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CHEMICAL MANUFACTURERS ASSOCIATION

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Langley A. Spurlock, Ph.D., CAE
Vice President, CHEMSTAR

September 13, 1995

Dr. Lynn Goldman
Assistant Administrator
Office of Prevention, Pesticides and Toxic Substances TS-7101
Environmental Protection Agency
401 M Street, SW, Room 637, East Tower
Washington, DC 20460

Dear Dr. Goldman:

The Chemical Manufacturers Association makes available to the public and appropriate government agencies final reports of environmental, health and safety research that it manages. In keeping with this policy, the following recently completed reports are enclosed:

- Test Atmosphere Development for Inhalation Exposures to Phthalic Anhydride Dust and Vapor
- Pulmonary Sensory Irritation Study (RD50) of Phthalic Anhydride Vapor in the Rat
- Pulmonary Sensory Irritation Study (RD50) of Phthalic Anhydride Dust in the Rat

These reports do not include confidential information.

If you have any questions, please call Marian Stanley of my staff at 202/887-1207.

Sincerely,

Charlie Auer
OPPT



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**TEST ATMOSPHERE DEVELOPMENT FOR INHALATION EXPOSURES TO
PHTHALIC ANHYDRIDE DUST AND VAPOR**

FINAL REPORT

Performing Laboratory:

**IIT Research Institute
Life Sciences Department
10 West 35th Street
Chicago, IL 60616**

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Sponser by:

**Chemical Manufacturers Association
Phthalate Ester Panel
2501 M Street, NW
Washington, DC 20037**

July 1995

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**TEST ATMOSPHERE DEVELOPMENT FOR INHALATION EXPOSURES TO
PHTHALIC ANHYDRIDE DUST AND VAPOR**

FINAL REPORT

Report date:

July 1995

IITRI Project No. L08552

Performing Laboratory:

**IIT Research Institute
Life Sciences Department
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Submitted to:

**Chemical Manufacturers Association
2501 M Street NW
Washington, DC 20037**



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COMMITMENT TO EXCELLENCE

FOREWORD

This report, entitled "Test Atmosphere Development for Inhalation Exposures to Phthalic Anhydride Dust and Vapor" (IITRI Project No. L08552), was conducted at IIT Research Institute for the Chemical Manufacturers Association (CMA), 2501 M Street NW, Washington, DC 20037. The Sponsor's Representative was Marian K. Stanley, Manager, Phthalate Esters Panel, Chemical Manufacturers Association. The technical advisor representing CMA was Dr. Michael W. Gili, Director of Toxicology, Toxicology/Regulatory Services (TRS). Principal contributors to the experimental studies and preparation of this report were Dr. Narayanan Rajendran, Aerosol Scientist, and Messrs. Stanley C. Vana and Scott Garthwaite, Inhalation Engineers.

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7-27-95

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7/27/95

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Date

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1. SUMMARY

A series of experiments were conducted to evaluate the feasibility of generating phthalic anhydride dust and vapor atmospheres for possible future inhalation toxicity studies in rats. For phthalic anhydride vapor atmospheres, phthalic anhydride flakes were heated in a round bottom flask and phthalic anhydride vapor in the head space of the flask was mixed with chamber supply air, filtered, and fed into the inhalation chamber. The vapor generation system operating parameters were adjusted to give the maximum attainable chamber concentration of phthalic anhydride vapor, absent of phthalic anhydride aerosols, while maintaining acceptable inhalation chamber temperature and air flow for animal toxicity studies. A maximum phthalic anhydride vapor concentration of 0.011 mg/l was achieved. No phthalic anhydride dust or phthalic acid were detected in the inhalation chamber.

For generating phthalic anhydride dust atmospheres, pneumatic dispersion and evaporation-condensation generation techniques were evaluated in an attempt to generate phthalic anhydride dust concentrations of 2 mg/l with mass median aerodynamic diameters (MMAD) $\leq 4 \mu\text{m}$. These parameters were selected to comply with the US EPA Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies (February 1994). While it was possible to generate aerosols with the pneumatic dispersion method, the highest dust concentration achievable was 0.6 mg/l with an MMAD of 4 to 5 μm . Efforts to increase the aerosol concentration output of the pneumatic dispersion technique by use of a jet mill and increased feed rate, and to reduce the MMAD to less than 4 μm by use of various grinding techniques were unsuccessful.

The evaporation-condensation method produced aerosols containing long needle-like particles (several hundred microns in length) which plugged up the inhalation chamber exhaust port in our one to two hour test runs. Therefore, the pneumatic generation technique was selected as the method for aerosolizing the phthalic anhydride test material.

2. MATERIALS, METHODS, AND RESULTS

2.1. Test Substance

Phthalic anhydride (CAS No. 85-44-9; supplied by Stepan Company, Northfield, IL, lot number 2282), in the form of white flakes, was received October 12, 1994 and was stored in the original container (5 gallon plastic pail) at room temperature (approximately 22 °C). Analyses of the test substance and attendant documentation pertaining to the characterization, purity, and stability of the test substance were the responsibility of the Sponsor. Information supplied by the Sponsor indicated that the phthalic anhydride was 99.9% pure.

2.2. Test Atmosphere Generation and Monitoring

2.2.1. Phthalic Anhydride Vapor

2.2.1.1. Vapor Generation

Phthalic anhydride flake was placed into a glass 3000 ml round bottom flask and heated to approximately 140 °C. The temperature was held constant using a temperature controller (Digi-Sense, Cole-Parmer Instrument Company, Chicago, IL) connected to a heating mantle which was located around the bottom of the flask. At 140 °C the test substance was completely melted and had the appearance of a clear liquid.

Three ground glass joints were located in the top of the glass round-bottom flask. One was used for a thermocouple which was connected to the temperature controller, one was used as an inlet for compressed air, and the third was connected to a glass elbow which was connected to the chamber supply air (Figure 1). The glass elbow was wrapped with heat tape to prevent condensation of the hot vapor/aerosol before it entered the chamber supply air. Chamber supply air was filtered through a HEPA and charcoal filter and then directed through a 2-inch diameter glass pipe where it mixed with the output of the test substance generator. After the generator, but before entering the chamber, a HEPA filter was used to collect test substance aerosol particulates. The supply air was then directed into the top of the chamber. Chamber exhaust was filtered through both HEPA and charcoal filters before being released to the outside environment.

The generation system operating parameters were adjusted to give the maximum obtainable chamber concentration of phthalic anhydride vapor with no aerosol component, and to use exposure chamber environmental conditions that would be realistic for possible longer-term studies, (i.e. the chamber temperature should not be significantly elevated above ambient levels). The chamber supply air inlet through the glass pipe was examined visually while the compressed air flowrate through the generator was adjusted. As the air flowrate was increased visible particulate aerosol was present in the supply air stream, indicating that a saturated vapor concentration of phthalic anhydride had been achieved. The air flowrate was set so that visible particulate was present in the supply air stream, but not so much that the HEPA filter located between the generator and the chamber would become clogged during exposure. The air flowrate through the generator was approximately 0.2 liters/min.

2.2.1.2. Vapor Monitoring

The test atmosphere was monitored for phthalic anhydride vapor and aerosol using a sampling train consisting of an open-face glass fiber filter (Number 66075, Gelman Sciences, Inc., Ann Arbor, MI) followed by a tenax adsorbent tube (ORBO 402, Supelco, Inc., Bellefonte, PA). The total sample volume was determined using a dry gas meter. Following collection, the filter and adsorbent tube were analyzed

separately. Using this sampling regimen, test substance aerosol/particulate is trapped on the glass fiber filter and vapor is trapped on the adsorbent tube. Phthalic anhydride and phthalic acid in the test atmosphere was measured using a modified gas chromatographic (GC) method (Kruglov EA, Tsypysheva LG, Kharlamovich GD and Portnova TV, 1980. *Zh. Anal. Khim.*, Vol. 35, 1980, pp. 122-127).

2.2.1.3. Results

The highest vapor concentration attainable by the vapor generation system was 0.01 mg/l (\approx 2 ppm). GC analysis of the vapor confirmed that there was no phthalic acid present.

2.2.2. Phthalic Anhydride Dust

2.2.2.1. Particle Size Reduction

When dispersing a powder to produce an aerosol suitable for inhalation exposure of rodents, the powder must consist of sufficiently fine particles to generate aerosols with the required MMADs (typically less than 4 μ m according to US EPA Interim Policy of February 1994). Because of the technical difficulties encountered in the milling of phthalic anhydride flakes, three grinding techniques were tried:

- Ambient temperature grinding with a Centrifugal Mill (F. Kurt Retsch GmbH & Co., Germany)
- Ambient temperature grinding with a Cutting Mill (F. Kurt Retsch GmbH & Co., Germany)
- Cold grinding with a Cutting Mill

Initially, a Centrifugal Mill (F. Kurt Retsch GmbH & Co., Germany) was used to grind the phthalic anhydride flakes. The heat generated in the centrifugal milling process promoted agglomeration of phthalic anhydride flakes in the grinding chamber with eventual plugging of the mill outlet. Therefore, an alternate grinder, a Cutting Mill (F. Kurt Retsch GmbH & Co., Germany), was evaluated next. The Cutting Mill pulverizes large particles with the use of rotating blades until the particles are small enough to pass through a separation screen. Phthalic anhydride flakes were fed directly into the Cutting Mill. The Cutting Mill functioned without any operational problems and produced a fine and free-flowing powder (characterization based on qualitative observations). The Cutting Mill was also used for cold grinding of phthalic anhydride as described later in this report.

2.2.2.2. Phthalic Anhydride Aerosol Generated by Pneumatic Powder Dispersion.

Aerosol Generation. The milled test material was aerosolized by a pneumatic powder dispersion system (Figure 2). Pneumatic dispersion is a commonly used technique to aerosolize fine powders. The two major components of the aerosol generation system are a screw feeder to feed the bulk phthalic anhydride

powder continuously at a constant rate, and a jet mill to disperse the metered powder and form the aerosol. A screw feeder (Model 101, Accurate, White Water, WI) with a 1/4" diameter helix screw was used in this study. The screw feeder operates by conditioning the test material to constant bulk density by a flexible hopper-paddle mechanism and is designed to provide uninterrupted feed at high rates and material-feeding accuracy. In addition a mechanical vibrator was attached to the feed tube to prevent powder bridging (i.e., formation of voids over the feed screw). The metered powder was aerosolized by a jet mill type disperser (Jet-o-Mizer; Fluid Energy Corp., Hatfield, PA). The jet mill uses compressed air to deagglomerate and aerosolize the bulk material from the screw feeder.

Aerosol leaving the jet mill was passed through a one-stage impactor and a vertical elutriator to eliminate large particles and agglomerates prior to introduction into a 0.5 m³ stainless steel and glass Rochester-type inhalation exposure chamber. Conditioned room air, used as dilution/supply air, was passed through HEPA filters before entering the chamber. Chamber exhaust was passed through HEPA and activated carbon filters before being discharged to the outside environment.

Monitoring Aerosol Mass Concentration. Aerosol mass concentration of the phthalic anhydride was determined gravimetrically by weighing filter-collected aerosol samples on an analytical balance and measuring the corresponding volume of chamber air sampled with a dry gas meter. The gravimetric sampling train consisted of a pre-weighed glass fiber filter in series with a dry-gas meter and a constant-flow vacuum pump.

Monitoring Aerosol Particle Size Distribution. Aerosol particle size distribution in the chamber was measured with a Quartz Crystal Microbalance (QCM) Cascade Impactor (California Measurements Inc., Sierra Madre, CA). QCM is a cascade of ten inertial impactors which classify the particles in the range of 0.07 to 35.4 μm diameter according to their aerodynamic sizes.

