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Studies were conducted to assess hazards to personnel exposed to hexachloroethane (HCE) in an industrial setting. Acute testing in laboratory animals indicated HCE was moderately toxic orally and could produce reversible eye irritation and mild skin irritation. Percutaneous absorption appeared to have no practical significance. The subchronic vapor inhalation toxicity was determined in rats, guinea pigs, Japanese quail and dogs in a 6-week test, monitoring pulmonary function changes, teratogenicity and operant behavioral changes in addition to clinical chemistry, hematology and histopathology. Concentrations of 260 ppm HCE vapor caused severe systemic effects in dogs, moderate effects in guinea pigs and rats and almost no responses in quail. No differences were found between exposed and control animals at 3 months after termination of these exposures. Minimal toxic effects were found following similar 6-week tests at levels of 48 ppm with no detectable changes at 15 ppm of HCE.

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The toxicity of hexachloroethane in laboratory animals

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introduction

Hexachloroethane, C_2Cl_6 (carbon hexachloride; perchloroethane; 1,1,1,2,2,2-hexachloroethane; phenohep, CAS Number 000067721) is a crystalline white solid with a molecular weight of 236.74, bp 186.8°C and density of 2.09. It has a camphoraceous odor, readily sublimes without melting and is soluble in alcohol, toluene, benzene, chloroform, ether and oil; insoluble in water. Saturated air at 20°C contains approximately 650-700 ppm hexachloroethane vapor. It is used as a solvent, a camphor substitute in the preparation of celluloid, a rubber vulcanizing accelerator and in explosives, pyrotechnics and smoke devices. It is used in veterinary practice as an anthelmintic for livestock and for treatment of liver fluke in sheep.[1] In a rabbit study using ^{14}C-labelled compound, numerous metabolites were formed following administration, some of which were excreted in the urine and others into the expired air. Approximately 5 percent of a 0.5 g/kg dose appeared in the urine in a period of 3 days and from 14 to 24 percent of the dose was eliminated into the expired air. The mechanisms for the formation of the metabolites are not clear.[2]

The lowest published lethal dosages for hexachloroethane were 325 mg/kg administered intravenously to the dog and 4000 mg/kg given subcutaneously to the rabbit.[3]

There has been some experience in industry to indicate that an excessive amount of HCE dust in the air can cause irritation. Fumes of the material when handled hot have been reported to be moderately irritating to the skin and mucous membranes.[4] One authority gives the material a moderate hazard rating which may involve both irreversible and reversible changes, but not severe enough to cause death or permanent injury.[5] He reports that liver injury has been described from exposure to this material. It is given a slight explosive hazard rating, but is considered a dangerous disaster hazard since, when heated to decomposition, it emits the highly toxic gas phosgene.[5] Others note that HCE is very toxic causing more potent central nervous effects than chloroform or carbon tetrachloride, but slower in action.[6] On ingestion, it was reported that severe mucosal injury and often liver necrosis occurs. A

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literature search using the data base of the National Library of Medicine revealed that hexachloroethane had weak therapeutic activity in guinea pigs with experimental opisthorchiasis. It has been used as carriers for evaporation of natural pyrethrins and pyrethroid insecticides.

The recommended 8-hour Occupational Safety and Health Administration (OSHA) Federal standard for hexachloroethane in the workplace is an 8-hour time weighted average of 1 ppm (9.7 mg/m^3) with a skin notation. The skin note refers to the potential contribution of the overall exposure by the cutaneous route including mucous membranes and eyes.[7]

The OSHA air standard of 1 ppm was recommended due to the serious injury potential to several organ systems of the body.[8] However, because of the paucity of reports of animal or human experience, it is not known whether the 1 ppm limit is too high to prevent chronic injury or is unrealistically too low causing compliance problems and economic hardships.

The U.S. Army is presently using large quantities of hexachloroethane in various types of pyrotechnics. Industrial hygiene surveys conducted by the U.S. Army Environmental Hygiene Agency (USAEHA) suggested that the use of the OSHA standard in risk assessments had little validity in onsite inspection surveys. In addition, lack of valid experimental support for this value was apparent from literature searches. Supportive evidence for additional studies was derived from range finding animal studies that did not demonstrate significant effects at very high dosages.[9] We have studied the toxicity of hexachloroethane in various species of animals when administered acutely and when repeatedly inhaled. These data will be used as a baseline for supportive toxicological evidence of hexachloroethane action relevant to the OSHA standard for air of 1 ppm. Data from these studies will help plan experiments to further evaluate the chronic inhalation effects of exposure to this compound.

materials and methods

The toxicological evaluation of hexachloroethane was conducted by USAEHA using male New Zealand White rabbits, male Hartley guinea pigs, male and female Sprague-Dawley rats, male and female Coturnix Japonica quail and male beagle dogs. Rabbits and pregnant rats were purchased from Marland Breeding Farms, Hewitt, New Jersey and the dogs from Hazelton Laboratories, Vienna, Virginia. The rats, guinea pigs and quail were selected from our breeding colonies.

