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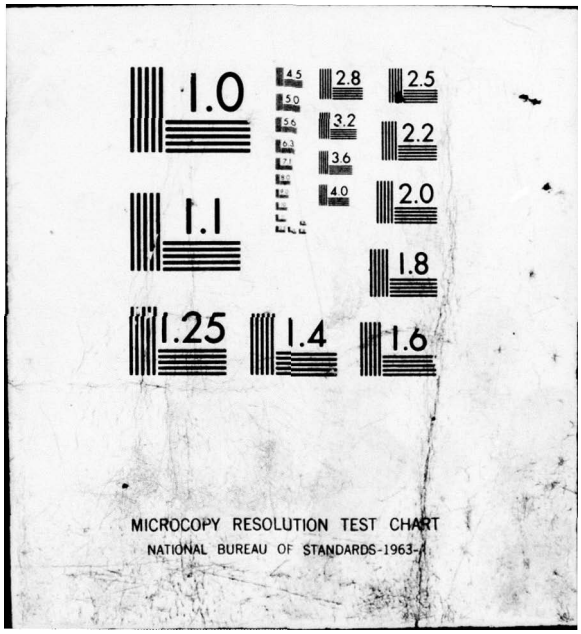
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Project No. 13E04

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ABSTRACT

A rapid gas chromatographic assay for urinary dimethylmorpholinophosphoramidate (DMMPA) has been developed which uses disposable containers and automatic dispensers. It has a sensitivity of five nanograms DMMPA per ml of urine. Measurable amounts have been detected in cumulative four hour post-test urines after 200 micrograms of DMMPA have been applied to the face in four small drops. Reduction of urine collection from 24 to 4 hours gives the possibility of more rapid verification of exposure to chemical contamination. The rapid assay will be useful for testing control urines when a yes or no answer is sufficient and for trials where the requirement for accuracy of results is not high.

(U)

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INTRODUCTION

Dimethylmorpholinophosphoramidate (DMMPA) has been used extensively to simulate persistent chemical agents in the assessment of the effectiveness of chemical defence procedures. In these assessments the intake of the simulant has been based on a gas chromatographic assay of a cumulative 24 hour post-test urine sample (1).

In the event that DMMPA would be used in large scale operations involving a great many participants, it was proposed to shorten the analytical procedures in two ways:

1) Urine Collection: Decrease the period of urine collection from 24 hours to 4 hours for the convenience of those participating and to facilitate a rapid assessment of a positive or negative intake of simulant.

2) Automation: Devise a rapid assay using disposable containers and automatic dispensers. The assay procedure which had been used for years to gather information used considerable glassware which required scrupulous cleansing.

PROCEDURES

Application of Simulant

Three series of tests were made on ten individuals with skin applications of two microliters of a 10% solutions of DMMPA in propylene glycol. This was applied as 2 drops on each cheek about 2 cm below the eyes.

Modified Automated DMMPA Assay in Urine

a) Equipment Required:

1) Gas Chromatograph - MT220 - Microtek - equipped with Tracor flame photometric detector - Phosphorus mode.
2) Dual Pen Westronic MT22 Recorder.
3) Wrist-Action Shaker - Burrel Corp., Pittsburgh, Pa.
4) Vortex Jr. Mixer - Scientific Industries Inc., Queens Village, N.Y.
5) Rotary Evapo-Mix - Buchler Instruments, New York 31, N.Y.
6) Repipet Dispenser - to deliver 0.5 ml (standard solution) - Labindustries, 1802 Second Street, Berkeley, Cal. 94710.
7) Dispensette - to deliver 0.5 ml hexane - Brinkmann Instruments, Inc., Westbury, N.Y. 11590.
8) Glass dispenser (25 ml) on 1000 ml Erlenmeyer flask (for $CHCl_3$) - and 1 ml dispenser for NaOH - Tech. Glass, Vineland, N.Y.
9) Polypropylene disposable Centrifuge Tube - 50 ml - Kimble, Canlab.
10) Evaporating Adaptor - Centrifuge tube to Evapo-Mix - Figure 1. DRES designed and fabricated.
11) Hydrometer - (1.000 - 1.060) - 100 mm in length (from Fisher Scientific Urinometer).
12) Filter Pumps - Aspirator - Glass, Kimax Brand.
13) Pipettes - a) 10 ml glass disposable - Corning - Laboratory Glassware.
    b) dispo pipets (Pasteur Type) - 9" long - Scientific Product, McGaw Park, Illinois 60085.
14) Pressure-Lok syringe - Precision Sampling Corporation, Baton Rouge, La. 70815.

