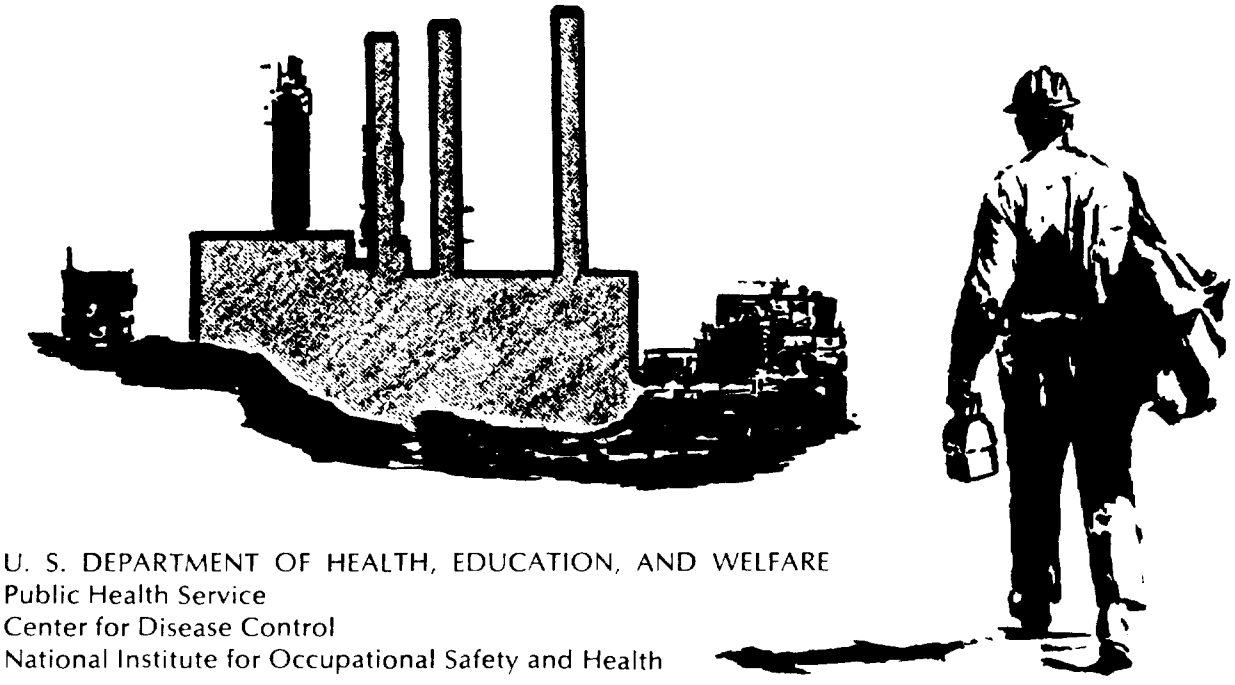
NIOSH

CRITERIA FOR A
RECOMMENDED STANDARD....

OCCUPATIONAL
EXPOSURE TO

o-TOLIDINE



U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health

criteria for a recommended standard....

OCCUPATIONAL EXPOSURE TO

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August 1978

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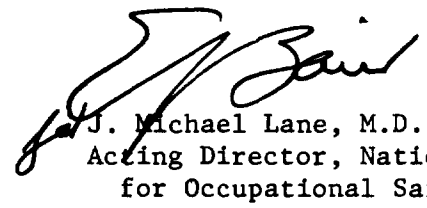
PREFACE

The Occupational Safety and Health Act of 1970 emphasizes the need for standards to protect the health and provide for the safety of workers occupationally exposed to an ever-increasing number of potential hazards. The National Institute for Occupational Safety and Health (NIOSH) evaluates all available research data and criteria and recommends standards for occupational exposure. The Secretary of Labor will weigh these recommendations along with other considerations, such as feasibility and means of implementation, in promulgating regulatory standards.

NIOSH will periodically review the recommended standards to ensure continuing protection of workers and will make successive reports as new research and epidemiologic studies are completed and as sampling and analytical methods are developed.

The contributions to this document on o-tolidine by NIOSH staff, other Federal agencies or departments, the review consultants, the reviewers selected by the Society of Toxicology, and Robert B. O'Connor, M.D., NIOSH consultant in occupational medicine, are gratefully acknowledged.

The views expressed and conclusions reached in this document, together with the recommendations for a standard, are those of NIOSH. They are not necessarily those of the consultants, the reviewers selected by professional societies, or other Federal agencies. However, all comments, whether or not incorporated, have been sent with the criteria document to the Occupational Safety and Health Administration for its consideration in setting the standard. The review consultants and the Federal agencies which received the document for review appear on pages v and vi.



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The Division of Criteria Documentation and Standards Development, National Institute for Occupational Safety and Health, had primary responsibility for the development of the criteria and recommended standard for o-tolidine. Herbert L. Venable of this Division served as criteria manager. SRI International developed the basic information for consideration by NIOSH staff and consultants under contract CDC-99-74-31.

The Division review of this document was provided by Douglas L. Smith, Ph.D. (Chairman), Keith H. Jacobson, Ph.D., and Frank L. Mitchell, D.O., with Larry K. Lowry, Ph.D. (Division of Behavioral and Biological Sciences) and Charles C. Hassett, Ph.D.

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Environmental Protection Agency
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    for Program Integration
  Health Effects Research Laboratory

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I. RECOMMENDATIONS FOR AN o-TOLIDINE STANDARD

NIOSH recommends that employee exposure to o-tolidine in the workplace be controlled by adherence to the following sections. The recommended standard is designed to protect the health and provide for the safety of employees for up to a 10-hour workshift, 40-hour workweek, over a working lifetime. Compliance with all sections of the recommended standard should prevent adverse effects of o-tolidine on the health of employees and provide for their safety. The standard is measurable by techniques that are reproducible and available to industry and government agencies. The employer should regard the recommended workplace environmental limit as a maximum limit for exposure and should make every effort to keep the exposure as low as is technically feasible. Sufficient technology exists to permit compliance with the recommended standard. The criteria and standard will be subject to review and revision as necessary.

o-Tolidine is widely used in the dye industry. It is also used in analytical chemistry procedures, including tests for chlorine in water, and in medical laboratory tests for sugar or occult blood. The biologic dye, trypan blue, contains the o-tolidine moiety. Animals exposed to o-tolidine have developed cancers at various sites, and results of bacterial tests indicate that o-tolidine may be mutagenic. Damage to mammalian DNA metabolism has also been reported. o-Tolidine is readily absorbed through the skin, and nasal irritation has been reported in humans exposed to it.

The term "o-tolidine" refers to various physical forms of the compound and its salts. Synonyms for o-tolidine include 3,3-dimethylbenzidine, 4,4-diamino-3,3-dimethylbiphenyl, diorthotoluidine, diaminoditolyl, azoic diazo reagent, gold diazo reagent, nitro coupling reagent, direct blue 63, fast blue R base, and benzo fast blue R. "Occupational exposure to o-tolidine" is defined as work in any place in which o-tolidine is produced, stored, used, packaged, or distributed. If o-tolidine is handled or stored only in intact, sealed containers, (eg, during shipment), adherence to the following sections, except for Sections 3, 5(a), 6(g), and 8(a), is not required. This recommended standard does not apply to users of test tapes or test kits containing o-tolidine. Employees shall avoid skin contact with o-tolidine, since skin absorption can be a significant source of exposure.

Section 1 - Environmental (Workplace Air)

(a) Concentration

Occupational exposure to o-tolidine shall be controlled so that employees are not exposed at a concentration greater than 20 micrograms per cubic meter of air (μg/cu m), determined as a ceiling concentration in a 1-hour sampling period. Skin contact with o-tolidine shall be avoided.

(b) Sampling and Analysis

Procedures for the collection and analysis of environmental samples shall be as provided in Appendix I or by any methods shown to be at least equivalent in precision, accuracy, and sensitivity to the methods specified.

Section 2 - Medical

Medical surveillance shall be made available as outlined below to all employees occupationally exposed to o-tolidine.

(a) Preplacement medical examinations shall include at least:

(1) Comprehensive medical and work histories with special emphasis directed towards the urinary tract.

(2) Comprehensive physical examination to include urinalysis with a microscopic examination of cells in urine. If the urinalysis is judged to be abnormal, a reexamination shall be performed. If the abnormal finding is confirmed, a comprehensive urologic evaluation should follow.

(3) A judgment of the worker's ability to use positive pressure respirators.

(b) Periodic examinations shall be made available at least annually to employees occupationally exposed to o-tolidine. Quarterly urine examinations are recommended. These examinations shall include at least:

(1) Interim medical and work histories.

(2) Comprehensive physical examination as described in (a)(2).

(c) Pertinent medical records shall be maintained for all employees exposed to o-tolidine in the workplace. Such records shall be kept for at least 30 years after the last occupational exposure to o-tolidine. Records of environmental exposures applicable to an employee shall be included in the employee's medical records. These records shall be made available to the designated medical representatives of the Secretary of Health, Education, and Welfare, of the Secretary of Labor, of the employer, and of the employee or former employee.

Section 3 - Labeling and Posting

All warning signs shall be printed both in English and in the predominant language of non-English-reading employees. Workers who cannot read the language used on labels or warning signs shall receive information regarding hazardous areas and shall be informed of the instructions printed on labels and signs.

All containers of o-tolidine shall be labeled and all areas where o-tolidine is stored, handled, used, produced, or distributed shall be posted in accordance with the following subsections.

(a) Containers of o-tolidine shall bear the following label in addition to, or in combination with, labels required by other statutes, regulations, or ordinances:

O-TOLIDINE
DANGER
CANCER SUSPECT AGENT
CAN BE ABSORBED THROUGH SKIN

Use only with adequate ventilation.
Handle with gloves resistant to o-tolidine.
Wash from skin immediately.

(b) The following warning sign shall be posted in readily visible locations at or near entrances to areas in which o-tolidine is stored, handled, used, produced, or distributed:

WARNING--HAZARDOUS AREA
O-TOLIDINE
CANCER SUSPECT AGENT
CAN BE ABSORBED THROUGH SKIN
AUTHORIZED PERSONNEL ONLY

Section 4 - Personal Protective Equipment and Clothing

Employers shall use engineering controls and safe work practices to keep exposure to o-tolidine as low as possible and to minimize skin contact. When necessary, these shall be supplemented by the use of personal protective equipment. All employees entering the regulated area shall be equipped with clean work clothing (long-sleeved shirts, trousers, underwear, and footwear) and with the necessary protective equipment. Full-body protection with appropriate head covering and air supply shall be used in weighing and charging operations in which there is occupational exposure to o-tolidine. At no time shall protective equipment be stored in the regulated area.

(a) Respirators may be used only:

(1) During the time necessary to install and test the required engineering controls.

(2) During nonroutine operations or maintenance and repair activities in which brief exposure to o-tolidine dust or vapor may occur.

(b) Respirators permitted or required by paragraph (a) of this section shall be supplied-air or self-contained positive-pressure respirators with full facepiece and shall comply with the standards jointly approved by NIOSH and the Mining Safety and Health Administration as specified in 30 CFR 11. All respiratory protective devices should be worn with full-body clothing resistant to penetration by o-tolidine. Employers shall provide respiratory protection for employees and shall establish and enforce a respiratory protection program meeting the requirements of 29 CFR 1910.134 and shall ensure that employees use the respiratory protective equipment when necessary.

(c) Employers shall ensure that respirators are properly cleaned and maintained. They shall also ensure that employees know the location of respirators assigned to them, how to use them, and how to test respirators for leaks, proper operation, and proper fit.

(d) Respirators shall be easily accessible. If respirators for more than one purpose are present, employees shall be taught to recognize the proper one.

Section 5 - Informing Employees of Hazards from o-Tolidine

(a) Employees who work in areas in which o-tolidine is stored, handled, used, produced, or distributed shall be informed at the beginning of their assignment and at least annually thereafter of the hazards of exposure to o-tolidine, including the information that o-tolidine is believed capable of causing cancer, that it can be absorbed through the skin, mouth, and respiratory tract, and that it may cause kidney damage. Employees shall also be informed of the value of continued periodic medical examinations. Information shall also be provided on the specific nature of the operation that could result in exposure and on how to recognize and evaluate conditions and situations that may result in the release of o-tolidine. The employer shall also inform the employees about cleanup, decontamination, and emergency procedures and their role in these activities. Employers shall post this information in the workplace and shall keep it on file, readily accessible to employees.

(b) Employers shall institute a continuing education program, conducted at least annually by persons qualified by experience or training, for employees whose jobs may involve occupational exposure to o-tolidine to ensure that all such employees have current knowledge of job hazards; relevant maintenance, cleanup, and decontamination methods; and proper respirator use. The instruction program shall include a description of the environmental and medical surveillance procedures and of the advantages to the employee of participating in these procedures. As a minimum, instruction shall include the information in Appendix III.

(c) Required information shall be recorded on the "Material Safety Data Sheet" shown in Appendix III or on a similar form approved by the Occupational

Safety and Health Administration, US Department of Labor, and kept on file, readily accessible to employees at all places of employment where there is occupational exposure to o-tolidine.

Section 6 - Work Practices

(a) Emergency Procedures

For all work areas in which emergencies involving o-tolidine may occur, employers shall ensure that employees are properly trained and follow the procedures specified below and any others appropriate for the specific operation or process.

(1) All employees involved in the emergency who may have had skin contact with o-tolidine shall wash affected parts promptly and thoroughly.

(2) Persons essential to emergency operations shall have the approved protective clothing and respirators, as specified in Section 4, readily available.

(3) Procedures shall be prepared for maintenance or cleanup and decontamination of areas where leaks or discharges of o-tolidine have occurred. Employees not essential to emergency operations shall be evacuated from the affected areas during emergencies. Perimeters of these areas shall be delineated, posted, and secured.

(4) Only personnel properly trained in emergency procedures and protected against the attendant hazards shall clean up and decontaminate spills and control and repair leaks. After cleanup and decontamination, protective clothing and equipment shall be decontaminated and removed and the employee required to shower.

(5) Emergency telephone numbers shall be prominently posted.

(b) Engineering Controls

Engineering controls shall be used to prevent the inhalation of, and to minimize skin contact with, o-tolidine by controlling the amount of o-tolidine that is emitted into the air and, indirectly, the amount present in the work area through settling. The most effective control measure is enclosure of unit operations and processes. For small operations, glove boxes or laboratory hoods may constitute sufficient enclosure. Local exhaust ventilation may also be effective when used at the source of o-tolidine emission.

Ventilation systems shall be inspected for corrosion, subjected to regular preventive maintenance, and cleaned at least every 6 months to ensure their effectiveness. The effectiveness of the system shall be verified by airflow measurement at least annually and a log showing the results of annual

inspections shall be kept. Exhaust ventilation systems shall discharge to the outside air through an appropriate filtering device and shall conform to applicable local, state, and Federal regulations. Contaminated exhaust air shall not be recirculated or discharged to other work areas, either regulated or unregulated.

Enclosures, exhaust hoods, and ductwork shall have pressure-failure alarms and shall be kept in good repair so that design airflows are maintained. Airflow at each hood shall be measured at least quarterly, but monthly measurements are recommended. Continuous airflow indicators, such as water or oil manometers properly mounted at the juncture of fume hood and duct throat (marked to indicate acceptable airflow), are recommended. A log showing design airflow and the results of quarterly inspections shall be kept and may be used in place of the annual inspection log of the ventilation system.

(c) Regulated Areas

Regulated areas shall be established and maintained where o-tolidine is manufactured, used, processed, or repackaged. Access to these areas shall be limited to authorized persons. This requirement for regulated areas includes the manufacturing and processing of o-tolidine test tapes and test kits, but it does not apply to the use of such tapes or kits for testing applications (such as for water analysis for chlorine or for measurement of occult blood) if skin contact with o-tolidine is avoided. An entry roster shall be kept of employees entering regulated areas. Toilets shall be located in regulated areas for use of employees in these areas and shall be separate from other toilet facilities.

(1) Access to the regulated area shall be limited to employees having assigned duties there.

(2) A daily entry roster shall be kept of all employees entering the regulated area and of their length of stay.

(3) Employees working in regulated areas shall wash their face, neck, hands, and forearms each time they leave the regulated area. Washing facilities shall be provided at each exit. Employees working in regulated areas shall wash their hands and forearms before and after using the toilet.

(4) Employees engaged in operations in which o-tolidine is transferred, charged, or discharged, or which involve using a laboratory-type hood, opening a closed system, or repackaging, shall be provided with gloves and aprons or coveralls or with full-body protective suits resistant to penetration by o-tolidine.

(5) As a backup precaution, employees using glove boxes to handle o-tolidine shall wash their hands and arms on completion of the assigned task.

(6) When employees use protective clothing and equipment, they shall remove it and leave it at the exit before they leave the regulated area; the

employees shall then wash their hands, forearms, face, and neck to remove accumulated o-tolidine before they enter nonregulated areas.

(d) Clean Room

A clean room shall be established and maintained that is free of o-tolidine contamination and that contains locker facilities.

(1) Shower facilities shall separate the clean room from the regulated area.

(2) The clean room shall be kept under positive pressure relative to the regulated area.

(3) Signs meeting the requirements of Section 3 shall be posted at the doorway separating the clean room and regulated area. Instructions informing employees of the procedures for entering and leaving the regulated area shall also be posted.

(4) Employees assigned to the regulated area shall change into clean work clothing (long-sleeved shirts, trousers, underwear, and footwear) each day before entering the regulated area. The necessary protective clothing and equipment shall also be put on at this time.

(5) At the end of each workday, protective clothing, work clothing, and protective equipment shall be removed and placed in clearly labeled containers located in the regulated area. The employee shall then proceed to the shower facility and shower and shampoo before entering the clean room to put on street clothing.

(e) Decontamination

Good housekeeping practices shall be observed to prevent contamination of areas and equipment with o-tolidine solids, solutions, and aerosols. The regulated area shall be washed thoroughly at the end of each shift to prevent such contamination.

Solids or solutions containing o-tolidine shall be removed from work areas by vacuum cleaning with a specially designated vacuum cleaner or by other methods, including wet methods, that do not increase the concentration of airborne o-tolidine. No dry sweeping, blowing by compressed air, or any method of dust removal that increases the concentration of airborne o-tolidine shall be allowed. After cleanup, the area shall be decontaminated and washed with water.

(f) Laundering

The employer shall provide for the daily decontamination and laundering of used work clothing. Clothing should be washed with soap or other detergent and water. Precautions shall be taken to protect personnel who handle and

launder soiled clothing. These employees shall be advised of the hazards of, and means of preventing, exposure to o-tolidine. If an outside laundry facility is used, the launderers shall be advised of the hazards and proper procedures involved in handling contaminated work clothing. Contaminated clothing that is to be transported to an outside laundry facility shall be placed in sealed containers.

