HAZARD ASSESSMENT OF BUTYLATED HYDROXYTOLUENE FROM URETHANE CARPET CUSHIONS

Val H. Schaeffer, Ph.D.
Toxicologist, *Division* ***of*** *Health Sciences*
U.S. Consumer Product Safety Commission
4330 East West Highway
Bethesda, ***MD*** *208* ***14***

Released March 2, ***1998***

EXECUTIVE SUMMARY

This report provides the results of two studies by the U.S. Consumer Product Safety Commission (CPSC) to assess the potential hazard from exposure to BHT emitted from newly installed polyurethane carpet cushion. CPSC Field staff collected 20 bonded urethane and 10 prime urethane cushion samples from different manufacturing mills around the country. An additional five bonded and four prime, urethane samples were collected from retail outlets. The samples were tested for BHT emissions by the CPSC Division of Chemistry (LSC).

The twenty-four hour BHT emission rates from all samples ranged from 0.024 to 0.648 mg $m^{-2} h^{-1}$. The average BHT emission rates from bonded urethane cushion samples (0.21t0.12 mg $m^{-2} h^{-1}$) and prime urethane cushion samples (0.19 ± 0.15 mg $m^{-2} h^{-1}$) are similar.

The BHT emission rates measured from the polyurethane cushion samples collected in 1997 were generally lower than the BHT emission rates from the same type of cushion samples collected and tested two to four years earlier. The average peak BHT emission rates for the 1993/95 samples was 0.611 ± 0.320 mg $m^{-2} h^{-1}$ and ranged from 0.23 to 0.99 mg $m^{-2} h^{-1}$. This is about three-fold higher than the current BHT emission rates.

CPSC also contracted with IIT Research Institute (IITRI) to conduct a sensory irritation study of BHT. The objective was to assess the potential of BHT emitted from carpet cushion to cause sensory irritation in humans using a standardized mouse bioassay. Based on the IITRI sensory irritation data, CPSC staff estimate that the Acceptable Air Limits for Sensory Irritation (AAL_{SI}) for B'KT ranged from 1.53-18.81ppm.

The amount of BHT emitted from the recently collected bonded urethane and prime urethane cushion samples are not expected to produce indoor air concentrations of sufficient magnitude to cause eye and upper respiratory irritation in most individuals as typically installed in a residence. The lowest plausible AAL_{SI} estimate derived from the IITRI sensory irritation data are 7-fold higher than the highest predicted residential BHT air concentration and 25-fold higher than the median BHT air concentration. All plausible IITRI-derived AAL_{SI}s are also higher than the highest predicted BHT air concentration based on emission rates measured from polyurethane cushions collected in 1993/95. The 1993/95 BHT emission rates were about three-fold higher than those measured from the 1997 cushions.

Thus, data from the IITRI study indicate that the levels of BHT emitted from recently tested carpet cushion samples would not be expected to cause sensory irritation.

1. Introduction

Over the years, the U.S. Consumer Product 'Safety Commission (CPSC) has received health complaints from consumers following installation of new carpet. Some of the most frequently cited symptoms associated with carpet installation are watery eyes, runny nose, and a burning sensation of the eyes, nose, and throat, reminiscent of sensory irritation caused by exposure to airborne chemicals (Schachter, 1990 and Inkster, 1995). The agency initiated a laboratory investigation to determine what chemicals are emitted from carpet and carpet cushion (the pad installed underneath the carpet), and if those chemicals could cause the symptoms reported by consumers. Carpet and carpet cushion are the basic materials involved in nearly all residential installations.

Butylated hydroxytoluene (BHT) was identified as a major compound emitted from urethane carpet cushion in a previous phase of the CPSC investigation (Schaeffer et al., 1995). There are two types of urethane carpet cushion. Prime urethane cushion is freshly manufactured flexible polyurethane foam. The more popular bonded urethane is produced from polyurethane foam scrap. BHT is used as a heat stabilizer in the production of the polyurethane foam. It was emitted from all prime urethane (six samples) and bonded urethane (four samples) cushion samples tested in small environmental chambers under conditions representative of indoor environments. These cushion samples were collected and tested by CPSC staff over a period from 1993 to 1995.

A study was initiated under contract with Air Quality Sciences (AQS) to evaluate the sensory irritation potential of BHT and other selected chemicals emitted from carpet and carpet cushions. After reviewing supplemental AQS laboratory data records on BHT, CPSC staff determined that considerable measurement errors may have occurred and the results could not be relied on for estimating the sensory irritation potential of BHT in humans. Staff also reviewed and analyzed a sensory irritation study of BHT sponsored by the Carpet and Rug Institute and conducted by DuPont (Stadler, 1997). In this study, BHT was found to be a relatively potent sensory irritant using a standardized mouse bioassay. The RD_{50} was estimated to be 3.6 ppm with a fairly wide 95% confidence interval of 2.1 ppm to 36 ppm. Staff determined that the RD_{50} was derived from three experiments in which the respiratory rate responses were not optimally spaced and produced a linear regression line that did not fit well.

In the last six months, CPSC undertook two additional studies in order to obtain more reliable information on which to assess the potential hazard to indoor occupants from exposure to BHT emitted from newly installed polyurethane carpet cushion. With the cooperation and assistance of the Carpet Cushion Council, CPSC Field staff collected 20 bonded urethane and 10 prime urethane cushion samples from different manufacturing mills around the country. An additional five bonded and four prime urethane samples were collected from retail outlets. The samples were tested for BHT emissions by the CPSC staff using the small environmental chamber test method (see attached report). The method recently underwent a successful comparison study with three other testing laboratories (Bhooshan and Chen, 1997). CPSC also contracted with IIT Research Institute (IITRI) to conduct another sensory irritation study of BHT. The objective was to confirm the previously

reported sensory irritation data and obtain more accurate estimates of the RD_{50}[1] and other benchmarks with narrower confidence: intervals.

This report summarizes the results of the new studies and uses the data to assess the potential risk of sensory irritation to consumers.

II. BHT Emission Study

The attached CPSC laboratory report describes the emission testing of recently collected polyurethane cushions for BHT. Briefly, a 36 square inch piece of cushion was placed in a 52 liter stainless steel environmental chamber maintained at 23^0 C. Humidified nitrogen was passed through the chamber at 0.85 liters min" (1 .O air changes per hour). After 24 hours, a known volume of chamber effluent is delivered to multisorbent tubes which trap the airborne BHT. The BHT is then desorbed onto a gas chromatograph-mass spectrometer for analysis. Past emission studies have shown that chamber concentrations of BHT typically reach near steady state levels by 24 hours and decline very slowly over several weeks.

The 24 hour emission rates of BHT for the 20 bonded urethane cushions and 10 prime urethane cushions obtained from the manufacturing line are presented in Figures 1A and 1B, respectively. BHT emission rates from all samples ranged from 0.024 to 0.648 mg $m^{-2} h^{-1}$. The average BHT emission rates from bonded urethane cushion samples (0.21 ± 0.12 mg $m^{-2} h^{-1}$) and prime urethane cushion samples (0.19 ± 0.15 mg $m^{-2} h^{-1}$) are similar. Two prime urethane samples were manufactured without BHT and, as a result, did not emit detectable amounts of this compound. The BHT emission rates for the nine retail polyurethane cushion samples (0.11 ± 0.06 mg $m^{-2} h^{-1}$) are presented in Figure 1C and tend to be lower than the production line cushion samples.

The homogeneity in the BHT emission rate from multiple pieces of the same sample was examined for one prime and one bonded urethane cushion (see attached report). The observed intra-sample variability in emission rate from these cushions was nearly as large as that between samples collected from different mills. This suggests that multiple pieces may need to be tested to accurately characterize the BHT emission rate from any particular carpet cushion sample. The emission rate variability is not explained by heterogeneity in the BHT content of the cushion matrix. Unlike the BHT emission rate, the bulk BHT content of several pieces of the same sample was relatively uniform for both the bonded and prime urethane cushion. However, there was no clear correlation between the BHT content and the BHT emission rate from the tested samples. This suggests that other factors contribute to the rate at which BHT is emitted from the cushions.

---

[1] The exposure concentration that produces a sensory irritation-induced 50 percent drop in respiratory rate of mice challenged with different concentrations of a test vapor.

The BHT emission rates measured from the polyurethane cushion samples collected in 1997 were generally lower than the BHT emission rates from the same type of cushion samples collected and tested two to four years earlier. This is illustrated by the cumulative frequency distributions in Figure 2. The average peak BHT emission rates for the 1993/95 samples was 0.611 ± 0.320 mg $m^{-2} h^{-1}$ and ranged from 0.23 to 0.99 mg $m^{-2} h^{-1}$. This is about three-fold higher than the current BHT emission rates. The reason for the apparent decline in BHT emission rates from 1993/95 to present is not known.

III. Sensory Irritation Study of BHT

Sensory irritation was evaluated using the standardized mouse bioassay, ASTM E981. These experiments are described in the attached Final Contract Report from IITRI. Briefly, four mice per experiment were restrained, head only, in an animal chamber. They were exposed to BHT vapor for a 30 minute period in which respiratory rate and pattern were measured. Sensory irritation was identified by a characteristic change in the breathing pattern and a concentration-dependent drop in the respiratory rate of the mice. The sensory irritant activity of airborne chemicals can be described by the RD_{50} and other benchmarks determined from the analysis of the log concentration-respiratory rate depression response curve. Using ASTM E98 1, it has been shown that chemicals that cause sensory irritation in mice are also able to cause sensory irritation in humans. However, the human symptoms occur at lower irritant concentrations than those required to trigger respiratory changes in mice.

The log concentration-respiratory response curve for four positive sensory irritation experiments with BHT is presented in Figure 3. BHT vapor concentrations between about 30 ppm (275 mg m^{-3}) and 85 ppm (765 mg m^{-3}) produced a concentration-dependant decrease in the respiratory rate of mice between 20 and 63 percent. The linear correlation between the log concentration and response is excellent with a correlation coefficient of 0.99. The RD_{50} is estimated to be 60 ppm (532 mg m"') the RD_{20}, is estimated to be 31 ppm (282 mg m"). The slope of the log concentration-response relationship is 109. This is steeper than most sensory irritants in which the slope is generally around 40. Experiments at two lower exposure concentrations showed decreases in the breathing rate of the mice by less than 10 percent. Respiratory depressions of less than 12 to 15 percent are considered within the normal variation in respiratory rate of the mice.

The RD_{50} for BHT determined by IITRI is substantially higher than the RD_{50} of 3.6 ppm (32 mg m") reported by DuPont. This is probably due to the poor collection efficiency of the vapor sampling method used to determine the BHT exposure concentrations in the DuPont study. In that study, BHT vapor concentrations were measured after trapping the vaporized BHT in acetone over a 10 minute period. IITRI demonstrated that acetone impinger trapping produced 10-20 fold lower BHT measurements than direct vapor sampling of a test atmosphere. Although the cause was not definitively determined, the most likely explanation was loss of BHT while drawing the air stream into the acetone. Once the BHT reached the acetone, the trapping efficiency was shown to be nearly 100 percent. During its

experiments, IITRI determined the BHT vapor concentration at the breathing zone of the mice by directly collecting and analyzing four to five air samples per experiment. This collection method was validated with a known BHT vapor concentration prior to animal testing. The IITRI-derived RD_{50} was determined from four vapor concentrations that produced respiratory rate responses that were well spaced over the range of interest (respiratory depressions between 15 and 70 percent). The result was a linear regression line with a good fit and benchmarks with tight confidence limits. The RD_{50} reported by DuPont was determined from three experiments in which the respiratory rate responses were not optimally spaced and produced a linear regression line that did not fit as well. Consequently, the confidence intervals around the RD_{50} estimated from this study are wider.

IV. Determination of Acceptable Air Limits for BHT

A multi-benchmark approach is used to determine a range of plausible air concentrations for BHT below which most individuals would not be expected to experience sensory irritation. The RD_{50} has been traditionally used as the benchmark of choice when utilizing sensory irritation data in mice to estimate air levels unlikely to cause sensory irritation in humans. This approach relies on a single point along the log concentration-response curve and does not factor in the slope (how quickly the degree of sensory irritation increases with increasing concentration) or how well the experimental data fit the regression line. This has particular relevance for BHT since the slope of the log concentration-response is higher than is typically encountered.

In order to address the weaknesses of the single benchmark approach, four benchmarks are employed to define a reasonable range of acceptable air limits values (AAL_{SI}s) in humans. Besides the RD_{50}, the RD_{20} was chosen as an additional benchmark to account for the slope of the log concentration-response curve. The RD_{20} approximates the minimum respiratory depression that represents a clear irritant response in the mice. Two other benchmarks were calculated that depend on how well the experimental data fit the regression line. These were the respective 95 percent lower confidence limits on the RD_{50} (LCL_{50}) and RD_{20} (LCL_{20}). The RD_{50} and LCL_{50} are divided by uncertainty factors (UFs) of 100 and 300. These UFs are among those commonly recommended to account for differences in sensory irritation between mouse and human and variation within the human population. The RD_{20} and LCL_{20} are divided by UFs of 15 and 45. These values are derived from the previous UFs (100 and 300) and adjusted for the log concentration-response slope of approximately 40 exhibited by most sensory irritants tested using ASTM E98 1.

