INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

THE TOXICITY OF AMYL NITRATE

A dissertation submitted to the
Graduate School of Arts and Sciences
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF INDUSTRIAL MEDICINE

1954

by

John Carpenter Duffy

A.B. Stanford University 1944
M.D. Stanford University School of Medicine 1947

ACKNOWLEDGEMENT

I wish to thank Dr. Joseph Treon for his aid and instruction in the preparation of experimental work and for his review of the work in this thesis.

I also wish to express appreciation to Mr. Sylvan Witherup for permitting the inclusion of his studies on the cutaneous, oral and parenteral toxicity of amyl nitrate; also, to Mr. Jacob Cholak for permitting the inclusion of his work on air pollution with amyl nitrate.

I also wish to acknowledge the contribution made by Dr. Raymond Suskind in reviewing the portions of the thesis which deal with the skin, and that made by Dr. A. Wesley Horton for assistance in writing the introduction. I express my appreciation to Dr. Frank Princi for reviewing the clinical applications. The counsel of Dr. Robert A. Kehoe, for which I am most grateful, made possible the final presentation.

TABLE OF CONTENTS

Table of Contents (cont'd)

SEQUENCE OF TABLES

1. Physical Properties of Ethyl Corporation's Ignition Improver.
2. Per Cent of Weight Loss in Relation to Duration and Concentration of Exposure (Inhalation).
3. Signs Observed During Amyl Nitrate Inhalation in Relation to Component Radicals of Amyl Nitrate.

SEQUENCE OF FIGURES

OBJECTIVE

Amyl nitrate has been found to serve the practical purpose of improving the ignition of diesel fuel under the ordinary conditions of its use in diesel engines. In addition, it can be made available in ample quantity by apparently economical means. Accordingly, the compound may well find extensive use and is worthy of toxicological investigation in relation to the potential hazards which it offers to those who may handle it and the fuel to which it is to be added.

Experimental observations on laboratory animals were made to obtain information concerning the toxicity of amyl nitrate by various routes of administration. The experimental exposure included cutaneous, oral, intravenous and intraperitoneal administration of amyl nitrate in graded dosages, and the inhalation of its vapor in air in varying concentrations. The extent of the contamination of the atmosphere with amyl nitrate was also determined under a variety of conditions, some experimental in type and others related to the actual handling of the compound. The efficiency of the removal of the vapor of amyl nitrate from air passed through the standard gas mask canister "for organic vapors" was tested. The effects of the exposure of human beings to amyl nitrate so far as they are known, have been described.

The foregoing information is correlated with the known behavior and the tolerable concentrations of related compounds.

On the basis of the assembled information, the author (1) makes recommendations for further experimental work; (2) proposes tentative maximal allowable concentrations; (3) suggests precautionary measures; and (4) advises that clinical observations be carried out systematically on exposed workmen.

INTRODUCTION

Since 1940, there has been a four-fold increase in demand for diesel fuel as well as an increased competition for oil stocks from such varied sources as heating units and jet engines. These competitive markets continue to increase the demand for the raw material; such products need less refining than diesel fuel. This has stimulated the search for an economical means of production of diesel fuel. Producers, engine manufacturers, and consumers of diesel fuel desire a product fulfilling certain basic requirements which will: (1) simplify refining processes; (2) supply quality fuel economically in quantity; (3) allow the upgrading of oil stocks to diesel fuel requirement; and (4) produce diesel fuel of uniform ignition quality. These specifications are met most adequately by the use of an additive to the oil. Many materials are suggested. Experimentally, the most effective additives appear to be acetone peroxide, isoamyl nitrate, commercial amyl nitrate and ethyl nitrate (1).

The quality of diesel fuel is measured primarily by the cetane number, a measure of the ignition value of a diesel fuel oil. It is the percentage by volume of cetane ($C_{16}H_{34}$, a colorless oil found in petroleum) in a mixture of cetane and 1-methyl naphthalene which gives the same lag in ignition as the oil tested. The higher the cetane number the better is the ignition value.

It is frequently necessary to refine the crude oils selectively and to blend them individually in order to obtain a standard product or to gain specifically desired qualities in the finished diesel fuel. Desirable ignition qualities are present in straight run distillates of paraffin base crude oils; however, naphthenic crudes, and distillates from cracking operations are usually deficient in these ignition qualities. With the increase in demand for diesel oil and in competitive uses of the same stocks, greater quantities of cracked products have been included in the marketed fuel. This has advantages of lower pour points and higher heating values per gallon, but introduces the problem of adequate ignition quality.

A variety of additives are suggested and have been found to be satisfactory, experimentally, in producing the desired ignition quality in diesel fuel. The reasonable cost (estimated at $0.001 per cetane number per gallon) and vapor pressure (the boiling point being in the range of the lighter fraction of diesel oil) of commercial amyl nitrate

either in concentrated form or when blended with fuel, made its selection economically sound. An advantage of amyl nitrate is a flash point of 130°F. which is high enough to have little effect on the finished diesel fuel. Experimental storage over the period of four and one-half months has not demonstrated deterioration in ignition acceleration or separation of the fuel and additive (2). Investigations by the Bureau of Explosives of the American Railroads have demonstrated the insensitivity of amyl nitrate to thermal or mechanical shock; and it has been classed as a nonflammable liquid by the Interstate Commerce Commission (3).

In order to use a wider range of oil products and to maintain the desired qualities in the finished diesel fuel, investigative work was initiated on the types of oil products and on the quantity of additive necessary to produce a commercially satisfactory diesel fuel. Experimental work on the quality of amyl nitrate as an ignition accelerator demonstrated that its greatest effect in increasing the cetane number of a diesel fuel occurred with the addition of the first one per cent (2); however, fuels of higher cetane number displayed a greater degree of improvement with the same percentage of additive. Disadvantages of loss of power or increased rate of fuel consumption have been observed with the use of the additive. As the percentage of additive is increased, the power output for a

given weight of fuel is decreased; this is apparently related to the lowering of the heat content of the fuel. There also occurs an increase in rate of fuel consumption at a constant power output, which is related to dilution of the fuel by the additive. Although the specific action of amyl nitrate as an ignition accelerator is not known, as an oxidant it apparently produces an increased activity at the centers from which combustion originates. For general use, not more than 0.5 per cent of amyl nitrate, as an additive, is suggested (4).

By better selection of basic oil stocks and by the use of amyl nitrate as an additive, it is possible to increase the cetane number of diesel fuel and thus allow engine manufacturers to design engines for maximum performance with reduced operational costs. It has been shown that with an increase in the cetane number of the fuel, exhaust odors and smoke, engine roughness, engine deposit, and starting difficulties decreased. A fuel of fifty cetane number has been established as a satisfactory minimum requirement for automotive type diesel engines in general service, although it is possible to design diesel engines for operation on low cetane fuel. The demands of automotive engine service are for reduced weight and minimum space requirements, together with simplification of design with anticipated reduction of maintenance problems. Improved engine design demands a fuel of higher cetane number.

When firing occurs with the piston in the top position, an adequate delay in ignition occurs if the fuel has the desired cetane number. Fuels of inadequate cetane number produce "knocking", uneven and incomplete combustion with subsequent smoke and loss of power, particularly under unfavorable conditions of ignition such as those associated with a "cold" engine, idling speed and high altitudes. Tests of full scale diesel engines have shown that engine deposits are not increased. The engines can not distinguish between fuels of the same cetane number, whether due to high cetane content or to addition of amyl nitrate (4).

This meets the refiner's requirement for a method of producing the desired diesel fuels without costly intermediate processes. The use of an ignition accelerator will allow the blending of diesel fuel for special purposes. The reasonable cost of amyl nitrate will permit its use as an additive to supply quality diesel fuel economically in quantity. The availability and distribution of a fuel of uniform and constant igntion quality will meet the demands of the automotive engineers in their efforts to manufacture an engine of greater efficiency and economy.

Under these conditions of commercial feasibility it is anticipated that amyl nitrate will probably find general use as an ignition accelerator for diesel fuel.

Section I

EXPERIMENTAL WORK ON AMYL NITRATE

EXPERIMENTAL MATERIAL

The experimental work reported in this paper used Ethyl Corporation Compound DB-26. This compound was a mixture of several normal, primary and branched-chain amyl nitrates withonot more than five per cent of secondary amyl nitrates and a trace of amyl alcohol. It was unstable above the boiling range and should not be distilled above fifty millimeters of mercury. The material was miscible with hydrocarbons and soluble in water to the extent of 0.3 per cent (5).

The vapor pressure of isoamyl nitrate was reported by Stull (6). The vapor pressure is plotted logarithmically against temperature in degrees Kelvin in Figure 1.

The physical properties of the material used in these experiments are shown in Table 1. (2)

INHALATION (5)

The potentialities for the inhalation of the vapors of amyl nitrate under uncontrolled conditions of handling prompted investigations of the toxicity and hazards of the source of exposure. A series of animals were selected for exposure. These groups consisted of two guinea pigs, five mice, two rabbits and four rats. In certain experiments a cat was added. Where the fatal outcome of the experiments was reasonably certain, only rats or mice were employed. This selection of animals was economical and consistent with the experience of other investigators, while the numbers employed were sufficient to provide a sound basis for statistical analysis of the results. The animals were subjected to the inhalation of varying concentrations of the material over varying periods of time. These conditions ranged from twenty minutes at 13.6 milligrams of amyl nitrate per liter of air (2549 ppm) to one-hundred forty hours at 1.4 milligrams per liter (262 ppm), the latter being accomplished by twenty periods of seven hours each. The highest concentration employed was 19.9 milligrams per liter (3730 ppm) for seven hours. Initially, periods of shorter duration at higher concentrations served to establish tolerable and lethal levels for the several species of animals. Subsequently, longer periods of exposure at lower concentrations

were employed to discover more insidious effects. These experiments, although few in number, provided reasonably adequate information concerning the several species.

The apparatus consisted of a wooden cage of 283 liter capacity, internally coated with chemically resistant paint, and equipped with a fan and fluorescent light. The behavior of the animals was observed through a hinged door in which was a glass window. The window was protected by a wire mesh screen on the interior surface. Four openings into the cage were available: one, for the introduction of vaporized amyl nitrate in a controlled air stream; another, for the removal of the mixed air in the cage; a third, for a vaporizing wick with which to provide quickly the desired initial concentration of amyl nitrate; and a fourth, for the removal of samples of the mixed air of the cage for analysis.

A Gast rotary blast and suction air pump passed a controlled volume of air through a calcium chloride tower and a calibrated rotometer, Figure #2. The measured flow of air was passed into a receiving chamber into which liquid amyl nitrate was delivered at a calibrated rate. In this receiving chamber the amyl nitrate was dropped upon glass wool and vaporized into the air by a heating unit, the temperature of which was controlled by a "Variac". The contaminated air was then passed into the animal cage. Preliminary to the introduction of the air-amyl nitrate stream into the cage, a calculated amount of amyl nitrate was added to the

vaporizing wick. The temperature of the wick was held at the boiling range of amyl nitrate (150° C.), and the quantity added was determined by the volume of the cage and the concentration desired for the duration of the experiment.

The liquid amyl nitrate was delivered from a twenty millimeter calibrated glass cylinder at a constant rate of flow, by means of a plunger actuated by a motor and suitable gears, the specific rate being determined by the gear ratio. The amyl nitrate in the cylinder was maintained at a constant temperature by means of a water bath at room temperature. The mechanism for the delivery of amyl nitrate was calibrated under the constant experimental conditions, by collecting increments from the cylinders at intervals of one minute in weighed receiving chambers which were immersed in an ice bath.

The delivery of amyl nitrate was varied to meet the experimental conditions desired in the most convenient manner, in association with the regulation of theair stream with which it was carried into the chamber.

The concentration of amyl nitrate in the air of the chamber was determined directly in samples of air collected in evacuated glass balloons. Several samples were collected during each experiment. The sample was shaken for five minutes with a measured quantity of redistilled ethyl alcohol, released into the balloon from a connected glass compartment

by opening the interposed stopcock. Themixture was then recovered, measured, and a portion was introduced into a ten millimeter Quartz cell. The percentage of transmission of ultra-violet radiation at 235 mu. was measured by comparison with a corresponding cell containing distilled ethyl alcohol, in a Beckman spectrophotometer set with a 2.0 centimeter opening. The readings were interpreted by means of a transmission curve prepared from solutions of known concentration (Figure #3). In experiments of prolonged duration the analytical results subjected to statistical analysis. In one of seventy hours duration the mean concentration was 3.20 milligrams of amyl nitrate per liter of air, and the standard deviation was $\pm$ 0.64, while in another of one hundred forty hours duration the corresponding values were 1.40 ($\pm$ 0.38).

The species mortality is summarized graphically in Figures #3 to 8, in which are shown the logarithmic relationships of time and concentration with mortality. The duration, in hours, is plotted along the abscissae and the concentrations in milligrams per liter along the axes of the ordinates. The total number of animals in each experiment is represented by a circle; the portion of each circle that is filled indicates the percentage of mortality under the experimental conditions. The straight lines separate the graphs into two regions, that above and to the right representing lethal conditions, and that below and to the left being compatible with survival. No deaths occurred among the exposed cats.

It is noted that the slope of the lines varies with the species, and that some change in the slope seems to occur as the period of exposure lengthens above fifteen hours.

At intervals during the course of each experiment and at its conclusion, observations were made on the experimental animals through the protected glass window in the door of the cage. The following paragraphs summarize the behavior patterns of the experimental animals during the periods of exposure.

CATS - Upon exposure to a concentration greater than 9.09 milligrams per liter (1703 ppm) the cats responded in a uniform manner. The severity of their reactions increased to the convulsive level at 19.17 milligrams per liter (3593 ppm). Tremors, salivation, rapid deep respiration, and prostration with a full cardiac pulse were observed below nineteen milligrams per liter. At a level of 3.20 milligrams per liter (599 ppm), the animals became ataxic and lethargic. They showed an increase in the rate and depth of respiration, and moderate salivation, but were not narcotized. No abnormalities of behavior were observed during or after exposure to the concentration of 1.40 milligrams per liter (262 ppm). No consistent pattern of weight loss was manifested, except that there was some tendency to greater weight loss in association with the more prolonged exposure to the higher concentrations.

GUINEA PIGS - Lethal conditions for guinea pigs were found to be near or slightly above 16.39 milligrams per liter (3972 ppm) for fourteen hours, and 12.7 milligrams per liter (2380 ppm) for twenty-one hours. At these levels the animals showed tremors, convulsive episodes, and labored respirations. The respiratory changes were more constant and were accompanied by tremors when the concentrations were in excess of 3.20 milligrams per liter (599 ppm). The guinea pigs were asymptomatic at a level of 1.40 milligrams per liter (262 ppm). Individual losses in weight were sustained, these being evidence of greater losses as the exposure increased in severity and duration.

RABBITS - The lethal level for rabbits was between 16.39 milligrams per liter (3072 ppm) or twenty-one hours to 19.17 milligrams per liter (3593 ppm) for three and one-half hours. At these levels the animals showed these signs: pawed their noses, attempted to escape, and developed cyanosis of the ears, labored respiration, and convulsions. These signs were less evident during exposure to lower concentrations. At a level of 1.40 milligrams (262 ppm), some slight increase in the depth and rate of respiration and slight hyperemia of the ears were noted. Themaximum losses of weight occurred in association with conditions of maximum severity.

RATS - The lethal concentrations for rats ranged from 16.39 milligrams per liter (3072 ppm) for fourteen hours to 19.90 milligrams per liter (3730 ppm) for seven hours. There were signs of ataxia, labored respirations,

and prostration. At 3.20 milligrams per liter (599 ppm), the ataxia was minimal, and the animals showed only a slight change in respiration. Signs of intoxication were absent at 1.40 milligrams per liter (262 ppm). In general, the losses in weight of these animals varied in accordance with the severity of the experimental conditions.

MICE - The lethal range was above 9.09 milligrams per liter (1703 pp,) for twenty-one hour exposure period. The animals were able to withstand exposures of 13.6 milligrams per liter (2549 ppm) for twenty minutes and 17.22 milligrams per liter (3227 ppm) for one hour. There were consistent signs of ataxia and respiratory distress. During exposure to the lower concentrations, the mice huddled and developed increases in the depth and rate of respiration, in inverse relationship to the level of concentration. The weight losses tended to vary directly with the severity of the conditions of exposure.

The rapidity of onset and the severity of the signs observed varied directly with the concentrations of amyl nitrate to which the animals were exposed regardless of the species, although a species variation in susceptibility was noted.

Following the exposure of animals to the higher concentrations for longer than brief periods of time, the surviving animals were lethargic on the next day thereafter. This lethargy decreased, and normal behavior patterns returned gradually. The duration of the lethargic state appeared to depend upon the intensity and duration of the exposure to amyl nitrate. No definitive observations or conclusions

were made in this respect, but the apparent persistence of lethargy tended to coincide, generally, with the extent of the losses in weight.

Blood was obtained from four cats by cardiac puncture for the determination of methemoglobin formation, using a modification of the method of Michael and Harris (8). There were no spontaneous deaths among these animals. Because of technical difficulties, we can only report the results obtained from the study of one cat which was exposed to an average concentration of 3.20 milligrams per liter (599 ppm); on eight separate occasions. During the control period, 10.3 per cent of the hemoglobin of this cat was found to have been converted to methemoglobin; the conversion rose to 35.6 per cent after the fifth period of exposure. After four days without exposure, the blood of the animal exhibited 11.2 per cent conversion. Immediately after three subsequent periods of exposure, 59.5 per cent of the hemoglobin had been converted to methemoglobin. Four days later the percentage of methemoglobin had decreased to 4.3 per cent.

Heinz bodies (9) were sought in the peripheral blood of cats subjected to inhalation of the vapors of amyl nitrate, the frequency of occurrences (percentages) of these bodies in the erythrocytes was determined before the animals were exposed. Subsequent counts followed the rise and return to the normal (control) range. Figures # 9 to 11. This response on the part of the cats varied somewhat under varying

experimental conditions, but the most pronounced early response was obtained by a single prolonged exposure to a relatively high concentration.

Thirty hours after exposure of one hour duration to the concentration of 19.9 milligrams per liter (3730 ppm), Heinz bodies were found in ninety per cent of the erythrocytes. The relative numbers decreased rapidly after this peak had been reached, but twenty-seven days were required for the return to normal numbers.

Exposure to the concentration of 19.17 milligrams (3593 ppm) for three and one-half hours resulted in the formation of these bodies in large numbers, the peak being reached only after six days, when forty per cent of the erythrocytes contained them. The return of the blood to a normal state in this regard occurred only after forty-one days.

Thirty hours after exposure to the concentration of 9.64 milligrams per liter (1807 ppm) over the period of seven hours, thirty-one per cent of the red cells contained Heinz bodies. This declined to the control range in sixty-eight days.

Exposure to the concentration of 17.22 milligrams per liter (3227 ppm) for one hour resulted in a rise in these inclusion body counts which reached a peak of twenty-nine per cent of the erythrocytes in nine days. The decline to control level occurred in sixty-four days.

On the fourth day after the last of a series of three periods of exposure (for seven hours per day on three successive days) to the concentration of 9.09 milligrams per liter (1703 ppm), one cat had been found to have Heinz bodies in seventy-nine per cent of its red cells. By the twenty-fifth day after the last period of exposure the blood had returned to normal.

All of the animals employed in these experiments were examined post mortem for gross pathological lesions and the tissues of representative animals of all species were examined microscopically.

Animals of all species that died during or after the inhalation of amyl nitrate were found to have similar but non-specific toxic changes in their tissues. These, as described in the more important organs, may be characterized as diffuse degenerative changes involving the hepatic cells, the epithelium of the renal convoluted tubules, and the nerve cells of the brain. Hyperemia and edema of the lungs were also regular in their occurrence.

With a single exception, the tissues of animals that survived following inhalation of the vapor of amyl nitrate and were killed for examination after they had regained their normal weight, were found to be essentially normal. The exception was that of a cat subjected to the inhalation of the vapor in the concentration of 1.4 milligrams per liter in air, for seven hours per day on each of twenty days. When the animal was killed on the eighth day after the

final exposure, the liver was found to be the site of an acute focal inflammatory process involving the peripheral zone of the lobules. One rat employed in this same experiment died of pneumonia after the seventh period of exposure.

