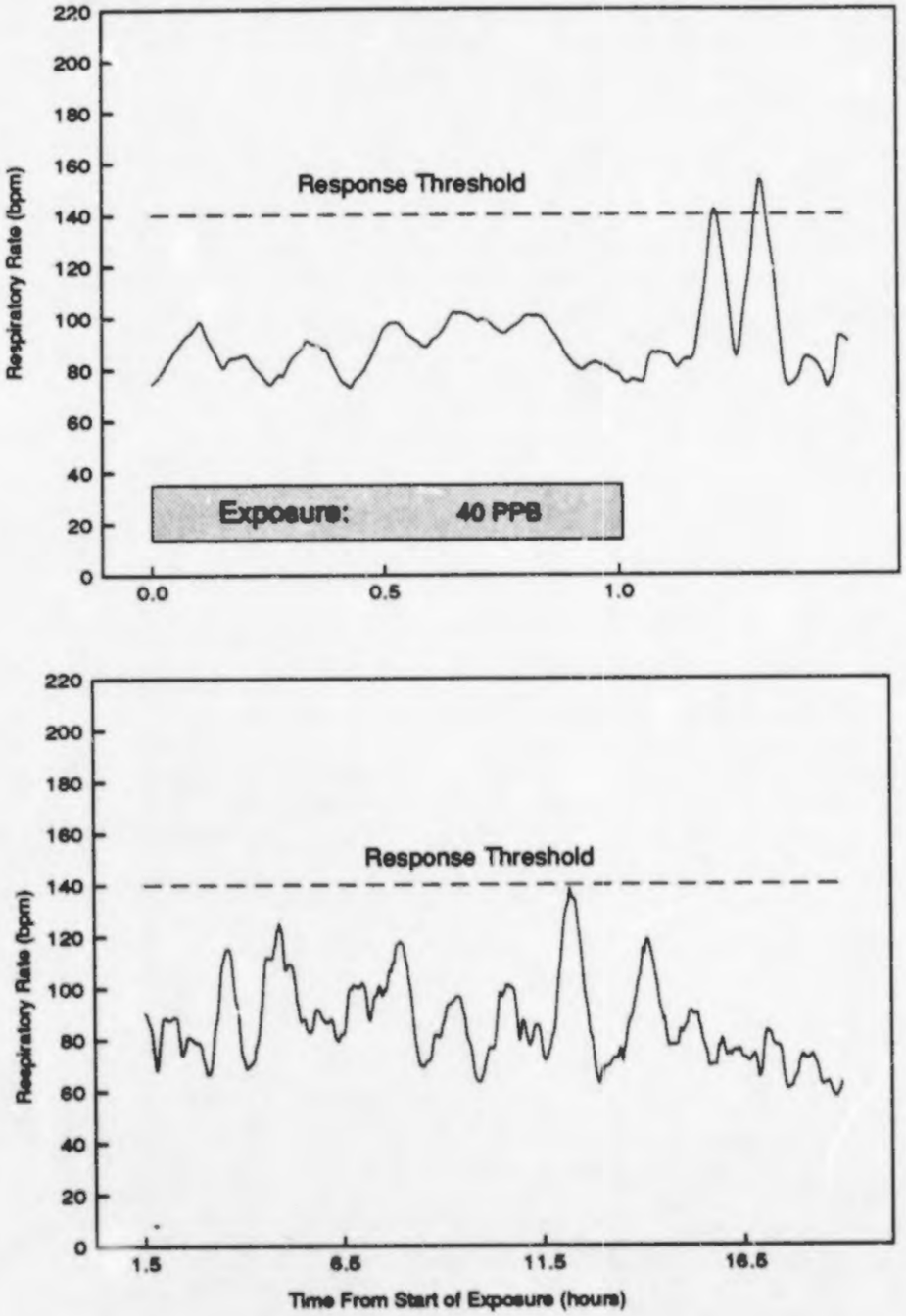




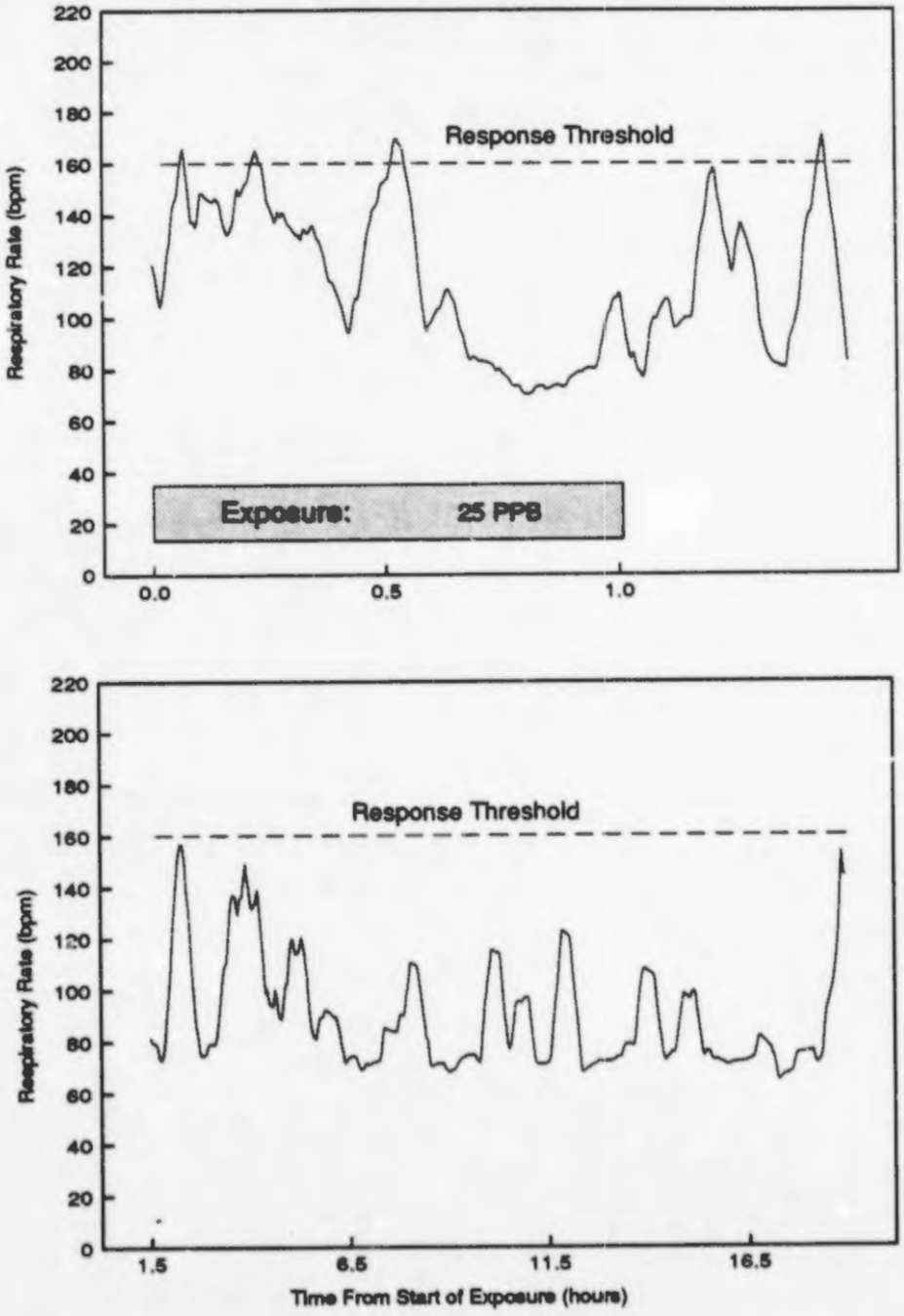
Study Number 92-344-PP

Animal # 215: Sham Sensitization

Figure: 81 2,6-TDI Challenge Week 1



Study Number 92-344-PP
Animal # 216: Sham Sensitization
Figure: 82 2,6-TDI Challenge Week 1



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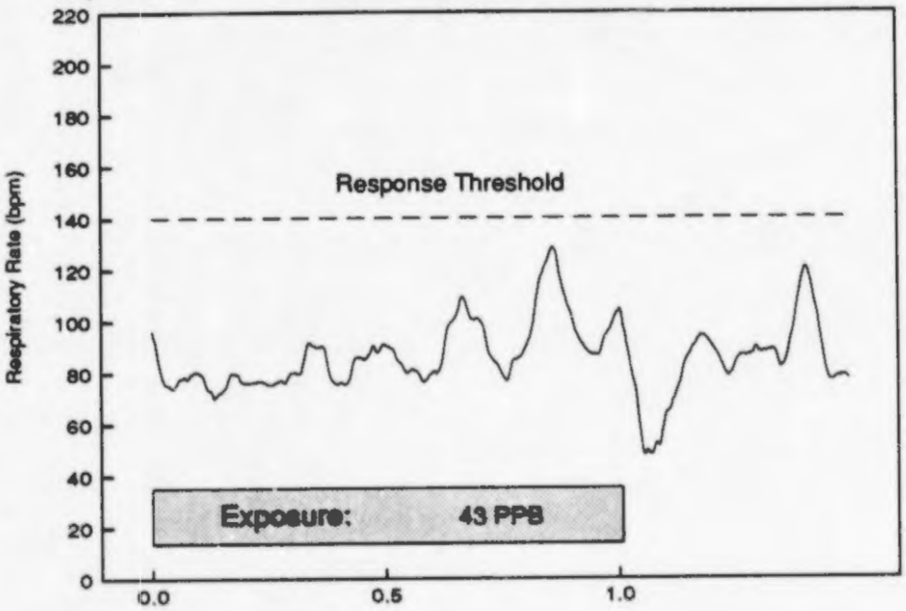
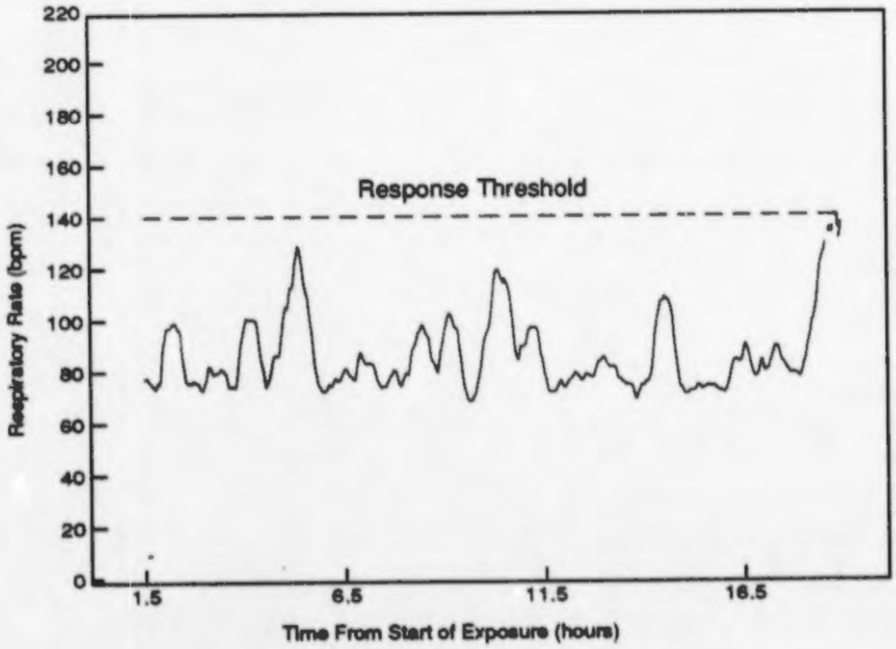
Study Number 92-344-PP
Animal # 217: Sham Sensitization
Figure: 83 2,6-TDI Challenge Week 1