The AAL_{SI} estimates for BHT range from 1.53 mg m^{-3} (based on the $LCL_{50}/300$) to 18.8 mg m^{-3} (based on the $RD_{20}/15$). This represents a range of scientifically plausible AAL_{SI}s (Table 1). The median AAL_{SI} ($RD_{50}/100$ and $LCL_{20}/45$) is 5.3 mg m^{-3}. 'It must be emphasized that this range of AALs for BHT are those reasonably expected to prevent sensory irritation in most healthy individuals and may not necessarily protect against other toxicities. Other than sensory irritation, the toxicity of BHT has not been extensively studied by the inhalation route.

Table 1. Range of Acceptable Air Levels (AAL_{SI}) for BHT-Induced Sensory Irritation

Benchmark	mg m^{-3}	UF	AAL_{SI} (mg m^{-3})
LCL_{50}	461	300	1.53
RD_{50}	531	300	1.77
LCL_{50}	461	100	4.61
RD_{50}	531	100	5.31
LCL_{20}	238	45	5.30
RD_{20}	282	45	6.27
LCL_{20}	238	15	15.89
RD_{20}	282	15	18.81

V. Exposure Assessment and Risk Characterization

Exposure concentrations of BHT following carpet and cushion installation in a residence can be predicted using an indoor mass balance model (Sparks et al., 1993). This application of the model treats the residence as a single compartment using the input parameters presented in Table 2 below. The air exchange rate, compartment volume, and loading factor (the carpeted area relative to the residence volume) are representative of a typical residence in an enclosed condition (windows and doors closed to the outside) and installed with wall to wall carpeting. The model assumes that the air throughout the residence is well mixed, the only indoor source of BHT is the carpet/cushion assembly, and BHT does not undergo reactive decay.

The emission function term, R(t), describes BHT emissions from carpet cushion placed underneath carpet, as normally installed in a residence. It contains two parts in the form; $R(t) = F_d(t)*F_c(t)$. The F,(t) describes the BHT emission from the cushion as a function of time, in the absence of a carpet overlay. Chamber emission experiments with polyurethane carpet cushion samples over an extended time period show that the BHT chamber concentrations reach a steady state condition by 24 to 48 hours. These levels are maintained over several weeks before any noticeable decline in chamber concentration takes place. The F,(t) for BHT over this time period can be approximated by the emission rate measured at 24 hours (R_{24}). The $F_d(t)$ empirically describes the time-dependant delay in chemical emissions as a result of the diffusion barrier provided by the overlaid carpet. It was determined from chamber experiments with carpet/cushion assemblies. Carpet placed over polyurethane cushion lowers the peak chamber concentrations, increases the time required to reach peak levels, and prolongs the time period over which BHT is emitted from the cushion. The

emission function that best fits the time-dependent delay in reaching the BHT steady state emission rate is $A*time^{k}*R_{24}$ where $A=0.013$ and $k=0.6$. The steady state emission rate of BHT from assembly experiments was 30 to 40 percent of that measured from the cushion alone. This was approximated by a steady state emission function of $0.35*R_{24}$.

Table 2. Indoor Air Model Parameters

Dimension and Air Flow Parameters	Value
Air Exchange Rate	0.35 h^{-1}
Residence Volume	400 m^3
Loading Factor	0.33 $m^2 m^{-3}$
BHT Source Emission Parameters	
Emission Model	$R(t) = F_d(t)*R_{24}$
Carpet Diffusion Function $[F_d(t)]$ prior to steady state	$0.013*time^{0.6}$
Carpet Diffusion Function $[F_d(t)]$ at steady state	$0.35*time^{0}$
BHT Emission Rate $[R_{24}]$	0.02-0.65 mg $m^{-2} h^{-1}$

The model predicts that peak BHT concentrations will not be reached until 8 to 10 days following cushion installation. Increases in residential ventilation are expected to reduce the BHT air level but the increased ventilation needs to be maintained over several weeks to months due to the prolonged emissions. Short-term fresh air ventilation is not expected to be an effective means of lowering indoor BHT concentrations. The residential BHT levels were estimated assuming negligible sink effects (adsorption onto surfaces). Significant BHT adsorption/desorption onto indoor surfaces can delay the time required to reach peak BHT air levels and prolong the time period over which BHT is emitted. However, the model does not predict a profound reduction in peak: BHT concentrations unless significant adsorption is combined with very low desorption rates.

Table 3. Comparison of Indoor Model-Predicted BHT Concentrations After Carpet Cushion Installation with AAL_{SI}s for BHT-Induced Sensory Irritation

Data Set	BHT Concentration (mg m") Range	Median
Levels Predicted From 1993/95 Cushion Emission Data	0.08 - 0.34	0.19
Levels Predicted From 1997 Cushion Emission Data	0.00 - 0.22	0.06
AAL_{SI}s Based on IITRI Sensory Irritation Data	1.53-18.81	5.30

The peak residential air concentrations of BHT estimated by the exposure model for the recently collected polyurethane carpet cushions ranged from 0.00 to 0.22 mg m^{-3} with a median value of 0.06 mg m^{-3} (6.3 ppb). This is more than 25-fold less than the most conservative AAL_{SI} estimate from the IITRI sensory irritation data (Table 3). Residential BHT concentrations predicted from the 1993/95 cushion emissions data are also well below the range of plausible AAL_{SI}s estimated from the IITRI data.

VI. Conclusion

The amount of BHT emitted from the recently collected bonded urethane and prime urethane cushion samples as typically installed in a residence are not expected to produce indoor air concentrations of sufficient magnitude to cause eye and upper respiratory irritation in most individuals. The lowest plausible AAL_{SI} estimate derived from the IITRI sensory irritation data are 7-fold higher than the highest predicted residential BHT air concentration and 25-fold higher than the median BHT air concentration. All plausible IITRI-derived AAL_{SI}s are also higher than the highest predicted BHT air concentration based on emission rates measured from polyurethane cushions collected in 1993/95. The 1993/95 BHT emission rates were about three-fold higher than those measured from the 1997 cushions.

VII. References

Bhooshan, B. and Chen, S.B. (1997). Report on the Inter-Laboratory Study to Measure BHT Emission Rates From a Prime Urethane Carpet Cushion. U.S. Consumer Product Safety Commission Memorandum, Washington, DC.

Inkster, S.E. (1995). Summary of Carpet Cushion Complaints In -Depth Investigations Data Base-Current Status. U.S. Consumer Product Safety Commission Report, Washington, DC.

Schachter, L. (1990). Carpet Related Health Complaints. U.S. Consumer Product Safety Commission Report, Washington, DC.

Schaeffer, V.H., Bhooshan, B.B., Chen, S.B., Sonenthal, J., and Hodgson, A.T. (1995). Volatile Organic Chemical Emissions From Carpet Cushion. U.S. Consumer Product Safety Commission Report, Washington, DC.

Schaper, M. (1993). Development of a Database for Sensory Irritants and Its Use in Establishing Occupational Exposure Limits. *Am. Ind. Hyg. Assoc. J. 54: 488-544.*

Sparks, L.E., Tichenor, B.A., and White, J.B. (1993). Modeling Individual Exposure From Indoor Sources. In: *Modeling of Indoor Air Quality and Exposure,* Nagda, N.L. (ed.), pp. 245-256, ASTM, Philadelphia, PA.

Stadler, J.C. (1997). Inhalation Sensory Irritation (RD_{50}) Studies in Mice with Selected Carpet Chemicals - Part II. Haskell Laboratory Report Number 299-96, E.I. du Pont de Nemours and Co., Newark, DE