More specific information regarding the cardio-respiratory response to amyl nitrate (7) was obtained by the study of a male albino rabbit. After being anesthetized with twenty milligrams of sodium barbital administered intra-peritoneally, the carotid artery and trachea of this animal were cannulated and connected to recording tambours. Air was bubbled through amyl nitrate kept at the temperature of 35° C., and supplied to the animal by means of a conventional apparatus over the period of six and one-half hours during which 10.6 grams of amyl nitrate were vaporized from the container.

The animal responded to the inhalation of the vapor with an initial increase in respiratory rate and volume, interspersed with periods of apnea and accompanied by a sharp fall in blood pressure. After twenty minutes, the respiratory rhythm became steady at about three times the initial rate and the systolic blood pressure remained de-pressed. During the following six hours, the blood pressure gradually stabilized at a lower level and the respiratory rate decreased.

Parallel observations (7) of the effects of the in-halation of amyl nitrate and amyl nitrite by the same species of animal, have shown that the latter induces a more rapid

fall in the systolic blood pressure and a lesser increase in the respiratory rate than does the former. The inhalation of the nitrate caused a gradual and moderate decline in the systolic pressure, while initially, the respiratory rate was markedly increased in rate and decreased in volume. When amyl nitrate was being inhaled over a period of less than one minute, the respiratory abnormalities tended to return to normal promptly and indeed before the end of the period of inhalation.

APPLICATION UPON THE INTACT SKIN (10)

Observations were made on the systemic and local effects induced by repeated applications of amyl nitrate, or of fuels containing amyl nitrate, upon the intact skin of rats and rabbits, under conditions which prevented the ingestion of the compound and the inhalation of the vapor.

Nine groups, each consisting of five male albino rabbits, were selected for the experiment. The hair and fur on the abdominal area of eight groups of these animals were shorn and the anjmals were confined supinely in stocks situated in a fume hood with their heads to the front in the stream of entering air. Four portions of the experimental material were placed upon the shorn surface and allowed to remain in contact with the skin, the intervening period between the applications was one hour, and after a specified period of time, usually one hour, following the fourth application of the material, the residue was removed from the skin as effectively as possible by washing the animal with

soap and warm water. This procedure was repeated on five successive days per week for one or more weeks. The control group of animals was not subjected to daily handling or to the periods of restraint described above but was employed only for the purpose of providing comparative data on changes in weight during the experimental period.

Three groups of animals were subjected to applications on each of forty-nine days (five days per week except for one week of four days). The groups of animals and the respective materials applied upon their skins are identified as follows:

Group 1. 2 ml. of diesel fuel (type not specified) containing 0.5 per cent of amyl nitrate. Four doses were applied in the manner previously specified, the total dose of amy nitrate per day being 0.04 milliliters.

Group 2. 2 ml. of a model airplane fuel; this fuel was composed of twenty per cent of amyl nitrate, sixty per cent of forty-five straight run gasoline, eighteen per cent of lubricating oil (S. A. E.), and two per cent of oleic acid. Four doses were applied, as indicated, the total dose of amyl nitrate per day being 1.6 milliliters.

Group 3. One ml. of amyl nitrate. Four doses were applied, the total dose of amyl nitrate per day being four milliliters.

Group 4. Control animals, as indicated.

None of the animals in the foregoing groups exhibited any gross signs of systemic illness; nor did their organs display any pathology attributable to the experimental material. However, some retardation of growth, in varying degree, was sustained by certain groups of test animals. Figure #12 (10).

The animals subjected to contact with the diesel fuel containing 0.5 per cent of amyl nitrate lost weight during the first week and gained slowly thereafter. The average gain was 200 grams per rabbit over the total period of sixty days. These animals sustained moderately severe injury of the skin, characterized by extreme erythema, moderate edema, lichenification and induration. Subsequently, fissures and marginal hemorrhages developed in the affected area. Figure # 13, W-6141 (10). Microscopically, the skin showed papillomatous hyperplasia, acanthosis, hyperkeratosis and chronic inflammation.

The animals subjected to contact with the model airplane fuel gained in weight, the average per rabbit being 520 grams over the total period. There was no grossly apparent injury to the skin. Figure #13, W-6148 (10). Microscopically, there was a minimum of chronic inflammation, slight hyperkeratosis and acanthosis.

Animals subjected to repeated applications of the undiluted amyl nitrate showed an average gain of 760 grams per rabbit during the total experimental period of sixty days.

Grossly, the skin exhibited only mild lichenification with some desquamation and occasional mild hyperemia. Figure #13, W-6150 (10). Microscopically, there was some slight evidence of chronic inflammation, slight hyperkeratosis and acanthosis.

The control group, not subjected to the periods of restraint, showed an average gain of 1130 grams per rabbit during the sixty days of observation.

Five additional groups of animals were subjected to applications of the experimental materials on each of seven days, the residue being removed as before but only after two hours had elapsed following the fourth application. The groups and materials applied were:

Group 1. Two ml. of catalytically cracked diesel fuel containing two per cent of amyl nitrate, the four doses separated from each other by hourly intervals resulting in the total daily dose of 0.16 ml. of amyl nitrate.

Group 2. Two ml. of straight run diesel fuel containing two per cent of amyl nitrate. Four doses were applied as above, the total daily dose being 0.16 ml. of amyl nitrate.

Group 3. Two ml. of catalytically cracked diesel fuel without the additive.

Group 4. Two ml. of straight run diesel fuel without the additive.

Group 5. Two ml. of refined mineral oil only.

Five deaths occurred among the animals in the five groups. Post mortem examination of these animals, and of two others that were killed shortly after the last application

on their skin, revealed diffuse degeneration of the liver and degeneration and necrosis of the renal convoluted tubules. These lesions occurred without regard to the material applied, being found in the animals subjected to contact with mineral oil alone.

They were regarded as typical of the effects produced by chemical irritants, showing no resemblance to those produced by pathogenic organisms. The animals remaining in each group were killed for examination on the sixty-fifth day after that of their last contact, and their tissues were examined microscopically. Their viscera were normal. The skin of all of the rabbits which had been subjected to applications of diesel fuels exhibited papillomatous hyperplasia, acanthosis, hyperkeratosis and chronic inflammation.

Few premonitory signs preceded the death of the rabbit exposed to mineral oil. Those subjected to contact to diesel fuels, with or without the additive, displayed similar sumptoms as: loss of appetite, loss of body weight, weakness, slow and labored respirations, lowered body temperature and terminal coma.

Injury to the local skin was produced by each of the four fuels. The injury was of about the same degree of severity in all instances, but more severe than that induced by the experimental procedures to which the first four groups had been subjected.

Slight gains in weight were exhibited by the group exposed to mineral oil, while all the other groups suffered

some loss in weight during the period of the application. Soon after the applications had been discontinued, the animals had regained the lost weight. Figure # 14 (10).

The animals subjected to contact with the catalytically cracked diesel fuel containing two per cent of amyl nitrate developed an intense irritation of the skin in the area of contact, and sustained losses in weight; and therefore, further applications were suspended after the seventh day. Two of these animals became so weak and emaciated that they were killed on the sixth day after the last period of contact. The others regained the lost weight in about eight weeks.

The animals subjected to contact with straight run diesel fuel containing two per cent of amyl nitrate displayed severe irritation of the skin but smaller losses in weight than those subjected to contact with the cracked diesel fuel. One death occurred in this group. Following the termination of applications, these animals grew at an accelerated rate, so that after four weeks their weight compared favorably with the group subjected to contact with mineral oil.

Contact with the catalytically cracked diesel fuel, without the additive, induced smaller losses in weight than did the same fuel containing the amyl nitrate. One animal of this group died on the fourth day after that of the last application. After about eight weeks of freedom from the experimental applications, the other animals of this group had regained their weight to an extent comparable to those in the group subjected to contact with mineral oil.

The animals subjected to contact with the straight run diesel fuel, without the additive, displayed weight losses comparable to those induced by contact with the same fuel containing the additive, but these animals regained their lost weight less rapidly than did those subjected to contact with the fuel containing the additive. One animal in this group died.

The group subjected to contact with the refined mineral oil gained in weight at a somewhat retarded rate. There was a single death in this group.

Four groups of rats were selected for further observations of this same type. Each group consisted of six eight-week-old male rats. On each of five days per week over the period of twelve weeks, one-half milliliter of an experimental material was placed upon the fur and skin over the scapular area of the back of each rat. The material was not removed by washing. The groups and materials applied were:

Group 1. Model airplane fuel containing twenty per cent of amyl nitrate, the daily dose being 0.1 ml. of amyl nitrate.

Group 2. Undiluted amyl nitrate, in the daily dose of 0.5 milliliter.

Group 3. Mineral oil containing 25 per cent of V/V amyl nitrate, the daily dose being 0.125 milliliter of amyl nitrate.

Group 4. Mineral oil.

These animals were so handled that other routes of absorption were not excluded. Some of the material was probably ingested, and inhalation of vapors of volatile components, including the amyl nitrate, may well have occurred to some extent. However, the animals were housed in wire mesh cages in a well ventilated room and the concentration of vapors cannot have been high; nor was such exposure prolonged.

There were no fatalities among the animals, nor did any of them exhibit signs of systemic illness, however, those subjected to contact with the amyl nitrate in any of the vehicles, failed to gain in weight at the control rate. Figure #15. (10)

Zucker (11) has represented the post-weaning growth curve of rats as a straight line described by general equation, $Y = K - bx$, in which "Y" is the logarithm of the body weight in grams, "K" is a constant, "b" the slope of the growth curve, and "x" the reciprocal of the age in weeks from birth.

Comparison of the curves of growth show that growth was inhibited, generally to an extent which correlated with the amount of amyl nitrate in the preparation applied upon the skin. The model airplane fuel retarded growth slightly more than mineral oil containing amyl nitrate but the difference was not statistically significant. Both of these curves differed significantly from the curve for the animals subjected to contact with mineral oil only ($P < 0.01$).

At the conclusion of the applications, the hemoglobin content and formed elements in the peripheral blood of the rats of the several groups were compared. The hemoglobin values were similar in all four groups. The numbers of erythrocytes were greater in those subjected to contact with mineral and with mineral oil containing amyl nitrate than in those subjected to contact with undiluted amyl nitrate or with the model airplane fuel, but the lower values were within the range of variability of groups of apparently normal rats. On the other hand, relatively low values with respect to erythrocytes went hand in hand with increases in the numbers of eosinophilic leucocytes, the two variables gaining somewhat in significance, perhaps, from their association.

The average weights of the lungs and spleens of the animals subjected to contact with amyl nitrate were significantly less than the average weights of the corresponding organs of animals subjected to contact with only mineral oil, after making the necessary correction for differences in the body weights of the respective groups.

No gross or micorscopic alterations were found in the organs of these animals, there being no demonstrable basis in pathology for the variations in the organ weights. The skins of the animals showed only slight and inconstant evidences of hyperkeratosis in the area of the applications.

In summary, rabbits andrats subjected to repeated cutaneous contact with either undiluted amyl nitrate or a model airplane fuel containing amyl nitrate showed some

inhibition in growth. This inhibition of the growth of the rats varied in degrees according to the concentration of the amyl nitrate in the material applied. Thelocal injury may have been a factor in influencing the rate of growth whether through its direct effect or through its effect upon the absorption of the materials applied. The potential absorption by the lungs can not be completely eliminated.

The toxic effects of the percutaneous absorption of both straight run and catalytically cracked diesel fuels were clearly demonstrable, and these effects as induced by contact of the skin with the catalytically cracked fuel were enhanced by the addition of amyl nitrate to this fuel.

Although hyperkeratotic and acanthotic changes were noted in the skin, these changes can not be attributed to the presence of amyl nitrate in the materials applied.

The observed losses in the weight, and the retardation in the rate of growth of these animals were not great, nor is there any evidence that they correlated with or resulted from the degenerative changes in the organs of the animals.

ORAL AND PARENTERAL ADMINISTRATION OF AMYL NITRATE (12)

Further investigations of the toxic characteristics of amyl nitrate were concerned with the effects of its oral and parenteral administration to experimental animals. Although little hazard would be expected to arise from any such measures of absorption of the material in an industrial

environment, the widespread use and availability of amyl nitrate provides good reason for observing its effects under a wide variety of conditions and especially after its ingestion.

Three groups of albino rats were selected for experimental use. These were made up of adult females, three to four months of age, young females, approximately six weeks of age, and young males approximately five weeks of age. Each rat was given one of a series of doses of undiluted amyl nitrate by means of an esophageal tube passed into the stomach.

The approximate lethal dose for adult female and young male rats was found to be 4.7 milliliters per kilogram of body weight; however, young females were killed by the smallest dose given (1.4 milligrams per kilogram of body weight.)

Initially, the animals exhibited an increase in respiratory rate and volume with mild hyper-excitability. The respiration gradually decreased in rate and volume, in association with a gradual decrease in motor activity, and finally failed completely. Coma, cyanosis and lowered body temperature appeared terminally. Although, the animals tended to survive longer and to lose more weight as the dose was reduced to the minimum required to kill, there was much individual variation with respect to length of survival after the administration of a lethal dose. Sublethal doses produced an initial weight loss, which progressed about one

weeks, after which the initial weight was regained and the normal rate of gain was resumed.

A group of male albino rabbits was selected and subjected to graded doses of a special preparation of amyl nitrate administered intravenously. This preparation was an emulsion made by homogenizing ninety milliliters of a one per cent solution of lecithin in physiological saline, with ten milliliters of a twenty per cent solution (V/V) of amyl nitrate in peanut oil (13). The emulsion was injected into the marginal ear vein at the rate of two ml. per minute. Dosages of 0.12 millileters of amyl nitrate per kilogram were tolerated, but 0.18 milliliters per kilogram precipitated death almost immediately after completion of the injection.

Intravenous injection of the emulsion induced marked and prompt increase in respiratory rate and volume, lachrymation, transitory miosis and physical weakness. Lethal doses induced tonic spasms and dyspnea. Within two to three hours after the injection of sublethal doses, the animals appeared to have recovered completely. No delayed effects were observed during the subsequent three weeks of observation.

Further details of the response of rabbits to the intravenous administration of this emulsion were investigated. Preliminary anesthesia was obtained by intraperitoneal injections of twenty milligrams of sodium barbital per kilogram of body weight. After anesthesia had been produced, the carotid artery and trachea were cannulated and connected to

recording tambours. When doses of amyl nitrate as small as 0.005 milligrams per kilogram were injected intravenously, a marked increase in the respiratory rate and volume was induced, accompanied by a sharp drop in the systolic blood pressure. Occasional episodes of apnea occurred, followed by gradual but incomplete recovery. The administration of a further dose of this size resulted in a diminished response, but reactions equivalent to the first could be elicited by increasing the dose sufficiently. Injections of multiple small doses induced respiratory changes without a fall in blood pressure. Lethal doses produced respiratory and circulatory failure.

Adult female rats, three to four months of age, were subjected to intraperitoneal injections of undiluted amyl nitrate. The lethal dose of amyl nitrate for those animals was approximately 3.2 milliliters per kilogram of body weight.

The fatally poisoned animals exhibited the initial increase in respiratory rate and volume and a subsequent decrease until respiration ceased; motor activity followed a similar pattern. Sublethal doses gave rise to weight loss during the first week, after which the normal pattern of growth was resumed.

CONTAMINATION OF THE ATMOSPHERE WITH AMYL NITRATE UNDER CERTAIN PRACTICAL CONDITIONS (14,15)

An experiment was conducted to determine the extent of the vaporization and dissemination of amyl nitrate under

a given set of conditions within a closed room. A jar, five and one-half inches in diameter, containing a one and one-half inch layer of amyl nitrate, was placed in the still atmosphere of a sealed chamber one-hundred cubic foot in capacity. Air samples were obtained from the chamber at the intervals indicated below and analyzed quantitatively for amyl nitrate; the volume of each sample was equivalent to one per cent of the volume of the chamber, and air was permitted to flow into the chamber from the room to replace that removed as a sample. The relative volumes of air involved in the interchanges, when balanced against the periods of time intervening between successive samples, was believed to be too small to give rise to a practicably significant variable.

The vapor pressure of amyl nitrate, at 82° F. (27.8° C.), is 4.2 millimeters of mercury; the concentration at saturation would approximate thirty milligrams per liter (5600 ppm). The analytical results obtained on samples taken at the specified intervals over a period during which the temperature of the chamber ranged from 80° to 86° F. were as follows; at the end of one hour, 0.18 milligrams per liter (33 ppm); at the end of seven hours, 0.46 milligrams per liter (85 ppm); at the end of thirty hours, 1.50 milligrams per liter (281 ppm); at the end of ninety-five hours, 2.70 milligrams per liter (525 ppm). It is evident that the rate of evaporation was slow under these conditions.

Conditions associated with the actual handling of the compound (15) were investigated by sampling and analyzing the atmosphere of a partially underground tank of 27,500 barrel capacity in which diesel fuel containing 0.25 per cent of amyl nitrate (16) was stored. The height of the tank was twenty-one feet, two and one-quarter inches; of which, ten feet were above ground. The contents of the tank were removed, except for seven inches of oil and three inches of underlying water. The vacuum created during the emptying had been released by opening the manhole over the pump pit. The tank had not been steamed or aerated. Samples of the atmosphere within the tank were collected, through each of the several man-holes, by means of a twelve foot length of rubber hose connected to a midget impinger containing alcohol and equipped with a scrubber. The results of the analyses of fifteen liter samples ranged from 0.12 to 0.19 milligrams per liter (22 to 36 ppm).

Observations were made to test the usefulness of the canister-type approved organic vapor respirator (MSA Type O-MA, containing charcoal) in protecting men in an atmosphere contaminated with significant concentrations of amyl nitrate. A stream of air containing amyl nitrate in the concentration of approximately 600 ppm (3.2 milligrams per liter) was passed through the canister at the rate of ten liters per minute for four hours. The efficiency of the canister in the removal of amyl nitrate from the air stream was better than 99.9 per cent (14).

With one exception, indicated below, amyl nitrate vapor in air was determined by the same technique employed for the analysis of air to which animals were exposed. This technique was based upon the absorption of ultraviolet radiations, as measured by the Beckman Spectrophotometer.

The samples obtained (in alcohol) from the tank of diesel fuel were subjected to a preliminary hydrolysis of the organic nitrate to the sodium salt, after which the total nitrogen in the alcohol was determined by a modified Kjeldahl method (17). Subsequent calculations were made to express the quantities of amyl nitrate in milligrams per liter of air or in parts per million, within the tank.

Section II

THE COMPONENT RADICALS OF AMYL NITRATE PHARMACOLOGICAL ACTION AND PHYSIOLOGICAL RESPONSE

THE COMPONENT PARTS OF AMYL NITRATE

In an orderly classification of the observed experimental results, the action of amyl nitrate may be expressed in terms of its pharmacological action and its physiological manifestations. Some correlation of the activities of the entire molecule with the specific activities of its component parts is desirable, if possible. From such correlation, the properties peculiar to amyl nitrate may better be understood.

Marshall (18) noted, in the experimental poisoning of rabbits with methyl nitrate, such signs of nitrate assimilation as, increase in heart rate, dyspnea, methemoglobin formation, and subnormal body temperature. The characteristic signs of methyl alcohol poisoning were unsteadiness, chewing, running movements, and unconsciousness. He further observed that the toxicity of the aliphatic nitrate series increased from the methyl homologue to the amyl homologue. Since immediate convulsive movements were common throughout the homologous series, he attributed them to the nitrate portion of the molecule.

The increasing toxicity in this homologous series of compounds is similar to the increase of toxicity in other homologous series as aliphatic alcohols, aliphatic nitroparaffins, and aliphatic esters.

Signs of unsteadiness, chewing, running movements, and unconsciousness are among the common effects induced by the aliphatic alcohols.

Ethyl nitrate is said to be capable of inducing signs of headache, narcosis, vomiting and decrease in blood pressure (19c, 19d).

Von Oettingen (19) states that the acute toxicity of the aliphatic nitrous and nitric acid esters is due mainly to their vasodilator action and to methemoglobin formation. Among the nitrous acid esters, it has been found that the higher homologues are more readily decomposed by hydrolysis than are the lower homologues (19a).

Consideration must be given to the amyl radical of amyl nitrate and to its metabolism within the body whereby amyl alcohol, valeraldehyde and valeric acid are formed. Secondary alcohols may form ketones (20). The amyl portion is partly oxidized and partly eliminated by way of the lungs (21). The metabolism of the nitrate may give rise to the formation of the oxides of nitrogen and the associated acids, as well as the nitrite.

