



VELSICOL CHEMICAL CORPORATION

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pg 20

December 17, 1985

FVI-CTS-1285-0454 SUPPLEMENT
SEQUENCE B

Document Control Officer
Chemical Information Division
Office of Pesticides and Toxic Substances (TS-793)
EPA
401 M Street S.W.
Washington, D.C. 20460

12/24/85
TGB/CSE

SUBJECT: Chlorendic Anhydride (CAS Registry No. 115-27-5)
Additional Toxicological and Epidemiological Studies -
For your Information.

Dear Sir or Madam,

Velsicol Chemical Corporation is herein submitting two copies of a number of toxicology and epidemiology studies which either directly or indirectly involve chlorendic anhydride for the Agency's information.

It is our opinion that these studies may be of interest to the Agency in the light of the results of the recently released NTP report on chlorendic acid and the fact that chlorendic anhydride can be converted to chlorendic acid.

The studies herein submitted are:

1. Shindell and Associates, Milwaukee, WI, "Report of Epidemiologic study of the Employees of Velsicol Chemical Corporation Plant Memphis, Tennessee, January 1952-December 1979", March 1981.
2. Leong, B. K., "Chlorendic Anhydride. Acute Inhalation Toxicity Study in Rats", International Research and Development Corp., Mattawan, MI, Report No. 163-512, August 10, 1978.
3. Wazeter, F. X., and Goldenthal, E. I., "Chlorendic Anhydride. Acute Toxicity Studies in Rats and Rabbits", International Research and Development Corp., Mattawan, MI, Report NO. 163-140, November 22, 1972.

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- ✓ 4. Witherup, S., Stemmer, K. L. and Schlecht, H., "The Toxicity of Chlorendic Acid and Chlorendic Anhydride", The Kettering laboratory, Cincinnati, OH, June 25, 1965.
- ✓ 5. Jagannath, D. R., "Mutagenicity Evaluation of Chlorendic Anhydride," Litton Bionetics, Inc., Kensington, MD, Project NO. 20838, October 1977.
- ✓ 6. Matheson, D. W., "Mutagenicity Evaluation of Chlorendic Anhydride in the Mouse Dominant Lethal Assay," Litton Bionetics, Kensington MD, Project 20862, February 1978, revised June 1978.
- ✓ 7. Goldenthal, E. I., "Chlorendic Anhydride. Teratology Study in Rats", International Research and Development Corp., Mattawan, MI, Report No. 163-535, November 8, 1978.
- ✓ 8. Goldenthal, E. I., "Chlorendic Anhydride. Twenty-Eight Day Range Finding Study in Rats", International Research and Development Corp., Mattawan, MI, Report No. 163-532, July 6, 1978.
- ✓ 9. Nair, K.P.C. and Gunderson, G., "Chlorendic Anhydride, Tech. 90-Day Subacute Toxicity Study in Rats", International Research and Development Corp., Mattawan, MI, Report no. 163-544, January 7, 1980.
- ✓ 10. Jefferson, N. D. and Goldenthal, E. I., "Chlorendic Anhydride. 90-Day Subacute Toxicity Study in Rats. Amendment to the Final Report", International Research and Development Corp., Mattawan, MI, Report No. 163-533, February 8, 1980 (amendment to reference 9)
- ✓ 11. Goldenthal, E. I., "Chlorendic Anhydride, Three Week Dermal Toxicity Study in Rabbits", International Research and Development Corp., Mattawan, MI, Report No. 163-530, January 24, 1979.
- ✓ 12. Ulrich, C. E., "Chlorendic Anhydride. Subacute Inhalation Study in Rats 20 Exposures in 28 Days," International Research and Development Corp., Mattawan, MI, Report No. 163-531, October 19, 1979.

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Chlorendic Anhydride has been produced at Velsicol's Memphis, Tennessee Plant since commercialization. The plant epidemiology study is, in our opinion, useful in assessing the effects of chlorendic anhydride to humans.

The NTP chlorendic acid study was done orally. Any exposure to chlorendic anhydride is likely to be via inhalation or dermal exposure rather than oral. The results of the enclosed inhalation and dermal studies would be more useful than the results of an oral study in assessing the effects on exposure.

Velsicol has already submitted a great deal of information on chlorendic anhydride to the Agency. A summary of these submissions is, for convenience, listed on the following page.

With regards to the confidentiality of these studies, we believe that the provision in TSCA, which provides that health and safety data is not a trade secret, is an unlawful taking of property under the constitution. However, we are a small company, not in a position to challenge the constitutionality of this provision.

Until such challenge is made, we will abide by the terms of the statute.

Accordingly, we assert an interest in these studies to the extent that there may be compensation due from other manufacturers and processors of chlorendic anhydride.

We will not resist the Agency's disclosure of these studies recognizing that the Agency proceeds at its own risk regarding the constitutionality of this provision of the Act.

If you have any questions on this submission, please contact me at (312) 670-4805.

Very truly yours,



Alfred A. Levin
Director, Toxic Substances Control

enc.
AAL:jmes

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Chlorendic Anhydride Information Previously Submitted to EPA

<u>Reference</u>	<u>Submitted To</u>	<u>Date Submitted</u>
Diaz, L. and Atallah, Y. H. "Pharmacokinetics of Chlorendic Anhydride in Rats" Velsicol Chemical Corporation, Chicago, IL Project 482348, Report No. 5 October 11, 1978	Document Control Officer	9/26/85
Brusick, D., "Mutagenicity Evaluation of Chlorendic Anhydride in the DNA Synthesis in Human WI-38 Cells Assay" Litton Bionetics, Inc., Kensington, MD, Project No. 20840 April, 1978	"	6/15/79
Dean, W. P. and Jessup, D. C. Tech. Ref. Std. Chlorendic Anhydride Dermal Sensitization in the Albino Guinea Pig" International Research and Development Corp., Mattawan Mattawan, MI, Report No. 163-529, May 11, 1978	"	6/15/79
Dean, W. P. "Tech. Ref. Std. Chlorendic Anhydride 98.81%." Acute Toxicity Studies in Rabbits and Rats International Research and Development Corp. Mattawan, MI, Report No. 163-512, March 21, 1978	"	4/17/78

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Brusick, D. "Mutagenicity Evaluation of Chlorendic Anhydride in the Mouse Lymphoma Forward Mutation Assay" Litton, Bionetics, Inc., Kensington, MD. Project No. 20839, February 1978	Document Control Officer	3/31/78
Dean, W. P. "Tech. Ref. Std., Chlorendic Anhydride. Acute Oral Toxicity (LD50) Study in Mice" International Research and Development Corp., Mattawan, MI, Report No. 163-513, February 15, 1978	"	3/6/78
Matheson, D. W. and Brusick, D. "Evaluation of Chlorendic Anhydride in Vitro Malignant Transformation in BALB/3T3 Cells" Litton Bionetics, Inc., Kensington, MD Project No. 20840, January 1978 April, 1978	"	3/6/78
Dean, W. P. and Jessup, D. C. Tech. Ref. Std. Chlorendic Anhydride Dermal Sensitization in the Albino Guinea Pig" International Research and Development Corp., Mattawan Mattawan, MI, Report No. 163-529, May 11, 1978	"	6/15/79
Dean, W. P. "Tech. Ref. Std. Chlorendic Anhydride 98.81%." Acute Toxicity Studies in Rabbits and Rats International Research and Development Corp. Mattawan, MI, Report No. 163-512, March 21, 1978	"	4/17/78

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Shindell AND ASSOCIATES
MILWAUKEE, WISCONSIN

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REPORT OF EPIDEMIOLOGIC STUDY
OF THE EMPLOYEES OF
VELSICOL CHEMICAL CORPORATION PLANT
MEMPHIS, TENNESSEE

JANUARY 1952 - DECEMBER 1979

MARCH 1981

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REPORT OF EPIDEMIOLOGIC STUDY
VELSICOL CHEMICAL CORPORATION
MEMPHIS, TENNESSEE
JANUARY 1952 - DECEMBER 1979

Introduction

The initial epidemiologic study by Shindell and Associates of health experience of employees of the Velsicol Chemical Corporation at its Memphis, Tennessee plant was conducted during the period July 1976 through May of 1977. Its purpose was to evaluate the overall health status of all former and current employees with three months or more employment at the Memphis plant between 1952 when manufacture of heptachlor began and the study cut-off date of 31 December 1976.

Mortality data on Velsicol employees were compared to the overall mortality experience of like segments (by age and sex) of the United States population at large to determine whether Memphis plant exposure produced any discernible variations from expected mortality from all causes and from selected significant causes. Analysis of these mortality data revealed no significant differences between Velsicol employees and the overall U.S. population. The degree of follow-up achieved in this study was less than optimal, however, the study having been prematurely terminated in order to meet a deadline imposed by the necessity to prepare a report for presentation at a hearing being conducted by the Environmental Protection Agency.

In spite of the relatively limited follow-up achieved in 1977, the observed deaths among Memphis plant employees from all causes and from the specific causes of primary interest (i.e., heart disease and cancer) were found to be essentially at the level of the calculated expected deaths among comparable segments of the overall U.S. population. These data were presented in testimony by Dr. Shindell on 9 June 1977 at the EPA hearing along with similar data

derived from a study of the chlordane plant in Marshall, Illinois.

Concomitant with this prior study, an independent investigation employing different follow-up and analytical techniques was performed by Drs. Wang and MacMahon of the Harvard School of Public Health. An account of their study was published in the *Journal of Occupational Medicine*, Volume 21, No. 11, November 1979 (pp. 745-748) and reported substantially similar results.

A second independent study involving the Memphis plant has been conducted by the University of Illinois School of Public Health under contract with the National Institute for Occupational Safety and Health (NIOSH). The NIOSH/University of Illinois contract was awarded in 1976 and Memphis is one of four plants included in the Mortality Study of Workers Employed at Organochlorine Pesticide Manufacturing Plants. This study is based on a longer minimum employment period (6 months) and covers a shorter overall time span, including only those qualified individuals whose employment terminated on or before 31 December 1964. The follow-up cut-off date was 31 December 1976 and a report in general agreement with our prior findings and those of Drs. Wang and MacMahon was completed in draft form in April 1980.

Velsicol renewed its contact with Shindell and Associates in the latter half of 1979, wishing to update its own study of the Memphis plant and achieve two objectives: (a) to aim for a more complete degree of follow-up, and (b) to extend the period of follow-up by including persons employed subsequent to 1976, thus resulting in an additional three years of experience compared to the initial Velsicol-sponsored study. Negotiations were formalized in Memoranda of Understanding in September and October of 1979; the follow-up work was begun in February 1980 and concluded in January 1981.

This report describes the methodology of investigation, the

techniques of analysis, and the findings and conclusions of the resulting updated study of the mortality of employees of the Velsic' Memphis plant during the twenty-eight year period of heptachlor manufacture from 1952 through 1979.

Design of the Study

The overall goal of the study is to determine the health status of all persons who completed a minimum of three months' employment at Velsicol's plant in Memphis, Tennessee at any time between 1 January 1952 and 31 December 1979. The employment experience of such employees prior to 1952 is included for individuals who terminated employment in that year or thereafter.

The methods employed in reconstructing the roster of hourly employees terminated prior to 1957 and salaried employees terminated prior to 1969 was described in the Summary Report issued in 1977. This roster of employees was the starting point of the current study and, using all collateral sources available, every individual who could be identified as having been employed for three months or more in the plant during the 28-year period of the study has now been included in the current study cohort.

The requirement that a period of three months' employment be necessary for inclusion in the study cohort was imposed primarily to eliminate transients from consideration. All persons who could be determined to have had the potential of experiencing significant exposure to the occupational environment of the Memphis plant have been subjected to the follow-up procedures employed.

After updating the prior cohort roster to include qualified employees hired after 31 December 1976, intensive effort was made to trace and contact all employees not determined to be dead at the time of the original study. Death certificates were obtained for individuals who died between 1 January 1977 and 31 December 1979 and attempts were made to determine the current health status of all survivors as of 31 December 1979.

In order to evaluate relative health effects of various occupational exposures among Memphis personnel, classifications of job types and products exposures, established at the time of the original

study, were reviewed and refined. Based on information obtained during and subsequent to the original study, the categories of job/product exposure were clarified to reflect distinguishable characteristics in groups large enough to permit valid statistical analysis. The job/product classifications ultimately employed are in accordance with the Project Specification attached (Appendix I-1).

A data sheet was created for each individual incorporating information as of 31 December 1976 for members of the original cohort and relevant information as of 31 December 1979 for subsequent additions to the cohort. The form used for data collection is attached (Appendix I-2).

All information collected was coded and/or transcribed for computer input in accordance with revised and updated Coding Specifications (Appendix I-3), and entered for analysis as required in accordance with the Data Field Layout (Appendix I-4).

The methods employed to contact former employees included the traditional approaches of searching company records, conferring with current employees and former employees still residing in the local communities, contacting relatives and personal references, inquiring of school alumni groups and professional associations, and following up with former neighbors and identified acquaintances.

Two general circumstances significantly enhanced our success in determining current status of former Memphis employees. The first was the generous cooperation received from local and state agency custodians of background data on individuals in the cohort. The Memphis Consumer Credit Association was particularly helpful in providing address information from its files; Mr. Harold E. Crawford responded with unusual speed to our requests for assistance. Similarly valuable help was provided by Major Charles W. Dunner, Director of Driver Control of the Tennessee Department of Safety, by Mr.

Thomas Overton, Assistant Director of the Division of Vital Records of the Tennessee Department of Public Health, and by Mr. Art Richardson, Management Information Systems Officer of the Mississippi Department of Public Safety. The second major factor contributing to the substantially increased achievement of contact compared to the 1977 report was the availability at reasonable cost of the cross-reference directories by street address for Memphis and environs published by Cole Publications of Lincoln, Nebraska. These directories were of inestimable value in tracing former employees by contacting their former neighbors.

Determination of survivor status by querying the Social Security Administration was also attempted. Through SSA assistance we were able to identify ten decedents whose deaths we had not discovered by other tracing efforts, and to confirm as alive during the first three months of 1980 an additional nine individuals not otherwise ascertained to be alive as of the 31 December 1979 cut-off date.

Although assistance from all these sources has been helpful in increasing success in locating many individuals whose status had not been determined in 1977, the reports did not permit resolution of the status of all the individuals identified as qualifying for inclusion in the study cohort. Overall, however, the status of 92.8% of the study cohort was determined.

The Study Universe

The Memphis plant cohort of employees who completed three months or more employment during the study period of 1 January 1952 through 31 December 1979 totaled 1,115 individuals. The group is comprised of 970 white males, 55 non-white males and 90 females.

Of the 1,115 in all classifications, 263 were persons employed as of 31 December 1979 and 852 were former employees of whom 80 were determined to have died before 1 January 1980. Of the 772 not known to be deceased, 692 were contacted either directly or indirectly and confirmed to be alive as of 31 December 1979. An individual is classified as a direct contact whenever (a) one of our staff actually spoke with him or her by telephone, confirmed all the data on the data sheet and received an answer concerning his or her current health status, or (b) a mailed questionnaire affirming the above information was completed, signed and returned by the employee. An indirect contact is (a) one in which the desired information was obtained from a family member or close associate, (b) in which current status as alive was confirmed by a personally signed receipt for a certified letter sent to an address reported by a reliable source to be the employee's current address, or (c) by report of SSA of a currently active account or by the Motor Vehicle Department of a current driver's licence. Eighty persons could not be confirmed alive or dead and are classified as status unknown.

Table I shows the white males (grouped according to primary job/product category), the non-white males and the females classified according to status: current (1), former contacted (2 & 3), former deceased (4 & 5), and former with status unknown (9). The degree of follow-up achieved for each sub-group is also shown. As was stated, 92.8% of the study cohort could be accounted for as of 31 December 1979.

TABLE I

STUDY UNIVERSE AS OF 31 DECEMBER 1979
 VELSICOL CHEMICAL CORPORATION--MEMPHIS, TENNESSEE PLANT
 BY SEX, RACE, EMPLOYMENT AND CONTACT STATUS
 AND BY JOB/PRODUCT CLASSIFICATION FOR WHITE MALES

EMPLOYEE GROUP	TOTAL	EMPLOYEES CURRENT 31 Dec 79 (1)		FORMER EMPLOYEES CONTACTED/ALIVE (2)		DECEASED EMPLOYEES With D.C. Obtained (4)		EMPLOYEES No D.C. Obtained (5)		EMPLOYEES W/ STATUS UNKNOWN (9)		PERCENT STATUS KNOWN
		Direct	Indirect	Direct	Indirect	Obtained	Obtained	Obtained	Obtained	Unknown		
<u>WHITE MALES</u>												
Opr.Heptachlor, Inter-med. & Multip.	207	58	78	49	78	3	0	19	0	19	90.8%	
Opr.Caustic Chlorine	60	19	17	18	17	5	0	1	0	1	98.3%	
Opr.Endrin	71	12	24	19	24	3	1	12	1	12	83.1%	
Maintenance	209	47	64	54	64	27	1	16	1	16	92.3%	
Shipping, Laborers & Other	161	20	70	51	70	14	0	6	0	6	96.3%	
Lab, Research	113	19	43	42	43	1	2	6	2	6	94.7%	
Engineers	69	11	22	22	22	7	1	6	1	6	91.3%	
Mgmt/Clerical	80	10	26	28	26	11	0	5	0	5	93.8%	
TOTAL WHITE MALES	970	196	344	283	344	71	5	71	5	71	92.7%	
NON-WHITE MALES	55	46	1	7	1	1	0	0	0	0	100.0%	
FEMALES	90	21	27	30	27	3	0	9	0	9	90.0%	
TOTAL	1,115	263	372	320	372	75	5	80	5	80	92.8%	

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The original Memphis plant study covering the period from 1 January 1952 through 30 December 1976 identified a total of 1,074 potential members of the then study cohort. This total included seven part-time employees shown later not to qualify for inclusion and a number of individuals who had been identified by only a name at the time of the original study. Review of Velsicol records during the current investigation failed to disclose any additional information regarding sex, race, dates of birth, hire or termination, nor any social security numbers. Being unable to obtain valid data for analysis or identification for review by the Social Security Administration, and being unable to confirm reliably their basic qualification for inclusion in the study, these persons were deleted from the current update cohort.

Of the remaining 161 originally qualified individuals whose status was not determined during the original study, we succeeded in ascertaining the status of 104 during the current study by direct or indirect contact. Only 57 of the 228 with undetermined status in 1976 remained in that category at the conclusion of the current update study.

Of the 534 individuals classified in the initial study cohort as former employees, confirmed alive, all but 15 were successfully contacted or otherwise confirmed to be deceased or still alive during the current update study. These 15, whose status was not confirmed during the current study, include six who were contacted directly and nine who were confirmed alive with unknown health status in 1976.

The update study added 115 individuals to the original study cohort. The status of all but six of these was determined. Of these six, three had been hired after completion of the previous study and the other three were identified as qualified former employees when a group of personnel records was discovered in October and November of 1980.

Of the 1,077 health status investigations which comprised the original study, all but the 54 individuals for whom death certificates were obtained at that time required complete reinvestigation for the current study. Thus, the present study involved retracing and/or additional data collection for 1,138 individuals as compared to 1,077 in the 1977 investigation. The death certificates on file from the original study were reviewed and classification by specific cause confirmed. Efforts to obtain death certificates for all individuals reported to be deceased were not successful in five cases. Table II summarizes the information available on those reported to be deceased but for whom no death certificates were obtained. These individuals are included in the analysis as deceased individuals even though final confirmation of their deaths was not obtained.

TABLE II

INFORMATION AVAILABLE ON EMPLOYEES REPORTED DECEASED

NO DEATH CERTIFICATE OBTAINED

VELSICOL CHEMICAL CORPORATION--MEMPHIS, TENNESSEE PLANT

I.D.	DATE HIRED BY VELSICOL	TERMINATION BY VELSICOL	***** SOURCE	***** REPORT OF LOCATION	DEATH DATE	***** CAUSE	STATES QUERIED FOR CERTIFICATES
W.S.C.	Apr '52	Mar 76	SSA	Mississippi	Jan 78	Cardiovascular	MS (not found)
D.B.I.	Jan 58	Dec 58	SSA	Louisiana	After 58	Unknown	LA,MS,TN (not found)
W.R.K.	Nov 55	Apr 67	Spouse	Tennessee	Nov 79	Stroke	TN (not found)
J.D.L.	Jan 63	Jun 63	SSA	Tennessee	Mar 72	Unknown	TN (not found)
M.E.W.	Aug 74	May 76	SSA	Tennessee	Jun 77	Suicide	TN (not found)

Analytic Technique

In order to analyze the mortality experience of the Memphis employees, it is necessary to compare the observed mortality in the employee groups with that of the comparable segments of the United States population over the same period of time. To perform these comparisons, the probability of each employee dying from the specific causes during the period of the study was calculated using the following formula:

$p_1 = 1 - (1-p_1)^{e_1}(1-p_2)^{e_2} \dots (1-p_k)^{e_k}$ = the probability of each individual employee dying over the total period of the study, where:

p_1, p_2, \dots, p_k = the probability of dying in the individual years of the study, and

e_1, e_2, \dots, e_k = the portion of a year in which the individual is represented in the study ("e" may be 0, 1, or a fraction of a yr.)

When calculating expected mortality, two important constraints are imposed. First, the period for calculation of risk of dying for each individual is precisely defined. Because the conditions for inclusion in the study included a minimum period of employment, the calculation of expected mortality began at the conclusion of the period of minimum employment rather than on the date on which employment began. The counting of time at risk terminates at the end of the study. However, where a person's status as of 31 December 1979 was not determined, time was counted only to the point of last known date alive rather than the cut-off date of the study.

The second constraint concerns the rates that were used in calculating expected mortality. Race- and sex-specific U.S. mor-

tality rates for the years 1945, 1950, 1955, 1960, 1965, 1970 and 1975 were employed. The rates for 1945 were applied in calculations for the period January 1946 - June 1947; rates for 1950 for July 1947 - June 1952; and similarly for each five-year period, using 1975 rates for the period July 1972 - December 1979 (the most recent detailed rates available).

Rates for 1960 and later are published by the National Center for Health Statistics for five-year age groups. In order to make data for 1945, 1950 and 1955 comparable, logarithmic interpolations between published rates for ten-year age groups were derived to obtain estimates for the intervening five-year intervals. Thus in calculating expected mortality, five-year age groups and five-year time periods were used for comparison purposes.

The causes of death considered in the analyses were classified according to the Eighth Revision of the International Classification of Diseases, Adapted. The categories of interest were deaths from: all causes (all ICDA), malignant neoplasms (ICDA 140-209), diseases of the heart and circulatory system except cerebrovascular (ICDA 390-429 & 440-458), cerebrovascular diseases (ICDA 430-438), and all trauma (accidents, suicide, homicide, etc. - ICDA E800-E999). For those cases in which death certificates were not obtainable, the cause of death was tabulated as "Other or Unknown" unless the source of the information was able to furnish a reliably classifiable cause.

Statistical significance was determined by comparing the observed number of deaths with that expected, and assuming that the distribution of the difference is Gaussian. This is expressed by the following formula:

$$Z = \frac{O - E}{S}, \text{ where}$$

$$E = \Sigma p_i, \text{ and}$$

$$S = \sqrt{\Sigma p_i(1-p_i)}$$

This technique results in statistical significance for smaller variations than is the case if the distribution is assumed to be Poisson and $S = \sqrt{E}$.

Summarizing the above: we use the published mortality rates for the U.S. population at large to calculate how many deaths would be expected to occur for all causes and for each specific cause among a group of individuals of the same numerical size, race, sex and age composition as the Memphis plant employee group during the time period in which the Memphis cohort was "at risk" during and after Velsicol employment. The comparison of these expected values to the observed deaths among the Memphis cohort provides a determination of whether working at the Memphis plant is likely to be accompanied by a mortality experience that is similar to that of people of comparable ages not working at the Memphis plant.

By eliminating periods of adult activity of Memphis workers prior to Velsicol employment from the time at risk calculations, we reduce the overall person-years base on which the expected deaths are calculated, and thus reduce the number of expected deaths to which we compare the number of actually observed deaths. While it can be argued that it is likely that Velsicol employees hired after employment elsewhere should be considered at risk during their entire working lives for purposes of establishing the overall person-years base for calculating expected deaths, we deem it more conservative to consider only the time after the required minimum exposure to the occupational environment whose effects we are attempting to evaluate.

The analysis includes 5,805.8 person-years of employment at Velsicol and 14,092.8 person-years of survival since commencement of such employment.

A second type of analysis was performed in which the actual mortality experienced and the expected mortality in the comparable

segment of the U.S. population as calculated above were used to develop survival curves, i.e. the proportion of a cohort surviving over the period of study, compared to the survival of the comparable segment of the U.S. population.

The results of these two types of analyses are presented in the following pages.

Summary of Findings

Comparisons of the observed mortality among the Memphis cohort to calculated expected values based on national statistics revealed a slightly lesser occurrence of death overall than would be expected in a group of similar size and age distribution in the U.S. as a whole. When viewed in detail for various job and product classifications and with respect to specific causes of death the results are generally consistent with the national experience. This can be seen in Table III and Figure I which are presented on the following pages.

Table III shows the number of deaths by major cause among the employees of the Memphis plant. Overall there were 80 deaths in this group compared to an expected number of 84.2. There were 30 deaths from heart disease as compared to 32.2 expected and 15 deaths from cancer as compared to an expected 16.3.

Both strokes and trauma showed an increase over expected, but the apparent excess of deaths from these causes is limited to employee groups who are at relatively low risk of exposure to the actual process of manufacturing the chemicals which are produced in this plant. These findings will be discussed in further detail in the next section.

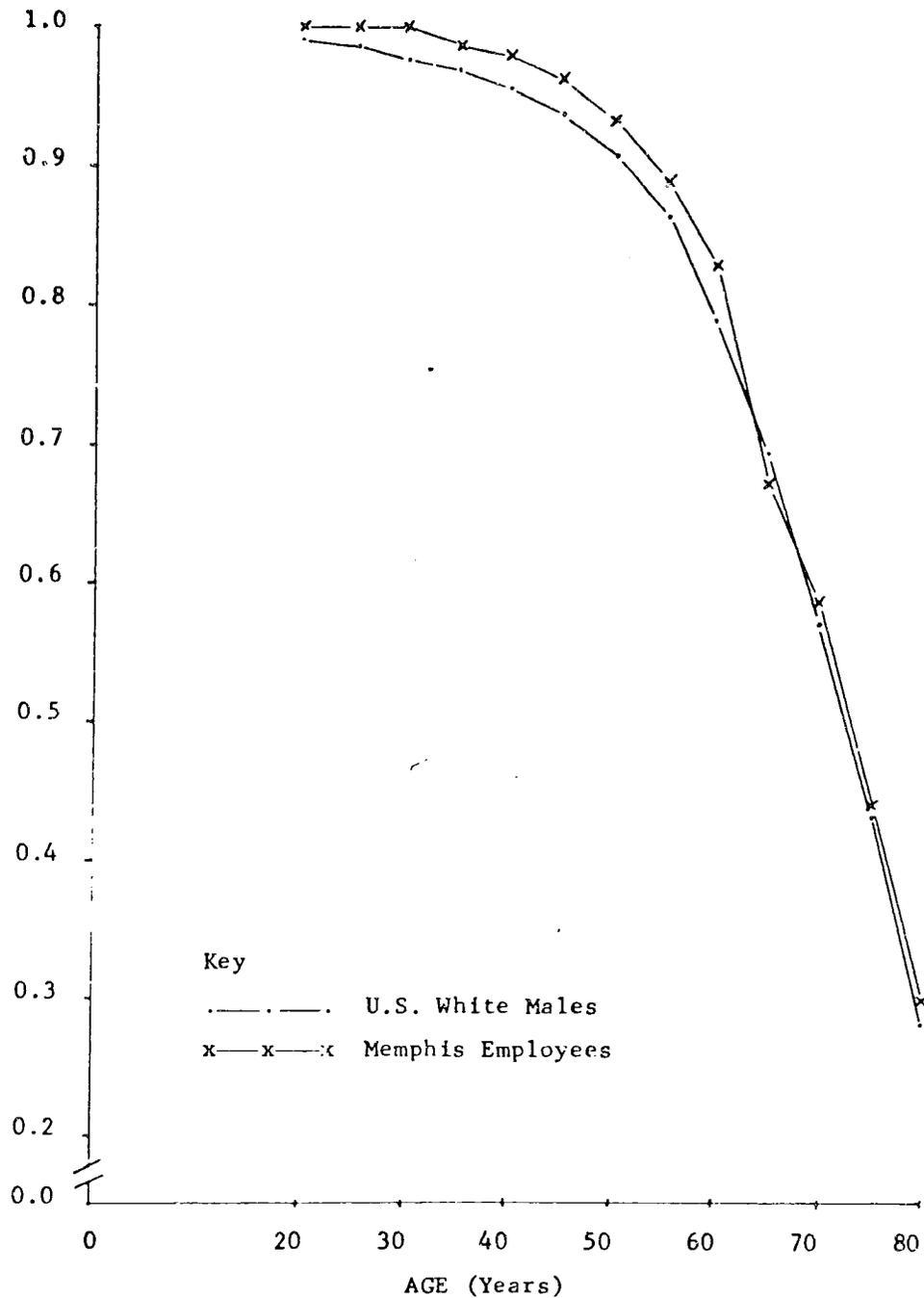
Figure I displays graphically the survival curve of the white male employees. Too few non-white males or female employees are in the cohort to permit a comparable analysis. As can be seen from this curve, the likelihood of survival among the employees of the Memphis plant is more favorable than the comparable portion of the U.S. population until about age 60 and from then on is essentially identical to that of the U.S. white male in the country as a whole.

TABLE III
 DEATHS IN VELSICOL EMPLOYEES COMPARED TO U. S. POPULATION DEATHS BY MAJOR CAUSE
 AND BY PRODUCT/JOB CLASSIFICATION, MEMPHIS PLANT, 1952-1979

	ALL CAUSES		HEART DISEASE		CANCER		STROKE		TRAUMA		OTHER	
	Memphis	U.S.	Memphis	U.S.	Memphis	U.S.	Memphis	U.S.	Memphis	U.S.	Memphis	U.S.
WHITE MALES												
Opr. Heptachlor, Inter-med. & Mulcip.	3	7.7	0	2.1	0	1.2	1	0.2	2	2.7	0	1.5
Opr. Caustic chlorine	5	8.9	3	3.6	0	1.8	1	0.4	1	1.4	0	1.7
Opr. Endrin	4	4.2	0	1.4	1	0.8	1	0.1	2	1.1	0	0.8
Maintenance	28	26.7	13	11.4	7	5.4	4*	1.4	3	3.3	1	5.2
Shipping, Laborers and Other	14	13.9	7	5.5	3	2.5	0	0.9	1	2.4	3	2.6
Lab, Research	3	4.9	1	1.4	0	0.8	0	0.2	0	1.6	2	0.9
Engineers	8	7.3	1	3.0	2	1.5	1	0.4	3*	1.0	1	1.4
Mgmt/Clerical	11	7.2	4	2.9	2	1.4	1	0.4	3	1.1	1	1.4
TOTAL WHITE MALES	76	80.8	29	31.3	15	15.4	9	4.0	15	14.6	8	15.5
NON-WHITE MALES	1	1.2	1	0.4	0	0.2	0	0.1	0	0.3	0	0.2
FEMALES	3	2.2	0	0.5	0	0.7	0	0.2	2*	0.3	1	0.5
TOTAL	80	84.2	30	32.2	15	16.3	9	4.3	17	15.2	9	16.2

* Statistically significant difference (p<0.05)

FIGURE I: Proportion of white male employees of the Memphis plant of the Velsicol Corporation surviving at specific ages when those dying from all causes are deducted, as compared to the comparable segment of the U.S. population (January 1952 to December 1979)



The morbidity experience among current and surviving former employees is entirely unremarkable. Although no reliable national statistics are available on the health of the U.S. population in general, we reviewed the occurrence of health problems among the Memphis employees confirmed to be alive and compared the results to similar findings of our prior studies in the chemical industry. Approximately 72% of all surviving Memphis employees reported no health conditions requiring medical attention. This "good health" index is substantially higher than we have found in previous studies where the overall absence of health problems has averaged about 66%.

In summary, we discern no relationships attributable to the Memphis occupational environment which would suggest any deleterious effect upon the life expectancy or general health of the Memphis employees. The detailed results of our analyses are presented in the following sections.

Mortality by Job and Product

Table III above showed the overall mortality experience of all Memphis employees who worked at the plant for three months or more since Velsicol began producing pesticides in that facility some twenty-seven years ago. It also displays the mortality experience with respect both to the kinds of exposure by job and product and to specific causes of death. The matrix of exposure categories and specific causes of death represents an attempt to be as specific as possible in distinguishing differences in potential exposures and types of illnesses given the constraint of considering groups of sufficient size to permit statistical validity.

The comparisons of observed deaths among Memphis employees with expected deaths based on U.S. rates for like segments of the general population reveal no significantly excessive differences with the exception of deaths from stroke among the maintenance employees and deaths from trauma among both the engineers and female employees. Other than these instances, the number of deaths observed for all causes and for each specific cause among the various employee groups are consistent with the national norms.

Because of the finding that the deaths from stroke among the former maintenance personnel exceeded the number expected in the comparable segment of the U.S. population over this period of time, this group was subjected to further analysis.

It should be noted that while more deaths from stroke were recorded generally for the Memphis employees than expected, this is offset by a lesser number of deaths from causes other than those specifically noted. The "Other" category included deaths from emphysema (3 cases), alcoholism (2 cases), and diabetes and

hypertension (1 case). In two instances, as was reported in Table II, the cause of death is unknown.

Table IV shows a listing of the nine former employees whose death was recorded as having been from a stroke. As can be seen, five of these persons worked for Velsicol two years or less and died an average of 13.8 years after leaving Velsicol. Three of the four maintenance workers who died of stroke are in this group. The other had worked for Velsicol 18 years and died five years after leaving the company's employ. There thus appears to be no relationship between the occurrence of stroke and the work experience at Velsicol.

The deaths from trauma of various forms were also subject to further exploration. Among the white male employee group, traumatic deaths occurred in the following fashion:

Auto accident	3
Industrial accident	3 (2 at Velsicol)
Suicide	3
Homicide	2
Household accident	1 (while employed at Velsicol)
Poisoning	1 (while employed at Velsicol)
Drowning	1
Asphyxiation	1

Four of the above people were employed by Velsicol at the time of death. An average of 7.8 years intervened between leaving Velsicol and time of death in the other individuals. Two of the three deaths listed as industrial accident resulted from an explosion at the Memphis plant which occurred in November 1955. The death from poisoning was in a painter employed in maintenance at Velsicol at the time of his death in May of 1955. Other than the three deaths from trauma occurring during employment at Velsicol, all of which

TABLE IV
 FORMER EMPLOYEES OF MEMPHIS PLANT OF VELSICOL CHEMICAL CORPORATION
 DYING OF STROKE, 1952-1979

I.D.	AGE AT HIRE	PERIOD EMPL. AT VELSICOL	TIME BETW. LEAVING VELSICOL & DEATH	AGE AT DEATH	JOB AT VELSICOL
J.L.J.	30	2 years	10 years	42	Oper. Other/Mult.
B.C.B.	36	12	0	48	Caustic Chlorine
J.R.L.	37	18	5	60	Maintenance
W.R.K.	39	11	13	63	Endrin
W.E.B.	44	2	23	69	Maintenance
L.M.P.	48	2	9	59	Maintenance
W.K.M.	49	4	17	70	Office
R.C.R.	52	<1	20	72	Engineer
A.M.R.	56	<1	7	63	Maintenance

occurred 25 years ago, none of the deaths in this category can be considered to be related to the work situation.

There were no deaths from trauma recorded among the non-white males of the study cohort and the two trauma deaths in the female employee group were both auto accidents seven and fourteen years respectively after leaving the employ of Velsicol.

It would appear, therefore, that both the trauma deaths and stroke deaths represent chance variations unrelated to the work environment at Velsicol.

While the incidence of cancer deaths was consistent with the expected number of deaths from this cause in the U.S. population, an analysis was nonetheless performed of the deaths from cancer by site and by job class. Table V shows the result of this analysis. The only concentration of cases is in the lung cancer category among maintenance personnel.

Only one of these individuals exhibited the disease while at Velsicol and died while in the employ of the company. A second individual in this group worked for Velsicol less than one year and died six years after leaving the company. The other two worked for Velsicol over ten years and died in their mid-60's after leaving Velsicol--one three years later and the other ten years later.

Among the current and former employees alive at the time of this study and reporting neoplastic disease, none report lung cancer. There are two with brain tumors, two with skin cancer and one each of the testicle, bladder and prostate.

Of the individuals affected by brain tumor, neither of the decedents was involved in the production process. The two surviving employees with this condition are a former maintenance employee and a currently employed operator who was involved at one time or another in multiple production processes.

TABLE V
 CANCER DEATHS IN FORMER EMPLOYEES: 1 JANUARY 1952 - 31 DECEMBER 1979
 VELSICOL CHEMICAL CORPORATION - MEMPHIS, TENNESSEE PLANT
 PRIMARY SITE OF CANCER BY JOB/PRODUCT CLASSIFICATION

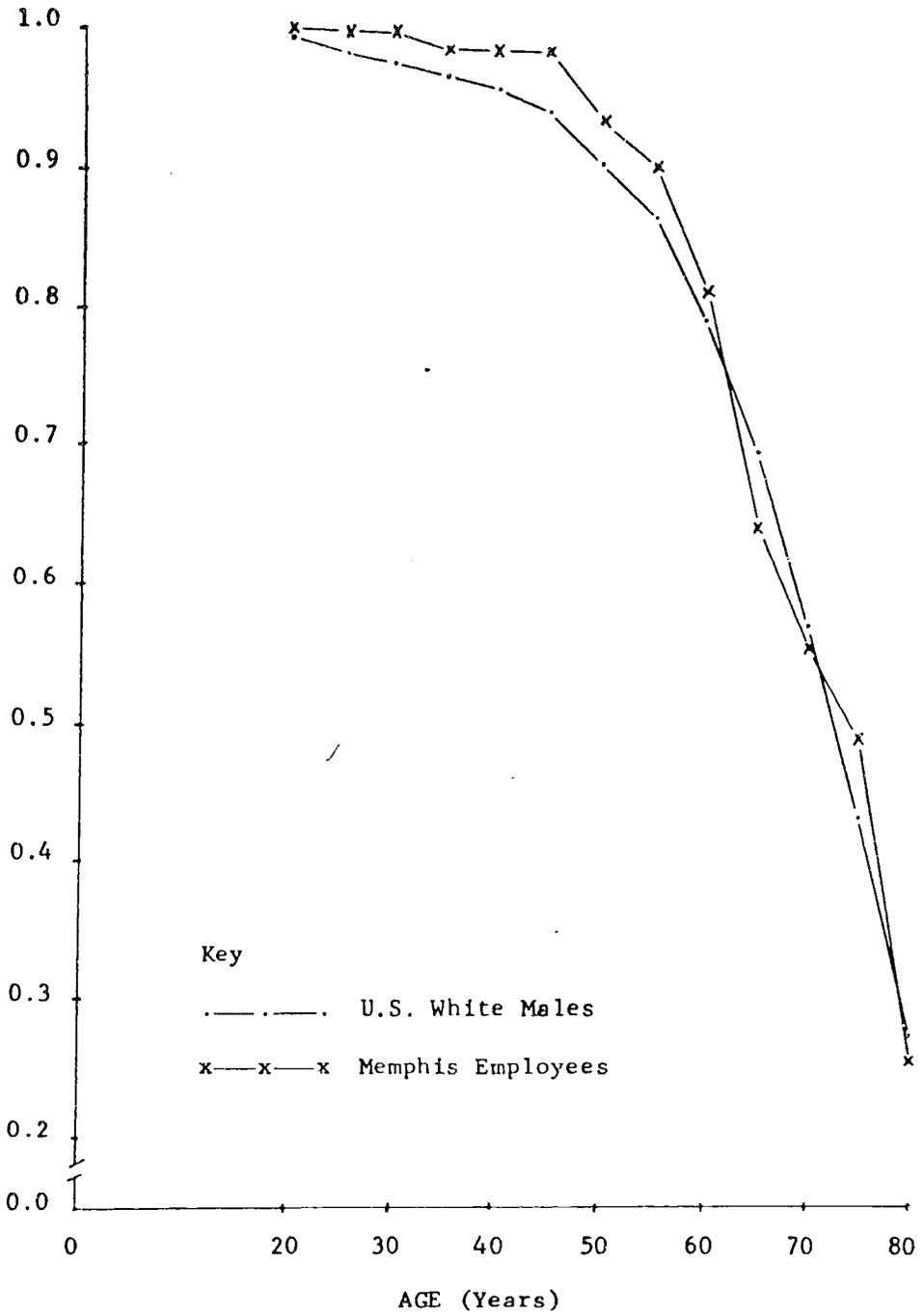
EMPLOYEE GROUP	ALL SITES	Bladder	Brain	Colon	Leukemia	Lung	Prostate	Stomach
Operator, Endrin	1					1		
Maintenance	7	1		2		4		1
Plant: Non-maintenance	3		2			1		
Engineer	2				1	1		
Management/Clerical	2						1	1
TOTAL	15	1	2	2	1	7	1	1

No pattern of neoplastic disease thus emerges that would raise any suspicion of a job-related risk factor unless, of course, the ability to smoke on the job may be considered job-related. A factor which may also be operative is the likelihood of being affected either by prior or subsequent employment. On the average, maintenance personnel in the cohort were employed at Velsicol at 35.7 years of age. The white male employees in the other job categories were employed at age 27.8 years on the average. Thus if the work-situation were an operative factor, the maintenance personnel had a substantially greater period of time prior to employment at Velsicol to be exposed to hazardous substances in other work settings.

Since the maintenance employees represent a somewhat dissimilar group from the remainder of the employees at Velsicol's Memphis plant, an analysis of the survival of this group of employees was performed separately.

Figure II shows the curve for overall survival of the maintenance employees as was presented for the total cohort of white male employees in Figure I. The curve demonstrates that even though hired at an older age than the remainder of the work force, their overall mortality experience does not vary significantly from the U.S. population which is plotted for comparison.

FIGURE II: Proportion of white male maintenance employees of the Memphis plant of the Velsicol Corporation surviving at specific ages when those dying from all causes are deducted, as compared to the comparable segment of the U.S. population (January 1952 - December 1979)



Mortality by Length of Employment

The matter of "latency" of influences present in the work environment is of constant concern. In an attempt to examine this issue, an analysis of standard mortality ratios was performed for the white male employees other than the maintenance group. The results are shown as standard mortality ratios in Table VI.

It will be noted that the analysis continues only through 15 years of employment. Because the period of the study extends over a 28-year period and the average non-maintenance worker was employed at age 27.8 years, only those employed in the early years have had time for death to occur in sufficient numbers for analysis. The average age at death for those that have died was found to be 55.2 years for this group.

It is evident that the analysis indicates the likelihood of mortality from exposure to conditions present in the plant primarily during the first half of the study period. The numbers are too small to permit analysis at this time beyond the 15-year period. Among people with a minimum of 20 years of employment, only ten deaths have thus far occurred, six from heart disease, two from cancer and two from trauma.

The analysis displayed in Table VI shows essentially no change in risk of death overall or from heart disease or cancer regardless of length of employment at Velsicol. It is curious to note that the risk of death from causes other than heart disease or cancer tends to decline as length of employment increases. This is undoubtedly affected by the fact that the chronic degenerative diseases have not had sufficient time to be manifested in this employee group and that trauma deaths, which are more likely to occur in younger persons, are already evident in the data available and are reflected in the SMR's for the briefer periods of employment.

TABLE VI
 STANDARD MORTALITY RATIOS IN WHITE MALE EMPLOYEES--OTHER THAN MAINTENANCE
 1 JANUARY 1952 - 31 DECEMBER 1979
 VELSICOL CHEMICAL CORPORATION - MEMPHIS, TENNESSEE
 EMPLOYED FOR VARYING MINIMUM LENGTHS OF TIME, BY MAJOR CAUSE

Minimum Length of Employment	Deaths from All Causes	Deaths from Cancer	Deaths from Heart Disease	Deaths from All Other Causes
3 Months	.89	.81	.80	.99
1 Year	.87	.98	.78	.91
5 Years	.79	.93	.82	.70
10 Years	.88	.83	.95	.83
15 Years	.80	1.20*	.82	.54

* Observed 3, Expected 2.5 (Not statistically significant)

SMR = Ratio of observed to expected: i.e., $\frac{\text{Memphis observed deaths}}{\text{Calculated expected deaths}}$

In summary, the analyses performed of the specific causes of death, of the specific job categories showing apparent variation from normal and of the relationship of mortality to duration of employment all tend to confirm that chance variation is responsible for the findings present. The overall death rate of individuals employed in the plant and the death rates by major causes are consistent with what one would expect in the population studied.

Health Status of Surviving Employees

In addition to the analyses of mortality, the health conditions warranting medical attention were recorded for all employees, current and former, who could be contacted and who agreed to supply the information.

Table VII shows the types of problems reported by the employees indicating a current medical condition or illness. Consistent with earlier studies in the chemical industry, a substantial majority of current and former employees reported no medical conditions warranting treatment. Of the 955 persons confirmed to be alive, the current health status of 822 was determined. Good health was reported by 683 of these, representing 83.1% of those reporting. The 139 who reported health problems identified the conditions shown in Table VII.

The most commonly reported problem was hypertension. Hypertension and other cardiac disease were reported by 6.5% of the surviving employee group, an incidence appreciably less than observed in other studies which averaged 10% of surviving employees reporting hypertension and cardiovascular disease. This may be due to the fact that follow-up only covers a 28-year period, while other studies covered periods of greater length.

Because of the interest in the possible relationship between work environment and the occurrence of cancer, the seven subjects alive at the time of contact who reported the presence of neoplastic disease were reviewed in detail. As was reported above, there were two individuals who reported having brain tumors. One is a currently employed operator, the other formerly employed in maintenance. Two individuals reported having skin cancer, a currently employed operator and a former laboratory worker. The cancers of the testicle, bladder and prostate are in a former

TABLE VII

HEALTH STATUS OF CURRENT AND FORMER EMPLOYEES ALIVE AS OF 31 DECEMBER 1979
VELSICOL CHEMICAL CORPORATION - MEMPHIS, TENNESSEE PLANT

EMPLOYEE GROUP	TOTAL EMPLOYEES	Number Without Health Problems	% Without Health Problems of Empl. Reporting Status	***** HEALTH PROBLEMS REPORTED *****												
				Hypertension	Cardiovascular	Arthritis	Musculoskeletal	Emphysema & Asthma	Diabetes, Thyroid	Allergy & Dermatitis	Gastro-Intestinal	Prostate	Hearing	Neoplastic	Other	Unknown Health Status
Opr. Heptochlor, Inter-med. & Multiple	226	156	80.4	8	4	7	3	2	1	3	1	3	3	3	8	32
Opr. Caustic Chlorine	54	37	84.1	-	-	-	-	2	1	1	1	1	1	-	1	10
Opr. Endrin	55	37	84.1	2	2	1	1	-	2	-	1	-	-	-	1	11
Maintenance	167	106	74.1	10	13	4	7	3	2	-	2	2	3	2	7	24
Shipping, Laborers & Others	159	113	83.1	8	1	1	-	2	2	3	2	1	1	1	6	23
Laboratory, Research	117	90	88.2	6	-	-	-	-	1	2	1	1	-	1	1	15
Engineers	55	42	91.3	1	1	-	-	1	-	1	-	-	-	-	1	9
Mgmt/Clerical	122	102	90.3	4	2	1	1	1	2	1	1	1	1	-	-	9
TOTAL	955	683	83.1	39	23	14	12	11	11	11	9	9	9	7	25	133

laborer, operator and maintenance employee respectively. The variety of tumors present in the numbers noted does not reveal an unusual concentration of a specific cancer type in any specific employee group.

Review of the information contained in Table VII revealed no pattern of disease or medical condition, by job or product exposure, that would suggest an adverse influence on morbidity from the work environment in the Memphis plant.

Summary and Conclusions

A study was conducted of a cohort of 1,115 current and former employees who worked three months or more at the Memphis, Tennessee plant of the Velsicol Chemical Corporation from 1 January 1952 through 31 December 1979. Of the study cohort, 93.1% of the males and 90.0% of the females were located and data on morbidity and mortality obtained.

The data indicate that overall mortality was lower (but not significantly) in the male employees than that expected in the comparable segment of the U.S. population as a whole. This was true also in the case of deaths from heart disease and cancer. Trauma deaths and deaths from stroke were generally consistent with U.S. experience. Trauma was the major cause of death among female employees. There were minor differences among specific classes of workers due to chance variation but none of the variations in specific employee groups could be related to the products handled. The relative mortality by cause was found to be consistent regardless of length of employment.

Morbidity data are likewise comparable to expectations in a work force of the age composition present in the Memphis facility and among those no longer employed and surviving at the time of the termination of the study.

There is, thus, no evidence of any long-term latent effect on health related in any way to employment at the Velsicol plant in Memphis, Tennessee, for the twenty-eight year period in which it has been engaged in the production of chlorinated hydrocarbon insecticides.

APPENDIX I

000042

Shindell AND ASSOCIATES

MILWAUKEE, WISCONSIN

SPECIFICATIONS FOR STUDYat Velsicol Chemical Corporation - Memphis Plant: 1979 UpdateMailing address: 1199 Warford, Memphis, TN 38108Phone: 901-324-4401

Plant Mgr. C. Lynn Sharpe Medical Dept. C. Lee Maglio
 Ind. Rel. Fred C. Billings Personnel Linda Medlin-Spitzer

DEFINITION OF SAMPLE:

Employees completing 3 months or more after 1 Jan 1952 to 31 Dec 1979COMPANY/PLANT CODE: VMEB

JOB CATEGORY CODE:

1. Operator, Shift Foreman
2. Labor
3. Shipping
4. Maintenance, High Risk
5. Maintenance, Low Risk
6. Laboratory
7. Engineering
8. Management/Admin./Clerical
9. Miscellaneous/Unknown
0. Absence/Leave/Layoff

PRODUCT/PROCESS CODE:

1. End products - EGH(exc.chlorine)
2. Intermediates - PCL
3. Caustic Chlorine
4. Heptachlor/Chlorendic
5. Endrin
6. not assigned
7. not assigned
8. Formulations/Laboratory
9. Multiple/Other/Unknown
0. Office

SPECIAL CODES:

Study approved on 18 October 1979Data collection commenced initial visit 31 Oct 1979
project start-up 7 Jan 1980FIELD SUPERVISOR: 000043
Slack Ulrich

Shindell AND ASSOCIATES

INFORMATION SHEET ON EMPLOYEES

NAME _____

SSN _____ Sex _____ Born _____

Last address _____ Phone _____

Subsequent _____ Phone _____

Subsequent _____ Phone _____

Major Job Category _____ Product _____

Date Employed _____ Date Terminated _____

Intervening Activity _____ Years _____

Status as of _____ 1 - Current 2 - Contacted 3 - Indirect

Deceased: Location _____ D.C? 4 - Yes 5 - No Date _____

Major Illness/Cause of Death _____

Other Illness/Cause of Death _____

Primary Cancer Site _____

Other Jobs within Plant

Category	Product	Months
_____	_____	_____
_____	_____	_____
_____	_____	_____

NOTES:

CODING SPECIFICATIONS

<u>COL</u>	<u>ENTRY</u>	<u>COL</u>	<u>ENTRY</u>
1-12	Last Name	54-55	Major Illness/Cause of Death
13-14	Initials	00	No illness reported
15-23	Social Security Number	01	Cancer
24	Sex and Race	02	Cardiovascular disease
25-22	Date of Birth (day, month, year - dd, mm, yyyy)	22	Hypertension
33	Major Job Category (Specify for plant)	03	Central nervous system/CVA
34	Product Code (Specify for plant)	04	Liver
35-38	Date Employed (month and year - mm, yy)	05	Pneumonia
39-42	Date Terminated (month and year - mm, yy)	25	Thyroid
43	First Intervening Activity	06	Renal disorder
44-45	Duration of Intervening Activity (in years)	07	Diabetes
46	Second Intervening Activity (See 43)*	27	Allergy
47-48	Duration of Second Intervening Activity (in years)*	08	Dermatologic
49	Status of Individual at Time of Study	09	Infectious disease
50-53	Date of Death (month and year - mm, yy)	28	Hearing disorder
54	If "9" in column 49 (status undetermined), the entries in cols. 46-48 record latest date of known status (last date known alive) as follows:	29	Other
55	Month (1 thru 9 for Jan thru Sept; A, B, C, for Oct, Nov, Dec.)	56-57	Second Illness/Cause of Death (See 54-55)
56	Year (yy) 00045	58-59	Third Illness/Cause of Death (See 54-55)
		60-61	Primary Cancer Site
		00	None (unknown primary)
		01	Lymphomas
		02	Sarcomata
		21	Leukemia
		22	Cartilage
		03	Central nervous system
		04	Liver
		24	Stomach/Esophagus
		05	Lung
		25	Mediastinum
		06	Kidney
		26	Prostate
		07	Pancreas
		08	Skin
		28	Oral
		09	Breast
		29	Ovaries
		62	Second Job within Plant (See 33)
		63	Product Code for Second Job (See 34)
		64-66	Months Worked at Second Job
		67-71	Third Job within Plant (See 62-66)
		72-76	Fourth Job within Plant (See 62-66)
		77-80	Project Identification Codes

- 10 - Accident
- 30 - Homicide
- 11 - Benign tumors
- 12 - Peripheral vascular disease
- 32 - MID
- 13 - Peripheral nervous disorder
- 33 - Parkinson's Disease
- 14 - Gastrointestinal disorder
- 15 - Emphysema, COPD
- 35 - Asthma
- 16 - Genitourinary disorder
- 17 - Arthritis
- 18 - Cataracts, Glaucoma, Blindness
- 19 - Alcoholism
- 39 - Hematologic problems
- 99 - Unknown

- 11 - Hodgkin's Disease
- 12 - Myeloma
- 32 - Bone
- 13 - Peripheral nervous system
- 14 - Gall bladder
- 34 - Colon/Rectum
- 15 - Nasopharynx
- 35 - Mesothelioma
- 16 - Bladder
- 36 - Testicle
- 46 - Penis
- 17 - Other endocrine
- 18 - Melanoma
- 19 - Cervix/Uterus
- 90 - Other

DATA FIELDS LAYOUT

Name		Social Security Number		Date of Birth		Employment		Interven ^g			Date of Illness or Death			Other Jobs in Plant						Project																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
Last	First Initial	Middle Initial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017

(2) (2)

International Research and Development Corporation

SPONSOR: Velsicol Chemical Corporation
COMPOUND: Chlorendic Anhydride
SUBJECT: Acute Inhalation Toxicity
Study in Rats.