Attachment

FIGURE 1A

BHT EMISSIONS FROM CARPET CUSHION

Bonded Urethane

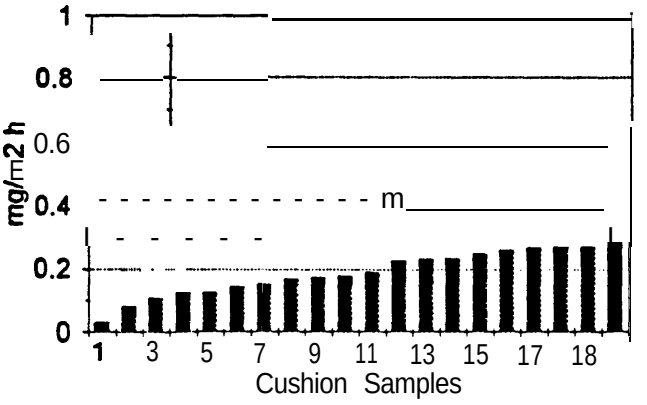


FIGURE 1B

BHT EMISSIONS FROM CARPET CUSHION

Prime Urethane

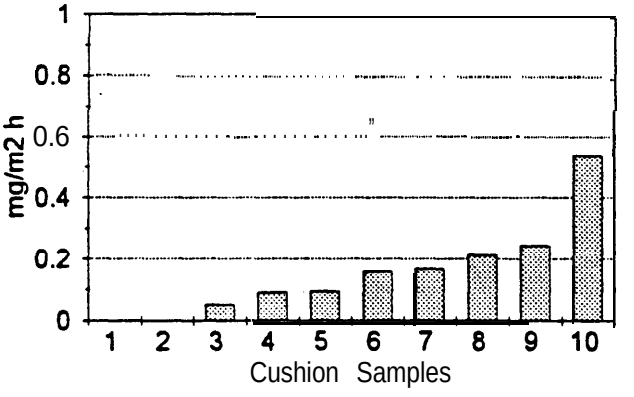


FIGURE 1C

BHT **EMISSIONS** FROM CARPET **CUSHION**

Retail

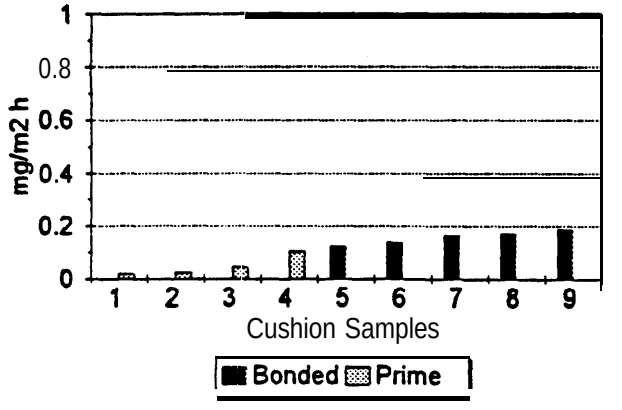


FIGURE 2

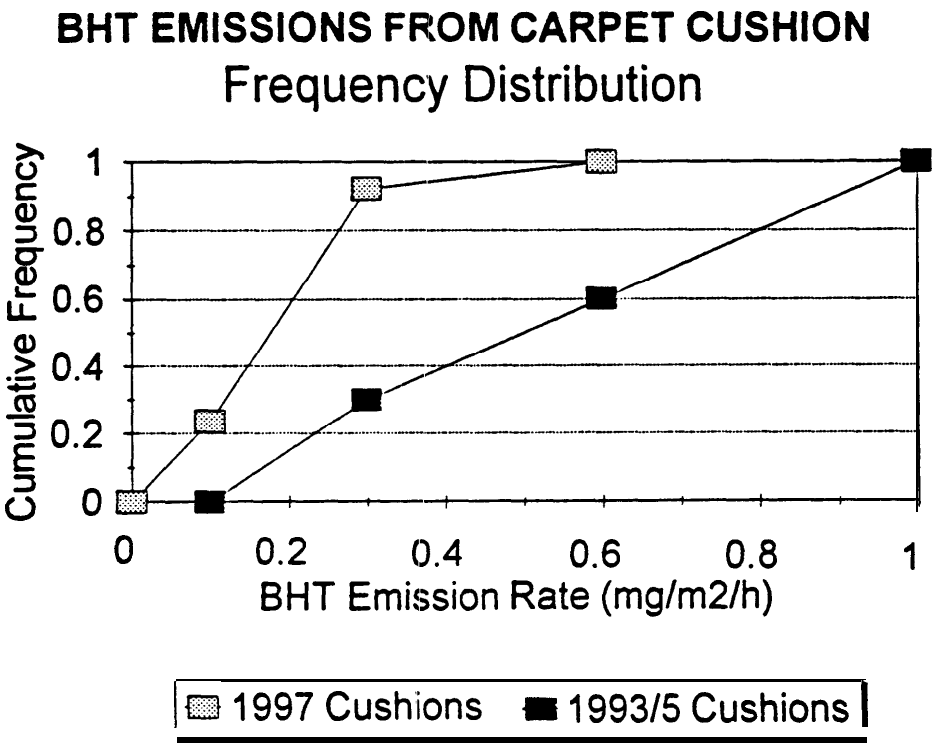
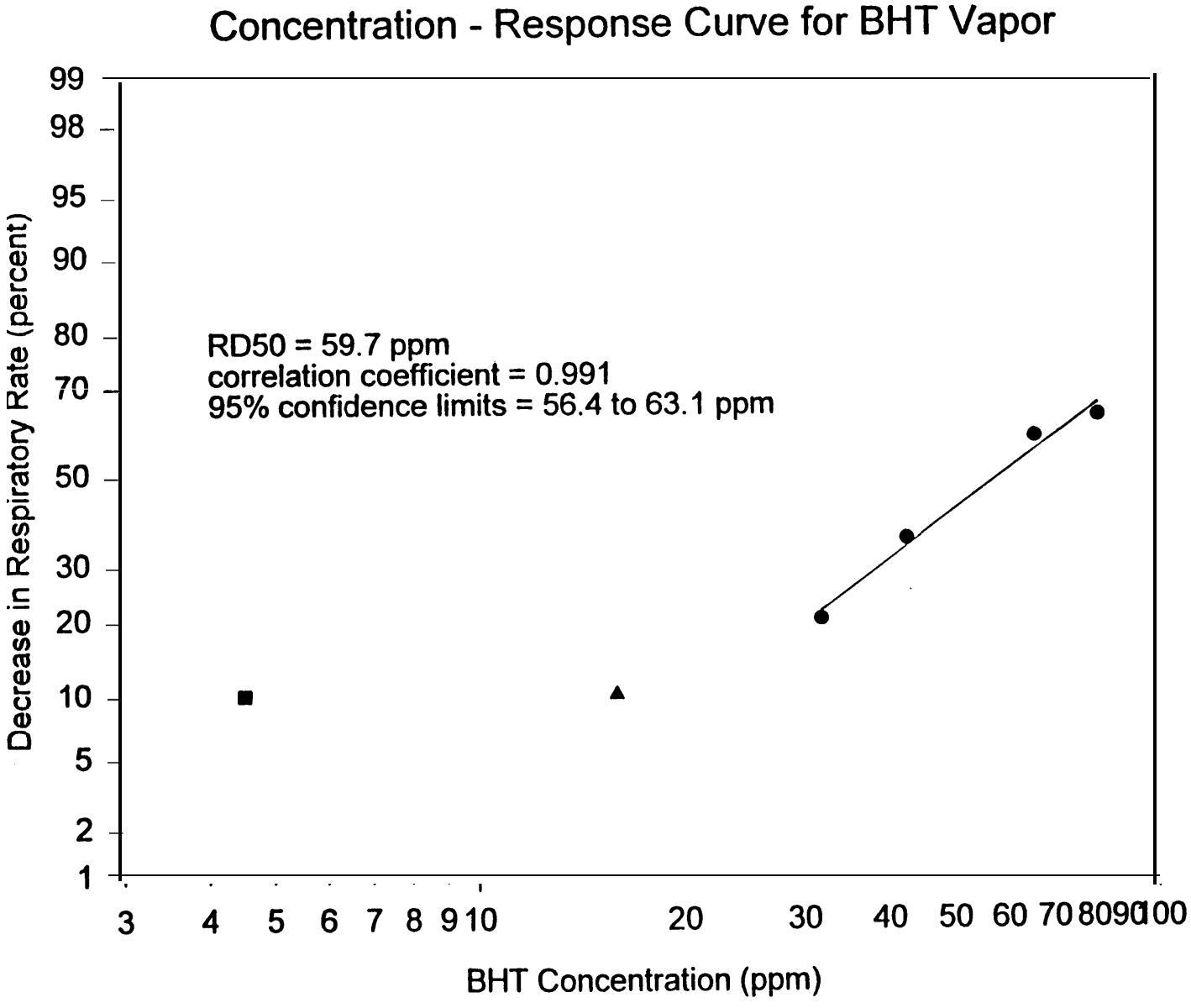

BHT EMISSIONS FROM CARPET CUSHION
Frequency Distribution
Cumulative Frequency
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
BHT Emission Rate (mg/m2/h)
1997 Cushions
1993/5 Cushions

Figure 3


Concentration - Response Curve for BHT Vapor
RD50 = 59.7 ppm
correlation coefficient = 0.991
95% confidence limits = 56.4 to 63.1 ppm
Decrease in Respiratory Rate (percent)
99
98
95
90
80
70
50
30
20
10
5
2
1
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
100
BHT Concentration (ppm)

ATTACHMENT

BHT Release from Carpet Cushions

Bharat Bhooshan
Chemist
U.S. Consumer Product Safety Commission
10901 Darnestown Road
Gaithersburg, MD 20878

February 27, 1998

I. BACKGROUND

The U.S. Consumer Product Safety Commission (CPSC) has identified butylated hydroxytoluene (BHT) as the major chemical released by urethane carpet cushions. It is used as a heat stabilizer in the production of flexible polyurethane foam. BHT has been reported to cause sensory irritation in mice and therefore, has the potential to cause similar effects in humans.

The Division of Chemistry (LSC) of the Laboratory Sciences Directorate has developed a small chamber methodology to measure emission rates of BHT emitted by urethane cushions. The method involves placing a carpet cushion sample in a simulated indoor environment and collecting air samples on sorbent tubes that are analyzed by gas chromatography/mass spectrometry (GUMS).

Recently, CPSC initiated a study to determine BHT emission rates from urethane carpet cushions available in the U.S. market. In addition, studies were conducted to address the question of BHT homogeneity in a carpet cushion sample. This report summarizes results obtained from both of these studies.

II. EXPERIMENTAL

A. CARPET/CUSHION SAMPLES

With the cooperation of the Carpet Cushion Council (CCC), the Field Offices of CPSC collected 30 urethane carpet cushions (20 bonded and 10 prime) directly from the manufacturing mills. Each sample, approximately 1 foot x 6 feet, was placed in a polyvinylfluoride (pvf) bag; the opening of the bag was folded twice and sealed with a heavy

duty tape. Upon arrival at the LSC, each sample was heat sealed in the same bag. Nine cushion samples (five bonded and four prime) were also collected from retail stores and analyzed for the emission of BHT.

The samples remained in the pvf bags until tested. The cushion sample to be analyzed was removed from its pvf bag and a 6 inches x 6 inches piece removed for testing. Immediate resealing the bag preserves the chemical integrity of the remaining unused cushion sample. The used piece was discarded at the end of each experiment.

B. EXPERIMENTAL PROTOCOLS

The 36 square inch piece of the cushion sample with mesh side ‘up was placed in an environmental chamber. Air samples were collected in duplicate at 0(blank) and 24 hours on clean sorbent tubes. The tubes were analyzed by GC/MS to determine the emission rates of BHT released by this cushion sample. Two cushion samples were tested simultaneously in the two environmental chambers available. Details of the environmental chamber operations, BHT analysis by GC/MS and sample calculations are given in APPENDIX A.

Two cushion samples were studied to examine the homogeneity of BHT emissions from carpet cushions. This task was accomplished in two ways. First, small pieces (1" x 4”) of cushion from

---

‘Cushions are typically installed with mesh side up. Studies conducted at LSC found similar levels of BHT emission rates whether the mesh side was up or down.

various locations (5 to 6) of a sample were extracted with methylene chloride by Soxhlet extraction and the extract analyzed for BHT content. Secondly, four 36 square inch pieces of a cushion were removed from four known locations of a sample and each piece studied for the emission of BHT in the environmental chamber. These results were used to correlate BHT emission rate with the BHT content.

III. RESULTS & DISCUSSION

A. BONDED CUSHIONS

Table 1 shows data for the 20 bonded cushion samples analyzed during this study. The BHT emission rates for all the bonded cushion samples ranged from 34 to 648 $\mu g/M^2.hr$ with an average emission rate of 208 ± 123.8 $\mu g/M^2.hr$.

B. PRIME CUSHIONS

Table 2 shows data for the 10 prime cushion samples collected from the manufacturing mills. Of these, two samples did not emit detectable levels of BHT. Information collected from the mills indicated that BHT was not used in the production of these cushions. The BHT emission rate for the remaining eight prime cushion samples ranged from 51 to 535 $\mu g/M^2.hr$ with an average emission rate of 194 ± 152 $\mu g/M^2.hr$.

C. CUSHION SAMPLES COLLECTED FROM RETAIL STORES

Table 3 gives data for the nine (four prime and five bonded) cushion samples obtained from the retail stores. The average BHT emission rate for prime and bonded cushions is 48 ± 39 and 157 ± 26 $\mu g/M^2.hr$, respectively. For prime cushions, these values are lower than the average value obtained for samples collected directly from the manufacturing mills (Table 2). The difference may be attributed to aging; however, the process of storage, transportation and sample handling may also contribute to it.

D. STUDIES RELATED TO THE HOMOGENEITY OF BHT IN CARPET CUSHIONS

Two samples (one bonded (97-896-7431) and one prime (97-896-7521) were studied for homogeneity. Both of these cushions had a thickness of 7/16". This study was performed in two ways (by extraction and by emission) as discussed in the protocol. These cushions showed the highest BHT emission rates during this testing.

1. BHT in cushion extracts-

Six small pieces (about 1"x 4") of a cushion sample from various known locations were extracted with methylene chloride, usiag Soxhlet extraction apparatus, and the extract analyzed for BHT content. The results are shown in Table 4. The data suggest that the concentration of BHT is fairly uniform throughout a cushion matrix in prime cushion as well as in bonded cushion. The average BHT concentration in prime cushion was found to be 900 $\mu g/g$ (454 mg/M^2) in prime cushion and 1864 $\mu g/g$ (3281 mg/M^2) in bonded cushion. On weight basis, the bonded cushion contains two times the level of BHT in prime cushion. But on surface area basis, this level increases to over seven times. Overall these data raise the possibility that bonded cushion may continue to emit BHT over a longer period of time.

2. BHT emission rates-

Four 36 square inch pieces of each sample (from known locations and having all fresh edges) are analyzed in environmental chambers. The results are shown in Table 5. The BHT emission rate from six pieces of the same prime cushion sample had a range from 182 to 535 $\mu g/M^2.hr$, with an average value of 315 ± 137 $\mu g/M^2.hr$ and indicating approximately a three-fold variation within a sample. Similarly, the BHT emission rate from nine pieces of the same bonded cushion sample had a range of 50 to 648 $\mu g/M^2.hr$, with an average value of 398 ± 190 $\mu g/M^2.hr$ and showing a thirteen fold variations within a sample. However, if the value 50 is dropped, the variation is approximately two-fold. These results suggest that two to three pieces of the same cushion sample have to be analyzed in order to adequately characterize the BHT emission rate.

V. CONCLUSIONS

A total of 39 carpet cushion samples were analyzed for BHT emission rates using stainless steel environmental chambers. Of these, 30 samples (20 bonded and 10 prime) were obtained from the manufacturing mills and nine samples were obtained from retail shops. The average BHT emission rate for the 20 bonded cushion samples and the 10 prime cushion samples, was 208 ± 124 $\mu g/M^2.hr$ and 194 ± 152 $\mu g/M^2.hr$, respectively. Two prime urethane cushion samples did not emit detectable amounts of BHT. In general, prime and bonded cushions gave similar BHT emission rates. Also, prime cushion samples obtained from retail stores had lower values of BHT emission rates than those collected directly from the manufacturing mills.

Two Samples (one bonded and one prime) were studied to determine the homogeneity of BHT emissions from carpet cushions. The extraction studies indicate that BHT is distributed more or less uniformly throughout a cushion sample. Additionally, on the basis of weight as well as surface area, the bonded cushion sample contains more BHT than the prime cushion sample. The amount of BHT emitted from multiple pieces of the same cushion sample could vary three to thirteen fold. This suggests that multiple pieces may have to be tested in order to adequately characterize the emission rate of a cushion sample.

TABLE 1

Emission rates of BHT from bonded cushions collected from various mills in the US

#	Sample #	BHT emission rates ($\mu g/M^2.hr$)	Avg ± SD
1	97-6336	34	208 ± 124
2	97-4117	83	
3	97-3359	109	
4	97-3743	126	
5	97-6113	128	
6	97-6114	145	
7	97-4145	154	
8	97-5807	169	
9	97-3508	175	
10	97-4286	179	
11	97-3744	192	
12	97-1537	228	
13	97-7388	233	
14	97-3505	236	
15	97-7312	249	
16	97-2353	261	
17	97-4013	269	
18	97-7522	271	
19	97-1538	271	
20	97-7431	648	

TABLE 2

Emission rates of BHT from prime cushions collected from various mills in the US

#	Sample #	BHT emission rates ($\mu g/M^2.hr$)	Avg ± SD
1	97-2352	51	194 ± 152
2	97-4012	90	
3	97-6337	94	
4	97-3509	158	
5	97-3506	166	
6	97-5806	213	
7	97-7389	243	
8	97-7521	535	
9*	97-6047	0	
10*	97-4146	0	

* During sample collection, the manufacturing mills reported that BHT was not used in the production of these cushions.

