BIOLOGICAL EFFECT OF SOUTH DAKOTA DEICER NUMBER 2 ON ENVIRONMENT

A Final Report

Submitted to
South Dakota Department of Transportation

By

Sookie S. Bang
Associate Professor
Principal Investigator

and

Jane A. Roseland
Assistant Professor
Co-Principal Investigator

Department of Chemistry and Chemical Engineering

South Dakota School of Mines and Technology
Rapid City, South Dakota 57701

AUGUST 1989

BIOLOGICAL EFFECT OF SOUTH DAKOTA DEICER NUMBER 2 ON ENVIRONMENT

A Final Report

Submitted to
South Dakota Department of Transportation

By

Sookie S. Bang
Associate Professor
Principal Investigator

and

Jane A. Roseland
Assistant Professor
Co-Principal Investigator

Department of Chemistry and Chemical Engineering

South Dakota School of Mines and Technology
Rapid City, South Dakota 57701

AUGUST 1989

DISCLAIMER

The contents of this report reflect the views of the authors who are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views or policies of the South Dakota Department of Transportation or State Transportation Commission. This report does not constitute a standard specification, or regulation.

ACKNOWLEDGEMENT

The study presented in this report is based upon research supported by the South Dakota Department of Transportation under Contract No. 3359. The authors are extremely grateful for this support. A deep appreciation is extended to Mr. Dan Johnston, Senior Engineer, and Mr. Dave L. Huft, Research Engineer, of the South Dakota Department of Transportation. They provided direction and helpful comments throughout the study.

Many personnel directly and indirectly contributed to the successful completion of the study. The authors are deeply indebted to Ms. Mary A. Verhelst, Mr. Warren Back, and Ms. Karen Tjaden of the South Dakota School of Mines and Technology, Mr. Larry Ferber of the State Fish Hatchery in Rapid City, and Mr. Craig Back and Mr. Charles Larson of the Rapid City Wastewater Treatment Plant.

Special thanks go to Dr. Scott A. Mischler, D.V.M., who has collaborated in the animal toxicity experiments of the Acute Dermal Toxicity Test, Primary Dermal Irritation Test, and Acute Inhalation Toxicity Test. He performed clinical examinations and gross necropsies on all test animals. Without his valuable participation, these experiments would not have been possible.

The authors also gratefully acknowledge Dr. Alfred R. Boyson, South Dakota School of Mines and Technology, who reviewed the draft of this report.

TABLE OF CONTENTS

INTRODUCTION

Researchers from the South Dakota Department of Transportation (SDDOT) and the South Dakota School of Mines and Technology (SDSM&T) have developed a deicer known as South Dakota Deicer Number 2 (SDD2). The physical and chemical characterizations of the compound have indicated its efficacy as a high performance deicer. The deicer is basically a processed cellulose, a mixture of sodium salts of organic acids.

It however needs to be understood what apprehensive concern would be present due to the introduction of a new compound in the environment. It may produce ecological impacts on various biological systems which are directly or indirectly related to the welfare of human beings.

The primary objective of this study is to determine the environmental impact of SDD2 by utilizing laboratory animals to evaluate its toxicity and by monitoring the behavior of SDD2 in the aquatic environment in terms of its degradation by microorganisms and adverse effect on aquatic animals. The investigation includes a series of short-term toxicity tests and a comparison of the data with the known deicer, Calcium Magnesium Acetate (CMA) to evaluate the capability of SDD2 as a potential deicer.

The following experimental tests have been performed to investigate the basic behavior of SDD2 in the biological system: Acute Dermal Toxicity Test, Primary Dermal Irritation Test, Acute Inhalation Toxicity Test, Fish Acute Toxicity Test, and Biochemical Oxygen Demand Test. It is hoped that the results of this study would provide detailed preliminary information of the basic effect of SDD2 on the biological system and of its feasibility as a deicer in the environment.

SECTION A: ACUTE DERMAL TOXICITY TEST

ABSTRACT

This study examines the acute dermal toxicity of the South Dakota Deicer Number 2 (SDD2) in animals and evaluates the toxic characteristics of the substance. The results are expected to provide information on health hazards which might arise from short-term exposure by the dermal route. Acute dermal toxicity, by definition, is the adverse effect occurring within a short time of dermal application of a substance given within 24 hours. The Limit Test, with a single high level of SDD2, has been performed on rats according to the procedure described by the Environmental Protection Agency in the Code of Federal Regulations (CFR) (1).

The dose level of SDD2 has been determined with the concentration recommended by the CFR procedure (i.e., 2g SDD2/kg of body weight of rat). Among ten animals tested, two females with the lowest body weights showed very mild skin irritation which was fully reversed within 18 hours. There was no serious toxic reaction or abnormality in the gross pathological findings.

Since there was no SDD2-related mortality observed, a study using three different dose levels has not been pursued. In conclusion the lethal concentration of SDD2, if present, would be much higher than that allowed by the Environmental Protection Agency.

EXPERIMENTAL PROCEDURES

Animals

Chosen were young adults (average weight of approx. 175g each) of white rats of strain Sprague Dawley (Charles River Laboratories, Inc.). Upon arrival, rats were allowed twelve days of rest before the test. Throughout the study, they were kept individually and provided with sufficient water and with two pellets (5g) per rat a day of the rodent laboratory chow (#5001, Purina Mills Inc.).

Animal Preparations

Prior to the test, the hair was shaved from the dorsal area of the trunk, approximately 10% of the body surface area. Used were rats with no abrasions and with healthy intact skin. They fasted overnight. Rats were identified by a standard earmarking system. Five males and five nonpregnant females were chosen for the experiment and weighed to the nearest whole gram at the time of dosing.

SDD2 Preparation and Administration

On test day, the powdered (less than 38 micrometers in diameter) SDD2 was dissolved into water to obtain a light paste, 0.8g SDD2/ml. The paste was then applied uniformly on the skin and covered with a cheesecloth patch with plastic backing. Administration volume was adjusted in order to deliver 2g/kg body weight of each rat. Each dose was delivered to the nearest tenth of a milliliter. To make direct contact with the skin and to secure the patch, the test site was covered by wrapping around the trunk. Wrapping was tight enough to secure the cover but not to immobilize the animal. Individual rats were kept in separate cages with an exposure period of 24 hours.

Observation Period

At the end of the exposure period, the cover was removed and the application site was gently wiped with a paper towel and the residual SDD2 was washed out with water. Animals were weighed this day, weekly thereafter, and at death.

Careful clinical examination was made within 30 minutes and 24 hours after the removal of the patch. Cage-side observation was made at least once a day for signs of toxicity. Evaluation of toxicity signs has followed the principles and methods provided in the CFR manual (1) and by Chan et al. (2). Surviving animals were observed for 14 days and sacrificed. Termination was carried out by the exposure of rats to dry ice in a closed chamber, which would lead to carbon dioxide intoxication. Gross necropsies were performed on all animals by Dr. Scott A. Mischler, D.V.M., of the National College, Rapid City, South Dakota.

RESULTS AND DISCUSSION

Right after exposure, two females showed mild reactions, one with slight erythema and the other with minimal wheals at the application site of SDD2. The signs, however, completely disappeared overnight. Other animals showed no reaction related to SDD2 application during the observation period. Interestingly, the reacting animals were all females with the lowest weights. All animals appeared active and healthy showing no SDD2 related abnormalities in their behavior throughout the observation period. Pathologically, there was no evidence of gross lesions or abnormalities except the sign of atelectiasis in focal areas of the lung. The atelectasis might have resulted from the carbon dioxide intoxication, which was the means of termination of rats.

Table A-1 details the results of the Acute Dermal Irritation test, including the number, sex, weight, clinical examination after exposure, and gross necropsy. It also includes information on the average weights and standard deviations of rats (0, 24 hours, 7, and 14 days after exposure), by gender and total.

The Limit Test, with a single large dose of SDD2 (2g/kg of body weight), indicates that there is no SDD2 related irreversible toxicity or mortality. Therefore, testing with higher doses or higher numbers of dose levels would not be necessary. SDD2 can be consequently regarded as a non-toxic substance in terms of its dermal absorption and mode of toxic reaction to laboratory animals.

REFERENCES

1. U.S. Environmental Protection Agency, Code of Federal Regulations, Title 40, Part 798, Section 1100 (1987).

2. Chan, P. K., G. P. O'Hara, and A. W. Hayes, "Principles and Methods for Acute and Subchronic Toxicity," In: Principles and Methods of Toxicology; edited by A. W. Hayes, Student edition, pp.17-19, Raven Press, New York, (1982).

Table A-1: Results of the Acute Dermal Toxicity Test

SEX	WEIGHT (g) AFTER (days) 0	1	7	14	CLINICAL EXAMINATION AFTER EXPOSURE	RESULTS OF GROSS NECROPSY 14 DAYS AFTER EXPOSURE
Male	174	171	172	186	No Reaction	No Gross Lesion
Male	183	179	164	179	No Reaction	No Gross Lesion
Male	185	180	180	187	No Reaction	No Gross Lesion
Male	179	179	170	174	No Reaction	No Gross Lesion
Male	180	177	174	181	No Reaction	No Gross Lesion
Female	172	171	171	175	No Reaction	No Gross Lesion
Female	160	157	160	169	Slight Erythema	No Gross Lesion
Female	174	168	179	180	No Reaction	No Gross Lesion
Female	168	165	171	174	Minimal Wheals	No Gross Lesion
Female	173	171	174	187	No Reaction	No Gross Lesion
Male Weight $\bar{X}$	180.2	177.2	172	181.4	Remarks: $\bar{X}$ = Average	
SD	4.21	3.63	5.83	5.32	SD = Standard Deviation	
Female Weight $\bar{X}$	169.4	166.4	171	177		
SD	5.73	5.81	6.96	6.82		
Both Sexes Weight $\bar{X}$	174.8	171.8	171.5	179.2		
SD	7.41	7.30	6.08	6.21		

SECTION B: PRIMARY DERMAL IRRITATION TEST

ABSTRACT

To evaluate the toxic characteristics of the South Dakota Deicer Number 2 (SDD2), the Primary Dermal Irritation test has been performed. It provides information on the existence of possible hazard that may arise from exposure of skin to SDD2. The dermal irritation is defined as the production of reversible inflammatory change in the skin following the application of a test substance. The principle of the test methods follows the guideline described in the Code of Federal Regulations (CFR) prepared by the Environmental Protection Agency (1).

The experiment was carried out by applying a single dose, 650 mg of SDD2, to healthy skin of eight healthy adult rats. Animals were exposed to SDD2 for four hours and observed for fourteen days. No test animals showed signs of skin irritation, even immediately after the exposure. Also, there were no abnormalities in gross pathological findings. By following the clinical examination and evaluation procedures for classification of the skin reaction, all animals were scored zero, indicating no erythema and edema formations on the exposed areas.

The test results strongly indicate the nontoxic characteristic of SDD2 in the environment as evidenced by the Dermal Irritation Test and the Acute Dermal Toxicity test which showed no adverse effects of the material on the animals' skin.

EXPERIMENTAL PROCEDURES

Animals and Preparations

Animal strains, conditions, and other preparations for the tests were the same as for the Acute Dermal Toxicity Test (Section A). The number of animals utilized was eight, with four from each sex.

SDD2 Preparation and Administration

The chemical and physical properties of SDD2 are described in detail in Appendix A. The same paste (0.8g/ml) prepared for the Acute Dermal Toxicity Test was used with different application volume. The final administered concentration of SDD2 was 650mg per each application site. All procedures for delivering and maintaining SDD2 on the animal skin were identical to those of the Acute Dermal Toxicity Test. The exposure duration of SDD2 was 4 hours.

Observation Period

At the end of exposure, the animals were cleaned and weighed. The signs of erythema and edema were carefully recorded and the responses were scored in 30 minutes, and then 24, 48, and 72 hours after the removal of the patch, following the evaluation guidelines provided by the references (1,2). Surviving animals were observed for 14 more days and sacrificed. The procedures for termination and performance of gross necropsies are described in Section A of this report.

RESULTS AND DISCUSSION

SDD2 was completely washed out by water after removal of patch. There was no apparent cutaneous absorption of the material even with thick

application. The complete results of the test are summarized in Table B-1. As indicated in the table, no skin irritation reaction was observed over the period of 72 hours. The dermal irritation scores were evaluated to be zero in erythema and edema formation. In addition, no lesions or other apparent toxic effects in the general area of the application site were observed. All animals were healthy and active throughout the entire observation period. In the gross necropsy examinations, there was no evidence of gross lesions or abnormalities in internal organs including the brain. The only noticeable finding was the signs of atelectasis appearing in focal areas of the lung apparently due to the carbon dioxide intoxication.

The test is essentially a short-term, high intensity dermal toxicity experiment compared to the Acute Dermal Toxicity test. It allows a large dose, 650 mg per animal, which is estimated to be 1.7 times the concentration per animal than that used for the Acute Dermal Toxicity test. It also has a shorter duration, 4 hours, compared to 24 hours for the other. Summary of the comparison between the two tests is shown in Table B-2.

Results of the dermal irritation scores, other general skin responses, and gross pathology findings indicate SDD2 is a relatively mild substance. Data obtained from both tests, the Primary Dermal Irritation and the Acute Dermal Toxicity test, strongly show that an insignificant amount of toxicity is produced by SDD2.

REFERENCES

1. U.S. Environmental Protection Agency, Code of Federal Regulations, Title 40, Part 798, Section 4470 (1987).

2. Daize, J.H., G. Woodard, and H. O. Calvery, "Methods of the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes," J. of Pharmacology and Experimental Therapeutics, 83: 377-390 (1944).

