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EFFECTS OF CHRONIC INHALATION OF PROPYLENE GLYCOL 1,2-DINITRATE ON THE CONDITIONED AVOIDANCE BEHAVIOR OF PRIMATES

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Four primates (Macaca mulatta) were trained to stable levels of performance on a multiple avoidance schedule of reinforcement. The schedule included alternating sessions of discrete trial cued avoidance and free operant avoidance, separated by 3-minute rest periods. Two of the animals were exposed to successive atmospheric concentrations of propylene glycol 1,2-dinitrate (PGDN) vapors of 1.8, 5.6, 11.0, and 28.2 mg/m^3. Exposure occurred on a 23-hour per day basis

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in a Rochester-type inhalation chamber at the Navy Toxicology Unit. Consecutive exposures lasted 35, 56, 20 and 14 days respectively. The other two animals were housed in an identical inhalation chamber but remained in a normal atmosphere for the duration of the testing period. These animals served as controls. Plasma concentrations of PGDN appeared to increase each time the chamber concentration was increased, however none of the four PGDN concentrations had a discernible effect on avoidance behavior. There was no measurable change in the overall behavior of either test animal which could have been attributed to general debilitation, sensory deficit, or motor dysfunction. Food and water consumption remained unchanged. Necropsy and histopathological examinations were negative. This study employs behavioral performance measures as indices of the presence or absence of toxic effects that could be important in exposure situations involving humans.

SUMMARY
(Nontechnical)

Two primates (Macaca mulatta) trained to stable levels of responding on a multiple avoidance schedule were exposed to atmospheric concentrations of 1.8, 5.6, 11.0 and 28.2 mg/m^3 of PGDN vapor on a 23-hour per day basis for 35, 56, 20 and 14 days, respectively. The multiple avoidance schedule consisted of 100-trial blocks of 1-second discrete trials separated from 10-minute sessions of free operant avoidance (response-shock interval = 10 seconds) by a 3-minute rest period. Two control animals in an ambient atmosphere were tested in a similar manner each day. Blood plasma levels of PGDN increased each time the nominal chamber concentration was increased. There were no significant differences in response rate or pattern of responding between test animals as a function of PGDN inhalation at concentrations up to 28.2 mg/m^3. These results indicate that PGDN produced no gross disruption of avoidance behavior, motor coordination, or sensory function.

PREFACE

This research was sponsored by the U. S. Naval Medical Research and Development Command under work unit MF 51.524.023.0001. The authors are grateful to their associates who participated in and contributed to various phases of this study. The PGDN exposures were carried out under the direction of R. A. Jones. L. Kurlansik monitored the atmospheric concentration as well as analyzed the plasma PGDN concentrations in the experimental animals. R. L. Brubaker trained and cared for the animals. B. A. Dennison, G. G. Kessell and P. Mannon tested the animals and collected the data.

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INTRODUCTION

Industrial experience with aliphatic nitrates, primarily glyceryl trinitrate (nitroglycerine) and ethylene glycol dinitrate (EGDN), has led to both anecdotal and experimental documentation of the "nitrate effect". The principal biological effects of nitrates are vasodilation and methemoglobin formation. Vasodilation is associated with hypotension, intense throbbing headache, flushing, palpitation and, less frequently, nausea, vomiting or abdominal distress. Although there is wide variability in individual susceptibility to nitrate effects, those occupationally exposed to these materials develop a tolerance to the headaches and exhibit no symptoms as long as some exposure to the material is maintained.[3] Clark and Litchfield[1] have reported vasodilation, lowered blood pressure and methemoglobin formation associated with exposure to propylene glycol 1,2-dinitrate (PGDN). They also report little difference in the metabolic and pharmacologic properties of PGDN and EGDN from oral, subcutaneous and percutaneous administration to rats, mice and cats. Kylin et al.[6] compared the toxicity of PGDN, EGDN and nitroglycerine and found PGDN and EGDN comparable in effect on mice, and both to be less toxic than nitroglycerine. Litchfield[7] has demonstrated that PGDN is a monoamine oxidase inhibitor which could alter behavior by affecting central nervous system catecholamines.