b) Material

1) Chloroform - Certified A.C.S. - 5 gal. drum - Fisher Scientific Co.

2) n-Hexane - Photrex Reagent - suitable for Ultraviolet Spectrophotometry - J.T. Baker Chemical Co.

3) Toluene - Special Purity for Scintillation Work - Matheson Coleman and Bell - (Preservative)

4) DMMPA - Dimethylmorpholinophosphoramidate - $C_6H_{14}NO_4P$ - with a chemical purity of at least 98% of theoretical and an intraperitoneal LD_{50} in mice of 3500 mg/kg or greater.

c) Measuring Volume

An extremely small amount of DMMPA may be contained in urine samples and a useful detectable level of DMMPA in urine has been considered to be as low as five nanograms per ml of urine. However, there may be very large quantities in urine after extended periods of vapour inhalation or skin exposure to liquid DMMPA without decontamination. Urinary excretion following skin applications depends on the contact site as the rates of skin absorption between different body sites are similar for VX and DMMPA, about 5 times more absorptive from face than arm (1). Following inhalation of DMMPA about 24 percent of the amount inhaled is excreted in urine during the immediate 24 hour period. Therefore, care must be taken when measuring total volumes not to introduce DMMPA from a urine sample containing a high concentration into one which contains a lower one. A simple method of measuring volume, which is accurate enough in most cases, is to compare the level of urine in the collection bottle to that of a similar bottle calibrated with water. This ensures that a DMMPA-free urine has no contact with a dirty measuring vessel before an aliquot is taken.

d) Specific Gravity (S.G.)

The S.G. of a healthy individual's urine is directly proportional to the concentration of its dissolved solids and varies with fluid intake (2). Our experience has included those with S.G.s from 1.010 to 1.030. Difficulties have been encountered during the extraction procedure when the S.G. has been in the higher range as the urine usually formed an emulsion when shaken with $CHCl_3$. It has been the practice in our laboratory to dilute the urine with distilled water to bring it to an approximate S.G. of 1.010, taking care to record the diluted volume as that required

in the formula for calculation of DMMPA. The few drops of toluene that are added to each sample on collection as a preservative do not cause interference. A graph (Figure 2) has been prepared from data gathered after a scientific phase of Trial Vacuum, when intake simulant was used to assess field procedures. Seventy-five 24 hour urine samples were processed. The original volume and S.G. were recorded along with the final volume of each sample at S.G. 1.010.

The specific gravity of each urine sample is determined as follows: A portion of the urine is poured into a 50 cc polycarbonate test tube and the S.G. is taken using a small hydrometer. The hydrometer is rinsed under running tap water and the urine in the test tube is returned to the original sample. The polycarbonate test tube is discarded, or saved for future thorough cleansing. The volume of urine to be diluted is multiplied by the conversion factor (taken from the graph in Figure 2) to obtain the final volume which is reached by adding distilled water.

e) Chromatographic Conditions Used

Pyrex Column - 6 ft in length and 1/4 inch O.D.
Packing Support - Chromasorb W, H.P., 80/100 mesh
Stationary Phase - OV101, 7.5%
Column Temperature - 225°C
Detector Temperature - 130°C
Inlet Block - 200°C; Outlet Block - 200°C
Carrier Gas - Nitrogen (super pure) - Flow Rate - 90 ml/min.
Oxygen (super pure) - Flow Rate - 20 ml/min., 20 psi.
Air (super pure) - Flow Rate - 40 ml/min., 20 psi.
Hydrogen (super pure) - Flow Rate - 200 ml/min., 30 psi.
Attenuation - 10^4 x 8

f) Step-by-Step Assay

The volume of 24 or 4 hour urine at an approximate S.G. of 1.010 was recorded. Ten ml of each urine sample were pipetted into two 50 ml plastic centrifuge tubes. An internal standard of 500 nanograms of DMMPA (0.5 ml of a 1 µg/ml solution of DMMPA in distilled water) was added to one of the tubes before adding 1 ml of 1N NaOH and 25 ml of $CHCl_3$ to both.