All animals were maintained on commercial chow* and water ad libitum with a 12 hour light-dark sequence. Ambient conditions were 24°C ± 2°C and 45-55 percent relative humidity. All rodents, rabbits and quail were housed in suspended wire cages while dogs were housed in individual indoor/outdoor runs.

The hexachloroethane used in these studies was obtained from Pine Bluff Arsenal, Arkansas, identified as technical grade and supplied by Hummel Chemical Company, Plainfield, NJ, under the specifications of MIL-H-235B, National Stock Number 6810-00-N00-001. The purity of hexachloroethane was ascertained by preparing a saturated solution of the solid hexachloroethane in reagent grade carbon disulfide (CS_2) and injecting this solution into gas chromatographs. Both a Hewlett-Packard 5700A GC equipped with a thermal conductivity detector and a Hewlett-Packard 5700A GC equipped with a flame ionization detector were used. Both gas chromatographs utilized a non-polar column (Supelcoport 80/100 mesh coated with 10 percent SP2100) and a polar column (Chromosorb W 80/100 mesh coated with 10 percent FFAP). Gas chromatographic conditions were varied. Chromatographic peaks were determined and integrated using a Hewlett-Packard 3352A Laboratory Data System. Impurity peaks added up to approximately 0.2 percent of sample, therefore hexachloroethane was considered to have a purity of approximately 99.8 percent.

Chemical analyses of samples from inhalation chambers were conducted using two gas chromatographic procedures. Samples were collected by drawing chamber air through a series of two midget impingers containing 10 mL of nanograde toluene at the rate of 1 liter per minute. Analyses for low concentrations (< 0.1 mg/mL) were run using a Hewlett-Packard

*Purina Lab Canine Diet, Ralston Purina Co., St. Louis, MO; Guinea Pig/Rabbit Ration and Rat and Mouse Ration, Zeigler Bros., Inc., Gardners, PA.

5730A gas chromatograph equipped with a ^{63}Ni electron capture detector. The instrument was fitted with a 91.44 cm x 0.635 cm glass column packed with Chromosorb G HP 80/100 mesh coated with 3 percent OV-1. The operating parameters were: oven temperature 125°C, inlet temperature 200°C, detector temperature 200°C, attenuation 2048, carrier gas 95 percent argon 5 percent methane, column flow rate 60 cc per minute. Peak integrations were conducted using Hewlett-Packard 3352A Laboratory Data System. Analyses for high concentrations (>0.1 mg/mL) were run using a Hewlett-Packard 5710A gas chromatograph equipped with a flame ionization detector. The instrument utilized a 3.66 m x 0.318 cm stainless steel column packed with Supelcoport 80/100 mesh coated with 10 percent SP 2100. The operating parameters were: oven temperature 170°C, inlet temperature 200°C, detector temperature 200°C, attenuation 100 x 1, carrier gas N_2, column flow rate 30 cc per minute, H_2 flow 30 cc per minute, air flow 240 cc per minute. Peak integrations were conducted using Hewlett-Packard 3352A Laboratory Data System.

toxicity in vertebrates

The toxicity evaluations of technical grade hexachloroethane using several species of animals were conducted in two study series using various parenteral administration techniques and by inhalation. They ranged from acute toxicity tests to subchronic inhalation and teratogenic studies.

parenteral administration

Hexachloroethane was tested in male rabbits for eye and skin irritation, acute oral and dermal lethality, and for clinical chemistry changes following repeated oral administration. Guinea pigs were used for skin sensitization and oral LD50 studies. Male and female rats were utilized for acute oral toxicity and female rats for teratology.[10,11]

inhalation toxicity

acute

Acute range-finding inhalation studies were conducted by exposing groups of rats to vapors of hexachloroethane for 6-8 hour periods. Vapors were generated by passing dried air over the solid material contained in a long heated tube. Groups of six male rats were exposed in a 20-liter glass chamber to room air and to vapors of hexachloroethane generated at 25°C and 50°C. Chamber flow was one L/min and nominal concentrations were calculated from the weight of material lost from the dispersion vessel. Surviving animals were necropsied 14 days after exposure and selected tissues and organs were examined.

subchronic

Subchronic inhalation studies were conducted using 2000-liter glass and stainless steel dynamic flow chambers operated at 750 liters of air per minute and under negative pressure of 0.2 cm of red oil. The hexachloroethane was vaporized into the chamber by passing dried air over reservoirs of the compound and sweeping the vapor into the entering air at the cylindrical top.

