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A REVIEW OF THE TOXICOLOGY OF HALOGENATED FIRE EXTINGUISHING AGENTS

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FOR THE COMMANDER

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Bromotrifluoromethane (Halon 1301), bromochlorodifluoromethane (Halon 1211), and chlorobromomethane (Halon 1011) were evaluated for toxicity and toxic hazards. The most important toxicological effects of these compounds are on the central nervous and cardiovascular systems. The neurological effects are manifested as alterations of perception and a reduction in reaction time and the ability to concentrate on complex intellectual tasks. The cardiovascular effects are manifested as changes in cardiovascular dynamics and the electrical activity

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of the heart. Clinically important central nervous system effects generally appear at lower levels of exposure than clinically important cardiovascular effects. Behavioral changes and performance decrements during exposure would undoubtedly have some effect on the interaction of the subject with his environment, and such consequences of exposure could be life-threatening. Likewise, certain manifestations of halogenated alkane toxicity, such as the occurrence of cardiac arrhythmias, also constitute readily identifiable hazards.

SUMMARY

This report has been prepared in response to the requirement by the AF Aero Propulsion Laboratory (Aeronautical Systems Division, Wright-Patterson Air Force Base, Ohio) for a toxicity and toxic hazards evaluation of three fire extinguishing chemicals, bromotrifluoromethane (Halon 1301), bromochlorodifluoromethane (Halon 1211), and chlorobromomethane (Halon 1011).

The report consists of 4 parts. Section I represents a brief review of 26 documents that have served as sources of the information upon which the report with its evaluation is based. Section II is a technical summary of the studies of the cardiovascular toxicology-pharmacology of the compounds conducted by personnel of the Toxicology Branch, Toxic Hazards Division, 6570 Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. Section III describes original studies of the effects of the fire extinguishing compounds on myocardial metabolism. Section IV is a comparative toxic hazards evaluation of the compounds based on available, relevant technical information.

The most important toxicological effects of these compounds are on the central nervous and cardiovascular systems. The neurological effects are manifested as alterations of perception and a reduction in reaction time and the ability to concentrate on complex intellectual tasks. The cardiovascular effects are manifested as changes in cardiovascular dynamics and the electrical activity of the heart.

Clinically important central nervous system effects generally appear at lower levels of exposure than clinically important cardiovascular effects. Behavioral changes and performance decrements during exposure would undoubtedly have some effect on the interaction of the subject with his environment and such consequences of exposure could be life-threatening. Likewise, certain manifestations of halogenated alkane toxicity, such as

the occurrence of cardiac arrhythmias, also constitute readily identifiable hazards.

This report does not represent a comprehensive definitive evaluation of halogenated alkane toxicology. It does represent the author's best judgement, based on current evidence, of the relative toxic hazards of short-term inhalation exposure to Halons 1301, 1211, and 1011.

PREFACE

This study was initiated and performed by personnel of the Toxicology Branch, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. This research was supported in part by the Air Force Aeropropulsion Laboratory, Aeronautical Systems Division, Wright-Patterson Air Force Base, Ohio and the National Institute of Occupational Safety and Health, Cincinnati, Ohio.

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SECTION I

REVIEW OF LITERATURE

Acute Toxicity

The popularly accepted indexes of acute toxicity of these compounds have been published by Engibous & Torkelson (1960) and Clayton (1967). Indexes such as median lethal concentration (LD_{50}) and approximate lethal concentrations (ALC) are of some comparative value for 1011 and 1211 when air was used as the diluent. One of the commonly cited figures for the ALC of 1301 in rats is 83%. The report indicated that air was used as the diluent in some of these studies which would invalidate the results since the 83% 1301 mixture in air would contain about 3-1/2% oxygen and the animals would have been severely hypoxic. Clayton did report, however, a mouse LD_{50} of 84% of 1301 and a guinea pig LD_{50} of 88% with the balance of the inspired gas mixture, oxygen. Svirbely et al. (1947) reported an LD_{50} for 1011 of 2.9% in mice. Engibous and Torkelson reported Army Chemical Center results of the exposure of one (!) animal to 1011 and arrived at a figure of 6.5% as the ALC.

The report by Clayton (1967) of the levels of 1301 and 1011 required to produce drowsiness in rats is more illuminating than the preceding. The ratio of 50% 1301 to .475% 1011 equals about 105. This is very close to the ratio of a composite of cardiovascular effects found by our group on dogs and summarized in Table 3.1., page 27 herein.

Engibous & Torkelson reached two inaccurate conclusions because of paucity of supportive data 15 years ago. They suggested a practical limit for human exposures to 1301 of 50% and based this on the observation that 1301 was apparently biologically inert and exerted its effects only through a displacement of oxygen. We now know this to be erroneous.

Neurological Effects

High brain levels of 1301 were achieved in rats exposed to a nominal concentration of 75% $CBrF_3$ in oxygen. The levels were 50% higher than heart and blood levels (Van Stee & Back, 1971). Aware that the compound reached the brain, Chikos et al. (1969) studied two basic cortical functions, the primary and direct evoked cortical responses. Both were depressed by exposure to 1301, indicating cerebrocortical depression. The relative lipid solubilities of the compounds are 1301 < 1211 < 1011 and, therefore, 1211 and 1011 would also be expected to accumulate in brain tissue, and probably to a greater extent than 1301. Presence of significant levels of the compounds in the brain would be related to the genesis of CNS dysfunction.

Carter et al. (1970) studied the performance of operant trained monkeys during exposure to 1301. Exposure to 20-25% resulted in performance decrements while exposure to higher concentrations resulted in a complete disintegration of operant behavior. Similar effects would be expected from exposure to 1211 or 1011 although the precise character of the expected responses is unknown.

Van Stee & Back (1970) reported that the electroencephalograms (EEG) of monkeys and dogs exposed to 70% of 130I were synchronized and had increased amplitude. The EEG could still be activated by sensory stimuli, however, and no seizure activity was detected. This is evidence of central nervous system depression without loss of consciousness. Since no seizure activity was elicited in the dogs that were paralyzed with curare during the recording sessions the genesis of the convulsions may require a functional somatic motor system.

Hine et al. (1968), Haskell Laboratory (1967), and Call (1973) conducted human exposures to 130I. Hine et al. reported that exposure to 10-15% of 130I decreased the subjects' (10) performance of 5 of 6 psychomotor tasks. The volunteers also reported subjective changes in the sensorium. Call reported only a slight increase in reaction time during 3 minute exposures to 4 and 7% of 130I. He further reported no significant interaction between the presence of 130I and hypoxia. Lightneadedness, paresthesias, and diminished performance during exposure to up to 10% were reported by the Haskell Laboratory.

Clark exposed human volunteers to 4 and 5% of 121I. Feelings of light-headedness and paresthesias were reported at 4% which were aggravated at 5%. Exposure to 5% of 121I caused marked symptoms of CNS depression.

Controlled exposure of human volunteers to 101I has not been reported. Rutstein has, however, published 3 case reports involving accidental human exposures in which the absorbed doses could not be estimated. The subjects initially lost equilibrium and then consciousness. The loss of consciousness is consistent with the observation by Svirbely et al. of the anesthetic potency of 101I in mice. This has been confirmed in other rodent species in our laboratory as well as others.

Human volunteers have reported feelings of impending unconsciousness during exposure to 15% of 1301 (Hine et al., 1968). Monkeys have been observed by Van Stee & Back (1968) to go into a trance-like state and Carter et al. (1970) reported behavioral depression in monkeys. Dogs, on the other hand, convulsed during exposure to 40-80% 1301 (Van Stee & Back, 1968; Hine et al. 1968).

Beck et al. (1973) reported tremors and convulsions in dogs exposed to 5% to 8.8% of 1211. The neurologically equivalent responses of dogs to 5-8.8% of 1211 were roughly equivalent to 40-80% of 1301, an approximately 1:10 relationship.

Myocardial Metabolism

Rhoden and Gabriel (1972) reported that exposure of rats to 1301 increased myocardial glycolysis by activating phosphofructokinase (PFK). Van Stee et al. (1973) provided the first report of a series now in various states of preparation or publication supporting this observation for 1301, 1211, and 1011. This is discussed in detail in Section III of this report and will not be reiterated here. Tabulated partially reduced data from the first of this series of experiments is shown in Table 3.1.

Cardiac Arrhythmias

Much attention has been directed toward the problem of the genesis of cardiac arrhythmias during exposure to the halogenated alkanes. Van Stee & Back (1971) demonstrated that in addition to the presence of 1301, arrhythmias appearing during exposure to this compound were sensitive to blood pressure, acid-base balance, and pressor amines such as epinephrine (by some mechanism other than their ability to raise blood pressure).

The interaction between the presence of the halogenated alkanes and epinephrine, the so-called "sensitization" of the heart to epinephrine has been thoroughly investigated for 130I and 121I. The concern is based on the supposed release of endogenous epinephrine from the adrenal medulla during excitement, fear, or other stressful stimuli. The IV infusion of exogenous epinephrine during exposure to the halogenated alkanes does not duplicate the sympathoadrenal activation of the stressful situation. Not only is epinephrine liberated from the adrenal medulla, but also adrenergic neurotransmitter (presumably norepinephrine) is elaborated at the adrenergic terminals of the sympathetic innervation of the heart (as well as other sympathetically innervated structures). Furthermore, the volumes of distribution of endogenous and exogenous catecholamines are not identical.

Hine et al. (1968) came closest to modeling the physiological situation when they exposed dogs to 130I and then frightened them by means of stroboscopic lights and noise. No dog in this study developed ventricular fibrillation. Van Stee & Back (1969) did report the death from ventricular fibrillation of one dog exposed to 40% 130I and not given any additional drugs. Marked excitement accompanied the event.

Beck et al. (1973) and Clark (1970) demonstrated the exogenous epinephrine-121I interaction on cardiac arrhythmias in dogs.

Hine et al. (1968) monitored cardiac electrical activity (EKG) during exposure of human volunteers to nominal concentrations of 5, 10, and 15% of 130I. A-V dissociation and premature ventricular contractions were recorded during exposure to the highest concentrations (maximum, 16.9%).

Neither Call (1973) nor Smith (1973) detected any cardiac arrhythmias during exposure to 1301. Call exposed human volunteers to 4 or 7% for 3 minutes in hypobaric chambers. Smith exposed crews to 5-7% for 5 minutes at pressurized altitudes of 1000-20,000 ft in aircraft flight tests.

Drug Metabolizing Enzyme Systems

Many organic compounds have the ability to stimulate enzyme systems in the liver that metabolize foreign chemicals in the body. Compounds may affect their own metabolism and/or the metabolism of other compounds. The duration of exposure required to elicit this phenomenon is much longer than the brief exposures considered in this report. Preliminary evidence has suggested, however, that 1301 and 1211 have little or no ability to induce hepatic microsomal enzyme systems. We would expect that 1011 would but know of no published reports to this effect.

Cardiovascular Dynamic Effects

This aspect of the toxicity of the halogenated alkanes is discussed in detail in Section II and will not be discussed further here.

SECTION II

TOXIC HAZARDS EVALUATION OF NEW AIR FORCE FIRE EXTINGUISHING AGENTS

Low molecular weight halogenated alkanes, particularly certain fluoroalkanes, are of interest to the United States Air Force as fire extinguishing agents, refrigerants, and solvents. Some fluoroalkanes also find use as aerosol propellants and are of interest to industrial and public health agencies because of wide-spread consumer use of pressurized household products. They also have a potential for abuse, particularly among drug-oriented youth. Another fluoroalkane, halothane, has been in common use in most hospitals since 1954 as an inhalation anesthetic.

Pharmacologically significant exposure of the human organism to these compounds, whether by design or accident, is usually by inhalation. Many compounds have a relatively high vapor pressure at ordinary conditions, so that they readily mix with air in pharmacologically significant concentrations. Fluoroalkanes readily diffuse through cell membranes because of their lipid solubility. Availability to the alveolar membrane, coupled with lipid solubility, results in a potential for quantitatively significant pulmonary absorption of fluoroalkanes.

Fluoroalkanes as a rule are not pulmonary irritants. In low concentrations inhalation is not an unpleasant experience acutely, nor does prolonged exposure result in pathological changes in the upper respiratory tract or lungs. In somewhat higher concentrations inspiration may be resisted, but this is likely to be a consequence of the activation of reflexes as the Kratschmer reflex, as suggested by Aviado (1973).

The USAF is currently most interested in $CBrF_3$ (Fluorocarbon 1301[a]), $CBrClF_2$ (F1211), and chlorobromomethane (1011). They are for use in air-

a. Fluorocarbons numbering system: 1st digit, no. of carbons; 2nd digit, no. of fluorines; 3rd digit, no. of chlorines; 4th digit, no. of bromines; 5th digit, no. of iodines; terminal zeros are dropped.

craft fire control systems. F1301 is under consideration for deployment in a total flooding system for use in crew and cargo compartments in the C5-A. A combination of F1301 and F1211 has been proposed for use in a new hand-held first aid fire extinguisher, referred to as the "Halonfoam" system. This is under consideration as a replacement for the standard A-20 which uses chlorobromomethane as the fire extinguishant.

METHODS

Dogs were anesthetized with fentanyl-droperidol[b] and pentobarbital (Hamlin et al., 1968), morphine-chloralose, or ethanol-morphine (Van Stee et al., 1973). Guinea pigs were anesthetized with fentanyl-droperidol and ketamine (0.4 ml/kg and 60 mg/kg, respectively). Monkeys were anesthetized with pentobarbital sodium. Rabbits were killed by cervical dislocation for the removal of hearts for the isolated heart studies, and the rats from which the mitochondria were prepared were killed by a blow to the head.

The general methods for the cross-circulation experiments (Van Stee et al., 1972), ganglionic transmission studies (Van Stee, 1970 and Van Stee et al., 1972), the cardiac arrhythmia studies (Van Stee and Back, 1971), the Langendorff heart studies (Rusy and Coulson, 1973), the dye-dilution studies (Dow, 1955), the mitochondrial respiration studies (Sordahl et al., 1971), and the electron micrographic studies (McNutt et al., 1973) have been published elsewhere.

The data were analyzed using one and two-way analyses of variance, analysis of covariance, Student's t-test for paired observations, Box's modification of the Bartlett test for homogeneity of variances, and the Mann-Whitney-Wilcoxon test for multiple comparisons when the assumptions underlying the application of parametric methods were known to be invalid (Li, 1964).

b. Innovar-Vet, McNeil Laboratories, Fort Washington, PA

RESULTS AND DISCUSSION

Some time ago our attention was directed to F1301 and so it has been subjected to the most detailed scrutiny. Investigation of other fluoroalkanes has followed and we are gradually acquiring enough information to be able to make certain generalizations concerning mechanisms of action and structure-activity relationships.

Exposure of human volunteers to 15% $CBrF_3$ was accompanied by irregularities in the electrocardiogram and impressions of impending unconsciousness (Hine et al., 1968 and Smith and Harris 1973). Exposure of monkeys suggested related central nervous system effects. Psychomotor performance of trained monkeys was impaired in the presence of concentrations of $CBrF_3$ higher than those to which humans have been exposed (Carter et al., 1970). Furthermore, cardiac arrhythmias have appeared in all species tested so far (Van Stee and Back, 1971).

The central nervous system effects of exposure of primates to $CBrF_3$ differ from those observed in dogs. Whereas in man the effects may be termed depressant on the basis of behavioral observations, dogs become agitated and almost half experience epileptiform convulsions (Van Stee and Back, 1969). It is of interest to note that another fluorinated compound, hexafluorodiethyl ether (Indoklon), has been used in the past for the therapeutic induction of convulsions.

The cardiovascular actions of the fluoroalkanes are considered to represent the most significant hazard incident to their use and so we have conducted detailed studies of the cardiovascular dynamic and myocardial metabolic effects of exposure to F1301, F1211, or chlorobromomethane.

Figure 2.1 illustrates 2 consequences of exposure of an anesthetized rhesus monkey to 80% $CBrF_3$. A similar picture would be seen during exposure to about 12% of $CBrClF_2$. During the exposures, the animals' blood pressure often fell and they developed a markedly elevated end diastolic pressure. This indicates that myocardial performance shifted up the Starling curve to a region approaching a state of compensated heart failure which would constitute a decrease of myocardial contractility.