(g) Storage

Storage areas shall be isolated, well ventilated, and fire-resistant. Containers of o-tolidine shall be tightly closed and stored safely away from strong oxidizing materials and corrosive liquids and gases, heat, explosives, and gases under pressure to minimize the possibility of accidental breakage or spills.

(h) Maintenance

Lines and fittings that may carry o-tolidine shall be made of materials resistant to penetration of o-tolidine and shall be inspected frequently for corrosion and leaks. All o-tolidine equipment, including valves, fittings, and connections, shall be checked for tightness and kept in good working order. Such inspections shall be made immediately after new connections are made and after o-tolidine is introduced. Repairs and adjustments shall be made promptly.

(i) Entry into Confined Spaces

Entry into confined spaces, such as tanks, pits, and process vessels, that have contained o-tolidine shall be controlled by a permit system. Permits shall be signed by an authorized employer representative, certifying that preparation of the confined space, precautionary measures, and personal protective equipment are adequate and that prescribed procedures will be followed.

(1) All lines shall be disconnected or blocked while a vessel is being cleaned. All valves or pumps leading to and from the vessel shall be locked out or tagged out.

(2) The vessel shall be either washed with water and purged with air or purged with nitrogen and then with air.

(3) The vessel shall then be checked by trained personnel for fire or explosion hazard, airborne o-tolidine, possible oxygen deficiency, and concentrations of other likely contaminants, to assure that no danger exists.

(4) If a respirator is necessary, a self-contained breathing apparatus as specified in Section 4 of this chapter shall be provided to the employee.

(5) Each employee entering the vessel shall be equipped with appropriate respiratory protection, a harness, and a lifeline. At least one

other person equipped with appropriate respiratory protection, harness, and lifeline shall watch at all times from the outside. At least one more person shall be available to assist in an emergency. Mechanical ventilation shall be provided continuously when workers are inside the vessel.

(j) Disposal

Waste material contaminated with o-tolidine and containers of o-tolidine shall be disposed of in a manner not hazardous to employees. The disposal method shall conform to applicable local, state, and Federal regulations and shall not constitute a hazard to the surrounding population or environment. Waste water shall be flushed to holding basins for decontamination.

Section 7 - Sanitation

(a) The pertinent requirements for plant sanitation, stated in 29 CFR 1910.141, shall be complied with. The subsections entitled General (a), Toilet Facilities (c), Washing Facilities (d), Change Rooms (e), and Consumption of Food and Beverages on the Premises (g) are especially relevant to o-tolidine.

(b) Washing facilities, conveniently located and near the exit, shall be provided to employees in the regulated area. Locker room facilities, including showers, shall be located outside the regulated area.

(c) Preparing, storing, dispensing (including that done through vending machines), or eating food shall be prohibited in regulated areas.

(d) Smoking shall be prohibited in regulated areas.

Section 8 - Monitoring and Recordkeeping Requirements

(a) Industrial Hygiene Surveys

Each employer who has a place of employment in which o-tolidine is stored, produced, processed, or otherwise handled shall determine by an industrial hygiene survey the areas in which occupational exposure to o-tolidine occurs. Records of these surveys shall be retained until the next survey has been completed. For areas where an employer concludes that there is no occupational exposure to o-tolidine, the records shall show the basis for this conclusion. Surveys shall be repeated at least annually and within 14 days after any process change likely to result in occupational exposure to o-tolidine.

(b) Personal Monitoring

If it has been determined that occupational exposure to o-tolidine occurs, the employer shall institute environmental monitoring.

(1) A program of personal monitoring shall be instituted to identify and measure, or permit calculation of, the exposure of each employee. Source and area monitoring may be used to supplement personal monitoring.

(2) In all personal monitoring, samples representative of exposure in the breathing zone of the employee shall be collected.

(3) For each determination of the concentration of o-tolidine, a sufficient number of samples shall be taken to characterize the employee's work and production schedules, location, or duties, and changes in production schedules shall be considered in deciding when samples are to be collected.

(4) Each operation in each regulated area shall be sampled at least once every 6 months while o-tolidine is produced or handled. For intermittent operations, ie, those lasting less than 6 months, at least one monitoring regimen shall be conducted during each operation period and monitoring should coincide with the periods of maximum potential exposure to o-tolidine during these intermittent operations.

If an employee is found to be exposed to o-tolidine at concentrations exceeding the recommended ceiling limit, the exposure of that employee shall be measured at least once every week, control measures shall be initiated, and the employee shall be notified of the exposure and of the control measures being implemented. Such monitoring shall continue until two consecutive determinations, at least 1 week apart, indicate that control measures are effective in that the employee's exposure no longer exceeds the recommended occupational exposure limit; routine semiannual monitoring may then be resumed.

(c) Recordkeeping

Records of environmental monitoring and other pertinent records shall be kept for at least 30 years after the employee's last occupational exposure to the o-tolidine. Records of environmental monitoring shall include an identification of the employee being monitored, duties and job locations within the worksite, time and dates of sampling and analysis, sampling and analytical methods used and available evidence of their precision and accuracy, the number, duration, and results of samples taken, environmental concentrations determined from these samples, and the type of personal protective equipment used by the employee. Entry rosters of authorized persons who enter regulated areas shall also be retained for at least 30 years. Environmental monitoring records and entry rosters shall be made available to designated representatives of the Secretary of Labor and of the Secretary of Health, Education, and Welfare. Employees shall have access to data on their environmental exposures. Medical records shall be made available to the designated medical representatives of the Secretary of Labor, of the Secretary of Health, Education, and Welfare, of the employer, and of the employee or former employee.

II. INTRODUCTION

This report presents the criteria and the recommended standard based thereon that were prepared to meet the need for preventing disease or injury arising from occupational exposure to o-tolidine. The criteria document fulfills the responsibility of the Secretary of Health, Education, and Welfare under Section 20(a)(3) of the Occupational Safety and Health Act of 1970 to "develop criteria dealing with toxic materials and harmful physical agents and substances which will describe...exposure levels at which no employee will suffer impaired health or functional capacities or diminished life expectancy as a result of his work experience."

After reviewing data and consulting with others, NIOSH formalized a system for the development of criteria on which standards can be established to protect the health and to provide for the safety of workers from exposure to hazardous chemical and physical agents. The criteria and recommended standard should enable management and labor to develop better engineering controls and more healthful work practices, and simply complying with the recommended standard should not be the final goal.

These criteria for a standard for o-tolidine are part of a continuing series of criteria developed by NIOSH. The proposed standard applies to the processing, manufacture, storage, handling, and use of o-tolidine. The standard was not designed for the population-at-large, and any extrapolation beyond occupational exposures is not warranted. It is intended to protect against injury to health from o-tolidine, be measurable by techniques that are valid, reproducible, and available to industry and governmental agencies, and be attainable with existing technology.

o-Tolidine is widely used in small quantities in chromatography and other analytical chemistry techniques, including water test kits for chlorine, and in biologic stains. It has been used as a direct dye for textiles but is most commonly used as an intermediate from which other dyes are made. It may be used as a precursor or as a curing agent in the manufacture of urethane resins. The use of o-tolidine in the United States is expected to decline.

Absorption of o-tolidine through the intact skin and from the respiratory and gastrointestinal tracts has been reported. Cancer and kidney damage have been reported in animals exposed to o-tolidine alone at high doses. Bacterial tests of mutagenicity and mammalian tests of DNA damage further support the findings of mammalian carcinogenicity. Furthermore, o-tolidine resembles benzidine, a known human carcinogen, in chemical structure, physical properties, and metabolism and excretion. o-Tolidine is, therefore, judged to pose a risk of cancer in workers. The recommended standard for occupational exposure to o-tolidine is based on keeping exposure at the lowest possible level through engineering controls and work practices. The recommended sampling and analytical methods have been evaluated for benzidine and are expected to be useful for o-tolidine.

The development of the recommended standard for occupational exposure to o-tolidine has revealed the need for additional data in several areas, especially on the effects of long-term occupational exposure to o-tolidine in the absence of other aromatic amines, possible carcinogenic, teratogenic, or mutagenic effects, and possible kidney damage.

III. BIOLOGIC EFFECTS OF EXPOSURE

o-Tolidine is a symmetrical biphenyl compound with an amine group and a methyl group attached to each ring. The amine groups are in the para position to the biphenyl link; the methyl groups are in the ortho position to the amine group [1,2]. The structures of several biphenyl amines, including o-tolidine and benzidine, are similar. These substances are sometimes called biphenyl amines, aminobiphenyl compounds, or simply diamines. Because they have been measured colorimetrically by the formation of colored holoquinone complexes [3], aminobiphenyl compounds have also been referred to as quinonizable substances. Their physical and chemical properties are also similar [1,2]. For example, o-tolidine melts at 129 C, is slightly soluble in water, and is soluble in alcohol and ether, whereas benzidine has a melting point of 126 C, is slightly soluble in water and alcohol, and is soluble in ether. The chemical structures of some aromatic amine compounds are given in Figure III-1 [4,5].

Extent of Exposure

o-Tolidine (3,3'-dimethyl,4,4'-diamino biphenyl, formula weight 212.32) is a crystalline solid. Salient physical and chemical properties of o-tolidine and benzidine are listed in Table XII-1 [1,2,5,6]. o-Tolidine is used both as a dye and as an intermediate in the production of other dyes [4]. It is widely used in small quantities as a laboratory analytical reagent and is a moiety of the commonly used biologic stain, trypan blue [7]. o-Tolidine is used in small quantities in chlorine test kits by water companies and swimming pool owners and in test tapes in clinical laboratories. Although other chemicals are preferred, o-tolidine has been used as a curing agent for urethane resins, in part because some of the other curing agents, eg, 4,4'-methylene bis (2-chloraniline)(MOCA), were proposed for stringent regulation as carcinogens (29 CFR 1910.93).

o-Tolidine is prepared from o-nitrotoluene by reduction to a hydrazo compound and rearrangement to the biphenyl amine in the same manner that benzidine is made from nitrobenzene [4]. Reduction is usually carried out with zinc dust and caustic soda in organic solvents [4]. Other reducing agents used in the manufacture of o-tolidine may also be used to prepare benzidine [4]. If m-nitrotoluene is the starting material, m-tolidine (2,2-dimethyl-4,4'-diamino biphenyl) is formed; dyes made from m-tolidine may be used for dyeing wool, but they have little affinity for cotton [4].

The major US manufacturer of o-tolidine makes an average of 200,000 pounds of o-tolidine salts, eg, hydrochloride, each year. Smaller quantities are produced by other companies. US production data on o-tolidine base are not available. Many chemical companies buy o-tolidine in bulk from other,

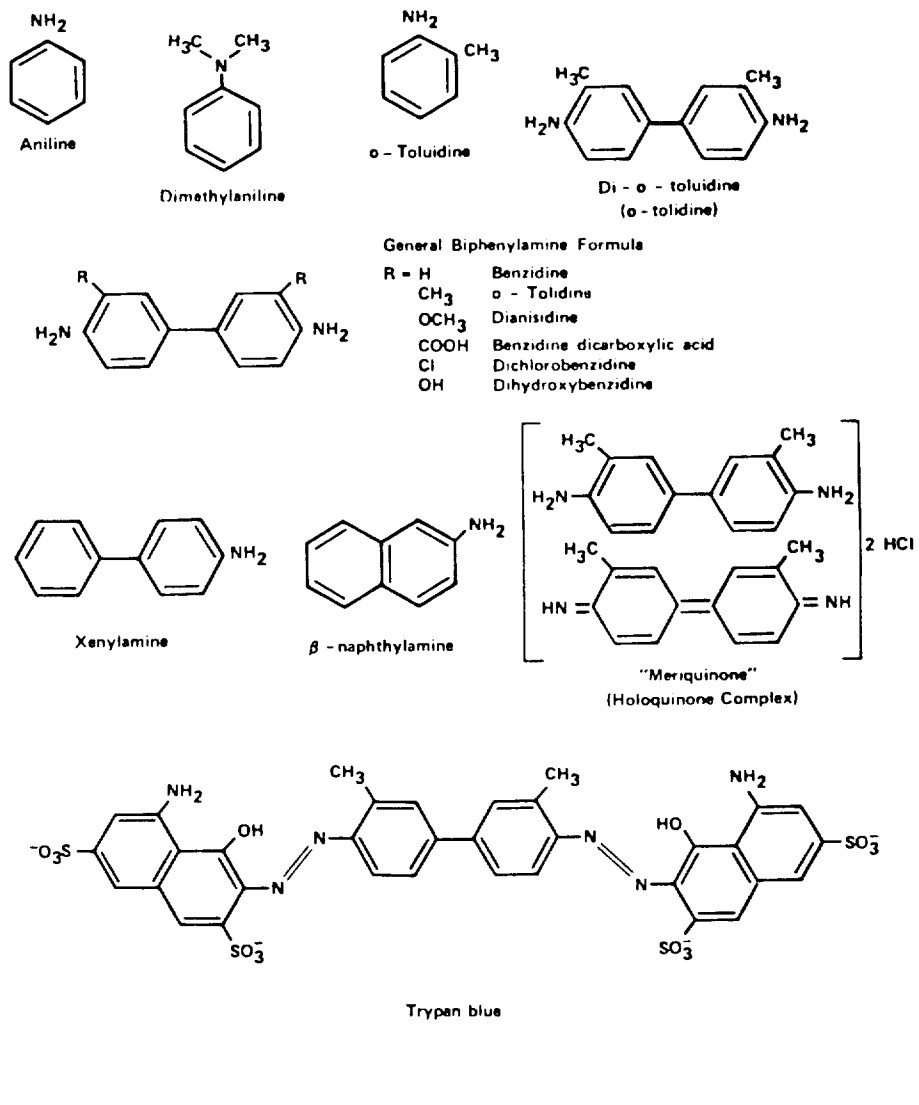

FIGURE III-1

CHEMICAL STRUCTURE OF SOME AROMATIC AMINE COMPOUNDS

principally foreign, manufacturers, repackage it, and sell it in smaller units; they sometimes refine it. About 300,000 pounds of o-tolidine base and 150,000 pounds of o-tolidine salts are imported annually [8,9].

Workers potentially exposed to o-tolidine are listed in Table XII-2 [4]. Workers exposed to the greatest amounts of o-tolidine are probably dye makers, toluene-diisocyanate makers, clinical or analytical chemistry laboratory workers, and repackagers [4,10]. Workers in a variety of occuptions may be exposed to small quantities of o-tolidine for analytical purposes, among them water and sewage plant attendants, sanitarians, forest service chemists, swimming pool service representatives, and chemical test tape or kit makers. It is estimated that less than 100 employees are exposed to large quantities of o-tolidine in the United States, but as many as 200,000 may be exposed to small quantities [11].

Historical Reports

In 1932, Berenblum [12] reviewed the incidence of "aniline cancer." The term "aniline cancer" was a general term used to refer to bladder cancer resulting from exposure to synthetic organic dyes derived from aniline, and to other similar dyes. Aniline was originally thought to cause cancer but more recently has been held to be noncarcinogenic [13]; use of the term "aniline cancer" has since been discontinued [12]. Berenblum concluded that the incidence of bladder tumors in dye-industry workers varied in different factories, districts, and countries. The data, however, did not point to any single reason for this variation. For example, the incidence of bladder tumors was higher in Germany than in other countries, even when the values were corrected for the greater size and age of the German dye industry. The variation in tumor incidence, however, was also found in different factories within Germany. It was observed that the incidence of bladder tumors decreased in one district after various protective measures were instituted in the work practices of these factories. Berenblum [12] suggested that other factors, eg, individual susceptibility, age, race, length of exposure, and type of industrial process for dye making, contributed to variation in tumor incidence.

Berenblum [12] also found reports of tumors localized in other parts of the body. Rehn, in 1895, described how one dye worker had carcinomas in the right ureter and kidney as well as of the bladder with metastases chiefly in the lumbar lymph nodes. Another worker developed a cancer of the renal pelvis of a hydronephrotic kidney. The bladder, however, appeared to be by far the most frequent tumor location. Both benign and malignant tumors were found, the malignant tumors developing as early as the benign tumors. Berenblum found suggestions, dating from 1920, that periodic urine testing and cystoscopic examinations of workers would aid in early diagnosis of bladder tumors.

Until 1932, few experimental studies were conducted to determine which individual chemicals in the dye industry were carcinogenic. The results

reported before then were inconclusive, but it was widely accepted that the causative agents were to be found among the many intermediates used to prepare the dyes [12].

Scott and Williams [14], in 1957, wrote work practice recommendations for the control of bladder cancer caused by occupational exposure. They suggested that large amounts of o-tolidine, dianisidine, and dichlorobenzidine should be treated like benzidine. Benzidine was by then an accepted human carcinogen [13,15].

Effects on Humans

Most investigations regarding o-tolidine have centered on its action on the urinary tract, but they have not developed evidence of chronic effects attributable solely to o-tolidine exposure. There have also been informal comments made to NIOSH regarding nasal irritation. This information is consistent with the observations of one researcher [16]. No other symptoms directly referable to o-tolidine were reported.

Brown and coworkers [17] found no o-tolidine in the urine of one human volunteer exposed to 100 mg of moistened crystalline o-tolidine for 48 hours. It was held against the skin under gauze patches according to the Draize technique [18]. They did detect urinary excretion of 0.113 mg of o-tolidine after 24 hours when 200 mg was applied to the palm of the hand for 8 hours under a rubber glove. No skin irritation was observed.

When one female volunteer was given three oral doses of 100 mg of o-tolidine (65.1% as base), she excreted 5.60-7.47 mg/day [17]. There were no quinonizable substances detected in the urine after 3-4 days.

Rye et al [19] mentioned in a review of diamine toxicity that they had found no cases of human cancer in two decades of experience with workers exposed to o-tolidine, dianisidine, and dichlorobenzidine, but specific data were not given to support this observation. There were, however, 23 cases of urinary system cancer reported in Japanese workers exposed to benzidine, o-tolidine, and dianisidine [20]. The length of exposure and the period between onset of exposure and the development of tumors were not reported, except that 14 workers (61%) had been exposed less than 6 years.