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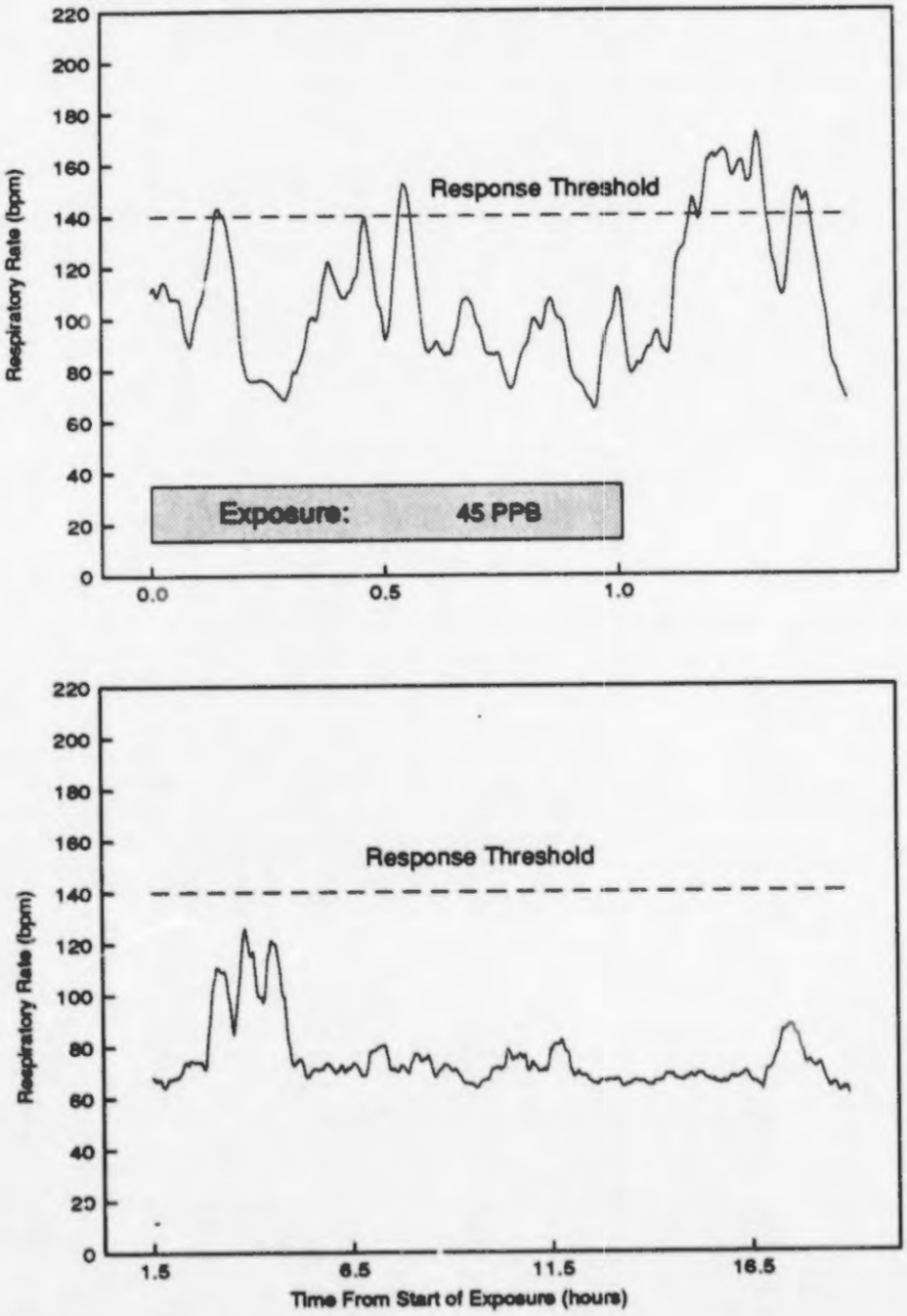
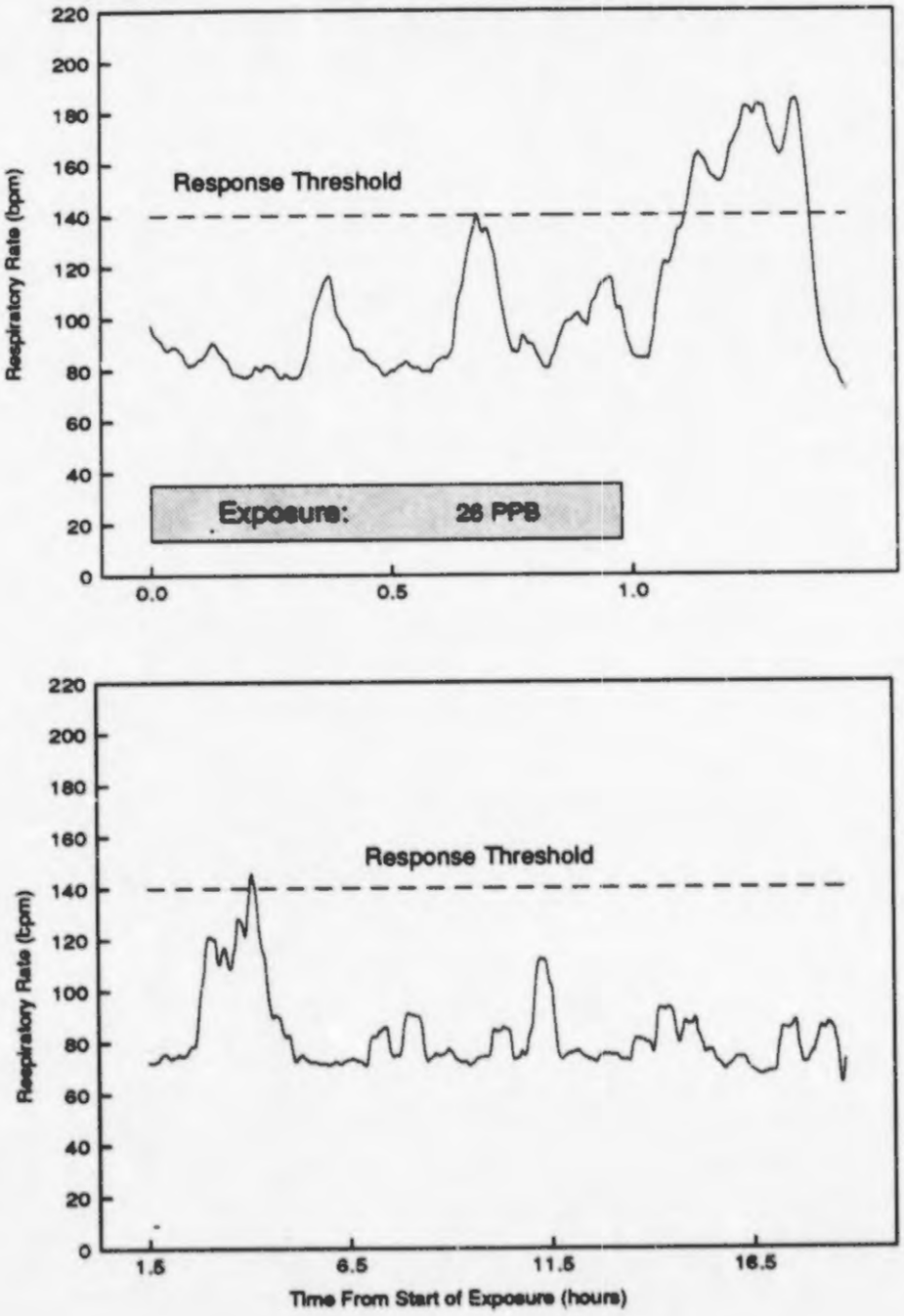
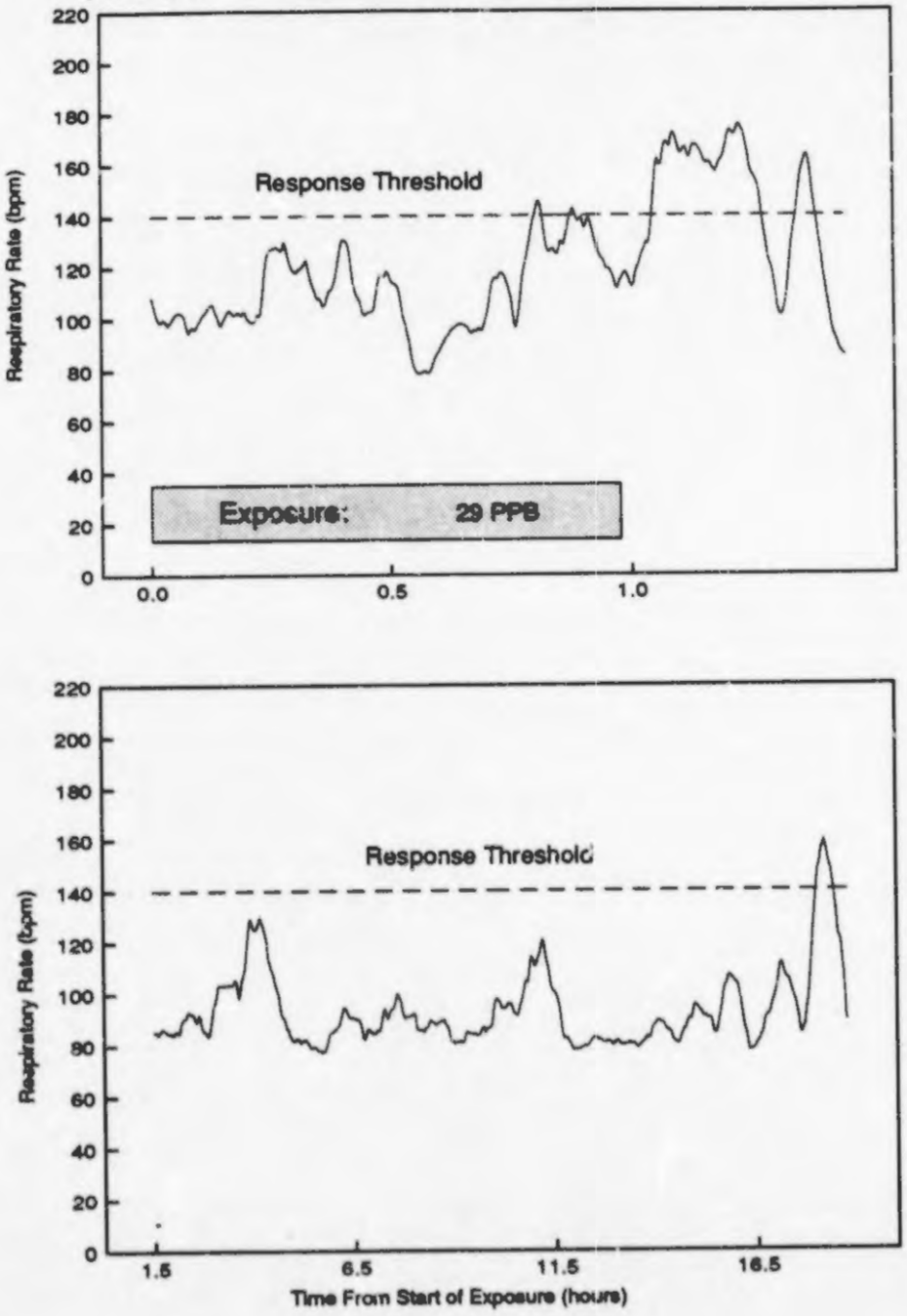
Study Number 92-344-PP
Animal # 218: Sham Sensitization
Figure: 84 2,6-TDI Challenge Week 1



Study Number 92-344-PP
Animal # 207: Sham Sensitization
Figure: 85 2,6-TDI Challenge Week 2



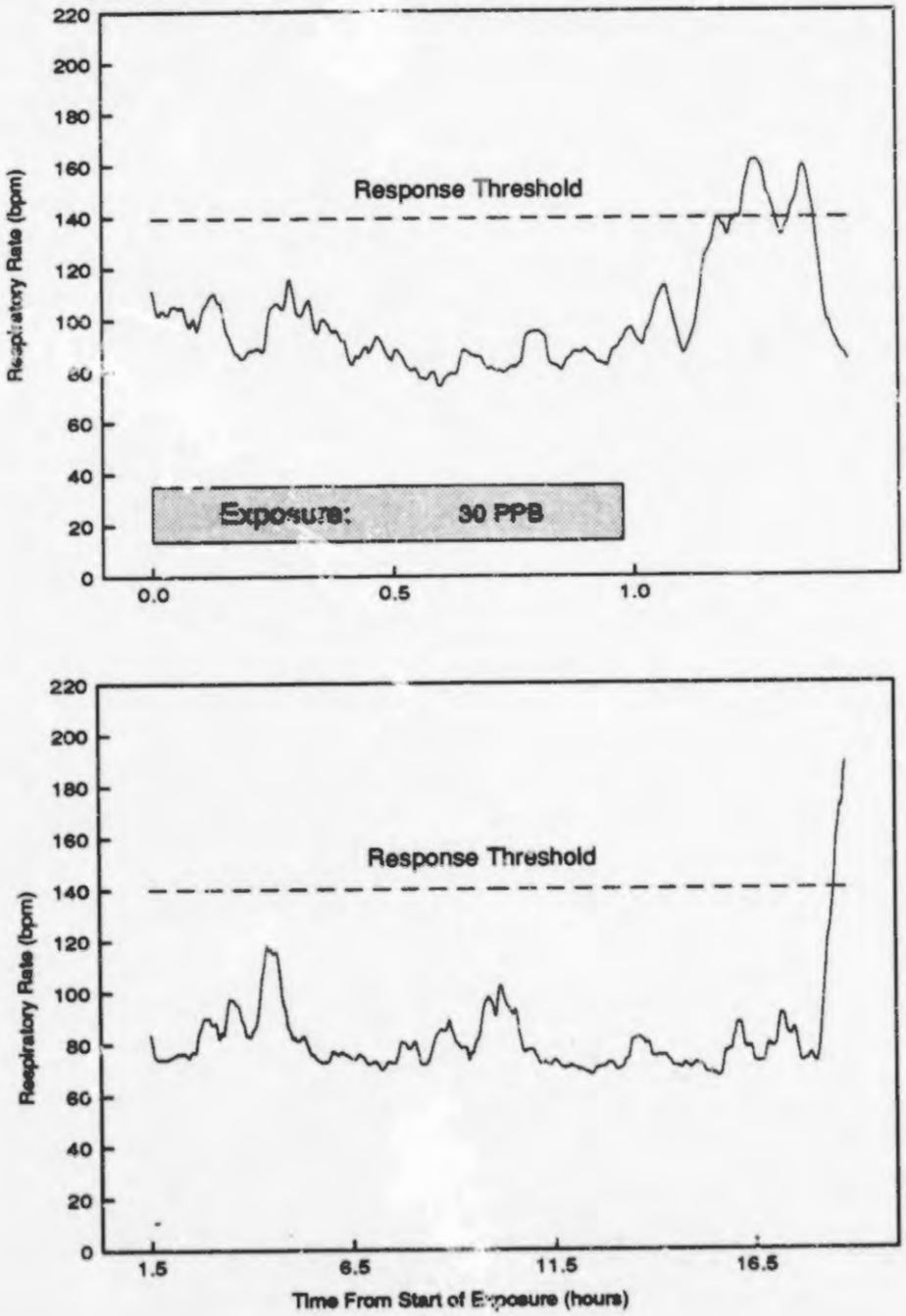
Study Number 92-344-PP
Animal # 208: Sham Sensitization
Figure: 86 2,6-TDI Challenge Week 2