ORIGINAL RECORD
DO NOT REMOVE

15964

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Date: August 10, 1978

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I. SYNOPSIS

Inhalation exposure of 10 rats for 1 hour to a dust atmosphere of Chlorendic Anhydride at a "metered" concentration of 203.0 mg/l resulted in salivation during the exposure. A slight body weight loss was observed in 6 rats for 1 day postexposure.

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II. COMPOUND

The compound was received from the Velsicol Chemical Corporation, Chicago, Illinois on September 2, 1977.

It was received and identified as follows:

<u>Compound Identification</u>	<u>Description</u>
Chlorendic Anhydride Technical Reference Standard 93.8% (E1) Lot No. 3-12-206	fine white powder containing some chunks

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III. PROCEDURE

A. ANIMAL EXPOSURE:

Five male and 5 female Charles River CD rats, which had been quarantined for at least 1 week, were used to study the compound. The male rats weighed from 211 to 226 grams, and the females from 213 to 232 grams. The rats were housed individually in wire-mesh cages and were kept throughout the pre- and postexposure periods in a temperature and humidity controlled room in accordance with standards outlined in the "Guide For the Care and Use of Laboratory Animals; DHEW No. (N.I.H. 74-23) 1974". Purina Laboratory Chow and water were supplied ad libitum except when the rats were in the exposure chamber.

The rats were exposed to a single concentration of test material.

During exposure, the rats were caged individually in compartmented wire-mesh exposure cages. The cages were placed in a 160-liter cubical, stainless steel and glass chamber. A constant chamber airflow was maintained by means of a rotary centrifugal air pump located at the exhaust side of the chamber. The chamber exhaust was filtered with an activated charcoal filter and a Cambridge Absolute® filter before being discharge outside of the laboratory.

B. OBSERVATIONS:

Observations for pharmacotoxic signs and mortality were made during and immediately following the 1 hour exposure period and twice daily thereafter for 14 days. Individual body weights were recorded prior to 1 hour exposure and periodically thereafter in order to detect any latent effects following the exposure. The rats were sacrificed and discarded at the end of the 14 day observation period.

C. DUSTS ATMOSPHERE:

The dust atmosphere of the compound was generated by dispersing the powder at a calculated rate, with a specially constructed dust generator located near the chamber air inlet at the top of the exposure chamber. This dust generator consisted of a revolving plate with calibrated "cups" for transporting a known quantity of powder per unit time from a reservoir to a "blowhole". At the "blowhole" the powders in a "cup" were dispersed into the chamber by a jet of air blowing at the rate of 7.5 liters per minute.

The actual quantity of powder disseminated was determined by weighing the quantity of powder in the reservoir before and after the experiment.

A total of 91.37 grams of the powders was disseminated during the 1 hour exposure period. The rate of powder dissemination was calculated to be 1.52 grams per minute. The rate of chamber airflow was 7.5 liters per minute and the chamber dust concentration was calculated to be 203.0 mg/l. The chamber atmosphere was extremely dusty.

IV. RESULTS

A. ANIMAL OBSERVATIONS:

The immediate response of the rats to the experimental atmosphere was an increase of activity in preening. After several minutes of exposure, this activity decreased. After 30 minutes of exposure six rats exhibited salivation. By the end of the exposure all the rats exhibited salivation and one rat exhibited nasal discharge. After the exposure, all the rats appeared normal.

B. BODY WEIGHTS:

A slight body weight loss was observed in 6 rats for 1 day postexposure (Table 1).

C. MORTALITY:

None of the rats died in the experiment.

All animals survived the observation period and were sacrificed and discarded.

Chlorendic Anhydride: Acute Inhalation Toxicity Study in Rats.

TABLE I.
Individual Body Weights, Grams.

Calculated Concentration, Rat Number	Sex	Postexposure (Days)						
		0	1	3	5	7	14	
202.0 mg/l:								
81861	M	212	214	237	250	250	306	
81862	M	224	218	264	264	269	324	
81863	M	226	222	252	264	270	290	
81864	M	211	213	236	252	254	289	
81865	M	218	220	242	260	260	306	
81866	F	214	205	222	218	220	243	
81867	F	220	213	228	225	225	246	
81868	F	232	233	248	246	240	250	
81869	F	213	202	212	222	212	223	
81870	F	221	216	224	232	228	252	

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Date Produced (6)	6	Date Rec'd (6)	7
11-2272-		1-22485-	8
Conf. Code •	N		
Check one: <input type="checkbox"/> Publication <input type="checkbox"/> Internally Generated <input checked="" type="checkbox"/> Externally Generated	9		
Pub/Journal Name	9		
Author(s)	10		
Organ. Name	11		
1/ELSTON CHEM CORP	12		
Dept/Div	12		
P.O. Box	13	Street No./Name	14
		341 E OHIO ST	
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ZIP	18	Country	18
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MID No. (7)	19	D & B NO. (11)	20
Contractor	21		
INTL RES & DELV CORP	22		
Doc Type	22		
• R.I. • U.P. • F.Y.I.V.S. — — — S.U.B.	23		
Doc Title	23		
ACUTE TOXICITY STUDIES IN RATS AND RABBITS	24		
Chemical Name (300 per name)	25	CAS No. (10)	24
CHLORANTIC AMIDE		115-27-5	

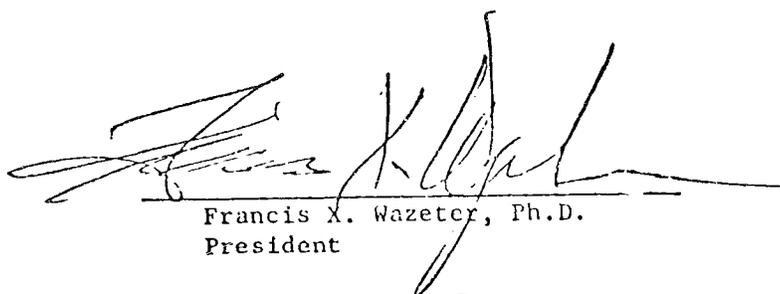
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SPONSOR: Velsicol Chemical Corporation
COMPOUND: Chlorendic Anhydride
SUBJECT: Acute Toxicity Studies in Rats
and Rabbits.



Francis X. Wazeter, Ph.D.
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Date: November 22, 1972

International Research and Development Corporation

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I. SYNOPSIS

The test compound was examined for acute toxicity in rats and rabbits in accordance with the regulations under the Federal Hazardous Substances Act. The following tests were conducted and the results as indicated obtained:

Eye Irritation Test in Albino Rabbits (Proposed regulation under 21 CFR Part 191, Hazardous Substances Test for Eye Irritants, Food and Drug Administration):

Group I - 5 minute wash:

A strongly irritant substance.

Group II - 24 hour wash.

An extremely irritant and corrosive substance.

Acute Dermal Toxicity in Albino Rabbits:

Not a toxic substance by the dermal route of administration.

Acute Inhalation Exposure in Male Albino Rats:

Not a highly toxic substance by the inhalation route of administration.

The test compound also was evaluated for primary skin irritation and corrosive hazard in accordance with Title 49 - Transportation, Chapter 1, Classification of Corrosive Hazards. Based upon the test data, the following result was obtained:

Not a primary skin irritant nor does this material present a corrosive hazard to the skin.

In addition, an acute oral toxicity (LD_{50}) was conducted in male and female albino rats. The acute oral LD_{50} values were calculated to be as follows:

Male Albino Rats: 1190 (875-1618) mg/kg

Female Albino Rats: 1098 (912-1320) mg/kg

Combined Male and Female Albino Rats: 1138 (971-1333) mg/kg

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II. COMPOUND

The test compound was received from the Velsicol Chemical Corporation, Chicago, Illinois on July 21, 1972

It was identified as "Chlorensic Anhydride, 72-785, C. Calo" and was received as a white powder.

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III. EYE IRRITATION IN THE ALBINO RABBIT

A. METHODS:

Four male and 4 female New Zealand White rabbits were used in this test. The rabbits weighed from 2171 to 2777 grams at the beginning of the study. Food and water were available ad libitum.

Prior to compound administration, the eyes of each rabbit were examined with ultraviolet light after instillation of one drop of a 2.0 percent sodium fluorescein solution. Rabbits exhibiting corneal lesions were discarded.

The 8 rabbits were divided into 2 groups. Group I consisted of 5 rabbits and Group II consisted of 3 rabbits. Rabbits in both groups received 0.1 milliliter by volume of the test compound.*

The test material was placed into the cupped conjunctival sac of the right eye of each rabbit following which the eyelids were gently held together for one second. The left eye served as the untreated control for each rabbit.

The 5 rabbits in Group I were exposed to the test compound for 5 minutes. The rabbits in Group II were exposed to the test compound for 24 hours. Following the exposure periods, the treated eyes were washed with a gentle, continuous stream of water, regulated to deliver a volume of 300 milliliters of water over a 2 minute period. The eyes of the rabbits in Group II were examined at 24 hours prior to the 24 hour wash.

The treated eyes were examined in accordance with the following schedule:

*Average weight of each milliliter test dose:
Group I - 104.3 milligrams
Group II - 94.9 milligrams

Group I	Group II
1 hour following wash at 5 minutes*	1 hour following instillation
24 hours*	24 hours*
48 hours	48 hours
72 hours*	72 hours*
7 days*	7 days*
14 days*	14 days*
21 days*	21 days*

*Sodium fluorescein examinations were conducted at these intervals. After grading the eye reaction, one drop of sodium fluorescein was instilled directly onto the corneal surface of each rabbit. After a few seconds, excess stain was flushed from the corneal surface with 20 milliliters of water and the eyes examined with ultraviolet light.

Fourteen and 21 day examinations were not required in this test as no ulceration, necrosis or corneal opacity was present at the 7 day examination interval.

The grading system employed in this test procedure is presented on the following page.

B. RESULTS:

Table 1 (Group I) and Table 2 (Group II) present a summary of the results obtained at each examination period.

Based upon the data derived, the following results were obtained:

Group I - 5 minute wash:

A strongly irritant substance.

Group II - 24 hour Wash:

An extremely irritant and corrosive substance.

Grading System for Scoring Ocular Lesions

<u>Area</u>	<u>Grade</u>
A. CORNEA:	
No ulceration or opacity	0
Scattered or diffuse areas of opacity, details of iris clearly visible	1 ^{a,c}
Easily discernible translucent areas of opacity, details of iris slightly obscured	2 ^{a,b}
Nacreous areas of opacity, no details of iris visible, size of pupil barely discernible	3 ^{a,b}
Complete corneal opacity, iris not discernible	4 ^{a,b}
Ulceration, absence of a gross patch of corneal epithelium	4 ^b
B. IRIS:	
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof). Iris still reacting to light	1 ^a
No reaction to light, hemorrhage, gross destruction (any of all of these)	2 ^a
C. CONJUNCTIVAE:	
1. Redness (palpebral/bulbar conjunctivae):	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	2 ^a
Diffuse, beefy red	3 ^a
2. Chemosis:	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2 ^a
Swelling with lids half closed	3 ^a
Swelling with lids more than half closed	4 ^a
3. Ulceration or necrosis of palpebral/bulbar conjunctivae or nictitating membrane	4 ^b

-
- a. considered positive for determining an eye irritant
 b. considered positive for determining a corrosive substance
 c. if a grade 1 opacity is evidenced for 6 or more days, considered positive for determining a corrosive substance

Chlorencic Anhydride: Eye irritation in the Albino Rabbit.

TABLE 1. Observations (Group I - 5 minute wash).

Area	Observation	Examination Interval (No. Positive/No. Dosed)				
		Hours				Days
		1	24	48	72	7
<u>CORNEA</u>						
	No ulceration or opacity	5/5	5/5	5/5	5/5	5/5
	Grade 1 opacity					
	Grade 2 opacity					
	Grade 3 opacity					
	Grade 4 opacity					
	Ulceration, absence of corneal epithelium					
<u>IRIS</u>						
	Normal	5/5	5/5	5/5	5/5	5/5
	Grade 1 iridal irritation					
	Grade 2 iridal irritation					
<u>CONJUNCTIVAE</u>						
	Redness:					
	Vessels normal			1/5	3/5	5/5
	Grade 1 redness	4/5		3/5	2/5	
	Grade 2 redness	1/5	5/5	1/5		
	Grade 3 redness					
<u>CHEMOSIS</u>						
	No swelling	1/5	4/5	5/5	5/5	5/5
	Grade 1 swelling	2/5	1/5			
	Grade 2 swelling	2/5				
	Grade 3 swelling					
	Grade 4 swelling					
	Ulceration or necrosis of conjunctivae or nictitating membrane					
<u>OTHER:</u>						
	Blanching	4/5	0/5	0/5	0/5	0/5
	Hemorrhage, conjunctivae					
	Purulent discharge					
	Vocalization following instillation					
	Sodium fluorescein examination, number exhibiting positive corneal damage	1/5	1/5	-	0/5	0/5

Chlorenic Anhydride: Eye Irritation in the Albino Rabbit.

TABLE 2. Observations (Group II - 24 hour wash).

Area	Observation	Examination Interval (No. Positive/No. Dosed)						
		Hours				Days		
		1	24	48	72	7	14	21
<u>CORNEA</u>								
	No ulceration or opacity	3/3	2/3	1/3	2/3	2/3	3/3	2/3
	Grade 1 opacity		1/3	2/3	1/3	1/3		1/3
	Grade 2 opacity							
	Grade 3 opacity							
	Grade 4 opacity							
	Ulceration, absence of corneal epithelium							
<u>IRIS</u>								
	Normal	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	Grade 1 iridal irritation							
	Grade 2 iridal irritation							
<u>CONJUNCTIVAE</u>								
	Redness:							
	Vessels normal	1/3				1/3	3/3	3/3
	Grade 1 redness	2/3			3/3	2/3		
	Grade 2 redness		3/3	3/3				
	Grade 3 redness							
<u>CHEMOSIS</u>								
	No swelling					2/3	2/3	2/3
	Grade 1 swelling						1/3	1/3
	Grade 2 swelling		2/3	2/3	3/3	1/3		
	Grade 3 swelling	1/3	1/3	1/3				
	Grade 4 swelling	2/3						
	Ulceration or necrosis of conjunctivae or nictitating membrane							
<u>OTHER:</u>								
	Blanching	3/3	3/3	1/3	0/3	0/3	0/3	0/3
	Hemorrhage, conjunctivae							
	Purulent discharge		2/3	1/3	0/3	0/3	0/3	0/3
	Vocalization following instillation							
	Sodium fluorescein examination, number exhibiting positive corneal damage	3/3			3/3	1/3	1/3	1/3

IV. ACUTE DERMAL TOXICITY IN ALBINO RABBITS

A. METHOD:

Two male and 2 female New Zealand White rabbits were used in this test. The rabbits weighed from 2410 to 2930 grams at the beginning of this study. Food and water were available ad libitum. Body weights were measured initially and at 14 days after compound application.

The hair was removed from the back of each rabbit with an electric clipper. The skin of 1 male and 1 female rabbit was abraded with a scalpel blade.

The test compound was applied once only to the back of each rabbit at a dosage level of 2000 mg/kg. The area of application was wrapped with a gauze bandage and occluded with Saran Wrap. Twenty-four hours later the bandages were removed and the backs were washed with tepid tap water. The rabbits were observed for mortality for a period of 14 days.

B. RESULTS:

All of the rabbits survived the 14 day period of study.

Two of four rabbits exhibited body weight gains during the study period; the remaining two rabbits showed body weight losses of 914 and 31 grams.

Based upon the results obtained, the test compound would not be considered a toxic substance by the dermal route of administration.

V. ACUTE INHALATION TOXICITY IN THE ALBINO RAT

A. METHOD:

1. General Procedure:

Ten male rats of the Spartan strain, weighing from 223 to 265 grams, were used in this test. The rats were housed in groups of 5 in metal cages above the droppings and maintained in temperature and humidity controlled quarters throughout the pre-exposure and post-exposure periods. Purina Laboratory Chow and water were available ad libitum.

During the 4 hour exposure to the test compound, the rats were observed continuously for changes in behavior and/or appearance. Immediately following the exposure, the rats were examined closely for pharmacodynamic and/or toxic signs. All rats which died on study were subjected to a gross necropsy examination. The rats were observed for a period of 14 days and then sacrificed.

2. Compound Administration:

The group of 10 rats was placed in a sealed 59.1 liter glass chamber and exposed for 4 hours to a dynamic atmosphere containing the aerosol mist of the test material. In order to prevent "piling up" during the exposure, the rats were separated into 4 units of 2 or 3 rats each.

Addition of the test compound to the test chamber atmosphere was controlled by a Wright Dust Feeder. Dried and filtered air was passed through the mechanism and directly into the exposure chamber. Airflow was regulated by means of a flowmeter¹.

¹Gelman Instrument Company, Ann Arbor, Michigan, Model No. 8221

The calculated atmospheric concentration administered was approximately 65 mg/L. of the test compound.

B. RESULTS:

None of the rats exposed to the 65 mg/L. atmospheric concentration died during the 4 hour exposure period or the subsequent 14 day observation period.

Signs seen during the exposure period included eye squint, dyspnea, prostration, salivation, lacrimation, ocular and nasal porphyrin discharge, erythema and generally decreased motor activity. At 24 hours 3 rats continued to exhibit ocular or nasal porphyrin discharge. At 48 hours all rats were normal and continued so for the remainder of the 14 day study period.

All rats exhibited normal body weight gains during the 14 day period of study.

In accordance with the results obtained and the regulations under the Federal Hazardous Substances Act, the test compound would not be considered a highly toxic substance by the inhalation route of administration.

VI. PRIMARY SKIN IRRITATION AND CORROSIVE HAZARD TEST IN ALBINO RABBITS

A. METHOD:

Three male and 3 female New Zealand White rabbits were used. The rabbits weighed from 2330 to 2595 grams at the beginning of the study.

The hair was removed from the back of each rabbit with an electric clipper. The skin of 3 of the rabbits was abraded with a scalpel blade. Food and water were available ad libitum.

500 milligrams of the test material were applied to the back of each rabbit. The area of application was then wrapped with a gauze bandage. Four hours later the bandages were removed and the area was washed with tepid tap water and examined for skin irritation in accordance with the scale on the following page. These examinations were repeated at 24 and 72 hours.

B. RESULTS:

No evidence of skin irritation was observed at any of the observation intervals.

Based upon the results obtained, Chlorencic Anhydride does not present a corrosive hazard to the skin nor is this material a primary skin irritant.

Due to the negative results obtained in this study, tables of a summation of observations and calculated primary irritation score have been deleted from this report.

	<u>Value*</u>
<u>Erythema and Eschar Formation:</u>	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
 <u>Edema Formation:</u>	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4

*The "Value" recorded for each reading is the average value of six or more animals subjected to the test.

The values for erythema and eschar formation at 24 hours and at 72 hours for the intact animals' skin were added to similar values obtained for the abraded skin animals (a total of 4 values).

Similarly, the values for edema formation at 24 and 72 hours for intact and abraded skin animals were added together (a total of 4 values). The primary irritant score is the sum of the 8 values divided by 4. As scored by this method, a primary irritant is a substance which is not corrosive, but which results in a score of 5 or more. (Section 191.1 (g) (2) of the regulations of the Federal Hazardous Substances Act.)

VII. ACUTE ORAL TOXICITY IN ALBINO RATS

A. METHOD:

Twenty-five male and 25 female Spartan rats weighing from 104 to 144 grams were used for this study. The rats were housed by sex in groups of 5 rats per cage, elevated above the droppings. They were maintained in temperature and humidity controlled quarters throughout the study period. The rats had food and water available ad libitum, except for an overnight period preceding compound administration during which food, but not water, was withheld.

The test compound was suspended in corn oil and administered orally at the following dosage levels to male and female rats: 500, 1250, 1984, 3150 and 5000 mg/kg.

Five rats of each sex were used at each dosage level. Volumes of 10 ml/kg of body weight were administered at all dosage levels.

All rats were observed for mortality continuously during the first 4 hours after dosing, at 24 hours, and once daily thereafter for a total of 14 days.

Body weights were recorded initially and at 14 days.

B. RESULTS:

1. Body Weight:

All surviving rats at each dosage level exhibited normal body weight gains during the 14 day observation period.

2. Calculated Acute Oral LD₅₀ Values:

a. Male Albino Rats (Table 3):

The acute oral LD₅₀ of Chlorencic Anhydride in male albino rats was calculated to be 1190 mg/kg with confidence limits of 875-1618 mg/kg.

b. Female Albino Rats (Table 4):

The acute oral LD₅₀ of Chlorencic Anhydride in female albino rats was calculated to be 1098 mg/kg with confidence limits of 912-1320 mg/kg.

c. Combined Male and Female Rats (Table 5):

The combined acute oral LD₅₀ of Chlorencic Anhydride in male and female albino rats was found to be 1138 mg/kg with confidence limits of 971-1333 mg/kg.

Chlorencic Anhydride:

TABLE 3. Acute Oral Toxicity (LD₅₀) in Male Albino Rats.

Dose mg/kg	No. Died								Total No. Died/ No. Dosed	LD ₅₀ and Confidence Limits (mg/kg)
	Hours	Days								
	0-4	1	2	3	4	5	6	7-14		
500									0/5	
1250									0/5	
1984			3		1				4/5	1190
3150			3	1					4/5	(875-1618)
5000		4	1						5/5	

Geometric Ratio: 1.588

Statistical Reference:

1. Thompson, W. R., Bact. Rev., 11: 115-145, 1947.

Chlorencic Anhydride:

TABLE 4. Acute Oral Toxicity (LD₅₀) in Female Albino Rats.

Dose mg/kg	Hours	No. Died							Total No. Died/ No. Dosed	LD ₅₀ and Confidence Limits (mg/kg)
		Days								
		0-4	1	2	3	4	5	6		
500									0/5	
1250									0/5	
1984			2	1				1	4/5	1098
3150			3	2					5/5	(912-1320)
5000		3	2						5/5	

Geometric Ratio: 1.588

Statistical Reference:

1. Thompson, W. R., Bact. Rev., 11: 115-145, 1947.

Chlorenic Anhydride:

TABLE 5. Acute Oral Toxicity (LD₅₀) in Male and Female Albino Rats.

Dose mg/kg	No. Died								Total No. Died/ No. Dosed	LD ₅₀ and Confidence Limits (mg/kg)
	Hours	Days								
	0-4	1	2	3	4	5	6	7-14		
500									0/10	
1250									0/10	
1984			5	1	1			1	8/10	1138
3150			6	3					9/10	(971-1333)
5000		7	3						10/10	

Geometric Ratio: 1.588

Statistical Reference:

1. Thompson, W. R., Bact. Rev., 11: 115-145, 1947.

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Organ. Name	11		
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MID No. (7)	19	D & B NO. (11)	20
Contractor	21		
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Chemical Name (300 per name)	25	CAS No. (10)	24

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CHLORENDIC ANHYDRIDE

CHLORENDIC ANHYDRIDE

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THE TOXICITY OF CHLORENDIC ACID
AND CHLORENDIC ANHYDRIDE



June 25, 1965

THE KETTERING LABORATORY
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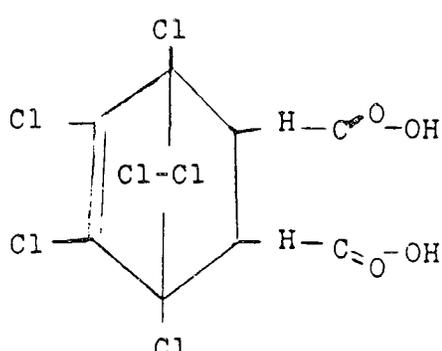
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THE TOXICITY OF CHLORENDIC ACID
AND CHLORENDIC ANHYDRIDE

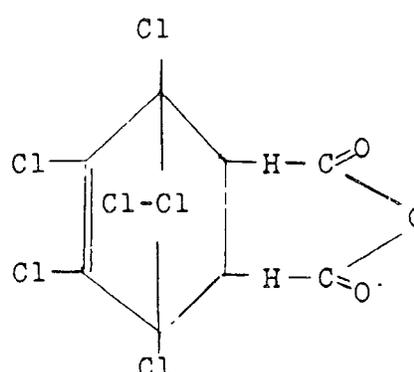
INTRODUCTION

Chlorendic acid and chlorendic anhydride are products of Velsicol Chemical Corporation. They may be used in the making of resins and plasticizers that retard the propagation of flame, in the syntheses of other organic chemicals, as additives in certain petroleum products, and for various other technical purposes.

The chemical structure of each of the 2 materials is shown below:



Chlorendic Acid



Chlorendic Anhydride

Investigation of the toxicological properties of the 2 compounds was undertaken in order to provide information which could serve in promoting methods whereby the compounds could be used safely. These investigations were concerned with 1), the immediate toxicity of chlorendic acid and chlorendic anhydride, when administered orally to rats; 2) the effects associated with the ingestion by rats of small amounts of either

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material on each of 5 days per week over periods of 7 weeks; 3) the physiological responses of rabbits, following the placement, repeatedly, of either the acid or the anhydride in contact with the intact abdominal skin; and 4) the irritating properties of the material when placed upon the surfaces of the ocular tissues of rabbits.

MATERIALS AND METHODS

Chlorendic anhydride (52-CS-83A) and chlorendic acid (52-CS-83B) were obtained from Velsicol Chemical Corporation, Chicago, Illinois. Each material is a white crystalline solid. The acid decomposes to the anhydride when heated. The anhydride melts at 240-241°C.; it sublimes between 90° and 100°C. when under a pressure of 0.5 mm. of mercury. The acid is formed when the anhydride is boiled in water. The acid is soluble in benzene to the extent of 1.1 per cent (w/w), as is the anhydride, to the extent of 40.4 per cent; they are but slightly soluble in water.

Each compound, as a 20 per cent aqueous suspension, was administered, on one occasion, in staged dosages, to separate groups of rats. Each group contained both sexes, ranging in age from 3 to 12 weeks.

Three groups, each composed initially of 6 male and 6 female rats, approximately 5 weeks of age, were given chlorendic anhydride orally in repeated dosages of 400, 200, or 100 mg. per kg. of bodily weight, respectively. An appropriate volume of an aqueous suspension containing the anhydride, in the concentration of 4 per cent (w/v), was given by means of a

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stomach tube to each animal on each of 5 days per week over the period of 7 weeks. In like manner and dosage, chlorendic acid was given orally to 3 other groups of rats; a 7th group (similarly constituted in respect to the sex, number and age of the rats) was given water only, in the corresponding manner.

The animals were observed regularly for signs of illness, and were weighed individually each week.

Terminally, samples of blood were taken from each rat, in which the relative numbers of erythrocytes and leukocytes, the content of hemoglobin and the percentage distribution of the differentiated leukocytes, were determined. The animals were killed, their organs were examined for gross pathological changes, the brain, heart, lungs, liver, kidneys, and spleen were weighed individually, and sections of the viscera were prepared for microscopic examination.

In preparation for topical applications of the acid or the anhydride, the hair was clipped closely from the abdominal skin of 18 male New Zealand rabbits (10 weeks of age), and the animals were restrained supinely in individual stocks. Powdered chlorendic anhydride, in the dosage of 1 g. per kg., was applied upon and allowed to remain for 4 hours in contact with the intact abdominal skin of 3 of the rabbits; a solution containing chlorendic anhydride in the concentration of 20 per cent in dimethyl phthalate, was applied upon the intact skin of 3 other rabbits, in volumes equivalent to 0.2 g. of the compound per kg. of bodily weight. After 4 hours, the residual materials were washed from the skin of the animals with soap and warm running water. The contact with the compound

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was repeated in like manner on each of 5 days per week, over the period of 8 weeks.

Similarly, 3 rabbits were subjected to daily contact with powdered chlorendic acid in dosages of 1 g. per kg., as were 3 others with a solution of the compound in dimethyl phthalate, the daily dose of the compound being 0.2 g. per kilogram. Dimethyl phthalate, containing no chlorendic acid or anhydride, was applied daily upon the skin of 3 rabbits; 3 rabbits were confined in the stocks regularly and were subjected to all of the manipulations required by the procedure, excepting that no material was applied upon their skin.

A solution containing either chlorendic anhydride or acid, in dimethyl phthalate in concentrations of 5, 10, or 20 per cent (w/v), was inserted beneath the eyelids, upon the surface of the ocular tissues of one eye of each of 4 rabbits; dimethyl phthalate was placed upon the surface of the other eye. The volume of the dose was uniformly 0.2 ml. regardless of the concentration of the chemical in the solution or the bodily weight of the rabbit. Powdered chlorendic anhydride (20 mg.) was placed upon the surface of the conjunctiva of one eye and a like amount of the powdered acid upon the conjunctiva of the other eye of each of 2 rabbits. The eyes were examined periodically during 5 days after the materials had been placed in contact with them, and the reactions were noted and recorded.

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RESULTS

1. Immediate Oral Toxicity.

The detailed data showing the numbers of rats of each sex that were given the specified dosage of either material, together with the number of deaths resulting at each level of dosage, are given in Table 1A in the Appendix. The LD₅₀ values estimated from the data are represented in Table 1.

Table 1

Chlorendic compound	LD ₅₀ (g. per kg.)		
	Males	Females	Both sexes
Anhydride	3.13 ± 0.37	3.16 ± 0.30	3.13 ± 0.34
Acid	2.92 ± 0.10	2.65 ± 0.48	2.79 ± 0.35

Within the limits of the experimental procedures, the sex of the animals and their age had little, if any, effect upon their responses to the absorption of the anhydride or the acid. The length of the period of survival, following the administration of lethal dosages, generally ranged from 10 hours to 15 days; most of the deaths occurred within 2 or 3 days. The signs of illness were vague and poorly defined. Rats that had been given lethal amounts of either the anhydride or the acid generally showed some mild degree of weakness and a continuous loss in bodily weight. An occasional animal had a bloody discharge from the anus. Animals that had been given sublethal amounts of either compound exhibited only mild transient losses in bodily weight.

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All rats that had been given any one of the several oral dosages of either the chlorendic anhydride or the chlorendic acid were examined post mortem. Animals that died after ingesting a sufficient amount of either compound exhibited marked passive hyperemia of the internal viscera, on gross examination. The brain and liver were swollen and soft. The kidneys were swollen, pale and cloudy.

Representative animals were chosen at random for microscopic examination of their viscera.

The viscera of animals that survived after ingesting chlorendic anhydride in dosages of 1.4, 2.1, 3.2, or 4.7 g. per kg. of bodily weight (these animals were killed for examination 1 month after ingesting any one of the stated doses), were found to be normal. The animals that died, within 3 days after ingesting the compound, had diffuse degenerative changes in the hepatic cells throughout the liver lobule. These changes increased in severity with increase in the dosage of chlorendic anhydride. There was degeneration in the epithelial cells of the renal tubules, with focal necrosis. This also increased in severity with increase in the dosage of the material. Neurophagia was encountered in the cerebral cortex of some of the animals to which 4.7 or 7.0 g. of chlorendic anhydride per kg. of bodily weight had been administered. There was no difference between male or female, or young or older rats, with respect to the morphologic manifestations of the effects of the absorption of chlorendic anhydride, upon the liver, kidney, or brain.

The rats that survived, after ingesting chlorendic acid in amounts ranging from 1.4 to 4.7 g. per kg., were killed 1 month later for pathological examination. The microscopic examination of their viscera revealed no alterations attributable to chlorendic acid. Rats that died within 3 days, after ingesting the acid in a single oral dose of 4.7 or 7.0 g. per kg., exhibited diffuse degeneration in the hepatic cells, degeneration of the epithelial cells of the renal tubules, and a slight degree of neurophagia. The pathologic alterations were more severe in the animals to which the compound had been administered in the higher concentration; they were not differentiated according to the age or sex of the animals.

2. The Effects Associated With the Ingestion, Repetitively, of Moderate Amounts of Either Compound.

The cumulative number of deaths among the 6 animals of each sex to which each level of the dosage of either compound was administered during the 7 weeks, is shown in Table 2A. The data with respect to mortality are summarized in Table 2 below, without respect to the time at which the fatalities occurred.

Table 2

Daily dosage (mg./kg.)	Number of deaths resulting			
	Chlorendic anhydride		Chlorendic acid	
	males	females	males	females
400	4	2	1	0
200	1	0	1	0
100	2	0	0	0
water only		1	2	

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The mortality was 50 per cent among the 12 rats that were given chlorendic anhydride at the level of 400 mg. per kg.; among the rats subjected to the dosages of 200 or 100 mg. of the anhydride per kg., and among those that were given the chlorendic acid at all 3 levels of dosage, the mortality was less than that which occurred among the rats that were given water only. Certain groups of male rats to which the anhydride had been given, showed marked reduction in their average rates of growth (Figure 1), the inhibition being apparent at the 100 mg. per kg. level of dosage, and increasing in degree with increased levels of intake. The female rats to which the anhydride was administered, showed only slight and inconclusive interferences with their rates of growth, irrespective of the level of dosage.

Male rats subjected to the oral administration of chlorendic acid also sustained marked reduction in their rates of growth (Figure 2), but this effect correlated poorly with the level of intake of the compound; males that were given 400 mg. per kg. per day, gained in weight as well, or better, than those that were given 200 mg. per kg. per day. The females sustained a mild degree of depression in their average rates of growth, which agreed but poorly with the magnitude of the dose.

The average content of hemoglobin, the relative numbers of erythrocytes and leukocytes, and the relative distribution of the differentiated leukocytes in the blood of the rats, after they had been given the specified doses of either the anhydride or the acid, are given in Tables 3A (males) and 4A (females).

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Figure 1

Average Rates of Growth of Groups of Rats Given
Chlorendic Anhydride, Orally, on Each of 5 Days per Week,
During the Period of 7 Weeks

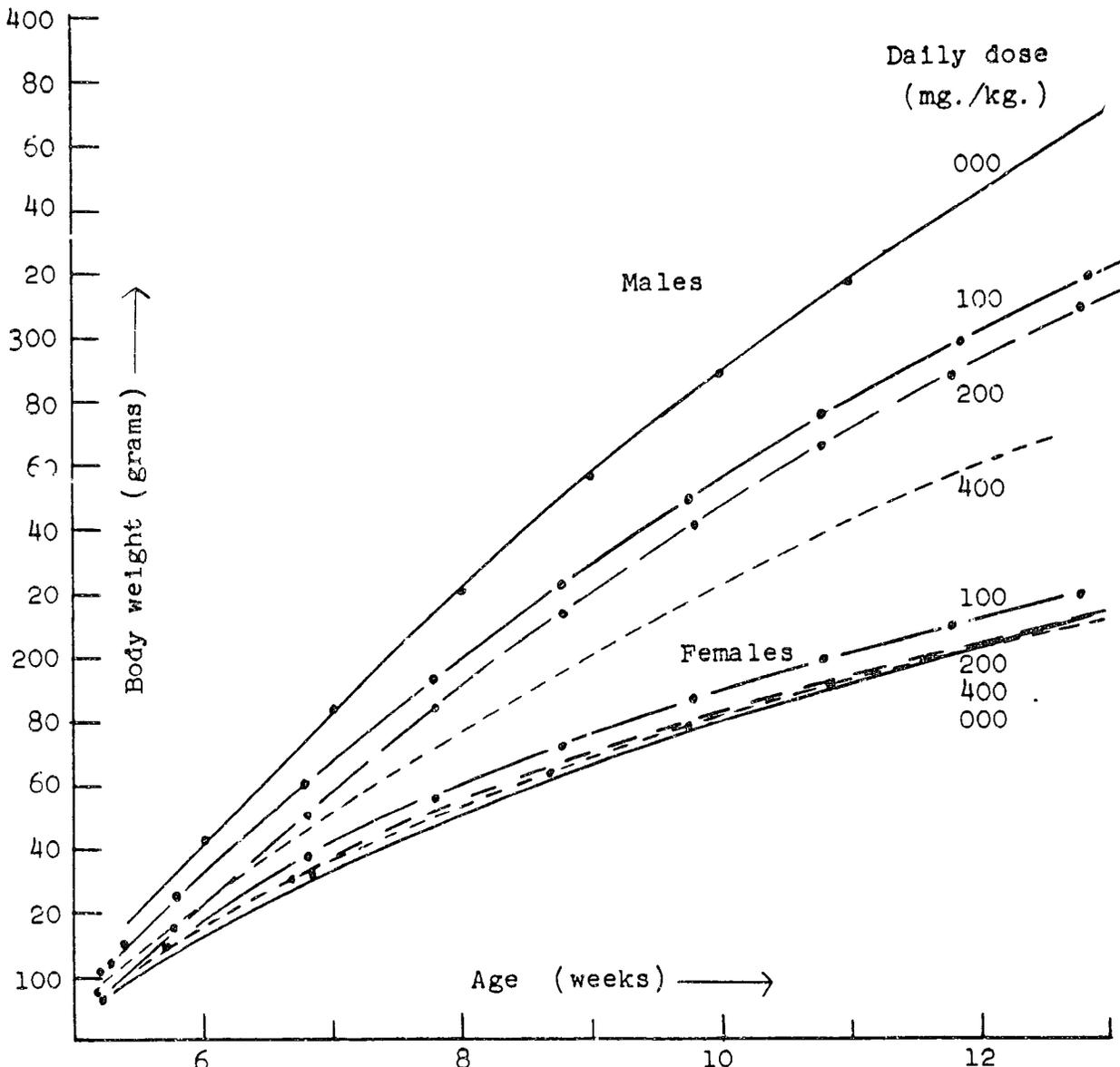
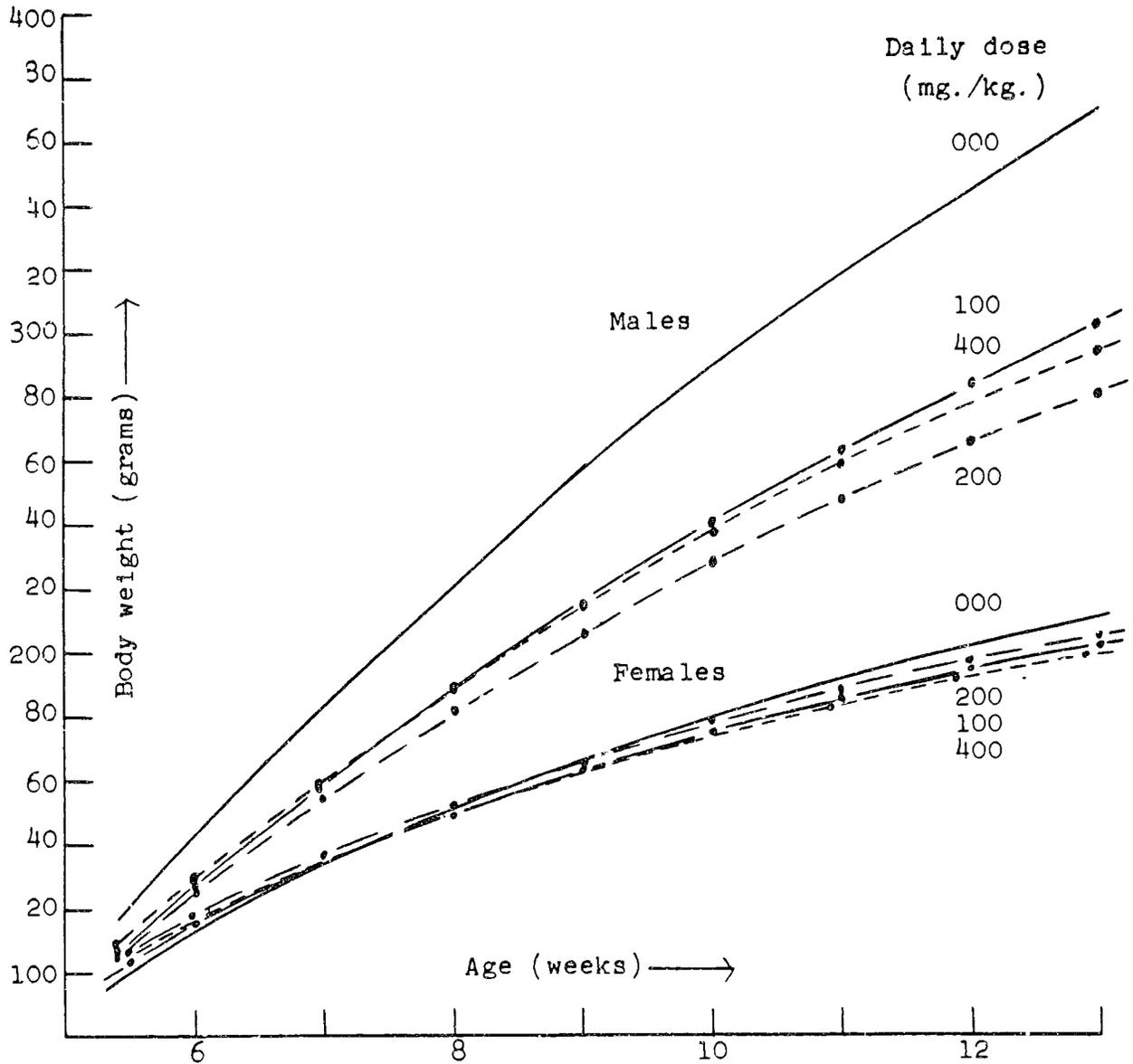


Figure 2

Average Rates of Growth of Groups of Rats Given
Chlorendic Acid, Orally, on Each of 5 Days per Week,
During the Period of 7 Weeks



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There was an increase in the relative numbers of leukocytes in the blood of the male rats to which 400 mg. per kg. of either the anhydride or the acid were given; occasionally, significantly elevated numbers were noted in individual animals at other levels of dosage. The content of hemoglobin and the relative numbers of erythrocytes remained essentially unchanged in the blood of both sexes.

The average weights of various organs of the rats in the respective groups (after having taken 35 doses of either material) are given in Table 5A in conjunction with the final bodily weights. The data indicate that the weights of the brain, heart, lungs, liver, kidneys, and spleen, respectively, were not affected by either compound that had been administered at any of the levels of dosage.

Of the rats to which chlorendic anhydride had been administered, repetitively, in oral dosages of 100 mg. per kg., 2 males died after the 15th and 36th days. The gross and microscopic examination of their viscera revealed diffuse degeneration in the liver, tubular renal degeneration, focal necrosis in the spleen, and hyperemia and slight edema in the lungs. The 4 males that survived for 7 weeks during the administration of this dosage had normal viscera, except for slight evidences of fatty metamorphosis of the hepatic cells. All the female rats survived; microscopic examination disclosed only comparable evidences of fatty changes in the liver.

Of the 6 male rats that had ingested chlorendic anhydride in the repetitive dosage of 200 mg. per kg., 1 died on the 10th day; microscopic examination of its viscera revealed

acute pneumonia, slight fatty metamorphosis in the hepatic cells, and tubular degeneration and focal necrosis in the kidneys. The 5 animals that survived at this level of dosage, had normal viscera, except for a slight to moderate evidence of fatty metamorphosis of the hepatic cells. All the female rats at this level of dosage had normal viscera excepting for corresponding alterations in the liver.

The rats that died during the period in which chlrendic anhydride had been administered to them in serial dosages of 400 mg. per kg., were found to have intercurrent infectious diseases, including diffuse myocarditis, encephalitis, and bilateral bronchopneumonia. A toxic effect induced by the absorption of the compound was manifested in the liver in the form of a moderate degree of fatty metamorphosis. Two female rats that died during the administration of the same dosage were found to have acute bilateral pneumonia; however, slight to moderate fatty changes were noted in the livers of all of the females within this group. The other viscera exhibited no abnormalities.

Except for slight degrees of chronic gastritis, no pathologic changes were present in the viscera of rats to which chlrendic acid had been administered repetitively in the dosage of 100 mg. per kilogram.

One of the male rats that had been given the acid in serial dosages of 200 mg. per kg., died of acute confluent bronchopneumonia. This animal, and 3 of the survivors showed slight degenerative changes in the centro-lobular hepatic cells. All of the 6 males in this group had chronic gastritis.

The 6 female rats survived during the period in which chlorendic acid had been given in the serial dosage of 200 mg. per kilogram. All of them had gastritis; 2 animals had slight degenerative changes in the hepatic cells.

Of the 12 rats to which chlorendic acid had been administered, repetitively, in oral dosages of 400 mg. per kg., 1 male died, incidentally, of acute peritonitis. Except for chronic gastritis, the 11 survivors had normal viscera.

One female and 2 male rats in the control group died of bilateral confluent pneumonia. The viscera of the survivors were normal.

The pathologic changes resulting from the ingestion of chlorendic anhydride, repetitively, were more severe than those resulting from the ingestion of chlorendic acid under comparable conditions of dosage. The latter compound caused only irritation of the gastric mucosa, without inducing toxic changes in the other viscera. The pathologic changes attributable to the absorption of chlorendic anhydride by the male rats were more prominent than those observed in the female rats. The deaths that occurred among the rats subjected to all levels of dosage of either compound, and as well as those which occurred in the control group, were caused, primarily, by intercurrent infectious diseases.

3. Effects Associated With Repeated Contact of Chlorendic Anhydride or Chlorendic Acid With the Skin of Rabbits.

All of the animals that were subjected, repetitively, to contact of their skin with the anhydride or the acid, survived without exhibiting signs of illness, but for some

fluctuation in their bodily weights. Contact of the skin of rabbits with either material, in solution in dimethyl phthalate, resulted in their failure to gain in bodily weight as rapidly as did those subjected to contact with the respective compounds as a dry powder. In each instance, however, the average rate of growth was better than that of the corresponding control group (Figure 3).

No local irritation resulted from repeated contact of the skin of the animals with either the anhydride or the acid in dry powder form. Corresponding periods of contact with a solution containing the anhydride in dimethyl phthalate produced mild, recurrent erythema, and occasional slight edema in the skin of one of 3 rabbits. Repeated contact with solutions of chlorendic acid in dimethyl phthalate caused mild to severe erythema, mild edema, vesiculation, and occasional fissures in the skin. In general, these changes did not appear until the 5th week; they were transient, but recurrent.

The gross and microscopic examination of the viscera of the rabbits at the time they were killed revealed no pathologic alterations that could be attributed to the absorption of either chlorendic acid or chlorendic anhydride. Some of the animals suffered from intercurrent diseases such as granulomatous encephalitis, chronic pyelonephritis, or pericholangitis.

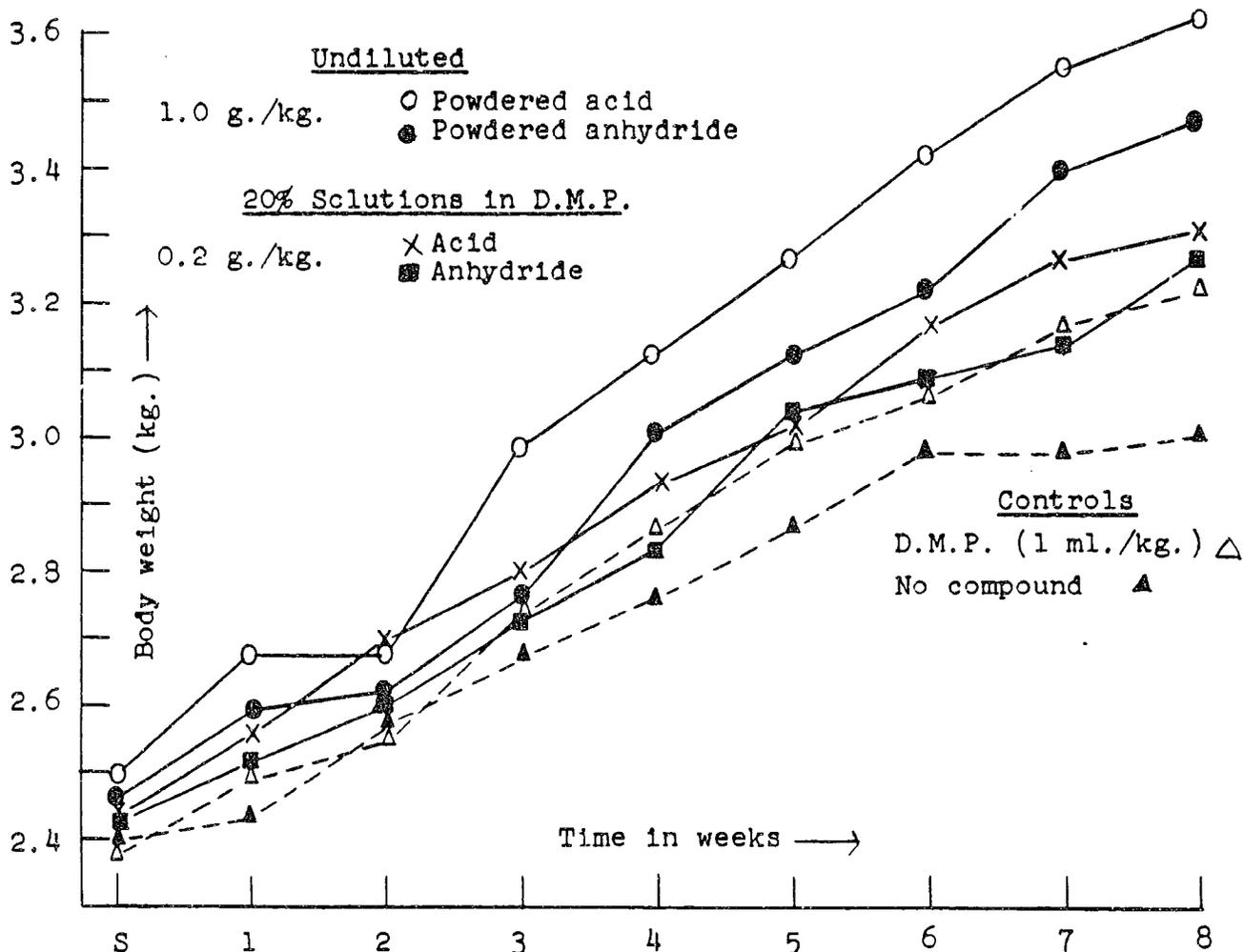
4. Ophthalmic Effects.

