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Filed under Laboratory Procedures

RCC1.960617.001

NAVAL MEDICAL RESEARCH INSTITUTE
NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND

*Laboratory Procedures
Nitrite (VPI X220)
Anti-Lactobacillus (VPI X220)
Toxicity (VPI X220)*

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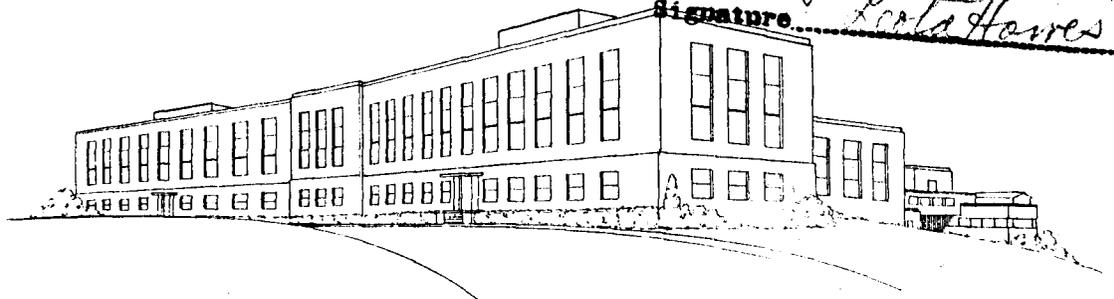
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PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES ON
DIISOPROPYLAMMONIUM NITRITE (V.P.I. X220)

Research Project X-545

Report No. 1

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NATIONAL NAVAL MEDICAL CENTER
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2 February 1946

PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES ON
DIISOPROPYLAMMONIUM NITRITE (V.P.I. X220)

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OBJECT

To determine the pharmacological and toxicological actions of V.P.I. X220 in experimental animals and man; and to determine whether this nitrite is an irritant or sensitizing agent on human skin.

SUMMARY AND CONCLUSIONS

1. Inhalation of the vapors of diisopropylammonium nitrite (V.P.I. X220), a vapor phase rust inhibitor, caused no blood pressure, pulse rate or respiratory changes in the anesthetized dog. When the dog received this compound by mouth, a moderate increase in respiratory rate occurred and was followed by a series of transient drops in both systolic and diastolic blood pressures; there was, however, no accompanying change in pulse rate.
2. Both inhalation of the vapor and exposure of the skin to a solution of the nitrite failed to result in circulatory changes in one human subject.
3. The blood of rats, which had been exposed for 24 hours to an atmosphere containing approximately 46 μ gm. per liter (8ppm.) of this nitrite, was found to contain no detectable quantities of methemoglobin or nitrite.
4. The oral LD-50 for mice was found to be 367.0 mg. \pm 6.7 mg/kg. Rats, which received this dose, were found to have an average of 10.7 gm. per 100 cc. of methemoglobin and 379 μ gm. per cc. of nitrite in their blood.
5. Seven of the eight subjects, whose skin was exposed to the nitrite powder, to an ointment and a solution containing the nitrite, and to cardboard impregnated with it, showed no reactions of any sort. The eighth subject showed no primary irritation but developed sensitization after repeated exposure.

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6. The results of this study show that V.P.I. X220 is definitely poisonous when administered by mouth, but suggest that, following other routes of administration, the nitrite effects are milder than from nitroglycerine or TNT.

7. Protective measures are listed, which may be observed in the handling of this material in bulk. However, it is considered that such precautions are probably unnecessary where naval personnel handle only the nitrite-impregnated cardboard.

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INTRODUCTION

Diisopropylammonium nitrite (V.P.I. X220), chemical formula $((\text{CH}_3)_2\text{CH})_2\text{-NH.HNO}_2$, is reported to be an effective vapor phase rust inhibitor (1). Cardboard tubes are impregnated with the material and then placed in metal systems.

Since laboratory workers are reported to have complained of severe headaches when working with this compound (1), it was submitted for pharmacological and toxicological tests (2).

PROCEDURE AND RESULTS

1. The pharmacological action was studied in the anesthetized dog, after both inhalation of the vapor and oral administration. A 24-hour fasted animal was anesthetized (sodium barbital, 250 mg/kg., supplemented with 20 mg/kg. pentobarbital, both intravenously); the carotid artery and trachea were both cannulated in order to obtain kymograph records of blood pressure, pulse rate and respiratory rate.

For varying periods of time, vapor from a heated aqueous solution of the nitrite was introduced through the inspiratory valve of the tracheal cannula. The time of administration of the vapor in each instance was the only quantitative estimate of the amount of nitrite given. Inhalation of the vapors thus, for periods ranging from one-half to one minute at a time, was followed by no change in blood pressure, pulse rate or respiratory rate.

