R1620

(6)

Protocol for Urine Monitoring Study of Workers Exposed to 4,4'-Methylene Dianiline

Prepared by: Mark Boeniger, M.S., C.I.H.

Collaborators: Rick Hornung, M.S.
Larry Lowry, Ph.D.
Edward Slick, B.S.
Anthony Smallwood, M.S.
Ana Maria Osorio, M.D.

Date: October, 1983

Final Revision: August, 1984

Industrial Hygiene Section
Industrywide Studies Branch
Division of Surveillance, Hazard Evaluations and Field Studies
National Institute for Occupational Safety and Health
Centers for Disease Control

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3 PB88-247291

4. Title and Subtitle Protocol for Urine Monitoring Study of Workers Exposed to 4,4'-Methylene Dianiline

5. Report Date 84/08/00

6.

7. Author(s) Boeniger, M.

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
Industrywide Studies Branch, Division of Surveillance, Hazard Evaluations and Field Studies, NIOSH, U.S. Department of Health and Human Services, Cincinnati, Ohio

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C)
(G)

12. Sponsoring Organization Name and Address

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

Efforts were directed toward characterizing worker exposure to 4,4'-methylene-dianiline (101779) (4,4'-MDA) and evaluate the usefulness of collecting worker urine samples to assess exposure to 4,4'-MDA on the job. Industrial uses for 4,4'-MDA include as a curing agent for epoxy resin laminates, in industrial coatings for abrasion and corrosion resistance, in the formulation of high temperature polyester-imide wire coating enamels, in the production of chemically related derivatives, as a coreactant with polyurethanes, and in the production of electrical circuit boards. A review of pharmacokinetic studies was included, along with considerations of importance in biological monitoring, study site and worker selection factors, identification of biological variables, choosing a nonexposed comparison group, survey procedures, sampling methods, environmental sampling techniques, urinalysis methods and sample collection and processing, analytical procedures used for the air, wipe, and dermal samples, statistical methods used for the field data analysis, quality assurance procedures, safety of the investigators during the study, records management and data reporting.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

NIOSH-Publication, NIOSH-Author, Carcinogens, Arylamines, Cancer-rates, Urine-chemistry, Curing-compounds, Epoxy-compounds, Sampling-methods

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages 72
	20. Security Class (This Page)	22. Price A04 $14.95

Contents

List of Figures

List of Tables

Appendices

Abstract

This protocol describes a study that is being conducted at NIOSH and directed by the Industrywide Studies Branch. The objectives of this protocol are to 1) fully characterize worker exposure to 4,4'-methylene dianiline (4,4'-MDA) and 2) develop and evaluate the collection of urine samples as a reliable means of assessing workers' exposure to 4,4'-MDA. In addition to pertinent background information, methods used to measure 4,4'-MDA in air, through personal contact, on workplace surfaces and in human urine are described.

Disclaimer

Mention of company name does not constitute endorsement by NIOSH.

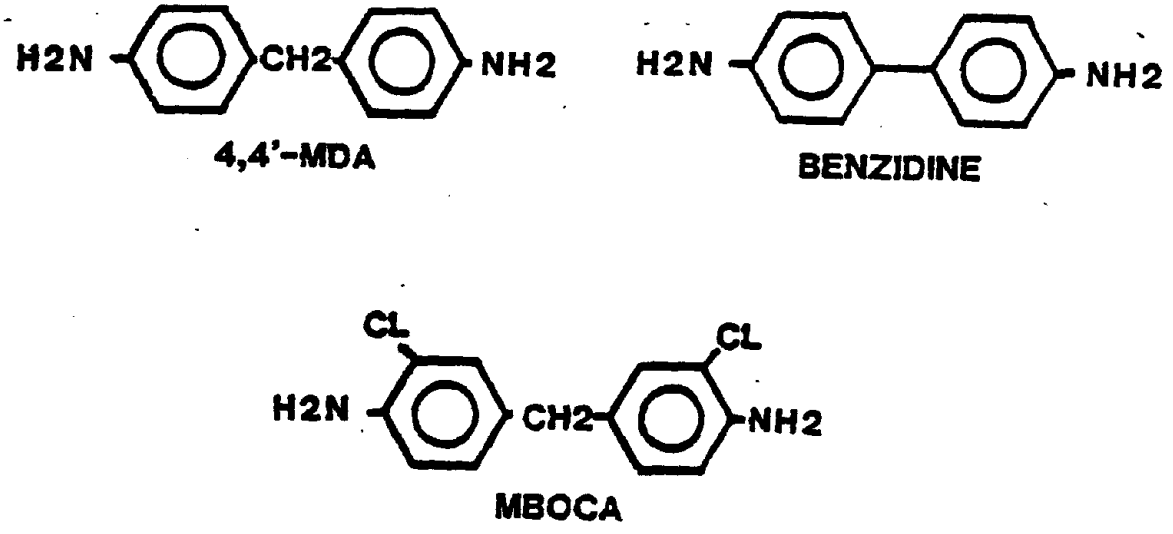
Protocol for Urine Monitoring Study

of 4,4'-Methylene Dianiline Exposed Workers

INTRODUCTION

The arylamine compound 4,4'-methylene dianiline (4,4'-MDA) is structurally similar to the known human carcinogen, benzidine, (refer to Figure 1). In June, 1982 the results of the National Toxicology Program bioassay on 4,4'-MDA firmly established it as carcinogenic in both rats and mice of both sexes (1). In addition, 4,4'-MDA has been found to be a potent hepatotoxic agent in man and has been shown to produce a pattern of illness characterized by epigastric or right-upper-quadrant pain, fever, jaundice, and rash.[2, 7, 23, 24]

Figure 1: Chemical Structures of Three Arylamines



While most (98-99%) of the 4,4'-MDA manufactured in the United States is consumed on-site for the production of methylene diphenyl methane 4,4'-diisocyanate (MDI) which in turn is used to produce rigid polyurethane foams, approximately 3 million pounds per year of technical grade MDA is used for non-MDI applications. Technical grade 4,4'-MDA contains a variety of dimers, trimers, higher polymers, and isomers of 4,4'-MDA but it may contain 70% or more monomeric 4,4'-MDA. An additional 1-2 million pounds of pure (97%) 4,4'-MDA is used for several purposes, notably as an epoxy resin hardener in the production of high temperature wire coating enamels and as the curing agent in filament wound piping and rocket casings.[3]

Because of the evidence for hepatotoxicity and other toxic effects in workers exposed to 4,4'-MDA, the American Conference of Governmental Industrial Hygienists (ACGIH) recommended in 1980 that airborne exposures to 4,4'-MDA not exceed 0.1 ppm (0.8 mg/m^3) averaged over an 8-hour day. Specific attention was given to cutaneous exposure.[4] NIOSH and the Occupational Safety and Health Administration have not set maximum allowable limits of exposure for MDA. Based upon experimental carcinogenicity data the Environmental Protection Agency (EPA) has tentatively determined that regular repeated exposures to 0.1 ppm of 4,4'-MDA in the air could constitute a substantial risk of human cancer.[5] Due to the possibility that MDA may be a human carcingoen, NIOSH is initiating studies to determine workplace exposure to 4,4'-MDA, to evaluate the possibility of using urine samples to monitor workers' exposure, and to determine if unusual mortality occurs among workers who had been exposed to 4,4'-MDA.

We are performing these studies under the mandated authority of the Occupational Safety and Health Act (Public Law 91-596, December 29, 1970). NIOSH has been designated responsibility for conducting field research studies in industry, to evaluate findings, and report on these findings. Section 20(a)7 states that NIOSH shall conduct and publish industrywide studies of the effects of chronic or low level exposure to industrial materials, processes, and stresses on the potential for illness, disease, or loss of functional capacity.

In contrast to airborne exposure, which may be quantitatively measured, exposures such as those occurring by skin absorption and ingestion are sometimes ignored in industrial hygiene studies, and often no attempt is made to estimate their significance. Excluding from this study the non-inhalational routes of exposure is unwarranted in light of the information indicating significant penetration of the skin by MDA in humans where clinical poisoning was seen to occur.[2, 7] Industrial hygiene air samples are by themselves of limited practical value when attempting to estimate the exposure dose because variables such as respiratory rate, personal respiratory protection, and exposed skin surface area can differ appreciably between workers. Thus, biological monitoring of body fluids from workers may provide a more quantitative and definitive estimate of the exposure dose.

The primary industrial uses of 4,4'-MDA include it as a curing agent for epoxy resin laminates, (nonmetalic pipe, tanks, rocket casings, laminates, castings and encapsulation) as well as industrial coatings for abrasion and

corrosion resistance. Another major use of 4,4'-MDA is in the formulation of high temperature polyester-imide wire coating enamels. Other minor uses are in the production of other chemically related derivatives, as a co-reactant with polyurethanes, and the production of certain electrical circuit boards.

CHEMICAL AND PHYSICAL PROPERTIES

MDA is a white or tan crystaline solid with a faint amine-like odor. It is commercially available in bulk as flakes, granules or solid blocks. Eutectic liquid blends with 40% w/w p-phenylenediamine are available, as well as pastes, powders, etc. A summary table including most of 4,4'-MDA's chemical and physical proterties is attached as Table I.

A crude estimate of the vapor pressure of 4,4'-MDA using the Clausius-clapeyson equation indicates that at room temperature (25°C) 4,4'-MDA should have a vapor pressure of about 1.5×10^{-7} torr. However, experimental determination by Uniroyal, Inc. with Tonox (a mixture of monomeric 4,4'-MDA with smaller amounts of higher molecular weight polyamine derivatives of MDA) indicates that at room temperature 4,4'-MDA has a vapor pressure around 6×10^{-2} torr (mm Hg). This same experimental data also indicates that at elevated temperature the vapor pressure increases in a linear relationship (v.p. about 1 mm Hg at 190°C).

Table 1: Physical Properties of MDA

Molecular Weight	198.2
Boiling range (768 mm* Hg), °C	398-399
Melting point, °C	91-92
Density at 100°C (cP)	1.056
Viscosity at 100°C (cP)+	8.04
Flash point, °C	221.1
Fire point, °C	248.9
Heat of vaporization, kJ/mole** (kcal/mole)	95.4 (22.8)
Specific heat at 29°C (solid), J/(g °C) [cal/(g °C]	1.46 [0.35]
Specific heat at 109°C (liquid), J/(g °C) [cal/(g °C)]	2.01 [0.48]
Heat of fusion, kJ/mol** (kcal/mole)	19.6 (4.7)
Approximate solubility, g/100g of solvent at 25°C	
Acetone	273.0
Benzene	9.0
Carbon tetrachloride	0.7
Ethyl ether	9.5
Methanol	143.0
Water	0.1

* Millimeters
\+ CentiPoise
** Kilojoules per mole.

