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**ACUTE TOXICITY IN RATS AND MICE
RESULTING FROM EXPOSURE TO HCl GAS
AND HCl AEROSOL FOR 5 AND 30 MINUTES**

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The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

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13. ABSTRACT Rats and mice have been subjected to HCl vapor and HCl aerosol for periods of 5 and 30 minutes to determine the acute toxicity of HCl. To simulate as nearly as possible the exposure conditions at rocket engine test firing sites, HCl vapor was mixed with a saturated water droplet mist in a Longley exposure chamber to obtain HCl aerosol atmospheres. The results indicate that HCl vapor and HCl aerosol have comparable toxicity in rats and mice, respectively. The results of the present study were compared to those obtained in another study of HCl vapor toxicity in rabbits and guinea pigs, and it was found that HCl had the same degree of toxicity in mice, rabbits, and guinea pigs, while rats were considerably more tolerant to the effects of HCl. Key Words: HCl Aerosol HCl Vapor Toxicity Rodents Rats Mice			

FOREWORD

This report on the comparison of HCl vapor and aerosol acute toxicity is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit (THRU). The experimental program has been accomplished on behalf of the Air Force by Systemed Corporation under Contract F33615-70-C-1046. The study was performed under Project 6302 "Toxic Hazards of Propellants and Materials," Task 630201 "Toxicology of Propellants and Materials," Work Unit Number 63020110. K. C. Back, PhD, Chief of the Toxicology Branch, was the technical contract monitor for the Aerospace Medical Research Laboratory.

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This technical report has been reviewed and is approved.

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TABLE OF CONTENTS

<u>Section</u>		<u>Page</u>
I	INTRODUCTION	1
II	METHODS AND MATERIALS	2
	GAS EXPOSURES	2
	AEROSOL EXPOSURES	2
	ANIMALS	3
	ANALYSIS OF CHAMBER HCl CONCENTRATIONS	3
	EVALUATION OF THE AEROSOL GENERATOR	3
III	EXPERIMENTAL RESULTS	5
	TOXIC SIGNS	5
	PATHOLOGY	5
IV	DISCUSSION	6
	REFERENCES	17

LIST OF TABLES

<u>Table</u>		<u>Page</u>
I	Particle Size Distribution of Aerosol Droplets	7
II	Summary of Acute Toxicity Data for Exposure to HCl Vapor and HCl Aerosol	8
III	HCl Vapor Toxicity in Rats (5-Minute Exposure)	9
IV	HCl Vapor Toxicity in Mice (5-Minute Exposure)	10
V	HCl Vapor Toxicity in Rats (30-Minute Exposure)	11
VI	HCl Vapor Toxicity in Mice (30-Minute Exposure)	12
VII	HCl Aerosol Toxicity in Rats (5-Minute Exposure)	13
VIII	HCl Aerosol Toxicity in Mice (5-Minute Exposure)	14
IX	HCl Aerosol Toxicity in Rats (30-Minute Exposure)	15
X	HCl Aerosol Toxicity in Mice (30-Minute Exposure)	16

SECTION I

INTRODUCTION

Hydrogen chloride (HCl) is one of the combustion products formed during the test firing of certain rocket and missile engines. Since the vicinity in which these firings take place is occasionally engulfed with a heavy fog, it was of interest to the Air Force to determine the exposure hazard to Air Force personnel conducting these tests as well as inhabitants of nearby towns who may be placed at risk during either clear or foggy days under prevailing weather conditions.

Very little information exists in the literature regarding inhalation toxicity due to HCl. Machle et al. (1942) described exposures of rabbits and guinea pigs to HCl gas in which 100% mortality was observed after exposure to 6.5 mg/liter (4416 ppm) for 30 minutes. They observed that deaths occurring either during or shortly after the exposures were due to acute respiratory damage, and that delayed deaths were caused by secondary infections resulting from the insult of exposure. They also observed hepatic lesions in both rabbits and to a greater extent in guinea pigs.