Four species of laboratory animals were exposed to analytically determined concentrations of hexachloroethane in air, 6 hours daily, 5 days per week for 6 weeks. Male and female rats, male guinea pigs, quail and dogs were observed for development of signs and histopathologic changes during the 6-week exposure and for 12 weeks following exposure. Blood from dogs was examined for development of changes in chemistries and hematology. An evaluation of pulmonary function in dogs was made before and following exposure as well as changes in oxygen consumption in rats. Groups of pregnant rats were exposed at three air concentrations each day from day 6 through day 16 of gestation while other groups were given daily oral dosages of hexachloroethane during this time period. Dams were observed for toxicity and fetuses examined for soft tissue and skeletal abnormalities. Guinea pigs were studied for sensitization potential following inhalation exposure.

behavior

Behavioral tests were performed in order to measure possible changes in both learned (conditioned avoidance) and unlearned (spontaneous motor activity) responses after hexachloroethane exposure. The behavioral

testing apparatus and procedures were the same as those described in detail earlier.[12]

Briefly, the avoidance testing apparatus consisted of a small translucent plexiglass start box with a grid bar floor which opened into a cardboard-lined safe compartment. The animals were trained to avoid shock 1-3 days prior to hexachloroethane exposure, tested for avoidance just before being placed in the chambers on day 1, and again tested immediately after removal from the chambers after 1 day, 3 weeks and 6 weeks in the high and middle dose levels or just 6 weeks in the low dose level of exposure. Training consisted of four brief exposures to unavoidable foot shock through the grid floor of the start compartment followed by two avoidance trials. During these trials individual animals were placed in the start box and allowed 20 seconds to avoid shock by running into the safe compartment before shock onset. The dependent measure of avoidance performance was the animal's latency to escape into the safe compartment after being placed in the start box.

Spontaneous motor activity was tested following 3 weeks exposure (high and middle dose levels) and 6 weeks exposure (high, middle and low dose levels). During this test, individual rats were placed inside an actophotometer (Lehigh Valley Electronics Model 1497), a darkened cylindrical drum with a metal grid floor. Activity counts were comprised of the total number of photo beam interruptions accumulated during individual 5 minute test sessions. The activity testing immediately followed avoidance testing (approximately 1 hour after removal from the exposure chambers) and required approximately 4 1/2 hours for the animals in the high and middle dose levels and 3 hours for those in the low dose levels. During these tests, representatives of each group were run in random sequence so that there were no systematic differences between the groups in order or time of testing.

teratology

Teratology studies were carried out in pregnant Sprague-Dawley rats given hexachloroethane by inhalation or by gavage from day 6 through day 16 of gestation. The day of finding sperm in the vaginal smears of rats was considered Day 0 of gestation. Twenty-two pregnant rats per dose were used and all adult animals were sacrificed on day 20 of gestation by intracardiac injection of sodium pentobarbital. The reproductive tracts were exposed by laparotomy and the number of corpora lutea, implantation sites, and resorption sites were recorded. The fetuses were removed, examined for viable conceptuses, gross abnormalities, sex, weight, and body length. All grossly abnormal fetuses as well as eight normal appearing fetuses from each dam were fixed in either Bouin's fluid[13] or 95 percent ethyl alcohol. These were further studied by freehand section for soft tissue abnormalities (2 male and 2 female)[14] or after Alizarin Red S staining for skeletal malformations (2 male and 2 female).[15]

Five groups of 22 pregnant female rats were given daily oral dosages from day 6 through day 16 of gestation of hexachloroethane (50, 100, 500 mg/kg), corn oil (5 mL/kg) and aspirin (250 mg/kg). Doses were adjusted according to individual daily body weights. Animals were housed in groups of 2 in suspended wire cages, food and water was freely available except for a daily 6-hour period when both were removed to emulate the groups receiving hexachloroethane by inhalation.

Three groups of 22 pregnant female rats were exposed from day 6 through day 16 of gestation to the vapors of hexachloroethane at concentration of 15, 48 and 260 ppm. All animals were observed for signs of toxicity and were weighed daily. Statistical treatment of the data was made using the Student's "t" test comparing the chemical treatment groups against the chamber/corn oil controls.

mutagenicity screening

A final toxicological study was performed to evaluate hexachloroethane for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. One yeast strain, *Saccharomyces cerevisiae* D4 and five bacterial strains of *Salmonella typhimurium*, TA-1535, TA-1537, TA-1538, TA-98 and TA-100 were used in evaluating mutagenic potential. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Sprague-

Dawley adult male rats pretreated five days prior to kill with Aroclor 1254, Monsanto Chemical Co., St Louis, MO. The work in this assay was performed under contract by Litton Bionetics Inc., Kensington, MD 20795 (LBI Project No. 2575).

blood chemistry

Serum clinical chemistries were performed on blood from rabbits and dogs using the Abbott Bichromatic Analyzer 100 (ABA-100),™ Abbott Diagnotics, South Pasadena, CA. Red blood cell and plasma cholinesterase activities of dog's blood were determined using the Technicon Autoanalyzer I System,™ Technicon Corporation, Tarrytown, NY. The Instrumentation Laboratory Flame Photometer Model 143, Instrumentation Laboratory, Inc., Lexington, MA, was used in analyzing for sodium and potassium.

histopathologic techniques

A complete necropsy was performed on all animals that died during the study and on all sacrificed animals. The brain, lungs, heart, liver, kidneys, spleen, eyes, bone marrow, trachea, nasal turbinates, thymus, stomach, small intestine, large intestine, pancreas, adrenal glands, urinary bladder, testes, skin, skeletal muscle and bone were taken from each animal and fixed in 10 percent neutral buffered formalin. The lungs were infused via the trachea and submerged in formalin for completion of fixation.