Sources: (18,19)

STUDY OBJECTIVES

The objectives of this study are: (1) (a) to determine if free monomeric 4,4'- MDA and the acid hydrolysate products of MDA are excreted in the urine at detectable levels and (b) to develop a basic understanding of MDA's elimination in humans; and (2) to determine if the urinary levels of the excreted compound are highly and significantly correlated with measurements of recent total exposure (see Figure 2). Each objective will be addressed in a separate phase. An affirmative determination to the second objective would enable biological samples to be used as an alternate monitoring tool for measuring workers' exposure to MDA.

It is important to remember that the results of this study are exploratory in nature and that the results from urinary sampling have no known relation to any abnormal clinical findings. The study procedures proposed may be solely utilized as a screening tool to evaluate worker exposure to MDA.

Figure 2

Outline of Study Objectives

Phase I

- Determine if 4,4'-MDA is excreted in human urine (n _ 20).
- Collect data to describe excretion rate in urine (n _ 12).

Phase II

- Correlate detailed exposure data with urine samples data (n = 50).

  Make determination of the usefulness of procedures for routine use.

PHARMACOKINETICS REVIEW

Experimental animal studies have shown that the 3,3'-dichloro analog of 4,4'-MDA (MBOCA) is predominantly excreted within 24 hours (see Figure 1 for chemical structure). After i.v. injection of MBOCA into beagle dogs, 46% was recovered in the urine within 24 hours.[11] After a dermal application of MBOCA to beagle dogs, an estimated 2.4% to 10% was absorbed into the systemic circulation within 24 hours.[11] Another study using rats concluded that no differences in the route of elimination was seen following several routes of administration. About 30% of the total MBOCA eliminated by all excretory routes was found in the urine with 80 to 90% of the MBOCA found in the urine being eliminated during the first 24 hours after oral gavage (12). A NIOSH study with rats showed that 4.8% of the MBOCA in acetone was absorbed into the skin, and 0.016% of the dose was present in the urine after 1 hour.[13] It was estimated that a 50 mg dose of MBOCA applied dermally on the rat and left for several hours would result in the same urinary concentration of parent MBOCA as 1 mg taken orally. NIOSH found that 0.225% $\pm$ 0.058 (N = 8) of the oral dose of MBOCA was eliminated as acid hydrolysates of the parent compound in the urine during the first 24 hours. In a recent unpublished study, rats dosed intraperitoneally with MDA eliminated 35% $\pm$ 5 (N = 4) of the administered dose as both metabolites (10 - 12%) and parent compound in the urine while rabbits excreted 80% of the dose as essentially unchanged parent compound (N = 2).[14]

It might be expected from the chemical and physical properties of MDA that it might be absorbed through the skin more readily than MBOCA since MDA is not chlorinated, therefore making it less ionic and more lipid soluble. However, this has not yet beem substantiated.

The presence of 4,4'-MDA in the urine of workers handling 4,4'-MDA has previously been reported. The Rhone-Poulenc Company in France has performed urine monitoring on its employees since 1977.[22] Methods of analysis and limits of detection (LOD) have changed from use of colorimetry with an LOD of 200 ppb to gas chromatography with electron capture detection (LOD = 20 ppb). The incidence of "positive" samples has ranged from 15% in 1970 to about 1% in 1980. The results from morning, midday and afternoon sample collection indicate that first morning void samples contain the greatest amount of the amine. Details of the analysis, collection procedures, and work operations are at present not known.

The British Industrial Biological Research Association however, has recently stated in a letter to the EPA that Ciba-Geigy-U.K. has analyzed workers' urine for the presence of MDA since 1967. Their use of the urinary screening test has been used as an indicator that liver function testing might be warranted in those workers with positive urinary amines, and this might similarly be used to indicate over-exposure to the carcinogen.

Details of the methods from that company have been made available to NIOSH and indicate a minimum limit of detection of 10 ppm as MDA. Reportedly,

detection of 4,4'-MDA in urine cannot be detected at these concentrations. NIOSH will not be using this method. This limit of detection is regarded as being inadequate for use by NIOSH in the forthcoming study. Colorimetric analyses as described in the Ciba-Geigy-U.K. letter, have frequently been used in the past by others to provide non-specific, non-sensitive determination of urinary aromatic amines.(6)

Human data regarding the elimination of a closely related arylamine, benzidine, indicated that the maximum urinary excretion rate occurred 2-3 hours after the end of the work shift. The biological half-life was calculated to be between 5 and 6 hours. An end of shift void represented about 50% of the total 24 hour elimination.(21)

Finally, a recent analysis by NIOSH of urine samples collected from workers handling MBOCA in several facilities found the compound in all samples. Peak concentrations of the parent amine in urine reached 200 ppb and 300 ppb of total MBOCA.(29)

BIOLOGICAL MONITORING CONSIDERATIONS

A potential means of overcoming the problem of better estimating total dose is to monitor the workers' excretion of the compound. Urine samples represent a noninvasive method of collecting a biological medium that may contain the compound of interest. An important hypothesis which must be initially tested, however, is whether the concentration found in the

biological sample significantly correlates to the estimated dose. There are reports in the literature where attempts to correlate exposure to other compounds with residue levels have failed.[30] However, lack of accuracy in the estimation of total exposure dose may have partially hindered these studies, causing wide variability in the results. Also, not understanding the elimination kinetics for the compound and biological variability for each individual may have introduced sizable errors.

Based upon the results of rough calculations which are supported by the results from Rhone-Poulenc, MDA should be detectable in the urine of presumably exposed workers. At the 0.8 mg/m^3 maximum recommended air standard proposed by the American Conference of Governmental Industrial Hygienists, a continually exposed, moderately active individual would inhale about 8 mg (0.8 mg/m^3 x 10 m^3) of MDA per 8 hour workday. If 10% of the absorbed dose is excreted as deconjugated MDA in the urine and 80% of the absorbed dose is eliminated during the first 24-hours after exposure then 0.64 mg (8 mg x 0.10 x 0.8) MDA per liter of urine would constitute an approximate concentration found in those voids from the average individual. The analytical limit of detection in our procedure is estimated to be 0.001 mg/l urine (1 ppb). Thus, if the above estimated calculations are reasonable, urine monitoring of workers exposed to 4,4'-MDA should be successful in detecting 4,4'-MDA and its metabolites in most exposed workers, even if dermal exposure is negligible.

Further assessment of urinary screening as a monitoring tool may therefore be appropriate. Biological monitoring, if feasible, could provide a better estimate of absorption, and through multivariate analysis possibly indicate the relative contribution of dermal exposure when compared to quantitative air sampling data and estimates of respiratory inhalation volume.

STUDY SITE AND WORKER SELECTION

In 1976, NIOSH estimated that about 2500 workers (processing as well as manufacturing) were potentially exposed to MDA. NIOSH had raised this estimate to 5000 in 1979, and most recent estimates indicates that between 12,000 and 13,000 workers may be exposed.[16]

MDA for non-isocyanate use is found chiefly in the production of polyimide-amide wire coating enamels; as a curing agent for epoxy resins used in coatings, filament wound structures, and castings; and as an intermediate for other derivatives. The above industries are judged to provide the greatest potential of finding suitable facilities to include in this study.

The chief plant selection criteria include (1) the number of potentially exposed workers, (2) the likelihood of a measurable environmental concentration of MDA (based on past sampling or existing engineering controls), (3) the frequency of operation or practices that could potentially expose workers to MDA, and (4) the proximity of the site to Cincinnati, Ohio.

Participants will be selected based on their likelihood of exposure to MDA. The likelihood of worker exposure will be estimated from exposure data collected during preliminary industrial hygiene sampling surveys. Details of the actual study methods are provided under "Field Sampling Procedures". This study is viewed as consisting of two phases of research.

Phase One

The objectives of Phase One are two-fold. Phase One, as stated earlier, requires that it first be determined if MDA is excreted in the urine of exposed workers. To determine if MDA is detectable with the proposed methods of analysis, an end-of-shift urine void will be collected from several workers who are repeatedly exposed to MDA on a daily basis. Regularly exposed workers, as measured by air, dermal or other assessment means, would be preferable to intermittently exposed workers for determining if 4,4'-MDA and its conjugates are being excreted, especially when single samples are to be collected.

Secondly, It would also be desirable to determine the peak excretion period of 4,4'-MDA after exposure. This information would later be used in Phase Two of this study to determine the optimal collection period for a single urine void. To determine this, intermittently exposed workers would be most suitable for study. These workers would be asked to collect their urine voids over an extended period of time, both at work and at home. To study the rate of elimination of 4,4'-MDA and its metabolites, favorable

participants would be chosen who are measurably exposed to 4,4'-MDA for a period of time less than or equal to one work shift and who are not subsequently exposed during the following two days. These persons would be asked to collect each of his or her urine voids in separate containers at prescribed times over a period beginning prior to the initial MDA exposure and ending approximately 48 hours after the termination of exposure. If 4,4'-MDA is excreted in detectable quantities in exposed workers then only a few participants may be required to enable a visual estimate (from the graphically represented data) as well as a calculation of the time from initial exposure to maximum excretion of 4,4'-MDA. We will also be able to gain a better understanding of the elimination pattern over time.

Given the dearth of existing data on the variability of occurence of MDA in urine of exposed workers or its rate of elimination, it is presently impossible to estimate the number of participants that will be required to determine the above. However, a sample of 12 subjects will initially be included since the decrease in the width of the confidence interval on the mean half time ($t_{1/2}$) and maximum elimination time (t_{max}) reaches a point of diminishing returns for each additional subject over 10.

The results of some initial plant sampling surveys will be needed before it can be determined if only a small number or if a large number of participants are needed to determine the pharmacokinetic excretion pattern. Epoxy resin and wire enamel formulators using 4,4'-MDA represent suitable

worker study populations that are generally intermittently exposed to 4,4'-MDA during batch processing, and several such sites have been identified.