With the animal's respiratory and circulatory record stable, the nitrite was administered by stomach tube (50 mg/kg. in 5 per cent aqueous solution). No immediate changes were observed in the recordings; however, 4 3/4 minutes after the stomach tube was withdrawn, the respiratory rate suddenly increased about five respirations per minute, and this increased rate, with slight transient variations, was maintained throughout the rest of the experiment. About 18 seconds after the change in respiration, there followed a drop in blood pressure; this was accompanied by a lowered pulse pressure due to a relatively greater drop in systolic as compared with diastolic pressure. There was no concurrent change in pulse rate, and, after about 20 seconds, the pressures returned to the previous levels. During the following ten minutes similar transient drops in the pressures were repeated at irregular intervals; thereafter, they were repeated with less frequency and intensity until they failed to occur at all. The kymograph record was followed several minutes to be certain that these phenomena had ceased, when the experiment was terminated and the animal was sacrificed.

2. The nitrite effect was also studied in a human subject who had no known acquired tolerance to nitrites. After a preliminary ten minute period of bed rest, control blood pressures and pulse rates were determined, and

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these were then followed at short intervals after inspiration of the vapor from an unheated jar of crystalline material for several successive periods increased progressively to a full minute. No changes were detectable in the blood pressure, pulse rate or respiratory rate.

In a second experiment, 64 square inches of body surface were covered with a gauze pad wetted with a strong aqueous solution of the nitrite. The pulse rate and blood pressure were determined at five minute intervals for half an hour, while the subject remained at rest. The initial control pulse rate was 80 per minute, and it remained unchanged throughout. The control blood pressure was 120/80 mm. Hg, and the five minute readings, after application of the gauze pad, were 122/80, 120/80, 114/80, 120/80, and 120/80 mm. Hg.

3. Groups of four rats, fasted 24 hours, were exposed for 24-hour periods in a gassing chamber to air which was first dried and then passed through loosely-packed layers of crystalline V.P.I. X220 in a gas absorption flask. Prior to placing the rats in the chamber, the rate of air flow through the chamber was measured and found to remain very nearly constant at 680 cc. per minute under the experimental conditions. Air samples were also taken to determine the nitrite content; a ten minute sample was found to contain 45.0 μ gm. per liter (8 ppm.), and a 40 minute sample, 47.0 μ gm. per liter (8 ppm.). It is assumed, therefore, that, during the experimental runs with the rats, the nitrite content of the air remained close to 45.0 μ gm. per liter (8 ppm.). This probably is not the saturation value for V.P.I. X220 in air since another air sample, taken during a slower rate of flow through the chamber, was found to contain 60.5 μ gm. per liter (10 ppm.).

The rats were killed by ether anesthesia upon removal from the chamber at the end of the 24-hour experiment. They were exsanguinated, and their bloods were heparinized, pooled and stored under liquid petrolatum. A portion of the pooled sample was analyzed for methemoglobin by the method of Evelyn and Malloy (3); the protein-free filtrate from the remainder was treated with potassium permanganate to oxidize nitrites to nitrate, and analysis was made for the latter by the method of Yagoda (4).

The collected bloods from two groups of four rats were thus analyzed. No methemoglobin was found in either case. No nitrite (determined as nitrate) was found in the sample from the first group; the second sample was found to contain 9.33 μ gm. per cc. This latter result must be considered doubtful since the experimental error of the method is 9.0 μ gm.

4. The acute oral toxicity was determined in a series of 60 (unfasted) albino Swiss mice, ranging in age from 30 to 60 days, to which the nitrite in a 2 per cent solution in distilled water was administered by stomach tube. Three groups of 20 mice each received 325, 350 and 400 mg/kg. respectively.

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The results were:

Dose mg/kg.	Mortality	Mortality per cent
325	7/20	35
350	8/20	40
400	13/20	60

From the mortalities at these dosage levels, the LD-50 was calculated by the method of Litchfield and Fertig (5) and found to be 367.0 mg. \pm 6.7 mg/kg.

In order to determine the approximate quantity of nitrite absorbed into the system from the gastrointestinal tract and as a check on the analytical method being employed, this same dosage level was chosen for administration to rats, animals from which a sufficiently large amount of blood could be drawn for chemical analysis. Six 24-hour fasted rats were given 367.0 mg/kg. by stomach tube. It was found, however, that they developed more rapid and greater toxic reactions than had been anticipated, so that between 15 and 20 minutes after the administration and as each animal appeared to be in extremis, it was etherized and exsanguinated by heart puncture. The bloods from the first three rats, being those most severely affected, were heparinized and pooled under paraffin oil (sample A); blood from the remaining animals was similarly collected in a second tube (sample B). Analyses, by the same methods given above, showed the following:

Sample	Methemoglobin gm/100 cc.	Nitrite (as nitrate) μ gm/cc.
A	9.8	423
B	11.6	335

5. In order to determine whether V.P.I. X220 is a primary skin irritant or skin sensitizer in man, a series of tests was made in a group of eight volunteers. The crystalline material was made into an ointment as follows:

40 per cent aqueous solution of V.P.I. X220	70.0 cc.
Aquaphor	70.0 cc.