The nitrite ion causes a relaxation of smooth muscle (22, 23, 24), which is more evident when the prior tonus of the muscle is increased (spasm). This action occurs after the administration of pilocarpine, a fact which suggests that the action on the muscle is direct. Amyl nitrite and sodium nitrite have essentially the same capacity for inducing this relaxation; however, amyl acetate induces no change in muscle tone. This fact suggests that the amyl ion is ineffectual in bringing about relaxation of smooth muscle (25).

Finally, amyl nitrate is able to bring about the formation of methemoglobin, and it also gives rise to the formation of Heinz bodies within the erythrocytes, The significance of these facts must be considered in their relationship to the signs observed in the experimental animals.

Amyl alcohol, known as fusel oil, is the most toxic of the commonly used alcohols (26). It follows Richardson's Law: "The toxicity of theaalcohols belonging to the fatty acid series increases in proportion to the molecular structure" (27, 28, 29, 30). Methyl alcohol appears to be an exception to this generalization.

Munch and Schwartz (27) give the relative toxicity of isoamyl alcohol, as compared to ethyl alcohol (a value of one), as:

6.8 to 7.3	-- orally, rabbits
6.2 to 9.7	-- subcutaneously, dogs
19.8 to 38.2	-- intravenously, rabbits
35.9 to ---	-- intravenously, cat
9.9 to ---	-- intravenously, dog.

Further investigations of isoamyl alcohol showed it to be less toxic than the straight chain alcohol (n-amyl alcohol). These investigations also found that the narcotic action of the alcohols increased to a greater extent than did their toxicity (as indicated by lethal consequences), with increase in molecular weight. They also demonstrated that the primary amyl alcohols were less narcotic than the secondary or tertiary alcohols (27,29).

Exposures to the isomers of amyl alcohol have resulted in irritation of the mucous membranes, headaches, vertigo, nausea and vomiting (30 - 35). Central nervous system symptoms of vertigo, diplopia, deafness and delirium have been described in connection with fatal poisoning (26,36, 37). Death has been attributed to respiratory failure of central origin (20, 26) and to pulmonary edema with cardiac insufficiency (38).

Some authors (30) characterize amyl alcohol intoxication by the appearance of coma, glycosuria, and on occasion, methemoglobinemia. Others concur, that methemoglobin occurs occasionally in this intoxication (39), they further describe the presence of metabolic products in conjugation with glycuronic acid in the urine (20a, 39).

The irritant action of amyl alcohol has been confirmed repeatedly (34,35,36). Following one period of exposure to the vapors of isoamyl alcohol, Nelson and others (34) found objectionable subjective phenomena at 200 ppm, irritation in the throat at 100 ppm, and ocular and nasal membrane irritation at 150 ppm. The possibility of the development of tolerance or of hypersensitivity was not investigated.

Study of the metabolism of amyl alcohol has shown that a small amount is excreted in conjugation with the glycuronic acid. Quantities of amyl alcohol ranging from a trace up to ten per cent of that administered have been

found in the urine and expired air (20a). The amounts eliminated reflected the height and duration of the blood level attained (20). There is a species variation in rates of elimination (36).

Haggard, Miller and Greenberg (20) administered one gram of the isomers of amyl alcohol per kilogram of body weight to rats intraperitoneally. Effective absorption was demonstrated and the following information was obtained:

Amyl Alcohol	Concentration in blood after one hour , in mg. per 100 grams	Disappearance from blood in hours
Primary	14 - 55	3.5 - 9
Secondary	51 - 65	13.0 - 16
Tertiary	123	50 +

As the coefficient of distribution between blood and air is similar for the isomers, the varying rates of elimination must be attributed to differences in the rates or mechanisms of the metabolic processes in which these isomers are involved.

The slow elimination of the tertiary alcohol is due to the slowness of the oxidation of the molecule. The prolonged period of elimination of the secondary alcohol is due to the formation of ketone in the body and to the slow oxidation of ketones.

The primary alcohols are oxidized to aldehydes and acids. Valeraldehyde has not been found in the blood after the administration of alcohol, and only small transitory amounts are demonstrable following the administration of

aldehyde itself. After the alcohol has disappeared from the blood, a persistent sedative effect has been noted. This effect is also obtained when valeric acid is administered. This sedative effect occurs only when the primary alcohol is given, and the valeric acid is formed only from the primary alcohol. (20)

The primary amyl alcohols are converted to the aldehyde in the liver; this conversion is inhibited in the partially hepatectomized animal (20). Further evidence of the importance of liver function in this process is the demonstration of conjugated glycuronic acid in the urine of rabbits, following the oral administration of the primary alcohol (20). Injury to the liver of the rabbit has been described following the oral administration of this alcohol repeatedly over periods of time (36).

The maximum allowable concentration of the vapor of amyl alcohol in the atmosphere to which workmen are exposed has not been agreed upon. Values ranging from 0.20 milligrams per liter (50-56 ppm) (34a) to 1.44 milligrams per liter (400 ppm) (32) have been recommended.

Amyl acetate is also a primary irritant to the skin and mucous membranes (36). It, too, is more toxic than its lower homologues (26), but its hydrolysis gives rise to non-toxic salts (26, 40).

The exposure of cats to the vapors of amyl acetate in the concentration of twenty-four milligrams per liter

(4500 ppm) over the period of thirty minutes resulted in narcosis. Guinea pigs died after five hours of exposure to about fifty-six milligrams per liter (10,500 ppm), while rabbits recovered. Both species of animals displayed lachrymation, salivation, convulsions with ataxia and motor paralysis. The animals suffered transient losses in weight (37a) and developed albuminuria after six periods of exposure of eight hours each, to the concentration of ten milligrams per liter (1900 ppm) (26b). The pathological changes described were those of low grade inflammation in the respiratory tract, fatty changes in the liver, and parenchymatous degeneration of the kidneys (26, 41). Similar signs and pathological changes have been described in association with exposure to secondary amyl acetate vapors, and all of them have been found to be reversible (42).

One human death is reported (41), following exposure to a high concentration of amyl acetate. Death was attributed to edema of the glottis. Post-mortem examination revealed diffuse irritation of the respiratory and gastrointestinal tracts. Workers exposed to non-lethal concentrations complained of "dopiness" (drowsiness), vertigo, tinnitus, dry cough, chest pain or tightness, irritation of the throat, tachycardia, nausea and gastric irritation (26, 37, 41). Prolonged exposure has been associated with headache, weight loss, and mild persistent irritation of the mucous membrane; possibly corneal irritation and the loss of the olfactory function may occur (26). Urobilinuria has been reported (26c).

These signs and symptoms are consistent with the observations of mucosal irritation, narcosis and hepatic damage. The maximum allowable concentration of 400 ppm has been suggested (45).

Briefly, amyl alcohol is six to forty times as toxic as ethyl alcohol, depending upon species of animal involved, the route of administration, and its isomeric composition. Intoxication is characterized by central nervous depression, glycosuria and, occasionally, methemoglobinemia. Exposure to the vapors gives rise to irritation of the mucous membranes. The primary alcohol is destroyed by oxidation to the aldehyde and acid in the liver. The persistent narcotic effect is attributed to valeric acid.

Amyl acetate is a primary irritant, which is also capable of inducing narcosis. Sublethal exposure to high concentrations results in reversible degenerative changes in both the liver and kidneys.

Consideration must be given to the oxides of nitrogen which may be produced in the oxidation of amyl nitrate. In this instance nitrous oxide (N_2O) is relatively unimportant, since its action as an asphyxiant, occurs only when its concentration is so high as to lower the uptake of oxygen in the pulmonary circuit. The formation, in vivo, of nitric oxide (NO) may give rise to methemoglobinemia. Nitric oxide also acts upon reduced hemoglobin to produce nitrosohemoglobin, thereby reducing the oxygen carrying capacity of the blood (44).

The oxides of nitrogen have two principle physiological effects; the non-specific irritant and corrosive effect and the specific drug action of the nitrite. The relative importance of the nitrogen dioxide or nitrite effects varies with the duration of the exposure and the concentration of the oxides in the air inhaled. Whereas pulmonary irritation is the dominant factor in situations involving brief periods of exposure to high concentrations, the nitrite effect must be considered in relation to prolonged exposure to lower concentrations (45). The maximum allowable concentration of nitrogen dioxide for prolonged occupational exposure, as promulgated by the American Standards Association is twenty-five parts per million.

Although there is not complete agreement as to the occurrence and extent of the reduction of nitrate to nitrite in the animal organism (46), the potentialities of this metabolic process must be considered. Whitemore (47) suggests that the intermolecular hydrolysis of nitrates occurs as follows: $R \cdot CH_2ONO_2 \longrightarrow R \cdot C\overset{O}{\underset{H}{<}} + HNO_2$. Marshall (18) and Rath and Krantz (48) question the nitrite action of the nitrates, since the depressor effects of nitrate are in excess of the amounts of nitrite recoverable from the blood. It has been suggested that the organic nitrates diffuse more readily than nitrites and are changed to nitrite within the cells (49). Animal tissues contain

enzyme systems capable of reducing nitrate to nitrite (48a). Bernheim and Dixon (50) showed that the liver is the main site of this reduction, but that some reduction occurs in muscle tissue.

Forty to sixty per cent of ingested nitrate is destroyed within the body (51), most of the remainder being excreted as nitrate (52a). Ingested nitrites are destroyed somewhat more readily (60to 70 per cent) within the body, the smaller residue being excreted in the urine (52).

Organic nitrates, of the composition $R \cdot O \cdot NO_2$, dilate the blood vessels and react, to some extent, with the hemoglobin of the blood. The latter action is highly variable and appears to be related to the solubility of the compound, and to its chemical stability (53), and to such external factors as the pH of the medium and the temperature (18). The formation of methemoglobin by the organic nitrates is attributed to this hydrolysis and the subsequent reduction of the nitrate to nitrite (54).

The degree and duration of the response, in vivo, to the organic nitrates are related to their vapor pressures and to their solubility coefficients in oil and water. The administration, by inhalation, of the nitrites of high molecular weight which, also are characterized by high vapor pressures, demonstrates the transitory quality and the greater depressant action of these compounds (56), as compared with those which have relatively low vapor pressures.

Krantz and others (55) demonstrated the relationship between octyl and amyl nitrites, which have, respectively, low and high vapor pressures. Compounds of relatively high vapor pressure are the more potent methemoglobin formers. Among the higher homologues, the branched chain molecules have the higher vapor pressures (56).

A high oil-to-water coefficient of solubility increases the duration of the depressant effect (46,54,55,56). By leaving the blood and entering the tissues, the action is extended. Under these conditions there is also less oxidation of hemoglobin to methemoglobin (55).

Generally, the compounds of the higher molecular weight (56) and those more refractory to hydrolysis (54) have the more prolonged depressant effect. The influence of these qualities is modified by the factors of solubility and volatility.

The administration of nitrite induces throbbing headache, some increase in the heart rate and usually a sharp decrease in the blood pressure (57). The use of alcohol by workers who absorb nitroglycerine induces flushing of the face, nausea, vomiting, increase in respiratory rate, loss in weight, polyuria and maniacal disturbances (37,58,59).

The administration of lethal or barely sublethal doses of sodium nitrite (dosage of eighty to ninety milligrams per kilogram of body weight) intravenously to rabbits

produced asphyxia. Initially, these animals had respiratory stimulation, then tonic convulsions which progressed to clonic, and they died of respiratory failure. When the dosage was of a slowly lethal magnitude, they exhibited an ascending paralysis with a secondary slowing of both respiratory and cardiac rates. Death was attributed to asphyxia from either circulatory failure or methemoglobin formation (60). The pathological findings in animals poisoned by sodium nitrite were limited essentially to hyperemia of the tissues, and varying amounts of methemoglobinemia (30).

Von Oettingen (19) describes an increase in respiratory rate, following the inhalation of amyl nitrate. Medullary stimulation produces this increase, the stimulus being the deficiency in the blood supply, or the reduced oxygen carrying capacity of the blood as the result of methemoglobinemia. When the anoxia is severe enough, respiratory depression occurs. High concentrations, when inhaled, may produce reflex changes from the upper respiratory tract.

The basic action of the nitrite ion within the body is the relaxation of smooth muscle, especially that of the smaller blood vessels. This action is independent of the innervation. The action of the smooth muscles is not paralyzing, since muscular stimulants and nerve constrictor impulses can exert their maximum effect, even though the tone of the muscle is reduced.

The drugs which liberate nitrite ions readily may produce marked cutaneous vasodilatation; however, following the use of slower reacting drugs, the cutaneous capillaries and venules do not display this tendency (61). Dilatation of the smaller meningeal vessels has been noted through intracranial windows and is the basis for the increase in intracranial pressure which accounts for the transitory pulsating headache. A similar action on the retinal vessels has been observed (62).

There is no direct action of the nitrite ion on the myocardium, the stroke volume may be diminished because of poor venous return from the post-arteriolar pool. The associated increase in heart rate maintains the cardiac output. The constancy of the diastolic pressure is a manifestation of continued peripheral arteriolar resistance, indicating little nitrite effect on these structures (61, 63).

There is also a loss in venous tone as manifested by an increase in the volume of the hand, and by susceptibility to syncope with change in position, after the administration of nitrite (64). A rapid fall in venous pressure is associated with circulatory collapse, which occurs when the blood flow is twenty to forty per cent of normal (63).

Following the fall in blood pressure, there is a vaso-constrictor response mediated through the carotid and

aortic sinuses (63) or by a secondary central stimulation due to anoxia (65). These reflexes produce an increase in arteriolar tonus, a response similar to that induced by epinephrine (64). Medullary ischemia per se may elicit both sympathetic and parasympathetic responses (63). The bradycardial response, preceding syncope, is of this character, as is shown by the fact that atropine abolishes this vagal response but does not inhibit the syncope. Pupillary dilatation is of the same pattern; although, there is species and individual variation in the dominant response.

The carotid sinus chemo-receptors are sensitive to the nitrite ion, and through this reflex mechanism a sympatho-adrenal discharge may produce a tachycardia and an increase in blood pressure. The latter soon drops because of the peripheral action. Injection of sodium nitrite into the carotid artery below the carotid sinus produces bradycardia and hyperpnea; this does not occur if the chemoreceptor area is denervated (21a).

A full pulse is also characteristic of nitrite ion action; this is independent of vaso-depression effect, and is apparently related to the relaxation of the smooth musculature of the large arteries.

Electrocardiographic changes, mainly an increase amplitude of the "t" wave of the chest leads, indicate the absence of myocardial anoxia and depression during extreme circulatory collapse after the administration of large doses

of nitrite ion (66). The coronary circulation appears to remain adequate. However, heart block has been reported (67).

Ordinary therapeutic doses of nitrite affect no change in the body temperature. There is no significant change in the basic metabolic rate; nor is a correlation observed between changes in renal clearance and blood pressure, following the administration of nitrites (61).

Nitrite syncope is the manifestation of peripheral circulatory failure due to the pooling of blood in the post-arteriolar vascular beds. Under these conditions, reflex constriction of the arterioles may contribute to the maintenance of the circulation. The post-arteriolar pooling reduces the venous return to the heart, thereby causing a fall in the systolic pressure, and a lowering of the pulse pressure. The essential cause of syncope in this instance, is therefore, a decrease in the effective blood volume. (63)

There is the danger of tissue anoxia, when the volume of the blood is inadequate. In the central nervous system this may be manifested by convulsions. Gross decreases in blood flow may result in damage to such organs as the kidneys and the liver, if the inadequacy persists for a prolonged period of time (66).

There may also be a relative pressure increase in the pulmonary artery and the left auricle, which may lead to pulmonary stasis and edema. (19)

Tolerance for doses of nitrite sufficient to induce headache is developed in about three days, and thereafter, the dosage can be maintained or increased without difficulty; the tolerance is not due to decreased absorption, since a sufficient increase in the dosage will elicit the typical response (59,68,69). An interval of freedom from the administration of nitrite will re-establish the original susceptibility(37). There is evidence of cross-tolerance among members of the nitrite group of compounds (69, 70). The cross-tolerance acquired through the use of oil soluble nitrites appear to be limited (46); these compounds are less rapidly hydrolyzed and they produce a more effective tolerance to the specific compound (69). Tolerance, as expressed by the disappearance of headache, is achieved more readily than that indicated by reduction of smooth muscle relaxation (69).

The mechanism of nitrite tolerance is not known. Histamine has a similar pharmacological action, but is unrelated chemically, and there is no evidence of cross-tolerance. The phenomenon of local tolerance is also absent (69).

The source of the nitrite effect associated with the absorption of amyl nitrate must be considered. It may be the result of an hydrolysis or the nitrate may be subject to an intracellular reduction. About fifty per cent of nitrate administered may be available for this

reduction. Amyl nitrate is a member of the effective nitrate group ($R \cdot O \cdot NO_2$), producing the nitrite effect and methemoglobinemia. Low water solubility and high oil solubility increases the duration of the nitrite effect, but reduced the formation of methemoglobin.

The basic action of nitrite (aside from its ability to form methemoglobin) is relaxation of smooth muscles. Physiologically, this produces post-arteriolar pooling of blood and a deficiency in the effective blood volume with secondary anoxia of the organ systems.

Methemoglobin formation by both the nitrate and amyl radicals must be evaluated. However, methemoglobin formation by the amyl radical is less probable than methemoglobin formation by the nitrate.

Hemoglobin can be oxidized by a number of drugs and toxic agents to form methemoglobin - a product which contains the same amount of oxygen as oxyhemoglobin, but does not release its oxygen when the pressure is reduced (71). Gamges (71a) in 1868, pointed out that amyl nitrite caused methemoblobin to be formed in blood.

Methemoglobinemia may be classified, either as the plasma or the cellular type (72). The methemoglobin, in the plasma type, is formed after liberation of the hemoglobin from the erythrocyte following hemolysis. It occurs, occasionally in eclampsia, in paroxysmal hemoglobinuria, and in anaerobic sepsis. In cellular methemoglobinemia the

hemoglobin remains within the erythrocyte. Methemoglobinemia may be classifies further as enterogenous, in which nitrite is produced in the alimentary tract in a sufficiently alkaline environment, by bacteria capable of reducing nitrates to nitrites (73). Following this reduction, there must be sufficient absorption through a damaged intestinal mucosa (74).

Nitrites produce the cellular type of methemoglobenemia. This type may also be produced by bacteria capable of axidizing hemoglobin to methemoglobin under conditions of decreased oxygen tension (75); this is referred to as autoxidation (76). However, hypoxemia does not affect methemoglobin formation from nitrite (77). Cellular methemoglobinemia due to chemicals is the result of the direct action of oxidants or of the action of hydrogen donors in the presence of oxygen.

Methemoglobin has been found in the normal blood of man and of experimental animals. Heubner (77a) found the following percentages of methemoglobin in the total blood pigment of the following species of animals; man - 0.7 per cent, cat - 1.1 per cent, rat - 0.8 per cent, rabbit - less than 0.2 per cent, guinea pig - less than 0.2 per cent. Paul and Kemp (78) in one hundred unselected humans, found that the methemoglobin ranged from 0.01 to 0.5 grams per one hundred grams of hemoglobin, the mean value being 0.09 gram. In this series, there was no correlation between the hemoglobin and the methemoglobin. The demonstration of the presence of methemoglobin in normal blood suggests that

there is a reversible equilibrium between methemoglobin and hemoglobin, which usually favors hemoglobin very greatly. This equilibrium is regulated by various oxidizing and reducing substances which are controlled by the glycolytic enzyme systems of the intact erythrocyte (75). The control mechanism appears to be inhibited under conditions of lowered body temperature (79). Under usual circumstances the equilibrium takes long periods to become established (76).

Aside from the intravenous administration of toxic agents (80), experimental work has shown that the maximum conversion of hemoglobin to methemoglobin is approximately seventy-five per cent (81). At this level equilibrium is reached between methemoglobin formation and reconversion to hemoglobin.

The degree of methemoglobinemia resulting from a toxic chemical is dependent upon a number of factors: the mode of administration, bacterial conversion, rate of absorption, pattern of metabolism, rate of excretion, and methemoglobin reducing mechanisms of the organism which vary with age and species (76). Themethemoglobin reducing mechanisms are also dependent upon the concentration of methemoglobin, the concentration of the toxic substance, and the pH of the blood (76a). Under these circumstances, equilibrium may be reached before the methemoglobin, so formed, reaches a significantly harmful concentration. It has been observed that

the concentration of methemoglobin increases with increasing dosage of a compound, but a point in the conversion is reached beyond which large doses of the drug produce no further significant increases in the methemoglobin. (76d)

The variation in the behavior of species and the appearance of delayed methemoglobinemia are attributed to the variations in the metabolism of the offending agent, whereby the resultant intermediate compounds in various species have different capacities for oxidizing hemoglobin, or inhibiting the reduction of methemoglobin (81, 82). In addition to the metabolic factors, the permeability of the erythrocyte to methemoglobin-forming substances is of importance (84). These factors explain the differences in the rates of conversion of hemoglobin to methemoglobin by the same drug in various species, as well as the variations in the rates of reduction of methemoglobin.

