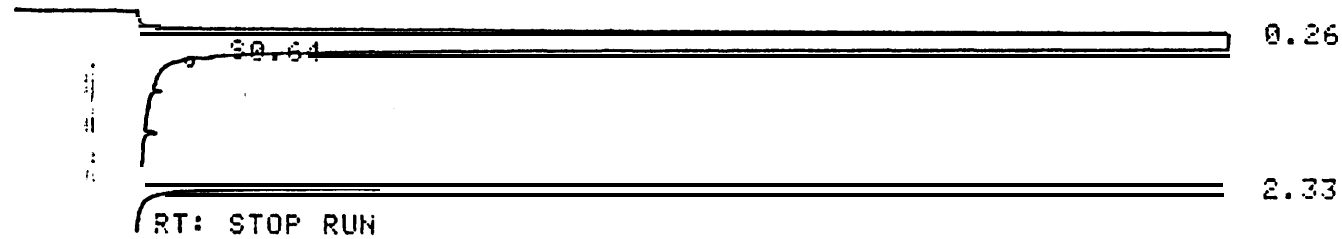
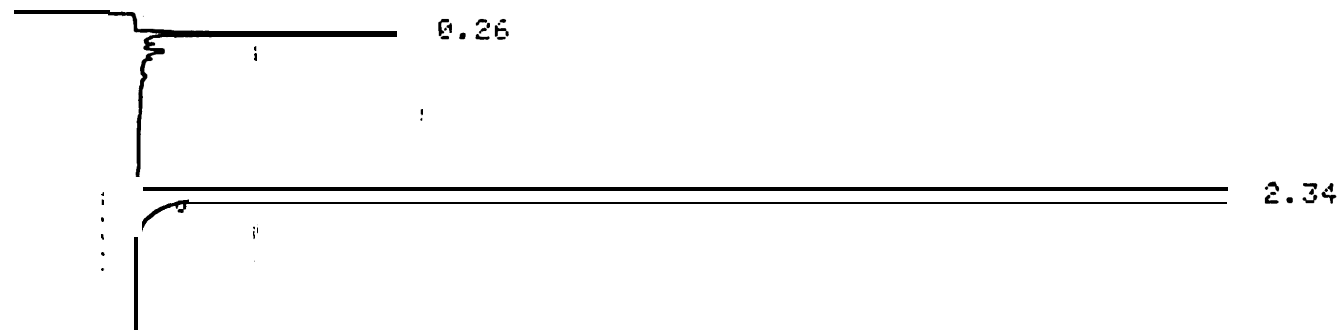
FIGURE 8

BHT Chromatograms



BHT Standard in Acetone (0.1052 mg/ml)



BHT Vapor Sample (30 ppm)

VIII. APPENDICES

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

Appendix 1

Individual Respiratory Rate Data

BHT Vapor Concentration = 4.54 ppm

Exposure Time (minutes)		Percent Change in Respiratory Rate				
		Animal Number 1	Animal Number 2	Animal Number 3	Animal Number 4	mean
baseline	-8	-1.5	-0.4	9.5	2.2	2.4
	-6	-2.0	-3.2	5.3	-6.9	-1.7
	-4	0.6	6.2	2.9	2.4	3.0
	-2	-4.1	-0.7	-8.9	12.1	-0.4
	0	7.0	-1.9	-8.9	-9.8	-3.4
exposure	0.25	15.5	21.3	-0.7	16.5	13.2
	0.5	28.8	49.6	1.1	74.8	38.6
	0.75	25.0	23.4	-2.4	4.1	12.5
	1	28.8	-2.9	-2.4	5.9	7.3
	1.25	8.0	-15.1	-0.7	5.9	-0.5
	1.5	6.1	-5.0	-7.8	-17.0	-5.9
	1.75	-1.5	25.4	-9.5	-15.3	-0.2
	2	8.0	-2.9	-16.6	14.7	0.8
	2.25	2.3	11.2	-7.8	5.9	2.9
	2.5	2.3	3.1	-9.5	-2.9	-1.8
	2.75	-3.4	1.1	-14.9	-10.0	-6.8
	3	-7.2	-2.9	-9.5	-15.3	-8.7
	6	8.6	-7.1	-11.3	-12.0	-5.5
	9	9.4	-2.8	-12.0	-7.3	-3.2
	12	-4.5	-1.8	-15.3	-3.6	-6.3
	15	-5.9	-3.6	-16.6	-12.3	-9.6
	18	-7.8	-6.0	-12.0	-10.0	-9.0
	21	-7.8	-11.2	-1.4	-8.2	-7.2
	24	-8.3	-9.3	-18.7	2.7	-8.4
	27	-5.6	-2.4	-17.7	2.4	-5.8
	30	-3.3	-6.8	-26.4	-3.2	-9.9
post exposure	2	-3.0	6.9	-11.8	-7.9	1.5
	4	2.0	3.6	-9.8	32.6	7.1
	6	-1.5	-10.3	-26.2	4.1	-8.4
	8	-12.6	-3.2	-19.7	6.8	-7.2
	10	-13.8	-11.5	-24.8	-0.5	-12.7

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

Individual Respiratory Rate Data

BHT Vapor Concentration = 16.0 ppm

Exposure Time (minutes)		Percent Change in Respiratory Rate				
		Animal Number 5	Animal Number 6	Animal Number 7	Animal Number 8	mean
baseline	-8	-5.0	6.6	2.2	4.5	2.1
	-6	1.5	4.5	-0.4	3.0	2.2
	-4	2.4	2.2	-0.4	0.3	1.1
	-2	0.0	-5.8	-2.2	-3.7	-2.9
	0	1.1	-7.5	0.7	-4.1	-2.5
exposure	0.25	6.3	-4.2	-4.5	-4.3	-1.7
	0.5	38.7	37.2	51.5	24.0	37.8
	0.75	33.3	-4.2	16.2	8.1	13.3
	1	17.1	10.9	1.7	-2.6	6.8
	1.25	4.5	-2.3	-4.5	2.7	0.1
	1.5	8.1	-9.8	3.8	-11.4	-2.3
	1.75	0.9	-11.7	1.7	-0.8	-2.5
	2	15.3	-9.8	-4.5	-6.1	-1.3
	2.25	6.3	-13.6	12.1	-7.9	-0.8
	2.5	6.3	-11.7	-2.4	-7.9	-3.9
	2.75	2.7	-13.6	1.7	1.0	-2.1
	3	4.5	-15.5	-2.4	-9.7	-5.8
	6	4.3	-10.0	-3.6	-8.9	-4.6
	9	5.8	-8.1	-4.7	-1.8	-2.2
	12	2.1	-9.0	-2.8	-1.5	-2.8
	15	-4.8	-11.2	-5.7	-4.6	-6.6
	18	1.9	-11.7	-7.3	-6.1	-5.8
	21	4.2	-13.9	-6.9	-9.2	-6.5
	24	1.9	-12.3	-4.9	-6.3	-5.4
	27	-4.7	-13.1	-7.3	-10.0	-8.8
	30	-3.5	-14.5	-6.1	-7.0	-7.8
post exposure	2	8.3	-10.1	0.4	0.3	-0.3
	4	-3.4	-4.4	-4.3	-4.1	-4.1
	6	-10.4	-7.5	-6.1	0.1	-6.0
	8	-12.4	-5.6	-3.7	-3.5	-6.3
	10	-8.4	-8.2	-5.6	-7.2	-7.3

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

Individual Respiratory Rate Data

BHT Vapor Concentration = 32.1 ppm

Exposure Time (minutes)		Percent Change in Respiratory Rate				
		Animal Number 9	Animal Number 10	Animal Number 11	Animal Number 12	mean
baseline	-8	-1.9	1.0	1.4	7.0	1.9
	-6	-2.2	0	6.6	-2.9	0.4
	-4	1.9	6.9	-0.3	0.7	2.3
	-2	1.6	1.0	-0.9	-3.4	-0.4
	0	0.6	-8.8	-6.9	-1.4	-4.1
exposure	0.25	-0.7	-13.5	-1.1	-3.4	-4.7
	0.5	39.9	74.9	42.5	58.5	54.0
	0.75	13.5	6.1	1.1	15.9	9.2
	1	31.8	-9.6	5.7	10.1	9.5
	1.25	13.5	-1.7	8.0	10.1	7.5
	1.5	-0.7	-19.4	-1.1	4.3	-4.2
	1.75	-6.7	-23.3	5.7	8.2	-4.0
	2	1.4	0.2	1.1	0.5	0.8
	2.25	-2.7	10.1	-5.7	-1.4	0
	2.5	-4.7	-21.4	-8.0	2.4	-7.9
	2.75	19.6	-9.6	-5.7	2.4	1.7
	3	5.4	4.2	-8.0	0.5	0.5
	6	1.5	-12.7	-8.6	-1.0	-5.2
	9	6.8	-11.2	-8.8	1.3	-3.0
	12	-7.1	-14.7	-12.5	-0.8	-8.7
	15	-4.0	-16.1	-12.8	-3.1	-9.0
	18	-13.3	-12.4	-15.3	1.4	-9.9
	21	-10.1	-15.0	-15.5	-3.5	-11.0
	24	-9.1	-12.9	-26.1	-4.8	-13.2
	27	-18.2	-20.9	-23.6	0.2	-15.6
	30	-17.2	-21.7	-30.3	-11.3	-20.1
post exposure	2	2.9	1.5	-26.7	6.8	-3.9
	4	-4.7	-16.0	-28.2	-3.6	-13.1
	6	-4.7	-17.0	-29.3	-4.1	-13.8
	8	-3.4	-9.8	-31.0	-5.8	-12.5
	10	-4.5	-16.7	-28.7	-2.2	-13.0

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

Individual Respiratory Rate Data

BHT Vapor Concentration = 82.6 ppm

Exposure Time (minutes)		Percent Change in Respiratory Rate				
		Animal Number 13	Animal Number 14	Animal Number 15	Animal Number 16	mean
baseline	-8	1.9	27.7	-2.3	2.8	7.5
	-6	3.2	-5.6	-0.1	17.2	3.7
	-4	-2.0	1.6	1.0	-4.5	-1.0
	-2	-2.3	-13.8	-0.6	-16.4	-8.3
	0	-0.7	-9.9	2.0	0.9	-1.9
exposure	0.25	-2.0	1.8	6.3	-15.1	-2.3
	0.5	58.4	49.4	51.8	68.0	56.9
	0.75	87.6	85.6	86.0	91.5	87.7
	1	8.4	51.1	-1.3	12.0	17.5
	1.25	-20.8	-16.3	-8.9	4.8	-10.3
	1.5	-33.3	-22.8	-50.7	-0.6	-26.9
	1.75	-22.9	-34.3	-50.7	-24.1	-33.0
	2	-18.7	-27.8	-46.9	-4.2	-24.4
	2.25	-27.0	-42.5	-45.0	-27.7	-35.6
	2.5	-47.9	-29.4	-20.3	-15.1	-28.2
	2.75	-37.5	-26.1	-29.8	-15.1	-27.1
	3	-56.2	-34.3	-24.1	-22.3	-34.2
	6	-52.1	-60.9	-57.6	-41.9	-53.1
	9	-57.8	-67.4	-67.6	-47.5	-60.1
	12	-57.6	-66.5	-68.5	-45.3	-59.5
	15	-61.4	-69.1	-68.7	-54.5	-63.4
	18	-60.7	-66.5	-66.5	-55.9	-62.4
	21	-57.3	-67.3	-68.7	-50.6	-61.0
	24	-58.1	-65.2	-65.5	-56.8	-61.4
	27	-55.0	-62.5	-63.8	-51.8	-58.3
	30	-55.9	-60.5	-64.7	-54.5	-58.9
post exposure	2	-48.7	-57.1	-54.5	-38.1	-49.6
	4	-34.1	-37.4	-38.3	-8.8	-29.6
	6	-27.3	-31.4	-31.0	-11.2	-25.2
	8	-24.2	-28.0	-37.6	-14.4	-26.0
	10	-15.8	-23.2	-34.1	-6.7	-20.0

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

Individual Respiratory Rate Data

BHT Vapor Concentration = 66.6 ppm

Exposure Time (minutes)		Percent Change in Respiratory Rate				
		Animal Number 17	Animal Number 18	Animal Number 19	Animal Number 20	mean
baseline	-8	-9.1	-3.9	-1.1	4.9	-2.3
	-6	3.0	-2.2	-2.0	-2.9	-1.0
	-4	-2.4	3.3	0	-2.0	-0.3
	-2	-4.0	2.7	1.4	0.1	0
	0	12.6	0.1	1.8	0.1	3.6
exposure	0.25	21.4	4.6	30.0	-6.3	12.4
	0.5	41.7	6.2	12.0	-6.3	13.4
	0.75	-16.2	-8.9	-16.9	-15.9	-14.5
	1	-33.5	-29.2	-35.0	-27.9	-31.4
	1.25	-56.6	-37.6	-45.8	-30.3	-42.6
	1.5	-33.5	-51.1	-51.2	-32.7	-42.1
	1.75	-29.2	-35.9	-56.7	-20.7	-35.6
	2	-45.1	-35.9	-54.9	-39.9	-43.9
	2.25	-29.2	-41.0	-47.6	-32.7	-37.6
	2.5	-46.5	-46.0	-42.2	-27.9	-40.7
	2.75	-69.6	-42.7	-44.0	-35.1	-47.9
	3	-71.1	-44.4	-56.7	-35.1	-51.8
	6	-63.7	-62.8	-61.2	-30.9	-54.6
	9	-64.7	-69.1	-66.1	-34.5	-58.6
	12	-46.0	-57.8	-51.2	-53.1	-52.1
	15	-36.4	-35.9	-35.1	-82.4	-41.3
	18	-45.9	-48.7	-55.6	-a	-50.1
	21	-53.4	-48.3	-53.5	-	-51.7
	24	-54.7	-48.8	-55.6	-	-53.1
	27	-50.6	-37.5	-51.4	-	-46.5
	30	-45.9	-32.8	-45.2	-	-41.3
post exposure	2	-25.0	-21.0	-29.8	-	-25.3
	4	-16.7	-23.9	-18.5	-	-19.7
	6	0.7	-26.9	-21.7	-	-16.0
	8	-16.0	-17.4	-21.4	-	-18.3
	10	-17.1	-25.0	-18.1	-	-20.0

