U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES ■ Public Health Service
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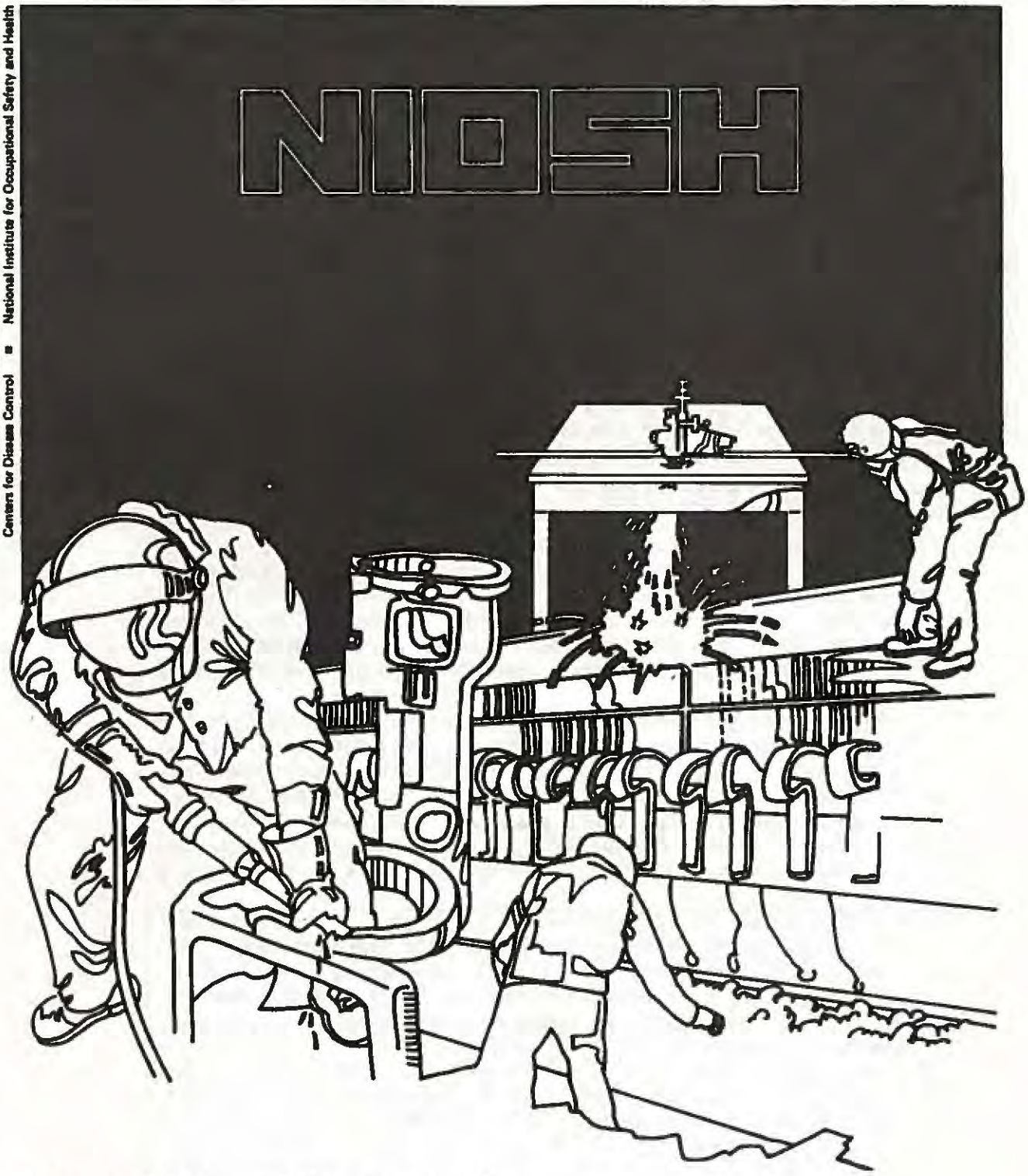

Health Hazard Evaluation Report

HETA 82-089-1213
IMMUNOLOGY BRANCH LABORATORY
CENTERS FOR DISEASE CONTROL
ATLANTA, GEORGIA

PREFACE

The Hazard Evaluations and Technical Assistance Branch of NIOSH conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employer or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

The Hazard Evaluations and Technical Assistance Branch also provides, upon request, medical, nursing, and industrial hygiene technical and consultative assistance (TA) to Federal, state, and local agencies; labor; industry and other groups or individuals to control occupational health hazards and to prevent related trauma and disease.

Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

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IMMUNOLOGY BRANCH LABORATORY
CENTERS FOR DISEASE CONTROL
ATLANTA, GEORGIA

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I. SUMMARY

On December 23, 1981, the National Institute for Occupational Safety and Health (NIOSH) received a request to investigate possible toxic environmental exposures for personnel working in the Immunology Branch (IB) laboratory, Center for Infectious Diseases, Centers for Disease Control (CDC), Atlanta, Georgia. Five of seven pregnancies of IB employees or of spouses of IB employees had ended in spontaneous first-trimester abortions within an 8 month period from May to December, 1981. In December, 1981, personnel from the Birth Defects Branch, Chronic Diseases Division, Center for Environmental Health (CEH), CDC, interviewed all 7 women concerned. On January 5, 1982, a NIOSH industrial hygienist conducted a walk-through survey of the laboratory. NIOSH reviewed potential mutagenic, teratogenic, and embryotoxic effects of 83 chemicals found in the lab and measured face velocities of laboratory hoods. On April 12-15, 1982, NIOSH collected personal and area environmental samples to determine exposures to airborne sodium azide and 2-mercaptoethanol. CEH collected urine samples to evaluate possible exposure and absorption of phenols (from use of a phenolic detergent, Amphyl™) and dimethyl sulfoxide (DMSO). These chemicals were selected for further study because most of the IB employees, and 4 of 5 women who spontaneously aborted, were potentially exposed.

Although many substances used in the laboratory were potentially mutagenic, teratogenic, or embryotoxic, no definite causal agent was identified. General laboratory ventilation was excellent. However, the exhaust face velocity on one laboratory hood, used for radioisotopes, was only half the NIOSH recommended rate. No exposures to the substances identified above were detected in the air samples or above the normal background mean in urine specimens.

This cluster of spontaneous abortions was an unexpected event for which we could not identify a definite causal agent or associated risk factor. The cluster may represent an "expected-unexpected" event, since one would expect that out of the many groups of people working together, a small number would have a cluster of some event, such as spontaneous abortion. However, we cannot exclude the possibility that employees' exposures to chemical substances or biologic agents in the laboratory increased their risk for spontaneous abortions. We recommend that CDC monitor future pregnancies of IB employees and of spouses of employees and consider instituting an ongoing registry of pregnancies of all CDC employees and their spouses to determine if they are at increased risk of having adverse reproductive outcomes. This registry would also serve as a model for other institutions and industries for use in evaluating the possible adverse reproductive effects of exposures in the workplace.

KEYWORDS: SIC 8922 (Noncommercial, Educational, Scientific, and Research Organizations), spontaneous abortions, miscarriage, laboratory workers, phenolic detergents, o-phenylphenol, CAS 90-43-7, p-tert- amylphenol, CAS 80-46-6, sodium azide, CAS 26628-22-8, DMSO, CAS 67-68-5, 2-mercaptoethanol, CAS 60-24-2

II. INTRODUCTION

On December 23, 1981, NIOSH received a request for technical assistance from the Chief, Birth Defects Branch (BDB), Chronic Diseases Division, Center for Environmental Health, Centers for Disease Control (CDC). On December 17, 1981, the BDB had been contacted by the Chief of the Immunology Branch (IB), Center for Infectious Diseases, CDC, concerning the occurrence of first-trimester abortions among employees and spouses of employees working in the IB labortories. In 8 months five of seven pregnancies had resulted in spontaneous abortions. Three of the women concerned were employees--the only employees who had been pregnant. In the same period spouses of male employees had two miscarriages and two normal births. The BDB was proceding with an investigation of this unexpected cluster of spontaneous abortions and asked NIOSH for industrial hygiene support to evaluate the potential for exposures to chemical substances or biological agents handled by IB personnel.

During the last two weeks of December, the BDB investigator had initially questioned all IB employees about their reproductive history and had completed interviews with all female employees (3 females) or spouses of male employees (4 spouses) who had been pregnant or had tried to become pregnant in 1981. A list of the chemicals used in the IB laboratory was compiled and forwarded to NIOSH for review.

On January 5, 1982, NIOSH conducted a walk-through survey of the laboratory facilities with the Chief of the Immunology Branch and the BDB investigator. The procedures and equipment used for handling chemicals and biological samples were discussed. Methods for storage of chemicals were determined, and exhaust flow rates on laboratory fume hoods were measured. A preliminary report summarizing the initial NIOSH evaluation was forwarded to BDB on January 18, 1982.

On January 20, 1982, a meeting was held in the office of the Director, Chronic Diseases Division, to discuss preliminary results of the initial investigations. At that time no specific environmental exposures or associated risk factors were identified which might account for the unexpected cluster of spontaneous abortions that had been reported. However, there were many potentially mutagenic or embryotoxic substances used in the laboratories.