Sections from organs and tissues taken at necropsy were prepared for histologic examination with hematoxylin and eosin stain and examined by light microscopy for evidence of abnormalities.

Upper respiratory evaluation was accomplished microscopically by examination of longitudinal H&E stained sections of nasal turbinates. Upper respiratory inflammation was judged on the presence of polymorphonuclear leukocytes in close association with excess mucus within the lumens of the nasal passages.

results

irritation studies

Crystalline hexachloroethane (0.1 g) applied to the corneas of rabbits and allowed to remain overnight caused moderate corneal opacity, iritis and severe swelling and discharge in 5 of 6 rabbits. No signs were seen 72 hours after administration. The potential for primary skin irritation was tested by 24 hour application of 0.5 g of either the dry crystalline material or of a water paste to the intact and abraded skin of six rabbits. The dry material caused no skin irritation, and the paste caused only slight redness that disappeared after 72 hours.

The inherent sensitization potential was studied in guinea pigs with ten 0.1 mL intradermal injections of a 0.1 percent suspension of hexachloroethane in propylene glycol and saline. A concurrent group of guinea pigs was similarly tested using the known sensitizer dinitrochlorobenzene (DNCB).

A three week exposure period followed by a two week rest then challenged with a single dose of the sensitizing solution showed that hexachloroethane produced no recognizable sensitization reactions in a group of 10 animals while DNCB produced definite sensitization reactions in 10 out of 10 animals.

acute toxicity

In acute oral toxicity studies, the test compound was dissolved in corn oil (50 percent w/v) or methylcellulose (5 percent w/v). Solutions were administered by stomach tube to male and female rats, male guinea pigs and male rabbits.

In acute dermal toxicity studies the test compound was mixed into a paste with equal quantities of distilled water. The paste was applied to the shaved skin of rabbits and covered with a plastic wrap for a 24-hour period. Signs in rats at lethal dosages were ataxia, tremors and convulsions, at nonlethal dosages, red discharge from eyes. No primary skin irritation was observed in dermal LD50 rabbits.

The rabbit oral and rat intraperitoneal approximate lethal dosages (ALD),[16] dermal LD50 for rabbits and the oral LD50 for guinea pigs and rats were calculated after a 14-day observation period,[17] and are found in Table I.

enzyme induction

Studies to determine the potential of hexachloroethane for inducing liver enzyme formation were conducted using three groups of 10 male rats with a mean body weight of 125 ± 15

TABLE I
Lethal Dosages for Animals Following Single Administration of Hexachloroethane

Animal	Treatment	Diluent	Lethal Value mg/kg	(95% C.L.)	Slope
Rabbit, male	Oral ALD	Methylcellulose	>1000		
Rat, male	I.P. ALD	Corn Oil	2900		
Rat, female	Oral LD50	Corn Oil	4460	(3900-5110)	9.3
		Methylcellulose	7080	(6240-8040)	19.9
Rat, male	Oral LD50	Corn Oil	5160	(4250-6270)	6.1
		Methylcellulose	7690	(6380-9250)	8.5
Guinea pig, male	Oral LD50	Corn Oil	4970	(4030-6150)	4.7
Rabbit, male	Dermal LD50	Water paste	≥32000		

g. Each was pretreated for four consecutive days with intraperitoneal injections of a positive control (sodium phenobarbital 100 mg/kg), hexachloroethane (500 mg/kg) or solvent control (2 mL/kg corn oil). On the fifth test day each rat received an intraperitoneal injection of hexobarbital (220 mg/kg). The sleeping times were started when a rat failed to right itself and continued until the rat was able to right itself in two consecutive attempts. The mean sleeping times for each of the groups are shown in Table II. The sleeping times of the hexachloroethane treated rats were not significantly different ($p < .05$) than the corn oil controls. The phenobarbital positive control times were significantly shorter than the other treated groups and indicated that, under the test conditions described, hexachloroethane was not an inducer of hepatic microsomal enzymes.

prolonged oral administration studies

Daily oral dosages of hexachloroethane suspended in 5 percent aqueous methylcellulose (1000, 320, 100 mg/kg and control 3 mL/kg) were given for 12 days to groups of five male rabbits each to monitor the development of toxic signs and changes in blood chemistry values.

Blood was taken from the central ear artery of rabbits on treatment days 1, 4, 8, 12 and on day 4 following termination of dosing. The following parameters were measured in serum: glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), alkaline phosphatase, bilirubin, total protein, potassium and sodium. Animals were necropsied on day 4 following the last dosing and samples of the following tissues and organs were examined for microscopic lesions: eye, brain, lung, kidney, liver, spleen, heart, stomach, pancreas, large intestine, skeletal muscle, bone, urinary bladder, small intestine and testes. Daily body weights were recorded and at necropsy organs were weighed (lungs, liver, kidneys, spleen, and testes) in order to compute relative organ-to-body weight ratios for each dosage level.