TABLE 3

Emission rates of BHT from cushions collected from various retail shops in the US

#	Sample #	Cushion Type	BHT emission rate ($\mu g/M^2.hr$)	Avg ± SD
1	98-3785	Prime	19	48 ± 39
2	98-0707	Prime	24	
3	98-5856	Prime	45	
4	984503	Prime	104	
1	984502	Esonded	124	157 ± 26
2	98-4252	Esonded	166	
3	984251	Esonded	187	
4	98-5850	Esonded	172	
5	98-5851	Esonded	138	

TABLE 4

BHT concentration in various pieces of two cushion samples

Sample #	Location of cushion piece	Cushion weight (g)	BHT found (µg)	BHT in cushion (µg/g)	BHT in cushion (mg/M^2)
97-7521" (prime)	Right upper	1.304	1161	890	450
	Right lower	1.312	1169	891	453
	Left upper	1.295	1183	914	459
	Left lower	1.298	1160	894	450
	Left Middle	1.302	1202	923	467
	Right Middle	1.291	1145	887	443
Average				900	454
97-7431** (bonded)	Right upper	4.546	8252	1815	3198
	Right lower	4.539	8364	1843	3241
	Left upper	4.543	9078	1998	3519
	Left lower	4.547	8474	1864	3284
	Left Middle	4.541	8179	1801	3170
	Right Middle	4.544	8451	1860	3275
Average				1864	3281

*Five µl of the methylene chloride extract analyzed.
**One µl of the methylene chloride extract analyzed.

'TABLE 5

Emission rates of BHT from two cushion samples analyzed to study the homogeneity of BHT emissions

Sample #	Cushion Type	Location of cushion piece under test	BHT emission rate ($\mu g/M^2.hr$)	Average
97-7521	Prime	Right upper	417	315 ± 137
		Right lower	535	
		Left upper	194	
		Left lower	273	
		Left Middle	182	
		Right Middle	290	
97-7431	Bonded	Right upper	398	398 ± 190
		Right lower	648	
		Left upper	315	
		Left lower	294	
		Middle	50	
		Middle right upper	338	
		Middle right lower	631	
		Middle left upper	346	
		Middle left lower	565	

APPENDIX A

I. CHAMBER OPERATIONS

An incubator maintains the temperature of the two 52 liters stainless steel environmental chambers at 23±1°C. A stainless steel tray holds the piece of cushion in the middle of the environmental chamber. Vaporized liquid nitrogen provides the carrier gas for the chamber. Part of the carrier gas passes via a loop through two bubblers that contain distilled water to provide humidity to the system. The chamber maintains a relative humidity (RH) of 50 ± 5 percent by means of adjusting nitrogen flow with a needle valve in the loop. A digital thermohygrometer (Cole-Parmer model # AH37950-10) measures the RH of the chamber effluent. A digital flow meter (J & W Scientific # ADM1000) is used to measure the flow rate of the chamber effluent at the start of each experiment. A mass flow controller (Model FC-260, Tylan General, 359 Van Ness Way, Torrance, CA 90501) maintains a gas flow of 0.85 ± 0.04 liters/minute through the chambers to provide an air exchange rate of one per hour. Placing a 36 square inch piece of cushion in a 52-liter chamber represents a loading factor of 0.45 square meter per cubic meter. Carpeting in a typical house has a loading factor in the range of.0.3 to 0.5 square meter per cubic meter. Other laboratories may use chambers that are larger or smaller but the loading factor of 0.45 M^2/M^3 shall be maintained.

Multi-sorbent tubes connected to the chamber effluent adsorb the BHT. The reusable multi-sorbent tubes used by LSC contain glass beads, Tenax, Ambersorb, and charcoal (ST-32, Envirochem, Inc., Kemblesville, Pa 19347). Heating the tubes at 300°C for 30 minutes with nitrogen gas flowing through them at 100 ml/minute removes the residual organics from the sorbents. The gas flow, during this cleaning process, is in reverse direction to the gas flow during sample collection. Storage of the conditioned tubes in a jar (containing desiccant and charcoal beads) at room temperature maintains their cleanliness lbefore use.

Samples for the analysis of BHT are collected by passing a known volume (using mass flow controllers from Tylan General and air pump model 107CA18 from Thomas Industries) of chamber effluent through a clean multi-sorbent tube. The sampling time will be 10 minutes with a flow rate set at 200 ± 3 ml/minute to give a total sampling volume of two liter. Immediately, another set of duplicate samples (reserve samples) will be collected for 5 minutes having a sampling volume of one liter. Reserve samples will be used for analysis when BHT levels are too high and fall out side the calibration curve. Normally, the sorbent tubes are analyzed within a week of sample collection. An earlier inter-laboratory study demonstrated that individual laboratories using a variety of packing material produced comparable results (Bhooshan and Chen, June 1997).

II. ANALYSIS of BHT by GC/MS

A UNACON model 810C Concentrator (from Envirochem, Inc.) transfers the trapped VOCs from multi-sorbent tubes to a Hewlett Packard 5890 Series II Gas Chromatograph (GC). A capillary column in the GC separates the VOCs into various components. A oven temperature program separates each compound present in the effluent. A Hewlett Packard 5971 MSD Mass Spectrometer (MS) analyzes each compound separated by the GC. The MS is run at mass range of *m/z* 33 to 300. The data are collected and stored on Chem Station. The operating parameters for the concentrator are:

- initial carrier flow time 7 minutes,
- secondary carrier flow time 4 minutes,

- trap to trap transfer time 3 minutes,
- trap to column transfer time 5 minutes, and
- desorption temperature 250°C.

The operating parameters for the GC are:

- column @B-l, 30 meter x 0.25 millimeter with film thickness 1.0 micrometer),
- oven temperature (160°C for 2 minutes, heat @ 4°C per minute to 210°C, and keep at 250°C for 5 minute),
- transfer line temperature 250°C, and
- carrier gas Helium at a flow of one milliliter/minute.

The mass spectrometer is calibrated using three point calibration curve (50 ηg, 100 ηg, and 200 ηg of BHT) for the quantitation of BHT. Additional calibration curves incorporating 300 ηg and 1000 ηg mass are prepared if needed. Elxtemal standard is used for quantitation. The sorbent tubes are loaded by injecting a standard BHT solution (in methanol) into a sorbent tube at room temperature while a carrier gas (nitrogen or Helium) is flowing through it. This is referred to as liquid phase loading. Some laboratories use vapor phase loading where a standard BHT solution is injected into a heated chamber (~250°C) and the resulting vapors are swept into a sorbent tube by a carrier gas. LSC has observed that both method give similar results.

A regression analysis of the peak areas corresponding to the three points of calibration curve provide the slope and the intercept of the calibration line. Each regression analysis also gives a value of R^2 which is typically between 0.95 and 0.99. Quantitation of BHT in samples collected from the chambers is accomplished by applying the regression equation to the peak ares measured for the unknowns. Dividing the amount (ηg) of BHT collected from the chamber effluent by the volume (liter) of the effluent sampled gives the concentration (ηg/L or μg/M^3) of BHT in the chamber.

III. CALCULATIONS

The emission rate of BHT is calculated from its concentration in the chamber as shown below.

$$\text{Emission Rate } (\mu g/M^2.hr) = \frac{V_{ch}\,(M^3) * \text{ACH (hi')} * C\,(\mu g/M^3)}{\text{Cushion surface area } (M^2)}$$

where V_{ch} = Chamber volume in M^3
ACH = Chamber air exchange rate (hr^{-1})
C = Chamber concentration of BHT $(\mu g/M^3)$

For a cushion sample six inches on a side and placed in a 0.052 M chamber (loading factor 0.447) with an air exchange rate of one, the above equation reduces to:

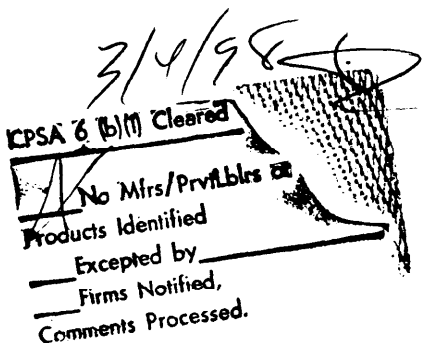
Emission Rate $(\mu g/M^2.hr)$ = Chamber concentration/Loading factor

= 2.232 * C

PULMONARY/SENSORY IRRITATION STUDY OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE

FINAL REPORT

IITRI Project No. LO6322
Study No. 1



Testing Facility:

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

Sponsor:

U.S. Consumer Product Safety Commission
4330 East West Highway
Bethesda, MD 20814

Sponsor Representative:
Val Schaeffer, Ph.D.

February 1998

This project has been funded with federal funds from the U.S. Consumer Product Safety Commission under contract number CPSC-R-97-5249. The content of the report does nor necessarily represent the views of the Commission, nor does mention of trade names, commercial products, or organizations imply endorsement by the Commission.

GLP COMPLIANCE STATEMENT

This study was conducted in accordance with U.S. Environmental Protection Agency (EPA) (TSCA) Good Laboratory Practice (GLP) Standards as set forth in the *Code of Federal Regulations (40* CFR 792), according to the Statement of Work (CPSC-R-97-5249) and appropriate modifications to ASTM Method E98 1-84 "Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals." All chemical analyses and attendant documentation pertaining to the characterization and stability of the bulk test substance were performed by the commercial supplier (Sigma Chemical Company). The raw data have been reviewed by the Study Director, who certifies that the results reported herein are consistent with and supported by the study raw data.

Scott Garthwaite 2-17-98

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

PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE

Study Initiation Date: December 1, 1997
Inhalation Exposure Date: December 1, 1997
Biophase Termination Date: December 23, 1997

SUMMARY

Butylated hydroxytoluene (BHT) vapor was administered by head-only inhalation exposures to six groups of four male Swiss-Webster mice each at graded vapor concentrations. The mice were held in plethysmograph tubes and their respiration monitored during a 30-minute exposure period, as well as for approximately 10 minutes prior to and following the exposure. The depressions in respiratory rates were <10%, <10%, 20.1%, 35.3%, 58.6% and 63.4% at vapor concentrations of 4.54 ppm, 116.0 ppm, 32.1 ppm, 42.9 ppm, 66.6 ppm, and 82.6 ppm, respectively. The calculated RD_{50} (concentration that produces a 50% decrease in respiratory rate) was 59.7 ppm (with a 95% confidence limit of 56.4 to 63.1 ppm). The RD_{20} (concentration that produces a 20% decrease in respiratory rate) was 30.9 ppm (with 95% confidence limits of 29.2 to 32.7 ppm).

The respiration pattern before, during, and after exposure was recorded on a chart recorder. A characteristic pause during expiration relative to control conditions was observed. At relatively high BHT concentrations, the pause became longer with a larger decrease in respiratory rate. This response confirms that exposure to BHT vapor caused a sensory irritation-induced respiratory response. No signs of pulmonary irritation or overt toxicity were found in the BHT exposed animals.

James M. Gerhart, Ph.D., D.A.B.T. — Date
Manager, Inhalation Toxicology
Life Sciences Research

Scott Garthwaite 2-17-98
Scott Garthwaite, B.S. Date
Study Director
Life Sciences Research

TABLE OF CONTENTS

I. INTRODUCTION .

The objective of this study was to determine the effect on respiratory rate in male mice following a 30-minute inhalation exposure of the test substance and, in the event of detectable irritation, to characterize the concentration-response including an estimation of the RD_{50} (the concentration that results in a 50 percent decrease in respiratory rate) and RD_{20} (the concentration that results in a 20 percent decrease in respiratory rate).

II. MATERIALS AND METHODS

A. Test Substance: The test substance, butylated hydroxytoluene (BHT, lot number 37H0294, purity > 99.9%), was received from Sigma Chemical Company (St. Louis, MO) on September 29. 1997. The test substance was a white, crystalline solid and was stored at room temperature. Analyses and attendant documentation pertaining to the characterization of the bulk test substance were supplied by Sigma.

B. Experimental Design: Six groups of four male mice were exposed to graded concentrations of the test substance via head-only inhalation. The test atmosphere was generated by lheating the test substance in a flask maintained in a water bath. The respiratory rate of each animal was measured prior to, during, and following a 3 O-minute exposuire. This method was based on the ASTM method E981-84, "Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals" and Alarie, Y., "Sensory Irritation of the Upper Airways by Airborne Chemicals," *Toxicol Appl Pharmacol* 42:279-297, 1973. Test substance exposures were performed on December 1, 1997 through December 23, 1997.