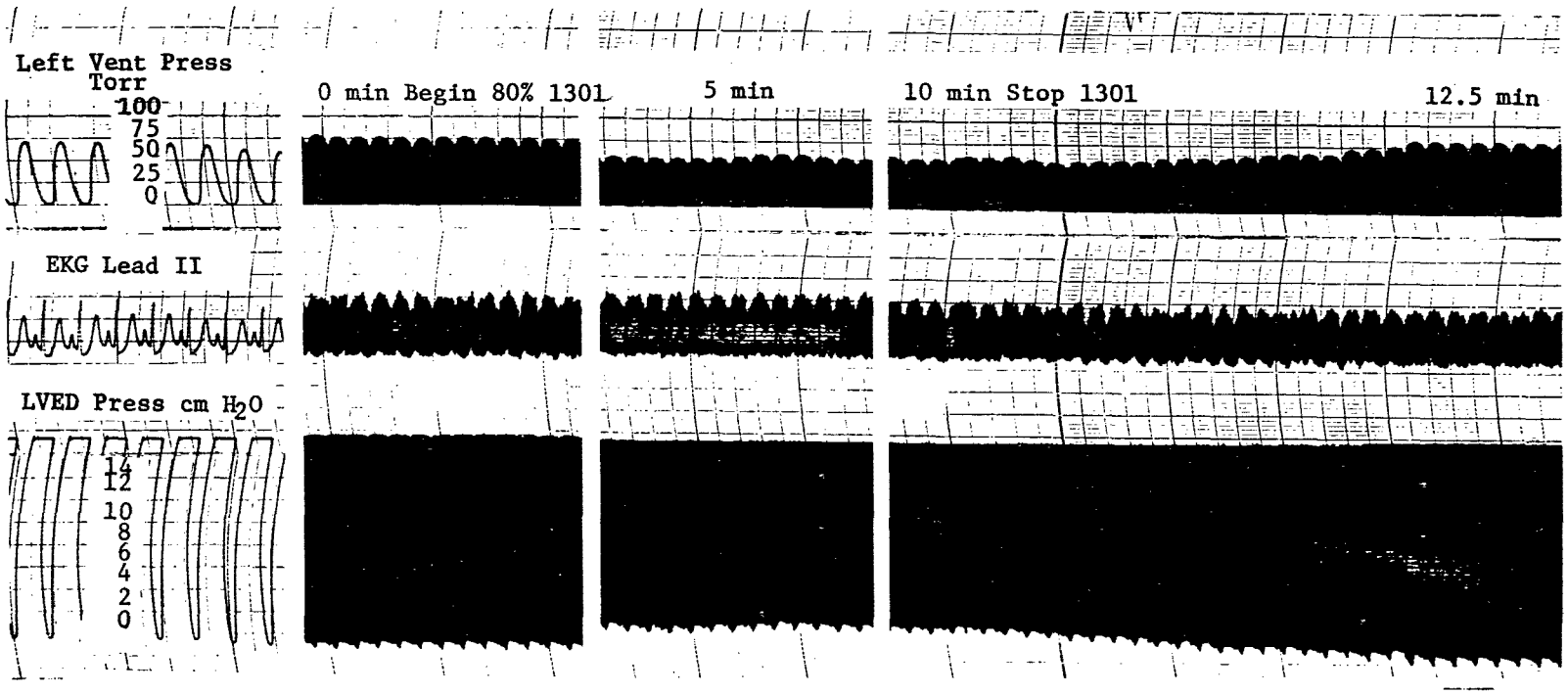

Figure 2.1 The effect of 80% $CBrF_3$ exposure on left ventricular blood pressure in an anesthetized, open-chested monkey. The fall in peak systolic blood pressure after 5 min exposure is represented in the top tracing. The bottom tracing represents the same ventricular pressure curve amplified to show the increase in left ventricular end diastolic pressure over the same exposure interval.

The negative inotropic effects of exposure to the halogenated alkanes have been quantified and compared (Van Stee et al., 1973). The decreases in such indexes as dP/dt_{max} and dP/dt_{max} divided by developed pressure were comparable during exposure to approximately 1% 1011, 12-14% 1211, and 75-80% 1301.

The arrhythmias, particularly premature ventricular contractions (PVC), are dependent on a number of factors in addition to the level of fluoroalkane (Van Stee and Back, 1971). Figure 2.2 illustrates the results of experiments conducted to evaluate the sensitivity of the arrhythmias to mean blood pressure changes. The exposures were constant throughout the periods during which the recordings were made. In the upper tracing the PVC appeared when pressure was elevated by expanding circulating blood volume by infusing 6% dextran. In the bottom tracing PVC appeared and disappeared when mean arterial pressure was raised and lowered by aortic construction.

In another experiment (Figure 2.3) the blood pressure was lowered and raised by exsanguination and reinfusion. PVC are indicated by the presence of the high peaks in these EKG run at slow speed. Other experiments were conducted that demonstrated that circulating catecholamine levels and acidosis altered the arrhythmia threshold independently of changes in blood pressure.

Exposure to the fluoroalkanes often caused a reversible, concentration-dependent fall in mean arterial blood pressure (Figures 2.1 and 2.5). Cross-circulation experiments have been performed in which the blood from donor dogs was used to perfuse the hind limbs of recipient dogs (Van Stee and Back, 1972). The hind limbs of the recipients were in vascular isolation from the dogs' general circulation but the autonomic innervation remained intact. Through the use of combinations of recipient and donor dog exposures, coupled with the administration of autonomic drugs, the mechanism of hypotensive response to the fluoroalkanes, 1301 and 1211, was determined to be a decrease of vasoconstrictor tone. The compounds were found not to have any direct vascular smooth muscle action.

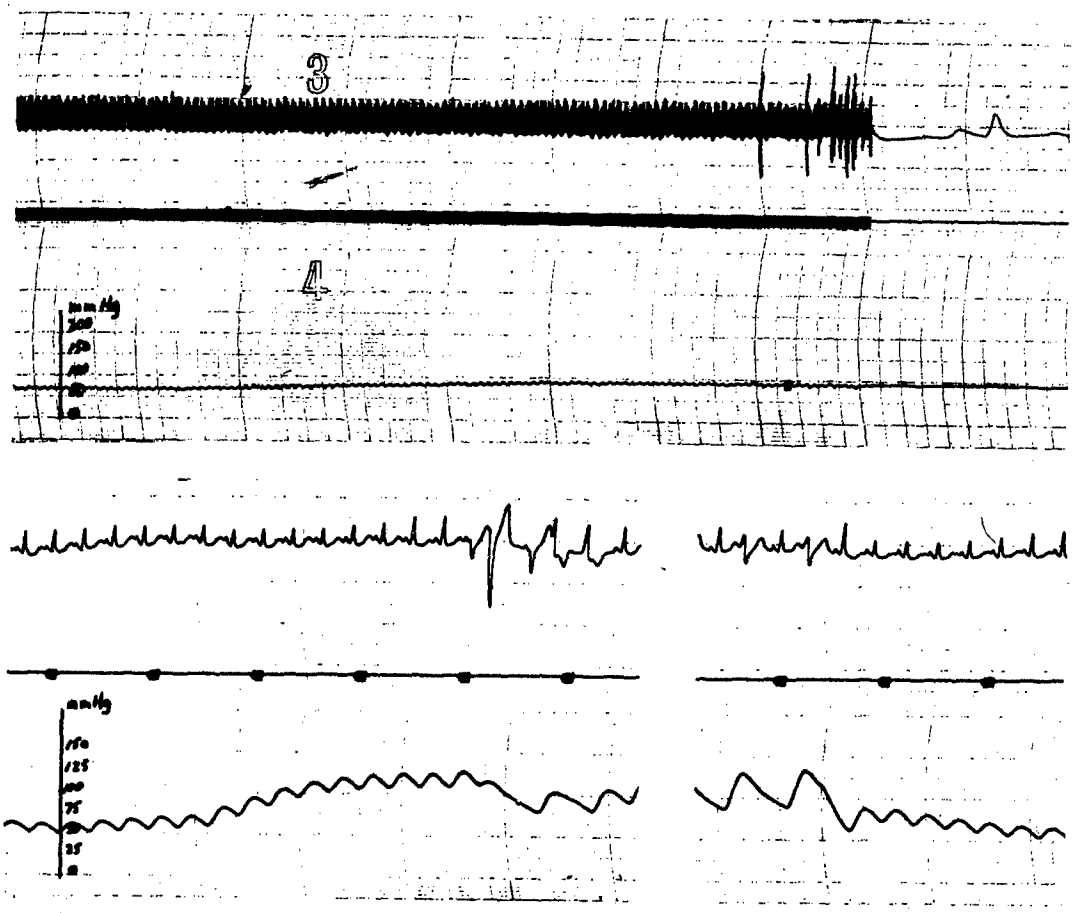

Figure 2.2 The upper tracing illustrates the triggering of cardiac arrhythmias in a monkey exposed to 70% $CBrF_3$ by the expansion of plasma volume with 6% dextran. The lower tracing illustrates the triggering and abolition of arrhythmias during a similar exposure by the constriction and release, respectively, of the thoracic aorta.

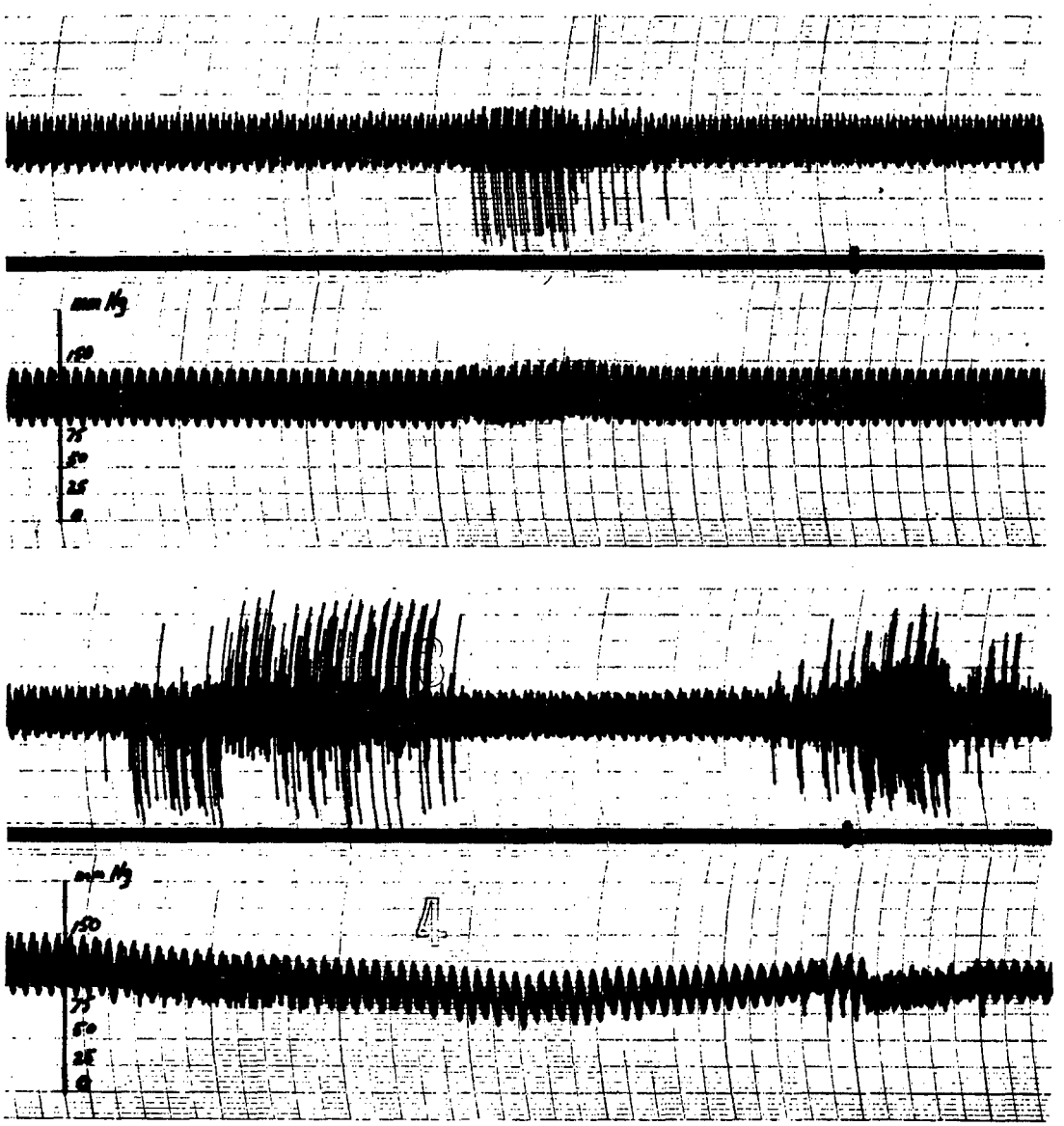

Figure 2.3 The appearance of cardiac arrhythmias during exposure to $CBrF_3$ depended on the maintenance of a minimal blood pressure. Arrhythmias (EKG, upper recording of each pair) were triggered by raising the blood pressure (upper pair) with IV epinephrine. Arrhythmias also were abolished and restored by the alteration of blood pressure by exsanguination and reinfusion (lower pair).

A series of experiments was conducted to test the hypothesis that the decrease in vasoconstrictor tone was the result, in part, of an impairment of ganglionic transmission[22].

The vagosympathetic trunk was severed in the midcervical region and the cut ends stimulated electrically. Nictitating membrane tissue tension was measured during stimulation of the central end and vagal inhibition of the heart was monitored during stimulation of the peripheral end. The fluoroalkanes, but not chlorobromomethane, were found to cause a partial ganglionic blockade.

In another set of experiments intravenous acetylcholine or norephinephrine were the autonomic stimuli[19]. A reduction in the hypotensive response to injected acetylcholine during exposure to 1301 suggested an anticholinergic effect of this compound.

In summary of the cardiovascular dynamic effects the conclusion was reached that the fall in blood pressure seen during exposure to the fluoroalkanes resulted from a combination of cardiodynamic functional impairment and ganglionic blockade. A reduction of cardiodynamic performance also was seen during exposure to 1011 but since the pressoreceptor reflexes remained functional in the absence of ganglionic, blockade, their activation resulted in the maintenance of normal or slightly elevated mean arterial blood pressure during exposure.

The cardiac arrhythmias were sensitive to changes in mean arterial blood pressure which implied that myocardial afterload was a determinant of the arrhythmia threshold as well as the presence of the halogenated alkanes. Tension on the myocardium has been demonstrated to alter both the electrical and mechanical properties of cardiac muscle[11]. This concept may be extended to include muscle preload and the authors suspect that this variable may affect the arrhythmias threshold as well as afterload. This hypothesis has not been tested in this laboratory but assumes some importance in view of the fact that end diastolic pressure (and presumably end diastolic volume, as well) may rise during exposure to the compounds.

Having determined that the hypotensive effect was primarily the consequence of a decrease in vasoconstrictor tone secondary to ganglionic blockade, a group of experiments was conducted to investigate the mechanism of the negative inotropic effect. Myocardial performance is influenced by factors that might be divided into extrinsic and intrinsic (Figure 2.4). Each of these must be controlled or eliminated as significant variables in studies of this type.

A general procedure was established in which dogs were anesthetized and instrumented for acute exposure to different gas mixtures under anesthesia (Van Stee, et al., 1973). Forty variables were either measured directly or computed from measured variables to provide a basis for an evaluation of cardiovascular dynamics and myocardial metabolism during exposure of anesthetized dogs to the halogenated alkanes. The measurements included arterial and coronary venous blood levels of O_2, glucose, lactate, pyruvate, non-esterified fatty acids, and the acid-base variables. Cardiovascular dynamic variables were monitored and Stewart-Hamilton indicator-dilution studies were performed using indocyanine green.

Some animals were pretreated with amine-depleting doses of reserpine 24 hours prior to examination. The results of these experiments indicated that the cardiovascular dynamic impairment that occurred during exposure to 1211 was independent of the integrity of the aminergic neural mechanisms. We have interpreted this to mean that the negative inotropic effect of the compound is independent of myocardial adrenergic postsynaptic activity.

Likewise, the determination was made that the myocardial effects of exposure to the halogenated alkanes was not the consequence of an altered availability to the myocardium of oxygen and oxidizable substrates. Delivery to, and extraction by, the myocardium of nutrients was not altered significantly.

