PNL-6672
UC-408

Inhalation Reproductive Toxicology Studies: Sperm Morphology Study of n-Hexane in B6C3F1 Mice

Final Report

T. J. Mast
P. L. Hackett
J. R. Decker
R. B. Westerberg
L. B. Sasser
B. J. McClanahan
R. L. Rommereim
J. J. Evanoff

August 1988

Prepared for the
National Institute of Environmental
Health Sciences, National Toxicology Program
under a Related Services Agreement
with the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Operated for the U.S. Department of Energy
by Battelle Memorial Institute

Battelle

PNL-6672

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor Battelle Memorial Institute, nor any or their employees, makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or Battelle Memorial Institute. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE MEMORIAL INSTITUTE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC06-76RLO 1830

Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22161

NTIS Price Codes
Microfiche A01

Printed Copy

Pages	Price Codes
001-025	A02
026-050	A03
051-075	A04
076-100	A05
101-125	A06
126-150	A07
151-175	A08
176-200	A09
201-225	A10
226-250	A11
251-275	A12
276-300	A13

3 3679 00056 0500

PNL-6672
UC-408

INHALATION REPRODUCTIVE TOXICOLOGY STUDIES: SPERM MORPHOLOGY STUDY OF n-HEXANE IN B6C3F1 MICE

Final Report
No. NIH-Y01-ES-70153

T.J. Mast
P.L. Hackett
J.R. Decker
R.B. Westerberg
L.B. Sasser
B.J. McClanahan
R.L. Rommereim
J.J. Evanoff

August 1988

Prepared for the
National Institute of Environmental
Health Sciences, National Toxicology
Program under a Related Services Agreement
with the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

SUMMARY

The straight-chain hydrocarbon, n-hexane, is a volatile, ubiquitous solvent routinely used in industrial environments; consequently, the opportunity for industrial, environmental or accidental exposure to hexane vapors is significant. Although myelinated nerve tissue is the primary target organ of hexane, the testes have also been identified as being sensitive to hexacarbon exposure. The objective of this study was to evaluate the epididymal sperm morphology of male B6C3F1 mice 5 weeks after exposure to 0, 200, 1000, or 5000 ppm n-hexane, 20 h/day for 5 consecutive days. Two concurrent positive control groups of animals were injected intraperitoneally with either 200 or 250 mg/kg ethyl methanesulfonate, a known mutagen, once each day for 5 consecutive days. The mice were weighed just prior to the first day of exposure and at weekly intervals until sacrifice. During the fifth post-exposure week the animals were killed and examined for gross lesions of the reproductive tract and suspensions of the epididymal sperm were prepared for morphological evaluations.

The appearance and behavior of the mice were unremarkable throughout the experiment and there were no deaths. No evidence of lesions in any organ was noted at sacrifice. Mean body weights of male mice exposed to n-hexane were not significantly different from those for the 0-ppm animals at any time during the study. Analyses of the sperm morphology data obtained 5 weeks post-exposure (the only time point examined) indicated that exposure of male mice to relatively high concentrations of n-hexane vapor for 5 days produced no significant effects on the morphology of sperm relative to that of the 0-ppm control group. Furthermore, there were no significant differences among classes of sperm abnormalities in mice exposed to increasing concentrations of n-hexane. A significant, dose-related reduction in the percentage of normal sperm 5 weeks post-exposure was demonstrated for the positive control agent, ethyl methanesulfonate. These results indicate that n-hexane inhalation exposures of short duration, 5 days, do not adversely affect spermatogonia or primary spermatocytes in the B6C3F1 mouse as determined by examining the morphology of mature sperm by light microsopy.

ACKNOWLEDGMENTS

Responsibility	Name
Study Director	T.J. Mast
Previous Study Director	P.L. Hackett
Exposure System	J.R. Decker
	M.L. Clark
	R.J. Weigel
	E.J. Rossignol
Monitoring/Analytical Chemistry	R.B. Westerberg
	M.M. McCullough
Animal Resources Section	M.G. Brown
	S.E. Rowe
	A.E. Jarrell
Reproductive Toxicology	T.J. Mast
	B.J. McClanahan
	R.L. Rommereim
Report Co-ordination	J.J. Evanoff
	L.B. Sasser

Terryl J. Mast, PhD
Study Director

This work was supported by the National Institute of Environmental Health Sciences, National Toxicology Program, under a Related Services Agreement with the U.S. Department of Energy under Contract DE-AC06-76RLO 1830 at the Pacific Northwest Laboratory operated for the U.S. Department of Energy by Battelle Memorial Institute.

In-life portions of this study were conducted under the supervision of the previous Study Director, Dr. P.L. Hackett and co-Investigator Dr. B.J. McClanahan. The excellent technical support in the Reproductive Toxicology Laboratory of B.J. Willemsen, M.L. Sours and B.L. Champion is appreciated.

CONTENTS

SUMMARY iii
ACKNOWLEDGMENT v
TABLE OF CONTENTS vii
INTRODUCTION 1
MATERIALS AND METHODS 3
EXPERIMENTAL DESIGN 3
VAPOR GENERATION AND CHEMICAL ANALYSES 4
ANIMAL HUSBANDRY 6
SPERM MORPHOLOGY 7
STATISTICAL ANALYSES 7
RESULTS 9
DISCUSSION 10
REFERENCES 12
APPENDIX A - ANALYTICAL CHEMISTRY NARRATIVE AND DATA ..... A. 1
APPENDIX B - EXPOSURE NARRATIVE AND DATA ..... B. 1
NARRATIVE ..... B. 1
EXPOSURE CHAMBER CONCENTRATIONS B.15
CHAMBER AIR FLOWS B.21
RELATIVE HUMIDITY B.25
TEMPERATURE B.29
EXCURSION REPORT B.33
APPENDIX C - DEVELOPMENTAL TOXICOLOGY DATA ..... C. 1
BODY WEIGHT DATA ..... C. 1
SPERM ABNORMALITY DATA ..... C. 7
CALENDAR OF EVENTS C.13
ANIMAL DISPOSITION SUMMARY C.14
APPENDIX D - HEALTH SCREEN ..... D. 1
APPENDIX E - QUALITY ASSURANCE STATEMENT ..... E. 1
APPENDIX F - STUDY PROTOCOL AND CAGE MAPS ..... F. 1

FIGURES

FIGURE 1. Buildup and Decay of Vapor Concentrations in the 200, 1000 and 5000 ppm Chambers Without Animals Present.....15

FIGURE 2. Cumulative Body Weight Gains for Post-Exposure Weeks 1 Through 5.....16

TABLES

TABLE 1a. n-Hexane Mouse Sperm Morphology Study: Average Daily Exposure Chamber Concentrations.....17

TABLE 1b. n-Hexane Mouse Sperm Morphology Study: Average Daily Exposure Chamber Concentrations.....17

TABLE 1c. n-Hexane Mouse Sperm Morphology Study: Average Daily Exposure Chamber Concentrations.....18

TABLE 1d. n-Hexane Mouse Sperm Morphology Study: Average Daily Exposure Chamber Concentrations.....18

TABLE 2. n-Hexane Mouse Sperm Morphology Study: Mean Body Weights of Male B6C3F1 Mice (g ± SD).....19

TABLE 3. n-Hexane Mouse Sperm Morphology Study: Morphologic Classification of Sperm as Percentage of Total Examined (Mean ± SD).....20

TABLE 4. n-Hexane Mouse Sperm Morphology Study: Distribution of Sperm Abnormalities (Mean ± SD).....20

INTRODUCTION

The straight-chain hydrocarbon, n-hexane, is commonly used as a solvent for the extraction of oil seeds, as a reaction medium in the production of polyolefins, elastomers and pharmaceuticals, and as a component of quick-drying cements, lacquers and adhesives. The production of n-hexane, which was estimated to be four billion pounds per year in 1979, utilizes stocks of straight-run gasoline and higher boiling liquid products stripped from natural gas or paraffinic fractions of refinery streams. Since it is also found as a minor component of gasoline and its combustion products, petroleum products are a major source of environmental hexane contamination. Due to the large-scale production and widespread use of hexane, including teaching laboratories, the opportunity for industrial, incidental, environmental, or volitional (glue-sniffing) exposure to hexane vapors is significant. This study was performed due to concern that exposure to n-hexane vapors may result in a negative impact on human reproductive function.

An excellent review concerning hexacarbon toxicity and metabolism is available in Experimental and Clinical Neurotoxicology (edited by Spencer and Schaumburg, 1980). In summary, polyneuropathies have been reported following exposure of workers to n-hexane. A metabolite, 2,5-hexanedione (2,5HD), has been shown to be responsible for most, if not all, of the neurotoxicity. Younger rats appear to be less sensitive to n-hexane neurotoxicity than are older animals. It has been suggested that this difference may be due to their having shorter axons with smaller diameters, or to a greater rate of growth and repair of peripheral nerves as compared to that of adults (Howd et al. 1983; Kimura et al. 1971). Likewise, Graham and Gottfried (1984) hypothesized that mice are less sensitive than rats to gamma-diketones, such as 2,5HD, because myelinated axons in mice are shorter and have smaller diameters than the corresponding axons in larger species.

Pharmacokinetic and distribution studies of inhaled n-hexane in the rat indicated that the saturation concentration of n-hexane in organs is directly proportional to their lipid content, and that blood contains more hexane in relation to its lipid content than do other organs (Andersen 1981; Bohlen et

al. 1973). Baker and Rickert (1981) found that both the metabolism and elimination of n-hexane in male Fisher-344 rats given a single 6-h exposure to 500, 1000, 3000 or 10000 ppm n-hexane were dependent upon the exposure concentration, but that the tissue concentration of the metabolite, 2,5-hexanedione (2,5HD), was not directly correlated to increasing n-hexane exposure concentration. Maximal 2,5HD levels in the blood and in the sciatic nerve were observed in the 1000-ppm exposure group, but did not increase for the two higher exposure groups. Bus et al. (1982), using ^{14}C-labeled n-hexane administered to rats in a single 6-hour inhalation exposure of 500, 1000, 3000 or 10000 ppm observed a similar phenomena. Tissue ^{14}C-concentrations measured in liver, lung, kidney, testes, brain, sciatic nerve and blood were found to be maximal at the 3000-ppm level and did not increase at the 10000-ppm level.

Although myelinated nerve tracts are the primary target organ, the testes have also been identified as being sensitive to hexacarbon toxicity. Krasavage et al. (1980) reported testicular atrophy following oral administration of n-hexane and several of its metabolites to male rats. Chapin et al. (1982) administered a 1% solution of the hexane metabolite, 2,5HD, to male rats in their drinking water and found a decrease in the activity of two Sertoli cell enzymes, β-glucuronidase and γ-glutamyl transpeptidase, after 3 weeks of exposure. No morphologic changes were noted at three weeks; however, the testes were essentially azospermic after 6 weeks of exposure, and the few primary or secondary spermatocytes that were observed exhibited severe degenerative changes. Since circulating levels of testosterone and the gonadotropins remained normal throughout the study, these workers concluded that 2,5HD does not act via the central gonadotropin regulatory system to induce azospermia, and furthermore, that changes in Sertoli cell biochemistry precede visible morphologic changes in the testes. In a later study, Chapin et al. (1983) indicated that the Sertoli cell is probably an initial target cell for 2,5HD action on the testis of rats.

Cavender et al. (1984) did not detect neurotoxicity or testicular toxicity in rats exposed to 3000, 6500 or 10000 ppm purified hexane vapor(99.3%) 6 h/day, 5 day/wk for 13 weeks. However, the only measurement of testicular toxicity obtained in this study was the organ weight; the possibility of his-

tological or biochemical changes in the testes was not addressed. Although the exposure concentrations used in this study were relatively high, the short daily exposure periods may not have permitted the concentration of the hexane metabolite, 2,5HD, to build up sufficiently in the blood or tissues to cause testicular toxicity.

The potential testicular toxicity of n-hexane was investigated in the sperm morphology assay in mice in light of the reported testicular toxicity of the hexane metabolite, 2,5HD, in rats. This mouse sperm morphology assay was originally developed as an *in vivo* test to detect mutagenic compounds (Wyrobek and Bruce, 1975; Wyrobek et al., 1979) and typically employed the B6C3F1 hybrid mouse as the test species. This hybrid mouse was chosen for this assay because of its low numbers of abnormal sperm and little inter-mouse variation (Wyrobek and Bruce, 1978). Accordingly, the objective of this study was to evaluate the sperm morphology of B6C3F1 male mice five weeks after exposure to relatively high concentrations of n-hexane vapor, 20 h/day for a 5-day period. An alternate approach to detecting mutagenic events in spermatogenesis, the male dominant lethal assay was conducted as a companion study, Mast, et al. (1988a). In this study the potential for n-hexane to cause male dominant lethal effects in the Swiss (CD-1) mouse was examined following the same exposure regimen.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Six groups of B6C3F1 male mice, each consisting of 20 randomly selected animals were exposed to 0 (filtered air), 200, 1000, or 5000 ppm n-hexane vapor for 5 consecutive days, 20 h/day, or to a positive-control agent, ethyl methanesulfonate (EMS). The long daily inhalation exposure periods were chosen in order to maximize n-hexane exposures since the maximum vapor concentration of n-hexane in the exposure chambers was not allowed to exceed 50% of the lower explosion limit, ≈10,000 ppm. Mice in the positive-control groups were given intraperitoneal injections of either 200 or 250 mg/kg EMS. The 0-ppm animals were housed in an exposure chamber in the same room, and were handled in the same manner as the animals exposed to n-hexane vapors. The

positive-control animals were housed in an exposure chamber in a separate room and were injected with EMS on each of 5 consecutive days concurrent with the n-hexane exposures.

After the 5-day exposure period, the mice were maintained in individual wire-mesh cages for 4 weeks and were killed during the 5th post-exposure week. Sperm morphology was evaluated at this time by light microscopy of stained epididymal sperm preparations (details follow). The sacrifice was performed 5 weeks post-exposure since previous work had shown that the peak incidence of abnormalities in sperm induced by mutagenic agents such as EMS occurred at about this time (Wyrobek and Bruce 1975).

VAPOR GENERATION AND CHEMICAL ANALYSES

Bulk chemical purity analyses were performed on the single lot of n-hexane used for the mouse exposures. Analytical procedures employed infrared spectroscopy and gas chromatography (GC) for the initial identity and purity determinations. The purity of the n-hexane used in this study was 99.1% (BNW Lot 50846-39; Phillips Lot # H-116). See Appendix A for details.

On-line measurements of the chamber n-hexane concentrations were performed using an HP5840 GC equipped with a flame ionization detector. A computer-controlled, rotating 8-port valve allowed measurement of n-hexane concentrations in the exposure room, the control chamber, the exposure chambers, and the on-line standard. All ports were sampled at least once every 40 minutes. The GC was equipped with a 1/8" o.d., 1-ft nickel column packed with 1% SP-1000 on 60/80 mesh Carbopack B. The oven operating temperature was 120°C. An on-line standard, 994 ppm n-hexane in nitrogen (MG Industries Scientific Gases, Los Angeles, CA), was used to check instrument drift throughout the exposure day. The minimum detectable limit of n-hexane was estimated from the decay profile of the 5000-ppm chamber and found to be 0.11 ppm. The calibration curve for this analysis was linear over an extended range and was monitored at intervals by routine analysis of bubbler-samplers.

Inhalation exposures were conducted in 2.3-m^3 stainless steel chambers (1.7-m^3 active-mixing volume) designed at Battelle Northwest Laboratories

(Brown and Moss 1981; Moss et al. 1982). Each chamber contained three levels of caging, each of which was split into two offset tiers. Air, filtered through HEPA and charcoal filters and uniformly mixed with the test material, flowed through the chamber at approximately 15 air changes per hour. See Appendix B for a more detailed description.

The n-hexane exposures were conducted using an automated data acquisition and control system which monitored and controlled the basic inhalation test system functions, including chamber air flow, vacuum, temperature, relative humidity, and test chemical concentrations. Conditions which may have been a threat to the health of the animals, or constituted an explosion hazard, triggered alarms to personnel on-call 24 h/day. All data acquisition and control originated from an executive computer which contained the exposure protocols and controlled a multiplexing interface system.

Generation of the n-hexane vapor was accomplished by metered pumping of the liquid chemical from a 5-gallon reservoir which was replenished daily. The test material was delivered through inert delivery tubes to a vaporizer located at the fresh air inlet of each animal exposure chamber. The vaporizer was comprised of a stainless steel cylinder wrapped with a glass fiber wick from which the liquid was vaporized. The operating temperature of the vaporizer was maintained below 50°C (the boiling point of n-hexane is ≈70°C). All generation equipment which came into contact with the n-hexane was stainless steel, Teflon®, or Viton®. All equipment was contained in a vented, explosion-proof generator cabinet. Chamber air flows were maintained by a computer-controlled pump in the exhaust line of each chamber. The exposure suite data acquisition and control computer automatically controlled the concentration of n-hexane in the chambers by adjusting the flow rate of dilution air through the individual chambers.

The buildup and decay of n-hexane concentrations, without animals in the chambers, were checked prior to the first week of the study, Figure 1. The time required to reach 90% of the target concentration (T_{90}) ranged from 11.0-13.0 min. The decay time, the time required to decline to 10% of the target concentration (T_{10}) after generators were shut off, ranged from 9.0-11.0 min. Uniformity of vapor concentration in the exposure chambers was

measured prior to the start of the study and found to be acceptable in all chambers (e.g. ±5%). A buildup and decay determination with animals present in the chambers was not performed due to the short total duration of the exposure—5 days. However, later evaluations showed that the presence of mice did not significantly affect build-up and decay times (Mast, et al 1988b).

ANIMAL HUSBANDRY

A total of 137 male B6C3F1 mice, 8 weeks of age (Charles River Laboratories, Portage, MI), were singly housed in wire-mesh cages in a quarantine room for 24 days. Mice selected for inhalation exposure were acclimated to the exposure chambers for 3 days prior to the initiation of the study. During the quarantine period 10 animals were killed and examined for bacterial pathogens. Serum from each animal was tested for antibodies to selected pathogens, and histopathologic examinations of lung, liver, kidney, ileum, colon, and heart were performed (Appendix D). At necropsy, serum from five animals from the high dose group and five from the control group was also tested for antibodies to selected pathogens. All results were negative for significant pathogens and gross lesions. All animals were observed daily throughout the study for morbidity, mortality, and overt signs of toxicity.

Pelleted NIH-07 diet (Ziegler Bros. Inc., Gardner, PA) was provided *ad libitum* during the entire study. Since the daily duration of exposure was so long, 20 h, food was left in place during the exposure period in order to prevent food deprivation and was replaced daily. Water was provided *ad libitum* by an automatic watering system at all times. Room lighting was maintained on a 12-h on-off cycle starting at 6 A.M.

During quarantine period the animal room was maintained at 72±3°F and 50±15% relative humidity (RH). During the exposure period daily mean chamber temperatures were between 73.0 and 74.8°F, within the normal operating range of 75±3°F. Actual chamber temperatures ranged between 71.7 and 78.5°F, and 93% of the measurements were within the specified range. Daily mean RH in all exposure chambers was between 49.9 and 59.8%; RH was within the normal operating range of 50±15% for more than 93% of the measurements. The average air flow in all chambers for the study was 15.0-15.1 CFM; the minimum and

maximum were 13.2 and 18.5 CFM, respectively. A detailed summary of the daily chamber environment is listed in Appendix B.

SPERM MORPHOLOGY

All mice were weighed, identified by numbered eartags, toe-clipped by exposure group, and randomized on the basis of body weight, during the week prior to exposure. Body weights were obtained on the 1st day of exposure and weekly during the post-exposure period. During the fifth post exposure week, the mice were killed with CO_2, weighed, and examined for gross lesions of the genital tract and for gross tissue abnormalities. See Appendix C for a complete tabulation of body weight and sperm morphology data.

Sperm suspensions were prepared immediately after sacrifice by mincing the cauda epididymis in 2 ml phosphate-buffered saline (PBS; Wyrobek and Bruce 1975). Minced tissues were agitated gently in PBS then filtered through an 80-100 µm stainless steel screen. Several drops of 1% Eosin Y stain solution were added to the filtrate and it was allowed to stand for 45 min. After resuspension of the cells, four drops were placed on a glass microscope slide and allowed to air dry overnight. All slides were labeled only with the animal number in order to conceal the identity of the treatment group from the examiner during evaluation. The morphology of at least 500 sperm from each slide was examined under a light microscope (40x) and classified as either normal or abnormal. The following types of abnormal sperm were also recorded: blunt-hook, banana, amorphous, pin-head, or two heads/two tails.

STATISTICAL ANALYSES

The SAS statistical software on a VAX 11/780 computer was used to calculate all means and standard deviations of animal data. Body weights were analyzed using the SAS General Linear Models (GLM) procedures (SAS 1985) with an analysis of variance (ANOVA) model for unbalanced data. Values for normal and abnormal sperm heads were expressed as a proportion of the total number of cells examined for each slide. The proportion of abnormal sperm of each type relative to the total number of abnormal sperm was calculated as well. These proportional data were subjected to an arcsin transformation and evaluated by

an ANOVA. Tukey's multiple-range test (two-tailed) was used to ascertain the presence of statistically significant differences between control and exposed groups. When appropriate, the data were also evaluated for orthogonal contrasts to determine significance of trend (Winer 1971).

RESULTS

The daily mean n-hexane concentrations for all exposure chambers are shown in Table 1. Grand means for all exposure concentrations were within 6% of the target concentrations. Detailed summaries of the concentration data, summaries of environmental data, and graphic illustrations of the daily means, and standard deviations for each chamber are included in Appendix B.

The appearance and behavior of the mice were unremarkable throughout the experiment and no evidence of n-hexane toxicity was observed. No deaths occurred in any of the four inhalation exposure groups. However, three ethyl methanesulfonate (EMS) positive-control animals (one in the 200 mg/kg group and two in the 250 mg/kg group) died from EMS toxicity between 4 and 10 days after treatment was initiated. At the scheduled sacrifice, 5 weeks post-exposure, no unusual clinical observations were observed for either of the EMS groups nor were gross lesions noted in any organ.

Significant differences in mean body weights were not observed between any treatment group at sacrifice (Table 2). Mean body weights of male mice exposed to n-hexane vapor concentrations as high as 5000 ppm for 5 consecutive days were not significantly different from the 0-ppm group at any time during the post-exposure period. However, the body weights of both EMS-treated positive-control groups were significantly lower than the 0-ppm group during 1st and 2nd post-exposure weeks. The mean body weight of the 250-mg/kg group was also significantly reduced relative to the 0-ppm group during 3rd post-exposure week. Cumulative weight gains for the 250-mg/kg EMS group were significantly less than all n-hexane groups throughout the entire post-exposure period (Figure 2).

Analyses of the sperm morphology data obtained from male mice 5 weeks post-exposure to 200, 1000 or 5000 ppm n-hexane vapor produced no statistically significant effects on the percentages of normal or abnormal sperm relative to the 0-ppm group (Table 3). Although the percentages of sperm classified as having a "blunt hook" were positively correlated to increasing n-hexane concentration, this is probably of no biological

significance since the 200 and 1000 ppm groups both had lower values than the 0-ppm group. Ethyl methanesulfonate was shown to be an effective positive-control agent at the doses used as evidenced by a significant, treatment-related reduction in the percentage of normal sperm observed at five weeks post-exposure (Table 3). The proportional distribution of the different types of abnormalities was not affected (Table 4).

DISCUSSION

Significant increases in the incidence of sperm abnormalities were not observed five weeks post-exposure (the only time point examined) in the B6C3F1 mice exposed to 200, 1000 or 5000 ppm n-hexane vapor for 5 days, 20 h/day. Nor were any significant treatment-related effects found in a companion study conducted to assess the potential for n-hexane exposure to cause male dominant lethality in mice (Mast et al. 1988a). Although the mechanisms producing abnormal sperm and male dominant lethality may be dissimilar, these two studies lend support to the conclusion that n-hexane exposures of short duration do not adversely affect sperm quality, even when administered at relatively high concentrations.

In contrast to the lack of observable affects on the sperm noted in this mouse study, others have reported toxic effects to the testes of rats after exposure to hexane or its metabolites. Atrophy of testicular germinal epithelium in rats following the oral administration of n-hexane or its metabolites was reported by Krasavage et al.(1980); however, n-hexane was much less potent than its metabolites. The oral administration of the hexane metabolite, 2,5HD, in drinking water caused demonstrable changes in Sertoli cell biochemistry which occurred prior to visible morphologic changes in the rat testes (Chapin et al. 1982). The activities of Sertoli cell ß-glucuronidase and γ-glutamyl transpeptidase were decreased at 3 and 6 weeks of exposure, whereas the testes, which appeared normal at 3 weeks, had severe lesions and were azospermic at 6 weeks. These results are not consistent with the studies of Cavender et al. (1984), who failed to detect testicular toxicity (e.g. as decreased testes weight) in rats exposed to atmospheric concentrations of n-hexane as high as 10,000 ppm, 6 h/day for 13 weeks.

The results of most of the studies reviewed above can not be directly compared because of differences in animal species and in experimental design. However, it is interesting to note that negative responses of the testes or the sperm have resulted with inhalation exposures whereas positive effects have been seen in studies employing oral routes of exposure. The differences in response in rats may be due to differences in effective dose or in the duration of exposure. The inhalation exposures were either for a short time period (1-5 days), or for a small fraction of the day (6 h), whereas the oral studies were generally continuous for up to 6 weeks. Krasavage et al. (1980) showed that a single, oral dose of n-hexane to the rat was rapidly metabolized to 2,5HD, and that the 2,5HD was completely cleared from the blood in about 18 h. Consequently, a sustained exposure may be required to cause adverse effects. Mice could be insensitive to testicular effects of n-hexane or respond differently than rats to n-hexane exposures.

The mouse sperm morphology assay was developed by Wyrobek and Bruce (1975) to detect mutagenic compounds and has been applied to a wide variety of industrial carcinogens and other compounds. However, it has shown only moderate correlation with the *in vitro* mutagenic assays (Bruce and Heddle, 1979). The mouse sperm morphology assay was also investigated by Osterloch et al. (1983) as a possible adjunct to assessing testicular toxicity. These workers examined a broad range of pesticides using the sperm morphology assay and found surprisingly few positive responses to known testicular toxins and/or carcinogens, perhaps due to the wide array of mechanisms which may cause testicular toxicity. Thus, Osterloh et al. (1983) concluded that the sperm morphology assay was not a suitable method to use in the detection of testicular toxins. In our study, sperm abnormalities were not detected following n-hexane exposure; however, we were able to demonstrate a dose-response effect of the positive-control material, EMS, on the incidence of sperm abnormalities occurring 5 weeks after the cessation of exposure. Under the conditions of this study, n-hexane inhalation exposures of short-duration, 5 days, did not adversely affect sperm morphology, suggesting the lack of an effect on spermatogonia or primary spermatocytes in the B6C3F1 mouse.

REFERENCES

Andersen, M.E. 1981. Pharmacokinetics of inhaled gases and vapors. Neurobehav. Toxicol. Teratol. 3:383-389.

Baker, T.S. and D.E. Rickert. 1981. Dose-dependent uptake, distribution, and elimination of inhaled n-hexane in the Fischer-344 rat. Toxicol. Appl. Pharmacol. 61:414-422.

Bohlen, P., U.P. Schlunegger and E. Lauppi. 1973. Uptake and distribution of hexane in rat tissues. Toxicol. Appl. Pharmacol. 25:242-249.

Brown, M.G. and O.R. Moss. 1981. An inhalation exposure chamber designed for animal handling. Labor. Anim. Sci. 31:717-720.

Bruce, W.R. and J.A. Heddle. 1979. The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella and sperm morphology assays. Can. J. Genet. Cytol. 21: 319-334.

Bus, J.S., D. Deyo, and M. Cox. 1982. Dose-dependent disposition of n-hexane in F-344 rats after inhalation exposure. Fund. Appl. Toxicol. 2:226-229.

Cavender, F.L., H.W. Casey, H. Salem, D.G. Grahem, J.A. Swenberg and E.J. Gralla. 1984. A 13-week vapor inhalation study of n-hexane in rats with emphasis on neurotoxic effects. Fund. Appl. Toxicol. 4:191-201.

Chapin, R.E., K.T. Morgan, and J.S. Bus. 1983. The morphogenesis of testicular degeneration induced in rats by orally administered 2,5-hexanedione. Exp. Molec. Path. 38:149-169.

Chapin, R.E., R.M. Norton, J.A. Popp, and J.S. Bus. 1982. The effects of 2,5-hexanedione on reproductive hormones and testicular enzyme activities in the F-344 rat. Toxicol. Appl. Pharmacol. 62:262-272.

Graham, D.G., and M.R. Gottfried. 1984. Cross-species extrapolation in hydrocarbon neuropathy. Neurobehav. Toxicol. Teratol. 6:433-435.

Howd, R.A., C.S. Rebert, J. Dickinson, and G.T. Pryor. 1983. A comparison of the rates of development of functional hexane neuropathy in weanling and young adult rats. Neurobehav. Toxicol. Teratol. 5:63-68.

Kimura, E.T., D.M. Ebert, and P.W. Dodge. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol. Appl. Pharm. 19:699-704.

Krasavage, W.J., J.L. O'Donoghue, G.D. DiVincenzo and C.J. Terhaar. 1980. The relative neurotoxicity of methyl-n-butyl ketone, n-hexane and their metabolites. Toxicol. Appl. Pharmacol. 52:433-441.

Mast, T.J., J.J. Evanoff, J.R. Decker, R.L. Rommereim, L.B. Sasser, R.B. Westerberg. 1988a. Inhalation Reproductive Toxicology Studies: Male Dominant Lethal Study of N-Hexane in Swiss (CD-1) Mice. PNL-6679. Pacific Northwest Laboratory, Richland, Washington.

Mast, T.J., J.R. Decker, K.H. Stoney, R.B. Westerberg, J.J. Evanoff, R.L. Rommereim, R.J. Weigel. 1988b. Inhalation Developmental Toxicology Studies: Teratology Study of n-Hexane in Mice. PNL-6590. Pacific Northwest Laboratory, Richland, Washington.

Moss, O.R., J.R. Decker and W.C. Cannon. 1982. Aerosol mixing in an animal exposure chamber having three levels of caging with excreta pans. Amer. Ind. Hyg. Assoc. J. 43:244-249.

Osterloh, J., L. Gideon, S. Pond, and C. Becker. 1983. An assessment of the potential testicular toxicity of 10 pesticides using the mouse sperm morphology assay. Mutation Res. 116: 407-415.

SAS Institute. 1985. SAS® User's Guide: Basics, Version 5 Edition. SAS Institute, Cary, North Carolina, pp 434-506.

Spencer, P.S., D. Couri and H.H. Schaumburg. 1980. n-Hexane and methyl n-butyl ketone. In Experimental and Clinical Neurotoxicology, P.S. Spencer and H.H. Schaumburg J. (eds.), Williams & Wilkins, Baltimore, MD, pp. 456-475.

Winer, B.J. 1971. Statistical Principles in Experimental Design, McGraw-Hill Book Co., New York, NY, pp 170-185.

Wyrobek, A.J. and W.R. Bruce. 1975. Chemical induction of sperm abnormalities in mice. Proc. Nat. Acad. Sci. 72, 4425-4429.

Wyrobek, A.J. and W.R. Bruce. 1978. The induction of sperm-shape abnormalities in mice and humans. Chemical Mutagens: Principles and Methods for Their Detection, A. Hollaender and F.J. De Serres (eds.), Plenum, New York, pp. 257-285.

Wyrobek, A.J. et al. (1979) The effect of 17 chemical agents including 6 carcinogen/noncarcinogen pairs on sperm shape abnormalities in mice, Proc. Symp. Int. Program for the Evaluation of Short-Term Tests for Carcinogenicity, St. Simon Island, GA.

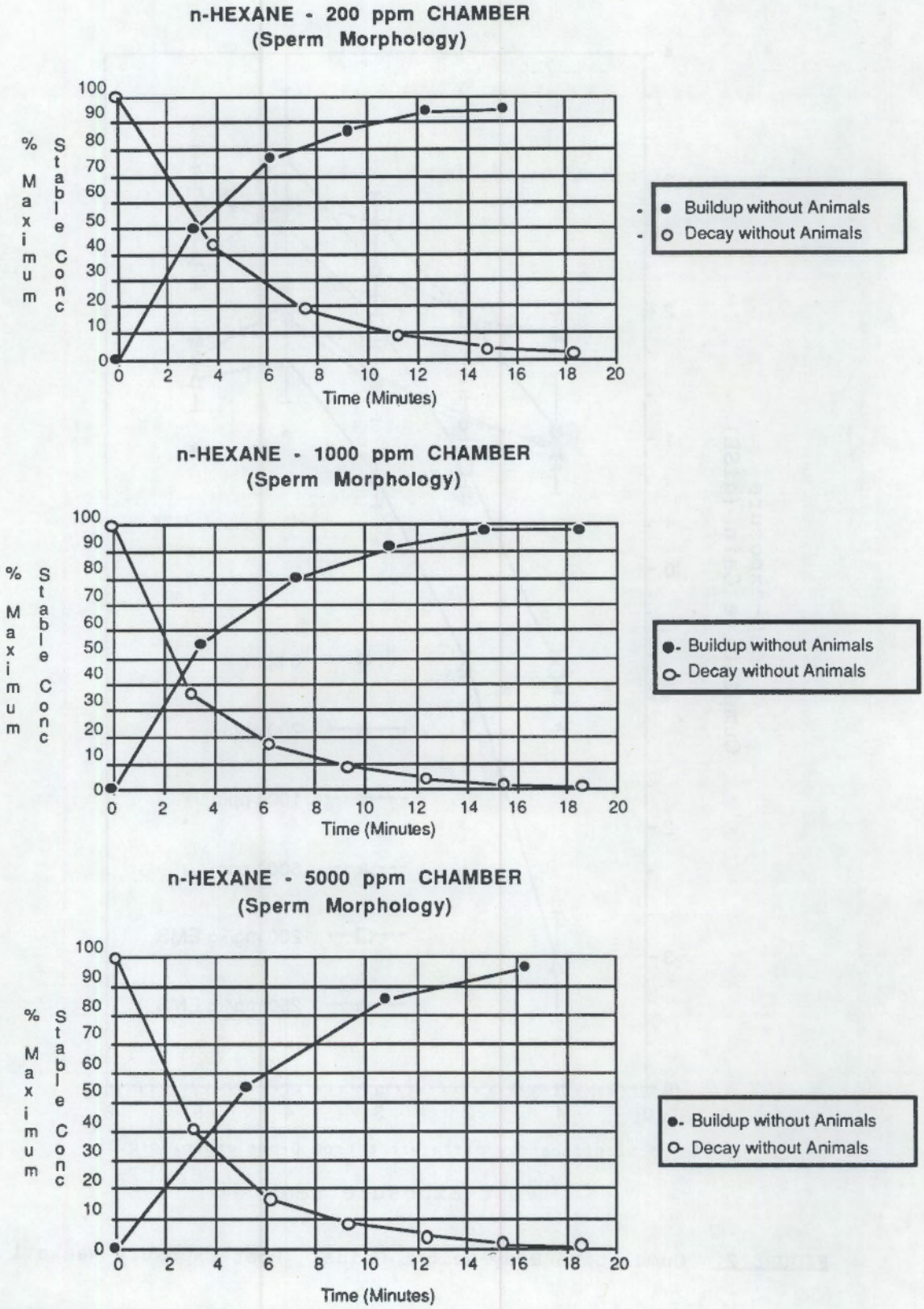


FIGURE 1. Buildup and Decay of 200, 1000, and 5000 ppm n-Hexane Vapor Concentrations in Chambers Without Animals Present.

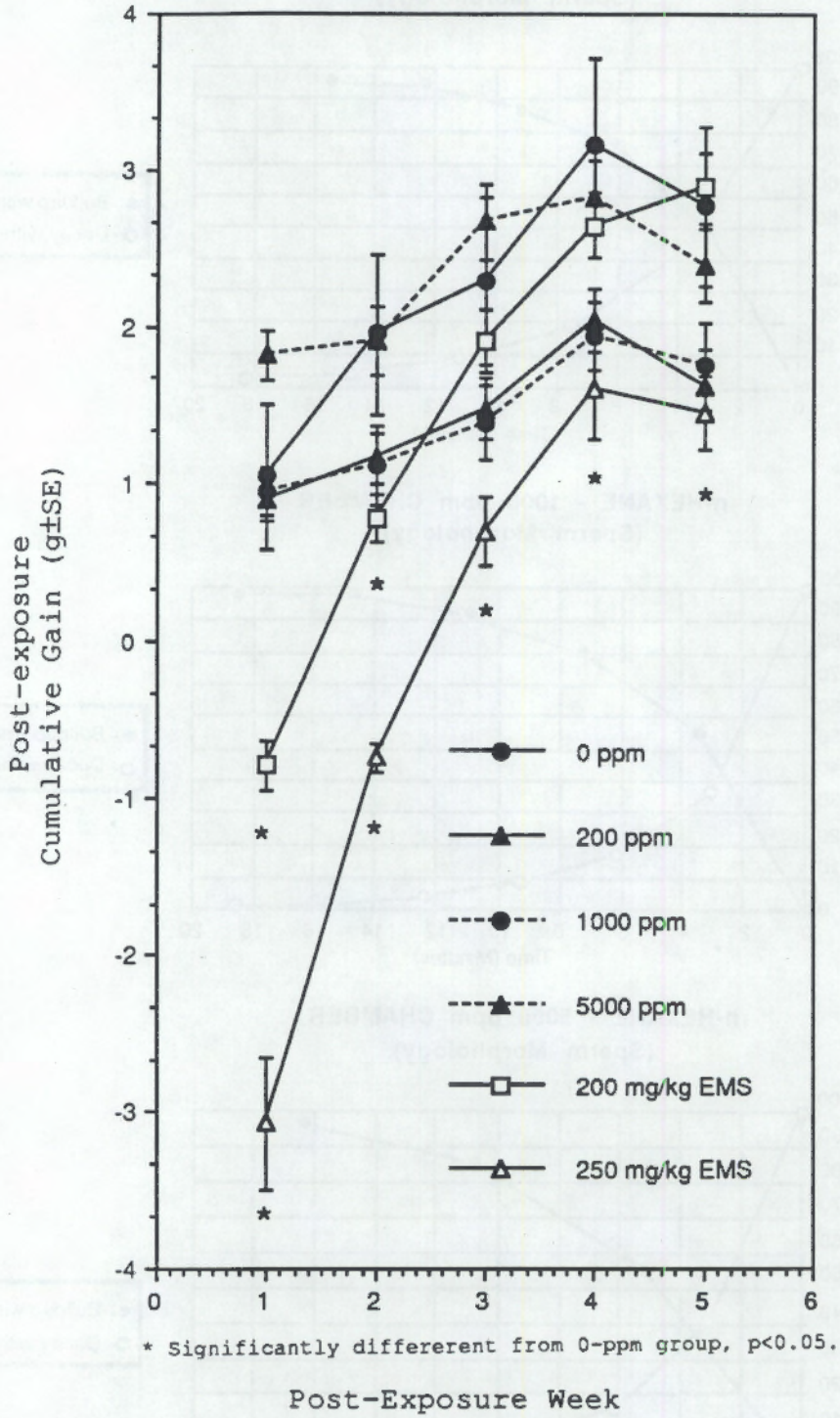


FIGURE 2. Cumulative Body Weight Gains: Post-Exposure Weeks 1 through 5.

TABLE 1a. n-Hexane Sperm Morphology Study: Average Daily Exposure Chamber Concentrations

0 ppm n-Hexane Vapor						
Exposure Day	Mean ±SD (ppm)	Max (ppm)	Min (ppm)	Number Samples	Number in Range (b)	Percent in Range
1	<MDL (a)	< MDL	<MDL	36	36	100
2	<MDL	< MDL	<MDL	41	41	100
3	<MDL	< MDL	<MDL	41	41	100
4	<MDL	< MDL	<MDL	35	35	100
5	<MDL	< MDL	<MDL	43	43	100
Summary	<MDL	< MDL	<MDL	196	196	100

(a) Minimum detectable Limit (MDL) = 0.11 ppm n-Hexane.
(b) Range = 0-1 ppm n-Hexane

TABLE 1b. n-Hexane Sperm Morphology Study: Average Daily Exposure Chamber Concentrations

200 ppm n-Hexane Vapor						
Exposure Day	Mean ±SD (ppm)	Max (ppm)	Min (ppm)	Number Samples	Number in Range (a)	Percent in Range
1	189 ± 33	219	0.3	36	34	94
2	200 ± 5	208	183	40	40	100
3	195 ± 8	210	181	41	41	100
4	197 ± 7	219	182	36	36	100
5	196 ± 4	209	188	42	42	100
Summary	196 ± 16	219	0.3	195	193	99

(a) Range = ±10% target.

TABLE 1c. n-Hexane Sperm Morphology Study: Average Daily Exposure Chamber Concentrations

1000 ppm n-Hexane Vapor						
Exposure Day	Mean ±SD (ppm)	Max (ppm)	Min (ppm)	Number Samples	Number in Range (a)	Percent in Range
1	995 ± 14	1030	962	36	36	100
2	1 010 ± 16	1040	975	40	40	100
3	988 ± 30	1070	938	41	41	100
4	997 ± 24	1100	930	36	35	97
5	997 ± 16	1030	972	42	42	100
Summary	998 ± 22	1100	930	195	194	99

(a) Range = ±10% target.

TABLE 1d. n-Hexane Sperm Morphology Study: Average Daily Exposure Chamber Concentrations

5000 ppm n-Hexane Vapor						
Exposure Day	Mean ±SD (ppm)	Max (ppm)	Min (ppm)	Number Samples	Number in Range (a)	Percent in Range
1	3450 ± 1160	4430	15	36	0 (b)	0 (b)
2	5130 ± 97	5280	4960	41	41	100
3	4870 ± 163	5190	4610	42	42	100
4	4920 ± 800	5910	516	37	34	92
5	4990 ± 66	5240	4880	42	42	100
Summary	4700 ± 848	5910	15	198	159	80

(a) Range = ±10% target.
(b) Pump failure.

TABLE 2. n-Hexane Mouse Sperm Morphology Study: Mean Body Weights of Male B6C3F1 Mice (g ±SD).

	n-Hexane Concentration (ppm)				EMS (mg/kg) (b)	
	0	200	1000	5000	200	250
Week No.	N=20	N=20	N=20	N=20	N=20	N=20
Exposure Day 1	26.7 ± 2.2	27.6 ± 1.2	27.2 ± 1.3	27.2 ± 1.5	26.4 ± 1.6	26.8 ± 1.4
Post-exposure Wk 1	27.8 ± 1.4	28.5 ± 1.3	28.2 ± 1.3	29.0 ± 1.6	25.6 ± 1.4 (b,d)	23.7 ± 2.1 (c,d)
Post-exposure Wk 2	28.7 ± 1.6	28.8 ± 1.3	28.4 ± 1.3	29.1 ± 2.0	27.2 ± 1.4 (b,d)	26.0 ± 1.4 (c,d)
Post-exposure Wk 3	29.0 ± 1.7	29.1 ± 1.4	28.6 ± 1.3	29.8 ± 2.1	28.3 ± 1.5 (b)	27.4 ± 1.9 (c,d)
Post-exposure Wk 4	29.9 ± 1.8	29.7 ± 1.4	29.2 ± 1.3	30.0 ± 2.0	29.1 ± 1.7 (b)	28.3 ± 2.3 (c)
Post-exposure Wk 5	29.5 ± 1.6	29.3 ± 1.4	29.0 ± 1.5	29.5 ± 1.8	29.3 ± 1.7 (b)	28.2 ± 2.0 (c)

(a) Positive control; ethyl methanesulfonate.
(b) N=19; (See Appendix C).
(c) N=18; (See Appendix C).
(d) Significantly different from control group, $p<0.05$.