• Animal Died

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

Individual Respiratory Rate Data

BHT Vapor Concentration = 42.9 ppm

Exposure Time (minutes)		Percent Change in Respiratory Rate				
		Animal Number 21	Animal Number 22	Animal Number 23	Animal Number 24	mean
baseline	-8	-2.2	-10.3	5.2	0.9	-1.6
	-6	11.2	-0.4	7.7	0.7	4.8
	-4	-0.2	1.3	-2.4	0.7	-0.2
	-2	-3.8	11.0	0.9	0.2	2.1
	0	-5.0	-1.6	-11.3	-2.4	-5.1
exposure	0.25	20.4	47.2	60.6	36.9	41.3
	0.5	82.8	72.6	97.2	63.1	78.9
	0.75	2.6	33.7	40.2	24.8	25.3
	1	20.4	20.1	-0.4	32.9	18.2
	1.25	0.3	11.7	-4.5	16.8	6.1
	1.5	4.8	40.4	32.1	10.7	22.0
	1.75	7.0	16.8	-16.7	-1.4	1.4
	2	4.8	10.0	-10.6	2.7	1.7
	2.25	0.3	11.7	-10.6	-3.4	-0.5
	2.5	2.6	4.9	-0.4	2.7	2.4
	2.75	2.6	11.7	-14.6	4.7	1.1
	3	24.9	13.4	32.1	22.8	23.3
	6	0.5	6.0	-2.6	3.8	1.9
	9	2.6	3.8	-8.5	-0.9	-0.8
	12	-4.1	-2.4	-12.1	-0.9	-4.9
	15	-9.7	-6.1	-14.8	-1.5	-8.0
	18	-14.2	-31.2	-37.3	-23.3	-26.5
	21	-14.9	-20.5	-28.0	7.4	-14.0
	24	-32.0	-34.3	-43.1	-31.9	-35.3
	27	-19.9	-23.7	-33.3	-2.2	-19.8
	30	-24.9	-24.0	-33.9	-17.8	-25.2
post exposure	2	-17.2	-19.6	-23.8	-11.7	-18.1
	4	-14.2	-21.7	-20.2	-5.1	-15.3
	6	-14.2	-23.2	-18.7	-5.6	-15.4
	8	-10.0	-11.4	-13.4	-4.4	-9.8
	10	-15.6	-19.4	-15.4	-4.4	-13.7

Appendix 2

PROTOCOL

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

2. Sponsor: U.S. Consumer Product Safety Commission
4330 East West Highway
Bethesda, MD 20814
attn: Dr. V. Schaeffer

3. Testing Facility: IIT Research Institute
10 West 35th Street
Chicago, IL 60616

4. Objective: The objective of the study is to characterize the sensory irritancy of BHT vapor by the ASTM E981 method.

5. Duration: The duration of the study will be a minimum of one day.

6. Proposed Study Dates:

a. Treatment Initiation: December 1, 1997
b. Biophase Termination: December 15, 1997
c. QA Audited Draft Report Completion: December 30, 1997

7. Protocol Approval:

a. Study Director: Scott Garthwaite Date: 12-1-97
Scott Garthwaite, B.S.

b. IITRI Section Head: Date: 12-1-97
James M. Gerhart, Ph.D., D.A.B.T.

c. Sponsor: Val Schaffe Date: 1-6-98

* revise ~~study~~ proposed study dates per IITRI letter of 12/18/97.

8. This protocol complies with specific -requirements of the Sponsor.

9. Test Substance:

a. Identification: The test substance is 2,6-di-tert-butyl-4-methylphenol or butylated hydroxytoluene (BHT). It is a white, crystalline solid at room temperature. It was purchased for use in this study from Sigma Chemical Company, St. Louis, MO. The certificate of analysis for this material indicates a purity of > 99.9 %.

b. Handling Precautions: When working with the test substance, study personnel must wear an organic vapor respirator, eye protection and two pairs of gloves (latex over polyethylene), or other protective clothing if required.

c. Assay: The test substance was characterized by the manufacturer at the time of analysis for this lot of test substance and a copy of the Material Safety Data Sheet (MSDS) will be maintained with the study data.

d. Storage: The test substance will be stored at room temperature (approximately 22 "C), unless otherwise noted by the Sponsor.

e. Dispensation/Disposition: Reserve samples of the test substance will not be retained. All quantities of the test substance which are dispensed will be documented. At the time of the acceptance of the report by the Sponsor, arrangements will be made to dispose of residual test substance. IITRI will not be required to retain any samples.

10. Test System:

a. Model: Male Swiss-Webster mice (Hilltop Lab animals, Scottdale, PA) will be used in this study. The animals will weigh 20-35 grams at exposure and will be approximately 4 weeks old upon arrival.

b. Selection of Test System: The RD50 in mice has been used as a model for quantitative prediction of sensory irritation in humans. This protocol is based upon ASTM method E981-84, Standard Test Method of Estimating Sensory Irritancy of Airborne Chemicals.

c. Housing: Animals will be housed in stainless steel wire cages suspended over excrement pans, or in polycarbonate cages, except during the inhalation exposure. Animals may be housed individually or group-housed up to 3 per cage.

d. Cleaning and Sanitation: Animal rooms and cages will be cleaned and sanitized prior to placing animals in them, and periodically thereafter in accordance with accepted animal care practices and relevant standard operating procedures.

e. Food: Certified Rodent Chow 5002 (PMI Feeds, Inc., St. Louis, MO) will be provided ad libitum except during the inhalation exposure. No known contaminants are expected to be present in the basal diet that would interfere with the test substance or test system and would confound the interpretation of the study.

f. Water: City of Chicago water will be provided ad libitum by means of an automatic watering system, except during the inhalation exposure. Supply water is periodically monitored for bacterial contamination and chemical composition (i.e., electrolytes, metals, etc.).

g. Animal Identification: Animals selected for the study will receive a unique tail marking using a permanent marker. Cage cards will also be provided.

h. Environmental Control: Animal rooms will be lighted automatically with fluorescent lights and maintained on a 12-hour light/12-hour dark cycle. Room temperature and humidity will be regulated to avoid extreme fluctuations (temperature range approximately 18-25 °C, humidity range approximately 30-70%).

11. Methods:

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

b. Assignment to Groups: Animals will be assigned randomly by weight to groups using a computer program.

c. Exposure Levels: Four to six exposure concentrations will be chosen to achieve a dose-response curve, if possible. The starting concentration will be 5 ppm. Target concentrations for the other groups will be chosen based upon respiratory rate responses as specified by the Sponsor.

d. Test Atmosphere Generation:

1. The test substance atmospheres will be generated by heating the test substance in a glass flask maintained in a constant-temperature water bath. Filtered compressed air will be directed over the surface of the heated test substance with the resulting vapor directed into the exposure chamber. Additional make-up air, if necessary, may be added. The concentration of test substance vapor in the exposure chamber will be controlled by adjusting the water bath temperature and/or the flowrate of air through the generator or the flowrate of make-up air. The chamber air flowrate will be high enough to allow rapid equilibration of the concentration (i.e., the time to achieve 99% of the, final concentration will be 5 minutes or less).

2. The nominal concentration of test substance in the exposure atmosphere will not be determined because the amount of material consumed during exposure will be too Iow to accurately measure.

3. The actual concentration of the test substance in the exposure atmosphere will be measured using gas chromatography. The analytical method will be characterized in terms of the limit of quantitation, accuracy and precision. Samples of the chamber atmosphere will be taken by collection using a glass

bubbler (using acetone or ethanol as solvent) or by direct vapor injection into the GC. If the impinger method is used, two impingers connected in series and analyzed separately will be used to insure complete trapping of the test substance. The GC will be calibrated weekly by analysis of at least four BHT standards covering the actual or anticipated analytical range and prepared in either acetone or ethanol and from the same source and lot of test substance as is used for inhalation exposure. Each exposure day, prior to sample analysis, the calibration of the GC will be checked by reanalysis of at least one previously prepared standard. If the prepared and analyzed concentrations are within ± 10% then the analyzer is considered calibrated. If the calibration check is outside the acceptable range, then it will be recalibrated. Measured exposure concentrations will, to the maximum extent possible, be in the calibrated instrument range. Details of the GC conditions used for analysis will be recorded in the data notebook. Adequate separation and freedom from interfering substances will be documented.

e. Justification for Route of Exposure: This inhalation test was chosen by the Sponsor due to the possibility of human exposure via this route.

f. Exposure Chambers: All exposures will be conducted via head-only inhalation in a 2.5 L glass chamber (figure 1). Temperature and dynamic flow conditions will be recorded at appropriate intervals. Total air flow will be adjusted as a means of controlling the concentration of the exposure atmosphere, but will provide enough air changes to maintain a safe oxygen level (greater than 19%) for the animals.

g. Chamber Loading: Four mice per group will receive a single 30-minute exposure.

h. Final Disposition of Animals: All animals surviving to the end of the exposure will be euthanized without necropsy using carbon dioxide.

12. Experimental Design: The analytical methods will be characterized prior to animal testing. Then, at least 4 or 5 groups of 4 male mice will be exposed to graded concentrations of the test substance via head-only inhalation (additional groups may be added at the request of the Sponsor). Only the heads of the animals will be exposed to the test substance; the body will be held in a glass holding port. Groups of animals will have an average resting, or baseline respiration rate recorded for approximately 10 minutes immediately prior to their collective exposure. Individual respiration rates will be determined from the measurements of a pressure transducer which will be attached to each animal's holding port. Mice will be exposed to the test atmosphere for 30 minutes. The average respiratory rate will be calculated after the respiratory rate stabilizes. Animals may remain in the plethysmographs at the end of exposure for at least 10 minutes to evaluate the potential for recovery from any irritant effects. This method is based on Alarie, Y ., Sensory irritation of the upper airways by airborne chemicals. *Toxicol Appl Pharmacol* 24:279-297, 1973 and ASTM method E981-84, "Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals".

13. Observations:

a. Mortality and Reactions: All animals which die during exposure will be noted. Animals shall be observed for clinical signs and abnormal behavior during and immediately following exposure to the test substance.

b. Body Weight: All animals in the study will be weighed prior to and following exposure.

c. Necropsy: Necropsy will not be performed.

14. Results and Statistical Treatment:

Each individual animal and each group of animals serves as its own control. The baseline respiratory rate shall be calculated as the average respiratory frequency during the preexposure period. The respiratory rate for each animal shall be calculated for three minute intervals during the exposure period. The individual percent decrease from the baseline respiratory rate for each animal and the mean percent decrease for the group shall be determined. The RD50 (the estimated exposure concentration required to reduce the average respiratory frequency by 50 percent), the RD20 (the estimated exposure concentration required to reduce the average respiratory frequency by 20 percent), and their 95 % confidence limits shall be calculated by linear regression analysis with the logarithm of the exposure concentration as the independent variable and the maximum average percent decrease in respiratory rate as the dependent variable. The slope and intercept of the regression line shall also be determined.

15. Report: The study report will include, but not be limited to:

1. Summary
2. Introduction
3. Experimental Design
4. Materials and Methods
5. Exposure Data
6. Description of the Generation System
7. Respiratory Rate Data
8. Conclusion
9. Q.A. Statement
10. GLP Compliance Statement

16. Data Notebooks:

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

1. the original signed protocol and all amendments
2. test substance information
3. animal receiving records
4. randomization procedures
5. exposure calculations
6. description of generation systems
7. chamber environment
8. monitoring data for vapor generation and chamber systems
9. respiratory rate measurements
10. dose-response calculations

b. Storage: At the completion of the study, all reports and raw data will be maintained in the Test Facility's Archives for two years following CPSC acceptance of the final report and will be available for inspection and review (photocopies if shipped offsite) by the Sponsor. After two years the Sponsor will be contacted to determine the storage location.

17. Personnel:

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

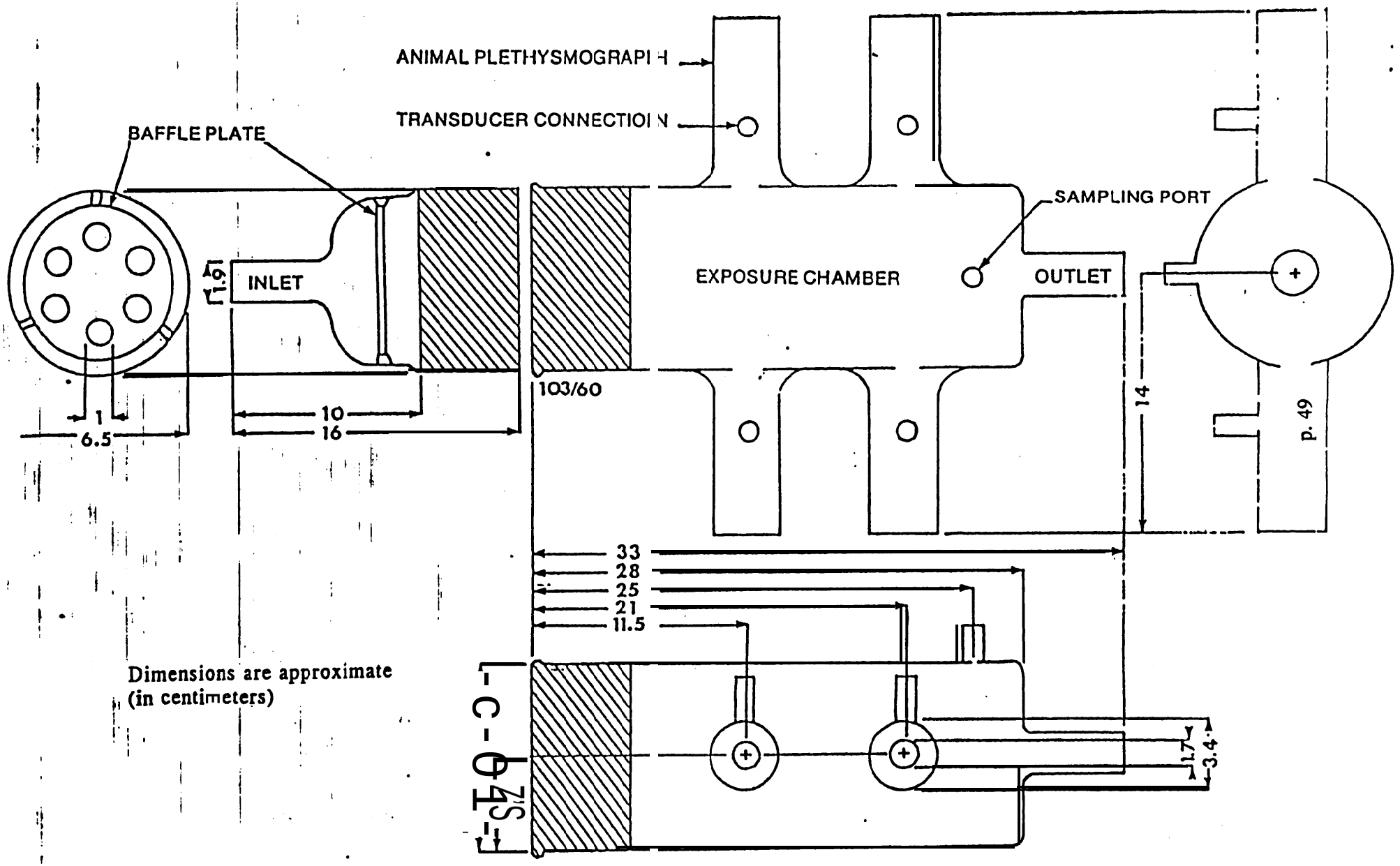
18. Compliance with Government Regulations:

This study will be conducted in compliance with the EPA (TSCA) Good Laboratory Practice Standards set for in Part 792 of Title 40 in the Code of Federal Regulations and according to the Statement of Work (CPSC-R-97-5249) and appropriate modifications to ASTM method E981-84, "Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals". At least one critical phase of the study will be audited by IITRI's Quality Assurance Unit.