Results. The pneumatic dispersion method resulted in an unstable aerosolization rate caused by solidification of phthalic anhydride in the jet mill probably due to the high dispersion energy imparted to the powder. Therefore, the jet mill was replaced with a venturi aspirator which provides a somewhat lower dispersion energy. The pneumatic aerosol generation system with the venturi aspirator performed satisfactorily in the test runs. However, the highest concentration obtainable, 0.6 mg/l, was significantly lower than the highest target level of 2 mg/l, as required by the EPA. The ranges of aerosol concentration and particle size distribution measurements obtained in the method evaluation test runs are shown in Table 1. The MMADs of the aerosol were larger than 4 μm (the targeted high limit for particle size) and individual measurements were in the range of 4 to 6 μm , with the Geometric Standard Deviations in the range of 2 to 3.5. These measurements showed that generally approximately 20 to 30% of the aerosol mass was distributed in the $\leq 3\mu\text{m}$ diameter range and 80 to 90% in the $\leq 10\mu\text{m}$ range.

Qualitative chemical analysis of selected filter samples demonstrated that the test aerosol was phthalic anhydride with very little ($\leq 1\%$) phthalic acid. Quantitative results of the chemical composition of

aerosol produced by pneumatic dispersion in a subsequent respiratory irritation study in rats (IITRI Project No. L08552, Study No. 3) confirmed that 99% of the dust atmosphere produced by this method was present as phthalic anhydride.

Cold Grinding. In an effort to reduce the MMAD of the dust, cold grinding of phthalic anhydride test material was conducted. The cold temperature was expected to increase the hardness of phthalic anhydride and therefore improve its grindability and produce a finer powder than ambient temperature grinding. For cold grinding, frozen plugs of phthalic anhydride obtained by solidifying molten material at -70°C were introduced into the Cutting Mill. The milling was performed at room temperature or in a walk-in freezer at -20°C . Aerosol mass concentration and particle size measurements demonstrated that the test aerosols generated with the cold ground material were low in concentration (0.20 to 0.33 mg/l) with MMADs in the range of 3.5 to 4.9 μm , similar to the test aerosol generated with material ground at ambient temperature. Therefore, the cold grinding method was not further evaluated.

2.2.2.3. Phthalic Anhydride Aerosol Generated by Evaporation-Condensation Method.

Aerosol Generation. After having encountered the technical limitations of the pneumatic generation method in aerosolizing phthalic anhydride, the alternate aerosol generation approach of evaporation-condensation was tried. This aerosol generation technique relies on condensation to produce copious quantities of submicron size particles by rapid cooling of a saturated vapor-laden gas stream (Figure 3). The phthalic anhydride vapor-laden air stream was generated by passing filtered compressed air over a bed of heated phthalic anhydride test material contained in a glass flask. The resulting saturated stream was transported to the inlet of the exposure chamber through a heated tube and was subsequently mixed with conditioned filtered air to produce the aerosols. Test runs were conducted at phthalic anhydride temperatures ranging from approximately 100 to 130°C . The evaporation surface area and airflow rate (25-35 lpm) were also varied. Evaporation surface area was changed by using a larger quantity of test material (surface area was not measured).

Monitoring Aerosol Mass Concentration and Particle Size Distribution. Aerosol mass concentration and particle size distribution were measured as described for the pneumatic dispersion method evaluation earlier in this report.

Results. The highest aerosol mass concentration obtained by the evaporation-condensation method was approximately 0.4 mg/l (Table 1). Chemical analysis of aerosol samples indicated that the test atmosphere contained mainly phthalic anhydride mixed with $\leq 1\%$ of phthalic acid. Photomicrographs were obtained from randomly selected samples for qualitative particle size evaluation. The aerosol obtained by the evaporation-condensation method consisted of long needle-like structures with submicron size dendrites as shown in the photomicrographs of Figure 4. Typically, the needles were several hundred microns in length and 5 to 10 μm in diameter (geometric size). The aerodynamic size as measured by the QCM cascade impactor, showed a bimodal distribution with $\sim 28\%$ of the mass at 0.6

μm mode and ~72% of the mass at 4.5 μm mode. Since the deposition characteristics of these long needle-like particles are based primarily on geometric size rather than aerodynamic size, these particles are not considered to be respirable in the rodent.

The phthalic anhydride needles generated by the evaporation-condensation method deposited on the interior surfaces of the exposure chamber (exposure cage, chamber walls, etc.) from the onset of test runs and led to airflow reduction and/or blockage in the chamber exhaust after approximately one to two hours of test run time (see photographs in Figure 5 for typical accumulation of needles after about 45 minutes of test run). Variations of the evaporation temperature, surface area of test material, and carrier air flow rate did not curtail the formation of these needles. Therefore, the evaporation-condensation technique was considered unsuitable to generate phthalic anhydride dust for inhalation exposure studies.

3. DISCUSSION

The maximum attainable vapor concentration of phthalic anhydride was approximately 0.01 mg/l (\approx 2 ppm). This maximum attainable vapor concentration was consistent with the calculated theoretical maximum concentration (1 ppm) based on phthalic anhydride vapor pressure and acceptable exposure chamber temperature for animal toxicity studies (see addendum for calculation). GC analysis of the vapor confirmed that there was no phthalic acid present.

Aerosolization of phthalic anhydride to generate test atmospheres suitable for acute inhalation exposure studies presented technical problems:

- unsuitable grinding properties (due to surface softness of the test material)
- sporadic aerosolization and plugging of the dispersion system (due to the flake-like structure of the test material)

Two candidate aerosol generation techniques were evaluated: pneumatic dispersion and evaporation-condensation. While aerosols could be generated with pneumatic dispersion method, the highest aerosol concentration achievable was 0.6 mg/l with particle size (MMAD) in the range of 4 to 5 μm , in contrast to the target concentration and particle size of 2 mg/l and \leq 4 μm required by the US EPA Interim Policy of February 1994. The evaporation-condensation method produced aerosols containing long needle-like particles (several hundred microns in length) which plugged up the inhalation chamber exhaust port in our one to two hour test runs. Based on this evidence, the pneumatic generation technique was selected as the method for aerosolizing the phthalic anhydride test material. Efforts to increase the aerosol concentration by use of a jet mill and increased feed rate, and to reduce the MMAD to less than 4 μm by use of various grinding techniques in combination with an appropriate aerosolization method were unsuccessful.

Theoretically, for a given set of exposure chamber operating conditions it should be possible to increase the aerosol concentration by increasing the generation system output (i.e. increasing the screw feeder rate in the pneumatic generation method, or increasing the evaporation rate in the evaporation-condensation method). However, such changes did not succeed with phthalic anhydride, probably because of its surface softness and hygroscopic nature. The highest attainable phthalic anhydride aerosol exposure concentration with pneumatic dispersion was 0.6 mg/l; when the feed rate to the generation system was further increased it promoted agglomeration in the venturi aspirator resulting in blockage of airflow through the disperser. Similarly, increased vapor output from the evaporator caused severe increase in formation of the needles and eventual plugging of exposure chamber exhaust making the evaporation-condensation technique unsuitable for generating phthalic anhydride test atmospheres. Of the two methods evaluated, the pneumatic powder dispersion technique worked better and was the more suitable for generating phthalic anhydride dust to produce a stable test atmosphere.

TABLE I

Summary of Test Airborne Development Data for Phthalic Anhydride Dust

Aerosol Generation Method	Particle Size Reduction Method	Test run	Aerosol Concentration, mg/l	Particle Size Distribution		Comments
				MMAD ^a , μ m	GSD ^b	
Pneumatic Dispersion	PA ^b flakes milled at ambient temperature	1 ^c	0.21	5.2	2.0	Sporadic aerosolization; system plugged with jet mill for both tests, and venturi aspirator at high concentration in one of two hours
		2 ^c	0.27	5.1	1.9	
		3	0.63	4.1	1.9	
		4	0.11	7.7	2.0	
		5	0.50	No data	No data	
	Frozen PA milled at ambient temperature	10	0.33	3.5	2.3	Sporadic aerosolization; no improvement over ambient temperature grinding
		11	0.20	3.9	2.8	
	Frozen PA milled in walk-in freezer (-20°C)	12	No data	4.9	2.6	Only particle size evaluation was performed
Evaporation-Condensation	Not Applicable	6 (130°C) ^d	0.53 0.25 0.16 0.10	Non-log normal size distribution; possibly Bimodal	Not applicable	Formation of needles in the chamber in all test runs
		7 (110°C)				
		8 (100°C) ^e				
		9 (120°C)				

^a MMAD: Mass Median Aerodynamic Diameter; GSD: Geometric Standard Deviation

^b Phthalic anhydride

^c Jet mill used to disperse the phthalic anhydride dust; all other tests used venturi aspirator