In considering species variation, the herbivores are exceedingly resistant to methemoglobinemia (85). Among carnivores, the order of sensitivity to methemoglobin formation is such that man is about half as sensitive as the cat, which is one of the most sensitive of the animals (82).

The oxidizing action of nitrite on hemoglobin produces methemoglobin, nitrosohemoglobin and nitroso-methemoglobin (86). The nitroso-methemoglobin is unstable and reverts to methemoglobin (76). The formation of nitroso-methemoglobin by the organic nitrates in the absence of a reducing agent is disputed (18, 44).

There is little agreement among investigators as to the ratio of nitrite administered to the quantity of methemoglobin formed (75d, 86). The rate, not the final extent of the formation of methemoglobin is dependent upon a number of factors. The maximum conversion occurs when there is excess of nitrite; however, before this maximum is attained the factors which tend to reverse the reaction are in action. In vitro, experiments have shown that one molecule of nitrate reacts with two molecules of hemoglobin to form two molecules of methemoglobin. Greenberg (87) suggests the action is:

$$HNO_2 + 2HbO_2 \longrightarrow HNO_3 + Hb_2O + O_2.$$

It has been demonstrated that the effects of tissue anoxia due to the simultaneous action of multiple methemoglobin forming drugs (76) and to other types of hemoglobin immobilization, such as carbon monoxide are additive (86).

The formation of methemoglobin subjects the tissues to anoxia in two ways: first, through some lowering of the capacity of the blood to take up fresh oxygen because of the reduction in the effective hemoglobin; and second, the decreased capacity of the blood to give up oxygen to the tissues. It is postulated that there are intermediate compounds between hemoglobin and methemoglobin. As an intermediate step in the formation of methemoglobin, one or more of the four ferrous atoms in the hemoglobin molecule are converted to ferric. The presence of ferric atoms increases the affinity of the remaining ferrous atoms for oxygen. This

thesis explains the leftward shift of the oxyhemoglobin dissociation curve observed in severe methemoglobinemia; a manifestation of the reduced ability for the residual oxyhemoglobin to dissociate (86).

From experimental observations on the oxygen tension of arterial blood containing methemoglobin, it appears that the unconverted hemoglobin continues as an oxygen carrier. Although the available oxygen in the arterial blood is decreases, the arterial oxygen tension is not sufficiently disturbed to initiate compensatory responses in the respiratory center. Effective respiratory stimulation is initiated through the carotid and aortic bodies; these organs are stimulated by a diminished arterial oxygen tension. Methemoglobinemia per se produces anoxia of the respiratory centers, which acts as a respiratory depressant (88). A decrease in the oxygen tension does not change the rate at which nitrite induces the formation of methemoglobin (77), although it is known to alter the rates at which certain other drugs affect this conversion.

Methemoglobinemia produces a definite increase in circulatory function, when that function is not overtaxed, as manifested by an increase in heart rate and cardiac output. These effects are probably related to tissue anoxia from a decrease in venous oxygen tension (88). Cardiac disturbances have also been described as the result of myocardial anoxia. The cardiac signs increase in severity

with increases in the content of methemoglobin in the blood.

It has been calculated (76), that when eighty-five to ninety per cent of the hemoglobin has been converted to methemoglobin, complete dissociation of the remaining oxyhemoglobin would occur if the venous oxygen tension was zero. Complete dissociation at this level would deliver an insufficient amount of oxygen to the organism. A sufficient increase in the partial pressure of oxygen in the atmosphere will permit an animal to survive despite the conversion of n ninety per cent of his hemoglobin to methemoglobin (60). The susceptibility of the central nervous system to oxygen deficiency is such that definite signs of hypoxia appear when sixty per cent of the hemoglobin is in the form of methemoglobin (76, 89), and conversion to the extent of seventy per cent may, perhaps, be lethal (90). The signs of cerebral hypoxia are ataxia, unconsciousness and prostration.

Concentrations of methemoglobinemia near fifty per cent cause an increase in pulse rate (80). The cardiac output increases in response to concentrations approximately forty per cent (16). At these levels the increase in heart rate and in cardiac output maintains tissue oxygenation. At levels below forty per cent, oxygen is supplied under the conditions which characterize the lower half of the oxygen dissociation curve (88). At the forty per cent level,

methemoglobinemia is not, in itself, a threat to survival (90), although, headaches, dizziness and exertional dyspnea appear when methemoglobinemia approaches this level (91). These signs and symptoms are relieved by increasing the partial pressure of oxygen in the respired air.

When less than thirty per cent of the hemoglobin is converted to methemoglobin the patient may be symptomless (90) or he amy display an euphoria. However, he will be markedly cyanotic. Thecyanosis is usually recognizable when the methemoglobin concentration is as low as ten to fifteen per cent. In acute poisoning, the severity of the clinical picture is proportional to the concentration of methemoglobin (81 85).

Frequently, during a period of intoxication associated with methemoglobin, it has been observed that there is a rise in the absolute numbers of erythrocytes and in the hemoglobin content of the peripheral blood; however, these values return to previous control levels within twenty-four hours after the disappearance of the methemoglobin. This secondary polycythemia is a common response in a number of conditions which reduce the available oxygen in the blood. Its precise mechanism is not fully understood. An adequate increase in the numbers of erythrocytes may inhibit the leftward shift of the oxygen dissociation curve. There does not appear to be substantial evidence that methemoglobin formation in itself is associated with hemolytic anemia. (16)

The association of methemoglobin formation with the appearance of Heinz bodies is inconstant. The administration of ethyl nitrite does not produce Heinz bodies whereas, the nitrate does (19b). Amyl nitrite has not been found capable of producing these bodies in erythrocytes (19), whereas, the experiments described previously herein have demonstrated the effectiveness of amyl nitrate in this regard.

Heinz bodies are multiple, round, refractile inclusions within the erythrocytes. These inclusion bodies usually appear in response to the absorption of materials that produce methemoglobinemia. However, the Heinz bodies persist after the methemoglobinemia has disappeared. Changes in the fragility of the erythrocytes and decreases in the amount of functional hemoglobin have not been observed, consistently, in the presence of heinz bodies (92). (93)

The number of Heinz bodies formed appears to be a function of the concentration of the agent which induces the phenomenon. There is insufficient evidence of a correlation between the capacities of materials to produce Heinz bodies and their chemical structure. The differences in species metabolism and in the resultant intermediate compounds, may explain the species variation in susceptibility to Heinz body production. (93)

The belief that is most widely held concerning the origin and nature of Heinz bodies is that they are composed

of denatured protein which separates out as discrete new particles within the mature erythrocyte. On this thesis, they are formed from the cell by its reaction with a toxic agent or its intracellular metabolite; the reaction being irreversible (9). As to the exact nature of these inclusion bodies, aside from their being a protein substance, there is no agreement among investigators (94). Some (93, 95, 96) believe that the appearance of Heinz bodies is morphological evidence of the splitting of the porphorin ring in hemoglobin to form verdohemochronogen, an early step in the degradation to bilirubin. This change is catalyzed by methemoglobin (95).

Heinz bodies are found only in mature erythrocytes (95). Under some circumstances, they may be found in small numbers in the blood of normal subjects. Erythrocytes containing Heinz bodies are thought to be removed by the spleen soon after these bodies are formed (93, 94, 97). The bodies are believed to be evidence of erythrocytic alteration, which may be associated with hemolysis and anemia (9, 96).

Heinz bodies appear in the erythrocytes during the exposure of animals to a large number of chemicals (98) of which many belong to the aromatic nitro and amino series. The aliphatic nitro compounds and nitrates shown to induce the formation of Heinz bodies are: 2-nitropropane, 2-2 dinitropropane, nitroglycol, and nitroglycerine (9, 94). The relative capacities of nitrates and nitrites to produce Heinz bodies are highly variable (97, 98).

The experimental production of Heinz bodies demonstrates such variable factors as species susceptibility, dosage, route of administration, and nature of the compound (9, 98).

Heinz bodies occur in the erythrocytes of man, in association with the absorption of a variety of chemical compounds (9). However, little is known about the phenomenon in man, excepting as it has been observed from time to time in connection with accidental exposure, or situations involving unusual types and intensities of exposure for short periods of time.

Section III

CRITIQUE AND DISCUSSION OF EXPERIMENTAL WORK

CRITIQUE AND DISCUSSION

Amyl nitrate, when vaporized and inhaled in sufficient concentrations by the experimental animals, produced an initial excitation followed by lethargy. In high concentrations it produced convulsions, salivation, pupillary dilatation, lachrymation and deep and rapid respirations. Although the compound is something of an irritant, no corneal injury was seen following the exposure of animals to its vapor. Lethal concentrations induced respiratory failure.

Among the surviving animals, there were weight losses which tended to vary with the duration and intensity of the exposure . Table #2. This weight loss was of gradual onset and was gradually regained. Since there was not a control group for each inhalation experiment, the contributory effects of daily handling and of the artificial environment on the test animals can only be assumed to have been generally insignificant. Other than minimal transient losses in weight, no signs of intoxication were observed among the animals during their prolonged period of exposure to the concentration of 1.4 milligrams of amyl nitrate per liter of air.

Animals which succumbed to the vapors of amyl nitrate in varying concentrations were found, consistently, to have undergone the following pathological changes: pulmonary hyperemia and edema, some lungs showed acute focal

inflammatory reaction; degeneration of hepatic cells; degeneration of the epithelial cells lining the renal convoluted tubules, and degeneration of the nerve cells of the brain. These changes were reversible, as was demonstrated by the fact that other animals which had survived following the exposure and had regained their lost weight, displayed no evidence of these pathological changes. The liver of one cat, which had been exposed to 1.4 milligram per liter on twenty days and killed eight days after the last exposure, had persisted in its damaged state, although it had regained the weight lost during the period of exposure. It is apparent therefore, that the recovery process requires time and that such histologic abnormalities as those observed are not, in themselves, satisfactory evidence of dysfunction.

The susceptibility of animals to the inhalation of the vapor of amyl nitrate may be seen in diminishing order as the animals are listed: mice, rabbits, guinea pigs, rats, and cats.

A glance at the curves of mortality among several species of animals (excluding cats, all of which survived) in the inhalation experiments shows that their extension would give them sharp deflection to the horizontal as the concentration of the vapor diminished and the duration of the exposure increased. The probable physiological interpretation of this deflection is that the break in the line

occurs when the maximum concentration of amyl nitrate in the tissues of the animals during the period of exposure on any one day undergoes no further significant progression from day to day; that is, when the rate of absorption associated with the atmospheric concentration, over the period of several hours, is balanced by the rates of metablic breakdown and excretion over the period of exposure and the period intervening before the initiation of the next exposure. When the concentration of the vapor in the atmosphere is potentially lethal, the fate of the animal is dependent upon the duration of exposure. The slopes of the mortality curves appear to vary fairly consistently with the weight relationships of the several species.

Analysis of possible synergistic and cumulative effects would necessitate more prolonged exposure of animals to the lowest concentration investigated in these experiments. The use of a control group of animals, under the same conditions of handling and confinement but excluding exposure to amyl nitrate, would be advantageous. Further investigation of the significance and of the rate and degree of disappearance of the pathological changes induced by exposure to the vapor of amyl nitrate should be carried out by killing and examining animals at appropriate intervals after the termination of the period of exposure.

In view of the suggestive but not altogether convincing evidence of the occurrence of systemic intoxication in association with the percutaneous absorption of amyl

nitrate under certain experimental conditions, further experiments along this line may well be carried out. In at least one experiment, which might be repeated and extended, the effects observed may be attributed to the absorption of amyl nitrate. In this experiment the losses in weight and the systemic signs of intoxication were more marked in the animals which were being subjected to applications of catalytically cracked diesel fuel containing two per cent of amyl nitrate, than they were in those whose contact was limited to the same fuel without the additive.

The anticipated concentration of amyl nitrate in diesel fuel is 0.25 per cent (V/V); experimental work with selected diesel fuels containing amyl nitrate in this concentration would be advantageous.

Animals exposed to diesel fuels, with or without amyl nitrate, displayed moderately severe injury of the skin. Theskin injury was characterized by extreme erythema, moderate edema, lichenification and induration; subsequently, fissures and marginal hemorrhages developed in the affected areas. Microscopically, there was papillomatous hyperplasia, acanthosis, hyperkeratosis and chronic inflammation. The severity of the injury to the skin induced by contact with a model airplane fuel was much less than that caused by contact with the diesel fuel, while that sustained by animals subjected to contact with undiluted amyl nitrate was even less.

These cutaneous lesions appear to be of the nature of the primary irritant type. Thepetroleum fractions contained in diesel fuel are within the boiling point range of petroleum products known to be irritating to human skin, and the experiments leave no doubt that the petroleum hydrocarbons were the principal factros in the cutaneous injury. However, undiluted amyl nitrate is a mild skin irritant.

The administration of amyl nitrate to rats and rabbits by the oral, intraperitoneal and intravenous routes produced a uniform group of signs of intoxication, composed essentially of the following: increased respiratory rate, which eventually failed with lethal doses; motor excitation preceding lethargy; terminal cyanosis; and lowered body temperature. Oral administration displayed a relatively low toxicity. Intravenous administration produced a transient miosis in contrast to the constant mydriasis noted with inhalation; intravenous injection also produced lachrymation, and spasms followed the administration of lethal doses. The intravenous administration of multiple doses of an emulsion containing amyl nitrate demonstrated a falling off in the effectiveness of successive doses in eliciting the response, that is, in order to adhieve the original effect, increasing dosages were required.

In experiments involving the oral administration of amyl nitrate, young female rats were found to be more

susceptible to the effects of the compound than were the adult rats. This observation is worthy of further investigation.

The irritant properties of amyl nitrate suggest that it may give rise to some hazard to the eyes. No severe conjunctival or corneal pathology was demonstrated among the experimental animals, but it suggested that the keratoconjunctival reaction be investigated further.

Any further experimental work should look into the factors of possible biological susceptibility related to sex. The possibility of deleterious effects from the absorption of amyl nitrate on the blood forming tissues should be investigated in any further prolonged experiments, at least to the extent of carrying out systematic observations on the leucocyte elements in the peripheral circulation. The feasibility of detecting functional changes in the livers and kidneys of experimental animals following exposure to high concentrations of amyl nitrate may well be explored.

Under experimental conditions, the inhalation of the vapor of amyl nitrate is lethal to exposed animals when the concentration exceeds seven milligrams per liter or thereabouts (1310 ppm), for the period of approximately fifteen hours. Below this level, there appears to be a break in thecurve which portrays the toxicity, in that animals survive following exposure for disproportionately prolonged periods of time at the lower concentrations.

Taking this fact into account and providing a safety factor of ten in relation to this critical concentration, the level of 0.7 milligrams per liter (131 ppm) may reasonably be regarded as probably safe for men who may be exposed to the vapor of amyl nitrate for no longer than seven hours per day. If men who work under these conditions were maintained under appropriate medical surveillance, no immediate risk would be involved, and the facts concerning the potential hazards of human exposure to the vapors of this compound can be obtained. This concentration is half of that to which the animals were exposed in the final experiment. In this experiment involving prolonged exposure to the concentration of 1.4 milligrams of amyl nitrate per liter of air (262 ppm), the experimental animals displayed a significant loss in weight. Moreover, evidence was obtained in the case of one animal, that minor damage to the liver, sustained, presumably, during the period of exposure, persisted after the animal had regained the weight lost during the period of exposure. It is by no means certain that these effects can be attributed to the toxic action of amyl nitrate, but it is safer, for practical purposes, to assume that they were.

In the concentration of 1.4 milligrams of amyl nitrate per liter of air, the component radicals of the compound occur in the following proportions: 53.4 per cent of amyl radical or 140 ppm, and 46.6 per cent of the nitrate radical or 122 ppm.

The accepted safe concentration of amyl alcohol ranges from 55 to 400 ppm. Eighty-one per cent of the alcohol is represented by the amyl radical, which therefore is represented over the range of 44 to 320 ppm. The correspondingly acceptable safe concentration of amyl acetate is 400 ppm; about fifty per cent of the compound is amyl radical, equivalent to 200 ppm. Therefore, in the case of amyl nitrate at 114 milligrams per liter, the equivalent concentration of amyl radical is within the usually accepted range for the amyl equivalents of amyl alcohol and amyl acetate.

Amyl nitrate contains 46.6 per cent of the nitrate radical, and 34.6 per cent of the amyl nitrate is potentially available as nitrogen dioxide, equivalent to a concentration of nitrogen dioxide of 90ppm. The accepted range for nitrogen dioxide is twenty to seventy parts per million, although the lower figure is the more generally acceptable as indicated by the value of 25 ppm recommended by the American Standards Association. Taking the concentration of nitrogen dioxide of 45 ppm as acceptable and assuming that somewhat less than all of the nitrate radical in amyl nitrate is available as nitrogen dioxide, the nitrate component is found to be within the acceptable range when the concentration of amyl nitrate is 0.7 milligrams per liter. This agreement with the terms of the tentative standard of safety previously proposed may be looked on as somewhat, if not definitely, confirmatory.

The observations on the rate and extent of the vaporization of amyl nitrate in an enclosed chamber suggest that the tentative standard for safe industrial practice should not be excessively difficult to meet. After thirty hours, the completely enclosed experimental chamber contained amyl nitrate in a concentration well above that suggested but the rate of evaporation was found to be slow, and the influence of the movement of air in any adequately or even partially ventilated space may be expected to offset such progressive accumulation of vapor. It is evident, on the other hand, that potentially dangerous concentrations of vapor may accumulate within an enclosed space. The observations made under the actual conditions of the use of amyl nitrate (0.25 per cent V/V) in diesel fuel, indicate that the concentrations of amyl nitrate was well below the suggested air concentration.

The efficiency of the respiratory equipped with the canister for organic vapor, was shown to be satisfactory for providing respiratory protection against the vapor of amyl nitrate.

The pathological changes observed in the livers and kidneys of animals exposed to amyl nitrate are related to the amyl portion of the radical. This is consistent with their reversibility and may contribute to the explanation of the observed lossed in weight on the part of the animals. The degenerative changes in the central nervous system are

related to the amyl portion of the molecule. The labored respiration, which appeared after a period of exposure, together with the ataxia, may be early evidences of this neuro-toxicity. The ataxia appears in animals relatively resistant to the formation of methemoglobin; this tends to exclude the implication that anoxia in the central nervous system from this source, was a factor in producing the altered gait.

Both components of the amyl nitrate molecule are irritants to mucous membranes. This property of amyl nitrate was manifested by lachrymation, salivation and pulmonary hyperemia or edema.

The occurrence of convulsions and coma depended primarily upon the concentration of vapor in the air breathed by animals; that is, upon the rate of administration of amyl nitrate to animals. The early appearance of these signs, following the administration of a large dose, is a secondary phenomenon associated with the disturbance of the circulation by the nitrate. Under these conditions, in which there is extensive post-arteriolar pooling of blood, there is a reflex arteriolar contraction. This reflex contraction is similar to the response to epinephrine. Therefore, under these circumstances, epinephrine is contraindicated therapeutically.

Convulsions and coma, follwoing the appearance of ataxia and further respiratory disturbances, are related to

the amyl portion of the molecule. The observed lethargy, subsequent to exposure, but not persisting during the entire period of weight loss, is also attributed to the amyl portion of the radical and its metabolism to valeric acid.

During the experiments involving inhalation of the vapor of amyl nitrate, it was necessary to remove the animals from the respiratory chamber before sweeping it free of the vapor-air mixture, this procedure contaminated the atmosphere of the experimental laboratory, and resulted in the exposure of the experimental staff. The latter experienced headaches which were throbbing in character, and some of them experienced nausea. These reactions are similar to those observed among workers exposed to nitroglycerine.

Headache of rapid onset is a result related to the nitrate portion of the molecule, resulting from the dilatation of the intracranial vessels. Appearing after repetitious and prolonged exposure, such a symptom is due to accumulation of the amyl radical. This is consistent with the relatively prolonged presence of the mayl radical in the blood, due to its relatively slow metabolism and excretion.

Some tolerance to the nitrate portion of the radical and to some of its effects appears to be developed. Should there be no such acquired tolerance to concentrations of the order of 0.7 milligrams per liter (131 ppm), this tentative value will require reduction.

