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Contractor	MILES INC
Document Title	INITIAL SUBMISSION: LETTER FROM INT ISOCYANATE INST INC TO USEPA REGARDING A RESPIRATORY INHALATION STUDY IN GUINEA PIGS WITH 2,4- AND 2,6-TOLUENE DIISOCYANATE WITH ATTACHMENTS
Chemical Category	2,4- AND 2,6-TOLUENE DIISOCYANATE

INTERNATIONAL ISOCYANATE INSTITUTE, INC.
119 CHERRY HILL ROAD
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8EHQ-92-8568
INIT 10/09/92

8EHQ-1092-8568

Attn: 8(e) Coordinator

Dear Sir or Madam:

The International Isocyanate Institute, Inc. (III) is currently sponsoring a study on Toluene Diisocyanate; 2,4- and 2,6: Respiratory Sensitization Study with 2,4- or 2,6-Toluene Diisocyanate (TDI) in Guinea Pigs - III Project AM-AB-89. This study is being conducted at Miles Inc., Agriculture Division Toxicology, 17745 South Metcalf, Stilwell, Kansas 66085-9104. The findings, detailed below, are being submitted to the TSCA 8(e) coordinator on behalf of BASF Corporation, The Dow Chemical Company, ICI Americas Incorporated, Miles Incorporated, and Olin Corporation. These findings will be communicated to our member companies.

Details of the Study:

A respiratory sensitization study of 2,4- and 2,6-Toluene diisocyanate (CAS: 584-~~80~~84-9 and 91-08-~~07~~7, respectively) was recently performed by Miles Inc. under the auspices of the International Isocyanate Institute, Inc. This study was performed using guinea pigs as the test model, to evaluate the potential for 2,4- and 2,6-Toluene diisocyanate (TDI) to induce pulmonary sensitization following respiratory exposure.

In the study, 11-12 male guinea pigs/group were exposed during the sensitization phase (exposures for three hours/day for five consecutive days) to either 2,4- or 2,6-TDI. All animals were then housed under standard conditions for two weeks. Animals were subsequently challenged three times (at approximately one-week intervals) with one isomer of TDI to assess hypersensitivity reactions as shown below.

	Sensitization	Challenge
Group 1	2,4-TDI	2,4-TDI
Group 2	2,4-TDI	2,6-TDI
Group 3	2,6-TDI	2,4-TDI
Group 4	2,6-TDI	2,6-TDI

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Note: The first two challenges in each case involved TDI vapor while third challenge was to the appropriate isomer of TDI conjugated to guinea pig serum albumin.

Increases in respiratory rate were taken as a primary indicator for hypersensitivity responses

2 pgs.

and the respiratory rate of each animal was monitored before, during, and after each challenge exposure. All animals were evaluated for suspected immediate-and delayed-type hypersensitivity reactions during each of the three challenges.

Immediate, delayed and combined (both) type responses were observed from all groups. A greater incidence of delayed-type responses were observed from all groups. A greater incidence of delayed-type rather than immediate-type suspected responses were apparent from each group. More suspected immediate-type responses tended to be observed after the third week of challenge (TDI-GSA conjugate exposure) than were observed during the first two weeks of challenge (TDI vapor). This trend was not followed, however, with respect to observations of suspected delayed-type responses.

These data suggest that guinea pigs may become sensitized to either isomer of TDI, and that each isomer of TDI may be used to assess hypersensitivity reactions after prior exposure to either TDI isomer. This study was conducted to compare isomer-specific response patterns from animals given exposures to either 2,4- or 2,6-TDI, however, in that no true controls were conducted during this study, none of the suspected hypersensitivity responses observed here may be authenticated. Nevertheless, because we believe that the results of this study mark the first time that the individual isomers of commercial grade Toluene diisocyanate have been shown to induce pulmonary sensitization, we are bringing these findings to your attention.

Very truly yours,

R. K. Rigger
Managing Director

CERTIFICATE OF AUTHENTICITY

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Date Produced	10/06/92
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TSCA section	8E
Submitting Organization	INT ISOCYANATE INST INC
Contractor	MILES INC
Document Title	SUPPORT: RESPIRATORY SENSITIZATION STUDY WITH 2,4- OR 2,6-TOLUENE DIISOCYANATE (TDI) IN GUINEA PIGS WITH COVER LETTER DATED 030894 AND ATTACHMENTS
Chemical Category	2,4- AND 2,6-TOLUENE DIISOCYANATE

8EHQ-0494-8568

INTERNATIONAL ISOCYANATE INSTITUTE, INC.
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401 M Street, S.W.
Washington, D.C. 20460

Attn: 8(e) Coordinator

Dear Sir or Madam:

As a follow- up to the attached previous filing (8EHQ-1092-8568) dated October 9, 1992, the International Isocyanate Institute Inc. (III) on behalf of its members (BASF Corporation, Dow Chemical Company, ICI Americas, Inc., Miles, Inc. and Olin Corporation) hereby submits a copy of the final report for the following study:

"Respiratory Sensitization Study with 2,4- or 2,6-TDI in guinea pigs."
III Project: AM-AB-89.

A copy of the report is also being sent to the section 8(d) Coordinator as a follow-up to a filing.

Very truly yous,

R. K. Rigger
Managing Director

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MILES

Agriculture Division

Miles Inc.
17745 South Metcalf
Stilwell, KS 66085-9104
Phone: 913 897-9100

MAR 8, 1994

Sponsor: International Isocyanates Institute (III)
119 Cherry Hill Road
Parsippany, NJ 07054

Regarding completion of III project AM-AB-89, "**Respiratory Sensitization Study with 2,4- or 2,6-TDI in Guinea Pigs**". This letter completes this project as well as presents a summary perspective for the information gathered.

This respiratory sensitization project was conducted in two phases (Miles study numbers 91-344-KR and 92-344-PP). The initial work (91-344-KR) generated comparative data for the sensitization potential of each common isomer of Toluene Diisocyanate (TDI), while the secondary work (92-344-PP) generated the extensive data from controls needed for interpretation and identification of the absolute incidence of compound-specific hypersensitive responses.

Summarily the data gathered does confirm a sensitization potential for TDI in Guinea Pigs. Both isomers of TDI were similar with respect to their potential to sensitize animals, and the incidence of sensitization was similar to literature values reported from similarly designed experiments using a mixture of these isomers. Hypersensitive responses could be elicited with similar frequency by provocation with the same isomer used for the initial sensitization as well as with the alternative isomer. Overall, with respect to this biological endpoint, few distinctions were apparent between the two isomers of TDI studied.

Work on this project is concluded and the attached reports describe in detail the approachs and methodologies used, as well as animal specific results.

3/8/94

Darren L. Warren, Ph.D., DABT (Study Director)
Miles Inc.
Agriculture Division / Toxicology

3/8/94

Ghona K. Sangha, Ph.D. (Director of Toxicology Lab. Sponsors Representative)
Miles Inc.
Agriculture Division / Toxicology

FILE
5183

Study Title

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

Author

D. L. Warren

Study Completion Date

October 6, 1992

Contains No CBI

Test Facility

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

OCT 5 1992

Study Number

91-344-KR

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: 9-23-92

SPONSOR

INTERNATIONAL ISOCYANATE INSTITUTE, INC.

G. K. Sangha: _____
Sponsor Representative

Date: 9-22-92

STUDY DIRECTOR

D. L. Warren: _____

Date: 9/22/92

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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 22, 1991
Terminal Sacrifice Date: November 22, 1991

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. W.
Study Conduct:	L. A. Berry	L.A. Be
	D. W. Sturdivant	David W. Sturdivant
	A. T. Tran	Anh Tran
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	Roger D. Moore
Quality Assurance:	C. A. Halder	Chris A. Halder

QUALITY ASSURANCE STATEMENT

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.

AUDITS			REPORT TO STUDY DIRECTOR/ MANAGEMENT
Date	Phases	Auditor	
	Phase of Study		
07/25/91	Balance Verification, Removal of Test Substance From Storage, Weighing of Test Substance, Return of Test Substance to Storage, Placement of Test Substance in Bubbler, Lab-Tech Software Operation/Data Collection, Use of Gilibrator, Verification of Generator and Exhaust Airflows, Water Bath Heater, Balance Verification, Body Weights, Clinical Signs, Placement of Animals in Restrainers/Chamber, Generator Operation, Chamber Temperature, Sampling of Chamber Atmosphere, Onset and End of Exposure, Removal of Animals From Chamber/Restrainers and Post-Exposure Observations	J. Ranjbar	08/05/91
08/13-15/91	Test Substance Retrieval From Storage, Balance Verification, Weighing, Dispensing of Test Substance, Reweighing, and Return to Storage, Pre and Post-Exposure Body Weights, Observations, Loading of Plethysmographs, Calibration of Mixing Chamber, Plethysmograph and Generator Airflows, Water Bath Temperature Verification, Start of Acclimation Period, BUXCO/Gould Respiratory Rate Recorder Operation, Airflow and Static Pressure Readings, Respiratory Measurements, End of Experiment, Removal of Animals From Plethysmograph, Generation Operation Start and Stop of Delivery System (Test Substance), Mixing Chamber and Plethysmograph Atmosphere Sampling	J. Hagensen	08/16/91

QUALITY ASSURANCE STATEMENT (Continued)

AUDITS			REPORT TO STUDY DIRECTOR/ MANAGEMENT
Date	Phases	Auditor	
	Phase of Study (Continued)		
08/26,27/91	Balance Verification, Protein Conjugate Preparation, Pitt No. 1 Nebulizer Operation, Razel Pump Operation, Calibration/Operation of Cahn 27 Electrobalance, Mixing Chamber/Plethysmograph Atmosphere Sampling, Animal Inventory, Anesthesia, Blood Collection, Serum Preparation, Sacrifice of Animals and Necropsy	J. Hagensen	08/29/91
01/27/92	Lab-Tech Software Operation and Collection of and Storage of Chamber Airflow, Temperature and Humidity Data, Calibration of Solomat Temperature and Humidity Probes, Balance Verification, Body Weights, Placement of Animals in Plethysmographs, Animal Inventory, Acclimation Start, Exposure Start and End, Removal of Animals from Plethysmograph and Clinical Observations	J. Hagensen	01/29/92
03/05/92	Use of Gilibrator, Verification of Chamber, Generator, and Plethysmograph Airflows, Pre-challenge Body Weights, Observations, Balance Verification, Calibration of Gould and BUXCO Systems, Placement of Animals in Plethysmographs, Acclimation, Preparation of Test Substance, Calibration of Cahn Balance and Wet Test Meter, Start of Pre-exposure, Onset of Challenge Exposure, Operation of Pump Delivery System and Nebulizer, Airflow Readings, Static Pressure Readings, Sampling of Chamber and Plethysmograph Atmosphere, End of Challenge Exposure, Feeding and Watering of Animals, Post-Exposure Termination, Post-Exposure Body Weights, Observations, Balance Verification, Anesthesia, Blood Collection, Sacrifice, and Necropsy	J. Hagensen	03/05/92
08/21-09/01/92	Final Report Review	K. Schroeder/ J. Hagensen	09/01/92

QUALITY ASSURANCE STATEMENT (Continued)

Date	AUDITS Phases	Auditor	REPORT TO STUDY DIRECTOR/ MANAGEMENT
	Clinical Laboratory Support Functions		
08/27/91	Hematology Parameters	J. Hagensen	08/29/91
10/24/91	Differential Counts	J. Ranjbar	10/24/91
	Analytical Support Functions		
10/07/91	Analysis, Method For Analysis, Analytical Standards, Standardization and Maintenance of Chromatographic Instruments	S. Bailey	10/17/91
	Animal Care Support Functions		
07/02/91	Animal Receiving, Examination, Randomization, Animal Inventory and Room Activity Checklist (T-F189)	J. Hagensen	07/02/91
07/03/91	Animal Identification	J. Hagensen	07/03/91
07/08/91	Release of Animal Shipment For Study Use	J. Hagensen	07/10/91
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	Quarantine, Feeding and Observations	C. A. Cox	02/26/92
03/02/92	Special Procedures For Guinea Pigs	C. A. Cox	03/02/92
03/06/92	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 91-344-KR 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, Quality Assurance

9/16/92
Date

ABSTRACT

The potential of 2,4- or 2,6-TDI to sensitize male guinea pigs after respiratory exposure was investigated. Eleven to 12 animals per group were exposed during the sensitization phase (exposures for three hours/day for five consecutive days) to either 2,4- or 2,6-TDI. All animals were then housed under standard conditions for two weeks. Animals were subsequently challenged three times (at approximately one-week intervals) with one isomer of TDI to assess hypersensitivity reactions as shown below.

	Sensitization	Challenge
Group 1	2,4-TDI	2,4-TDI
Group 2	2,4-TDI	2,6-TDI
Group 3	2,6-TDI	2,4-TDI
Group 4	2,6-TDI	2,6-TDI

Note: The first two challenges in each case involved TDI vapor while third challenge was to the appropriate isomer of TDI conjugated to guinea pig serum albumin.

Increases in respiratory rate were taken as a primary indicator for 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.

Two animals died during the sensitization exposures. A small number of clinical signs were observed from groups 2 and 3 after the first day of exposure and one animal from group 4 reacted strongly during the third challenge exposure. No signs were observed from other groups. Analysis of body weight data showed few notable differences between groups. Although few gross lesions were observed animals from group 4 showed an incidence of red zones in the lungs at necropsy that was elevated from the other groups.

All animals were evaluated for suspected immediate- and delayed-type hypersensitivity reactions during each of the three challenges. Immediate, delayed, and combined (both) type responses were observed from all groups. A greater incidence of delayed-type rather than immediate-type suspected responses was apparent from each group. More suspected immediate-type responses tended to be observed after the third week of challenge (TDI-GSA conjugate exposure) than were observed during the first two weeks of challenge (TDI vapor). This trend was not followed, however, with respect to observations of suspected delayed-type responses.

This study was performed to compare response patterns elicited by exposures to 2,4- or 2,6-TDI, and few differences were observed here between groups. In that sham-sensitized but TDI-challenged control groups were not included in this study, the true incidence of authentic hypersensitivity reactions cannot be identified by this study.

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:

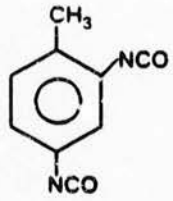
Test Substance:

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

Active Ingredient:

Chemical Name: 2,4-Toluene Diisocyanate

Structures:



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:

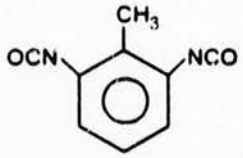
Test Substance:

Identification:	2,6-Toluene Diisocyanate
Physical Appearance:	Clear liquid
Catalog. No.:	22,386-7
Purity:	97%
Stability Under Storage Condition:	Not available*

Active Ingredient:

Chemical Name: 2,6-Toluene Diisocyanate

Structures:



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 2,4- and 2,6-TDI to sensitize guinea pigs by respiratory exposure. 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, exposures to much lower concentrations of the same test material for a much shorter time (the "challenge" phase). 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.

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, or Sasco, Inc., Omaha, Nebraska, 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 47 animals are reported. Body weights prior to the first sensitization exposure ranged from 261 to 361 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 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 [7]. 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. Each animal experienced each phase. Four groups of 11 to 12 animals were used and each group had a different

isomer-specific course of exposure during the sensitization and challenge exposures. These groups were designated as:

Group	Exposure	
	Sensitization	Challenge
Group 1	2,4-TDI	2,4-TDI
Group 2	2,4-TDI	2,6-TDI
Group 3	2,6-TDI	2,4-TDI
Group 4	2,6-TDI	2,6-TDI

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

Phase	Regimen	Description
Sensitization	Week 1	5 x 3 hours/day exposures to TDI vapor
Induction	Weeks 2,3	Conventional housing
Challenge	Week 4	Challenge #1 TDI vapor
	Week 5	Challenge #2 TDI vapor
	Week 6	Challenge #3 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

Exposures with the appropriate TDI isomer as a vapor were administered to each group for three hours per day on each of five consecutive days (days 0-4).

Induction Phase

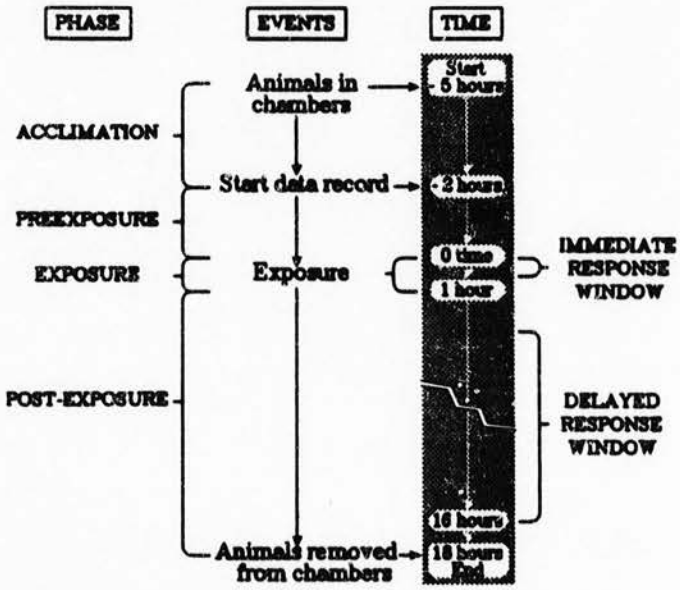
Each animal was conventionally housed for approximately two weeks.

Challenge Phase

This phase was conducted identically for all four groups and animals were exposed to the same isomer of TDI during each challenge exposure. These exposures were started between 12:00 p.m. and 4:00 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 (maximum) were placed individually into whole-body plethysmographs on day 21. Subsequent subgroups (from the original group of 11 or 12 animals that were given sensitization exposures) were challenged on days 22, 23 or 24. Animals were allowed to acclimate for 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). Food and water were available for ad libitum consumption 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 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 [8]). An exhaust airflow of approximately 29 - 30 Lpm (23 - 24 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 food reservoir and source of drinking water (ad libitum after exposure). Plethysmographs were operated in a dynamic mode (constant airflow of 1.7 Lpm and a static pressure of approximately -0.3 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 30°C during sensitization exposures and 25°C for challenge exposures. Dried compressed air was supplied with a flow of approximately 4 Lpm during sensitization or 0.3-0.5 Lpm during challenge. The TDI-GSA conjugates were generated as a liquid aerosol in borate buffer (50 mM, pH=9.4, 1% w/v solution) using an air-blast type glass nebulizer. This protein solution was delivered by a syringe pump (Razel Model A-99).

Generator output was mixed with conditioned room air at the chamber (sensitization) or mixing chamber (challenge) inlet and total airflows were approximately 29 - 30 Lpm for sensitization and 10 - 18 Lpm for challenge. During sensitization exposures airflows were monitored continuously and were recorded at approximately five-minute intervals (Labtech software [8]). 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 derivatization with N-4-nitrobenzyl-N-n-propylamine [9]. 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 ppm or 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 [10]. 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 [11], and the results consistently indicated a percent of substitution of approximately 85% for each conjugate.

Particle Size Distribution

The particle size distribution from exposures with 2,4- or 2,6-TDI was monitored by a TSI [12] Aerodynamic Particle Sizer (Model 3310) and Diluter (Model 3302) interfaced to an IBM PS/2-50. The particle size character of TDI-GSA atmospheres during challenge was assessed during preliminary runs in that it is not possible to conduct these measurements during actual exposures involving animals (sampling and flow requirements are substantially disparate). The mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) were calculated from the values of cumulative percent mass.

Monitoring of Temperature and Relative Humidity

Our desired ranges for chamber temperature and relative humidity were 20-24°C and 40-60%, respectively. During sensitization exposures temperatures were taken by hand with a National Institute of Standards and Technology (NIST) certified thermometer at 30-minute intervals. Relative humidity monitoring was not done during exposures in that our Hygroscopic-Dielectric type sensors are incompatible with isocyanates. No suitable substitutes for these sensors are available.

During the challenge phase neither temperature or RH were routinely monitored.

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 [13].

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 [14]) and electronically transferred into a Lotus 1-2-3 [15] 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 sensitization by 2,4- or 2,6-TDI. Comparison between responses from controls and those from animals exposed to 2,4- or 2,6-TDI during the sensitization and challenge exposures will be used to identify true hypersensitivity reactions.

<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 [15], while graphs were prepared using a Mass-11 Draw package (Microsystems Engineering Corporation) after transferral 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

The respiratory parameters were transferred to dBase IV [14] files to delete those parameters not relevant to this study. These dBase files were then transferred to a Lotus 1-2-3 [15] spreadsheet to compute means and descriptive statistics. Other descriptive statistics were generated by Lotus 1-2-3 [15] 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 seven separate days the desired range for RH (40-70%) was exceeded by 1-3%. These excursions were from 1 to 64 minutes in duration.

Chamber Parameters

Temperature

Weekly mean values for chamber temperature during the sensitization phase (days 0-4) from all groups ranged from 22 to 23°C as indicated below.

Group 1: Weekly mean 22°C (daily mean values ranged from 22-23°C)
Group 2: Weekly mean 22°C (daily mean values ranged from 22-23°C)
Group 3: Weekly mean 22°C (daily mean values were 22°C)
Group 4: Weekly mean 23°C (daily mean values ranged from 22-24°C)

These chamber temperatures were all within our desired range of 20-24°C.

No routine monitoring of temperature was done during the challenge phase of this study.

Exposure Concentrations

Sensitization Phase

Individual analytically confirmed TDI concentrations are presented in Appendix I while daily and weekly average values are presented in Table 1A. Weekly averages ranged from 1.29 to 1.40 ppm for all groups and day-to-day chamber concentrations showed minimal variance. These data indicate the operation of a stable and reproducible generation system.

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 [4,1].

Challenge concentrations for vaporous and GSA-conjugated TDI are presented in Table 1B while individual values may be found in Appendix III. Weekly mean values were very consistent and ranged from 18 to 46 ppb for TDI vapor and from 18 to 25 mg/m³ for the GSA-conjugate. The challenge concentrations achieved are roughly 30 to 70 times lower than the mean sensitization concentrations.

Particle Size Characteristics

In that it is not physically possible to measure the aerosol particle size distribution during actual challenge phase exposures, these measurements were performed during preliminary studies. The conditions used for aerosol generation were varied so as to investigate the array of particle size distributions that were available. A method for generation was ultimately identified that produced a small particle size distribution, with the appropriate concentration, and this method was used during all challenge exposures involving the TDI-GSA conjugate.

A solution of 1% GSA in borate buffer (50 mM, pH=9.4) was generated for calibration and the TSI Aerodynamic Particle Sizer was used to monitor particle size distributions from a plethysmograph. The airflow dynamics (delivery from mixing chamber) for operation of this plethysmograph were similar to those used during challenge exposures to animals. MMAD values appeared to be concentration dependent and ranged from 0.87 to 0.94 μm (GSD values were all in the range of 1.3 to 1.4). Thus the TDI-GSA aerosol generated was easily respirable by guinea pigs.

Biologic Observations and Measurements

Clinical Observations

All signs observed were considered to be exposure-related and with the exception of one animal from group 4, all signs were observed during the sensitization exposures (days 0-4). Rapid difficult breathing was observed on day 2 from two animals (#46, #51) of group 3. Ataxia was observed on day 0 from one animal (#59) of group 2, and tremors were observed from three animals (#59, #61, #65) of group 2 and one animal (#51) of group 3 at various times from day 0 to day 4.

Contrasting with all other observations, one animal from group 4 (#105) showed clinical observations shortly after the start of the third challenge exposure. These signs included labored abdominal breathing and a yellowish-brown discharge about the rostrum and mouth. This condition rapidly progressed to what appeared to be a comatose state and at this point the animal was removed from the plethysmograph.

Body Weight

A summary of the body weight data collected is presented in Table 2, while individual values are presented in Appendix II. These data indicate that with the exception of animals from group 3 on day 4 of sensitization, all groups showed weight gain at each timepoint through day 14. 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

Two exposure-related mortalities occurred; one each on days 0 and 1 (groups 1 and 3, respectively). A total of two animals were replaced; one each from groups 1 and 4 (the group 4 animal was moribund after the first exposure and therefore was replaced). These animals were replaced before the start of exposure on day 1. Replacement animals were taken from the same original shipment of animals, and had been given identical care and acclimation to our facility as original group members. It is noted that the replacement animals received only four out of five sensitization exposures; however, responses from these animals upon challenge were indistinguishable from other animals from these groups.

Gross Pathology

Each animal was gross necropsied after the final challenge and the table below presents each observation.

Observation	Group 1 n=12	Group 2 n=12	Group 3 n=11	Group 4 n=12
No Gross Lesions	12	10	10	6
Red Zones in Lungs		1		5
Pink/Tan Zones in Liver		2	1	1
Colon Reddened				1

These findings from groups 1, 2, and 3 were unremarkable. The increase in multiple red zones from lungs of animals from group 4 may suggest that 2,6-TDI is slightly more irritating than 2,4-TDI.

Physiological Indications of Hypersensitivity

Response threshold values were not disparate between groups. Average response threshold values from each of the four groups (means of all animals from all three weeks of challenge) were 146, 146, 144, and 144 bpm (groups 1-4, respectively). Individual response threshold values are presented in Appendix III. This information indicates that the criteria used to assess hypersensitivity reactions was not dissimilar between groups.

A summary of the incidence of suspected hypersensitivity responses from all groups is presented in Table 3, while individual responses are identified in Appendix III. 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 - 141 (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 suspected immediate-type hypersensitivity reactions were observed from each group on each week of challenge. Differences between groups with respect to the incidences of these suspected reactions were minimal. For each group the challenge on week 3, involving exposure to the TDI-GSA conjugate, provoked an equal or greater incidence of suspected immediate hypersensitivity reactions as did challenge either on week 1 or week 2 (TDI vapor). It is noted that one animal from group 4 (#105) showed what appeared to be an anaphylactoid-type response upon exposure during challenge for the third time (see Clinical Observations section). This animal was removed from the plethysmograph nine minutes after the start of exposure in what appeared to be a comatose-like condition. This animal was revived and replaced into the plethysmograph after the end of the exposure period for monitoring of delayed-type responses. This episode was taken to indicate a positive immediate-type response.

A substantial incidence of suspected delayed-type hypersensitivity reactions were observed from each group of animals on each week of challenge with one exception; no responses were observed from group 3 animals on the first week of challenge. In contrast to the incidence of suspected immediate-type reactions, the incidence of suspected delayed-type reactions was not consistently higher on challenge week 3 by comparison with weeks 1 and 2. No substantial differences between groups with respect to the incidence of suspected delayed-type hypersensitivity responses was noted.

One indication that a suspected 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 suspected hypersensitivity responses from each of the four groups. To be included in this incidence table a particular animal must have shown the same type of suspected response on at least two out of the three challenges. This table indicates that more animals consistently showed delayed-type rather than immediate-type reactions and that few differences in incidence were apparent between groups.

Summarily these data suggest that both 2,4- and 2,6-TDI may have the potential to sensitize guinea pigs and that either of these TDI-isomers may be used to assess hypersensitivity reactions from guinea pigs that were previously exposed to (and potentially sensitized with) either 2,4- or 2,6-TDI. Few differences were observed in the extent of suspected hypersensitivity reactions between groups that were exposed to 2,4 or 2,6-TDI. The true incidence of hypersensitivity reactions may only be authenticated by comparison of data from the current groups with information from appropriate control groups (including groups that were sham-sensitized with room air and challenged with either 2,4- or 2,6-TDI in a manner that is identical to those presented in this report).

SUMMARY AND CONCLUSIONS

The potential for 2,4- and 2,6-TDI to induce respiratory sensitization in male guinea pigs was evaluated. Four groups were tested including animals that were initially given sensitization or induction exposures with one isomer and were subsequently challenged with either the same or the alternative isomer. Each animal was challenged three times (at one week intervals) with the same isomer - twice as a vapor and once (the final challenge) as the GSA conjugate. Sustained increases in respiratory rates were taken as indicators for immediate-type and delayed-type responses. Few clinical signs or substantial differences in weight-gain between groups were noted. The group given sensitization as well as challenge exposures to 2,6-TDI showed the only outstanding incidence of lesions at necropsy; red zones in lungs. A similar incidence of suspected immediate- and delayed-type hypersensitivity responses were observed from each group. Animals appeared to demonstrate more delayed-type rather than immediate-type suspected responses. These data suggest that guinea pigs may become sensitized to either isomer of TDI, and that each isomer of TDI may be used to assess hypersensitivity reactions after prior exposure to either TDI isomer. This study was conducted to compare isomer-specific response patterns from animals given exposures to either 2,4- or 2,6-TDI, however, in that no true controls were conducted during this study, none of the suspected hypersensitivity responses observed here may be authenticated.

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14. Ashton - Tate Corporation, Torrance, California.

15. Lotus Development Corp., Cambridge, Massachusetts.

Table 1A

Chamber Concentrations of 2,4- or 2,6-TDI During Sensitization

Study Number 91-344-KR

Sensitization Day	Group 1 2,4-TDI (ppm)	Group 2 2,4-TDI (ppm)	Group 3 2,6-TDI (ppm)	Group 4 2,6-TDI (ppm)
0	1.51	1.80	1.79	1.57
1	1.35	1.02	1.73	1.43
2	1.23	1.08	1.25	1.13
3	1.20	1.20	1.19	1.28
4	1.27	1.33	1.04	1.33
Mean	1.31	1.29	1.40	1.35
SD	0.12	0.31	0.34	0.16

SD = Standard Deviation

Table 1B

Summary of Plethysmograph 2,4- or 2,6-TDI Concentrations During Challenge

Study Number 91-344-KR

Group	Challenge Atmosphere	Challenge Week	n=	Concentration (Mean + SD)
1	2,4-TDI	1	12	21 ± 11 ppb
	2,4-TDI	2	12	18 ± 10 ppb
	2,4-TDI-GSA	3	12	18 ± 4 mg/m³
2	2,6-TDI	1	12	32 ± 11 ppb
	2,6-TDI	2	12	19 ± 6 ppb
	2,6-TDI-GSA	3	12	20 ± 8 mg/m³
3	2,4-TDI	1	11	28 ± 17 ppb
	2,4-TDI	2	11	25 ± 15 ppb
	2,4-TDI-GSA	3	11	20 ± 5 mg/m³
4	2,6-TDI	1	12	46 ± 18 ppb
	2,6-TDI	2	12	32 ± 12 ppb
	2,6-TDI-GSA	3	12	25 ± 6 mg/m³

SD = Standard Deviation

Table 2

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

Study Number 91-344-KR

Group	Initial Body Weight Range		Analysis of Body Weights (g) Day of Study[1] 0	4	7	14	Mean Weight Change During Challenge (g)[2] Week 1	Week 2	Week 3
1	261-280	Mean	269	283	311	363	-27	-28	-38
		SD	6	8	10	12			
		MC		14	28	52			
2	294-345	Mean	311	315	331	382	-28	-28	-36
		SD	18	17	27	28			
		MC		4	17	51			
3	332-361	Mean	350	348	381	437	-37	-39	-44
		SD	9	11	14	22			
		MC		-2	33	56			
4	262-289	Mean	279	288	326	372	-30	-36	-34
		SD	8	13	16	20			
		MC		9	37	46			

SD = Standard Deviation.
MC = Mean Change.
[1] = Day 0 denotes first day of exposure.
[2] = Averages are presented for all animals challenged in a given week. Values indicate the body weight change that occurs during the time that animals are housed in plethysmographs. For individual data see Appendix I.

Table 3

Incidence of Suspected Hypersensitivity Responses Observed During Challenge

Study Number 91-344-KR

	Incidence of Suspected Response*					
	Wk. 1	Wk. 2	Wk. 3	Wk. 1	Wk. 2	Wk. 3
	Immediate			Delayed		
Group 1 (2,4-TDI/2,4-TDI)**	1/11	1/12	4/12	5/10	3/11	4/12
Group 2 (2,4-TDI/2,6-TDI)	2/12	3/11	4/11	4/12	3/12	4/12
Group 3 (2,6-TDI/2,4-TDI)	1/11	5/10	5/10	0/9	5/11	7/11
Group 4 (2,6-TDI/2,6-TDI)	1/12	3/12	5/11	7/12	3/10	6/12

* Responses during each week of challenge: number of animals showing response/group size.

** Isomer used for sensitization/challenge exposures.

Table 4

Incidence of Repeated Hypersensitivity Responses Observed During The Challenge Phase

Study Number 91-344-KR

	Incidence*	
	Immediate	Delayed
Group 1 (2,4-TDI/2,4-TDI)**	1 [8%]	3 [25%]
Group 2 (2,4-TDI/2,6-TDI)	2 [18%]	4 [33%]
Group 3 (2,6-TDI/2,4-TDI	3 [30%]	5 [45%]
Group 4 (2,6-TDI/2,6-TDI)	2 [18%]	6 [50%]

* Number of animals showing the same type of response during two out of the three challenge episodes.

** Isomer used for sensitization/challenge exposures.

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 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.

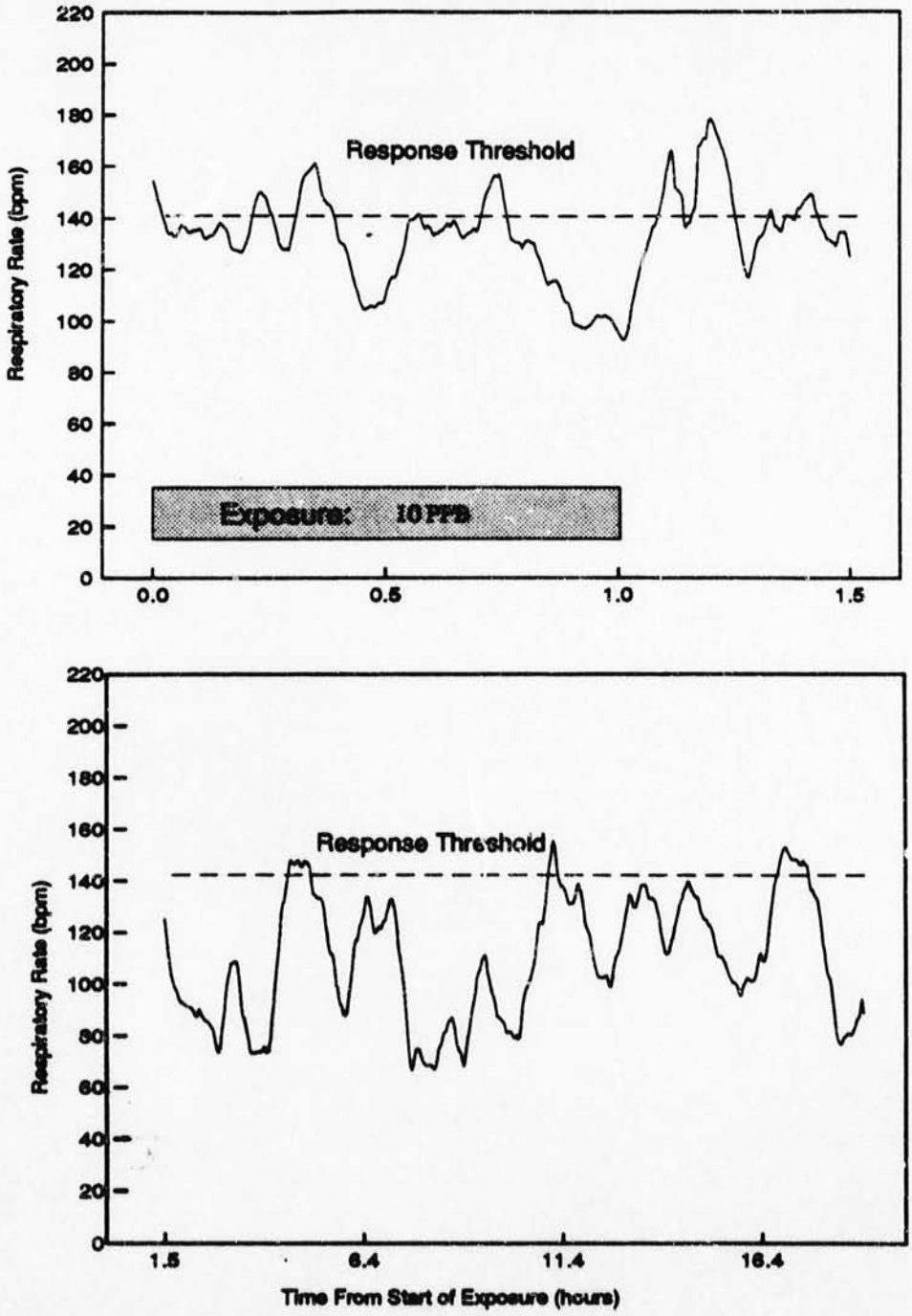
Each Figure is separately numbered from 1 to 141. They are presented in the order of groups 1 - 4, and, for each group data for the first week of challenges are presented in sequence and these are followed by data from the second and then finally the third week of challenge. The following table identifies figure numbers that are associated with each group.

Group	Figure No.
1	1 - 36
2	37 - 69
3	70 - 105
4	106 - 141

Study Number 91-344-KR

Animal # 28 : 2,4-TDI Sensitization

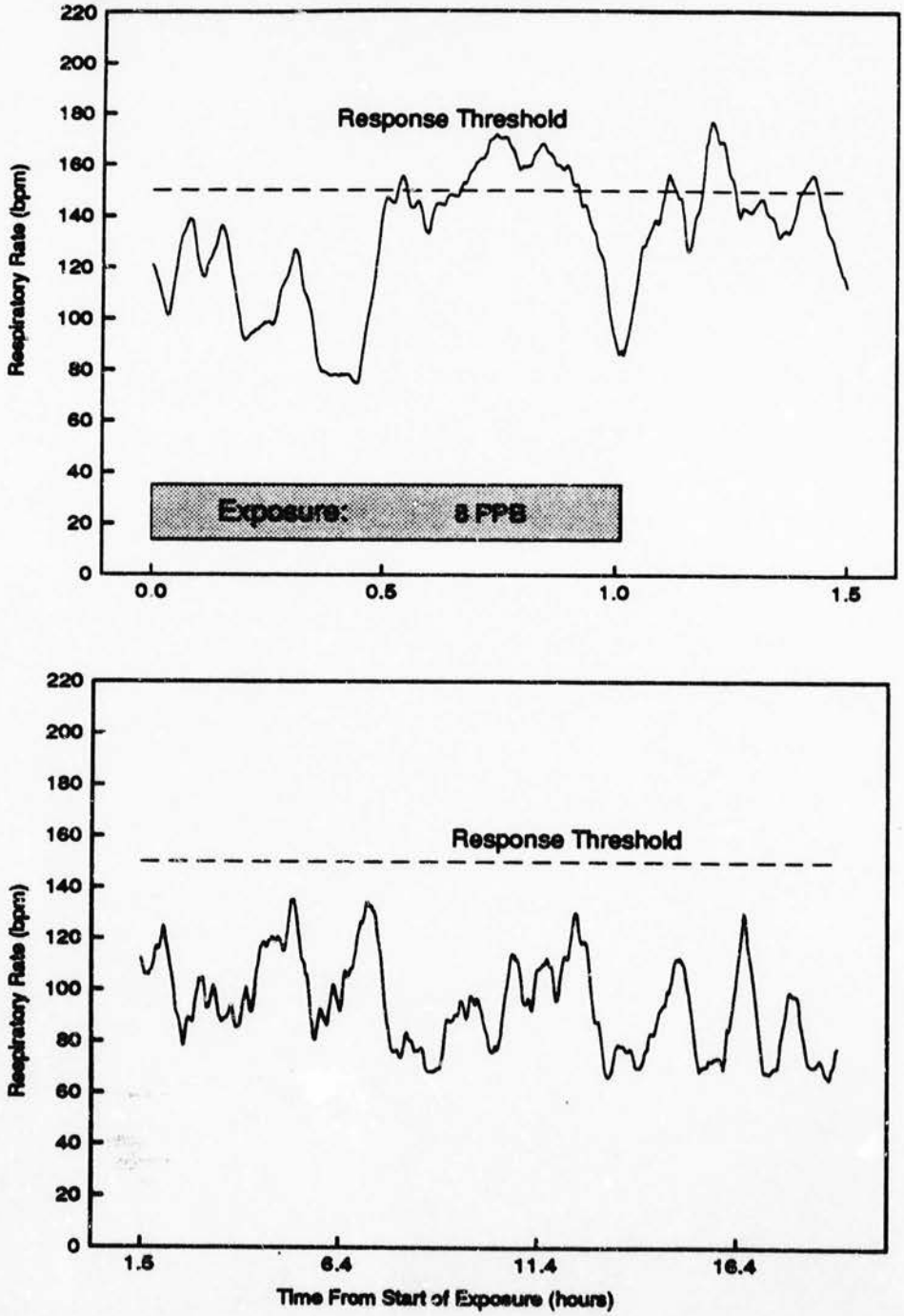
Figure: 1 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 29 : 2,4-TDI Sensitization

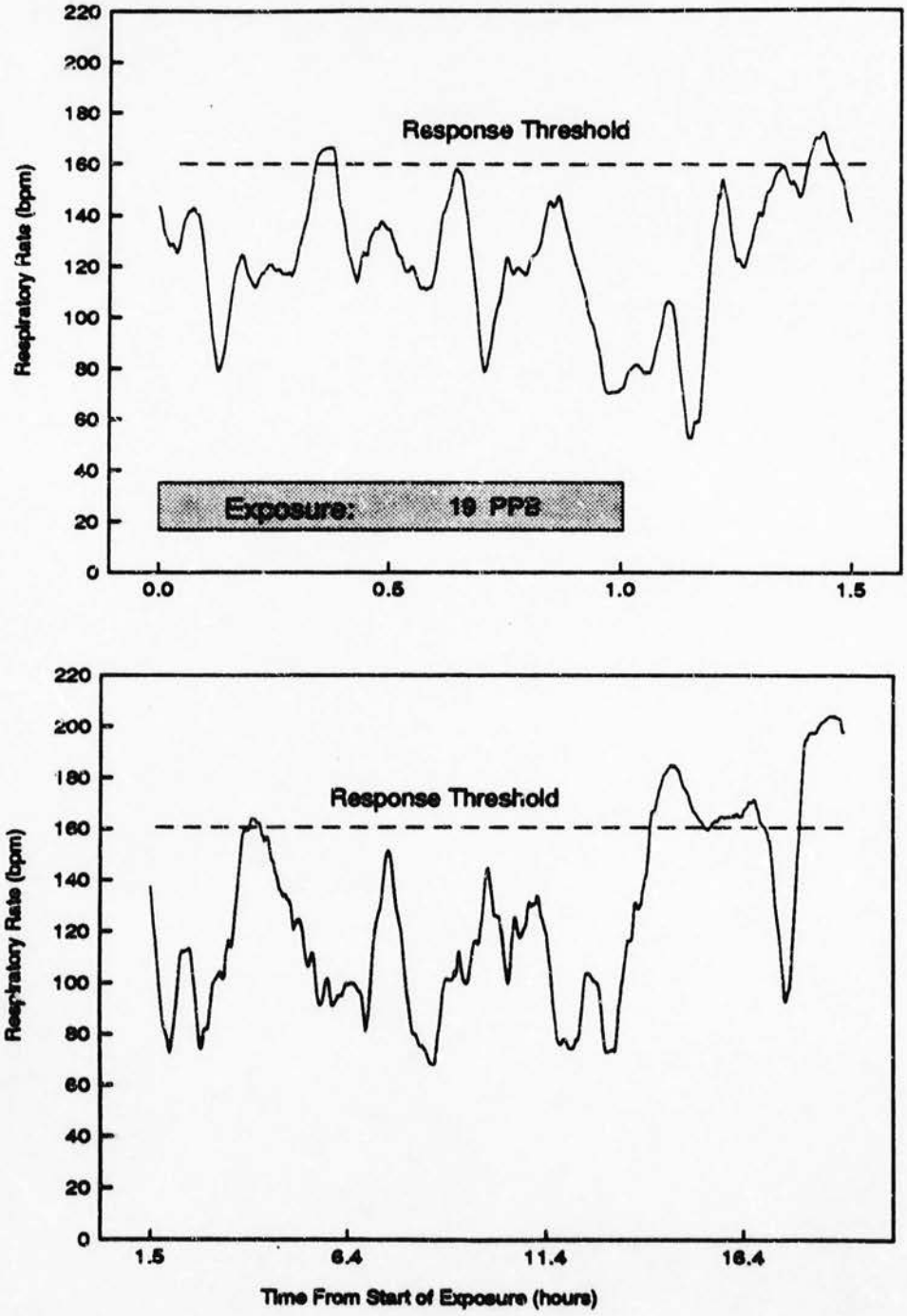
Figure: 2 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 30 : 2,4-TDI Sensitization

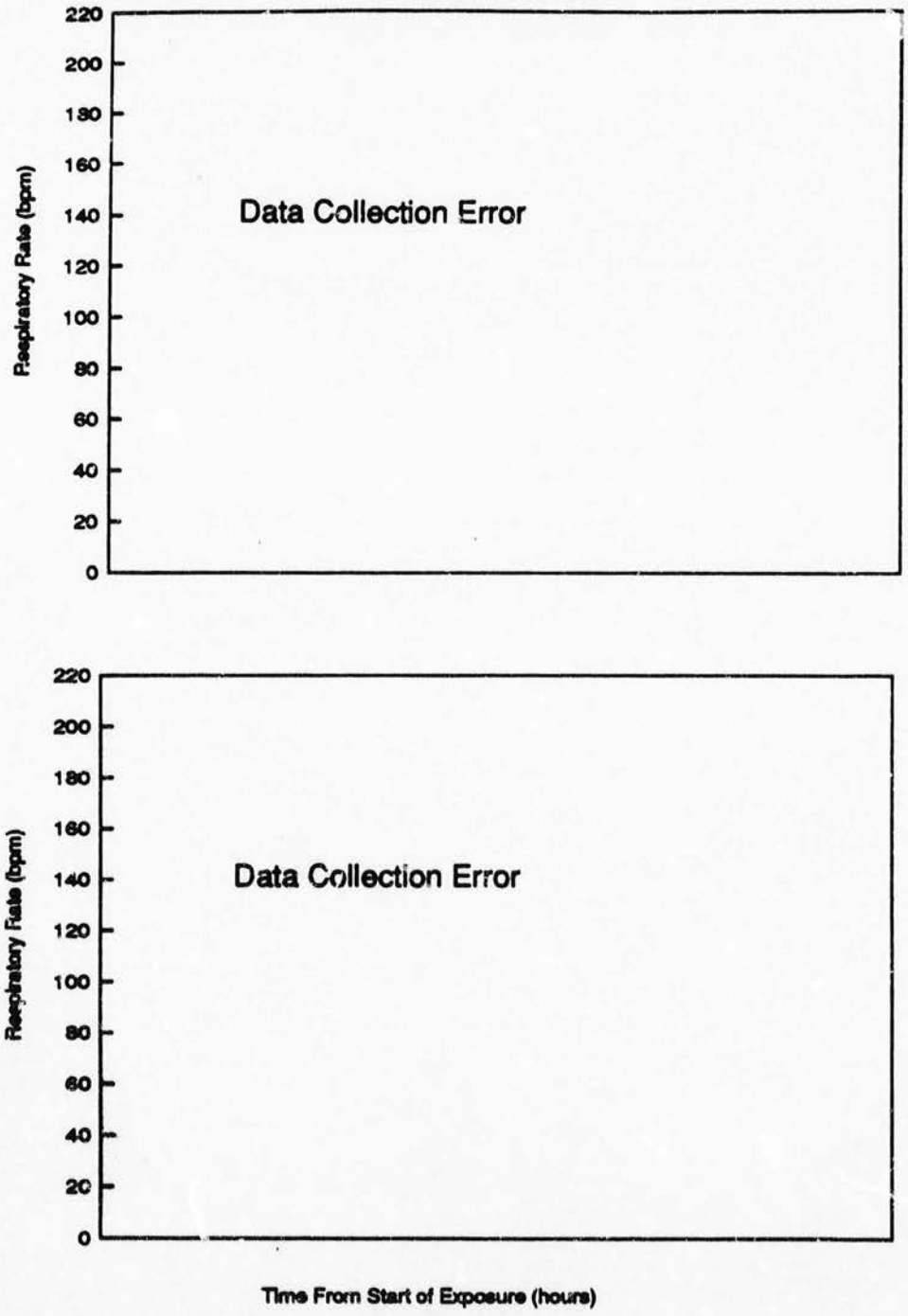
Figure: 3 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 31 : 2,4-TDI Sensitization

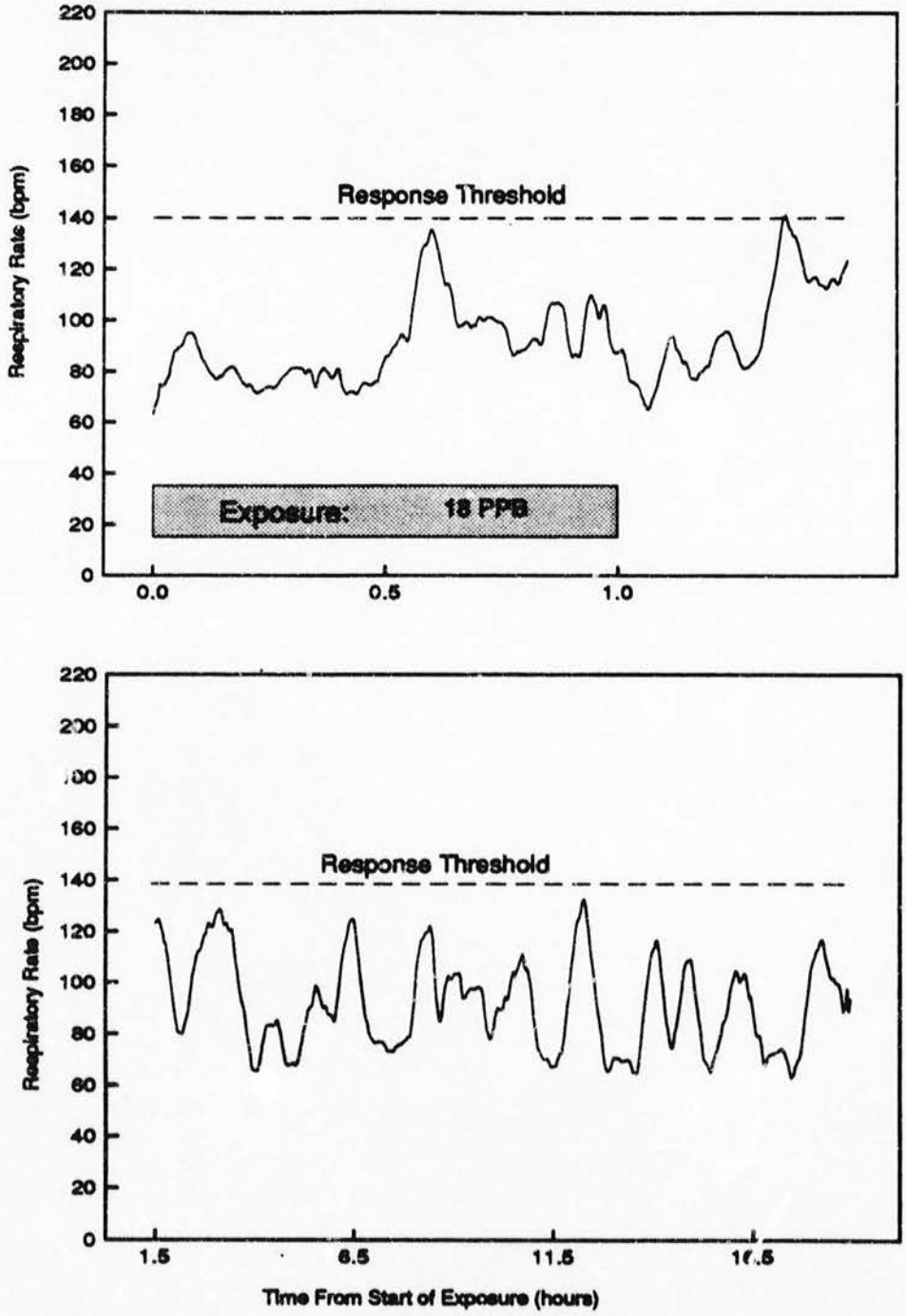
Figure: 4 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 32 : 2,4-TDI Sensitization

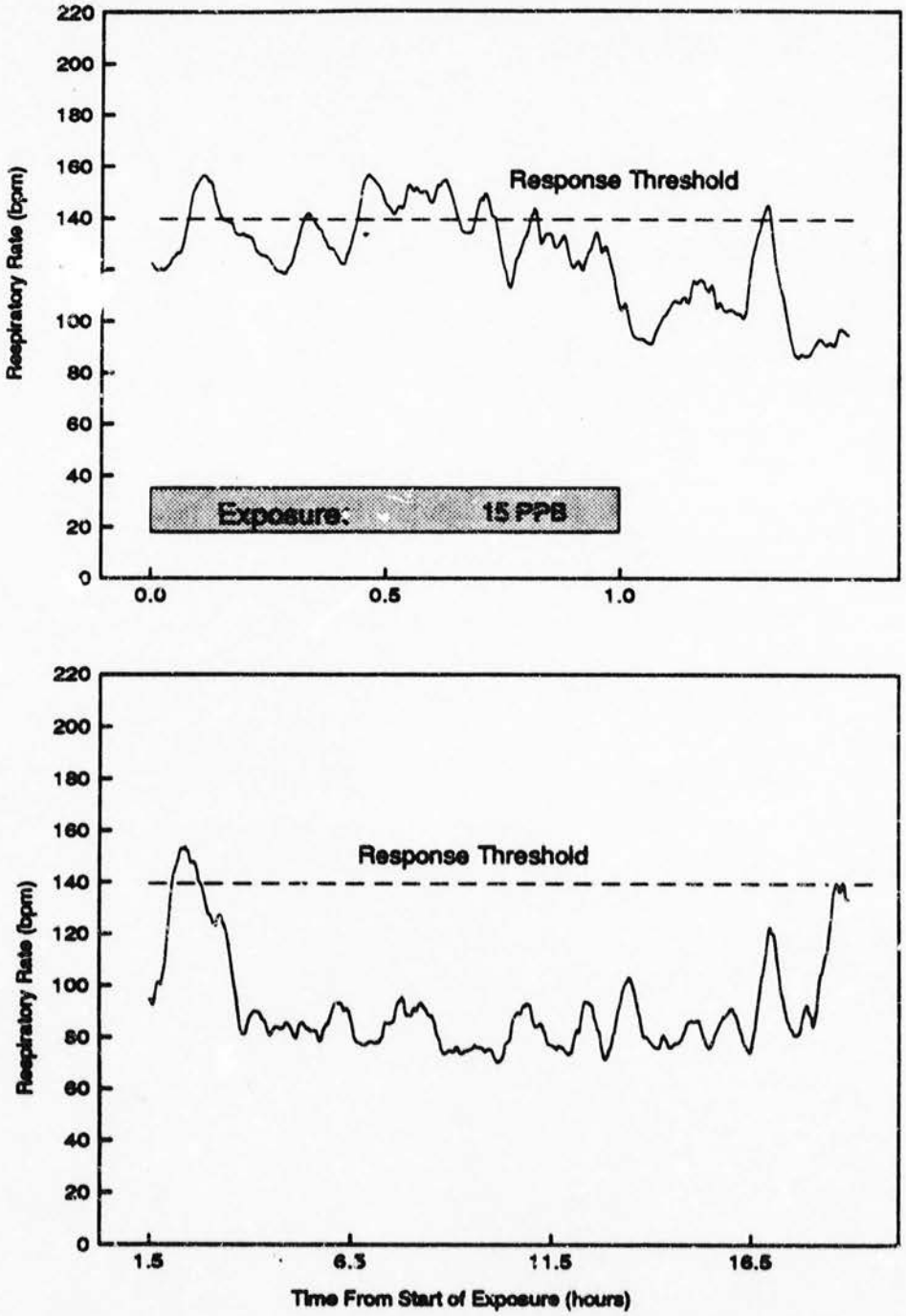
Figure: 5 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 33 : 2,4-TDI Sensitization

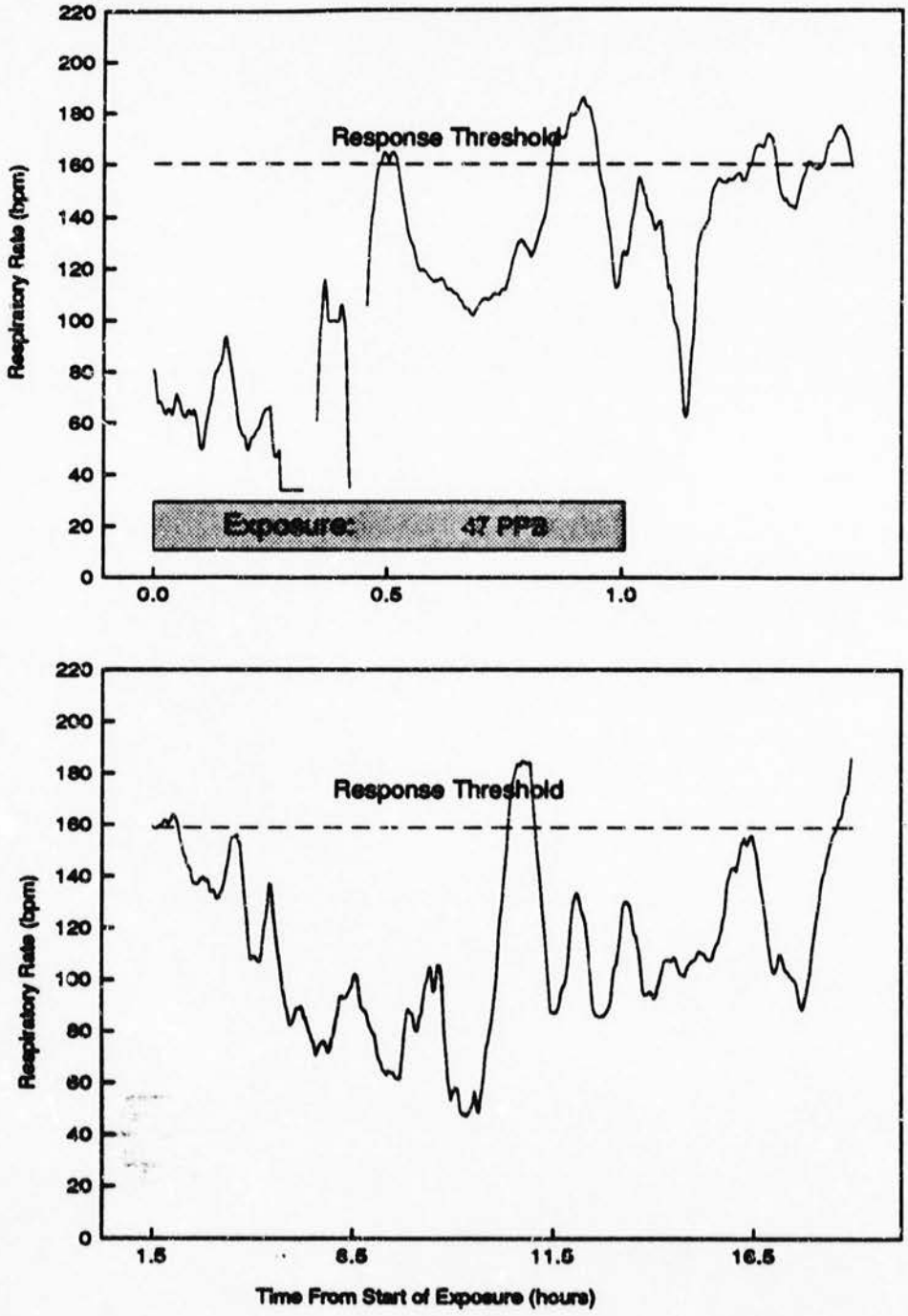
Figure: 6 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 34 : 2,4-TDI Sensitization

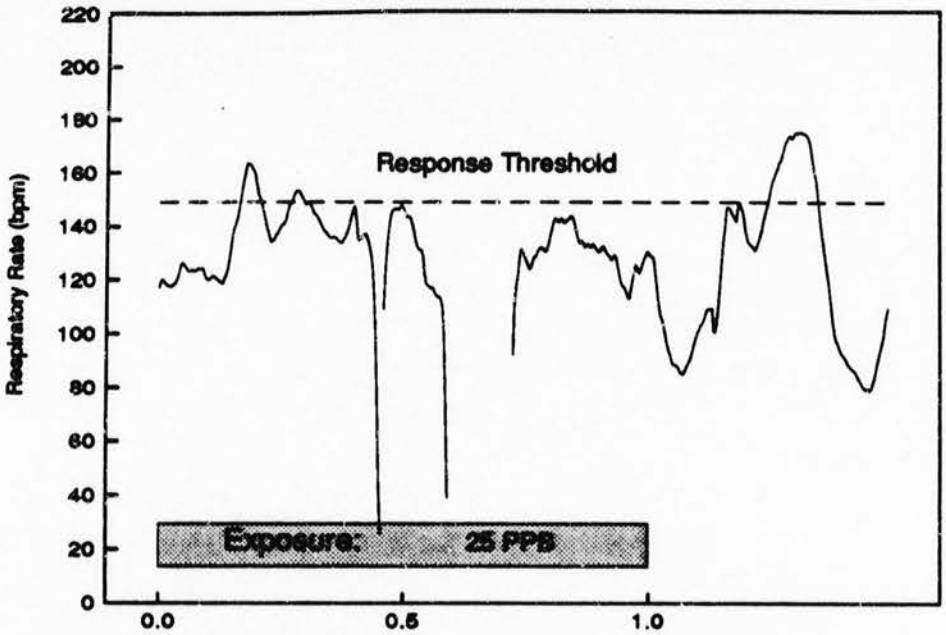
Figure: 7 2,4-TDI Challenge Week 1

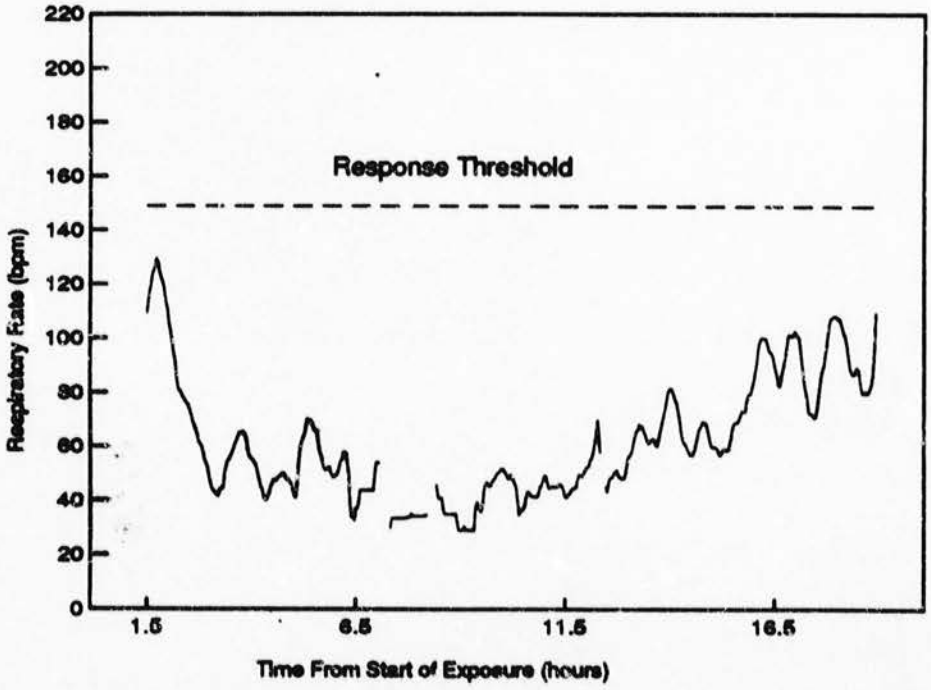


Study Number 91-344-KR

Animal # 35 : 2,4-TDI Sensitization

Figure: 8 2,4-TDI Challenge Week 1

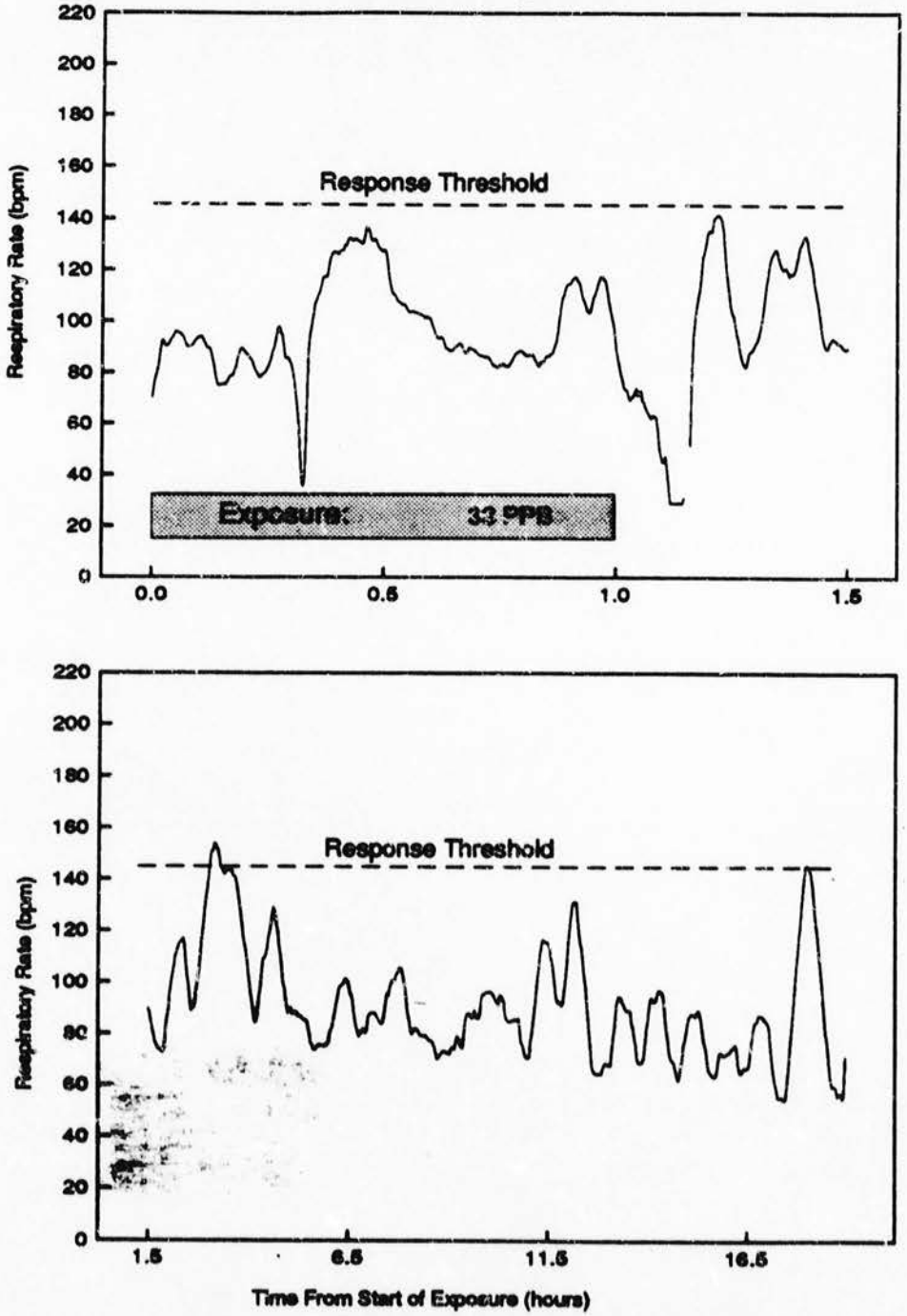




Study Number 91-344-KR

Animal # 36 : 2,4-TDI Sensitization

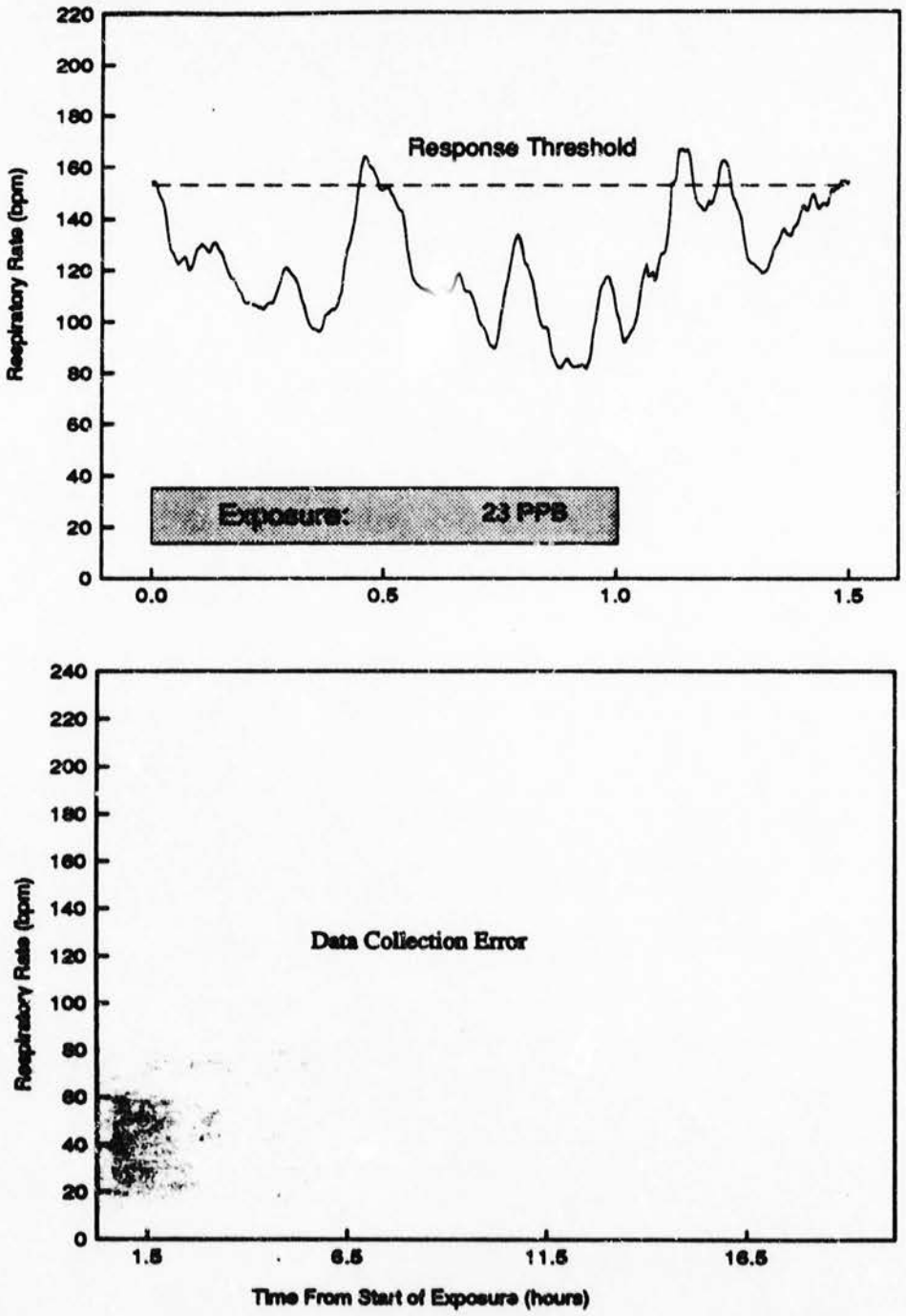
Figure: 9 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 38 : 2,4-TDI Sensitization

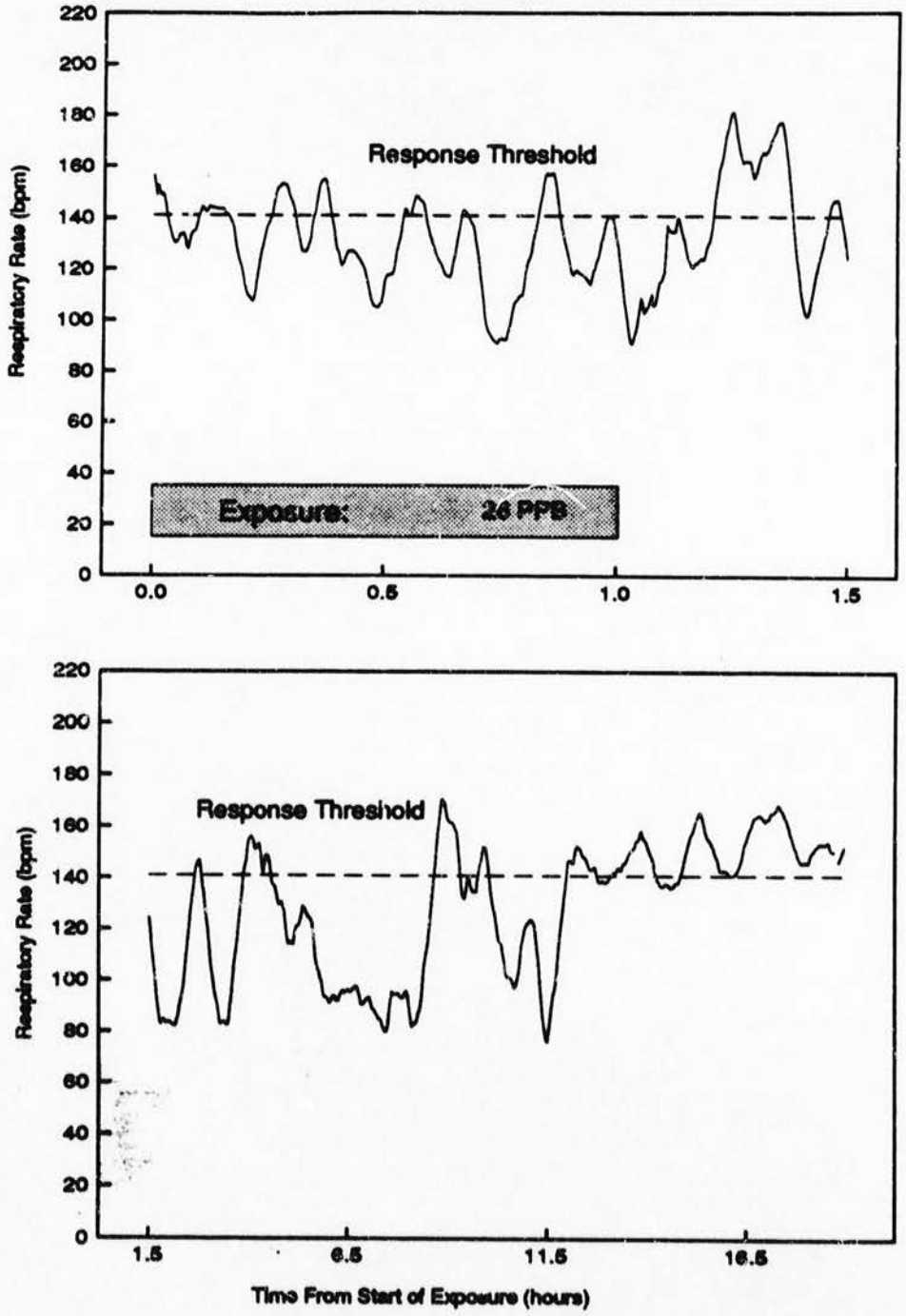
Figure: 10 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 39 : 2,4-TDI Sensitization

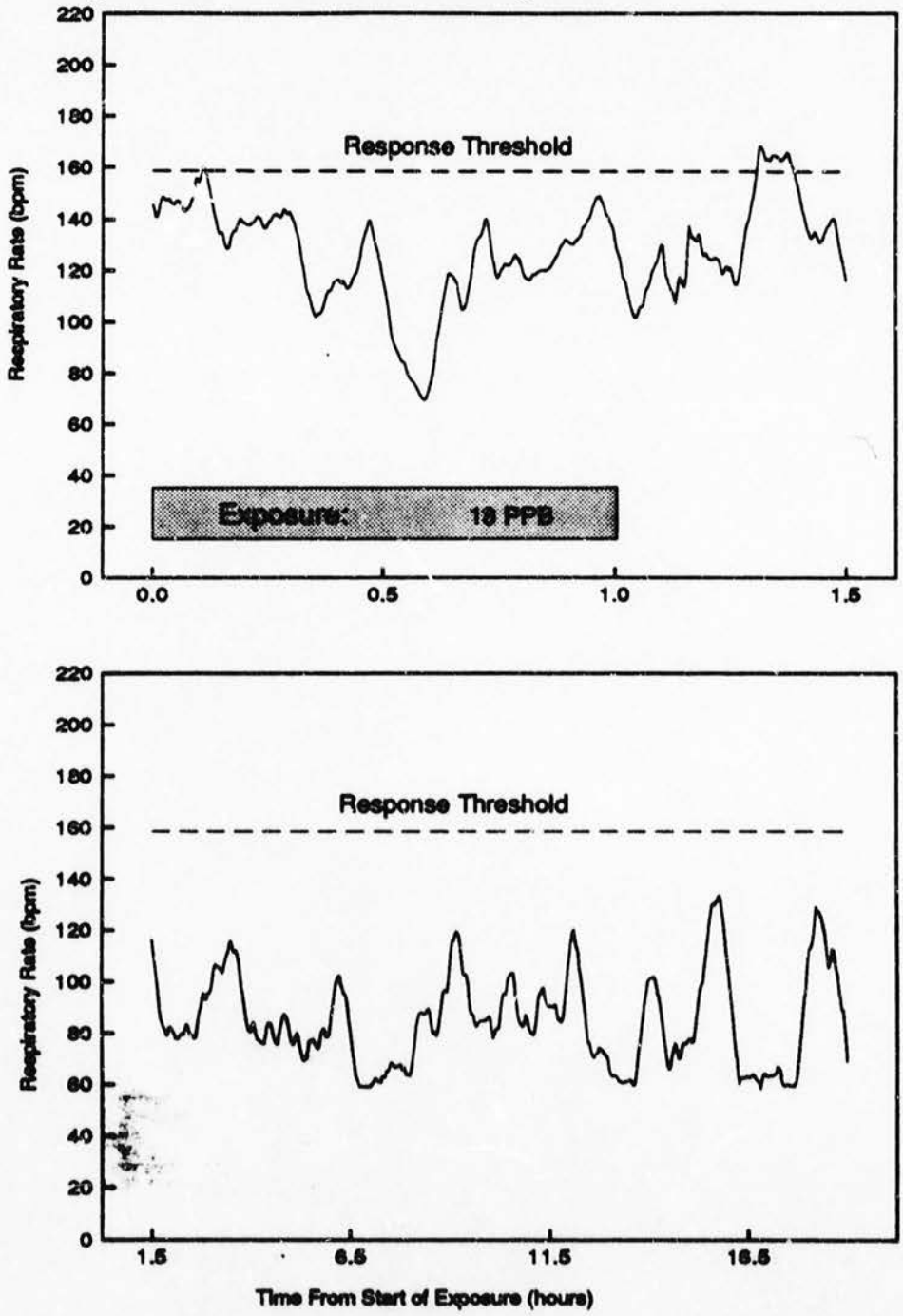
Figure: 11 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 40 : 2,4-TDI Sensitization

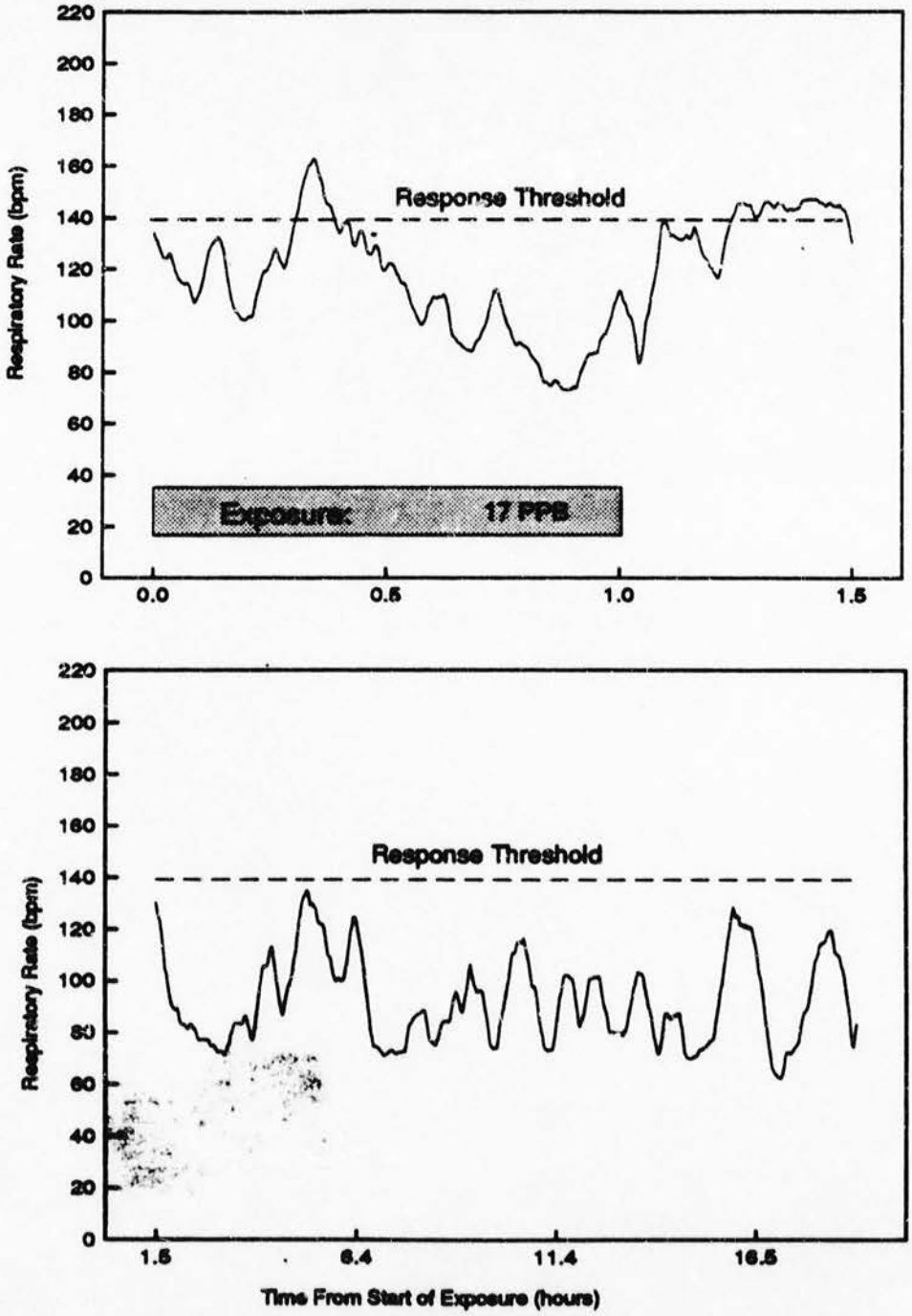
Figure: 12 2,4-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 28 : 2,4-TDI Sensitization

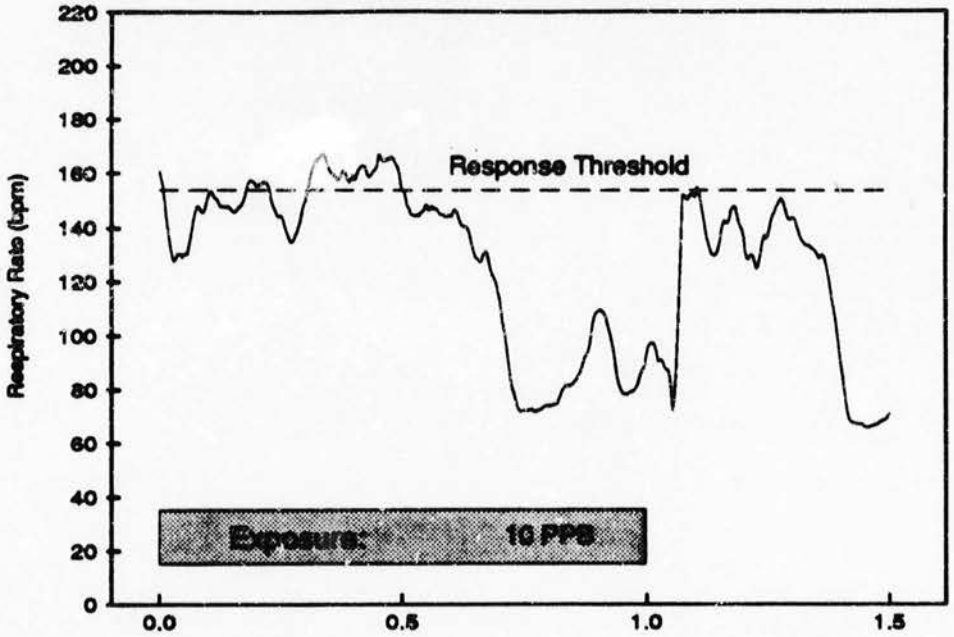
Figure: 13 2,4-TDI Challenge Week 2

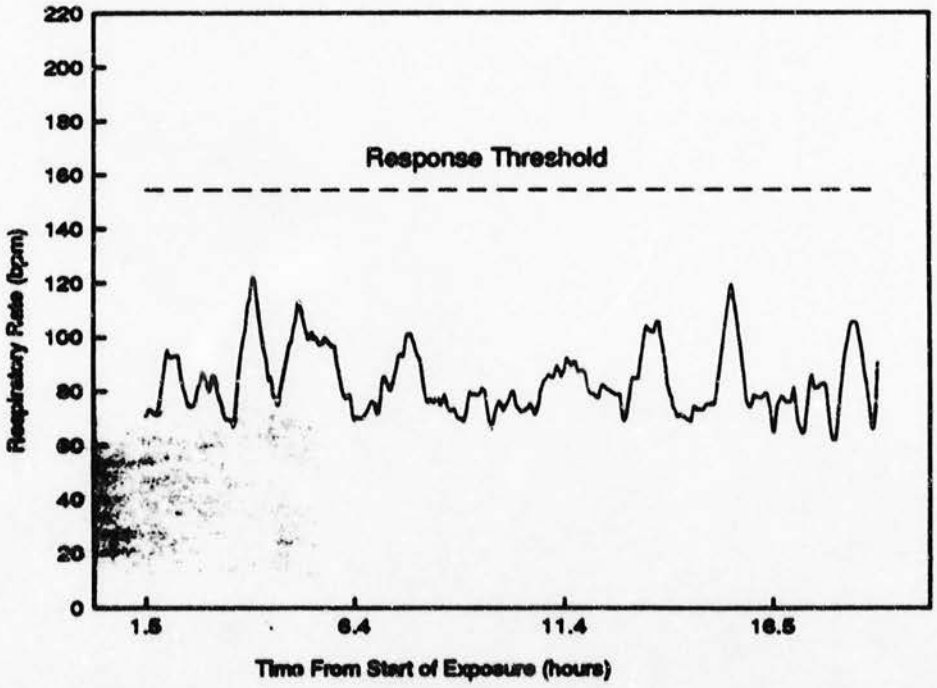


Study Number 91-344-KR

Animal # 29 : 2,4-TDI Sensitization

Figure: 14 2,4-TDI Challenge Week 2

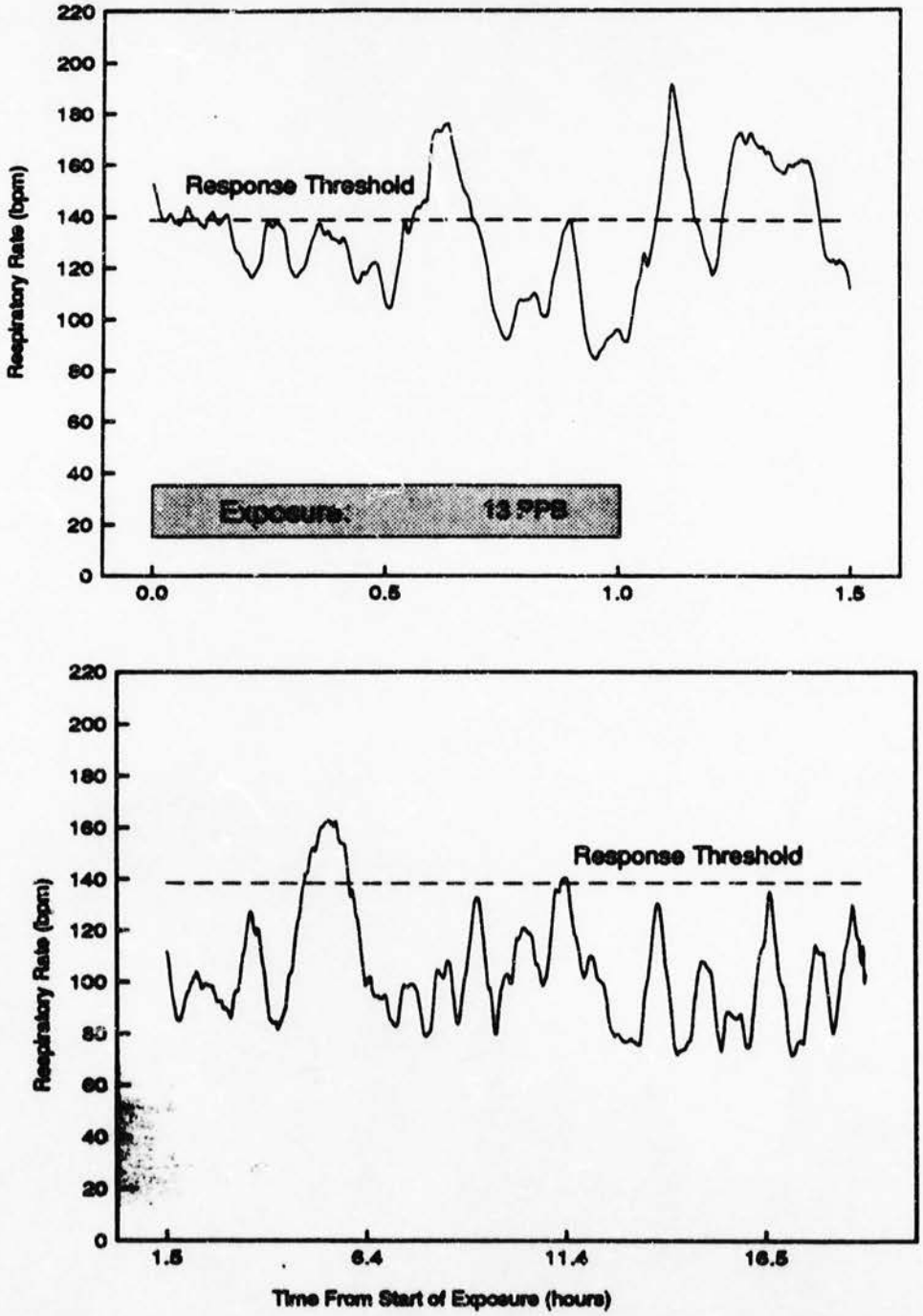




Study Number 91-344-KR

Animal # 30 : 2,4-TDI Sensitization

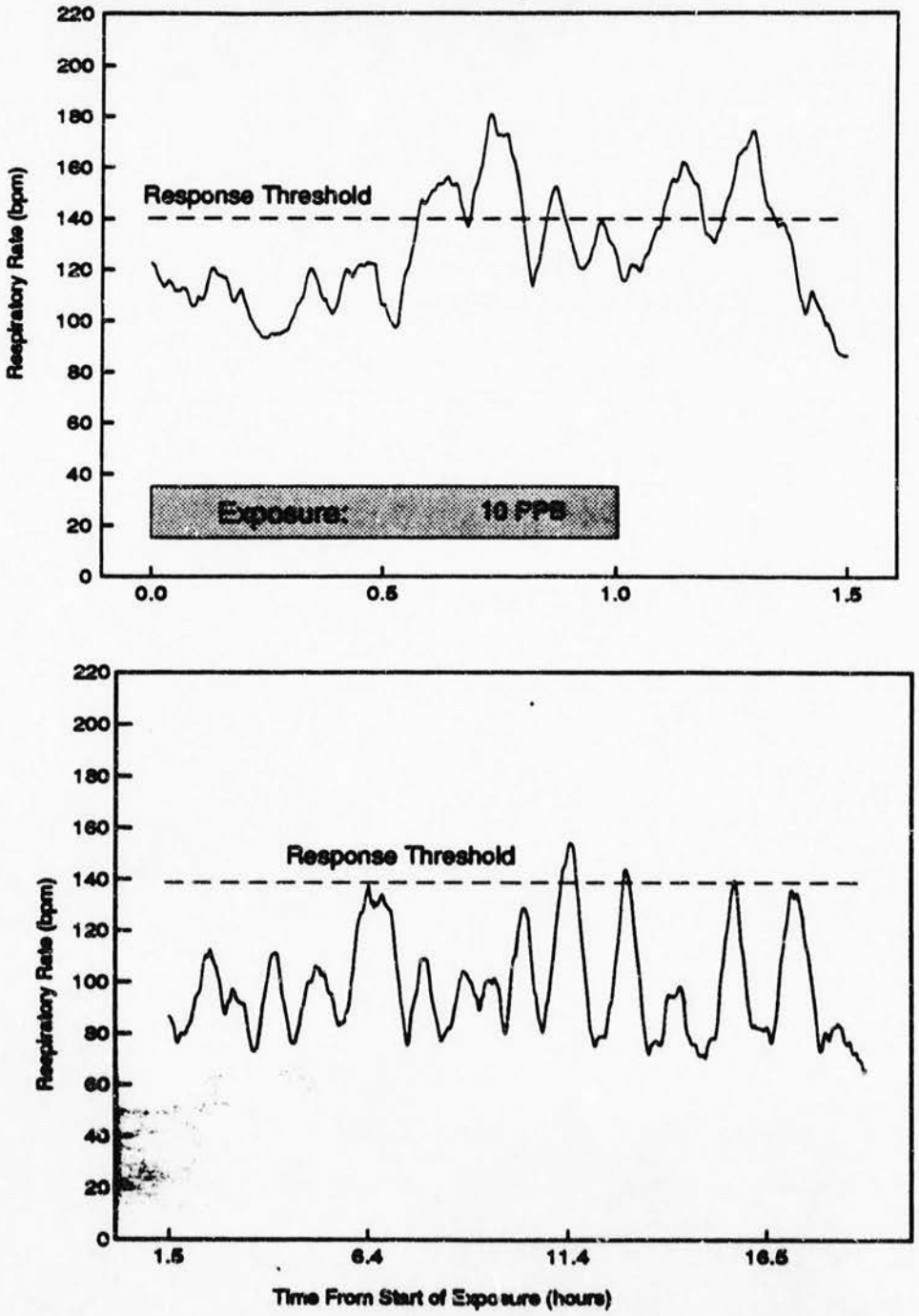
Figure: 15 2,4-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 31 : 2,4-TDI Sensitization

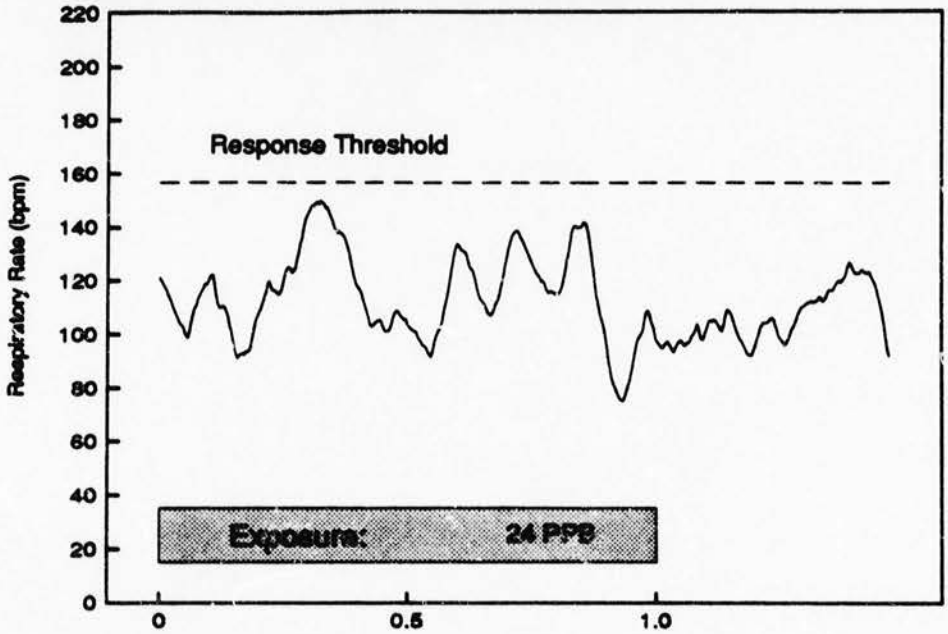
Figure: 16 2,4-TDI Challenge Week 2

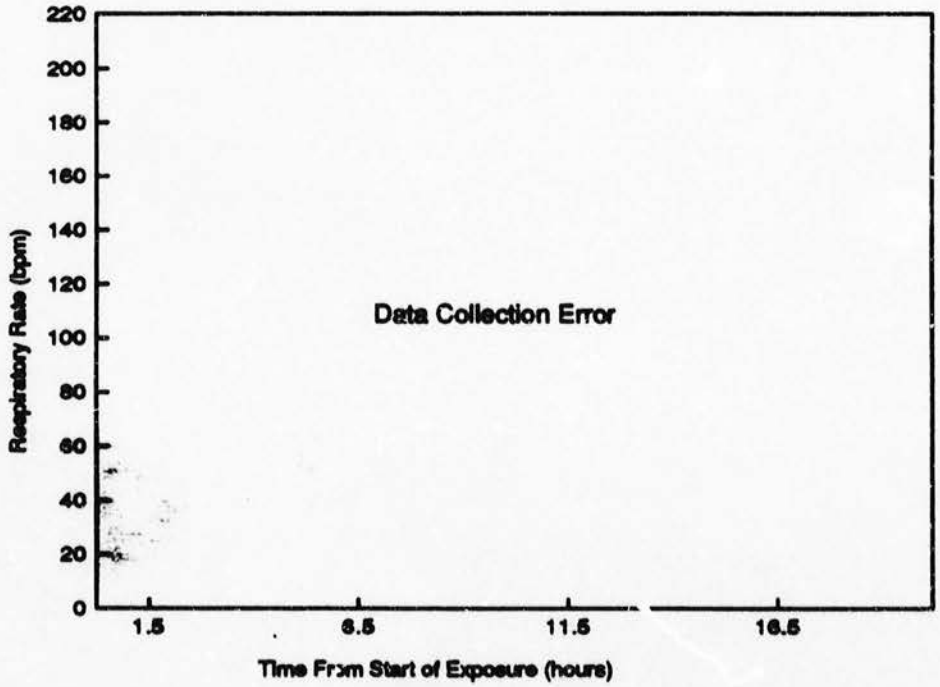


Study Number 91-344-KR

Animal # 32 : 2,4-TDI Sensitization

Figure: 17 2,4-TDI Challenge Week 2

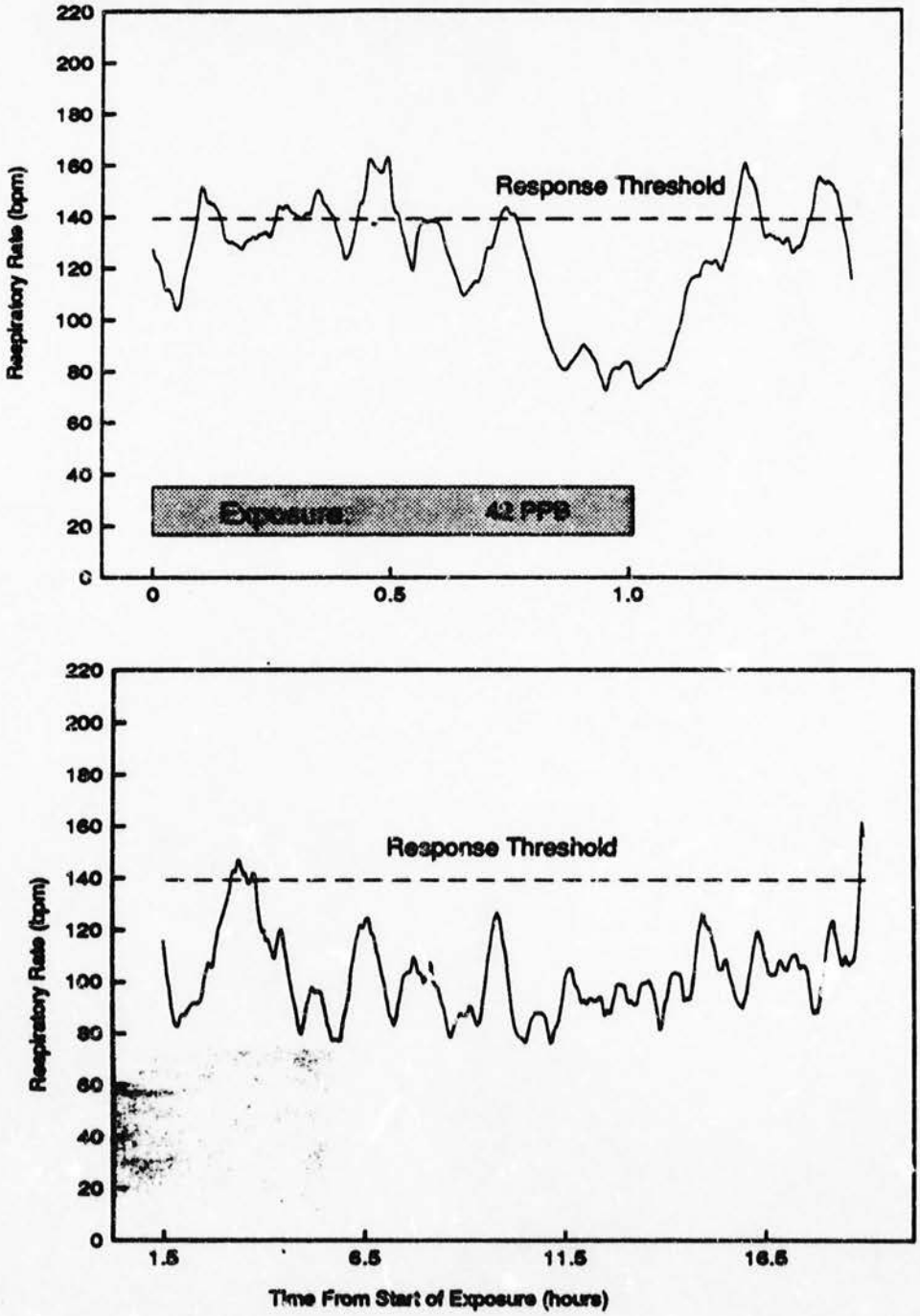




Study Number 91-344-KR

Animal # 33 : 2,4-TDI Sensitization

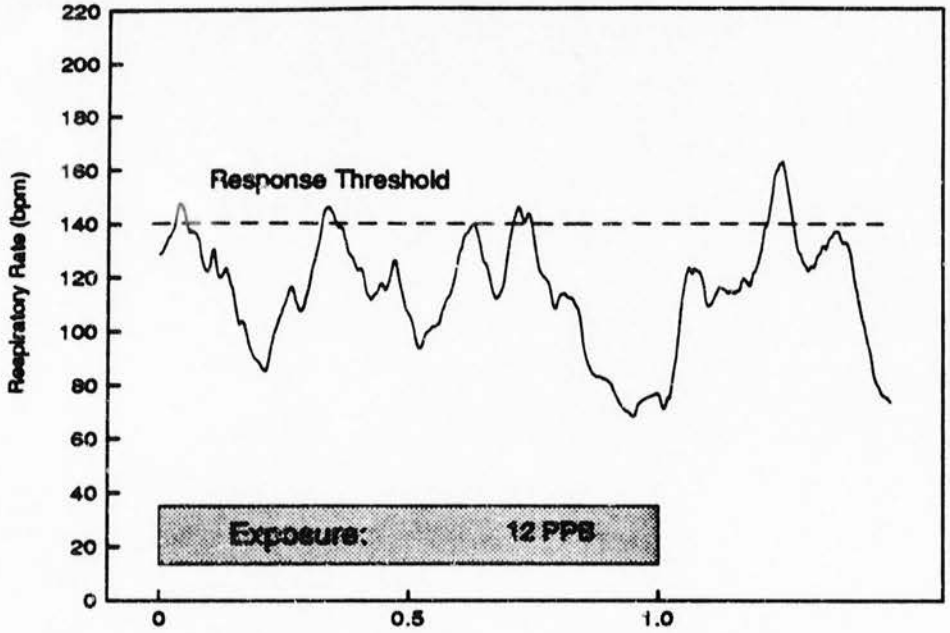
Figure: 18 2,4-TDI Challenge Week 2

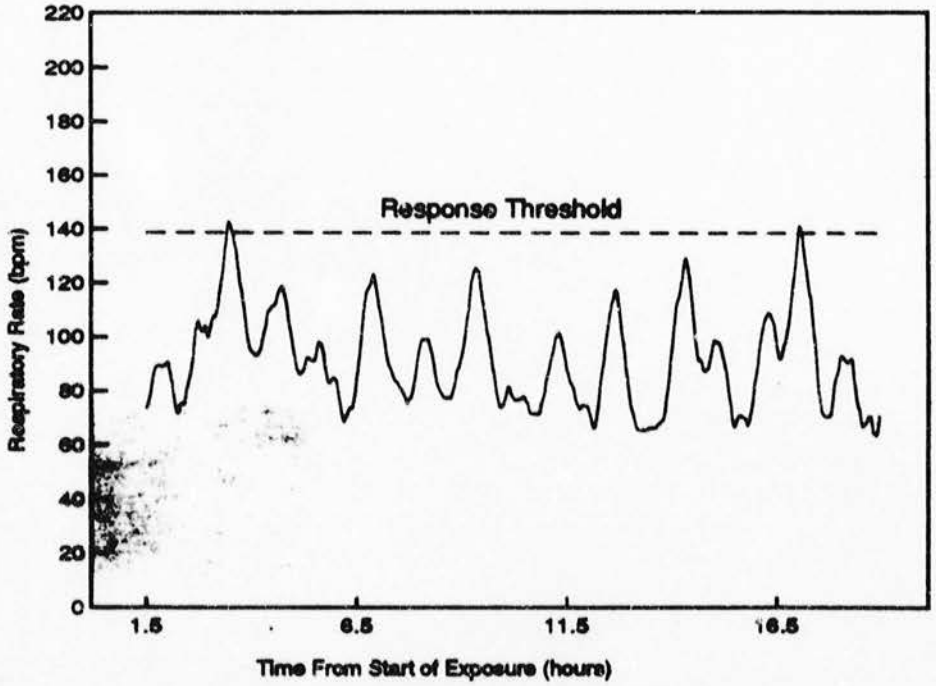


Study Number 91-344-KR

Animal # 34 : 2,4-TDI Sensitization

Figure: 19 2,4-TDI Challenge Week 2

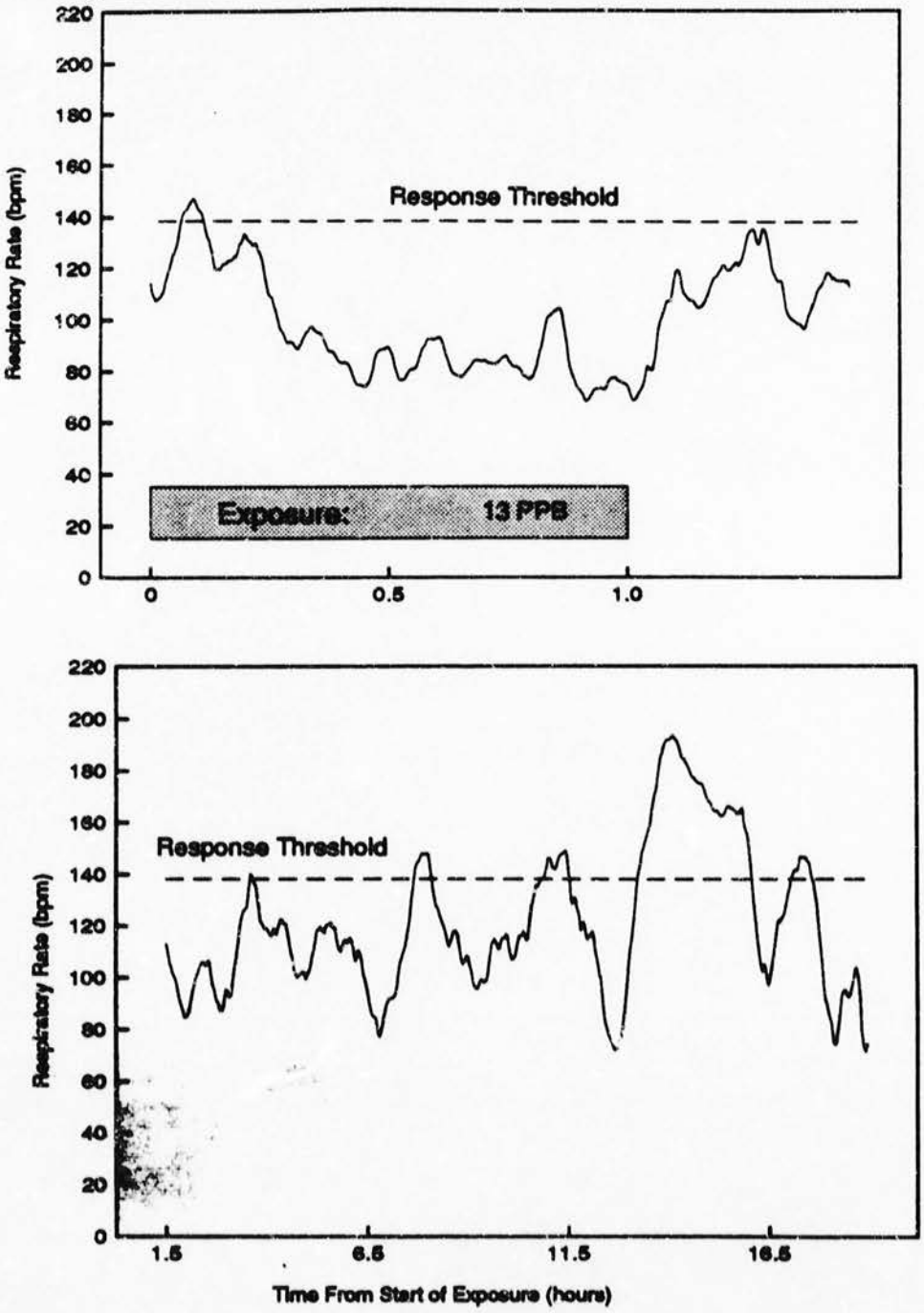




Study Number 91-344-KR

Animal # 35 : 2,4-TDI Sensitization

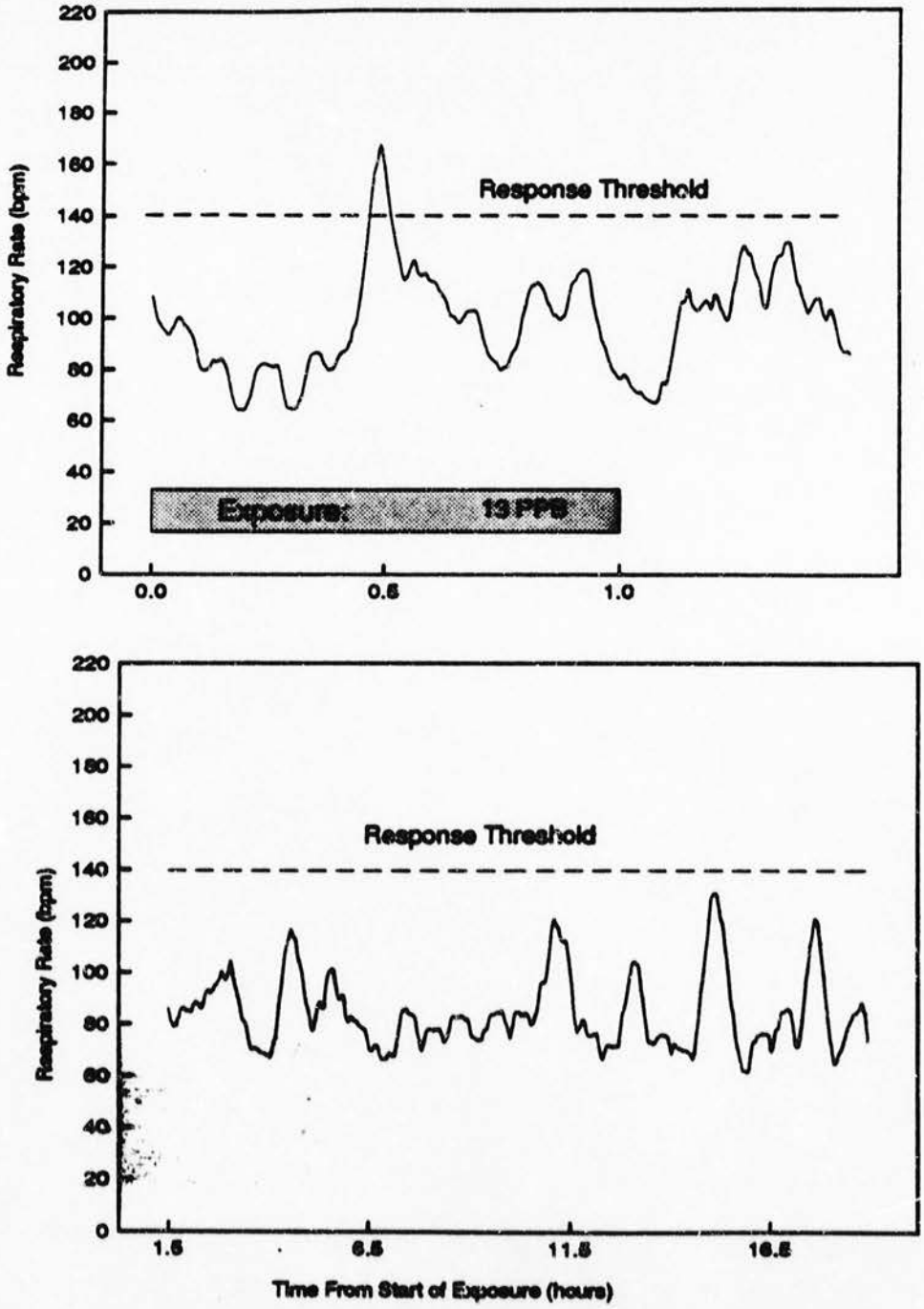
Figure: 20 2,4-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 36 : 2,4-TDI Sensitization

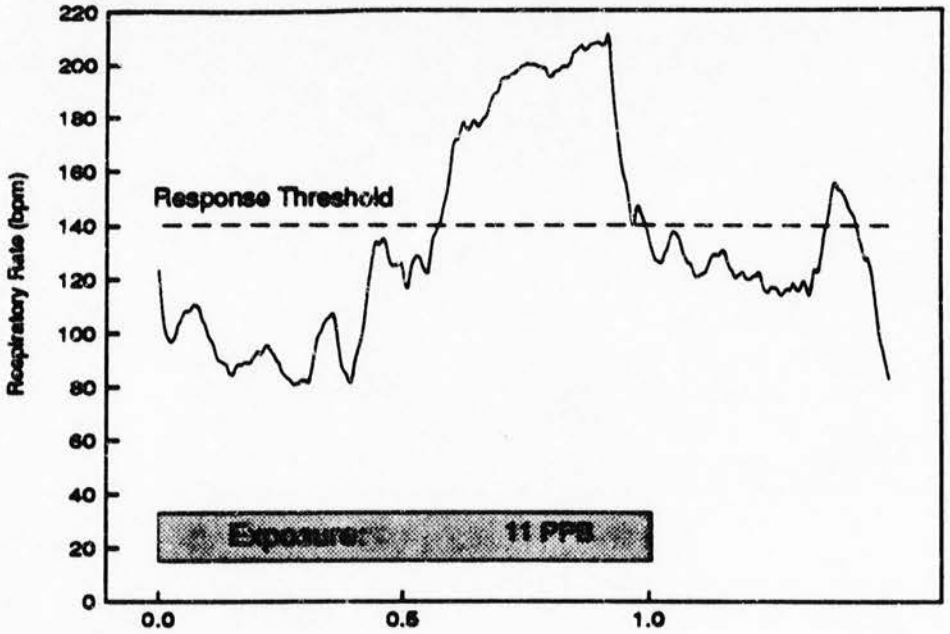
Figure: 21 2,4-TDI Challenge Week 2

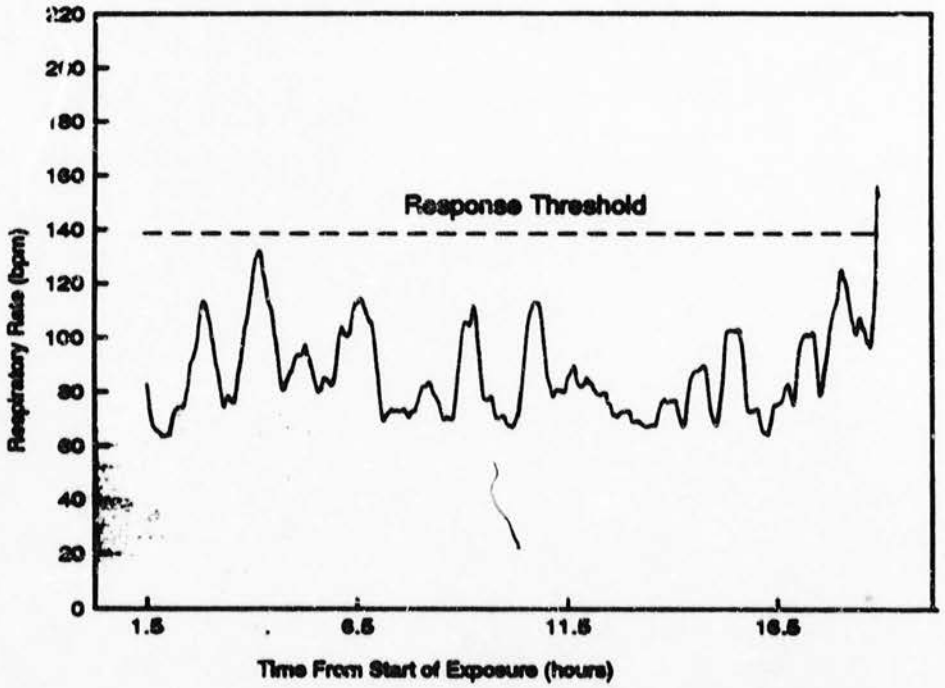


Study Number 91-344-KR

Animal # 38 : 2,4-TDI Sensitization

Figure: 22 2,4-TDI Challenge Week 2

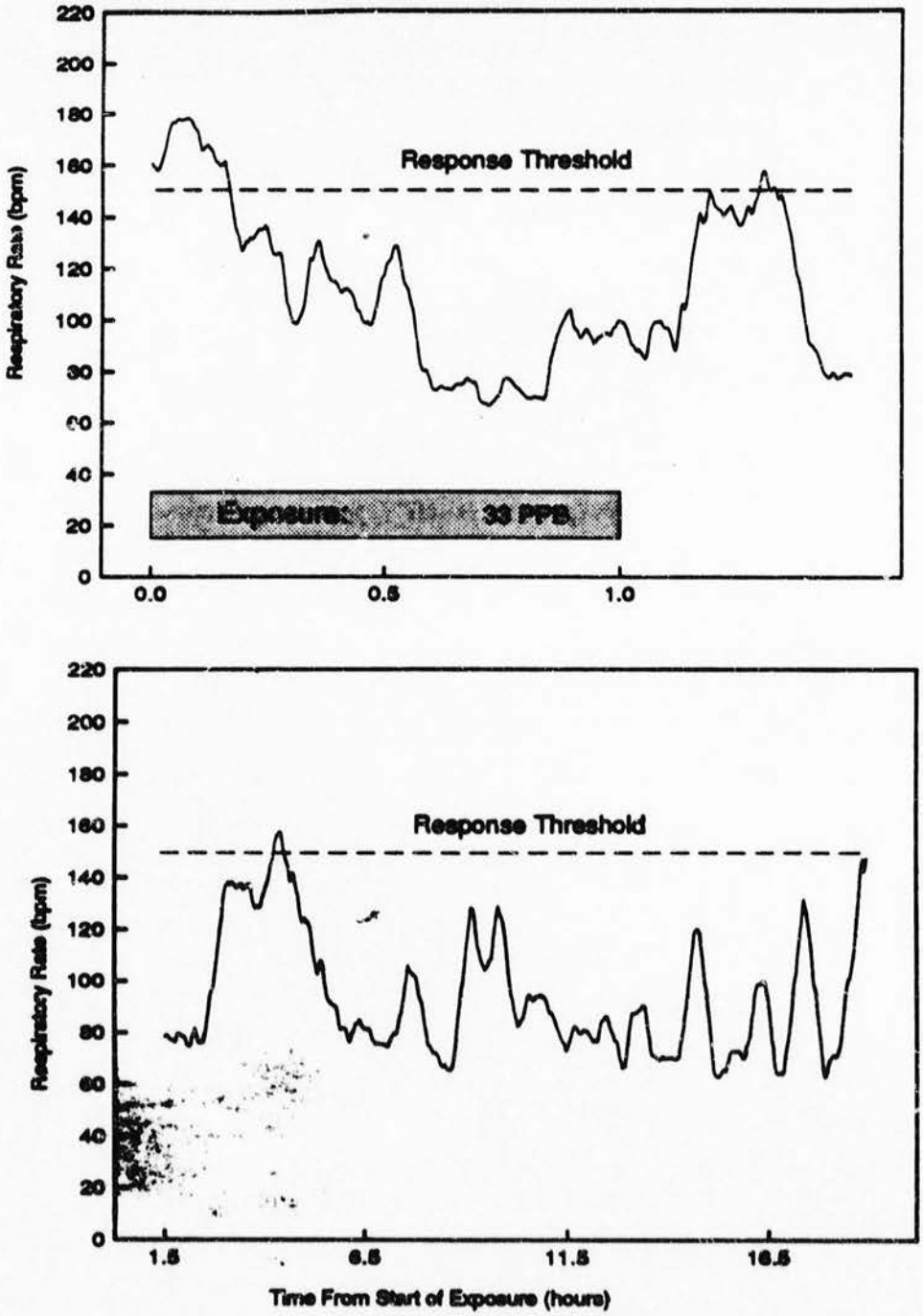




Study Number 91-344-KR

Animal # 39 : 2,4-TDI Sensitization

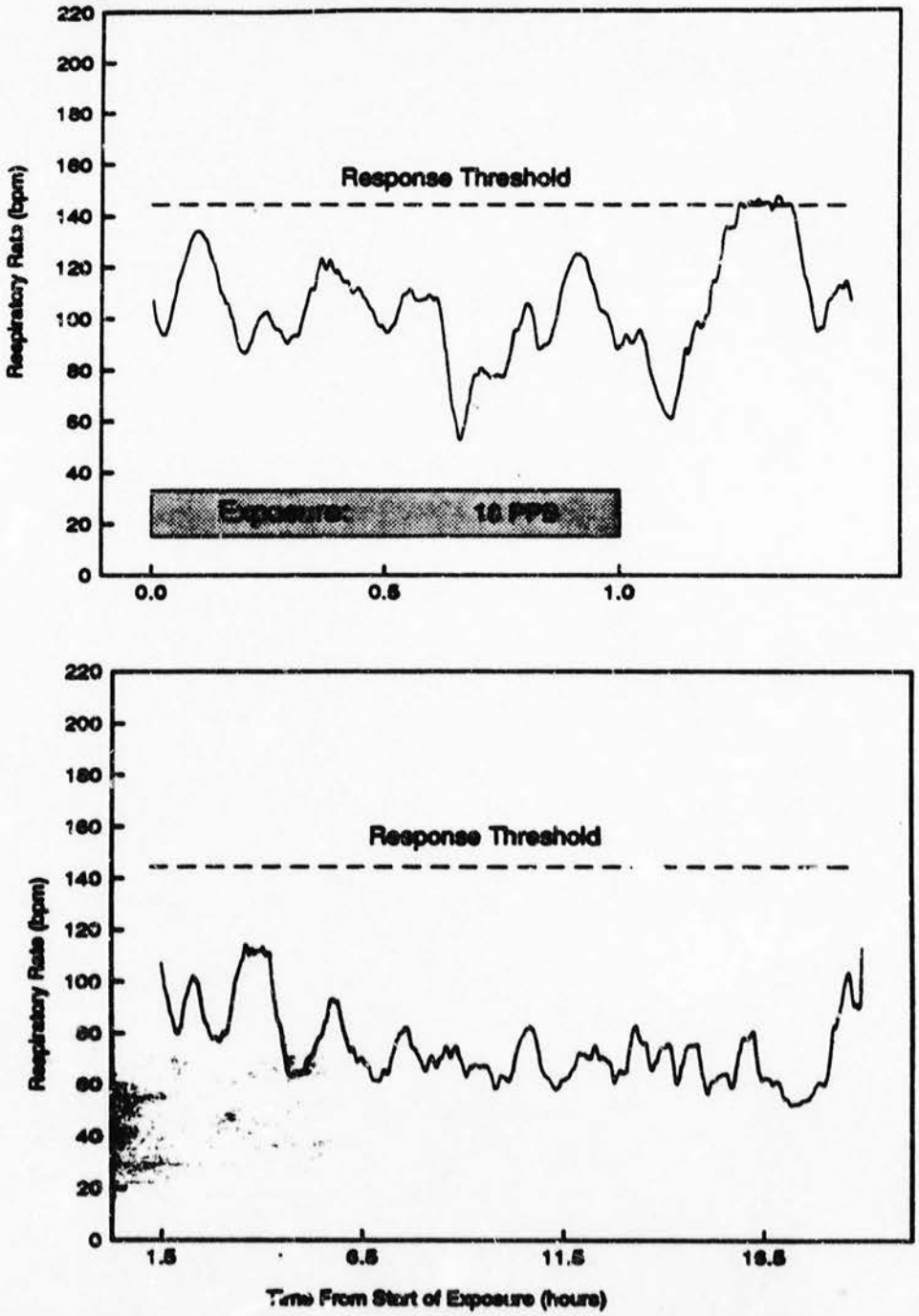
Figure: 23 2,4-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 40 : 2,4-TDI Sensitization