TABLE 3. n-Hexane Mouse Sperm Morphology Study: Percentages of Normal and Abnormal Sperm (Mean ± SD). (a)

	n-Hexane Concentration (ppm)				EMS (mg/kg) (b)	
	0	200	1000	5000	200	250
Number Examined	20	20	20	20	19	17 (c)
Type of Abnormality	%	%	%	%	%	%
Normal	99.02 ± 0.99	99.60 ± 0.44	99.16 ± 0.68	99.11± 0.78	96.38 ± 2.05 (e)	86.91 ± 5.68 (e)
Blunt Hook (d)	0.44 ± 0.65	0.16 ± 0.22	0.36 ± 0.44	0.53 ± 0.47	1.22 ± 0.91	6.29 ± 3.87 (e)
Banana	0.06 ± 0.15	0.04 ± 0.14	0.06 ± 0.17	0.09 ± 0.21	0.03 ± 0.12	0.49 ± 0.72 (e)
Amorphous	0.49 ± 0.62	0.21 ± 0.33	0.43 ± 0.32	0.28 ± 0.34	2.32 ± 1.45 (e)	6.32 ± 2.44 (e)
Pinhead	0.01 ± 0.03	0	0	0.01 ± 0.04	0.05 ± 0.19	0
Two heads/two tails	0	0	0.01 ± 0.03	0	0.01 ± 0.03	0.01 ± 0.03
Total abnormal	0.99 ± 1.17	0.40 ± 0.45	0.85 ± 0.68	0.90 ± 0.78	3.62 ± 2.05	13.09 ± 5.68

(a) Approximately 1000 sperm examined per male (2 slides, 500 per slide).
(b) Positive control; ethyl methansulfonate.
(c) One male in this group was azospermic.
(d) Values for n-hexane groups significantly correlated with increasing exposure concentration, $p<0.05$.
(e) Significantly different from 0-ppm group following arcsin transformation, $p<0.05$.

TABLE 4. n-Hexane Mouse Sperm Morphology Study: Distribution of Classes of Sperm Abnormalities as Percent of Total Abnormalities (Mean ± SD). (a)

	n-Hexane Concentration (ppm)				EMS (mg/kg) (b)	
Type of Abnormality	0	200	1000	5000	200	250
Blunt Hook	44.0 ± 34.0	43.2 ± 41.43	38.4 ± 31.7	62.1± 29.0	33.0 ± 19.6	46.0 ± 11.4
Banana	5.6 ± 14.7	7.9 ± 20.4	6.0 ± 18.2	4.7 ± 11.0	0.9 ± 3.5	3.6 ± 5.2
Amorphous	49.8 ± 34.4	48.9 ± 40.9	55.0 ± 32.3	32.2 ± 30.3	65.0 ± 19.4	50.2 ± 12.4
Pinhead	0.7 ± 4.2	0	0	0.9 ± 3.8	0.8 ± 2.6	0
Two heads/two tails	0	0	0.7 ± 4.1	0	0.2 ± 1.4	0.1 ± 0.3

(a) (Mean percent abnormal of individual class of abnormality/mean percent of total abnormal sperm) x 100.
(b) Positive control; ethyl methansulfonate.

APPENDIX A

ANALYTICAL CHEMISTRY NARRATIVE AND DATA FOR N-HEXANE

ANALYTICAL CHEMISTRY NARRATIVE AND DATA FOR n-HEXANE

1. Test Material Receipt and Usage

n-Hexane, manufactured by Phillips Chemical Company, was received from Research Triangle Institute (RTI), P.O. Box 12194, Research Triangle Park, NC 27709-9981. The test material for this study, identified as BNW Lot 50846-39 (Phillips Lot#H-116) was received on 2/12/86 and consisted of thirty 4-liter bottles.

The bulk chemical was stored in its original shipping container at ≈65°F in a flammable storage cabinet and maintained under a blanket of nitrogen. All chemical transfers to the reservoir took place under a blanket of nitrogen to avoid the introduction of air into the bulk chemical. Approximately 11.2 kg of test material were required for each exposure day. The usage of n-hexane for the mice dominant lethal study is summarized in Table 1.

TABLE 1. Mice Sperm Morphology Study with n-Hexane - Chemical Usage

Exposure Period	Phillips Lot#	BNW Lot#	Test Material Used
3/24/86 - 3/28/86	H-116	50846-39	~56 kg

2. Bulk Chemical Analysis

Bulk chemical analysis was performed using infrared spectroscopy and gas chromatography (GC) for identity and purity determinations. The gas chromatographic system used for purity analysis employed a 1.8mm x 4mm glass column packed with 0.1% SP-1000 on 80/100 Carbopack C. BNW Lot 50846-39 was analyzed for bulk purity and found to be 99.1% pure relative to the frozen reference material.

3. Vapor Concentration Monitoring

A Hewlett-Packard 5840 gas chromatographic system (employing a 1/8" o.d., one-foot nickel column packed with 1% SP-1000 on 60/80 mesh Carbopack B; oven temperature was 120°C) was used to monitor animal exposures. This instrument was equipped with an 8-port stream select valve and measured n-hexane in the three exposed chambers, the control chamber, the exposure room, and the on-line standard.

a. Calibration of the On-Line Chamber Monitor

The calibration of the on-line chamber monitor was based on analysis of bubbler grab samples. Thus, the calibration of the on-line monitor was tied to gravimetrically prepared standard solutions in dodecane through a second directly calibrated GC which was off-line. The analysis depended upon quantitative preparation of gravimetric standards and careful grab sampling. The gravimetrically calibrated GC was used to measure the quantity of n-hexane collected from exposure chambers in dodecane filled bubblers. The relationship between the peak area observed with the on-line GC and the concentration of n-hexane in the chamber was then defined using chamber concentrations determined by the gravimetrically calibrated GC.

The analysis of bubbler grab samples was performed using a HP 5830A GC with a 6' x 4mm glass column with 10% Carbowax 20m (TPA) on 80/100 Chromosorb WAW. The temperature program was 40°C for 3 minutes to 150°C for 10 minutes at the rate of 15°C/minute. A set of three standards was run for each analysis session. The concentration range of the standards bracketed the concentration range of interest.

The calibration procedure required quantitatively prepared gravimetric standards and carefully collected grab samples of a measured volume. The collection efficiency of a single bubbler was less than 100%, some hexane broke through the primary bubbler. Breakthrough was typically <4-6%. Breakthrough was measured each time bubblers were collected by acquiring back-up bubblers for the high concentration chamber. The calculation for chamber concentration by the grab sampling method included a breakthrough correction.

b. Detection of Monitor Drift Using an On-Line Standard

An on-line standard was used to check instrument drift throughout the exposure day. The on-line standard was 994 ppm n-hexane in nitrogen (MG Industries Scientific Gases, 11705 South Alameda St., Los Angeles, CA). The standard was checked before the start of any given exposure day, then monitored every 8th sample throughout the exposure period. The measured concentration for the standard had to be within ±10% of the assigned target value before any exposure could begin without consultation with the Exposure Control Task Leader. During the course of the exposure, if the on-line standard was within 5% of the target value, no change in calibration was required. If the on-line standard was between 5% and 10% of its assigned target, the calibration could be updated immediately by an Exposure or Chemistry Specialist. Such a correction was based upon the on-line standard. If the cumulative drift exceeded 15%, then the calibration was checked by quantitative analysis of grab samples.

c. Demonstration of Sensitivity and Specificity

The sensitivity of the GC was estimated from the decay profile for the highest concentration chamber. The minimum detectable limit (MDL) was estimated as 0.15 ppm. A measure of chromatographic specificity was defined by determination of the analytes partition coefficient. The retention time of methane, assumed to be non-retained was 0.19 min.; the retention time for n-hexane was 1.49 minutes. Thus, the partition ratio was about 6.8.

d. Precision, Linearity and Absolute Recovery Evaluation

Precision for the on-line GC was estimated from 5 consecutive measurements made on the 994 ppm on-line certified standard; a 0.4% coefficient of variation (CV) was observed (all values fell within ±1 ppm of the mean). Linearity of the on-line GC was assured by calibrating the on-line GC against a gravimetrically calibrated GC (also see comments in the "Calibration of the On-Line Chamber Monitor" section). This was basically accomplished by analyzing a series of bubbler grab samples acquired during exposure generation and then implementing the appropriate on-line GC calibration curve in the data acquisition and control system.

Achievement of linearity for the on-line monitor was therefore dependent upon defining a linear method for analysis of bubbler samples. The calibration curve for this analysis showed good linearity over an extended range. Routine analysis of bubblers was performed using midrange, high and low level standards in order to assure linearity.

4. n-Hexane Degradation Studies

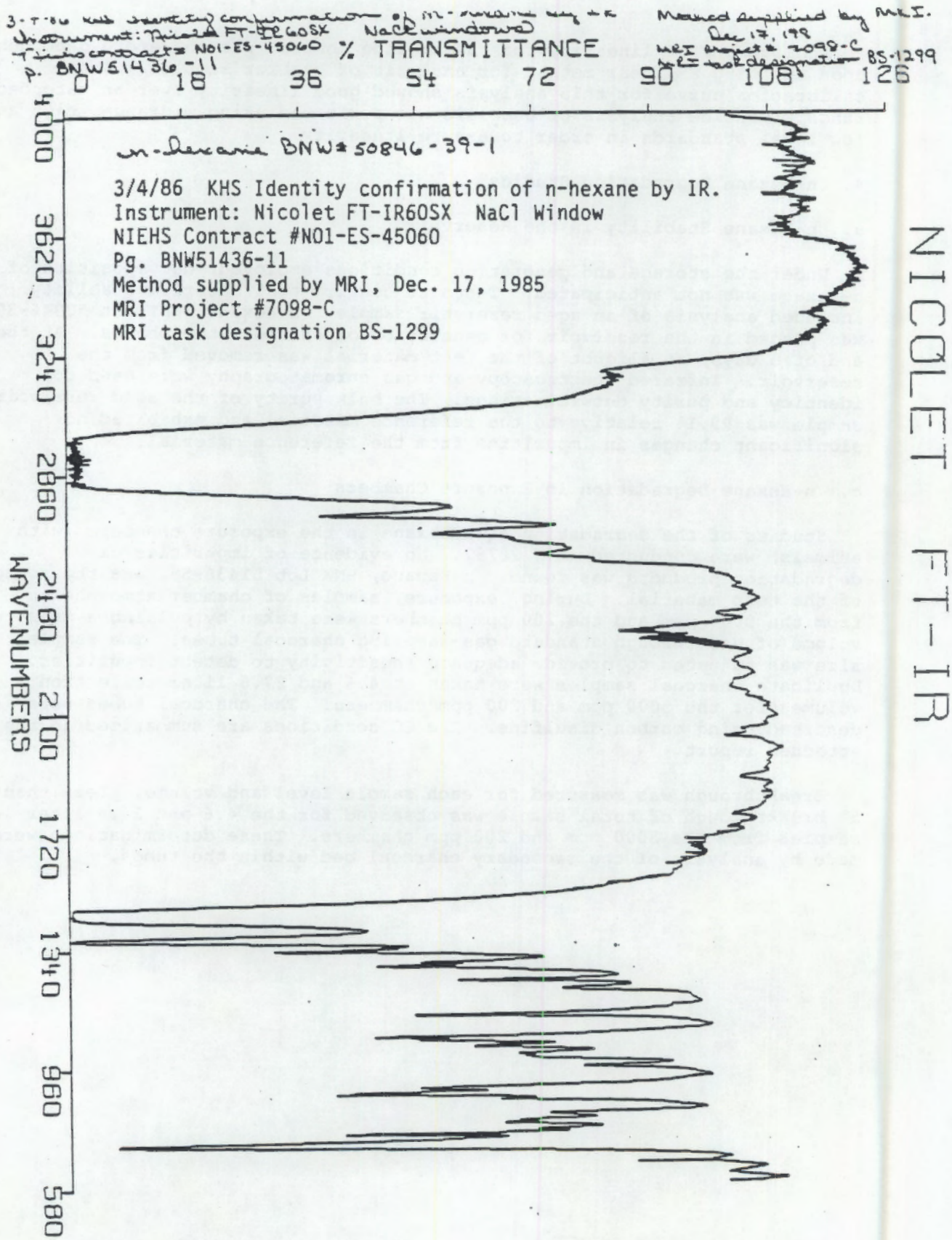
a. n-Hexane Stability in the Reservoir

Under the storage and generation conditions employed, decomposition of n-hexane was not anticipated. Tests to confirm test material stability included analysis of an aged reservoir sample. n-Hexane (BNW Lot 50846-39) was placed in the reservoir for generation of chamber atmospheres. At the end of 5 days, an aliquot of the test material was removed from the reservoir. Infrared spectroscopy and gas chromatography were used for identity and purity determinations. The bulk purity of the aged reservoir sample was 99.1% relative to the reference material and exhibited no significant changes in impurities from the reference material.

b. n-Hexane Degradation in Exposure Chambers

Studies of the degradation of n-hexane in the exposure chambers (with animals) were conducted on 3/28/87. No evidence of impurities or degradation products was found. n-Hexane, BNW Lot 51436-58, was the source of the test material. During exposure, samples of chamber atmospheres from the 5000 ppm and the 200 ppm chambers were taken by pulling a measured volume of gas through standard gas-sampling charcoal tubes. The sample size was adjusted to provide adequate sensitivity to detect impurities. Duplicate charcoal samples were taken at 4.6 and 27.6 liter collection volumes for the 5000 ppm and 200 ppm chambers. The charcoal tubes were desorbed using carbon disulfide. The GC conditions are summarized in the attached report.

Breakthrough was measured for each sample level and volume. Less than 5% breakthrough of total sample was observed for the 4.6 and 27.6 liter samples from the 5000 ppm and 200 ppm chambers. These determinations were made by analysis of the secondary charcoal bed within the tubes.


% TRANSMITTANCE
0
18
36
54
72
90
108
126
4000
3620
3240
2860
2480
2100
1720
1340
960
580
WAVENUMBERS
n-hexane BNW# 50846-39-1
3/4/86 KHS Identity confirmation of n-hexane by IR.
Instrument: Nicolet FT-IR60SX NaCl Window
NIEHS Contract #N01-ES-45060
Pg. BNW51436-11
Method supplied by MRI, Dec. 17, 1985
MRI Project #7098-C
MRI task designation BS-1299
NICOLET FT-IR

3-6-92 KBL

n-hexane purity analysis - p. BNW51436-(10-11) (1)

Bottle # BNW50846-39-1

H.C. HP5830A, N807630

Column: 80/100 Carbopack C/0.1% SP-1000 (1.8 m x 4 mm ID, glass)

Method supplied by MRI: Contract # N01-ES-45060

HC Range # 7098-C

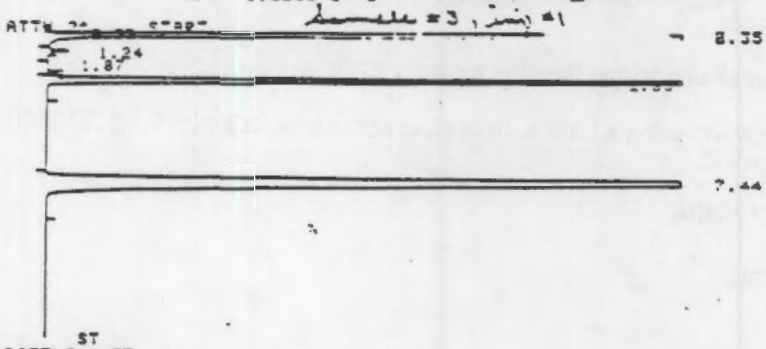
MRI Task Description BS-199

Dec. 17, 1984

TEMP1	150	150
TIME1	20.2	
RATE	4.90	
TEMP2	150	
TIME2	0.2	
INJ TEMP	200	200
FID TEMP	250	250
OVEN MAX	400	
CHT SPD	0.50	
ATTN 2↑	10	
FID SCHL	-8	
SLP SENS	0.10	
AREA REJ	1	
FLOW A	2	
FLOW B	62	
OPTN	22	

XF: 1.0000 E- 0 n-hexane BNW50846-39-1

bottle #3, inj #1



BOTTLE ST 33

** 5830A

NO METHOD

RT	AREA	AREA %
0.33	311000	0.072
0.35	401200000	93.137
1.24	121600	0.029
1.87	9520	0.002
2.65	10460000	2.428
7.44	18660000	4.332

Blank, inj #1 - Toluene

BOTTLE ST 34

** 5830A

NO METHOD

RT	AREA	AREA %
0.26	688	0.000
0.33	294800	0.070
0.35	403400000	95.534
1.25	108700	0.026
2.00	2935	0.001
2.65	10100	0.002
3.01	784	0.000
3.49	214	0.000
7.47	19440000	4.367

(1)

N-hexane purity analysis - p. BNW51436-(10-11)
Bottle #BNW50846-39-1
GC HP5830A N807630
Column: 80/100 Carbopack C/0.1% SP1000(1.8 m * 4 mm id, glass)
(BNW50846 - 146)
Method supplied by MRI: NIEHS Contract #N01-ES-45060
MRI Project #7098-C
MRI Task Description BS-199
Dec. 17, 1984.

BULK CHEMICAL REANALYSIS

COMPOUND:	n-HEXANE
CAS#	110-54-3
LOT#	Phillips lot# H-116 (BNW 50846-39, sample removed from reservoir 3/29/86 - last day of study)
APPEARANCE:	Clear liquid
RECEIPT DATE:	2/12/86
ANALYSIS PERIOD:	Last usage day
STORAGE TEMPERATURE:	Room Temperature
SAMPLE SUBMITTAL DATE:	3/29/86
SAMPLE ANALYSIS DATE:	4/3,4/86 & 5/5/86
ANALYSIS PROCEDURE:	ØB-AC-3A15-ØØ
NOTEBOOK REFERENCE:	BNW 51436-33 & BNW 51436-45

IDENTITY: Infrared spectroscopy using a Nicolet FT-IR 60SX with 4mm NaCl windows and 0.1mm spacer.

RESULTS: The spectra was similar to that found in previous BNW analysis.

ASSAY: Gas chromatography using a 1.8m x 4mm glass column packed with 0.1% SP-1000 on 80/100 Carbopak C.

Instrument: HP 5840A

RESULTS: Relative % Purity

Date	Bulk
4/86	99.1

Retention time of n-Hexane ~2.8 minutes.
Retention time of internal standard ~8.0 minutes.

ASSAY: Gas chromatography using a 1.8m x 2mm glass column packed with 0.1% SP-1000 on 80/100 Carbopack C.

Instrument: HP5840A

RESULTS: Impurity Profile

Date	Area %		
		Reference	Test
5/86	~RT	Material	Material
	9.62	0.113	0.112
	11.64	0.043	0.044
	11.98	0.003	0.003
	16.92	0.001	
	24.90	0.003	0.005

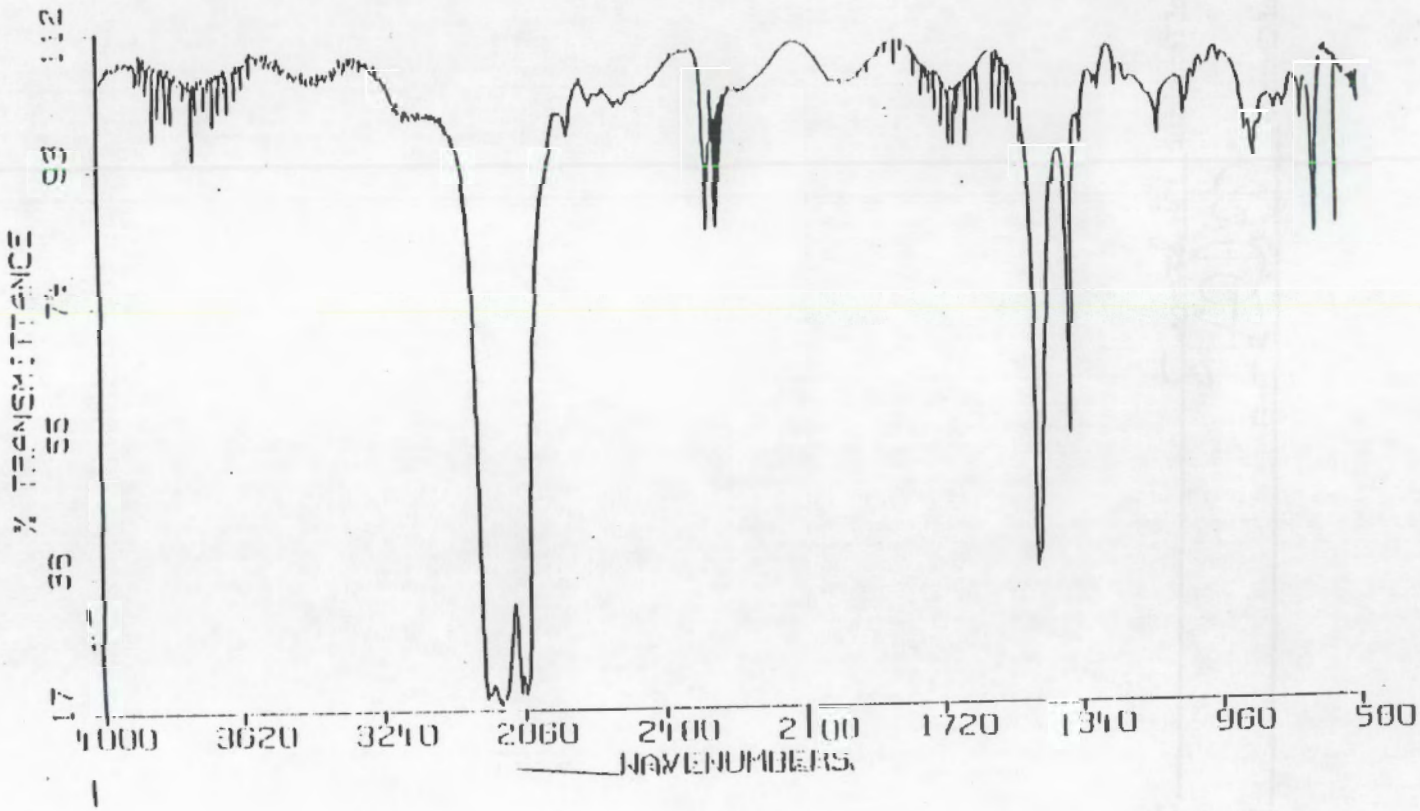
A major peak of 99.84% area was observed at a retention time of ~13.3 minutes for both the reference and test material. The reference material showed 5 impurity peaks and the test material showed 4 impurity peaks all ≥ 0.001%.

CONCLUSION: Gas chromatography shows this test material to be 99.1% pure by area ratio of an internal standard. The impurity profile showed four impurities greater than 0.001% for the test material. An infrared spectrum was obtained between 4000 cm-1 and 600 cm-1. The spectrum was similar to previous BNW analysis.

Signature of Technician: KH Stone Jr Date: 5/8/86

Signature of Chemist: [illegible] Date: 5/8/86

4-4-86 KHB
Reference n-Hexane BNW50846-145-1
Instrument: Nicolet FT-IR 60SX WA36074 / 4mm NaCl windows / 0.1mm spacers
~~Method provided by MRE, December 17, 1984~~ wrong information
Method of ØB-AC-3A15-ØØ 5-15-86 KHB
T. BNW51436-34



4-4-86

n-Hexane collected 3-29-86 (Day five of study)

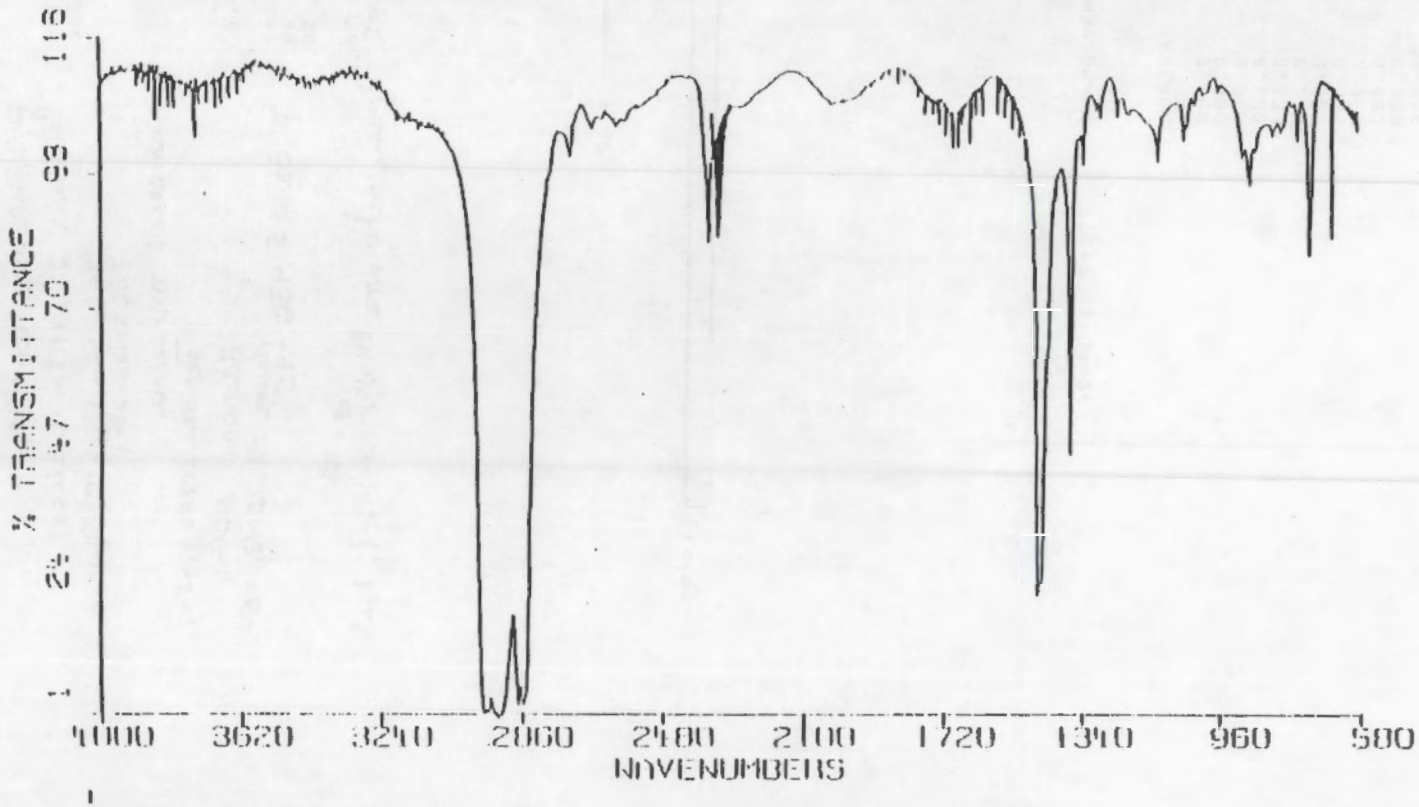
~~Dipole provided by MRI dated December 17, 1984 wiring information~~

Instrument: Nicolet FT-IR 60SX WA36074 5-15-86 RHS

4mm NaCl windows, 0.1mm Spacers

Method # ØB-AC-3A15-ØØ

p. BNW51436-34



%1 400 50 65

STOP

HP RUN # 34 MAY/05/86 TIME 10:45:08
ESCAPE
ESCAPE

5-5-86 KH

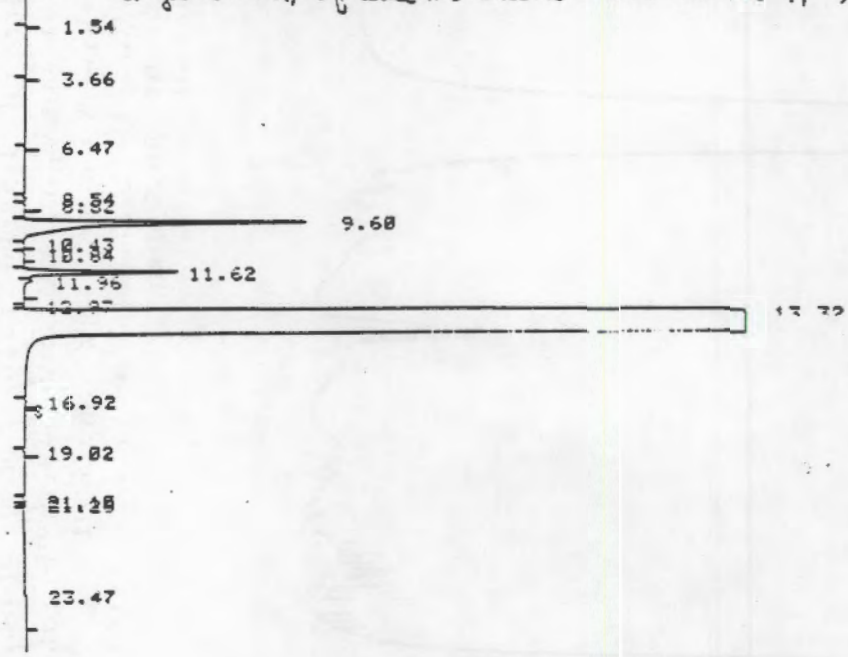
Purity Analysis of n-Hexane by G.C. Impurity Profile

Method# ØB-AC-3A15-ØØ
G.C. HP5840A WA 10706
Columns: 2mm x 1.8m ID glass
5/100 Carbopack C/0.1% SP-1000
BNW51436-38-1
Samples: n-hexane
Ref BNW50846-145-1
Last day 3-29-86
drum BNW51436-5-1
P. BNW51436-45

TEMP1	408	50	56
TIME1	5.00		
RATE	18.00		
TEMP2	480	225	
TIME2	5.00		
INJ TEMP	400	200	199
FID TEMP	400	250	250
CHT SPD	0.50		
ZERO	10.0		
ATTN 2↑	10		
FID SGNL	+0		
SLF SENS	[illegible].10		
AREA REJ			
FLOW A	[illegible].0	31.6	
FLOW B	[illegible].0	3.7	
TEMP1	400	50	49
TEMP1	400	50	49
TEMP1	400	50	50

? TEMP1 400 50 50
? START

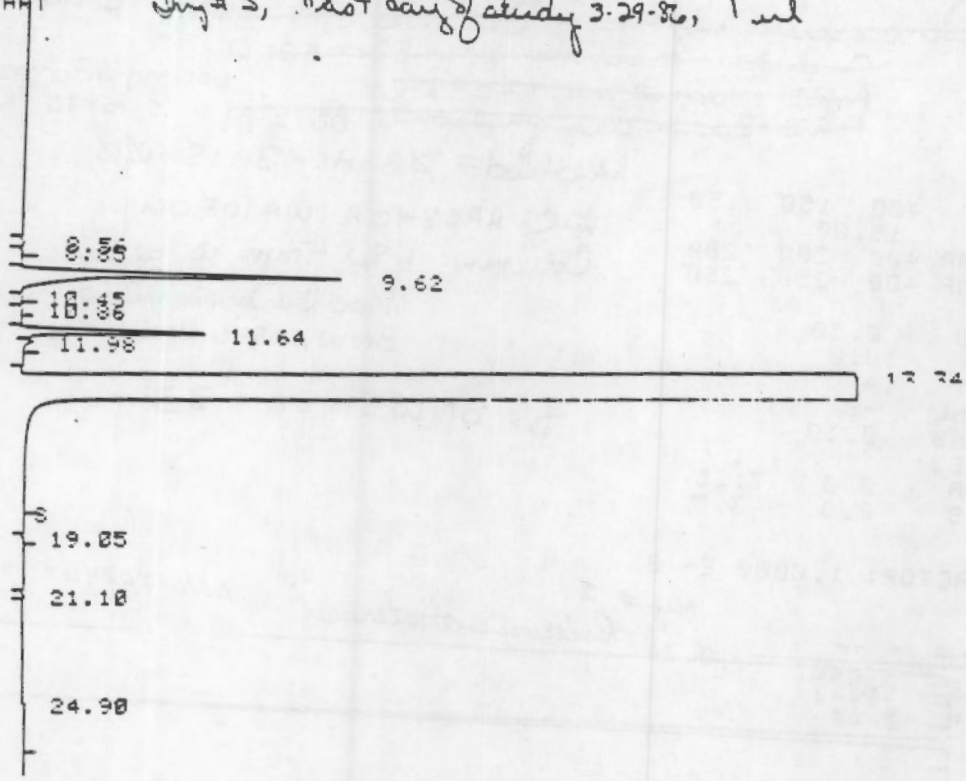
Injection #2, Reference n-hexane BNW51436-145-1, 1 µl.



HP RUN # 36 MAY/05/86 TIME 11:34:26
ID:10706
AREA %

RT	AREA	AREA %
1.54	234	0.000
3.66	133	0.000
6.47	225	0.000
8.54	2050	0.000
8.82	1388	0.000
9.60	598200	0.113
10.43	547	0.000
10.84	1393	0.000
11.62	229900	0.043
11.96	17910	0.003
12.97	28	0.000
13.32	528900000	99.836
16.92	[illegible]	[illegible]

? TEMP1 400 50 50 Reservoir sample
? START Inj #3, last day of study 3-29-86, 1 ml



HP RUN # 37 MAY/05/86 TIME 12:23:24
ID:10706
AREA %

RT	AREA	AREA %
8.56	2417	0.000
8.85	435	0.000
9.62	594900	0.112
10.45	543	0.000
10.86	725	0.000
11.64	233000	0.044
11.98	16420	0.003
13.34	528200000	99.835
19.05	196	0.000
21.10	474	0.000
24.90	26420	0.005

DIL FACTOR: 1.0000 E+ 0

? TEMP1 400 50 50

4-3-86 KHL

Purity Analysis of n-hexane by G.C. major peak

~~Method supplied by MRI: December 17, 1984~~ Comparison

~~Niehs contract no: N01-ES-45060~~

~~MRI Project no: 7093-C~~ wrong information

~~MRI Task designation: BS1299~~ 5-15-86 KHL

ESCAPE

Method # ØB-AC-3A15-ØØ

TEMP1	400	150	150
TIME1	15.00		
INJ TEMP	400	200	200
FID TEMP	400	250	250

GC: HP5840A WA10706

Column: 1.8m x 4mm ID glass

80/100 Carbopack C/0.1% SP-1000

BNW50846-146

CHT SPD	0.50	
ZERO	10.0	
ATTN 2↑	14	
FID SGNL	+B	
SLP SENS	0.10	
AREA REJ		1
FLOW A	0.0	71.5
FLOW B	0.0	3.7

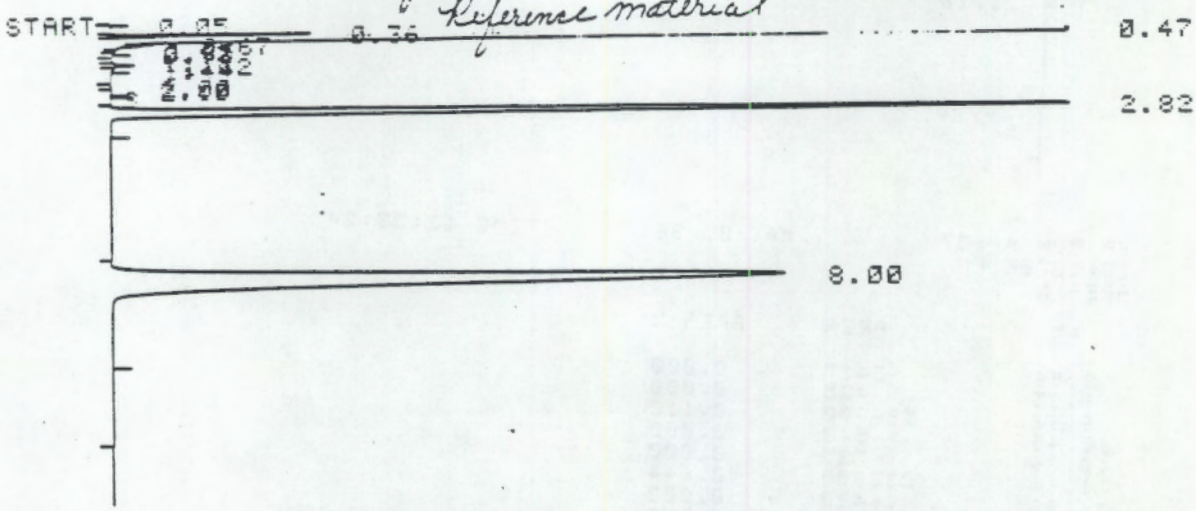
p. BNW51436-33

OIL FACTOR: 1.0000 E+ 0

Ref # 2

Reference material

BNW50846-145-1



HP RUN # 4 APR/03/86 TIME 18:02:52

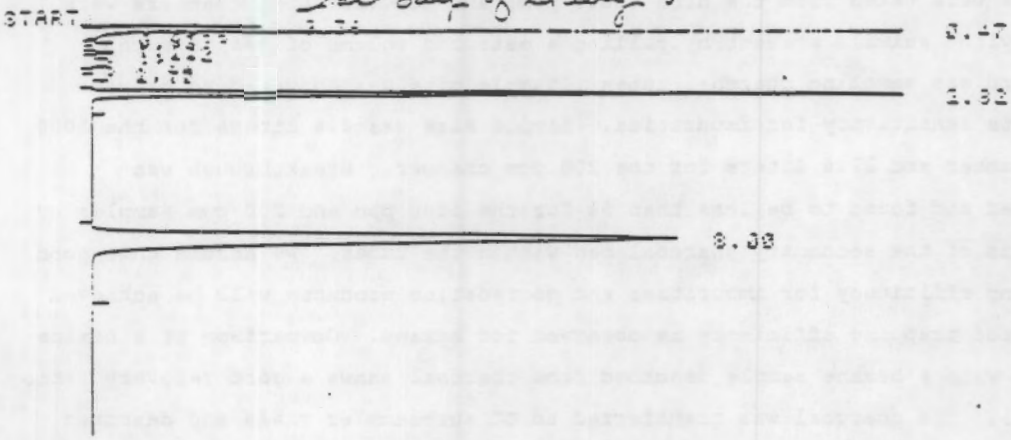
ID:10706 BOTTLE 7

AREA %

RT	AREA	AREA %
0.05	7	0.000
0.36	155100	0.115
0.47	121100000	90.098
0.67	29430	0.022
0.85	2671	0.002
0.93	880	0.001
1.18	4923	0.004
1.32	46930	0.035
1.44	7314	0.005
2.00	3191	0.002
2.10	1343	0.001
2.82	4791000	3.565
8.00	8266000	6.150

DIL FACTOR: 1.0000 E- 0

#6 n-hexane collected from reservoir 3-29-86 last day of study



HP RUN # 11 APR/03/86 TIME 20:01:00
ID:10706 BOTTLE 21
AREA %

RT	AREA	AREA %
0.36	156800	0.117
0.47	120600000	89.871
0.66	30420	0.023
0.85	2741	0.002
0.93	931	0.001
1.19	5012	0.004
1.32	47720	0.036
1.44	7492	0.006
1.99	3238	0.002
2.10	1296	0.001
2.82	4895000	3.648
8.00	8442000	6.291

RT	AREA	AREA %
0.36	156800	0.117
0.47	120600000	89.871
0.66	30420	0.023
0.85	2741	0.002
0.93	931	0.001
1.19	5012	0.004
1.32	47720	0.036
1.44	7492	0.006
1.99	3238	0.002
2.10	1296	0.001
2.82	4895000	3.648
8.00	8442000	6.291

JPM
5/20/87

n-Hexane degradation in Exposure Chamber with Animals Present

Degradation Study

Studies of the degradation on hexane in the exposure chambers were conducted for the test run performed on March 28, 1987 Test material BNW Lot No. 51436-58 was used as the source of test material. The bulk purity using gas chromatography by major peak comparison was approximately 99.2% .

Samples were taken from the high (5000 ppm) and low (200 ppm) chambers were taken with animals present by pulling a measured volume of gas through standard gas sampling charcoal tubes. Sample size was adjusted to provide adequate sensitivity for impurities. Sample size was 4.6 liters for the 5000 ppm chamber and 27.6 liters for the 200 ppm chamber. Breakthrough was measured and found to be less than 5% for the 5000 ppm and 200 ppm samples by analysis of the secondary charcoal bed within the tubes. We assume that good trapping efficiency for impurities and degradation products will be achieved when good trapping efficiency is observed for hexane. Comparison of a hexane sample with a hexane sample desorbed from charcoal shows a good recovery ratio (-100%). The charcoal was transferred to GC autosampler vials and desorbed using carbon disulfide with approximately 1 minute of ultrasound treatment.

Samples were analyzed using an HP5890 chromatographic system with a DB-5, 30 m x 0.52 mm ID, 1.5 micron film thickness, fused silica megabore column and a temperature program of 35°C for 5 minutes to 250°C for 5 minutes at a rate of 20°C/minute.

Composition of Degradation Samples by GC Area %

-RT(minutes)	Low Chamber	High Chamber	Bulk Hexane on Charcoal	Identity
2.2	-	0.10	0.11	unknown
2.4	99.58	99.51	99.50	n-hexane
2.8	0.41	0.38	0.39	unknown

An additional peak less than 0.004 % of total observed peak areas was observed for the high and low chamber charcoal samples at 14.5 minutes. This study shows no evidence for decomposition products exceeding 0.004% of the hexane concentration in the high and low chambers. The impurities at ~2.2 and ~2.8 minutes were found in equivalent amounts in a standard of bulk test material prepared with charcoal. Thus, these impurities are not formed by decomposition in the generator or chamber. The unknown peaks are probably other hexane isomers.

Technical Specialist: K.H. Stoney Date: 5/7/87

Chemist: R.B. Westberg Date: 5/7/87

APPENDIX B

EXPOSURE NARRATIVE AND DATA

FOR N-HEXANE

EXPOSURE DATA AND NARRATIVE FOR n-HEXANE

Animal Exposure Chamber

The Battelle-designed inhalation exposure chamber (commercially available from Harford Systems/Lab Products, Inc., Aberdeen, MD) was used for the inhalation exposures. The 2.3 m^3 (1.7 m^3 active-mixing volume) stainless steel chamber contains three levels of caging, each level split into two offset tiers (Figure B.1). The drawer-like stainless steel cage units comprise individual animal cages, feed troughs and automatic watering. Stainless steel catch pans for the collection of urine and feces are suspended below each cage unit.

The catch pans, which remained in the chamber during exposure, were designed to aid in mixing to maintain uniform concentrations of aerosol, dust or vapors throughout the chamber. Incoming air is HEPA and charcoal filtered before addition of the test article. A uniform mixture of incoming air and the test article is diverted to flow along the inner surfaces of the chamber. A portion of the flow is "peeled off" by each catch pan thus creating mixing eddies. Exhaust from each tier is cleared through the space between the tiers.

Exposure Suite System Description

The n-hexane exposures were conducted using an automated data acquisition and control system in an exposure suite (Figures B.2 and B.3). This system monitors and controls the basic inhalation test system functions including chamber air flow, vacuum, temperature, relative humidity and test chemical concentration. The system computers, printers, magnetic data storage devices, interface equipment, and monitoring instruments were located in a central control room and interfaced with monitoring and control elements in three exposure rooms. All data acquisition and control originated from an executive computer which controlled a multiplexing interface system. All experimental protocols related to data acquisition and control resided in this computer and were entered into software tables accessed by menus.

Data were printed and stored immediately upon completion of the measurement on separate diskettes in the exposure control center. Data and comments from each exposure room were printed on separate printers. At the end of the 24 hour period, the daily data were analyzed, and summary and data outlier reports were printed.

A dual point alarm system with user-defined set points was available for each parameter measured. Action taken upon alarm depended on the cause and severity of the alarm and ranged from audio/visual alert to automatic shutoff of the exposure generator. Alarm conditions which might be a threat to the health of the animals alerted a building power operator who was on duty 24 hours per day.

