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ABSTRACT

All fires occurring in air produce carbon dioxide (CO_2). Fires involving nitrogen-containing products will also generate nitrogen dioxide (NO_2), a pulmonary irritant. In Fischer 344 male rats, the LC_{50} (30 minute exposure plus 14 day post-exposure observation period) for NO_2 was 200 ppm (with 95% confidence limits ranging from 190-210 ppm); the LC_{50} for CO_2 was 47%[3] (with 95% confidence limits of 43 to 51%); whereas, the LC_{50} for NO_2 in the presence of 5% CO_2 was 90 ppm (with 95% confidence limits ranging from 70-120 ppm). Exposure to NO_2 increased the methemoglobin (MetHb) levels in the arterial blood. At the end of the 30 minute exposures, the MetHb levels were 2 - 3 times higher in the animals exposed to the combination of NO_2 (200 ppm) and CO_2 (5%) than in those exposed to NO_2 only. Deaths from NO_2 were all post-exposure and occurred earlier in the presence of NO_2 plus 5% CO_2 than in the absence of the CO_2. The time of death was concentration-dependent when both gases were present. At death, evidence of hemorrhage and extensive edema was observed in the lungs. The mean lung wet weight/body weight ratio from rats exposed to 200 ppm NO_2 with and without 5% CO_2 was 3-4 times that of non-exposed rats. More edema was noted with NO_2 and CO_2 than with NO_2 alone.

Key words: carbon dioxide, fire gases, inhalation, LC_{50}, lung edema, methemoglobin, nitrogen dioxide, rats, synergism, toxicology.

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[1]This paper is a contribution of the National Institute of Standards and Technology and is not subject to copyright.

[2]This material was presented in part at the annual meeting of the Society of Toxicology, Atlanta, GA, February, 1989.

[3]1% is equal to 10,000 ppm.

1. INTRODUCTION

The National Institute of Standards and Technology (NIST) is developing a model to predict the toxic interactions of the primary gases generated in fires [1][4]. This model (called the N-Gas Model) is designed to determine if the toxicity of a material's combustion products can be explained by the toxic interactions of the primary gases or if minor or more obscure gases need to be considered. At the present time, the model is based on four major fire gases - carbon monoxide (CO), carbon dioxide (CO_2), hydrogen cyanide (HCN) and low oxygen (O_2) [1-5]. The N-Gas Model is being used in a screening test for toxic potency and unusual toxicity and also in the development of a more extensive computer model of fire hazard.

The next gas that we are incorporating in the N-Gas Model is NO_2, a highly toxic gas. The generation of NO_2 might be expected in fires due to atmospheric nitrogen fixation (material independent) or the oxidation of nitrogen from nitrogen-containing products. Nitrogen oxides (NO_x)[5] have been implicated in deaths and injuries from fires. The most famous example was the 1929 Cleveland Clinic fire in which 50,000 nitrocellulose x-ray films ignited [6]. Ninety-seven people died during this fire, 26 survived at least 2 hours but not more than one month, and 92 sustained non-fatal injuries. It was postulated that the post-exposure deaths and injuries were primarily due to the poisonous effects of NO_x.

In order to include NO_2 as the fifth fire gas in the N-Gas model, the acute inhalation toxicity (as determined by lethal concentrations to rats) of NO_2 as an individual gas as well as in combination with the other major fire gases (e.g., CO_2, CO, HCN, and reduced O_2) needs to be examined. Since previous work at NIST has shown the synergistic effects of CO_2 when combined with any or all of the other three gases [2-5], it was decided to investigate the lethal and physiological effects in rats of NO_2 alone and in combination with 5% CO_2 (in each case mixed with air). The results of this investigation, which has been partially funded by The Society of the Plastics Industry, Inc., are presented here.

[4]Numbers in brackets refer to the references at the end of this paper.

[5]NO_x refers to those cases where both NO and NO_2 were probably generated and were measured as one entity by the experimental technique used.

2. MATERIALS AND METHODS

2.1 Gases

Gas cylinders of NO_2 in air were obtained from the Gas and Particulate Science Division, NIST, and from a commercial supplier. The concentration of the calibration tank was 248 ppm, whereas, the source tanks were 2450, 2500 and 5048 ppm. According to the Gas and Particulate Science Division, the NO_2 tanks (prepared as standard reference materials) contained 3 to 5% nitric acid (HNO_3) and no detectable nitric oxide (NO) (the minimum detection level of NO was 0.3%). Information from the commercial supplier stated that their tanks contained 5 ppm of water. Since the balance gas in these tanks was air, any NO would be expected to be oxidized to NO_2.

The CO_2 utilized in this study was commercially supplied in gas cylinders containing 99.5% CO_2 (Technical grade). The specifications of the supplier stated that these CO_2 cylinders have a very low moisture content (dew point: -40°F) and may contain CO (<10 ppm), hydrogen sulfide (<1 ppm), NO_x (<5 ppm), total hydrocarbons (<20 ppm), phosphene (<0.3 ppm), and sulfur dioxide (<5 ppm). The balance is air.

2.2 Animals

Fischer 344 male rats, weighing 200-300 grams, were obtained from Taconic Farms (Germantown, NY)[6]. They were allowed to acclimate to laboratory conditions for at least 7 days prior to experimentation. Animal care and maintenance were performed in accordance with the procedures outlined in the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals." Each rat was housed individually in suspended stainless steel cages and provided with food (Ralston Purina Rat Chow 5012) and water ad libitum. Twelve hours of fluorescent lighting per day were provided using an automatic timer.

[6]Certain commercial equipment, instruments, materials or companies are identified in this paper in order to specify the experimental procedure adequately. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the equipment or material identified is necessarily the best available for the purpose.

2.3 Animal Exposure System

All exposures were conducted using the chemical analysis system and the animal exposure system that was designed for the NBS Toxicity Test Method [7]. The exposure chamber and a schematic of the experimental arrangement are illustrated in Figures 1 and 2, respectively. The NBS Toxicity Test Method consists of a closed design in which all the gases and smoke (except NO_2, see Section 2.4) are kept in a 200 liter rectangular chamber for the duration of the exposure.

Before the animal exposures, steady-state atmospheric conditions, were achieved by adding various gases to the chamber until the given concentrations (monitored as described below) were reached. A fan in the chamber was used to ensure adequate mixing of the gases prior to and during the animal exposures. The animals were exposed for 30 minutes. During these exposures, the concentration of NO_2 decreased approximately 8% which was probably due to wall and animal adsorption, while the concentration of CO_2 increased about 5% due to the respiration of the animals. Low levels of NO_2 were added to the chamber during the exposures to help maintain constant atmospheric concentrations.

2.4 Gas Analysis

The concentration of NO_2 was measured continuously by a chemiluminescent NO_x analyzer Model 14A (Thermo Electron Instruments, Hopkinton, MA). The analyzer is equipped with a stainless steel converter (set at 700-800°C) which catalytically reduces NO_2 to NO before reacting with ozone. The reaction of NO with ozone produces electronically excited NO_2 which relaxes, emitting light that is detected by the instrument. In order to minimize the total gas volume removed during the animal exposure experiments, the chemiluminescent analyzer was modified so that the sample flow was reduced to about 25 ml/min. The calculated NO_2 concentrations are accurate to within 5%. The interference from NO present in combustion atmospheres would not be a problem under the present conditions in which only NO_2 and CO_2 from individual gas cylinders were mixed.

Carbon dioxide was measured continuously using a non-dispersive infrared (NDIR) analyzer. The calculated CO_2 concentrations are accurate to within 500 ppm. Oxygen concentrations were monitored continuously with a paramagnetic analyzer and were never lower than 20% in the NO_2 exposures nor lower than 19% in the NO_2 and CO_2 exposures. All gases (except NO_2) that were removed for chemical analysis were returned to the chamber. The NO_2, CO_2 and O_2 data were recorded by an on-line computer every 15 seconds. All reported gas concentrations are the average exposure values which were calculated by integrating the area under the instrument response curve and dividing by the exposure time.

2.5 Blood Analyses

Animals designated for blood analysis were cannulated. This procedure involved anesthetizing the animals with pentobarbital sodium (0.08 g/kg) and surgically inserting a cannula into the animal's femoral artery. Sixteen to 24 hours later, blood samples (0.7 mℓ) were taken during and following the exposures from unanesthetized cannulated rats with syringes flushed with heparin. Methemoglobin (MetHb) as well as oxyhemoglobin (O_2Hb), and vol % O_2 were measured by a CO-Oximeter 282 (Instrumentation Laboratory Inc., Lexington, MA). The pH, partial pressure of CO_2 (pCO_2), bicarbonate (HCO_3^-), and base excess (BE) were measured with a Corning 168 pH/Blood Gas Analyzer (Corning Medical, Medfield, MA). Blood samples taken prior to exposure served as the control values.

2.6 Lung Weights

Rats were exposed for 30 minutes to approximately 200 ppm of NO_2 with or without 5% CO_2 and continuously monitored until death occurred. At the time of death, the lungs were removed, rinsed in heparinized saline, externally dried with paper towels, and weighed (wet weight). The lungs were then placed in an incubator set at 37°C and weighed daily until they reached their lowest steady-state weight (dry weight). The pulmonary extravascular water was calculated as the difference between the wet weight and dry weight [8]. Control rats were anesthetized with pentobarbital sodium (0.08 g/kg), their lungs were removed and treated the same as the experimental rat lungs.

2.7 Test Procedure

Six rats were exposed to a single atmospheric gas concentration in each experiment. To achieve steady-state exposures, the portholes of the animal exposure chamber were fitted with rubber stoppers during the time that the gas concentrations were reaching equilibrium. Each animal was placed in a restrainer and inserted into one of the portholes such that the rubber stoppers fell into the chamber. Simultaneously, the heads of the animals were exposed to the chamber atmosphere.

Animal were exposed to the experimental atmospheric environment for 30 minutes. In each of the multiple experiments conducted to determine the LC_{50} values, six non-cannulated animals were used. The LC_{50} in these cases is defined as the concentration of NO_2 (ppm) with and without CO_2 which caused 50% of the animals to die during and/or following the 30 minute exposure. The LC_{50} values and their 95% confidence limits were calculated by the statistical method of Litchfield and Wilcoxon [9]. All animals (including the controls) were weighed daily from the day of arrival until the end of the 14 day post-exposure observation period.

In each of the experiments conducted to examine the blood parameters, the femoral artery of one to three of the six exposed animals was cannulated. Blood was sampled from each of the cannulated animals before the exposure (control blood) and at specified times or as often as possible from sequential animals during the 30 minutes of exposure and during the following two hours of post-exposure time until the animals stopped giving blood or died. The cannulated animals were sacrificed after the last blood sample.

3. RESULTS

3.1 LC_{50} Values and Time of Deaths

The LC_{50} value for NO_2 was 200 ppm with 95% confidence limits of 190 to 210 ppm. This value was based on the results of 9 experiments in each of which six animals were exposed to NO_2 for 30 minutes. All deaths from NO_2 exposures occurred during the post-exposure period between 90 minutes and 24 hours. The animals appeared normal immediately following exposure, although some occasional rapid breathing was noted. In some cases, the animals were observed to become extremely agitated just prior to death; and after death, foamy liquid was noted coming from the nares.

The LC_{50} value for CO_2 was 470,000 ppm (47%) with 95% confidence limits from 430,000 to 510,000 ppm. Seven experiments were conducted to determine this LC_{50} value. All deaths occurred either during the 30 minute exposure period or the following 2 minutes. The highest concentration tested at which no deaths occurred was 260,000 ppm.

The LC_{50} value for NO_2 in the presence of 5% CO_2 decreased to 90 ppm with 95% confidence limits of 70 to 120 ppm. This LC_{50} was also based on 9 experiments during which the animals initially appeared unaffected except for the rapid breathing noted in most of the animals. Deaths occurred much sooner in the presence of the NO_2 and CO_2 than with NO_2 alone (i.e., the earliest death was noted at 24 minutes post-exposure with NO_2 and CO_2, but not before 90 minutes with NO_2 alone). No deaths occurred after the first 24 hours of the post-exposure period. A concentration-dependency on NO_2 was noted with regard to the time of the post-exposure deaths only when the CO_2 was present (Fig. 3).

3.2 Lung Edema

Lung edema was very evident in the animals that died from exposure to NO_2 with and without CO_2. In some cases, foamy liquid was seen coming from the nares following death; and in all cases, foamy liquid was observed coming from the trachea when the lungs were removed from the exposed rats at the time of death (see procedure in Section 2.5). The lungs from the exposed rats were also hemorrhagic. Both exposure to NO_2 (200 ppm) and NO_2 (200 ppm) plus CO_2 (5%) caused a significant increase in the lung wet weight, dry weight, extravascular fluid, and the wet weight/body weight ratio over that of the controls (Table 1). The exposure to NO_2 plus 5% CO_2 increased all four values compared to the NO_2 alone, but the differences were not statistically significant based on two standard deviations of the mean. The increase in dry weight of the exposed animals compared to the controls is believed due to the blood cells at the sites of hemorrhage.