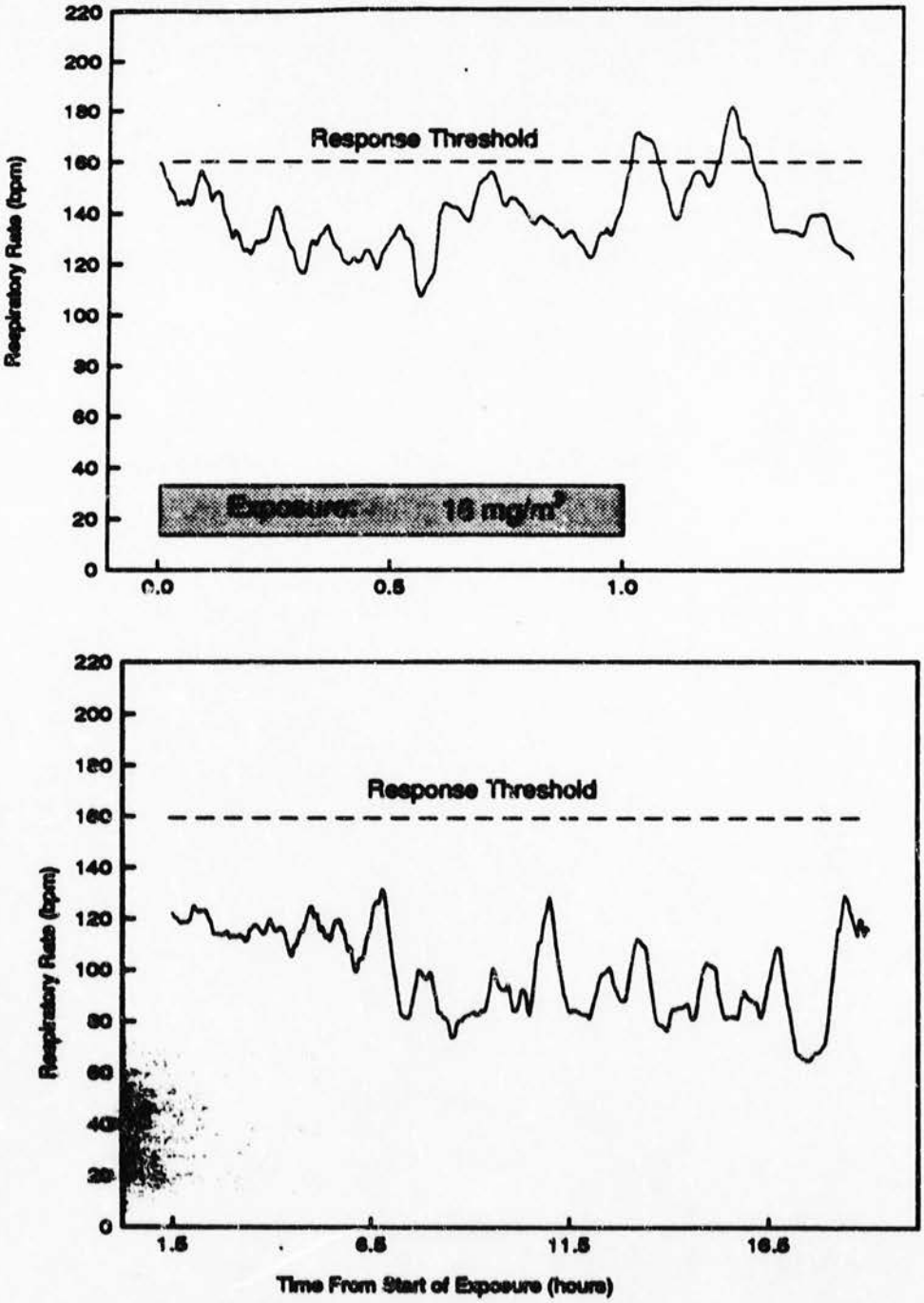
Figure: 24 2,4-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 28 : 2,4-TDI Sensitization

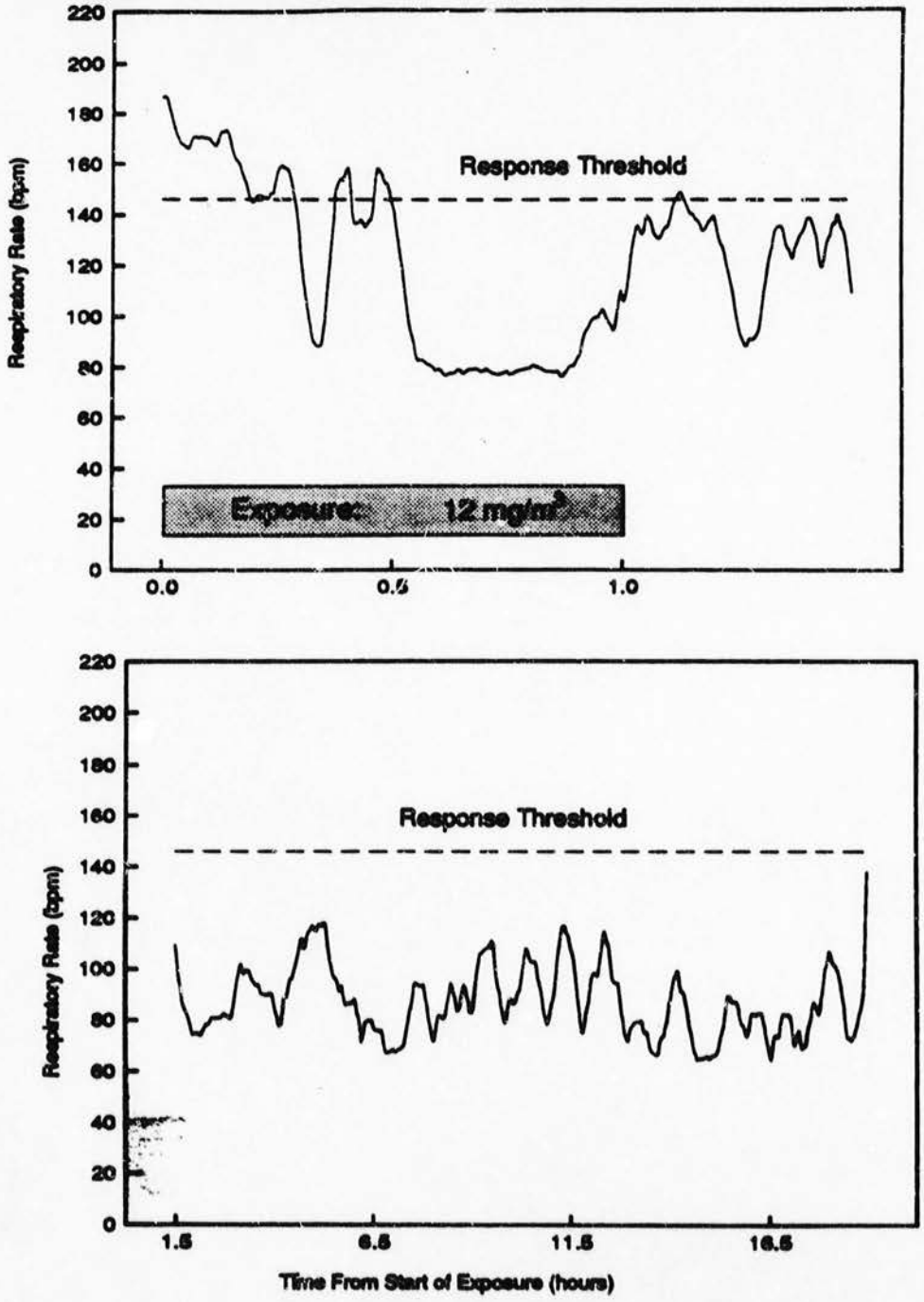
Figure: 25 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 29 : 2,4-TDI Sensitization

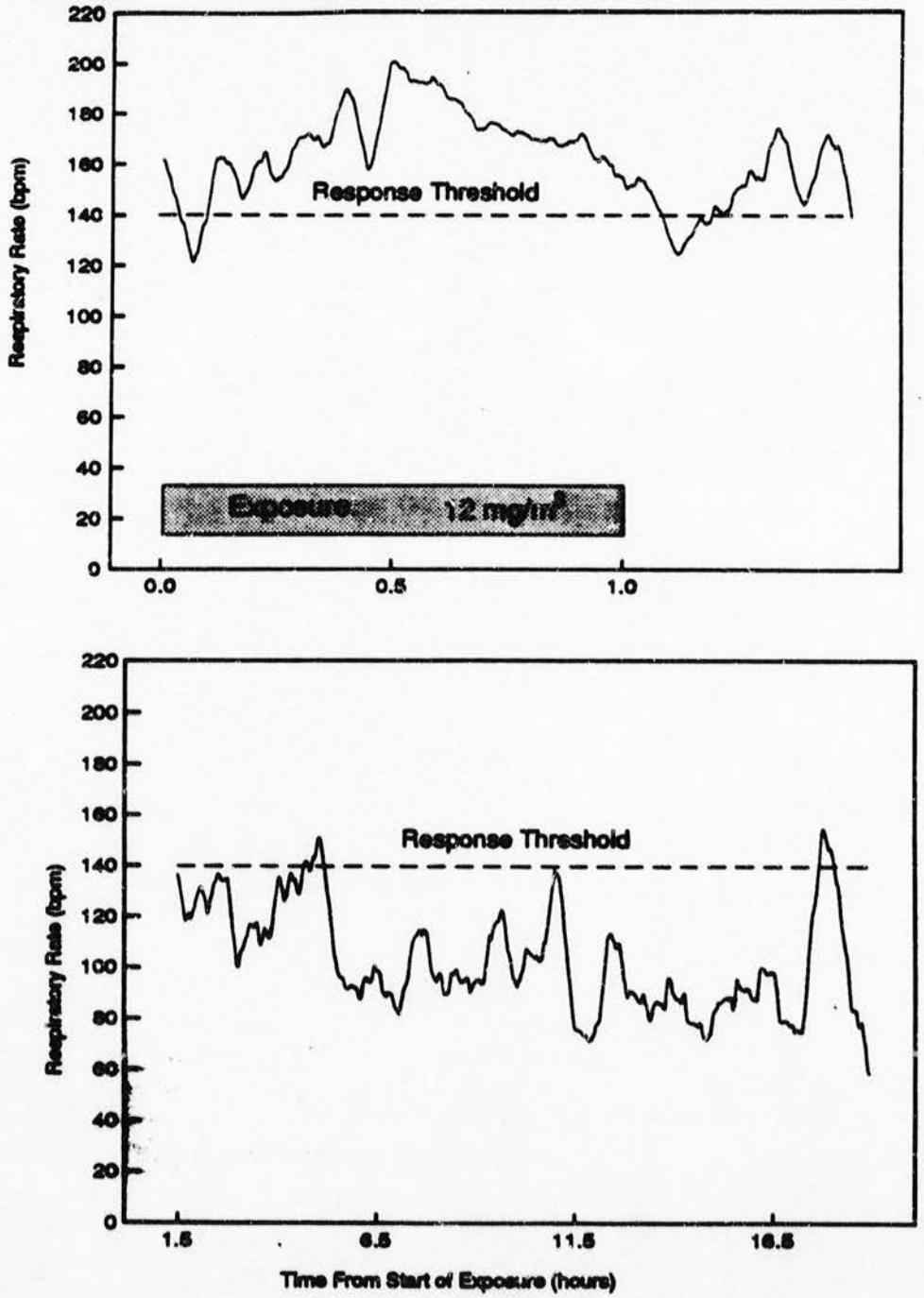
Figure: 26 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 30 : 2,4-TDI Sensitization

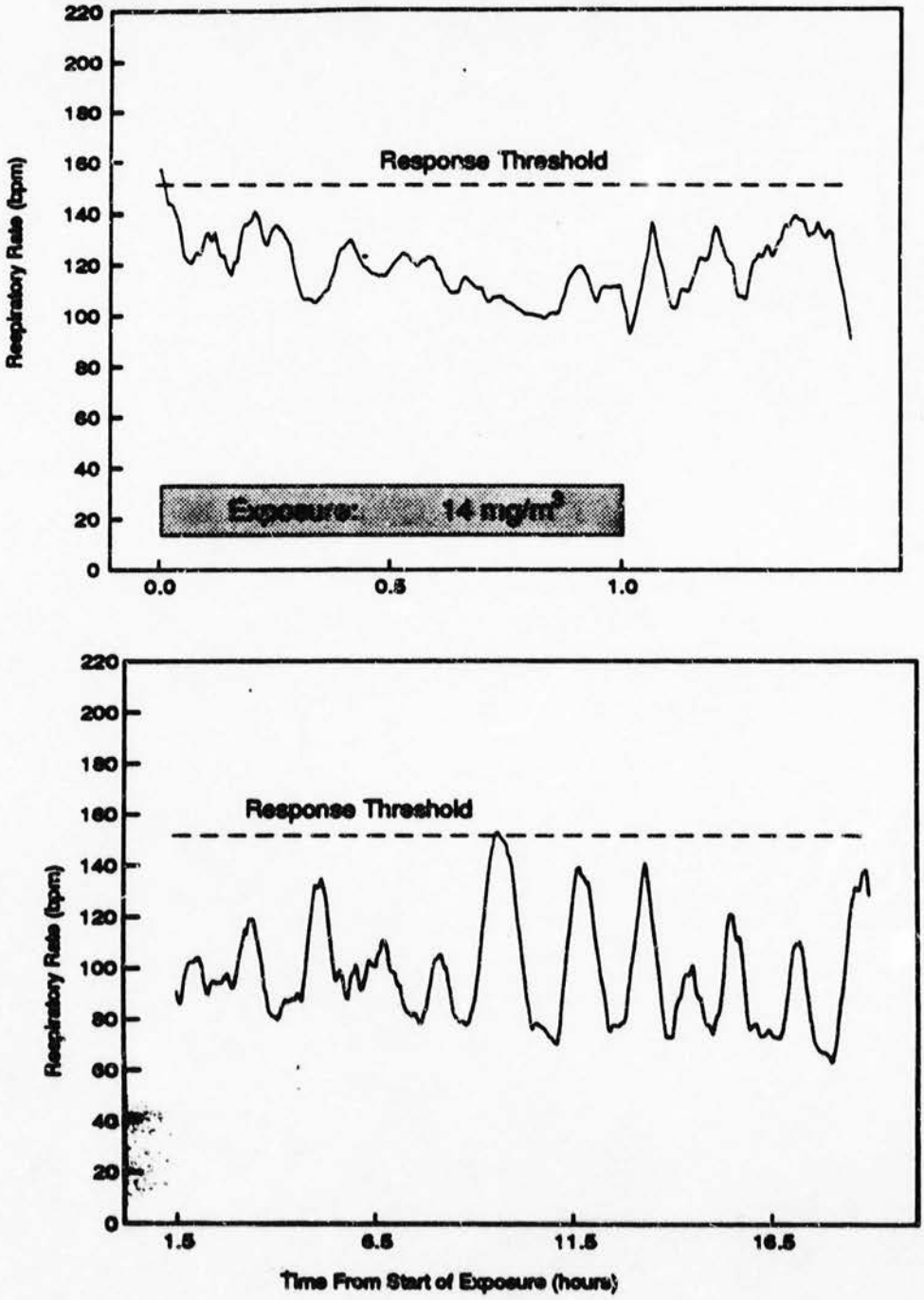
Figure: 27 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 31 : 2,4-TDI Sensitization

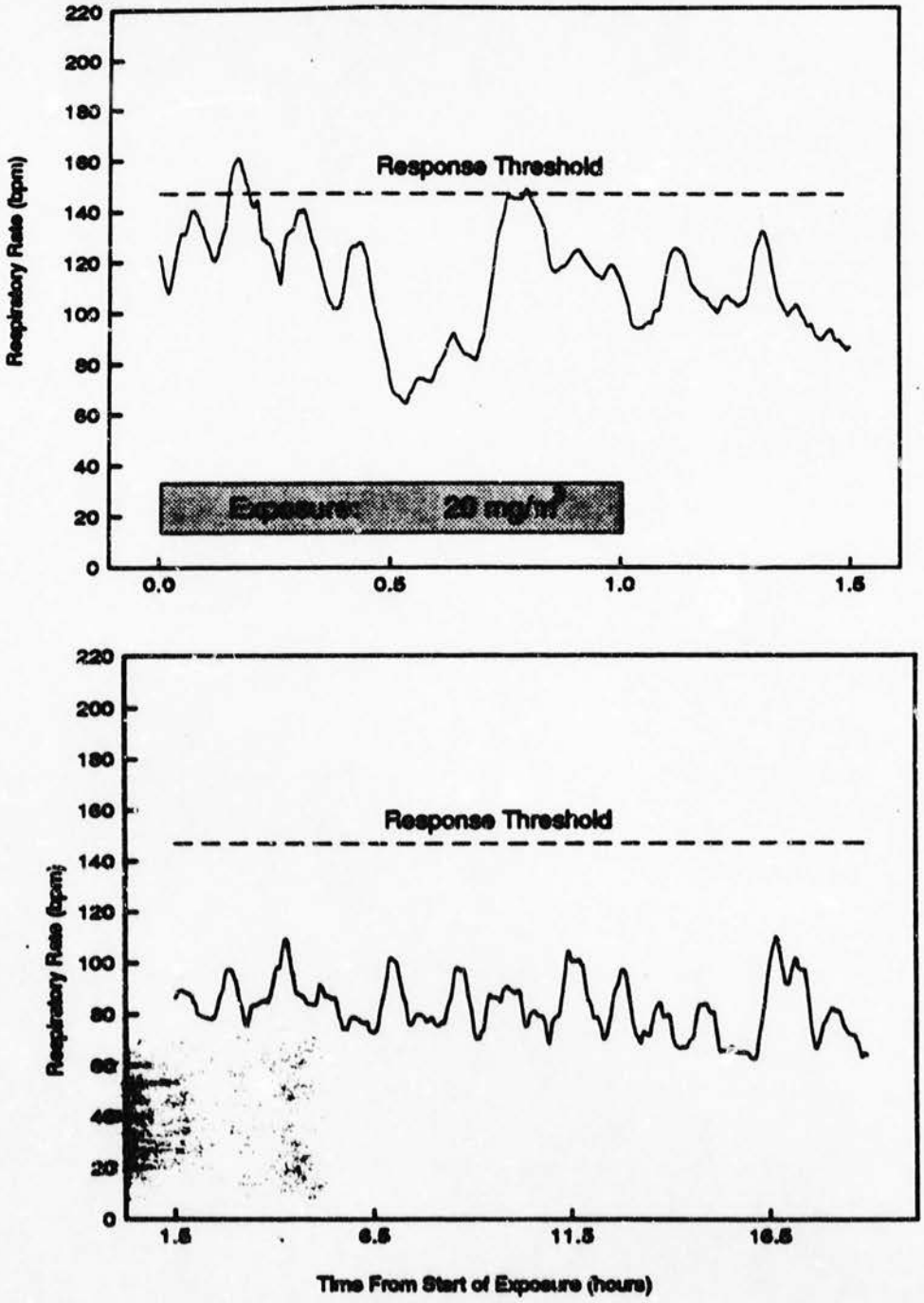
Figure: 28 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 32 : 2,4-TDI Sensitization

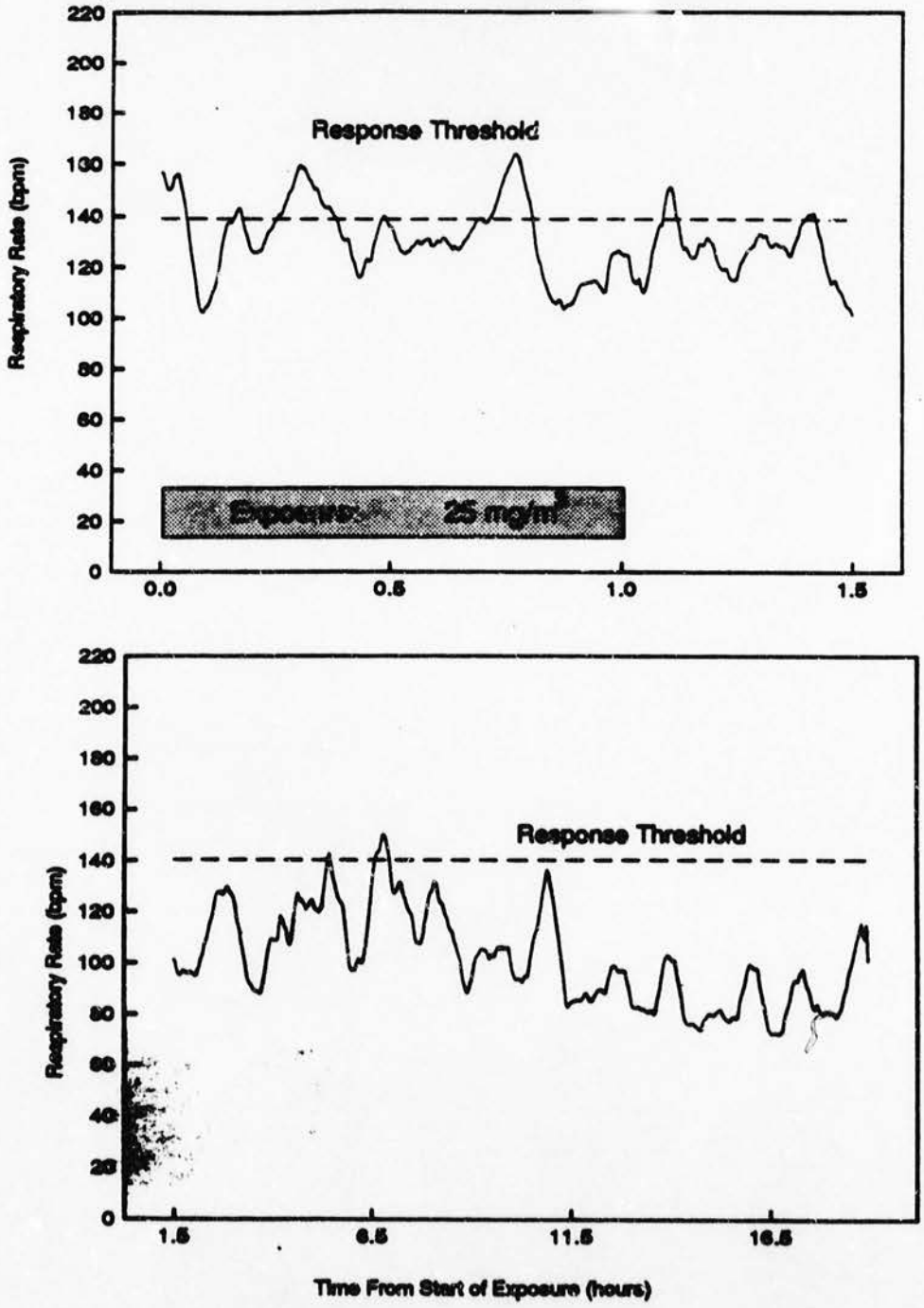
Figure: 29 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 33 : 2,4-TDI Sensitization

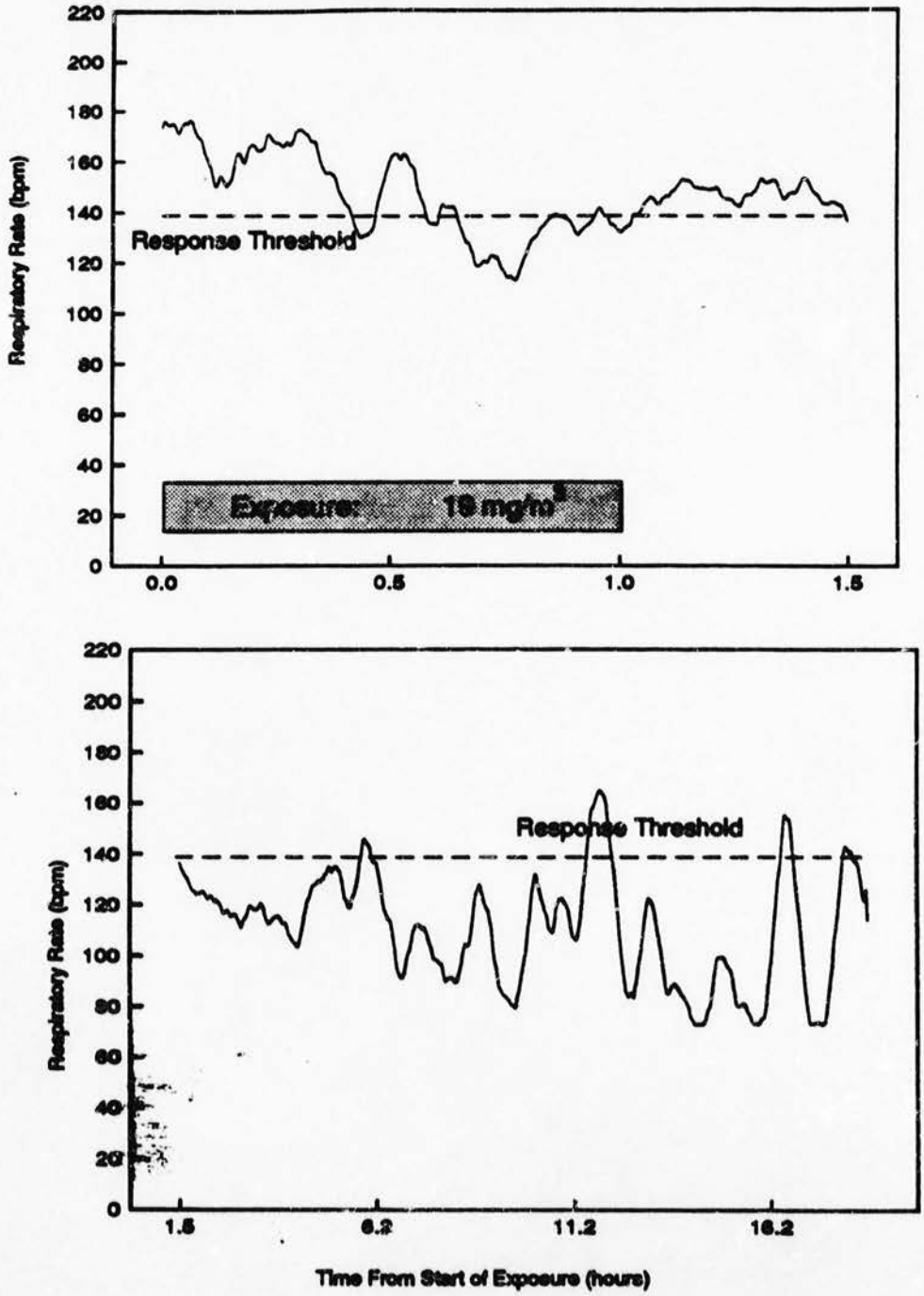
Figure: 30 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 34 : 2,4-TDI Sensitization

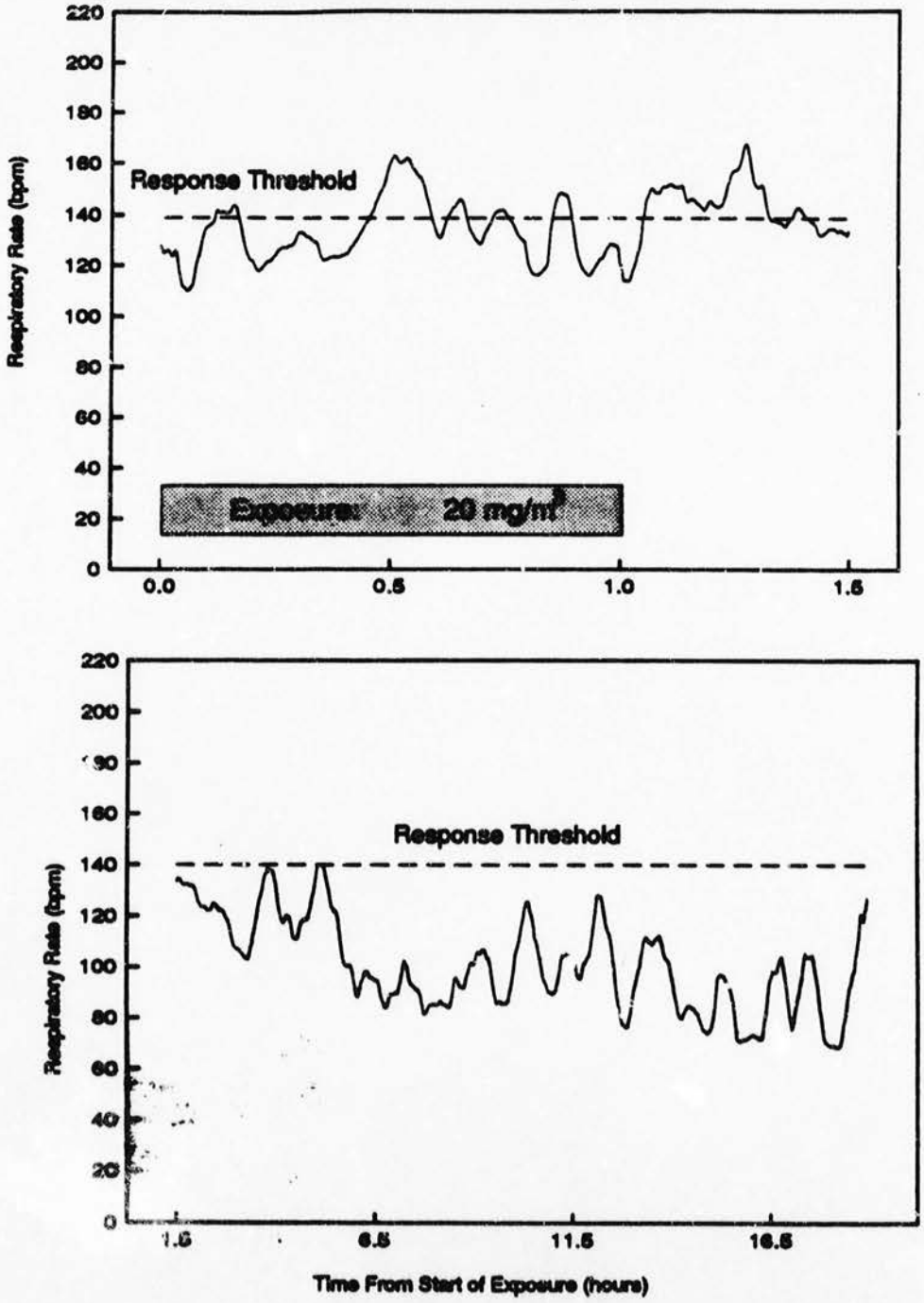
Figure: 31 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animzal # 35 : 2,4-TDI Sensitization

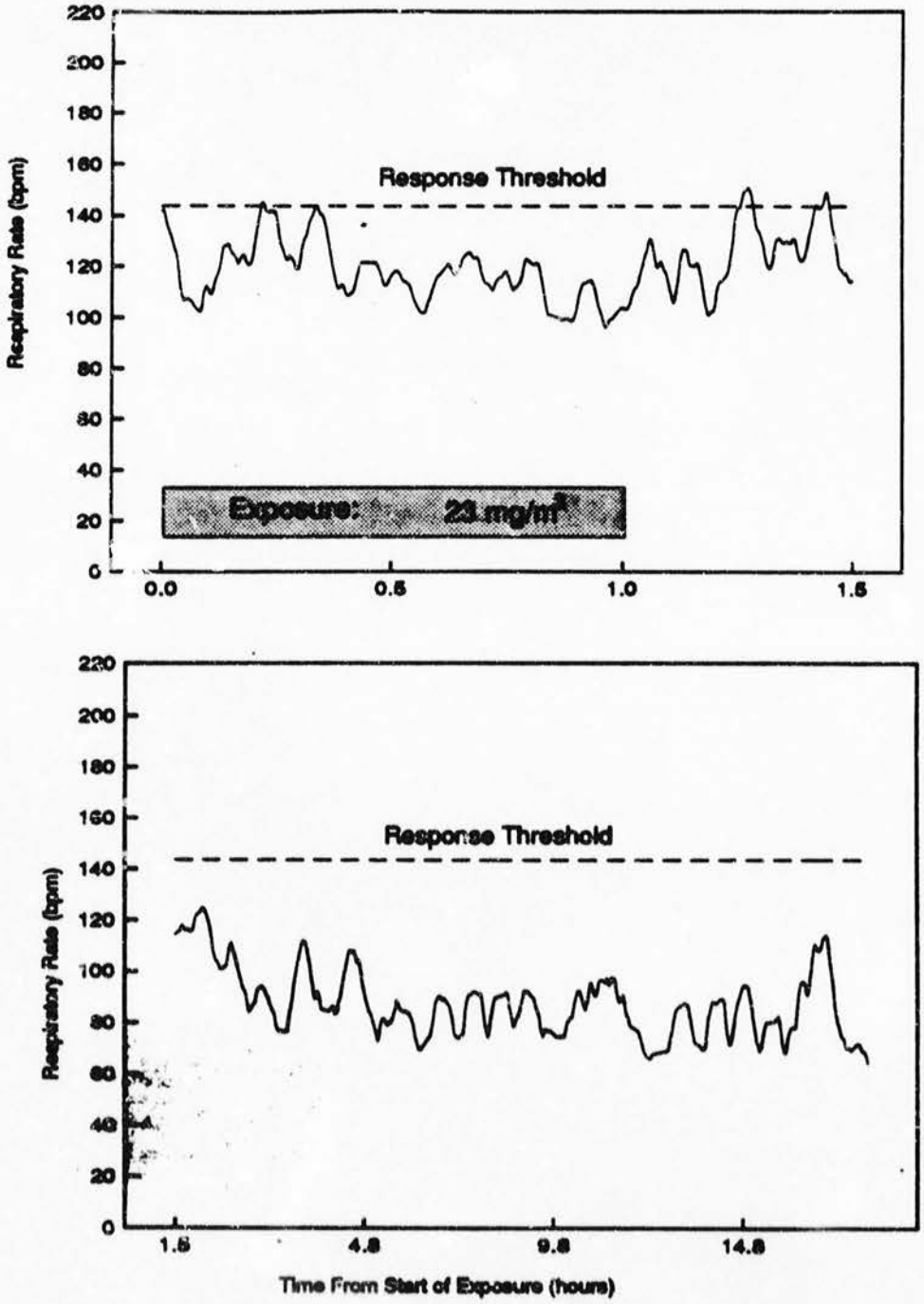
Figure: 32 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 36 : 2,4-TDI Sensitization

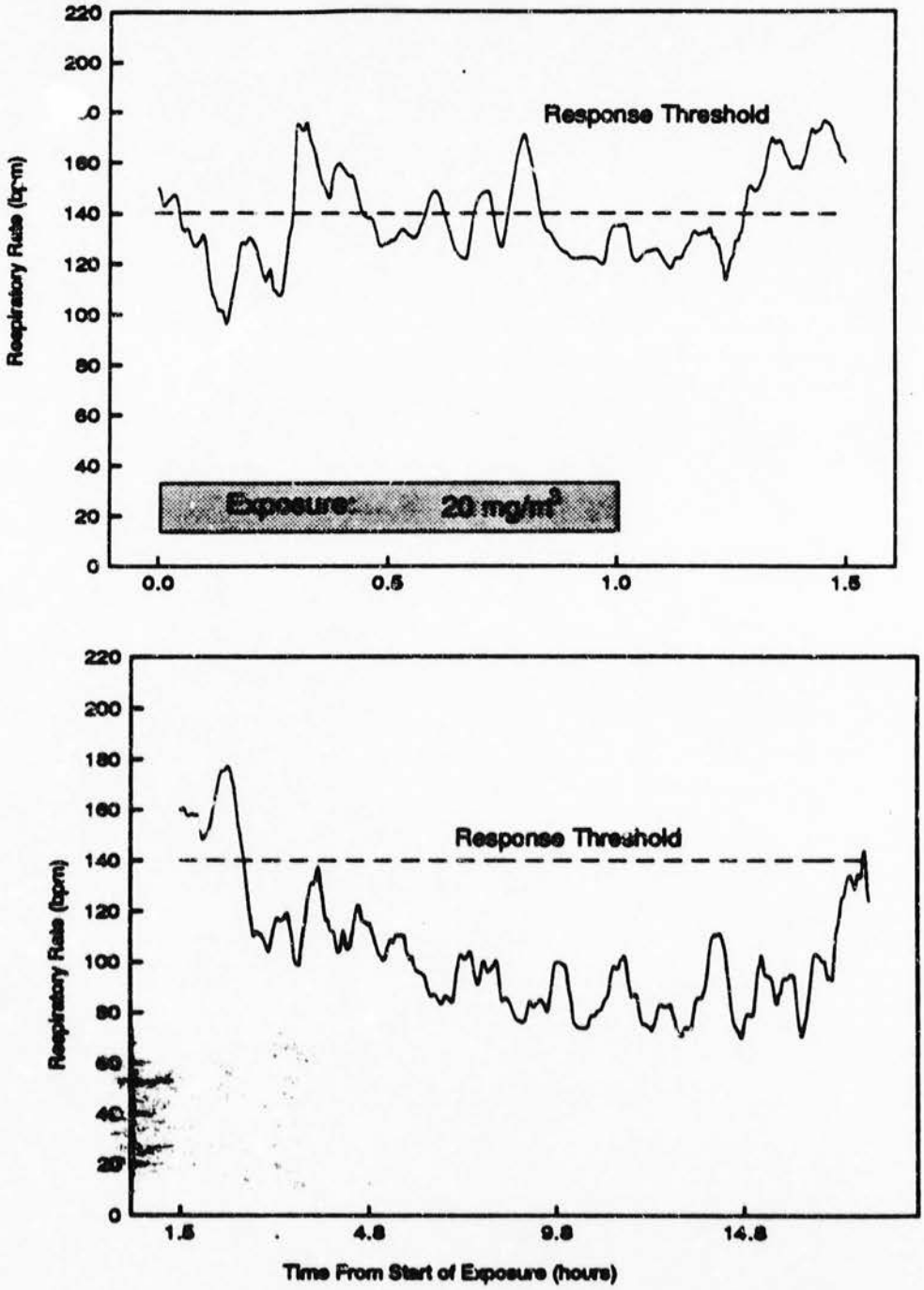
Figure: 33 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 38 : 2,4-TDI Sensitization

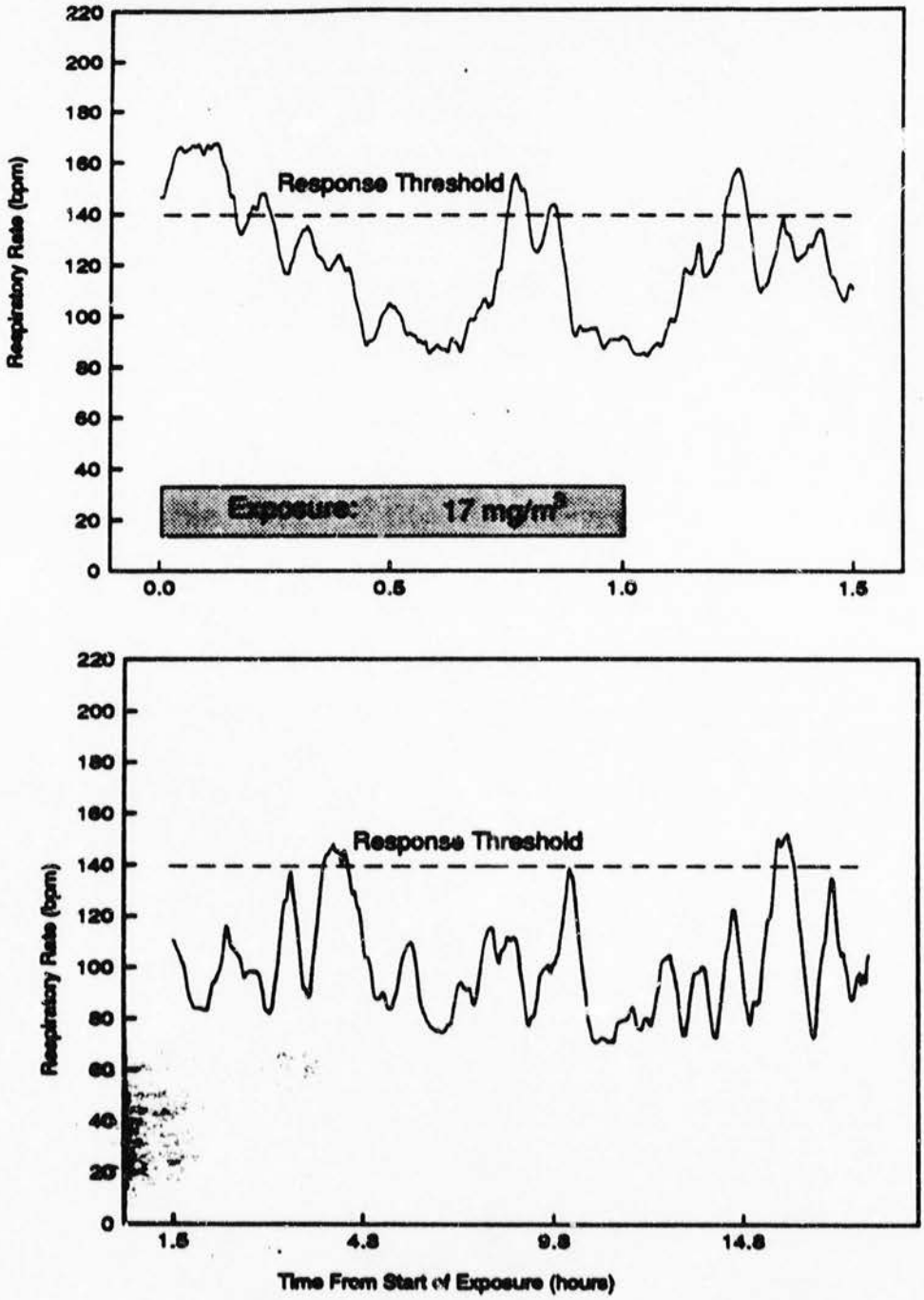
Figure: 34 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 39 : 2,4-TDI Sensitization

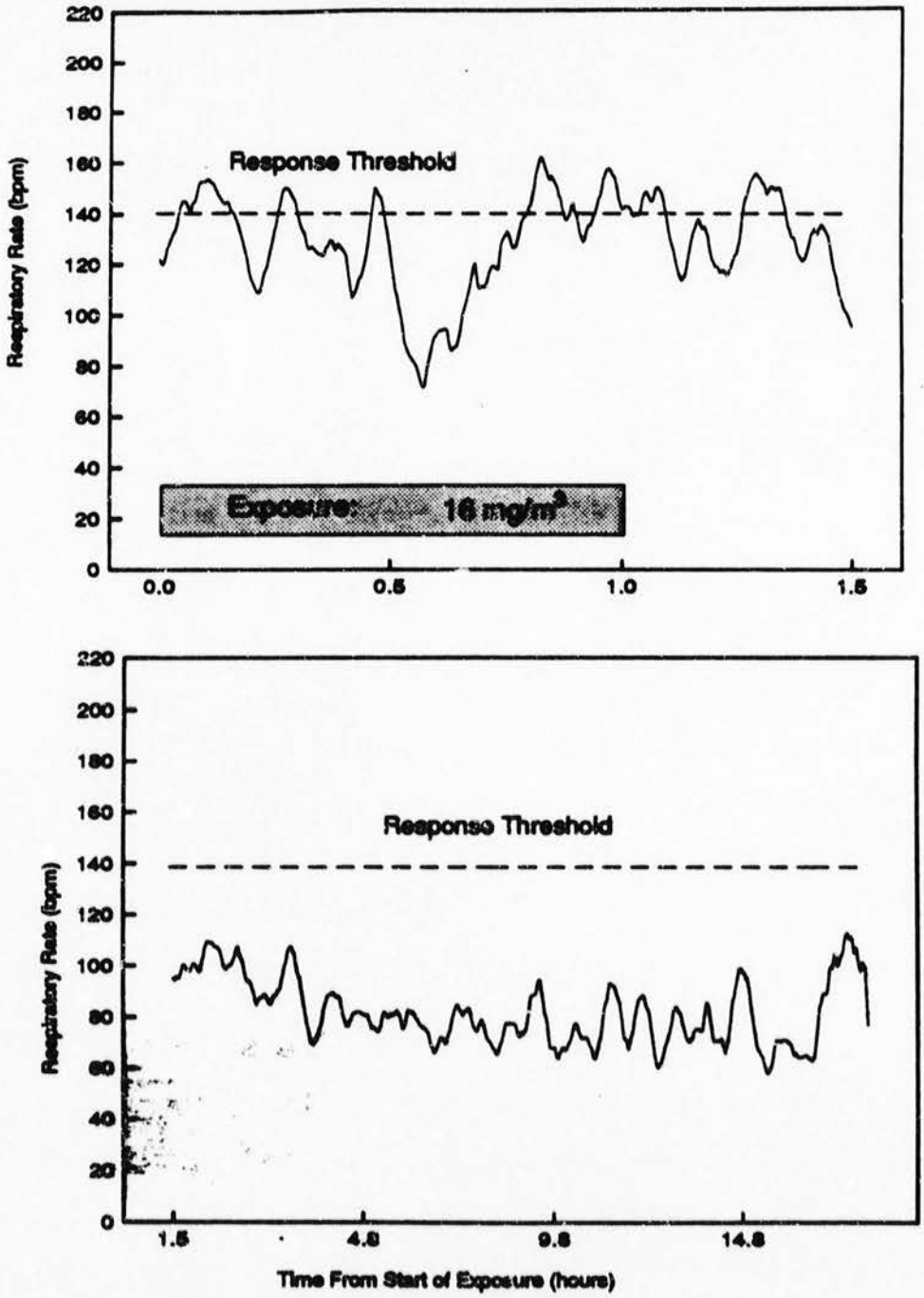
Figure: 35 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 40 : 2,4-TDI Sensitization

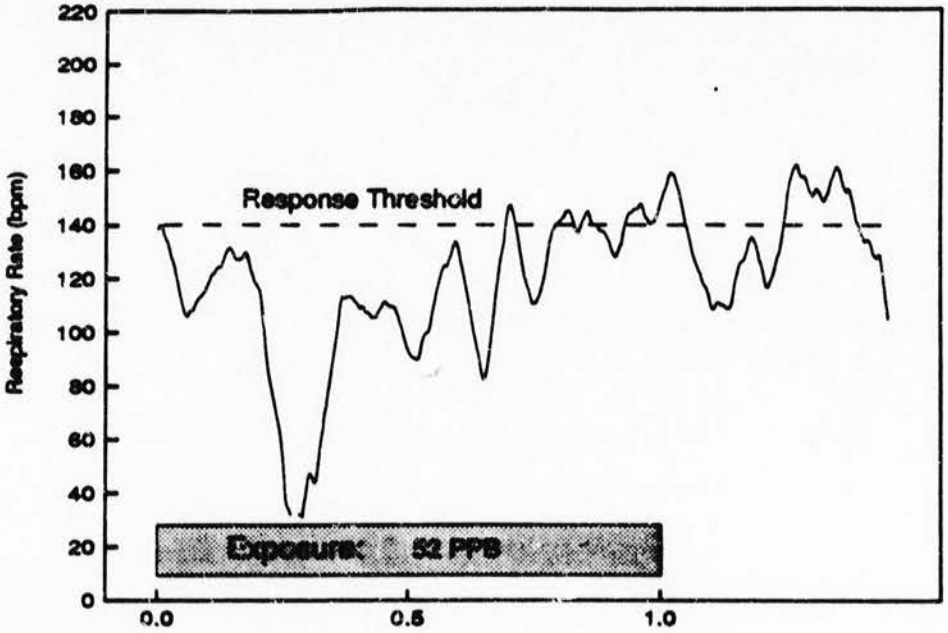
Figure: 36 2,4-TDI-GSA Challenge Week 3

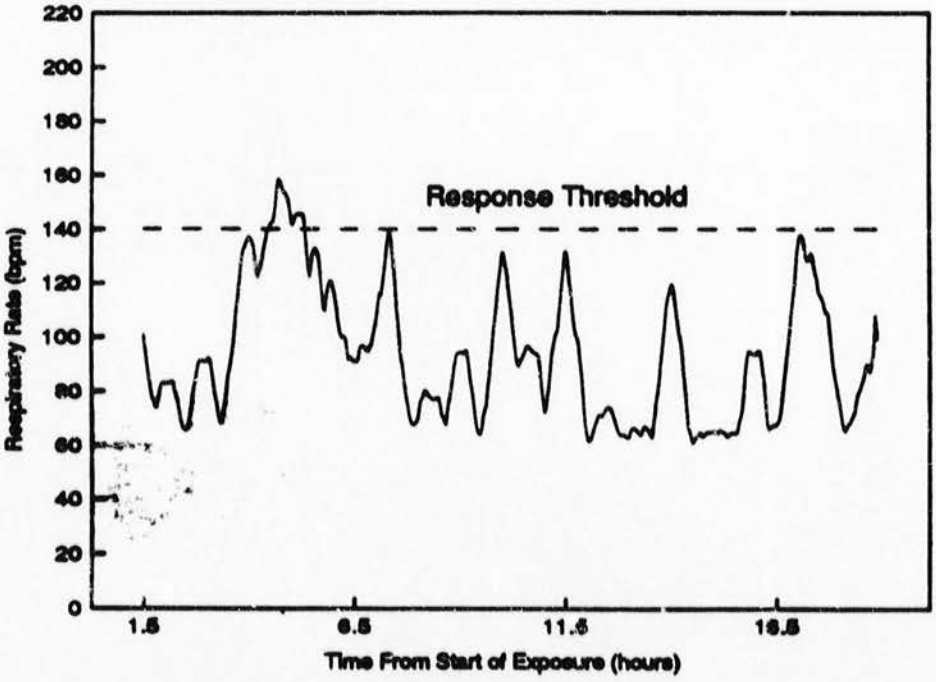


Study Number 91-344-KR

Animal # 59: 2,4-TDI Sensitization

Figure: 37 2,6-TDI Challenge Week 1

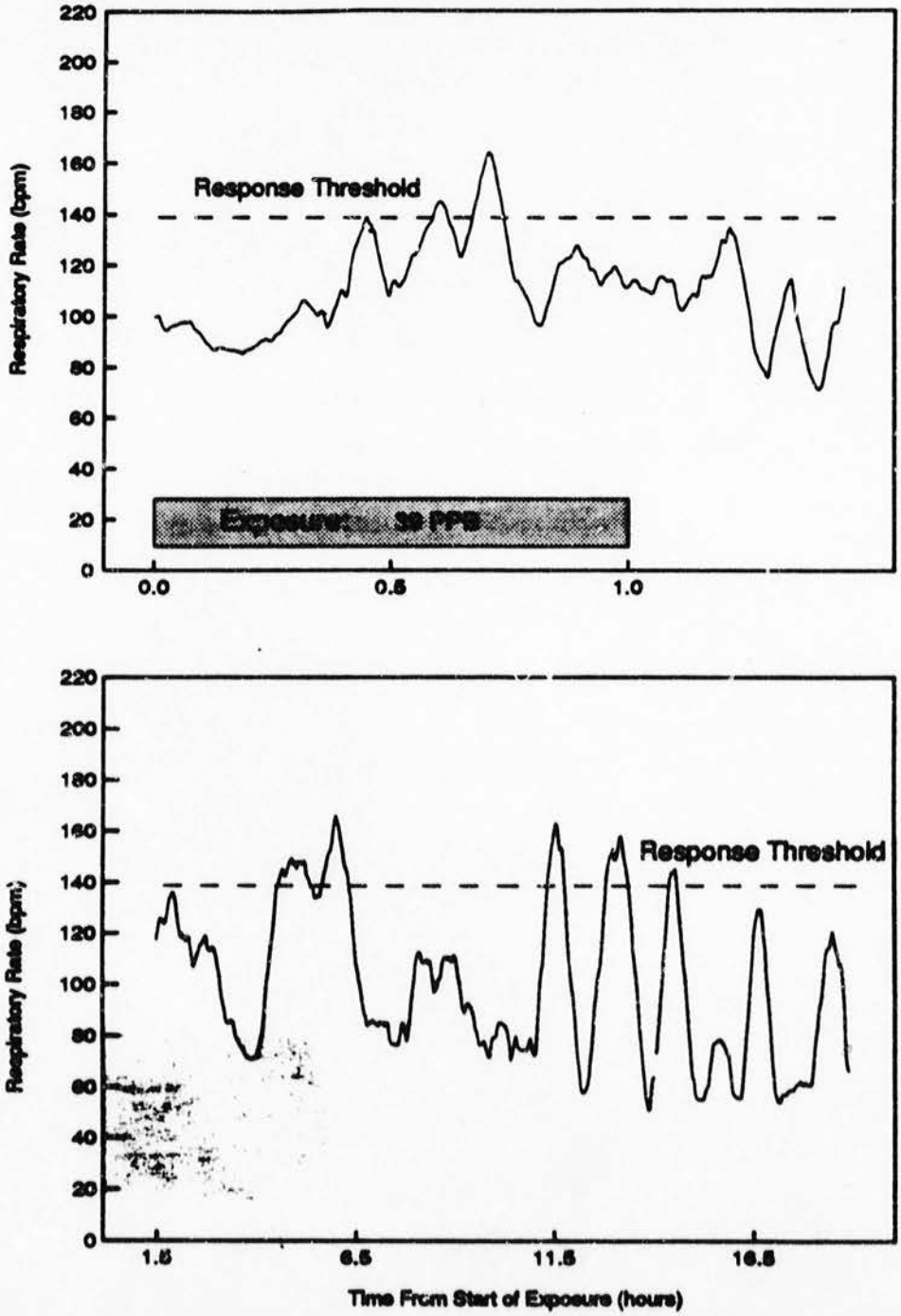




Study Number 91-344-KR

Animal # 60: 2,4-TDI Sensitization

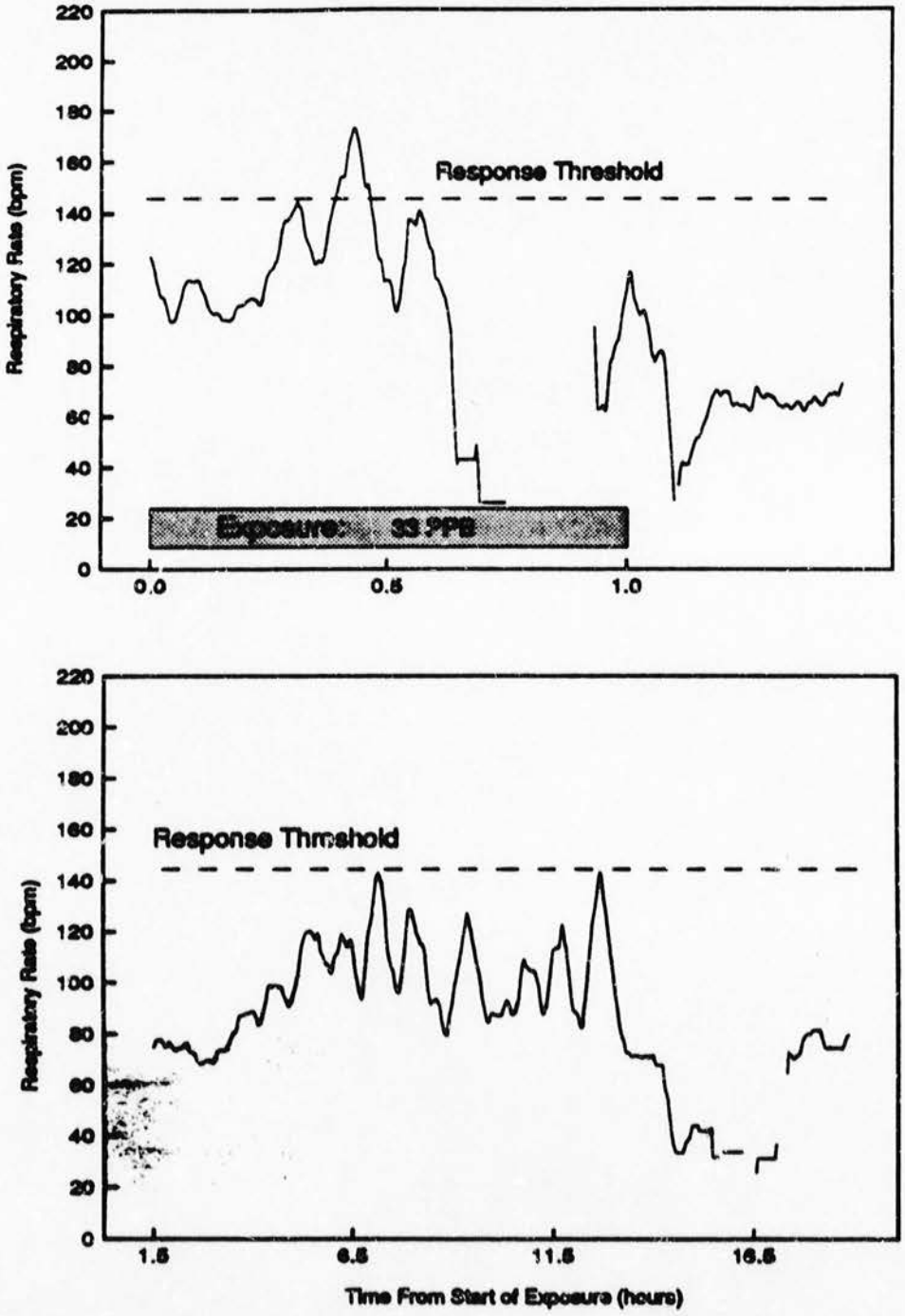
Figure: 38 2,6-TDI Challenge Week 1



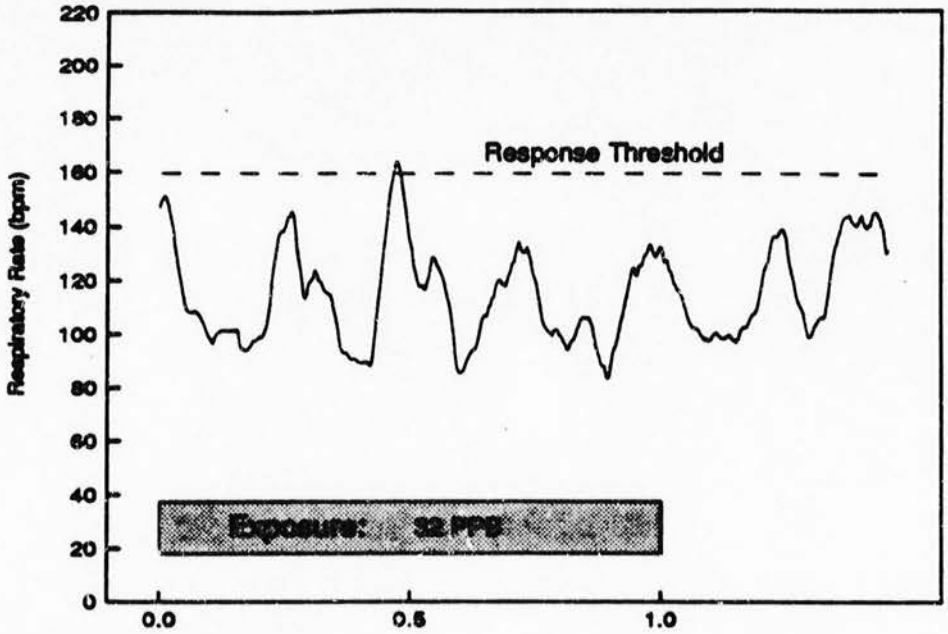
Study Number 91-344-KR

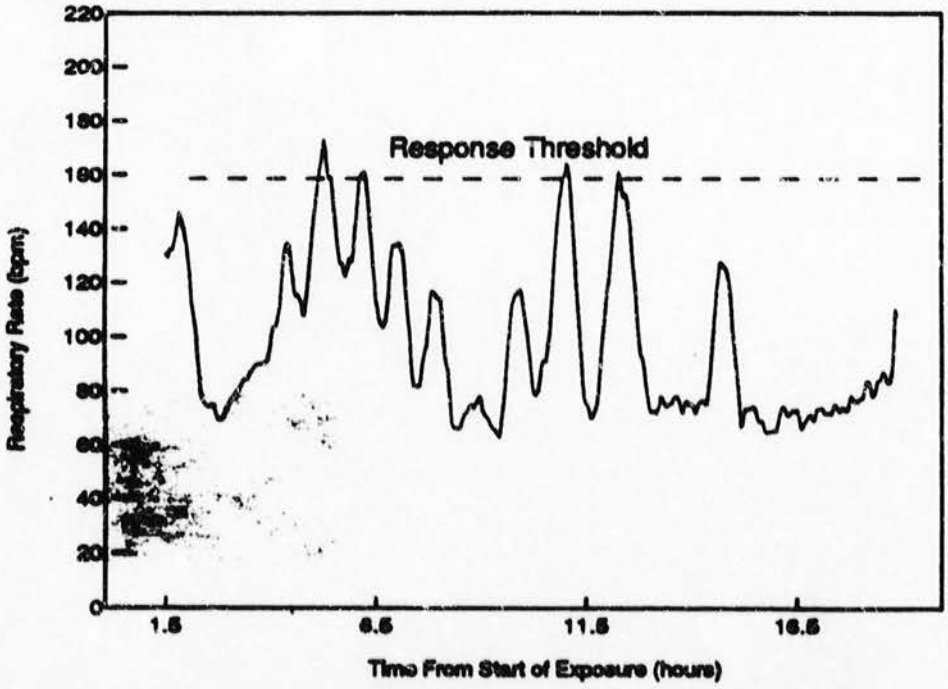
Animal # 61: 2,4-TDI Sensitization

Figure: 39 2,6-TDI Challenge Week 1



Study Number 91-344-KR
Animal # 62: 2,4-TDI Sensitization
Figure: 40 2,6-TDI Challenge Week 1

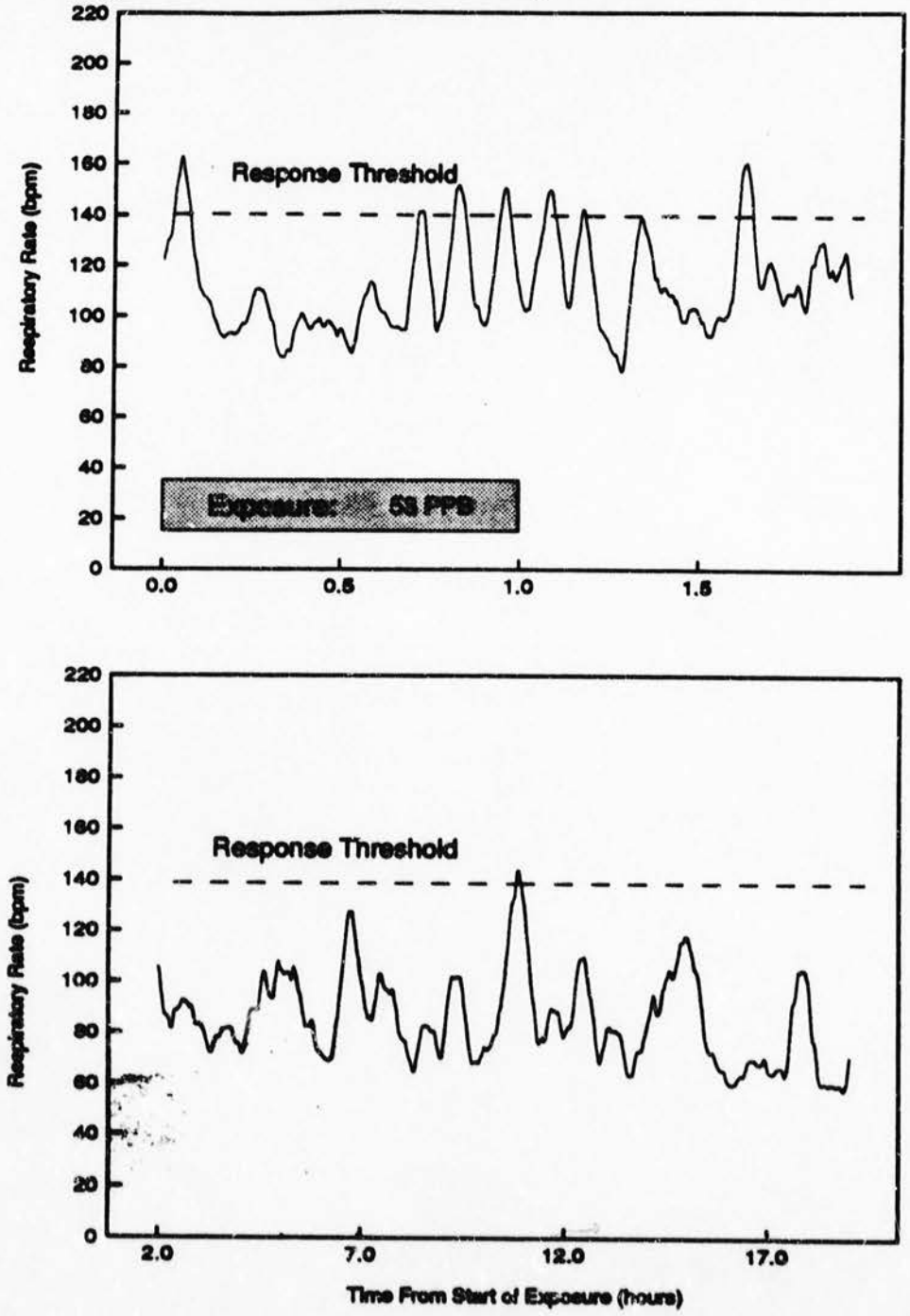




Study Number 91-344-KR

Animal # 63 : 2,4-TDI Sensitization

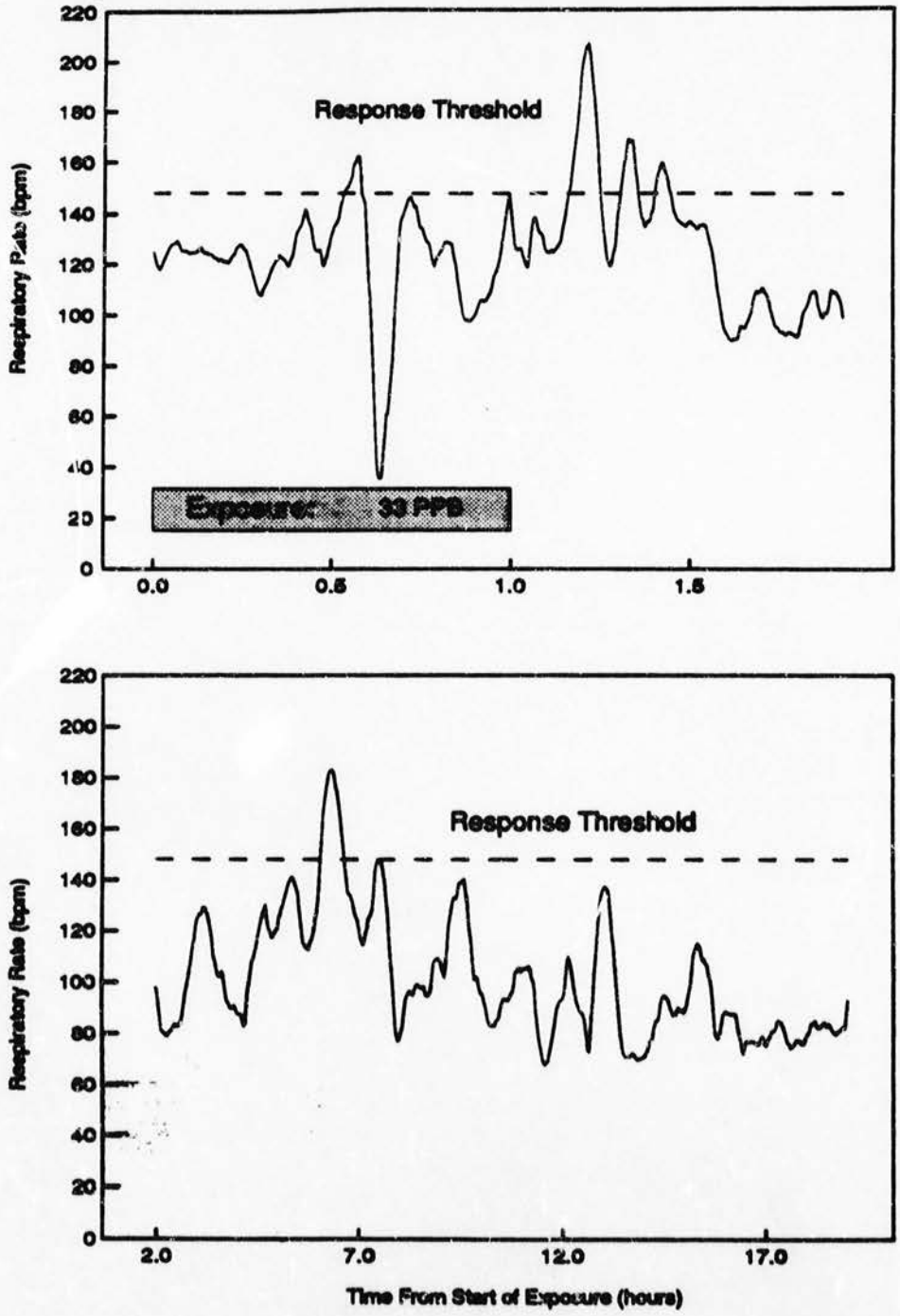
Figure: 41 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 64 : 2,4-TDI Sensitization

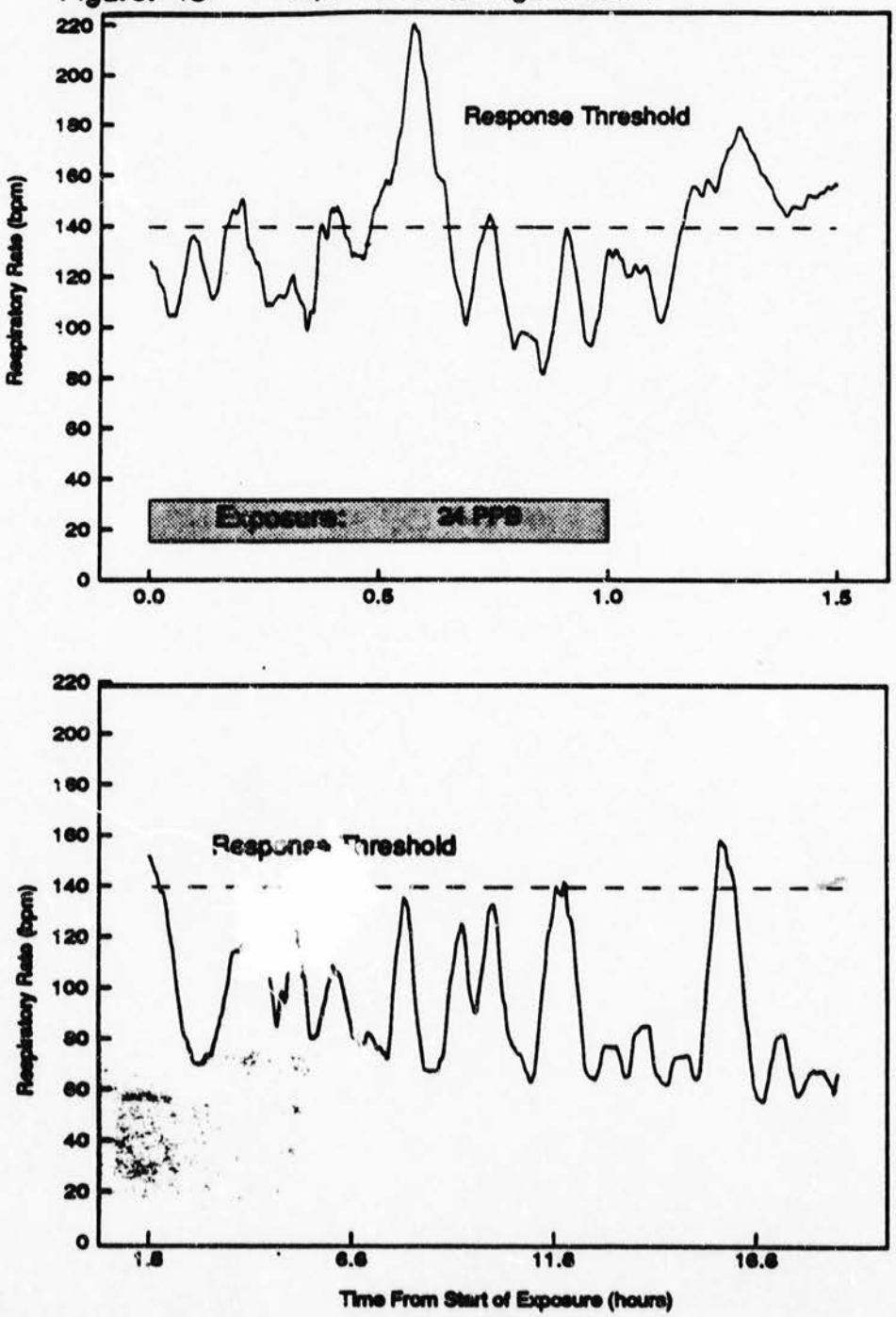
Figure: 42 2,6-TDI Challenge Week 1



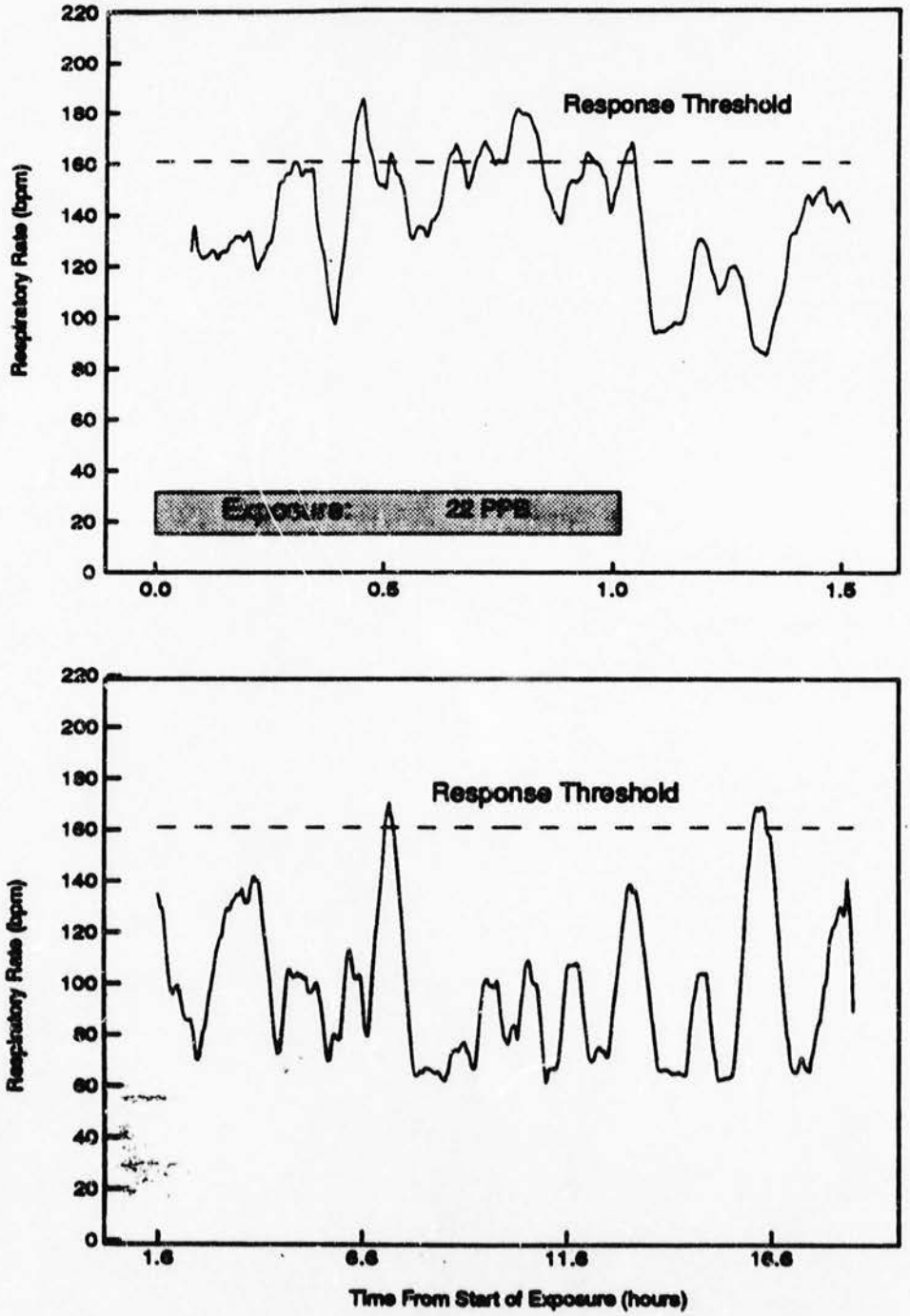
Study Number 91-344-KR

Animal # 65 : 2,4-TDI Sensitization

Figure: 43 2,6-TDI Challenge Week 1



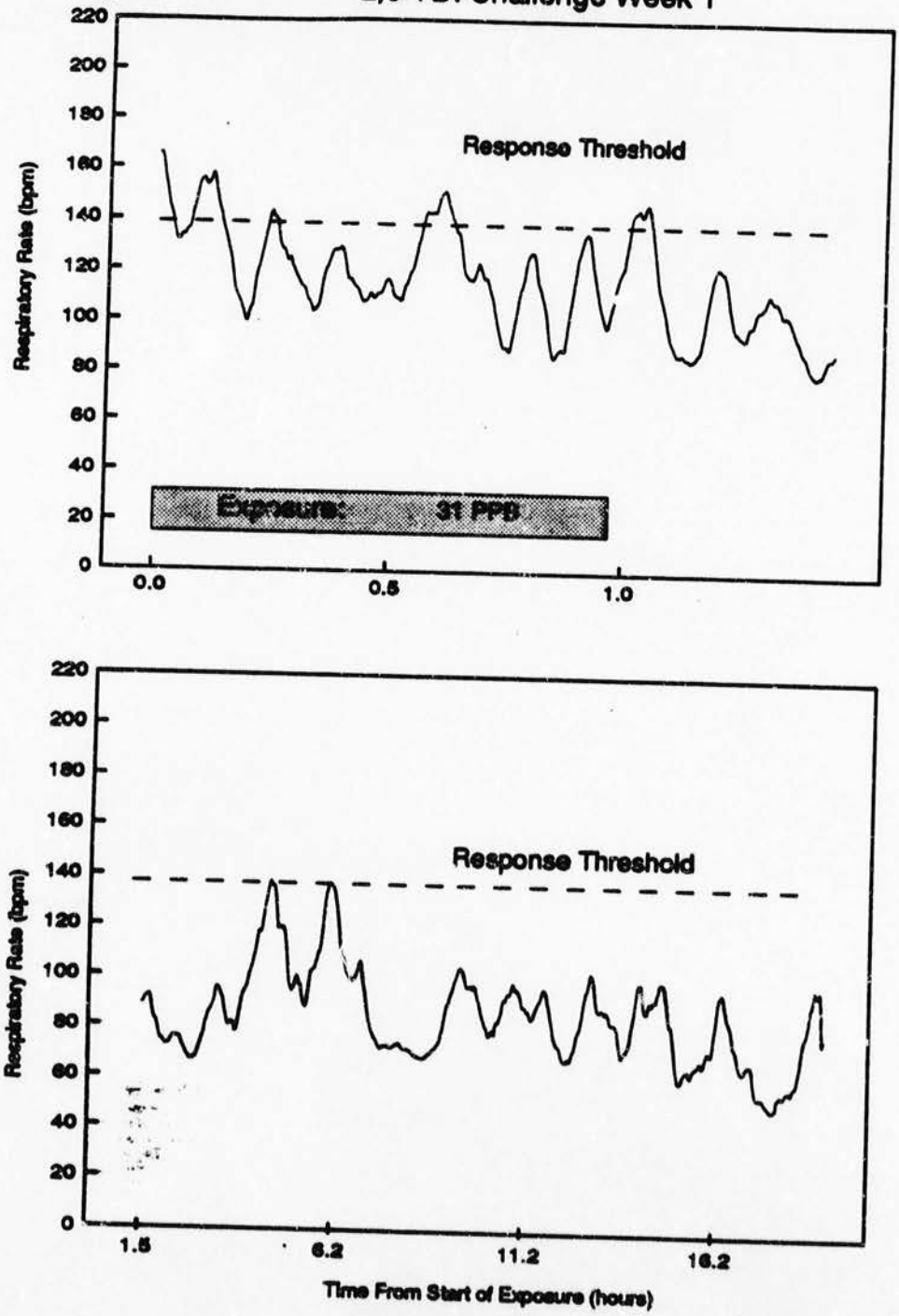
Study Number 91-344-KR
Animal # 66 : 2,4-TDI Sensitization
Figure: 44 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 67 : 2,4-TDI Sensitization

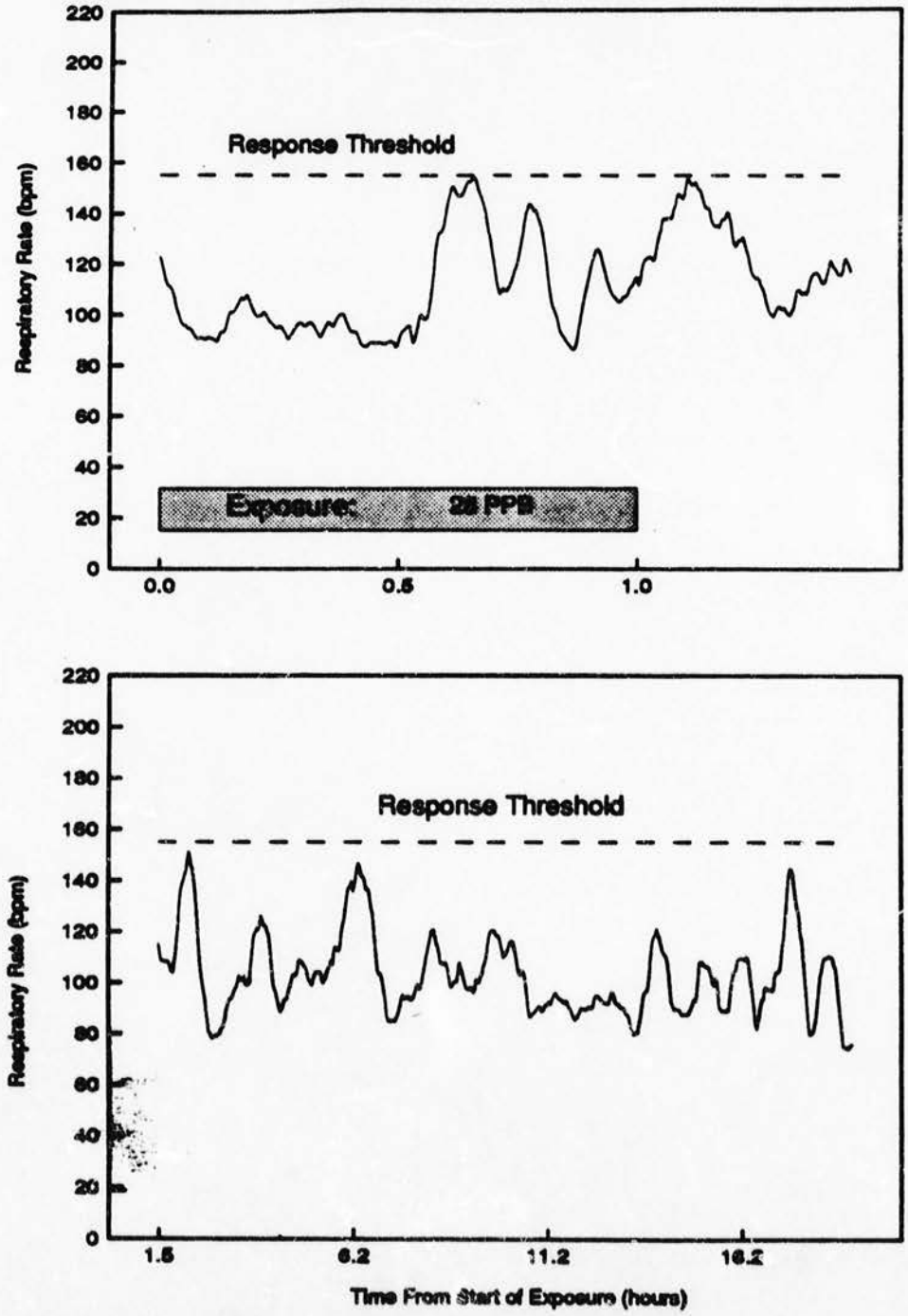
Figure: 45 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 68 : 2,4 -TDI Sensitization

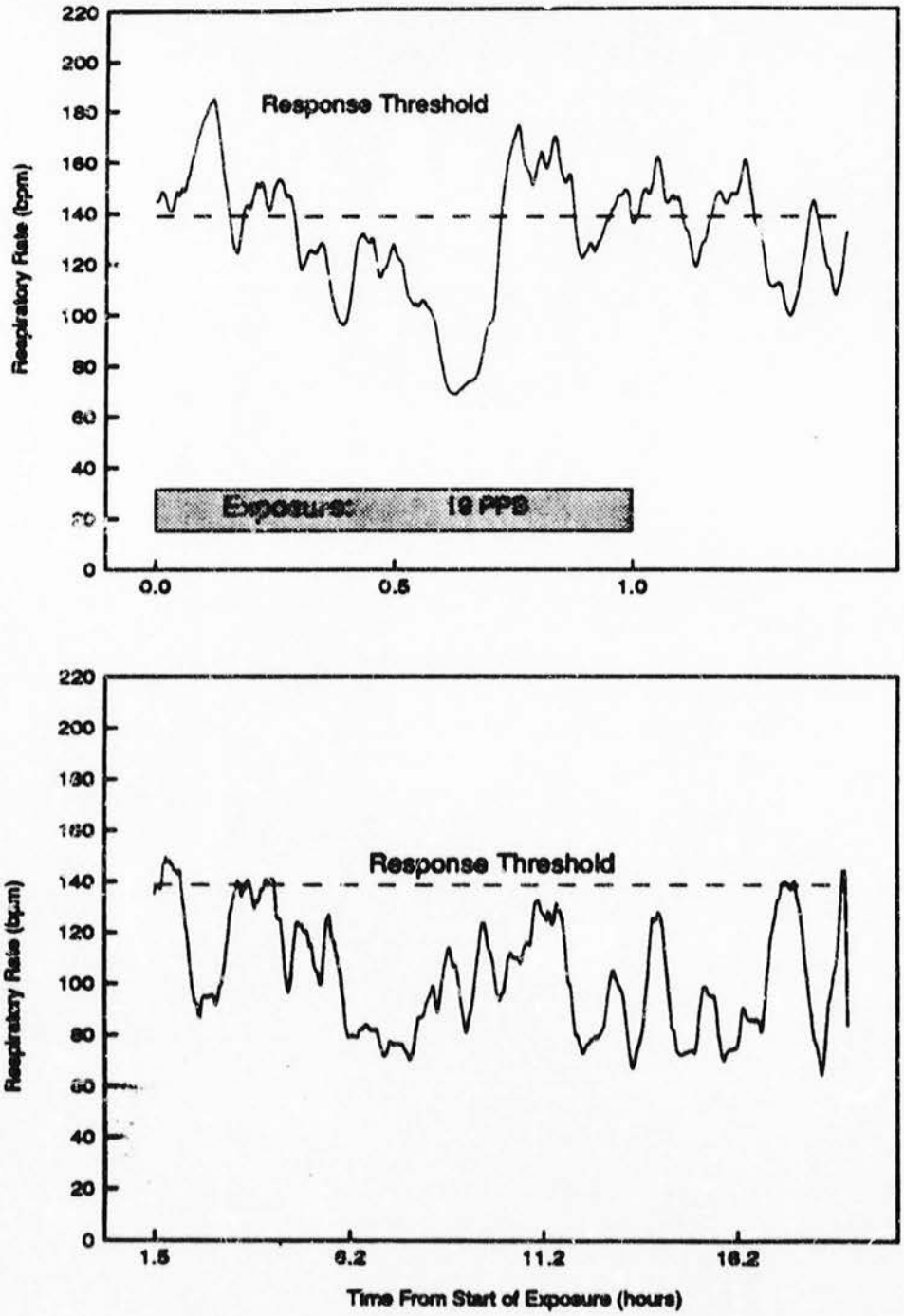
Figure: 46 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 69 : 2,4-TDI Sensitization

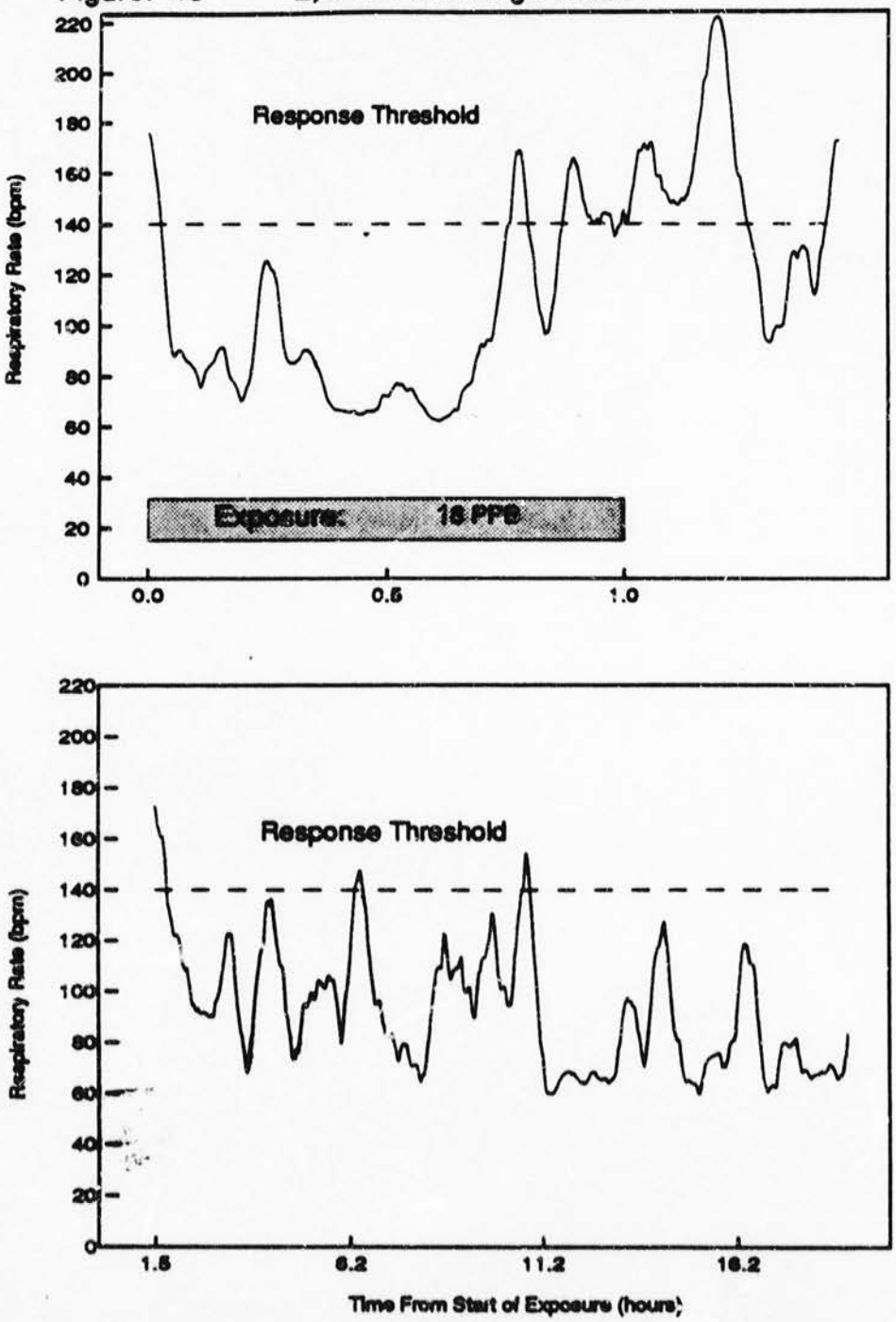
Figure: 47 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 70 : 2,4-TDI Sensitization

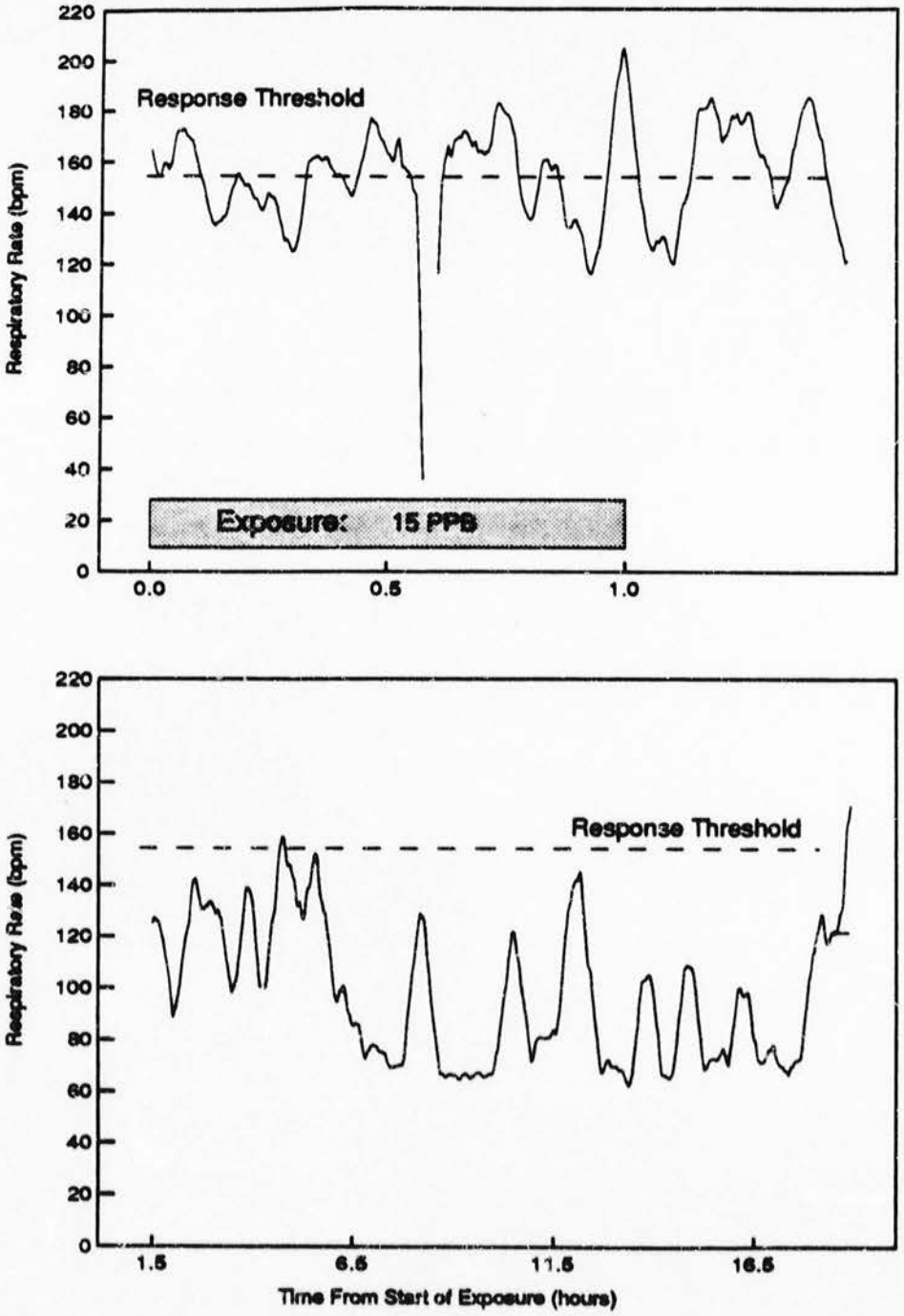
Figure: 48 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 59 : 2,4-TDI Sensitization

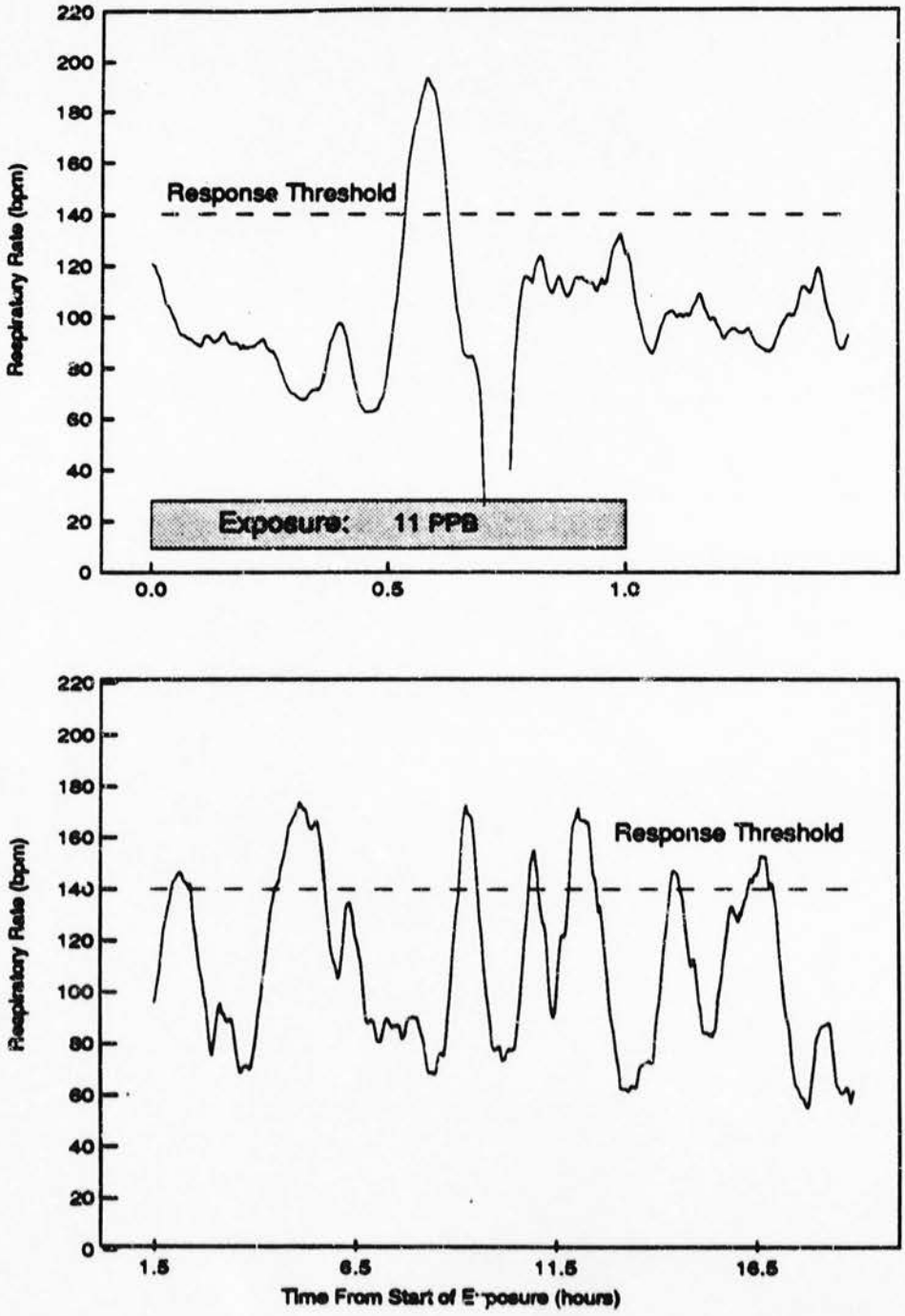
Figure: 49 2,6-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 60 : 2,4 -TDI Sensitization

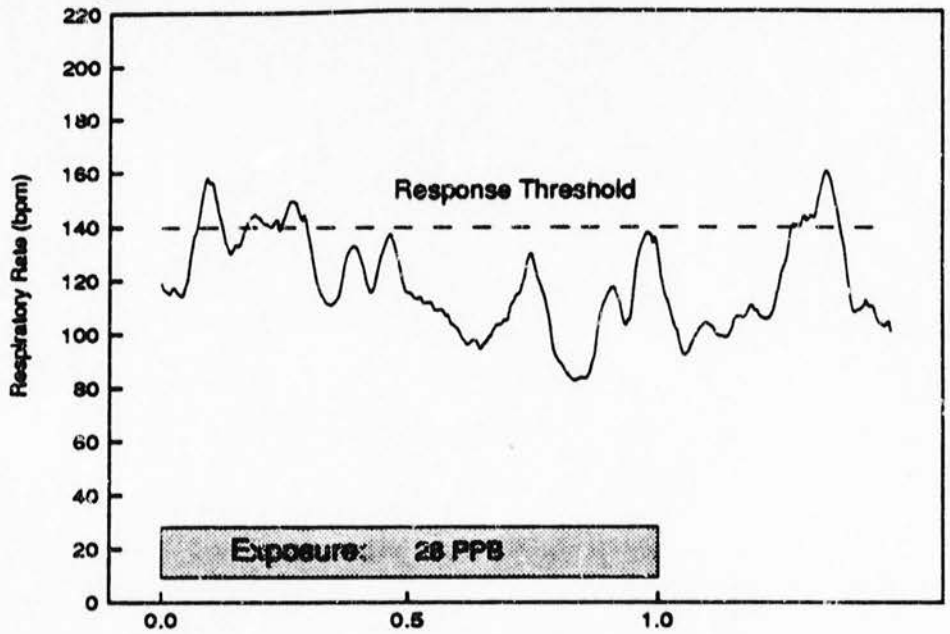
Figure: 50 2,6-TDI Challenge Week 2

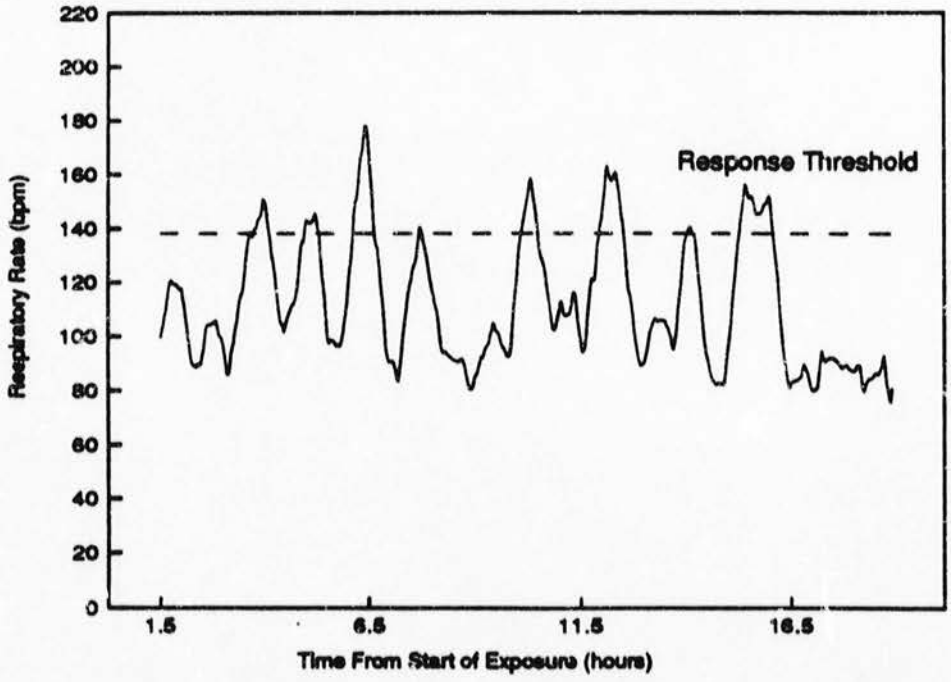


Study Number 91-344-KR

Animal # 61 : 2,4-TDI Sensitization

Figure: 51 2,6-TDI Challenge Week 2

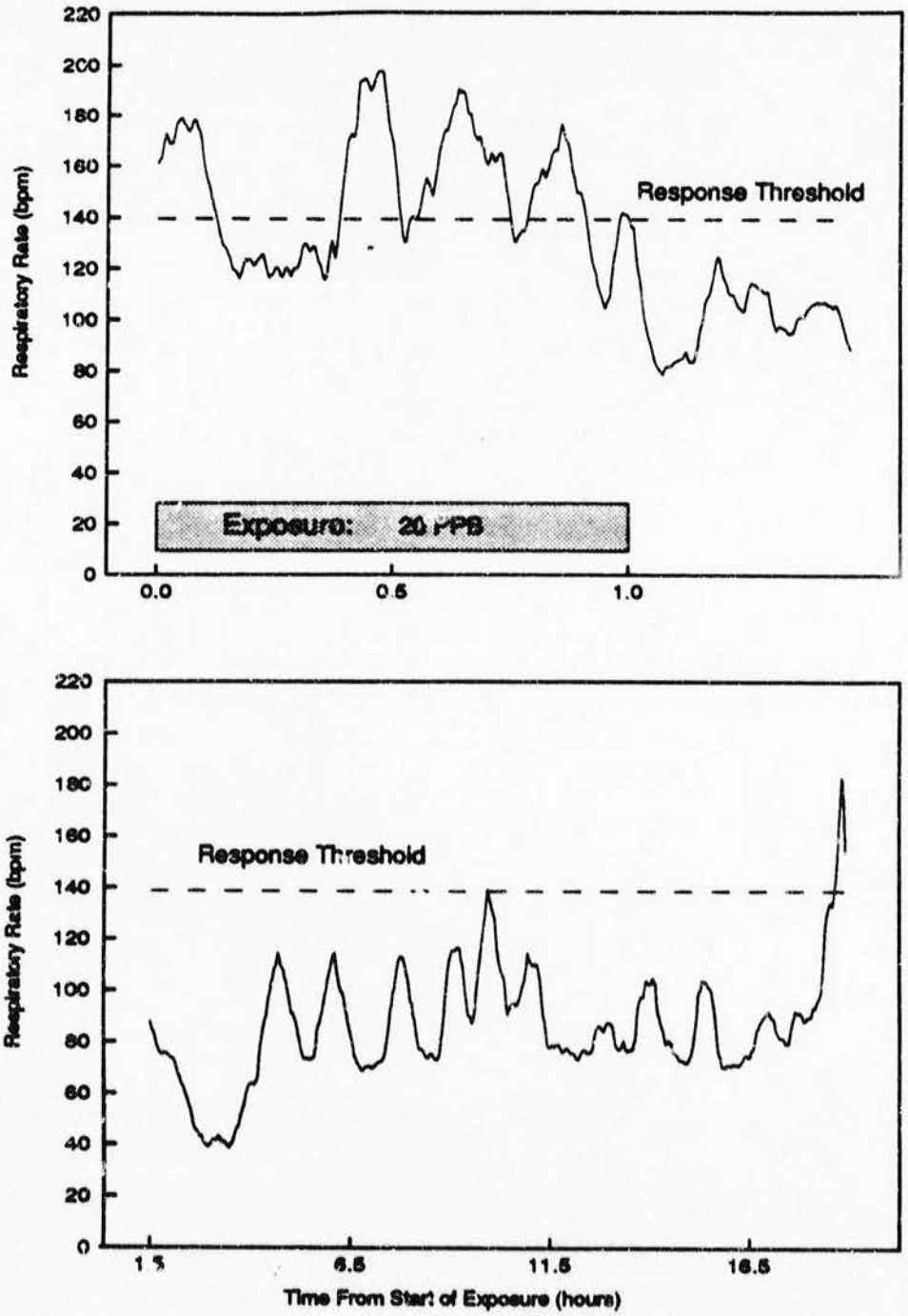




Study Number 91-344-KR

Animal # 62 : 2,4-TDI Sensitization

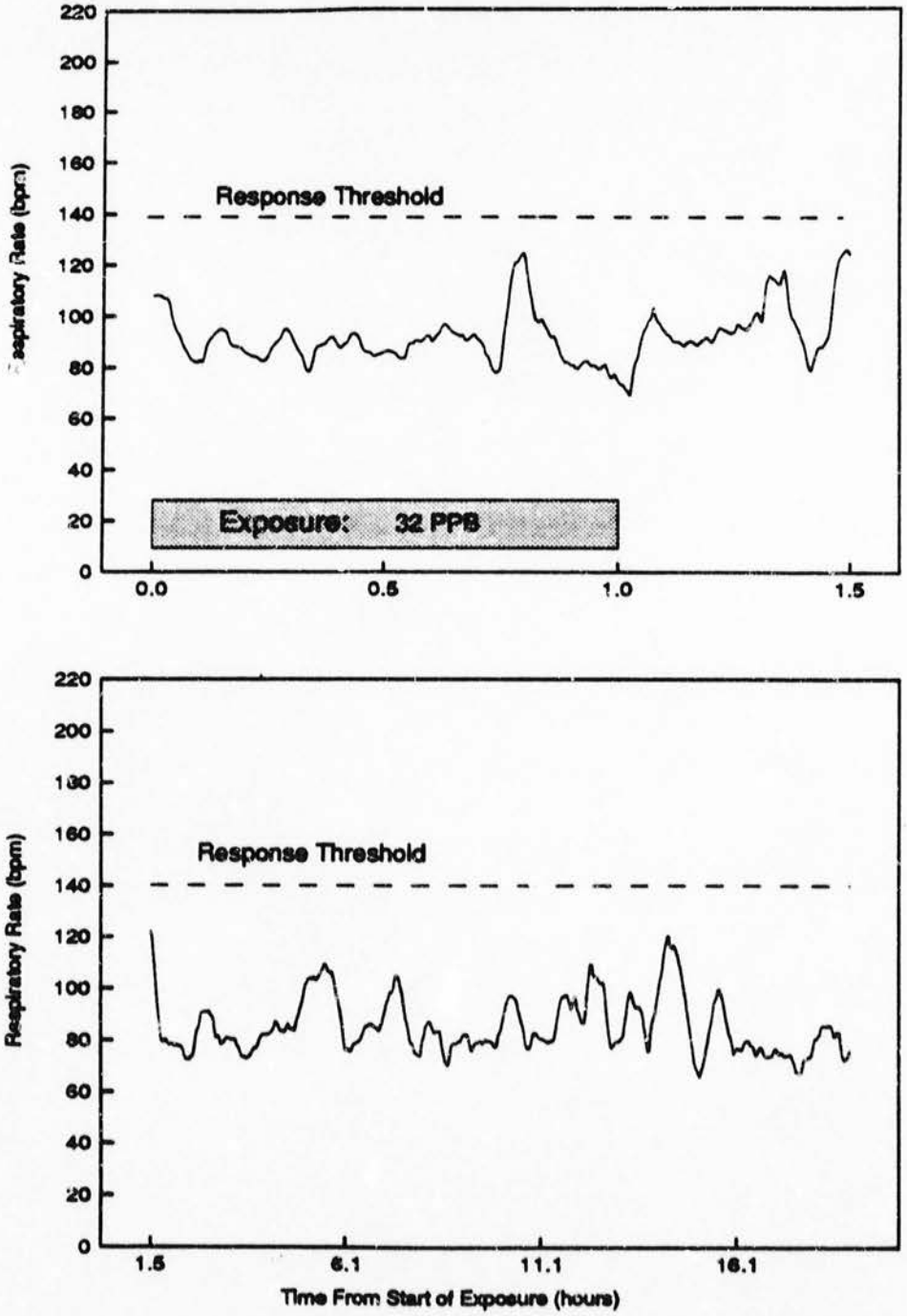
Figure: 52 2,6-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 63 : 2,4-TDI Sensitization

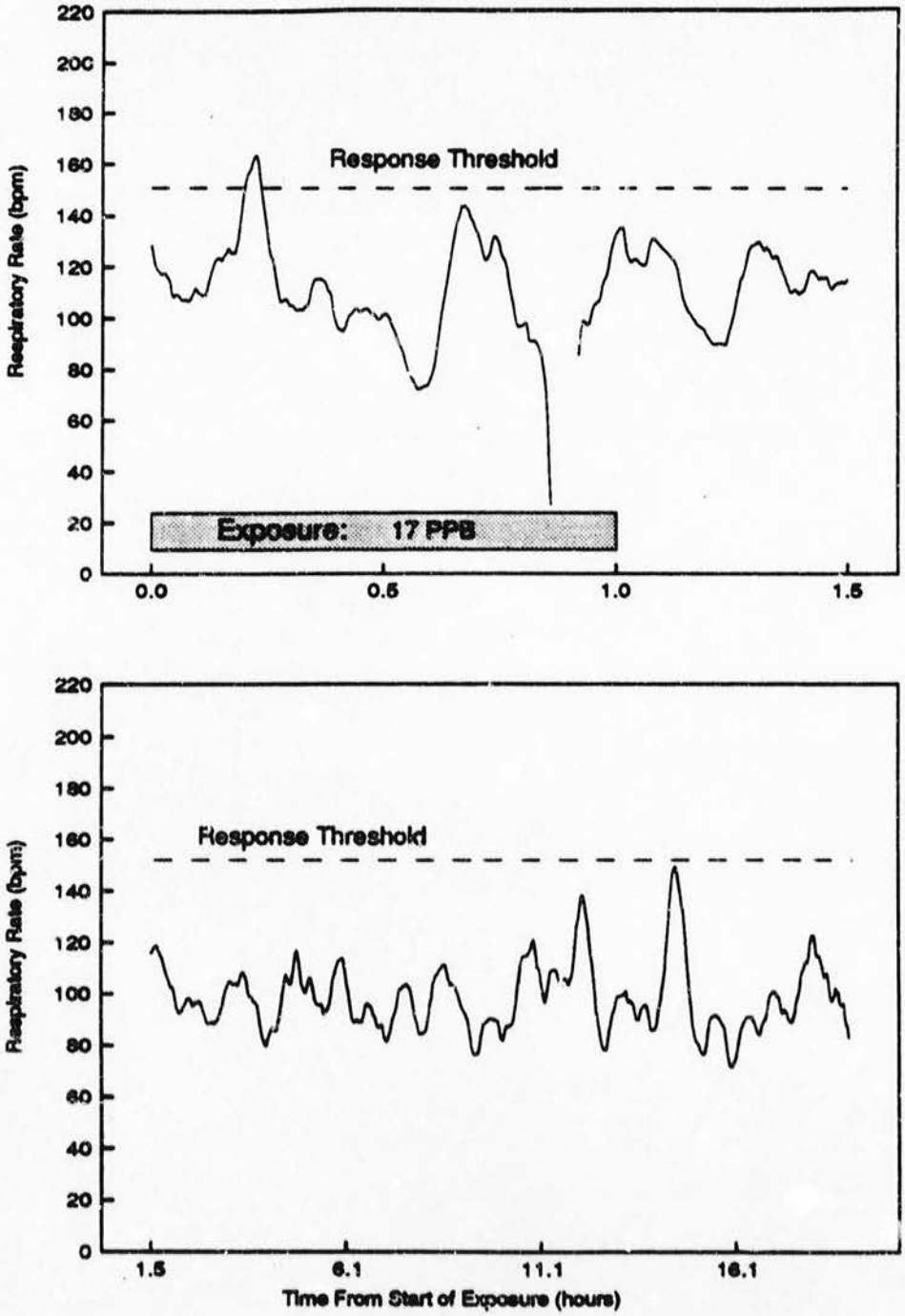
Figure: 53 2,6-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 64 : 2,4-TDI Sensitization

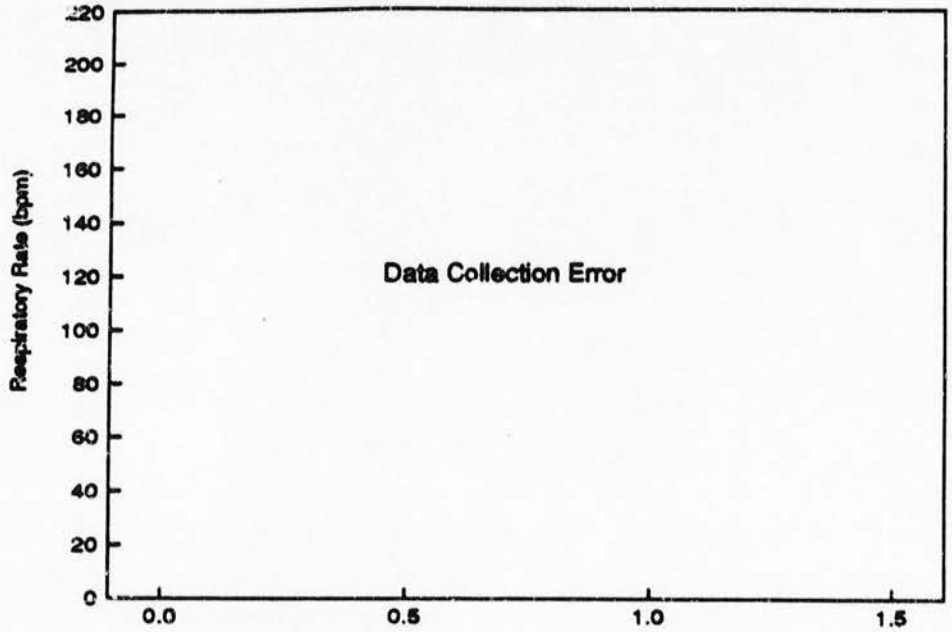
Figure: 54 2,6-TDI Challenge Week 2

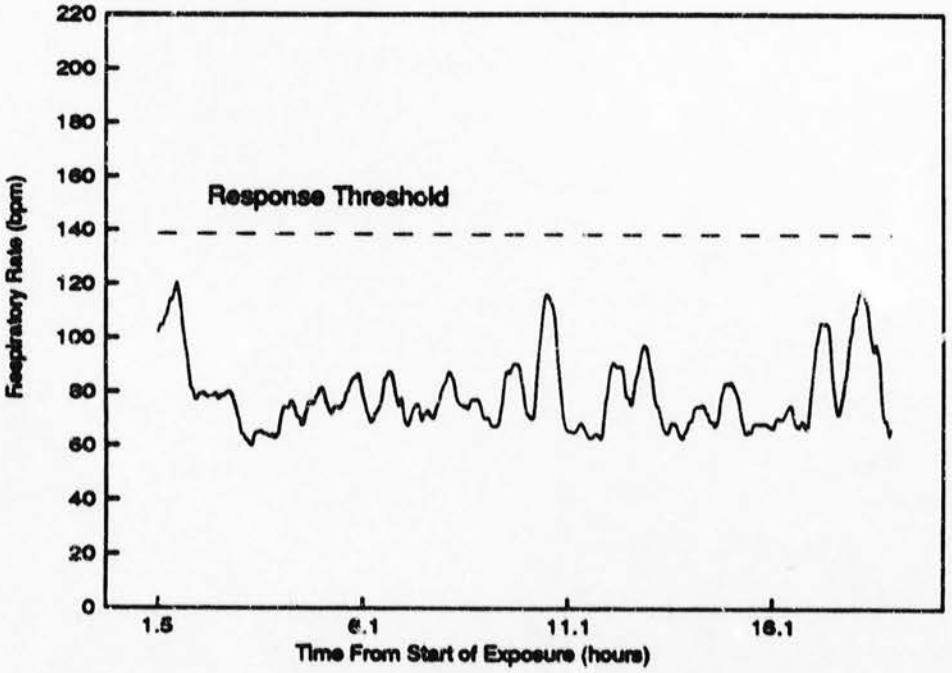


Study Number 91-344-KR

Animal # 65 : 2,4-TDI Sensitization

Figure: 55 2,6-TDI Challenge Week 2

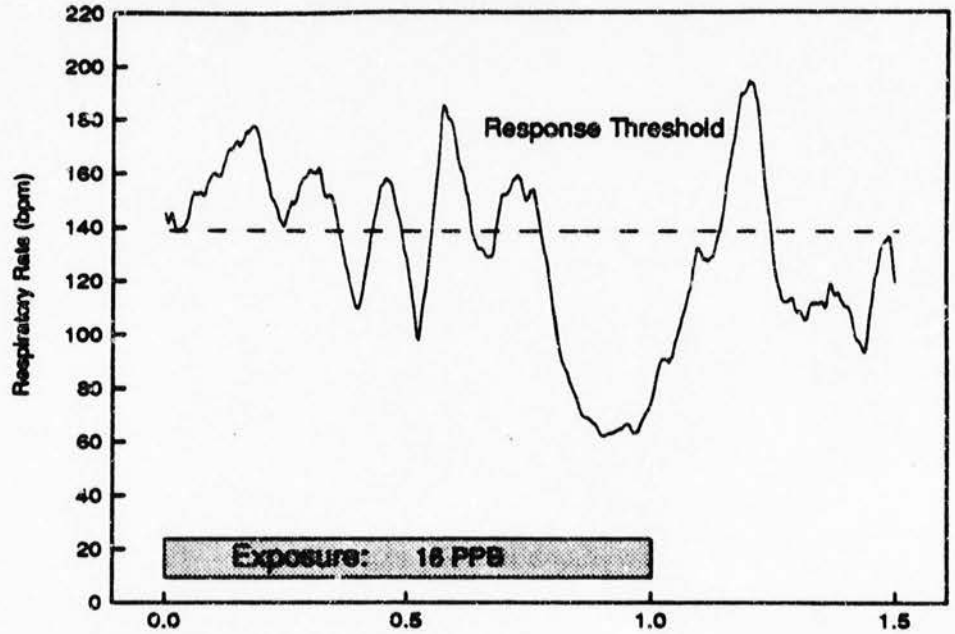


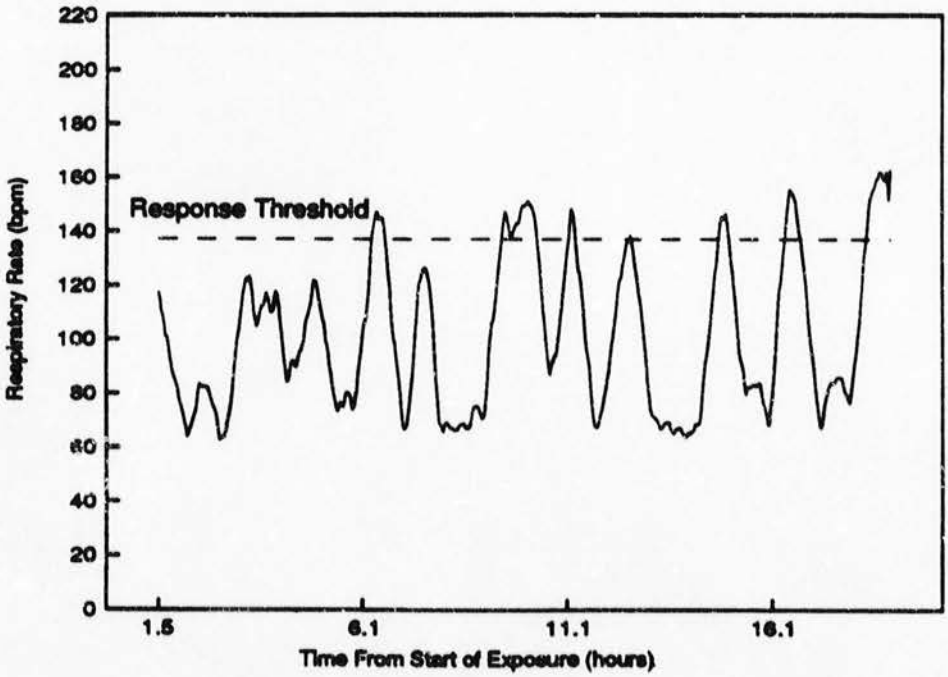


Study Number 91-344-KR

Animal # 66 : 2,4- TDI Sensitization

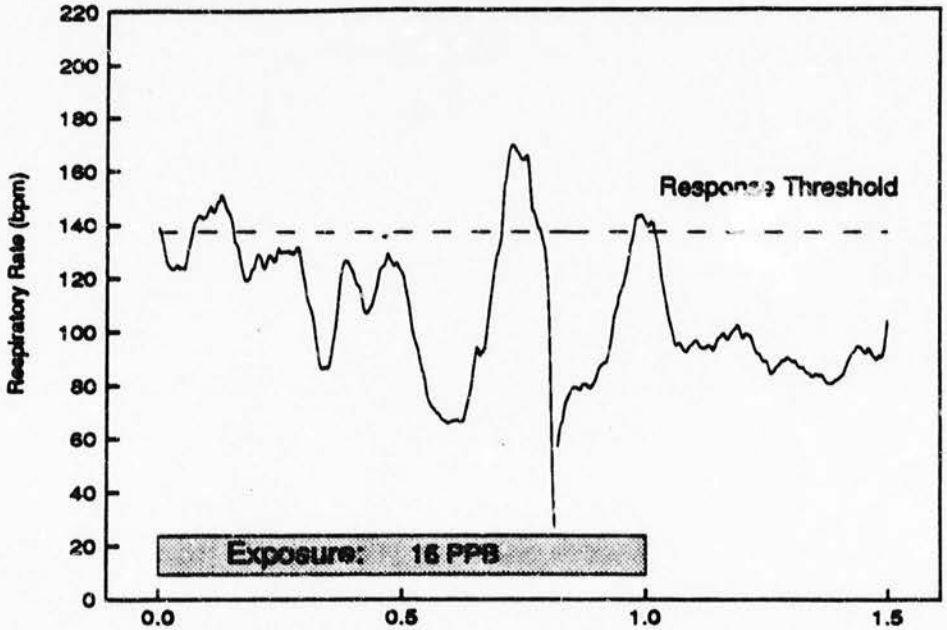
Figure: 56 2,6-TDI Challenge Week 2

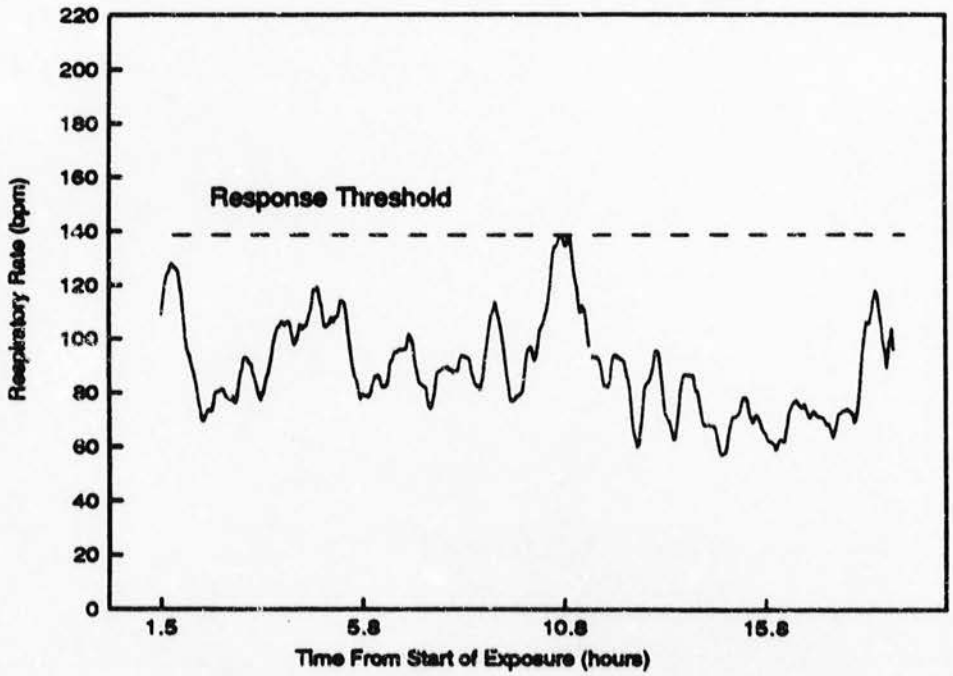




Study Number 91-344-KR
Animal # 67 : 2,4-TDI Sensitization

Figure: 57 2,6-TDI Challenge Week 2

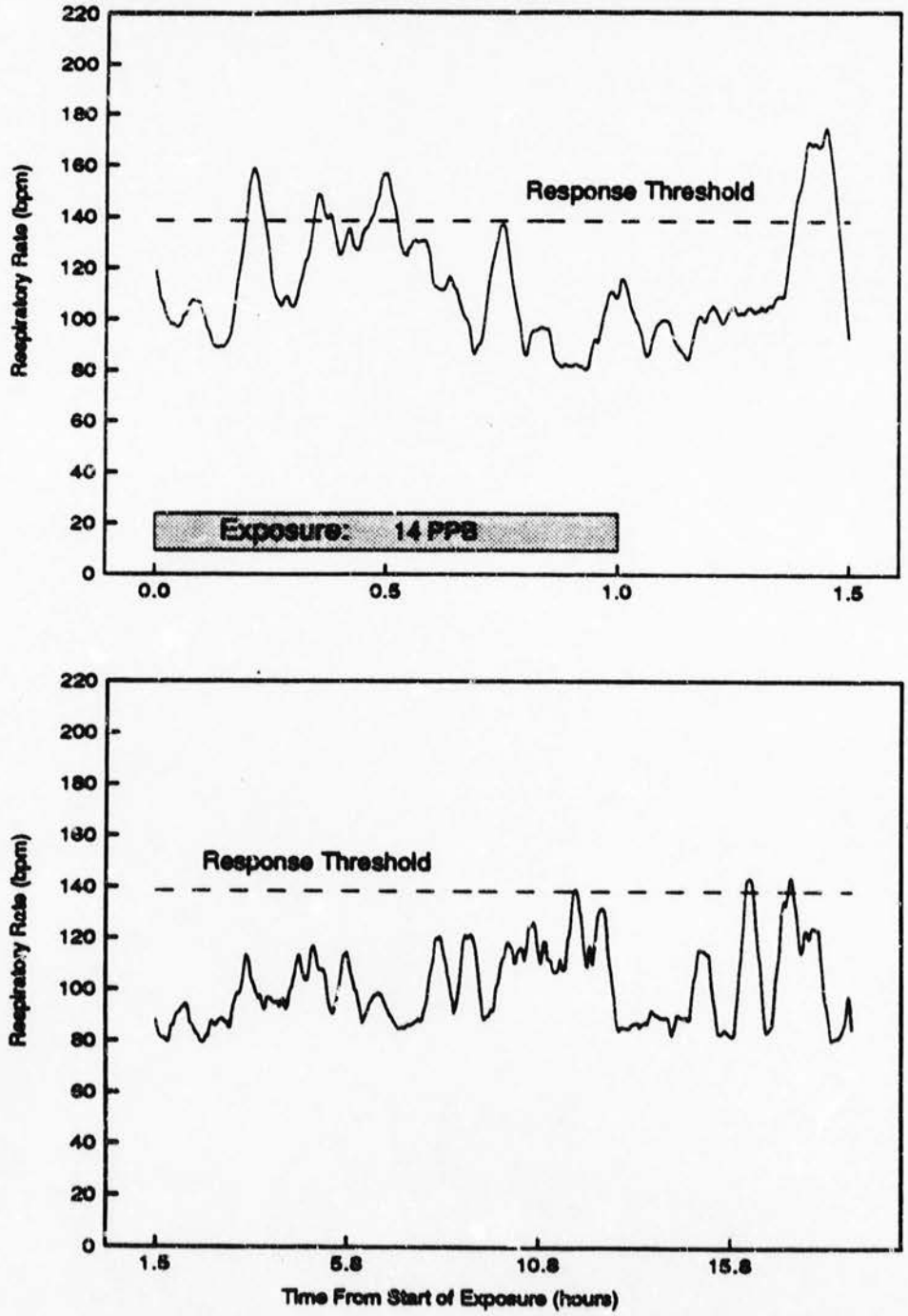




Study Number 91-344-KR

Animal # 68 : 2,4-TDI Sensitization

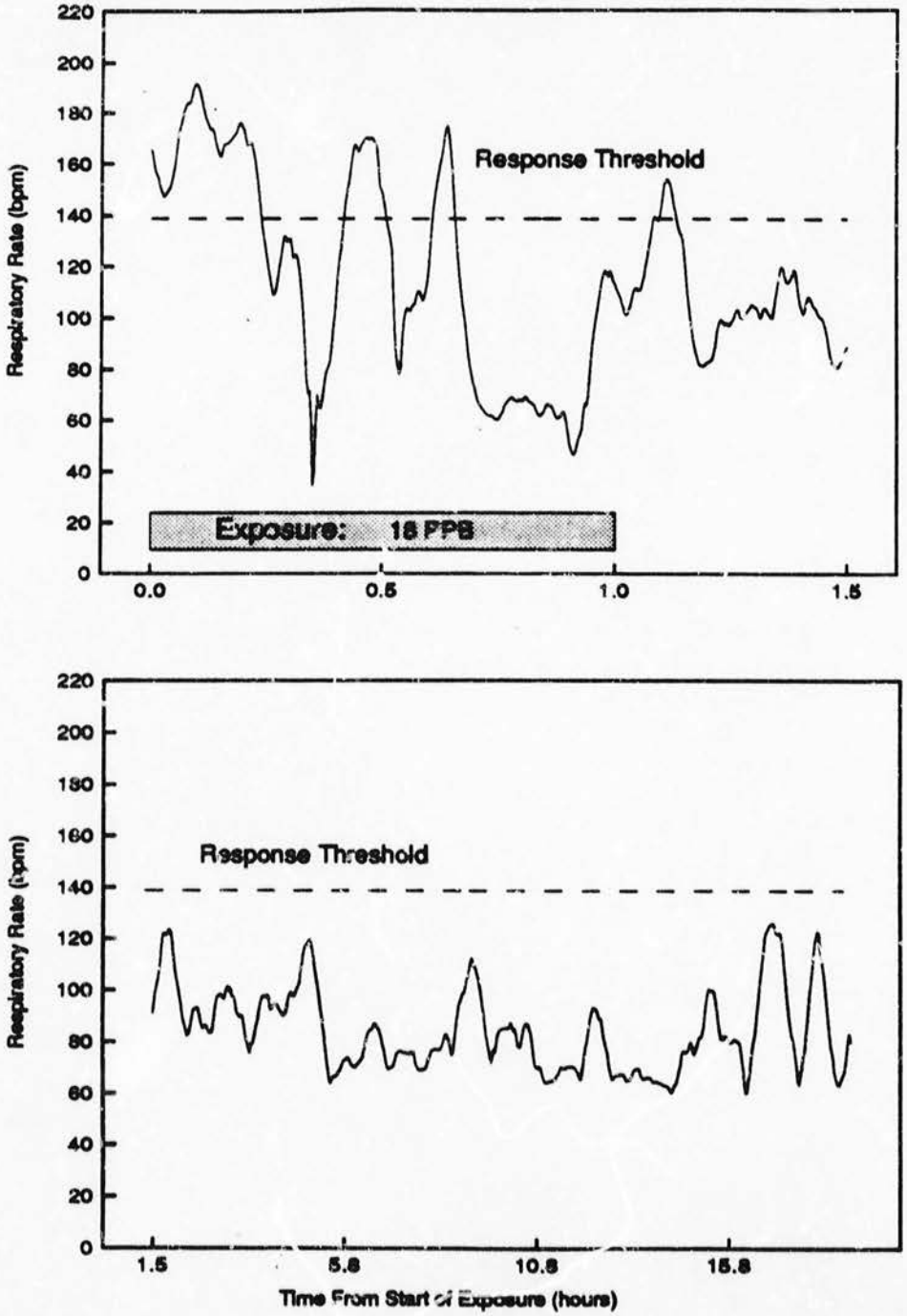
Figure: 58 2,6-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 69 : 2,4-TDI Sensitization