Before preceeding to Phase Two, only enough data is required from Phase One to provide supportive evidence that MDA and its hydrolyzable metabolites are excreted within a given time interval and that interpersonal excretion variability is within an acceptable range. A more detailed study of the human kinetics of elimination, including determining intrapersonal variability, is impractical in this study due to the excessive resource requirements for repeat visits to each of the intermittent user facilities chosen for this task. Between individual differences are expected to be greater than intrapersonal variability. Therefore, intrapersonal variability will, for the purposes of this study, be assumed to be insignificant.

Phase Two

The second phase of this study, to determine the correlation between concentrations of MDA in the environment and in workers' urine, will be conducted using regularly exposed workers. Filament winding facilities could provide a suitable study population. However, walk-through surveys have not yet been performed at all facilities under consideration.

The justification for selecting regularly exposed individuals during the second phase of this study is that 1) the largest numbers of workers who are exposed in single locations appear to be those that use MDA regularly and thus offer the best likelihood of finding an adequate number of study participants, 2) regularly exposed individuals may be at greater risk to their health than intermittently exposed workers and therefore have the greatest need for a reliable exposure monitoring method, and 3) it is assumed that regularly exposed workers have reached a more stable or steady state condition than intermittently exposed workers so that a single sample, taken at a given specified time of the day (i.e. end of shift) may be satisfactorily used to correlate urine concentrations with estimates of environmental exposure.

The time of collection of a single urine void will have been determined in Phase One. The collection of scheduled single samples are more technically practical to obtain than diurnal and 24-hour samples. This would be so for both NIOSH as well as others who may wish to later adopt the procedures defined here. During a period of several days, single daily urine voids shall be collected at the optimally prescribed time from each worker participating in this study.

Phase One environmental and urine monitoring data from participants of this study may also be used separately for a preliminary regression/correlation analysis. However, more participants will be involved in Phase Two of the study than in Phase One and therefore the second phase of the study would

more adequately meet the needs of the task to relate environmental exposure data with urinary excretion data.

BIOLOGICAL VARIABLES

While variables such as age, race, and sex are not known to significantly affect the levels of MDA excreted we have tentatively chosen to include only male workers in the statistical analysis for this study to eliminate any possible bias due to sex. Controlling for additional personal variables would be beyond the objectives of this study. No questionnaire will be used since the use of assorted personal data (i.e. drug use, diet, etc.) would be of no known utility in this study and could constitute an unnecessary burden to the worker. Each participant's medical status will be checked against company medical records.

NON-EXPOSED COMPARISON GROUP

For the purpose of identifying the occurance of positive chemical interferences, an equal number of presumeably non-exposed clerical and administrative employees at each facility will be asked in Phase One to voluntarily submit urine samples for comparison to the concentration of 4,4'-MDA in the urine of exposed workers. To confirm and document the control groups' lack of respiratory and dermal exposure to MDA, area air samples and wipe samples will be collected in their work area. Equal numbers of exposed and non-exposed workers who are matched by sex and

apparent age will be used since this provides the most efficient design in terms of maximum power for a given total sample size. No 4,4'-MDA is expected to be found in control workers since it is not present in nature or in pharmaceuticals (8, 9). Non-production employees are preferred for the control group due to the suspected ubiquitous nature of 4,4'-MDA in the plant environment. Smoking is not expected to directly influence the concentrations of 4,4'-MDA found in the urine though it may contribute to 4,4'-MDA dosing through accidental ingestion if the smokers hands were contaminated with 4,4'-MDA. Periodic visual observation and documentation of each participants activity in this study will be recorded (see Figure 3). This shall be performed so that personal and work related factors could be later accounted during the interpretation of the biological data.

METHODS

a. Survey Procedures

All surveys of places of employment will be performed under authority of the Occupational Safety and Health Act of 1970 and conducted as described by Code of Federal Regulations, Title 42, Part 85a, 41 FR 45003, October 14, 1976. After a minimum of 10 working days notification of the intended visit to the facility using or manufacturing MDA, a NIOSH survey team consisting of the project director, an industrial hygiene technician, and additional industrial hygienists will arrive at the facility.

At each place of employment visited and selected for inclusion in these studies an opening meeting will be held with appropriate representatives from both management and labor. The functions of NIOSH and the scope and design of the present study will be discussed. During the meeting a list of the materials which are handled by the workers who will be included in this study will be requested. Although no known interferences in the analysis of MDA exist, this information will help to determine if any materials other than MDA could interfere or conflict with the analysis to be conducted. Employees will be asked to volunteer as participants in this study. The purpose, procedures and benefits of this study will be explained individually to workers or in small groups. Informed written consent will be obtained from each participant prior to their involvement in this study.

A brief review with the management and union representative(s) (if applicable) of existing health, safety, industrial hygiene, and medical programs at each facility will be conducted. A walk-through survey will be performed with management and employee representatives of the processes where MDA is used or produced. This will allow the survey team to familiarize themselves with the facility and its processes and enable them to identify those workers who are in areas where exposure is likely to occur. Space will be requested for equipment set up and use during the stay. This should preferably be a small area that has electrical outlets and can be secured at night. The survey team will remain at the facilities for varying lengths of time, usually 3 to 5 days. However, in all cases several days of industrial hygiene/ biological monitoring will be conducted

to determine the extent of interday variability and to enable the pooling of some data (if applicable) for statistical analysis. The NIOSH investigator will determine from the walk-through survey and process parameters which job areas will be monitored, and the workers to be included in the study. The company medical records of all potentially exposed employees, identified by name during the walk-through survey, will be checked by a NIOSH physician for known renal or liver disease or chronically prescribed drug use. Any participants having known renal or hepatic disease will be exclused since these factors might abnormally affect the way that MDA is eliminated in the urine. Prescribed medication shall be noted to possibly aid in the later identification of analytical interferences.

Observations will be made of engineering controls, workers, and administrative practices. Photographs will be taken to help document and describe the process. Observations and photographs will be used only for our research purposes during the interpretation of our results, and this will be explained to each volunteer in the study.

b. Sampling Methods

Laboratory and field validation of all methods used to measure exposure or elimination of MDA will be performed prior to conducting sampling to establish the precision, accuracy and bias. These data are described in the section on Ouality Assurance.

Environmental

Environmental monitoring will consist of collecting personal air samples using a sulfuric acid treated 47 mm Gilman Type AE glass fiber filter. To collect these samples, air will be drawn through the sampling media at a flow rate of two liters per minute using a portable sampling pump attached to the workers' belts. Area or process air samples of a similar nature will be taken to measure incidental exposure to other workers and detect sources of MDA contaminant release. The samplers will be changed at 4 hour intervals, or if excess dust build-up warrants, at 2 hour intervals.

To assess previously used MDA-in-air sampling procedures a comparison of these prescribed methods to the acid-treated filter method will be performed. The comparison would help to estimate the relative error associated with previously collected sample data. The procedure will entail collecting side-by-side air samples located in stationary positions in areas likely to contain MDA. The Marcali method (NIOSH P&CAM 142), as well as glass fiber filters not treated with sulfuric acid, will be used for comparison to the acid treated filter collection method described above and used currently by NIOSH. The Marcali sample data will be used to determine the influences of interfering compounds and/or collection efficiency in this older method. Untreated glass fiber filters will be used to evaluate the degree of sample loss through volatilization and/or oxidation using this method. Replicate samples of each type will be obtained to determine method variability.

In addition to the above, area samples will be obtained to determine MDA aerosol particle size fractions using a cascade impactor. Aerosol particle size data will suggest the likelihood of rapid or slow respiratory and dermal absorption as well as the sedimentation velocity of the aerosol in air.

Environmental monitoring of potential dermal contact with MDA will be conducted by using a hand pad sampler (Figure 3). This device, consisting of two glycerin dampened cotton gauze pads affixed with two sided adhesive tape to the front and back of an elastic glove-like design, will be worn on the hand of the worker to assess dermal contact. This device can be worn with or without protective gloves and does not interfere with normal operation of the hand. The back of the hand pad is intended to measure exposure from fallout while the palm pad should determine direct skin contact from (for example) griping objects. The area of the sample pad is approximately 25 cm^2 and analytical results can be adjusted to micrograms per square centimeter when presented. Additional cotton pads shall be consistently attached to the workers in Phase One (only) in such locations as the shoulder, arm, and lower leg to assess the relative deposition or contact with 4,4'-MDA at anatomical sites other than the hand.

Hand pad sampling data has been used to calculate total dermal exposure to pesticides using anthropometric measurements.[25, 26, 27] Studies have indicated that under the conditions evaluated, hand contact can contribute between 40% of the total dermal exposure when protective gloves are worn (25) to up to 90% without protective gloves.[28] Since a number of

variables that have not been adequately determined could influence both contact and absorption of 4,4'-MDA no adjustment for total exposure quantity or absorbed dose will be attempted. Rather, the quantitative hand pad data will be used in multiple regression analysis and a fractional contribution of the total collected sample shall be applied that produces the best fit among all other variables tested.

The hand pad samples will be replaced with fresh pads at two hour intervals. The pads will be removed from the hand device by a NIOSH investigator who is wearing fresh disposable gloves while using cleaned tweezers. The cotton pads will be placed in small marked glass vials for laboratory analysis. Additional wipe samples using dry Whatman cellulosic filter paper will be used to assess environmental contamination of work surfaces with 4,4'-MDA. These samples are intended only to identify the dispersion of 4,4'-MDA and general housekeeping for the workplace. To identify areas contaminated with MDA, a quick, inexpensive, and sensitive on-site screening method using a fluorogenic coupling reagent will precede the collection of wipe samples for laboratory quantitation.[17] This method produces a yellow color under untraviolet light that we have found to be distinct for a few aromatic amines such as 4,4'-MDA. Comparison results for the two methods will be reported.

Detailed records of worker movements and adherence to personal protection measures and personal hygiene will be recorded on time sheets (see Figure 4). Observations shall be recorded at the end of each 15 minute interval of time.

Detailed records of worker movements and adherence to personal protection measures and personal hygiene will be recorded on time sheets (see Figure 4). Observations shall be recorded at the end of each 15 minute interval of time.