Table B-1: Results of the Primary Dermal Irritation Test

SEX	WEIGHT (g) AFTER (days) 0	1	7	14	CLINICAL EXAMINATION ERYTHEMA AND EDEMA AT (hr) 0.5, 24, 48, 72	GROSS NECROPSY 14 DAYS AFTER EXPOSURE
Male	200	192	192	211	0, No Reaction	No Gross Abnormality
Male	188	178	174	186	0, No Reaction	No Gross Abnormality
Male	194	184	177	174	0, No Reaction	No Gross Abnormality
Male	202	197	203	207	0, No Reaction	No Gross Abnormality
Female	165	161	159	169	0, No Reaction	No Gross Abnormality
Female	175	168	164	166	0, No Reaction	No Gross Abnormality
Female	189	179	178	178	0, No Reaction	No Gross Abnormality
Female	185	180	175	181	0, No Reaction	No Gross Abnormality
Male Weight $\bar{X}$	196	187.5	186.5	194.5		
SD	6.32	8.42	13.53	17.52		
Female Weight $\bar{X}$	178.5	172	169	173.5		
SD	10.75	9.13	8.98	7.14		
Both Sexes Weight $\bar{X}$	187.2	179.9	177.7	184		
SD	12.42	11.70	14.16	16.72		

Remark: $\bar{X}$ = Average
SD = Standard Deviation

Table B-2: Comparison of Test Conditions between the Acute Dermal Toxicity Test and the Primary Dermal Irritation Test

TEST	AVERAGE WEIGHT OF RATS (g)	NUMBERS OF RATS	DOSE OF SDD2 PER RAT (g/kg)	EXPOSURE DURATION (hours)
Acute Dermal Toxicity	175	10	2	24
Primary Dermal Irritation	187	8	3.5	4

SECTION C: ACUTE INHALATION TOXICITY TEST

ABSTRACT

Detailed study on the inhalation toxicity of the South Dakota Deicer Number 2 (SDD2) is essential to identify its effect on living organisms which need to breath to survive. The Acute Inhalation Toxicity Test is designed to evaluate the adverse effect caused by a substance following a single uninterrupted exposure by inhalation over a short period of time. The Limit Test with maximum attainable concentration of SDD2 has been performed by following the procedure outlined by the Environmental Protection Agency in the Code of Federal Regulations (CFR) (1).

Since the dose level proposed in the CFR guideline, 5mg/l, was practically unattainable due to the nature of physical and chemical properties of SDD2, a nominal concentration of 4.48mg/l was used. This factor contributed to the result that only 83% of the nominal concentration of SDD2 was recovered as the actual concentration in the breathing zone.

The administered concentration of SDD2 showed no adverse effect on the test rats during and after the exposure period. Furthermore, there were no abnormal gross pathological changes. Since the Limit Test produced no SDD2-related mortality, a full study with three dose levels has not been pursued.

The results of the test further assure that SDD2 is a safe and relatively nontoxic substance and unlikely to cause any malicious impact on living organisms.

EXPERIMENTAL PROCEDURES

Animals and Preparations

Used were five nonpregnant females and five males of white rats (strain Sprague Dawley, Charles River Laboratories, Inc.). They were allowed 10 days of post-shipping rest in the laboratory before the test. Throughout the study, individual rats were kept separately and fed twice a day with a pellet per each feeding of the rodent laboratory chow (#5001, Purina Mills Inc.). Sufficient amount of water was provided all the time. Animals fasted overnight prior to the test. Untreated animals were kept separately for test control.

Inhalation Apparatus

A 20 liter glass tank was used as an exposure chamber. The deicer prepared by a wet-sieving process (less than 10μm of particle size) was aerosolized through the nebulizer (model #1408, Hudson, Temecula, CA). The nebulizer was designed to optimally aerosolize less than 10 micrometer-size particles (personal communication with the technical service of the company). The compressed air containing 19% oxygen was supplied by a local distributor (Air Products and Chemicals, Inc., Rapid city). A diagram of the apparatus for the inhalation experiment is schematically shown in Figure C-1.

Chamber Operation

The flow rate of air was adjusted to 5l/min which resulted in 15 air changes in the 20 liter chamber every hour or 0.25 chamber volume each minute. The practical maximum concentration of SDD2 for nebulizing was determined to be approximately 0.224g/ml (discussed in detail in the section of Dose Selection and Administration).

The volume of nebulized SDD2 was measured to be 6ml/hour, from which the weight of SDD2 introduced could be calculated as 6ml/hr x 0.224g/ml = 1.344g/hr. Since the air flow rate was set 30Ol/hr (5l/min), the nominal concentration of SDD2 in the exposure chamber could be expected to be 4.48mg/l.

The necessary time to attain the maximum concentration in the chamber was calculated from the equation introduced by Kennedy and Trochimowicz (2);

$$C = (w/b)\ [1 - \exp(-bt/a)]$$

where C: chamber concentration,

W: weight of SDD2/unit time

b: total air flow rate

t: time

a: chamber volume

From this equation, the time to reach 99% (maximum percentage) of the concentration is calculated to be approximately 18 minutes.

Preparation of SDD2

SDD2 was dried thoroughly at 50 C for more than 48 hours after being supplied by the South Dakota Department of Transportation. It was ground finely in the ball mill and filtered through a 38 micrometer opening sieve (#400, U.S.A. Standard Testing Sieve, W.S. Tyler, Inc., Ohio). This filtrate was used for all sets of concurrent toxicity experiments. It was kept at 50°C or in the desiccator until needed.

On test day, it was suspended into the distilled water and then passed through a sieve with the mesh size of 10 micrometer (BMC Micro Mesh, Buckbee-Mears, St. Paul, MN). A value of 0.224g/ml was obtained as the density of the final suspension of the filtrate with less than 10 micrometers in particle

sizes. This suspension was used for the inhalation test. The physical and chemical nature of SDD2 is described in detail in Appendix A.

Dose Selection and Administration

Numerous attempts have been made to obtain the concentration (5mg/l) of aerosolized SDD2 allowed for the Limit Test. The maximum concentration of SDD2 was determined to be 0.224g/ml in the original suspension for nebulization. When more than this concentration of SDD2 was suspended, the degree of nebulization decreased drastically. This was mainly due to the build-up of a large amount of foam in the nebulizer which interfered with the aerosolizing action on the surface of SDD2 suspension. At this concentration, the uniform nebulization for at least 30 minutes was confirmed. However, a new suspension was introduced every 30 minutes to assure the consistent action of aerosol.

Atmosphere Analysis

Quantitative measurement of retained dose was conducted by following the modified procedure of Kennedy and Trochimowicz (2). The actual concentrations of SDD2 were determined at the beginning, middle, and end of the exposure period. The actual dose of the deicer administered was collected in two layers of preweighed filter papers; the first was a glass fiber filter (0.7μm, Whatman) and the second a cellulose nitrate membrane (0.2μm, Whatman). After drying in the desiccator for approximately 4 hours, the collected deicer was weighed.

Inhalation Test

Ten animals were weighed before the experiment. Average weight of both sexes was 93g. In the laboratory, the average volume versus weight of rats

was determined to be approximately 0.9. Therefore the total volume of 10 animals was less than 1 liter (930g x 0.9ml/g = 840ml), which was within 5% of the test chamber volume. Water and food were withheld during the exposure time. Woodchip bedding was layered on the floor of the exposure chamber to prevent animals from sliding.

Slightly negative pressure was maintained by connecting to the exhaust pump. Both hygrometer and thermometer were installed in the chamber. Humidity and temperature were read and recorded every 30 minutes.

The actual test was commenced after 18 minutes which was required for the equilibration of the maximum concentration level achieved in the chamber. Air flow rate (5l/min) was maintained to accommodate 0.25 chamber volume per minute or 15 air changes per hour. The rate was checked and recorded every 30 minutes. As an added precaution, the SDD2 solution in the nebulizer was changed every 30 minutes and the output was checked 15 minutes after the change.

Observation Period

After the exposure period (4 hours), the test was terminated by disconnecting the airflow with the exhaust pump running. Animals were allowed to sit in the chamber for 30 minutes and their behavior and toxic signs were observed. After examination, animals were sent to their cages and supplied with food and water. Individual weights of animals were measured after 24 hours and weekly thereafter and at death. Cage-side observations were made daily until the termination day. The gross necropsy was performed at day 17 after the exposure.

RESULTS AND DISCUSSION

The exposure data are summarized in Table C-1. As discussed previously, it was not possible to obtain the deicer concentration of 5mg/l in the chamber. This was mainly due to the hydroscopic nature of SDD2 (Appendix A). Determination of the particle sizes from Stokes' Law (3) was not applicable because of its deliquescent property in the air.

During the 4-hour exposure period, a total of 6 ml (0.224g SDD2/ml) was aerosolized. The nominal concentration of the deicer in the chamber was therefore expected to be 4.48mg/l throughout the exposure. However, it was tried without success to recover the amount of aerosolized deicer in the chamber. The actual concentration recovered on the GF/F paper and cellulose nitrate membrane was an average of 3.7mg/l. The recovery rate was approximately 83% of the nominal concentration of SDD2.

The chemical nature of SDD2, a mixture of sodium salts of organic acids, might contribute to the problems encountered during the inhalation experiment. In particular, unknown chemical characteristics of SDD2 might be the main cause of the difficulties in the generation of the optimum atmosphere condition and its recovery.

After the exposure duration, animals were kept in the chamber for 30 minutes. After this holding period, a careful clinical examination was made. All the animals resumed normal activities and showed no signs of toxicity-related behavior.

At day 8 after exposure, one male rat (male #5) was found dead. One more male (male #4) died at day 16. Gross necropsy was performed on the first animal by Dr. Mischler immediately after death. It was confirmed that the death was caused by crystal blockage of the urinary tract and not apparently related to the toxicity of SDD2. No other abnormalities in internal organs including the brain were found. The autopsy report of male #5 is included in

Appendix B-1. The second dead male was refrigerated overnight and examined on day 17 with other sacrificed animals. Gross necropsy indicated an enlarged bladder and blockage of the urethra, and some red consolidation in the lung, without any abnormality in other internal organs and the brain. The report of male #4 is included in Appendix B-2. Even though the unusual type of abnormality discovered in the lung did not appear to be related to the toxicity of SDD2 inhalation, the rat was sent to the South Dakota State University for further examination.

Both animals were lethargic prior to death. During this period, a similar behavior was observed in a male goat at the Animal Health Unit of the National College. All these animals showed red and enlarged penises and werediagnosed as having urolithiasis. It was suspected that water supply and/or nutritional imbalances resulted in the blockage of the urinary tract. Therefore the mortality of rats was ruled out as being SDD2 related.

All surviving animals displayed no signs of toxicity and acted normally and healthy throughout the observation period. All the test animals including the control group were sacrificed by exposure to the dry ice in the closed chambers at day 17 after the inhalation test. The gross necropsy showed no gross lesions in all animals except the atelectasis, a type of collapsed or airless condition of the lung which would be commonly encountered in the death by carbon dioxide intoxication. The control animals showed the same type of consolidation in the lung. Table C-2 summarizes the animal data which include number, sex, and the response of weights (average and standard deviation) as well as the results of gross necropsies. The resulting data reveal repeatedly that SDD2 would not cause any acute inhalation toxicity to animals.

REFERENCES

1. U.S. Environmental Protection Agency, Code of Federal Regulations, Title 40, Part 798, Section 1150 (1987).

2. Kennedy, G. L. Jr. and H. J. Trochimowicz, "Inhalation Toxicity," In: Principles and Methods of Toxicology; edited by A. W. Hayes, Student edition, pp. 185-207, Raven Press, New York (1982).

3. Metcalf and Eddy, Inc., "Wastewater Engineering: Treatment, Disposal, Reuse," 2nd Ed. McGrow-Hill, Inc., New York, pp. 203-207 (1979).

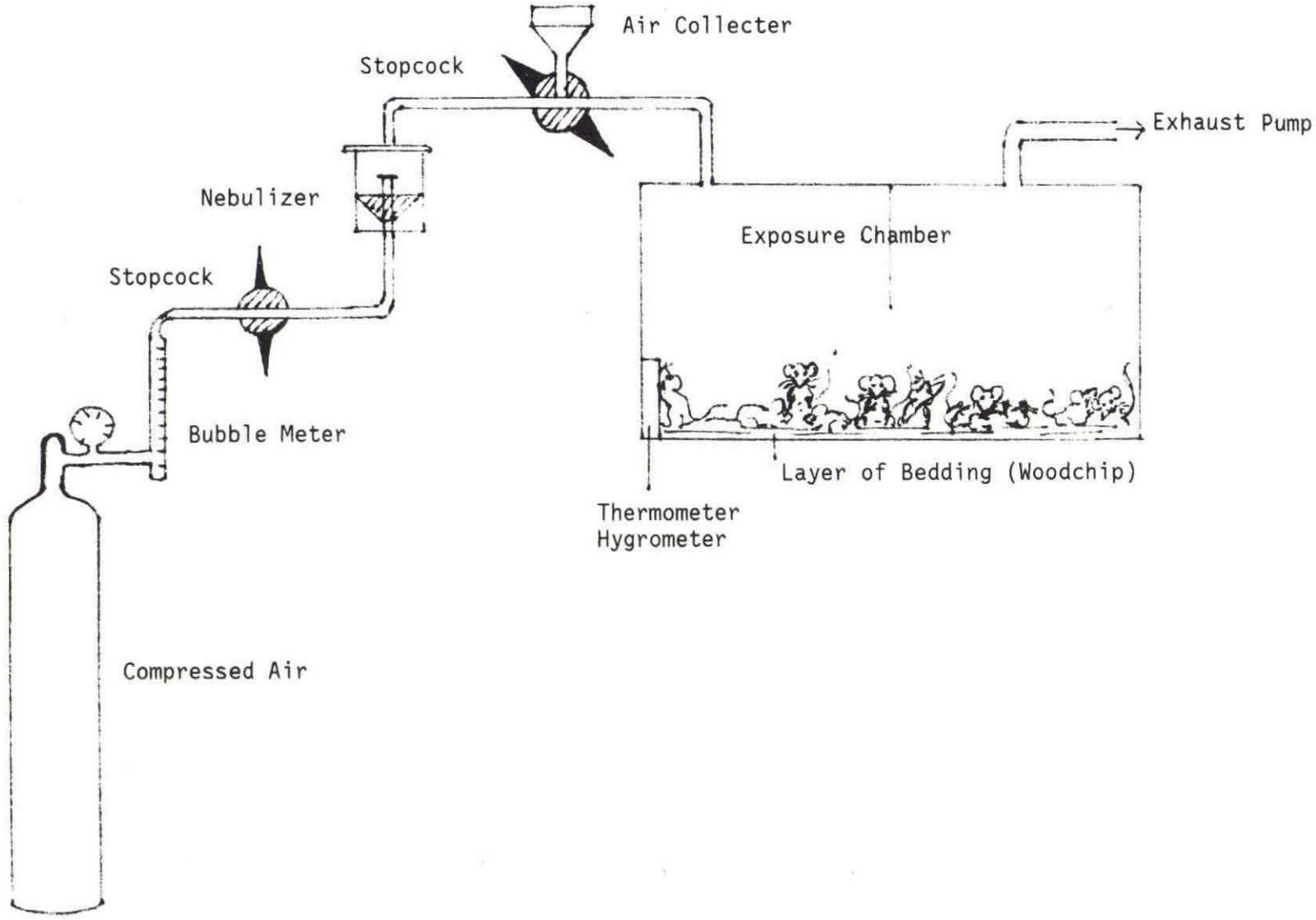


Figure c-1: Schematic of Inhalation Apparatus

Table C-1: Summary of Inhalation Exposure Data

TIME (hr)	RATE OF AIR FLOW (l/min)	TEMPERATURE (°C)	HUMIDITY (%)	NOMINAL CONCENTRATION OF SDD2 (mg/l)	ACTUAL CONCENTRATION OF SDD2 (mg/l)
0	5	26	76	4.48	3.7
0.5	5	26	76		
1.0	5	26	76		
1.5	5	26.5	76		
2.0	5	26	76	4.48	3.8
2.5	5	26	76		
3.0	5	26	78		
3.5	5	26	78		
4.0	5	26	82	4.48	3.6