Macalpine [21] described two cases of papilloma of the renal pelvis in workers manufacturing o-tolidine and benzidine. Other chemicals occasionally handled included azobenzol, nitrobenzol, and o-nitrotoluol. The first worker's urine contained red blood cells, and a red, coarsely nodular papilloma was discovered in the vault of the bladder by cystoscopy. It was not necrotic. A smaller papilloma with fronds was located on the left wall of the bladder near the urethra. The man was treated by diathermy, and nine cystoscopic examinations over the next 3.5 years showed him to be tumor free. He then complained of difficulty in urinating, although there was no hematuria. Papillomas found at the neck of the bladder were treated by

diathermy. Several months later, the bladder tumors had grown larger and a large mass was felt in the area of the left kidney. The enlarged left kidney and ureter were removed surgically, and the bladder was treated by diathermy. The patient was discharged, but he was readmitted within 4 months with hematuria. Papillomas were found again at the neck of the bladder; they were larger than before and treated by surgery and diathermy. The patient died suddenly of hemorrhage during the course of treatment; no autopsy was permitted.

The second worker described by Macalpine [21] had a similar occupational and medical history. Eight cystoscopic examinations in 3.5 years were negative, then papillomas recurred in the kidney while the bladder remained free of tumors. He experienced recurrent anuria and died of uremia. At autopsy both kidneys were found to be affected. The right kidney was greatly enlarged by a papillary carcinoma. The left kidney cortex had atrophied; a papilloma obstructed part of the upper calyx. The bladder and both ureters appeared normal. The effects attributable to benzidine could not be distinguished from those of tolidine, and the isomer of tolidine was not identified. This report suggests the need for comprehensive urologic evaluation, including kidney function tests and microscopic examination for red blood cells and abnormal epithelial cells in urine. Repeated cystoscopy did not detect tumors in the upper part of the urinary tract.

In Italy, Barsotti and Vigliani [13] collected data on bladder lesions in 200 workers in the dye industry. Exposure to o-tolidine may have occurred but was not mentioned specifically. The workers had received routine cystoscopic examinations between 1931 and 1948. The authors found that beta-naphthylamine and benzidine were the most dangerous carcinogens of the aromatic amines to which the workers were exposed, whereas aniline had no appreciable carcinogenic potential. Tumors appeared 4-28 years after initial contact with beta-naphthylamine or benzidine.

In 1954, Meigs et al [22] analyzed urine samples from workers over a 5-year period at a plant manufacturing benzidine, dichlorobenzidine, dianisidine, and o-tolidine. About 900 samples from production workers and about 250 samples from non-production controls located in the same building were analyzed by the chloramine-T test. In addition, 17 control urine specimens were obtained from an employee in a different building 500 yards away, and 35 control specimens were obtained from men and women working in a nearby university and hospital. The number of individuals participating was not stated. Workers were required to shower daily after the workshift and provide a clean urine specimen. Air samples from production areas contained "quinonizable" (holoquinone-forming) substances at concentrations of 2-87 μg/cu m, with a mean of 18 ±2 μg/cu m. Air from other areas in the same building contained less than 1 μg/cu m. The chloramine-T test did not distinguish among aminobiphenyl compounds; separation was accomplished by paper chromatography. The results were generally reported in units of benzidine.

Urine samples from workers producing benzidine and substituted benzidines contained quinonizable substances in mean concentrations of 144-1,482 μg/liter and averaged about 500 μg/liter [22]. Samples from workers not making benzidine and related amines contained less than 15 μg/liter of quinonizable substances. Specimens from a worker in another building had a mean of 6 μg/liter, and the control samples from the university and hospital workers contained no detectable biphenyl diamines. Urine specimens from foremen had lower biphenyl diamine concentrations than did those from workers who actually handled the substances. Operators who were directly exposed excreted about 500 μg/liter of o-tolidine and foremen excreted about 50 μg/liter. Biphenyl diamine concentrations in urine were lower in the winter and higher in the summer, although the specific gravity of the urine did not differ appreciably. Higher concentrations of excreted biphenyl diamines were also associated with higher humidity. This is consistent with Meigs' speculation that increased skin moisture from sweating increased diamine absorption. A downward trend was noted in urinary excretion of biphenyl diamines over 3 years. The summer mean declined from 1,482 to 570 μg/liter, the winter mean from 433 to 144 μg/liter. This decrease was related to improved work practices and personal hygiene.

Brief but heavy exposure to a substituted benzidine resulted in a 1-day elevation of excreted quinonizable substance [22]. A worker was accidentally drenched with a slurry containing dichlorobenzidine hydrochloride in acid solution. Although he took a shower and changed his clothes within 5 minutes of the accident, his urinary excretion of diamines went from 43 to 1,130 μg/liter for 1 day and then returned to baseline levels.

Results of urinalyses from other workers led to the discovery of previously unrecognized sources of exposure, such as contaminated boots or gloves [22]. One worker had made only o-tolidine, dianisidine, and dichlorobenzidine, but chromatographic analysis of his urine sample for that day showed benzidine as well as the three diamines to which he was known to have been exposed. Investigation revealed that his boots were contaminated with benzidine. Similarly, o-tolidine was found in urine samples of two workers who had made only dichlorobenzidine on the day in question and in another sample from a worker who had that day made only m-aminophenol.

When both the skin and the clothing were carefully decontaminated, the urinary concentrations of biphenyl amines decreased markedly [22]. Before the initiation of a personal hygiene program at the workplace, one worker's specimens contained quinonizable substances in concentrations averaging 117 μg/liter, the concentration tending to increase slightly as the week progressed. After hygienic work practices were adopted, his average urinary output of quinonizable substances had fallen 80%, to 21 μg/liter, at the end of 14 months [22].

The authors [22] concluded that absorption of aminobiphenyl compounds, such as o-tolidine, through the skin was a more important hazard than was inhalation of airborne particles of these compounds. They based this conclusion on several observations. First, the concentrations of the

compounds in the workplace air were too low to produce the reported urinary concentrations. Also, foremen in the production areas excreted very little quinonizable substance compared with the workers actually handling the substances in the same areas, and the substances excreted corresponded to the substances to which the workers were exposed. Because diamine excretion increased with larger exposures (splashes) and on hot, humid days, Meigs et al suggested that moist skin was conducive to absorption of benzidine and substituted benzidines. They suggested that producing substituted benzidines in the winter or in air-conditioned facilities would reduce absorption through the skin by keeping the skin dry. It should be noted again that the chloramine-T test is not specific and that positive results were followed up by separation by paper chromatography. Whether this detection of unexpected diamines may have been the result of mixed exposures, chromatographic error, or biotransformation is not clear, but the latter is hard to demonstrate. The explanation offered by Meigs et al, that there were occult exposures to other chemicals deposited in boots or clothing, is the most probable. When this study was performed in the early 1950's, skin contact was generally considered the greatest hazard. With the subsequent improvement of engineering controls and work practices, skin contact with o-tolidine could be effectively controlled, and concern shifted to the inhalation hazard of airborne o-tolidine.

A study of occupational bladder tumors in the Japanese dye industry, by Tsuchiya et al [23] in 1975, reported that 1 worker of 107 exposed to "other amines, e.g. orthotolidine, including mixed exposures," excluding benzidine and alpha- and beta-naphthylamine, had a positive urinary Papanicolaou test. The workers' ages and the type and extent of exposure were not reported. Both active and retired workers were tested. Probably all workers exposed to o-tolidine are also exposed to other amines that may be carcinogenic.

Animal Toxicity

While experimental evidence of the effects of exposure to o-tolidine is meager, a few animal studies have attempted to elucidate the toxicologic properties of o-tolidine.

(a) General

Rye et al [19] stated in a review of diamine toxicity that o-tolidine base penetrates the skin more readily than its salts, but no supporting data were given.

In studies of acute toxicity in various species of animals, the toxicity of o-tolidine appears to be similar to, perhaps slightly greater than, that of benzidine. In the rat, the oral LD50 of o-tolidine was 404 mg/kg [24]; for benzidine, it was 566 mg/kg [25]. LD50's of 90.9 mg/kg and 199.4 mg/kg for o-tolidine and benzidine, respectively, were determined after albino mice were given intraperitoneal (ip) injections [17]. Renal toxicity of o-tolidine from a single dose administered orally to the rabbit was said to be greater than

that of benzidine [16], but no quantitative comparison was made.

In 1908, Adler [16] reported that three rabbits weighing about 2 kg each were given o-tolidine suspended in water orally at a daily dose of 1 g. The results, anuria, lethargy, and death within a few days, were said to be similar in all three rabbits tested. According to Adler, effects which he termed "urinary excretion disorders" were produced in rabbits by o-tolidine but not by benzidine.

Brown et al [17] evaluated skin irritation in six albino rabbits. The fur was clipped close to the skin and 0.5 g of o-tolidine, 65.1% base and the rest dihydrochloride, was kept on the skin under small gauze patches for 24 hours. No irritation was noted with intact skin, but very slight erythema appeared when o-tolidine was applied to abraded skin. This suggests that any cutaneous absorption of o-tolidine will probably not be accompanied by significant irritation; thus, it might not be noticeable.

Maruya [26] fed o-tolidine, at a 5% concentration in olive oil, mixed with rice at 20 cc of olive oil solution/kg of rice, to nine adult albino rats for 21-111 days. Control rats were not reported, but a total of 311 other rats were administered 4 other aromatic amines or 14 azo pigments. The ratio of males to females in each group was not reported, but half of the total of 320 rats were male. The kidneys of each rat were microscopically examined when the animal died during, or was killed at the end of, the experiment. Six of the rats exposed to o-tolidine had swelling and thickening of the cells lining the uriniferous tubules (two mild, two moderate, and two severe), with an accumulation of giant cells with abnormally large nuclei in the distal straight portions. The tubular lumen was narrowed. Two other rats showed a mild accumulation of pigment derived from hemolysis in the tubular epithelial cells. The degeneration of tubular cells was generally more severe when pigment granules were found in the cells, but it was not stated whether this finding occurred in rats fed o-tolidine. No results were reported for the ninth rat given o-tolidine. Maruya considered it significant that chemicals like o-tolidine, with a slight tendency to induce hemolysis and pigmentation of the uriniferous tubule cells, strongly provoked swelling of the tubular epithelium and massing of giant cells with heteromorphous giant nuclei.

(b) Carcinogenesis, Mutagenesis, and Teratogenesis

Early attempts to induce tumors of the urinary system experimentally by injecting animals with suspensions of o-tolidine at an unreported dose for 18 months did not succeed [27]. Feeding o-tolidine to dogs similarly yielded no bladder tumors [28]. In an experiment performed by Brown and Franz and reported by Ferber [29], there was one case of bladder cancer in dogs fed o-tolidine. In a brief commentary on the use of hamsters in studying induction of bladder cancer by aromatic amines, Saffiotti and colleagues [30] said they found no carcinogenic activity in hamsters fed o-tolidine (or 2-naphthylamine or benzidine as well as some other aromatic amines) at 0.1% in the diet. No supportive details were given.

Experiments, primarily on dogs, were reported in 1948 by Gehrmann and colleagues [28]. The following compounds were fed to dogs in daily doses 5 times a week for the time period indicated: aniline (300 mg/3.5 years), benzidine (117 mg/5 years), dianisidine (291 mg/3.5 years), alpha-naphthylamine (technical, 330 mg/4.5 years), alpha-naphthylamine (pure, 301 mg/4.5 years), phenyl-alpha-naphthylamine (290 mg/3 years), phenyl-beta-naphthylamine (540 mg/4.5 years), tolidine (isomer not specified, 230 mg/3 years), and beta-naphthylamine (300 mg/50 days). Only beta-naphthylamine was found to produce bladder tumors. No bladder tumors were induced in the three female dogs fed tolidine. The total dosage of tolidine was almost 180 g. Benzidine did not induce detectable bladder tumors or papillomas in these dogs.

As previously mentioned, Ferber [29], in 1970, prepared an unpublished report of an experiment designed to indicate whether bladder cancer could be induced by o-tolidine in dogs and whether it would continue to develop after a normal diet was resumed. Four young mongrel bitches weighing 40-48 pounds (18-22 kg) each were given 200 mg of o-tolidine in gelatin capsules daily for 8-9 months, a total dose of almost 50 g per dog. The findings of cystoscopic examinations before and after o-tolidine administration were negative. One dog died of bladder cancer (papillary tumor and cystitis) 8 years after the last cystoscopic examination. Another dog died of natural causes soon after the first, but no tumors were found at autopsy. The last two dogs were then killed and examined, but no tumors were found. Ferber concluded that o-tolidine may be a weak carcinogen but is not as carcinogenic as benzidine. This conclusion could not be supported statistically, because too few dogs were studied. Old dogs may develop tumors spontaneously; however, bladder tumors in dogs are rare [31].

Spitz et al [15], in 1950, reported the carcinogenic potential of benzidine. Clinical evidence had been lacking until then, because workers exposed to benzidine had frequently also been exposed to beta-naphthylamine, a recognized carcinogen [15]. The experiments of Spitz et al on rats dealt with the individual effects of benzidine; other compounds encountered during its manufacture, such as azoxybenzene, hydrazobenzene, and benzidine sulfate; and those closely allied to it chemically, such as tolidine, azobenzene, and benzidine disulfonic acid. All chemicals were injected subcutaneously (sc). Benzidine sulfate was also fed to rats. Results showed that benzidine did not produce bladder cancer in rats, but it did produce hepatomas and tumors of the Zymbal glands (the specialized sebaceous glands of the rat's auditory canal). o-Tolidine also produced tumors of the Zymbal glands. Technical grade benzidine caused more tumors than pure benzidine; however, the reason for this difference was not discussed. o-Tolidine caused fewer tumors in spite of injection of four times more o-tolidine (60 mg) than benzidine (15 mg) each week, suggesting that o-tolidine is a less potent carcinogen than benzidine. Fifty control rats did not develop tumors.

Holland et al [32] administered o-tolidine sc in peanut oil to 21 Alderly Park strain rats for 241 days (cumulative dose, 5.4 g/kg). The rats survived 94-703 days following the first dose of o-tolidine; tumors were not detected

at necropsy until day 325. A total of 18 rats developed tumors, including tumors of the gastrointestinal tract (11), hepatomas (7), tumors of the bone and associated tissues (including hematopoietic tissues) (4), and Zymbal gland tumors (40. Similarly, 22 rats were administered benzidine sc for 150 days (cumulative dose, 0.75 g/kg). Treated rats survided for 24-387 days following the first dose of benzidine. Tumors were not detected at necropsy until day 84 as compared with day 325 for o-tolidine. A total of 20 of 22 rats developed tumors (compared with 18/21 for o-tolidine), notably hepatomas (19), cholangiomas (18), intestinal tumors (7), and tumors of the Zymbal glands (4).

Holland et al [32] also tested 3,5,3',5'-tetramethylbenzidine in peanut oil administered sc to 24 male rats for 32 weeks at doses varying from 100 to 25 mg/kg during the testing period. A control group of 12 rats was given peanut oil sc only. Gross and microscopic post-mortem examinations revealed a few tumors but none that the authors could definitely relate to 3,5,3',5'-tetramethylbenzidine. Holland et al regarded the tumors associated with the administration of o-tolidine and benzidine as having been induced by these chemicals.

In 1970, Pliss and Zabezhinsky [33] reported the results of sc administration of o-tolidine to rats either in oil suspension or in glycerin pellet implants. The results were also discussed in two other publications [34,35]. White rats of both sexes weighing 100-120 g were used in five experiments. The only general statement about control animals was that 2.5% of an unreported number of untreated old virgin female rats developed fibroadenomas of the mammary glands.

In the first experiment, 27 males and 26 females were injected once a week with 20 mg of o-tolidine in sunflowerseed oil for 13 months [33]. The total dose for each rat was 1.16 g. The appearance of tumors was noted, and necropsies were ultimately performed on all animals. Tumors and abnormal organs were examined microscopically. Tumors appeared in rats of both sexes in 8 months; two males and one female died before the appearance of tumors [33]. Seventeen males and 13 females developed tumors, some more than one [33-35]. Tumors were found in Zymbal glands in 14 males and 6 females in 8-22 months, in preputial glands in 2 males in 20-22 months and in 1 female in 13 months, in the skin in 2 males in 18-23 months and 1 female in 16 months, in the mammary gland in 5 females in 13-22 months, in the forestomach in 3 males in 21-25 months, in the liver of 1 male in 23 months, and in the uterus of 1 rat in 22 months. Four other tumor sites were reported for male rats: one tumor of the small intestine in an unreported induction time, two tumors of the hematopoietic system in 12 and 23 months, one thyroid tumor in 25 months, and one lung tumor in 22 months. Various multiple tumors were found. No sarcomas were found at the site of injection. Only one of 50 control rats injected sc with sunflowerseed oil developed a tumor, a sarcoma associated with a parasitic cyst [34].

In the second experiment, pellets of o-tolidine were used [33]. In this experiment, 20 males and 20 females received weekly sc implants of one pellet containing 20 mg of o-tolidine and 10 mg glycerin for 14 months.

Approximately the same site was used each time for implantation, and the total dose for each rat was 1.22 g. Autopsy procedures were the same as those used in the first experiment. It took 12 months for the first tumor to appear in rats of either sex, at which time 16 males and 20 females were still living. The tumor that appeared earliest and affected the greatest number was, again, in Zymbal glands; six males and five females developed tumors of the Zymbal glands in 12-18 months. Seven females had mammary tumors in 12-23 months. One male and one female showed skin tumors in 17 and 18 months. Three males developed liver tumors in 15-20 months. One male had angiosarcoma in the area of the forelimb in 14 months, another showed neurosarcoma around the ear in 15 months, and a third had a tumor of the hematopoietic system after 20 months. One female had a lymphangioma in the neck after 20 months. Only one rat, a male, showed a tumor, diagnosed as a rhabdomyosarcoma, at the site of implantation in 20 months. In all, neoplasms were found in 23 rats (64%). Six rats had multiple tumors involving chiefly the skin and the liver. By the 18th month, three males and five females were alive.

The third experiment [33] was similar to the second, except that the o-tolidine in the implanted pellet was subjected to ultraviolet irradiation to "oxidize" the o-tolidine photochemically. It was not stated why the authors concluded that application of ultraviolet radiation would oxidize o-tolidine. Twenty-four males and 24 females were used. As in the first and second experiments, the onset and characteristics of tumors were noted and confirmed when necropsies were performed. Tumors were first observed during month 11. Thirty-two rats were then alive, 18 males and 14 females. Six males and two females survived for 18 months. Neoplasms appeared in 25 rats (52%), 15 males and 10 females, and 6 of these had combinations of tumors at different sites. Among the males, nine had tumors of Zymbal glands in 11-19 months, four had tumors of the skin in 16-19 months, one had an intestinal tumor in 15 months, one had a tumor of the submaxillary salivary gland in 18 months, and one had a subcutaneous sarcoma, diagnosed as a rhabdomyosarcoma, at the site of implantation. Among the females, seven had Zymbal gland tumors in 12-16 months, one had a tumor of the mammary gland in 19 months, three had skin tumors in 14-19 months, and one had a tumor of the hematopoietic system in 17 months.