Figure 3

HAND CONTACT MONITOR

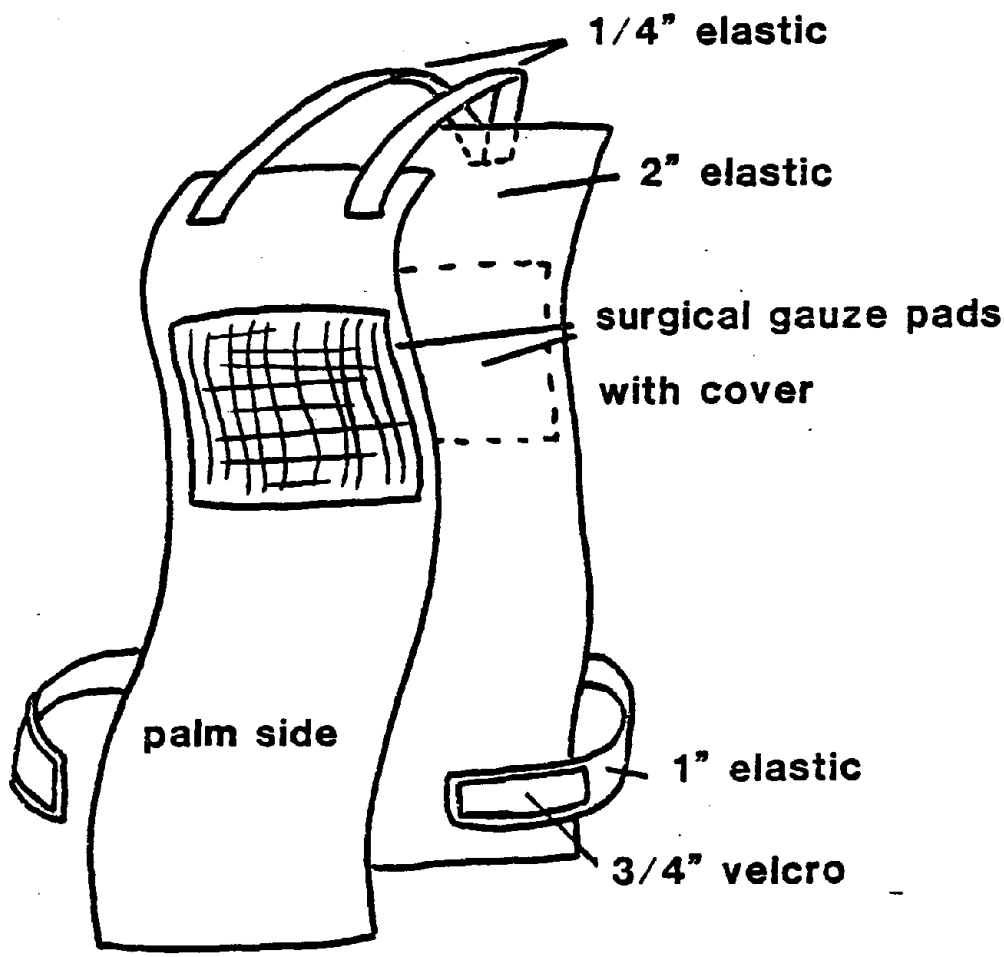


Figure 4
Participant Activity Record

Participants Name:

Dates:

Locations:

Interval	Time	Observations	Measured Respiratory Rate	Estimation of Activities Effect on Exp.
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				

Because respiratory rate can vary substantially due to work activity and this can influence respiratory dose accordingly, it will be necessary to

establish individual rates. Timed respiratory volume (and thus rate) can be monitored by using a flow meter. A Wright respirometer attached to the exhalation part of a modified half-face respirator (without filter cartridges) will be worn by the participant for three minute periods while performing various activities during the work shift. The respiratory volume on exhalation shall be recorded and the activity described. The recorded respiratory volumes will be used in estimating the respiratory rate for all other periods when the participant is observed but when the respiratory rate is not measured.

Urine

Urine (and environmental) samples will be collected from exposed workers in two distinct phases.

Phase One:

In Phase One, environmental characterization of each participant's respiratory and dermal exposure will be performed over the workday (as described above). Frequent changes of the samples will allow determination of the changes of exposure concentration over time. Phase One will attempt to determine whether MDA can be detected in the urine of exposed workers. This phase will consist primarily of the collection of partial shift urine samples (i.e. one or two voids near the end of the work shift) from a few

workers* in facilities studied for environmental characterization but not where there are sufficient numbers of workers to meet the statistical requirements of Phase Two.

However, these facilities are also likely to involve intermittently exposed workers, some of whom have not recently worked with MDA and who may not be exposed to MDA through contact with contaminated work areas. Such workers also offer a unique opportunity to study the rate of partitioning of MDA into the urine and would be of further value to this study since it is not currently known when excretion of MDA reaches its maximum rate after human exposure nor for how long detectable clearance will occur. In such circumstances attempts would be made to collect continuous urine voids beginning before MDA processing and ending 48 hours after last exposure. Environmental exposures would be fully documented while the worker is on the job. The participants would be asked to collect each urine void in a separate bottle and record the time of the void. It would be requested that

---

* Although only three or so samples having MDA in the urine would probably suffice to meet the objective of Phase One it is likely that 10 or more samples will be collected since this would entail only marginal cumbrance to either the NIOSH investigators or the participants and could strengthen the statistical power of the results.

voids be obtained at four hour intervals, where possible, for up to 48 hours after termination of exposure. In any case, an end of shift, end of day, and morning void are presumed from the literature [21, 22] to be most reflective of recent exposure while the times of all other voids should be at least recorded. Participants shall be cautioned about possibly contaminating urine voids with MDA dust and as a minimum precaution they will be asked to wash their hands prior to voiding. Urine containing MDA has been found to be stable at room temperatures so that immediate refrigeration of participants urine samples is necessary.[10]

The urine samples will be returned to the NIOSH investigator at the earliest opportunity. The participant's name, date, time of voiding, and urine volume shall be recorded. To reduce urine volume during transport the urine volume in excess of 200 ml will be discarded. These aliquots will then be frozen in dry ice to prevent putrefaction.

The results will be presented graphically as time dependent concentrations per fluid volume of urine voided, corrected and uncorrected for creatinine. Both the free parent amine and the total amount of MDA hydrolysates excreted per time period may be related as a percent of estimated exposed dose using a combination of air and dermal samples. Acid hydrolysis of the urine samples will serve the function of deconjugating the conjugated form of the parent amine as well as hydrolyzing the acetylated derivatives. Acid hydrolysis should increase the detectable concentration of MDA in each urine sample. This has been demonstrated in recent experimental studies with

MBOCA [11,12] as well as in animals dosed with MDA [14] and shall be performed in this study (see Analytical Section for details). The statistical analysis of data obtained from Phase One is discussed in the Statistical Analysis section of this protocol.

Phase Two:

In Phase Two, we will assume, until the results from Phase One are available, that the maximum excretion period occurs near the end of the work shift. Modification of this assumption shall be made pending additional data. Participants in the Phase Two study will similarly be asked to collect their urine in several large polyethylene sample bottles. The same collection protocol shall be followed over three successive days. In order to allow the calculation of time-dependent excretion rates, participants will be asked to urinate, without sample collection, upon arriving at work. The success of the participants being able to produce a urine void shall be noted in a sample log by the NIOSH surveyor. The participants will be asked to collect all subsequent urine voids at 2 hour intervals or during scheduled company breaks, as well as an end-of-shift void. This schedule shall result in the collection of a maximum of four samples per participant per 8-hour period.

Laboratory studies have demonstrated that 4,4'-MDA is stable at room temperature for at least 8 hours (10). Regular collection of urine sample bottles at the workers' work stations will be performed by the NIOSH

investigator and the urine samples shall be kept until the end of the shift. As an alternative for the collection of urine samples from mobile workers, samples shall be brought to the NIOSH investigator by the worker. Specific arrangements shall be made with each participant on a case-by-case basis within each plant.

The original urine volume, period of collection, participants name and identification number will be recorded for each urine void. Failure to provide a urine sample shall also be noted. Using the original four samples collected during an 8 hour period, adjustments in their sample volume shall be made, as described below, so that only two 100 ml urine samples are transported to the laboratory. One sample will represent a composite, volume adjusted, aliquot of the 8-hour work shift. The second sample will represent a single end-of-shift aliquot. Both an 8-hour time integrated and end-of-shift sample are desired so that we may determine which sample provides the closest correlation to a participants current work exposure to MDA. The reduction in urine volume will be performed to provide a manageable transport weight while retaining an adequate volume to produce a reasonable analytical limit of detection. The need for both a timed integrated sample adjusted for excretion volume per unit time versus a short term sample based on a constant dose dependent excretion rate reflects the need to address the present uncertainty in the mechanism of elimination for MDA and its metabolites. The proposed dual approach should consider the two possible mechanisms which might impact the elimination rate - elimination via a blood:urine concentration dependent diffusion gradient versus

elimination via a dose dependent facilitated mechanism which is irrespective of urinary concentration. The two prepared urine aliquots shall be prepared in the following manner.

Each urine sample will be obtained by the NIOSH survey team and retained until the end of the 8-hour period. After the last void sample is collected the total volume shall be measured by weighing each sample bottle on a balance and recorded. From each sample bottle 25 ml of urine will be removed by a mechanical pipette with disposable tips and transferred to a fresh 100 ml polyethylene bottle. If a two hour void is not provided the next voided sample shall be used and 50 ml will be removed. A second aliquot shall be prepared by decanting 100 ml of urine from the last voided sample into a fresh polyethylene bottle. This will produce two 100 ml samples. All other urine shall be discarded. A sample of the recording sheet for each sample is presented as Figure 4. Quantitative chemical analysis and statistical analysis will be performed separately for each sample pair. Chemical analysis shall be performed to report free parent compound and/or total hydrolyzable 4,4'-MDA, depending upon the information determined in Phase One. These procedures are described in the following section.

The aliquots will then be frozen in dry ice. The frozen samples will be secured in foam packing containers then placed in a large polyfoam shipping case with dry ice for shipment to the laboratory for analysis. Included in the shipment of urine samples will be 'blank' control specimens collected

from non-exposed NIOSH investigators just prior to each field survey. Also included will be 100 ml urine samples from non-exposed that have been spiked with 10 micrograms of 4,4'-MDA. The samples will be shipped so that they arrive at a NIOSH laboratory within 24 hours where they will be put into a freezer until being analyzed.

Figure 5
Log Sheet for Urine Voids
Phase Two

Participant Name:

ID:

Job Duties:

Date of Collection:

Sample #	Code No.	Time of Coll.	Orig. Vol.	Aliq. Vol.	Sp. Gr	Creatinine
1		(beginning of shift)				
2		(mid shift)				
3		(late shift)				
4		(end of shift)				
5		(composite)				

Written and verbal instructions on the procedures for collecting urine samples will be provided to each participant. An example of the participant instruction guide for the Phase Two study (see A1-A2) and a participant notification letter for reporting the results (Item III) are attached. Every effort will be made to minimize interference of normal work practices at the facility during the NIOSH survey.