Daily oral dosages of 1000 mg/kg caused a significant ($p < .05$) reduction in body weight beginning at day 7 of dosing and relative organ weight increases in liver and kidneys.

The 320 mg/kg dosages caused a significant ($p < .05$) reduction in body weight gain beginning on day 10. No other toxic signs were noted. A dosage of 100 mg/kg/day produced no changes in the criteria studied.

Liver degeneration and necrosis were observed in the two highest dosed groups (320 and 1000 mg/kg). These changes included fatty degeneration, coagulation necrosis, hemorrhage, ballooning degeneration, eosinophilic change and hemosiderin-laden macrophages and giant cells. Similar liver degeneration as described above was not seen in controls or in

TABLE II
Hexobarbital Sleeping Times of Rats Following Various Treatments

Treatment	Sleeping Time Minutes Mean ± SD
Positive control	
Sodium Phenobarbital (100 mg/kg)	38 ± 17
Test compound	
Hexachloroethane (500 mg/kg)	111 ± 32
Solvent control	
Corn Oil (2 mL/kg)	128 ± 39

TABLE III
Subchronic Inhalation Toxicity Study: Exposure Concentrations, Designated Signs of Toxicity Observed, Species and Numbers of Animals

	Designated Signs of Toxicity Observed						
	Histopathology			Behavior	Sensitization	Teratology	
Exposure Concentrations Mean ± SD	Male Rats	Female Rats	Male Dogs	Male Rats	Male G. Pigs	Rats	Male/Female Quail
Control (air only)	25	25	4	15	10	22	20
15 ± 4 ppm HCE	25	25	4	15	10	21	20
48 ± 15 ppm HCE	25	25	4	15	10	22	20
260 ± 48 ppm HCE	25	25	4	15	10	22	20

rabbits dosed at 100 mg/kg. The liver lesions in the 1000 mg/kg rabbits were more severe than those observed in the 320 mg/kg group and thus followed a dose response pattern.

An exposure-related toxic tubular nephrosis of the convoluted tubules in the corticomedullary region of the kidney was present in the 320 mg/kg and 1000 mg/kg dosed rabbits. This change was not present in the controls or 100 mg/kg dosed rabbits. A dose-response relationship was not observed between the 320 mg/kg and 1000 mg/kg dosed rabbits.

Tubular nephrocalcinosis of a minimal degree was observed in the 320 and 1000 mg/kg rabbits. This change was not seen in the controls or 100 mg/kg dosed rabbits.

Blood chemical parameters were affected only in that the potassium and glucose values were decreased significantly at the 1000 and 320 mg/kg dosage levels. A trend toward increase in alkaline phosphatase, SGOT and bilirubin levels was seen at the 1000 mg/kg dosage but was not statistically significant when compared to controls.

range finding acute inhalation vapor exposures

Rats exposed to a nominal concentration of 2.5 mg/L (260 ppm) for 8 hours showed no toxic signs during exposure or for 14 days thereafter. Body weight gain and organ-to-body weight ratios of liver, spleen, kidney, lung and testes from exposed rats were not significantly different from chamber controls. No exposure-related gross or histopathologic changes were noted in tissues and organs.

Rats exposed to a nominal concentration of 57 mg/L (5900 ppm) for 8 hours showed severe toxic signs including death. At 6 hours, one exposed rat showed a staggered gait, 2 out of 6 were dead at eight hours. Surviving rats showed a reduced body weight gain over the 14 day observation period as compared with controls. No gross lesions were observed although two of the four remaining exposed animals showed exposure-related microscopic changes. The lesions in both animals were a subacute diffuse interstitial pneumonitis of minimal to moderate severity. The vapor concentrations in this study were derived from a heated container of hexachloroethane and when this supersaturated air passed into the cooler chamber, as expected, crystals of the chemical formed throughout the exposure chamber.

subchronic inhalation toxicity

The subchronic inhalation toxicity of hexachloroethane vapor was studied in male and female rats, male dogs, guinea pigs, quail and pregnant rats.

The subchronic inhalation toxicity for hexachloroethane vapor was tested by exposing animals to control air and 3 concentrations of HCE for six hours a day, five days a week for six weeks. The groups and numbers of animals exposed and the analytical air concentrations are shown in Table III. Chamber air samples for all concentrations were analyzed by gas chromatograph as previously described. All animals used in these experiments were observed during a preliminary period and control groups exposed to chamber air were matched with each treatment group in respect to number, age, sex and body weight. Animals were weighed weekly and observed daily for general appearance and attitude. Periodic clinical and hematological examinations were made to establish baselines on dogs for pre-exposure control values and during the course of the experimental period.

