TABLE IV

HIGH-PRESSURE PROPELLANTS OF LOW TOXICITY (MEAN ± SEM)

Propellant	% Conc. v/v	Monkey No.	Pulm. resistance			Pulm. compliance			Resp. min. vol.		
			Control cm H_2O/ LPS	Response	%Δ	Control ml/cm H_2O	Response	%Δ	Control ml/min	Response	%Δ
FC 115 (Chloropenta-fluoroethane)	10.0	3	23.36 ± 2.16	23.36 ± 2.16	0.00 0.00	6.07 ±0.07	6.07 ±0.07	0.00 0.00	1473 ± 93	1473 ± 93	0.00 0.00
	20.0	3	22.90 ± 2.03	23.90 ± 1.19	+2.02 ±2.02	6.07 ±0.07	6.10 ±0.21	+0.59 ±3.87	1390 ± 123	1322 ± 156	−5.40 ±2.70
FC 152a (Difluoroethane)	10.0	3	26.90 ± 1.60	26.90 ± 1.60	0.00 0.00	7.80 ±1.10	7.80 ±1.10	0.00 0.00	783 ± 140	767 ± 142	−2.00 ±1.00
	20.0	4	29.30 ± 1.90	29.70 ± 1.80	+2.00 ±2.00	7.10 ±0.60	7.10 ±0.60	0.00 0.00	633 ± 96	587 ± 117	−9.00 ±6.00

TABLE IV (continued)

Propellant	% Conc. v/v	Monkey No.	Heart rate			Aortic blood pressure		
			Control beats/min	Response	%Δ	Control mm Hg	Response	%Δ
FC 115 (Chloropenta-fluoroethane)	10.0	3	183.33 ± 11.79	182 ± 10.58	+1.49 ±0.94	120.66 ± 4.33	121.66 ± 5.24	+0.80 ±1.67
	20.0	3	185.33 ± 13.13	186.00 ± 12.49	+0.42 ±0.42	120.66 ± 3.38	119.33 ± 3.84	−1.05 ±2.73
FC 152a (Difluoroethane)	10.0	3	183 ± 3	183 ± 3	0.00 0.00	97.30 ± 7.90	97.30 ± 7.90	0.00 0.00
	20.0	4	182 ± 5	182 ± 5	0.00 0.00	98.00 ± 7.70	98.00 ± 7.70	0.00 0.00

difference in pattern of action between the two groups of propellants. The low-pressure propellants influence predominantly circulation and the high-pressure ones affect respiration. The extent of action is not limited to one function, because there are a few propellants from each group that also influence those of the other group. For instance, the most potent low-pressure propellant that causes tachycardia and hypotension, *i.e.*, FC 11, also depresses respiration. On the other hand, the most potent high-pressure propellant, which depresses respiration, increases resistance and decreases compliance, *i.e.*, FC 12, also causes tachycardia and hypotension. About half of the low-pressure propellants also influence respiration, and a third of the high-pressure propellants also affect circulation.

The second conclusion can be derived from an examination of the four tables which list the classes of propellants. For each pressure class, there are two levels of toxicity. The highly toxic class of low-pressure propellants includes FC 11 and trichloroéthane, which are suspected of causing deaths [2,5,9] following the use and abuse of aerosols. The low-pressure propellants of intermediate toxicity include 4 possible substitutes in the event that a decision is made to dispense with one or all 5 propellants that are in the highly toxic class.

The high-pressure propellants consist of four propellants of intermediate toxicity. One of them is vinyl chloride, which has been banned for use in aerosols because of the reports of liver cancer among workmen exposed to this chemical [6]. The two high-pressure propellants in the low-toxicity class can be used as substitutes. It should be emphasized that the level of toxicity is based on results in monkeys reported in this publication. The results obtained from earlier experiments are commented upon in the next two papers which conclude this series.

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TOXICITY OF AEROSOL PROPELLANTS IN THE RESPIRATORY AND CIRCULATORY SYSTEMS

X. PROPOSED CLASSIFICATION*

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SUMMARY

The 15 propellants are grouped into four classes on the basis of results of investigation reported in this series of publications. *Class 1* low-pressure propellants of high toxicity; *Class 2* low-pressure propellants of intermediate toxicity; *Class 3* high-pressure propellants of intermediate toxicity; and *Class 4* high-pressure propellants of low toxicity.

INTRODUCTION

This series of investigation concludes by proposing a classification of propellants used in aerosols that is based on their acute inhalational toxicity. The grouping of propellants used in the experiments in the anesthetized monkey [8] is being extended to the previous investigation in the same species with open chest [4], as well as in the dog heart-lung preparation [5], the rat [2,6,7] and the mouse [1,3]. As this series is being concluded, the aerosols used to dispense bronchodilator drugs have been studied in the dog and are being published elsewhere [10,11]. The classification that is presented takes into consideration all these results. The most toxic propellant, trichlorofluoromethane (FC 11) is used as the basis for the comparison of the 14 other propellants. The details of the toxicity of FC 11 reported in the literature and uncovered in this series of investigation are reviewed in the preceding publication [9].

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LOW-PRESSURE PROPELLANTS

The basic feature of the proposed classification is the separation of the low-pressure from the high-pressure propellants [8]. In addition, the level of toxicity has been grouped into high, intermediate and low. The low-pressure propellants are divided into Class 1 with high toxicity and Class 2 of intermediate toxicity. The toxicologic features of the propellants are summarized in Table I relating to circulation and Table II relating to respiration.