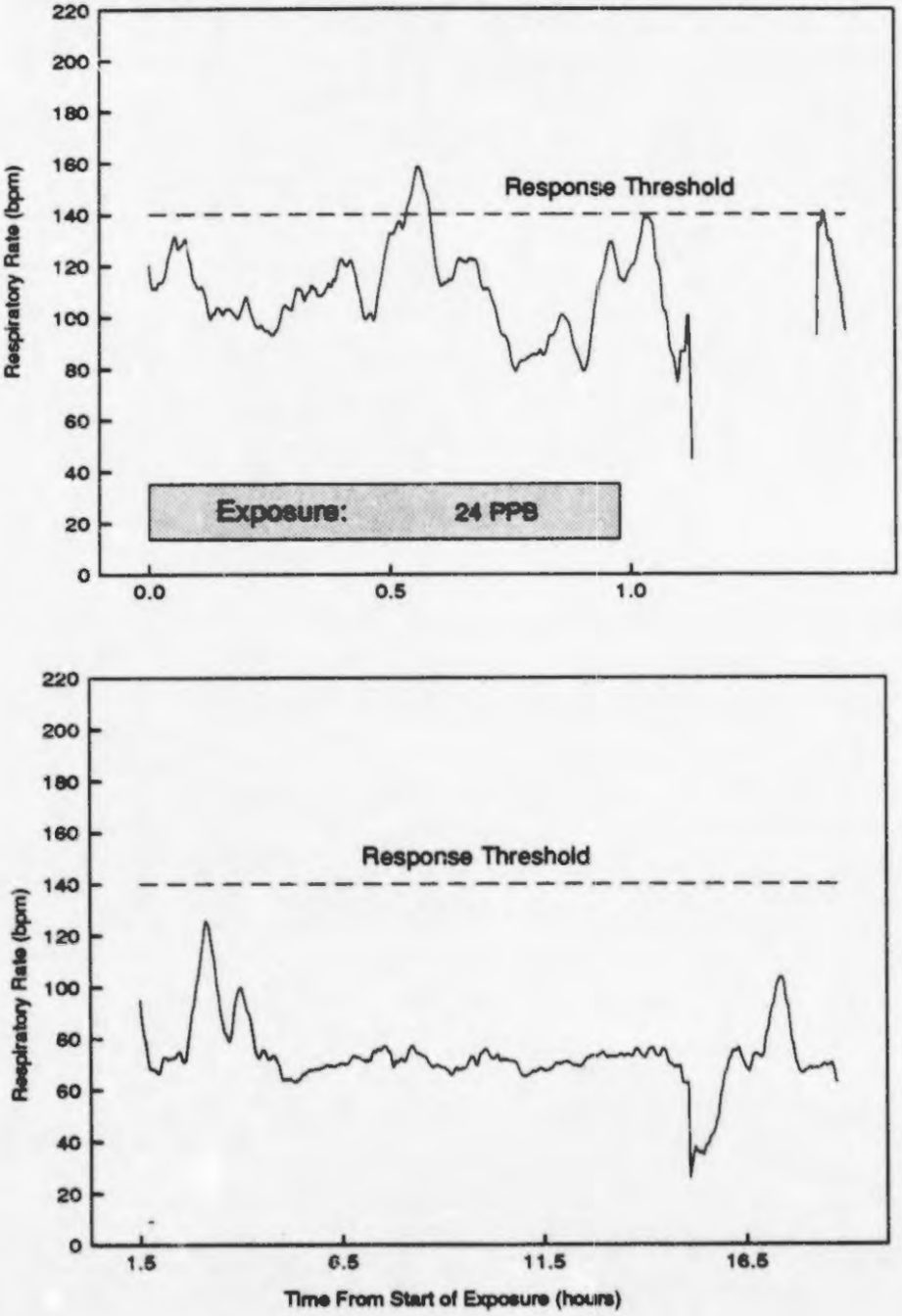
Study Number 92-344-PP

Animal # 209: Sham Sensitization

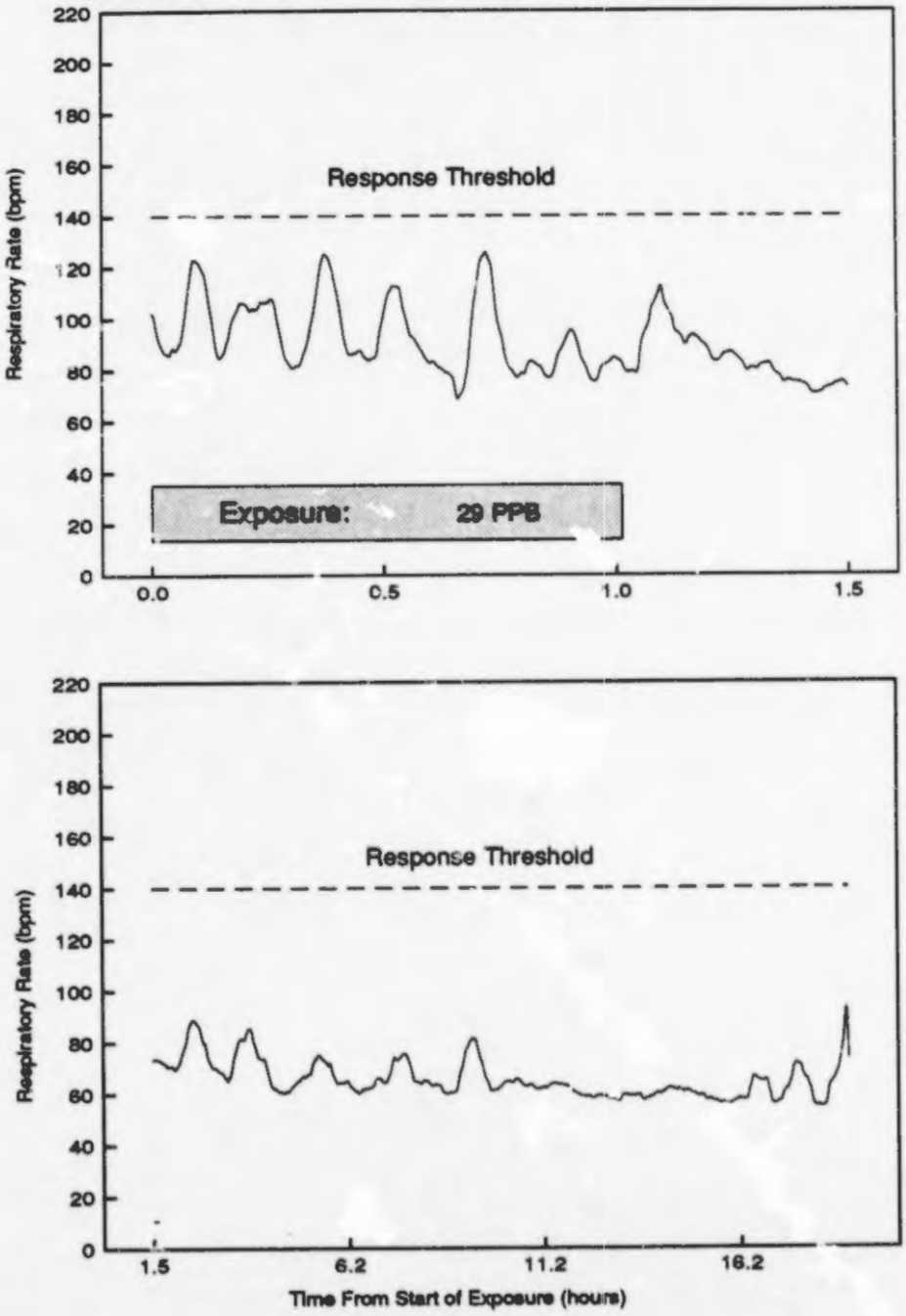
Figure: 87 2,6-TDI Challenge Week 2



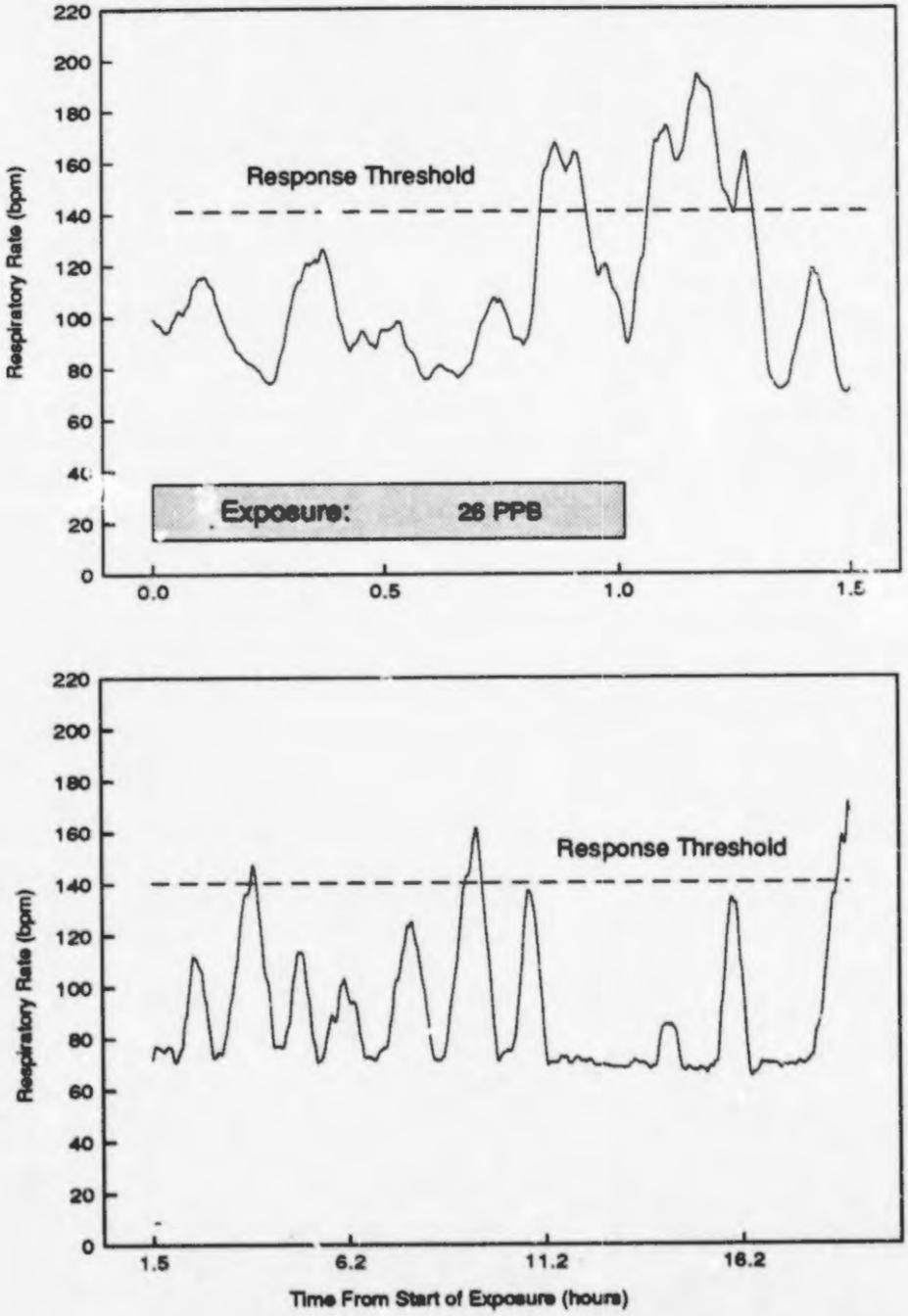
Study Number 92-344-PP
Animal # 210: Sham Sensitization
Figure: 88 2,6-TDI Challenge Week 2



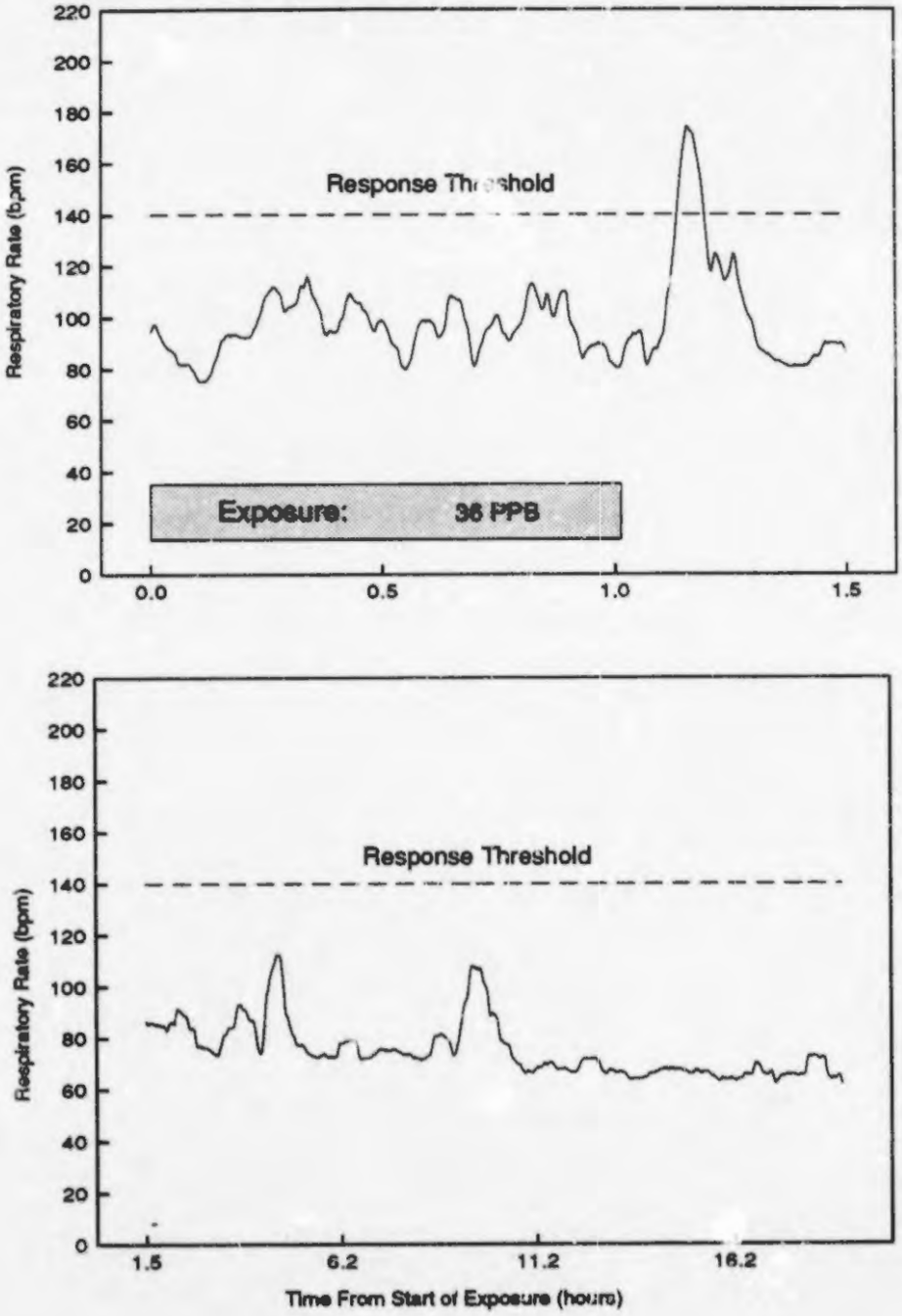
Study Number 92-344-PP
Animal # 211: Sham Sensitization
Figure: 89 2,6-TDI Challenge Week 2