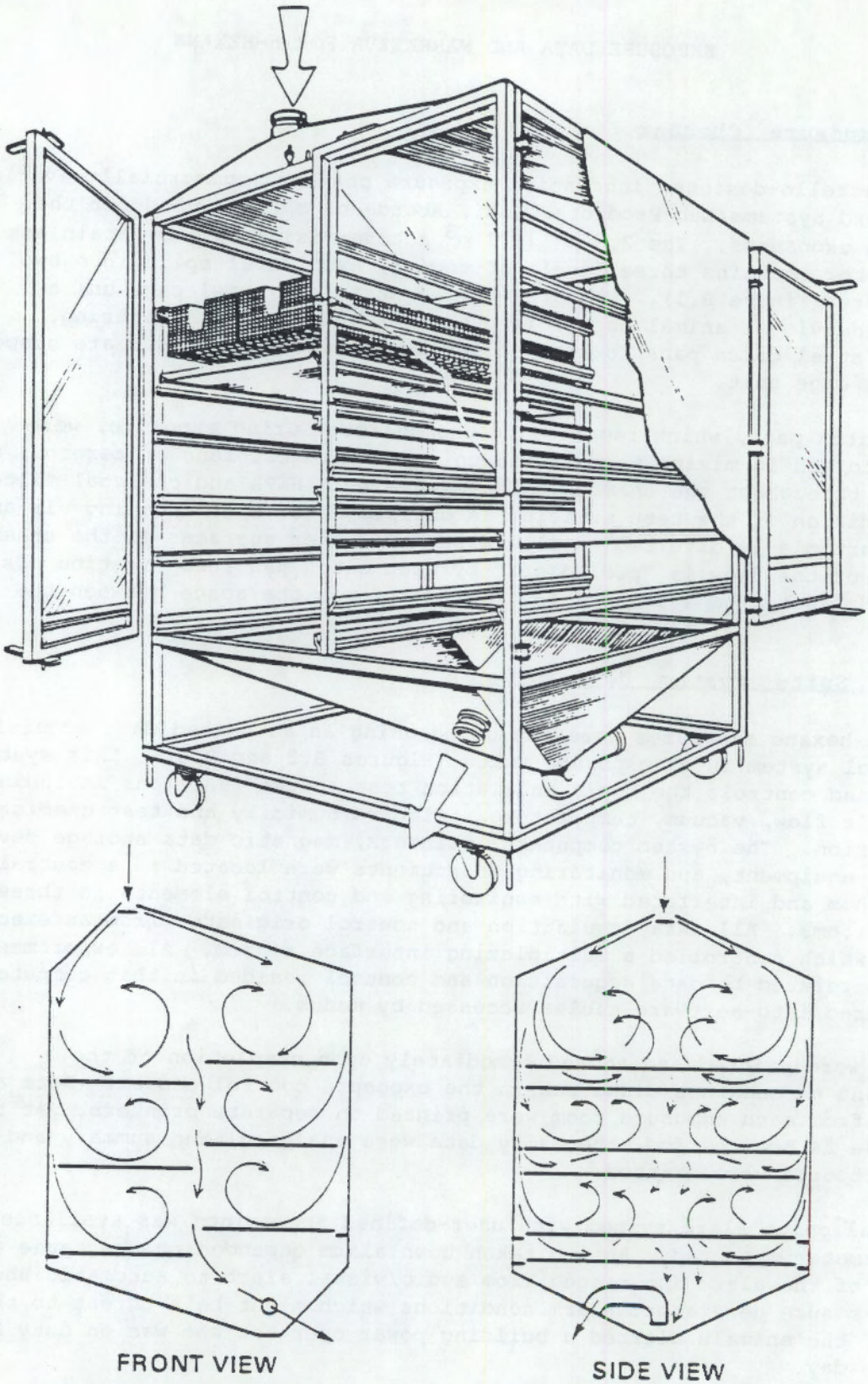


FIGURE B.1. Inhalation Exposure Chamber

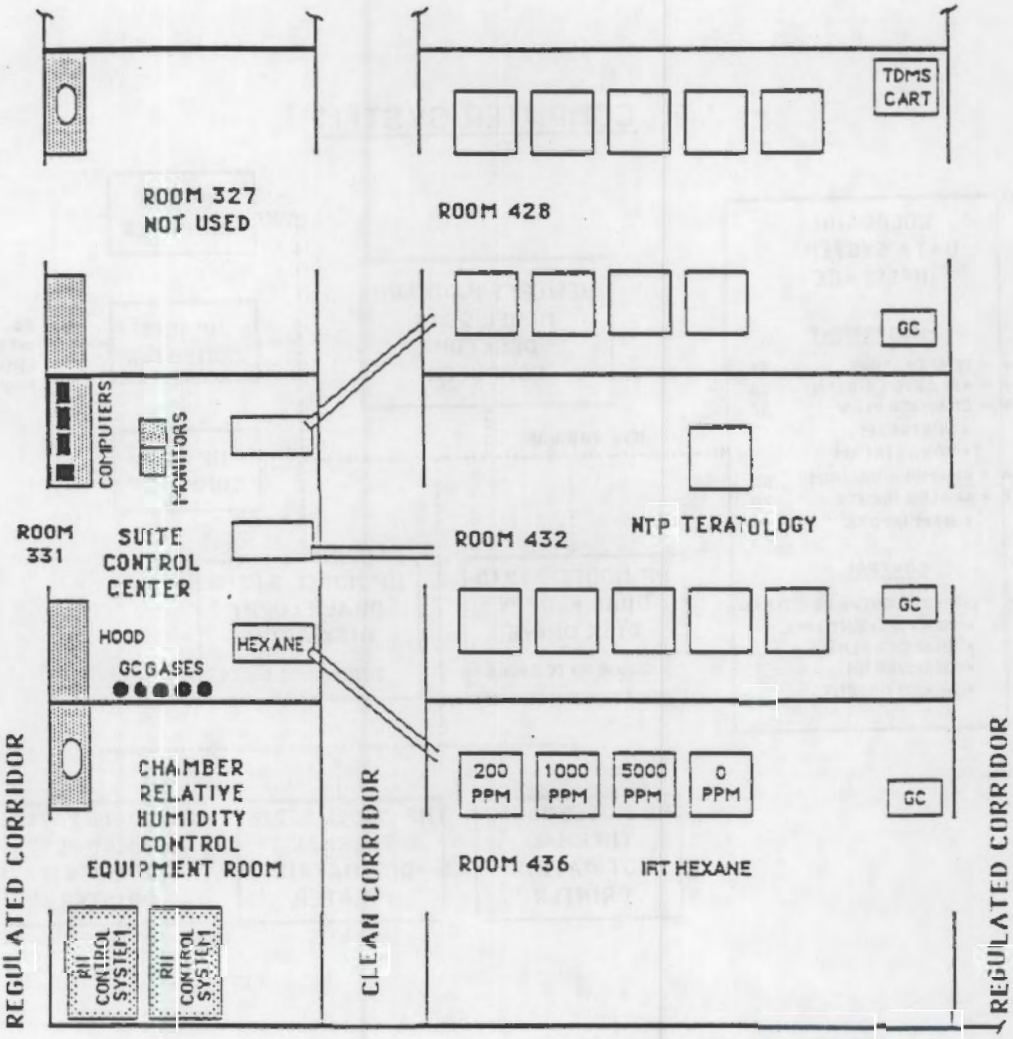


FIGURE B.2. Data Acquisition System for n-Hexane Exposures

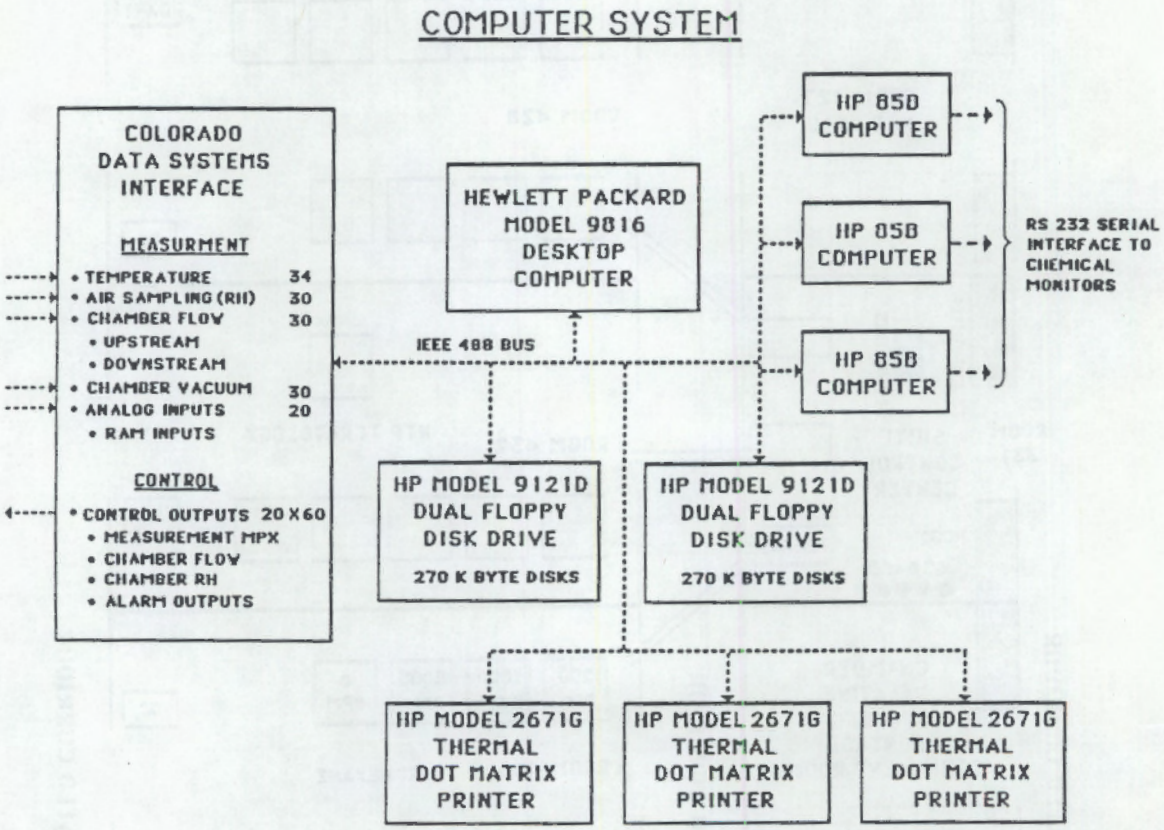


FIGURE B.3. Data Acquisition System for n-Hexane Exposures

Temperature was measured with an accuracy of approximately ±0.5°F by Resistance Temperature Devices (RTDs) located at the measurement site. The RTDs were multiplexed to a digital thermometer which was interfaced to the computer. Chamber temperature was controlled primarily by controlling the temperature of the room housing the chambers.

Percent relative humidity (%RH) was calculated with an accuracy of approximately ±6% by pulling a sample from the measurement location through a Teflon® tube into a dewpoint hygrometer located in the control center. Measurements were made from different locations by a valving system which multiplexes the tubes to the hygrometer. Percent RH was calculated by the executive computer from temperature and dewpoint measurements. Chamber %RH was maintained by a "wet/dry" air source supplied to each chamber. The ratio of "wet" to "dry" air, determined by a computer-controlled mixing valve, determined the chamber %RH.

Chamber air flow was calculated with an accuracy of approximately ±15 liters/min by measurement of the pressure drop across calibrated orifices located at the inlet and exhaust of each chamber. The desired flow orifice was attached by means of a multiplexed valve system to a calibrated pressure transducer located in the control center. Small leaks in the chambers could be detected by comparison of the measurement of inlet flow with that of the exhaust. Flow was maintained by a computer controlled pump in the exhaust line of each chamber.

Chamber vacuum, relative to the control center, was measured with an accuracy of approximately ±0.2 cm H_2O using the same pressure transducer system which measures chamber air flows. Chamber vacuum was maintained at approximately (-)1" H_2O primarily by inlet resistance provided by the HEPA and charcoal filters.

Hexane Generation System

A schematic diagram of the hexane generation system is shown in Figure B.4. Most of the generator was housed in a vented cabinet located in the Suite Control Center. The cabinet was vented to the building exhaust. The hexane to be vaporized was contained in a 19-liter stainless steel reservoir. This reservoir was filled daily from the original shipping container by the following method which was designed to prevent explosion during transfer. All oxygen in the reservoir was displaced with nitrogen through a purge port. The nitrogen pressure in the shipping container forced hexane through a filter and into the reservoir. The reservoir was on an electronic scale during filling so that the correct level was readily obtained. All metal containers were grounded. The filled reservoir was then transferred and installed into the generator cabinet.

During exposure the hexane was pumped from the reservoir through a stainless steel eductor tube and delivery tubes to vaporizers located at the fresh air inlet of each animal exposure chamber. Stable micrometering pumps with adjustable drift-free pump rates ranging from less than 1×10^{-3} to greater than 20 ml per minute were used.

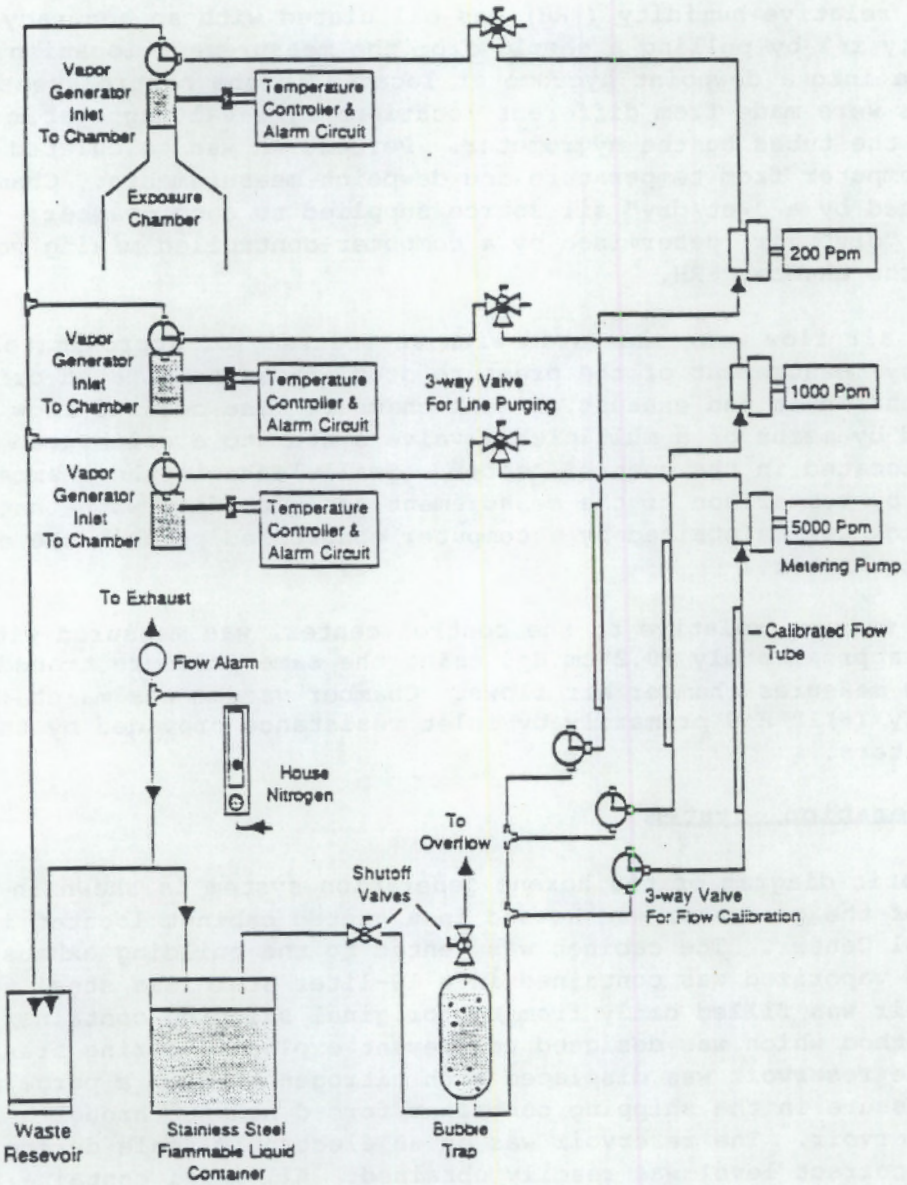


FIGURE B.4. n-Hexane Generation and Delivery System

The vaporizer comprised a stainless steel cylinder covered with a glass fiber wick from which the liquid was vaporized. The wick could be easily and inexpensively replaced if residue buildup occurred. An 80-watt heater and a temperature sensing element were incorporated within the cylinder and connected to a remotely located temperature controller. A second temperature monitor was incorporated in the vaporizer allowing the operating temperature to be recorded by the automated data acquisition system. The operating temperature of the vaporizer was maintained below 50°C (the boiling point of hexane is about 70°C). The cylindrical vaporizer was positioned in the fresh air duct leading directly to the inlet of the exposure chamber.

A clear Teflon® tube of measured volume, preceded by a three-way valve was attached downstream of the pump to facilitate measurement of the flow rate of the vapor generator. Measurement was accomplished by momentarily switching the three-way valve from the run position to the test position. A small bubble of air was pulled by the pump from the cabinet through the valve and into the clear tube. The progress of this bubble from one end of the tube to the other (calibrated volume) was timed with a stop watch. Flow rate was calculated by dividing the volume by the time. The concentration in the exposure chamber was calculated from the flow measurements of liquid and dilution air. This value was then compared to the chamber concentrations as measured by the gas chromatograph (on-line monitor).

All generation equipment which came in contact with the hexane was stainless-steel, Teflon® or Viton®. All equipment contained in the vented generator cabinet was explosion proof.

The exposure suite data acquisition and control computer automatically controlled the concentration of n-hexane in the animal exposure chambers by adjusting the flow rate of dilution air through the chamber over a narrowly-limited flow range. This was accomplished by adjusting the dilution air flow pump which was mounted in the exhaust duct of the chamber. This air-multiplier-type pump was controlled by adjusting the air pressure by a computer-controlled motor attached to the air pressure regulator.

Adjustments were made to the air flow only if the concentration was beyond the Non-Critical Limit (±10% of target concentration). The concentration adjustment was limited to assure that the chamber dilution air flow was not adjusted beyond the non-critical flow limits (12 to 18 air changes per hour). If the allowed adjustment was not sufficient to bring the concentration back into the desired operating range, the computer made the maximum adjustment possible within the flow limits, then set the alarm and indicated to the operator that a manual adjustment of the generation system had to be made.

The following conditions for alarms and concentration adjustments applied:

- Concentration ≤ Target + 10% and ≥ Target - 10%

  No action necessary

- Concentration > Target + 10% and ≤ Target + 20%
  or
  < Target - 10% and ≥ Target - 20%

  Set no alarms.
  Adjust chamber air flow rate to bring concentration as close to target as possible within air flow limits (12 to 18 air changes per hour).

- Concentration > Target + 20% and ≤ Target + 30%
  or
  < Target - 20% and ≥ Target - 30%

  Set audible alarm in control room and exposure room. If after normal working hours or if weekend, also set power operator alarm. Adjust chamber air flow rate to bring concentration as close to target as possible within air flow limits (12 to 18 air changes per hour).

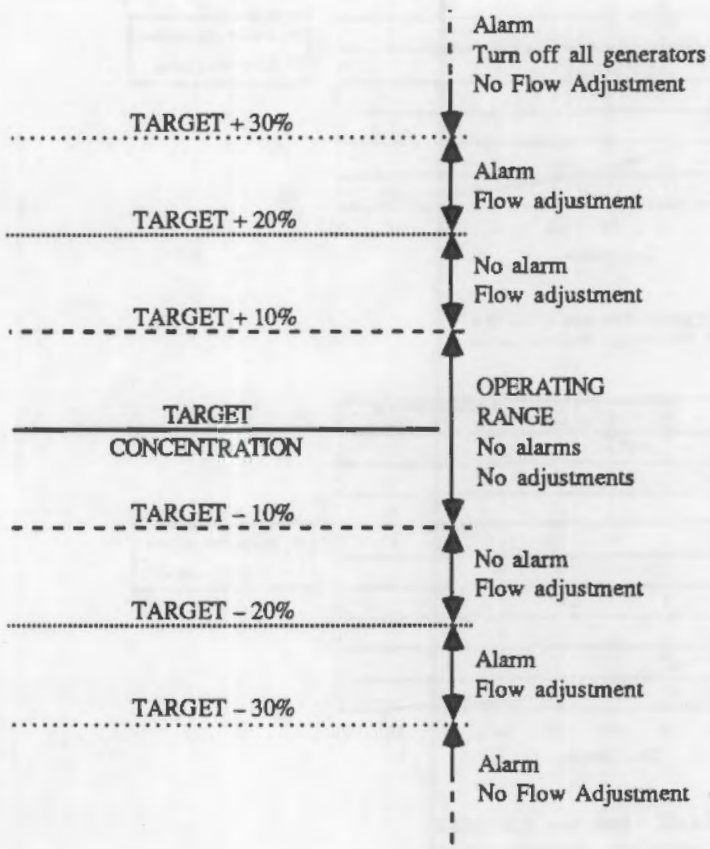
- Concentration > Target + 30%

  Turn off all generators.
  Set audible alarm in control room and exposure room. If after normal working hours or if weekend, also set power operator alarm. Make no adjustment of chamber air flow.

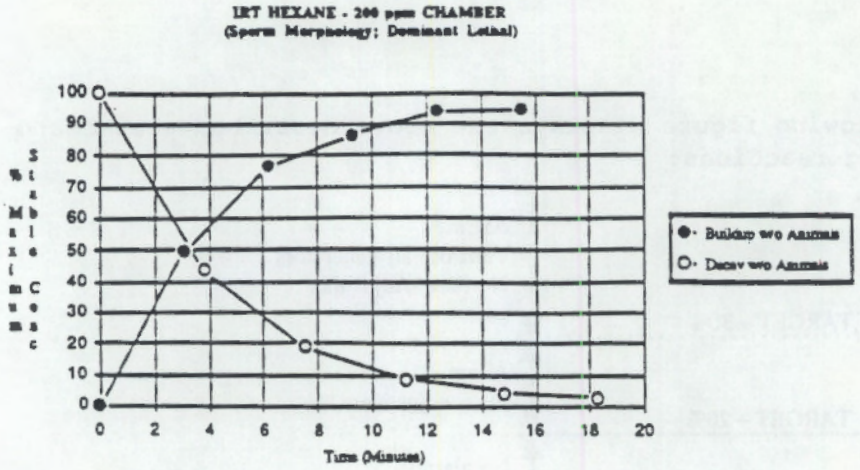
- Concentration < Target - 30%

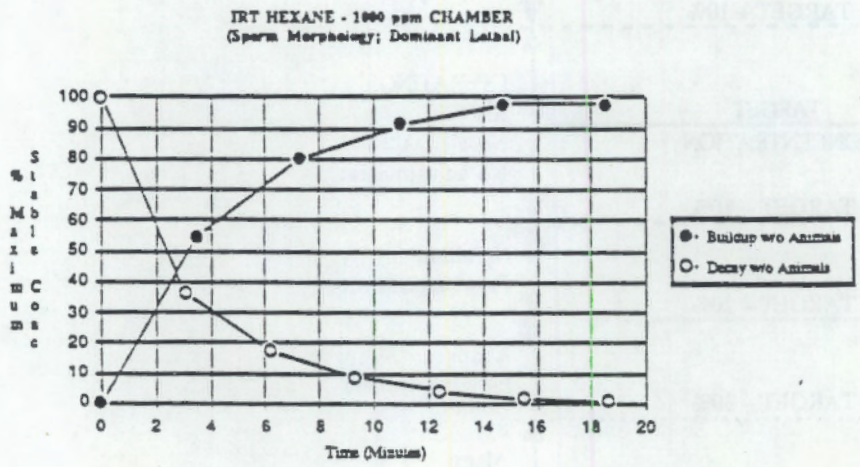
  Set audible alarm in control room and exposure room. If after normal working hours or if weekend, also set power operator alarm. Make no adjustment of chamber air flow.

The following figure displays the above described alarms and the corresponding reactions:



The time (T_{90}), following the start of generation, for the concentration to build up to 90% of the final stable concentration in the chamber and the time (T_{10}), following the stop of generation for the vapor concentration to decay to 10% of the stable concentration were determined before animals were placed in the chambers. The resulting curves for all chambers are shown in Figure B.5. The value of T_{90} was found to range from approximately 11 to 13 minutes. At a chamber air flow rate of 15 air changes per hour, the theoretical value for T_{90} was approximately 12.5 minutes. A T_{90} of 12 minutes was chosen for this study. The value of T_{10} ranged from 9 to 11 minutes. Due to the short total duration of the exposure, a buildup and decay determination with animals present in the chambers was not performed.





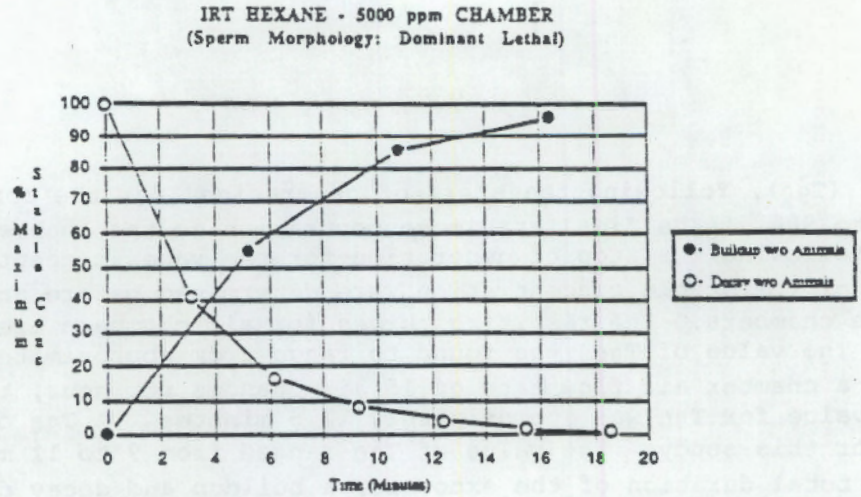


FIGURE B.5. Buildup and Decay of Vapor Concentrations in Chambers Without Animals Present

Vapor Concentration Uniformity in Chambers

Uniformity of vapor concentration in the exposure chambers was measured prior to the start of the study, but not during the study due to its short duration. The vapor concentration was measured using the on-line GC with the automatic 8-port sample valve disabled to allow continuous monitoring from a single input line. Twelve chamber positions (two positions, one in front (F) and one in back (B), for each of the six possible animal cage unit positions per chamber) were measured.

The sample point was just above and about 10 cm in from the front or back center of each cage unit. The uniformity data for each chamber during prestart testing is summarized in Table B.1. Uniformity in all chambers was found acceptable. To provide easier interpretation of the results, the concentration readings at each port is also expressed as a percentage of the mean measurement at all ports measured. The possible variation of chemical concentration measured from one sample port to another during the chamber balance procedure is termed the Total Port Variability (TPV). Three factors contribute to the TPV. The first, the Between Port Variability (BPV), represents the variation of chemical distribution within the chamber. This factor is of interest because it is the measure of the uniformity of distribution of the chemical in the chamber. The second factor, the Within Port Variability (WPV), represents the fluctuation of the average chemical concentration within the chamber during the time the uniformity measurements are made. The third is the variability of the measurement instrument itself.

TABLE B.1. Summary of Pre-Start Chamber Uniformity Data

Chamber	TPV (%RSD)	WPV (%RSD)	BPV (%RSD)
200 ppm	2.5	1.8	1.8
1000 ppm	1.2	0.2	1.2
5000 ppm	1.0	1.8	0

Chamber Uniformity Limits

WPV ≤ 5% RSD
BPV ≤ 5% RSD
TPV ≤ 7% RSD

Environmental Data During Exposure

Summations of chamber air flow, temperature and relative humidity data for the study are shown in Table B.2. This table includes the mean, standard deviation, mean expressed as a percentage of the target, the percent relative standard deviation (SD/Mean), maximum, minimum readings, number of readings and the percent of readings for which the value was within the specified operating range.

TABLE B.2. Inhalation Sperm Morphology Study of n-Hexane in Mice—Summation of Environmental Data for the Period When Animals Were Housed in the Exposure Chambers. (Acceptable Ranges are Also Shown.)

Temperature (°F)
(Acceptable Range = 72 to 78 °F)

Target Chamber Conc. (ppm)	Mean ± SD	Target ±%RSD	Maximum	Minimum	Number of Samples	Percent of in Range
1 0	73.0±0.8	97±1%	75.5	71.7	71	93
200	74.1±0.9	99±1%	77.8	72.8	49	100
1000	74.8±0.9	100±1%	78.5	73.5	49	98
5000	73.5±0.7	98±1%	76.4	72.1	49	100

Relative Humidity (% RH)
(Acceptable Range = 40 to 70 %RH)

Target Chamber Conc. (ppm)	Mean ± SD	Target ±%RSD	Maximum	Minimum	Number of Samples	Percent of in Range
1 0	59.8±5.0	109± 8%	68	44	71	93
200	57.0±4.3	104± 8%	65	45	50	100
1000	56.8±4.8	103± 8%	65	41	50	100
5000	49.9±4.6	91± 9%	56	35	50	96

Air Flow (CFM)
(Acceptable Range = 12 to 18 CFM)

Target Chamber Conc. (ppm)	Mean ± SD	Target ±%RSD	Maximum	Minimum	Number of Samples	Percent of in Range
1 0	15.1±0.3	101±2%	17.5	13.8	70	100
200	15.0±0.6	100±4%	17.9	13.6	49	100
1000	15.1±0.5	100±3%	17.9	13.5	49	100
5000	15.0±0.7	100±4%	18.5	13.2	49	98

Period Covered: 3/24/86 - 3/30/86 except [1]:3/21/86 - 3/30/86.

The mean values of temperature in all chambers for the entire study were between 73.0 and 74.8°F, all within the specified limits of 72 to 78°F. Temperature extremes ranged from 71.7 to 78.5°F. The percent of temperature readings within the operating range for all chambers was greater than 93%. No reading exceeded the critical operating limits.

The mean values of relative humidity in all chambers for the study were between 49.9 and 59.8%, all within the specified limits of 40 to 70%. Relative humidity extremes (considering all chambers) ranged from 35 to 68% and at least 93% of all relative humidity readings were within specified limits throughout the study. No reading exceeded the critical operating limits.

The mean values of chamber air flow in all chambers for the study were between 15.0 and 15.1 CFM (1 CFM = 1 air change per hour), all within the specified limits of 12 to 18 CFM. Flow extremes (considering all chambers) ranged from 13.2 to 18.5 CFM. Most of the variation was due to the use of air flow to adjust concentrations during the nighttime hours. No reading exceeded the critical operating limits.

A complete summary of the daily chamber environmental data follows.

Exposure Data

The study protocol called for daily exposure means to be within ±10% of target levels, % RSD's (relative standard deviation) ≤10% and at least 90% of samples within ±10% of the target. Due to a compatibility problem between hexane and the construction materials in the pump (see Exposure Operation Discussion Sheet in this appendix), we had a great deal of difficulty attaining and maintaining the target concentration, especially in the 5000 ppm chamber. As a result, for the 5000 ppm chamber, the overall %RSD was 18% with 80% of samples within the normal operating range. Concentration mean was 94% of target.

The daily mean concentrations for all chambers were within 6% of the target concentrations except for Day 1 for the 5000 ppm chamber (69%). Standard deviations were outside the 10% protocol-defined limits on 1 day for the 200 ppm chamber and 2 days for the 5000 ppm chamber. The percent of concentration readings within the operating range for the 5000 ppm chamber was 80%, the other chambers were greater than 99%

Summaries of the concentration data for all chambers and the exposure room are included in Table B.3. Summaries of concentration by exposure day are included in this appendix along with graphic illustrations of the daily mean and standard deviation for each chamber. A complete discussion of all concentration excursions is included.

TABLE B.3. Inhalation Sperm Morphology Study of n-Hexane in Mice — Summation of Concentration Data for the Period When Animals Were Housed in the Exposure Chambers.[1]

Concentration (PPM)
Acceptable Range = Target ± 10%

Target Conc. (ppm)	Mean ± SD	Percent Target ±RSD	Max	Min	Number Samples	Number In Range	% Samples in Range
Room	0.00±0.03	--------	0.2	0	197	*197	*100
0	0.00±0.00	--------	0	0	196	*196	*100
200	196±15.7	98± 8%	219	0.3	195	193	99
1000	998±22.0	100± 2%	1100	930	195	194	99
5000	4700±848	94±18%	5910	14.5	198	159	80
St. Gas	1030±37.7	103± 4%	1170	992	206	200	97

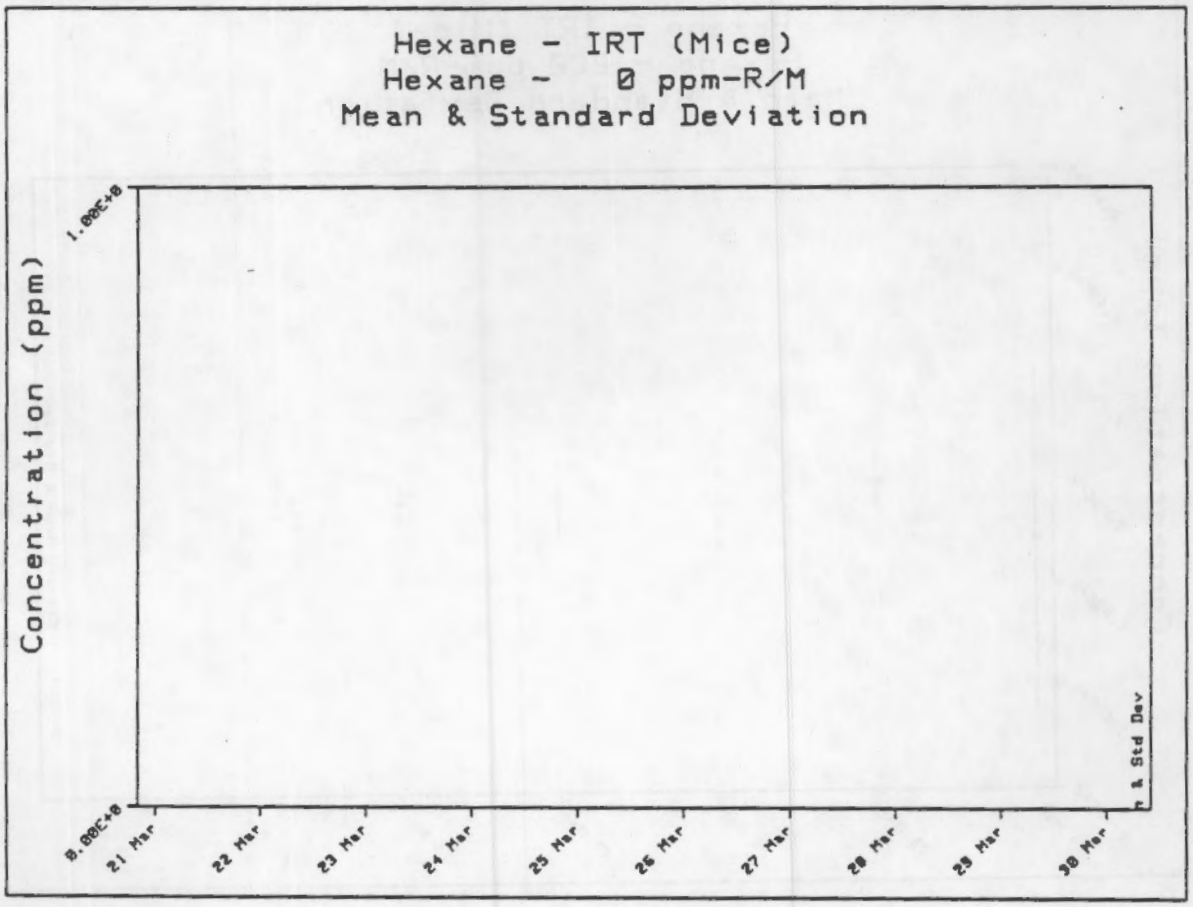
* Samples with concentration less than 1 ppm

[1]: 3/24/86 - 3/28/86

Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 28 Mar 1986

Summary Data for: Hexane - 0 ppm-R/M/Concentration 0.00E+0 to 1.00E+0

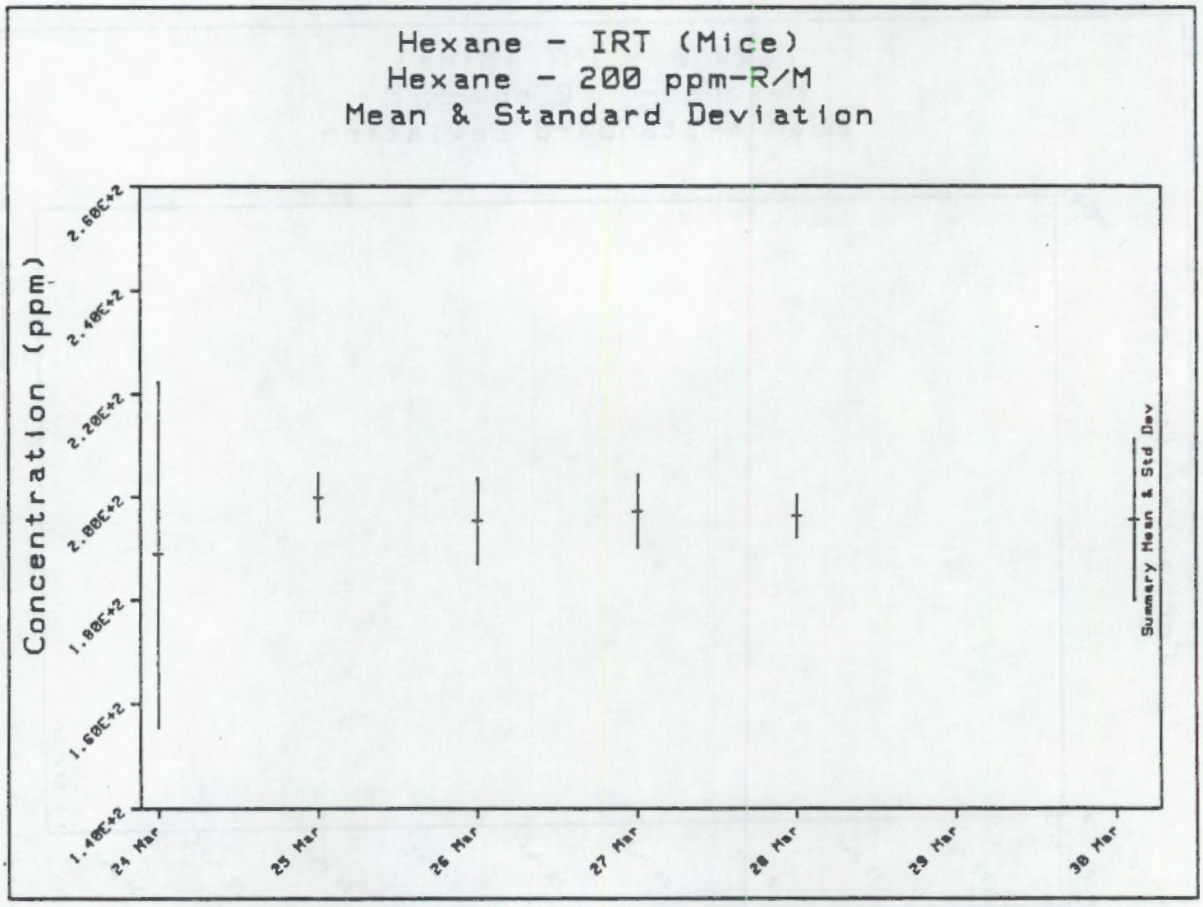
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	36.	36.	100%
25 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	41.	41.	100%
26 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	41.	41.	100%
27 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	35.	35.	100%
28 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	43.	43.	100%
Summary	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	196.	196.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 28 Mar 1986

Summary Data for: Hexane - 200 ppm-R/M/Concentration 1.80E+2 to 2.20E+2

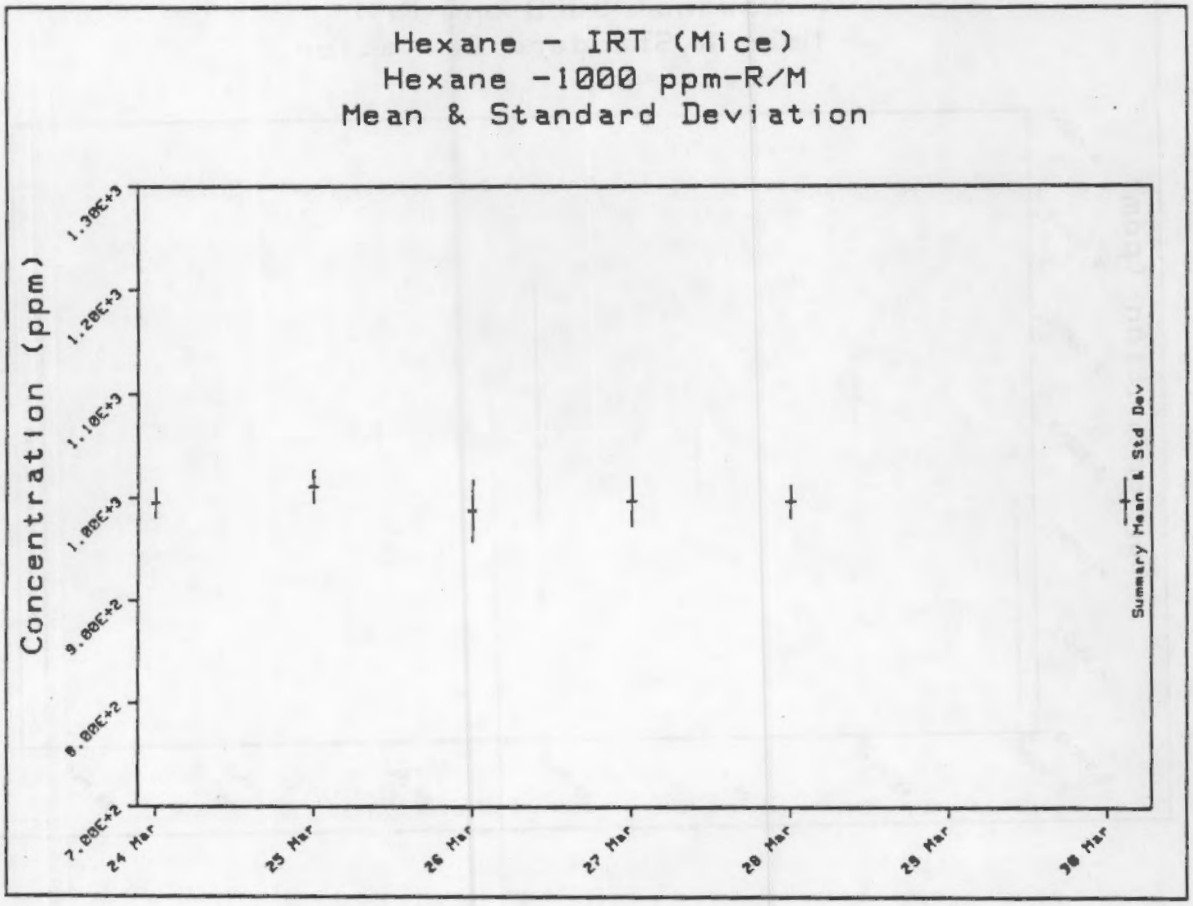
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	1.89E+2	94%	3.344E+1	18%	2.19E+2	2.72E-1	36.	34.	94%
25 Mar 1986	2.00E+2	100%	4.753E+0	2%	2.08E+2	1.83E+2	40.	40.	100%
26 Mar 1986	1.95E+2	98%	8.358E+0	4%	2.10E+2	1.81E+2	41.	41.	100%
27 Mar 1986	1.97E+2	99%	7.159E+0	4%	2.19E+2	1.82E+2	36.	36.	100%
28 Mar 1986	1.96E+2	98%	4.180E+0	2%	2.09E+2	1.88E+2	42.	42.	100%
Summary	1.96E+2	98%	1.569E+1	8%	2.19E+2	2.72E-1	195.	193.	99%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 28 Mar 1986