TABLE I
CIRCULATORY EFFECTS OF PROPELLANTS
+++, ++, +, Intensity of response (minimal inhaled concentration %)[a]

	Cardiac arrhythmia				
	Induced	Sensitized epinephrine		Induced	
	Monkey	Dog	Mouse	Mouse	Rat
Class 1: Low-pressure propellants of high toxicity					
Trichlorofluoromethane (FC 11)	++(2.5)	+++(0.3)	++(5)	++(10)	++(2.5
Dichlorofluoromethane (FC 21)	+(5.0)		+(10)	++(10)	
Trichlorofluoromethane (FC 113)	+(5.0)	+++(0.5)	++(5)	++(10)	
Trichloroethane	+(5.0)	+++(0.5)	+(40)	+(40)	
Methylene Chloride	+(5.0)	+++(0.5)	+(20)	+(40)	
Class 2: Low-pressure propellants of intermediate toxicity					
Dichlorotetrafluoroethane (FC 114)	+(10)	++(5.0)	+(20)	No	
Monochlorodifluoroethane (FC 142b)	No	++(5.0)	No	No	
Isobutane	+(10)	++(5.0)	+(20)	No	
Octafluorocyclobutane (FC C-318)	No	+(25)	+(20)	No	
Class 3: High-pressure propellants of intermediate toxicity					
Dichlorodifluoromethane (FC 12)	+(10)	++(5.0)	No	No	No
Monochlorodifluoromethane (FC 22)	No	++(5.0)	+(40)	No	
Propane	No	+(10)	+(10)	No	
Vinyl chloride	No	++(5)	+(10)	+(20)	
Class 4: High-pressure propellants of low toxicity					
Chloropentafluoroethane (FC 115)	No	+(25)	+(40)	No	
Difluoroethane (FC 152a)	No	+(15)	No	No	No

[a] Results from papers I to VIII of series [1—8] except dog sensitization to epinephrine which was reported by C.F. Reinhardt, A. Azar, M.E. Maxfield, P.F. Smith and L.S. Mullin, Cardiac arrhythmias and aerosol "sniffing". Arch. Environ. Health, 22 (1971) 265—279.

Class 1. Low-pressure propellants of high toxicity

The 5 propellants belonging to Class 1 exert their toxicity in the following concentrations: from 0.5 to 5% in the monkey and the dog, and from 1 to 10% in the rat and the mouse. Their identifying features are: induction of cardiac arrhythmias, sensitization to epinephrine-induced arrhythmia, tachycardia, myocardial depression, and hypotension. The effects on respiration are variable. It should be noted that the predominant influence of low-pressure propellants belonging to Class 1 (as well as to Class 2) is on circula-

Tachycardia			Myocardial depression		Hypotension	
Anesthetized		Unanest.	Open-chest	Heart-lung	Closed-chest	
Monkey	Dog	Rat	Monkey	Dog	Monkey	Dog
++(2.5)	+++(1)	++(2.5)	++(2.5)	++(2.5)	++(2.5)	+++(2.5)
+(5.0)	++(2.5)		+(5.0)		+(5.0)	+(10)
+(5.0)			+(5.0)	+(5.0)	+(5.0)	
+(5.0)			++(2.5)	+(5.0)	++(2.5)	
+(5.0)			+(5.0)		+(5.0)	
+(10)	+(10)		+(10)		+(10)	+(20)
No	+(20)		+(10)		+(10)	+(20)
No	No		+(10)		+(10)	No
No	No		No	+(20)	No	No
+(10)	+(10)	No	+(10)		+(10)	No
+(20)			+(20)		+(20)	
No			No		No	
No			+(10)		No	
No	+(20)		No	+(20)	No	+(20)
No	No		No	+(20)	No	No

TABLE II

RESPIRATORY EFFECTS OF PROPELLANTS

+++, ++, +, Intensity of response (minimal inhaled concentration %)

	Early respiratory depression			
	Monkey	Dog	Rat	Mouse
Class 1: Low-pressure propellants of high toxicity				
Trichlorofluoromethane (FC 11)	+(5.0)	+(10)	++(2.5)	++(2.5)
Dichlorofluoromethane (FC 21)	++(2.5)	No	++(2.5)	
Trichlorofluoromethane (FC 113)	No			
Trichloroethane	++(2.5)			
Methylene chloride	No			
Class 2: Low-pressure propellants of intermediate toxicity				
Dichlorotetrafluoroethane (FC 114)	+(20)	No	No	
Monochlorodifluoroethane (FC 142b)	No	No		
Isobutane	+(10)	+(10)	+(10)	
Octafluorocyclobutane (FC C-318)	No	No	+(10)	
Class 3: High-pressure propellants of intermediate toxicity				
Dichlorodifluoromethane (FC 12)	No	+(20)	+(10)	+(5.0)
Monochlorodiflurormethane (FC 22)	+(20)			
Propane	+(20)			
Vinyl chloride	+(10)			
Class 4: High-pressure propellants of low toxicity				
Chloropentafluoroethane (FC 115)	No	No	No	
Difluoroethane (FC 152a)	No	No		+(5.0)

tion rather than on respiration. The specific characteristics of the five propellants in Class 1 are as follows:

Trichlorofluoromethane (FC 11). This propellant is the most potent in its toxicity to the cardiovascular system. The sensitization of the heart to arrhythmic action of epinephrine or ischemia or of both appears when 0.3% FC 11 is inhaled in the unanesthetized dog or 0.5% in the anesthetized monkey [4]. Higher concentrations are needed, *i.e.*. 5%, to induce arrhythmia in the mouse that has received an injection of epinephrine [1]. For the induction of spontaneous arrhythmias without injection of epinephrine, the following concentrations of FC 11 are required: 2.5% in the rat [6], 10% in the mouse [1] and 2.5% in the monkey [4]. These last-mentioned species also show tachycardia, myocardial depression and hypotension in response to the same concentration that elicits cardiac arrhythmia [4]. In the anesthetized dog, the minimal concentration is 1%, which induces tachycardia and hypotension [10]. The depression of contractility appears at 2.5% in the heart-lung preparation [5]. This does not necessarily imply that the contractility of the heart is less sensitive than the heart rate in the dog because