^d Temperature of test material

^e Mean of four samples collected under different operating conditions

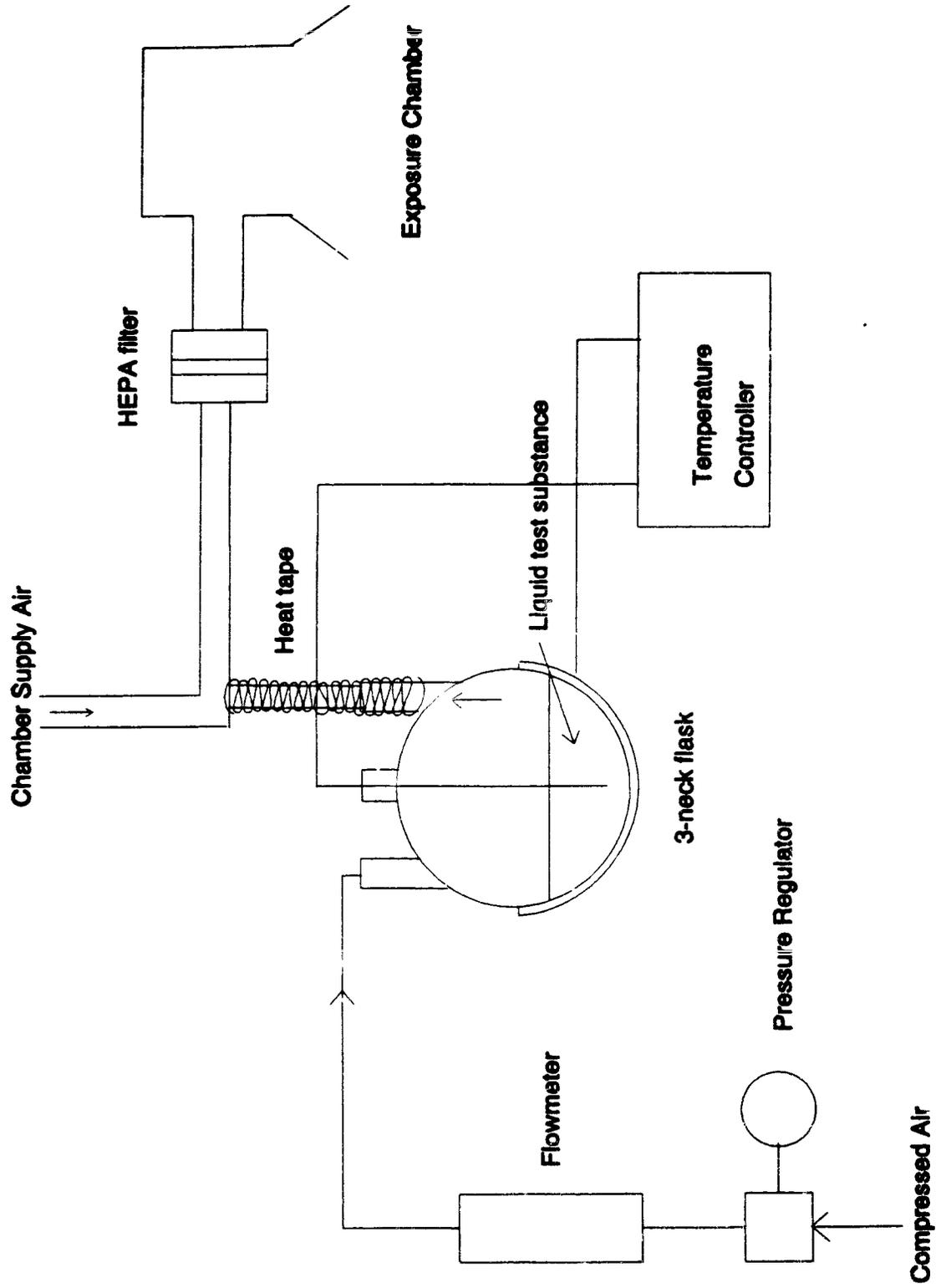


Figure 1. Vapor Generation System

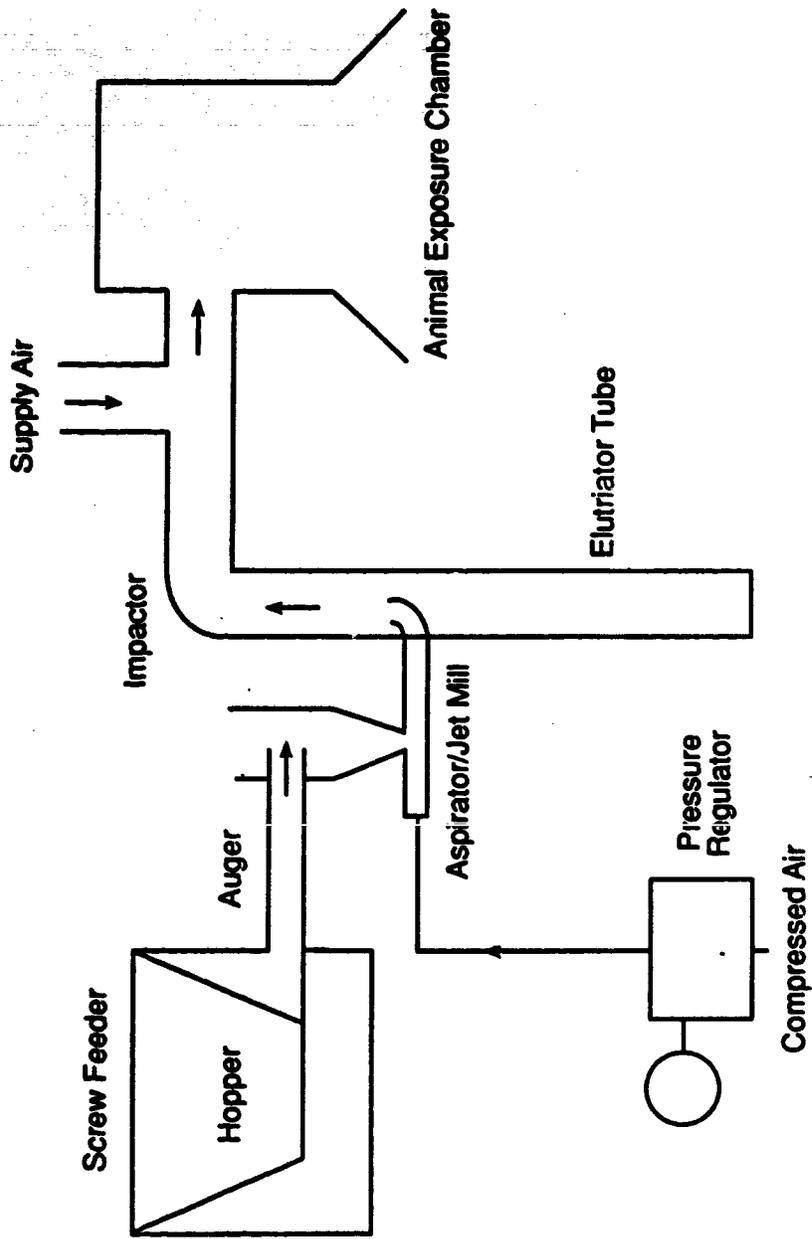


Figure 2. Pneumatic Dispersion System for Phthalic Anhydride Powder

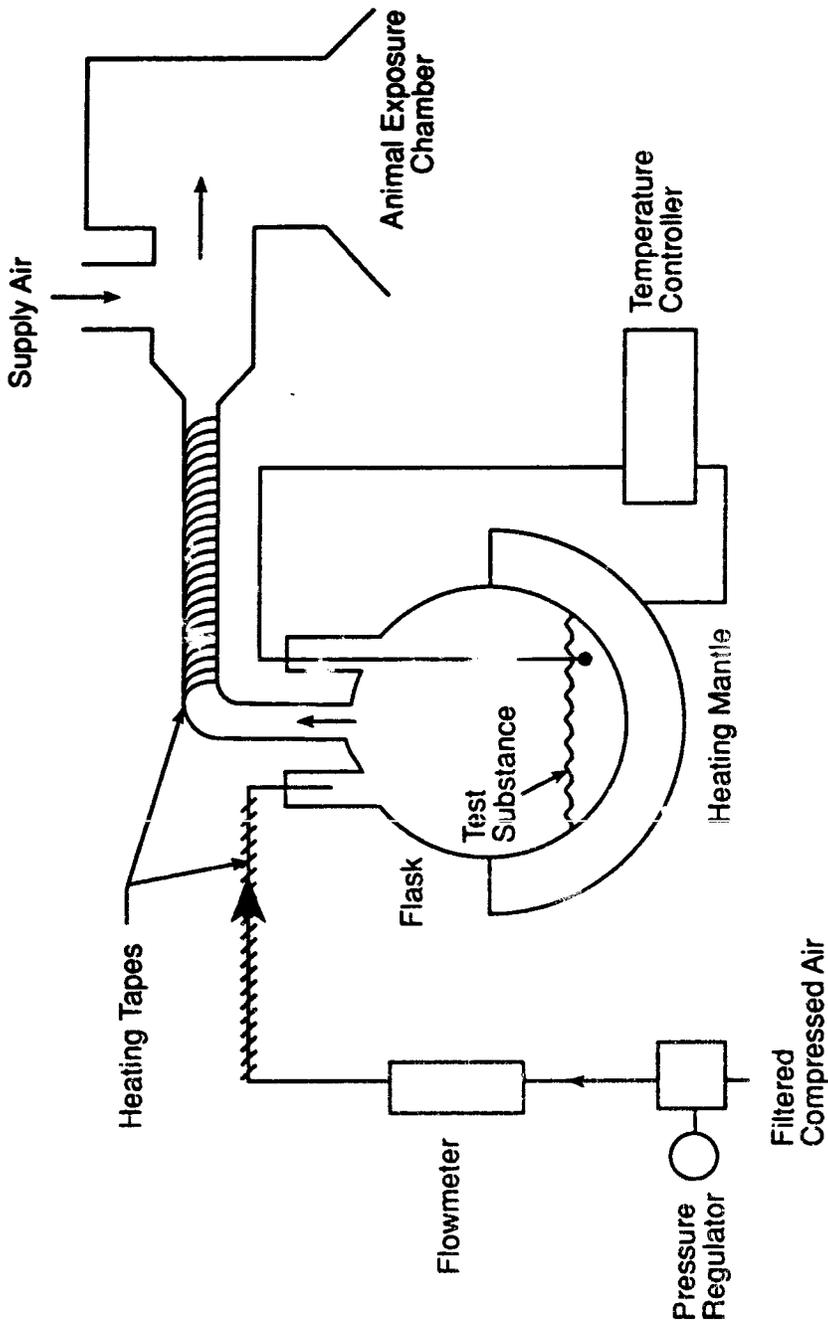
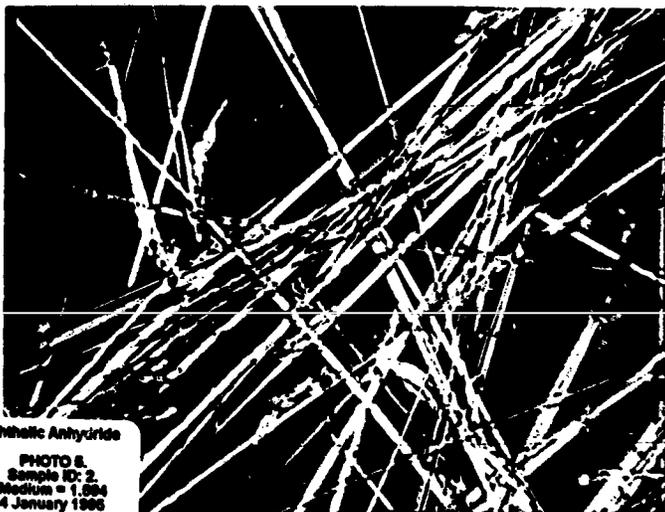


Figure 3. Evaporation-Condensation Aerosol Generation System



Phthalic Anhydride
PHOTO 3.
Sample ID: 1.
Medium = 1.894
4 January 1986
MAG = 100x

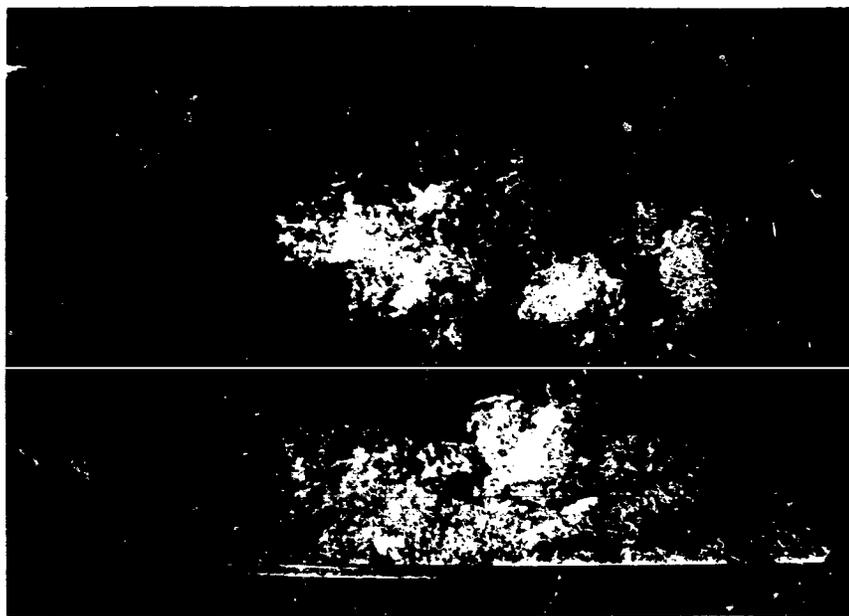


Phthalic Anhydride
PHOTO 6.
Sample ID: 2.
Medium = 1.894
4 January 1986
MAG = 100x

Figure 4. Photomicrographs of Phthalic Anhydride Aerosol Generated by Evaporation-Condensation



a) Deposition on the animal cage (run time \approx 45 minutes)



b) Accumulation at the bottom of the exposure chamber (run time \approx 45 minutes)

Figure 5. Deposition of Phthalic Anhydride Needles Generated by Evaporation-Condensation Technique at Dust Concentration of 0.25 mg/l

ADDENDUM

Calculation of the Theoretical Saturated Vapor Concentration of Phthalic Anhydride

The theoretical saturated vapor concentration of phthalic anhydride was calculated by dividing the vapor pressure of the test substance by the total pressure (760 mmHg) and multiplying by 1×10^6 (ppm conversion factor). Since data for the vapor pressure of phthalic anhydride at room temperature was not available, an estimate of the vapor pressure was obtained by extrapolation of data obtained from Perry's Chemical Engineers Handbook, 6th ed., McGraw-Hill, 1984. Vapor pressure versus temperature data was plotted as the logarithm of the vapor pressure versus $1/\text{absolute temperature (}^\circ\text{K)}$. Extrapolation of this data to room temperature (21°C or 294°K) indicated an approximate vapor pressure of 0.001 mmHg. At this vapor pressure the theoretical saturated vapor concentration is: $0.001/760 \times 1 \times 10^6 = 1.3$ ppm. Since this concentration was calculated by extrapolation, the 1.3 ppm should be considered a rough estimate of the saturated vapor concentration at room temperature.