The present study was undertaken to determine LC_{50} values for exposure to HCl gas for 5 and 30 minutes, and to an HCl aerosol for the same exposure periods. This would accomplish two objectives; first, to define short-exposure toxicity levels for HCl in either state and second, to determine whether the aerosol form is more or less dangerous than the vapor itself.

SECTION II

METHODS AND MATERIALS

GAS EXPOSURES

HCl gas was supplied in a cylinder obtained from the Matheson Company. For the exposures to HCl vapor, the HCl was metered through a corrosion resistant gas regulator and a flowmeter, then introduced into a modified Rochester Chamber which has been previously described (Haun et al., 1969). The input air to this chamber was pre-dried and metered at a constant rate of 10 cfm for all exposures. The desired concentration of HCl was established within the chamber and the animals to be exposed were then introduced into the chamber by means of sliding cage "drawers" in the walls of the chamber (DiPasquale and Davis, 1971). This technique was particularly important in the case of the 5-minute exposures since exact timing of the exposure could be carefully controlled. The concentration of HCl within the exposure chamber was monitored continuously during all exposures.

The animals were observed for visible signs of toxicity and mortality during exposure, and for a 7-day postexposure observation period. Gross and histopathological examinations were made of representative samples of animals used in the exposures to both the HCl gas and HCl aerosol.

AEROSOL EXPOSURES

To simulate as nearly as possible the conditions under which the HCl aerosol might be formed at the rocket test site, HCl gas was introduced into an exposure chamber which was filled with a saturated water droplet mist. The chamber used for the exposures to HCl aerosol was a Longley Chamber, which has four equal quadrants. Only one of these quadrants, having a volume of approximately 16 cubic feet, was used in creating the aerosol. To produce the saturated water droplet mist (simulating a heavy fog cloud) a DeVilbiss ultrasonic nebulizer Model 800, with modifications, was used. Using a three-inch diameter tubing instead of the tubing provided with the nebulizer and attaching it over the entire top of the nebulizer chamber, a greater output of aerosol mist could be produced without significantly altering droplet size. With this technique it was possible to introduce a sufficient quantity of water droplets to reach the saturation point. This occurred with a water flow rate of 6.8 ml/minute.

The water aerosol was introduced into the chamber directly below the air input opening at the top of the quadrant. Pre-dried air was used to exclude any source of water other than that coming from the nebulizer. A chamber flow rate of 10 cfm was used for all exposures. The HCl gas was introduced into the air input line just prior to its entry into the chamber. The aerosol appeared to be very uniform in size and in chamber distribution.

The Longley Chamber used in this study was also equipped with sliding cage drawers in its wall, and the same exposure technique as described for HCl vapor was also employed when animals were exposed to HCl aerosol.

ANIMALS

The animals used in these experiments were 25 to 30 gram male CF1 mice (ICR derived) and 250 to 300 gram male CFE rats (Sprague-Dawley derived) obtained from Carworth Farms, Incorporated. Exposure groups consisted of 10 rats or 10 mice per exposure to HCl aerosol and 10 rats or 15 mice per exposure to HCl vapor. Quality control studies on both rats and mice during the quarantine period showed the animals to be in good health.

ANALYSIS OF CHAMBER HCl CONCENTRATIONS

The HCl concentrations within the exposure chamber were continuously monitored throughout all animal tests. In both gas and mist exposures a measured air sample flow was passed through a gas scrubber column located adjacent to the sliding animal cage. The HCl was collected in distilled water and analyzed in a flow cell using a chloride ion specific electrode. Collection of the HCl in distilled water was shown to give complete recovery using gas bag standards. The electrode was calibrated with bag standards before each animal run and was suitable for use in the concentration range of 1000-100,000 ppm (1.47-147 mg/liter) HCl. All animal exposures were well within this range.