Table C-2: Results of the Acute Inhalation Toxicity Test

SEX	weight (g) AFTER (days)					CLINICAL EXAMINATION	GROSS NECROPSY 17 DAYS
	0	1	7	14	17	AFTER EXPOSURE	AFTER EXPOSURE
Male	98	92	83	106	98	No Toxicity Sign	No Abnormality
Male	79	72	80	97	90	No Toxicity Sign	No Abnormality
Male	78	71	78	93	97	No Toxicity Sign	No Abnormality
Male	103	92	78	80 (died at day 16, 80g)		No Toxicity Sign	(See Appendix B-2)
Male	104	91	73 (died at day 8, 79g)			No Toxicity Sign	(See Appendix B-1)
Female	96	87	92	102	108	No toxicity Sign	No Abnormality
Female	79	76	82	97	95	No Toxicity Sign	No Abnormality
Female	102	92	89	100	97	No Toxicity Sign	No Abnormality
Female	99	95	97	111	112	No Toxicity Sign	No Abnormality
Female	90	81	80	109	108	No Toxicity Sign	No Abnormality
Male Weight							
$\bar{X}$	92.4	83.6	78.4	94.0	95.0		
SD	12.90	11.06	3.65	10.80	4.36		
Female Weight							
$\bar{X}$	93.2	86.2	88.0	103.8	104.0		
SD	9.09	7.79	7.04	5.97	7.52		
Both Sexes Weight							
$\bar{X}$	92.8	84.9	83.2	99.4	100.6		
SD	10.53	9.12	7.32	9.40	7.71		

Remark: $\bar{X}$ = Average
SD = Standard Deviation

SECTION D: FISH ACUTE TOXICITY TEST

ABSTRACT

The acute bioassay with rainbow trout, Salmo mykiss, was conducted in the laboratory to assess the effect of the South Dakota Deicer Number 2 (SDD2) on aquatic systems in the environment. The static Acute Toxicity Test was carried out for this purpose, following the guideline prepared by the Environmental Protection Agency (1). The toxicity of SDD2 was expressed by the median lethal concentration (LC_{50}) after a 96-hour test period.

It was not possible to determine LC_{50} of SDD2 directly, mainly due to the lack of data points in the calculation range. However, it was concluded that the LC_{50} value might reside between 12.78g SDD2/l (10% mortality) and 23g SDD2/l (100% mortality), suggesting low toxicity of the substance on fish. A similar value (17.5g/l) has also been reported with the other known highway deicer, CMA. At higher concentrations, SDD2 was observed to reduce the amount of dissolved oxygen in water which would induce a potential environmental impact, since rainbow trout could be easily harmed by low oxygen level rather than by high concentration of SDD2.

The test results indicate low toxicity of SDD2 in aquatic system, since a realistic concentration of SDD2 is much lower in the environment. However, in order to further understand the direct effect of SDD2 on aquatic ecosystems, an extensive study with a variety of other aquatic animals of different developmental stages needs to be conducted.

EXPERIMENTAL PREPARATIONS

The experiment was prepared and carried out following the basic procedures described by the Environmental Protection Agency (EPA) in the Code of Federal Regulations (CFR) (1) and the EPA research reports (2,3).

Test chambers

For the Static Test, used were the glass chambers (10 gallon size) with glass covers. Chambers were cleaned by following the procedures described by the EPA guideline (1) and the EPA research reports (2,3). Other accessories including air diffusion stones, thermometers, and tubing were washed and thoroughly rinsed with the dilution water prior to the test.

Dilution Water

For dilution water, used was the glass distilled water, quality of which has been analyzed (Table D-1). Appendix C includes a summary of the analysis data from the Travis Laboratory, Rapid City, SD. The average specific conductivity of the distilled water was 1.3 micromhos/cm. The contents of total organophosphorus pesticides and total organochlorine pesticides plus polychlorinated biphenyls (PCB's) were not determined.

Reconstituted Water

Reconstituted water was prepared with the glass distilled water by adding Calcium Chloride, Magnesium sulfate, Sodium Bicarbonate, and Potassium Chloride, in which the sum of the calcium and magnesium ions was 2.5mmol/l with the ratio of Ca:Mg-ions 4.13 and of Na:K-ions 10:1 (1). The basic quality of the reconstituted water is summarized in Table D-2.

Fish

Juvenile rainbow trout (Salmo mykiss) were supplied by the State Fish Hatchery in Rapid City, where they had been raised at 11°C with supersaturated dissolved oxygen of around 14mg/L. Upon delivery, fish were approximately 9 weeks old (batch from the same hatch) and weighed between 0.3 and 1.5g each. The fish hatchery also furnished their diet, a type of granule which consisted of ground herring, soy meals, wheat, fish oil, vitamin pack and a trace of minerals.

Acclimation

An acclimation tank, holding over 200 rainbow trouts, was filled with reconstituted water and aerated through porous stones connected to air pumps. The dissolved oxygen (DO) was maintained above 8.7mg/L and the temperature was maintained at 12 ± 2 C. The amount of oxygen available in water is critical for the survival of all respiring aquatic organisms including fish. Coldwater fish, e.g., rainbow trout, specially require more dissolved oxygen than other warmwater fish. Fish were held for 22 days before the Definitive Test. Twice a day, they were lightly fed, the metabolic excreta of which were cleaned, and DO and pH of the water were checked. An alternating 14-hour light and 10-hour dark photoperiod was maintained. Less than 1% mortality per day had been observed throughout the holding period.

Chemical and Physical Measurements

The dissolved oxygen in the tank was measured with the DO meter (YSI model 57, #5750 Probe, Yellow Springs Instrument Co., Yellow Springs, OH). The specific conductivity and total dissolved solids (TDS) were measured by the conductivity/TDS meter (Hach Co., Loveland, CO). The measurements of total hardness and alkalinity of tank water were conducted by following the standard methods described by the American Public Heath Association (4,5).

Concentration of SDD2

The toxicant used was the pulverized SDD2 with less than 38 micrometers diameter. Chemical and physical properties are described in Appendix A. A complete analysis of SDD2 by the High Performance Liquid Chromatography (HPLC) is also described in Appendix D. The one-point range-finding test indicated that a concentration of 25g SDD2/l caused 100% lethality within 24 hours. In comparison, CMA has shown an LC_{50} of 17.5g/l (6). Based on the above information and amount of SDD2 available, it was decided to use the highest concentrations of 23, 12.78, 7.10, 3.94, and 2.19g/l. These concentrations of SDD2 have a factor of 1.8 between a successive pair.

Calculation of LC_{50}

The LC_{50} indicates the concentration of the test substance that is lethal to 50% of the test population during continuous exposure over a specified period. From the experiment data, efforts were made to calculate the 96-hour LC_{50} and its 95% confidence limits by following the method of Litchfield-Wilcoxon (7).

EXPERIMENTAL PROCEDURES

Due to the shortage of supplied SDD2, the Definitive Test was somewhat limited in its replication. The toxicity experiment consisted of 5 different concentrations of SDD2 and a control. It was decided to use a 96 hour exposure duration.

Two days prior to the Definitive Test, 37 liters of reconstituted water were added into each of six tanks, which had been placed inside a 60-quart cooler. Each tank was provided with a thermometer and two air diffusion stones connected to two air pumps. Temperature was maintained by refurbishing

ice cubes and freeze bottles between the tank and the cooler. Dissolved oxygen and temperature were maintained at over 9mg/L and approximately 12°C, respectively.

One day prior to the testing, 20 fish were transferred into each tank. A careful attempt was made to choose the right size of fish, i.e., 5.0 ± 1.0cm in length and less than 0.9g in weight. After being transferred into the tanks, fish were not fed throughout the test period. Separately, five different concentrations of SDD2 were dissolved into aliquots of each tank water and kept in the refrigerator overnight.

On test day, the Definitive Static Test was commenced by adding the prepared concentrations of SDD2 into individual tanks. One tank was kept without the deicer for control. Immediately measured were the specific conductivity, the pH, the dissolved oxygen, and the temperature. Approximately 250ml of sample was taken from each tank to measure the initial total alkalinity and hardness which were determined later on the same day. Number of fish, DO, pH, and temperature were checked every 12 hours, while specific conductivity, total alkalinity, and total hardness were checked at the beginning and end of the test.

During the exposure period of the Static Test, the temperature of each chamber was closely monitored by adding ice and changing the freeze bottles. Behavior and death of fish were checked as often as possible. Dead animals were removed immediately and their length and weight were measured. Every 12 hours, readings of pH, number of fish, DO, and temperature were recorded.

At the end of the 96 hour exposure period - the end of the fish toxicity test - surviving fish were sacrificed by placing into deaerated warm water. Length and weight of all fish were measured to obtain the averages and the standard deviations of each tank. Last readings were made on all parameters

including the specific conductivity. Aliqots of the water samples were taken from each tank to obtain the hardness and the alkalinity.

RESULTS AND DISCUSSION

As indicated in Table D-1, the analysis test of dilution water was not complete. Note that relatively high COD values were obtained in the glass distilled water, which might have resulted from technical error. The quality of dilution water was, however, considered acceptable, since there had been no death in the control tank for a 96-hour period.

Tables D-3a through D-3f summarize the experimental data from individual tanks, including the results of DO, pH, temperature, number of fish, average weight and length of fish with standard deviations, conductivity, total hardness, and total alkalinity.

The Definitive Test (Tables D-3's) was completed without major difficulties. Relatively constant temperature was maintained throughout the test. Gradual decrease in pH however was observed during the experiment, which could be explained by the accumulation of metabolic waste products of fish.

It was anticipated that the saturated level of dissolved oxygen would be in the vicinity of 9.5mg/L at 12°C at the altitude of the laboratory. The concentrations of dissolved oxygen were maintained close to 100%, but always above 90%, as a minimum saturation in individual tanks except Tank 6. The concentration of DO in Tank 6 decreased to a minimum of close to 60% saturation toward the end, which might have been contributed by the high concentration of SDD2 as well as its interaction with waste products of fish. Depletion of oxygen was clearly evident by the addition of SDD2 as expected.

CMA showed a similar result on the surface water (6).

It was difficult to obtain a uniform size of fish for the experiment, resulting in relatively high values in standard deviation of fish weight. However, the loading in the test chamber was 0.46g/l on average with an average fish length of 4.3cm, which met the criterion for coldwater fish (1).

The measurements of the total hardness versus concentrations of SDD2 are depicted in Figure D-1. The measurements are expressed as mg $CaCO_3$/l. Results of the hardness measurements indicated a linearity of the values with changes in SDD2 concentration without a major disagreement. As shown in the figure, the total hardness changed little after the test period.

Some difficulties were observed in titration during the alkalinity measurement. Due to the buffering nature of SDD2, inflection points were not obtained in titrations for Tanks 2 - 6. The determination of an inflection point is crucial in order to calculate an alkalinity value. Thus, it was practically impossible to calculate alkalinity values. The lowest pKa value of the known organic acids (whose salts are present in significant quantities in SDD2) is 3.1 for lactic acid. Calculating the number of moles of sulfuric acid titrant necessary to reach a pH of 3.0 with 10 ml of sample would generate meaningful data with regard to total alkalinity. The data are presented in Figure D-2.

Overall, the total alkalinity at pH 3.0 increased with increasing concentration of SDD2. Also, in general, the total alkalinty at pH 3.0 increased by a constant amount after 96 hours of fish habitation, reflecting the addition of alkaline products due to excretion.

The chemical characteristics of SDD2 generated a unique problem. Immediately after the addition of SDD2 in Tanks 5 and 6, a significant amount of foam built up in the tanks. Extra care was made to remove the foam which

had interfered with the regular experimental readings. In addition, test water with higher concentration darkened in color, resulting in difficulties in monitoring the fish behavior.

Throughout the test period, fish from the control and tanks with low concentrations of SDD2 (Tanks 2, 3, 4) behaved normally, showing no death. As shown in Tables D-3e and D-3f, 16 fish were lost in first 12 hours and 4 more in the next 24 hours in Tank 6, while 2 fish were lost eventually in Tank 5.

Based on the mortality data summarized in Table D-4, an attempt was made to calculate the 96-hour LC_{50} following the Litchfield and Wilcoxon method (7). Due to the lack of data points in the range of 16% to 84% mortality, it was not possible to compute the LC_{50} as well as its confidence limits. It could be estimated to be approximately in the range of 12.78 to 23g/l of SDD2, which is comparable to that of CMA, 17.5g/l (6).

Relatively higher concentrations of LC_{50} of SDD2 would provide a low acute toxicity of the substance in aquatic system in the environment. As a result, unless unrealistic spill occurs on the highway, SDD2 concentrations expected in most highway runoff would not likely cause any harmful toxic effect on aquatic animals.

To obtain a better understanding of the effect of SDD2 on fresh water animals, it is imperative to conduct a further in-depth comprehensive study using a variety of aquatic animals so that the toxicity of the substance can be fully identified.

REFERENCES

1. U.S. Environmental Protection Agency, Code of Federal Regulations, Title 40, Part 797, Section 1400 (1987).

2. U.S. Environmental Protection Agency, Committee on Methods for Toxicity Tests with Aquatic Organisms, "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians," EPA Report No. 660/3-75-009, Corvallis, OR (1975).

3. U.S. Environmental Protection Agency, "Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms," EPA Report No. 600/4-78-012, Cincinnati, OH (1978).

4. American Public Health Association, "Standard Methods for the Examination of Water and Wastewater," 16th Ed., American Public Health Association, Washington, D.C. pp. 209-214 (1985).