The significant finding in this series of experiments was that, whereas the myocardium was presented with adequate oxygen, the animals exposed to 1211 and 1011,

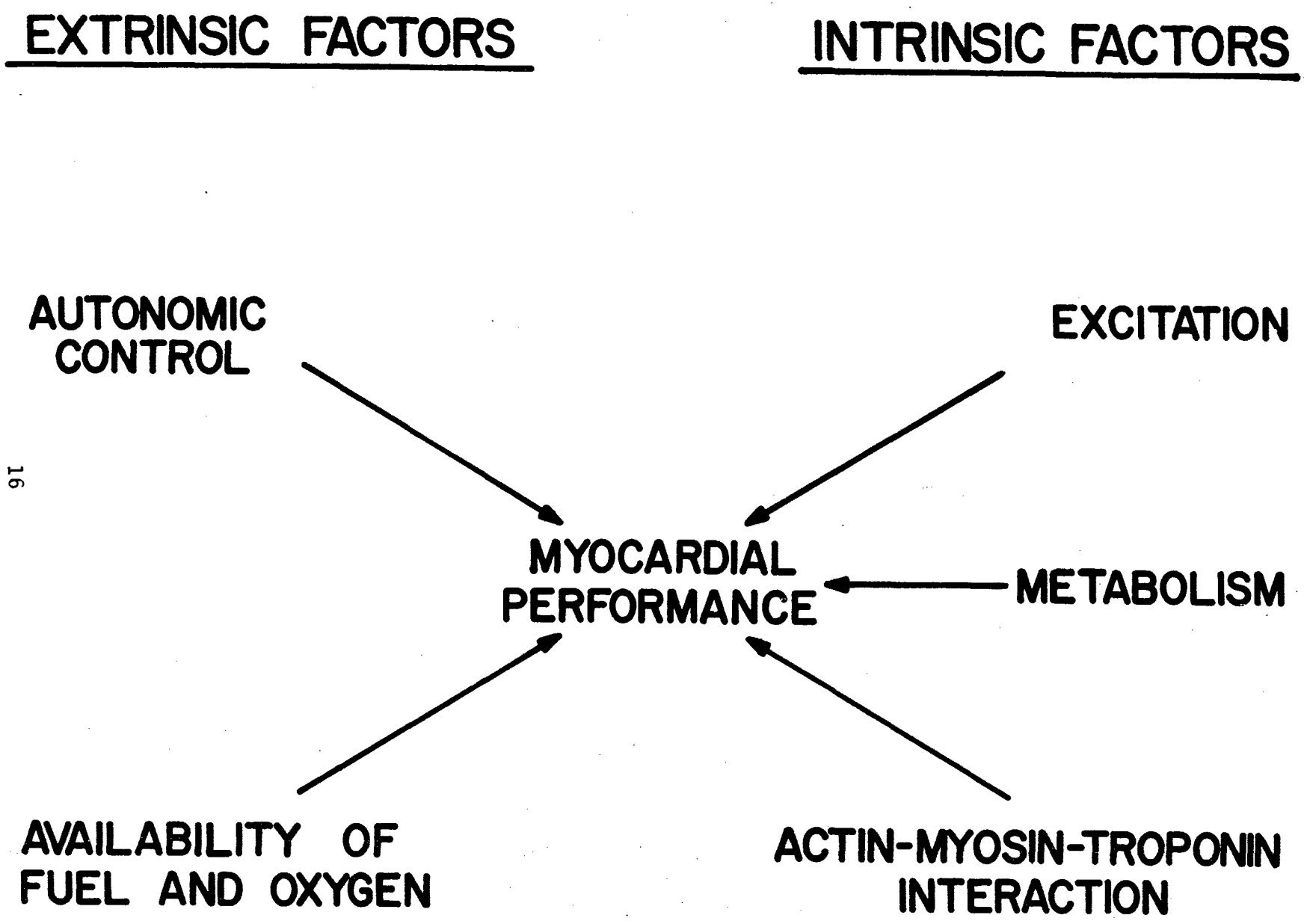

Figure 2.4 The extrinsic and intrinsic factors that control myocardial performance.

failed to extract a normal amount. A rise in the oxygen content of coronary sinus blood was measured that was correlated with the concentrations of bromchlorodifluoromethane or bromochloromethane to which the animals were exposed. This observation led to an investigation of the role of the intrinsic variables of Figure 2.4

More and more evidence has accumulated to reinforce the concept that in the myocardium the primary determinant of coronary blood flow is the tissue oxygen requirement (Dempsey and Cooper, 1972). Coronary flow can, in turn, be a major determinant of myocardial contractile force. As a result of these relationships, when the myocardial oxygen requirement decreases, the coronary flow would be expected to decrease correspondingly, thus maintaining a relatively constant coronary venous blood oxygen content. This relationship was obviously disturbed in the animals exposed to the two compounds in question since as oxygen consumption fell, coronary flow remained undiminished or fell, but coronary venous blood oxygen content rose.

The normal clinical determinants of myocardial oxygen consumption are heart rate, blood pressure and myocardial wall tension (Pitt, 1974). The latter two were demonstrated to be reduced to a certain extent which would account for a reduced oxygen demand by the myocardium, but could not account for a failure of a normal readjustment of the coronary flow-venous oxygen-myocardial oxygen demand mechanism.

A series of experiments was initiated to study the effects of the halogenated alkanes on the respiration of isolated mitochondria (Sordahl et al., 1971). The equilibration of isolated rat liver mitochondria with the same concentrations of the halogenated alkanes to which the intact animals were exposed resulted in decreases in the rate of respiration without an uncoupling of oxidation from phosphorylation. The order of potency in slowing mitochondrial respiration was the same as that for producing a rise in coronary venous blood oxygen content. $CBrF_3$ was the weakest, CH_2BrCl was the most effective, and $CBrClF_2$ was intermediate between the two.

Given sufficient time, in all cases, all of the ADP added to the isolated mitochondrial suspensions was phosphorylated to ATP.

Since mitochondrial respiration was slowed during exposure to the halogenated alkanes we conducted a series of experiments to determine the effects of exposure on myocardial ATP content. Anesthetized guinea pigs have been exposed to 50-60% 1301 in oxygen and their hearts freeze-clamped for phosphate compound analysis. ATP levels were found to begin to fall after 3.75 minutes exposure. As may be seen in Figure 2.5 the rise in end diastolic pressure began, in this experiment in a dog, 1.75 minutes after beginning a similar exposure. Mean arterial blood pressure was monitored in the guinea pigs from which the hearts were removed, and the marked hypotensive response to exposure began within a minute of the beginning of the exposure. The evidence clearly fails to support the hypothesis that the cardiovascular dynamic effects are a direct response to myocardial ATP depletion.

The complete significance of the myocardial metabolic derangement has yet to be assessed, but it would be expected to be of some biological significance. The salient features are represented schematically in Figure 2.6.

The observation that the rate of mitochondrial respiration was slowed in the presence of the haloalkanes, was corroborated by electron-micrographic studies of guinea pig hearts exposed to 1301 and perfused in situ (McNutt et al., 1973). The cristal arrangement within the mitochondria of the exposed animals was changed from a condensed to orthodox configuration which was consistent with the phenomenon of slowed respiration. Evidence for hypoxia such as mitochondrial swelling was not detected.

The decrease in the rate of mitochondrial respiration was consistent with the observation by Rhoden and Gabriel (1972) of an activation of myocardial phosphofructokinase (PKF) in rats exposed to 1301. Activation of PKF would be expected to result from a reduced inhibition of this enzyme by ATP and phosphocreatine as they were depleted.

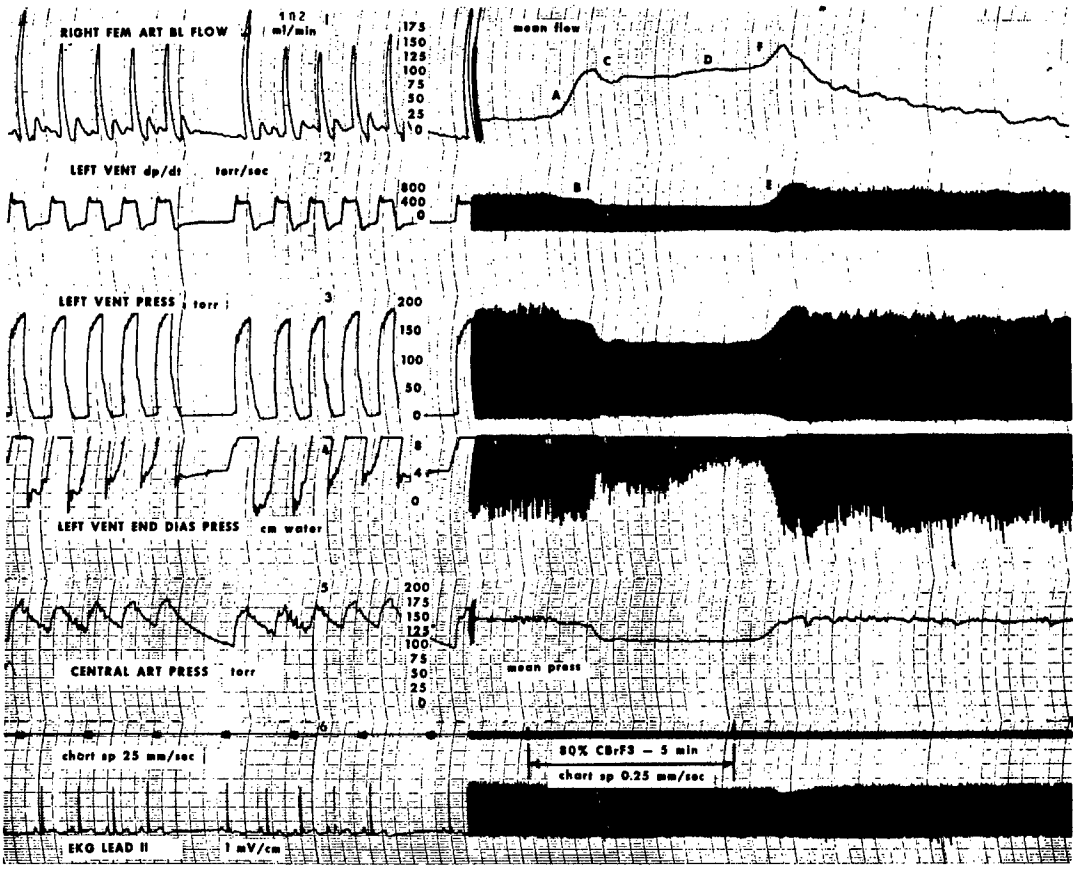

Figure 2.5 A left lumbar sympathectomy was performed on the dog used in this experiment one week prior to experimentation. Exposure of the dog to 80% $CBrF_3$ resulted in a large increase in right femoral arterial blood flow. Flow through the contralateral artery (not shown) was unchanged. Concurrently, mean arterial blood pressure, peak systolic blood pressure and dP/dt_{max} fell, while left ventricular end diastolic pressure rose. The onset of these phenomena was 1.75 min after beginning the exposure.

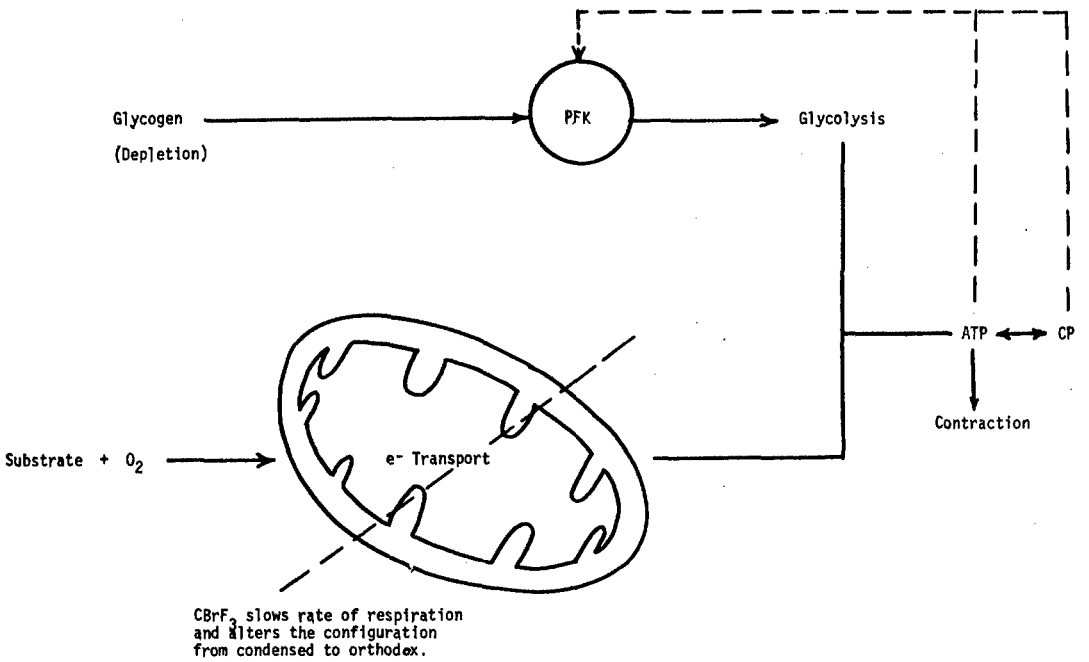

Figure 2.6 Proposed scheme for the alteration of myocardial metabolism during exposure to halogenated alkanes. The rate of mitochondrial respiration falls which results in a decrease of intracellular ATP levels. ATP is in equilibrium with phosphocreatine (CP) which likewise falls as it is dephosphorylated to rephosphorylate ADP. ATP and CP are normal inhibitors of phosphofructokinase (PKF). When intracellular levels of ATP and CP fall, PKF is activated which results in an acceleration of anaerobic metabolism. A net intracellular decrease of the levels of "high-energy" phosphate compounds occurs which contributes to the development of a myocardial performance deficit.

The failure of the detection of an immediate decrease in ATP to account for the decreased myocardial performance led to the study of the effects of the halogenated alkanes on mechanical performance and electrical activity of isolated rabbit hearts perfused with Krebs-Henseleit solution. Preliminary experiments with 1301 and dichlorodifluoromethane, a related compound, indicated that changes in the configuration of the ventricular action potential, conduct velocity, and mechanical activity all were correlated with fluoroalkane level.

The maximal rate of voltage change (dV/dt_{max}) or the slope of phase 0 of the action potential was reduced and the phase 4 plateau abbreviated during exposure to these compounds. The consequences of such alterations of the sarcolemmal electrical activity are represented schematically in Figure 2.7. Myocardial contractility has been demonstrated to vary with dV/dt_{max} and the duration of phase 2 (Miller and Gilmore, 1972; Morad and Trautwein, 1968; Pruett and Woods, 1967). This is thought to be the consequence of a coupling of calcium translocation to the membrane electrical activity (excitation-contraction coupling). An alteration in sarcolemmal electrical activity during haloalkane exposure could provide the functional basis for the negative inotropic action of these compounds. Furthermore, changes in dV/dt_{max} affect the velocity of propagation of the action potential (Rosen and Hoffman, 1973), a phenomenon measured independently during these studies.

The contribution of the changes in electrical activity of the myocardium during exposure to the haloalkanes to the genesis of the cardiac arrhythmias has yet to be fully investigated.

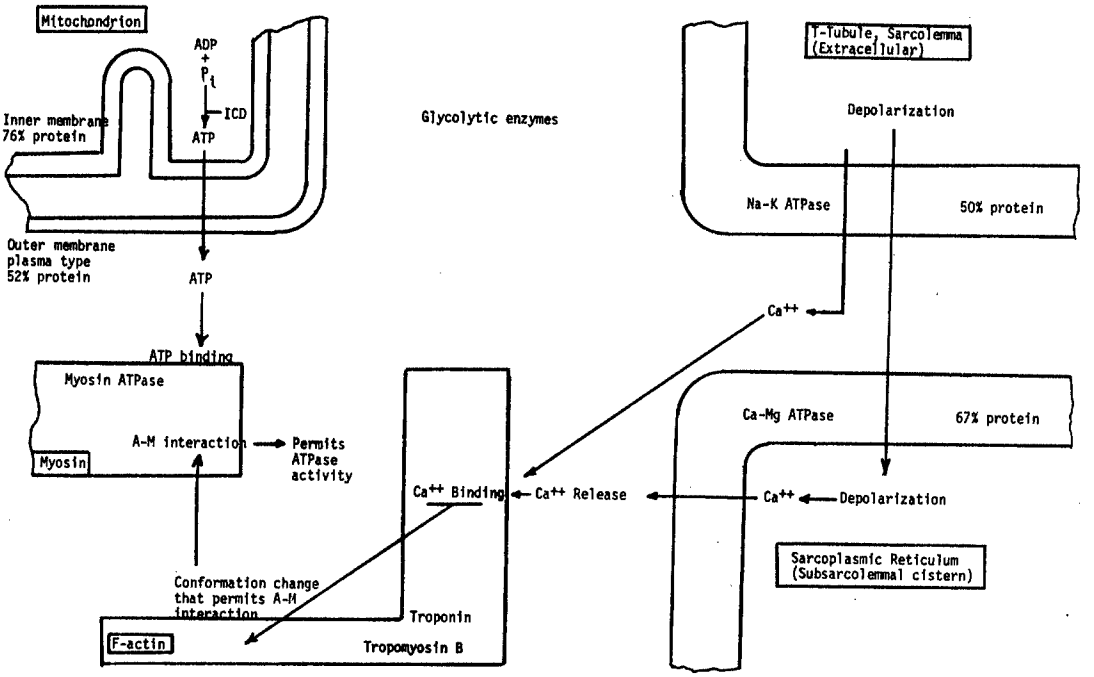

Figure 2.7 A synopsis of myocardial metabolic and electromechanical events leading to the excitation-contraction sequence. Mitochondrial respiration (upper left) maintains ADP phosphorylation at a rate sufficient to meet the demands of the cell's exergonic processes, principally, the actin-myosin interaction (lower left). The actin-myosin interaction is permitted through an alteration of the configuration of the actin component that accompanies binding of intracellular calcium to troponin. The calcium is liberated from sarcolemmal stores and translocated to the contractile machinery as a function of sarcolemma depolarization (upper right). Relaxation accompanies the reuptake of calcium by the sarcoplasmic reticulum and/or the sarcolemma (lower right).