A code that was used to score the responses of the external tissues of the eye to contact with the material, and a summary of the results, are given in Tables 6A and 6B,

Figure 3

Average Rates of Growth of Groups of Rabbits Subjected, Repetitively, to Contact of Their Skin With Chlorendic Anhydride or Chlorendic Acid

(The compounds were applied upon the skin of the respective animals, in the stated dosages, either as undiluted powders or as 20 per cent solutions in dimethyl phthalate, and allowed to remain for 4 hours on each of 38 days.)



respectively, in the Appendix. Contact of the ocular tissues with chlorendic acid resulted in severe palpebral conjunctivitis, marked edema in the palpebra, and, frequently, a mild purulent discharge. The severity of the responses and the duration of their persistence, varied with the concentration in which the acid had been applied to the tissues. The eyes were generally normal in appearance and in the pupillary responses when stimulated with light after the 4th day.

The responses to the anhydride were generally milder and less prolonged. There was rarely any evidence of irritation in the palpebra 24 hours after contact with the anhydride.

DISCUSSION

With respect to the immediate toxicity of the 2 compounds, there was no significant difference in the quantity of the anhydride as compared with that of the acid required to produce a specific degree of mortality, when given orally to rats. The 2 compounds were not differentiated by the nature and severity of the responses which they elicited when given orally in comparable dosages.

The anhydride and the acid were not cumulative in their toxic properties; at the levels of dosage of 100, 200 and 400 mg. per kg. the total quantities administered to rats that survived for 7 weeks were approximately 1, 2, and 3 times the magnitude of the respective immediate lethal dosages (LD_{50}). The mortality among the several groups of rats that

were given serial dosages of either material was affected, primarily, by extraneous diseases, without apparent relationship to the amounts, either of the anhydride or of the acid, that were ingested by the animals. This conclusion is supported, in part, by the deaths of 3 rats in the control group that had ingested neither chlorendic acid nor the anhydride; it is supported further by the nature and extent of the pathological changes found in the viscera of the fatalities.

Neither material was absorbed through the skin of rabbits in amounts sufficient to cause serious illness or death. Solutions of the acid, and to a lesser extent, solutions of the anhydride in dimethyl phthalate, were moderately irritating to the skin. Both the acid and the anhydride were irritating to the palpebra and the conjunctival membrane of rabbits, but they induced no marked injury in the eye itself.

SUMMARY

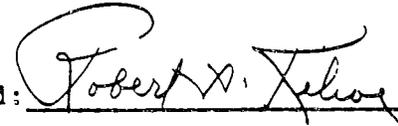
1. When given orally as aqueous suspensions to rats, the LD_{50} of chlorendic anhydride was 3.13 ± 0.34 g. per kg. of bodily weight; the LD_{50} of chlorendic acid was 2.79 ± 0.35 g. per kilogram.
2. The mortality was 50 per cent among rats given chlorendic anhydride orally in dosages of 400 mg. per kg. on each of 35 days; however, the deaths have been attributed to infectious diseases. Reduced rates of growth were evident in the male rats but not in the females that were given the compound repetitively in dosages of 200 or 100 mg. per kilogram.

3. Retardation of growth occurred among male rats that were given chlorendic acid orally in amounts of 400, 200, or 100 mg. per kg. on each of 35 days. Rates of growth of the females given comparable amounts of the acid were not affected. Occasional deaths at all levels of oral administration, without reference to the dosage, were attributed to extraneous disease.
4. Neither chlorendic anhydride nor chlorendic acid was absorbed in quantities sufficient to cause severe illness or death when kept in contact with the intact skin of rabbits for 4 hours on each of 36 days. Local irritation resulted from repeated contact of the skin with solutions containing either compound in dimethyl phthalate.
5. Chlorendic acid was severely irritating to the palpebra and the conjunctival tissue of rabbits; chlorendic anhydride was moderately irritating.

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Director

Date: June 25, 1965

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Table 1A

The Immediate Toxicity of Chlorendic Anhydride and Chlorendic Acid, When Administered Orally to Rats as 20 Per Cent Aqueous Suspensions

Chlorendic compound given	Dose (g./kg.)	3 to 4 wks. old		8 to 12 wks. old		All groups						
		Males		Females		Males		Females		D * T	Per cent Mortality	
		D	T	D	T	D	T	D	T			
Anhydride	0.94	0	3	0	5	0	3	0	2	0	13	0.0
	1.4	1	4	0	3	0	2	0	4	1	13	7.7
	2.1	2	4	0	3	0	4	1	4	3	15	20.0
	3.2	1	4	3	4	2	3	2	4	8	15	53.3
	4.7	2	4	3	4	4	4	3	4	12	16	75.0
	7.0	2	2	1	1	1	1	3	3	7	7	100.0
Acid	0.94	0	4	0	3	0	3	0	2	0	12	0.0
	1.4	0	5	1	3	0	3	1	4	2	15	13.3
	2.1	1	4	0	4	2	3	1	4	4	15	26.7
	3.2	1	4	3	4	2	3	2	4	8	15	53.3
	4.7	3	4	4	5	3	3	4	4	14	16	87.5
	7.0	2	2	1	1	2	2	2	2	7	7	100.0

*D = Number of deaths

T = Number of rats given the specified dosage

Table 2A

The Mortality Among Groups, Each Composed Initially of
6 Male and 6 Female Rats, to Which Either
Chlorendic Anhydride or Chlorendic Acid Was Administered,
Orally, on Each of 5 Days per Week During a Period of 7 Weeks

Sex of the rats and the compound given	Daily dose (mg./kg.)	Number of deaths	Length of the periods of survival (days)
<u>MALES</u>			
Anhydride	400	4	14, 20, 32, 45
	200	1	10
	100	2	15, 36
Acid	400	1	17
	200	1	13
	100	0	
Water only		2	6, 13
<u>FEMALES</u>			
Anhydride	400	2	23, 31
	200	0	
	100	0	
Acid	400	0	
	200	0	
	100	0	
Water only		1	41

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Table 3A

The Content of Hemoglobin, the Relative Numbers of Erythrocytes and Leukocytes, and the Relative Distribution of Differentiated Leukocytes in the Peripheral Blood of Male Rats, to which Chlorendic Anhydride or Chlorendic Acid was Administered, Orally, in the Stated Dosages, on Each of 35 Days

Concentration of the compound (mg./kg.)	Erythrocytes 10^6 /c.mm.	Hemoglobin (g./100 ml.)	Leukocytes 10^3 /c.mm.	Distribution of the differentiated leukocytes (per cent)					
				Poly-morpho-nuclears	Lympho-cytes	Band cells	Mono-cytes	Eosin-ophils	Baso-phils
				<u>Chlorendic Anhydride</u>					
400	7.88	14.0	24.0	11.5	87.0	0.0	0.5	1.0	0.0
200	7.80	15.2	18.5	8.7	89.6	0.0	0.7	1.0	0.0
100	8.84	15.9	13.6	12.7	82.6	0.7	2.0	2.0	0.0
				<u>Chlorendic Acid</u>					
400	8.58	14.8	23.6	9.8	87.4	0.4	1.2	1.2	0.0
200	7.63	15.4	14.0	10.0	86.7	0.0	2.3	0.7	0.3
100	7.96	14.9	19.5	10.7	88.3	0.0	0.0	1.0	0.0
Controls	8.84	15.3	16.1	10.5	85.0	0.2	1.5	2.8	0.0

Table 4A

The Content of Hemoglobin, the Relative Numbers of Erythrocytes and Leukocytes, and the Relative Distribution of Differentiated Leukocytes in the Peripheral Blood of Female Rats, to which Chlorendic Anhydride or Chlorendic Acid was Administered, Orally, in the Stated Dosages, on Each of 35 Days

Concentration of the compound (mg./kg.)	Erythrocytes 10^6 / c.mm.	Hemoglobin (g./100 ml.)	Leukocytes 10^3 / c.mm.	Distribution of the differentiated leukocytes (per cent)					
				Poly-morpho-nuclears	Lymphocytes	Band cells	Mono-cytes	Eosinophils	Basophils
				<u>Chlorendic Anhydride</u>					
400	7.74	14.6	14.5	12.7	85.3	0.0	1.0	1.0	0.0
200	7.58	15.1	18.5	15.3	83.0	0.7	0.0	1.0	0.0
100	8.36	15.2	13.5	9.7	86.3	0.3	1.3	2.0	0.3
				<u>Chlorendic Acid</u>					
400	8.06	14.3	17.6	17.7	79.0	0.5	1.0	1.8	0.0
200	7.46	14.8	16.4	18.3	80.3	0.0	0.7	0.7	0.0
100	8.47	15.2	21.4	9.7	86.7	0.3	0.3	1.7	0.3
Controls	8.30	15.6	19.9	10.2	86.0	0.0	1.0	2.5	0.3

Table 5A

Average Weights of Various Organs of Groups of Rats
To Which Either Chlorendic Anhydride or Chlorendic Acid
Was Administered, Orally, on Each of 5 Days per Week
During a Period of 7 Weeks

Compound and con- centration given (mg./kg.)	Average weights (grams)						
	Body	Brain	Heart	Lungs	Liver	Kidneys	Spleen
<u>MALES</u>							
Anhydride							
400	289	1.94	0.96	1.83	10.56	2.18	1.63
200	297	1.97	1.21	1.92	9.94	2.33	1.58
100	312	1.97	1.21	1.78	11.64	2.40	1.40
Acid							
400	288	1.72	1.03	1.58	11.24	2.14	1.57
200	292	1.88	1.06	1.85	12.32	2.27	1.53
100	289	1.82	1.03	1.60	11.46	2.13	1.48
Controls	354	2.16	1.36	1.96	13.88	2.42	1.62
<u>FEMALES</u>							
Anhydride							
400	215	1.72	0.88	1.42	9.16	1.67	1.33
200	208	1.75	0.86	1.30	8.75	1.54	1.08
100	207	1.75	0.82	1.34	8.26	1.56	1.07
Acid							
400	202	1.64	0.70	1.36	8.35	1.53	1.01
200	215	1.78	0.81	1.28	9.68	1.62	1.10
100	194	1.75	0.75	1.28	7.96	1.53	1.17
Controls	230	1.73	0.84	1.44	9.48	1.65	1.31

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Table 6A

Code Used in Scoring the Responses of the Ocular
and Conjunctival Tissues of Rabbits, Following the
Instillation of Either Chlorendic Acid or Anhydride

Irritation

0. No remarkable signs of injury
1. Injection of the palpebral vessels
2. Mild general palpebral conjunctivitis
3. Moderate general palpebral conjunctivitis
4. Severe general palpebral conjunctivitis

Edema

- A. Mild
 - B. Moderate
 - C. Severe
 - D. Puriform secretion
-

Table 6B

The Severity and Extent of Local Irritation Resulting From the Contact of Chlorendic Anhydride or Chlorendic Acid With the Conjunctiva of Rabbits, Either as Solutions in Dimethyl Phthalate or as Undiluted Powders

Time (hours)	Chlorendic acid in D.M.P.				Chlorendic anhydride in D.M.P.				Dimethyl phthalate
	5%	10%	20%		5%	10%	20%		
0.5	2	4-A	2-B		1	2	2	1	1
1.0	3-A	4-A	3-B		1	1-A	1-A	1	0
2.0	3-B	4-A	4-C-D		1	1-A	1-A	1	1
4.0	2-B	4-A	4-C-D		1	1-A	1-A	0	1
8.0	2-B	4-A	4-C-D		1	1-A	1-A	0	0
24.0	2-A	3-B	3-C		0	1-A	0	0	0
30.0	2-A	2-A	3-B		0	0	0	0	1
48.0	2-A	2-A	2-A		0	0	1	0	0
72.0	2-A	2-A	2-A		0	0	0	0	0
96.0	0	1	1		0	0	0	1	0
120.0	0	0	0		0	0	0	0	0

Time (hours)	20 mg. powdered chlorendic acid				20 mg. powdered chlorendic anhydride			
	W-261 (L.E.)	W-262 (L.E.)	W-261 (R.E.)	W-262 (R.E.)	W-261 (L.E.)	W-262 (R.E.)	W-261 (R.E.)	W-262 (R.E.)
0.5	1	1	1	1	1	1	1	1
1.0	2-A	2-A	2-A	2-A	1	1	2	2
2.0	4-C	3-B	3-B	3-B	2	2	3-A	3-A
4.0	4-C-D	4-C	4-C	4-C	3-A	3-A	3-A	3-A
8.0	4-C-D	4-C	4-C	4-C	3-A	3-A	3-A	3-A
24.0	4-C-D	4-C-D	4-C-D	4-C-D	2	2	1	1
48.0	2-D	2-B	2-B	2-B	1	1	0	0
72.0	2-A	2-A	2-A	2-A	0	0	0	0
96.0	0	0	0	0	0	0	0	0

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LITTON BIOMETICS INC

R.E. U.P. FYI.V.S. S.U.B.

MUTAGENICITY EVALUATION OF
CHLORENDIC ANHYDRIDE FINAL REPORT

CHLORENDIC ANHYDRIDE

115-27-5

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MUTAGENICITY EVALUATION
OF
CHLORENDIC ANHYDRIDE
FINAL REPORT

SUBMITTED TO

VELSICOL CHEMICAL COMPANY
341 EAST OHIO STREET
CHICAGO, ILLINOIS 60611

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SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20838

OCTOBER, 1977



BIONETICS

000002

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SPONSOR: Velsicol Chemical Company

MATERIAL: Chlorendic Anhydride

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: September 6, 1977

2. Description: White powder

B. Indicator Microorganisms

Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

<u>Component</u>	<u>Final Concentration/ml</u>
TPN	4 μ moles
Glucose-6-phosphate	5 μ moles
Sodium phosphate (dibasic)	100 μ moles
MgCl ₂	8 μ moles
KCl	33 μ moles
Homogenate fraction equivalent to 25 mg of wet tissue	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

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2. MATERIALS (Continued)

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL</u> ^a	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS ^b
	2-Nitrofluorene (NF)	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard (QM)	Water or saline	FS ^b
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide ^c	BPS ^b
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide ^c	FS ^b

^a Concentrations given in Results Section

^bBPS = Base-pair substitution

FS = Frameshift

^cPreviously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.

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3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the $9,000 \times g$ liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

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IV RESULTS

LITTON BIOMETRICS, INC.

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: CHLOROINDIC ANHYDRIDE
 B. SOLVENT: DMSO
 C. TEST INITIATION DATE: SEPT. 15, 1977
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	ISSUE	18-19354	1A-1537	1A-1538	1A-98	1B-100	20	21	16	24	26	115	28
ROIPATZORARD	---	---	936	711	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	944
SOLVENT CONTROL	---	---	26	18	26	24	24	24	26	18	26	24	124	31
POSITIVE CONTROL	---	---	27	16	28	29	29	29	27	16	28	29	124	25
TEST COMPOUND	---	---	20	18	23	35	39	39	20	18	23	35	131	20
	---	---	22	14	29	29	25	25	22	14	29	29	126	25
	---	---	20	18	25	28	28	28	20	18	25	28	120	21

ROIPATZORARD	RAT	LIVER	18	22	26	31	27	33	120	6
SOLVENT CONTROL	RAT	LIVER	18	22	26	31	27	33	120	6
POSITIVE CONTROL	RAT	LIVER	238	317	317	>1000	>1000	>1000	655	98
TEST COMPOUND	RAT	LIVER	18	26	24	36	31	38	105	46
	RAT	LIVER	16	24	24	36	31	43	137	45
	RAT	LIVER	19	27	27	49	25	49	133	53
	RAT	LIVER	18	24	24	27	27	46	135	43
	RAT	LIVER	15	20	20	32	32	38	134	54

*** TA-1535 ANTH 100 UG/PLATE
 TA-1537 ANH 100 UG/PLATE
 TA-1538 ASF 100 UG/PLATE
 TA-98 AAF 100 UG/PLATE
 TA-100 ANTH 100 UG/PLATE
 D4 DMNA 100 MICROMOLES/PLATE
 SOLVENT DMSO 50 UL/PLATE

*** IB. CONVERTANTS PER PLATE
 TA-1535 MNNG 10 UG/PLATE
 TA-1537 OH 10 UG/PLATE
 TA-1538 NF 10 UG/PLATE
 TA-98 NF 100 UG/PLATE
 TA-100 MNNG 10 UG/PLATE
 D4 MNNG 10 UG/PLATE
 SOLVENT DMSO 50 UL/PLATE

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5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity Test Results

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.1 μ g to 500 μ g per plate.

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

D. Conclusions

The test compound, Chlorendic Anhydride, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation.

Submitted by:

for Shoulla Wecker 10/31/77
D.R. Jagannath, Ph.D. Date
Section Chief
Submammalian Genetics
Department of Molecular
Toxicology

Reviewed by:

David J. Brusick 10/31/77
David J. Brusick, Ph.D. Date
Director
Department of Molecular
Toxicology

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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a

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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.

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STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

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VELSICOL
REPORT REQUEST

Compound: Chlorendic Anhydride

ASSAY NO. 2293 TEST TYPE Plate

REPORT: N/A, ACTIVATION, SPECIAL

STRAINS: 35, 37, 28, (398), (3100), G46, WP₂, WP₂UVTA⁺, POL A⁺, POL A⁻, D4, others....

ACTIVATION SYSTEM IRL

CONCENTRATION 1000 1500 2000 µg

Requested by Orzde DATE 12/7

TEST RESULTS

CONCENTRATION <u>µg / PLATE</u>	INDICATOR ORGANISMS (REVERTANTS/PLATE)					
	TA-1535	TA-1537	TA-1538	TA-98	TA-100	CG*
SOLVENT CONTROL				33	257	
POSITIVE CONTROL				257	172	
1000				1272	911	
1500				15	205	
2000				6	176	
				11	185	
SOLVENT CONTROL				37	276	
POSITIVE CONTROL				578	1337	
1000				31	227	
1500				33	239	
2000				22	245	

*rev⁺ revertants per plate

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Negative
Orzde
12/28

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MID No. (7)	19	D & B NO. (11)	20
Contractor	21		
Doc Type	22		
Doc Title	23		
Chemical Name (300 per name)	25	CAS No. (10)	24

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MUTAGENICITY EVALUATION OF CHLORINDIC ANHYDRIDE IN THE MOUSE DOMINANT LETHAL ASSAY FINAL REPORT

CHLORINDIC ANHYDRIDE

115-27-5

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MUTAGENICITY EVALUATION OF
CHLORENDIC ANHYDRIDE
IN THE
MOUSE DOMINANT LETHAL ASSAY
FINAL REPORT

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CHICAGO, ILLINOIS 60611

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KENSINGTON, MARYLAND 20795

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LBI PROJECT NO. 20862

FEBRUARY 1978

REVISED JUNE 1978



BIONETICS

000002

PREFACE

This report contains a summary of the data compiled during the evaluation of the test material. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-IV and provides sponsor and compound identification information, type of assay and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V identifies the tables and/or figures containing the data used by the Study Director in interpreting the test results (item VI).

The second part of the report, entitled PROTOCOL, describes, in detail, the materials and procedures employed in conducting the evaluation. This part of the report also contains evaluation criteria, standard operating procedures and any appendices. These are included to acquaint the sponsor with the methods used to develop and analyze the test results. All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology. Copies of raw data will be supplied to the sponsor upon request.



BIONETICS

Litton

000003

- I. SPONSOR: Velsicol Chemical Corporation
- II. MATERIAL*
 - A. Identification: Chlorendic Anhydride
 - B. Date Received: September 6, 1977
 - C. Physical Description: White powder
- III. TYPE OF ASSAY: Mouse Dominant Lethal Assay
- IV. PROTOCOL NO.: DMT-110
- V. RESULTS

The results are presented in Tables 1-7. Table 1 provides information on toxicity and dose selection. The remaining tables summarize the test results and statistical analysis.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Chlorendic Anhydride was examined for its ability to induce dominant lethality in male mice. The test material was evaluated at 0.223 g/kg, 0.074 g/kg and 0.022 g/kg. The vehicle for this test was DMSO and the route of administration was oral (PO). Following dosing, the males were mated sequentially for 7 weeks to virgin female mice. Pregnant females were scored for dominant lethal indexes at mid-pregnancy.

The results of the assay are presented in Tables 2-7. The data did not indicate any evidence of compound-induced dominant lethality. Some reduction in fertility was observed at Week 5 but none of the other parameters produced significant trends during the same week.

The positive control, TEM, was active over the first three mating weeks indicating induction of alterations in sperm and spermatids.

*Information was supplied by the sponsor. If this information was not indicated by the sponsor, N.I. was entered.



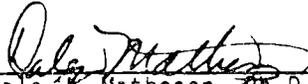
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VI. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

Chlorendic Anhydride was considered to be inactive in this test and did not induce dominant lethality in mice under the test conditions employed in this evaluation.

Submitted by:

Study Director


Dale W. Matheson, Ph.D. 3/2/78
Associate Director and Date
Section Chief
Mammalian Genetics
Department of Genetics
and Cell Biology

Reviewed by:


David J. Brusick, Ph.D. 2/17/78
Director Date
Department of Genetics
and Cell Biology

TABLE 1

Test Mouse Dominant LethalLBI RANGE FINDING AND LD₅₀ DATA SHEET

Compound Chlorendic Anhydride Assay# 2293
 Client Velsicol Chemical Corporation Project # 20862
 Test Initiated September 30, 1977 By Va, AT
 Solvent DMSO Species/PO# CD-1 Male Mice
 Route of Admin. Oral (PO) Dose Units g/kg
 File Reference _____
 Comments _____

Deaths/ 6 Animals

Date	Dosage	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
9/30/77	1 .3 ml 5 g/kg		1	0	5	0	0	0	0	0	0	0	0	0	0	0
"	2 .3 ml 1 g/kg		1	0	0	1	0	0	1	0	0	0	0	0	0	0
"	3 .03 ml .5g/kg		0	0	0	0	1	0	0	0	0	0	1	0	0	0
"	4 .03 ml .1g/kg		0	0	0	0	0	0	0	0	0	0	0	0	0	0
"	5 .03 ml .05 g/kg		0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6															

Calculated Values:

LD₅₀ 0.835 LD₁ 0.129 LD₅ 0.223
 upper 2.610 upper 0.322 upper 0.455 slope 2.8705
 lower 0.361 lower 0.000 lower 0.001 intercept 5.2241

Reviewed by: *David B...*Date: 4/30/78

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Table 2

WEEK	COMPOUND: CHLORFENIC ANHYDRIDE		FERTILITY INDEX		STUDY: SURCHRONIC		SPECIES: MICE		LOG DOSE	ARITH DOSE
	HIST. CONTROL	NEG. CONTROL	POS. CONTROL	0.072 G/KG	0.072 G/KG	0.074 G/KG	0.223 G/KG			
1	371/ 640 = 0.58	6/ 20 = 0.30	2/ 20 = 0.10	6/ 20 = 0.30	9/ 20 = 0.45	8/ 20 = 0.40	8/ 20 = 0.40	I		
2	452/ 640 = 0.71	13/ 20 = 0.65	2/ 20 = 0.10**	8/ 20 = 0.40	13/ 20 = 0.65	10/ 20 = 0.50	10/ 20 = 0.50	I		
3	446/ 640 = 0.70	12/ 20 = 0.60	9/ 20 = 0.45	8/ 20 = 0.40	8/ 20 = 0.40	8/ 20 = 0.40	8/ 20 = 0.40	I		
4	443/ 640 = 0.69	14/ 20 = 0.70	7/ 20 = 0.35	9/ 20 = 0.45	3/ 20 = 0.15**	10/ 20 = 0.50	10/ 20 = 0.50	I		
5	421/ 639 = 0.66	13/ 20 = 0.65	10/ 20 = 0.50	4/ 20 = 0.20*	4/ 20 = 0.20*	4/ 20 = 0.20*	4/ 20 = 0.20*	I		3
6	431/ 640 = 0.67	12/ 20 = 0.60	8/ 20 = 0.40	10/ 20 = 0.50	7/ 20 = 0.35	11/ 20 = 0.55	11/ 20 = 0.55	I		
7	430/ 640 = 0.67	14/ 20 = 0.70	10/ 20 = 0.50	10/ 20 = 0.50	11/ 20 = 0.55	10/ 20 = 0.50	10/ 20 = 0.50	I		

NOTE: THE SYMBOLS \$ AND * DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL OR HISTORICAL CONTROL GROUP.

* INDICATES SIGNIFICANT DIFFERENCE FROM CONTROL.

\$ INDICATES SIGNIFICANT LINEAR RELATIONSHIP WITH APITH OR LOG DOSE.

ONE \$ OR * INDICATES SIGNIFICANCE AT P LESS THAN 0.05.

TWO \$ OR * INDICATES SIGNIFICANCE AT P LESS THAN 0.01.

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Table 3

WEEK	COMPOUND: CHLORENDIC ANHYDRIDE		POS. CONTROL		STUDY: SURCHRONIC		SPECIES: MICE		LOG DOSE	ARITH DOSE
	HIST. CONTROL	NFC. CONTROL	64/ 6 = 10.7	117/ 13 = 10.5	18/ 2 = 9.0	0.022 G/KG	0.074 G/KG	0.223 G/KG		
1	4307/ 371 = 11.6				58/ 6 = 9.7	95/ 9 = 10.6	96/ 8 = 10.8			
2	5341/ 452 = 11.8				85/ 8 = 10.6	141/ 13 = 10.8	104/ 10 = 10.4			
3	5294/ 446 = 11.9				85/ 8 = 10.6	81/ 8 = 10.1	91/ 8 = 11.4			
4	5156/ 443 = 11.6				105/ 9 = 11.7	34/ 3 = 11.3	114/ 10 = 11.4			
5	4907/ 421 = 11.7				41/ 4 = 10.3	46/ 4 = 11.5	42/ 4 = 10.5			
6	5278/ 431 = 12.2				110/ 10 = 11.0	80/ 7 = 11.4	110/ 11 = 10.0			
7	5228/ 430 = 12.2				105/ 10 = 10.5	113/ 10 = 11.3	108/ 10 = 10.8			

NOTE: THE SYMBOLS * AND ° DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL OR HISTORICAL CONTROL GROUP.

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TWO * OR ° INDICATES SIGNIFICANCE AT P LESS THAN 0.01.

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Table 4

WEEK	CHLORDANE ANHYDRIDE		PREGNANT FEMALE STUDY: SUBCHRONIC		SPECIES: MICE	
	MEG. CONTROL	POS. CONTROL	0.022 G/KG	0.074 G/KG	0.223 G/KG	ARITH DOSE
1	215/ 371 = 0.63	1/ 2 = 1.50	5/ 6 = 0.83	7/ 9 = 0.78	9/ 11 = 1.13	
2	189/ 452 = 0.86	1/ 2 = 3.50	11/ 11 = 1.38	12/ 13 = 0.92	12/ 10 = 3.20	
3	330/ 444 = 0.76	23/ 9 = 2.56*	6/ 8 = 0.75	7/ 8 = 0.25	2/ 8 = 0.25	
4	157/ 443 = 0.81	17/ 7 = 2.43	6/ 9 = 0.67	5/ 3 = 1.67	2/ 10 = 0.20	
5	258/ 421 = 0.61	29/ 10 = 2.90	9/ 4 = 2.25	11/ 4 = 2.75	8/ 4 = 2.00	
6	336/ 431 = 0.78	1/ 8 = 0.13	3/ 10 = 0.30	3/ 7 = 0.43	6/ 11 = 0.55	
7	260/ 420 = 0.63	8/ 10 = 0.80	4/ 10 = 0.40	6/ 11 = 0.55	6/ 10 = 0.60	

NOTE: THE SYMBOLS \$ AND * DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL OR HISTORICAL CONTROL GROUP.

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* INDICATES SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE.

ONE \$ OR * INDICATES SIGNIFICANCE AT P LESS THAN 0.05.
TWO \$ OR * INDICATES SIGNIFICANCE AT P LESS THAN 0.01.

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Table 5

AFFECT	COMPOUND: CHLORENDIC ANHYDRIDE			STUDY: SIBICHRONIC			SPECIFICS: MICE		
	NEG. CONTROL	POS. CONTROL	0.072 G/KG	0.074 G/KG	0.223 G/KG	LOG DOSE	ARITH DOSE		
1	158/ 371 = 0.43	2/ 2 = 1.00	4/ 6 = 0.67	5/ 9 = 0.56	5/ 8 = 0.63				
2	167/ 452 = 0.37	2/ 2 = 1.00	2/ 8 = 0.25	9/ 13 = 0.69	6/ 10 = 0.60				
3	200/ 446 = 0.45	6/ 9 = 0.67	5/ 8 = 0.63	2/ 8 = 0.25	1/ 8 = 0.13	§			
4	214/ 443 = 0.48	4/ 7 = 0.57	4/ 9 = 0.44	3/ 3 = 1.00	2/ 10 = 0.20				
5	161/ 421 = 0.38	4/ 10 = 0.40	3/ 4 = 0.75	1/ 4 = 0.25	2/ 4 = 0.50				
6	195/ 431 = 0.45	1/ 8 = 0.13	1/ 10 = 0.30	2/ 7 = 0.29	5/ 11 = 0.45				
7	187/ 430 = 0.43	4/ 10 = 0.40	1/ 10 = 0.30	5/ 11 = 0.45	3/ 10 = 0.30				

NOTE: THE SYMBOLS § AND * DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL OR HISTORICAL CONTROL GROUP.

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TWO § OR * INDICATES SIGNIFICANCE AT P LESS THAN 0.01.

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Table 6

WEEK	COMPOUND: CHLORGENIC ANHYDRIDE		POS. CONTROL		NEG. CONTROL		STUDY: SURCHRONIC		SPECIES: MICE		LOG DOSE	ARITH DOSE
	HIST. CONTROL	NEG. CONTROL	POS. CONTROL	POS. CONTROL	NEG. CONTROL	NEG. CONTROL	0.022 G/KG	0.074 G/KG	0.223 G/KG			
1	60/ 371 = 0.16	1/ 6 = 0.17	2/ 2 = 1.00	1/ 6 = 0.17	1/ 9 = 0.11	3/ 13 = 0.23	5/ 10 = 0.50	3/ 8 = 0.38				
2	81/ 452 = 0.18	1/ 13 = 0.08	2/ 2 = 1.00*	1/ 8 = 0.13	3/ 13 = 0.23	5/ 10 = 0.50						
3	66/ 446 = 0.15	3/ 12 = 0.25	6/ 9 = 0.67	1/ 8 = 0.13	0/ 8 = 0.0	1/ 8 = 0.13						
4	81/ 443 = 0.18	2/ 14 = 0.14	3/ 7 = 0.43	1/ 9 = 0.11	1/ 3 = 0.33	0/ 10 = 0.0						
5	54/ 421 = 0.13	1/ 13 = 0.08	3/ 10 = 0.30	1/ 4 = 0.25	1/ 4 = 0.25	1/ 4 = 0.25						
6	65/ 431 = 0.15	2/ 12 = 0.17	0/ 8 = 0.0	0/ 10 = 0.0	1/ 7 = 0.14	1/ 11 = 0.09						
7	52/ 430 = 0.12	1/ 14 = 0.07	2/ 10 = 0.20	1/ 10 = 0.10	1/ 11 = 0.09	2/ 10 = 0.20						

NOTE: THE SYMBOLS * AND • DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL OR HISTORICAL CONTROL GROUP.

* INDICATES SIGNIFICANT DIFFERENCE FROM CONTROL.

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TWO * OR • INDICATES SIGNIFICANCE AT P LESS THAN 0.01.

Table 7

WEEK	COMPOUND:		DEAD IMPLANTS / TOTAL IMPLANTS		STUDY: SURCHRONIC		SPECIES: MICE	
	HIST. CONTROL	CHLORENDIC ANHYDRIDE	NEG. CONTROL	POS. CONTROL	0.022 G/KG	0.074 G/KG	0.223 G/KG	
1	235/307 = 0.05	5/64 = 0.08	7/19 = 0.39*	5/58 = 0.09	7/95 = 0.07	9/86 = 0.10		
2	380/534 = 0.07	15/137 = 0.11	7/11 = 0.64*	11/45 = 0.13	12/141 = 0.09	12/104 = 0.31		
3	330/5204 = 0.06	8/124 = 0.06	23/97 = 0.24*	6/85 = 0.07	2/81 = 0.02	2/91 = 0.02		
4	351/5156 = 0.07	21/155 = 0.14	17/74 = 0.23	6/105 = 0.06	5/34 = 0.15	2/114 = 0.02		
5	258/4907 = 0.05	13/135 = 0.10	29/107 = 0.27	9/41 = 0.22	11/46 = 0.24	8/42 = 0.19		
6	336/5278 = 0.06	24/137 = 0.18	1/69 = 0.01	1/110 = 0.03	3/80 = 0.04	6/110 = 0.05		
7	260/5228 = 0.05	7/155 = 0.05	8/105 = 0.08	4/113 = 0.04	6/108 = 0.06	6/104 = 0.06		

NOTE: THE SYMBOL * DENOTES SIGNIFICANT DIFFERENCE USING THE NEGATIVE CONTROL GROUP.
 ONE * INDICATES SIGNIFICANCE AT P LESS THAN 0.05.
 TWO * INDICATES SIGNIFICANCE AT P LESS THAN 0.01.

PROTOCOL

1. PURPOSE

The purpose of this study was to evaluate the test material for its ability to induce dominant lethality in mice.

2. OVERVIEW AND RATIONALE

The dominant lethal assay is designed to determine the ability of a compound to induce genetic damage in the germ cells of treated male mice leading to fetal wastage. Chromosome aberrations including breaks, rearrangements, and deletions are believed to produce the dominant lethality although ploidy changes and chromosome nondisjunction may also be detected in this assay. Male mice are exposed to several dose levels of the test compound for 5 days and then mated over the entire period of spermatogenesis to unexposed virgin females. At mid-pregnancy, the females are killed and scored for the number of living and dead implants as well as the level of fertility. These results are then compared to data from control animals and used to determine the degree of induced dominant lethality.

Evidence of dominant lethality emphasizes that the compound is able to reach the developing germ cells and induce genetic damage. It also suggests, but does not measure directly, that in addition to the detected gross chromosomal lesions, more subtle balanced lesions or specific locus gene mutations may be produced. These latter types have a good chance of being transmitted to the gene pool of future offspring.

3. EXPERIMENTAL DESIGN

Ten (10) random bred, male mice from a closed colony were assigned to 1 of 5 groups. Three of these groups received different dose levels of the test compound; a fourth group received only the solvent or vehicle; and the fifth group received a known mutagen and served as the positive control group. The test compound and control compounds were administered as specified in Table 1. Triethylenemine (TEM) was used as the positive control and was given as a single intraperitoneal injection. Following treatment, each male was rested for 2 days and then caged with 2 unexposed virgin females on the third day. At the end of 5 days, these females were removed. This weekly mating sequence was continued for 7 weeks. Each pair of mated females was transferred to a fresh cage, and approximately 14 days after the midweek of being caged with the male, the females were killed with CO₂. At necropsy, their uteri were examined for dead and living implants, and total implantations.



3. EXPERIMENTAL DESIGN (Continued)

A. Animals

Random bred, adult male and female mice, strain CD-1 were purchased from the Charles River Breeding Laboratories, Inc. Male and female mice were at least 8 weeks of age upon initiation of dosing.

B. Animal Husbandry

Males were housed individually and females housed in pairs (except during mating) in shoe box cages on AB-SORB-DRI bedding.

All animals were quarantined prior to being used in the study to acclimate them to the new laboratory conditions. Purina Lab Chow was used as the basic diet and water was offered ad libitum. Light was provided on a 12-hour light/dark cycle.

Personnel handling animals or working within the animal facility wore suitable protective laboratory garments, including face masks or respirators.

C. Dosage Determination

Dosage information was calculated on the basis of range finding studies using 6 groups of 6 mice each. The high dose level was selected from these data. One-third and one-tenth of the high dose were used as the intermediate and low dose levels, respectively. For nontoxic compounds, a maximum high dose level of 5 g/kg (or equivalent) is generally chosen.

D. Records

The number of dead and living implants and total implantation sites were recorded on a standardized record form. Data were keypunched directly from these forms to computer entry cards, and analyzed for statistical significance as outlined in Appendix A. Original copies of all data are stored in the Litton Bionetics, Inc. archival system.



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4. EVALUATION CRITERIA

Both pre- and post-implantation losses contribute to dominant lethality. The former is reflected in the total number of implantation sites per pregnant female and strictly measured by the difference between the number of corpora lutea gravidus and the number of implantation sites. Toxic or physiologic effects on sperm may also reduce the number of implantation sites. Therefore, unless subtle physiological effects on sperm can be discounted, pre-implantation loss is not as rigorous an indication of dominant lethality as post-implantation loss. Corpora lutea can not be reliably counted in mice and, therefore, pre-implantation loss is not evaluated in studies using mice. Post-implantation losses are measured as early and late fetal deaths plus the number of resorption sites.

Dominant lethality is typically determined from: a mutation index derived from the ratio of dead to total implants; or the number of dead implants per pregnant female. In interpreting these values, it must be remembered that the former measurement reflects both pre- and post-implantation losses and that the ratio is affected by changes in either the numerator or the denominator. For this reason the second parameter is perhaps a better indicator of post-implantation loss. This becomes especially so if one concurrently examines the number of living embryos per pregnant female. The two sets of data should be inversely related. In other words, if true dominant lethality is being observed, then a significant increase in the number of dead implants per pregnant female should be accompanied by a significant decrease in the number of living implants per pregnant female.

These ratios are compared with both concurrent and historical control data for significant statistical differences. Dose-related trends are also looked for, but may not always be found. For example, some compounds such as EMS tested in mice show a threshold value and then a very steep rise. Certain portions of the response might be missed, depending on the spacing of the dose levels used.

True, as opposed to spurious, dominant lethality also tends to cluster according to the stage of spermatogenesis affected and typically would not be expected to appear in widely spaced weeks or blocks of weeks.

All data which are indicated as being statistically significant must also be strongly evaluated for their biological significance. By bringing both statistical and biological selective pressures to bear on the data gathered, an estimate of dominant lethality and of risk to the gene pool should be obtainable.



REFERENCES

Epstein, S.S., Arnold, E., Andrea, J., Bass, W., and Bishop, Y. (1972). Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. 23: 288-325.

Ray, V.A. and Hyneck, M.L. (1973). Some primary considerations in the interpretation of the dominant-lethal assay. Environmental Health Perspective 6: 27.

Bateman, A.J. (1960). The induction of dominant lethal mutations in rats and mice with triethylenemelamine (TEM). Genet. Res. Camb. 1: 381.

STANDARD OPERATING PROCEDURES

The test compound will be registered in a bound log book recording the date of receipt, complete client identification, physical description, and Litton Bionetics, Inc., code number.

Complete records of weights and dilutions associated with the testing of the submitted material will be entered into a bound notebook.

Raw data information will be recorded on special printed forms that will be dated and initialed by the individual performing the data collection at the time the observations are made. These forms will be filed as permanent records.

All data will be entered in ink (no pencil).

All changes or corrections in entries will be made with a single line through the change, and an explanation for the change must be written.

All calculations (weights, dilutions, dose calculations, etc.) will be shown on data records.

All data entries will be dated and initialed.

All laboratory operations will be written out in standard protocol manuals. These manuals will be present in each laboratory area.

Deviations from any established protocol will be described and justified.

Data will be stored in bound form (notebooks or binders). These bound data books will be reviewed by the appropriate Section Heads.

Chemicals submitted for testing will have date of receipt and initials of entering person.

Lot numbers for all reference mutagens, solvent, or other materials used in assays will be recorded.

Animal orders, receipts, and identification will be recorded and maintained such that each animal can be traced to the supplier and shipment. All animals on study will be properly identified.

A copy of the final report plus all raw data and support documents will be permanently stored in the archival system of Litton Bionetics, Inc.

Current curricula vitae and job descriptions will be maintained on all personnel involved in the study.



APPENDIX A

This analysis describes the statistical treatment of dominant lethal data. The entire analysis is applied to studies in rats and only parts are applicable to mice since corpora lutea counts are not made in this species. The respective table numbers are given where applicable.

APPENDIX A

Analysis of Data

1. Fertility Index - Table 2 (Mice and rats)

- a. The fertility index is defined as F.I. = # of pregnant females / # of mated females. It is calculated for each week (in subacute study) or at the end of 8 weeks (in acute study) and for each dose level, negative control, and positive control.
- b. A chi-square test is used to compare each treatment group and positive control to negative control.

$$\chi^2 = \frac{(N_0 + N_i) (|n_0(N_i - n_i) - n_i(N_0 - n_0)| - (N_0 + N_i)/2)^2}{(n_0 + n_i)(N_0 - n_0 + N_i - n_i)N_0N_i}$$

where

n_i = # impregnated in i-th test group

n_0 = # impregnated in negative control group

N_i = # of females mated in the i-th test group

N_0 = # of females mated in negative control group

A 2 x 2 table is formed as follows:

	control	test
# impreg	n_0	n_i
# not impreg	$N_0 - n_0$	$N_i - n_i$

Significance at the 5 and 1% levels is indicated with asterisks on Table 1.

- c. Armitage's trend for linear proportions is used to test whether the fertility index is linearly related to arithmetic or log dose.

The following table is set up:

	-control	dose 1	dose 2	dose 3	dose k	totals
# impreg	n_0	n_1	n_2	n_3	n_k	t
# not impreg	$T_0 - n_0$	$N_1 - n_1$	$N_2 - n_2$	$N_3 - n_3$	$N_k - n_k$	$T - t$
totals	N_0	N_1	N_2	N_3	N_k	T

and Armitage's chi-square is calculated:

$$x_A^2 = x_{(k-1)}^2 - x_1^2$$

where

$$x_1^2 = \frac{T(\sum_{i=0}^k n_i x_i - t \sum_{i=0}^k N_i x_i)^2}{t(T-t)(\sum_{i=0}^k N_i x_i^2 - (\sum_{i=0}^k N_i x_i)^2)}$$

$$x_{(k-1)}^2 = \frac{T^2(\sum_{i=0}^k n_i^2/N_i - t^2/T)}{t(T-t)}$$

and the x_i are the dose levels. This calculation is repeated with x replaced by $\log_{10} x$. The 5 and 1% significance levels are indicated by dollar signs on the data table.



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2. Total Number of Implantations - Table 3 (Mice and rats)

- a. The total number of implantations is evaluated by the Student's t-test to determine whether the average number of implantations per pregnant female for each treatment group and the positive control group differs significantly from the negative control group.

n_i = # of pregnant females at dose level i .

u_{ij} = # of implantations for pregnant female j in dose group i .

$$\bar{u}_i = 1/n_i (\sum_{j=1}^{n_i} u_{ij})$$

$$s_i^2 = \sum_{j=1}^{n_i} (u_{ij} - \bar{u}_i)^2$$

$$t_i = \bar{u}_0 - \bar{u}_i / \left(\frac{s_0^2 + s_i^2}{n_0 + n_i - 2} \left(\frac{1}{n_0} + \frac{1}{n_i} \right) \right)^{1/2}$$

$$\text{d. f.} = n_0 + n_i - 2$$

Significance at the 5 and 1% levels is indicated by asterisks in the data table.



b. A regression fit of the average number of implantations, \bar{u}_i , is made for both the arithmetic and logarithmic dose (x_i and $\log x_i$). The doses x_i are used as independent variables and the fit includes data from the three treatment groups and the control group.

N = total # of pregnant females in all groups.

x_i = dose/log (dose) for the i -th female.

U_i = # of implantations for the i -th female.

$$\bar{x} = \frac{1}{N} \sum_{i=1}^N x_i$$

$$SS_x = \sum_{i=1}^N (x_i - \bar{x})^2$$

$$\bar{U} = \frac{1}{N} \sum_{i=1}^N U_i$$

$$SS_U = \sum_{i=1}^N (U_i - \bar{U})^2$$

$$S_{xu} = \sum_{i=1}^N (x_i - \bar{x})(U_i - \bar{U})$$

B = estimate of slope of regression line = S_{xu}/SS_x

A = estimate of intercept of regression line = $\bar{U} - B\bar{x}$

$VARU$ = variance of U about regression line

$$= \frac{SS_U - S_{xu}^2/SS_x}{N-2}$$

$VARB$ = variance of B = $\frac{VARU}{SS_x}$



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VARA = variance of A = $\text{VARU} \left(\frac{1}{N} + \frac{\bar{x}^2}{SS_x} \right)$
 TB = $B/(\text{VARB})^{1/2}$ = t-statistic for testing the hypothesis
 that the regression slope is zero
 DF = $N-2$ = # of degrees of freedom for T B
 CVUX = coefficient of variation of U about x
 = $(\text{VARU.X})^{1/2}/\bar{U}$
 VARU.X = $\frac{1}{N-2} (SS_U - s_{XU}^2/SS_X)$
 SDY = standard deviation of U about the regression line
 = $(\text{VARU.X})^{1/2}$
 SDS = standard deviation of the slope
 = $(\text{VARB})^{1/2}$
 SDA = standard deviation of intercept
 = $(\text{VARA})^{1/2}$

Significant difference of the slope from zero is indicated at the 5 and 1% levels in Table 2. Table 2A shows detailed results of the regression analysis.

3. Total Number of Corpora Lutea - Table 4 (Rat studies only)

- a. The average number of corpora lutea per pregnant female is evaluated by t-test to determine whether each treatment group differed significantly from the control group. Use the equation described in Step 2 above with

u_{ij} = # of corpora lutea for pregnant female j in dose group i.

- b. A regression fit of the average number of corpora lutea per pregnant female is made for both the arithmetic and logarithmic dose. Use the equations described in Step 2 above with

$$u_i = \# \text{ of corpora lutea for the } i\text{-th female}$$

4. Preimplantation Losses - Table 5 (Rat studies only)

- a. The number of preimplantation losses is the number of corpora lutea minus the number of implantations.

Y_{ij} = preimplantation losses for j -th female in i -th group

V_{ij} = # of corpora lutea for j -th female in the i -th group

- b. The Freeman-Tukey transformation is applied to the Y_{ij} as follows:

$$f_{ij} = \sin^{-1} \sqrt{\frac{y_{ij}}{V_{ij} + 1}} + \sin^{-1} \sqrt{\frac{y_{ij} + 1}{V_{ij} + 1}}$$

The t -test is then applied to the f 's, comparing the test groups to the negative control. Let

$$\bar{f}_i = \frac{1}{n_i} \sum_{j=1}^{n_i} f_{ij}$$

$$s_i^2 = \sum_{j=1}^{n_i} (f_{ij} - \bar{f}_i)^2$$

where n_i = # of pregnant females at dose level i .

$$\text{Then } t = (\bar{f}_0 - \bar{f}_i) / \left[\frac{s_0^2 + s_i^2}{n_0 + n_i - 2} \left(\frac{1}{n_0} + \frac{1}{n_i} \right) \right]^{1/2}$$

- c. Regression analysis is used to determine whether the average number of preimplantation losses per female is related to the arithmetic or the log dose. The method is as used in Step 2 above substituting

$$U_i = \# \text{ of preimplantation losses for the } i\text{-th female.}$$



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5. Dead Implantations - Table 5 in rat studies
Table 4 in mouse studies

The dead implants were evaluated by the same statistical techniques that were used in evaluating the total number of implantations.

Substitute

u_{ij} = # of dead implants for j-th female in the i-th group in the equations in Step 2 above.

6. Proportion of Females with One or More Dead Implantations - Table 7 in rat studies
Table 5 in mouse studies

The proportion of females with one or more dead implants is the number of females with dead implants/number of pregnant females. These proportions are analyzed by the same method used to analyze the fertility indices, i.e., by a chi-square test and Armitage's trend.

Substitute n_i = # of pregnant females with one or more dead implants at dose level i and

N_i = # of pregnant females at dose level i in Step 1 above.

Also a probit regression analysis is done using these proportions, p_i , to determine whether the probit of p_i is linearly related to the log or arithmetic dose. The Biomedical Computer Program BMD03S is used to compute A and B and the χ^2 statistic for the regression equations $y = A + B x$ and $y = A + B \log x$.

7. Proportion of Females with Two or More Dead Implantations - Table 8 in rat studies
Table 6 in mouse studies

The proportion of females with two or more dead implantations is the number of females with two or more dead implants/number of pregnant females. The data are evaluated by the same method used for evaluating the proportion of females with one or more dead implants.

8. Dead Implants/Total Implants - Table 9 in rat studies
Table 7 in mouse studies

Dead implants/total implants were computed for each female and transformed by way of the Freeman-Tukey arc-sine transformation prior to being evaluated by t-test to compare each treatment group and positive control to negative control.

Use y_{ij} = # dead implants for j-th female in i-th group

v_{ij} = # of total implants for j-th female in i-th group

in the equations in Step 4 above.



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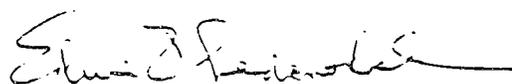
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International Research and Development Corporation

SPONSOR: Velsicol Chemical Corporation
COMPOUND: Chlorendic Anhydride
SUBJECT: Teratology Study in Rats.

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Date: November 8, 1978

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I. SYNOPSIS

Pregnant Charles River CD rats were used to evaluate the teratogenic potential of Chlorendic Anhydride in this study. The compound was administered by gastric intubation at dosage levels of 25, 100 and 400 mg/kg/day from days 6 through 15 of gestation. A control group received the vehicle, corn oil, at 10 ml/kg/day.

During gestation the females were observed for clinical signs of effect, for mortality and for changes in body weight gains. Cesarean sections were performed on gestation day 20. The number of viable and nonviable fetuses, early and late resorptions, corpora lutea and total implantations were recorded. The fetuses were weighed, sexed and examined for external, soft-tissue and skeletal anomalies and variations.

There were no differences in maternal body weights or changes in appearance or behavior for rats in the 25 or 100 mg/kg/day dosage group when compared to the control group. There was a slight increase in matted fur, red nasal discharge and anogenital staining in the 400 mg/kg/day dosage group when compared to the control group. A slight mean body weight loss during the first 3 days of treatment and reduced mean body weight gains throughout treatment were seen for the rats in the 400 mg/kg/day dosage group when compared to the control group. Survival was 100% for all groups. There were no biologically meaningful differences in the mean number of viable fetuses, total implantations, corpora lutea or male to female sex ratio in any of the Chlorendic Anhydride treated groups when compared to the control group. There was an increase in the mean number of postimplantation losses in the 100 and 400 mg/kg/day dosage groups when compared to the control group, however this increase was only slightly higher than the historical control mean. The number of developmental or genetic variations were comparable for all Chlorendic Anhydride dosage groups and the control group. The increase in malformations in the

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Chlorendic Anhydride groups were not biologically meaningful when compared to the control group. Chlorendic Anhydride is not considered teratogenic in rats in dosage levels up to and including 400 mg/kg/day.

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II. COMPOUND

The compound was received from Velsicol Chemical Corporation, Chicago, Illinois on October 10, 1977 as indicated below:

<u>Label</u>	<u>Description</u>
Tech. Ref. Std. Chlorendic Anhydride 93.81% (Titr.) Lot No. 3-12-206	white somewhat chunky powder

III. METHODS AND PROCEDURES

A. ANIMALS:

One hundred female Charles River CD rats (The Charles River Breeding Laboratories, Inc., Canada) were used in this teratology study. These rats were approximately 3 months old at the time of mating and had been acclimated in this laboratory for a minimum of 10 days prior to mating. All rats were individually housed, except during mating, in wire mesh cages and maintained in a temperature, humidity and light controlled room. Purina® Laboratory Chow® and tap water were available ad libitum.

Mating was initiated on February 13, 1978 and the last cesarean section was performed on March 10, 1978.

B. MATING:

Male rats of the same strain were used for mating. One female and one male were placed together for mating. The day of mating was determined by daily inspection for a copulatory plug or vaginal smear for sperm. The day when a plug or sperm was found, was designated day 0 of gestation and the female was returned to an individual cage.

C. ORGANIZATION OF TEST GROUPS AND TREATMENT:

Bred females were consecutively assigned to 4 groups in a block design, one control and 3 treatment groups of 25 rats each.

Chlorendic Anhydride was suspended, using a tissue homogenizer, in Mazola® corn oil at varying concentrations to permit the administration of 10 ml/kg/day at dosage levels of 25, 100 and 400 mg/kg/day. Compound administration was by gavage from day 6 through day 15 of gestation. The control females were given the vehicle on a comparable regimen at 10 ml/kg/day. Individual dosages were based on the body weights on gestation days 6, 9 and 12.