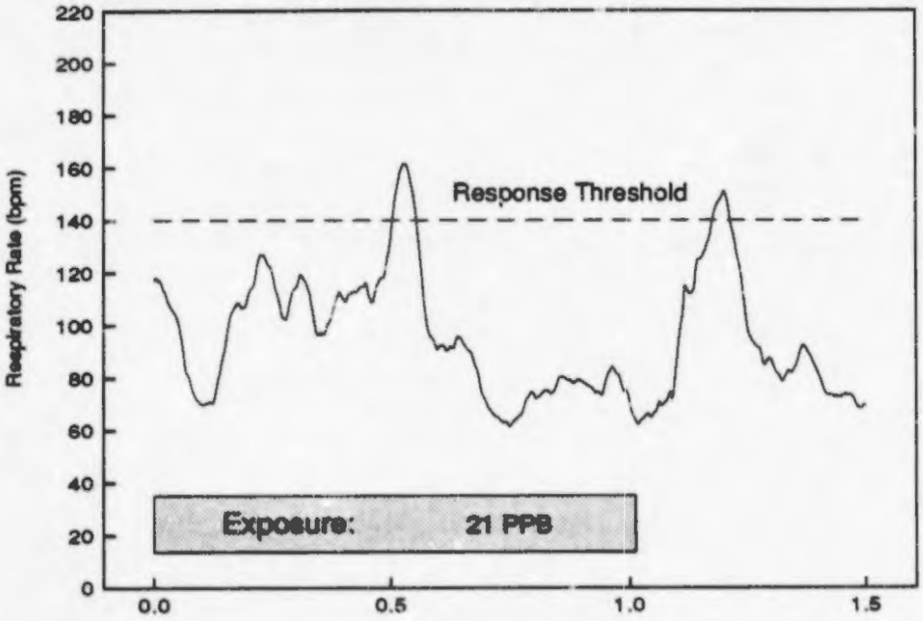
Study Number 92-344-PP
Animal # 212: Sham Sensitization
Figure: 90 2,6-TDI Challenge Week 2

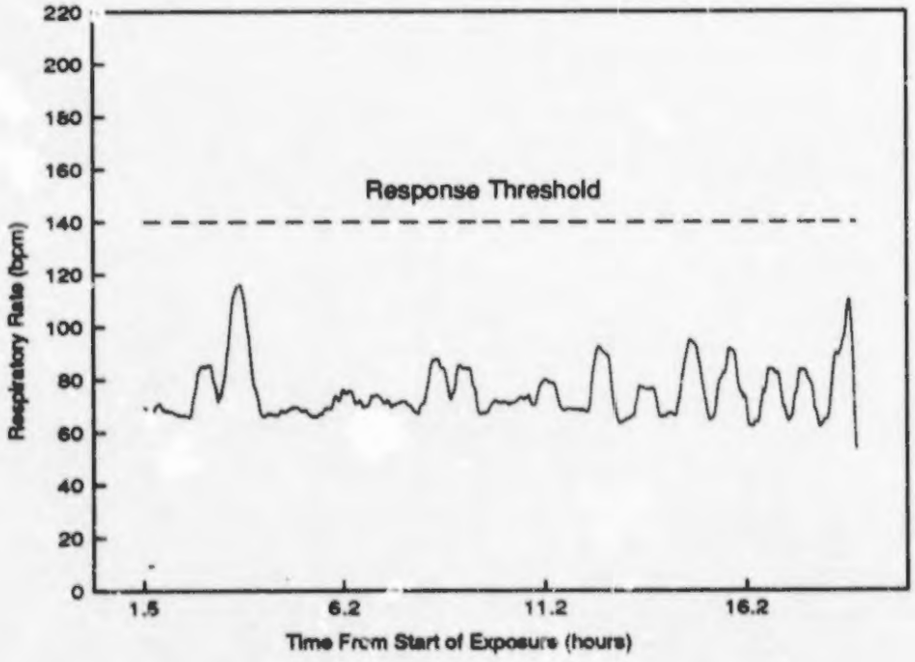


Study Number 92-344-PP
Animal # 213: Sham Sensitization
Figure: 91 2,6-TDI Challenge Week 2

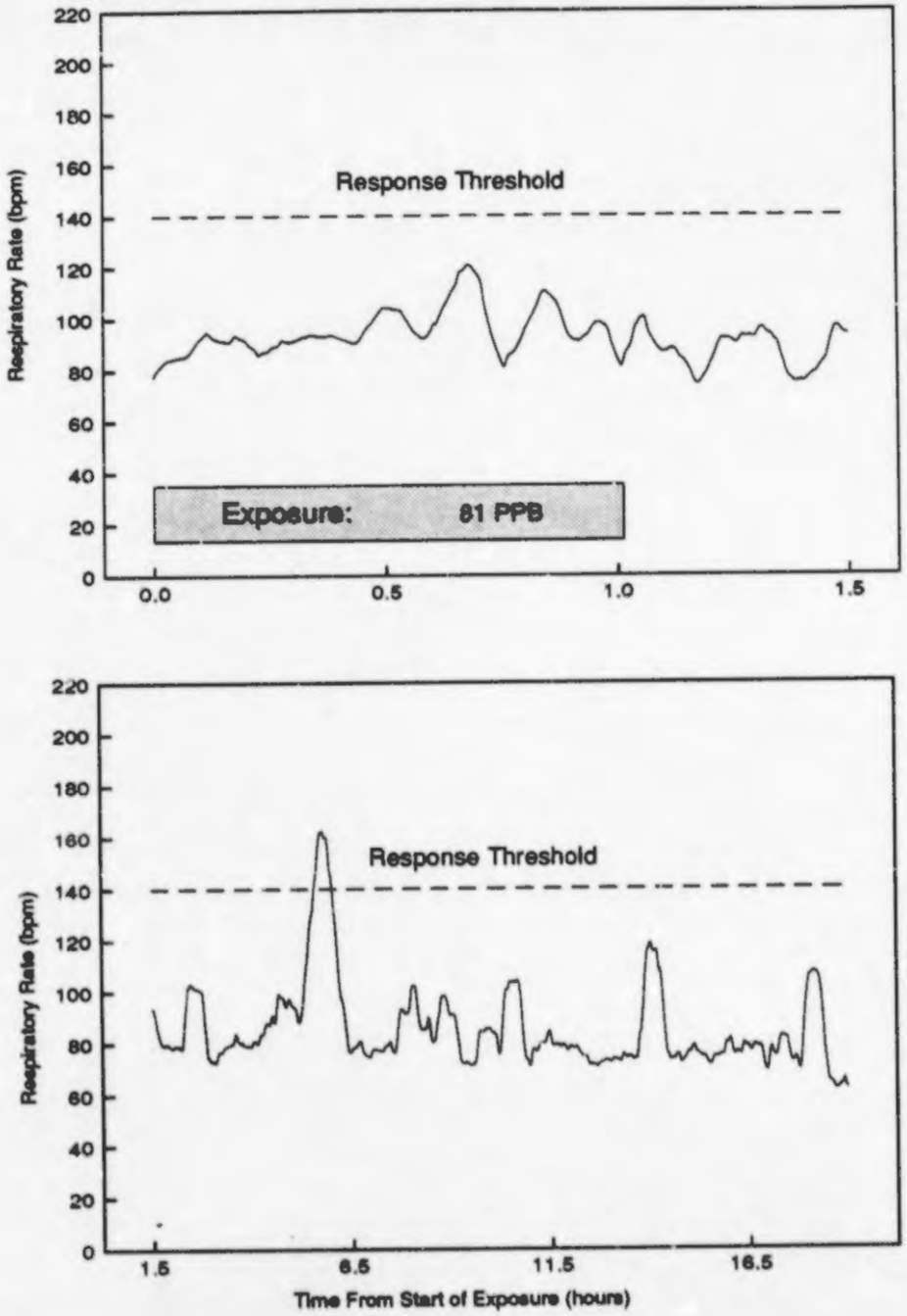


Study Number 92-344-PP
Animal # 214: Sham Sensitization
Figure: 92 2,6-TDI Challenge Week 2

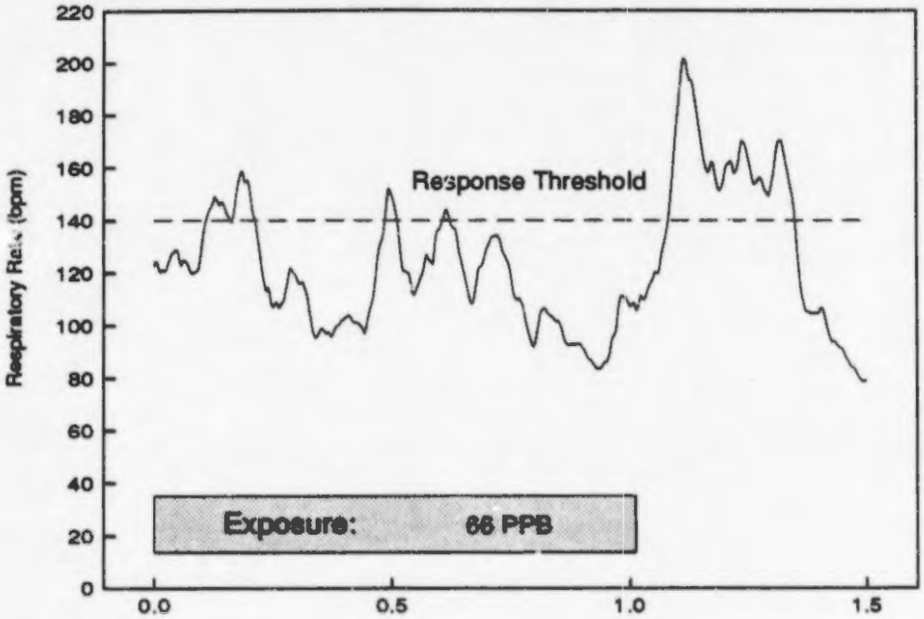


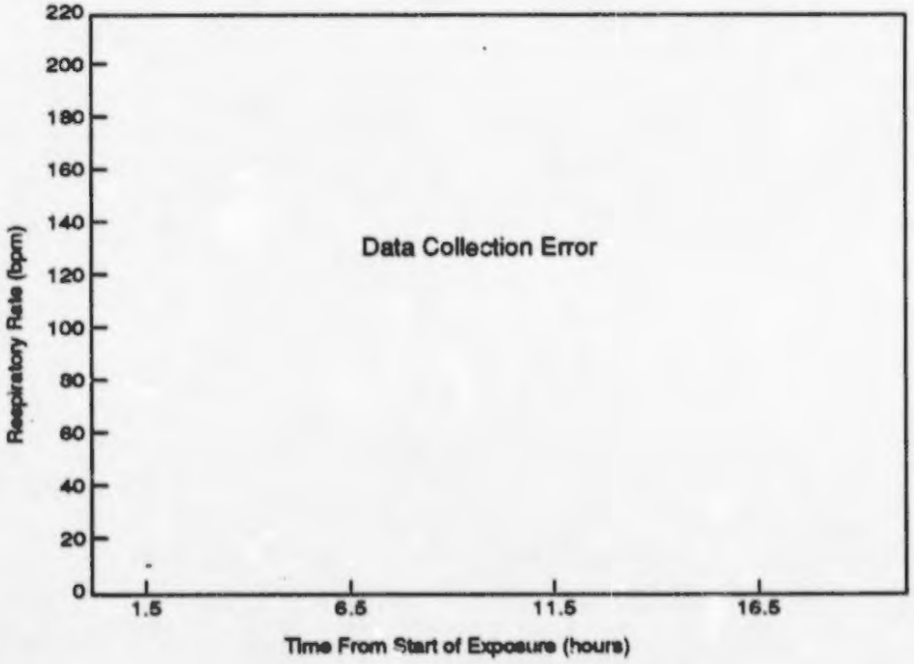


Study Number 92-344-PP
Animal # 215: Sham Sensitization
Figure: 93 2,6-TDI Challenge Week 2



Study Number 92-344-PP
Animal # 216: Sham Sensitization
Figure: 94 2,6-TDI Challenge Week 2