Bronchoconstriction				Decreased compliance			
Monkey	Dog	Rat	Mouse	Monkey	Dog	Rat	Mouse
No	No	++(2.5)	++(1)	No	No	++(2.5)	++(1)
No	++(2.5)	No		No	++(2.5)	No	
No				No			
No				No			
No				No			
+(20)	+(10)	+(15)		No	+(5)	+(10)	
No	+(10)			No	No		
+(10)	++(2.5)	++(2.5)		No	++(2.5)	++(2.5)	
No	++(2.5)	+(10)		No	+(10)	++(2.5)	
+(10)	+(10)	No	+(2)	+(10)	+(20)	+(10)	+(2)
+(20)				No			
+(20)				No			
+(10)				+(10)			
No	+(10)	++(10)		No	+(20)	+(10)	
No	No		+(2)	No	No	+(2)	

the observations were made in two separate experiments, *i.e.*, a canine heart-lung preparation for contractility, and a dog with closed chest for heart rate.

The primary effect of FC 11 on respiration is depression of minute volume. In the dog [10] and the monkey [8], the minimal dose that depressed breathing is higher then the dose that produced hypotension: in the monkey 2.5% reduced blood pressure and 5% depressed respiratory minute volume; in the dog 1.0% and 10% did so respectively. The dose that depressed respiration was 2.5% for the rat [7] and the mouse [3]. The influence on the airways is variable when the four species are compared, namely, none in the monkey [8], bronchodilation in the dog [10] and bronchoconstriction in the rat [2] and the mouse [3]. The constriction can be prevented by pretreatment of the rodents with atropine, suggesting that the vagus nerve is responsible for the bronchomotor reaction [3]. There is also a reduction in pulmonary compliance in the rodents although not in the dog and the monkey, but this effect is not prevented by atropine, indicating a change in the elasticity of the lung, most probably resulting from congestion of the lungs.

Dichlorofluoromethane (FC 21). This low-pressure propellant, which has

one less chlorine atom than FC 11, is less toxic than FC 11. The minimal concentration of FC 21 which induces cardiac arrhythmia, tachycardia, myocardial depression and hypotension in the monkey is about half that of FC 11. In the dog [10], the hypotensive dose of FC 21 is 10 times that of FC 11, whereas the tachycardiac dose is 2.5 times that of FC 11. The most significant differences between both propellants relate to respiration. In the dog, FC 21 causes bronchoconstriction and decreased compliance, whereas FC 11 does not [10]. It should be noted that the effects of FC 21 are opposite in these two species: bronchodilation, no decreased compliance and early respiratory depression in the monkey; bronchoconstriction, reduced compliance and no respiratory depression in the dog. The response of the rat is more like that of the monkey, so that it is more reasonable to accept the pattern of action for the monkey as characteristic of FC 21.

Trichlorotrifluoroethane (FC 113). The circulatory effects of FC 113 are about equal in degree to those of FC 21 [4,10]. The only difference is in minute volume of respiration, *i.e.*, FC 113 has no influence in the monkey [8].

Trichloroethane. Although the basic features of trichloroethane are similar to those of FC 11, there are differences in potency among various species. The proarrhythmic activity of trichloroethane in the mouse [1] is 1/8 that of FC 11. The myocardial depression of trichloroethane in the dog [5] is 1/2 that of FC 11. In the monkey, trichloroethane is as potent as FC 11 in producing myocardial depression and hypotension [4] and half as potent in causing cardiac arrhythmia, tachycardia and early respiratory depression [8].

Methylene chloride. This, the fifth and last member of Class 1, has essentially the same pattern of effects as FC 21 with one exception [1,4]. Methylene chloride does not cause respiratory depression in the monkey [8].

Class 2. Low-pressure propellants of intermediate toxicity

The four propellants of Class 2 have been separated from those of Class 1 because of the following differences: (*a*) The concentrations of Class 2 that sensitize the dog to epinephrine-induced arrhythmias ranging from 5 to 25%, whereas those of Class 1 are 0.5% or less. (*b*) The propellants of Class 2 do not induce arrhythmias in the mouse but all of Class 1 do so in concentrations of 10 to 40%. (*c*) The propellants of Class 2 influence circulation in the anesthetized dog and monkey in concentrations of 10 of 20% but those of Class 1 do so in concentrations of 0.5 to 2.5%. (*d*) The propellants of Class 2 cause bronchoconstriction in the dog in concentrations of 2.5 to 10%, whereas there is at least one propellant of Class 1 that does not produce this effect. With the exception of (*d*), items (*a*), (*b*) and (*c*) indicate that the Class 2 propellants are safer than those of Class 1, thus the notations of *intermediate* and *high* toxicity are used respectively for the two classes.

Dichlorofluorotetrafluoroethane (FC 114). This can be regarded as the prototype of a low-pressure propellant of intermediate toxicity. More specifically, FC 114 causes cardiac arrhythmia in the monkey [4] and the dog but not in the mouse [1], tachycardia and hypotension in the monkey [8]

and the dog [10], and myocardial depression in the monkey [4] but this has not been determined in the dog. In addition to causing bronchoconstriction in the monkey [8] dog [10] and rat [2], FC 114 has varied action on other respiratory parameters. It causes respiratory depression in the monkey [8] and stimulation of this parameter in the rat [2], but has no effect in the dog [10]; it decreases compliance in the dog and the rat but not in the monkey. For purposes of generalization, the complete profile for FC 114 is that it reduces compliance and increases airway resistance (bronchoconstriction).

Monochlorodifluoroethane (FC 142b). The range of effect of FC 142b is less than that of FC 114. The following characteristics of FC 114 are *not* observed when FC 142b is administered: cardiac arrhythmia and tachycardia in the monkey [4], epinephrine-induced arrhythmia in the mouse [1], decreased compliance in the dog [10] and bronchoconstriction and early respiratory depression in the monkey [8]. To the contrary, FC 142b is a respiratory stimulant in the monkey [8] and the dog [10], and is the only propellant in all four classes that exerts such action in these two species.