C. Animals: Male Swiss-Webster mice, approximately 1 month of age, were received from Hilltop Lab animals, Inc., Scottdale, PA on November 11, 1997, and weighed 18.2-21.3 g the next day. The mice were held in quarantine for at least one week during which time they were examined carefully to ensure their health and suitability as test subjects. Mice selected for the study were identified by an unique tail markings and by a cage card bearing the study number and corresponding animal number.

D. Food and Water: Purina Rodent Chow 5002 (PMI Feeds, Inc., St. Louis, MO) and City of Chicago water, supplied by water bottles, were available *ad libitum*, except during the exposure period.

E. Environment: The mice were group housed in polycarbonate cages, except during the inhalation exposure. The animal room temperature and relative humidity ranged

from 19.5 to 23°C and 35 to 61%, respectively. Fluorescent lighting was provided automatically for 12 hours followed by 12 hours of darkness.

F. Mortality and Body Weight: The animals were observed during the exposure for mortality and overt signs of toxicity. Body weights of the mice were collected prior to and after exposure. The pre-exposure weights were used for randomization.

G. Assignment to Groups: The mice were selected by an in-house developed computerized randomization procedure constrained by body weight and assigned to groups of four mice for each of six exposures.

H. Test Atmosphere Generation, Exposure and Monitoring:

Inhalation Exposure Six groups of four mice each were exposed to graded concentrations test substance vapor via head-only inhalation in a glass chamber. The volume of the chamber was approximately 2.5 I. Chamber temperature, humidity, dynamic flow conditions, aerosol concentration, and chamber oxygen concentration were monitored at about 5 minute intervals during the exposure period. The bodies of the mice were contained in plethysmograph tubes (Crown Glass Company, Somerville, NJ) that were connected to a pressure transducer that detects changes in pressure created by the inhalation and exhalation of the animal. While the animals were held in the plethysmograph tubes, an average resting or baseline respiration rate was recorded for approximately 10 minutes prior to exposure. Mice were then exposed to the test atmosphere for 30 minutes and respiration recorded. At the end of the exposure, the mice remained in the plethysmographs for approximately 10 minutes.

Test Atmosphere Generation The test atmosphere generation system consisted of a glass flask containing the liquid test substance, held in a temperature-controlled water bath. Filtered compressed air was directed through the flask, over the surface of the heated test substance. Additional ambient air was added for dilution. The temperature of the water bath and air flow rate through the generator and chamber were adjusted to produce the desired target concentrations. Six exposure atmospheres of the test substance were generated at mean concentrations of 4.54 ppm to 82.6 ppm.

Test Atmosphere Monitoring The concentration of test substance vapor in the exposure atmosphere was measured using gas chromatography (GC). Samples of the chamber atmosphere were taken directly from the chamber using gas-tight syringes and injected into a calibrated GC. The GC was calibrated using standards of the test

substance (same lot as was used for the inhalation exposures) prepared in acetone. Three GC calibration curves were used for exposure concentration determination, two typical calibration curves are in Table 2. Calibration checks were performed prior to exposure. Chamber samples were collected at approximately the beginning, middle, and end of the 30-minutes exposure period (3 to 5 samples per exposure). Details of the GC parameters are in Table 2. In addition, the concentration of aerosol particulates, if present, was determined using a Portable Continuous Aerosol Monitor (PCAM; PPM, Inc., Knoxville, TN).

I. Respiratory Rates: Respiratory rates were measured prior to, during, and following exposure by a computer program which determined the respiratory rates from the plethysmograph signal from each animal. The respiratory pattern for each animal was displayed in a chart recorder. Pressure transducers (physiological microphones) were used to detect pressure changes in the plethysmography tubes created by the inhalation and exhalation of the animal. This pressure signal was amplified by the chart recorder, displayed as a strip chart trace, and was also directed into the computer for determination of individual animal respiratory rates. Respiratory rates were determined at 15-second intervals during the pre-exposure, exposure, and post-exposure periods.

J. Necropsv: Animals were euthanized by carbon dioxide overdose immediately foilowing exposure. No necrospy was performed.

K. RD50 Calculation: The maximum decrease in respiratory rate during the 30-minute exposure period was determined at each of 4 (decreases greater than 10%) exposure concentrations from their averaged 15-second measurements. A linear regression using the log concentration versus the probit-transformed percentage decrease in respiratory rate was performed. The RD_{50}, RD_{20}, confidence limits, and linear correlation coefficient were calculated from the regression results.

L. Archives: All raw data generated at IITRI during the study and a copy of the final report will be kept in the IITRI archives for 2 years following the date of this report.

III. RESULTS

A. Test Atmosphere:

Chamber Conditions. Exposure chamber conditions are presented in Table 1. Chamber airflow, generator airflow, and generation temperature were adjusted to

control the concentration of the exposure atmosphere. The T_{99} equilibration time ranged from 0.3 to 0.6 to minutes (1% to 2% of the exposure duration). The average chamber temperature ranged from 71.5°F to 74.4°F for the all exposures conducted for this study.

Exposure Concentrations: Mean BHT vapor concentrations and individual sample concentrations during exposure are presented in Table 3. The aerosol concentration was at ambient levels during exposure (less than 10 $\mu g/m^3$). Therefore, the exposure was to test substance vapor only. The GC calibration curve was linear (Table 2) and no interference from other compounds was present in the GC chromatogram (Figure 8).

Preliminary Test Vapor Experiments: Several experiments were performed to characterize the GC sampling procedure.

(a) *Impinger Sampling*: Initially, two methods of chamber atmosphere sampling (direct vapor injections of the chamber atmosphere into the GC and impinger sampling) were considered. Impinger trapping was used in a previous BHT irritation study.

The trapping efficiency of the impinger method was determined (Table 5). During two trials, the front/rear impingers (two impingers in series) were analyzed for BHT concentration. Complete trapping of BHT in the front trap was achieved, no measurable BHT breakthrough into the second trap was found. The total trapping efficiency was 100%.

During test runs, not during the actual exposures, chamber samples were collected for simultaneous analysis by both direct vapor injection and with impinger samples. Four sets of impinger samples (two impingers in series, front and rear impingers analyzed separately) were collected. While the impinger samples were being collected, direct vapor samples were collected and analyzed.

The direct vapor samples show a stable vapor concentration but the impinger samples had variable results with measured concentrations much below the direct vapor results (Table 6) possibly due to poor collection efficiency as a result of BHT vapor loss (adsorption onto tubing, condensation, etc.) prior to being trapped by the acetone in the impinger.

The direct vapor injection method was considered to be the vapor sampling method of choice since there are fewer intermediate steps, less chance of sample loss, better precision, and more samples could be collected/analyzed during exposure.

(b) *Maximum Vapor Concentration: The* vapor pressure of BHT at 20°C is 0.01 mmHg according to the MSDS. Sigma Chemical Company (the supplier) indicated that this value was obtained from a standard reference source and was not measured by Sigma. Data from the NIST (Standard Reference Database 69 - August 1997)

indicated a vapor pressure based on the Antoine equation over a temperature range of 358.9 to 535.65 K as referenced by Ohe. S., **Computer Aided Data Book of Vapor Pressure,** Data Book Publishing Company, Tokyo, 1976, 2000, based on data by Stull, D.R., *Vapor Pressure of Pure Substances Organic Compounds,* **Ind. Eng. Chem., 1947, 39, 5 17-540.**

If a temperature of 20°C (293 K) is used in the Antoine equation a vapor pressure of 0.007 mmHg (approximately 0.01 mmHg) is obtained. However, this is an extrapolated value beyond the listed temperature range (358.9 K to 535.65 K; 85.9°C to 263°C). The temperature range of the Antoine equation corresponds to liquid BHT (melting point of 69°C, boiling point of 265 °C).

The vapor pressure of BHT at ambient conditions (20°C) is based on extrapolated data from liquid BHT which was originally published in 1947. As a result, the quality of vapor pressure data is insufficient to accurately calculate the theoretical maximum vapor concentration in the exposure atmospheres and the maximum vapor concentration was, therefore, determined experimentally.

The maximum vapor concentration of BHT in the chamber was experimentally determined by increasing the BHT chamber concentration to the point at which a slight, but measurable, amount of aerosol was present (about 100 $\mu g/m^3$). Then the concentration was slowly reduced until the aerosol just disappeared (maximum vapor concentration without aerosol present) and GC samples were taken and analyzed. The mean analyzed concentration was 127.1 ± 24.0 ppm.

Animal exposures (maximum concentration of 82.6 ppm, Group 4) were below the maximum vapor concentration. Aerosol concentrations during exposure were at background ambient levels (Table 1). The lack of measurable aerosol during exposure correlates with the fact that all of the animal exposures were at concentrations below the experimentally determined maximum vapor concentration.

(c) Vapor Sampling of Known BHT Concentration: An experiment was performed to verify that the direct vapor injection technique would yield quantitative results. A known amount of BHT dissolved in acetone and injected into a glass sampling bulb (250 cc volume). After equilibration, a vapor sample was removed from the bulb using a gas-tight syringe and injected into the GC. The analyzed vapor concentration was 0.884 ± 0.07 ppm and the prepared concentration (determined from the concentration of BHT in the spiking solution and the volume injected into the gas bulb) was 0.933 ppm, indicating that the sampling procedure was quantitative.

B. Mortality and Body Weight: One mouse died during the exposure to 66.6 ppm of BHT vapor. No other mice died during exposure to any other concentration. This animal most likely died from neck trauma combined with decreased ventilation from the irritation response and was probably not due to overt toxicity of the test

substance. Surviving animals were euthanized immediately following their respective exposures. Body weights were used for randomization purposes only. The mean body weights of animals selected for exposure were 27.9 g to 34.5 g. Post-exposure body weight loss that occured during test substance exposures was normal (typical of control animals).

C. Respiratory Rates: Mean respiratory rates calculated during the pre-exposure, exposure, and post-exposure periods for all exposure groups are presented in Table 7 and individual animal data is presented in Appendix A. This data was collected at 15-second intervals and averaged over 2-minute periods during the pre-exposure baseline period, 15-second intervals during the first 3 minutes of exposure, during 3-minute intervals during the rest of the exposure period, and for 2 minute intervals during the post-exposure period. Graphs of mean respiratory rate vs. exposure time are contained in Figures l-5.

D. Irritation Response: The respiration pattern before, during, and after exposure was recorded on a chart recorder. A characteristic pause during expiration relative to control conditions was observed. At higher BHT concentrations the pause became longer with a larger decrease in respiratory rate. This confirms that exposure to BHT vapor caused a sensory irritation-induced respiratory response. There was no evidence of a pulmonary irritation-induced respiratory response.

E. RD_{50} Calculation: The exposures at 4.54 ppm and 16.0 ppm caused no measurable effect (<10%) on respiratory rate so these two concentrations were not used in the RD_{50} and RD_{20} calculations. The linear regression concentration response curve for the four concentrations used to calculate the RD values is presented in Table 4. The calculated RD_{50} was 59.7 ppm, with 95% confidence limits of 56.4 ppm to 63.1 ppm. The calculated RD_{20} was 30.9 ppm, with 95% confidence limits of 29.2 ppm to 32.7 ppm. The slope of the dose-response curve was 2.924, with a Y-intercept of -0.193 and correlation coeffkient of 0.991.

Under the conditions of this study, exposure to BHT vapor caused sensory irritation in male mice. The depressions in respiratory rates were <10%, <10%, 20.1%, 35.3%, 58.6% and 63.4% at vapor concentrations of 4.54 ppm, 16.0 ppm, 32.1 ppm, 42.9 ppm, 66.6 ppm, and 82.6 ppm, respectively. The respiration pattern before, during, and after exposure was recorded on a chart recorder. A characteristic pause during expiration relative to control conditions was observed. At higher BHT concentrations the: pause became longer with a larger decrease in respiratory rate. This confirms that exposure to BHT vapor caused a sensory irritation-induced respiratory response.

IV. DISCUSSION

Under the conditions of this study, exposure to BHT vapor caused sensory irritation in male mice. The depressions in respiratory rates were <10%, <10%, 20.1%, 35.3%, 58.6% and 63.4% at vapor concentrations of 4.54 ppm, 16.0 ppm, 32.1 ppm, 42.9 ppm, 66.6 ppm, and 82.6 ppm, respectively. The respiration pattern before, during, and after exposure was recorded on a chart recorder. A characteristic pause during expiration relative to control conditions was observed. At higher BHT concentrations the pause became longer with a larger decrease in respiratory rate. This confirms that exposure to BHT vapor caused a sensory irritation-induced respiratory response. No evidence of pulmonary irritation or overt toxicity in the BHT exposed animals was found.

V. QUALITY ASSURANCE STATEMENT

Study Title: Pulmonary/Sensory Irritation Study of Butylated Hydroxytoluene (BHT) in Mice

Project Number: LO6322

Study Number: 1

Study Director: Scott Garthwaite, B.S.