SECTION III

Bromotrifluoromethane[a] (1301), bromochlorodifluoromethane[b] (1211), and bromochloromethane[c] (1011) are vaporizable fire extinguishing agents of interest to the United States Air Force. The requirement for a comparative toxicologic-pharmacologic evaluation of these agents led to a 2-stage program in which the agents were first studied in open-chested anesthetized dogs at concentrations that were progessively increased to levels above those tolerated by conscious dogs (this report). The study is being continued with chronically instrumented, conscious, unrestrained dogs, the results of which will be the subject of a later report.

METHODS

Thirty-four mongrel dogs that weighed over 25 kg each and that represented both sexes were used. Dogs were housed individually and fed a standard laboratory diet[d]. Each dog was examined and determined to be free from obvious diseases and defects.

The dogs were divided into 5 groups. Six dogs served as O_2 controls. Eight dogs were exposed to 27-75% 1301 in O_2. Four dogs were exposed to 27-75% N_2 in O_2. Comparison of the results of the O_2 and N_2 exposures permitted the identification of the variables dependent on inspired PO_2. The PO_2-dependent variables from the 1301 exposures were compared with the corresponding results from the N_2 exposures. The remainder of the 1301 results were compared with the O_2 controls.

a. Bromotrifluoromethane, Freon 1301, E.I. DuPont De Nemours, Inc., Freon Products Div., Wilmington, DE. Analysis: 99% $CBrF_3$, < 0.1% contaminant #1, < 0.1% contaminant #2 (contaminants not identified).
b. Bromochlorodifluoromethane, BCF, ICI America, Inc., Charlotte, NC. Analysis: 99% $CBrClF_2$,<0.1% contaminant #1,<0.1% contaminant #2, < 0.1% contaminant #3 (contaminants not identified).
c. Bromochloromethane, technical grade, Spec. MIL-B-4394C. Analysis: 95% CHBrCl,<3% contaminant #1,<1% contaminant #2,<2% contaminant #3 (contaminants not identified).
d. Purina Dog Meal, Ralston-Purina Co., St. Louis, MO.

Nine dogs were exposed to 4-12% 1211 in O_2 and 7 were exposed to 0.3-1.0% 1011 in O_2. Results were compared with those from the O_2 controls.

The exposures were divided into 5 periods. Pre-exposure measurements were made (I). Dogs were then exposed to the low ranges of the respective compounds for approximately 30 min after which the second set of measurements was made (II). The exposure levels were increased without interruption to the middle and high ranges for approximately 30 min each and the third and fourth sets of measurements were made (III, IV) accordingly. Final measurements were made 30 min postexposure (V).

Results from the exposures were compared with those from the respective O_2 and N_2 controls. The pre- and post-exposure results were compared by 2-way analysis of variance with replication (Sokal and Rohlf, 1969). The test for interaction was the test for a postexposure residual effect of the compounds. Results from periods I-IV were compared by the analysis of covariance (Li, 1964) for the detection of significant treatment effects. The variances of some of the groups of data were unequal (Box's modification of Bartlett's test, Li, 1963) and so the groups were compared using the non-parametric Mann-Whitney-Wilcoxon test (Natrella, 1963).

Surgical Preparation and Instrumentation

Dogs were given morphine, 4 mg/kg, and a vagolytic dose of atropine sulfate (0.2 mg/kg) subcutaneously. Basal anesthesia was provided by the IV injection of α-chloralose (70 mg/kg) in polyethylene glycol 200.

Dogs were ventilated mechanically[e]. Respiratory rate and tidal volume were adjusted according to individual requirements to maintain the mean arterial pH at 7.34-7.43, PCO_2 at 27-35 torr, and PO_2 > 70 torr.

e. Respiration pump, Model 614, Harvard Apparatus Co., Dover, MA.

Thoracotomy was performed on the left side at the 5th intercostal space. Left ventricular pressure (LVP) was recorded using a catheter-tip pressure transducer[f] inserted via the left atrial appendage. Arterial pressure was recorded through a 13 ga stainless steel cannula inserted into a brachial artery and connected to a strain-gage pressure transducer[g]. All recordings were performed on an oscillographic physiological recorder[h].

The dP/dt was derived electronically and dP/dt max divided by developed pressure was determined by a method described previously (Van Stee et al., 1973).

Flow through the left coronary circumflex artery was monitored using an electromagnetic flowmeter[i] with a 2-3 mm probe as required. Venous blood was obtained through a catheter placed into the vessel wall just proximal to the ostium of the coronary sinus.

Blood lactate and pyruvate levels were determined according to the method of Marbach and Weil (1967)[j] and glucose by a hexokinase method[k]. Non-esterified fatty acids (NEFA) were determined by a method using thymolphthalein indicator (Tietz, 1970).

Blood O_2 and CO_2 partial pressures and pH were determined using a blood gas analyzer[l]. Blood gases were corrected for hemoglobin concentration and body temperature according to equations derived from a large beagle colony by Pickrell and Schluter (1973). An electronic calculator[m] was programmed for all numerical data processing.

f. Model P866-01, Statham Instrument Co., Oxnard, CA.
g. Model P23Db, Statham Instrument Co., Oxnard, CA.
h. Model DR-12, Electronics for Medicine, White Plains, NY.
i. Model SP7518, Statham Instrument Co., Oxnard, CA.
j. Technical Bull. 726-UV and 826-UV, Sigma Chemical Co., St. Louis, MO.
k. Glucose Stat-Pack, Calbiochem, LaJolla, CA.
l. BMS 3 Mk 2 and PHM 72 Mk 2, Radiometer, Copenhagen, Denmark.
m. Model 9810A, Hewlett-Packard, Cupertino, CA.

At the conclusion of each experiment India ink was injected into the coronary circulation at the site of placement of the electromagnetic flow probe. The unstained tissue was dissected away and the remaining, stained tissue weighed as representative of the perfused myocardial mass.

The complete electronic calculator program required the following inputs: blood hemoglobin concentration, ventricular weight (perfused myocardial mass), body temperature at the time of blood sampling, arterial and venous PO_2, PCO_2, pH and mean, left coronary circumflex arterial blood flow at the time of sampling. The program output provided the following: arterial and venous PO_2 and PCO_2 corrected for hemoglobin concentration and body temperature, arterial and venous O_2 content, bicarbonate concentration and "base excess," (Anderson 1962) and myocardial oxygen extraction.

The halogenated alkanes were diluted with O_2 and the exposures were carried out by introducing the appropriate gas mixtures into the respirator inlet as described earlier (Van Stee & Back, 1972). Gas mixtures and compound purity were analyzed by gas chromatography.

RESULTS

A summary of the results is given in Table 3.1. The levels of halogenated alkanes to which the animals were exposed are given in the first row of the table. The results of the N_2 exposures were not included in the table, but were compared with the results of the 6 O_2 experiments by the analysis of covariance. Only arterial PO_2 and O_2 content were found to differ significantly because of the differences in inspired PO_2. Arterial PO_2 and O_2 content during the 1301 exposures were, therefore, compared with the N_2 exposures. All other variables for all other exposures were compared with the O_2 controls.

TABLE 3.1

Summary of Myocardial Metabolic and Cardiovascular Dynamic Studies
of Dogs Exposed to Haloalkanes by Inhalation

Determination	Compound	Period I	Period II	Period III	Period IV	Period V	Sig.[a] I vs V	Sig.[b] I - IV
Haloalkane Concentration, %	O_2	0	0	0	0	0		
	1301	0	27.0±2.3	51.6±2.2	74.6±2.9	0		
	1211	0	4.33±.43	8.03±.58	12.31±.76	0		
	1011	0	.29±.14	.67±.19	.98±.28	0		
Arterial PO_2, torr	O_2	251±156	219±164	164±100	225±118	233±137	-	-
	1301[d]	262±149	139±80	98±29	71±23	313±146	NS	NS
	1211	333±133	213±111	320±163	386±127	325±154	NS	NS
	1011	261±107	234±83	332±117	337±104	339±116	NS	NS
Venous PO_2,[c] torr	O_2	28±6	29±7	29±8	29±6	31±5	-	-
	1301[d]	30±9	30±9	31±9	26±5	33±11	NS	NS
	1211	30±16	35±18	45±20	68±32	69±38	$p<.05$	$p<.005$
	1011	29±3	32±6	49±11	61±23	42±8	$p<.05$	$p<.03$
Arterial O_2 Content, ml/l	O_2	301±28	299±35	284±35	302±55	302±52	-	-
	1301	257±56	216±33	205±28	183±32	286±57	NS	NS
	1211	330±42	285±43	311±46	333±38	320±55	NS	NS
	1011	290±71	299±54	354±57	336±87	348±59	NS	NS
Venous O_2 Content, ml/l	O_2	101±32	93±29	97±42	97±34	110±34	-	-
	1301	98±44	93±40	97±42	82±34	107±54	NS	NS
	1211	113±69	124±54	163±58	198±46	197±55	$p<.05$	$p<.01$
	1011	99±47	116±43	172±66	201±85	151±65	NS	$p<.03$

TABLE 3.1 (continued)

Summary of Myocardial Metabolic and Cardiovascular Dynamic Studies
of Dogs Exposed to Haloalkanes by Inhalation

Determination	Compound	Period I	Period II	Period III	Period IV	Period V	Sig.[a] I vs V	Sig.[b] I - IV
EO_2[e] µl/min/g	O_2	126±48	125±54	102±47	106±46	105±54	-	-
	1301	116±48	96±42	78±28	71±37	124±45	NS	NS
	1211	188±77	130±71	106±70	91±66	96±55	NS	NS
	1011	95±34	87±25	70±18	67±31	78±30	NS	NS
Arterial PCO_2, torr	O_2	29±7	31±8	29±7	28±11	27±10	-	-
	1301	29±7	31±6	32±6	29±5	31±4	NS	NS
	1211	31±9	29±9	27±8	24±4	28±8	NS	NS
	1011	35±6	33±7	33±5	33±6	32±6	NS	NS
Venous PCO_2, torr	O_2	38±14	45±6	41±8	40±8	44±12	-	-
	1301	40±7	41±7	42±8	38±8	44±7	NS	NS
	1211	40±11	40±10	37±10	36±9	38±10	NS	NS
	1011	51±8	49±8	54±6	54±8	55±5	NS	NS
Arterial $[HCO_3^-]$ mEq/l	O_2	18.2±4.9	18.6±4.8	17.9±6.3	17.8±8.9	16.1±7.9	-	-
	1301	17.6±2.8	18.2±2.4	17.5±1.7	16.6±2.8	16.5±2.3	NS	NS
	1211	19.2±4.3	17.5±4.9	17.6±4.3	15.0±3.4	16.6±3.4	NS	NS
	1011	22.4±4.2	20.8±3.8	20.3±4.1	18.8±4.2	19.0±5.6	NS	NS

TABLE 3.1 (continued)

Summary of Myocardial Metabolic and Cardiovascular Dynamic Studies
of Dogs Exposed to Haloalkanes by Inhalation

Determination	Compound	Period I	Period II	Period III	Period IV	Period V	Sig.[a] I vs V	Sig.[b] I - IV
Venous [HCO_3^-] mEq/l	O_2	23.1±9.3	24.2±3.9	22.6±6.8	21.8±6.7	22.1±8.2	-	-
	1301	21.9±2.7	22.1±2.9	21.4±3.0	20.2±4.2	20.8±3.3	NS	NS
	1211	23.2±5.3	22.7±5.4	22.0±5.3	21.1±5.1	21.2±5.1	NS	NS
	1011	28.9±4.9	26.8±4.6	27.1±4.9	26.0±6.1	25.9±5.6	NS	NS
Arterial BE[f] mEq/l	O_2	-4.14±4.90	-4.28±3.10	-4.75±6.47	-4.78±9.14	-2.25±10.91	-	-
	1301	-4.96±2.05	-5.00±2.37	-6.34±2.61	-6.55±4.05	-7.50±3.66	NS	NS
	1211	-0.30±3.66	-5.00±4.68	-4.11±3.76	-6.77±5.21	-5.79±3.44	NS	NS
	1011	-0.42±5.05	-2.05±4.41	-2.70±4.94	-4.01±4.97	-4.17±6.23	NS	NS
Venous BE mEq/l	O_2	-1.26±8.86	-1.51±4.35	-2.68±6.88	-3.38±7.60	-4.00±8.68	-	-
	1301	-2.80±1.77	-3.45±1.33	-3.98±3.29	-5.50±3.51	-5.39±3.48	NS	NS
	1211	-1.06±4.08	-1.89±4.67	-1.65±4.4	-2.42±4.42	-3.00±4.35	NS	NS
	1011	1.72±3.24	0.81±4.82	-1.04±4.49	-2.37±5.58	-2.56±3.35	NS	NS
Arterial pH	O_2	7.41±.06	7.39±.08	7.39±.08	7.39±.10	7.36±.10	-	-
	1301	7.41±.06	7.39±.06	7.36±.08	7.38±.09	7.34±.08	NS	NS
	1211	7.43±.07	7.40±.08	7.43±.05	7.41±.11	7.40±.07	NS	NS
	1011	7.42±.09	7.41±.09	7.40±.09	7.36±.09	7.38±.09	NS	NS
Venous pH	O_2	7.38±.08	7.34±.06	7.34±.08	7.34±.10	7.31±.12	-	-
	1301	7.36±.04	7.35±.04	7.33±.07	7.34±.09	7.30±.07	NS	NS
	1211	7.39±.04	7.37±.04	7.40±.04	7.40±.05	7.37±.04	NS	NS
	1011	7.36±.08	7.35±.06	7.31±.07	7.29±.08	7.28±.07	NS	NS

TABLE 3.1 (continued)

Summary of Myocardial Metabolic and Cardiovascular Dynamic Studies
of Dogs Exposed to Haloalkanes by Inhalation