EVALUATION OF THE AEROSOL GENERATOR

To determine that the modified DeVilbiss ultrasonic nebulizer Model 800 produced a droplet size of 0.5 to 9.0 microns as specified by the manufacturer, aqueous droplets were collected on a cellulose acetate filter and the size determined by a microphotograph of the particles following the procedure of Vooren and Meyer (1971).

To render the droplets visible on the filter, a 5% congo red solution was generated using the assumption of Vooren and Meyer that the 5% solution would have the same particle size as water alone because of the very slight differences in density, viscosity, and surface tension. The aerosol was generated into one quadrant of a Longley Chamber having a volume of 16 cubic feet. The nebulizer was modified by attaching a three-inch flexible hose over the top of the nebulizing chamber and the aerosol generated from the nebulizer while on setting #1. The air was exhausted from the chamber at 10 cfm.

The air sample was drawn through a Metricel filter (Type GA-4) with an average pore size of 0.8μ . A second filter inserted into the sampling line showed no signs of congo red which indicated that the first filter collected 100% of the droplets. The sample was drawn through the filter at 4 liters/minute (296 cm/minute), a rate calculated to be capable of collecting droplets up to 30 microns in diameter.

After sampling, the filter was covered with immersion oil to prevent evaporation and a consequent change in droplet size. The filter was then placed under the microscope and photographs of the filter and of the stage micrometer were enlarged to the same degree.

The droplets were measured by use of a plastic overlay on which concentric circles had been drawn corresponding to droplet diameters of 1, 2, 5 and 10 microns. These measurements were calculated directly from the microphotograph of the stage micrometer. A pinhole was made in the center of the circles on the template, enabling the technician to pierce each droplet image counted.

A total of 456 droplets were measured by the above method (table I)¹. No attempt was made to classify the droplets below one micron in diameter and they were included in the one micron group. Sixty-four percent of all of the droplets were one micron or less in diameter. Only 6.5% of all droplets measured exceeded two microns, and none was found larger than five microns in diameter.

The results shown in table I indicate that this nebulizer does produce droplets in the range specified by the manufacturer. Because no droplets larger than five microns in diameter were found, we can assume that all droplets produced by this nebulizer, under these conditions, are within a respirable range for rodents.

¹Tables are located on pages 7-16.

SECTION III

EXPERIMENTAL RESULTS

The results of 5 and 30 minute exposures of rats and mice to HCl vapor alone are shown in tables III through VI and to HCl aerosol in tables VII through X. To facilitate comparison of the data, the LC_{50} values for all exposures are summarized in table II. The values listed for all HCl aerosol data are expressed not only in mg/liter but also in parts per million (ppm) for comparison with the vapor results.

TOXIC SIGNS

Toxic signs during exposure to HCl vapor and HCl aerosol were essentially identical. The HCl was extremely irritating to the eyes, mucous membranes, and exposed areas of skin. Ulceration of the scrotum was a common finding in both rats and mice. The animals were observed to groom and preen excessively, and usually exhibited a rapid, shallow breathing pattern by the end of the exposure. The fur had a "singled" appearance and texture which was more noticeable with the aerosol than with the vapor alone. There was evidence of corneal erosion and clouding in both species when exposed to either the vapor or the aerosol.

PATHOLOGY

Gross examination of the animals that died during or shortly following exposure showed that the respiratory tract was the primary target for the HCl damage. Moderate to severe alveolar emphysema, atelectasis, and edema of the lung were observed, and occasional "spotting" of lung tissue was also found. The upper respiratory tract was severely irritated, and the epithelial tissue of nasal and tracheal passages was badly damaged in both species. Animals surviving up to 14 days postexposure showed at necropsy that recovery from the exposure was not complete. Often the lungs, which had an abnormal gray color, failed to collapse upon opening of the chest cavity. There was also evidence of consolidation of lung tissue and some residual alveolar damage.

The death patterns observed with HCl vapor and HCl aerosol were similar, with delayed death being observed in both cases. In general, more delayed deaths occurred in mice than in rats, but there was no significant shift between the vapor and aerosol exposures in the pattern of time to death. The actual death patterns may be seen by referring to tables III through X, where times of death are detailed for each exposure.