3.3 Blood Values

3.3.1 Methemoglobin (MetHb)

The most dramatic blood difference noted when the animals were exposed to NO_2 was the increase in MetHb. During the exposures to 200 ppm of NO_2, the MetHb levels increased from control values of approximately 0.2% to 4 - 5% (Fig. 4). The MetHb continued to increase for about 10 minutes into the post-exposure period. Then the MetHb decreased slowly and returned to the control value in about 2 hours. Both the rate of formation and the ultimate MetHb level were dependent upon the NO_2 concentration (Figs. 5 and 6). A very good linear relationship (correlation coefficient of 0.99) was found when the 30 minute MetHb values were plotted against the NO_2 concentration.

In the presence of 200 ppm NO_2 and 5% CO_2, the rate of increase of MetHb was greater than with NO_2 alone and the ultimate levels reached during the 30 minute exposures were 2.5 times higher than those measured in the animals exposed to NO_2 only (Fig. 4). As with NO_2 alone, the MetHb continued to increase during the first 10 minutes of the post-exposure period. During this time, in the presence of both gases, the MetHb reached levels that were 3 - 4 times those observed in animals exposed to NO_2 alone.

3.3.2 Oxyhemoglobin

In general, the oxyhemoglobin (O_2Hb) appeared unaffected during and following exposures (up to 2 hours) to 200 ppm of NO_2 alone, although a few values fell below two standard deviations of the control mean after 20 minutes of the 30 minute exposure (Fig. 7). In the post-exposure period, the O_2Hb values were not significantly different from the mean of the controls.

The O_2Hb in the rats exposed to 200 ppm NO_2 plus 5% CO_2 started to decrease about 15 minutes into the 30 minute exposure and continued to decrease in the post-exposure period (Fig. 7). Although there was more scatter of the data in the post-exposure period, all values were significantly lower than those of the control and of NO_2 alone.

A plot of the 30 minute O_2Hb values vs. various concentrations of NO_2 ranging from 50 to 500 ppm showed a reasonably good linear relationship (correlation coefficient of -0.94) (Fig. 8). As the NO_2 increased, the 30 minute O_2Hb values decreased.

3.3.3 pH

During the exposures to 200 ppm NO_2 with and without 5% CO_2, the pH was lower in the presence of the 5% CO_2 than in its absence (Fig. 9). The lowest pH noted during the 30 minute exposures to 200 ppm of NO_2 was 7.31. In the case of the NO_2 alone, the pH started to drop below the control mean about 20 minutes into the exposure. In the presence of 200 ppm NO_2 plus 5% CO_2, the lowest pH was 7.14; and the values were all below the control mean after 10 minutes of exposure. During the post-exposure period, the pH continued to decrease following the NO_2 exposures; however, in the case of NO_2 plus CO_2, the pH showed much more variability.

When the 30 minute pH values were determined for the various NO_2 concentrations ranging from 50 to 500 ppm, a fairly good linear relationship was noted (correlation coefficient of -0.895). As the NO_2 exposure increased, the pH decreased (Fig. 10).

3.3.4 Bicarbonate and Partial Pressure of CO_2

As expected, both the bicarbonate concentration (HCO_3^-) and pCO_2 were higher during the exposures to 200 ppm of NO_2 plus 5% CO_2 than in the absence of CO_2 (Fig. 11 and 12). There was no significant difference in the post-exposure period between the two types of exposures (NO_2 with and without CO_2), although in both cases, the values dropped below that of the controls.

Increasing the concentrations of NO_2 (ranging from 50 to 500 ppm) did not affect the bicarbonate concentration (Fig. 13), although it seemed to increase the pCO_2 (correlation coefficient of 0.83) (Fig. 14).

3.3.5 Volume % O_2 and Base Excess

Although both the volume % O_2 and base excess (BE) dropped below the control values during and following the exposures to 200 ppm NO_2 with and without 5% CO_2, there was no detectable difference when the CO_2 was present (Figs. 15 + 16).

Exposure to increasing concentrations of NO_2 (up to 250 ppm) caused a decrease in the 30 minute volume % O_2 values (Fig. 17). There appeared to be little change in this parameter between exposures of 250 ppm and 500 ppm.

The base excess showed a decrease as the animal exposure to NO_2 increased from 50 to 500 ppm (Fig. 18). However, this parameter showed a lot of variability even in the unexposed controls and the experimental values were not significantly different from the controls. The linear correlation coefficient was only -0.67.

4. DISCUSSION

Nitrogen dioxide is about as toxic as HCN and may be produced in fires involving both nitrogen and non-nitrogen containing materials. Whether NO_2 should be considered a major factor of toxicological concern depends upon the concentrations that would be produced in real fires. The results from a preliminary examination of nitrogen fixation conducted at NIST indicated that the levels of NO_x were low [10]. The maximum concentrations of NO_x found in two large-scale room burns containing polystyrene-covered walls were 10 and 25 ppm. In controlled laboratory fires involving nitrogen-containing products, NO_x values ranging from less than 1 ppm to 1000 ppm have been noted [10-12]. In a study of real structural fires, 90 samples of fire atmospheres were taken by two Boston Fire Department units and analyzed for O_2, CO_2, CO, NO_2, HCℓ, HCN and total particulates [13]. In these 90 samples, NO_2 was detected 8 times and the highest concentration was 0.89 ppm. The authors noted, however, that these structural fires were in the older, dilapidated residential sections of Boston and may have contained fewer synthetic materials. The NO_2 could have also reacted with the water vapor produced in the fires forming nitrous and nitric acid. The above limited studies indicate that more work needs to be done to determine the degree to which NO_2 is produced in real fires and whether it is an important toxicological factor in the deaths that occur.

While most studies have concentrated on examining the chronic effects of low levels of nitrogen oxides typically found in areas troubled by air pollution, high concentrations of NO_x have also been shown to be a problem in industrial and agricultural accidents involving welding, explosives, chemicals and silos ("silo-fillers" disease) [14]. Patients who have been exposed to large concentrations of NO_x (probably predominantly NO_2) have experienced some of the following symptoms: 1. hypoxia from asphyxia and methemoglobinemia (immediate, but rare, reaction); 2. dyspnea (difficult or labored breathing), bronchospasm, cough, diffuse weakness, nausea, headache, conjunctivitis, tachycardia, and chest pain (common acute reactions); 3. pulmonary edema (common delayed reaction following symptom-free interval); and 4. bronchitis, pneumonia, and increased susceptibility to upper respiratory illness (delayed reactions) [14,15]. The immediate and acute reactions could impact the ease of escape and lead to deaths due to other factors.

In our animal studies of NO_2 exposures, deaths appeared to be predominantly due to pulmonary edema, although the formation of non-lethal levels of methemoglobin was an indicator of NO_2 exposure. Neither immediate deaths from hypoxia or methemoglobinemia nor delayed deaths from respiratory illnesses were observed. No deaths were observed earlier than 90 minutes nor later than 24 hours following exposure to concentrations of NO_2 ranging from 50 - 500 ppm. Within that time period, the time of death was variable and may depend upon the activity of the animals following the exposures. Studies [16] have shown that the effects of NO_2 are potentiated by exercise undertaken one hour post-exposure. Twelve hours after the NO_2 exposures, neither exercise nor exposure to 5% CO_2 (a respiratory stimulant) had potentiating effects. Although the authors [16] were unable to explain the mechanism of this exercise effect, it may be due to the pharmacological action of nitrite (a metabolite of NO_2). Nitrite has been shown to produce vasodilation and

a decrease in central venous pressure which is extremely sensitive to gravity (i.e., the blood pressure may fall to negative values if the patient is brought to an upright position; this is also termed postural hypotension) [17].

In the experiments reported here, the LC_{50} (30 minute exposure plus 14 day post-exposure observation period) for NO_2 was calculated as 200 ppm. The 95% confidence limits were 190-210 ppm. This value agrees with the results of Gray et al. who found the 30 minute LC_{50} value in rats was 174 ppm, with 95% confidence limits of 154 - 197 ppm [18]. Gray et al. also examined the toxicity of NO_2 for other exposure times. That data plus some 5 minute exposure data from the literature [19] and the 30 minute LC_{50} values determined for this study are given in figure 19.

Although the in vivo biochemistry of the reactions of NO_2 is still under active investigation, studies under in vitro conditions indicate that in the presence of H_2O, NO_2 will form nitric acid (HNO_3) and nitrous acid (HNO_2) [14,20]. These two acids are most likely responsible for the lung damage leading to the massive pulmonary edema and subsequent deaths. Nitrite ion (NO_2^-) is formed in the blood when the nitrous acid dissociates. The nitrite ion oxidizes the ferrous iron in oxyhemoglobin to ferric iron to produce MetHb [21] as follows:

$$2H^+ + 3NO_2^- + 2O_2Hb(Fe^{++}) \longrightarrow 2MetHb(Fe^{+++}) + 3NO_2^- + H_2O$$

Thus, when the animals are exposed to higher concentrations of NO_2, the 30 minute MetHb levels increase and the 30 minute O_2Hb levels decrease. No O_2 is released, therefore, the vol. % O_2 also decreases. The rate of the conversion of oxyhemoglobin to MetHb by nitrite has been shown to be faster when the pH is lower [21]. Since the pH of the blood also decreases with exposures to increasing concentrations of NO_2 (Fig. 10), the overall effect is to increase the rate of production and ultimate levels of MetHb. Since MetHb will not bind O_2, it further compromises the health of the exposed organisms. The increase in pCO_2 that occurs with exposure to increasing levels of NO_2 may indicate that higher concentrations of NO_2 are causing (1) some sensory irritation which is resulting in breath holding or some respiratory rate depression or (2) lung injury or buildup of edema which is preventing the expiration of CO_2.

When the animals were exposed to both NO_2 plus 5% CO_2, a synergistic effect was noted. The LC_{50} value of NO_2 was reduced from 200 ppm to 90 ppm (95% confidence limits were 70-120 ppm). Five percent CO_2 (50,000 ppm), by itself, is not lethal. The LC_{50} for CO_2 is 470,000 ppm with 95% confidence limits were 430,000-510,000 ppm. Carbon dioxide does, however, have profound physiological effects on respiratory rate, tidal volume, and the cardiovascular and central nervous systems [22-24]. Detrimental synergistic effects were observed previously in this laboratory when rats were exposed to combinations of CO_2 and other gases. For example, the LC_{50} values of CO decreased when various concentrations of CO_2 were also present. Five percent CO_2 plus hydrogen cyanide (HCN) decreased the LC_{50} value of HCN and 5% CO_2 plus oxygen increased the LC_{50} value of reduced oxygen atmospheres[4,5]. (In this latter case, an increased LC_{50} is indicative of a more toxic situation, since the animals are dying at higher levels of O_2 which normally would be non-lethal.)

Carbon dioxide is probably increasing the toxic potency of NO_2 through its action as a respiratory stimulant. It is increasing the rate of uptake of NO_2 and causing the final dose of NO_2 in the body to be higher. These effects can be observed in figure 4 denoting the formation of MetHb from exposure to 200 ppm of NO_2 in the presence and absence of 5% CO_2. In our previous experiments on combinations of CO plus 5% CO_2, the CO_2 increased the rate of uptake of CO (as indicated by the faster rate of COHb formation), but, contrary to the NO_2 plus CO_2 results, did not change the final concentration of COHb [4].

The deaths that occur as a result of NO_2 plus CO_2 are also primarily due to pulmonary edema. When the animals are exposed to NO_2 plus 5% CO_2, the rate of uptake and dose of NO_2 in the body is increased. This increase is indicated by the greater degree of pulmonary edema as evidenced by the lung weights (Table 1).

One question that remains is why does the exposure to the combined gases produce more rapid death than the exposures to NO_2 alone? The possibility that the edema is formed earlier is indicated by the decrease in O_2Hb seen when the animals were exposed to 200 ppm NO_2 and 5% CO_2, but not when they were exposed to NO_2 alone. This decrease in O_2Hb may be due to the presence of edema which prevents the oxygen from being transferred from the lungs to the blood or simply due to the increase in MetHb. The pH is also lower when CO_2 is present and this could cause greater formation of nitric and nitrous acid, more edema, and more rapid formation of MetHb from nitrite - all leading to a much more toxic situation.

5. SUMMARY

NITROGEN DIOXIDE IN AIR

1. LC_{50} value (30 minute exposures) was 200 ppm.
2. MetHb levels reached 4-5% at end of 30 minute exposure to the LC_{50} concentration.
3. NO_2 caused increase in the wet lung weight/body weight ratio that was 3.7 times that of the controls.
4. Deaths were primarily due to pulmonary edema and occurred post-exposure between 90 minutes and 24 hours.
5. The time of the post-exposure deaths was not concentration-dependent.