19. Changes or Revisions of the Protocol:

No changes will be made without the verbal approval of the Sponsor. Any changes or revisions of the protocol shall be documented with accompanying explanations, signed by the Study Director and Sponsor, dated and maintained with the Protocol.

Figure 1
Anima l Exposure Chamber



Dimensions are approximate
(in centimeters)

U.S. CONSUMER PRODUCT SAFETY COMMISSION
WASHINGTON, D.C. 20207

IMPORTANT NOTICE

The U.S. Consumer Product Safety Commission (CPSC) contracted with Air Quality Sciences (AQS) to investigate the potential for chemicals emitted from carpet and carpet cushions to produce sensory and pulmonary irritation in mice. These data were needed to evaluate the potential for health effects that might occur in humans following exposure to carpet and carpet cushion chemicals that might be released after new installations in homes.

The attached final report from Air Quality Sciences (AQS) represents work conducted by them under CPSC contract number CPSC-C-94-1122. After reviewing supplemental data records associated with this study, the CPSC staff determined that considerable measurement errors may have occurred during the performance of at least some of the work conducted under this contract. Therefore, the staff believes that some of the information contained in the report may be inaccurate and misleading and no conclusions can be drawn from this study.

The following discussion describes the nature of the errors that led the staff to conclude that the report contains inaccurate and misleading information.

On April 9, 1997, CPSC staff received study data that were not made available during the performance of the contract, CPSC-C-94-1122. The records revealed that considerable measurement error may have occurred in the determination of butylated hydroxytoluene (BHT) exposure concentrations during the sensory irritation experiments with that compound. Because of the uncertainty in BHT vapor concentrations, the RD_{50}, RD, and other exposure measurements for BHT cited in this report are considered unreliable.

AQS used a total hydrocarbon (THC) analyzer calibrated with propane gas to measure BHT exposure concentrations during the sensory irritation experiments. AQS converted the THC analyzer response to mass units of BHT by calculating a series of response factors using a gas chromatograph/mass spectrometer (GC/MS) that was routinely calibrated with toluene. The only direct measurement of BHT was a single GC/MS calibration using three mass loadings performed nearly a year before the sensory irritation experiments with BHT were initiated. As a result, AQS never properly calibrated the analytical instruments for BHT, creating uncertainty with regard to the BHT levels during the sensory irritation testing. The analytical instruments were also calibrated for other sensory irritants only once during the contract study.

The Final Report cites stable and accurate mean BHT vapor concentrations during animal exposures with a relative standard deviation of 5 to 20 percent. However, CPSC staff subsequently learned that only the THC response in ppm propane was stable and accurate. The GC/MS measurements during the same exposures had extremely high relative standard deviations ranging from 20 to 100 percent. This degree of variability indicates that either the GC/MS was not accurately measuring BHT, or the BHT vapor concentrations were not as stable as suggested by the THC analyzer. As the GC/MS data were used to determine the THC response factor, the accuracy and stability of the BHT exposure concentrations are in question. The measurement precision of the other sensory irritants, reported in the same way as BHT, are also in question.

CPSC staff examined the correlation between the THC analyzer output and GC/MS data for seven sensory irritation experiments with BHT. A linear regression analysis of the data shows an exceptionally weak correlation between these two variables ($r^2 = 0.165$). This finding casts further doubt about the accuracy of the BHT exposure determinations. Therefore, CPSC staff decided to repeat the BHT sensory irritation study at a different testing laboratory.

Exposure-related data for other tested chemicals may also be in error for the same reasons as BHT. Therefore, the staff is unable to fully evaluate and verify the accuracy of the RD_{50} s and RD_{20}s and exposure measurements for these other compounds.

At the request of Air Quality Sciences, Inc. ("AQS"), the Consumer Product Safety Commission ("CPSC") has permitted it to respond to the CPSC Notice that accompanies this report. The fact that the CPSC has permitted this response does not mean that the CPSC agrees with any statement explicit or implicit in it:

RESPONSE OF AIR QUALITY SCIENCES TO CPSC NOTICE DATED MARCH 2, 1998 RE REPORT ON CARPET SYSTEM CHEMICALS

Air Quality Sciences ("AQS") stands by the conclusions contained in the Report on Carpet Systems Chemicals that it submitted to the Consumer Product Safety Commission ("CPSC"), the procedures utilized in the course of its report, and strongly objects to the characterizations of its work as contained in the CPSC Notice Dated March 2, 1998 accompanying release of its report. All. study procedures and results had been monitored and reviewed by the CPSC prior to its acceptance of the AQS final report.

AQS submitted its final report entitled "Sensory and Pulmonary Irritation Studies of Carpet System Materials and their Constituent Chemicals" to the CPSC on January 3 1, 1996. As directed by the terms of the contract, this report presented limited research studies on the sensory and pulmonary potential of 17 chemicals, selected by CPSC for study. As anticipated by the contract, AQS' work resulted in the presentation of limited data on the sensory and pulmonary irritation characteristics of the 17 chemicals studied and some mixtures of these chemicals. AQS conducted the study following its standard IS0 quality assurance procedures for animal testing and environmental chamber chemical measurements. All scientific procedures followed by AQS are fully documented and are presented in the final report.

AQS presented its completed studies along with discussions of how data were obtained and potential limitations of the data. Summary exposure data are presented in Table 24 for BHT and a discussion of the data is presented in Section 4.2.7 of the report. The analytical data is valid, defined by the methodologies by which it was achieved. The BHT exposure concentrations showed variations from 5.2 to 19.6% relative standard deviation and mass spectrometric determinations of BHT were accurate with recovery of 98.5 +/ - 9.6%, as presented in the final report. The range of estimated RD_{50} for BHT is 8.1 mg/m^3 to 17.6 mg/m^3 with an average of 12.9 mg/m^3.

In January of 1997, one year following completion of the AQS work. CPSC held a meeting with industry groups to discuss their study efforts relative to potential health concerns from carpet and carpet related chemicals. The AQS study was cited during this meeting along with other data generated by CPSC. Industry scientists presented their independent studies of certain chemicals including BHT, which were similar to that found in the AQS study.

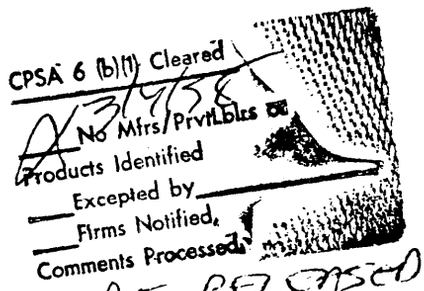
All analytical instrumentation, including the THC and mass spectrometer, were calibrated to the actual chemical being measured. AQS has conducted BHT analysis for over a 6 year period and has a fully documented and validated mass spectrometric method. Breakthrough measurements, analytical recoveries, calibrations, and methodology operating range are clearly documented and were made available to CPSC, in Tables 11 and 12 of the report. The accuracy and precision of the methodology for measuring emissions from carpet related chemicals

including BHT were revalidated prior to the start of this study and confirmed both during and after completion of this study. Summary exposure data during the animal studies measured with the THC are presented in Table 24, showing the good precision of the BHT vapor concentration.

CPSC made numerous changes to the original scope of work as the study progressed. CPSC was aware of the difficulties in generating high concentrations of chemicals, especially with chemicals such as BHT which are not volatile (this is a solid at room temperature). AQS discussed the use of a THC and received approval from CPSC to use the analyzer. The THC, calibrated to BHT and the other specific chemicals, was used to monitor the original concentrations being used to supply the animals. Mass spectrometric measures were also made at the end of the animal train to compensate for potential losses in the glass exposure apparatus. All of these data are fully documented.

In conclusion, AQS stands by its procedures and the analysis as set forth in its final report, and urges those who review the document to also consider the explanation in this Response.

FINAL REPORT



MUST BE RELEASED WITH IMPORTANT NOTICE. 3/4/96

U.S. CONSUMER PRODUCTSAFETYC OMMISSION

Contract **CPSC-C-94-1122**

"SENSORY AND PULMONARY IRRITATION STUDIES OF CARPET SYSTEM MATERIALS AND THEIR CONSTITUENT CHEMICALS"

NOTE

CPSC's position on this report is explained in the accompanying "Important Notice Concerning Air Quality Sciences January 31, 1996 Contract Report on Carpet System Chemicals."

This project has been funded with federal funds from the VS. *Consumer Product Safety Commission under Contract number CPSC-C-94-1 722. The content of the report does not* necessarily *represent the views of the Commission, nor does mention of trade names, commercial products, or organizations imply endorsement by the Commission.*

Released by Air Quality Sciences, Inc.
AQS Report No. **01890-06**
January **31, 1996**

AIR QUALITY SCIENCES. INC. 1337 Capital Circle Atlanta. Georgia 30067 770-933-0638 • Fax 770-933-0641

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1.0 INTRODUCTION

1.0 INTRODUCTION

This report presents the results of Air Quality Sciences, Inc., (AQS) investigation of sensory and pulmonary irritation resulting from exposure to compounds emitted from carpet and carpet-related products. This project was carried out under Contract CPSC-C-94-1122, "Sensory and Pulmonary Irritation Studies of Carpet System Materials and Their Constituent Chemicals," from the United States Consumer Product Safety Commission (CPSC). The goal of this study is the further understanding of the potential for emissions from carpet and associated products to cause health effects in humans. The principal health effects of interest in this study include respiratory tract irritation.

A standard test method, approved by the American Society for Testing and Materials (ASTM, Method E 981), was applied in this study to the evaluation of specific chemicals and chemical mixtures for their sensory and pulmonary irritation properties. This method involves the head-only exposure of mice to the atmospheres of interest, and has been demonstrated to show high correlation between the observed animal responses and subjective human responses to individual chemicals.

The specific objectives of this research project are:

Task 1

- identification of those compounds emitted by carpets and associated products for which the potential for respiratory irritation is unknown;
- generation of vapors from the identified chemicals at air concentrations of 500 mg/m^3 or the maximum achievable air concentration, whichever is lower;
- determination of the respiratory irritation characteristics (sensory and/or pulmonary irritation) of these chemicals at the above concentrations; and,
- determination of the concentration-response (and, if possible, the RD_{50}) relationship for the chemicals which show a response at the above concentrations;

Task 2

- development of testing conditions for the evaluation of potential irritation due to chemical mixtures associated with emissions from carpet system products, at concentrations 10 to 100 times higher than concentrations determined in chamber tests under standard conditions of loading, temperature, and relative humidity;

- determination of the respiratory irritation characteristics and chemical atmospheres during this testing; and,

- identification and implementation of a strategy for understanding which compounds emitted by the product samples contribute to any respiratory irritation observed during the above tests.

Results from this study are intended to be used by CPSC staff to conduct a screening level risk assessment to human health of exposures to the emissions from carpet and associated products in indoor settings, with an overall goal of determining whether there are specific chemicals which could contribute to complaints associated with product emissions.

1.1 BACKGROUND

Numerous accounts in the popular media and consumer complaints to industry and government regulatory agencies have suggested a possible connection between volatile emissions from new carpet installation and the occurrence of complaints [1]. Reported complaints include eye and upper respiratory tract irritation, headache, nausea, and memory loss, and are similar to health effects associated with "sick building syndrome" (SBS)[2]. Eye and upper respiratory tract irritation are consistent with human physiological responses upon exposure to chemicals classified as sensory and pulmonary irritants. Additionally, many volatile organic compounds may cause both irritation and the non-specific symptoms (headache and nausea) described in SBS [3].

As a means of establishing the contribution of chemical emissions from carpet and associated products to these health complaints, the CPSC has implemented a study with two general goals:

- determination of the sensory and pulmonary irritation characteristics of selected chemicals known to be emitted from carpets and associated products, and

- investigation of the sensory and pulmonary irritation characteristics of mixtures of chemicals associated with the emissions from selected product samples, for which complaints about adverse health effects have been recorded.

The current report summarizes the data generated from this project.

Sensory irritation is a physiological response to chemical exposure which has been quantified in mice for individual chemicals and defined mixtures by a standardized bioassay [4]. This bioassay has been applied to determine the degree of irritation associated with a large number of different

airborne chemicals, in the form of vapors and aerosols [5,6]. Sensory irritation in mice produces a reflexive change in breathing pattern, resulting in a concentration dependent decrease in respiration rate caused by stimulation of the trigeminal nerve endings in the nasal mucosa [6]. While normal waveforms are nearly sinusoidal, waveforms during exposure to sensory irritants show a pause during the expiratory phase of breathing, a distinguishing characteristic of sensory irritation, The degree of irritation is quantified by the decrease from baseline of the mean respiratory frequency of the exposed mice. Exposure to varied concentrations of an individual chemical allows determination of the concentration at which a 50% decrease in mean respiratory rate results, or the RD_{50} of a specific chemical atmosphere. RD, values for many chemicals have been shown to correlate with human irritation [6] and with threshold limit values for industrial exposures [5].