**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC
ANHYDRIDE DUST IN THE RAT**

FINAL REPORT

Performing Laboratory:

**IIT Research Institute
Life Sciences Department
10 West 35th Street
Chicago, IL 60616**

Sponser by:

**Chemical Manufacturers Association
Phthalate Ester Panel
2501 M Street, NW
Washington, DC 20037**

August 1, 1995



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**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
DUST IN THE RAT**

FINAL REPORT

**IITRI Project No. LO8552
Study No. 3**

Performing Laboratory:

**IIT Research Institute
Life Sciences Department
10 West 35th Street
Chicago, IL 60616**

Author:

Scott Garthwaite, B.S.

Submitted to:

**Chemical Manufacturers Association
2501 M Street NW
Washington, DC 20037**

Study Completion Date:

August 1, 1995

iitri
since 1936

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L08552 SN3

GLP COMPLIANCE STATEMENT

This study was conducted in accordance with U.S. Environmental Protection Agency (EPA) TSCA Good Laboratory Practice (GLP) Standards as set forth in the Code of Federal Regulations (Part 792). All chemical analyses and attendant documentation pertaining to the characterization, stability, and purity of the test substance were the responsibility of the Sponsor. The raw data were reviewed by the Study Director, who certifies that the results reported herein are consistent with and supported by the study raw data.

Scott Garthwaite 8-1-95 Date
Scott Garthwaite, B.S.
Study Director
Life Sciences Department

Marian Stanley Date
Chemical Manufacturers Association
Manager, Phthalate Esters Panel
Sponsor Representative

L03552 SN3

**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
DUST IN THE RAT**

Study Initiation Date: December 21, 1994
In-life Initiation Date: February 15, 1995
In-life Termination Date: February 15, 1995

SUMMARY

This study was conducted in preparation for possible repeated exposure inhalation toxicity studies in the rat and was designed to generate information regarding the potential for phthalic anhydride dust to produce pulmonary sensory irritation in this species. A major consideration in the decision to conduct this study was the concern for a potential pulmonary sensory irritation response in the rat at the maximum concentration that could be achieved.

Phthalic anhydride dust was administered by a head-only inhalation exposure at a gravimetric chamber concentration of 0.574 mg/l (the maximum obtainable dust concentration) to a group of 4 male Sprague-Dawley rats for a minimum of 10 minutes, on a single day. The nominal concentration [i.e., total test substance consumed (mg) divided by the total air flow (l)] was 42 mg/l. Analysis of the phthalic anhydride atmospheres included gas chromatographic analysis of dust and vapor, dust particle size analysis and gravimetric analysis of dust. The results of these analyses indicated that greater than 98% of the chamber atmosphere was phthalic anhydride dust. Negligible concentrations of phthalic acid (a potential hydrolysis product of phthalic anhydride) and phthalic anhydride vapor were detected. The average particle size of phthalic anhydride dust was 4.95 microns (mass-median aerodynamic diameter) with a geometric standard deviation of 1.87; 21.7% of the mass was less than 3 microns and 92.7% of the mass was less than 9 microns.

The respiratory rate of each rat was measured before, during and after exposure. The animals were placed in ventilated plexiglass plethysmographs and the individual respiratory rates were determined from the measurements of a pressure transducer which was attached to the plethysmographs. Calculation of respiratory rates was made on a real-time basis using a computer. No significant changes in respiratory rates occurred during exposure. Therefore, under the conditions of this study, a maximum attainable phthalic anhydride dust concentration of 0.574 mg/l did not produce pulmonary sensory irritation in the rat.

Scott Garthwaite 8-1-95
Scott Garthwaite, B.S. Date
Study Director
Life Sciences Department

Catherine Aranyi 8/1/95
Catherine Aranyi, M.S. Date
Head, Inhalation Toxicology Program
Life Sciences Department

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**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
DUST IN THE RAT**

I. INTRODUCTION

The objective of this study was to determine the dust concentration of test substance that would cause a 50% decrease in the respiratory rate (RD50) in male rats when administered by inhalation. This study was conducted because of a concern over a potential sensory irritation response in the rat in possible future repeated exposure inhalation toxicity studies to be conducted with phthalic anhydride dust in this species.

II. MATERIALS AND METHODS

- A. Test Substance:** Phthalic anhydride (CAS No. 85-44-9; supplied by Stepan Company, Northfield, IL, lot number 2282), in the form of white flakes, was received October 12, 1994 and was stored in the original container (5 gallon plastic pail) at room temperature (approximately 22 °C). Analyses of the test substance and attendant documentation pertaining to the characterization, purity, and stability of the test substance were the responsibility of the Sponsor. Information from the Sponsor indicated that the test-substance was 99.9% pure.
- B. Test Atmosphere Generation:** Ground phthalic anhydride was delivered at a constant rate through a dry materials feeder (AccuRate, Inc., Whitewater, WI) into an air dispersion device which was connected to the chamber air supply inlet. Chamber supply air was filtered through a HEPA and charcoal filter before the generator inlet. The supply air with dispersed test substance was then directed into the top of the chamber. The terminology for the physical form of the airborne material is particulate, dust, or aerosol. In this report, the airborne material will be referred to as a dust. Details of the test atmosphere development can be found in a separate report, "Test Atmosphere Development of Phthalic Anhydride Dust and Vapor (LO8552)".

The exposure atmospheres were generated in a 0.5 m³ stainless steel and glass chamber (Unifab Corp., Kalamazoo, MI) designed to accommodate a plethysmograph (Buxco Electronics, Inc., Sharon, CT). The plethysmograph consisted of two compartments separated by a yolk; one compartment for the animal's head and one for its body. Only the head of the animal was exposed to the test substance. During exposure to the test substance, the exposure atmosphere was bled from the 0.5 m³ chamber into the head compartment of the plethysmograph at approximately 4 l/min. Air flow through the 0.5 m³ chamber was maintained at 122 l/min during exposure. The animals were acclimated to the plethysmograph prior to exposure.

Chamber exhaust was filtered through both HEPA and charcoal filters before being released to the outside environment.

- C. **Maximum Obtainable Chamber Concentration/Minimum Obtainable Particle Size:** The generation system operating parameters were adjusted to give the maximum obtainable chamber concentration of phthalic anhydride dust. The output rate of the dry materials feeder was adjusted until it was just lower than the output rate which caused plugging of the generator.

The minimum obtainable particle size was determined during preliminary chamber development. The phthalic anhydride supplied by the Sponsor for inhalation toxicity testing was in the form of "flakes". Several mills/grinders were used to reduce the particle size prior to generation of the test atmospheres. All of the grinders/grinding techniques yielded about the same results. The grinder that gave the best results was a Retsch Impeller-Type Cutting Mill (F. Kurt Retsch GmbH & Company, Germany) equipped with a 0.25 mm screen. Several grinding techniques were tried with this mill. The test substance flakes were ground at room temperature, the test substance was frozen at - 70 °C and ground at room temperature, and the test substance was frozen at - 70 °C and ground at subambient conditions (-20 °C). All three techniques gave a fine white powder with an aerodynamic particle size of approximately 4-5 microns. Consequently, the test substance was ground at room temperature for use in the exposure phase of the study.

- D. **Animals:** Male Sprague Dawley rats (CrI:CD®BR) were used in this study. Rats were selected because this study was conducted in preparation for possible repeated exposure inhalation toxicity studies in the rat and there was concern that in these studies the rat may develop pulmonary sensory irritation. Rats were purchased from Charles River Laboratories, Kingston, NY at approximately 8 weeks of age. Animals weighed 246 to 272 g one day after arrival (February 8, 1995) and were held in quarantine until exposure on February 15, 1995. All rats were examined carefully during the quarantine period to ensure their health and suitability as test subjects. Each rat selected for the study was identified by a study-unique numbered metal tag, which was inserted through the pinna of the right ear and by a cage card bearing the corresponding study-unique animal number.

- E. **Food and Water:** Purina Rodent Chow 5002 (Ralston Purina Company, St. Louis, MO) and reverse osmosis-purified water, supplied by an automatic watering system, were available *ad libitum*, except during the exposure period.

- F. **Environment:** During the quarantine observation period, the rats were housed individually in suspended stainless steel cages. Deotized animal cage boards (Bunzl Paper, Cincinnati, OH) were provided beneath the suspended cages, except during the inhalation exposure, for the collection and absorption of urine and feces. During the quarantine period, the animal room average temperature and relative humidity were 22.8°C and 48%, respectively.

Fluorescent lighting was provided automatically for 12 hours followed by 12 hours of darkness.

G. Methods:

1. **Assignment to Groups:** Study rats were randomly selected via computer from healthy quarantined rats and were assigned to a single group of four male rats. Each rat acted as its own control, therefore there was no control group. The body weight range of animals selected for the study was 290 to 305 g.
2. **Exposure:** The rats were exposed to a test atmosphere generated from a single batch (lot number 2282) of test substance on February 15, 1995.
3. **Test Atmosphere Monitoring:** The test atmosphere was monitored for phthalic anhydride dust using a sampling train consisting of an open-face glass fiber filter (Number 66075, Gelman Sciences, Inc., Ann Arbor, MI) followed by a tenax adsorbent tube (ORBO 402, Supelco, Inc., Bellefonte, PA). The total sample volume was determined using a dry gas meter. Following collection, the filter and adsorbent tube were analyzed separately. Using this sampling regimen, test substance dust is trapped on the glass fiber filter and vapor is trapped on the adsorbent tube.

Phthalic anhydride and phthalic acid in the test atmosphere was measured using a modified gas chromatographic (GC) method [Kruglov EA, Tsypysheva LG, Kharlamovich GD and Portnova TV (1980). *Zh. Anal. Khim.*, Vol. 35, 1980, pp.122-127].

Chamber temperature, humidity and airflow were monitored continuously and recorded periodically during the exposure. Temperature was measured using a thermocouple connected to an Omega DP-11 Panel Meter (Omega Engineering, Inc., Stamford, CT). Humidity was monitored using an Airguide Humidity Indicator (Airguide Instrument Company, Chicago, IL). Airflow was measured by monitoring the pressure drop across an orifice using a Magnehelic Pressure Gauge (Dwyer Instruments, Inc., Michigan City, IN).

4. **Respiratory Rate Measurement:** Each animal was placed in a ventilated plexiglass plethysmograph. The rats were previously acclimated to the plethysmographs for at least approximately one-half to one hour on the day before exposure. An average resting or baseline respiration rate was recorded immediately prior to exposure. Individual respiratory rates were determined from the measurements of a pressure transducer which was attached to the plethysmograph. Chest wall excursions cause a flow through a low resistance screen pneumotachograph which is attached to the plethysmograph. Calculation of respiratory rates was made in real time, at 20 second intervals, using a computer program (LS-20, Buxco Electronics, Inc. Sharon, CT). In addition, the analog signal of flow through the pneumotachograph was displayed on the computer screen.