NITROGEN DIOXIDE PLUS CARBON DIOXIDE (5%) IN AIR

1. The toxicity of NO_2 doubles; LC_{50} value (30 minute exposures) was 90 ppm.
2. Methemoglobin levels are 2-3 times higher than with NO_2 alone.
3. Lung edema was 1.3 times higher than with NO_2 alone.
4. Deaths were primarily due to pulmonary edema and occurred earlier than with NO_2 alone. The earliest death was observed at 24 minutes post-exposure.
5. The time of the post-exposure deaths was concentration-dependent.

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TABLE 1

LUNG WEIGHTS FROM RATS EXPOSED TO NO_2 WITH AND WITHOUT CO_2

EXPOSURE	NUMBER ANIMALS	NO_2 (ppm)	CO_2 (%)	WET WEIGHT (g)	DRY WEIGHT (g)	EXTRA-VASCULAR FLUID[a] (g)	WET LUNG WT. / BODY WEIGHT ($\times 10^{-3}$)
CONTROLS	8	0	0	1.19 ± 0.12[b]	0.25 ± 0.02	0.94 ± 0.10	3.5 ± 0.2
NO_2	8	200	0.4[c]	2.59 ± 0.24*	0.37 ± 0.03*	2.22 ± 0.21*	10.0 ± 0.8*
$NO_2 + CO_2$	12	197	5.1	3.53 ± 0.34*	0.45 ± 0.06*	3.08 ± 0.30*	13.2 ± 1.2*

a. wet weight - dry weight
b. mean ± S.D.
c. CO_2 from respiration of animals.
*. Significantly different from controls based on two standard deviations.