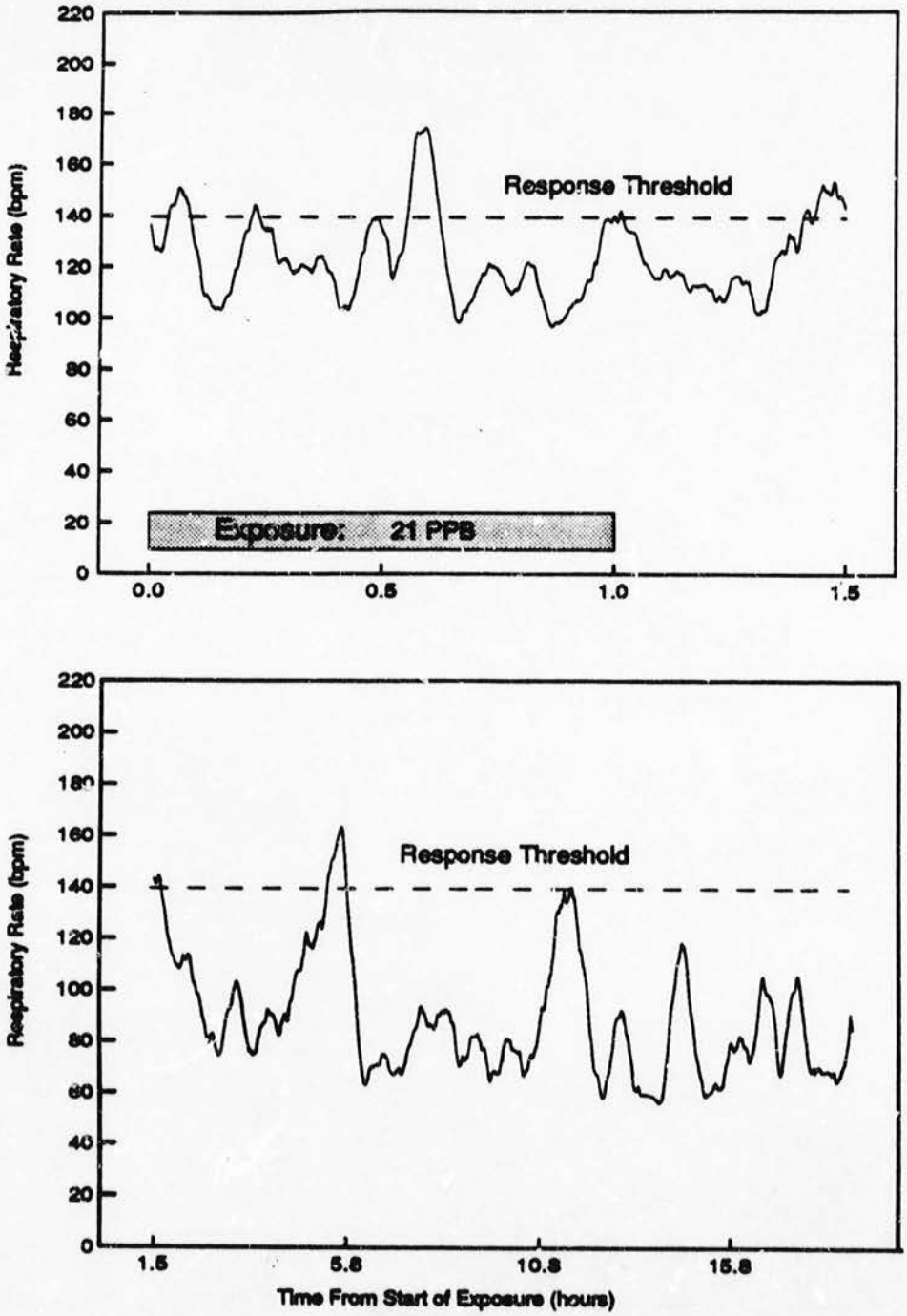
Figure: 59 2,6-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 70 : 2,4-TDI Sensitization

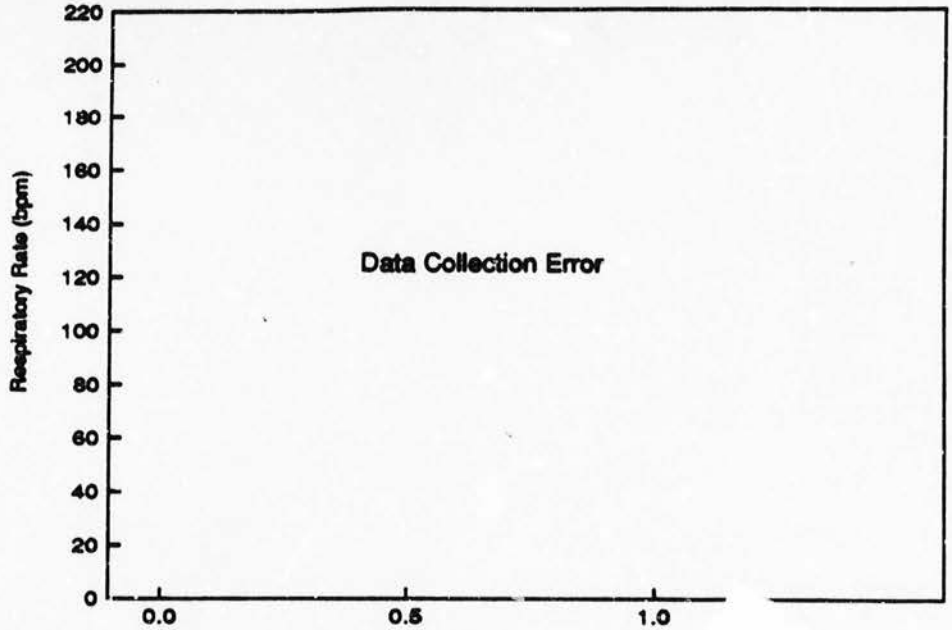
Figure: 60 2,6-TDI Challenge Week 2

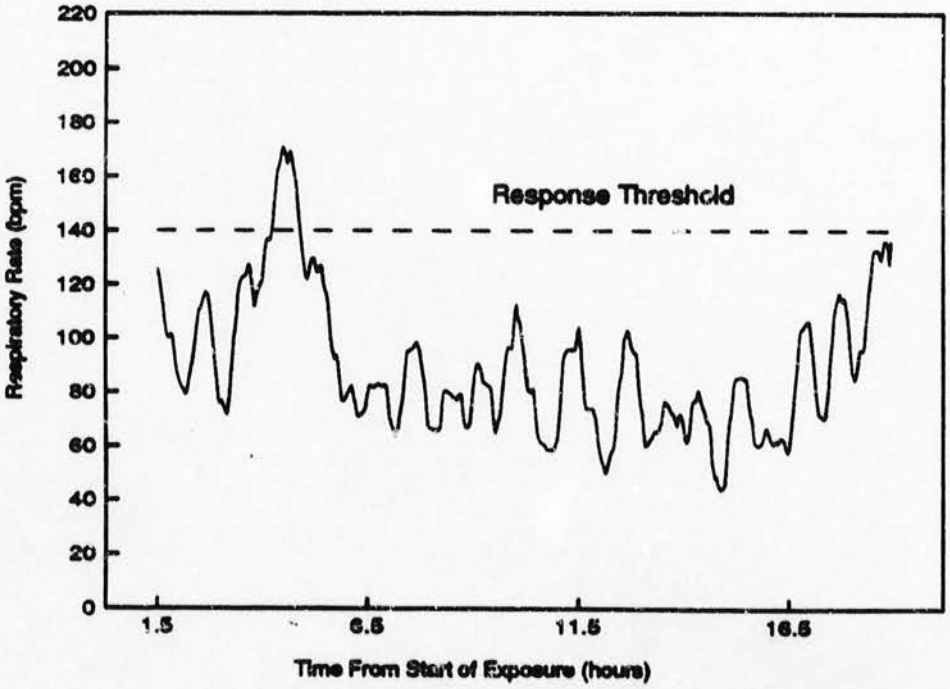


Study Number 91-344-KR

Animal # 59 : 2,4 -TDI Sensitization

Figure: 61 2,6-TDI-GSA Challenge Week 3

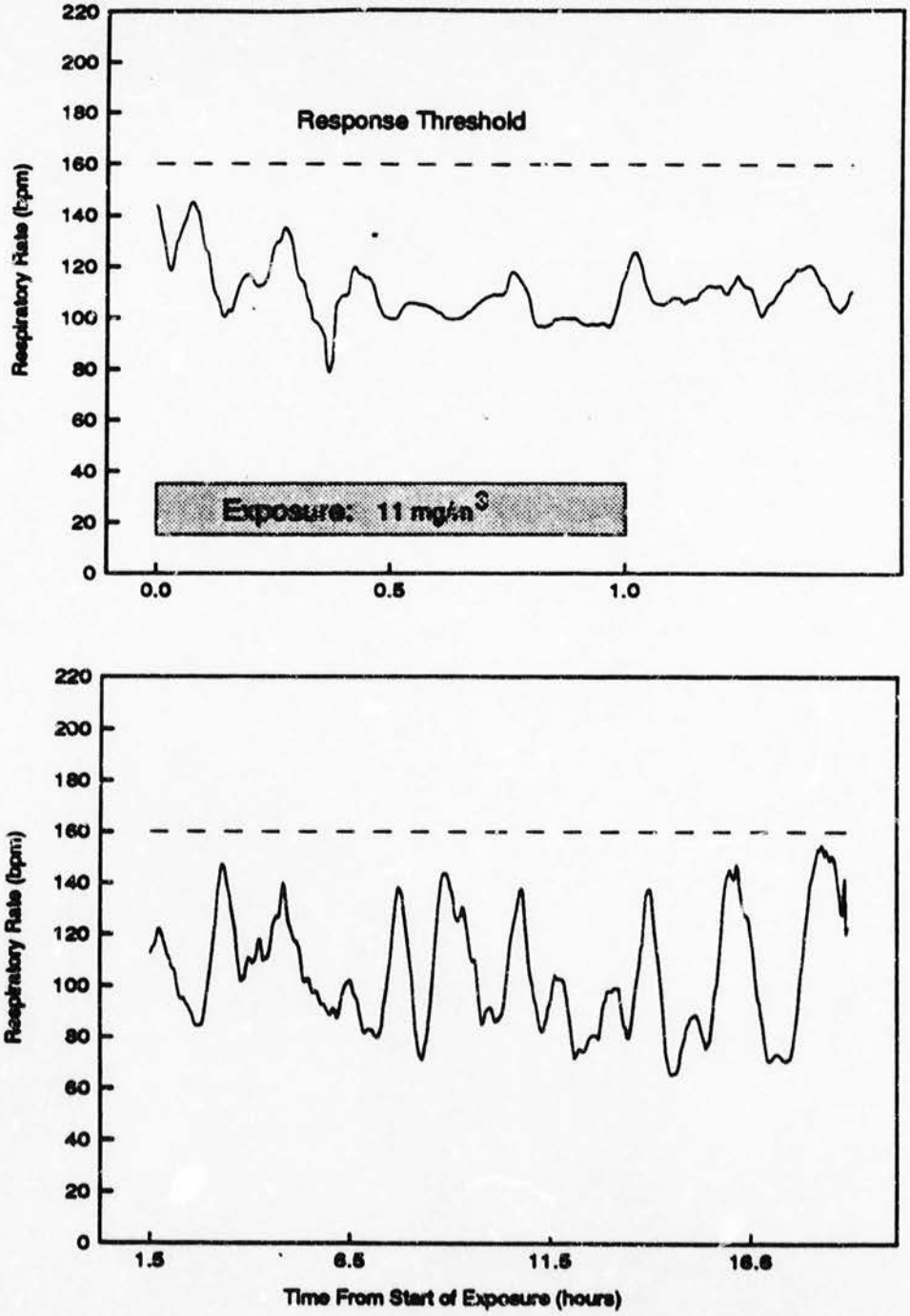




Study Number 91-344-KR

Animal # 60 : 2,4-TDI Sensitization

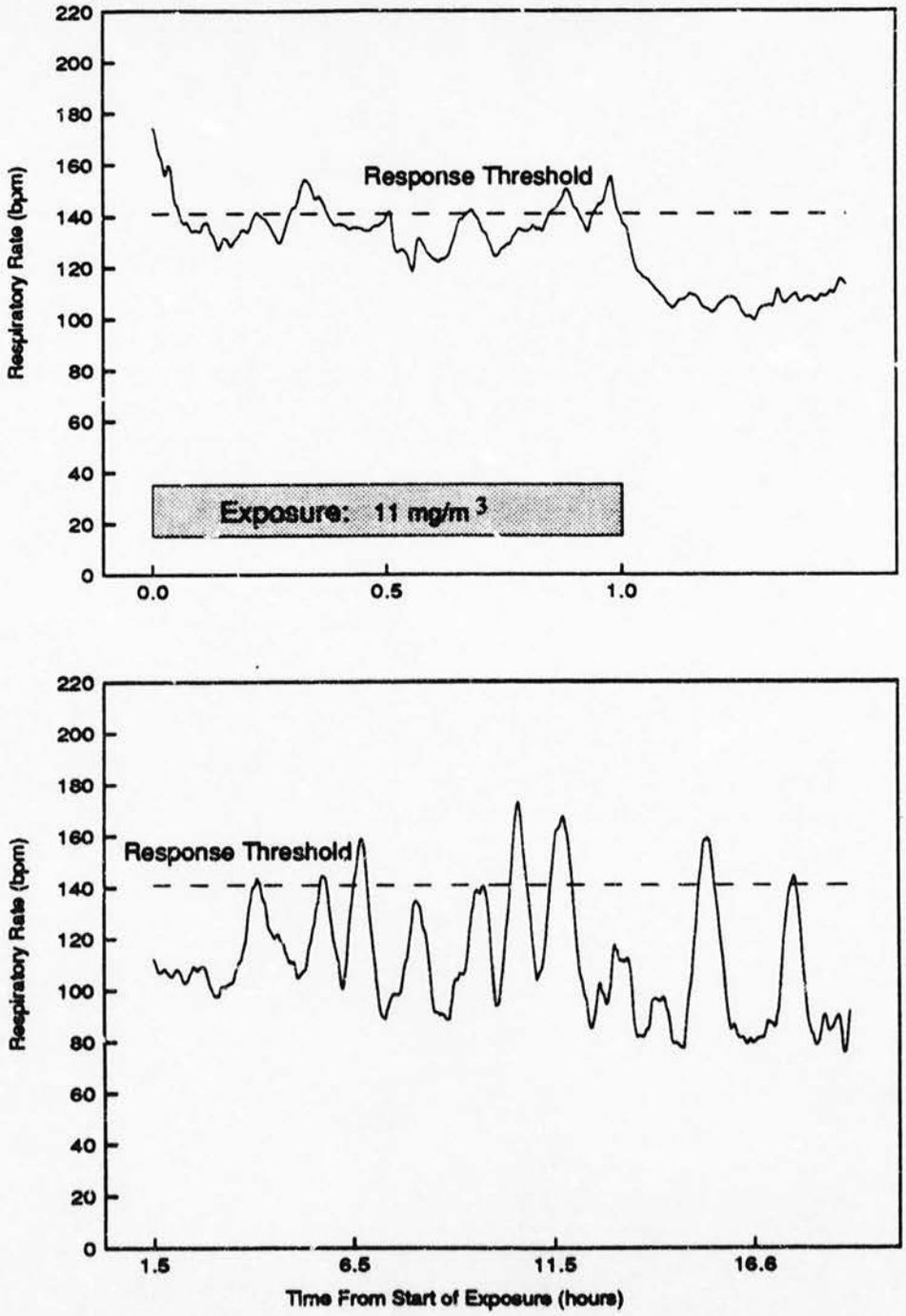
Figure: 62 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 61 : 2,4-TDI Sensitization

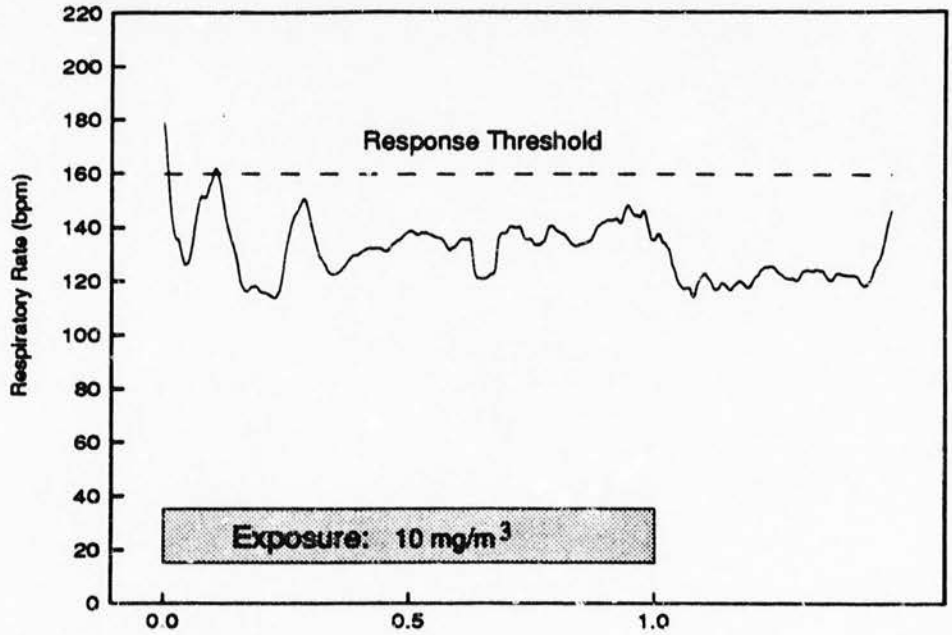
Figure: 63 2,6-TDI-GSA Challenge Week 3

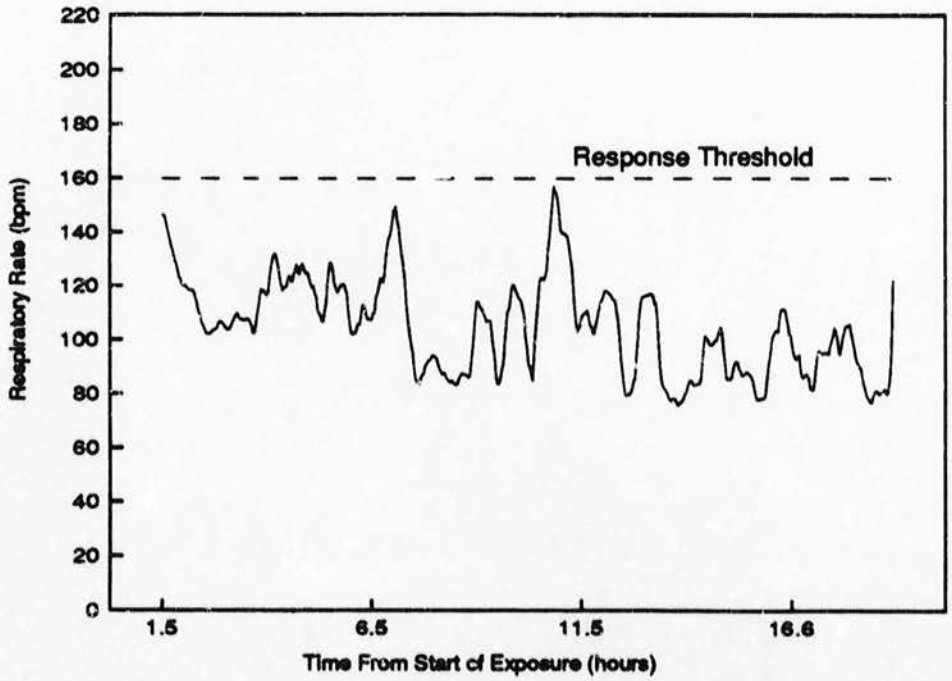


Study Number 91-344-KR

Animal # 62 : 2,4-TDI Sensitization

Figure: 64 2,6-TDI-GSA Challenge Week 3

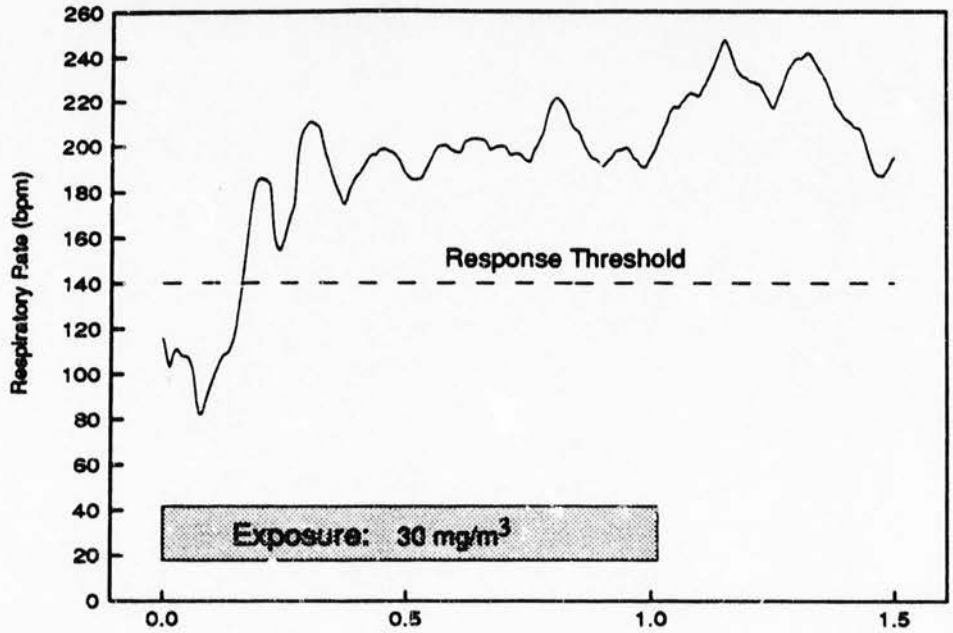


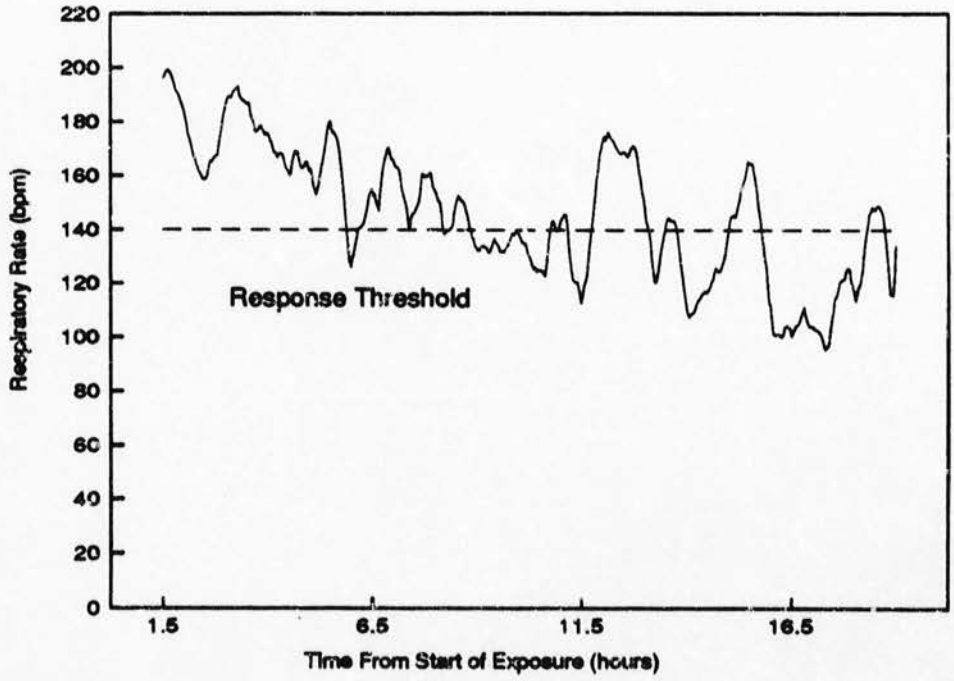


Study Number 91-344-KR

Animal # 63 : 2,4 -TDI Sensitization

Figure: 65 2,6-TDI-GSA Challenge Week 3

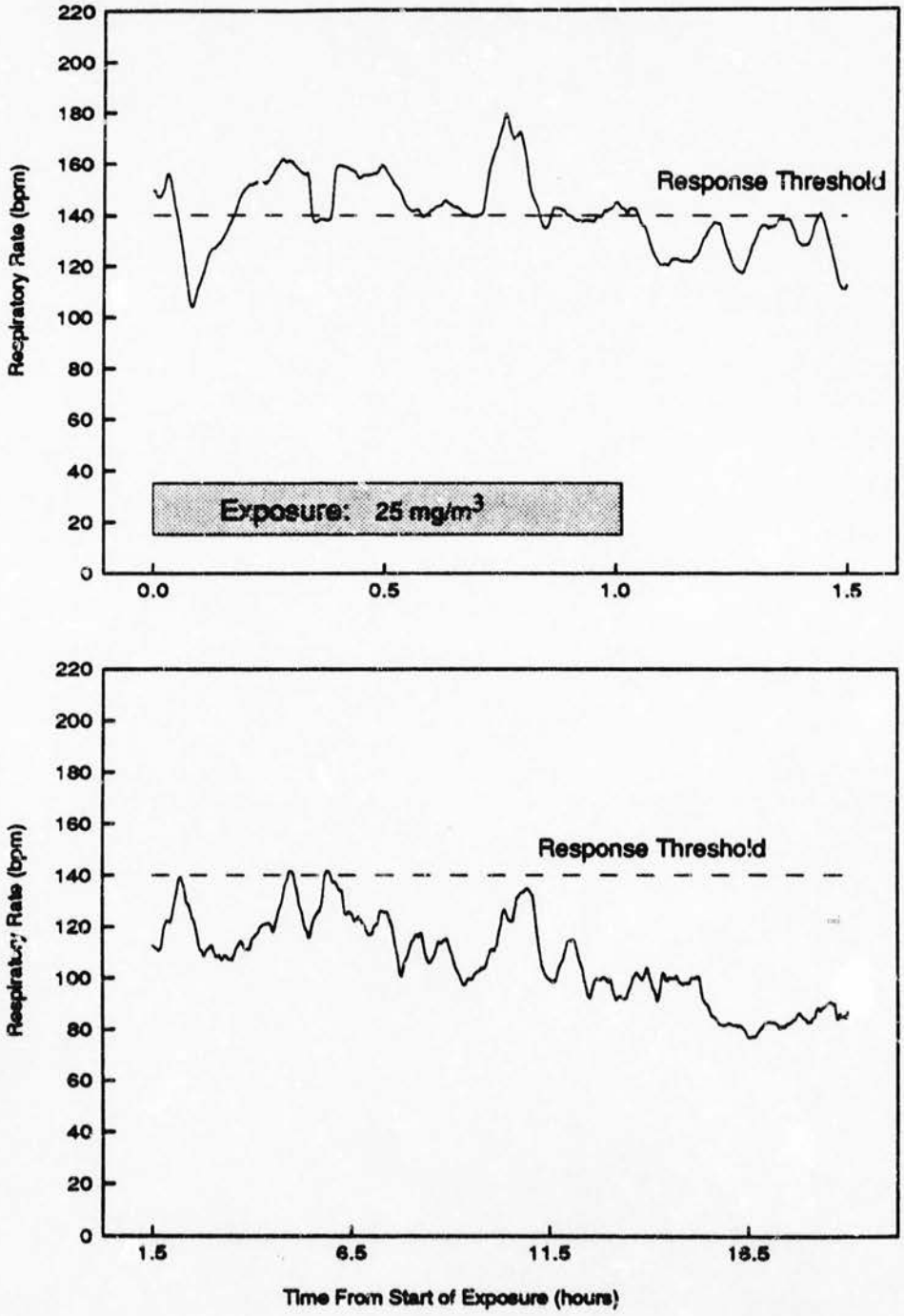




Study Number 91-344-KR

Animal # 64 : 2,4-TDI Sensitization

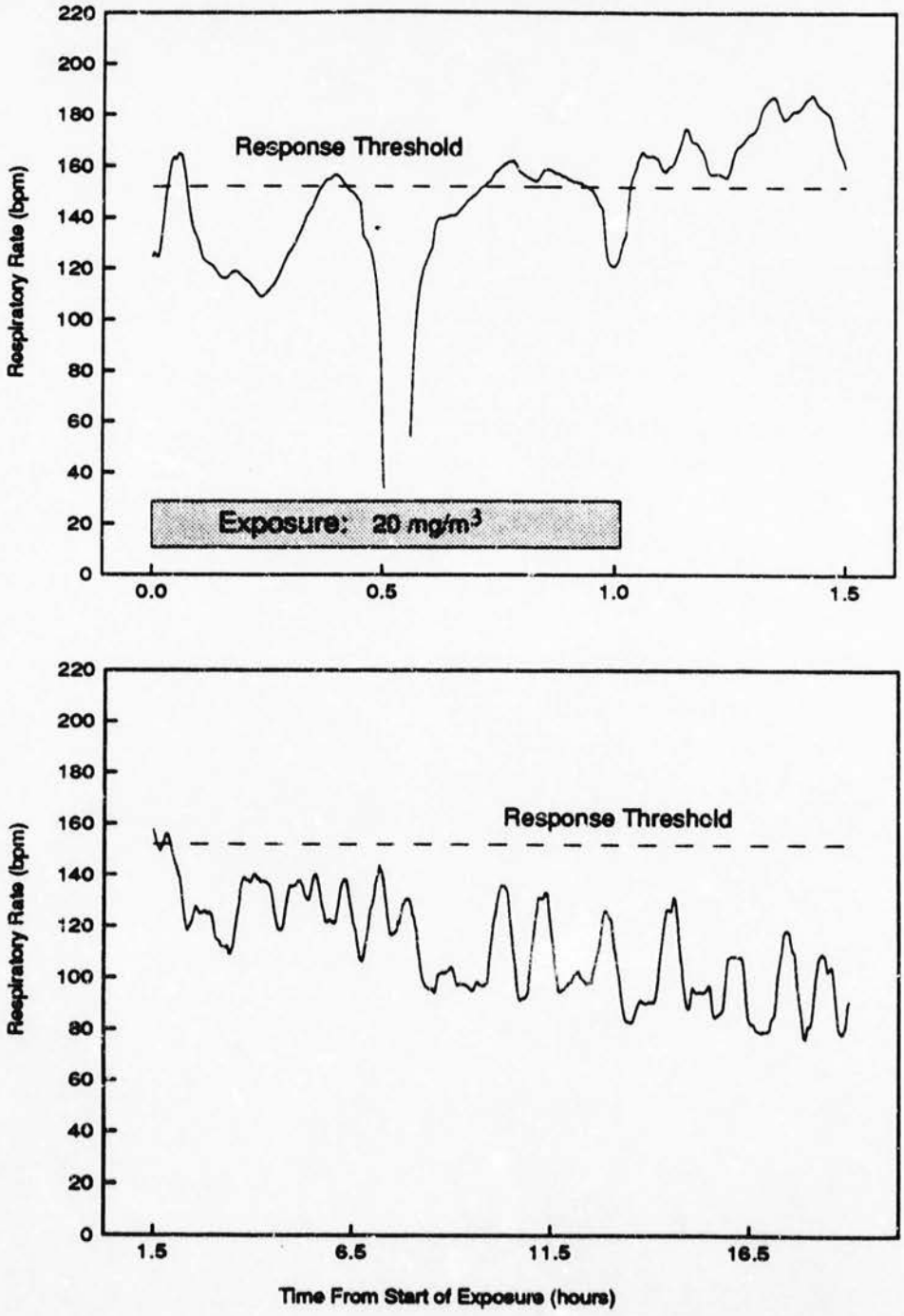
Figure: 66 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 65 : 2,4-TDI Sensitization

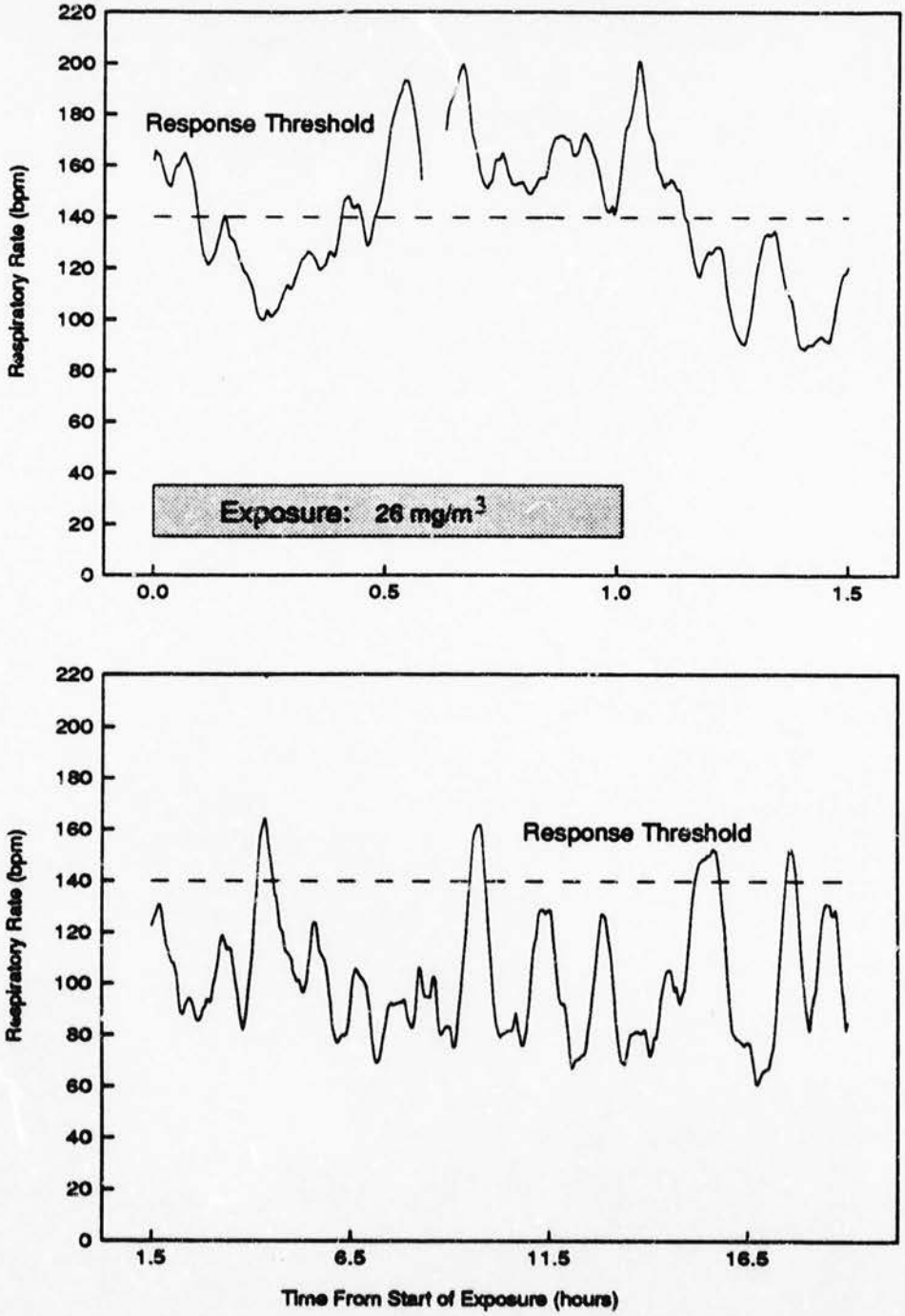
Figure: 67 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 66 : 2,4-TDI Sensitization

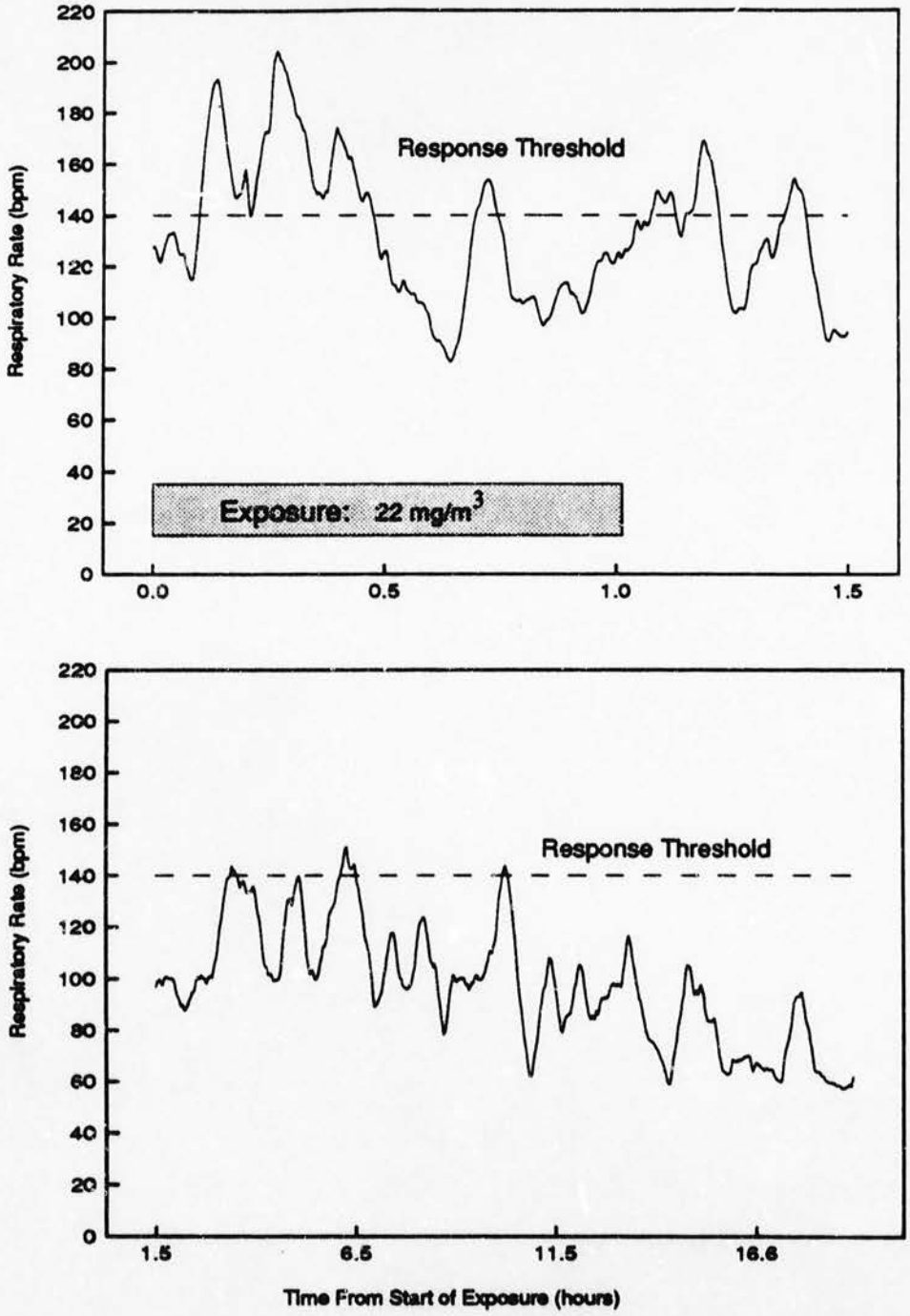
Figure: 68 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 67 : 2,4-TDI Sensitization

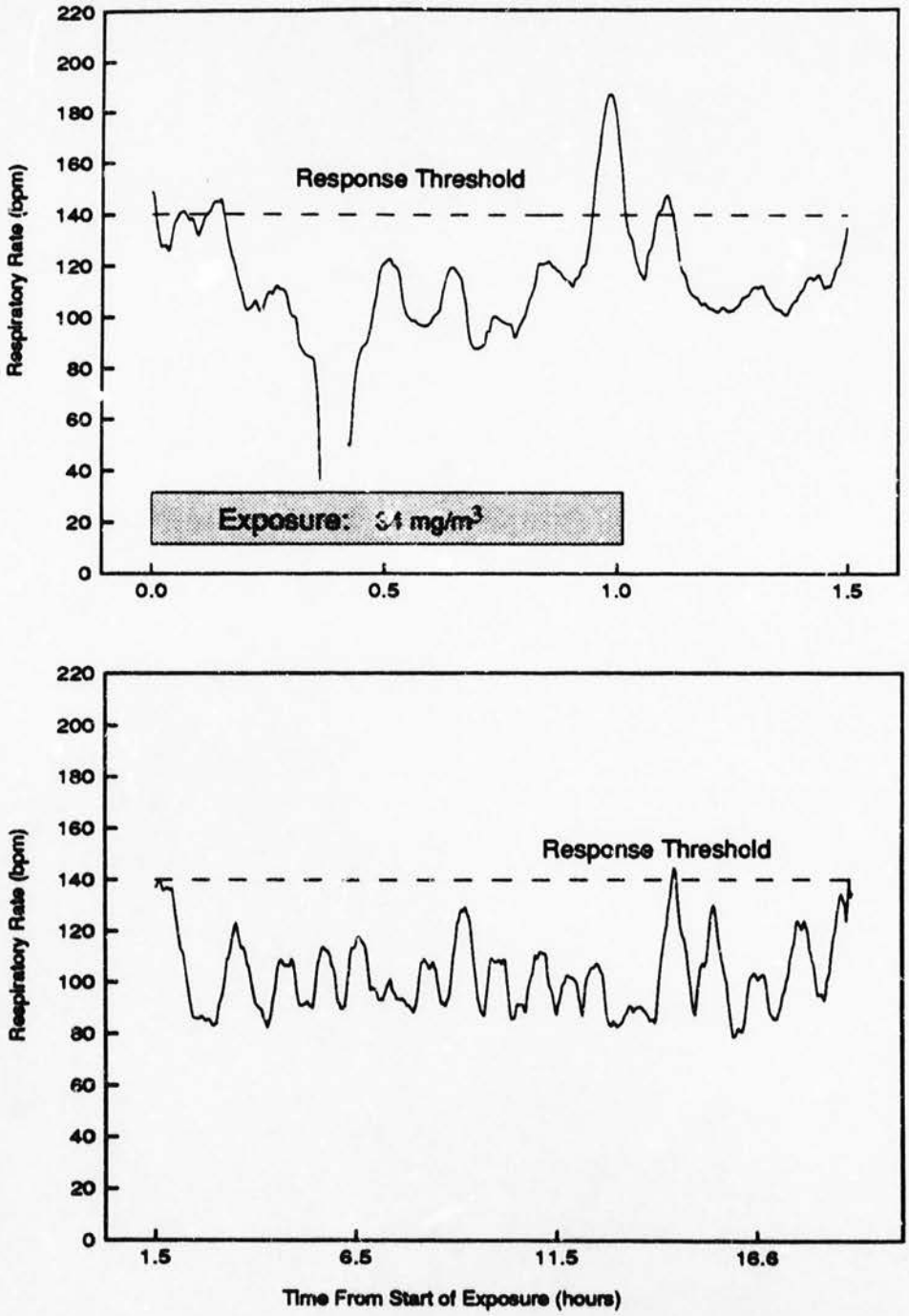
Figure: 69 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 68 : 2,4-TDI Sensitization

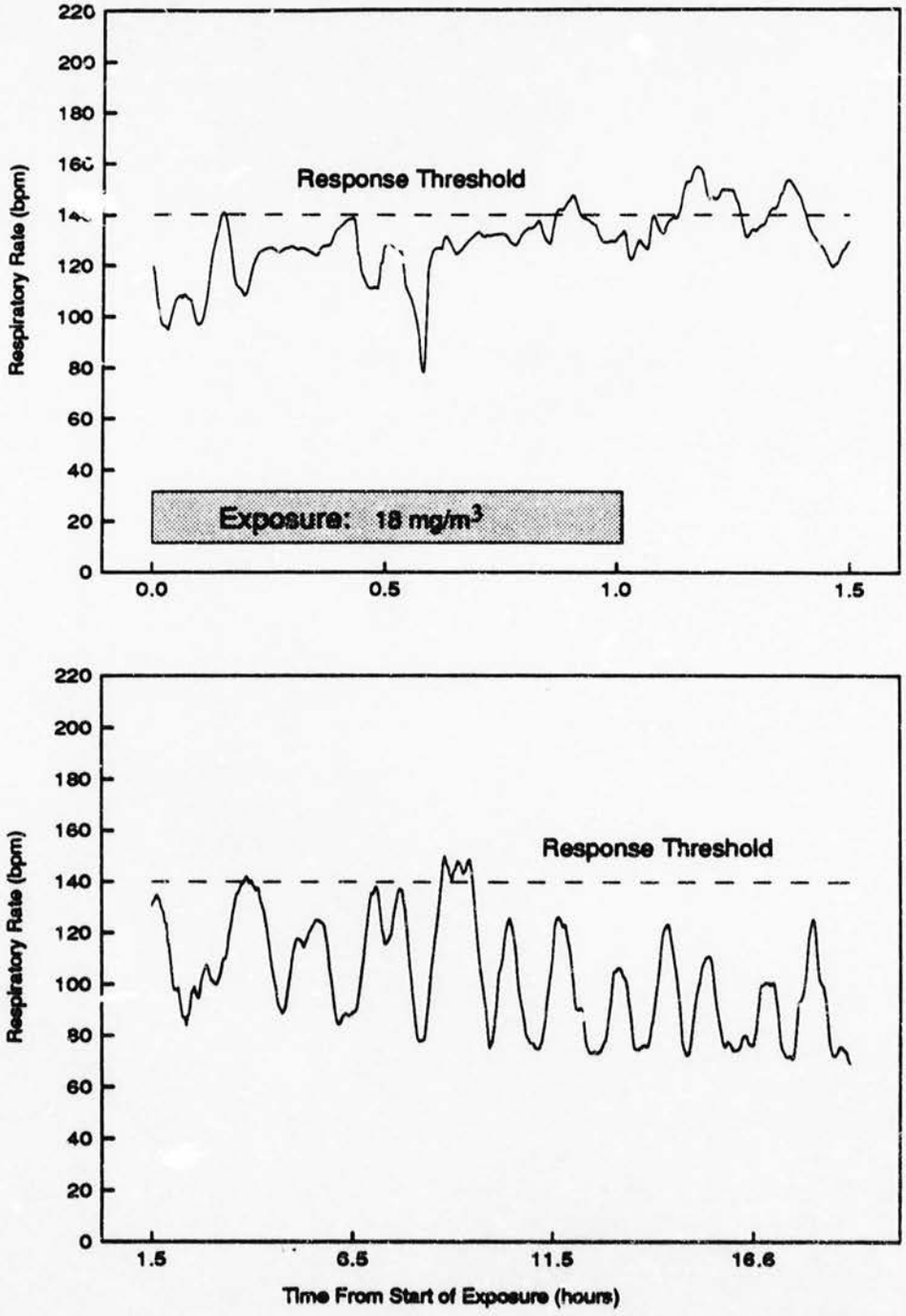
Figure: 70 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 69 : 2,4-TDI Sensitization

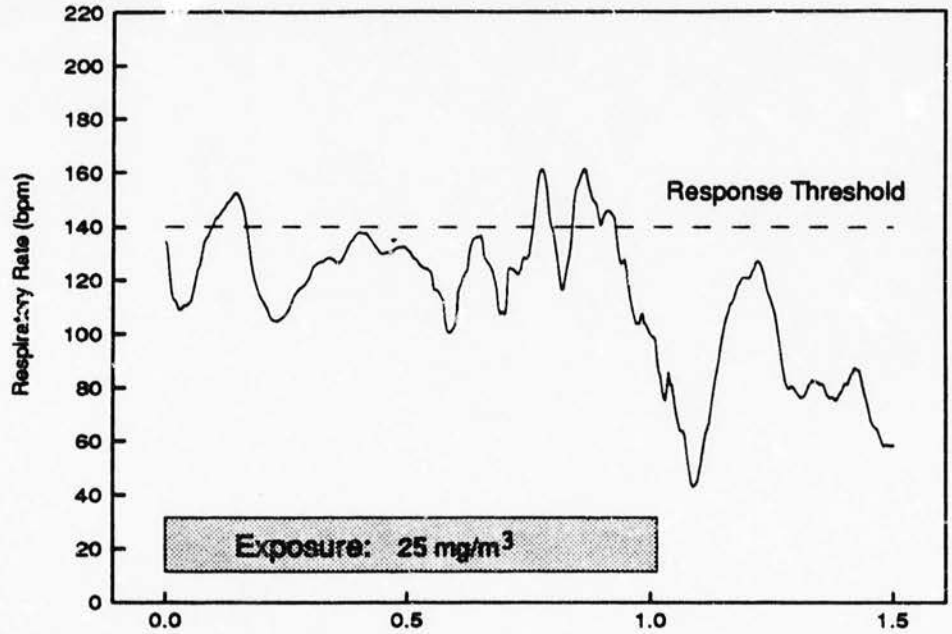
Figure: 71 2,6-TDI-GSA Challenge Week 3

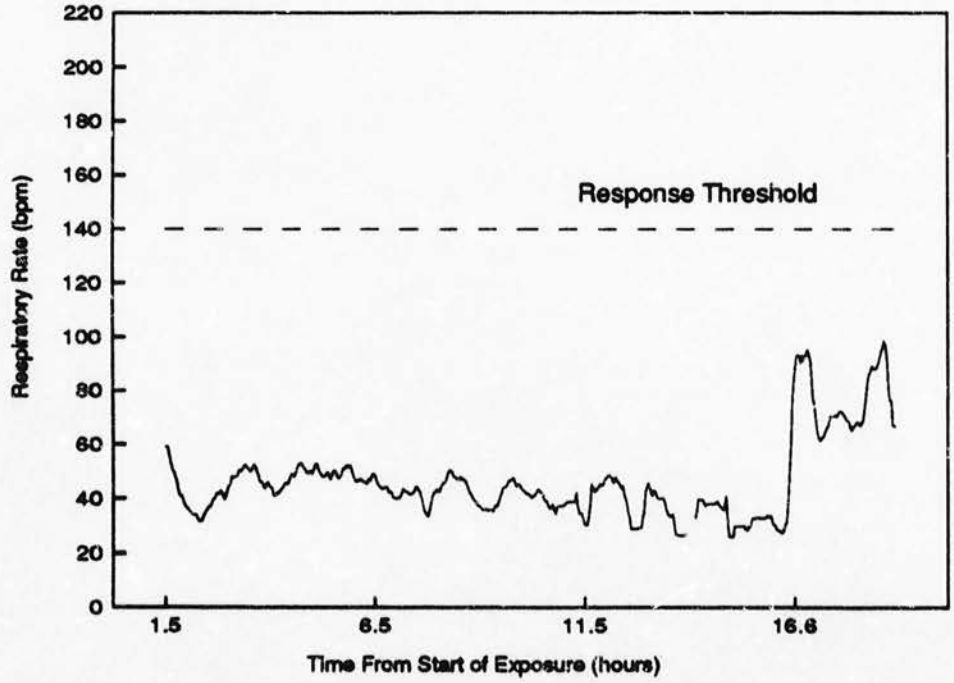


Study Number 91-344-KR

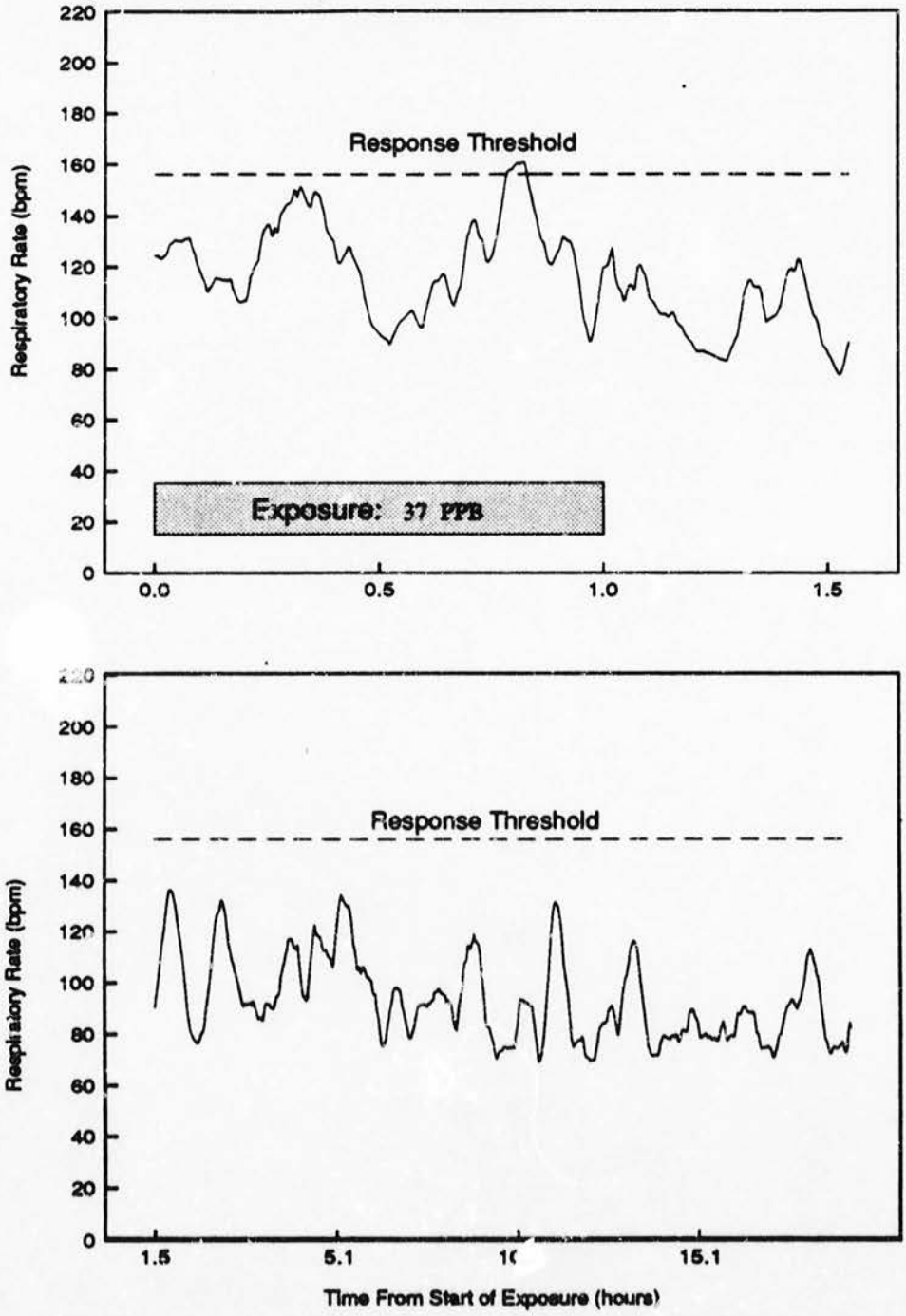
Animal # 70 : 2,4-TDI Sensitization

Figure 72 2,6-TDI-GSA Challenge Week 3





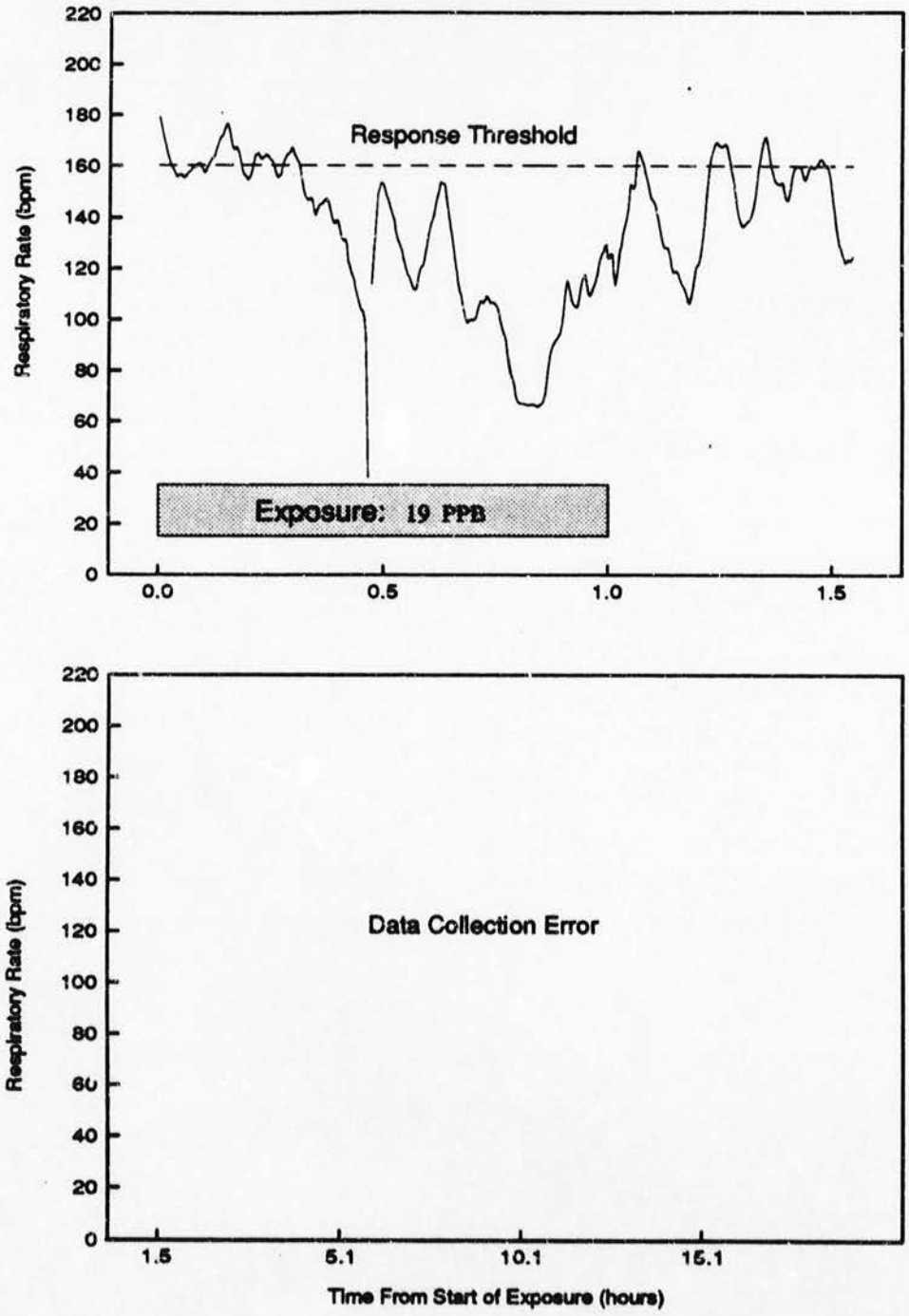
Study Number 91-344-KR
Animal # 44: 2,6-TDI Sensitization
Figure: 73 2,4-TDI Challenge Week 1



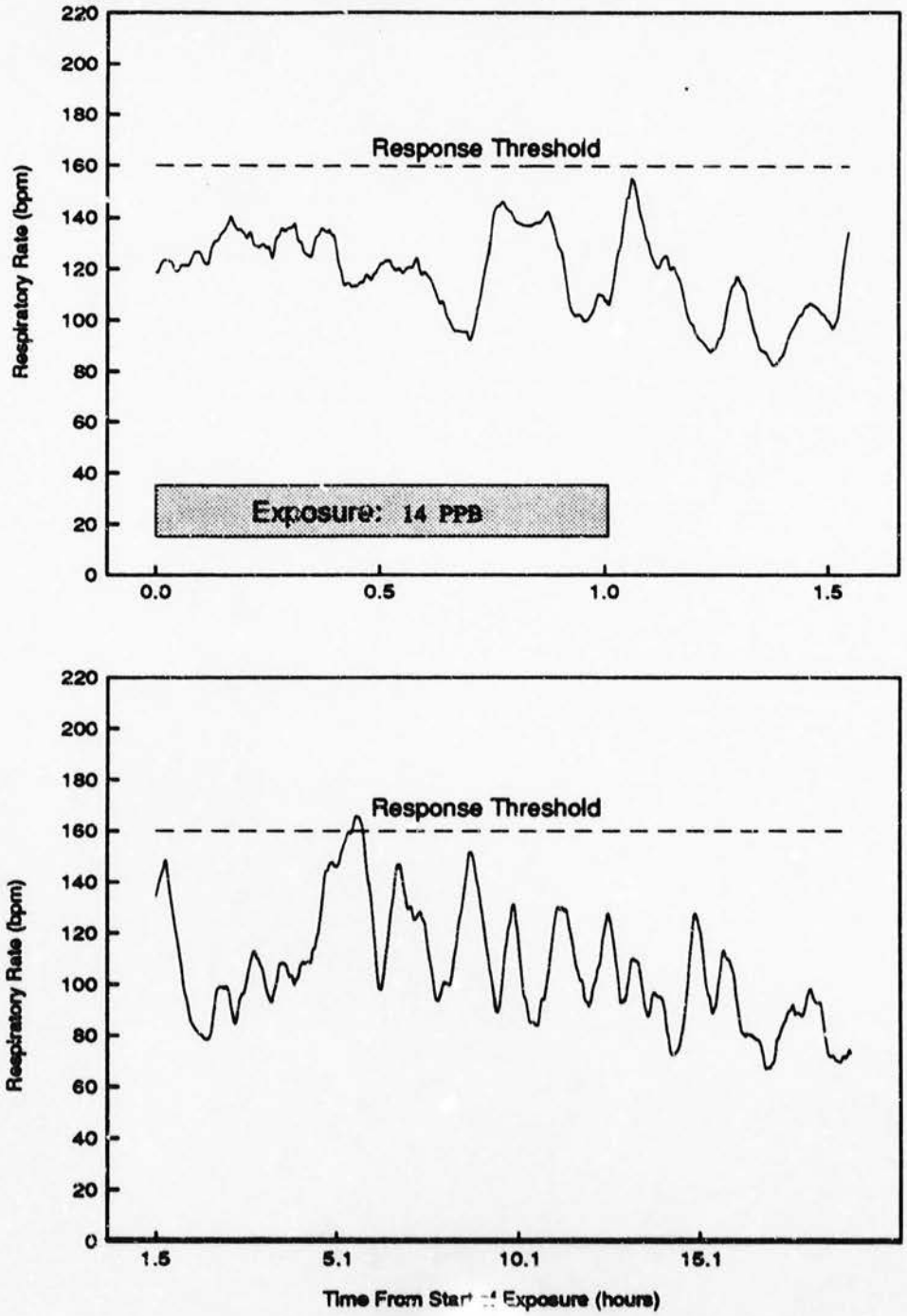
Study Number 91-344-KR

Animal # 45: 2,6-TDI Sensitization

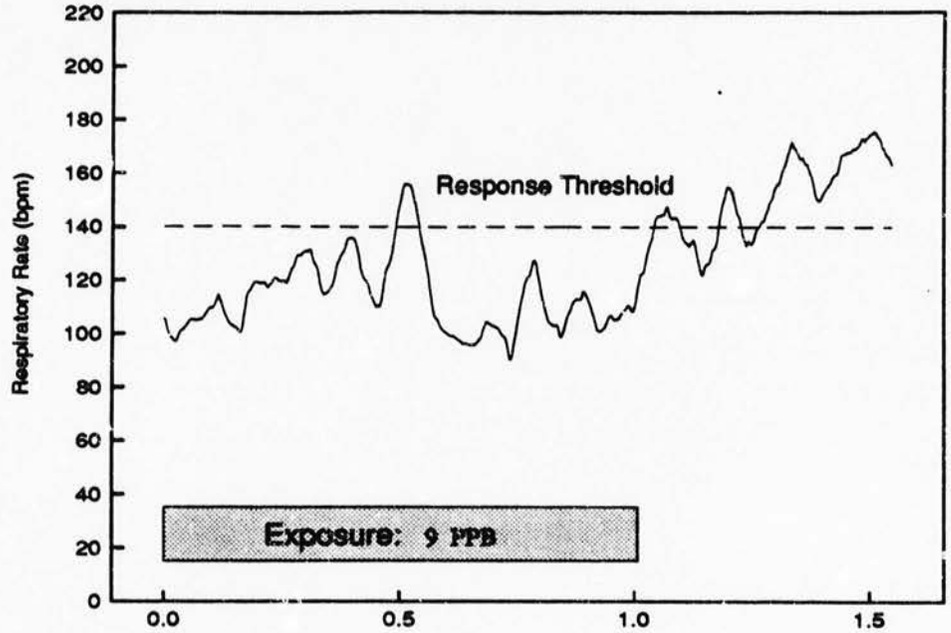
Figure: 74 2,4-TDI Challenge Week 1

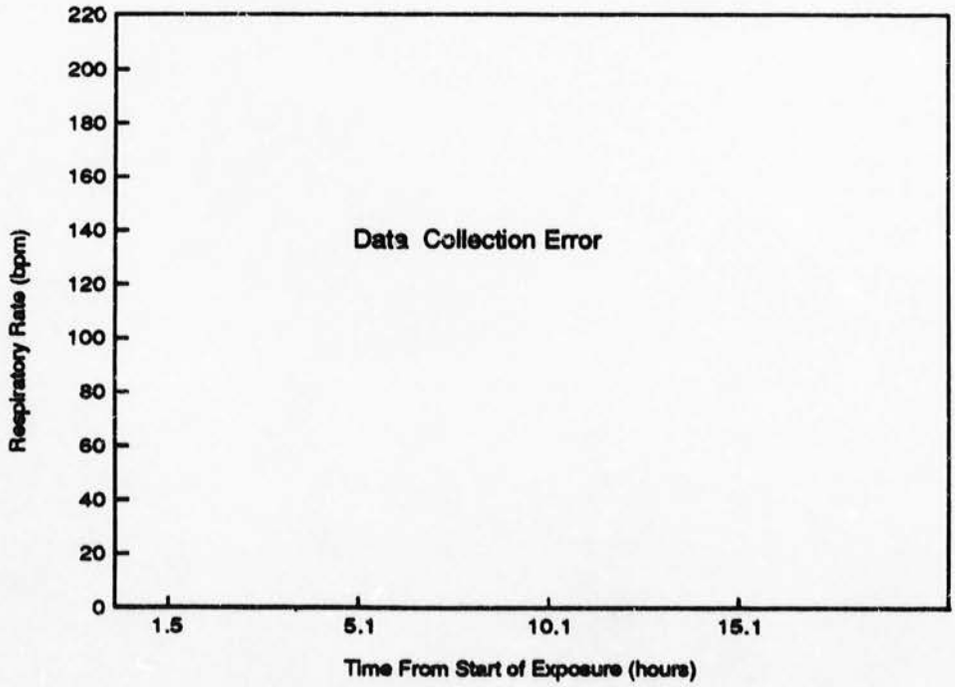


Study Number 91-344-KR
Animal # 46: 2,6-TDI Sensitization
Figure: 75 2,4-TDI Challenge Week 1



Study Number 91-344-KR
Animal # 47: 2,6-TDI Sensitization
Figure: 76 2,4-TDI Challenge Week 1

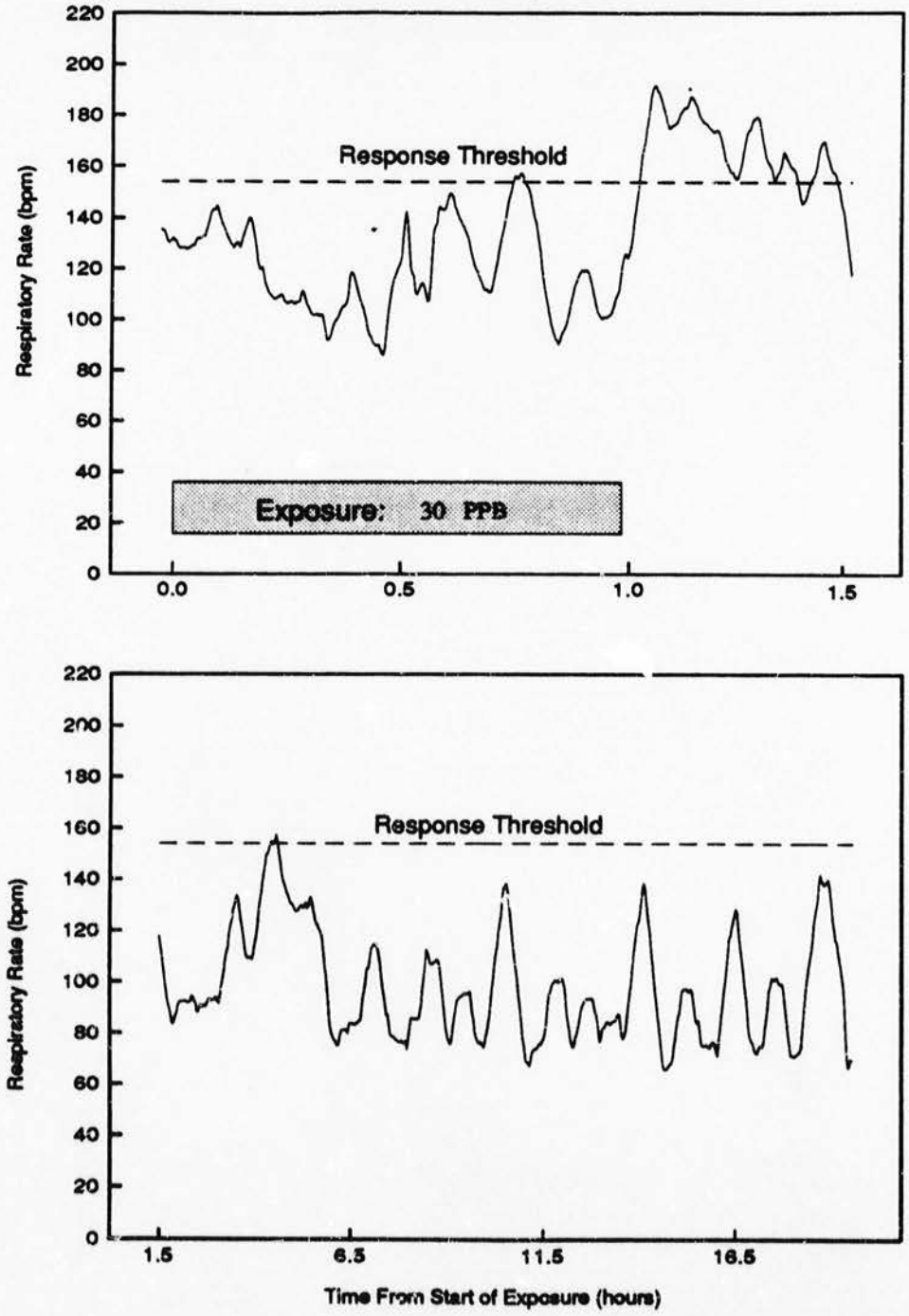




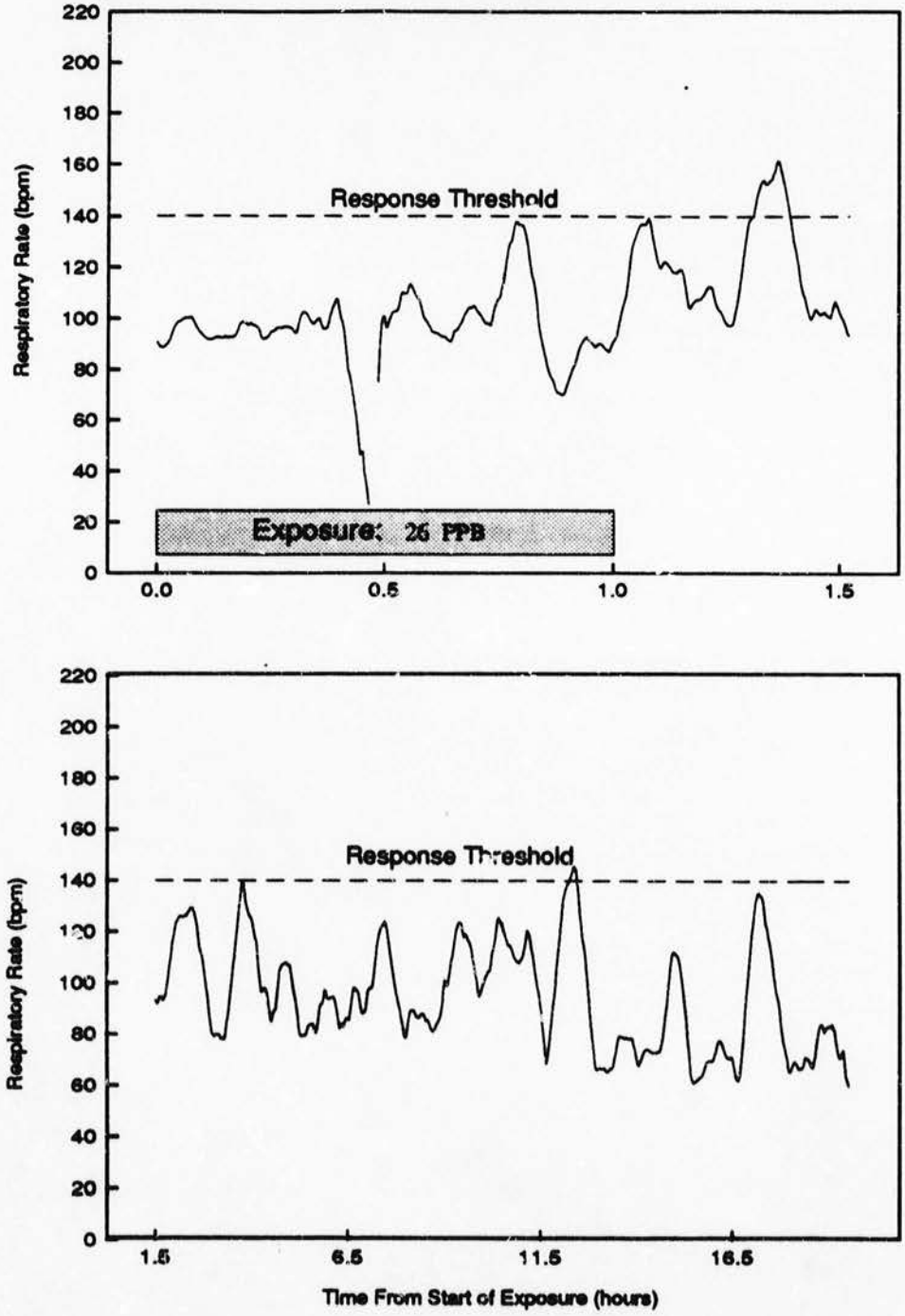
Study Number 91-344-KR

Animal # 48: 2,6-TDI Sensitization

Figure: 77 2,4-TDI Challenge Week 1



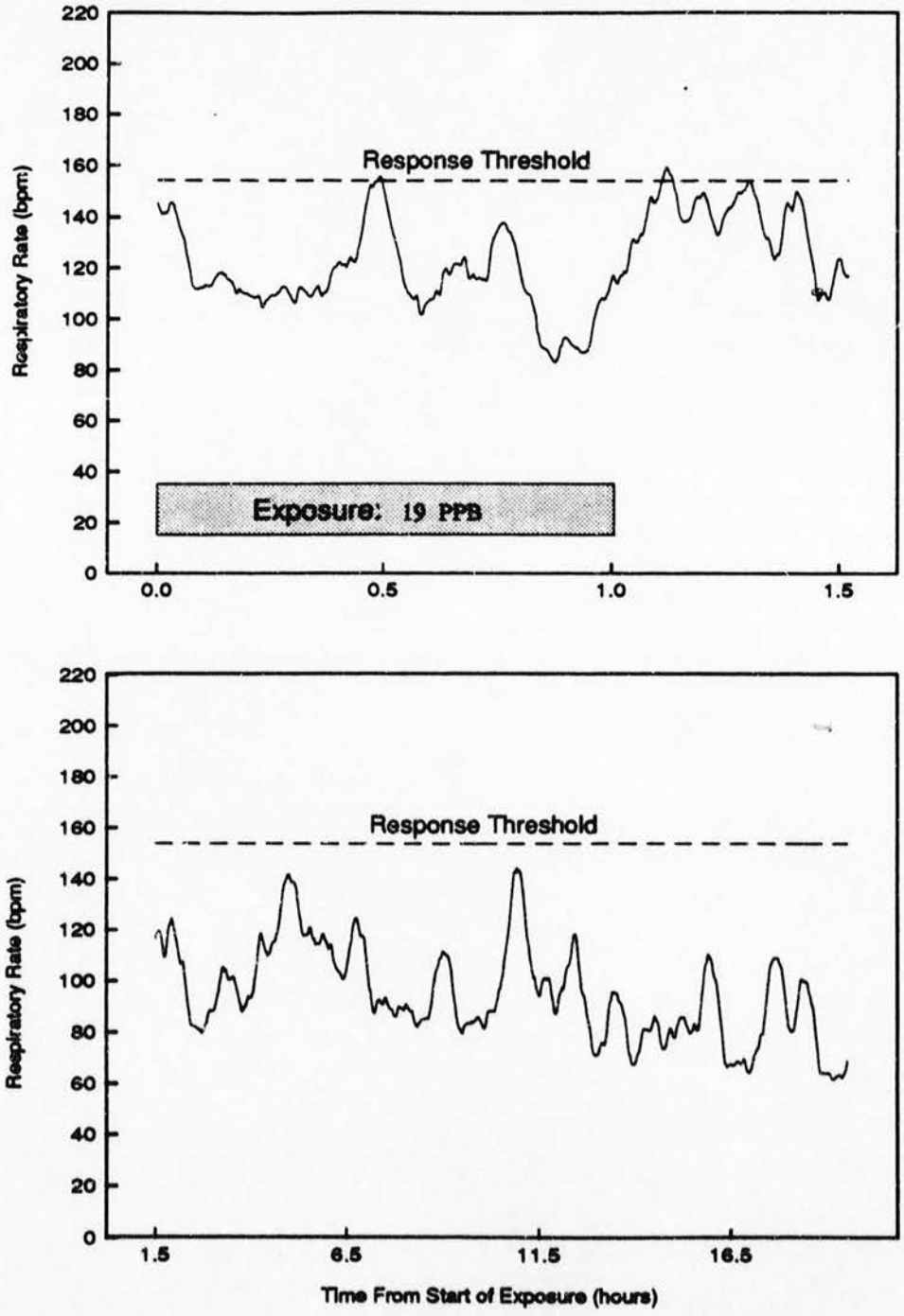
Study Number 91-344-KR
Animal # 49: 2,6-TDI Sensitization
Figure: 78 2,4-TDI Challenge Week 1



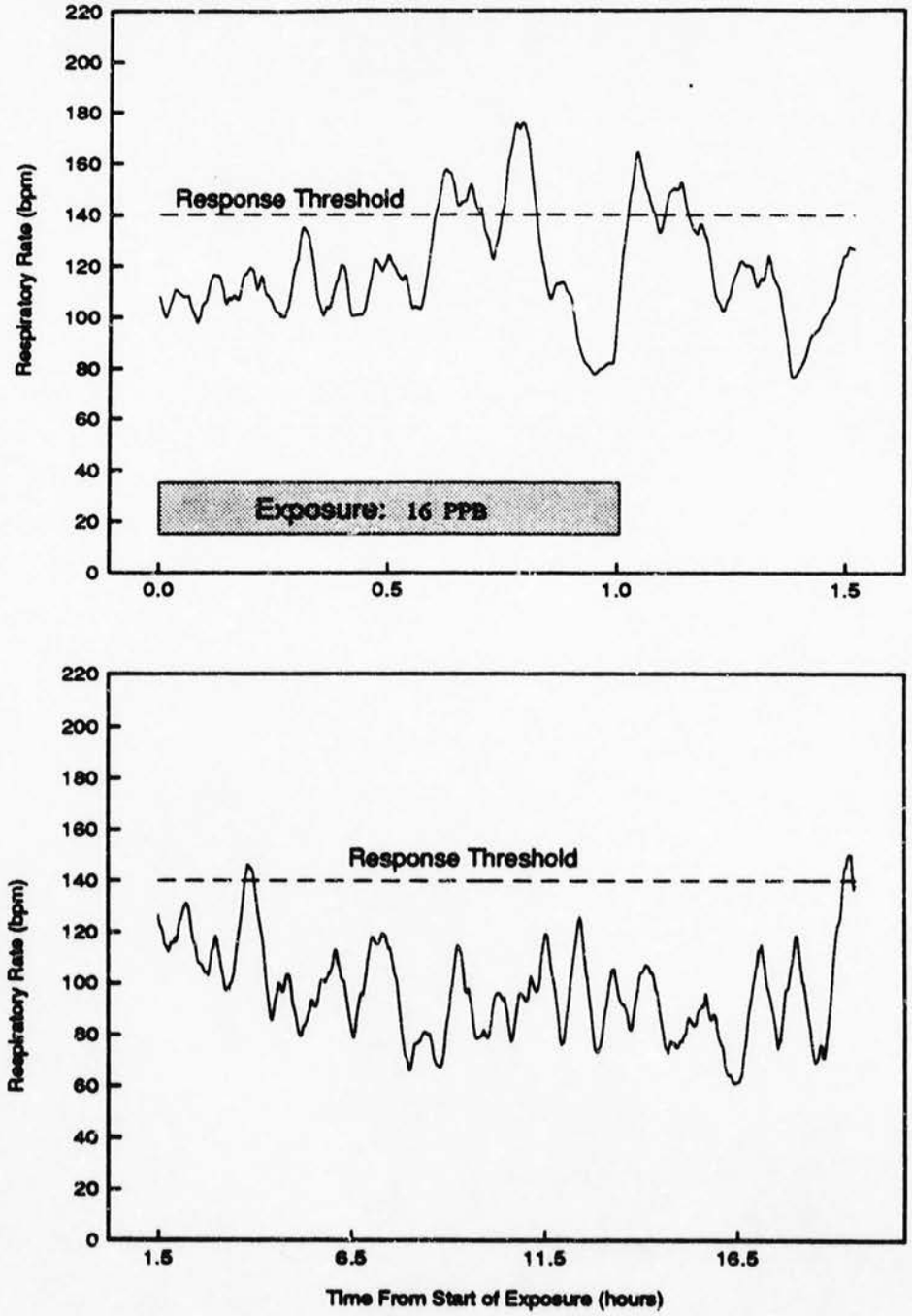
Study Number 91-344-KR

Animal # 51: 2,6-TDI Sensitization

Figure: 79 2,4-TDI Challenge Week 1

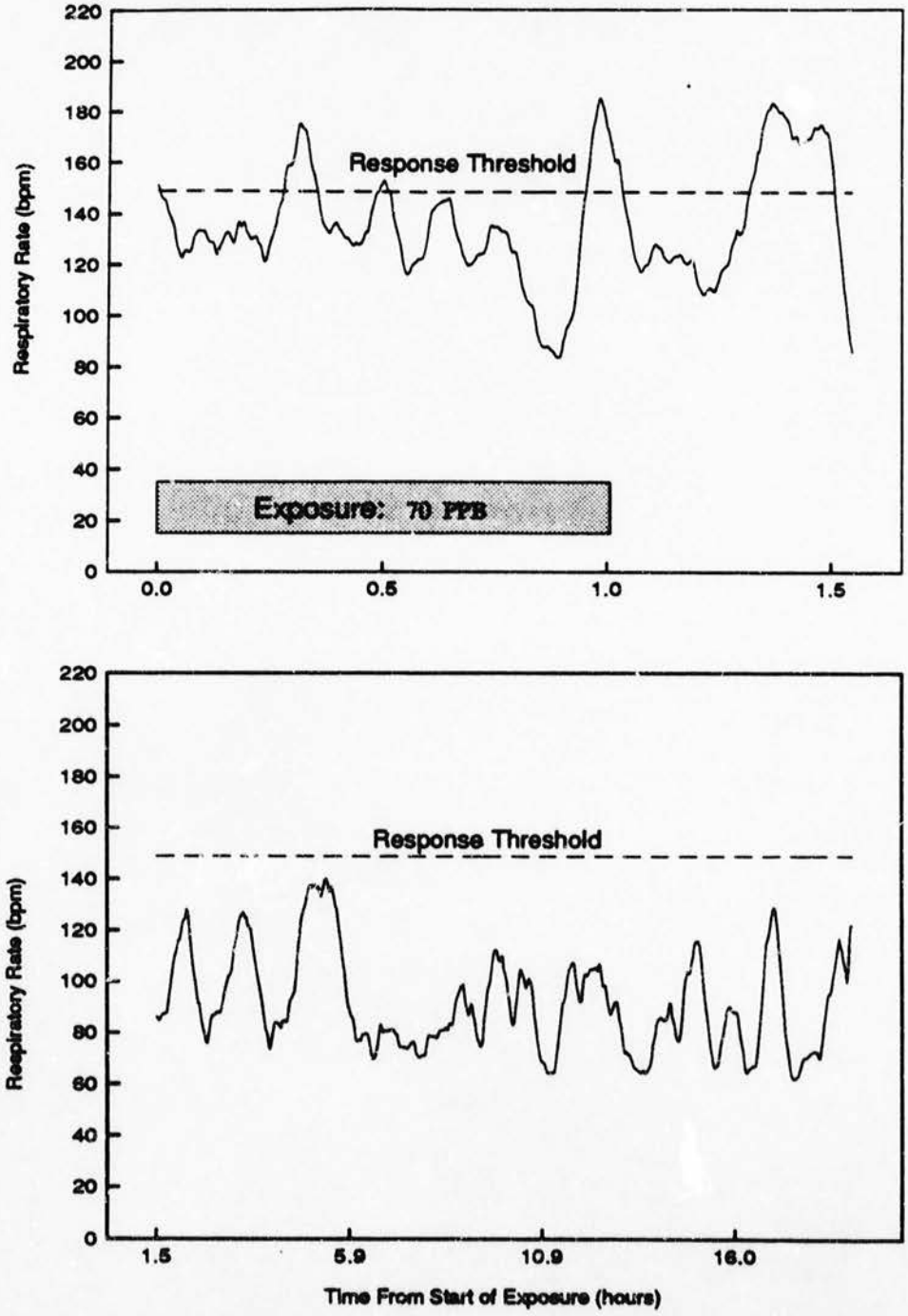


Study Number 91-344-KR
Animal # 52: 2,6-TDI Sensitization
Figure: 80 2,4-TDI Challenge Week 1



Study Number 91-344-KR
Animal # 53: 2,6-TDI Sensitization
2,4-TDI Challenge Week 1

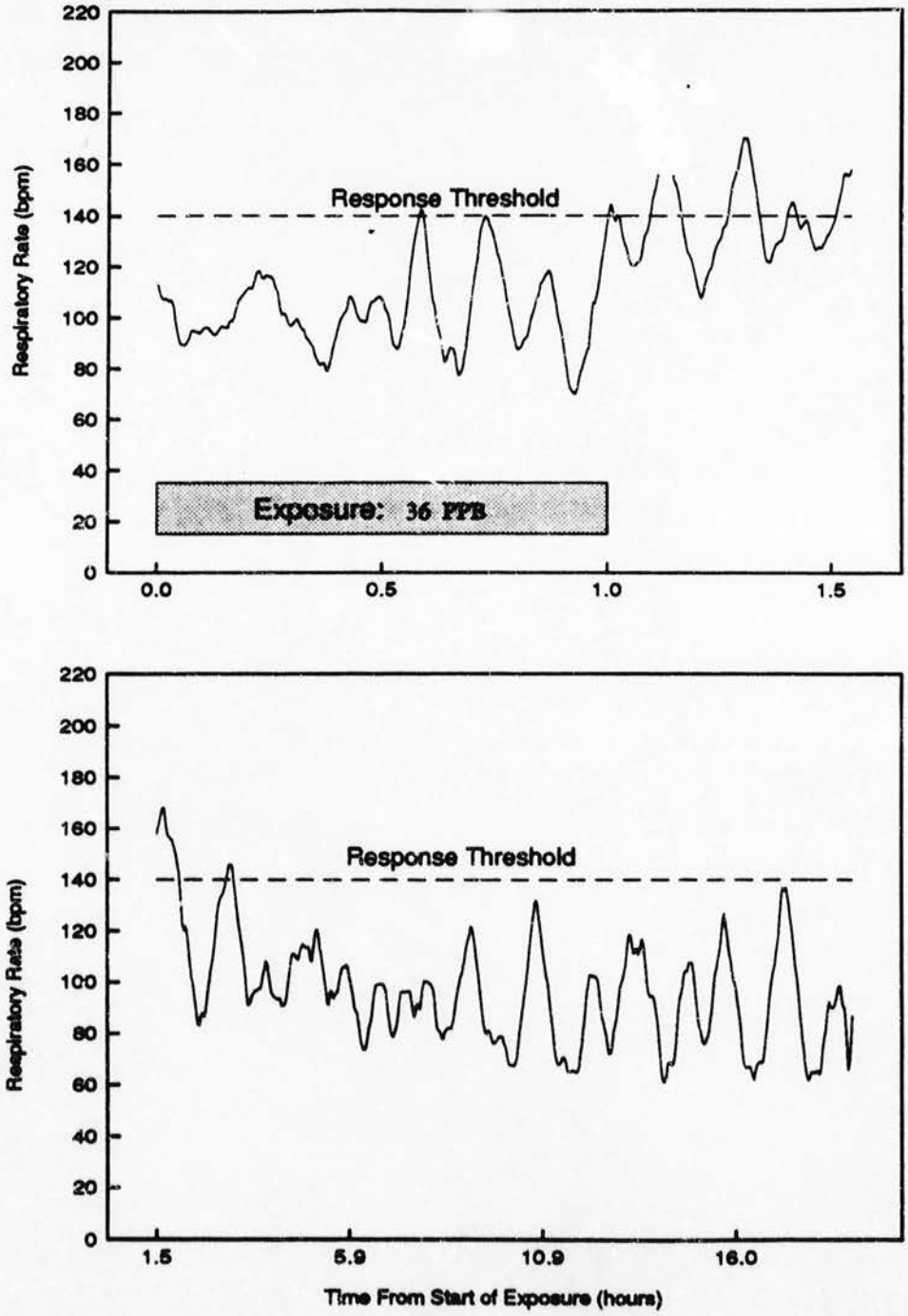
Figure: 81



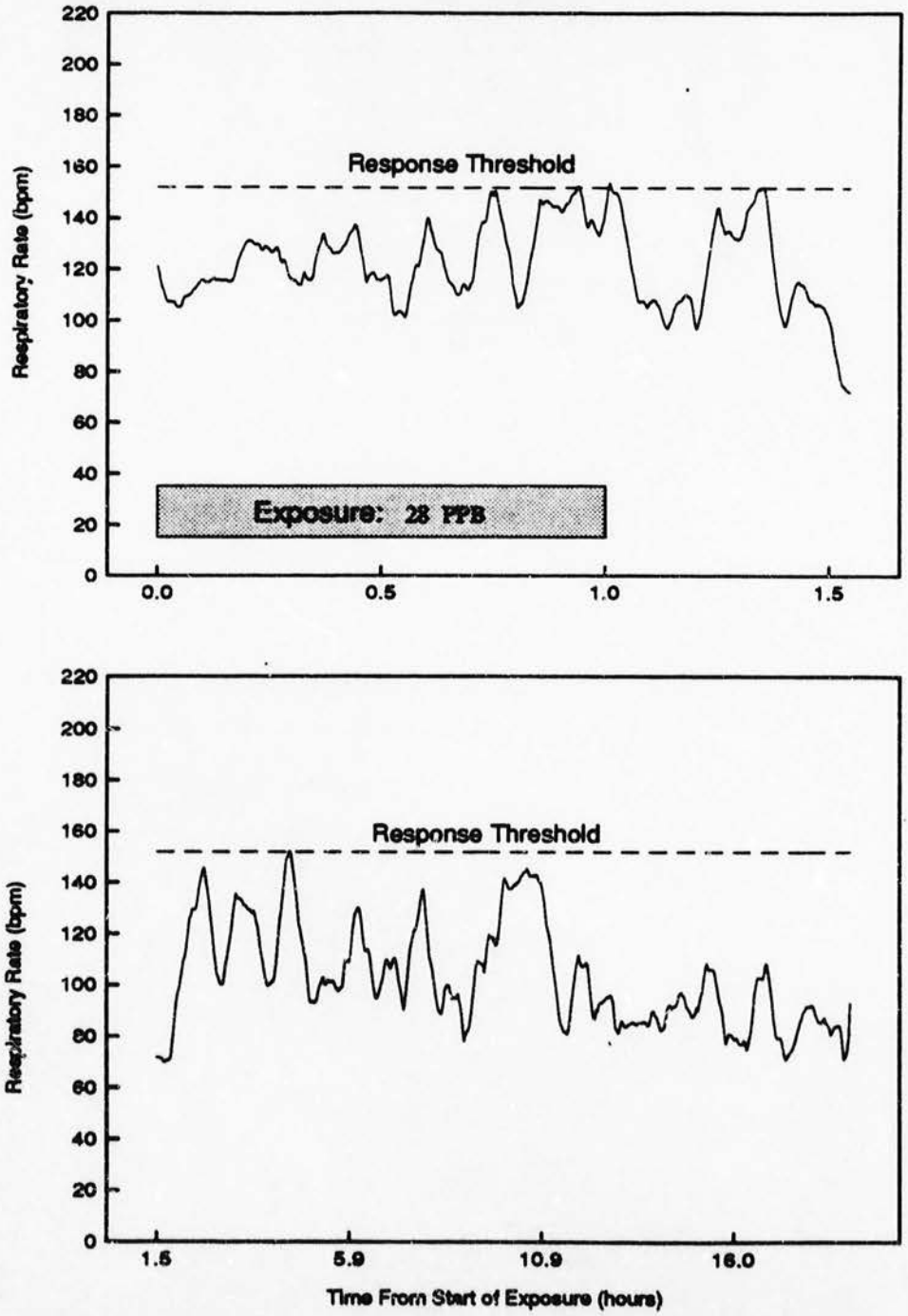
Study Number 91-344-KR

Animal # 54: 2,6-TDI Sensitization

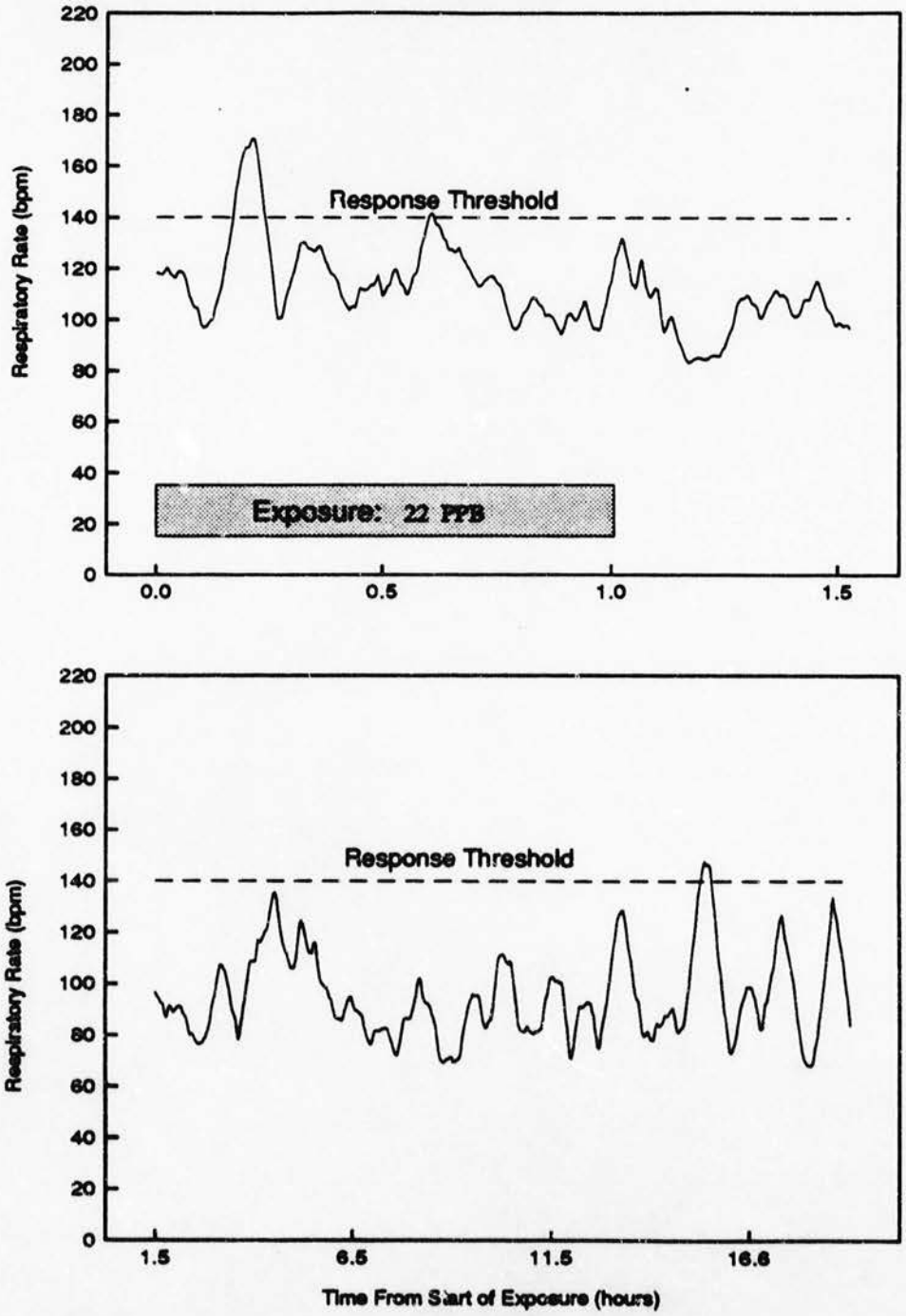
Figure: 82 2,4-TDI Challenge Week 1



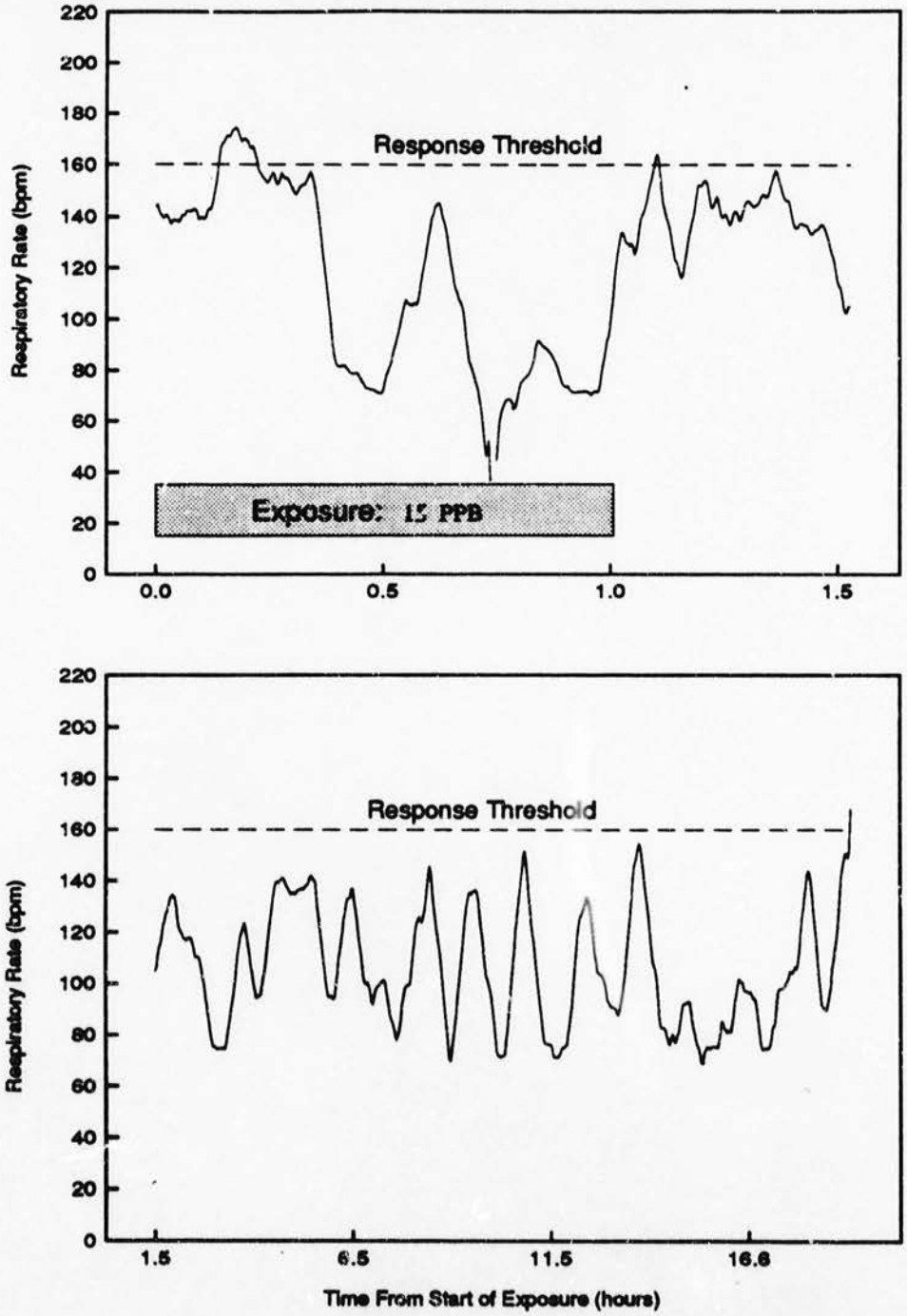
Study Number 91-344-KR
Animal # 55: 2,6-TDI Sensitization
Figure: 83 2,4-TDI Challenge Week 1



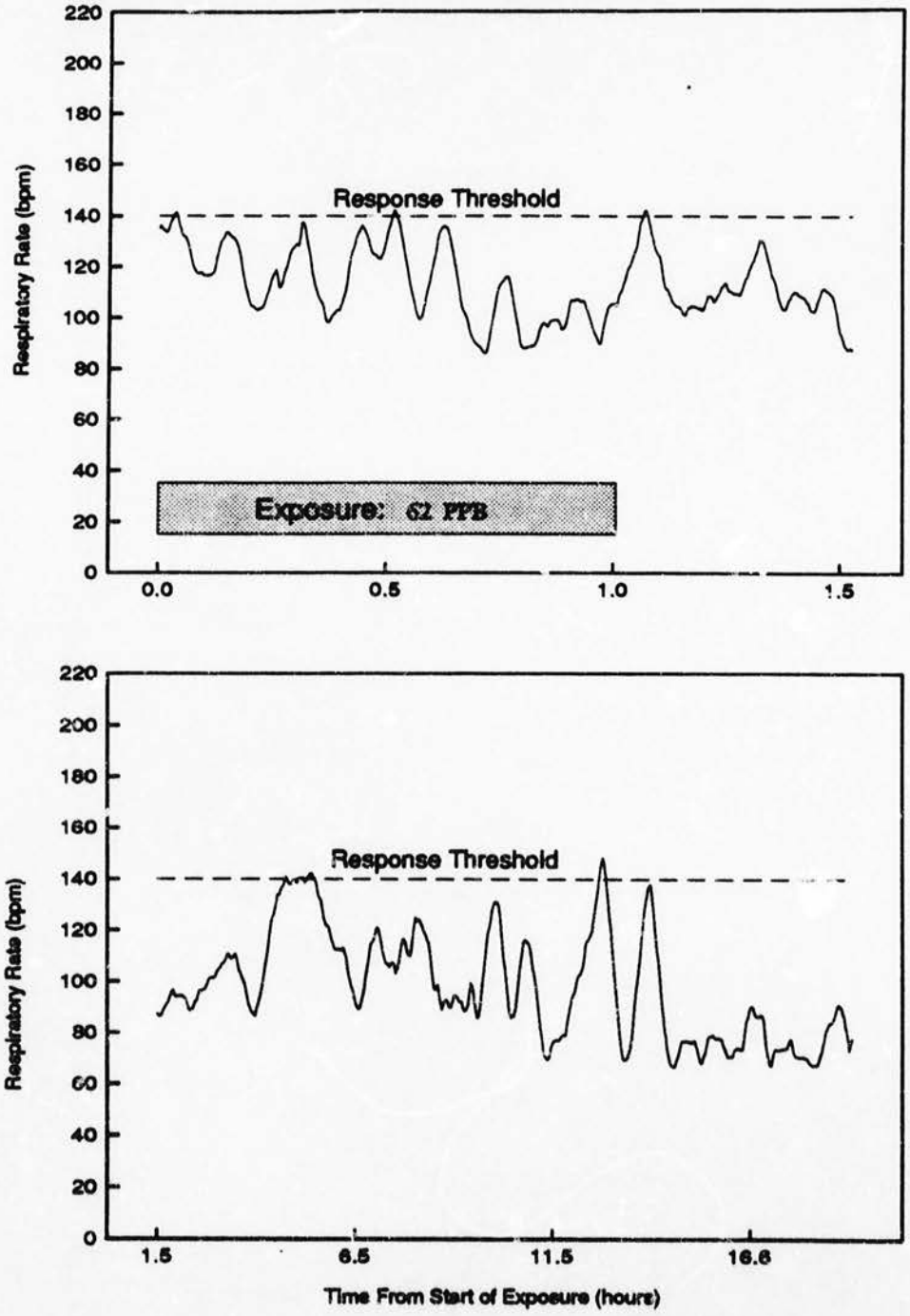
Study Number 91-344-KR
Animal # 44: 2,6-TDI Sensitization
Figure: 84 2,4-TDI Challenge Week 2



Study Number 91-344-KR
Animal # 45: 2,6-TDI Sensitization
Figure: 85 2,4-TDI Challenge Week 2



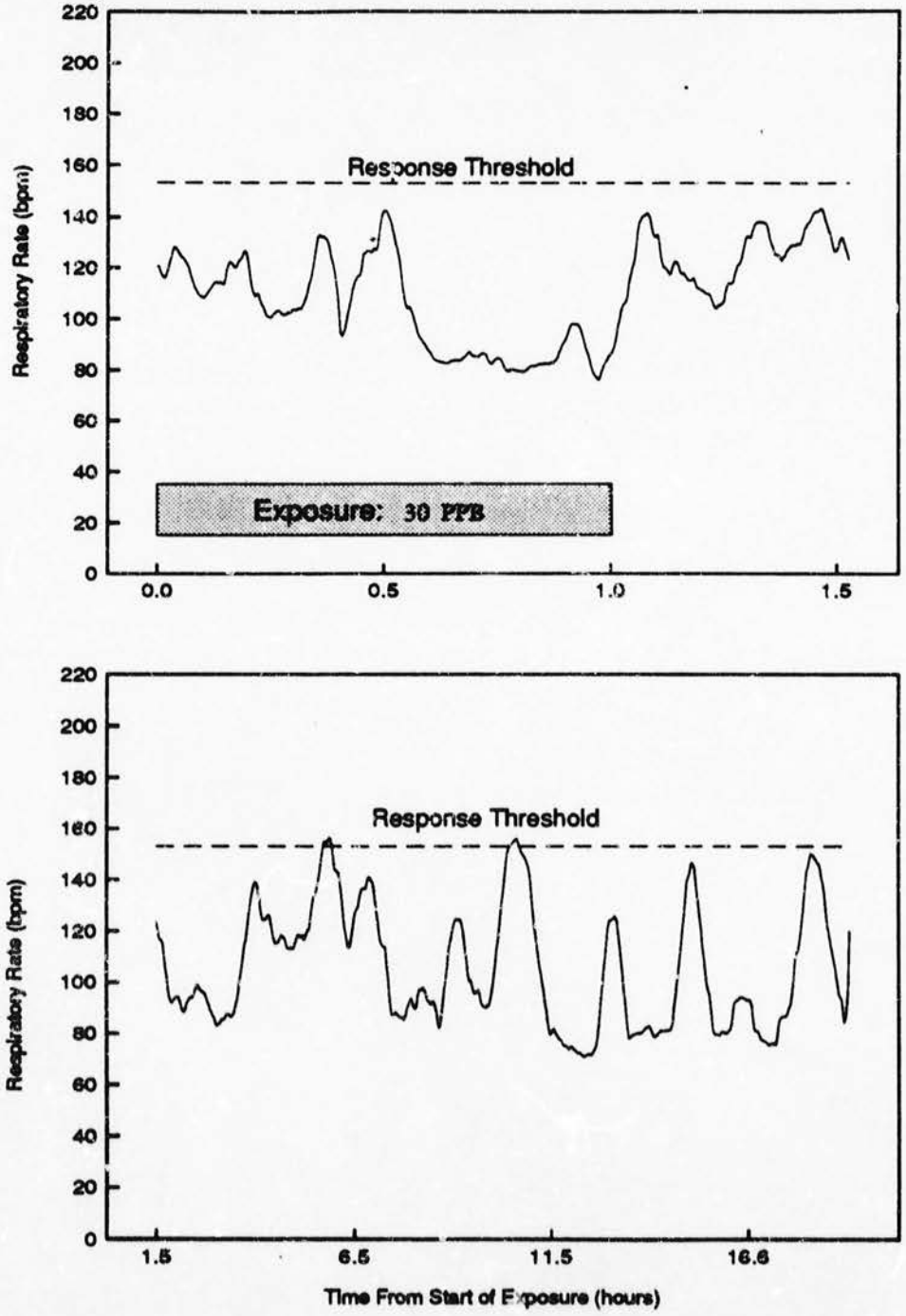
Study Number 91-344-KR
Animal # 46: 2,6-TDI Sensitization
Figure: 86 2,4-TDI Challenge Week 2



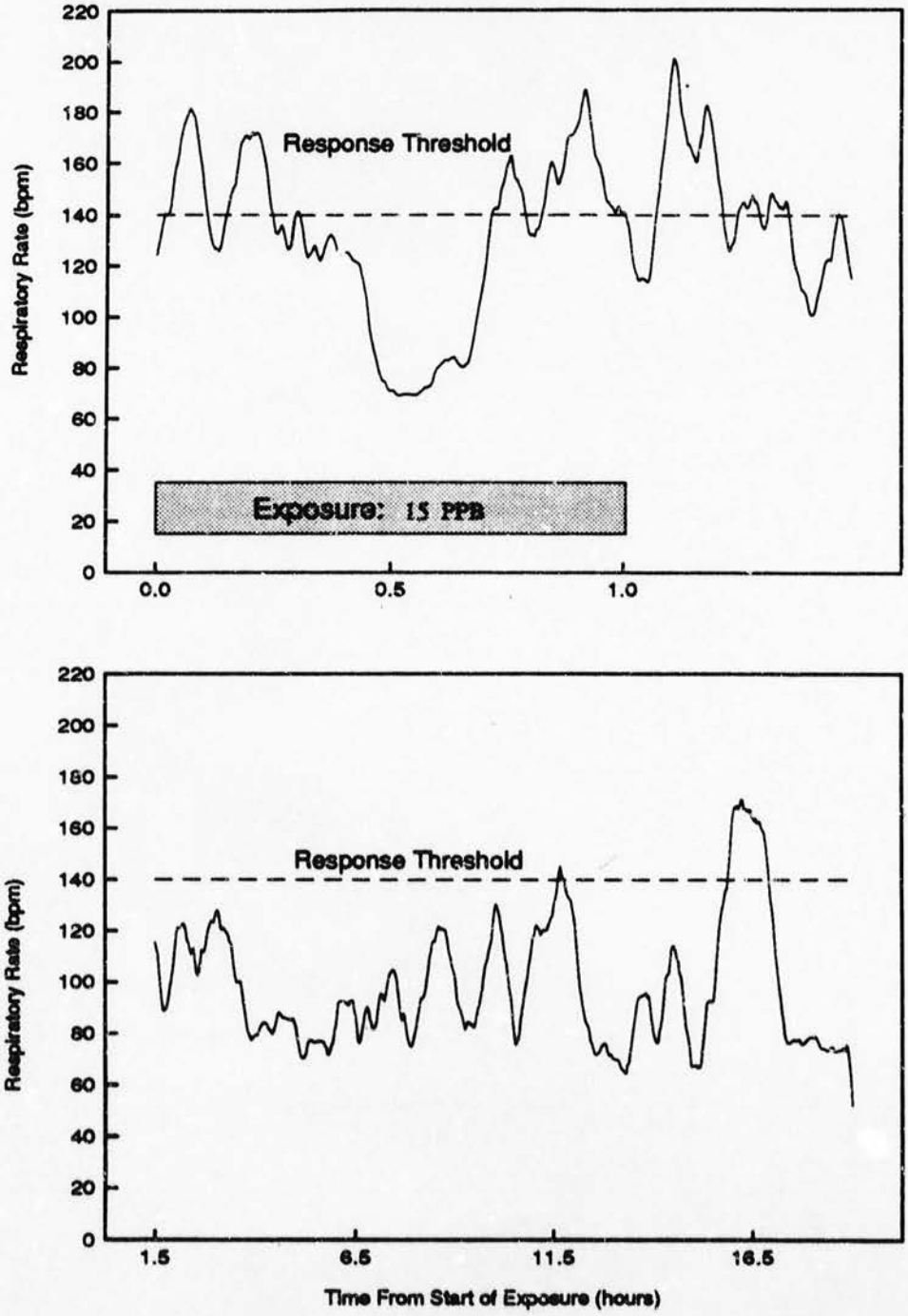
Study Number 91-344-KR

Animal # 47: 2,6-TDI Sensitization

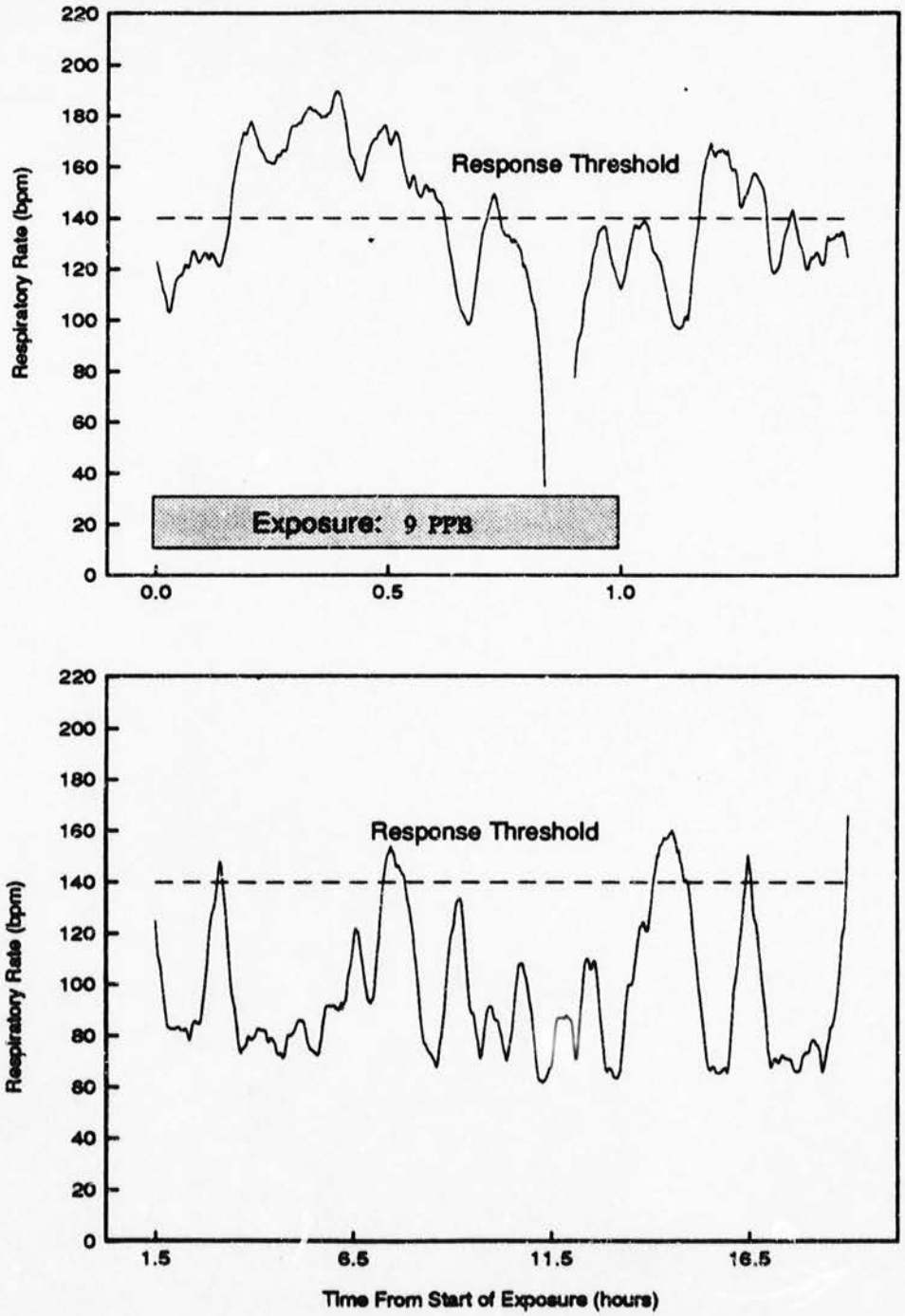
Figure: 87 2,4-TDI Challenge Week 2



Study Number 91-344-KR
Animal # 48: 2,6-TDI Sensitization
Figure: 88 2,4-TDI Challenge Week 2