L0852 SN3

Rats were exposed to the test atmospheres for a minimum of approximately 10 minutes. The average respiratory rate over an approximately three minute period during exposure was calculated. Measurements associated with animal movement were excluded from the calculated average respiratory rate. To evaluate the potential for recovery from possible irritant effects, the animals remained in the plethysmographs at the end of exposure. This modified method is based on that of Alarie (Sensory irritation of the upper airways by airborne chemicals. *Toxicol. Appl. Pharmacol.* 24:29-297, 1973).

H. Archives: All original data generated at IITRI and a copy of the final report will be kept in the IITRI archives.

III. RESULTS

- A. **Exposure Concentration:** The nominal concentration [i.e., total test substance consumed (mg) divided by the total air flow (l)] was 42 mg/l. The gravimetric chamber concentration was 0.574 mg/l. Negligible concentrations of phthalic acid (a potential hydrolysis product of phthalic anhydride) and phthalic anhydride vapor were detected. The average particle size of phthalic anhydride dust was 4.95 μm (mass-median aerodynamic diameter) with a geometric standard deviation of 1.87; 21.7% of the mass was less than 3 μm and 92.7% of the mass was less than 9 μm . The analytical concentration of phthalic anhydride and phthalic acid (Table 1) was 0.491 mg/l; with 0.93% of the total collection being phthalic acid. Most of the chamber atmosphere was present as dust, only 0.05% was vapor.
- B. **Chamber Conditions:** The average exposure chamber temperature and relative humidity were 23.6 °C and 51%, respectively. The average ambient room temperature was 23.4 °C.
- C. **Respiratory Rates:** The average respiratory rate during the pre-exposure period was 132.1 breaths/minute (Table 2). The average rate during exposure was 128.8 breaths/min, and the average rate following exposure was 130.0 breaths/min.

IV. CONCLUSION

Under the conditions of this study, a maximum attainable phthalic anhydride gravimetric dust concentration of 0.574 mg/l did not alter respiratory rate in the rat and did not produce pulmonary sensory irritation.

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V. QUALITY ASSURANCE STATEMENT

Study Title: PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE DUST IN THE RAT
Project Number: L08552 SN3
Study Director: Scott Garthwaite, B.S.

This study was subjected to inspections and the report was audited by the IITRI Quality Assurance Unit in accordance with the Environmental Protection Agency's TSCA "Good Laboratory Practice Standards" - "CFR Part 792". The report describes the methods used in the study and the reported results accurately reflect the raw data of the study.

The following are the inspection dates and the dates inspection findings were reported:

<u>Date(s) of Inspection(s)</u>	<u>Findings Reported to:</u>	
	<u>Study Director</u>	<u>Management</u>
2/14/95	2/14/95	2/14/95
2/15/95	2/15/95	2/15/95
4/10/95	4/11/95	4/11/95
6/20/95	6/21/95	6/21/95

 R. A. Boyne 8-1-95

Ronald Boyne, B.S. Date
Manager, Quality Assurance

L08552 SN3

VI. TABLES

L08552 SN3

**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
DUST IN THE RAT**

TABLE 1

Individual Analytical Chamber Concentration Measurements

Sample Number	Sample Duration (min)	Total Volume (l) ^a	Test Substance Concentration (mg/l)	
			Phthalic Anhydride	Phthalic Acid
1	20	78.0	0.442	0.00231
2	10	39.0	0.458	0.00385
3	10	39.0	0.397	0.00385
4	10	39.0	0.645	0.00931
		mean	0.486	0.00483
		± S.D. ^b	0.109	0.00307

- ^a Sample flow rate was 4 l/min
- ^b S.D. = Standard Deviation

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**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
DUST IN THE RAT**

TABLE 2

Effect of Phthalic Anhydride on Respiratory Rates in Male Rats

<u>Animal Number</u>	<u>Respiratory Rate (breaths/minute)</u>		
	<u>pre-exposure</u>	<u>during exposure</u>	<u>post-exposure</u>
885	116.1 ± 3.2 ^a	111.6 ± 6.3	120.3 ± 7.7
886	151.4 ± 9.4	143.7 ± 16.1	138.3 ± 5.0
887	118.7 ± 11.2	106.6 ± 3.9	118.0 ± 4.8
888	142.2 ± 5.8	153.1 ± 12.0	143.3 ± 5.0
mean ^b	132.1 ± 17.4	128.8 ± 23.1	130.0 ± 12.7
% of pre-exposure mean	100	97.5	98.4

^avalues are the mean respiratory rate ± standard deviation calculated during the measurement period (n=6 to 11 twenty second intervals per animal).

^bmean ± standard deviation of the average respiratory rate during the measurement period (n=4 animals).

VII. APPENDIX



IIT RESEARCH INSTITUTE
STUDY PROTOCOL

1. Title **Pulmonary Sensory Irritation Study (RD50) of Phthalic Anhydride Dust in the Rat**
2. IITRI Project Number **L08552; Study No. 3**
3. Sponsor **Chemical Manufacturers Association
2501 M Street NW
Washington, DC 20037
202-887-1100**
4. Testing Facility **IIT Research Institute
Life Sciences Department
10 West 35th Street
Chicago, IL 60616**
5. Objective **The objective of this study is to determine the concentration of test article that causes a 50% decrease in the respiratory rate (RD50) in rats when administered by inhalation exposure.**

6. Proposed Study Dates

- a. Experimental Start: To be determined
b. Experimental Termination: To be determined
c. Draft Report Submission: To be determined

7. Protocol Approval

- a. Study Director: Scott Garthwaite Date: 12-21-94
Scott Garthwaite, BS (312-567-4353)
- Manager of
b. Research: Catherine Aranyi Date: 12-21-94
Catherine Aranyi, MS (312-567-4864)
- c. Sponsor's
Representative: Marian K. Stanley Date: 12/9/94
Marian Stanley (202-887-1207)
Manager, Phthalate Esters Panel

8. Compliance This protocol is a modified method of that reported by Alarie, Y. (Sensory irritation of the upper airways by airborne chemicals. *Toxicol. Appl. Pharmacol.* 24:29-297, 1973). This protocol complies with the minimum requirements of the Sponsor and all current TSCA Good Laboratory Practice Standards.

9. Test Substance

a. Test Article Identification:	phthalic anhydride
CAS No. --	85-44-9
Physical state --	solid at room temperature
Color --	white flake
Odor --	not applicable
Vapor pressure --	0.05 torr at 20°C
Sublimation point --	285°C
Flash point --	152°C
Lot number --	2282
Supplier --	Stepan Company, Northfield, IL

Documentation of the methods of test article synthesis, fabrication or derivation will be the responsibility of the Sponsor. The chemical characterization and stability of the test article will be also the responsibility of the Sponsor.

- b. Handling Precautions:** Prior to working with the test article, study personnel will be familiarized with and will have access to the test article's Material Safety Data Sheet (MSDS). When working with the test article, personnel must wear:

Gloves --	yes
Face mask --	no
Respirator --	yes
Lab Clothing --	yes
Other --	no

- c. Storage:** The test article will be stored at room temperature (approximately 22°C).
- d. Dispensation:** Reserve samples of the test article will be retained by the Sponsor. All quantities of the test article which are dispensed will be documented. At the time of the acceptance of the report by the Sponsor, arrangements will be made to return any residual material to the Sponsor. IITRI will not be required to retain any samples.

10. Test System

- a. Model:** Male Sprague-Dawley rats (Charles River Laboratories, Inc., Portage, MI) can be used in this study. The rats will be approximately 6 weeks of age and will weigh approximately 125-150 grams on arrival.
- b. Selection of Test System:** Rodent models are widely used in acute toxicity testing. IITRI has collected control data in this species which can be possibly used for comparison.

- c. **Housing:** Animals will be housed individually in stainless steel wire cages suspended over excrement pans, except during the inhalation exposure.
- d. **Cleaning and Sanitation:** Animal rooms and cages will be cleaned and sanitized prior to placing animals in them, and periodically thereafter, in accordance with accepted animal care practices and relevant standard operating procedures.
- e. **Food:** Purina Certified Rodent Chow 5002 (Ralston Purina Company, St. Louis, MO) will be provided *ad libitum* except during the inhalation exposure. To the best of our knowledge, no known contaminants are expected to be present in the basal diet that would interfere with the test article or test system and would confound the interpretation of the study.
- f. **Water:** Water from a reverse osmosis purifier will be provided *ad libitum* by means of an automatic watering system, except during the inhalation exposure. Supply water is periodically monitored for bacterial contamination and chemical composition (i.e., electrolytes, metals, etc.).
- g. **Animal Identification:** Animals selected for the study will receive a unique permanent identification number tag which will be inserted through a pinna of an ear. Individual cage cards will also be provided.
- h. **Environmental Control:** Animal rooms will be lighted automatically with fluorescent lights and maintained on a 12-hour light/12-hour dark cycle. Room and chamber temperature and relative humidity (RH) will be regulated to avoid extreme fluctuations. The target environmental conditions for animal room and exposure chamber are:

	T ^o C	RH %
Animal Room	22 ± 3	50 ± 25
Exposure Chamber	22 ± 2	50 ± 20

If either the chamber or the room is out of target range for more than 12 hours, then corrective action will be taken. If either the chamber or the room is out of target range for 24 hours, then the Sponsor will be notified. The Sponsor will be notified in all cases of extreme environmental fluctuation. Extreme fluctuation is defined as values outside the range of 22 ± 5°C for temperature and 50 ± 25 % for relative humidity.

11. Methods

- a. **Quarantine:** The animals will be held in quarantine for at least one week prior to study initiation. During the quarantine period the animals will be observed at least daily, and at the end of the period they will receive a thorough physical examination to ensure their suitability for use as test animals.

b. Assignment to Groups: Animals will be assigned to the appropriate number of groups based on a computerized randomization program which stratifies animals across groups based on body weight.

c. Exposure Levels: The range of target concentrations will be made in conjunction with the Sponsor and will be based on the results of LC50 studies with the test article. The highest exposure concentration is expected to be the highest achievable chamber concentration.

d. Test Atmosphere Generation:

(1) Details of test atmosphere generation will be appropriately documented in the raw data and reflected in the report. Generally, dusts will be generated by metering the solid material through a solid material feeder and dispersing the airborne material using compressed air. More specifically for this test article, an Accurate Dry Materials Feeder (Accurate, Inc., Whitewater, WI) is proposed for metering the solid dust into the chamber supply air inlet. Other dust feeders will be tested if the output rate of the Accurate is not sufficient to produce the targeted chamber concentrations. In the event of this approach is deemed inappropriate upon methods development, an alternative generation method will be used. The method employed will depend ultimately upon the physical/chemical characteristics of the test article.

(2) The nominal concentration of the test article in the exposure atmosphere will be determined by recording the amount of material consumed in the generation of a measured volume of atmosphere.

(3) The vapor concentration in a generated dust atmosphere will be reported. The actual exposure chamber concentration of phthalic anhydride, whether dust or vapor, and phthalic acid will be measured using a modified gas chromatographic (GC) method [Kruglov EA, Tsypysheva LG, Kharlampovich GD and Portnova TV (1980). *Zh. Anal. Khim.*, Vol. 35, 1980, pp.122-127]. The GC method will distinguish between and measure both phthalic anhydride and phthalic acid.