Summary Data for: Hexane -1000 ppm-R/M/Concentration 9.00E+2 to 1.10E+3

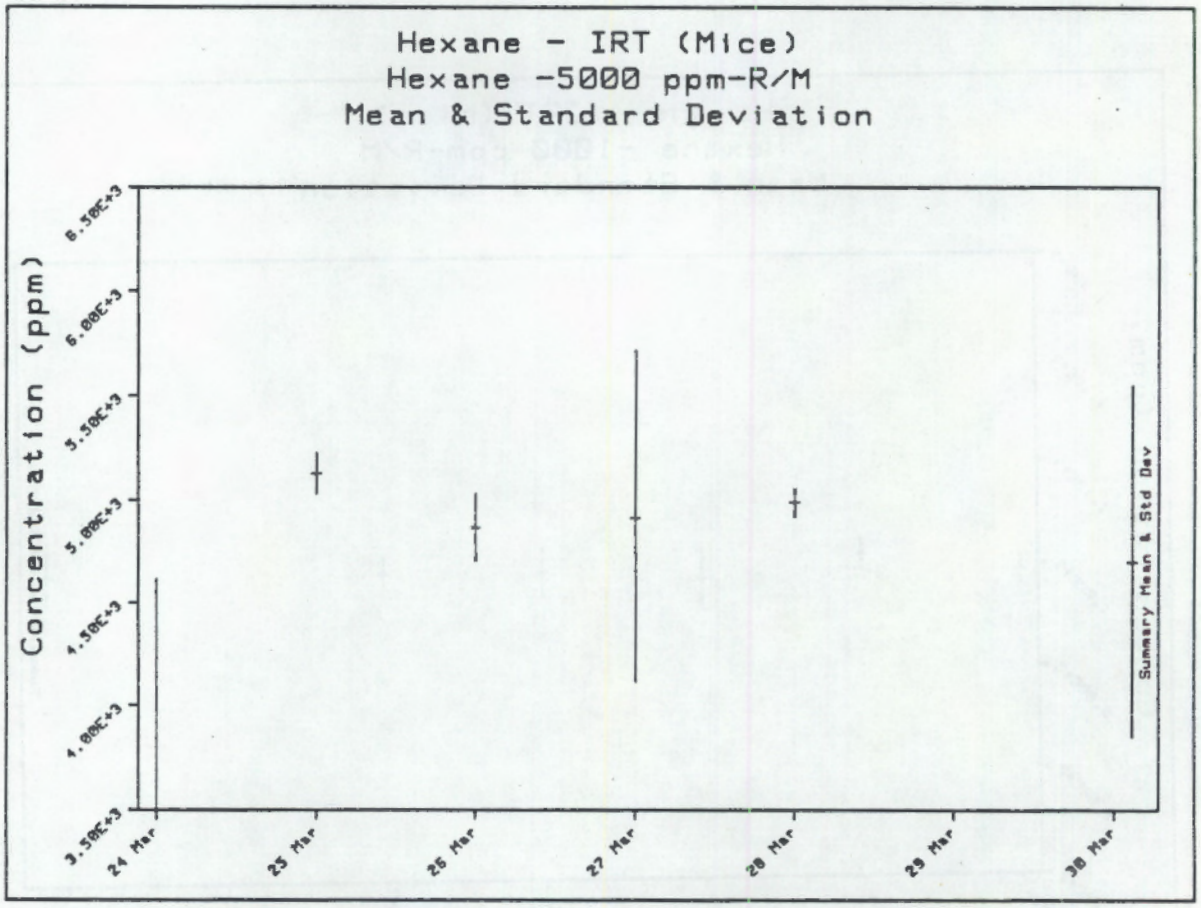
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	9.95E+2	100%	1.441E+1	1%	1.03E+3	9.62E+2	36.	36.	100%
25 Mar 1986	1.01E+3	101%	1.591E+1	2%	1.04E+3	9.75E+2	40.	40.	100%
26 Mar 1986	9.88E+2	99%	2.971E+1	3%	1.07E+3	9.38E+2	41.	41.	100%
27 Mar 1986	9.97E+2	100%	2.375E+1	2%	1.10E+3	9.30E+2	36.	35.	97%
28 Mar 1986	9.97E+2	100%	1.582E+1	2%	1.03E+3	9.72E+2	42.	42.	100%
Summary	9.98E+2	100%	2.202E+1	2%	1.10E+3	9.30E+2	195.	194.	99%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 28 Mar 1986

Summary Data for: Hexane -5000 ppm-R/M/Concentration 4.50E+3 to 5.50E+3

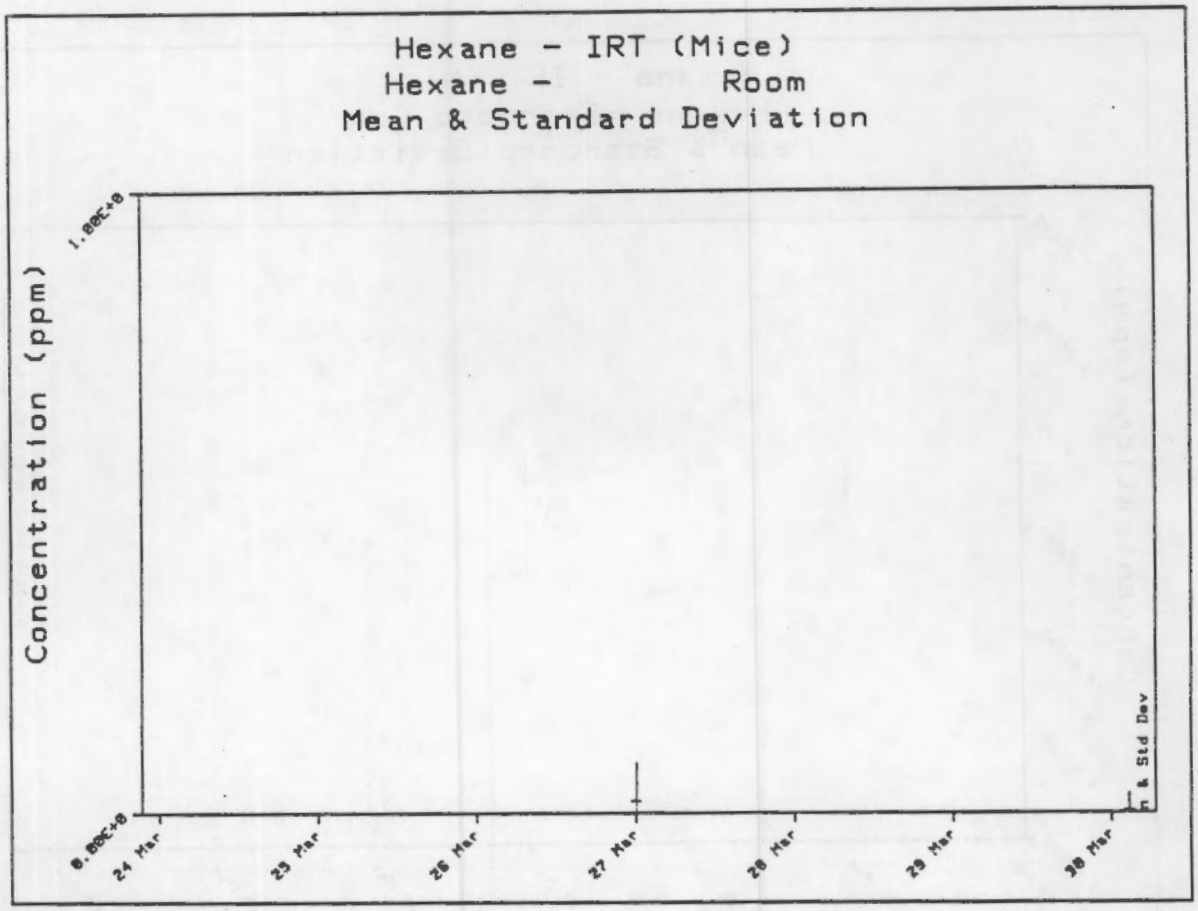
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	3.45E+3	69%	1.160E+3	34%	4.43E+3	1.45E+1	36.	0.	0%
25 Mar 1986	5.13E+3	103%	9.737E+1	2%	5.28E+3	4.96E+3	41.	41.	100%
26 Mar 1986	4.87E+3	97%	1.631E+2	3%	5.19E+3	4.61E+3	42.	42.	100%
27 Mar 1986	4.92E+3	98%	8.003E+2	16%	5.91E+3	5.16E+2	37.	34.	92%
28 Mar 1986	4.99E+3	100%	6.569E+1	1%	5.24E+3	4.88E+3	42.	42.	100%
Summary	4.70E+3	94%	8.475E+2	18%	5.91E+3	1.45E+1	198.	159.	80%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 28 Mar 1986

Summary Data for: Hexane - Room/Concentration 0.00E+0 to 1.00E+0

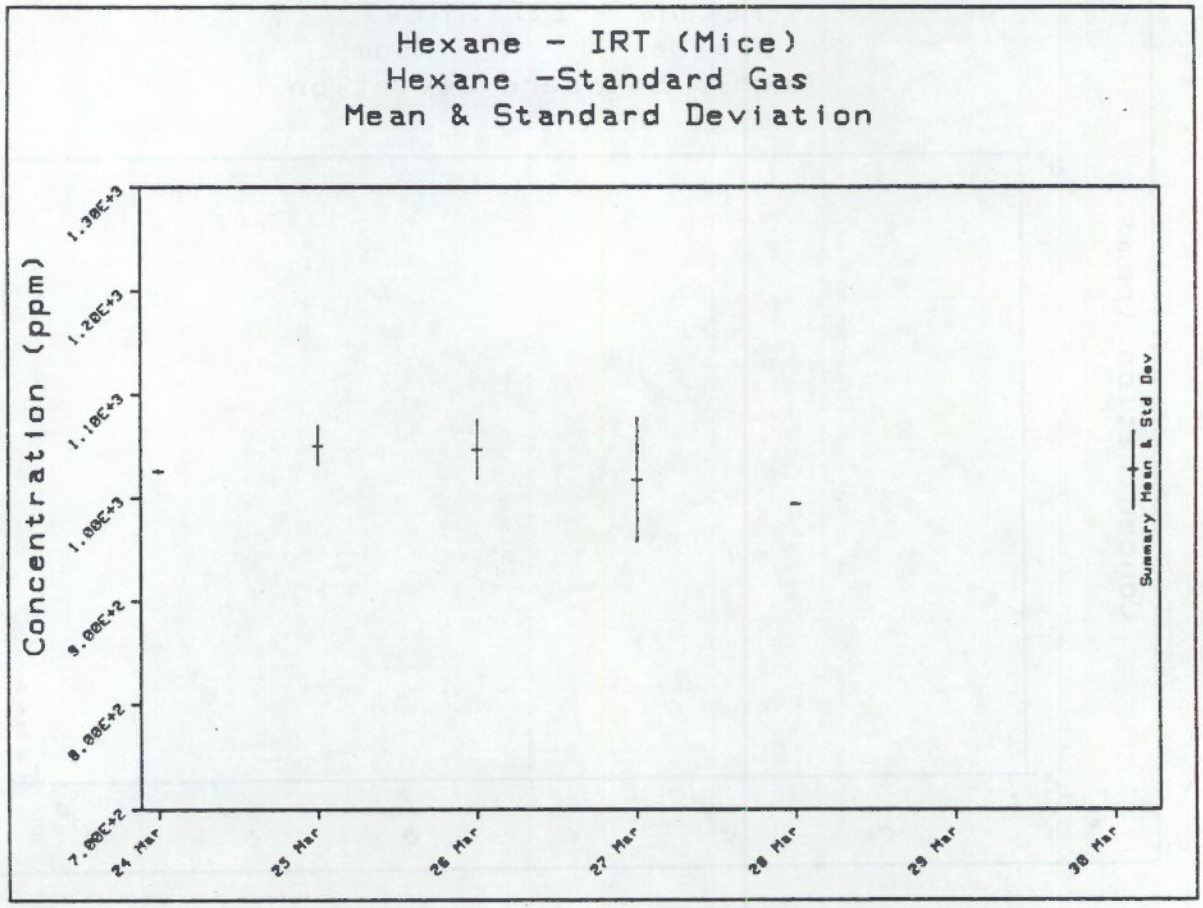
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	36.	36.	100%
25 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	41.	41.	100%
26 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	41.	41.	100%
27 Mar 1986	1.97E-2	2%	6.052E-2	307%	2.38E-1	0.00E+0	36.	36.	100%
28 Mar 1986	0.00E+0	0%	0.000E+0	0%	0.00E+0	0.00E+0	43.	43.	100%
Summary	3.60E-3	0%	2.669E-2	741%	2.38E-1	0.00E+0	197.	197.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 28 Mar 1986

Summary Data for: Hexane -Standard Gas/Concentration 9.00E+2 to 1.10E+3

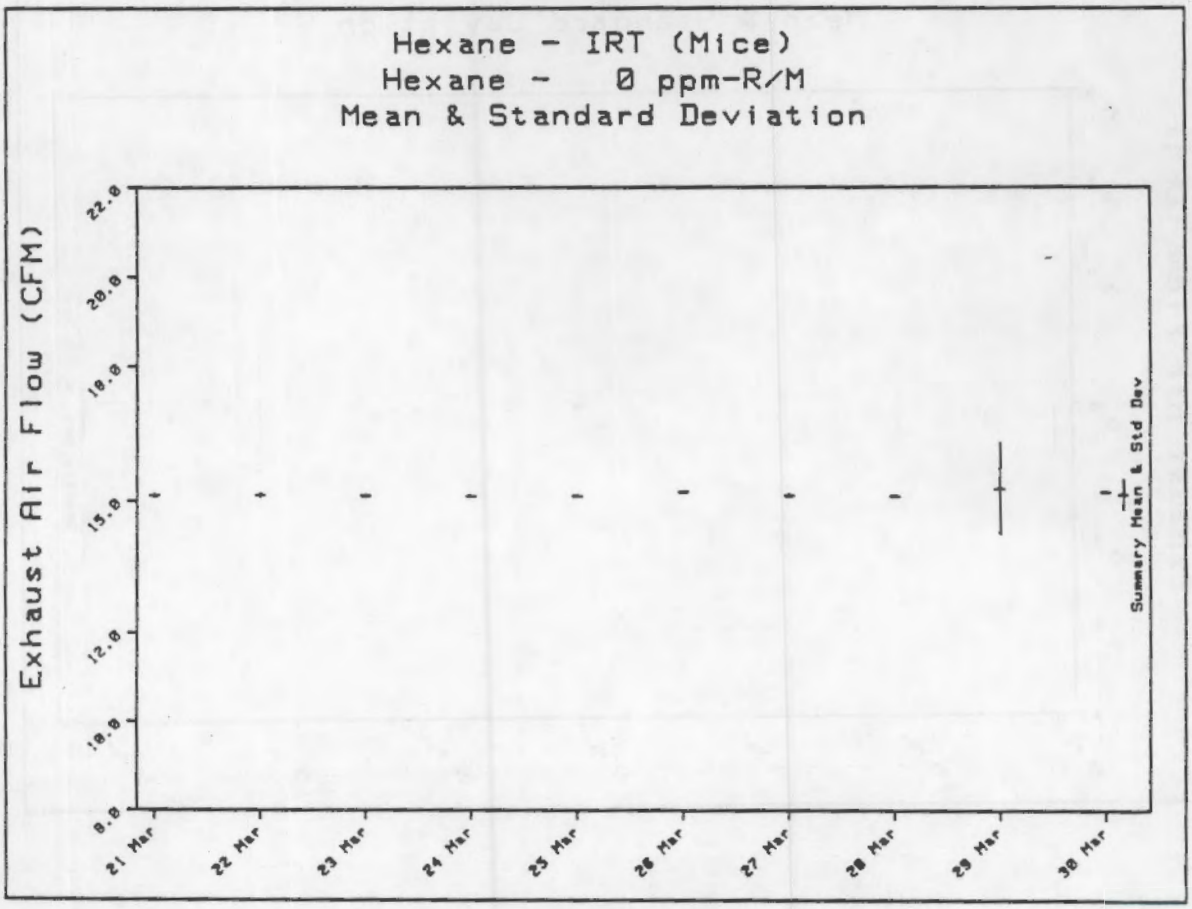
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	1.03E+3	103%	8.929E-1	0%	1.03E+3	1.02E+3	37.	37.	100%
25 Mar 1986	1.05E+3	105%	1.943E+1	2%	1.07E+3	1.03E+3	41.	41.	100%
26 Mar 1986	1.05E+3	105%	2.891E+1	3%	1.07E+3	1.00E+3	42.	42.	100%
27 Mar 1986	1.02E+3	102%	6.007E+1	6%	1.17E+3	9.92E+2	43.	37.	86%
28 Mar 1986	9.94E+2	99%	7.541E-1	0%	9.95E+2	9.92E+2	43.	43.	100%
Summary	1.03E+3	103%	3.773E+1	4%	1.17E+3	9.92E+2	206.	200.	97%



Daily Summation For Hexane - IRT (Mice) From 21 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane - 0 ppm-R/M/Exhaust Air Flow 12.0 to 18.0

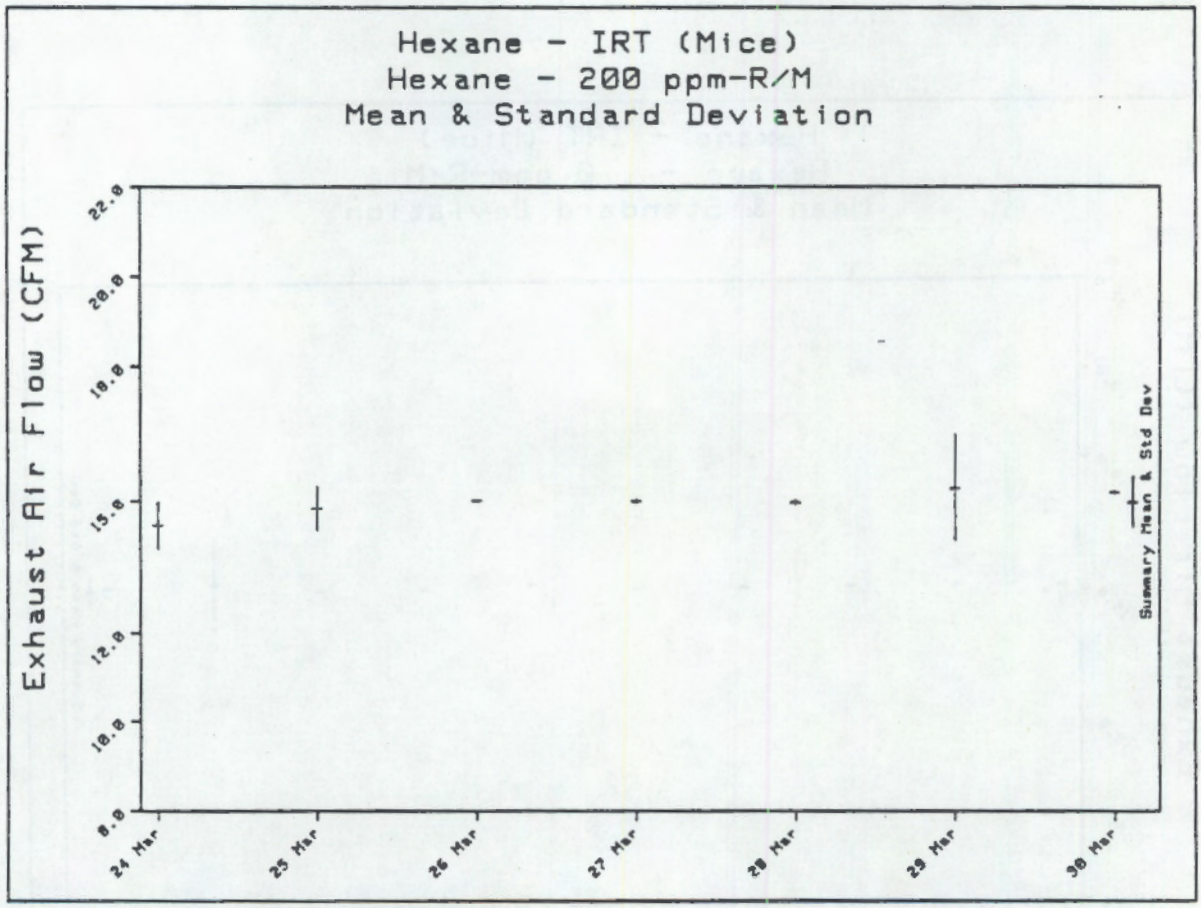
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
21 Mar 1986	15.1	101%	.04	0%	15.2	15.1	5.	5.	100%
22 Mar 1986	15.1	101%	.05	0%	15.2	15.1	8.	8.	100%
23 Mar 1986	15.1	101%	.04	0%	15.2	15.1	8.	8.	100%
24 Mar 1986	15.1	101%	0.00	0%	15.1	15.1	6.	6.	100%
25 Mar 1986	15.1	101%	0.00	0%	15.1	15.1	7.	7.	100%
26 Mar 1986	15.2	101%	0.00	0%	15.2	15.2	7.	7.	100%
27 Mar 1986	15.1	101%	.04	0%	15.2	15.1	7.	7.	100%
28 Mar 1986	15.1	101%	0.00	0%	15.1	15.1	7.	7.	100%
29 Mar 1986	15.3	102%	1.02	7%	17.5	13.8	8.	8.	100%
30 Mar 1986	15.2	101%	0.00	0%	15.2	15.2	7.	7.	100%
Summary	15.1	101%	.33	2%	17.5	13.8	70.	70.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane - 200 ppm-R/M/Exhaust Air Flow 12.0 to 18.0

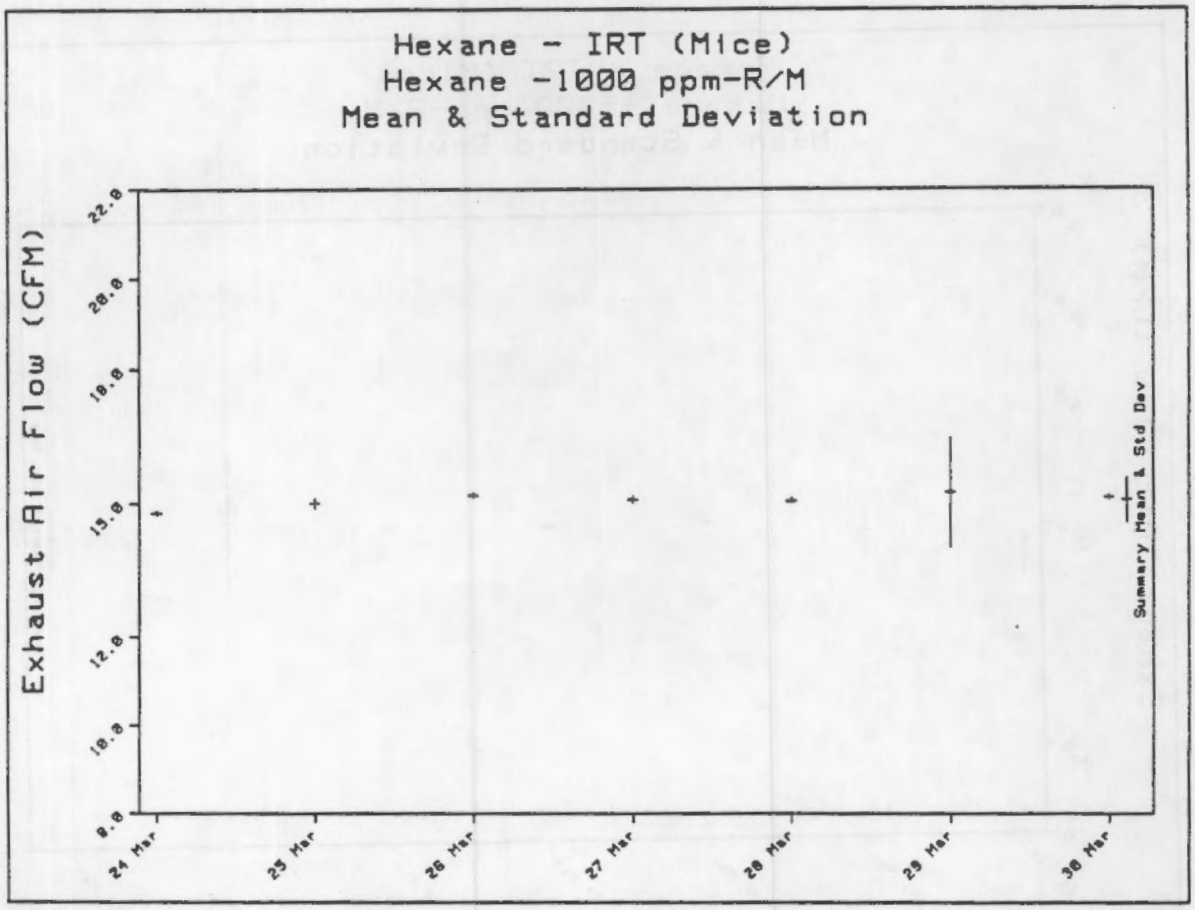
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	14.4	96%	.54	4%	14.9	13.7	6.	6.	100%
25 Mar 1986	14.8	99%	.49	3%	15.0	13.7	7.	7.	100%
26 Mar 1986	15.0	100%	0.00	0%	15.0	15.0	7.	7.	100%
27 Mar 1986	15.0	100%	.04	0%	15.0	14.9	7.	7.	100%
28 Mar 1986	15.0	100%	.05	0%	15.0	14.9	7.	7.	100%
29 Mar 1986	15.3	102%	1.19	8%	17.9	13.6	8.	8.	100%
30 Mar 1986	15.2	101%	.04	0%	15.2	15.1	7.	7.	100%
Summary	15.0	100%	.57	4%	17.9	13.6	49.	49.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane -1000 ppm-R/M/Exhaust Air Flow 12.0 to 18.0

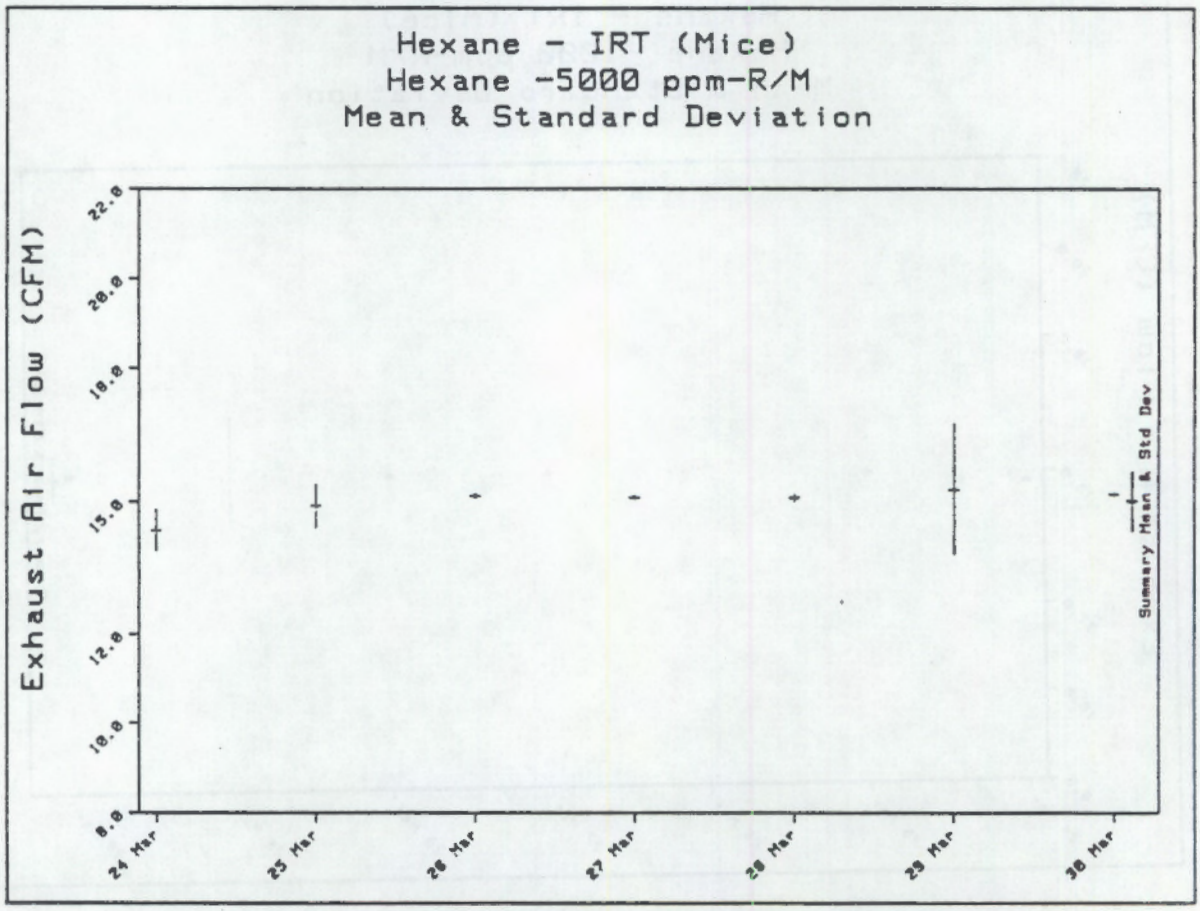
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	14.8	99%	.04	0%	14.8	14.7	6.	6.	100%
25 Mar 1986	15.0	100%	.10	1%	15.1	14.8	7.	7.	100%
26 Mar 1986	15.2	101%	.05	0%	15.2	15.1	7.	7.	100%
27 Mar 1986	15.1	100%	.08	1%	15.2	15.0	7.	7.	100%
28 Mar 1986	15.0	100%	.05	0%	15.1	15.0	7.	7.	100%
29 Mar 1986	15.2	102%	1.21	8%	17.9	13.5	8.	8.	100%
30 Mar 1986	15.1	101%	.05	0%	15.2	15.1	7.	7.	100%
Summary	15.1	100%	.48	3%	17.9	13.5	49.	49.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane -5000 ppm-R/M/Exhaust Air Flow

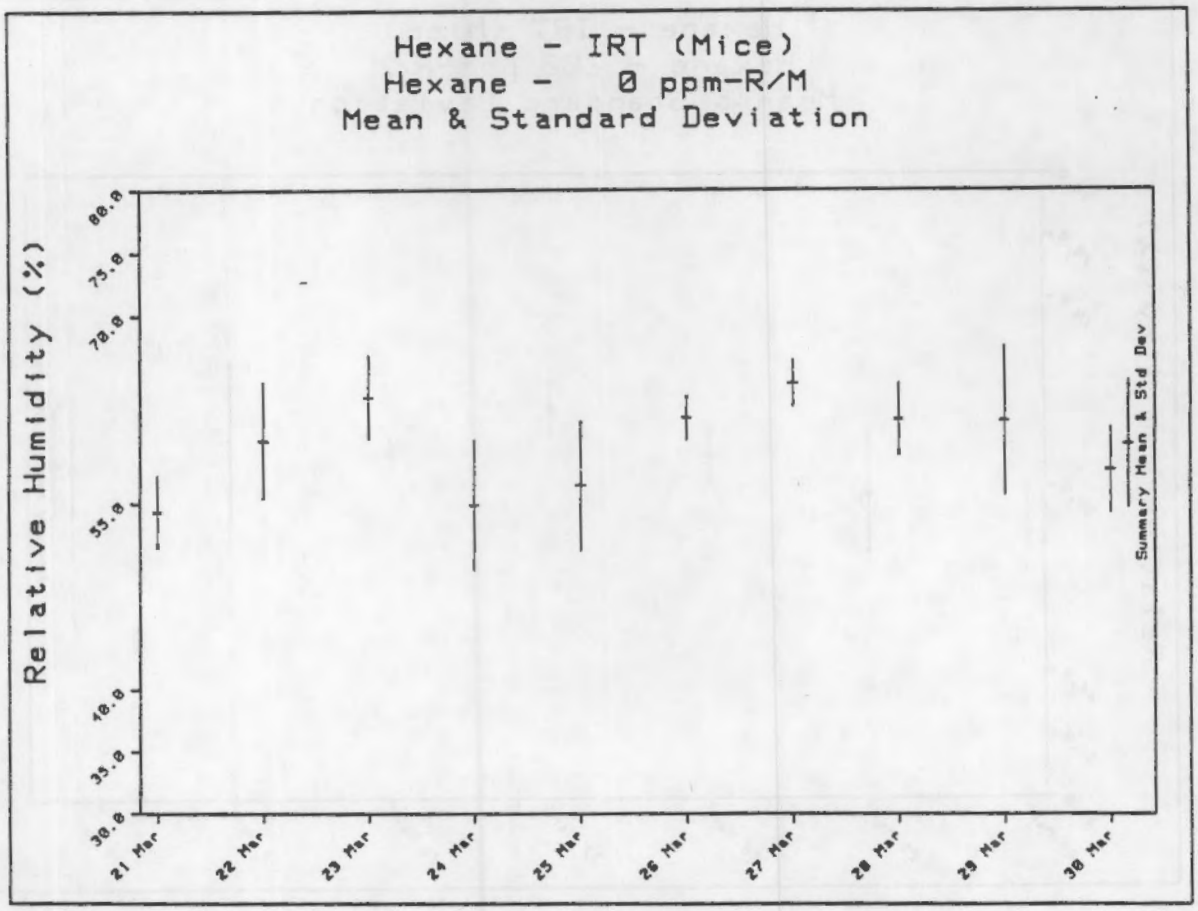
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	12.0 to 18.0 N in	% N in
24 Mar 1986	14.4	96%	.45	3%	14.9	13.9	6.	6.	100%
25 Mar 1986	14.9	99%	.49	3%	15.1	13.8	7.	7.	100%
26 Mar 1986	15.1	101%	.05	0%	15.2	15.1	7.	7.	100%
27 Mar 1986	15.1	101%	.04	0%	15.1	15.0	7.	7.	100%
28 Mar 1986	15.1	101%	.07	0%	15.2	15.0	7.	7.	100%
29 Mar 1986	15.3	102%	1.46	10%	18.5	13.2	8.	7.	88%
30 Mar 1986	15.2	101%	.05	0%	15.2	15.1	7.	7.	100%
Summary	15.0	100%	.66	4%	18.5	13.2	49.	48.	98%



Daily Summation For Hexane - IRT (Mice) From 21 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane - 0 ppm-R/M/Relative Humidity 40.0 to 70.0

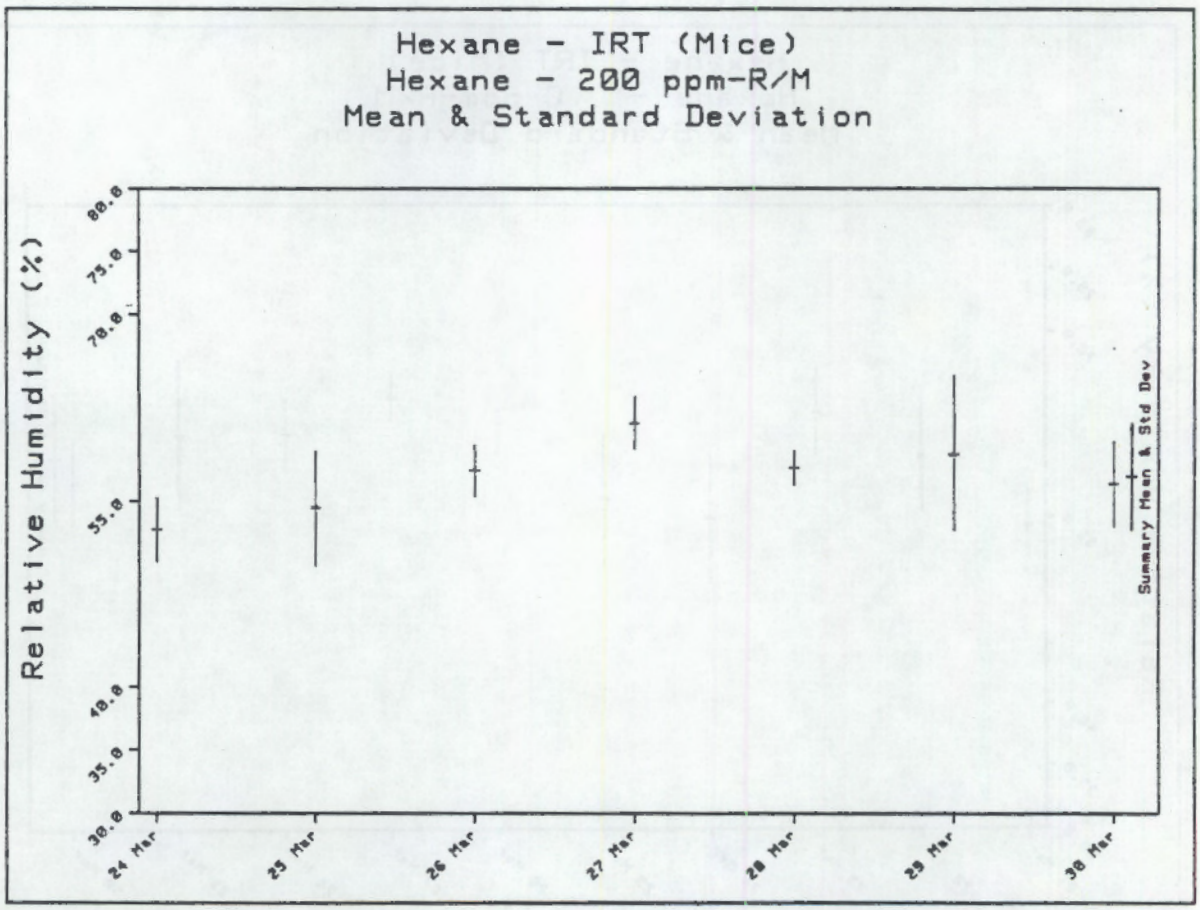
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
21 Mar 1986	54.3	99%	2.88	5%	58.0	50.0	6.	6.	100%
22 Mar 1986	60.0	109%	4.63	8%	65.0	51.0	8.	8.	100%
23 Mar 1986	63.4	115%	3.31	5%	68.0	59.0	7.	7.	100%
24 Mar 1986	54.9	100%	5.21	9%	60.0	44.0	7.	7.	100%
25 Mar 1986	56.4	103%	5.13	9%	64.0	52.0	7.	7.	100%
26 Mar 1986	61.9	112%	1.77	3%	64.0	60.0	7.	7.	100%
27 Mar 1986	64.6	117%	1.81	3%	67.0	63.0	7.	7.	100%
28 Mar 1986	61.7	112%	2.87	5%	65.0	57.0	7.	7.	100%
29 Mar 1986	61.6	112%	5.90	10%	67.0	48.0	8.	8.	100%
30 Mar 1986	57.7	105%	3.40	6%	63.0	53.0	7.	7.	100%
Summary	59.8	109%	5.03	8%	68.0	44.0	71.	71.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane - 200 ppm-R/M/Relative Humidity 40.0 to 70.0

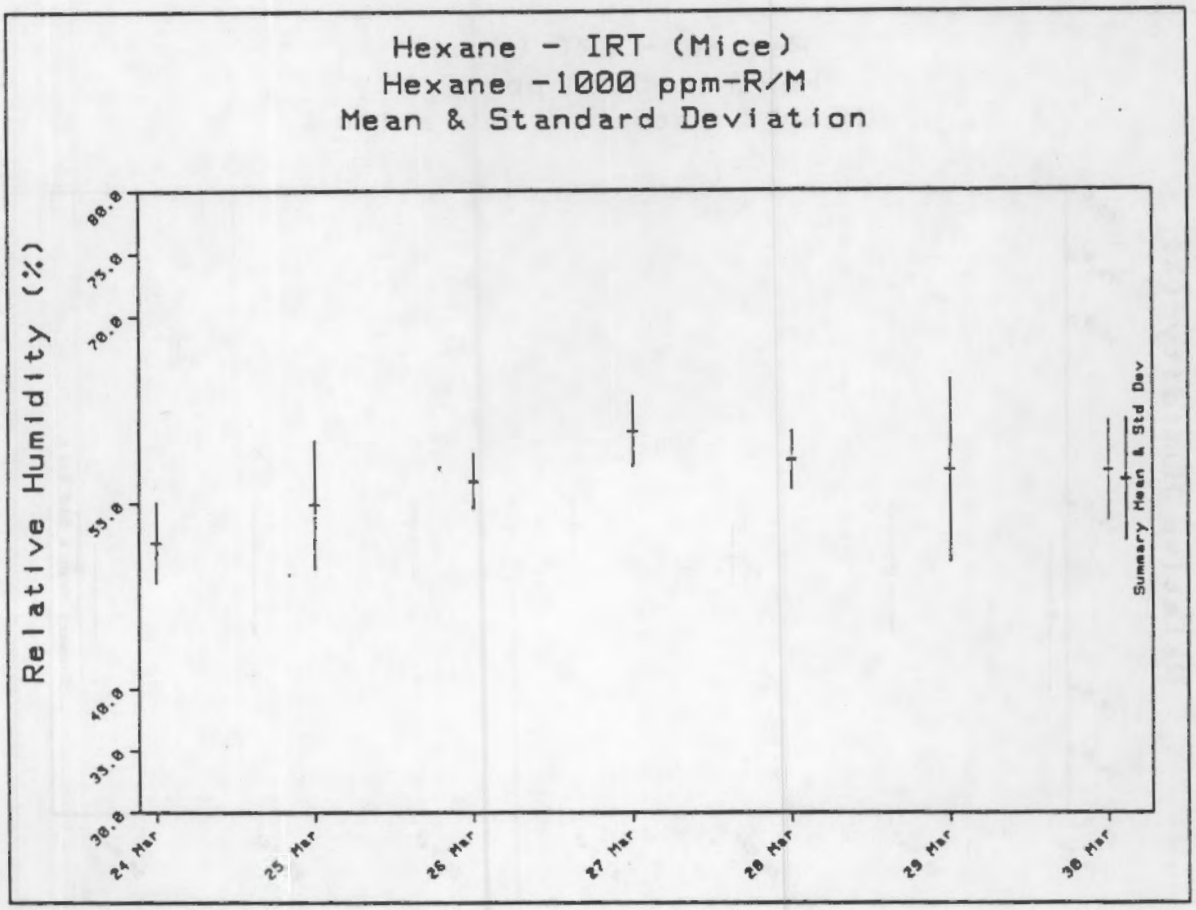
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	52.7	96%	2.56	5%	56.0	49.0	7.	7.	100%
25 Mar 1986	54.4	99%	4.65	9%	60.0	49.0	7.	7.	100%
26 Mar 1986	57.4	104%	2.07	4%	60.0	55.0	7.	7.	100%
27 Mar 1986	61.3	111%	2.06	3%	64.0	59.0	7.	7.	100%
28 Mar 1986	57.7	105%	1.38	2%	60.0	56.0	7.	7.	100%
29 Mar 1986	58.9	107%	6.20	11%	65.0	45.0	8.	8.	100%
30 Mar 1986	56.4	103%	3.46	6%	60.0	52.0	7.	7.	100%
Summary	57.0	104%	4.31	8%	65.0	45.0	50.	50.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane -1000 ppm-R/M/Relative Humidity 40.0 to 70.0