Determination	Compound	Period I	Period II	Period III	Period IV	Period V	Sig.[a] I vs V	Sig.[b] I - IV
Arterial Glucose	O_2	6.37±1.05	6.18±.71	6.08±1.29	6.56±1.25	6.36±.98	-	-
mM/l	1301	6.13±.35	6.39±.33	6.80±.81	7.81±1.64	8.26±1.52	p<.05	p<.05
	1211	6.06±.41	5.71±.61	5.54±.58	5.76±.27	6.74±.32	NS	NS
	1011	6.13±.66	6.26±.47	6.43±.76	6.59±.96	5.92±.72	NS	NS
Venous Glucose	O_2	5.75±1.13	5.64±.78	5.83±1.11	5.70±1.25	5.88±1.07	-	-
mM/l	1301	5.71±.37	5.63±1.14	6.65±.85	7.41±1.71	7.93±1.63	p<.05	p<.05
	1211	5.54±.47	5.44±.61	5.25±.49	5.41±.27	6.43±.27	NS	NS
	1011	5.49±.93	5.53±.54	5.12±1.72	5.47±.86	5.35±.67	NS	NS
E Glucose	O_2	.416±.374	.315±.234	.203±.176	.425±.429	.247±.266	-	-
μM/min/g	1301	.309±.289	.411±.380	.311±.244	.266±.258	.216±.187	NS	NS
	1211	.425±.271	.199±.252	.181±.238	.194±.129	.220±.153	NS	NS
	1011	.295±.166	.342±.332	.582±.950	.453±.410	.238±.168	NS	NS
Arterial Lactate	O_2	1.62±.73	1.66±.77	1.91±.91	2.46±1.61	2.44±1.66	-	-
mM/l	1301	3.14±1.45	2.58±1.24	2.77±.97	3.89±1.86	3.37±1.28	NS	NS
	1211	1.69±.75	1.58±.49	1.56±.38	1.67±.63	1.90±.52	NS	NS
	1011	1.59±.54	1.67±.66	1.67±.66	1.79±.69	1.85±.62	NS	NS
Venous Lactate	O_2	0.87±.38	0.97±.43	1.16±.70	1.47±1.07	1.54±1.11	-	-
mM/l	1301	2.04±1.51	1.89±1.02	1.97±.77	3.19±1.88	2.38±1.18	NS	NS
	1211	0.92±.46	0.92±.38	1.00±.44	1.16±.40	1.25±.33	NS	NS
	1011	0.80±.24	0.78±.24	0.84±.30	0.87±.25	0.91±.20	NS	NS

TABLE 3.1 (continued)

Summary of Myocardial Metabolic and Cardiovascular Dynamic Studies
of Dogs Exposed to Haloalkanes by Inhalation

Determination	Compound	Period I	Period II	Period III	Period IV	Period V	Sig.[a] I vs V	Sig.[b] I - IV
E Lactate	O_2	.510 +.278	.474+.322	.482+.362	.649+.499	.510+.367	-	-
µM/min/kg	1301	.721+.587	.760+.605	.599+.410	.485+.311	.738+.438	NS	NS
	1211	.681+.358	.480+.279	.349+.178	.294+.178	.467+.287	NS	p<.05
	1011	.374+.185	.363+.159	.350+.149	.322+.212	.358+.145	NS	NS
Arterial Pyruvate	O_2	49+55	102+35	121+40	145+42	138+37	-	-
µM/l	1301	170+56	164+58	179+44	221+70	216+64	NS	NS
	1211	97+38	96+27	102+24	99+27	108+30	NS	p<.05
	1011	83+36	78+34	88+34	92+33	108+33	NS	NS
Venous Pyruvate	O_2	38+22	42+21	79+52	94+75	70+43	-	-
µM/l	1301	133+67	133+63	143+50	182+75	187+107	NS	NS
	1211	49+24	49+19	55+24	63+17	66+18	NS	NS
	1011	36+15	36+13	39+20	37+11	48+17	NS	p<.03
E Pyruvate	O_2	38+18	36+16	29+35	27+21	32+16	-	-
nM/l	1301	38+34	38+30	39+18	35+30	34+27	NS	NS
	1211	41+24	35+19	30+14	21+12	30+22	NS	NS
	1011	22+13	20+10	22+12	22+11	24+11	NS	NS
Arterial L/P[g]	O_2	16.3+3.2	15.6+2.9	15.5+4.6	17.7+10.7	17.1+9.5	-	-
	1301	18.4+6.8	15.5+3.5	16.2+7.5	18.8+11.1	16.3+6.2	NS	NS
	1211	17.4+3.4	16.5+2.7	15.2+3.1	16.8+3.2	18.1+3.1	NS	NS
	1011	21.3+7.4	21.0+5.1	19.8+5.3	20.0+4.5	17.6+4.1	NS	NS

TABLE 3.1 (continued)

Summary of Myocardial Metabolic and Cardiovascular Dynamic Studies
of Dogs Exposed to Haloalkanes by Inhalation

Determination	Compound	Period I	Period II	Period III	Period IV	Period V	Sig.[a] I vs V	Sig.[b] I - IV
Venous L/P	O_2	22.5±4.3	23.8±2.7	20.6±6.7	23.1±1.8	20.7±3.0	-	-
	1301	14.4±5.1	13.9±3.0	13.9±3.5	17.5±8.6	14.7±6.5	NS	NS
	1211	19.4±5.1	19.2±4.5	19.2±4.6	18.7±4.9	19.1±4.6	NS	NS
	1011	23.2±4.7	22.7±5.7	23.2±6.2	24.6±7.5	18.1±1.9	NS	NS
Arterial NEFA[h] mM/l	O_2	.558±.203	.681±.285	.706±.276	.630±.250	.628±.288	-	-
	1301	.460±.090	.529±.302	.651±.418	.613±.338	.485±.274	NS	NS
	1211	.506±.253	.553±.335	.555±.304	.509±.195	.785±.405	NS	NS
	1011	.379±.190	.518±.393	.390±.160	.517±.291	.497±.253	NS	NS
Venous NEFA mM/l	O_2	.305±.082	.269±.096	.355±.132	.351±.170	.332±.097	-	-
	1301	.356±.106	.411±.211	.499±.271	.425±.161	.330±.173	NS	NS
	1211	.285±.107	.300±.181	.346±.165	.312±.075	.485±.346	NS	NS
	1011	.186±.064	.191±.108	.157±.065	.193±.055	.200±.077	NS	NS
E NEFA nM/min/g	O_2	161±73	213±138	180±99	134±63	127±67	-	-
	1301	77±54	82±113	115±122	143±160	106±99	NS	NS
	1211	200±164	212±175	171±165	134±112	238±134	NS	NS
	1011	82±59	149±137	78±54	137±123	116±94	NS	NS
Mean Arterial Pressure, torr	O_2	119±12	115±15	107±20	105±30	102±29	-	-
	1301	100±13	100±20	103±22	82±26	94±31	NS	NS
	1211	125±27	109±24	98±26	85±26	116±28	NS	NS
	1011	111±20	119±13	125±14	123±17	113±13	NS	NS

TABLE 3.1 (continued)

Summary of Myocardial Metabolic and Cardiovascular Dynamic Studies
of Dogs Exposed to Haloalkanes by Inhalation

Determination	Compound	Period I	Period II	Period III	Period IV	Period V	Sig.[a] I vs V	Sig.[b] I - IV
Cardiac Output ml/min/g	O_2	38.9±16.4	38.3±12.4	36.1±7.3	30.5±6.0	29.1±7.8	-	-
	1301	38.2±14.6	39.0±15.4	42.4±17.3	39.7±22.9	41.3±24.0	NS	NS
	1211	60.1±24.3	53.3±22.1	47.5±22.8	34.2±14.6	45.2±26.7	NS	NS
	1011	34.5±13.3	31.5±8.5	30.5±10.3	27.1±6.5	27.4±10.4	NS	NS
Mean Transit Time, s	O_2	6±1	7±2	8±2	8±2	9±2	-	-
	1301	7±2	8±2	7±1	8±2	8±2	NS	NS
	1211	8±2	9±2	10±3	13±4	9±3	NS	p<.05
	1011	7±2	8±2	9±1	10±1	11±2	NS	NS
Central Venous Volume, l	O_2	.194±.074	.207±.061	.233±.072	.203±.069	.212±.078	-	-
	1301	.213±.068	.222±.067	.245±.084	.246±.130	.248±.114	NS	NS
	1211	.317±.108	.326±.111	.322±.113	.305±.111	.282±.100	NS	NS
	1011	.219±.068	.218±.065	.227±.072	.244±.080	.280±.150	NS	NS
Total Peripheral Resistance, PR units	O_2	60±34	67±20	61±21	68±17	72±21	-	-
	1301	58±19	57±26	54±23	51±28	54±23	NS	NS
	1211	54±21	54±23	55±22	64±27	71±30	NS	NS
	1011	65±19	78±28	85±30	91±30	88±34	NS	NS
Heart Rate beats/min	O_2	188±28	197±37	195±37	188±45	190±37	-	-
	1301	178±25	177±25	175±32	175±53	171±39	NS	NS
	1211	212±30	212±48	190±45	181±37	207±45	NS	NS
	1011	197±53	198±49	183±45	159±33	165±48	NS	NS

TABLE 3.1 (continued)

Summary of Myocardial Metabolic and Cardiovascular Dynamic Studies of Dogs Exposed to Haloalkanes by Inhalation

Determination	Compound	Period I	Period II	Period III	Period IV	Period V	Sig.[a] I vs V	Sig.[b] I - IV
Stroke Volume, ml	O_2	10.3±3.6	9.6±3.1	9.6±2.6	8.6±2.7	8.2±3.2	-	-
	1301	11.7±5.9	12.1±6.5	13.6±7.9	10.9±9.6	14.4±11.3	NS	NS
	1211	12.6±5.3	11.3±4.7	11.4±6.1	8.4±3.6	9.9±5.4	NS	NS
	1011	9.9±4.5	9.1±3.9	9.2±3.4	9.6±3.5	9.8±5.4	NS	NS
Stroke Work, g-m/g	O_2	.335±.121	.296±.092	.273±.086	.246±.106	.221±.092	-	-
	1301	.308±.148	.317±.172	.379±.239	.337±.342	.403±.382	NS	NS
	1211	.520±.314	.404±.264	.368±.237	.234±.149	.381±.253	NS	NS
	1011	.300±.162	.280±.33	.299±.128	.291±.099	.281±.157	NS	NS
dP/dt_{max} torr/s	O_2	2470±420	2530±320	2390±330	2120±480	2050±400	-	-
	1301	2223±305	2310±470	2269±486	1721±389	2254±434	NS	NS
	1211	2933±750	2306±433	1828±365	1439±355	2606±505	NS	p<.01
	1011	2500±700	2290±420	2160±290	1950±270	1800±180	NS	NS
$dp/dt_{max} \div P$ sec^{-1}	O_2	29±6	31±7	29±4	28±5	27±5	-	-
	1301	31±5	32±6	31±7	28±6	33±11	NS	NS
	1211	30±11	31±13	28±8	28±11	33±10	NS	NS
	1011	28±5	26±3	26±3	24±2	24±3	NS	p<.01
L. Cor. Circ $\dot{Q}^i$ ml/min/g	O_2	.63±.19	.59±.18	.55±.19	.52±.19	.49±.18	-	-
	1301	.74±.22	.78±.25	.71±.18	.68±.20	.69±.18	NS	NS
	1211	.86±.16	.77±.29	.68±.27	.62±.26	.78±.27	NS	p<.05
	1011	.47±.09	.50±.12	.45±.14	.43±.11	.40±.10	NS	NS

a. One-way analysis of variance
b. Analysis of covariance
c. Coronary sinus blood
d. Compared with N_2 controls
e. Myocardial extraction
f. Base excess
g. Lactate/Pyruvate
h. Nonesterified fatty acids
i. Left coronary circumflex arterial blood flow

Seven dogs were in the control (O_2) group, 8 in the 130I group, 9 in the 121I group, and 7 in the 101I group.

Exposure to 1211 or 1011 resulted in significant increases in coronary venous PO_2 and O_2 content. These elevations persisted for at least 30 min postexposure after 1211, but not after 1011.

No significant differences among the acid-base variables were detected for any of the compounds.

Exposure to 1301 resulted in significant elevations of arterial and coronary venous glucose levels which persisted for at least 30 min postexposure.

Lactate extraction by the myocardium and arterial pyruvate decreased significantly during exposure to 1211 and venous pyruvate decreased significantly during exposure to 1011. None of these changes persisted postexposure.

Mean transit time was prolonged significantly during exposure to 1211. The dP/dt max and left coronary circumflex arterial blood flow were decreased significantly during exposure to 1211 and the dP/dt max divided by developed pressure was decreased significantly during exposure to 1011.

The triple product of heart rate times mean arterial blood pressure times left ventricular dP/dt max was used as an index of left ventricular myocardial oxygen demand. Mean oxygen extraction and mean left coronary circumflex arterial blood flow were adjusted for differences in oxygen demand (triple product) and the oxygen exposed group was compared with the respective halogenated alkane exposed groups by means of the analysis of covariance. A summary of these results is presented in Table 3.2.

TABLE 3.2

THE RESPONSES OF LEFT VENTRICULAR MYOCARDIAL OXYGEN EXTRACTION AND LEFT CORONARY CIRCUMFLEX ARTERIAL BLOOD FLOW TO MYOCARDIAL OXYGEN DEMAND AS AFFECTED BY $CBrF_3$, $CBrClF_2$, AND CH_2BrCl

	Adjusted Mean ± SE				Sig Elev Cor Ven O_2?[c]
	EO_2[a]		Cor $\dot{Q}$[b]		
$CBrF_3$ (1301)	(C)[d] 113 ± 8.4	NS[f]	(C) .56 ± .04	p<.01	No
	(E)[e] 92 ± 8.4		(E) .72 ± .04		
$CBrClF_2$ (1211)	(C) 118 ± 13	NS	(C) .59 ± .05	p<.05	Yes
	(E) 126 ± 11		(E) .72 ± .04		
CH_2BrCl (1011)	(C) 114 ± 7.6	p<.01	(C) .57 ± .03	p<.05	Yes
	(E) 80 ± 7.5		(E) .46 ± .03		

a. Left Ventricular Myocardial Oxygen Extraction, µl/min/g
b. Left Coronary Circumflex Arterial Blood Flow, ml/min/g of Left Ventricular Myocardium
c. Was the coronary sinus blood oxygen content elevated significantly during exposure? See Table 1.
d. Oxygen Control Group
e. Halogenated Alkane Exposed Group
f. Difference between adjusted means was not significant.

DISCUSSION

The most significant observation was the elevation of the coronary sinus blood PO_2 and O_2 content during exposure to 1211 or 1011. Both of these variables increased progressively during the exposures. The persistence of the effect postexposure was greater for 1211 than for 1011.

Functional relationships are known to exist among coronary flow rate and perfusion pressure, the vigor of myocardial contraction, and myocardial oxygen demand and consumption (Dempsey and Cooper, 1972; Koyama and Nakagawa, 1972; Templeton et al., 1972; Fisher et al., 1969; Arnold et al., 1968; Opie, 1968). The precise causal sequences are not completely understood. In summary of what is usually accepted as physiological performance of normal systems, myocardial oxygen extraction is very nearly maximal which provides little cardiac reserve through this mechanism. A variable myocardial oxygen demand is met by corresponding variations in coronary flow rate and thus delivery of oxygen to the myocardium. That this relationship was somehow disturbed during exposure to 1211 or 1011 was reflected in the elevation of the coronary venous blood oxygen content.

The relationship among myocardial oxygen demand (heart rate x mean arterial blood pressure x left ventricular dP/dt max), myocardial oxygen extraction (EO_2) and coronary arterial blood flow as it is affected by the respective haloalkanes was illustrated in Table 3.2.

EO_2 followed demand during exposures to $CBrF_2$ and $CBrClF_2$. The same two variables, on the other hand, were apparently significantly dissociated during the CH_2BrCl exposures. This could have been the consequence of a slowing of mitochondrial respiration. All three haloalkanes were found to slow state 3 respiration without evidence of uncoupling in isolated rat

liver mitochondria (Van Stee et al., 1974). The effectiveness of the compounds was $CBrF_3$ < $CBrClF_2$ < CH_2BrCl. In the absence of measurements of mitochondrial levels of the compounds during the in vivo and in vitro experiments only limited inferences may be made concerning the treatment-response relationship. The absence of the measurable O_2 demand-EO_2 dissociation during exposure to $CBrF_3$ or $CBrClF_2$ may only have reflected a failure to achieve mitochondrial levels of the compounds sufficient to affect respiration significantly.

Coronary flow was higher than controls for any given oxygen demand during $CBrF_3$ exposure. This was attributable to the mild hypoxemia that accompanied exposure of open-chested dogs to the relatively high levels of the compounds that displaced a significant amount of oxygen from the inspired gas mixture. The failure of $CBrF_3$ to dissociate O_2 demand and EO_2, and the normal response of coronary flow to mild hypoxemia during the exposure, support the conclusion that $CBrF_3$ had no significant effect on the myocardial O_2 demand-EO_2-coronary flow relationship in these experiments.

Coronary flow was somewhat higher than controls for any given oxygen demand during $CBrClF_2$ exposure. The element of hypoxia was not present in these experiments, however. Therefore, a coronary vasodilation was implied which may have explained the elevated coronary sinus blood oxygen level. Arterial hyperoxemia has been shown normally not to increase coronary venous PO_2 significantly in conscious, unmedicated, chronically instrumented dogs (Van Stee, unpublished observations).