TABLE IV
Avoidance and Motor Activity
Following Hexachloroethane Exposure

Exposure	Control	Hexachloroethane 15 ppm	48 ppm	260 ppm
	Avoidance Latency (median & IQ range, secs)			
Pre-Exposure	0.9 (0.7-1.6)	0.7 (0.4-2.1)	0.6 (0.5-1.4)	0.9 (0.6-1.4)
1 Day	0.8 (0.6-1.5)	*	0.6 (0.4-1.6)	1.1 (0.3-4.7)
3 Weeks	1.3 (0.6-4.2)	*	0.8 (0.4-2.5)	1.7 (0.6-8.6)
6 Weeks	2.2 (0.6-12.4)	2.5 (0.5-20.4)	3.9 (1.0-14.9)	3.3 (0.6-22.8)
	Spontaneous Motor Activity (mean ± SD)			
3 Weeks	265 (±46)	*	245 (±75)	251 (±89)
6 Weeks	163 (±74)	231 (±77)	183 (±109)	201 (±102)

*Not tested at this time interval.

Half of all animals were sacrificed and necropsied at the end of the six week exposure while the remainder were necropsied 12 weeks after termination of exposure.

subchronic biological findings: body weight and survival

260 ppm Group - Dogs exposed to this concentration developed tremors, were ataxic, hypersalivated, showed severe head bobbing, facial muscular fasciculations and held their eyelids closed during the exposure. One dog convulsed during the first exposure and died after 5 hours of exposure. Cause of death for the animal was not grossly evident. All surviving dogs showed similar signs intermittently throughout the remaining exposures with signs disappearing overnight. Evaluation of body weights of the dogs revealed no effects related to the exposures. No clinically significant changes were found in any of the blood parameters measured in dogs.

Guinea pigs showed a reduction in body weight gain beginning at the second week of exposure. Two guinea pigs died during each of the fourth and fifth week. Challenging these animals with an intradermal injection of 0.1 percent suspension of hexachloroethane in saline produced no sensitization response. The liver-to-body weight ratio was significantly higher than control.

Body weight gain of the male, but not the nonpregnant female rats, was reduced starting with the third week of exposure. All rats showed tremors, ruffled pelt and red exudate around the eyes following exposure during the fourth week with one male and one female rat found dead. During the post exposure period all signs disappeared and the body weight gain reflected that of the controls. No exposure-related gross changes were evident at necropsy. The kidney-, spleen- and testes-to-body ratios in the male rat and the liver ratio in the female rats were significantly larger than controls. The quail showed no signs, no effects in body weight and no gross organ or tissue changes at necropsy. All animals sacrificed 12 weeks after termination of exposure showed no gross exposure-related changes in tissues and organs. All signs had disappeared and body weight changes of all animals were comparable to the control.

The older male rates (12-14 weeks, 300-350 g) in the behavior group showed no toxic signs during exposure. The body weight gain was reduced compared to controls and at necropsy the relative weights of lung, liver, kidney and testes were increased over the controls.

15 ppm - 48 ppm Groups - Evaluation of body weights, gross necropsy changes and organ weights of all groups of male and nonpregnant female rats, quail, guinea pigs and dogs revealed no effects related to hexachloroethane exposure.

TABLE V
Mean Body Weights of Rats in Behavioral Tests (g ± SD)

Treatment Group	Pre-Test	2nd Week	3rd Week	4th Week	5th Week	6th Week	4 Days After Last Exposure
Control	310	335	346	374	395	408	429
	±17	±13	±19	±34	±39	±37	±44
15 ppm HCE	327	348	347	378	390	399	410
	±26	±30	±28	±31	±31	±34	±37
48 ppm HCE	313	327	344	355	377	388	386
	±17	±20	±17	±26	±28	±30	±38
260 ppm HCE	318	323	336	345*	353*	369*	380*
	±13	±15	±17	±20	±20	±22	±26

*Significantly different from control at $p < .05$.

No signs were seen in any of the above. No sensitization response was found following challenge doses of 0.1 percent in guinea pigs.

behavior

None of the rats tested displayed obvious signs of toxicity. The results of the behavioral tests are shown in Table IV. The avoidance latencies of the exposed group were compared with those of controls tested on the same day using the Mann-Whitney U test. Similar comparisons were conducted on the motor activity scores using Student's "t" test. No significant differences were found. Thus it appears that these hexachloroethane exposures produced no measurable effects on either avoidance performance or spontaneous motor activity in rats. Table V presents the mean body weights of the behavior groups of male rats throughout the study.

pulmonary function studies

Changes in compliance and resistance functions of the lungs due to inhalation of hexachloroethane vapor were determined in all groups of dogs.