Retention of o-tolidine in the subcutaneous tissues at the sites of injection and implantation was studied in the fourth experiment [33]. Fifteen rats of unreported sex were injected sc once with 20 mg of o-tolidine in sunflowerseed oil; after 1-7 days, the subcutaneous tissue was excised for determination of o-tolidine content. Similarly, a pellet containing 20 mg of o-tolidine was implanted in each of 10 rats (number of each sex not reported), and tissue from the implant site was taken for o-tolidine determination after 7 days. In 2 samples, 0.24-1.65 mg of o-tolidine were found at the site of administration of the oil suspension 24 hours after sc injection; trace amounts were found after 48 hours in 2 samples and 168 hours in 10 samples. The locus of injection was not described. Seven days after implanting a pellet containing 20 mg of o-tolidine, 5-13.5 mg were found at the site of implantation in 10 samples.

In the fifth experiment, the amounts of free and bound aromatic amines were determined by diazotization from samples of the liver, kidneys, spleen, omentum, and Zymbal glands of 12 rats that had received daily sc 20-mg injections of o-tolidine in oil for 8 months [33]. The analytical method was reportedly capable of distinguishing aromatic amines from naturally occurring primary and secondary amines [36]. Eleven rats were used as controls. The highest amine content, expressed as μg/g of organ weight, was found in Zymbal glands, 246 bound and 26 free. The kidneys contained less total amines, 117 bound and 38 free, followed by the omentum with 120 bound and 19 free, the spleen with 90 bound and 35 free, and the liver with 90 bound and 32 free. In the control animals, Zymbal glands contained 31 bound and 11 free, the kidney had 18 bound and 17 free, the omentum had 31 bound and 11 free, the spleen contained 15 bound and 19 free, and the liver had 31 bound and 11 free. The standard error associated with all these weight averages was around 5 μg/g. The low amine content of the omentum suggested to the authors that o-tolidine distribution does not depend primarily on its lipid solubility.

Results of microscopic studies of all tumors found in rats from the first three experiments were discussed together [33]. Most of the tumors of Zymbal glands were diagnosed as squamous-cell carcinomas, some were adenocarcinomas or sarcomas. Lung cancer in one rat was attributed to metastasis from a squamous-cell carcinoma of Zymbal glands. Tumors found in the preputial sebaceous gland were diagnosed as one squamous-cell carcinoma, one adenoma, and one sebaceous carcinoma. Most of the skin tumors were epitheliomas arising from hair follicles, rarely from sebaceous or sweat glands. Tumors of mixed types occurred frequently, and both basal-cell carcinoma and spinocellular carcinoma were reported. Both benign and malignant mammary tumors were found. Most malignant mammary tumors were papillary cystadenocarcinomas. A few carcinomas were cribriform in appearance. Two rats had squamous metaplasia of the glandular epithelium. Five rats from the second experiment had benign fibromas arising from fibroadenomas. One rat in the third experiment had a fibroadenoma of the mammary gland. Tumors of the liver occurred in single or multiple nodules. Both benign hepatomas and hepatocellular carcinomas were found. A few rats had cystadenomas of the bile ducts. In one rat, liver carcinoma metastasized to the lung. The tumors of the forestomach were multiple, squamous-cell, cornified papillomas with a cauliflower appearance. Intestinal tumors appeared in two rats; one developed adenocarcinoma of the small intestine and the other had carcinosarcoma of the large intestine with multiple peritoneal metastases. One mixed tumor of the submaxillary salivary gland occurred. Four rats had tumors of the hematopoietic system, one had reticulosarcoma of the liver with multiple peritoneal visceral metastases, two had reticulosis with multiple visceral foci, and one had myeloid leukemia with enlargement of the liver and spleen. The tumors found at the site of administration were polymorphous subcutaneous neoplasms with giant cells; they were suggested to be rhabdomyosarcomas. Various solitary tumors were found, including pulmonary cystadenocarcinoma, uterine leiomyosarcoma, thyroid adenoma, angiosarcoma in the region of the forelimbs, lymphangioma of the neck, and neurosarcoma of the ear [33]. In another experiment, the one rat tested had a tumor of the heart that the authors suggested was a rhabdomyosarcoma [37].

Pliss and Zabezhinsky [33] concluded that o-tolidine is a carcinogen and suggested that its carcinogenic effects may be caused by unidentified metabolites rather than by the compound itself. The high frequency of tumors in structures of ectodermal origin, they suggested, was related to the accumulation of o-tolidine or its metabolites in excretory organs, such as sebaceous glands. Pliss [34] also concluded that a single methyl group ortho to the amino group reduced the likelihood of liver tumors, but that substituting additional methyl groups in the aminobiphenyl molecule increased the possibility of rats developing liver tumors. The evidence in this paper [34] to support these conclusions is weak. Pliss [35] inferred that the carcinogenic properties of o-tolidine were manifested after its conversion to carcinogenic metabolites because all tumors except one formed in a number of distant tissues and did not form at the sc injection sites. He also observed that more males than females developed tumors, particularly of specialized sebaceous glands, eg, Zymbal glands.

Saffiotti et al [30] and Sellakumar et al [38] conducted similar experiments on hamsters, administering o-tolidine in oral doses of 0.1-1%. Their results were negative.

The papers by Spitz et al [15] and Pliss and Zabezhinsky [33] present considerable evidence of the carcinogenicity of o-tolidine in rats. No bladder tumors were found, but they were not expected because even benzidine does not cause bladder tumors in rats [31].

In 1973, Freeman and coworkers [39] tested the use of cell culture transformation to indicate the carcinogenic potential of several chemicals, one of which was o-tolidine. The chemicals were tested in cell cultures from the F111 pool of rat embryo cells inoculated with Rauscher leukemia virus (RLV). The cultures showed no spontaneous transformation for at least 50 passes by RLV, or by chemical carcinogens alone, but did show transformation with both the virus and the chemical. One set of cultures showing transformation was held indefinitely; another was subdivided at 2-week intervals, with half being held indefinitely and half being subdivided again. When transformation occurred frequently, it could be detected in the original cultures and was more pronounced in daughter cultures. When transformation was rare, the cultures had to be subdivided at least once before it was detected. Transformation was recognized by macroscopic foci of spindle cells without polar orientation or contact inhibition; these cells were tumorigenic when transplanted into newborn Fischer rats.

o-Tolidine was found to be toxic to the cell cultures at 199 μg/ml [39]. At 50 μg/ml, there were well-defined foci of spindle cells. At 10 and 5 μg/ml, transformation was still apparent, but the foci were less well defined. No effects were seen at 1 μg/ml. Although this test uses an in vitro system and is not well established, its usefulness in this case is supported by the findings of tumorigenesis when the transformed cells were transplanted into rats. Similar findings with known carcinogenic chemicals, eg, 4-aminobiphenyl and dichlorobenzidine, lend additional support.

Seiler [40] applied the Friedman-Staub screening test to a variety of chemical mutagens and carcinogens. This procedure uses the amount of tritiated thymidine incorporated into DNA as an indication of DNA damage, and, in turn, of carcinogenicity or mutagenicity. The smaller the amount of radiolabeled thymidine incorporated into DNA, the greater the indication of damage. Adult male mice (23-28 g) were given oral 100 mg/kg doses of o-tolidine. Controls were given only thymidine. o-Tolidine administration resulted in incorporation of 77.6% of the amount of thymidine incorporated by controls, a decrease reported to be statistically significant. Overall, 86% of the known carcinogenic and mutagenic chemicals tested depressed DNA synthesis, while only 10% of noncarcinogenic and nonmutagenic compounds did so. Among the chemicals that demonstrated carcinogenicity in vivo, all the polycyclic hydrocarbons tested, all three azo dyes tested, and six of the seven aromatic amines tested were also transforming agents on this assay. This study [40] supports the finding of Freeman and coworkers [39] that o-tolidine damages DNA in mammalian cells.

In 1976, Shimizu and Takemura [41] reported that o-tolidine at an unreported concentration exerted a positive mutagenic effect on two strains of bacteria used in the Ames test, Salmonella typhimurium TA 98 and TA 100. The Ames test indicates mutation by testing a chemical's ability to change a microorganism's requirement for a specific nutrient, usually histidine. The magnitude of this effect was not reported. The authors compared their results favorably with the positive evidence for carcinogenicity of o-tolidine reported by Pliss in 1970, but did not cite the specific reference.

o-Tolidine base was evaluated for mutagenic activity in another study [42]. Microbial plate assays were performed with Saccharomyces cerevisiae D4 and Salmonella typhimurium TA 1535, TA 1537, and TA 1538, both with and without metabolic activation from tissue homogenate prepared from the lungs, liver, and testes of ICR random-bred mice, Sprague-Dawley rats, and rhesus monkeys. Tests were done in duplicate with positive and negative controls. A test was considered positive if spontaneous reverse mutations in Salmonella and mitotic recombination in Saccharomyces increased tenfold or more above background levels. The initial results were inconclusive, but the nonactivation assays and the activation assays with mouse liver indicated that the number of revertants with Salmonella typhimurium TA 1538 was slightly increased. Additional tests were performed using the same protocol, except that a fivefold or greater increase was considered positive. This time, no mutagenic activity was detected. The experimenters concluded that o-tolidine base was not mutagenic.

In 1977, Ferretti et al [43] tested the mutagenic effects of o-tolidine and several other compounds on Salmonella typhimurium TA 1538. The standard Ames procedure was followed. The results, expressed as a ratio of the average number of revertants/plate with activation by liver microsomal enzymes to the revertants/plate without activation, were 371:18 for benzidine, 422:12 for o-dianisidine, and 80:6 for o-tolidine. The authors judged this to be evidence that benzidine, dianisidine, and o-tolidine were mutagenic. None of the compounds showed mutagenic activity in the absence of microsomal activation;

this is consistent with suggestions that the carcinogenic effect of o-tolidine in rats is due to a metabolite of o-tolidine, rather than to o-tolidine itself [33].

Golub [44] administered o-tolidine to pregnant BALB/c mice at a daily sc dose of 2 mg in 0.1 ml of sunflowerseed oil. The mice were killed after 19-20 days, and the embryonic kidneys were obtained for organ culture. A total of 55 organ cultures were obtained from controls and 25 from mice given o-tolidine. Organ cultures were examined for 20 days. Epithelial hyperplasia and other undescribed cellular changes were noted in the experimental cultures but not in controls, suggesting that o-tolidine had transplacental effects on the embryos of pregnant mice.

Wilson [45], in 1955, reported on the teratogenic effect of o-tolidine and dyes with o-tolidine nuclei, eg, trypan blue. A total of 30 mg of o-tolidine was administered by sc injections on 3 successive days to each of 10 young adult albino rats after pregnancy had proceeded for 1 week. No control group was reported. Pregnancy continued until the 20th day, when the mothers were killed and the offspring examined. A total of 109 implantations occurred in the 10 rats given o-tolidine; of these, no embryos were malformed, but 8% were resorbed. Basing their conclusion on these data, the authors stated that "o-tolidine alone, without azo linkages and naphthalene side rings, was totally ineffective" as a teratogen.

Korotkova and Tokin [46], in 1968, immersed colonial or single sponges in 100 ml of sea water containing 300 mg of o-tolidine for 45 minutes. Control sponges were treated similarly, but India ink was substituted for o-tolidine. All sponges were then observed for 11 days in running sea water. Water temperature was held at 7-9 C at all times. Both living and fixed specimens were studied. Sponges immersed in o-tolidine showed epithelial damage and mitotic activity accelerated to 2-3 times the rate of regenerating tissue. Colonial sponges were more susceptible to o-tolidine than were single sponges, as shown by earlier budding and abnormal growth. Mitotic activity and budding of control sponges were unaltered.

(c) Metabolism

The metabolism of o-tolidine has been investigated in dogs [47,48], rabbits [48], rats [49], and in occupationally exposed workers [48,50]. Although the experimental data are sparse, the available evidence allows a comparison between humans and other mammalian species with respect to the metabolic fate of o-tolidine in vivo.

Urine of workers occupationally exposed to undefined quantities of o-tolidine was reported to contain o-tolidine, N,N'-diacetyl-o-tolidine, and 5-hydroxy-o-tolidine and its conjugates [22,50]. Although N-acetyl-o-tolidine, a known metabolite in animals [48], was not identified in the urine of workers, it probably occurs as an intermediate during the biosynthesis of N,N'-diacetyl-o-tolidine. Benzidine metabolites found in the urine of occupationally exposed workers are reported to include benzidine (3.5-5.6%),

N-acetylbenzidine (5.1-10%), N,N'-diacetyl benzidine (5.1-10.0%), and 3-hydroxybenzidine and conjugates (78.5-89.7%) [51]. These data suggest that the metabolic pathways for o-tolidine (3,3'-dimethylbenzidine) and benzidine are qualitatively similar in the human organism. The first steps in the pathways appear to be acetylation of the amino groups and introduction of the phenolic group on the aromatic ring.

Engelbertz and Babel [48] measured the concentration of aromatic amines after acid hydrolysis in the urine of workers occupationally exposed to o-tolidine. The hydrolysis was carried out to convert any N-acetyl-o-tolidine or N,N'-diacetyl-o-tolidine present into free aromatic amines. The urine samples from eight workers in o-tolidine production units contained 0.050-0.250 mg of aromatic amines, reported as o-tolidine equivalents, per 100 ml. The authors also measured the concentrations of aromatic amines in 12 urine samples collected intermittently from one worker during a 24-hour period. The highest concentrations of aromatic amines were measured within 6 hours after beginning work. The concentration in the last sample was about one-fifth of the maximum value, which indicates that o-tolidine had a half-life of a few hours in this individual. Despite the limited nature of this study, it confirms that o-tolidine was absorbed by occupationally exposed workers and was rapidly metabolized and excreted.

The metabolites of o-tolidine reported to occur in the blood or urine of dogs include o-tolidine, N-acetyl-o-tolidine, N-N'-diacetyl-o-tolidine, 5-hydroxy-o-tolidine or its conjugates, and o-tolidine-5-sulfate or o-tolidine-5-glucuronide [47,48,52]. A pharmacokinetic study of o-tolidine in dogs following exposure by different routes of administration was reported by Engelbertz and Babel in 1956 [52]. The analytical technique they employed was based on colorimetric determination of the diazotization products of the free aromatic amines. Consequently, the method might not distinguish between o-tolidine and any of its aromatic metabolites containing free amino groups.

The maximum color intensity of free aromatic amines in the blood was reached about 2 hours after a 100-mg iv dose was administered [52]. Color intensity then decreased continuously with time. The half-lives of o-tolidine and its metabolites in canine blood appeared to be less than 8 hours. After the dogs received 200 mg in their food, the maximum concentrations of o-tolidine and related amines in the blood also occurred in about 2 hours, and a similar half-life was observed. These data indicate that o-tolidine is rapidly absorbed through the gut following oral ingestion. The measured rates of clearance of aromatic amines and acetylated derivatives from the blood are consistent with a rapid metabolism and excretion of o-tolidine and its metabolites. The analytical techniques employed did not allow for assessment of the fraction of the total dose of o-tolidine or its metabolites in the blood, so that conclusions on the pharmacokinetics of o-tolidine and all of its metabolites are not possible. In parallel experiments, the investigators observed similar rates of absorption, metabolism, and excretion of benzidine and its metabolites.

Engelbertz and Babel [48] also conducted feeding studies to investigate the metabolic fate of N-acetyl-o-tolidine and N,N'-diacetyl-o-tolidine in dogs and rabbits. They devised an extraction procedure to separate the urinary metabolites. They observed that orally administered N-acetyl-o-tolidine was a precursor of both o-tolidine and N,N'-diacetyl-o-tolidine in the urine. Similarly, they reported that N,N'-diacetyl-o-tolidine was a precursor of both N-acetyl-o-tolidine and o-tolidine. The report suggests that a facile metabolic interconversion of o-tolidine and its acetylated derivatives may occur in dogs and rabbits.

An investigation of the metabolism of benzidine in vitro has recently been reported by Morton et al [53,54]. Using extracts prepared from rat, mouse, guinea pig, and hamster livers, the investigators demonstrated the formation of diacetylbenzidine from acetyl CoA and benzidine. Subsequent oxidation of diacetylbenzidine to N-hydroxydiacetylbenzidine and 3-hydroxydiacetylbenzidine was found to be catalyzed by microsomal enzymes. The shift of the acetyl moiety from the nitrogen to the oxygen atom, catalyzed by N-O-acetyltransferase, yielded a highly reactive species which was shown to bind to nucleic acids covalently. This reaction sequence for benzidine parallels the reaction sequence for other carcinogenic aromatic amines, such as 2-aminofluorene [55]. Consequently, these in vitro studies suggest that a fraction of the absorbed o-tolidine will also be metabolized to a similar reactive intermediate.

After the administration of a single ip dose of o-tolidine to a dog, Sciarini and Meigs [47] recovered only about 40% of the dose in the urine as o-tolidine or its known metabolites. The fraction of the dose that remained and was excreted in the feces and the fraction that was covalently bound were not determined. The binding of aromatic amines to tissues during long-term exposure of rats to o-tolidine was investigated by Pliss and Zabezhinsky [33]. After weekly sc injections of 20 mg were given to each animal for 8 months, the authors measured the distribution of aromatic amines using a diazotization method. The content of free and bound aromatic amines in different organs was determined 3 days after the last administration of o-tolidine. Bound aromatic amines were measured after hydrolysis of tissue homogenates in 2N hydrochloric acid. Relative to the control tissues, an increased amount of both free and bound aromatic amines was observed in all examined tissues--the liver, kidneys, spleen, omentum, and Zymbal glands. The largest increase of bound aromatic amines (more than eightfold) occurred in the kidneys and Zymbal glands. Since the increase in aromatic amines was observed 3 days after administration of the last dose and since the in vivo half-life of o-tolidine and its metabolites is only a few hours, these observations are consistent with the formation of a reactive intermediate that covalently binds to cellular macromolecules.

The available evidence [22,47,48,50-52,55,56] indicates that the fates of o-tolidine and benzidine in biologic systems are closely related, probably because of similar metabolic mechanisms. Although quantitative differences in metabolism do occur, these differences are insufficient to allow for postulation of major differences in the mechanisms of action of o-tolidine and benzidine.

Correlation of Exposure and Effect

There is some evidence of carcinogenicity in rats [33,57], possible carcinogenicity in dogs [29], altered enzyme activity in rats [49], and possible evidence of mutagenicity and teratogenicity in mammalian cell cultures. Little information is available on the toxic effects of o-tolidine on humans. The information that is available for humans and animals can only be used as a qualitative index of effects.

