EFFECTS OF HEAT STRESS ON NITROGEN DIOXIDE TOXICITY IN MONKEYS

by

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ABSTRACT. In order to assess the effects of hot environments on nitrogen dioxide toxicity, groups of 10 male cynomolgus monkeys were exposed continuously for 90 days to 5 and 10 ppm of NO_2 at 72° E.T. and at 88° E.T. Air control groups were maintained at the respective effective temperatures. Hematological studies included CBC, methemoglobin, plasma volume, and red cell survival. Serum chemistry, blood gases, chest x-rays, and body weights were monitored. Pulmonary function tests were conducted. The results indicated that NO_2 at 10 ppm impaired distribution of ventilation of the lungs, increased respiratory rate, and decreased tidal volume. The addition of heat stress to this level of NO_2 exposure did not further impair distribution of ventilation but it decreased dynamic compliance of the lungs whereas NO_2 alone did not. The combination of 10 ppm NO_2 and heat stress resulted in a mean loss in body weight over the 90-day exposure whereas all other groups gained weight. No effects attributable to NO_2 or heat stress were found on hematological or serum chemistry variables. No deleterious effects were found on blood gases from either factor. No effects on airway resistance were obtained. General observation indicated that the animals were extremely hypoactive in the chambers at 88° E.T. with and without NO_2. No synergistic effects of heat stress were found at 5 ppm NO_2. Nitrogen dioxide related microscopic alterations were seen in the respiratory bronchioles, alveolar ducts and adjacent interalveolar septa. No microscopic morphological effect of heat stress was seen in the lungs from either the nitrogen dioxide exposed or control monkeys.

INTRODUCTION. Nitrogen dioxide (NO_2) has been widely studied as an irritant of the lower respiratory tract and is recognized as a potential health hazard, both as an air pollutant and in industrial operations wherever incomplete combustion occurs. Since such operations as welding, brazing, and soldering often occur in relatively poorly ventilated areas, the ambient local air concentrations of NO_2 may easily approach the current TLV. Furthermore, since these work areas can easily become hotter than normal room temperature, there arises the question of a possible temperature potentiation of NO_2 toxicity in a manner parallel to that found, for example, for ozone[1]. The present study was undertaken to investigate this possibility under "worst case" conditions; i.e., chronic continuous exposure of monkeys to NO_2, at levels known to have toxic sequelae, under continuous maximum tolerable heat stress. The cynomolgus monkey was chosen as the species to be evaluated because of its relative freedom from lung parasites and because it was expected to be fairly susceptible to NO_2 effects, both functional and morphological.

MATERIALS AND METHODS. Animals. Sixty healthy sexually mature male cynomolgus monkeys (Macaca fascicularis) weighing between 2.8 and 6.2 kg at Week 0 were used in this study. A total of 70 monkeys which had been quarantined for 8 weeks were screened in order to obtain a healthy sample. During the 8-week period following quarantine the animals were twice skin-tested negative for tuberculosis, radiographed (thoracic anterior-posterior and lateral views), and passed through a health screening procedure consisting of routine hematology and serum chemistry determinations. Sixty acceptable animals were then grouped into six groups of 10 each equated for body weight distributions as closely as possible. (One back-up monkey was assigned to pass through the battery of pre-exposure tests which followed). The six groups and their exposure conditions are presented in Table I. The singly caged animals had drinking water ad libitum and were fed once daily.

Exposure Conditions. The animals were exposed to their respective experimental environments in Rochester-type cubical 6000-liter glass and stainless steel chambers with pyramidal tops and bottoms operated with a constant through-put of 1000 liters of air per minute. This air was filtered and conditioned by passing it through a conventional air conditioning system to bring it to 72 ± 3° F and 50 ± 5% relative humidity. Additional heat, as required, and additional moisture as needed, to maintain the relative humidities required to sustain the targeted effective temperatures in the chambers within set limits, were provided to the inlet air ducts by in-line heaters and steam injection, respectively. These heaters and steam valves were controlled by thermostats and hygrostats mounted inside the chambers. The resulting dry bulb temperatures and relative humidities were continuously recorded on Rustrak Model 200 recorders calibrated against both a sling

psychrometer and a calibrated Lab-Line Electro-Hygrometer. The respective values were read six times per day for purposes of reporting. Effective Temperature (ET) was read from the normal scale of ET - a nomogram relating dry bulb to wet bulb temperature -, the latter being read from the nomogram relating dry bulb temperature to relative humidity. Due to heat loss to the chamber room, a dry bulb temperature difference of 5° F existed between the pyramidal top of the chambers where the sensors were mounted and the bottom of the stainless steel wire cages in the chambers maintained at high temperature; therefore, since the calibration dry bulb temperatures were measured at the middle of the cages while the chamber was monitored near the top of the chamber, a correction factor of 5° F was applied to the recorded data.