Methemoglobinemia has been observed following the absorption of compounds and compounds containing the amyl radical. The nitrates are more consistent and more potent methemoglobin forming drugs. The effects of the radicals are additive with respect to each other and to any other source of impairment of the oxygen supplying function of hemoglobin. In the single experience reported, there was an increase susceptibility to methemoglobin formation with repeated exposure. It may be postulated that an equivalent exposure of a healthy man would not be deleterious, although the individual would appear cyanotic.

Characterized by a relatively low vapor pressure and low solubility in water, amyl nitrate follows the pattern of the organic nitrates, diffusing readily into the cells and being converted to nitrite within the cell. Under these circumstances, amyl nitrate is of a relatively low order of potency as a methemoglobin forming compound. However, the persistent presence of the nitrate, under the conditions associated with its sources in this compound (the properties of which are indicated above) provides for a prolonged vasodepression.

The circulatory changes of relatively rapid onset are attributed to the nitrate. These changes are manifested by alteration in pulse, appearance of cyanosis and early collapse.

Respiratory stimulation may be attributed to irritation with reflex stimulation in the respiratory tract, or

to the nitrite action on the carotid and aortic bodies. Subsequent respiratory depression can be the result of central depression induced either by the amyl radical or by the anoxemia associated with deficient circulatory volume or methemoglobinemia or some combination of these two factors. The change in body temperature are secondary to the circulatory disturbances.

The pupillary changes are due to the nitrate portion of the molecule. Late changes, associated with coma and convulsions, may be an effect of the central nervous system depression by the amyl radical.

Repeated exposure to amyl nitrate did not produce hemolysis or anemia.

These signs and their relationship to compounds with either the amyl or the nitrate radical are summarized graphically in Table #3.

There is some correlation between amyl nitrate and the known action of related compounds. Adapting this to the experimental work, it appears most probable that the action of lethal doses of amyl nitrate is a combination of both components of the radical. The pulmonary irritation in the inhalation series was a significant feature of the lethal process where it terminated fatally. Delayed deaths are attributed primarily to the pulmonary pathology, the contributory effects of the hepatic and renal disorders being, in all probability, essentially unimportant.

Administration of sublethal doses of amyl nitrate displayed initial effects attributable to the nitrate portion of the radical and the products of its metabolism. Delayed effects, as manifested by weight loss, may be related to the amyl radical.

With one exception, which has been referred to previously, but can not be credited with much significance, no relevant pathology was observed in the tissues of animals that had survived and had regained the weight lost during periods of respiratory exposure to amyl nitrate, when the animals were killed for examination. It is evident that the pathological changes associated with the exposure of the animals to amyl nitrate (as found in those that died or were killed during the period of exposure) were reversible, as indeed, from their histological characteristics, they would be expected to be.

Amyl nitrate displays evidence of being a mild skin irritant, and the repetitious application of amyl nitrate upon the skin of animals results in retardation of growth. The additive, in catalytically cracked diesel fuel also increases the toxicity of the fuel.

Section IV

RECOMMENDATIONS CONCERNING AMYL NITRATE

RECOMMENDATIONS CONCERNING AMYL NITRATE

In consideration of the results of the experimental work described herein, it is recommended, in the event that amyl nitrate should find wide spread use, that:

1. Experimental animals be subjected to the inhalation of atmospheric contamination of amyl nitrate in the concentration of 1.4 milligrams per liter over a prolonged period of time, in simulation of human occupational exposure.

2. Animals be subjected to further applications of amyl nitrate in selected diesel fuels in the concentrations of 0.25 per cent (V/V).

3. Control groups of animals be subjected to the same conditions of handling and confinement so as to better gauge the minor effects of the absorption of amyl nitrate.

4. The experimental groups of animals be sufficient in number so that representative animals can be killed for examination at staged intervals during and at the conclusion of the periods of exposure.

5. The effects of contact of the eyes of animals with liquid and vaporized amyl nitrate be investigated.

6. In further experimental work, biological susceptibility, with reference to age and sex, be determined.

7. In further experimental work, the variation in the leucocyte responses, if any, of exposed animals be investigated.

8. In further appropriate experiments, the influence of amyl nitrate on hepatic and renal function be investigated.

9. In further experimental work, the relationship of weight loss and hepatic damage be investigated.

The recommendation is made, tentatively, that the concentration of amyl nitrate in the atmosphere to which men are to be exposed in the normal course of their work should not exceed 0.7 milligrams per liter (131 ppm); this figure may be revised upward as experience is obtained. The low volatility of the material allows it to be handled safely in a well ventilated area under conditions of moderate temperature.

The suggested precautionary measures for handling amyl nitrate or mixtures containing it are the following:

1. The material should be stored in a segregated area. Containers should be closed tightly when not in use. There should be adequate ventilation in the storage area.

2. The material should not be subjected to temperatures near to, or in the range of, its boiling point (150°F.).

3. Amyl nitrate which has been spilled or permitted otherwise to escape from closed containers should be reclaimed promptly into closed containers. Small quantities may be removed by flooding the area with water into a drainage system.

4. Enclosed contaminated areas should be entered only with protective respiratory equipment. The circumstances or pertinent safety regulations will determine whether canister

masks (for organic vapor) are to be used, or whether air line hose masks with air supplied under positive pressure, are to be used.

5. The concentration of amyl nitrate in the atmosphere of regular working places should be determined by representative sampling and analysis.

6. Contact of the skin of workmen with amyl nitrate, and with products containing it, should be limited by appropriate precautionary measures, and if necessary, by impermeable garments.

7. If amyl nitrate should be ingested, vomiting should be induced.

8. The eyes should be protected against contact with amyl nitrate. Chemical goggles should be used in areas where splashing is probable. The therapeutic principles of chemical contamination of the eye apply in the case of amyl nitrate.

9. Adequate facilities for personal cleanliness and hygiene should be available (washing facilities, showers and extra clothing and equipment for replacing that contaminated).

The use of amyl nitrate and the potential human exposure associated therewith, make it necessary to emphasize the importance of making and recording comprehensive comprehensive clinical observations as a means of refining our knowledge of its potential hazards, and of ensuring their

control. Pre-employment and periodic medical examinations of workers will contribute this valuable information.

1. Clinical observations of workers exposed to amyl nitrate, must begin with the employment of individuals in suitable physical condition. Following medical examination, the physician should suggest selected job placement, with an appropriately restricted exposure to amyl nitrate, for individuals with significant historical or clinical evidence of the following:

   a. chronic alcoholism
   b. meningitis or uveitis
   c. pulmonary pathology
   d. cardiac pathology
   e. anemia
   f. liver pathology
   g. renal pathology
   h. dermatitis.

2. Periodic physical examination should note the:

   a. general well being
   b. weight change
   c. persistance of headache
   d. fundiscopic findings
   e. pulmonary status
   f. depression of the systdic blood pressure
   g. appearance of anemia
   h. urinalysis
   i. cutaneous lesions.

3. Opportunities for acute severe exposure of workers may necessitate medical supervision. Whereas epinephrine is contraindicated, the physician should have available positive pressure oxygen and whole blood. Skin contamination should be removed by washing.

4. Demonstration of symptoms or signs attributable to amyl nitrate may necessitate rotation of the worker to an unexposed job.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Bogen, J. S. and Wilson, G. C.; Ignition Accelerators for Compression-Ignition Engine Fuels; Petroleum Refiner, July 1944 (reprint).

2. Robertson, F.; Effect of Amyl Nitrate on Diesel Engine Combustion and Fuel Economy. PhD. Thesis, University of Wisconsin, 1940.

3. Ethyl Corporation, Research and Engineering Dept.; An Ignition Improver for Diesel Fuel, Dec. 13, 1951.

4. Anderson, M. J. and Wilson, G. C.; Effectiveness of Amyl Nitrate in a Full Scale Diesel Engine; Ethyl Corporation, October, 1951.

5. Treon, J. F., Duffy, J. C., Cleveland, F. P., and Shaffer, F. E.; The Toxicity of the Vapor of Amyl Nitrate; Kettering Laboratory Report, March 1953.

6. Stull, D. R.; Vapor Pressure of Pure Substances, Organic Compounds, Ind. Eng. Chem. 39:517 1947

7. Witherup, S.; Personal Communication.

8. Michael, H. O., Harris, J. S. and Durham, N. C.; The Blood Pigments; The properties and Quantitative Determination with Special Reference to the Spectrophotometric Methods, J. Lab. Clin. Med. 25:445 1940.

9. Webster, S. H.; Heinz Body Phenomenon in Erythrocytes; Blood, 4:479 Reprint, 1949.

10. Witherup, S., Cleveland, F. P. and Schlecht, H.; The Physiological Response of Animals to a Series of Periods of Cutaneous Contact with Various Motor Fuels Containing Cetane Improver DB-26; Kettering Laboratory Report, April 1953.

11. Dunn, M. S., Murphy, E. A. and Rockland, L. B.; Optimal Growth of the Rat; Physiological Reviews 27:72 1947, Quoting Zucker et al.

12. Witherup, S. and Schlecht, H,; the Immediate Toxicity of Amyl Nitrate; Kettering Laboratory Report, April 25, 1951.

13. Philips, F. S. and Gilman, A.; the Acute Toxicity of DDT Following Intravenous Injection in Mammals with Observations on the Treatment of Acute DDT Poisoning; J. Pharmacol. and Exper. Therap., 86:213 1946

14. Cholak, J.; Atmospheric Pollution with Amyl Nitrate (Experimental); Kettering Laboratory Report, August 6, 1952.

15. Cholak, J.; Atmospheric Pollution with Amyl Nitrate (Conditions of Use); Kettering Laboratory Report, August 31, 1952.

16. Guy, D. M.; Ethyl Corp. Research Lab., Detroit; Personal Communication, January 1953.

17. Association of Official Agricultural Chemists; Official and Tentative Methods of Analysis, 6th Ed., 1945, p.27; Pub. A. O. A. C., Washington, D. C.

18. Marshall, C. R.; The Mode of Action of Nitric Esters; J. Pharmacol. and Exper. Therap., 83:106 1945.

19. Von Oettingen, W. F.; The Effects of Aliphatic Nitrous and Nitric Acid Esters on the Physiological Functions with Special References to Their Chemical Constitution, Nat'l Inst. Health Bull., 186 1946.

19a. citing:
Cash, J. T. and Dunstan, W. R.; The Physiological Action of the Nitrites of the Paraffin Series, Considered in Connection with Their Chemical Constitution., Phil. Trans. Roy. Soc. London; Series B; 184:505 1894.

19b. citing:
Pulina, B.; Studies on Methemoblobin Formation (XXIV), Effect of Nitrous and Nitric Acid Esters on the Blood, Arch. Expth. Path. Pharmacol., 200:324 1942.

19c. citing:
Lewin, L.; Gifte und Vergiftungen,; G. Stilke; Berlin, 1929.

19d citing:
Keeser, E.; On the Mode of Action of Organic Nitro Compounds, Arch. Expth. Path. Pharmacol. 22;77 1887.

20. Haggard, H. W., Miller, D. P. and Greenberg, L. A.; The Amyl Alcohols and Their Ketones: Their Metabolic Fates and Comparative Toxicities; J. Indust. Hyg. and Toxicol; 27:1, 1945.

20a citing:
Neubauer, O.; Ueber Glykurousäurepaarung Bei Stoffen der Fettrehe; Arch, F. Exper. Path. u. Pharmakol., 46:133 1901.

21. Goodman, L. S. and Gilman, A.; Pharmacological Basis of Therapeutics, MacMillan, 1943.

21a. citing:
Heymans, C., Bouckaert, J. J., and Dartrebande, L.; Sinus Carotidiens et Reflexes Respiratoires; Action Reflexogène Respiratoire et Cardiaque des Nitrites; Compt. Rend. Soc. de Biol.; 106:1279 1931.

22. Butsch, W. L., McGowan, J. M. and Walters, W.; Clinical Studies on Influence of Certain Drugs in Relation to Pressure; S. G. and O., 63:451 1936.

23. Beams, A. J.; Effect of Nitrites on Pain and on Mobility of Gastro-intestinal Tract, Clinical Study, Arch. Ind. Med., 49:270 1932.

24. Stieglitz, E. J.; Arterial Hypertension, Arch. Ind. Med., 46:227 1930.

25. Beams, A. J. and Barlow, O. W.; Effects of Nitrites on Mobility of Gastro-intestinal Tract, Experimental Study, Arch. Ind. Med., 49:276, 1932.

26. Browning, E., Toxicity of Indust. Organic Solvents, His Majesty's Stationary Office, London 1937.

26a. citing:
Dauterbande, L., La Paralysie du Système Vasomoteur par les Solvants Volatils Industriels; Pr. Med. 43:1085, 1935; Rev. Path. Physiol. Trav. 12:273 1936.
Paralysie du Système Vaso-moteur par Les Vapeurs d'acetate d'amyle et de Vernis dit Cellulosique; C. R. Soc. Biol., Paris 119:314 1935

26b. citing:
Flury, F. and Wirth; Zur Toxikologie der Lösungsmittel; Arch. Gewerbepath. Gewerbehyg., 5:34, 1934.

26c. Burger, G. C. S. and Stockmann, R. H.; Uber Urobilinuria als Folge der Einatmung von Organischen Losungsmitteln in Geringer Konzentration; Zbl. Gew. Hyg. 19:31 1932

27. Munch, J. C. and Schwartze, E. W.; Narcotic and Toxic Potency of Aliphatic Alcohols upon Rabbits; J. Lab. Clin. Med., 10:985 1925

28. Lehman, A. J. and Newman, H. W., Comparative Intravenous Toxicity of Some Monohydric Saturated Alcohols; J. Pharmacol. and Exper. Therap; 61:103 1937

29. Macht, D. I., A Toxicological Study of Some Alcohols, with Especial Reference to Isomers; J. Pharmacol., 16:1 1920

30. Underhill, F. P., Toxicology; p.212, 2nd Ed.; Blakiston, Phila., 1928

31. Carozzi, L., Amyl Alcohol, Occupation and Health, International Labor Office, Geneva 1930

32. Ley, E. B. and Vintinner, F. J.; The Toxicology and Prevention of Industrial Disease, p.13, 3rd Ed., U. S. War Dept.; 8th Service Command 1944

33. U. S. Dept Labor; Occupation Hazards and Diagnostic Signs; Bur. Labor Statistics; Bull. #41:33 1942

34. Nelson, K. W., Ege, J. F. Jr., Ross, M., Woodman, L. E. and Silverman, L.; Sensory Response to Certain Industrial Solvent Vapors; J. Ind. Hyg. and Toxicol. 25:282 1943

34z. citing:
Lehmann, K. B. and Flury, F.; Toxicology and Hygiene of Industrial Solvents; Julius Springer, Berlin, 1938

35. Resnick, L.; Eye Hazards in Industry; p. 251, Columbia University Press; New York 1941

36. Treon, J. F.; Industrial Hygiene and Toxicology, Frank A. Patty, Editor. Interscience Pub. Inc., N. Y., p. 864 1949

37. Hamilton, A. and Hardy, H. L.; Industrial Toxicology; 2nd Ed., Paul Howber Inc., N. Y. 1949

37a. citing:
Joachmigolu, G. and Heffter, A.; Amyl Acetate; Vrtljrschz. f. Gerichtl. Med.; 48:161 1914

38. McNally, D.; Toxicology; Ind. Med. Pub. Co., Chicago, 1937

39. Fuchter, T. B.; Fusel Oil Poisoning, with Special Reference to the Copper Reducing Substances Eliminated in the Urine; Am. Med.; 2:210 1901

40. Henderson, Y., and Haggard, H. W.; Noxious Gases and the Principles of Respiration Influencing Their Action., Chemical Catalog Co., N. Y. , 1927, p. 135

41. St. George, A. V.; The Pathology of the Newer Commercial Solvents; Am. J. Clin. Path. 7:69 1937

41a. citing:
Oberarzt, Crecelius; Schadigung Durch Amylacetat.; Klin. Wchsft. 9:452 1930

42. Patty, F. A., Yant, W. P. and Schrenk, H. H.; Acute Response of Guinea Pigs to Vapors of Some New Commercial Organic Compounds; U. S. Pub. Health Report. 51:811 1936

43. Sterner, J. H.; Industrial Hygiene and Toxicology; Frank A. Patty, Editor; Interscience Pub. Inc., N. Y., 1949 p. 904

44. Marshall, W. and Marshall, C. R.; The Action of Nitrites on Blood; J. Biol. Chem. 158:187 1945

45. Patty, F. A.; Industrial Hygiene and Toxicology, Interscience Pub. Inc., N. Y. 1949 pp. 605-609

46. Krantz, J. C. Jr., Carr, C. J., Forman, S. E. and Cone, N.; Alkyl Nitrites (VI), J. Pharmacol and Exper. Therap. 70:323 1940

47. Whitmore, F. C.; Organic Chemistry, Van Norstrand Co., N. Y. 1937 p. 490

48. Rath, M. and Krantz, J. C. Jr.; Nitrites (VIII, IX); J. Pharmacol and Exper. Therap., 76:27, 33 1942

48a. citing:
Stephanow; Arch. f. Exptl. Path. u. Pharmakol; 47:4;; 1902

49. Sollmann, T.; Manual of Pharmacology; W. B. Saunders Co., Phila. 7th Ed. 1948

50. Bernheim, F. and Dixon, M.; The Reduction of Nitrates in Animal Tissues; Biochem. J. 22:125 1928

51. Mitchell, H. H., Schonle, H. A. and Grindley, H. S.; Origin of the Nitrates in the Urine; J. Biol. Chem. 24:461 1916

52. Macht, D. I.; Action of the Nitrates andNitrites; J. Pharmacol. and Exper. Therap. 9:427 1916

52a. citing;
Abderhalden and Hirsch; Z. Physiol. Chim. lxxxiv, 189 1913

53. Bradbury, J. B.; Some New Vaso-dilators; Lancet, p. 1205, Nov. 16, 1895

54. Krantz, J. C. Jr., Carr, C. J., and Ellis, F. W.; Alkyl Nitrites (III,IV); J. Pharmacol and Exper. Therap. 67:187, 191 1939

55. Krantz, J. C. Jr., Carr, C. J. and Forman, S. E.; Alkyl Nitrites (I, II); J. Pharmacol and Exper. Therap. 64:298, 302 1938

56. Krantz, J. C., Carr, C. J. and Forman, S. E.; Alkyl Nitrites (V); Proc. Soc. Exp. Biol. Med. 42:472 1939

57. Miller, J. L.; Hypertension and Value of Various Methods for its Reduction, J. A. M. A., 54:1666 1910

58. Ebright, G. E.; The Effects of Nitroglycerine on Those Engaged in its Manufacture; J. A. M. A. 62:201 1914

59. Heitz, J.; Cardiovascular Status in Nitroglycerine Workers, Archives des Maladies du Coeur; 17:578 1924: Abstract; J. A. M. A., 83:1279 1924

60. Oltman, T. V. and Crandall, L. A. Jr.; The Acute Toxicity of Glyceryl Trinitrate and Sodium Nitrite in Rabbits; J. Pharmacol. and Exper. Therap. 41:121 1931

61. Weiss, S. and Ellis, L. B.; Influence of Sodium Nitrite on Cardiovascular System and on Renal Health in Arterial Hypertension and in Renal Disease; Arch. Int. Med. 52:105 1933

62. Hirschfelder, A. D.; Effects of Drugs upon the Vessels of the Pia Mater and Retina; J. Pharmacol. and Exper. Therap. 6:597 1914

63. Wilkins, R. W., Haynes, F. W. and Weiss, S.; Role of Venous System in Circulatory Collapse Induced by Sodium Nitrite; J. Clin. Invest., 16:85 1937

64. Wilkins, R. W., Weiss, S. and Haynes, F. W.; Effect of Epinephrine in Circulatory Collapse Induced by Sodium Nitrite; J. Clin. Invest. 17:41 1938

65. Pilcher, J. D. and Sollmann, T.; Studies on the Vasomotor Centre Effects of the Nitrite Group; J. Pharmacol. and Exper. Therap. 6:323 1915

66. Weiss, S. and Wilkins, R. W.; Syncope, Collapse and Shock, Their Medical Significance and Treatment; M. Clin. N. Am. 21:481 1937