ANALYTICAL PROCEDURES

The air, wipe, and dermal pad samples will be extracted directly from the individual glass vials in which they are transported. The following analytical procedure shall be used:

Extract the samples in 4 ml of a mixture that is 5% acetonitrile in 0.26N aqueous NaOH. Cap the vial (Teflon lined septum in cap) and shake. After 15 minutes, add 1 ml of acetic anhydride to the vial, cap and shake. After another 15 minutes place the capped vial in a heater block (80°C) for 15 minutes, remove and allow to cool to room temperature. For HPLC analysis, transfer the contents of the vial into a 5-ml syringe which is fitted with a 13-mm Swinnex filter holder unit that contains a 5 um pore size Teflon® filter. Transfer does not need to be quantitative but the mixture must not be diluted. Filter this mixture into a clean vial. Inject 100-ul aliquot into a High Performance Liquid Chromatograph equipped with a Waters C-18 Radial Pak column or equivalent. Use a 40/60 acetonitrile/water eluent that is run isocratically at 1 ml/minute. The approximate elution time for 4,4'-MDA should be about 7.3 minutes during a total run time of 18 minutes.

Calibration is performed by making up stock solutions of the analyte in methanol. Spike aliquots (about 10 ul) of the stock standards onto acid coated glass fiber filter blanks that have been placed into a reaction vial. Follow the procedure for analysis.

Analysis by high pressure liquid chromatography (HPLC) should be linear over an approximate range of 1 to 150 ug. The acidified filters have been shown to have close to a 100% collection efficiency for several aromatic diamines. Possible interferences may be corrected by adjusting the eluent solvent composition. The yield of acetylated diamines is greater than 90% for MDA over a range of 1 to 150 ug. A small amount of the analyte (less than 10% of the total) may be found on the stainless steel support screen if the air is 80% relative humidity or greater and the sampling time is long. The samples are stable on the collection filter for at least 1 week. The derivatives are stable in the reaction vial for at least 12 hours. The limit of quantitation for this procedure is approixmately 0.1 ug per sample.

Urine samples collected in this study shall be analyzed by the following procedure. Samples shall be thawed and run through a sorption column containing about two grams of XAD-1 resin. The urine passing through the column is discarded. The sorption column is eluted with 25 ml acetone. Evaporate the eluent while in a warm water bath to a volume of one milliliter. Add 1 ml of 0.1N sodium hydroxide. Extract three times with 5 ml benzene. At this point the samples may be analyzed on either a HPLC or a gas chromatograph with an electron capture detector.

If analysis is to proceed using HPLC, again evaporate the extract solution to one milliliter. One milliliter of 0.12N hydrochloric acid solution is added and agitated for one hour. Add 5 ml of hexane and lightly centrifuge the samples. The ageous phase is removed and from this 100 microliters (ul)

is injected into a dual-phase HPLC with a linear gradient programming from 100 percent pH4.7 sodium acetate buffer to 100 percent acetylnitrile. The limit of detection for this method is approximately 25 ppb of 4,4'-MDA in 100 ml of urine. Extraction efficiency (recovery) and precision is 76% $\pm$ 7% for the concentration range 25 to 250 ppb.

If greater sensitivity is required the urine samples may be analyzed by the following procedure which starts with the 15 ml benzene extract from the initial extraction procedure described above. First anhydrous sodium sulfate is added to the extract solution to remove moisture. The extract solution is evaporated to 1.5 ml and 0.5 ml trimethylamine is added. Next 100 ul of hexafluorobutyric acid is added and the solution is placed in a warm water bath for 20 minutes. The solution is then extracted three times with 2 ml of pH6 1 molar phosphate buffer. Discard the agueous layer and the resulting nonaqueous layer is shook with sodium sulfate. One milliliter of a 200 ppb 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE)solution is added as an internal standard.

The final sample solution is diluted to 5 ml with benzene. Three microliter injections are used for analysis on a 15 meter SE54 capillary column gas chromatograph with an electron detector. The overall extraction efficiency for this latter procedure has not yet been determined. The limit of detection is about 1 ppb 4,4'-MDA in 100 ml urine.

Some 4,4'-MDA may be metabolized by N-acetylation and/or N-O-conjugation and the methodology described above will not detect these metabolites. Increased detection of exposure in workers urine might be enhanced if these metabolites could be determined. One scheme involves the chemical conversion of metabolites present in the urine to the parent compound (4,4'-MDA), via hydrolysis, with subsequent analysis as described by one of the two methods above. Currently we do not know if the limit of detection of parent compound alone in the urine of exposed workers is sufficient, or if determining the metabolites would substantially increase the limit of detection. Determining urine concentrations of 4,4'-MDA metabolites in human participants shall be performed during the first phase of this research as well as the concentrations of unmetabolized 4,4'-MDA. Urine samples from monkeys that were dosed with radiolabeled 4,4'-MDA shall also be used to trace urinary elimination of 4,4'-MDA and its metabolites. From these data a decision regarding the importance of determining 4,4'-MDA hydrolyzable metabolites can be made and changes in the analytical procedure can be incorporated in the protocol to reflect this need at the appropriate time.

Urine creatinine will be determined on each sample void using a GEMSAEC centrifugal analyzer based upon the Jaffee Alkaline Picrate Reaction. Specific gravity shall be determined on each sample using a refractometer.

STATISTICAL ANALYSIS OF FIELD DATA

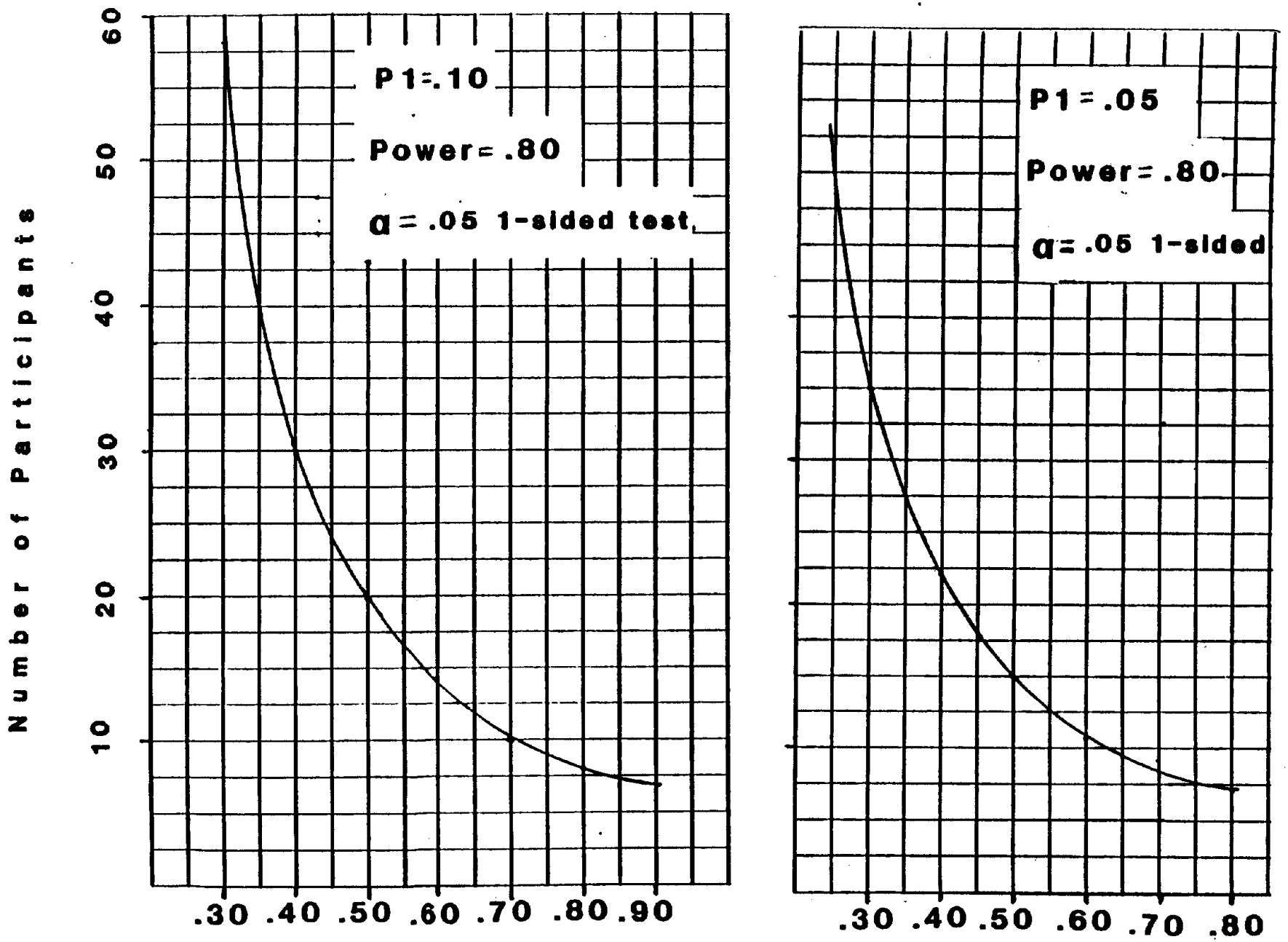
Environmental field sampling data will be reported in its entirety within tables, grouped and displayed in appropriate ways using histograms and/or graphs, etc. Personal identifiers will be omitted from the report.

Phase One:

MDA is not expected to be found in control urines. Since both MBOCA and benzidine have been found to be excreted in exposed workers it is anticipated that a difference in occurrence of MDA in the urine of control vs. exposed workers urines will be easily determined by a non-parametric statistical analysis. To be liberal in our sample size requirements, if the background of detectable MDA in urine were 10% (we do not expect any) in the control group and the proportion is 50% or greater detectable in the exposed group, we will have 80% power with a sample size of 20 per group. Sample size requirements for differenct outcomes are shown in Figure 6. The data from Phase One may also be used to indicate the time to maximum excretion after a workshift exposure, and the time dependent concentrations. A profile analysis of the curves for each participant will be performed which will generate a best fit for all the data. Continuous long term urine monitoring data that is collected during Phase One and is intended to describe the elimination kinetics for MDA in man will be used to calculate an elimination half-time average, and a peak elimination time with a range and standard deviation. If elimination follows an exponential decay then

Figure 6

Sample Size Requirements For Phase One Study



plotting concentration values, expressed after normalizing and adjusting for various co-factors, versus time on semi-log paper will produce a linear relationship.