SECTION IV

DISCUSSION

The acute effects of exposure to HCl were similar to those observed with exposure to other pulmonary irritants such as OF_2 (Davis, 1970), HF (DiPasquale and Davis, 1971), ClF_6 (Darmer, 1971) and ClF_3 (Dost et al., 1967). Deaths due to HCl were attributed primarily to its effect on the respiratory tract. There were few differences in observed symptomatology between exposure to HCl vapor and HCl aerosol. The results of this study seem to indicate little difference in the acute toxicity of HCl vapor and HCl aerosol in rats and mice, as the 95% confidence limits for each LC_{50} overlap in every case when comparing vapor to aerosol for the same length of exposure.

The effects of HCl vapor and HCl aerosol on rats and mice were similar to those reported by Machle et al. (1942) for exposure of rabbits and guinea pigs to HCl vapor. These workers reported that an exposure to 6.5 mg/liter (4416 ppm) for 30 minutes was 100% fatal to all rabbits and guinea pigs exposed. In the present study, exposure to HCl aerosol of 6.52 mg/liter (4432 ppm) for 30 minutes was fatal to 100% of the mice exposed (see table X).

An exposure of mice to 4076 ppm (6.0 mg/liter) HCl vapor for 30 minutes resulted in the death of 13 of 15 animals (see table VI). This indicates that the toxicity of HCl as either a vapor or an aerosol is very similar in mice, rabbits, and guinea pigs; however, the actual exposures of rabbits and guinea pigs to HCl aerosol would have to be conducted to validate this premise. On the other hand, none of the rats exposed to 6.6 mg/liter (4481 ppm) HCl aerosol for 30 minutes died (see table IX), and exposure to an HCl vapor concentration in this same range would be expected to kill only a small percentage of rats also (see table V). Therefore, rats are considerably more tolerant to HCl than any of the other three species mentioned here.

These experiments have shown that there is no significant difference in the acute toxic response of rodents exposed to the vapor and aerosol forms of HCl.

Table I

Particle Size Distribution of Aerosol Droplets

(456 Droplets Measured)

<u>Droplet Size Range (microns)</u>	<u>Number of Droplets</u>	<u>Percentage of Droplets</u>	<u>Cumulative Percentage of Droplets</u>
≤1	292	64.0	64.0
1-2	134	29.4	93.4
2-5	30	6.5	99.9
>5	0		

Table II

Summary of Acute Toxicity Data for Exposure to
HCl Vapor and HCl Aerosol

Aerosol

	<u>5-Minute LC₅₀</u>		<u>30-Minute LC₅₀</u>	
	<u>ppm</u>	<u>mg/liter</u>	<u>ppm</u>	<u>mg/liter</u>
Rat	31,008	45.6	5,666	8.3
Mouse	11,238	16.5	2,142	3.2

Vapor

Rat	40,898	--	4,701	-
Mouse	13,750	--	2,644	-

Table III

HCl Vapor Toxicity in Rats
(5-Minute Exposure)

Concentration (ppm)	Mortality Ratio	Time of Deaths (No. /Day)							
		<u>0</u> *	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
30,000	0/10								
32,255	1/10			1					
39,850	6/10		2	3			1		
45,200	7/10		5	2					
57,290	9/10		5	4					

Calculated LC₅₀ 40,989 ppm (34,803-48,272)

*Deaths during exposure.

Table IV

HCl Vapor Toxicity in Mice
(5-Minute Exposure)

Concentration (ppm)	Mortality Ratio	Time of Deaths (No. /Day)							
		<u>0</u> *	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
3,200	1/10						1		
5,060	1/10				1				
6,145	2/10			1		1			
6,410	0/10								
7,525	6/10			1	2			3	
8,065	2/10				2				
9,276	5/10				2	3			
13,655	6/10		2		3			1	
26,485	13/15	1	3	2	1	2	4		
30,000	13/15	3	4		1	1	4		

Calculated LC₅₀ 13,745 ppm (10,333-18,283)

*Deaths during exposure.

