The Toxicological Assessment of a Fire Suppressant and Potential Substitute for Ozone Depleting Sustances.

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INTRODUCTION

in 1974, both the environmental and scientific communities expressed concern regarding the decrease in stratospheric ozone due to the release of chlorofluorocarbons (CFCs) into the atmosphere. CFCs are perhalogenated alkanes that are used as refngerants, blowing agents in the manufacture of foam plastics, cleaning fluids and propellants. These applications exploit the physical properties of CFCs which include low boiling points, specific heats and heats of vaporization, high insulating value, low surface tension and viscosity, and high vapor densities (Anders, 1991). Moreover, CFCs are nonflammable and relaUvely free of adverse health effects (Clyton, 1967; Aviado, 1974, Back and Van Stee, 1977) Howeve', CFC interaction or effects on stratospheric ozone is of increasing concern (MaIms and Rowland, 1974).

Ozone (03) is present in two layers of the earth's atmospheres the stratosphere and troposphere. The tropospheric layer envelopes the earth's surface. Ozone is a noxmous pollutant in the troposphere and exposure to it has been associated with lung damage and respiratory problems. However, in the stratospheric layer, ozone acts as a protective shield preventing much of the sun's ultraviolet radiation (UVR) from reaching the earth's surface (Longstreth, 1991). The range of possible wavelength radiation from the sun that reaches the earth's surface extends from the infrared (>700 rim), through the visible (400-700 rim), and into the ultraviolet spectrum (4400 rim). However, the presence of ozone produces ar~ atmospheric cut off between 286-300 rim by absorbing wavelengths below 300 rim (Crosby, 1976), thus protecting chemicals in the troposphere from potential photodegradaton.

Under the protective ozone shield, CFCs are stable chemicals that are capabie or acting as affective absorbers of infrared radiation, which makes them important greennousa gases. The half-life of CFCs in the troposphere is 10-50 years (Piver, 1991a1 However, n the stratosphere, CFCs found above the protective ozone shield, undergo photodegradatmcn from exposure to UVR (Piver, 1991b). Thus, the absence of short wavelength UVR prevents the photodegradation of CFCs, allowing them to remain longer in the troposphere and diffuse to the stratosphere. During photodegradation, chlorine atoms are released and react wmtfl stratospheric ozone, forming chlorine monoxide. This monoxide does not absorb short wavelength UVR (Last, 1993; Lashof and Ahuga, 1990). Thus, chlorine monoxide does rot compensate for the loss in UVR's absorbing capability of ozone. This depletion ir stratospheric ozone concentrations results in more short wavelength UVR (UV-B. 280-320 and UV-C, <280) penetrating the protective ozone shield, striking the earths surface and increasing human exposure. Prolonged human exposure to short wav~ength UVR has been associated with increased tnc~dences of non-melanoma skin cancers, cataracts and possible adverse effects on immune function (Urtach, 1991; van Kuijk, 1991; Shope. 1991) Hence,

stratospheric ozone is a significant barrier against UVR reaching the earth's surface and thus prevents various exposure-related health effects from UVR.

Halons are a variation of CFCs and have similar physical properties, industrial uses and environmental . characteristics. Halons have a half-life in the troposphere of 20 - 65 years. They undergo photodegradatiofl in the stratosphere, releasing bromine atoms that react with ozone and

forming bromine monoxide. Bromine monoxide does not absorb short wavelength UVR. Thus, Halons, like CFCs, decrease stratospheric ozone when released into the atmosphere. CFCs and Halons are substantially different in that the bromine atoms released from Halons are 20 to 60 times more effective at destroying ozone than the chlorine atoms from CFCs (Cicerone, 1994).

As a result of this concern, in September of 1987, twenty-six countries met in Montreal. Canada and agreed to the Montreal Protocol on Substances that Deplete Ozone Layer. The Montreal Protocol called for a 50% reduction in CFC production (CFC production not to exceed 1986 levels). This list also included Halons. The phase-out of CFCs and Halons were accelerated in June of 1990, when the Montreal Protocol was revised in London, Great Britain, as the London Amendment. This revision provided for deeper cuts in production levels for Class I ozone depleting substances (CFCs and Halons; a total production phase-out by the year 2000), it placed additional chemicals on the phase-out list (methyl chloroform and carbon tetrachloride), and it established a phase-out schedule for hydrochlorofluorocarbons (HCFCs: temporary alternatives to CFCs). In November of 1992, the Montreal Protocol was revised again in Copenhagen, Denmark, increasing the accelerated production phase-out dates for all ozone depleting substances (Halons by the year 1994; CFCs, methyl chloroform and carbon tetrachloride by the year 1996; and HCFCs by the year 2030). Because of this acceleration the development of acceptable substitutes for CFCs and Halons has produced several new technologies in the areas of refrigeration, climate control systems and fire suppression.