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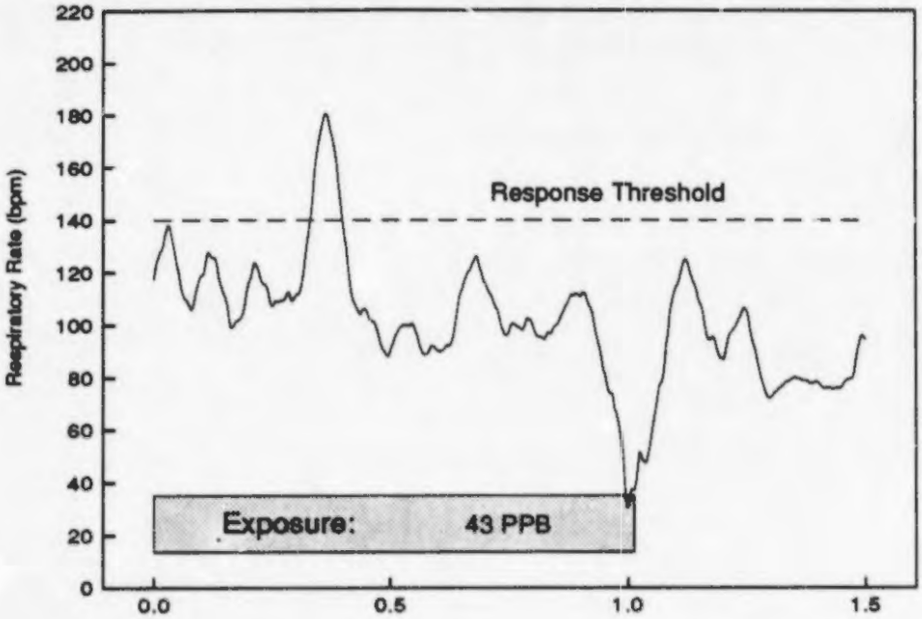
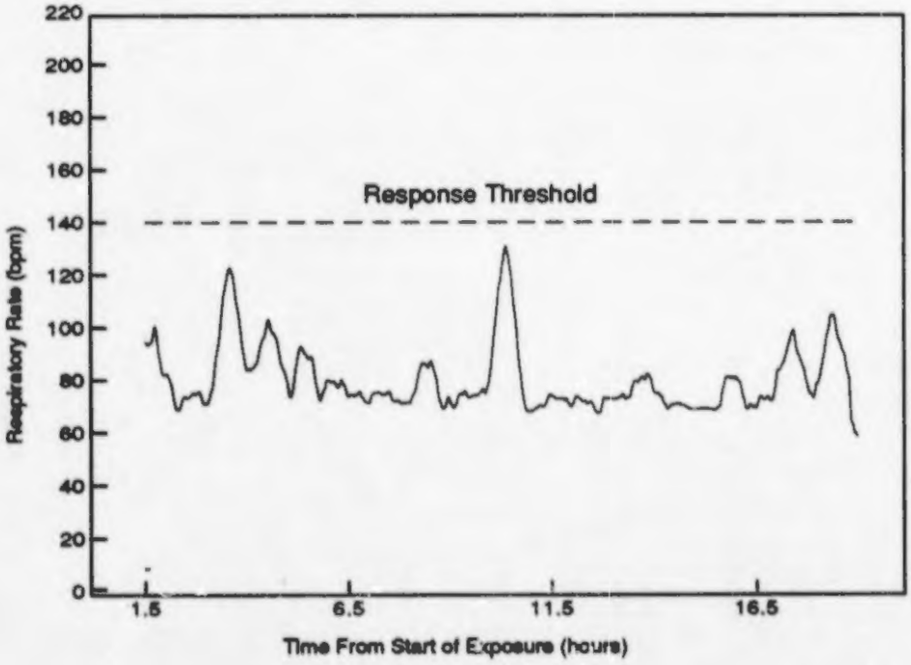
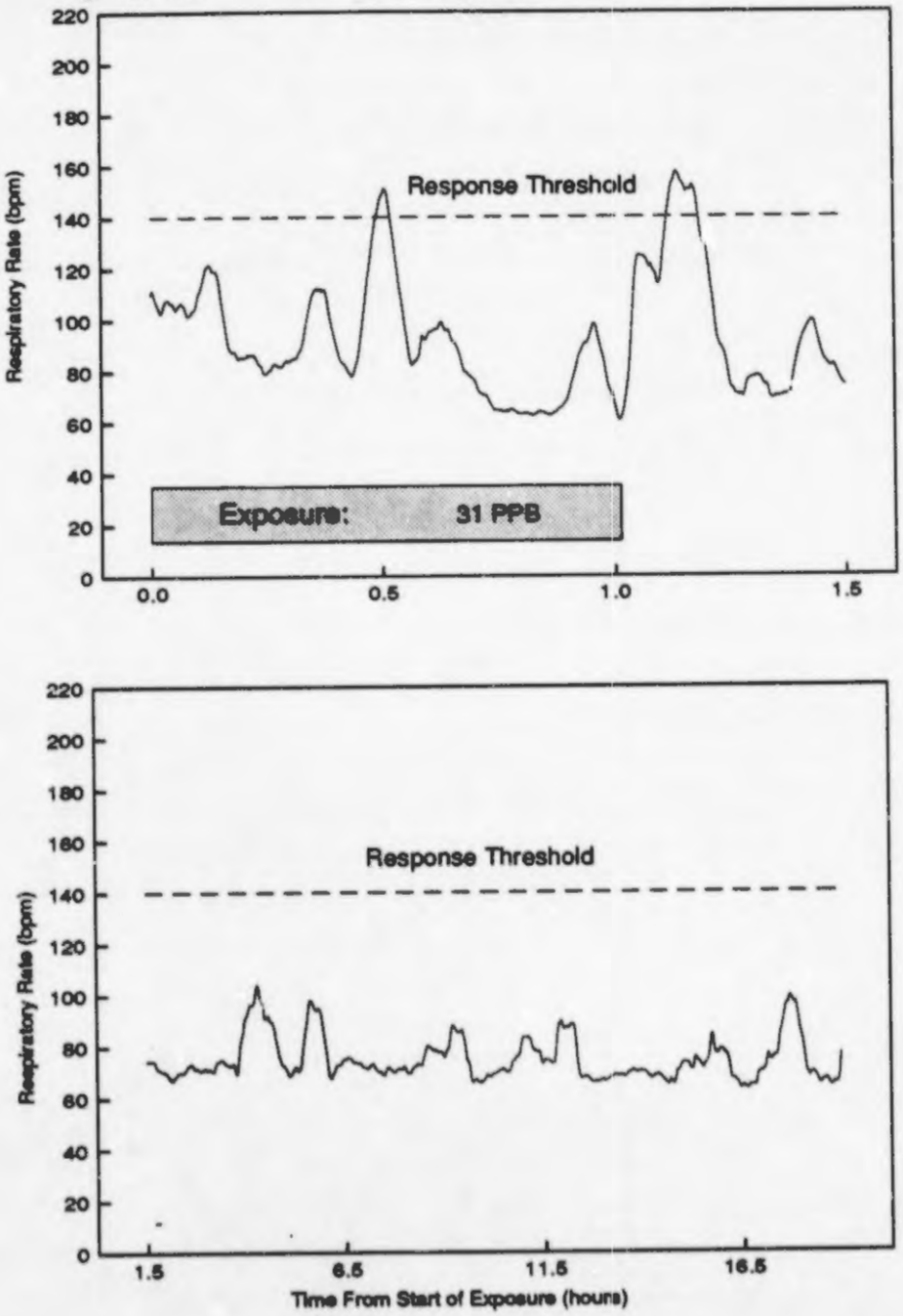
Study Number 92-344-PP
Animal # 217: Sham Sensitization
Figure: 95 2,6-TDI Challenge Week 2





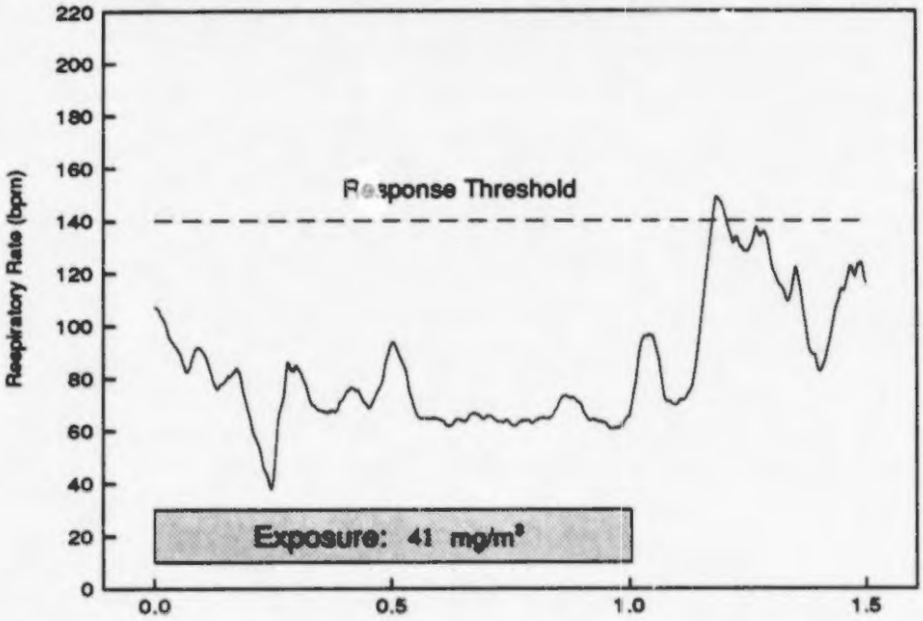
Study Number 92-344-PP
Animal # 218: Sham Sensitization
Figure: 96 2,6-TDI Challenge Week 2

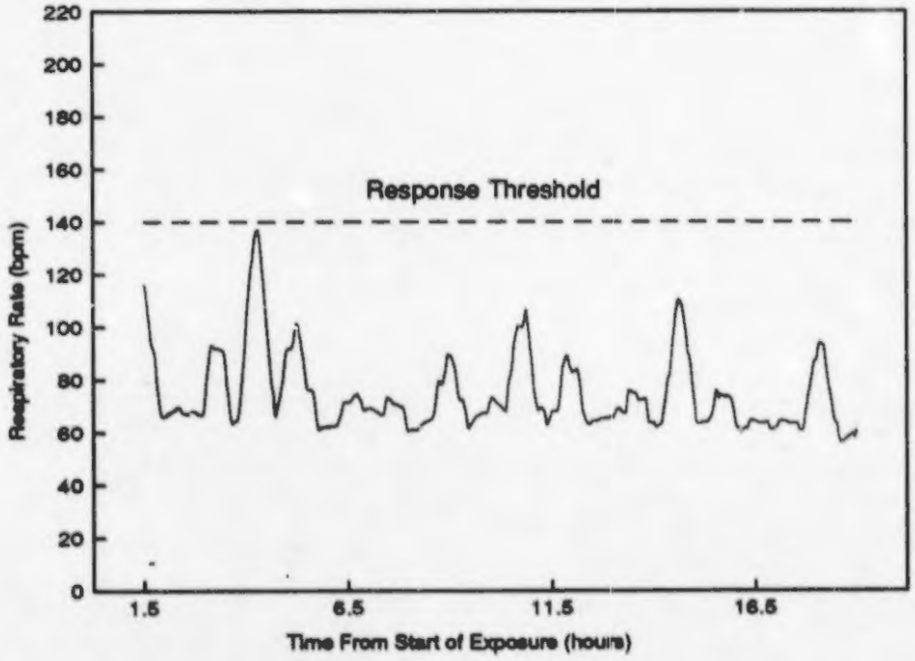


Study Number 92-344-PP

Animal # 207: Sham Sensitization

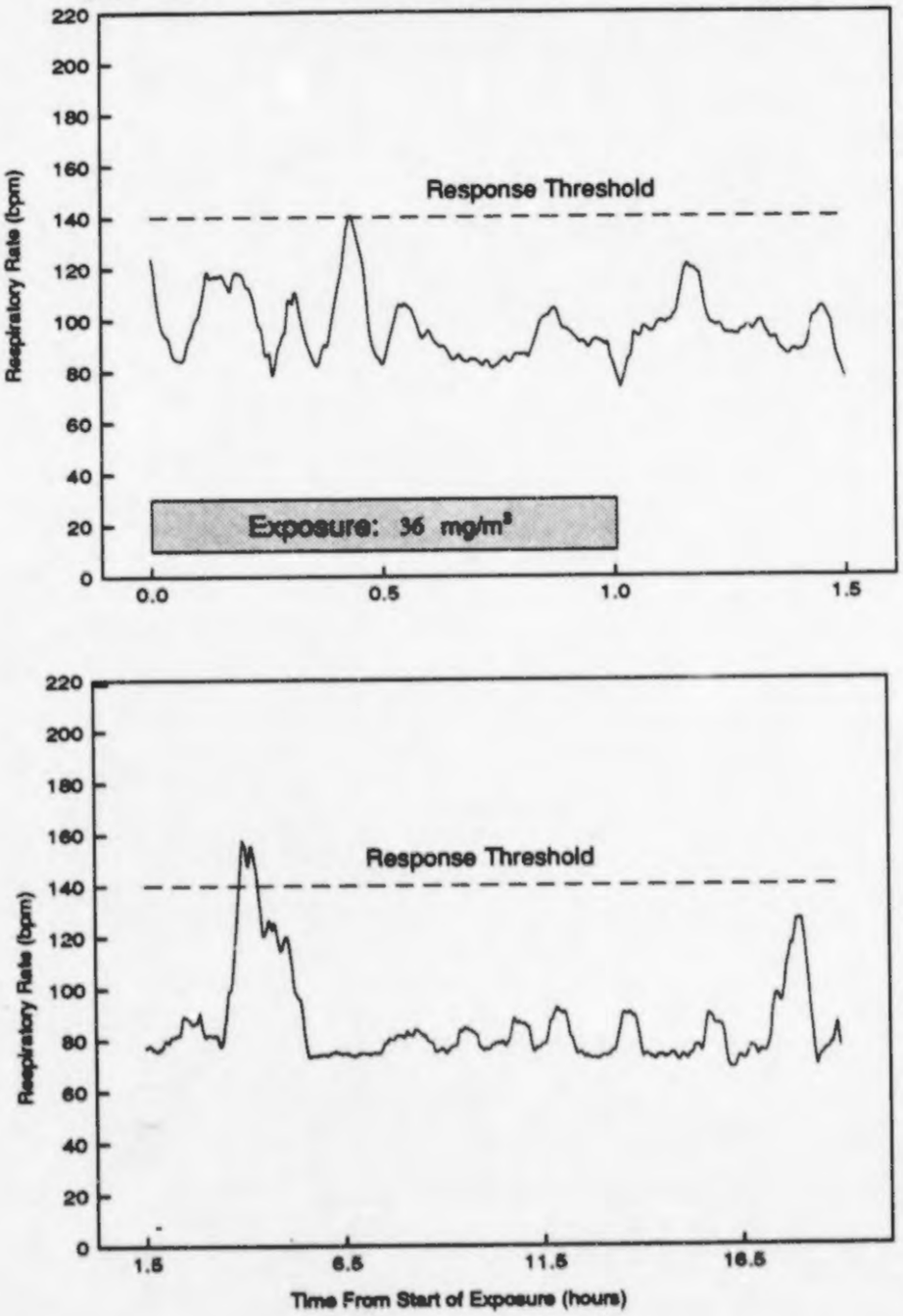
Figure: 97 2,6-TDI-GSA Challenge Week 3



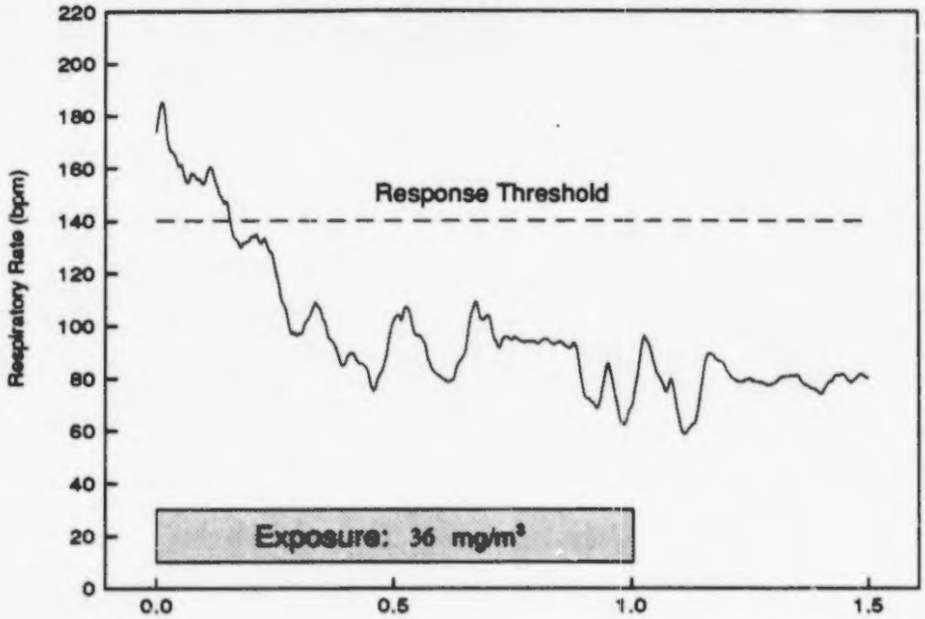


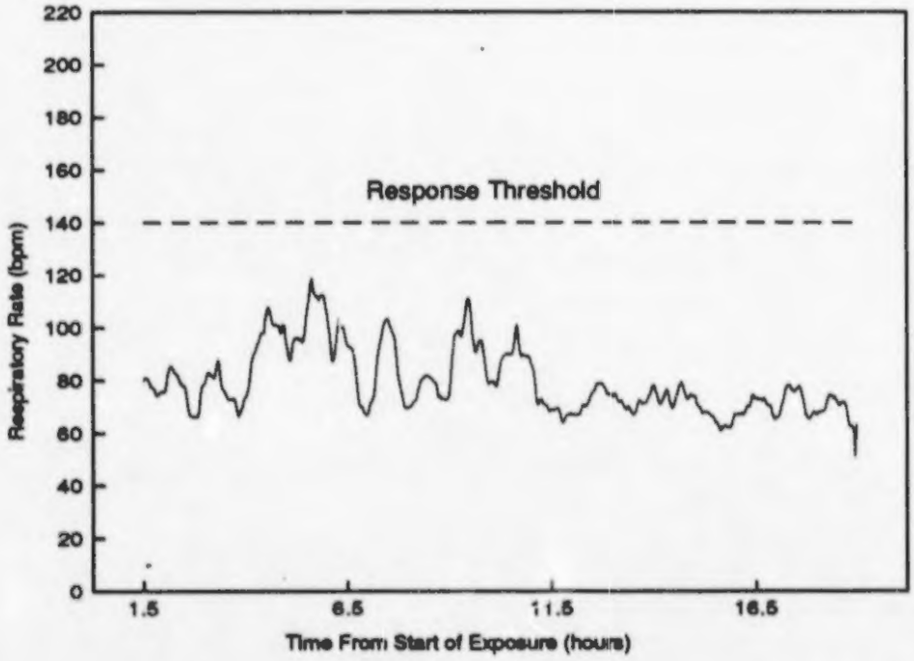
0129

Study Number 92-344-PP
Animal # 208: Sham Sensitization
Figure: 98 2,6-TDI-GSA Challenge Week 3