D. MATERNAL OBSERVATIONS:

Prior to compound administration females were observed daily for mortality and overt signs of toxicity. The females were observed daily for changes in appearance, behavior, mortality and clinical signs of toxicity from gestation days 6 through 20. Individual female body weights were recorded on days 0, 6, 9, 12, 16 and 20 of gestation.

E. CESAREAN SECTION OBSERVATIONS:

On day 20 of gestation, all female rats were sacrificed by an overdose of carbon dioxide, the abdominal and thoracic cavities were examined, and the fetuses delivered by cesarean section. The number and location of viable and nonviable fetuses, early and late resorptions, total implantations and corpora lutea were recorded. The sex and body weight were recorded for each fetus.

F. MORPHOLOGICAL OBSERVATIONS:

All fetuses were subjected to gross examination to determine sex and any external abnormalities. Approximately one-third of the fetuses was placed in Bouin's fixative and later sectioned by the method described by Wilson¹ to examine viscera. The remaining fetuses were fixed in alcohol, macerated with potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson² and examined for skeletal anomalies and variations.

G. STATISTICAL ANALYSES:

All statistical analyses compared the treatment groups with the control group, with the level of significance at $p < 0.05$. Male to female fetal sex ratio, number of litters with anomalies and number of fetuses with anomalies were compared using the Chi-square test criterion with Yates correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described by Siegel³ to judge significance of differences.

The proportion of early and late resorbed fetuses, nonviable fetuses and postimplantation losses were compared by the Mann Whitney U-test as described by Siegel³ and Weil⁴ to judge significance of differences.

Mean number of corpora lutea, total implantations and viable fetuses were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie⁵ using Dunnett's⁶ multiple comparison table to judge significance of differences.

Fetal body weights were compared by analysis of variance (hierarchal classification) and t-test as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

IV. RESULTS

A. MATERNAL OBSERVATIONS:

Survival was 100% for all groups.

Individual maternal body weights are presented in Table 1. There was a slight loss in mean body weight during the first 3 days of treatment and reduced mean body weight gains throughout the entire treatment period for the 400 mg/kg/day dosage group when compared to the control group. Mean body weight gains for the 25 and 100 mg/kg/day dosage groups were comparable to the control group.

There were no changes in appearance or behavior for the females in the 25 and 100 mg/kg/day dosage group when compared to the rats in the control group. Matted fur, anogenital staining and red nasal discharge, were seen for some rats in all groups, however there was a slight increase in the 400 mg/kg/day dosage group when compared to the rats in the control group.

B. CESAREAN SECTION OBSERVATIONS:

A summary of maternal and fetal observations is presented in Table 2 and individual cesarean section data is presented in Table 3. There were no biologically meaningful or statistically significant differences in the mean number of viable or nonviable fetuses, corpora lutea or in the mean fetal body weights for rats in the 25, 100 or 400 mg/kg/day dosage groups when compared to the control group. The male to female sex ratio in the 25 mg/kg/day dosage group was statistically significantly different from the control group, this difference is attributable to random occurrence and not considered compound related. The increase in the mean number of postimplantation losses in the 100 and 400 mg/kg/day dosage group was statistically significant when compared to the vehicle control. However, this increase is only slightly higher than the mean for the historical control.

C. FETAL MORPHOLOGICAL OBSERVATIONS:

Summarized findings of all fetal external, soft tissue and skeletal examinations are presented in Table 4. The findings are listed as malformations or developmental and genetic variations. There were no malformed fetuses in the control group, one malformed fetus in the 25 mg/kg/day group, 2 malformed fetuses in two litters in the 100 mg/kg/day group and 1 malformed fetus in the 400 mg/kg/day group. These malformations were not statistically significant and not considered to be treatment related. The variations observed were similar for all groups.

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Chlorendic Anhydride:

Teratology Study in Rats.

TABLE 1. Individual Body Weights, Grams.

Dosage Level, Dam Number	Day of Gestation					
	0	6	9	12	16	20
<u>Control:</u>						
81195	234	257	269	278	311	350
81201	236	265	267	286	320	372
81206	239	275	265	280	326	393
81211	235	269	266	283	301	362
81212	237	252	245	252	286	343
81216	265	298	285	309	340	404
81220	236	258	265	279	318	355
81225 ^a	244	256	255	255	264	256
81232	242	271	270	279	316	356
81233	234	257	256	274	311	366
81237	239	270	276	294	312	358
81241	240	263	266	276	313	355
81250	237	264	268	291	321	384
81251	236	257	258	291	304	354
81255	245	271	266	290	320	377
81256	232	274	279	287	319	376
81262	239	265	267	286	316	319
81269	234	261	264	292	313	370
81270	252	278	285	303	331	405
81272	226	249	259	265	293	339
81277	234	274	263	296	331	330
81290	249	270	277	304	320	389
81300	260	276	286	296	320	366
81310 ^a	246	265	258	269	275	280
81318	250	282	278	303	342	410
Mean	240	268	269	287	317	367

^aNongravid, not used in calculation of means.

Chlorendic Anhydride:

Teratology Study in Rats.

TABLE 1. Cont.

Individual Body Weights, Grams.

Dosage Level, Dam Number	Day of Gestation					
	0	6	9	12	16	20
<u>25 mg/kg/day:</u>						
81196	231	257	259	273	309	352
81197	246	271	272	296	315	378
81202	251	261	264	260	292	354
81207	237	263	256	284	310	368
81213	245	278	269	294	321	366
81218	244	267	268	285	311	361
81222	256	276	284	286	320	365
81227	251	286	292	302	354	425
81231	252	281	284	301	330	386
81235	234	258	263	276	316	365
81238	246	270	282	306	326	382
81239	249	281	287	298	331	394
81245	239	269	274	286	317	371
81252	225	266	256	275	301	359
81254	256	287	303	317	331	392
81257	228	259	259	268	296	351
81264	225	259	263	279	316	359
81268	253	284	283	307	337	406
81271	247	273	285	309	321	344
81275	246	272	276	287	319	375
81279	257	288	292	306	348	399
81281	253	288	284	303	334	390
81293	229	267	269	289	315	375
81308	264	273	283	306	319	363
81319	232	255	254	276	302	349
Mean	244	272	274	291	320	373

Chlorendic Anhydride:

Teratology Study in Rats.

TABLE 1. Cont.

Individual Body Weights, Grams.

Dosage Level, Dam Number	Day of Gestation					
	0	6	9	12	16	20
100 mg/kg/day:						
81198	243	268	265	284	302	342
81204	246	271	271	276	305	375
81208	233	253	257	272	301	354
81209	235	274	268	274	315	382
81214	246	266	258	271	290	346
81221	249	285	281	279	311	380
81223	222	246	254	269	311	353
81228	268	292	296	313	338	408
81234	252	287	270	288	320	385
81236	242	270	275	286	314	304
81242	248	280	280	301	324	386
81243	245	280	287	281	314	383
81247 ^a	235	272	271	258	271	274
81253	251	273	271	294	314	374
81259	240	257	266	290	312	381
81260	249	279	277	291	324	375
81265	243	277	285	304	336	396
81273	228	261	255	266	291	341
81276	233	255	256	259	388	361
81278	245	262	267	289	309	364
81280	256	289	299	306	353	420
81284	257	297	296	307	331	386
81295	252	273	279	297	307	369
81309	262	279	282	298	316	357
81321	263	287	294	321	351	408
Mean	246	273	275	288	320	372

^a Nongravid, not used in calculation of means.

Chlorendic Anhydride:

Teratology Study in Rats.

TABLE 1. Cont.

Individual Body Weights, Grams.

Dosage Level, Dam Number	Day of Gestation					
	0	6	9	12	16	20
400 mg/kg/day:						
81200	244	273	274	258	246	295
81203	228	261	256	268	281	361
81205	236	264	259	276	299	375
81210 ^a	236	250	238	245	246	256
81215	265	301	293	294	306	376
81217	242	276	270	257	298	361
81219	244	281	286	308	334	386
81224	229	250	257	266	278	335
81226	246	283	284	284	301	351
81229 ^a	246	283	283	270	271	270
81240	244	281	269	275	301	350
81244	243	275	277	255	264	312
81246 ^a	233	255	239	241	237	251
81248	254	294	282	302	309	380
81249	233	262	263	269	299	356
81258	227	263	238	255	276	336
81261	227	256	250	265	289	341
81266	228	254	259	258	286	347
81267	231	251	256	242	276	326
81274	233	278	266	278	298	370
81288	242	266	269	286	311	320
81289 ^a	235	251	228	244	251	260
81297	266	282	276	284	282	343
81299 ^a	259	276	254	273	294	371
81316 ^a	259	266	265	268	242	254
Mean	241	271	267	273	291	350

^aNongravid, not used in calculation of means.

Chlorendic Anhydride:

Teratology Study in Rats.

TABLE 2. Summary of Maternal and Fetal Observations at Cesarean Section.

	(Corn Oil) Control	Historical Control	Chlorendic Anhydride mg/kg/day		
			25	100	400
No. of animals on study:	25	126	25	25	25
No. of animals examined at cesarean section:	25	126	25	25	25
No. gravid	23	118	25	24	20
No. nongravid	2	8	0	1	5
No. of dams with live fetuses:	23	117	25	24	20
No. of dams with resorptions only:	0	1	0	0	0
No. of live fetuses/dam:	12.9	12.5	12.4	12.5	11.8
No. of postimplantation losses/dam:	0.5	1.0	0.8	1.1**	1.2*
No. of implantations/dam:	13.3	13.6	13.3	13.6	13.0
No. of corpora lutea/dam:	14.3	15.3 ^a	14.4	14.2	13.7
Sex ratio - male:female:	132:164	752:726	166:145*	153:147	121:115
Mean fetal body weight (g):	3.7	3.6	3.7	3.8	3.7

*Significantly different from the Control group, $p < 0.05$.**Significantly different from the Control group, $p < 0.01$.^aBased on 95 dams.

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Chlorendic Anhydride:

Teratology Study in Rats.

TABLE 3. Cesarean Section Data for Individual Females.

Dosage Level, Dam Number	Fetuses Live Dead	Resorptions		Post- Implantation Loss	Implanta- tions	Corpora Lutea	Sex Distribution		Mean Body Weight (g)
		Late	Early				Male	Female	
Control:									
81195	12	0	2	2	14	14	4	8	3.7
81201	10	0	0	0	10	11	4	6	4.0
81206	16	0	1	1	17	19	7	9	3.8
81211	13	0	0	0	13	13	4	9	3.4
81212	12	0	0	0	12	12	4	8	3.9
81216	15	0	1	1	16	16	7	8	3.7
81220	12	0	1	1	13	14	3	9	3.6
81225	nongravid								
81232	8	0	2	2	10	12	2	6	3.8
81233	14	0	0	0	14	14	7	7	3.2
81237	10	0	0	0	10	15	6	4	3.9
81241	14	0	0	0	14	16	3	11	3.6
81250	14	0	0	0	14	14	9	5	3.6
81251	12	0	1	1	13	13	10	2	3.7
81255	12	0	0	0	12	12	3	9	4.0
81256	13	0	1	1	14	14	4	9	3.6
81262	13	0	0	0	13	14	6	7	3.6
81269	12	0	0	0	12	12	7	5	3.6
81270	13	0	1	1	14	14	6	7	4.3
81272	13	0	0	0	13	13	4	9	3.4
81277	15	0	0	0	15	19	7	8	3.4
81290	14	0	1	1	15	15	8	6	3.8
81300	14	0	0	0	14	16	9	5	3.4
81310	nongravid								
81318	15	0	0	0	15	16	8	7	4.2
Total	296	0	11	11	307	328	132	164	
Mean	12.9	0.0	0.0	0.5	13.3	14.3			3.7

Chlorendic Anhydride:

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TABLE 3. Cont. Cesarean Section Data for Individual Females.

Dosage Level, Dam Number	Fetuses Live Dead	Resorptions		Post- Implantation Loss	Implanta- tions	Corpora Lutea	Sex Distribution		Mean Body Weight (g)
		Late	Early				Male	Female	
25 <u>ug/kg/day</u> :									
81196	10	0	0	0	10	11	6	4	3.9
81197	14	0	0	0	14	14	9	5	3.9
81202	13	0	0	0	13	14	5	8	3.5
81207	13	0	0	0	13	14	8	5	3.6
81213	13	0	0	2	15	16	6	7	3.4
81218	10	0	0	0	10	13	4	5	3.5
81222	12	0	1	1	13	13	9	3	3.4
81227	15	0	0	0	15	16	9	6	4.0
81231	15	0	1	1	16	20	11	4	3.6
81235	15	0	0	0	15	15	6	9	3.7
81238	12	0	0	0	12	12	5	7	3.9
81239	8	0	3	3	11	16	5	3	3.6
81245	13	0	2	2	15	17	7	6	3.7
81252	13	0	2	2	15	15	5	8	3.4
81254	12	0	3	3	15	15	9	3	3.7
81257	14	0	0	0	14	16	7	7	3.6
81264	11	0	0	0	11	11	4	7	3.7
81268	15	0	0	0	15	15	6	9	3.9
81271	9	0	4	4	13	18	5	4	3.5
81275	12	0	1	1	13	13	7	5	3.7
81279	13	0	0	0	13	13	8	5	4.2
81281	13	0	0	0	13	14	6	7	3.8
81293	12	0	1	1	13	13	5	7	3.5
81308	12	0	1	1	13	13	8	4	3.7
81319	12	0	0	0	12	12	6	6	3.5
Total	311	0	21	21	332	359	166	145	3.7
Mean	12.4	0.0	0.0	0.8	13.3	14.4			

Chlorendic Anhydride:

Teratology Study in Rats.

TABLE 3. Cont. Cesarean Section Data for Individual Females.

Dosage Level, Dam Number	Fetuses Live Dead	Resorptions Late Early	Post- Implantation Loss	Implanta- tions	Corpora Lutea	Sex Distribution		Mean Body Weight (g)
						Male	Female	
100 mg/kg/day:								
81198	9 0	0 0	0	9	11	7	2	3.5
81204	14 0	0 2	2	16	16	5	9	3.8
81208	12 0	0 1	1	13	13	4	8	3.8
81209	16 0	0 1	1	17	18	10	6	3.8
81214	10 0	0 1	1	11	13	5	5	3.9
81221	13 0	0 2	2	15	16	10	3	3.5
81223	12 0	0 1	1	13	14	8	4	3.7
81228	14 0	0 1	1	15	15	8	6	3.9
81234	14 0	0 0	0	14	15	6	8	3.7
81236	11 0	0 1	1	12	12	5	6	3.8
81242	13 0	0 0	0	13	13	7	6	4.0
81243	16 0	0 0	0	16	16	9	7	3.5
81247	nongravid							
81253	15 0	0 0	0	15	18	9	6	3.9
81259	15 0	0 1	1	16	17	7	8	3.6
81260	10 0	0 2	2	12	12	6	4	4.5
81265	14 0	0 0	0	14	14	5	9	3.5
81273	9 0	0 3	3	12	12	6	3	3.6
81276	12 0	0 1	1	13	13	6	6	3.9
81278	13 0	0 1	1	14	14	4	9	3.7
81280	14 0	0 2	2	16	16	5	9	3.4
81284	12 0	0 3	3	15	15	6	6	4.0
81295	12 0	0 1	1	13	13	7	5	3.7
81309	9 0	0 2	2	11	12	4	5	4.1
81321	11 0	0 1	1	12	12	4	7	3.3
Total	300 0	0 27	27	327	340	153	147	3.8
Mean	12.5 0.0	0.0 1.1**	1.1**	13.6	14.2			

**Significantly different from the Control group, p<0.01.

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Chlorethidic Anhydride:

Teratology Study in Rats.

TABLE 3. Cont. Cesarean Section Data for Individual Females.

Dosage Level, Dam Number	Fetuses Live	Fetuses Dead	Resorptions		Post- Implantation Loss	Corpora Lutea	Sex Distribution		Mean Body Weight (g)
			Late	Early			Male	Female	
<u>400 mg/kg/day:</u>									
81200	11	0	0	1	1	13	7	4	3.6
81203	13	0	0	1	1	14	8	5	3.9
81205	12	0	0	0	0	14	6	6	4.0
81210	nongravid								
81215	10	0	0	2	2	13	5	5	3.9
81217	14	0	0	0	0	15	10	4	3.3
81219	13	0	0	0	0	13	6	7	3.7
81224	12	0	0	2	2	14	5	7	3.7
81226	13	0	0	2	2	15	7	6	3.7
81229	nongravid								
81240	10	0	0	2	2	14	2	8	3.1
81244	10	0	0	0	0	11	6	4	4.0
81246	nongravid								
81248	13	0	0	0	0	13	8	5	3.8
81249	11	0	0	2	2	13	4	7	3.6
81258	14	0	0	1	1	16	9	5	3.5
81261	9	0	0	4	4	13	5	4	4.2
81266	11	0	0	0	0	12	2	9	3.7
81267	11	0	0	1	1	12	3	8	3.8
81274	12	0	0	2	2	14	6	6	4.0
81288	13	0	0	1	1	14	6	7	3.6
81289	nongravid								
81297	10	0	0	3	3	13	6	4	3.5
81299	14	0	0	0	0	15	10	4	3.4
81316	nongravid								
Total	236	0	0	24	24	273	121	115	3.7
Mean	11.8	0.0	0.0	1.2*	1.2*	13.0			

*Significantly different from Control group, $p < 0.05$.

Chlorendic Anhydride:

Teratology Study in Rats.

Table 4. Summary of Incidence of Malformations and of Developmental and Genetic Variations.

	Chlorendic Anhydride (mg/kg/day)		
	Control	25	100
No. of litters examined:	23	25	24
Total no. of fetuses examined externally:	296	311	300
Total no. of fetuses examined skeletally:	196	204	197
Total no. of fetuses examined for soft tissue:	100	107	103
	No. of Fetuses (No. of Litters)		

Malformations Observed

Cleft lip with other head anomaly:			1(1)
Bent ribs:			1(1)
Adrenals absent:	1(1)		
Supernumerary digit:		1(1)	
Total Malformations	0(0)	1(1)	2(2)

Variations - Developmental and Genetic Observed

12 full pair of ribs:			1(1)
27 presacral vertebrae:	1(1)	2(2)	1(1)
14th full rib(s):		1(1)	3(3)
14th rudimentary rib(s):	37(15)	40(13)	33(15)
7th cervical rib(s):	1(1)	1(1)	2(1)
Sternebrae no. 5 and/or 6 unossified:	18(8)	16(12)	8(6)
Other sternebrae unossified:	4(3)		1(1)
Misaligned sternebrae:			1(1)
Reduced ossification of skull:		2(2)	3(3)
Hyoid unossified:		1(1)	1(1)
Renal papilla not developed with/or without ureters distended:	1(1)	2(2)	2(2)

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163-535

^aOther head anomaly includes: cleft palate, left premaxilla malformed and fusion of left frontal to left parietal with associated accessory bone.

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TWENTY-EIGHT DAY RANGE FINDING
STUDY IN RATS

CHLORENDIC ANHYDRIDE

115-27-5

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Chemical Name (300 per name)	25 CAS No. (10)	24
4,7-METHANISOBENZOFURAN-1,3-DIONE, 4,5,6,	115-27-5	
7,8,8-HEXACHLORO-3A,4,7,7A-TETRAHYDRO-		
BICYCLO(2.2.1)HEPT-5-ENE-2,3-DICARBOXYLIC,	115-28-6	
ACID, 1,4,5,6,7,7-HEXACHLORO-		
CHLORINDIC ACID	115-28-6	

JB SFG 11/27/86

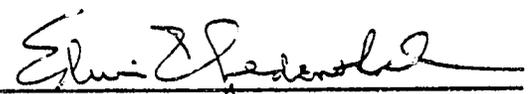
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International Research and Development Corporation

SPONSOR: Velsicol Chemical Corporation
COMPOUND: Chlorendic Anhydride
SUBJECT: Twenty-eight Day Range Finding Study in Rats.

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Edwin I. Gozdenhal, Ph.D.
Vice President and
Director of Research

Collaborators:

- D. C. Jessup, Ph.D., Associate Director of Research
- R. G. Geil, D.V.M., Director of Pathology
- B. W. Benson, B.S., Director of Small Animal Toxicology

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Date: July 6, 1978

International Research and Development Corporation

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I. SYNOPSIS

In a twenty-eight day range finding study in Charles River CD rats, Chlorendic Anhydride was administered at dosage levels of 500, 1,000, 2,500, 5,000 and 10,000 ppm. Five male and five female rats were used at each dosage level and also in a control group. The rats were observed daily for overt signs of toxicity and for mortality. Detailed observations were recorded weekly. Individual body weights and food consumption were recorded weekly. Plasma and red blood cell cholinesterase activities were determined at the termination of the study for all rats in the control group and the 2,500, 5,000 and 10,000 ppm dosage levels.

No changes considered to be related to compound were seen in general behavior and appearance. Group mean body weights were slightly to markedly lower for rats at the 500, 2,500, 5,000 and 10,000 ppm dosage levels as compared to control rats. The average food consumption during the study was slightly lower for male rats at the 5,000 and 10,000 ppm dosage level as compared to male control rats, but was similar for control and treated female rats. No rats died during the 4 weeks of compound administration; however, 1, 2 and 2 rats at the 2,500, 5,000 and 10,000 ppm dosage levels, respectively, died following the collection of blood for cholinesterase determinations. Plasma and red blood cell cholinesterase levels were similar for control and treated rats.

No gross lesions considered to be compound related were seen in rats at necropsy.

II. COMPOUND

The compound was received from Velsicol Chemical Corporation, Chicago, Illinois, on October 10, 1977, and November 7, 1977, as described below:

<u>Label</u>	<u>Description</u>
Tech. Ref. Std. Chlorendic Anhydride 93.81% (titr) Lot No. 3-12-206	white somewhat chunky powder.
Tech. Ref. Std. Chlorendic Anhydride 93.81% Lot No. 3-12-206	white somewhat chunky powder.

III. CLINICAL STUDIES

A. METHODS:

1. General Procedure:

Thirty male (weighing from 80 to 96 grams) and thirty female (weighing from 72 to 90 grams) Charles River CD rats, obtained from The Charles River Breeding Laboratories, Inc., Portage, Michigan were used in this study. The rats were distributed among the groups by use of a computer generated table of random numbers. Upon receipt, the rat numbers and their corresponding body weights were entered onto magnetic tape which was used as the data source for the following computer calculated procedures. After calculating the body weight mean (by sex) and standard deviation, a revised list was generated from those rats whose body weight was within the interval of the body weight mean \pm 1.5 standard deviations. A computer generated randomization selected from the revised list the required number of rats and assigned them to the desired groups. Bartlett's chi-square test for homogeneity of variances was performed on these groups. If the group variances were judged heterogeneous, then new randomizations were generated until they were homogeneous, at which time the randomization was accepted. The rats were housed individually in hanging wire mesh cages and maintained in a temperature, humidity and light controlled environment. Water and the control and test diets were available ad libitum.

This study was initiated on December 7, 1977 and terminated on January 4, 1978.

2. Compound Administration:

Chlorendic Anhydride was administered in the diet at dosage levels of 500, 1,000, 2,500, 5,000 and 10,000 ppm. Five male and five female rats were used at each dosage level and also in a control group. The control rats received the basal laboratory diet, ground Purina[®]

Laboratory Chow[®], on the same regimen as treated rats.

The compound was ground with a mortar and pestle and the appropriate amount added to a portion of ground Purina[®] Laboratory Chow[®] and mixed in a Hobart mixer. This premix was then added to the remainder of the required amount of Purina[®] Laboratory Chow[®] and mixed in a twin shell blender with an intensifier bar.

3. Observations:

The rats were observed daily for overt signs of toxicity and for mortality. Detailed observations were recorded weekly.

Individual body weights and food consumption were recorded weekly.

4. Cholinesterase Activity:

Plasma and red blood cell cholinesterase activities* were determined for all rats in the 2,500, 5,000 and 10,000 ppm dosage levels as well as the control group at 28 days of study.

B. RESULTS:

1. General Behavior, Appearance and Survival:

No changes considered to be related to compound were seen in general behavior and appearance.

No rats died during the 4 weeks of compound administration; however, 1, 2 and 2 rats at the 2,500, 5,000 and 10,000 ppm dosage levels, respectively, died following the collection of blood for cholinesterase determinations.

2. Body Weights (Tables 1-2):

Group mean body weight gains were moderately lower for male rats at the 2,500 and 5,000 ppm dosage levels and markedly lower for male rats at the 10,000 ppm dosage level as compared to male control rats.

Group mean body weight gains were slightly lower for female rats at the 500 ppm dosage level, moderately lower for female rats at the 2,500 and 10,000 ppm dosage levels and markedly lower for female rats at the 5,000 ppm dosage level as compared to female control rats.

*Levine, Scheidt and Nelson - An Automated Micro Determination of Serum Cholinesterase, Technicon Corporation, Ardsley, New York.

Group mean body weights and the percent difference from the control group at the end of the study were as follows:

<u>Dosage Level</u> ppm	<u>Group Mean Body Weights, grams</u> (% difference)	
	<u>Male</u>	<u>Female</u>
0	302	200
500	292 (-3.3)	185 (-7.5)
1,000	297 (-1.7)	195 (-2.5)
2,500	270 (-10.6)	177 (-11.5)
5,000	258 (-14.6)	154 (-23.0)
10,000	240 (-20.5)	172 (-14.0)

3. Food and Compound Consumption (Table 3):

The average food consumption during the study was slightly lower for male rats at the 5,000 and 10,000 ppm dosage levels as compared to male control rats, but was similar for control and treated female rats.

The average food consumption and compound consumption during the study were as follows:

<u>Dosage Level</u> ppm	<u>Average Food Consumption</u> g/rat/day		<u>Average Compound Consumption</u> mg/kg/day	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
0	24.0	18.1	0	0
500	23.0	17.5	53	59
1,000	23.7	18.1	108	115
2,500	22.7	17.1	282	287
5,000	20.5	15.7	529	606
10,000	20.2	18.2	1113	1242

4. Cholinesterase Activity (Table 4):

Plasma and red blood cell cholinesterase levels were similar for control and treated rats.

IV. PATHOLOGICAL STUDIES

Necropsy observations in rats which died after terminal blood collection included the following:

2,500 ppm

no gross lesions

1/1 female

5,000 ppm

no gross lesions

2/2 females

10,000 ppm

no gross lesions

1/2 females

alopecia, both flanks

1/2 females

No gross lesions were seen in any of the remaining 5 male and 3 female rats from the 10,000 ppm dosage level which were sacrificed and necropsied.

All other remaining test and control rats were sacrificed and discarded.

No tissues were saved.

Tech. Ref. Std.
Chlordane Anhydride:

TABLE I.
Group Mean Body Weights, Grams; Weight Ranges; and Survival.

Week of Study	Control			500 ppm			1,000 ppm			2,500 ppm			5,000 ppm			10,000 ppm		
	Mean Body Wt.	Weight Ranges	Sur- vival															
0	87	80-94	5/5	88	80-94	5/5	92	84-96	5/5	85	80-90	5/5	88	82-94	5/5	87	84-90	5/5
1	146	116-160	5/5	147	114-160	5/5	148	138-160	5/5	143	130-150	5/5	136	120-144	5/5	131	118-140	5/5
2	202	190-218	5/5	199	184-218	5/5	201	180-220	5/5	182	162-200	5/5	180	170-190	5/5	162	144-180	5/5
3	244	234-254	5/5	243	228-264	5/5	248	224-278	5/5	228	210-246	5/5	222	206-234	5/5	207	186-220	5/5
4	302	296-306	5/5	292	272-304	5/5	297	266-330	5/5	270	248-292	5/5	258	240-272	5/5	240	220-266	5/5
FEMALES:																		
0	83	80-86	5/5	78	74-82	5/5	84	78-90	5/5	83	76-90	5/5	80	72-88	5/5	84	80-90	5/5
1	125	120-130	5/5	117	100-124	5/5	126	114-136	5/5	125	114-136	5/5	108	100-118	5/5	121	114-128	5/5
2	150	140-160	5/5	142	110-156	5/5	149	130-170	5/5	141	124-150	5/5	120	110-130	5/5	135	126-154	5/5
3	168	156-178	5/5	158	130-174	5/5	167	144-186	5/5	160	142-170	5/5	141	130-154	5/5	156	140-178	5/5
4	200	178-210	5/5	185	150-204	5/5	195	164-220	5/5	177	152-188	5/5	154	144-160	5/5	172	156-188	5/5

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Tech. Ref. Std.
Chlorendic
Anhydride:

Twenty-eight Day Range Finding Study in Rats.

TABLE 2. Individual Weekly Body Weights, Grams.

Group, Rat No.	Sex	Control	Week of Study			
		0	1	2	3	4
<u>Control:</u>						
77353	M	86	140	200	242	304
77354	M	80	136	190	234	296
77355	M	90	152	204	244	302
77356	M	94	160	218	254	306
77357	M	86	144	198	246	304
77358	F	86	130	160	178	206
77359	F	84	122	150	168	200
77360	F	80	126	150	174	210
77361	F	84	120	140	154	178
77362	F	80	126	152	164	204
<u>500 ppm:</u>						
77363	M	86	140	196	234	272
77364	M	80	134	184	228	274
77365	M	90	156	208	256	304
77366	M	94	160	218	264	324
77367	M	90	144	190	234	288
77368	F	78	124	156	174	204
77369	F	80	120	152	170	200
77370	F	82	124	150	168	190
77371	F	74	116	142	154	180
77372	F	76	100	110	130	150
<u>1,000 ppm:</u>						
77373	M	96	160	220	278	330
77374	M	94	156	216	262	320
77375	M	94	138	180	226	272
77376	M	94	148	204	248	298
77377	M	84	140	184	224	266
77378	F	78	118	130	144	164
77379	F	90	136	170	186	220
77380	F	78	114	142	160	188
77381	F	84	130	154	180	204
77382	F	90	130	150	164	198

Tech. Ref. Std.
Chlorendic
Anhydride:

Twenty-eight Day Range Finding Study in Rats.

TABLE 2. Cont. Individual Weekly Body Weights, Grams.

Group, Rat No.	Sex	Control	Week of Study			
		0	1	2	3	4
<u>2,500 ppm:</u>						
77383	M	84	150	200	246	284
77384	M	90	150	194	240	292
77385	M	80	130	162	210	248
77386	M	86	140	176	222	264
77387	M	86	144	178	224	260
77388	F	80	122	136	156	170
77389	F	76	114	124	142	152
77390	F	90	136	150	170	188
77391	F	82	124	144	164	186
77392	F	86	130	150	168	188
<u>5,000 ppm:</u>						
77393	M	86	140	176	220	260
77394	M	92	134	170	218	248
77395	M	94	144	190	234	270
77396	M	86	140	186	234	272
77397	M	82	120	178	206	240
77398	F	72	100	110	130	144
77399	F	80	100	114	134	152
77400	F	88	118	130	150	160
77401	F	80	114	128	154	160
77402	F	78	108	120	138	152
<u>10,000 ppm:</u>						
77403	M	90	134	162	218	240
77404	M	86	124	154	204	234
77405	M	90	140	180	220	266
77406	M	84	118	144	186	220
77407	M	84	138	168	206	238
77408	F	80	124	138	164	178
77409	F	90	128	154	178	188
77410	F	84	118	126	148	156
77411	F	80	114	126	140	164
77412	F	84	120	130	150	174

Tech. Ref. Std.
Chlorendic Anhydride: Twenty-eight Day Range Finding Study in Rats.

TABLE 3.

Mean Food Consumption

Week of Study	Control		500 ppm		1,000 ppm		2,500 ppm		5,000 ppm		10,000 ppm	
	g/ rat/ day	g/ kg/ day										
MALES:												
1	19.3	132.3	18.0	122.4	18.8	127.0	19.0	132.7	17.6	129.4	16.5	126.1
2	20.9	103.5	20.6	103.7	20.9	104.1	20.1	110.5	18.9	105.1	18.6	115.0
3	26.4	108.2	25.4	104.6	25.0	100.7	24.3	106.8	21.0	94.5	20.5	99.1
4	29.5	97.5	28.0	96.0	29.9	100.5	27.5	101.8	24.3	94.2	25.2	104.9
FEMALES:												
1	16.5	132.1	16.2	138.5	16.6	131.5	16.3	130.3	14.8	137.0	15.3	126.6
2	16.1	107.0	15.7	110.3	17.2	115.4	16.5	117.1	15.2	126.7	16.2	120.2
3	18.8	111.9	17.1	107.8	18.0	107.8	16.0	100.0	15.3	108.2	18.2	116.5
4	21.0	104.9	20.8	112.3	20.5	105.1	19.7	111.1	17.4	112.8	22.5	133.4

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Tech. Ref. Std.
Chlorendic
Anhydride:

Twenty-eight Day Range Finding Study in Rats.

TABLE 4. Cholinesterase Activity.

Group, Rat Number	Sex	Units of Plasma Cholinesterase Activity	Units of Cell Cholinesterase Activity
<u>Control:</u>			
77353	M	1.9	10.1
77354	M	2.3	7.4
77355	M	2.2	11.1
77356	M	2.0	10.4
77357	M	2.1	11.7
Mean		2.1	10.1
77358	F	4.4	11.4
77359	F	4.6	11.1
77360	F	3.6	10.7
77361	F	5.1	11.1
77362	F	6.7	12.7
Mean		4.9	11.4
<u>2,500 ppm:</u>			
77383	M	2.3	11.4
77384	M	2.0	11.7
77385	M	3.1	11.4
77386	M	2.8	11.1
77387	M	2.0	10.7
Mean		2.4	11.3
77388	F	4.2	13.7
77389	F	3.8	10.7
77390	F	3.4	9.0
77391	F	2.7	10.1
77392	F	3.2	11.4
Mean		3.5	11.0

Tech. Ref. Std.
Chlorendic
Anhydride:

Twenty-eight Day Range Finding Study in Rats.

TABLE 4. Cont. Cholinesterase Activity.

Group, Rat Number	Sex	Units*of Plasma Cholinesterase Activity	Units*of Cell Cholinesterase Activity
<u>5,000 ppm:</u>			
77393	M	3.0	9.0
77394	M	3.4	12.7
77395	M	2.5	10.4
77396	M	2.9	11.7
77397	M	2.8	11.7
Mean		2.9	11.1
77398	F	2.9	11.4
77399	F	3.6	10.1
77400	F	3.1	11.4
77401	F	3.1	8.7
77402	F	4.4	8.0
Mean		3.4	9.9
<u>10,000 ppm:</u>			
77403	M	4.0	9.7
77404	M	4.2	10.1
77405	M	3.1	11.4
77406	M	3.1	11.7
77407	M	2.6	9.7
Mean		3.4	10.5
77408	F	3.4	8.7
77409	F	3.0	10.1
77410	F	3.3	10.4
77411	F	3.4	9.7
77412	F	3.0	11.4
Mean		3.2	10.1

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*Micromoles of SH groups liberated in 3 minutes
from 1 ml of plasma or erythrocytes.

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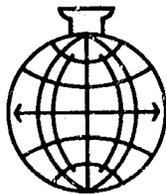
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CHLORENDIC ANHYDRIDE		115-27-5		

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**International Research
and Development Corporation**

MATTAWAN, MICHIGAN, U.S.A. 49071 TELEPHONE (616) 668-3336

SPONSOR: Velsicol Chemical Corporation

COMPOUND: Chlorendic Anhydride, Tech.

SUBJECT: 90-Day Subacute Toxicity Study
in Rats

DATE OF SUBMISSION: January 7, 1980

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APPENDIX I

Ophthalmoscopic Examination Summary

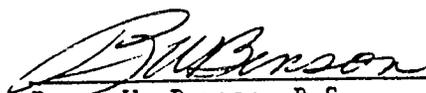
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I. QUALITY ASSURANCE STATEMENT

Based on a quality assurance review, it was concluded that this report accurately reflects the data for the 90-Day Subacute Toxicity Study in Rats with Chlorendic Anhydride, Tech.

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Submitted By:



Barry W. Benson, B.S.
Director of Quality Assurance

2/4/80
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II. SYNOPSIS

Chlorendic Anhydride was fed in the diet at levels of 100-, 500- and 2500-ppm to three groups of Charles River CD® rats in a 90-day subacute toxicity study; there were 15 rats/sex/group. Control rats, also 15/sex/group, received the basal laboratory diet only. The rats were observed twice daily for signs of overt toxicity and mortality. Detailed observations, individual body weights and individual food consumption were recorded weekly. Ophthalmic examinations were conducted during the pretest period and at 3 months of study. Hematologic and biochemical tests and urinalyses were performed at 1, 2 and 3 months of study for five rats/sex/groups.

Mid- and high-dose males and all three groups of treated females had decreased group mean body weights when compared with controls. Mid- and high-dose males and high-dose females had decreased food consumption over the 90-day study when compared with controls. Both treated males and females had elevated SAP activities at 1, 2 and 3 months of study.

Statistically significant decreases in the mean absolute weights of hearts of male rats ($p < 0.05$) and in the mean absolute and relative weights of livers of male and female rats ($p < 0.01$) at all dosage levels, appeared to be treatment related.

No compound-related gross lesions were seen in any of the rats from the treatment groups.

No compound-related microscopic lesions were seen in any of the tissues from rats that were examined from the 2500-ppm group.

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III. COMPOUND

The test material was received from Velsicol Chemical Corporation, Ann Arbor, Michigan on April 17, 1978 as follows:

<u>Label</u>	<u>Description</u>
Chlorendic Anhydride, Tech. #8093-1	White somewhat chunky powder

IV. CLINICAL STUDIES

A. METHOD

1. General Procedure

Sixty male (75 to 106 grams) and 60 female (71 to 97 grams) weanling Charles River CD® rats from The Charles River Breeding Laboratories, Inc., Portage, Michigan were initiated in this subacute toxicity study. The rats were housed individually in suspended wire-mesh cages and maintained in a temperature-, humidity- and light- (12-hour light/12-hour dark) controlled room. Water and appropriate diets were available ad libitum throughout the study.

The rats used in the study were selected from a larger group of 160 animals after observation and examination during the pretest period. Any rat with physical abnormalities or signs of disease was discarded, and the remaining animals were distributed among the four groups in accordance with the randomization described in IV. A.

The study was initiated on July 25, 1978; all surviving rats were sacrificed and necropsied on October 23-24, 1978.

2. Compound Administration

The test material, Chlorendic Anhydride, was fed to the rats at the following concentration:

<u>Treatment Level (ppm)</u>	<u>No. of Rats/Group</u>	
	<u>Male</u>	<u>Female</u>
0 (Control)	15	15
100	15	15
500	15	15
2500	15	15

The test diets were prepared by first blending known amounts of test material (ground with mortar and pestle) and basal laboratory diet, ground Purina® Laboratory Chow®, in a Hobart mixer to yield a premix. This premix was then mixed with larger amounts of the basal laboratory diet in a twin-shell blender (with an intensified bar run

for 2 minutes at the beginning and end of each mixing period) that resulted in test diets of the desired concentrations. Control rats received only the basal laboratory diet and water.

The diets were prepared fresh weekly. Dietary samples were collected immediately after mixing and on day 7 at week 1 and at 1, 2 and 3 months of study. The samples were frozen and shipped to the sponsor on October 30, 1978 (after termination of the study).

3. Observations

The rats were observed twice daily for signs of overt toxicity, moribundity and mortality. Detailed observations were recorded weekly. Individual body weights and individual food consumption also were recorded weekly.

4. Ophthalmoscopy

Ophthalmic examinations by a veterinary ophthalmologist were performed on all rats once during the pretest period and again at 3 months of study.

Ophthalmic examination of the rats was performed following pupillary dilation with 1% Tropicamide solution. The binocular indirect ophthalmoscope was used with a positive 20-diopter focusing and magnifying lens. This instrumentation allowed for the evaluation of the ocular tissues with stereoscopic vision at a magnification of approximately 4-5 power. The clarity of the ocular media (precorneal tear film, cornea, aqueous humor, lens and vitreous humor) and fundic reflex were initially evaluated. The ocular adnexa and iris were viewed under magnification and the lens focal distance changed to view the fundic tissues (approximately the equator of the globe).

5. Laboratory Tests

Blood and urine samples were collected from five rats/sex/group at 1, 2 and 3 months of study. Selection of rats for these tests was from a computer-generated table of random numbers. Prior to sample

collection, the rats were housed overnight in metabolism cages; also food and water were withheld during this time period. Blood was collected via the orbital sinus technique.

Samples of liver were taken from five rats/sex/group at terminal sacrifice, frozed and stored for possible future analysis of the bromine content.

a. Hematology

Hematologic tests included: total erythrocyte count¹, total¹ and differential leucocyte counts, hemoglobin concentration² and hematocrit value³.

b. Biochemistry

Biochemical tests included: fasting blood glucose⁴, blood urea nitrogen (BUN)⁴, serum alkaline phosphatase activity⁴, serum glutamic oxalacetic transaminase activity⁴ and serum glutamic pyruvic transaminase activity⁵.

c. Urinalysis

Urinalyses included: description of appearance; measurement of volume, pH⁶ and specific gravity; and qualitative tests for albumin⁶, glucose⁶, bilirubin⁶ and occult blood⁶; and microscopic examination of the sediment.

6. Statistical Analysis

All statistical analyses compared the treatment groups with the control group by sex. Body weights (week 13), food consumption (week 13), hematological, biochemical and urinalysis parameters (1, 2 and 3 months) and absolute and relative organ weights (terminal sacrifice) were compared by analysis of variance (one-way classification) Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie⁷ using Dunnett's⁸ multiple comparison tables to judge significance of differences.

7. Randomization Procedure

Animal numbers and corresponding body weights were entered onto magnetic tape which was used as the data source for the following randomization procedure. First, the mean body weight and standard deviation was calculated by sex, and a computer-generated edit developed a listing of those animals whose body weights were within ± 1.5 standard deviations of the mean. From the qualifying animals, the randomization procedure selected and assigned the required number of animals. Bartlett's Chi-square test for homogeneity of variances was applied to the groups. If the groups were not judged to be homogeneous, new randomizations were applied until homogeneity was established.

B. RESULTS

1. General Behavior, Appearance and Survival

There were no signs of overt toxicity observed for the treated rats. Some incidental signs seen in a few control and/or treated rats were malaligned upper incisors, soft stools, skin lesions, hair loss, lacrimation, corneal opacity, redness around eye. Eye problems, such as pale coloration of eye, decrease in size of eyeball, dilated pupil unresponsive to light, clear or white internal eye and increased distance between pupil and cornea, occurred most frequently in rats that had had blood drawn via the orbital sinus technique.

Three high-dose females died between the 5th and 13th week of study; no other rats died during the study. Survival at week 13 was as follows:

<u>Treatment Level (ppm)</u>	<u>No. Survivors/No. Initiated</u>	
	<u>Male</u>	<u>Female</u>
0 (Control)	15/15	15/15
100	15/15	15/15
500	15/15	15/15
2500	15/15	12/15

2. Body Weights (Tables 1-3)

The mid- and high-dose males and all three groups of treated females had a decreased rate of weight gain that was compound related. The group mean body weights of the mid- and high-dose males and high-dose females were significantly less than controls at week 13. At week 13 of the study, the group mean body weights were as follows:

<u>Treatment Level (ppm)</u>	<u>Group Mean Body Weight, g (% difference from control)</u>	
	<u>Male</u>	<u>Female</u>
0 (Control)	502	285
100	509 (+ 1.4)	274 (- 3.9)
500	464 (- 7.6)	269 (- 5.6)
2500	440 (-12.4)	224 (-21.4)

3. Food and Compound Consumption (Tables 4-5)

Mid- and high-dose males and high-dose females had decreased food consumption values over the 90-day study when compared with controls; however, only the food consumption of the high-dose females was significantly less than the controls. Food and compound consumption over the entire study were as follows:

<u>Treatment Level (ppm)</u>	<u>Food and Compound Consumption (g/rat/day)</u>		<u>Compound Consumption (mg/kg/day)</u>	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
0 (Control)	25.7	18.2	-	-
100	25.8	18.3	8	8
500	24.7	18.5	39	45
2500	24.6	16.4	202	226

4. Ophthalmoscopy

There were no compound-related effects in the 13-week ophthalmic examinations.

5. Laboratory Tests (Tables 6-17)

a. Hematology

No compound-related effects were observed in the results of the hematologic tests. An incidental finding was the slightly elevated number of leucocytes seen for the high-dose males at 1, 2 and 3 months of study. Other occasional statistically significant values were of no physiologic importance.

b. Biochemistry

Both treated males and females had elevated SAP activities at 1, 2 and 3 months of study (only the 2- and 3-month values of the high-dose females and the 2-month value of the mid-dose females were significantly greater than controls). At 3 months of study, all groups had greater SAP activities than controls. Mid- and low-dose males and females had SGOT activities significantly lower than controls at 3 months of study. However, these activities as well as other occasional statistically significant values were of no physiologic importance. No other compound-related effects were seen in the results of the biochemical tests.

c. Urinalysis

No compound-related effects were seen in the results of the urinalyses. An incidental finding at 3 months of study was the elevated urinary pH of two of five high-dose females tested. The few statistically significant values (specific gravity) were of no physiologic importance.

V. PATHOLOGICAL STUDIES

A. METHODS

1. Gross Pathology

At the termination of the 90-day period of study, all rats were sacrificed using carbon dioxide gas and necropsied. Selected organs were weighed and representative tissues were preserved in buffered 10% neutral formalin.

Three rats from the 2500-ppm group, that died following blood collection during the course of the study, were also necropsied and representative tissues were preserved in 10% formalin.

2. Histopathology

The following tissues from 10 males and 10 females from the control and the 2500-ppm groups were paraffin embedded, sectioned, stained with hematoxylin and eosin and examined microscopically:

adrenals	pituitary
aorta	prostate/uterus
brain	sternum (bone marrow)
eye	skin/mammary gland
heart	spinal cord
large intestine (colon)	stomach
small intestine (3 levels)	testis/ovary
kidneys (2)	thymus
liver	thyroid
lung and bronchi	urinary bladder
mesenteric lymph node	nerve (sciatic)
spleen	skeletal muscle
pancreas	any other tissues with lesions

In addition, frozen sections of liver and kidneys from 10 male and 10 female rats from the control and the treatment groups were stained for fat with oil red O and examined microscopically.

B. RESULTS

1. Gross Pathology (Table 18)

The three rats from the 2500-ppm group that died during the course of the study did not show any compound-related lesions. None of the rats that were sacrificed at the termination of the study had any compound-related lesions.

2. Organ Weights (Tables 19-20)

Statistically significant ($p < 0.05$) decreases in the mean absolute weight of spleens of female rats at the 2500-ppm dosage level, mean absolute weight of kidneys of male rats at the 2500-ppm dosage level, mean absolute weight of hearts of male rats at all dosage levels and the female rats at the 500-ppm dosage level and an increase in the mean weight of testes of male rats at the 500-ppm dosage level, were noted. In respect of liver, statistically significant ($p < 0.01$) decreases in the mean absolute and relative weights at all dosage levels were observed. Of these variations, decreases in the mean absolute weights of heart of male rats ($p < 0.05$) and in the mean absolute and relative weights of livers ($p < 0.01$) at all dosage levels, appeared to be treatment related. In the absence of any significant histomorphologic changes in these organs in the test groups, these decreases probably might be due to the overall reduction in the body weights resulting from a reduction of body fat and/or extracellular body fluid.

3. Histopathology (Table 21)

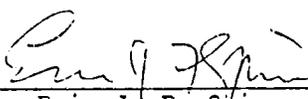
No compound-related microscopic lesions were observed in any of the tissues from rats that were examined from the 2500-ppm group.

The microscopic lesions seen were considered spontaneous and incidental in nature.

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Chlorendic Anhydride,
Tech. 90-Day Subacute Toxicity Study in Rats.

TABLE 1. t-Test Comparison Between Means of Control and Treated Groups,
Body Weights, grams.

Week of Study	Sex	Control	100 ppm	500 ppm	2500 ppm
13	M	508	504	463*	435**
	F	277	275	266	226**

*p<0.05
**p<0.01

Chlorendic Anhydride, Tech.