5. American Public Health Association, "Standard Methods for the Examination of Water and Wastewater," 16th Ed., American Public Health Association, Washington, D.C. pp. 269-273 (1985).

6. Horner, R. R., "Environmental Monitoring and Evaluation of Calcium Magnesium Acetate (CMA)," National Cooperative Highway Research Program (NCHRP) Report 305 (1988).

7. Litchfield, J. T., and F. Wilcoxon, "A Simplified Method of Evaluating Dose-Effect Experiments," J. of Pharmacology and Experimental Therapeutics, 96: 99-113 (1947).

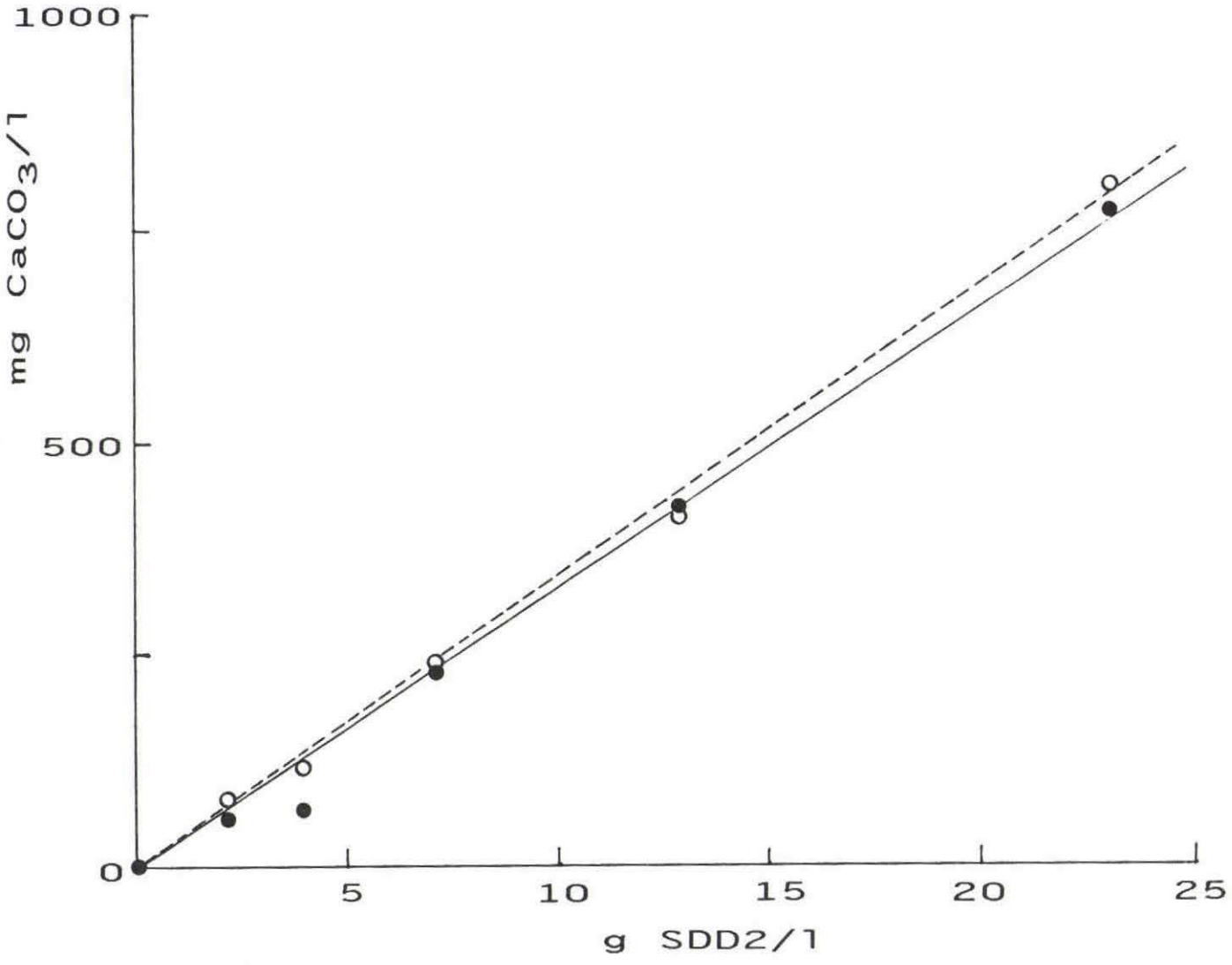


Figure D-1: Total hardness versus SDD2 concentration
(—●—); 0 hour, (--o--); 96 hour

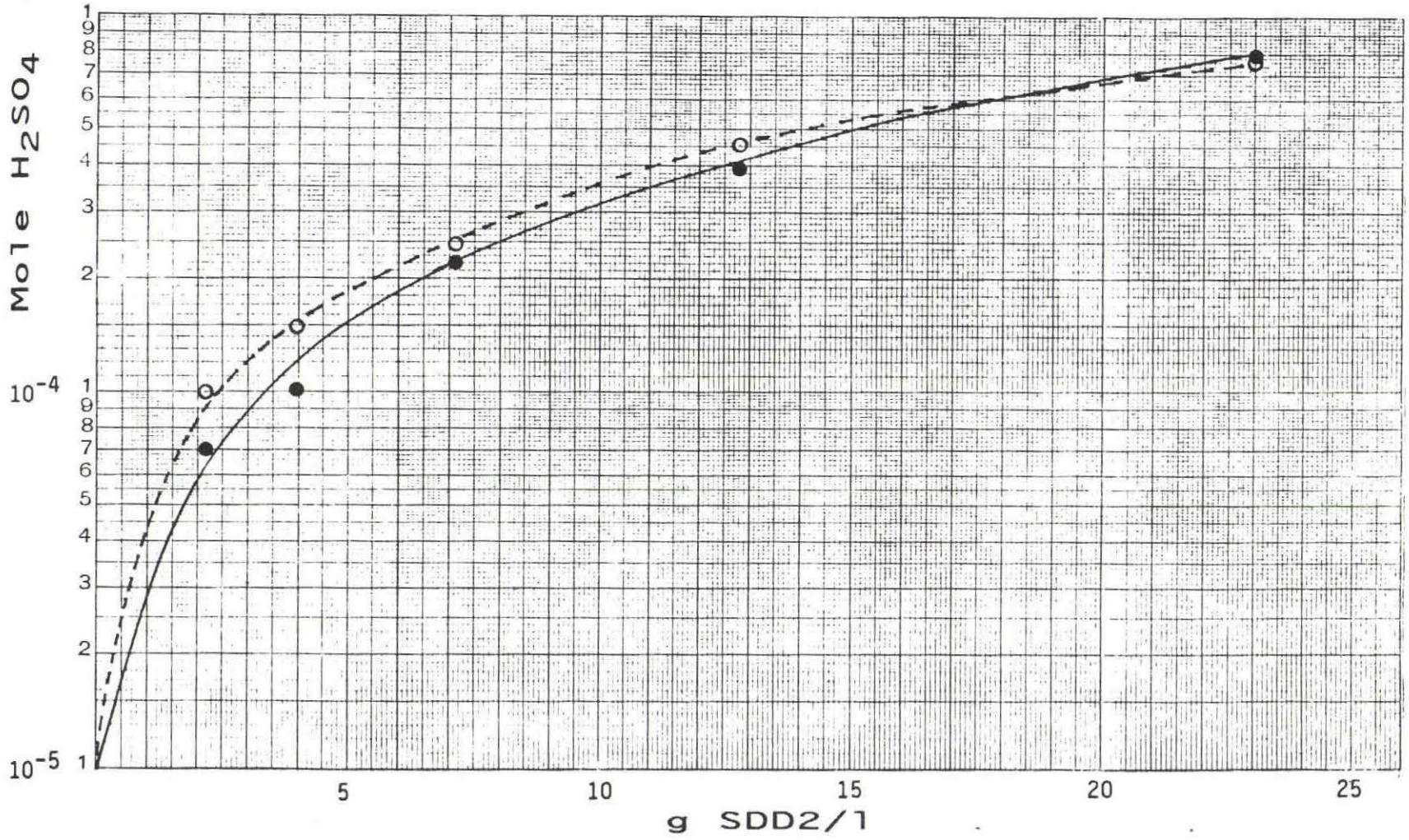


Figure D-2: Concentration of H_2SO_4 titrant (in the Total Alkalinity Measurement) necessary to reach pH 3.0 with 10 ml of sample (—●—); 0 hour, (--o--); 96 hour

Table D-1: Quality of Dilution Water

Substance	Quantity
Conductivity	1.3 micromhos/cm
Total Dissolved Solid (TDS)	0.7 mg/l
Chemical Oxygen Demand (COD)	5.6 mg/l*
Boron	< 200 µg/l*
Fluoride	< 100 µg/l*
Un-ionized ammonia	< 0.1 µg/l*
Arsenic, Chromium, Cobalt, Copper, Lead, Nickel, Zinc	< 5 µg/l each*
Iron	< 50 µg/l*
Mercury	< 0.2 µg/l*
Cadmium, Silver	< 0.5 µg/l each*
Total Residual Chlorine (TRC)	< 100 µg/l*
Total organophosphorous pesticides	ND
Total organochlorine pesticides plus polychlorinated biphenyls (PCB's)	ND

* Determined by the Travis Laboratories, Rapid City, SD.

ND: Not Determined

Table D-2: Quality of Reconstituted Water

pH	CONDUCTIVITY (millimho/cm)	HARDNESS (mg $CaCO_3$/l)	ALKALINITY (mg $CaCO_3$/l)	CHEMICALS FOR RECONSTITUTION (g/100l)
7.15	0.384	140	110	$CaCO_3.2H_2O$; 7.422 $MgSO_4.7H_2O$; 3.109 $NaHCO_3$; 1.625 KCl; 1.625

Table D-3a: Summary of test data from Tank 1

TIME (hr)	# OF LIVE FISH	DISSOLVED OXYGEN (mg/L)	TEMPERATURE (°C)	pH	CONDUCTIVITY (mmhos/cm)	ALKALINITY (mg $CaCO_3$/l)	HARDNESS (mg $CaCO_3$/l)	CONCENTRATION OF SDD2 (g/l)
0	20	9.6	12.0	7.15	0.348	110	176	0
12	20	9.7	12.1	7.5				
24	20	9.8	12.0	7.35				
48	20	9.5	12.0	7.25				
72	20	9.5	12.0	7.25				
96	20	9.5	12.5	7.2	0.350	140	192	

Average Length of Fish: 4.570 cm (SD; 0.409)

Average Weight of Fish: 0.956 g (SD; 0.245)

Table D-3b: Summary of Test Data from Tank 2

TIME (hr)	# OF LIVE FISH	DISSOLVED OXYGEN (mg/L)	TEMPERATURE (°C)	pH	CONDUCTIVITY (mmhos/cm)	ALKALINITY (mg $CaCO_3$/l)	HARDNESS (mg $CaCO_3$/l)	CONCENTRATION OF SDD2 (g/l)
0	20	9.7	12.0	8.95	2.28	420	228	2.19
12	20	9.6	12.8	8.40				
24	20	9.5	12.0	7.95				
48	20	9.4	11.8	8.00				
72	20	9.2	12.7	8.00				
96	20	8.9	14.0	7.95	2.35	530	268	

Average Length of Fish: 4.335 cm (SD; 0.372)

Average Weight of Fish: 0.855 g (SD; 0.209)

Table D-3c: Summary of Test Data from Tank 3

TIME (hr)	# OF LIVE FISH	DISSOLVED OXYGEN (mg/L)	TEMPERATURE (°C)	pH	CONDUCTIVITY (mmhos/cm)	ALKALINITY (mg $CaCO_3$/l)	HARDNESS (mg $CaCO_3$/l)	CONCENTRATION OF SDD2 (g/l)
0	20	9.7	11.8	8.85	3.68	620	246	3.94
12	20	9.8	11.8	9.10				
24	20	9.6	10.5	8.35				
48	20	9.3	10.7	8.05				
72	20	9.1	11.2	8.00				
96	20	9.0	11.8	8.05	3.85	1,250	312	

Average Length of Fish: 4.400 cm (SD; 0.381)

Average Weight of Fish: 0.948 g (SD; 0.288)

Table D-3d: Summary of Test Data from Tank 4

TIME (hr)	# OF LIVE FISH	DISSOLVED OXYGEN (mg/L)	TEMPERATURE (°C)	pH	CONDUCTIVITY (mmhos/cm)	ALKALINITY (mg $CaCO_3$/l)	HARDNESS (mg $CaCO_3$/l)	CONCENTRATION OF SDD2 (g/l)
0	20	9.7	11.8	9.0	6.0	1,050	400	7.10
12	20	9.7	12.8	9.0				
24	20	9.5	11.0	8.35				
48	20	9.3	12.3	8.2				
72	20	8.7	13.1	8.25				
96	20	8.8	14.8	8.32	6.29	1,550	436	

Average Length of Fish: 4.325cm (SD; 0.313)

Average Weight of Fish: 0.826g (SD; 0.180)

Table D-3e: Summary of Test Data from Tank 5

TIME (hr)	# OF LIVE FISH	DISSOLVED OXYGEN (mg/L)	TEMPERATURE (°C)	pH	CONDUCTIVITY (mmhos/cm)	ALKALINITY (mg $CaCO_3$/l)	HARDNESS (mg $CaCO_3$/l)	CONCENTRATION OF SDD2 (g/l)
0	20	9.7	12.0	9.0	9.84	1,150	596	12.78
12	20	9.6	12.5	9.25				
24	20	9.7	10.9	8.5				
48	19	8.6	12.2	8.25				
72	19	8.2	12.9	8.3				
96	18	8.7	13.8	8.3	10.15	2,250	608	

Average Length of Fish: 4.137 cm (SD; 0.315)

Average Weight of fish: 0.736 g (SD; 0.195)

Table D-3f: Summary of Test Data from Tank 6

TIME (hr)	# OF LIVE FISH	DISSOLVED OXYGEN (mg/L)	TEMPERATURE (°C)	pH	CONDUCTIVITY (mmhos/cm)	ALKALINITY (mg $CaCO_3$/l)	HARDNESS (mg $CaCO_3$/l)	CONCENTRATION OF SDD2 (g/l)
0	20	9.5	11.8	8.9	16.18	1,950	952	23
12	16	9.6	12.0	9.35				
24	1	9.6	11.0	8.8				
48	0	7.8	11.9	8.3				
72	0	6.9	12.5	8.2				
96	0	6.8	13.0	8.13	16.57	5,000	992	

Average Length of Fish: 4.150 cm (SD; 0.324)

Average Weight of Fish: 0.751 g (SD; 0.185)

Table D-4: Results of the Acute Toxicity of Rainbow Trout

	NUMBER OF TANK					
	1	2	3	4	5	6
CONCENTRATION OF SDD2 (g/l)	0	2.19	3.94	7.10	12.78	23
# LIVE FISH	20	20	20	20	18	0
% MORTALITY	0	0	0	0	10	100

SECTION E: BIOCHEMICAL OXYGEN DEMAND TEST

ABSTRACT

Having recognized the chemical nature of SDD2, a mixture of sodium salts of organic acids, it is imperative to study the microbial decomposition characteristics of the compound in the environment. To assess the impact on the aquatic environment by SDD2, a basic laboratory determination of biochemical oxygen demand (BOD) was conducted through two sets of experiments. The first consisted of determining oxygen depletion with four different concentrations of SDD2, whereas the second was to determine the effect of different temperatures on oxygen depletion at fixed SDD2 concentration.