The tubes were capped tightly with the screwtops provided and put on the shaker for 5 minutes. They were then allowed to settle 15 minutes before the aqueous layer was aspirated off and discarded. The tubes were attached to a Rotary Evapo-Mix with the adapter (rinsed with solvent after each use) shown in Figure 1. The $CHCl_3$ was evaporated at approximately 25 mm of Hg and 35°C. To the residue, 0.5 ml of n-hexane was added and the tightly capped tube was agitated 15 seconds on the Vortex Mixer. One microliter of the hexane was injected into the Gas Chromatograph, allowing 40 seconds for the solvent to vent before turning the 4-way valve to the "on column" position. The retention time of DMMPA was approximately 51 seconds. Three injections were made from each sample and the average height in millimeters used in the following formula to calculate the amount of DMMPA in the sample.

g) Calculations

$$X = \frac{V_{TU}}{V_{AU}} \times W_D \times \frac{V_{TH}}{V_{IH}} \times \frac{H_S}{H_T - H_S}$$

X - DMMPA in 24 or 4 hour sample (nanograms)
V_{TU} - total volume of urine in 24 or 4 hours
V_{AU} - volume of urine aliquot (10 ml)
W_D - quantity of added DMMPA in injected sample (1 ng)
V_{TH} - total volume of n-hexane (500 µl)
V_{IH} - volume of n-hexane injected (1 µl)
H_S - peak height of sample (mm)
H_T - peak height of sample plus added DMMPA (mm)
} measured at same attenuation

RESULTS

The assay of 30 cumulative 4 hour urines following application of 2 microliters of 10% solution of DMMPA to facial skin showed that an average of 2.3% of the amount applied was recovered. In other words, there was an average of 4.3 micrograms of DMMPA in an average 300 ml 4 hour urine sample.

When checking reproducibility of the rapid assay, five analyses were made of 700 ml of urine containing 20 micrograms of DMMPA and the average of the results was 19.28 µg with a S.D. of 1.13 and S.E. of 0.50.

DISCUSSION

It appeared that 4 hour urine samples contained measurable DMMPA after exposure of a very small area of facial skin to 200 micrograms of the chemical. These results are qualitative and cannot be related to actual intake until tests have been done relating total intake of DMMPA to 4 hour post intake urine content. This will be done by assaying 4 hour samples after intramuscular injection of DMMPA and will be reported elsewhere. From previous experience it is felt that inhalation of vapour and skin contamination by realistic liquid sprays would also give very detectable amounts of DMMPA in post-exposure 4 hour urine samples. It is recognized that the reproducibility of this method is not as good as in the original method with 25 ml of urine and separatory funnels; however, the disposal of containers eliminates any possibility of cross-contamination. The automatic dispensers were checked for accuracy and were excellent. The reproducibility of the method from one operator to another should improve with extensive use. This method is excellent for use on control urines when a yes/no content is required, when an internal standard is unnecessary and the need to assay a duplicate urine is eliminated..

Single urine samples can also be used for rapid estimation of DMMPA in urine, and peak heights can be compared with peak heights of standard solutions extracted from urine of S.G. 1.010 when injected under identical G.C. conditions. This is usually the best approach when handling a hundred or more samples, many of which may contain no DMMPA. An initial survey will determine the urines without DMMPA and the approximate content of those containing it.

Special consideration must be given to samples containing extremely large amounts of DMMPA when determining accurate quantities, as the internal standard and the volume of hexane diluent used to

dissolve the dried sample should be increased so that injections from the paired tubes will record at the same electrometer attenuation.