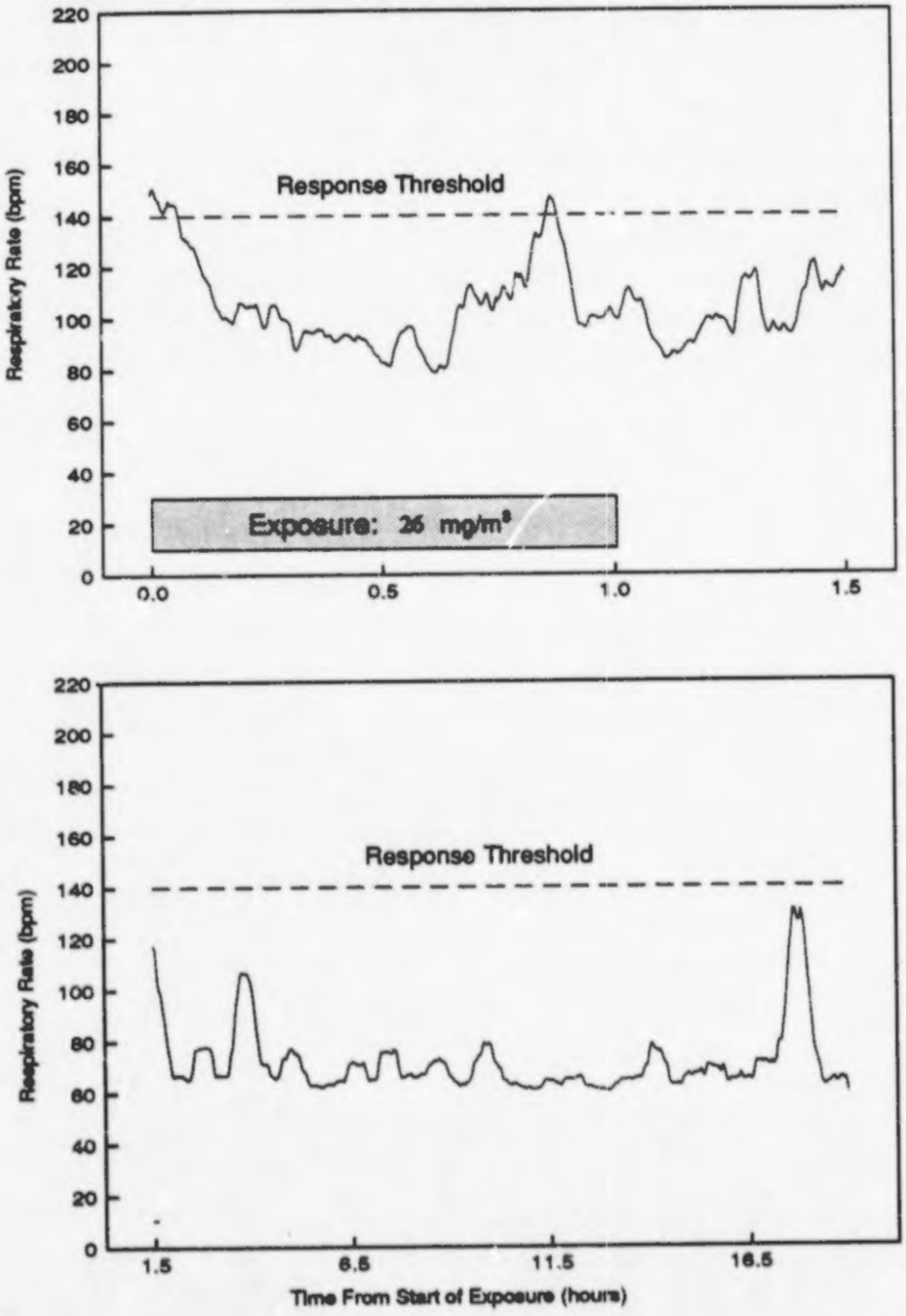


Study Number 92-344-PP
Animal # 209: Sham Sensitization
Figure: 99 2,6-TDI-GSA Challenge Week 3





Study Number 92-344-PP
Animal # 210: Sham Sensitization
Figure: 100 2,6-TDI-GSA Challenge Week 3