Pulmonary irritation is a less well-defined physiological response which may be assessed by application of the same methodology [4]. Pulmonary irritation produces a qualitative change in the respiratory pattern distinct from sensory irritation, involving a post-expiratory bradypnea which has been associated with peripheral irritation in the respiratory tract [7]. While it is generally thought to be preferable to do more extensive testing, including assessing respiratory characteristics during tracheal cannulation (to bypass the nerve endings of the upper respiratory tract) [8-11], the potential for a chemical to cause pulmonary irritation may be assessed by this waveform change.

Environmental chamber testing has identified many chemicals emitted by carpets and associated products [12-13]. These chemicals are generally volatile organics; however, the potential irritating properties are not established for a!! of these chemicals. Several tests have indicated the potential for product emissions (or other chemicals associated with indoor air) to cause irritating effects under some conditions [14-17]. More detailed information about the specific properties of the emitted chemicals is necessary to fully establish the potential for any irritation caused by these products.

This report describes the application of the methodology for determination of sensory and pulmonary irritation to organic vapors emitted by carpet and associated products. The term "respiratory irritation" is used in this report to refer to sensory and/or pulmonary irritation, as described in the standard test method [4]. The data resulting from this study can be used to evaluate the potential for emissions from these products to cause human irritation.

2.0 SUMMARY

2.0 SUMMARY

2.1 TASK 1: Determination of the sensory and pulmonary irritation characteristics of selected chemicals known to be emitted from carpets and carpet cushions

A total of 17 target compounds associated with carpet and carpet cushion emissions were selected for Task 1 testing. Of these compounds, 10 were identified to result in measurable sensory irritation at levels below 500 mg/m^3. None of the target compounds was found to induce pulmonary irritation at the tevets studied. The sensory irritation caused by these compounds was compared based on the levels which were predicted to result in 50%, 20%, and 12% respiratory depression (RD_{50}, RD_{20}, and RD,,), as measured by the ASTM E 981 bioassay [4]. These levels were also compared to data for formaldehyde vapor, a known irritant of relatively high potency.

Predicted RD_{50} values for Task 1 testing ranged from 7.8 mg/m^3 to 320 mg/m^3 (1.3 ppm to 60 ppm). Three of the Task 1 irritants were found to cause irritation at levels comparable to formaldehyde vapor, which had a measured RD,, in this study of 12.9 mg/m^3 (10.5 ppm). These were 2,6-di-*tert*-butyl-4-methylphenol (BHT), 2-methylnaphthalene, and 1,4-dimethylpiperazine. The RD_{20} and RD,, data showed these same three compounds having comparable potency to formaldehyde, with 1,3-dichloro-2-propanol also having similar potency.

Exposure-response characteristics of the Task 1 compounds were evaluated over a range of concentrations. Comparison of the slopes of the log (exposure concentration) vs. respiratory response curves showed that most of the Task 1 compounds had similar slopes. Notable exceptions were BHT, which had a steeper slope, and 1,3-dichloro-2-propanol, which had a more shallow slope. The slope of the formaldehyde log (exposure concentration) vs. respiratory response curve was also more shallow than the majority of the Task 1 compounds, though it was not as shallow as 1,3,-dichloro-2-propanol. The significance of the slopes of the exposure-response curves is not completely understood, though the range over which an irritant will exert its effects is likely to be related to this slope.

The characteristics of the respiratory response during each exposure were also investigated. Most compounds were found to cause an immediate respiratory depression (within 10 minutes) upon *onset* of the exposure at high concentrations. At concentrations below the RD_{20}, the onset of respiratory depression and sensory irritation waveforms became more delayed. N,N-Dimethylacrylamide was a notable exception. Respiratory depression and waveform changes were delayed (> 20 minutes) upon exposure to this compound at all concentrations studied. Again, the significance of these observations is not clear, but may be related to the manner in which the compounds are transported to their sites of action, how they interact with a putative receptor, and whether there is any biotransformation prior to the exertion of their effects.

Details of the Task 1 data are provided in Chapter 4.

2.2 TASK 2: Investigation of the sensory and pulmonary irritation characteristics of mixtures of chemicals associated with the emissions from selected product samples

2.2.1 Testing of defined mixtures

Defined mixtures of two or three compounds identified as irritants in Task 1 testing were evaluated for potential interactions. The compounds chosen to be studied for interaction effects were selected based on their occurrence in emissions from common product types or systems studied previously in environmental chambers. Three defined mixtures were evaluated, with target levels established to determine their interactions. Unfortunately, it was difficult to consistently obtain exposure conditions which were close enough to target levels to satisfactorily evaluate the interaction effects. However, there was some suggestive evidence for antagonistic effects between certain combinations of compounds at some of the tested levels. A much broader evaluation, with more focus on generation of the target levels within tight tolerances, would be required. Additionally, the individual chemical exposure-response characteristics would need to be established within known confidence limits to completely evaluate any interactions.

2.2.2 Testing of mixtures representing emissions from product types

Testing of "synthesized mixtures" was done to evaluate the respiratory irritation potential of more complex mixtures associated with product emissions. The test mixtures were created based on chamber evaluations of different product types, and generated at levels up to 100 times higher than the highest concentrations determined in the chamber tests. This was because respiratory irritation measured in mice usually occurs at levels 10 to 100 times higher than levels which result in irritation in humans.

The mixtures were designed to represent emissions from seven product types tested at CPSC: styrene-butadiene latex rubber (SBR) backed carpet, two types of prim6 urethane carpet cushion, sponge rubber carpet cushion, bonded urethane carpet cushion, and two systems of cushion and carpet associated with consumer complaints. Irritation was detected in synthesized mixture tests simulating all product types, with the exception of the SBR carpet mixture, which did not cause measurable irritation at the tested levels. In all cases, removal of one or two compounds expected to cause irritation based on 'Task 1 testing, was able to abolish the observed irritation at these levels. Irritation was generally not detected for any mixtures at levels less than 10 - 20 times higher than the chamber test levels.

3.0 METHODOLOGY

3.0 METHODOLOGY

The two principal tasks for this project included the assessment of the respiratory irritation characteristics of specific individual volatile organic chemicals (VOCs), and the irritation testing of mixtures of compounds associated with the emissions from product samples, to determine those chemicals which could contribute to any observed irritation. These tasks have been divided into subtasks, in order to describe the approach for the project.

3.1 TASK 1: Determination of the respiratory irritation characteristics of selected chemicals known to be emitted from carpets and carpet cushions

3.1.1 Compound identification

At the inception of the project, CPSC staff provided a list of 28 compounds identified in emissions from carpet and associated materials [13], which were considered most likely to potentially contribute to sensory and/or pulmonary irritation. Those chemicals most likely to cause sensory or pulmonary irritation were determined1 based on structure-activity relationships [18], and on previous evaluations of similar compounds for sensory and pulmonary irritation? The prevalence of identification for some compounds, and the levels at which they were observed in emissions studies, also were accounted for in creating the target list. Structural attributes common to many sensory irritants include the presence of aldehyde groups, allyl compounds, halogens, and amines [5,18]. Pulmonary irritation is often associated with compounds having nitrogen-containing groups, such as arnines [8-10] and isocyanates [11, 19-20].

The target compounds are listed in Table 1, along with their boiling points (°C), molecular weights, densities, and physical states at standard temperature and pressure, when these data were available. Some existing toxicological information from these compounds, as summarized from the Toxicology Data Network (Hazardous Substances Data Base) available through the National Library of Medicine, is provided in Appendix A.

3.1.2 Generation of exposure atmospheres for individual chemicals

The target compounds identified in Table 1 were prioritized for exposure testing. Seventeen of the 28 compounds listed were eventually tested for irritation properties. Prior to exposure testing, development work determined the appropriate conditions and methodology required to generate these chemicals as vapors of the desired concentrations (500 mg/m^3 or the maximum achievable air concentration, whichever was lower). Depending on the chemical and its volatility, different methods were necessary to attain the desired vapor concentrations. Two principal methods were applied to the target compounds, depending on their physical state at room temperature- Those which existed as a liquid at room temperature were tested using the J-tube

methodology, while those existing as a solid were tested using a flask method. Both methods are described below.

Table 2 fists the compounds which were tested, and the methodology used to generate these compounds as vapors.

J-tube method

The J-tube method uses a 25 mm inner diameter J-shaped glass tube for vapor generation [21]. This method has been previously used in toxicology studies to generate stable vapor concentrations over 6 hour periods. Glass beads are held within the J-tube and the test compound is metered as a liquid into a 2 mm inner diameter side inlet on the J-tube via a syringe pump. Carrier gas (air or nitrogen) is heated and passed through the J-tube, with the glass beads providing a large surface area for liquid volatilization. The vapor concentration is dependent upon the carrier gas flow rate, the rate of liquid delivery, and the air temperature and pressure,

A schematic of the J-tube device is given in Figure 1.

Advantages of the J-tube device include:

- minimization of thermal decomposition of test liquid;
- minimal hazard when vaporizing potentially explosive compounds, since the highest concentration of the compound exists only in a short length of tube;
- stable generation rate over an extended period of time; and
- high efficiency of liquid vaporization.

flask method

A relatively simple method for generation of high concentrations of vapors involves placing samples of the chemical into a round-bottom flask. The flask is placed in a heating mantle or a water bath to control the temperature of the liquid, and a carrier gas (air) is directed through the flask to generate the vapor containing stream. The rate of volatilization of the vapor from the liquid is controlled by the carrier gas flow rate and composition, and the temperature of the surrounding heating mantle or water bath. A schematic of a vapor generation device operating under these principles is given in Figure 2. Maintaining the volume in the flask at a constant temperature and large enough volume produces a relatively constant rate of vaporization.

The advantages of the flask method include:

- a potentially large area for volatilization of the compound;
- the method is applicable to compounds which exist as a solid at room temperature; and
- the apparatus is relatively inexpensive, and easy to clean and re-use for a different chemical.

Disadvantages of the flask method include the fragile apparatus (glassware and support stands), the need to ensure tightly sealed connections between the glass generation device and any carrier or dilution gas lines, and the possibility of uneven heating, which may cause thermal degradation of the target compound.

3.1.3 Chemical monitoring

Once vapors were generated from a given compound, monitoring of the vapor atmospheres for stability of the concentration during each test was performed using a total hydrocarbon analyzer {Model 51, Thermo Environmental Instruments, Inc., Franklin, MA). This instrument contains a flame ionization detector, and measures total VOCs over a 0.1 to 10,000 ppm range relative to propane, For this study, the instrument was calibrated for exposures to each of the target chemicals using concentration data obtained by gas chromatography/mass spectrometry (GC/MS, described below), where the relative GC/MS responses were determined from standard solutions of the target compounds.

The GC/MS method consists of collection of VOCs on triple bed sorbent tubes containing Carbotrap C, Carbotrap 20/40 mesh, and Carbosieve SIII (Supelco, Bellefonte, PA). Air is pulled through the tubes at between 0.01 anclO.2 L/min, and any chemicals contained in the air are adsorbed onto the traps. Sampled tubes are subsequently analyzed by GC/MS. A NuTech 8533 universal sample concentrator (NuTech, Durham, NC), is used to desorb the collected chemicals from the traps, and concentrate the sample into a cryotrap. The cryotrap is flash-heated to transfer the sample to the GC column,, where it is cryofocussed prior to chromatographic separation Chromatography is done using a Hewlett-Packard 5890 Series II gas chromatograph, and mass speciation and quantitation with a Hewlett-Packard 5971 mass selective detector (Houston, TX). Total volatile organic compound concentrations and identifiable specific volatile organic compounds are characterized by this method. The detector is operated at a scan rate of approximately 2.4 Hz, over a total ion mass scan range between 25 and 450 m/z. Detector response is maintained on a daily basis, using a bromofluorobenzene (BFB) tuning procedure.

For target chemicals, calibration standards were used to quantify the mass of a given air sample, by determination of the mass spectral response factor of external standards of the individual chemicals themselves. An external toluene calibration standard is used to quantify the mass of any other identified chemical. Generation of the response factors involves injection of a known amount of a liquid standard onto a sorbent tube mounted to the desorption unit, and analysis as for a normal sample. A range of three mass levels were injected through the sorbent and analytical systems to generate the recovery, sensitivity, and breakthrough data for each target compound. Internal toluene standards were used in generating these data, to determine relative response factors for quantitation of exposure atmosphere data.

A daily standard of a single toluene mass is used to track instrument stability, and weekly multipoint standards are used to confirm instrument linearity over a range of masses. Other relevant analytical characteristics of the target compounds were also determined, including the suitability of the chromatographic method for separation of the target compounds, the recovery of the compound from the sorbent tube, and the existence of breakthrough under the sampling conditions used.

The analytical technique used has been shown to be generally applicable for compounds in the $C_5 - C_{16}$ hydrocarbon range, and has a detection limit of 16 ng for most individual VOCs [22]. It corresponds to EPA Method IP-1B. The precision of this method at AQS has been consistently within 10% RMD (relative mean deviation), based on replicate determinations of toluene spikes. For complex mixtures, an average precision of within 20% RMD has been determined.

Individual VOCs are identified based on mass spectral comparisons with the AQS proprietary mass spectral library and, secondarily, with a library made commercially available by the U.S. National Institute of Standards and Technology (NIST). As a result of its extensive product testing, AQS has also developed an "in-house" mass spectral library. For evaluation of product emissions, the entire chromatogram is analyzed to determine the identity and amount of the chemical emissions. Identifications based on the AQS mass spectral library are made with greater confidence, since the spectra were obtained on the AQS instruments and the retention time information is available. Identifications based on the NIST library are typically made with slightly less confidence, as the retention time information is not available.

3.1.4 Respiratory irritation testing of individual chemicals

Sensory and pulmonary irritation are associated with characteristic changes in the respiratory breathing pattern of an animal during head-only exposure [4]. For sensory irritation, the change in the waveform is caused by a pause in breathing during expiration, and is accompanied by a decrease in the breathing frequency. Pulmonary irritation is associated with a pause at the end of expiration, and may not include a change in breathing frequency. The characteristic waveforms which are used to assess irritation are shown in Figure 3.

Exposure system

The AQS animal exposure system used during this study is shown schematically in Figure 4. The 2.7 L animal exposure chamber was identical to that described by the ASTM standard [4], and was manufactured from Pyrex@ glass. Purified (contaminant-free) air or exposure air was supplied to the animal chamber, depending on the position of the valving. The chamber was designed to ensure a well-mixed atmosphere, and minimal surface adsorption or other chemical interactions. Experimental test conditions of supply air flow rate, temperature, and relative humidity were monitored during each test. Supply air to the volatilization system was maintained at the same temperature and relative humidity conditions as the chamber supply air, although this air was sometimes pre-heated prior to entering the volatilization unit, to provide more efficient volatilization of the test compounds. In these cases, supply air to the animal chamber was maintained at ambient temperature during exposures. Mass flow controllers and rotameters were used for airflow measurement, and Vaisala temperature and relative humidity sensors were used for monitoring of chamber temperature and relative humidity.