This study was subjected to inspections and the report has been audited by the IITRI Quality Assurance Unit in accordance with the Environmental Protection Agency's (TSCA) "Good Laboratory Practice Standards" - 40 CFR 792. The report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

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

	Findings Reported To:	
Dates of Inspections:	Study Director	Management
11/13/97	11/13/97	11/13/97
12/1/97	12/2/97	12/2/97
2/11-13198	2/13/98	2/13/98

R. A. Boyne 2-17-98

Ronald Boyne, B.S. Date

Manager, Quality Assurance

VI. TABLES

PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE

TABLE 1

Individual Chamber Exposure Conditions

Exposure No. Group No.	Reading	Chamber Air Flow (l/min)	Chamber Temperature (°F)	Chamber Oxygen (percent)	Chamber Humidity (% RH)	Generator Air Flow (l/min)	Generator Temperature (°C)	Aerosol Concentration (µg/m³)
1	1	34	72.5	20.9	50.3	0.4	69.0	0
	2	34	72.8	20.9	49.9	0.4	69.1	0
4.54 ppm	3	34	73.0	20.9	40.4	0.4	69.1	0
	4	34	73.1	20.9	49.9	0.4	69.1	0
	5	34	73.1	20.9	50.0	0.4	69.1	0
	6	34	73.0	20.9	49.4	0.4	69.1	0
	mean	34	72.9	20.9	49.8	0.4	69.1	0
2	1	30	71.4	20.9	42.0	0.5	74.2	0
	2	30	71.5	20.8	48.4	0.5	74.3	0
16.0 ppm	3	30	71.5	20.9	50.3	0.5	74.3	0
	4	30	71.5	21.0	45.4	0.5	74.2	0
	5	30	71.6	20.9	44.8	0.5	74.3	0
	6	30	71.4	20.9	48.1	0.5	75.0	0
	mean	30	71.5	20.9	46.5	0.5	74.4	0
3	1	20	72.3	21.0	46.4	0.5	75.2	
	2	20	72.1	20.9	47.3	0.5	75.2	0
32.1 ppm	3	20	72.6	20.9	48.1	0.5	76.8	0
	4	20	72.7	20.9	47.6	0.5	76.9	0
	5	20	72.7	20.9	48.6	0.5	78.1	0
	6	20	72.7	21.0	47.5	0.5	78.1	0
	mean	20	72.5	20.9	47.6	0.5	76.7	0

TABLE 1 (cont)

Individual Chamber Exposure Conditions

Exposure Group No.	Reading No.	Chamber Air Flow (l/min)	Chamber Temperature (°F)	Chamber Oxygen (percent)	Chamber Humidity (% RH)	Generator Air Flow (l/min)	Generator Temperature (°C)	Aerosol Concentration (µg/m³)
4	1	20	73.5	20.9	54.5	1.5	85.4	9
	2	20	74.1	21.0	52.0	1.5	85.3	0
82.6 ppm	3	20	74.5	21.0	52.3	1.5	85.4	2
	4	20	74.7	21.0	52.4	1.5	85.4	4
	5	20	74.8	20.9	51.3	1.5	85.3	0
	6	20	75.0	20.9	51.5	1.5	85.4	4
	mean	20	74.4	20.9	52.3	1.5	85.4	3
5	1	20	73.5	21.1	47.6	0.5	82.3	0
	2	20	73.9	21.1	47.4	0.5	74.6	0
66.6 ppm	3	20	74.1	21.1	47.3	0.5	74.3	0
	4	20	74.2	21.1	47.4	0.5	78.2	0
	5	20	74.2	21.1	47.4	0.5	78.2	1
	6	20	74.0	21.1	47.1	0.5	78.2	1
	mean	20	74.0	21.1	47.4	0.5	77.6	0
6	1	20	73.1	21.1	46.5	0.4	73.1	1
	2	20	73.0	21.1	46.4	0.4	73.0	1
42.9 ppm	3	20	73.2	21.1	46.5	0.4	73.2	1
	4	20	73.1	21.1	46.3	0.4	73.1	1
	5	20	73.1	21.1	46.3	0.4	73.1	1
	6	20	73.1	21.1	46.3	0.4	73.1	1
	mean	20	73.1	21.1	46.4	0.4	73.1	1

TABLE 2

Gas Chromatography Parameters and Calibration

Instrument: HP5880
Column: Restek Stabilwax, 0.53 mm x 30 M, 1 micron
Detector: FID
Carrier: Helium
Column Temperature: 135 °C
Detector Temperature: 260 °C
Injector Temperature: 250 °C
Injection Volumes: 1 microliter for liquid, 0.25 to 1.0 cc for vapor

Typical Calibration Curve (low concentration range)

Concentration (mg/ml)	Peak Area
0.00526	44.52
0.02103	176.63
0.06309	517.94
0.1052	872.83
0.2103	1779.83

Slope = 8461.2
Y-intercept = -6.802
correlation = 0.99992

Typical Calibration Curve (high concentration range)

Concentration (mg/ml)	Peak Area
0.202 1	2637.85
0.6064	833 8.24
1.011	13991 .o
2.0212	27576.9

Slope=13686 .
Y-intercept =5.32 1
correlation = 0.99993

TABLE 3

Exposure Chamber Concentrations

Group Number	Sample Number	Vapor Concentration (ppm)	Mean ± Standard Deviation
1	1	4.95	
(12-1-97)	2	4.73	4.54 ± 0.85
	3	5.63	
	4	3.91	
	5	3.48	
2	1	20.6	
(12-4-97)	2	15.5	16.0 ± 3.2
	3	13.3	
	4	14.5	
3	1	33.2	
(12-9-97)	2	33.3	32.1 ± 2.6
	3	33.7	
	4	28.3	
4	1	91.9	
(12-16-97)	2	72.2	82.6 ± 9.9
	3	83.8	
5	1	74.0	
(12-23-97)	2	52.7	66.6 ± 8.8
	3	71.4	
	4	71.5	
	5	63.2	
6	1	34.9	
(12-23-97)	2	40.5	42.9 ± 9.4
	3	53.2	

TABLE 4

RD50 Calculation

Concentration (ppm)	Percent Decrease in Respiratory Rate
32.1	20.1
42.9	35.3
66.6	58.6
82.6	63.4

RD_{50} = 59.7 ppm

slope = 2.924

Y-intercept = -0.193

conelation coefficient = 0.99 1

95% confidence limits = 56.4 to 63.1 ppm

RD_{20} = 30.9 ppm

95% confidence limits = 29.2 to 32.7 ppm

The values were calculated from a linear regression of log concentration versus probit values obtained from the percent decrease in respiratory rate.

TABLE 5

Impinger Trapping Efficiency

	Peak Area	BHT Conc. (%)(ml)	sample volume (ml)	Amount BHT in trap (µg)	breakthrough	total trapping efficiency
run 1 front	3726.51758	107.9	10	1079		
run 1 back	0	0	10	0	0	100%
run 2 front	3708.28125	107.4	10	1074		
run 2 back	0	0	10	0		100%

TABLE 6

Direct Vapor Sampling versus Impinger Sampling

Direct Vapor Analysis

Sample Number	Peak Area	BHT Concentration (ppm)
1	1575.49	25.4
2	2184.56	35.2
3	2071.84	33.4
4	1556.57	25.1
		mean = 29.8 ± 5.3

Impinger Samples

Sample Number	trap	volume (ml)	peak area	amount (µg)	air volume (L)	BHT concentration (ppm)
1	front	15	358.50479	0.178	7.70	2.6
	back	15	0	0	7.70	BDL'
2	front	15	117.02579	0.075	7.87	1.1
	back	15	0	0	7.87	BDL
3	front	15	0	0	7.90	BDL
	back	15	0	0	7.90	BDL
4	front	15	0	0	7.58	BDL
	back	15	0	0	7.58	BDL

BDL = below detection limit (<1 ppm)

TABLE 7
Mean Respiratory Rate Data
Exposure 1
BHT Vapor Concentration = 4.54 ppm

Exposure Time (minutes)		Respiratory Rate (breaths/minute)	Percent Change
baseline	-8	236.6	2.4
	-6	227.1	-1.7
	-4	238.0	3.0
	-2	230.1	-0.4
	0	223.2	-3.4
	mean	231.0	
exposure	0.25	261.4	13.2
	0.5	320.1	38.6
	0.75	259.9	12.5
	1	247.9	7.3
	1.25	229.9	-0.5
	1.5	217.3	-5.9
	1.75	230.5	-0.2
	2	232.8	0.8
	2.25	237.7	2.9
	2.5	226.9	-1.8
	2.75	215.3	-6.8
	3	210.8	-8.7
	6	218.4	-5.5
	9	223.6	-3.2
	12	216.4	-6.3
	15	208.8	-9.6
	18	210.3	-9.0
	21	214.5	-7.2
	24	212.1	-8.2
	27	217.5	-5.8
	30	208.1	-9.9
post exposure	2	234.5	1.5
	4	247.5	7.1
	6	211.5	-8.4
	8	214.4	-7.2
	10	201.7	-12.7

TABLE 7 (cont)
Mean Respiratory Rate Data
Exposure 2
BHT Vapor Concentration = 16.0 ppm

Exposure Time (minutes)		Respiratory Rate (breaths/minute)	Percent Change
baseline	-8	233.8	2.1
	-6	233.9	2.1
	-4	231.6	1.1
	-2	222.3	-2.9
	0	223.4	-2.5
	mean	229.0	
exposure	0.25	225.1	-1.7
	0.5	315.7	37.8
	0.75	259.6	13.3
	1	244.5	6.8
	1.25	229.2	0.1
	1.5	223.6	-2.3
	1.75	223.3	-2.5
	2	226.0	-1.3
	2.25	227.2	-0.8
	2.5	220.0	-3.9
	2.75	224.3	-2.1
	3	215.8	-5.8
	6	218.6	-4.6
	9	224.0	-2.2
	12	222.5	-2.8
	15	213.9	-6.6
	18	215.7	-5.8
	21	214.2	-6.5
	24	216.7	-5.4
	27	208.9	-8.8
	30	211.2	-7.8
post exposure	2	228.4	-0.3
	4	219.7	-4.1
	6	215.3	-6.0
	8	214.6	-6.3
	10	212.2	-7.3

TABLE 7 (cont)
Mean Respiratory Rate Data
Exposure 3
BHT Vapor Concentration = 32.1 ppm

Exposure Time (minutes)		Respiratory Rate (breaths/minute)	Percent Change
baseline	-8	212.9	1.9
	-6	209.8	0.4
	-4	213.8	2.3
	-2	208.1	-0.4
	0	200.3	-4.2
	mean	209.0	
exposure	0.25	199.2	-4.7
	0.5	321.8	54.0
	0.75	228.2	9.2
	1	228.9	9.5
	1.25	224.7	7.5
	1.5	200.2	-4.2
	1.75	200.6	-4.0
	2	210.7	0.8
	2.25	209.1	0
	2.5	192.4	-7.9
	2.75	212.5	1.7
	3	210.1	0.5
	6	198.2	-5.2
	9	202.7	-3.0
	12	190.7	-8.7
	15	190.2	-9.0
	18	188.3	-9.9
	21	185.9	-11.0
	24	181.4	-13.2
	27	176.3	-15.6
	30	166.9	-20.1
post exposure	2	200.9	-3.9
	4	181.6	-13.1
	6	180.2	-13.8
	8	182.8	-12.5
	10	181.8	-13.0

TABLE 7 (cont)
Mean Respiratory Rate Data
Exposure 4
BHT Vapor Concentration = 82.6 ppm

Exposure Time (minutes)		Respiratory Rate (breaths/minute)	Percent Change
baseline	-8	249.4	7.5
	-6	240.5	3.7
	-4	229.8	-1.0
	-2	212.8	-8.3
	0	227.5	-1.9
	mean	232.0	
exposure	0.25	226.7	-2.3
	0.5	364.0	56.9
	0.75	435.4	87.7
	1	272.7	17.5
	1.25	208.1	-10.3
	1.5	169.7	-26.9
	1.75	155.5	-33.0
	2	175.4	-24.4
	2.25	149.5	-35.6
	2.5	166.7	-28.2
	2.75	169.1	-27.1
	3	152.6	-34.2
	6	108.8	-53.1
	9	92.6	-60.1
	12	94.0	-59.5
	15	84.8	-63.4
	18	87.2	-62.4
	21	90.6	-61.0
	24	89.5	-61.4
	27	96.8	-58.3
	30	95.3	-58.9
post exposure	2	117.0	-49.6
	4	163.2	-29.6
	6	173.4	-25.2
	8	171.6	-26.0
	10	185.7	-20.0

TABLE 7 (cont)
Mean Respiratory Rate Data
Exposure 5
BHT Vapor Concentration = 66.6 ppm