In the standard BOD experiment at 20°C, SDD2 was rapidly degraded, as expected, by microorganisms. Complete oxygen depletion occurred within a day with SDD2 concentrations of over 50mg/l. With the concentration of 10mg SDD2/l, sufficient BOD (79%) was exerted within the first five days, with almost all (99%) in 20 days. However, an undesirable noncarbonaceous oxygen demand was observed after day 16.

In the multiple-temperature BOD experiment, the rate of oxygen consumption decreased as the temperature was lowered. The decomposition rate at 10°C was two thirds of that at 20°C, while the rate constant at 2°C was approximately one third of that at 20°C. Comparison with the results from similar experiments with CMA demonstrated that overall decomposition rates of SDD2 at different temperatures were faster than for CMA.

Results of the BOD test indicate that in the typical environment the decomposition of SDD2 takes place in a relatively short time period in warm surface water, while relatively longer in cold water.

EXPERIMENTAL DESIGN

Apparatus

1) Incubators and equipment: Incubators for different temperatures (2, 10, 20°C) were installed with thermo-controllers (IncuTrol/2, Hach Co. Loveland, CO) to maintain constant temperatures. The temperatures were maintained within ± 1°C throughout the experiment. Light was prevented to eliminate the possibility of any DO production by photosynthetic microorganisms. Equipment needed for the experiment were checked for proper operation and replaced when necessary.

2) Incubation bottles: Prior to the experiment, BOD glass bottles (300 ml capacity) and glass stoppers were cleaned with acid, thoroughly rinsed with tap water and distilled water in order, and dried overnight in the oven at 120°C. Plastic cups were cleaned with soap, rinsed with water, and completely dried before use. Assembled bottles were kept at three different constant temperatures overnight before the test.

3) Dissolved oxygen (DO) meter: The membrane of the DO meter probe (DO meter; YSI model 57, Probe; YSI 5720A, Yellow Springs Instrument Co., Yellow Springs. OH) was checked and changed, whenever necessary, prior to the experiment. The meter was equipped with a probe that allowed readings to be made directly in the bottle without sacrificing its contents. Standard membrane appeared to work more accurately than high sensitivity membrane even at low temperatures (2 and 10°C).

Reagent

All reagents for dilution water and the Winkler titration method (1)

were supplied by the Banco, Anderson Laboratories Inc. (EPA approved reagents). Glass distilled water (specific conductivity of 1.3 micromhos/cm) was used for the preparation of dilution water. The distilled water was aerated at three different temperatures for one full day prior to the experiment.

Seeding

The influent of the untreated domestic wastewater from the Rapid City Wastewater Treatment Plant was taken and settled at 20°C for 24 hours prior to the experiment. The supernatant was used as a microbial seed the following day.

EXPERIMENTAL PROCEDURES

Preparation of Dilution water and Sample

Except the bottles for unseeded blanks, 1ml of microbial seed was added to all BOD bottles prior to the addition of dilution water. The fully aerated distilled water at different temperatures was reconstituted with 1 ml each of phosphate buffer, ferric chloride, magnesium sulfate, and calcium chloride solution per liter.

For the blanks (with and without seed), only dilution water was added up to the neck of the bottles. After installing stoppers, more dilution water was added to fill up to the rim and then covered with plastic cups to minimize the evaporation of dilution water. Concentrations of the deicer used in the experiment were 10, 50, 100, and 1000mg SDD2/l, prepared by dissolving the dried deicer powder (less than 38 micrometers in diameter) into the reconstituted dilution water. All sets were prepared in triplicate samples.

Standard BOD Experiment

The basic experimental procedure closely followed the guideline provided by the American Public Health Association (2) with some modification. The experiment was carried out at 20°C for twenty days rather than the standard five days. At this fixed temperature, used were 4 different concentrations of 10, 50, 100, and 1000mg SDD2 per 1 liter of dilution water. In addition, two sets of blanks were prepared; one with dilution water only (unseeded blanks) and the other with dilution water and microbial seed (seeded blanks).

Sample bottles were placed in an incubator at 20°C. To determine the rate of decomposition, triplicate samples from each of the four concentrations and both blanks were analyzed for DO concentrations on each day during the 20-day experiment period. The dissolved oxygen meter was calibrated everyday by using the modified Winkler, Full-Bottle Technique (1) and air calibration (3). Both methods have shown almost identical calibration values every day.

To minimize any unnecessary exposure to light and room temperature, DO readings were made one bottle at a time, close to the incubator, in the order of unseeded blanks, seeded blanks, 10, 50, 100, and 1000mg SDD2/l BOD bottles. Rate constant values of decomposition reaction (K) were determined by the Thomas method (4), with the intercept (a) and the slope (b) computed from the least-squares error minimizing technique (4).

Multiple-Temperature BOD Experiment

This experiment was run at three different temperatures (2, 10, 20°C) with a fixed concentration of SDD2 of 10mg/l. The set-up for the test was basically the same as that of the standard BOD experiment. To reduce the internal temperature changes at the first day, the BOD bottles and distilled water with aeration were kept at the same temperatures for one full day prior

to the experiment. The test at each temperature included two sets of controls; one with seed and the other unseeded. DO readings were taken every day at the same time (±2 hours), starting from the blank BOD bottles of lowest temperature. The DO meter was calibrated as described previously.

RESULTS

Standard BOD Experiment

Figure E-1 summarizes the results of the standard BOD experiment with four different SDD2 concentrations. As shown in the figure, the DO uptake in 5 days shows almost no change in unseeded blanks, while 0.07mg/L in seeded blanks has been observed. This indirectly assures the quality of dilution water guided by the Standard Methods (1).

Oxygen was completely depleted within one day in 100, and 1000mg/l SDD2 concentrations, and after two days in 50mg/l concentration. In order to achieve a valid BOD test, the guideline proposes that at least one sample dilution meets the criteria of residual DO of at least 1mg/L and DO depletion of at least 2mg/L after 5 days. The 10mg SDD2/l concentration met these criteria.

BOD determination is based on the measurement of DO depletion caused by microorganisms in the biochemical oxidation of carbonaceous matter. However as indicated by an arrow in Figure E-1, a significant amount of noncarbonaceous BOD was exerted from 10mg SDD2/l after 16-day incubation. Only first-stage BOD, carbonacous demand, was therefore used for the calculation of the reaction rate constant (K) and the ultimate BOD (L).

Using data points from the curve without those of second-stage BOD (Figure E-1), the BOD rate constant of the decomposition reaction (K) was calculated to be 0.136/day. In addition, the ultimate BOD was determined as

57% of the initially applied SDD2 concentration. Biochemical oxidation expressed as the ultimate BOD is a slow process and theoretically requires an infinite amount of time to be completed. Data obtained from this experiment has shown that approximately 79% of the ultimate BOD would be exerted in the first 5 days, 96% in 10 days, and 99% in 20 days. Detailed data are tabulated in Table E-1.

Multiple-Temperature BOD Experiment

Complete results of the experiment are detailed in Figure E-2. Significant differences of the decomposition rate of 10mg SDD2/l can be found at three different temperatures. The amount of oxygen depletion in BOD bottles incubated at each temperature was calculated by subtracting the mean of the triplicate SDD2 samples from that of the triplicate seeded blanks.

Dissolved oxygen was depleted considerably within a 24 hour-incubation at 10 and 20°C, while there was a 5-day lag period before any significant oxygen depletion at 2°C. Similar patterns of oxygen depletion were observed at 10 and 20°C, except the low reading at the first day for 10°C. After day 8, the oxygen depletion of both samples more or less reached a plateau, which was sustained until day 20 at 10°C, whereas at 20°C it was interrupted by second-stage oxygen depletion begining at day 16. However, a plateau was obtained after day 13 in the 2°C samples because of a slow start with a low decomposition rate.

The results from oxygen depletion at 20°C clearly indicate the presence of noncarbonaceous BOD exertion, which were ruled out for the calculation of K and L values. The calculated BOD rate constants of the oxygen depletion reactions at 2, 10, and 20°C were 0.053, 0.092, and 1.42/day, respectively. Table E-2 summarizes the values of K, L, and the percentages of ultimate BOD of different days at three temperatures.

DISCUSSION

During the range-finding experiments using the standard five day procedure, some oxygen depletion, 0.6mg/L in dilution water (unseeded blanks), was noticed. For a valid BOD test, the DO depletion in 5 days at 20°C, should not be more than 0.2mg/L and preferably not more than 0.1mg/L. However, this problem was quickly resolved by preparing, with extra care, the clean glassware and distilled water. As indicated in Figure E-1, there occurred almost no oxygen depletion in unseeded blanks.

Two major BOD experiments were performed based on the results of the range-finding experiments. There were minor variations in rate constants and ultimate BOD's from two experiments. It could have resulted due to the different batches and amount of microbial seed used. Two ml of seed was used in the first experiment and 1 ml in the second. However, the patterns of the curves were similar except for the unseeded blanks which have shown a relatively fast oxygen depletion throughout the test. The results of a second BOD test were presented in this report.

In the standard BOD experiment, SDD2 of higher than 10mg/l in concentration was quickly decomposed, rapidly removing oxygen from water. With a concentration of 10mg SDD2/l, a significant amount (approximately 79%) was exerted in the first 5 days and almost the entire amount (99%) in 20 days.

In particular, oxygen became depleted at faster rates after day 16, which positively resulted from the nitrogenous oxygen demand caused by nitrifying bacteria. In BOD bottles which would contain a mixed culture of microogranisms, most heterotrophic microorganisms would utilize carbonaceous matter at the beginning, showing BOD exertion. As organic matter becomes depleted, the heterotrophic microorganisms would die and become hydrolyzed to

produce nitrogenous matter. Inorganic nitrogen compounds would be readily utilized by slow growing autotrophic nitrifying bacteria which were shown as a second-stage BOD after day 16. This interference could be easily overcome by pretreatment of seed or addition of a nitrification inhibitor such as 2-chloro-6-(trichloro methyl) pyridine. A slight hint of this phenomenon was observed during the first set of the BOD experiments. However, since the unseeded blanks exhibited unrealistic DO depletion at that time, the problem of seed was paid more attention and consequently the possibility of nitrification was overlooked. Therefore, it is recommended that any future investigation on the BOD test should include the elimination of interference of noncarbonaceous oxygen demand by the pretretment of sample or the use of inhibitors.

In the multiple-temperature experiment, the decomposition rate at 10°C (0.092) was two thirds of that at 20°C (0.142), while the rate at 2°C (0.053) was approximately one third of that at 20°C. It was determined that the ultimate BOD's at 2, 10, and 20°C would be exerted approximatedly within 57, 33, and 21 days, respectively.

A similar BOD experiment has been carried out by Horner (5) with CMA, which is chemically known as calcium magnesium acetate. SDD2 basically shares similar characteristics with CMA. However, CMA is a chemically pure organic salt, while SDD2 is a mixture of sodium salts of organic acids. The resulting data from the BOD experiments for the two materials are compared in Table E-3. These two materials have shown similar characteristics in the standard BOD experiment at 20°C, with a slightly higher rate constant for SDD2 than that of CMA. At low temperatures, the rate constants for SDD2 were much higher than that of CMA, indicating that SDD2 would be decomposed readily in cold water when compared to CMA.

Based on the results of the BOD experiments with SDD2, the following conclusions can be drawn:

1. SDD2 is a readily decomposable substrate for microbial respiration, showing an overall faster decomposition rate than that of CMA.
2. With higher concentrations at warm temperature, the rapid decomposition might result in a drastic oxygen depletion in surface water. This poses a possible detrimental situation when melting starts in the environment.

In order to evaluate the detailed characteristics of SDD2 on the decomposition that may accompany the oxygen depletion, further in-depth study needs to be conducted with different types of surface water.

REFERENCES

1. American Public Health Association, "Standard Methods for the Examination of Water and Wastewater", 16th edition, American Public Health Association, Washington, D.C., pp. 418-419 (1985).

2. American Public Health Association, "Standard Methods for the Examination of Water and Wastewater," 16th edition, American Public Health Association, Washington, D.C., pp. 525-532 (1985).

3. Instrumental Manual for YSI Model 57 Dissolved Oxygen Meter (and further personal consultation with technical service of the company), Yellow Springs Instrument Co., Yellow Springs, OH.

4. Metcalf and Eddy, Inc., "Wastewater Engineering: Treatment, Disposal, Reuse," 2nd Ed., McGrow-Hill, Inc., New York, NY, pp. 86-95 (1979).

5. Horner, R. R., "Environmental Monitoring and Evaluation of Calcium Magnesium Acetate (CMA)," National cooperative Highway Research Program (NCHRP) Report 305 (1988).