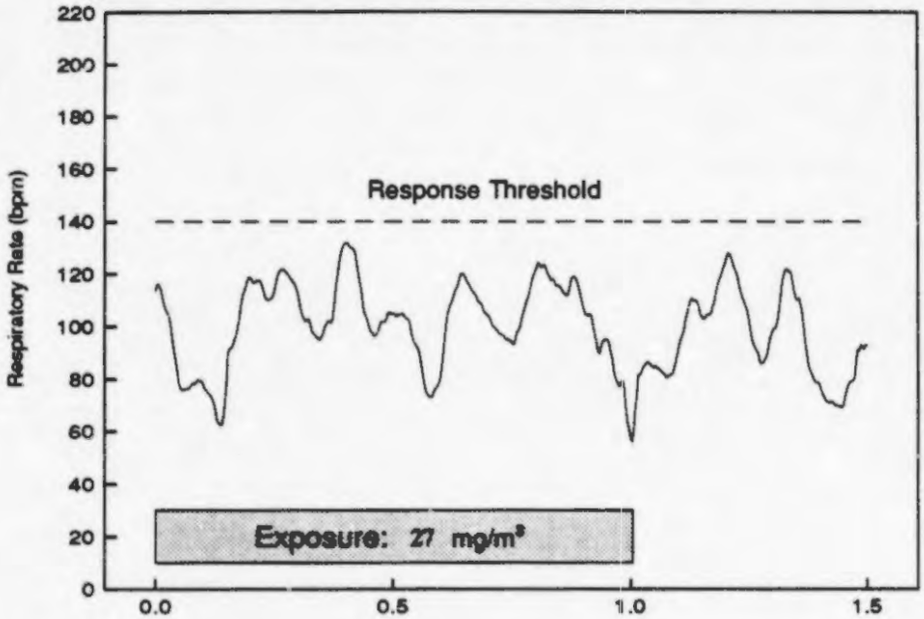


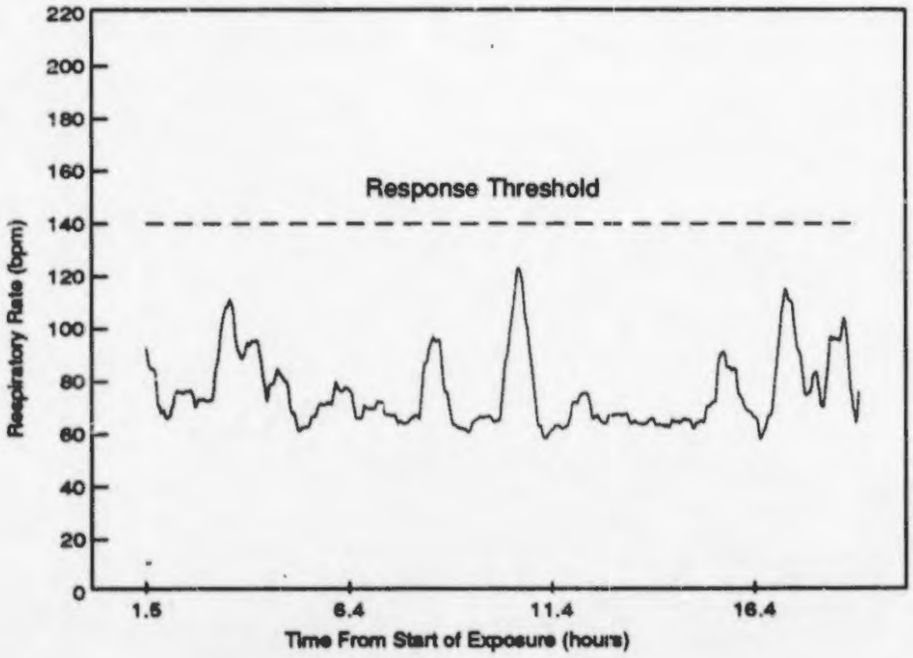
0132

Study Number 92-344-PP

Animal # 211: Sham Sensitization

Figure: 101 2,6-TDI-GSA Challenge Week 3

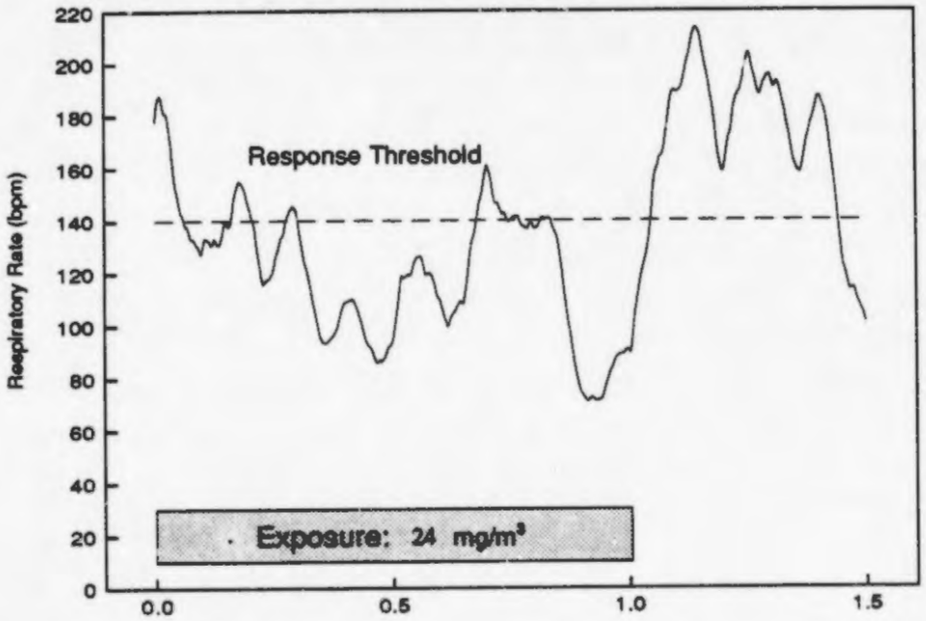


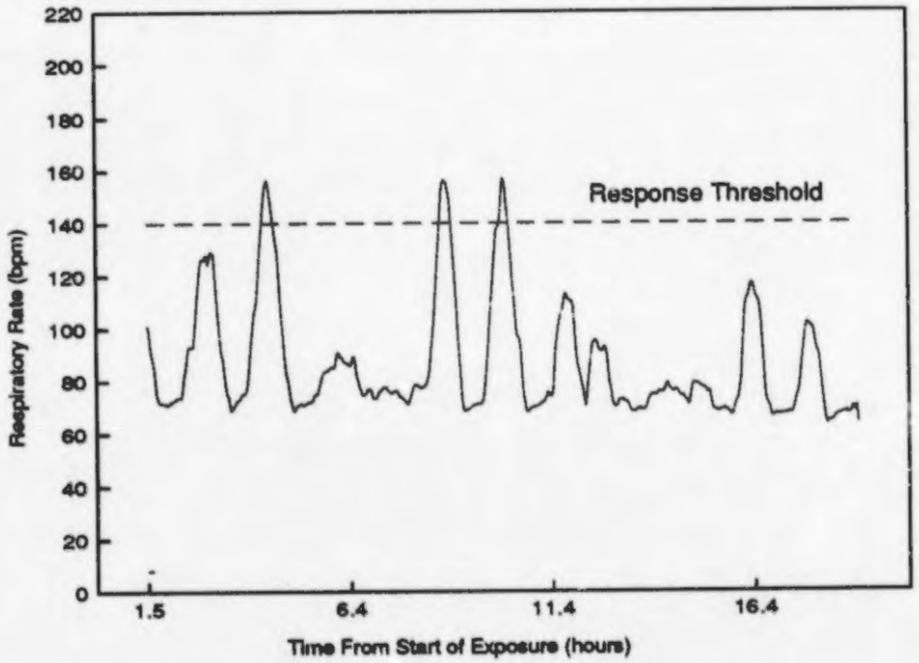


Study Number 92-344-PP

Animal # 212: Sham Sensitization

Figure: 102 2,6-TDI-GSA Challenge Week 3

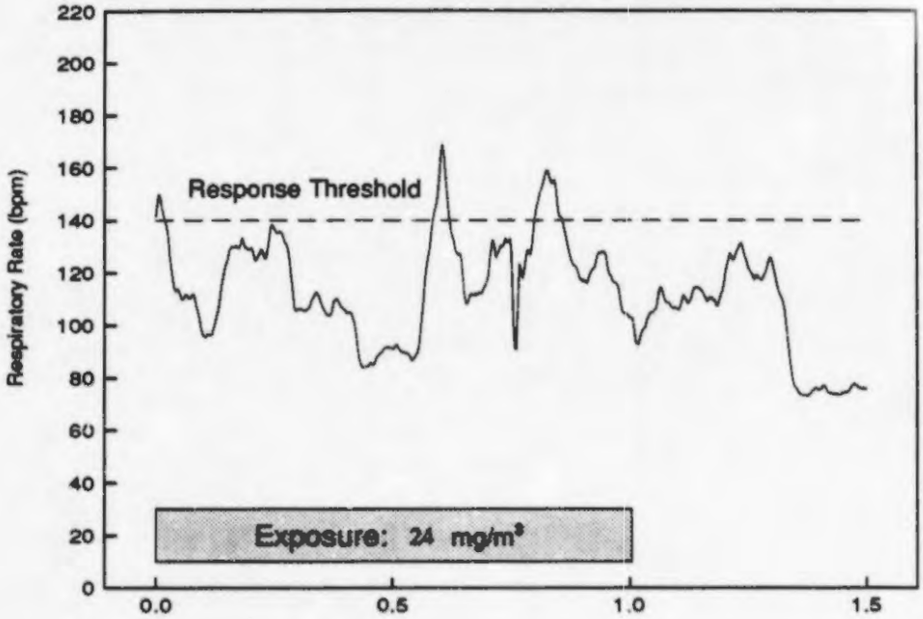


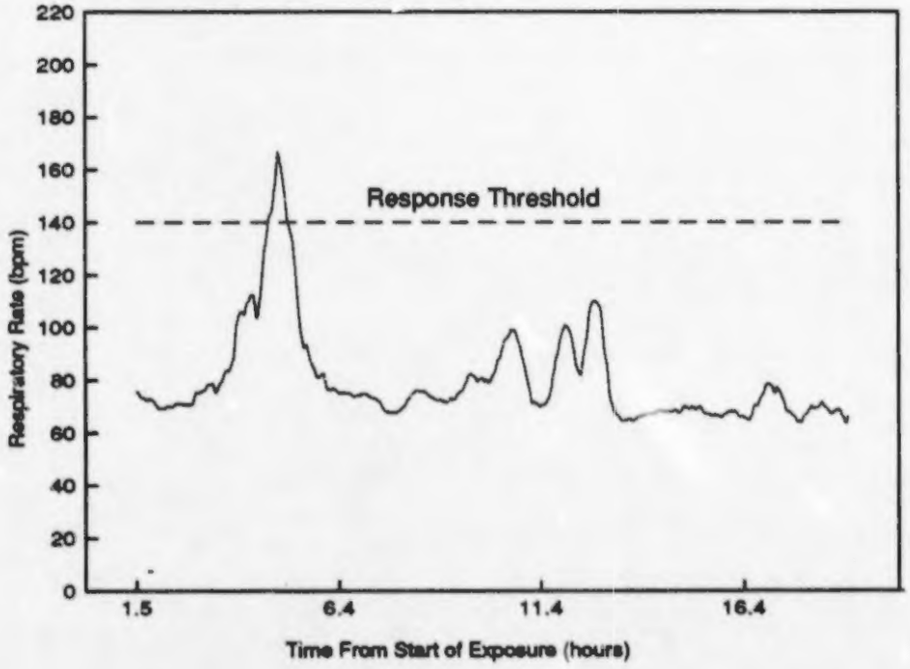


Study Number 92-344-PP

Animal # 213: Sham Sensitization

Figure: 103 2,6-TDI-GSA Challenge Week 3





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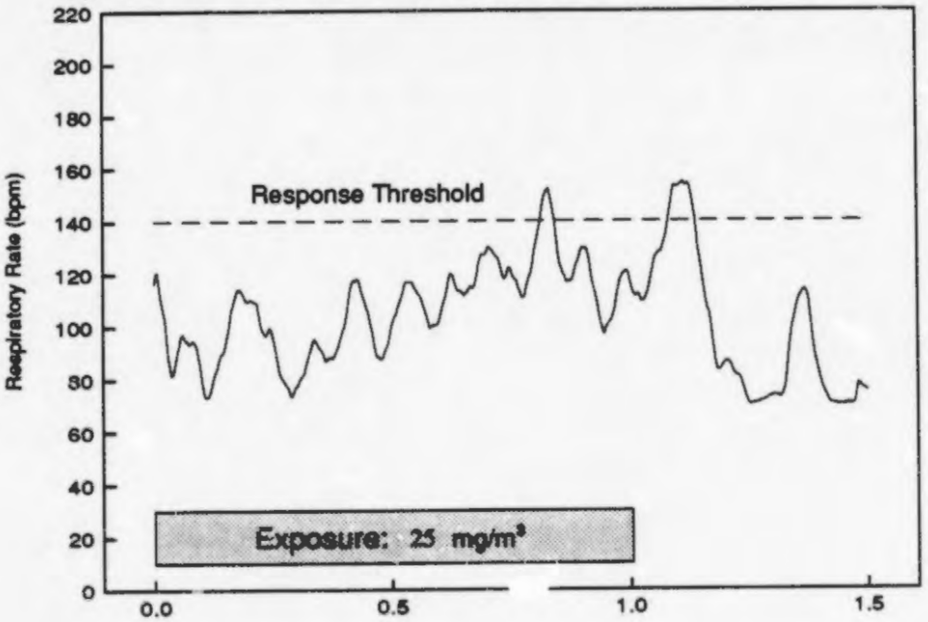
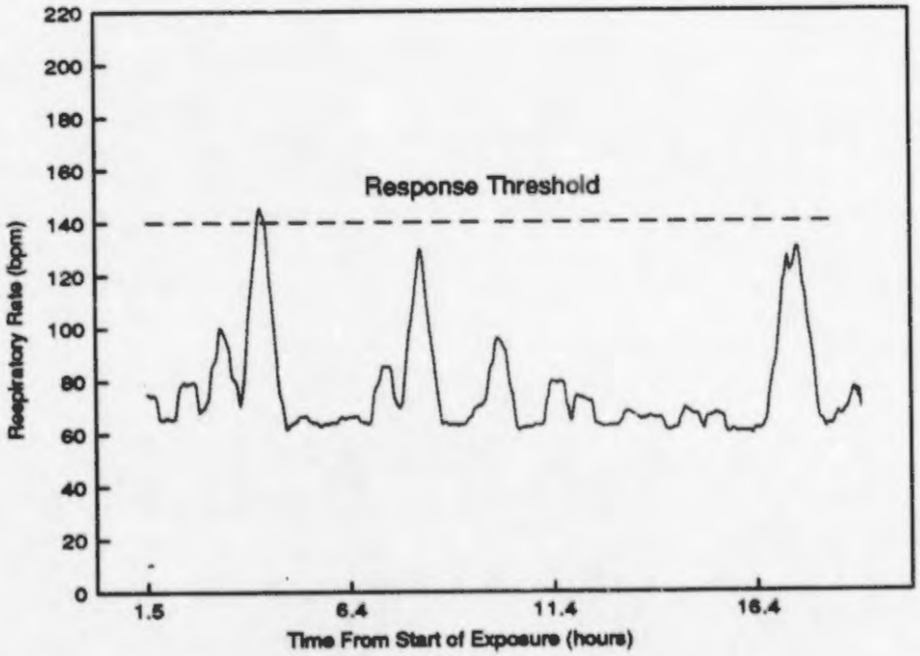
Study Number 92-344-PP