NO_2 was metered via a fail-safe system into the air input duct leading into the turret on top of the chamber and the air was exhausted from the bottom. Chamber concentration analyses were performed on samples drawn from a standard probe approximately 18 inches above the top of the middle cage. Analyses were made at least once daily by the method of Saltzman[2] and the chambers were monitored continuously using two Atlas Electric Devices Company NO_2 Analyzers automatically time sharing two chambers each at 15-minute intervals. NO_2 was provided as a 5 ± .5% mixture of 99.5% certified pure NO_2 in nitrogen from cylinders. The generation of NO_2 into the chambers was continuous except for approximately 60 minutes per day during which time the chambers were opened for cleaning and feeding the animals and/or for removing them for testing, weighing, etc. The temperature and humidity conditions were similarly maintained in parallel. NO_2 samples were drawn after at least one hour of chamber operation.

The nominal 10 ppm groups were treated somewhat differently than the other groups. Initially, what was planned to be Group 5 (Group 5') was started at ≈ 10 ppm and 72° E.T., but after just under nine hours exposure (during which time the chamber NO_2 concentration was analyzed to be between 10.22 and 11.56 ppm in four determinations beginning one-hour after startup), three monkeys appeared to be dying or dead. When the chamber was opened 10 hours after start-up, the three animals were dead and a fourth (No. 280) was in serious respiratory distress. Inasmuch as what was planned to be Group 6 (Group 6') had not yet been started, the six healthy appearing survivors of Group 5' and the members of Group 6' were combined and then divided to form two new groups of eight each, equated as to prior exposure histories and body weights. The new Group 5 was then started-up two days later at ≈ 5.0 ppm and gradually brought up to 10 ppm over a 5-day period. New Group 6 exposures were similarly graduated. Group 2 suffered a death, apparently from heat prostration, on Day 13. The extra monkey (No. 715) was used as his replacement for the remainder of the study. Because he was somewhat lighter in weight than the original animal, the group mean was lowered. The remaining survivor from the original Group 5' (No. 280) was carried as a ninth member of the new Group 6, but was not included in any of the group analyses. He survived to the end of the study. No further deaths occurred during the study.

Animal Measurements. Body weights were recorded weekly before and during the 13-week exposure period. The animals were inidividually observed for signs of toxicity and well-being, frequently during the days and occasionally during the nights. Hematological determinations (consisting of RBC, WBC, differential leukocyte counts, reticulocyte count, hemoglobin concentration, and hematocrit) and serum chemistry determinations (consisting of Na, K, Cl,

CO_2, Ca, protein, albumin, total bilirubin, BUN, glucose, alkaline phosphatase, SGOT, and SGPT) were made prior to and after 13 weeks on study. In addition, hematocrit, hemoglobin, and methemoglobin (Evelyn - Malloy method) were measured after 4, 8, and 13 weeks and plasma volume (Evans blue dye dilution method) was determined prior to and after 13 weeks of exposure.

Also, prior to and starting after eight weeks of exposure, an erythrocyte half-life determination (^{51}Cr-tagged red cell method) was made on each animal. To a 10 ml ACD solution pre-warmed to 37°C, 15 ml of heparinized blood was added and mixed gently with 30μCi of sodium chromate ^{51}Cr. After incubation for 20 minutes, 100 mg of sodium ascorbate were added and 10 ml of the blood sample reinjected intravenously into the donor animal. Thirty minutes later, 6-7 ml of arterial blood was drawn from the opposite leg and duplicate 3 ml aliquots prepared in heparinized counting tubes as 100% standards. Similar blood samples were drawn on Days 1, 4, 9, 15, 22, and 29 and counted against the 100% standard to correct for radioactivity decay. Five-minute counts were taken using a Nuclear-Chicago Mark I liquid scintillation counter. These counts were individually plotted against days since tagging and the best-fit curve drawn for each animal. The number of days to 50% initial (100%) count was estimated for each animal from these curves. Percent red cell survival was corrected for the hematocrit of each sample.