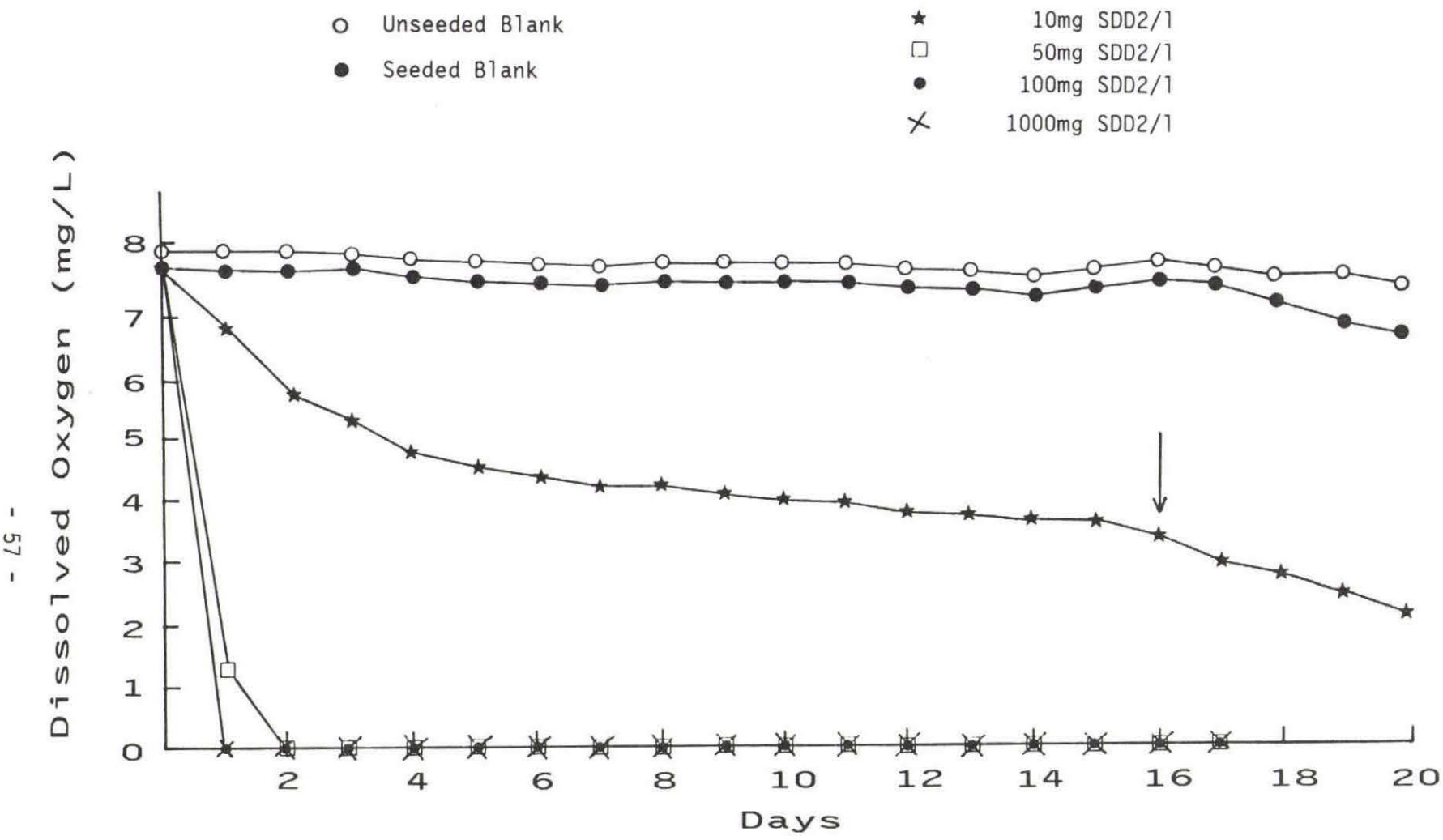


Figure E-1: Oxygen remaining over Time in Standard BOD Experiment with Different SDD2 Concentration (↓ indicates the Starting Point of Non-carbonaceous Oxygen Demand)

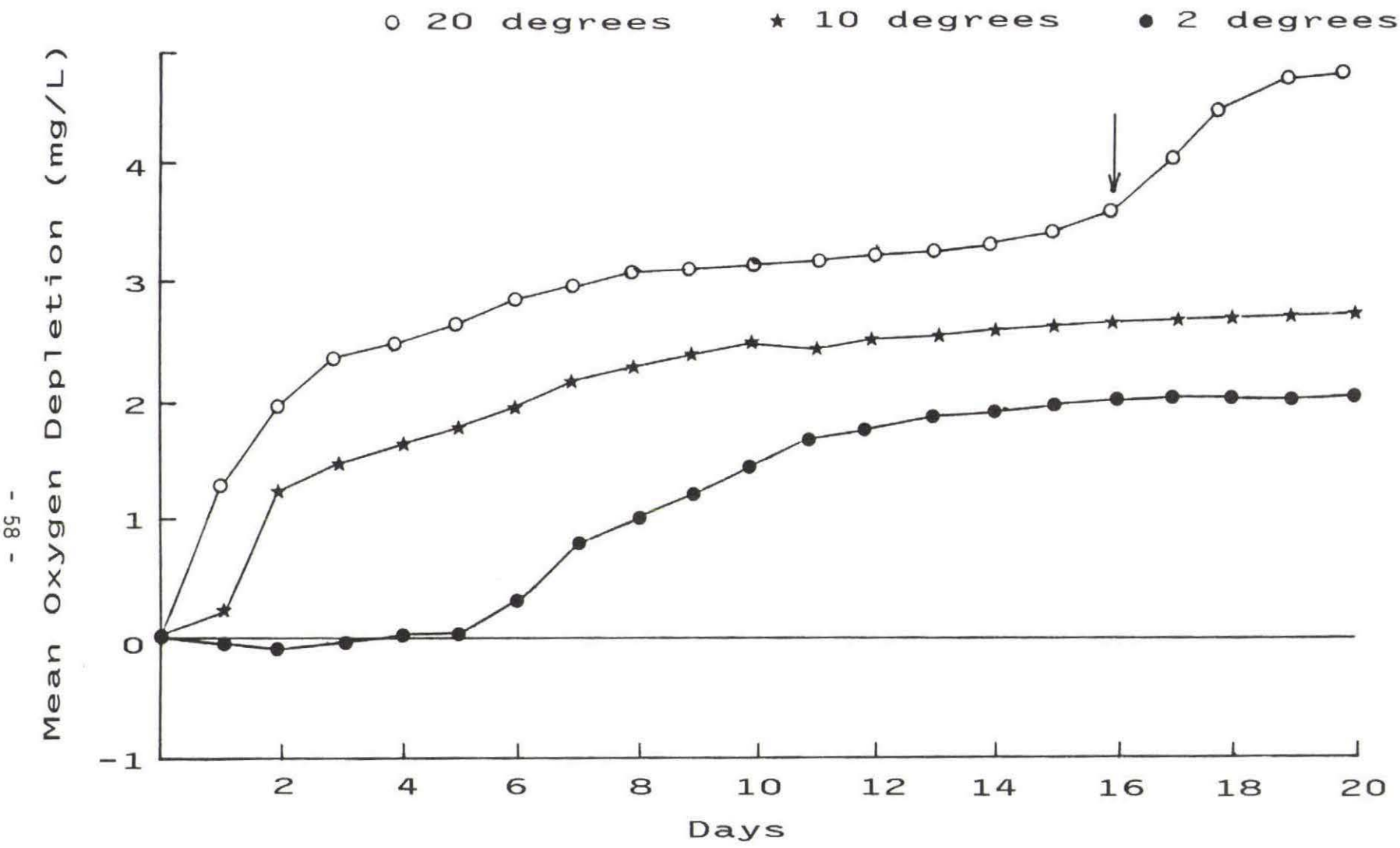


Figure E-2: Oxygen Depletion over Time in Multiple-Temperature BOD Experiment (10mg SDD2/l; ↓ indicates the Starting Point of Non-carbonaceous Oxygen Demand.)

Table E-1: Results of the Standard BOD Experiment

RATE CONSTANT K	ULTIMATE BOD L	% ULTIMATE BOD EXTERTED IN 5 DAYS	10 DAYS	20 DAYS
0.136/DAY	4.451 mg/L	79.1%	99.1%	99.9%

Table E-2: Results of the Multiple-Temperature BOD Experiment

	2°C	10°C	20°C
RATE CONSTANT (K, day^{-1})	0.053	0.092	0.142
ULTIMATE BOD (L, mg/L)	2.259	2.869	3.681
% ULTIMATE BOD EXERTED IN			
5 days	45.8	65.3	80.5
10 days	70.6	87.9	96.2
20 days	91.4	98.5	99.9

Table E-3: Comparison of Rate Constants (K) of SDD2 and CMA at Different Temperatures

	K VALUE (DAY^{-1}) AT		
	2°C	10°C	20°C
SDD2	0.053	0.092	0.142
CMA	0.020	0.064	0.130

CONCLUSIONS

The main objective of the study is to identify the potential environmental impact that might be caused by the application of SDD2. Performed experiments, following well-standardized laboratory procedures, include Acute Dermal Toxicity Test, Primary Dermal Irritation Test, Acute Inhalation Toxicity Test, Fish Acute Toxicity Test, and Biochemical Oxygen Demand Test.

The findings from the experiments lead to the following conclusions;

1. SDD2 would not cause any immediate health hazard in laboratory animals by inhalation or dermal absorption.
2. As evidenced by the value of LC_{50}, tolerance of aquatic animals (rainbow trout) to SDD2 is relatively high.
3. SDD2 could be readily decomposed at warm temperature and relatively slowly at near freezing temperature. However, at higher concentrations, it could reduce the concentration of available oxygen in the aquatic system, which might indirectly cause detrimental effects on aquatic animals.

The overall results from this preliminary experimental investigation suggest a relatively low toxicity of SDD2 in the terrestrial and aquatic ecosystem, which promotes the potential of SDD2 as a highway deicer. However, full-scale environmental studies need to be conducted before the actual application of SDD2 on highways to verify the findings obtained from this study.

APPENDICES

A: CHEMICAL AND PHYSICAL PROPERTIES OF THE SOUTH DAKOTA DEICER NUMBER 2 (SDD2)

B-1: RESULTS OF GROSS NECROPSY OF MALE #5 FROM THE ACUTE INHALATION TOXICITY TEST (PREPARED BY SCOTT A. MISCHLER, D.V.M.)

B-2: RESULTS OF GROSS NECROPSY OF MALE #4 FROM THE ACUTE INHALATION TOXICITY TEST (PREPARED BY SCOTT A. MISCHLER, D.V.M.)

C: RESULTS OF ANALYSIS OF DILUTION WATER FOR THE FISH ACUTE TOXICITY TEST (PREPARED BY THE TRAVIS LABORATORIES, INC.)

D: RESULTS OF QUANTITATIVE ANALYSIS OF SDD2 (LOT 6) BY TWO-POINTS STANDARD ADDITION (PREPARED BY JANE A. ROSELAND)

APPENDIX A: CHEMICAL AND PHYSICAL PROPERTIES OF THE SOUTH DAKOTA DEICER NUMBER 2 (SDD2)

CHEMICAL AND PHYSICAL PROPERTIES OF THE SOUTH DAKOTA DEICER NUMBER 2 (SDD2)

SDD2 is basically a processed cellulose which consists of a group of sodium carboxylates, including Na-acetate, Na-formate, Na-lactate, Na-glycolate, Na-glycerate, Na-oxalate, and unidentified solids. Much of the components are not yet fully identified. The proportions of these organic salts vary from one lot to the other. SDD2 from Lot 6 was used for the toxicity and BOD studies. The results of quantitative analysis of SDD2 by means of the High Performance Liquid Chromatography (HPLC) are included in Appendix D.

Due to the chemical treatment during the manufacturing process, a relatively high pH was expected from the raw deicer, close to 11. SDD2 from Lot 6 was further treated by adding 1) sulfuric acid to reduce pH, 2) calcium hydroxide to precipitate Ca-oxalate, and 3) acetic acid to adjust the final pH to approximately 9.0.

Upon delivery from the South Dakota Department of Transportation (SDDOT), SDD2 showed light and dark brown color in lumps. It exhibited very high hydroscopic characteristics, resulting in liquidification at room condition. SDD2 was therefore kept constantly at 50°C or in a desiccator. The hydroscopic nature of SDD2 might have contributed to unsuccessful determination of particle sizes of SDD2 from Stokes' law.

APPENDIX B-1: RESULTS OF GROSS NECROPSY OF MALE #5
FROM THE ACUTE INHALATION TOXICITY TEST
(PREPARED BY SCOTT A. MISCHLER, D.V.M.)

Accession No. _____ Necropsy No. ___P___ Date of Necropsy 7/17/89

Date submitted 7/17/89
Submitted by _____
Address NC
Phone _____

Pathologist _____
Owner _____
Phone _____

Veterinarian Mischler Address _____ Phone _____
Species Rat Breed Sprague Dawley Age Juvenile (78) Sex M
or
Specimen _____ Weight ~~80~~ gms Markings white
No. in group _____ No. submitted _____
Dead 7/15/89 Euthanatized _____ Hours interval to necropsy 48
Disease duration < 12hrs Morbidity — Mortality —
Management and nutrition Standard
Medication and vaccination None
Previous history of disease on premises No
Clinical signs Lethargic 7/14/89 - Dead 7/15/89

Gross observations:

General appearance and physical condition Normal
Integument Normal
Natural body openings: Eyes — Ears — Mouth — Nostrils —
Anus — Vulva — Prepuce Hemorrhagic

Subcutis: NGL
Superficial lymphatics: —
Musculature: NGL
Skeletal system and articulations: NGL

Circulatory system:
Heart: NGL
Pericardium: "
Blood vessels: "

Respiratory system:
Nasal cavity: Mild Erythema
Larynx: NGL
Trachea: NGL
Bronchi: NGL
Lungs: Small atelectic Area Apical Rt lobe
Lymphatics: —
Diaphragm: NGL

Digestive system:
Oral cavity: NGL
Pharynx: "
Esophagus: "
Stomach: Filled w/ingesta
Small intestine: Posthortem Degeneration
Cecum: "

Colon: PM degeneration
Mesentery: NGL
Liver: NGL
Gallbladder: —
Pancreas: NGL
Lymphatics: "
Peritoneum: "
Spleen: "

Urinary system:
Kidneys: NGL
Bladder: Hemorrhagic - Hematuria
Other: Urethra hemorrhagic - No crystals Found

Reproductive system: NGL

Nervous system:
Cerebrum: NGL
Cerebellum: "
Medulla oblongata: "
Spinal cord: "
Other: _____

Endocrine glands: —
Other: —

Tissues for histopathology: None
Photographs: _____

Most Probable Dx: Urolithiasis

APPENDIX B-2: RESULTS OF GROSS NECROPSY OF MALE #4
FROM THE ACUTE INHALATION TOXICITY TEST
(PREPARED BY SCOTT A. MISCHLER, D.V.M.)