90-Day Subacute Toxicity Study in Rats

TABLE 2. Group Mean Body Weights, Grams; Weight Ranges; and Survival

Week of Study	0 ppm (Control)			100 ppm			500 ppm			2500 ppm		
	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival
MALES:												
0	95	77-106	15/15	90	75-103	15/15	91	75-100	15/15	90	76-100	15/15
1	158	136-174	15/15	150	134-172	15/15	146	110-166	15/15	142	119-162	15/15
2	209	197-220	15/15	202	183-222	15/15	193	148-218	15/15	184	158-204	15/15
3	262	240-278	15/15	254	232-287	15/15	240	179-269	15/15	228	165-261	15/15
4	317	288-336	15/15	312	288-336	15/15	290	225-331	15/15	269	214-315	15/15
5	352	326-374	15/15	346*	316-370	15/15	320*	242-364	15/15	289*	236-338	15/15
6	383	335-429	15/15	374	340-411	15/15	347	276-402	15/15	328	254-383	15/15
7	404	330-456	15/15	402	366-432	15/15	373	306-427	15/15	352	271-420	15/15
8	428	360-489	15/15	425	389-459	15/15	393	312-444	15/15	369	286-444	15/15
9	451*	393-513	15/15	441*	405-485	15/15	417*	375-460	15/15	396*	344-457	15/15
10	464	375-538	15/15	455	420-500	15/15	420	332-477	15/15	397	306-480	15/15
11	486	404-564	15/15	483	444-532	15/15	439	344-496	15/15	415	318-515	15/15
12	494	410-568	15/15	496	467-541	15/15	452	360-510	15/15	431	336-538	15/15
13	502**	430-539	15/15	509**	457-558	15/15	464**	382-522	15/15	440**	336-558	15/15
FEMALES:												
0	83	74- 97	15/15	88	79- 95	15/15	83	71- 96	15/15	86	77- 95	15/15
1	125	112-140	15/15	128	114-140	15/15	124	112-140	15/15	125	112-136	15/15
2	153	134-171	15/15	155	130-175	15/15	151	131-170	15/15	140	129-156	15/15
3	171	152-185	15/15	172	145-192	15/15	165	148-183	15/15	153	135-175	15/15
4	196	168-215	15/15	199	163-225	15/15	189	171-216	15/15	168	144-192	15/15
5	208	182-230	15/15	211	180-244	15/15	202*	183-230	15/15	172*	154-200	14/15
6	224	201-249	15/15	224	190-256	15/15	218	198-242	15/15	187	162-217	14/15
7	236	214-265	15/15	238	202-274	15/15	229	214-253	15/15	196	171-226	14/15
8	244	219-276	15/15	244	210-279	15/15	237	220-269	15/15	202	180-228	14/15
9	254*	228-285	15/15	253*	215-292	15/15	242*	220-270	15/15	202*	178-228	12/15
10	255	230-282	15/15	256	222-296	15/15	248	230-276	15/15	210	187-240	12/15
11	268	239-300	15/15	267	234-306	15/15	257	236-290	15/15	218	198-241	12/15
12	276	246-311	15/15	276	238-318	15/15	265	242-308	15/15	220	200-244	12/15
13	285**	259-309	15/15	274**	238-317	15/15	269**	248-309	15/15	224**	199-251	12/15

*Food withheld 5 rats/sex/group; not included in mean

**Food and water withheld 5 rats/sex/group; not included in mean

165-533

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Chloromide Anthelmintic, Tech.
 90-Day Subacute Toxicity Study in Rats
 Individual Weekly Body Weights, Grams

Group, Rat No.	Sex	Pretest	Week of Study												
			0	1	2	3	4	5	6	7	8	9	10	11	12
0 ppm. (Control):															
96256	M	100	160	254	308	336	369	392	407	422	442	456	469	482	
96257	M	100	164	278	329	413	441	471	471	493	518	536	538	556*	
96258	M	100	164	265	321	382	419	449	449	464	496	532	536	540*	
96259	M	80	142	197	303	340	404	404	429	442	475	497	506	532	
96260	M	77	136	197	296	347	364	383	383	394	402	428	428	428*	
96261	M	99	160	240	332	429	456	489	489	513	538	564	568	580*	
96262	M	92	158	273	313	374	429	456	456	473	487	497	475	497	
96263	M	93	154	258	320	398	421	438	438	463*	468	487	490	508	
96264	M	100	164	212	324	396	421	434	434	445	460	488	500	523	
96265	M	106	174	277	332	393	417	440	440	442*	472	492	498	507*	
96266	M	93	160	275	336	407	429	453	453	462*	487	512	518	539	
96267	M	98	156	260	308	368	393	419	419	438	455	476	490	506	
96268	M	90	148	242	288	335	365	387	387	340*	375	404	410	430	
96269	M	100	164	269	325	400	428	455	455	478	495	502	510	524	
96270	M	98	160	265	316	377	330	360	360	393	426	450	470	475	
96271	F	97	134	180	205	234	243	255	255	263	269	273	284	292	
96272	F	90	132	168	183	218	227	236	236	243	250	260	270	274	
96273	F	75	122	148	189	216	228	229	229	228	236	250	260	259	
96274	F	85	128	159	211	235	252	259	259	266	273	290	293	295	
96275	F	74	126	158	211	240	254	263	263	267	280	300	310	309	
96276	F	75	126	163	215	230	249	265	276	285	282	296	311	308	
96277	F	75	120	147	186	208	217	223	223	229	232	246	248	248*	
96278	F	81	114	152	168	201	214	220	220	225*	235	250	254	265	
96279	F	81	114	152	168	201	214	220	220	225*	235	250	254	265	
96280	F	95	136	163	211	231	239	249	249	257	265	270	280	289	
96281	F	78	112	140	183	210	226	232	232	237	242	251	256	256*	
96282	F	79	120	152	201	227	236	249	249	254*	255	270	284	279	
96283	F	84	128	157	198	225	243	253	253	253*	262	280	284	282	
96284	F	95	140	171	214	241	244	255	255	266	272	284	292	291*	
96285	F	61	130	151	192	223	231	239	239	243*	246	258	264	261*	
96286	F	82	114	137	175	206	216	219	219	221*	230	239	246	248*	

*Food withheld: not included in mean

Chloronilic Anhydride, Tech.
 TABLE 3. Cont.
 Individual Weekly Body Weights, Grams

Group	Sex	Pretest	1	2	3	4	5	6	7	8	9	10	11	12	13
160 ppm:															
96286	M	85	134	183	232	288	310*	340	368	389	416	422	457	465	465*
96287	H	85	156	211	264	322	347	376	406	429	433*	450	484	496	512
96288	M	83	144	203	260	321	358	390	426	453	468	490	518	530	540
96289	M	99	160	211	264	323	365	397	419	441	453	462	494	510	518*
96290	M	78	145	209	261	324	369	411	432	459	485	500	525	539	507*
96291	M	107	156	195	240	288	316	346	369	393	405	420	446	460	457
96292	H	88	149	203	257	327	370	401	432	456	469*	498	532	541	558
96293	M	93	154	207	259	310	340	368	390	413	427	438	460	470	499
96294	H	88	138	191	236	293	328	359	390	412	410*	430	452	478	465
96295	M	84	142	191	242	305	334*	384	409	437	455	473	501	519	532
96296	M	87	144	190	242	296	320*	351	377	397	409	432	454	473	481*
96297	M	101	172	222	287	335	356*	402	423	445	459	468	500	510	532
96298	M	75	136	198	255	311	336	353	386	419	435	460	486	500	508
96299	M	98	160	202	245	300	330	357	384	403	396*	425	444	457	470
96300	M	100	162	221	273	336	318*	374	416	436	439*	464	485	508	497*
96301	F	90	134	167	190	216	228	250	267	270	280	290	306	315	315
96302	F	94	140	175	191	225	244	256	274	279	292	296	303	318	310*
96303	F	87	138	165	190	221	232*	249	264	270	277*	287	300	312	317
96304	F	95	138	167	192	222	230	237	250	254	252*	273	280	286	288
96305	F	90	130	161	171	199	216	225	236	245	248	255	270	284	269*
96306	F	83	130	151	158	186	200	214	228	233	238	242	255	265	251*
96307	F	88	130	158	180	205	220	240	254	260	271	282	294	302	300*
96308	F	87	128	156	175	204	208*	226	247	240	240	246	258	258	258
96309	F	87	115	136	145	175	180	192	204	210	215	225	235	240	238
96310	F	88	118	150	161	188	198*	213	228	237	244*	240	252	265	257*
96311	F	92	130	154	169	200	206*	220	230	237	240	247	260	266	273
96312	F	80	117	146	160	184	196	205	210	219	214*	222	236	242	251
96313	F	87	128	161	177	209	217	219	241	253	264	268	278	285	292
96314	F	86	128	152	173	192	206*	217	230	237	237	240	253	260	265
96315	F	79	114	130	149	163	182	190	202	210	210*	226	234	238	244

*Food withheld; not included in mean

Chloroform Anhydride, Tech. 90-Day Subacute Toxicity Study in Rats

TABLE 3. Cont.

Individual Weekly Body Weights, Grams

Group, Rat No.	Sex	Pretest (0)	1	2	3	4	5	6	7	8	9	10	11	12	13
500 Ppm:															
96316	M	99	160	207	257	321	350	384	405	429	443	460	442	466	470*
96317	N	93	168	198	233	286	318	346	373	399	415	430	452	471	472*
96318	M	97	162	201	261	315	348	397	419	442	460	477	496	510	522
96319	N	94	144	190	236	277	310	342	349	371	384	402	415	422	425*
96320	M	78	122	165	204	242	260*	291	328	354	374	398	403	398	403
96321	M	100	160	208	260	297	320*	351	385	401	420	430	454	465	469*
96322	N	98	166	218	269	331	364	402	427	444	448	467	489	494	502
96323	M	91	150	197	247	287	332	367	400	418	409*	434	462	470	486
96324	M	78	130	187	231	286	310	343	368	395	396*	415	449	464	487
96325	N	94	152	206	262	316	336*	367	377	403	415	430	450	470	486
96326	M	93	144	188	231	280	302*	327	352	363	375	390	407	412	425
96327	M	95	156	211	261	312	324*	351	378	396	400	418	450	454	458
96328	M	83	134	188	231	281	310	338	364	389	409	424	455	467	488
96329	M	75	110	148	179	225	242	276	300	312	315*	332	344	360	382
96330	M	90	146	183	233	289	320	350	379	398	405*	430	448	460	479*
96331	F	72	116	144	159	183	200	223	236	240	248	255	266	275	282
96332	F	87	126	159	167	189	202*	221	232	243	245	256	259	266	268
96333	F	87	122	146	160	181	188	206	214	220	220	230	242	246	248
96334	F	76	114	131	148	171	182*	198	214	220	221	232	236	242	254
96335	F	87	124	146	155	176	183	210	225	234	237	247	258	266	266*
96336	F	89	124	152	165	195	205	217	224	232	225*	244	248	254	250*
96337	F	96	138	169	183	200	219	230	239	246	247	260	270	274	285
96338	F	71	112	139	160	184	199	215	224	233	231*	233	247	262	256
96339	F	95	138	168	183	216	230	242	253	269	270	276	290	308	309
96340	F	84	120	152	160	183	194	201	220	230	226*	237	248	254	254
96341	F	75	129	157	176	201	210*	229	239	248	252	260	268	278	268*
96342	F	78	122	146	163	180	190*	212	226	237	230*	244	254	262	260
96343	F	73	112	140	155	181	192	208	220	229	223*	240	254	258	259*
96344	F	94	140	170	175	200	202*	231	238	239	245	252	258	266	258*
96345	F	81	125	148	170	196	210	223	232	239	237	248	254	262	267

*Food withheld; not included in mean

Chloroform Anhydride, Tech.
 90-Day Subacute Toxicity Study in Rats
 Individual Weekly Body Weights, Grams

Group, Rat No.	Sex	Pretest 0	Week of Study												
			1	2	3	4	5	6	7	8	9	10	11	12	13
2500 Ppm:															
96346	M	89	134	182	231	260	286	323	349	360	366*	394	414	439	440*
96347	N	80	132	180	218	256	284	313	337	353	363	385	402	420	430
96348	N	99	162	204	261	306	338	378	400	421	435	452	477	490	479
96349	N	87	150	192	237	294	326	366	392	412	424	440	460	478	466*
96350	M	98	157	201	253	298	314*	354	384	404	417	440	460	478	466*
96351	M	87	126	158	185	214	236	254	271	286	288*	306	318	338	336
96352	M	92	154	202	257	315	340*	383	420	444	457	480	515	538	558
96353	M	93	137	171	200	231	255	288	306	316	310*	329	345	365	364
96354	M	100	160	201	253	296	322	359	375	400	413	430	437	448	436*
96355	N	87	140	181	223	265	286*	319	343	360	372	388	408	424	433
96356	M	87	138	181	220	256	280	310	329	341	344	358	373	386	400
96357	M	99	157	200	252	292	315*	355	384	399	405*	430	448	458	475
96358	M	76	119	160	200	242	278	299	319	332	333*	354	368	380	358*
96359	M	81	127	171	212	251	284	296	328	353	381	394	406	420	432*
96360	M	94	142	182	223	262	280*	322	340	356	368	385	400	412	424
96361	F	83	119	133	141	162	168	178	186	193	190	198	206	206	215*
96362	F	83	123	129	135	144	154	162	175	184	183	188	202	206	206
96363	F	95	125	146	154	170	178	186	198	206	Died				
96364	F	77	120	133	145	157	165	180	187	190	187*	197	207	209	228
96365	F	78	114	134	152	171	176	191	201	204	204	213	214	216	220*
96366	F	89	131	145	155	165	180	183	193	194	192*	211	210	214	216*
96367	F	91	126	139	149	164	166	177	184	194	192	200	210	210	220
96368	F	86	123	141	153	177	182*	200	211	223	220	226	237	241	244*
96369	F	91	125	142	159	173	180*	193	203	213	213	220	228	228	240
96370	F	84	134	156	169	187	186*	212	222	223	228	232	241	242	251
96371	F	85	136	152	175	192	200	217	226	228	223*	240	240	244	240*
96372	F	89	128	144	147	169	176	185	190	197	Died				
96373	F	86	124	139	155	170	176*	190	198	200	211	212	221	221	227
96374	F	77	112	130	140	144	154	169	171	180	178	187	198	200	199
96375	F	88	128	143	161	182	Died								

*Food withheld; not included in mean

TABLE 4. t-Test Comparison Between Means of Control and Treated Groups
Food Consumption, g/rat/day

Week of Study	Sex	0 ppm (Control)	100 ppm	500 ppm	2500 ppm
1-13	M	25.7	25.8	24.7	24.6
	F	18.2	18.3	18.5	16.4*

*Significantly different from control group mean, $p < 0.05$.

Chlorzoxazone Antacid, Test.

30-Day Subacute Toxicity Study in Rats

TABLE 5.

Mean Food and Compound Consumption

Week of Study	0 ppm (Control)			100 ppm			200 ppm			2500 ppm		
	Food		Compd	Food		Compd	Food		Compd	Food		Compd
	g/rat/day	g/kg/day		g/rat/day	g/kg/day		g/rat/day	g/kg/day		g/rat/day	g/kg/day	
MALES:												
1	17.5	112.7	17.6	117.4	12	17.6	120.4	60	13.9	111.8	280	
2	22.9	108.4	22.9	113.4	11	21.7						
3	21.5	95.0	23.7	95.3	9	23.5	122.5	56	21.5	117.0	292	
4	26.5	85.6	27.6	88.6	9	23.4	97.9	49	22.3	97.9	243	
5	24.9*	84.7	29.2*	84.5	8	27.8*	87.6	44	23.8	88.4	221	
6	25.9	67.6	24.3	65.3	7	24.5	86.8	43	26.4*	91.5	224	
7	27.9	69.0	28.9	71.9	7	24.5	70.7	35	25.9	72.9	182	
8	24.3	56.9	26.0	61.3	6	24.5	72.2	36	26.1	74.1	185	
9	29.9*	66.0	28.8*	65.3	6	26.2	66.5	33	26.6	77.5	194	
10	27.1	38.4	27.9	61.4	6	28.3*	67.9	34	26.6*	72.3	181	
11	26.1	53.7	27.1	56.0	6	26.1	62.1	31	26.9	67.9	170	
12	26.8	54.3	26.8	54.0	5	24.9	56.8	28	25.7	61.9	155	
13	24.3**	48.8	24.1**	47.4	5	25.4	56.2	28	26.1	60.6	152	
						23.3**	50.2	25	24.6**	35.9	140	
FEMALES:												
1	14.5	114.2	15.3	120.9	12	15.0	120.6	60	13.3	111.3	278	
2	17.7	115.4	16.7	120.4	12	16.5	122.7	61	17.2	122.8	307	
3	17.0	94.7	16.7	97.3	10	16.8	102.6	51	16.0	104.3	261	
4	17.3	89.2	18.7	93.9	9	17.7	93.5	47	15.9	94.8	237	
5	20.0*	96.4	19.7*	93.2	9	20.4*	100.8	50	17.1*	98.3	248	
6	17.4	77.9	16.2	72.2	7	17.0	78.1	39	14.0	74.9	187	
7	19.9	84.3	20.0	84.2	8	20.0	87.4	44	16.1	82.2	205	
8	18.0	73.9	18.6	76.2	8	19.2	81.1	41	16.8	82.9	232	
9	21.0*	83.0	20.7*	81.9	8	20.8*	86.1	43	17.9*	88.6	221	
10	17.8	70.0	19.0	74.3	7	19.2	77.4	39	17.0	81.2	203	
11	19.1	71.3	18.3	68.7	7	19.1	74.2	37	16.9	77.7	194	
12	19.4	70.1	19.9	72.3	7	19.5	73.7	37	17.1	77.7	194	
13	16.9**	59.5	16.2**	59.0	6	17.6**	65.3	33	15.0**	66.9	167	

*Food withheld 5 rats/sex/group; not included in mean.
 **Food and water withheld 3 rats/sex/group; not included in mean.

Chloroform Anhydride,
Tech. 90-Day Subacute Toxicity Study in Rats

TABLE 6. MALES: Means and Significance of Hematological Values

Hematology	Study Month	0 ppm (Control)	100 ppm	500 ppm	2500 ppm
Erythrocytes, $10^6/\text{cmm}$	1	6.47	6.47	6.30	6.38
	2	7.37	7.27	7.38	7.47
	3	7.69	7.30	7.43	7.13
Hemoglobin, g/100 ml	1	15.1	15.6	15.5	15.3
	2	17.0	16.4	16.0	15.9*
	3	17.3	16.9	16.7	16.3*
Hematocrit, %	1	47	48	48	47
	2	50	48	47	47*
	3	54	51	50*	50*
Leucocytes, $10^3/\text{cmm}$	1	12.34	11.56	12.16	13.39
	2	13.38	10.64	12.18	18.46**
	3	13.93	13.82	12.07	14.00
Neutrophils ^A , %	1	10	11	8	10
	2	13	14	9	11
	3	12	11	6	18
Lymphocytes ^B , %	1	88	88	91	86
	2	86	84	81	86
	3	87	87	94	82
Eosinophils ^B , %	1	0	0	1	1
	2	1	1	0	1
	3	1	2	0	0
Monocytes ^B , %	1	2	1	0	1
	2	0	1	4	2
	3	0	0	0	0
Basophils ^B , %	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0

*Significantly different from control group mean; $p < 0.05$

**Significantly different from control group mean; $0 < 0.01$

^ANo statistical analysis performed on this parameter.

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000027

90-Day Subacute Toxicity Study in Rats

TABLE 6. Cont. FEMALES: Means and Significance of Hematological Values

Hematology	Study Month	0 ppm (Control)	100 ppm	500 ppm	2500 ppm
Erythrocytes, 10^6 /cmm	1	6.38	6.33	6.82	6.90*
	2	6.83	7.12	7.13	7.11
	3	7.27	7.03	7.3*	7.41
Hemoglobin, g/100 ml	1	15.3	15.0	16.3	16.2
	2	15.9	16.3	16.3	15.6
	3	16.6	16.5	17.2	17.5
Hematocrit, %	1	46	46	49	49
	2	47	47	48	45
	3	51	50	52	52
Leucocytes, 10^3 /cmm	1	9.03	9.57	10.03	12.26
	2	11.22	9.36	11.87	15.92*
	3	11.42	10.94	9.81	12.25
Neutrophils ^a , %	1	16	8	10	7
	2	13	11	9	10
	3	13	10	8	8
Lymphocytes ^a , %	1	82	92	87	92
	2	86	87	88	86
	3	86	89	91	92
Eosinophils ^a , %	1	1	0	2	1
	2	1	1	1	1
	3	1	1	1	0
Monocytes ^a , %	1	1	0	2	0
	2	0	1	2	3
	3	0	0	0	0
Basophils ^a , %	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0

*Significantly different from control group mean: $p < 0.05$ ^aStatistical analysis performed on this parameter.

TABLE 1. Individual Hematology, Serum Values & 1 Month
 90 Day Evaluation Study in Pats

Group	Sex	Weight (kg)	ESR (mm/hr)	ESR (mm/hr) Appr.	Hemoglobin (g/100 ml)	Hematocrit (%)	Leucocytes (10 ³ /mm ³)	Neutrophils (Sup. Mem. %)	Lymphocytes (Sup. Mem. %)	Platelets (10 ³ /mm ³)					
0658	M	6.36		Normal	15.0	47	12.61	16	0	67	1	1	76	76	57
0659	M	7.10		Normal	15.1	48	13.71	11	0	95	1	1	68	74	43
0660	M	6.64		Normal	15.9	66	10.37	8	0	88	0	7	77	71	57
0661	F	6.13		Normal	15.5	68	13.36	9	0	91	0	0	78	73	57
0662	M	6.39		Normal	16.9	66	10.69	6	0	97	0	2	77	73	57
0663	M	6.61		Normal	15.1	67	12.36	10	0	88	0	7	74	73	57
0664	F	6.75		Normal	16.0	65	6.97	10	0	87	7	1	77	76	53
0665	F	6.36		Normal	15.6	66	9.95	19	0	78	1	0	73	72	56
0666	F	6.56		Normal	15.6	67	11.35	11	0	88	1	0	77	76	53
0667	F	6.37		Normal	15.3	66	7.67	18	0	80	1	1	86	76	56
0668	F	6.66		Normal	15.3	66	10.36	20	0	78	1	1	71	76	51
0669	F	6.38		Normal	15.3	66	9.93	16	0	87	1	1	77	76	53
0670	M	6.80		Normal	16.1	69	10.03	15	0	85	0	0	77	76	53
0671	M	6.51		Normal	15.9	69	16.33	16	0	84	0	0	75	76	52
0672	M	6.65		Normal	15.6	67	13.61	9	0	91	0	0	73	76	53
0673	M	6.19		Normal	15.5	68	9.58	6	1	89	0	6	75	76	53
0674	M	6.76		Normal	17.0	66	10.68	7	0	89	1	7	73	76	53
0675	F	6.67		Normal	15.6	68	11.66	11	0	88	0	1	76	76	51
0676	F	5.83		Normal	13.9	63	10.69	7	0	93	0	0	76	76	57
0677	F	7.02		Normal	16.7	69	8.66	7	0	93	0	0	73	73	53
0678	F	6.10		Normal	15.7	66	10.36	6	0	93	1	0	75	75	53
0679	F	6.15		Normal	16.8	65	7.86	13	0	86	0	1	71	71	53
0680	F	6.13		Normal	15.0	66	10.31	5	0	93	1	1	71	76	53
0681	F	6.13		Normal	15.0	66	9.77	8	0	97	0	0	71	76	53

*Spun off RA - Not applicable

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Chloroacetic Substrate Toxicity Study in Rats
TABLE 1. Cont.

Category	Sex	Weight (g)	Eye color (10/100 mm)	Eye color (400x)	Eye color (400x) app. or.	Blow pl. dia (g/100 ml)	Hematocrit (%)	Leucocyte count (10 ³ /mm ³)	Basophilic seg. (%)	Neutrophilic seg. (%)	Monocyte seg. (%)	Platelets (x 10 ⁹ /mm ³)			
5000 ppm:															
76320	M	6.76			Normal	14.8	66	13,710	0	0	0	0	0	0	0
76321	M	6.65			Normal	16.7	50	9,97	0	0	0	0	0	0	0
76322	M	6.88			Normal	16.0	50	8,03	0	0	0	0	0	0	0
76323	M	6.60			Normal	15.3	67	16,65	0	0	0	0	0	0	0
76324	M	6.37			Normal	15.6	62	16,52	0	0	0	0	0	0	0
76325	M	6.50			Normal	15.5	68	17,36	0	0	0	0	0	0	0
76326	F	7.17			Normal	17.1	52	9,56	0	0	0	0	0	0	0
76327	F	7.16			Normal	17.0	51	11,73	0	0	0	0	0	0	0
76328	F	6.56			Normal	15.2	66	11,07	0	0	0	0	0	0	0
76329	F	6.70			Normal	16.0	67	10,91	0	0	0	0	0	0	0
76330	F	6.80			Normal	16.7	69	6,98	0	0	0	0	0	0	0
Mean		6.87				16.3	69	10,05	0	0	0	0	0	0	0
7500 ppm:															
76331	M	6.58			Normal	15.6	67	17,53	0	0	0	0	0	0	0
76332	M	6.60			Normal	15.0	66	16,97	0	0	0	0	0	0	0
76333	M	6.18			Normal	16.7	66	11,69	0	0	0	0	0	0	0
76334	M	6.80			Normal	15.6	67	16,00	0	0	0	0	0	0	0
76335	M	6.96			Normal	15.9	68	17,07	0	0	0	0	0	0	0
76336	M	6.58			Normal	15.3	67	15,39	0	0	0	0	0	0	0
76337	F	6.91			Normal	17.1	51	12,31	0	0	0	0	0	0	0
76338	F	6.69			Normal	15.8	69	16,66	0	0	0	0	0	0	0
76339	F	6.86			Normal	15.9	69	8,52	0	0	0	0	0	0	0
76340	F	7.10			Normal	15.7	68	13,30	0	0	0	0	0	0	0
76341	F	6.98			Normal	16.5	69	10,69	0	0	0	0	0	0	0
76342	F	6.90			Normal	16.7	69	17,76	0	0	0	0	0	0	0

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000030

Chloroacetic Anhydride, 10.0%

TABLE 7.

NO. Dry Culture no. Toxicity Study in Rats

Intraperitoneal Hematological Values - 3 Months

Group	Sex	Age	Weight (g)	Hemoglobin (g/100 ml)	Mean corpuscular volume (cc)	Mean corpuscular hemoglobin (g/cc)	Mean corpuscular hemoglobin concentration (g/cc)	Red blood cell count (x 10 ⁶ /mm ³)	White blood cell count (x 10 ³ /mm ³)	Platelet count (x 10 ³ /mm ³)	Relative lymphocyte count (%)	Relative neutrophil count (%)	Relative monocyte count (%)	Relative eosinophil count (%)	Relative basophil count (%)	Relative platelet count (%)	Relative reticulocyte count (%)	Relative erythrocyte count (%)	Relative hemoglobin count (%)	Relative hematocrit count (%)			
Normal	F	7.67	14 Anison ^a	17.7	56	11.66	20.7	27	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	17.6	57	17.83	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	18.7	56	16.38	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			14 Anison ^a	16.7	55	16.68	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			14 Anison ^a	18.0	55	16.78	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			14 Anison ^a	17.5	56	13.93	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			14 Anison ^a	16.7	55	16.86	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			14 Anison ^a	16.9	53	13.65	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			14 Anison ^a	16.5	49	8.85	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			14 Anison ^a	16.3	49	8.86	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Normal	F	7.77	14 Anison ^a	17.3	57	11.17	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	16.4	51	11.67	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	16.7	49	11.65	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	17.6	57	17.75	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	17.1	55	16.68	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	16.3	47	7.85	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	16.8	51	8.35	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	16.9	51	13.87	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	15.2	47	9.56	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			14 Anison ^a	17.3	57	17.16	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Normal	F	7.84	14 Anison ^a	16.2	50	8.79	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	17.0	50	13.79	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	16.7	50	17.63	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	16.5	50	10.96	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	16.9	51	13.87	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	15.2	47	9.56	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	17.3	57	17.16	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	16.2	50	8.79	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	17.0	50	13.79	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			14 Anison ^a	16.7	50	17.63	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

^a Mice only

^b Mice only

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000033

Chlorendic Anhydride,
Tech. 90-Day Subacute Toxicity Study in Rats

TABLE 10. MALES: Means and Significance of Biochemical Values

Biochemistry	Study Month	0 ppm (Control)	100 ppm	500 ppm	2500 ppm
Glucose, mg/100 ml	1	109	126*	118	84**
	2	114	137	124	107
	3	132	137	126	125
B.U.N., mg/100 ml	1	14.7	14.2	16.6	16.3
	2	18.9	15.2	13.5	19.9
	3	15.2	15.0	12.5	15.8
Alkaline Phosphatase, int'l u/i	1	357	334	377	415
	2	196	225	179	223
	3	151	168	175	184
S.G.P.T., sigma u/ml	1	25	25	24	26
	2	29	20**	18**	20**
	3	30	29	23	22*
S.G.O.T., int'l u/l	1	234	232	192	277
	2	213	160	181	195
	3	206	152*	136*	235

*Significantly different from control group mean; $p < 0.05$

**Significantly different from control group mean; $p < 0.01$

90-Day Subacute Toxicity Study in Rats

TABLE 10. Cont. FEMALES: Means and Significance of Biochemical Values

Biochemistry	Study Month	0 ppm (Control)	100 ppm	500 ppm	2500 ppm
Glucose, mg/100 ml	1	129	138	127	92**
	2	140	134	133	104**
	3	127	142	136	106
B.U.N., mg/100 ml	1	18.2	19.8	16.2	24.4
	2	17.0	21.0	17.4	15.3
	3	17.0	16.4	15.6	21.6
Alkaline Phosphatase, int'l u/l	1	199	179	238	295
	2	114	115	193*	185*
	3	86	94	127	156*
S.G.P.T., sigma u/ml	1	23	20	19	25
	2	23	19	20	18
	3	37	26	21	26
S.G.O.T., int'l u/l	1	205	202	169	259**
	2	170	163	192	169
	3	199	158*	143**	176

*Significantly different from control group mean; $p < 0.05$
 **Significantly different from control group mean; $p < 0.01$

Chlorandi, Ashford, Tech.

90 Day Subacute Toxicity Study in Rats
 Individual Biochemical Values - 1 Month

TABLE II.

Group, Rat Number	Sex	Glucose mg./100 ml	BUN mg./100 ml	Alkaline Phosphatase Int'l U/ml	Urea mg./100 ml	Urea mg./100 ml
(0 ppm (Control)):						
9675R	M	111	15.7	465	73	179
96767	M	110	13.8	377	72	223
96763	M	114	15.0	445	76	290
96765	M	106	16.9	553	76	299
96768	M	102	16.1	757	77	266
Mean		109	16.7	357	75	185
96776	F	116	18.5	199	19	177
96775	F	117	25.2	199	17	191
96780	F	121	17.7	205	19	251
96781	F	161	17.0	176	15	290
96786	F	150	13.7	217	63	221
Mean		129	18.7	199	23	205
1000 ppm:						
96786	M	116	13.1	467	35	267
96795	M	128	16.1	775	73	288
96796	M	131	16.1	402	76	195
96797	M	128	12.1	333	19	229
96800	M	126	17.8	261	76	222
Mean		126	16.7	336	75	227
96803	F	149	20.2	195	29	189
96808	F	120	22.8	239	19	217
96810	F	150	18.8	115	17	171
96811	F	126	18.1	165	19	228
96816	F	137	19.1	181	15	196
Mean		138	19.8	179	20	202

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Chloroform Anhydride, Tech.

90-Day Subacute Toxicity Study in Rats

TABLE 11. Cont. Individual Biochemical Values - 1 Month

Group, Rat Number	Sex	Glucose mg/100 ml	BUN mg/100 ml	Alkaline Phosphatase Int'l U/l	SGPT (SGOT) U/ml	SGFP Int'l U/l
500 ppm:						
96320	M	124	22.0	366	79	174
96321	M	115	11.9	311	77	237
96325	M	106	15.2	474	75	197
96326	M	116	18.0	303	77	181
96327	M	128	15.1	431	77	177
Mean		118	16.6	377	74	197
96337	F	122	16.9	236	19	167
96334	F	127	16.3	189	19	160
96341	F	143	19.1	275	19	170
96342	F	124	16.3	274	19	179
96344	F	117	12.3	215	19	160
Mean		127	16.2	234	19	169
2500 ppm:						
96350	M	101	17.0	319	76	297
96352	M	90	18.1	462	28	288
96355	M	79	18.0	560	30	260
96357	M	74	16.2	261	19	270
96360	M	74	12.1	474	27	268
Mean		86	16.3	415	26	277
96368	F	92	51.6	354	73	236
96369	F	91	18.8	185	74	259
96370	F	86	17.6	387	29	243
96373	F	101	18.7	343	22	292
96375	F	88	15.5	207	27	263
Mean		92	24.4	295	25	259

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161-531

Chloroethyl Acrylate, Tech.

90 Day Subacute Toxicity Study In Pigs

Individual Biochemical Values - 2 Months

Group, Rat Number	Sex	Glucose mg/100 ml	BUN mg/100 ml	Alkaline Phosphatase Int'l U/l	Urea Nitrogen mg/dl	Creatinine Int'l U/l
0 ppm (Control):						
96260	M	110	13.1	177	23	169
96263	M	106	17.9	276	23	753
96265	M	111	12.2	273	37	249
96266	M	116	17.9	215	60	237
96268	M	111	67.5	166	78	176
Mean		116	18.9	196	79	253
96278	F	139	19.2	79	71	176
96281	F	17	18.9	106	15	141
96282	F	137	13.0	135	18	170
96286	F	166	15.8	166	62	196
96285	F	165	18.1	106	19	179
Mean		166	17.0	114	23	170
100 ppm:						
96287	M	139	16.0	237	71	162
96292	M	163	16.1	271	71	188
96296	M	120	16.8	196	16	179
96299	M	163	17.0	276	71	160
96300	M	118	16.1	199	71	171
Mean		137	16.2	225	70	160
96303	F	132	17.8	135	20	152
96306	F	136	26.2	199	15	158
96310	F	161	18.9	87	70	168
96312	F	136	22.0	70	18	163
96315	F	126	22.1	163	71	192
Mean		136	21.0	115	19	163

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161-513

Chelonioides, Aedes, di Eber, Toxic.
 TABLE 1. 7. Cont.
 90 Day Subacute Toxicity Study in Rats

Group, Sex	Spec	Clotting mg./100 ml	PHH mg./100 ml	Alkaline phosphatase int. u./l	serum protein g./100 ml	serum albumin g./100 ml
5000 ppm:						
96370	M	170	16.9	166	18	138
96371	M	176	13.9	176	19	179
96376	M	138	16.9	170	20	196
96379	M	177	12.0	179	16	196
96380	M	171	17.0	233	21	170
Mean		176	13.5	179	18	181
96386	F	179	17.1	237	25	196
96388	F	166	21.0	92	16	177
96390	F	136	16.1	203	17	189
96407	F	136	16.8	216	22	211
96363	F	171	16.2	206	17	209
Mean		173	17.6	193	20	192
7500 ppm:						
96366	M	180	26.6	253	16	158
96351	M	159	20.8	303	23	219
96353	M	136	17.2	195	23	203
96357	M	137	17.0	180	18	166
96358	M	106	19.9	186	18	209
Mean		137	19.9	223	20	195
96363	F	105	15.1	198	20	158
96364	F	116	19.6	200	16	172
96366	F	100	15.0	176	21	209
96371	F	110	12.9	185	18	190
96372	F	97	14.0	168	14	156
Mean		106	15.3	185	18	169

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163-511

Chironomid, AnhydroLab., Tech.

90 Day Subacute Toxicity Study in Rat
Individual Biochemical Values - 3 Months

TABLE II.

Group	Sex	Chlorure mg/100 ml	BUN mg/100 ml	Alkaline phosphatase int'l u/l	SALT mg/m ² /m	SALT int'l u/l
0 ppm (Control):						
96251	M	136	13.9	135	38	730
96258	M	126	16.7	156	35	169
96260	M	119	15.1	162	30	169
96261	M	166	17.0	156	78	739
96265	M	115	15.8	165	71	722
Mean		132	15.7	151	30	706
96277	F	128	17.0	111	31	388
96280	F	121	15.7	89	76	170
96281	F	133	16.7	60	50	208
96286	F	135	16.7	85	62	261
96285	F	170	21.2	85	77	388
Mean		127	17.0	86	37	199
100 ppm:						
96286	M	130	14.9	160	72	117
96289	M	135	16.8	216	31	168
96290	M	136	17.1	166	29	169
96296	M	160	13.2	169	30	179
96303	M	166	15.1	129	36	183
Mean		137	15.0	168	29	152
96302	F	160	15.8	51	77	167
96305	F	119	14.9	122	78	366
96306	F	167	18.6	96	23	152
96307	F	150	16.0	136	77	162
96310	F	136	17.0	66	27	169
Mean		162	16.4	96	76	158

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167-511

Chloroacetic Anhydride, Tech.

90-Day Subcutaneous Toxicity Study in Rats
Individual Pharmacokinetic Values - 3 Months

Group, Rat Number	Sex	Glucose mg/100 ml	BUN mg/100 ml	Alkaline Phosphatase int'l u/l	Serum Cholesterol mg/dl	Serum Triglyceride mg/dl
5000 ppm:						
96116	M	119	11.1	148	77	171
96117	M	175	13.0	231	18	97
96119	M	139	16.2	165	25	171
96121	M	175	17.2	161	22	168
96130	M	171	10.1	100	77	160
Mean		176	17.5	175	73	166
96135	F	169	16.9	91	19	166
96136	F	176	18.0	205	26	152
96161	F	136	13.9	135	16	136
96163	F	166	15.0	131	18	171
96166	F	179	16.1	91	18	154
Mean		136	15.6	177	23	138
2500 ppm:						
96156	M	115	17.0	210	17	160
96150	M	106	16.7	157	21	181
96154	M	111	19.1	180	71	218
96158	M	131	15.0	186	28	437
96159	M	166	13.8	196	21	200
Mean		175	15.8	186	27	235
96161	F	78	46.9	178	26	191
96165	F	116	15.1	113	78	136
96166	F	99	15.0	196	25	209
96168	F	112	18.1	169	29	181
96171	F	170	13.1	175	26	167
Mean		106	21.6	156	26	176

161-511

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Chlorendic Anhydride,
Tech. 90-Day Subacute Toxicity Study in Rats

TABLE 14. MALES: Means and Significance of Urinalysis Values

Urinalysis	Study Month	0 ppm (Control)	100 ppm	500 ppm	2500 ppm
Volume, ml	1	5.0	6.2	5.8	6.9
	2	4.6	5.8	4.2	5.4
	3	3.4	3.4	4.4	4.0
pH	1	6.7	6.8	7.0	6.3
	2	6.6	7.0	7.3	7.2
	3	6.8	6.6	6.6	6.3
Specific Gravity	1	1.051	1.043	1.031**	1.031**
	2	1.048	1.045	1.041	1.043
	3	1.055	1.062	1.054	1.064

*Significantly different from control group mean; $p < 0.05$
 **Significantly different from control group mean; $p < 0.01$

90-Day Subacute Toxicity Study in Rats

TABLE 14. Cont. FEMALES: Means and Significance of Urinalysis Values

Urinalysis	Study Month	0 ppm (Control)	100 ppm	500 ppm	2500 ppm
Volume, ml	1	4.2	4.1	3.9	4.8
	2	3.5	1.8	3.3	2.5
	3	0.5	1.6	1.7	2.0
pH	1	6.8	6.8	6.5	6.8
	2	6.4	6.6	6.9	7.3
	3	6.6	6.5	6.2	7.2
Specific Gravity	1	1.040	1.041	1.036	1.032
	2	1.041	1.059*	1.044	1.041
	3	1.088	1.064	1.069	1.056*

*Significantly different from control group mean; $p < 0.05$

Chlorinated Biphenyls, Tech.

90 Day Subacute Toxicity Study in Rats
Individual Hematology Values - 1 Month

TABLE 1. Cont.

Group Rat Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Albu- min	Glu- cose	Bill. rubin	Occult Blood	Leuco- cytes	Plate- lets	Red Cells	White Cells	Triple Plate.	Calc. Coef.	Wbc Rate
5000 ppm:																
96170	M	6.0	LS-C	6.8	1.078	N	N	N	N	-	-	occ	-	P	-	M
96171	M	7.0	LS-C	6.8	1.080	N	N	N	N	-	-	occ	-	P	-	M
96175	M	6.0	LS-C	6.8	1.035	N	N	N	N	-	-	-	-	P	-	F
96176	M	6.5	LS-c1	7.8	1.077	N	N	N	Tr*	-	-	-	-	P	-	M
96177	M	5.5	LS-C	6.8	1.035	N	N	N	N	-	-	-	-	P	-	M
96137	F	3.0	LS-C	6.7	1.038	N	N	N	N	-	-	-	-	P	-	M
96134	F	7.5	LS-C	7.0	1.060	N	N	N	Tr*	-	-	-	-	P	-	F
96143	F	5.0	LS-C	7.3	1.033	N	N	N	N	-	-	-	-	P	-	M
96157	F	3.0	LS-C	6.0	1.060	N	N	N	N	-	-	-	-	P	-	M
96166	F	6.0	LS-C	6.0	1.077	N	N	N	N	-	-	occ	-	P	-	F
2500 ppm:																
96150	M	6.0	LS-c1	6.6	1.033	N	N	N	N	-	-	-	-	P	-	M
96157	M	9.5	LS-C	6.0	1.077	N	N	N	N	-	-	-	-	P	-	M
96155	M	8.5	LS-C	6.6	1.077	N	N	N	N	-	-	-	-	P	-	M
96157	M	5.5	LS-C	6.3	1.033	N	N	N	N	-	-	-	-	P	-	M
96160	F	5.0	LS-C	6.0	1.036	N	N	N	N	-	-	occ	-	P	-	M
96168	F	3.0	LS-C	6.0	1.038	N	N	N	N	-	-	occ	-	P	-	F
96169	F	5.0	LS-C	6.5	1.077	N	N	N	N	-	-	occ	-	P	-	F
96310	F	6.0	LS-C	6.5	1.036	N	N	N	N	-	-	-	-	P	-	M
96373	F	6.0	LS-C	9.0	1.037	N	N	N	N	-	-	-	-	P	-	F
96375	F	8.0	LS-c1	6.7	1.077	N	N	N	N	-	-	occ	-	P	-	M

161 511
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 14 - Trace
 16 - Trace to slight
 74 - Slight to moderate
 36 - Moderate
 44 - Marked
 8 - Severe
 10 - Slight Strain
 100 - Dark Strain
 41 - cloudy
 C - Clear
 N - Normal
 P - Few
 L - Leukoid
 M - Murky
 R - Rare
 10M - Dark Amber
 10M - Light Amber
 occ - Occasional
 98C - Slightly not sufficient
 10M - Normal serum

Chloroform: Acetone 10:1, Tech.

70 Day Subacute Toxicity Study in Rats

TABLE 16.

Individual Analysis Values - 7 Months

Group	Rat Number	Sex	Volume ml	Color and Appearance	pH	Spec. Grav.	Alloy. in case	Clk. in case	RBCs	Hct	Hemoglobin	Occult Blood	Leucocytes	Platelets	Triple Phase	Col. Ex.	Base Acid	Base	
10 ppm (Control):	96760	M	5.5	LS-C	6.8	1.047	N	N	N	N	74	occ	occ	F	-	-	-	M	
	96763	M	5.0	LS-C	6.5	1.066	N	N	N	N	N	N	occ	F	F	-	-	-	M
	96765	M	6.5	LS-C	6.5	1.060	N	N	N	N	N	N	-	F	F	-	-	-	F
	96766	M	3.0	LS-C	6.5	1.074	N	N	N	N	N	N	-	F	F	-	-	-	M
	96768	M	3.0	LS-C	6.5	1.050	N	N	N	N	N	N	-	F	F	-	-	-	M
	96778	F	5.0	LS-C	6.5	1.035	N	N	N	N	N	N	occ	occ	M	-	-	-	M
	96781	F	6.0	LS-C	6.5	1.038	N	N	N	N	N	N	occ	occ	M	-	-	-	M
	96787	F	3.5	LS-C	6.0	1.069	N	N	N	N	N	1+	-	-	occ	-	-	-	F
	96786	F	3.0	LS-C	6.8	1.070	1+	N	N	N	N	2+	-	-	F	-	-	-	F
	96785	F	7.0	LS-C	6.0	1.067	N	N	N	N	N	N	-	-	F	-	-	-	F
100 ppm:	96787	M	7.0	LS-C	8.5	1.032	N	N	N	N	N	N	-	F	F	-	-	-	M
	96797	M	7.0	LS-C	6.5	1.037	N	N	N	N	N	N	-	F	F	-	-	-	M
	96796	M	5.5	LS-C	6.8	1.066	N	N	N	N	1+	N	-	F	F	-	-	-	M
	96799	M	3.0	LS-C	6.9	1.070	N	N	N	N	N	N	-	F	F	-	-	-	M
	96800	M	6.5	LS-C	6.5	1.038	N	N	N	N	N	N	-	F	F	-	-	-	M
	96803	F	2.5	S-C	6.5	1.066	N	N	N	N	N	N	-	F	F	-	-	-	M
	96806	F	0.3	LS-C	6.5	1.050	N	N	N	N	N	N	-	F	F	-	-	-	M
	96810	F	2.0	LS-C	6.0	1.076	N	N	N	N	N	N	-	occ	occ	-	-	-	M
	96817	F	1.0	LS-C	6.0	1.070	N	N	N	N	N	N	-	-	-	-	-	-	F
	96835	F	3.0	S-C	8.0	1.037	N	N	N	N	N	N	-	occ	occ	-	-	-	F

Codes: 1+ - Trace
 1+ - Trace to slight
 2+ - Slight to moderate
 3+ - Moderate
 4+ - Marked
 S - Strong
 LS - Light Strong
 MS - Dark Strong
 C1 - Cloudy
 C - Cloudy
 N - Normal
 F - Few
 1 - Isolated
 M - Many
 R - Rare
 Diff - Diff. Ambry
 1.0m - 1.1m Ambry
 occ - Occasional
 abs - Absent
 PMS - Quantity not sufficient
 - - - - - None seen

103-111

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Cholesterol, Anticholesterol, Test.

TABLE 16. Cont.

70-Day Subacute Toxicity Study in Rats
Individual Hemolytic Values - 7 Months

Group Rat Number	Sex	Volume ml	Color and Appearance	pH	Spec. Grav.	Albu. min conc	Glu. conc	Bili. mg/dl	Occult Blood	Leucocytes	Erythrocytes	Erythrocyte Count	Hemoglobin	Hematocrit	Col. Coeff.	Viscosity	Red Cell Count	Red Cell Volume
700 ppm:																		
96370	M	6.0	LS-C	7.8	1.037	N	N	N	N	-	occ	-	P	P	-	-	-	M
96373	M	3.0	LS-C	6.5	1.030	N	N	N	N	-	1-3	-	P	P	-	-	-	M
96376	M	6.5	DS-c1	9.0	1.037	N	N	N	tr	-	occ	-	P	P	-	-	-	F
96379	M	6.0	LS-C	6.5	1.067	N	N	N	N	-	-	-	P	P	-	-	-	M
96380	M	3.5	LS-C	6.5	1.066	N	N	N	N	-	-	-	P	P	-	-	-	M
96386	F	5.5	LS-C	6.5	1.036	N	N	N	N	-	-	-	P	P	-	-	-	M
96388	F	3.0	LS-C	6.7	1.060	N	N	N	N	-	-	-	P	P	-	-	-	M
96390	F	7.0	LS-C	6.0	1.060	N	N	N	N	-	-	-	P	P	-	-	-	M
96397	F	1.5	DS-c1	9.0	1.066	N	N	N	tr	-	-	-	P	P	-	-	-	F
96393	F	6.	LS-C	7.0	1.036	N	N	N	N	-	-	-	P	P	-	-	-	M
7500 ppm:																		
96366	M	7.0	LS-C	8.0	1.036	N	N	N	N	-	-	-	P	P	-	-	-	M
96351	M	5.5	LS-C	7.5	1.033	N	N	N	tr	-	-	-	P	P	-	-	-	M
96353	M	8.5	LS-C	7.0	1.031	N	N	N	N	-	-	-	P	P	-	-	-	M
96357	M	6.0	LS-c1	7.0	1.066	N	N	N	N	-	-	-	P	P	-	-	-	M
96358	M	7.0	LS-C	6.5	1.076	N	N	N	N	-	-	-	P	P	-	-	-	M
96363	F	3.0	LS-C	6.5	1.068	N	N	N	N	-	-	-	P	P	-	-	-	F
96364	F	1.0	DS-c1	9.0	1.066	N	N	N	2+	-	8-10	-	P	P	-	-	-	F
96366	F	6.0	LS-C	8.0	1.037	N	N	N	N	-	-	-	P	P	-	-	-	M
96371	F	3.0	LS-C	6.5	1.038	N	N	N	N	-	-	-	P	P	-	-	-	M
96377	F	1.5	LS-C	6.5	1.062	N	N	N	N	-	-	-	P	P	-	-	-	F

Codes:
 tr - Trace
 1+ - Trace to slight
 2+ - Slight to moderate
 H - Hemolytic
 44 - Masked
 S - Slight
 LS - Light Stroma
 DS - Dark Stroma
 cl - Cloudy
 C - Clear
 R - Regenerative
 P - Few
 I - Isolated
 M - Many
 N - None
 Wm - Dark Anisocytosis
 Lm - Light Anisocytosis
 occ - Occasional
 abs - Absent
 Quantify red cells

000048

Cholesterol Anhydride, Tech.

90 Day Subacute Toxicity Study in Rats

TABLE 17. Cont.

Individual Hematology Values - 3 Months

Group, Rat Number	Sex	Volume, ml	Color and Appear.	pH	Spec. Grav.	Albu- min conc	Glo- bulin conc	Hemoglobin	Leuco- cytes	Plate- letes	Pro- tein	WBC	Acid Phos- phatase	Bar- ium
500 ppm:														
96316	M	4.0	LS-C	7.3	1.064	N	N	N	14	-	-	-	-	M
96317	M	4.5	LS-C	6.5	1.060	N	N	N	occ	occ	occ	occ	occ	F
96319	M	8.0	LS-C	6.5	1.060	N	N	N	-	-	-	-	-	F
96321	M	4.0	LS-C	6.5	1.065	N	N	N	-	-	-	-	-	F
96320	M	1.5	LS-C	6.0	1.080	N	N	N	occ	occ	occ	occ	occ	F
96315	F	0.5	LS-C	6.5	1.080	N	N	N	-	-	-	-	-	F
96316	F	1.5	LS-C	6.0	1.080	N	N	N	-	-	-	-	-	F
96311	F	1.0	LS-C	6.0	1.080	N	N	N	-	-	-	-	-	F
96313	F	1.0	LS-C	6.5	1.070	N	N	N	occ	occ	occ	occ	occ	M
96314	F	4.5	LS-C	6.0	1.077	N	N	N	-	-	-	-	-	F
7500 ppm:														
96316	M	5.0	LS-C	6.5	1.065	N	N	N	-	-	-	-	-	M
96350	M	2.0	LS-C	6.0	1.080	N	N	N	-	-	-	-	-	F
96354	M	4.0	LS-C	6.5	1.050	N	N	N	-	-	-	-	-	F
96358	M	6.0	LS-C	6.5	1.080	N	N	N	occ	occ	occ	occ	occ	F
96359	M	3.0	LS-C	6.0	1.066	N	N	N	-	-	-	-	-	F
96361	F	1.5	LS-C	6.5	1.070	N	N	N	-	-	-	-	-	F
96365	F	4.0	LS-C	6.5	1.037	N	N	N	-	-	-	-	-	F
96366	F	0.5	S-c1	9.0	1.056	N	N	N	-	-	-	-	-	F
96368	F	2.0	S-c1	8.0	1.050	N	N	N	1-3	occ	occ	occ	occ	M
96371	F	2.0	LS-C	6.0	1.068	N	N	N	occ	occ	occ	occ	occ	F

Code: tr - Trace
 14 - Trace to slight
 24 - Slight to moderate
 34 - Moderate
 44 - Marked
 S - Severe
 1S - Light Severe
 1S - Dark Severe
 c1 - Cloudy
 C - Clear
 N - Negative
 P - Few
 1 - Loaded
 M - Many
 R - Rare
 DM - Dark Amber
 1Am - Light Amber
 occ - Occasional
 ORS - Quantitatively not sufficient
 - - None seen

000050
 161-533

Culturable Antibiotic Tech.

90 Day Subacute Toxicity Study In Rats

TABLE 18.

Necropsy Observations, Terminal Sacrifices,
Deaths and Unscheduled Sacrifices

Site Location	0 ppm (Control)		100 ppm		500 ppm		2500 ppm		0 ppm (Control)		Deaths and Unscheduled Sacrifices		2500 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number necropsied	15	15	15	15	15	15	12	12	0	0	0	0	0	0
No gross lesions	3	0	2	2	3	2	4	1	0	0	0	0	0	0
External alopecia			1			2		1						
Tip of tail scabbed														
Eyes														
Infernal eye white/cataract				1	1			1						
corneal opacity/eye cloudy														
area around eye red/red														
material around eye	2													
excess lacrimation	1			1										
eye small														
conjunctivitis	1					2								
Thymus														
dark red foci	1		2	1	1									
dark red areas/congestion			1		1			3						
Lungs														
dark red foci	6		5	1				1						
yellow/gray/white foci	2	7	5	3				6						
congestion/dark red areas	4	13	6	10	9	9	4	8						
yellow area														
apoptoly					1									
Stomach														
dark red foci, glandular mucosa													1	
Small intestine														
dark red contents													1	
wall red														1
Pancreas														
nodules														1

163-533

000051

TABLE 19. Absolute (Grams) and Relative (% Body Weight) Organ Weights, Terminal Sacrifice

Group Sex	Body Wt. g	Spleen		Liver		Kidneys		Testes/ Ovaries		Heart	
		g	%	g	%	g	%	mg	%	g	%
<u>0 ppm (Control):</u>											
M	500	0.84	0.17	23.11	4.63	4.17	0.84	3.75	0.75	1.66	0.33
F	269	0.59	0.22	17.75	4.74	2.45	0.91	133	0.49	1.04	0.39
<u>100 ppm:</u>											
M	492	0.86	0.17	19.65**	3.98**	4.02	0.82	3.69	0.75	1.54*	0.31
F	266	0.59	0.22	11.22**	4.22**	2.31	0.87	134	0.51	1.00	0.38
<u>500 ppm:</u>											
M	452	0.80	0.18	18.82**	4.15**	3.96	0.87	3.63	0.81*	1.54*	0.34
F	258	0.56	0.22	10.63**	4.13**	2.30	0.89	133	0.52	0.98*	0.38
<u>2500 ppm:</u>											
M	426	0.75	0.18	17.74**	4.14**	3.73*	0.88	3.62	0.86**	1.46*	0.34
F	217	0.46*	0.21	9.35**	4.32**	2.03**	0.94	108**	0.49	0.83**	0.38

Group mean relative organ weights shown in this table were calculated by averaging the individually calculated relative organ weights.

*Significantly different from Control group mean, $P < 0.05$.

**Significantly different from Control group mean, $P < 0.01$.

TABLE 19. Cont. Absolute (Grams) and Relative (% Body Weight) Organ Weights, Terminal Sacrifice

Group Sex	Body Wt. g	Brain		Adrenals		Pituitary		Thyroid	
		g	%	mg	%x10 ²	mg	%x10 ²	mg	%x10 ²
<u>0 ppm (Control):</u>									
M	500	2.17	0.44	71	1.41	14	0.29	26	0.53
F	269	1.98	0.74	82	3.06	25	0.91	20	0.73
<u>100 ppm:</u>									
M	492	2.10	0.43	66	1.35	15	0.30	25	0.50
F	266	1.95	0.74	75	2.84	15	0.55	20	0.76
<u>500 ppm:</u>									
M	452	2.04	0.45	60**	1.33	14	0.30	24	0.53
F	258	1.97	0.76	77	2.97	15	0.57	18	0.70
<u>2500 ppm:</u>									
M	426	2.13	0.51**	58**	1.37	14	0.32	23	0.55
F	217	1.98	0.92**	64**	2.96	13	0.58	16**	0.72

Group mean relative organ weights shown in this table were calculated by averaging the individually calculated relative organ weights.