Animal # 214: Sham Sensitization

Figure: 104 2,6-TDI-GSA Challenge Week 3

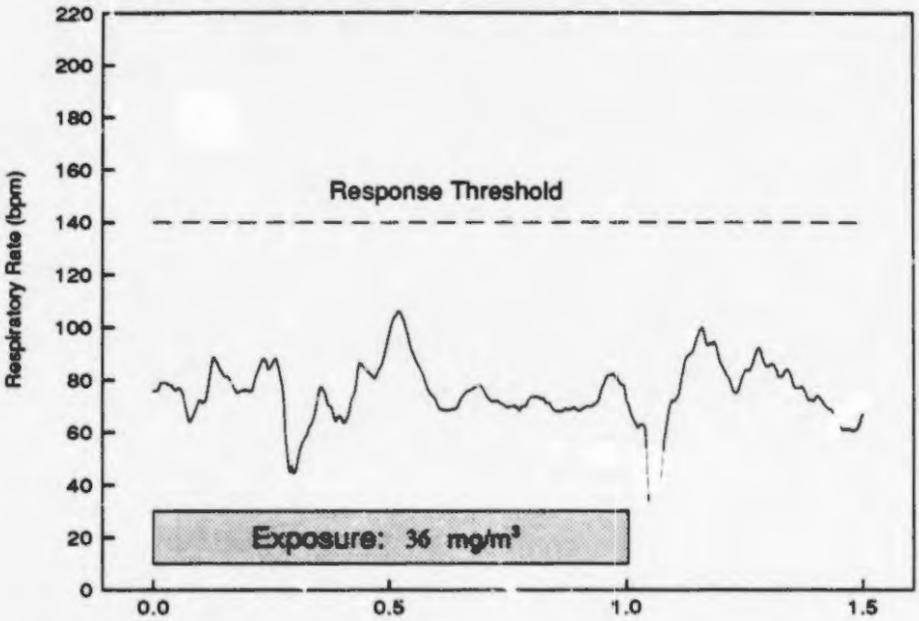


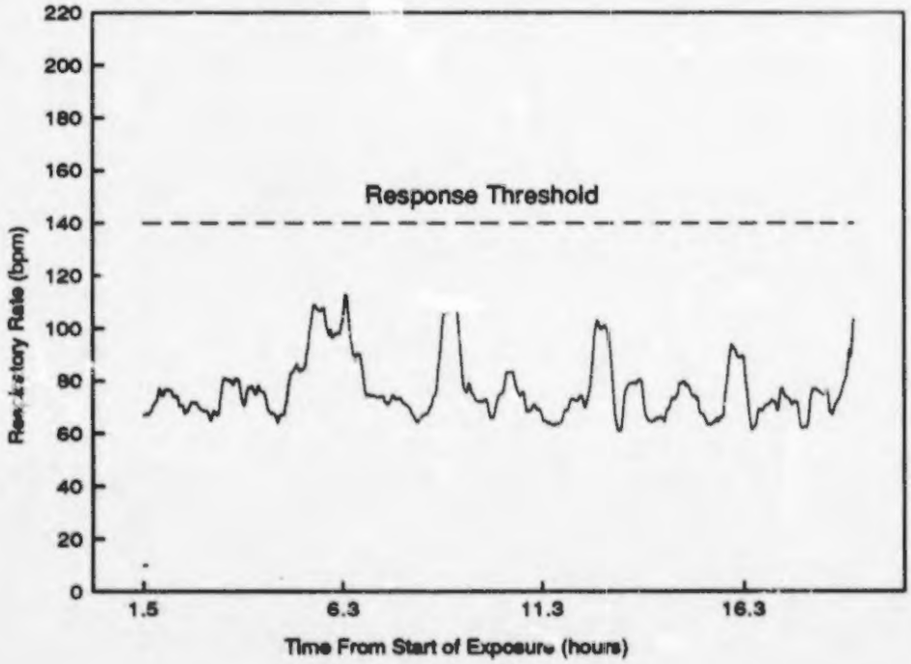


Study Number 92-344-PP

Animal # 215: Sham Sensitization

Figure: 105 2,6-TDI-GSA Challenge Week 3

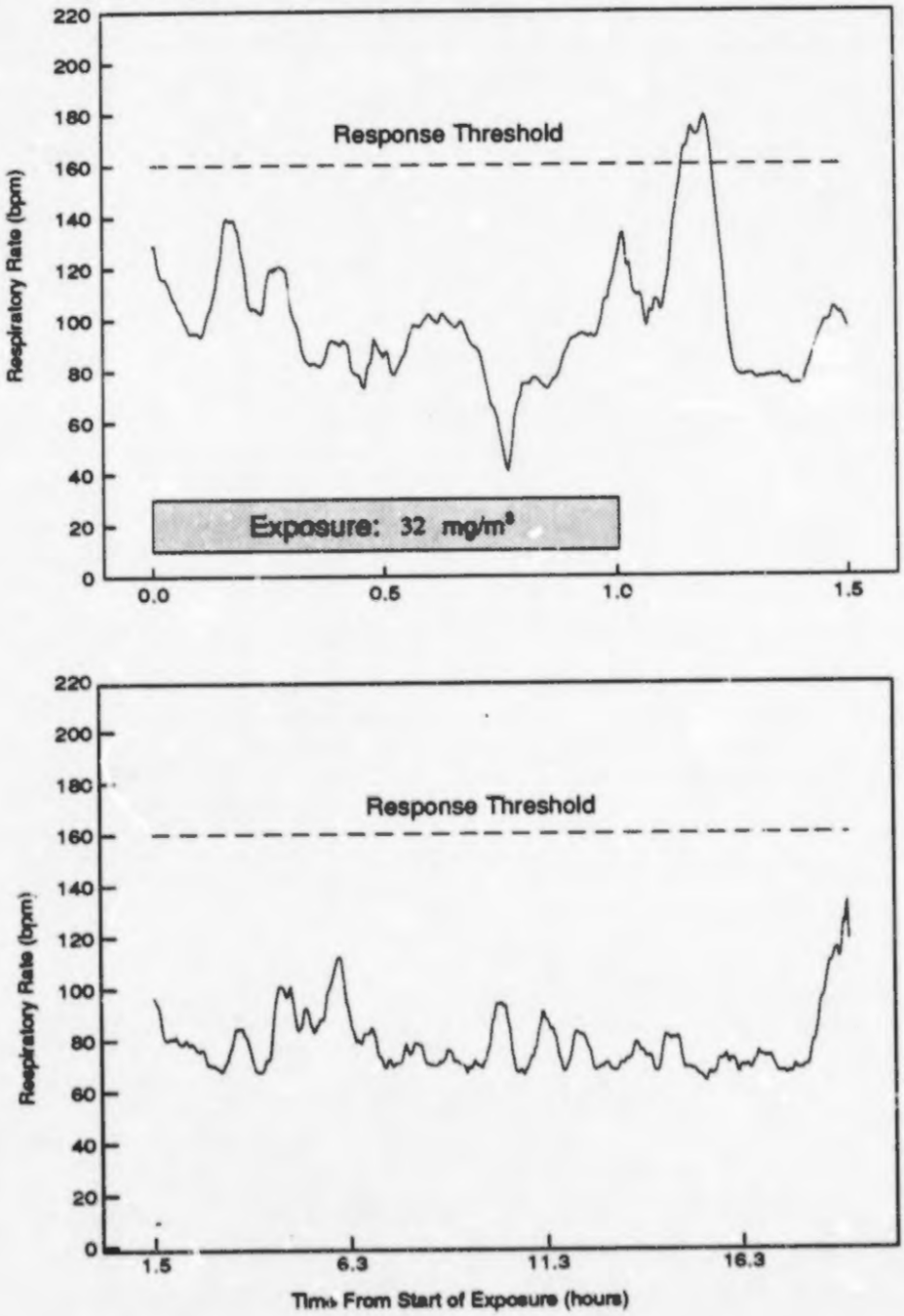




Study Number 92-344-PP

Animal # 216: Sham Sensitization

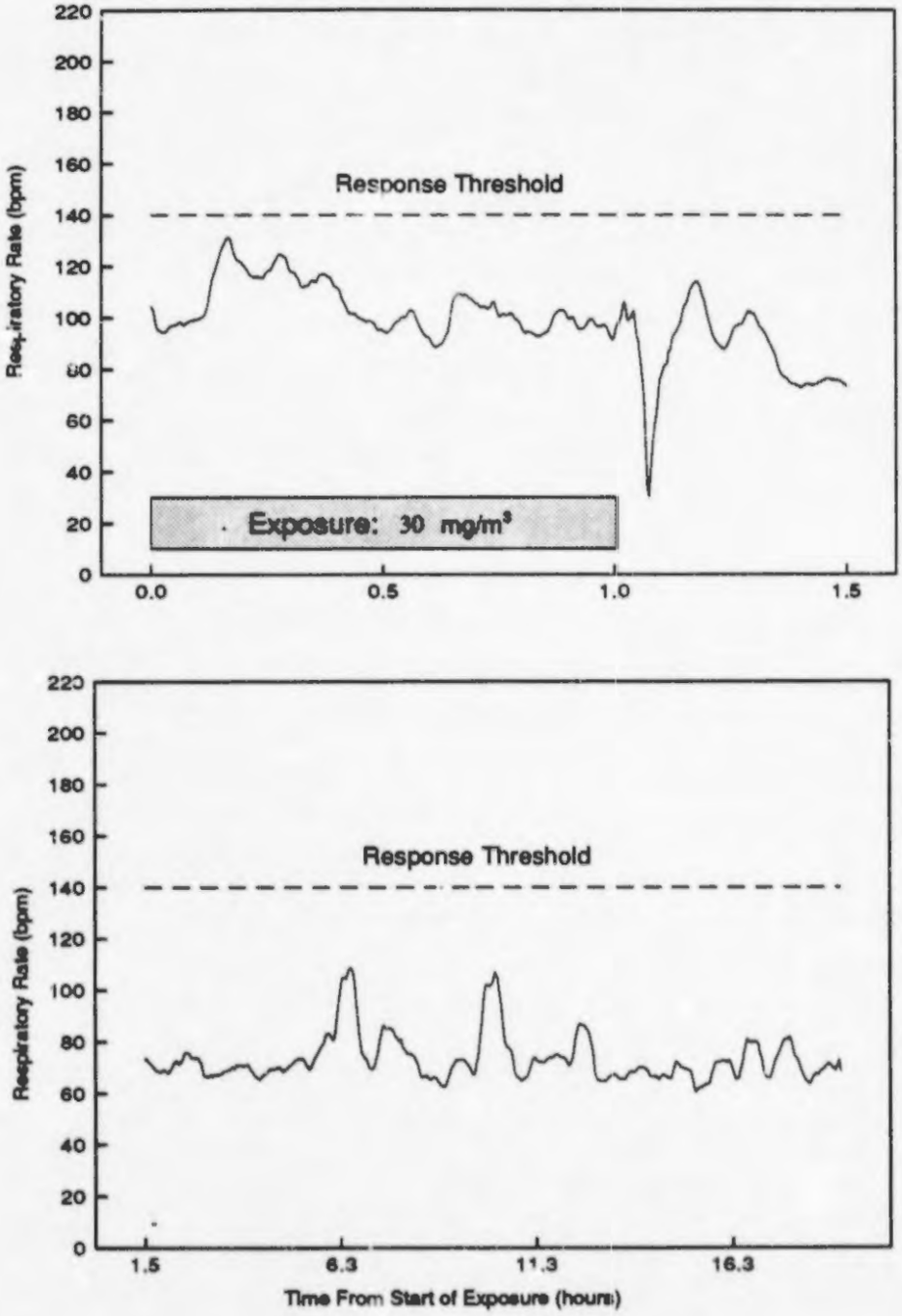
Figure: 106 2,6-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 217: Sham Sensitization

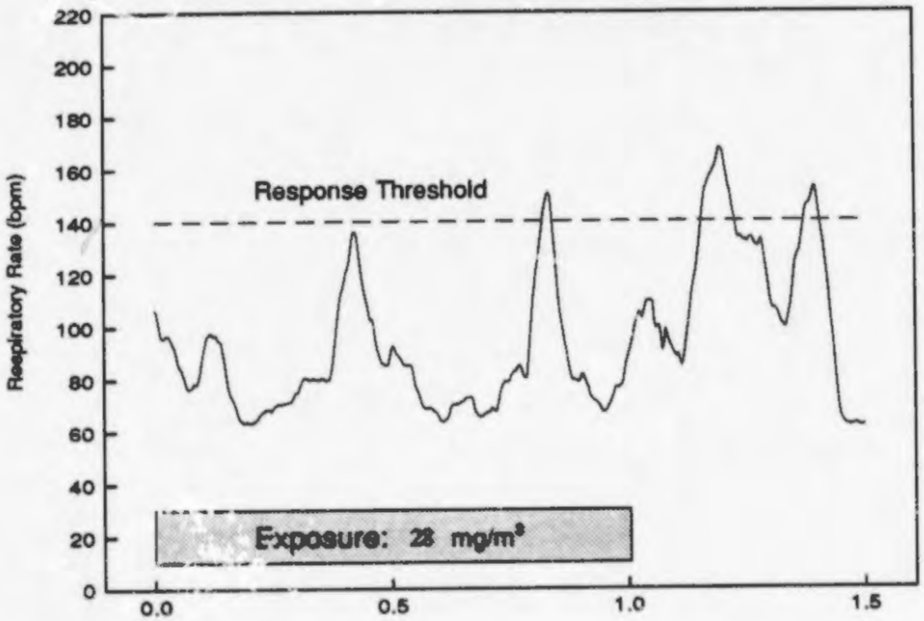
Figure: 107 2,6-TDI-GSA Challenge Week 3

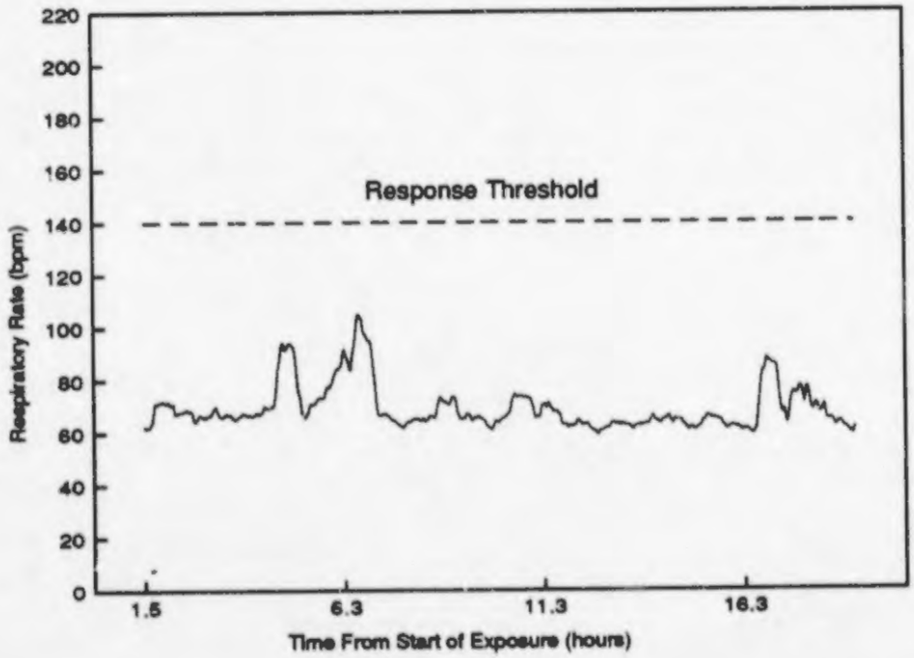


Study Number 92-344-PP

Animal # 218: Sham Sensitization

Figure: 108 2,6-TDI-GSA Chailenge Week 3





Appendix I

Individual Body Weights (g) of Guinea Pigs Used During This Respiratory Sensitization Study

Study Number 92-344-PP

Animal Number	Post Exposure Day 0	4	7	14	Challenge Week* 1		2		3	
Group I (Sham Sensitization, Sham Challenge)										
193	253	275	298	348	384	365	427	405	485	445
194	243	274	298	346	401	363	448	415	492	450
195	262	287	294	339	394	360	447	408	495	448
196	263	273	290	329	350	332	371	364	413	383
197	263	292	310	384	466	406	531	465	601	525
198	272	289	313	359	397	383	446	411	492	453
199	275	317	332	399	483	444	540	503	612	561
200	248	283	299	351	389	351	433	406	471	424
201	248	287	308	367	439	403	496	457	566	513
202	253	288	311	370	462	423	503	467	544	495
203	269	302	331	392	474	439	526	489	569	524
204	244	275	294	344	396	383	435	417	451	434
Group II (Sham Sensitization, 2,4-TDI Challenge)										
222	244	266	293	343	381	354	427	387	472	43[illegible]
223	270	299	342	398	459	408	570	460	571	509
224	247	271	285	318	350	317	382	339	417	372
225	259	295	314	369	415	378	464	427	524	474
226	241	258	284	329	371	345	396	369	448	415
227	264	297	326	375	426	383	474	424	528	466
228	268	299	331	399	464	419	520	459	580	514
229	273	304	341	405	457	408	502	452	566	499
230	273	302	324	378	437	393	491	434	525	479
231	265	305	349	420	525	464	584	516	623	556
232	280	316	334	391	454	409	507	446	558	487
233	270	298	322	365	436	387	475	429	522	463

*Pre-challenge and post-challenge weights are shown.

Appendix I (Continued)

Individual Body Weights (g) of Guinea Pigs Used During This Respiratory Sensitization Study

Study Number 92-344-PP

Animal Number	Post Exposure Day 0	4	7	14	Challenge Week* 1		2		3	
Group III (Sham Sensitization, 2,6-TDI Challenge)										
207	272	301	330	376	321	322	448	395	483	430
208	266	274	296	327	264	255	377	330	423	382
209	258	279	299	322	321	312	373	339	401	365
210	281	306	332	370	307	295	422	375	457	414
211	279	292	315	345	388	353	426	392	463	424
212	287	311	339	372	424	370	478	415	527	470
213	255	270	292	334	334	308	376	350	390	356
214	275	295	320	353	399	361	420	390	456	421
215	296	322	347	406	465	401	513	459	552	504
216	259	287	317	352	411	365	451	420	483	442
217	262	291	318	356	403	362	454	408	486	440
218	270	291	324	376	439	394	510	466	560	512

*Pre-challenge and post-challenge weights are shown.

0 1 4 3

Appendix II

Animal Dependent Hypersensitivity Response Incidence, Response Threshold Values, and Exposure Concentrations During Challenge

Group 1 - Sham Sensitization/Sham Challenge

Study Number 92-344-PP

Immediate or Delayed Responses

	Animal No.											
Challenge Wk.	193	194	195	196	197	198	199	200	201	202	203	204
Week 1	-/D	I/D	-/D	-/D	-/0	-/-	-/D	-/D	I/D	-/0	-/D	-/-
Week 2	-/-	-/0	-/-	-/-	-/0	-/-	-/D	-/-	-/-	-/D	-/0	-/D
Week 3	-/-	I/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

Response Threshold Values

Week 1	140	140	143	140	141	160	140	140	140	140	140	140
Week 2	148	140	140	140	140	140	140	140	140	140	140	140
Week 3	140	151	140	146	140	154	140	140	140	140	140	140

Exposure Concentrations

Week 1, Week 2 Sham Exposures												
Week 3 (mg/m³)	41	29	45	51	42	34	28	44	22	27	39	48

- = No response
I = Immediate response
D = Delayed response
0 = No data

Appendix II (Continued)

Animal Dependent Hypersensitivity Response Incidence, Response Threshold Values, and Exposure Concentrations During Challenge

Group 2 - Sham Sensitization/2,4-TDI Challenge

Study Number 92-344-PP

Immediate or Delayed Responses

	Animal No.											
Challenge Wk.	222	223	224	225	226	227	228	229	230	231	232	233
Week 1	-/D	-/D	-/-	-/-	-/-	-/-	-/D	-/-	-/-	-/0	-/-	-/D
Week 2	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/D	-/-	-/D
Week 3	-/-	-/-	-/-	-/-	-/D	-/-	-/-	-/-	-/-	-/-	-/-	-/D
Response Threshold Values												
Week 1	140	158	147	140	159	160	159	140	140	149	140	141
Week 2	160	152	140	149	160	144	150	140	152	148	140	140
Week 3	160	160	140	160	140	140	140	140	140	154	159	146
Exposure Concentrations												
Week 1 (ppb)	10	22	38	18	16	19	24	27	21	12	13	22
Week 2 (ppb)	23	21	34	20	6	*	*	23	35	*	47	32
Week 3 (mg/m^3)	68	28	46	26	33	20	29	35	28	29	28	14

- = No response
I = Immediate response
D = Delayed response
0 = No data
* = Sampling error

Appendix II (Continued)

Animal Dependent Hypersensitivity Response Incidence, Response Threshold Values, and Exposure Concentrations During Challenge

Group 3 - Sham Sensitization/2,6-TDI Challenge

Study Number 92-344-PP

Immediate or Delayed Responses

	Animal No.											
Challenge Wk.	207	208	209	210	211	212	213	214	215	216	217	218
Week 1	-/0	-/-	-/0	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Week 2	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/0	I/-	-/-
Week 3	-/-	-/D	-/-	-/-	-/-	I/D	-/-	-/-	-/-	-/-	-/-	-/-

Response Threshold Values

Week 1	140	160	140	140	156	140	140	140	140	160	140	140
Week 2	140	140	140	140	140	140	140	140	140	140	140	140
Week 3	140	140	140	140	140	140	140	140	140	160	140	140

Exposure Concentrations

Week 1 (ppb)	49	29	17	12	52	76	31	24	40	25	43	45
Week 2 (ppb)	26	29	30	24	29	26	36	21	81	66	43	31
Week 3 (mg/m^3)	41	36	36	26	27	24	24	25	36	32	30	28

- = No response
I = Immediate response
D = Delayed response
O = No data

Contains No CBI

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