Coronary flow was somewhat lower than controls for any given O_2 demand during CH_2BrCl exposure. EO_2 was profoundly lower, however. These results implied that this compound, in contrast to $CBrClF_2$, caused little or no coronary vasodilation. The elevated coronary sinus oxygen was apparently the consequence of a marked reduction of EO_2.

In summary, $CBrF_3$ did not affect significantly the responses of either EO_2 or coronary arterial blood flow to oxygen demand. Neither was the coronary sinus blood oxygen level elevated. $CBrClF_2$, on the other hand, elevated coronary flow which resulted in a delivery of oxygen to the myocardium in excess of demand. The excess appeared in the coronary sinus effluent. CH_2BrCl impaired oxygen extraction in response to demand without markedly affecting the coronary flow response which resulted in an elevation of coronary sinus blood oxygen.

The metabolic basis for the disturbance in myocardial oxygen utilization has been suggested to reside at the mitochondrial level. A correlate at the ultrastructural level was provided by McNutt et al. (1973) who exposed intact, free-roaming guinea pigs to 1301 followed by in situ perfusion and fixation of the heart while the animals were under anesthesia in the 1301-O_2 exposure gas mixture. The mitochondrial cristal configuration was observed to be changed from the energized to the orthodox configuration without evidence of hypoxia such as mitochondrial swelling. The cristal configuration change was consistent with a depression of mitochondrial metabolism.

Whereas mitochondrial changes have been reported in response to 1301 exposure, significant pathophysiological correlates were not detected in this study. This reflected a combination of a relative insensitivity of the in vivo methodology and the ability of the intact organism to undergo compensatory adaptive change to the relatively mild insult of 1301 exposure.

The cardiovascular and hemodynamic variables monitored during these exposures did not change as dramatically as had been reported earlier (Van Stee and Back, 1969, 1972; Van Stee et al., 1973). Whereas total peripheral resistance and mean arterial blood pressure fell markedly during the first several minutes of exposure, they tended to rise with prolonged exposure. A similar phenomenon has been observed in the dog during prolonged halothane and halothane-N_2O anesthesia (Staffey et al., 1974). Apparently circulatory adjustments (adaptation) tending to raise blood pressure take place during prolonged exposure to pharmacologically non-specific hemodynamic depressants. Such changes could result from vascular smooth and cardiac muscle plasticity alterations that would cause changes in both vascular compliance and myocardial performance (Blinks and Koch-Weser, 1963).

The 1301 exposures, but not the 1211 or 1011 exposures, resulted in a progressive rise of plasma glucose levels that persisted for at least 30 min postexposure. Halothane, also a halogenated alkane, has been demonstrated to inhibit glucose-stimulated insulin secretion from isolated rat pancreas (Gingerich et al., 1974). Why 2 halogenated alkanes (halothane and 1301) should have a diabetogenic action while the others do not is unknown. Indeed, the endocrinological basis for the 1301 action has not been demonstrated and, therefore, cannot be assumed to be similar to that of halothane at this time.

In summary, exposure to 1301, 1211, or 1011 may result in disturbances of myocardial energy metabolism that are connected to myocardial performance. Based on the concentration to which dogs must be exposed to elicit such responses, 1301 was least effective and 1011 was most effective. The 1211 was intermediate between the 2 but closer to the 1011 than to the 1301.

SECTION IV

CONCLUSIONS AND RECOMMENDATIONS

Six factors have been selected for the purpose of comparing the toxicity of 1301, 1211, and 1011. The factors are identified in Table 4.1. Table 4.1 should be read horizontally. The numbers in the body of the table represent percentages of the respective halogenated alkanes in oxygen to which experimental animals were exposed that elicited approximately equivalent responses. The data of Engibous and Torkelson are presented but they are not directly comparable since the diluent for the compounds was air rather than oxygen. This introduced the variable of hypoxia into their design, a variable that was scrupulously controlled in the studies conducted in AMRL.

Acute vapor toxicity. The general considerations of acute vapor toxicity in the Engibous & Torkelson study were discussed on page 51 (WADC TR 59-643). The numbers in Table 4.1 were extracted from Figures 21 and 22 of the Engibous & Torkelson paper. They represent approximate concentrations of the respective compounds to which rats and guinea pigs were exposed causing essentially no mortality. The duration of the exposures considered for this comparison was about midrange (one hour). The numbers would be slightly higher for durations of exposure less than one hour and slightly lower for durations greater than one hour. Since the slopes of the lines in their Figures 21 and 22 were approximately equal the relationship among the 3 compounds remained essentially unchanged over the range of duration of exposure in this study.

The exposures in the Engibous & Torkelson study were conducted on conscious animals. This factor, in addition to the diluent factor (air vs oxygen), provided the basis for the differences observed between their acute toxicity evaluations and those from AMRL.

TABLE 4.1

A COMPARISON OF PERCENTAGES OF HALOGENATED ALKANES REQUIRED TO ELICIT APPROXIMATELY EQUIVALENT RESPONSES FROM EXPERIMENTAL ANIMALS

	1301	1211	1011
Acute Vapor Toxicity (Engibous & Torkelson, 1960)[1]	60[2]	20	2
Acute Vapor Toxicity (AMRL/THT)	80[3]	15	1
Coronary Sinus Blood Oxygen Content Elevation (AMRL/THT)	--[4]	15.5	1
Myocardial Contractility (AMRL/THT)	57.9	5.79	.68
Blood Pressure (AMRL/THT)	57.9	9.6	--[4]
Coronary Blood Flow (AMRL/THT)	62.2	7.0	.51
Cardiac Arrhythmias (AMRL/THT)	80	14	.7

1. References to sources of data in parentheses.
2. Percentage of compounds in air (Engibous & Torkelson only).
3. Other numbers in body of table are percentage of compound in oxygen.
4. Compound without measurable effect on this variable.

The evaluation of acute vapor toxicity was performed according to different criteria in the AMRL studies as compared with those of Engibous & Torkelson. The criteria in the AMRL study embodied a sum of the physiological effects of the respective compounds that was somewhat subjectively determined and rather less accurately quantified. Many of the experiments were performed on anesthetized dogs and monkeys, most of which were ventilated mechanically, a procedure that eliminated the possibility of respiratory failure. The numbers represent concentrations of the halogenated alkanes to which the animals were exposed for 10-30 min from which they would recover completely with no residual effects within a period of 5-15 minutes. Exposure to concentrations much in excess of these led to irreversible cardiovascular depression with either a failure of recovery altogether or only a partial recovery characterized by a severe residual cardiovascular dynamic deficit.

Coronary sinus blood oxygen content elevation. Exposure to 1211 and 1011 caused a concentration-dependent increase in the oxygen content of the coronary venous blood. This appeared to be a direct consequence of the inhibition of mitochondrial respiration by the compounds. Studies of isolated mitochondria in vitro revealed that all three compounds slowed the rate of mitochondrial respiration in the following order of effectiveness: 1301 < 1211 < 1011. $CBrF_3$ (1301) was the least effective in this regard which probably accounted for the failure to detect an increase in coronary sinus blood oxygen in the intact animals. It is also possible that the concentration of 1301 that the mitochondria required to slow respiration to a biologically significant degree was not achieved in vivo. Figure 4.1 is a graph of the relationship between the log of the coronary venous oxygen content versus the log of the halogenated alkane concentration. From this graph it may be seen that 1211 and 1011 were highly effective whereas 1301 and the oxygen control were ineffective. The decrease in coronary venous

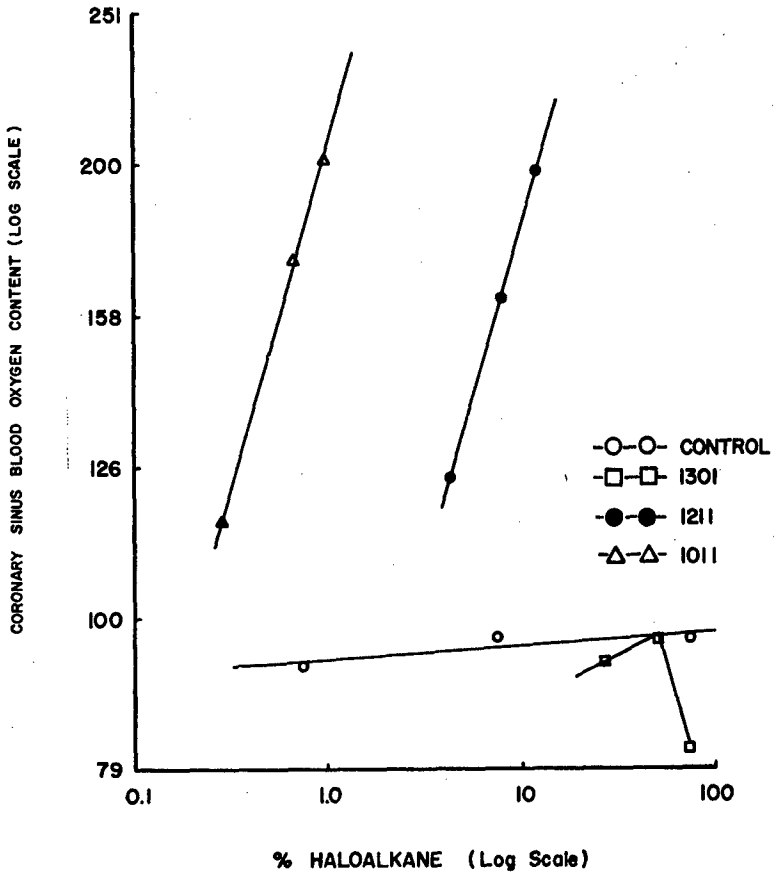

Figure 4.1 The logarithm of the coronary sinus blood oxygen content versus the logarithm of the concentration of the haloalkane to which the animals were exposed. Each point represents the mean of several experiments (see text). Points represent determinations made during exposures to the haloalkanes at low, medium, and high concentrations. The concentration-response relationships were analyzed by the analysis of covariance. The elevations of coronary venous blood oxygen content during exposure to 1211 and 1011 were significant ($p<.005$, $p<.03$, respectively). 1301 was without a statistically significant effect on this variable.

blood oxygen content measured during exposure to the highest concentrations of 130I was the consequence of a lowered arterial blood oxygen. The statistical significance of this observation was determined by comparing the coronary venous blood oxygen measurements on the 130I-exposed animals with measurements made on animals exposed to control gas mixtures containing concentrations of N_2 equivalent to the 130I concentration. No significant difference was detected between controls and 130I-exposed animals. Note that the percentages in Table 4.1 compared favorably between acute vapor toxicity and the potential for elevating coronary venous blood oxygen content.

This phenomenon may or may not be of clinical significance in the individual with a normal cardiac reserve. Such may not obtain, however, in those individuals, diagnosed or undiagnosed, who have a coronary reserve limited by atherosclerosis and who may be either suffering from intermittent bouts of angina pectoris, or be potential candidates for anginal attacks in the near future.

Furthermore, angina is known to be aggravated by any factor that increases myocardial oxygen demand such as psychological stress and exercise, both of which would be encountered in fire-fighting and escape situations. Although an interaction between 121I and 101I and angina pectoris has not been demonstrated in the laboratory the pharmacologic-toxicologic basis has been established by these studies and warrants consideration.

Myocardial contractility and blood pressure. The force with which the heart contracts is one of the determinants of the efficacy of the heart as a mechanical pump. A decrease in myocardial contractility (Figure 4.2) may or may not result in decreases in cardiac output or arterial blood pressure depending on the functioning of the pressoreceptor reflexes. The pressoreceptor

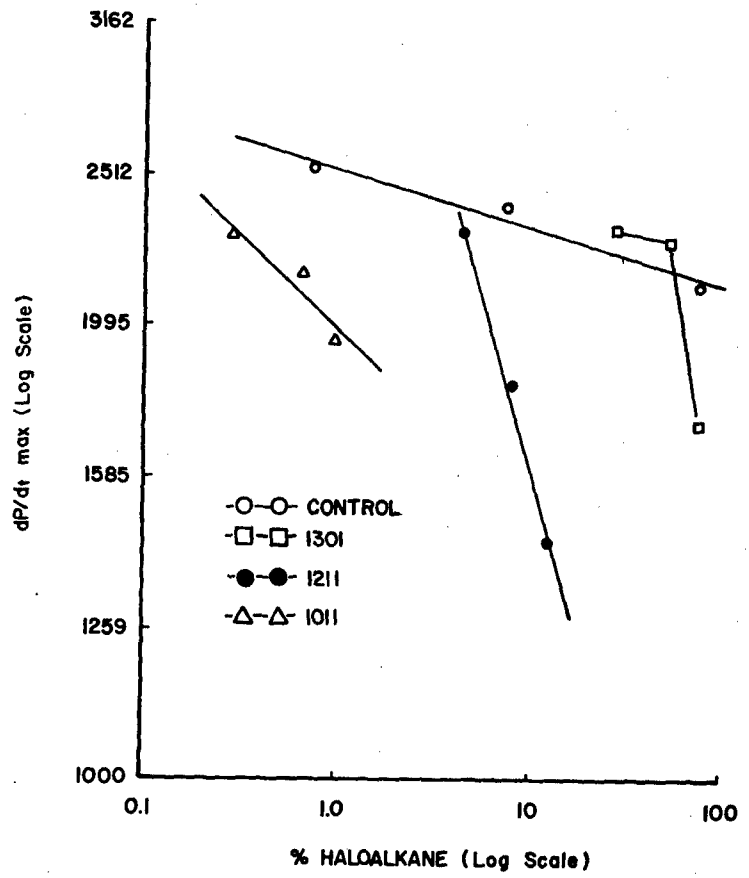

Figure 4.2 The logarithm of the left ventricular dP/dt_{max} versus the logarithm of the concentration of the haloalkane to which the animals were exposed. Each point represents the mean of several experiments (see text). The concentration-response relationships were analyzed by the analysis of covariance. The decrease in contractility measured by this index was determined to be statistically significant ($p<.01$) only during the 1011 exposure. The decreases were not statistically significant during exposures to 1301 or 1211 during this series, but were statistically significant in other types of experimental preparations (see text). The decrease in contractility is a biologically significant phenomenon in all cases.

reflexes were apparently partially disabled during exposure to 130I and 121I which resulted in a fall of arterial blood pressure without a predictable change of any biological significance of the cardiac output. The pressoreceptor reflexes remained nearly normally intact during exposure to 101I which resulted in the maintenance of a normal blood pressure as seen in Figure 4.3. This could be of clinical significance in relation to 1) perfusion of the body, particularly the head, and 2) the genesis of cardiac arrhythmias (discussed below).

A transitory hypotension associated with exposure to 130I or 121I could increase the probability of syncope (fainting). We have not studied cardiovascular dynamics in unrestrained, conscious animals. The hypotensive response may be significantly modified by anesthesia. (Chronically instrumented dogs are being prepared for this evaluation.)

Coronary arterial blood flow. Relationships among coronary blood flow, myocardial oxygen demand, and myocardial contractility were discussed on page 38.

