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Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460
Attn: Section 8(e) Coordinator (CAP Agreement)

Re: TSCA Section 8(e) Reporting - CAP Agreement 8ECAP

Gentlemen:

submitting this study pursuant to the Compliance Audit Program (CAP) under Agreement No. 8ECAP. This study is categorized as a Unit II.B.2.b CAP Agreement submission. The report is entitled Acute LC₅₀ of Bromochlorodimethylhydantoin Dust in Sprague-Dawley Rats. The identity of the chemical being tested is Bromochloro-5,5-dimethyl-2,4-imidazolidione (CASRN 126-06-7). This letter confirms that is the substance being cited.

This study is being submitted to complete an earlier TSCA Section 8(e) submission and we believe it should not be considered in the CAP Consent Agreement since the results are similar to those submitted earlier for DBDMH and should be expected by the Agency.

The Acute Inhalation Toxicity Study was performed on ten Sprague-Dawley rats (m/f) at each of four nominal concentrations (0.62, 1.03, 1.20 and 2.60 mg/l). The four hour inhalation exposure determined the acute LC₅₀ to be 1.88 mg/l. Necropsies indicated no lesions attributable to the test substance exposure.

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If I may be of further assistance, my phone number is

Sincerely yours,

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FOOD AND DRUG
Research LABORATORIES, INC.

REPORT

ACUTE LC₅₀ OF BROMOCHLORODIMETHYLHYDANTOIN DUST
IN SPRAGUE-DAWLEY RATS

Kenneth A. Voss, Ph.D.
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Study Director

FDRL Study No.: 6711_A

Submitted to:

Peter J. Fucci, Ph.D.
Director of Toxicology

Date: August 10, 1981

Richard S. Parent, Ph.D.
Vice President, Director
Haverly Research Center

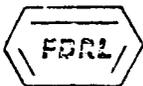
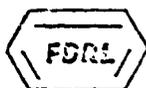


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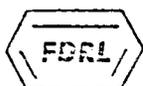
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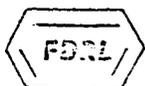


FDRL Study No. 6711_A

ABSTRACT

The purpose of this study was to determine the acute LC_{50} of Bromochlorodimethylhydantoin on the basis of nominal concentration. Five groups of Sprague-Dawley rats (five animals per sex per group) were exposed to the test article at nominal concentrations of 0.00, 0.62, 1.03, 1.20 and 2.60 mg/L for four hours. Average particle size (mass median diameter) ranged from 4.1 to 5.4 μm and the geometric standard deviation ranged from 2.0 to 2.1. The average fraction of particles less than 10 μm in diameter ranged from 80 to 91 percent for the test article exposures.

Under conditions of the study, the LC_{50} of Bromochlorodimethylhydantoin was 1.88 mg/L. The upper and lower 95% confidence intervals were 2.77 and 1.48 mg/L, respectively and the slope of the curve was 5.42. Exposure to the test article caused transient decreased body weight gain in animals exposed to 1.03 and 1.20 mg/L. Labored breathing was observed in all animals exposed to the test article. Nasal discharge and decreased activity were seen in animals exposed to 1.03 mg/L or more Bromochlorodimethylhydantoin.



INTRODUCTION

The purpose of this study was to determine the acute LC₅₀ (based on nominal concentration) of an aerosol of Bromochlorodimethylhydantoin dust. The study was run according to the Regulations for Good Laboratory Practices as defined by the FDA (21 CFR Part 58) and FDRL Standard Operating Procedures. The study was authorized on September 22, 1980

The study was conducted from June 18, 1981 through July 15, 1981 under the supervision of Kenneth A. Voss, Ph.D., Staff Toxicologist/Pathologist. All data and pertinent records are retained in the FDRL archives and are available upon request.

MATERIALS AND METHODS

Test Article

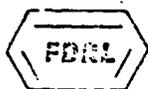
The test article was received at the Waverly Research Center on December 11, 1980.

Description: white powder

Spencer's Identification: Bromochlorodimethylhydantoin

FDRL Identification: 80-0177

The test article was ground to a particle size of $\leq 10 \mu\text{m}$ at Fluid Energy Processing and Equipment Company, Hatfield, PA) prior to receipt at FDRL. The test article was packed into 500 mL bottles in 10 g (0.135 U.S. cup) or 50 g (1.0 L) increments. The density of the particles was determined by a laboratory press. The density of the particles was found to be approximately 1.1 g/cm³.