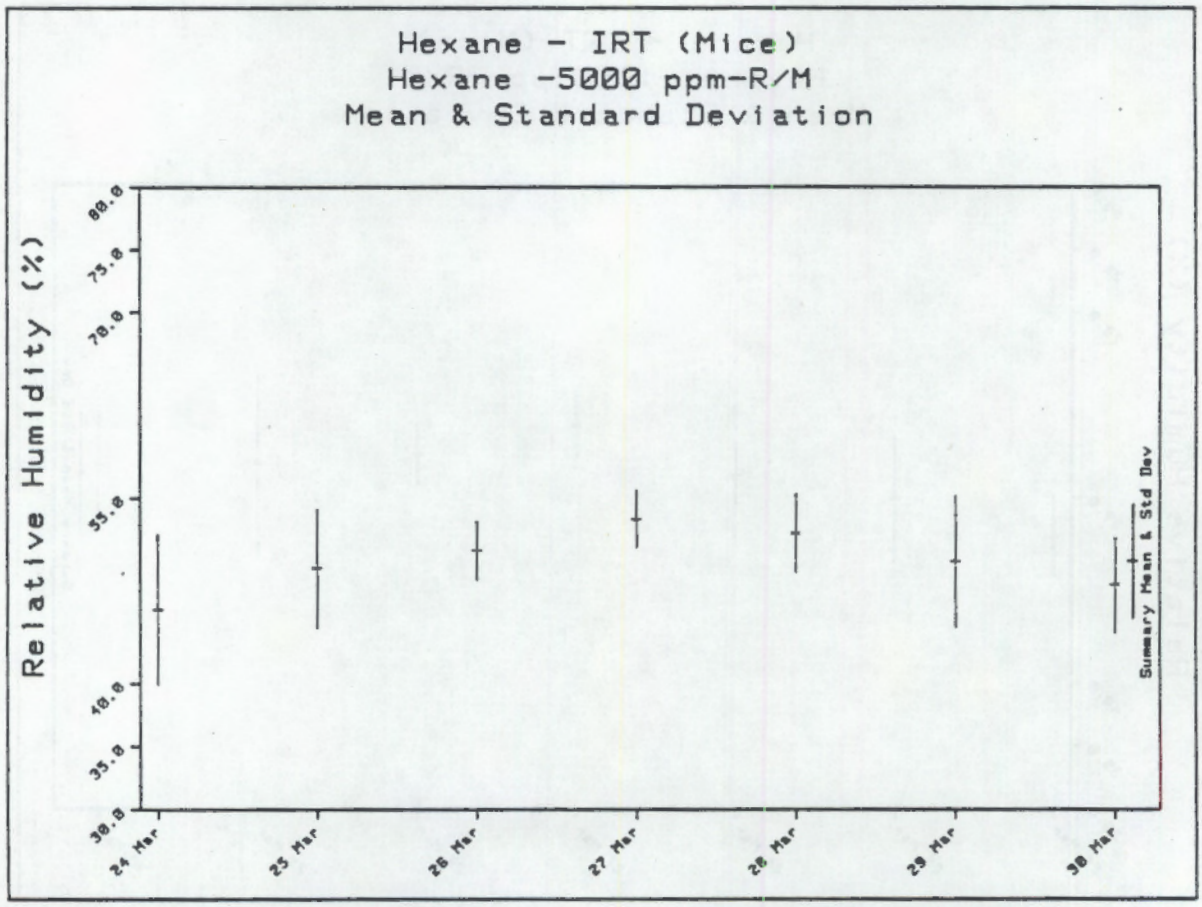
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	51.9	94%	3.24	6%	56.0	46.0	7.	7.	100%
25 Mar 1986	54.9	100%	5.11	9%	61.0	49.0	7.	7.	100%
26 Mar 1986	56.7	103%	2.21	4%	60.0	54.0	7.	7.	100%
27 Mar 1986	60.7	110%	2.81	5%	63.0	56.0	7.	7.	100%
28 Mar 1986	58.4	106%	2.30	4%	61.0	54.0	7.	7.	100%
29 Mar 1986	57.6	105%	7.29	13%	65.0	41.0	8.	8.	100%
30 Mar 1986	57.6	105%	3.95	7%	62.0	52.0	7.	7.	100%
Summary	56.8	103%	4.80	8%	65.0	41.0	50.	50.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane -5000 ppm-R/M/Relative Humidity 40.0 to 70.0

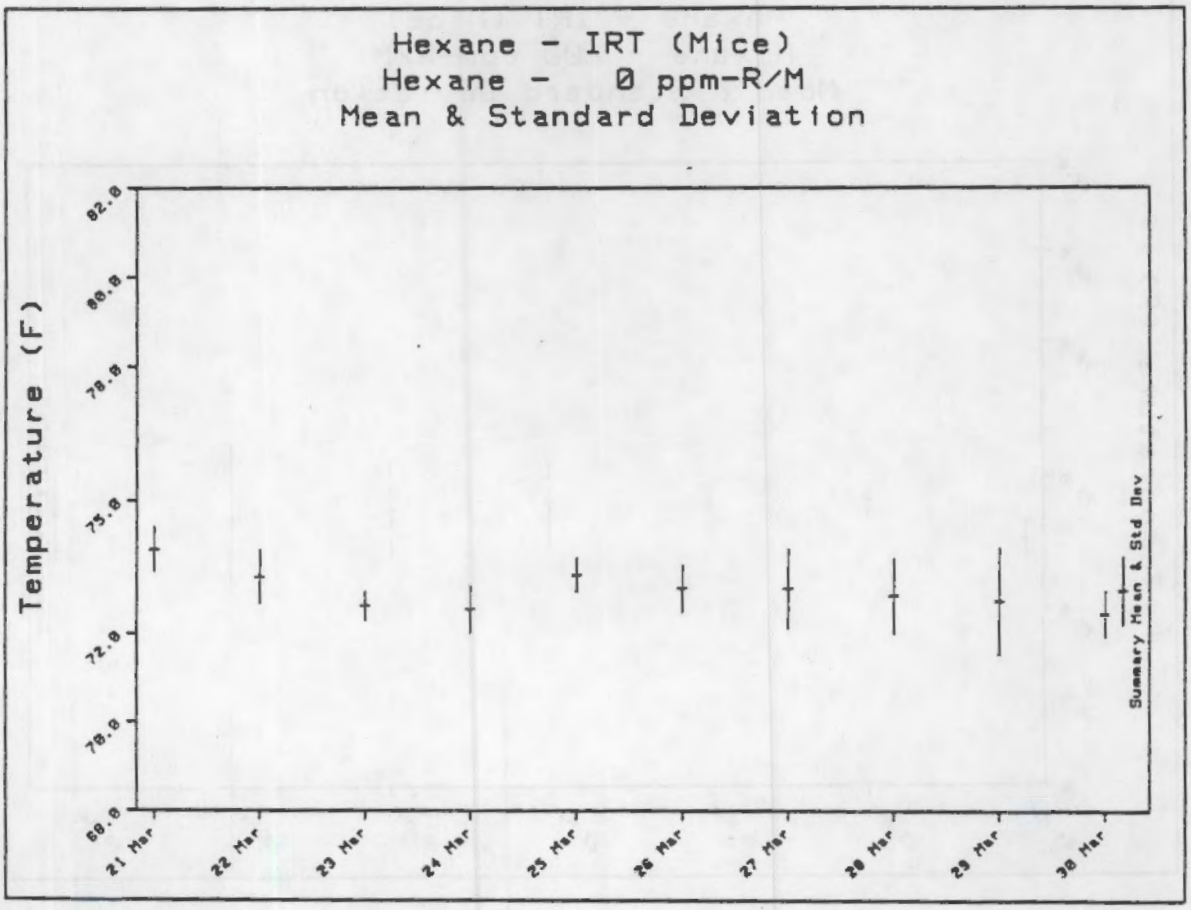
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	46.0	84%	6.06	13%	54.0	35.0	7.	6.	86%
25 Mar 1986	49.3	90%	4.75	10%	54.0	44.0	7.	7.	100%
26 Mar 1986	50.7	92%	2.36	5%	54.0	49.0	7.	7.	100%
27 Mar 1986	53.3	97%	2.29	4%	56.0	50.0	7.	7.	100%
28 Mar 1986	52.1	95%	3.13	6%	56.0	47.0	7.	7.	100%
29 Mar 1986	49.9	91%	5.28	11%	55.0	38.0	8.	7.	88%
30 Mar 1986	48.0	87%	3.83	8%	53.0	44.0	7.	7.	100%
Summary	49.9	91%	4.55	9%	56.0	35.0	50.	48.	96%



Daily Summation For Hexane - IRT (Mice) From 21 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane - 0 ppm-R/M/Temperature 72.0 to 78.0

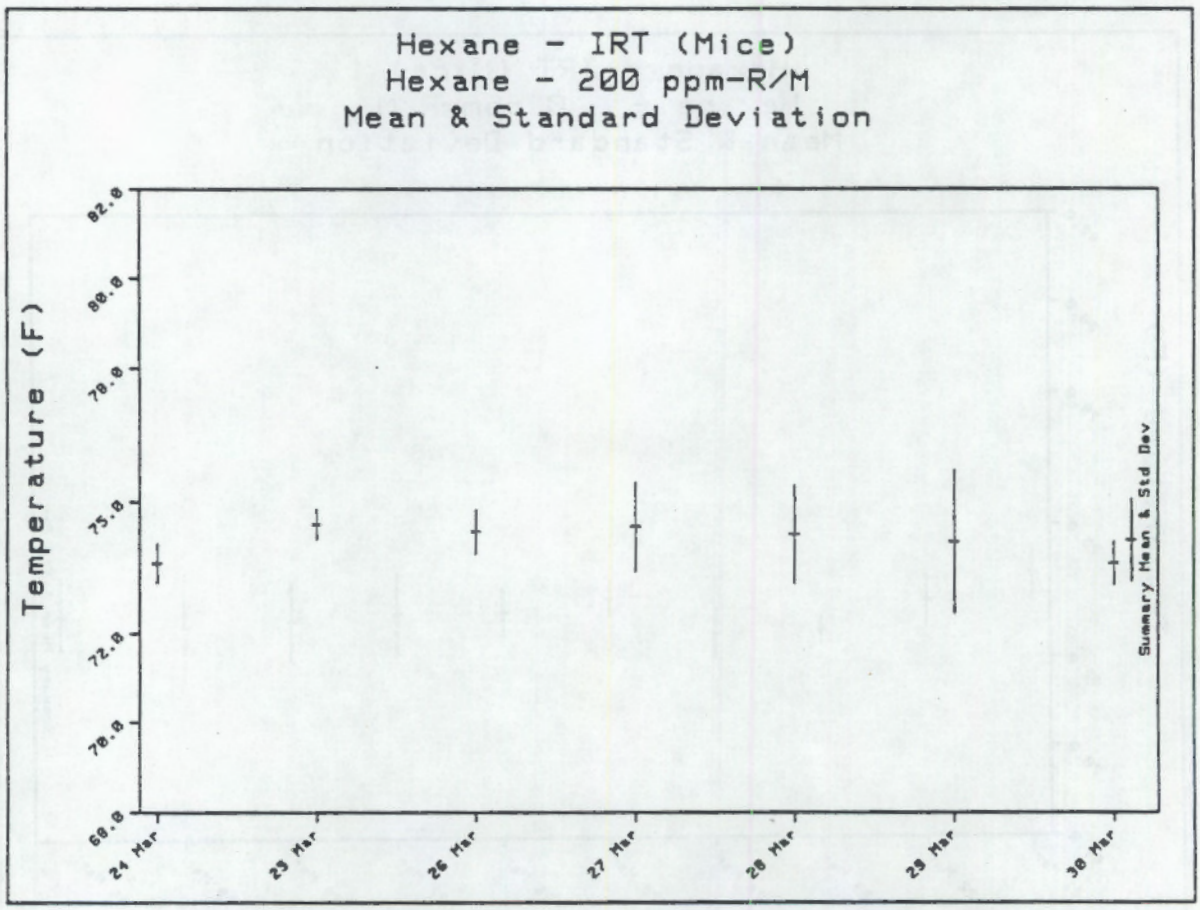
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
21 Mar 1986	73.9	99%	.49	1%	74.7	73.4	6.	6.	100%
22 Mar 1986	73.3	98%	.61	1%	74.4	72.8	8.	8.	100%
23 Mar 1986	72.6	97%	.32	0%	73.2	72.2	7.	7.	100%
24 Mar 1986	72.6	97%	.55	1%	73.3	72.0	6.	6.	100%
25 Mar 1986	73.3	98%	.39	1%	73.8	72.8	7.	7.	100%
26 Mar 1986	73.0	97%	.53	1%	73.9	72.1	8.	8.	100%
27 Mar 1986	73.0	97%	.90	1%	74.5	72.1	7.	7.	100%
28 Mar 1986	72.9	97%	.86	1%	74.2	71.8	7.	6.	86%
29 Mar 1986	72.7	97%	1.23	2%	75.5	71.7	8.	6.	75%
30 Mar 1986	72.4	97%	.53	1%	73.2	71.7	7.	5.	71%
Summary	73.0	97%	.77	1%	75.5	71.7	71.	66.	93%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane - 200 ppm-R/M/Temperature 72.0 to 78.0

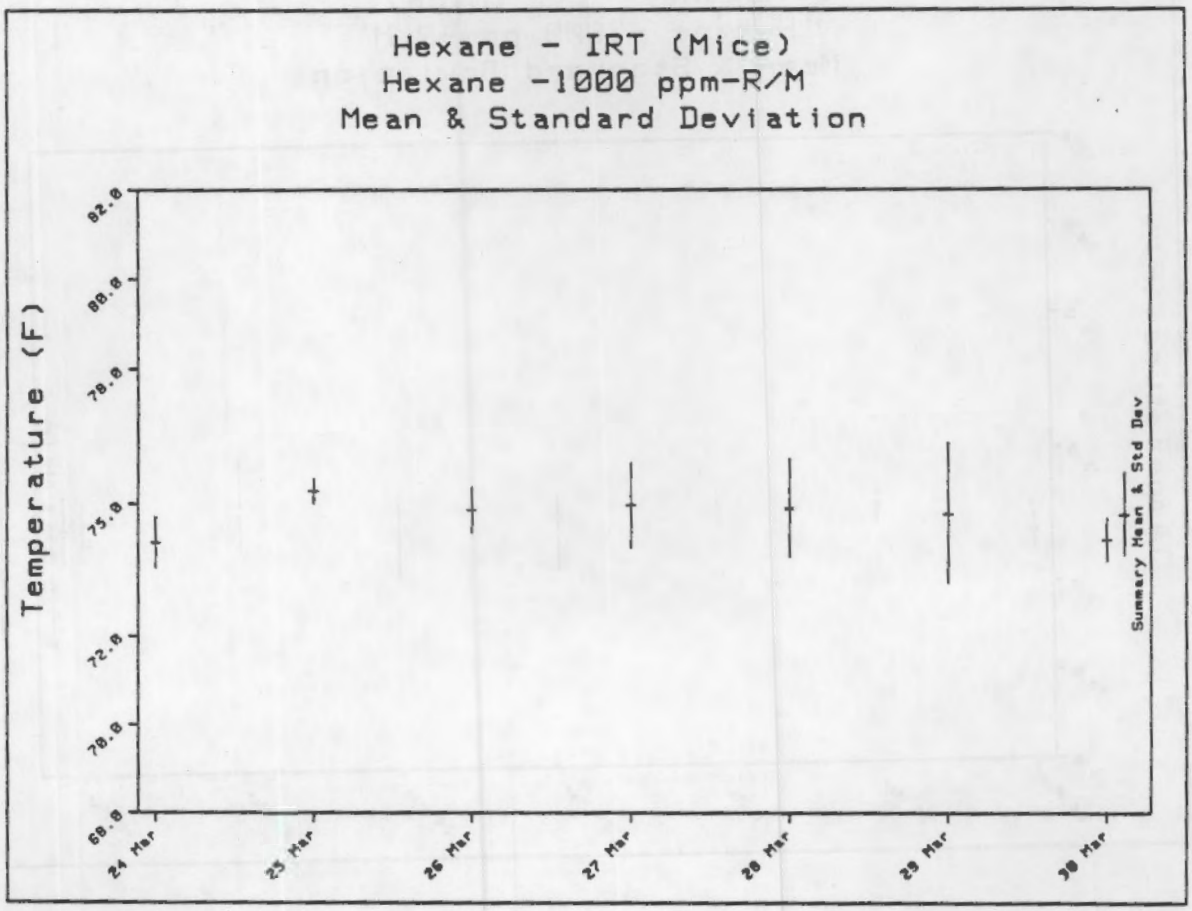
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	73.6	98%	.45	1%	74.3	73.2	5.	5.	100%
25 Mar 1986	74.5	99%	.34	0%	75.0	73.9	7.	7.	100%
26 Mar 1986	74.3	99%	.51	1%	75.1	73.5	8.	8.	100%
27 Mar 1986	74.4	99%	1.01	1%	76.1	73.3	7.	7.	100%
28 Mar 1986	74.3	99%	1.11	1%	75.9	72.9	7.	7.	100%
29 Mar 1986	74.1	99%	1.62	2%	77.8	72.8	8.	8.	100%
30 Mar 1986	73.6	98%	.48	1%	74.2	72.9	7.	7.	100%
Summary	74.1	99%	.93	1%	77.8	72.8	49.	49.	100%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane -1000 ppm-R/M/Temperature 72.0 to 78.0

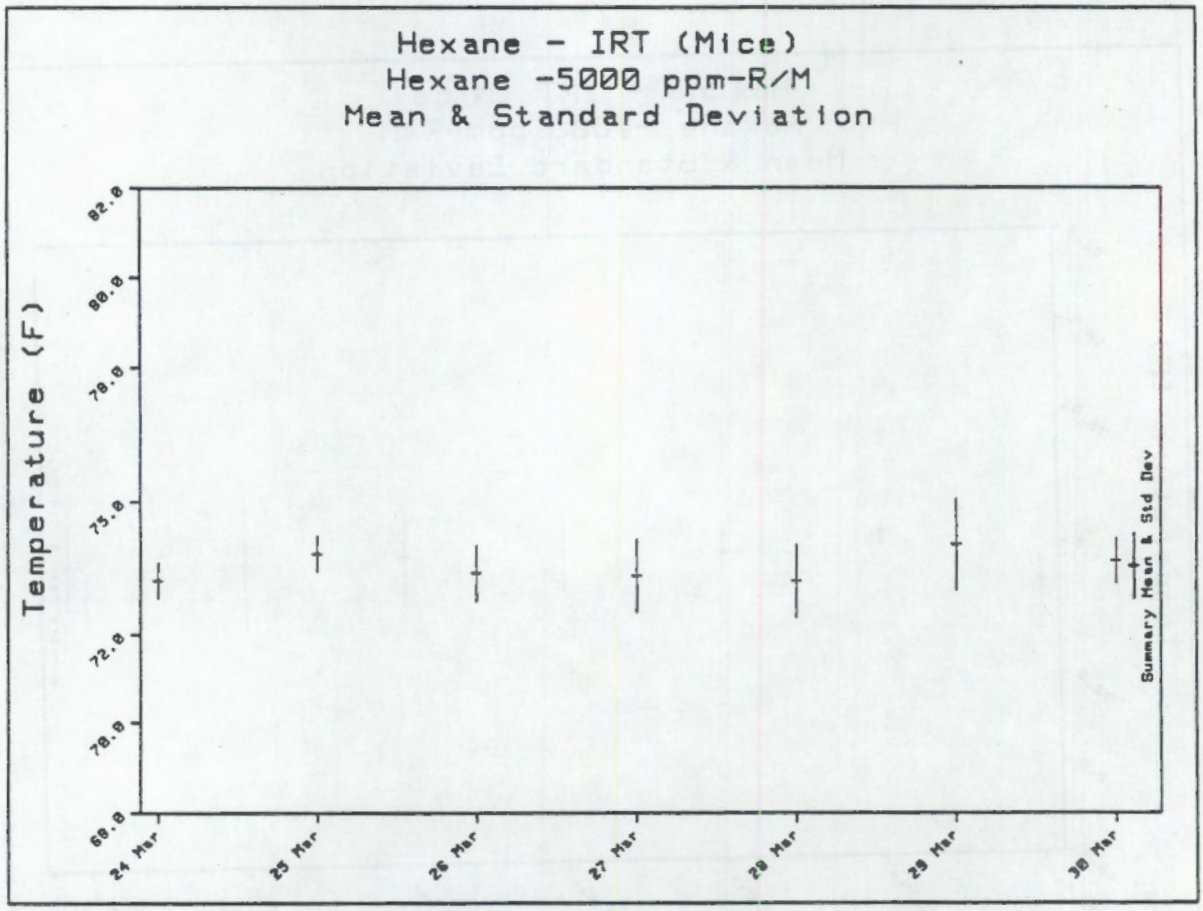
Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	74.1	99%	.57	1%	75.0	73.6	5.	5.	100%
25 Mar 1986	75.3	100%	.27	0%	75.7	74.8	7.	7.	100%
26 Mar 1986	74.9	100%	.51	1%	75.7	74.0	8.	8.	100%
27 Mar 1986	75.0	100%	.94	1%	76.4	73.8	7.	7.	100%
28 Mar 1986	74.9	100%	1.09	1%	76.6	73.5	7.	7.	100%
29 Mar 1986	74.8	100%	1.59	2%	78.5	73.6	8.	7.	88%
30 Mar 1986	74.2	99%	.49	1%	74.9	73.5	7.	7.	100%
Summary	74.8	100%	.93	1%	78.5	73.5	49.	48.	98%



Daily Summation For Hexane - IRT (Mice) From 24 Mar 1986 through 30 Mar 1986

Summary Data for: Hexane -5000 ppm-R/M/Temperature 72.0 to 78.0

Date	Mean	% Target	Std Dev	% RSD	Maximum	Minimum	N	N in	% N in
24 Mar 1986	73.2	98%	.38	1%	73.8	72.8	5.	5.	100%
25 Mar 1986	73.8	98%	.40	1%	74.2	73.1	7.	7.	100%
26 Mar 1986	73.4	98%	.63	1%	74.1	72.4	8.	8.	100%
27 Mar 1986	73.3	98%	.81	1%	74.5	72.4	7.	7.	100%
28 Mar 1986	73.2	98%	.81	1%	74.3	72.1	7.	7.	100%
29 Mar 1986	74.0	99%	1.03	1%	76.4	73.2	8.	8.	100%
30 Mar 1986	73.7	98%	.50	1%	74.4	72.9	7.	7.	100%
Summary	73.5	98%	.73	1%	76.4	72.1	49.	49.	100%



EXPOSURE OPERATION DISCUSSION SHEET

INCLUDES DISCUSSIONS AND/OR EXPLANATIONS OF PROBLEMS AFFECTING ANIMAL ENVIRONMENT AND EXPOSURES. EXPLANATIONS ARE INCLUDED FOR DATA IN WHICH THERE WERE EXCURSIONS OF DAILY MEAN OR STANDARD DEVIATION BEYOND ALLOWABLE OPERATING LIMITS OR EXCURSIONS OF INDIVIDUAL DATUM BEYOND CRITICAL LIMITS.

STUDY: IRT n-Hexane Sperm Morphology/Dominant Lethal Exposure

REPORTING PERIOD: March 21-30,1986

NOTE: 24 Hour Data Collection Period extends from ~8:00 a.m. to ~8:00 a.m.

COMPILED BY: [illegible] DATE: 5/5/86

CHAMBER CONCENTRATION

DATE	DISCUSSION OR EXPLANATION
3/24/86	We were unable to reach the high dose level (i.e. 5000 ppm) using the two pumps originally installed. The Operator was advised to maintain a concentration level of 4000 ppm throughout the exposure period until the situation could be resolved the following day by the installation of pumps with higher pumping capacity. At 22:45 the high dose chamber exceeded the lower critical operating limit (3200 ppm) because of a pump failure. Chamber concentration dropped to ~1450 ppm, the level maintainable by the second pump. At 00:26 the Specialist on call discovered a loose coupling on the failed pump and tightened. By 00:35 the chamber concentration had returned to specifications. Between 02:26 and 02:54 the second pump failed. The alarming system failed as well, so the Specialist on call was not notified. Chamber concentration dropped to ~2000 ppm. At 03:25 the computer communication failed and data was lost until the problem was discovered and corrected at 06:32.
3/25/86	New pumps were installed on the 5000 ppm dose chemical delivery system. This required establishing new pump setpoints so chamber concentration buildup took longer than normal, i.e. 45 minutes to achieve T_{90} vs the usual 12 minutes, this because the Operator was advised to approach the concentration level conservatively. No additional excursions were noted during this 20-hour exposure period.
3/27/86	Between 21:19 and 21:46 the pumps to the 5000 ppm chamber began to fail. Chamber concentration dropped to 516 ppm. The Exposure Specialist was alerted and changed out the pumps at 21:55. By 22:14 chamber concentration had returned to specs. The second pump was replaced a short time later, at 22:29, as a precautionary measure. Following this replacement, chamber concentration jumped to 5914 ppm, at 23:38, before the Specialist was able to set the proper flowrates and regain the operating concentration level. By 00:49 the exposure was again under control and no further excursions were reported during the remainder of the exposure period.

TEMPERATURE & RELATIVE HUMIDITY

DATE	DISCUSSION OR EXPLANATION
	No problems or excursions during this reporting period.

CHAMBER FLOW & VACUUM

DATE	DISCUSSION OR EXPLANATION
	No problems or excursions during this reporting period.

CHAMBER UNIFORMITY DATA SHEET

COMPOUND: IRT HEXANE

EXPOSURE ROOM NUMBER: 436

TPV MEASUREMENTS

CHAMBER:	5000 ppm		1000 ppm		200 ppm					
DATE:	3/12/86		3/13/86		3/14/86					
SAMPLE PORT	MONITOR READING	%	MONITOR READING	%	MONITOR READING	%	MONITOR READING	%	MONITOR READING	%
BACK: 1B	791	99.6%	367	100.0%	232	103.9%				
2B	801	100.8%	368	100.2%	222	99.4%				
3B	791	99.6%	371	101.0%	224	100.3%				
4B	804	101.2%	361	98.3%	211	94.5%				
5B	803	101.1%	371	101.0%	225	100.8%				
6B	805	101.3%	370	100.8%	223	99.9%				
FRONT: 1F	792	99.7%	374	101.9%	227	101.7%				
2F	780	98.2%	359	97.8%	220	98.5%				
3F	795	100.1%	363	98.9%	230	103.0%				
4F	783	98.6%	368	100.2%	218	97.6%				
5F	798	100.5%	366	99.7%	227	101.7%				
6F	790	99.4%	368	100.2%	220	98.5%				
MEAN:	794.4	100.0%	367.2	100.0%	223.3	100.0%				
TPV:	8.10	1.0%	4.37	1.2%	5.67	2.5%				
BPV:	//////////////	≤0%	//////////////	1.2%	//////////////	1.8%	//////////////		//////////////	

WPV MEASUREMENTS

IN-LINE 1st	801	100.5%	368	99.9%	232	101.0%				
2nd	808	101.4%	368	99.9%	225	98.0%				
3rd	781	98.0%	369	100.2%	232	101.0%				
MEAN:	796.7	100.0%	368.3	100.0%	229.7	100.0%				
WPV:	14.01	1.8%	0.58	0.2%	4.04	1.8%				

MONITOR TYPE: GC SERIAL # : N809569

MONITOR DATA LOCATION:

COMMENTS: No animals in chambers, pre-exposure chamber balance, no deflector plate in chamber inlets
BNW 51192 pg 102

ENTERED BY: RJW DATE: 3/15/87 REVIEWED BY: Richard J Weigel DATE: 3/15/87

APPENDIX C

DEVELOPMENTAL TOXICOLOGY DATA

Sperm Morphology n-Hexane Study: Body Weights (g) for Male Mice 1

0 ppm n-Hexane

Male Id	Pre-Study Wt	Day 1 of Exposure	Week 1 (Post-Exposure)	Week 2 (Post-Exposure)	Week 3 (Post-Exposure)	Week 4 (Post-Exposure)	Sacrifice Wt
10	26.0	26.8	26.4	27.4	27.6	28.4	28.1
13	27.6	29.0	29.2	30.4	30.8	31.4	31.4
19	25.0	26.0	27.2	27.8	26.4	29.0	27.9
30	24.0	26.6	26.2	27.2	27.2	26.2	27.8
32	27.8	29.0	29.0	29.6	29.8	30.2	29.6
33	30.2	31.0	31.4	32.6	32.8	34.4	33.2
37	27.2	28.2	28.2	29.2	29.4	30.0	28.6
56	23.0	24.8	26.2	27.8	28.2	29.4	29.7
60	26.8	27.8	28.4	29.0	29.6	30.6	30.5
78	26.4	27.6	28.8	29.6	30.6	31.8	31.6
81	24.2	26.0	25.8	26.2	26.2	27.2	27.5
91	27.0	28.2	28.2	28.6	29.4	30.0	29.8
93	25.2	25.8	26.0	26.2	26.6	26.8	27.0
98	24.4	25.0	26.6	27.2	28.2	26.8	28.9
105	26.2	21.2	29.0	30.6	31.6	32.0	31.0
107	27.4	22.8	28.4	29.6	29.8	30.8	29.8
118	26.6	27.6	28.4	29.2	29.4	30.6	30.3
121	28.2	27.6	28.2	29.6	29.8	30.8	30.4
123	25.2	26.8	26.8	27.8	27.6	29.0	28.5
124	25.8	27.2	27.6	28.4	27.8	29.0	28.5

200 ppm n-Hexane

Male Id	Pre-Study Wt	Day 1 of Exposure	Week 1 (Post-Exposure)	Week 2 (Post-Exposure)	Week 3 (Post-Exposure)	Week 4 (Post-Exposure)	Sacrifice Wt
1	27.6	28.6	30.2	30.4	31.0	31.8	31.0
2	26.2	27.4	27.8	27.8	28.2	29.0	27.8
25	24.6	25.8	26.2	26.6	26.6	27.2	26.5
27	29.8	30.6	31.4	31.6	32.0	32.4	31.0
43	25.2	27.0	28.8	28.8	28.6	29.6	29.1
44	24.4	26.2	27.0	27.0	27.2	27.8	27.2
46	25.6	27.0	28.6	29.2	28.8	28.8	28.3
49	23.4	26.6	27.8	27.8	28.2	28.6	29.3
58	27.0	28.4	28.2	29.2	28.6	29.2	28.9
64	27.0	28.8	29.4	29.0	29.0	29.4	29.3
68	25.0	27.8	28.6	28.4	29.2	29.4	29.8
69	26.0	27.6	27.2	27.4	27.4	28.0	28.1
70	23.8	25.2	26.6	27.2	28.2	28.4	28.2
80	26.0	27.6	29.6	29.4	30.0	30.8	31.7
88	26.4	28.2	30.2	30.8	30.8	31.2	31.1
89	26.6	28.0	28.6	29.4	29.4	30.8	29.6
104	28.2	29.2	30.0	30.2	31.2	31.2	31.1
106	27.4	28.4	29.0	29.0	29.6	30.0	29.2
109	26.4	26.8	27.6	28.2	28.6	29.4	28.6
111	25.4	27.4	28.4	28.8	29.8	30.6	29.6

1000 ppm n-Hexane

Male Id	Pre-Study Wt	Day 1 of Exposure	Week 1 (Post-Exposure)	Week 2 (Post-Exposure)	Week 3 (Post-Exposure)	Week 4 (Post-Exposure)	Sacrifice Wt
4	26.0	26.8	27.2	26.8	26.8	27.6	27.3
18	25.4	27.6	28.0	27.2	27.8	27.8	27.5
23	26.4	27.8	28.8	28.6	28.8	29.2	29.4
31	28.2	28.2	28.6	28.2	28.6	29.2	28.8
34	24.8	25.8	26.0	26.2	26.6	27.2	26.2
52	27.2	27.4	28.8	28.4	28.4	29.2	29.0
62	26.2	28.2	28.2	27.6	28.0	29.0	28.5
63	26.2	27.0	27.8	28.2	28.4	29.2	29.9
67	24.4	25.2	25.8	26.6	26.6	27.6	27.1
72	23.2	25.0	26.4	27.0	27.6	28.2	28.1
73	27.6	29.0	28.8	28.6	29.0	29.2	29.4
75	27.4	29.4	30.8	31.6	32.0	32.4	33.3
83	27.0	27.4	29.8	29.2	30.0	31.0	30.7
88	25.8	27.0	28.2	28.8	28.8	29.0	28.9
94	26.6	28.0	29.0	29.0	29.8	30.0	29.5
95	25.2	26.2	27.6	27.8	28.0	29.0	26.6
103	23.6	25.4	27.4	29.2	28.4	28.6	28.7
108	26.4	28.0	29.0	29.0	29.8	30.2	29.6
113	25.2	26.4	28.0	28.8	29.4	29.2	29.2
115	28.6	29.2	30.0	30.6	30.0	31.0	30.2

5000 ppm n-Hexane

Male Id	Pre-Study Wt	Day 1 of Exposure	Week 1 (Post-Exposure)	Week 2 (Post-Exposure)	Week 3 (Post-Exposure)	Week 4 (Post-Exposure)	Sacrifice Wt
5	25.0	25.6	26.6	28.2	27.4	27.0	26.8
7	26.0	25.8	28.0	26.8	26.8	27.2	26.9
12	23.6	25.0	28.6	27.8	28.4	20.8	28.1
16	26.2	27.2	29.4	29.0	29.4	29.2	28.8
22	24.4	25.0	26.6	26.6	27.2	27.2	26.8
28	26.6	27.8	30.0	30.0	30.2	30.6	30.1
36	25.2	26.2	27.2	27.2	27.4	28.2	27.4
38	25.0	27.4	28.6	29.6	30.0	30.4	30.4
40	26.4	28.6	31.0	31.4	31.8	31.6	31.3
42	27.8	29.0	30.4	30.2	31.0	31.0	30.9
48	27.2	28.2	30.2	29.2	30.2	30.8	30.5
51	25.4	26.6	28.4	28.0	28.8	29.0	28.6
79	24.0	24.8	26.8	27.6	29.0	29.8	29.6
87	25.8	27.0	28.4	28.2	29.0	28.6	26.4
96	25.0	25.6	27.0	26.4	27.6	28.2	28.2
100	28.0	28.8	30.2	30.8	31.0	32.4	31.5
112	27.6	28.4	31.8	32.4	33.2	33.6	32.7
116	26.4	27.8	29.8	31.8	32.6	32.0	31.6
117	28.4	29.8	31.0	32.0	33.4	33.0	31.9
119	27.0	28.8	29.6	30.4	31.4	31.2	30.8

Sperm Morphology n-Hexane Study: Body Weights (g) for Male Mice 5

200 mg/kg EMS

Male Id	Pre-Study Wt	Day 1 of Exposure	Week 1 (Post-Exposure)	Week 2 (Post-Exposure)	Week 3 (Post-Exposure)	Week 4 (Post-Exposure)	Sacrifice Wt
6	27.6	27.9	25.8	28.4	29.6	30.6	30.6
9	28.8	27.6	25.9	27.0	27.8	28.4	28.4
11	26.2	25.0	23.5	25.6	26.6	26.6	27.1
17	24.2	23.8	23.6	25.2	26.8	27.6	27.1
20	26.4	26.5	26.3	28.2	29.2	29.4	29.4
26	24.4	24.8	25.4	26.6	27.6	28.4	28.7
36	24.8	25.0	24.3	26.2	27.8	28.4	28.1
39	25.6	25.6	24.3	26.6	27.2	28.8	29.9
41	25.2	25.8	25.4	27.2	28.2	29.2	30.2
45	27.0	27.5	27.1	28.8	30.2	31.2	31.6
54	28.4	28.5	27.3	28.6	29.4	30.2	30.6
57	29.2	30.4	28.9	31.2	32.8	34.2	33.9
59	25.4	26.6	.	.	.	.	.
61	26.4	26.9	26.5	27.4	28.6	29.4	29.5
71	23.4	24.1	24.2	25.2	26.4	26.8	28.8
82	25.0	25.3	25.1	26.6	28.2	28.0	29.4
102	26.0	26.7	25.7	27.2	28.0	29.2	28.5
110	26.2	26.2	24.9	26.4	27.2	28.6	28.7
114	27.2	27.9	26.6	28.0	28.4	28.8	29.5
120	27.4	26.8	26.5	27.0	28.4	28.8	29.2

250 mg/kg EMS

Male Id	Pre-Study Wt	Day 1 of Exposure	Week 1 (Post-Exposure)	Week 2 (Post-Exposure)	Week 3 (Post-Exposure)	Week 4 (Post-Exposure)	Sacrifice Wt
3	26.0	26.3	23.9	25.4	26.8	27.4	27.4
8	26.4	26.6	24.8	25.6	26.8	27.6	27.2
14	25.8	26.8	24.2	26.4	28.8	29.4	28.4
21	24.4	24.2	21.6	24.0	25.6	26.4	26.1
47	27.0	28.2	.	.	.	.	.
50	26.2	27.5	25.4	26.8	28.6	30.0	28.5
53	24.8	25.1	21.8	25.2	26.0	27.8	27.2
55	28.8	29.5	26.6	28.8	32.2	33.8	32.2
65	27.4	27.5	17.7	.	.	.	.
66	25.2	26.5	22.1	25.6	27.8	28.4	28.2
74	27.0	28.0	24.8	26.6	28.4	28.6	28.8
76	23.6	24.6	22.4	23.4	23.6	23.8	24.6
77	28.2	28.9	24.9	28.2	29.6	30.8	31.6
84	27.8	28.6	26.8	28.0	29.8	31.6	31.7
85	26.6	26.7	25.6	25.8	26.8	27.4	28.3
90	25.4	26.1	24.4	25.2	26.8	28.2	28.9
92	24.2	25.4	22.6	24.8	25.4	25.8	26.1
99	26.8	27.5	22.6	26.4	26.6	26.8	27.0
101	26.2	27.1	24.3	26.0	27.2	28.6	28.4
122	25.0	25.2	23.1	25.0	26.4	27.2	26.3

n-Hexane Sperm Morphology Study: Abnormalities in Sperm Observed in Male Mice

0 ppm n-Hexane

Male Id	Normal	% Normal	Blunt Hook	% Blunt Hook	Banana	% Banana	Amorphous	% Amorphous	Pin Head	% Pin Head	Double Head/ Double Tail	% Double Head/ Double Tail
10	487	97.4	8	1.6	0	0.0	5	1.0	0	0.0	0	0.0
10	494	98.8	3	0.6	0	0.0	3	0.6	0	0.0	0	0.0
13	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
13	496	99.2	0	0.0	0	0.0	4	0.8	0	0.0	0	0.0
19	498	99.6	0	0.0	1	0.2	1	0.2	0	0.0	0	0.0
19	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
30	497	99.4	1	0.2	1	0.2	1	0.2	0	0.0	0	0.0
30	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
32	497	99.4	2	0.4	0	0.0	1	0.2	0	0.0	0	0.0
32	494	98.8	1	0.2	2	0.4	3	0.6	0	0.0	0	0.0
33	494	98.8	0	0.0	0	0.0	6	1.2	0	0.0	0	0.0
33	494	98.4	2	0.4	0	0.0	6	1.2	0	0.0	0	0.0
37	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
37	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
58	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
58	497	99.4	2	0.4	0	0.0	1	0.2	0	0.0	0	0.0
60	493	98.6	3	0.6	0	0.0	4	0.8	0	0.0	0	0.0
60	487	97.4	3	0.6	0	0.0	10	2.0	0	0.0	0	0.0
78	490	98.0	5	1.0	0	0.0	5	1.0	0	0.0	0	0.0
78	497	99.4	1	0.2	0	0.0	2	0.4	0	0.0	0	0.0
81	495	99.0	2	0.4	0	0.0	3	0.6	0	0.0	0	0.0
81	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
91	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
91	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
93	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
93	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
98	496	99.2	1	0.2	0	0.0	2	0.4	1	0.2	0	0.0
98	494	98.8	4	0.8	0	0.0	2	0.4	0	0.0	0	0.0
105	493	98.6	2	0.4	0	0.0	5	1.0	0	0.0	0	0.0
105	470	94.0	14	2.8	0	0.0	16	3.2	0	0.0	0	0.0
107	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
107	495	99.0	2	0.4	3	0.6	0	0.0	0	0.0	0	0.0
118	481	96.2	13	2.6	3	0.6	3	0.6	0	0.0	0	0.0
118	489	97.8	8	1.6	1	0.2	2	0.4	0	0.0	0	0.0
121	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
121	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
123	496	99.2	1	0.2	0	0.0	3	0.6	0	0.0	0	0.0
123	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
124	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
124	498	99.6	2	0.4	0	0.0	0	0.0	0	0.0	0	0.0

n-Hexane Sperm Morphology Study: Abnormalities in Sperm Observed in Male Mice

200 ppm n-Hexane

Male Id	Normal	% Normal	Blunt Hook	% Blunt Hook	Banana	% Banana	Amorphous	% Amorphous	Pin Head	% Pin Head	Double Head/ Double Tail	% Double Head/ Double Tail
1	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
1	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
25	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
25	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
27	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
27	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
43	492	98.4	3	0.6	0	0.0	5	1.0	0	0.0	0	0.0
43	498	99.6	1	0.2	1	0.2	0	0.0	0	0.0	0	0.0
44	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
44	496	99.2	0	0.0	0	0.0	3	0.6	0	0.0	0	0.0
46	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
46	496	99.2	2	0.4	0	0.0	2	0.4	0	0.0	0	0.0
49	498	99.6	2	0.4	0	0.0	0	0.0	0	0.0	0	0.0
49	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
58	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
58	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
64	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
64	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
68	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
68	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
69	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
69	491	98.2	1	0.2	0	0.0	8	1.6	0	0.0	0	0.0
70	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
70	494	98.8	2	0.4	0	0.0	4	0.8	0	0.0	0	0.0
80	497	99.4	2	0.4	0	0.0	1	0.2	0	0.0	0	0.0
80	496	99.2	1	0.2	0	0.0	3	0.6	0	0.0	0	0.0
88	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
88	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
89	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
89	497	99.4	3	0.6	0	0.0	0	0.0	0	0.0	0	0.0
104	498	99.6	2	0.4	0	0.0	0	0.0	0	0.0	0	0.0
104	496	99.2	1	0.2	1	0.2	2	0.4	0	0.0	0	0.0
106	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
106	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
109	496	99.2	3	0.6	0	0.0	1	0.2	0	0.0	0	0.0
109	496	99.2	4	0.8	0	0.0	0	0.0	0	0.0	0	0.0
111	495	99.0	0	0.0	4	0.8	1	0.2	0	0.0	0	0.0
111	498	99.6	0	0.0	1	0.2	1	0.2	0	0.0	0	0.0

n-Hexane Sperm Morphology Study: Abnormalities in Sperm Observed in Male Mice

1000 ppm n-Hexane

Male Id	Normal	% Normal	Blunt Hook	% Blunt Hook	Banana	% Banana	Amorphous	% Amorphous	Pi Head	% Pin Head	Double Head/ Double Tail	% Double Head/ Double Tail
4	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
4	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
18	491	98.2	5	1.0	0	0.0	4	0.8	0	0.0	0	0.0
18	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
23	494	98.8	3	0.6	0	0.0	3	0.6	0	0.0	0	0.0
23	496	99.2	2	0.4	0	0.0	1	0.2	0	0.0	1	0.2
31	497	99.4	0	0.0	0	0.0	3	0.6	0	0.0	0	0.0
31	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
34	496	99.2	1	0.2	0	0.0	3	0.6	0	0.0	0	0.0
34	496	99.2	0	0.0	0	0.0	4	0.8	0	0.0	0	0.0
52	495	99.0	3	0.6	0	0.0	2	0.4	0	0.0	0	0.0
52	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
62	495	99.0	1	0.2	0	0.0	4	0.8	0	0.0	0	0.0
62	495	99.0	2	0.4	0	0.0	3	0.6	0	0.0	0	0.0
63	495	99.0	0	0.0	1	0.2	4	0.8	0	0.0	0	0.0
63	494	98.8	2	0.4	1	0.2	3	0.6	0	0.0	0	0.0
67	491	98.2	7	1.4	0	0.0	2	0.4	0	0.0	0	0.0
67	485	97.0	8	1.6	1	0.2	6	1.2	0	0.0	0	0.0
72	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
72	494	98.8	1	0.2	1	0.2	4	0.8	0	0.0	0	0.0
73	492	98.4	4	0.8	0	0.0	4	0.8	0	0.0	0	0.0
73	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
76	487	97.4	8	1.6	0	0.0	5	1.0	0	0.0	0	0.0
76	497	99.4	1	0.2	0	0.0	2	0.4	0	0.0	0	0.0
83	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
83	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
86	492	98.4	5	1.0	0	0.0	3	0.6	0	0.0	0	0.0
86	496	99.2	0	0.0	0	0.0	4	0.8	0	0.0	0	0.0
94	498	99.2	1	0.2	0	0.0	3	0.6	0	0.0	0	0.0
94	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
95	491	98.2	2	0.4	4	0.8	3	0.6	0	0.0	0	0.0
95	497	99.4	0	0.0	3	0.6	0	0.0	0	0.0	0	0.0
103	498	99.6	2	0.4	0	0.0	0	0.0	0	0.0	0	0.0
103	496	99.2	4	0.8	0	0.0	0	0.0	0	0.0	0	0.0
108	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
108	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
113	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
113	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
115	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
115	494	98.8	2	0.4	1	0.2	3	0.6	0	0.0	0	0.0