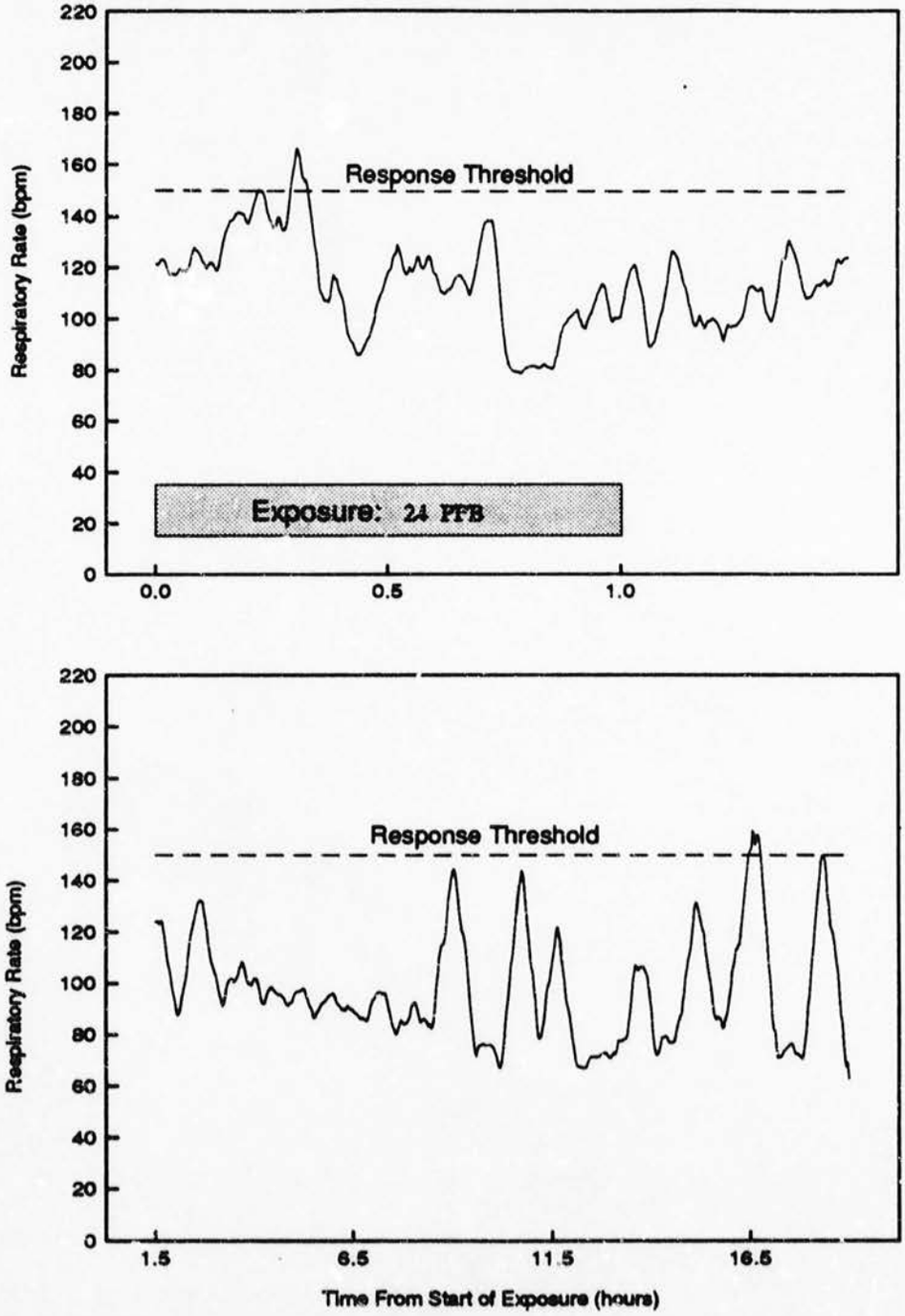
Study Number 91-344-KR
Animal # 49: 2,6-TDI Sensitization
Figure: 89 2,4-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 51: 2,6-TDI Sensitization

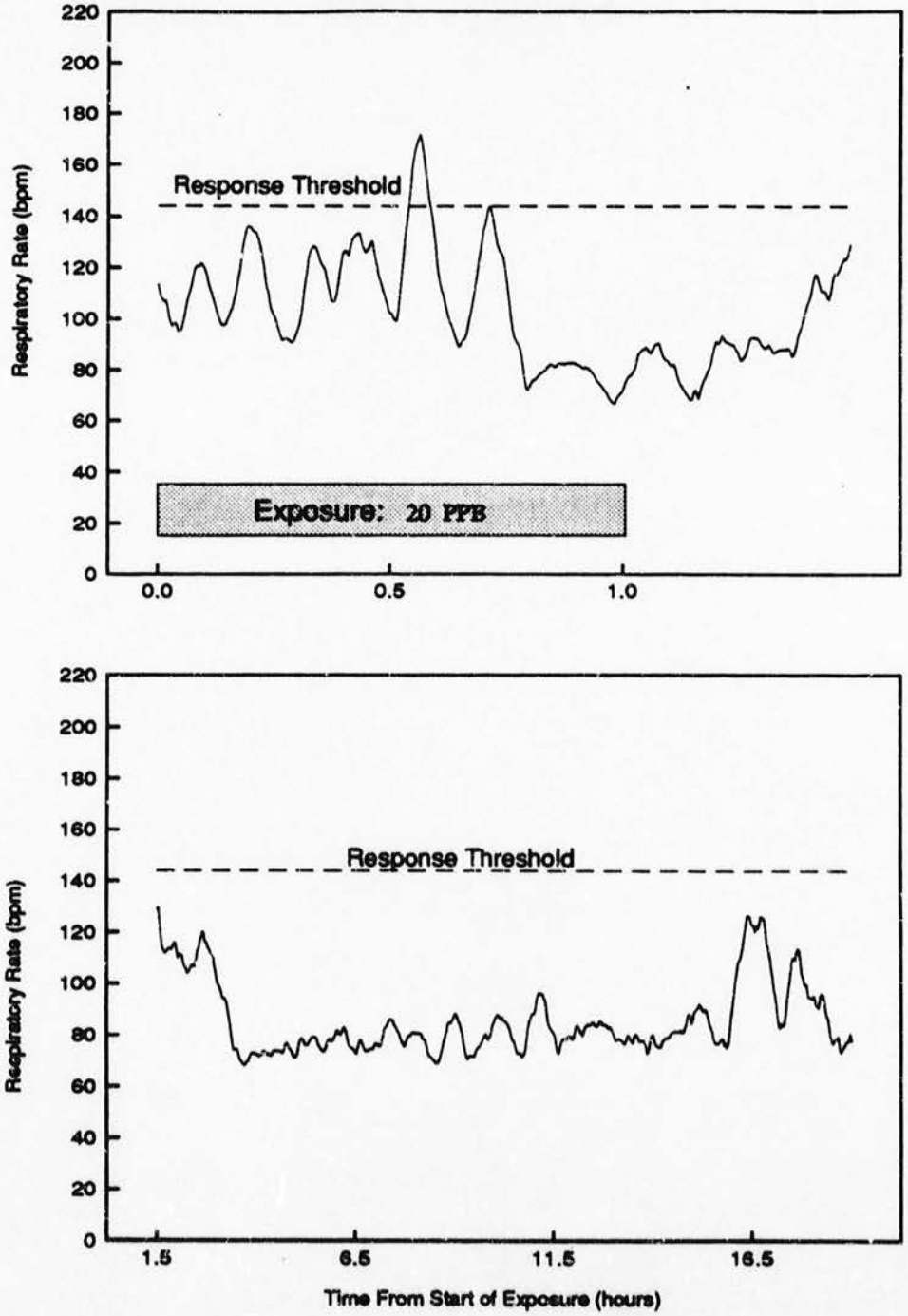
Figure: 90 2,4-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 52: 2,6-TDI Sensitization

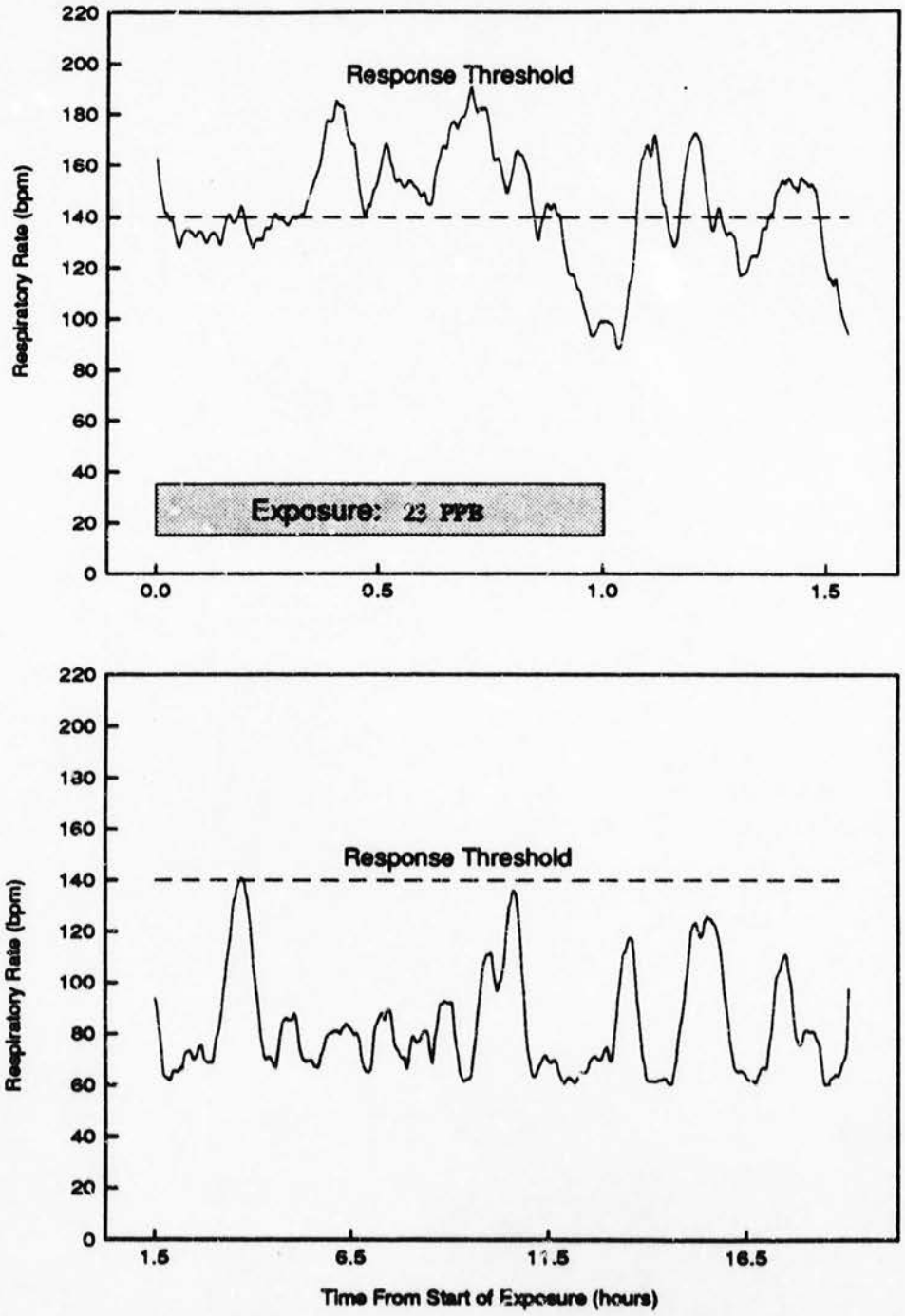
Figure: 91 2,4-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 53: 2,6-TDI Sensitization

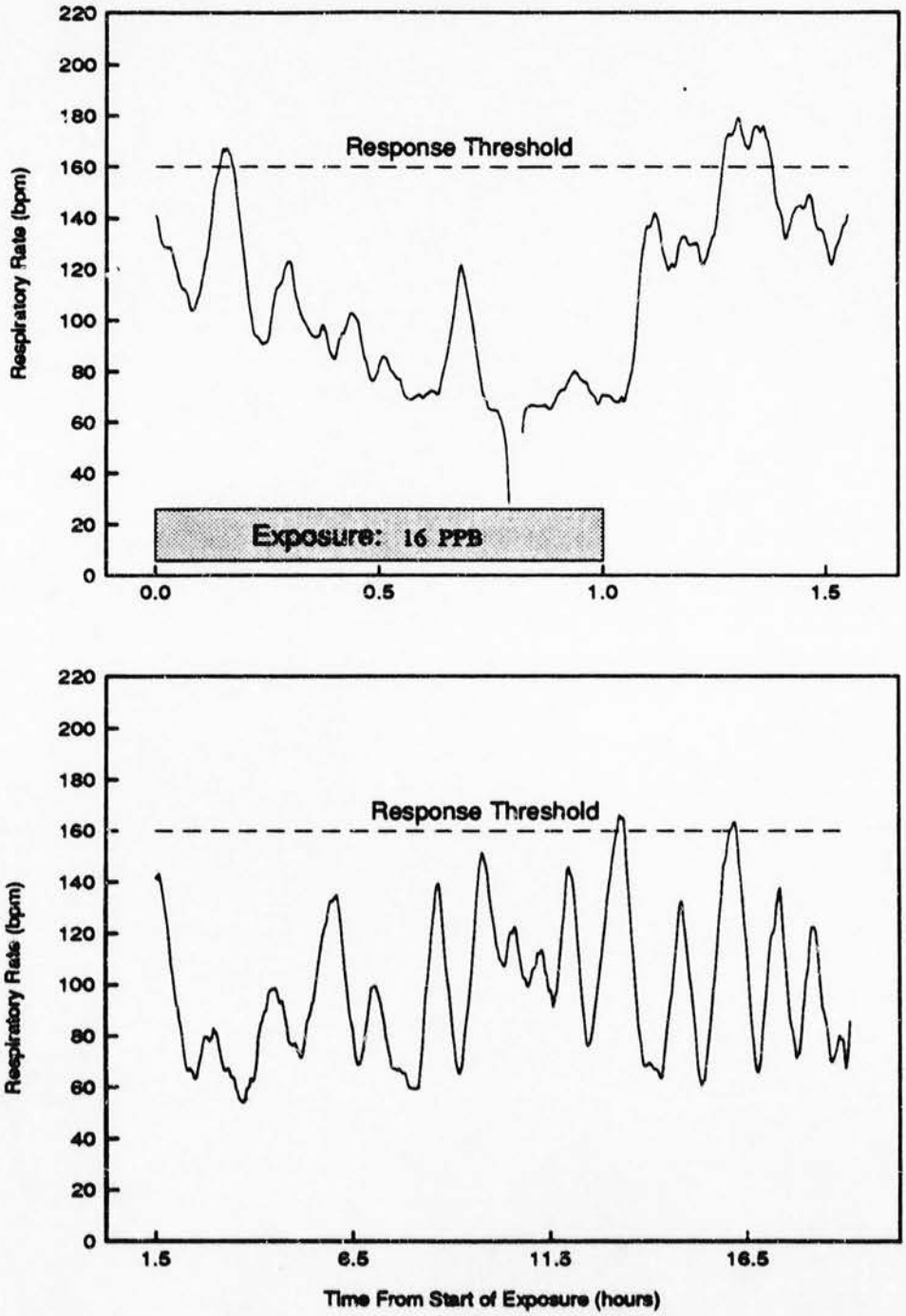
Figure: 92 2,4-TDI Challenge Week 2



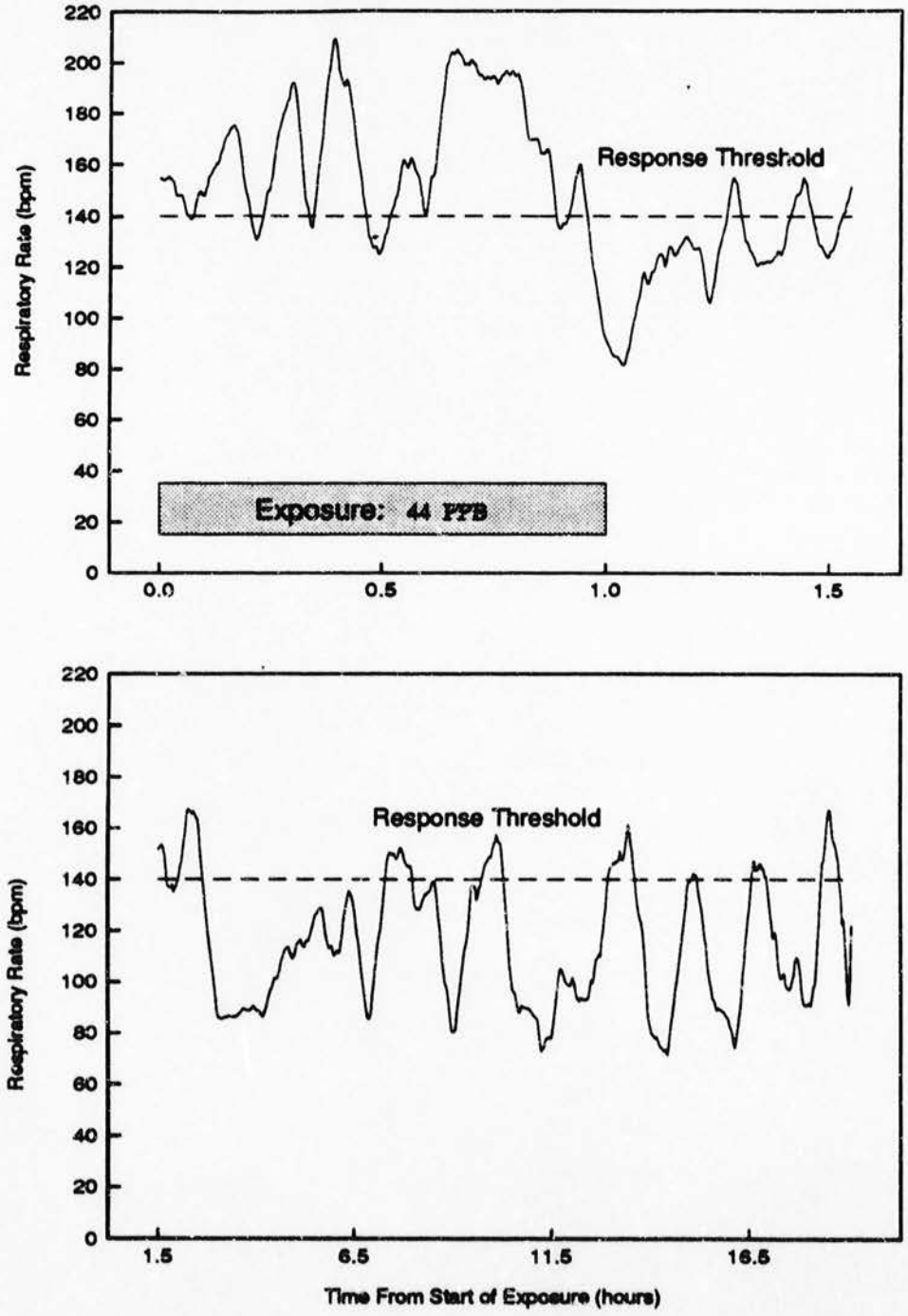
Study Number 91-344-KR

Animal # 54: 2,6-TDI Sensitization

Figure: 93 2,4-TDI Challenge Week 2



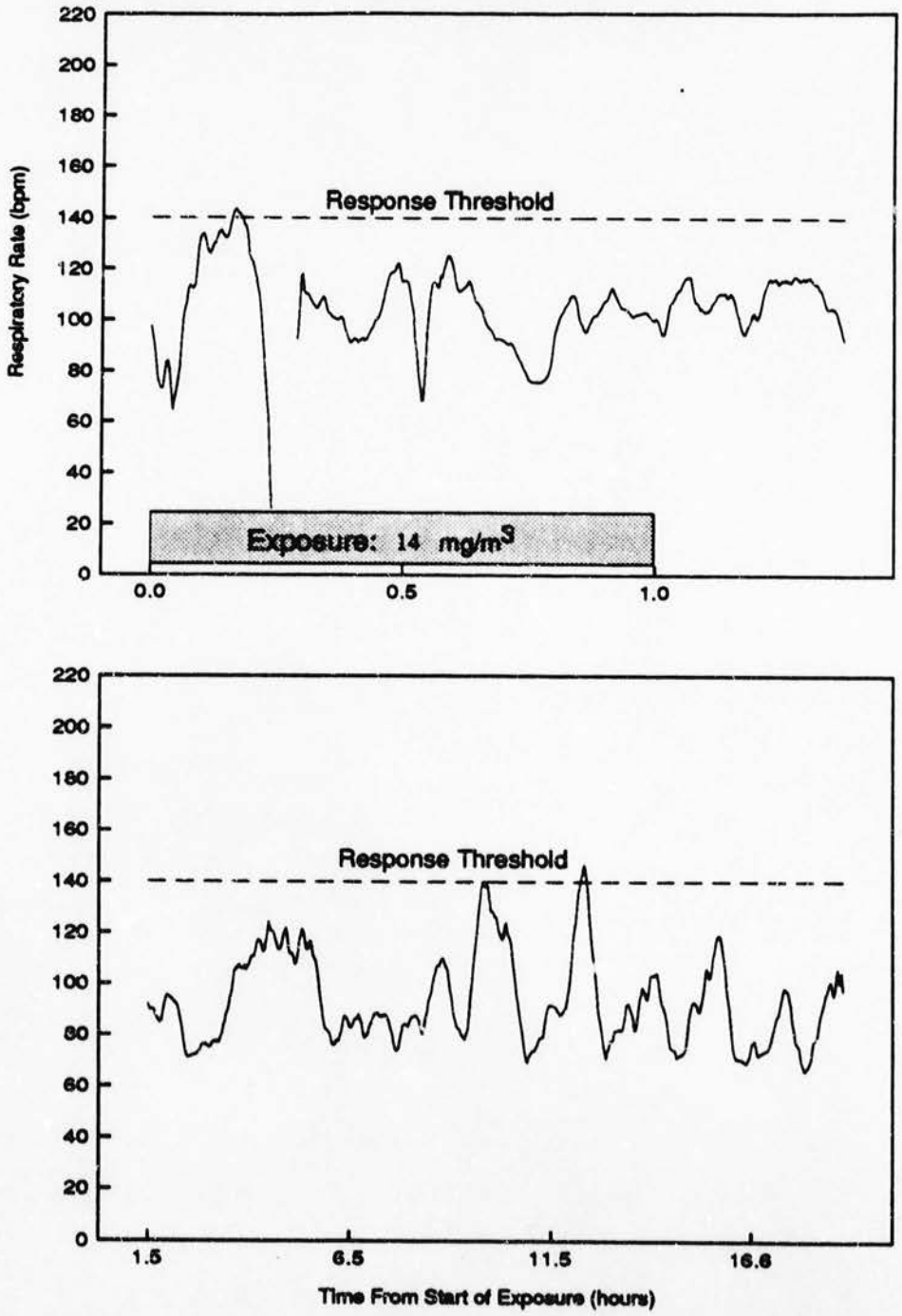
Study Number 91-344-KR
Animal # 55: 2,6-TDI Sensitization
Figure: 94 2,4-TDI Challenge Week 2



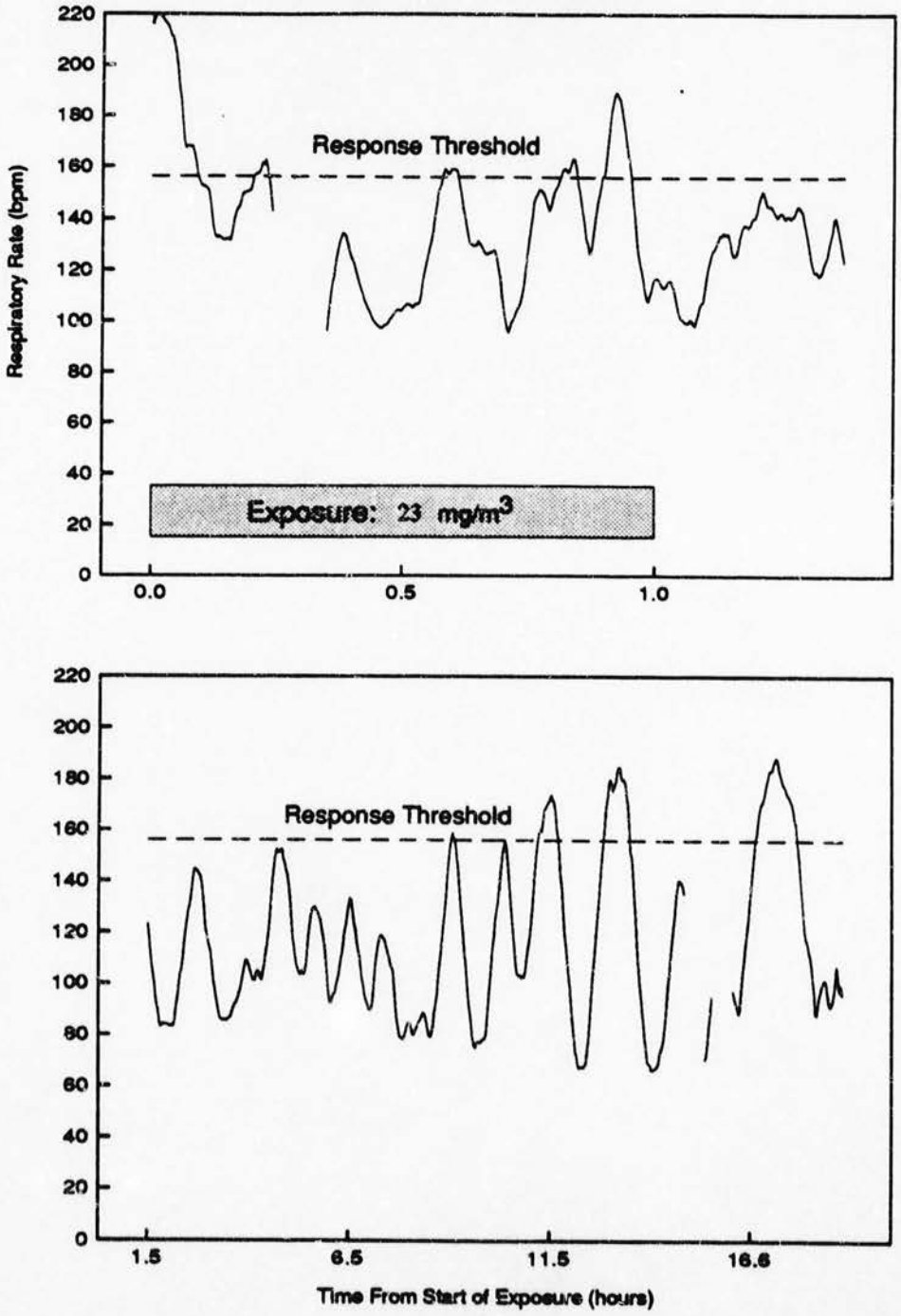
Study Number 91-344-KR

Animal # 44: 2,6-TDI Sensitization

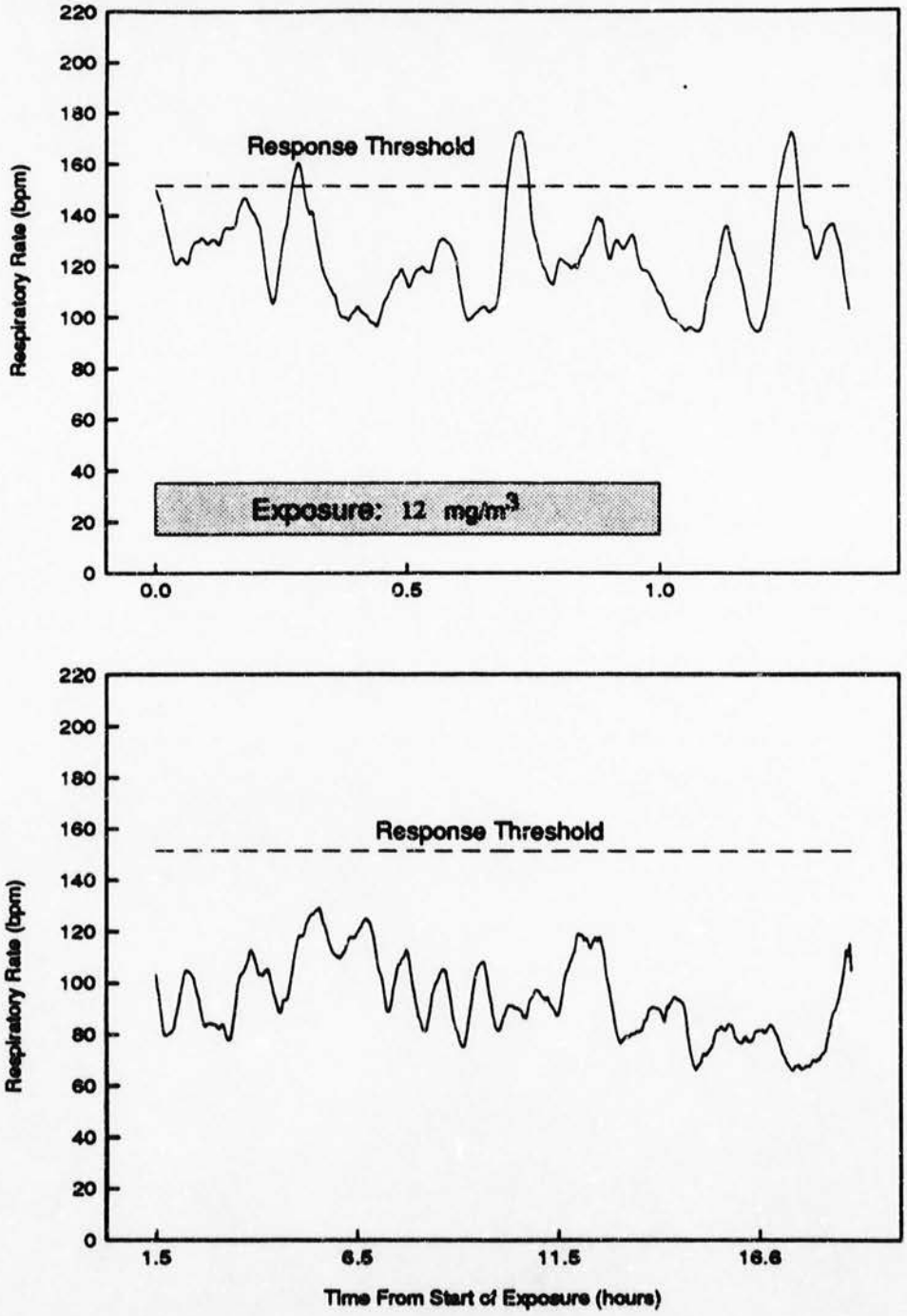
Figure: 95 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR
Animal # 45: 2,6-TDI Sensitization
Figure: 96 2,4-TDI-GSA Challenge Week 3



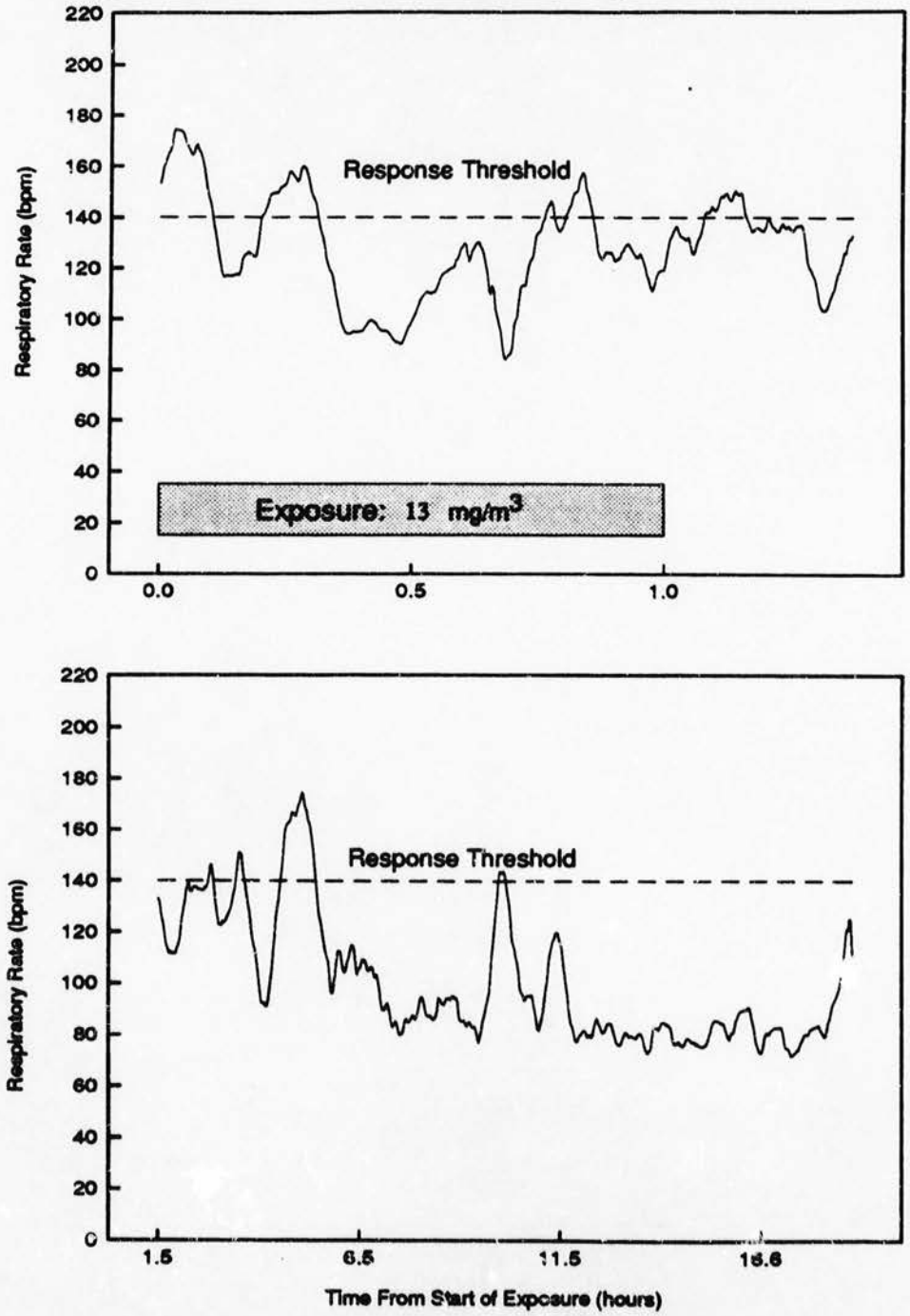
Study Number 91-344-KR
Animal # 46: 2,6-TDI Sensitization
Figure: 97 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 47: 2,6-TDI Sensitization

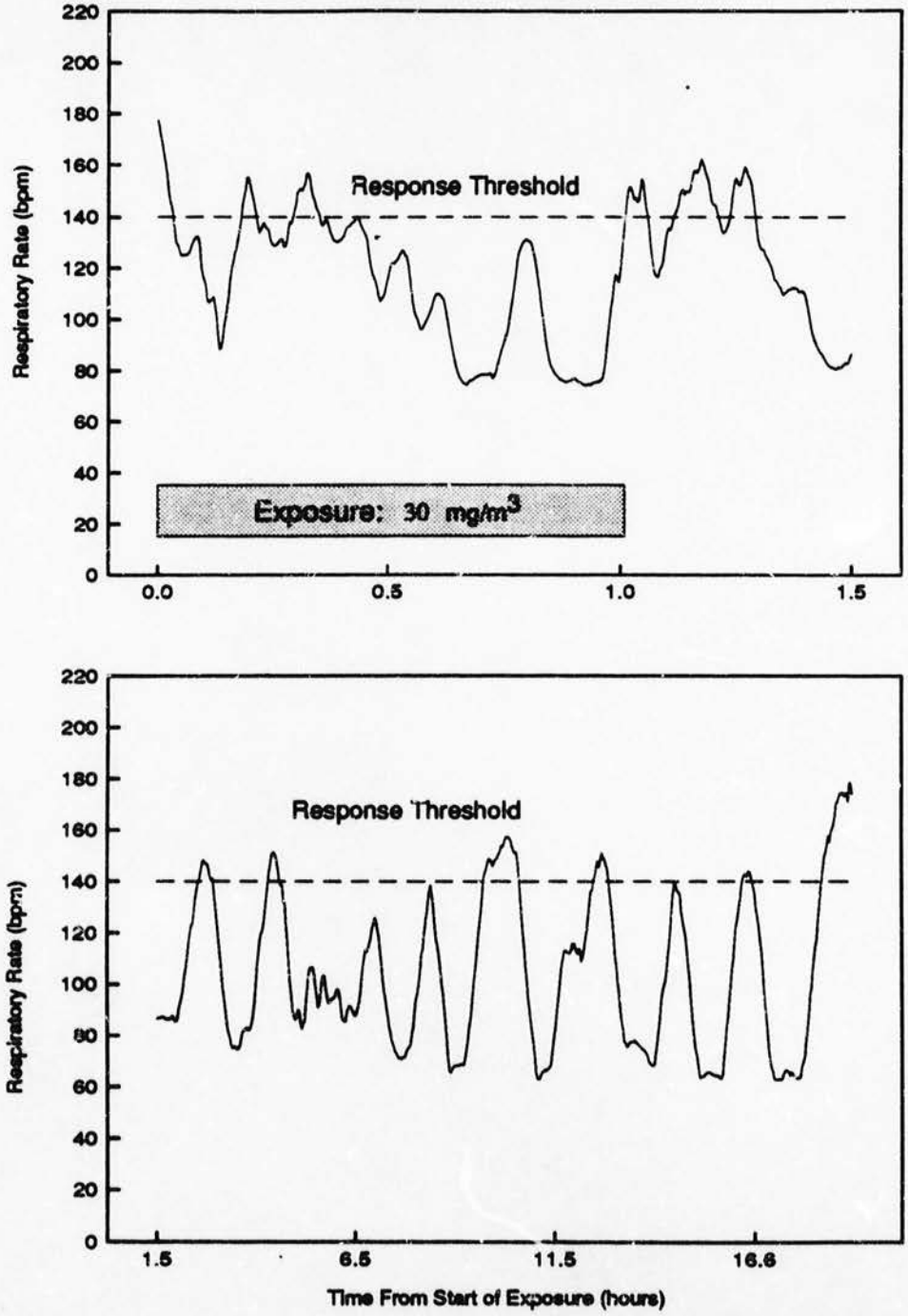
Figure: 98 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 48: 2,6-TDI Sensitization

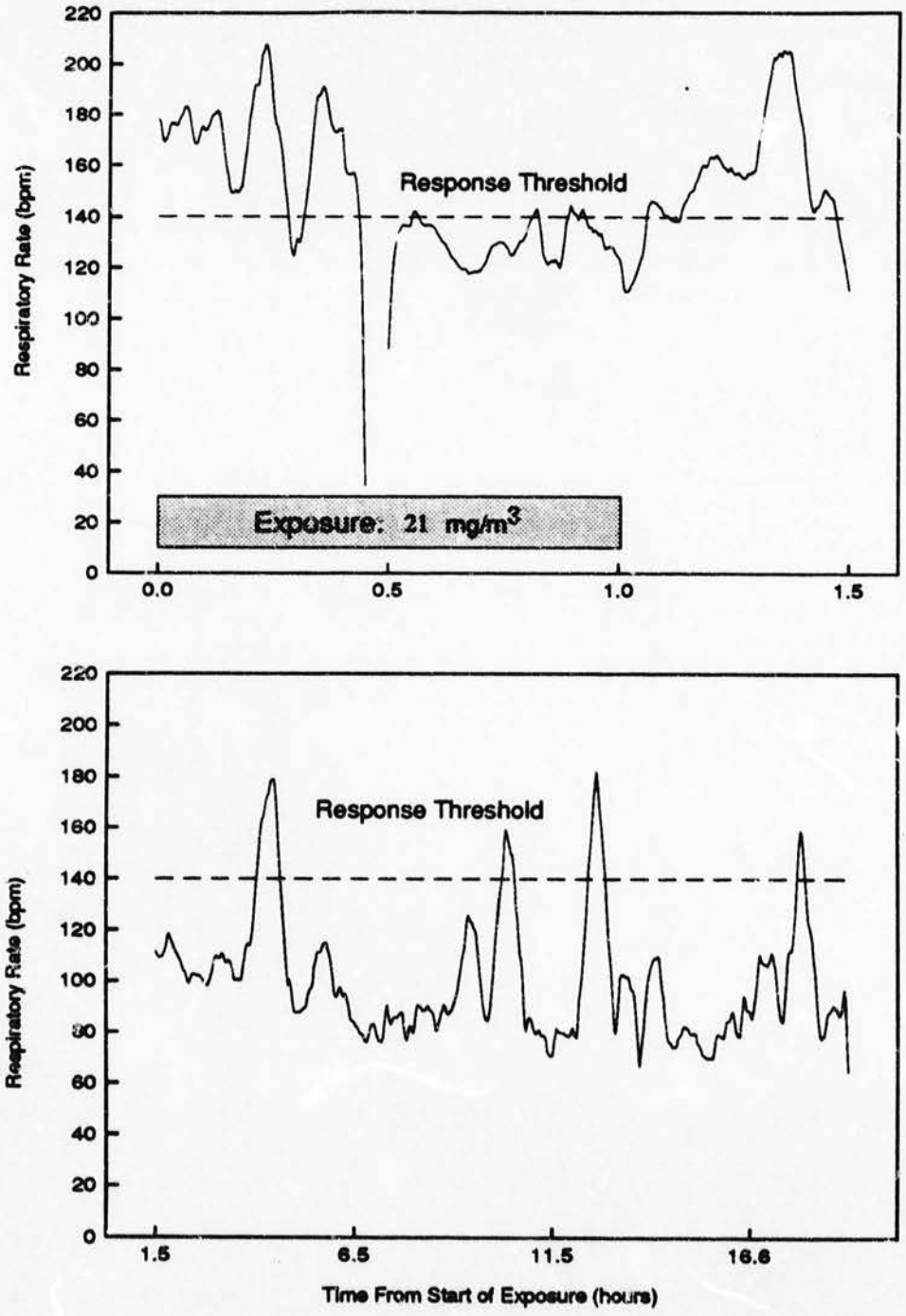
Figure: 99 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

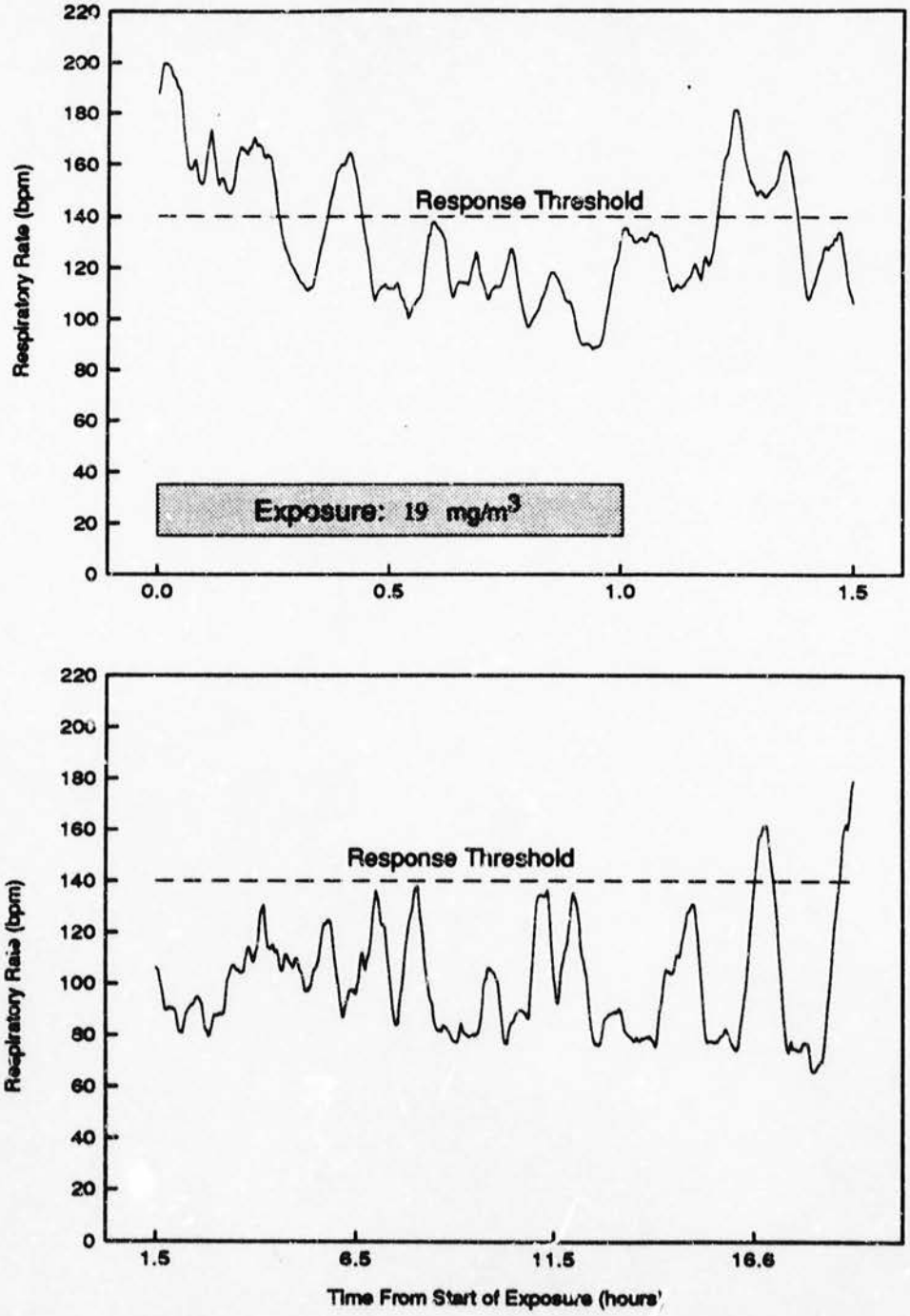
Animal # 49: 2,6-TDI Sensitization

Figure: 100 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR
Animal # 51: 2,6-TDI Sensitization

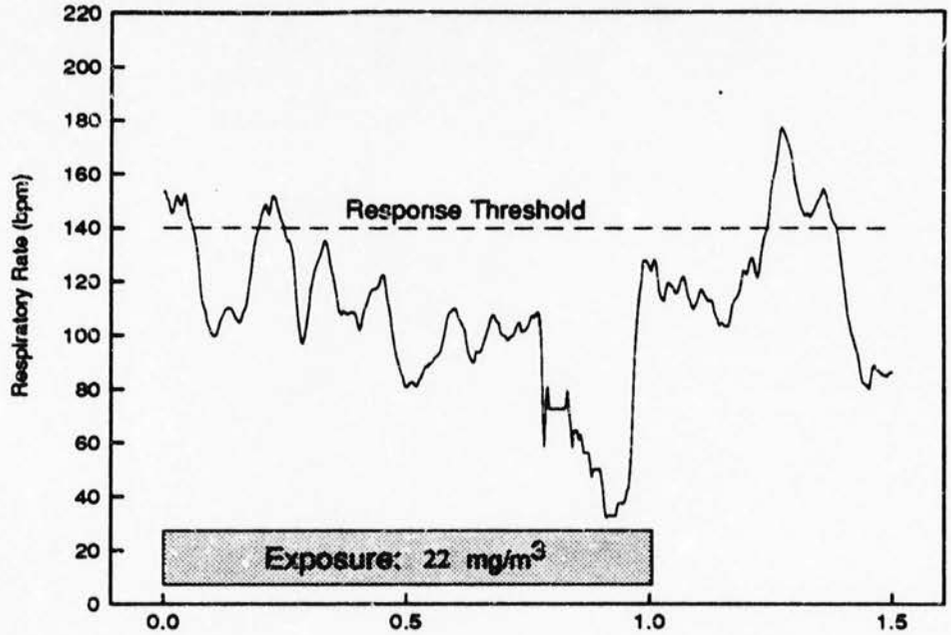
Figure: 101 2,4-TDI-GSA Challenge Week 3

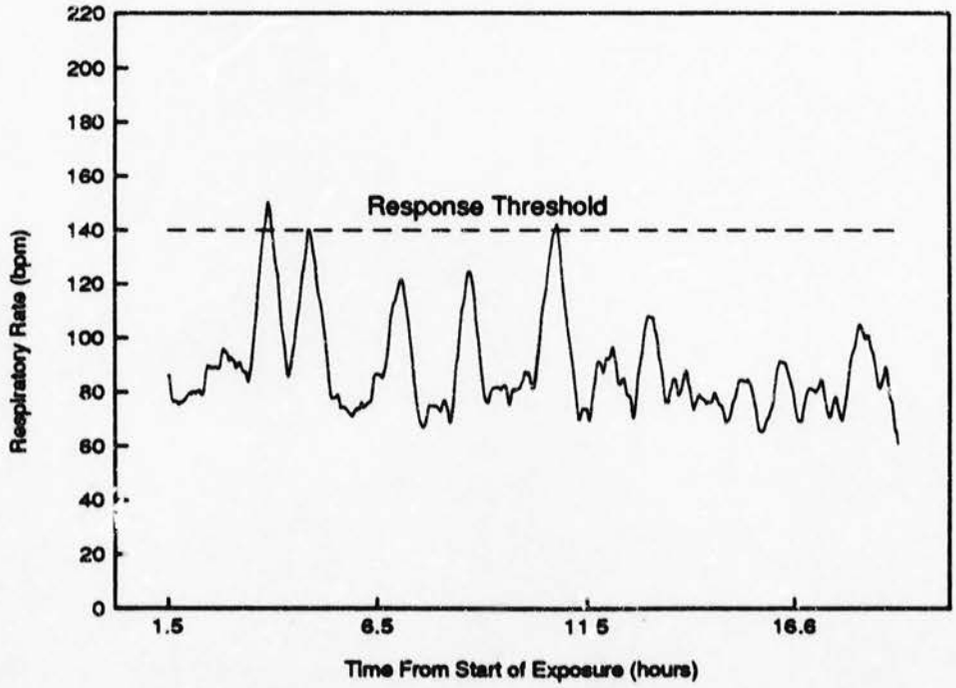


Study Number 91-344-KR

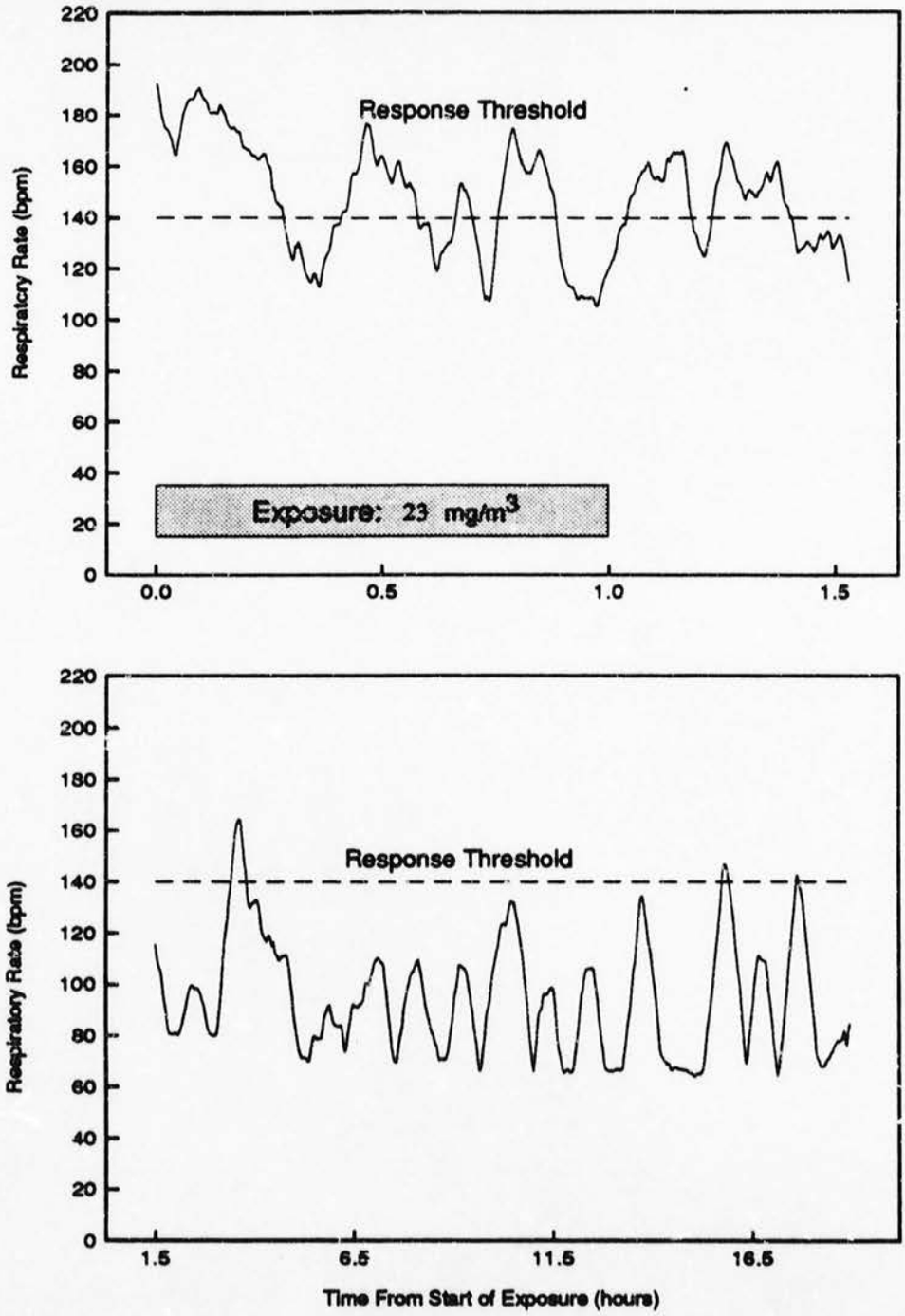
Animal # 52: 2,6-TDI Sensitization

Figure: 102 2,4-TDI-GSA Challenge Week 3





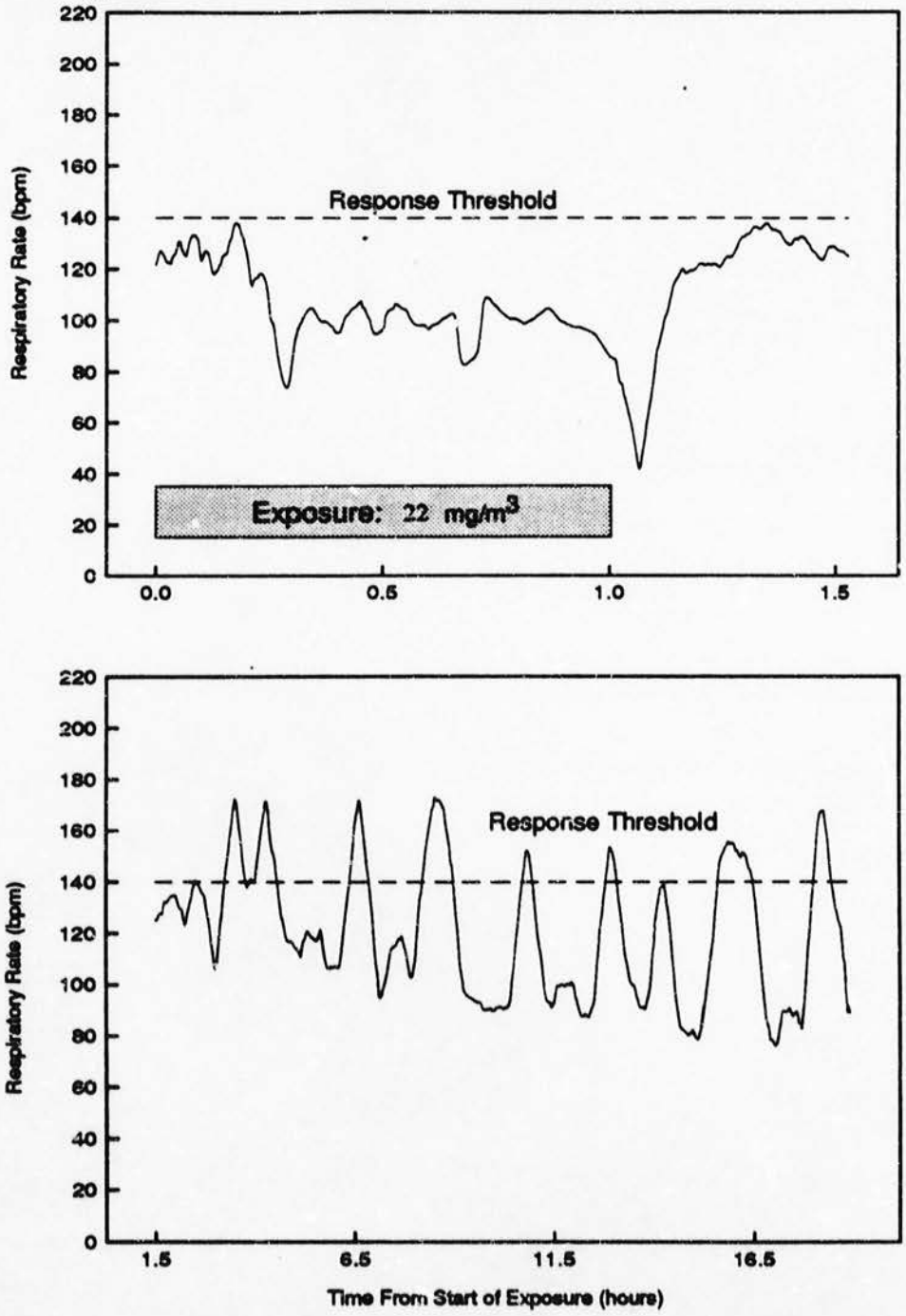
Study Number 91-344-KR
Animal # 53: 2,6-TDI Sensitization
Figure: 103 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 54: 2,6-TDI Sensitization

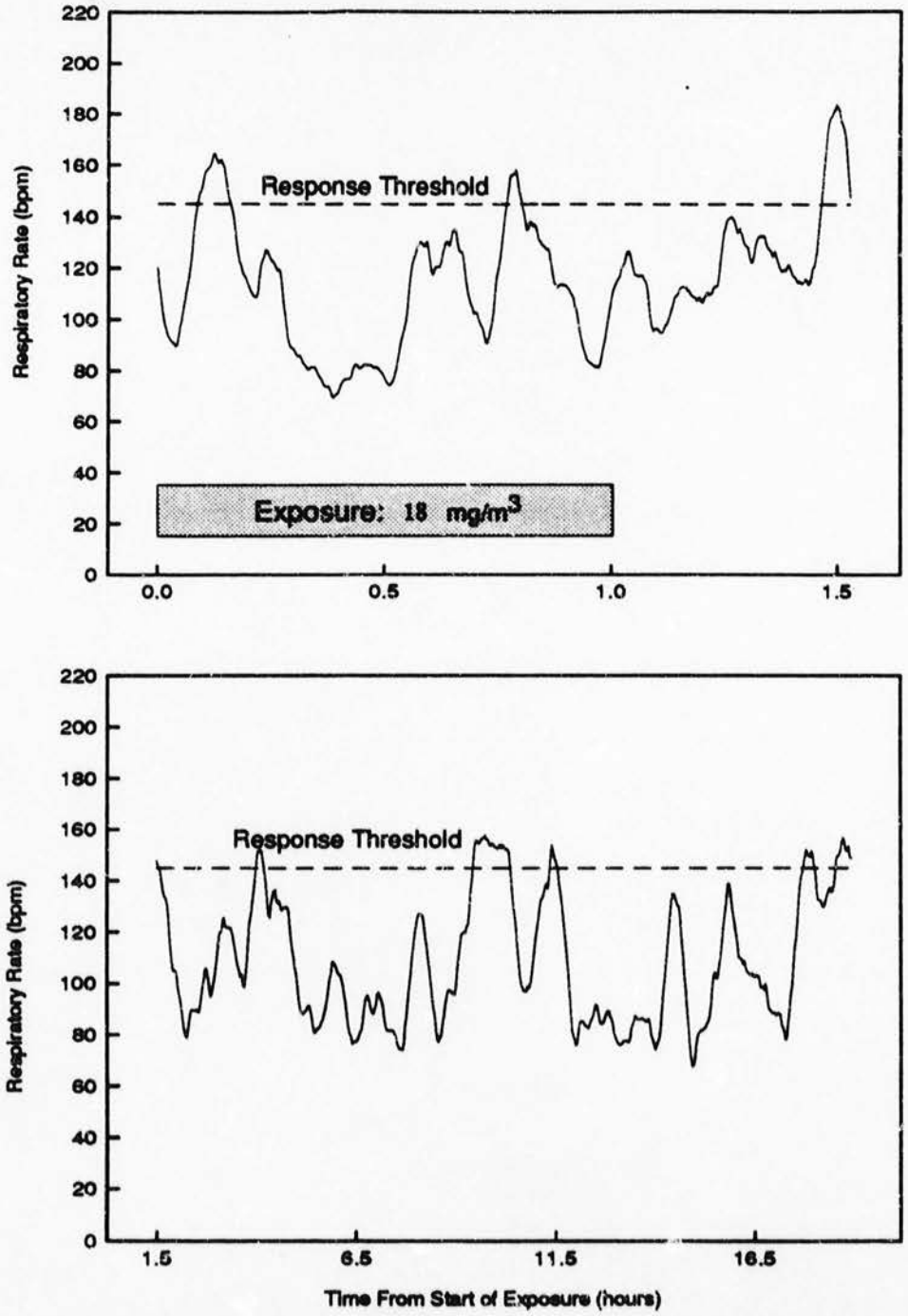
Figure: 104 2,4-TDI-GSA Challenge Week 3



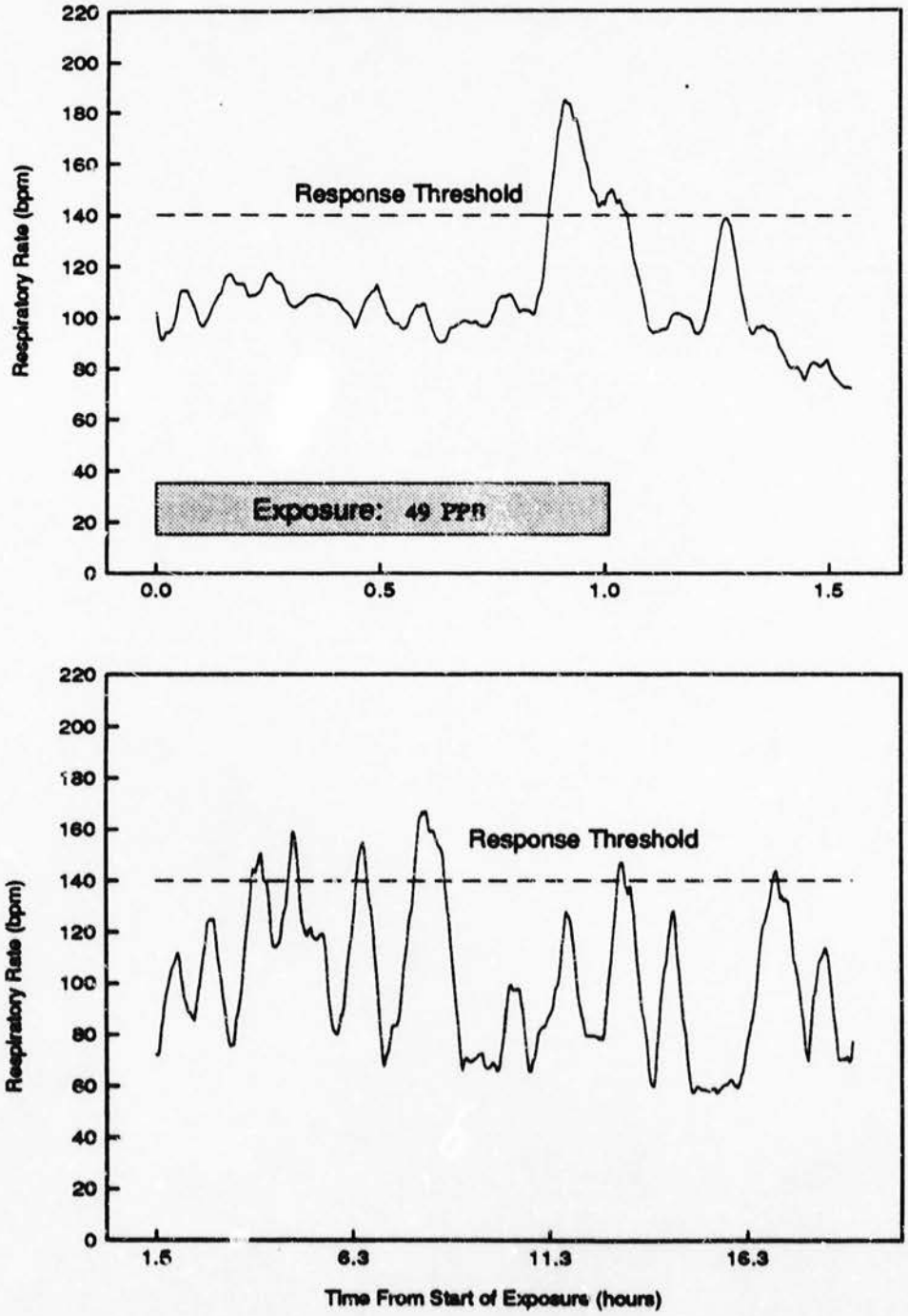
Study Number 91-344-KR

Animal # 55: 2,6-TDI Sensitization

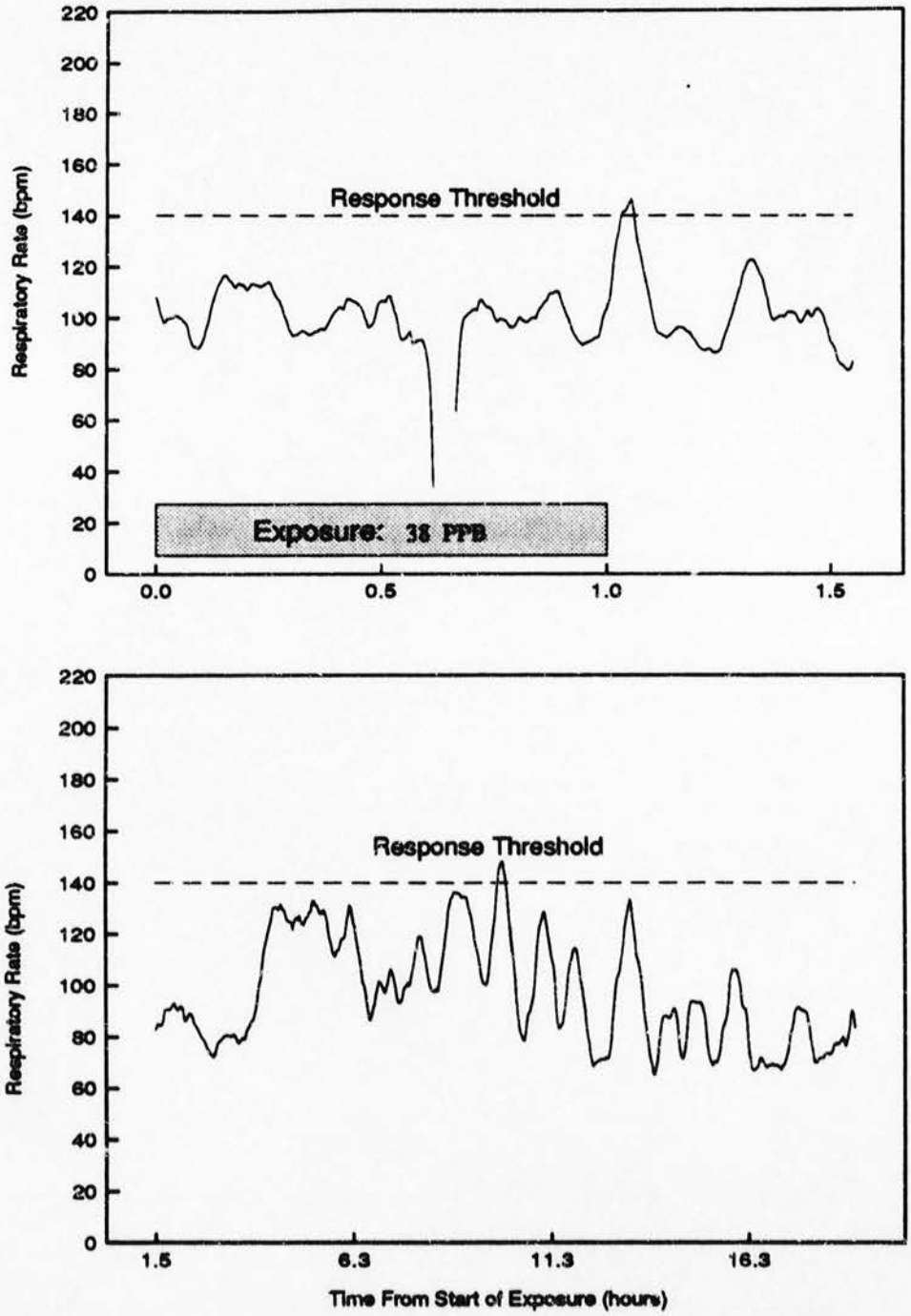
Figure: 105 2,4-TDI-GSA Challenge Week 3



Study Number 91-344-KR
Animal # 93: 2,6-TDI Sensitization
Figure: 106 2,6-TDI Challenge Week 1



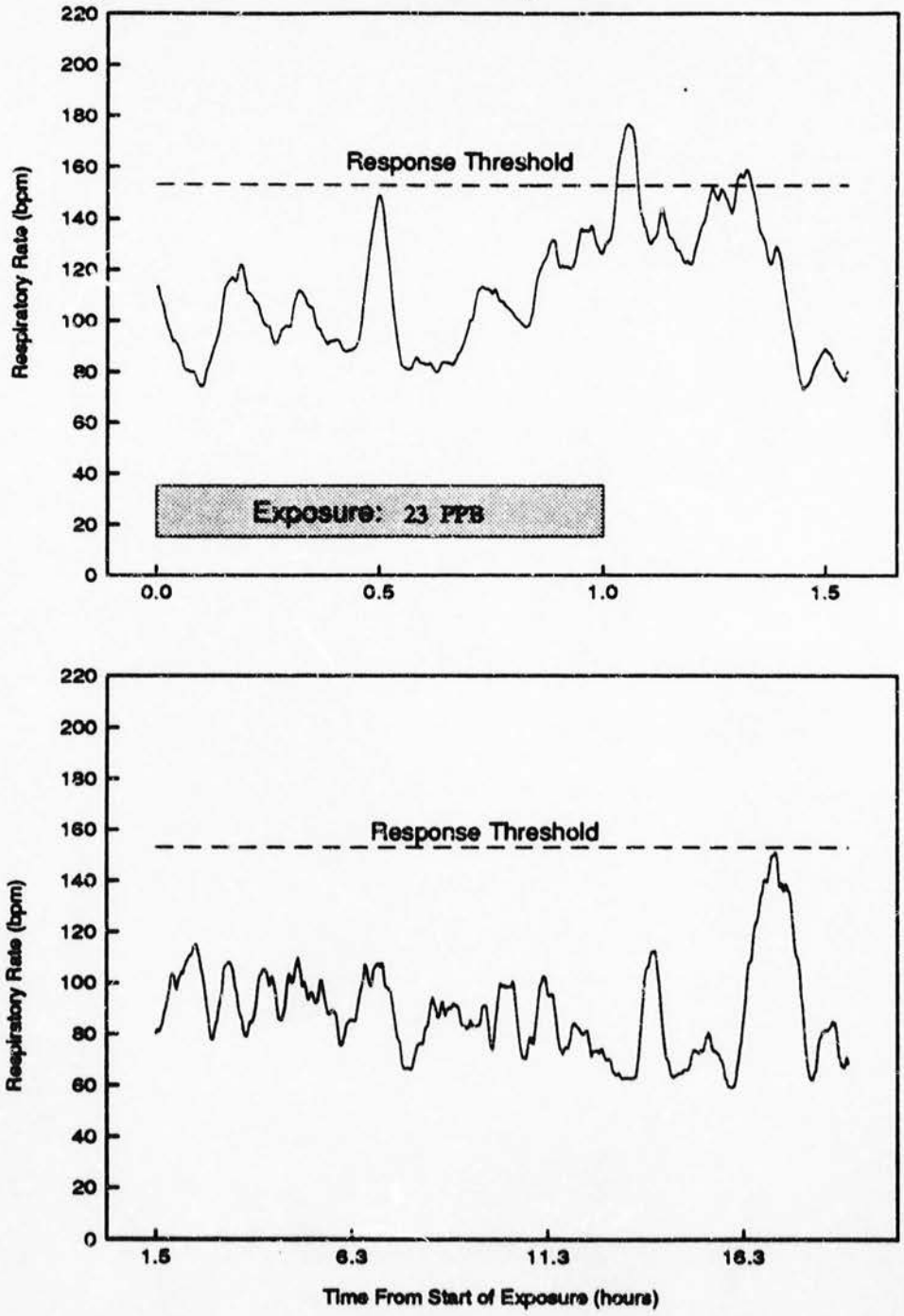
Study Number 91-344-KR
Animal # 94: 2,6-TDI Sensitization
Figure: 107 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 95: 2,6-TDI Sensitization

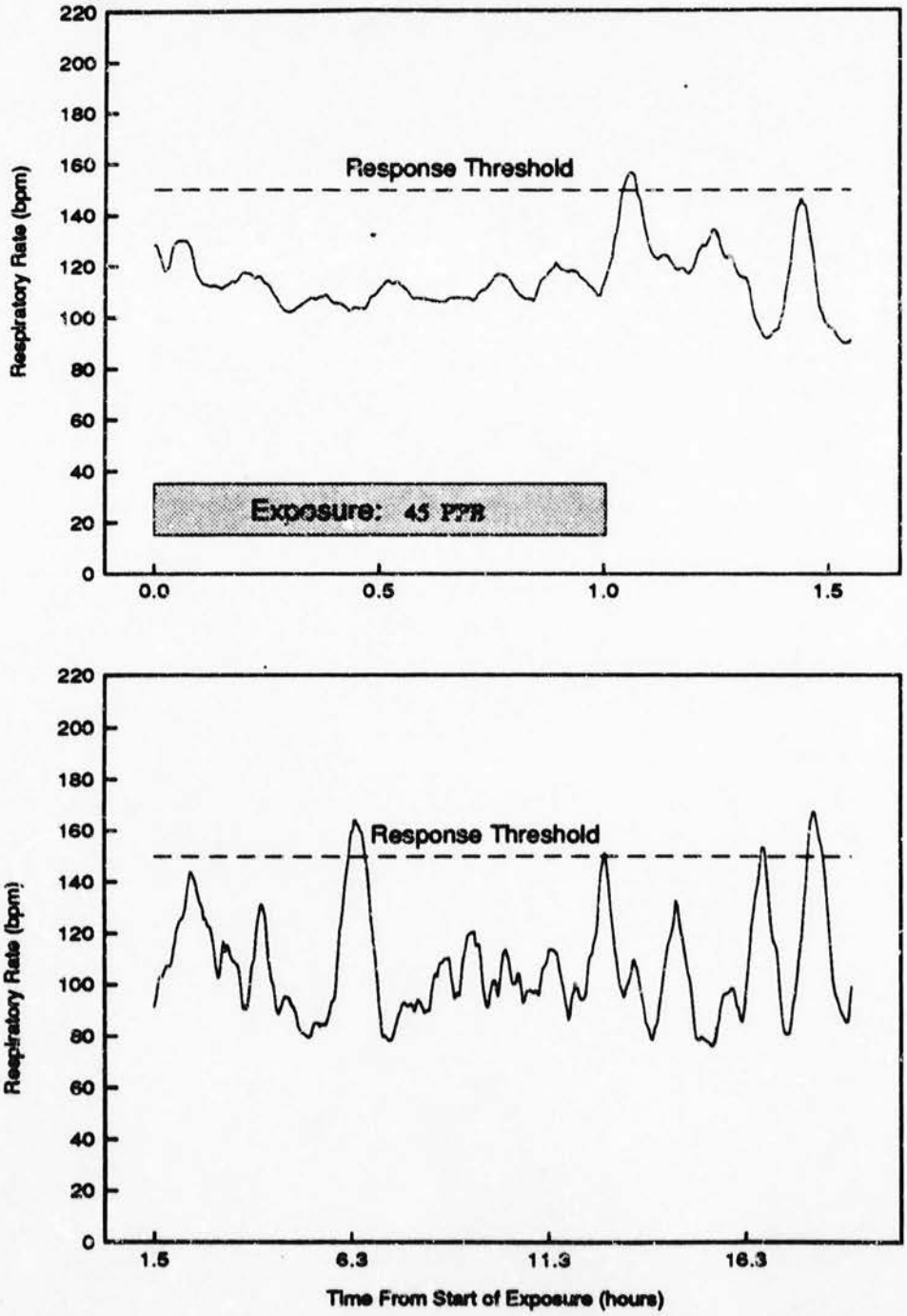
Figure: 108 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 97: 2,6-TDI Sensitization

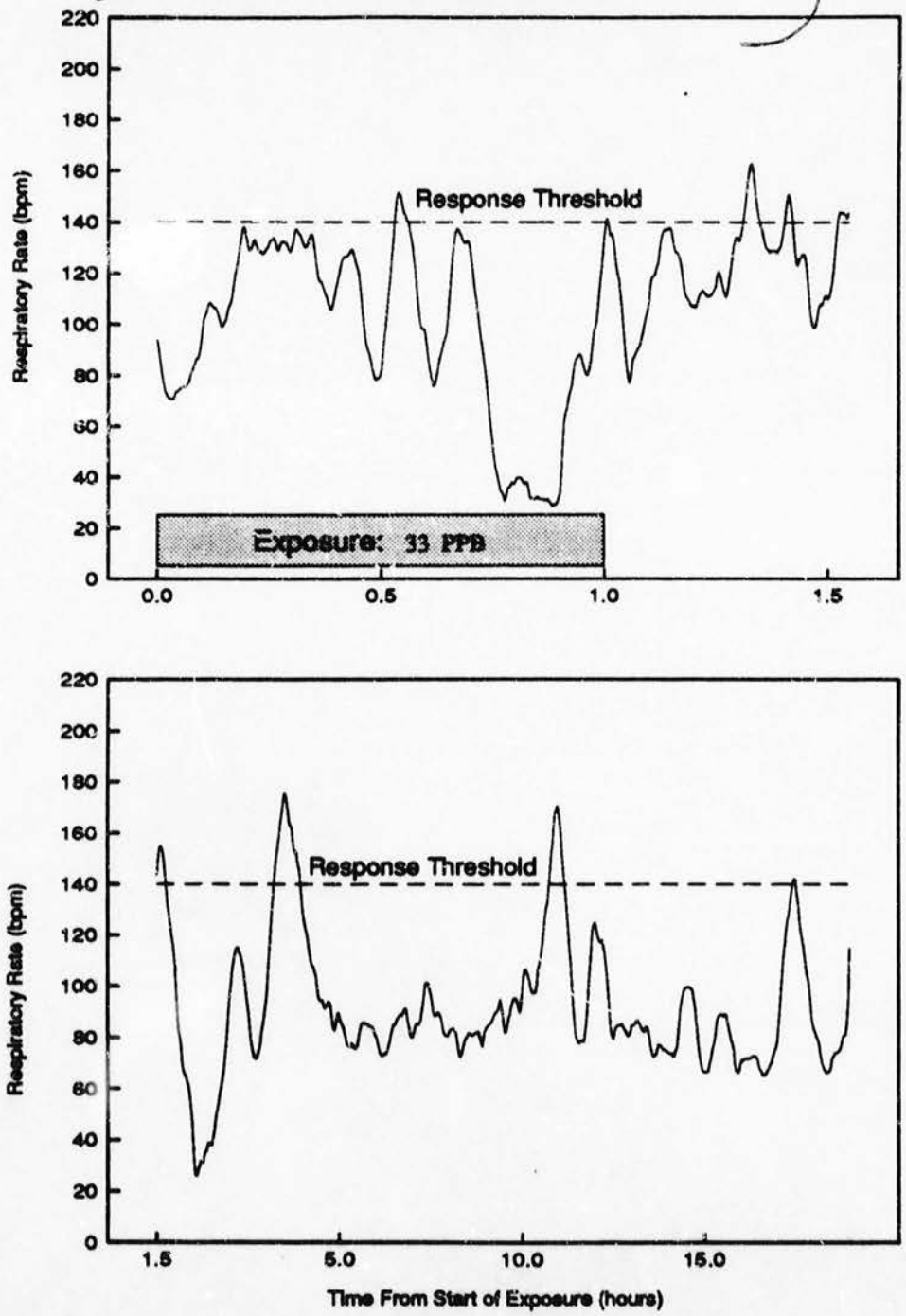
Figure: 109 2,6-TDI Challenge Week 1



Study Number 91-344-KR

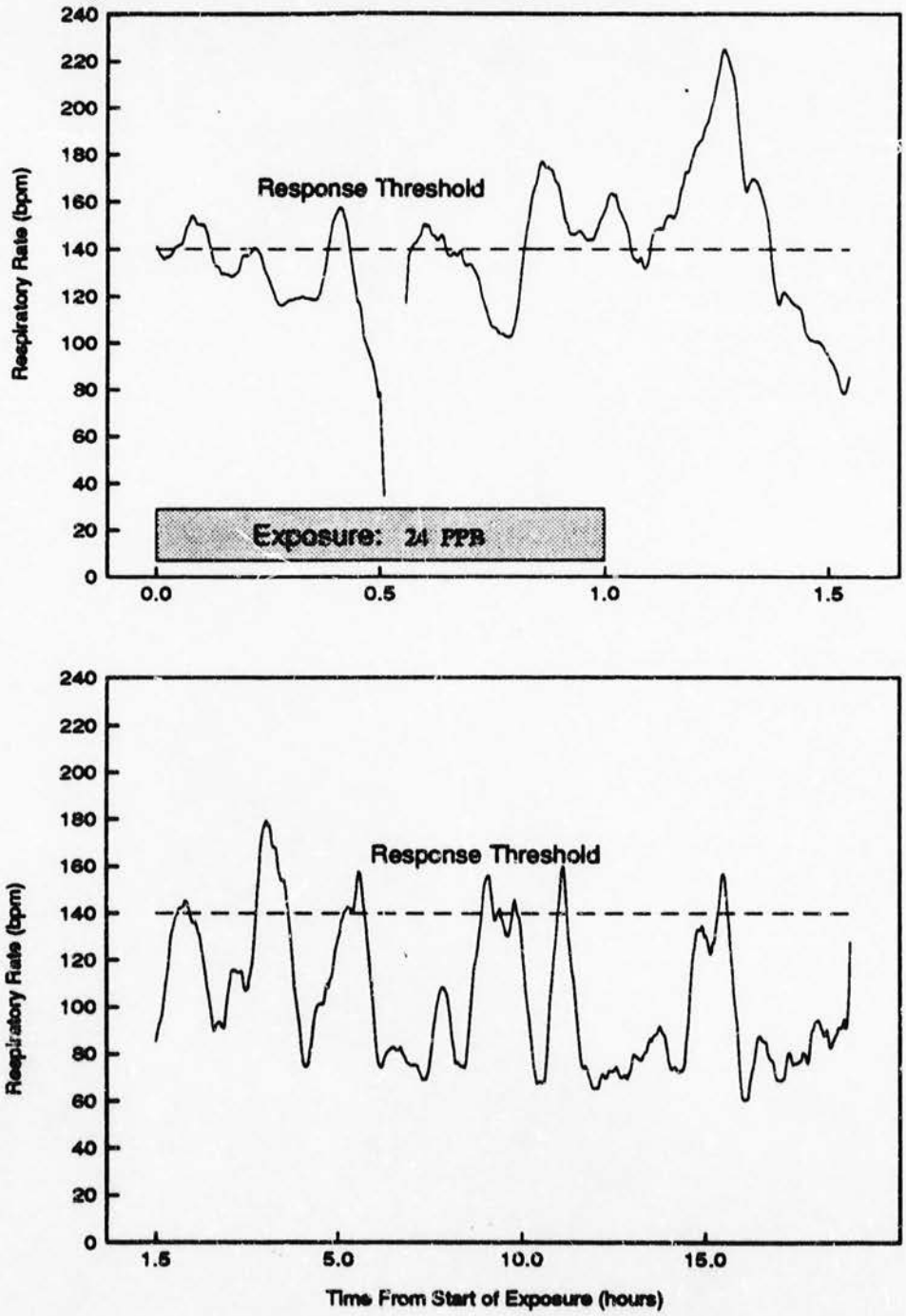
Animal # 98: 2,6-TDI Sensitization

Figure: 110 2,6-TDI Challenge Week 1



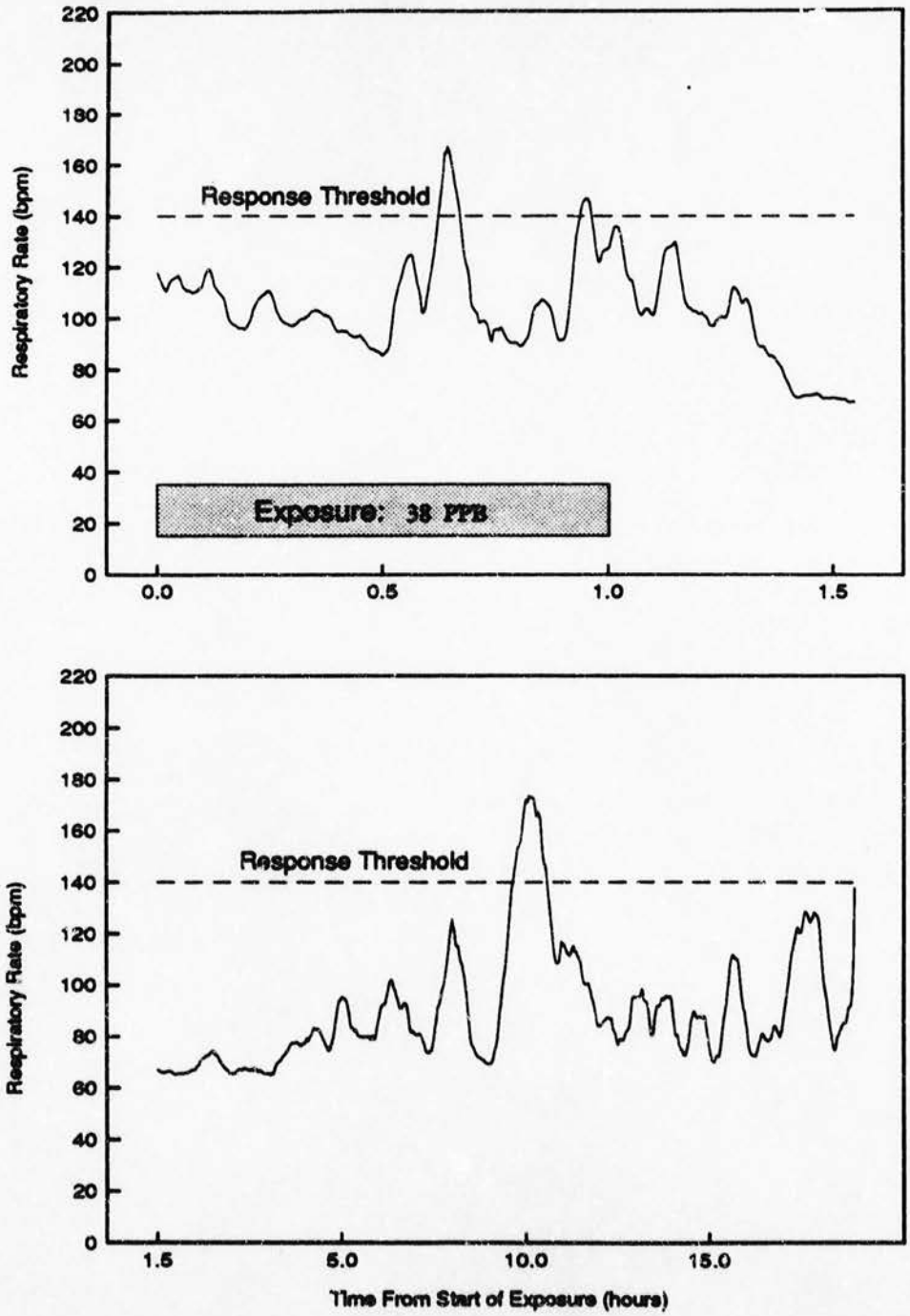
Study Number 91-344-KR
Animal # 99: 2,6-TDI Sensitization

Figure: 111 2,6-TDI Challenge Week 1



Study Number 91-344-KR
Animal # 100: 2,6-TDI Sensitization

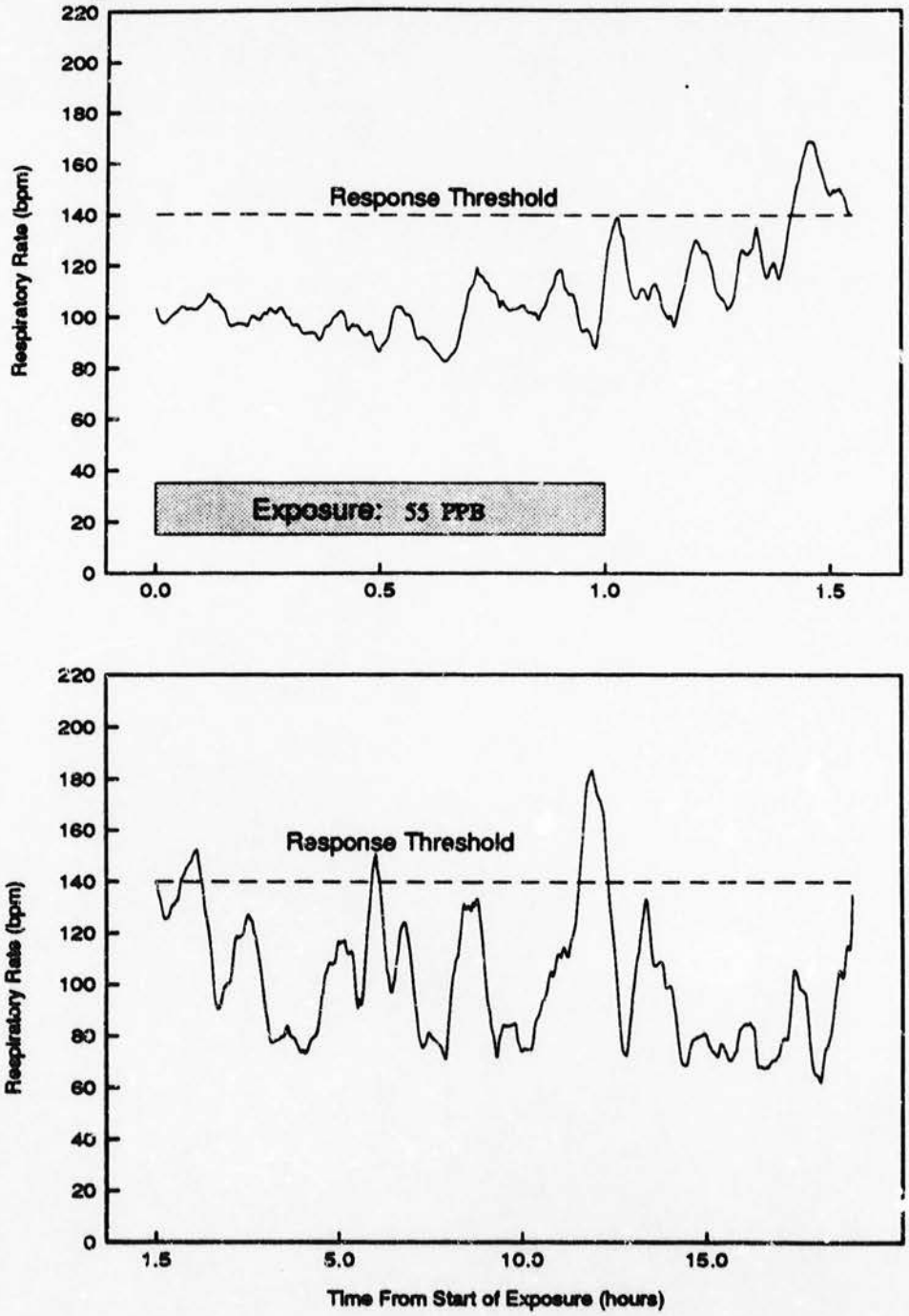
Figure: 112 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 101: 2,6-TDI Sensitization

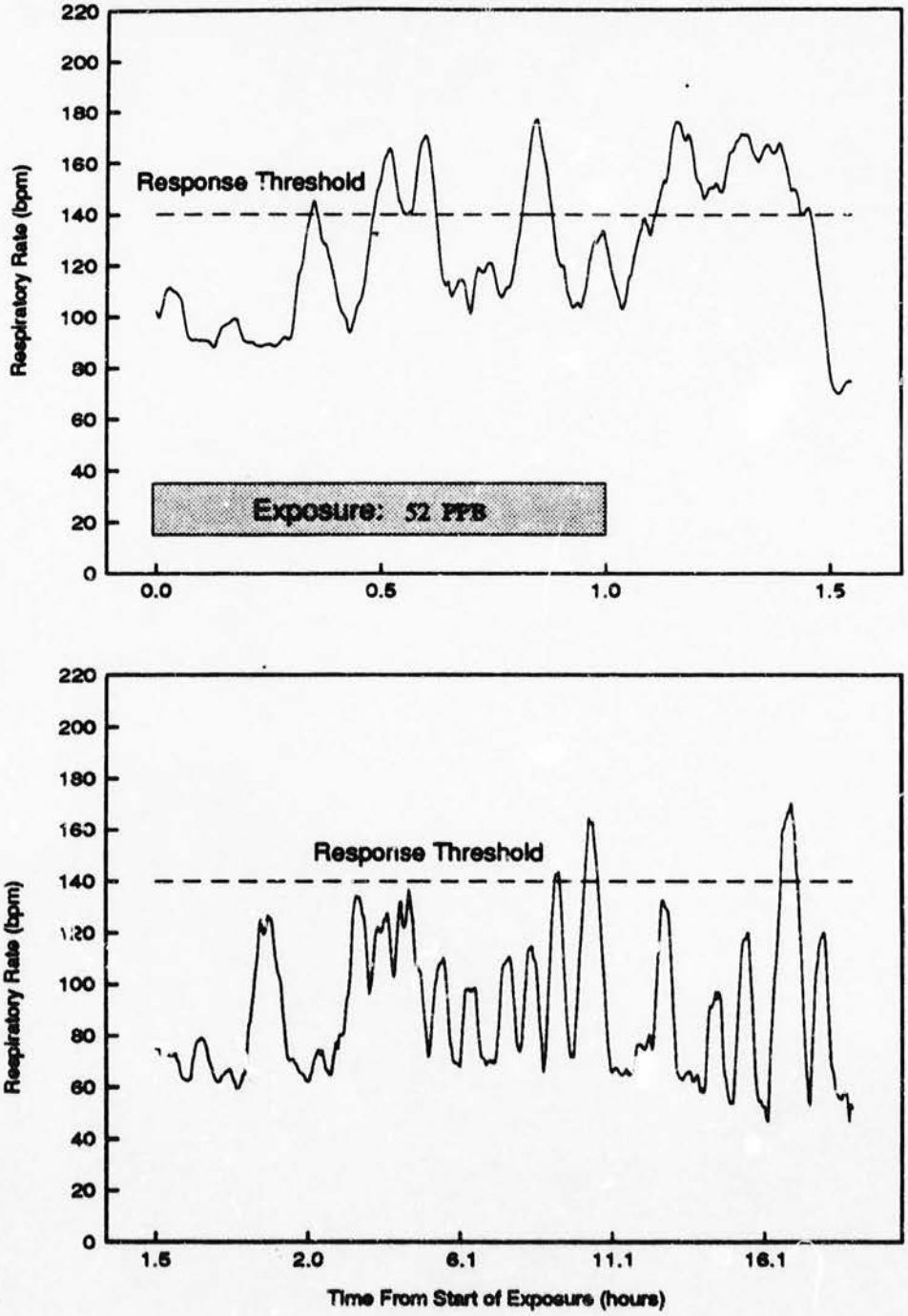
Figure: 113 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 102: 2,6-TDI Sensitization

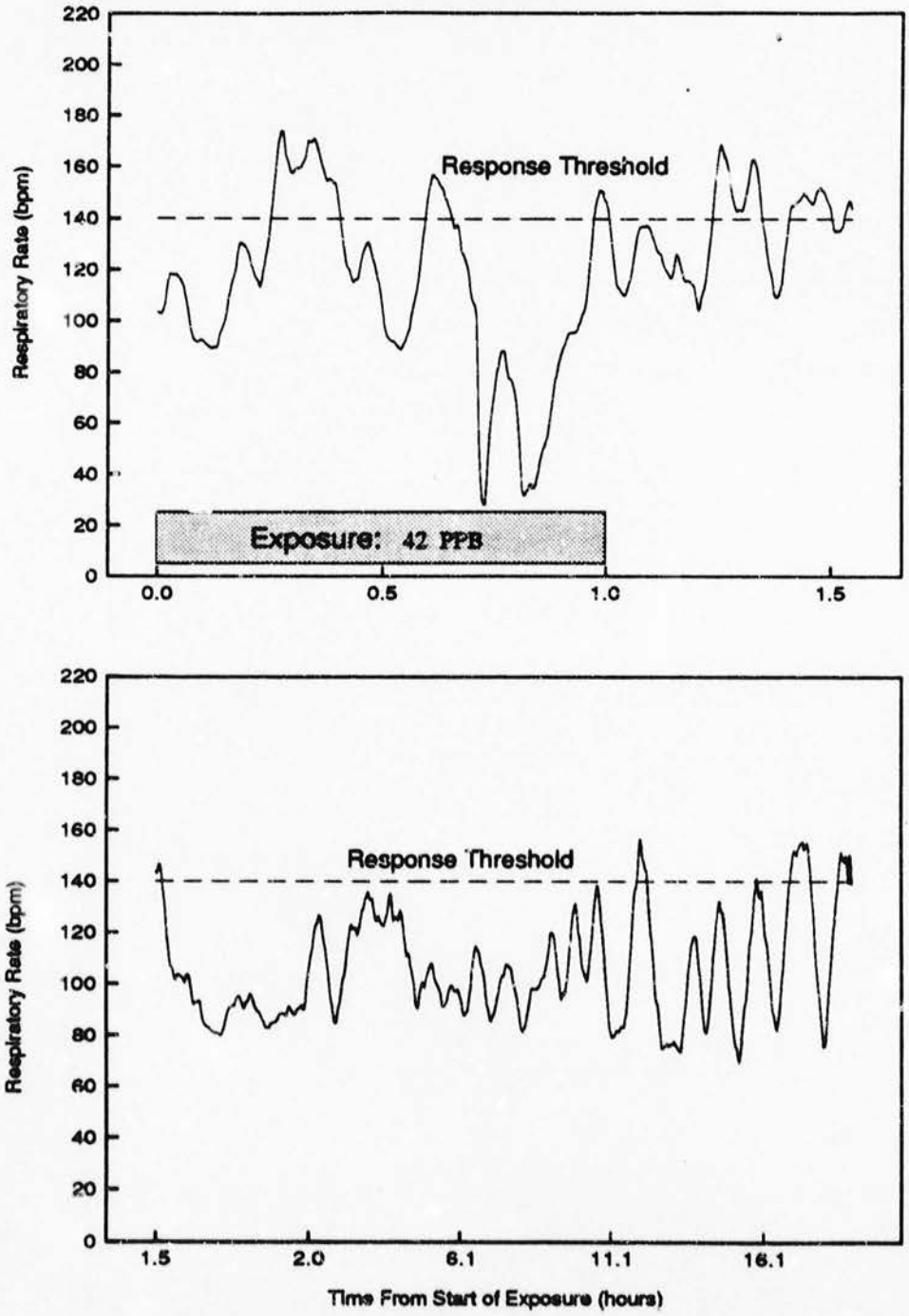
Figure: 114 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 103: 2,6-TDI Sensitization

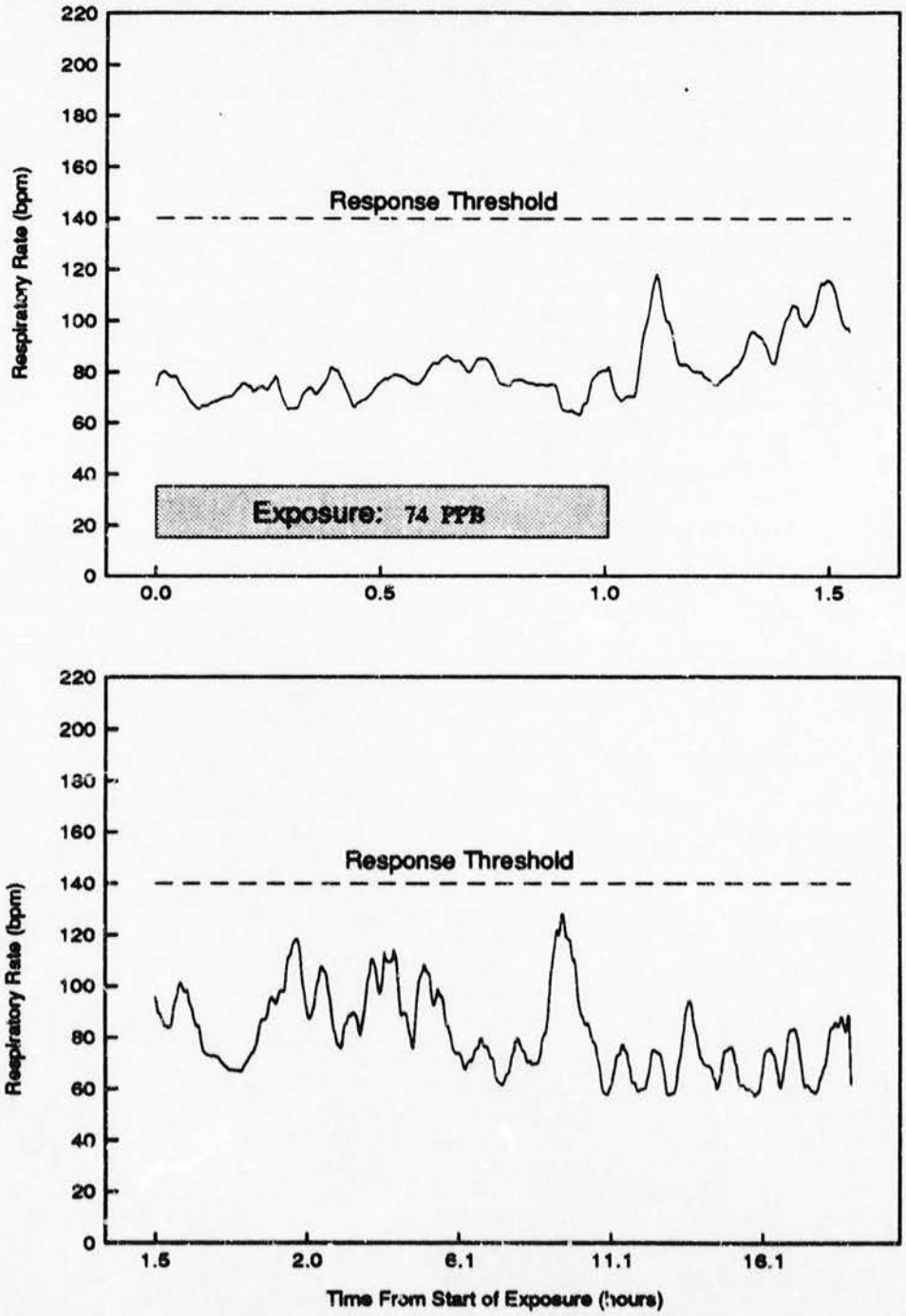
Figure: 115 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 104: 2,6-TDI Sensitization

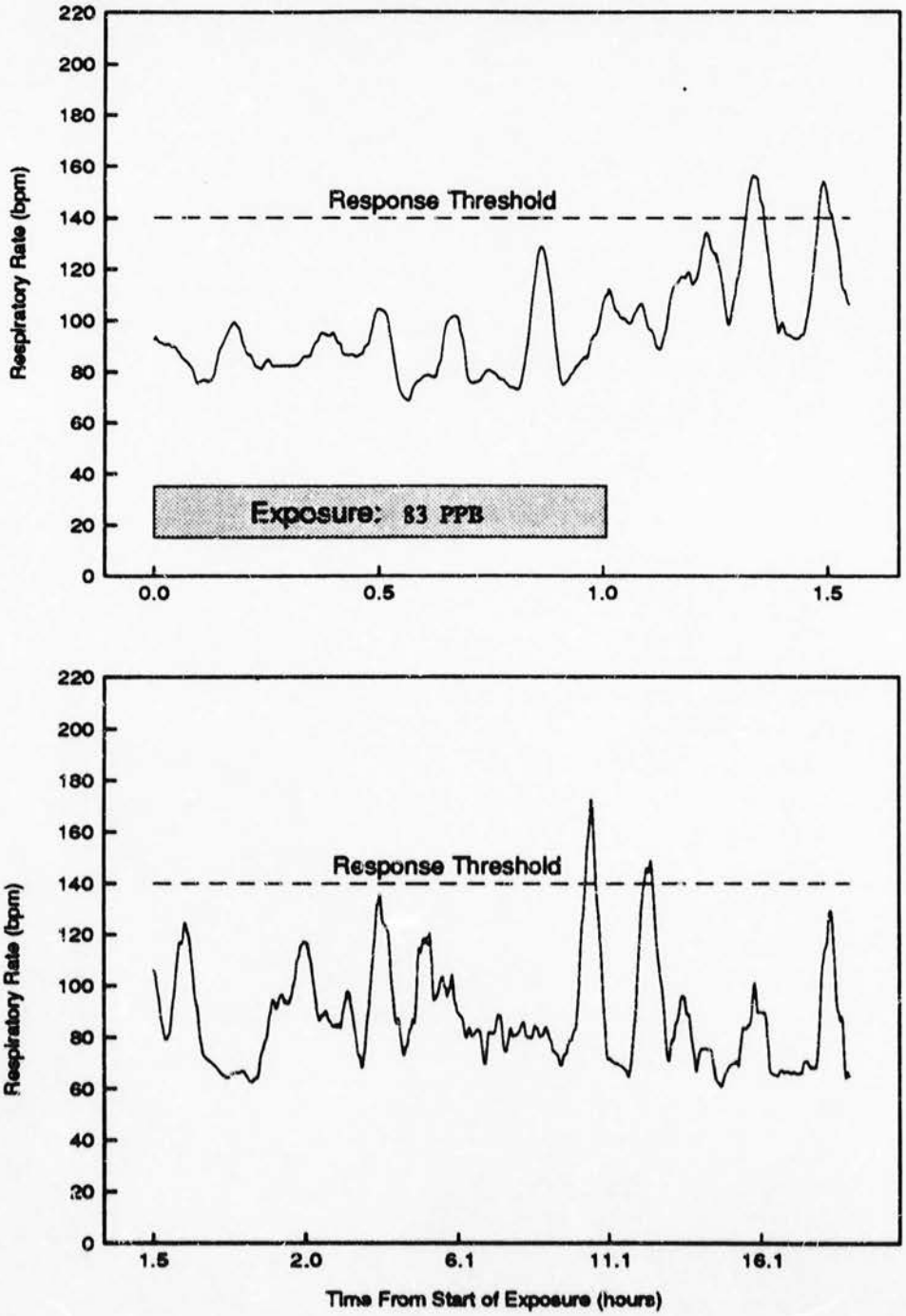
Figure: 116 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 105: 2,6-TDI Sensitization

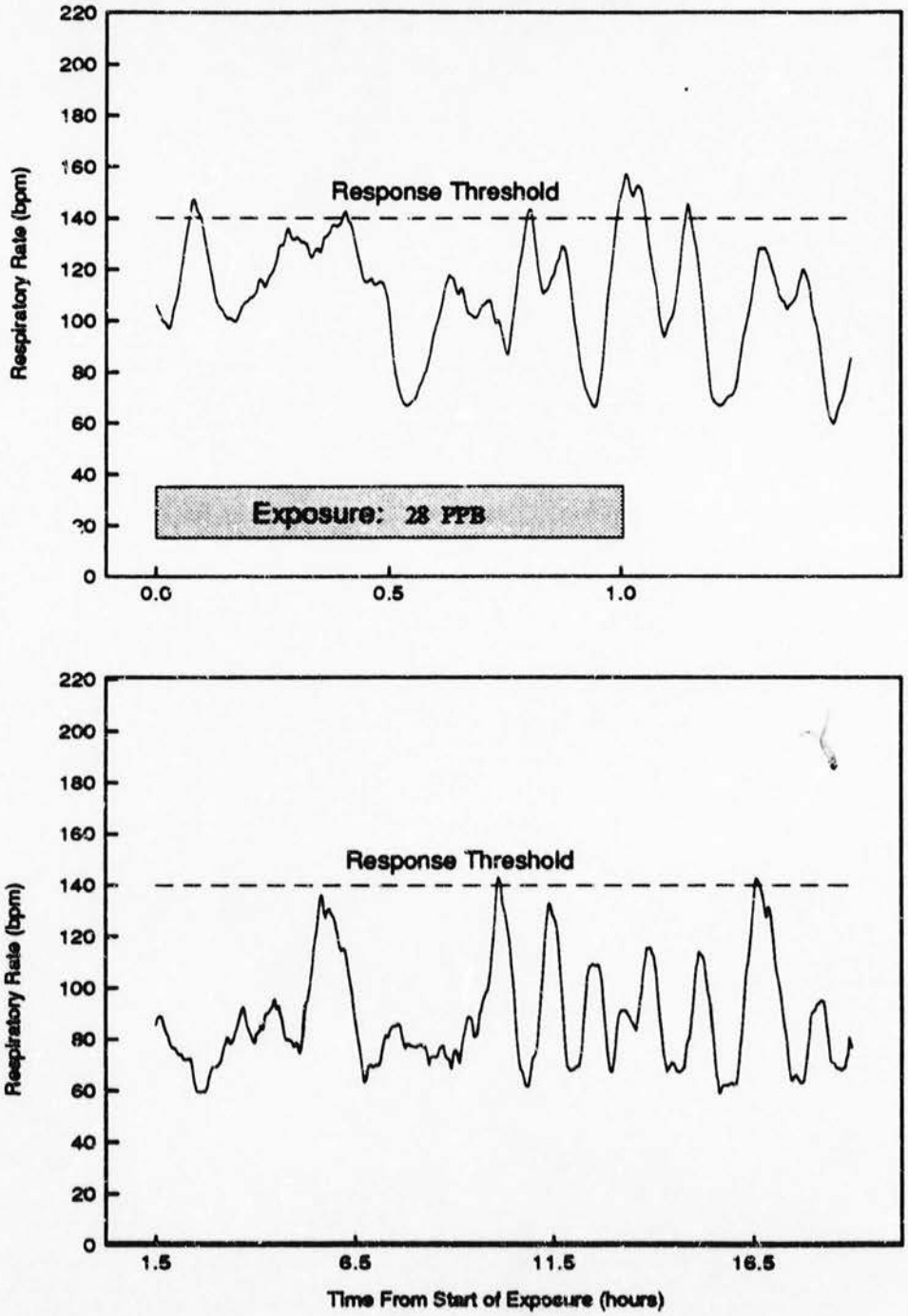
Figure: 117 2,6-TDI Challenge Week 1



Study Number 91-344-KR

Animal # 93: 2,6-TDI Sensitization

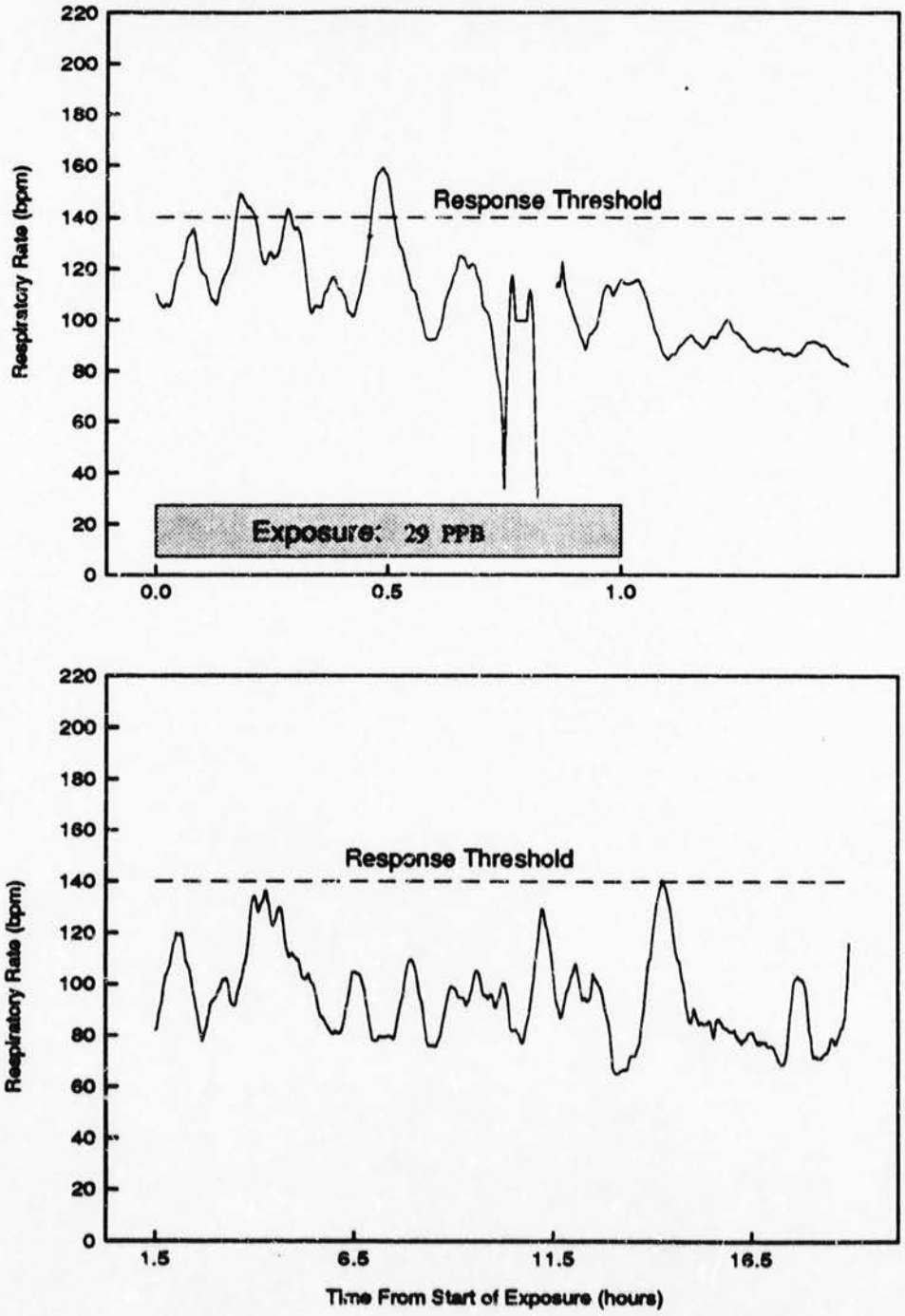
Figure: 118 2,6-TDI Challenge Week 2



Study Number 91-344-KR

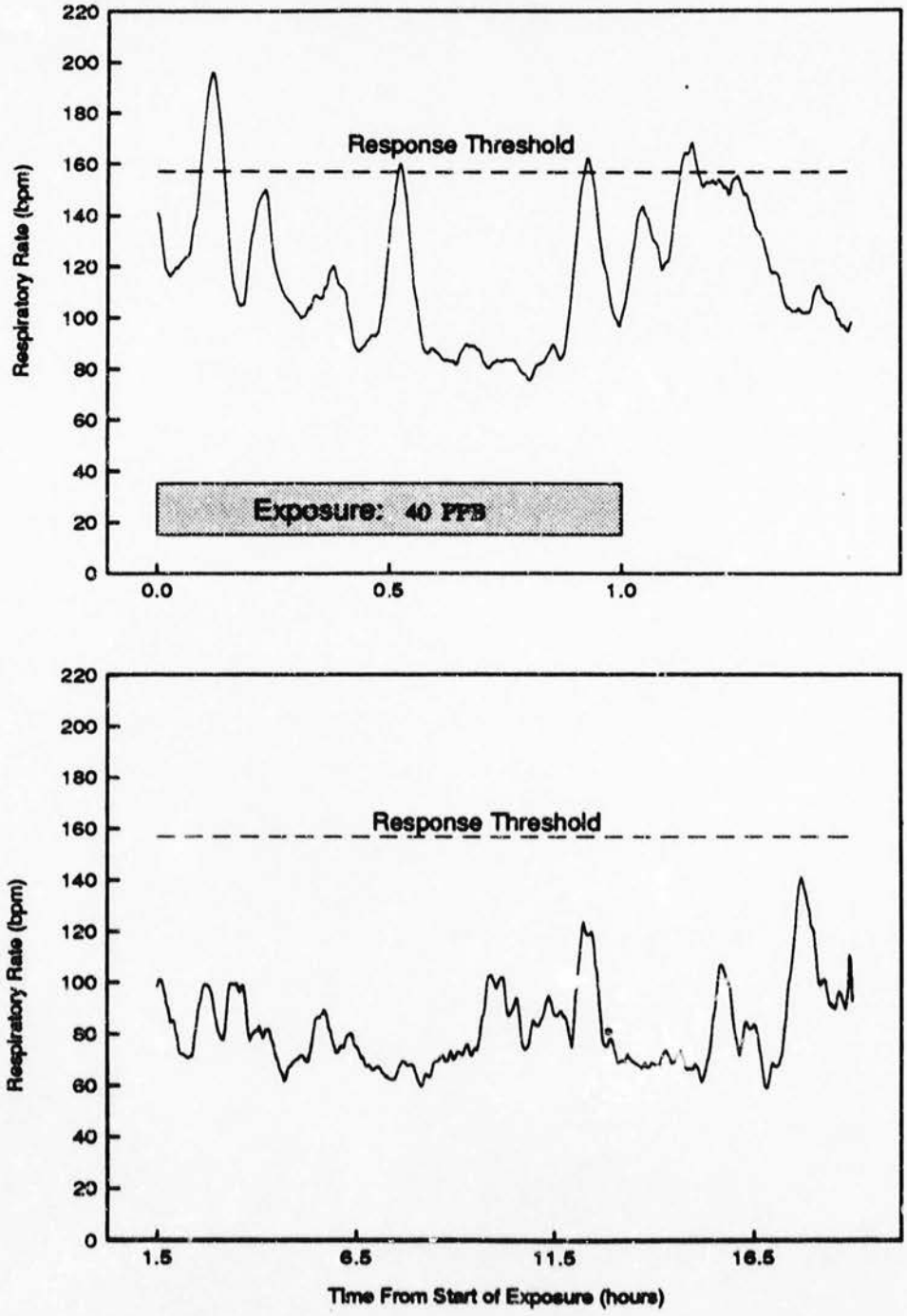
Animal # 94: 2,6-TDI Sensitization

Figure: 119 2,6-TDI Challenge Week 2

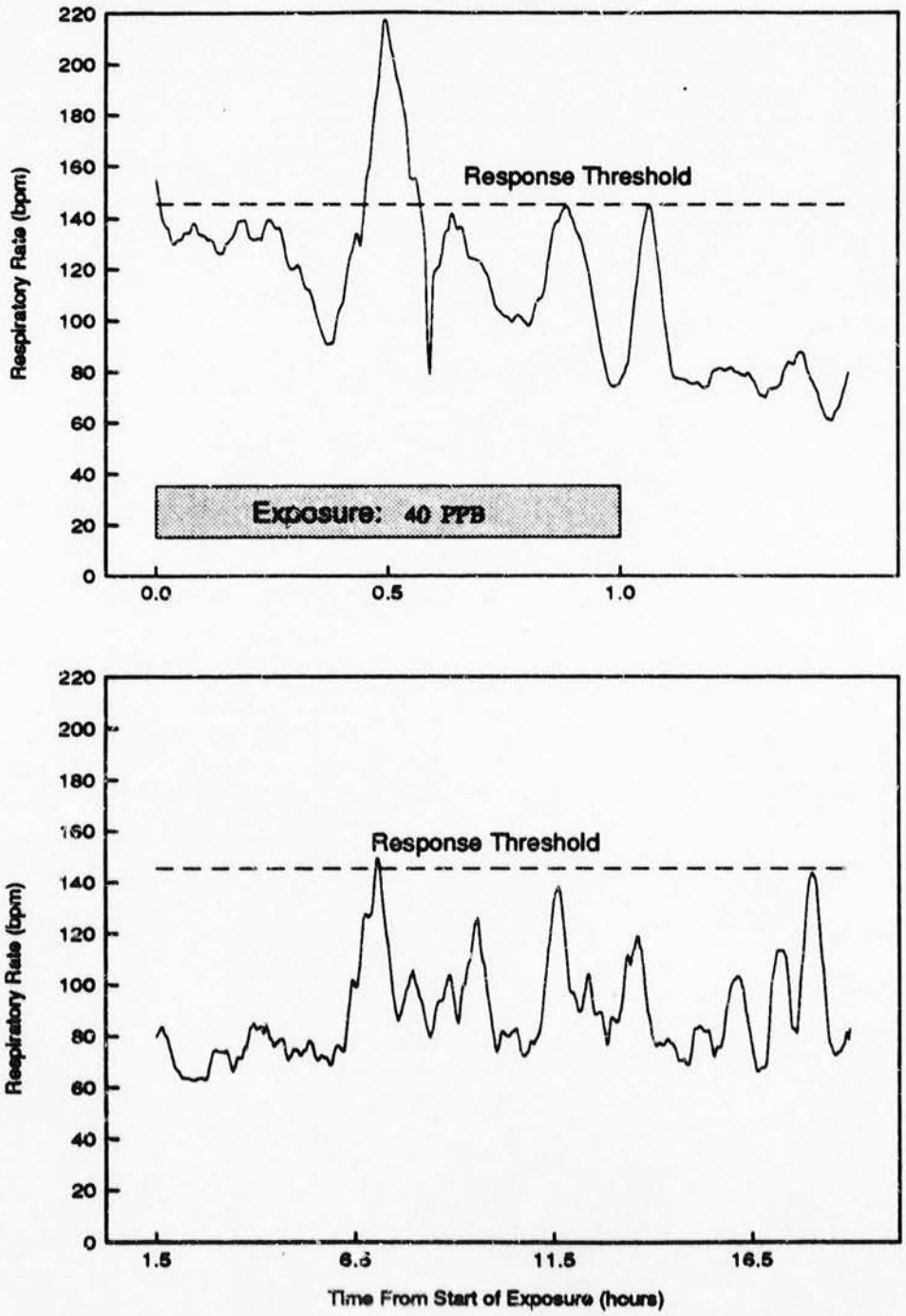


Study Number 91-344-KR
Animal # 95: 2,6-TDI Sensitization

Figure: 120 2,6-TDI Challenge Week 2

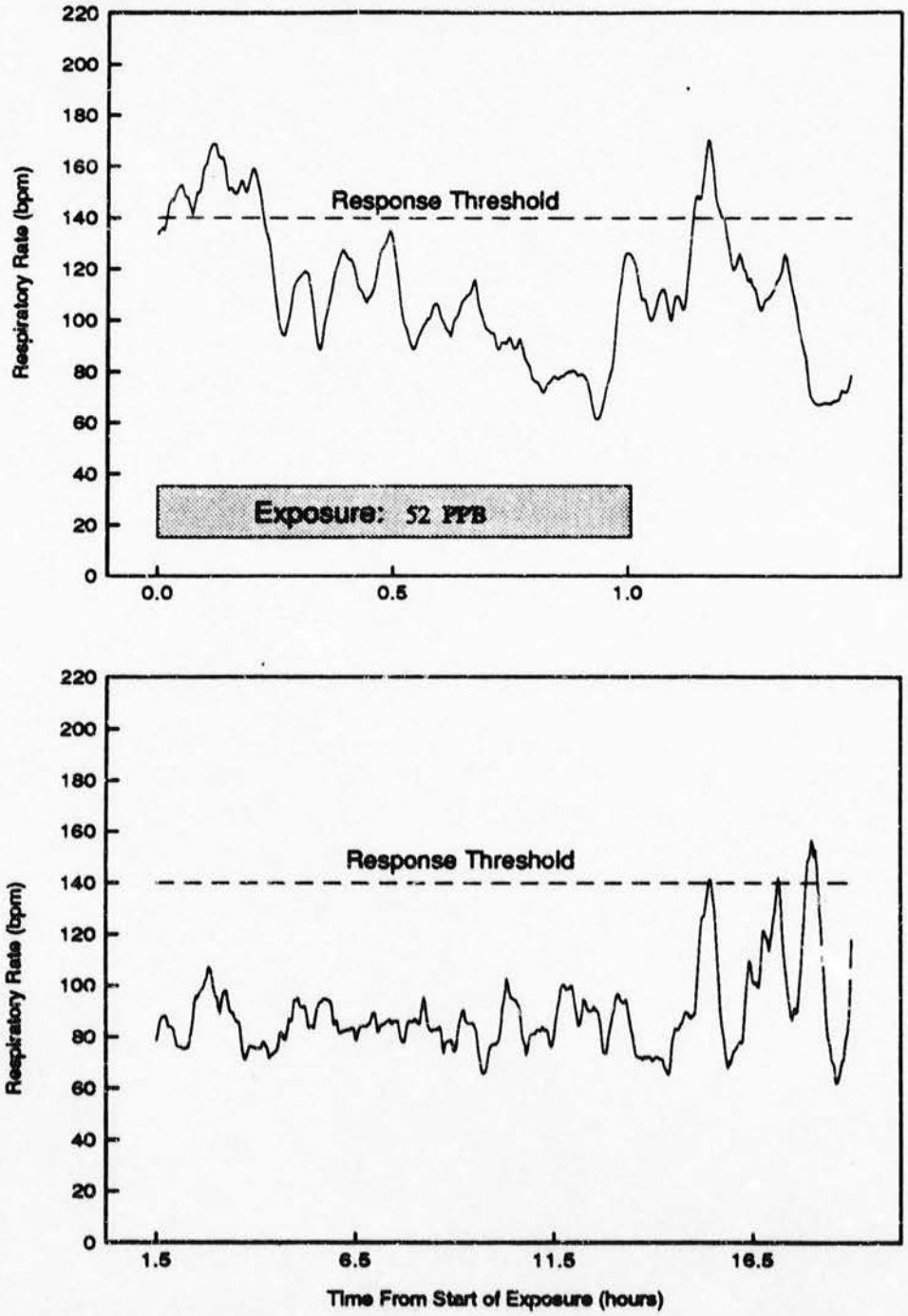


Study Number 91-344-KR
Animal # 97: 2,6-TDI Sensitization
Figure: 121 2,6-TDI Challenge Week 2