Animals

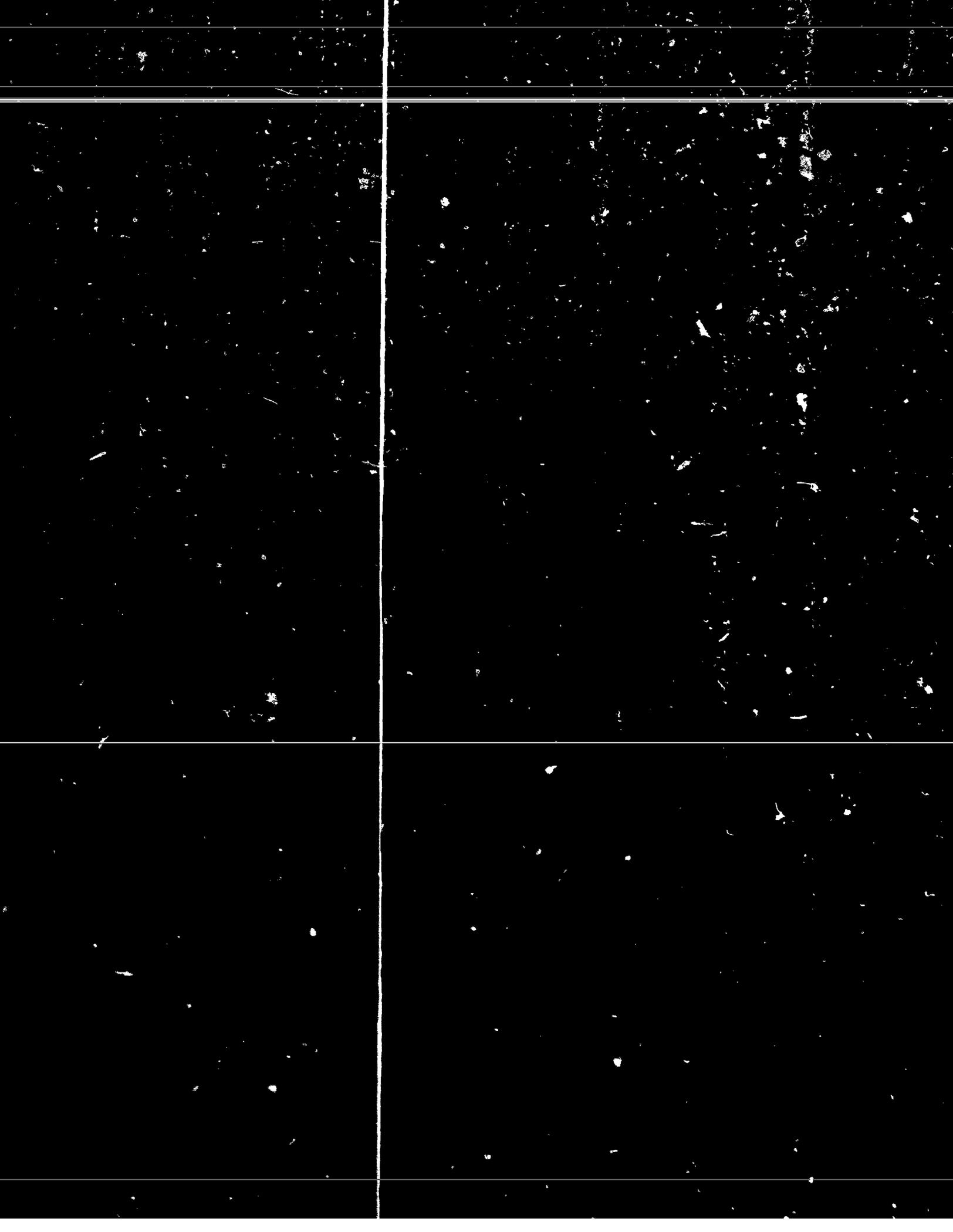
Twenty-five male and twenty-five female Sprague-Dawley derived rats were used in this study. They were obtained from Blue Spruce Farms, Altamont, NY, and upon receipt were housed in groups of three. Food (NIH Open Formula 07, Zeigler Brothers, Gardners, PA) and fresh tap water were available ad libitum. The animals were acclimated to the laboratory environment for a minimum of five days prior to exposure. Animals with evidence of disease or abnormality were discarded prior to exposure and replaced by healthy, fully acclimated animals.