67. Prodger, S. H. and Ayman, D.; Harmful Effects of Nitroglycerin; Am. J. Med. Sci. 184:480 1932

68. Stewart, D. D.; Tolerance to Nitroglycerine; J. A. M. A. 44:1678 1905

69. Crandall, L. A., Leake, C. D. and Loevenhart, A. S. and Muehlberger, C. W.; Acquired Tolerance to and Cross Tolerance Between the Nitrous and Nitric Acid Esters of Sodium Nitrite in Man; J. Pharmacol and Exper. Therap. 41:103 1931

70. Myers, H. B. and Austin, V. T.; Nitrite Toleration, J. Pharmacol and Exper. Therap. 36:227 1929

71. Harrow, B.; Textbook of Biochemistry, Saunders Pub. Co. 1943

71a. citing:
Gangee, A. ; Phil. Tr. Roy. Soc., London, 158:589 1868

72. Marcus, H. and Jaffe, J. R.; Nitrate Methemoglobinemia; New Eng. Jour. Med. 240:599 1949

73. Cornblath, M. and Hartmann, A. F.; Methemoglobinemia in Young Infants; J. Pediatrics 33:421 1948

74. Walton, G.; Survey of Literature Relating to Infant Methemoglobinemia Due to Nitrate Contaminated Water, Am. J. Pub. Health 41:986 1951

75. Neill, J. M.; Studies on the Oxidation-reduction of Hemoglobin and Methemoglobin, J. Exp. Med. 41:299 1925

76. Bodansky, O.; Methemoglobinemia and Methemoglobin-Producing Compounds, Pharmacol Reviews, 3:144 1951

76a. citing:
Heubner, W., Kiese, M., Stuhlmann, M. and Schwartzkopff-Jung, W.; Arch. F. Exper. Path. u. Pharmakol. 204:313 1947

76b. citing:
Kiese, M.; Die Reduktion des Hämiglobins; Biochem. Ztschr. 316:264 1943

76c. citing:
Von Issekutz, B. V.; Studien über Methämoglobinbildung; "Normales" Methämoglobin bei Katzen; Arch. F. Exper. Path. u. Pharmakol. 193:551 1939

76d. citing:
Petersen, C.; Studien über Methämoglobindung, othoroaminophenol und nitrosobenzol, Arch. F. Exper. Path. u. Pharmakol. 198:675 1941

77. Spicer, S. S. and Neal, P. A.; Effect of Hypoxia on in Vivo Formation of Methemoglobin by Aniline and nitrite; J. Pharmacol. and Exper. Therap. 95:438 1949

78. Paul, W. D. and Kemp, C. R.; Methemoglobin: A Normal Constituent of Blood, Proc. Soc. Exper. Biol. and Med. 56:55 1944

79. Cox, W. W. and Wendell, W. B.; Normal Rate of Reduction of Methemoglobin in Dogs, J. Biol. Chem. 143:331 1942

80. Hamblin, D. O.; Industrial Hygiene and Toxicology, Frank A. Patty, editor, Interscience Pub. Inc., N. Y. 1949, p.987

81. Clark, B. B., Van Loon, E. J. and Morrissey, J.; Acute Experimental Aniline Intoxication, J. Ind. Hyg. Toxicol. 25:1 1943

82. Lester, D.; Formation of Methemoglobin, J. Pharmacol. 77:154, 160 1943

84. Spicer, S. S.; Species Differneces in Susceptibility to Methemoglobin Formation, J. Pharmacol. and Exper. Therap. 99:185 1950

85. Hamblin, D. O. and Mangelsdorff, A. F.; Methemoglobinemia and its Measurement, J. Ind. Hyg. Toxicol. 20:523 1938

86. Darling, R. C. andRoughton, F. J. W.; The Effect of Methemoglobin on the Equilibrium between Oxygen and Hemoglobin, Am. J. Physiol. 137:56 1942

87. Greenberg, L. A., Lester, D. and Haggard, H. W.; The Reaction of Hemoglobin with Nitrite, J. Biol. Bhem. 151:665

88. Clark, B. B., Van Loon, E. J. and Adams, W. L.; Respiratory and Circulatory Responses to Acute Methemoglobinemia Produced by Aniline, Am. J. Physiol.; 139:64 1943

89. Vandenbelt, J. M., Pfeiffer, C., Kaiser, M. and Sibert, M.; Methemoglobinemia after Administration of P-amine-acetophenone and P-amino-propiophenone, J. Pharmacol. and Exper. Therap. 80:31 1944

90. Bodansky, O. and Gutmann, H.; Treatment of Methemoglobinemia, J. Pharmacol. and Exper. Therap. 90:46 1947

91. Finch, C. A.; Medical Progress, Methemoglobinemia and Sulfhemoglobinemia, New Eng. J. Med. 239:470 1948

92. Treon, J. F., Deichmann, W. B., Sigmon, H. E., Wright, H., Witherup, S. O., Heyroth, F. F., Kitzmiller, K. V. and Keenan, C.; The Toxic Properties of Xylidine and Mono-methylaniline, J. Indust. Hyg. and Toxicol. 31:1 1949

93. Buckell, M. and Richardson, J. D.; The Significance of Heinz Bodies in the Erythrocyte, British J. Ind. Med. 7:131 1950

94. Hughes, J. P.; The Significance of Erythrocytic Inclusion Bodies; Thesis I. M. D., Kettering Laboratory, University of Cincinnati, 1952

95. Gajdos, A. and Tiprez, G.; Biological Significance of Heinz Bodies, C. A. 40:5123 (4) 1946

96. Editorial: Inclusion Bodies of the Erythrocyte; British Medical Journal, p. 431 August 28, 1948

97. Fertman, M. H. and Doan, O. A.; Irreversible Toxic "Inclusion Body" Anemia; a Clinical and Experimental Study, Proc. Central Soc. Clinical Research 18:66 1945

98. Webster, S. H., Liljegren, E. J. and Zimmer, D. J.; Heinz Body Formation by Certain Chemical Agents, J. Pharmacol. and Exper. Therap. 95:201 Reprint 1949

TABLES AND FIGURES

Table #1

PHYSICAL PROPERTIES OF THE IGNITION IMPROVER$_2$

Molecular formula	$C_5H_{11}NO_3$
Molecular weight	133.146
Density: d_4^{20} (68 F)	0.998
A. P. I. Gravity (60 F)	9.5
Weight per gallon, lbs.	8.3
Boiling range (extrapolated to 760 mm Hg)	302-311
Pour point, F	-139
Coefficient of expansion, per °F at 68 F	0.00056
Solubility in 100 parts of: Water at 68 F	0.34
Diesel fuel	miscible
Index of refraction, n_D^{20}	1.415
Flash point, F Open cup	130
Closed cup	107
Viscosity, centistokes at 100 F	0.774
Odor	Ethereal (Non-offensive)
Color	Water white to light straw

Table #2

PER CENT OF WEIGHT LOSS IN RELATION TO DURATION OF EXPOSURE AND CONCENTRATION OF AMYL NITRATE

Exposure Time in Hours	Concentration mg/l	Max. Wt. Loss as % of Initial Wt.
7 - 21	16	13.6
7 - 21	9 - 11	12.6
3.5	19	9
7	9.6	8
70	3.2	7.8
7	19.9	7.6
140	1.4	4

Table #3

SIGNS OBSERVED DURING AMYL NITRATE INHALATION IN RELATION TO COMPONENT RADICALS OF AMYL NITRATE

Signs Observed	Amyl Radical		Nitrate Radical	
	Alcohol	Acetate	Acids + Oxides	Nitrite
Labored Resp.	+			
Liver Degen.	+			
Kidney Degen.	+ -			
Tremors	+	+		
Ataxia(vertigo)	+	+		
C.N.S. Degen.	+ -	+		
Weight Loss		+		
Lachrymation	+	+	+	
Salivation	+	+	+	
Lung Edema		+	+	
Lung Hyperemia		+	+	
Headache	+			+
Convulsions	+		+ -	+
Coma	+		+	
Tolerance		+		+
Methemoglobin	+ -			+
Transitory Miosis				+
Mydriasis				+
Hyperemia (skin)				+
Cyanosis				+
Deep Rapid Resp.				+
Full Pulse				+
Dec. Systol. B/P				+
Dec. Body Temp.				+