Phase Two:

The data collected in Phase Two will be statistically analyzed according to the following procedures. To determine the relationship between environmental parameters and urinary excretion of MDA a multiple regression analysis using normalized independent variables (e.g. air and skin exposure), including an investigation of possible non-linear response may be appropriate. Correlation analysis would further quantitate the strength of association between pairs of variables. Statistical evaluations of variation between individuals that are pooled into like exposure groups will be made.

Since urine samples will be collected from each participant on three successive days, a proportional comparison of the urine concentrations found each day can be reported to show if a cumulative effect of exposure is operating on the elimination rate.

Three facilities that are continually using MDA have been identified, with about 50, 60, and 28 potentially exposed workers, respectively. Other large worker groups should be identified in the near future. All of these individuals who are suitable and who volunteer to participate will be included in the second phase of this evaluation. Thus, sufficient numbers of potential participants do exist to perform this study. This exploratory study will help to demonstrate whether a measurable association exists between estimated exposure to MDA and its excretion in urine.

QUALITY ASSURANCE PROCEDURES

In order to minimize to an expected acceptable level all methodological errors, each method used in this study has been thoroughly evaluated. Industrial hygiene sampling and chemical analytical methods have been evaluated for such considerations as collection efficiency (air samples only), recoverability, storage stability, and precision.

For instance, the acid treated glass fiber air collection method has been evaluated in three separate laboratories. NIOSH has evaluated the sample recoverability, storage stability, and analyte stability during air sample

collection. Stanford Research Institute, Menlo Park, California, had provided a full evaluation of the method, including the collection efficiency, in dynamic aerosol generation chambers. An independent method evaluation was undertaken by the Midwestern Research Institute, Kansas City, Missouri, where the method was challenged to the vapor state of 4,4'-MDA only. In both laboratories the method demonstrated that filter collection of the analyte was essentially complete. The storage stability of acid treated glass fiber filters in polystryene cassettes as assessed after a known liquid amount of 4,4'-MDA in methanol was added. Storage stability for up to one week at room temperature was essentially complete. The analytical coefficient of variation (CV) for laboratory prepared samples was found to be 0.053.

Both blank samples and liquid spiked samples with known amounts of 4,4'-MDA shall be included with the field collected samples for laboratory analysis. Spiked samples will be prepared at 5 times the limit of quantitation (about 1 ug/sample). To determine the precision, accuracy, and bias of the air samples collected in the field, sampling stations shall be used. Air samples using three liquid spiked, and three unspiked acid, treated filters will be collected where MDA is judged to be present. Accurately spiked samples, which will be sampled side-by-side with normal samples, will be used to determine the recovery and whether the recovery has been affected by chemical or environmental interferences. Unused spiked samples will also be shipped from the sampling sites to assess the effect of transporting and storing the samples on recovery of the analyte. Blank samples shall be

shipped with field collected samples to determine migration of analyte between samples and as a check of analytical quality. Initial sampling performed in the field presently indicates that no unusual problems shall be encountered and additional evaluation of the air sampling method will be performed prior to Phase Two of this study. Procedures for statistical analysis of field sample evaluation data can be found elsewhere.[31]

Precision shall be determined by comparing the deviation of the individual sample results from the means of spiked and unspiked triplicate sample sets that were collected in the field. These control samples will not be identified to the laboratory prior to the analysis.

All field air samples shall be collected using constant flow regulated pumps which are calibrated prior to each survey. A random sample of at least 25% of the pumps used will be recalibrated at the end of the workshift with a Kurz calibration meter, model 541. This meter will be calibrated using a primary calibration standard in our industrial hygiene laboratory just prior to the survey.

The sample stability for cellulosic wipe taps and glycerin dampened cotton pads has been assessed in our laboratories. Twenty samples of each media were initially spiked with 4,4'-MDA from a microsyringe. The recovery at day one and times thereafter was determined by analyzing four samples per sample set.

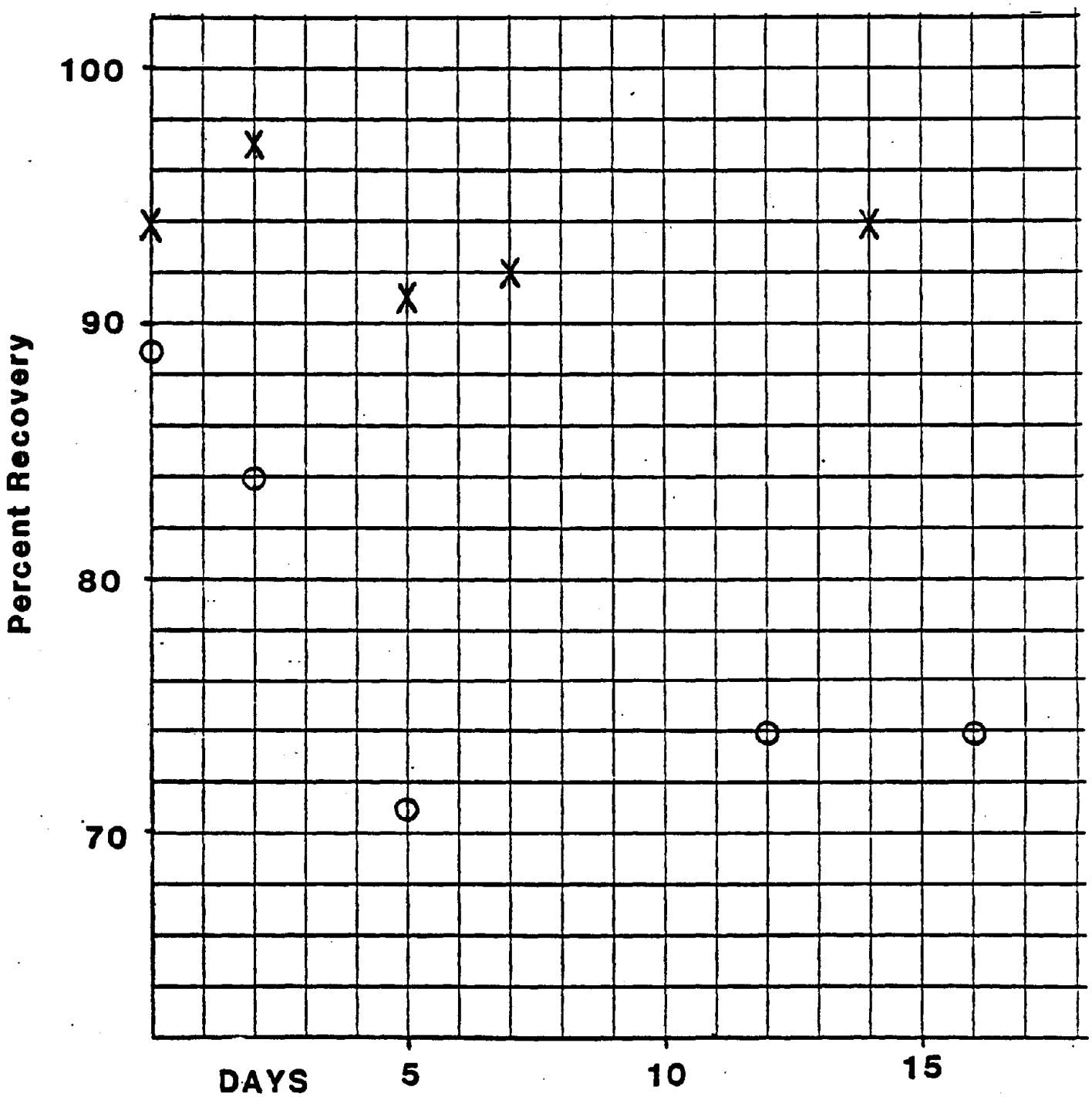
Initial results of analyte recovery from wipe tabs and cotton pads that were spiked with 4,4'-MDA and were then stored at room temperature were unsatisfactory. While initial analyte recoveries for the wipe and pad samples were 92% and 88%, respectively, recoveries deteriorated to 43% and 53% after twenty days. In subsequent trials, four milliliters of the dilute sulfuric acid solution that was used to treat the air sample filters was added to the wipe and pad samples after sample collection. Sample stability during storage at ambient temperature was substantially improved. Recovery of the analyte on the wipe tabs remained above 90% for up to 14 days. No decline in stability was seen. The stability of the analyte on cotton gauze pads showed an initial decline from 89% to 71% after five days but remained stable thereafter through day 16. Figure 7 shows the performance of these two sampling media at five storage periods.

The Wright respirometer used to measure respiration volumes on worker participants in this study has been evaluated in our laboratory. The respirometer was connected in line with a primary air volume calibration bell. Air was drawn through the respirometer at various flow rates ranging from 4.5 to 31 liters per minute at constant flow. The percent deviation in measured volume versus actual volume were plotted in Figure 8. These results show negligible deviation between flow rates of 7-20 lpm. Simulated breathing with flow rates between zero and 20 lpm indicated perfect agreement in most trials. Peak expiratory flow rates under low to moderate physical demand usually do not exceed 20 lpm.