Preliminary patch tests were made with this ointment and the powdered nitrite, and these were found to cause no reactions. Thereafter, 2 cc. of the ointment was rubbed once daily for ten successive days into the groin of each subject. The skin was examined daily for signs of irritation.

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By W77 NARA Date 3/1/96

Two weeks, and again three weeks after the first application, in order to determine whether skin sensitization had occurred, patch tests were conducted with the following:

- a. the ointment which had been originally applied
- b. a 40 per cent solution of the nitrite in a saturated solution of sodium bicarbonate
- c. cardboard impregnated with the nitrite (applied dry)
- d. cardboard impregnated with the nitrite (applied moist)

In these tests seven of the eight subjects showed no reactions of any sort to any of the materials tested. The eighth man reacted adversely as follows: after the seventh daily application he developed diffuse redness, maceration, induration and pain at the site of application to the groin. The inunctions were stopped, and the irritation persisted for three days. Patch tests on this man, at two weeks, showed a very faint reaction to the ointment. At three weeks another application of the ointment was made to the groin, some bicarbonate solution was applied to the back, and another series of patch tests was performed on back and arm. Within a few hours the skin of the groin reacted violently to the application of the ointment, whereas the application of the nitrite in bicarbonate solution to the back elicited no reaction. The patch tests with the original ointment were weakly positive, while those with the nitrite in bicarbonate solution reacted more sharply.

DISCUSSION

On the basis of past experience with nitroglycerine (6) and TNT (7), it is reasonable to expect similar nitrite effects to develop in workers handling large quantities of V.P.I. X220. There is a suggestion in the present findings, although no positive evidence, that this last material causes these effects to a lesser degree than the former two; this might be related to the number of nitrite radicals in the respective molecules, i.e., one in the last compared with three in the others.

The oral toxicity of V.P.I. X220 indicates that, when taken by this route of administration, this material may possess specific toxic properties apart from the nitrite effects. This may or may not be true when the substance enters the body by other routes.

The skin tests show that this nitrite, even in strong concentration, is not a primary irritant. On the other hand, it apparently does have some capacity for sensitizing skin.

To avoid recurrence of symptoms when handling V.P.I. X220 in bulk, certain precautions may be observed (6):

1. Physical examinations to exclude workers with a history of organic cardiovascular disease
2. Acceptance of workmen only with blood pressures within the ranges of 100 to 149 mm. Hg systolic and 65 to 89 mm. Hg diastolic (du Pont recommendation (6))
3. Periodic physical examinations for all workmen
4. Daily laundered coveralls and showers at the end of the work shift (use of canvas or rubber impregnated gloves is also suggested)
5. Maximum personal and environmental cleanliness
6. Maintenance of adequate exhaust ventilation
7. Transfer to other work of any individual showing evidence of sensitization

In all probability, such precautions are unnecessary for naval personnel handling only the nitrite-impregnated cardboard. It is well, however, to keep them in mind should complaints arise or should naval personnel be obliged to handle this material in bulk.

ACKNOWLEDGMENTS

The co-operation of Commander Marion E. Sulzberger, (MC), USNR, is gratefully acknowledged for performing the tests for skin irritation and sensitization at U. S. Naval Disciplinary Barracks, Harts Island, New York.

Acknowledgment is also made to the following for assistance in various phases of this study: Lieut. R. Snyder, H(W), USNR, Lt. (jg) N. M. Clausen, MC(S), USNR, S. E. Mobily, PhM2c, USNR, H. L. Williams, PhM2c, USNR, and H. J. Mark, PhM3c, USNR.

J. McCULLOUGH TURNER
Lieutenant, H(S), USNR

APPROVED;

E. G. HAKANSSON
Captain, (MC), USN
Commanding

J. M. STEELE
Commander, MC(S), USNR
Research Executive

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REFERENCES

1. Memo to Capt. E. W. Brown, (MC), USN, BUMED-X-BLW:II, from Lt. Comdr. Jack Basman, (MC), USNR, dated 16 Mar 1945.
2. NRL ltr ChBuMed, Attn: Research Div., C-JJ14(458-JKW), C-458-38/45, sbg, dated 1 Mar 1945.
3. Evelyn, K. A., and Malloy, H. T., Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood, J. Biol. Chem., 126: 655-662, Dec 1938.
4. Yagoda, H., Determination of aliphatic nitrate ester - a colorimetric method, Indust. & Engin. Chem. (Indust. Ed.), 15: 27, 15 Jan 1943.
5. Litchfield, J. T., and Fertig, J. W., On a graphic solution of the dosage-effect curve, Bull. Johns Hopkins Hosp., 69: 276-286, Sep 1941.
6. Minutes of conference on the physiological effects of nitroglycerine, BUMED-X-MW:II, by Lt. Comdr. Jack Basman, (MC), USNR, dated 19 May 1945.
7. von Cettingen, W. F., et al, Experimental studies on the toxicity and potential dangers of trinitrotoluene (TNT), Pub. Health Bull. No. 285, 1944.

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Review of 4 April 1996 and 8 May 1996

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