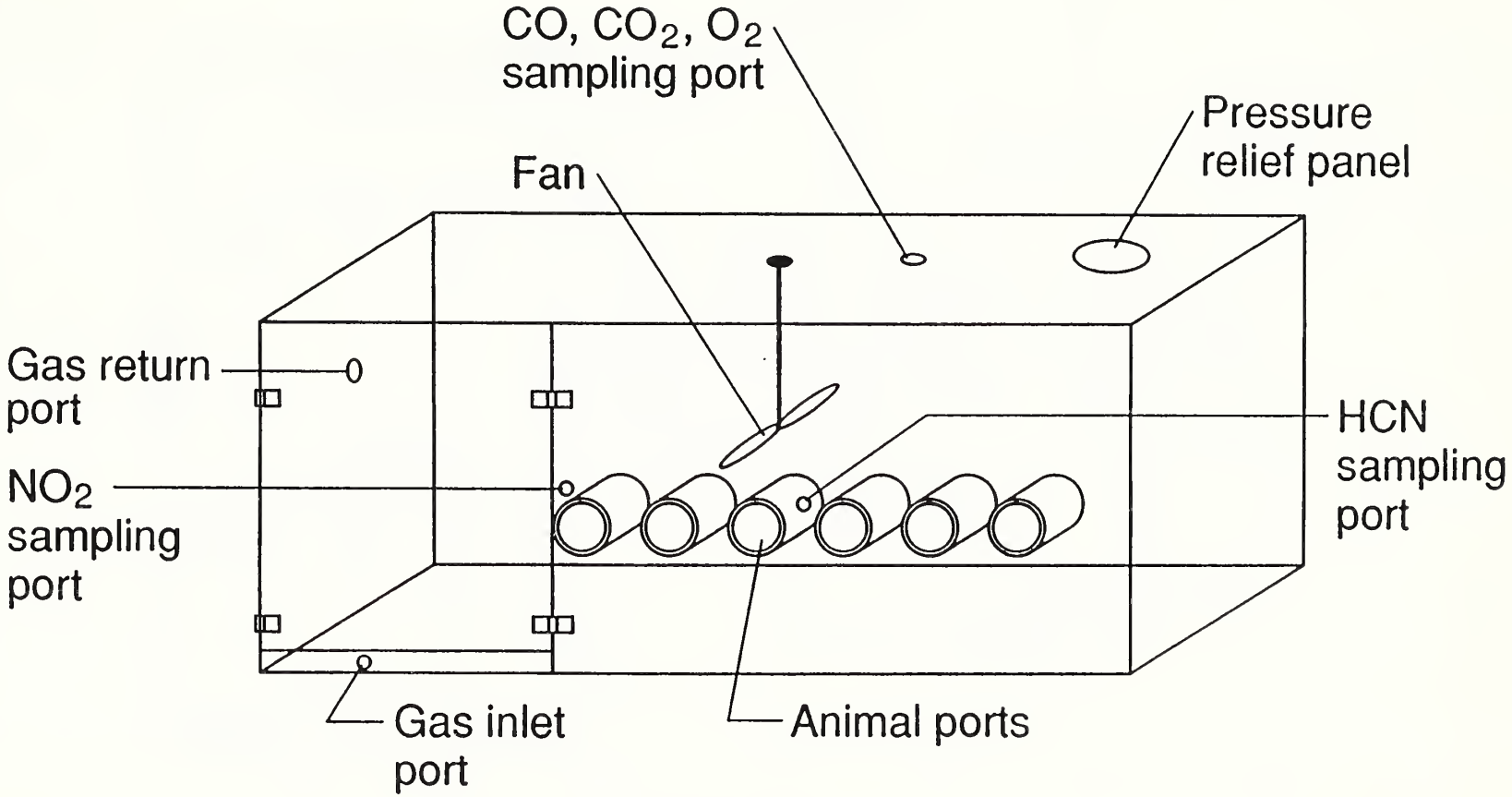

Figure 1. Exposure chamber

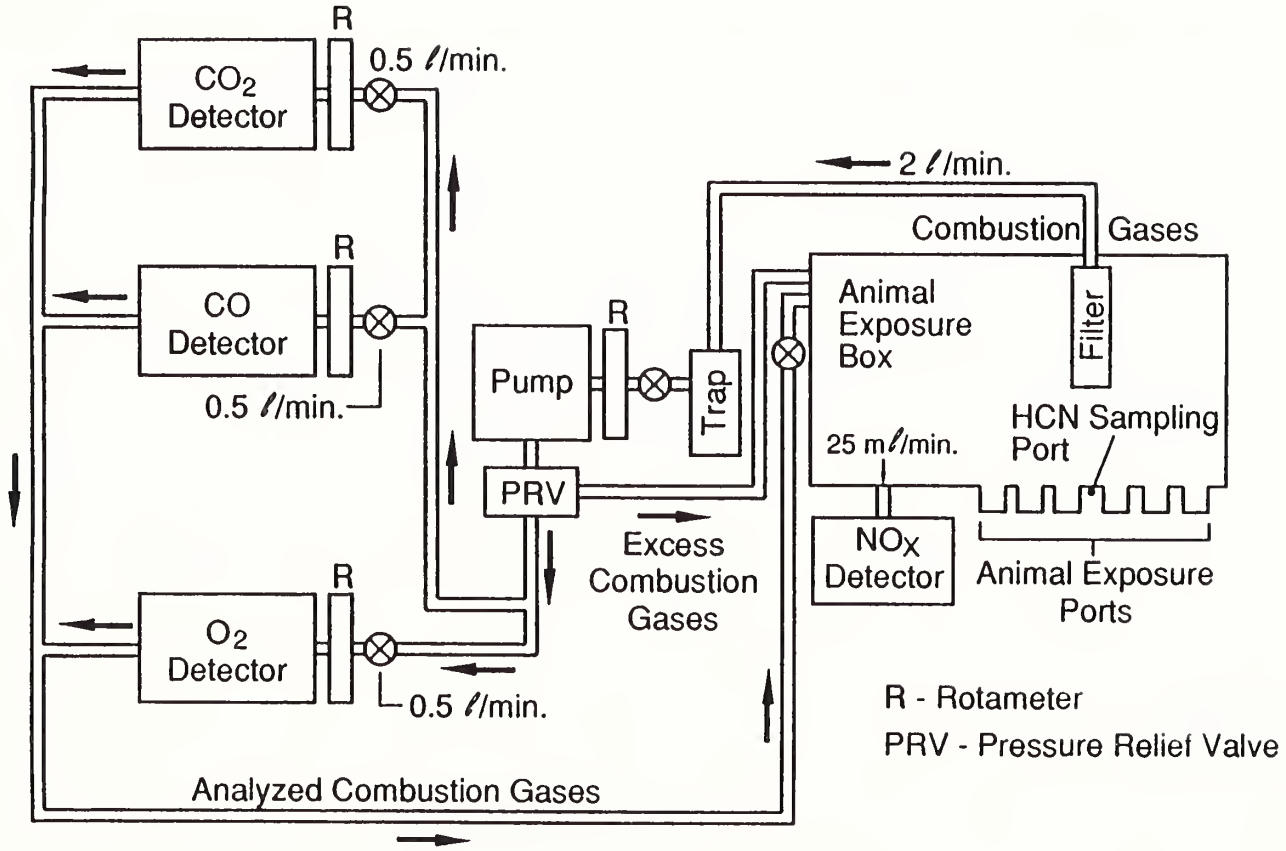

Figure 2. Schematic of exposure chamber and analytical equipment

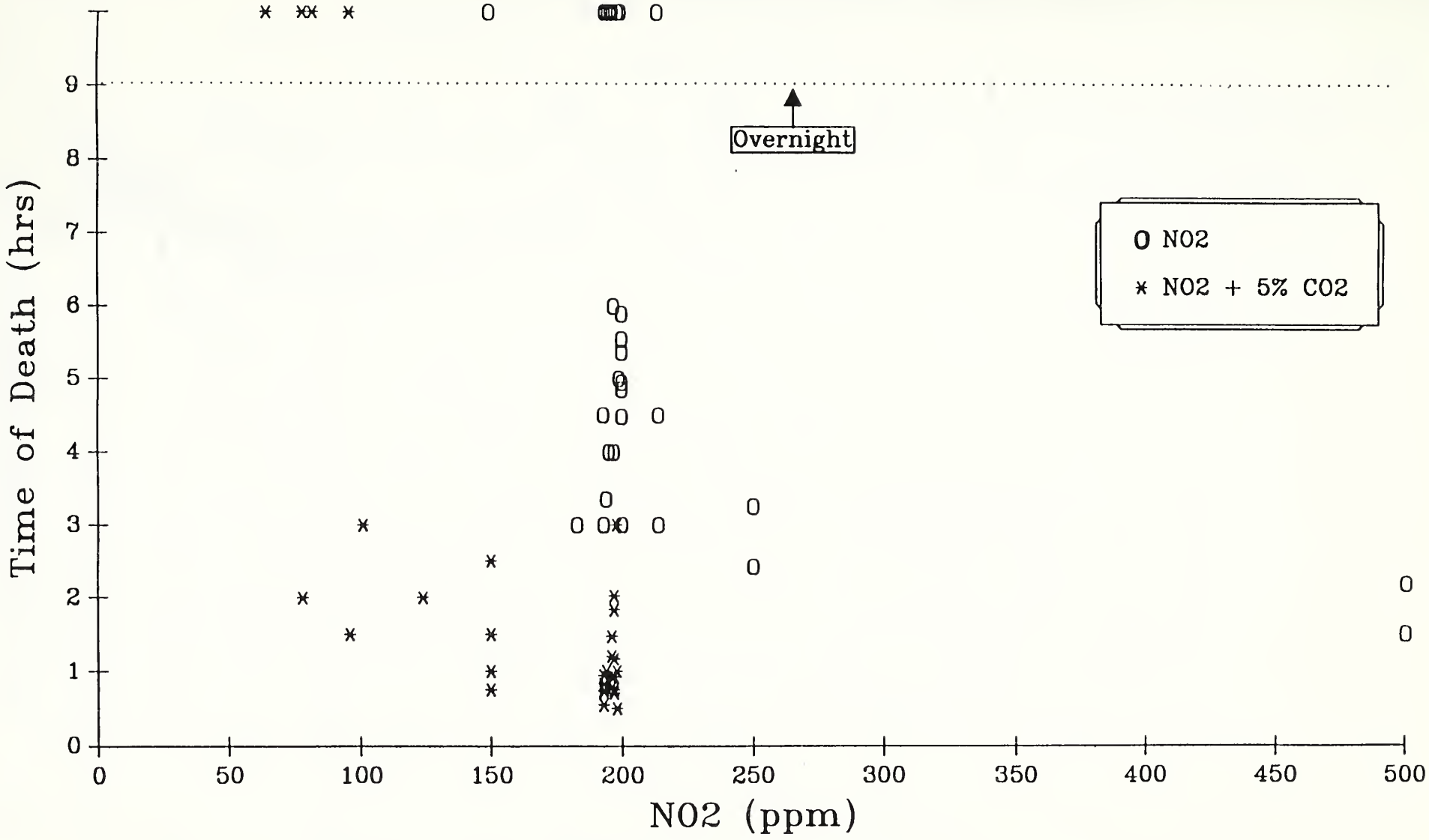

Figure 3. Time of death following exposure to $NO_2 \pm CO_2$

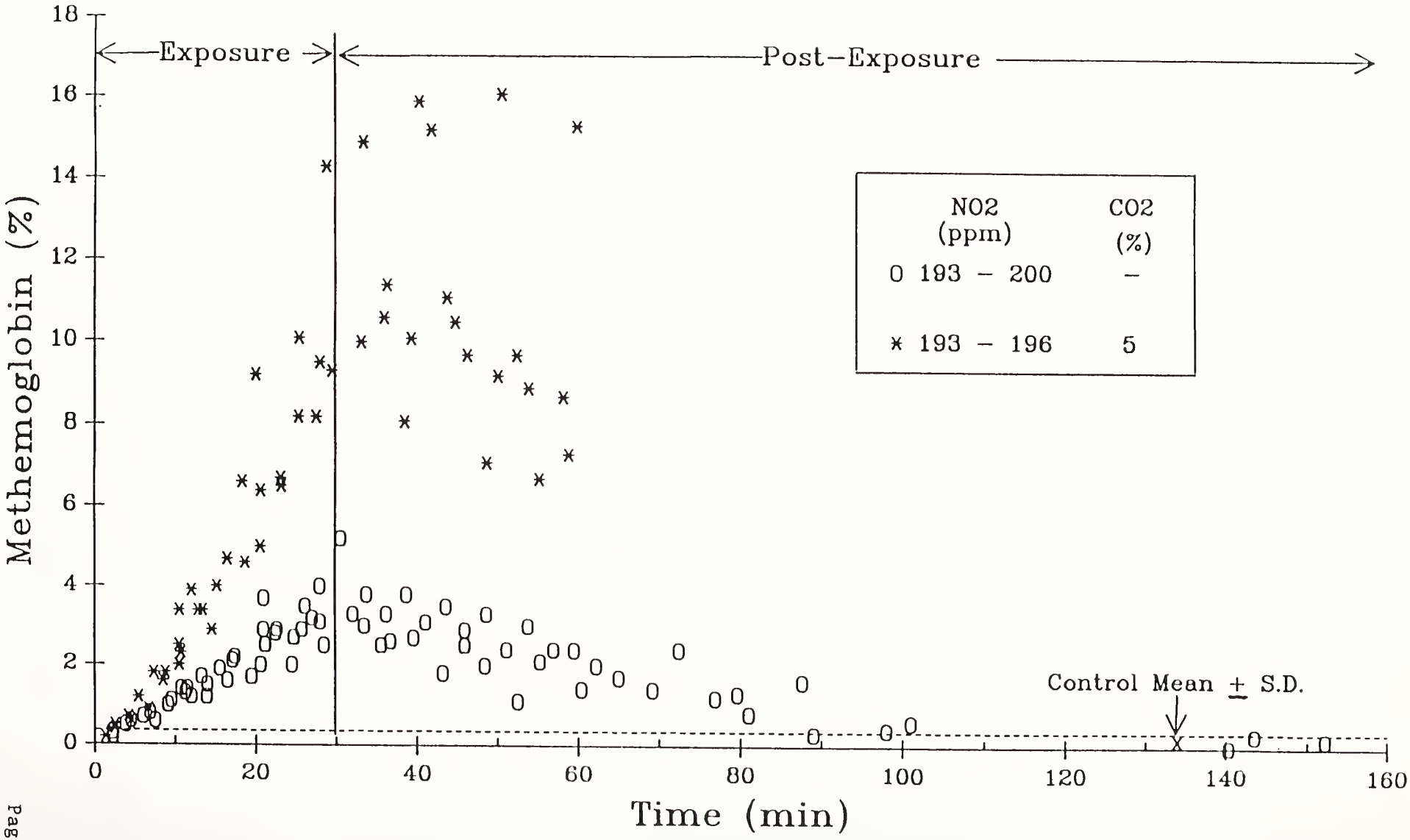

Figure 4. Methemoglobin generation during and following exposure to $NO_2 \pm CO_2$. Control mean ± the standard deviation of 32 animals was 0.2 ± 0.2.

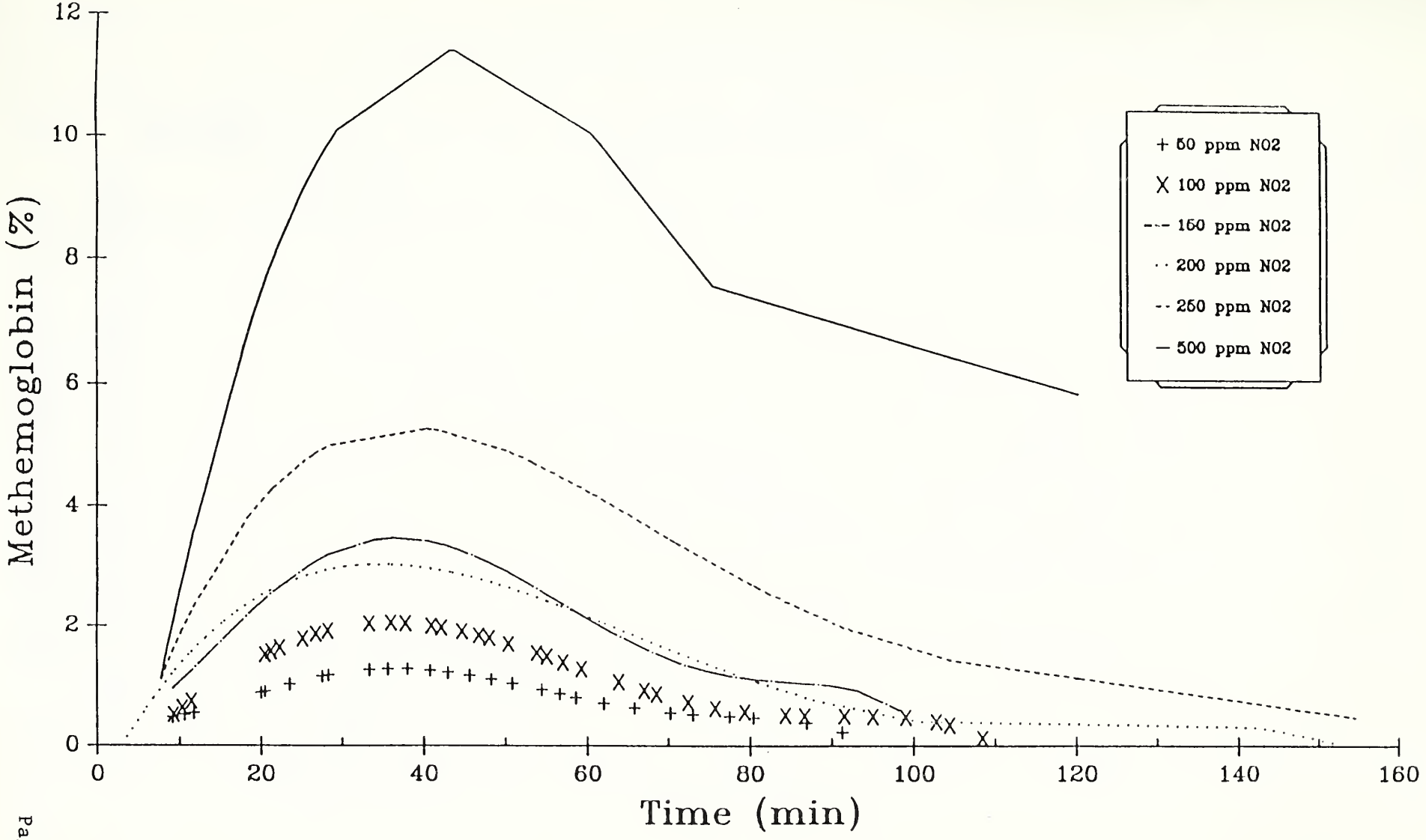

Figure 5. Methemoglobin values from various exposures to NO_2 vs. time of exposure.

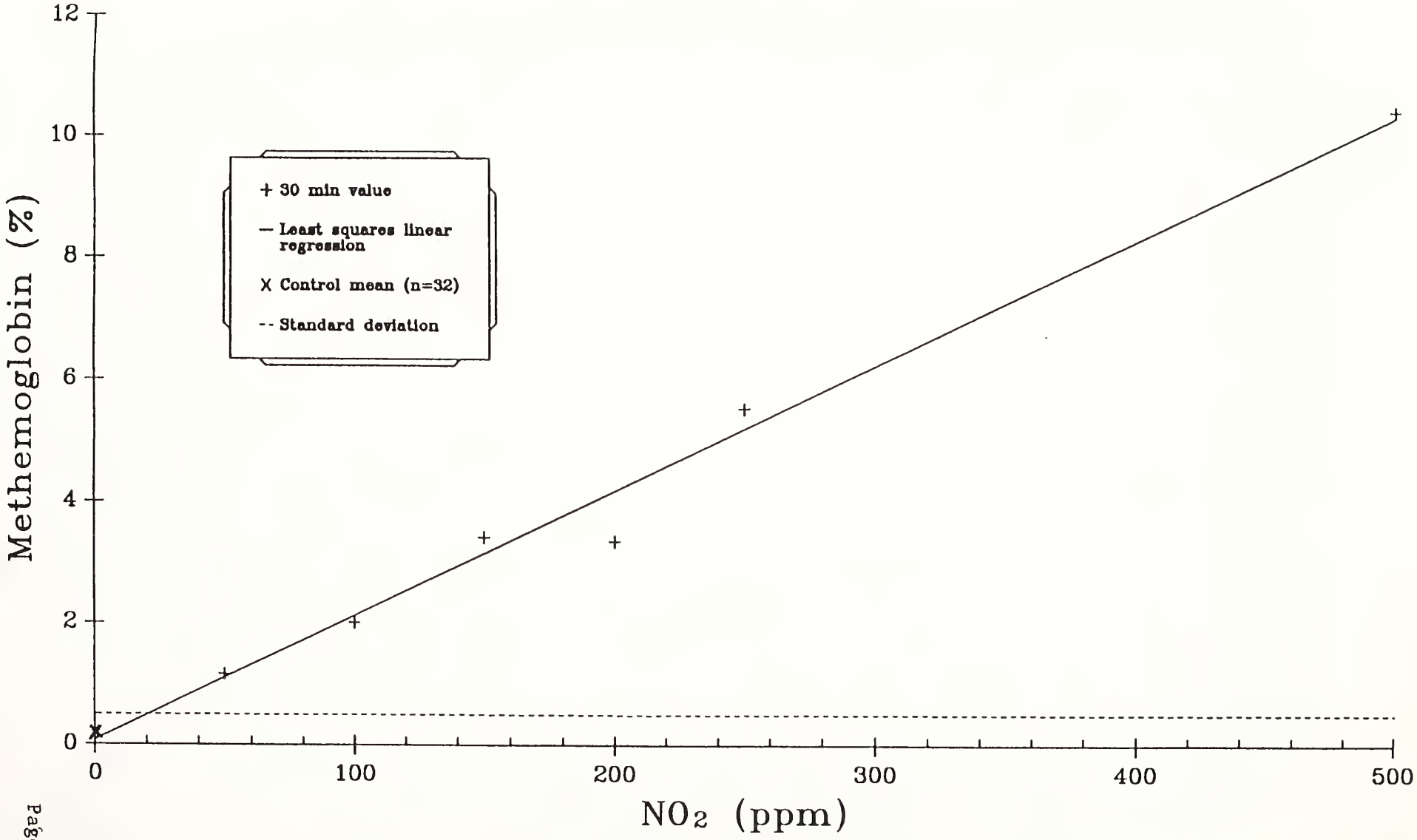

Figure 6. Methemoglobin values (30 minute) from various exposures to NO_2. Control mean $\pm$ the standard deviation of 32 animals was 0.2 $\pm$ 0.2.