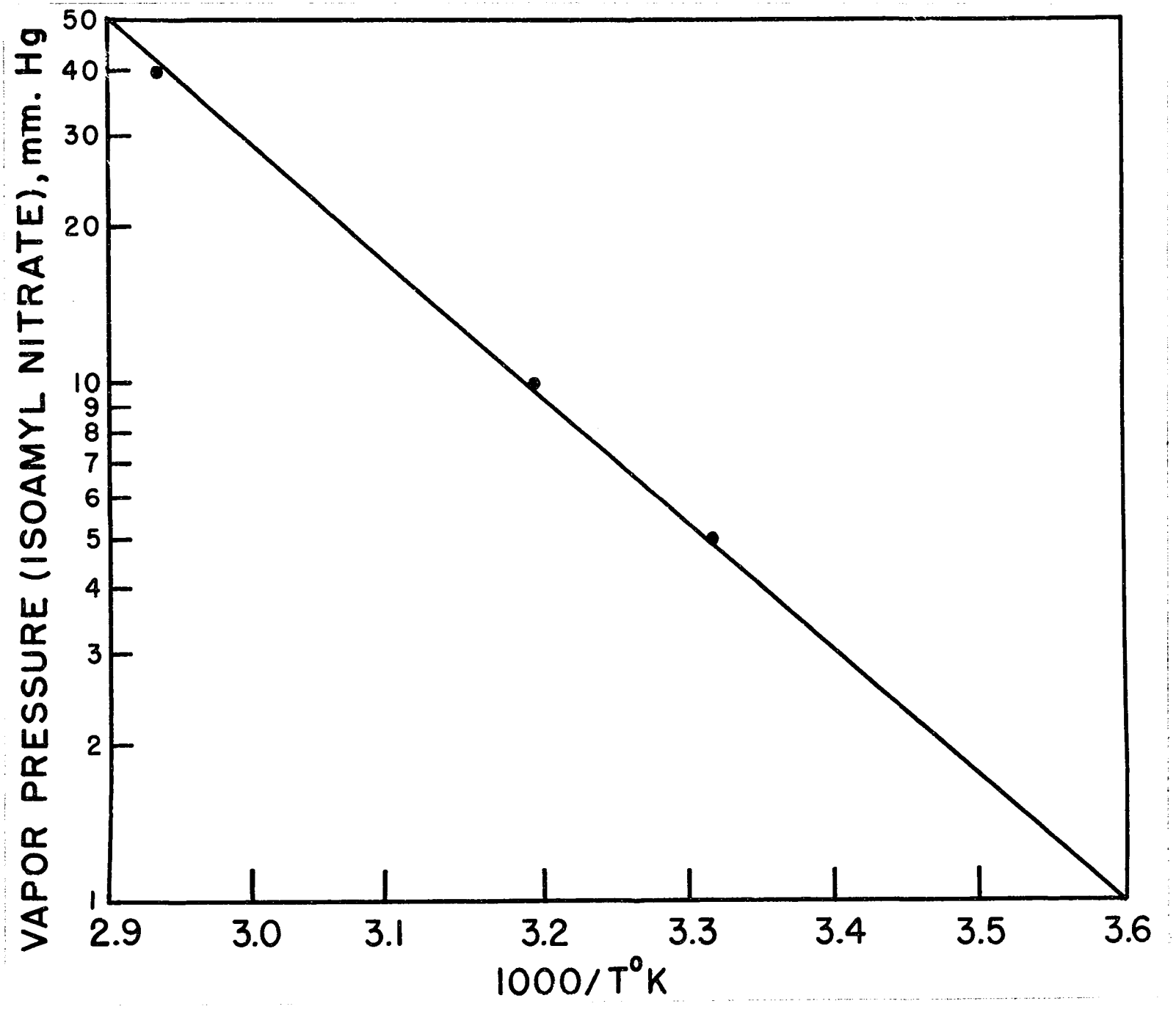

FIGURE 1

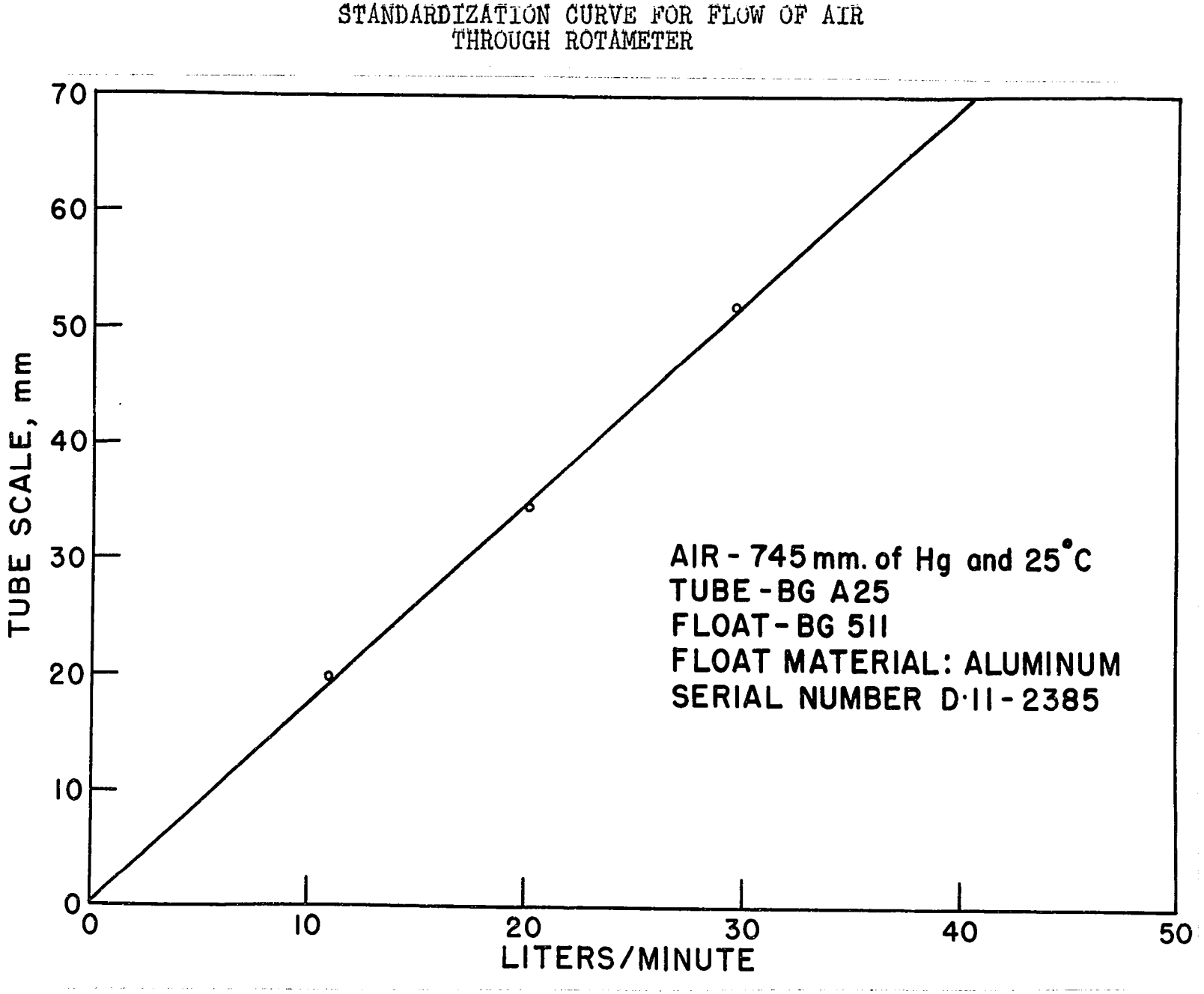

FIGURE 2

THE STANDARDIZATION CURVE FOR THE DETERMINATION OF AMYL NITRATE

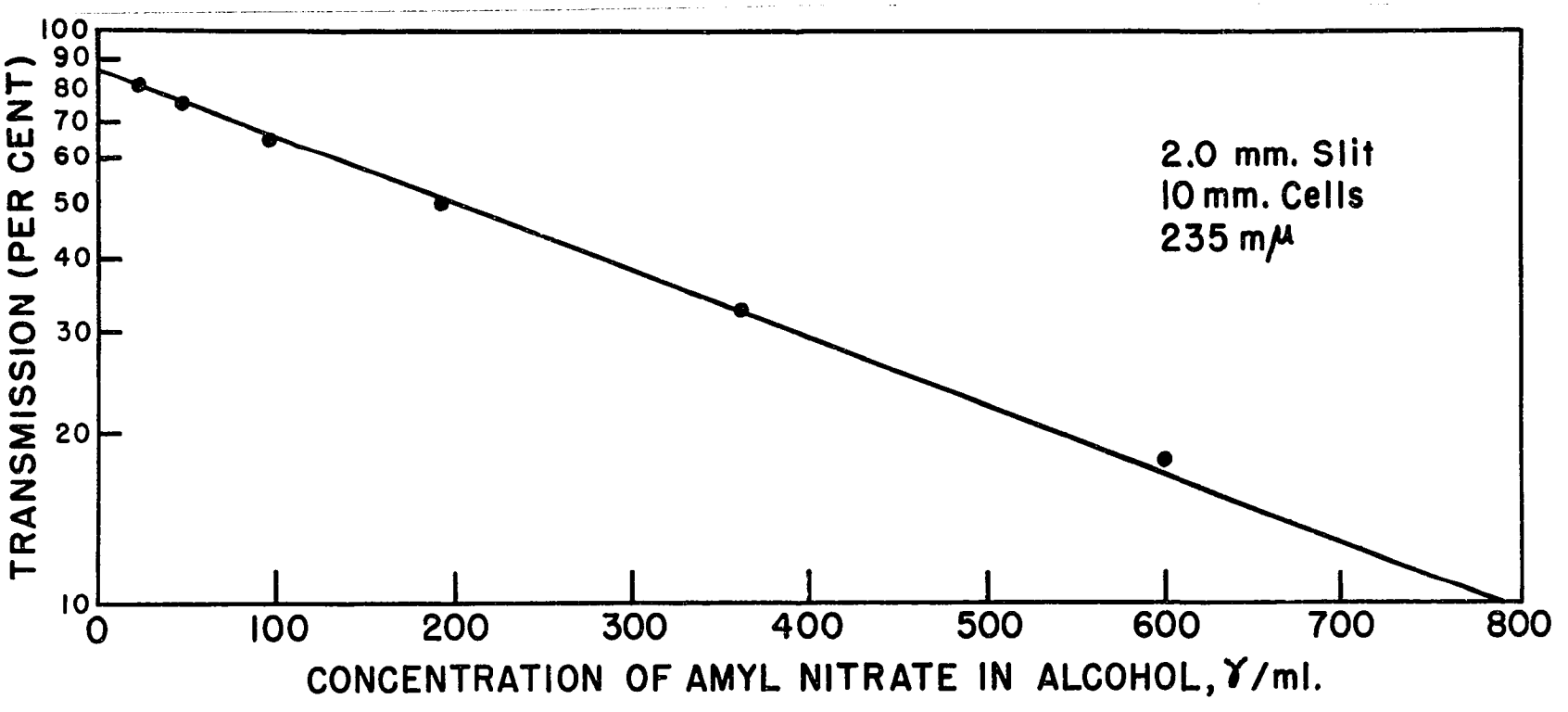

FIGURE 3

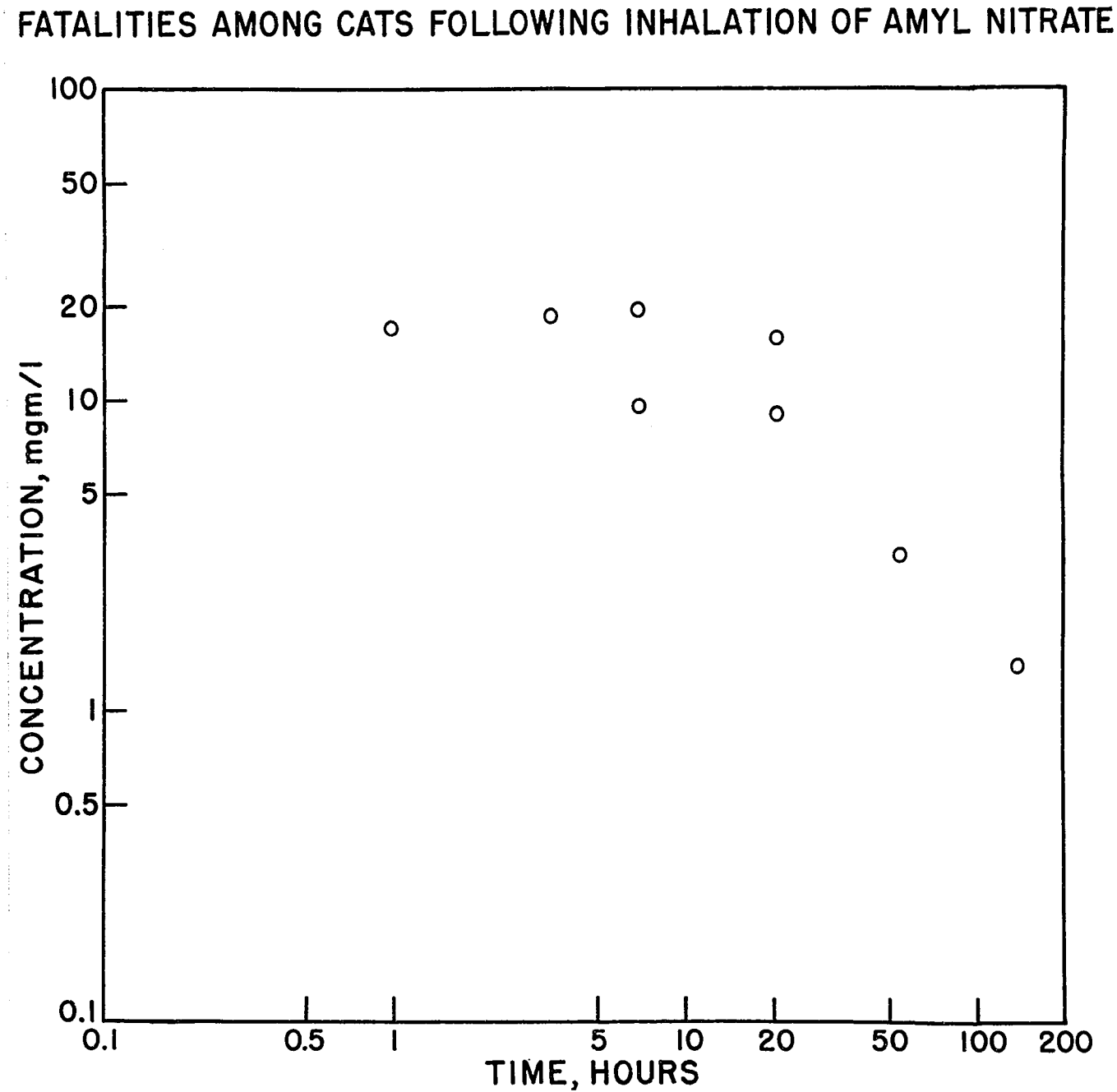

FIGURE 4

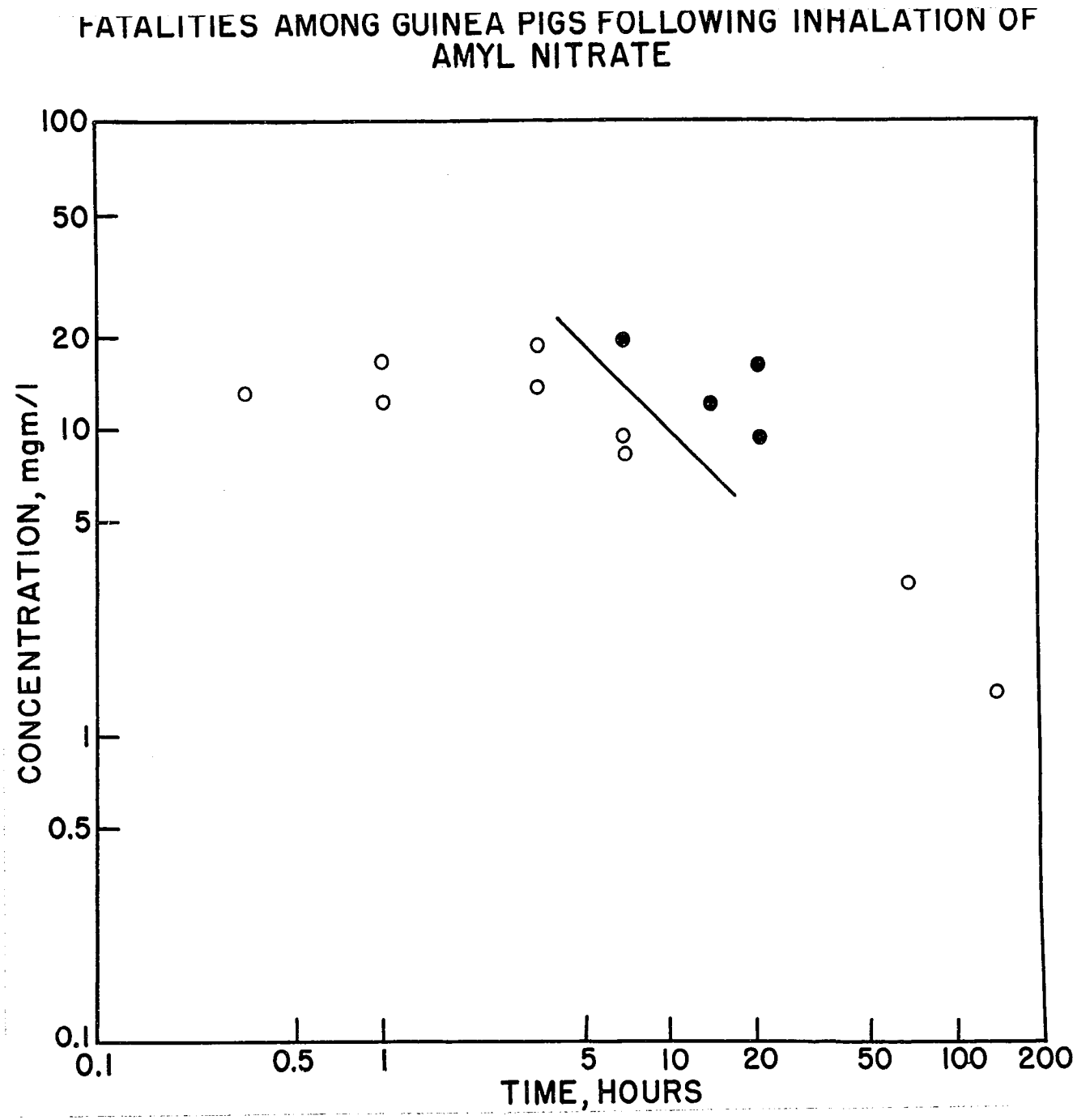

FIGURE 5

FATALITIES AMONG RABBITS FOLLOWING INHALATION OF AMYL NITRATE

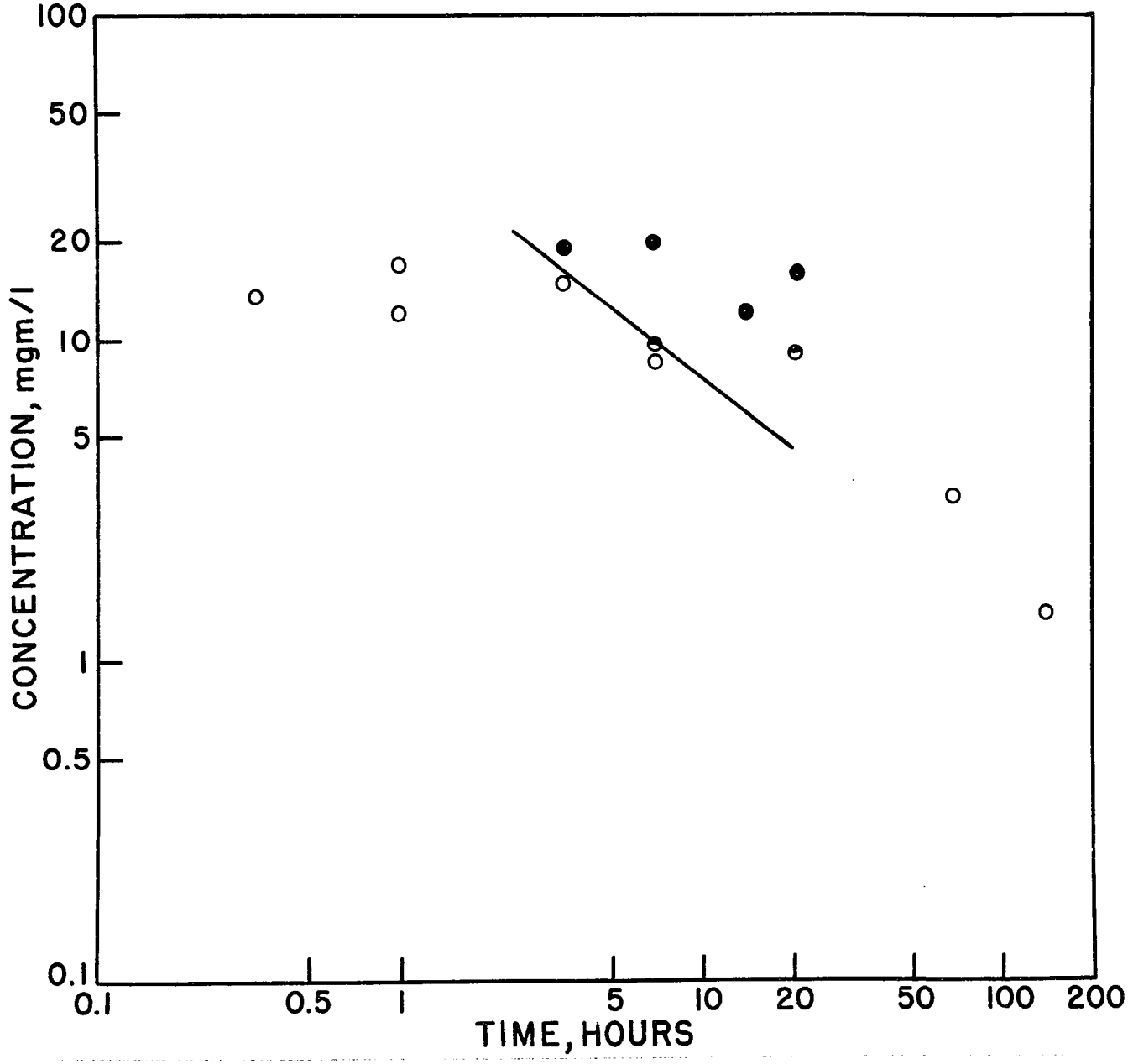

FIGURE 6

FATALITIES AMONG RATS FOLLOWING INHALATION OF AMYL NITRATE

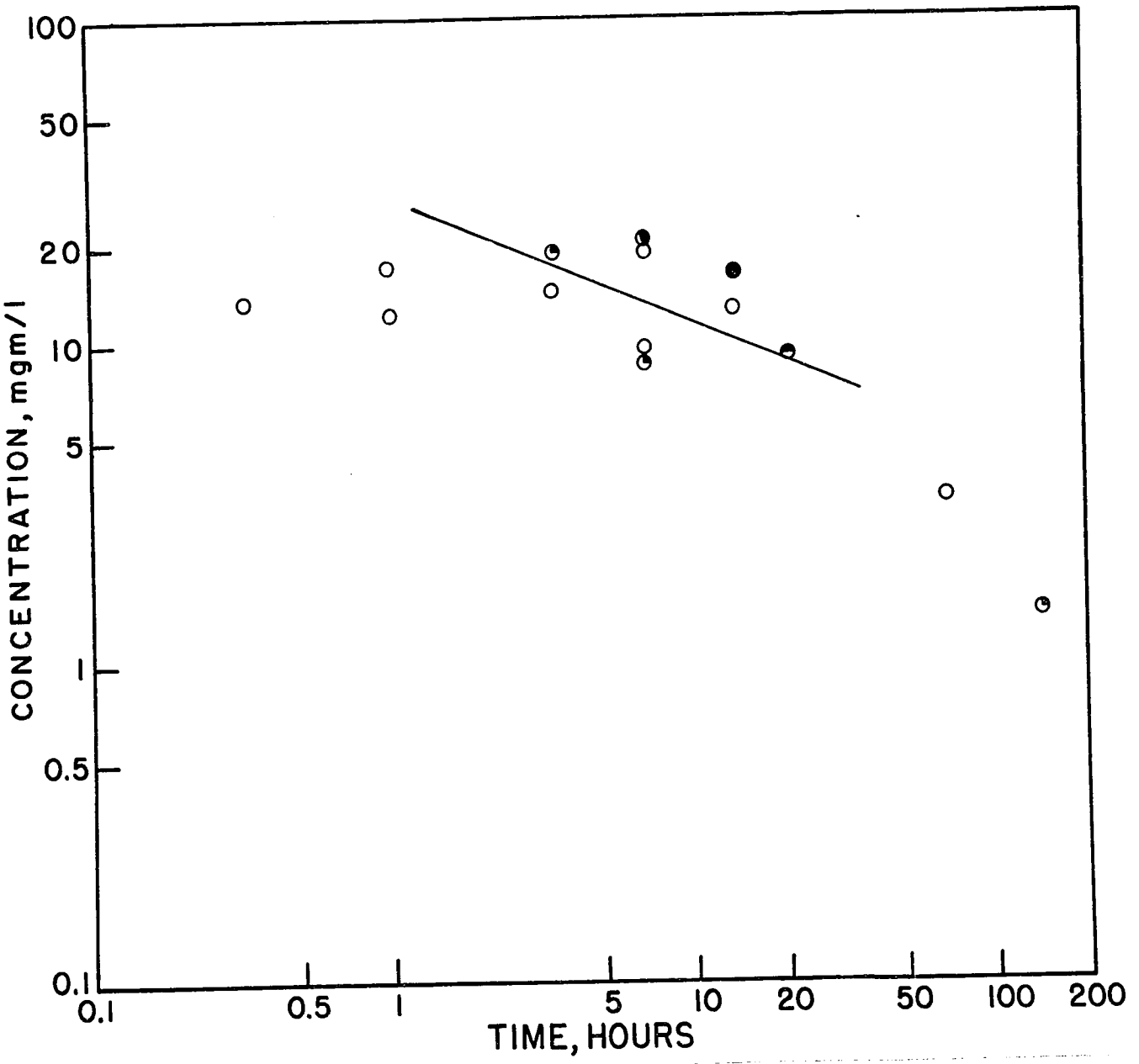

FIGURE 7