Pyrotechnically Generated Aerosol (PGA) fire extinguishment is a newly emerging technology in the area of fire suppression. Spectrex Fire Extinguishant (SFE) is a product employing this technology and is a potential replacement for certain applications of Halon 1301. SFE is a solid formulation that is comprised of various potassium-based compounds. Its fire suppression capabilities are derived from the resulting aerosol created during the pyrolysis of the parent material. A temperature of 500^0C or greater is needed for pyrolyzation of the parent material to occur. Once reached, SFE produces a finely-dispersed, optically-dense soluble aerosol by-product of potassium alkali salts. Based upon the stoichiometry of SFE parent material chemical composition, the alkali salts of potassium chloride (KCI) and/or potassium hydroxide (KCH) are predicted to be present in the aerosol cloud. These potassium alkali salts have no known atmospheric/environmental impacts (Ozone Depleting Potential or Global Warming Potential). The amount of parent SEE required to suppress a fire is estimated at 50 - 80 g per cubic meter of exposure area. Theoretical calculations indicate that aerosol particles of KCI and/or KOH range widely in diameter with a median size of 1 to 2 µm. Aerosols of this size are capable of reaching the deep lung or alveolar sacs (Menzel and Amdur, 1986). The alveolar sacs are anatomical structures highly sensitive to chemical insult, and chemicals reaching the alveolar sacs may readily be absorbed into the blood system and earned throughout the body. Thus, the potential physiological and/or toxic effects due to the inhalation of SFE aerosol may include; 1) respiratory system irritancy, resulting in discomfort and/or altered respiration and oxygen intake, 2) irritation of the skin, nasal area and/or mucous membranes of the eye, resulting in discomfort and/or impaired

visual capacity, 3) the possibility of high aerosol concentration, producing a pulmonary dust overload phenomenon, resulting in incapacitation and possible death, and 4) the potential presence of carbon monoxide (C0), resulting in increased carboxyhemoglobin levels and decreased oxygen uptake, thus, leading to disorientation and possible incapacitation.

To begin safety/toxicity assessment of SFE aerosol by-products, animals were exposed to atmospheres of two load concentrations of SFE Formulation A for 15 or 60 minutes. At the conclusion of the exposure, the following parameters were evaluated: nasal, ocular and dermal irritation, presence of pulmonary edema, blood gases and pH, hemoglobin, general toxicology and survivability. The exposure atmospheres were characterized for aerosol concentration, particle size distribution (mass median aerodynamic diameter, MMAD) and particle size analysis (geometric standard deviation, σ_g).

MATERIALS AND METHODS:

Animals: Twenty male Fisher CDF (F-344)/Cr1 BR rats, weighting between 20O and 250 g, were obtained from Charles River Breeding Labs (Wilmington, MA). Upon arrival the animals were tail tattooed and quarantined for two weeks. The animals used in this study were handled in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals , prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHS, National Institute of Health Publication #85-23, 1985, and the Animal Welfare Act of 1966, as amended. Animals were housed in suspended shoe box type cages. They were provided Formula Lab Chow 5008 (Purina Milles Inc., St. Louis, MO) and reverse osmosis filtered water *ad libiturn.*

Experimental Design: To examine the potential toxic effects of SFE Formulation A aerosol by-products, animals were placed in a whole body inhalation chamber. The experimental design consisted of 5 exposure groups. Animals were randomized prior to exposure and were examined for ocular and dermal irritation or abnormalities. Each exposure group contained 4 animals. Exposures were:

Group	**Load Concentration**	Length of Exposure
1	Control	60 minutes
2	50 g/m^3 of Formulation A	15 minutes
3	**80** g/m^3 of Formulation A	15 minutes
4	50 g/m^3 of Formulation A	60 minutes
5	80 g/m^3 of Formulation A	60 minutes

Inhalation Chamber To conduct the inhalation study, a delivery system for the aerosol was constructed. The system consisted of a 700 L inhalation chamber with a supply/exhaust system, a specially designed aerosol generator and an exhaust scrubber. The generator was connected to the inlet side of the system. Exposures were conducted under static chamber conditions. Air flow through the generator and chamber was halted when 2-5 gIm^3 **was** detected in the exhaust flow via a MIE, Inc. RAM-S aerosol photometer (Bedford100:1 diluter was placed in-line prior to the aerosol photometer. A pneumatic valve in the chamber exhaust line and a solenoid valve on the generator air line were activated simultaneously to capture the aerosol inside the chamber. Sampling ports for aerosol collection were placed in the rear of the chamber. Samples collected were analyzed for aerosol concentration (MMAD) and particle size (ag). The system was exhausted through a scrubber.