Pulmonary function tests were performed before and after exposures as follows:

Mechanical Properties of the Lung. Measurements of total respiratory system flow resistance during inspiration [Rrs(i)] and during expiration [Rrs(e)] were made on unanesthetized monkeys according to the procedure of Alarie et al.[3]

after the method of Mead[4]. Using the method described by Alarie et al[5], measurements were made of tidal volume (V_T), respiratory rate (RR), minute volume (MV), pulmonary flow resistance (Rl), airway resistance during inspiration [Raw(i)] and during expiration [Raw(e)], and dynamic compliance of the lung (Cdyn). At each measurement period, each monkey was measured at least twice for the first two parameters.

Distribution of Ventilation. The distribution of pulmonary ventilation was inferred from the results of a multiple breath nitrogen-washout test as described for use with awake monkeys by Alarie et al[6]. Time, number of breaths (BR), and cumulative tidal volume (CV_T) to reach 1% N_2 during pure oxygen breathing were measured twice at each interval as was respiration rate during pure oxygen breathing (RR-V). The above measurements were made on fully awake monkeys seated in restraining chairs.

Functional Residual Capacity. Functional residual capacity (FRC) was determined on anesthetized monkeys by the helium equilibration technique as described by Meneely and Kaltreider[7] modified to employ a cuffed endotracheal tube. The animals were anesthetized with pentobarbital sodium, and tested in the supine position. A lubricated endotracheal tube of a size appropriate to each monkey was inserted per orally after administration of a local anesthetic spray (tetracaine hydrochloride) into the larynx. After a few minutes to assure quiet, even breathing, the test was commenced. Since the test was a before-and-after exposure comparison, no dead-space corrections were made to the determined FRC. In any case, dead-space was a very small percentage of the total measured volume.

Arterial Blood Gases. Arterial blood, obtained from the femoral artery while the monkeys were tranquilized with phencyclidine hydrochloride was measured for oxygen tension (Pa_{O_2}), carbon dioxide tension (Pa_{CO_2}), and

acidity (pH). Measurements were made at 37.5°C utilizing P_{O_2}, P_{CO_2}, and pH electrodes on a radiometer as described by Banerjee et al[8].

Sacrifice Procedures and Histopathological Evaluation. Following the 13-week exposure period, all animals were sacrificed by exsanguination under pentobarbital sodium anesthesia. Complete necropsies were performed on all animals which died on study and on all animals sacrificed. A detailed histopathological evaluation of the lower respiratory tract was made on each animal. The lungs were fixed while inflated at expiratory volume in 10% neutral buffered formalin. Six micron sagittal plane sections were made of each of the seven lobes of the lung, mounted, and after staining with hematoxylin-eosin, were examined microscopically.

Statistical Analysis. All the data at each measurement interval were analyzed on a Datapoint 2200 Minicomputer programmed to perform a multiple group analysis of covariance using the pre-exposure value for the given parameter as covariate. In all cases, if the resulting "treatment/error" F-ratio was significant with $P \leq .05$, t-tests for significant differences between pairs of group means were performed with the means adjusted for pre-exposure differences. Again, the .05 level of probability was chosen for rejecting the null hypothesis. Two-tailed tests were used for all analyses; however, judgments as to impairment of the animals usually rested on a unidirectional assumption.

RESULTS. Body Weight. There was a clear impairment of normal body weight gain due to the exposures to 9.64 ppm and 9.63 ppm NO_2 under both E.T. conditions, with a significantly greater effect from the combination of 9.63 ppm NO_2 with 88.4° E.T. This group, in fact, lost weight over the first eight weeks and never gained its starting weight while all other groups gained weight over the course of the study, as may be seen in Figure 1. This mean weight loss was not specifically due to the contribution of the animals which were initially exposed to ≈ 10 ppm as members of Group 5' and then subdivided, as survivors, into new Groups 5 and 6. These animals, with the exception of No. 588, gained weight; No. 588 lost weight, as did four other members of Group 6 while only one member of Group 5 lost weight. Repeated tranquilization with phencyclidine HCl during Week 8, necessitated by the red-cell survival test, resulted in weight losses in all groups which were only gradually reversed.