Exposure Time (minutes)		Respiratory Rate (breaths/minute)	Percent Change
baseline	-8	236.4	-2.3
	-6	239.5	-1.0
	-4	241.3	-0.3
	-2	242.0	0
	0	250.8	3.6
	mean	242.0	
exposure	0.25	272.1	12.4
	0.5	274.4	13.4
	0.75	207.0	-14.5
	1	166.0	-31.4
	1.25	138.9	-42.6
	1.5	140.0	-42.1
	1.75	155.8	-35.6
	2	135.7	-43.9
	2.25	151.0	-37.6
	2.5	143.6	-40.7
	2.75	126.2	-47.9
	3	116.6	-51.8
	6	109.8	-54.6
	9	100.2	-58.6
	12	116.0	-52.1
	15	142.0	-41.3
	18	120.8	-50.1
	21	116.8	-51.7
	24	113.6	-53.1
	27	129.5	-46.5
	30	142.0	-41.3
post exposure	2	180.9	-25.3
	4	194.3	-19.7
	6	203.4	-16.0
	8	197.8	-18.3
	10	193.5	-20.0

TABLE 7 (cont)
Mean Respiratory Rate Data
Exposure 6
BHT Vapor Concentration = 42.9 ppm

Exposure Time (minutes)		Respiratory Rate (breaths/minute)	Percent Change
baseline	-8	213.5	-1.6
	-6	227.4	4.8
	-4	216.6	-0.2
	-2	221.5	2.1
	0	206.0	-5.1
	mean	217.0	
exposure	0.25	306.5	41.3
	0.5	388.2	78.9
	0.75	272.0	25.3
	1	256.6	18.2
	1.25	230.2	6.1
	1.5	264.8	22.0
	1.75	220.1	1.4
	2	220.7	1.7
	2.25	216.0	-0.5
	2.5	222.3	2.4
	2.75	219.3	1.1
	3	267.5	23.3
	6	221.2	1.9
	9	215.3	-0.8
	12	206.4	-4.9
	15	199.6	-8.0
	18	159.5	-26.5
	21	186.6	-14.0
	24	140.4	-35.3
	27	174.1	-19.8
	30	162.4	-25.2
post exposure	2	177.8	-18.1
	4	183.8	-15.3
	6	183.5	-15.4
	8	195.8	-9.8
	10	187.3	-13.7