Jones et al.[5] exposed several groups of monkeys to PGDN vapors in both short- and long-term inhalation studies. One of nine squirrel monkeys (Saimiri sciureus) died after 31 days of exposure to 236 mg/m^3 PGDN vapor. At 500-700 mg/m^3 rhesus monkeys (Macaca mulatta) exhibited such signs of toxicity as vomiting, pallor, cold extremities, semiconsciousness and clonic convulsions within 6 hours, while other rhesus monkeys continuously exposed to 262 mg/m^3 of PGDN for 90 days showed no signs of toxicity including no change in the performance of a visual discrimination or visual acuity threshold test.

Stewart et al.[9] reported that PGDN concentrations of 1.3 mg/m^3 or greater disrupted the organization of the visual evoked response (VER) and produced headache in a majority of human volunteers tested. However, tolerance to headache induction developed when the subjects were repeatedly exposed to 1.3 mg/m^3 for 8 hours on a

daily basis. At 3.3 mg/m^3, 6- to 8-hour exposures markedly impaired the performance of the heel-to-toe and modified Romberg tests. Eye irritation occurred at 9.9 mg/m^3. The overall effects were interpreted as being consistent with the VER changes produced by central nervous system (CNS) depression. Based on the work reported by Clark and Litchfield,[1] Jones et al.[5] and Stewart et al.,[9] the Threshold Limit Values (TLV) Committee of the American Conference of Governmental Industrial Hygienists has proposed a TLV for PGDN of 0.35 mg/m^3.

The present study was undertaken to determine if any evidence of general CNS change could be detected in the behavior of monkeys chronically exposed to PGDN.

METHODS

Four male rhesus monkeys (Macaca mulatta) 28 to 36 months of age and weighing 5.1 to 8.3 kg were used in the study (Table 1). Food and water were available throughout training and testing on an ad libitum basis.

Table 1. Age and Weight at Start of Study

	Animal Number	Age (months)	Weight (kg)
Control Animals	B2-49	33	8.3
	HI-46	32	5.4
Test Animals	W-26	36	5.4
	A2-35	28	5.1

The animals were trained to respond to a multiple avoidance schedule in a test cage constructed of Lexan and aluminum. Two levers and two lights (one over each lever -- used for visual stimulus presentation) were installed on a Lexan interface panel. The panel served as one side of the test cage and also contained a spring-loaded door, a plastic water bottle, and a plastic food box. The other five sides of the

test cage were constructed of aluminum bars 3/8 in. (.95 cm) in diameter and 1-1/8 in. (2.86 cm) apart on center. The bars served as a grid for scrambled shock presentation. The outside dimensions, 20 x 20 x 36 in. (50.8 x 50.8 x 91.4 cm), were chosen to permit installation of two cages in each of the Navy Toxicology Unit inhalation chambers and still provide maximum available living space for the primates. The necessary electronic control equipment was housed outside the training or test cages. Data were recorded on a 14-channel Hewlett-Packard analog tape recorder for later computer analysis.

The multiple avoidance schedule was composed of three response contingencies: (1) a 100-trial block of discrete, cued avoidance trials; (2) a rest period; and (3) a 10-minute session of free operant avoidance. Figure 1 presents the order in which the avoidance schedules occurred during a daily testing session.

Avoidance Schedule	Cued	Rest	Free	Rest	Cued	Rest	Free	Rest	Cued	Rest	Free	Rest	Cued	Rest	Free
Time (Minutes)	10	3	10	3	10	3	10	3	10	3	10	3	10	3	10

Figure 1. Daily test session

Cued avoidance. In each discrete trial of cued avoidance, an animal was given 1 second to respond to the lever cued correct by a red light directly above the lever. If the animal operated the correct lever, a 0.2-second, 6-mA shock was avoided for that trial. If the animal operated the incorrect lever, a brief shock was administered immediately. If the animal failed to operate either lever, a shock occurred at the end of the 1-second trial period. Trials were separated by 5 seconds of time-out. The red cue light was terminated by a correct or incorrect response or by the end of the trial; the cue light was not illuminated during the time-out period. Trials on each lever were alternated in a nonsystematic fashion. An equal number of trials were presented on each lever during each 100-trial block of testing. Neither lever was ever cued correct more than three times consecutively.

Rest. A 3-minute rest period separated all cued avoidance and free operant avoidance test periods. No cue lights were provided and the animals were given no warning of the onset of the next test period.