*Significantly different from Control group mean, $P < 0.05$.

**Significantly different from Control group mean, $P < 0.01$.

C. brendis Anhydride, Tech. 1

90-Day Subacute Toxicity Study in Rats.

TABLE 20.

Individual Organ Weights, Terminal Sacrifice.

Group, Rat Number	Sex	Body Wt. g	Spleen g	Liver g	Kidneys g	Testes/ Ovaries		Heart g	Thymus g	Adrenals mg	Pituitary mg	Thyroid/ Parathyroid mg
						g	mg					
[rats (Control):												
96254	M	474	0.91	20.19	3.87	3.71	1.36	2.17	59	16		
96257	M	346	0.92	21.36	4.57	3.64	1.69	2.24	80	13		22
96258	M	323	0.79	22.76	4.19	4.22	1.79	1.98	80	11		24
96259	M	321	0.73	23.88	4.48	3.72	1.69	2.07	75	14		33
96260	M	414	0.99	18.76	3.88	3.57	1.37	2.17	75	14		24
96261	M	374	0.78	28.18	4.76	3.90	1.73	2.21	65	13		30
96262	M	487	0.59	23.24	3.65	3.60	1.49	2.29	72	18		24
96263	M	302	0.87	23.11	4.11	3.82	1.67	2.28	83	11		27
96264	M	502	0.84	23.82	3.99	3.78	1.80	2.08	67	14		27
96265	M	500	0.96	23.22	4.09	4.11	1.68	2.17	81	13		26
96266	M	324	0.86	23.28	4.23	3.48	1.78	2.18	71	8		28
96267	M	497	0.80	23.57	4.43	3.78	1.58	2.06	68	22		28
96268	M	420	0.86	20.74	4.43	3.35	1.48	2.13	47	13		28
96269	M	317	0.98	23.87	3.86	3.93	1.91	2.26	66	16		28
96270	M	469	0.77	24.64	3.92	3.59	1.71	2.22	75	15		24
96271	F	281	0.60	13.16	2.33	159	1.06	2.15	88	18		22
96272	F	265	0.55	12.21	2.33	153	0.98	1.98	83	11		22
96273	F	251	0.46	11.74	2.69	121	1.00	1.91	78	14		13
96274	F	287	0.51	13.23	2.34	155	1.00	1.94	75	17		19
96275	F	300	0.46	12.17	2.36	164	1.06	1.97	104	18		24
96276	F	298	0.53	15.06	2.49	173	1.06	1.44	67	13		22
96277	F	242	0.84	10.65	2.11	121	0.98	1.92	68	14		15
96278	F	256	0.49	12.53	2.45	143	1.07	1.98	88	13		23
96279	F	278	0.43	13.59	2.41	145	1.09	1.99	81	15		22
96280	F	247	0.75	12.51	2.40	91	1.02	1.97	90	16		12
96281	F	272	0.52	11.62	2.54	126	1.00	2.01	71	16		20
96282	F	274	0.34	12.74	2.52	109	1.04	2.13	91	17		22
96283	F	288	0.77	15.08	2.57	140	1.24	1.96	86	14		24
96284	F	258	0.85	13.77	2.84	66	1.13	1.94	82	18		13
96285	F	240	0.57	11.24	1.99	127	0.90	1.88	75	15		21

Chloroform Anhydride, Tech. 1

90-Day Subacute Toxicity Study in Rats.

Table 20. Cont.

Individual Organ Weights, Terminal Sacrifice.

Group, Rat Number	Sex	Body Wt. g	Testes								
			Spleen g	Liver g	Kidneys g	Ovaries mg	Heart g	Brain g	Adrenals mg	Pituitary mg	Thyroid/ Parathyroid mg
100 ppm:											
96286	M	458	0.86	17.34	3.20	3.13	1.35	2.05	70	11	24
96287	M	498	0.97	20.64	3.90	3.78	1.72	2.25	76	14	26
96288	M	531	0.94	24.51	4.74	3.61	1.56	2.23	78	17	33
96289	M	496	0.83	19.69	3.86	3.97	1.70	2.30	67	14	25
96290	M	496	0.78	18.43	3.75	4.00	1.71	2.21	74	13	24
96291	M	451	0.63	16.30	3.43	3.66	1.24	2.11	50	14	24
96292	M	545	0.85	21.14	4.34	3.54	1.43	2.38	72	18	23
96293	M	483	0.58	18.21	4.18	4.20	1.43	2.01	63	15	22
96294	M	470	0.77	19.33	4.01	3.76	1.58	2.15	55	12	23
96295	M	516	0.84	22.95	4.22	3.67	1.58	2.10	60	19	26
96296	M	486	1.32	28.86	4.17	3.54	1.43	2.17	63	16	22
96297	M	549	0.88	19.74	4.22	3.52	1.54	2.15	28*	16	22
96298	M	446	0.89	20.75	4.47	3.44	1.66	1.98	72	15	21
96299	M	457	0.75	16.97	3.63	3.86	1.67	1.39	61	14	21
96300	M	-	0.29	20.10	4.46	3.75	1.93	1.63	80	15	20
96301	F	305	0.53	11.54	2.79	163	0.99	1.94	74	18	18
96302	F	297	0.67	13.02	2.74	154	1.10	1.91	84	14	24
96303	F	306	0.65	12.98	2.62	146	1.09	2.04	75	17	22
96304	F	280	0.60	12.35	2.55	114	0.99	1.81	77	17	24
96305	F	260	1.01	11.73	2.35	146	1.05	1.98	89	11	23
96306	F	241	0.55	8.62	2.16	105	0.78	1.92	78	14	22
96307	F	290	0.75	12.43	2.32	137	1.09	2.07	75	15	19
96308	F	253	0.34	10.26	2.21	127	0.91	1.83	60	14	19
96309	F	229	0.53	9.75	2.15	129	0.93	1.97	62	15	19
96310	F	255	0.60	11.48	2.17	106	1.01	2.02	64	13	17
96311	F	262	0.63	11.15	2.15	165	1.04	2.04	79	15	21
96312	F	242	0.53	10.69	1.87	115	0.94	1.77	62	-	18
96313	F	266	0.49	11.66	2.13	157	1.22	1.99	84	13	22
96314	F	253	0.55	9.60	2.01	128	1.05	1.95	78	14	19
96315	F	235	0.55	11.06	2.36	123	0.96	2.02	86	15	14

*Weight of one organ only; not included in statistics.
- Data not available.

165-553

000056

Chlorendic Anhydride, Tech.:

90-Day Subacute Toxicity Study in Rats.

TABLE 20. Cont.

Individual Organ Weights, Terminal Sacrifice.

Group, Rat Number	Sex	Body Wt. g	Spleen g	Liver g	Kidneys g	Testes/ & Ovaries mg	Heart g	Brain g	Adrenals mg	Pituitary mg	Thyroid/ Parathyroid mg
500 ppm:											
96316	M	454	0.68	20.15	4.01	3.48	1.62	2.29	62	13	30
96317	M	461	0.81	18.30	3.90	4.33	1.45	2.11	62	12	19
96318	M	506	0.69	21.91	4.62	3.58	1.69	2.20	63	16	20
96319	M	416	0.74	16.04	3.53	3.26	1.41	2.08	52	13	27
96320	M	392	0.69	16.13	3.23	3.46	1.35	1.92	50	12	19
96321	M	461	1.16	17.58	3.74	3.60	1.62	2.27	70	14	24
96322	M	486	0.95	19.10	4.66	3.62	1.70	2.27	69	15	25
96323	M	478	0.72	20.78	3.99	3.61	1.67	2.16	56	16	23
96324	M	478	0.77	20.32	3.66	3.65	1.63	0.64	55	12	22
96325	M	469	0.66	17.24	3.72	3.72	1.45	2.14	63	-	27
96326	M	417	0.76	17.78	3.92	3.21	1.48	1.99	47	-	27
96327	M	448	1.08	17.92	3.70	3.53	1.53	2.20	63	13	18
96328	M	476	0.81	23.92	5.24	3.91	1.56	2.30	63	13	27
96329	M	368	0.62	13.63	3.08	3.59	1.46	1.98	47	10	24
96330	M	466	0.74	21.49	4.38	3.93	1.49	2.04	81	15	16
96331	F	263	0.56	10.81	1.97	135	0.96	1.89	70	14	21
96332	F	258	0.42	10.11	2.36	135	0.97	1.96	81	15	20
96333	F	244	0.54	10.69	2.47	120	0.96	2.11	46	15	16
96334	F	242	0.63	10.73	2.30	128	0.96	2.03	73	16	23
96335	F	256	0.73	10.79	2.20	139	0.90	1.90	84	14	16
96336	F	245	0.64	8.62	1.91	116	1.02	1.97	84	14	16
96337	F	272	0.42	11.08	2.47	124	0.98	2.03	76	13	17
96338	F	247	0.41	11.38	2.18	152	1.01	1.63	81	14	19
96339	F	301	0.37	11.66	2.73	109	1.09	2.00	78	15	16
96340	F	252	0.43	11.99	2.38	141	1.04	1.99	80	13	18
96341	F	263	0.75	10.60	2.47	165	0.99	2.00	70	19	16
96342	F	258	0.53	9.78	2.01	134	1.00	1.99	83	11	15
96343	F	250	0.55	10.03	2.32	142	1.00	1.98	77	14	20
96344	F	259	0.71	10.60	2.66	134	1.01	2.05	87	17	18
96345	F	257	0.58	10.53	2.09	117	0.85	1.95	78	15	18

- Not available

Chloroform Anhydride, Test 11 90-Day Subacute Toxicity Study in Rats.

TABLE 20. Cont. Individual Organ Weights, Terminal Sacrifice.

Group, Rat Number	Sex	Body Wt. g	Testes								
			Spleen g	Liver g	Kidneys g	Ovaries mg	Heart g	Brain g	Adrenals mg	Pituitary mg	Thyroid/ Parathyroid mg
2500 ppm:											
96344	M	421	0.75	17.65	3.70	3.37	1.32	2.19	58	13	20
96345	M	419	0.74	20.39	4.12	3.57	1.38	1.99	62	14	22
96346	M	476	0.83	20.46	4.27	3.80	1.62	2.32	52	13	23
96348	M	486	0.77	20.86	4.13	3.85	1.63	2.18	55	16	24
96350	M	461	0.78	18.10	3.82	3.53	1.47	2.17	57	13	24
96351	M	327	0.39	11.31	2.71	2.72	1.11	2.02	47	11	22
96352	M	548	0.96	24.53	4.32	3.52	1.99	2.09	71	17	23
96353	M	353	0.68	14.44	2.81	3.59	1.16	2.11	59	12	18
96354	M	429	0.67	17.22	3.79	4.06	1.93	2.11	62	13	20
96355	M	422	0.81	20.83	3.79	3.29	1.17	1.93	53	13	21
96356	M	367	0.70	15.14	3.39	3.40	1.17	2.18	58	12	22
96357	M	464	1.02	17.67	3.94	4.57	1.71	2.20	58	17	27
96358	M	350	0.64	12.29	3.17	3.71	1.32	2.07	44	11	20
96359	M	421	0.84	17.71	4.10	3.45	1.52	2.10	64	14	22
96360	M	410	0.70	17.48	3.94	3.53	1.42	2.22	63	13	23
96361	F	201	0.42	9.08	2.07	101	0.80	2.06	63	11	18
96362	F	199	0.33	8.31	2.07	79	0.83	2.04	56	14	12
96364	F	214	0.43	10.48	1.98	101	0.83	2.00	62	11	13
96365	F	211	0.47	8.44	1.86	120	0.78	1.89	56	14	9
96366	F	206	0.44	9.47	1.84	118	0.84	2.05	63	12	16
96367	F	210	0.51	8.97	1.75	106	0.82	1.98	58	13	14
96368	F	235	0.62	8.99	1.97	134	0.85	2.05	77	13	19
96369	F	229	0.46	9.13	2.18	123	0.80	1.87	49	11	20
96370	F	244	0.37	11.54	2.35	112	0.87	2.00	64	12	14
96371	F	236	0.67	9.26	2.20	123	0.92	2.03	90	14	16
96373	F	223	0.35	9.75	2.24	100	0.79	1.91	70	12	22
96374	F	194	0.39	8.75	1.85	74	0.80	1.92	63	13	14

Gibberellic Anhydride, Tech. 1
 90-Day Subacute Toxicity Study in Rats.
 TABLE 21
 Histomorphological Observations.

Tissue	0 ppm (Control)										7500 ppm									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Fluorescence																				
Liver																				
Brain																				
Spinal cord																				
Peripheral nerve (sciatic)																				
Eye																				
Characteristical atrophy and degeneration of cartilage/mineralized foci, bone																				
Pituitary cyst(s)																				
Thyroids																				
Adrenals																				
Lung/Bronchi																				
perivascular lymphoid infiltrate																				
interstitial congestion																				
interstitial inflammatory cell infiltrate																				
mineralized foci, pulmonary artery																				
Heart																				
degeneration, myocardial fibers																				
Aorta																				
Spleen																				
extramedullary hematopoiesis																				
Spleen (bone marrow)																				
Stomach																				
Small intestine (3 levels)																				
leukocytic infiltrate, lamina propria, mucosa																				

Code: X - condition present
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - extreme
 - - not available

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Chloroform Anhydride, Tech. 1
90-Day Subacute Toxicity Study in Rats

TABLE 71. Cont. Histomorphological Observations.

Tissue Location	0 ppm (Control)										7500 ppm									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Large intestine (colon) cut sections of nematode in lumen	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Thymic congestion	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Benign reactive lymph node erythrophagocytosis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pancreas inflammatory cell infiltrate periductal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Liver cytoplasmic vacuolation, hepatocytes extramedullary hematopoiesis Kupffer cell proliferation inflammatory cell infiltrate, portal scattered inflammatory foci bile duct proliferation congestion	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kidneys interstitial inflammatory cell infiltrate pyelitis hyperplasia, epithelium of pelvic vasculature, tubular epithelium microcalcifi, pelvis dilated pelvis, atrophy of medulla	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Urinary bladder polypoid cystitis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Testes/ovaries	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Prostate/uterus prostatitis endometrial atrophy, dilated lumen lymphocytic infiltrate, interstitial	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Muscle (skeletal) degeneration of muscle fibers	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SP/inflammatory gland	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Code: 8 - condition present
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - extreme
 7 - not available
 8 - applied on study

000060

Chloroacetic Anhydride, Tech. 2

TABLE 71. (cont.)

Histomorphological Observations

Tissue Location	0 ppm (Control)		100 ppm	
	Sex	Code	Sex	Code
Liver Oil Red O stain	96256	1	96298	2
	96268	1	96297	1
	96269	1	96296	1
	96253	1	96295	1
	96270	2	96294	1
	96257	1	96293	1
	96261	1	96292	1
	96265	1	96291	1
	96264	1	96290	1
	96252	1	96289	1
Oil Red O Positive	96285	1	96310	1
	96276	1	96311	1
	96284	1	96312	1
	96283	1	96313	1
	96274	1	96314	1
	96275	1	96315	1
	96280	1	96316	1
	96273	1	96317	1
	96278	1	96318	1
	96272	1	96319	1
Kidneys Oil Red O Stain	96285	1	96320	1
	96276	1	96321	1
	96284	1	96322	1
	96283	1	96323	1
	96274	1	96324	1
	96275	1	96325	1
	96280	1	96326	1
	96273	1	96327	1
	96278	1	96328	1
	96272	1	96329	1

Code: 1 - condition present
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - ext range
 7 - not available

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Chloromel Anhydride, Tech.:

90-Day Subacute Toxicity Study in Rats.

TABLE 71. Cont.

Micromorphological Observations.

Tissue Location	500 ppm										7500 ppm									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Liver Oil Red O stain	96328	96329	96330	96331	96332	96333	96334	96335	96336	96337	96338	96339	96340	96341	96342	96343	96344	96345	96346	96347
Oil Red O positive	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kidney Oil Red O stain	96348	96349	96350	96351	96352	96353	96354	96355	96356	96357	96358	96359	96360	96361	96362	96363	96364	96365	96366	96367
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Code: 1 - condition present
 2 - very slight
 3 - light
 4 - not remarkable
 5 - moderate
 6 - marked
 7 - extreme
 8 - not available

APPENDIX I
Ophthalmoscopic Examination Summary

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TABLE

MALES: Ophthalmologic Examination Summary

Group, Rat Number	Pretest		13 Weeks	
<u>Control (Control):</u>				
96256	N			
96257	N		OU-Chorioretinal hypoplasia, NOP	
96258	N		N	
96259	N		N	
96260	N		N	
96261	N		N	
96262	N		N	
96263	N		N	
96264	N		N	
96265	N		N	
96266	N		N	
96267	N		N	
96268	N		N	
96269	N		OU-Cataract; phthisis bulbi, NOP	
96270	N		OS-Chorioretinal hypoplasia, NOP	
			OD-Conjunctivitis, NOP	
<u>100 EPE:</u>				
96286	N			
96287	N		N	
96288	N		N	
96289	N		N	
96290	N		N	
96291	N		N	
96292	N		N	
96293	N		N	
96294	N		N	
96295	N		N	
96296	N		N	
96297	N		N	
96298	N		N	
96299	N		N	
96300	N		N	

Code: N - Normal, no observed ocular pathology
 NOP - No other pathology
 OU - Both eyes
 OD - Right eye
 OS - Left eye

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TABLE

MALES: Ophthalmoscopic Examination Summary

Group, Rat Number	Pretest	13 Weeks
<u>500 ppm:</u>		
96316	N	
96317	N	OD-Conjunctivitis, NOP
96318	N	N
96319	N	N
96320	N	N
96321	N	N
96322	N	N
96323	N	OS-Conjunctivitis, NOP
96324	N	OD-Cataract; retinal vessel attenuation, NOP
96325	N	N
96326	N	OD-Cataract; phthisis bulbi, NOP
96327	N	N
96328	N	OS-Chorioretinal hypoplasia
96329	N	OD-Retinal vessel attenuation, NOP
96330	N	N
96330	N	N
<u>2500 ppm:</u>		
96346	N	
96347	N	N
96348	N	N
96349	N	N
96350	N	N
96351	N	N
96352	N	N
96353	N	N
96354	N	N
96355	N	N
96356	N	OD-Cataract; phthisis bulbi, NOP
96357	N	N
96358	N	N
96359	N	N
96360	N	N

Code: N - Normal, no observed ocular pathology
 NOP - No other pathology
 CU - Both eyes
 OD - Right eye
 OS - Left eye

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TABLE

FEMALES: Ophthalmoscopic Examination Summary

Group, Rat Number	Pretest	13 Weeks
<u>0 ppm (Control):</u>		
96271	N	
96272	N	N
96273	N	N
96274	N	N
96275	N	N
96276	N	N
96277	N	OU-Chorioretinal hypoplasia, NOP
96278	N	N
96279	N	N
96280	N	N
96281	N	N
96282	N	N
96283	N	N
96284	N	N
96285	N	N
<u>170 ppm:</u>		
96301	N	
96302	N	N
96303	N	N
96304	N	N
96305	N	N
96306	N	N
96307	N	N
96308	N	N
96309	N	OD-Cataract; phthisis bulbi, NOP
96310	N	N
96311	N	N
96312	N	N
96313	N	N
96314	N	N
96315	N	N

Code: N - Normal, no observed ocular pathology
 NOP - No other pathology
 OU - Both eyes
 OD - Right eye
 OS - Left eye

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Chlorthalidate Anhydride, Tech.

90-Day Subacute Toxicity Study in Rats

TABLE

FEMALES: Ophthalmoscopic Examination Summary

Group,
Sex
Number

Pretest

13 Weeks

500 ppm:

96331	N	
96332	N	N
96333	N	N
96334	N	N
96335	N	N
96336	N	OD-Chorioretinal hypoplasia, NOP
96337	N	N
96338	N	N
96339	N	N
96340	N	N
96341	N	N
96342	N	N
96343	N	OS-Chorioretinal hypoplasia, NOP
96344	N	N
96345	N	N

2500 ppm:

96361	N	
96362	N	N
96363	N	N
96364	N	Died
96365	N	N
96366	N	N
96367	N	N
96368	N	N
96369	N	N
96370	N	N
96371	N	OD-Cataract; phthisis bulbi, NOP
96372	N	N
96373	N	Died
96374	N	N
96375	N	OD-Chorioretinal hypoplasia, NOP
		Died

Code: N - Normal, no observed ocular pathology
 NOP - No other pathology
 OU - Both eyes
 OD - Right eye
 OS - Left eye

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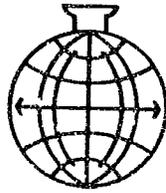
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MATTAWAN, MICHIGAN, U.S.A. 49071 TELEPHONE (616) 668-3336

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Amendment
to the
Final Report

SPONSOR: Velsicol Chemical Corporation

TEST ARTICLE: Chlorendic Anhydride

SUBJECT: 90-Day Subacute Toxicity Study in Rats

DATE OF SUBMISSION: February 8, 1980

International Research and Development Corporation

The following changes in the final report have been made by request of the sponsors' representative, Mr. Dennis W. Arnold:

1. Section II. SYNOPSIS, page 2, paragraph 2, last sentence should read as follows:

Males and females treated at 2500 ppm had elevated serum alkaline phosphatase values at 1, 2 and 3 months of study.

2. Section III. COMPOUND, page 3, should read as follows:

<u>Label</u>	<u>Description</u>
Chlorendic Anhydride Tech #8093-1	white, somewhat chunky powder

3. Section IV. CLINICAL STUDIES, B. RESULTS, 1. General Behavior, Appearance and Survival, page 7, second paragraph, first sentence should read as follows:

Three high-dose females died between the 5th and 13th week of study; no other rats died during the study.

B. RESULTS, 5. Laboratory Tests b. Biochemistry, page 9 should read as follows:

Both males and females treated at 2500 ppm had consistently elevated SAP activity at 1, 2 and 3 months of study. However, the only SAP values showing statistical significance were the high-dose females at 2 and 3 months of study. In addition the mid-dose females at 2 months showed statistically significant elevated SAP values. Other statistically significant changes were noted. However, these values were of no physiological importance. No other compound related effects were seen in the results of the biochemical tests.

B. RESULTS, 5. Laboratory Tests c. Urinalysis, page 9, second sentence should read as follows:

An incidental finding at 3 months of study was the elevated urinary pH of two of five high-dose females tested.

International Research and Development Corporation

4. Section IV. PATHOLOGICAL STUDIES, B. RESULTS, 2. Organ Weights, first sentence should read as follows:

. mean absolute weight of hearts of male rats at all dosage levels and the female rats at the 500-ppm dosage level were noted.

5. Table 1. t-Test Comparison Between Means of Control and Treated Groups, Body Weight, gross, page 15.

This table presents mean body weights for all animals in each sex group. The mean body weights presented on page 8 and 16 are the means of animals excluding animals fasted for clinical pathology determinations. Statistics were run only at week 13.

6. Table 2. Group Mean Body Weights, Grams, Weight Ranges and Survival, page 16, should be clarified as follows:

a. Week 5, an asterisk(*) should be placed after the control male body weight; asterisks should be placed after the control female and 100 ppm female body weights.

7. Table 3, Individual Weekly Body Weight, Grams, page 17 should be corrected as follows:

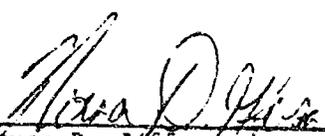
Animal 96260, Control female, was bled during study week 9. An asterisk(*) was inadvertently not recorded on this body weight table.

8. Table 3, Individual Weekly Body Weight, Grams, page 20, should be clarified as follows:

A footnote (a) should be placed after the word "died" for animal 96375, 2500 ppm female, study week 5. The (a) should be coded, died following week 5 blood collection.

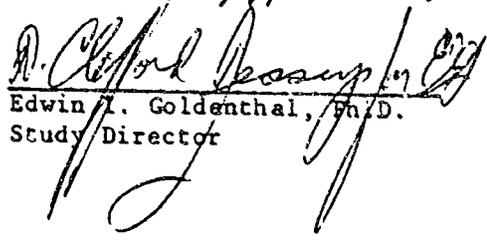
A footnote (b) should be placed after the word "died" for animals 96363 and 96372, 2500 ppm female, week 9. The (b) should be coded, died following week 9 blood collection.

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Norman D. Jefferson
Director of Chronic Toxicity

2/7/80
Date



Edwin L. Goldenthal, Ph.D.
Study Director

2/9/80
Date

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STUDY IN RABBITS

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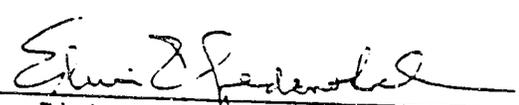
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COMPOUND: Chlorendic Anhydride
SUBJECT: Three Week Dermal Toxicity Study
in Rabbits.

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I. SYNOPSIS

Chlorendic Anhydride was administered to the backs of New Zealand White rabbits at dosage levels of 100, 500 and 2500 mg/kg/day, 5 days a week during this 3 week dermal study. Four male and four female rabbits were used at each dosage level and in the control group. The rabbits were observed daily for signs of overt toxicity, general behavior, dermal irritation, moribundity or mortality. Body weights were recorded weekly. Hematologic, biochemical and urinalysis studies were conducted during the control period and following the 21-day treatment period.

One or more of the following signs of dermal irritation were noted for all treated rabbits: erythema, edema, atonia, desquamation, coriaceousness and fissuring. The number of signs observed, severity of the conditions (barely perceptible to moderate) and duration were dose-related. Incidental findings (primarily at the 2500-mg/kg/day dosage level) included: diarrhea, nasal or ocular discharge, hypoactivity, anorexia and dehydration. Male and female rabbits at the high dosage level had decreases in weight when compared with the controls. All rabbits survived the treatment period. No changes considered related to compound were seen in the hematologic and biochemical studies. Urinalyses were considered normal.

Stomach mucosal lesions, described as erosions, ulcerations, or light foci and areas at necropsy in rabbits from the 2500 and 500 mg/kg/day were the only gross findings at terminal sacrifice which were considered compound-related. No compound-related organ weight variations were observed. Microscopically, grossly described stomach changes were confirmed in several rabbits from the 500- and 2500-mg/kg/day groups. These changes were attributed to compound effect.

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Page 2

Evidence of mild skin irritation, characterized by hyperkeratosis, acanthosis and dermal inflammatory cell infiltrate was seen at the application site in most rabbits from the 100-, 500- and 2500-mg/kg/day groups and was considered compound related. Overall skin response based on microscopic examination of the application site was characterized as mild.

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Page 3

II. COMPOUND

The compound was received from Velsicol Chemical Corporation, Chicago, Illinois as indicated below:

<u>Date Received</u>	<u>Label</u>	<u>Description</u>
October 10, 1977	Tech. Ref. Std. Chlorendic Anhydride 93.81% (titr) Lot No. 3-12-206	white somewhat-chunky powder

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III. CLINICAL STUDIES

A. METHODS:

1. General Procedure.

Sixteen male (2124 to 2781 g) and 16 female (2187 to 2602 g) New Zealand White rabbits (Sweetwater Farms, Hillsboro, Ohio) were housed individually in suspended wire-mesh cages and maintained in a temperature-, humidity- and light-controlled room. Water and Purina® Rabbit Chow® were available ad libitum.

The rabbits were selected from sex groups of 25 rabbits each following a preconditioning period of approximately 2 weeks and evaluation of baseline hematologic, biochemical and urinalysis values. Assignment to either control or treatment groups was determined by a computer-generated table of random numbers.

The study was initiated on January 13, 1978 and terminated by sacrifice of all animals on February 3, 1978.

2. Compound Administration:

Chlorendic Anhydride was applied at dosage levels of 100, 500 and 2500 mg/kg/day, 5 days per week for 3 weeks, for a total of 15 applications. Four male and four female rabbits were assigned to each treatment group and to the control group. The control rabbits were administered 1.2 ml/kg/day of 0.9% physiological saline on the same regimen as treated rabbits. The compound was wetted with a comparable amount of saline prior to dosing.

The dorsal skin (approximately 10% of body surface) was prepared for treatment using electric clippers, as necessary, for close cropping of the fur. In addition, the skin of the first two rabbits of each sex-group was abraded twice weekly using a scapel blade.

The compound administration period was 6 hours, 5 days a week during which time the rabbits were restrained using an Ejay Saf-T Shield (W. A. Butler Company). A glass rod was used to evenly distribute the

compound over the entire prepared area. Following each exposure period, the excess compound was wiped off the animals and they were returned to their cages.

3. Observations:

The rabbits were observed daily for changes in general behavior, signs of overt toxicity, moribundity and mortality. Signs of dermal irritation were scored and recorded prior to, and following, each 6-hour treatment period. Individual body weights were recorded weekly.

4. Laboratory Tests:

Once in the control period and at three weeks of study, blood and urine samples were obtained from all rabbits for appropriate analysis. The rabbits were fasted overnight prior to collection of samples.

a. Hematology:

Hematological studies included hemoglobin¹, hematocrit², total erythrocyte count³ and total³ and differential leucocyte counts.

b. Biochemistry:

Biochemical studies on serum included glucose⁴, blood urea nitrogen (B.U.N.)⁴, alkaline phosphatase activity⁴, serum glutamic oxalacetic (S.G.O.T.)⁵ and pyruvic (S.G.P.T.)⁴ transaminase activities, calcium⁶, inorganic phosphorus⁷, total protein⁷ and albumin⁴.

c. Urinalysis:

Urinalysis included measurement of volume, pH⁸ and specific gravity; description of color and appearance; and qualitative tests for albumin⁸, glucose⁸, bilirubin⁸ and occult blood⁸.

5. Statistical Analysis:

All statistical analyses compared the treatment groups with the control group by sex. At termination of the study, body weights,

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hematologic, biochemical and urinalysis parameters and absolute and relative organ weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie⁹ using Dunnett's¹⁰ multiple comparison tables to judge significance of differences.

B. RESULTS:

1. General Behavior, Appearance and Survival:

One or more signs of dermal irritation were present for all treated rabbits. The level of intensity and time-of-onset were primarily dose-related.

At the 100-mg/kg/day dosage level, a barely perceptible erythema was noted for all rabbits beginning in weeks 2 or 3 of treatment. At the 500-mg/kg/day dosage level, the onset of erythema (barely perceptible to slight) was usually evident by day 4 and persisted throughout most, or all, of the treatment period. Other signs of dermal irritation (barely perceptible to slight) included: edema, atonia, desquamation, coriaceousness and fissuring. These signs were evident for most treated rabbits during either the second or third week of treatment.

At the 2500-mg/kg/day dosage level, erythema (barely perceptible to moderate) was evident as early as day 2 and persisted throughout the study for most rabbits. Other signs of dermal irritation as noted previously (barely perceptible to moderate) were evident by, or on, day 7 and for most rabbits persisted throughout the treatment period.

Incidental findings (primarily at the 2500-mg/kg/day dosage level) included: diarrhea, nasal or ocular discharge, hypoactivity, anorexia and dehydration. None of the rabbits died.

2. Body Weights (Table 1):

Female rabbits in the 2500-mg/kg/day group had decreased body weight gains when compared to controls. Males had slight, but statistically significant, weight losses. Percent change in sex-group mean body weight from week 0 to week 3 are as follows:

<u>Dosage Level</u> <u>(mg/kg/day)</u>	<u>Change in Group Mean Body Weight, %</u>	
	<u>Males</u>	<u>Females</u>
Control	+15.1	+12.2
100	+13.4	+13.6
500	+10.0	+13.0
2500	- 1.1	+ 4.7

3. Laboratory Tests (Tables 2-10):

a. Hematology:

No changes considered to be related to compound were seen in the hematologic studies.

b. Biochemistry:

Values for biochemical parameters measured were within the expected physiologic range for this species.

c. Urinalysis:

No urine was collected from one male and one female rabbit in the 2500-mg/kg/day treatment group. All other urinalyses were similar to the control.

IV. PATHOLOGICAL STUDIES

A. METHODS:

1. Gross Pathology:

After 3 weeks of compound administration, all rabbits were sacrificed with an intravenous overdose of sodium pentobarbital and necropsied.

At necropsy the spleen, liver, adrenals, ovaries/testes, thyroid/parathyroid, brain and kidneys were weighed and representative tissues were collected in buffered neutral 10% formalin.

2. Histopathology:

Hematoxylin and eosin stained paraffin sections of the following tissues from rabbits from the control and 2500-mg/kg/day groups were prepared by Medical Pathfinder Labs, Inc; Fennville, Michigan:

skin (treated and untreated)	urinary bladder
regional lymph node	prostate/uterus
spleen	ovaries/testes
pancreas	nerve, muscle
stomach	bone marrow
duodenum	thymus
ileum	heart
jejunum	trachea
cecum	lung
colon	thyroid, parathyroid
mesenteric lymph node	eye
liver	brain (cerebrum, cerebellum and pons)
gallbladder	pituitary
adrenals (2)	kidneys (2)
spinal cord	and any unusual lesions

Selected tissues from some of the above rabbits were also processed histologically by International Research and Development Corporation. Hematoxylin and eosin stained paraffin sections of treated and untreated skin and stomach from all rabbits in the 100- and 500-mg/kg/day groups were prepared by International Research and Development Corporation. All microscopic examinations were performed by R. G. Geil D.V.M., International Research and Development Corporation.

B. RESULTS:

1. Gross Pathology (Table 11) and Organ Weights (Table 12):

Six rabbits from the 2500-mg/kg/day group and 2 rabbits from the 500-mg/kg/day group had stomach lesions which may have been compound related. These stomach changes were described as ulcerations, erosions and yellow, gray or white foci or areas in the mucosa. They were not seen in rabbits from the control or 100-mg/kg/day groups. No compound-related organ weight variations were observed.

2. Histopathology (Table 13):

Stomach erosions noted grossly and confirmed microscopically in several rabbits from the 2500-mg/kg/day group were shallow and did not extend the full thickness of the mucosa. The stomach mucosa away from the grossly noted erosions was completely normal. Erosions or other stomach alterations could not be confirmed microscopically in all stomachs in which a gross description of a lesion was made. Stomach lesions were not seen microscopically in rabbits from the control- or 100-mg/kg/day groups; their occurrence in rabbits from the 500- and 2500-mg/kg/day groups was probably compound related.

Evidence of very slight to slight compound related dermal irritation was seen in most rabbits from the 2500-, 500-, and 100-mg/kg/day groups. These skin changes included epidermal acanthosis and hyperkeratosis and inflammatory cell infiltrate in the dermis. The severity of these skin changes appeared somewhat dose related and the overall skin response to this compound could best be characterized as mild.

Other microscopic lesions were considered spontaneous and unrelated to treatment and were typical of the usual lesions seen in untreated rabbits. Brain lesions, characterized by perivascular lymphocytic cuffing, glial nodules and lymphocytic meningitis were considered due to infestation by Encephalitozoan Cuniculli, a common protozoan parasite of laboratory rabbits.

References

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9. Steel, R. G. D. and Torrie, J. H. (1960), Principles and Procedures of Statistics, McGraw-Hill, New York, N. Y.
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Chlorendic Anhydride: Three Week Dermal Toxicity Study in Rabbits.

TABLE 1. Individual and Group Mean Body Weights, Grams.

Group, Rabbit Number	Sex	Skin Preparation	Control			Week of Study		
			-2	-1	0	1	2	3
<u>Control:</u>								
32021	M	I	1999	2122	2263	2296	2603	2558
32027	M	A	1953	2253	2639	2749	2915	2884
32049	M	A	1984	2186	2266	2470	2689	2729
32057	M	I	2036	2152	2435	2543	2854	2883
Mean				2178	2401	2515	2765	2764
32022	F	I	1843	2003	2273	2478	2749	2528
32036	F	A	1943	2135	2187	2357	2355	2293
32040	F	I	1923	1972	2203	2312	2539	2488
32064	F	A	1850	2028	2318	2488	2815	2770
Mean				2035	2245	2409	2615	2520
<u>100 mg/kg/day:</u>								
32033	M	I	1833	1997	2292	2378	2306	2318
32043	M	A	2044	2140	2219	2373	2648	2681
32047	M	A	1908	2031	2472	2796	3079	2948
32059	M	I	1842	1965	2124	2248	2489	2384
Mean				2035	2277	2449	2631	2583
32024	F	A	1924	2078	2338	2456	2729	2754
32026	F	A	1944	2073	2280	2457	2685	2663
32028	F	I	2167	2324	2602	2689	2959	2932
32034	F	I	1896	2077	2336	2387	2540	2506
Mean				2138	2389	2497	2728	2714

A - Abraded
I - Intact

Chlorendic Anhydride: Three Week Dermal Toxicity Study in Rabbits.

TABLE 1. Cont. Individual and Group Mean Body Weights, Grams.

Group, Rabbit Number	Sex	Skin Preparation	Control			Week of Study		
			-2	-1	0	1	2	3
<u>500 mg/kg/day:</u>								
32025	M	A	2188	2326	2620	2736	2946	2928
32035	M	I	1891	1964	2272	2365	2756	2733
32039	M	A	2225	2398	2781	2717	2930	2814
32045	M	I	1976	2158	2366	2319	2569	2567
Mean				2212	2510	2534	2800	2761
32032	F	A	1884	2103	2297	2548	2775	2797
32052	F	I	1895	1978	2308	2400	2498	2406
32054	F	A	2098	2181	2483	2627	2757	2734
32056	F	I	1923	2209	2418	2607	2847	2808
Mean				2118	2377	2546	2719	2686
<u>2500 mg/kg/day:</u>								
32019	M	I	1973	2063	2372	2376	2310	2277
32023	M	A	1958	2009	2402	2066	2019	2094
32031	M	A	1952	2118	2452	2510	2398	2302
32065	M	I	1818	1880	2135	2296	2576	2582
Mean				2018	2340	2312	2326	2314*
32020	F	I	1975	2102	2425	2495	2700	2679
32048	F	A	1720	1850	2252	2556	2633	2707
32050	F	I	1906	2078	2409	2349	1786	2131
32068	F	A	1849	2216	2442	2657	2679	2456
Mean				2062	2382	2514	2450	2493

A - Abraded

I - Intact

*Significantly different from Control group mean, $p < 0.05$.

Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits.

TABLE 2.

Means and Significance of Hematological Values.

Hematology	Study Week	Control	100 mg/kg/day	500 mg/kg/day	2500 mg/kg/day
MALES:					
Erythrocytes 10 ⁶ /cmm	Control	5.10	5.25	5.29	4.96
	Terminal	5.71	5.23	5.84	5.32
Hemoglobin g/100 ml	Control	11.7	12.0	12.1	11.5
	Terminal	13.1	13.5	12.8	11.8
Hematocrit %	Control	38	40	38	37
	Terminal	40	40	40	38
Leucocytes 10 ³ /cmm	Control	7.77	7.31	6.55	8.51
	Terminal	8.02	8.29	9.26	7.15
FEMALES:					
Erythrocytes 10 ⁶ /cmm	Control	5.42	5.48	5.33	5.29
	Terminal	5.99	5.98	5.34	5.66
Hemoglobin g/100 ml	Control	12.3	12.2	12.2	12.1
	Terminal	12.7	13.4	12.4	12.5
Hematocrit %	Control	40	39	39	38
	Terminal	42	41	39	40
Leucocytes 10 ³ /cmm	Control	8.14	7.24	7.62	6.44
	Terminal	7.01	8.18	7.74	7.79

Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits.

TABLE 3.

Group, Rabbit Number	Sex	Individual Hematological Values - Control.										
		Erythro- cytes 10 ⁶ /cmm	Hemo- globin g/100 ml	Hemato- crit %	Leuco- cytes 10 ³ /cmm	Neutrophils Seg. Non-Seg. %	Lympho- cytes %	Eosino- phils %	Mono- cytes %	Baso- phils %		
32021	M	4.59	10.7	36	8.60	38	0	60	0	0	0	2
32027	M	4.84	11.2	37	8.67	57	0	42	0	0	0	1
32049	M	5.55	12.8	41	6.49	49	0	48	0	1	0	2
32057	M	5.41	12.1	39	7.30	43	0	56	0	0	0	1
Mean		5.10	11.7	38	7.77							
32022	F	4.54	11.7	37	11.62	35	0	64	0	0	0	1
32036	F	6.00	13.8	44	5.99	28	0	71	0	0	0	1
32040	F	5.35	11.4	39	7.74	43	0	55	0	1	0	1
32064	F	5.80	12.1	40	7.20	27	0	73	0	0	0	0
Mean		5.42	12.3	40	8.14							
<u>100 mg/kg/day:</u>												
32033	M	5.69	13.0	44	6.78	45	0	53	0	0	0	1
32043	M	5.36	12.7	43	6.93	47	0	51	0	0	0	2
32047	M	5.44	11.7	38	8.46	30	0	69	0	1	0	0
32059	M	4.49	10.6	35	7.05	38	0	60	0	0	0	2
Mean		5.25	12.0	40	7.31							
32024	F	5.56	12.2	40	6.40	61	1	38	0	0	0	0
32026	F	5.44	12.0	38	8.76	39	0	59	0	0	0	2
32028	F	5.38	11.9	38	6.95	38	0	60	0	0	0	2
32034	F	5.54	12.5	39	6.84	48	0	51	0	0	0	1
Mean		5.48	12.2	39	7.24							

Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits.

TABLE 3. Cont. Individual Hematological Values - Control.

Group, Rabbit Number	Sex	Erythro- cytes 10 ⁶ /cmm	Hemo- globin g/100 ml	Hemato- crit %	Leuco- cytes 10 ⁶ /cmm	Neutrophils Seg. %	Non-Seg. %	Lympho- cytes %	Eosino- phils %	Mono- cytes %	Baso- phils %	
<u>500 mg/kg/day:</u>												
32025	M	5.21	12.2	37	5.40	33	0	66	0	0	1	
32035	M	5.12	11.5	37	7.18	48	0	51	0	0	1	
32039	M	5.49	12.2	37	8.08	34	0	64	0	0	2	
32045	M	5.35	12.6	41	5.53	24	0	73	1	0	2	
Mean		5.29	12.1	38	6.55							
32032	F	4.87	10.5	36	8.99	36	0	63	0	0	1	
32052	F	5.58	13.5	41	4.92	22	0	77	1	0	0	
32054	F	5.87	13.2	42	8.91	54	0	46	0	0	0	
32056	F	4.98	11.4	36	6.85	24	0	76	0	0	0	
Mean		5.33	12.2	39	7.42							
<u>2500 mg/kg/day:</u>												
32019	M	5.52	12.4	38	9.90	56	0	39	0	2	3	
32023	M	4.62	10.7	35	8.43	55	0	43	0	0	2	
32031	M	4.82	11.3	38	8.74	28	0	72	0	0	0	
32065	M	4.89	11.4	37	6.97	22	0	77	0	0	1	
Mean		4.96	11.5	37	8.51							
32020	F	4.72	11.7	35	9.62	26	0	74	0	0	0	
32048	F	5.53	11.1	37	4.79	32	0	64	2	0	2	
32052	F	5.58	13.5	41	4.92	22	0	77	1	0	0	
32068	F	5.31	12.2	40	6.42	35	0	64	0	0	1	
Mean		5.29	12.1	38	6.44							

Chlorendic Anhydride;

Three Week Dermal Toxicity Study in Rabbits.

TABLE 4.

Individual Hematological Values - Terminal.

Group, Rabbit Number	Sex	Erythrocytes 10 ⁶ /cmm	Hemoglobin g/100 ml	Hemato-crit %	Leuco-cytes 10 ³ /cmm	Neutrophils Seg. %	Non-Seg. %	Lympho-cytes %	Eosino-phils %	Mono-cytes %	Baso-phils %
<u>Control:</u>											
32021	M	5.08	11.3	36	8.45	14	0	86	0	0	0
32027	M	5.71	13.4	40	9.00	26	0	72	0	2	0
32049	M	5.65	13.8	42	7.15	35	0	64	0	0	0
32057	M	6.41	13.7	43	7.47	33	0	66	1	0	1
Mean		5.71	13.1	40	8.02				1	0	0
32022	F	5.40	12.5	41	9.64	65	0	34	0	0	1
32036	F	6.88	12.9	44	4.91	19	0	81	0	0	0
32040	F	5.76	13.1	41	7.42	43	0	54	1	1	1
32064	F	5.90	12.1	40	6.08	23	0	74	3	0	0
Mean		5.99	12.7	42	7.01						
<u>100 mg/kg/day:</u>											
32033	M	5.20	11.9	39	7.33	17	0	78	3	0	2
32043	M	5.61	16.0	41	10.08	18	0	79	1	0	2
32047	M	4.96	13.6	40	9.02	17	0	81	1	1	0
32059	M	5.14	12.3	38	6.71	14	0	83	1	1	1
Mean		5.23	13.5	40	8.29						
32024	F	5.66	12.8	38	8.21	36	0	63	1	0	0
32026	F	5.91	13.9	42	10.19	19	0	79	1	0	1
32028	F	5.94	12.9	40	7.49	23	0	76	0	0	1
32034	F	6.40	14.1	45	6.83	17	0	81	0	0	2
Mean		5.98	13.4	41	8.18						

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Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits.

TABLE 4. Cont.

Individual Hematological Values - Terminal.

Group, Rabbit Number	Sex	Erythro- cytes 10 ⁶ /cmm	Hemo- globin g/100 ml	Hemato- crit %	Leuco- cytes 10 ⁶ /cmm	Neutrophils Seg. Non-Seg. %	Lympho- cytes %	Eosino- phils %	Mono- cytes %	Baso- phils %
<u>500 mg/kg/day:</u>										
32025	M	6.13	14.5	44	11.19	12	82	4	0	2
32035	M	5.60	12.9	40	7.69	25	71	0	2	2
32039	M	6.10	12.1	38	10.73	32	62	0	0	6
32045	M	5.52	11.5	37	7.41	25	72	1	0	2
Mean		5.84	12.8	40	9.26					
32032	F	5.69	12.7	40	5.65	32	67	1	0	0
32052	F	4.08*	9.5	31	10.01	18	76	4	1	1
32054	F	6.12	14.3	44	8.86	36	58	6	0	0
32056	F	5.46	12.9	40	6.45	41	57	1	1	0
Mean		5.34	12.4	39	7.74					
<u>2500 mg/kg/day:</u>										
32019	M	5.81	12.7	41	8.24	27	67	5	0	1
32023	M	5.48	12.1	38	7.81	38	60	2	0	0
32031	M	5.13	11.0	38	7.25	39	59	0	0	2
32065	M	4.84	11.4	35	5.30	12	87	0	0	1
Mean		5.32	11.8	38	7.15					
32020	F	5.56	12.4	39	8.86	24	74	2	0	0
32048	F	5.99	13.1	42	8.90	44	55	0	0	1
32050	F	5.12	10.8**	36	8.27	25	74	0	0	1
32068	F	5.97	13.5	43	5.11	35	61	2	0	2
Mean		5.66	12.5	40	7.79					

**Sample checked.
2+Anisocytosis
*3+Polychromasia

Code: 3+ - 50% to 75% cells with sign indicated.
2+ - 25% to 50% cells with sign indicated.

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Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits,

TABLE 5.

MALES: Means and Significance of Biochemical Values.

Biochemistry	Study Week	Control	100 mg/kg/day	500 mg/kg/day	2500 mg/kg/day
Glucose, mg/100 ml	Control	136	139	128	139
	Terminal	135	137	140	149
B.U.N., mg/100 ml	Control	15.4	17.5	16.6	14.8
	Terminal	15.6	16.7	16.0	21.0
Alkaline Phosphatase, int'l u/l	Control	173	195	194	160
	Terminal	182	199	175	138
S.G.O.T., Sigma u/ml	Control	26	19	24	25
	Terminal	35	31	35	25
S.G.P.T., int'l u/l	Control	73	74	53	70
	Terminal	57	43	39	80
Calcium mg/100 ml	Control	15.1	15.6	14.3	14.7
	Terminal	11.2	11.2	11.0	11.2
Inorganic Phosphorus, mg/100 ml	Control	6.8	6.9	6.4	6.4
	Terminal	7.6	7.8	7.5	6.9
Total Protein, g/100 ml	Control	6.48	6.63	5.92	4.02
	Terminal	6.58	6.58	6.23	6.46
Albumin, g/100 ml	Control	4.48	4.27	4.02	4.10
	Terminal	4.56	4.25	4.01	3.77

Chlorendic Anhydride:

Three Week Dermal Toxicity Study In Rabbits.

TABLE 5. Cont. FEMALES: Means and Significance of Biochemical Values.

Biochemistry	Study Week	Control	100 mg/kg/day	500 mg/kg/day	2500 mg/kg/day
Glucose, mg/100 ml	Control	141	144	137	137
	Terminal	131	151	147	155
B.U.N., mg/100 ml	Control	20.6	17.5	19.5	17.8
	Terminal	20.4	18.3	19.5	17.4
Alkaline Phosphatase, int'l u/l	Control	150	165	156	180
	Terminal	154	203	160	160
S.G.O.T., Sigma u/ml	Control	17	17	18	27
	Terminal	36	26	28	16
S.G.P.T., int'l u/l	Control	75	73	65	66
	Terminal	46	92	42	62
Calcium mg/100 ml	Control	14.0	14.7	14.2	14.4
	Terminal	10.8	11.4	11.0	11.7
Inorganic Phosphorus, mg/100 ml	Control	7.2	6.7	7.9	6.6
	Terminal	6.7	7.3	7.8	6.4
Total Protein, g/100 ml	Control	6.57	6.78	6.31	6.16
	Terminal	6.66	6.78	6.10	5.95
Albumin, g/100 ml	Control	4.07	4.24	4.19	3.93
	Terminal	4.60	4.62	3.86	3.75*

*Significantly different from Control group mean, p<0.01.

Chloroethyle Anhydride

TABLE 6. Three Week Dermal Toxicity Study In Rabbits.

Group, Rabbit Number	Sex	Individual Biochemical Values - Control								
		Glucose mg/100 ml	B.U.N. mg/100 ml	Alkaline Phosphatase (μmole/l) u/l	S.G.O.T. (μmole/ml)	S.G.P.T. (μmole/l) u/l	Calcium mg/100 ml	Inorganic Phosphorus mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml
Control:										
32024	H	171	17.8	176	19	71	16.7	6.0	6.54	4.28
32027	H	179	16.0	156	46	86	14.8	7.8	6.39	4.60
32049	H	150	13.9	200	18	60	15.0	6.9	7.02	4.82
32057	H	165	14.0	209	27	75	14.2	6.6	5.97	4.62
Mean		136	15.4	173	26	73	15.1	6.8	6.48	4.48
32022	F	135	22.1	137	20	72	13.5	6.9	6.48	3.69
32016	F	153	20.9	158	19	81	13.7	7.5	7.05	4.10
32060	F	149	18.4	127	12	66	14.4	6.0	7.02	4.42
32064	F	178	21.0	181	17	86	14.4	8.6	5.73	3.86
Mean		161	20.6	150	17	75	14.0	7.7	6.57	4.07
100 mg/kg/day:										
32033	M	141	18.1	190	24	94	16.0	8.6	6.87	4.40
32063	M	136	15.0	208	16	46	15.2	6.9	7.02	4.70
32067	M	165	11.0	170	18	80	15.8	6.0	6.09	4.19
32059	H	132	25.9	210	19	79	15.2	6.3	6.54	3.77
Mean		139	17.5	195	19	74	15.6	6.9	6.63	4.27
32024	F	148	15.8	141	28	75	14.6	6.3	6.27	3.90
32026	F	146	20.0	152	13	76	16.5	6.6	6.87	4.37
32028	F	154	16.1	215	8	60	14.5	6.3	7.08	4.38
32014	F	129	17.9	150	19	79	15.2	7.5	6.90	4.30
Mean		144	17.5	165	17	73	14.7	6.7	6.78	4.74

B.U.N. - Blood Urea Nitrogen
 S.G.O.T. - Serum Glutamic Oxalacetate Transaminase
 S.G.P.T. - Serum Glutamic Pyruvic Transaminase

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Chloroethyl Anhydride:

TABLE 6. Cont. Three Week Dermal Toxicity Study in Rabbits.