Aerosol Concentration; Aerosol mass concentration within the chamber was determined via filter samples. Samples were collected on a 47 mm Gelman 61631 A/E glass fiber filter placed in a brass filter holder (IN-TOX Products, Albuquerque, NM). Filters were stored in a decicator until prior to sampling and weighed on a Cahn C-31 Microbalance (Cerritos, CA). Samples were collected at 1, 5, and 15 minutes for 15 minute exposures and 1, 5, 15, 30, 45, and 60 minutes for 60 minute exposures. The flow rate through a filter was at 5 L/min. with a sampling time of 15 seconds.

Particle Size Analysis; Mass weighted particle size distribution was determined using a cascade impactor (design based on Marple's criteria; Marple, 1978; IN-TOX Products. Albuquerque, NM). Aerosol particles were collected on 37 mm stainless steel substrates coated with a layer of an apiezon/toluene mixture and allowed to dry for 1 hour. A 47 mm Gelman 61631 A/E glass fiber filter was used as a final filter. Substrates and filter were weighed on a Cahn 0-31 Microbalance

(Cerritos, CA). Samples were collected at the beginning and end of each exposure. The flow rate through the impactors was at 20 L/min with a sampling time of 4 to 15 sec. depending upon the time the sample was collected. Particle size distribution was reported as MMAD and c~g.

Aerosol size within the exposure chamber was also analyzed with a TSI model 3300 Aerodynamic Particle Size Analyzer (APS; TS1 Corp, St.Paul, MN). The APS obtained the MMAD and particle counts. Two TSI model 3302 diluters each with a 100:1 dilution probe proceeded the APS providing a final dilution of 10,000:1. The real time aerosol analysis was performed on a Zenith Data System 286 RC with 1St model 390041 APS Advanced Software, version B using a density value of 2 g/cc. Samples were collected for 10 seconds. The aerosol was sampled at 1,15, 30, 45, and 60 minutes.

Clinical Obseriations, Postmortem, Histopathology, and Blood Collection; Animals were observed throughout the duration of the exposure (15 or 60 minutes). At the conclusion of each exposure, an ocular and dermal irritation examination was performed. Animals were euthanized within 30 minutes of exposure by intraperitoneal injection of a euthanasia mixture consisting of Ketamine HCI (Vetalor Parke-Davis, Moms Plains, NJ) and Xylazine (Rompun: Mobay Corporation, Shawnee, KS) at a mix ratio of 7 mL to 3 mL, respectively. Once euthanized, the abdominal and thoracic cavities were opened and blood samples collected from the left ventricle oi the heart with a 10 cc syringe containing heparin. A portion of each blood sample was transferred to a 3 mL Vacutainer containing heparin (green top) for serum chemistry, while the remaining portion was analyzed for blood gases and pH (see Blood Gases and pH, Hemoglobin Analysis and Serum Chemistry). Gross examination was performed on the trachea, lung, heart and abdominal organs. The trachea and lung were removed from the thoracic cavity and trimmed for wet/dry weight determinations. The head was removed and transversely cut at the level of the incisive papilla and second palatal ridge using a Buehler lsomet low speed saw with diamond wafering blade (Evanston, IL) to examine nasal turbinate. Each nasal turbinate section was placed in 10% neutral buffered formalin and decalcified for 3 days in 10% ethylenediamjnetetraacetic acid (EDTA: Sigma. St. Louis, MO). The nasal turbinates were processed for histological examination (light microscopy). Each section was embedded in paraffin, sectioned at 3-4 microns, and stained with hematoxylin and eostn.

Ocular and Dermal Irritation Examination: Clippers were used to shave a 2 cm by 6 cm strip on the dorsal side of all animals approximately 24 hours prior to the exposure. The dermis of each animals was examined before and after exposure and scored according to Draize(Draize, 1959). Eyes were examined before and after exposure by the application of a flourscein solution on each eye and scored according to Draize (Draize and Keller. 1944). The flourscein solution contained 2% flourescein in phenyl mercuric nitrate. The eyes were observed for comeal opacity using a ultraviolet (UV) light.

Blood Gases and pH, Hemoglobin Analysis and Serum Chemistry: Blood gases and pH were performed on a Ciba-Coming 288 Blood Gas Analyzer (Coming Diagnostics Corp., Medfied MA). Parameters analyzed were pH, partial pressure of oxygen and carbon dioxide, bicarbonate, sodium, potassium and calcium. Hemoglobin analysis was performed on a Ciba-Coming 2500 CO-oximeter (Coming Diagnostics Corp., Medfied MA). Hemoglobin parameters were total hemoglobin, carboxyhemoglobin, methemoglobin. oxyhemoglobin and deoxyhemoglobin. Serum chemistries were performed on a Kodak Ektachem 700 Analyzer(Rochester, NY). Serum chemistry parameters were glucose, sodium. potassium, chloride, carbon dioxide, calcium, magnesium1 and phosphorus.