n-Hexane Sperm Morphology Study: Abnormalities in Sperm Observed in Male Mice

5000 ppm n-Hexane

Male Id	Normal	% Normal	Blunt Hook	% Blunt Hook	Banana	% Banana	Amorphous	% Amorphous	Pin Head	% Pin Head	Double Head/ Double Tail	% Double Head/ Double Tail
5	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
5	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
7	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
7	492	98.4	5	1.0	0	0.0	3	0.6	0	0.0	0	0.0
12	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
12	494	98.0	3	0.6	1	0.2	2	0.4	0	0.0	0	0.0
16	493	98.6	6	1.2	0	0.0	1	0.2	0	0.0	0	0.0
16	496	99.2	4	0.8	0	0.0	0	0.0	0	0.0	0	0.0
22	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
22	489	97.8	9	1.8	1	0.2	1	0.2	0	0.0	0	0.0
28	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
28	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
35	492	98.4	4	0.8	1	0.2	3	0.6	0	0.0	0	0.0
35	500	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
38	498	99.2	2	0.4	0	0.0	2	0.4	0	0.0	0	0.0
38	494	98.8	5	1.0	0	0.0	0	0.0	1	0.2	0	0.0
40	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
40	492	98.4	5	1.0	0	0.0	3	0.6	0	0.0	0	0.0
42	494	98.8	4	0.8	0	0.0	1	0.2	1	0.2	0	0.0
42	497	99.4	2	0.4	0	0.0	1	0.2	0	0.0	0	0.0
48	482	96.4	8	1.6	4	0.8	6	1.2	0	0.0	0	0.0
48	489	97.8	8	1.6	3	0.6	0	0.0	0	0.0	0	0.0
51	493	98.6	4	0.8	2	0.4	1	0.2	0	0.0	0	0.0
51	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
79	497	99.4	2	0.4	0	0.0	1	0.2	0	0.0	0	0.0
79	492	98.4	4	0.8	4	0.8	0	0.0	0	0.0	0	0.0
87	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
87	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0
96	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
96	498	99.6	2	0.4	0	0.0	0	0.0	0	0.0	0	0.0
100	494	98.8	4	0.8	0	0.0	2	0.4	0	0.0	0	0.0
100	489	97.8	3	0.6	1	0.2	7	1.4	0	0.0	0	0.0
112	497	99.4	2	0.4	0	0.0	1	0.2	0	0.0	0	0.0
112	495	99.0	4	0.8	0	0.0	1	0.2	0	0.0	0	0.0
116	492	98.4	3	0.6	0	0.0	5	1.0	0	0.0	0	0.0
116	499	99.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
117	498	99.6	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
117	499	99.8	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
119	495	99.0	1	0.2	0	0.0	4	0.8	0	0.0	0	0.0
119	498	99.6	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0

n-Hexane Sperm Morphology Study: Abnormalities in Sperm Observed in Male Mice

200 mg/kg EMS

Male Id	Normal	% Normal	Blunt Hook	% Blunt Hook	Banana	% Banana	Amorphous	% Amorphous	Pin Head	% Pin Head	Double Head/ Double Tail	% Double Head/ Double Tail
6	488	97.6	3	0.6	0	0.0	9	1.8	0	0.0	0	0.0
6	489	97.8	1	0.2	0	0.0	10	2.0	0	0.0	0	0.0
9	488	97.6	0	0.0	0	0.0	11	2.2	0	0.0	1	0.2
9	490	98.0	0	0.0	0	0.0	10	2.0	0	0.0	0	0.0
11	484	96.8	2	0.4	0	0.0	14	2.8	0	0.0	0	0.0
11	484	96.8	3	0.6	0	0.0	12	2.4	1	0.2	0	0.0
17	493	98.6	0	0.0	0	0.0	7	1.4	0	0.0	0	0.0
17	487	97.4	0	0.0	0	0.0	13	2.6	0	0.0	0	0.0
20	494	98.8	1	0.2	0	0.0	5	1.0	0	0.0	0	0.0
20	480	96.0	8	1.6	0	0.0	12	2.4	0	0.0	0	0.0
26	476	95.2	11	2.2	0	0.0	13	2.6	0	0.0	0	0.0
26	488	97.6	4	0.8	0	0.0	7	1.4	1	0.2	0	0.0
36	481	96.2	12	2.4	0	0.0	7	1.4	0	0.0	0	0.0
36	489	97.8	8	1.6	0	0.0	3	0.6	0	0.0	0	0.0
39	81	91.0	1	1.1	0	0.0	6	6.7	1	1.1	0	0.0
39	74	90.2	3	3.7	0	0.0	5	6.1	0	0.0	0	0.0
41	487	97.4	8	1.6	0	0.0	5	1.0	0	0.0	0	0.0
41	477	95.4	12	2.4	0	0.0	10	2.0	1	0.2	0	0.0
45	483	96.6	7	1.4	0	0.0	10	2.0	0	0.0	0	0.0
45	487	97.4	4	0.8	0	0.0	9	1.8	0	0.0	0	0.0
54	469	93.8	12	2.4	0	0.0	19	3.8	0	0.0	0	0.0
54	477	95.4	7	1.4	0	0.0	16	3.2	0	0.0	0	0.0
57	488	97.6	6	1.2	0	0.0	6	1.2	0	0.0	0	0.0
57	494	98.8	2	0.4	1	0.2	3	0.6	0	0.0	0	0.0
61	491	98.2	4	0.8	0	0.0	5	1.0	0	0.0	0	0.0
61	489	97.8	2	0.4	0	0.0	9	1.8	0	0.0	0	0.0
71	468	93.6	11	2.2	0	0.0	21	4.2	0	0.0	0	0.0
71	471	94.2	11	2.2	0	0.0	18	3.6	0	0.0	0	0.0
82	496	99.2	1	0.2	0	0.0	3	0.6	0	0.0	0	0.0
82	493	98.6	3	0.6	0	0.0	4	0.8	0	0.0	0	0.0
102	485	97.0	7	1.4	0	0.0	8	1.6	0	0.0	0	0.0
102	486	97.2	10	2.0	0	0.0	4	0.8	0	0.0	0	0.0
110	485	97.0	8	1.6	0	0.0	7	1.4	0	0.0	0	0.0
110	475	95.0	12	2.4	3	0.6	10	2.0	0	0.0	0	0.0
114	473	94.6	3	0.6	2	0.4	22	4.4	0	0.0	0	0.0
114	478	95.6	8	1.6	0	0.0	14	2.8	0	0.0	0	0.0
120	470	94.0	12	2.4	0	0.0	18	3.6	0	0.0	0	0.0
120	473	94.6	5	1.0	0	0.0	22	4.4	0	0.0	0	0.0

n-Hexane Sperm Morphology Study: Abnormalities in Sperm Observed in Male Mice

250 mg/kg EMS

Male Id	Normal	% Normal	Blunt Hook	% Blunt Hook	Banana	% Banana	Amorphous	% Amorphous	Pin Head	% Pin Head	Double Head/ Double Tail	% Double Head/ Double Tail
3	464	92.8	15	3.0	0	0.0	21	4.2	0	0.0	0	0.0
3	457	91.4	15	3.0	1	0.2	27	5.4	0	0.0	0	0.0
14	463	92.6	18	3.6	0	0.0	19	3.8	0	0.0	0	0.0
14	471	94.2	9	1.8	0	0.0	20	4.0	0	0.0	0	0.0
21	456	91.2	25	5.0	0	0.0	19	3.8	0	0.0	0	0.0
21	449	89.8	29	5.8	1	0.2	20	4.0	0	0.0	0	0.0
50	445	89.0	14	2.8	1	0.2	40	8.0	0	0.0	0	0.0
50	455	91.0	10	2.0	1	0.2	34	6.8	0	0.0	0	0.0
53	404	80.8	35	7.0	7	1.4	54	10.8	0	0.0	0	0.0
53	394	78.8	54	10.8	3	0.6	48	9.6	0	0.0	0	0.0
55	409	81.8	43	8.6	12	2.4	36	7.2	0	0.0	0	0.0
55	404	80.8	41	8.2	1	0.2	54	10.8	0	0.0	0	0.0
66	430	86.0	29	5.8	0	0.0	41	8.2	0	0.0	0	0.0
66	440	88.0	19	3.8	1	0.2	40	8.0	0	0.0	0	0.0
74	436	87.2	38	7.6	1	0.2	25	5.0	0	0.0	0	0.0
74	443	88.6	35	5.0	0	0.0	32	6.4	0	0.0	0	0.0
78	360	72.0	94	18.8	1	0.2	45	9.0	0	0.0	0	0.0
78	351	70.2	91	18.2	0	0.0	58	11.6	0	0.0	0	0.0
77	457	91.4	24	4.8	0	0.0	19	3.8	0	0.0	0	0.0
77	463	92.6	18	3.6	0	0.0	19	3.8	0	0.0	0	0.0
84	428	85.6	40	8.0	5	1.0	27	5.4	0	0.0	0	0.0
84	442	88.4	28	5.6	4	0.8	26	5.2	0	0.0	0	0.0
85	437	87.4	39	7.8	12	2.4	12	2.4	0	0.0	0	0.0
85	438	87.6	30	6.0	8	1.6	24	4.8	0	0.0	0	0.0
90	453	90.6	15	3.0	7	1.4	25	5.0	0	0.0	0	0.0
90	453	90.6	13	2.6	4	0.8	30	6.0	0	0.0	0	0.0
92	454	90.8	18	3.6	3	0.6	25	5.0	0	0.0	0	0.0
92	460	92.0	20	4.0	0	0.0	20	4.0	0	0.0	0	0.0
99	449	89.8	27	5.4	0	0.0	23	4.6	0	0.0	1	0.2
99	445	89.0	22	4.4	0	0.0	33	6.6	0	0.0	0	0.0
101	429	85.8	44	8.8	0	0.0	27	5.4	0	0.0	0	0.0
101	430	86.0	29	5.8	0	0.0	41	8.2	0	0.0	0	0.0
122	404	80.8	43	8.4	0	0.0	54	10.8	0	0.0	0	0.0
122	409	81.8	45	9.0	10	2.0	36	7.2	0	0.0	0	0.0

n-Hexane Sperm Morphology Study: Calendar of Events

Exposed concurrent with dominant lethal study males

Hexane exposure levels; tmt1-4; 20M each	(5 day; 20 hr/day)	0,200,1000,5000 ppm
EMS positive controls; tmt 5 & 6; 20M each	(5 day dose period)	200 mg/Kg 250 mg/Kg
Ordered B6C3F1 mice		2-14-86
Received males (ARS#860034)		2-25-86
Individually caged males		3-05-86
Health screen 10 males		3-17-86
Eartagged, weighed & randomized males		3-19-86
Toe-clipped by treatment group		3-20-86
Moved study males to exposure room		3-21-86
Released for study		3-22-86
Exposure; 5 days 20hr/day		3-24 to 3-28-86
Body weights		
Hexane exposure day 1		3-24-86
EMS dose period- daily, 5 days		3-24 to 3-28-86
Hexane & EMS week 1 postexposure		3-31-86
Hexane & EMS week 2 postexposure		4-07-86
Hexane & EMS week 3 postexposure		4-14-86
Hexane & EMS week 4 postexposure		4-21-86
Sacrificed & weighed; post-exposure week 5: 117 males (3 dead prior to sacrifice)		4-28-86 terminal serology
Completed slide evaluations		9-17-87

n-HEXANE MOUSE SPERM MORPHOLOGY STUDY DISPOSITION

Exposure Group	Treatment Code	Number of Mice		
			Removed	
		On Study	From Study	Sacrificed
Control (0 ppm)	1	20	0	20
200 ppm	2	20	0	20
1000 ppm	3	20	0	20
5000 ppm	4	20	0	20
200 mg/kg EMS(a)	5	20	1(b)	19
250 mg/kg EMS(a)	6	20	2(c)	18

(a) ethyl methanesulfonate (EMS), positive control animals
(b) 1 died from EMS toxicity
(c) 2 died from EMS toxicity

APPENDIX D

ANIMAL HEALTH SCREEN

ARC DIAGNOSTIC LABORATORY REPORT

INVESTIGATOR Hackett
EXPERIMENT n-Hexane - Sperm Morph.
COST CODE —
BUILDING LSL II
PEN, ROOM 336

LAB NO. 840
DATE 3/17/86
ANIMAL OR SHIPMENT NO. 860034
SOURCE Chari River Analogue PO2 REC'D 2-25-86
SPECIES & STRAIN mice B6C3F1
SEX males AGE B.D. 1-2-86
① wrote wrong AEJ 3/26/86

SPECIMEN SUBMITTED AND CLINICAL HISTORY:

10 mice for pre-exposure health screening including anal tapes, gross, nasopharyngeal washing for culture, viral serology & histology

LABORATORY RESULTS

Anal tape preps: #1-10 - All negative, no pinworm ova seen 3/17/86 AEJ

Gross necropsy: No significant findings 3/17/86 AEJ

Culture results: Nasopharyngeal washings #1-10

#1 - BAP - 3+ white gr. gpc cat ⊕ tube coag ⊖ Staphylococcus sp.
1+ Beta white gs gpc cat ⊕ coag ⊕ Staphylococcus sp.
MAC - NG

#2 - BAP - 3+ small gray gpr cat ⊖ prob lactobacillus sp.
MAC - NG

#3 - BAP - 1+ mucoid gray API # 3305572 - Enterobacter cloacae
2+ small gray - gpr - cat ⊖ prob lactobacillus sp.
1+ raised white gpc cat ⊖ BE ⊕ Group D streptococcus
MAC - 2+ slow LF - see BAP - same

#4 BAP - 1+ white gpc cat ⊕ coag ⊕ Staphylococcus sp.
1+ yellow gpc cat ⊕ coag ⊖ Staphylococcus sp.
1+ gray small gpr - cat ⊖ prob lactobacillus sp.
1+ gray mucoid - A/A LIA = K/A ⊕ U +r.w MIO + - + prob Ent. cloacae
MAC - 1+ slow LF - same as BAP

LAB NO. 040

RESULTS

ARC DIAGNOSTIC LABORATORY

#5 BAP - 1+ white - same morph. as # 40-1 prob. Staph sp.
1+ small grey cat ⊖ same morph. as 40-2 prob lactobacilli
MAC - No growth

#6 BAP - 2 colonies (+) mucoid - same as MAC
2+ small white - gpc cat ⊖ BE ⊕ Group D streptococcus
MAC - 1 col. LF - A/A, K/K E ⊖ U ⊖ MO +++ - prob. E. coli

#7 BAP - 1+ white cat ⊕ gpc - prob. Staph. sp.
3+ clear sm cat ⊖ gpc - prob. lactobacillus sp.
1+ raised white gpc cat ⊕ coag ⊕ Staphylococcus sp.
1+ small grey gpc cat ⊖ BE ⊖ nonhemolytic strep not Group D
MAC - No growth

#8 BAP - 3+ white Nonhemolytic cat ⊕ gpc - prob. Staph sp.
1+ gray mucoid - see MAC
3+ gray gpc cat ⊖ BE ⊕ Group D streptococcus
MAC - 3+ slow LF API 3305572 - Ent. cloacae

#9 BAP
MAC } No growth

#10 BAP - 1+ white gpc, slight Beta coag ⊕ Staphylococcus sp.
MAC - NG

REG 3/26/86

RESULTS

ARC DIAGNOSTIC LABORATORY ELISA REPORT

LAB NO. 040

TEST: MRM
Positive Control: + Lot: A6056
Negative Control: — Exp.: 5/86

Animal #	Result
1	—
2	QNS
3	—
4	—
5	—
6	—
7	—
8	—
9	—
10	—

TEST: MVM
Positive Control: + Lot: 06751
Negative Control: — Exp.: 3/86

Animal #	Result
1	QNS
2	QNS
3	—
4	QNS
5	—
6	—
7	QNS
8	QNS
9	—
10	—

TEST: MHV
Positive Control: + Lot: 07173
Negative Control: — Exp.: 5/86

Animal #	Result
1	—
2	QNS
3	—
4	—
5	—
6	—
7	—
8	—
9	—
10	—

TEST: PVM
Positive Control: + Lot: A6392
Negative Control: — Exp.: 5/86

Animal #	Result
1	—
2	QNS
3	—
4	—
5	—
6	—
7	—
8	—
9	—
10	—

TEST: Sendai
Positive Control: + Lot: 07970
Negative Control: — Exp.: 12/86

Animal #	Result
1	—
2	QNS
3	—
4	—
5	—
6	—
7	—
8	equivocal
9	—
10	—

TEST: GD7
Positive Control: + Lot: 07768
Negative Control: — Exp.: 11/86

Animal #	Result
1	—
2	QNS
3	—
4	—
5	—
6	—
7	—
8	—
9	—
10	—

TEST: Ro 3
Positive Control: + Lot: 06733
Negative Control: — Exp.: 5/86

Animal #	Result
1	—
2	QNS
3	—
4	QNS
5	—
6	—
7	QNS
8	QNS
9	—
10	—

Initial: a Ef

Date: test performed 3/20/86

HEALTH EVALUATION

HISTOPATHOLOGY

ARC Lab. # C9-40 Histo Lab. # 286-1084

Animal #	1	2	3	4	5	6	7	8	9	10
Lung	NSL	NSL	NSL	NSL	NSL	NSL	(1)	NSL	NSL	(2) ~~NSL~~
Trachea	NSL	NE	NSL	NSL	NSL	NSL	NE	NSL	NSL	NE
Harderian Gl.	NE	→								
Salivary Gl.	NE	→								
Submand. L.N.	NE	→								
Heart	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Liver	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Kidney	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Ileum	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Colon	NE	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL

NSL = No Significant lesion

NE = tissue not submitted or didn't make it on slide

(1) Frequent perivascular cuffing with mixed inflammatory cells.

(2) Occasional slight perivascular cuffing with mixed inflammatory cells. SCR 3/22/86

Lung lesions in 7 and 10 are of some concern. Should do more histo/path and viral antibody tests on additional mice from that room if available. The mice are cell-released for study that the room (group) that 7 and 10 are from should remain on quarantine status until more checks are examined. 3/22/86 SCR

#8 exhibited reaction to Sendai by ELISA. No Sendai like lesions in #8 so presumably this is a false reaction. However, follow up, particularly for Sendai and PVM is indicated on the basis of reactions in #7 and #10.

SCR 3/22/86

ARC DIAGNOSTIC LABORATORY ELISA REPORT

INVESTIGATOR Hackett
EXPERIMENT Hexane Sperm Morphology
COST CODE —
BUILDING LSL II
PEN. ROOM

LAB NO. 080
DATE 4/29/86
ANIMAL OR SHIPMENT NO. 860034
SOURCE CR-~~Raleigh~~ Portage O wrong nomenclature REC'D 2-25-86
SPECIES & STRAIN mice B6C3F1
SEX male AGE BD-1-2-86

TESTS REQUESTED

10 blood specimens submitted at terminal sacrifice for viral serology.

SEROLOGY RESULTS

TEST: MHV lot #07173 exp. 5/86
Positive Control: +
Negative Control: —

Animal #	Result
1	—
2	—
3	—
4	—
5	—
6	—
7	—
8	—
9	—
10	—

TEST: GD 7 lot 07768 exp 11/86
Positive Control: +
Negative Control: —

Animal #	Result
1	—
2	—
3	—
4	—
5	—
6	—
7	—
8	—
9	—
10	—

TEST: Sendai lot 07970 exp. 12/86
Positive Control: +
Negative Control: —

Animal #	Result
1	—
2	—
3	—
4	—
5	—
6	—
7	—
8	—
9	—
10	—

TEST: PVM Lot 08190 exp 2/87
Positive Control: +
Negative Control: —

Animal #	Result
1	—
2	—
3	—
4	—
5	—
6	—
7	—
8	—
9	—
10	—

All tests negative - MHV, GD VII, Sendai, PVM, and Mycoplasma pul. SEN 5/29/86

test performed 5/13/86 AEJ
Comm to Hackett

RESULTS

ARC DIAGNOSTIC LABORATORY ELISA REPORT

LAB NO. 080

TEST: mem

Positive Control: + Lot: 08489

Negative Control: − Exp.: 2/87

Animal #	Result
1	−
2	−
3	−
4	−
5	−
6	−
7	−
8	−
9	−
10	−

TEST: _____

Positive Control: _____ Lot:

Negative Control: _____ Exp.:

Animal #	Result

TEST: _____

Positive Control: _____ Lot:

Negative Control: _____ Exp.:

Animal #	Result

TEST: _____

Positive Control: _____ Lot:

Negative Control: _____ Exp.:

Animal #	Result

Initial: AEJ

Date: test performed 5/29/86

SEP 5/29/86

APPENDIX E

QUALITY ASSURANCE STATEMENT

SPERM MORPHOLOGY STUDY OF B6C3F1 MICE
EXPOSED TO n-HEXANE

Quality Assurance Statement

Listed below are the phases and/or procedures included in the study described in this report which were reviewed by the Quality Assurance Unit during the period, 2/15/86 -5/31/86, specifically for this study and the dates the reviews were performed and findings reported to management. (Findings were reported to the study director or his designee at the time of the review.)

Phase/Procedure Reviewed	Review Date	Date Findings Submitted in Writing to Study Director/Management
Animal Receipt	2/25/86	3/03/86
Health Screen	3/17&18/86*	3/20/86
Animal Identification	3/19/86*	3/20/86
Body Weights	3/19/86*	3/20/86
Dosing	3/25&27/86*	3/28/86
Data	3/28/86*	4/18/86
Necropsy	4/29/86*	4/30/86
Data	2/03/88*	5/04/88
Data	4/19&20/88*	5/04/88
Draft Report	4/20 & 5/3/88	5/04/88
Final Report	8/22/88	8/22/88

* Reviewed specifically for this study.

R. A. Gelman
Quality Assurance Specialist

8/22/88
Date

APPENDIX F

PROTOCOL AND CAGE MAPS

PROTOCOL FOR INHALATION REPRODUCTIVE STUDIES
n-HEXANE

I. TITLE

Sperm Morphology Study of B6C3F1 Mice Exposed to n-Hexane.

II. PURPOSE OF STUDY

The straight-chain hydrocarbon, n-hexane, is commonly used as a solvent for the extraction of oil seeds, as a reaction medium in the production of polyolefins, elastomers and pharmaceuticals, and as a component of quick-drying cements, lacquers and adhesives. The production of n-hexane, which was estimated to be four billion pounds per year in 1979, utilizes stocks of straight-run gasoline and higher boiling liquid products stripped from natural gas or paraffinic fractions of refinery streams. It is also found as a minor component of gasoline and its combustion products, hence petroleum products are a major source of environmental hexane contamination. Due to the large-scale production and widespread use of hexane, including teaching laboratories, the opportunity for industrial, incidental environmental, or volitional (glue-sniffing) exposure to hexane vapors is significant. The studies described herein are proposed as a result of a concern that this exposure may result in a negative impact on human reproductive function.

Several excellent reviews concerning hexacarbon toxicity and metabolism are available in Experimental and Clinical Neurotoxicology (edited by Spencer and Schumburg, 198Ø) and in CRC Critical Reviews in Toxicology (Spencer, Schaumburg, Sabri, and Veronesi, 198Ø). In summary, polyneuropathies have been reported following exposure of workers to n-hexane contained in adhesives or used as an industrial solvent as well as following repeated exposure by glue sniffing. A metabolite, 2,5-hexanedione, has been shown to be responsible for most, if not all, of the neurotoxicity.

Pharmacokinetic and distribution studies indicated that the hexane saturation concentration of organs following inhalation is directly proportional to their lipid content, and that blood contains more hexane in relation to its lipid content than do organs (Andersen, 198l; Bohlen et al., 1973). Baker and Rickert (1981) found that metabolism and elimination of n-hexane were dependent upon exposure concentration, but that the tissue concentration of the metabolite, 2,5-hexanedione, was not directly related to n-hexane exposure concentration. Bus et al. (1982), using 6-hour exposures to ^{14}C-labeled n-hexane, found that the distribution of radioactivity was dose-dependent.

Although myelinated nerve tracts are the primary target organ, the testes have been identified as being sensitive to hexacarbon toxicity. Krasavage et al. (198Ø) reported testicular atrophy following oral administration of n-hexane and several of its metabolites. Chapin et al. (1982) administered a 1% solution of the hexane metabolite, 2,5-hexanedione, to male rats in their drinking water and found a decrease in the activity of two Sertoli cell enzymes, b-glucronidase and g-transferase, after 3 weeks of exposure. No morphologic changes were detected at this time; however, after 6 weeks of exposure the testes were essentially azospermic. The few primary or secondary spermatocytes that were observed exhibited severe degenerative changes. Since circulating levels of testosterone and the gonadotropins remained normal throughout the study, these workers concluded that 2,5-hexanedione does not act via the central gonadotropin control systems to induce azospermia and that demonstrable changes in Sertoli cell biochemistry precede visible morphologic changes in the testes.

Cavender et al. (1984) were unable to detect neurotoxicity or testicular toxicity in rats exposed to purified hexane (99.3%) via inhalation at 1Ø,ØØØ ppm, 6 hr/day for 13 weeks. The possibility of histological or biochemical changes in the testes was not addressed in this study. Although the exposure concentrations used in this study were relatively high, Ø, 3ØØØ, 65ØØ, and 1Ø,ØØØ

ppm, the short exposure periods—6 hr/day—may not have allowed significant buildup of major metabolites in blood and tissues.

Because of apparent differences in the induction of reproductive deficits between males exposed to n-hexane orally and by inhalation, it is important that more sensitive measures be employed to assess the effects of exposure by the latter route. Evaluation of test animals for the presence of dominant lethal mutations and/or changes in sperm morphology may serve as a means of detecting testicular effects produced in males by inhalation exposure. Accordingly, sperm morphology studies will be performed on B6C3F1 mice which have been exposed to Ø, 2ØØ, 1ØØØ, or 5ØØØ ppm n-hexane, 2Ø hr/dy for 5 days, following standard timing and evaluation procedures. Dominant lethal mutation studies will be performed concurrently on CD-1 male mice. As a supplement to the standard tests, the testes of these animals will be preserved at necropsy and supplied to NIEHS for examination.

III. SPONSOR AND SPONSOR'S REPRESENTATIVE

A. Sponsor
National Institute of Environmental Health and Safety
National Toxicology Program (NTP)
P.O. Box 12233; Research Triangle Park, NC 277Ø9

B. Sponsor's Representatives
Dr. Bryan Hardin
Dr. Bernard Schwetz

IV. TESTING LABORATORY

A. Facility
Pacific Northwest Laboratories (PNL)
P.O. Box 999; Richland, WA 99352

B. Study Co-Directors
Dr. Patricia L. Hackett
Dr. Beatrice J. McClanahan
[Dr. Terryl J. Mast][+A]

V. PROPOSED SCHEDULE OF EVENTS (This proposed schedule may be altered. All changes will be appended to the protocol.)

A. Prestart audit for GLP compliance: 3/17/86.
B. The B6C3F1 male mice will arrive the week of 2/24/86.
C. Quarantine will commence upon arrival of the shipment and continue for at least 4 weeks. Health evaluation will proceed within 3 weeks of arrival.
D. Exposure interval: 3/24/86 - 3/28/86.
E. Necropsies will be performed during the week of 4/28/86 (4 weeks post-exposure).
F. Evaluation of data: 4/28/86 - 7/15/86.
G. Completion of draft report: ~~7/3Ø/86~~ [5/15/88][&A]
~~H. Completion of final report: 9/3Ø/86~~ [-A]

VI. TEST SYSTEM

A. Species: mouse.
B. Strain: B6C3F1.
C. Number of Animals and Supplier: 13Ø male animals will be purchased from the Charles River Laboratories, Portage Facility.
D. Age of Animals Upon Arrival: 7-8 weeks.

[+A] Added 5/1/88 by Amendment A.
[&A] Corrected 5/1/88 by Ammendment A.
[-A] Deleted 5/1/88 by Amendment A.

E. Strain: B6C3F1 males.

F. Number of Animals in Study: 2Ø males will comprise each of the four treatment groups exposed to air or one of three levels of n-hexane for 5 consecutive days. A fifth group, consisting of 15 males will serve as a positive control group and will receive 5 daily doses of 2ØØ mg/kg of ethylmethanesulfonate (EMS) by intraperitoneal administration during the same interval as the hexane inhalation exposure. [A sixth group of 15 males was given 250 mg/kg EMS.][+A]

G. Test System Justification: The assay for induction of sperm abnormalities has been developed using mice for many chemicals classified, by means of other assays, as mutagens, carcinogens or teratogens.

VII. TEST SYSTEM HOUSING, HANDLING AND ENVIRONMENTAL CONDITIONS

A. Quarantine and Acclimatization:

1. Upon arrival at PNL, the animals will be quarantined (ØB-AA-3FØ3) for 4-5 weeks in the LSL-II Building.
2. Temperatures in all rooms will be maintained at 72°±3 F and relative humidities at 5Ø±15% during the quarantine, acclimatization and exposure periods. These values will be measured and recorded twice daily.
3. During the quarantine period the animals will be housed singly in wire-mesh cages.
4. Prior to exposure the males will be acclimated for 2-3 days in individual compartments of wire-mesh cages within exposure chambers (with chamber doors open).

B. Feed: NIH-Ø7 Open Formula Diet (pellets) will be provided ad libitum during the acclimation and experimental period. Feed will remain in place during the exposure period and will be changed daily.

C. Water: Water will be supplied ad libitum at all times during the study using an automatic watering system.

D. Randomization: During the week prior to exposure, male mice will be weighed; their weights will be ranked from lightest to heaviest and each animal will be randomly assigned to a treatment group by means of a computer-assisted randomization program which is based on a single blocking factor; body weight (ØB-DT-3BØB).

E. Identification:

1. All experimental male mice will be individually identified by metal ear tags (ØB-DT-3BØ1).
2. Exposure groups will be designated by distinctive toe clipping and by placement within the individual compartments of the chamber cage units (ØB-DT-3BØ1).
3. Cage maps (ØB-DT-3BØ3) showing placement of individual animals in each cage unit of the exposure chamber will be prepared and updated as needed. Each exposure chamber will be identified by chamber number and exposure level. The proposed arrangement of the exposure chambers is included in Attachemnt 2.

G. Animal Disease Screening Program (ØB-AA-3FØ2): Approximately 2-3 weeks after receipt of the animal shipmen t, ten males will be examined for internal and external parasites and bacterial pathogens; their sera will be tested for antibodies to selected pathogens and histopathologic examinations of lung, liver, kidney, ileum, colon and heart will be performed. At necropsy, serum from 5 animals in the control group and 5 from the high dose goup will be tested for antibodies to selected pathogens.

VIII. TEST ARTICLE

A. Chemical name: n-hexane.

B. Formula: $CH_3(CH_2)_4 CH_3$

C. Manufacturer: Phillips Chemical Company

D. Source: Research Triangle Institute, Research Triangle Park, NC.

[+A] Added 5/1/88 by Amendment A.

E. CAS No.: 11Ø-54-3
F. NTP No.: 1Ø189-N
G. LOT No.: RTI log number: 4911-1ØØ-Ø1
PNL 1st Shipment BNW 5Ø846-39
H. Date of Receipt: 1st Shipment 2/12/86
I. Test Article Preparation and Storage Areas: 2-day reserve in rms 311 and 315 LSL-II; the remainder in the Research Technology Laboratory (RTL) chemical storage facility.
J. The vehicle control will be filtered air.
K. Analytical Chemistry

1. Upon receipt, identity and gross purity analyses of the bulk chemcial were performed by infrared spectroscopy; gas chromatography (GC) was used to determine purity by major peak comparison and also to generate an impurity profile (ØB-AC-3A15). Subsequent bulk assays, upon completion of the animal exposures, will use GC to determine test material purity and an impurity profile.

2. n-Hexane concentrations within the exposure chambers will be monitored (ØB-AC-3B1P) using an HP-584Ø gas chromatograph calibrated by the method detailed in ØB-AC-3CØW (see Attachment 2).

IX. DESCRIPTION OF INHALATION EXPOSURE SYSTEM

Inhalation exposure was selected by the sponsor as the route of administration since it is the most commonly encountered route of occupational exposure. The inhalation chambers will be located in room 436 of the LSL-II building. A detailed description of the inhalation exposure system to be used in this study is included in Atttachment #2 of this protocol.

X. EXPERIMENTAL DESIGN AND DOSE LEVELS

A. Experimental Design: Four groups of animals, consisting of 2Ø male mice in each group, will be exposed to air or to the test chemical on 5 consecutive days. Simultaneously, a fifth group of 15 males will be injected (IP) with 2ØØ mg of ethyl methanesulfonate (EMS)/kg daily on five days (ØB-DT-3B14) to serve as a positive control goup. This dose level of EMS is expected to induce excess sperm abnormalities and minimal mortality. All males will be held for 4 weeks and sacrificed during the 5th post-exposure week for sperm morphology evaluations. Previous studies (Wyrobek and Bruce, 1975) have indicated that the peak incidence of sperm abnormalities induced by chemical mutagens is observed at this time during the post-treatment interval.
[A sixth group of 15 males was given 250 mg/kg EMS][+A]
B. Exposure Regimen: Chamber atmospheric concentrations of n-hexane will be Ø (filtered air), 2ØØ, 1ØØØ and 5ØØØ ppm. Male mice will be exposed for 2Ø hr/day for 5 consecutive days. Control mice (Ø ppm) will be housed in an exposure chamber in the same room, and will be handled in the same manner as the mice that are expcsed to the test chemical. The exposure chamber doors will be closed throughout the exposure and nonexposure periods, except during animal care procedures. Exposure chamber temperatures will be maintained at 75±3 °F and relative humidities at 55 ±15%. Air flow will be maintained at 15±33 cfm and the chamber pressure at approximately 1" water negative with respect to the room pressure.

C. Selection of Atmospheric Concentrations: The maximum exposure chamber atmospheric concentration of hexane, 5ØØØ ppm, is 5Ø% of the LEL (lower explosion limit). In order to maximize exposure the expsoure time will be 2Ø hr/day for all doses; exposure concentrations were approved by the Co-Project Officers.

+A Added 5/1/88 by Amendment A.

XI. EXPERIMENTAL OBSERVATIONS

A. Clinical Observations: The animals will be observed once daily for mortality, morbidity, and signs of toxicity. The date and approximate time of death or euthanasia of moribund animals will be recorded and the animals will be necropsied according to ØB-DT-3BØF.

B. Body Weights: The male mice will be weighed during the week prior to exposure, on the first day of exposure, once each week during the four-week holding period, and at necropsy.

C. Scheduled Necropsy: The twenty males in each dose group will be euthanized with CO_2 and necrospied during the fifth postexposure week. At necropsy (ØB-DT-3B12) all males will be weighed and examined for lesions of the reproductive tract and for gross tissue abnormalities. To document the presence of lesions which may be due to chemical exposure, any organs or tissues with lesions will be preserved in neutral buffered formalin (NBF); in this case, comparable organs or tissues from approximately 2Ø% of the control animals will be preserved in NBF; all other tissues will be discarded.

The right cauda epidiymis will be removed and processed to obtain the sperm suspensions. The sperm suspensions will be stained with 1% Eosin Y and smears will be prepared on microscope slides (4 slides/animal). The dried smears will be mounted under coverslips with mounting medium. During microscopic examination of these preparations, the quality of the slides will be noted (qualitative observations on the maount of debris, degree of crystallization, clumping of sperm) and at least 5ØØ sperm from each mouse will be examined for abnormalities (NTP Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice: revised 1Ø/13/83).

One testes and corresponding epididymis from each of these animals will be treated as follows: fixed in 1Ø% buffered formalin (24 hr), transected and transferred to 4% glutaraldehyde in Ø.1 M. cacodylate buffer at pH 7.4 (at least 24 hr), imbedded in glycol methacrylate , cut into 2-micron sections and stained with PAS-hematoxylin. Slides will be sent to Dr. Chapin at NIEHS for evaluation of germinal epithelium.

D. Indices of Effects: The following parameters, expressed as mean ± SE, when appropriate, will be computed from data for males will be presented in the Final Report for each treatment group:
- Number of dead animals, animals removed from the study and reason for removal.
- Summary of toxicity, including incidence of changes detected during clinical observations.
- Body weight of males before and after exposure, on each of the 4 postexposure weeks and at necropsy.
- Number and percentage of normal sperm heads and each observed category of abnormality, such as blunt hook, banana, amorphous, pin-head, two heads, two tails, and short sperm head.
- Summary of qualitative observations of the sperm preparations.

XII. PROSPOSED STATISTICAL METHODS

Body weights will be analyzed by repeated measures analysis of variance (Steel and Torrie, 198Ø). The number sperm (and sperm with morphologic alterations) will be expressed as a percentage of the total cells examined for each animal, transformed by an arcsin transformation and analyzed by an analysis of variance. Orthogonal contrasts will be employed to test for dose response trends (Winer, 1971).

XIII. STORAGE OF STUDY MATERIAL

All raw data and study records will be retained in the Project Office (room 1519); all tissues and slides will be termporarily stored in the Teratology Laboratory (room 1428). Both of these rooms are located in Life Sciences Laboratory II, Battelle, Pacific Northwest Laboratories. All tissue specimens will be shipped to the NTP Archives. Records generated in the conduct of the study will be microfiched. Computer tapes of biological data, the original and one copy of the microfiche, and the microfiche index will be sent to Dr. Schwetz (NIEHS) for storage in the NTP Archives. One copy of the microfiche and the microfiche index will be sent to Dr. Hardin (NIOSH). The Quality Assurance Unit at PNL will retain the following materials:

- Bound PNL laboratory notebooks, which are required to remain at PNL.
- QAU master schedule and audit records.
- Personnel training and experience records and job descriptions for persons participating in the study is sent to NTP archives.
- Maintenance and calibration records of equipment used on the study. (Exception: if the equipment is government-owned, the records would accompany the equipment.)

XIV. RECORDS RETENTION

The following records, generated during the course of the study, will be maintained at PNL until they are shipped to the NTP archives. Some of these records may be presented in the protocol or in study reports.

A. Personnel Records:
   1. Current professional resume and job description for each person recording data.
   2. Safety Training records, including respirator and hazardous material, and specific-task training records.
   3. Accident/injury reports for personnel in contact with the test material or test system.
   4. Record of removal of any individual, because of illness, from direct contact with the test system.

B. Study Protocol:
   1. Study protocol prepared prior to the initiation of the study and approved by the PNL Study Director(s), the PNL QAU Officer and the NTP Project Officer(s).
   2. All amendments to the study protocol resulting from modifications in the study or time schedule.
   3. A record of any deviations from the protocol and corrective actions taken.

C. Equipment Records:
   1. Title(s) of person(s) assigned to clean, inspect, and maintain equipment.
   2. Schedule for cleaning, calibrating, inspecting and maintaining equipment.
   3. Documentation of routine cleaning, inspection, calibraiton, and maintenance of equipment.
   4. Documentation of any nonroutine maintenance:
      - Description of malfunction

- Description of remedial action.

D. Test Materials Records:
1. Test materials identity records including manufacturer, quantity, lot number(s) and purity grade.

2. Records from NTP analytical contractor concerning characterization, bulk stability and shipment.

3. PNL records for receipt and storage of material, including storage conditions.

4. PNL records for bulk analysis and degradation.

5. PNL records of inventroy, usage and shipment of unused test material to the NTP repository.

F. Animal records:
1. Animal receiving records including supplier, species, strain, birth week, sex, number of animals of each sex, receiving date and condition upon receipt.

2. Health evaluation records of findings, written release from quarantine/acclimatization or reasons for refection for use in the study and results of serologic examination at sacrifice.

3. Housing records for quarantine, acclimation, mating and exposure to the test material, including room location, temperature, relative humidity, lighting cycle, caging type, number of animals per cage, location of chambers within the exposure room, cage assignment of individual animals within the exposure chamber and sanitation procedures (frequency and methods of cage and rrom cleaning/sterilization).

4. Feed records of commercial source and product information (feed tags, lot numbers and milling dates), analyses and mode and frequency of feeding.

5. Records of mode and frequency of watering, annual analysis and weekly water hardness tests (records are maintained in offices of the building engineer or building manager.

6. Animal disposition records.

G. Study Implementation and Conduct Records:
1. Records of assignment of animals to treatment groups.

2. Body weights.

3. Dates of exposure intervals for individual animals.

4. Daily observations.

5. Time of death/euthanasia of animals occurring prior to scheduled sacrifice and results of gross necropsy.

6. At scheduled sacrifice, gross necropsy findings in male animals and results from microscopic examinations of sperm preparations.

H. All relevant correspondence.

I. Reports:
   1. Literature survey and recommendations for studies.

   2. Monthly progress reports.

   3. Draft final and final reports.

J. Internal Computer Generated Forms and Tables:
   1. Study data and statistical analyses.

   2. Analytical data.

   3. Exposure suite control center computer printouts.

K. Standard Operating Procedures: The list of SOP's to be used in this study appears in Attachment 1. A file of these SOP's is maintained in the QAU office.

L. Health and Safety Records:
   1. NTP safety and toxicity package.

   2. PNL Biohazard Protocol and Health and Safety Plan.

   3. Personnel repirator and hazardous material training records: accident/injury reports.

   4. Monitoring records of ventilation system, hoods and exhaust systems used in this study.

   5. Relevant sections of the Health and Safety Monthly Progress Reports.

   6. NTP site visit reports, attention items and related correspondence concerning health and safety.

XV. OTHER SPECIFICATIONS

A. This study will be performed in compliance with the FDA Good Laboratory Practice Regulations for Non-Clinical Laboaratory Studies (21 CFR 58).

B. This Protocol will be the controlling document in case of discrepancies between the Protocol and SOP's. If discrepancies are noted, the Study Director is to be notified immediately to resolve and document the variance between the Protocol and SOP.

XVI. HEALTH AND SAFETY

PNL's Health and Safety Plan, which has been submitted for NTP approval, is detailed in ØB-HS-3S1C. In addition, a repiratory program is outlined in ØB-HS-3S1B. This is supplemented by an SOP (ØB-HS-3S19) which covers the use of supplied-air respirators which will be worn by personel during periods of animal care while the chambers are open, and by an SOP (ØB-HS-3S1A) which covers the use of a self-contained breathing apparatus for use when entering a room under emergency conditions following an accidental release of the chemical.

Personnel training, protective equipment and facilities are designed to conform with DOE health and safety requirements and with Health and Safety Minimum Requirements for Laboratories under Contract to the NTP Systemic Toxicology Branch, dated November 19, 1984 and consisting of a basic document of eight pages, Appendix I of ten pages and Appendix II of two pages.

XVII. APPROVAL BY PNL

R L Hackett Date: 3/4/86
Co-Study Director

B J McClanahan Date: 3/4/86
Co-Study Director

R. A. Gelman Date: 3/5/86
Quality Assurance Auditor

XVIII. APPROVAL BY NTP

B A Schwetz Date: 20 Mar 86
Co-Project Officer

Bryan D Hardin Date: 21 March 1986
Co-Project Officer

XIX. REFERENCES

Andersen, M.E. 1981. Pharmacokinetics of inhaled gases and vapors. Neurobehavioral Toxicology and Teratology 3: 383-389.

Armitage, P. 1955. Tests for linear trends in proportions and frequencies. Biometrics 11: 375-386.

Baker, T.S. and D.E. Rickert. 1981. Dose-dependent uptake, distribution, and elimination of inhaled n-hexane in the Fischer-344 rat. Toxicol. Appl. Pharmacol. 61: 414-422.

Bohlen, P., U.P. Schlunegger and E. Lauppi. 1973. Uptake and distribution of hexane in rat tissues. Toxicol. Appl. Pharmacol. 25: 242-249.

Bus, J.S., D. Deyo and M. Cox. 1982. Dose-dependent disposition of n-hexane in F-344 rats after inhalation exposure. Fund. and Appl. Toxicol. 2: 226-229.

Cavender, F.L., H.W. Casey, H. Salem, D.G. Grahem, J.A. Swenberg and E.J. Gralla. 1984. A 13-week vapor inhaltion study of n-hexane in rats with emphasis on neurotoxic effects. Fund. App. Toxiccol. 4: 191-201.