Free operant avoidance. The free operant avoidance schedule permitted an animal to avoid the 0.2-second shock indefinitely as long as the interval between successive responses (interresponse time, or IRT) on the right-hand lever never equalled or exceeded 10 seconds. Each response automatically reset the shock avoidance period to 10 seconds (response-shock or RS interval = 10 seconds). If the time between responses reached 10 seconds, brief shocks were administered once every second until the animal made a response to the right lever (shock-shock or SS interval = 1 second). The left lever was inoperative during the free operant avoidance periods. The red cue light directly above the right lever remained on continuously for the 10-minute duration of the period in which the free operant avoidance schedule was in effect.

A detailed discussion of discrete trial and free operant avoidance schedules may be found in Honig.[4]

A 10-ml blood sample was drawn from each animal on the experimental days indicated in Figure 2. At the end of the study, the animals were euthanatized and necropsied. Tissue samples were also submitted for histopathologic examination.

Procedure. Initial training was performed in isolation boxes with the animals confined in restraint chairs. When performance during a daily session met a predetermined criteria of stability, the animals were transferred to the test cages and conditioned to perform the multiple avoidance task in an open environment. After the animals again reached a stable level of performance, they were transferred with the primate test cages to the inhalation chambers. Two animals were housed in separate cages in each of the two chambers and were visually isolated from each other by a plywood barrier. The animals lived in the test cages on a continuous basis. The chambers were opened for 1 hour each morning for feeding, watering, and general maintenance. Stabilization training (35 days) was conducted for 5 additional weeks after the animals were placed in the inhalation chambers. The criteria for performance stability which had to be met before exposure to PGDN could begin were a median

of 95 percent correct for at least 20 consecutive days on the cued avoidance schedule coupled with a free operant avoidance response rate for the same 20 days within ± 3 standard deviations of the mean. During stabilization training and exposure to PGDN, the animals were tested for 1 hour and 41 minutes each day, 7 days a week. The 20 days prior to the beginning of PGDN exposure were recorded as base-line performance data for purposes of preexposure and postexposure comparisons.

The two animals in the chamber with a normal atmosphere (HI-46, B2-49) served as controls and were maintained and tested in a manner similar to the two animals exposed to PGDN. The two animals housed in the experimental chamber (W-26, A2-35) served as test subjects. These animals were exposed to four increasingly higher atmospheric concentrations of PGDN for 23 hours per day. Table 2 presents a schedule of PGDN concentrations and the duration of exposure at each dose level. Exposure to PGDN lasted a total of 125 days. Following the final exposure, the animals in the test chamber were returned to normal atmosphere and tested for an additional 16 days.

Exposure chamber. The Rochester-type inhalation exposure chamber was approximately 2 m^3 in volume and was modified for continuous use.[2] Atmospheric air was passed through the PGDN mixture, contained in a gas washing bottle, and into the chamber. Dilution air flow through the chamber varied from 0.5 to 1.0 m^3/min to achieve the desired PGDN concentrations. The chamber atmosphere was monitored chromatographically by drawing an air sample through a Micro Tek seven-part automatic switching valve using vacuum, and sending the contents of a calibrated sample loop through a Dorhmann 2460 chromatograph equipped with a 6 ft x 1/4 in. (1.8 m x .64 cm) glass column containing 2.92 percent OV-17 on Anakrom Q 70 '80 mesh at a temperature of 110°C using nitrogen at 70 ml/min and an electron capture detector at 150°C with a voltage of 20 V dc. Long lines and switching valves were heated to prevent condensation. A second independent method of analysis was used by drawing a known volume of the atmosphere through a bubbler equipped with a coarse frit and containing ethyl alcohol as the absorbing media. The sample was then read at 220 nm on a Beckman DU spectrophotometer (A = 1650).

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There were no behavioral changes in the animals exposed to PGDN which would suggest toxicity. No indication of significant deviation in avoidance performance, appearance or nutrient consumption occurred for exposures of PGDN up to an atmospheric concentration of 28.2 mg/m^3.

Free operant avoidance. The average interresponse times (and standard error of the mean) for the test and control subjects are presented in Table 2. The average IRT for each subject was determined for preexposure testing, testing at each PGDN concentration and for postexposure testing. Tested with a Friedman Analysis of Variance (ANOV) for repeated measures, there was no significant difference between the treated and nontreated control subjects ($p < .8$).