Group, Rabbit Number	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alkaline Phosphatase Int'l U/l	S.G.O.T. (U/ml)	S.G.P.T. (Int'l U/l)	Calcium mg/100 ml	Inorganic Phosphorus mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml
500 mg/kg/day:										
32025	M	174	15.0	174	22	62	16.6	6.0	6.17	4.05
32035	M	171	15.1	267	15	18	13.6	6.0	5.28	3.60
32039	M	176	17.0	166	28	65	13.6	6.6	5.55	3.69
32065	M	139	19.1	186	11	65	15.8	6.9	6.72	4.71
Mean		178	16.6	194	24	53	14.3	6.4	5.92	4.02
32032	F	130	15.0	155	15	65	15.0	6.3	6.15	4.11
32052	F	141	26.0	189	18	66	14.2	7.8	6.39	4.62
32054	F	121	23.1	150	17	63	13.0	8.7	6.48	3.83
32056	F	154	16.0	129	22	87	14.5	8.7	6.21	4.18
Mean		137	19.5	156	18	65	14.2	7.9	6.31	4.19
2500 mg/kg/day:										
32019	M	160	11.2	197	11	93	15.0	6.6	6.06	4.17
32023	M	176	15.2	181	12	66	15.2	5.7	6.63	4.01
32031	M	130	20.0	146	17	60	14.8	6.9	6.75	4.56
32065	M	160	12.9	116	19	60	13.6	6.3	5.26	3.67
Mean		139	14.8	160	25	70	14.7	6.4	6.10	4.10
32020	F	128	20.1	198	16	53	14.5	5.7	5.94	3.82
32068	F	131	16.5	169	15	54	14.1	6.0	6.18	3.50
32050	F	128	13.0	154	17	61	14.1	6.9	5.82	4.10
32068	F	161	21.4	217	60	94	14.7	7.8	6.69	4.30
Mean		137	17.8	180	27	66	14.4	6.6	6.16	3.93

B.U.N. - Blood Urea Nitrogen
 S.G.O.T. - Serum Glutamic Oxalacetic Transaminase
 S.G.P.T. - Serum Glutamic Pyruvic Transaminase

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Chloroform Anhydride:
 TABLE 7.
 Three Week Normal Toxicity Study in Rabbits.
 Individual Biochemical Values - Terminal.

Group, Rabbit Number	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alkaline Phosphatase Int'l u/l	S.G.O.T. S/pmc u/m	S.G.P.T. Int'l u/l	Calcium mg/100 ml	Inorganic Phosphorus mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml
Control:										
32021	M	179	12.0	135	31	81	10.6	6.0	6.62	4.18
32027	M	117	20.2	160	52	74	10.6	8.8	6.51	4.22
32069	M	157	16.1	216	28	35	11.8	7.6	6.88	5.00
32057	M	135	16.1	260	30	17	11.8	8.0	6.50	4.82
Mean		135	15.6	187	35	57	11.2	7.6	6.58	4.56
32022	F	149	17.2	113	26	62	10.2	5.0	6.79	4.60
32036	F	110	28.0	158	72	33	10.3	8.2	7.30	4.61
32060	F	130	18.5	109	28	46	11.5	6.2	6.53	4.61
32064	F	133	17.8	236	17	43	11.2	7.7	6.00	4.56
Mean		131	20.6	156	36	46	10.8	6.7	6.66	4.60
100 mg/kg/day:										
32033	M	138	18.5	193	28	47	10.5	7.6	6.98	3.99
32063	M	121	13.0	150	41	38	11.4	8.8	6.79	4.62
32047	M	166	13.4	225	28	42	12.1	7.0	6.67	4.89
32059	M	131	22.0	229	26	66	10.8	7.6	5.91	3.70
Mean		137	16.7	199	31	43	11.2	7.8	6.58	4.25
32024	F	135	16.0	167	29	70	11.3	7.0	6.65	4.69
32026	F	129	19.0	171	21	78	11.6	7.0	6.71	4.52
32078	F	155	19.3	231	30	69	11.8	7.4	6.90	4.74
32034	F	186	18.8	244	23	152	11.0	7.8	6.86	4.52
Mean		151	18.1	203	26	92	11.6	7.7	6.78	4.67

B.U.N. - Blood Urea Nitrogen
 S.G.O.T. - Serum Glutamic Oxaloacetic Transaminase
 S.G.P.T. - Serum Glutamic Pyruvic Transaminase

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Chlordane Antidote:
 TABLE 7. Cont.
 Three Week Dermal Toxicity Study in Rabbits.

Group, Rabbit Number	Sex	Individual Biochemical Values - Terminal								
		Glucose mg/100 ml	B.U.N mg/100 ml	Alkaline Phosphatase Int'l u/l	S.G.O.T. (Sigma) u/ml	S.G.P.T. (Int'l) u/l	Calcium mg/100 ml	Inorganic Phosphorus mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml
500 mg/kg/day:										
32025	H	136	14.9	185	56	73	11.1	6.8	6.70	4.48
32035	H	115	13.0	266	25	21	11.0	7.6	5.78	4.01
32039	H	166	17.0	86	36	23	10.4	8.0	6.13	3.59
32045	H	164	19.0	166	25	41	11.6	7.6	6.31	3.95
Mean		160	16.0	175	35	39	11.0	7.5	6.23	4.01
32032	F	166	20.0	191	26	37	10.5	7.6	5.98	4.09
32052	F	125	20.0	99	16	15	10.4	7.6	4.89	2.78
32054	F	155	18.2	176	43	38	11.5	8.2	7.26	4.51
32056	F	161	19.9	174	79	59	11.6	7.6	6.28	4.06
Mean		147	19.5	160	28	47	11.0	7.8	6.10	3.86
2500 mg/kg/day:										
32019	H	161	19.6	167	44	108	11.6	7.0	7.21	3.88
32023	H	145	21.9	182	21	108	11.9	5.8	6.81	1.71
32031	H	139	27.8	67	17	34	10.6	7.2	6.03	3.31
32065	H	151	14.8	136	16	70	10.7	7.4	5.80	4.16
Mean		149	21.0	138	25	80	11.2	6.9	6.46	3.77
32020	F	166	20.1	206	19	117	11.7	6.8	6.50	3.98
32068	F	147	15.5	152	11	54	11.6	7.2	6.01	3.67
32050	F	160	13.2	105	16	36	11.1	6.6	5.08	3.45
32068	F	167	18.6	175	14	40	12.5	5.0	6.19	3.88
Mean		155	17.4	160	16	62	11.7	6.4	5.95	3.75

B.U.N. - Blood Urea Nitrogen
 S.G.O.T. - Serum Glutamic Oxaloacetic Transaminase
 S.G.P.T. - Serum Glutamic Pyruvic Transaminase

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Chlorendic Anhydride: Three Week Dermal Toxicity Study in Rabbits.

TABLE 8.

Means and Significance of Urinalysis Values.

Urinalysis	Study Week	Control		100 mg/kg/day		500 mg/kg/day		2500 mg/kg/day	
		Control	Terminal	Control	Terminal	Control	Terminal	Control	Terminal
MALES:									
Volume, ml	Control	166		115		169		55	
	Terminal	126		93		111		98	
pH	Control	8.9		8.9		8.6		8.6	
	Terminal	8.6		9.0		9.0		9.0	
Spec. Grav.	Control	1.014		1.016		1.010		1.024	
	Terminal	1.023		1.021		1.018		1.019	
FEMALES:									
Volume, ml	Control	200		110		244		191	
	Terminal	185		118		219		110	
pH	Control	8.9		8.9		8.9		8.9	
	Terminal	9.0		9.0		9.0		8.2	
Spec. Grav.	Control	1.013		1.015		1.010		1.011	
	Terminal	1.013		1.020		1.011		1.015	

Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits.

TABLE 9.

Individual Urinalysis Values - Control.

Group, Rabbit Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Albumin	Glucose	Bili-rubin	Occult Blood	Ketones
Control:										
32021	M	85	LS-cl	8.8	1.014	N	N	N	tr	N
32027	H	305	LS-cl	8.8	1.010	N	N	N	tr	N
32049	M	110	S-cl	8.9	1.015	N	N	N	N	N
32057	M	165	LS-cl	8.9	1.015	N	N	N	2+	N
Mean		166		8.9	1.014					
32022	F	130	LS-cl	8.9	1.016	N	N	N	1+	N
32036	F	225	LS-cl	8.9	1.016	N	N	N	1+	N
32040	F	265	LS-cl	8.9	1.010	N	N	N	tr	N
32064	F	180	LS-cl	8.9	1.010	N	N	N	tr	N
Mean		200		8.9	1.013				tr	N
<u>100 mg/kg/day:</u>										
32033	M	150	LS-cl	8.8	1.010	N	N	N	N	N
32043	M	85	S-cl	8.9	1.031	N	N	N	N	N
32047	M	170	LS-cl	8.8	1.005	N	N	N	N	N
32059	M	55	LS-cl	8.9	1.019	N	N	N	tr	N
Mean		115		8.9	1.016					
32024	F	85	LS-cl	8.9	1.018	N	N	N	tr	N
32026	F	0.0								
32028	F	175	LS-cl	8.9	1.015	N	N	N	tr	N
32034	F	70	LS-cl	8.9	1.013	N	N	N	tr	N
Mean		110		8.9	1.015					

N - Negative
 F - Few
 L - Loaded
 M - Many
 R - Rare
 occ - Occasional

S - Straw
 LS - Light Straw
 DS - Dark Straw
 LAM - Light Amber
 DAM - Dark Amber
 cl - Cloudy
 c - Clear

1+ - Trace to slight
 2+ - Slight to moderate
 3+ - Moderate
 4+ - Marked

Code:

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Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits.

TABLE 9. Cont. Individual Urinalysis Values - Control.

Group, Rabbit Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Albumin	Glucose	Bili-rubin	Occult Blood	Ketones
<u>500 mg/kg/day:</u>										
32025	M	160	LS-cl	8.8	1.011	N	N	N	tr	N
32035	M	160	LS-cl	7.8	1.007	N	N	N	tr	N
32039	M	90	LS-cl	8.9	1.015	N	N	N	tr	N
32045	M	265	LS-cl	8.8	1.005	N	N	N	N	N
Mean		169		8.6	1.010					
32032	F	280	LS-cl	8.9	1.010	N	N	N	tr	N
32052	F	210	LS-cl	8.9	1.011	N	N	N	N	N
32054	F	235	LS-cl	8.9	1.009	N	N	N	N	N
32056	F	250	LS-cl	8.9	1.008	N	N	N	3+	N
Mean		244		8.9	1.010					
<u>2500 mg/kg/day:</u>										
32019	M	75	LS-cl	8.0	1.022	N	N	N	1+	N
32023	M	25	LS-cl	8.8	1.032	N	N	N	N	N
32031	M	45	S-cl	8.8	1.032	N	N	N	tr	N
32065	M	75	LS-cl	8.9	1.009	N	N	N	tr	N
Mean		55		8.6	1.024					
32020	F	140	LS-cl	8.9	1.010	N	N	N	tr	N
32048	F	255	LS-cl	8.9	1.005	N	N	N	tr	N
32050	F	70	S-cl	8.9	1.020	N	N	N	tr	N
32068	F	300	LS-cl	8.9	1.010	N	N	N	tr	N
Mean		191		8.9	1.011					

Code:

tr - Trace
 1+ - Trace to slight
 2+ - Slight to moderate
 3+ - Moderate
 4+ - Marked

S - Straw
 LS - Light Straw
 DS - Dark Straw
 LAm - Light Amber
 DAm - Dark Amber
 cl - cloudy
 C - clear

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N - Negative
 F - Few
 L - Loaded
 M - Many
 R - Rare
 occ - Occasional

Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits.

TABLE 10.

Individual Urinalysis Values - Terminal.

Group Rabbit Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Albumin	Glucose	Bili-rubin	Occult Blood	Ketones
<u>Control:</u>										
32021	M	50	norm	9.0	1.032	N	N	N	tr	N
32027	M	225	norm	7.4	1.015	N	N	N	N	N
32049	M	70	norm	9.0	1.029	N	N	N	N	N
32057	M	160	norm	9.0	1.017	N	N	N	N	N
Mean		126		8.6	1.023					
32022	F	25	norm	9.0	1.025	N	N	N	N	N
32036	F	285	norm	9.0	1.008	N	N	N	N	N
32040	F	175	norm	9.0	1.011	N	N	N	tr	N
32064	F	255	norm	9.0	1.007	N	N	N	N	N
Mean		185		9.0	1.013					
<u>100 mg/kg/day:</u>										
32033	M	25	norm	9.0	1.036	N	N	N	N	N
32043	M	100	norm	8.9	1.016	N	N	N	tr	N
32047	M	110	norm	9.0	1.012	N	N	N	N	N
32059	M	135	norm	8.9	1.018	N	N	N	N	N
Mean		93		9.0	1.021					
32024	F	75	norm	9.0	1.023	N	N	N	N	N
32026	F	255	norm	8.9	1.008	N	N	N	N	N
32028	F	105	norm	9.0	1.023	N	N	N	N	N
32034	F	35	norm	9.0	1.027	N	N	N	N	N
Mean		118		9.0	1.020					

Code:

tr - Trace
 1+ - Trace to slight
 2+ - Slight to moderate
 3+ - Moderate
 4+ - Marked

S - Straw
 LS - Light Straw
 DS - Dark Straw
 LAm - Light Amber
 DAm - Dark Amber
 cl - Cloudy
 C - Clear

N - Negative
 F - Few
 L - Loaded
 M - Many
 R - Rare
 occ - Occasional
 norm - Normal

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Chlorendic Anhydride:

Three Week Dermal Toxicity Study in Rabbits.

TABLE 10. Cont.

Group, Rabbit Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Albumin	Glucose	Bili- rubin	Occult Blood	Ketones	Individual Urinalysis Values - Terminal.	
											500 mg/kg/day:	2500 mg/kg/day:
32025	M	155	norm	9.0	1.011	N	N	N	N	N		
32035	M	200	norm	8.9	1.010	N	N	N	N	N		
32039	M	18	norm	8.9	1.027	N	N	N	N	N		
32045	M	70	norm	9.0	1.025	N	N	N	N	N		
Mean		111		9.0	1.018							
32032	F	275	norm	9.0	1.007	N	N	N	N	N		
32052	F	185	norm	9.0	1.012	N	N	N	N	N		
32054	F	200	norm	9.0	1.014	N	N	N	N	N		
32056	F	215	norm	9.0	1.009	N	N	N	N	N		
Mean		219		9.0	1.011							
2500 mg/kg/day:												
32019	M	40	norm	9.0	1.021	N	N	N	tr	N		
32023	M	0.0										
32031	M	100	norm	9.0	1.013	N	N	N	N	N		
32065	M	155	norm	9.0	1.022	N	N	N	N	N		
Mean		98		9.0	1.019							
32029	F	110	norm	8.9	1.022	N	N	N	I+	N		
32048	F	136	norm	9.0	1.012	N	N	N	N	N		
32050	F	0.0										
32068	F	90	norm	6.8	1.012	N	N	N	N	N		
Mean		110		8.2	1.015							

Code:

tr - Trace
 1+ - Trace to slight
 2+ - Slight to moderate
 3+ - Moderate
 4+ - Marked

S - Straw
 LS - Light Straw
 DS - Dark Straw
 LAm - Light Amber
 DAm - Dark Amber
 cl - Cloudy
 C - Clear

N - Negative
 F - Few
 L - Loaded
 M - Many
 R - Rare
 occ - Occasional
 norm - Normal

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Chloramide Anhydride:
 TABLE II.
 Three Week Dermal Toxicity Study in Rabbits.

Site Lesion	Necropsy Observations															
	Control				100 mg/kg/day				500 mg/kg/day				2500 mg/kg/day			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
No gross lesions	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
External purulent nasal discharge																
Application Site white moist/pasty material matted on surface																
Submaxillary lymph Nodes enlarged				X												X
Lungs dark red foxy congestion/edema consolidation, abscesses					X	X	X	X								
Liver yellow/white foel								X								
Stomach ulcerations/erosions, fundic mucosa yellow foxy, foallic mucosa yellow/gray/white areas, fundic mucosa																
Kidneys left absent, right large dark red pitted foel, cortical surface pale																
Oviduct cystic, unilateral																
Bladder few cysts of Tenia platiformis, omentum yellow necrotic fat nodule with adjacent hemorrhage, sublumbar region																

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Chloroacetic Anhydride:
 TABLE 17.
 Three Week Dermal Toxicity Study In Rabbits.
 Absolute (Grams) and Relative (~ Body Weight) Organ Weights.

Group/ Rabbit Number	Sex	Body Wt. g	Adrenals P x10 ⁷	Brain P Z	Kidney P Z	Liver P Z	Spleen P Zx10 ⁷	Testes/ Ovaries R 7x10 ⁷	Thyroid R Zx10 ²
Control:									
32021	M	2558	0.28	9.81	22.31	88.18	1.57	2.80	0.27
32027	M	2684	0.64	9.48	21.95	85.01	2.12	6.14	1.06
32069	M	2279	0.18	1.39	20.87	178.77	0.76	4.11	0.61
32057	M	2883	0.28	8.99	19.96	106.30	0.76	3.76	1.49
Mean		2764	0.35	9.06	21.27	101.93	0.86	3.08	0.70
32022	F	2578	0.13	7.91	17.48	59.86	1.37	3.16	1.01
32016	F	2293	0.63	9.06	16.67	74.60	0.86	3.40	1.06
32060	F	2488	0.47	8.35	16.02	77.99	0.77	3.16	1.50
32066	F	2770	0.23	9.10	16.97	103.72	0.80	3.27	0.78
Mean		2520	0.37	8.61	16.24	86.57	0.86	3.10	1.49
100 mg/kg/day:									
32033	M	2318	0.15	8.71	16.10	84.96	0.98	4.23	1.41
32063	M	2681	0.30	8.70	20.38	107.75	0.93	3.67	0.31
32067	M	2968	0.33	8.95	20.56	96.90	1.05	3.58	0.86
32059	M	2384	1.17	9.67	15.13	71.15	1.45	6.08	1.63
Mean		2583	0.32	8.96	17.53	90.19	1.10	4.34	1.17
32024	F	2754	0.30	9.35	15.69	70.28	0.91	3.70	1.17
32026	F	2661	0.16	1.28	16.50	95.92	1.71	6.62	1.34
32028	F	2932	0.43	8.18	17.13	101.36	1.09	3.72	1.01
32034	F	2506	0.36	8.51	20.48	102.06	0.83	3.31	0.85
Mean		2714	0.35	8.64	17.50	91.61	1.14	4.19	1.12
500 mg/kg/day:									
32025	M	2978	0.16	8.57	20.34	92.82	0.98	3.35	1.08
32035	M	2737	0.65	9.19	19.36	81.49	0.71	2.67	0.82
32039	M	2814	0.14	9.21	18.14	93.97	1.69	3.68	0.88
32065	M	2567	0.10	1.17	22.26	105.61	1.67	5.29	1.31
Mean		2761	0.36	9.10	20.07	93.92	1.17	4.26	1.01
32052	F	2606	0.27	8.26	15.67	78.87	0.97	4.03	1.01
32056	F	2734	0.37	9.32	16.04	78.96	1.01	3.69	1.21
32056	F	2808	0.36	8.89	19.23	90.22	0.86	4.66	0.99
32037	F	2797	0.15	9.39	14.97	99.88	1.90	6.91	0.75
Mean		2686	0.34	9.09	17.67	86.98	1.18	4.17	1.34

Group mean relative organ weights shown in this table were calculated by averaging the individually calculated relative organ weights.

Chloroethic Anhydride:

TABLE 12. cont.

Three Week Dermal Toxicity Study in Rabbits.
 Absolute (Grams) and Relative (2 Body Weight) Organ Weights.

Group/ Rabbit Number	Sex	Body WT. g	Adrenals		Brain		Kidneys		Liver		Spleen		Testes/ Ovaries		Thyroid	
			R	Z	R	Z	R	Z	R	Z	R	Z	R	Z	R	Z
2500 mg/kg/day:																
32019	M	2277	0.53	2.33	8.51	0.37	21.39	0.94	80.17	3.52	2.19	9.67	3.75	0.17	0.72	0.97
32021	M	2096	0.66	2.79	9.55	0.65	17.66	0.86	80.82	3.86	1.40	6.69	2.17	0.16	0.75	1.19
32031	M	2107	0.76	1.33	8.42	0.37	17.00	0.76	86.68	3.68	1.05	11.75	2.14	0.09	0.66	1.91
32065	M	2582	0.76	1.01	8.35	0.32	17.83	0.69	102.57	3.97	1.53	5.91	3.68	0.14	0.33	1.28
Mean		2314	0.38	1.67	8.68	0.38	18.67	0.80	87.06	3.76	2.04	8.87	2.92	0.13	0.31	1.36
32070	F	2679	0.25	0.93	9.49	0.15	18.86	0.70	118.84	4.44	0.98	3.66	0.23	0.86	0.40	1.69
32068	F	2707	0.42	1.55	9.21	0.34	20.65	0.76	108.06	3.99	1.98	7.31	0.21	0.78	0.30	1.11
32050	F	2131	0.73	1.08	9.32	0.64	17.17	0.82	81.66	3.83	1.69	7.93	0.19	0.89	0.25	1.17
32068	F	2656	0.60	1.63	7.71	0.31	18.85	0.77	105.70	4.30	0.63	2.57	0.21	0.86	0.25	1.02
Mean		2493	0.33	1.30	8.93	0.36	18.93	0.76	103.57	4.14	1.32	5.37	0.21	0.85	0.30	1.20

Group mean relative organ weights shown in this table were calculated by averaging the individually calculated relative organ weights.

Chloroethyl Antihydroxide:
Three Week Dermal Toxicity Study In Rabbits

TABLE 11.
Histomorphological Observations

Tissue Lesion	Group											
	32022	32023	32024	32025	32026	32027	32028	32029	32030	32031	32032	32033
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Skin (Treated)												
dermal inflammatory cell foci												
acanthosis												
hyperkeratosis												
dermal inflammatory cell infiltrate												
Skin (Untreated)												
dermal inflammatory cell infiltrate												
dermal inflammatory cell foci												
acanthosis												
necrotic exudate, epidermal surface												
Brain												
acute suppurative meningitis												
focal gliosis												
lymphocytic meningitis												
perivascular lymphocyte cuffing												
Spinal Cord												
acute suppurative meningitis												
Peripheral Nerve												
Eye												
Pituitary												
Thyroid												
Parathyroid												
Adrenals												
Trachea												
Lung												
multifocal nonsuppurative interstitial pneumonia												
Heart												
multifocal nonsuppurative myocarditis												
Thymus												
Bone Marrow												

Code: x - condition present
 - = not available
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - extreme

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Chlorendic Anthraquinone
 TABLE 13. Cont.
 Three Week Dermal Toxicity Study in Rabbits.
 Histomorphologic Observations.

Tissue Location	7500 mg/kg/day															
	32021	32027	32049	32050	32022	32036	32040	32064	32019	32023	32031	32065	32020	32048	32050	32068
Stomach																
mucosal erosion(s) with fibroplasia of lamina propria																
submucosal edema, lymphocytic infiltrate																
Small Intestine																
nematoide																
Large Intestine																
nematoide																
coccidiosis																
Pancreas																
ectopic splenic tissue																
ectopic splenic tissue																
lymph Node, Mesenteric																
lymph Node, Prefemoral (Regional)																
erythrophagocytosis																
Spleen																
granuloma, capsule																
Liver																
portal lymphocytic infiltrate																
multiple necrotic foci																
lysinic bodies, hepatocyte cytoplasm																
focal fibrosis, subcapsular and portal																
Gallbladder																
Kidneys																
multifocal interstitial nephritis																
scattered tubules with vacuolated epithelium																
ectatic tubules, cortex																
scattered mineralized tubules, cortex																
Urinary Bladder																
Testes/Ovaries																
areas of ectatic, aspermic tubules																
Prostate/Uterus																

Code: x - condition present
 - - - - - not available
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - extreme

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Chloroacetic Anhydride:

TABLE 11. Cont.

Histomorphologic Observations.

Tissue Lesion	Control		2500 mg/kg/day														
	Group	Sex	32021	32027	32029	32037	32038	32040	32064	32019	32023	32031	32065	32020	32048	32050	32068
Skeletal Muscle focal fiber degeneration																	
Miscellaneous w/dm, cysts, lipomatous cysticercosis, omentum																	

Grade: x - condition present
 - - not available
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - extreme

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Chloroacetic Anhydride: Three Week Dermal Toxicity Study in Rabbits.

TABLE 11. Cont.

Histomorphologic Observations

Tissue Lesion	100 mg/kg/day				500 mg/kg/day			
	Group, Rabbit Number, Sex	K	M	F	K	M	F	K
Skin (Treated)	dermal inflammatory cell foci	2	1	2	1	1	1	1
	acanthosis							
	hyperkeratosis		2	2	2	2	2	2
dermal inflammatory cell infiltrate			4	3	4	3	4	2
		1	1	1	1	1	1	1
Skin (Untreated)	dermal inflammatory cell infiltrate							
	acanthosis							
	necrotic exudate, epidermal surface							
dermal inflammatory cell foci					2	2		2
Stomach	minimal erosion(s) with fibroplasia of lamina propria	1	1	1	1	1	1	1
	submucosal edema, lymphocytic infiltrate							

Code: K - condition present
 - = not available
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - extreme

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CODING FORM FOR GLOBAL INDEXING

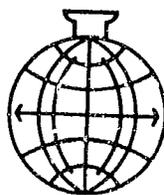
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(31) (5)

Microfiche No. (7) •	0454	1	No. of Pages	2
Doc I.D.	FYI-OTS-1285-0454 SM	3	Old Doc I.D.	4
Case No. (s)				5
Date Produced (6)	10 19 79	6	Date Rec'd (6)	122485
			7	Conf. Code •
				N
Check One:	<input type="checkbox"/> Publication	<input type="checkbox"/> Internally Generated	<input checked="" type="checkbox"/> Externally Generated	
Pub/Journal Name				9
				9
Author(s)				10
Organ. Name	VELSTICOL CHEM CORP			11
Dept/Div				12
P.O. Box		13	Street No./Name	341 E OHIO ST
City	CHICAGO	15	State	IL
			16	Zip
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MID No. (7)		19	D & B NO. (11)	
Contractor	INTL RES & DEV CORP			21
Doc Type	R.I. U.P. FYI.V.S. S.U.B.			22
Doc Title	SUBACUTE INHALATION TOXICITY STUDY IN RATS 20 EXPOSURES IN 28 DAYS			23
Chemical Name (300 per name)	CHLORINDIC ANHYDRIDE	25	CAS No. (10)	115-27-5

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International Research
and Development Corporation

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SPONSOR: Velsicol Chemical Corporation

TEST ARTICLE: Chlorendic Anhydride

SUBJECT: Subacute Inhalation Toxicity Study in
Rats 20 Exposures in 28 Days

DATE OF SUBMISSION: October 19, 1979

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I. QUALITY ASSURANCE STATEMENT

Based on a quality assurance review, it was concluded that this report accurately reflects the data for the Subacute Inhalation Toxicity Study in Rats 20 Exposures in 28 Days with Chlorendic Anhydride.

Approved And
Submitted By:


Barry W. Benson, B.S.
Director of Quality Assurance

12/18/79
Date

II. SYNOPSIS

Male and female rats were exposed to the dusts of chlorendic anhydride for 6 hours per day, 5 days per week for 20 exposures during a 28 day experimental period. The average nominal exposure concentrations were 0, 0.11, 0.99 and 9.97 mg/liter. The aerodynamic equivalent mass medium diameter was calculated to be 6.0 micrometers with a geometric standard deviation of 3.16.

Immediately after the 6 hour exposure period, the rats exhibited varying degrees of ocular and nasal irritation, and salivation in relation to the chlorendic anhydride concentrations. By the following morning these symptoms had generally disappeared. The rats also exhibited alopecia ranging from a slight thinning on the abdomen to large patches lost on the back of some of the rats in the high concentrations group.

The low- and medium-concentration groups and the female rats of the high-concentration group demonstrated weight gains comparable to the rats of the control group. The male rats of the high-concentration exhibited decreased weight gains as compared to the controls.

Statistical differences in hematocrit, erythrocytes and hemoglobin concentrations were observed in male rats while statistical differences in hematocrit, erythrocytes and leucocytes were seen in female rats. Similarly, statistical differences in glucose, alkaline phosphatase and SGPT levels were observed in male rats while statistical differences in alkaline phosphatase levels were observed in female rats.

Increased incidence of dark red foci and dark red area/discoloration in the lungs and dark red or brown foci in glandular part of the stomach seen at necropsy in the treated groups, were probably compound-related.

Statistically significant decreases ($p < 0.05$), probably compound-related, were noted in the mean relative weights of livers of males

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1-31-80
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from all treated groups and in the mean absolute and relative weights of thyroids in female rats from the 1.0-mg/l and 10.0-mg/l groups.

Compound-related microscopic changes of a hemorrhagic inflammatory nature in the lungs and of an inflammatory nature in the trachea, nasal turbinates and stomach mucosa, occurred in rats from all treated groups.

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III. TEST MATERIAL

The compound was received from Velsicol Chemical Company, Chicago, Illinois on October 10, 1977. It was received and identified as follows:

<u>Test Material Identification</u>	<u>Description</u>
Sponsors': Tech. Ref. Std. Chlorendic Anhydride 93.81% (titr.) Lot # 3-12-206	white somewhat chunky powder 7 jars x 300 grams. according to packing slip.

IRDC'S: 6398

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IV. TEST PROCEDURES

A. ANIMALS:

Forty male and 40 female Charles River CD® rats, 6-8 weeks of age, were used in this study. The male rats weighed from 191 to 211 grams. The female rats weighed from 182 to 206 grams. The 40 males were randomized into 4 groups of 10 rats each, in accordance with the IRDC computer randomization program for 40. The females were likewise randomized.

After the randomization process, the rats were individually identified with ear punches in numerical order, from 1 to 40 for the males and from 41 to 80 for the females. Each animal was also given a "permanent" IRDC number which were used in all records. The rats were housed in individually numbered wire-mesh cages and were kept throughout the one week quarantine and the 28 day experimental periods in a temperature- and humidity-controlled room in accordance with standards outlined in the "Guide for the Care and Use of Laboratory Animals, DHEW No. (N.I.H. 74-23) 1974". Purina® Laboratory Chow® and water were supplied ad libitum except when the rats were in the exposure chamber. During exposure, the rats were caged individually in numbered stainless steel cages.

The study was initiated on June 7, 1978 and was terminated on July 5, 1978.

B. EXPOSURE:

All exposures were conducted in a 1-cubic meter cubical stainless steel and glass chamber, with pyramidal top and bottom. A constant chamber airflow was maintained by means of a rotary centrifugal air pump located at the exhaust side of the chamber. The chamber exhaust was filtered through an activated charcoal filter and a Cambridge

Absolute® filter before being further diluted with air and discharged outside of the laboratory. The desired chamber concentrations were 0, 0.1, 1.0 and 10.0 mg/l.

C. DUST ATMOSPHERE GENERATION:

IRAD dust generators were used for generating the dust atmospheres. The DUSTGUN® consisted of a revolving plate with calibrated "cups" for transporting a known quantity of powder per unit time from a reservoir to air jet. At the air jet the powders in a "cup" were dispersed into the chamber by blowing at a rate of 8 liters per minute.

The dusts emerging from the dust generators were directed into the exposure chamber air inlet and were further diluted by the incoming make-up air to the desired concentration.

D. MONITORING OF CHAMBER DUST CONCENTRATIONS:

1. Calculated Concentration:

The concentrations are based upon the empirical chamber airflow rate and the powder dissemination rate. The quantity of powder disseminated was determined by weighing the quantity of powder in the reservoir of the dust generator before and after the experiment. The concentration of the dusts in the chamber atmosphere was calculated from the ratio of the average rates of powder dissemination to the rate of total chamber airflow (the volume of air ejected from the dust generator plus the volume of make-up air passing through the chamber per unit time).

2. Analytical Concentration of Airborne Particles:

The concentration of the airborne dusts in the chamber atmosphere was determined gravimetrically using the fiberglass filter, sampling technique. The chamber atmosphere was drawn through a 37 mm,

fiberglass filter at the rate of 2 liters/min for a suitable duration. A critical orifice was used for regulating the air flow rate. Three samples were taken from each chamber during the 6-hour exposure period. The weight of chlorendic anhydride collected on the filter was determined and the quantity of the chlorendic anhydride powder per unit volume of chamber air was calculated in milligrams/liter.

3. Particle Size Distribution Analysis:

The particle size distribution of the chlorendic anhydride powder in a sample of the chamber atmosphere was determined once daily using an Andersen® 8 stage cascade impactor. The chamber atmosphere was drawn through the sampler at the rate of 28.3 l/min for a suitable duration. The weight of the particles collected on each stage was determined gravimetrically and the weight percent of each size category was calculated. The cumulative weight-percent of each size category was calculated. The cumulative weight-percent of particles smaller than a certain indicated diameter were plotted on a logarithmic probability graph. The equivalent aerodynamic diameter were determined graphically.

E. CLINICAL STUDIES:

1. Animal Observations:

The rats were observed daily for ocular and nasal irritation, respiratory distress and any pharmacotoxic signs before and immediately after the 6-hour exposure.

Individual body weights were recorded periodically before the initiation of the study and twice weekly during the four weeks of exposure.

2. Laboratory Tests:

Clinical laboratory tests were conducted on all male and all

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fiberglass filter at the rate of 2 liters/min for a suitable duration. A critical orifice was used for regulating the air flow rate. Three samples were taken from each chamber during the 6-hour exposure period. The weight of chlorendic anhydride collected on the filter was determined and the quantity of the chlorendic anhydride powder per unit volume of chamber air was calculated in milligrams/liter.

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Approved And
Submitted By:

Charles E. Ulrich
Charles E. Ulrich, B.S.
Study Director

1-31-80
Date

female rats from the four groups at the termination of the exposure period. The rats were fasted overnight and the blood was obtained by the orbital sinus puncture technique. The following clinical laboratory test were conducted:

a. Hematology:

Hematological studies included hemoglobin, hematocrit, erythrocyte count and morphology, leucocyte count/total and differential, bone marrow smear/differential count only if indicated by blood hematologic evaluation.

b. Biochemistry:

Biochemical studies included alkaline phosphatase, blood urea nitrogen, serum glutamic pyruvic transaminase and fasting blood sugar.

F. GROSS AND HISTOPATHOLOGICAL EXAMINATION:

At the termination of the study the rats from all groups were sacrificed for gross and histopathological examinations.

G. STATISTICAL ANALYSIS:

All statistical analyses compared the treatment groups with the control group, by sex.

The hematological and biochemical parameters (1 month) and absolute and relative organ weights (terminal) were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie¹ using Dunnett's² multiple comparison tables to judge significance of differences.

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a. Hematology:

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Approved And
Submitted By:

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Charles E. Ulrich, B.S.

Study Director

1-31-80
Date

163-531

V. RESULTS

A. CHAMBER DUST CONCENTRATION:

1. Nominal and Analytical Concentrations:

Nominal concentrations of chlorendic anhydride are presented in Table 1. These concentrations are only slightly different from the desired chamber concentrations of 0.1, 1.0 and 10.0 mg/l, respectively. Gravimetric analysis using fiberglass filters resulted in mean analytical chamber concentrations of 0.004, 0.01 and 0.08 mg/l as shown in Table 1. These values represent 4.0%, 1.0% and 0.8% of the respective desired concentrations. The daily variations in concentrations and discrepancy between nominal and analytical concentration is due in part to: 1) the number of large particles in the dust which were too heavy to remain air borne; and 2) the quantity of fine dust that adhered to chamber walls because of the electrostatic charge produced in generating fine dust atmosphere.

2. Dust Particle Size Distribution Analysis:

Figure 1 presents a composite aerosol size distribution graph for the three exposure concentrations. For all three levels the equivalent aerodynamic diameter was 6.0 micrometers with a geometric standard deviation of 3.16.

B. CLINICAL STUDIES:

1. Appearance and General Behavior:

The rats exhibited varying degrees of ocular and nasal irritation, salivation and hair loss in relation to the chlorendic anhydride concentrations.

1: the group exposed to 9.97 mg/liter, all the rats, except one, exhibited at various times and in varying degrees, red-tinged nasal and ocular discharge when observed immediately after exposure. Salivation was also noted in some of the rats. When observed the

V. RESULTS

A. CHAMBER DUST CONCENTRATION:

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Nominal concentrations of chlorendic anhydride are presented in Table 1. These concentrations are only slightly different from the desired chamber concentrations of 0.1, 1.0 and 10.0 mg/l, respectively. Gravimetric analysis using fiberglass filters resulted in mean analytical chamber concentrations of 0.004, 0.01 and 0.08 mg/l as shown in Table 1. These value represent 4.0%, 1.0% and 0.8% of the respective desired concentrations. The daily variations in concentrations and discrepancy between nominal and analytical concentration is due in part to : 1) the number of large particles in the dust which were too heavy to remain air borne; and 2) the quantity of fine dust that adhered to chamber walls because of the electrostatic charge produced in generating fine dust atmosphere.

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B. CLINICAL STUDIES:

1. Appearance and General Behavior:

The rats exhibited varying degrees of ocular and nasal irritation, salivation and hair loss in relation to the chlorendic anhydride concentrations.

In the group exposed to 9.97 mg/liter, all the rats, except one, exhibited at various times and in varying degrees, red-tinged nasal and ocular discharge when observed immediately after exposure. Salivation was also noted in some of the rats. When observed the

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2-14-80
Date

following morning, before exposure, these toxic signs had generally disappeared. Also, in half of the animals, alopecia was observed, ranging from slight thinning on the abdomen to large patches on the back of the animals in the high-concentration group.

These symptoms appeared in the low and intermediate groups but with less severe effects and were exhibited by fewer animals.

It should be noted that 2 of the control rats also displayed slight alopecia.

2. Body Weights:

Group mean body weights are presented in Table 2. The male rats of the high-concentration group showed depressed weight gains as compared to the controls. The low- and medium-concentration groups and the female rats of the high-concentration gained weight comparable to the rats of the control group. Individual body weights for each group are presented in Tables 3-6.

3. Laboratory Tests:

a. Hematology:

Group mean hematological values for male rats are presented in Table 7. Hematocrit values are significantly depressed from control values in all three exposure groups. In addition, the number of erythrocytes and hemoglobin levels were significantly depressed in the low-concentration group.

Group-mean hematological values for female rats are given in Table 8. The hematocrit value for the low-concentration group was significantly depressed from control values. The number of erythrocytes was significantly increased for the medium- and high-concentration groups while the number of leucocytes was significantly increased in the high-concentration group. Individual hematological values for all four groups are given in Tables 9-12.

Even though these changes in values for both males and females are statistically significant, these values probably do not represent any biological significance since they fall within the normal range of biological variation for the species.

b. Biochemistry:

Group-mean blood chemistry values for male rats are given in Table 13. For male, the fasting blood glucose level was significantly depressed from control values in the high-concentration group. Also for males, alkaline phosphatase values were significantly increased in the intermediate- and high-concentration groups while the SGPT level was significantly increased in the intermediate-concentration group. Group-mean values for female rats are presented in Table 14. For females, alkaline phosphatase was significantly increased in the intermediate- and high-concentration groups. Even though the changes in alkaline phosphatase and SGPT values were statistically significant, these values probably do not represent an exposure related effect since these values are within the normal range of biological variation for the species. Individual blood chemistry values for all four groups are given in Tables 15-18.

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Even though these changes in values for both males and females are statistically significant, these values probably do not represent any biological significance since they fall within the normal range of biological variation for the species.

b. Biochemistry:

Group-mean blood chemistry values for male rats are given in Table 13. For males, the fasting blood glucose level was significantly depressed from control values in the high-concentration group. Also for males, alkaline phosphatase values were significantly increased in the intermediate- and high-concentration groups while the SGPT level was significantly increased in the intermediate-concentration group. Group-mean values for female rats are presented in Table 14. For females, alkaline phosphatase was significantly increased in the intermediate- and high-concentration groups. Even though the changes in alkaline phosphatase and SGPT values were statistically significant, these values probably do not represent an exposure related effect since these values are within the normal range of biological variation for the species. Individual blood chemistry values for all four groups are given in Tables 15-18.

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VI. PATHOLOGICAL STUDIES

A. METHODS:

1. Gross Pathology:

At the termination of 4 week period of study, all rats were sacrificed using carbon dioxide gas and were necropsied. One rat each from the 1.0-mg/1 and the 10.0-mg/1 group that died after bleeding at necropsy were also included under the terminal sacrifice. Selected organs were weighed and representative tissues were preserved in 10% neutral formalin.

2. Histopathology:

The following tissues from 20 rats from the control group and the 10.0-mg/1 group were paraffin embedded, sectioned, stained with hematoxylin and eosin and examined microscopically:

adrenals	nasal turbinates
aorta	pancreas
brain (2 sections)	pituitary
bone - rib junction	salivary gland
gonad	mammary gland
heart (with coronary vessels)	skin
duodenum	spleen
jejunum	stomach
ileum	thymus
esophagus	thyroids
colon	urinary bladder
kidneys	uterus
liver (2 sections)	trachea
lungs (2 sections)	gross lesions
mesenteric lymph node	

Additionally, from 20 rats each from the 0.1-mg/1 and 1.0-mg/1 groups, nasal turbinates, trachea, lungs and stomach were processed similarly and examined. (Microscopic examination of tissues from the high dose group, 10.0-mg/1, did not suggest possible treatment related lesions in any other organs.)

B. RESULTS:

1. Gross Pathology and Organ Weights (Tables 19-22):

Gross lesions that were considered probably treatment related were seen in the lung and stomach.

In the lungs, these consisted of increased incidence of dark red foci and dark red areas/discoloration and in the stomach, dark red or brown foci on the glandular mucosa.

Other gross lesions observed in the control and the treatment groups were considered spontaneous or incidental in nature and were not treatment related.

Statistically significant decreases ($p < 0.05$) in mean relative weights of livers in males in all treated groups and the mean absolute and relative weights of thyroids in female rats in the 1.0-mg/l and 10.0-mg/l groups, were considered probably compound-related. The variations in the mean weights seen in adrenals and pituitary were not consistent and their biological significance is unknown.

2. Histopathology (Tables 23-25):

Compound-related microscopic changes were observed in treated groups at all levels in the lungs, trachea, nasal turbinates and glandular part of the stomach.

In the lungs, the lesions consisted of increased incidence and intensity in interstitial inflammatory cell infiltration, peribronchial lymphoid hyperplasia, perivascular lymphocytic infiltration, interstitial fibrosis and scattered hemorrhages. Interstitial pneumonia occurred only in the treated groups.

In the trachea, the changes included inflammatory cell infiltration in mucosa and submucosa and focal epithelial hyperplasia.

In the nasal turbinates, changes seen were inflammatory cell infiltrate in mucosa and dark brown pigment and/or inflammatory exudate in the nasal cavity.

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In the glandular part of the stomach, inflammatory cell infiltrate in mucosa and submucosa and focal mucosal congestion in the 10.0-mg/1 group and focal mucosal congestion in the 1.0-mg/1 and 0.1-mg/1 group were seen.

Other organs in which microscopic lesions occurred included adrenals (excessive vacuolation in cortical cells), liver (portal inflammatory cell infiltration, scattered inflammatory foci), kidney (interstitial lymphocytic infiltration, healed infarct), uterus (hydrometra), and cervical lymph node (congestion, erythrophagocytosis). These lesions were of low or equal frequency in both the control and the high dose groups and were considered spontaneous and incidental in nature and not related to treatment.

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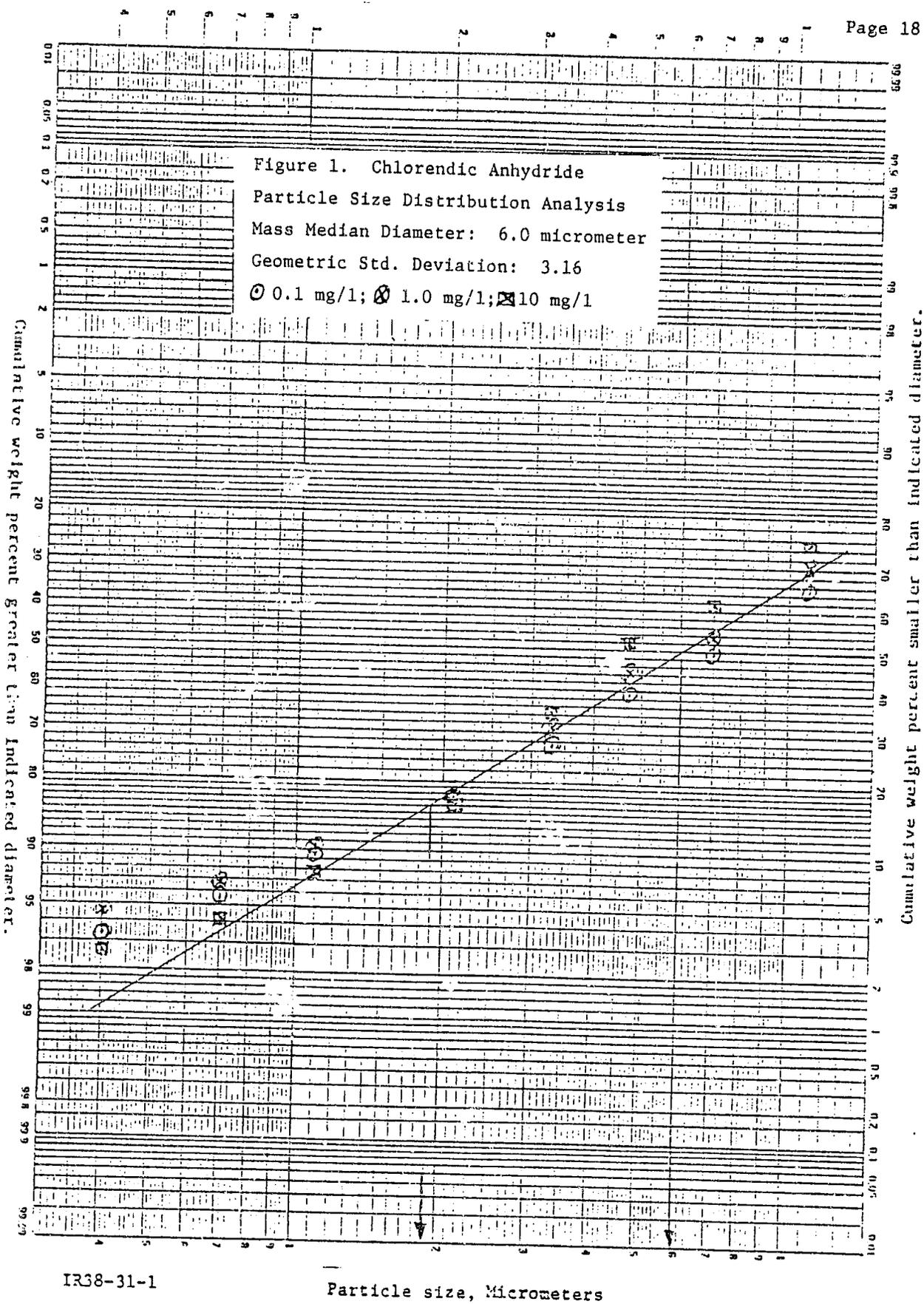
Chlorendic Anhydride:

Subacute Inhalation Toxicity Study in Rats - 4 Weeks

TABLE 1. Means and Standard Deviations of the Nominal and Analytical Chamber Concentrations

Exposure Day	0.0 mg/l (Control)	0.1 mg/l		1.0 mg/l		10.0 mg/l	
		Nominal (mg/l)	Analytical (mg/l) N=3	Nominal (mg/l)	Analytical (mg/l) N=3	Nominal (mg/l)	Analytical (mg/l) N=3
1	0	0.098	0.0027 + 0.0006	1.240	0.0088 + 0.0045	10.41	0.051 + 0.007
2	0	0.085	0.0022 + 0.0009	0.946	0.0200 + 0.0225	8.55	0.155 + 0.103
3	0	0.124	0.0073 + 0.0065	0.767	0.0109 + 0.0017	9.72	0.031 + 0.005
4	0	0.149	0.0030 + 0.0011	0.958	0.0126 + 0.0015	5.79	0.082 + 0.094
5	0	0.116	0.0022 + 0.0011	0.932	0.0058 + 0.0014	12.04	0.142 + 0.160
6	0	0.109	0.0026 + 0.0003	1.098	0.0107 + 0.0018	11.34	0.100 + 0.041
7	0	0.124	0.0047 + 0.0008	0.964	0.0263 + 0.0135	12.00	0.082 + 0.063
8	0	0.060	0.0017 + 0.0006	0.983	0.0067 + 0.0007	11.87	0.043 + 0.007
9	0	0.321	0.0103 + 0.0009	0.953	0.0133 + 0.0073	10.05	0.017 + 0.006
10	0	0.122	0.0032 + 0.0007	1.096	0.0091 + 0.0027	10.18	0.050 + 0.033
11	0	0.063	0.0016 + 0.0003	0.830	0.0056 + 0.0027	9.48	0.022 + 0.001
12	0	0.102	0.0045 + 0.0047	0.935	0.0117 + 0.0017	10.43	0.123 + 0.162
13	0	0.094	0.0032 + 0.0004	1.094	0.0070 + 0.0042	9.05	0.022 + 0.005
14	0	0.066	0.0027 + 0.0012	0.927	0.0063 + 0.0009	9.45	0.034 + 0.009
15	0	0.068	0.0097 + 0.0051	0.992	0.0142 + 0.0025	9.15	0.370 + 0.116
16	0	0.057	0.0044 + 0.0023	0.993	0.0062 + 0.0025	10.45	0.054 + 0.009
17	0	0.105	0.0123 + 0.0073	1.072	0.0112 + 0.0050	9.70	0.056 + 0.017
18	0	0.127	0.0059 + 0.0020	1.115	0.0087 + 0.0009	9.91	0.038 + 0.005
19	0	0.069	0.0026 + 0.0009	1.137	0.0092 + 0.0038	10.06	0.030 + 0.010
20	0	0.133	0.0018 + 0.0009	0.679	0.0052 + 0.0004	9.86	0.048 + 0.012
Mean ± Std. Dev.		0.11±0.06	0.004 + .004	0.99±0.13	0.01 ± 0.007	9.97 ± 1.38	0.08 ± 0.01
No. of determinations		N=20	N=60	N=20	N=60	N=20	N=60

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IR38-31-1

Particle size, Micrometers

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Chloroacetic Anhydride:

TABLE 2. Subacute Inhalation Toxicity Study in Rats
Group Mean Body Weights, Grams; Weight Ranges; and Survival

Days on Exposure	Days Exposed	0.0 mg/l. (Control)			0.1 mg/l.			1.0 mg/l.			10.0 mg/l.		
		Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival
MALES:													
1	0	200	197-211	10/10	199	193-208	10/10	198	191-206	10/10	203	197-211	10/10
1	7	216	207-224	10/10	210	203-218	10/10	208	202-218	10/10	208	202-218	10/10
6	3	239	228-251	10/10	231	220-239	10/10	213	224-244	10/10	227	205-238	10/10
10	7	262	245-282	10/10	251	235-269	10/10	256	237-292	10/10	264	226-257	10/10
11	8	283	256-322	10/10	268	246-292	10/10	276	259-294	10/10	271	236-278	10/10
17	12	309	281-350	10/10	289	256-319	10/10	297	274-318	10/10	299	255-306	10/10
20	13	327	300-375	10/10	305	266-340	10/10	317	293-336	10/10	301	265-323	10/10
24	17	346	318-404	10/10	327	285-362	10/10	339	310-364	10/10	304	275-334	10/10
27	18	360	330-418	10/10	336	291-373	10/10	348	316-378	10/10	314	284-345	10/10
28	19	363	334-422	10/10	335	294-375	10/10	352	322-379	10/10	316	285-349	10/10
29	20	326	292-373	10/10	300	260-339	10/10	317	290-344	10/10	283	255-370	10/10
FEMALES:													
1	0	197	182-200	10/10	195	188-202	10/10	195	183-206	10/10	192	183-201	10/10
1	7	197	187-204	10/10	198	190-206	10/10	197	183-213	10/10	192	180-198	10/10
6	3	203	188-214	10/10	206	199-214	10/10	205	190-218	10/10	201	188-213	10/10
10	7	210	197-217	10/10	210	201-216	10/10	214	201-227	10/10	204	186-215	10/10
13	8	217	208-223	10/10	221	217-234	10/10	218	207-230	10/10	208	188-223	10/10
17	12	226	220-234	10/10	229	215-252	10/10	222	207-235	10/10	210	187-224	10/10
20	13	230	226-237	10/10	237	217-260	10/10	229	218-242	10/10	221	199-241	10/10
24	17	235	230-242	10/10	236	206-255	10/10	231	220-247	10/10	223	200-243	10/10
27	18	242	235-253	10/10	244	226-262	10/10	239	226-250	10/10	210	206-243	10/10
28	19	241	234-252	10/10	243	227-257	10/10	236	226-248	10/10	229	208-250	10/10
29	20	218	209-229	10/10	218	200-237	10/10	216	201-230	10/10	206	190-236	10/10

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Chloroethic Anhydride:
Subacute Inhalation Toxicity Study in Rats

TABLE 1.