Isobutane. The important differences between isobutane and the prototype FC 114 are identifiable only in the dog [10]. Unlike FC 114, isobutane does not cause tachycardia and hypotension. On the other hand, isobutane induces early respiratory depression and more intense bronchospasm and decreased compliance, as compared with FC 114.

Octafluorocyclobutane (FC C-318). This last member of Class 2 is the least toxic of all low-pressure propellants. A glance at Tables I and II indicates 11 "No" entries for FC C-318, and 4 "No's" each for FC 114 and FC 11. The actions that still remain with FC C-318 are as follows: bronchoconstriction and reduced compliance in the dog [10] and the rat [2], early respiratory depression in the rat only [2], myocardial depression in the monkey [4], and sensitization to epinephrine-induced arrhythmia in the dog and the mouse [1]. However, it should be emphasized that FC C-318 produces these actions in doses considerably larger than those used for FC 11. The differences are 250-fold in the unanesthetized dog for arrhythmia, 4-fold in the anesthetized mouse also for arrhythmia, 8-fold in the anesthetized monkey for myocardial depression, 4-fold in the anesthetized rat for respiratory depression and bronchoconstriction. On the other hand, FC C-318 causes bronchoconstriction and reduced compliance in the dog which are not induced by FC 11..

HIGH-PRESSURE PROPELLANTS

The levels of toxicity of the high-pressure propellants are lower than those of the low-pressure ones. There are no high-pressure propellants of the high toxicity level; there are only *Class 3*, intermediate level and *Class 4*, low level of toxicity. The toxicologic features of these classes are summarized in Tables I and II.

Class 3. High-pressure propellants of intermediate toxicity

The Class 3 high-pressure propellants that have the notation of *inter-*

mediate toxicity have two features in common with the Class 2 low-pressure propellants, also grouped as intermediate: (*a*) The concentrations of Class 2 and Class 3 propellants that sensitize the unanesthetized dog to epinephrine-induced arrhythmias ranging from 5 to 25%. (*b*) The concentrations that influence circulation in the anesthetized dog and monkey range from 10 to 20%. The difference between Class 2 and Class 3 is in the extent of respiratory action. The high-pressure propellants of Class 3 cause early respiratory depression and bronchoconstriction which predominate over the influence on circulation. It should be noted that this is opposite to the effects of low-pressure Class 1 and Class 2 propellants that act predominantly on circulation.

Dichlorodifluoromethane (FC 12). This can be regarded as the prototype of Class 3 because it influences all parameters relating to respiration, namely, depression of respiratory minute volume, bronchoconstriction and reduction in compliance. The dog [10] and the mouse [1] show all three effects but the monkey [8] lacks the respiratory depression and the rat [2] bronchoconstriction. For circulation, all parameters are also affected in some species but not in others. The monkey shows cardiac arrhythmia, tachycardia, myocardial depression and hypotension [4], and the dog lacks hypotension [10]. The mouse [1] and the rat [7] did not show alteration in heart rate and lacked proarrhythmic activity. However, the rat with emphysema [7], cardiac necrosis [6], or pulmonary arterial thrombosis [6] developed arrhythmia when exposed to FC 12, indicating that this propellant is cardiotoxic.

Monochlorodifluoromethane (FC 22). Like the prototype FC 12, FC 22 causes early respiratory depression, bronchoconstriction, tachycardia, myocardial depression and hypotension. The difference between the two is that FC 22 does not induce cardiac arrhythmia in the monkey [4], although it sensitizes the heart to epinephrine in the mouse [1], and that FC 22 does not decrease compliance in the monkey [8].

Propane. The respiratory effects of propane are different from those of FC 12 in that propane does not decrease compliance but causes early respiratory depression in the monkey [8]. As a matter of fact, the depression of breathing is the only toxicity in the monkey, because there are no accompanying changes in heart rate, myocardial contractility and blood pressure when 10% propane is administered.

Vinyl chloride. This last member of Class 3 has an effect on circulation limited to depression of contractility without hypotension, tachycardia and arrhythmia in the monkey [4]. However, the dog and the mouse are sensitized to epinephrine-induced arrhythmia [1]. The respiratory pattern of vinyl chloride involves all three parameters and therefore is similar to that of FC 12.

Class 4. High-pressure propellants of low toxicity

The major difference between Class 4 propellants of *low* toxicity and Class 3 propellants of *intermediate* toxicity is that the Class 4 propellants do

not cause bronchoconstriction, nor early respiratory depression, and do not decrease compliance in at least one animal species. The extent of action of Class 4 propellants is less than that of Class 3 propellants on circulation.

Chloropentafluoroethane (FC 115). The monkey does not show any effect on respiration or circulation when exposed to 20% FC 115 [4,8]. The dog shows no respiratory depression when exposed to 20% but shows bronchoconstriction, decreased compliance, sensitization of the heart to epinephrine, tachycardia, myocardial depression and hypotension when inhaling 10 to 25% FC 115 [5,10]. Nevertheless, the potency is considerably less than that of FC 11, which produces most of these effects when inhaled in concentrations of 0.3 to 2.5%. The rat responds with bronchospasm and decreased compliance and respiratory stimulation to 10% FC 115, whereas inhalation of 2.5% FC 11 causes bronchoconstriction, decreased compliance and respiratory depression [2].

Difluoroethane (FC 152a). This propellant has the most limited action on both respiration and circulation. In Tables I and II, there are 15 "No" entries for FC 152a. It can be regarded as the least toxic propellant. Although it has no detectable effect in the monkey, it causes sensitization to epinephrine in the dog. The mouse still shows bronchoconstriction, respiratory depression and decreased compliance but no cardiac arrhythmia [1,3]. In the mouse that has developed bronchitis [3], and in the rat with pulmonary emphysema [7], the administration of FC 152a provokes abnormalities in the electrocardiogram.