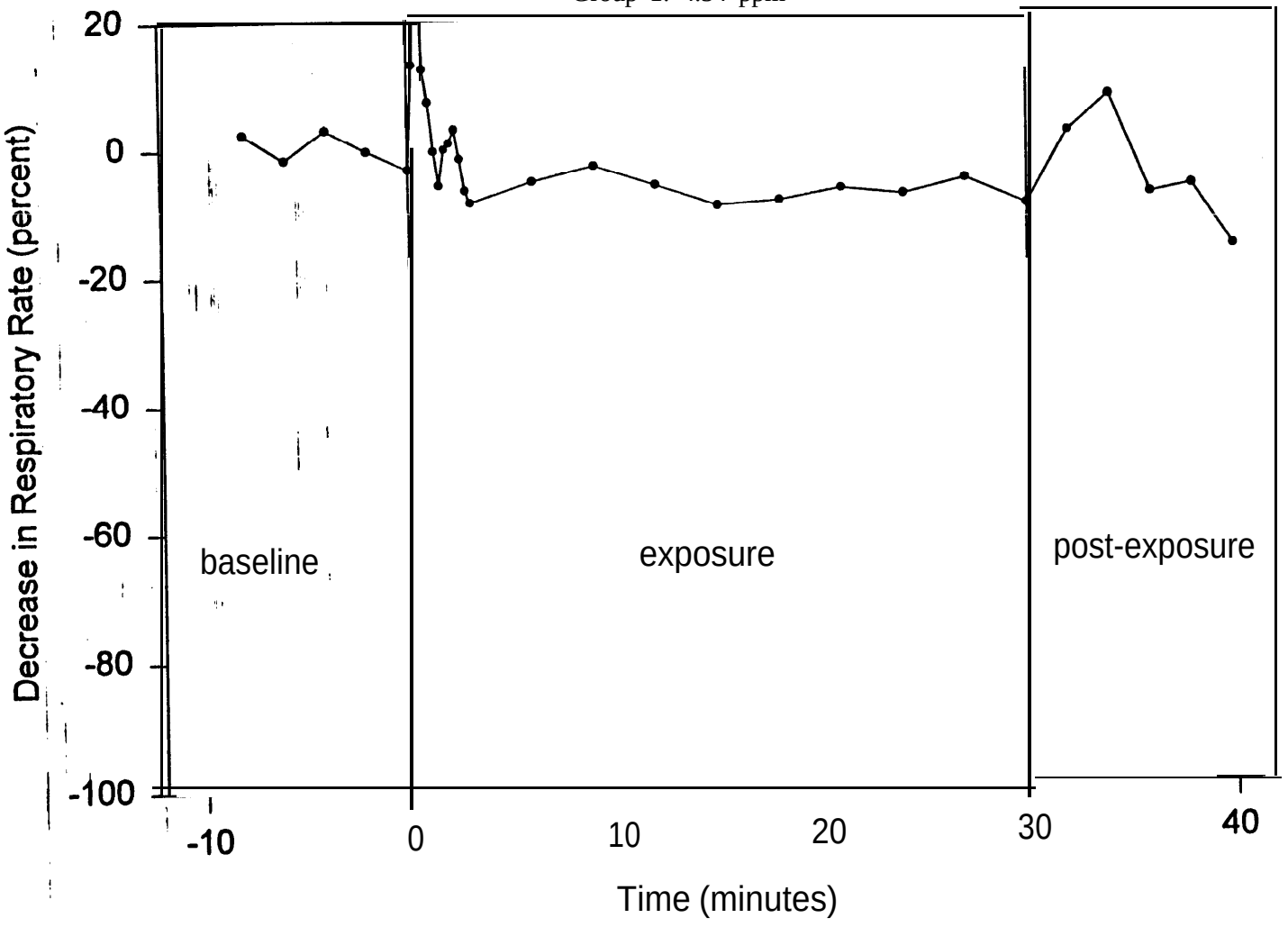
VII. FIGURES

PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE

FIGURE 1

Respiratory Rate vs. Exposure Time

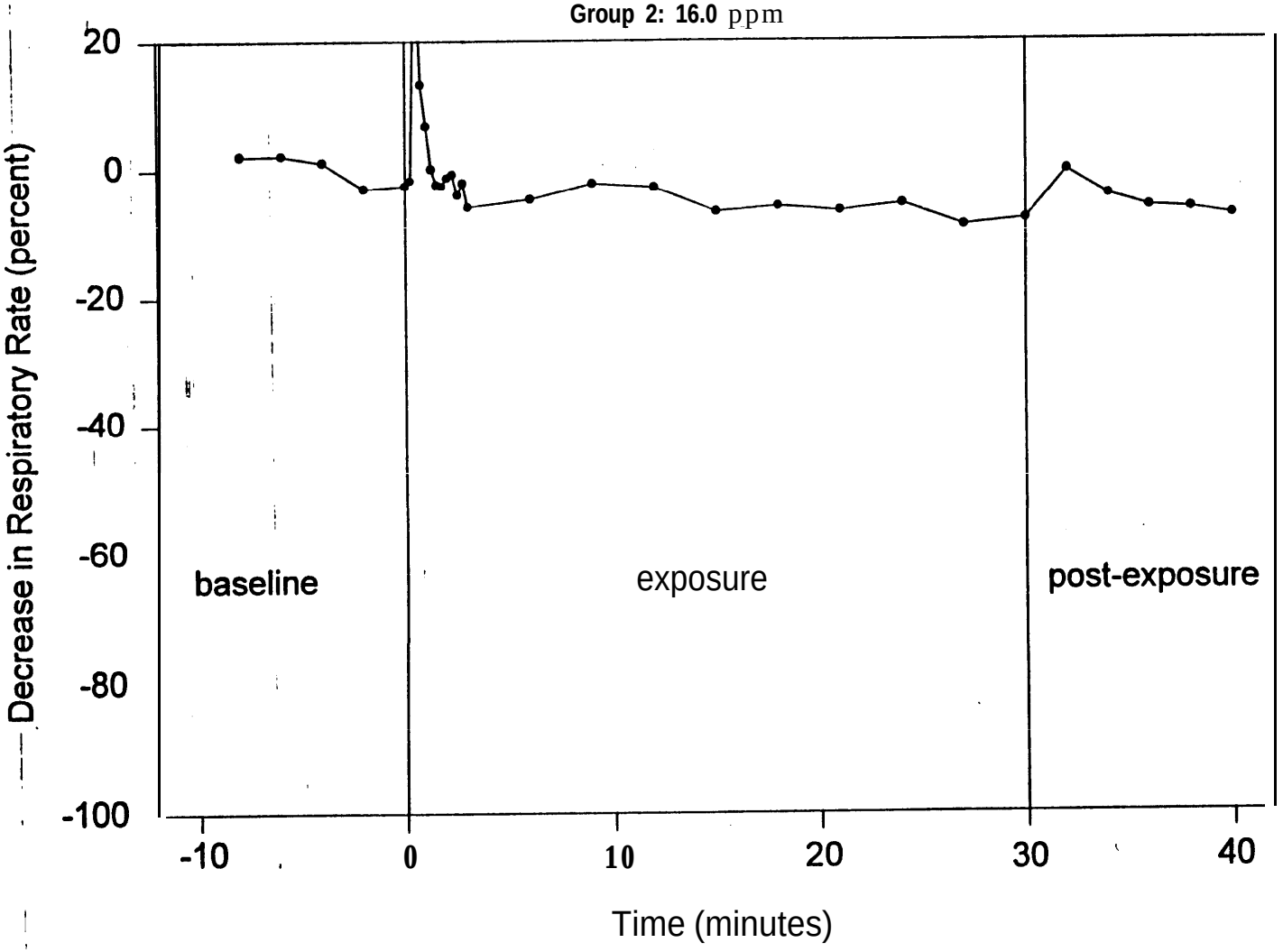
Group 1: 4.54 ppm



PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE

FIGURE 2

Respiratory Rate vs. Exposure Time

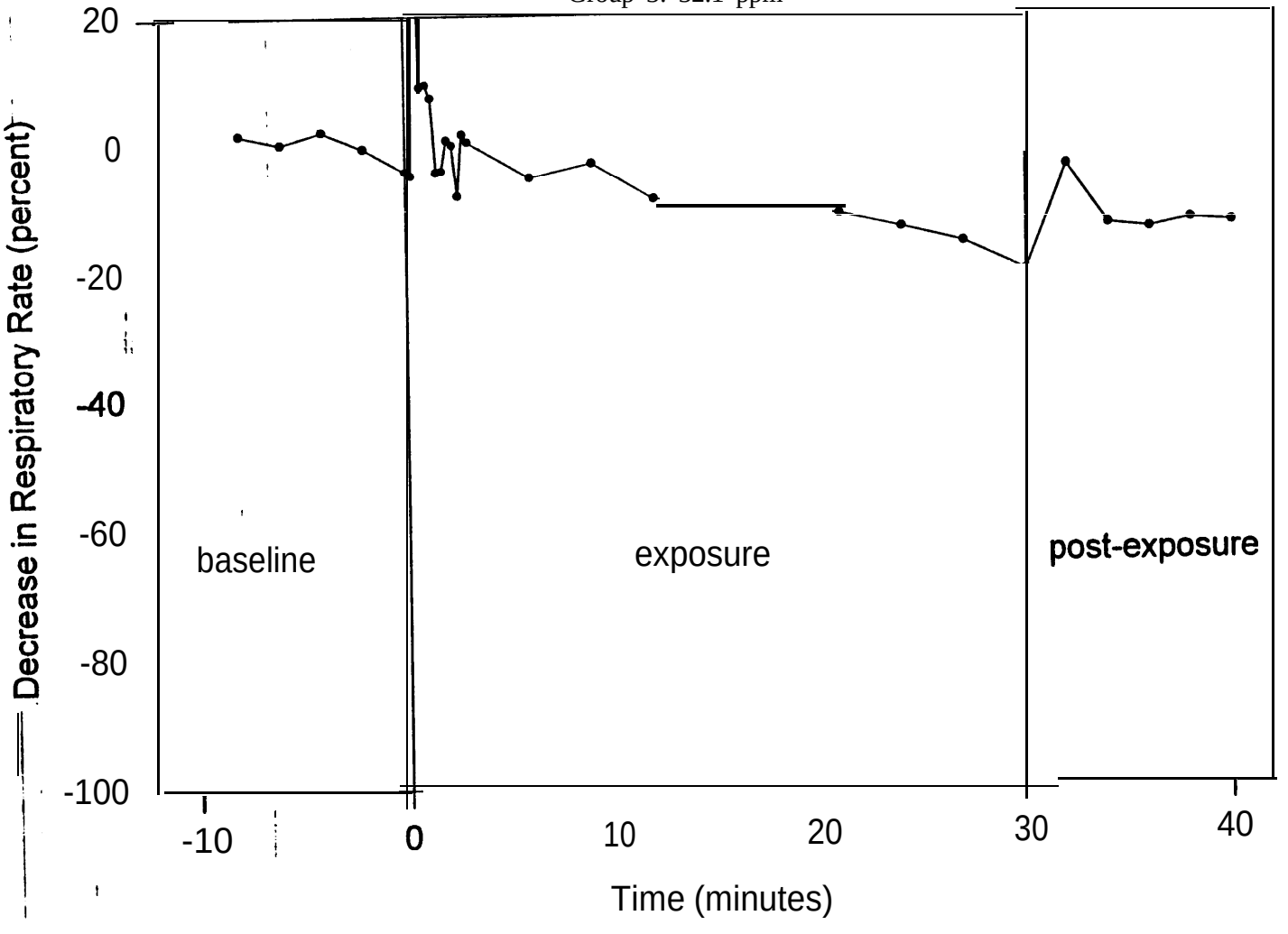


PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE

FIGURE 3

Respiratory Rate vs. Exposure Time

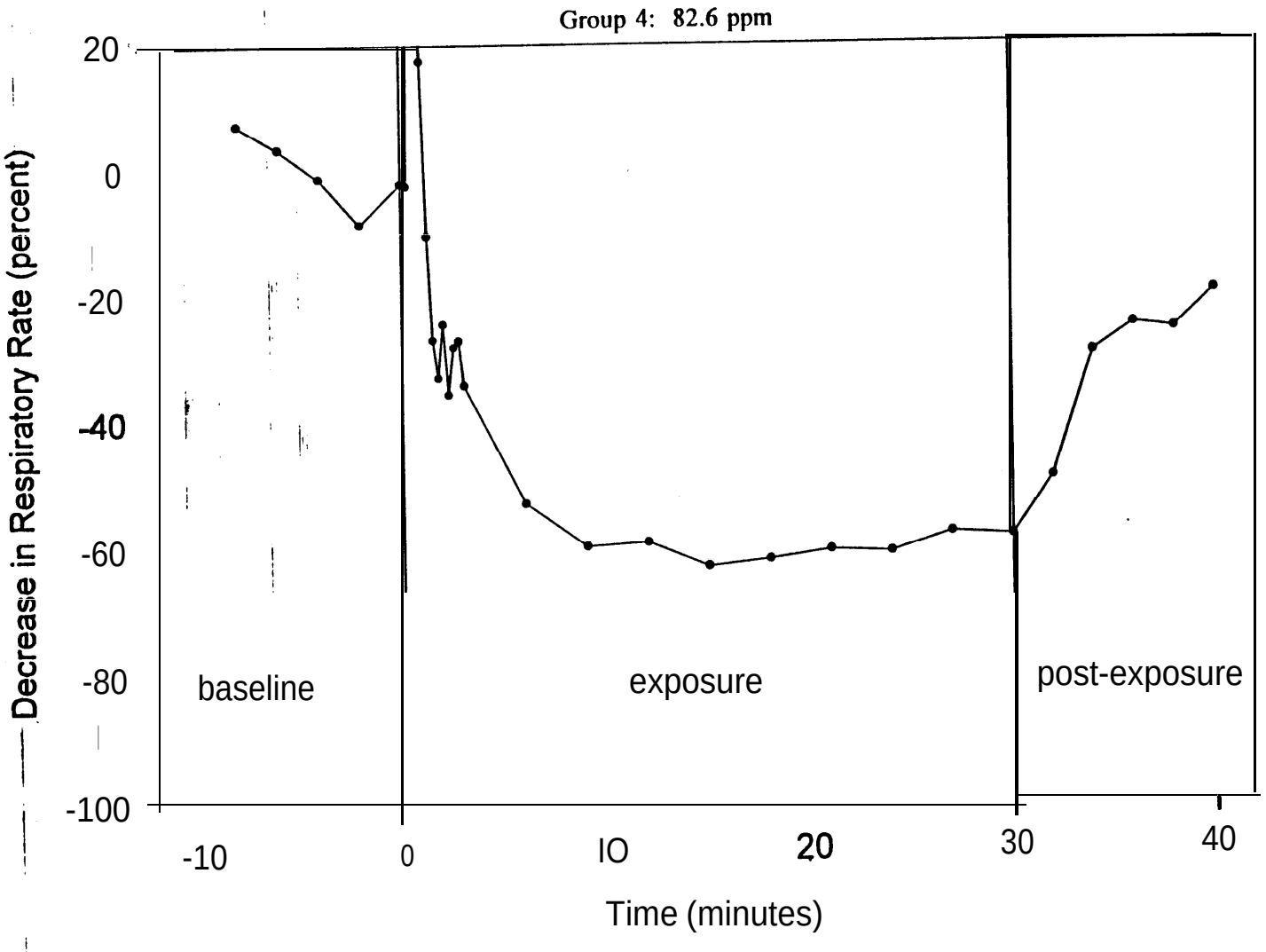
Group 3: 32.1 ppm



PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED IIYDROXYTOLUENE (BHT) IN MICE

FIGURE 4

Respiratory Rate vs. Exposure Time

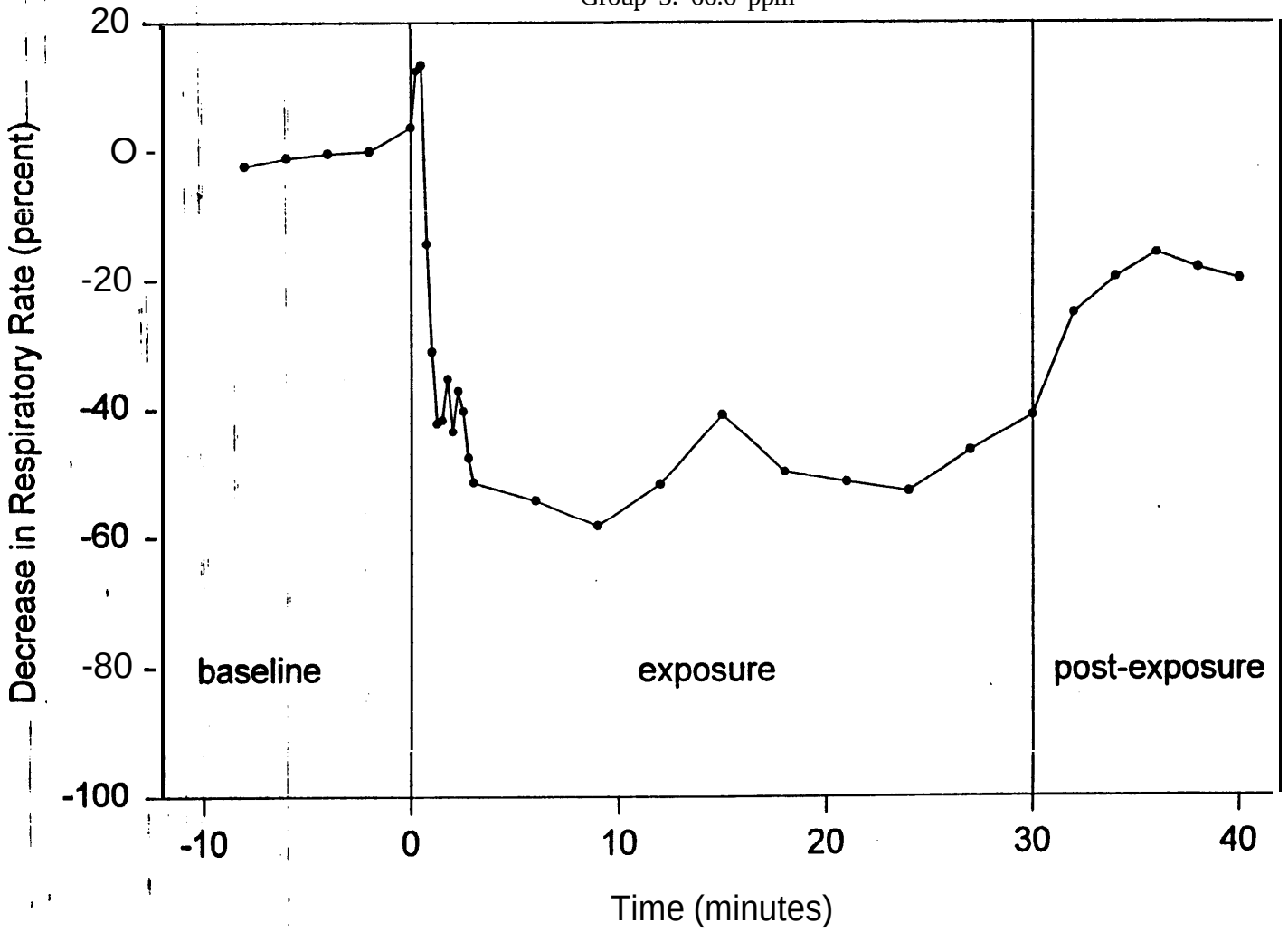


PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE

FIGURE 5

Respiratory Rate vs. Exposure Time

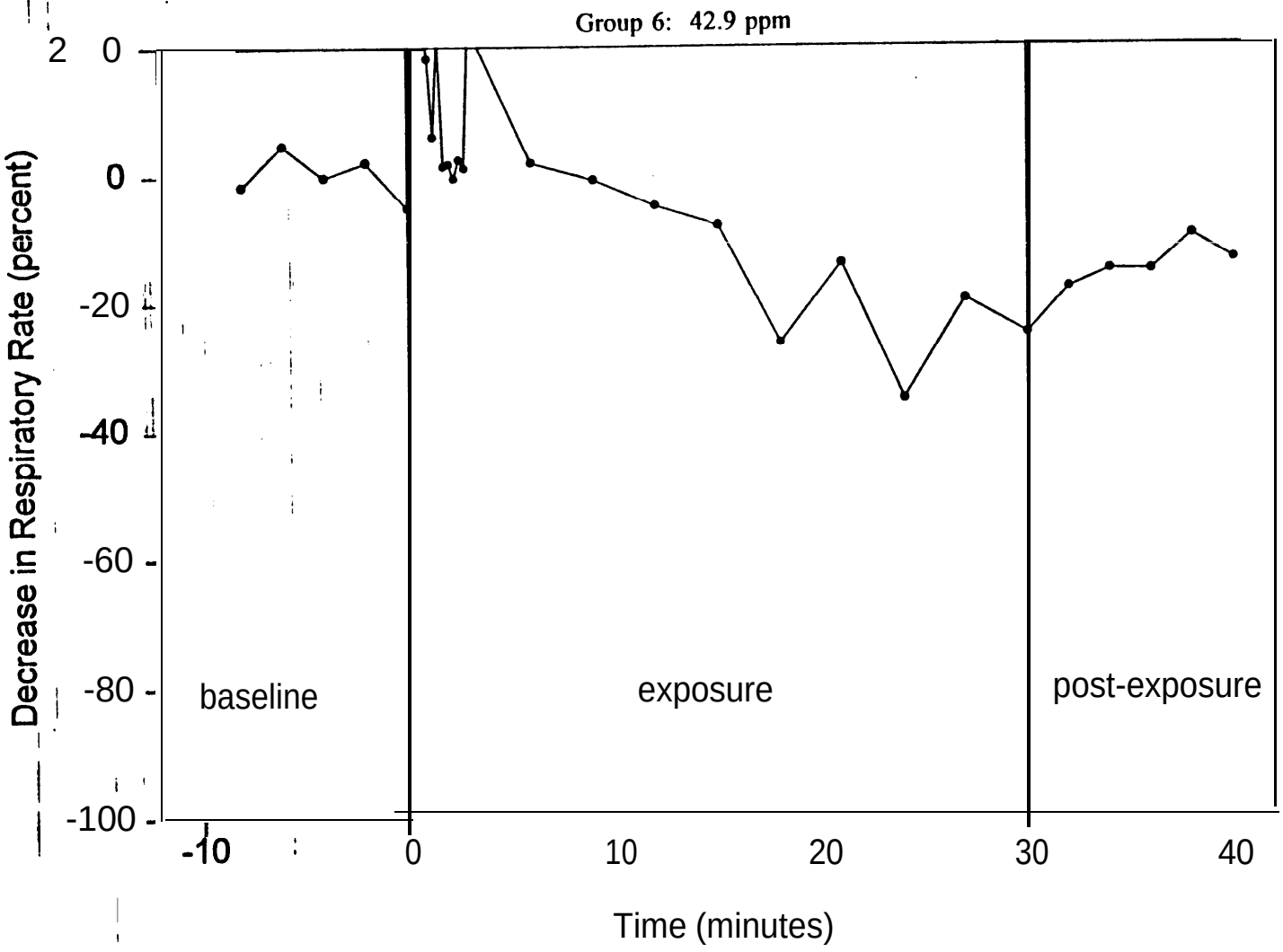
Group 5: 66.6 ppm



PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE

FIGURE 6

Respiratory Rate vs. Exposure Time



PULMONARY/SENSORY IRRITATION STUDY
OF BUTYLATED HYDROXYTOLUENE (BHT) IN MICE
FIGURE 7