Dogs were anesthetized with Surital, 4 percent, (thiamylal sodium) by intravenous injection through a pediatric catheter secured in the cephalic vein. A cuffed endotracheal tube was inserted in the trachea and the distal end connected to a Fleisch pneumotach for the measurement of airflow. Intrapleural pressure was measured by means of an esophageal balloon. Transpulmonary pressure (the difference between esophageal pressure and airway pressure derived from a lateral top at the distal end of the endotracheal tube) was used for all calculations. Both flow and transpulmonary pressure were recorded with Statham Model PM 15 differential pressure transducers. Flow and pressure signals were processed in a Buxco Electronics Inc., Pulmonary Function Computer and the following parameters were recorded on a Honeywell Model 1858 Fiber Optic recorder: flow, tidal volume, transpulmonary pressure, compliance and resistance. These parameters were measured or computed dynamically on a breath to breath basis and averaged every fifth breath. Table VI shows the pre- and post- test values for compliance and resistance in treated dogs. The mean values in all groups of dogs were not different from pre-test control values and were within reported normal limits.[18]

oxygen consumption studies

Groups of male rats were monitored for oxygen consumption as a means of estimating changes

TABLE VI
Pulmonary Functions of Compliance and Resistance in Exposed Dogs

Treatment Group	Pre-Test Compliance mL/cm H_2O/kg Mean ± SD	Pre-Test Resistance cm H_2O/mL/sec/kg Mean & Range x 10^{-1}	Post Test Compliance mL/cm H_2O/kg Mean ± SD	Post Test Resistance cm H_2O/mL/sec/kg Mean & Range x 10^{-1}
Controls	2.71 ± 0.23	0.8 (1.0-1.4)	3.57 ± 0.91	0.3 (0.1-0.4)
15 ppm HCE	3.57 ± 0.91	0.3 (0.1-0.4)	4.08 ± 2.12	0.3 (0.1-0.5)
48 ppm HCE	3.80 ± 0.92	1.2 (0.6-1.6)	3.75 ± 0.94	0.3 (0.3)
260 ppm HCE	3.37 ± 0.18	1.7 (1.6-1.8)	5.38 ± 0.72	0.7 (0.1-1.6)

in the overall metabolic state of animals inhaling hexachloroethane vapors. Rats in groups of five were tested in fifteen minute periods, three days per week prior to and following exposure.

A small water-sealed 22 liter plexiglass chamber connected to a 1 liter Collins Spirometer was used to test the rats. The total oxygen consumed expressed as liters/kg/day was calculated by dividing the volume of oxygen by total body weight and projecting to a 24-hour period. Exhaled CO_2 was contained by continuously cycling the chamber air through a container of Baralyme. The mean of each treatment group's pre-test values compared to their post-test values obtained after 6 weeks exposure are shown in Table VII.

Comparison of the grouped data indicates that a 6 week exposure to 260 ppm hexachloroethane produces a significant decrease in oxygen consumption, i.e., lowered basal metabolism rate. The test is nonspecific, but indicates an alteration in general metabolism. This change in the absence of other supportive pathology does not appear to portend a serious health hazard but may be a normal response to the inhalation of an upper respiratory irritant.

TABLE VII
Comparison of Oxygen Consumption in Rats

Treatment Group	Oxygen Consumption Liters/kg/day Mean ± SD	
	AM	PM
Control	39 ± 3	36 ± 4
15 ppm HCE	38 ± 8	32 ± 7
48 ppm HCE	39 ± 3	32 ± 5
260 ppm HCE	33* ± 3	28* ± 5

*Significantly different from control at $p < .05$.

teratogenicity

Hexachloroethane caused slight to moderate tremors during days 15 and 16 of gestation in pregnant rats receiving 500 mg/kg hexachloroethane orally. Tremors were also observed during days 12 through 16 of gestation in pregnant rats receiving hexachloroethane, 260 ppm, by inhalation.

Body weight gain of pregnant rats was significantly lower than the controls starting at day 8 of gestation for those receiving 500 mg/kg hexachloroethane orally and 260 ppm by inhalation, and day 14 for those exposed to 48 ppm.

There were no significant skeletal or soft tissue anomalies in fetuses exposed *in utero* to hexachloroethane. Fetuses from aspirin treated dams showed lower body weights and such malformations as hydrocephalus, spina bifida, and cranioschesis.

Fetuses from females treated with either 500 mg/kg hexachloroethane orally or aspirin showed significantly lower gestation indices and number of alive fetuses per dam. These groups also showed higher fetal resorption rates when compared with corn oil controls. Maternal and fetal parameters of all other groups of treated animals appeared to be within normal limits. These studies showed that hexachloroethane at dosages toxic to the dams did not produce a teratogenic effect. Dosages of hexachloroethane that were toxic to the dams resulted in a slight slowing of fetal development.

histopathologic findings

Teratology rats receiving oral (500 mg/kg) or respiratory exposure (48 and 260 ppm) to HCE had an increased incidence of a mucopurulent nasal exudate when compared with controls. The inflamatory exudate within the lumen of the nasal turbinates was observed in 85 percent of the 48 ppm group and in 100 percent of the 260 ppm exposed animals. About 70 percent of the orally exposed rats had evidence of upper respiratory tract irritation with approximately 20 percent having subclinical pneumonitis. Controls showed approximately 10 percent with a similar upper respiratory irritation and with subclinical pneumonitis.

No exposure related lesions were observed in dogs or guinea pigs.