Table 2. Average Free Operant Avoidance Interresponse Times for Both Control and Chronically Exposed Monkeys

PGDN CONCENTRATION (mg/m^3) MEAN ± SE	NO. DAYS TESTED AT EACH CONCENTRATION	PGDN EXPOSED ANIMALS MEAN ± SE		UNEXPOSED CONTROL ANIMALS MEAN ± SE	
		W-26	A2-35	B2-49	HI-46
0	21	3.212 ± .128	3.001 ± .136	2.350 ± .096	2.339 ± .137
1.8 ± .11	35	3.556 ± .138	3.366 ± .071	1.748 ± .059	1.764 ± .052
5.6 ± 1.0	56	3.968 ± .084	3.450 ± .069	1.709 ± .043	2.184 ± .067
11.0 ± .20	20	3.479 ± .112	3.493 ± .162	1.414 ± .096	2.318 ± .076
28.2 ± 1.30	14	3.606 ± .164	3.543 ± .148	1.754 ± .069	2.074 ± .084
0	16	3.624 ± .124	3.431 ± .213	1.543 ± .036	2.144 ± .077

Discrete-trial cued avoidance. The average response latencies (and standard error of the mean) for the test and control subjects are presented in Table 3. The average response latency for each subject was determined for preexposure responding, testing at each PGDN concentration and during the postexposure period. Tested with a Friedman ANOV, there was no significant difference across all conditions in the treated and nontreated control subjects ($p < .5$).

Table 3. Average Cued Avoidance Response Latency for Both Control and Chronically Exposed Monkeys

PGDN CONCENTRATION (mg/m^3) MEAN ± SE	NO. DAYS TESTED AT EACH CONCENTRATION	PGDN EXPOSED ANIMALS MEAN ± SE		UNEXPOSED CONTROL ANIMALS MEAN ± SE	
		W-26	A2-35	B2-49	HI-46
0	21	.750 ± .011	.645 ± .014	.782 ± .006	.651 ± .013
1.8 ± .11	35	.813 ± .004	.705 ± .006	.749 ± .009	.640 ± .007
5.6 ± .10	56	.809 ± .004	.709 ± .004	.743 ± .004	.690 ± .001
11.0 ± .20	20	.828 ± .005	.702 ± .006	.769 ± .014	.743 ± .025
28.2 ± 1.30	14	.804 ± .007	.690 ± .006	.761 ± .019	.731 ± .021
0	16	.785 ± .006	.679 ± .010	.734 ± .005	.687 ± .005

Rest period. There were no significant differences between the test and control animals in the number of responses emitted before, during, or after response to PGDN. Intra-animal comparisons of the number of responses emitted during the rest periods by W-26 and A2-35 showed no difference between base-line, test, and recovery performance.

Pathological findings. Necropsy and histopathologic findings were within normal limits.

DISCUSSION

The results of this study indicate that inhalation of PGDN vapor at nominal chamber concentrations as high as 28.2 mg/m^3 does not disrupt avoidance behavior as tested by cued and free operant avoidance schedules. Base-line levels of responding were maintained on both schedules during exposure to all chamber concentrations and after termination of the PGDN exposure. There appeared to be no disruption of the ability to discriminate between the two avoidance schedules as evidenced by the differences in the distributions of response latencies and interresponse times. Further, there were no changes in the rate of responding of either test animal during the rest periods throughout the experiment. Water and food consumption appeared to remain

unchanged. Thus, this test produced no evidence of general CNS disruption. It must be emphasized that these findings do not preclude the ability of other reinforcement schedules, which assess different aspects of behavior, to exhibit changes in responding during exposure to low vapor concentrations of PGDN.

Had the response of the test animals changed significantly on either avoidance schedule, the results would have suggested that PGDN inhalation has a specific differential effect on one avoidance schedule as opposed to the other. An increased response rate on the affected schedule would indicate an excitatory effect while a decrease would have suggested that PGDN inhalation was depressive in nature. A significant decrease in avoidance responding on both schedules would have indicated a general interference with sensory function, motor debilitation, or even a change in motivation. A loss of discriminative capacity would be indicated if the pattern of responding exhibited by the animals during base-line testing on one of the avoidance schedules appeared in the cumulative records of either the other schedule or the rest period. None of the changes discussed above appeared in the data. A detailed discussion of multiple schedules in behavioral toxicology analyses may be found in Sidman.[8]

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