ANIMAL MODELS FOR EVALUATION OF ACUTE TOXICITY

Although this series of investigations was conducted to compare the toxicity of propellants used in aerosols, the results are of value in the evaluation of chemical agents in general and of inhalants in particular. After application of various techniques to measure responses of four animal species, it has become apparent that certain generalizations can be made regarding the sensitivity of each animal model. The following discussion points out the specific application of the mouse, rat, dog and monkey in the determination of potential hazard of an inhalant to the circulatory and respiratory systems.

Mouse

Cardiac arrhythmia. The proarrhythmic activity of the inhalant has been examined by administering it in various concentrations while recording the electrocardiogram [1]. The compound was also inhaled while injecting epinephrine to determine sensitization of the heart to develop arrhythmia. As a rule, there are propellants (6 of 15) that provoke spontaneous arrhythmia and also sensitize the heart to epinephrine. There are also propellants (6 of 15) that do not induce spontaneous arrhythmia but cause sensitization. The remaining propellants (3 of 15) that do not exert both are not proarrhythmic, provided that a lack of activity is confirmed in one other species. The comparative results would have been more significant if lethal concen-

trations were determined to express the proarrhythmic index, *i.e.* ratio of toxic dose to lethal dose.

There is a wide range in concentrations (5 to 40%) for the various propellants to cause arrhythmia. The sensitivity of the mouse can be increased by experimental induction of bronchitis [3], indicating that the minimal concentration can be reduced by disease. Compared to the two other species, the mouse is least sensitive for determination of threshold concentration that would provoke cardiac arrhythmia.

Respiratory depression and bronchoconstriction. In the course of this investigation, a technique for measuring airway resistance and pulmonary compliance was developed for application to the mouse [3]. This was not hitherto possible so that for the first time the sensitivities of the respiratory and circulatory systems have been compared in this species. The minimal concentration to produce bronchoconstriction is 1% of FC 11, and to depress respiration is 2.5% FC 11. For the same propellant, 10% is needed to produce arrhythmia and this indicates that the airways are more sensitive than the heart in the mouse.

Rat

Heart rate and arrhythmia. A comparison of the rat with and without general aneathesia indicated that the unanesthetized state is more suitable for the investigation of cardiotoxicity [7]. Anesthesia blocks the cardioaccelerator response and even converts it to bradycardia. Although the unanesthetized rat is more sensitive than the mouse for the demonstration of cardiotoxicity, the dog and monkey show effects when exposed to even lower concentration of the propellant.

Importance of adrenal medulla and adrenergic receptors. The cardiotoxicity of the propellant is reduced by adrenalectomy or prior treatment with β-adrenergic blocking drugs [6]. This observation is significant because it demonstrates that the sympathetic nervous system participates in the cardiac effects of inhalants.

Pulmonary compliance and experimental pulmonary lesion. The rat is less sensitive than the mouse in showing respiratory toxicity [2]. However, there are forms of experimentally induced diseases applicable to the rat. In addition to pulmonary emphysema, thrombosis of the pulmonary artery and necrosis of the heart have been induced in the rat. In emphysematous animals, it has been demonstrated that the lungs become more sensitive to a reduction in pulmonary compliance [7]. The rats with cardiac necrosis or pulmonary arterial thrombosis show an increase in sensitivity to cardiotoxicity of the propellants. [6]

Dog

Circulatory depression. The dog is the most sensitive animal for eliciting hypotension, depression of myocardial contraction, tachycardia and cardiac arrhythmia, [10]. For the last-mentioned effect, the unanesthetized dog with epinephrine injection is 5 to 10 times more sensitive to propellants than

the anesthetized dog without injection of epinephrine. This preparation is so sensitive that the proarrhythmic activity of one propellant (FC 152a) can be demonstrated even though the 3 other animal species fail to do so. The heart-lung preparation can be used to demonstrate direct depression of contractility of the ventricles, without participation of adrenal glands and autonomic innervation to the heart [4].

Respiratory depression. As a rule, the concentration that influence respiration are higher than those for circulation. The dog appears to be the least sensitive animal for demonstrating respiratory toxicity. However, in the investigation of mode of action, there are several techniques applicable to the dog. The bronchodilation induced by some propellants is mediated by β-adrenergic receptors [10]. In addition, the propellants stimulate the receptors in the upper and lower respiratory tract which in turn influence respiration, bronchomotor tone and heart rate [11]. Additional experiments relating to pulmonary and bronchial blood vessels are in progress by techniques applicable only to the dog.

Monkey

The advantages and disadvantages of the monkey have not been fully appreciated because of its limited application in the author's laboratory. As a rule, the circulatory system is more responsive than the respiratory system [4,8]. However there are instances in which the monkey's response is opposite to those of the other species so that the relevance to humans is unsettled (Tables I and II). There are propellants that produce cardiotoxicity in all three species but not in the monkey, some that cause myocardial depression and hypotension in the dog but not in the monkey, some that cause early respiratory depression, bronchoconstriction or decreased compliance in the dog, rat and mouse but not in the monkey. The opposite results have been encountered, *i.e.*, propellants that are toxic to the monkey but not to the other species. It has been generally assumed that the monkey closely resembles the human response but there has been no definitive comparison. When human studies become available, it will be possible to decide the significance of the various animal models specifically for the prediction of toxicity in man. Meanwhile, the classification proposed in this publication has been adapted in other reviews by the author [12,13]. Other investigators are invited to modify and supplement the information summarized in Tables I and II.