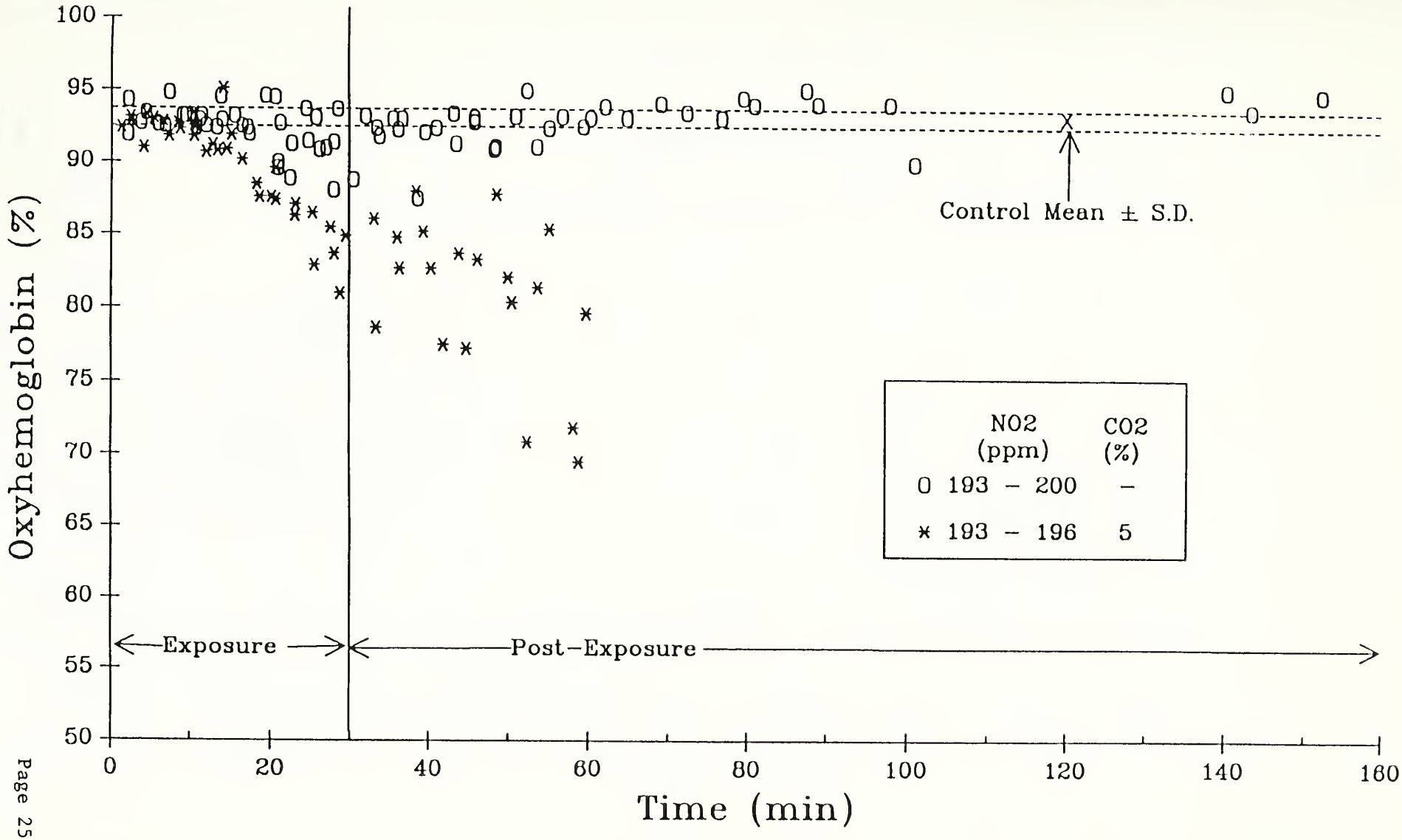

Figure 7. Oxyhemoglobin during and following exposure to $NO_2 \pm CO_2$. Control mean ± the standard deviation of 32 animals was 93.2 ± 0.7.

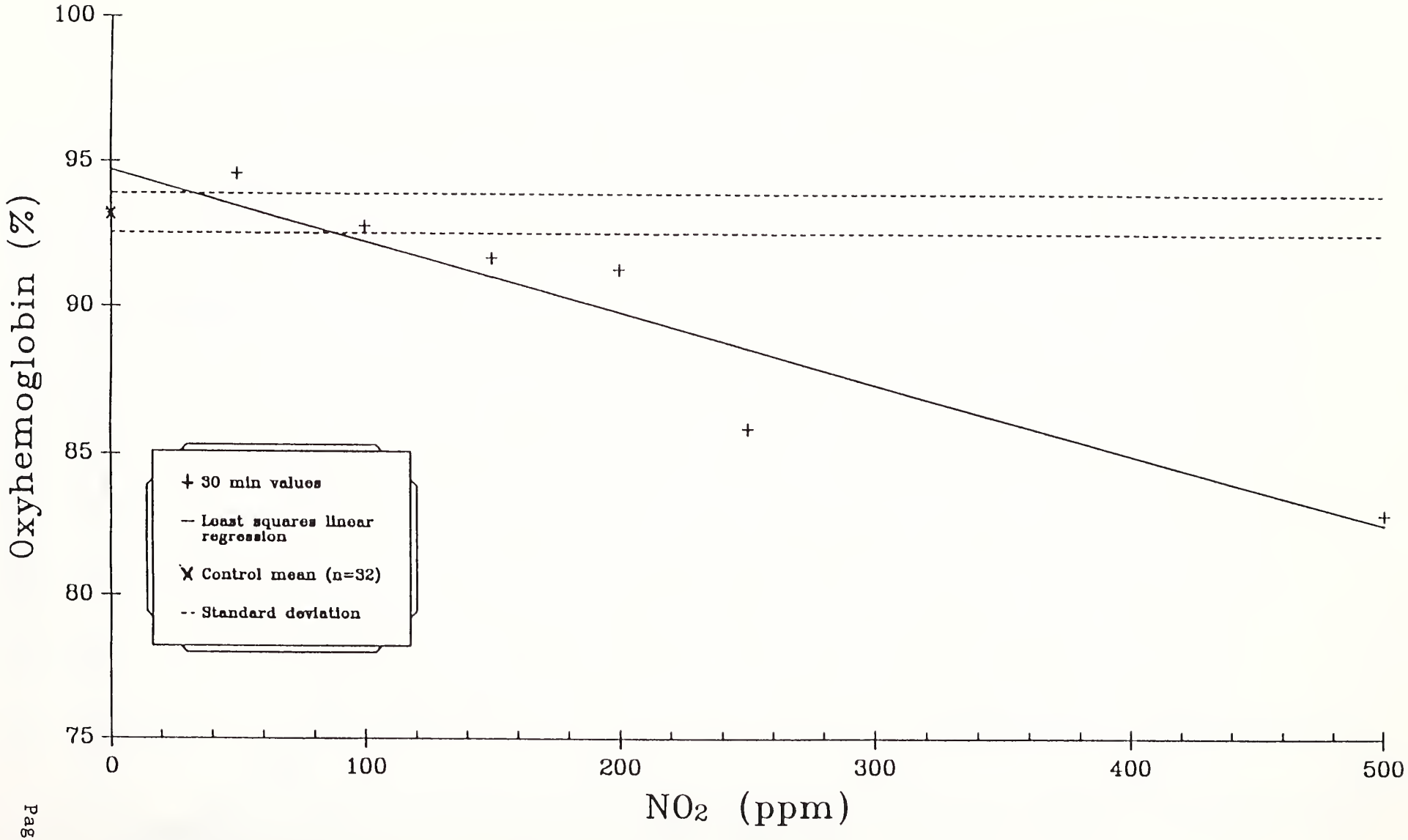

Figure 8. Oxyhemoglobin values (30 minute) from various exposures of NO_2. Control mean ± the standard deviation of 32 animals was 93.2 ± 0.7.

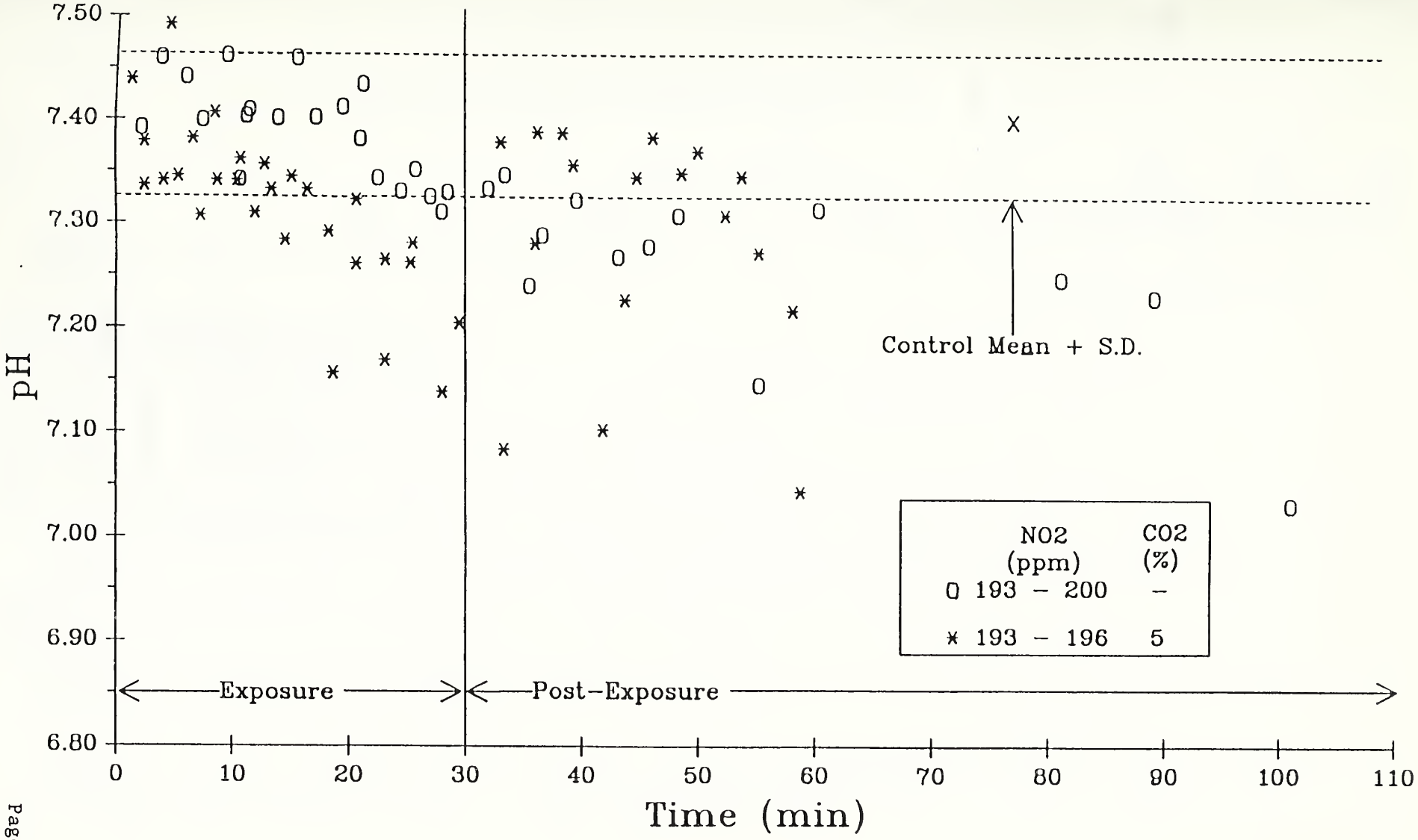

Figure 9. pH during and following exposure to $NO_2 \pm CO_2$. Control mean $\pm$ the standard deviation of 24 animals was 7.40 $\pm$ 0.07.

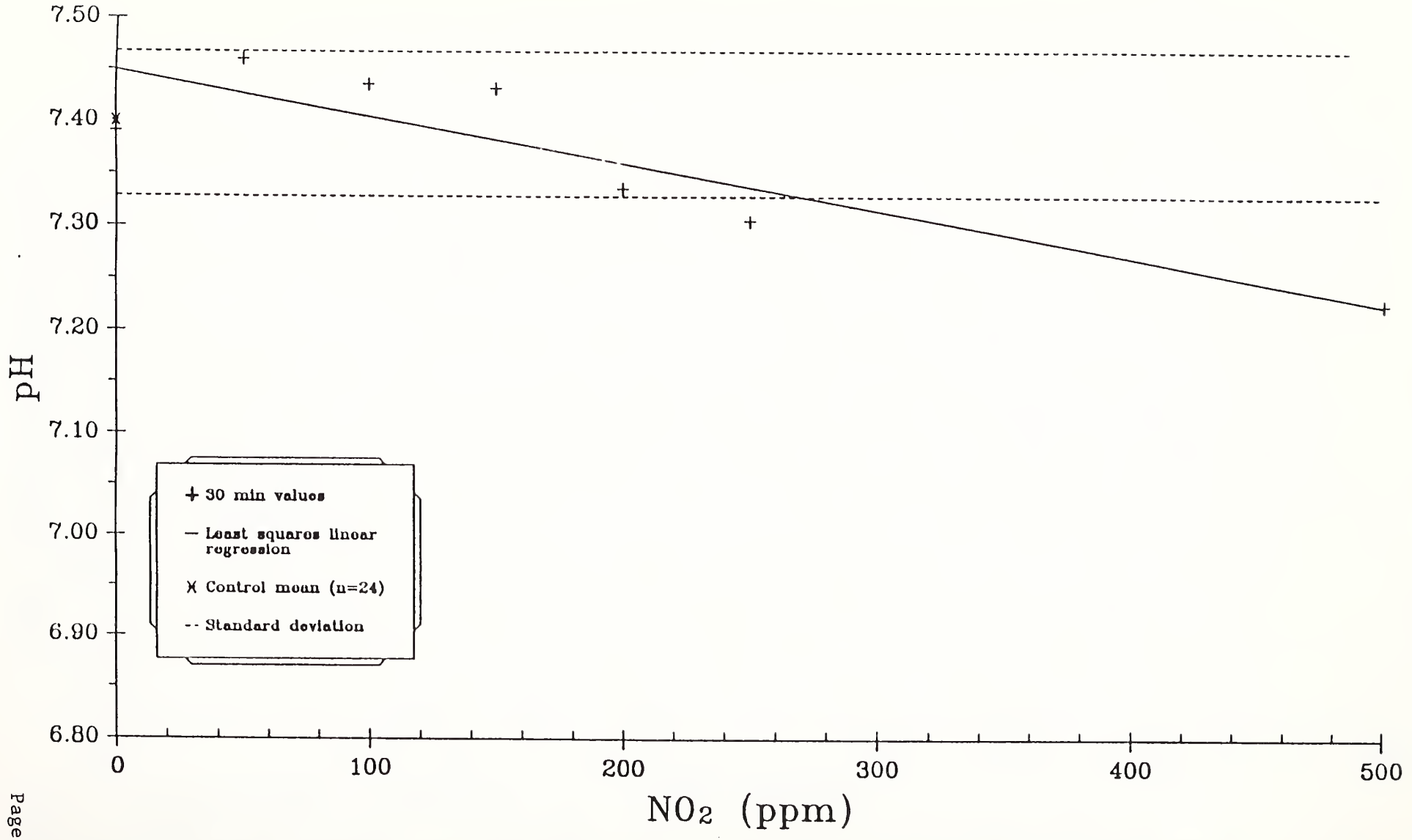

Figure 10. pH values (30 minute) from various exposures of NO_2. Control mean $\pm$ the standard deviation of 24 animals was 7.40 $\pm$ 0.07