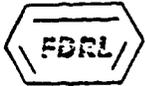
The animals were divided into five groups, each containing five males and five females, and exposed to the test article for four hours as outlined below:

Group	Animal Nos.		Test Article Exposure (mg/L nominal concentration)
	Males	Females	
1	6711001-005	6711006-010	0.62
2	6711011-015	6711016-020	2.60
3	6711021-025	6711026-030	0.00
4	6711031-035	6711036-040	1.20
5	6711041-045	6711046-050	1.00

Each animal was identified by ear notching, as well as a color coded cage tag.

During exposure, the animals were housed in wire mesh cages and provided food and water. The animals were again provided food and water subsequent to exposure.





Observations

The animals were observed for pharmacotoxic signs during exposure, subsequent to exposure on day one, and twice daily for fourteen additional days (2-15). Body weights were recorded prior to exposure (day 1) and on days 3, 4, 5, 8, and 15. Animals dying during the study and all animals sacrificed at termination were subjected to gross necropsy. All major organs were examined for signs of abnormalities and the following tissues excised and preserved in 10% neutral buffered formalin:

- whole head with nasal passages
- trachea
- bronchi
- lungs
- liver
- kidneys

Generation and Exposure

A schematic of the exposure system is presented in Figure 1. The test article was delivered into a 1311 L cubic stainless steel and glass exposure chamber (Basil Equipment Company, Wilson, NY) by means of a Spengler Particulate Generator (Charles Spengler and Associates, Cincinnati, OH) fitted with either a 0.33 or 1.00 L particulate cup. Ambient air was drawn through the chamber by means of the exhaust system driven by a 3-phase Baldor Industrial Motor (Baldor Electric Company, Fort Smith, AR). Air flow was monitored by a magnetic gauge (Dwyer Instruments, Inc., Michigan City, IN) calibrated by a mass flowmeter (Teledyne Hastings Raydon Mass Flow Meter).



element 8060, Transducer and counter 689, Hampton, VA) and was sufficient to provide the animals with fresh air. After passing through the chamber, the aerosol was vented through a filtration system consisting of a glass-fiber pre-filter, micretain[®] HEPA filter (Cambridge Filter Corporation, Syracuse, NY) and an activated charcoal bank (Fisher Scientific, Pittsburgh, PA).

The animals remained in the chamber for a minimum of thirty minutes following exposure. During this period the chamber was operated at the same flow rate using clean air only.

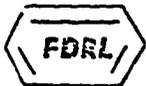
Exposure Concentrations

The nominal and actual exposure concentrations were determined. Nominal concentration was calculated by dividing the net weight of test article used by the total air utilized for the exposure.

Actual chamber concentrations were determined by gravimetric analysis. A known volume of chamber air was drawn through a preweighed filter (Gelman 25 mm glass fiber filter, 99.7% efficient at 0.3 μ m) held in an open face filter holder. The actual concentration was then calculated by dividing the filter-weight gain by the sample volume. Two samples per hour were taken and the time weighted average concentration calculated for each exposure (Tables 2-5).

Particle Size Determination

Particle size determinations (12-14 per exposure) were made using a Collis-Cordell Cascade Impactor. Gelman metricel



membranes (25 mm) were used as a collection surface on each stage of the impactor. The change in weight of each filter was determined subsequent to sampling, the mean cumulative percent for each stage calculated, and a lognormal distribution curve plotted for each exposure group (Tables 6-3 and Figures 2-5). The geometric mean size, geometric standard deviation, and percent of the particles less than 10 μm in size were estimated from the curve.

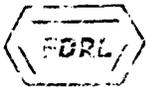
Chamber Temperature and Relative Humidity

A Taylor Instrument dry bulb/wet bulb hygrometer was used to monitor chamber temperature and relative humidity. Readings were taken prior to initiation of exposure and hourly thereafter.

Statistical Analysis

The LC_{50} was computed on the basis of nominal concentration, with males and females combined. Computations were done using Finney's Probit Analysis (Statistical Methods in Biological Assay, second edition. London: Griffin Press, 1971).