Chapin R.E., R.M. Norton, J.A. Popp, and J.S. Bus. 1982. The effects of 2,5-hexanedione on reproductive hormones and testicular enzyme activities in the F-344 rat. Toxicol. Appl. Pharamcol. 62: 262-272.

XX. CHANGES AND/OR REVISIONS TO THE PROTOCOL

Revision A:

Page2 IV.
Add Dr. Terryl J. Mast as Study Co-Director.
Reason: See attached memo.

Page 2 V.
Change completion date of Draft Report to 5/15/88 and delete V.H since it is an unknown quantity.
Reason: To correct calender dates.

Page 3.VI.
Add a sixth group as a second positive-control group, 250 mg/kg EMS.
Reason: Requested by Project Officers.

Page 4. X.
Add a sixth group as a second positive-control group, 250 mg/kg EMS.
Reason: Requested by Project Officers.

Attachment 2. Page 7. D.2
Reprinted percent relative humidity formula to clarify.

Terryl J. Mast 5/1/88

Krasavage, W.J., J.L. O'Donoghue, G.D. DiVincenzo and C.J. Terhaar. 198Ø. The relative neurotoxicity of methyl-*n* -butyl ketone, n-hexane and their metabolites. Toxicol. Appl. Pharmacol. 52: 433-441.

NTP. 1983. NTP Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice (Revised 1Ø/31/83).

Spencer, P.S., D. Couri and H.H. Schaumburg. 198Ø. n-Hexane and methyl n-butyl ketone. In Experimental and Clinical Neurotoxicology, P.S. Spencer and H.H. Schaumburg J. (eds.), Williams & Wilkins, Baltimore, MD, pp. 456-475.

Spencer, P.S., H.H. Schaumburg, M.I. Sabri and B. Veronesi. 198Ø. The enlarging view of hexacarbon neurotoxicity. CRC Critical Reviews in Toxicology 3: 279.

Steel, R.D.G. and J.H. Torrie. 198Ø. Principles and Procedures of Statistics, McGraw-Hill, New York, NY.

Winer, B.J. 1971. Statistical Principles in Experimental Design, McGraw-Hill, New York, NY

Wyrobek, A.J. and W.R. Bruce. 1975. Chemical induction of sperm abnormalities in mice. Proc. Nat. Acad. Sci. 72, 4425-4429.

ATTACHMENT 1

STANDARD OPERATING PROCEDURES FOR INHALATION
REPRODUCTIVE TOXICOLOGY STUDIES

STANDARD OPERATING PROCEDURES FOR INHALATION
REPRODUCTIVE TOXICOLOGY STUDIES

EXPOSURE SYSTEM	
CDS DMM Card Calibration	ØB-BE-3CØT
Bubbler Sample Collection via the Critical Orifice Sample System	ØB-BE-3CØQ
Inhalation Exposure Chamber Balance	ØB-BE-3B24
Model 1 Chamber Leak Tester	ØB-BE-3DØ6
Calibration and Check of Chamber Airflow Using Digital Anemometer	ØB-BE-3CØV
Digital Anemometer Calibration	ØB-BE-3CØS
Dwyer Manometer Calibration Check	ØB-BE-3CØX
Validyne Pressure Transducer Calibration	ØB-BE-3CØW
Filling Out Data Sheets	ØB-BE-3BØ7
EG&G Hygrometer: Operation, Maintenance and Calibration	ØB-BE-3CØJ
Relative Humidity Determination Via Use of Dewpoint Hygrometer	ØB-BE-3B1X
Exposure Suite Computer Program Documentation	ØB-BE-5EØ1
Exposure Suite Data Analysis Program Documentation	ØB-BE-5EØ3
Exposure Suite Data Analysis Program Operation	ØB-BE-3EØB
Exposure Suite Routine Computer Operation	ØB-BE-3GØ4
Exposure Suite Routine Data Disk Operation	ØB-BE-3EØA
Software Change Protocol	ØB-BE-5EØ2
Study Protocol Entry into Exposure Suite Computers	ØB-BE-3EØ9
Exposure Suite Emergency Evacuation Procedure	ØB-BE-3SØ1
Exposure Suite QC, Maintenance and Calibration	ØB-BE-3DØE
Selection of RTD's and Digital Thermometer Calibration	ØB-BE-3CØD
Omega RTD Thermometer Calibration	ØB-BE-3CØL
ERDCO FGD Maintenance & Calibration	ØB-BE-3CØU
Flammable Gas Detector (ERDCO) Checkout Prcedures	ØB-BE-3CØB
Hexane Exposure System Daily Operating Procedure	ØB-BE-3B2Y
Hexane Exposure System Quality Control, Maintenance and Calibration	ØB-BE-3DØM

ANALYTICAL CHEMISTRY AND MONITORING	
Operation of HP584Ø Gas Chromatograph for Monitoring n-Hexane in Inhalation Chamber	ØB-AC-3B1P
Calibration of n-Hexane Inhalation Chamber Monitor	ØB-AC-3CØW
Bulk Chemical Analysis of n-Hexane	ØB-AC-3A15
Use of Mettler H51 Analytical Balance	ØB-AC-3BØP
Special Operating Procedure for Care and Use of Volumetric Glassware	ØB-AC-3BØR
Use of Pipets	ØB-AC-3BØS
Ordering, Receipt, Recording Use and Returning Chemicals	ØB-AC-3EØ5
Labeling of Reagents and Chemicals	ØB-AC-3B12

Operation of Toledo Scale (Model 2120) to Weigh Large Containers of Test Material	ØB-AC-3B1A
ANIMAL RESOURCE CENTER	
Job Orientation and Training	ØB-QA-3BØ7
Barrier Procedures for LSL II Animal Facility	ØB-AR-3BØG
Operation and Maintenance of the Clean Corridor Area	ØB-AR-3BØ5
Operation of the Regulated Corridor	ØB-AR-3B1Y
Operation and Maintenance of the Street Corridor	ØB-AR-3BØ6
Moving Animals from LSL II Animal Resources Center	ØB-AR-3BØN
Management of Animal Feed	ØB-AR-3FØ5
Pre-cleaning Equipment and Operation of Cage, Bottle and Rack Washers	ØB-AR-3GØ1
Operation of Steam, Gas and Bulk Sterilizers	ØB-AR-3GØ2
Kaye Digistrip-III Room-Temperature Recorder	ØB-AR-3GØ3
Operation of Garb-El Waste Disposal	ØB-AR-3GØ4
Operation of Clark-A-Matic Floor Scrubber	ØB-AR-3GØ5
Operating Procedures for Pathological Incinerator	ØB-AR-3GØ7
Calibration/Service of Balances	WØ-SL-3CØ1
Biweekly Deep Cleaning of Exposure Rooms and Occupied Animal Rooms	ØB-AR-3HØ1
Deep-Cleaning and Sanitizing Empty Animal and Exposure Rooms	ØB-AR-3HØ3
Processing Laundry for the LSL II Animal Facility	ØB-AR-3BØ7
Sanitizing Operations Monitoring	ØB-AR-3HØA
Handling and Changing Out Exposure Chamber and Cage Units	ØB-AR-3BØ3
Handling, Changing and Storage of Animal Cages and Racks	ØB-AR-3BØD
Cage and Rack Change-Out and Rotatation for LSL II	ØB-AR-3B1U
Changing Out Racks Having Individual-Compartment Cage Units	ØB-AR-3B1V
Pre-exposure Health Screening for Rodents	ØB-AR-3FØ2
Quarantine of Animals	ØB-AR-3FØ3
Daily Care of Bioassay Animals and Cleaning of Exposure Rooms	ØB-AR-3FØA
Daily Care of Rodents Housed in Cage Units and Cleaning of Animal Holding or Exposure Rooms	ØB-AR-3FØN
Daily Care of Animals Housed in Holding Cages and Cleaning of Animal Holding Rooms	ØB-AR-3BØC
Handling Escaped Small Animals	ØB-AR-3BØ8
Determination of Ammonia Levels Within the Exposure Chambers	ØB-AR-3AØ1
Handling of Animal Death Records and ARC Daily Observation Records	ØB-AR-3FØ6
Moribund Sacrifice	ØB-AR-3FØB
Weighing Rodents with Toledo Semi-Automatic Weighing System Using the 733 ASR Terminal	ØB-AR-3GØ6
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	
Identification of Animals	ØB-DT-3BØ1
Cage Location Maps and Daily Observations	ØB-DT-3BØ3

Randomization of Animals	ØB-DT-3BØB
Animal Body Weights	ØB-DT-3BØC
Rodent Mating Procedures	ØB-DT-3BØD
Necropsies for Health Evaluation and of Dead and Moribund Animals	ØB-DT-3BØF
Positive Control Treatment for Sperm Morphology Studies	ØB-DT-3B14
Sacrifices for Sperm Morphology Study	ØB-DT-3B13
Photography	ØB-DT-3BØJ
Data Acquistion and Transfer with a Microcomputer	ØB-DT-3BØK
Data Handling and Storage	ØB-DT-3BØL

HEALTH AND SAFETY

Biohazard Protocol n-Hexane	ØB-HS-3S1S
Bioassay Studies: Health and Safety Plan	ØB-HS-3S1C
The 3M Brand W-2869 Hardcap, Continuous-Flow Air-line Respirator	ØB-HS-3S19
Scott-Presur Pak II Self-contained Breathing Apparatus	ØB-HS-3S1A
Bioassay Respiratory Protection Program	ØB-HS-3S1B

ATTACHMENT 2

DESCRIPTION OF THE EXPOSURE SYSTEM FOR INHALATION REPRODUCTIVE TOXICOLOGY STUDIES

CONTENTS

Page

INHALATION EXPOSURE SYSTEM DESCRIPTION. . . . . .

A. ANIMAL EXPOSURE CHAMBER. . . . . .

B. EXPOSURE SUITE CONTROL CENTER. . . . . .

C. TEST ARTICLE GENERATION AND MONITORING . . . . . .

1. Hexane Generation System. . . . . .
2. Test Article Concentration Monitoring . . . .
3. Explosive-Level Detector. . . . . .

D. ENVIRONMENTAL MONITORING . . . . . .

1. Temperature Measurements. . . . . .
2. Relative Humidity (RH) Measurements . . . . .
3. Chamber Air-Flow Measurements . . . . . .
4. Chamber Vacuum Measurements . . . . . .

E. ENVIRONMENTAL CONTROLS . . . . . .

1. Animal Facility Air-Handling System . . . . .
2. Animal Room Air-Handling System . . . . . .
3. Chamber Relative Humidity (RH) Control. . . .
4. Chamber Air-Flow Control. . . . . .
5. Chamber Temperature Control . . . . . .

F. CHAMBER EXHAUST WASTE TREATMENT. . . . . .

G. DATA HANDLING. . . . . .

H. EQUIPMENT OR POWER-FAILURE PROTECTION SYSTEM . . .

I. REFERENCES . . . . . .

INHALATION EXPOSURE SYSTEM DESCRIPTION

A. ANIMAL EXPOSURE CHAMBER

The Battelle-designed stainless steel chamber (U.S. Patent #4,216,741) available from Hazleton Systems, Inc., Aberdeen, MD, is used for inhalation exposures (Figure 1A). The total volume of the chamber is 2.3 m^3, the chamber has an active mixing volume of 1.7 m^3, the remainder being the non-mixing inlet and exhaust volumes. There are three levels of caging, each level split into two tiers which are offset from each other and from the chamber walls (Figure 1B). Drawer-like, stainless steel cage units composed of individual animal cages, are suspended in the space above each tier. Stainless steel catch pans for collection of urine and feces are suspended below each cage unit. Catch pans are left in position during each exposure period. Instructions for maintenance of these chambers is detailed in SOP# ØB-BE-3DØ6.

The chamber was designed so that uniform aerosol or vapor concentrations can be maintained throughout the chamber when the catch pans are in position. Incoming air containing a uniform mixture of test material is diverted so that it flows vertically along the inner surfaces of the chamber. Waves are formed (Figure 1B) at each tier as the aerosol or vapor flows past the catch pans. Stagnant zones that would normally exist above each pair of catch pans are cleared by exhaust flow through the space between the tiers. Aerosol or vapor reaching the lowest level is deflected across the bottom tiers by metal strips in the space between the catch pan and wall. Tests have shown that aerosol or vapor concentrations uniform to within 8% throughout the chamber can be obtained repeatedly provided the aerosol or vapor is uniformly mixed before passing through the chamber inlet.

Rats and mice are housed in individual cages having feed troughs and automatic watering. During exposure the feed will be removed from each cage unit. The floor area of an individual mouse cage is 106 cm^2 and of a rat cage 270 cm^2 (representing dimensions 14.0 cm by 7.6 cm with height 15.0 cm, and 27.9 cm by 9.7 cm with height 20.0 cm, respectively). There are 60 mice or 24 rat individual cages per cage unit. Up to six cage units can fit in a chamber.

B. EXPOSURE SUITE CONTROL CENTER

A computer located in the Suite Control Center interfaces with system monitors and provides control of the basic functions (e.g., chamber air flow, test chemical concentration, vacuum, and relative humidity) in three exposure rooms (Figure 2). The arrangement of computer control and interface instrumentation is shown in Figure 3. The executive computer is an Hewlett Packard Model (HP) 9816. All data acquisition and automated system control originates from this computer. All experimental protocols related to the data acquisition and control system (such as data channel assignments, monitoring frequencies, and alarm settings) reside in the executive computer and are entered into tables accessed by menus.

Data input to the executive computer is accomplished through several interface instruments. All gas chromatographic (GC) data is collected and preconditioned by Hewlett Packard Model 85B computers, one for each of the exposure rooms. Conditioned data is transferred to the executive computer for analysis, storage, printing and concentration control. Up to two GCs can be attached to each HP85B computer. Data from all monitoring equipment other than the GCs are inputted through a Colorado Data Systems (CDS) Model 53A-IbX Intelligent Interface System.

System control is provided from the computer by means of control relays in the CDS Intelligent Interface System. These relays control such devices as valves, drive motors, audible alarms, indicator lamps, etc.

A complete description of the software for this system is contained in document ØB-BE-5EØ1. Maintenance of the system is detailed in SOP #ØB-BE-3DØE. Routine operation of the computer system is detailed in SOP #ØB-BE-3GØ4. Routine daily operation of the system hardware is detailed in SOP #ØB-BE-3B2Y.

C. TEST ARTICLE GENERATION, MONITORING

1. Hexane Vapor Generation System

A Schematic diagram of the hexane vapor generation and delivery system is shown in Figure 4. Most of the hexane generator system will be enclosed within a vented cabinet locatd in the Exposure Suite Control Center. The hexane to be vaporized will be contained in an 19 liter stainless steel reservoir. This reservoir will be filled daily from the original shipping container by the following method which is designed to prevent explosion during transfer. All oxygen in the reservoir will be displaced with nitrogen. A vacuum will be applied to the reservoir to suck hexane through an eductor tube placed in the shipping container into the reservoir. All metal containers will be properly grounded. Transfer will take place in a vented vapor hood and the filled reservoir will then be transferred and installed into the generator cabinet.

During exposure the hexane will be pumped from the reservoir through a stainless steel eductor tube and delivery tubes to vaporizers located at the fresh air inlet of each animal exposure chamber. Stable micrometering pumps with adjustable drift-free pump rates ranging from less than 1×10^{-3} to greater than 20 ml per minute will be used.

The vaporizer (Figure 5) comprises a stainless steel cylinder covered with a glass fiber wick from which the liquid is vaporized. The wick can be easily and inexpensively replaced if necessitated by residue buildup. An 80-watt heater and a temperature sensing element are incorporated within the cylinder and connected to a remotely located temperature controller. A second temperature monitor is incorporated in the vaporizer allowing the operating temperature to be recorded by the automated data acquisition system. The operating temperature of the vaporizer will be maintained below 50°C (the boiling point of hexane is about 70°C). The cylindrical vaporizer will be positioned in the fresh air duct leading directly to the inlet of the exposure chamber.

A clear teflon® tube of measured volume, preceded by a three-way valve will be attached just upstream of the pump to facilitate measurement of the liquid flow rate of the vapor generator. Measurement will be accomplished by momentarily switching the three-way valve from the run position to the test position. A small bubble of air will be pulled by the pump from the cabinet through the valve and into the clear tube. The progress of this bubble from one end to the other of the tube (calibrated volume) will be timed with a stop watch. Flow rate will be calculated by dividing the volume by the time. The concentration in the exposure chamber can be calculated from the flow measurements of liquid and dilution of air.

All generation equipment which comes in contact with the hexane will be atainless-steel, teflon® or viton®. All equipment contained in the vented generator cabinet will be explosion proof.

Detailed operating instructions for this system are contained in SOP's ØB-BE-3B2Y and ØB-BE-3DØM.

2. Test Article Concentration Monitoring

An HP Model 5840 gas chromatograph with a flame ionization detector (FID) will be used to monitor the exposure chambers, the control chamber, the exposure room and a hexane standard gas. Sampling from multiple positions will be accomplished by means of an automated multiplexed eight-port sampling valve. The sampling system (Figure 6) is incorporated into the relative humidity (RH) sampling system. Samples of the atmosphere from each sample location are continuously drawn by a vacuum pump through polytetrafluoroethylene-lined, stainless-steel sample lines to a location near the input to the eight-port sample valve. This assures fresh samples at the monitor. The sample lines, which continue from the point where they "T" off to the eight-port valve to the dew point monitor, are polytetrafluoroethylene.

Sample values are accumulated and printed by an HP model 85B computer until samples from all eight ports of the sample valve have been measured. These values are then sent to the executive computer for printing and storage. As each value is sent to the HP 85B, it is compared with limit values for that particular location. If the value is beyond the control limits, the HP 85B will immediately send the information to the executive computer, which will then take the appropriate action as follows:

- Concentration ≥ non-critical low limit and ≤ non-critical high limit:

  No action

- Concentration < non-critical low limit but ≥ critical low limit:

  Increase concentration by decreasing chamber air flow.

- Concentration < critical low limit:

  Increase concentration by decreasing chamber air flow and activate audible alarm.

- Concentration > non-critical high limit but ≤ critical high limit:

  Decrease concentration by increasing chamber air flow.

- Concentration > critical high limit:

  Turn off generation system and activate audible alarm.

The monitor will be calibrated by quantitative analysis of grab samples. Additionally, the operation of the chamber-monitoring gas chromatograph will be checked daily against an on-line standard. This check provides a measure of day-to-day instrument drift. Additional calibration checks with grab samples will be performed to check the monitor calibration when drift of the on-line standard response factor is detected. Under normal circumstances, the calibration check will be performed once monthly (SOP #ØB-AC-3CØW).

Daily operating procedures for the concentration monitoring system are contained in SOP #ØB-AC-3B1P. Routine maintenance of the gas chromatograph is covered in SOP #ØB-AC-3DØ2.

The uniformity of the distribution of test chemicals in the chamber will be checked before the start of the study following SOP #ØB-BE-3B24.

3. Explosive-Level Detector

Figure 6 shows the explosive-level detection system. Sample lines from all chambers containing test chemicals "T" off from the chamber sample stream to the dewpoint hygrometer. Equal sample rates from each of these lines are controlled by flow meters incorporating five metering valves. Sample flow from each line is mixed in a plenum containing the explosive-level detector head. The detector will be set to alarm if the level in any one chamber reaches 20% of the lower explosive limit while the level in all other chambers is zero (SOP #ØB-BE-3CØU) and ØB-BE-3CØB). An alarm condition will automatically shut off the flow of test compound to all chambers.

D. ENVIRONMENTAL MONITORING

1. Temperature Measurements

Temperatures of the exposure chambers, exposure rooms and, if necessary, test chemical generators, are measured by Resistance Temperature Devices (RTDs). The RTDs will be placed in a representative location in each chamber (a top sample port on the back side). Each RTD can be connected to an Omega Model 412B

digital thermometer by a manual select switch or by computer controlled scanner relays in the CDS IIS (Figure 7). This allows temperature to be read manually or to be recorded automatically. All temperature measurement equipment except the RTDs will be located n the Suite Control Center. Temperatures will be automatically recorded at regular periods during each 24-hour day.

The RTD will be calibrated at least once every 2 months (SOP ØØB-BE-3CØD and ØB-BE-3CØL). Calibration will generate values for offset and slope, which will be entered into the computer for each RTD. Calibration data will be included as part of the study archives.

2. Relative Humidity Measurements

Relative humidity (RH) will be measured using a EG&G Model 910 chilled-mirror dewpoint hygrometer located in the Suite Control Center. Samples of the air from each measurement location will be pulled through individual polytetrafluoroethylene sample lines to a central location in the Suite Control Center (Figure 6). This assures a fresh sample of the air at the point of measurement. Air from exposure chambers will be sampled from a representative location (a top port on the back side). Sample air from a particular location passes through a three-way valve to the system exhaust. When the RH is to be measured at that location, the three-way valve is switched to divert the flow to the dewpoint hygrometer. The valve can be controlled by either a manual switch or by a computer-controlled relay in the CDS IIS. This allows RH to be measured manually or automatically. Once the dewpoint has been determined by the hygrometer, the RH is automatically calculated by the executive computer using the dewpoint value (T_1) and the drybulb temperature (T_2), measured simultaneously at that measurement location.

The following equation is used for this calculation:

$$\%RH = \frac{10^{\left[9.91 - \frac{2714.55}{(5/9)(T_1 - 32) + 293.3}\right]}}{10^{\left[9.91 - \frac{2714.55}{(5/9)(T_2 - 32) + 293.3}\right]}} \times 100$$ &A

$$\cancel{\%\ RH = \frac{10^{9.91 - \frac{2714.55}{(5/9)(T_1 - 32) + 293.3}}}{10^{9.91 - \frac{2714.55}{(5/9)(T_2 - 32) + 293.3}}} \times 100}$$

where: T_1 = dewpoint temperature, °F
T_2 = drybulb temperature, °F

Calibration of the dewpoint hygrometer will be checked before the start of the study and at least once every two months thereafter (ØB-BE-3CØJ and ØB-BE-3BIX). The procedure requires comparison of the RH calculated by the system monitor to measurements made by calibrated dewpoint hygrometer at the sample location. Calibration of the system monitor can be accomplished by inserting a value for offset and slope in the computer for each measurement location. Calibration data will be included as part of the study archive. RH will be recorded at regular periods during each 24-hour day.

&A Corrected 5/1/88 by Ammendment A.

3. Chamber Air-Flow Measurements

Chamber air flow measured by a multiplexed orifice-meter system (Figure 8). Calibrated flow orifices are installed at the inlet and exhaust of each chamber. The desired flow orifice is attached to a Validyne Model DP-45 pressure transducer and CD-18 carrier demodulator pressure-measurement system through Tygon® tubes by means of solenoid valves. The valves can be operated either by a manual switch or by computer activated relays in the CDS IIS. This allows flow to be measured either manually or automatically. Pressure is read manually on a Validyne Model PM-12 voltmeter. Usually chamber flow will be measured using the exhaust flow orifice; however, after closing of the chamber doors, both inlet and exhaust flow measurements will be made and compared to determine if there are leaks in the chamber. If leaks are present, the executive computer will notify the operator and will not allow exposures to proceed until the leak is repaired.

All flow measurement equipment, except the multiplexed solenoid valves, is located in the Suite Control Center. Flow will be automatically recorded at regular intervals during the 24-hour day. The Validyne pressure transducer will be calibrated once each week (ØB-BE-3CØW and ØB-BE-3CØX). Calibration of the flow orifices will be checked once every two months (SOPs #ØB-BE-3CØS and ØB-BE-3CØV). Calibration of each orifice will generate coefficients that will be inserted into the computer flow equation for each orifice. Calibration data will be included as part of the study archive.

4. Chamber Vacuum Measurements

The same Validyne pressure transducer system used to measure chamber flows will be used to measure chamber vacuum (Figure 8). Vacuum in the chamber will be measured relative to atmospheric pressure in the Suite Control Room. Vacuum will be automatically recorded at regular intervals during the 24-hour day.

Vacuum will also be continuously monitored by a pressure switch mounted near each chamber. If the chamber should develop a leak (for example, a door inadvertently opened or a sample port stopper jarred loose), the pressure switch will immediately shut off the flow of compound to the chamber and alert the executive computer of the condition. The computer will activate an audio alarm and print and display a comment for the operator.

E. ENVIRONMENTAL CONTROLS

1. Animal Facility Air Handling System

Supply air enters the building through two identical parallel air handling systems (Figure 9). Each system consists of a pre-heat coil, a filter system, a heating coil, a chilling coil, and a supply fan. The

pre-heat coil heats the air to a minimum of 45°F. The filter system - which includes a roll filter, pre-filter, and a bag filter - rids the air of most particles. The heating and chilling coils maintain the temperature of the air exiting the air conditioning system at about 53°F. The chilling coils also dry the air to a dewpoint not greater than 53°F.

2. Animal Room Air Handling System

The air from the two building air handling systems is then mixed together by an air mixing unit and is divided into two ducts which feed the rooms on East and West sides of the animal quarters. If necessary, steam is injected into the air in these ducts to maintain the RH of the room at between 35% and 65%.

3. Chamber Relative Humidity (RH) Control

Figure 10 shows a schematic diagram of the system used to control the relative humidity in the exposure chambers. Equipment located in the RH Control Equipment Room (Room 335) provides separate ducts of dry and moist air to each exposure chamber. A mixing valve, controlled by the computer, mixes the proper proportions of the moist and dry air to maintain the proper RH in each chamber.

Filtered air with a maximum dewpoint of about 53°F is supplied to the RH control equipment by the building air handling system. This air is evenly delivered to two ducts. Air from the first duct passes into a plenum where steam is injected to bring the air to a dewpoint of about 65°F. This provides moist air to the mixing valves. Steam is generated from city tap water with no additional additives. The air from the second duct passes through a refrigeration coil which reduces the moisture content of the air to a dewpoint of about 38°F. This provides "dry" air to the mixing valves.

Chamber RH is measured by the multiplexed dewpoint hygrometer. If the RH is found to be beyond the RH control range, the computer will calculate and make the appropriate adjustment to the mixing valve to bring the chamber RH to the desired target value.

4. Chamber Air-Flow Control

Flow of air through the chamber is maintained by an AIR-VAC Engineering Model TDRH 1000 air- multiplier pump located in the exhaust duct of the chamber (Figure 11). This air-pressure-driven pump is stable, contains no moving parts, and is very reliable. Exhaust air from the chamber is HEPA-filtered before passing through this pump to remove particles which may reduce pump reliability. The pressure regulator, which controls the pump rate, is operated by a motor drive system. The motor drive can be controlled by a manual switch or automatically by the computer through a relay in the CDS IIS. Fine control of exposure concentration will be accomplished by automatically adjusting the chamber air flow within the allowable flow limits. Gross

adjustments of concentration must be done manually by adjustment of the generation system. Maintenance of the chamber air flow control system is covered in SOP #ØB-BE-3DØE.

Exhaust from all chambers is collected into a central chamber exhaust duct within the exposure room. The exhaust from the chamber pump is rigidly attached to the central chamber exhaust duct. This rigid attachment prevents the possible escape of test compound into the room. The vacuum level in the central duct is regulated by a motor-driven feedback damper to prevent variations in building exhaust pressure from affecting chamber air-flow rates.

The air-flow rate in the central chamber exhaust duct is continuously monitored and alarmed. If the flow in this duct falls below 50% of the normal flow, the monitor trips the alarm which immediately shuts off the test compound generator system. Maintenance and calibration of the exhaust duct monitor is covered in SOP #ØB-BE-3DØE.

5. Chamber Temperature Control

Nearly all of the heat load contributed to the exposure chamber by the animals is dissipated from the chamber by radiation through the chamber walls (Bernstein and Drew, 1980). Consequently, temperature of the air supplied to the chamber has little effect on the temperature of the chamber while, on the other hand, the temperature of the room housing the chamber has a great deal of effect. For this reason, the major method of chamber temperature will be control of the room temperature. However, some cooling of chambers full of animals will be affected by the cool incoming air from the chamber's RH control system. Typically, a chamber full of animals will require the addition of dry air to maintain the proper RH. The dry air from the RH control system is cooler than room temperature. On the other hand, some warming of a chamber containing few animals will be affected by the warm air from the chamber's RH control system. Typically, a chamber with few animals will require the addition of wet air to maintain the proper chamber RH. The wet air is equal to or warmer than the room temperature.

F. CHAMBER EXHAUST WASTE TREATMENT

The exhaust from the central chamber exhaust duct is mixed with the exhaust from the entire animal facility (75,000 cfm) prior to being exhausted from the building stack. Dilution of chamber exhaust with building exhaust results in an acceptable stack concentration of less than 10% of the threshold limit value (TLV) for the test article.

G. DATA HANDLING

Data from each exposure room are stored in the Exposure Suite Control Center on separate magnetic diskettes by Hewlett Packard Model 9121 micro-floppy disk drives. Data and comments from each exposure room are printed on separate thermal dot-matrix printers (Hewlett

Packard Model 2171G). Data are printed and stored immediately upon completion of the measurement to a Daily Log (example, Figure 12). At the end of the day (24-hour period), the daily data are analyzed and a summary is printed (Figure 13). This summary includes the mean, standard deviation, maximum, minimum and target values for each set of data for the 24-hour period. A second printout (Figure 14) provides a list of outliers(i.e., all data points which were beyond the defined critical limits). This printout will allow quick review of the data.

Data handling and analysis procedures are described in the SOPs ØB-BE-5EØ3, ØB-BE-3EØA, and ØB-BE-3EØB.

H. EQUIPMENT OR POWER FAILURE PROTECTION SYSTEMS

In the event of equipment failure, or of a short-term power failure, two parameters must be considered most important to the well-being of the animals - temperature and air flow. To understand the factors protecting against either of these two parameters becoming life-threatening to the animals, one must understand both the emergency power system and the emergency air handling equipment.

Power is provided to the Battelle complex from two separate city substations through an automatic switching device. This significantly reduces the possibility of losing city power. Power from the city is routed to equipment in LSL-II through two types of motor control centers. One type can switch power to the equipment from either city power or emergency power from the LSL-II diesel generator. The other has access only to city power. The emergency-power-type motor control center has a low voltage detector on each leg of the three-phase input power. If the city-supplied power should fail or "brown out", these detectors automatically start the emergency power diesel generator, and route the emergency power to the equpiment supplied by the motor control center.

All equipment critical to the well-being of the animals is connected to the emergency-power-type motor control centers. A list of this equipment is as follows:

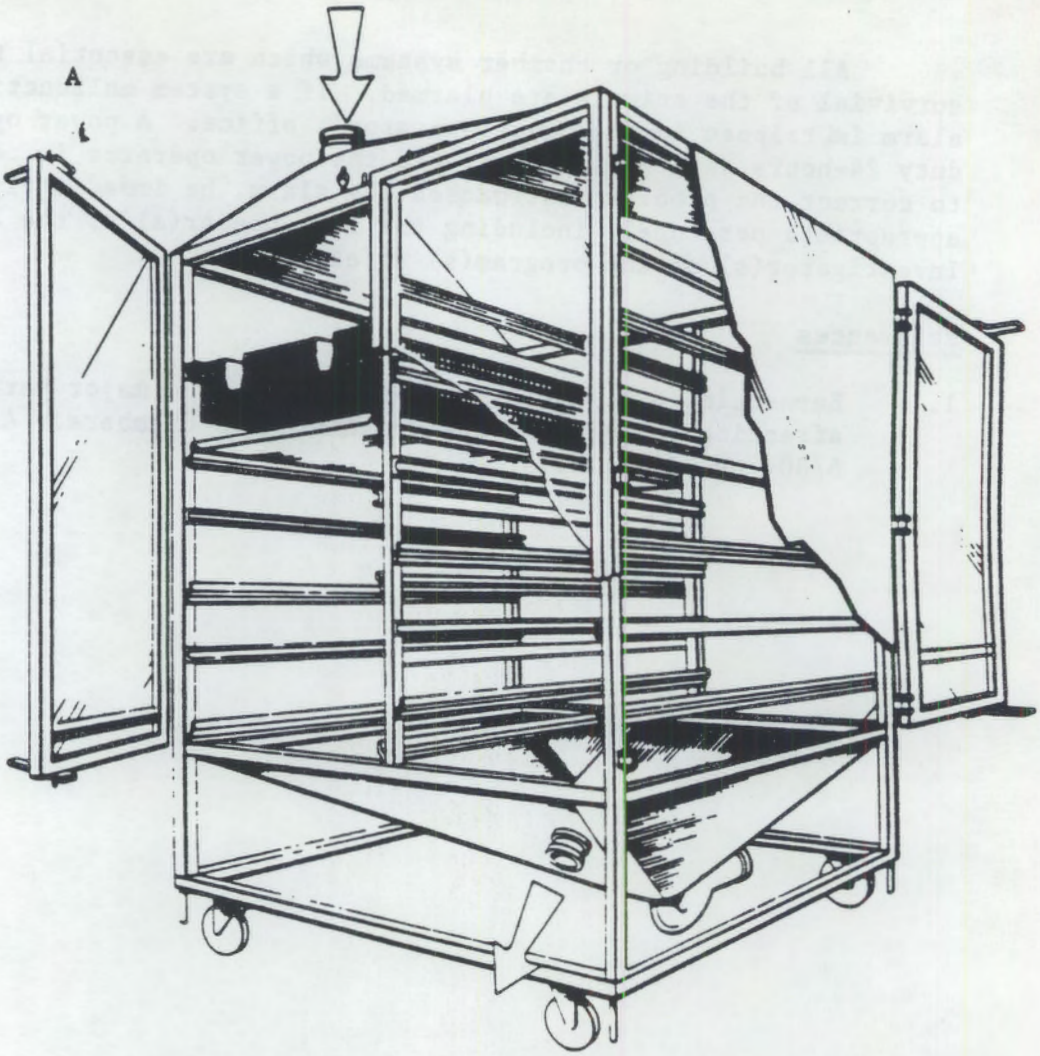
- Emergency lighting and electrical outlets
- Chillers #1 and #2
- Boiler and feedwater pump systems #1 and #2
- Air compressors #1 and #2
- Air supply fans #1 and #2
- Air exhaust fans #1 and #2

It should be noted that there are two identical units of all of the equipment that is vital to the well-being of the animals (heating, cooling, supply air, exhaust air, and compressed air). Either of the two units has sufficient capacity to maintain the animal environment within a safe range. In all cases, the emergency power system will operate one of the two identical units. If, during a power outage, the unit of equipment that is on emergency power should happen to fail, the other unit of identical equipment can be manually switched to run on emergency power.

All building or chamber systems which are essential to the survivial of the animals are alarmed. If a system malfunctions, an alarm is tripped in the power operator's office. A power operator is on duty 24-hours/day, 7 days/week. If the power operator is not authorized to correct the problem that caused the alarm, he immediately calls the appropriate personnel, including the Task Leader(s) or the Principal Investigator(s) of the program(s) affected.

References

1. Bernstein, D.M. and R.T. Drew. 1980. The major parameters affecting temperature inside inhalation chambers. AIHAJ, (41) 6/80, pp. 420-426.



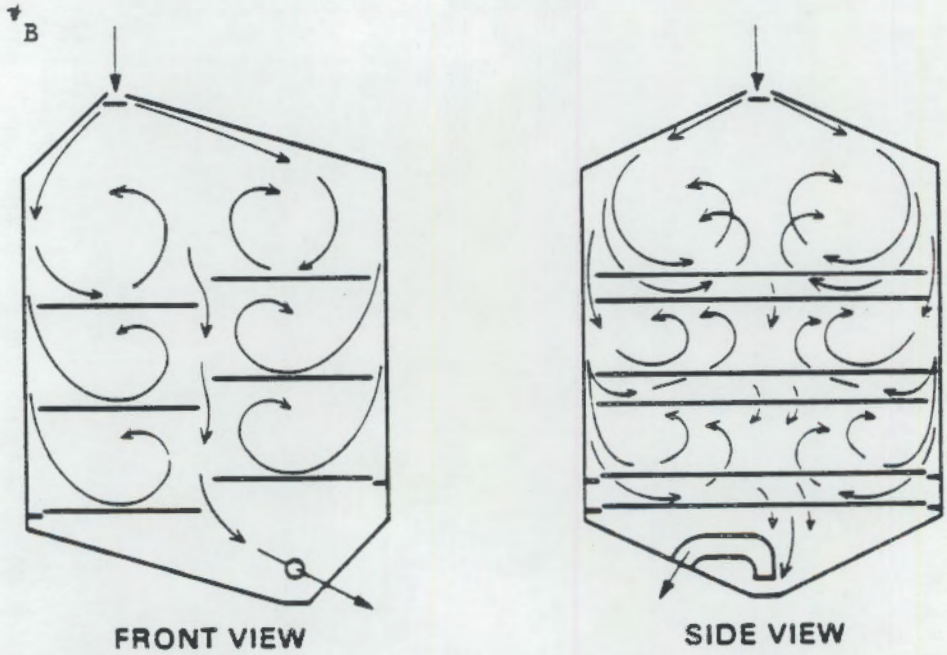


FIGURE 1. Inhalation Exposure Chamber Designed at BNW
(A. Oblique cutaway view of the chamber;
B. Airflow patterns)

EXPOSURE SUITE #1

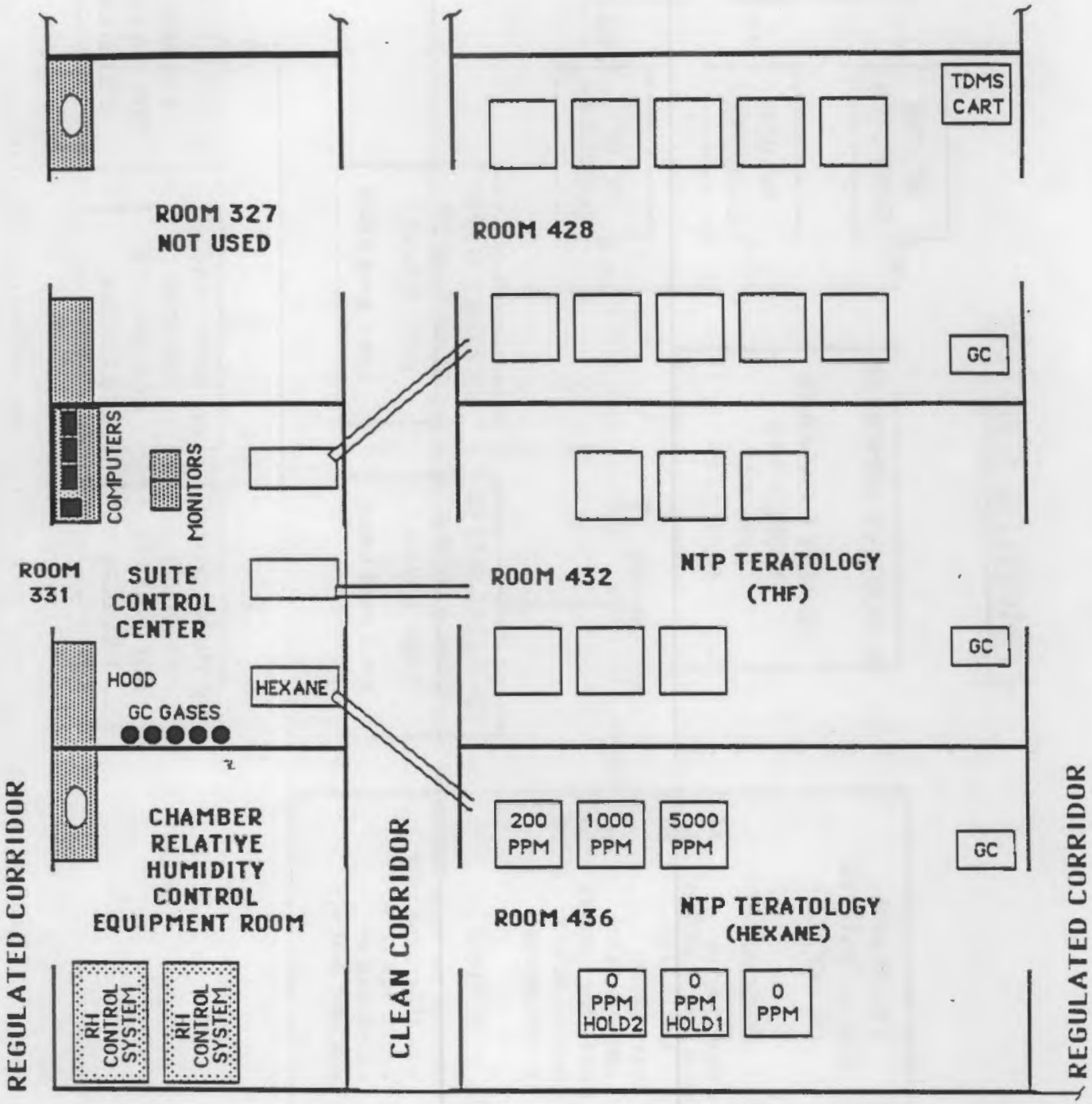


FIGURE 2. Schematic Diagram of the Three Exposure Rooms in the Automated Inhalation Exposure Suite.