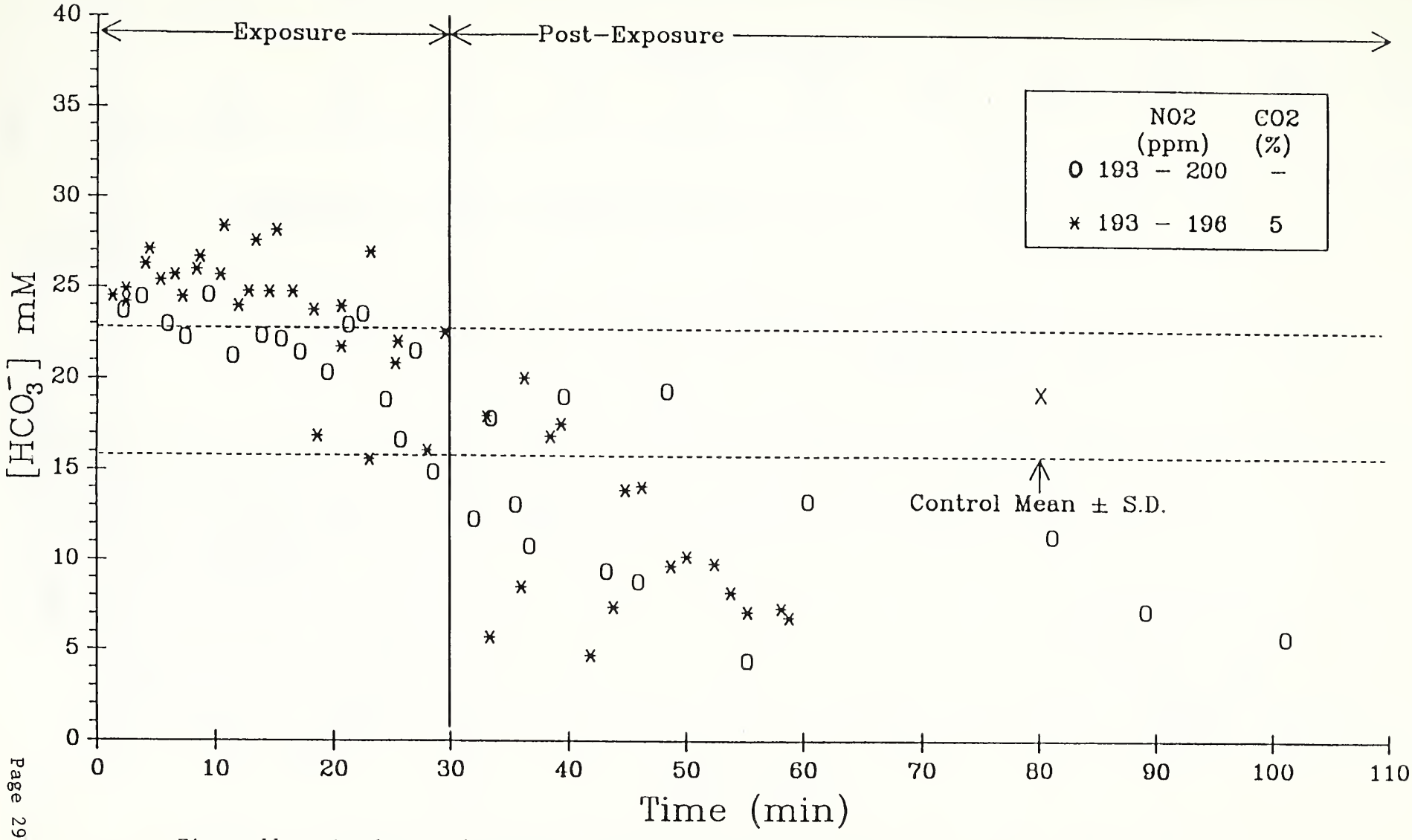

Figure 11. Bicarbonate during and following exposure to $NO_2 \pm CO_2$. Control mean $\pm$ the standard deviation of 24 animals was 19.3 ± 3.5.

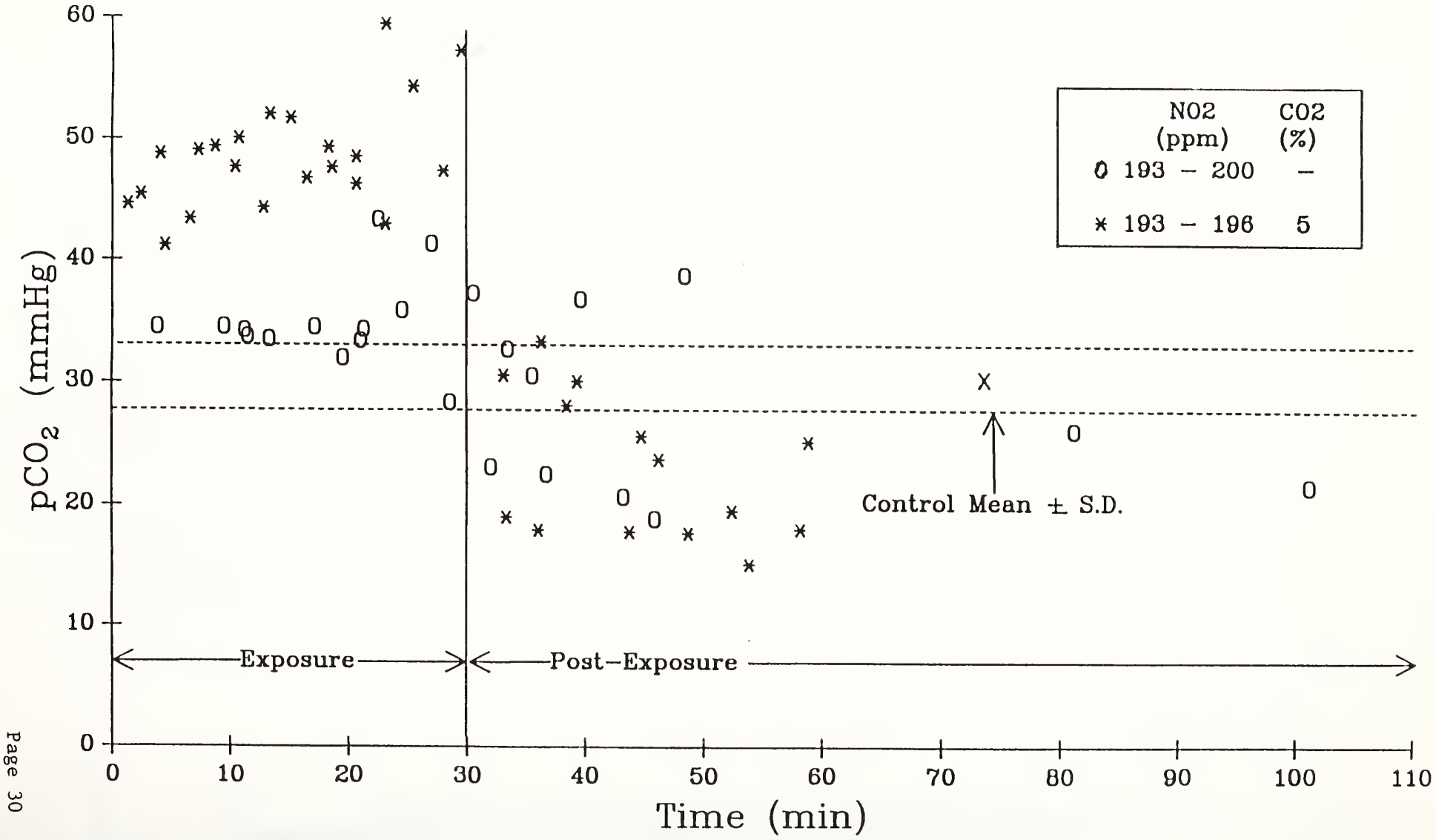

Figure 12. Partial pressure of CO_2 during and following exposure to $NO_2 \pm CO_2$. Control mean $\pm$ the standard deviation of 23 animals was 30.3 ± 2.6

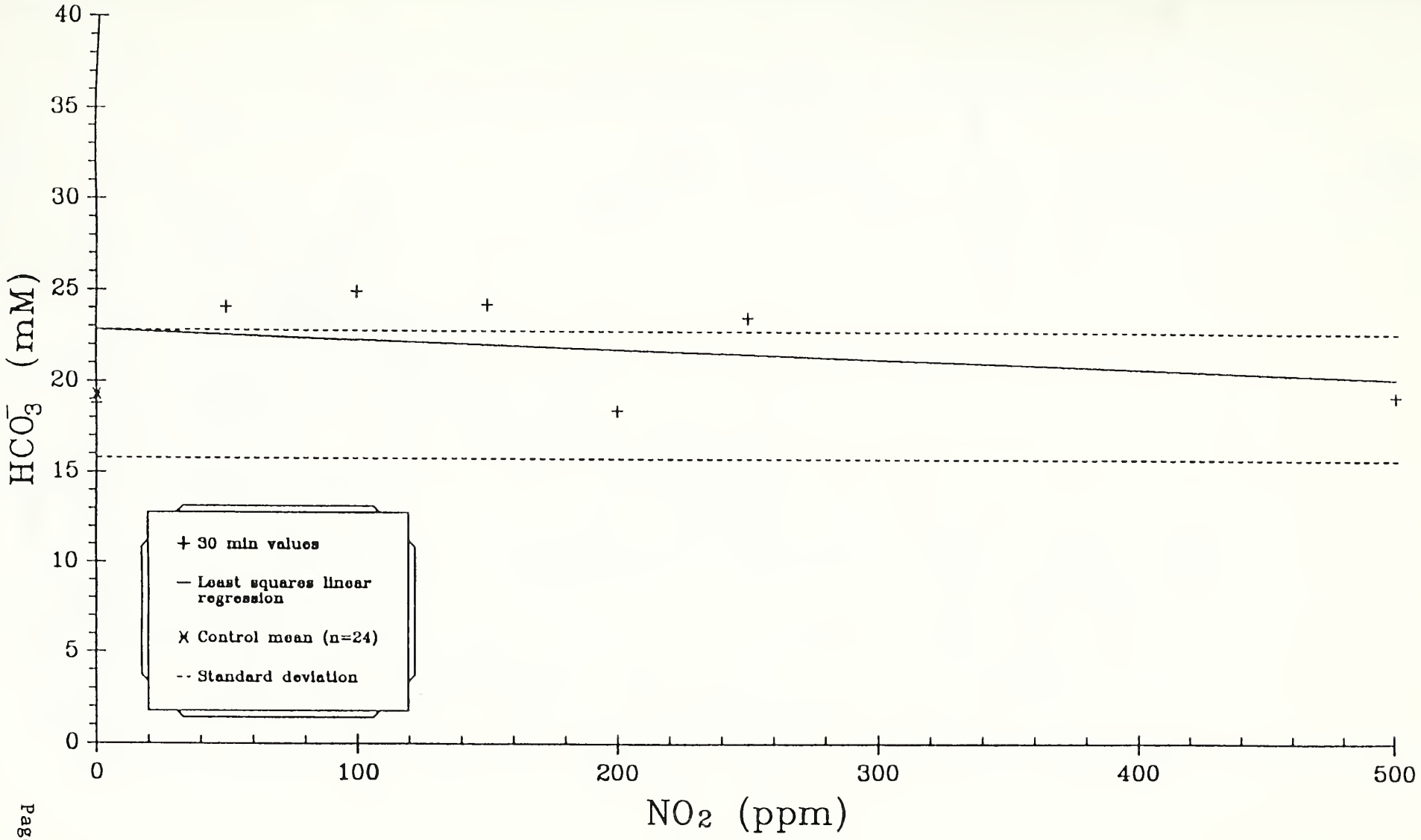

Figure 13. Bicarbonate values (30 minute) from various exposures of NO_2. Control mean ± the standard deviation of 24 animals was 19.3 ± 3.5