Respiratory activity durina exposure

Four animals were used for each exposure. Each animal was placed in a glass plethysmograph sleeve, which fit into the glass plethysmograph arm of the exposure chamber. A Teflon®/glass method was used to seal the animals in the plethysmograph. This method utilized glass inserts in the side arms of the chamber. A Teflon' collar was cut from 0.065 in. thick Teflon@ sheet, and a hole with diameter approximately 1.6 cm was cut into the center of the collar. The collar was attached to the end of the glass sleeve (dimensions about 7.4 cm length and 2.9 cm outer diameter) with Teflon® tape, with some of the tape molded around the inner surface of the collar, to aid in sealing around the neck of the animal..

The glass sleeve was inserted into the side arm of the plethysmograph in such a way that the head protruded into the central cylinder of the exposure chamber. A rubber stopper was used to seal the sleeve within the side arm. Pressure from behind the sleeve, by the rubber stopper, compressed the Teflon* tape to seal the front of the plethysmograph. Normal respiratory waveforms were verified by inspection of the pressure tracings [4]. A schematic of the Teflon® sealing method is shown in Figure 5.

Each plethysmograph was connected to an individual differential pressure transducer (Validyne Engineering, Model DP45-14, Northridge, CA). These transducers have a manufacturer-specified range of il.4 to 225 cm H_2O. The transducers were connected to a computer-based data acquisition system (based on LabView®, National Instruments, Austin, TX) which displayed in real time the respiratory activity of all four animals and digitally recorded the individual respiratory waveforms. Respiratory frequencies (individual and the mean of the four animals) were continuously calculated and digiitally recorded during the course of the exposure.

Exposure Time Course

Animals were exposed to each target vapor concentration for a single exposure period. Initially, animals were loaded into the animal chamber and exposed to purified air for a 30 minute baseline determination of respiratory characteristics. After the baseline period, the valves were switched to direct the exposure vapor through the animal exposure chamber. individual animal respiratory activity was then monitored throughout an exposure period of 60 minutes. A 15 minute recovery period, with the animals exposed to purified air, followed the 60 minute exposure period.

A 60 minute exposure period was used rather than the shorter 30 minute exposure suggested by the standard [4] to detect any irritation occuring after a delayed onset. For example, a delay in the onset of pulmonary irritation, with plateauing of the response occurring after 60 minutes, has been observed for exposures to methyl isocyanate [11].

If at any time an individual animal reached a respiratory depression of more than 85%, the exposure was stopped, and purified air re-introduced into the animal chamber for a recovery period, This was intended to avoid unnecessary exposures, and to avoid exposing animals to concentrations which could not be used to generate an exposure-response curve.

Analysis of respiratory response

Respiratory frequencies were determined digitally from the pressure traces recorded from the plethysmograph. Frequencies were determined from the averages of 15 second periods throughout the course of the test. The baseline respiratory frequencies were determined from the final 15 minutes of the 30 minute acclimation period, to eliminate abnormal frequencies associated with the initial stress of animal confinement. The effects of body and head movements on the respiratory waveforms were removed from the pressure traces by calculating a running time-averaged frequency of each individual mouse over a three minute interval [4].

Average frequencies for each group of four animals were determined from the individual frequency data for each time interval. Sensory irritation is indicated when the characteristic change in respiratory waveform is accompanied by a decrease in the mean respiratory frequency of the group of mice of 12% or greater [6]. In exposures classified as positive for sensory irritation, at least three mice were verified to have sustained waveform changes lasting at least three minutes during the exposure, A positive classification for pulmonary irritation was indicated only from the change in waveform, although for some compounds, pulmonary irritation has been shown to be accompanied by respiratory depression [8-11]. The criterion for a positive pulmonary irritation response in these tests was the presence of sustained waveforms exhibiting the characteristic post-expiratory bradypnea in at least 3 animals, for periods of at least 30 seconds. However, no exposures were found to result in pulmonary irritation.

Analysis of respiratory response during initial exposure testing

The results of the initial exposure testing are reported in subsequent sections as a mean percent respiratory depression. The respiratory depression may be interpreted in terms of a degree of response (slight, moderate, or extreme), as described in the ASTM standard [4].

Other observations

All test animals were routinely weighed before testing, immediately following testing, the day following testing, and at several intervals over a 7 to 14 day observation period. General clinical observations relating to appearance and behavior were made before and during testing, and during the post-test observation period.

Vapor and gas monitoring

The exposure atmosphere was monitored at the outlet of the animal exposure chamber, to ensure that the target concentrations measured during the vapor generation development work were maintained during the exposure. Continuous monitoring was performed using the total hydrocarbon analyzer, and additional samples were collected onto sorbent tubes for further analysis, At least three different measurements of vapor concentration were made during the exposure: one corresponding to the initial part of the exposure (approximately the first 1 O-l 5 minutes), one near the middle of the anticipated exposure (spanning the 30 minute point), and the last near the end of the exposure (the last IO-1 5 minutes of the exposure). This was done to establish that the target compounds were the principal compounds during exposures, and to confirm the maintenance of a steady exposure concentration over the test interval. The methods to be used for chemical monitoring are discussed in Section 3.1.3.

Other gases relevant to animal physiology were monitoredduring each exposure. CO_2 levels in the animal chamber were monitored continuously with a Gas Tech CO, sensor (Model RI-41 1A, Newark, CA) or a Brüel and Kjær Multi-gas monitor (Model 1302, Brüel and Kjær, Marlborough, MA), and recorded at regular intervals. CO, levels were generally maintained under 1500 ppm at the 6.7 L/min exposure flow rate used. O_2, CO, and NH_3 concentrations in animal chamber outlet air were monitored and electronically recorded continuously during testing with Sensor Stik flow-through gas sensors (EIT, Exton, PA). These sensors have ranges of 0 to 25%, 0 to 100 ppm, and 0 to 50 ppm, respectively, for O_2, CO, and NH,.

3.1.5 Concentration-response characteristics of target chemicals

The initial test of any individual chemical involved the exposure of the animals to a concentration of 500 mg/m^3 *or,* if that was not achievable, the maximum reachable concentration. If sensory

and/or pulmonary irritation was noted during the initial test, further testing characterized the concentration dependence of the irritation. Since most irritants have been characterized over a logarithmic range of exposure concentrations [6], the subsequent exposure concentrations were chosen to minimize the number of exposures needed to determine an exposure-response curve.

Characterization of the No Observable Effect Level (NOEL)

For each compound with a positive response, the relationship between exposure concentration and respiratory irritation response was determined. Regression analysis was applied to determine the mathematical relationship between the logarithm of the exposure concentration and the maximum percent respiratory depression measured. A NOEL could be defined from the regression equation as the concentration which would be predicted to result in a 12% respiratory depression (RD_{12}), since this is the minimum depression needed to classify an exposure as having a positive sensory irritation response [6].

3.2 TASK 2: Determination of the respiratory irritation characteristics of selected carpet material samples associated with reported human health complaints

Seven test samples were evaluated for chemical emissions, including two carpet and five cushion samples. These samples were supplied by the CPSC, and included new and previously installed samples. The samples were identified only as: Carpet A, Carpet B, and Cushions A through D. The chemical emissions were evaluated to establish exposure conditions for irritation testing,

3.21 Chamber testing of product samples

Storage, handlina. and emission characterization of samples

Upon receipt from CPSC, the sealed Tedlar bags used for shipment of the product samples were inspected for possible mishandling during shipment. They were stored in a controlled space, at 23 ± 2 °C and 50 ± 5% relative humidity, until evaluation.

After any test sample was opened, it was handled with latex gloves on a table prepared with fresh heavy brown Kraft paper. Samples were freshly cut to the desired size prior to testing, and placed within the test chamber either supported on stilts (both sides exposed) or in an appropriately sized stainless steel tray. Any unused portions of the sample are stored wrapped in the original Tedlar, with an outer wrapping of foil.

Emission characterization of samples

The test materials were evaluated by environmental chamber testing using typical-use conditions. A sample of the product was cut to provide a loading within the chamber of 0.4 m^2 of test material area per m^3 of environmental chamber volume. This corresponds to the loading of a carpet product within a room having an 8 foot ceiling height and a floor fully covered with material. The characterization test was done with the chamber maintained at 23 ± 2 °C, 50 ± 5% relative humidity, and air flow through the chamber of 1.0 ± 0.05 air changes per hour. Chamber environmental conditions of temperature and relative humidity were monitored continuously using factory-calibrated sensors. Air flow rates were controlled with either calibrated mass flow controllers or rotameters. Supply air to the environmental test chambers was monitored to ensure that less than 2 $\mu g/m^3$ of any VOC could be detected.

Chambers from 50 - 90 L in volume were used for emission testing. These chambers had an electropolished stainless steel interior surface, rounded comers, and tightly sealed penetrations to minimize surface adsorption and sink effects of chemicals. Supply and exhaust manifolds in these chambers were aerodynamically designed, and verified to ensure adequate internal mixing according to sulfur hexafluoride tracer decay measurements, as described in ASTM D-51 16 [23]. The product evaluation tests followed the guidelines of ASTM D-51 16 [23] and the *Standard Test Method for Determining* Total *Volatile* ***Organic Compound*** *Emission Factors* ***from Carpet under Defined Test Conditions Using*** *Environmental Chambers* (or the Carpet Test Method, provided as Appendix B).

Analysis of the product emissions was done by sampling air at 1, 6, 24, 48, 72, and 96 hours following the beginning of dynamic product ventilation. Data from these tests was used to characterize the emissions of the product samples, to determine the extent to which the emitted chemicals corresponded to the Task 1 test chemicals, and to identify those chemicals with a potential to cause irritation. These data were also used to identify testing conditions for irritation testing,

Determination of exposure test conditions

Emissions from carpets and associated products generally will result in chamber concentrations between about 20 and 1000 $\mu g/m^3$. Since target concentrations for Task 2 testing were required to be 10 to 100 times higher, environmental chamber conditions were varied to evaluate methods to enhance the sample emissions for respiratory irritation testing.

The variables used in the generation of increased emissions from the product samples included product loading, chamber temperature, and air flow rate. Two samples were tested with the product loaded at twice the normal loading, with both sides of the product exposed to the airflow by elevating it on short, stainless steel stilts. This effectively quadrupled the amount of surface

available for chemical emission. Additionally, since previous testing found that carpet emissions may increase by a factor of 30 when the product is heated to 70 °C [14], these tests were conducted with the chamber maintained at 70 °C, to evaluate any changes in the emissions at high-end conditions.

Initial product sample testing indicated that the elevated conditions required to create exposures at high concentrations could result in a significant change in the chemical character of the exposure atmosphere. That is, under conditions of elevated temparature and increased loading, some compounds were detected which were not detected in tests at ambient temperature and typical loading, and other compounds were detected in significantly different relative proportions. As a result, synthetic mixtures were created to evaluate the potential of these materials to cause irritation. These mixtures were designed to "mimic" the product emissions in terms of the content and relative amount of volatile organic compounds at ambient conditions, but at elevated concentrations. The vapor generation methodology described in Section 3.1.2 was used to create these exposure atmospheres. The mixtures themselves are described in further detail in Section 3.2.2.

Chemical measurements

The triple-bed sorbent sampling method combined with GC/MS analysis (described in Section 3.1.3) was used to evaluate the product sample emissions.

3.2.2 Respiratory irritation testing of chemical mixtures representing emissions from product samples

Chemicals identified in emissions from specific product types were determined at ambient conditions. "Characteristic' compounds were associated with each of the product types, and the approximate relative composition of these compounds was-determined by the CPSC (in consultation with AQS). Synthetic mixtures were created based on these chemicals in the same relative amounts, but at concentrations 10 to 100 times higher than those measured during chamber testing. Seven different synthesized mixtures were created to simulate the "enhanced" emissions from different products. These mixtures simulated one type of carpet, four types of cushion, and two 'systems" based on combined emissions from assemblies of carpet and cushion. The mixtures used to simulate the "systems" included chemicals which had previously been identified in samples associated with consumer complaints of irritation. Sensory irritation from these mixtures was evaluated, and further testing done to isolate specific irritants of concern.

Exposure atmosphere compositions

Table 3 summarizes the different test systems which were modeled using synthesized mixtures. The target ranges for exposure concentrations are provided in Tables 4 - 10. Also indicated are tie concentrations measured for the compounds of interest in chamber tests of these types of materials, and those compounds which were observed to decay within 24 hours during chamber testing.

Exposure time course

Exposures were conducted identically to the single chemical exposure testing. After a 30 minute baseline respiratory determination, the valves connecting the product and animal chambers were switched to direct exposure air through the animal exposure chamber. The respiratory response of the animals was monitored for a 60 minute period, which was followed by a 15 minute recovery period with animals exposed to purified air.

General observations

As for the Task 1 testing, all test animals were routinely weighed before and the day after testing, and general clinical observations relating to appearance and behavior were made before, during, and after testing. Additionally, animals exposed to the synthesized mixtures were held for a period of seven days, for further observation of general clinical appearance. Weight changes over this time were also monitored.

Evaluation/identification of emitted compounds contributing to irritation from product samples

The strategy used for evaluating which of the chemicals in the synthesized mixtures caused any observed irritation involved testing of the initial mixtures, followed by subsequent tests in which a suspected irritant was removed, while other compounds of the mixture were retained at similar levels. Suspected irritants were identified based on results of Task 1 testing. If irritation was observed in an initial test, and this second test eliminated the observed irritation, it suggested that the removed chemical was a principal contributor to the observed irritation. If the second test did not eliminate the irritation, further testing was done using a "synthetic" mixture which also eliminated the next most likely contributor to irritation, until the most likely irritants were identified.

If the first tests to any synthetic mixture resulted in irritation, testing was also done at a lower exposure concentration (while maintaining the same approximate relative composition of each compound). These tests were to establish the mixture concentration which would be below that which caused any irritation.