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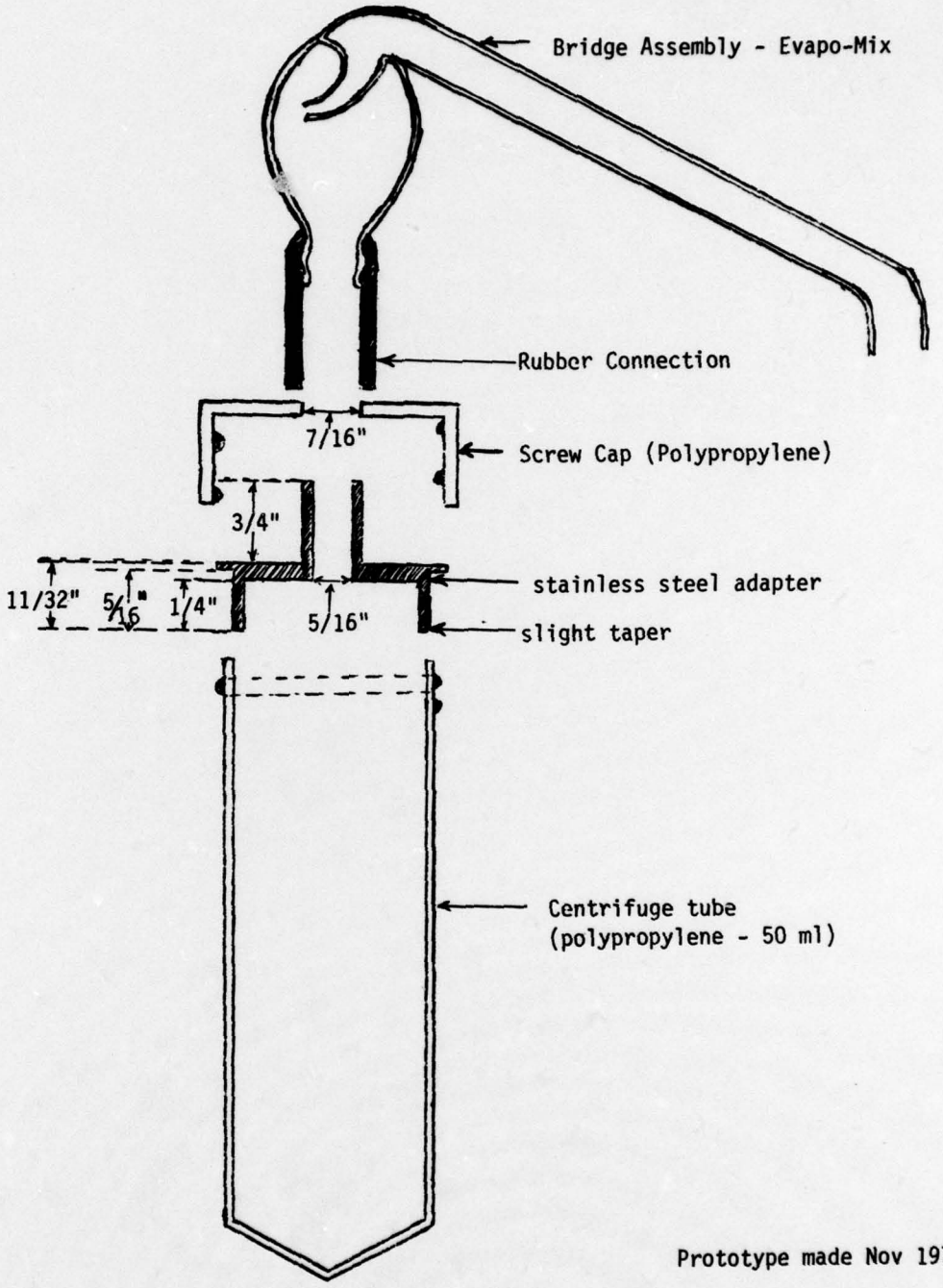