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文献 20

Acute and repetitive human exposure to isobutane

by RICHARD D. STEWART, M.D., M.P.H.,[1] ANTHONY A. HERRMANN, M.D.,[2] EDWARD D. BARETTA, M.Sc.,[2] HUBERT V. FORSTER, Ph.D.,[1] JEANNE J. SIKORA, R.N.,[2] PAUL E. NEWTON, M.Sc.,[1] and RICARDO J. SOTO, M.Sc.[2]

STEWART, R. D., HERRMANN, A. A., BARETTA, E. D., FORSTER, H. V., SIKORA, J. J., NEWTON, P. E. and SOTO, R. J. Acute repetitive human exposure to isobutane. *Scand. j. work environ. & health* 3 (1977) 234—243. Eight adult volunteers of both sexes were exposed to isobutane in a controlled-environment chamber for the purpose of monitoring their physiological responses to a series of gas concentrations ranging from 250 to 1,000 ppm. First, the response to exposure periods of 1 min, 2 min, 1 h, 2 h, and 8 h were studied. There being no untoward responses to these acute exposures, the eight volunteers were exposed repetitively to isobutane at concentrations of 500 ppm, 1, 2 or 8 h per day, five days per week for two weeks. Then exposures to two mixtures of isobutane and propane for 1, 2 or 8 h per day for two days were studied. During the investigation all subjects were kept under comprehensive medical surveillance. No untoward subjective responses or abnormal physiological responses occurred during or following these exposures. Special emphasis was placed on evaluating the cardiac and pulmonary response to these exposures through the use of continuous ECG telemetry and serial computerized spirometric measurements. The following serial laboratory studies were unaltered by the exposures: complete blood count, urinalysis, serum alkaline phosphatase, SGOT, LDH, serum bilirubin, blood sugar, serum calcium, serum phosphorus, BUN, spontaneous electroencephalogram, visual evoked response, a battery of cognitive tests, and an ACTH stimulation test.

Key words: human response, isobutane inhalation.

The increased use of isobutane and propane as substitutes for fluorocarbons in aerosol products raises the question of the potential of these gases to injure humans. The abuse of fluorocarbon propellants and organic solvents to obtain a "high" has resulted in the sudden death of approximately 300 American teenagers, presumably because of epinephrine sensitization of the heart and the development of fatal cardiac arrhythmia (2, 3, 6, 7, 8, 9, 12, 15, 24). However, few persons were concerned with the possible health hazard to consumers in normal situations until after Zuskin and Boyhuys suggested that aerosol propellants might be responsible for the transient increase in airway resistance observed after the use of hair sprays (25). Any remaining complacency was shattered by Speizer, Wegman, and Ramirez, who reported that brief exposures to fluorocarbon-22 in the 300 ppm range resulted in the development of severe palpitation in pathology residents in Boston and suggested that exposure to "normal-use" concentrations of certain aerosol propellants might pose health problems not previously recognized (16).

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The paucity of information regarding the human inhalation toxicology of isobutane at concentrations encountered both in home-use situations and in the industrial setting prompted this investigation.

EXPERIMENTAL PROCEDURE

Eight healthy volunteers of both sexes were exposed to isobutane or a mixture of isobutane and propane in concentrations ranging from those encountered in the home environment to those permitted in the industrial setting (1). First, a series of single exposures to 250, 500, and 1,000 ppm for periods from 1 min to 8 h were conducted in a controlled-environment chamber (19). There being no untoward health effects, the subjects were exposed repetitively to 500 ppm of isobutane five days per week for two weeks. Then exposures to two mixtures of isobutane and propane for 1, 2, or 8 h per day for two days were studied. These experiments were designed so that the absorption, excretion and physiological effect of isobutane inhalation could be studied. Special emphasis was placed on the monitoring of cardiac and pulmonary performance. The exposure schedule is presented in table 1.

The investigation was performed with strict adherence to the ethical and technical requirements for human inhalation experimentation previously detailed (17, 22), and an informed consent was obtained from each subject after the nature of the procedure had been fully explained.

Subjects

The subjects were selected from the Caucasian, middleclass, college student population. Each subject who completed the study received $ 2.50 per hour spent at the laboratory.

The ages of the four male subjects ranged from 20 to 22 years, height from 178 to 187 cm, and weight from 70.0 to 81.5 kg. None of them was obese. Two of these subjects were assigned to group I (8-h exposure), one to group II (2-h exposure), and one to group III (1-h exposure).

The ages of the four females ranged from 20 to 21 years, height from 155 to 174 cm, and weight from 57.2 to 72.9 kg. The division of the subjects into groups was identical to that for the study with male subjects.

All subjects were cautioned to abstain from the use of drugs and to limit their use of alcohol to very moderate amounts. Subjects who were smokers were not allowed to smoke during their stay in the controlled-environment chamber. Subjects who underwent behavioral testing (2 h and 8 h) were asked to refrain from consuming any caffeine prior to the end of each day's study (1-h postexposure).

Exposure schedule

Table 1 lists the exposure sequence, the number of subjects, the gas concentration investigated, and the duration of each exposure.

Exposure chamber

The experiments were conducted in a controlled-environment chamber. It had a 6 x 6 x 2.4 m testing room with an attached shielded room and an attached toilet facility. The air flow through the suite of rooms to the exhaust was approximately 42 m^3/min, which created a slight negative pressure within the chamber. The ambient temperature within the chamber was maintained at 22—23°C, while the relative humidity ranged between 45—55 %.

The propellant gas was mixed with the air supplying the chamber, entering through four diffusors in the ceiling of the testing room. For the desired concentration, the gas was metered from a cylinder into the return air duct of the air conditioner.

Analysis of exposure chamber atmosphere

The gases used in these experiments were purchased from the Phillips Petroleum Company. The isobutane had a boiling point of — 11.73°C, a vapor pressure of 3,733 mm Hg (about 37.7°C), a vapor density of 2.068 (about 15.5°C and 60 mm Hg), and a specific gravity of 0.563 (about 15.5/15.5°C).

The propane had a boiling point of — 42.49°C, a vapor pressure of 6,612 mm Hg, a vapor density of 1.549, and a specific gravity of 0.509 (15.5/15.5°C).