Testing of defined binary or ternary mixtures

Additional exposures to defined mixtures of compounds were done using compounds known to be emitted from specific carpet system materials based on previous work at CPSC. These mixtures focused on chemicals also shown to be sensory and/or pulmonary irritants. The exposures were designed to characterize the interaction (i.e., additive, synergistic, antagonistic) among the different irritating chemical components. Generation of the exposure mixtures involved the methods described in Section 3.1.2. Further details on the composition of these mixtures are provided in Section 5.

4.0 RESPIRATORY IRRITATION TESTING OF SELECTED CHEMICALS KNOWN TO BE EMITTED FROM CARPETS AND CARPET CUSHIONS: TASK 1

4.0 RESPIRATORY IRRITATION TESTING OF SELECTED CHEMICALS KNOWN TO BE EMITTED FROM CARPETS AND CARPET CUSHIONS: TASK 1

4.1 Analytical Evaluation of Target Compounds

4.1.1 Previous methods

Existing analytical methods were checked to determine if specific methods existed to measure the target chemicals vapors in air. Manuals of analytical methods from the National Institute for Occupational Health and Safety (NIOSH) [24] and the U.S. Environmental Protection Agency (EPA) [22,25] were consulted to determine whether specific methods were developed. Specific NIOSH methods were identified for three of the target chemicals: N,N-dimethylacetamide {Method 2004), N,N-dimethylformamide (Method 2004), and vinyl acetate (Method P&CAM 278). However, the sorbent sampling and thermal desorption methodology described in Section 3.1.3 was found to be satisfactory for the target chemicals.

4.1.2 Analytical evaluation

An analytical evaluation was completed for each of the seventeen target list chemicals. An additional 24 compounds used in the synthesized mixture tests for Task 2 were also evaluated. Specific evaluations for each chemical included:

- mass spectral and chromatographic characteristics (including retention time and peak shape);
- ability of the desorption instrument to effectively recover chemicals of interest from the sorbent tubes used for sampling;
- sensitivity of the mass selective detector (relative to toluene) to a range of masses representative of the target range for the analysis; and
- determination of the potential for breakthrough of the chemicals of interest through the sorbent collection system, under nominal sampling conditions (18 L of air at room temperature, 70-75°F).

Three mass levels were used to evaluate the recovery, sensitivity, and breakthrough information for each compound. Table 11 provides data for the analytical evaluations of compounds tested in Task 1. Data for the additional compounds tested for Task 2 are provided in Table 12.

Recovery of the Task 1 target chemicals from the triple-bed sorbent media was above 95% for 13 of the 17 compounds, indicating that this methodology was excellent for these compounds. Of the other four compounds, one (2-methylnaphthalene) was recovered at above 85%, which is satisfactory. The others (2-ethylhexanoic acid, 1,3-dichloro-2-propanol, 1-dodecanol) were

recovered at between 60 and 80%, which generally indicates that another methodology should be investigated. However, the current methodology was considered acceptable for the exposure testing of these compounds.

The analytical evaluations of the additional compounds used in synthesized mixture testing of Task 2 demonstrated generally good recovery; however, the methodology was found to be unsuitable for several of these compounds. Poor recovery of 4-morpholineethanamine (used in the synthesized mixture tests simulating Complaint System 'B"') indicates that the quantitation of this compound in the Task 2 testing should be considered suspect. Additionally, inconsistent chromatography led to high relative standard deviations for evaluation of 1,4-dimethylnaphthalene, tridecene, acetic: acid, and decanol. Precise quantitation of these compounds may be compromised by the lack of precision in their recovery.

4.2 Respiratory Irritation Testing of Target Compounds

A complete summary of the exposure-response data for the 17 target compounds is provided in Table 13, The table includes the concentration of each target compound required to produce a 50% decrease in respiratory rate (RD_{50}), the estimated concentration which would produce a 20% decrease in respiratory rate (RD_{20}), and the estimated concentration which would produce a 12% decrease in respiratory rate (RD_{12}, which is the minimum respiratory depression required for a positive sensory irritation response). Also shown for each compound are the slope of the exposure-response curve, the correlation coefficient (f) for the log(exposure concentration) vs. response data, and the number of exposures used to estimate the RD_{50}, RD_{20}, and RD_{12}. Of the 17 compounds evaluated, 10 were found to result in a positive sensory irritation response at the exposure concentrations tested.

Figure 6 compares the predicted exposure-response characteristics of the ten compounds which were found to cause measurable sensory irritation. Data for formaldehyde, which was tested as a positive control on sensory irritation, is also included in this figure. In general, most of the compounds have similar slopes of the log (exposure concentration) vs. response curves. However, 1,3-dichloro-2-propanol is noted to have an exceptionally shallow slope (21.7), while 2,6-di-*tert*-butyl-4-methylphenol (BHT) has a steeper slope (117.7) relative to the other compounds. The full exposure-response characteristics of a compound may be important when evaluating the potential of a compound to cause irritation.

Table 14 ranks those compounds which were found to have a positive sensory irritation response *in* order of increasing RD_{50} (expressed as mg/m^3). Concentrations in ppm are also shown. The RD_{50} is often used as an indication of the relative potency of a compound for the irritation response [5]. These responses may be compared with the response measured for formaldehyde, a known, relatively potent irritant tested as a positive control, and with the RD, values for known

irritants. Of 167 compounds tested in Swiss-Webster mice for sensory irritation [5], 72 compounds (43% of those reported) were found to have RD_{50} values under 5 ppm. In the current testing, three of the ten compounds with positive responses had RD_{50} values below 5 ppm. Additionally, 53 compounds (32% of those reported) were found to have RD_{50} values under 20 mg/m^3; in the current testing, three of the ten compounds with positive responses had RD_{50} values below 20 mg/m^3. It should be noted, though, that many of the compounds reported in previous studies were tested because of anticipated irritating properties, which may result in a higher percentage of relatively strong irritants being tested.

The RD_{50} is often extrapolated to human responses; for example, 0.03 x RD_{50} has been compared to threshold limit values for industrial exposures [5]. Table 15, taken from a study by Alarie [26], shows predicted human responses to different multiples of the RD,, value in mice. Further details of the approach used to generate this table are provided in a study by Kane et al.[27].

Table 16 ranks the compounds which were found to have a positive sensory irritation response in order of increasing RD_{20}, while Table 17 ranks these compounds in order of increasing RD_{12}. The RD_{20} separates slight from moderate irritation [4], while the RD_{12} is the minimum respiratory depression needed to classify an exposure as sensory irritation. The general patterns of the rankings are similar to that for the RD_{50}; however, some compounds are found to have comparably lower RD_{20} or RD_{12} values relative to the RD,, values, and vice versa. This may indicate that the RD_{50} is not sufficient in itself to evaluate the potential for a compound to cause irritation: since some compounds may have a lower threshold for initiation of irritation in mice than would be predicted from the RD_{50} value alone, the threshold level in humans may also be related to the full exposure-response characteristics for a given compound.

Details of the data for the individual chemical vapors studied are provided in the subsequent sections,

4.21 1,2,3-Trichloropropane

Vapors of 1,2,3-trichloropropane were generated using the J-tube methodology described in Section 3.1.2. Eight total exposures were conducted to this compound, each of which were used to estimate the sensory irritation characteristics of this compound. Exposure data are summarized in Table 18.

Figure 7 shows the maximum respiratory depression vs. the exposure concentration (log scale) for the exposures to 1,2,3-trichloropropane. Regression of the data results in a correlation coefficient (r^2) of 0.733, with the RD_{50} calculated to be 119 mg/m^3 and the RD_{12} determined to be 9 mg/m^3. The slope of the regression line is 33.7.

Figure 8 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of 1,2,3-trichloropropane tested. The characteristics of the respiratory response appear to differ, depending on the concentration of the compound tested. At the higher exposure concentrations (above 200 mg/m^3), the respiratory depression was reached more quickly than at lower concentrations (within about 15 minutes). Additionally, there appeared to be some recovery over the course of the exposures at the higher concentrations, while at the lower concentrations the maximum respiratory depression appeared to be more sustained, once reached. In all cases, recovery toward baseline frequency was relatively rapid at the end of the exposure period.

Figures B-1 through B-8 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). As shown in Table 18, all exposures were above 98% 1,2,3-trichloropropane by mass concentration.

4.2.2 N,N-Dimethylacrylamide

Vapors of N,N-dimethylacrylamide were generated using the J-tube methodology described in Section 3.1.2. Five exposures were conducted to this compound, four of which were determined to clearly result in sensory irritation. The exposure at the lowest concentration (44 mg/m^3) resulted in intermittent sensory irritant waveforms and a respiratory depression consistent with slight sensory irritation, but was not classified as sensory irritation due to the lack of sustained waveforms. Applying the criteria established in the ASTM standard (sensory irritation waveforms must be sustained for at least a three minute period in at least three animals over the course of the exposure)[4], the exposure at this concentration was not classified as causing sensory irritation. The four positive exposures were used to generate an estimate of the sensory irritation characteristics of this compound. Exposure data are summarized in Table 19.

figure 9 shows the maximum respiratory depression vs. the exposure concentration (log scale) for the exposures to N,N-dimethylacrylamide. The respiratory irritation characteristics (and the least-squares best fit regression line) were determined from the four exposures with positive sensory irritation response. Regression of the data results in a correlation coefficient (r^2) of O-849, with the RD_{50} calculated to be 234 mg/m^3 and the RD_{12} determined to be 19 mg/m^3. The slope of the regression line is 34.6.

figure 10 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of N,N-dimethylacrylamide tested The onset of sensory irritation was gradual for all exposures to this compound. At all exposure concentrations, the maximum respiratory depression was reached gradually over the duration of the exposure, with the response appearing to plateau by the end of the exposure.

Recovery toward baseline frequency at the end of the exposure period was very gradual in all cases, with no group of animals completely recovering to baseline frequency by the end of the 15 - 30 minute recovery period.

Figures B-9 through B-13 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). As shown in Table 49, between 66 and 100% of the exposure concentration was determined to be N,N-dimethylacrylamide in these tests. Methanol and acetone were the major other compounds (by concentration) identified in the exposures found to be less than 99% N,N-dimethylacrylamide by mass. These compounds were used in cleaning the exposure apparatus, and were likely carried over into the test. The measured concentrations of the hydrocarbon analyzer were not adjusted for the presence of these chemicals; however, the response of the analyzer is expected to be significantly lower for these lighter compounds relative to the N,N-dimethylacrylamide. Additionally, the RD_{50}'s for methanol and acetone are 54392 mg/m^3 and 183824 mg/m^3, respectively[5]. As these values are well above the concentations of these compounds in the exposures in which they were identified (44 and 72 mg/m^3), observed sensory irritation is likely due solely to the N,N-dimethylacrylamide, and not the contaminating compounds.

4.2.3 1 ,4-Dimethylpiperazine

Vapors of 1,4-dimethylpiperazine were generated using the J-tube methodology described in Section 3.1.2. Six exposures were conducted to this compound. One exposure was found to cause greater than 80% respiratory depression in one of the four animals exposed, resulting in premature termination of this exposure (see Section 3.1.4). Although the data from this exposure are provided, this exposure was not used in the calculations of the irritation characteristics of this *compound. The four other exposures were used to estimate the sensory irritation characteristics of this compound. Exposure data are summarized in Table 20.

Figure 11 shows the maximum respiratory depression vs. the exposure concentration (log scale) for exposures to 1,4-dimethylpiperazine. The respiratory irritation characteristics (and the least-squares best fit regression line) were determined from the four complete (60 minute) exposures. Regression of the data for these five exposures results in a correlation coefficient (r^2) of 0.785, with the RD_{50} calculated to be 20 mg/m^3 and the RD,, determined to be 3 mg/m^3. The slope of the regression line is 44.1.

Figure 12 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of 1,4-dimethylpiperazine tested. The onset of sensory irritation was virtually immediate for all exposures to this compound. At all exposure concentrations but the lowest, the maximum respiratory depression was reached within

the first 15 minutes of the exposure, with the frequency beginning to recover over the rest of the exposure, At the lowest exposure concentration, the frequency data varied over the course of the exposure. Recovery toward baseline frequency at the end of the exposure period was almost immediate in all cases.

Figures B-14 through B-19 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). As shown in Table 20, more than 92% (by mass) of the exposure atmospheres consisted of 1,4-dimethylpiperazine.

4.2.4 N,N-Dimethylbenzylamine

Vapors of N,N-dimethylbenzylamine were generated using the J-tube methodology described in Section 3.1.2. Five exposures were conducted for this compound, of which four were identified as resulting in measurable sensory irritation. Exposure data are summarized in Table 21.

Figure 13 shows the maximum respiratory depression vs. the exposure concentration (log scale) for ail exposures to N,N-dimethylbenzylamine. Regression of the data for the four exposures resulting in measurable sensory irritation results in a correlation coefficient (r^2) of 0.993, with the RD_{50} calculated to be 292 mg/m^3 and the RD,, determined to be 25 mg/m^3. The slope of the regression line is 35.4.

Figure 14 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of N,N-dimethylbenzylamine tested Respiratory depression and the onset of sensory irritation was immediate for all exposures to this compound except at the lowest concentration, for which sensory irritation was not observed. An apparent recovery during the exposure period was observed for this compound, up to the highest exposure concentration (averaging 907 mg/m^3), for which the recovery appeared to plateau. Following exposure, a rapid recovery toward baseline was indicated for all but the highest exposure concentration.

figures B-20 through B-24 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). As shown in Table 21, exposure compositions of identified volatile organic compounds (VOCs) averaged between 98 and 100% N,N-dimethylbenzylamine by mass concentration.

4.2.5 N,N-Dimethylacetamide

Vapors of N,N-dimethylacetamide were (generated using the J-tube methodology described in Section 3.1.2. A single exposure was conducted to this compound, at the highest attainable exposure concentration 440 mg/m^3. This exposure did not result in measurable sensory irritation to this compound. Table 22 summarizes the exposure conditions for this test, and Figure B-25 shows the respiratory response (as a percent of baseline) vs. time for this exposure, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). N,N-dimethylacetamide was the only VOC identified in air samples collected during this exposure. No further exposures to N,N-dimethylacetamide were conducted.

4.2.6 2-Methyleneglutaronitrile

Vapors of 2-methyleneglutaronitrile were generated using the J-tube methodology described in Section 3.1.2. Five exposures were conducted to this compound. The exposure at the lowest concentration (18 mg/m^3) did not result in sustained sensory irritation waveforms for at least three animals over at least three minutes, although intermittent sensory irritation waveforms were observed. As a result, data only from the other four exposures were used in the calculation of the sensory irritation characteristics. Exposure data are summarized in Table 23.