Investigators exposed to o-tolidine at unknown concentrations have experienced nasal irritation [16]. Humans absorb o-tolidine through intact skin and excrete o-tolidine and its acetylated and hydroxylated metabolites in urine [17,22,50]. Urinary metabolites of o-tolidine were detected in the urine of a volunteer after an 8-hour application of 200 mg under a glove, but when 100 mg of o-tolidine were applied to the skin for 48 hours there was no detectable urinary excretion of quinonizable substances [17]. Skin irritation has not been reported in humans.

The results of animal experiments have provided some limited data on the uptake of o-tolidine. The half-life of o-tolidine in blood after 100 milligrams of the compound was administered iv, seems to be less than 8 hours [52]. When o-tolidine was ingested with food, maximum blood concentrations of o-tolidine were reached in 2 hours, indicating that o-tolidine is rapidly absorbed through the gut. Similar findings were observed in dogs in parallel experiments conducted with benzidine [52]. After weekly sc injections of o-tolidine in rats [33], the highest observed increase in bound aromatic amines occurred in the kidneys and Zymbal glands. These findings are of interest because of the reports of kidney damage in rats and rabbits [16,26] and carcinoma of the Zymbal glands in rats [16,33] as summarized in the discussion which follows.

The possibility of kidney damage should be considered, but not much information is available on this aspect of o-tolidine toxicity. There is one report of renal failure in rabbits fed o-tolidine at 1 g/day for 3 days [16] and one report of kidney damage, especially in the uriniferous tubules, in rats fed o-tolidine at a 5% concentration mixed in olive oil mixed with rice at 20 cc olive oil solution/kg of rice [26].

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

Cancer of the urinary bladder [15] has been found in workers making dyes from a variety of hazardous substances, o-tolidine among them, but, because exposures have been to two or more biphenyl amines, there is no strong evidence either to indicate that o-tolidine alone causes cancer in humans or to absolve it as a human carcinogen. Cancer has not been reported in humans exposed to o-tolidine alone, although cancer of the urinary tract has developed in workers exposed to both benzidine and o-tolidine [20,21]. The single report of bladder cancer in one of four dogs fed 200 mg of o-

tolidine/day for 8-9 months [29] does not establish, but is consistent with, the hypothesis that o-tolidine, like benzidine, may induce urinary tract cancer in animals.

Cancer has been found in the Zymbal glands, skin, forestomach, lungs, bone tissues, and hematopoietic system in rats exposed to o-tolidine [32,33], but it has not been demonstrated in hamsters according to a brief commentary by Saffiotti et al [30]. Most of the experiments with rats have used repeated high doses (0.001-1.29 mg) to induce tumors. Single doses (100 mg/kg) have altered enzyme activities in rats [49], but tumors have not been reported.

It has been suggested that a single methyl group ortho to the amino group in the diphenyl diamine molecule reduces the likelihood of liver tumors in rats, but the substitution of additional methyl groups would increase the possibility of developing liver tumors [62]. However, the administration of 3,5,3',5'-tetramethylbenzidine to rats [32] did not produce tumors of the liver.

o-Tolidine resembles benzidine in structure, in physical and chemical properties [1,2,58], and in metabolism [47,48,50-52,55,56,58-60]. The sites and microscopic appearance of tumors observed in rats following o-tolidine administration [33] and dihydroxybenzidine administration [61] are similar. Benzidine, an aromatic amine which is accepted to be a human carcinogen (29 CFR 1910.1010), has not produced bladder cancer in rats [31]. Both o-tolidine and benzidine administration to rats do, however, result in cancer of the Zymbal glands [32,62]. The similarity between o-tolidine and benzidine in chemical structure, in absorption of both compounds by all routes (including the skin), in metabolism to acetylated and hydroxy compounds, in excretion preferentially by the urinary route, and in the induction of tumors of the Zymbal glands in rats suggests that o-tolidine can cause bladder tumors in dogs and humans as does benzidine. In addition, o-tolidine probably is a factor, along with benzidine, in cases of human bladder cancer following mixed exposures to these chemicals, such as those reported by Macalpine [21].

Pliss [62] suggested that the accumulation of o-tolidine or its metabolites in excretory organs, such as sebaceous glands, is related to the high frequency of tumors in structures of ectodermal origin. The results of research by Holland et al [32] lend support to the suggestion.

Although one study [42] did not agree, the indications of mutagenicity of o-tolidine in bacteria [41,43], the influence on DNA synthesis in mouse testes [40], the influence on organ cultures derived from pregnant mice treated with o-tolidine, and in vitro transformation of rat embryo cells [39] support the hypothesis that o-tolidine may adversely affect fundamental cellular control mechanisms.

TABLE III-1

EFFECTS OF EXPOSURE TO o-TOLIDINE ON ANIMALS

Route	Species	Dose	Duration	Observed Effects	Reference
oral	Rat	5%	21-111 d	Changes in uriniferous tubules	26
"	Hamster	0.3%	-	None	30
"	"	"	-	"	38
"	Rabbit	1 g	3 d	Renal failure, death	16
"	Dog	230 mg	3 yr	No bladder tumors	28
"	"	200 mg	9 mo	Bladder cancer in one/four after 8 yr	29
sc (injection)	Rat	100 mg/kg	1 hr	Changes in enzyme activities	49
"	"	"	2 mo	"	49
"	"	5.4 g/kg total	8 mo	Tumors in 85%	32
"	"	20 mg 1.16 g total	13 mo	Tumors in 60%	33, 35
"	Mouse	2 mg	-	Fetal kidney damage	44
sc (implantation)	Rat	20 mg 1.2 g total	14 mo	Tumors in 64% of animals	33
"	"	20 mg oxidized 1.22 g total	"	Tumors in 78%	33
dermal	Rabbit	0.5 g	24 hr	Slight erythema with abraded skin	17

IV. ENVIRONMENTAL DATA

Environmental Concentrations

The concentration of biphenyl amines in the air at the plant manufacturing o-tolidine around 1950 ranged from 2 to 87 μg/cu m and averaged about 20 μg/cu m [22], but specific o-tolidine levels were not given. Additional information on o-tolidine levels in the workplace environment was not found. However, environmental concentrations of o-tolidine for manufacturers and users can be only roughly estimated from current processing information. Because batch processing is used by both manufacturers and users, with the result that environmental levels of o-tolidine fluctuate from zero during off-production periods to a maximum when o-tolidine is produced or used, worker exposure to o-tolidine is expected to be intermittent, with the frequency of exposure depending on the frequency of production.

For manufacturing facilities, except for accidental spills or leaks, environmental levels would probably be highest near operating filter presses and driers. Bulk quantities of o-tolidine are also handled in dye manufacturing; 1,500-2,000 pounds are used for each batch operation. For these facilities, high levels can be expected when o-tolidine is weighed and when the reactor is being charged with o-tolidine. Other users of o-tolidine handle less than 200 pounds of o-tolidine per year in small quantities, generally milligrams [63]. It is expected that their exposure to airborne o-tolidine would be intermittent and possibly negligible.

o-Tolidine can enter the body by any route, particularly inhalation and skin contact [17,22]. A significant hazard comes from contaminated clothing [10,22,64]. Personal hygiene, removal of contaminated work clothing, separation of work clothing from street clothing, and showering at the end of the workshift have greatly reduced the absorption of o-tolidine [10,22].

Control of Exposure

Inhalation and skin absorption of o-tolidine can be minimized by proper ventilation, cleaning operations not requiring direct exposure of workers, prompt cleanup and decontamination procedures using liberal amounts of water, employee education and attention to personal hygiene, and full-body protective equipment when necessary. In one case, a worker's urinary excretion of aminobiphenyl compounds was reduced 80% by attention to personal hygiene [22].

Proper ventilation is an important means of controlling respiratory exposure to o-tolidine and will help to minimize skin exposure by reducing the amount of o-tolidine available to settle out of the air. Guidelines for ventilation systems may be found in Industrial Ventilation--A Manual of Recommended Practice of the American Conference of Governmental Industrial

Hygienists (ACGIH) [65] and in Fundamentals Governing the Design and Operation of Local Exhaust Systems of the American National Standards Institute (ANSI) [66].

The most effective engineering control measure is enclosure of unit operations and processes. Closed systems are most effective if they are operated under negative pressure with respect to surrounding uncontaminated areas, and they are only effective if their integrity is maintained. This can be ensured by a program of periodic maintenance, especially of equipment parts, eg, gaskets and seals that are subject to wear, by frequent inspections for and immediate repair of leaks, and by the use of pressure-failure alarms in enclosures, hoods, and ductwork.

An alternative or supplement to closed processes is local exhaust ventilation used at all sources of o-tolidine emission. The hood should be as close to the emission source as possible and shaped to control the area of contamination. Airflow into the exhaust hood should be directed away from the worker, and the capture velocity of the exhaust hoods should be high enough to overcome opposing air currents and the kinetic velocity of generated dust particles within the working environment, including those caused by movements of the workers. A minimum airflow of 150 fpm is recommended, but final determination should be made by an industrial hygienist. An industrial hygiene survey should be performed to determine the proper hood design and capture velocity for the facility.

With the exception of o-tolidine manufacturers and dye makers, each user facility generally consumes less than 200 pounds/year. For these facilities, local exhaust ventilation in the form of a laboratory-type hood or glove box may be effectively used for batch operations in which o-tolidine is weighed, converted into a concentrated acid solution, or diluted; in repackaging operations; and in laboratory activities involving research or quality control. A properly designed and maintained laboratory hood or glove box can confine the area of contamination to the hood or glove box itself, thereby simplifying control procedures for preventing skin contact with and inhalation of o-tolidine. The hood or glove box should be located away from heavy traffic aisles, doorways, and supply grills. A minimum exhaust volume for the laboratory hoods of 150 cu ft/minute/sq ft (45 cu m/minute/sq m) of door area is recommended [65], but final determination should be made by an industrial hygienist. Hoods should be inspected monthly for the first 6 months and quarterly thereafter [65].

o-Tolidine is used in some batch operations as an intermediate in dye, pigment, and urethane production. Bulk quantities up to 1,500-2,000 pounds are weighed and then dumped into reactors to be chemically converted into the final product. In such operations, o-tolidine is generally handled for less than 1 hour. Dust emissions from the weighing operation should be controlled by the use of local exhaust ventilation. The hood should enclose as much of the scale as possible without interfering with the worker's performance.

Local exhaust ventilation can also be used to control dust in the charging or reaction vessels. However, an alternative approach is to operate the

reactor under negative pressure relative to the working environment. Negative pressure within the system should usually control a billowing effect that has been reported during the charging of reaction vessels in dye production [10].

Handling o-tolidine in the wet-cake or paste form provides additional safeguards by reducing the amount of dust liberated [10]. The liberation of dust may also be reduced by converting the o-tolidine base to its larger crystalline salt structure [10]. If possible, premeasured units of dry o-tolidine base or salts should be opened under water or other suitable liquid to reduce the amount of dust released when the package is opened.

Environmental Sampling and Analytical Methods

(a) Sampling

Because airborne o-tolidine is typically an aerosol, filters or impingers are most appropriate for personal air sampling, although no studies have been found on this point. Filters are effective for particulates. Glass-fiber filters are recommended to minimize pressure drop. They are clean, self-contained, and convenient. It is recommended that the filter be backed in series by silica gel to minimize losses from vaporization of the sample. This system of a filter backed by silica gel has been used successfully in sampling for benzidine [67] and other related biphenyl amines. Alternatively, impingers may be substituted for filters or filter and silica gel tandem devices for sampling. Hydrochloric acid [68] and distilled water [69] have been used as collecting media, but because recovery rates were not provided, their effectiveness in capturing o-tolidine cannot be evaluated. Because of the difficulties involved in obtaining breathing zone samples with impingers, filters are recommended to sample air in the breathing zone of workers. However, stationary impingers may be used for supplementary area sampling. Sampling with impingers should be avoided unless precautions are taken to prevent sample losses through foaming and solvent loss.

(b) Analysis

Concentrations of airborne o-tolidine should be kept as low as possible to minimize worker exposure through both inhalation and skin absorption. The following discussion reviews several methods of analyzing o-tolidine and related compounds in air for environmental monitoring and in urine for biologic monitoring.

Matrka et al [70] described an oxidimetric (potentiometric) titration method for o-tolidine determination using cerium (IV) sulfate. Standards were prepared with 42-106 mg of o-tolidine dissolved in 45 ml of 0.1 N hydrochloric acid at 20 C. The indicator electrode was glossy platinum, the comparison electrode saturated calomel. The o-tolidine solution was titrated with 0.05 N cerium disulfate solution. The error between amounts of o-tolidine weighed and calculated after titration varied between 0.2% and 0.4%. Titration usually overestimated the actual amount. The authors mentioned other techniques of titration for o-tolidine, including titration with p-toluene sulfonic acid, but did not quantitatively compare those techniques with their

own. This oxidimetric method has a clearly marked end point but lacks specificity. The sensitivity of the method was not given, but this method probably is not sensitive at the microgram level.

Fluorometric [6,71,72] and colorimetic [68,69,73-76] methods have been developed for the analysis of biphenyl amines, including o-tolidine. The fluorometric techniques depend on the property of aromatic amines to fluoresce [6] or form fluorescent derivatives with a suitable reagent [71,77].

One method that uses the fluorescence of the amines has been described by Bowman et al [6]. The method has been successfully used to analyze biologic samples, such as blood and urine, for o-tolidine and required extraction of the compound with suitable solvents. For urine, 10 N NaOH was added to the sample, which was then extracted with benzene. A recovery of 90% was reported. The limits of detection for o-tolidine and its salts were 2 ng/ml (2 μg/liter). The major disadvantage of the method is its inability to separate o-tolidine from such related compounds as benzidine in a mixture.

Techniques in which a fluorescent derivative is formed generally use either o-phthalaldehyde or fluorescamine [71]. However, o-phthalaldehyde could not be applied to o-tolidine analyses because it failed to form a fluorescent derivative when reacted with o-tolidine [71]. Fluorescamine forms an unstable fluorescent derivative with aromatic amines [71], so it is probably unsuitable for o-tolidine fluorometry, but could be suitable for conventional colorimetry as discussed later.

In colorimetry, the biphenyl amine is reacted with a suitable reagent to form a colored complex, which is measured spectrophotometrically. A variety of colorimetric techniques have been developed, primarily for benzidine analysis of biologic specimens, and differ mainly in the reagent used. These methods have been used successfully with other related biphenyl amines and in the analysis of air samples, but only a few have actually been adapted to o-tolidine analysis. These include a qualitative method [75], a chloramine-T test [69,76], a sodium hypochlorite test [68,74], an N-(1-naphthyl)-ethylenediamine dihydrochloride test [73], and the fluorescamine reagent test [71]; they all use naphthoquinone potassium sulfonic acid as a reagent [48].

In the qualitative method [75], a bromocyanogen solution is mixed with the sample, giving rise to an orange to red color in the presence of o-tolidine. The limit of detection of this method is 50 μg of o-tolidine/liter of urine. The method is quick and simple but lacks specificity and sensitivity for monitoring o-tolidine in the air and in urine. Another disadvantage of the method is that bromocyanogen is highly toxic.

The diazotization and coupling of o-tolidine and N-(1-napthyl)-ethylenediamine dihydrochloride has been used in the analysis of o-tolidine [73]. A violet-rose complex is obtained, and the method has a reported limit of sensitivity of 2-3 μg/8 cc. The maximum concentration that can be detected without dilution is 50 μg/8 cc (6.25 μg/cc). The diazotization method may be difficult to use at very low concentrations, because the diazo compound may rapidly decompose [78].

Fluorescamine forms a stable yellow derivative with o-tolidine. It has been successfully used in the colorimetric analysis of o-tolidine and has a reported lower limit of 2 nmoles (0.3 μg) [71]. Advantages of this method over other colorimetric methods are that the reagent is colorless, thereby eliminating the need for removing the unreacted reagents, and that the colored complex is stable.

1,2-Naphthoquinone-4-potassium sulfonic acid forms a bluish-pink complex in the presence of o-tolidine and has a limit of detection of 0.05 mg/100 cc [47]. This method is not sensitive enough to monitor o-tolidine in urine samples.

Of the colorimetric methods that have been used for o-tolidine analysis, the qualitative method [75] and the 1,2-naphthoquinone-4-potassium sulfonic acid method [47] do not have the sensitivity required to adequately monitor o-tolidine in urine in the microgram range. Of the remaining four methods, the chloramine-T test [69], the sodium hypochlorite method [68], and the N-(1-naphthyl)-ethylenediamine dihydrochloride reagent method [73] are sensitive to o-tolidine in the microgram range but share the common problem of having an unstable color complex, thereby requiring quick and rapid reading.

The methods described above are nonspecific. Because o-tolidine is generally found in the same environment as benzidine and other biphenyl amines, the proper monitoring of o-tolidine in air requires a method that is both sensitive and specific for o-tolidine. Some investigators have coupled the fluorometric and colorimetric techniques discussed above with either paper [22,68,73] or thin-layer [6,71] chromatography. These chromatographic methods differ mainly in the solvents used to separate the biphenyl amines. Meigs et al [22] used reagent grade petroleum ether to separate o-tolidine from other biphenyl amines in paper chromatography but did not quantitate the o-tolidine present. Paper chromatography using an isobutanol, glacial acetic acid, and water system was coupled with the N-(1-naphthyl)-ethylenediamine dihydrochloride colorimetric method to obtain quantitative separation of o-tolidine with a level of detection of 5 μg/liter of urine [68,73]. Although these techniques can reach the desired level of sensitivity for monitoring o-tolidine in air, the procedures involved are tedious and time consuming.

Thin-layer chromatography has been used with fluorometry to obtain a qualitative separation of o-tolidine using a methanol solvent system [6] and with colorimetry using a chloroform-ethanol solvent system and 1,2-naphthoquinone-4-sulfonate reagent [71]. Quantitative separation of o-tolidine with thin-layer chromatography was accomplished using a chloroform, glacial acetic acid, and methanol solvent system to obtain separation followed by fluorescamine colorimetry [71]. The limit of sensitivity was 2 nmoles (0.3 μg). As with paper chromatography, the primary disadvantage of this method [71] is the tediousness of the operation and the time required to complete analysis.

Bowman et al [6] used a gas chromatograph equipped with a flame-ionization detector to obtain separation. The column packing was 10% OV-101 (w/w) on Gas Chrom Q (80-100 mesh) and operated with a helium flow of 100 ml/minute.

Injection port and detector temperatures were 275 C and 290 C, respectively. A temperature-programmed chromatograph (200-280 C) was used to obtain separation; o-tolidine appeared at 235 C. The sensitivity of the method was not given, but the authors did report that the method lacked the sensitivity and specificity required for trace analysis of o-tolidine.