Study Number 91-344-KR
Animal # 98: 2,6-TDI Sensitization

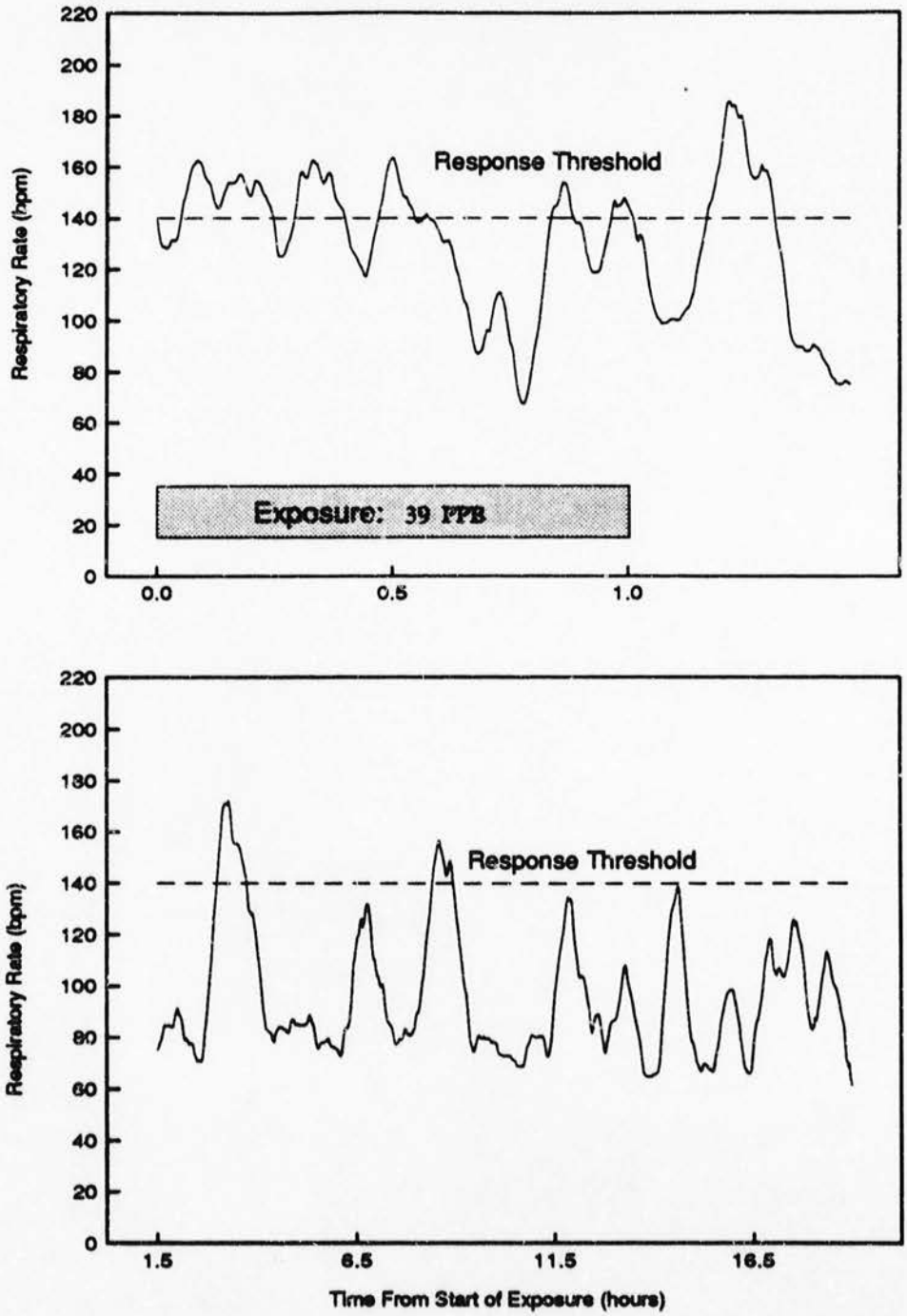
Figure: 122 2,6-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 99: 2,6-TDI Sensitization

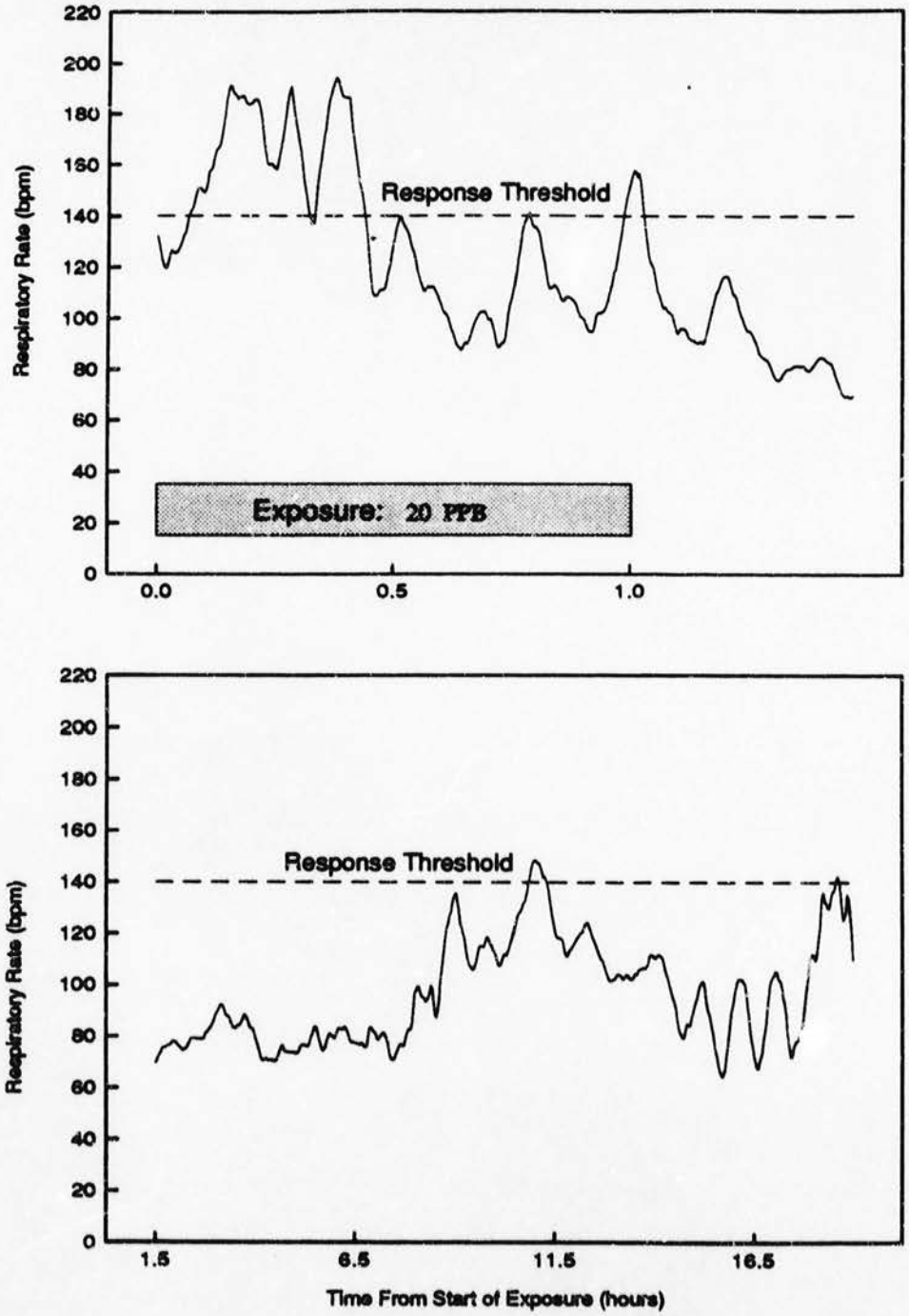
Figure: 123 2,6-TDI Challenge Week 2



Study Number 91-344-KR

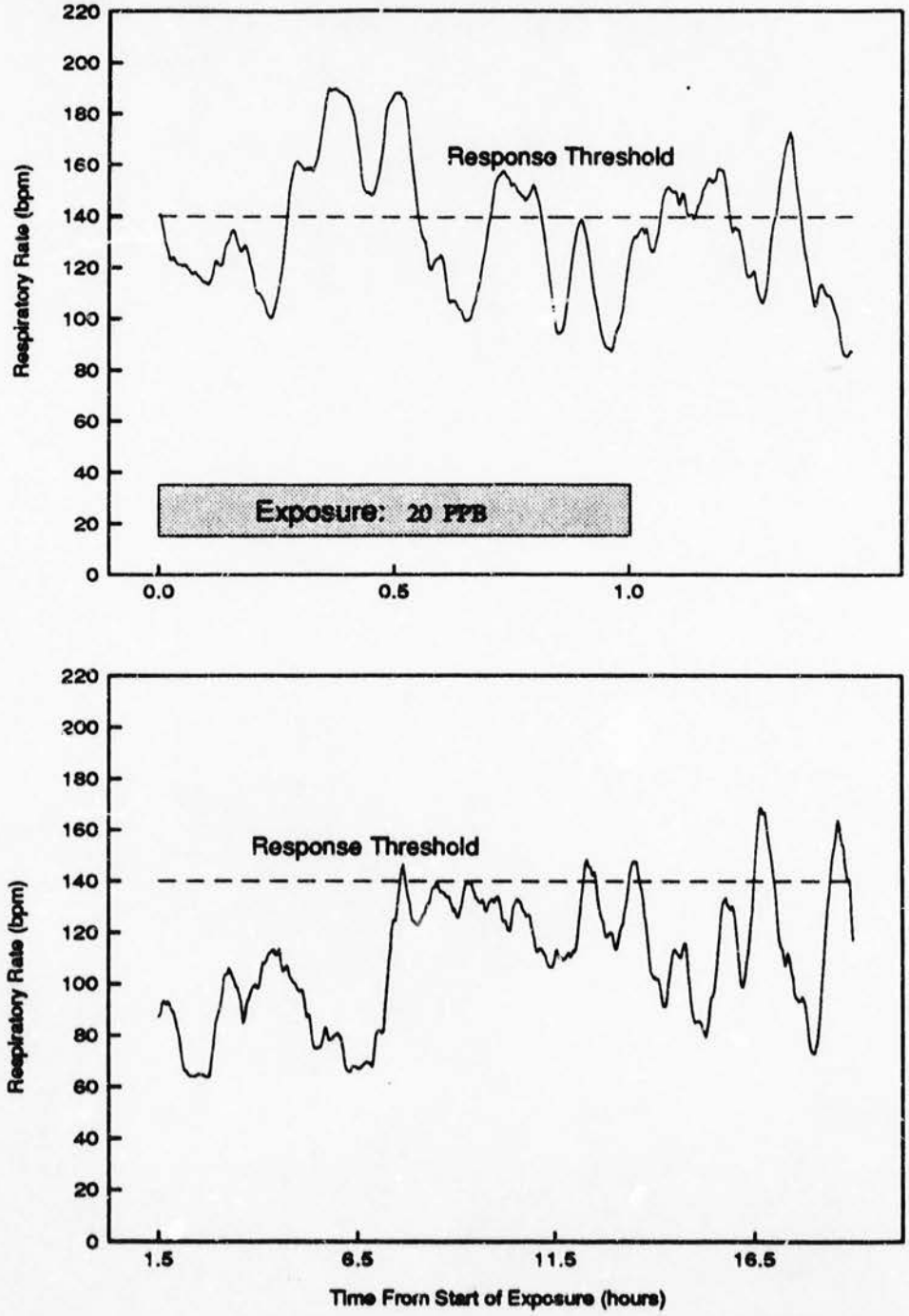
Animal # 100: 2,6-TDI Sensitization

Figure: 124 2,6-TDI Challenge Week 2



Study Number 91-344-KR
Animal # 101: 2,6-TDI Sensitization

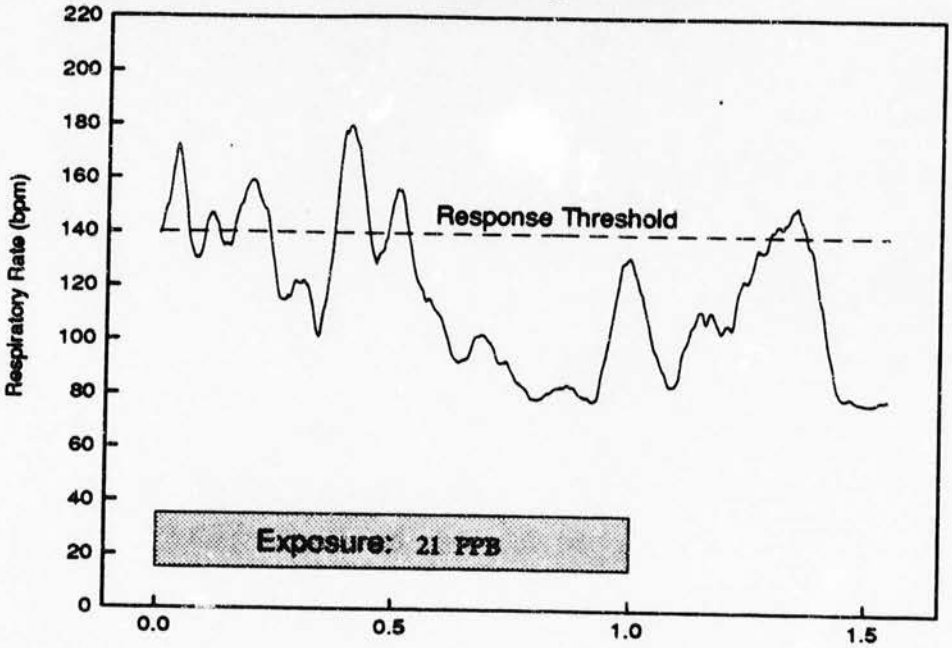
Figure: 125 2,6-TDI Challenge Week 2

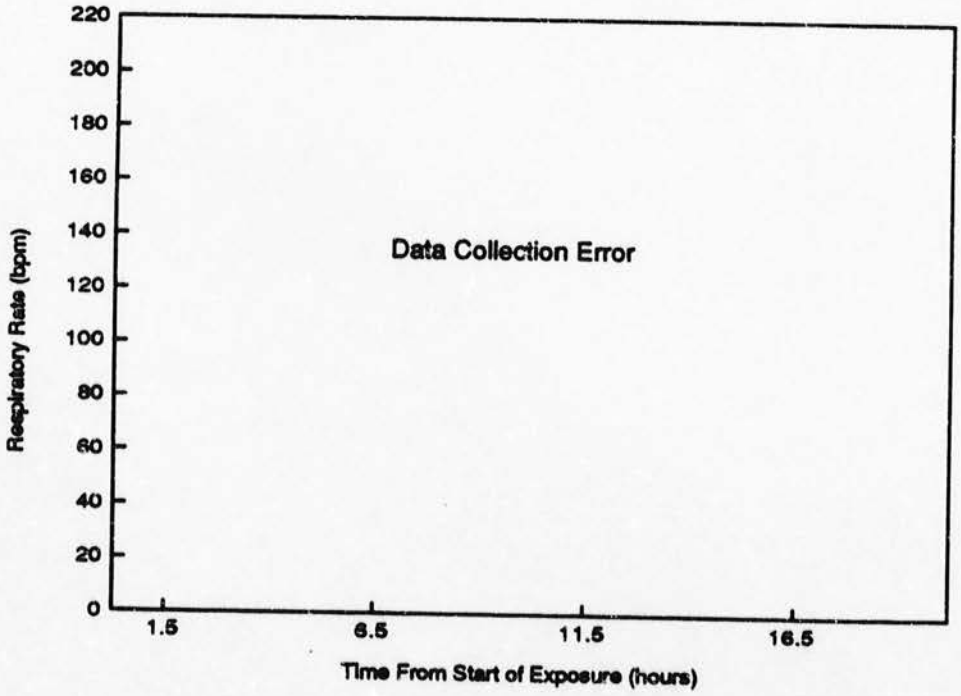


Study Number 91-344-KR

Animal # 102: 2,6-TDI Sensitization

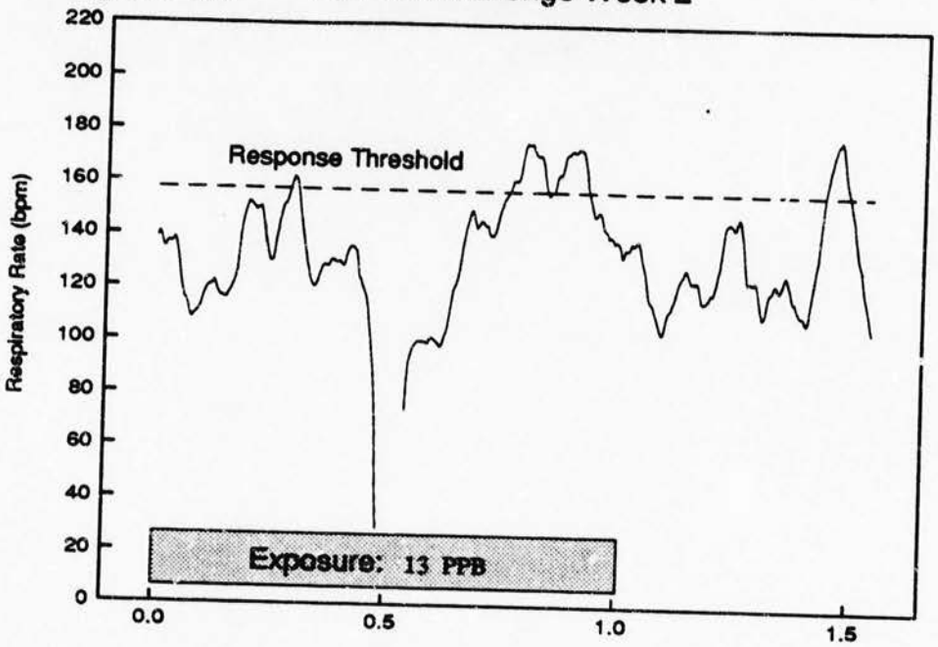
Figure: 126 2,6-TDI Challenge Week 2

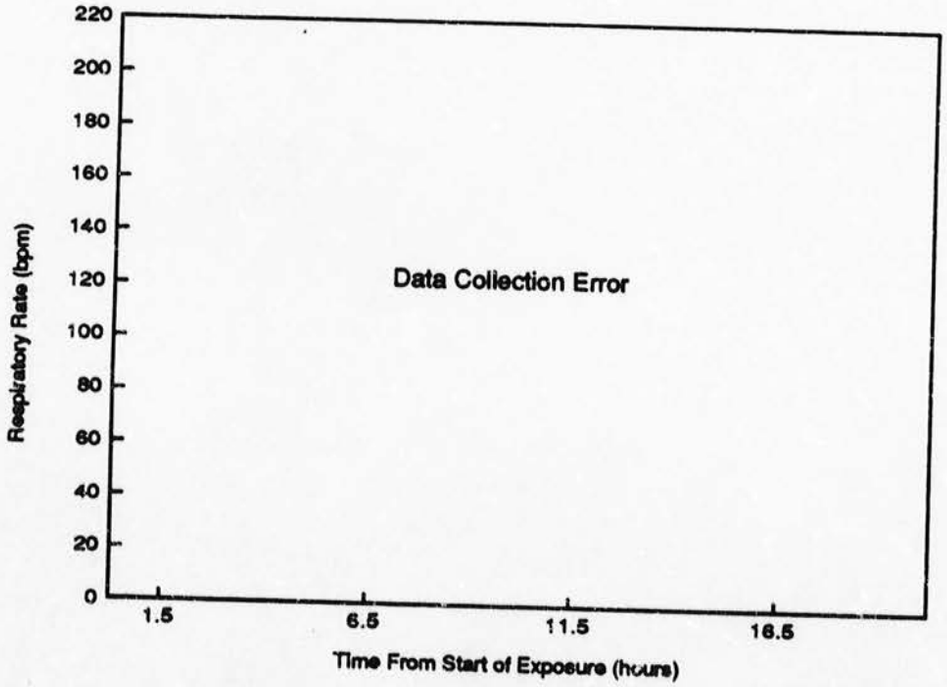




Study Number 91-344-KR
Animal # 103: 2,6-TDI Sensitization

Figure: 127 2,6-TDI Challenge Week 2

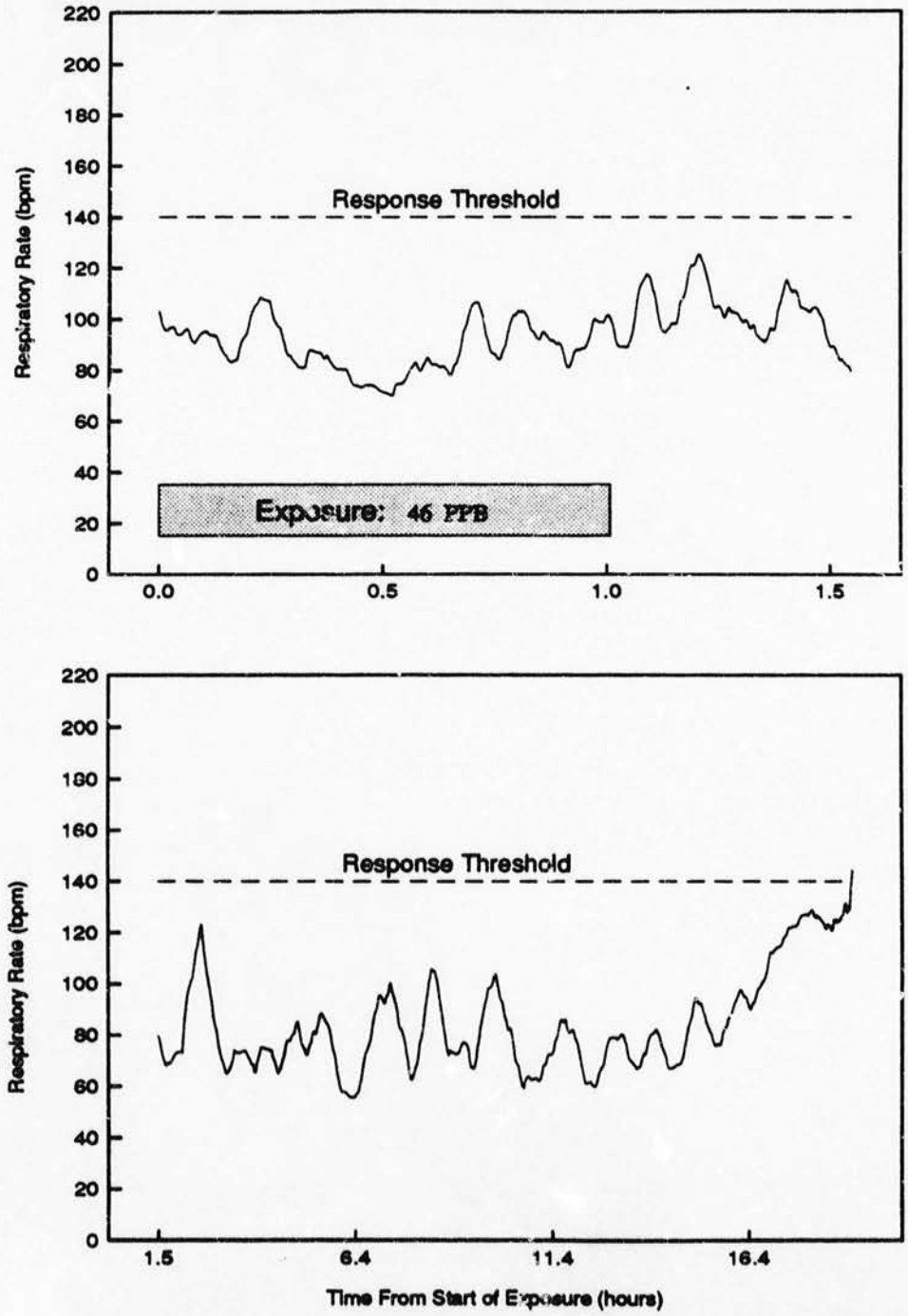




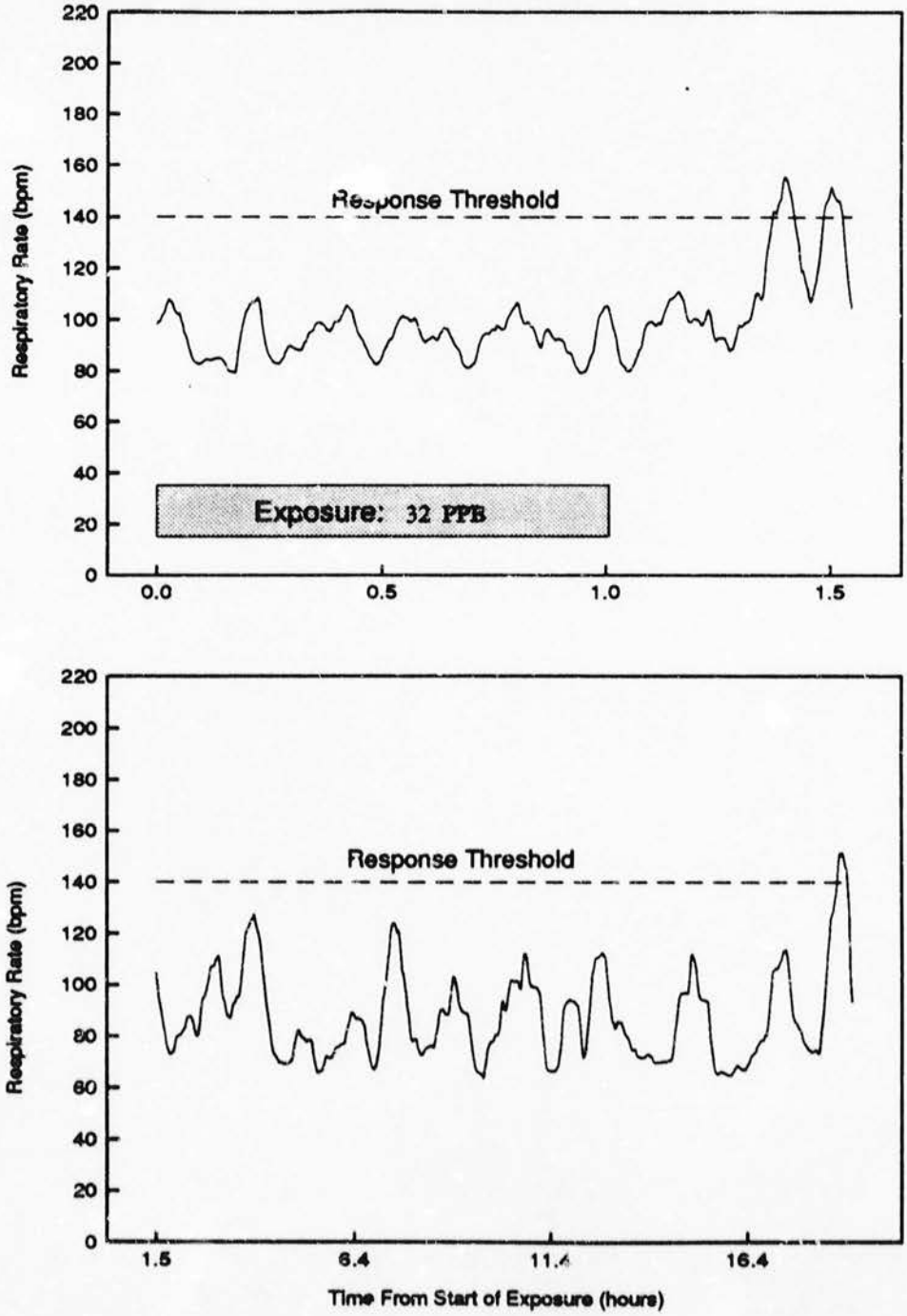
Study Number 91-344-KR

Animal # 104: 2,6-TDI Sensitization

Figure: 128 2,6-TDI Challenge Week 2



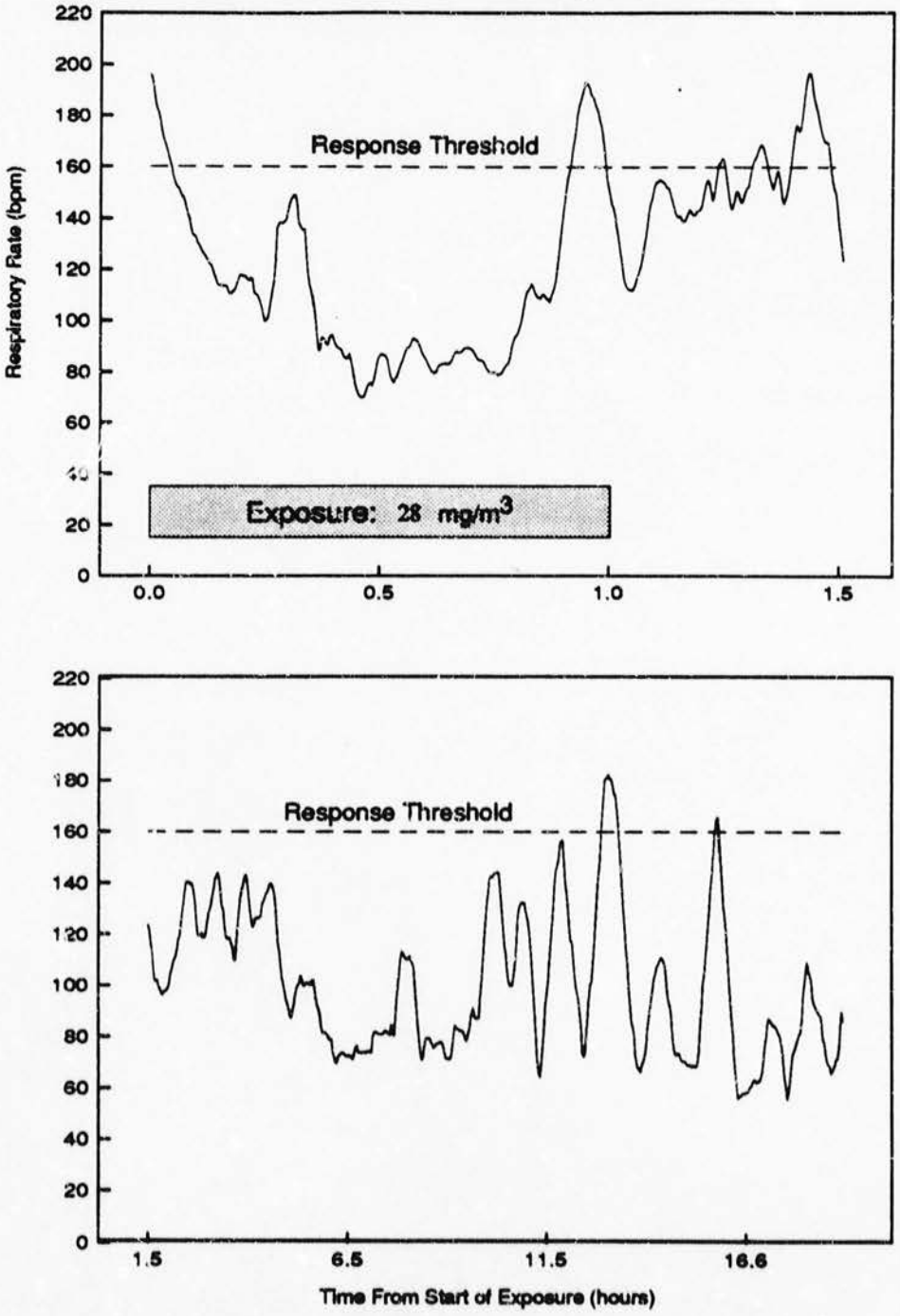
Study Number 91-344-KR
Animal # 105: 2,6-TDI Sensitization
Figure: 129 2,6-TDI Challenge Week 2



Study Number 91-344-KR

Animal # 93: 2,6-TDI Sensitization

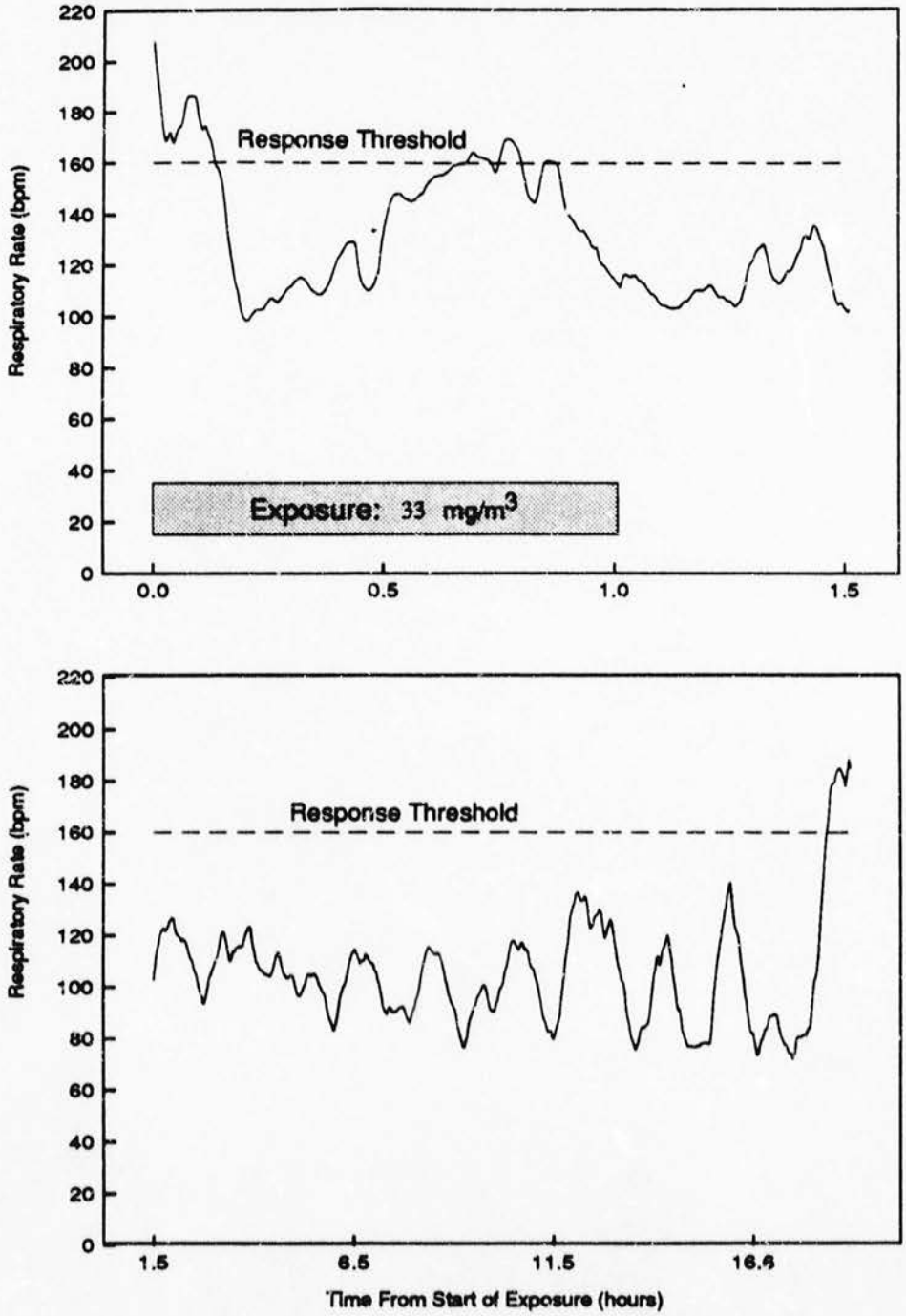
Figure: 130 2,6-TDI- GSA Challenge Week 3



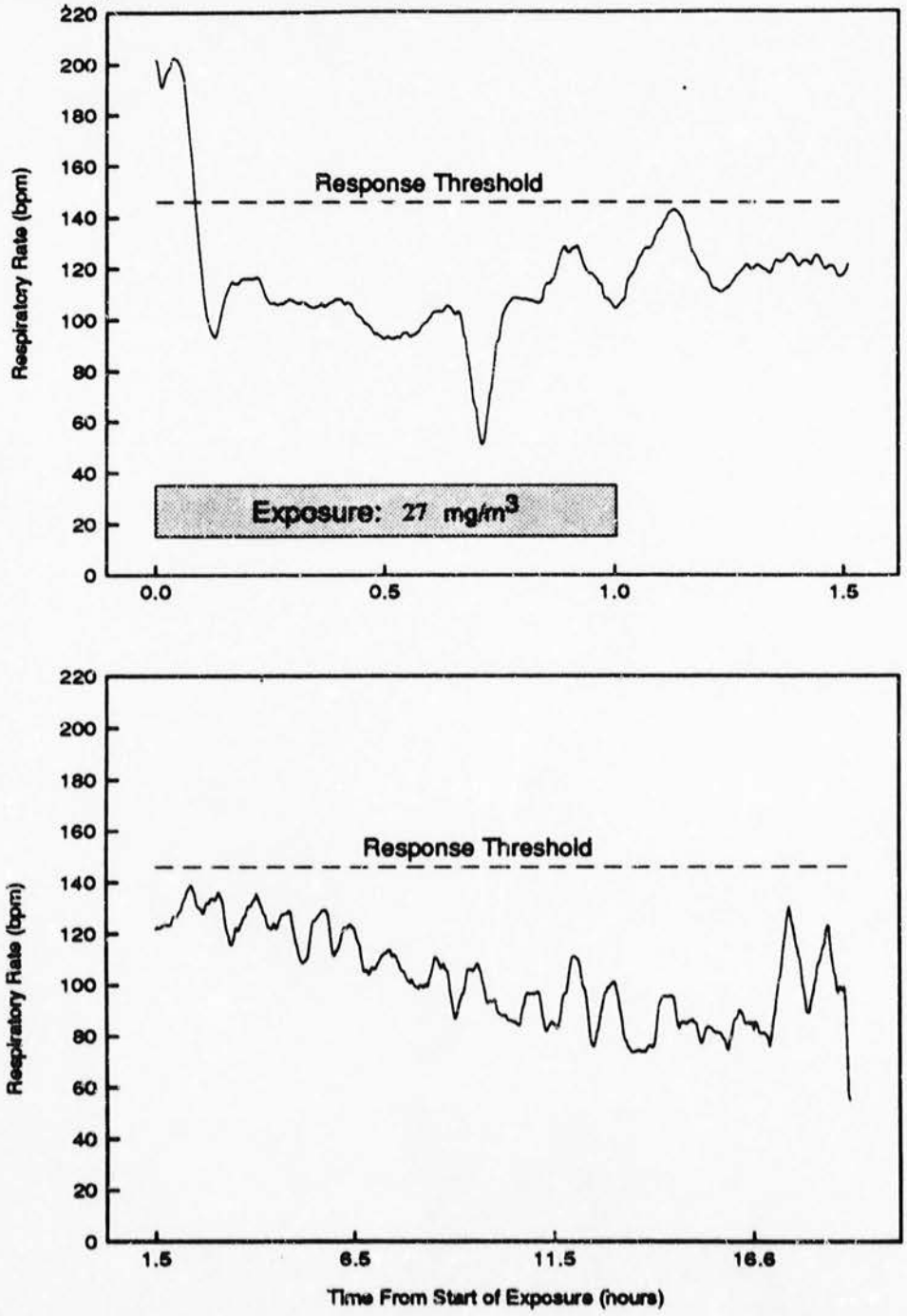
Study Number 91-344-KR

Animal # 94: 2,6-TDI Sensitization

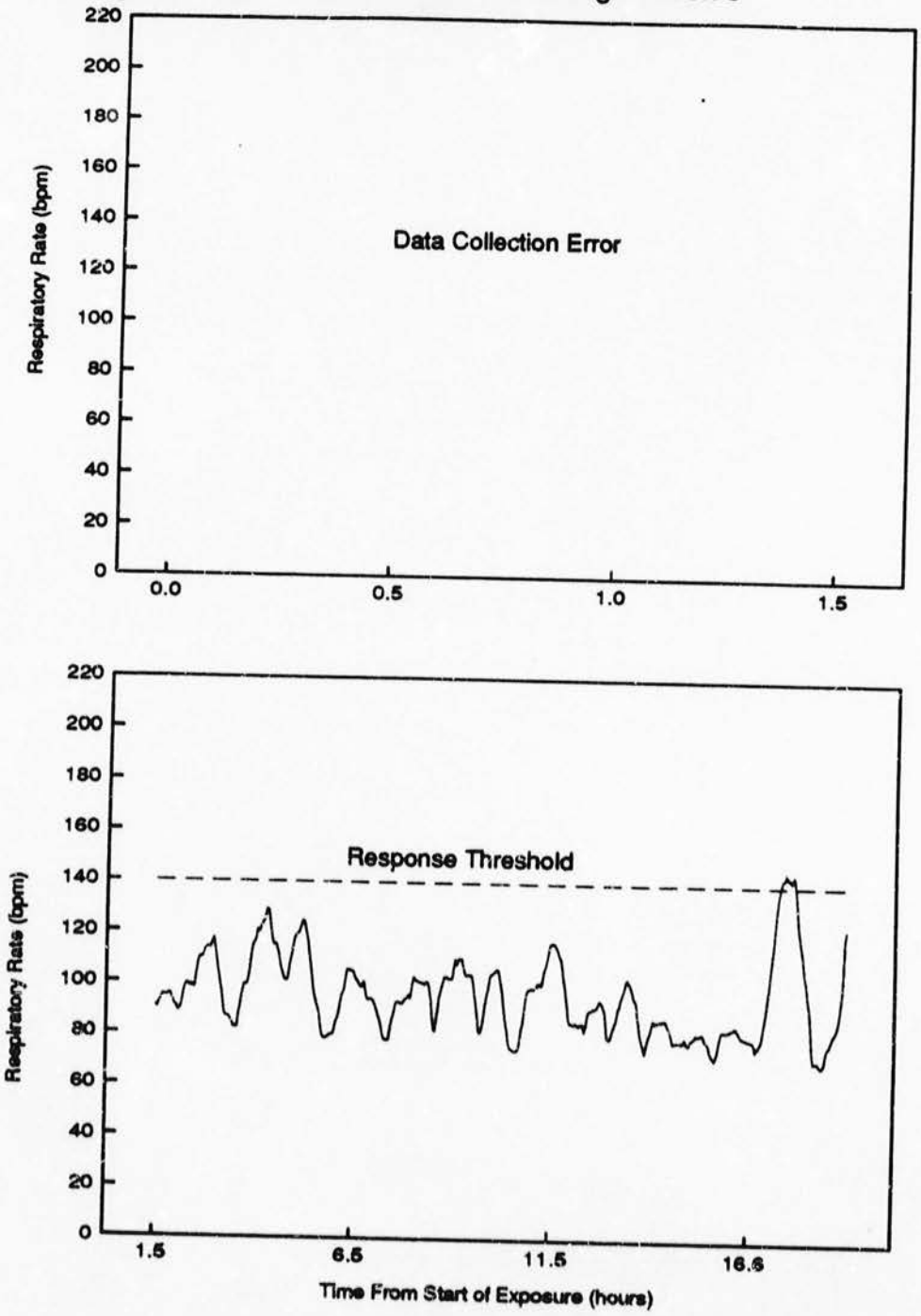
Figure: 131 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR
Animal # 95: 2,6-TDI Sensitization
Figure: 132 2,6-TDI-GSA Challenge Week 3



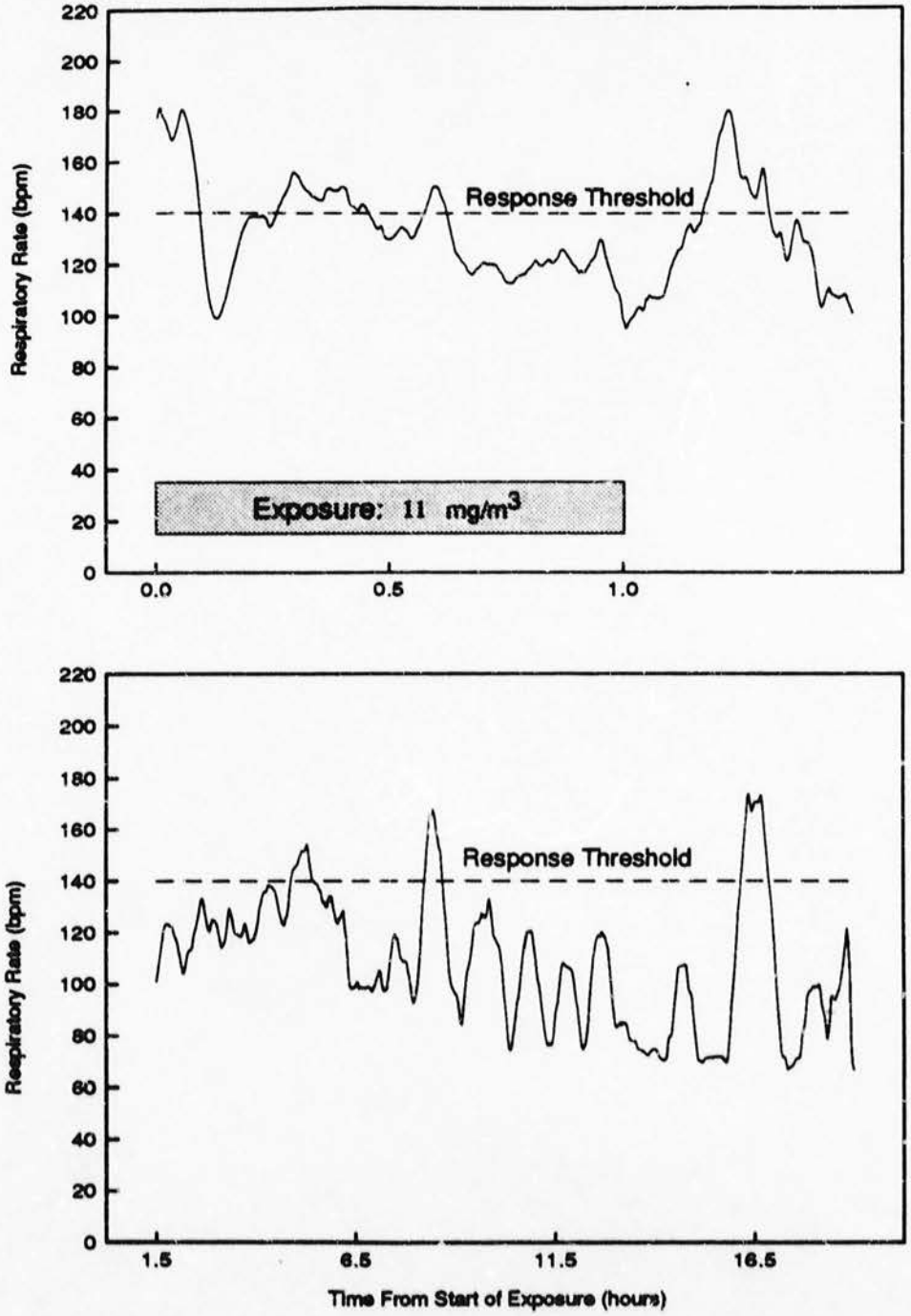
Study Number 91-344-KR
Animal # 97: 2,6-TDI Sensitization
Figure: 133 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 98: 2,6-TDI Sensitization

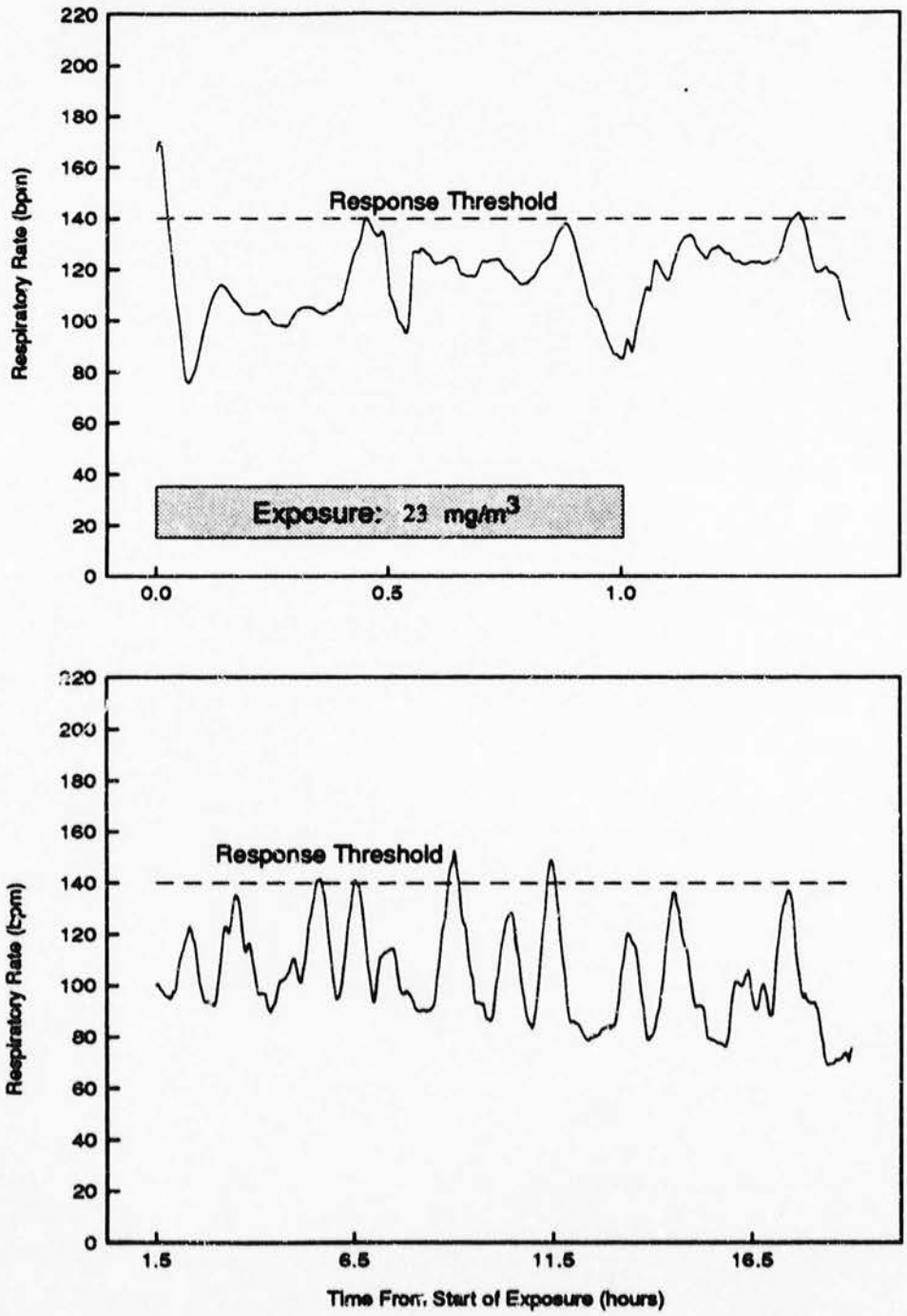
Figure: 134 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 99: 2,6-TDI Sensitization

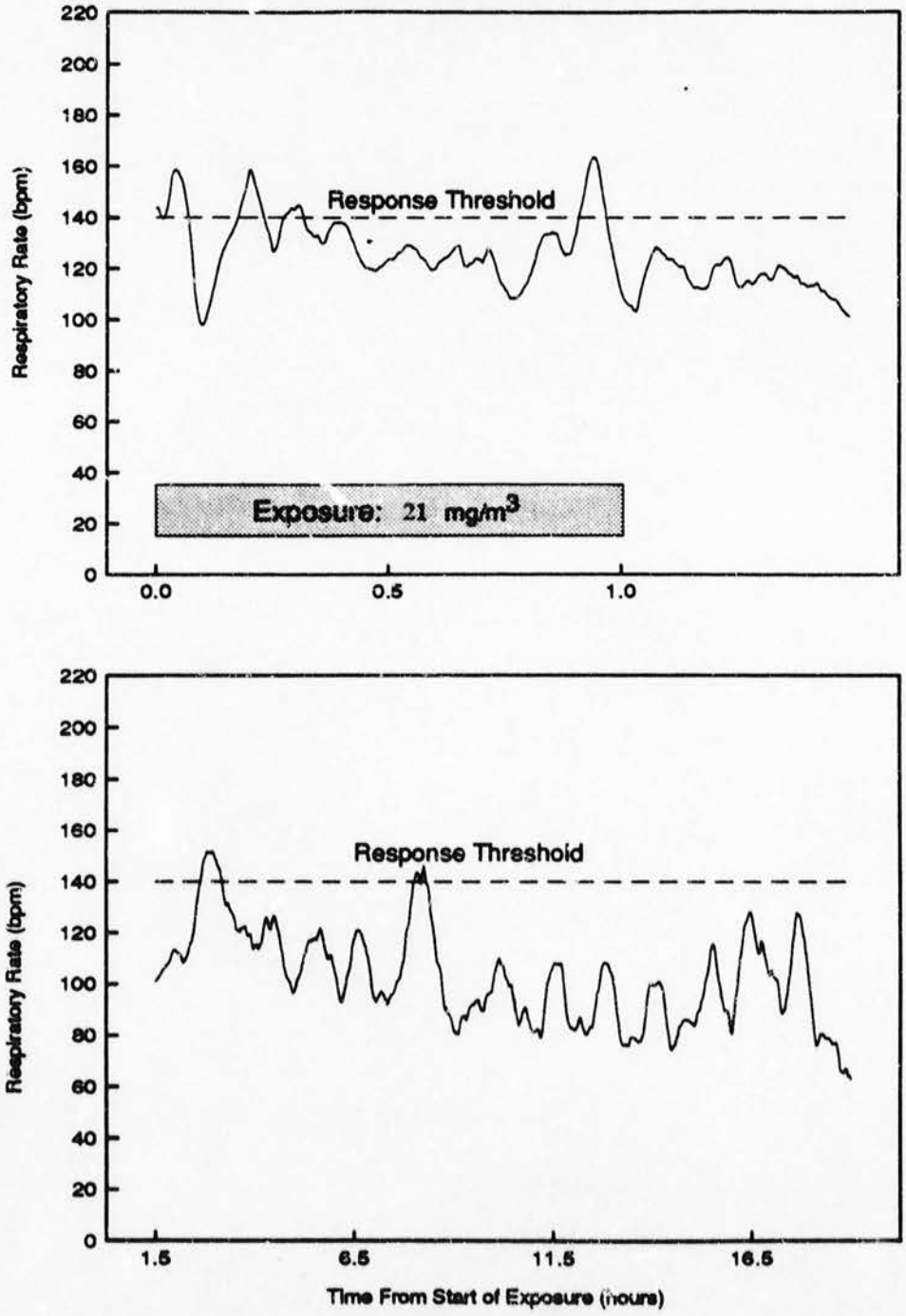
Figure: 135 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 100: 2,6-TDI Sensitization

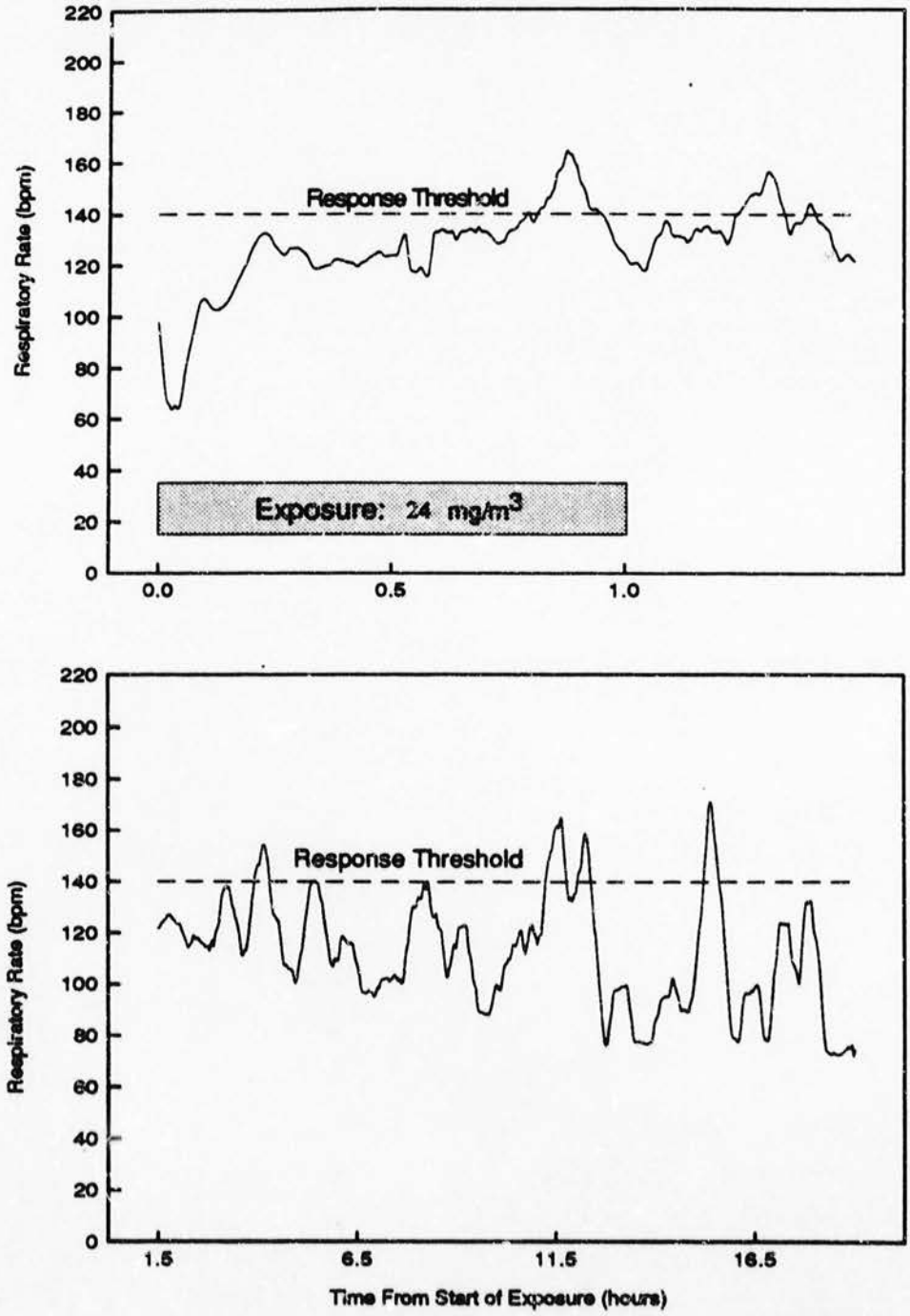
Figure: 136 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 101: 2,6-TDI Sensitization

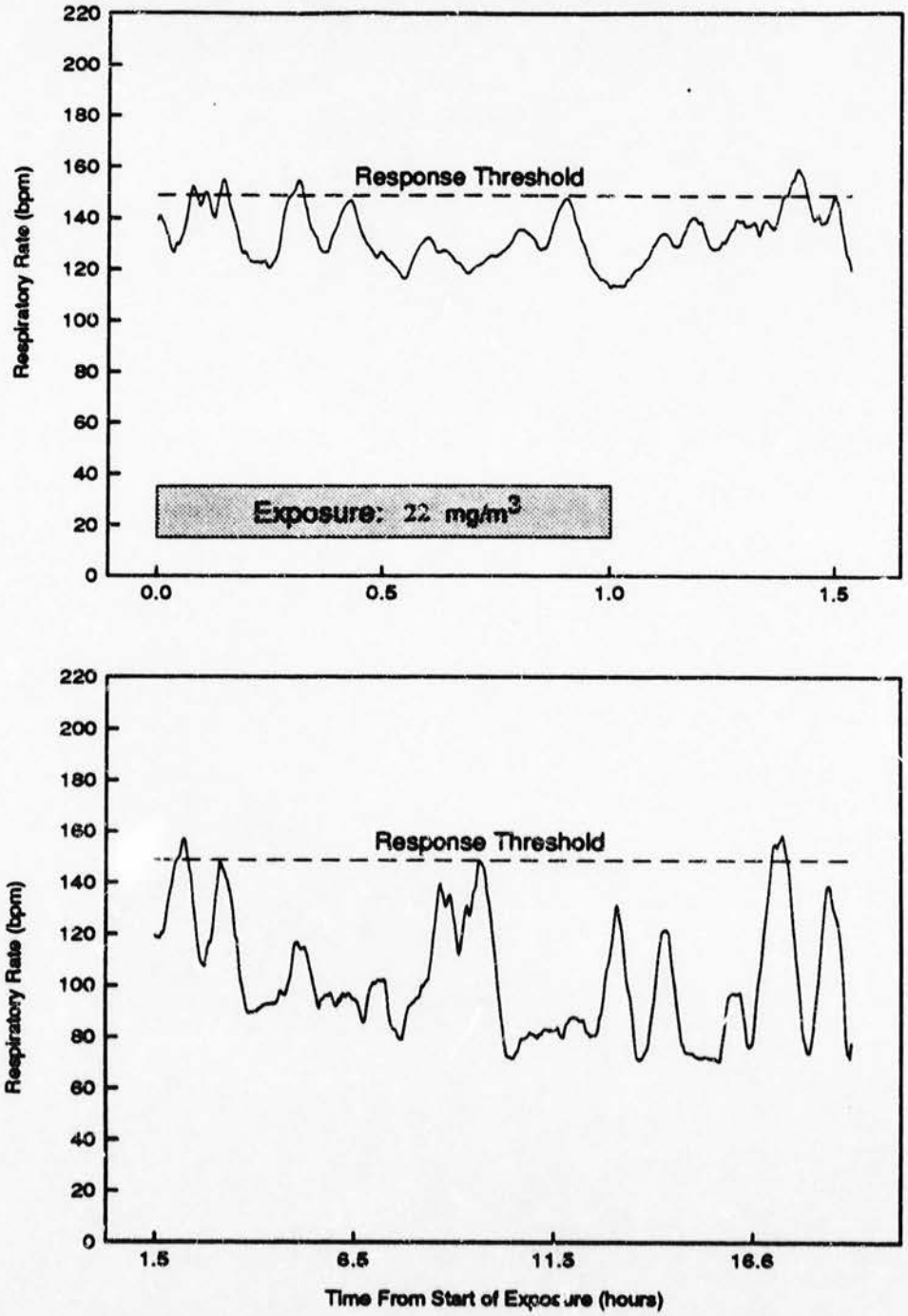
Figure: 137 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 102: 2,6-TDI Sensitization

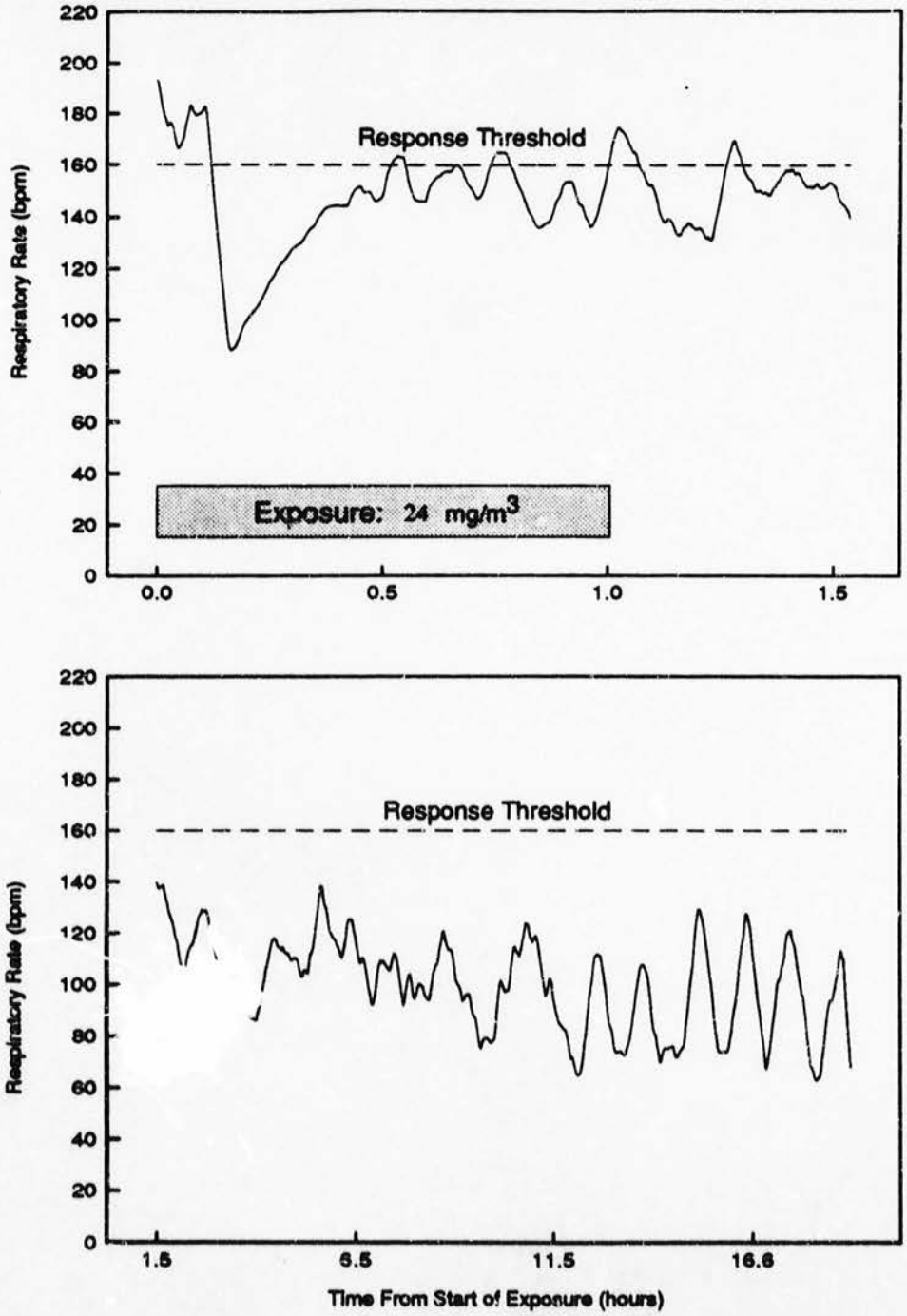
Figure: 138 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 103: 2,6-TDI Sensitization

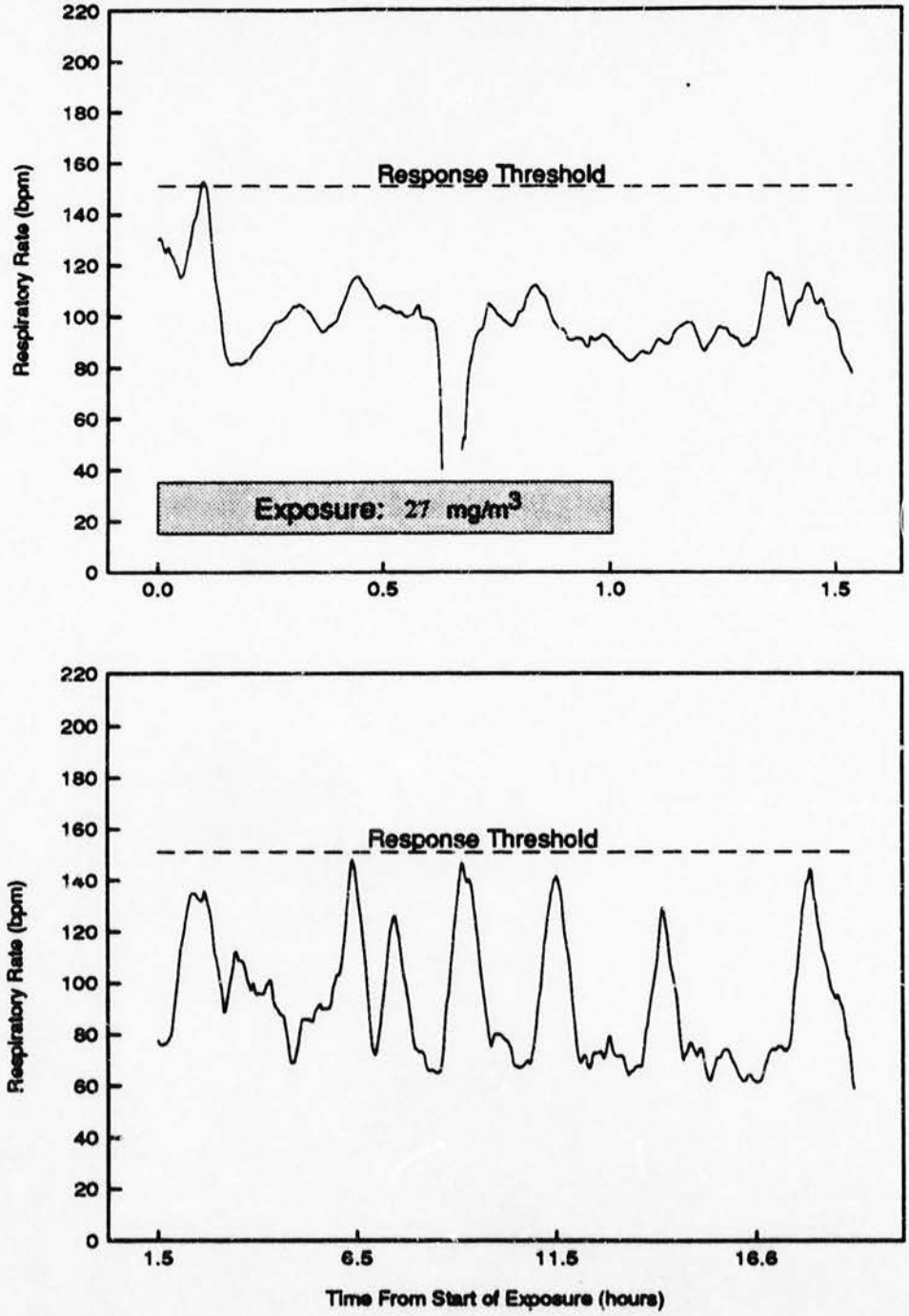
Figure: 139 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 104: 2,6-TDI Sensitization

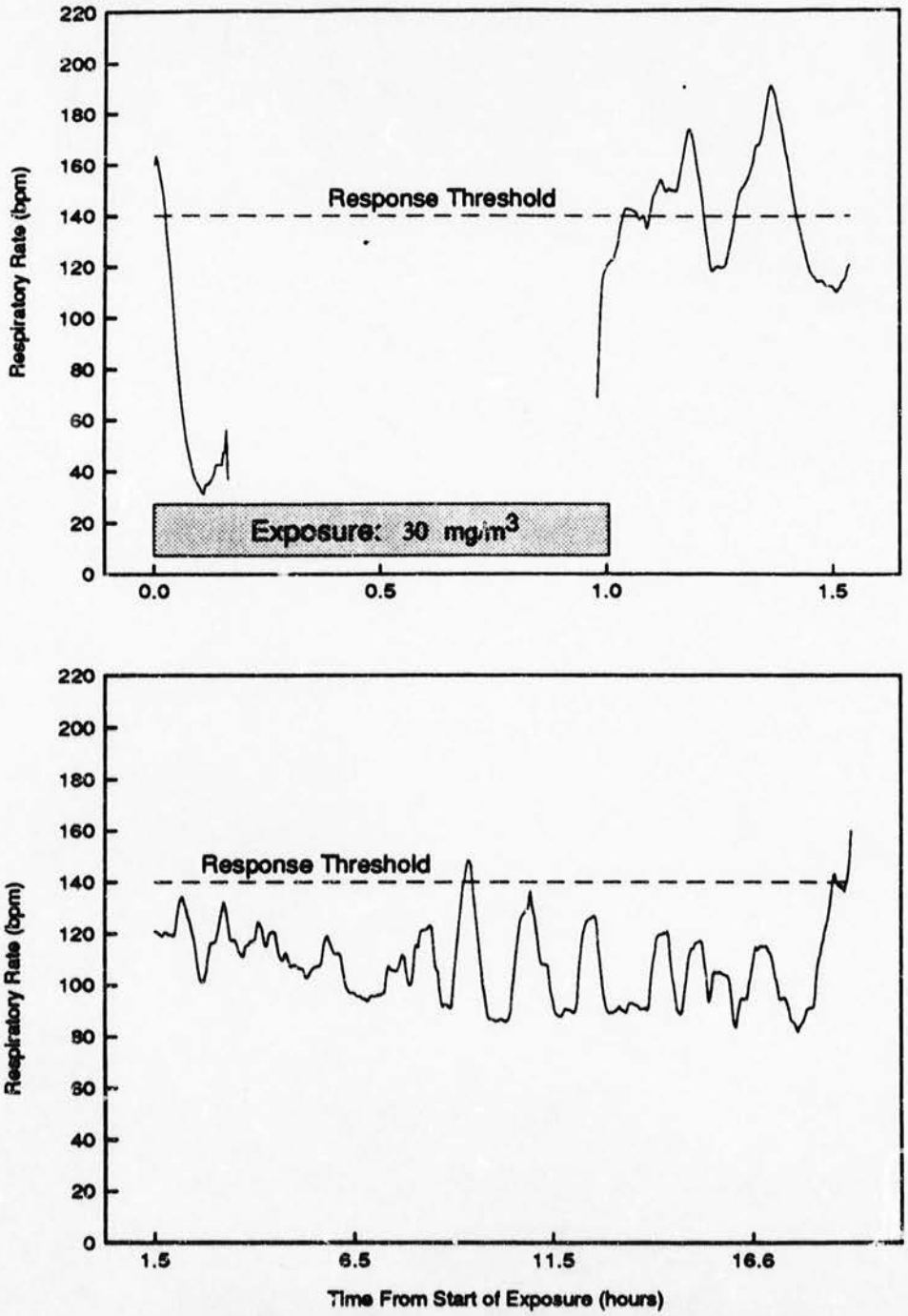
Figure: 140 2,6-TDI-GSA Challenge Week 3



Study Number 91-344-KR

Animal # 105*: 2,6-TDI Sensitization

Figure: 141 2,6-TDI-GSA Challenge Week 3



* Animal Removed from plethysmograph at .2 hours of exposure and returned to plethysmograph at end of exposure period.

Appendix I
Chamber Concentrations of TDI During Sensitization

Study Number 91-344-KR

Concentration (ppm)	Day of Study 0	1	2	3	4
Group 1 (2,4-TDI)					
	1.26	1.23	1.16	1.14	1.21
	1.43	1.32	1.20	1.13	1.22
	1.53	1.35	1.28	1.20	1.23
	1.60	1.29	1.26	1.27	1.25
	1.64	1.44	1.20	1.23	1.31
	1.63	1.44	1.28	1.22	1.38
Daily Mean	1.51	1.35	1.23	1.20	1.27
Daily SD	0.15	0.09	0.05	0.05	0.07
Group 2 (2,4-TDI)					
	1.75	1.00	1.02	1.11	1.36
	1.80	1.06	1.06	1.15	1.27
	1.98	1.02	1.06	1.14	1.36
	1.45	1.01	1.18	1.17	1.35
	1.89	.97	1.10	1.28	1.32
	1.93	1.05	1.08	1.37	1.34
Daily Mean	1.80	1.02	1.08	1.20	1.33
Daily SD	0.19	0.03	0.05	0.10	0.03
Group 3 (2,6-TDI)					
	1.83	1.53	1.15	1.18	.99
	1.83	1.54	1.20	1.15	1.02
	1.59	1.42	1.18	1.27	1.03
	1.85	1.48	1.22	1.14	1.07
	1.78	2.14	1.41	1.23	1.06
	1.88	2.28	1.25		
			1.30	1.22	1.06
			1.17	1.12	1.04
			1.33		1.04
Daily Mean	1.79	1.73	1.25	1.19	1.04
Daily SD	0.10	0.38	0.09	0.05	0.02
Group 4 (2,6-TDI)					
	1.52	1.34	1.04	1.20	1.33
	1.57	1.48	1.08	1.24	1.35
	1.61	1.44	1.18	1.28	1.37
	1.56	1.41	1.13	1.35	1.14
	1.60	1.40	1.18		1.39
	1.55	1.48	1.15	1.31	1.42
Daily Mean	1.57	1.43	1.13	1.28	1.33
Daily SD	0.03	0.05	0.06	0.06	0.10

SD= Standard Deviation

Appendix II

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

Study Number 91-344-KR

Animal Number	Post Exposure Day 0	4	7	14	Challenge Week* 1		2		3	
Group I (2,4-TDI Sensitization, 2,4-TDI Challenge)										
28	266	275	305	355	411	375	457	422	480	457
29	262	280	308	347	392	368	426	399	474	438
30	267	272	301	353	390	356	427	395	463	437
31	275	287	314	376	425	386	477	452	535	501
32	274	286	312	354	402	378	442	413	484	449
33	267	282	307	370	447	429	488	472	536	501
34	273	286	322	386	453	420	518	478	590	541
35	280	298	328	371	436	416	494	465	541	501
36	263	273	301	350	429	400	474	458	535	491
38	273	288	311	370	450	426	504	478	564	514
39	261	274	296	355	429	405	486	453	536	492
40		291	321	367	446	433	513	485	561	519
Group II (2,4-TDI Sensitization, 2,6-TDI Challenge)										
59	298	303	319	367	409	393	452	439	498	457
60	305	309	333	384	426	412	488	464	524	500
61	297	303	327	371	420	390	480	436	528	490
62	294	291	319	363	414	380	463	439	506	482
63	308	317	335	380	442	412	497	477	565	531
64	332	322	349	398	469	425	523	503	582	542
65	345	341	374	425	485	446	545	494	594	547
66	302	299	308	364	417	400	476	456	532	496
67	296	306	315	354	394	361	443	399	479	436
68	300	309	278	340	398	384	442	430	508	480
69	315	328	348	405	471	441	493	470	556	518
70	340	348	372	434	512	474	550	514	602	564

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

Appendix II (Continued)

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

Study Number 91-344-KR

Animal Number	Post Exposure Day 0	4	7	14	Challenge Week* 1		2		3	
Group III (2,6-TDI Sensitization, 2,4-TDI Challenge)										
44	350	357	380	445	495		563	530	627	592
45	344	350	380	427	494		556	513	620	571
46	332	333	360	413	464		514	482	580	542
47	358	356	404	479	563		628	564	692	602
48	348	350	389	447	547	501	602	563	658	627
49	344	335	363	417	496	451	569	510	598	550
51	358	354	387	461	556	498	620	576	668	618
52	352	345	372	437	499	467	536	528	585	555
53	343	332	372	411	490	463	550	511	589	554
54	359	351	384	422	502	476	538	508	581	541
55	361	368	400	451	552	527	614	576	627	591
Group IV (2,6-TDI Sensitization, 2,6-TDI Challenge)										
93	283	301	327	369	420	393	468	430	531	476
94	278	291	345	404	479	425	540	493	603	547
95	289	303	335	356	412	386	450	423	475	469
97	289	308	351	397	450	428	508	470	560	532
98	262	269	305	348	404	378	465	442	522	481
99	287	294	336	381	430	401	495	450	541	507
100	280	287	309	364	405	390	470	437	525	506
101	285	297	332	394	461	426	522	478	574	545
102	273	279	321	373	453	426	522	492	559	534
103	271	282	332	370	444	417	521	470	578	528
104	275	275	311	367	443	403	479	443	516	476
105		272	303	335	393	362	434	417	482	461

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

Appendix III

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

Group 1 - 2,4-TDI Sensitization/2,4-TDI Challenge

Study Number 91-344-KR

Immediate or Delayed Responses

Animal No.	28	29	30	31	32	33	34	35	36	38	39	40
Challenge Wk.												
Week 1	I/D	-/-	-/D	0/0	-/-	-/D	-/D	-/-	-/-	-/0	-/D	-/-
Week 2	-/-	-/-	-/D	-/D	-/0	-/-	-/-	-/D	-/-	-/-	I/-	-/-
Week 3	-/-	-/-	I/D	-/-	-/-	-/-	I/D	-/-	-/-	I/D	I/D	-/-

Response Threshold Values

Week 1	140	150	160	-	140	140	160	149	143	152	141	160
Week 2	140	155	140	140	158	140	140	144	140	140	150	145
Week 3	160	143	140	153	147	140	140	140	143	140	140	140

Exposure Concentrations

Week 1 (ppb)	10	8	19	10	18	15	47	25	33	23	26	18
Week 2 (ppb)	17	10	13	10	24	42	12	13	13	11	33	18
Week 3 (mg/m^3)	16	12	12	14	20	25	19	20	23	20	17	16

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

Appendix III (Continued)

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

Group 2 - 2,4-TDI Sensitization/2,6-TDI Challenge

Study Number 91-344-KR

Immediate or Delayed Responses

Animal No.	59	60	61	62	63	64	65	66	67	68	69	70
Challenge Wk.												
Week 1	-/D	-/D	-/-	-/-	I/-	-/D	-/-	I/-	-/-	-/-	-/-	-/D
Week 2	-/-	-/D	-/D	I/-	-/-	-/-	0/-	I/D	-/-	-/-	I/-	-/-
Week 3	0/D	-/-	-/D	-/-	I/D	I/-	I/-	-/D	I/-	-/-	-/-	-/-

Response Threshold Values

Week 1	140	140	144	160	140	148	140	160	140	155	140	140
Week 2	154	140	140	140	140	152	140	140	140	140	140	140
Week 3	140	160	141	160	140	140	152	140	140	140	140	140

Exposure Concentrations

Week 1 (ppb)	52	39	33	32	53	33	24	22	31	28	19	18
Week 2 (ppb)	15	11	28	20	32	17	18	16	16	14	18	21
Week 3 (mg/m^3)	11	11	11	10	30	25	20	26	22	34	18	25

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

Appendix III (Continued)

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

Group 3 - 2,6-TDI Sensitization/2,4-TDI Challenge

Study Number 91-344-KR

Immediate or Delayed Responses

Animal No.	44	45	46	47	48	49	51	52	53	54	55
Challenge Wk.											
Week 1	-/-	-/0	-/-	-/0	-/-	-/-	-/-	-/-	I/-	-/-	-/-
Week 2	I/-	I/-	-/-	-/D	I/D	I/D	-/-	-/-	0/-	-/D	I/D
Week 3	-/-	0/D	-/-	I/D	-/D	I/D	I/-	-/-	I/D	-/D	I/D

Response Threshold Values

Week 1	156	160	160	140	154	140	154	140	149	140	152
Week 2	140	160	140	153	140	140	150	144	140	160	140
Week 3	140	156	152	140	140	140	140	140	140	140	145

Exposure Concentrations

Week 1 (ppb)	37	19	14	9	30	26	19	16	70	36	28
Week 2 (ppb)	22	15	62	30	15	9	24	20	23	16	44
Week 3 (mg/m^3)	14	23	12	13	30	21	19	22	23	22	18

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

Appendix III (Continued)

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

Group 4 - 2,6-TDI Sensitization/2,6-TDI Challenge

Study Number 91-344-KR

Immediate or Delayed Responses

Animal No.	93	94	95	97	98	99	100	101	102	103	104	105
Challenge Wk.												
Week 1	-/D	-/-	-/-	-/-	-/D	-/D	-/D	-/D	-/D	I/-	-/-	-/D
Week 2	-/-	-/-	-/-	-/-	-/-	I/D	I/D	I/D	-/0	-/0	-/-	-/-
Week 3	-/D	I/-	-/-	0/-	I/D	-/D	I/D	-/D	-/-	I/-	-/-	I/D

Response Threshold Values

Week 1	140	140	153	150	140	140	140	140	140	140	140	140
Week 2	140	140	157	146	140	140	140	140	140	158	140	140
Week 3	160	160	146	140	140	140	140	140	149	160	151	140

Exposure Concentrations

Week 1 (ppb)	49	38	23	45	33	24	38	55	52	42	74	83
Week 2 (ppb)	28	29	40	40	52	39	20	20	21	13	46	32
Week 3 (mg/m³)	28	33	27	28	11	23	21	24	22	24	27	30

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

FILE
7-37

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

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.

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: 3-8-94

SPONSOR

INTERNATIONAL ISOCYANATE INSTITUTE, INC.

G. K. Sangha: _____
Sponsor Representative

Date: 3/8/94

STUDY DIRECTOR

D. L. Warren: _____

Date: 3/8/94

Note: Replacement page, original sponsor was incorrect.

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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	K.D. Moore for K.D. Moore
Quality Assurance:	C. A. Halder	C.A. Halder 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.

AUDITS			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:

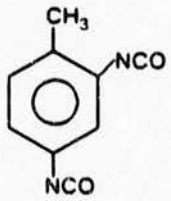
Test Substance:

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:

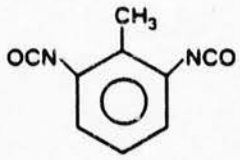
Test Substance:

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

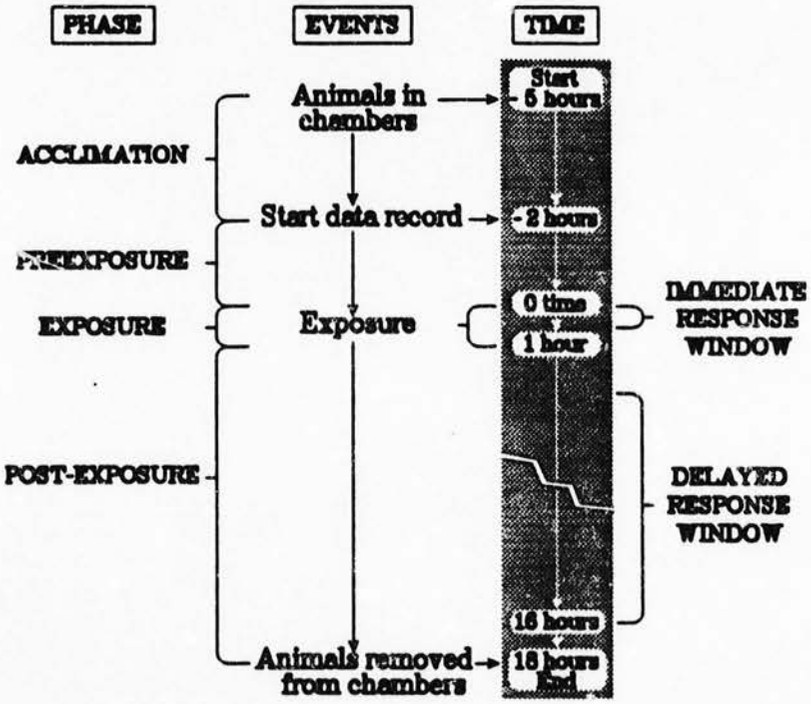
Each animal was conventionally housed for approximately two weeks (vide supra).

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 8 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/m^3 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 will be 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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14. BUXCO (Sharon, Conn). Equipment used: flow derived parameter analyzer and data logger Model #12/24, interfaced with an IBM PS/2 - 50 personal computer.

15. Ashton - Tate Corporation, Torrance, California.

16. Lotus Development Corp., Cambridge, Massachusetts.

Table 1

Summary of Plethysmograph Concentrations During Challenge

Study Number 92-344-PF

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.

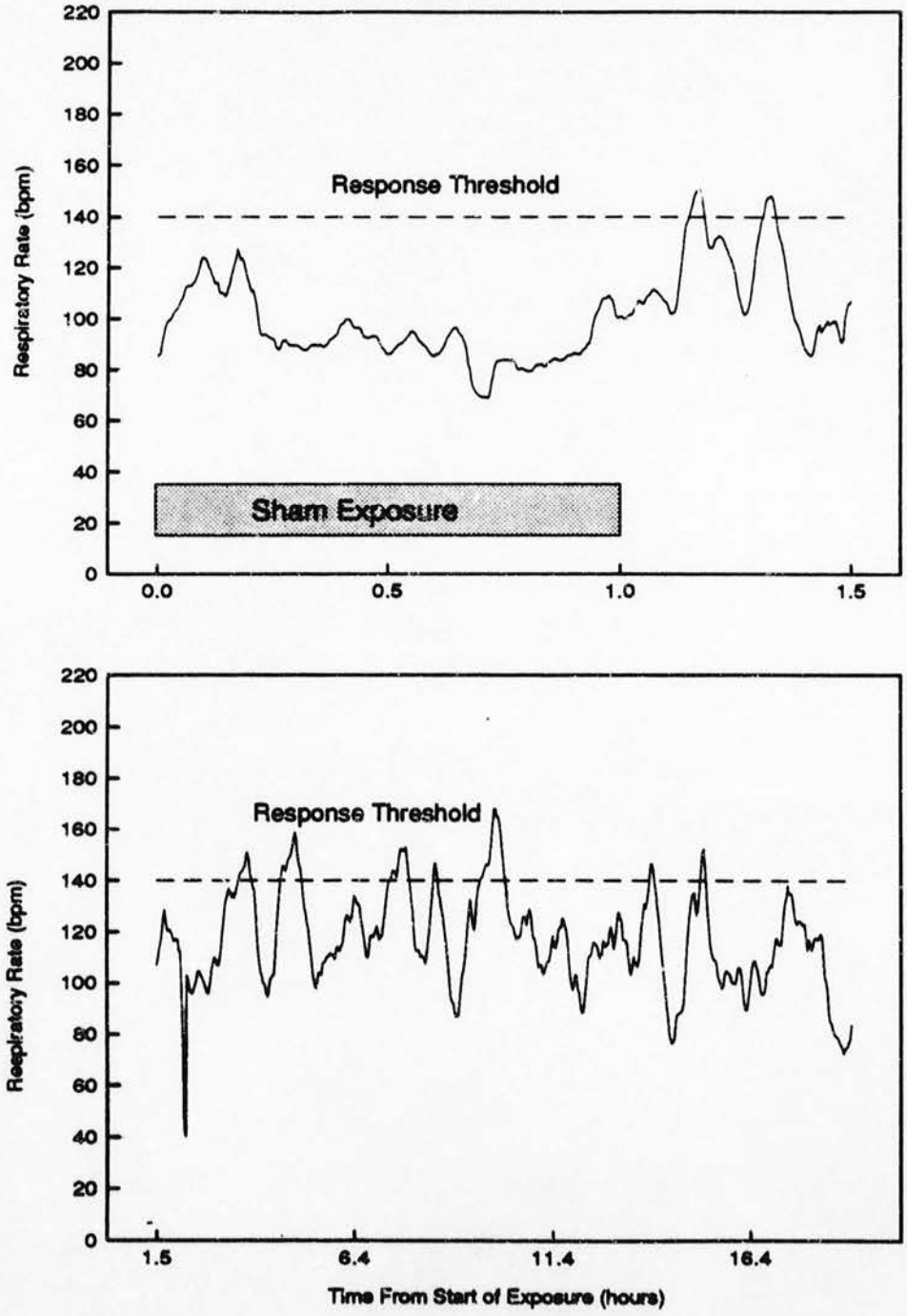
Each Figure is separately numbered from 1 to 108. They are presented in the order of groups 1 - 3, and, for each group data for the first week of challenges are presented in sequence and these are followed by data from the second and then finally the third week of challenge. The following table identifies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

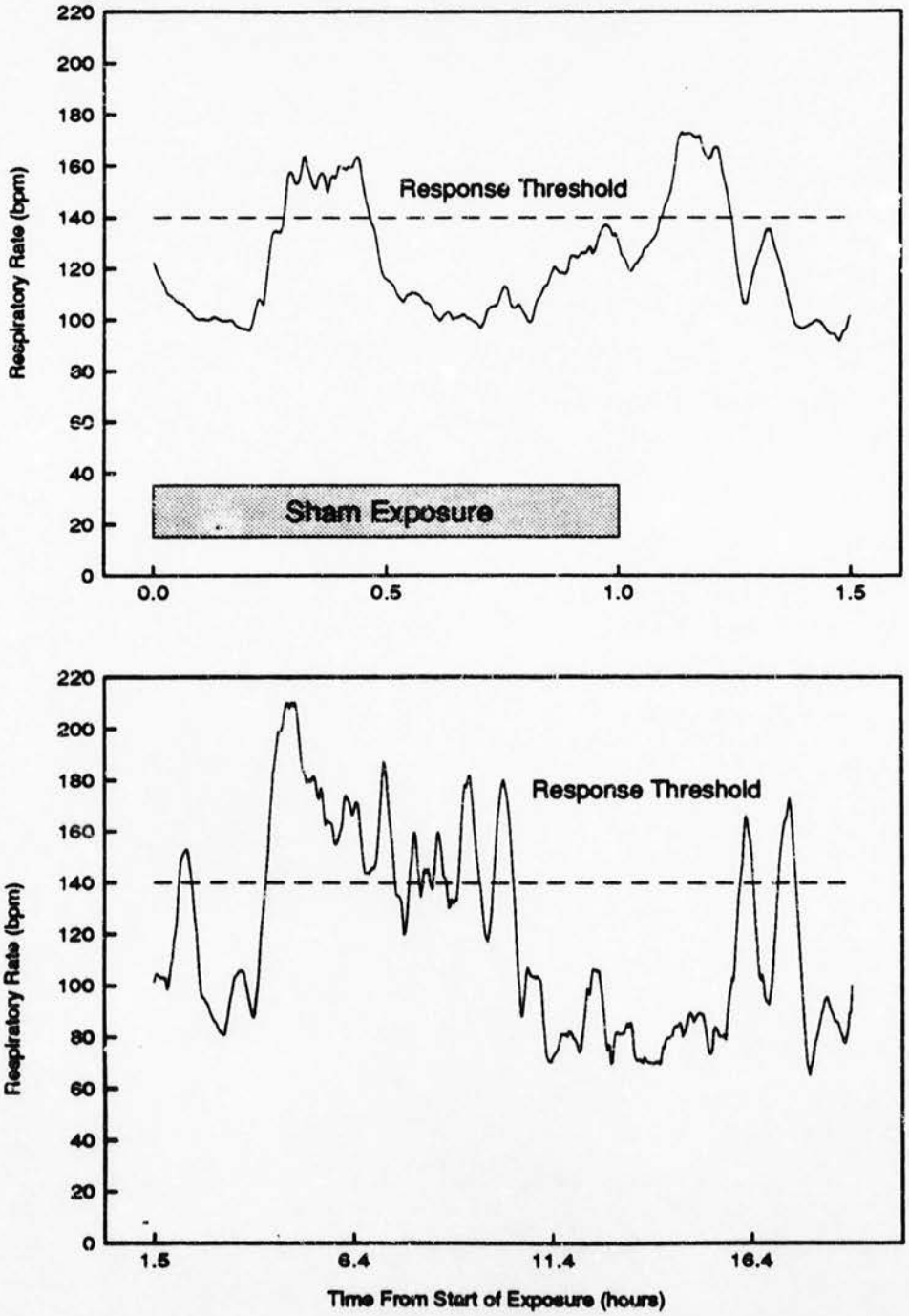
Figure: 1 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 194: Sham Sensitization

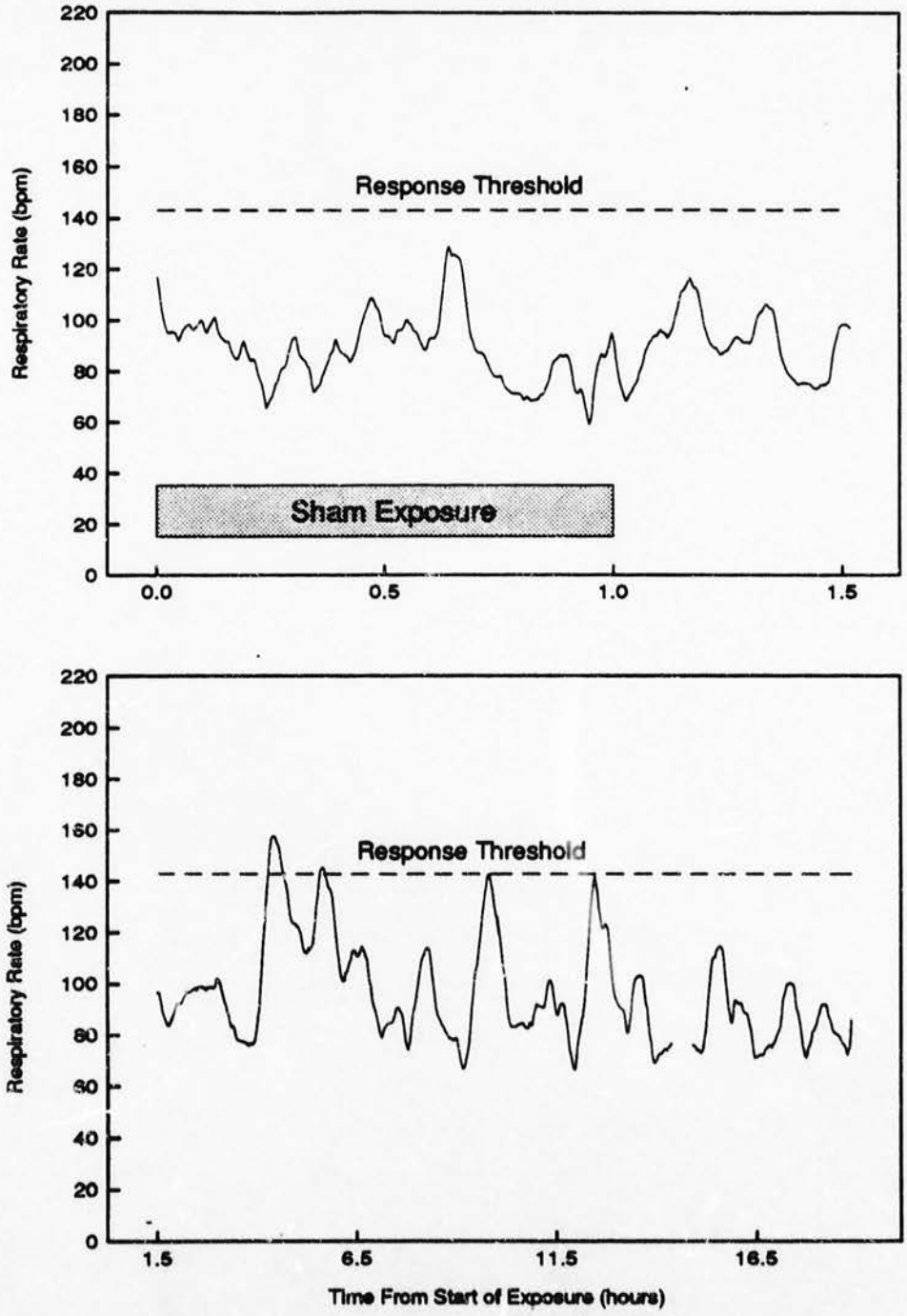
Figure: 2 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 195: Sham Sensitization

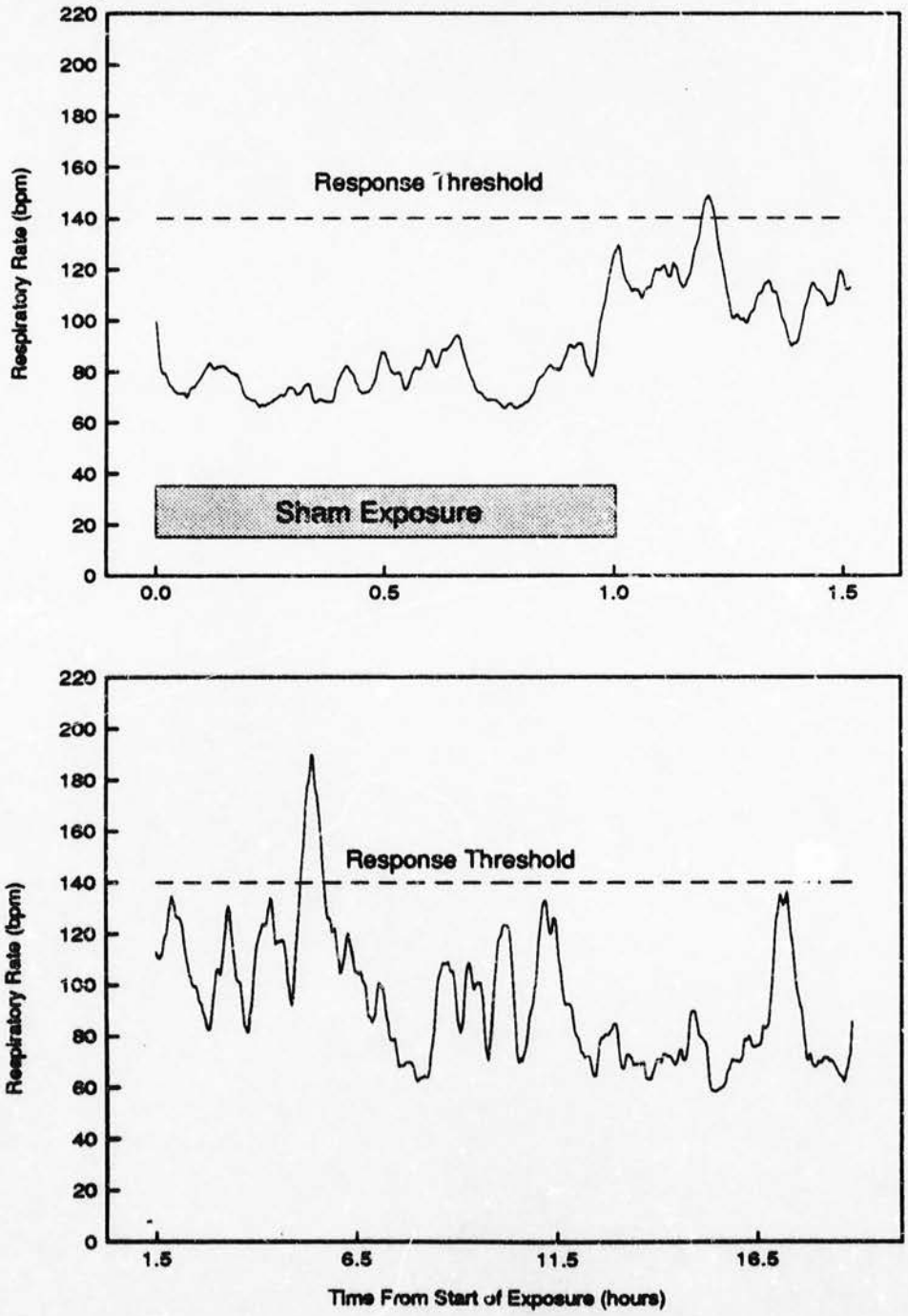
Figure: 3 Sham Challenge Week 1



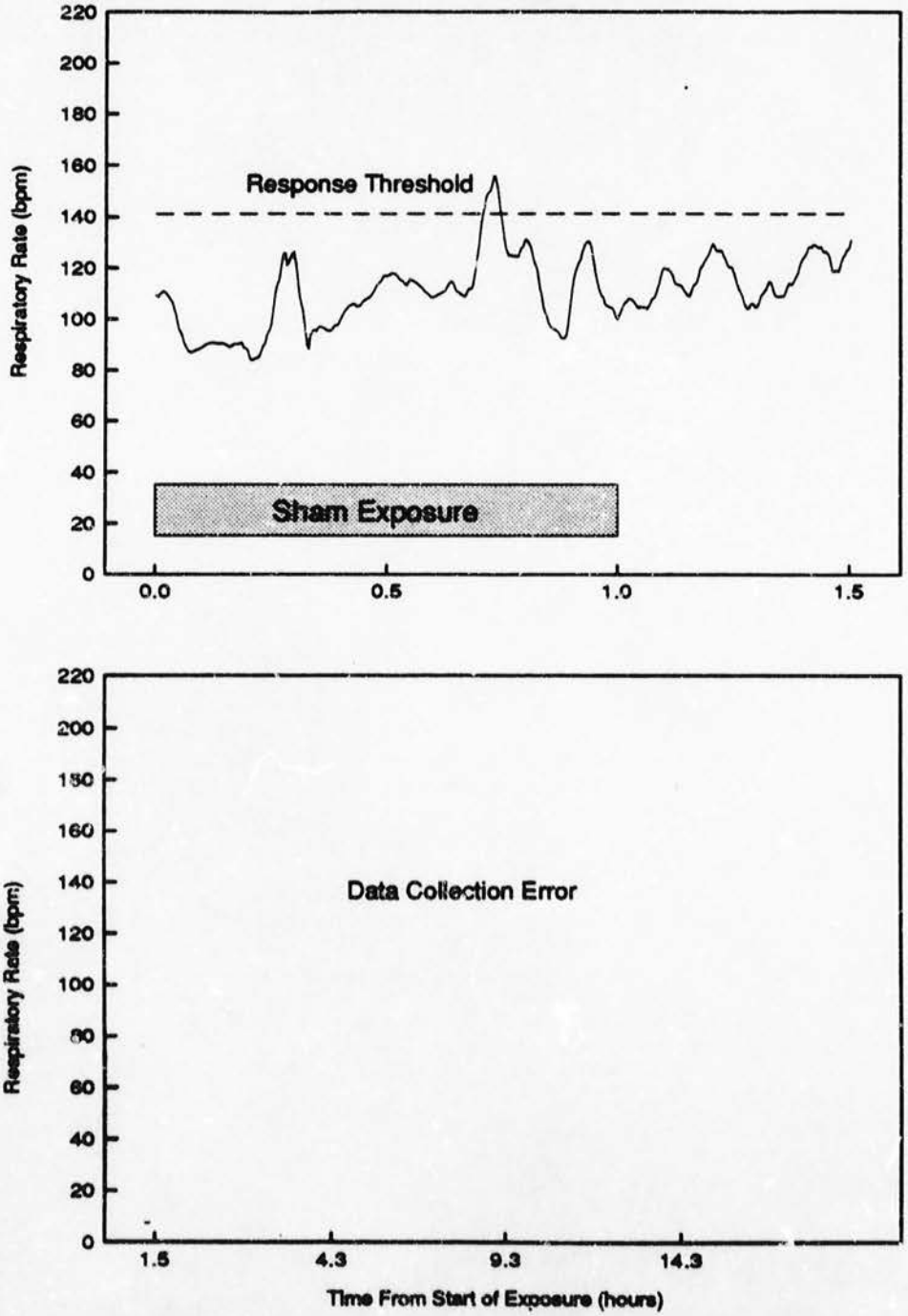
Study Number 92-344-P?

Animal # 196: Sham Sensitization

Figure: 4 Sham Challenge Week 1



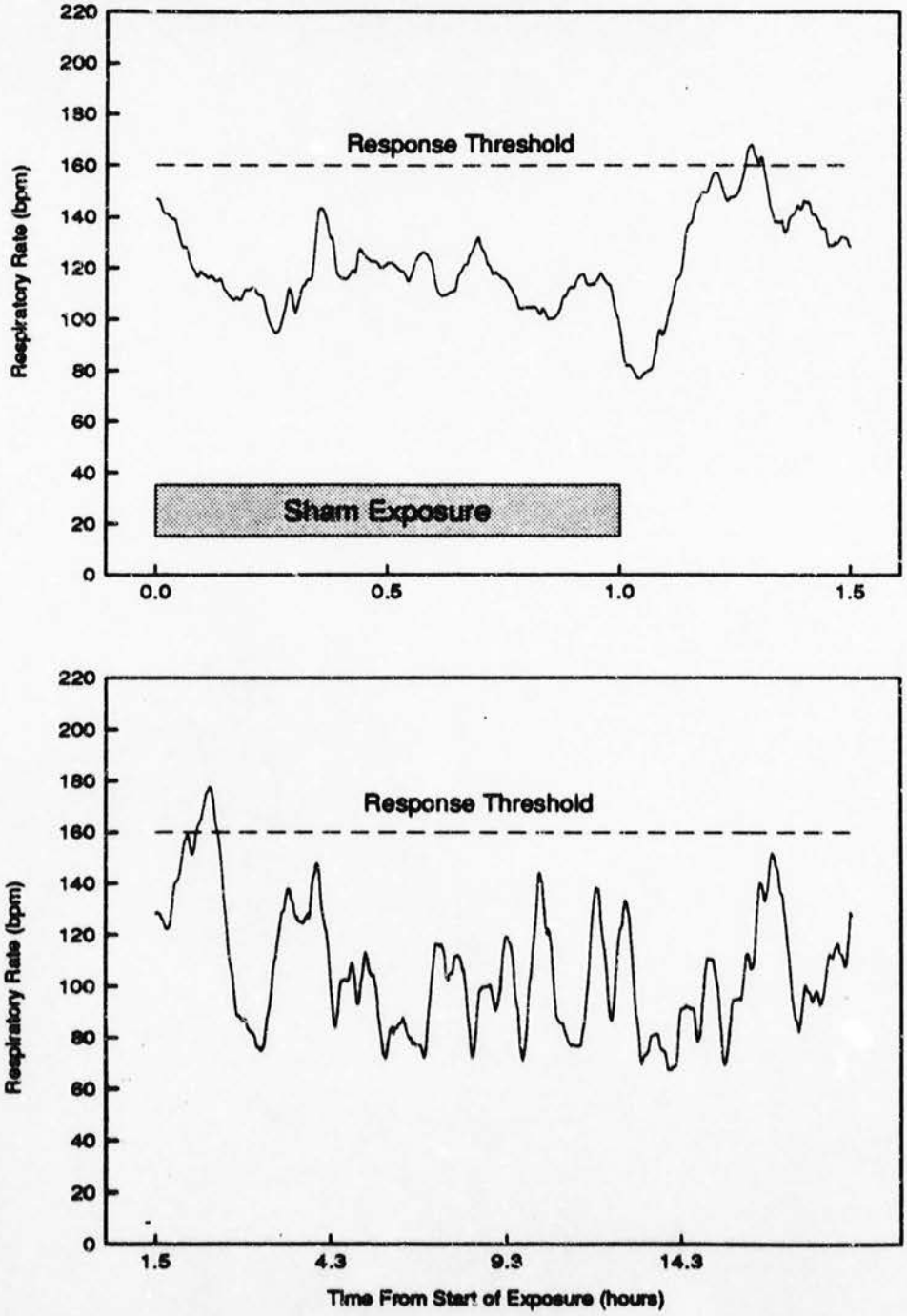
Study Number 92-344-PP
Animal # 197: Sham Sensitization
Figure: 5 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 198: Sham Sensitization

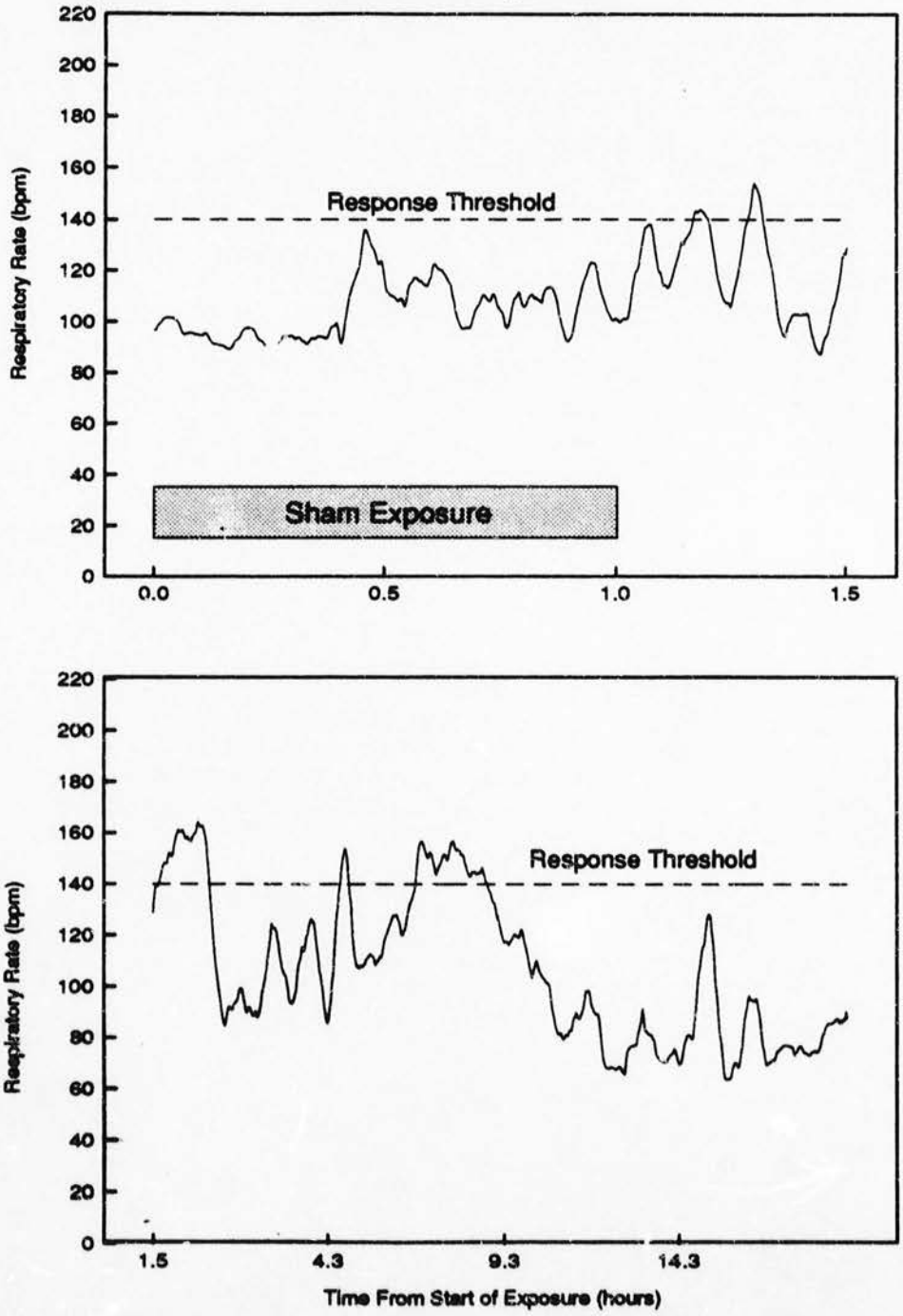
Figure: 6 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 199: Sham Sensitization

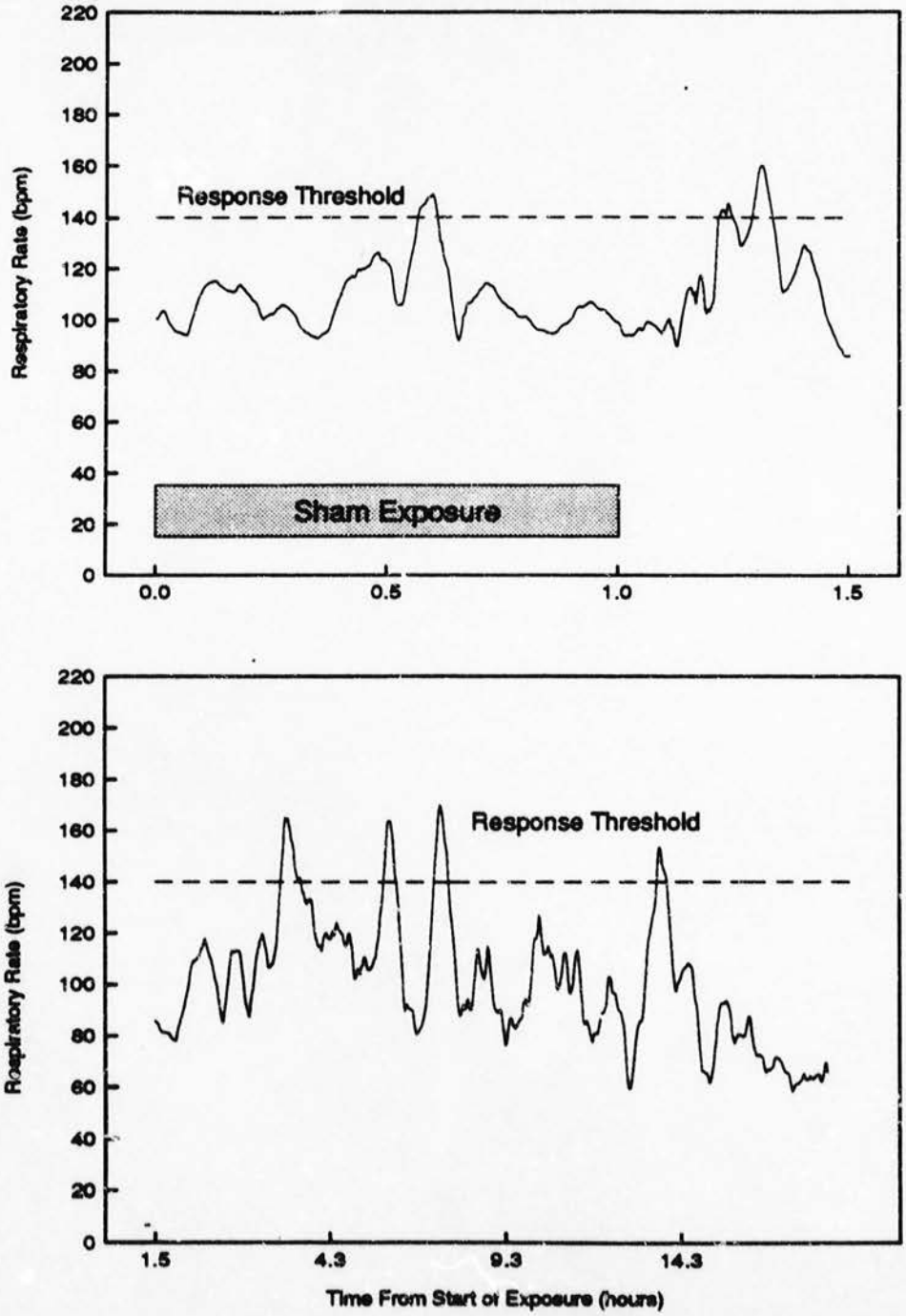
Figure: 7 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 200: Sham Sensitization

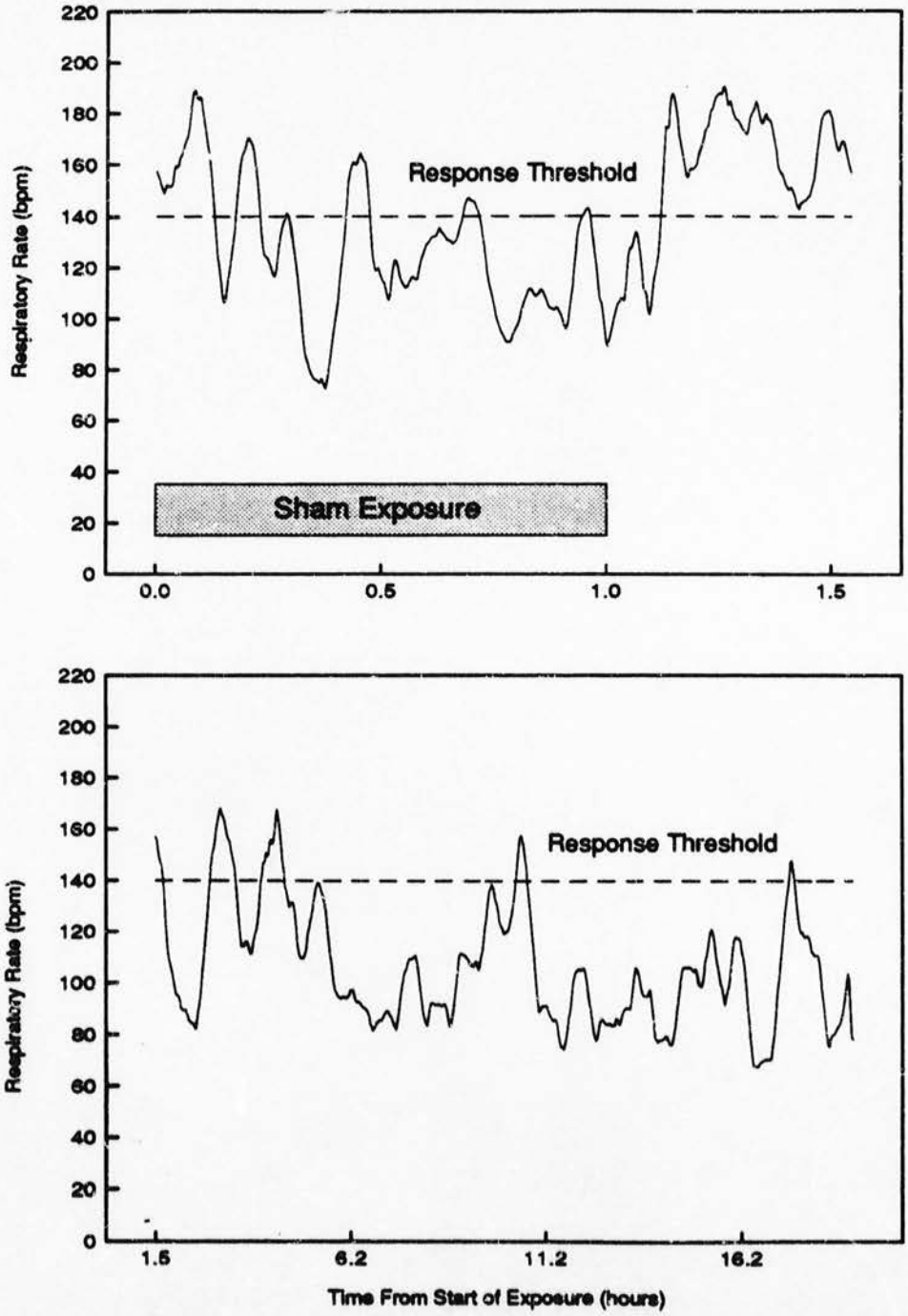
Figure: 8 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 201: Sham Sensitization

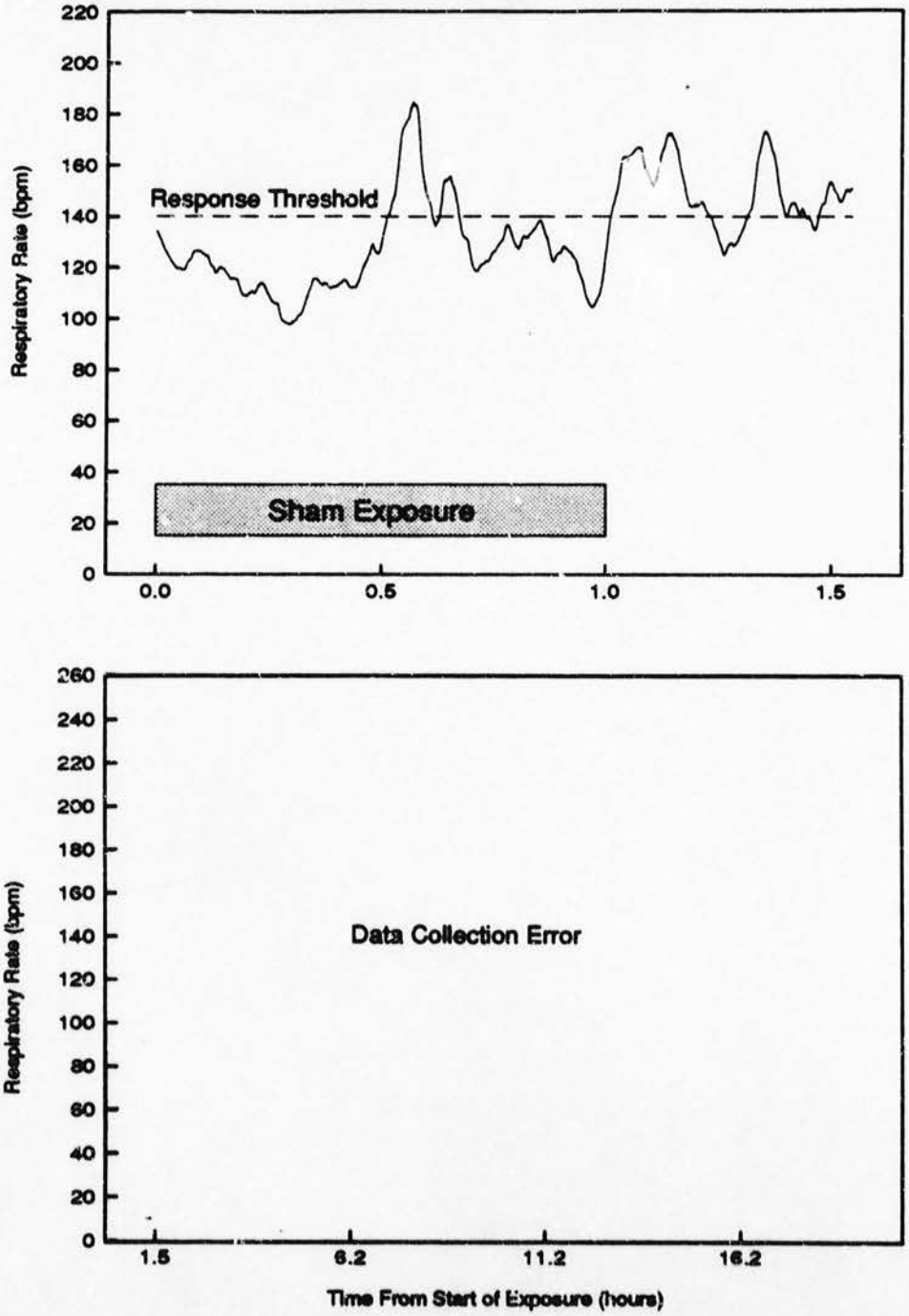
Figure: 9 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 202: Sham Sensitization

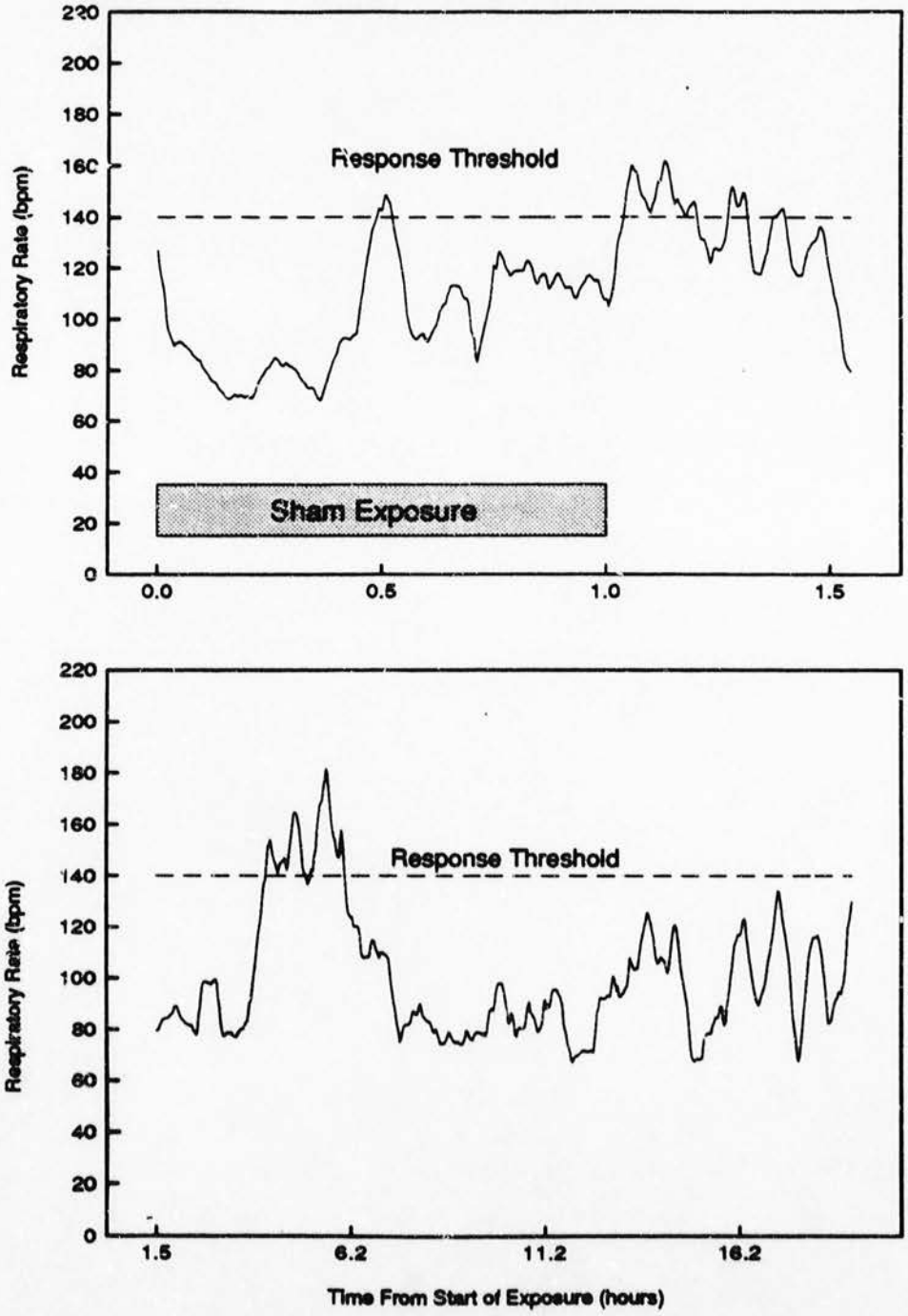
Figure: 10 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 203: Sham Sensitization