Group, Rat Number	Sex	Individual Body Weights, Grams										
		Days on Experiment					Days Exposed					
		1	3	6	10	13	17	20	26	27	28	29
90848	H	195	215	243	267	290	307	327	365	354	354	318
90851	H	200	217	219	260	277	312	330	353	361	354	318
90857	H	205	224	242	262	278	296	313	336	360	372	360
90858	M	195	208	228	252	276	300	320	336	344	344	313
90861	H	211	226	251	282	322	350	375	404	422	422	322
90866	M	197	212	230	250	256	281	300	318	330	334	373
90868	M	205	220	247	275	302	326	337	367	388	388	292
90874	H	204	219	247	273	296	323	339	360	376	376	360
90877	M	192	209	234	265	273	310	325	338	352	352	322
90884	M	195	207	232	251	262	290	306	323	333	336	305
90897	F	182	194	197	208	215	223	228	237	243	243	218
90898	F	197	203	214	215	222	233	229	238	250	243	226
90903	F	185	187	188	197	209	220	228	233	235	236	213
90905	F	192	204	205	216	218	226	232	240	244	244	224
90910	F	192	198	208	217	221	234	237	242	253	252	229
90911	F	188	188	201	203	208	222	228	230	240	240	213
90918	F	199	202	207	214	220	227	232	235	242	246	217
90920	F	191	201	204	206	223	225	227	230	238	236	216
90935	F	200	199	209	215	218	225	226	231	235	236	209
90940	F	190	196	200	213	217	228	237	246	246	243	218

0.0 mg/l (Control):

Chloroform Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 4.

Group, Rat Number	Sex	Individual Body Weights, Grams											
		Days on Experiment					Days Exposed						
		1	2	3	6	10	13	17	20	24	26	27	28
08854	M	198	206	232	259	280	295	322	324	331	324	331	291
08862	H	201	208	229	270	298	314	335	365	334	373	375	317
08867	N	205	214	236	289	319	360	362	373	375	375	375	339
08869	M	196	209	230	256	272	281	305	310	308	310	308	275
08880	H	193	204	227	265	281	305	320	332	345	345	345	299
08882	M	201	211	228	255	277	293	320	324	333	332	333	292
08885	M	208	217	239	292	313	329	362	354	338	354	338	303
08887	M	194	205	226	264	279	299	324	330	338	330	338	297
08888	M	194	203	220	246	256	266	285	291	294	291	294	260
08894	H	200	218	239	283	310	329	353	355	358	355	358	325
09006	F	194	203	211	234	235	242	240	248	250	248	250	226
09012	F	191	193	201	222	222	233	232	236	236	236	236	214
09023	F	196	202	214	223	229	239	235	243	246	246	246	225
09025	F	202	202	209	222	228	233	236	235	236	235	236	200
09026	F	193	193	201	219	226	233	236	247	243	247	243	215
09028	F	190	190	199	214	216	234	267	248	250	248	250	218
09030	F	188	192	200	212	215	217	220	226	227	226	227	200
09031	F	199	199	209	222	234	238	260	268	260	268	260	230
09034	F	202	201	207	213	228	236	234	242	252	242	237	215
09044	F	195	206	212	233	252	260	255	262	257	262	257	237

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Chloroform Anhydride: Subacute Inhalation Toxicity Study in Rats
 TABLE 5.

Group Rat Number	Sex	Individual Body Weights, Grams												
		Days on Experiment					Days Exposed							
		1	3	6	10	13	17	20	24	27	31			
90852	M	191	203	224	245	260	276	293	317	342	360	377	393	407
90855	M	198	203	230	266	262	282	293	316	340	360	377	393	407
90865	M	193	202	226	237	259	276	296	316	340	360	377	393	407
90871	F	195	205	230	253	278	303	326	352	378	400	425	450	475
90873	M	199	210	235	256	274	295	318	340	362	385	407	430	452
90876	M	194	203	231	269	276	305	332	353	378	400	425	450	475
90878	M	203	218	237	292	292	308	332	353	378	400	425	450	475
90881	M	206	216	246	267	294	318	336	359	368	390	415	440	465
90883	M	200	212	237	265	293	311	331	353	368	390	415	440	465
90889	M	198	208	233	251	271	292	314	337	358	382	405	428	451
90906	F	197	206	209	218	228	235	236	244	250	257	264	271	278
90908	F	206	213	218	227	230	232	238	243	250	257	264	271	278
90915	F	185	190	198	207	208	210	218	220	226	230	238	248	258
90917	F	200	205	210	213	215	221	223	220	226	230	238	248	258
90926	F	194	196	201	203	207	208	221	220	226	230	238	248	258
90933	F	197	193	202	214	224	226	221	226	230	238	248	258	268
90936	F	181	183	190	201	211	205	210	211	218	227	234	241	248
90938	F	191	198	211	219	218	224	234	235	244	251	258	265	272
90942	F	192	194	201	209	223	228	234	235	244	251	258	265	272
90945	F	200	189	210	224	217	226	234	237	246	253	260	267	274

1.0 mg/l:

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Chloroethane Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 6. Individual Body Weights, Grams

Group, Rat Number	Sex	Days on Experiment										
		1	2	3	6	7	8	10	13	17	20	
90866	M	206	214	217	257	271	299	308	325	324	332	310
90867	M	197	204	205	226	236	251	265	275	284	288	255
90868	H	203	207	227	267	252	260	275	277	295	285	258
90869	M	205	210	228	246	263	270	288	300	307	311	276
90870	M	197	202	218	233	251	261	281	295	313	320	275
90871	M	211	212	234	257	270	286	317	312	345	369	309
90872	M	206	213	238	250	278	306	323	336	362	364	320
90873	M	199	204	224	233	265	251	277	288	301	300	274
90874	M	201	215	234	254	263	274	300	308	323	326	288
90875	M	204	203	228	241	255	260	280	293	302	302	266
90896	F	190	192	206	215	223	270	229	227	238	231	218
90901	F	190	189	198	199	198	195	204	216	223	218	200
90907	F	191	180	188	186	188	187	199	200	206	208	190
90909	F	201	196	213	215	221	224	241	239	243	250	202
90914	F	183	188	189	195	198	208	216	220	223	223	205
90919	F	197	198	205	210	216	223	233	263	241	243	226
90927	F	188	197	203	200	204	197	200	217	230	225	206
90929	F	197	191	198	203	209	213	229	223	234	232	206
90937	F	196	196	202	206	205	208	220	223	215	222	200
90941	F	193	195	208	209	222	222	229	234	243	238	214

10.0 mg/l:

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Chlorendic Anhydride:

Subacute Inhalation Toxicity Study in Rats

TABLE 7.

MALES: Means and Significance of Hematological Values - Terminal

Hematology	0.0 mg/l (Control)	0.1 mg/l	1.0 mg/l	10.0 mg/l
Erythrocytes, 10 ⁶ /cmm	7.15	6.69*	7.03	7.43
Hemoglobin, g/100 ml	15.8	14.3**	15.4	15.8
Hematocrit, %	50	45**	47**	48*
Leucocytes, 10 ³ /cmm	12.61	11.94	13.31	13.81

* Significantly Different from Control Group Mean; p<0.05

** Significantly Different from Control Group Mean; p<0.01

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Chloroform Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 8.

FEMALES: Means and Significance of Hematological Values - Terminal

Hematology	0.0 mg/l (Control)	0.1 mg/l	1.0 mg/l	10.0 mg/l
Erythrocytes, 10 ⁶ /cmm	6.73	6.97	7.18**	7.06*
Hemoglobin, g/100 ml	15.0	14.8	15.4	15.0
Hematocrit, %	48	45*	47	46
Leucocytes, 10 ³ /cmm	10.79	10.08	11.02	13.63*

* Significantly Different from Control Group Mean; p<0.05

** Significantly Different from Control Group Mean; p<0.01

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Subacute Inhalation Toxicity Study in Rats

Chloroacetic Anhydride (4):

TABLE 9.

Group, Rat Number	Sex	Individual Hematological Values - Treatment										
		Erythrocytes 10 ⁶ /cmm	Erythrocyte Appear.	Nucleated Erythrocytes	Hemo-globin g/100 ml	Hemato-crit %	Leucocytes 10 ³ /cmm	Segm. Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophilis
0.0 mg/l. (Control):												
908548	M	7.42	Normal	-	16.2	50	11.23	11	0	0	0	0
908551	M	7.22	Normal	-	16.0	51	13.42	18	0	0	0	0
908557	M	6.91	Normal	-	15.2	49	11.36	22	1	0	0	0
908558	M	6.89	Normal	-	15.7	49	12.00	10	0	0	0	0
908611	M	7.31	Normal	-	16.6	51	13.24	26	1	2	0	0
908666	M	7.20	Normal	-	16.3	51	11.91	22	0	0	0	0
908668	M	7.33	Normal	-	15.4	50	12.28	13	0	0	0	0
908776	M	7.00	Normal	-	15.5	48	13.67	31	0	2	1	0
908777	M	6.96	Normal	-	16.0	51	11.17	8	0	5	1	0
908836	M	7.24	Normal	-	15.4	48	15.83	71	0	1	1	0
Mean		7.15			15.8	50	12.61	23	0	1	1	0
90897	F	6.88	Normal	2	15.4	49	7.38	32	2	1	1	0
90898	F	6.96	Normal	1	15.3	49	8.46	11	1	0	0	0
90903	F	6.87	Normal	-	14.3	45	9.17	9	0	2	0	0
90905	F	7.28	Normal	-	14.5	48	6.86	12	0	0	0	0
90910	F	6.57	Normal	-	15.9	49	14.16	23	1	1	0	0
90911	F	6.55	Normal	-	14.7	46	12.87	18	1	2	1	0
90918	F	6.58	Normal	-	14.6	47	14.62	21	3	0	0	0
90920	F	6.85	Normal	-	15.7	50	12.23	15	0	0	0	0
90935	F	6.40	Normal	-	14.7	47	10.04	14	0	1	1	0
90940	F	6.41	Normal	-	15.0	48	12.13	16	0	2	2	0
Mean		6.73			15.0	48	10.79	17	1	1	1	0

Code: - None Seen

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Chlorendic Acrylate:

Subacute Inhalation Toxicity Study In Rats

TABLE 10.

Group, Rat Number	Sex	Erythrocytes 10 ⁶ /cmm	Erythrocyte Appear.	Nucleated Erythrocytes	Hemoglobin g/100 ml	Reticulocyte %		Leucocytes 10 ⁶ /cmm	Neutrophils		Lymphocytes		Eosinophils		Monocytes		Basophils		
						Z	Z		Z	Z	Z	Z	Z	Z	Z	Z	Z	Z	
0.1 mg/l:																			
90854	M	5.46	11 Poly	4	11.7	37		9.72	13	1	84	2	0	0	0	0	0	0	
90862	M	6.76	Normal	-	15.1	44		13.14	10	0	90	0	0	0	0	0	0	0	
90867	M	6.65	Normal	-	13.8	42		12.52	20	0	79	0	0	0	0	0	0	0	
90869	M	6.76	Normal	-	14.3	47		13.60	21	0	77	2	0	0	0	0	0	0	
90880	M	6.71	Normal	-	15.1	49		12.57	24	1	73	1	0	0	0	0	0	0	
90882	M	7.07	Normal	-	14.4	46		12.38	9	0	89	0	0	0	0	0	0	0	
90885	M	6.94	Normal	-	15.2	48		10.70	10	0	90	0	0	0	0	0	0	0	
90887	M	6.67	Normal	-	14.7	47		15.96	19	0	80	0	0	0	0	0	0	0	
90888	M	6.48	Normal	-	13.0	45		7.27	25	0	73	2	0	0	0	0	0	0	
90894	M	7.42	Normal	-	15.8	49		11.72	12	1	85	0	0	0	0	0	0	0	
Mean		6.69			14.3	45		11.94	16	0	82	1	0	0	0	0	0	0	
90906	F	7.23	Normal	-	15.0	47		7.12	36	3	60	0	0	0	0	0	0	0	
90912	F	6.98	Normal	-	13.7	43		10.67	7	0	92	0	0	0	0	0	0	0	
90923	F	7.33	Normal	-	15.7	47		9.47	12	0	88	0	0	0	0	0	0	0	
90925	F	6.44	Normal	-	13.5	40		12.00	17	0	80	2	0	0	0	0	0	0	
90926	F	6.87	Normal	-	14.7	45		7.52	17	1	80	1	0	0	0	0	0	0	
90928	F	6.72	Normal	-	14.7	45		8.29	20	0	78	1	0	0	0	0	0	0	
90930	F	7.05	Normal	-	14.5	44		14.03	16	0	81	2	0	0	0	0	0	0	
90931	F	7.16	Normal	-	15.5	48		10.00	12	0	87	0	0	0	0	0	0	0	
90934	F	6.94	Normal	-	14.9	48		11.00	10	0	87	2	0	0	0	0	0	0	
90944	F	6.95	Normal	-	15.3	47		10.72	30	1	62	5	0	0	0	0	0	0	
Mean		6.97			14.8	45		10.08	18	1	79	1	0	0	0	0	0	0	

Code: Poly - Polychromasia
- None Seen

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000036

Chloroendic Anhydride:

Subacute Inhalation Toxicity Study In Rats

TABLE II:

Individual Hematological Values - Terminal

Group, Rat Number	Sex	Erythro-cytes 10 ⁶ /cmm	Erythro-cyte Appear.	Nucleated Erythro-cyte	Hemo-globin g/100 ml	Hemato-crit %	Leuco-cytes 10 ³ /cmm	Neutrophils		Lympho-cytes		Eosino-philis		Mono-cytes		Baso-philis	
								Seg.	Non-Seg.	Z	Z	Z	Z	Z	Z	Z	Z
90852	M	6.64	Normal	-	14.5	45	17.78	10	0	90	0	0	0	0	0	0	0
90855	M	6.87	Normal	-	16.0	49	15.74	19	0	78	1	1	0	2	0	0	0
90865	M	6.77	Normal	-	14.7	46	11.73	4	0	96	0	0	0	0	0	0	0
90871	M	6.65	Normal	-	14.7	47	12.58	23	0	74	0	0	0	3	0	0	0
90873	M	7.22	Normal	-	14.9	46	11.39	12	0	87	0	0	0	1	0	0	0
90876	M	7.26	Normal	-	16.2	69	11.62	30	0	69	0	0	0	1	0	0	0
90878	M	6.91	Normal	-	14.8	44	16.84	38	0	60	0	0	0	2	0	0	0
90881	M	7.53	Normal	-	16.5	50	14.26	14	0	84	0	0	0	2	0	0	0
90883	M	7.33	Normal	-	16.0	48	12.86	7	0	91	0	0	0	2	0	0	0
90889	M	7.10	Normal	-	15.2	47	11.30	20	1	76	2	0	0	1	0	0	0
Mean		7.03			15.4	47	13.31	18	0	81	0	0	0	1	0	0	0
90904	F	7.05	Normal	-	15.8	48	10.07	16	0	84	0	0	0	0	0	0	0
90908	F	6.89	Normal	-	13.9	43	9.11	18	0	80	2	2	0	0	0	0	0
90915	F	7.46	Normal	-	15.6	46	12.72	19	0	79	2	2	0	0	0	0	0
90917	F	6.98	Normal	-	15.5	47	9.92	19	0	78	2	2	0	0	0	0	0
90924	F	7.18	Normal	-	15.8	47	8.99	8	0	90	0	0	0	2	0	0	0
90933	F	7.32	Normal	-	16.4	50	12.00	15	0	84	0	0	0	0	0	0	0
90936	F	7.44	Normal	-	15.1	46	11.79	11	0	89	0	0	0	0	0	0	0
90938	F	6.77	Normal	-	14.7	46	12.67	18	2	78	1	1	0	1	0	0	0
90942	F	7.22	Normal	-	15.6	46	12.60	10	0	90	0	0	0	0	0	0	0
90945	F	7.47	Normal	-	15.5	46	10.28	12	0	86	0	0	0	2	0	0	0
Mean		7.18			15.4	47	11.02	15	0	83	1	1	0	1	0	0	0

Code: - None Seen

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000037

Chloroform Anhydride:

Subacute Inhalation Toxicity Study in Rats

TABLE 11.

Individual Hematological Values - Terminal

Group, Rat Number	Sex	Erythro-cytes 10 ⁶ /cmm	Erythro-cyte Appear.	Nucleated Erythro-cyte	Hemo-globin g/100 ml	Hemato-crit %	Leuco-cytes 10 ⁶ /cmm	Neutrophils		Lympho-cytes %	Eosino-phil %	Mono-cytes %	Baso-phil %
								Seg.	Non-Seg.				
90852	M	6.64	Normal	-	14.5	45	12.78	19	0	90	0	0	0
90855	M	6.87	Normal	-	16.0	49	15.74	19	0	78	1	2	0
90865	M	6.77	Normal	-	14.7	46	13.73	4	0	96	0	0	0
90871	M	6.65	Normal	-	14.7	47	12.58	23	0	74	0	3	0
90873	M	7.22	Normal	-	14.9	46	11.39	12	0	87	0	1	0
90876	M	7.26	Normal	-	16.2	49	11.62	30	0	69	0	1	0
90878	M	6.91	Normal	-	14.8	44	16.84	38	0	60	0	2	0
90881	M	7.53	Normal	-	16.5	50	14.26	14	0	84	0	2	0
90883	M	7.33	Normal	-	16.0	48	12.84	7	0	91	0	2	0
90889	M	7.10	Normal	-	15.2	47	11.30	20	1	76	2	1	0
Mean		7.03			15.4	47	13.31	18	0	81	0	1	0
90904	F	7.05	Normal	-	15.8	48	10.07	16	0	84	0	0	0
90908	F	6.89	Normal	-	13.9	43	9.11	18	0	80	2	0	0
90915	F	7.46	Normal	-	15.6	46	12.72	19	0	79	2	0	0
90917	F	6.98	Normal	-	15.5	47	9.92	39	0	78	2	1	0
90924	F	7.18	Normal	-	15.8	47	8.99	8	0	90	0	2	0
90931	F	7.32	Normal	-	16.4	50	12.00	5	0	84	1	0	0
90936	F	7.44	Normal	-	15.1	46	11.79	11	0	89	1	0	0
90938	F	6.77	Normal	-	14.7	46	12.67	18	2	78	1	1	0
90942	F	7.22	Normal	-	15.6	46	12.00	10	0	90	0	0	0
90945	F	7.47	Normal	-	15.5	46	10.28	12	0	86	0	2	0
Mean		7.18			15.4	47	11.02	15	0	83	1	1	0

Approved And Submitted By:

Charles E. Birch
Charles E. Birch, B.S.
Study Director

1-31-80
Date

Code: - None Seen
Spin - Sample lost during centrifugation

16J-511

000038

Chlorethide Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 12.

Group, Rat Number	Sex	Erythrocytes 10 ⁶ /cmm	Erythrocyte Appear.	Nucleated Erythrocytes	Hemoglobin g/100 ml	Hematocrit	Leucocytes 10 ⁶ /cmm	Neutrophils		Lymphocytes		Eosinophils		Monocytes		Basophils	
								Seg.	Non-Seg.	Z	Z	Z	Z	Z	Z	Z	Z
10.0 mg/l:																	
90866	M	7.58	Normal	-	15.5	48	10.76	9	0	89	1	1	0	0	0	0	0
90867	M	7.63	Normal	-	15.2	45	10.74	14	0	86	0	0	0	0	0	0	0
90856	M	8.07	Normal	-	16.5	49	14.93	13	0	84	3	0	0	0	0	0	0
90863	M	6.92	Normal	-	15.7	47	13.57	12	0	87	1	0	0	0	0	0	0
90864	M	7.67	Normal	-	15.2	46	11.58	25	0	75	0	0	0	0	0	0	0
90870	M	7.64	Normal	-	16.0	48	14.44	27	0	73	0	0	0	0	0	0	0
90872	M	6.81	Normal	-	15.7	47	18.13	18	2	80	0	0	0	0	0	0	0
90879	M	7.46	Normal	-	16.3	51	14.16	14	1	85	0	0	0	0	0	0	0
90890	M	7.16	Normal	-	15.5	47	16.36	33	3	63	0	0	0	0	0	0	0
90895	M	7.51	Normal	-	16.6	50	13.42	20	0	77	1	2	0	0	0	0	0
Mean		7.63			15.8	48	13.81	18	1	80	1	0	0	0	0	0	0
90896	F	7.04	Normal	-	15.1	44	15.99	10	0	90	0	0	0	0	0	0	0
90901	F	6.89	Normal	-	14.3	45	13.23	7	1	90	0	0	0	0	0	0	0
90907	F	6.91	Normal	-	14.8	46	9.91	9	0	91	0	0	0	0	0	0	0
90909	F	7.16	Normal	-	15.1	47	9.92	10	0	90	0	0	0	0	0	0	0
90914	F	6.77	Normal	-	14.5	44	12.13	17	1	80	2	0	0	0	0	0	0
90919	F	7.05	Normal	-	15.0	45	15.70	13	2	85	0	0	0	0	0	0	0
90927	F	7.31	Normal	-	15.2	47	16.52	18	0	77	0	0	0	0	0	0	0
90929	F	6.76	Normal	-	15.0	46	13.46	16	0	83	1	0	0	0	0	0	0
90937	F	7.32	Normal	-	16.1	48	14.80	14	0	85	1	0	0	0	0	0	0
90943	F	7.23	Normal	-	15.2	46	14.59	13	0	86	1	0	0	0	0	0	0
Mean		7.06			15.9	46	13.63	13	1	85	1	0	0	0	0	0	0

Code: - None Seen

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000039

Chlorethidic Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 13.

MALES: Means and Significance of Biochemical Values - Terminal

Biochemistry	0.0 mg/l (Control)	0.1 mg/l	1.0 mg/l	10.0 mg/l
Glucose, mg/100	105	110	100	85**
BUN, mg/100	11.8	12.2	13.3	14.7
Alkaline Phosphatase, int'l u/l	213	230	271*	277*
SGPT, Sigma u/ml	18	18	23*	20

* Significantly Different from Control Group Mean; $p < 0.05$ ** Significantly Different from Control Group Mean; $p < 0.01$

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000040

Chlorethidic Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 14. FEMALES: Means and Significance of Biochemical Values - Terminal

Biochemistry	0.0 mg/l (Control)	0.1 mg/l	1.0 mg/l	10.0 mg/l
Glucose, mg/100	106	114	111	101
BUN, mg/100	14.8	16.4	15.8	15.8
Alkaline Phosphatase, int'l u/l	94	108	132**	129**
SGPT, Sigma u/ml	20	17	19	20

* Significantly Different from Control Group Mean; $p < 0.05$ ** Significantly Different from Control Group Mean; $p < 0.01$

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Chlorenic Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 15. Individual Biochemical Values - Terminal

Group, Rat Number	Sex	Glucose mg/100	BUN mg/100	Alkaline Phosphatase int'l u/l	SGPT Sigma u/ml
0.0 mg/l (Control):					
90848	M	104	10.1	191	17
90851	M	122	13.0	195	19
90857	M	99	13.1	195	15
90858	M	91	9.7	183	19
90861	M	116	11.2	189	16
90866	M	91	12.8	189	18
90868	M	125	12.9	195	19
90874	M	95	11.4	196	27
90877	M	105	12.2	295	19
90884	M	97	11.1	305	15
Mean		105	11.8	213	18
90897	F	94	15.0	119	19
90898	F	108	17.1	81	22
90903	F	115	15.1	94	12
90905	F	109	16.1	91	15
90910	F	105	15.8	106	23
90911	F	97	16.1	70	22
90918	F	98	13.1	102	19
90920	F	124	14.0	87	30
90935	F	100	13.0	83	22
90940	F	107	12.9	106	19
Mean		106	14.8	94	20

Chlorendic Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 16. Individual Biochemical Values - Terminal

Group, Rat Number	Sex	Glucose mg/100	BUN mg/100	Alkaline Phosphatase int'l u/l	SGPT Sigma u/ml
0.1 mg/l:					
90854	M	101	11.9		
90862	M	106		219	15
90867	M	111	11.0	276	30
90869	M	119	12.0	278	17
90880	M	106	14.0	207	17
90882	M	114	11.2	195	18
90885	M	112	10.9	291	17
90887	M	125	13.1	240	16
90888	M	112	13.1	180	18
90894	M	95	11.9	260	16
Mean		110	13.3	150	16
90906	F	110	12.2	230	18
90912	F	113	14.1	80	16
90923	F	110	19.9	104	16
90925	F	106	17.0	122	19
90926	F	118	13.7	109	16
90928	F	116	13.4	105	15
90930	F	106	19.9	103	24
90931	F	126	17.8	104	19
90934	F	114	16.9	140	16
90944	F	116	18.9	136	15
Mean		114	12.1	79	13
			16.4	108	17

Chlorendic Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 17. Individual Biochemical Values - Terminal

Group, Rat Number	Sex	Glucose mg/100	BUN mg/100	Alkaline Phosphatase int'l u/l	SGPT Sigma u/ml
<u>1.0 mg/l:</u>					
90852	M	96	13.2	274	22
90855	M	100	14.2	344	25
90865	M	98	14.3	243	22
90871	M	91	11.4	387	24
90873	M	100	11.0	267	19
90876	M	96	11.9	246	34
90878	M	120	14.0	283	20
90881	M	99	18.1	238	18
90883	M	100	12.9	211	22
90889	M	97	12.1	212	25
Mean		100	13.3	271	23
90904	F	114	14.0	125	18
90908	F	115	19.2	84	17
90915	F	101	13.5	149	23
90917	F	118	17.2	144	17
90924	F	106	14.5	169	19
90933	F	111	12.9	101	15
90936	F	100	13.5	161	33
90938	F	121	17.9	134	18
90942	F	110	17.0	163	15
90945	F	117	18.1	90	18
Mean		111	15.8	132	19

Chlorendic Anhydride: Subacute Inhalation Toxicity Study in Rats

TABLE 18. Individual Biochemical Values - Terminal

Group, Rat Number	Sex	Glucose mg/100	BUN mg/100	Alkaline Phosphatase int'l u/l	SGPT Sigma u/ml
<u>10.0 mg/l:</u>					
90846	M	94	13.8	259	21
90847	M	81	25.0	313	19
90856	M	84	20.1	214	15
90863	M	87	12.2	261	18
90864	M	82	14.2	362	22
90870	M	79	9.1	304	18
90872	M	85	10.1	270	17
90879	M	84	12.2	327	25
90890	M	88	13.8	200	22
90895	M	85	16.9	258	24
Mean		85	14.7	277	20
90896	F	92	12.0	155	17
90901	F	92	16.0	102	16
90907	F	116	13.0	131	39
90909	F	91	17.1	119	16
90914	F	105	16.1	143	16
90919	F	100	19.0	123	16
90927	F	103	15.9	96	20
90929	F	92	13.0	150	18
90937	F	117	18.4	123	22
90943	F	105	17.9	144	15
Mean		101	15.8	129	20

Chloromide Anhydride: Subacute Inhalation Toxicity Study in Rats
 TABLE 19. Gross Necropsy Observations, Terminal Sacrifice

Site Lesion	0.0 mg/l (Control)		0.1 mg/l		1.0 mg/l		10.0 mg/l	
	M	F	M	F	M	F	M	F
Number Necropsied	10	10	10	10	10	10*	10	10*
No Gross Lesions	7	3	0	0	0	0	0	0
External								
alopecia								
bloody nasal discharge						2		1
Lungs								
dark red foci	5	1	6	9	9	9	8	10
gray red area/foci	1	2	1	1	2	2	2	10
dark red area/dyscoloration	1		5	2	2	1		4
Cervical Lymph Nodes								
dark red	9	5	6	2	7	8	7	4
enlarged					1			
Abdominal Cavity								
mesoperitoneum								1
Stomach								
dark red/brown foci	1		3	2	4	3		5
Liver								
yellow foci		1		1				
yellow tissue attached				1				
Kidneys								
hydronephrosis	1							
small	1							1
enlarged	1							
Uterus								
hydrometra		3		1		1		3

*Includes one animal which died following terminal blood collection.

Chloroacetic Anhydride:

Subacute Inhalation Toxicity Study in Rats

TABLE 20. Absolute (Grams) and Relative (% Body Weight) Organ Weights, Terminal Sacrifice

Group, Sex	Body Wt. R	Adrenals		Brain		Ovaries/ Testes/Z		Kidneys		Liver		Pituitary		Spleen		Thyroid	
		R	Z	R	Z	R	Z	R	Z	R	Z	R	Z	R	Z	R	Z
<u>0.0 mg/l (Control):</u>																	
M	326	0.073	2.22	2.02	0.62	3.09	0.95	3.03	0.93	14.51	4.44	0.012	0.36	0.62	0.19	0.029	0.87
F	218	0.089	4.10	1.94	0.89	0.150	0.69	1.92	0.88	8.62	3.95	0.016	0.73	0.52	0.24	0.028	1.29
<u>0.1 mg/l:</u>																	
M	300	0.067	2.24	2.00	0.67	2.95	0.99	2.84	0.95	11.79**	3.92*	0.013	0.45*	0.65	0.22	0.011	1.02
F	218	0.080	3.66	1.92	0.88	0.154	0.70	1.97	0.90	8.81	4.05	0.014	0.66	0.54	0.25	0.025	1.13
<u>1.0 mg/l:</u>																	
M	317	0.060	1.90	1.98	0.62	3.26	1.03	3.00	0.95	12.57*	3.95*	0.014	0.43	0.67	0.21	0.019**	0.61**
F	216	0.074*	3.44	1.94	0.90	0.133	0.62	1.84	0.85	8.12	3.76	0.016	0.74	0.49	0.23	0.018*	0.84*
<u>10.0 mg/l:</u>																	
M	275	0.053**	1.96	2.00	0.74**	3.27	1.20**	2.71	0.99	10.25**	3.77*	0.011	0.42	0.53	0.20	0.016**	0.60**
F	206	0.071**	3.43	1.95	0.95	0.134	0.65	1.89	0.92	7.71*	3.74	0.013*	0.63	0.45	0.22	0.016*	0.79*

Group mean relative organ weights shown in this table were calculated by averaging the individually calculated relative organ weights.
 *Significantly different from control group mean, P<0.05.
 **Significantly different from control group mean, P<0.01.

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Chlorendic Anhydride:

Subacute Inhalation Toxicity Study in Rats

TABLE 21. Individual Organ Weights, Grams, Terminal Sacrifice

Group, Rat Number	Sex	Body Wt.	Adrenals	Brain	Gonads	Kidneys	Liver	Pituitary	Spleen	Thyroid
0.0 mg/l (Control):										
90848	M	318	0.059	2.06	3.04	3.23	15.75	0.007	0.51	0.019
90858	M	322	0.067	1.90	3.15	3.32	14.44	0.016	0.65	0.026
90857	M	313	0.065	1.37	2.96	2.62	12.15	0.012	0.58	0.028
90851	M	346	0.075	1.94	2.72	3.30	18.20	0.017	0.51	0.034
90866	M	373	0.120	2.03	3.00	3.38	16.63	0.012	0.62	0.027
90868	M	292	0.065	2.16	2.99	2.47	12.75	0.013	0.58	0.027
90874	M	339	0.080	2.13	3.27	3.32	12.97	0.012	0.77	0.032
90877	M	340	0.073	2.17	3.55	3.35	15.83	0.014	0.71	0.025
90884	M	322	0.069	2.07	3.13	2.66	14.04	0.006	0.58	0.043
90884	M	305	0.057	1.90	3.05	2.68	12.36	0.010	0.73	0.024
90897	F	213	0.079	1.34	0.195	1.93	3.57	0.012	0.34	0.056
90898	F	225	0.076	1.95	0.128	1.89	3.70	0.019	0.59	0.027
90903	F	213	0.070	2.08	0.146	1.99	3.42	0.019	0.46	0.021
90905	F	224	0.103	2.04	0.134	2.15	7.38	0.014	0.45	0.037
90910	F	229	0.094	2.10	0.183	1.96	9.77	0.017	0.47	0.032
90911	F	213	0.081	1.91	0.146	1.91	8.66	0.015	0.53	0.023
90918	F	217	0.076	1.92	0.119	1.84	8.41	0.013	0.47	0.023
90920	F	214	0.100	1.80	0.139	1.61	7.99	0.018	0.41	0.012
90935	F	209	0.114	1.82	0.130	1.76	8.15	0.018	0.40	0.029
90940	F	218	0.100	1.96	0.181	2.17	9.68	0.014	0.60	0.022
0.1 mg/l:										
90854	M	291	0.078	1.74	2.54	2.60	11.28	0.013	0.65	0.034
90862	M	317	0.053	2.13	2.98	2.66	13.49	0.012	0.66	0.023
90867	M	339	0.070	2.11	3.58	3.40	14.15	0.013	0.37	0.045
90869	M	275	0.086	1.90	2.97	2.60	9.88	0.012	0.57	0.038
90880	M	299	0.067	1.97	2.99	2.80	11.55	0.014	0.64	0.023
90882	M	292	0.070	1.96	1.96	2.96	11.34	0.012	0.65	0.029
90885	M	303	0.056	2.11	3.19	2.87	11.54	0.016	0.61	0.020
90887	M	297	0.063	1.99	2.92	3.03	10.98	0.014	0.73	0.044
90888	M	250	0.066	1.97	3.24	2.36	10.29	0.013	0.51	0.026
90894	M	325	0.056	2.12	3.16	3.12	12.87	0.015	0.63	0.023
1.0 mg/l:										
90911	F	214	0.080	1.33	0.176	1.74	7.68	0.016	0.50	0.040
90906	F	226	0.082	1.38	0.115	2.11	8.91	0.015	0.57	0.029
90923	F	225	0.064	2.06	0.120	1.87	8.77	0.015	0.55	0.021
90925	F	200	0.092	1.91	0.081	1.96	9.40	0.013	0.55	0.028
90926	F	215	0.108	1.86	0.266	2.04	9.38	0.020	0.79	0.032
90928	F	218	0.085	1.93	0.177	2.17	9.10	0.016	0.54	0.023
90930	F	200	0.062	1.97	0.125	1.78	7.58	0.012	0.47	0.017
90931	F	230	0.063	1.90	0.133	1.94	3.03	0.013	0.37	0.018
90934	F	215	0.071	1.94	0.149	1.89	3.20	0.013	0.46	0.020
90944	F	237	0.089	1.89	0.193	2.17	10.58	0.010	0.63	0.017
1.0 mg/l:										
90852	M	298	0.067	1.94	3.31	3.18	11.24	0.013	0.45	0.015
90855	M	305	0.053	1.38	3.20	2.85	10.70	0.013	0.66	0.024
90865	M	290	0.060	0.53*	2.39	2.80	12.02	0.014	0.63	0.019
90871	M	322	0.083	1.37	3.36	3.22	13.09	0.016	0.63	0.022
90873	M	312	0.048	1.90	3.40	2.91	11.54	0.012	0.61	0.013
90876	M	332	0.063	2.05	3.61	2.98	14.48	0.014	0.66	0.020
90878	M	344	0.067	2.05	3.30	3.11	14.07	0.011	0.72	0.016
90881	M	334	0.044	2.03	2.86	3.03	13.41	0.014	0.81	0.021
90883	M	330	0.057	2.00	3.39	2.92	13.22	0.014	0.79	0.022
90889	M	307	0.058	2.10	3.27	3.01	11.93	0.015	0.75	0.015
1.0 mg/l:										
90904	F	230	0.070	1.34	0.147	1.86	3.32	0.021	0.52	0.018
90908	F	228	0.073	1.75	0.166	1.93	7.38	0.016	0.59	0.023
90915	F	203	0.063	1.38	0.104	1.73	7.58	0.012	0.52	0.017
90917	F	205	0.099	1.37	0.138	1.72	8.92	0.012	0.39	0.019
90924	F	208	0.087	2.00	0.163	1.79	7.59	0.016	0.32	0.017
90933	F	215	0.079	2.04	0.122	1.98	9.46	0.016	0.60	0.017
90936	F	205	0.057	2.02	0.115	1.86	6.23	0.015	0.57	0.019
90938	F	213	0.060	2.03	0.126	1.74	8.07	0.017	0.45	0.017
90942	F	213	0.073	2.00	0.138	1.83	8.00	0.016	0.42	0.018
90945	F	228	0.080	1.98	0.113	1.97	3.93	0.018	0.48	0.016

*Not included in statistics; questionable weight.

Chloroform Anhydride:

TABLE 21. Subacute Inhalation Toxicity Study in Rats
Histomorphologic Observations, Terminal Sacrifice

Site Lesion	0.0 mg/l (Control)					10.0 mg/l				
	Group	Number	Sex	+	-	Group	Number	Sex	+	-
Brain	90848	1	M			90846	1	M		
Pituitary	90858	1	M			90847	1	M		
Thyroids	90857	1	M			90856	1	M		
Adrenals	90861	1	M			90863	1	M		
Increased vasculation, cortical cells	90866	1	M			90864	1	M		
Nasal turbinates	90874	1	M			90872	1	M		
Inflammatory cell infiltrate, mucosa	90877	2	M			90890	2	M		
Trachea	90884	1	M			90895	1	M		
Lymphocytic infiltrate, submucosa	90897	1	M			90896	1	M		
Inflammatory cell infiltrate, submucosa	90903	3	M			90901	3	M		
Inflammatory cell infiltrate, muscularis/externa	90905	1	M			90906	1	M		
epithelial hyperplasia, focal	90910	1	M			90907	1	M		
Lungs	90918	1	M			90916	1	M		
Interstitial inflammatory cell infiltrate	90920	2	M			90927	2	M		
perivascular lymphocytic infiltrate	90930	2	M			90935	2	M		
peribronchial lymphoid hyperplasia	90935	3	M			90940	3	M		
interstitial fibrosis	90940	3	M							
atelectasis	90945	1	M							
scattered hemorrhages	90950	3	M							
interstitial pneumonia	90955	2	M							
Heart (with coronary vessels)	90960	3	M							
scattered inflammatory foci	90965	2	M							
Aorta	90970	1	M							
Spleen	90975	1	M							
Mesenteric lymph node	90980	1	M							
Thymus	90985	1	M							
Bone/marrow - rib junction	90990	1	M							
Salivary gland	90995	1	M							

Code: x - condition present
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - extreme
 - - not available
 * - Animal died after bleeding

Chloroendic Anhydride:
 TABLE 24.
 Subacute Inhalation Toxicity Study In Rats
 Histomorphologic Observations, Terminal Sacrifices

Site	Group, Sac Number	0.0 mg/l (Cont rol)										10.0 mg/l									
		90848	90851	90857	90858	90861	90866	90868	90874	90877	90884	90897	90898	90903	90905	90910	90911	90918	90920	90933	90940
Esophagus																					
Stomach																					
Inflammatory cell infiltrate, mucosa/submucosa/plandular congestion, mucosa, focal, glandular																					
Duodenum																					
Jejunum																					
Ileum																					
Colon																					
Pancreas																					
Liver (2)																					
Inflammatory cell infiltrate, portal scattered inflammatory foci																					
Lymphocytic infiltrate, portal																					
Kidneys																					
Lymphocytic infiltrate, interstitial																					
healed infarct																					
Urinary bladder																					
Testis/Ovary																					
Uterus																					
hydrometra, atrophy of endometrium																					
Skin																					
Mammary gland																					
Miscellaneous																					
congest ion/erythroplagocytosis, cervical lymph node																					

Code: x - condition present
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - extreme
 - not available

Chloroform Anhydride:

Subacute Inhalation Toxicity Study in Rats

TABLE 24.

Histomorphologic Observations, Terminal Sacrifice

Site Lesion	0.0 mg/l (Control)										10.0 mg/l																							
	90848	90851	90857	90858	90861	90866	90874	90877	90884	90897	90898	90903	90905	90910	90911	90916	90920	90935	90940	90846	90855	90856	90863	90870	90872	90879	90895	90901	90906	90917	90919	90926	90936	90949
Group, Rat	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Sex	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Esophagus																																		
Stomach inflammatory cell infiltrate, mucosa/submucosa/glandular congestion, mucosa, focal, glandular																																		
Duodenum																																		
Jejunum																																		
Ileum																																		
Colon																																		
Pancreas																																		
Liver (2) inflammatory cell infiltrate, portal scattered inflammatory foci lymphocytic infiltrate, portal																																		
Kidneys lymphocytic infiltrate, interstitial healed infarct																																		
Urinary bladder																																		
Testis/ovary																																		
Uterus hydrometra, atrophy of endometrium																																		
SKIN																																		
Primary gland																																		
Miscellaneous congestion/erythrocytosis, cervical lymph node																																		
*Animal died after bleeding, prior to necropsy																																		

Approved And Submitted by: *Charles E. Zwick* Date: *1-31-80*
 Charles E. Zwick, B.S.
 Study Director

Scale: x - condition present
 1 - not remarkable
 2 - very slight
 3 - slight
 4 - moderate
 5 - marked
 6 - not available

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APPENDIX I
Protocol

163-531

000054

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

Lab Project No. 143-531 Sheet 1 Date 10/5/77 Authorized by [Signature]

DISTRIBUTION

Wazeter	<u>X</u>	Mr. Cain	<u>X</u>	Mrs. Finlay	<u>X</u>	Dr. Goldenthal	
Goldenthal	<u>X</u>	Mr. Vollmar	<u>X</u>	Dr. Leong	<u>X</u>	Dr. Cookson	
Dr. Geil	<u>X</u>	Miss Emmons	<u>X</u>	Mr. Benson		Miss Lehrberg	
Dr. Jessup	<u>X</u>	Ms. French	<u>X</u>	Mr. Rodwell		Mr. Urbancic	
Dr. Griffith	<u>X</u>	Mr. Pangburn	<u>X</u>	Mrs. Schwartz		Mr. Dean	
				Miss Morseth		Mr. Thompson	
						Dr. Thorstenson	
						<u>Inhalation Lab</u>	<u>X</u>

<u>Compound</u>	<u>Identification Number</u>	<u>IRDC No.</u>
Chlorendic Anhydride		6398

TITLE: SUBACUTE INHALATION TOXICITY STUDY IN RATS

See attached protocol. Study Director: Dr. Leong.
 Sponsor should be called to determine dosage levels.

PROTOCOL MODIFICATION:

Clinical Laboratory Test: To be performed on 10 male and 10 female rats from all four groups at approximately one month of study.

Pathology: Histopathology will be conducted on 10 tissues from the low and mid dosage level. Tissues will be determined after examination of high dosage level animals.

Sponsor: Velsicol Chemical Corporation

Subject: Subacute Inhalation Toxicity - Rats
20 Exposures in 28 Days

Test Material: Chlorendic Anhydride

Purpose: The purpose of this study is to assess the toxicity from multiple inhalation exposure to a test material. The information permits: (a) more detailed delineation of signs of toxic response, (b) the detection of the accumulated effects and the rate of their development, (c) the estimation of the concentrations to be used in a TLV chronic exposure, (d) the selection of most responsible species and the toxicity criteria to be monitored and (e) the most appropriate intervals for periodic examination of the exposed animals.

Experimental Design:

Group	Dose (ppm)	Exposure			Total	Number of Animals ^a		
		Ers/Day	Days/Week	Weeks		Lab Studies 1 Mo.	Sacrifice 1 Mo.	Histopathology
1	0 (Control)	6	5	4	20	20	all survivors	20
2	Low	6	5	4	20	as required ^b	all survivors	as required ^b
3	Medium	6	5	4	20	as required ^b	all survivors	as required ^b
4	High	6	5	4	20	20	all survivors	20

^aNumber of animals equally divided between sexes.

^bTo be performed if indicated by high dose-group evaluation

METHODS AND MATERIALS

Compound: Chlorendic Anhydride

Animals: Rats, 4-6 weeks of age, Charles River CD

Number: 80 total - 40 males, 40 females

Husbandry: Housing - individual
Food - ad libitum except during exposure
Water - ad libitum except during exposure unless by automatic watering system

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International Research and Development

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Exposure Procedure: The animals will be exposed in an aerodynamic designed inhalation chamber to the dusts six (6) hours per day, five (5) days per week for a period of not less than 4 weeks, 20 exposures.

Dust Generation: The dust atmosphere of the test material will be generated by dispersing the powder at a calculated rate, with a specially constructed dust generator located near the chamber air inlet at the top of the exposure chamber. This dust generator consists of a revolving plate with calibrated "cups" for transporting a known quantity of powder per unit time from a reservoir to a "blowhole". At the "blowhole" the powders in a "cup" were dispersed into the chamber by a jet of air blowing at the rate of 8 liters per minute.

The actual quantity of powder disseminated will be determined by weighing the quantity of powder in the reservoir before and after the experiment. The concentration of the dusts in the chamber atmosphere will be calculated from the ratio of the rates of powder dissemination to the total chamber airflow (the volume of air ejected from the dust generator).

Particle Size
Distribution
Analyses:

The particle size distribution of the dust will be determined using an Anderson[®] 8-stages Particle Fractionating Sampler. The Mass Median Diameter (MMD) will be determined graphically using the logarithmic-probability plot technique.

Duration of Study: This study is designed as a one month experiment. Animals will be exposed for a minimum of 20 exposures over a 1 month period.

EXPERIMENTAL EVALUATION

In Life

Time Interval

Observation of Pharmacologic
and Toxicologic effects:

Daily

Physical Examination;
Body Weights:

Twice weekly, beginning one week prior
to treatment

Laboratory Studies:

To be performed on 10 males and 10 females of control and surviving high dose groups at one month. Studies of affected measurements will be performed on lower dose groups.

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International Research and Development

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Experimental Evaluation (Continued)

Hematologic: 1 month
 hemoglobin
 hematocrit
 erythrocyte count and morphology
 leukocyte count (total and differential)
 bone marrow smear (differential count
 only if indicated by blood hematologic
 evaluation)

Clinical Chemistry: 1 month
 alkaline phosphatase
 blood urea nitrogen
 serum glutamic pyruvic transaminase
 fasting blood sugar

Post Mortem

Gross necropsy will be performed on all animals. All body cavities will be examined and hollow organs will be opened for inspection.

Spontaneous deaths as occur
 Moribund animals to be sacrificed
 Terminal sacrifice 1 month
 all living

Organ Weights:

adrenals	liver
brain	pituitary (fixed weight)
gonads	spleen
kidneys	thyroid (after fixation)

Histopathologic Examination:

Tissues listed below from all animals are to be fixed in 10% buffered formalin; eyes and testes are to be fixed in an appropriate fixative as defined by the examining pathologist.

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At termination, slides (H & E stained) will be prepared and histopathologic examinations performed on all tissues of each control and high dose group. Histopathologic examinations will be performed on target organ(s) of the lower dose groups to define a subthreshold level.

adrenals
aorta
bone (rib junction)
brain (2 sections)
esophagus
gonad
heart (with coronary vessels)
intestine
 colon
 duodenum
 ileum
 jejunum
kidneys
gross lesions
liver (2 sections)
lung (2 sections)
 (fix intact by intratracheal
 infusion)

lymph nodes
 mesenteric
mammary gland
nasal turbinates
pancreas
pituitary
nerve peripheral
muscle skeletal
spinal cord
salivary gland
skin
spleen
stomach
thymus
thyroid
urinary bladder
uterus
trachea

} tissues will be fixed
but examined only if
clinical examinations
and physical signs so
indicate

Statistical Analyses:

All hematological (except WBC differential counts), biochemical and organ weight data will be analyzed statistically using an analysis of variance and Dunnett's test. The level of significance in all cases will be $p < 0.05$.

Reporting:

The report will describe in detail the experimental procedures including ^{dust}~~vapor~~ generation, chamber atmosphere analyses, animal observations, mortality and gross necropsy. Findings in hematology, clinical chemistry and histopathology together with statistical evaluation of various parameters will also be presented and discussed.

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DISTRIBUTION

Wazeter	<u>X</u>	Mr. Cain	<u>X</u>	Mrs. Finlay	<u>X</u>	Dr. Goldenthal	_____
Goldenthal	<u>X</u>	Mr. Vollmar	<u>X</u>	Dr. Leong	<u>X</u>	Dr. Cookson	_____
Gail	<u>X</u>	Miss Emmons	<u>X</u>	Mr. Benson	_____	Miss Lohrberg	_____
Dr. Jessup	<u>X</u>	Ms. French	<u>X</u>	Mr. Rodwell	_____	Mr. Urbancic	_____
Dr. Griffith	<u>X</u>	Mr. Pangburn	<u>X</u>	<u>Inhalation Lab</u>	<u>X</u>	Mr. Dean	_____
						Mr. Thompson	_____
						Dr. Thorstenson	_____

<u>Compound</u>	<u>Identification Number</u>	<u>IRDC No.</u>
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

TITLE: SUBACUTE INHALATION TOXICITY STUDY IN RATS.

PROTOCOL MODIFICATION

Pathology

Organ weights of adrenals and ovaries will be obtained on fixed tissues. The hollow organs to be opened are the stomach and cecum. The urinary bladders will be inflated with formalin and left unopened for examination before fixation.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

Study No. 163-531

Sheet 3

Date 11/20/78

Authorized by J. Goldenthal
Dr. Goldenthal

DISTRIBUTION

Mr. Waseter	<u>X</u>	Dr. Geil	<u>X</u>	Mr. Vollmar	<u>X</u>	Mr. Dean	<u> </u>
Dr. Goldenthal	<u>X</u>	Mr. Cain	<u>X</u>	Dr. Leong	<u>X</u>	Dr. Kahn	<u>X</u>
Dr. Jessup	<u>X</u>	Dr. Griffith	<u>X</u>	Dr. Mehring	<u> </u>	Dr. Cookson	<u> </u>
Dr. Spicer	<u>X</u>	Dr. Laveglia	<u>X</u>	Mr. Rodwell	<u> </u>	Dr. Tucek	<u> </u>
Dr. Blair	<u>X</u>	Mr. Sanson	<u>X</u>	Mr. Jefferson	<u> </u>	Dr. Thorstenson	<u> </u>
						Mr. Kjeldgaard	<u>X</u>

Compound	Identification Number	IRDC No.
Chlorendic Anhydride		6398

TITLE: SUBACUTE INHALATION TOXICITY STUDY IN RATS

No clinical neurological effects were observed in the animals during the inhalation exposure. Therefore, the nerve, spinal cord and skeletal muscle need not be processed for histological examination at this time.

This protocol was approved by N/A representative for the sponsor on

APPENDIX II
Personnel

163-531

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PERSONNEL RECORD

Study No. 163-231

The IRDC personnel listed below have participated in the technical activities of the study indicated.

Employee Name (Print)	Employee Signature	Employee Initials	Date
T.M. Spuler (pathologist)	<i>T.M. Spuler</i>	TMS/SP	7/5/53
Judy L. Stively (prosector)	<i>Judy L. Stively</i>	J.L.S./J.S.	7/5/53
Kim Liebowen (technician)	<i>Kim Liebowen</i>	K.L./K	7/5/53
Erwin Ludwig (prosector)	<i>Erwin Ludwig</i>	E.L./E	7/5/53
Ken Howard (technician)	<i>Ken Howard</i>	K.H./K	7/5/53
Lisa Fabatz (prosector)	<i>Lisa Fabatz</i>	L.F./L	7/5/53
Cindy Sleeman (technician)	<i>Cindy Sleeman</i>	C.S./C	7/5/53
Sharon Teal (prosector)	<i>Sharon Teal</i>	S.T./S	7/5/53
Angel Heclov (technician)	<i>Angel Heclov</i>	A.H./A	7/5/53
Cornie Pallen (prosector)	<i>Cornie Pallen</i>	C.P./C	7/5/53
Rosemarie Denney (technician)	<i>Rosemarie Denney</i>	R.D./R	7/5/53