Figure 15 shows the maximum respiratory depression vs. the exposure concentration (log scale) for the exposures to 2-methyleneglutaronitrile. Regression of the data for the four exposures resulting in a positive sensory irritation response results in a correlation coefficient (r^2) of 0.721, with the RD_{50} calculated to be 105 mg/m^3 and the RD_{12} determined to be 11 mg/m^3. The slope of the regression line is 38.3.

Figure ‘I6 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of 2-methyleneglutaronitrile tested. The onset of respiratory depression is observed to differ depending on the exposure concentration, becoming more rapid for increasing concentrations. Additionally, although the initial response was relatively rapid for some exposures, the response did not generally reach a stable plateau for any exposure; respiratory frequency appeared to continue to decrease throughout each exposure period.

Figures B-26 through B-30 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). As shown in Table 23, the VOC composition of the exposure atmospheres averaged greater than 99% 2-methyleneglutaronitrile.

4.2.7 2,6-Di-*tert*-butyl-4-methylphenol

Vapors of 2,6-di-*tert*-butyl-4-methylphenol (BHT) were generated using the flask methodology described in Section 3.1.2. A total of ten exposures were conducted for this compound. The need for additional exposures was due to the presence of contaminating compounds in some samples of exposure air. For the three tests in which the lowest exposure percentage of BHT was measured, additional compounds detected included carryover of previous target compounds and a solvent (acetone) used to clean the test chamber. Each of these tests were found to contain less than 65% BHT by mass concentration in the exposure atmosphere. Although the exposure data for these exposures are included in Table 24, these data were rejected in the further analysis.

The exposure-response data, using only those exposures which were more than 65% BHT, are shown in Figure 17 as the maximum respiratory depression vs. the exposure concentration (log scale). Regression of the data for these exposures results in a correlation coefficient (r^2) of 0.824, with the RD_{50} calculated to be 11 mg/m^3 and the RD,, determined to be 5 mg/m^3. The slope of the regression line is 117.7.

Figure 18 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of 2,6-di-*tert*-butyl-4-methylphenol tested and used for exposure-response analysis. In general, the respiratory depression response appeared to happen early during the exposure, with some attenuation (recovery toward baseline) during the course of the exposure period. Recovery continued toward baseline following the exposure.

Figures B-31 through B-39 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). The two exposures with positive irritation responses which were rejected in the prior analysis are included among these figures; the single exposure without a positive response is not included.

As shown in Table 24 and mentioned above, the VOC composition of the exposure atmospheres did not always consist of predominantly BHT. Those exposure atmospheres which were rejected consisted of 23%, 34% and 41% BHT by mass concentration. In one of the cases, benzothiazole (another target compound) was the principal contaminant; in the other two cases, acetone was the principal contaminant. Although these compounds were present at concentrations not expected to cause significant irritation, these exposures were rejected on the basis that they were not primarily composed of BHT.

Of the remaining exposures, compositions of the exposure atmospheres averaged from 69% to 99% 2,6-di-*tert*-butyl-4-methylphenol. For one of these exposures (conducted 01/31/95), 2-methyleneglutaronitrile was present at levels below the RD_{12} (approximate average concentrtaion of2 mg/m^3) and did not appear to influence the data obtained. The principal additional compound in the other exposures has not been positively identified; however, it appears to be a breakdown and/or reaction product of BHT, created during thermal desorption of the sorbent tubes during the analysis of the air samples. Temperatures during thermal desorption reach 250 °C, while those used during vapor generation of BHT were less than 75 °C. Further investigation into the nature of this thermal reaction is continuing. The exposure data should not be influenced by the presence of this compound; since it is generated during the analysis, it should not be present at significant levels during the actual exposures. Also, since it is formed during the analytical step and not the generation step, and the calibration of the hydrocarbon monitor used atmospheres for which this contaminant was not measured, the presence of this contaminant does not affect the determination of the BHT exposure concentrations.

4.2.8 Benzothiazole

Vapors of benzothiazole were generated using the J-tube methodology described in Section 3.1.2. Four exposures were conducted to this compound, all of which were used to generate an estimate of the sensory irritation characteristics of this compound. Exposure data are summarized in Table 25.

figure 19 shows the maximum respiratory depression vs. the exposure concentration (log scale) for the four exposures to benzothiazole. Regression of the data for all responses results in a correlation coefficient (r^2) of 0.931, with the RD_{50} calculated to be 235 mg/m^3 and the RD_{12} determined to be 21 mg/m^3. The slope of the regression line is 35.9.

Figure 20 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of benzothiazole tested. Respiratory depression was observed to occur eariy in the exposure period for all exposures, with slight attentuation (recovery towards baseline) of the response during the exposure period for all but the highest exposure (150 mg/m^3). Recovery towards baseline following the exposure period was more gradual in the exposure to the highest concentration than it was in the lower concentrations (where there was a sharper recovery to baseline).

figures B-40 through B-43 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). As shown in Table 25, exposure compositions were between 95 and 100% benzothiazole by mass concentration.

4.2.9 2-Ethylhexanoic acid

Vapors of 2-ethylhexanoic acid were generated using the J-tube methodology described in Section 3.1.2. Although a total of 5 exposures were conducted to this compound, subsequent inspection of the respiratory waveforms determined that these exposures did not contain sustained sensory irritation waveforms, as required for classification as a sensory irritant. Therefore, this compound was determined to have not caused sensory irritation at the exposure concentrations which were able to be generated (up to 199 mg/m^3). Only data for the highest concentration exposure are presented. Table 26 summarizes the exposure conditions for this test, and Figure B-44 shows the respiratory response (as a percent of baseline) vs. time for this exposure, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). The exposure concentration was 97% 2-ethylhexanoic acid by mass for this test.

4.2.10 4-Phenylcyclohexene

Vapors of 4-phenylcyclohexene were generated using the J-tube methodology described in Section 3.1.2. Nine exposures were conducted to this compound. The exposure at the lowest concentration (23 mg/m^3) did not result in significant respiratory depression to be classified as sensory irritation. As a result, data only from the other eight exposures were used in the calculation of the sensory irritation characteristics. Exposure data are summarized in Table 27.

Figure 21 shows the maximum respiratory depression vs. the exposure concentration (log scale) for the exposures to 4-phenylcyclohexene. For one of the exposures (138 mg/m^3), a computer malfunction led to a premature termination of the exposure. However, the data did not appear to be affected by this, and this exposure was used in the calculation of the RD_{50}. Regression of the data for the eight exposures resulting in a positive sensory irritation response results in a correlation coefficient (r^2) of 0.912, with the RD_{50} calculated_ to be 319 mg/m^3 and the RD,, determined to be 38 mg/m^3. The slope of the regression line is 41.2.

Figure 22 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of 4-phenylcyclohexene tested. Respiratory depression and the onset of sensory irritation was relatively rapid for all exposures to this compound except at the lowest concentrations. Only mild attenuation (recovery toward baseline) was noted during the exposure period. Following exposure, a recovery toward baseline was indicated for all exposures.

Figures B-45 through B-53 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals (including that with less than 12% respiratory depression), as well as the exposure concentration (based on total hydrocarbon analyzer

measurements, corrected for the instrument response). As shown in Table 27, the VOC composition of the exposure atmospheres which resulted in a positive sensory irritation response averaged greater than 90% 4-phenylcyclohexene by mass concentration.

4.21 1 1,3-Dichloro-2-propanol

Vapors of 1,3-dichloro-2-propanol were generated using the J-tube methodology described in Section 3.1.2. Six exposures were conducted for this compound. Exposure data are summarized in Table 28.

Figure 23 shows the maximum respiratory depression vs. the exposure concentration (log scale) for the exposures to 1,3-dichloro-2-propanol. All exposures were classified as positive for sensory irritation. Regression of the data for the exposures resulting in measurable sensory irritation results in a correlation coefficient (r^2) of 0.874, with the RD_{50} calculated to be 130 mg/m^3 and the RD,, determined to be 2 mg/m^3. The slope of the regression line is 21.7.

Figure 24 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of 1,3-dichloro-2-propanol tested. Respiratory depression and the onset of sensory irritation was relatively gradual at lower concentrations, but more rapid for exposures at higher concentrations. The respiratory depression generally plateaued during exposure. Following exposure, a relatively rapid but incomplete recovery toward baseline was indicated for all exposures.

Figures B-54 through B-59 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). As shown in Table 28, the VOC composition of the exposure atmospheres averaged greater than 78% 1,3-dichloro-2-propanol by mass concentration. Acetone was the principal contaminant in the three exposures which had less than 90% 1,3-dichloro-2-propanol by mass concentration. All exposures had low but measurable levels of I-chloro-2-propanone and (chloromethyl)oxirane, which may be related to the temperatureconditions required for exposure atmosphere generation

4.212 1-Dodecanol

Vapors of I-dodecanol were generated using the J-tube methodology described in Section 3.1.2. A single exposure was conducted to this compound, at the highest attainable exposure concentration of 0.5 mg/m^3. This exposure did not result in measurable sensory irritation to this compound. Table 29 summarizes the exposure conditions for this test, and Figure B-60 shows the respiratory response (as a percent of baseline) vs. time for this exposure, as well as the

exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). The exposure atmosphere was determined to consist of 95% 1-dodecanol. No further exposures to 1-dodecanol were conducted.

4.213 ε-Caprolactam

Vapors of s-caprolactam were generated using the flask methodology described in Section 3.1.2. A single exposure was conducted to this compound, at the highest attainable exposure concentration of 13.5 mg/m^3. This exposure did not result in measurable sensory irritation to this compound. Table 30 summarizes the exposure conditions for this test, and Figure B-61 shows the respiratory response (as a percent of baseline) vs. time for this exposure, as well as the' exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). The exposure atmosphere was determined to consist of 100% ε-caprolactam. No further exposures to s-caprolactam were conducted.

4,214 limonene

Vapors of limonene were generated using the J-tube methodology described in Section 3.1.2. A mixture of 50% (S)-(-)-limonene (CAS # 5989-54-8) and 50% (R)-(+)-limonene (CAS # 5989-27-5), by mass, was created from neat standards of the two isomers. A single exposure was conducted to this mixture, at the highest attainable exposure concentration of 76.4 mg/m^3. This exposure did not result in measurable sensory irritation to this compound. Table 31 summarizes the exposure conditions for this test, and Figure B-62 shows the respiratory response (as a percent of baseline) vs. time for this exposure, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). The exposure atmosphere was determined to consist of 78% limonene. Other VOCs identified in the exposure atmosphere included p-cymene (4-isopropyltoluene), 1-methyl-4-(1-methylethylidene)cyclohexene, and 3-carene. No further exposures to limonene were conducted.

4.2.15 2-Methylnaphthalene

Vapors of 2-methylnaphthalene were generated using the flask methodology described in Section 3.1.2. Nine exposures were conducted for this compound. Of these exposures, eight were identified as resulting in measurable sensory irritation. Exposure data are summarized in Table 32

figure 25 shows the maximum respiratory depression vs. the exposure concentration (log scale) for the exposures to 2-methylnaphthalene. A non-linear response to this compound was observed at exposure concentrations above 20 - 40 mg/m^3. The cause of this non-linearity is not clear, as the respiratory depression of a group of animals can reach as high as 85% [5]. Because

of this non-linearity, only those exposures below 25 mg/m^3 were used in the determination of the RD_{50} and RD_{12}. Regression of the data for these six exposures results in a correlation coefficient (r^2) of 0.874, with the RD_{50} calculated to be 8 mg/m^3 and the RD_{12} determined to be 2 mg/m^3. The slope of the regression line is 60.3.

Figure 26 shows the average respiratory frequency of the group of mice (as a percent of the baseline frequency of the group) exposed to each concentration of 2-methylnaphthalene tested. Respiratory depression and the onset of sensory irritation was relatively rapid at higher exposure concentrations, but was delayed at lower concentrations. The response generally plateaued during exposure, and a recovery toward baseline was observed following the exposure for all tests.

Figures B-63 through B-71 in Appendix B show the respiratory response (as a percent of baseline) vs. time for each group of animals (including that with less than 12% respiratory depression), as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrurnent response). As shown in Table 32, the VOC composition of the exposure atmospheres averaged greater than 96% 2-methylnaphthalene by mass concentration.

4,216 Hexanedinitrile (Adiponitrile)

Vapors of hexanedinitrile were generated using the J-tube methodology described in Section 3.1.2. A single exposure was conducted to this compound, at the highest attainable exposure concentration of 8.2 mg/m^3. This exposure did not result in measurable sensory irritation to this compound. Table 33 summarizes the exposure conditions for this test, and Figure B-72 shows the respiratory response (as a percent of baseline) vs. time for this exposure, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). The exposure atmosphere was determined to consist of 100% hexanedinitrile. No further exposures to hexanedinitrile were conducted.

4.217 Octamethylcyclotetrasiloxane

Vapors of octamethylcyclotetrasiloxane were generated using the J-tube methodology described in Section 3.1.2. A single exposure was conducted to this compound, at the highest attainable exposure concentration of 303 mg/m^3. This exposure did not result in measurable sensory irritation to this compound. Table 34 summarizes the exposure conditions for this test, and Figure B-73 shows the respiratory response (as a percent of baseline) vs. time for this exposure, as well as the exposure concentration (based on total hydrocarbon analyzer measurements, corrected for the instrument response). The exposure atmosphere was determined to consist of

more fhan 99% octamethylcyclotetrasiloxane. No further exposures to octamethylcyclotetrasiloxane were conducted.

4.3 Positive Control Data

Formaldehyde vapor was tested as a positive control on sensory irritation. These data are included in Figure 6 and Tables 14, 16, and 17, and discussed in Chapter 6. In brief, the regression of the data results in an RD_{50} of 12.9 mg/m^3 (10.5 ppm), RD_{20} of 1.2 mg/m^3 (0.9 ppm), and RD, of 0.6 mg/m^3 (0.5 ppm).

5.0 RESPIRATORY CHARACTERISTICS OF DEFINED MIXTURES OF CHEMICALS KNOWN TO CAUSE RESPIRATORY IRRITATION

5.0 RESPIRATORY CHARACTERISTICS OF DEFINED MIXTURES OF CHEMICALS KNOWN TO CAUSE RESPIRATORY IRRITATION

Irritation testing of defined mixtures of the Task 1 target compounds was conducted to investigate potential interactions from the combined irritation effects of selected compounds anticipated to be emitted by common types of carpet assemblies (combinations of carpet and cushion). One binary and two ternary mixtures were evaluated to determine if the irritation effects from the combination of chemicals could be classified as additive, synergistic, or antagonistic. The approach chosen was to conduct irritation testing at combinations of exposure concentrations predicted to result in a similar level of response, if the suspected irritants interact in an additive manner. Significant deviations from a line of additivity (see Figures 27 - 29) were used to indicate synergistic or antagonistic interactions.