FIGURE 7

Sample Recovery From Smear Tabs

and Hand Pad Samples Spiked With 4,4'-MDA

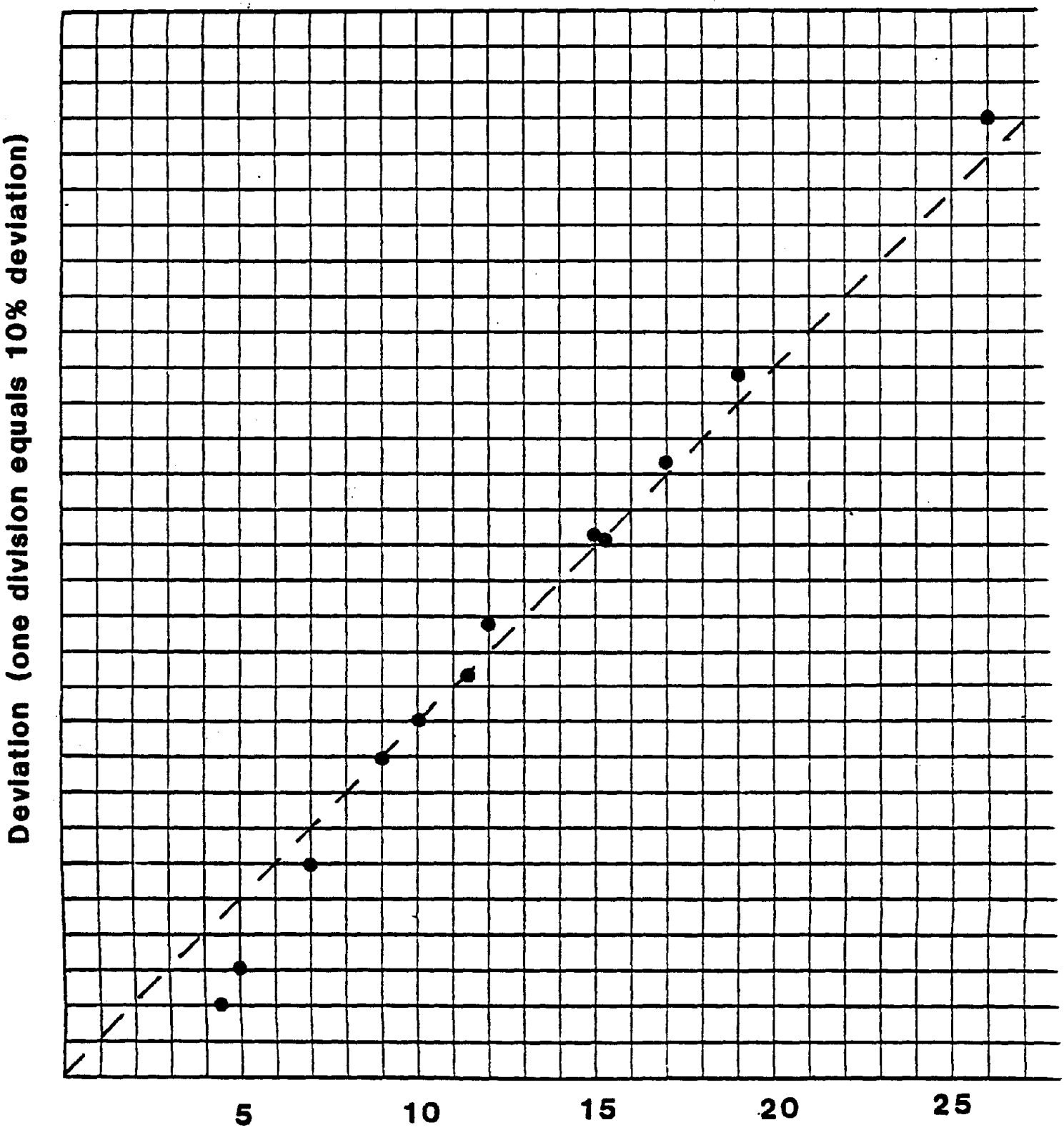


Legend: x indicates cellulosic smear tabs

O indicates cotton pad samples

FIGURE 8

Determination of Measured Flowrate at Various Known Velocities for the Wright Respirometer



The stability of free 4,4'-MDA in human urine has been assessed. One hundred milliliter aliquots of a one liter standard solution of human urine containing 100 nanograms of 4,4'-MDA per milliliter (about 100 ppb) were prepared and analyzed at 24 hour without refrigeration, at 48 hours with refrigeration and freezing, and at 96 hours with freezing and refrigeration in polyethylene storage bottles. Samples frozen for over a month were also analyzed. No appreciable loss or interferences to the determination of the analyte was found under any of the above storage conditions. The extraction efficiency (recovery) and precision was 76% $\pm$ 7% for the concentration range 25 to 250 ppb.[10]

SAFETY

For the safety of the investigators involved with this study the project officer shall be responsible for the assignment of personal protective equipment to the study members when necessary. This equipment may include use of respirators, gloves, or protective garments as described in the personal protection program of the Industrywide Studies Branch. Preliminary walk-through surveys of each facility will be used to assess the need for such protection.

The analytical staff responsible for the determination of 4,4'-MDA in environmental and biological samples will follow good work practices as prescribed for the handling of such samples.

Each member of the NIOSH research teams participates in a medical monitoring program for health maintenance.

ROLE OF NIOSH COLLABORATORS

General management of this overall study will be performed by the Project Officer, Mark Boeniger. In addition to coordinating the effort's of the other collaborators, the Project Officer shall supervise the survey team engaged in the collection of all samples during the field surveys. The project officer shall also be responsible for the writing of all reports. All of the collaborators working on this study are employed by NIOSH. Mr Rick Hornung (513/684-4211) will b available as needed for the statistical analysis and interpretation of the data. Dr. Larry Lowry and Mr. Anthony Smallwood (513/684-8338) will be responsible for the chemical analysis of all urine samples and the analytical procedures described herein. Mr. Edward Slick (513/684-4219) has been very instrumental in the development and evaluation of the environemtnal sampling methods. Ana Maria Osorio, M.D. will serve as medical records reviewer and consultant.

RECORDS MANAGEMENT AND REPORTING OF THE DATA

Information regarded as trade secret will be retained by NIOSH in accordance with the NIOSH Sensitive Data Security Program. Also, personal identified urine sampling results cannot be distributed to third parties without the volunteer's written consent, as required under the Privacy Act. Workers

participating in the urine monitoring portion of this study will be mailed their individual results directly to their residence or to a health care provider of their choosing. The nature in which the results will be used by NIOSH and the limited significance of their individual results to them will be explained. Data record sets shall be prepared that contain information on each participant in this study. These will include an activity record, urine collection record, and exposure measurement record. Hand written data shall be transferred to a personal computer for data management and statistical analysis. The hand written data and computerized records shall be stored in a locked file when not in use. All individuals shall be initially identified by a code number which will be used for the computerization and management of his records. Draft reports of each survey will be written and sent to company management for their review to allow for deletion of trade secret information before finalizing. The final reports will be forwarded to the management and the union (if applicable), OSHA Headquarters, EPA Headquarters, and the appropriate NIOSH regional consultant. Final reports will be obtainable from NIOSH or through the National Technical Information Service (NTIS).

A composite report, published by NIOSH, will include all relevant background information and specific information dealing with the methodologies used in this study, including the results, conclusions, and recommendations for future study. The identities of individual workers participating in this study will not be divulged in this document or any other report except by written consent from that individual.

For further information, contact Mark Boeniger, NIOSH Project Officer at (513) 684-2876 or write to the address below:

National Institute for Occupational Safety and Health

4676 Columbia Parkway

Mail Stop F-7

Cincinnati, Ohio 45226

Bibliography

1. Draft Report. NTP Technical Report on the Carcinogenesis Bioassay of 4,4'-Methylene Dianiline Dihydrochloride in F384/N Rats and B6C3F/N Mice. NTP. NIH Publication No. 82-2504, 1982.

2. Dunn, GW and Guirguis, SS. Methylene Dianiline (MDA) as an Occupational Health Problem A Suggested Time-Weighted Average Exposure Level and Medical Program. Arh. hig. rada toksikol. 30; 639-45 1979.

3. Non-Isocyanate Uses of 4,4'-methylene dianiline, Prepared by Springborn Regulatory Services, Inc. for Office of Pesticide and Toxic Substances, Regulatory Impacts Branch, TS-779, EPA, Washington D.C., 1983

4. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1980, American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio p. 23.

5. Federal Register, April 27, 1983 (48 FR 19078).

6. Benzidine in Urine (screening test) in NIOSH Manual of Analytical Methods, 2nd ed., Vol. 5, P&CAM 315, 1979, DHEW (NIOSH) Publication No. 79-141.

7. McGill, DB and Mott JD. An Industrial Outbreak of Toxic Hepatitis due to Methylene Dianiline. N Eng. J. Med 296: 278-282, 1974.

8. 4,4'-Methylene Dianiline, IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Volume 4, International Agency for Research on Cancer, Lyon, p. 79-85, 1974.

9. Sittig, M. Pharmaceutical Manufacturing Encyclopedia, Noyes Data Corporation, Park Ridge, New Jersey, 1979.

10. Smallwood, A., Debord, K. Laboratory Evaluation of the Stability of 4,4'-MDA in human urine. Unpublished data, Chemical and Biochemical Support Section, DBBS, NIOSH.

11. Manis, M and Braselton, E. Pharmocokinetics of MBOCA in Dogs. MBOCA - 1982: Research Results and Recommendations for Environmental and Occupational Levels. Toxic Substance Control Commission, Lansing, Michigan, June, 1982, p. 7.

12. Morton, K. Excretion and Mutagenicity of MBOCA and its Metabolites in Rats and Humans, Ibid., p. 6.

13. Groth, DW, Weigel WW, Tolos WP, Brewer DE, Cheever KL and Burg JR. 4,4'methylene-bis (ortho-chloro-aniline) (MBOCA): Absorption and Excretion After Skin Application and Gavage. Environmental Research, in press, 1983.

14. Margott, D. Personal communication, Univ. of Michigan, Ann Arbor, (313) 764-1316.

15. National Institute for Occupational Safety and Health 4,4' Methylene bis (o-chloroaniline) (MOCA) in Urine, NIOSH Manual of Analytical Methods, Vol. 7, P&CAM 342-1 to 342-9, DHHS Publication No. (NIOSH) 82-100, 1981.

16. NIOSH, 1980. National Institute for Occupational Safety and Health, Computer Printout: Projected numbers by employment. Washington, DC: Office of Pesticides and Toxic Substances.

17. Weeks, R.W. et al. Detection Limits of Chemical Spot Tests Toward Certain Carcinogens on Metal, Painted, and Concrete Surfaces. Anal. Chem. 48, 2227, Dec., 1976.

18. Moore, W.M. Methylenedianiline. In: Kirk-Othmer Encylclopedia of Chemical Technology, pp 338-348. Third Edition. Interscience Publishers, New York, 1978.

19. Windholz M (Ed). Merck Index. Ninth Edition, Merck and Company, Inc., Rahway, New Jersey, 1976.

20. Product Information Bulletin on Tonox, Uniroyal, Inc., Naugatuck, Connecticut.

21. Ader, D, 1957. Cited In Exposure Tests for Organic Compounds in Industrial Toxicology, J.K. Piotrowski, (ed.). U.S. Department of Health, Education, and Welfare (NIOSH) Publication No. 77-144, 1977.