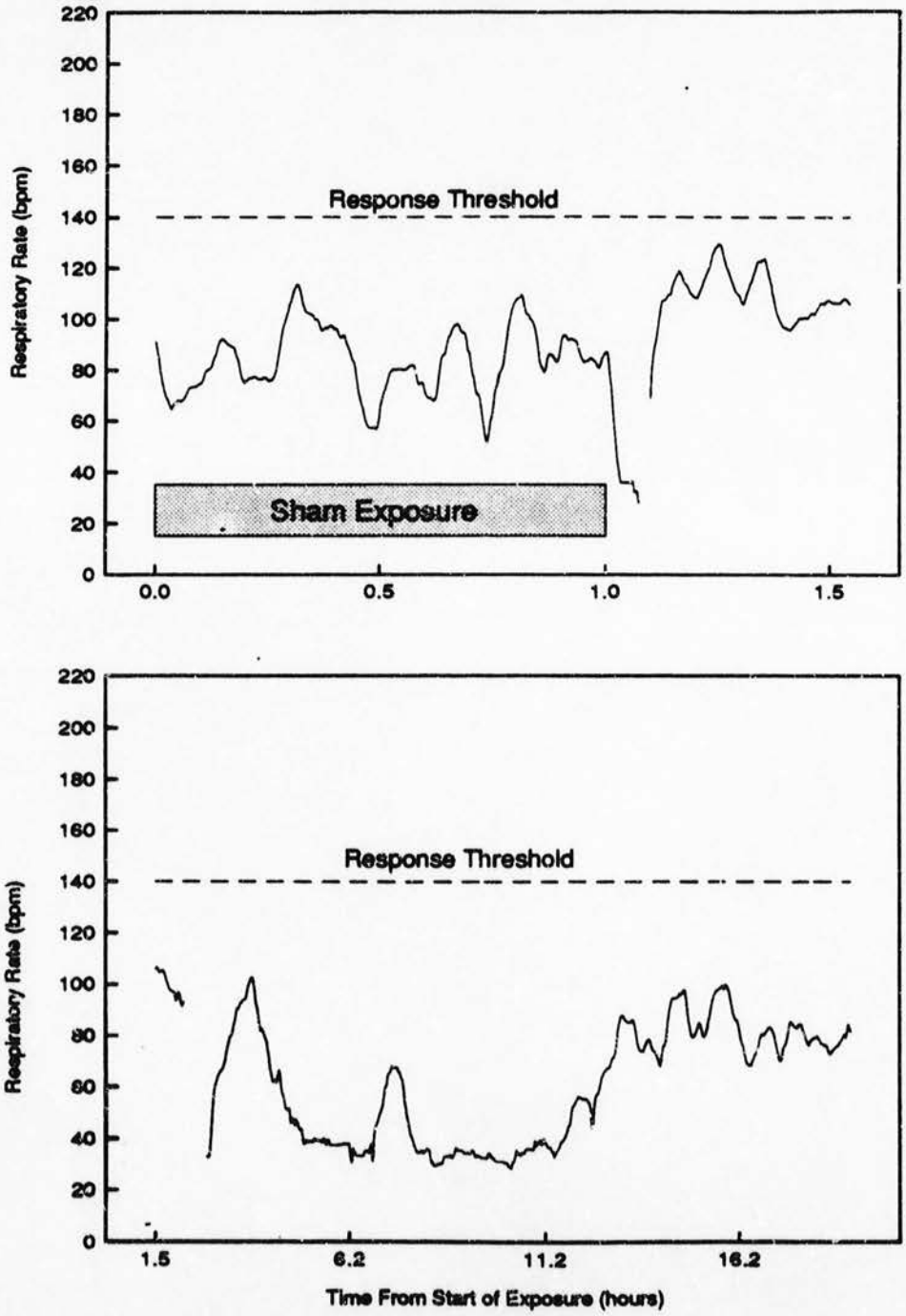
Figure: 11 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 204: Sham Sensitization

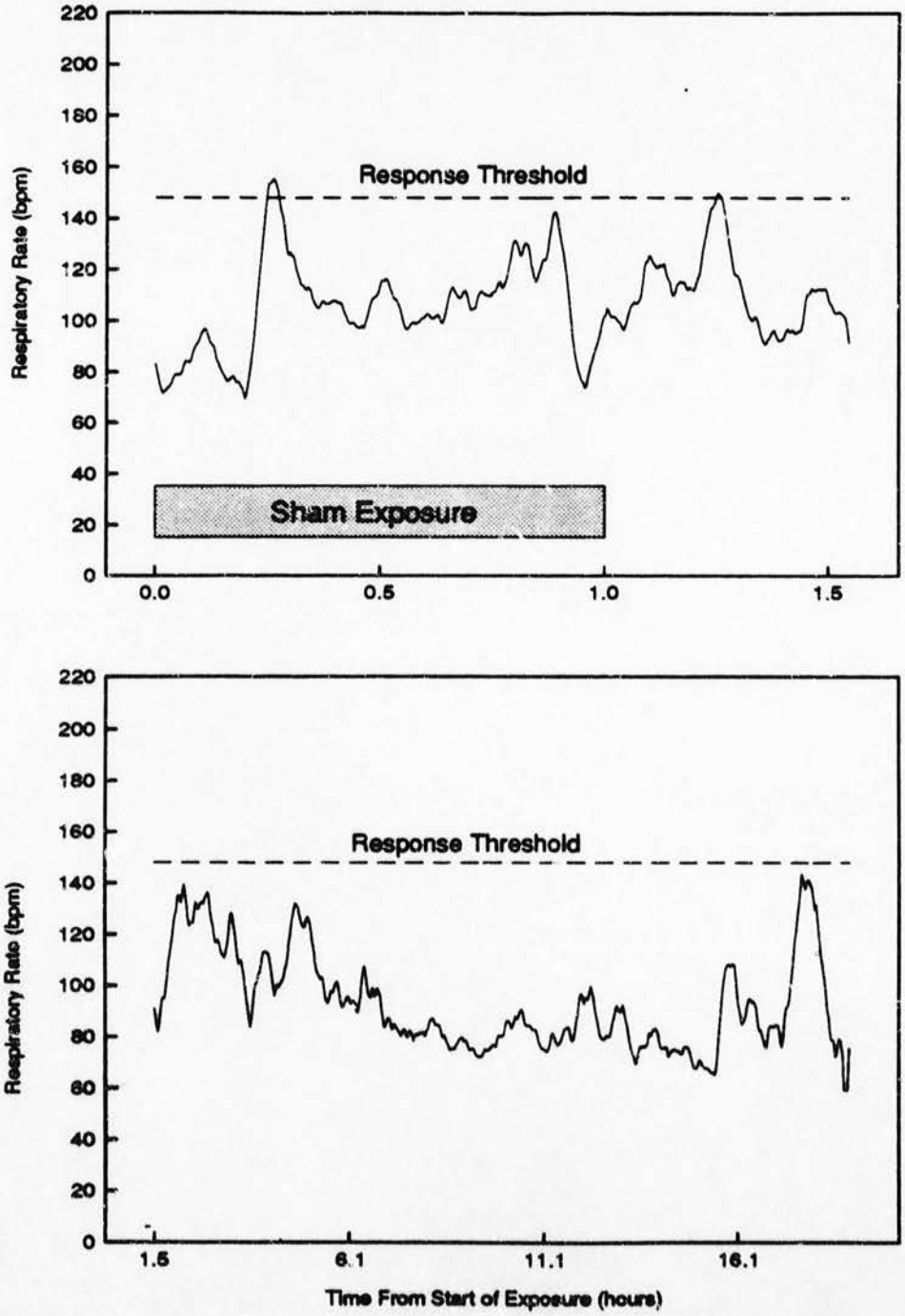
Figure: 12 Sham Challenge Week 1



Study Number 92-344-PP

Animal # 193: Sham Sensitization

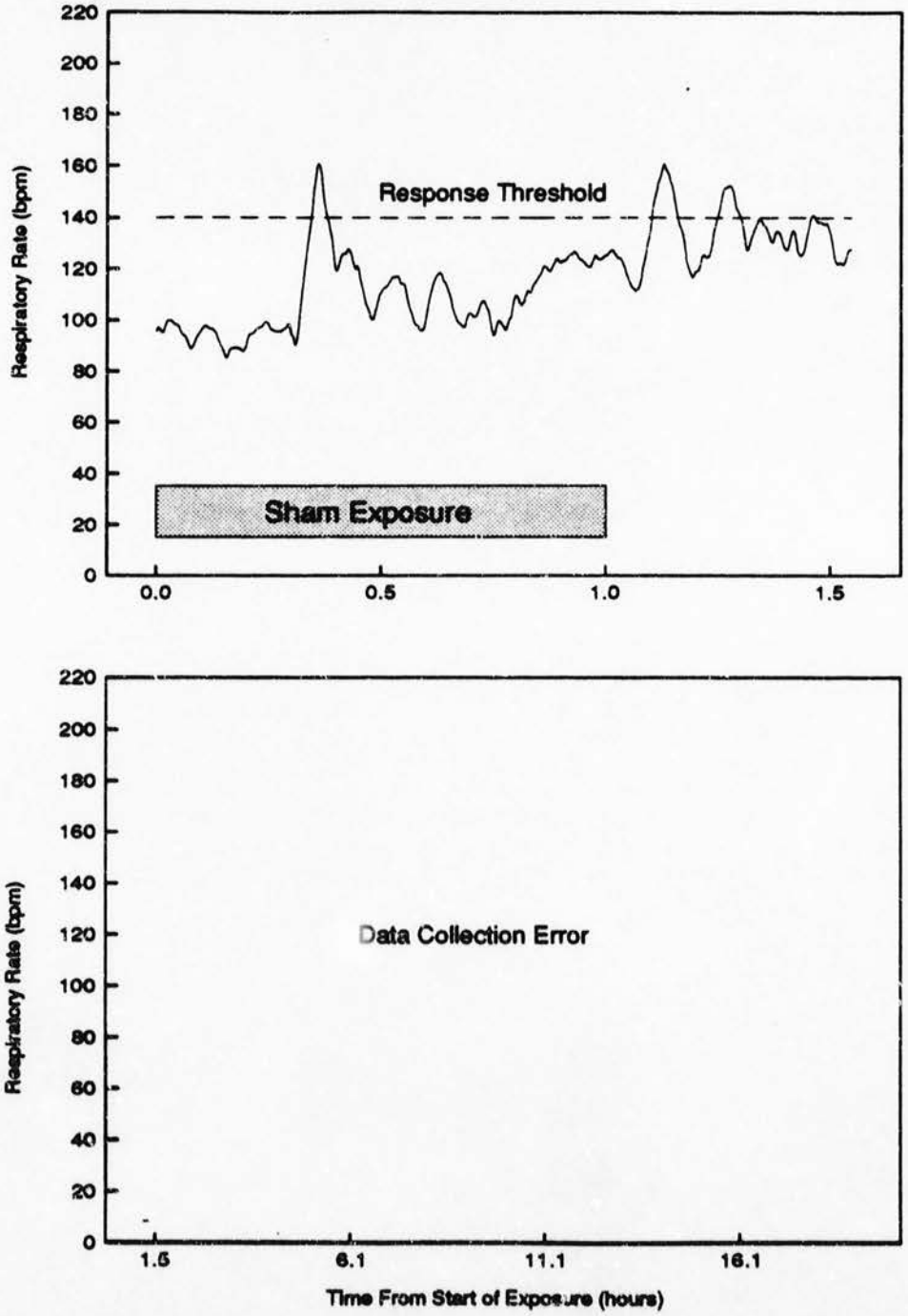
Figure: 13 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 194: Sham Sensitization

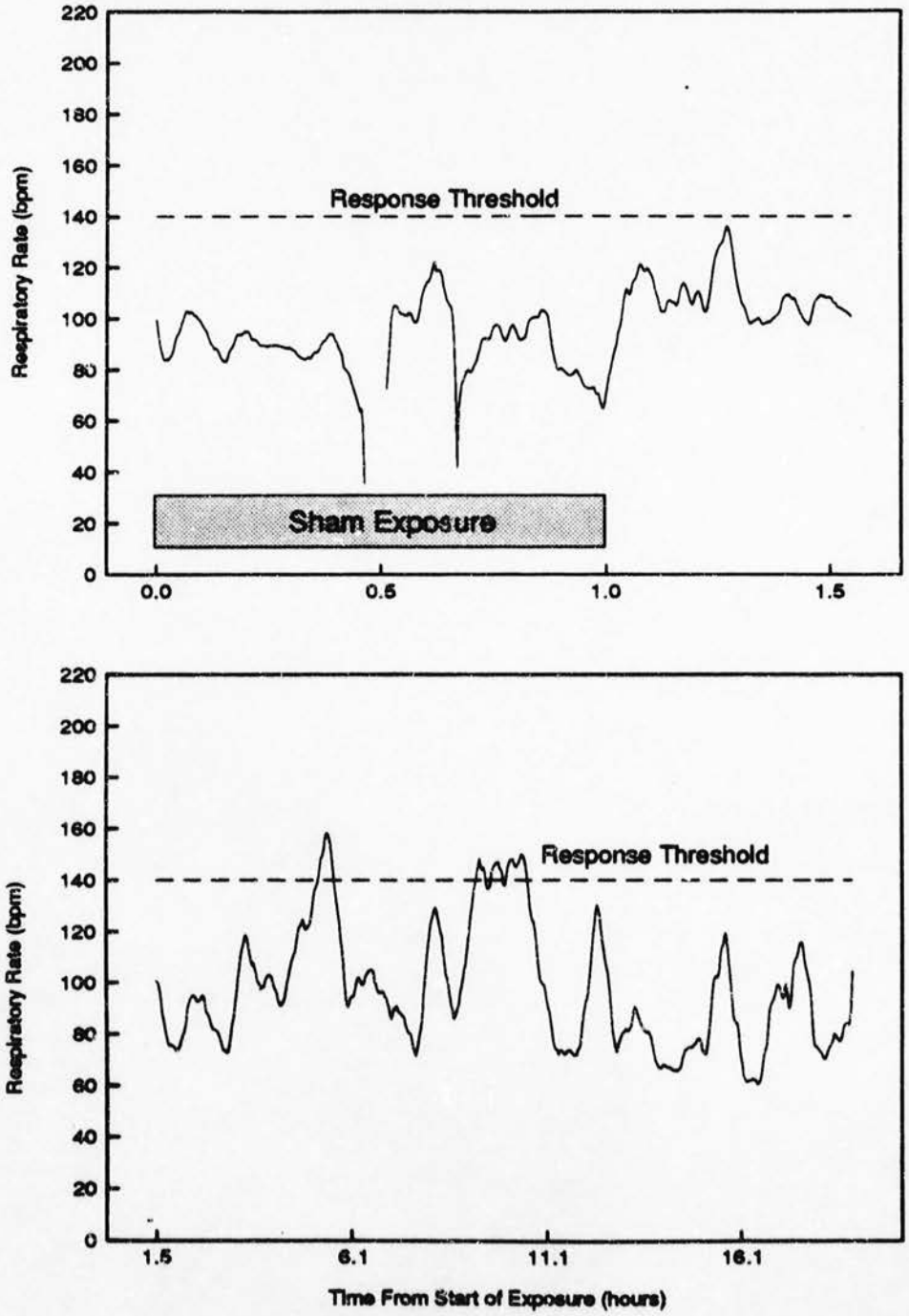
Figure: 14 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 195: Sham Sensitization

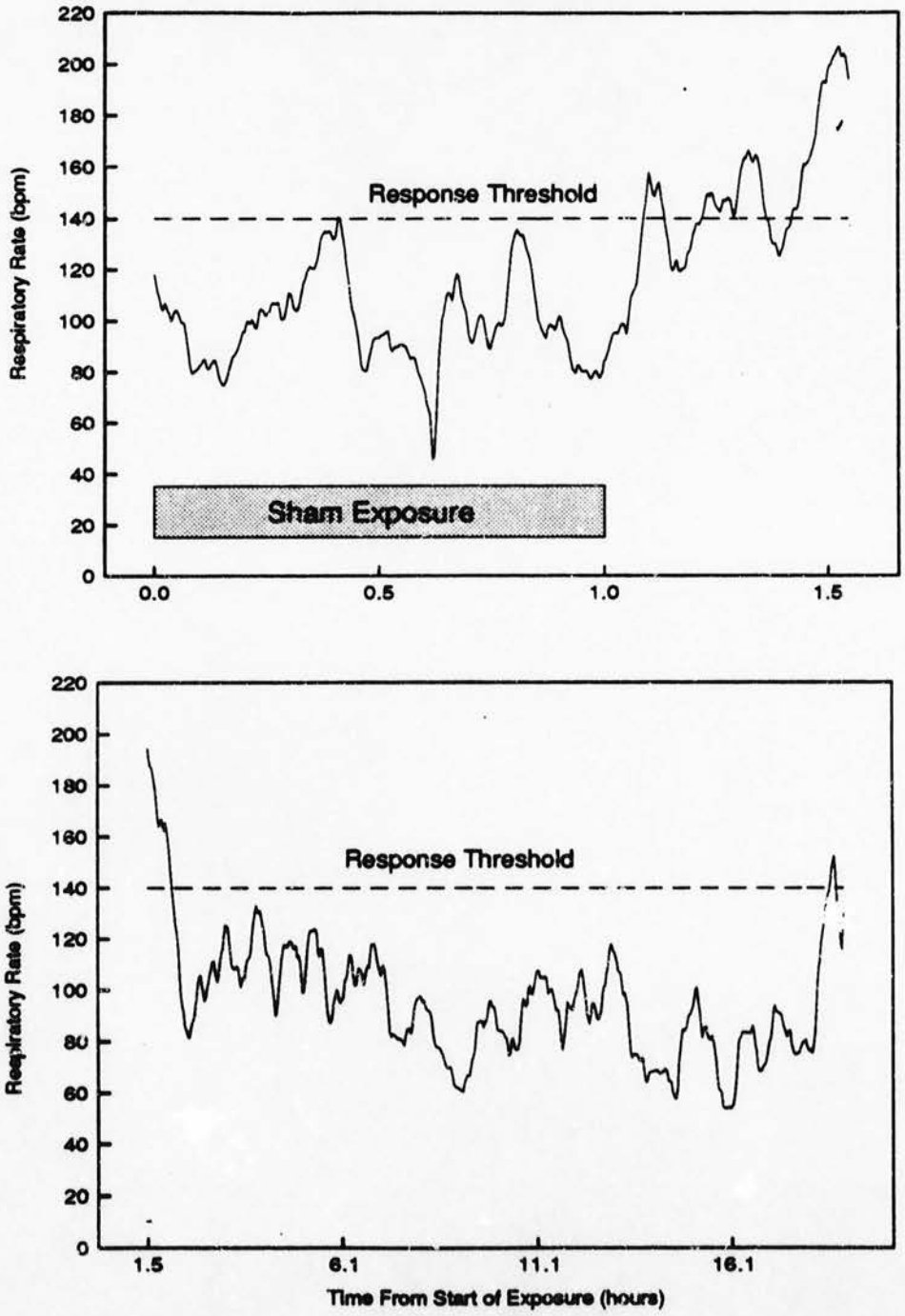
Figure: 15 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 196: Sham Sensitization

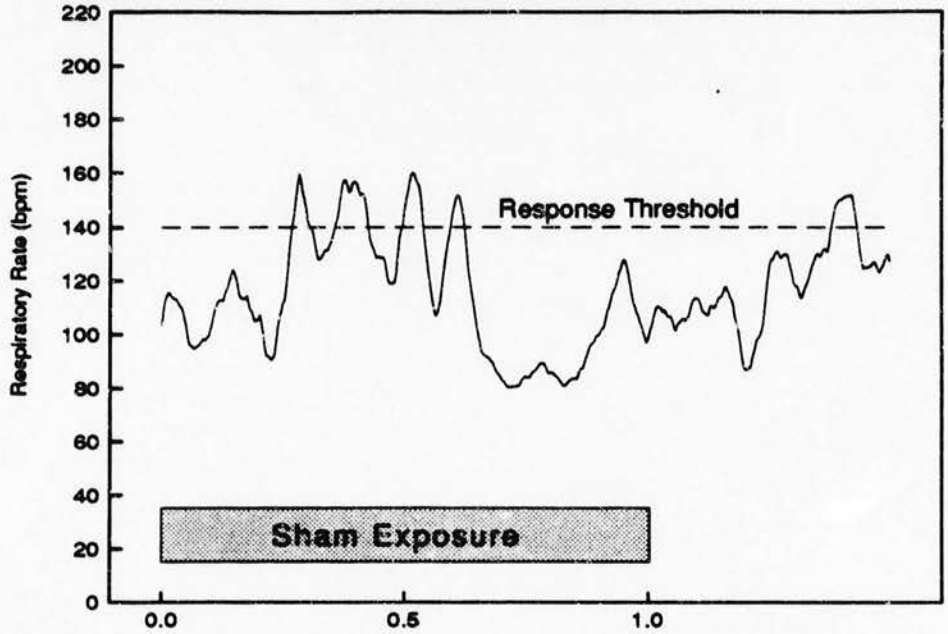
Figure: 16 Sham Challenge Week 2

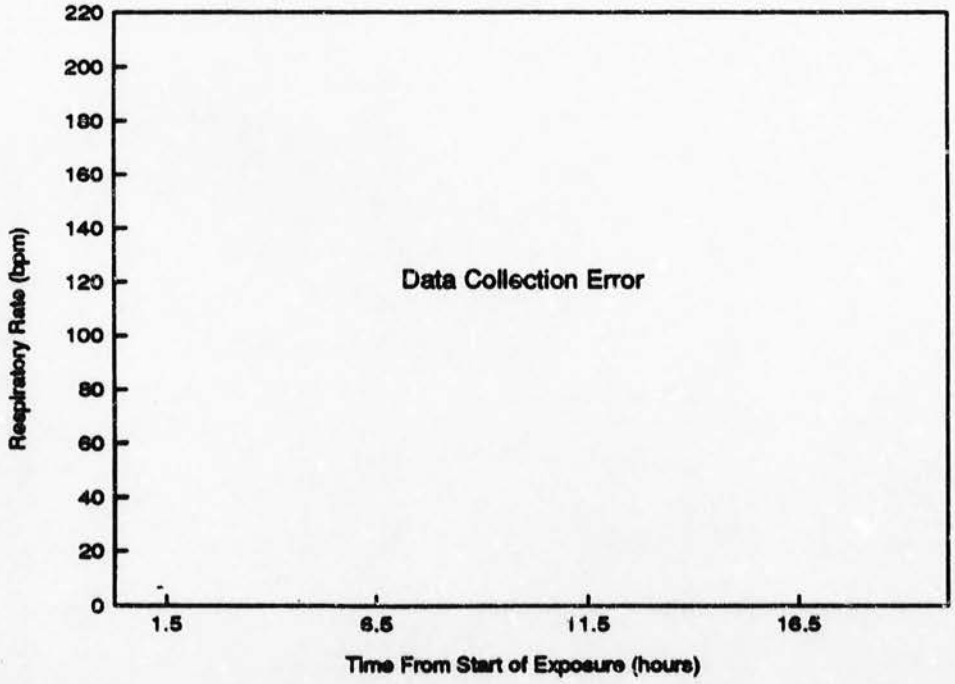


Study Number 92-344-PP

Animal # 197: Sham Sensitization

Figure: 17 Sham Challenge Week 2

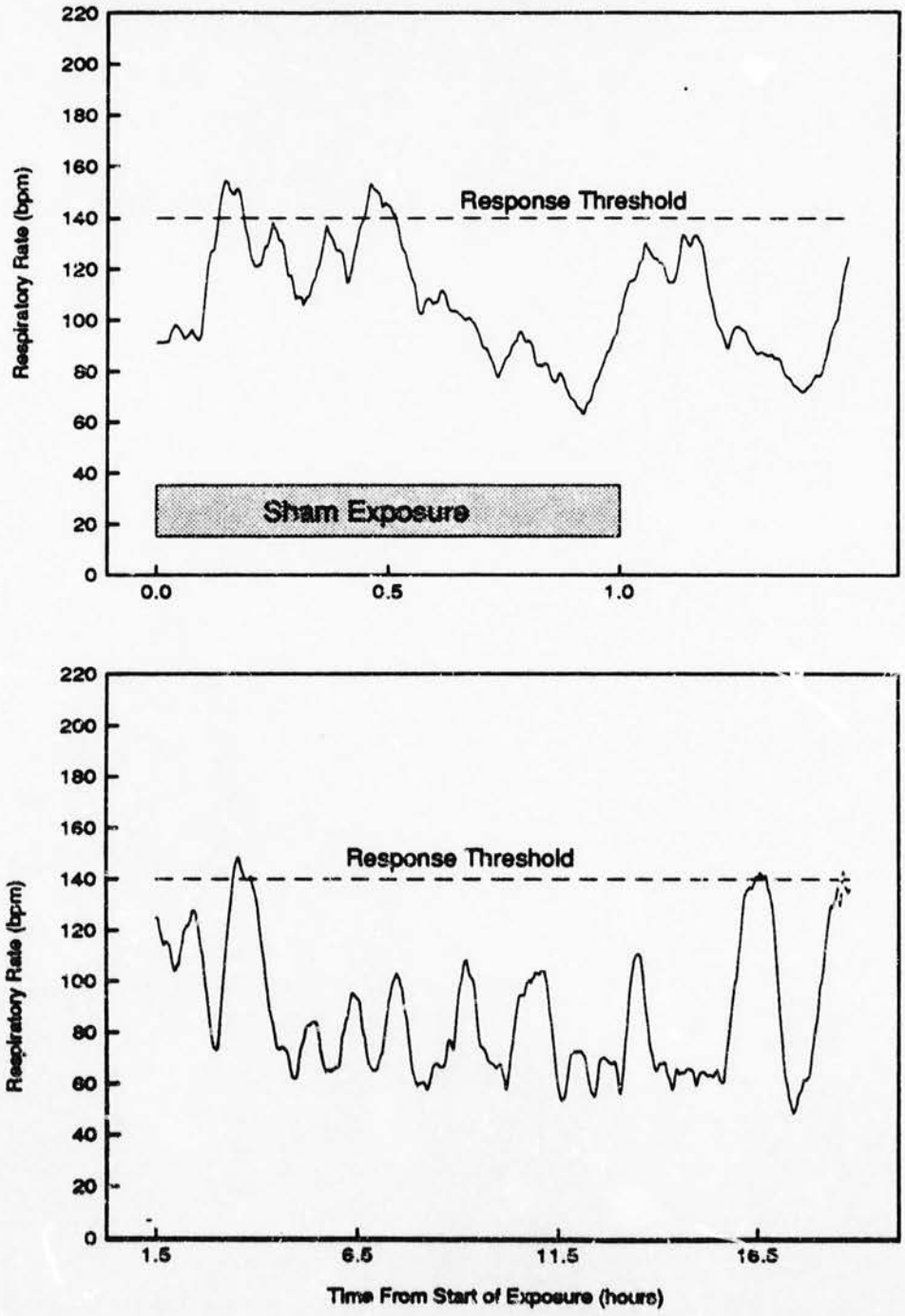




Study Number 92-344-PP

Animal # 198: Sham Sensitization

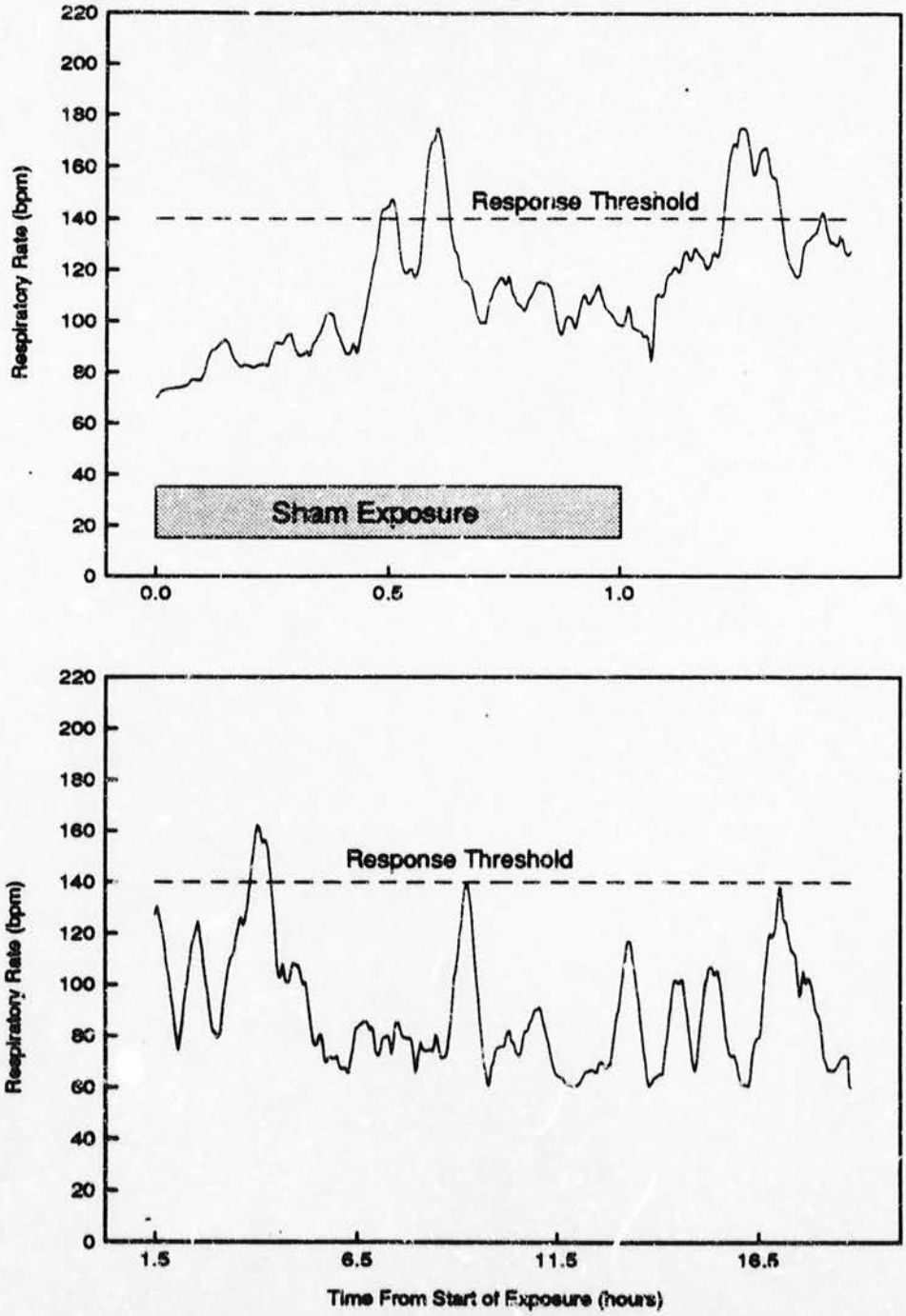
Figure: 18 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 199: Sham Sensitization

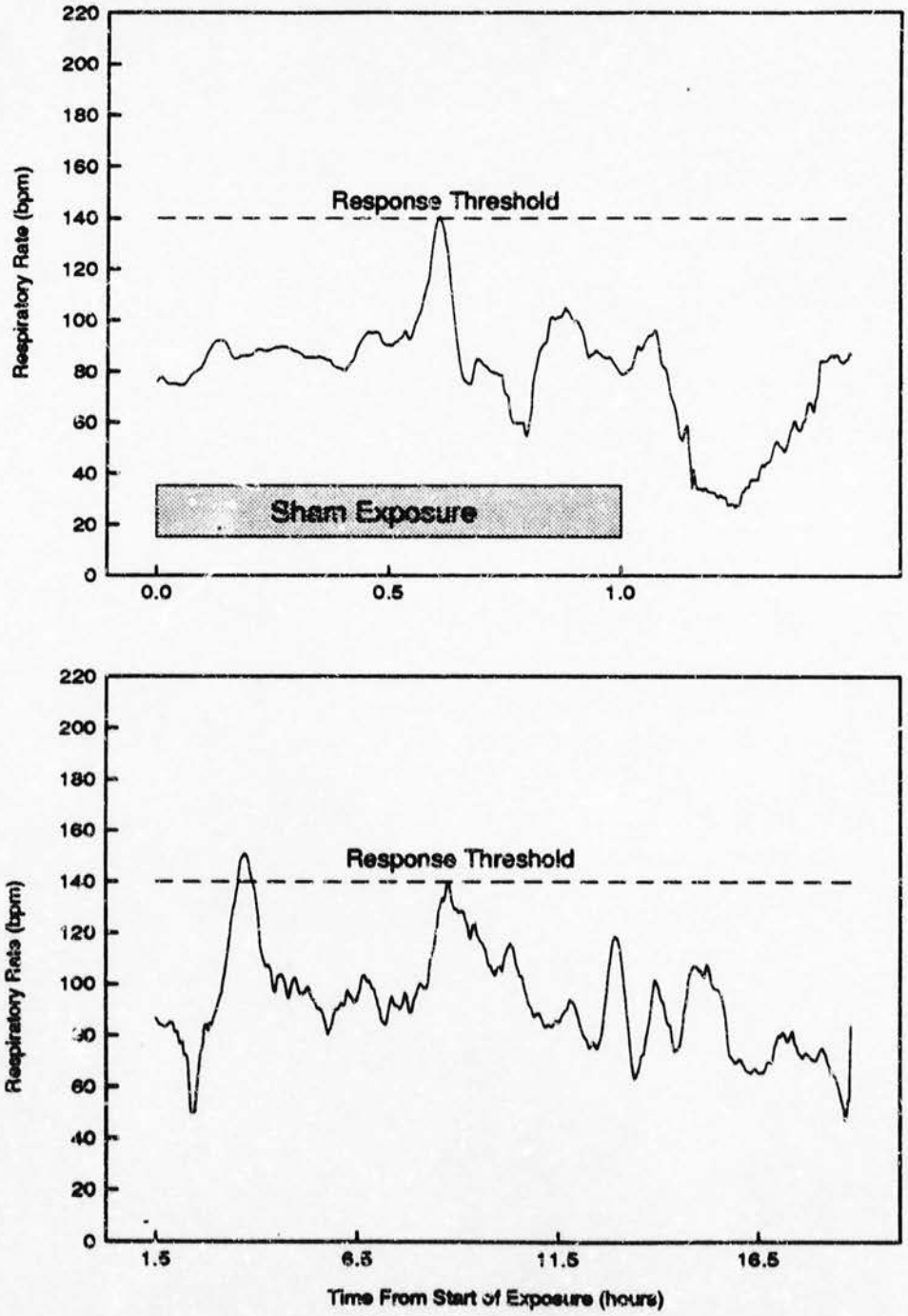
Figure: 19 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 200: Sham Sensitization

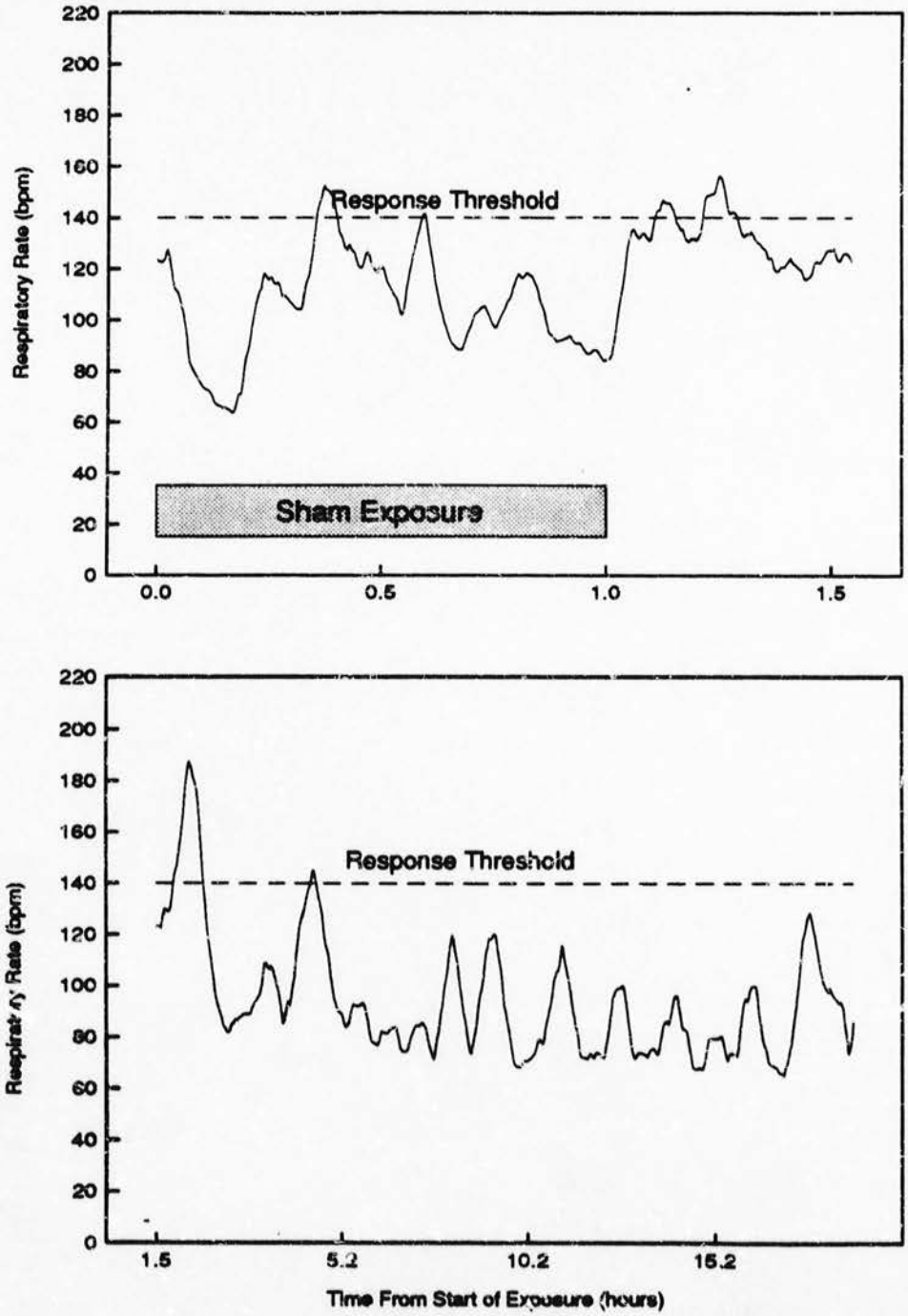
Figure: 20 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 201: Sham Sensitization

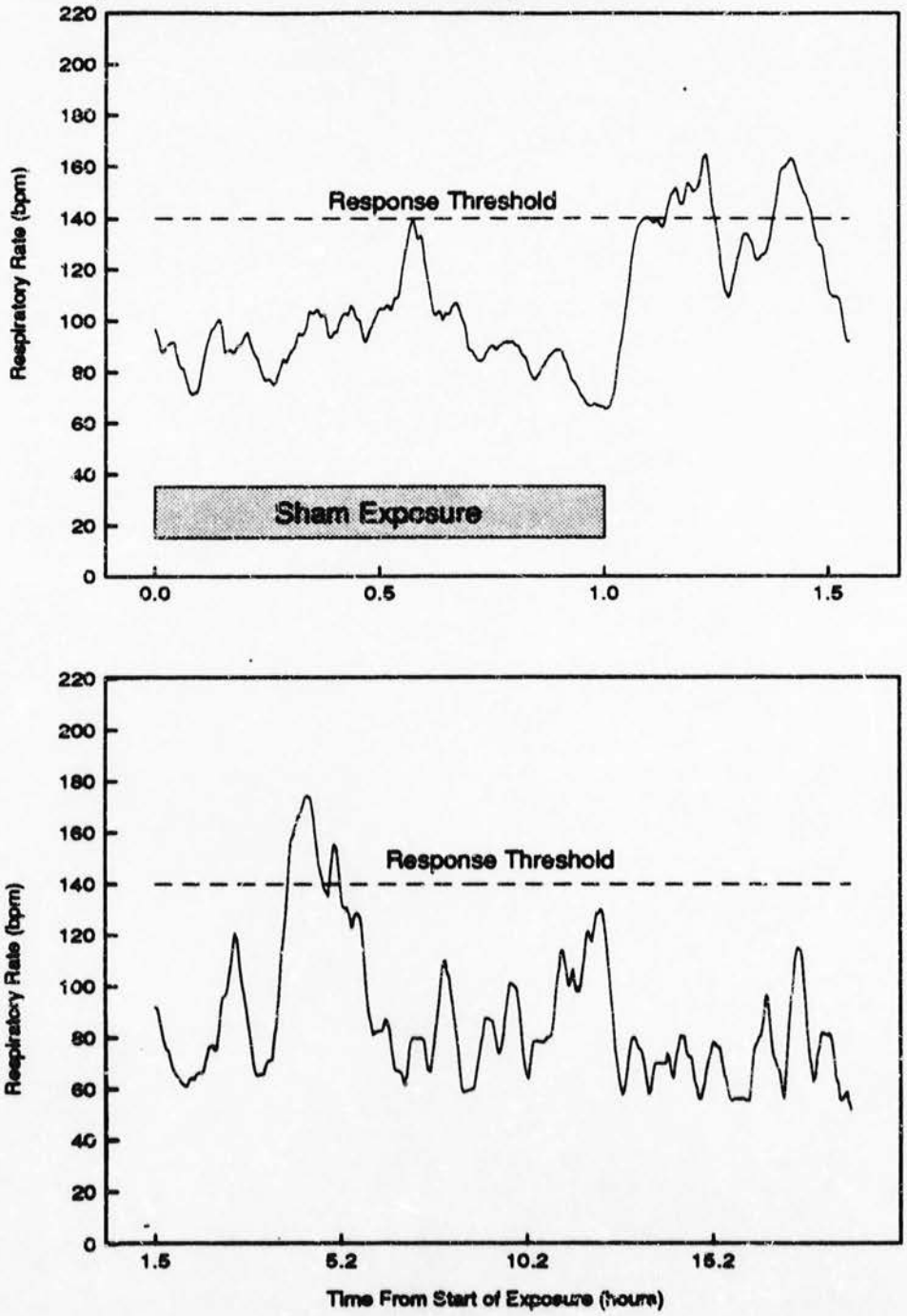
Figure: 21 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 202: Sham Sensitization

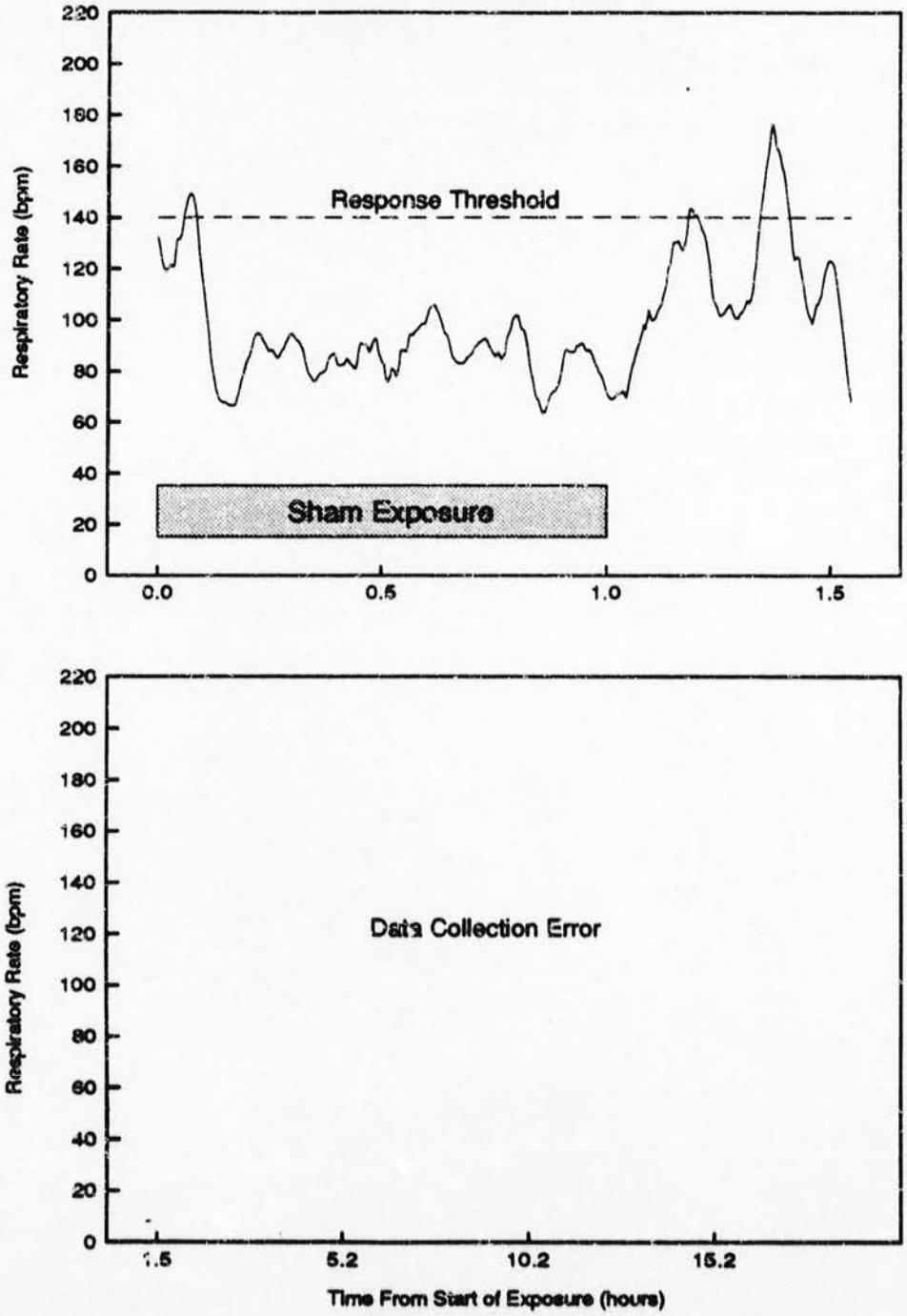
Figure: 22 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 203: Sham Sensitization

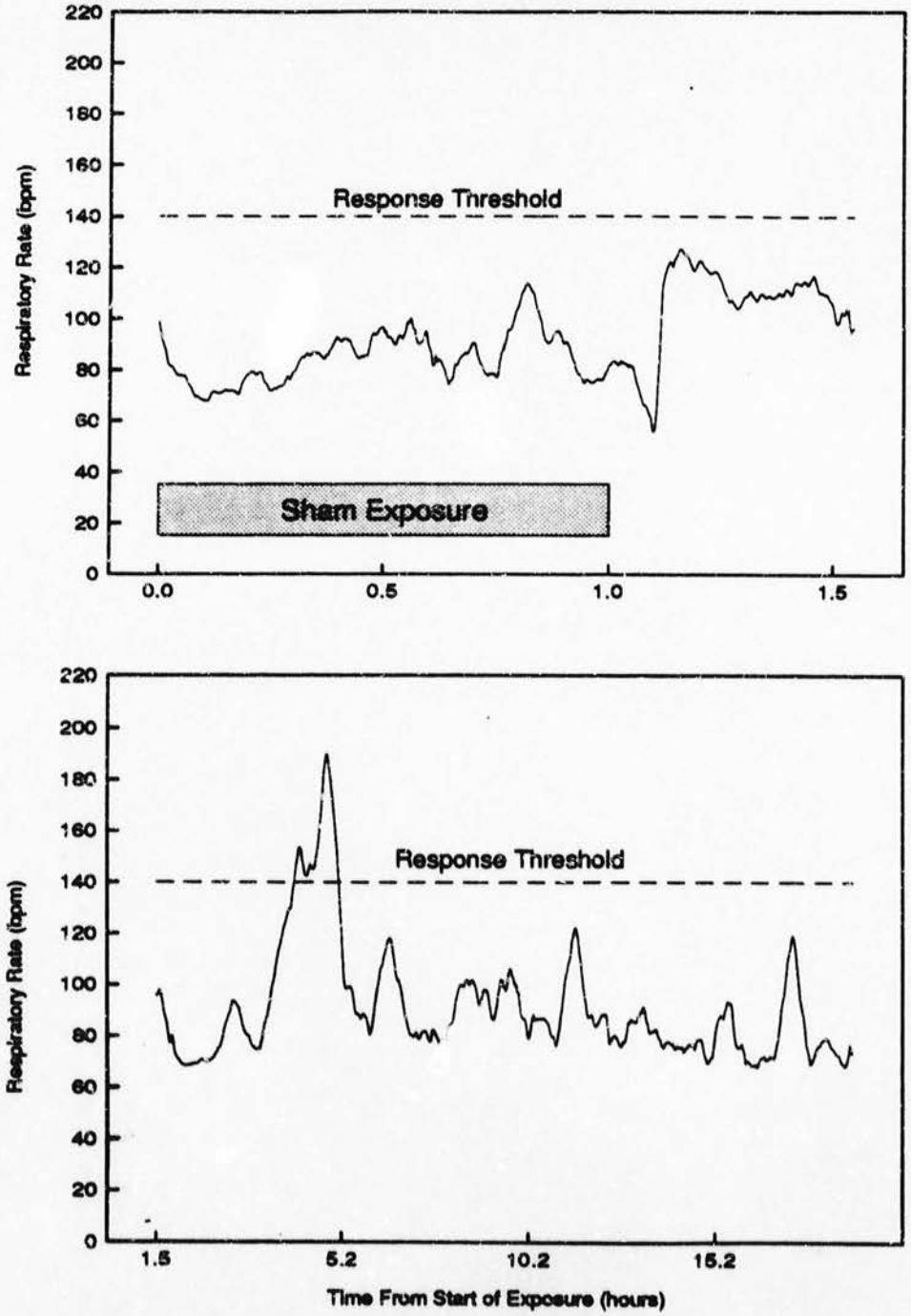
Figure: 23 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 204: Sham Sensitization

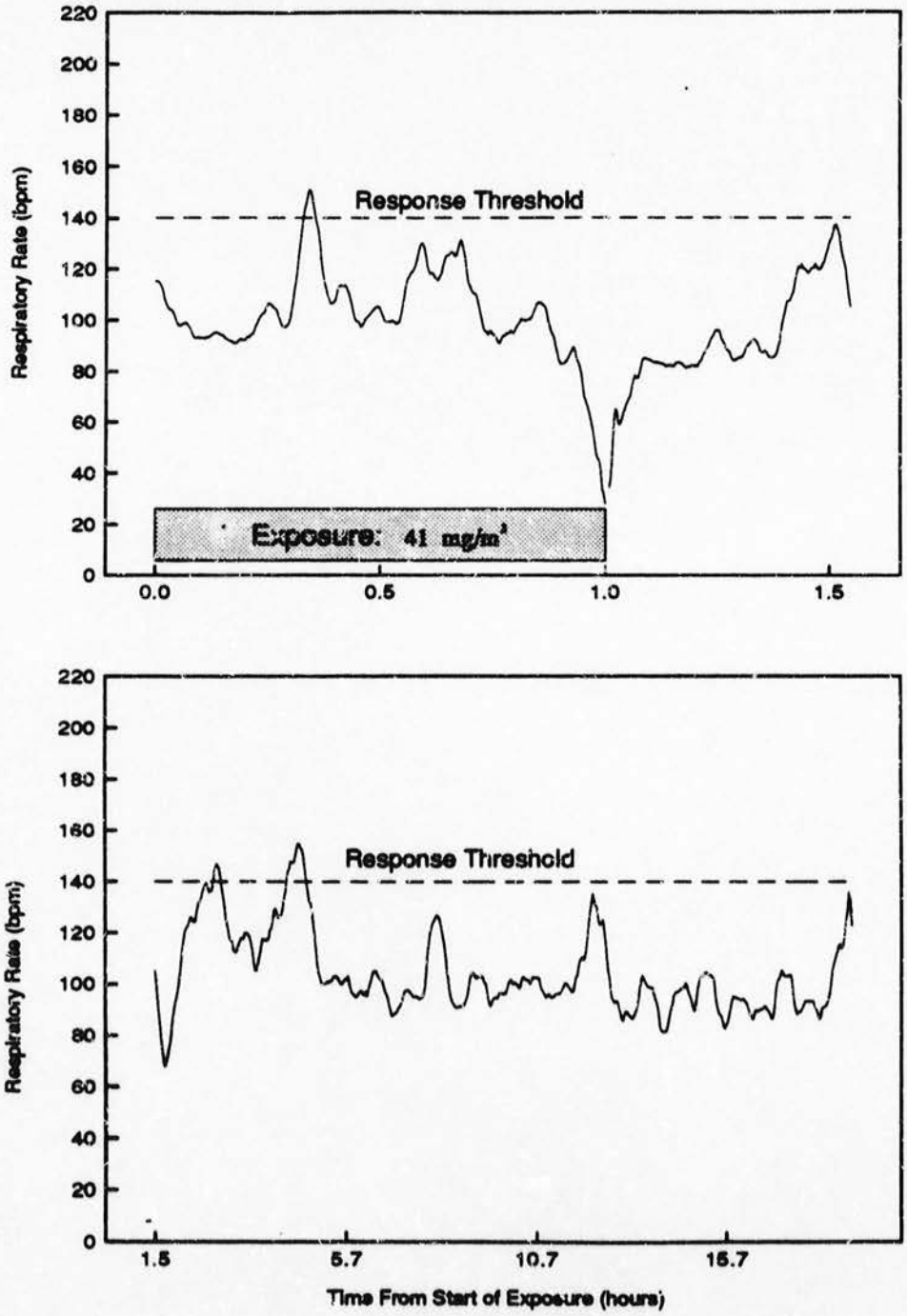
Figure: 24 Sham Challenge Week 2



Study Number 92-344-PP

Animal # 193: Sham Sensitization

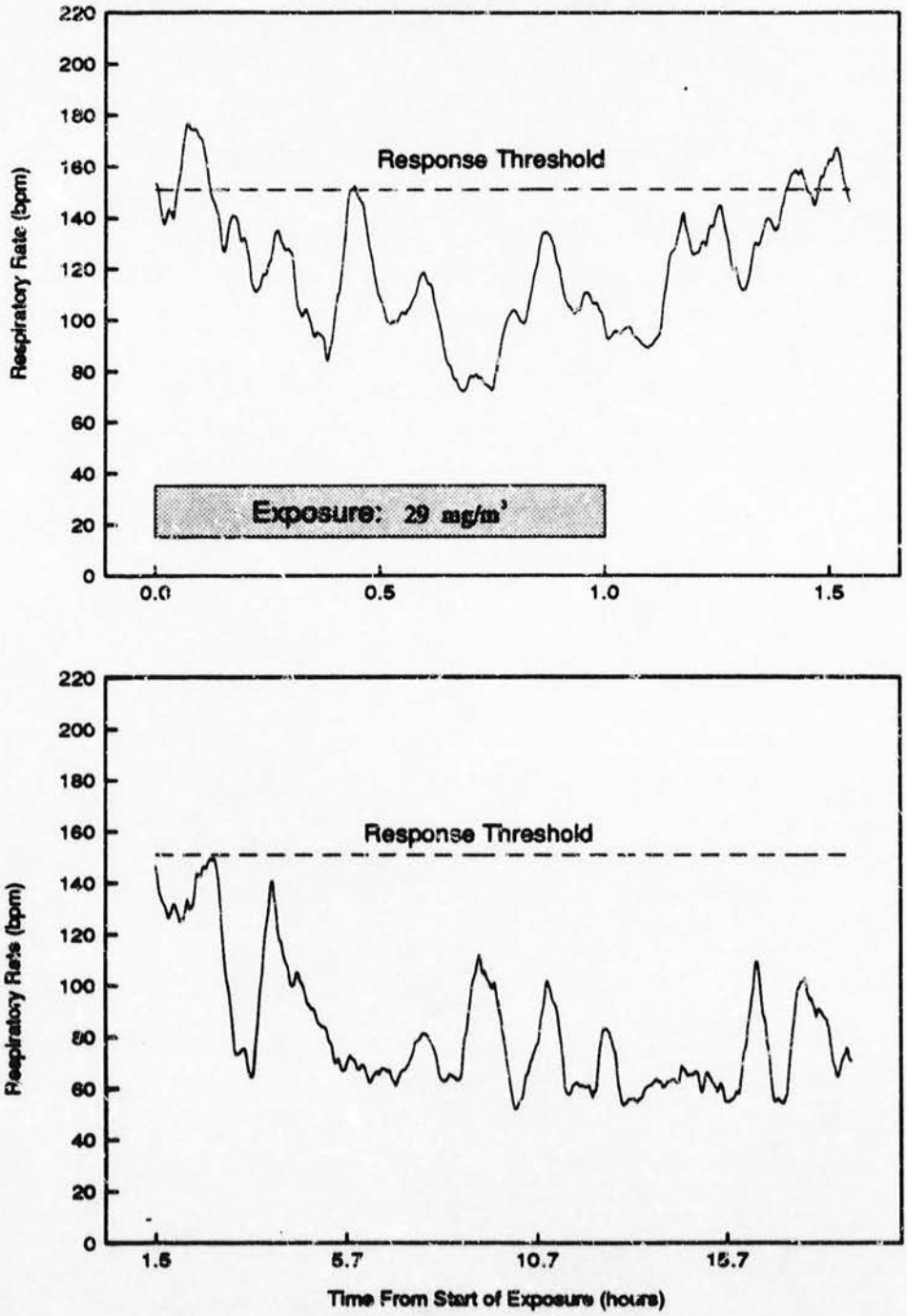
Figure: 25 GSA Challenge Week 3



Study Number 92-344-PP

Animal # 194: Sham Sensitization

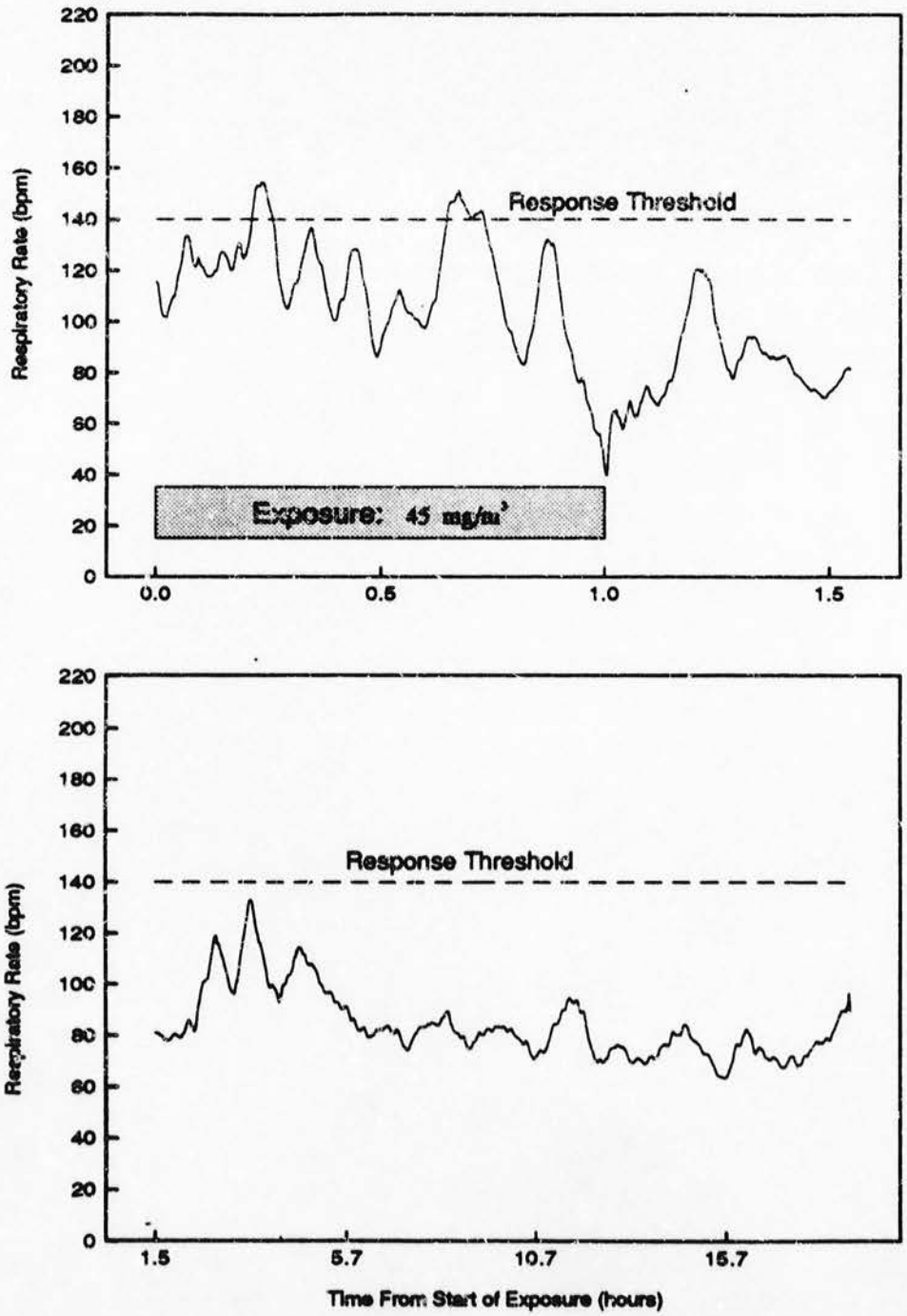
Figure: 26 GSA Challenge Week 3



Study Number 92-344-PP

Animal # 195: Sham Sensitization

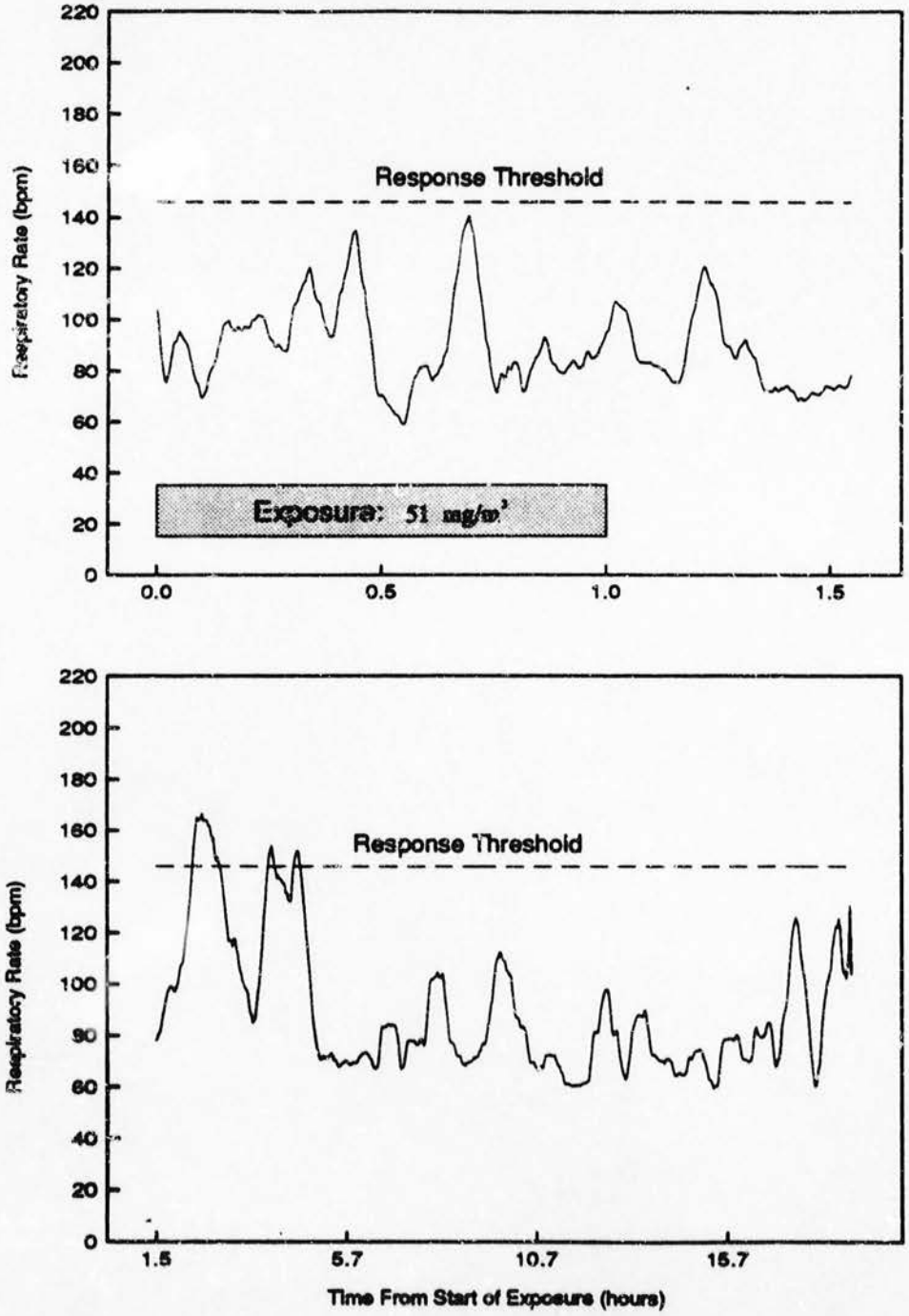
Figure: 27 GSA Challenge Week 3



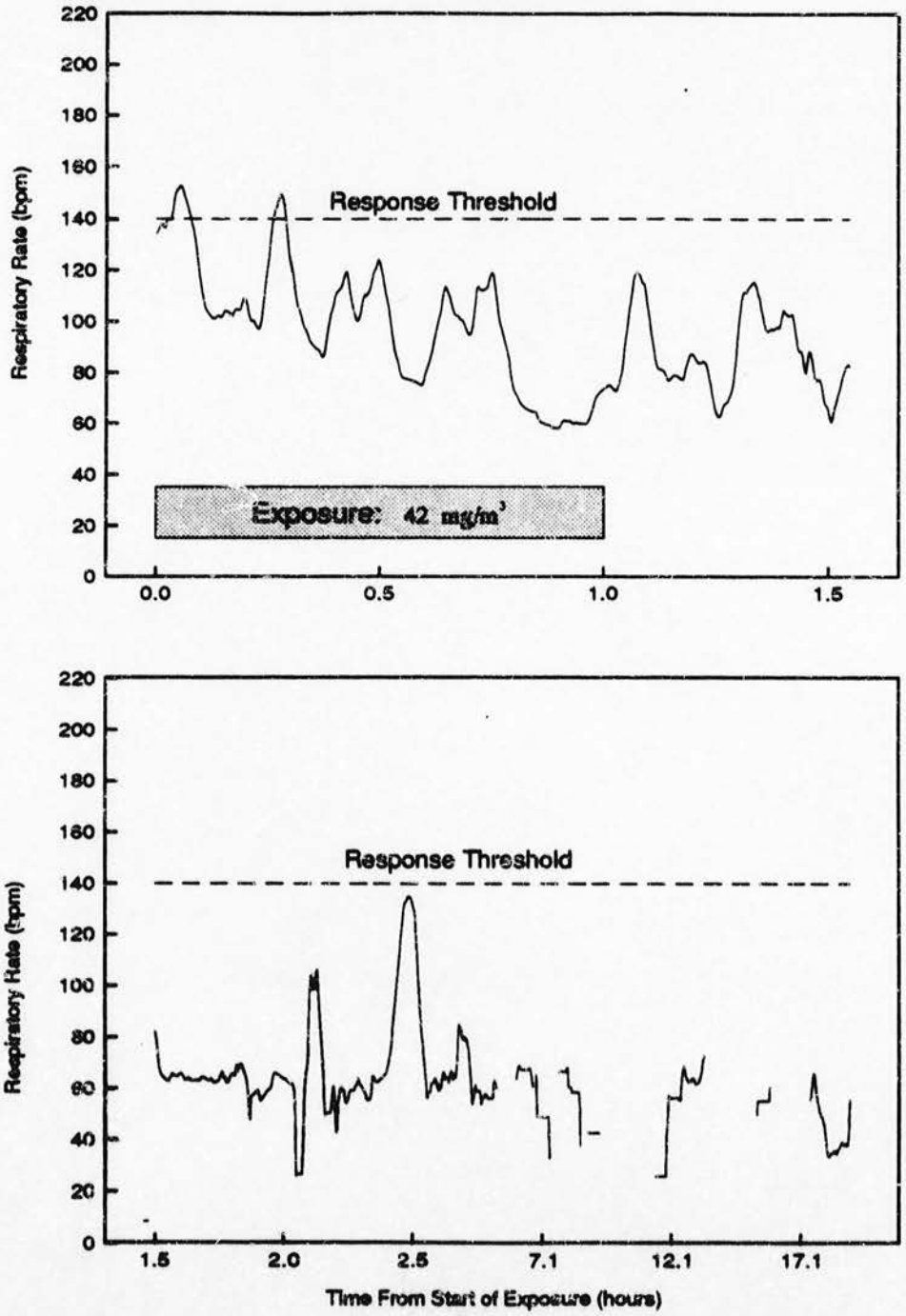
Study Number 92-344-PP

Animal # 196: Sham Sensitization

Figure: 28 GSA Challenge Week 3



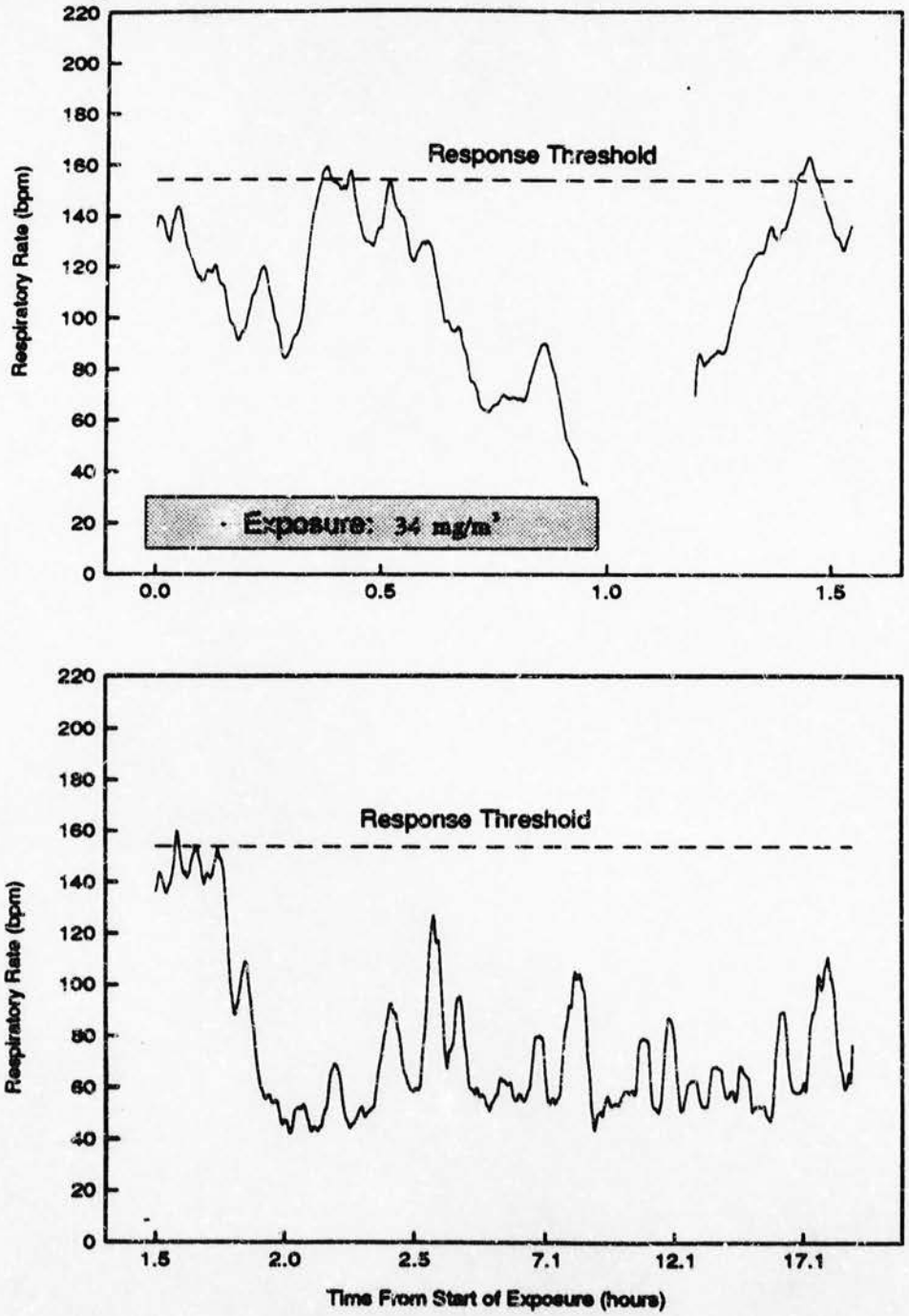
Study Number 92-344-PP
Animal # 197: Sham Sensitization
Figure: 29 GSA Challenge Week 3



Study Number 92-344-PP

Animal # 198: Sham Sensitization

Figure: 30 GSA Challenge Week 3



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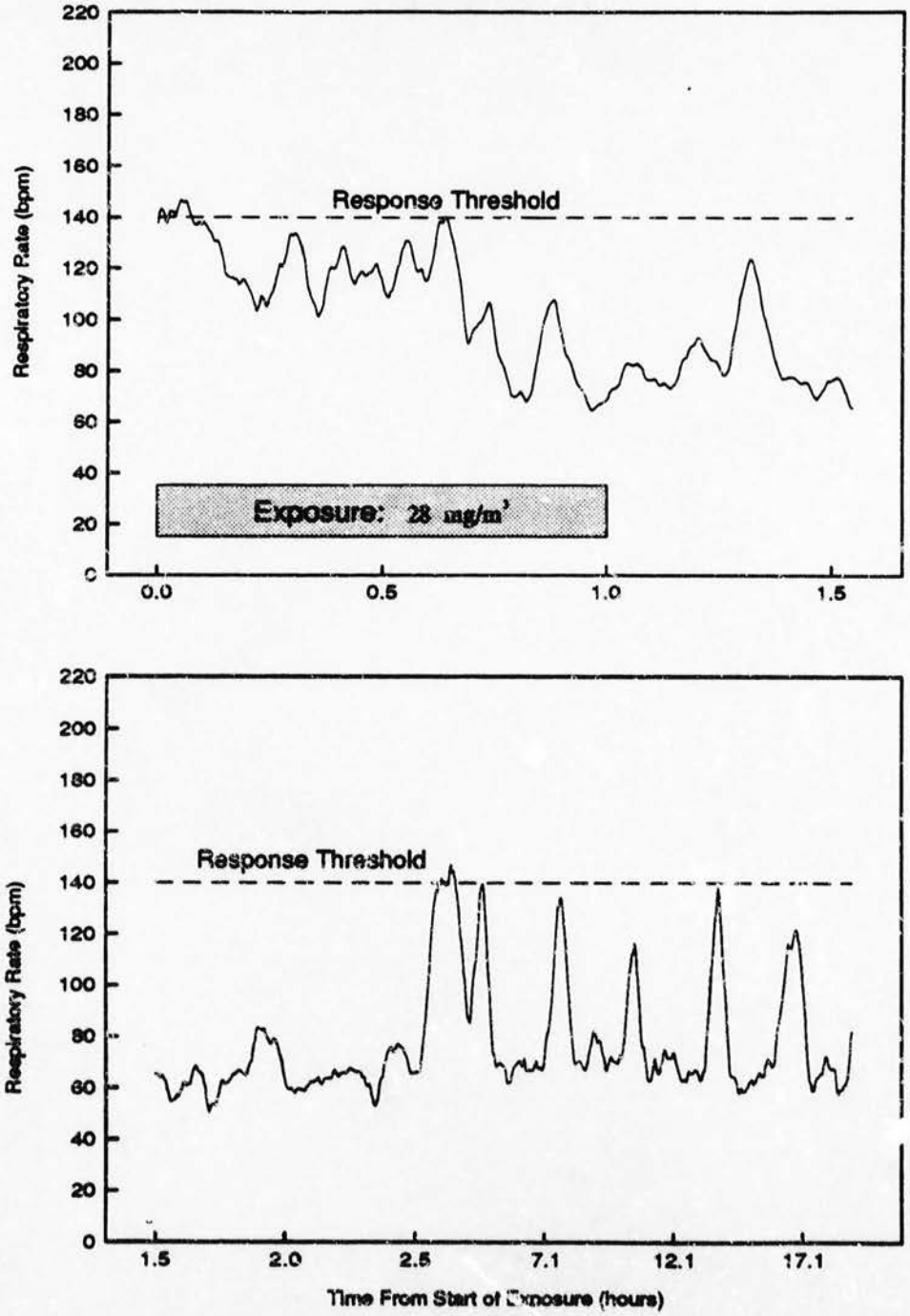
Animal # 199: Sham Sensitization

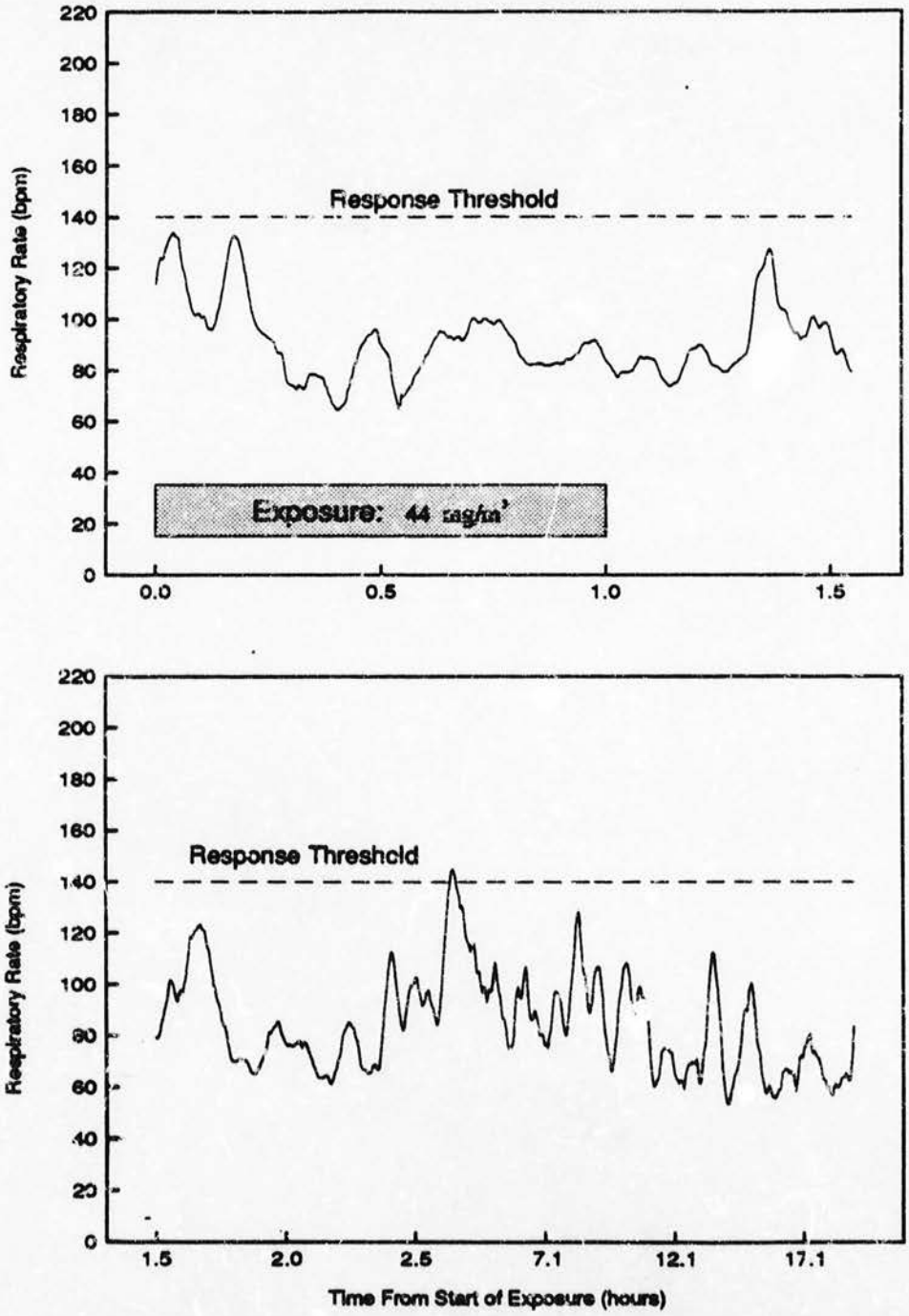
Figure: 31 GSA Challenge Week 3



Study Number 92-344-PP

Animal # 200: Sham Sensitization

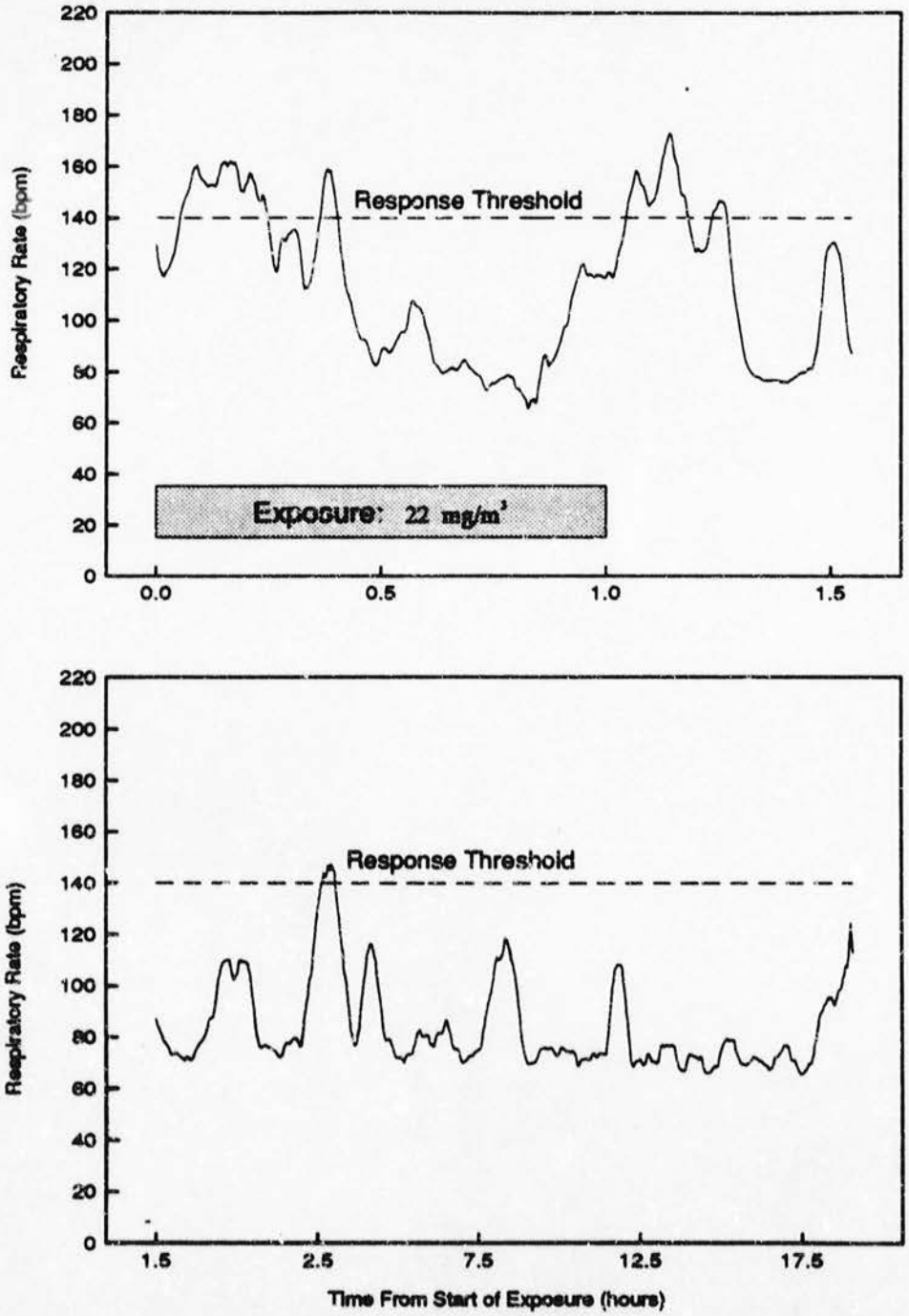
Figure: 32 GSA Challenge Week 3



Study Number 92-344-PP

Animal # 201: Sham Sensitization

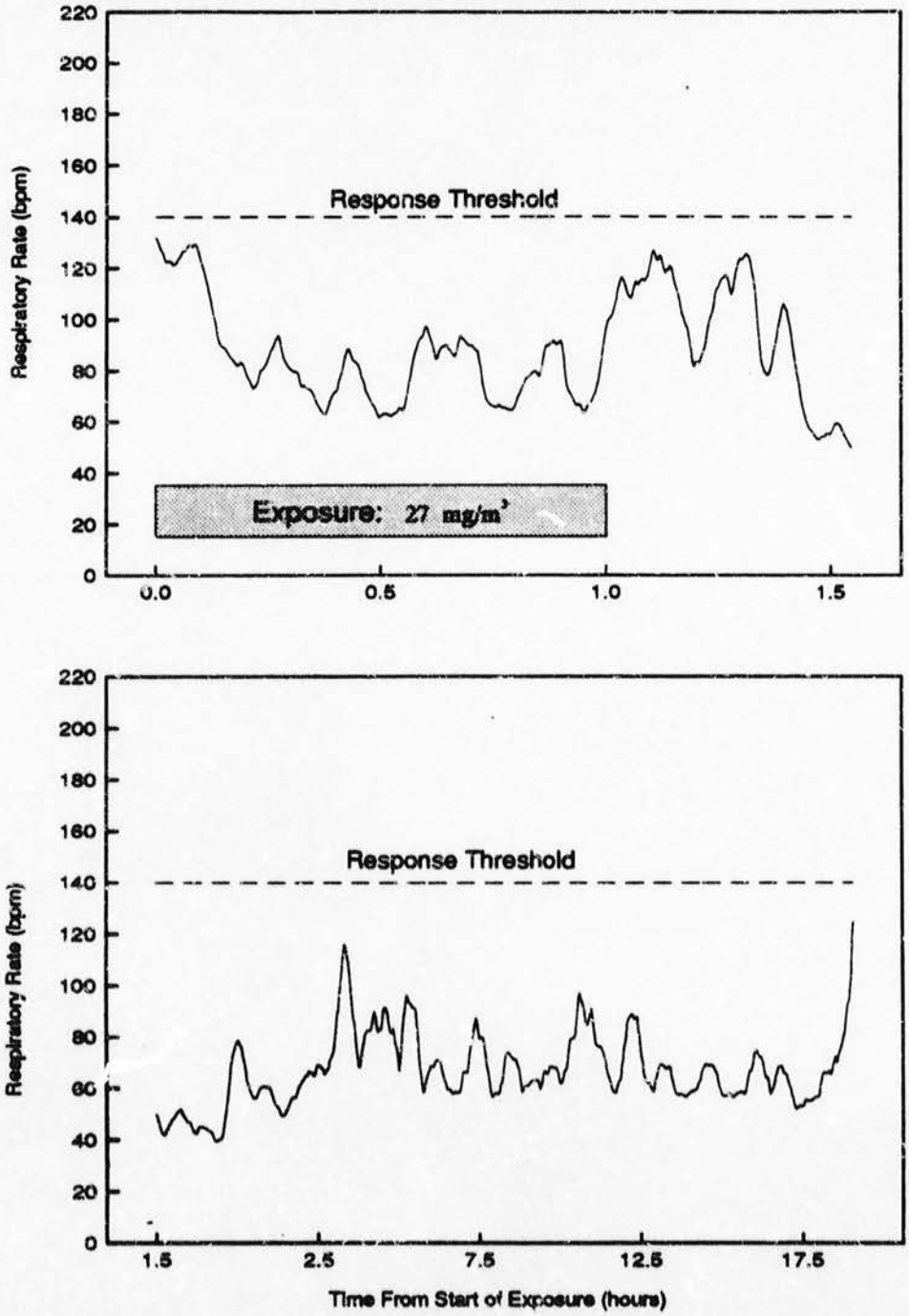
Figure: 33 GSA Challenge Week 3



Study Number 92-344-PP

Animal # 202: Sham Sensitization

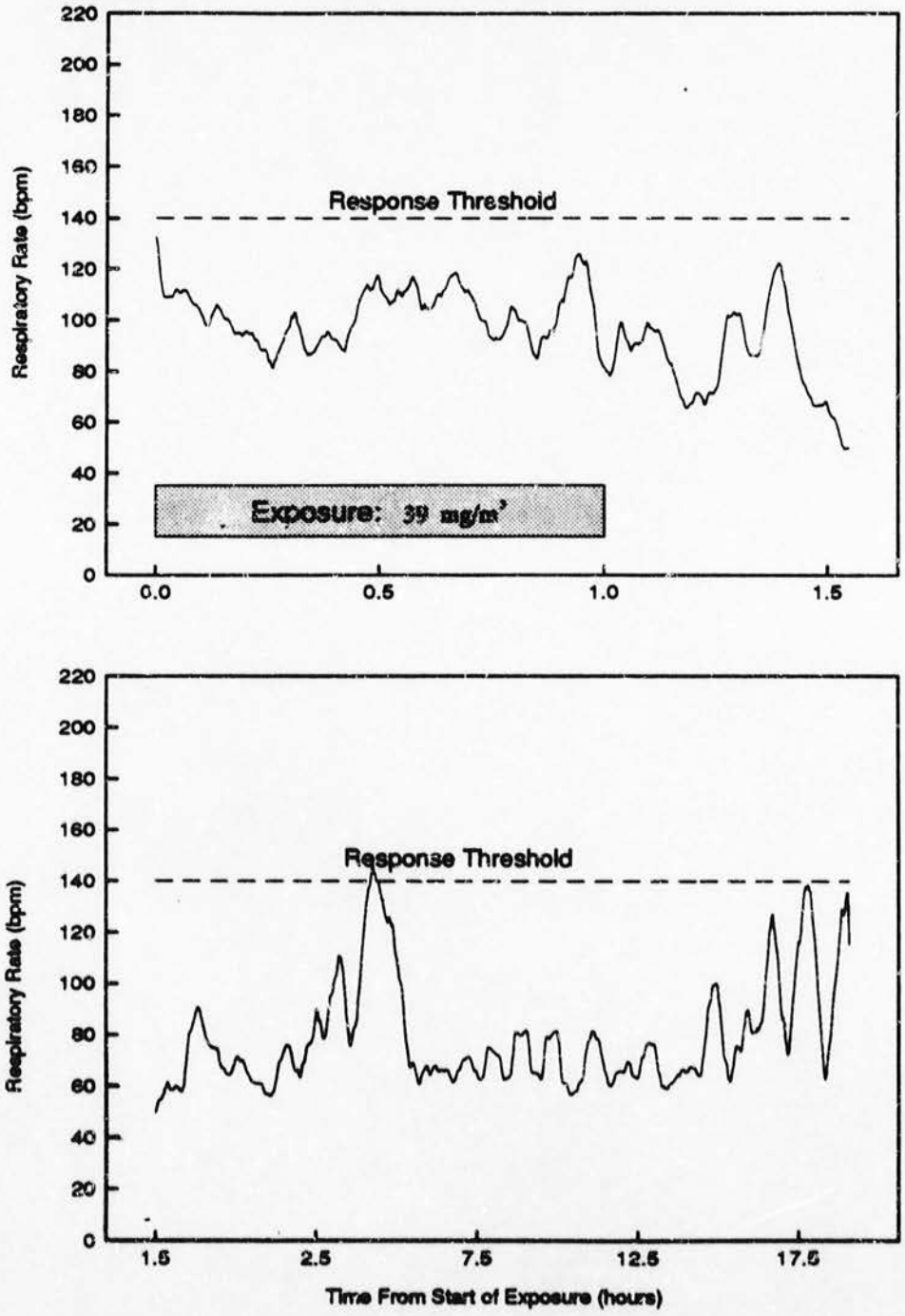
Figure: 34 GSA Challenge Week 3



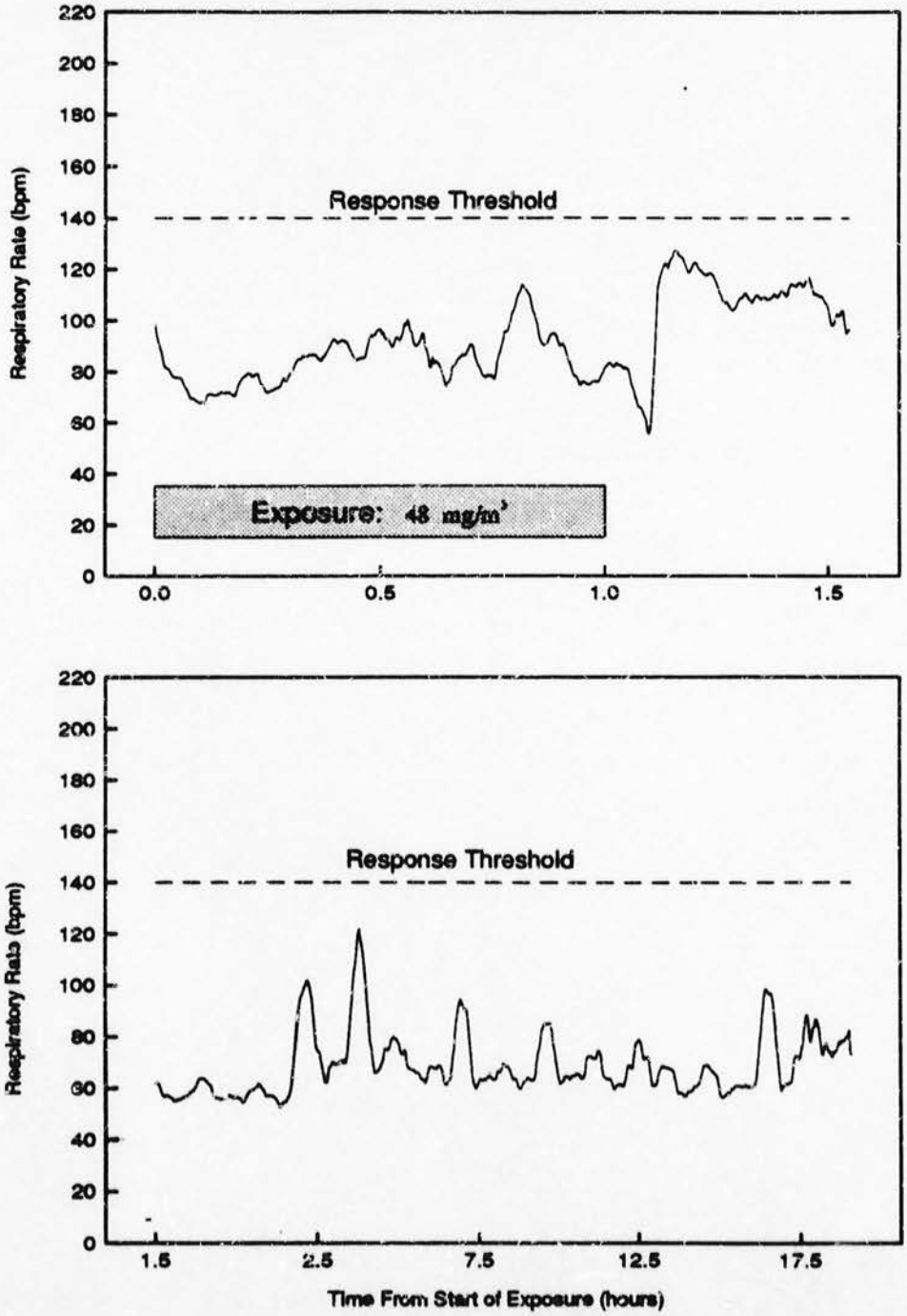
Study Number 92-344-PP

Animal # 203: Sham Sensitization

Figure: 35 GSA Challenge Week 3



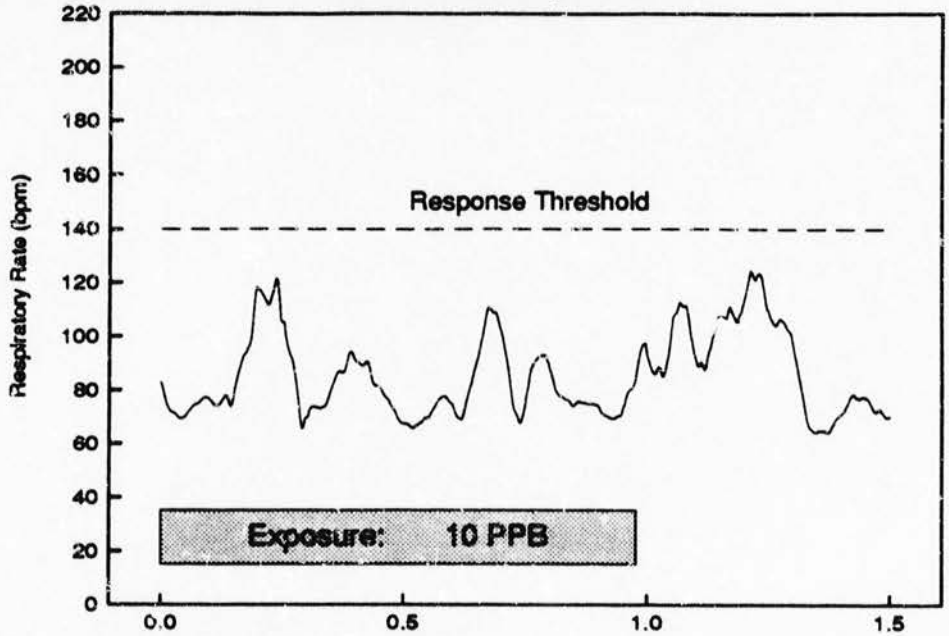
Study Number 92-344-PP
Animal # 204: Sham Sensitization
Figure: 36 GSA Challenge Week 3

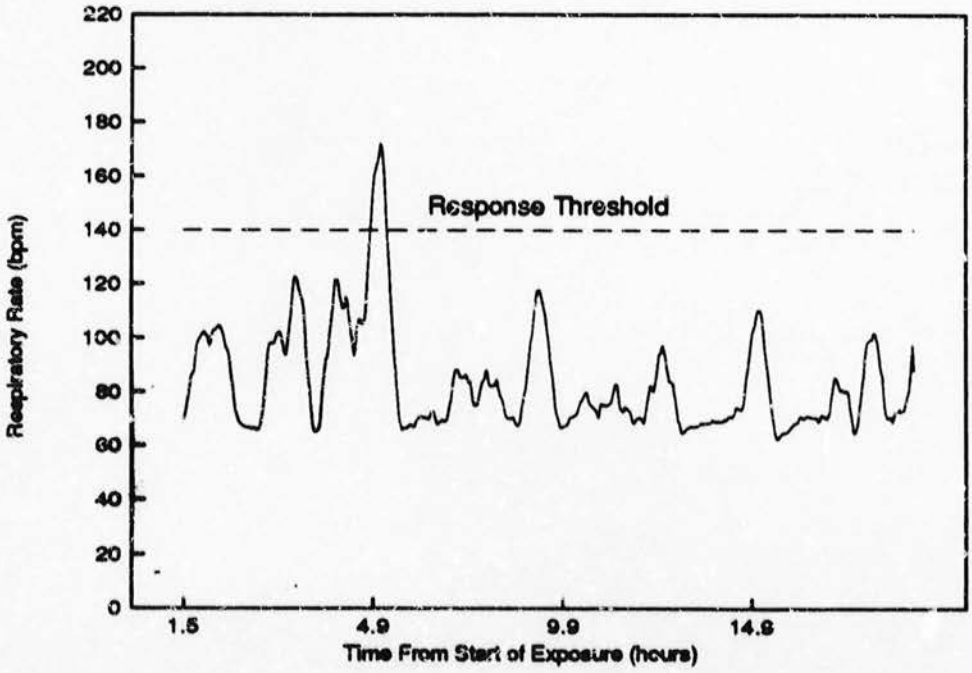


Study Number 92-344-PP

Animal # 222: Sham Sensitization

Figure: 37 2,4-TDI Challenge Week 1

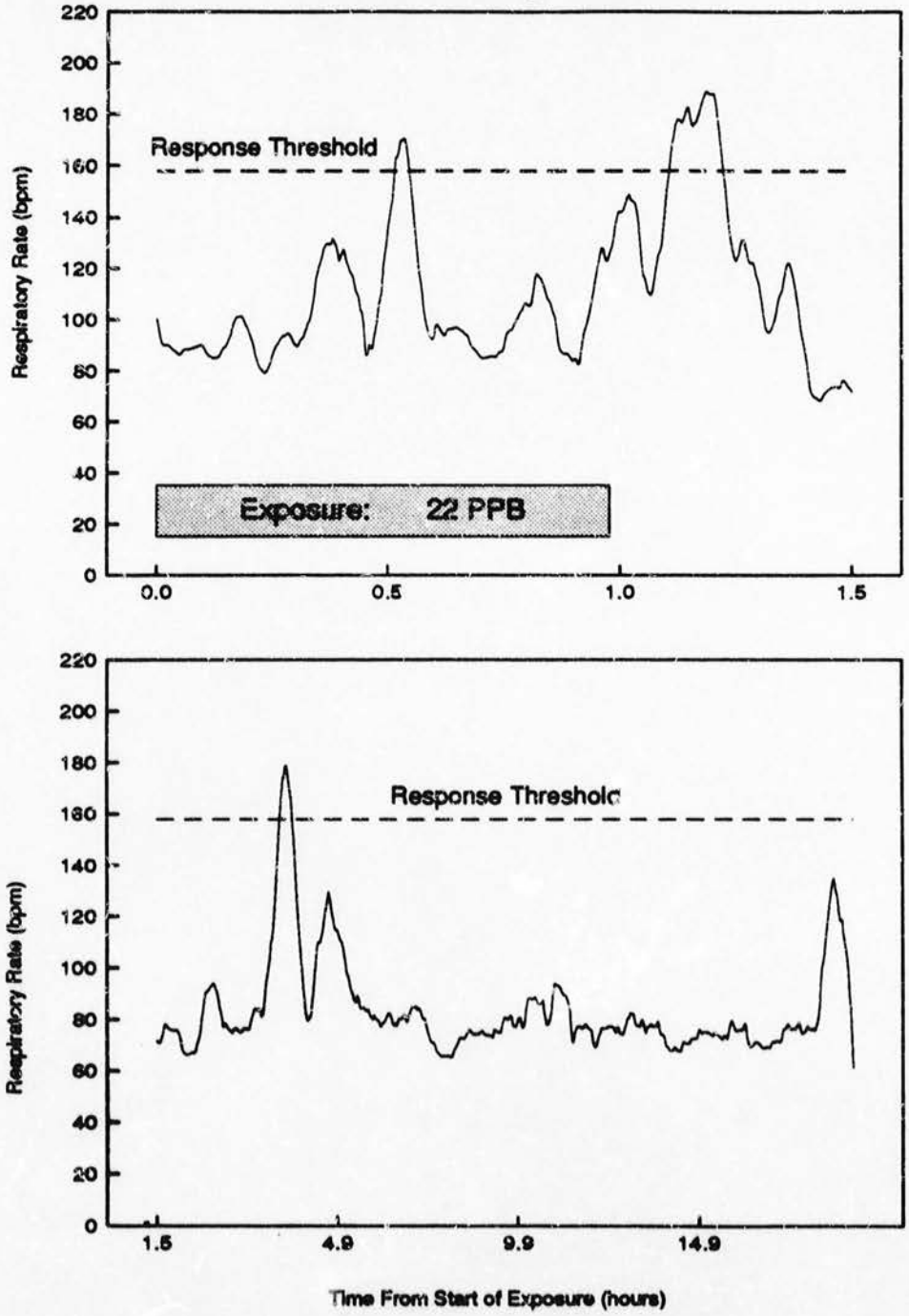




Study Number 92-344-PP

Animal # 223: Sham Sensitization

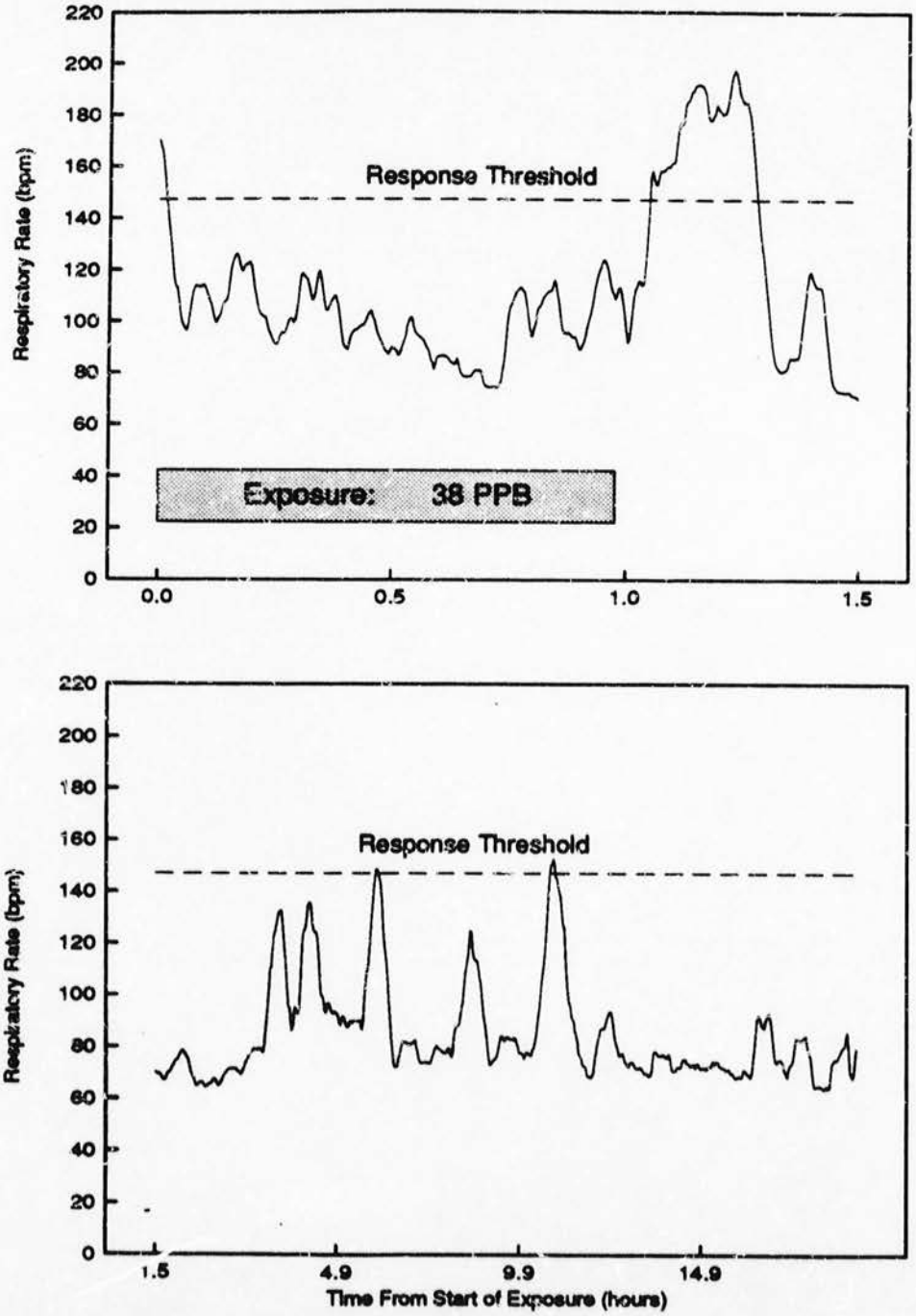
Figure: 38 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 224: Sham Sensitization

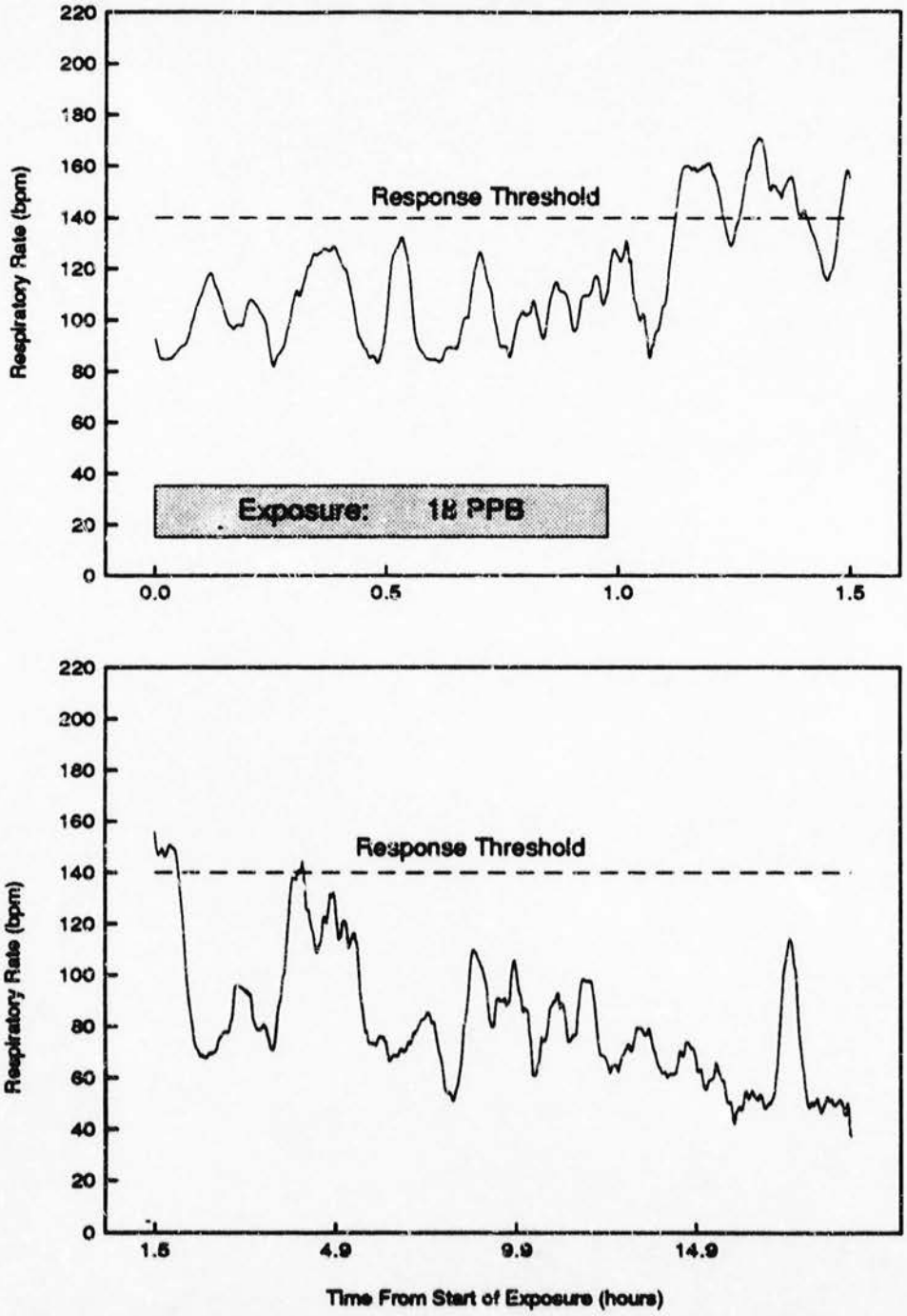
Figure: 39 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 225: Sham Sensitization

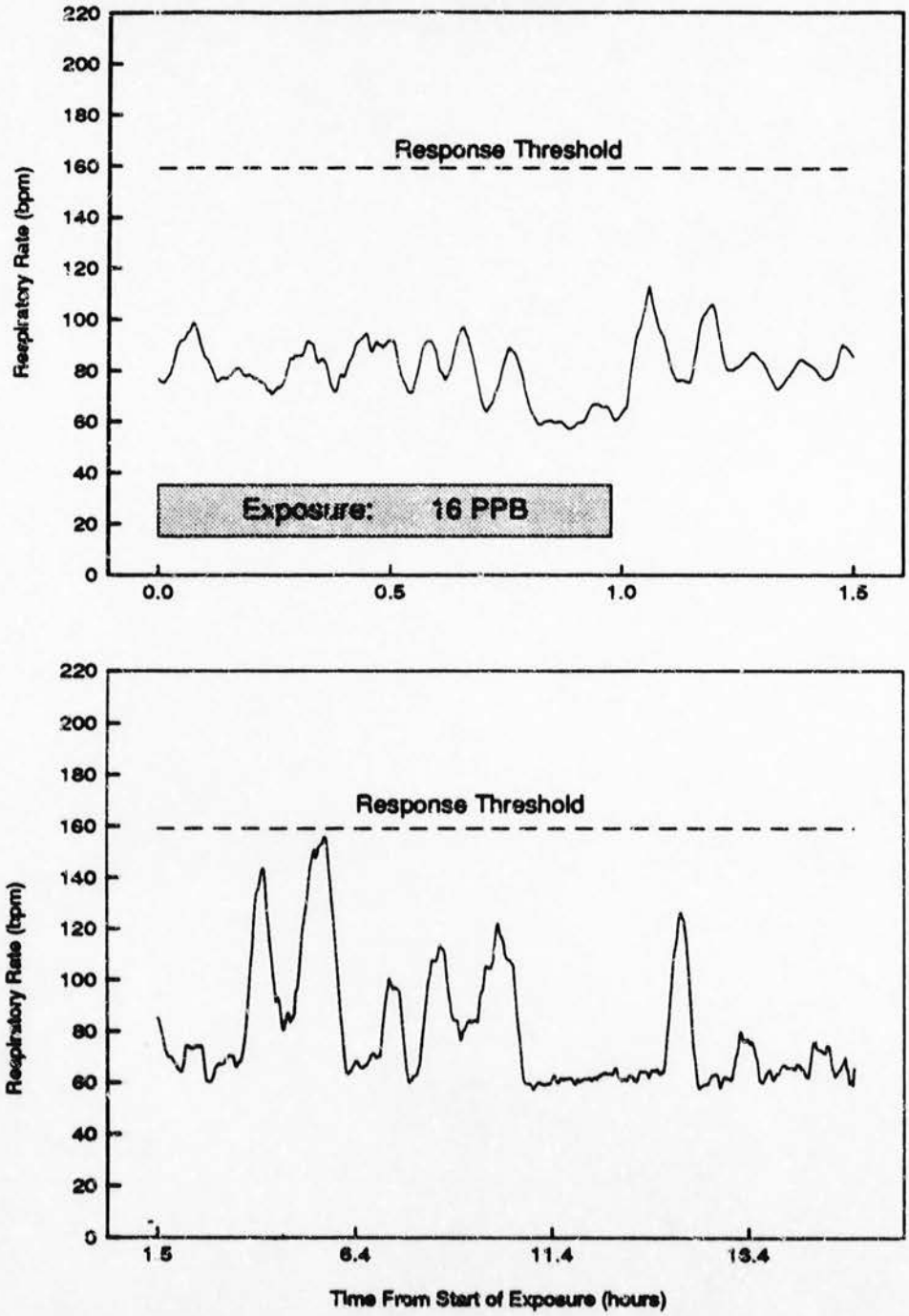
Figure: 40 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 226: Sham Sensitization

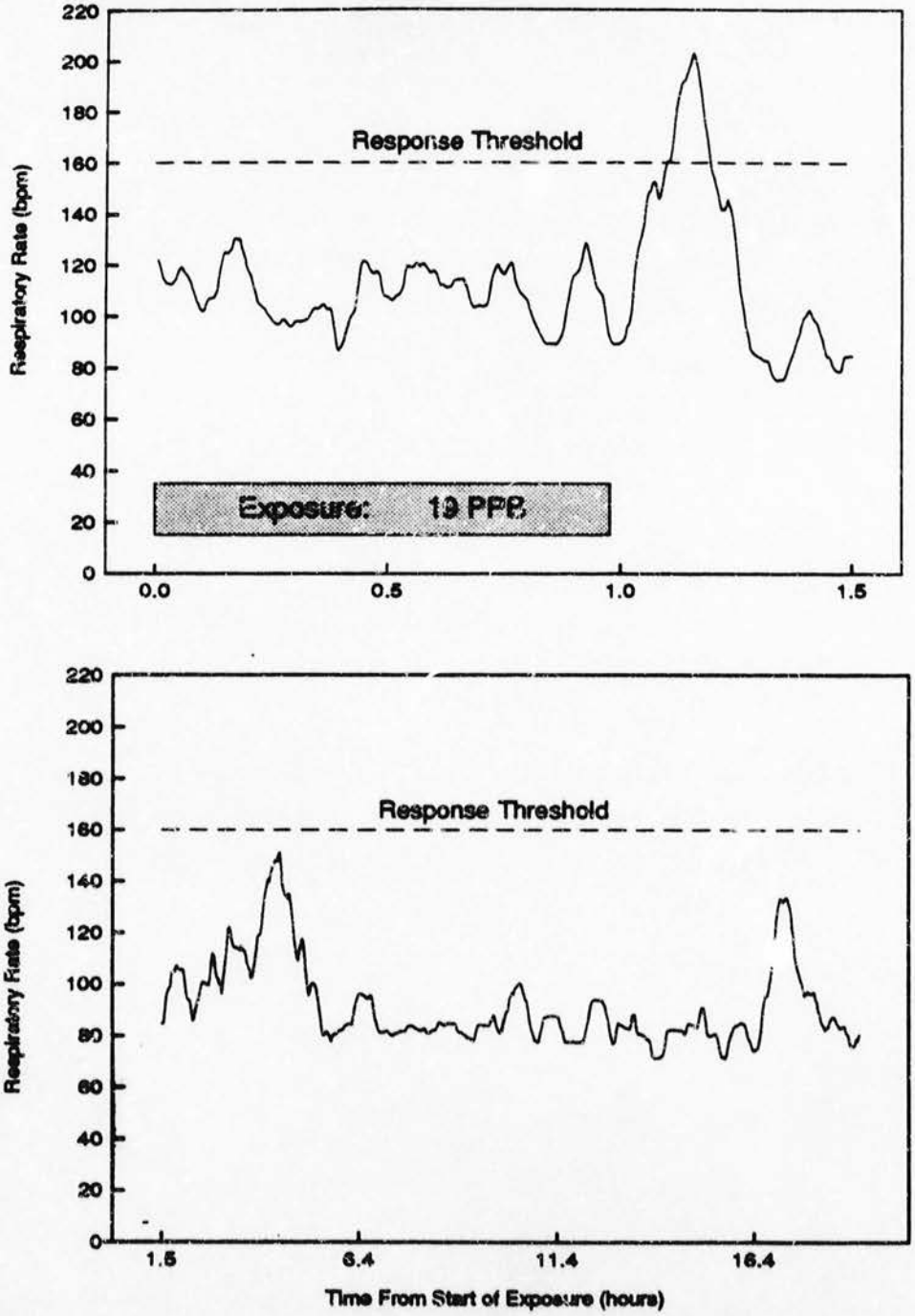
Figure: 41 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 227: Sham-Sensitization

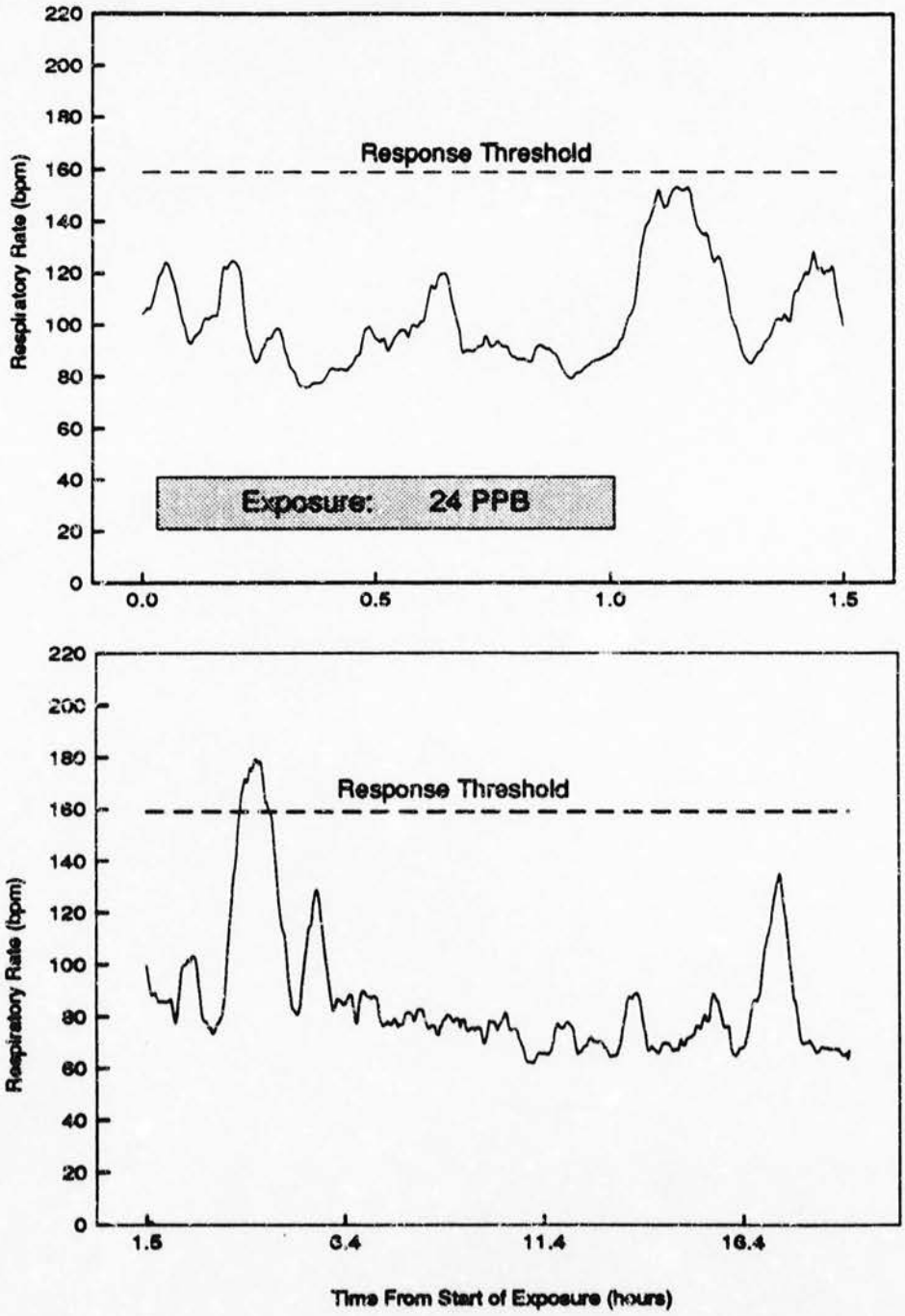
Figure: 42 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 228: Sham Sensitization

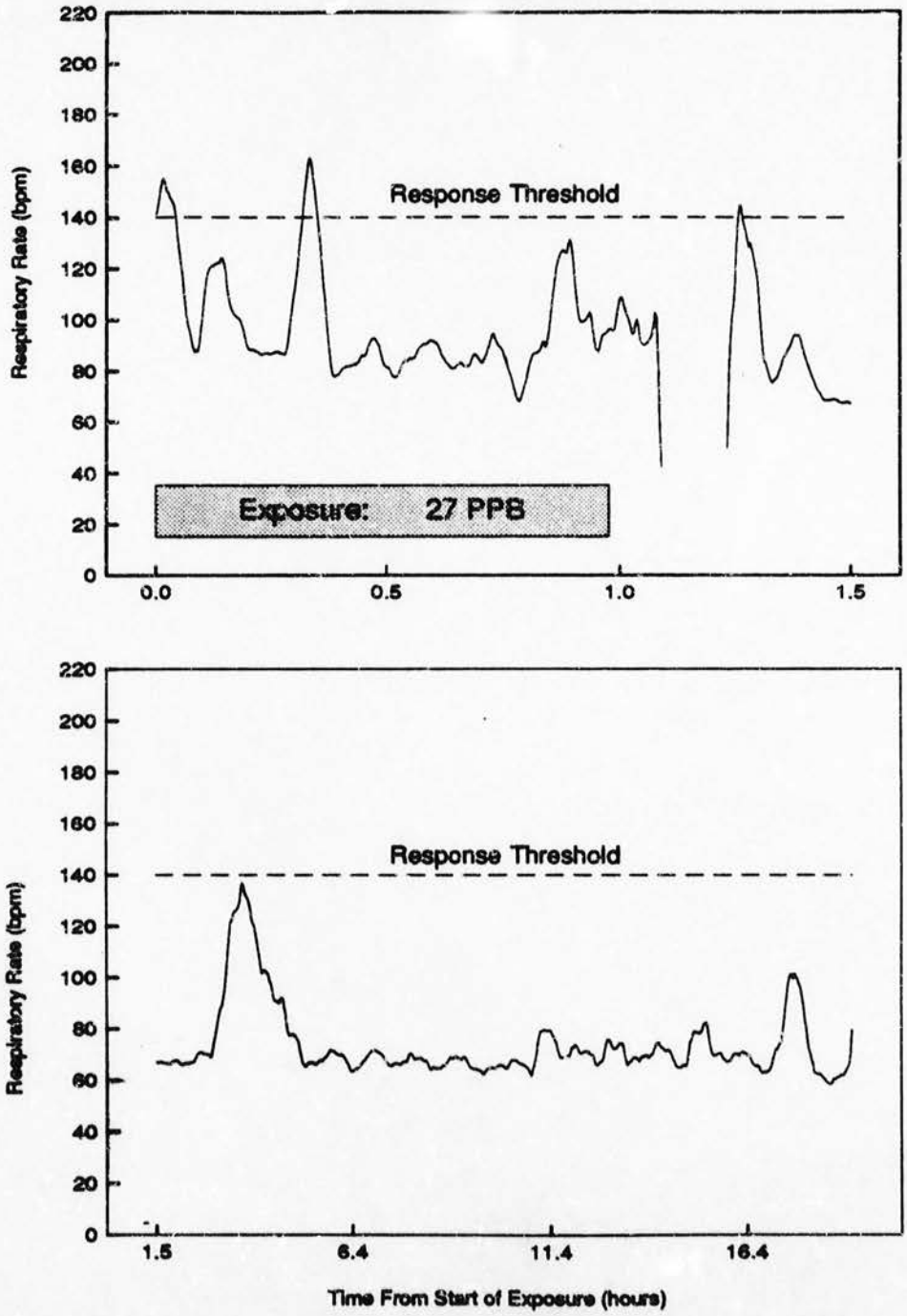
Figure: 43 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 229: Sham Sensitization