FIGURE 1: ROTA-VAP ADAPTER

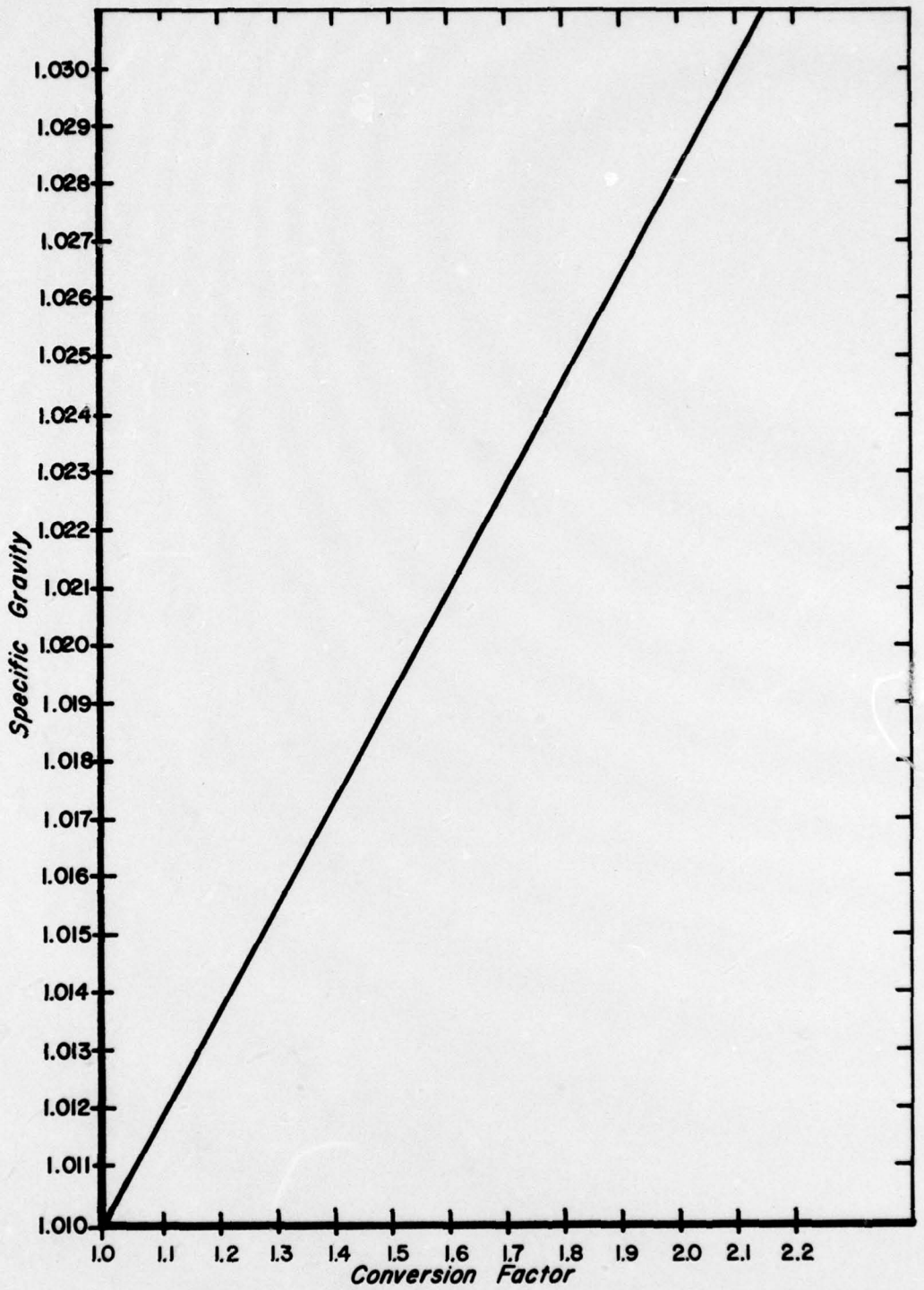

Figure 2 CONVERSION FACTOR FOR URINE DILUTION

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DMMPA

Rapid Assay

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