Observations. Aside from the acute mortalities from exposure to ≈ 10 ppm and the death in Group 2 on Day 13, the observations grouped around two phenomena: (a) all the animals in Groups 2, 4, and 6 (hot chambers) were grossly hypoactive and spent a large portion of their time either prone or supine on the cage floor and (b) occasional vomiting was observed when first exposed to the heat stress.

Hematology and Clinical Chemistry. None of the hematological or clinical chemistry values indicated any consistent deleterious effects of either NO_2 or high effective temperature alone or in combination, although Group 6 had a slightly low RBC at 13 weeks exposure. Plasma volume was not affected by the conditions of exposure nor was red cell survival as indicated by half-life determination. Table II presents the group means for hematocrit,

hemoglobin, methemoglobin, and plasma volume before and after 13 weeks of exposure, and for erythrocyte half-life before and after eight weeks of exposure.

Pulmonary Function. The group means for pre- and post- exposure pulmonary function measurements are shown in Table III. Evaluation of the pulmonary function data obtained revealed no effects on total respiratory system resistance. Group 6 displayed a severe reduction in V_T and a slight increase in RR relative to Group 1 and also to Group 4 after 13 weeks of exposure to 9.63 ppm NO_2 and 88.4° E.T. Group 5 also increased RR significantly, as did Group 3 to a lesser degree, but showed only a slight, nonsignificant decrease in V_T. Minute volume (MV) measurements showed the following effects: Group 6 had a significant decrease relative to Groups 3, 4, and 5 as expected, and a not quite significant decrease relative to Group 1. There thus appeared to be a dose-related effect on respiration pattern from NO_2 exposure which was aggravated by the addition of heat stress only at 9.63 ppm NO_2. Pulmonary flow resistance and airway resistance measurements were not adversely affected either by NO_2, by heat stress, or by the combinations. Cdyn was significantly reduced in Group 6 relative to all other groups. Every animal in Group 6 showed a decrease at Week 13 compared to pre-exposure. Respiration rate measured during the nitrogen washout test (RR-V) showed that these results paralleled the results for RR during mechanics testing above. The number of breaths to 1% N_2 increased as a function of NO_2 concentration although significantly only at the high level (Groups 5 and 6). Heat stress did not appear to exacerbate this effect. Cumulative tidal volume to 1% nitrogen was unaffected by either type of treatment or by the combination of NO_2 and heat stress. FRC was not significantly affected by the treatments. There were no meaningful effects on Pa_{O_2}, Pa_{CO_2}, or blood pH.

X-Rays. No changes in thoracic radiographs attributable to exposure conditions were discerned.

Histopathology. Sections from each lobe of the lungs from all the animals were examined microscopically and alterations related to the exposures to ≈ 5 and to ≈ 10 ppm of nitrogen dioxide were seen at the level of the respiratory bronchioles.

A slight to moderate hyperplasia of the epithelium of the respiratory bronchioles was seen in all of the animals exposed to ≈ 5 ppm of nitrogen dioxide, both with and without heat stress. A minimal to slight bronchiolitis, characterized by a mucosal and submucosal infiltration of mononuclear macrophages, lymphocytes, and polymorphonuclear granulocytes was also seen. The alveolar walls adjacent to affected respiratory bronchioles were somewhat thickened and infiltrated with mononuclear macrophages and lymphocytes. Occasionally, some Type II pneumonocyte hyperplasia was also evident in the alveoli adjacent to affected respiratory bronchioles. No differences were noted between the non-heat stressed and heat stressed animals.

A moderate to moderately severe bronchiolitis and bronchiolar epithelial hyperplasia was seen in all of the animals exposed to 10 ppm of nitrogen dioxide, both with and without heat stress. An accompanying moderate to marked alveolitis adjacent to the affected respiratory bronchioles was also seen. The bronchiolitis was characterized by a mucosal and submucosal infiltration of mononuclear macrophages, lymphocytes and polymorphonuclear granulocytes. The alveolitis was characterized by an infiltration of mononuclear macrophages and lymphocytes into the alveolar walls. In several animals, focal pulmonary edema, ranging in severity from minimal to moderate, was also seen.