Table 1. Exposure of human subjects to isobutane and propane.

Planned exposure			Number of subjects		Duration of exposure (h)	Exposure (ppm)
			Male	Female		Mean ± SD
Isobutane	500 ppm		4	4	1/60	514
			4	4	2/60	506
			4	4	10/60	504 ± 15
Isobutane	250 ppm		1	1	1	246 ± 7
			1	1	2	246 ± 19
			2	2	8	245 ± 24
Isobutane	1,000 ppm		1	1	1	1,008 ± 29
			1	1	2	1,006 ± 27
			2	2	8	1,000 ± 24
Isobutane	500 ppm		1	1	1	500 ± 35
			1	1	2	489 ± 15
			2	2	8	493 ± 20
Isobutane	500 ppm, fluctuating concentration		1	1	1	389 ± 92
			1	1	2	650 ± 200
			2	2	8	531 ± 259
Isobutane	500 ppm, 10 repetitive exposures	1.	1	1	1	509 ± 15
			1	1	2	510 ± 21
			2	2	8	507 ± 24
		2.	1	1	1	500 ± 58
			1	1	2	486 ± 20
			2	2	8	489 ± 33
		3.	1	1	1	499 ± 9
			1	1	2	505 ± 23
			2	2	8	503 ± 26
		4.	1	1	1	509 ± 30
			1	1	2	513 ± 17
			2	2	8	502 ± 32
		5.	1	1	1	505 ± 25
			1	1	2	498 ± 16
			2	2	8	503 ± 20
		6.	1	1	1	488 ± 31
			1	1	2	497 ± 22
			2	2	8	504 ± 28
		7.	1	1	1	493 ± 30
			1	1	2	504 ± 24
			2	2	8	504 ± 30
		8.	4	4	8	499 ± 27
		9.	4	4	8	507 ± 23
		10.	4	4	8	496 ± 30
	Mean concentration of the 10 repetitive exposures				1	500
					2	502
					8	501
Isobutane/propane mix (82.5 %/17.5 %)			1	1	1	
Isobutane						462 ± 75
Propane						107 ± 15
Isobutane/propane mix (82.5 %/17.5 %)			1	1	1	
Isobutane						537 ± 38
Propane						77 ± 7
			1	1	2	
Isobutane						483 ± 74
Propane						102 ± 11

Table 1. (continued)

Planned exposure	Number of subjects		Duration of exposure (h)	Exposure (ppm)
	Male	Female		Mean ± SD
(82 %/17.5 % mix cont.)	2	2	8	
Isobutane				502 ± 82
Propane				100 ± 18
Propane/isobutane mix (89 %/11 %)	2	2	8	
Propane				962 ± 55
Isobutane				111 ± 6
Propane/isobutane mix (87.5 %/12.5 %)	2	2	8	
Propane				1,030 ± 39
Isobutane				143 ± 6

Two independent systems were used to monitor the chamber atmosphere. In both cases, air was withdrawn from the chamber through a polyethylene tube (inner diameter 0.64 cm) at approximately 7 l/min, through or past the analytical device, to a small diaphragm pump that discharged back into the chamber.

The concentration of the gases in the chamber atmosphere was recorded continuously by a Wilks MIRAN-I infrared spectrometer equipped with a 20-m pathlength gas cell. The absorbance at 3.4 μ was measured. The voltage output was connected to a strip-chart recorder, and a voltage proportional to the pen position of that recorder was conducted to the analog-to-digital input of a PDP-12 (DEC) computer. The computer sampled the pen position voltage each second, averaged those voltages every 30 s, recorded the average on magnetic tape, and used the average to write on a cathode ray tube the concentration over that 30-s interval and the cumulative or time-weighted average concentration since the beginning of the exposure session.

Gas chromatography was the second and the most sensitive method of chamber air analysis employed. A Varian Aerograph Series 2700 gas chromatograph was equipped with a column packed with Porapak Q operated at 87°C. Nitrogen was used as the carrier gas to a hydrogen flame detector operated at 185°C. An automatic device injected a sample of air into the gas chromatograph every 170 s. Output of the gas chromatograph was connected to a strip-chart recorder. After each exposure ended, a calibration curve for the obtained values was established with a computer using regression analysis on the standards that had been analyzed during the day. With that equation, peak-height values read manually were transformed into concentrations which were then used to calculate time-weighted averages and standard deviations for exposure increments for comparison with the values obtained using the infrared spectrometer. Concentrations determined by the two methods were in agreement throughout the study.

For the standards, saran bags were filled with room air pumped in sequence through a charcoal column, wet test meter, a Drierite column, and a type N all-service gas mask canister. After a bag was filled with a known amount of clean, dry air, a known volume of isobutane and/or propane was injected into the bag. For the calibration of the analytical devices the saran bag standard was attached to the sampling probe within the chamber. At least three standards were analyzed before subjects were allowed to enter the chamber each day, and then standards were analyzed at approximately 1-h intervals throughout the day.

Clinical testing

All exposures with a duration of one or more hours were conducted according to a double-blind format.

Prior to commencing the actual exposures, the subjects underwent a training program in the controlled-environment chamber; during this time they became accustomed to the chamber setting and the testing procedures.

The subjects were given a repeat physical examination prior to each exposure. At this time each of them completed a "symptom checklist." This form had designated spaces for noting the presence of headache; nausea; dizziness; abdominal pain; eye nose, throat irritation; or other subjective symptoms. Each subject reviewed this list of symptoms immediately upon entering the chamber and each hour during and for 5 h following each exposure. The adjectives "mild, moderate, and strong" appeared on the sheet as cue words, and the phrase "only abnormalities recorded" was prominently typed at the bottom. The home telephone numbers of each of the department physicians appeared on the form, and the subjects were encouraged to phone if they became ill while away from the laboratory.