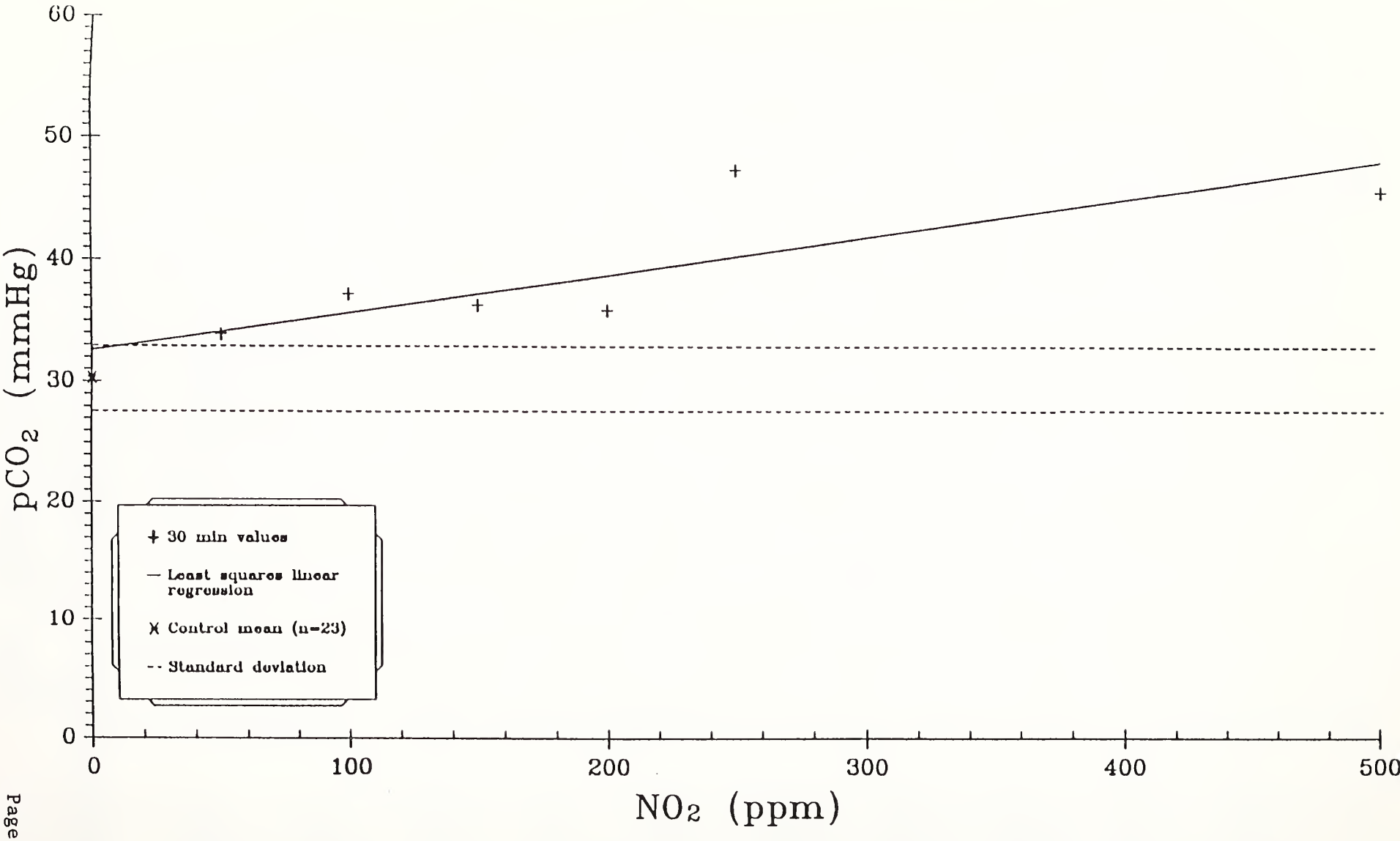

Figure 14. Partial pressure of CO_2 (30 minute values) from various exposures of NO_2. Control mean $\pm$ the standard deviation of 23 animals was 30.3 $\pm$ 2.6

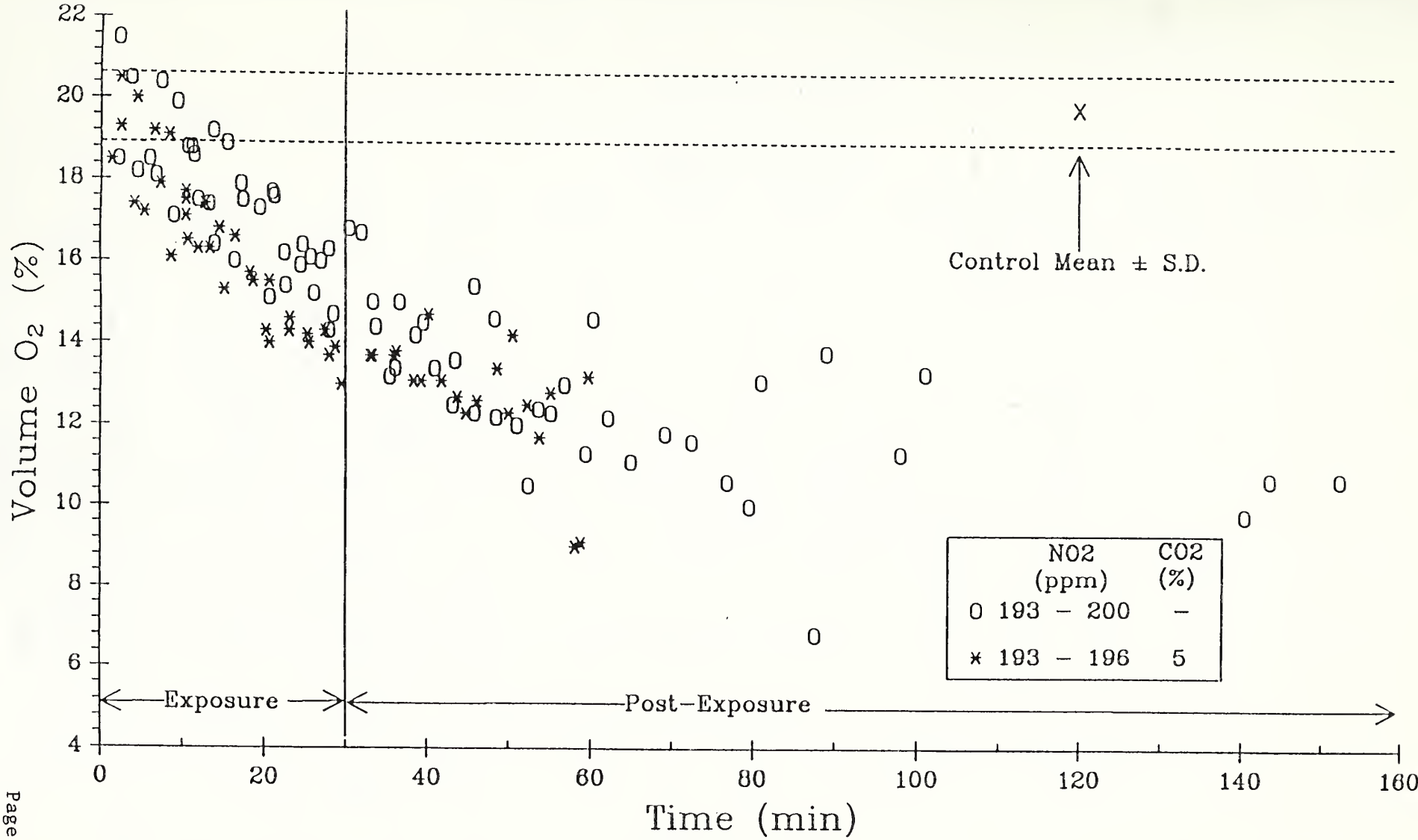

Figure 15. Volume percent oxygen during and following exposures to $NO_2 \pm CO_2$. Control mean $\pm$ the standard deviation of 32 animals was 19.8 $\pm$ 0.9.

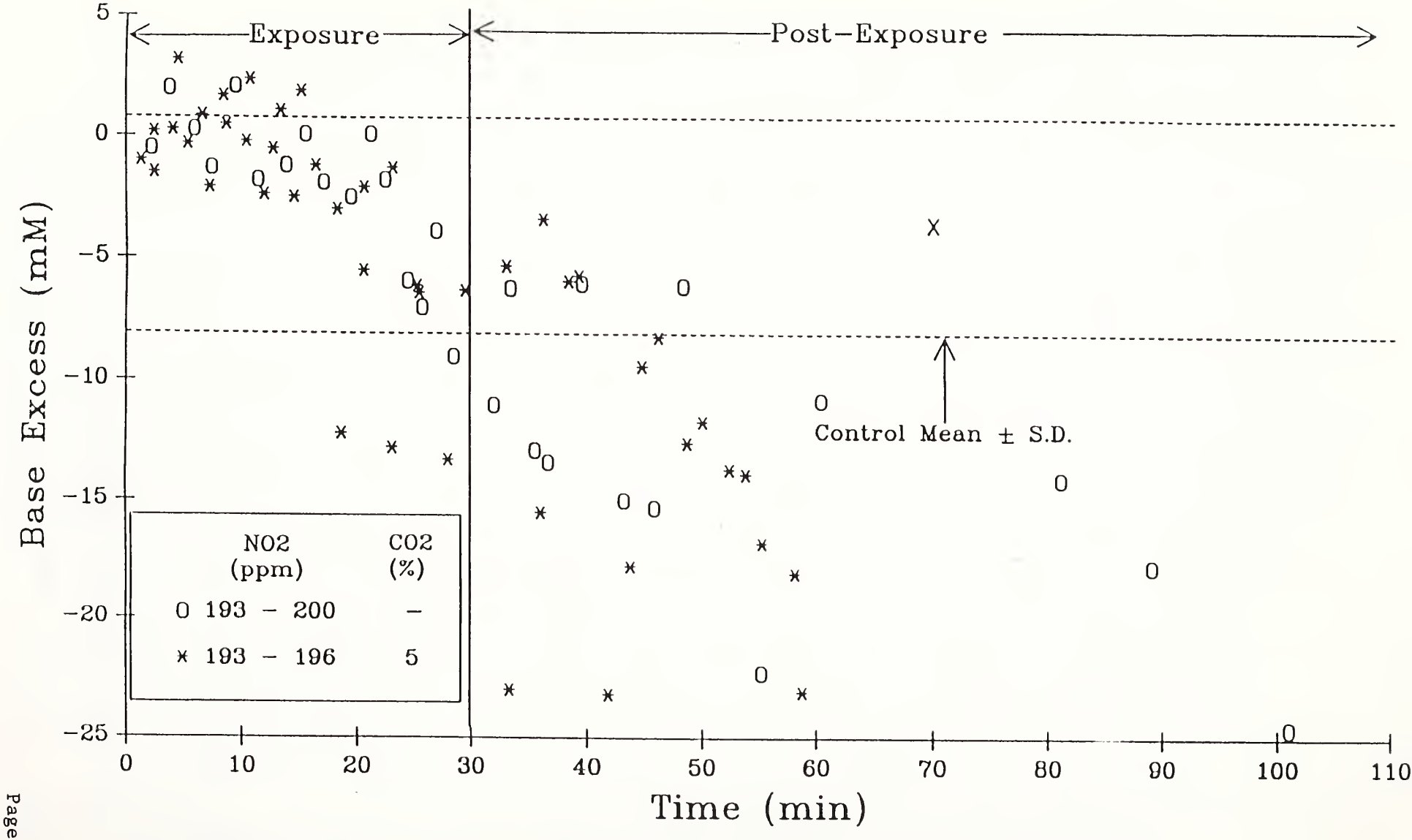

Figure 16. Base excess during and following exposure to $NO_2 \pm CO_2$. Control mean $\pm$ the standard deviation of 24 animals was -3.6 ± 4.5