5.1 Target Exposure Conditions

The binary mixture which was evaluated consisted of different relative concentrations of 2,6-di-*tert*-4-butyl-methylphenol and 1,4-dimethylpiperazine. Emission testing at CPSC identified these compounds in the same prime urethane cushion sample, and Task 1 testing determined these to be among the more irritating compounds tested (see Tables 14, 16, and 17). Table 35 summarizes the target levels for testing these compounds, and the expected respiratory depression for the individual compounds at that concentration, based on the Task 1 tests. Five exposure combinations were chosen for these tests, in combinations which would produce 40% respiratory depression if the responses were additive. One additional exposure was conducted at conditions under which neither compound would be expected to result in significant irritation alone, to determine whether the combination could produce irritation. Finally, single component exposures conducted as part of Task 1 are included in Table 35 for completeness.

One of the ternary mixture tests was an extension of the binary mixture testing of 2,6-di-*tert*-4-butyl-methylphenol (BHT) and 1,4-dimethylpiperazine, created by the addition of different concentrations of 4-phenylcyclohexene to the mixtures. 4-Phenylcyclohexene is a compound frequently identified in emissions from, new residential (SBR-backed) carpets, and would be anticipated to be included with BHT and 1,4-dimethylpiperazine in the emissions from an assembly of this type of carpet and a prime urethane cushion. Table 36 summarizes the target levels for testing these compounds, and the expected respiratory depression for the individual compounds at that concentration, based on the Task 1 tests. Exposure combinations for these tests were again chosen in combinations which would produce 40% respiratory depression if the responses were additive. Again, an additional exposure was conducted at conditions under which none of the compounds would be expected to result in significant irritation alone, to determine whether the combination could produce irritation. Finally, single component exposures

conducted as part of Task 1 are again iincluded in the table for completeness, as are some binary exposures from the series of binary mixture tests.

The second ternary mixture tested consisted of different relative concentrations of 2,6-di-*tert*-4-butyl-methylphenol (BHT), N,N-dimethylacrylamide, and 1,2,3-trichloropropane. Emission testing at CPSC identified these compounds in the same prime urethane cushion sample, and Task 1 testing determined these to be sensory irritants. Table 37 summarizes the target levels for testing these compounds, and the expected respiratory depression for the individual compounds at that concentration, based on the Task 1 tests. Exposure combinations for these tests were chosen in combinations which would produce 45% - 50% respiratory depression if the responses were additive. As for the previous mixtures, an additional exposure was conducted at conditions under which none of the compounds would be expected to result in significant irritation alone, to determine whether the combination could produce irritation. Single component exposures conducted as part of Task 1 are again included in the table for completeness.

5.2 Results of Mixture Testing

5.27 Binary mixture testing of 2,6-Di-tert-4-butyl-methylphenol and 1,4-Dimethylpiperazine

Table 38 summarizes the measured average concentrations of each compound during each exposure, the expected respiratory depression for the measured concentration based on the Task 1 data, the respiratory depression which would be predicted if the responses to a mixture of individual compounds were additive, and the actual measured respiratory depression during the test. The exposure numbers in the first column were intended to correspond to the exposure numbers in Table 35. The summed responses in all cases include contribution from compounds at levels below a "significant* level; i.e., if a compound is below the RD_{12} but would be predicted from the Task 1 testing to cause a respiratory depression between 0 and 11%, this contribution is included in the summed response.

Comparison of the measured average concentrations (Table 38) with the target concentrations (Table 35) indicates significant deviatiion between intended and actual exposure conditions. Exposure concentrations of BHT were often difficult to control within narrow tolerances, resulting in generated exposure concentrations which differed from the target concentrations more than was desirable. Although measurements of the individual component concentrations were made **before** and following testing, any drift in the individual concentrations would not be detected by the total hydrocarbon analyzer during a given exposure, since the analyzer measured the combined response of the mixture. This difficulty affected all the binary and ternary mixture **testing** conducted.

The expected responses for the individual components of each mixture differed from the target responses due to the lack of control of exposure concentration, and also to the characteristics of the compounds themselves. Most significantly, the exposure-response curve for BHT was determined to be relatively steep, resulting in a wide range of predicted responses over a rather narrow range of concentration changes. As a result, relatively small changes from the target concentration could result in significant changes in the predicted and measured responses.

Comparison of the observed responses with the sum of the responses based on single chemical testing does not provide a strong indication of the type of irritation interactions which would be expected from exposures to mixtures of these two compounds. Figure 27 plots the sum of expected responses based on single chemical tests against the measured responses for each test, along with the identity line (data would fall on the identity line if the interaction of the two compounds was purely additive). Although three of the exposures (including two for single component tests, where the concentration of one of the components was zero) result in data close to the identity line, four fall below the responses expected based on additivity of the irritation response. It is noteworthy that the three exposures with measurable levels of both target compounds all resulted in lower measured responses than predicted by additivity. These data support the possibility that BHT and 1,4-dimethylpiperazine may interact in an antagonistic manner at certain concentrations; however, more data, with generated concentrations closer to the target concentrations in Table 35, is required to confirm this observation.

Figures B-74 through B-78 provide the exposure response vs. time data for exposures numbered 1-4 in Table 38. Data for the other exposures are available elsewhere in the report. In general, these exposures produced immediate respiratory depression, which was either sustained through the exposure or attenuated slightly (recovered toward baseline) during the exposure. Recovery to baseline following the exposure was evident. This type of response is consistent with the behavior of either compound.

Further details on each of the exposures to these defined mixtures are provided in Table 39.

5.2.2 Ternary mixture testing of 2,6-Di-tert-4-butyl-methylphenol (BHT), 1,4-Dimethylpiperazine, and 4-Phenylcyclohexene

Table 40 summarizes the measured average concentrations of each compound during each exposure, the expected respiratory depression for the measured concentration based on the Task 1 data, the respiratory depression which would be predicted if the responses to a mixture of individual compounds were additive, and the actual measured respiratory depression during the test. The exposure numbers in the first column were intended to correspond to the exposure numbers in Table 36.

Comparison of the measured average concentrations (Table 40) with the target concentrations (Table 36) indicates that only two of the four mixtures (Exposure numbers 1 and 3) intended to include significant levels of BHT had BHT levels which would result in significant irritation (respiratory depression >12%), based on BHT alone. Also, none of the four exposures intended to include 4-phenylcyclohexene had concentrations of 4-phenylcyclohexene which would be predicted to result in significant irritation.

The three target compounds were all detected in the two exposures (Exposure numbers 1 and 3) having levels of BHT sufficient to result in significant irritation from BHT alone; however, the measured response was significantly less than the sum of the predicted responses based on BHT alone. Additionally, Exposure number 2, which had a measurable BHT concentration below that which would be expected to result in respiratory depression and a significant concentration of 1,4-dimethylpiperazine, produced a response lower than predicted from the 1,4-dimethylpiperazine concentration alone. Taken together, these data would suggest some antagonistic interaction between these compounds. However, since the 4-phenylcyclohexene concentrations were in all cases below that expected to produce a measureable response, its role in the observed responses is not clear.

Figure 28 plots the sum of expected responses based on single chemical tests against the measured responses for each test, along with the identity line (data would fall on the identity line if the interaction of the two compounds was purely additive). The four exposures which result in data closest to the identity line include the three exposures of single components, where the concentration of the other two target compounds was zero, and one exposure (number 5A) where the response was probably due to only one of the components (1,4-dimethylpiperazine). Of the other five exposures, all responses fell below that which would be expected based on additivity of the irritation response, and all were below or close to the 12% response band. This would support the hypothesis that the combination of these compounds at certain concentration levels may result in an antagonistic or attentuated irritation effect, though more data would be needed to confirm this observation.

Figures B-79 through B-82 provide the exposure response vs. time data for exposures numbered 1 - 4 in Table 40. Data for the other exposures are available elsewhere in the report. None of these responses was especially dramatic; the respiratory depression appeared to occur gradually, with animals recovering to baseline at the end of the exposure. This type of response was observed with low-level irritation for many of the Task 1 compounds.

Further details on each of the exposures to these defined mixtures are provided in Table 41.

5.2.3 Ternary mixture testing of 2,6-Di-tert-4-butyl-methylphenol, N,N-Dimethylacrylamide, and 1,2,3-Trichloropropane

Table 42 summarizes the measured average concentrations of each compound during the exposure, the expected respiratory depression for the measured concentration based on the Task 1 data, the respiratory depression which would be predicted if the responses to a mixture of individual compounds were additive, and the actual measured respiratory depression during the test The exposure numbers in the first column were intended to correspond to the exposure numbers in Table 37.

Comparison of the measured average concentrations (Table 42) with the target concentrations (Table 37) indicates significant deviation between intended and actual exposure conditions. Comparison of the observed responses with the sum of the responses based on single chemical testing indicates that two of the three tests with combinations of all three target compounds and the single test combining N,N-dimethylacrylamide and 1,2,3-trichloropropane (Exposure numbers 2 - 4) resulted in measured respiratory depression significantly less than the sum of the predicted responses based on the individual components. However, one of the three component mixtures (Exposure number 1) produced a respiratory depression consistent with the sum of the predicted responses from the individual components. The binary exposure to BHT and N,N-dimethylacrylamide resulted in a respiratory depression which would be consistent with the N,N-dimethylacrylamide alone; however, if the sub-threshold contribution (11% respiratory depression) of BHT were included in the sum of responses, the measured respiratory depression would be slightly less than predicted.

Figure 29 plots the sum of expected responses based on single chemical tests against the measured responses for each test, along with the identity line (data would fall on the identity line if the interaction of the two compounds was purely additive). The five exposures which result in data closer to the identity line include the three exposures of single components, where the concentration of the other two target compounds was zero. The two points lying above the additive response identity line include one exposure where two components were predicted to have no response (RD <12%), and one where one of the components (N,N-dimethylacrylamide) was just below a level which would result in a response. Of the other three exposures, all responses fell below that which would be expected based on additivity of the irritation response (and outside the 12% response band); each of these was for binary mixtures of the three components (i.e., one of the components of the ternary mixture was omitted). Since this is such a limited data set, it is difficult to draw conclusions about the interaction of these compounds at these levels. Further testing, using combinations of binary and ternary mixtures of these components closer to the target levels, would be required to more completely evaluate the interaction.

Figures B-83 through B-87 provide the exposure response vs. time data for exposures numbered 1- 5 in Table 42. Data for the other exposures are available elsewhere in the report. In general, these exposures appeared to result in a gradual onset of respiratory depression, with incomplete recovery following the exposure. This type of response is more consistent with N,N-dimethylacrylamide than with either of the other two components of the mixture. In fact, Table 42 indicates that for all but Exposure number 1, the observed response is consistent with (within 12% of) the response from the concentration of N,N-dimethylacrylamide alone. It is possible that this compound is controlling the response to the mixture; additional testing would be needed to confirm this observation.

Further details on each of the exposures to these defined mixtures are provided in Table 43.

5.2.4 Summary of defined mixture testing

In all cases, it was found to be difficult to obtain exposure conditions which were close enough to target levels to satisfactorily evaluate the interaction effects. Although evidence suggests there may be some antagonism between some of these compounds at the levels tested, a much broader evaluation, with more focus on generation of the target levels within tight tolerances, would be required. Finally, the variability in the data used to determine the target levels would need to be taken into account to fully interpret the responses; that is, it would be necessary to know the confidence limits around the target concentrations, to know if the exposure conditions would clearly be expected to result in the predicted RD used to estimate the target concentrations for a given compound.

6.0 RESPIRATORY CHARACTERISTICS OF SYNTHESIZED MIXTURES OF CHEMICALS DESIGNED TO MIMIC EMISSIONS FROM CARPETS AND ASSOCIATED PRODUCTS

6.0 RESPIRATORY CHARACTERISTICS OF SYNTHESIZED MIXTURES OF CHEMICALS DESIGNED TO MIMIC EMISSIONS FROM CARPETS AND ASSOCIATED PRODUCTS

6.1 Chamber Evaluations of Test Samples

Seven test samples were submitted for chamber evaluation of chemical emissions. These samples included two carpet samples and five cushion samples, and included uninstalled and previously installed samples. The samples were identified only as: Carpet A, Carpet B, and Cushions A through D. The chemical emissions were evaluated to establish exposure conditions for irritation testing. Each sample was evaluated over a 96 hour period at 23 °C and 50% relative humidity; two samples (Carpet B and Cushion B) were additionally tested at an increased loading and with both sides exposed, over a 6 hour period. These additional tests were at 70 °C and about 8% relative humidity (same absolute water content as 23 °C and 50% rh), to evaluate the enhancement in emissions at elevated conditions.

Data from these tests are supplied as Appendix C. These and similar data available at CPSC were used in identifying compounds and levels to be used in the synthesized mixture testing.

6.2 Synthesized Mixture Testing

Synthesized mixtures were created for exposure testing, to evaluate the irritation potential of selected product types. The mixture compositions were based on characteristic compounds and relative concentrations of the components, which were determined based on the data from emissions testing using environmental chambers. As mentioned in Section 3.2.2, seven general product types were simulated, including one type of carpet (styrene-butadiene latex rubber (SBR)-backed 'softback" carpet), four types of cushion (two types of prime urethane, sponge rubber, and bonded urethane), and two "systems" based on combined emissions from assemblies of carpet and cushion. These "systems" were materials which were associated with consumer complaints of irritation. The target ranges for exposure concentrations were chosen to be approximately 10 to 100 times the concentations measured in the chamber tests. These ranges were necessary, since sensory irritation in humans generally occurs at 10 to 100 times lower concentrations than those which result in measurable sensory irritation (see, for example, Table 15). Tables 4 -10 provide the target concentrations for exposure testing, the concentrations measured for the compounds of interest in the chamber tests of these types of materials, and those compounds which were observed to decay within 24 hours during chamber testing. Initial exposures to any synthesized mixture were attempted at the high end of these concentration ranges; subsequent exposures at the low end of the concentration ranges were also conducted for all but the "Complaint System" mixtures, to establish a level below which a response could not be measured.