The three animals (Animal Nos. 272F, 279F, and 498F) in the group exposed to 10-12 ppm of nitrogen dioxide without heat stress which died during the first day of exposure were examined separately. In these animals there was moderate to marked pulmonary edema and an acute necrotizing bronchiolitis characterized by necrosis of the respiratory bronchiolar walls and an infiltration of polymorphonuclear granulocytes into the walls and lumen of the respiratory bronchioles.

COMMENT. It had not been anticipated that 10 ppm NO_2 would be acutely lethal to the monkeys since beagle dogs had survived up to 191 days at 26 ppm[9] and rabbits survived a chronic exposure to 8-12 ppm NO_2[10]. The threshold of acute NO_2 response for most species studied was estimated by Stokinger and Coffin[11] to be somewhere above 50 ppm; with the 4-hour rat LC_{50} being around 80 ppm[12]. On the basis of the present study it would appear that the cynomolgus monkey is a sensitive species to the acute effects of NO_2. Of interest, therefore, was the fact that, following an initial 9-hour exposure to an NO_2 concentration (10-12 ppm) which was lethal to three out of 10 monkeys within 10 hours, the survivors were able to survive an additional 90 days of nearly continuous exposure to 9.63 ± 0.44 ppm NO_2 under conditions either of 72° E.T. or 88° E.T. Also of interest was the fact that these survivors could not be clearly differentiated functionally or morphologically from those monkeys which began their 90-day exposures with gradually incremented concentrations from ≈ 5 to ≈ 10 ppm over a five-day period, although the former all had moderate to severe subacute bronchiolitis, whereas a few of the latter had only a slight degree of bronchiolitis. Thus, the monkey, like other species, appears to adapt readily to continuous NO_2 exposure if he is not killed by it meanwhile. The pulmonary edema seen in the acute mortalities was reduced in severity in the chronic specimens while bronchiolar epithelial hyperplasia was well developed.

Ninety days exposure to 5.09 ± 0.17 ppm NO_2 at 74.2 E.T. produced no significant pulmonary function changes except a slightly increased respiratory rate. This effect was not seen in Group 4 (88.2 E.T.) exposed to 4.87 ± 0.42 ppm NO_2, perhaps because the hypoactivity induced by the heat stress reduced oxygen demand and hence allowed for a relatively lower

MV in order to ventilate adequately. These lower level NO_2 exposures caused slight degrees of bronchiolitis and alveolitis in both groups as well as slight to moderate bronchiolar epithelial hyperplasia. No hematological or serum chemistry changes were seen and their were no significant effects on body weight in these groups. Neither in functional nor structural terms was there any adverse effect of added heat stress observed.

Exposure to 9.64 ± 0.92 ppm NO_2 at 73.3 E.T. also resulted in a significant increase in RR and RR-V; changes not as clearly manifested by the group exposed to 9.63 ± 0.44 ppm at 88.4 E.T. Both groups required a significantly greater number of breaths to washout to 1% N_2. The addition of heat stress to ≈ 10 ppm NO_2 did produce one functional impairment not suggested by the data on the non-heat stressed group: a severe reduction in dynamic compliance of the lung. Since Cdyn is relatively immune to small changes in RR - which were not significant in Group 6 as compared to Group 5, in any case - this decrease in Cdyn was inferred to be the direct result of a decreased elasticity of the lung. Inasmuch as V_T in Group 6 was also significantly reduced (and Cdyn is directly proportional to V_T), it is possible that the decrease in Cdyn merely reflected this change. However, despite a lower V_T, six of the eight animals in the group showed an increase in intrapleural pressure. This might be expected following a decrease in lung elasticity since the lung would be inhibited in its ability to expand and relax in a synchronous manner with the chest wall. Therefore, it is concluded that the V_T decrease was more likely a consequence of an elasticity decrease rather than that the Cdyn change was simply the arithmetic result of a V_T decrease.