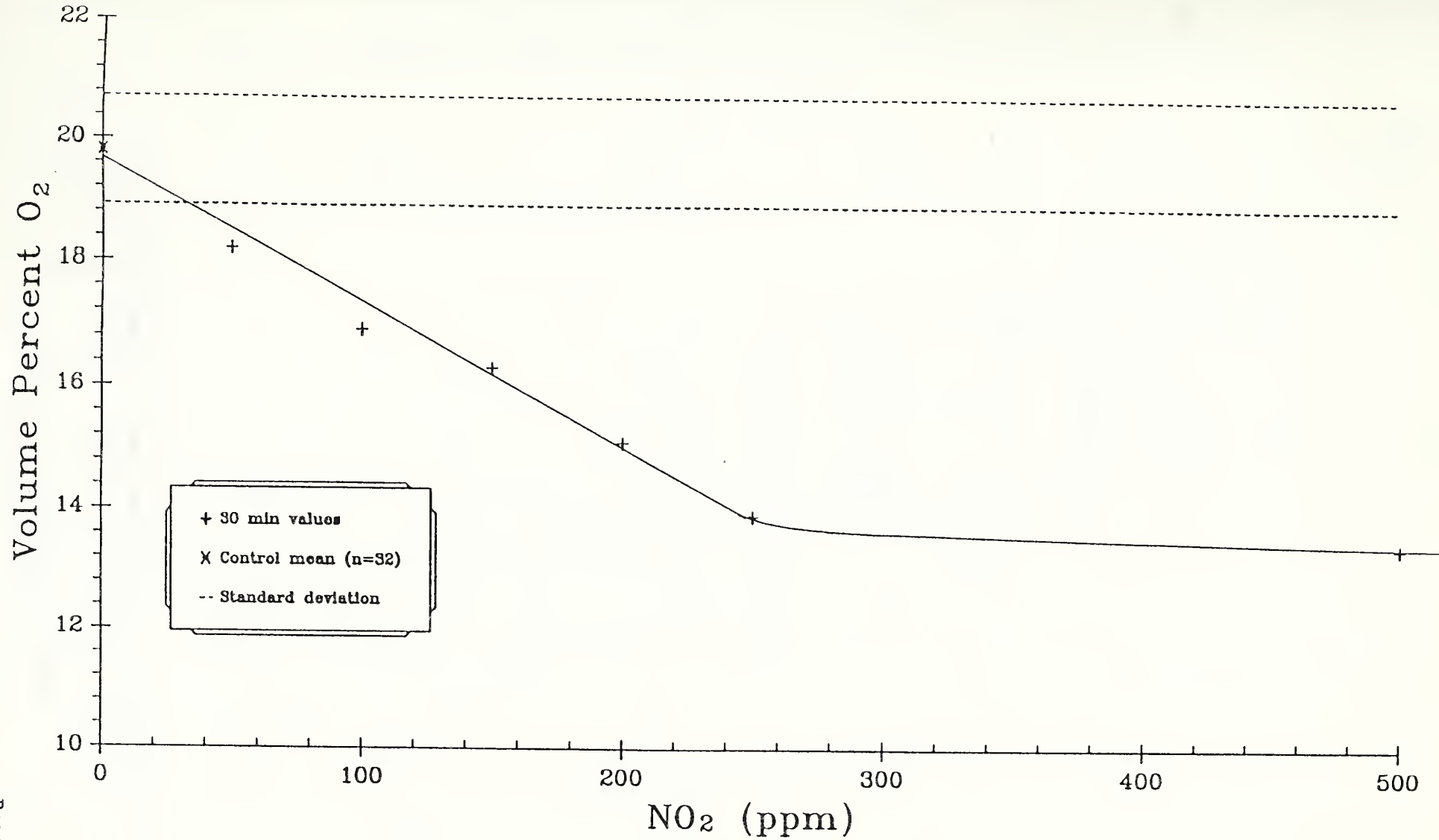

Figure 17. Volume percent oxygen (30 minute values) from various exposures of NO_2. Control mean $\pm$ the standard deviation of 32 animals was 19.8 $\pm$ 0.9

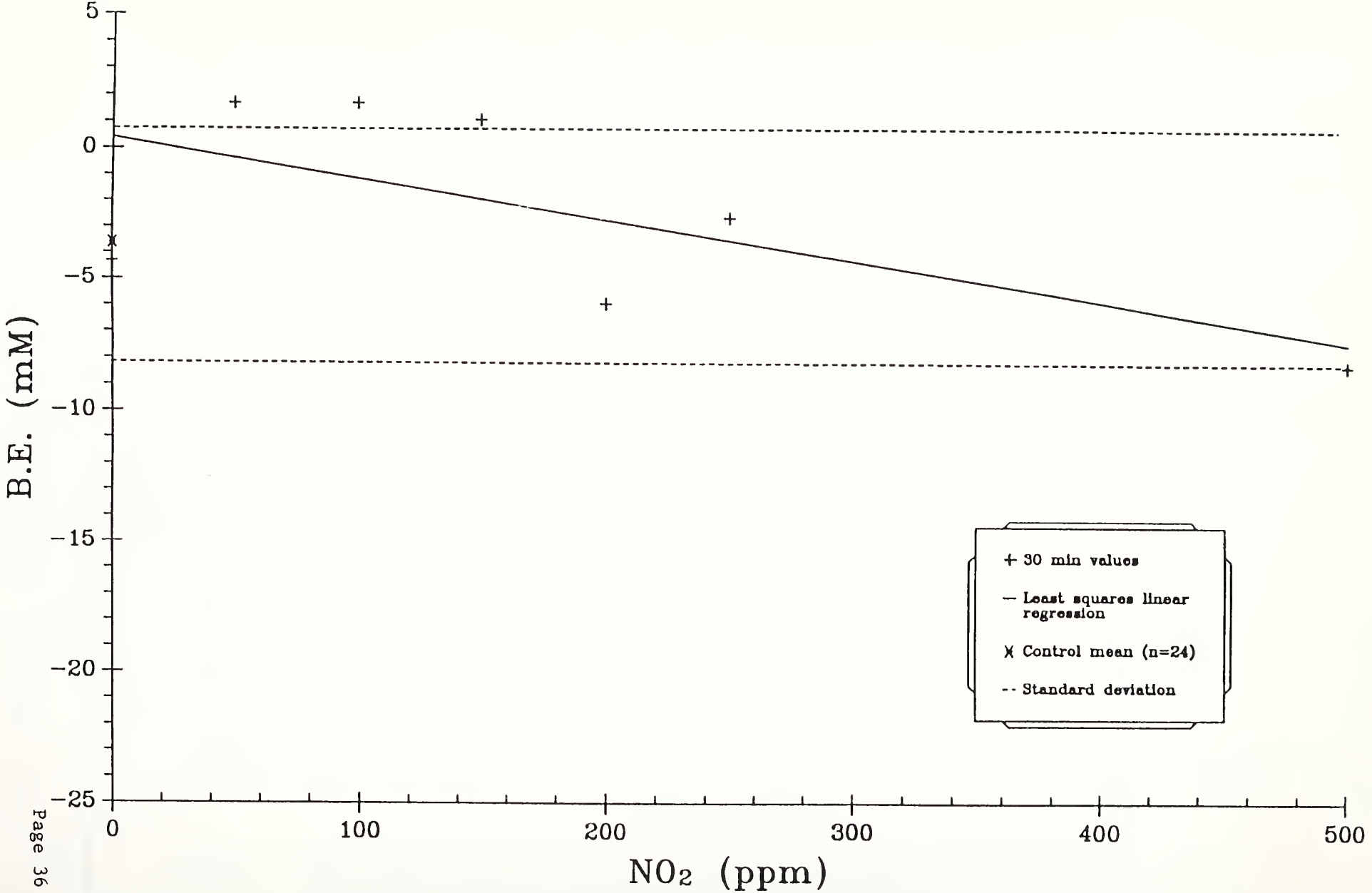

Figure 18. Base excess values (30 minute) from various exposures of NO_2. Control mean $\pm$ the standard deviation of 24 animals was -3.6 ± 4.5

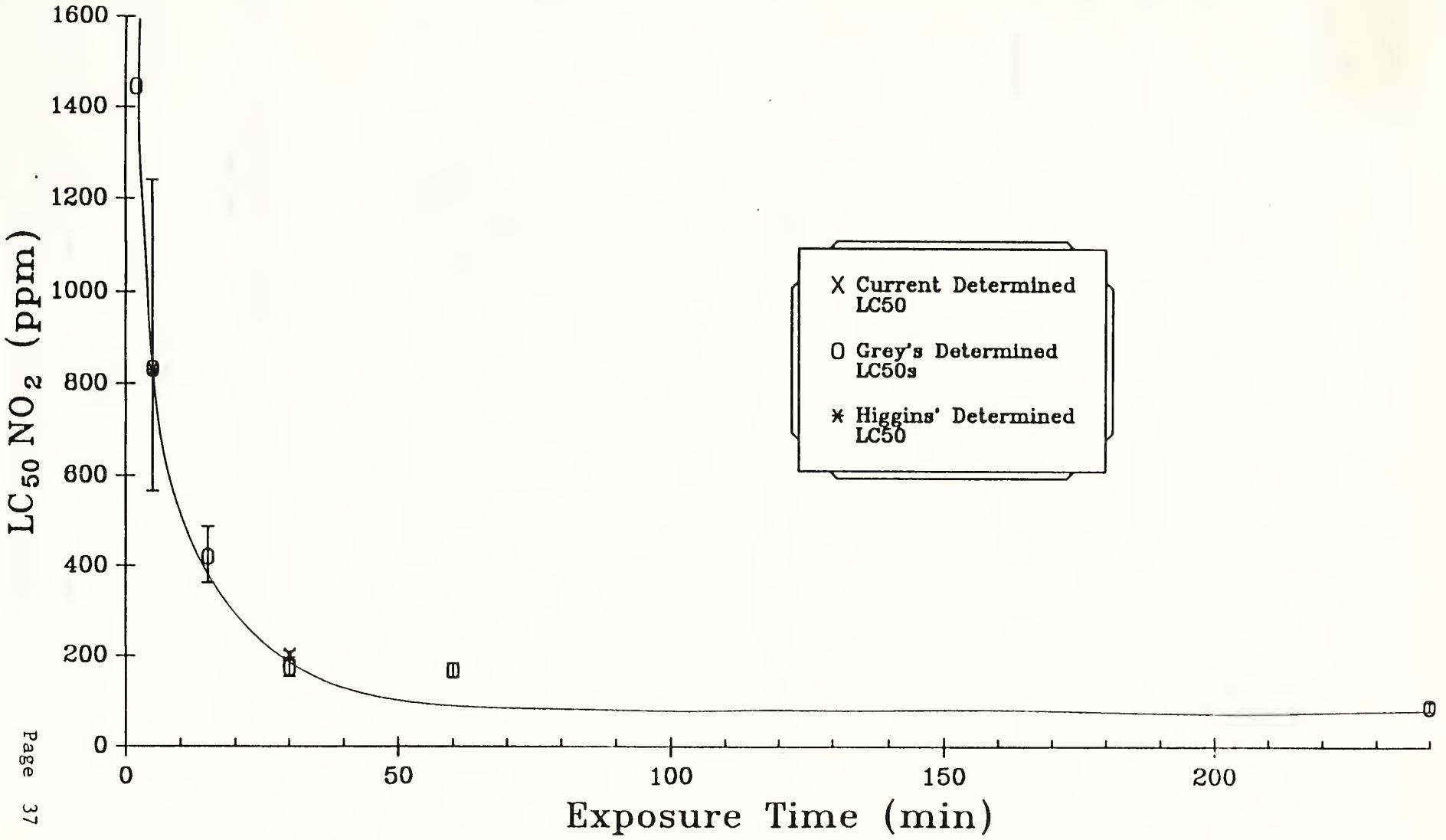

Figure 19. Comparison of literature and NIST LC_{50} values for NO_2.

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11. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)

All fires occurring in air produce carbon dioxide (CO_2). Fire involving nitrogen-containing products will also generate nitrogen dioxide (NO_2), a pulmonary irritant. In Fischer 344 male rats, the LC_{50} (30 minute exposure plus 14 day post-exposure observation period) for NO_2 was 200 ppm (with 95% confidence limits of 43 to 51%); whereas, the LC_{50} for NO_2 in the presence of 5% CO_2 was 90 ppm (with 90% confidence limits ranging from 70-120 ppm). Exposure to NO_2 increased the methemoglobin (MetHb) levels in the arterial blood. At the end of the 30 minute exposures, the MetHb levels were 2-3 times higher in the animals exposed to the combination of NO_2 (200 ppm) and CO_2 (5%) than in those exposed to NO_2 only. Deaths from NO_2 were all post-exposure and occurred earlier in the presence of NO_2 plus 5% CO_2 than in the absence of the CO_2. The time of death was concentration-dependent when both gases were present. At death, evidence of hemorrage and extensive edema was observed in the lungs. The mean lung wet weight/body weight weight ratio from rats exposed to 200 ppm NO_2 with and without 5% CO_2 was 3-4 times that of non-exposed rats. More edema was noted with NO_2 and CO_2 than with NO_2 alone.

12. KEY WORDS (Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)

Carbon dioxide, fire gases, inhalation, LC_{50}, lung edema, methemoglobin, nitrogen dioxide, rats synergism, toxicology.

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