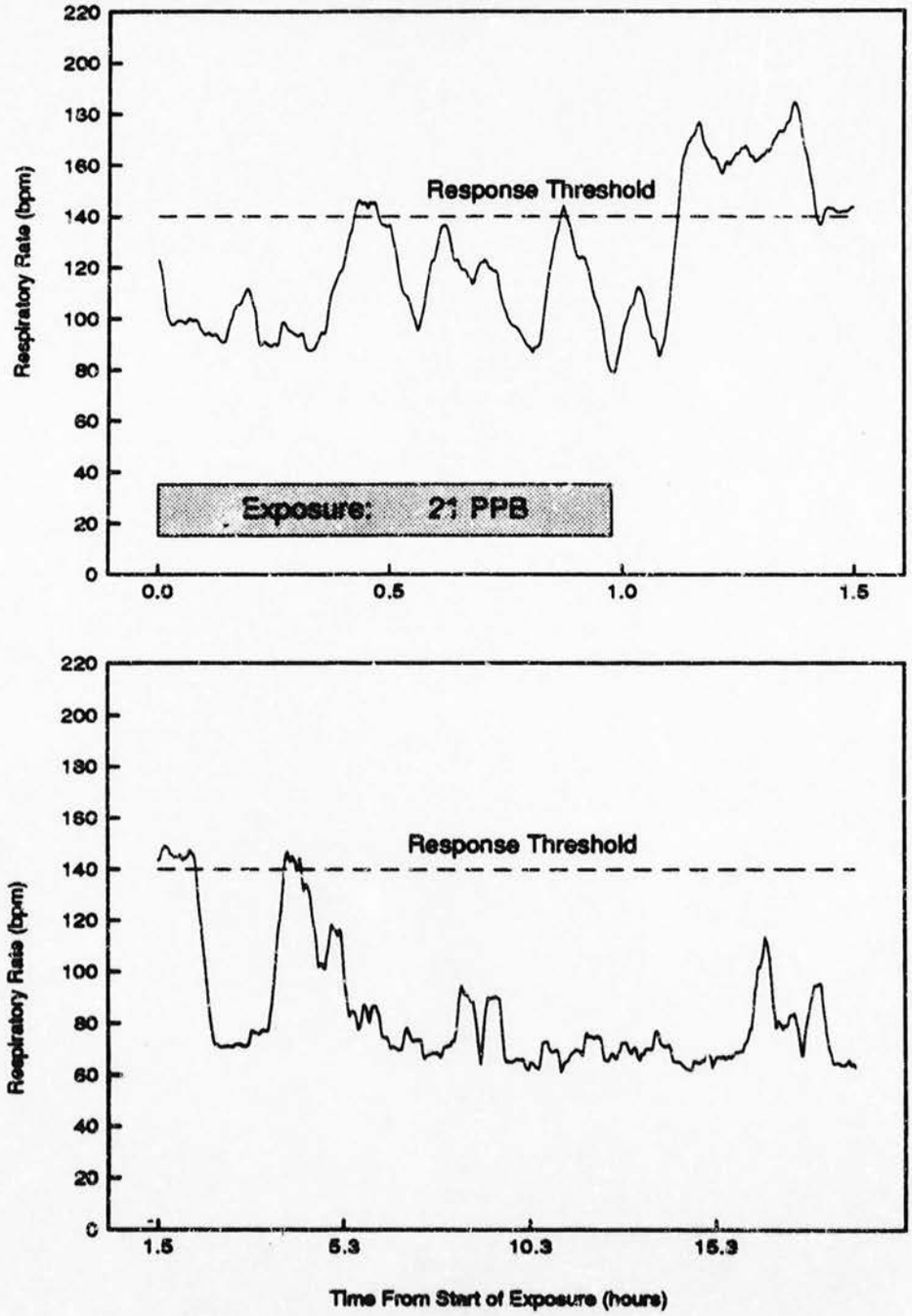
Figure: 44 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 230: Sham Sensitization

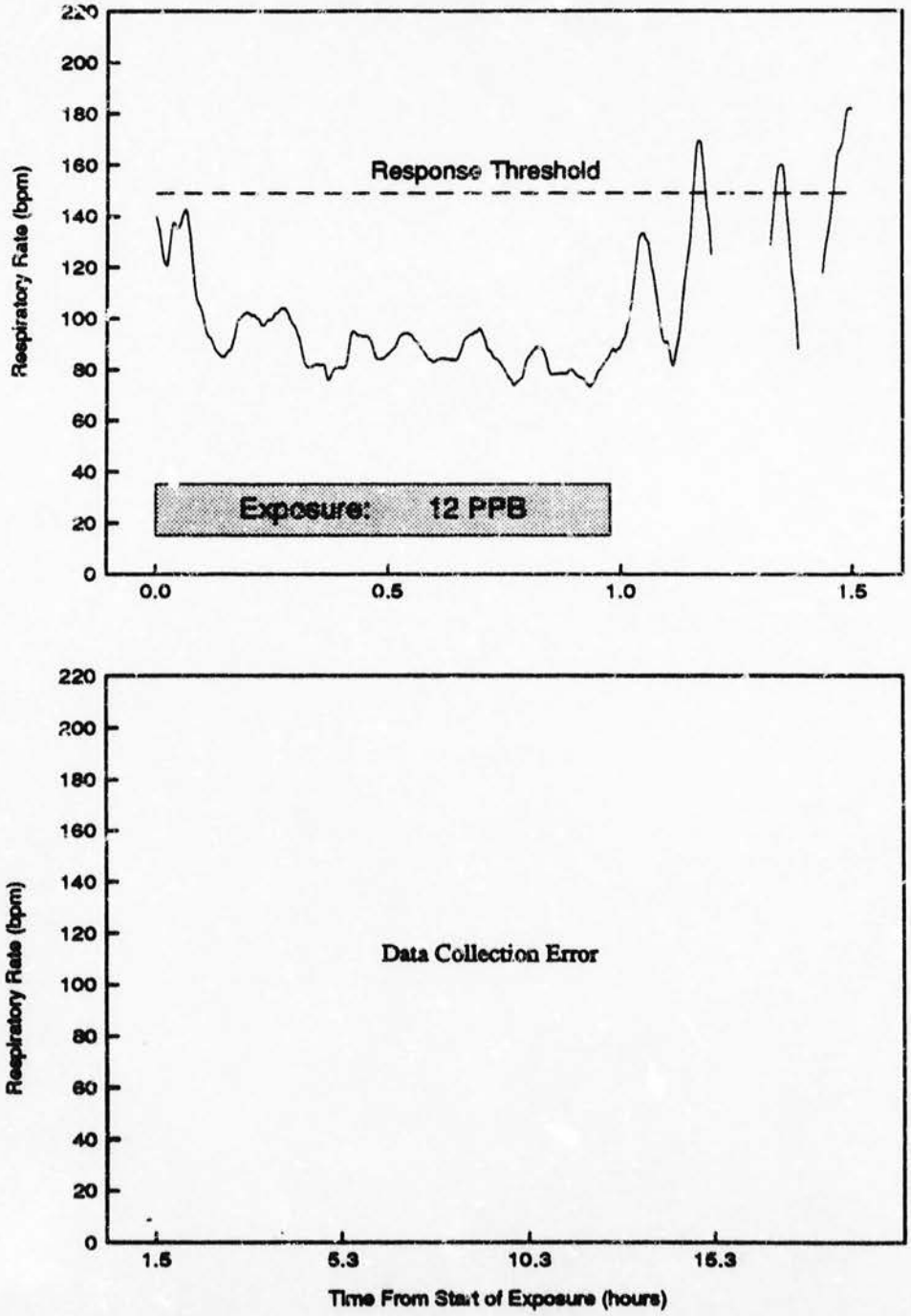
Figure: 45 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 231: Sham Sensitization

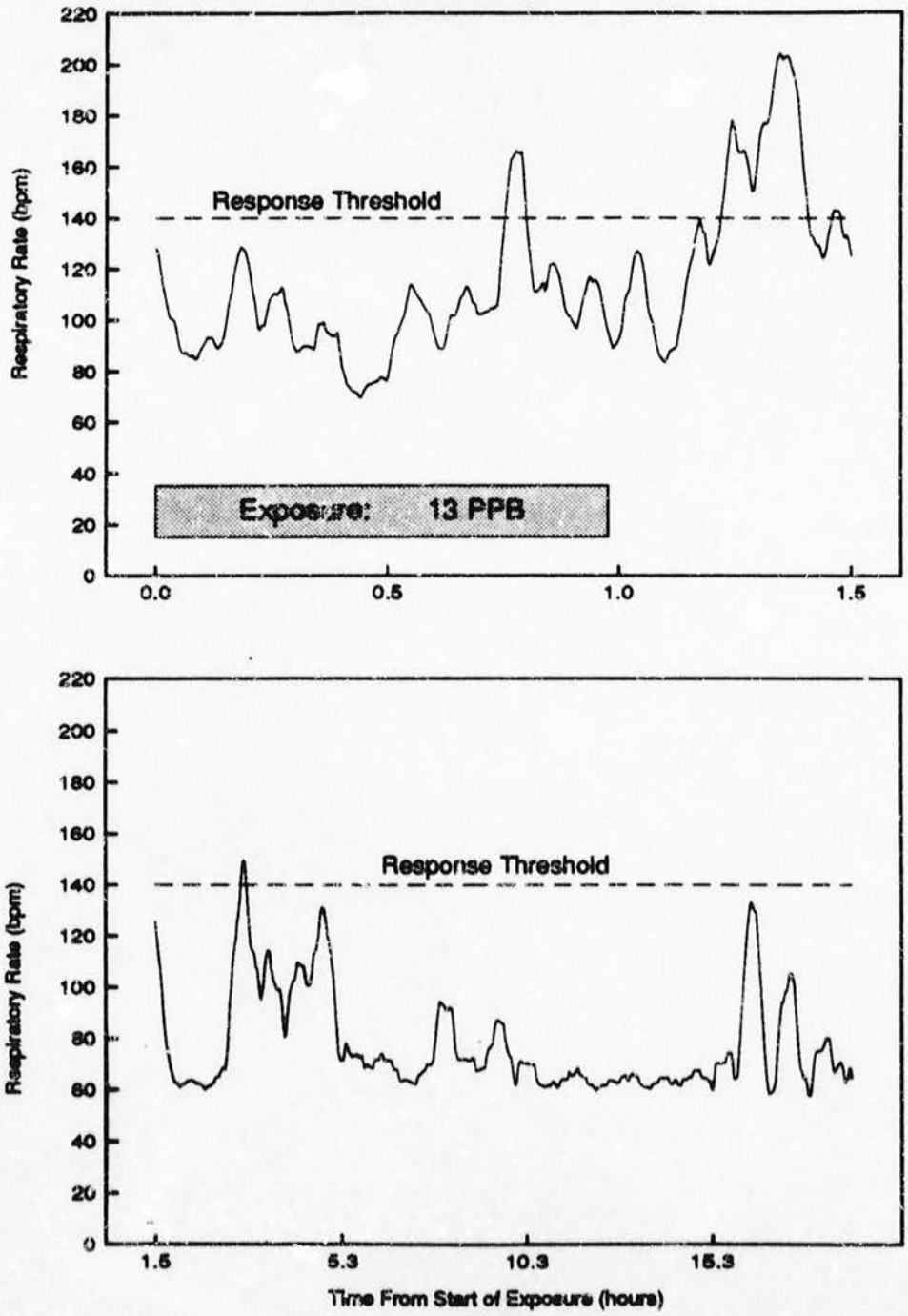
Figure: 46 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 232: Sham Sensitization

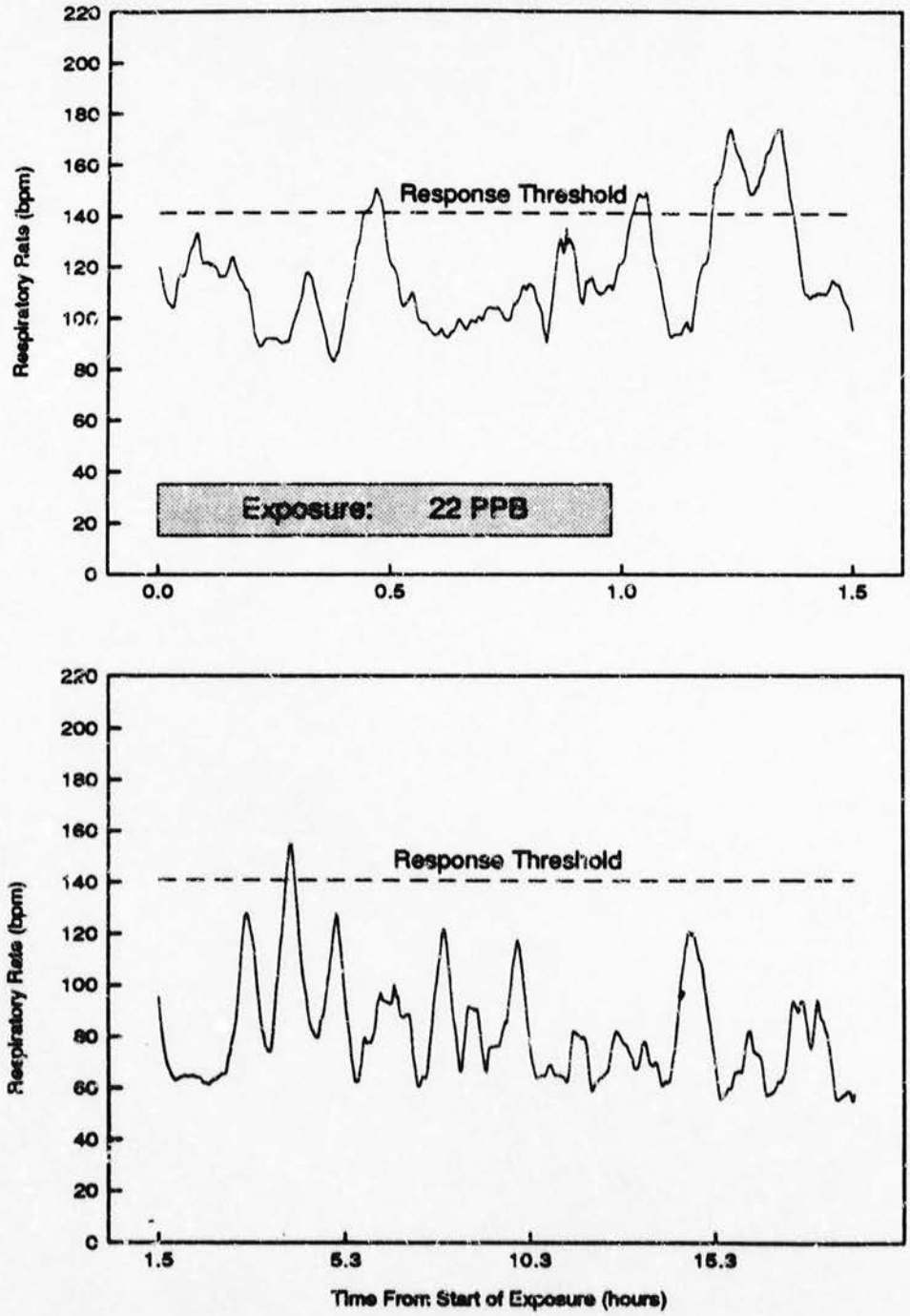
Figure: 47 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 233: Sham Sensitization

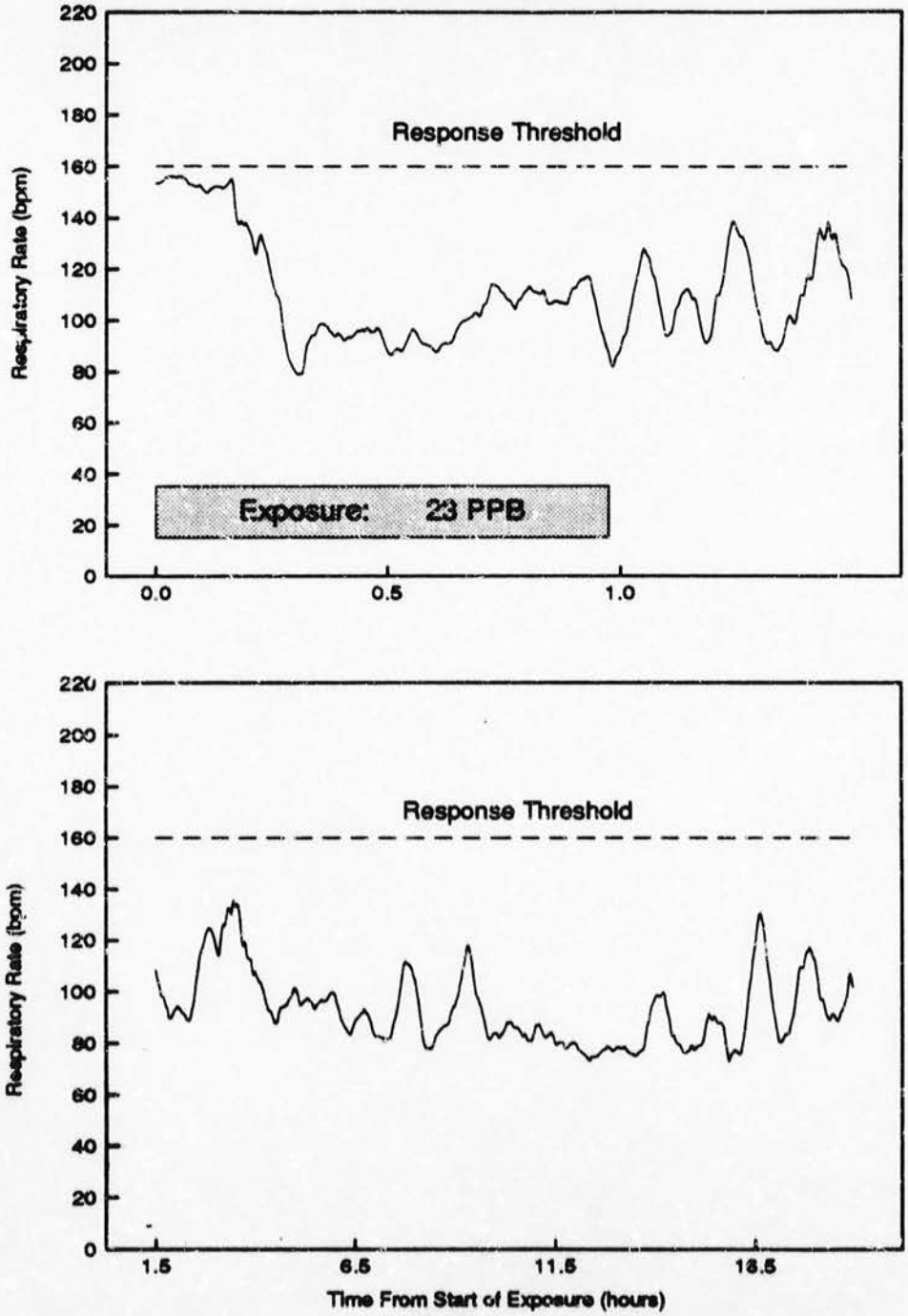
Figure: 48 2,4-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 222: Sham Sensitization

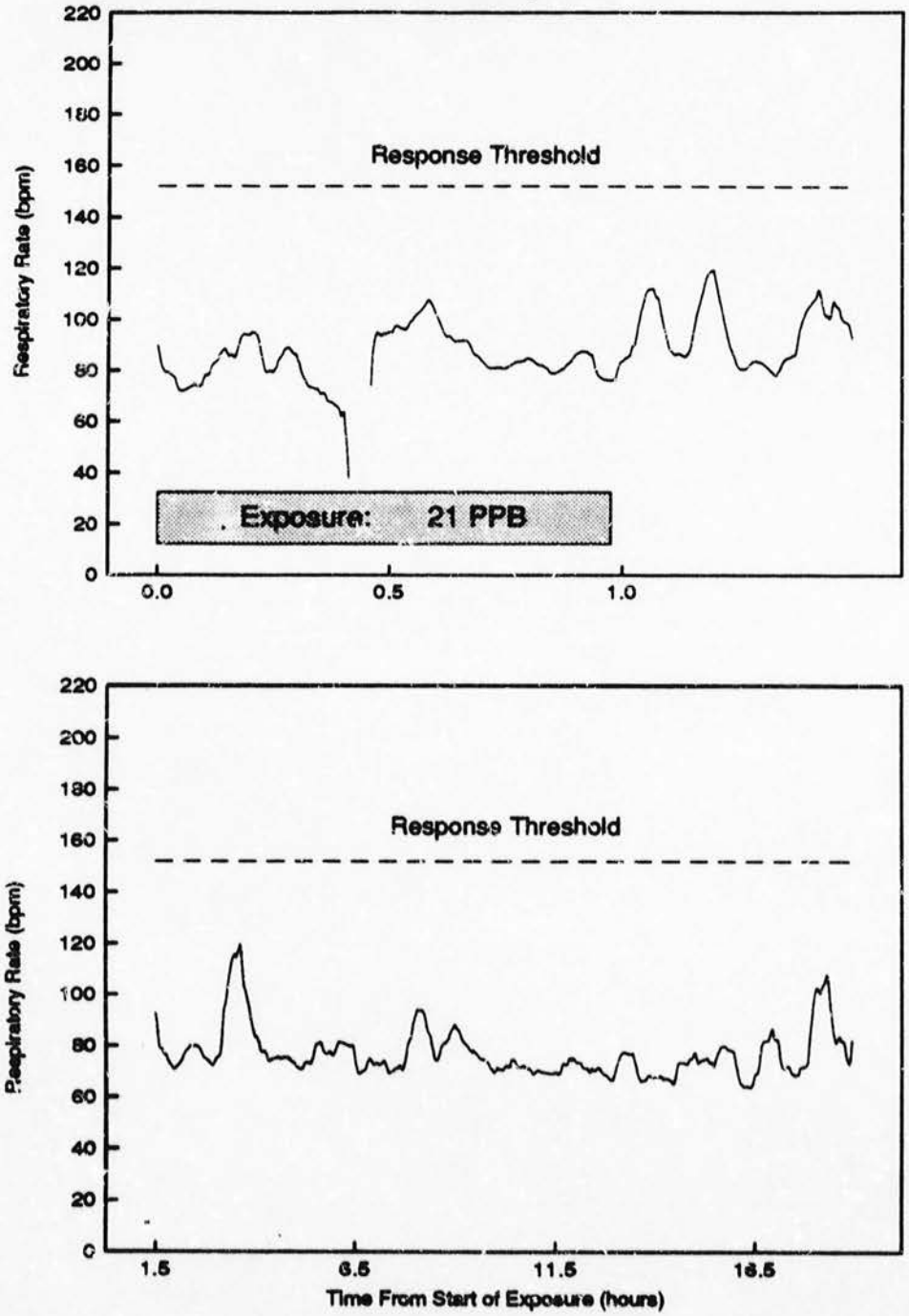
Figure: 49 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 223: Sham Sensitization

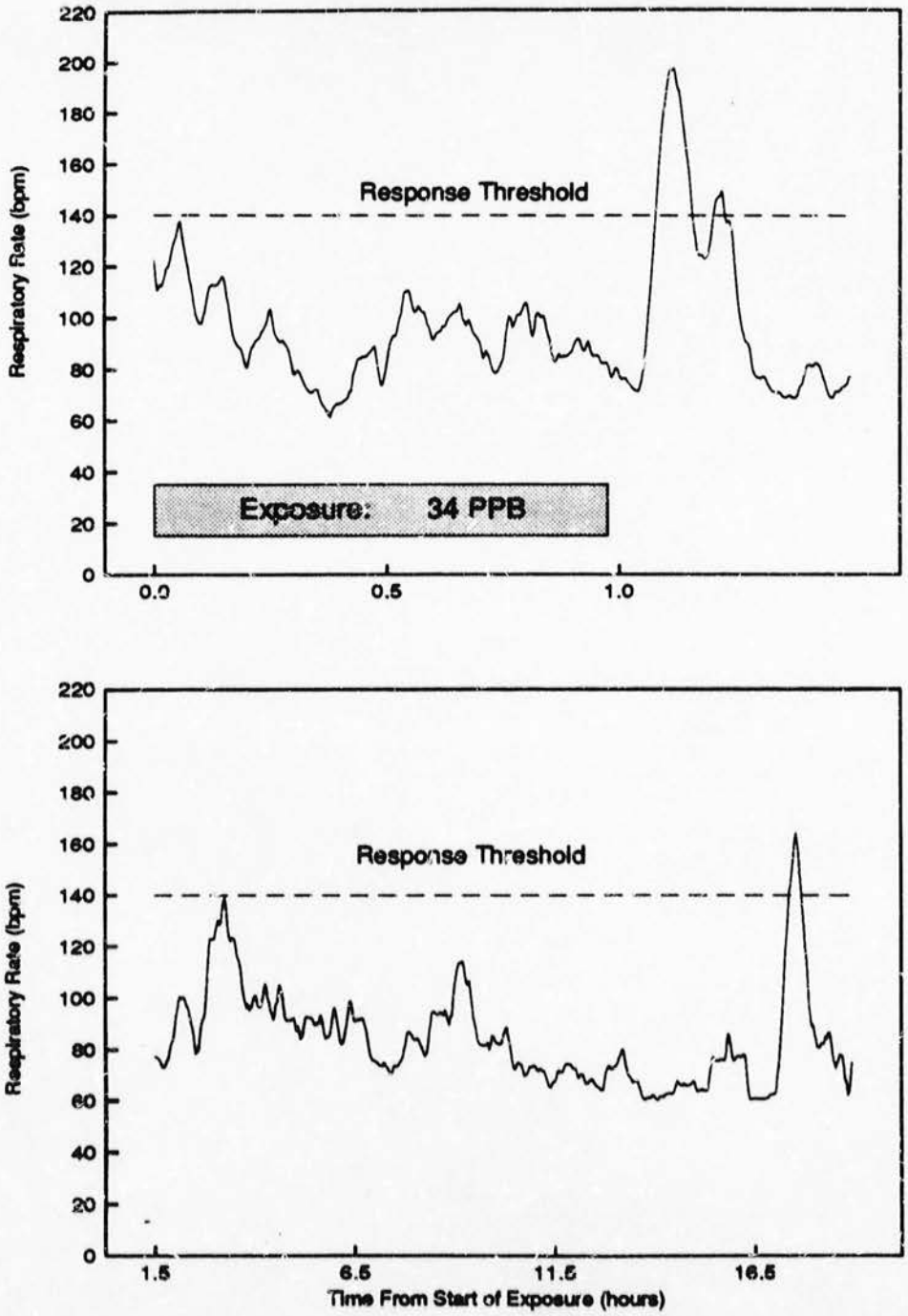
Figure: 50 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 224: Sham Sensitization

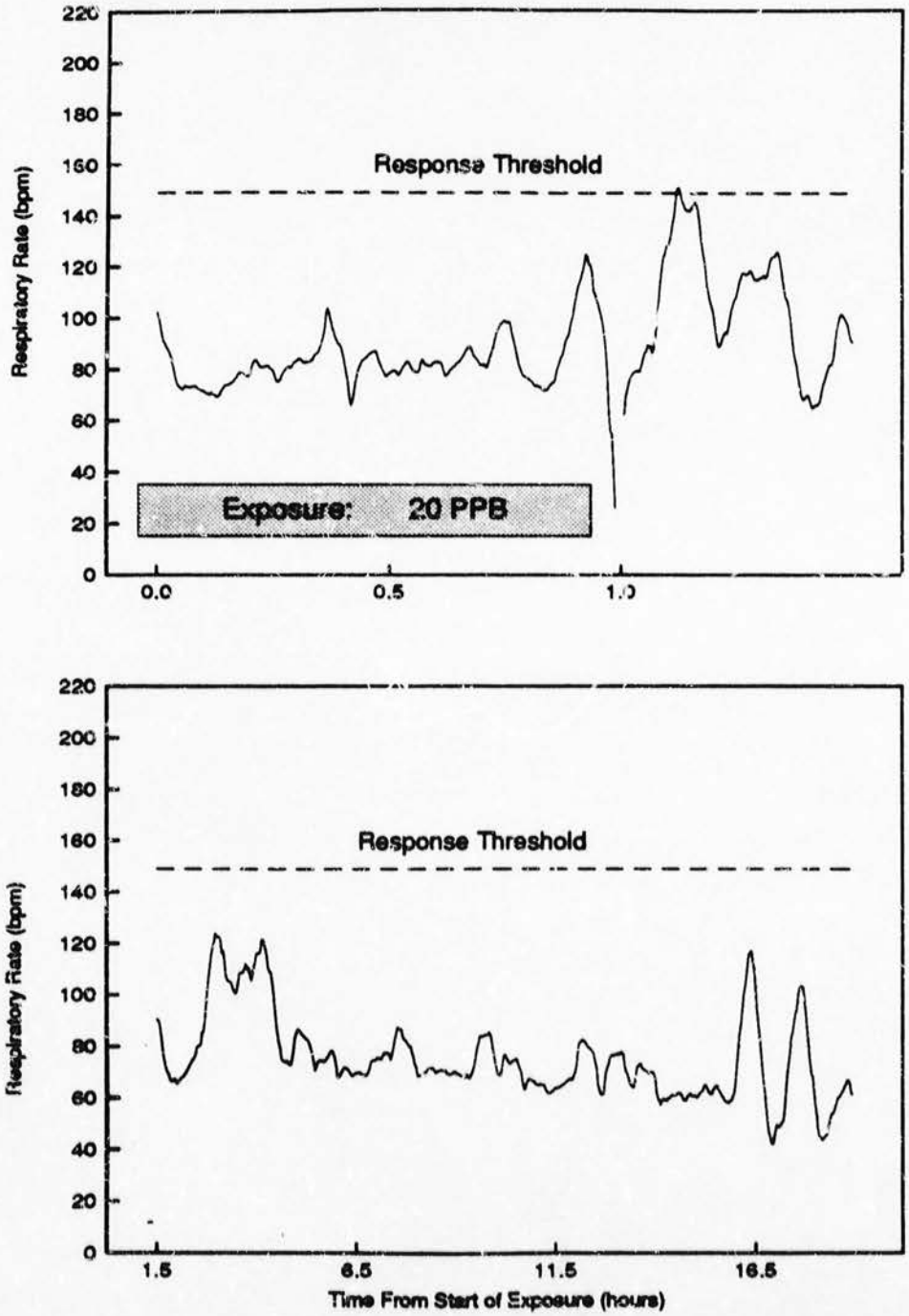
Figure: 51 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 225: Sham Sensitization

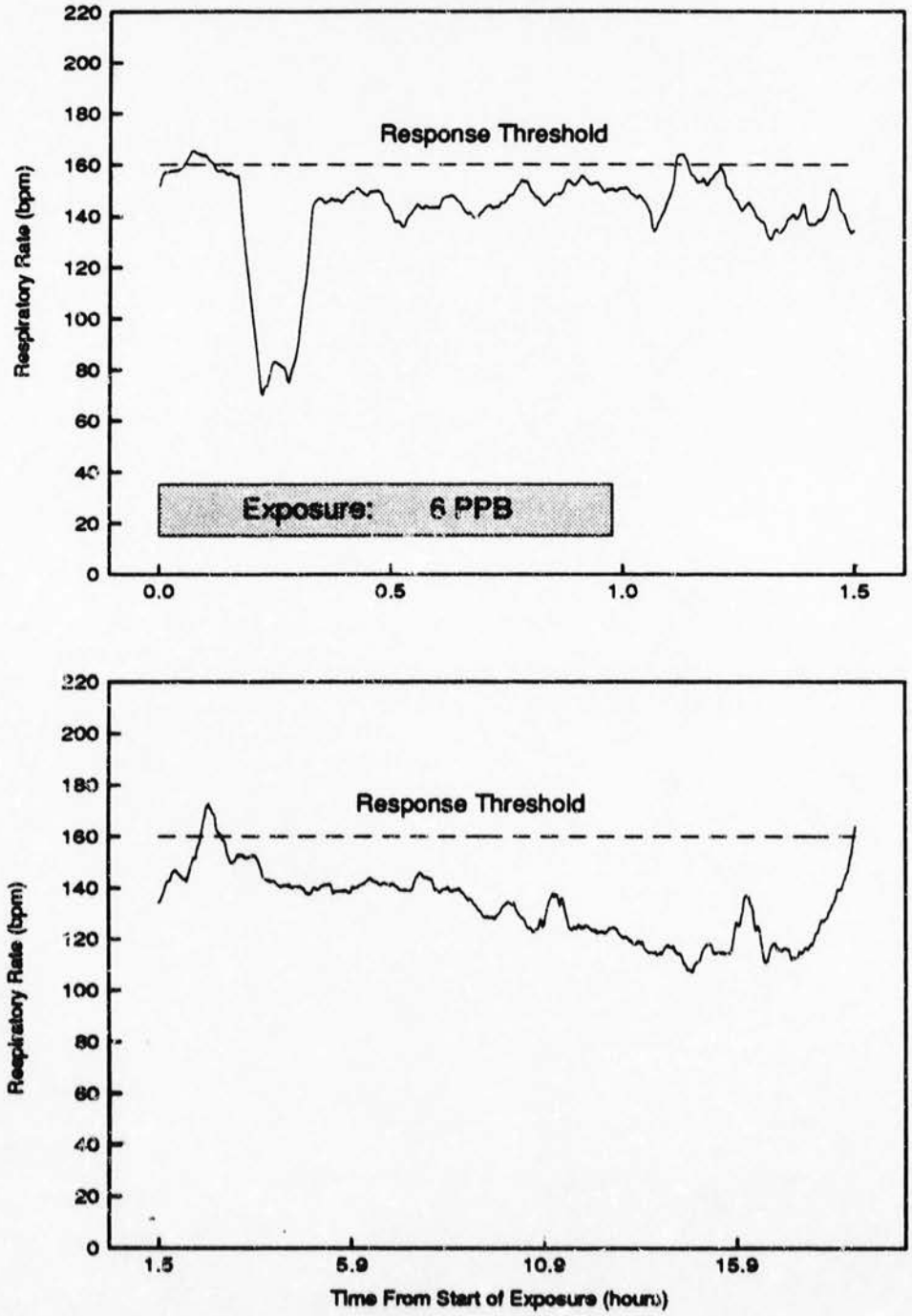
Figure: 52 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 226: Sham Sensitization

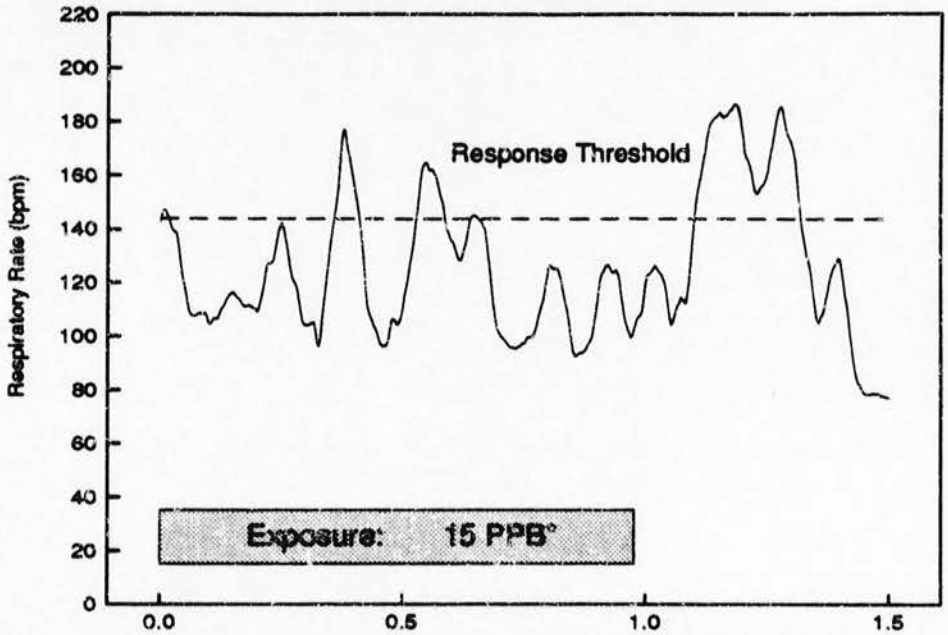
Figure: 53 2,4-TDI Challenge Week 2



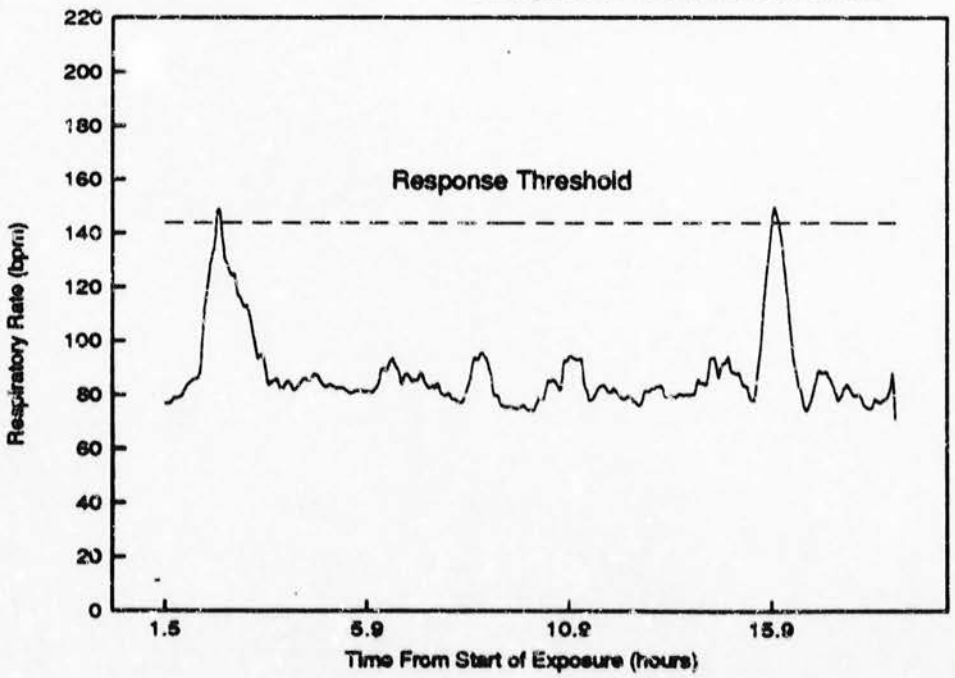
Study Number 92-344-PP

Animal # 227: Sham Sensitization

Figure: 54 2,4-TDI Challenge Week 2



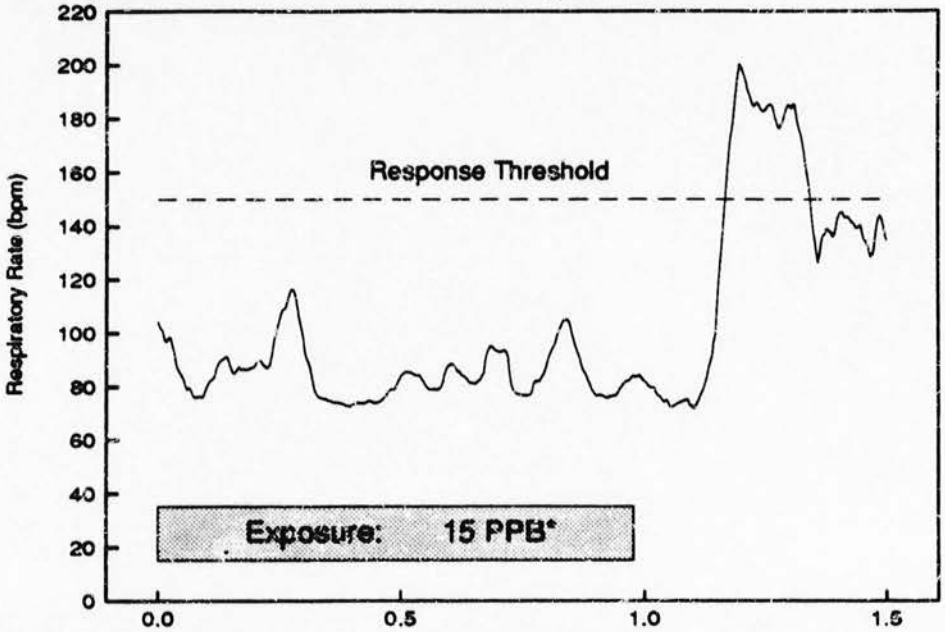
* Data Collection Error - Daily Average Used To Represent Individual Concentration



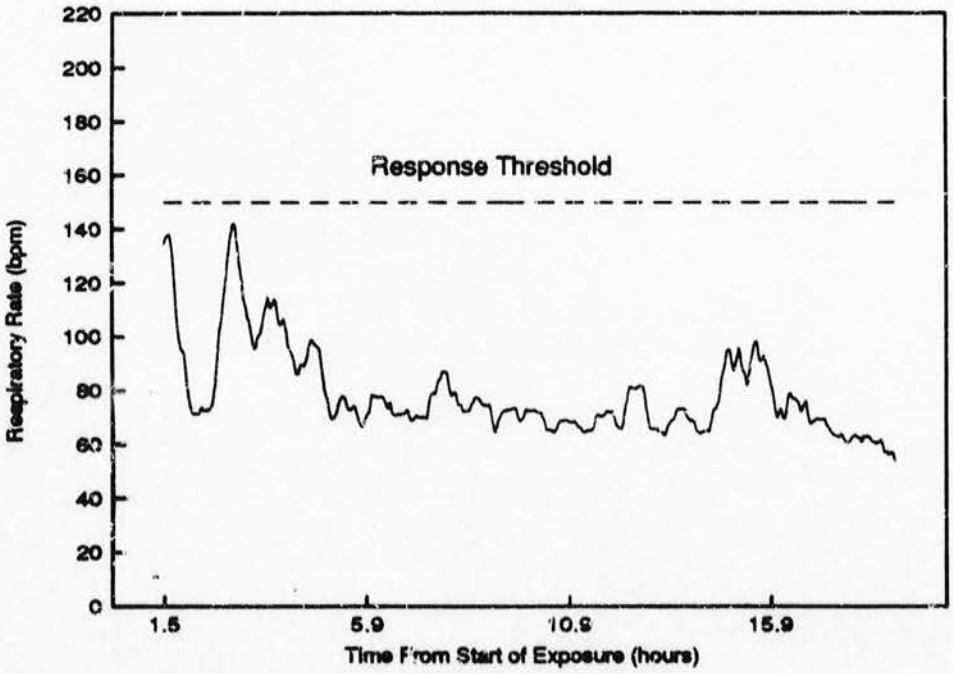
Study Number 92-344-PP

Animal # 228: Sham Sensitization

Figure: 55 2,4-TDI Challenge Week 2



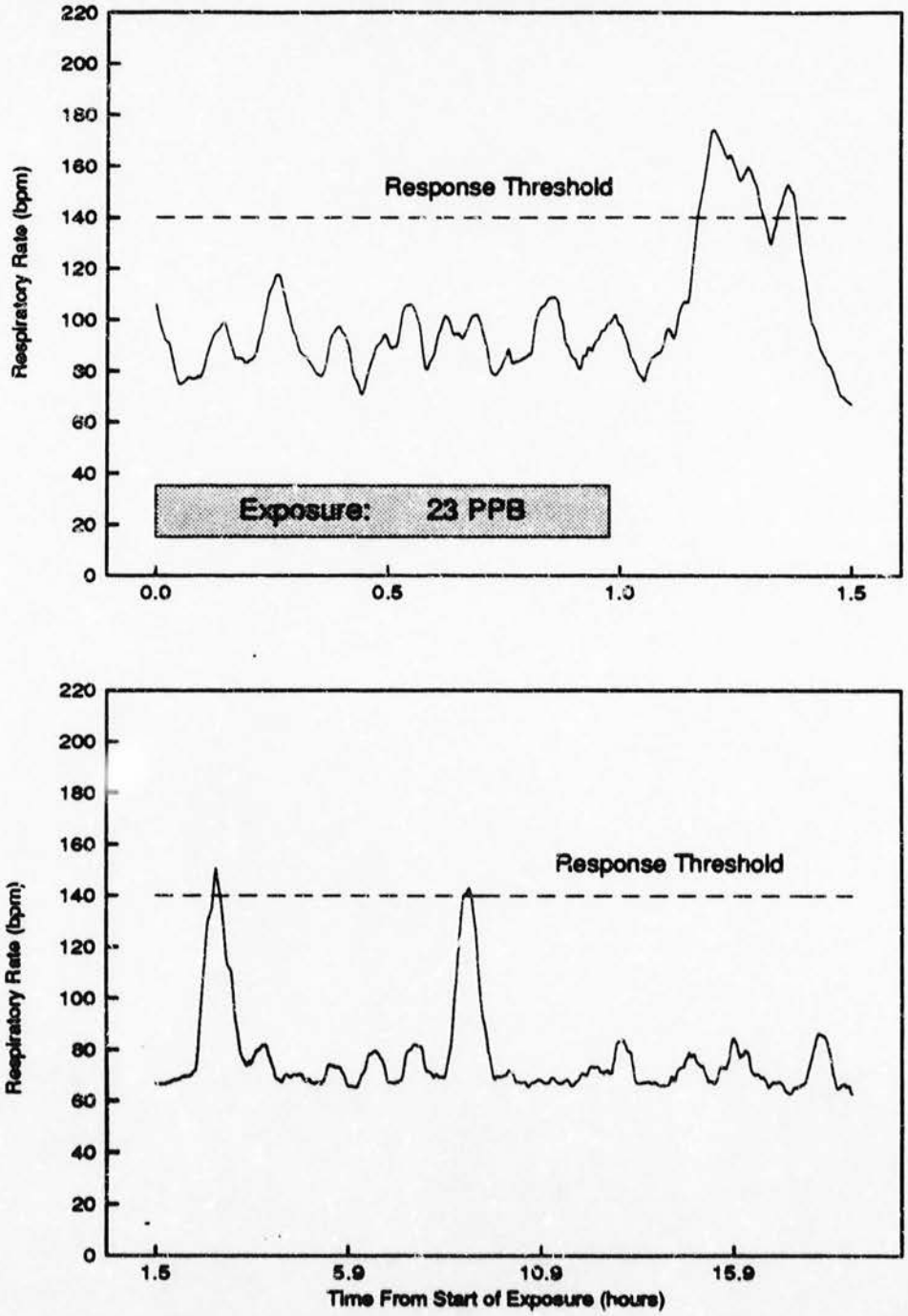
* Data Collection Error - Daily Average Used To Represent Individual Concentration



Study Number 92-344-PP

Animal # 229: Sham Sensitization

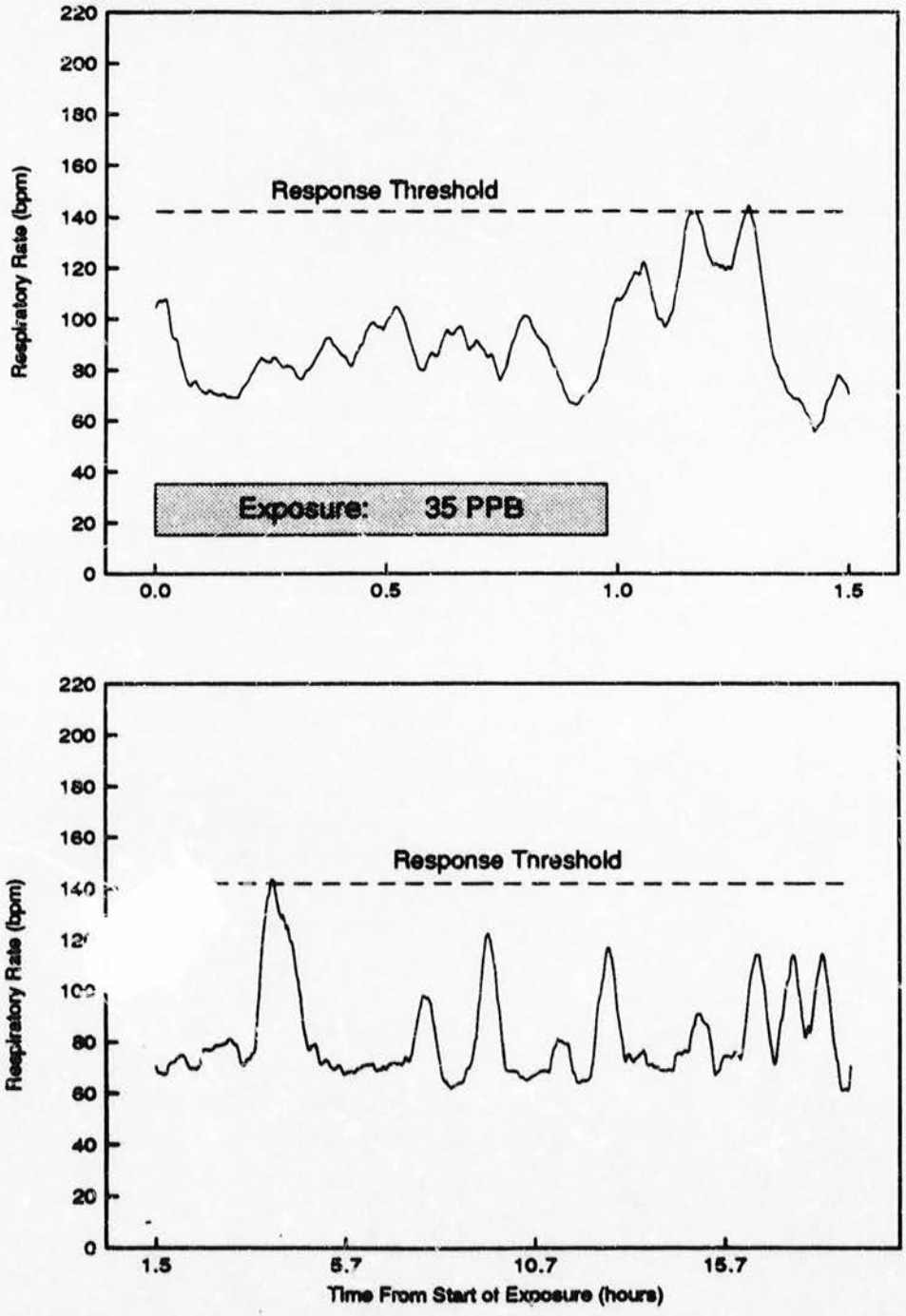
Figure: 56 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 230: Sham Sensitization

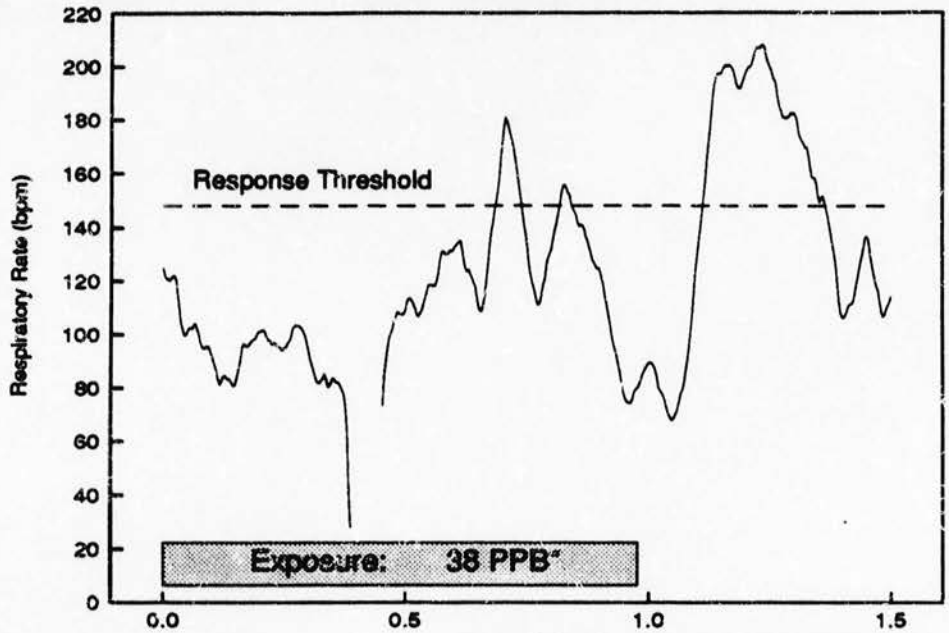
Figure: 57 2,4-TDI Challenge Week 2



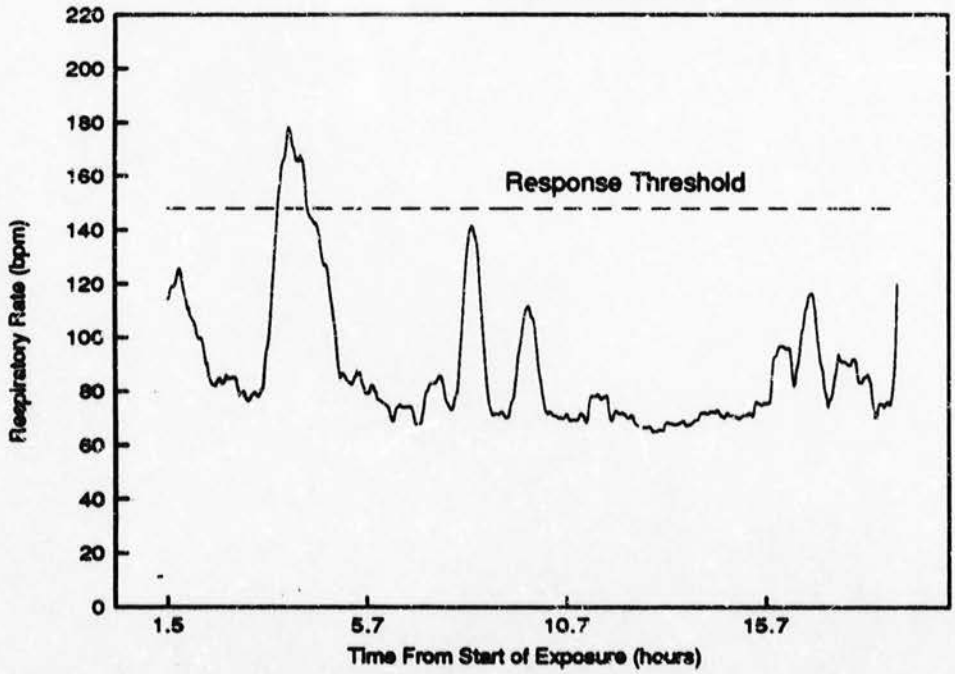
Study Number 92-344-PP

Animal # 231: Sham Sensitization

Figure: 58 2,4-TDI Challenge Week 2



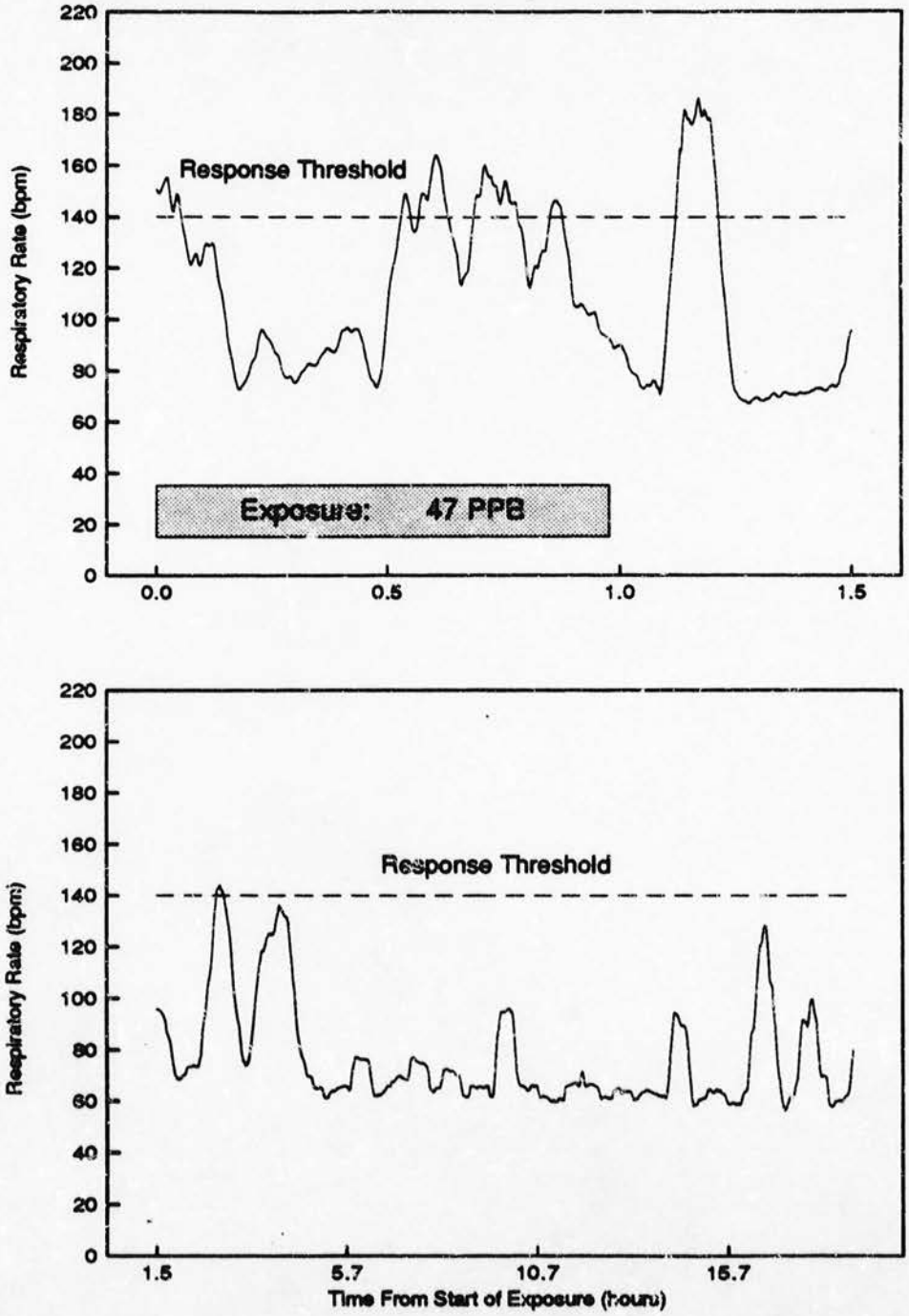
* Data Collection Error - Daily Average Used To Represent Individual Concentration



Study Number 92-344-PP

Animal # 232: Sham Sensitization

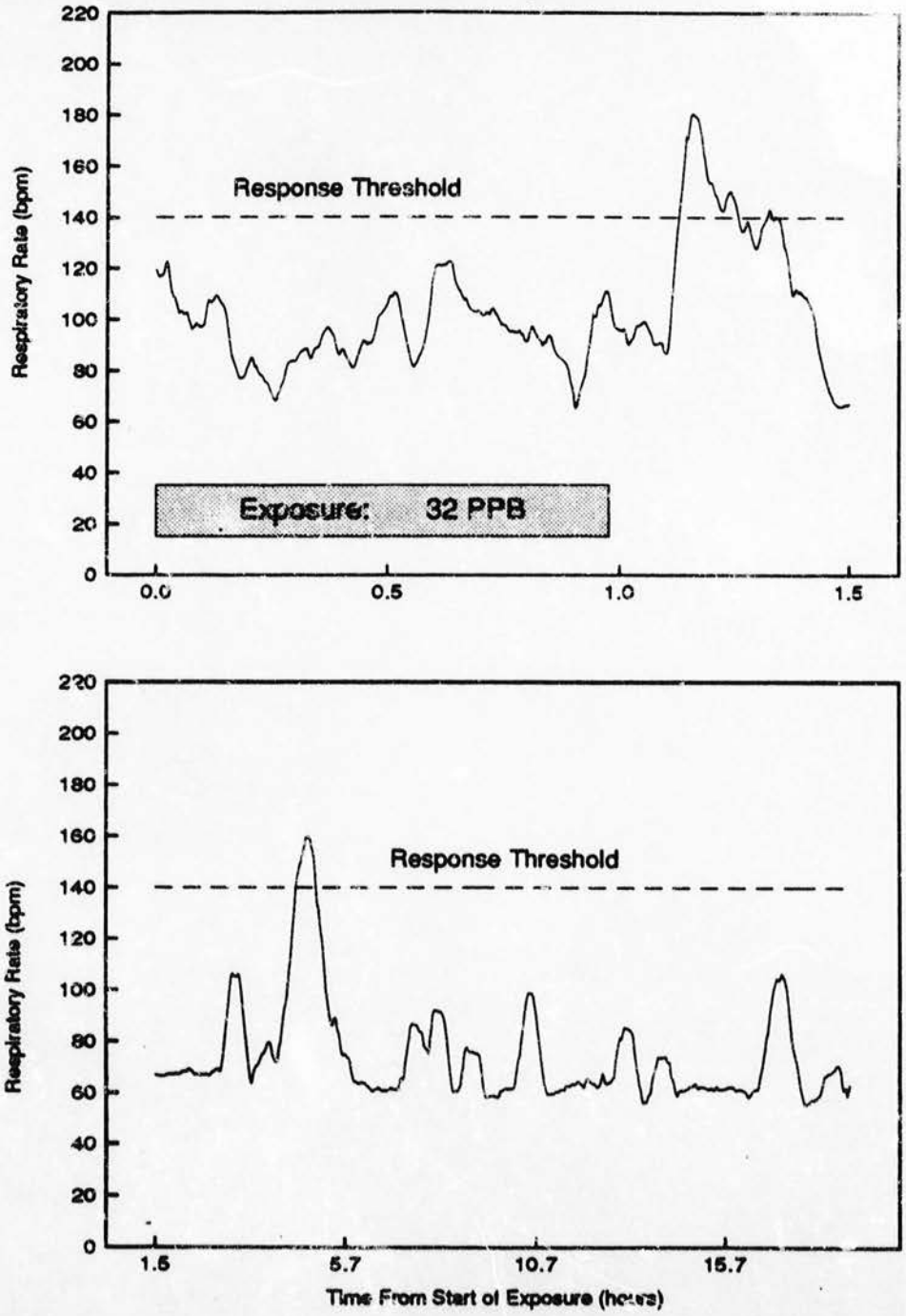
Figure: 59 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 233: Sham Sensitization

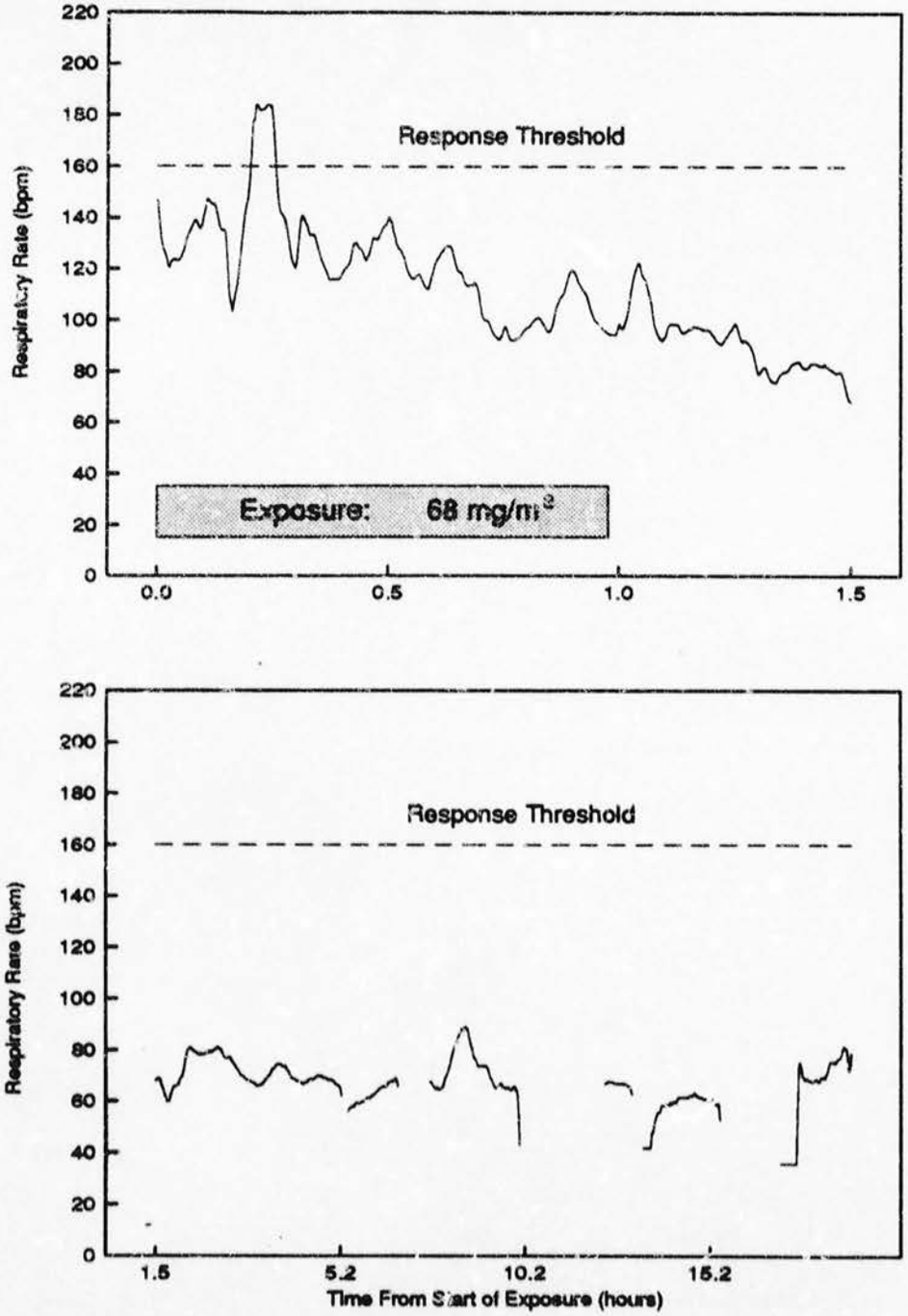
Figure: 60 2,4-TDI Challenge Week 2



Study Number 92-344-PP

Animal # 222: Sham Sensitization

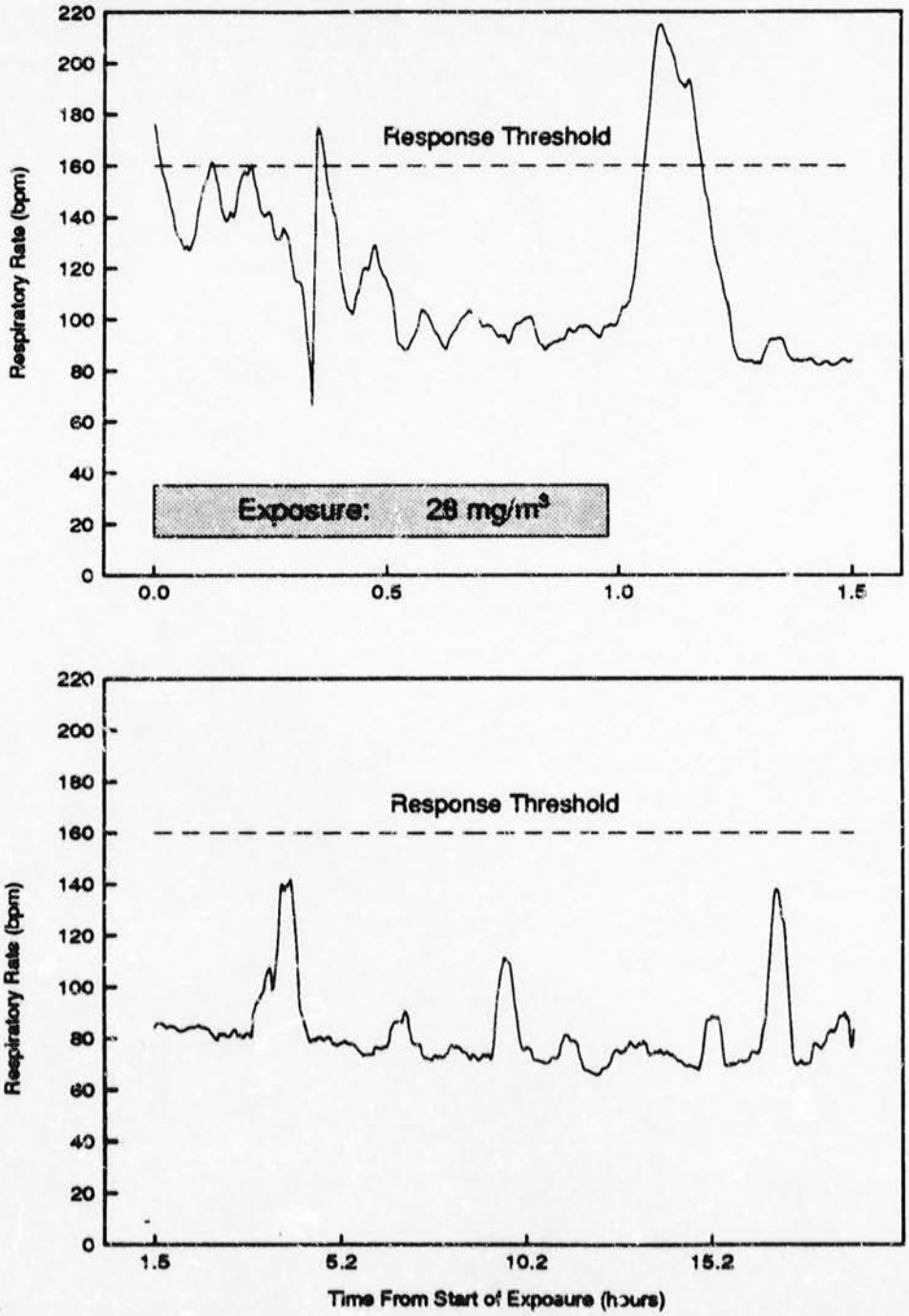
Figure: 61 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 223: Sham Sensitization

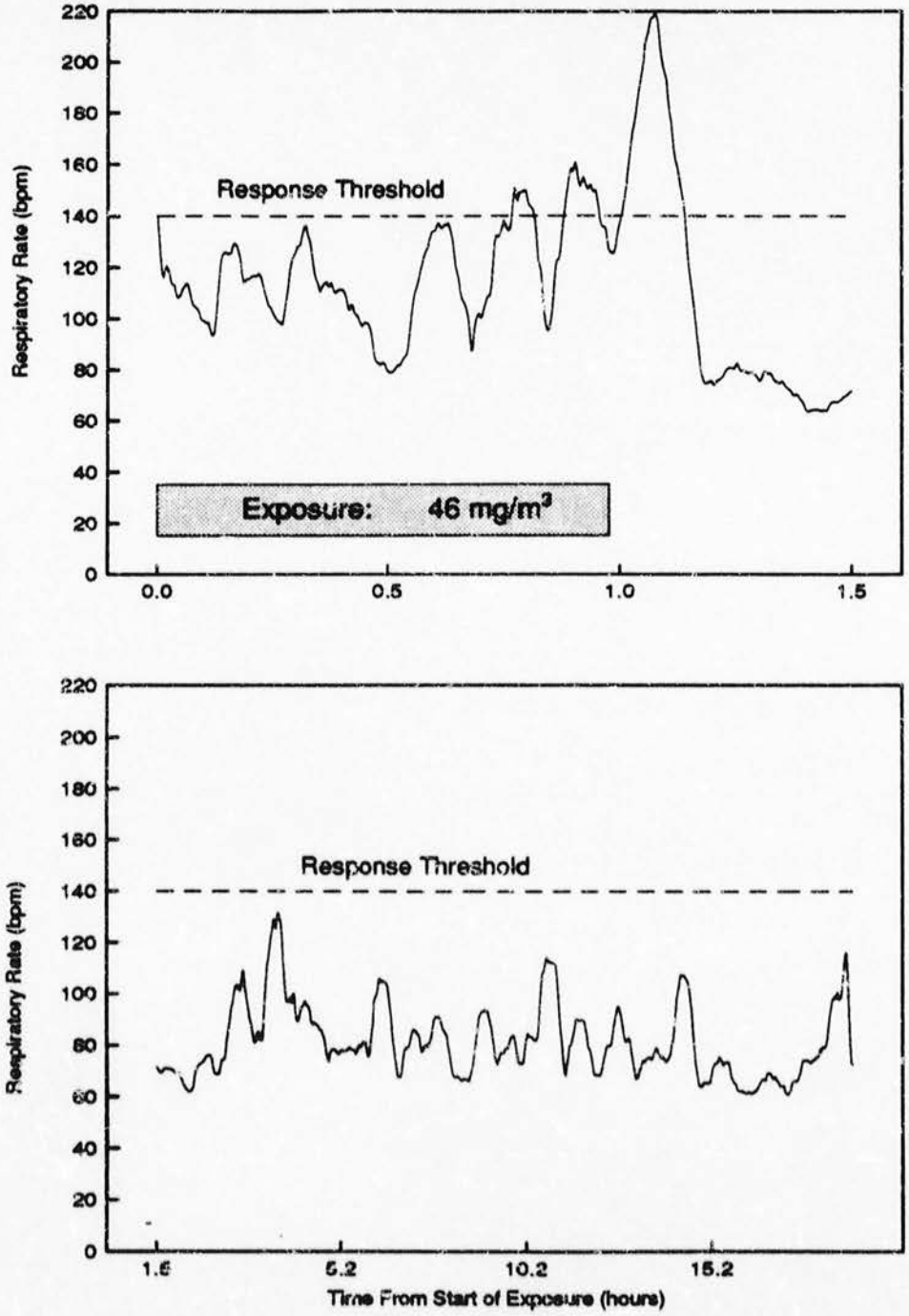
Figure: 62 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 224: Sham Sensitization

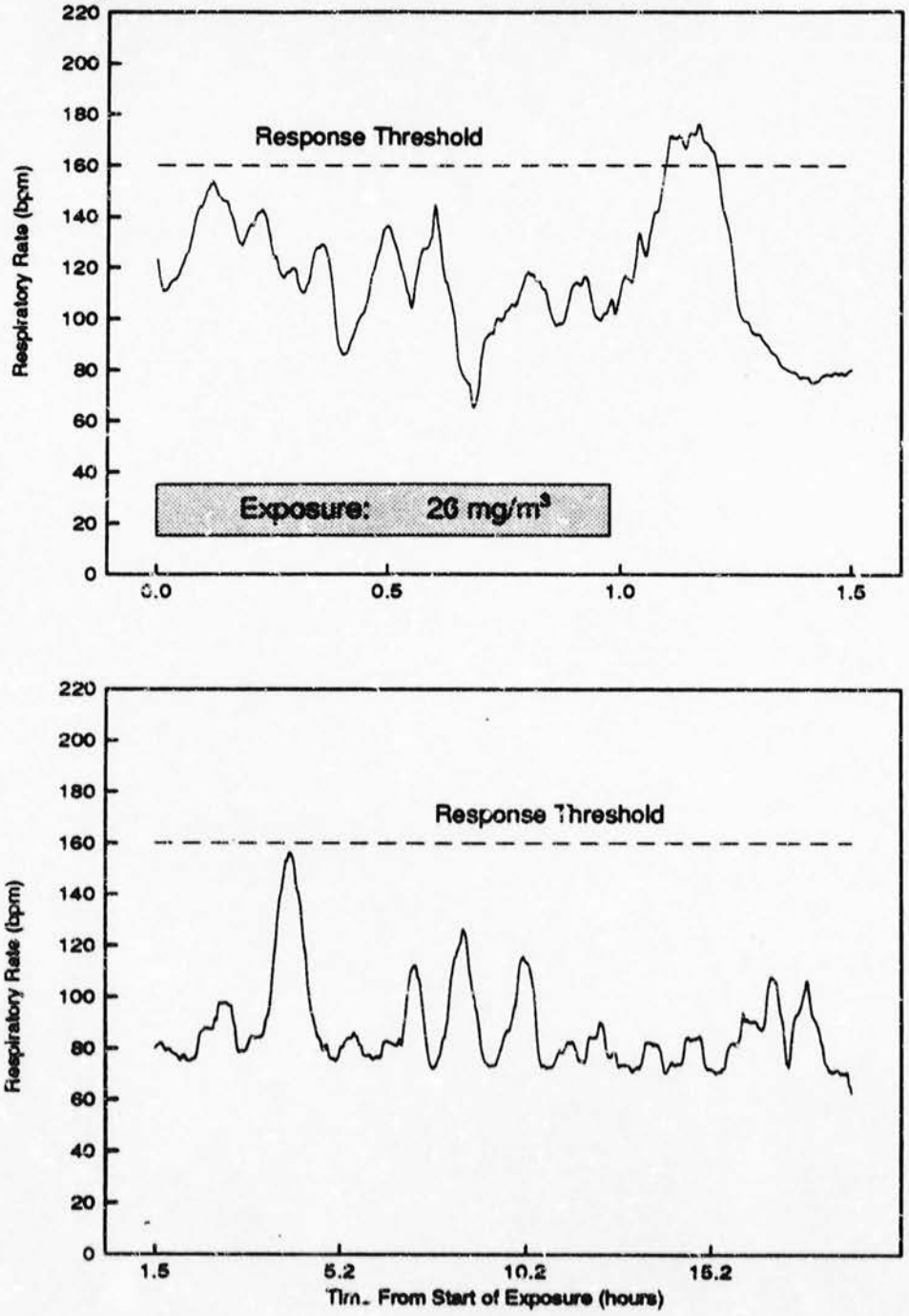
Figure: 63 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 225: Sham Sensitization

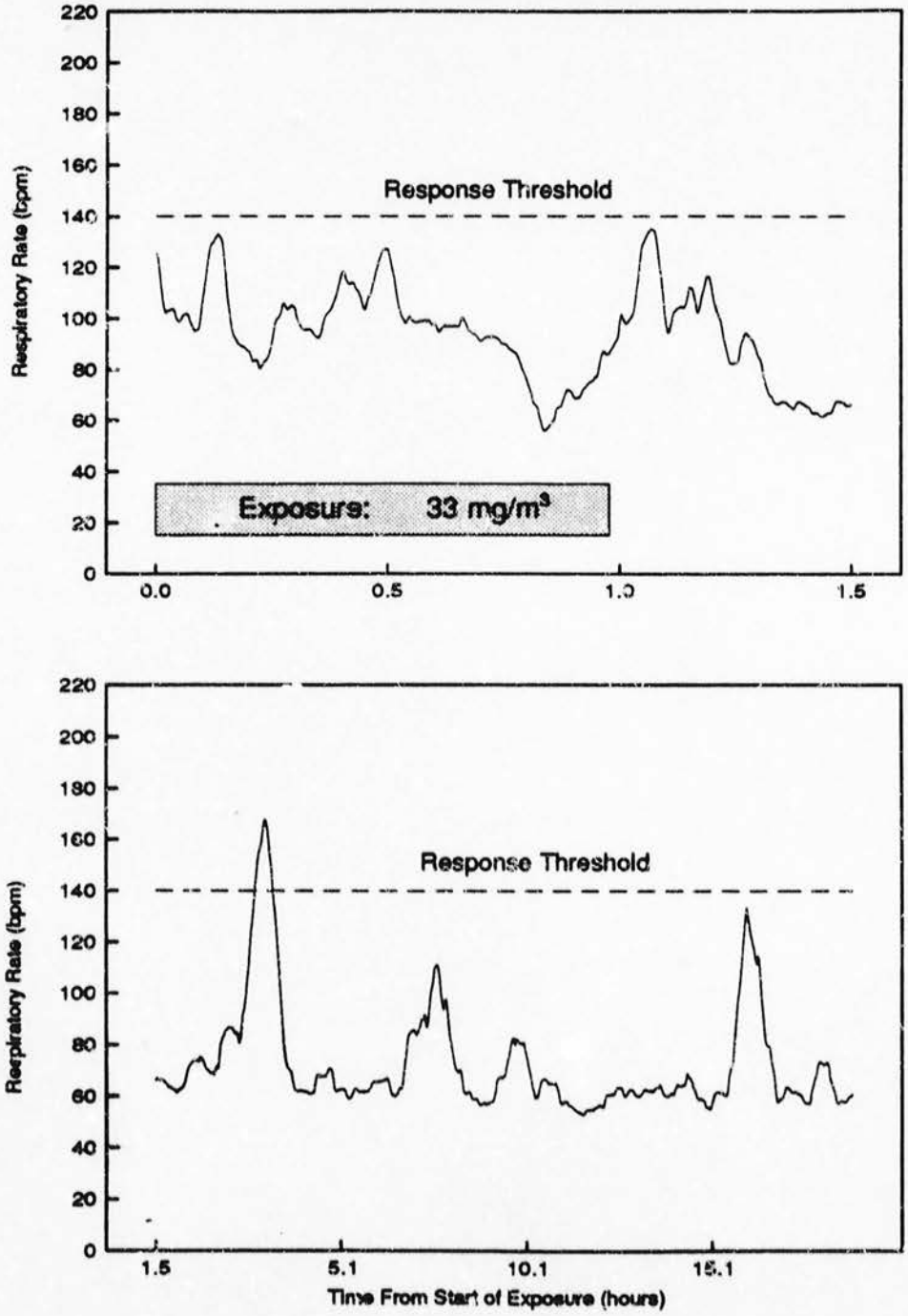
Figure: 64 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 226: Sham Sensitization

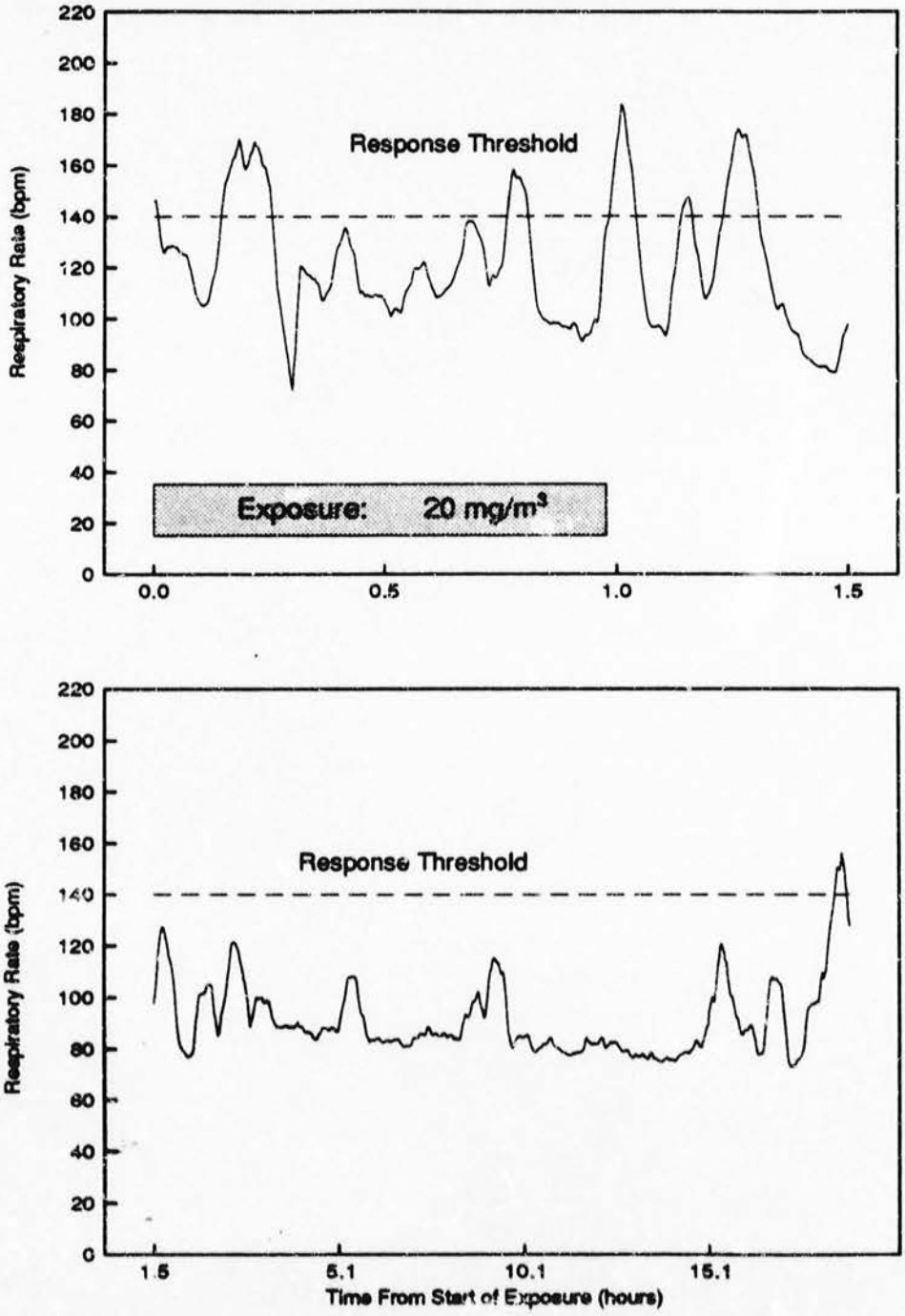
Figure: 65 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 227: Sham Sensitization

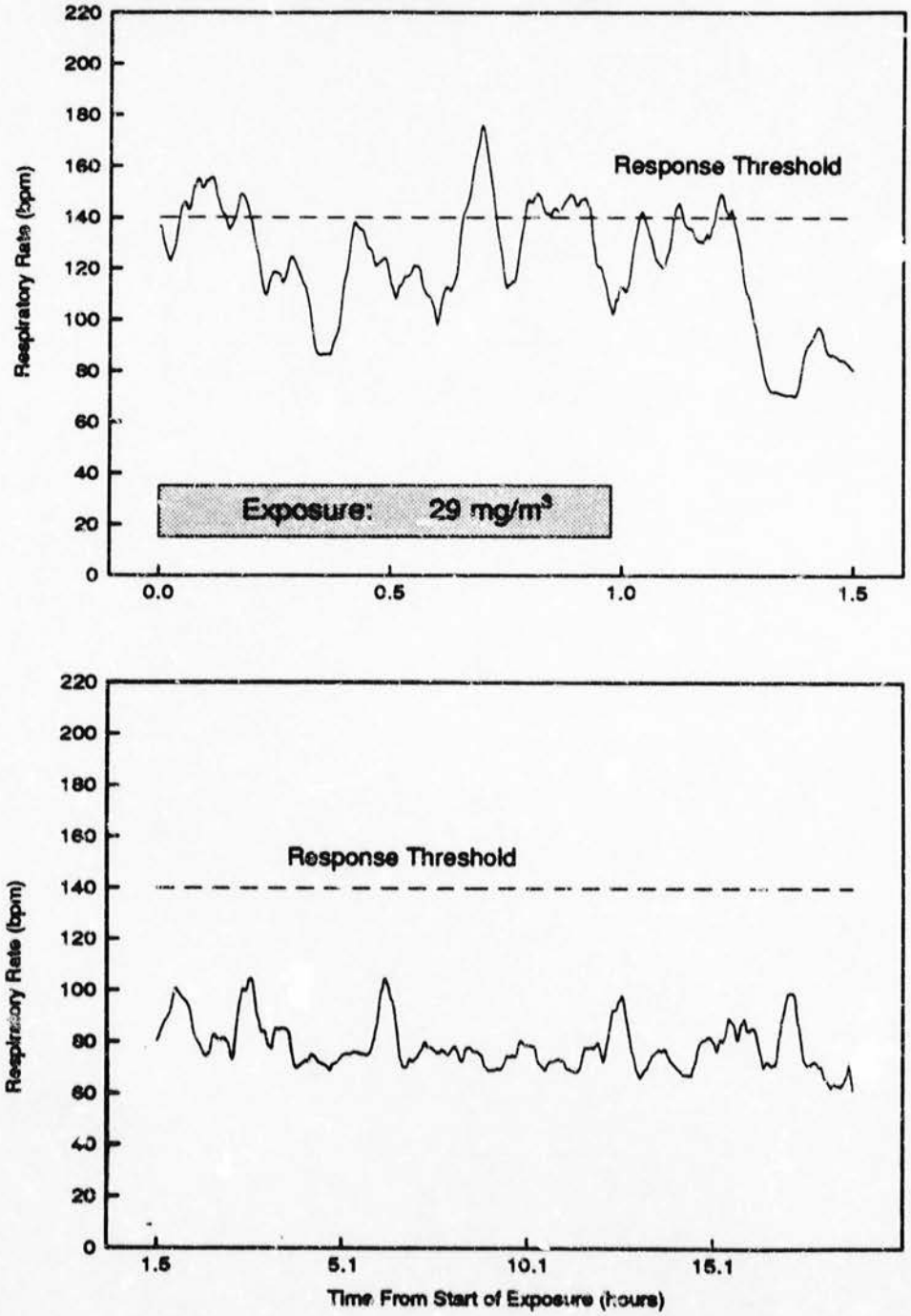
Figure: 66 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 228: Sham Sensitization

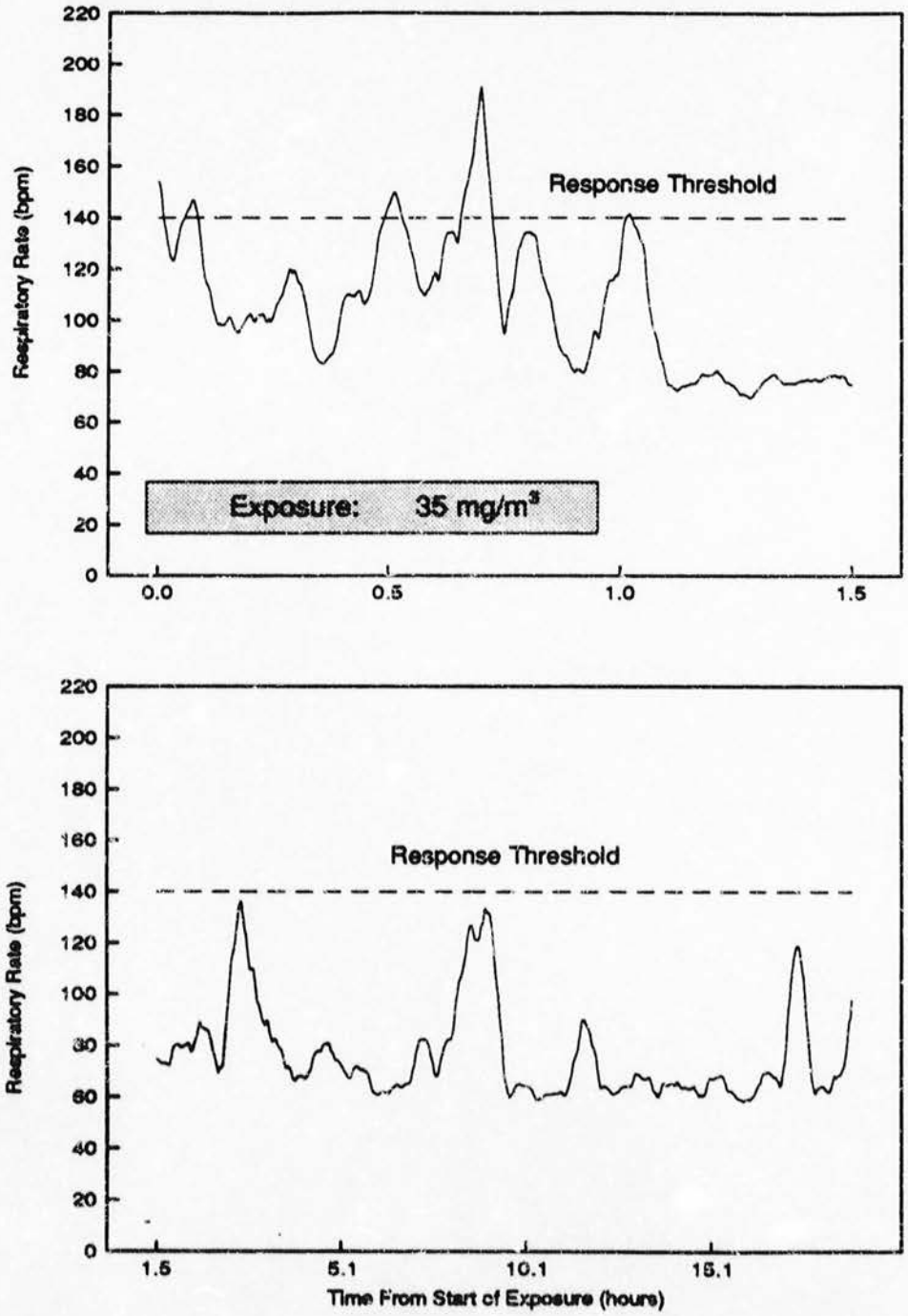
Figure: 67 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 229: Sham Sensitization

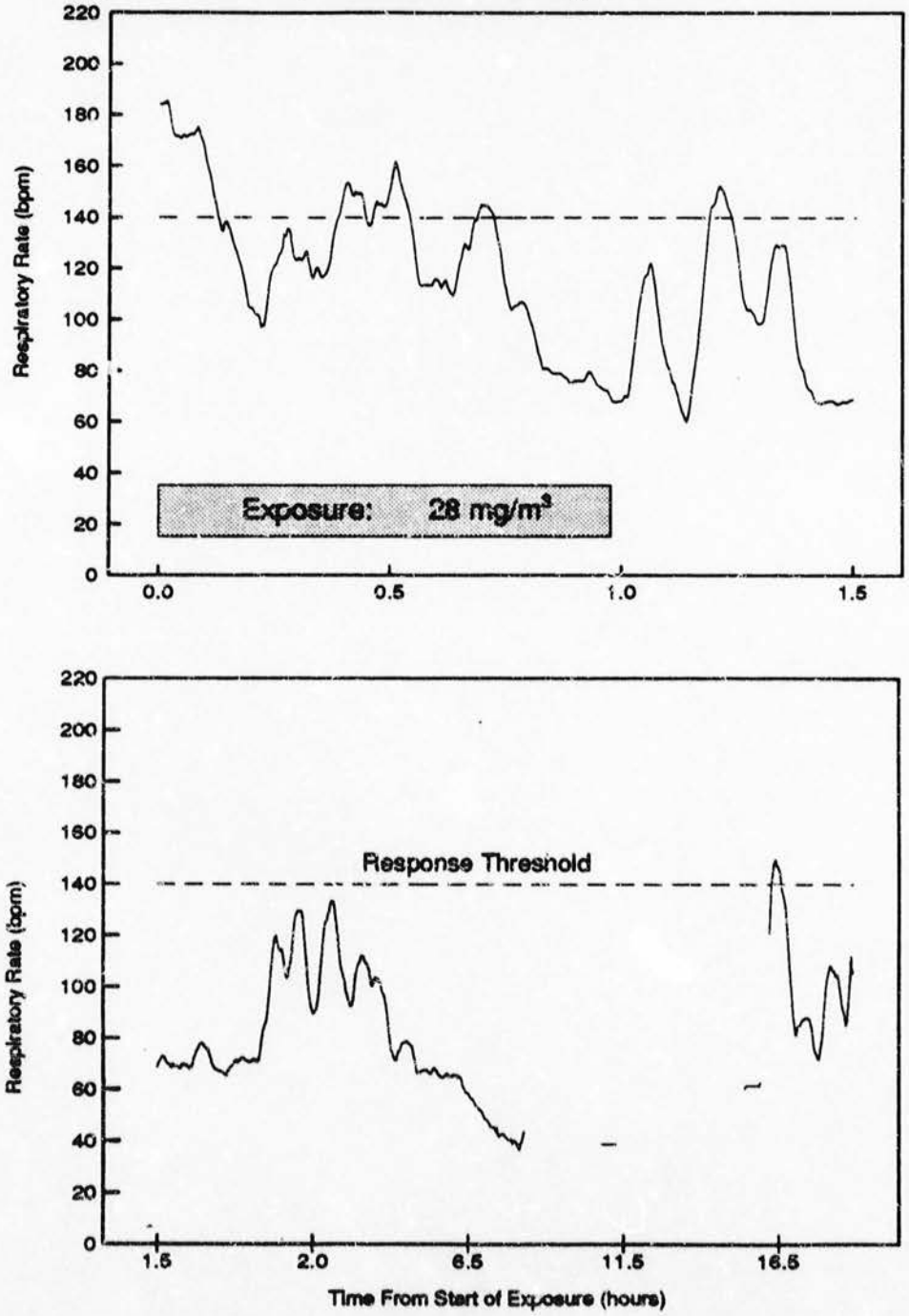
Figure: 68 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 230: Sham Sensitization

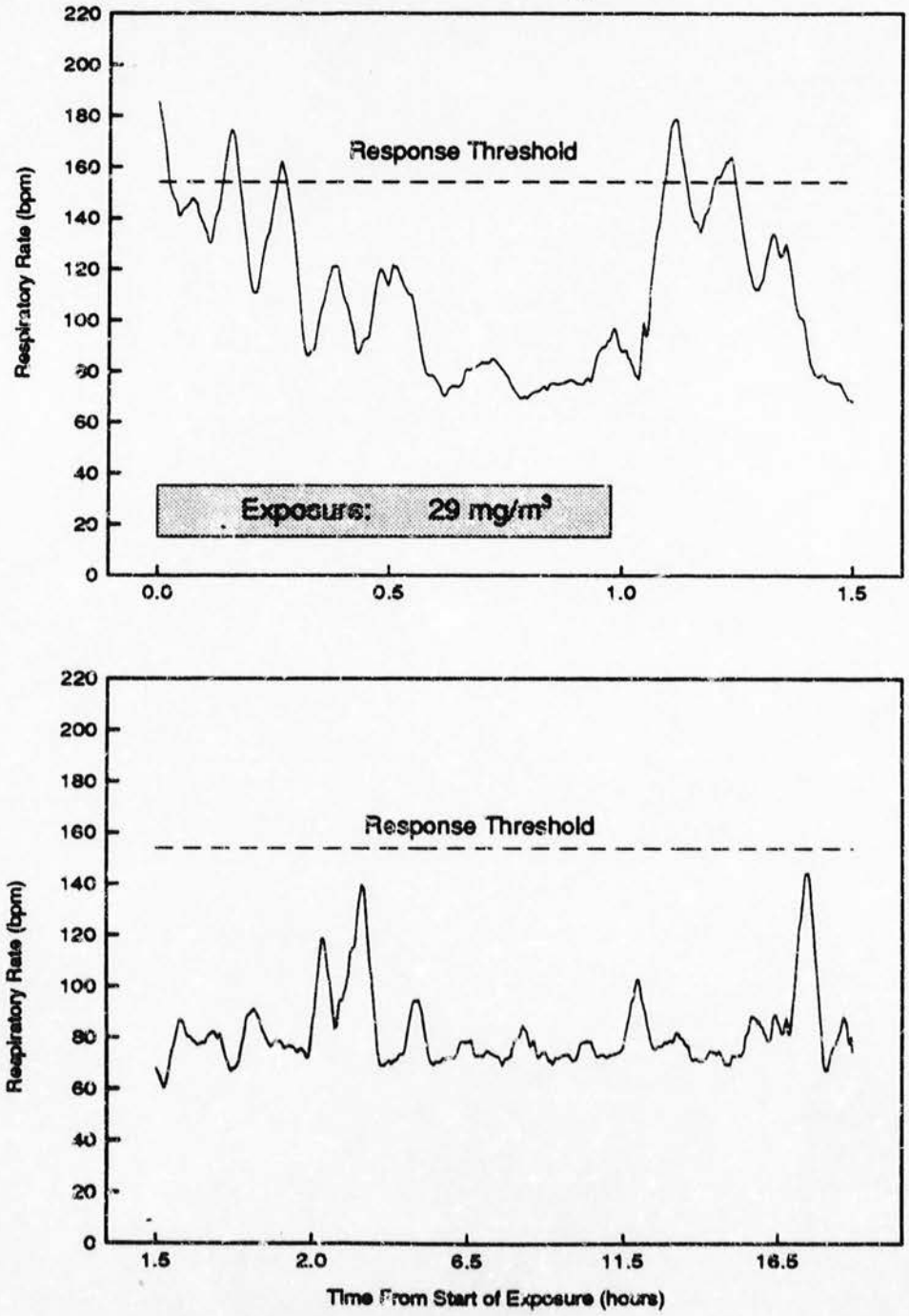
Figure: 69 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 231: Sham Sensitization

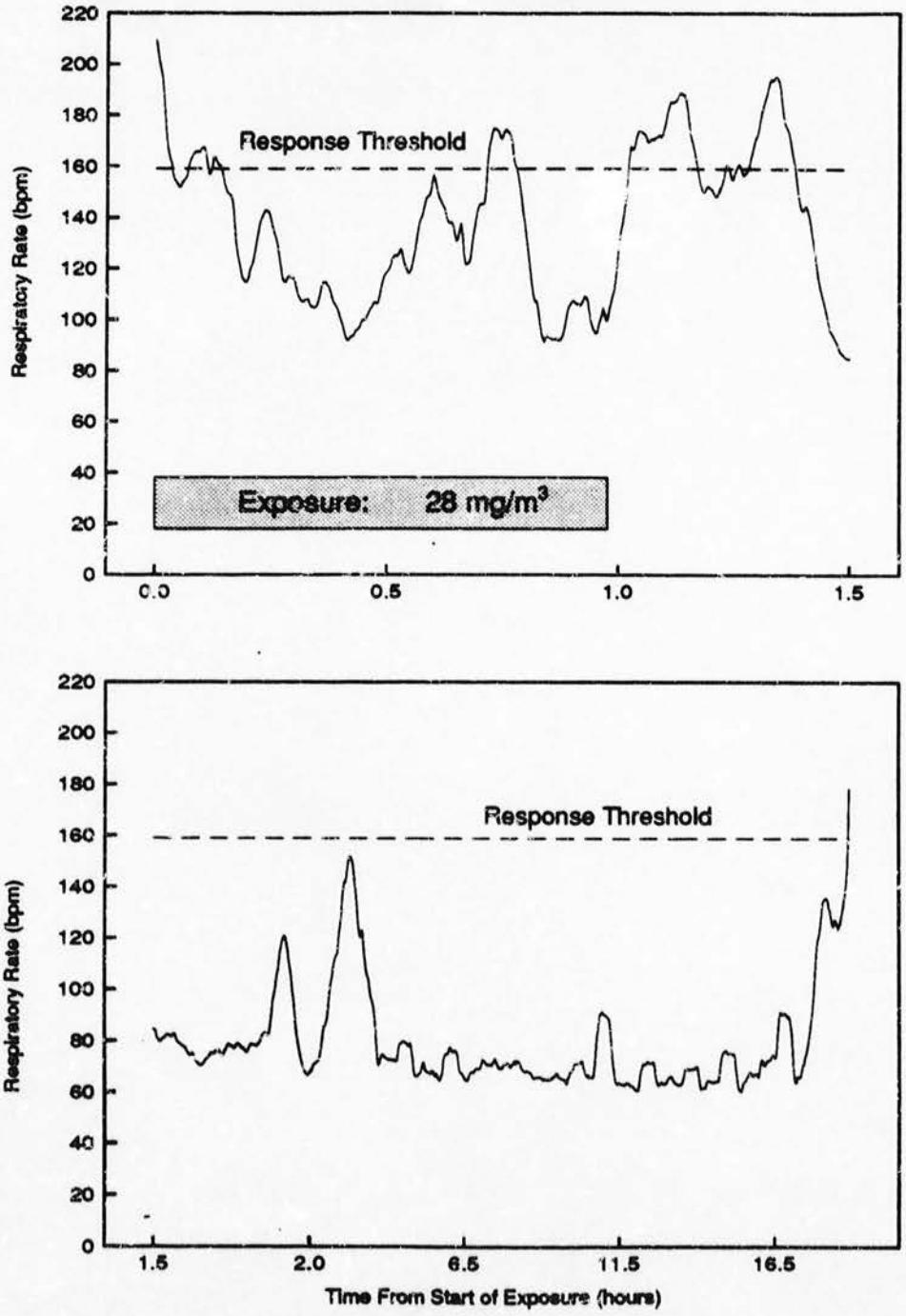
Figure: 70 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 232: Sham Sensitization

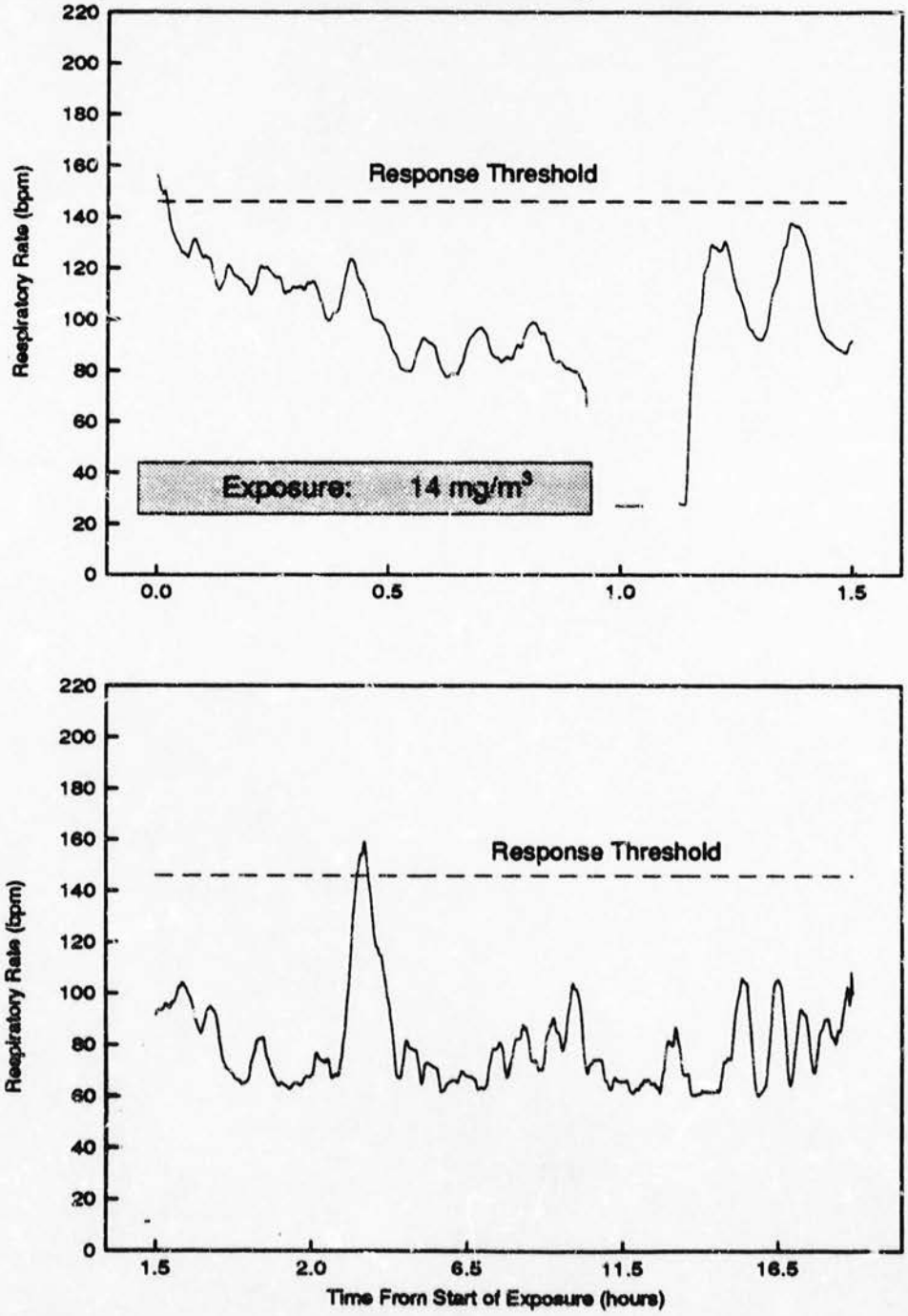
Figure: 71 2,4-TDI-GSA Challenge Week 3



Study Number 92-344-PP

Animal # 233: Sham Sensitization

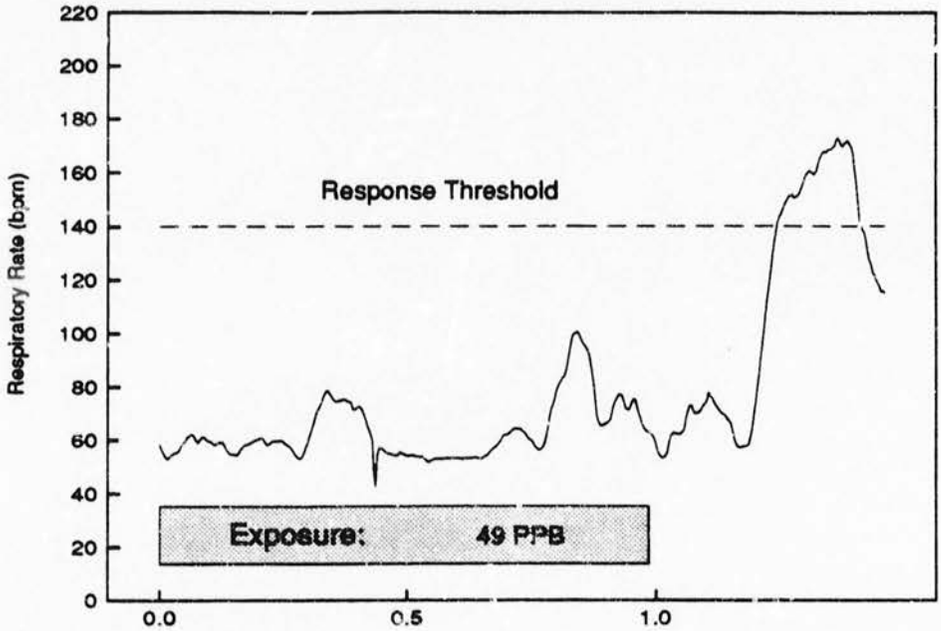
Figure: 72 2,4-TDI-GSA Challenge Week 3

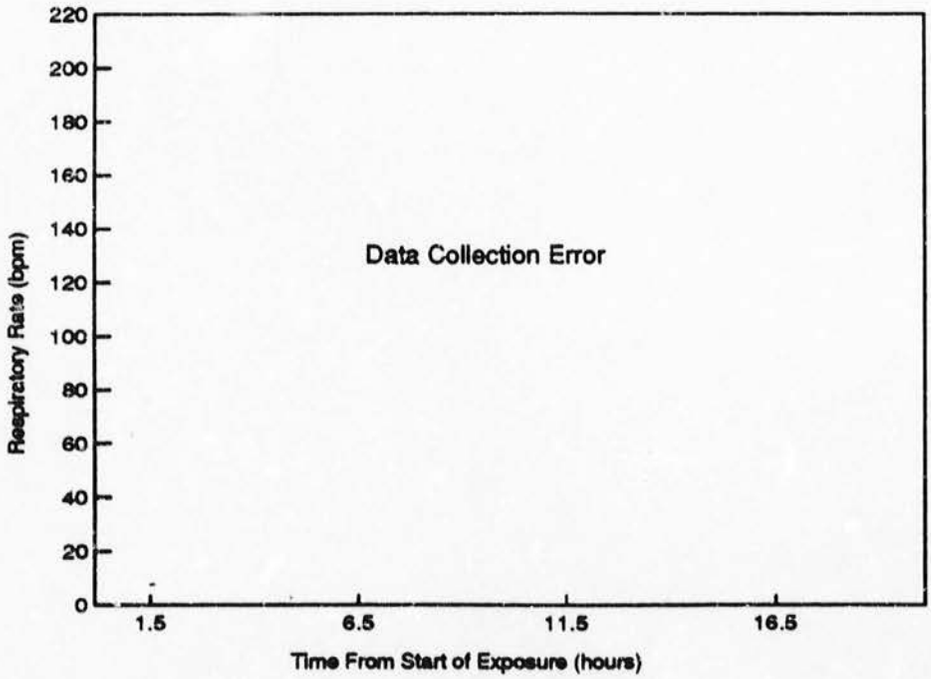


Study Number 92-344-PP

Animal # 207: Sham Sensitization

Figure: 73 2,6-TDI Challenge Week 1

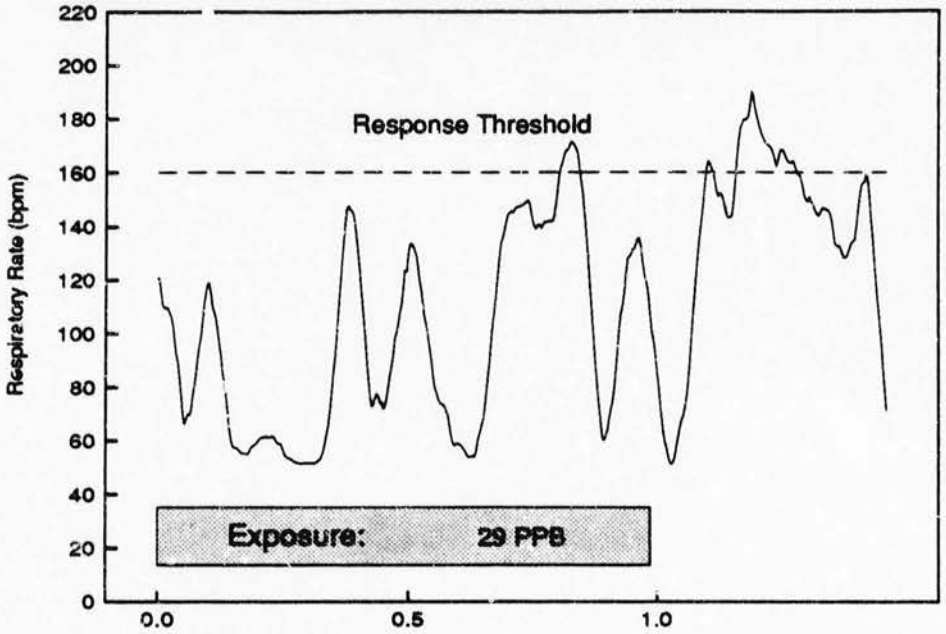


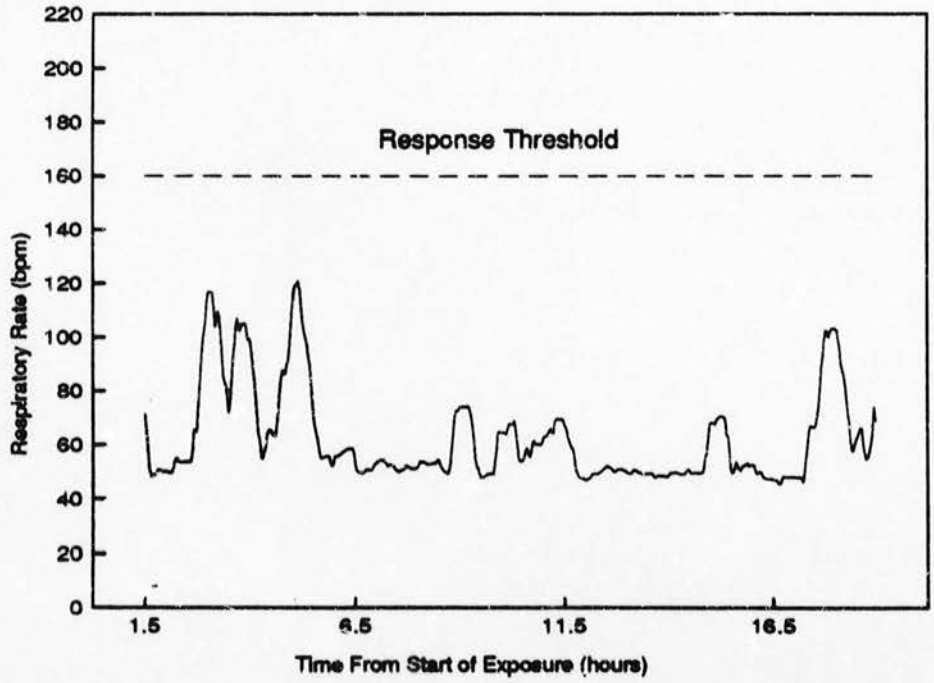


Study Number 92-344-PP

Animal # 208: Sham Sensitization

Figure: 74 2,6-TDI Challenge Week 1

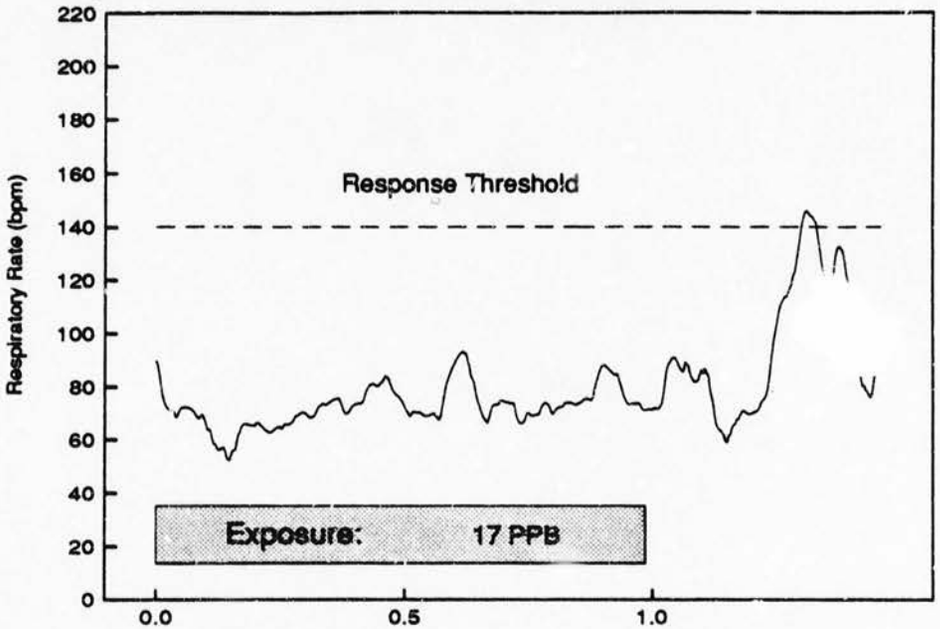


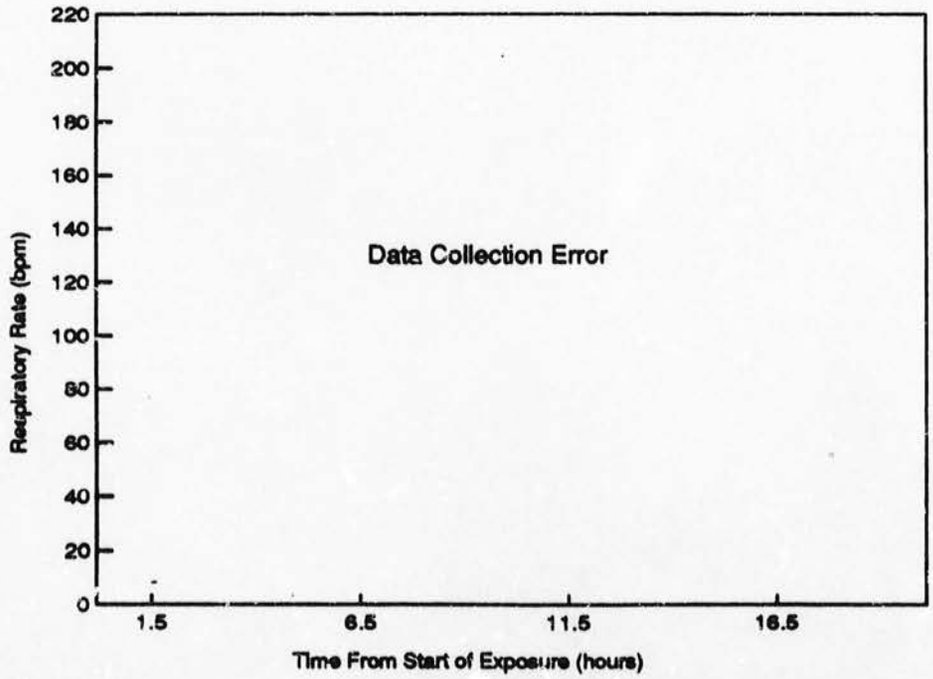


Study Number 92-344-PP

Animal # 209: Sham Sensitization

Figure: 75 2,6-TDI Challenge Week 1

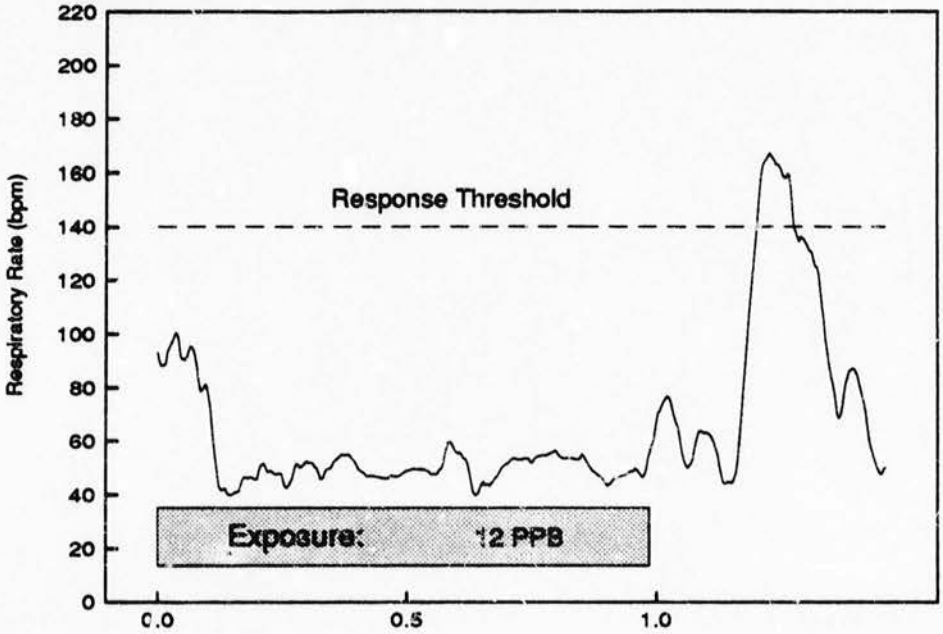


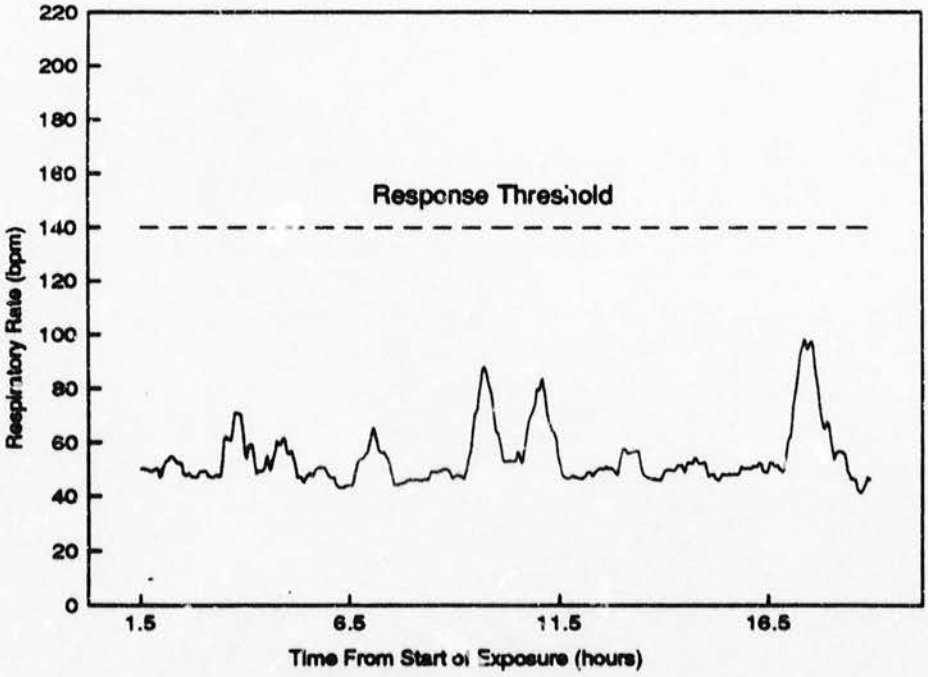


Study Number 92-344-PP

Animal # 210: Sham Sensitization

Figure: 76 2,6-TDI Challenge Week 1

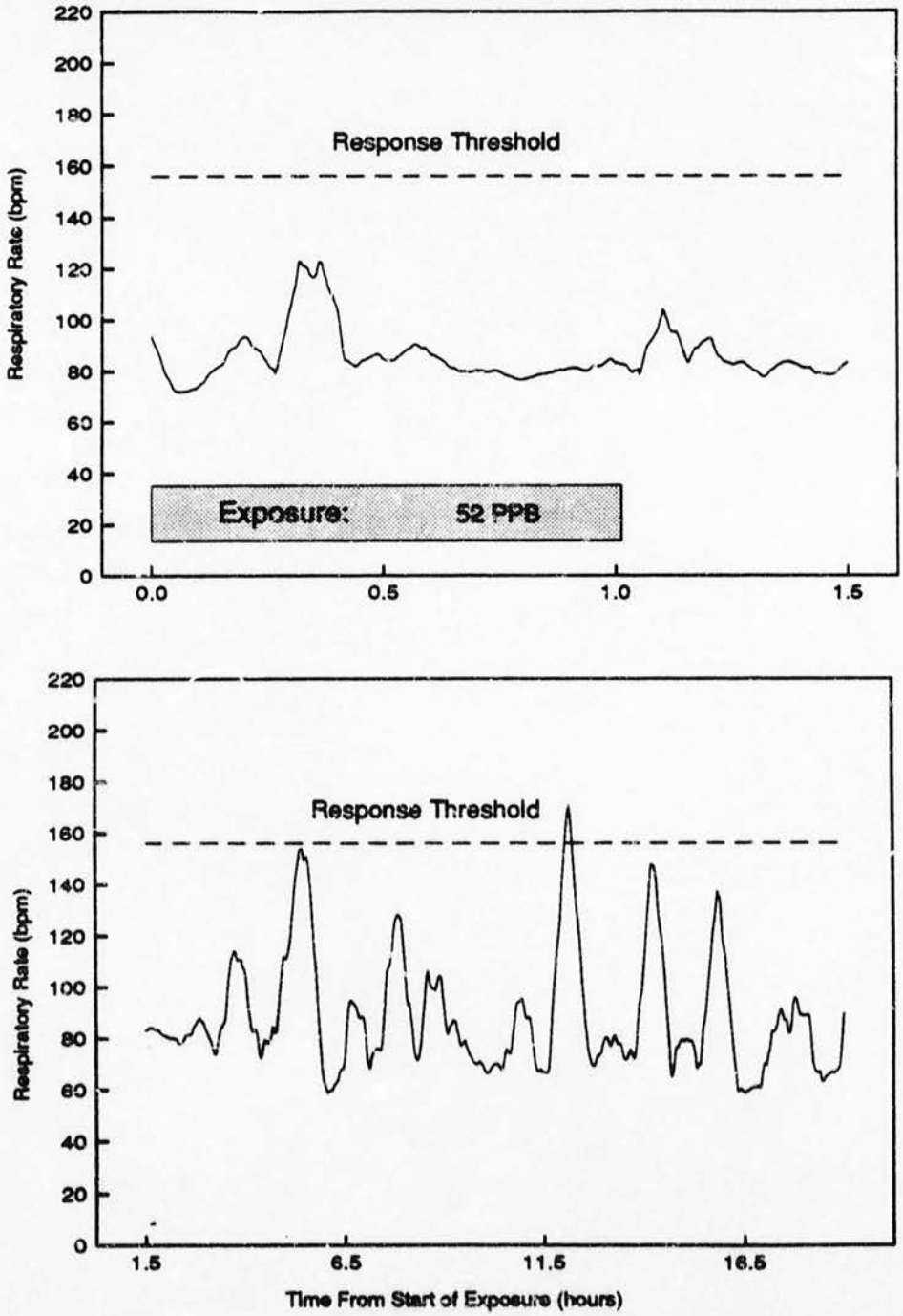




Study Number 92-344-PP

Animal # 211: Sham Sensitization

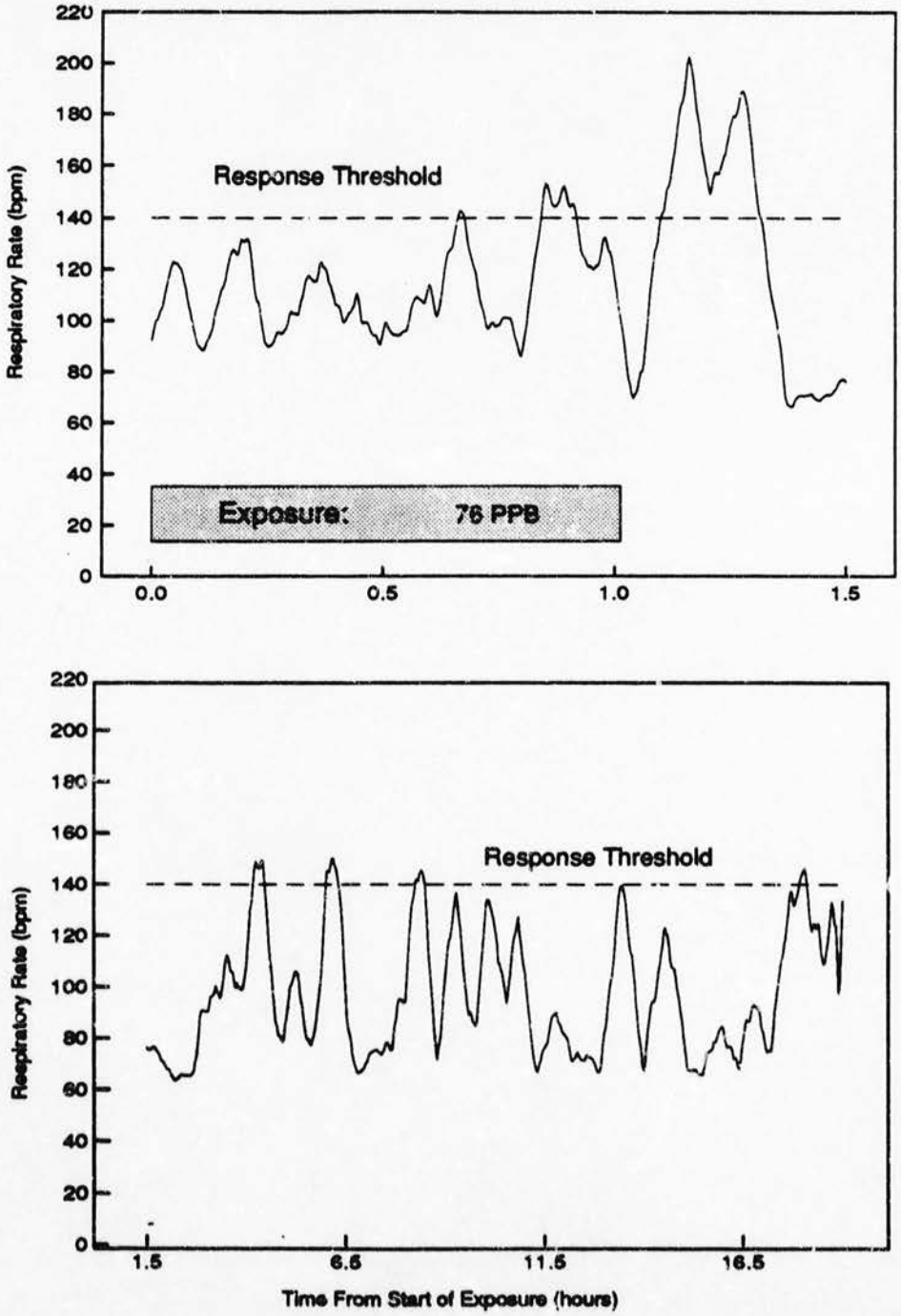
Figure: 77 2,6-TDI Challenge Week 1



Study Number 92-344-PP

Animal # 212: Sham Sensitization

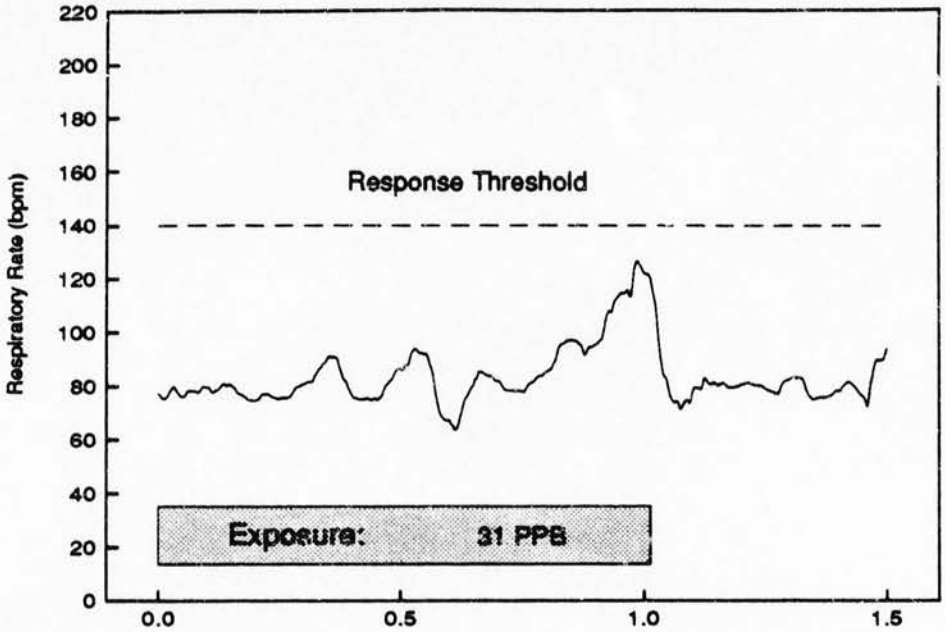
Figure: 78 2,6-TDI Challenge Week 1

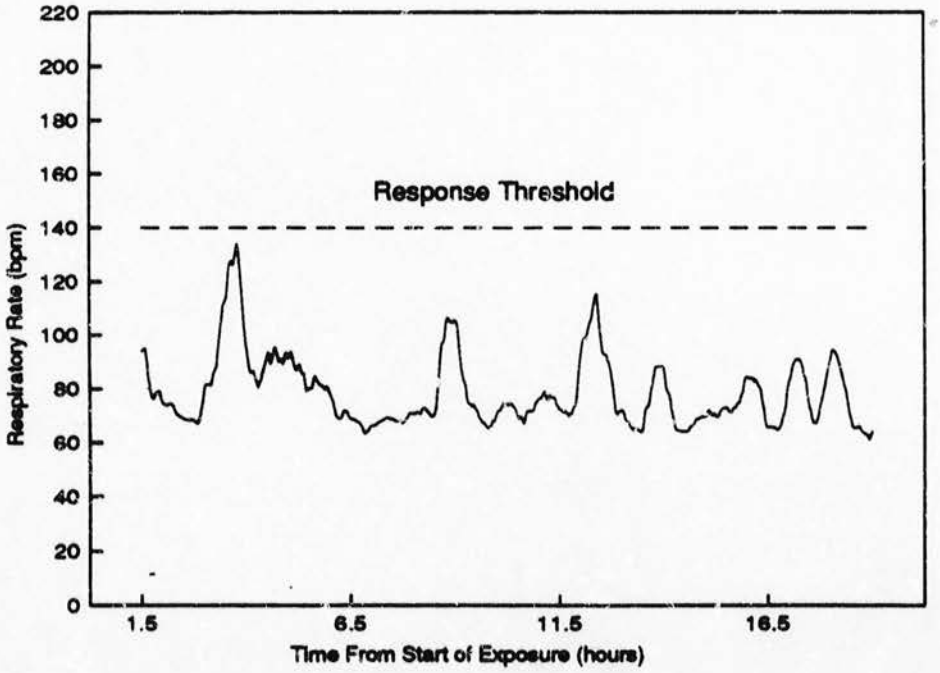


Study Number 92-344-PP

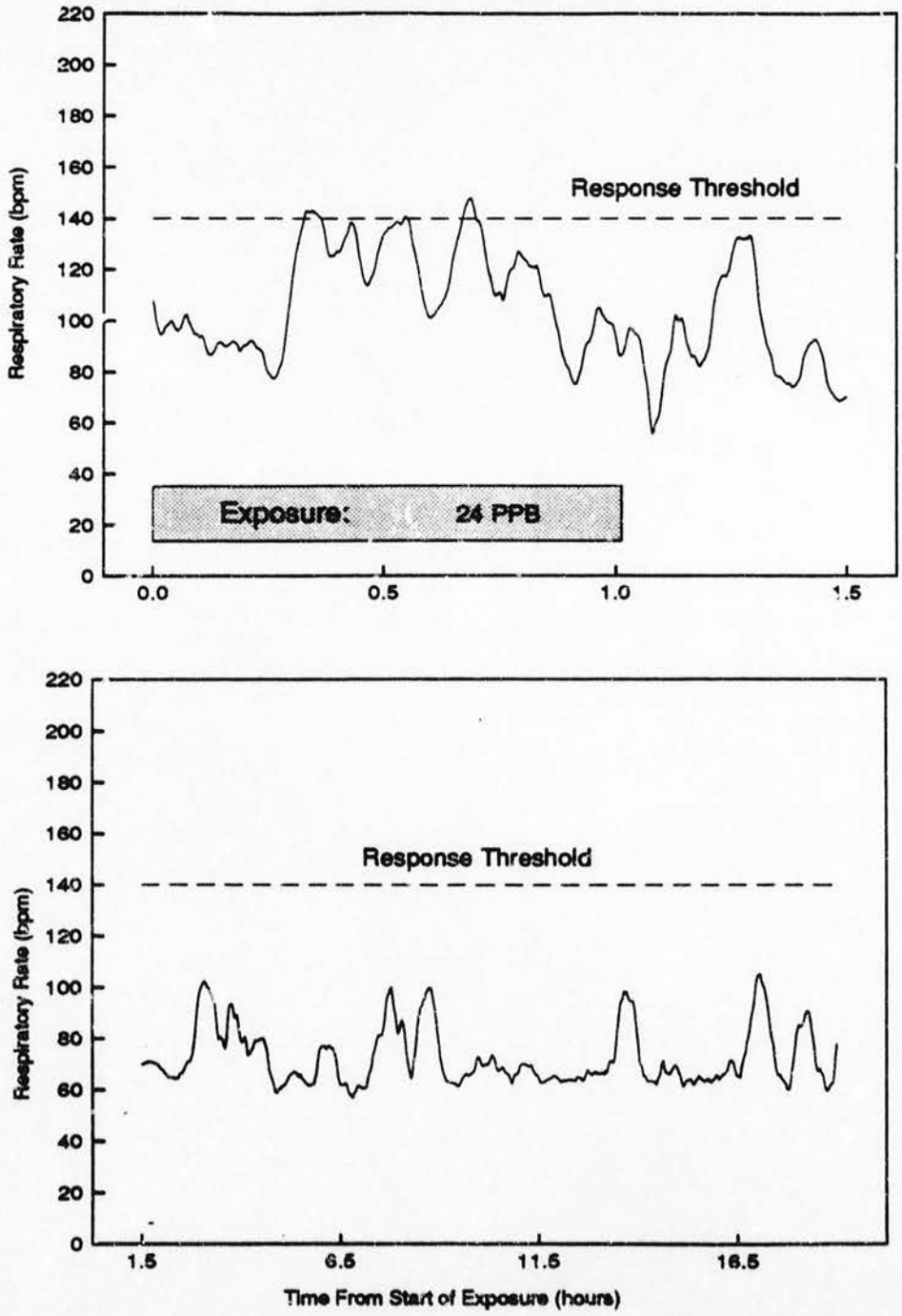
Animal # 213: Sham Sensitization

Figure: 79 2,6-TDI Challenge Week 1

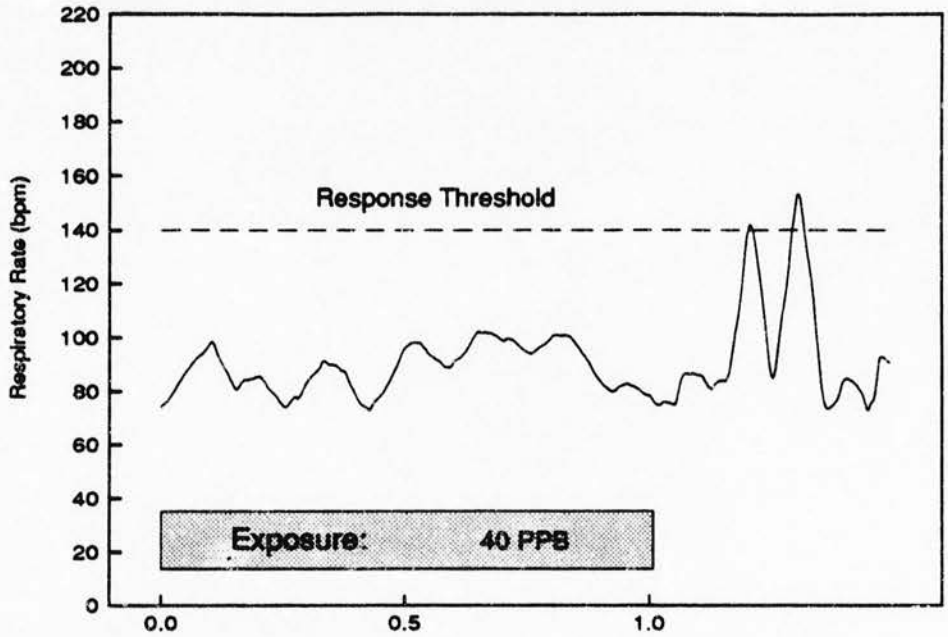


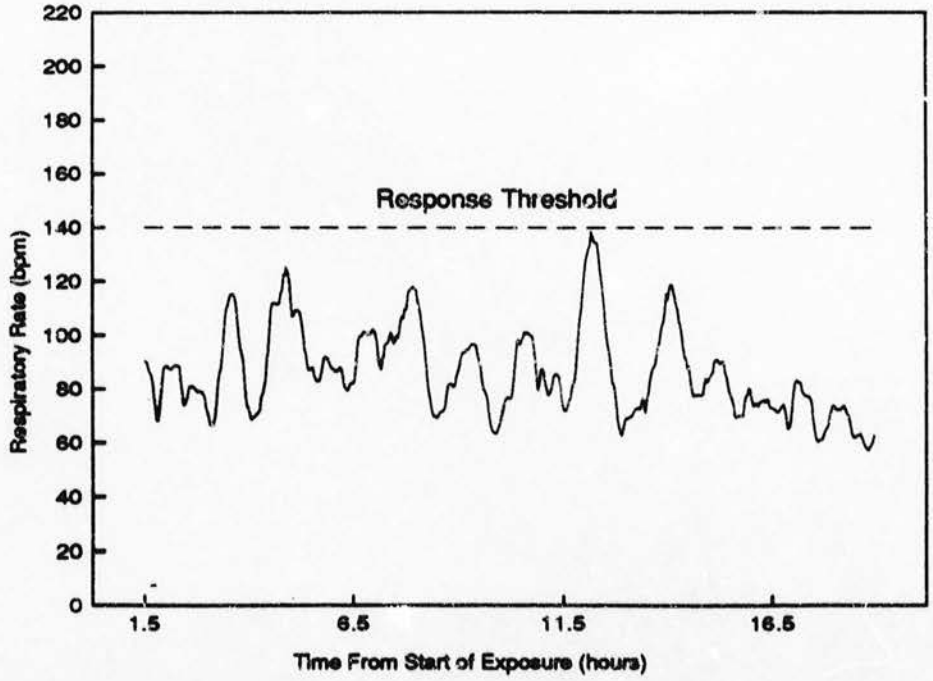


Study Number 92-344-PP
Animal # 214: Sham Sensitization
Figure: 80 2,6-TDI Challenge Week 1