A high-pressure liquid chromatography method has been developed and validated for benzidine in air [67]. The method has a working range of 3-130 μg/cu m and a limit of detection of 0.05 μg/sample. The method is simple and rapid compared with paper and thin-layer chromatography. Potential interferences can be overcome by changing the composition of the mobile phase or by solvent programming. This method has not been used for the analysis of o-tolidine, but because of its success in the analysis of other related biphenyl amines (those with similar physical and chemical properties) this method is judged to be acceptable for the separation and quantification of o-tolidine at the same level of detection as benzidine.

High-pressure liquid chromatography is a simple and rapid analytical procedure compared with paper and thin-layer chromatography coupled with fluorometry or colorimetry. It has a limit of detection of 0.05 μg/sample, which is four times more sensitive than the most sensitive currently available fluorometric (0.2 μg/sample) [6] or colorimetric (0.3 μg/sample) [71] method adapted for o-tolidine analysis. High-pressure liquid chromatography is therefore recommended for the analysis of o-tolidine in air.

The fluorescamine colorimetric method [71] does not have the disadvantages of the other colorimetric methods discussed above. It forms a very stable color complex with o-tolidine and is the most sensitive of these methods. This additional sensitivity would be advantageous for employee protection. The method also uses a colorless reagent, which eliminates the need for extracting the unreacted reagent. For these reasons, the fluorescamine colorimetric method of urinary analysis of o-tolidine is recommended.

The fluorometric method of Bowman et al [6] is comparable with the fluorescamine colorimetric method [71] and may be used in the analysis of urine samples. The fluorescamine colorimetric method of analysis is preferred because it is easier to perform.

Biologic Monitoring

Aromatic diamines, such as o-tolidine and benzidine, are not normally found in the body; therefore, the detection of o-tolidine or its metabolites in the urine should be attributed to exposure to this compound. o-Tolidine may be absorbed through the lungs, the skin, or the digestive tract.

It is important to minimize or eliminate the absorption of o-tolidine by workers. This is done by adhering to stringent engineering controls and work practices. If o-tolidine or other aromatic diamines are detected by urinalysis, it may signal inadequacies in either engineering controls or work

practices; however, it should be remembered that a positive indication of diamines in the urine may result from nonoccupational sources such as medication.

Meigs et al [22,64] reported a correlation between the magnitude of exposure to biphenyl diamines, including o-tolidine, and the amount of diamines detected in the urine of workers. Following an 8-hour workshift, workers directly exposed to biphenyl diamines excreted an average of 500 μg of diamine/liter of urine. Greater quantities were excreted during the summer months than during the winter months. Foremen excreted an average of 50 μg of diamine/liter of urine following a workshift. In some instances, the authors specifically identified which diamines were in the urine. Investigation of several cases revealed the presence in the urine of some diamines to which the worker had not knowingly been exposed, thus indicating exposure of unknown origin to diamines that were either previously produced or produced in other areas of the plant. Contaminated work clothing was discovered to be the source of unexpected exposure. Improved work practices and personal hygiene were reported to have been associated with decreased urinary excretion of biphenylamine compounds. Throughout the testing period, concentrations of biphenylamines in the workplace air ranged from 2 to 87 μg/cu m, averaging 18 μg/cu m.

Further evidence of the effectiveness of improved work practices and personal hygiene in minimizing exposure of workers to diamines was reported by PF Woolrich (written communication, December 1977). Three employees worked 5 successive days wearing the same underclothing and using the same gloves. At the end of the 5th day, aromatic amine concentrations of 30, 125, and 330 mg were determined in the work clothing of the three workers. At the end of the 5th day, the corresponding concentrations of aromatic amines, measured as quinonizable substances, in the urine of the three workers were 274, 501, and 602 μg/liter, respectively, suggesting a relationship between the absorption of diamines (including o-tolidine) and skin contact with contaminated clothing.

The time span of urinary diamine excretion may be estimated from results reported by Meigs and coworkers [22]. After an accidental drenching with dichlorobenzidine, one worker had an excretion rate of urinary diamines which peaked on the day of exposure (1.13 mg/liter) and which returned to a baseline level (.043 mg/liter) the next day. This suggested to the authors that urine samples should be obtained near the end of the workshift.

Although these studies are very limited, it is concluded that the presence of biphenylamines in the urine of employees working with o-tolidine represents absorption of the compound. The measurement of urinary diamines is more of a diagnostic practice than one of compliance. Biologic monitoring provides employers with a valuable measurement technique that can be used to verify o-tolidine exposure in the individual employee.

V. WORK PRACTICES

Health hazards from o-tolidine can be controlled by minimizing exposure through inhalation and skin contact and by a health and safety program that combines good work practices and engineering controls.

The area where o-tolidine is manufactured, processed, used, repackaged, released, handled, or stored should be designated a regulated area. This is not intended to include areas where o-tolidine tapes or kits are used for testing purposes. Skin contact with o-tolidine should be avoided. Access to the regulated area should be limited to employees having assigned duties within the area. A daily entry roster should be kept of all employees entering the regulated area and of their length of stay. This entry roster should be maintained as a part of the environmental records.

o-Tolidine should be transported or stored in sealed, intact containers. A "sealed container" is one that has been closed and kept closed to the extent that there is no release of o-tolidine. An "intact container" is one that has not deteriorated or been damaged to the extent that o-tolidine is released. It is concluded that sealed, intact containers would pose no threat of exposure to employees; therefore, it should not be necessary to comply with required monitoring and medical surveillance requirements in operations involving such containers. If, however, containers are opened or broken so that o-tolidine may be released, then all provisions of the recommended standard should apply.

Personnel working in the regulated area should be informed at least annually of the nature of the hazard of the compound, the specific nature of the operation that could result in exposure, and how to recognize and evaluate conditions and situations that may result in the release of o-tolidine. The employees should also be informed of all decontamination and emergency procedures that apply to their duties and location and especially of their role in emergency situations, such as fires or massive spills.

To prevent ingestion of o-tolidine, employees should not be allowed to eat, drink, or smoke in the regulated area, and smoking materials, food, or beverages should not be stored in this area. To counter unsuspected contamination from the container surface and process leaks, employees working where o-tolidine is stored in intact, sealed containers or contained in closed processes should wash their face, neck, hands, and forearms each time they leave the area before they engage in other activities because, even though they are wearing clothing that generally provides an effective barrier, leaks around sleeves and collars may negate some of the protective value of the clothing. Before leaving the regulated area at the end of the workday, employees should shower, shampoo, and scrub their fingernails. Users of o-tolidine test tapes and test kits need not comply with these provisions. Washing facilities should be located close to each exit. If glove boxes are used to handle o-tolidine, employees should wash their hands and arms on

completion of the assigned tasks, as a precautionary measure against possible defects in the gloves of the glove box, before engaging in activities not related to o-tolidine.

Test tapes, impregnated or coated with o-tolidine, are widely used in medical tests, such as monitoring glucose levels in the control of diabetes and for the testing of occult blood. In addition to frequent monitoring performed by patients themselves, tests are regularly performed by technicians in hospitals and clinical laboratories. The concentration of o-tolidine in test tapes is reported to be about 0.1 mg/2.5 cm (1 inch) of tape. Assuming approximately 0.5 cm of tape comes in contact with the fingers with each test, then a maximum amount of 0.02 mg might be absorbed, which is very unlikely. Some manufacturers have attached small plastic handles to the test tapes to further minimize dermal contact with o-tolidine.

Kits containing o-tolidine in solution are also used extensively by the public for testing the chlorine content in water in private swimming pools. Occupational use of similar test kits occurs in waste water treatment plants, potable water supply companies, and companies that service public and private swimming pools on a commercial basis. These test kits are usually prepared by taking 10 ml aliquots from 1- to 2-liter acidic (HCl) stock solutions containing 0.1% o-tolidine. From the test kit solution, 2-10 drops are added to 10 ml of water to be tested, the solution is capped and mixed by inverting the container, and then the developed color is compared with that on a standard color chart. Assuming there are about 20 drops/ml of water, if 5 drops of a 0.1% test solution were added to 10 ml of water, there would be approximately 0.00125 mg/drop of water being tested. In mixing water solutions improperly, an employee sometimes uses the tip of the thumb or finger, or possibly the forearm, rather than a cap to cover the opening of the container. Exposures are limited to the low o-tolidine concentration encountered during the mixing process. On rare occasions, test solutions containing 0.5% o-tolidine are used in water sanitizing procedures.

Although o-tolidine is present in test tapes and test kits, it is not believed that an occupational hazard exists from dermal contact with o-tolidine because of the low quantities available for skin absorption during the conduct of these tests.

It is essential that clothing, footwear, and headgear be clean each workday to reduce prolonged skin contact with o-tolidine. Each employee in the regulated area should therefore put on, at the beginning of each workshift, a complete set of clean work clothing (long-sleeved shirt, trousers, underwear, and footwear) before entering the regulated area. One manufacturer of o-tolidine preferred synthetic fibers to cotton because they have found that o-tolidine tends to adhere to cotton even after washing [42]. Footwear, including rubber shoes, should be cleaned inside and out at least daily and should not be taken home. Hard hats, if worn, should be over disposable or washable head covers and should be cleaned daily. In addition to this work clothing, protective clothing, including waterproof gloves and aprons or overalls, or a full-body protective suit, should be provided to employees engaged in operations in which o-tolidine is transferred from a

closed system or charged or discharged into other normally closed containers, in activities involving the opening of a closed system, or in activities involving laboratory hoods. This additional protection is believed necessary because these operations involve a greater risk of exposure to o-tolidine.

Waterproof gloves and aprons or overalls that are resistant to penetration by o-tolidine should be used while working with filters and dryers, while handling or decontaminating the exterior of filled barrels of o-tolidine, in dropping presses and filters, for taking process samples for quality checks, and for performing other routine tasks not involving maintenance. Protective clothing requirements for maintenance activities, including cleaning and decontamination of equipment and material, should be determined for each job by an industrial hygienist and the regulated-area supervisor.

Entry into confined spaces, such as tanks, pits, and process vessels that have contained o-tolidine, shall be controlled by a permit system. Permits shall be signed by an authorized employer representative certifying that prescribed precautionary measures and procedures have been followed. All lines should be disconnected or blocked off, and all valves or pumps leading to and from the vessel should be locked or tagged out while the vessel is being cleaned. The vessel should also be either washed with water and purged with air or purged with nitrogen and then with air.

The vessel should then be checked by trained personnel for fire or explosion hazard, airborne o-tolidine, possible oxygen deficiency, and concentrations of other likely contaminants to assure that no danger exists. Mechanical ventilation should be provided continuously when employees are inside the vessel. Each employee entering the vessel should be equipped with appropriate respiratory protection, a harness, and a lifeline. At least one other person, similarly equipped, should observe from outside and should maintain effective communication with the employee inside the vessel at all times.

As a supplement to engineering controls, a full-body protective suit with appropriate respiratory protection and head covering should be provided to each employee in such operations as weighing o-tolidine or charging reaction vessels for dye, pigment, and urethane manufacture [10] because of the higher risk of inhalation and skin contact. Positive pressure supplied-air respirators with full facepiece should be used whenever necessary to keep worker exposure to the lowest level possible. Full facepieces afford better fit to minimize leakage and obviate any possible problems of eye irritation. However, because contaminated clothing and equipment are primary sources of skin contact with o-tolidine, full-body protective clothing with necessary head and face protection should be used with these respirators.

Employees engaged in operations involving o-tolidine should leave their protective clothing and equipment at the exit. When workers leave the regulated area at the end of the workday, the used protective clothing and equipment should be placed in clearly labeled containers for decontamination or disposal.

Each employee involved in cleaning up leaks and spills should wear a completely enclosed full-body protective suit with either a self-contained breathing apparatus or a supplied-air respirator with auxiliary self-contained air supply. After the cleanup, the area should be decontaminated and washed, the protective clothing and equipment should be decontaminated and removed, and the employee should shower. Potassium permanganate and sodium hypochlorite have been recommended for decontamination of clothing and equipment, but data on their effectiveness as decontaminating agents were not provided (JW Meigs, written communication, December 1977). To minimize concentrations of airborne o-tolidine, employees should be prohibited from dry sweeping and dry mopping in the regulated area. The regulated area should be washed thoroughly at the end of each shift. The waste water should be collected in holding basins for decontamination with potassium permanganate or sodium hypochlorite. One o-tolidine manufacturer wipe tests work surfaces in the regulated area (PF Woolrich, written communication, December 1977). Quantitative information relating wipe test results with air levels of o-tolidine or with toxic effects of o-tolidine is not available, so its usefulness as a monitoring tool is unknown.

A clean change room, free of o-tolidine contamination and containing locker facilities, should separate regulated and nonregulated areas. The clean change room should be separated from the regulated area by a shower room. Therefore, with the exception of emergency exits, the movement of workers from clean room to regulated area should occur only through the shower area. The clean room should be under positive pressure relative to the regulated area to prevent accidental contamination of the clean area when workers move from one area to the other. Appropriate signs should be posted at the entrance informing workers of the procedures for entering and leaving the regulated area.

All workers must put on clean work clothing, including underwear, trousers, and shirt, along with footwear and the necessary protective equipment in the clean room before entering the regulated area. At no time should the regulated area be used to store protective equipment because accidental contamination by o-tolidine is possible. Before leaving the regulated area, workers should remove all protective clothing and equipment and wash their hands, forearms, face, and neck. Upon reentering the regulated area, the worker must put on the same protective clothing and equipment. To minimize traffic from regulated areas to clean areas, employers should locate toilets in rooms within regulated areas. Employees in regulated areas should wash their hands and forearms before and after using the toilet.

At the end of the workshift, protective clothing and equipment should be placed in clearly labeled containers in the regulated area for decontamination or disposal. The employees should then proceed to the shower facilities, remove all work clothing, and place it in labeled containers for laundering. Employees should shower and shampoo with soap or other detergent before entering the clean room to put on street clothing.

Laundry contaminated with o-tolidine should be transported only in sealed containers. Soap or other detergent should be used to clean work clothing.

Sodium hypochlorite has been suggested for decontamination of work clothing (PF Woolrich, written communication, December 1977). Plant personnel involved in laundering should be equipped with aprons and gloves, warned of the hazards of o-tolidine, and trained to handle contaminated clothing safely. If an outside laundry facility is used, the laundry employer must be advised of the hazards involved in handling clothing contaminated with o-tolidine and of the requirements to ensure that the laundry employees are not exposed to o-tolidine.

Employees who handle only very small quantities of o-tolidine (such as users of test tapes and water analysis kits) are considered to be at only minimal risk, and therefore the requirements for regulated areas and clean room facilities are not deemed necessary. These workers should be adequately protected from skin contact and inhalation if they perform all activities involving o-tolidine in a laboratory hood or glove box and use long-sleeved coveralls or coats and gloves of materials resistant to penetration by o-tolidine. Workers should be required to wash their hands, forearms, neck, and face with soap and water after working with o-tolidine to guard against accidental contamination.

In emergencies, the contaminated area should be evacuated immediately. Only personnel trained in emergency procedures and equipped with full-body protective clothing and proper respirators should enter the contaminated area to make repairs and decontaminate the site. All employees within the affected area at the time of emergency should be required to shower promptly. If biologic monitoring is performed, it should be conducted within 24 hours.

VI. DEVELOPMENT OF STANDARD

Basis for Previous Standards

No country has yet published a workplace environmental limit for o-tolidine. However, maximum allowable concentration (MAC) limits have been set by the Polish government for the chemically related substances benzidine and dianisidine (0.01 mg/cu m) [79]. Documentation supporting this value has not been found. Belgium, Great Britain, Sweden, and Japan have published work practice recommendations for o-tolidine. In Belgium, exposure to o-tolidine base (and to benzidine and its salts) is prohibited because o-tolidine is considered a carcinogen [80]. The basis for this decision was not given. Great Britain established stringent regulations, still in effect, concerning the manufacture and use of o-tolidine, which appeared with dianisidine and dichlorobenzidine on that country's 1967 controlled substances list [80,81]. These regulations are still in effect. The regulations state that all precautions must be taken to prevent workers' exposure, and they provide for extensive medical examinations every 6 months. The National Board of Occupational Safety and Health of Sweden included o-tolidine on a list of substances that could be manufactured or used only if special instructions by the labor inspectorate were followed [80,82,83]. Similarly, in Japan, special permission must be obtained from the labor department to use or manufacture o-tolidine. When this permission is granted, strict procedures that prevent worker exposure and provide for regular medical examinations must be followed [80]. The Soviet Union reportedly no longer produces o-tolidine [84].

Basis for the Recommended Standard

(a) Permissible Exposure Limits

A few studies have been found that give results of human exposure to o-tolidine, usually in conjunction with exposure to other related diamines, eg, benzidine, dichlorobenzidine, and dianisidine [16,19,21-23,85]. A number of animal studies have been found in which the effects of exposure to o-tolidine by itself were investigated [17,26,28-30,33,35,38,44,49]. Most occupational exposures involve a mixture of biphenyl amine compounds, including benzidine, o-tolidine, dichlorobenzidine, and dianisidine [19,21-23].

There is a report stating that inhalation of o-tolidine hydrochloride causes nasal irritation in humans [16]. o-Tolidine is absorbed through the skin of humans without apparent skin irritation [17] and is subsequently eliminated in the urine [17,22].

Although cancer has not been observed in humans exposed to o-tolidine alone, workers exposed to a combination of benzidine and o-tolidine have developed bladder cancer [20,21]. Benzidine is a known human bladder carcinogen [15], but these reports [20,21] do not give sufficient data to determine if the cancers were induced by benzidine alone, o-tolidine alone, or both compounds.

o-Tolidine (or its metabolites) is carcinogenic in some animals [29,33-35]. The majority of rats developing cancers had carcinomas of the Zymbal glands, mammary gland adenocarcinomas, and hepatocarcinomas [33-35]. Tumors of lesser frequency included skin cancers, stomach papillomas, small intestine adenocarcinomas, one uterine leiomyosarcoma, preputial sebaceous gland tumors, and reticulosarcomas. One of four dogs fed o-tolidine for 8-9 months developed bladder cancer after 8 years [29].