Accession No. _____ Necropsy No. ___P___ Date of Necropsy 7/24/89

Date submitted 7/24/89
Submitted by _____
Address NC

Phone _____

Pathologist _____
Owner _____

Phone _____

Veterinarian Mischler Address _____ Phone _____
Species Rat Breed Sprague Dawley Age Juvenile Sex M
or
Specimen _____ Weight 280 gms Markings white
No. in group _____ No. submitted _____
Dead 7/23/89 Euthanatized _____ Hours interval to necropsy 24 hrs
Disease duration < 12 hours Morbidity _____ Mortality _____
Management and nutrition Standard
Medication and vaccination None
Previous history of disease on premises None
Clinical signs hemorrhagy / Death

Gross observations:

General appearance and physical condition Normal
Integument Normal
Natural body openings: Eyes N GL Ears N GL Mouth N GL Nostrils N GL
Anus N GL Vulva N GL Prepuce extended inflamed, Hemorrhagic

Subcutis: N GL
Superficial lymphatics: —
Musculature: N GL
Skeletal system and articulations: —

Circulatory system:
Heart: N GL
Pericardium: "
Blood vessels: "

Respiratory system:
Nasal cavity: N GL
Larynx: N GL
Trachea: N GL
Bronchi: N GL
Lungs: Red Consolidation
Lymphatics: —
Diaphragm: N GL

Digestive system:
Oral cavity: N GL
Pharynx: #
Esophagus: #
Stomach: Normal ingesta Fill
Small intestine: N GL
Cecum: "

Colon: N GL
Mesentery: "
Liver: "
Gallbladder: —
Pancreas: N GL
Lymphatics: "
Peritoneum: "
Spleen: "

Urinary system:
Kidneys: Apparently Normal
Bladder: Thickened, Mild Hematuria
Other: Extended hemorrhagic
Reproductive system: Penis

Nervous system:
Cerebrum: N GL
Cerebellum: "
Medulla oblongata: "
Spinal cord: "
Other: _____

Endocrine glands: _____
Other: _____
Tissues for histopathology: Whole Carcass
Photographs: _____

Suggestive of Urolithiasis

APPENDIX C: RESULTS OF ANALYSIS OF DILUTION WATER
FOR THE FISH ACUTE TOXICITY TEST
(PREPARED BY THE TRAVIS LABORATORIES, INC.)

Business Phone 605-341-7284
Home Phone 605-341-2458
Hours 8:00 — 4:30 M — F

Travis Laboratories

COAL — ORES — ENVIRONMENTAL WATERS — FEEDS — SOILS

1854 Lombardy Dr.
Rapid City, South Dakota 57701

JAMES H. TRAVIS
Laboratory Director

REPORT DATE: 7/13/89

COMPANY: Sookie Bang SDSM&T

ADDRESS: Chemistry & Chemical Eng. Dept.

CITY-STATE: Rapid City, So. Dak. 57701

SAMPLE #:

SAMPLE DATE-TIME: 6/27/89

DATE RECEIVED: 6/27/89

Acct.: # 4-30700

LABORATORY #: 3517

PARAMETER -- Minerals	RESULT
Alkalinity (CaCO3), mg/l	
Bicarbonate (HCO3), mg/l	
Carbonate (CO3), mg/l	
Chloride (C1), mg/l	
Fluoride (F), mg/l	<0.10
Sulfate (SO4), mg/l	
Calcium (Ca), mg/l	
Magnesium (Mg), mg/l	
Potassium (K), mg/l	
Sodium (Na), mg/l	
Major Anions, MEQ/L	
Major Cations, MEQ/L	
Cation - Anion Balance, %	
PARAMETER -- MISCELLANEOUS	
pH, Units	
Conductivity, umhos/cm	
Cyanide, Total, mg/l	
Cyanide, WAD, mg/l	
Cyanide, Free, mg/l	
Hardness (CaCO3), GPG: mg/l	
Oil & Grease, mg/l	
Solids, Dissolved, mg/l	
Solids, Suspended, mg/l	
Turbidity, NTU	
COD	5.6
TRC	<0.10

METALS:
Tot ___ Tot Rec ___ Diss X EP Tox ___

Antimony (Sb), mg/l	
Aluminum (A1), mg/l	<0.10
Arsenic (As), mg/l	<0.005
Barium (Ba), mg/l	
Beryllium (Be), mg/l	
Boron (B), mg/l	<0.20
Cadmium (Cd), mg/l	<0.0005
Chromium (Cr), mg/l	<0.005
Cobalt (Co), mg/l	<0.005
Copper (Cu), mg/l	<0.005
Gold (Au), mg/l	
Iron (Fe), mg/l	<0.05
Lead (Pb), mg/l	<0.005
Lithium (Li), mg/l	
Manganese (Mn), mg/l	
Mercury (Hg), mg/l	<0.0002
Molybdenum (Mo), mg/l	
Nickel (Ni), mg/l	<0.005
Selenium (Se), mg/l	
Silicon (Si), mg/l	
Silver (Ag), mg/l	<0.0005
Strontium (Sr), mg/l	
Vanadium (V), mg/l	
Zinc (Zn), mg/l	<0.005
PARAMETER --Nutrients	
Nitrogen, Ammonia, mg/l	<0.03
Nitrogen, Nitrate, mg/l	
Nitrogen, Nitrite, mg/l	
Nitrogen, Total Kjeldahl, mg/l	
Phosphorus, Ortho, mg/l	
Phosphorus, Total, mg/l	

REVIEWED BY J. M. Travis

APPENDIX D: RESULTS OF QUANTITATIVE ANALYSIS OF SDD2 (LOT 6)
BY TWO-POINTS STANDARD ADDITION
(PREPARED BY JANE A. ROSELAND)

QUANTITATIVE ANALYSIS OF THE SOUTH DAKOTA DEICER SOLIDS LOT 6 BY TWO-POINTS STANDARD ADDITION

a study done in conjunction with the acute toxicity testing of SD-2, Lot 6

by
Prof. J.A. Roseland

Chem/ChemE Dept.
South Dakota School of Mines and Technology

presented to the
South Dakota Department of Transportation

INSTRUMENTATION

Beckman Model 112 HPLC pump
20uL sample loop, Altex Model 210 sample injector
Beckman Model 421 controller
Biorad Aminex Ion Exclusion HPX-87H (300 X 7.8mm) column
Altex Model 156 refractive index detector
Fiatron CH-30 column heater
Fischer Recordall Series 5000 recorder

INSTRUMENTAL PARAMETERS

The column was given at least 45 minutes equilibration time using a mobile phase of filtered, sep-paked 0.013N H_2SO_4 at a flow rate of 0.8mL/minute. The refractive index was set at range = 16, except during the analysis of acetic acid, where it was necessary to set at the less sensitive range of 32. The refractive index detector was not connected to a waterbath. The column heater was set at 38°C. The detector was allowed at least 45 minutes warmup time. The recorder was set at 10mV and 0.2 inch/minute chart speed.

SAMPLE PREPARATION

Lot 6 was received form SDDOT, grounded to a fine homogeneous powder and dried at 50°C for 48 hours. Two sample preparations were made for analysis in the following manner:

Approximately 0.35 gram of deicer was weighed out accurately and dissolved in 10mL of distilled water by sonicating. The resulting solution was frequently agitated while three 3mL aquilots were taken. Two mL of distilled water was added to the first aquilot. One mL of distilled water plus one mL of spiking solution was added to the second aquilot. Two mL of spiking solution was added to the third aquilot. Acidification of all aquilots was accomplished by adding concentrated H_3PO_4 until production of CO_2 ceased (2 drops). Each acidifed aquilot was then quantitatively filtered, sep-paked and made up to a final volume of 10mL with 0.01M sodium dihydrogen phosphate buffer(pH 2.5).

The concentrations of the organic acids in the spiking solution alongwith the conversion factors to obtain the sodium salts are given below:

ACID	CONCENTRATION (WT/VOL)	CONVERSION FACTOR
glyceric acid	0.051%	1.2072
glycolic acid	0.2%	1.2894
lactic acid	0.04248%	1.2444
formic acid	0.214%	1.4783
acetic acid	0.2038%	1.3666

There were two sample preparations of lot 6 for analysis and three aquilots per sample preparation. Each final prepared aquilot was analyzed twice and peak heights were determined. Peak heights for glycolic, formic and acetic acids were measured from the apex of each peak to the baseline. Peak heights for glyceric and lactic acids were measured from the apex of each peak to the trough of the next peak. Peak heights in millimeters are shown below (Peak height of spiked sample - peak height of unspiked sample is indicated by a Δ):

Lot 6 0.3405gram sample

	Unspiked Lot 6		Lot 6 + 1mL spike			Lot 6 + 2mL spike		
	pk hts	$\bar{x}$	pk hts	$\bar{x}$	Δ	pk hts	$\bar{x}$	Δ
glyceric acid	18,19	18.5	26,25	25.5	7	33,31	32	13.5
glycolic acid	51,49	50	76,74	75	25	99,96	97.5	47.5
lactic acid	15,14	14.5	20,21	20.5	6	27,26	26.5	13
formic acid	56,58	57	74,73	73.5	16.5	90,87	88.5	31.5
acetic acid	— 87	87	— 95	95	8	— 105	105	18

Lot 6 0.3540gram sample

	Unspiked Lot 6		Lot 6 + 1mL spike			Lot 6 + 2mL spike		
	pk hts	$\bar{x}$	pk hts	$\bar{x}$	Δ	pk hts	$\bar{x}$	Δ
glyceric acid	19,18	18.5	23,23	23	4.5	30,31	30.5	12
glycolic acid	49,47	48	70,68	69	21	93,90	91.5	43.5
lactic acid	14,15	14.5	20,20	20	5.5	27,26	26.5	12
formic acid	56,56	56	71,69	70	14	87,86	86.5	30.5
acetic acid	— 94	94	— 103	103	9	— 112	112	18

The calculation used to determine the percent sodium salts in the solid deicer sample lot 6 (wt/wt) is shown below:

$$\left(\frac{\text{pk ht sample}}{\text{pk ht spike}}\right)\left(\frac{\text{\% conc spike}}{1}\right)\left(\frac{\text{mL spike added}}{\text{wt of sample}}\right)\left(\frac{\text{Conversion Factor}}{1}\right)\left(\frac{10\text{mL}}{3}\right) =$$

% sodium salt

The mean percent concentration of sodium salts (n = 4) in lot 6 deicer solids is shown below:

Salt	Percent Concentration (wt/wt)
sodium glycerate	1.9 ± 0.4%
sodium glycolate	5.3 ± 0.3%
sodium lactate	1.3 ± 0.1%
sodium formate	11.2 ± 0.5%
sodium acetate	27.7 ± 1.4%

DISCUSSION

The concentrations of acids in the spiking solution were plotted against the millimeters peak heights corresponding to those acids. These plots, shown on pages 4 and 5 of this report, show fairly good

linearity, supporting the validity of the results obtained by two-points standard addition. Linear regression was applied and R squared values of 0.974 to 1.00 were obtained, again supporting the validity of the results. Linear regression results are shown on pages 6 and 7 of this report.

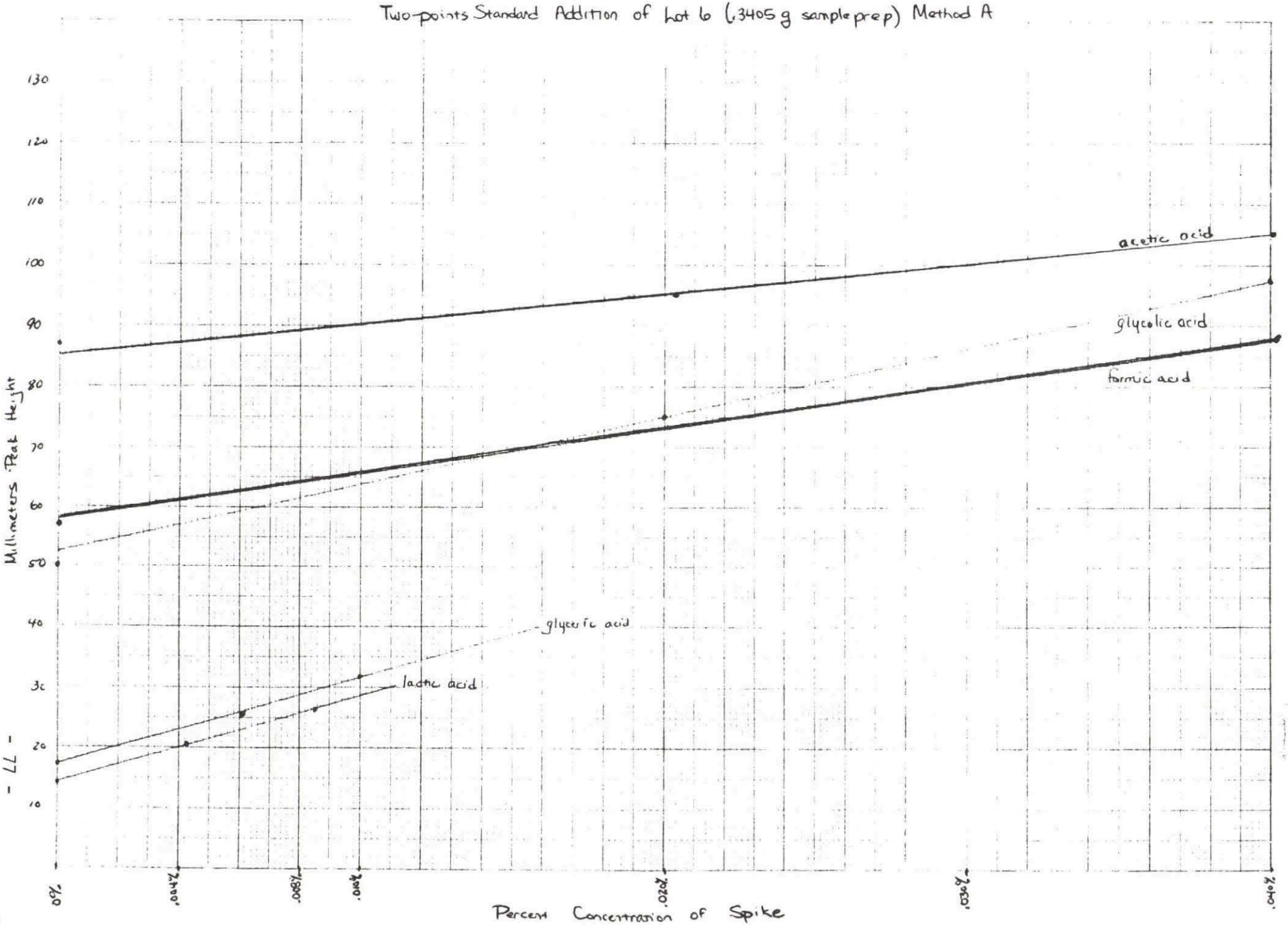
The purpose of standard addition is to subject the standard(s) to the same matrix as the sample. In the procedure described in this report (Method A), organic acids in the spiking solution are added to the organic salts of the sample in basic solution (pH 9-11), and thus are converted to the organic salts. Upon subsequent acidification, all organic salts (of the original sample plus those generated by the spiking solution) are then converted to the protonated organic acids. This procedure is straight-forward and time efficient.