Oxygen demand appears to be the chief determinant of coronary flow. The myocardium normally extracts most of the oxygen from the blood that passes through its capillary bed and the demand for oxygen is met by variations in coronary perfusion rate. This relationship was obviously disturbed in animals exposed to 121I and 101I. A complete causal sequence of events is impossible to establish based on the present data. Although coronary flow appeared to fluctuate in a manner that cannot be diagnosed as abnormal at this time, the elevation of coronary venous oxygen content discussed earlier represents a decidedly abnormal phenomenon. Therefore, at this time we have elected to include the concentration-dependent decrease in coronary blood flow illustrated in Figure 4.4as a biologically

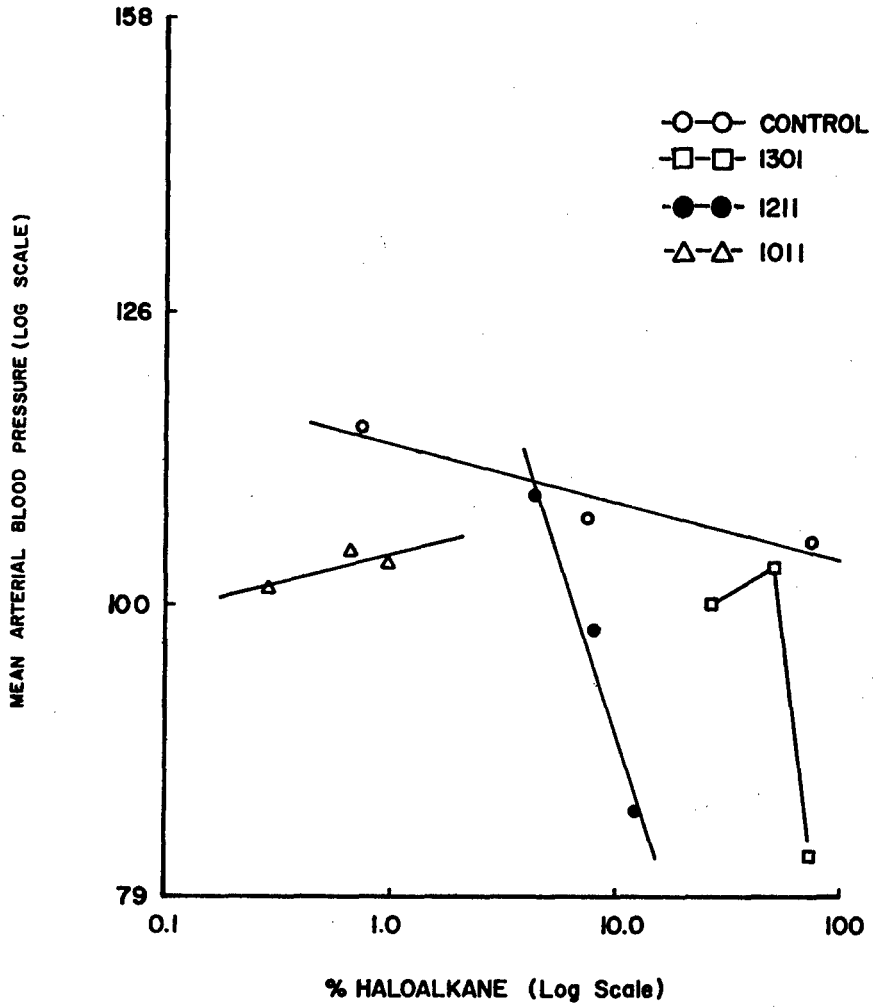

Figure 4.3 The logarithm of the mean arterial blood pressure versus the logarithm of the concentration of the haloalkane to which the animals were exposed. Each point represents the mean of several experiments (see text). The concentration-response relationships were analyzed by the analysis of covariance and were not statistically significant in this series of experiments. This effect was statistically significant in other studies (see text) and is known to be biologically significant.

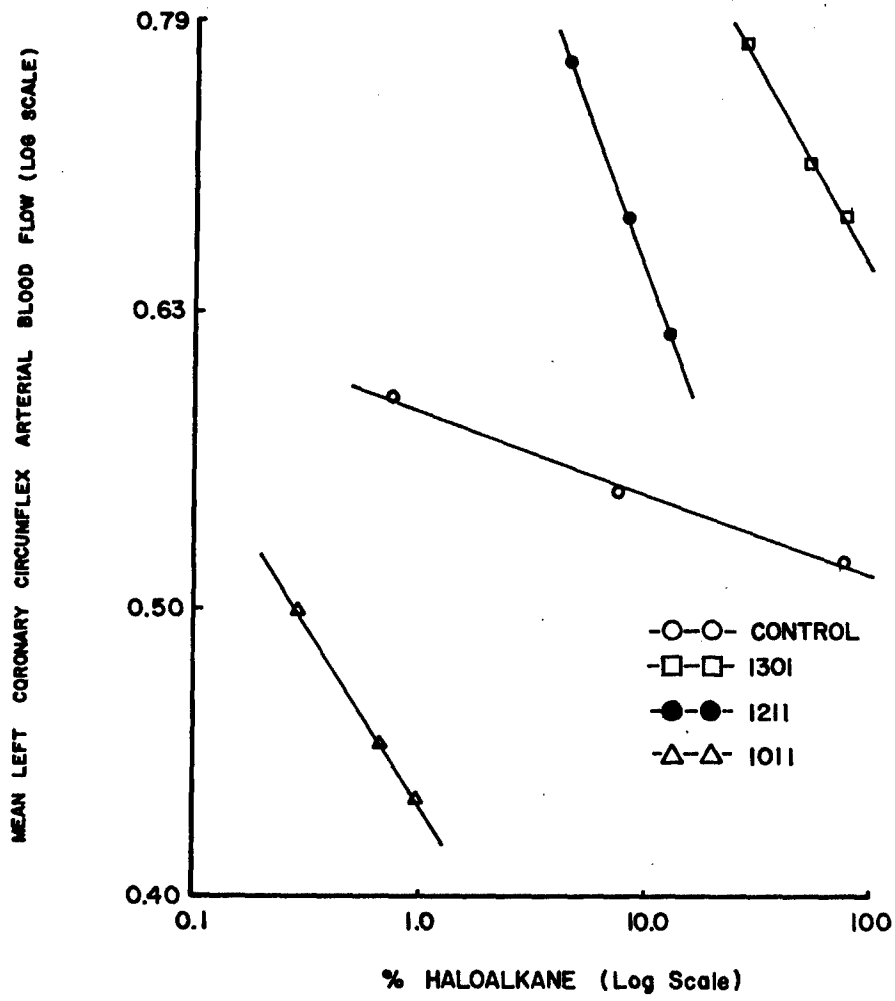

Figure 4.4 The logarithm of the left coronary circumflex arterial blood flow versus the logarithm of the concentration to which the animals were exposed. Each point represents the man of several experiments (see text). The concentration-response relationships were analyzed by the analysis of covariance. The effect of 1211 was statistically significant ($p<.05$). The effects of 1301 and 1011 on this variable were not statistically significant but were considered to be of possible biological significance (see text).

significant phenomenon without proposing a specific pathophysiologic role at this time. That this phenomenon was not statistically significant will be noted in the appendix of the preceding section, Table 4.1 "Summary of . . . studies . . .".

Cardiac arrhythmias. The potential for causing cardiac arrhythmias represents a significant hazard associated with the use of the halogenated fire extinguishing agents. The nature of the arrhythmias as well as interacting factors were discussed in the preceding section. The probability that arrhythmias will accompany exposure to the compounds is increased by increasing levels of circulating catecholamines and greatly decreased with falling blood pressure. A minimal blood pressure threshold was required to sustain the arrhythmias and a significant interaction between the hypotensive (blood pressure lowering) effect of the halogenated alkanes and the appearance of arrhythmias has been demonstrated. Indeed, the abolition of an arrhythmias episode was recorded during exposure of an anesthetized dog to 1301 (Figure 5 page 7 of Van Stee & Back, 1971 (AMRL-TR-68-188), Spontaneous Cardiac Arrhythmias Induced by Bromotrifluoromethane). The abolition of the arrhythmias was the direct consequence of the fall in blood pressure that accompanied the exposure. This phenomenon has not been evaluated in the conscious animal. The fall of blood pressure during exposure to 1301 and 1211 might even be interpreted as a favorable consequence of the exposure within this context.

The numbers in Table 4.1 of this section represent concentrations of the compounds in oxygen to which anesthetized animals were exposed that elicited episodes of comparable cardiac arrhythmias, e.g. premature ventricular contractions.

A comparison of the toxicities of the halogenated alkanes would be of little value in a toxic hazards assessment. It is not enough to consider toxicity in such terms as those of Table 4.1 without also considering some index of the availability of the toxic material to the organism.

This study has dealt with exposures to the compounds by inhalation, which implies that the exposures were to the compounds in the gaseous state. Therefore, toxic hazard within this context must contain a term that provides an index of availability to the organism (man) of the halogenated alkanes as gases. The choice of factors representing a function of availability to the user depends on the operation of factors that would tend to disperse the agents, thus removing them from the breathing zone. Dispersal factors would be more significant in open environments than closed spaces such as rooms or aircraft cockpits. Therefore, 2 different indexes of availability are used to estimate comparative toxic hazards depending on the intended application.

The toxic hazard indexes were computed as . . .

$$\frac{\text{vapor pressure, 25°C}}{\text{toxicity, \%}}$$

. . . when the agents were to be used outdoors where a maximum opportunity exists for dispersion of the released material. The reasoning behind this selection of numerator was that the tendency to vaporize would increase the possibility of a significant accumulation of compound in the gas phase in the breathing zone. On the other hand, a tendency to remain in the liquid state would favor gravitation from the breathing zone.

The toxic hazard indexes were computed as . . .

$$\frac{\text{\% saturation of air @ 25°C}}{\text{toxicity, \%}}$$

. . . when the agents were to be used in closed environments. The reasoning here was that the compounds are not as subject to dispersal in closed spaces. The fluoroalkanes, for example, vaporize completely at 25°C and displace a volume of air equal to the volume of fluoroalkane gas. Therefore, the concentration of fluoroalkanes in the breathing zone will be higher than that of 1011 for the same amount (molar quantity) of compound released.

The index of toxic hazard in Table 42represents vapor pressure divided by toxicity (percentage of halogenated alkane in the inspired mixture). This table was meant to be read horizontally. The vapor pressures were obtained from standard references.

Compound	Vapor Pressure psia, 25°C
1301	220
1211	34
1011	2.9

This toxic hazards index provides a rational basis for the comparison of the three compounds for use outdoors. One approach is that of a statistical analysis of ranked data. Table 4.3represents the hazard indexes of Table 4.2as ranked data. Included at the end of Table 4.3 are 4 additional factors similarly ranked.

Prior to making the computations it is necessary to change the ranks to "normal scores" (Table 4.4). An intermediate summary is presented in Table 4.5.

TABLE 4.2

RELATIVE HAZARD INDEXES[1] FOR HALOGENATED ALKANES

	1301	1211	1011
Acute Vapor Hazard (Engibous & Torkelson, 1960)	3.7	1.7	1.5
Acute Vapor Hazard (AMRL/THT)	2.8	2.3	2.9
Coronary Sinus Blood Oxygen Content Elevation (AMRL/THT)	0[2]	2.2	2.9
Myocardial Contractility (AMRL/THT)	3.8	5.9	4.2
Blood Pressure (AMRL/THT)	3.8	3.5	0[2]
Coronary Blood Flow (AMRL/THT)	3.5	4.9	5.6
Cardiac Arrhythmias (AMRL/THT)	2.8	2.4	4.1

1. Relative hazard index = vapor pressure (25°C)/toxicity. In this computation the index of toxicity used was the percentage cited in table 1. See text for further explanation.
2. Compound without measurable effect on this variable.

TABLE 4.3

RANKED RELATIVE HAZARD INDEXES FOR HALOGENATED ALKANES

	1301	1211	1011
Acute Vapor Hazard (Engibous & Torkelson, 1960)	3	2	1
Acute Vapor Hazard (AMRL/THT)	2	1	3
Coronary Blood Oxygen Content Elevation (AMRL/THT)	1	2	3
Myocardial Contractility (AMRL/THT)	1	3	2
Blood Pressure (AMRL/THT)	3	2	1
Coronary Blood Flow (AMRL/THT)	1	2	3
Cardiac Arrhythmias (AMRL/THT)	2	1	3
(RANKED ADDITIONAL FACTORS)			
Underwriter's Laboratory Classification	1	2	3
Fire Fighting Effectiveness (Engibous & Torkelson, 1960)	1	2	3
Storage Stability (Engibous & Torkelson, 1960)	1.5	1.5	3
Cost	3	2	1

TABLE 4.4

NORMAL SCORE TRANSFORMATION[1] OF RANKED RELATIVE HAZARD INDEXES FOR HALOGENATED ALKANES

	1301	1211	1011
Acute Vapor Hazard	0	.85	-.85
Coronary Blood Oxygen Content Elevation	.85	0	-.85
Myocardial Contractility	.85	-.85	0
Blood Pressure	-.85	0	.85
Coronary Blood Flow	.85	0	-.85
Cardiac Arrhythmias	0	.85	-.85
(ADDITIONAL FACTORS)			
Underwriter's Laboratory Classification	.85	0	-.85
Fire Fighting Effectiveness	.85	0	-.85
Storage Stability	.425	.425	-.85
Cost	-.85	0	.85

1. J.C.R. Li, Statistical Inference, Edwards Bros., Inc., Ann Arbor, Michigan, 1964, p. 517.

At this point in the analysis it was necessary to decide on what weight will be placed on the various indexes of toxic hazard. A mean hazard index can be computed with each hazard index given equal weight (Table 4.5 Mean, Unweighted Hazard). The hazard comparison on this basis would be 1301 < 1011 < 1211. Because of the way data are distributed in reality, however, this is a less accurate evaluation of comparative toxic hazard than one based on the normal score transformed data (Table 4.6). Thus, a truer relationship becomes 1301 < 1211 < 1011. Whereas the ranking of the unweighted indexes suggested a difference among the compounds, the analysis of variance suggested that the hypothesis that a significant difference exists among the compounds must be rejected.

Suggested weighting factors are listed in the middle of Figure 4.5. Acute vapor hazard represents the net effect of the interaction of a large number of individual variables, some of which have been defined, and some of which have not. If one considers this factor as an index of the overall toxic hazard it is not unreasonable to assign to it a weight of 3. The next 4 factors were assigned a weight of 1. It might also be entirely appropriate to assign a weighting factor of greater than 1 to one or more of the 4 factors, coronary venous blood oxygen, myocardial contractility, arterial blood pressure, and coronary arterial blood flow, in consideration of subjects with compromised coronary circulation. But, for purposes of this analysis, weighting factors will be assigned in consideration of entirely normal individuals only. Furthermore, it is doubtful that enough is known at this time to justify weighting these factors differently.

TABLE 4.5

SUMMARY OF COMBINED HAZARD INDEXES OF SIX FACTORS DESCRIBING EFFECTS OF HALOGENATED ALKANES

	1301	1211	1011
Mean, Unweighted Hazard Index	2.8	3.5	3.3
Rank	1	3	2

WEIGHTING FACTORS

Acute Vapor Hazard	3
O_2, Contractility, BP Coronary Flow	1
Cardiac Arrhythmias	6

	1301	1211	1011
Mean, Weighted Hazard Index	2.8	2.9	3.5
Rank	1	2	3

Since cardiac arrhythmias are considered to be of especial importance this hazard index was assigned a weighting factor of 6.

The mean weighted hazard indexes are at the bottom of Table 4.5. The ranking is seen to be changed from that of the mean, unweighted indexes, 1301 < 1211 < 1011.

The mean indexes are not as faithful a reflection of the comparative hazards as the sums of the normal score transformations (middle of Table 4.6). The ranking on this basis is changed to 1211 < 1301 < 1011. Furthermore, using these weighting factors, the differences among the compounds are statistically significant.

This exercise can be carried a step further with the incorporation of the 4 ranked additional factors listed at the bottom of Table 4.4. The 6 toxic hazard indexes were considered together with the 4 additional factors and they were all given equal weight. The sum of the normal scores of the compounds: 1301 < 1211 < 1011. The F ratio was smaller than the critical value indicating that no statistically significant difference was detected among the 3.

Acute toxicity hazard indexes for gases of varying toxicity are given in Table 47 for purposes of comparing the hazard indexes of the fire extinguishing agents with those of known compounds. The expected hazard incident to the use of these agents compared favorably with that of halothane and carbon tetrachloride (it is of utmost importance to recognize that the acute CCl_4 hazard does not include the hepatotoxic effect of the compound) and is 3 orders of magnitude less than that of such toxic compounds as sulfur dioxide, cyanide, or methyl bromide.

TABLE 4.6

ANALYSIS OF VARIANCE OF NORMAL SCORE TRANSFORMED HAZARD INDEXES

SIX HAZARD INDEXES, UNWEIGHTED

	1301	1211	1011
Sum of Normal Scores	1.7	.85	-2.55
Rank	1	2	3

F Ratio = 1.207 (Not Sig)

SIX HAZARD INDEXES, WEIGHTED

	1301	1211	1011
Sum of Normal Scores	1.7	6.8	-8.5
Rank	2	1	3

F Ratio = 11.86 ($p<.01$)

SIX HAZARD INDEXES, UNWEIGHTED, PLUS FOUR ADDITIONAL FACTORS

	1301	1211	1011
Sum of Normal Scores	3.0	1.3	-4.3
Rank	1	2	3

F Ratio = 2.29 (Not Sig)

TABLE 4.7

COMPARATIVE TOXIC HAZARD INDEXES OF KNOWN GASES COMPUTED ON THE SAME BASIS AS THE HALOGENATED ALKANES

	Acute Tox, ppm (A)	Acute Tox, % (A/10,000=B)	Vapor Press, psi, 25°C (C)	Hazard Index (C/B)
Sulfur Dioxide	100	.01	51	5100
Hydrocyanic Acid	100	.01	15	1500
Methyl Bromide	100	.01	27	2700
Carbon tetrachloride (exclusive of hepatotoxic effect)	10,000	1	2.2	2.2
Halothane	30,000	3	4.7	1.6

Tables 4.8-4.11 represent an analysis similar to the foregoing in which the hazard indexes were computed based on the use of the compounds in closed environments such as smaller rooms and aircraft cockpits. The relative hazard indexes are listed in Table 4.8. These numbers are not to be compared with the hazard indexes of Table 4.2. Since each set has a different numerator they are not comparable.