Ames assays showed o-tolidine to be mutagenic in two studies [41,43]. However, another, more comprehensive, study [42] reported negative findings for o-tolidine. o-Tolidine was shown to depress thymidine incorporation into testicular DNA [40], indicating a diminution of DNA synthesis directly related to o-tolidine exposure. o-Tolidine had mutagenic effects on rat embryo cells in cell culture; when the transformed cells were transplanted into rats, they developed into tumors [39].

o-Tolidine did not cause deformed rat embryos in dams receiving a total dose of 30 mg in three successive daily sc injections [45], but 8% of the fetuses were resorbed. Organ cultures of embryonic kidneys from pregnant mice given sc injections of 2 mg/day for 19-20 days exhibited epithelial hyperplasia and "other cellular changes" that were not seen in the controls [44]; this suggests that o-tolidine may have transplacental effects on embryogenesis.

There are important similarities between o-tolidine and benzidine, a known human bladder carcinogen. Structurally, o-tolidine is the 3,3'-dimethyl substituted form of benzidine, and its physical and chemical properties closely parallel those of benzidine [5,6]. Both are absorbed through human skin and are excreted in the urine either unchanged or as structurally analogous conjugates [22,47,58]. The metabolic pathways of both are similar; both undergo acetylation, hydroxylation, or sulfate esterification [22,47,50,56,58]. Neither compound has induced bladder cancer in rats [15,62], but each has caused cancer in other tissues and organs [15,62]. Bladder cancer in dogs has been observed with each compound [29,86], although the evidence for o-tolidine consists of only one dog with cancer of four dogs exposed, 8 years after an 8- to 9-month feeding period [29].

Because tests with o-tolidine, like benzidine, have resulted in mutagenic effects in test systems and because cancer has occurred in rodents as a result of o-tolidine absorption, there is substantial reason to believe that o-tolidine will induce bladder cancer in humans. Therefore, it is recommended that o-tolidine be handled as a suspect human carcinogen. Because of this conclusion and since it is not possible at this time to establish an exposure level at which o-tolidine is known to be harmless, it is recommended that exposure to o-tolidine be kept as low as possible through strict adherence to a program of monitoring, engineering controls, and stringent work practices. The recommended sampling and analytical method for measuring o-tolidine in air is one that has been experimentally confirmed for benzidine and, because of the physical and chemical similarities of the two compounds, the method is judged to be suitable for o-tolidine. The lowest amount of o-tolidine reliably measured quantitatively by this method is estimated to be 0.2

μg/sample. If an air sample is collected at 0.2 liters/minute for 60 minutes, this quantity of o-tolidine is equivalent to 20μg/cu m, so this concentration represents the lowest level at which a reliable quantitative estimate of exposure to o-tolidine can be determined. Therefore, it is recommended that occupational exposure be controlled so that no worker will be exposed at a concentration of o-tolidine in excess of 20μg/cu m in air determined from an air sample collected at 0.2 liters/minute for 60 minutes.

It is of interest that the International Agency for Research on Cancer (IARC) has also listed o-tolidine as a cancer suspect agent in humans [87,88].

Although o-tolidine is present in test tapes and test kits used for the determination of glucose in blood or urine, blood in urine or feces, or chlorine in water, it is not believed that an occupational hazard exists either from airborne o-tolidine or from dermal contact that may result from such use. It is concluded that users of test tapes and test kits containing o-tolidine should be excluded from the monitoring and surveillance requirements of this recommended standard. However, all provisions of the standard should apply where o-tolidine is used in the manufacturing and formulating of test tapes, test kits, and test solutions.

The presence of o-tolidine or its metabolites in the urine provides a means for the biologic monitoring of o-tolidine exposure, even though such monitoring has not been sufficiently investigated to warrant requiring it as part of medical monitoring. However, it may still be a useful adjunct to monitoring of the workplace air to gain information on unknown sources of exposure, to identify unanticipated excursions, and to recognize poor work practices.

(b) Sampling and Analysis

A filter is suitable for capturing the o-tolidine aerosol. The filter should be made of glass fibers to minimize pressure drop. The filter should be backed by a silica gel tube because o-tolidine has a low but distinct vapor pressure. High-pressure liquid chromatography has been shown to be a good method for benzidine and should be similarly useful for the analysis of o-tolidine.

(c) Medical Surveillance

Comprehensive preplacement and annual examinations should be made available to all workers occupationally exposed to o-tolidine. Workers should be informed that o-tolidine administration has resulted in nasal irritation in humans and that o-tolidine has caused kidney damage [16] or cancer of the skin, glands, or internal organs [29,33] in animals.

Because of the possibility of kidney damage [16,26], workers occupationally exposed to o-tolidine should have quarterly urine examinations, including a complete urinalysis and microscopic examination of the urine for evidence of abnormal cells indicative of kidney damage or neoplasms. The

test should be repeated within 1 week to confirm any abnormal results found. If the abnormalities are confirmed, the worker should be referred to a physician for a comprehensive urologic evaluation.

(d) Personal Protective Equipment and Clothing

Employees working with o-tolidine change clothes at the beginning and end of work shifts. Work clothes may become contaminated with dust, so they must be kept in a separate locker, away from street clothes. Clean work clothes should be worn each shift. If work clothes are not changed after each shift and laundered, absorption of o-tolidine through the skin may be facilitated by both the increased time in which skin contact is maintained and by the buildup of o-tolidine in the clothing [22]. Measurement of o-tolidine in the urine of workers confirmed this in one operation [22]. Soiled work clothes should be stored in covered containers until they are laundered either at the plant or, if an outside laundry facility is used, the launderers should be advised of the hazards and proper procedures involved in handling contaminated work clothing. Sodium hypochlorite has been used for decontamination prior to laundering, but no data supporting its effectiveness have been found. The employer in charge of laundering should emphasize precautionary measures to avoid exposure to o-tolidine from handling contaminated work clothes. Gloves and aprons resistant to o-tolidine should be worn, and skin contact with o-tolidine should be avoided. When exposure to dust or mist containing o-tolidine occurs during emergencies, maintenance operations, or special processes, use of the respirators specified in Chapter I, Section 3 (b) and full-body skin protection should be observed.

(e) Informing Employees of Hazards

Continuing education is an important part of a preventive hygiene program for employees exposed to o-tolidine. Workers should be instructed periodically by properly trained persons about possible sources of exposure to o-tolidine, engineering and work practice controls in use or being planned to limit exposure, and on monitoring procedures used to check control procedures. It should also be explained that o-tolidine may be readily absorbed through the intact skin as well as by inhalation or ingestion and that exposure to it poses a risk of damage to the urinary tract, primarily cancer of the urinary bladder. The function of environmental monitoring equipment, such as personal samplers, should be explained, so that employees understand their part in environmental monitoring. Medical monitoring procedures and their importance in detecting possible adverse health effects should be explained.

(f) Work Practices

Because o-tolidine can be readily absorbed through intact skin and mucous membranes, special work practices are essential. It is especially important that the use of regulated areas and clean rooms and the procedures for sanitation, maintenance, and emergencies in the control of airborne o-tolidine be understood and followed by workers occupationally exposed to the chemical.

If o-tolidine is handled or stored in intact, sealed containers, the requirements of regulated areas should not be necessary. However, if containers are opened, the requirement for a regulated area applies.

(g) Monitoring and Recordkeeping Requirements

Industrial hygiene surveys should be conducted after the promulgation of a standard based on these recommendations and within 14 days of any process change.

If the concentration of airborne o-tolidine in a regulated area exceeds the recommended occupational exposure limit, proper engineering and work practice control measures should be initiated. Sampling should be repeated until two consecutive determinations at least 1 week apart show that airborne concentrations of o-tolidine are below the recommended occupational exposure limit. If this survey reveals that the airborne concentration is below the recommended occupational exposure limit, then the survey need only be repeated annually.

Medical records for all employees occupationally exposed to o-tolidine should be kept for 30 years after the termination of employment. Records of environmental exposures to o-tolidine should be included with the worker's medical records. These records should be available to the designated medical representatives of the employer, employee, Secretary of Health, Education, and Welfare, and of the Secretary of Labor.

VII. RESEARCH NEEDS

Although o-tolidine is not a new compound and has been used for a century as an indicator in analytical chemistry and as an intermediate in the synthesis of approximately 100 dyes [4], relatively little has been published about its effects on biologic systems. In particular, there is a scarcity of information on how o-tolidine affects humans.

Before better dose-response relationships can be delineated, more experiments with animals are needed. As presented in Chapter III, the currently available experimental data on rats exposed to o-tolidine indicate that, as with benzidine, it can cause cancer in a number of organ systems in the rat, but it does not cause bladder cancer. This is also apparently true for the hamster. Attempts should be made to find an animal model in which bladder cancer could be identified from administration of o-tolidine, benzidine, or other diphenyl amines. This would be helpful in evaluating bladder cancer in humans associated with these amines. Epidemiologic studies on workers exposed to o-tolidine are especially needed to investigate the problem of o-tolidine-related bladder tumors in humans. Cell- and organ-culture studies, including host-mediated assays, would be useful to evaluate the significance of genetic alterations from o-tolidine. The renal effects from o-tolidine, both alone and associated with exposure to other chemicals, especially other aromatic amines, should be addressed.

The metabolism of o-tolidine resembles that of benzidine, a known human bladder carcinogen. Additional studies are needed to further elucidate o-tolidine metabolism, to compare it to that of benzidine, and to identify those metabolites that may be toxic or carcinogenic. The possibility exists that dyes made from o-tolidine might release free o-tolidine in the body. This should be investigated.

Workers who use o-tolidine coated or impregnated test tapes or water analysis test kits containing o-tolidine are judged at this time to be at negligible risk from the chemical. (This is based on professional estimates and calculations because investigative data are lacking.) Studies should be conducted to ascertain whether such users absorb o-tolidine from skin contact in quantities sufficient to pose a risk to worker health.

Validation of the sampling and analytical methods recommended for o-tolidine is needed, even though they have been tested for benzidine and are judged to be effective for o-tolidine.

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IX. APPENDIX I

SAMPLING AND ANALYSIS

This method for sampling and analysis of o-tolidine is adapted from the NIOSH Manual of Analytical Methods, Method No. 243, for benzidine and benzidinium sulfate [67]. Although the method has not been validated for o-tolidine, it has been used successfully to analyze for other biphenyl amines and is judged to be acceptable for o-tolidine.

Principle of the Method

A known volume of air is drawn through a two-stage sampler consisting of a high-efficiency glass fiber filter followed by a bed of silica gel to collect o-tolidine and its salts. The glass fiber filter is recommended to minimize pressure drop. The filter and sorbent sections of the sampler are transferred to stoppered tubes and the o-tolidine desorbed. A solution of triethylamine in methyl alcohol is used for benzidine and should be successful for o-tolidine also. An aliquot of this solution is injected into a high-pressure liquid chromatograph (HPLC). Peak areas are determined and compared with a calibration curve obtained from injections of standard solutions of o-tolidine.

Range and Sensitivity

This method can detect 0.15-6.5 μg of benzidine/sample (3-130 μg/cu m for a 50-liter air sample) using 0.5 ml of desorbing solution and a 10-μl injection into the liquid chromatograph. The range of detection for o-tolidine should be similar. The upper limit can be extended by increasing the volume of desorbing solvent as a diluent, decreasing the sensitivity of the detector, or decreasing the aliquot injected into the liquid chromatograph. The limit of detection for o-tolidine is not known but is expected to be similar to that of benzidine, which is 0.05 μg/sample.

Interferences

Any compound sampled with o-tolidine and having the same retention time as o-tolidine interferes with the analysis. This type of interference often can be minimized by changing the operating conditions of the chromatograph, eg, by changing the composition of the mobile phase or by solvent programming.

Precision and Accuracy

Filters spiked with benzidine or its salts and stored at -15 C showed 97-98% recovery after 11 days. Recovery tended to decrease with increased

storage time. o-Tolidine recovery should be similar to that of benzidine. The precision of this method for benzidine is 7% relative standard deviation for concentrations of benzidine of 21-63 μg/cu m of air. The accuracy of the method has not been determined.

Advantages and Disadvantages of the Method

The sampler is small, portable, contains no liquids, and can be used to sample the air in the breathing zone of a worker. Temperature and humidity do not significantly affect the method. Desorption of the collected sample is simple and the analysis is accomplished by a rapid instrumental technique. Possible interferences can be obviated by changing the composition of the mobile phase or by solvent programming.

At a flowrate of 0.2 liter/minute, the linear velocity through the 4-mm inlet of the filter holder is 26.5 cm/second. It is not known if this is sufficient to capture all important particles. The method has not been field tested.

Apparatus

(a) Calibrated personal air sampling pump that can be maintained for 60 minutes at a flowrate of 0.2 liter/minute with the sampler in line.

(b) Sampler (Figure IX-1). The sampler consists of two sections, a high-efficiency glass fiber filter and a 50-mg bed of silica gel. The first section, a 13-mm type A-E glass fiber filter, is contained in a 13-mm filter holder. The sorbent tube is a 30-mm section of Pyrex glass, 6.4-mm O.D. x 4-mm I.D., flared on one end, containing 50 mg of GC grade silica gel (D-08, 30/60 mesh, 720-760 sq m/g, 4.3 g/cc). The sorbent is held by 3.5-mm diameter, 100-mesh stainless steel screens and 4-mm O.D. Teflon rings. To connect the two stages, the filter holder is pressed into the flared end of the sorbent tube. Plastic caps of 6-mm I.D. seal the ends of the sampler.

(c) Insulated container suitable for transporting samples packed in dry ice.

(d) Dry ice.

(e) HPLC equipped with an ultraviolet detector (254 nm) and injection valve.

(f) μBondapak C18 column (10-μm), 4.0-mm I.D. x 30-cm or equivalent.

(g) Potentiometer strip chart recorder.

(h) Test tubes, 1-ml, fitted with polyethylene stoppers.

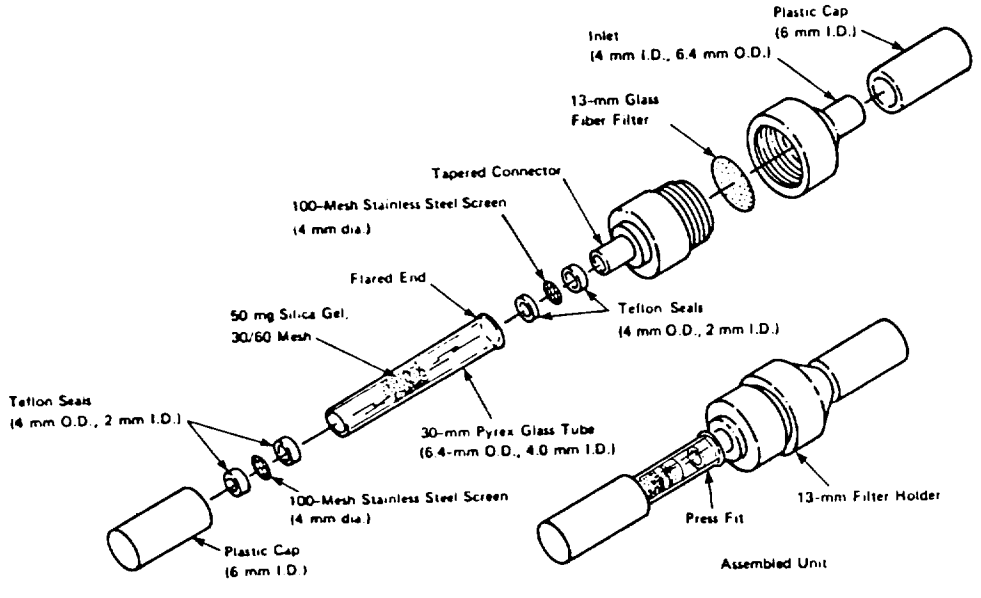

FIGURE IX-1

TWO-STAGE SAMPLER

(i) Glass syringes, 10 μl.

(j) Pipets of convenient sizes for the preparation of standard solutions.

(k) Volumetric flasks of convenient sizes for the preparation of standard solutions.

(l) Clinical centrifuge.

(m) Analytical balance.

(n) Test-tube shaker (vortex type).

Reagents

All reagents should be of analytical reagent quality or better.

(a) Methyl alcohol (UV grade), distilled in glass.

(b) Water, distilled.

(c) Mobile phase: 3/2 (v/v) methyl alcohol in water.

(d) Triethylamine.

(e) Desorbing solution: triethylamine in methyl alcohol, 0.17% (v/v).

(f) o-Tolidine, analytical standard quality.

Procedure

(a) Cleaning of Equipment

All glassware used for the laboratory analysis is washed with detergent, rinsed with tap water, distilled water, and methyl alcohol, and dried in an oven.

(b) Calibration of Personal Sampling Pumps

Each pump should be calibrated with a representative sampler in line to minimize errors in volume measurement. A bubble flowmeter or other suitable flow measuring device may be used.

(c) Collection and Shipping of Samples

(1) Immediately before sampling, the plastic caps are removed from the end of the sampler and saved for resealing after sampling.

(2) The sorbent end of the sampler is connected to the pump with plastic or rubber tubing. The sampler is positioned vertically during sampling. Sampled air must not pass through any tubing before entering the sampler.

(3) The atmosphere is sampled at a flowrate of 0.2 liter/minute for 60 minutes. The flowrate and sampling time, or the volume of sampled air, must be measured as accurately as possible.

(4) The temperature, pressure, and humidity of the atmosphere being sampled is measured and recorded.

(5) The sampler is resealed with the plastic caps immediately after sampling.

(6) Blank samples are obtained by handling random collection devices in the same manner as in sampling, except that no air is drawn through them.

(7) Samples shipped to the laboratory should be packed tightly to minimize breakage and cooled with dry ice.

(8) If samples of bulk material associated with the process under investigation are to be shipped to the laboratory, they should not be placed in the same container as the air samples or blanks.

(9) The samples should be stored at temperatures at or below -15 C prior to analysis. Because of the possible instability of the analyte, the samples should be analyzed as soon as possible after collection.

(d) Analysis of Samples

(1) Preparation of samples. Place the glass fiber filters and silica gel in separate 1-ml test tubes or other suitable vials. Teflon rings and stainless steel screens associated with the samplers may be analyzed with the appropriate stages or rinsed with the desorbing solution. Sampler caps can be discarded.

(2) Desorption of samples. Add 0.5 ml of desorbing solution to each vial containing a sampler stage. Cap the vials and shake them with a test-tube shaker. Allow them to stand for 1 hour with intermittent shaking. Centrifuge for 10 minutes. Treat blanks in the same manner.

(3) Liquid chromatographic conditions. The following conditions work for benzidine and may have to be modified to obtain proper separation of o-tolidine.

(A) Column: μBondapak C18 (30 cm x 4.0 mm I.D.).

(B) Mobile phase: Methyl alcohol/water, 3/2(v/v).

(C) Flowrate: 1.5 ml/minute, 1200 psi.

(D) Temperature: 23 C.

(E) Detector: UV (254 nm), 0.04 absorbance units full scale.