The addition of heat stress to the high level NO_2 exposure had an effect on body weight not attributable to either factor alone, thus indicating a significant interaction. There were no interactions observed, however, on

any of the hematological or serum chemistry parameters measured, including erythrocyte survival, plasma volume, cell volume, and methemoglobin concentration. Furthermore, no NO_2 effects on these parameters were seen. Thus, the effects reported for mice by Ehrman et al[13] were not found in the monkey at 10 ppm. The present negative hematological findings parallel the results for dogs reported by Wagner et al[14].

Despite the evidence of a decrease in lung compliance in Group 6, there were no histopathological concomitants observed to explain it. Group 6 animals were indistinguishable from Group 5 which did not show decreased compliance, and neither group showed evidence of fibrosis or other structural changes which might account for decreased elasticity of the lung.

Table I - Exposure Groups and Treatments in Chronic Study

Group No.	No. Monkeys	Treatment			
		NO_2 ppm		Effective Temperature E.T. Units	
		Target	Actual*	Target	Actual**
1	10	0	0	72	72.5 ± 0.5
2	10	0	0	88	88.2 ± 0.8
3	10	5	5.09 ± 0.17	72	74.2 ± 1.3
4	10	5	4.87 ± 0.42	88	88.2 ± 0.8
5	8	10	9.64 ± 0.92	72	73.3 ± 0.8
6	8	10	9.63 ± 0.44	88	88.4 ± 0.7

* Mean ± S.D. of 91 daily averages of $\geq$ 1 daily wet chemistry determinations.

** Mean ± S.D. of weekly averages based on 91 daily dry bulb and relative humidity readings.

Table II - Means for Hematocrit, Hemoglobin, Methemoglobin, Plasma Volume, and Erythrocyte Half-life Measurements: Pre- and Post- Exposure

Measurements (Units)	Interval	Groups (Nominal ppm NO_2, Effective Temperature) 1(0,72°)	2(0,88°)	3(5,72°)	4(5,88°)	5(10,72°)	6(10,88°)
Hematocrit (%)	Pre-	37.9	38.5	38.7	38.8	38.9	39.6
	Post-	37.3	37.3	38.4	36.0	36.9	35.2
Hemoglobin (g%)	Pre-	11.1	11.3	11.1	11.5	11.2	11.4
	Post-	11.8	11.7	12.2	11.3	11.4	11.1
Methemoglobin (%)	Pre-	1.1	1.2	1.7	1.1	1.3	1.1
	Post-	2.1	0.8	1.2	2.3	1.1	1.9
Plasma Volume (ml/kg)	Pre-	44.2	47.8	47.5	44.2	49.2	47.8
	Post-	47.8	45.8	45.1	44.5	48.2	45.9
Red Cell Half-life (Days)	Pre-	11.1	11.9	12.8	12.6	11.5	10.6
	Post-	10.9	10.6	11.4	12.1	10.9	11.1

Table III. Means for Pulmonary Function Pre- and Post- Exposure

Measurement (Units)	Interval	Groups (Nominal ppm NO_2, Effective Temperature) 1(0,72°)	2(0,88°)	3(5,72°)	4(5,88°)	5(10,72°)	6(10,88°)
Rrs(i) (cm H_2O/ml/sec)	Pre-	.034	.039	.041	.045	.029	.037
	Post-	.058	.059	.061	.064	.065	.059
Rrs(e) (cm H_2O/ml/sec)	Pre-	.039	.043	.040	.043	.033	.039
	Post-	.044	.045	.043	.041	.044	.042
RR (breaths/min)	Pre-	39	33	38	35	35	36
	Post-	37	39	44	40	50	44
V_T (ml)	Pre-	46	43	48	45	46	50
	Post-	50	44	56	48	44	29
MV (ml)	Pre-	1807	1443	1852	1580	1635	1793
	Post-	1841	1686	2640	1968	2163	1233
RI (cm H_2O/ml/sec)	Pre-	.030	.035	.037	.031	.041	.038
	Post-	.035	.032	.036	.029	.020	.044
Raw(i) (cm H_2O/ml/sec)	Pre-	.027	.037	.035	.027	.039	.038
	Post-	.027	.027	.023	.019	.011	.039
Raw(e) (cm H_2O/ml/sec)	Pre-	.021	.023	.025	.020	.030	.026
	Post-	.037	.035	.029	.023	.017	.038
Cdyn (ml/cm H_2O)	Pre-	8.7	7.6	8.0	8.8	7.4	8.6
	Post-	7.7	8.7	8.2	7.9	7.9	4.4
RR-Vent (breaths/min)	Pre-	41	42	37	42	39	37
	Post-	40	41	47	42	53	44