Excess mucus without inflammatory cells was present within tissue sections of nasal turbinates in 2 of 10 (20 percent) quail in the 260 ppm exposure group. The change was not observed in tissue sections of nasal turbinates taken from 10 control quail.

Male and nonpregnant female rats sacrificed immediately after 6 week's exposure to 260 ppm had a higher incidence and severity of myocoplasma related lesions in the nasal turbinates, trachea and lung when compared to

nonexposed controls. The lesions in the exposed rats were subclinical and consisted of a mucopurulent exudate in the nasal cavities, lymphoid hyperplasia in the lamina propria of the trachea and pneumonitis rarely associated with bronchiectasis. No exposure influenced lesions were observed in male or female rats exposed at 48 and 15 ppm HCE.

The lesions of respiratory mycoplasmosis were more advanced in the 6-week exposed rats when compared with teratology rats exposed for 11 days.

No exposure-related lesions were observed in quail or dogs sacrificed 12 weeks post exposure.

No histopathologic difference was observed between control and exposed rats sacrificed 12 weeks post exposure. No inflammatory exudate was present in the nasal turbinates. The degree of subclinical tracheal lymphoid hyperplasia and pneumonitis was similar for both exposed and control animals.

mutagenicity screening

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effect at the high dose level. The low dose was in all cases, below a concentration that demonstrated any toxic effect. DMSO was used to prepare 2.5 percent stock solutions of this compound. The dose range employed for the evaluation was from 0.1 μg to 500 μg per plate. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay. The results were presented as revertants per plate for each indicator strain employed in the assay. The results of the tests conducted in the absence or the presence of the rat liver activation were all negative.

discussion

Our studies have demonstrated dose dependent systemic toxic effects resulting from inhalation exposures of animals to hexachloroethane vapor. A species difference to the toxic action of HCE vapor was evident in the inhalation study where 40 percent of the guinea pigs and one of four dogs died at 260 ppm; whereas death occurred in only 4 percent of the rats and none of the quail. Dogs were probably the more susceptible of the species exhibiting signs of intoxication at 260 ppm on day 1 of exposure and on subsequent days. The major physiological effect of HCE seemed to be stimulation of the central nervous system rather than depression as previously reported.[6] The vapors of HCE were noticeably irritating to the eyes of dogs and rats at 260 ppm.

The increased incidence of upper and lower respiratory lesions observed in rats at the end of the 6 week exposure was interpreted as resulting from test exposure potentiation of an endemic mycoplasia infection. This was apparent as the oral exposed rats also had an increased incidence of similar lesions that are characteristically associated with respiratory mycoplasmosis in rodents. This respiratory irritation appeared to be transient as no histological difference was observed between the respiratory tracts of exposed and control rats 12 weeks post exposure.

The excess mucus present in the nasal passages of quail seen histologically is interpreted as a direct transient effect of the test exposure. The change is considered transient as no inflammatory cells or tissue damage were apparent. This finding is in concert with the transient clinical findings observed during and after test exposure.

From interpretation of the oral study in rabbits it would be expected that excess chronic oral exposure would result in liver degeneration and necrosis with less severe degenerative lesions in the kidneys.

Signs were seen sooner and more frequently in pregnant female than in male or in nonpregnant female rats. This suggests that stress may be an unquantitative factor in HCE's metabolism and toxicity. Although an adaptive mechanism to the toxic effects did not express itself, e.g., enzyme induction in rats, it may indicate again only a difference in species sensitivity and response to HCE.

Hexachloroethane did not demonstrate a teratogenic effect in the fetuses from orally or vapor exposed rats. The stress of exposure was evident in the fetuses from the higher dosage levels where some slowing in total fetal development was evident.

A recent NCI bioassay study showed that ingestion of high dosages of HCE was associated with the formation of hepatocellular tumors in B6C3F1 mice but not in Osborne-Mendel rats.[19] Estimation of the true relative risk of carcinogenicity to humans can not be estimated from these studies and must wait on further confirmatory animal and epidemiological evidence.

Percutaneous absorption appears to have no practical significance. It was also only moderately toxic orally, producing reversible acute eye irritation and little or no skin irritation.

Overall, the acute and subchronic data from our studies show that hexachloroethane does not pose a hazard from skin exposure and suggest that HCE vapor may be less toxic than that suggested in the literature and implied by the OSHA standard for air of 1 ppm with a skin notation.

Although our data are in conflict with existing reports, the results from the subchronic studies can be used as a baseline for supportive toxicological evidence of HCE action relevant to the OSHA standard for air of 1 ppm. Data from our studies will also help in planning experiments to further evaluate the chronic inhalation effects of this compound. These data are also suggestive that repeated exposure to the vapors of hexachloroethane in the range of 10-20 ppm would probably not be detrimental. In the absence of data to show that human beings are more or less sensitive to HCE vapors than various species of animals, the OSHA standard may be well served with consideration of a higher permissible exposure level.

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In conducting the studies described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals (1972), U.S. Department of Health, Education and Welfare Publication No. (NIH) 74-23, revised 1972 - second printing 1974.

The experiments reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.