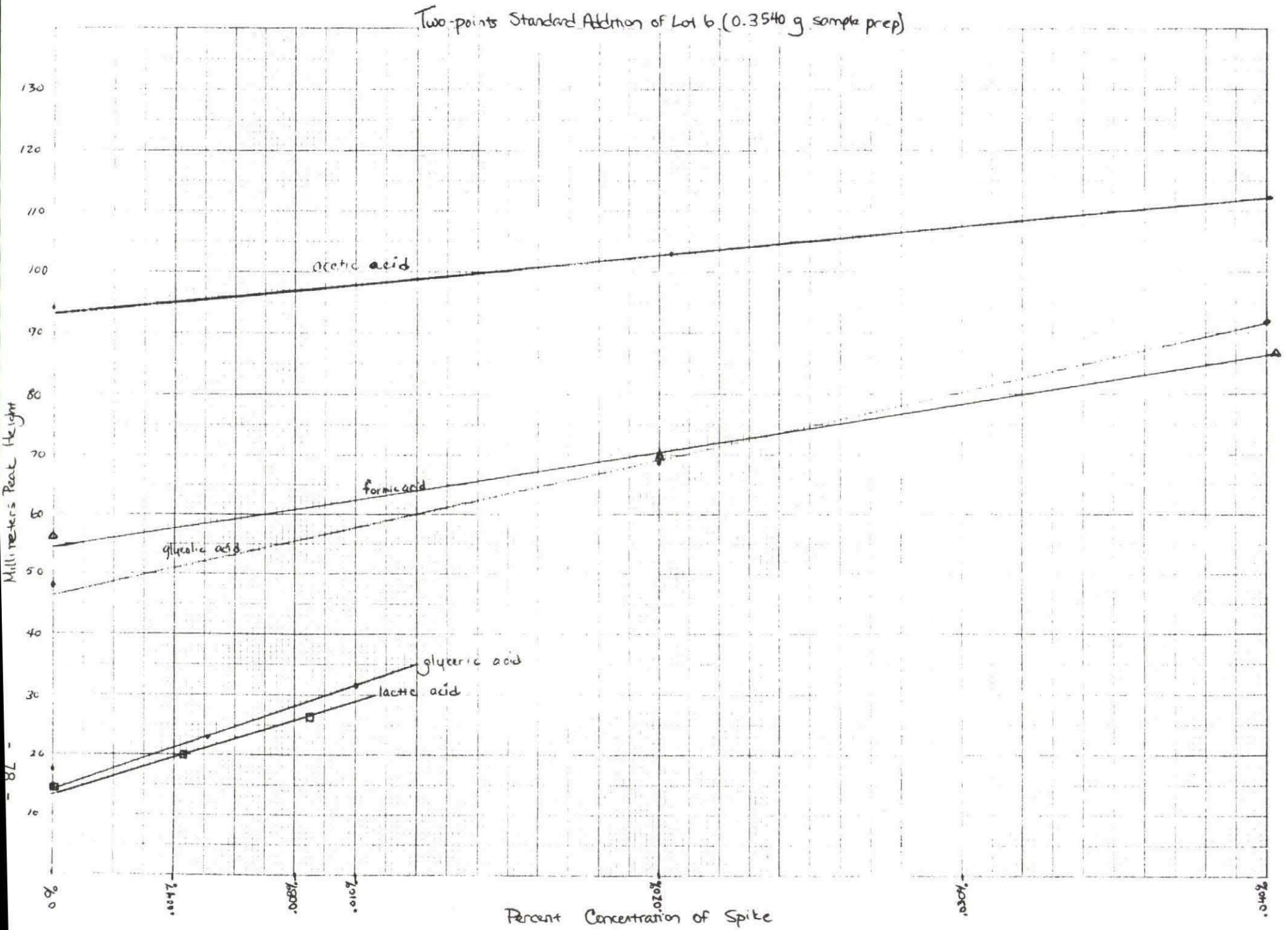
Other procedures of standard addition have been tried with lot 6. A two-points standard addition was done using a spiking solution of acid standards taken to a basic pH and then back to an acidic pH (Method B). This was done to try to duplicate more nearly the sample's matrix in the spiking solution. Method B was more time-consuming than Method A. The concentrations of acids in the spiking solution were plotted against the millimeters peak heights corresponding to those acids. This plot generated by Method B's data is shown on page 8. Linear regression was applied to the data generated by Method B and is shown on page 9.

A one-point standard addition was also done on lot 6, using a spiking solution of acid standard which was added after acidification of the sample (Method C). Method C did not subject the standard to the sample's matrix as completely as Methods A or B.

All standard addition methods used to analyze lot 6 produced similar results, which were approximately twice those obtained by the old HPLC method (Method D, reference J.A. Roseland's technical reports to SDDOT dated from May 1988 through April 1989). These results generated by the various methods are shown below:

	Method A 2 pt std add adding acid stds before acidification	Method B 2 pt std add adding acid stds made basic, then acidic again	Method C 1 pt std add adding acid stds after acidification	Method D old method
Na glycerate	1.9 ±0.4	2.2 ±0.4	2.0	0.7 ±0.0
Na glycolate	5.3 ±0.3	4.9 ±0.2	4.3	2.4 ±0.0
Na lactate	1.3 ±0.1	1.1 ±0.1	0.8	0.6 ±0.0
Na formate	11.2 ±0.5	9.1 ±0.3	8.6	5.6 ±0.1
Na acetate	27.7 ±1.4	26.6 ±1.7	off-scale	15.4 ±0.3


Two-points Standard Addition of Lot 6 (.3405 g sample prep) Method A
Millimeters Peak Height
130
120
110
100
90
80
70
60
50
40
30
20
10
acetic acid
glycolic acid
formic acid
glyceric acid
lactic acid
0%
.010%
.020%
.030%
.040%
Percent Concentration of Spike


Two-points Standard Addition of Lot b (0.3540 g sample prep)
Millimeters Peak Height
130
120
110
100
90
80
70
60
50
40
30
20
10
0%
.2440%
.0880%
.0160%
.0320%
.0480%
.0640%
Percent Concentration of Spike
acetic acid
formic acid
glycolic acid
glyceric acid
lactic acid

METHOD A
Lot 6 , 0.3405g sample preparation

glyceric acid

x	y
0	18.5
0.0051	25.5
0.0102	32

Regression Output:

Constant	18.58333
Std Err of Y Est	0.204124
R Squared	0.999542
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	1323.529
Std Err of Coef.	28.30148

formic acid

x	y
0	57
0.0214	73.5
0.0428	88.5

Regression Output:

Constant	57.25
Std Err of Y Est	0.612372
R Squared	0.999244
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	735.9813
Std Err of Coef.	20.23423

glycolic acid

x	y
0	50
0.02	75
0.04	97.5

Regression Output:

Constant	50.41666
Std Err of Y Est	1.020620
R Squared	0.999077
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	1187.5
Std Err of Coef.	36.08439

acetic acid

x	y
0	87
0.0238	95
0.04076	105

Regression Output:

Constant	86.31012
Std Err of Y Est	2.040102
R Squared	0.974413
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	434.7834
Std Err of Coef.	70.45371

lactic acid

x	y
0	14.5
0.004248	20.5
0.008496	26.5

Regression Output:

Constant	14.5
Std Err of Y Est	0
R Squared	1
No. of Observations	3
Degrees of Freedom	1

::
lot6 data

METHOD A
Lot 6, 0.3540g sample preparation

glyceric acid

x	y
0	18.5
0.0051	23
0.0102	30.5

Regression Output:

Constant	18
Std Err of Y Est	1.224744
R Squared	0.979591
No. of Observations	3
Degrees of Freedom	1
Std Err of Coef.	169.8089

glycolic acid

x	y
0	48
0.02	69
0.04	91.5

Regression Output:

Constant	47.75
Std Err of Y Est	0.612372
R Squared	0.999603
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	1087.5
Std Err of Coef.	21.65063

lactic acid

x	y
0	14.5
0.004248	20
0.008496	26.5

Regression Output:

Constant	14.33333
Std Err of Y Est	0.408248
R Squared	0.997690
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	1412.429
Std Err of Coef.	67.95554

formic acid

x	y
0	56
0.0214	70
0.0428	86.5

Regression Output:

Constant	55.58333
Std Err of Y Est	1.020620
R Squared	0.997765
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	712.6168
Std Err of Coef.	33.72373

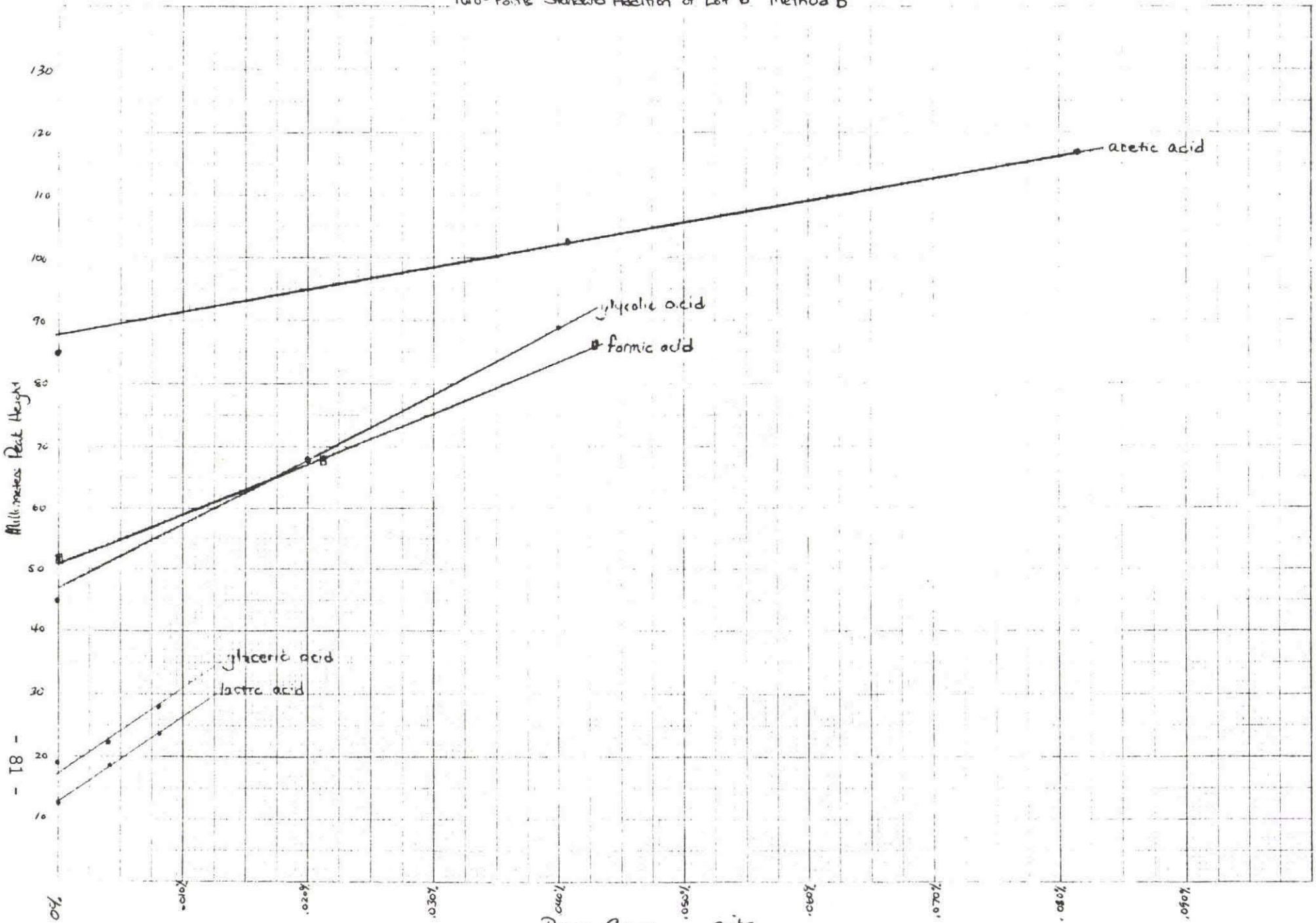
acetic acid

x	y
0	94
0.0238	103
0.04076	112

Regression Output:

Constant	93.58494
Std Err of Y Est	1.227410
R Squared	0.990700
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	437.5026
Std Err of Coef.	42.38790

Two-Point Standard Addition of Lot 6 Method B



METHOD B
Lot 6

glyceric acid

x	y
0	19
0.004	22.5
0.008	28

Regression Output:

Constant	18.66666
Std Err of Y Est	0.816496
R Squared	0.983805
No. of Observations	3
Degrees of Freedom	1
Std Err of Coef.	144.3375

glycolic acid

x	y
0	45
0.02	68
0.04	89

Regression Output:

Constant	45.33333
Std Err of Y Est	0.816496
R Squared	0.999311
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	1100

lactic acid

x	y
0	12.5
0.004248	18.5
0.008496	23.5

Regression Output:

Constant	12.66666
Std Err of Y Est	0.408248
R Squared	0.997252
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	1294.726
Std Err of Coef.	67.95554

formic acid

x	y
0	51.5
0.0214	68
0.0428	86

Regression Output:

Constant	51.25
Std Err of Y Est	0.612372
R Squared	0.999370
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	806.0747
Std Err of Coef.	20.23423

acetic acid

x	y
0	85
0.04076	102.5
0.08152	117

Regression Output:

Constant	85.5
Std Err of Y Est	1.224744
R Squared	0.997078
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	747.6635
Std Err of Coef.	40.46847