Prior to and following the exposures, the following laboratory determinations were made: complete blood count, urinalysis, alkaline phosphatase, SGOT, LDH, bilirubin, blood sugar, calcium, phosphorus, BUN. Blood and alveolar breath samples were collected for hydrocarbon gas analysis. The following studies completed the preexposure evaluation: computerized spirometry, 12-lead ECG, and a modified V_5 ECG rhythm strip by telemetry.

After entering the environmental chamber, the subjects were under continual visual surveillance by medical personnel and all important chamber activities were videotaped by closed circuit TV. The subjects immediately performed a modified Romberg test followed by a heel-to-toe test. These tests were first performed with the eyes open and then repeated with the eyes closed. Then, each subject completed his subjective symptom checklist as previously discussed. Five minutes prior to exiting from the exposure chamber, each subject repeated the modified Romberg test and the heel-to-toe test. Lead V_5 telemetry hard copy was obtained from each subject after 30 min of exposure and hourly thereafter. An additional telemetry strip was obtained each time an ECG change was observed.

During the final 40 min of exposure subjects exposed for 2 and 8 h performed the following: computerized spirometry measurements, maximum midexpiratory flow rate, the Flanagan coordination test, the Flanagan arithmetic test, the Marquette time estimation test (21), and the random number inspection test. During the repetitive studies these tests were performed twice a week during the final 2 h of exposure.

Spontaneous electroencephalograms (EEG) and visual evoked responses (VER) were recorded four times each Monday, Wednesday, and Friday of the two-month period of exposure. All recordings were obtained while the subjects were seated in a comfortable upholstered chair in the shielded room in which the hydrocarbon concentrations were identical to those in the controlled-environment chamber (11, 23). The time required to perform the EEG and VER precluded studying more than four subjects per day.

Alveolar breath samples were obtained daily from each subject prior to entry into the environmental chamber, and serially following each exposure. These samples were each collected in 5-l saran bags with the technique previously described in detail (18, 19).

Blood samples for hyrocarbon analysis were obtained from an antecubital vein of each subject before exposure, 15 min before exit from the chamber, and 15 min after exposure in Vacutainer® tubes with edetic acid anticoagulant. The preexit sample was obtained from the exposed subject's arm after it had been stuck through an armport in the chamber wall into the uncontaminated adjacent laboratory.

Electroencephalography

Gold-plated silver disk electrodes were oriented on the scalp according to the 10—20 International Electrode System (13). The pastefilled disk electrode at the inion was cemented with collodion to the scalp to prevent shifting. An eight channel Grass polygraph fitted with EEG amplifiers was utilized for recording. EEG

activity was recorded for 15—30 s before, periodically during, and 15—30 s after acquisition of the VER. The amplitude, frequency and wave form of the recordings were compared to those of control tracings.

Visual evoked response

A complete description and illustration of the EEG-VER monitoring system can be found in a previous publication (11). The VER was recorded from the electrode at the inion referred to the left ear. An EEG channel was used to amplify the VER, and the output was fed to an on-line averaging computer (Nicolet, 1074). The VER was triggered by a strobe flash (3 μs) at the rate of 1 per second for 128 s. The strobe was operated to deliver 18 million beam candles at 1 m from the subject's eyes, which were closed throughout the period of strobe flashing. Analysis time was 400 ms. The flash delay from the synchronizing pulse which initiated the computer sweep was 50 ms. The computer averaged the response to the 128 flashes, and the resultant VER was recorded on an X-Y plotter for analysis.

It has been shown that VER amplitude might be altered by varying levels of attention, cortical desynchronization, and sleep (4, 10, 14). Accordingly, standardized conditions were used throughout each exposure day and immediately preceding the actual recordings. A rigid schedule for food intake, physical activity, and additional testing procedures was followed. In addition, after entering the booth, the subject was always allowed 3—5 min to achieve a relaxed state; and then immediately prior to initiating the strobe flash, the subject was asked to clap his hands five times slowly and forcibly to insure a wakeful, attentive state.

The most prominent reproducible portions of the VER complex are the third, fourth, and fifth waves (designation by Gastaut) (5, 10, 11). The analysis was thus restricted to these waves. Wave 3 was identified as proceeding in a positive direction 80—120 ms after the initiation of the strobe flash. Waves 4 and 5 were the succeeding negative and positive segments of the VER. Analysis involved: (a) measuring the amplitude of these waves and (b) measuring whether changes had occurred in the latency and wave form of the VER complex.

Adrenocortical function

Upon the completion of the repetitive exposures the subjects underwent the standard 2-day ACTH stimulation test for the assessment of the ability of the adrenal gland to respond to stress. The reason for this testing was the observation that the organ with the highest hydrocarbon concentration following exposure is the adrenal cortex.

Analysis of ambient air, expired breath and blood

Air and breath samples for hydrocarbon analysis were injected directly onto a Porapak Q column of a Varian Aerograph Series 2700 gas chromatograph equipped with a hydrogen flame ionization detector. The column and operating conditions were: column: 45.72 cm × 0.32 cm O.D. stainless steel; column packing: Porapak Q, 50—80 mesh; oven temperature: 87° C; injector temperature: 150° C; detector temperature: 185° C; carrier gas: nitrogen, 20 ml/min.

Air standards were prepared by direct injection of the appropriate quantity of isobutane or propane into a saran bag containing a measured volume of air.

A headspace sampling technique was utilized for measuring the concentration of the hydrocarbons in blood. Blood standards were prepared by the direct addition of an appropriate quantity of an air standard containing 1,000 ppm propane or isobutane to a Vacutainer tube containing a known quantity of blood. The freshly drawn anticoagulated blood sample was allowed to equilibrate with the hydrocarbon by agitation. The headspace sample was injected directly into a gas chromatograph for analysis. The technique was far superior to the solvent extraction methods used previously.

Medical surveillance

The subjects were under continuous visual surveillance by a physician and by nursing personnel during each exposure. The pre-