22. Vaudaine M, et al. Diamino diphenyl methane: An example of toxic agent monitoring in the workplace at Rhone-Poulene Industry. J. de Toxicol. Med. 2 (3): 207-213, 1982.

23. Williams, S V, et al. Toxic hepatitis and methylene dianiline. New Eng. J. Med. Dec 5, p. 1256, 1974.

24. Brooks, L J, et al. Acute Myocardiopathy Following Tripathway Exposure to Methylene Dianiline. JAMA, 242 (14): 1527-8, 1979.

25. Maddy, K T et al. Dermal exposure monitoring of mixers, loaders, and applicators of pesticides in California. Worker Health and Safety Unit, California Department of Food and Agriculture, Sacramento, CA. Report HS-1069, 1983.

26. Gold, R E, et al. Exposure of urpan applicators to carbaryl. Arch. Environ. Toxicol. 11: 63-67.

27. Franklin, C A et al. Correlation of urinary pesticide metabolite excretion with estimated dermal contact in the course of occupational exposure to Guthion. J. Tox. Environ. Health 7: 715-31, 1981.

28. Davis, J E Minimizing occupational exposure to pesticide: Personnel monitoring. Residue Rev. 75: 33-50, 1980.

29. Clapp, D. Personal Communication. Industrial Hygiene Section, Industrywide Studies Branch, DSHEFS, NIOSH.

30. Linch, A.L. Biological monitoring for industrial chemical exposure control. CRC Press, Cleveland, OH 1974.

31. Borders, R. et al Improved methodology for field validation of industrial hygiene monitoring methods. Am. Ind. Hyg. Assoc. J 45(5): 299-305, 1984.

ATTACHMENT I

PARTICIPANTS CONSENT FORM

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH)
CENTERS FOR DISEASE CONTROL
U. S. PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

You have been asked to participate in a NIOSH research study. We explain here the nature of your participation, describe your rights, and specify how NIOSH will treat your records

---

I. Description

1. TITLE: Extent of Worker Exposure to 4,4'-methylene dianiline (MDA)

2. PROJECT OFFICER AND SPONSOR:

Mark F. Boeniger

Industrial Hygiene Section

Industrywide Studies Branch

Division of Surveillance, Hazard Evaluations and Field Studies

Robt. A. Taft Laboratories

National Institute for Occupational Safety and Health

4676 Columbia Parkkway.

Cincinnati, Ohio 45226

513/684-4363

3. PURPOSE AND BENEFITS:

The purpose of this study is to compare two methods that measure workers' exposure to MDA. In the course of this study we shall be able to determine the extent of your personal exposure to MDA.

We are concerned about MDA because overexposure has been shown to cause (1) damage to the liver, (2) skin rash (as in an allergic response), and (3) various ill effects such as abdominal pain, fever and nausea. Also, MDA has caused cancer in studies with animals. However, the significance of these animal studies to humans is not known at this time.

If you are exposed to MDA, the benefit to you as a participant is that a full evaluation is performed of your job exposure to MDA. If you are identified as a control or non-exposed participant, then you will not directly benefit from this study but you will be benefiting your fellow workers who may be exposed. Your personal results shall be reported to you in a way that will allow you to compare them to the general findings of other participants in this study. In addition to the benefits to you, workers who are not involved in this study will benefit from these findings which should help to reduce exposure to MDA.

II. CONDITIONS OF THE STUDY

1. Procedures: If you are identified as having exposure to MDA, you will be asked to wear a small light-weight personal air sampling pump and possibly a glove-like sampler on your hand to help NIOSH evaluate your exposure to MDA. Also you will be asked to collect urine samples in plastic bottles provided to you during the work day. Your name will be written on your bottle(s) and you will be requested to record the time during which you collected the urine sample. You may be asked to collect urine at predetermined times. This shall be explained fully to you by the NIOSH Project Officer.

   During the time of your participation in this study we will be observing and making notes on your work practices. Written records of the exposure measurements, urine samples, and other notes shall also be collected. Occasionally we may take photographs. Later, these notes and photographs shall only be used to help us in the interpretation of the data. Finally, your company medical file will be checked to identify any medical condition that could affect the interpretation of our measurements.

   If you are identified as a non-exposed or control participant, your only direct involvement will be to collect an end-of-work urine sample.

2. No medical risks or discomforts are foreseen, but should you have any reaction to the tests/procedures, you should contact Mark Boeniger,, Project Officer, 513/684-4363.

3. There is no alternative test that is recognized as being able to answer the research objectives of this study.

4. Injury from this project is unlikely. But if injury results, medical care is not provided, other than emergency treatment. If you are injured through negligence of a NIOSH employee or an agent of NIOSH, you may be able to obtain compensation under the Federal Tort Claims Act. If an injury should occur to you as the result of your participation, you should contact Mark Boeniger, 513/684-4363.

5. If you have questions about this research or your rights as a member of this study, contact Mark Boeniger, Project Officer 513/684-4363.

6. Your participation is voluntary and you may withdraw your consent and end your participation in this study at any time without penalty or loss of benefits to which you are otherwise entitled.

III. USE OF INFORMATION

1. NIOSH's authority for collecting information in this study is the Occupational Safety and Health Act of 1970.

2. You do not have to furnish any informaton. Nothing will happen to you if you don't answer our questions, except that we may not use you in the study.

3. NIOSH will provide you and your doctor (if you wish) with all findings from your tests (and any other examinations). We will do this when the study is finished, or sooner if appropriate. Your personal results shall be reported to you in a way that will allow you to compare them to the general findings of other participants in this study.

4. The information you and other persons give us will be used by us to help answer research questions about the extent of your exposure to MDA and the best means of protecting you from excessive exposures. This information will be provided to the Occupational Safety and Health Administration (OSHA) and to the Environmental Protection Adminstration (EPA) to help develop guidelines and standards to monitor worker exposure to MDA.

5. The information you provide us is covered by the Privacy Act, a federal law. We will not reveal your information in identifiable form to anyone without your permission, unless it is permitted by the Privacy Act. If requested, the Privacy Act permits the release of informaton in identifiable form under several specific conditions. All 11 conditions possible are stated on the Back Side of this page. These releases are infrequently used, in general. When these kinds of requests occur, each is reviewed by us to ensure that a person would not reasonably object. The reasons most often used to seek records subject to the Privacy Act are:

   (1) When the records are needed to protect the health and safety of other persons.

   (2) When a researcher (for example, at a university) asks for information and it will be used only for statistical purposes.

6. Your records are also covered by the Freedom of Information Act, a federal law. This law permits persons to request information held in our files. All Freedom of Information requests are reviewed by NIOSH to ensure that a person would not reasonably object to the release of the records.

IV. SIGNATURES

I have read this consent form and I agree to participate in this study.

PARTICIPANT _____ Age _____
(and Guardian, (signature)
(if required) Date _____

I, the NIOSH representative, have accurately described this study to the participant.

REPRESENTATIVE _____ Date _____
(signature)

page I-6 is blank

REQUEST AND AUTHORIZATION FOR RELEASE OF INFORMATION

I, _____, request and permit the project officer to inform the following physicians or health care facilities (whose names and addresses I have entered below) of any significant findings from this study which concern me. (Do not leave blank. Write "NO" where you do not wish to give a name and address.)

1. My personal physician(s):

   Dr._____

   Street_____

   City_____State_____Zip_____

2. Other physicians or health care facilities:

   Name_____

   Street_____

   City_____State_____Zip_____

_____

Participant

(and Guardian if required)_____Date_____

1 copy to participant
1 copy to project officer

ATTACHMENT II

PARTICIPANTS NOTIFICATION LETTER

Example of Participant Notification Letter

Dear_____:

Thank you for your participation in the research study of occupational exposure to methylene dianiline MDA which was performed by the National Institute for Occupational Safety and Health on _____. Your participation has provided useful information which will be used in evaluating the extent of exposure to MDA in workplaces in the United States. This may result in providing you, as well as others, with a safer workplace.

As we explained to you when we conducted the study, MDA can cause damage to the liver if overexposure occurs. At present, there are no exposure limits enforced by law. However, a recommendation has been made by occupational health researchers, based on the effects to the liver, that air levels should not exceed 0.1 ppm or 0.8 mg of MDA per cubic meter of air*. As we mentioned when we conducted the study, MDA can cause cancer in animals, and NIOSH is now attempting to investigate if MDA can cause cancer in humans. However, we do not have the answer yet. In this regard, air exposure levels probably should be minimized as much as possible.

The following results are provided to you for your information. It should be noted that the following are only indicative of your exposure to MDA and have no known direct clinical meaning as related to your state of health. It is recommended that these exposure results be kept for future reference.

Sampling Results for MDA

1 Air Sample: _____ppm* or _____mg/m^3

(average over _____minutes).

Hand Sample: _____mg - palm

_____mg - back of hand

Urine Sample: _____ppb**

By comparison, non-exposed workers have _____MDA in their urine. The highest level of MDA that was found among other exposed workers at your facility was _____ppb and the lowest was _____ppb the group average was _____ppb.

The company management will be informed of our general conclusions regarding the extent of exposure to MDA at your facility, and specific recommendations will be provided to them, if necessary, to reduce exposure.

Absorption of MDA into the body can occur by breathing it, by eating it in contaminated food, and by absorbing it through the skin. These routes of absorption can be minimized if proper attention is given to personal

practices, proper ventilation of your work area, or personal protection (including non-absorbant gloves, and dust respirator). Work clothes should be changed often and left at work to prevent contaminating your home. Cigarettes or food should not be handled with contaminated hands.

Thank you for your participation in this study. If you wish to discuss these results further, please call me at (513) 684-4363 or William E. Halperin, M.D., at (513) 684-4203, or send your inquiries to the above mailing address.

The confidentiality and protection of personal information will be maintained as required under the Privacy Act of 1974, Title 5, United States Code, Section 552 (a)(e)(3). The files will be kept in a secure storage area and disposed of after 5 years or when no longer deemed useful.

Footnotes:

* One part MDA per million parts of air is equivalent approximately to 8 milligrams MDA per cubic meter of air, in other words it is proportional in quantity to 1 teaspoon in 4350 gallons.

** One part MDA per billion parts of urine is equivalent in proportion to one foot in 189 thousand miles or one second in 1903 years. There is currently no maximum recommended limit or legal standard for MDA in the urine.