Wet/Dry Lung Weight Deteiminations: Interstitial edema in lung tissues can be determined via histopathological techniques; however, total pulmonary edema including alveolar edema is best analyzed via wet/dry lung weight ratio determinations. Wet/dry lung ratio were measured by the method described by Staub (1974), with minor modifications. After the animals were euthanized, the thoracic cavity was opened to expose the lung and heart, the cervical trachea was exposed,

and the tissue between the thoracic and cervical trachea was excised. The trachea was ligated I to 2 тnm below the pharynx. After transection of the aort and vena cava the heart was excised, the lung extracted en bloc, and the esophagus removed. The lungs were rinsed with saline and blotted dry with gauze pads. A small, preweighed S shaped hook was inserted through the trachea just above the ligature and lung wet weight recorded. The lung was then suspended from a drying rack via the S-hook and placed in a drying oven at 11 00C for 24 hours. At the conclusion of 24 hours. the lung dry weight was recorded. Corrected lung wet weight (WVV) and dry weight (DWÆ) was determined by subtracting the weight of the S-hook. Pulmonary edema formation was quantified by comparison of % water (H20), g of water in the lung/kg of body weight (BW). and g of solid (dehydrated) lung/kg of body weight between the exposed and control groups using the formulas:

$$\% \ H20 = (WW-DW)/WW \times 10^2$$
$$g \ H20/kg \ BW = (WW-DW)/BW \text{ in } g \times 10^3$$
$$g \ solids/kg \ 6W = DW/BW \text{ in } g \times 10^3$$

Statistical Analysis: A one factorial analysis of variance with Bonferroni Multiple Comparison was performed on all blood gases, pH, hemoglobin and serum chemistry parameters. The equality of variance was tested using Levene's test.

RESULTS:

Clinical Observations: No deaths were reported during the study. Animals expose SFE Formulation A exhibited signs of irregular breathing cycles, lack of coordination, lethargy,and coughing/sneezing. Head pulling or straining was observed frequently; that is, the animal would extent the head back, up and away from the body. As loads and length of exposure increased, these signs became more pronounced. All animals appeared to recover once placed in fresh air

Postmortem: No lesions were noted on the trachea, lung, heart and abdominal organs after gross examination.

Histopathology: Nasal turbinates exhibited no abnormalities under histopathological examination. The only lesions observed grossly were red foci around the hilar region of the lungs of all animals exposed to a load concentration of 80 g/m3 for 60 minutes.

Ocular and Dermal Irritation: All animals were free of ocular or dermal irritation and abnormalities prior to exposure. Post exposure examination showed no ocular or dermal irritation in the animals.

Blood Gases and pH: The pCO2 increased for groups 2, 3 and 4 with increased loading and length of exposure, whereas group 5 (80 g/m3 for 60 minutes), had a level similar to that of the control group (control; Fig. 1). The p02 decreased for all exposure groups with increased loading and length of exposure (Fig 1). The concentration of bicarbonate ion increased for groups 2, 3 and 4 with increased loading and length of exposure ,whereas group 5 (80 g/m3 for 60 minutes), had a level lower than the control group (control). Blood pH decreased for all exposure groups with increased load and length of exposure (Fig 2). The cations measured during blood gas analysis were within their respected biological ranges.

Hemoglobin Analysis: Hemoglobin analyses are shown in Figure 3. Total hemoglobin significantly increased ($p<Q.01$) in all exposure groups with the highest concentration observed in group 5. Methemogtobin was the same for groups 2 and 3, increased in group 4 and significantly increased ($p<0.01$) in group 5.Deoxyhemoglobin was decreased in groups 2 and 3, and significantly decreased ($2<0.01$) In groups 4 and 5. Oxyhemoglobin was depressed in all exposure groups

Serum Chemistry: Glucose levels increased in groups 2. 3 and 4. and was significantly increased ($p<0.01$) in group 5, as concentration and time increased (Fig 4). The remaining serum chemistry parameters were within normal ranges.

Wet/Dry Lung Weight Determinations: There was no significant difference between control and exposure groups for % of water in the lung, g of water in the lunglkg of body weight, and g of solid (dehydrated) lung/kg of body weight.

Aerosol Concentration: Aerosol concentrations are listed in Ta~4e 1. Initial aerosol concentrations were approximately 6.6 g/m3 for the low load arid 8.5 gJm3 for the high load. By the end of exposure, aerosol concentrations were approximately 0.88 g]m3 for the low load and 0.86g/m3 for the high load.