Group body weight data were computed for each sex as group mean percent of initial body weight and analyzed using a one-way analysis of variance (ANOVA) (Snedecor, G.W. and Cochran, W.C. Statistical Methods, Iowa State University Press, Ames, Iowa, 1967). Differences between groups were established using the Least Significant Differences Test.



RESULTS AND DISCUSSION

Generation and Exposure Data

Generation and exposure data are summarized in Table 1. All exposures lasted 240 minutes. One exposure (group 1) was run at an air flow of 500 L/minute. The other groups were run at 350 L/minute. This change was necessary to balance test article utilization with air flow, so that the desired nominal concentrations could be more easily obtained. In all cases, the relative equilibrium time to reach maximum concentration was less than ten percent of the exposure duration. Gravimetric analyses indicated that the measured chamber concentrations were similar for groups 2, 4, and 5. Because the range of nominal concentrations tested was narrow, small variations in sampling could hide any real differences in actual chamber concentrations among these groups. Secondly, insensitivity of gravimetric analysis at low chamber concentrations, (0.1-0.3 mg/L) probably contributed to this observation.

Mortality and LC₅₀

A summary of group mortality appears below:

Nominal Concentration (mg/L)	Group	Mortality ^a		
		Males	Females	Combined
1.00	1	0/5	0/5	0/10
1.5	2	0/5	0/5	0/10
2.0	3	0/5	2/5	2/10
3.0	4	0/5	0/5	0/10
4.0	5	3/5	3/5	8/10

^aNumber of animals dead/total number of animals.

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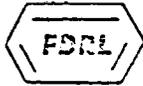
Probit analysis of the data computed the combined LC₅₀ to be 1.88 mg/L (nominal concentration). The upper and lower 95% confidence intervals were 2.77 and 1.48 mg/L, respectively. The slope of the curve was 5.42. Probit data is given in Appendix IV.

Daily Observations

Significant daily observations included labored breathing, nasal discharge (including apparently dried blood), and decreased activity. Following exposure, labored breathing was observed in five males and four females exposed to 1.03 mg/L and all animals exposed to 1.20 mg/L. Labored breathing was also noted during exposure in animals of group 2 (2.6 mg/L nominal concentration). Nasal discharge was noted among two males and two females exposed to 1.03 mg/L and five male and five females exposed to 1.20 mg/L. This probably resulted from test article induced irritation of the nasal mucosa. Decreased activity of the animals was apparent during exposure in all groups, except controls (group 3). Incidental findings included dried blood around the eyes (three animals), urinary incontinence (four animals), and ataxia (one animal). All individual daily observations are given in Appendix II.

Body Weight

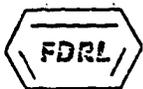
The group mean body weight data is summarized as Table 10. Slight decreases in body weight were initially noted in males and females, most likely resulting from the mechanics of



exposure (animal handling, placement in chambers, etc.). Significantly decreased body weights were observed in both males and females exposed to both 1.03 and 1.20 mg/L on days three and four. Females exposed to 1.03 mg/L also had significantly decreased body weights on days five and eight. No statistically significant differences were observed at termination of the study in group mean percent initial body weight of any group, indicating that the effect was transient. Due to the deaths of the animals in the high dose group, no statistical analysis of body weight data could be performed. All individual body weights are given in Appendix III.

Gross Pathology

Gross necropsies were performed on all animals. Post mortem examination did not ascertain the presence of any lesions attributable to test article exposure. Several incidental findings including hollow kidney (one animal), red or swollen cervical lymph nodes (seven animals) and red spots on the thymus (one animal) were reported. Hollow kidney corresponds to hydropelvis, a condition occasionally observed in the laboratory rat. Discoloration of the tissues caused by post mortem blood distribution is a common post mortem observation of no toxicological significance. All individual necropsy data are given in Appendix



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FDRL Study No. 6711_A

CONCLUSION

The LC_{50} of Bromochlorodimethylhydantoin was 1.88 mg/L (nominal concentration) for males and females combined. The upper and lower 95% confidence intervals were 2.77 and 1.43 mg/L, respectively and the slope of the curve was 5.42. Signs of toxicity among surviving animals during the first week following exposure included decreased weight gain, observed labored breathing, and nasal discharge.



FDRL Study No. 6711_A

Appendix Ia
Generation and Exposure Data
(Group 1)