(F) Injection volume: 10 µl.

(G) Power of separation: 784 theoretical plates.

(H) Capacity ratio: 2.4.

(4) Injection. Flush the 10-µl syringe first with methyl alcohol and then with the sample solution. Carefully draw 10 µl of sample solution into the syringe to minimize the intake of particulate matter and inject into the HPLC. No more than 3% difference in peak areas for replicate injections is to be expected.

(5) Measurement of peak area. The area of the sample peak is either measured manually or with an electronic integrator. Preliminary results are read from a standard curve prepared as discussed below.

(e) Desorption Efficiency

(1) Importance of determination. The desorption efficiencies for o-tolidine and its salts can vary from one laboratory to another and also from one batch of silica gel or filters to another. Thus, it is necessary to determine the percentage of o-tolidine that can be recovered from these matrices. The desorption efficiency may also vary with the amount of o-tolidine present; therefore, measurements should be made for at least two amounts in the range of the sample size.

(2) Procedure for determining desorption efficiency. Place the filters and silica gel from unused samplers in separate 1-ml test tubes. Add to each a known amount of o-tolidine in 10-µl of methyl alcohol using the same microliter syringe for each. Five filter and silica gel samples are thus prepared at each of two different levels in the range of interest. Cap the tubes and allow them to stand overnight to assure complete adsorption of the amine on the matrix. Treat a parallel blank in the same manner. Desorb and analyze samples and blanks as described above. Prepare two or three standards at these same levels by injecting 10 µl (same syringe as above) of the o-tolidine solutions into 0.5 ml of desorbing solution. Analyze these solutions and determine the desorption efficiency.

Calibration and Standards

For accuracy in the preparation of standards, it is recommended that one standard be prepared in a relatively large volume and at a high concentration. The initial standard is prepared by weighing a selected amount of o-tolidine, eg, 125 mg, into a 250-ml volumetric flask and adding the desorbing solution to the calibration mark. Dilutions encompassing the concentration range of interest down to 0.3 ng/µl are then made from the stock solution.

CAUTION: o-Tolidine is a suspected carcinogen. Appropriate precautions must be taken in handling this compound to avoid personnel exposure and area contamination.

The standard solutions should be analyzed under the same liquid chromatographic conditions and during the same time period as the samples. This will minimize the effect of variations of the detector response or the mobile phase. A standard curve for o-tolidine is prepared by plotting the average peak area for a standard against the concentration of the standard in μg/0.5 ml.

Calculations

If the blank produces a peak with the same retention time as o-tolidine, the analyst should determine the source of the interference and eliminate or compensate for it. Read the mass (M) in μg of o-tolidine present in the sample from the calibration curve. The corrected mass (W) is determined by:

$$W = \frac{M}{D}$$

where D is the desorption efficiency for that sampler stage.

Add the masses of o-tolidine found in the two sampler stages to determine the total mass (W) collected. The concentration of o-tolidine in air (C) in μg/cu m is given by:

$$C = \frac{W \times 1{,}000}{V}$$

where W is the total mass collected in μg and V is the sampling volume in liters.

This procedure calculates air concentrations as the mass of o-tolidine per volume of air.

X. APPENDIX II

ANALYSIS OF URINE SAMPLES USING FLUORESCAMINE

This method for analyzing o-tolidine in urine samples is adapted from the method described by Rinde [71].

Principle of the Method

A known volume of urine sample is extracted first with chloroform, then with 0.01 M hydrochloric acid to concentrate the o-tolidine. Fluorescamine solution is added to the sample followed by methanol to produce a yellow product. The optical density is determined at the wavelength of maximum absorption, 390 nm, and compared with the calibration curve obtained from standard solutions of o-tolidine.

Range and Sensitivity

This method can detect 0.2-5.0 μg of o-tolidine/sample of urine. For amounts greater than 5 μg, suitable dilutions of the sample are required. The limit of detection of the method is 0.2 μg/sample.

Interferences

The method is nonspecific, so any biphenyl amine other than o-tolidine may interfere with the analysis. This interference can be eliminated by using thin-layer chromatography to separate the biphenyl amines before adding the fluorescamine reagent.

Advantages and Disadvantages of the Method

Cleanup of the collected urine sample is simple, and analysis is accomplished by a rapid, instrumental technique. The fluorescamine reagent is colorless, so there is no need to extract the unreacted reagent.

The method is nonspecific without prior separation; the various compounds cannot be distinguished when o-tolidine occurs in a mixture with other biphenyl amines. This can be overcome by using paper or thin-layer chromatography to separate the biphenyl amines. However, this procedure is tedious and time consuming.

Apparatus

(a) Spectrophotometer with quartz cuvettes.

(b) Vortex mixer.

(c) Pipetes of convenient sizes to prepare standard solutions.

(d) Volumetric flasks of convenient sizes to prepare standard solutions.

(e) Glass test tubes, 12 x 75 mm.

Reagents

All reagents should be of analytical reagent quality or better.

(a) Fluorescamine.

(b) Glacial acetic acid.

(c) Methanol.

(d) Chloroform.

(e) Hydrochloric acid, 0.01 M.

(f) o-Tolidine, analytical standard quality.

(g) Water, distilled.

(h) Nitrogen gas.

Procedure

(a) Cleaning of Equipment

All glassware used for the laboratory analysis is washed with detergent, rinsed with tap water, distilled water, and methanol, and dried in an oven.

(b) Analysis of Samples

(1) Extraction of samples. Adjust the pH of the sample (100 ml) to 5-6, extract first with chloroform, then with 0.01 M hydrochloric acid. Readjust the pH of the HCl solution, and extract again with chloroform.

(2) Preparation of fluorescamine solution. Dissolve fluorescamine in glacial acetic acid (1 mg reagent/ml acid) to make the fluorescamine solution.

(3) Preparation of assay cuvettes. Evaporate the chloroform extract to dryness in 12 x 75 mm tubes using nitrogen gas. Add 50 μl of fluorescamine solution and mix for 30 seconds. After 10 minutes, add 0.5 ml methanol. Wait 10 minutes for full color development, then transfer to cuvette.

(4) Spectrophotometry. Set the spectrophotometer to 390 nm. Using a suitable blank of chloroform, adjust the optical density scale to zero.

(5) Measurement of o-tolidine concentration. Place the sample cuvete in the spectrophotometer and measure the optical density. Read preliminary results from a standard curve prepared as discussed below.

Calibration and Standards

For accuracy in the preparation of standards, it is recommended that one standard be prepared in a relatively large volume and at a high concentration. Prepare the initial standard by pouring a selected amount of o-tolidine, eg, 125 mg, into a 250-ml volumetric flask and adding chloroform to the calibration mark. Make dilutions encompassing the concentration range of interest down to 0.3 ng/μl from the stock solution.

CAUTION: o-Tolidine and chloroform are suspected carcinogens. Appropriate precautions must be taken in handling these compounds to avoid personnel exposure and area contamination.

The standard solutions should be analyzed at the same time as the samples. This will minimize the effect of variations of the spectrophotometer's response. Prepare a standard curve for o-tolidine by plotting the optical density for a standard against the concentration of the standard in μg.

XI. APPENDIX III

MATERIAL SAFETY DATA SHEET

The following items of information that are applicable to a specific product or material shall be provided in the appropriate block of the Material Safety Data Sheet (MSDS).

The product designation is inserted in the block in the upper left corner of the first page to facilitate filing and retrieval. Print in upper case letters as large as possible. It should be printed to read upright with the sheet turned sideways. The product designation is that name or code designation which appears on the label, or by which the product is sold or known by employees. The relative numerical hazard ratings and key statements are those determined by the rules in Chapter V, Part B, of the NIOSH publication, An Identification System for Occupationally Hazardous Materials. The company identification may be printed in the upper right corner if desired.

(a) Section I. Product Identification

The manufacturer's name, address, and regular and emergency telephone numbers (including area code) are inserted in the appropriate blocks of Section I. The company listed should be a source of detailed backup information on the hazards of the material(s) covered by the MSDS. The listing of suppliers or wholesale distributors is discouraged. The trade name should be the product designation or common name associated with the material. The synonyms are those commonly used for the product, especially formal chemical nomenclature. Every known chemical designation or competitor's trade name need not be listed.

(b) Section II. Hazardous Ingredients

The "materials" listed in Section II shall be those substances that are part of the hazardous product covered by the MSDS and individually meet any of the criteria defining a hazardous material. Thus, one component of a multicomponent product might be listed because of its toxicity, another component because of its flammability, while a third component could be included both for its toxicity and its reactivity. Note that a MSDS for a single component product must have the name of the material repeated in this section to avoid giving the impression that there are no hazardous ingredients.

Chemical substances should be listed according to their complete name derived from a recognized system of nomenclature. Where possible, avoid using common names and general class names such as "aromatic amine," "safety solvent," or "aliphatic hydrocarbon" when the specific name is known.

The "%" may be the approximate percentage by weight or volume (indicate basis) which each hazardous ingredient of the mixture bears to the whole mixture. This may be indicated as a range or maximum amount, ie, "10-40% vol" or "10% max wt" to avoid disclosure of trade secrets.

Toxic hazard data shall be stated in terms of concentration, mode of exposure or test, and animal used, eg, "100 ppm LC50-rat," "25 mg/kg LD50-skin-rabbit," "75 ppm LC man," or "permissible exposure from 29 CFR 1910.1000," or, if not available, from other sources of publications such as the American Conference of Governmental Industrial Hygienists or the American National Standards Institute Inc. Flashpoint, shock sensitivity, or similar descriptive data may be used to indicate flammability, reactivity, or similar hazardous properties of the material.

(c) Section III. Physical Data

The data in Section III should be for the total mixture and should include the boiling point and melting point in degrees Fahrenheit (Celsius in parentheses); vapor pressure, in conventional millimeters of mercury (mmHg); vapor density of gas or vapor (air = 1); solubility in water, in parts/hundred parts of water by weight; specific gravity (water = 1); percent volatiles (indicated if by weight or volume) at 70 F (21.1 C); evaporation rate for liquids or sublimable solids, relative to butyl acetate; and appearance and odor. These data are useful for the control of toxic substances. Boiling point, vapor density, percent volatiles, vapor pressure, and evaporation are useful for designing proper ventilation equipment. This information is also useful for design and deployment of adequate fire and spill containment equipment. The appearance and odor may facilitate identification of substances stored in improperly marked containers, or when spilled.

(d) Section IV. Fire and Explosion Data

Section IV should contain complete fire and explosion data for the product, including flashpoint and autoignition temperature in degrees Fahrenheit (Celsius in parentheses); flammable limits, in percent by volume in air; suitable extinguishing media or materials; special firefighting procedures; and unusual fire and explosion hazard information. If the product presents no fire hazard, insert "NO FIRE HAZARD" on the line labeled "Extinguishing Media."

(e) Section V. Health Hazard Information

The "Health Hazard Data" should be a combined estimate of the hazard of the total product. This can be expressed as a TWA concentration, as a permissible exposure, or by some other indication of an acceptable standard. Other data are acceptable, such as lowest LD50 if multiple components are involved.

Under "Routes of Exposure," comments in each category should reflect the potential hazard from absorption by the route in question. Comments should

indicate the severity of the effect and the basis for the statement if possible. The basis might be animal studies, analogy with similar products, or human experiences. Comments such as "yes" or "possible" are not helpful. Typical comments might be:

> Skin Contact--single short contact, no adverse effects likely; prolonged or repeated contact, possibly mild irritation.
>
> Eye Contact--some pain and mild transient irritation; no corneal scarring.

"Emergency and First Aid Procedures" should be written in lay language and should primarily represent first-aid treatment that could be provided by paramedical personnel or individuals trained in first aid.

Information in the "Notes to Physician" section should include any special medical information which would be of assistance to an attending physician including required or recommended preplacement and periodic medical examinations, diagnostic procedures, and medical management of overexposed employees.

(f) Section VI. Reactivity Data

The comments in Section VI relate to safe storage and handling of hazardous, unstable substances. It is particularly important to highlight instability or incompatibility to common substances or circumstances, such as water, direct sunlight, steel or copper piping, acids, alkalies, etc. "Hazardous Decomposition Products" shall include those products released under fire conditions. It must also include dangerous products produced by aging, such as peroxides in the case of some ethers. Where applicable, shelf life should also be indicated.

(g) Section VII. Spill or Leak Procedures

Detailed procedures for cleanup and disposal should be listed with emphasis on precautions to be taken to protect employees assigned to cleanup detail. Specific neutralizing chemicals or procedures should be described in detail. Disposal methods should be explicit including proper labeling of containers holding residues and ultimate disposal methods such as "sanitary landfill" or "incineration." Warnings such as "comply with local, state, and Federal antipollution ordinances" are proper but not sufficient. Specific procedures shall be identified.

(h) Section VIII. Special Protection Information

Section VIII requires specific information. Statements such as "Yes," "No," or "If necessary" are not informative. Ventilation requirements should be specific as to type and preferred methods. Respirators shall be specified

as to type and NIOSH or MSHA (Mine Safety and Health Administration) approval class, ie, "Supplied air," "Organic vapor canister," etc. Protective equipment must be specified as to type and materials of construction.

(i) Section IX. Special Precautions

"Precautionary Statements" shall consist of the label statements selected for use on the container or placard. Additional information on any aspect of safety or health not covered in other sections should be inserted in Section IX. The lower block can contain references to published guides or in-house procedures for handling and storage. Department of Transportation markings and classifications and other freight, handling, or storage requirements and environmental controls can be noted.

(j) Signature and Filing

Finally, the name and address of the responsible person who completed the MSDS and the date of completion are entered. This will facilitate correction of errors and identify a source of additional information.

The MSDS shall be filed in a location readily accessible to employees exposed to the hazardous substance. The MSDS can be used as a training aid and basis for discussion during safety meetings and training of new employees. It should assist management by directing attention to the need for specific control engineering, work practices, and protective measures to ensure safe handling and use of the material. It will aid the safety and health staff in planning a safe and healthful work environment and in suggesting appropriate emergency procedures and sources of help in the event of harmful exposure of employees.

MATERIAL SAFETY DATA SHEET

I PRODUCT IDENTIFICATION	
MANUFACTURER'S NAME	REGULAR TELEPHONE NO EMERGENCY TELEPHONE NO
ADDRESS	
TRADE NAME	
SYNONYMS	

II HAZARDOUS INGREDIENTS		
MATERIAL OR COMPONENT	%	HAZARD DATA

III PHYSICAL DATA		
BOILING POINT, 760 MM HG		MELTING POINT
SPECIFIC GRAVITY (H_2O=1)		VAPOR PRESSURE
VAPOR DENSITY (AIR=1)		SOLUBILITY IN H_2O, % BY WT
% VOLATILES BY VOL		EVAPORATION RATE (BUTYL ACETATE=1)
APPEARANCE AND ODOR		

IV FIRE AND EXPLOSION DATA

FLASH POINT (TEST METHOD)		AUTOIGNITION TEMPERATURE			
FLAMMABLE LIMITS IN AIR, % BY VOL.		LOWER		UPPER	
EXTINGUISHING MEDIA					
SPECIAL FIRE FIGHTING PROCEDURES					
UNUSUAL FIRE AND EXPLOSION HAZARD					

V HEALTH HAZARD INFORMATION

HEALTH HAZARD DATA

ROUTES OF EXPOSURE

INHALATION

SKIN CONTACT

SKIN ABSORPTION

EYE CONTACT

INGESTION

EFFECTS OF OVEREXPOSURE

ACUTE OVEREXPOSURE

CHRONIC OVEREXPOSURE

EMERGENCY AND FIRST AID PROCEDURES

EYES

SKIN

INHALATION

INGESTION

NOTES TO PHYSICIAN

VI REACTIVITY DATA

CONDITIONS CONTRIBUTING TO INSTABILITY

INCOMPATIBILITY

HAZARDOUS DECOMPOSITION PRODUCTS

CONDITIONS CONTRIBUTING TO HAZARDOUS POLYMERIZATION

VII SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED

NEUTRALIZING CHEMICALS

WASTE DISPOSAL METHOD

VIII SPECIAL PROTECTION INFORMATION

VENTILATION REQUIREMENTS

SPECIFIC PERSONAL PROTECTIVE EQUIPMENT

RESPIRATORY (SPECIFY IN DETAIL)

EYE

GLOVES

OTHER CLOTHING AND EQUIPMENT

IX SPECIAL PRECAUTIONS

PRECAUTIONARY
STATEMENTS

OTHER HANDLING AND
STORAGE REQUIREMENTS

PREPARED BY _____

ADDRESS _____

DATE _____

XII. TABLES

TABLE XII-1

PHYSICAL AND CHEMICAL PROPERTIES OF o-TOLIDINE AND BENZIDINE

Property	o-Tolidine	Benzidine
Molecular formula	$(CH_3C_6H_3NH_2)_2$	$(C_6H_4NH_2)_2$
Formula weight	212.32	184.32
Appearance	White-to-brownish leaves or crystals, often used in paste or wet cake form	White or reddish crystalline powder
Melting point (base)	129-131 C	115-128 C
Solubility in water at 25 C		
Base	1.3 mg/ml	0.52 mg/ml
Dihydrochloride salt	76.7 mg/ml	61.7 mg/ml
Excitation wavelength		
Base	300 nm	295 nm
Dihydrochloride salt	310 nm	302 nm
Emission wavelength		
Base	384 nm	396 nm
Dihydrochloride salt	410 nm	410 nm
p-Value* with		
Hexane-acetonitrile	0.01	0.02
Hexane-80% acetone, 20% water	0.16	0.08
Hexane-dimethylformamide	0.00	0.00
Chloroform-water	1.0	1.0
Chloroform-60% methanol, 40% water	0.96	0.85
Chloroform-aqueous sodium hydroxide	1.0	1.0
Chloroform-aqueous hydrochloric acid	0.0	0.0
TLC Rf value with		
Chloroform	14	10
Chloroform-methanol (9/1, v/v)	80	70
Benzene	2	2
Benzene-methanol (9/1, v/v)	45	33

*Fractional amount partitioning into the nonpolar phase of an equal-volume, two-phase system.

Adapted from references 1,2,5,6

TABLE XII-2

OCCUPATIONS WITH POTENTIAL EXPOSURE TO o-TOLIDINE

Analytical chemistry workers
Artists
Chemical distributors
Dyemakers
Forest service chemists
Glucose diagnostic tape makers
Leather dye workers
Medical laboratory workers
Organic chemists
o Tolidine makers
Sanitarians
Sewage treatment plant workers
Swimming pool test kit makers
Swimming pool service personnel
Textile dryers
Toluene diisocyanate makers
Urethane curers
Waterworks attendants

Adapted from reference 4

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