Study Number 92-344-PP
Animal # 215: Sham Sensitization
Figure: 81 2,6-TDI Challenge Week 1

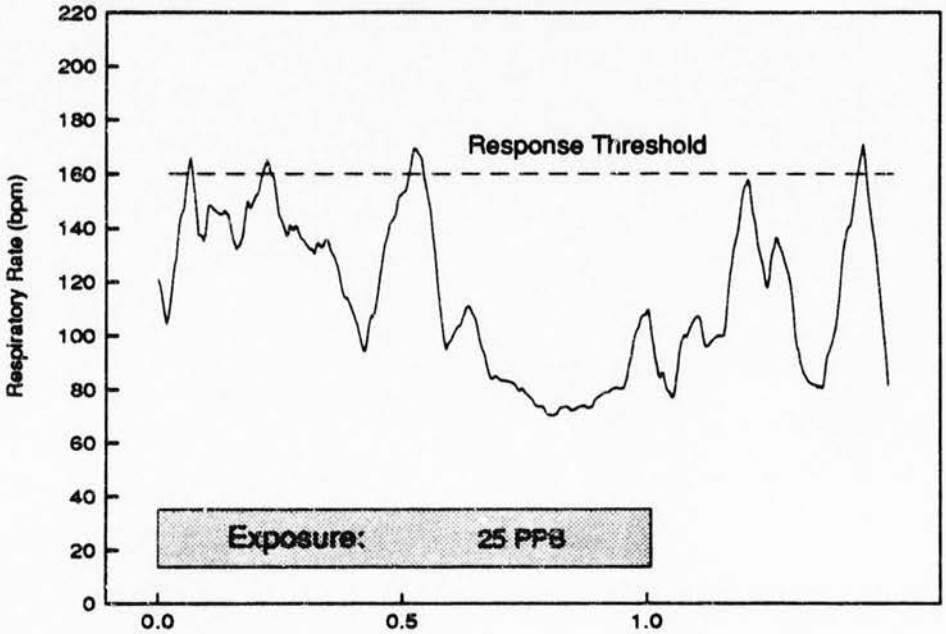


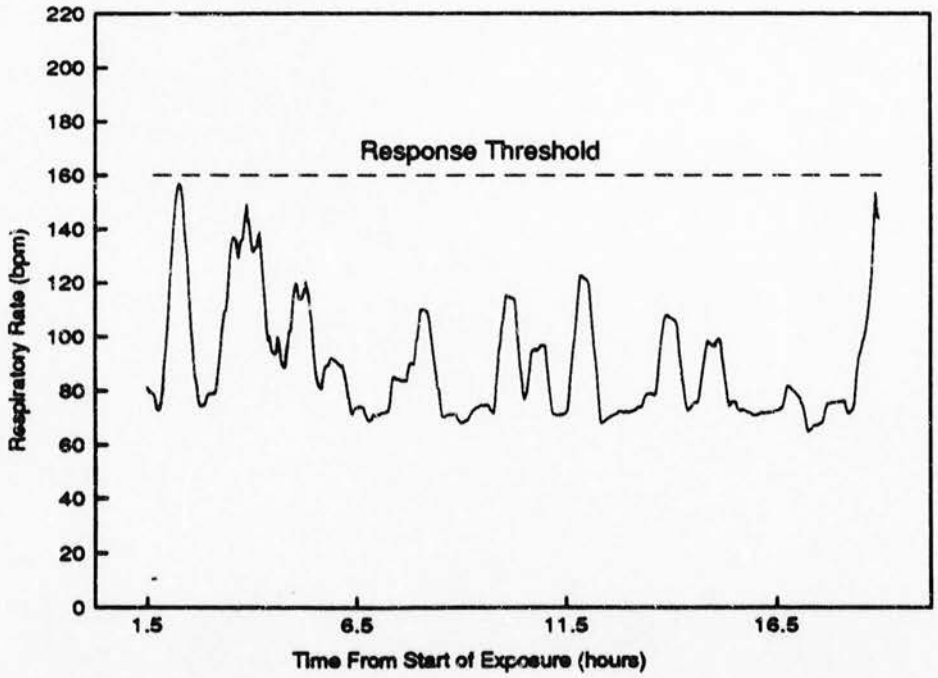


Study Number 92-344-PP

Animal # 216: Sham Sensitization

Figure: 82 2,6-TDI Challenge Week 1

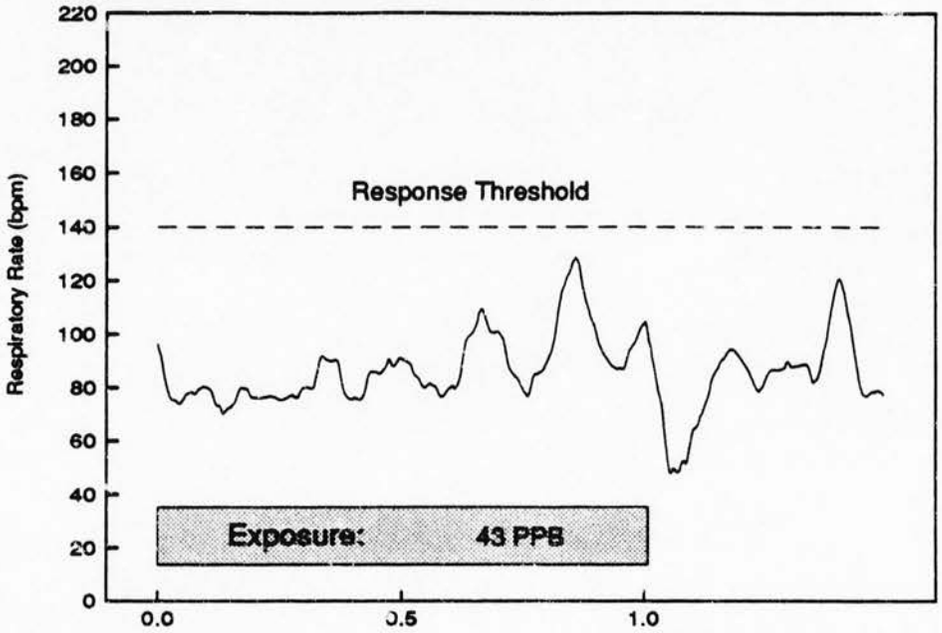


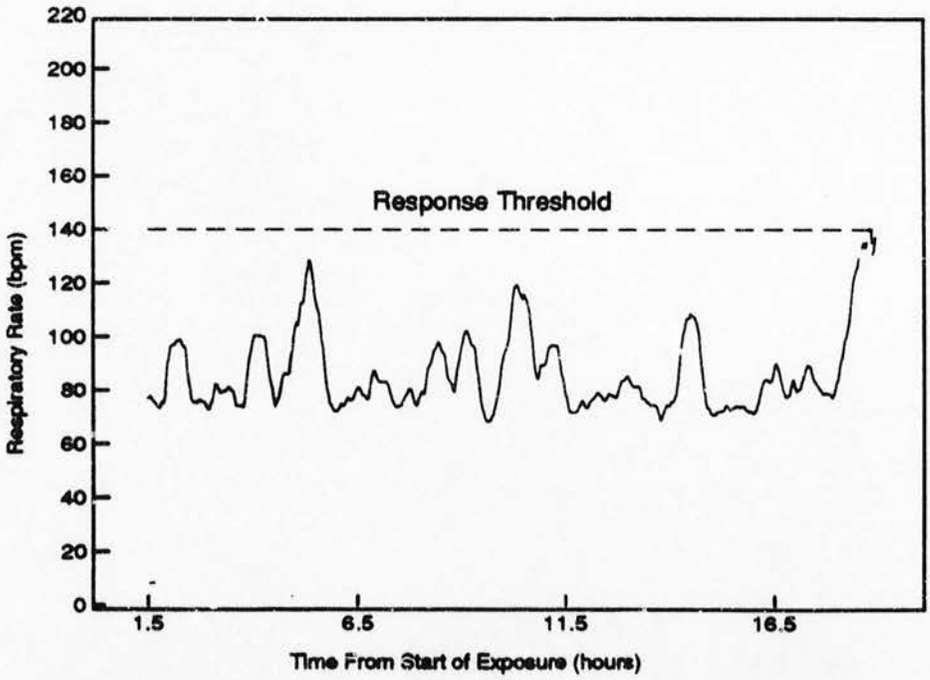


Study Number 92-344-PP

Animal # 217: Sham Sensitization

Figure: 83 2,6-TDI Challenge Week 1

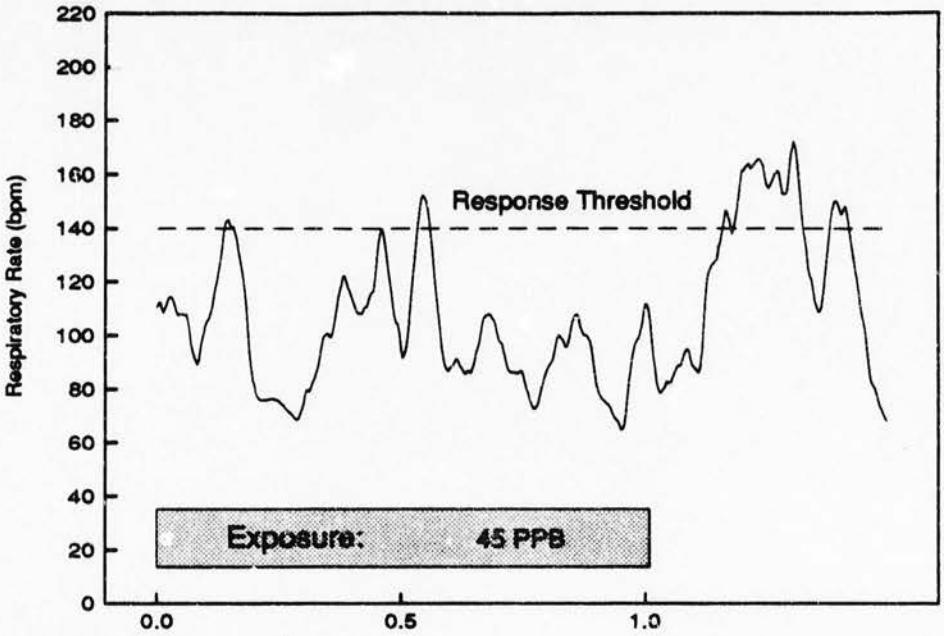


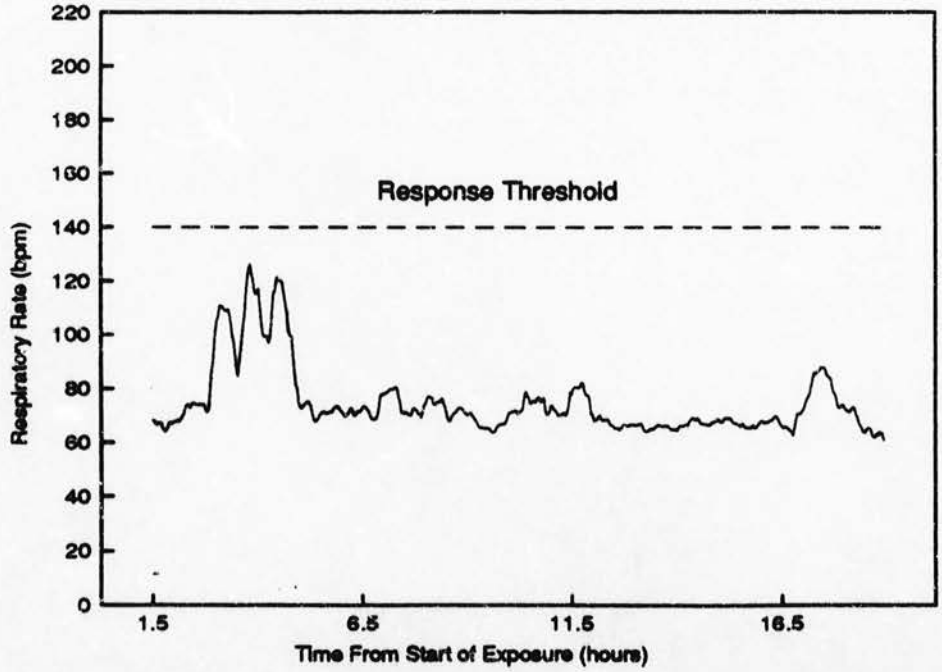


Study Number 92-344-PP

Animal # 218: Sham Sensitization

Figure: 84 2,6-TDI Challenge Week 1

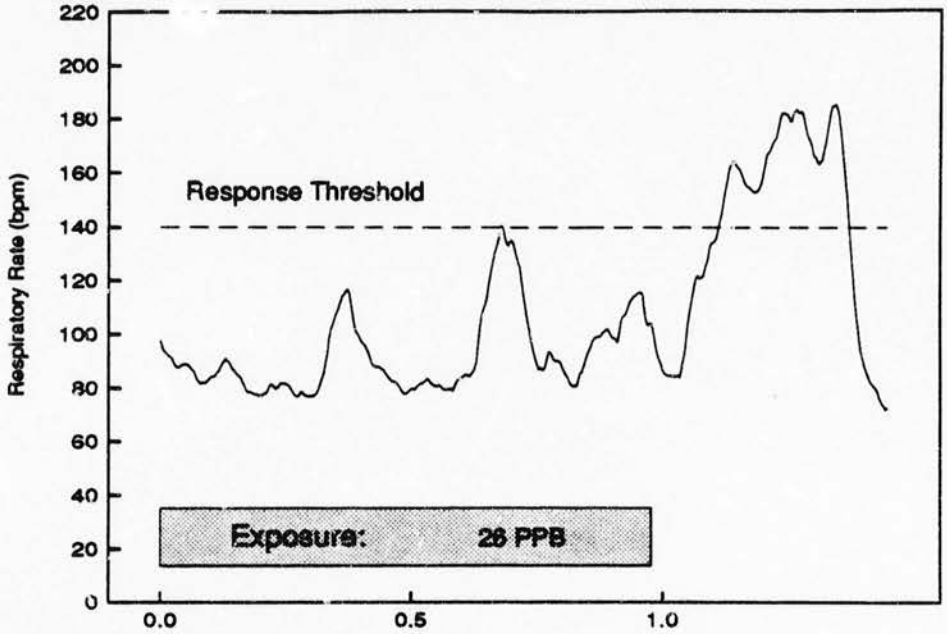


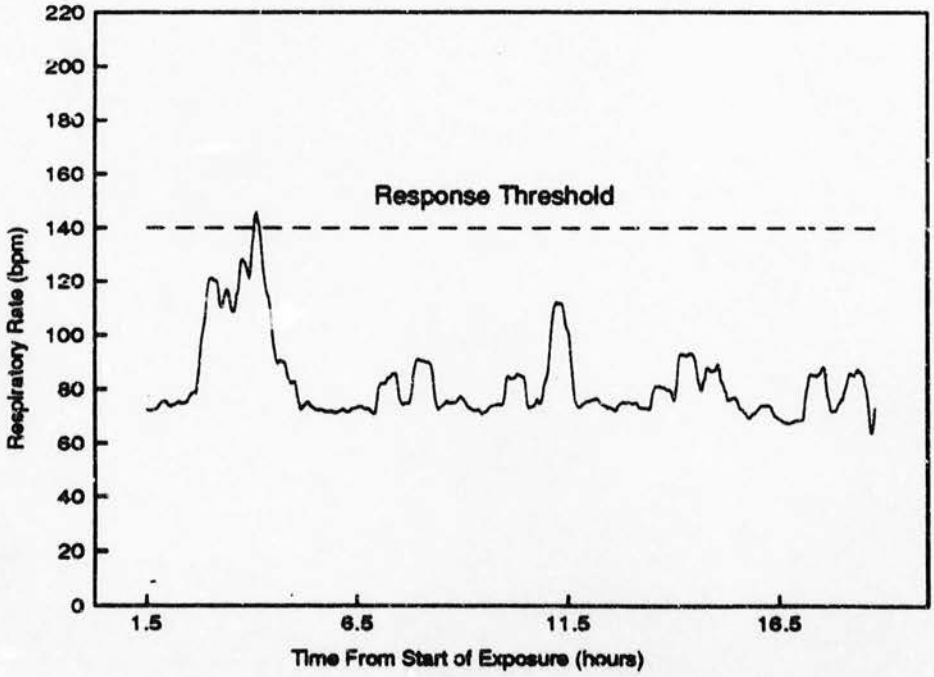


Study Number 92-344-PP

Animal # 207: Sham Sensitization

Figure: 85 2,6-TDI Challenge Week 2

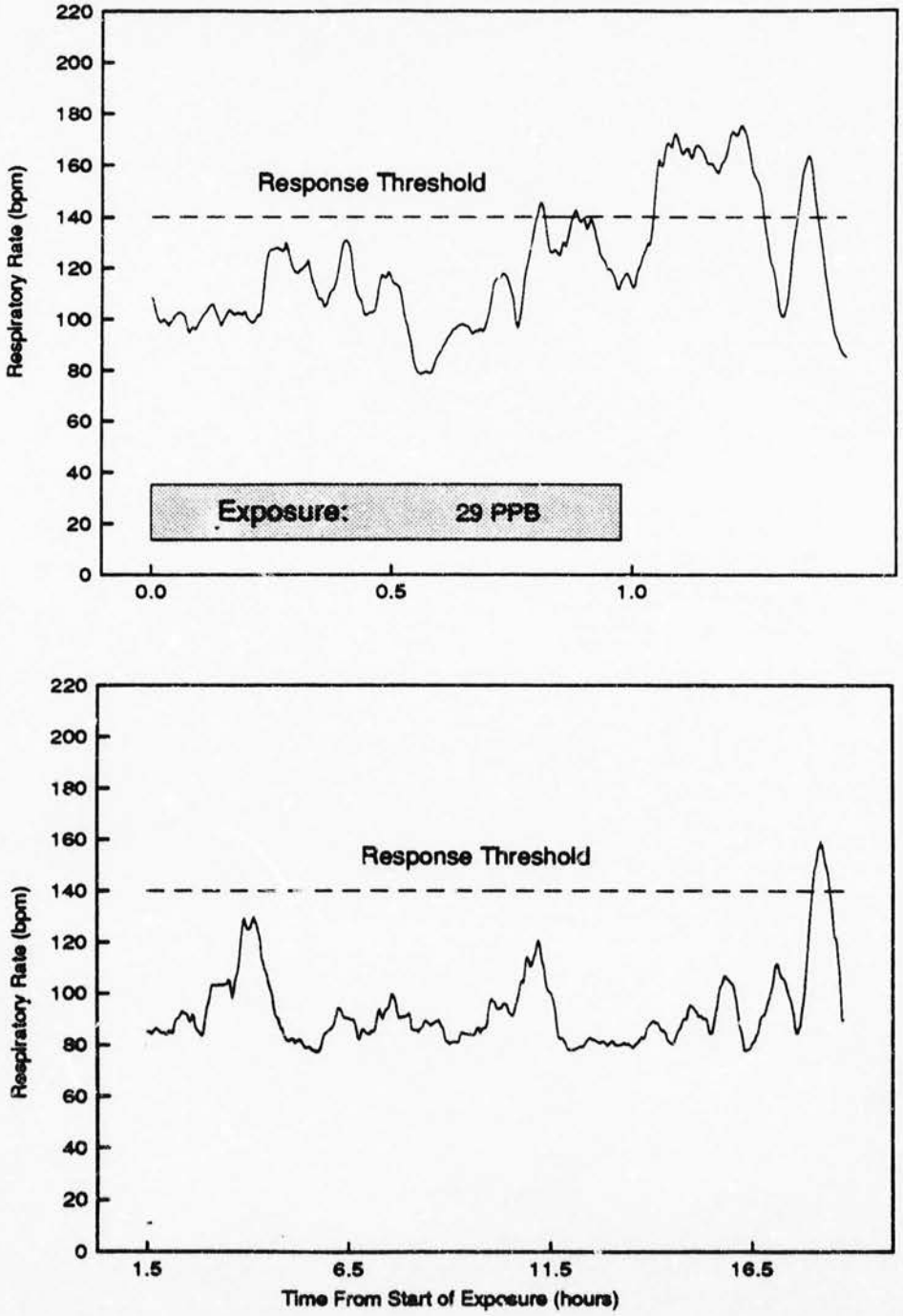




Study Number 92-344-PP

Animal # 208: Sham Sensitization

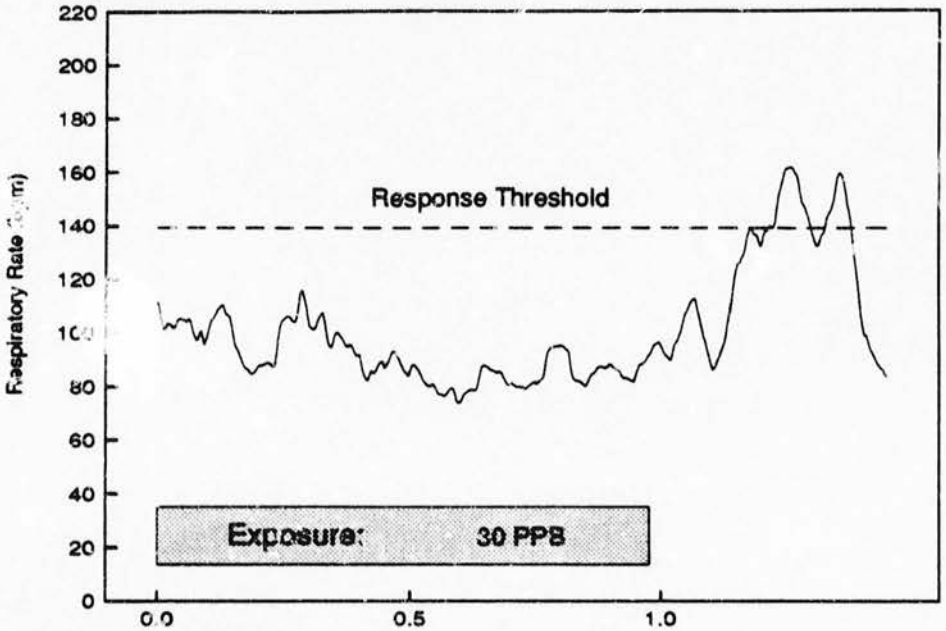
Figure: 86 2,6-TDI Challenge Week 2

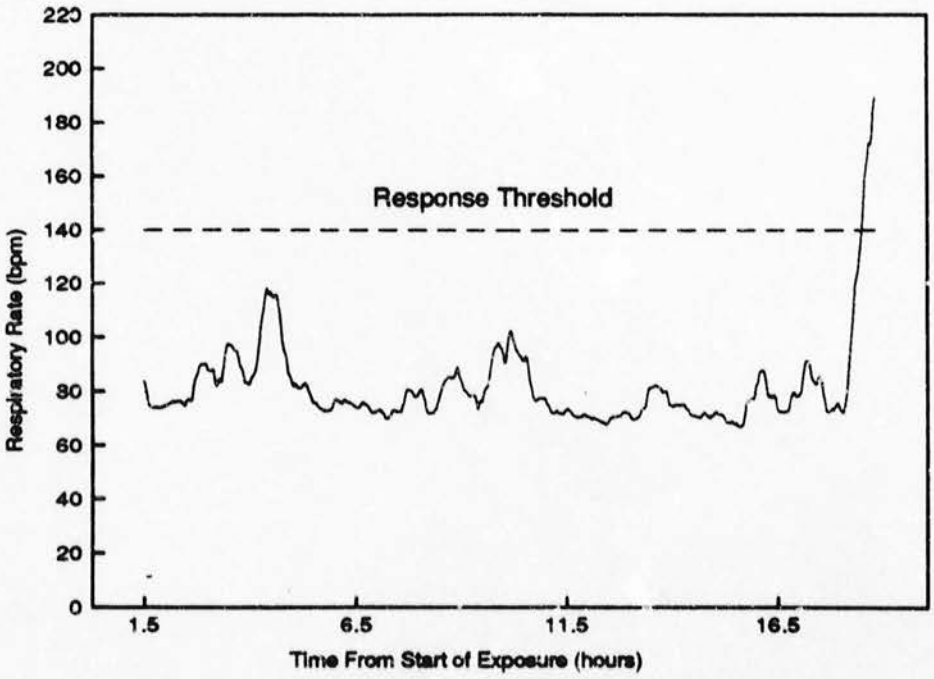


Study Number 92-344-PP

Animal # 209: Sham Sensitization

Figure: 87 2,6-TDI Challenge Week 2

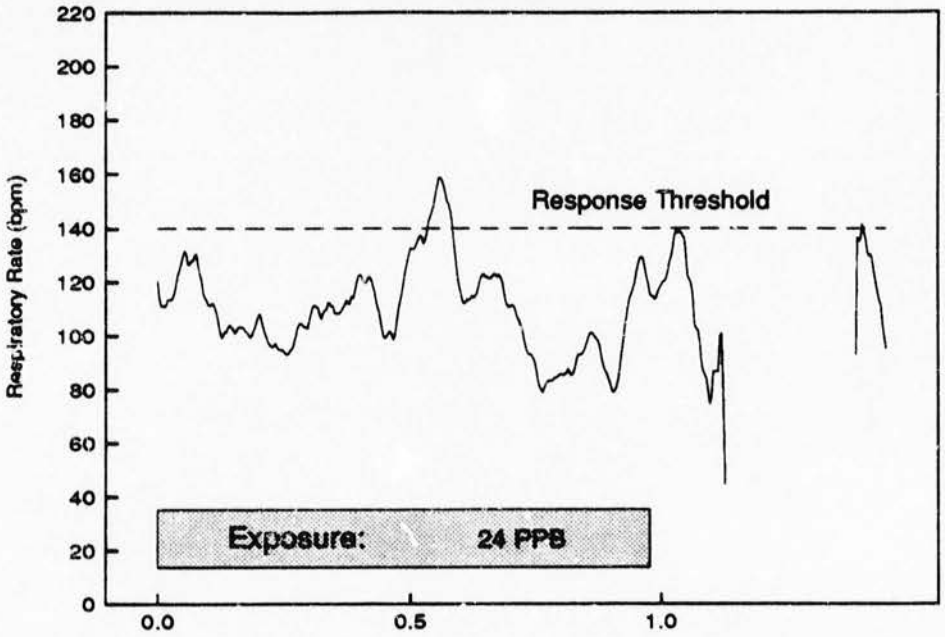


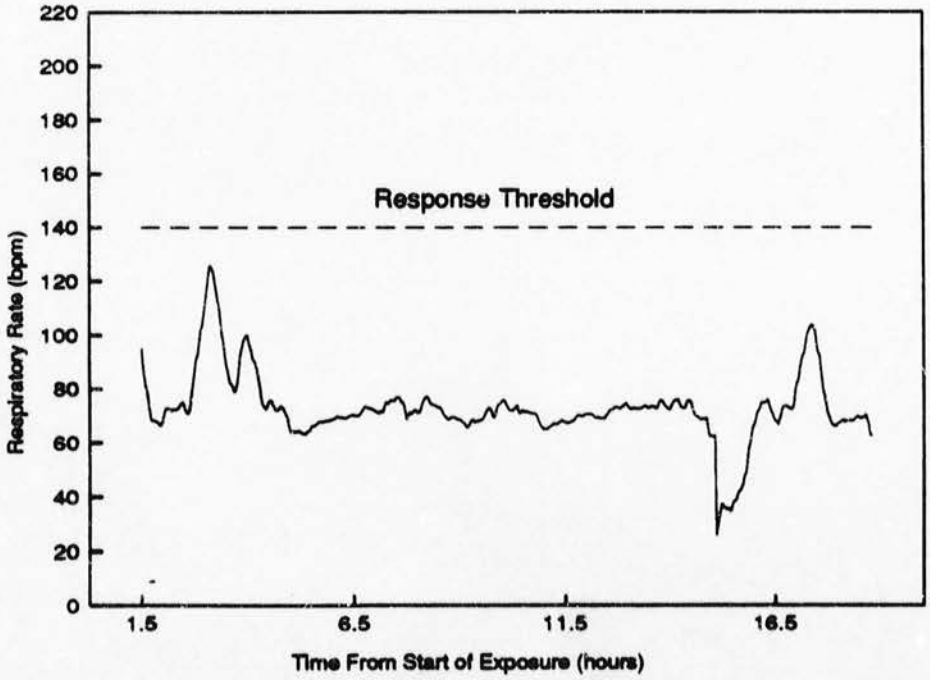


Study Number 92-344-PP

Animal # 210: Sham Sensitization

Figure: 88 2,6-TDI Challenge Week 2

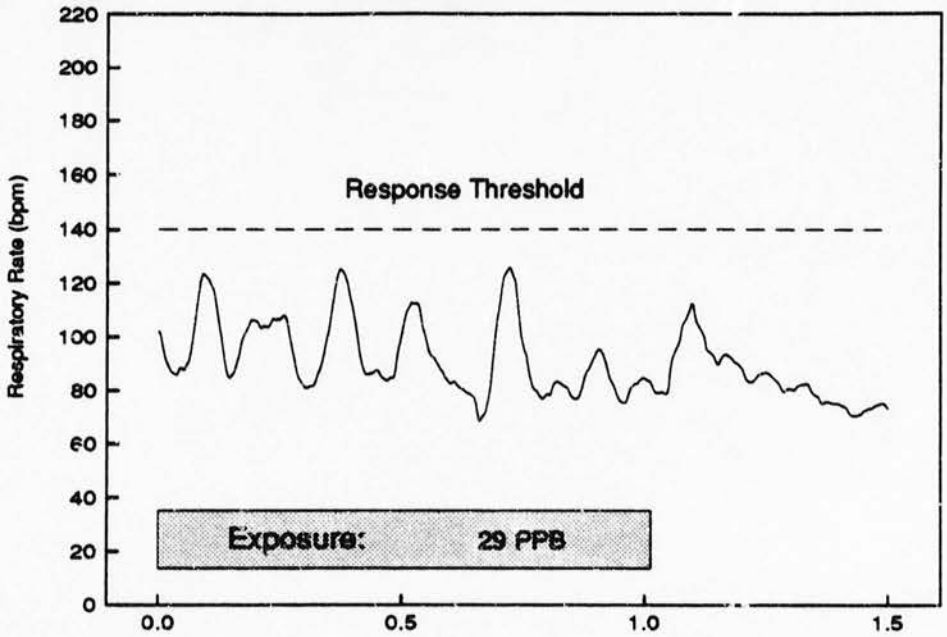


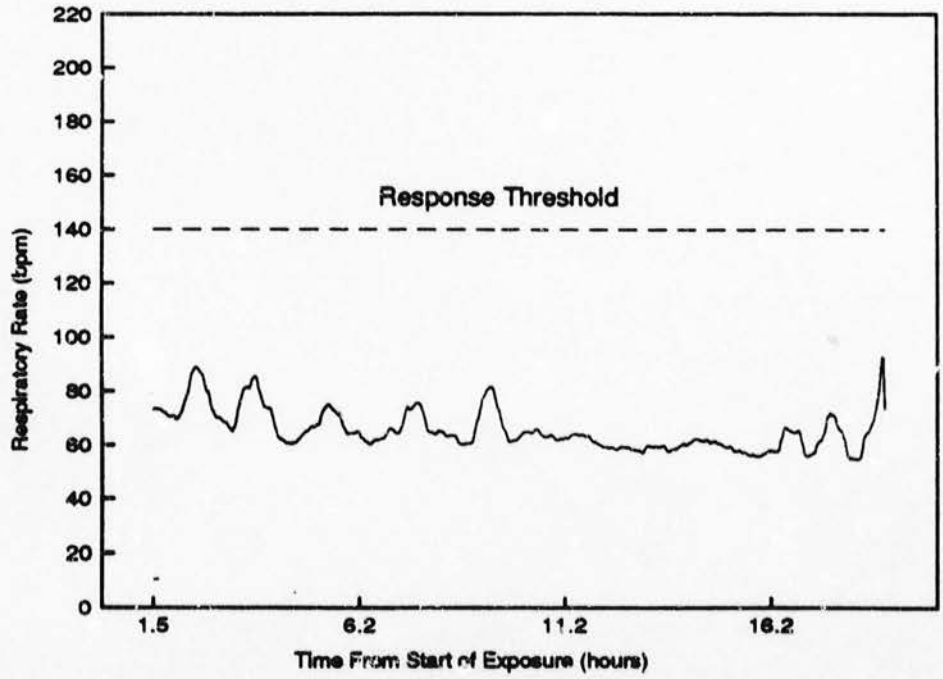


Study Number 92-344-PP

Animal # 211: Sham Sensitization

Figure: 89 2,6-TDI Challenge Week 2

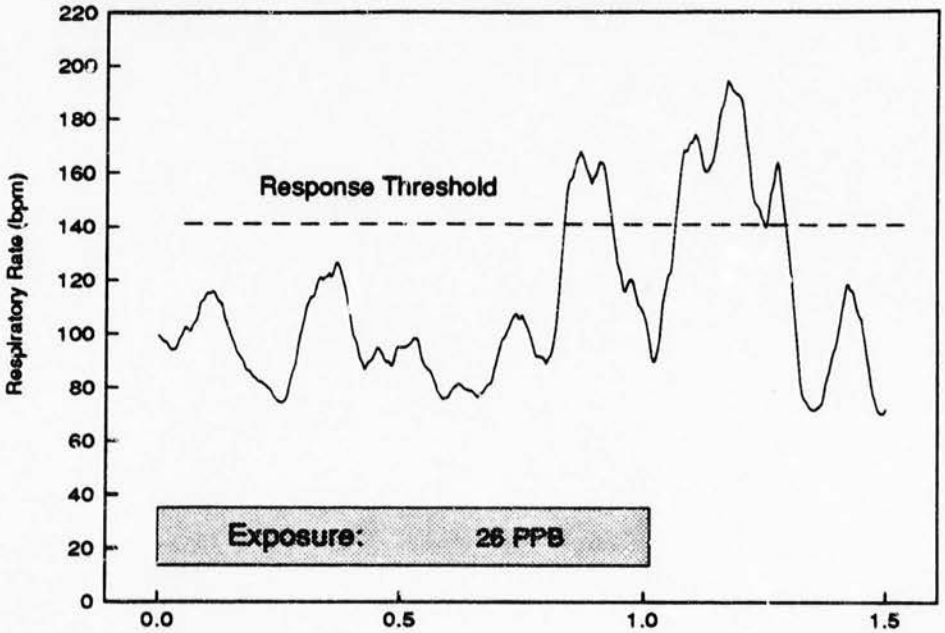


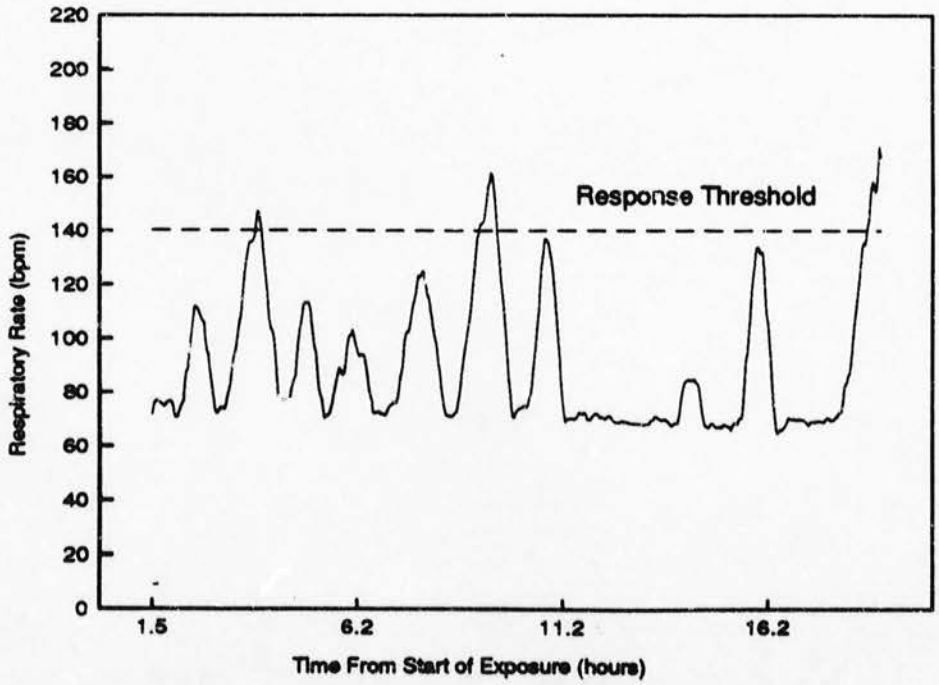


Study Number 92-344-PP

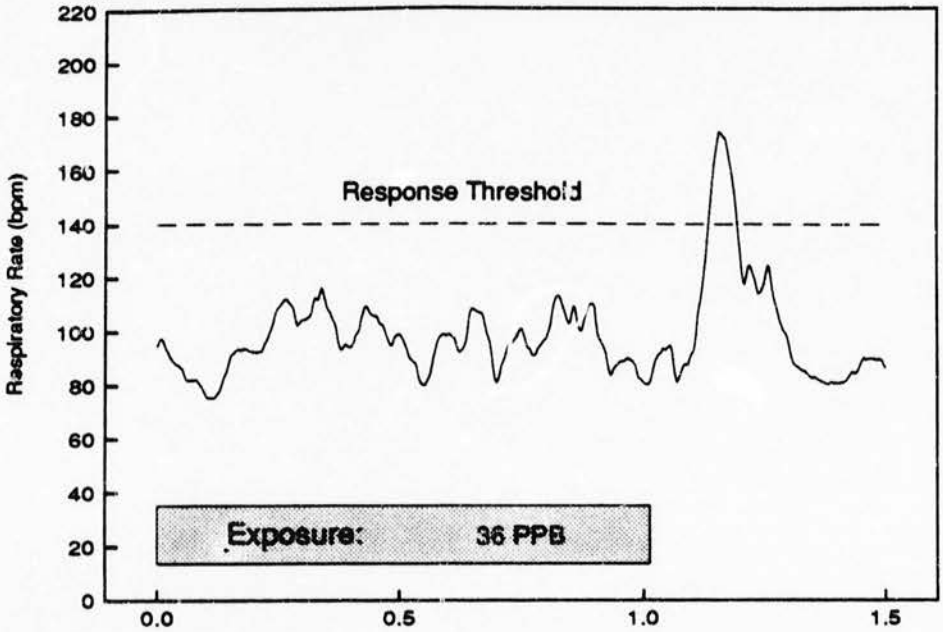
Animal # 212: Sham Sensitization

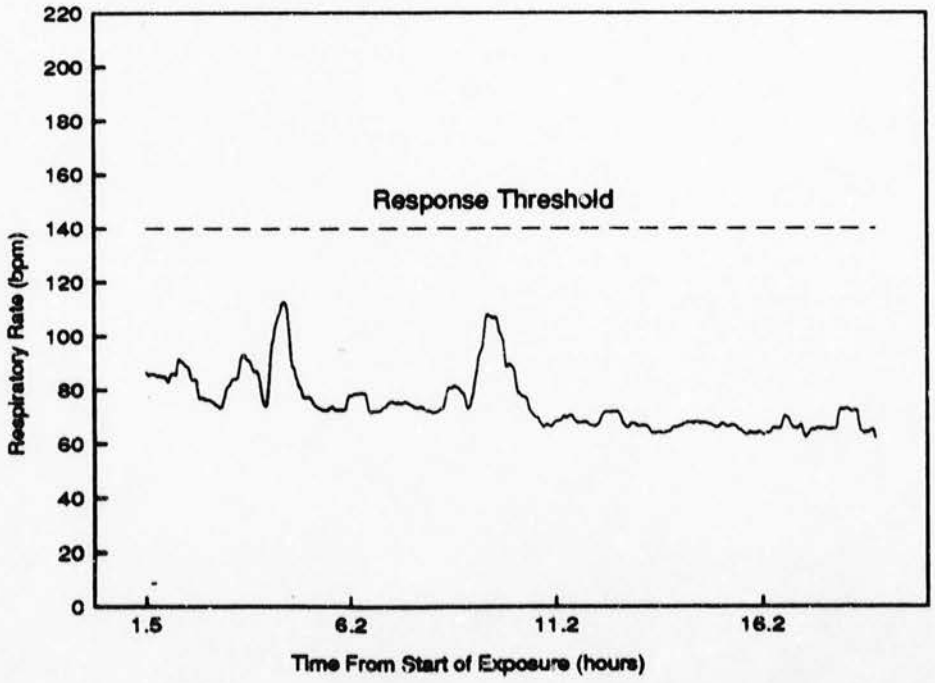
Figure: 90 2,6-TDI Challenge Week 2





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

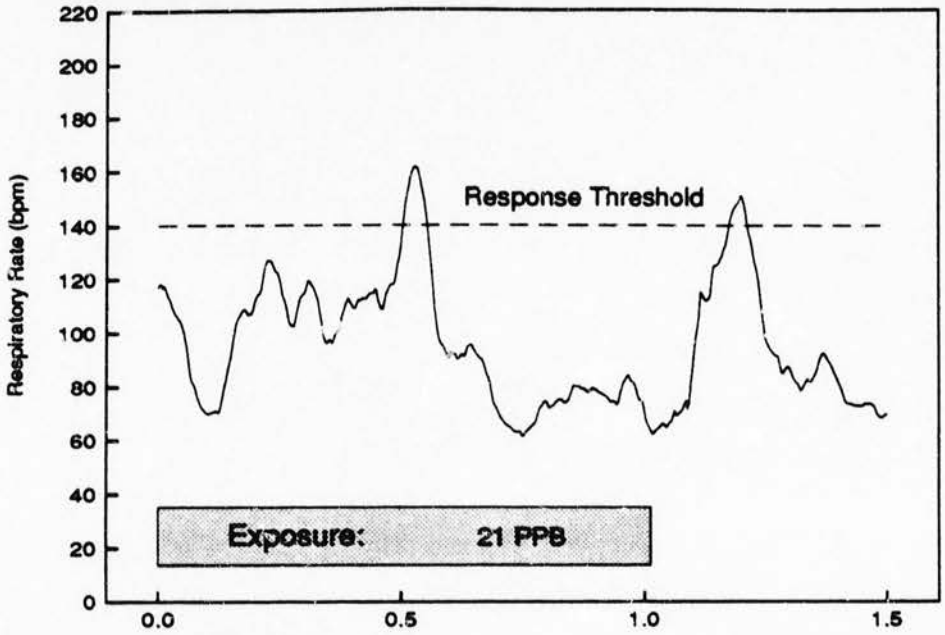


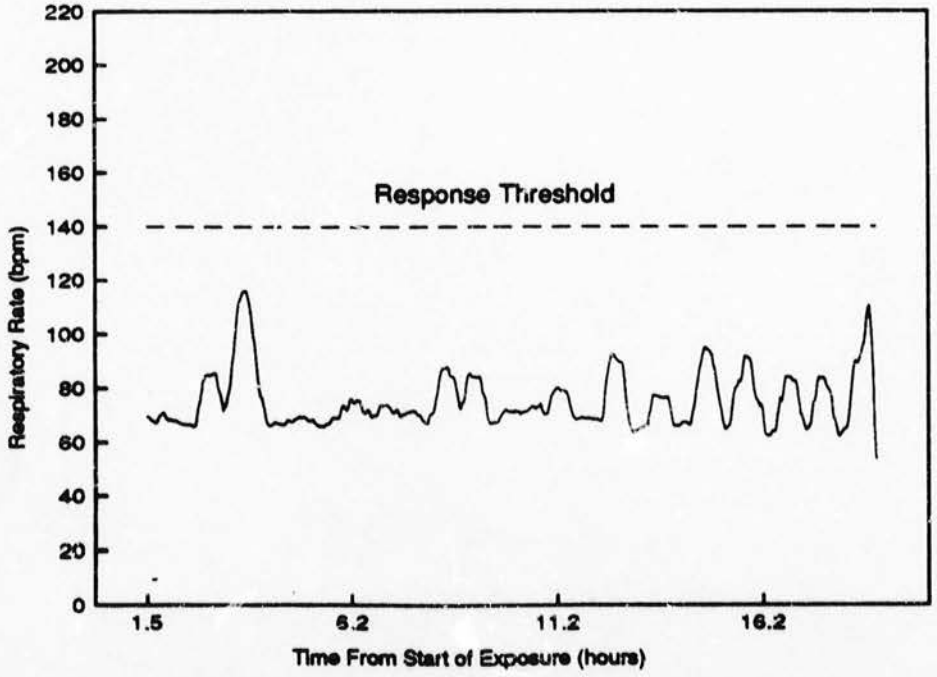


Study Number 92-344-PP

Animal # 214: Sham Sensitization

Figure: 92 2,6-TDI Challenge Week 2

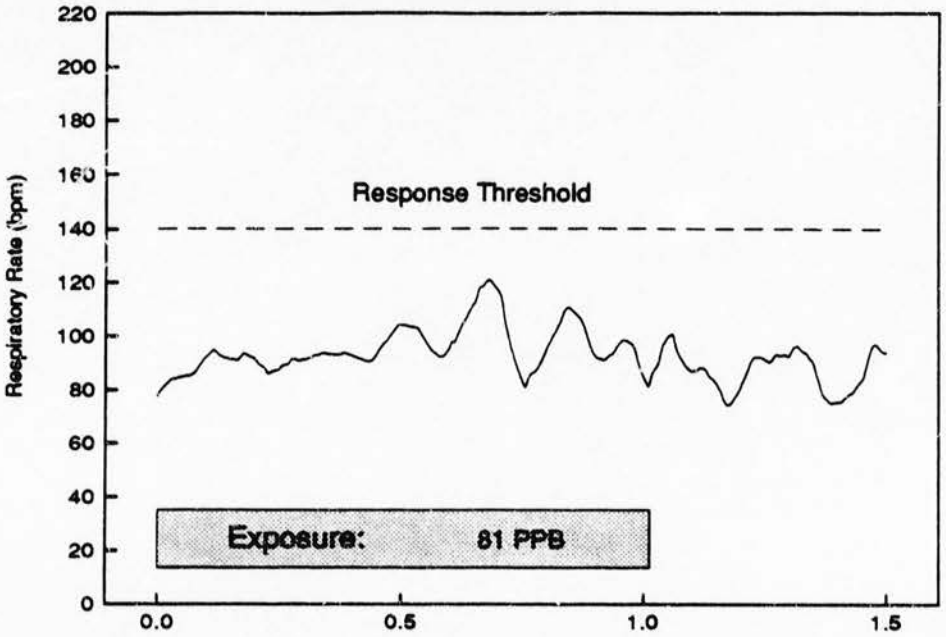


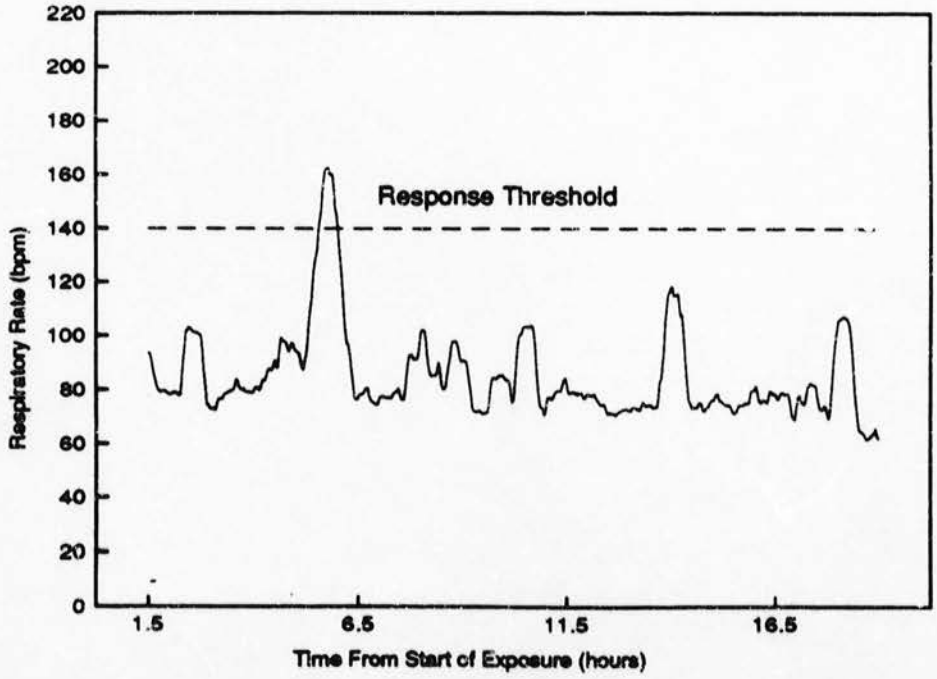


Study Number 92-344-PP

Animal # 215: Sham Sensitization

Figure: 93 2,6-TDI Challenge Week 2

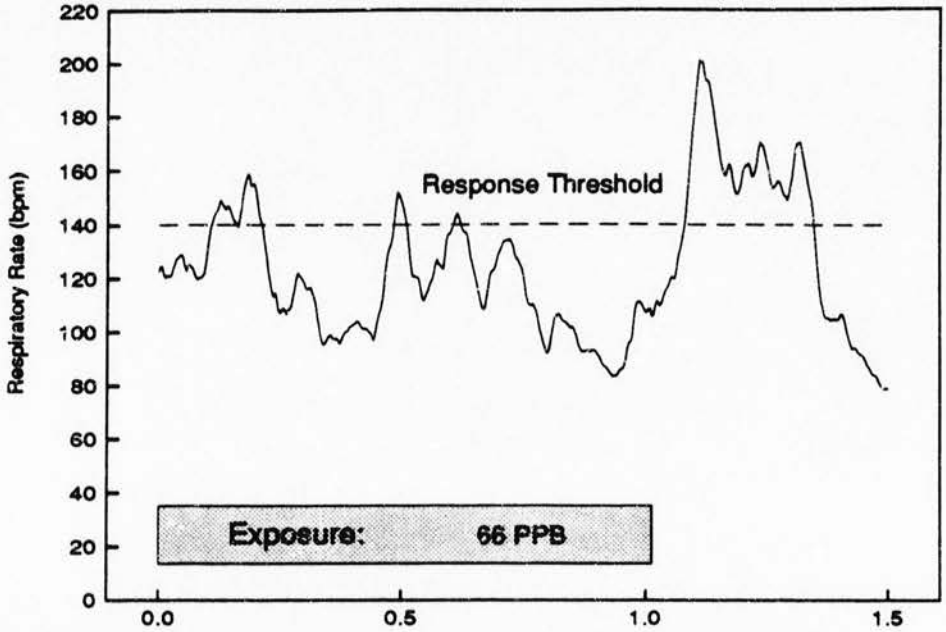


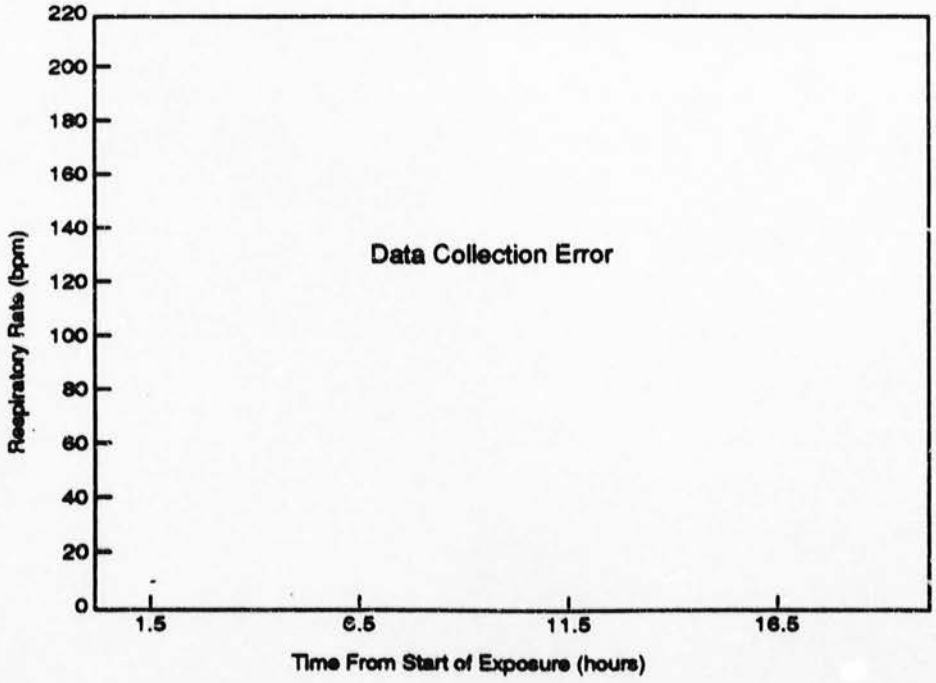


Study Number 92-344-PP

Animal # 216: Sham Sensitization

Figure: 94 2,6-TDI Challenge Week 2

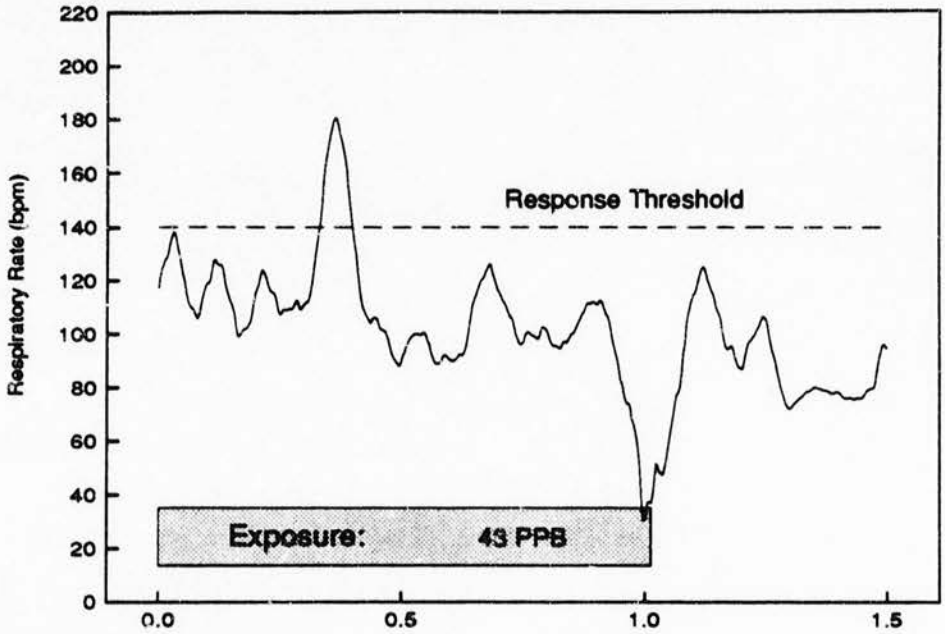


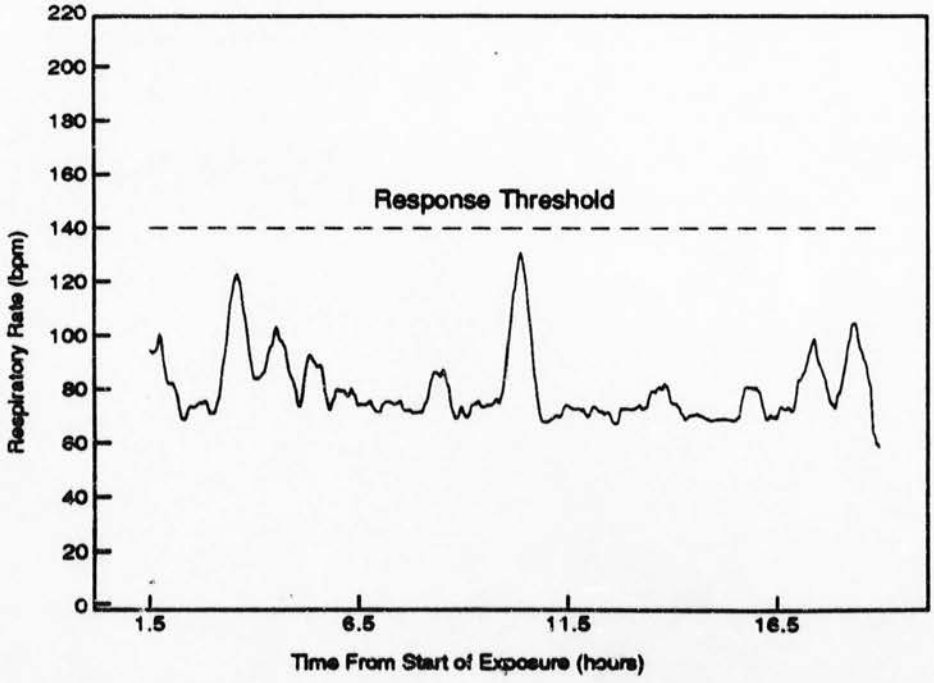


Study Number 92-344-PP

Animal # 217: Sham Sensitization

Figure: 95 2,6-TDI Challenge Week 2

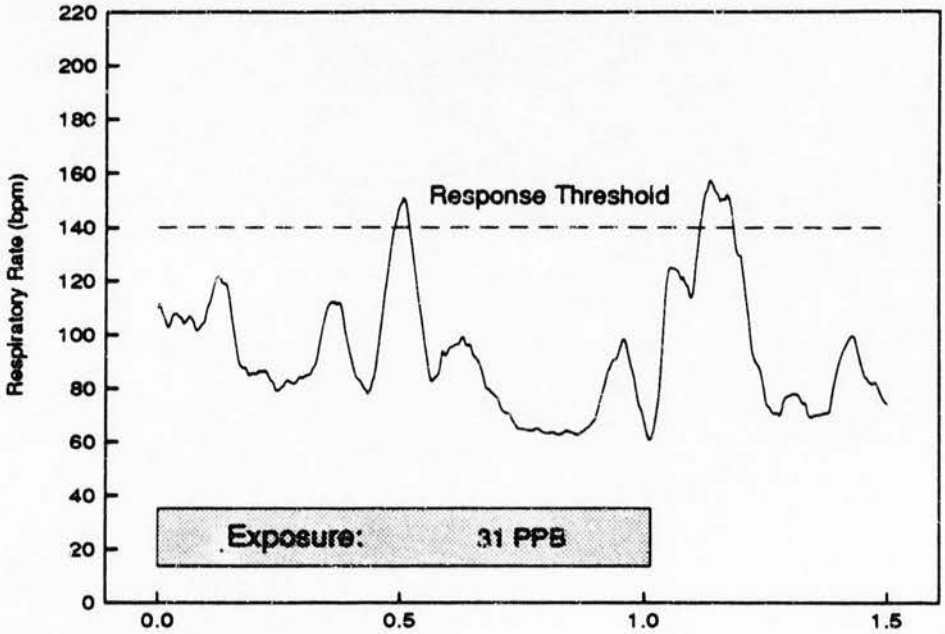


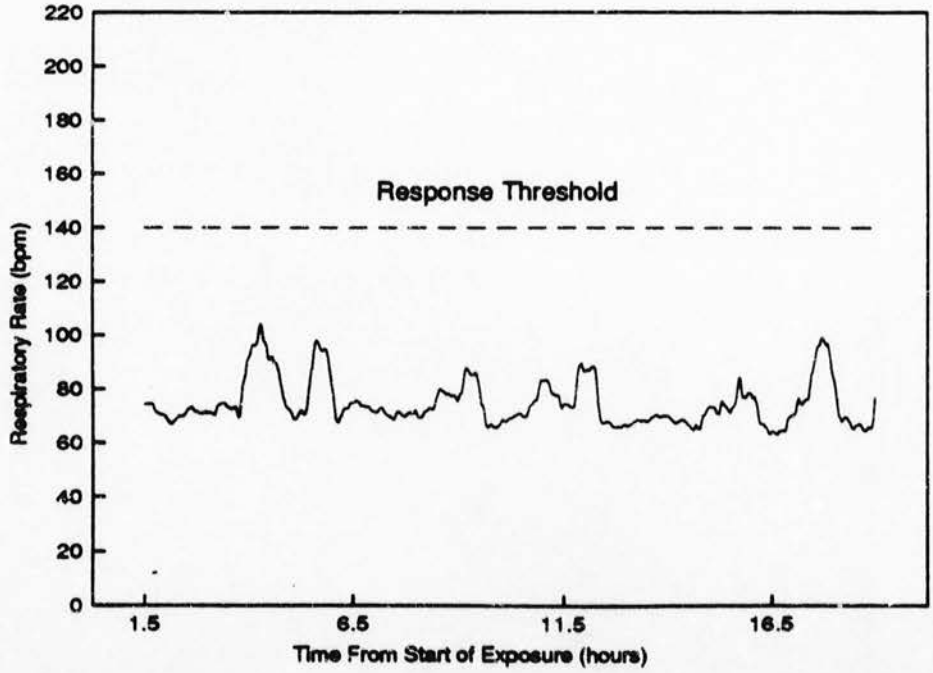


Study Number 92-344-PP

Animal # 218: Sham Sensitization

Figure: 96 2,6-TDI Challenge Week 2

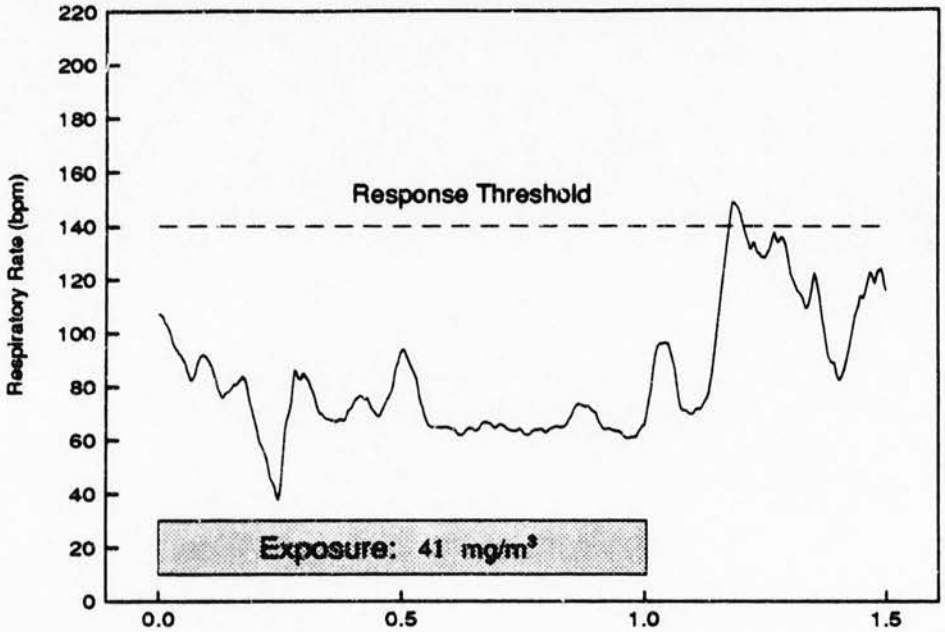


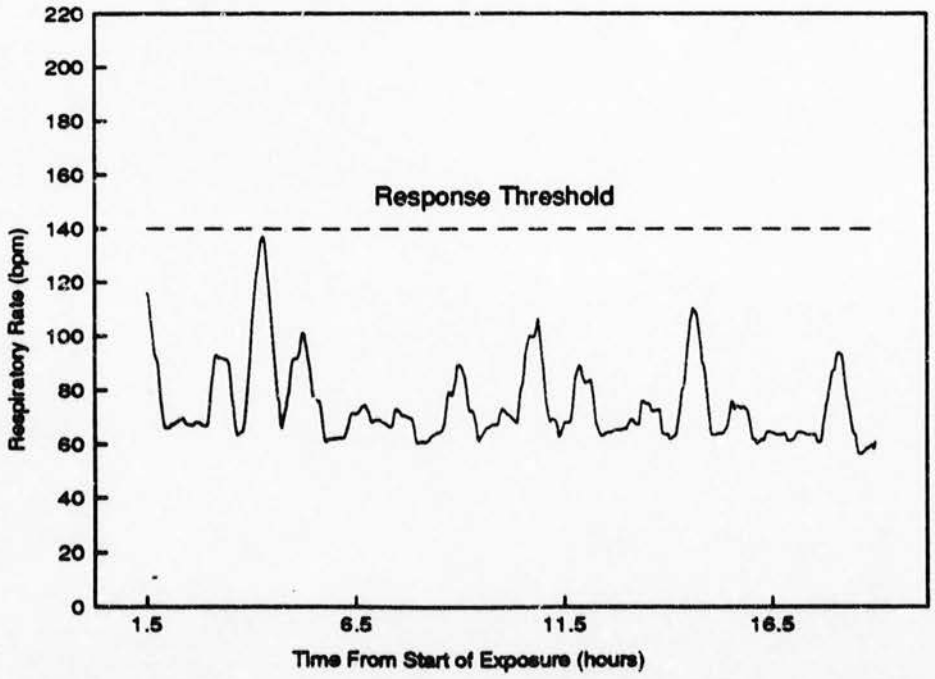


Study Number 92-344-PP

Animal # 207: Sham Sensitization

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

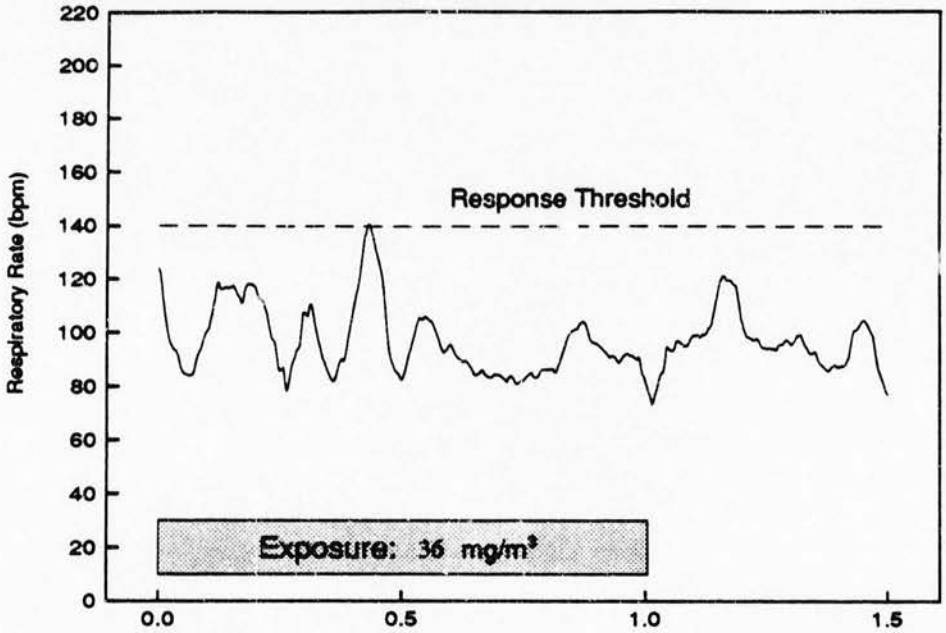


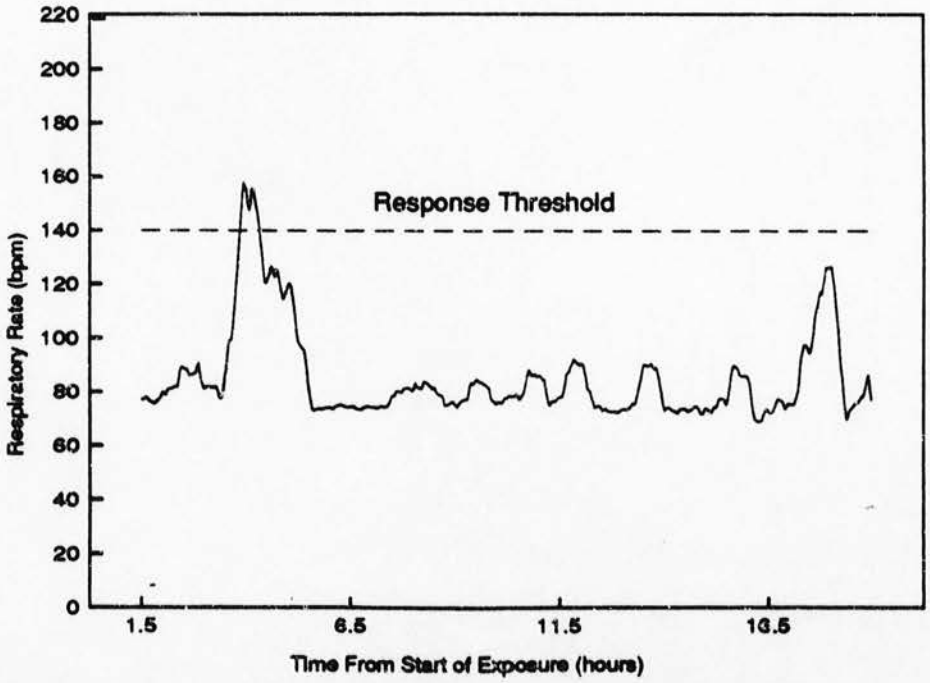


Study Number 92-344-PP

Animal # 208: Sham Sensitization

Figure: 98 2,6-TDI-GSA Challenge Week 3

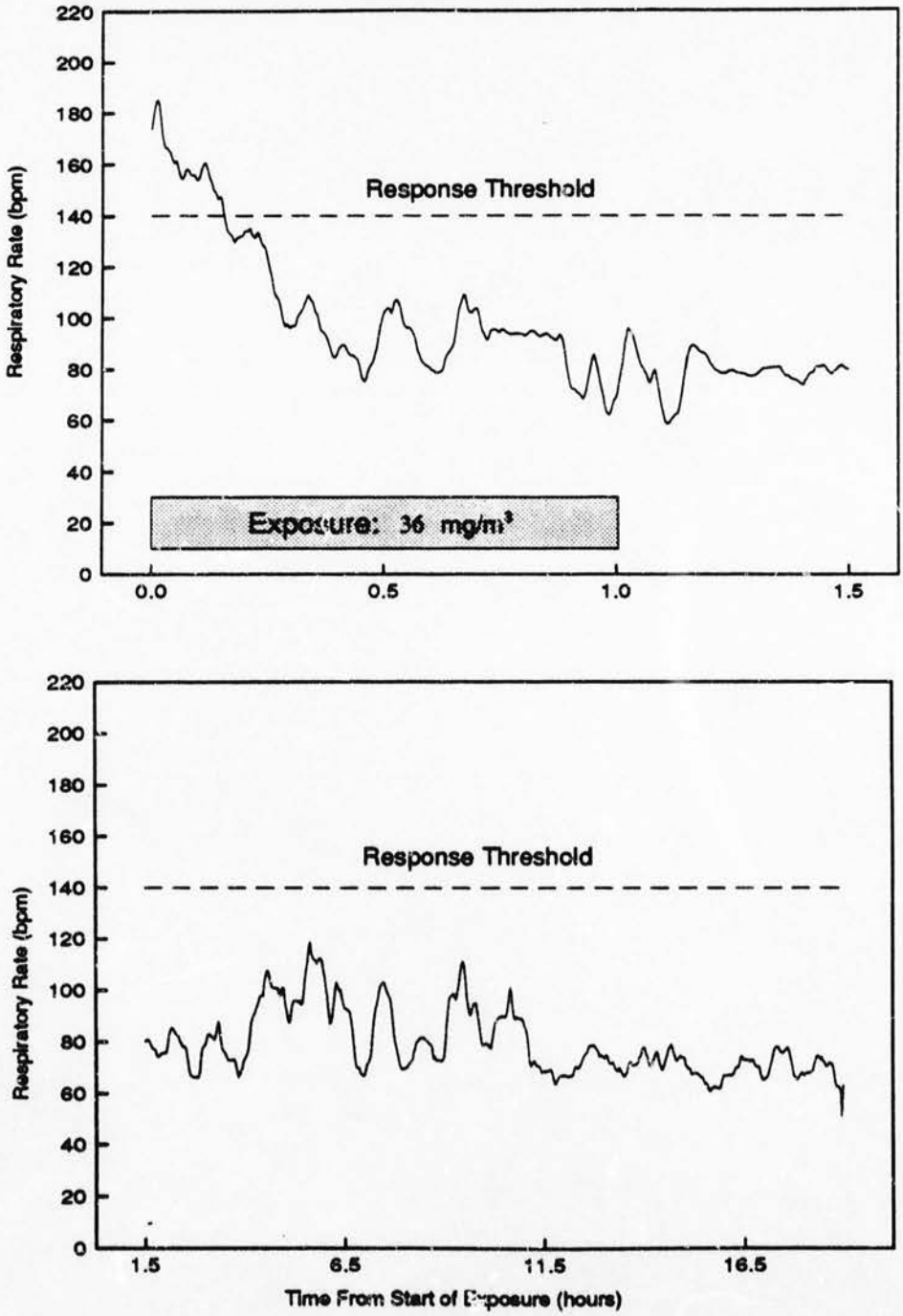




Study Number 92-344-PP

Animal # 209: Sham Sensitization

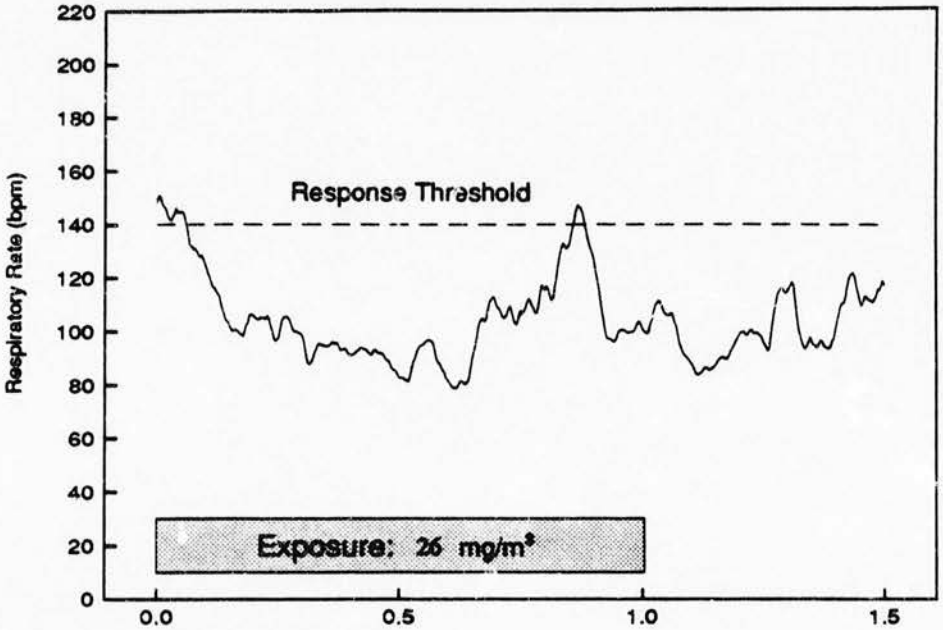
Figure: 99 2,6-TDI-GSA Challenge Week 3

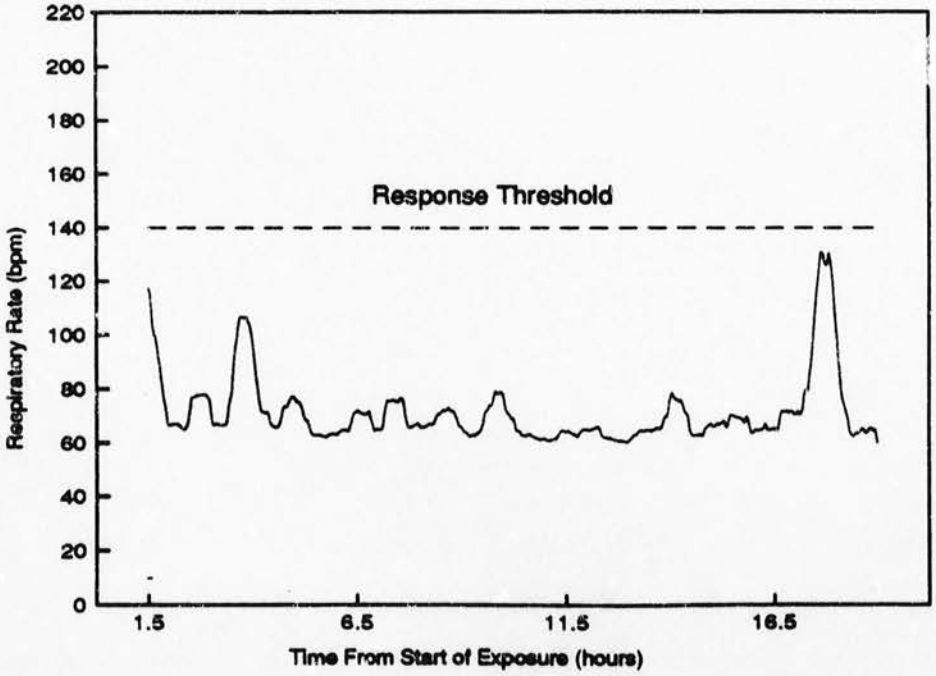


Study Number 92-344-PP

Animal # 210: Sham Sensitization

Figure: 100 2,6-TDI-GSA Challenge Week 3

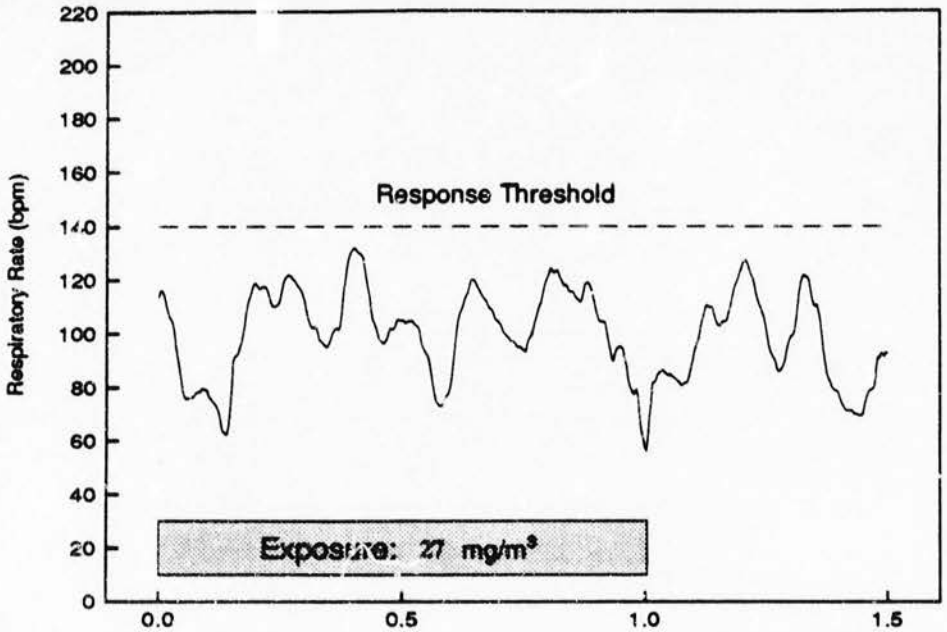


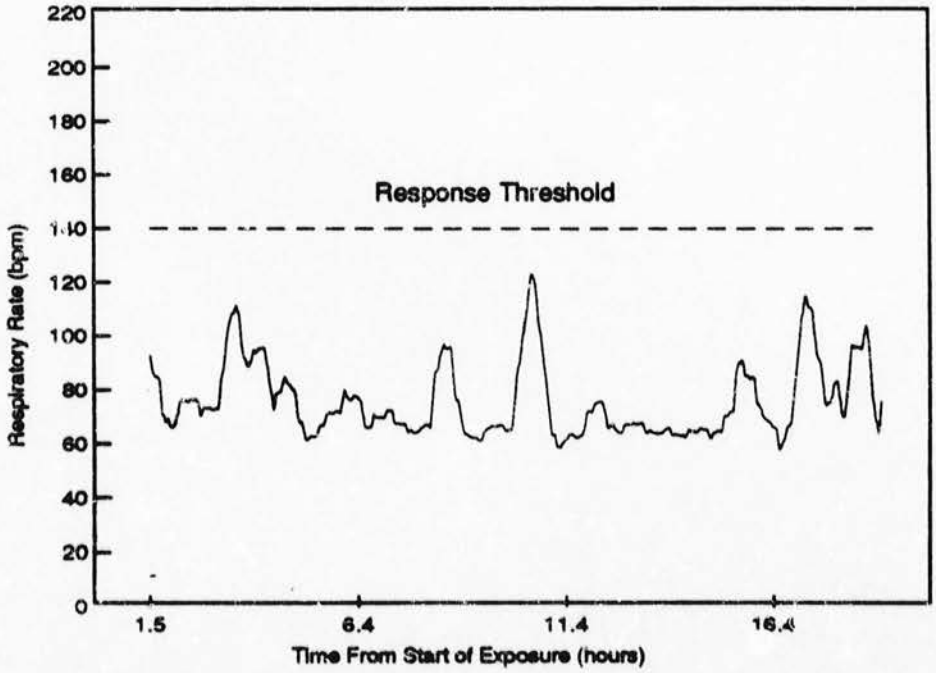


Study Number 92-344-PP

Animal # 211: Sham Sensitization

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

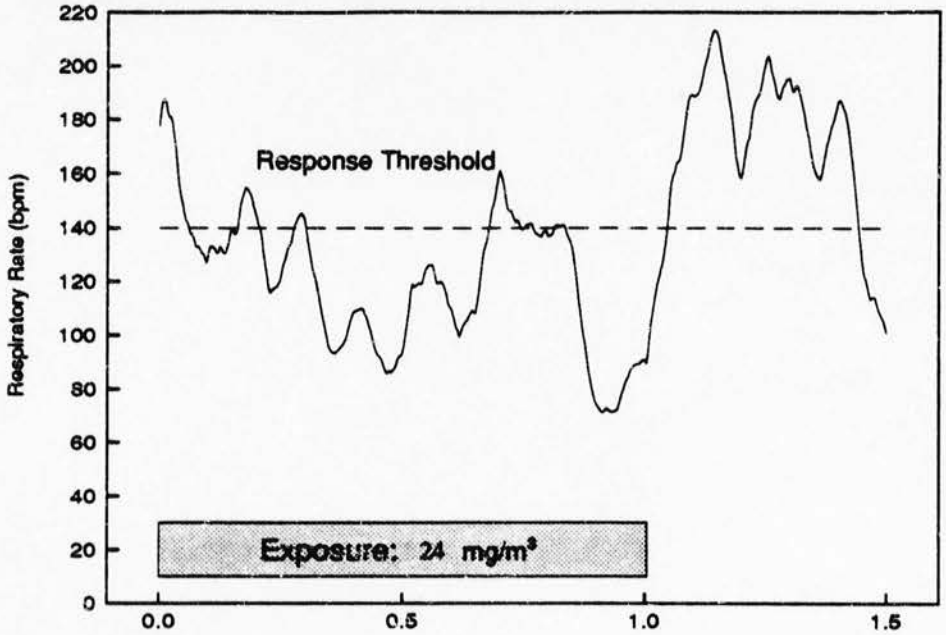


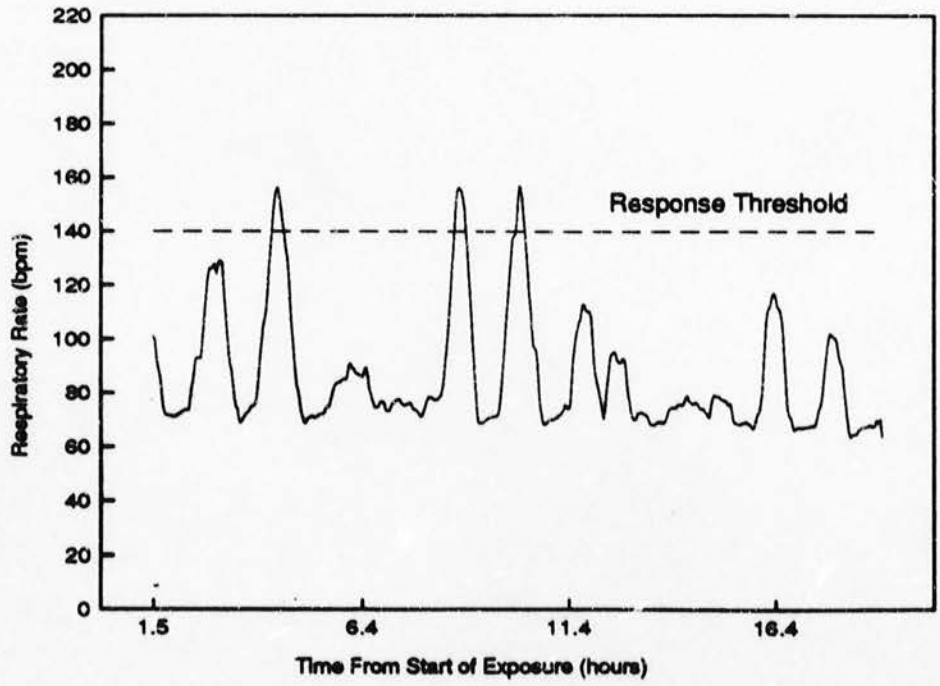


Study Number 92-344-PP

Animal # 212: Sham Sensitization

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

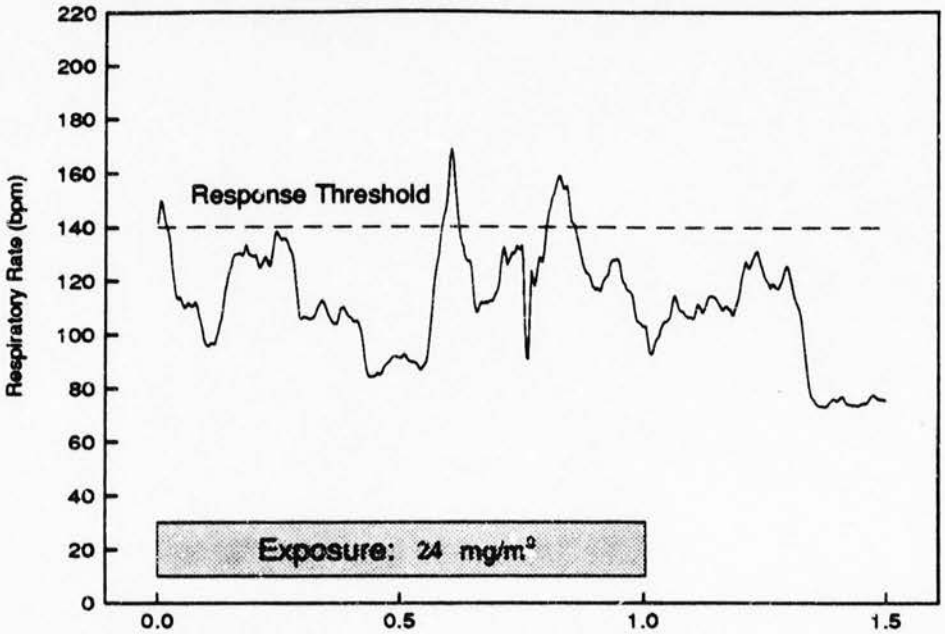


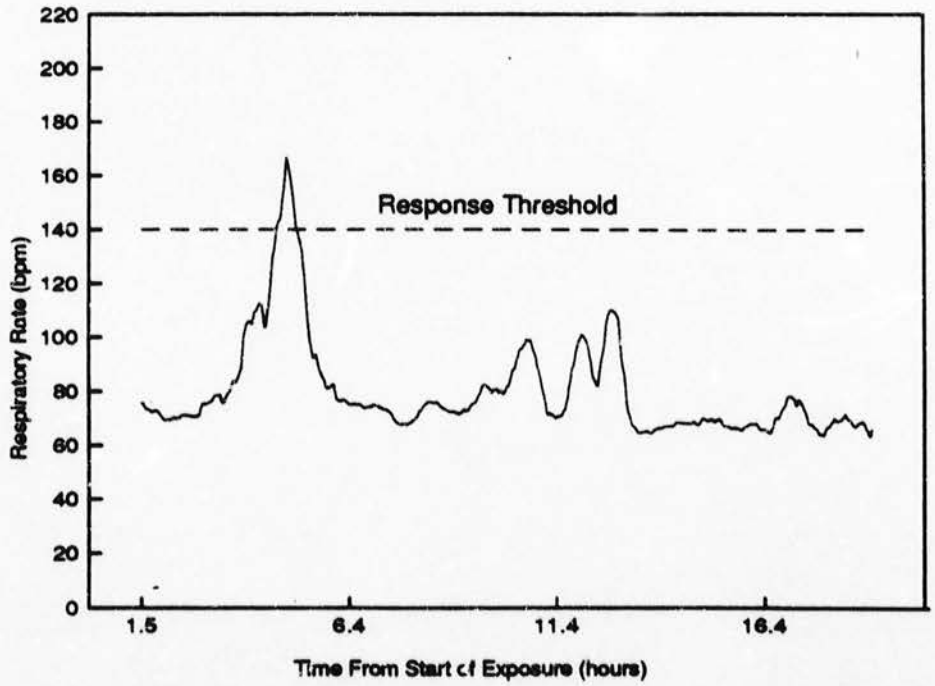


Study Number 92-344-PP

Animal # 213: Sham Sensitization

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

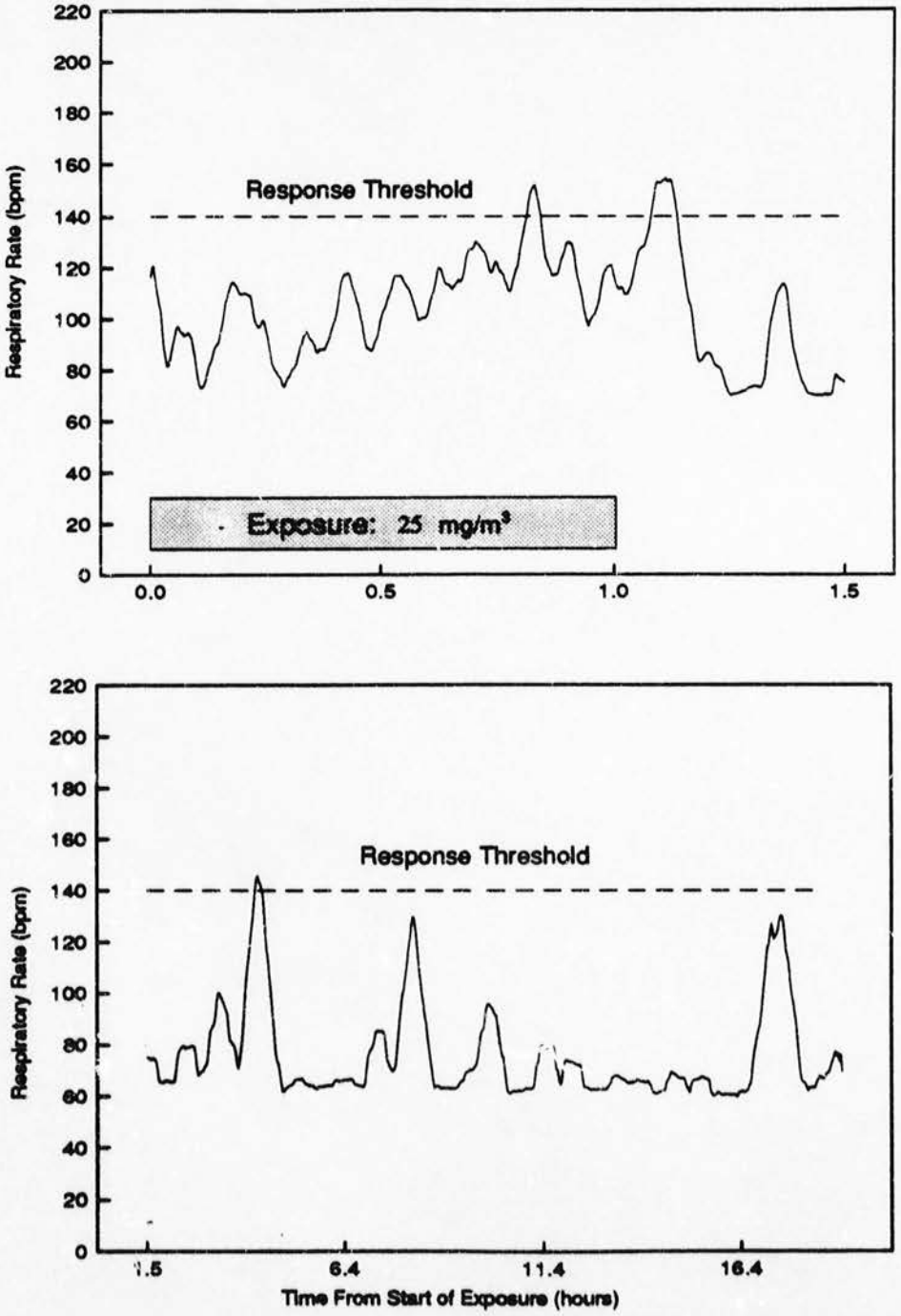




Study Number 92-344-PP

Animal # 214: Sham Sensitization

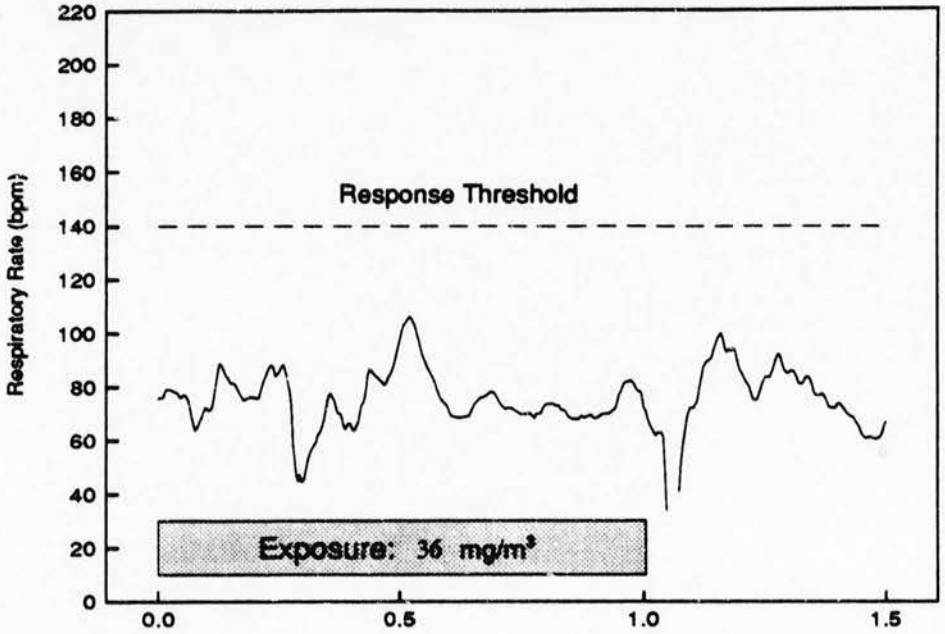
Figure: 104 2,6-TDI-GSA Challenge Week 3

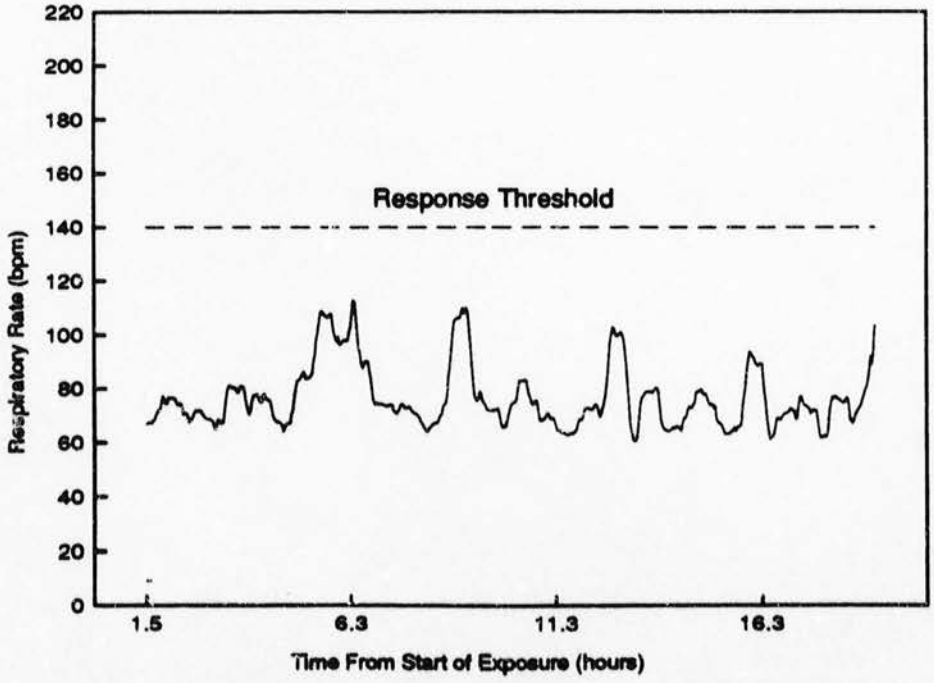


Study Number 92-344-PP

Animal # 215: Sham Sensitization

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

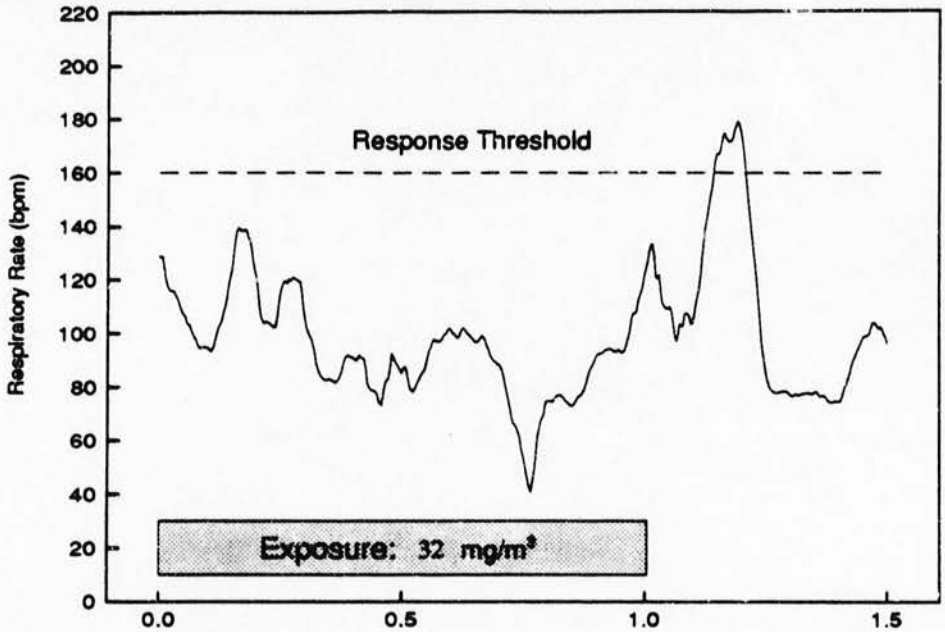


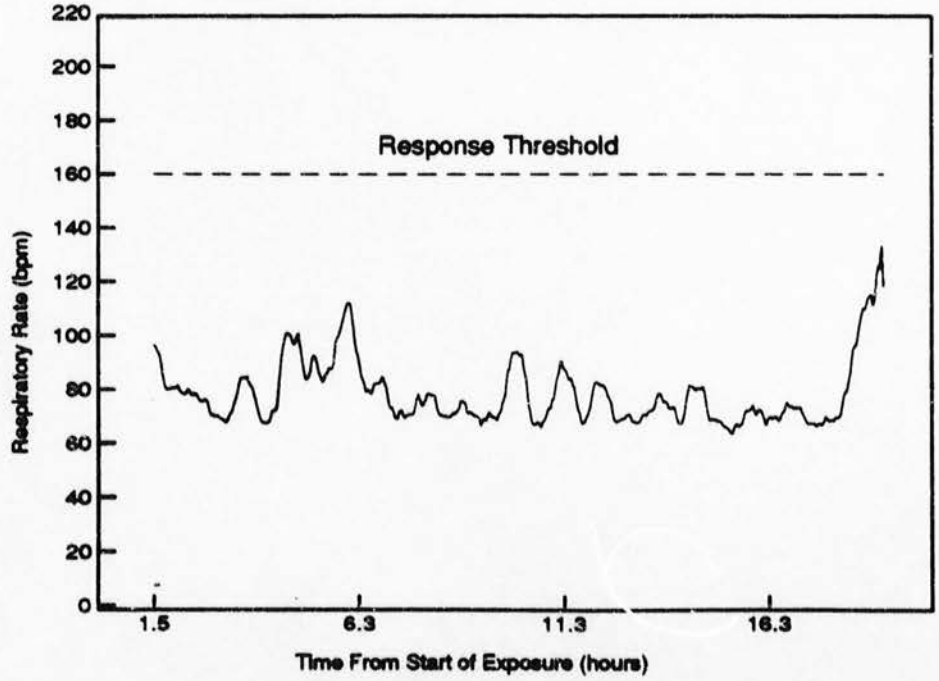


Study Number 92-344-PP

Animal # 216: Sham Sensitization

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

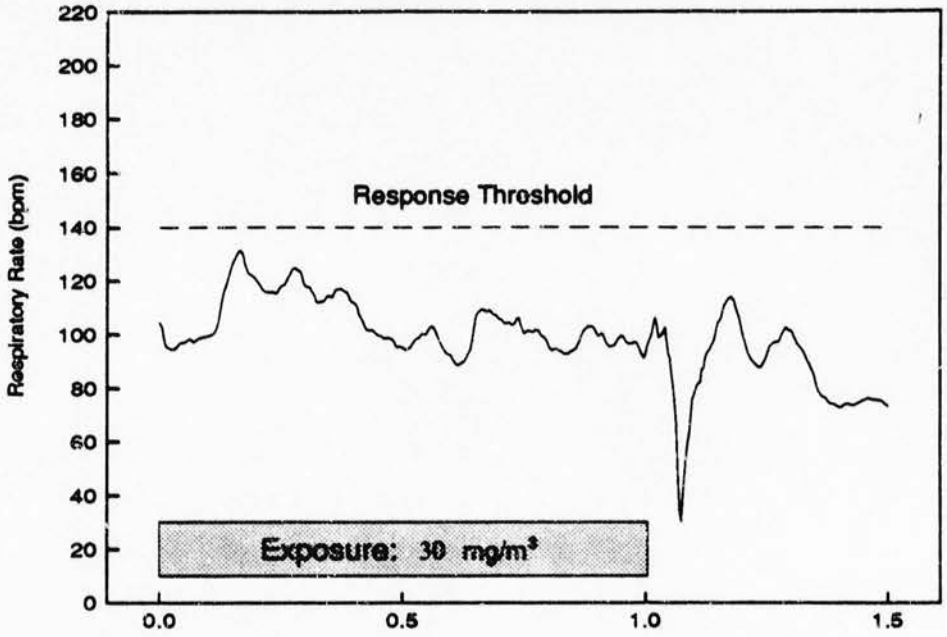


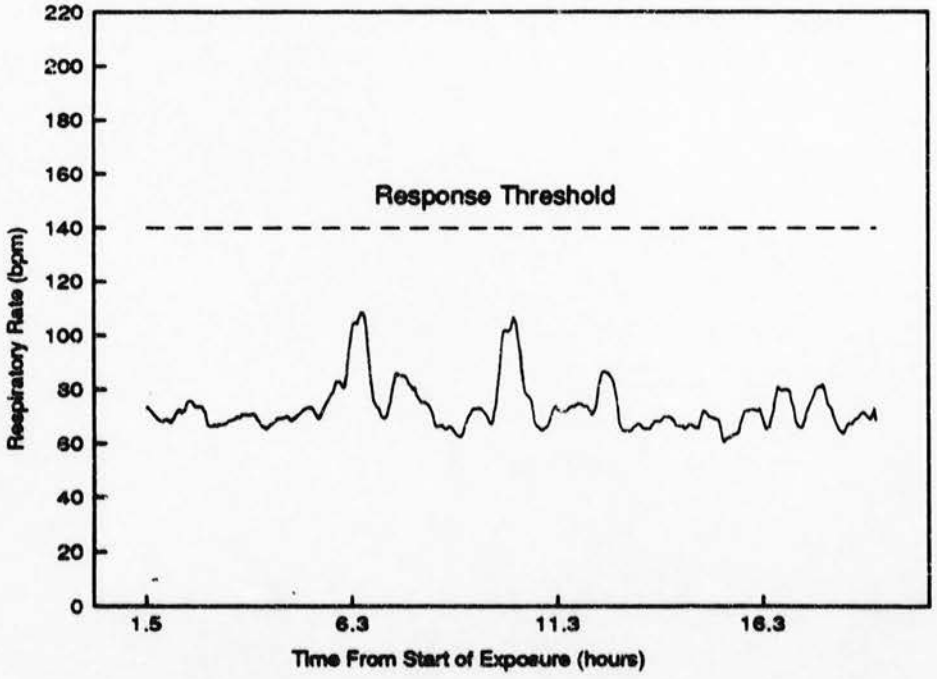


Study Number 92-344-PP

Animal # 217: Sham Sensitization

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

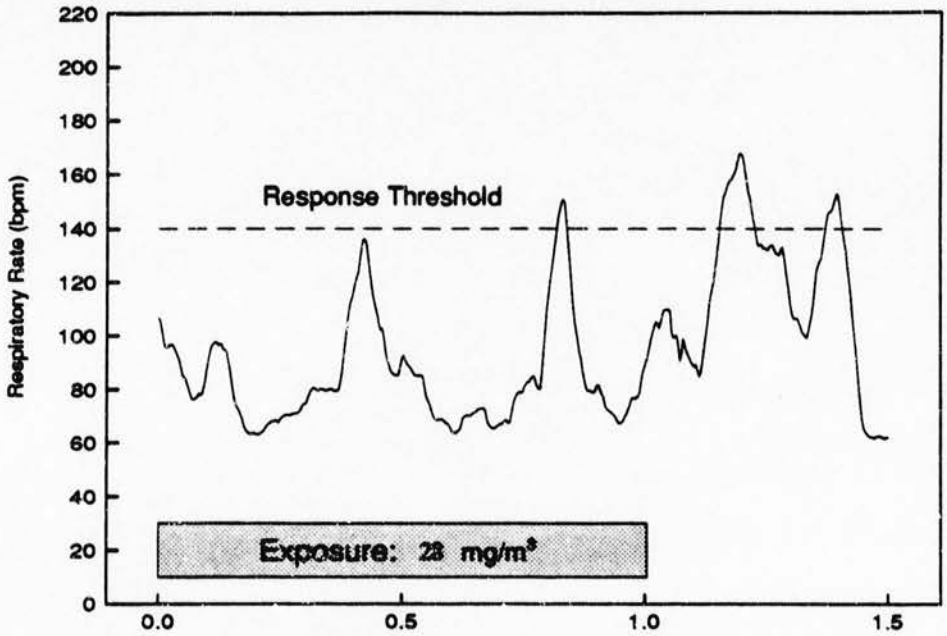


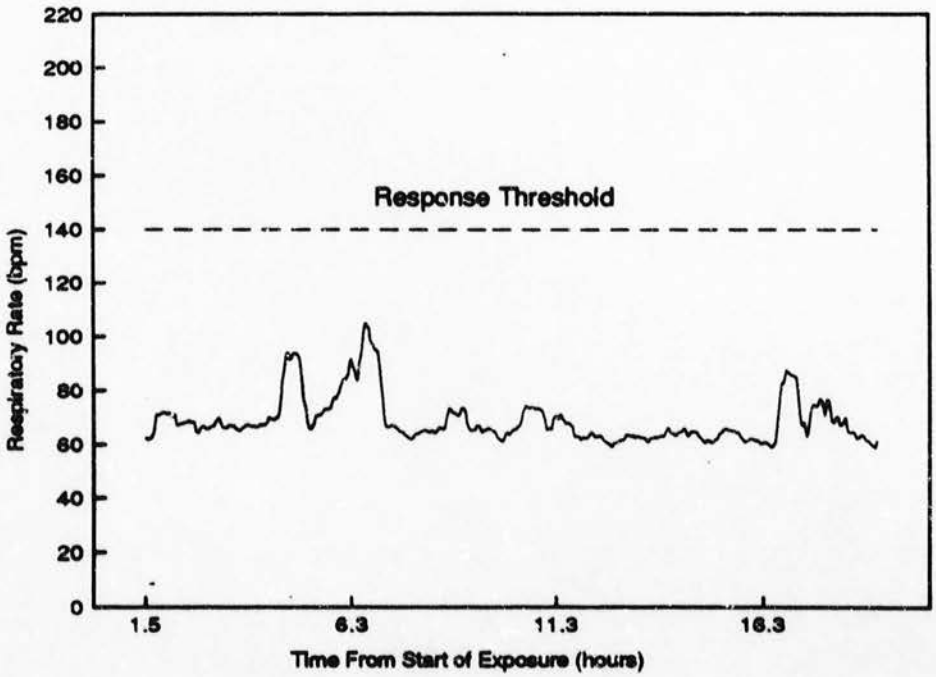


Study Number 92-344-PP

Animal # 218: Sham Sensitization

Figure: 108 2,6-TDI-GSA Challenge 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	434
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-PF

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.

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/m^3)	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

	Animal No.											
Challenge Wk.	222	223	224	225	226	227	228	229	230	231	232	233
Immediate or Delayed Responses												
Week 1	-/D	-/D	-/-	-/-	-/-	-/-	-/D	-/-	-/-	-/O	-/-	-/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
O = 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
0 = No data

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