It was subsequently learned that two of the five women who had aborted in 1981 had aborted again in 1982, a spouse of a male employee in January 1982 and a female employee in March 1982. These were the 6th and 7th spontaneous abortions in 10 pregnancies since the beginning of 1981. As a result, NIOSH and the BDB agreed to conduct a more extensive investigation concerning the use of several chemicals to which almost every IB laboratory worker, including 4 of the 5 women who spontaneously aborted, was potentially exposed. On April 12-15, NIOSH and BDB collected air and urine samples in order to evaluate possible exposures to these chemicals.

A report of the original analysis of the first 5 spontaneous abortions (which occurred in 1981) was issued by CDC on June 11, 1982 (EPI-82-13-2). This report also contained a summary of initial NIOSH findings and recommendations as provided to BDB on January 18, 1982.

III. BACKGROUND

The Immunology Branch is located on the first floor of Building 1 at 1600 Clifton Road, N.E., Atlanta, Georgia. Activities of the laboratory include immunological research and service work involving immune response in animal models, hybridoma tissue culture, HLA typing, human immune response evaluation, and testing for auto-immune diseases. Before CDC was reorganized in 1981, the Branch was not a separate organizational entity at the Clifton Road facility, but in the summer of 1981 the staff began to move into new quarters, a restricted area being vacated by the Clinical Chemistry Division. Most of the move was completed by October 1981. Since that time the space, consisting of 8 lab rooms, one storage room for freezers, and 2 office spaces, has been occupied only by Immunology Branch personnel, except for one person who occupies half of an office.

The 21 employees (14 women and 7 men) have individually assigned duties and workplaces, but they all work in other areas as well. Substances in the laboratory include organic and inorganic chemicals; cytochemical stains; radioactive isotopes; killed bacterial and viral antigens; living bacteria and viruses; human blood, urine, and lymph nodes; and animal blood and tissues. No major accidents have occurred within the past year, and the only physical malfunction was a blocked sewer which flooded one room on November 20, 1981.

IV. EVALUATION DESIGN AND METHODS

A. Epidemiology Study

After initially questioning all branch employees about their reproductive history, BDB interviewed all female employees or spouses of male employees who had been pregnant or who had tried to become pregnant in 1981, using the Metropolitan Atlanta Congenital Defects Program questionnaire. BDB also interviewed a woman who delivered a normal child in October 1981, two months after her husband ended his employment with the laboratory. One employee was excluded from the analysis because she was 4 months pregnant when she joined the branch in October 1981. BDB defined a case as a spontaneous abortion within the first trimester of a pregnancy which had been confirmed by a pregnancy test. Data from a life-table analysis of spontaneous abortions, adjusted for maternal age, was used to calculate the expected number of miscarriages.

Of the original five women who had spontaneous abortions, two became pregnant again in 1982. These two later pregnancies were not included in the analysis because at the time of the investigation they were not past the first trimester.

In only one case were the products of conception available to CDC for cytogenic analysis.

B. Initial Environmental Survey

During the initial walk-through survey on January 5, 1982, NIOSH evaluated the exhaust efficiency of the three laboratory "fume" hoods by measuring the hood face velocities with a Kurz, Model 441, electronic air velocity meter. The average of six measurements taken at the face of the hood opening, with the window sash fully open, was determined for each of the three hoods and compared to NIOSH ventilation guidelines.

Over 180 different chemical substances and biological agents were used in the IB laboratory, making characterization of all environmental exposures exceedingly complex. In order to focus the investigation on only those substances classified as mutagens, teratogens, or embryotoxins, a computer search of the NIOSH Registry of Toxic Effects of Chemical Substances (RTECS) was conducted for 83 different chemicals. In this way all chemicals with known or suspected potential to cause adverse reproductive outcomes could be given special consideration for a subsequent environmental investigation.

The RTECS search identified 39 chemicals which were reported to have mutagenic, teratogenic, or embryotoxic effects (Table 1). The 5 women who had spontaneous abortions in 1981 had minimal or no exposure to 31 of the 39 chemicals. Five of the remaining 8 chemicals, sodium chloride, hydrochloric acid, methyl alcohol, ethyl alcohol, and streptomycin, were considered unlikely candidates because of the doses required to produce effects. The three other chemicals, sodium azide, 2-mercaptoethanol (2-ME), and dimethyl sulfoxide (DMSO) were used by nearly every employee working in the IB.