The mean hazard indexes were omitted for reasons discussed earlier. The ranks of the hazard indexes are listed in Table 4.9 and the ranks transformed to normal scores are listed in Table 4.10.

The analysis of the normal scores is presented in Table 4.11. The sums of the normal scores were used to rank the compounds. The analyses of variance of the data grouped in the 3 ways discussed earlier revealed that statistically significant differences were detected among the hazard indexes of the 3 compounds. The ranking remained the same no matter what combination of factors was used and regardless of the incorporation of the weighting scheme. The ranking of the toxic hazard indexes of the 3 halogenated alkanes was . . .

$$1301 < 1211 < 1011$$

. . . for use in closed spaces.

Bioavailability of the halogenated alkanes is, in part, a function of the solubility of the compounds in body tissues. We have measured the solubility of the 3 compounds in blood and olive oil (a traditional model of the lipid compartments of the body, e.g. adipose tissue and nervous tissue). The order of solubilities is the same for the 3 compounds: 1301 < 1211 < 1011. The solubility determines the quantity of compound that will be absorbed during any given period of exposure was well as the rate of removal from the body. Thus, the more highly lipid soluble that

TABLE 4.8

RELATIVE HAZARD INDEXES[1] FOR HALOGENATED ALKANES

	1301	1211	1011
Acute Vapor Hazard (Engibous & Torkelson, 1960)	1.7	5.0	10
Acute Vapor Hazard (AMRL/THT)	1.3	6.7	20
Coronary Sinus Blood Oxygen Content Elevation (AMRL/THT)	0[2]	6.5	20
Myocardial Contractility (AMRL/THT)	1.7	17	29
Blood Pressure (AMRL/THT)	1.7	10	0[2]
Coronary Blood Flow (AMRL/THT)	1.6	14	39
Cardiac Arrhythmias (AMRL/THT)	1.3	7.1	29

1. Relative hazard index = % saturation (25°C)/toxicity. In this computation the index of toxicity used was the percentage cited in Table 4.1. See text for further explanation.
2. Compound without measurable effect on this variable.

TABLE 4.9

RANKED RELATIVE HAZARD INDEXES FOR HALOGENATED ALKANES

	1301	1211	1011
Acute Vapor Hazard (Engibous & Torkelson, 1960)	1	2	3
Acute Vapor Hazard (AMRL/THT)	1	2	3
Coronary Blood Oxygen Content Elevation (AMRL/THT)	1	2	3
Myocardial Contractility (AMRL/THT)	1	2	3
Blood Pressure (AMRL/THT)	2	3	1
Coronary Blood Flow (AMRL/THT)	1	2	3
Cardiac Arrhythmias (AMRL/THT)	1	2	3
(RANKED ADDITIONAL FACTORS)			
Underwriter's Laboratory Classification	1	2	3
Fire Fighting Effectiveness (Engibous & Torkelson, 1960)	1	2	3
Storage Stability (Engibous & Torkelson, 1960)	1.5	1.5	3
Cost	3	2	1

TABLE 4.10

NORMAL SCORE TRANSFORMATION[1] OF RANKED RELATIVE HAZARD INDEXES FOR HALOGENATED ALKANES

	1301	1211	1011
Acute Vapor Hazard	.85	0	-.85
Coronary Blood Oxygen Content Elevation	.85	0	-.85
Myocardial Contractility	.85	0	-.85
Blood Pressure	0	-.85	.85
Coronary Blood Flow	.85	0	-.85
Cardiac Arrhythmias	.85	0	-.85
(ADDITIONAL FACTORS)			
Underwriter's Laboratory Classification	.85	0	-.85
Fire Fighting Effectiveness	.85	0	-.85
Storage Stability	.425	.425	-.85
Cost	-.85	0	.85

1. J.C.R. Li, Statistical Inference, Edwards Bros., Inc., Ann Arbor, Michigan, 1964, p. 517.

TABLE 4.11

ANALYSIS OF VARIANCE OF NORMAL SCORE TRANSFORMED HAZARD INDEXES

SIX HAZARD INDEXES, UNWEIGHTED

	1301	1211	1011
Sum of Normal Scores	4.3	-.85	-3.4
Rank	1	2	3

F Ratio = 7.00 ($p<.05$)

SIX HAZARD INDEXES, WEIGHTED[1]

	1301	1211	1011
Sum of Normal Scores	11.0	-.85	-9.4
Rank	1	2	3

F Ratio = 44.3 ($p<.01$)

SIX HAZARD INDEXES, UNWEIGHTED, PLUS FOUR ADDITIONAL FACTORS

	1301	1211	1011
Sum of Normal Scores	5.5	-4.3	-5.10
Rank	1	2	3

F Ratio = 6.06 ($p<.01$)

1. See Table 4.5.

any foreign compound is, the longer will be its residence time in the body.

The solubility in lipids (fats, etc.) provides the physicochemical basis for the explanation of the residual effects that were monitored following exposure to the compounds. Venous PO_2 remained elevated for at least 30 minutes after the end of 90 minute exposures to 1211 and 1011 (see Table 4.1,"Summary of . . . studies . . ."). This was presumed to be directly related to the relative lipid solubilities of the compounds although complete pharmacokinetic studies have not been performed. We have measured the rate of elimination of 1301 ("Brain Heart Accumulation of Bromotrifluoromethane," AMRL-TR-70-139), but not of the others. The elimination half-time ($t_{1/2}$) for 1301 was determined to be a matter of only a minute or two. It would be expected to be much higher for the other compounds.

The clinical significance of bioavailability in these terms increases with increasing duration of exposure. The total body burden would increase more rapidly with duration of exposure in the case of 1011 than 1211; the total body burden of 1211 would increase more rapidly than 1301. A clinically significant difference in uptake would not be expected during exposures to nominal concentrations of the respective agents for durations less than 10 minutes, although this remains to be verified in the laboratory.

RECOMMENDATION

The ranking of the toxic hazards incident to the use of the halogenated fire extinguishing agents differed depending on the intended use.

Outdoor Use. The ranking of the expected toxic hazards for use in open environments such as outdoors or inside large buildings where the compounds are subject to dispersion factors is given in the middle of Table 4.6.

1211 < 1301 < 1011

This comparison is based on a set of weighting factors that gives the most emphasis to the potential that these agents have for precipitating cardiac arrhythmias, but also considers several other biologically significant effects of exposure by inhalation to these compounds. The difference among the compounds was statistically significant.

If it is desired to consider matters of cost, storage stability, etc. along with toxic hazard the ranking may be changed somewhat depending on the selection of weighting factors. The weighting factors that would be chosen by this investigator would be most likely to affect the relationship of 1301 to 1211 and not the relationship between the fluoroalkanes and 1011. Thus, the ranking would be most likely to be approximately the following:

1211 = 1301 < 1011 . . .

. . . based on a cardiovascular toxic hazards assessment plus 4 additional factors.

Use In Closed Spaces. The ranking of the expected toxic hazards for use in closed spaces is best represented by the ranking in the middle of Table 4.11.

1301 < 1211 < 1011

Weighting and the addition of the extra factors did not influence the ranking.

TENTATIVE EXPOSURE CRITERIA

The establishment of exposure criteria required the estimation of toxicity. In contrast to the evaluation of toxic hazard, no index of availability entered the calculation since, by definition, exposure criterion implies that the exposure takes place.

A complete set of quantitative human exposure data for the 3 compounds is not available and it was therefore necessary to establish a general relationship among the compounds from an incomplete set of experimental data.

The data in Table 4.12 were extracted from the references cited. They represent concentrations of the respective compounds that elicited approximately equivalent responses from the experimental subjects.

A function of drug response is usually a linear function of the logarithm of the dose or concentration to which the subjects are exposed. A variation of the usual log concentration-response relationship was found empirically to be approximately linear: the log of the concentration versus the log of the oil solubility (Figure 4.5). This was not surprising since a cogent argument for the relationship between oil (lipid) solubility and biological action of anesthetics was proposed many years ago by Meyer and Overton. Variations such as that of Mullins followed.

The apparently linear relationship is evident from visual inspection of Figure 4.5. The next step in establishing the mathematical relationship among the compounds was inductive. The line that fit the data best was determined by the method of least squares:

$$\text{Log conc} = -0.62\ (\text{log oil sol}) + 0.76$$

TABLE 4.12

EXPERIMENTAL DATA FROM WHICH RATIOS OF BIOLOGICAL ACTIVITY ESTIMATED

	1301	1211	1011
Essentially no mortality in 1 hr, rat (Engibous & Torkelson, 1960)	60[1]	20	1.3
Composite cardiovascular toxicity, dog 68 (Table 1, this report)	68	11	0.8
Serious neurological disturbance, man (Hine, 1968, Clark, 1970)	16.9	4-5	
Serious neurological disturbance, dog (Hine, 1968, Clark, 1970)	40	8.8	
Moderate neurological disturbance, dog (Hine, 1968, Clark, 1970)	20	5	
Mild neurological disturbance, dog (Hine, 1968, Clark, 1970)	10	2	

1. Numbers in body of table are percentages of compound in air (or oxygen when the exposure was to higher levels of 1301). When read across, percentages are those eliciting approximately equivalent biological responses in test subjects.

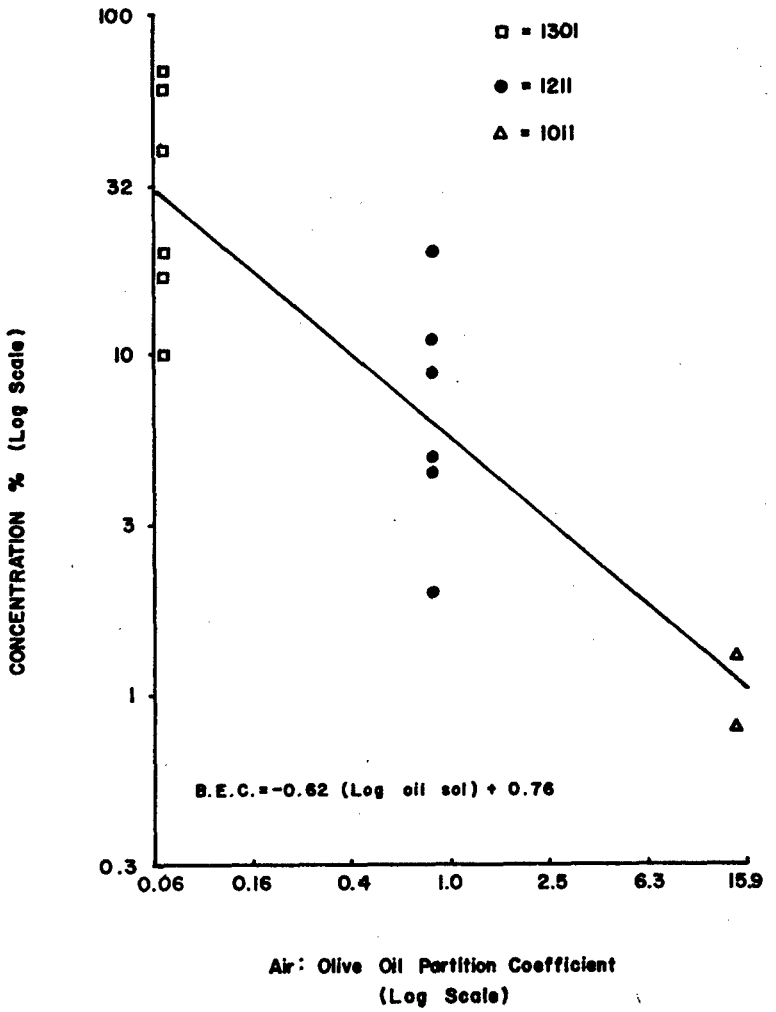

Figure 4.5 Concentrations of halogenated alkanes from Table 4.12are represented along the ordinate. Air:olive oil partition coefficients (a function of lipid solubility) are represented along the abscissa. Sets of coordinates from the horizontal rows of Table 4.12 represent concentrations of the respective compounds that elicited approximately equivalent biological responses from the experimental subjects. The biologically equivalent concentrations (B.E.C.) of the halogenated alkanes calculated from their respective lipid solubilities using the linear equation are listed in the 3rd column ("Halon Conc") of Table 4.13.

A log concentration for each compound was computed by substituting the oil solubilities of the respective compounds in the formula. The antilogs represent numbers that are related to each other as the biological activity of the compounds.

TABLE 4.13

RELATIVE BIOLOGICAL ACTIVITIES OF 1301, 1211, AND 1011

COMPOUND	REL OIL SOL[1]	HALON CONC[2]	REL BIOL ACT[3]
1301	0.07	29.9	30
1211	0.85	6.4	6
1011	14.4	1.1	1

1. Relative olive oil solubility = air:oil partition coefficient.
2. Halogenated alkane concentration computed from preceding formula.
3. Approximate relative biological activities from Halon concentrations of preceding column.

The last column of Table 4.13may be read, in consideration of the sum of the toxicologic manifestations of acute exposure: the concentration of 1301 that can be tolerated is approximately 5 times that of 1211, the concentration of 1211 that can be tolerated is approximately 6 times that of 1011, and the concentration of 1301 that can be tolerated is approximately 30 times that of 1011.

The ratio of biological activities represents one-half of the information necessary to establish exposure criteria. The other half is the point of reference to which the activity ratios will be applied.

Bromotrifluoromethane has been studied most extensively of the 3 compounds and we elected to use the results of human exposures to this compound to establish the exposure criteria for all. The data for 1301 of

Call (1973) and Hine (1968) were used and are included in Table 4.14.

TABLE 4.14

EXPOSURE CRITERIA FOR HALONS 1301, 1211, AND 1011

	1301	1211	1011
Biological Activity Ratio	30	6	1
Little or No Effect, 3-5 Min, %	7	1.2	0.23
Little or No Effect, 20 Min, %	5	0.8	0.17
Moderate Effect, 20 Min, %	10	1.7	0.33

Little or No Effect

This is defined as a slightly perceptible feeling of lightheadedness with the possibility of occasional slight tingling sensations in the extremities. No cardiovascular effects, with the possible exception of a slight increase in heart rate, would be expected.

Moderate Effect

This is defined as a definite feeling of lightheadedness that might be perceived by some individuals as a symptom of impending unconsciousness. Tingling sensations (paresthesia) would be expected to be felt by some. Heart rate would be expected to accelerate moderately and but few individuals would be expected to develop serious electrocardiographic abnormalities. The onset of those symptoms should alert the subject to be prepared to discontinue further exposure. Hine et al. (1968) have published detailed narrative descriptions of the sensations accompanying exposure to 1301.

These exposure criteria must be understood to be tentative and subject to revision. They are most accurate for 1301, less likely to be accurate for 1211, and only estimates for 1011. The final judgment must await complete, comprehensive studies of controlled exposures of human volunteers to both 1211 and 1011 at least to the extent that this has been done with 1301.

FINAL STATEMENT

Biological availability of the halogenated fire extinguishing agents is a function of . . .

1. temperature
2. air turbulence
3. openness of the use environment
4. solubility of the agents in body tissues
5. density of the agents in the states to which users would be exposed.

1. The rate of volatilization of 1211 and 1011 would be markedly affected by the heat encountered.

2. Turbulence of the air would enhance the availability in closed spaces whereas it would reduce the availability in open environments (wind).

3. The hazards would be lower in open than in closed environments.

4. The solubility of the agents in body tissues was a factor partially embodied in the "toxicity" evaluation and was included in the discussion of residual effects.

5. The density of the agents in the form to which the user would be exposed would affect the rate at which the agents would gravitate from the user's breathing zone. Thus, liquid 1011 would rapidly fall to earth subsequent to contact with the user thus removing the source of the vapor

from the breathing zone. The fluorocarbons would likewise fall to earth because of their greater density than air in the gas phase, but it is likely that the temporal courses of these gravitational events would be different for the different compounds. We cannot quantify this relationship in the absence of experimental data, but based on the physical properties of the respective compounds, the expectation would be that the availability to the organism as a function of their density would probably be 1011 < 1211 < 1301. Incorporation of this factor into the statistical hazards evaluation would have the effect of narrowing the range between the fluoroalkanes and 1011.

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