Table III - Continued

BR (1% N_2) (number)	Pre-	38	30	41	40	35	36
	Post-	43	56	57	69	89	81
CV_T (1% N_2) (liters)	Pre-	1.56	1.19	1.49	1.68	1.33	1.35
	Post-	1.70	1.57	*	1.91	1.79	1.68
FRC (ml)	Pre-	95	86	81	87	85	75
	Post-	95	77	85	88	99	93
Pa_{O_2} (mm Hg)	Pre-	106	98	101	101	103	108
	Post-	95	109	92	98	89	99
Pa_{CO_2} (mm Hg)	Pre-	41	39	39	39	42	42
	Post-	45	42	40	40	42	42
pH (units)	Pre-	7.40	7.37	7.41	7.37	7.40	7.39
	Post-	7.38	7.35	7.40	7.40	7.38	7.36

* Invalid measurements due to technical error.

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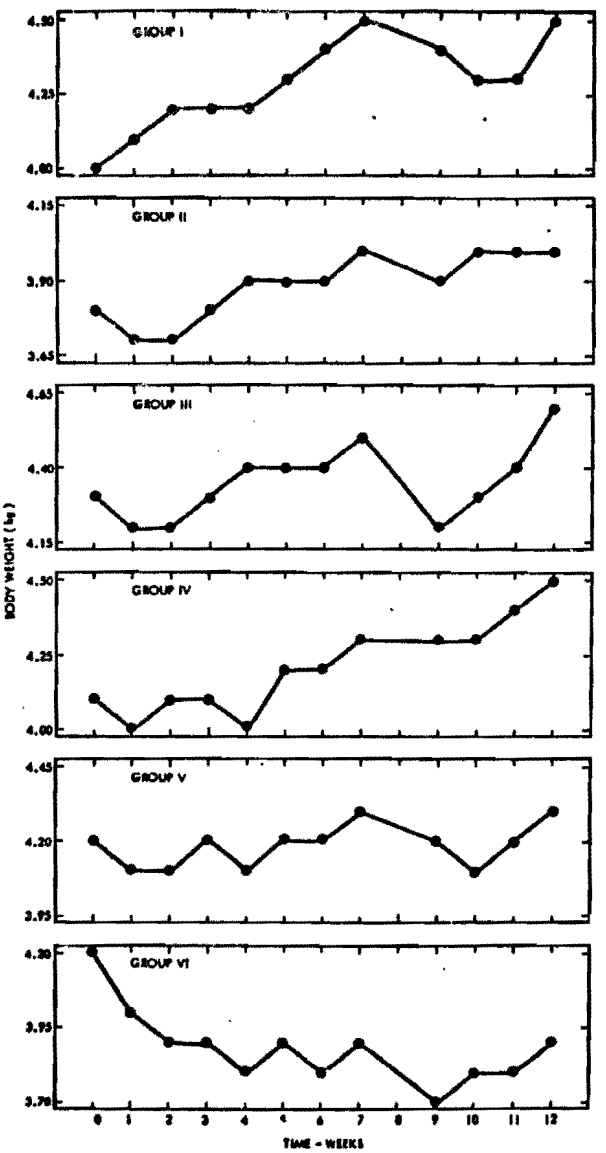
LEGENDS FOR FIGURES

Figure 1 - Body weight prior to and during exposure to nitrogen dioxide with and without heat stress. Each point represents the mean for each group of animals. Values for Week 8 are omitted due to repeated tranquilization of the animals during this interval.

Phencyclidine hydrochloride - Sernalyn®

Tetracaine hydrochloride - Cetacaine®


GROUP I
GROUP II
GROUP III
GROUP IV
GROUP V
GROUP VI
BODY WEIGHT (kg)
TIME - WEEKS
0 1 2 3 4 5 6 7 8 9 10 11 12