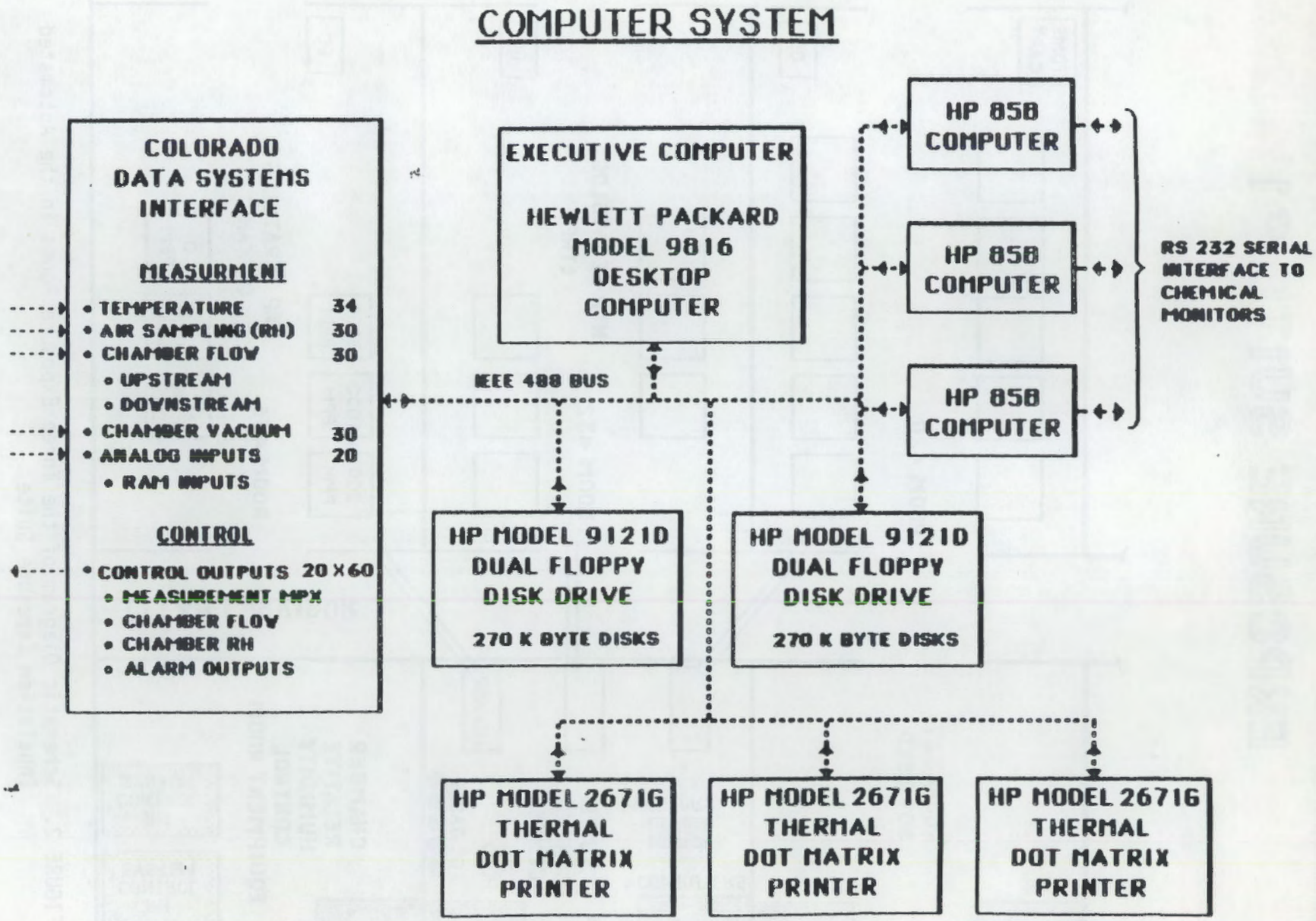


FIGURE 3. Block Diagram of Data Acquisition andControl Computers and Interface Instrumentation

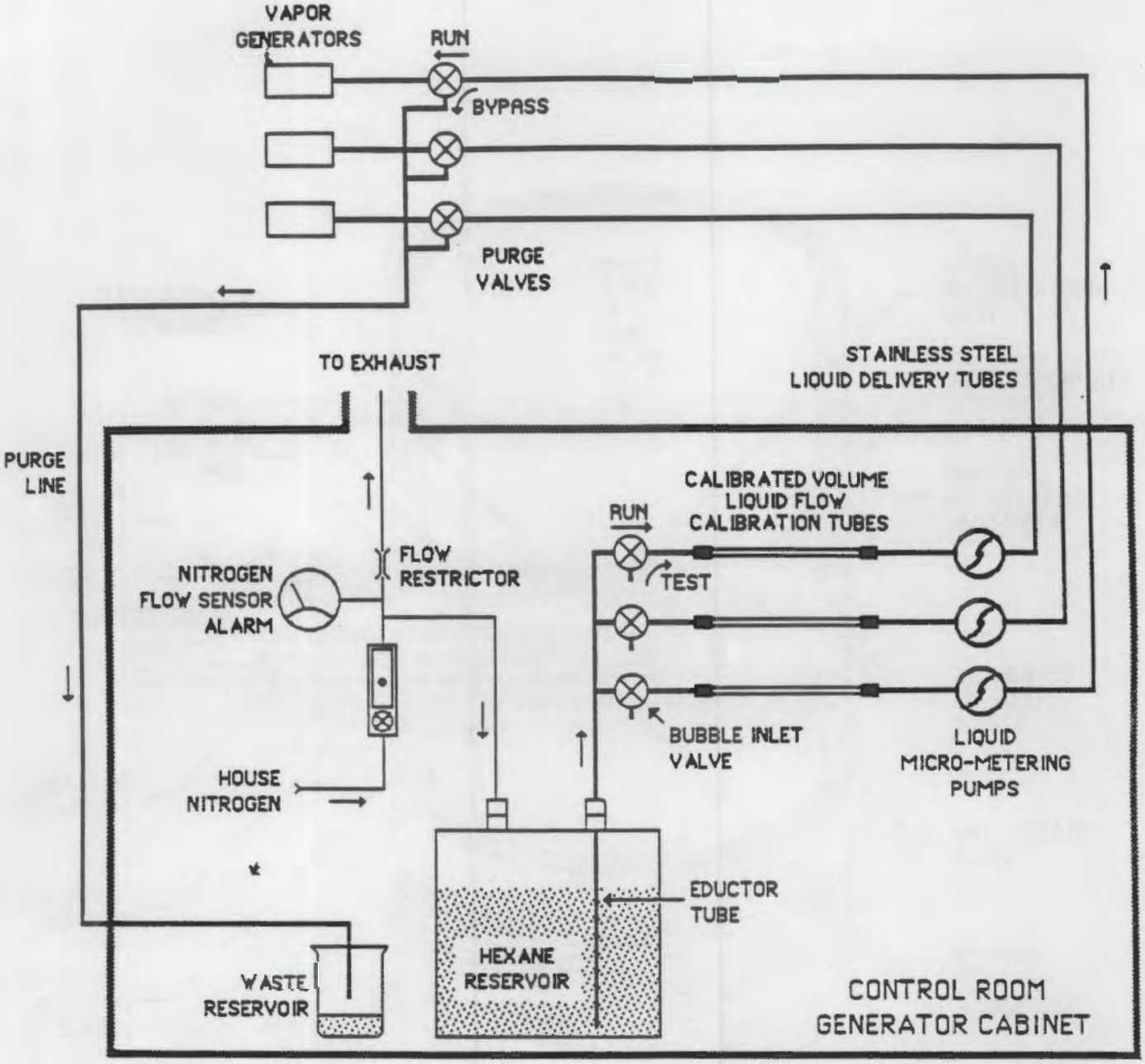


FIGURE 4. Schematic Diagram of the Hexane Vapor Generation System.

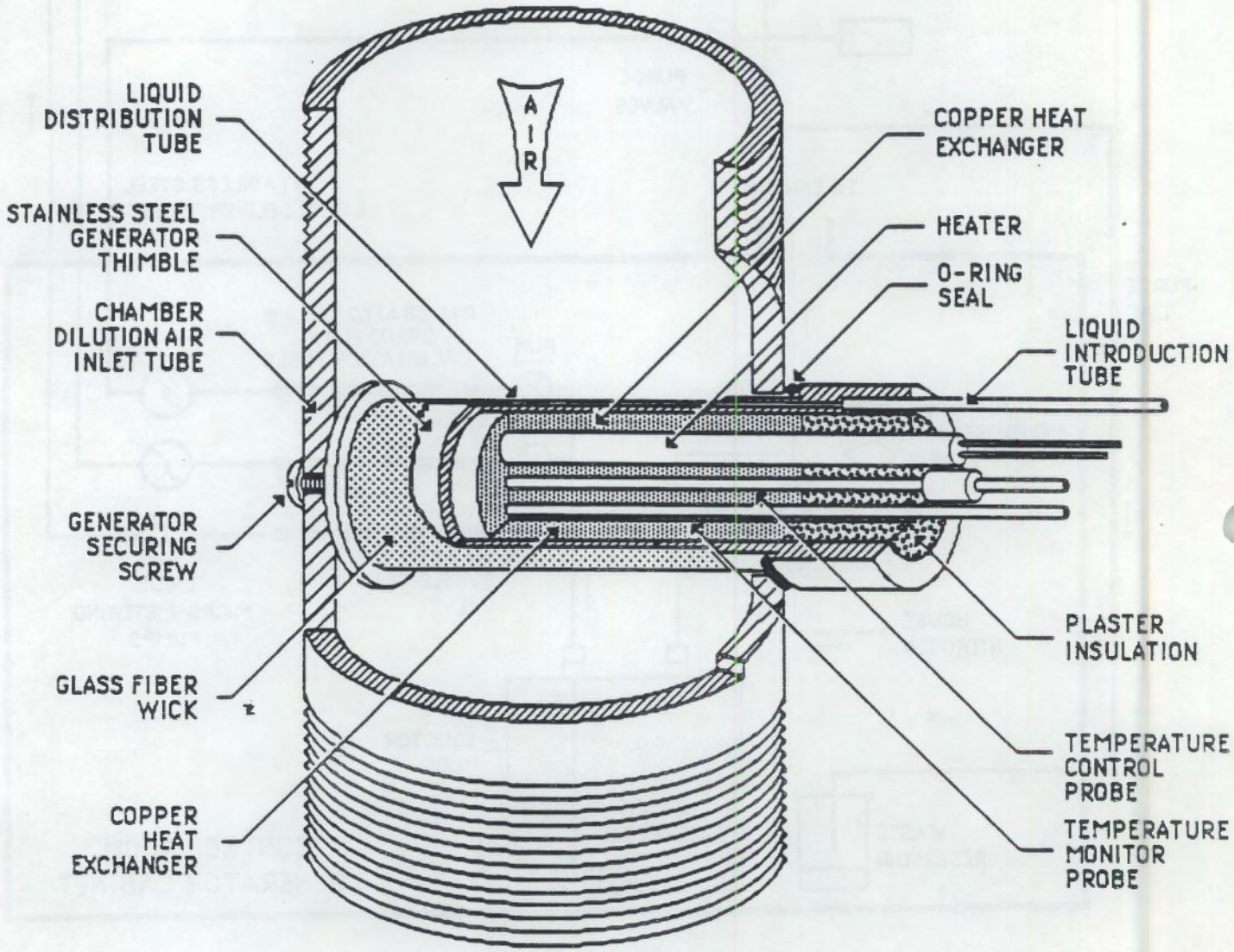


FIGURE 5. Cutaway Drawing of the Hexane Vapor Generator Located in the Fresh-Air Inlet Tube of the Exposure Chamber.

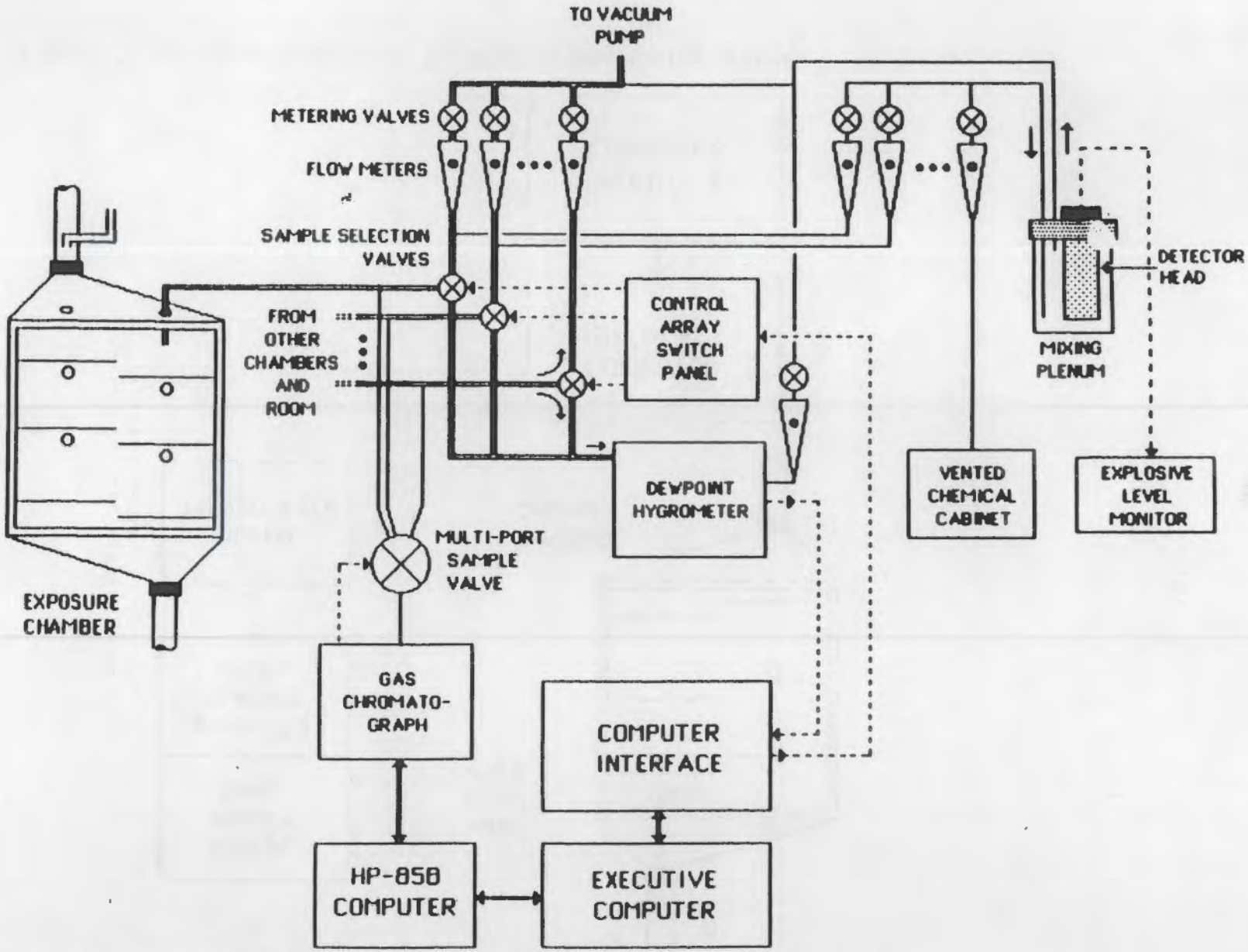


FIGURE 6. Schematic Diagram of the Dewpoint, Chemical Concentration, and Explosive Level Monitoring Systems

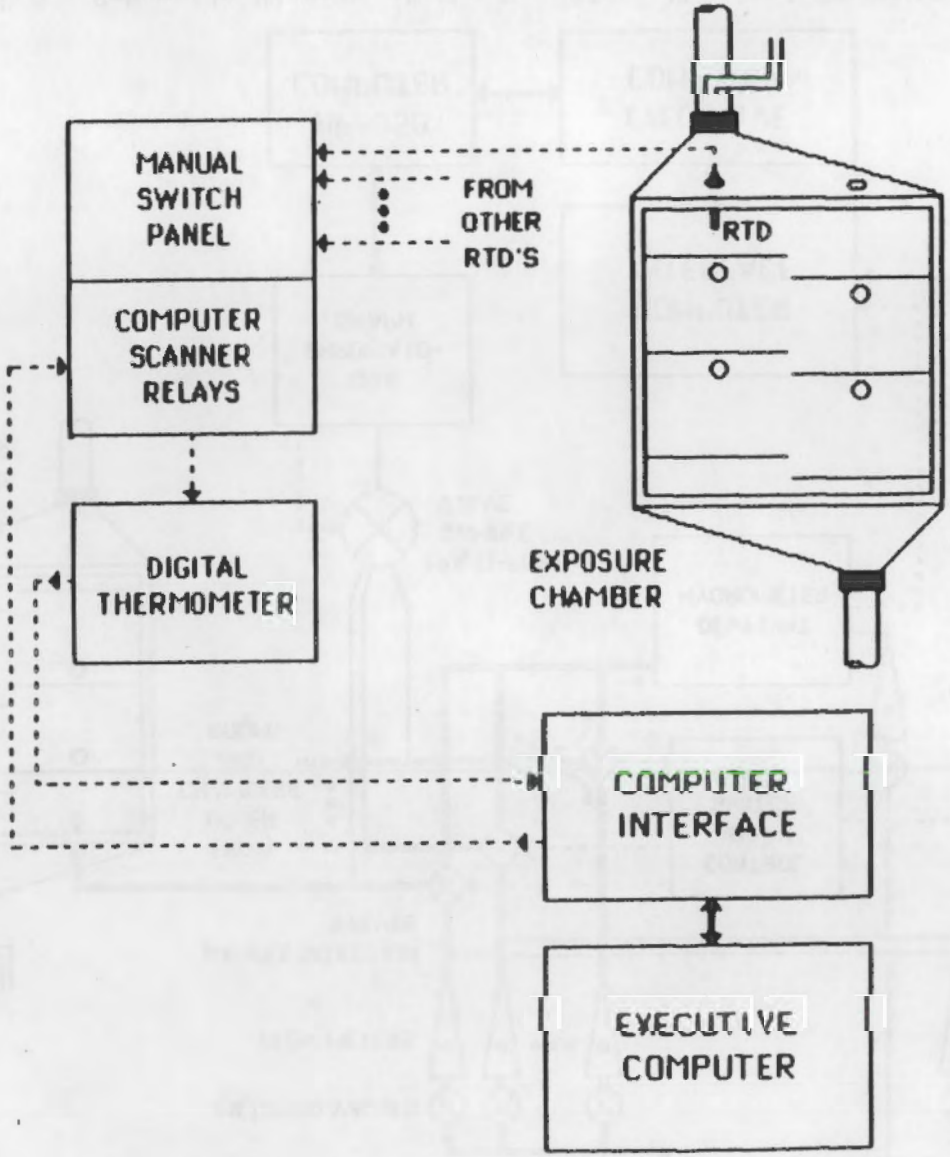


FIGURE 7. Schematic Diagram of Temperature Monitoring System

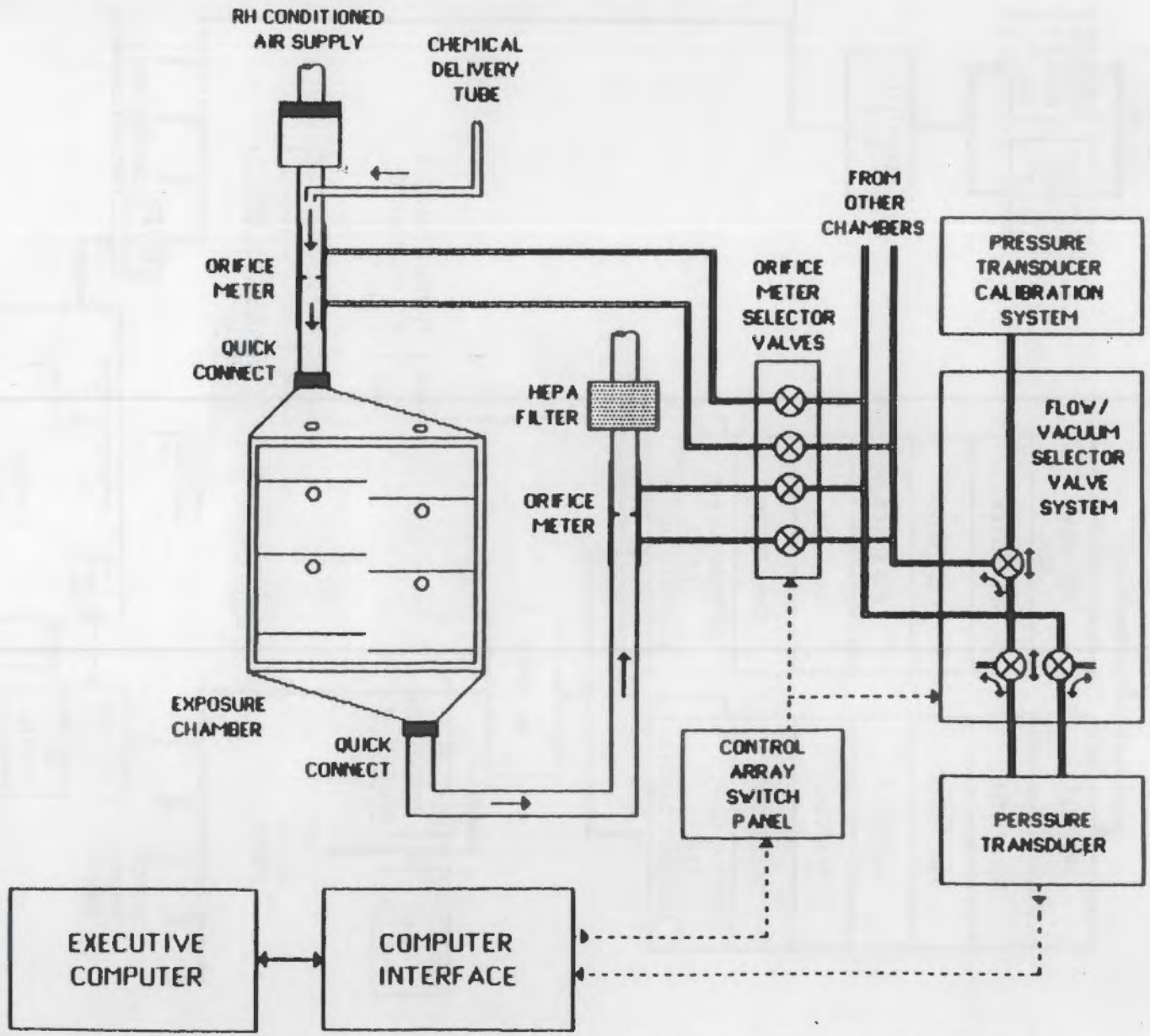


FIGURE 8. Schematic Diagram of the Chamber Flow and Vacuum Monitoring System

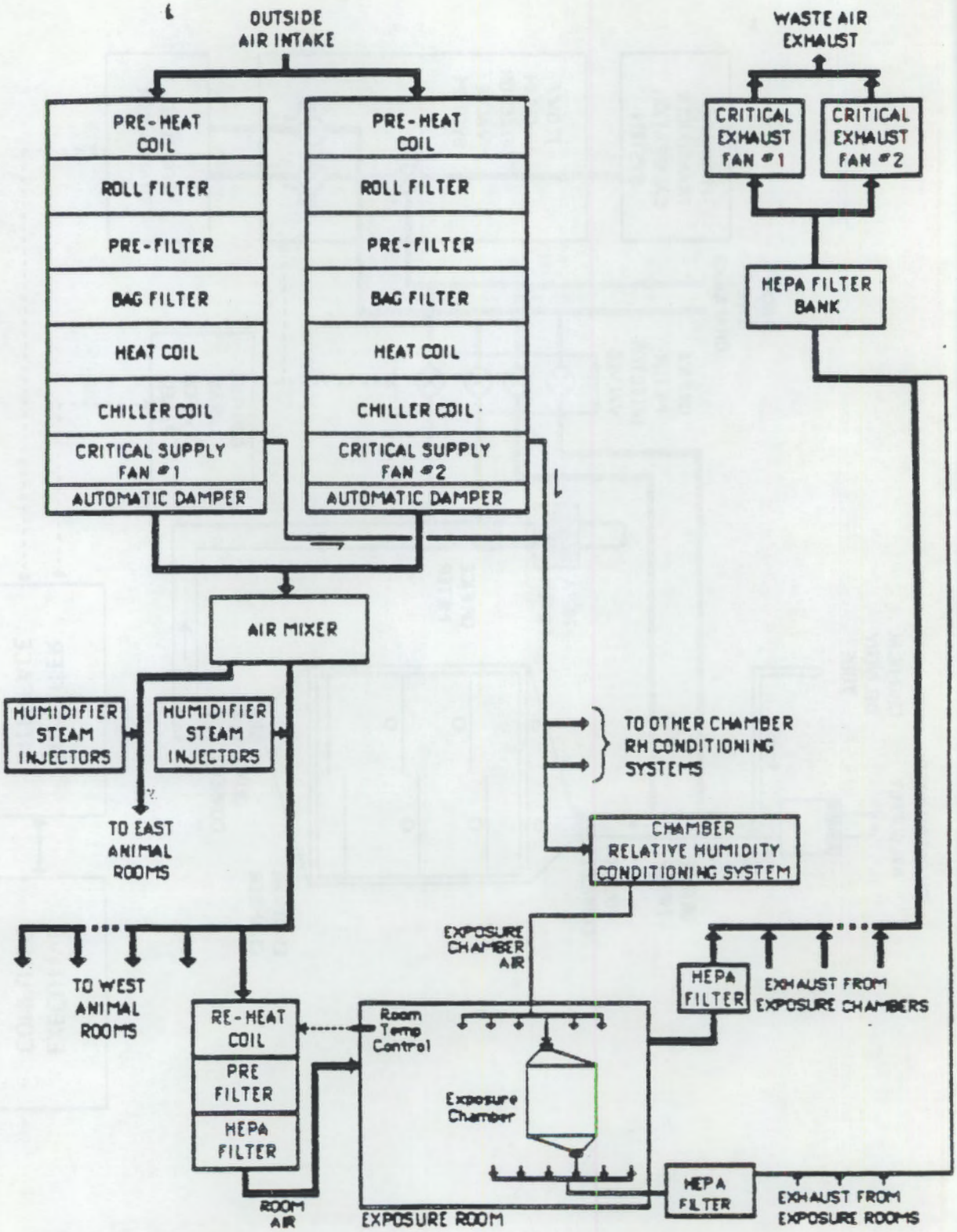


FIGURE 9. Air Handling System for Animal Rooms of Life Sciences II Building

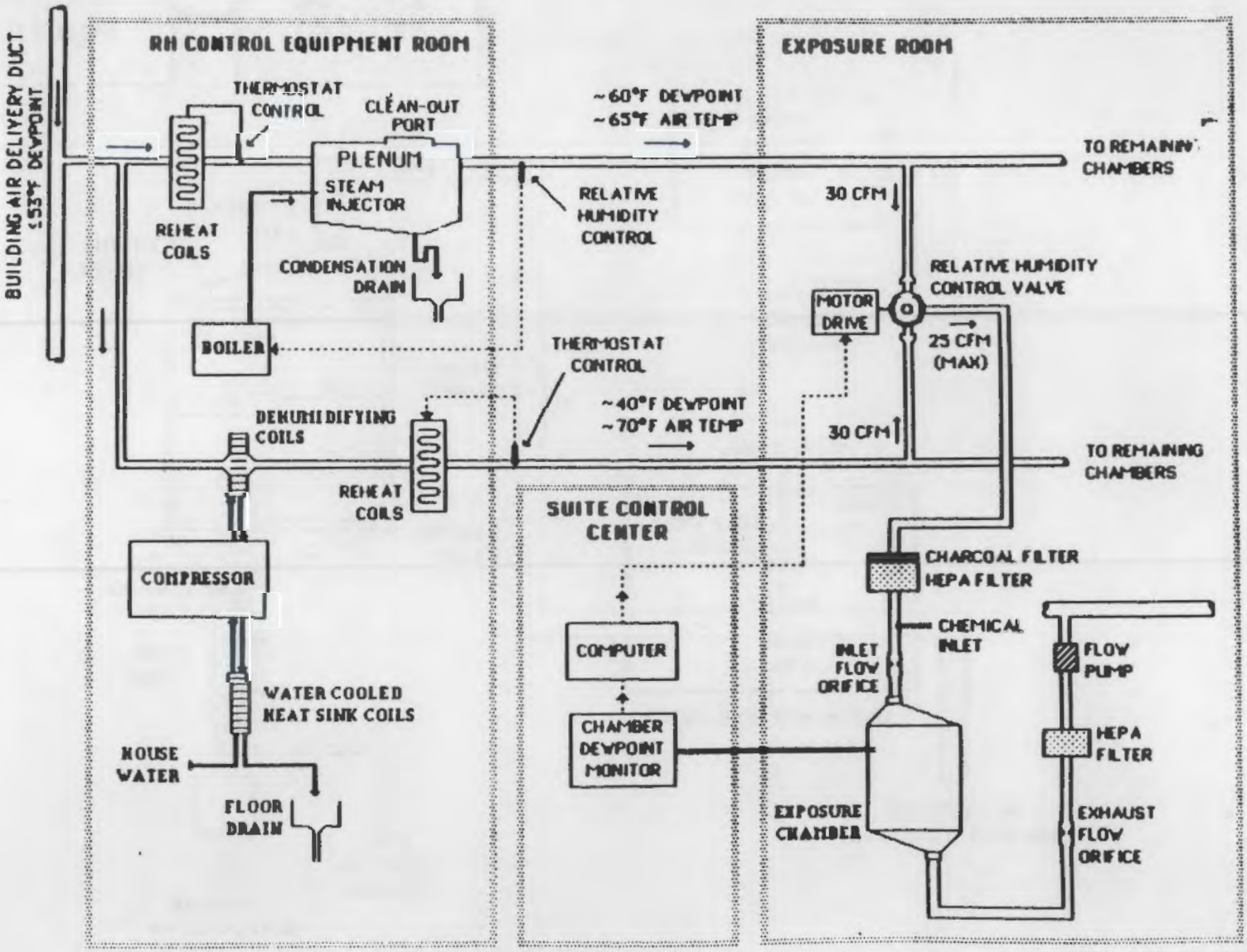


FIGURE 10. Schematic Diagram of Chamber Relative Humidity Control System

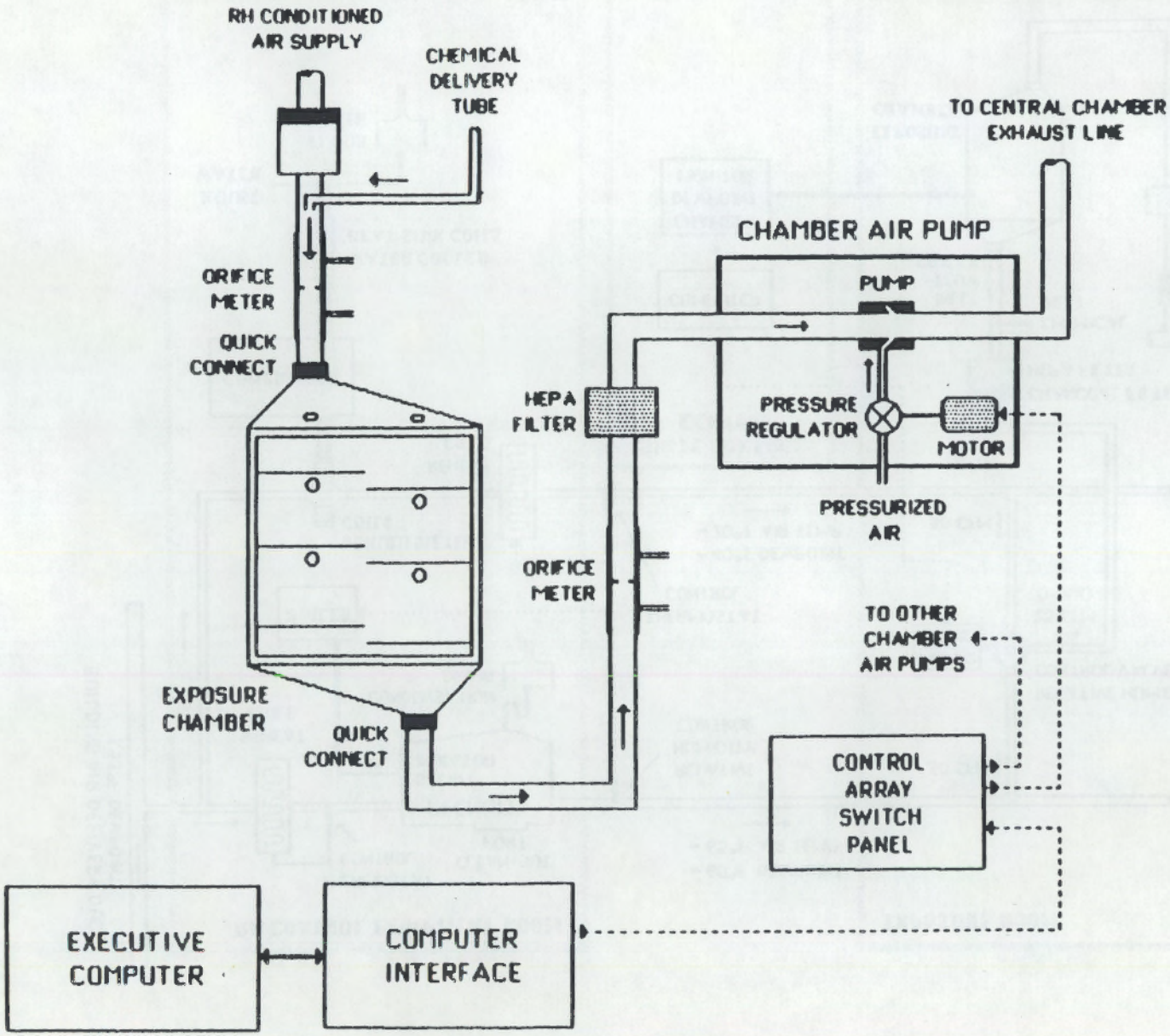


FIGURE 11. Schematic Diagram of the Chamber Air Flow Pump and Air Flow Control System

Exp #1: Demonstration Program: 85.01 24 July 1985

Time	Location	Function	Data		
21:01	Ch #1 -- Room 324	Temperature	(BSI	79.1	F
21:02	Ch #2 -- Room 324	Relative Humidity	OKI	40.	%
21:03	Ch #3 -- Room 324	Flow	OKI	16.3	CFM
21:06	Ch #2 -- Room 436	Relative Humidity	(BSE	65.	%
21:07	Ch #2 -- Room 436	Vacuum	<OKE	1.5	HOH
21:08	Ch #1 -- Room 324	Vacuum	<BSI	.8	HOH
21:10	Ch #2 -- Room 324	Relative Humidity	<OKI	35.	%
21:13	Ch #3 -- Room 324	Concentration	OKI	5.000E+1	PPM
21:16	Ch #2 -- Room 436	Relative Humidity	(BSE	65.	%
21:16	Ch #2 -- Room 436	Vacuum	<OKE	2.5	HOH
21:17	Ch #1 -- Room 324	Temperature	<BSI	81.1	F
21:18	Ch #2 -- Room 324	Relative Humidity	OKI	46.	%
21:18	Ch #3 -- Room 324	Flow	OKI	16.3	CFM
21:19	Ch #2 -- Room 436	Relative Humidity	(BSE	65.	%
21:20	Ch #2 -- Room 436	Vacuum	OKE	1.4	HOH
21:21	Ch #1 -- Room 324	Vacuum	<BSI	.8	HOH
21:25	Ch #2 -- Room 324	Relative Humidity	<OKI	35.	%
21:26	Ch #3 -- Room 324	Concentration	OKI	5.000E+1	PPM
21:26	Ch #2 -- Room 436	Relative Humidity	(BSE	65.	%
21:26	Ch #2 -- Room 436	Vacuum	<OKE	1.3	HOH
21:27	LJF				
	This is a demonstration of the comment routine. This routine is available from every menu.				
21:40	Ch #2 -- Room 436	Relative Humidity	(BSE	65.	%
21:46	Ch #2 -- Room 436	Vacuum	<OKE	.8	HOH
21:48	Ch #1 -- Room 324	Vacuum	<BSI	.8	HOH
21:50	Ch #2 -- Room 324	Relative Humidity	<OKI	35.	%
21:53	Ch #3 -- Room 324	Concentration	OKI	5.000E+1	PPM
21:56	Ch #2 -- Room 436	Relative Humidity	(BSE	65.	%
21:06	Ch #2 -- Room 436	Vacuum	OKE	1.5	HOH

FIGURE 12. Example of "Daily Log" Printout from Data Acquisition and Control Computer. See following page for explanation of columns.

DESCRIPTION COMPUTER "LOG BOOK" OUTPUT

The exposure number, exposure name, program version and exposure date will be printed at the top of every report page.

Time--This is the far left column. This is the time that the measurement was taken.

Location--This identifies where the data came from. Also referred to in the menus as "Location". This column allows for 20 characters.

Function--This identifies which function was used to take the reading. This column allows for 20 characters.

Data--This is the raw data. This column includes an alarm code, a status code, the data value and a units label.

Alarm code--"(" means that the data has exceeded non-critical alarm limits.
"<" means that the data has exceeded critical alarm limits.

Status code--OK1 - Okay and calibrated. Data is included in summary.
OKE - Okay and calibrated. Data is not included in summary.
BS1 - Beyond service time. Data is included in summary.
BSE - Beyond service time. Data is not included in summary.

Data format--Data will be expressed as four significant digits with non significant zeros suppressed. Number of decimal points was determined in the menus. (Function Assignments Menu.)
Examples: DDDD.
DDD.D
DD.DD
D.DDD
.DDDD
D.DDDESZ

Units label--This column allows 9 characters. Examples: ppm, °F, °C, HOH.

NOTE: At almost any time during the exposure day, a comment can be entered from the keyboard. Because our report is generated as events occur, comments can appear in the middle of the logbook printout. This first line will show only the time and the operator's full name. The next lines will contain the body of the comment.

FIGURE 12. (Continued)

Summation for the File: 23 July 1985 Exposure: Demonstration

Temperature	Mean	% Targ	Std Dev	% RSD	Maximum	Minimum	N	Target
Ch #01	73.20	101	.125	1	74.6	70.7	10	72.0
Ch #02	74.30	103	.128	2	78.3	72.7	15	72.0
Ch #03	73.20	101	.134	3	75.3	70.7	15	72.0
Ch #04	68.20	95	.131	2	75.6	65.7	15	72.0
Ch #05	73.20	101	.131	2	76.3	68.7	15	72.0
Ch #06	73.40	102	.139	2	75.3	72.7	15	72.0
Ch #07	69.40	96	.150	1	74.3	68.7	10	72.0
Ch #08	70.20	98	.130	2	75.3	72.7	15	72.0
Room	74.20	103	.130	2	75.3	72.7	15	72.0
Flow	**Mean**	**% Targ**	**Std Dev**	**% RSD**	**Maximum**	**Minimum**	**N**	**Target**
Ch #01	14.10	94	.300	3	17.0	12.0	8	15.0
Ch #02	17.90	118	.500	4	19.0	14.0	12	15.0
Ch #03	15.90	105	.400	3	17.0	12.0	15	15.0
Ch #04	13.80	92	.300	2	15.0	12.0	16	15.0
Ch #05	10.80	72	.200	3	12.0	8.0	18	15.0
Ch #06	14.90	99	.400	3	17.0	12.0	15	15.0
Ch #07	16.80	112	.300	4	18.0	14.0	14	15.0
Ch #08	14.60	91	.400	3	17.0	12.0	15	15.0
Relative Humidity	**Mean**	**% Targ**	**Std Dev**	**% RSD**	**Maximum**	**Minimum**	**N**	**Target**
Ch #01	51.0	102	5.10	10	70.	41.	14	50.
Ch #02	48.0	96	5.20	11	65.	45.	14	50.
Ch #03	52.0	106	5.30	10	70.	45.	14	50.
Ch #04	51.0	102	5.10	10	70.	41.	14	50.
Ch #05	48.0	96	5.20	11	65.	45.	14	50.
Ch #06	52.0	106	5.30	10	70.	45.	14	50.
Ch #07	51.0	102	5.10	10	70.	41.	14	50.
Ch #08	48.0	96	5.20	11	65.	45.	14	50.
Room	52.0	106	5.30	10	70.	45.	14	50.

FIGURE 13. Example of 24-Hour Data "Summation" Printout from Data Acquisition and Control Computer. Data are organized by data type.

Outlier Table for the File : 24 July 185 Exposure: Demonstration

Date	& Origin	Function	Time	Data	Lower	Target	Higher
23 Jul	Temperature	Ch #01	16:45	69.3	70.0	72.0	74.0
23 Jul			16:48	69.2	70.0	72.0	74.0
23 Jul			16:51	69.0	70.0	72.0	74.0
23 Jul			16:55	69.1	70.0	72.0	74.0
23 Jul			16:59	69.3	70.0	72.0	74.0
23 Jul		Ch #02	16:47	68.1	70.0	72.0	74.0
23 Jul			16:49	68.3	70.0	72.0	74.0
23 Jul		Ch #03	16:40	69.0	70.0	72.0	74.0
23 Jul		Ch #04	16:59	75.1	70.0	72.0	74.0
23 Jul			17:09	74.8	70.0	72.0	74.0
23 Jul		Ch #05	14:59	74.3	70.0	72.0	74.0
23 Jul		Ch #08	16:01	67.1	70.0	72.0	74.0
23 Jul			16:20	68.1	70.0	72.0	74.0
23 Jul		Room	16:23	69.0	70.0	72.0	74.0
23 Jul			16:41	69.8	70.0	72.0	74.0
23 Jul	Flow	Ch #01	12:45	11.2	12.0	15.0	17.0
23 Jul			15:23	18.1	12.0	15.0	17.0
23 Jul		Ch #05	15:33	9.1	12.0	15.0	17.0
23 Jul		Ch #08	10:23	20.1	12.0	15.0	17.0
23 Jul			16:41	20.2	12.0	15.0	17.0
23 Jul	Concentration	Ch #03	10:45	4.560E+0	5.000E+0	7.500E+0	1.000E+1
23 Jul			10:50	4.350E+0	5.000E+0	7.500E+0	1.000E+1
23 Jul			11:01	4.200E+0	5.000E+0	7.500E+0	1.000E+1
23 Jul			11:14	4.130E+0	5.000E+0	7.500E+0	1.000E+1
23 Jul			11:29	4.580E+0	5.000E+0	7.500E+0	1.000E+1
23 Jul		Ch #06	9:06	1.143E+1	5.000E+0	7.500E+0	1.000E+1
23 Jul			9:21	1.194E+1	5.000E+0	7.500E+0	1.000E+1
23 Jul			9:46	1.053E+1	5.000E+0	7.500E+0	1.000E+1
23 Jul		Ch #08	11:46	1.001E+1	5.000E+0	7.500E+0	1.000E+1
23 Jul			12:07	1.003E+1	5.000E+0	7.500E+0	1.000E+1

FIGURE 14. Example of 24-Hour Data "Outlier Table" Printout from Data Acquisition and Control Computer. Table shows data which were beyond the defined operating limits.

EXPOSURE CHAMBER CAGE LOCATION

SPONSOR: NTP/IRT

STUDY: SPERM MORPHOLOGY

ROOM: 436

DATE: 3-24-86 to 3-31-86 RLR

CHEMICAL: n-HEXANE

CHAMBER: Treatment #1

CONCENTRATION: 0 ppm (Experimental)

LEVEL 4

Cage		Cage		Cage		Cage	
15		30		45		60	
14		29		44		59	
13		28		43		58	
12		27		42		57	
11		26		41		56	
10	78	25	124	40		55	
9	60	24	123	39		54	
8	56	23	121	38		53	
7	37	22	118	37		52	
6	33	21	107	36		51	
5	32	20	105	35		50	
4	30	19	98	34		49	
3	19	18	93	33		48	
2	13	17	91	32		47	
1	10	16	81	31		46	

EXPOSURE CHAMBER CAGE LOCATION

SPONSOR: NTP/IRT

STUDY: SPERM MORPHOLOGY

ROOM: 436

DATE: 3-24-86 to 3-31-86 RJR

CHEMICAL: n-HEXANE

CHAMBER: Treatment #2

CONCENTRATION: 200ppm (Low)

LEVEL 4

Cage		Cage		Cage		Cage	
15		30		45		60	
14		29		44		59	
13		28		43		58	
12		27		42		57	
11		26		41		56	
10	64	25	111	40		55	
9	58	24	109	39		54	
8	49	23	106	38		53	
7	46	22	104	37		52	
6	44	21	89	36		51	
5	43	20	88	35		50	
4	27	19	80	34		49	
3	25	18	70	33		48	
2	2	17	69	32		47	
1	1	16	68	31		46	

EXPOSURE CHAMBER CAGE LOCATION

SPONSOR: NTP/IRT

STUDY: SPERM MORPHOLOGY

ROOM: 436

DATE: 3-24-86 to 3-31-86

CHEMICAL: n-HEXANE

CHAMBER: Treatment #3

CONCENTRATION: 1000ppm (Medium)

LEVEL 4

Cage		Cage		Cage		Cage	
15		30		45		60	
14		29		44		59	
13		28		43		58	
12		27		42		57	
11		26		41		56	
10	72	25	115	40		55	
9	67	24	113	39		54	
8	63	23	108	38		53	
7	62	22	103	37		52	
6	52	21	95	36		51	
5	34	20	94	35		50	
4	31	19	86	34		49	
3	23	18	83	33		48	
2	18	17	75	32		47	
1	4	16	73	31		46	

EXPOSURE CHAMBER CAGE LOCATION

SPONSOR: NTP/IRT

STUDY SPERM MORPHOLOGY

ROOM: 436

DATE: 3-24-86 to 3-31-86

CHEMICAL: n-HEXANE

CHAMBER: Treatment #4

CONCENTRATION: 5000ppm

LEVEL 4

Cage		Cage		Cage		Cage	
15		30		45		60	
14		29		44		59	
13		28		43		58	
12		27		42		57	
11		26		41		56	
10	42	25	119	40		55	
9	40	24	117	39		54	
8	38	23	116	38		53	
7	35	22	112	37		52	
6	28	21	100	36		51	
5	22	20	96	35		50	
4	16	19	87	34		49	
3	12	18	79	33		48	
2	7	17	51	32		47	
1	5	16	48	31		46	

DISTRIBUTION

No. of
Copies

OFFSITE

2 DOE/Office of Scientific
and Technical Information

M.L. Minthorn
U.S. Department of Energy
ER-72, GTN
Washington, D.C. 20545

10 R.E. Morrissey and
B.A. Schwetz
National Toxicology Program - NIEHS
Alexander Drive
Bldg. 101, Room D440
Research Triangle Park, NC 27709

E.B. Ford
National Institute of Environmental Health Sciences
Contracts Management Office, OAM
79 Alexander Drive, Bldg. 4401
P.O. Box 12874
Research Triangle Park, NC 27709

ONSITE

DOE Richland Operations Office

E.C. Norman/D.L. Sours

20 Pacific Northwest Laboratory

E.M. Crow (2)
R.A. Gelman
T.J. Mast (11)
Publishing Coordination
Technical Report Files (5)