(4) For particulates, the particle size distribution will be determined at least once during the exposure using an Andersen Cascade Impactor, Portable Continuous Aerosol Monitor (PCAM) or a Quartz Crystal Microbalance, depending upon the chamber concentration. The test article dust will be ground using an ultra-centrifugal mill (Retsch GmbH, Germany). Ideally, the measured particle size distribution in the chamber will have 95% of all particle sizes less than 10 microns, and 50% less than a 3 microns. However, the minimal obtainable particle size for dusts is frequently greater than 3 microns. Therefore, the Sponsor will be notified of the particle size distribution obtained during chamber development prior to study start.

(5) Other instrumentation (e.g., a light gathering aerosol monitor or total hydrocarbon analyzer) may be used for qualitative measurements of chamber concentrations. These

instruments will be used to assist in the setting of desired atmosphere concentrations, but will not be used for quantitative measurements.

- e. **Justification for Route of Exposure:** This route of exposure was selected to provide meaningful data to aid in the selection of exposure concentrations for subsequent inhalation toxicology studies employing this same test article.
- f. **Exposure Chambers:** Test atmospheres will be appropriately generated in a stainless steel and/or glass chamber. Temperature, humidity, and dynamic flow conditions will be recorded at approximate half-hour intervals. Test atmospheres will be pulled through either glass or Plexiglas exposure chambers having ports which hold individual animals. Only the heads of animals will be exposed to test article. Total air flow will be adjusted as a means of controlling the concentration of the exposure atmosphere but will provide enough air changes to maintain a safe oxygen level for the animals. The concentration of oxygen that will be regarded as safe is that greater than 19%. Oxygen levels will be determined by either an elemental mass balance of oxygen within the exposure chamber, or when expected oxygen levels approach 19%, an oxygen analyzer (Servomex, Model 1400, Norwood, MA). A pressure transducer will be attached to each animal port to monitor that animal's respiratory rate.
- g. **Chamber Loading:** The test animals will comprise no more than 5% of the total chamber volume (one kilogram equals approximately one liter).
- h. **Final Disposition of Animals:** All animals surviving to the end of the exposure period will not undergo an observation period, will not be subjected to a gross necropsy, but instead, will be euthanized and discarded.

12. Experimental Design

Four groups of rats, each consisting of four male rats, will be exposed to one of four graded concentrations of test article. Only the head of the animal will be exposed to test article; the body of the animal will be held in a ventilated Plexiglas holding port. Groups of animals will be acclimated to the testing apparatus and an average resting or baseline respiration rate will be recorded immediately prior to their collective exposure. Individual respiration rates will be determined from the measurements of a pressure transducer which will be attached to each animal's holding port. The shape of the respiratory rate profile will be evaluated on a few animals during preliminary chamber development to set appropriate limits on the length of the exposure period. Rats will be exposed to the test atmospheres for a minimum of 10 minutes. The average respiratory rate over an approximately three minute period during exposure will be calculated after the respiratory rate stabilizes. If necessary, the exposure period will be extended beyond 10 minutes until a stable respiratory rate is achieved. However, the exposure period will not exceed 30 minutes. Animals will remain in the plethysmographs for a 10 minute period at the end of exposure to evaluate the potential for recovery from the irritant effects. During this post exposure period, animals will be exposed to either room air or filtered air. This modified method is based on that of Alarie (Sensory irritation of the upper airways by airborne chemicals. *Toxicol. Appl. Pharmacol.* 24:29-297, 1973).

13. Observations

- a. **Mortality and Observations:** All animals dying during the course of the study will be noted in the study record. All animals will be observed during the exposure period, when possible, and periodically during the remainder of the first day. Gross signs of toxicity will be generally noted in the study record, but formal observation periods will not be scheduled.
- b. **Body Weight:** All quarantine animals in the study will be weighed prior to exposure for group selection purposes.
- c. **Necropsy:** Animals will be euthanized, but no necropsies will be performed.

14. Results

A dose response curve will be generated from the study data and an RD50 will be calculated according to the method of Alarie (1973). These results will be presented in a formal written report. The written report will include but is not limited to animal information, exposure calculations, a description of generation systems/methods, chamber measurements, analytical methods, and respiratory measurements.

15. Data Notebooks

- a. **Contents:** All original data will be maintained in notebooks and will include, but not necessarily be limited to, the following:
 - (1) the original signed protocol and all amendments
 - (2) test article analysis and supporting documentation
 - (3) animals purchase and receiving records
 - (4) randomization procedures
 - (5) exposure calculations
 - (6) description of generation systems/methods
 - (7) chamber environment
 - (8) body weights
- b. **Storage:** All original data and a copy of the final report will be kept in the IITRI archives for a period of one year after the submission of the signed final report. At that time, the Sponsor will be contacted in order to determine the final disposition of the raw data and will be responsible for all costs associated with continued storage of the raw data in the IITRI Archives or for the shipment of these materials to a new storage facility.

16. Personnel

Curricula vitae for all personnel involved in the execution of the study are on file at IITRI.

PROTOCOL AMENDMENT

Title Pulmonary Sensory Irritation Study (RD50) of Phthalic Anhydride Dust in the Rat

Protocol Amendment Number: 1

Project Number: LO8552

Study Number: 3

Effective Date: February 15, 1995

Revisions:

1. **Section 6.** The proposed study dates are as follows:

- a. **Experimental Start:** February 15, 1995
- b. **Experimental Termination:** February 15, 1995
- c. **Draft Report Submission:** April 3, 1995

These dates were not yet determined when the protocol was signed.

2. **Section 10a.** The protocol specified that male rats from Charles River Labs, Portage, MI were to be used and they would weigh 125-150 g upon arrival. The animals which were ordered for the study were approximately 225-250 grams and were supplied by Charles River - Kingston.

The receipt weight range was increased so that the body weights at the time of exposure would be comparable to other studies. In order to receive animals in time for the proposed start of the study, they were shipped from Charles River in Kingston.

3. **Section 11c.** The exposure levels for this study were to be determined based upon results from LC50 studies. These studies were not conducted. Therefore, the exposure level was set at the maximum obtainable chamber concentration.

4. **Section 11d(4).** The test substance was ground using a cutting mill (Retsch GmbH, Germany). This mill was found to give a better particle size distribution than the ultra-centrifugal mill.

5. Section 11f. The chamber temperature, humidity, and airflow were recorded on four occasions during the exposure, but not necessarily at half-hourly intervals.

6. Section 12. At the request of the Sponsor, only one group of animals was exposed to the test substance because there was no significant change in respiratory rate at the maximum obtainable concentration.

7. Section 13a. The protocol inadvertently contained conflicting information regarding a post-exposure observation period. Animals were not maintained for a post-exposure observation period as indicated in the protocol section 13a but, rather, were sacrificed following exposure as indicated in the protocol section 11h.

8. Section 14. There was no change in respiratory rate at the maximum obtainable concentration so an RD50 could not be calculated.

Approval

a. Study Director:

Scott Garinwaite Date: 7-26-95
Scott Garinwaite, BS

Manager of

b. Research:

Catherine Aranyi Date: 7/26/95
Catherine Aranyi, MS

c. Sponsor's
Representative:

Marian K. Stanley Date: 7/27/95
Marian Stanley
Manager, Phthalate Esters Panel

**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC
ANHYDRIDE VAPOR IN THE RAT**

FINAL REPORT

Performing Laboratory:

**IIT Research Institute
Life Sciences Department
10 West 35th Street
Chicago, IL 60616**

Sponser by:

**Chemical Manufacturers Association
Phthalate Ester Panel
2501 M Street, NW
Washington, DC 20037**

August 1 1995

CM^A
CHEMICAL
MANUFACTURERS
ASSOCIATION


Responsible Care •
A Public Commitment

**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
VAPOR IN THE RAT**

FINAL REPORT

**IITRI Project No. LO8552
Study No. 4**

Performing Laboratory:

**IIT Research Institute
Life Sciences Department
10 West 35th Street
Chicago, IL 60616**

Author:

Scott Garthwaite, B.S.

Submitted to:

**Chemical Manufacturers Association
2501 M Street NW
Washington, DC 20037**

Study Completion Date:

August 1, 1995

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since 1936

COMMITMENT TO EXCELLENCE

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L08552 SN4

GLP COMPLIANCE STATEMENT

This study was conducted in accordance with U.S. Environmental Protection Agency (EPA) TSCA Good Laboratory Practice (GLP) Standards as set forth in the Code of Federal Regulations (Part 792). All chemical analyses and attendant documentation pertaining to the characterization, stability, and purity of the test substance were the responsibility of the Sponsor. The raw data were reviewed by the Study Director, who certifies that the results reported herein are consistent with and supported by the study raw data.

Scott Garthwaite 8-1-95
Date
Scott Garthwaite, B.S.
Study Director
Life Sciences Department

Date
Marian Stanley
Chemical Manufacturers Association
Manager, Phthalate Esters Panel
Sponsor Representative

LA0352 SN4

**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
VAPOR IN THE RAT**

Study Initiation Date: December 21, 1994
In-life Initiation Date: January 5, 1995
In-life Termination Date: January 5, 1995

SUMMARY

This study was conducted in preparation for possible repeated exposure inhalation toxicity studies in the rat and was designed to generate information regarding the potential for phthalic anhydride vapor to produce pulmonary sensory irritation in this species. A major consideration in the decision to conduct this study was the concern for a potential pulmonary sensory irritation response in the rat at the maximum concentration that could be achieved.

Phthalic anhydride vapor was administered by a head-only inhalation exposure at 0.011 mg/l (the maximum obtainable vapor concentration) to a group of 4 male Sprague-Dawley rats for a minimum of 10 minutes, on a single day. Analysis of samples collected during exposure that included gas chromatographic analysis, showed that the exposure atmosphere contained phthalic anhydride vapor and that no phthalic anhydride dust or phthalic acid (a possible hydrolysis product of phthalic anhydride) was formed.

The respiratory rate of each rat was measured before, during and after exposure. The animals were placed in ventilated plexiglass plethysmographs and the individual respiratory rates were determined from the measurements of a pressure transducer which was attached to the plethysmographs. Calculation of respiratory rates was made on a real-time basis using a computer. Data for one animal was excluded from the calculation of the mean respiratory rate since a stable baseline for this animal could not be attained. No significant changes in respiratory rates occurred during exposure. Therefore, under the conditions of this study, a maximum attainable phthalic anhydride vapor concentration of 0.011 mg/l did not produce pulmonary sensory irritation in the rat.

Scott Garthwaite 8-1-95

Scott Garthwaite, B.S. Date
Study Director
Life Sciences Department

Catherine Aranyi 8/1/95

Catherine Aranyi, M.S. Date
Head, Inhalation Toxicology Program
Life Sciences Department

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PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE VAPOR IN THE RAT

I. INTRODUCTION

The objective of this study was to determine the vapor concentration of test substance that would cause a 50% decrease in the respiratory rate (RD50) in male rats when administered by inhalation. This study was conducted because of a concern over a potential sensory irritation response in the rat in possible future repeated exposure inhalation toxicity studies to be conducted in this species.

II. MATERIALS AND METHODS

A. Test Substance: Phthalic anhydride (CAS No. 85-44-9; supplied by Stepan Company, Northfield, IL, lot number 2282), in the form of white flakes, was received October 12, 1994 and was stored in the original container (5 gallon plastic pail) at room temperature (approximately 22 °C). Analyses of the test substance and attendant documentation pertaining to the characterization, purity, and stability of the test substance were the responsibility of the Sponsor. Information from the Sponsor indicated that the test substance was 99.9% pure.

B. Test Atmosphere Generation: Phthalic anhydride flake was placed into a glass 3000 ml round bottom flask and heated to approximately 140 °C. The temperature was held constant using a temperature controller (Digi-Sense, Cole-Parmer Instrument Company, Chicago, IL) connected to a heating mantle which was located around the bottom of the flask. At 140 °C the test substance was completely melted and had the appearance of a clear liquid.