Table V

HCl Vapor Toxicity in Rats
(30-Minute Exposure)

<u>Concentration</u> (ppm)	<u>Mortality</u> Ratio	<u>Time of Deaths (No. /Day)</u>							
		<u>0*</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
2078	0/10								
2678	1/10	1							
3071	0/10								
5180	5/10	2	1			1	1		
6068	8/10	7	1						
6681	10/10	7		1		2			

Calculated LC₅₀ 4701 ppm (4129-5352)

*Deaths during exposure.

Table VI

HCl Vapor Toxicity in Mice
(30-Minute Exposure)

<u>Concentration</u> (ppm)	<u>Mortality</u> Ratio	<u>Time of Deaths (No. /Day)</u>								
		<u>0*</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>
410	0/15									
1134	2/15				2					
2678	8/15		4		2		1		1	
2721	4/15		1	2	1					
2942	12/15	1			2	4	5			
3071	6/15		2	2	2					
4045	11/15		4			1	1		3	2
4076	13/15		4	1	1		3	4		
5363	14/15	3	5	2		1	2	1		

Calculated LC₅₀ 2644 ppm (2264-3086)

*Deaths during exposure.

Table VII

HCl Aerosol Toxicity in Rats
(5-Minute Exposure)

Concentration		Mortality Ratio	Time of Deaths (No. /Day)							
<u>mg/liter</u>	<u>ppm</u>		<u>0*</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
9.7	6,571	0/10								
28.4	19,312	1/10			1					
37.3	25,324	3/10		2		1				
43.6	29,648	6/10		1	4	1				
57.0	38,746	6/10		2	1			3		
60.1	40,810	7/10		2	4					1
91.3	62,042	10/10		8	1	1				

Calculated LC₅₀ 45.6 mg/liter (39.5-52.8)
31,008 ppm (26,824-35,845)

*Deaths during exposure.

Table VIII

HCl Aerosol Toxicity in Mice
(5-Minute Exposure)

Concentration		Mortality Ratio	Time of Deaths (No. /Day)							
<u>mg/liter</u>	<u>ppm</u>		<u>0*</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
13.3	9,058	3/10				1	2			
14.8	10,059	3/10						2	1	
17.7	12,104	5/10			1		1	1	2	
22.0	14,913	9/10		1		2		1	4	1
25.0	17,000	9/10		2	1	2	4			
27.6	18,773	10/10		2	1	5		2		

Calculated LC₅₀ 16.5 mg/liter (14.8-18.5)
11,238 ppm (10,006-12,547)

*Deaths during exposure.

Table IX

HCl Aerosol Toxicity in Rats
(30-Minute Exposure)

Concentration		Mortality Ratio	Time of Deaths (No./Day)							
<u>mg/liter</u>	<u>ppm</u>		<u>0*</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
4.3	2910	1/10			1					
6.6	4481	0/10								
9.0	6078	6/8	1	3	1			1		
9.8	6640	8/10	3	1	2		2			

Calculated LC₅₀ 8.3 mg/liter (7.2-9.7)
5,666 ppm (4,855-6,614)

*Deaths during exposure.

Table X

HCl Aerosol Toxicity in Mice
(30-Minute Exposure)

Concentration		Mortality Ratio	Time of Deaths (No. /Day)							
<u>mg/liter</u>	<u>ppm</u>		<u>0*</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
1.8	1204	2/10				1		1		
3.1	2127	5/10		3				1		1
3.8	2557	5/10					3		2	
4.0	2720	5/10		3		2				
4.3	2910	9/10	1	4	1		1	2		
4.5	3036	7/10	1	4	1	1				
6.5	4432	10/10	4	3	2		1			

Calculated LC₅₀ 3.2 mg/liter (2.6-3.8)
2142 ppm (1779-2580)

*Deaths during exposure.

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