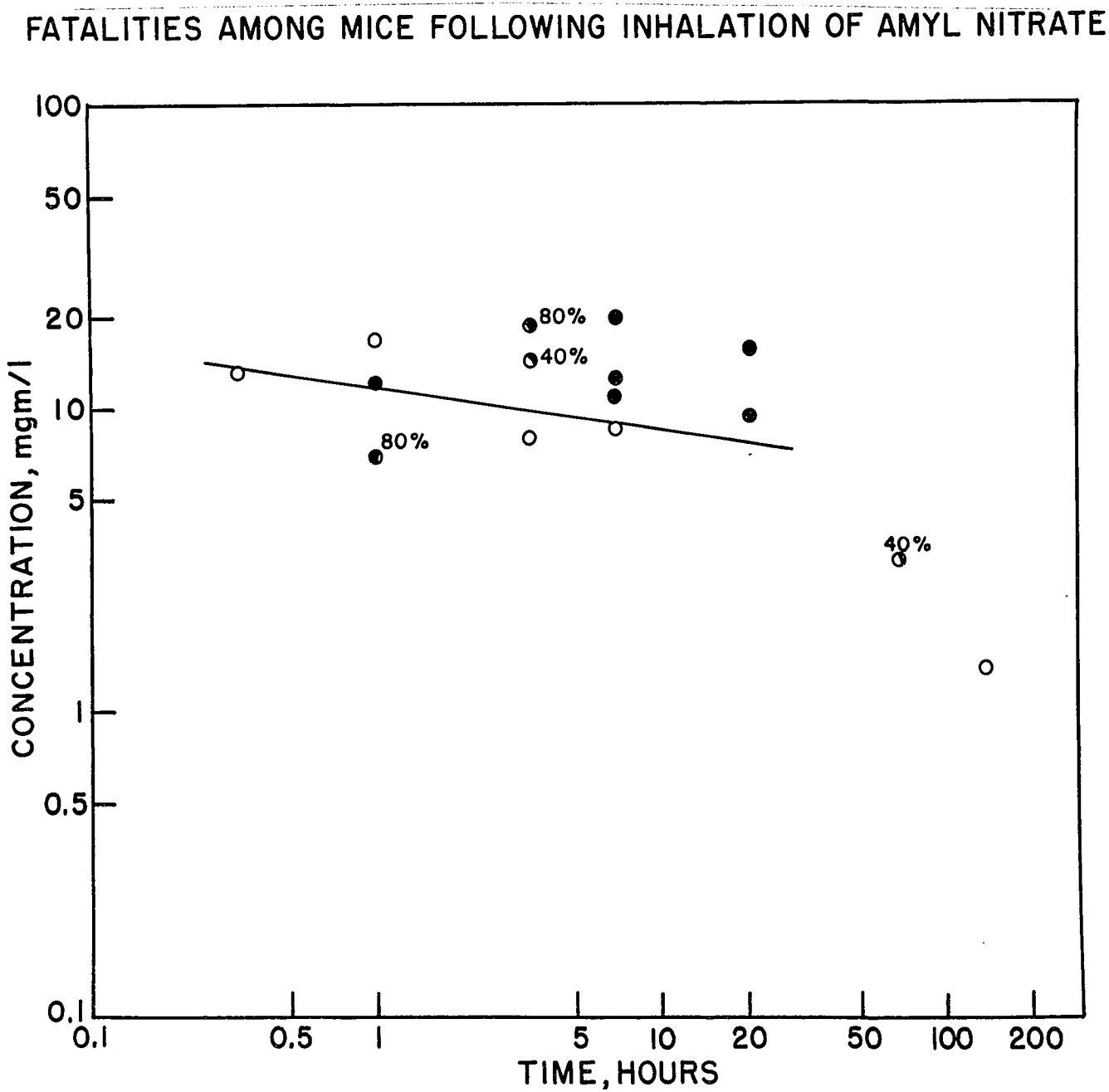

FIGURE 8

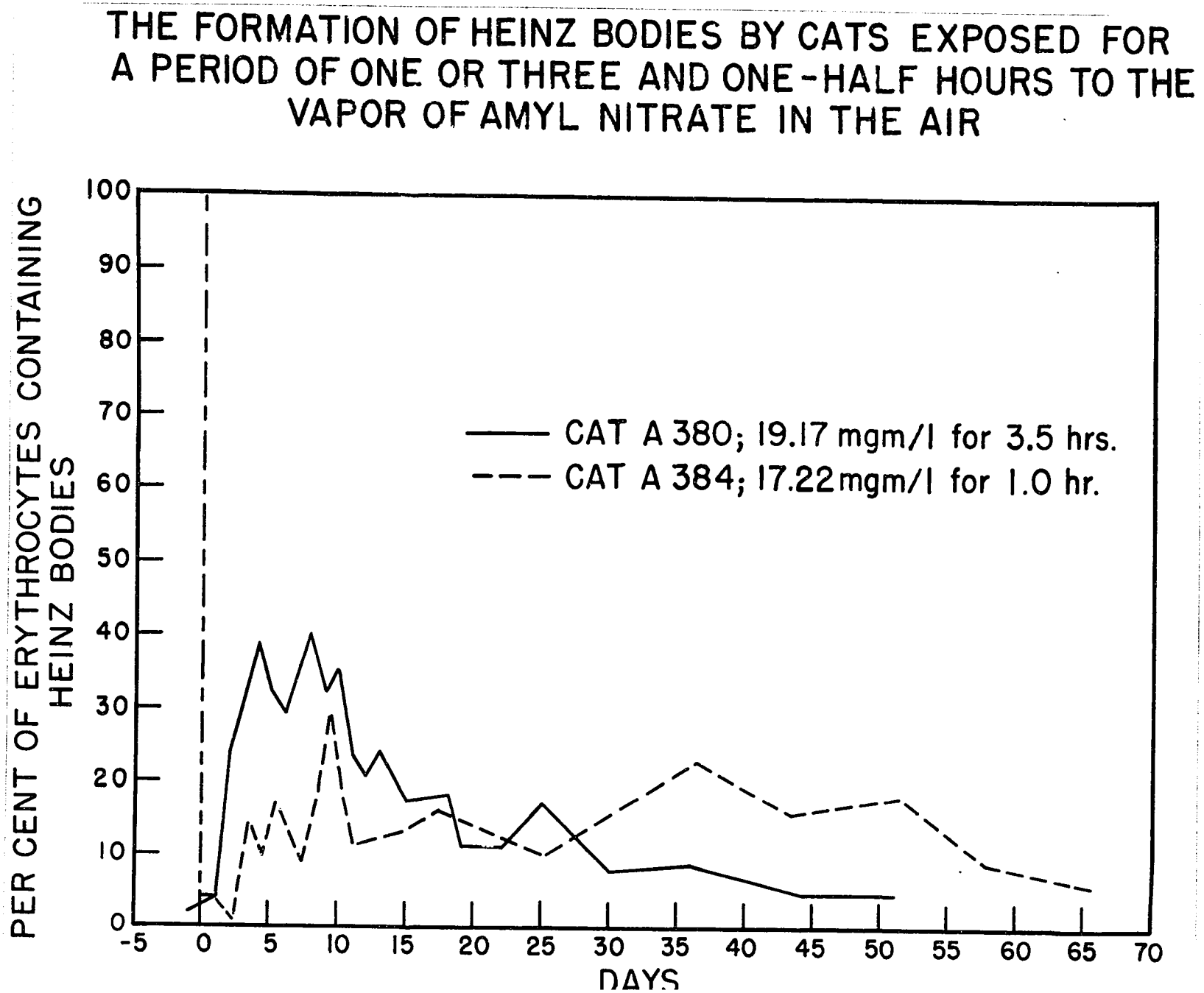

FIGURE 9

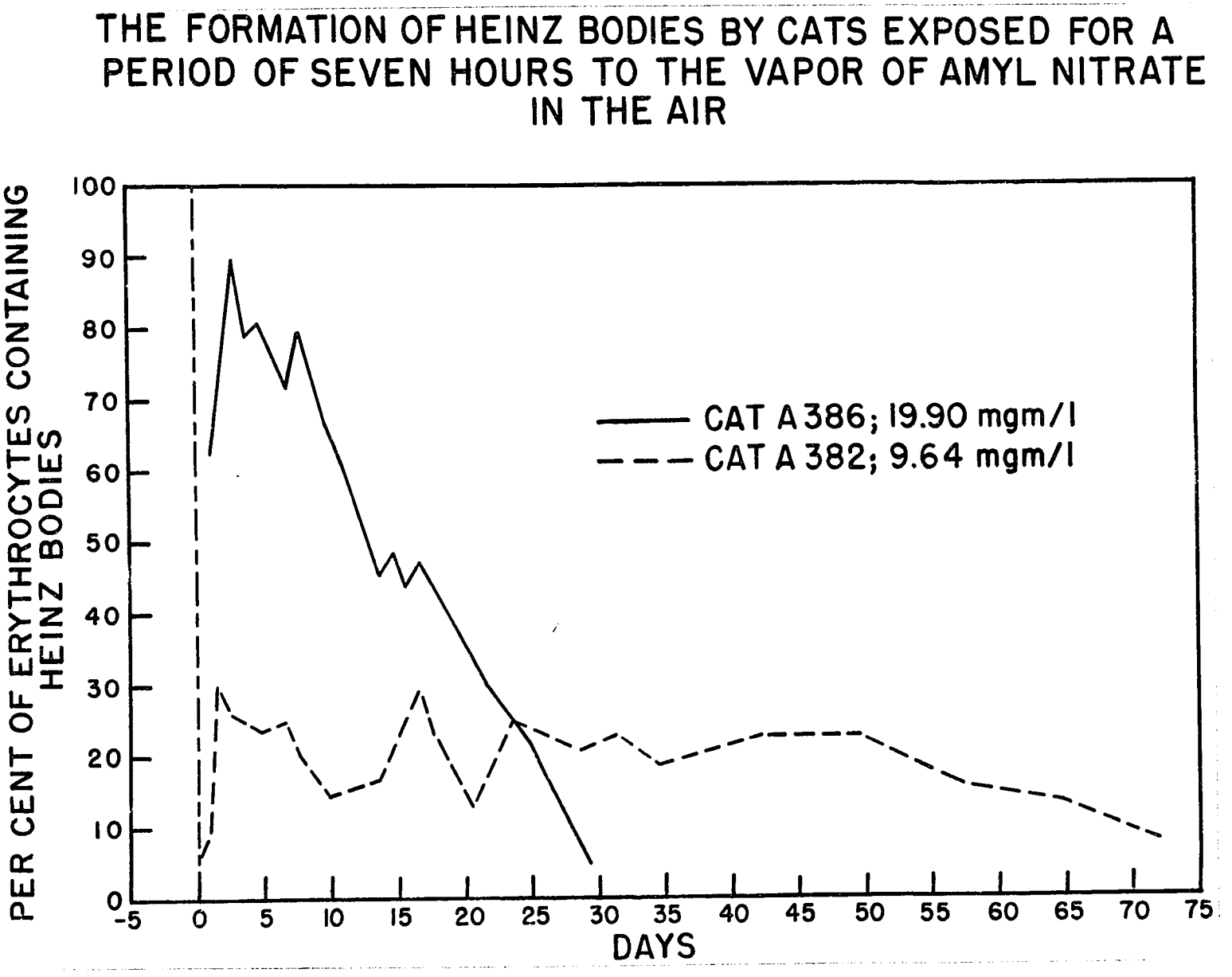

FIGURE 10

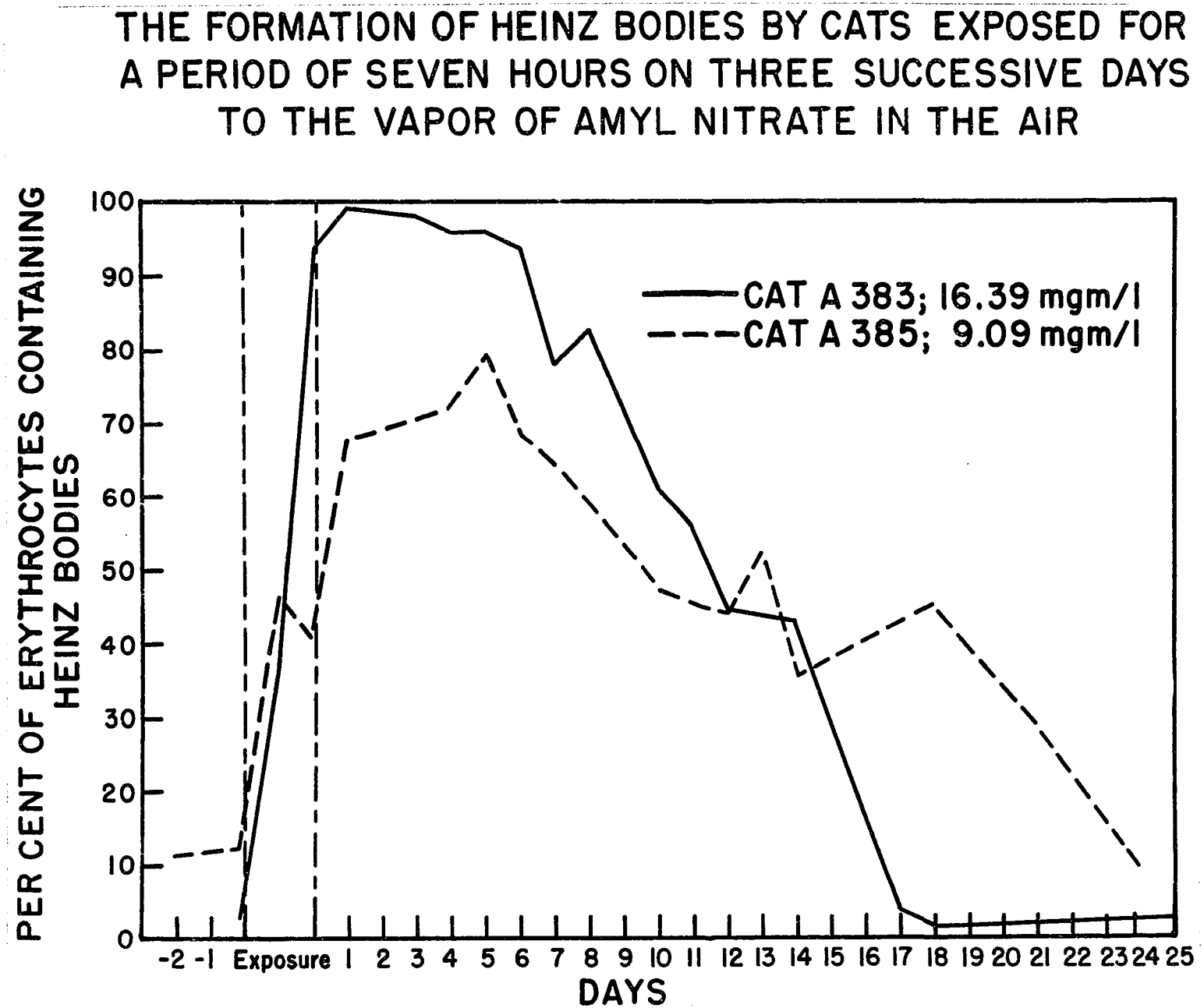

FIGURE 11

Average Growth of Groups of Rabbits During a Period in Which The Animals Were Repeatedly Subjected to Skin Contact With Cetane Improver (DB-26) or Fuels Containing It. (Animals Were Exposed for 4 Hours on Each of 5 Days)

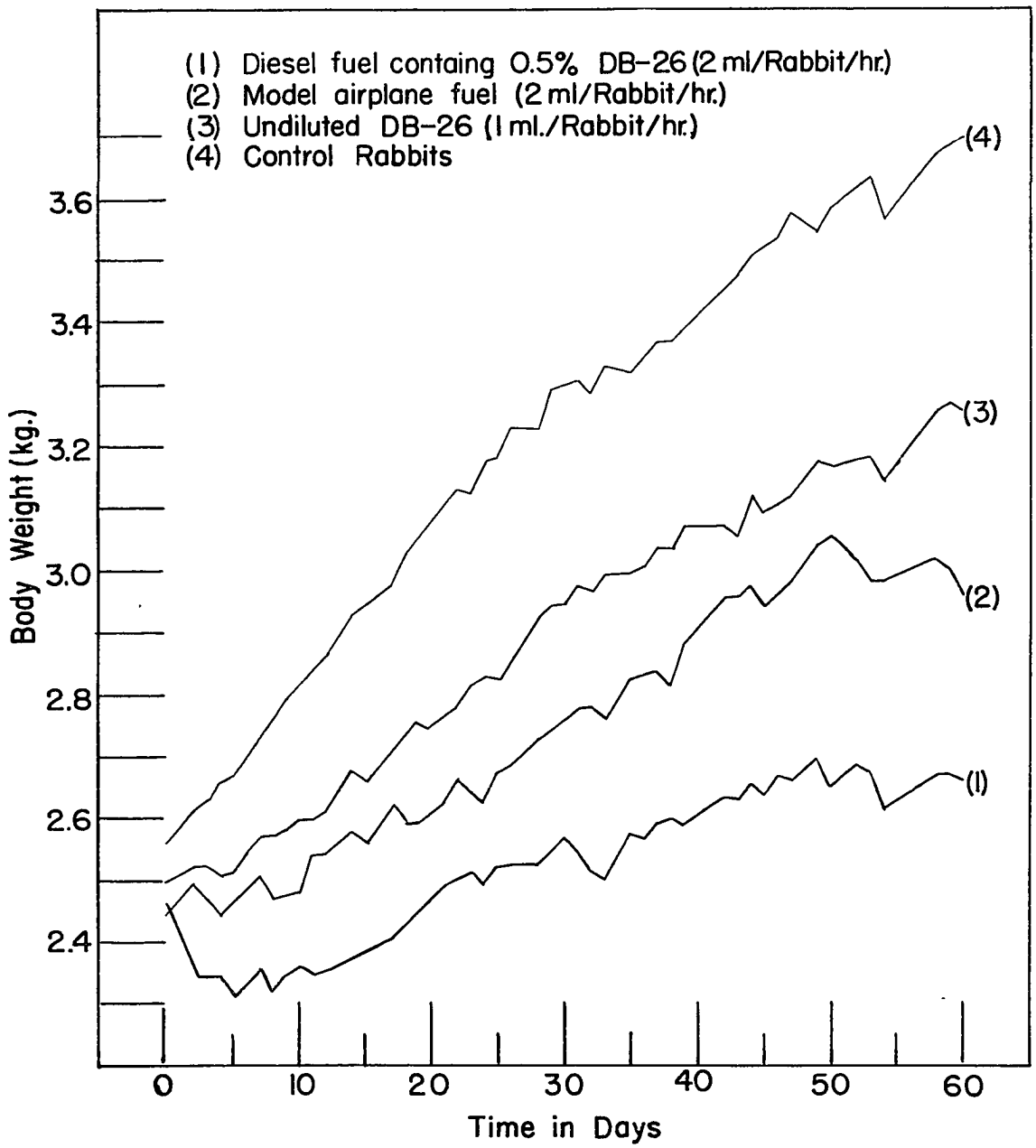

FIGURE 12

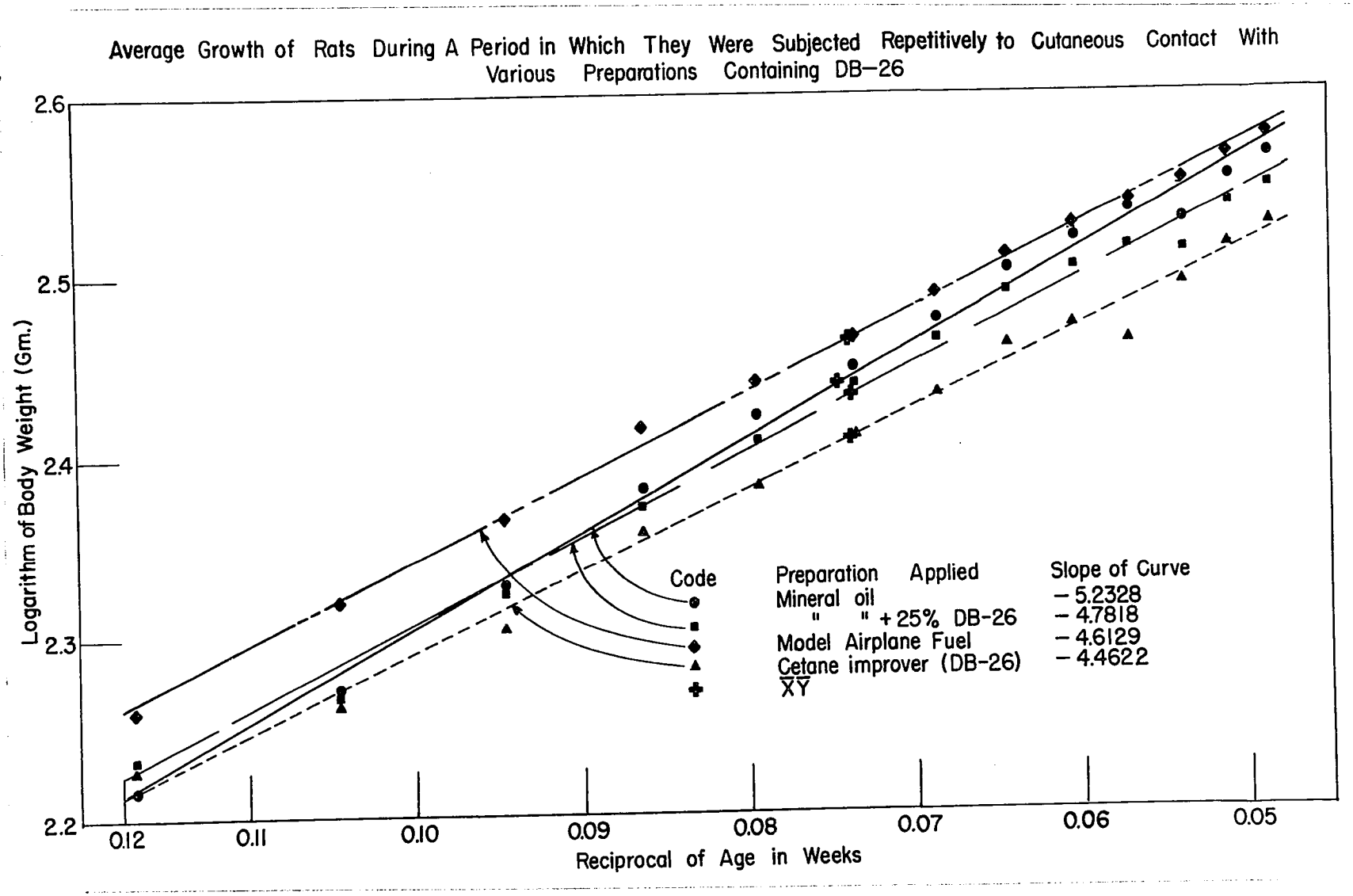

FIGURE 14

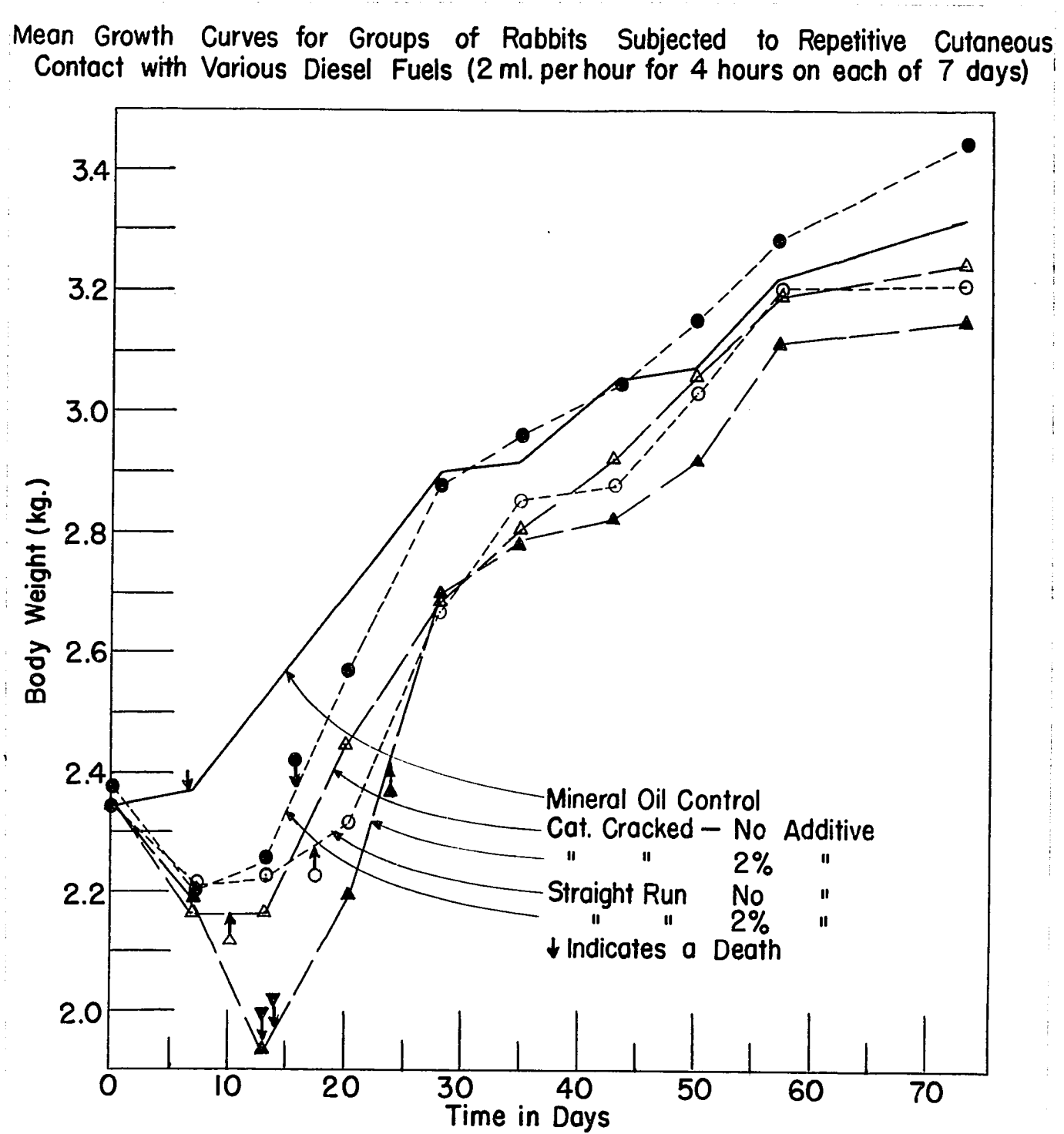

FIGURE 15