Three ground glass joints were located in the top of the glass round-bottom flask. One was used for a thermocouple which was connected to the temperature controller, one was used as an inlet for compressed air, and the third was connected to a glass elbow which was connected to the chamber supply air. The glass elbow was wrapped with heat tape to prevent condensation of the hot vapor/aerosol before it entered the chamber supply air. Details of the test atmosphere development can be found in a separate report, "Test Atmosphere Development of Phthalic Anhydride Dust and Vapor (LO8552)".

Chamber supply air was filtered through a HEPA and charcoal filter and then directed through a 2 inch diameter glass pipe where it mixed with the output from the test substance generator. After the generator, but before entering the chamber, a HEPA filter was used to collect test substance aerosol particulates. The supply air was then directed into the top of the chamber.

The exposure atmospheres were generated in a 0.5 m³ stainless steel and glass chamber (Unifab Corp., Kalamazoo, MI) designed to accommodate a plethysmograph (Buxco

Electronics, Inc., Sharon, CT). The plethysmograph consisted of two compartments separated by a yolk; one compartment for the animal's head and one for its body. Only the head of the animal was exposed to the test substance. During exposure to the test substance, the exposure atmosphere was bled from the 0.5 m³ chamber into the head compartment of the plethysmograph at approximately 4 l/min. Air flow through the 0.5 m³ chamber was maintained at 156 l/min during exposure. The animals were acclimated to the plethysmograph prior to exposure.

Chamber exhaust was filtered through both HEPA and charcoal filters before being released to the outside environment.

- C. **Maximum Obtainable Chamber Concentration:** The generation system operating parameters were adjusted to give the maximum obtainable chamber concentration of phthalic anhydride vapor with no aerosol component, and to use exposure chamber environmental conditions that would be realistic for possible longer-term studies, (i.e. the chamber temperature should not be significantly elevated above ambient levels).

The chamber supply air inlet was examined visually while the compressed air flowrate through the generator was adjusted. As the air flowrate was increased, visible particulate aerosol was present in the supply air stream. The air flowrate was set so that visible particulate was present in the supply air stream, but not so much that the HEPA filter located between the generator and the chamber would become clogged during exposure. The air flowrate through the generator was approximately 0.2 liters/min.

- D. **Animals:** Male Sprague Dawley rats (CrI:CD[®]BR) were used in this study. Rats were selected because this study was conducted in preparation for possible repeated exposure inhalation toxicity studies in the rat and there was concern that in these studies the rat may develop pulmonary sensory irritation. Rats were purchased from Charles River Laboratories, Portage, MI at approximately 6 weeks of age. Ten percent of the animals weighed 149 to 170 g two days after arrival (December 23, 1994) and were held in quarantine until exposure on January 5, 1995. All rats were examined carefully during the quarantine period to ensure their health and suitability as test subjects. Each rat selected for the study was identified by an study-unique numbered metal tag, which was inserted through the pinna of the right ear and by a cage card bearing the corresponding study-unique animal number.
- E. **Food and Water:** Purina Rodent Chow 5002 (Ralston Purina Company, St. Louis, MO) and reverse osmosis-purified water, supplied by an automatic watering system, were available *ad libitum*, except during the exposure period.
- F. **Environment:** During the quarantine observation period, the rats were housed individually in suspended stainless steel cages. Deotized animal cage boards (Bunzl Paper, Cincinnati, OH) were provided beneath the suspended cages, except during the inhalation exposure, for the collection and absorption of urine and feces. During the quarantine period, the animal

room average temperature and relative humidity were 23.9°C and 58%, respectively. Fluorescent lighting was provided automatically for 12 hours followed by 12 hours of darkness.

G. Methods:

1. **Assignment to Groups:** Study rats were randomly selected via computer from healthy quarantined rats and were assigned to a single group of four male rats. Each rat acted as its own control, therefore there was no control group. The body weight range of animals selected for the study was 210 to 243 g.
2. **Exposure:** The rats were exposed to a test atmosphere generated from a single batch (lot number 2282) of test substance on January 5, 1995.
3. **Test Atmosphere Monitoring:** The test atmosphere was monitored for phthalic anhydride vapor and aerosol using a sampling train consisting of an open-face glass fiber filter (Number 66075, Gelman Sciences, Inc., Ann Arbor, MI) followed by a tenax adsorbent tube (ORBO 402, Supelco, Inc., Bellefonte, PA). The total sample volume was determined using a dry gas meter. Following collection, the filter and adsorbent tube were analyzed separately. Using this sampling regimen, test substance aerosol/particulate is trapped on the glass fiber filter and vapor is trapped on the adsorbent tube.

Phthalic anhydride and phthalic acid in the test atmosphere was measured using a modified gas chromatographic (GC) method [Kruglov EA, Tsypysheva LG, Kharlamovich GD and Portnova TV (1980). *Zh. Anal. Khim.*, Vol. 35, 1980, pp.122-127].

Chamber temperature, humidity and airflow were monitored continuously and recorded periodically during the exposure. Temperature was measured using a thermocouple connected to an Omega DP-11 Panel Meter (Omega Engineering, Inc., Stamford, CT). Humidity was monitored using an Airguide Humidity Indicator (Airguide Instrument Company, Chicago, IL). Airflow was measured by monitoring the pressure drop across an orifice using a Magnehelic Pressure Gauge (Dwyer Instruments, Inc., Michigan City, IN).

4. **Respiratory Rate Measurement:** Each animal was placed in a ventilated plexiglass plethysmograph. The rats were previously acclimated to the plethysmographs for approximately one-half to one hour on the day before exposure. An average resting or baseline respiration rate was recorded immediately prior to exposure. Individual respiratory rates were determined from the measurements of a pressure transducer which was attached to the plethysmograph. Chest wall excursions cause a flow through a low resistance screen pneumotachograph which is attached to the plethysmograph. Calculation of respiratory rates was made in real time, at 10 second intervals, using a computer program (LS-20, Buxco Electronics, Inc. Sharon, CT). In addition, the analog

signal of flow through the pneumotachograph was displayed on the computer screen. Rats were exposed to the test atmospheres for a minimum of approximately 10 minutes. The average respiratory rate over an approximately three minute period during exposure was calculated after the respiratory rate stabilized. Measurements associated with animal movement were excluded from the calculated average respiratory rate. To evaluate the potential for recovery from possible irritant effects, the animals remained in the plethysmographs at the end of exposure. This modified method is based on that of Alarie (Sensory irritation of the upper airways by airborne chemicals. *Toxicol. Appl. Pharmacol.* 24:29-297, 1973).

H. **Archives:** All original data generated at IITRI and a copy of the final report will be kept in the IITRI archives.

III. RESULTS

- A. **Exposure Concentration:** The nominal concentration [i.e., total test substance consumed (mg) divided by the total air flow (l)] was 0.03 mg/l. GC analysis (Table 1) showed a mean phthalic anhydride concentration of 0.011 mg/l (1.83 ppm). No phthalic anhydride aerosol was detected in the chamber atmosphere. No phthalic acid was detected in the chamber atmosphere. A particle size measurement was not performed because no aerosol was detected.
- B. **Chamber Conditions:** The average exposure chamber temperature and relative humidity were 22.2 °C and 49%, respectively. The average ambient room temperature was 22.2 °C.
- C. **Respiratory Rates:** The average respiratory rate during the pre-exposure period was 118.9 breaths/minute (Table 2). The average rate during exposure was 116.0 breaths/min, and the average rate following exposure was 119.8 breaths/min. Retrospective analysis of the respiratory rate data for animal number 984 indicated that a stable baseline was not reached during the pre-exposure measurement period. Consequently, the data for this animal was not included in the calculation of the mean respiratory rate for the study.

IV. CONCLUSION

Under the conditions of this study, a maximum attainable phthalic anhydride vapor concentration of 0.011 mg/l did not alter respiratory rate in the rat and did not produce pulmonary sensory irritation.

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V. QUALITY ASSURANCE STATEMENT

Study Title: PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE VAPOR IN THE RAT

Project Number: L08552 SN4
Study Director: Scott Garthwaite, B.S.

This study was subjected to inspections and the report was audited by the IITRI Quality Assurance Unit in accordance with the Environmental Protection Agency's TSCA "Good Laboratory Practice Standards" - "CFR Part 792". The report describes the methods used in the study and the reported results accurately reflect the raw data of the study.

The following are the inspection dates and the dates inspection findings were reported:

<u>Date(s) of Inspection(s)</u>	<u>Findings Reported to:</u>	
	<u>Study Director</u>	<u>Management</u>
1/4/95	1/4/95	1/4/95
1/5/95	1/5/95	1/5/95
4/7/95	4/11/95	4/11/95
6/20/95	6/21/95	6/21/95

R. A. Boyne 8-1-95

Ronald Boyne, B.S. Date
Manager, Quality Assurance

L08552 SN4

VI. TABLES

L00352 SN4

**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
VAPOR IN THE RAT**

TABLE 1

Individual Analytical Chamber Concentration Measurements

Sample Number	Sample Duration (min)	Total Volume (l) ^b	Test Substance Concentration (mg/l)	
			Phthalic Anhydride	Phthalic Acid
1	60 ^a	30.0	0.0107	BDL ^c
2	60	30.0	0.0113	BDL
		mean	0.0110	
		± S.D. ^d	0.00042	

^aSample durations were greater than exposure durations for individual animals in order to collect enough material for chemical analysis

^bSample flow rate was 0.5 l/min

^cBelow detection limit

^dS.D. = Standard Deviation

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**PULMONARY SENSORY IRRITATION STUDY (RD50) OF PHTHALIC ANHYDRIDE
VAPOR IN THE RAT**

TABLE 2

Effect of Phthalic Anhydride on Respiratory Rates in Male Rats

Animal Number	Respiratory Rate (breaths/minute)		
	pre-exposure	during exposure	post-exposure
981	96.5 ± 6.8 ^a	99.5 ± 13.3	104.0 ± 6.8
982	137.7 ± 4.4	131.9 ± 15.3	124.2 ± 8.8
983	122.5 ± 10.3	116.6 ± 7.0	131.3 ± 8.2
984	- ^b	-	-
mean ^c	118.9 ± 20.8	116.0 ± 16.2	119.8 ± 14.2
% of pre-exposure mean	100	97.6	100.8

^avalues are the mean respiratory rate ± standard deviation calculated during the measurement period (n=7 to 19 of ten second intervals per animal).

^bdata for animal number 984 not used because of unstable pre-exposure readings

^cmean ± standard deviation of the average respiratory rate during the measurement period (n=3 animals)

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VII. APPENDIX



IIT RESEARCH INSTITUTE

STUDY PROTOCOL

1. Title **Pulmonary Sensory Irritation Study (RD50) of Phthalic Anhydride Vapor in the Rat**
2. IITRI Project Number L08552; Study No. 4
3. Sponsor Chemical Manufacturers Association
2501 M Street NW
Washington, DC 20037
202-887-1100
4. Testing Facility IIT Research Institute
Life Sciences Department
10 West 35th Street
Chicago, IL 60616
5. Objective The objective of this study is to determine the concentration of test article that causes a 50% decrease in the respiratory rate (RD50) in rats when administered by inhalation exposure.
6. Proposed Study Dates
 - a. Experimental Start: To be determined
 - b. Experimental Termination: To be determined
 - c. Draft Report Submission: To be determined
7. Protocol Approval
 - a. Study Director: Scott Garthwaite Date: 12-21-94
Scott Garthwaite, BS (312-567-4353)
 - b. Manager of Research: Catherine Aranyi Date: 12-21-94
Catherine Aranyi, MS (312-567-4868)
 - c. Sponsor's Representative: Marian K. Stanley Date: 12/9/94
Marian Stanley (202-887-1207)
Manager, Phthalate Esters Panel
8. Compliance This protocol is a modified method of that reported by Alarie, Y. (Sensory irritation of the upper airways by airborne chemicals. *Toxicol. Appl. Pharmacol.* 24:29-297, 1973). This protocol complies with the minimum requirements of the Sponsor and all current TSCA Good Laboratory Practice Standards.

9. Test Substance

a. Test Article Identification:

	phthalic anhydride
CAS No. --	85-44-9
Physical state --	solid at room temperature
Color --	white flake
Odor --	not applicable
Vapor pressure --	0.05 torr at 20°C
Sublimation point --	285°C
Flash point --	152°C
Lot number --	2282
Supplier --	Stepan Company, Northfield, IL

Documentation of the methods of test article synthesis, fabrication or derivation will be the responsibility of the Sponsor. The chemical characterization and stability of the test article will be also the responsibility of the Sponsor.

b. **Handling Precautions:** Prior to working with the test article, study personnel will be familiarized with and will have access to the test article's Material Safety Data Sheet (MSDS). When working with the test article, personnel must wear:

Gloves --	yes
Face mask --	no
Respirator --	yes
Lab Clothing --	yes
Other --	no

c. **Storage:** The test article will be stored at room temperature (approximately 22°C).

d. **Dispensation:** Reserve samples of the test article will be retained by the Sponsor. All quantities of the test article which are dispensed will be documented. At the time of the acceptance of the report by the Sponsor, arrangements will be made to return any residual material to the Sponsor. IITRI will not be required to retain any samples.

10. Test System

a. **Model:** Male Sprague-Dawley rats (Charles River Laboratories, Inc., Portage, MI) can be used in this study. The rats will be approximately 6 weeks of age and will weigh approximately 125-150 grams on arrival.

b. **Selection of Test System:** Rodent models are widely used in acute toxicity testing. IITRI has collected data in this species which can be possibly used for comparison.

- c. **Housing:** Animals will be housed individually in stainless steel wire cages suspended over excrement pans, except during the inhalation exposure.
- d. **Cleaning and Sanitation:** Animal rooms and cages will be cleaned and sanitized prior to placing animals in them, and periodically thereafter, in accordance with accepted animal care practices and relevant standard operating procedures.
- e. **Food:** Purina Certified Rodent Chow 5002 (Ralston Purina Company, St. Louis, MO) will be provided *ad libitum* except during the inhalation exposure. To the best of our knowledge, no known contaminants are expected to be present in the basal diet that would interfere with the test article or test system and would confound the interpretation of the study.
- f. **Water:** Water from a reverse osmosis purifier will be provided *ad libitum* by means of an automatic watering system, except during the inhalation exposure. Supply water is periodically monitored for bacterial contamination and chemical composition (i.e., electrolytes, metals, etc.).
- g. **Animal Identification:** Animals selected for the study will receive a unique permanent identification number tag which will be inserted through a pinna of an ear. Individual cage cards will also be provided.
- h. **Environmental Control:** Animal rooms will be lighted automatically with fluorescent lights and maintained on a 12-hour light/12-hour dark cycle. Room and chamber temperature and relative humidity (RH) will be regulated to avoid extreme fluctuations. The target environmental conditions for animal room and exposure chamber are:

	T°C	RH %
Animal Room	22 ± 3	50 ± 25
Exposure Chamber	22 ± 2	50 ± 20

If either the chamber or the room is out of target range for more than 12 hours, then corrective action will be taken. If either the chamber or the room is out of target range for 24 hours, then the Sponsor will be notified. The Sponsor will be notified in all cases of extreme environmental fluctuation. Extreme fluctuation is defined as values outside the range of 22 ± 5°C for temperature and 50 ± 25 % for relative humidity.

11. Methods

- a. **Quarantine:** The animals will be held in quarantine for at least one week prior to study initiation. During the quarantine period the animals will be observed at least daily, and at the end of the period they will receive a thorough physical examination to ensure their suitability for use as test animals.

- b. Assignment to Groups:** Animals will be assigned to the appropriate number of groups based on a computerized randomization program which stratifies animals across groups based on body weight.
- c. Exposure Levels:** The range of target concentrations will be made in conjunction with the Sponsor and will be based on the results of LC50 studies with the test article. The highest exposure concentration is expected to be the highest achievable chamber concentration.
- d. Test Atmosphere Generation:**
- (1) Details of test atmosphere generation will be appropriately documented in the raw data and reflected in the report. More specifically for this study, the test article vapor will be generated by passing an air or nitrogen stream over the heated liquid test article. The resultant fume will be passed through a HEPA filter to remove particulates, and then diluted into the chamber supply air inlet. In the event of this approach is deemed inappropriate upon methods development, an alternative generation method will be used. The method employed will depend ultimately depend upon the physical/chemical characteristics of the test article.
 - (2) The nominal concentration of the test article in the exposure atmosphere will be determined by recording the amount of material consumed in the generation of a measured volume of atmosphere.
 - (3) The dust concentration in a generated vapor phase atmosphere will be reported. The actual exposure chamber concentration of phthalic anhydride, whether dust or vapor, and phthalic acid will be measured using a modified gas chromatographic (GC) method [Kruglov EA, Tsypysheva LG, Kharlampovich GD and Portnova TV (1980). *Zh. Anal. Khim.*, Vol. 35, 1980, pp.122-127]. The GC method will distinguish between and measure both phthalic anhydride and phthalic acid.
 - (4) Other instrumentation (e.g., a light gathering aerosol monitor or total hydrocarbon analyzer) may be used for qualitative measurements of chamber concentrations. These instruments will be used to assist in the setting of desired atmosphere concentrations, but will not be used for quantitative measurements.
- e. Justification for Route of Exposure:** This route of exposure was selected to provide meaningful data to aid in the selection of exposure concentrations for subsequent inhalation toxicology studies employing this same test article.
- f. Exposure Chambers:** Test atmospheres will be appropriately generated in a stainless steel and/or glass chamber. Temperature, humidity, and dynamic flow conditions will be recorded at approximate half-hour intervals. Test atmospheres will be pulled through either glass or Plexiglas exposure chambers having ports which hold individual animals. Only the heads of animals will be exposed to test article. Total air flow will be adjusted as a means of controlling the concentration of the exposure atmosphere, but will provide enough air changes to maintain a safe oxygen level for the animals. The concentration

of oxygen that will be regarded as safe is that greater than 19%. Oxygen levels will be determined by either an elemental mass balance of oxygen within the exposure chamber, or when expected oxygen levels approach 19%, an oxygen analyzer (Servomex, Model 1400, Norwood, MA). A pressure transducer will be attached to each animal port to monitor that animal's respiratory rate.

- g. Chamber Loading:** The test animals will comprise no more than 5% of the total chamber volume (one kilogram equals approximately one liter).
- h. Final Disposition of Animals:** All animals surviving to the end of the exposure period will not undergo an observation period, will not be subjected to a gross necropsy, but instead, will be euthanized and discarded.

12. Experimental Design

Four groups of rats, each consisting of four male rats, will be exposed to one of four graded concentrations of test article. Only the head of the animal will be exposed to test article; the body of the animal will be held in a ventilated Plexiglas holding port. Groups of animals will be acclimated to the testing apparatus and an average resting or baseline respiration rate will be recorded immediately prior to their collective exposure. Individual respiration rates will be determined from the measurements of a pressure transducer which will be attached to each animal's holding port. The shape of the respiratory rate profile will be evaluated on a few animals during preliminary chamber development to set appropriate limits on the length of the exposure period. Rats will be exposed to the test atmospheres for a minimum of 10 minutes. The average respiratory rate over an approximately three minute period during exposure will be calculated after the respiratory rate stabilizes. If necessary, the exposure period will be extended beyond 10 minutes until a stable respiratory rate is achieved. However, the exposure period will not exceed 30 minutes. Animals will remain in the plethysmographs for a 10 minute period at the end of exposure to evaluate the potential for recovery from the irritant effects. During this post exposure period, animals will be exposed to either room air or filtered air. This modified method is based on that of Alarie (Sensory irritation of the upper airways by airborne chemicals. *Toxicol. Appl. Pharmacol.* 24:29-297, 1973).

13. Observations

- a. Mortality and Observations:** All animals dying during the course of the study will be noted in the study record. All animals will be observed during the exposure period, when possible, and periodically during the remainder of the first day. Gross signs of toxicity will be generally noted in the study record, but formal observation periods will not be scheduled.
- b. Body Weight:** All quarantine animals in the study will be weighed prior to exposure for group selection purposes.
- c. Necropsy:** Animals will be euthanized, but no necropsies will be performed.

14. Results

A dose response curve will be generated from the study data and an RD50 will be calculated according to the method of Alarie (1973). These results will be presented in a formal written report. The written report will include but is not limited to animal information, exposure calculations, a description of generation systems/methods, chamber measurements, analytical methods, and respiratory measurements.

15. Data Notebooks

a. **Contents:** All original data will be maintained in notebooks and will include, but not necessarily be limited to, the following:

- (1) the original signed protocol and all amendments
- (2) test article analysis and supporting documentation
- (3) animals purchase and receiving records
- (4) randomization procedures
- (5) exposure calculations
- (6) description of generation systems/methods
- (7) chamber environment
- (8) body weights

b. **Storage:** All original data and a copy of the final report will be kept in the IITRI archives for a period of one year after the submission of the signed final report. At that time, the Sponsor will be contacted in order to determine the final disposition of the raw data and will be responsible for all costs associated with continued storage of the raw data in the IITRI Archives or for the shipment of these materials to a new storage facility.

16. Personnel

Curricula vitae for all personnel involved in the execution of the study are on file at IITRI.

PROTOCOL AMENDMENT

Title Pulmonary Sensory Irritation Study (RD50) of Phthalic Anhydride Vapor in the Rat

Protocol Amendment Number: 1

Project Number: LO8552

Study Number: 4

Effective Date: January 5, 1995

Revisions:

1. **Section 6.** The proposed study dates are as follows:

- | | |
|-------------------------------------|-----------------|
| a. Experimental Start: | January 5, 1995 |
| b. Experimental Termination: | January 5, 1995 |
| c. Draft Report Subr.ission: | April 3, 1995 |

These dates were not yet determined when the protocol was signed.

2. **Section 11c.** The exposure levels for this study were to be determined based upon results from LC50 studies. These studies were not conducted. Therefore, the exposure level was set at the maximum obtainable chamber concentration.

3. **Section 11f.** The chamber temperature, humidity, and airflow were recorded on four occasions during the exposure, but not necessarily at half-hourly intervals.

4. **Section 12.** At the request of the Sponsor, only one group of animals was exposed to the test substance because there was no significant change in respiratory rate at the maximum obtainable concentration.

5. Section 13a. The protocol inadvertently contained conflicting information regarding a post-exposure observation period. Animals were not maintained for a post-exposure observation period as indicated in the protocol section 13a but, rather, were sacrificed following exposure as indicated in the protocol section 11h.

6. Section 14. There was no change in respiratory rate at the maximum obtainable concentration so an RD50 could not be calculated.

Approval

a. Study Director:

Scott Garthwaite Date: 7-26-95
Scott Garthwaite, BS

b. Research:
Manager of

Catherine Aranyi Date: 7/26/95
Catherine Aranyi, MS

c. Sponsor's
Representative:

Marian K. Stanley Date: 7/27/95
Marian Stanley
Manager, Phthalate Esters Panel