NIOSH also learned, through supplemental interviews, that many of the employees had been using a disinfecting detergent containing phenols (15% o-phenylphenol and 6.3% p-tert-amylphenol as a concentrated solution 50 to 100 times the strength recommended by the manufacturer for general disinfecting. The NIOSH RTECS classifies o-phenylphenol as mutagenic. Use of phenolic detergents in hospital nurseries has also been suggested as the causal agent in three episodes of neonatal hyperbilirubinemia[1,2].

Based on the above findings, a further study of the potential exposures to phenols, 2-ME, DMSO, and sodium azide was undertaken by NIOSH and BDB.

C. Follow-up Environmental/Biological Monitoring

The follow-up study conducted by NIOSH and BDB April 12-14, 1982 involved investigation of work practices, biological monitoring of personnel, and collection of environmental air samples.

Laboratory procedures performed by IB personnel believed to have the highest potential for exposure to the chemicals of concern were identified by the Branch Chief and discussed with IB personnel during interviews conducted by the NIOSH project officer on March 16, 1982. Listed below are the substances for which biological or environmental samples were collected, as well as the sampling and analytical methods used for their quantitation.

SODIUM AZIDE--Airborne sodium azide released during the preparation and use of a 0.1% or 0.2% sodium azide solution was monitored by collecting both personal and area samples on 37 mm PVC filters mounted in 2 piece plastic cassettes. Personal exposure samples were collected by attaching the cassette to the lab coat worn by the individual being monitored. A known volume of air was pulled through the filter using a battery powered pump operated at a flow rate of 2 liters per minute (LPM). The collected azide was removed from the filters with distilled water and then analyzed for total azides by ion chromatography using procedures recently developed by the NIOSH Inorganic Methods Development Section, Measurements Research Branch .

2-MERCAPTOETHANOL--Exposures to 2-ME were determined by collecting personal and area samples on 2-section silica gel tubes (130 mg and 65 mg of silica gel). Using battery powered air sampling pumps, a known volume of air was pulled through the tube at a flow rate of 50 cc/min. A maximum sample volume of 12 liters (L) was recommended. The airborne concentration of 2-ME was measured during the preparation of an acrylamide gel electrofreeze, involving the use of a 0.1% or 0.2% solution of 2-ME. The silica gel tube was attached to the collar of the lab coat when collecting a personal sample. The 2-ME which was collected on the silica gel was desorbed with methanol and analyzed by gas chromatography utilizing a flame photometric detector (GC/FPD) with a sulfur filter. The samples were analyzed by a NIOSH contract laboratory (Arthur D. Little, Inc.) using a modified version of the method developed by Chaudhary[3].

DIMETHYL SULFOXIDE--DMSO is readily absorbed through the skin, and its vapor pressure is only 0.37 mm at 20° C. In order to detect DMSO absorption resulting from exposure, collecting of air samples was rejected in favor of collecting urine specimens. Urine specimens were collected before, during, and after 2 employees prepared biological tissue samples for freezing. A solution of 20% DMSO and RPMI (a cell nutriant media) was used to protect the integrity of cells when freezing and thawing biological tissue samples. Urine specimens from 2 non-exposed IB employees were

collected as controls. The urine specimens were extracted with chloroform and analyzed by GC/FPD for DMSO and its metabolite dimethyl sulfone $DMSO_2$ at the NIOSH contract laboratory (Arthur D. Little Inc.) using a modified version of a method developed by Ogata et.al.[4]

PHENOLS--The urinary phenols for two employees using a concentrated solution of Amphyl™ (the phenolic disinfecting detergent) were monitored by collecting urine specimens prior to, 2 hours after, and 4 hours after use of Amphyl detergent. Since it had been common practice by many IB employees to use Amphyl in solutions of greater concentration than recommended by the manufacturer, the exposed individuals were instructed to use this stronger concentration in order to monitor the effect on urinary phenols. Monday morning urine specimens were collected from two other non-exposed employees as controls. The samples were analyzed by the CDC, Clinical Chemistry Division, CEH by hydrolyzing 5 mL samples with acids, extracting with ethyl acetate and quantitating by gas chromatography using a flame ionization detector. The sensitivity of the method was reported to be 0.5 ug phenol/5mL of urine.

V. EVALUATION CRITERIA

A. Environmental Criteria

The primary sources of environmental evaluation criteria selected for this study were: 1) NIOSH criteria documents and recommendations, 2) the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLV's),[5] and 3) the U.S. Department of Labor (OSHA) federal occupational health standards.[6] For those compounds with established occupational exposure limits, the various criteria proposed by OSHA, ACGIH, and NIOSH for airborne concentrations of the chemical substances measured in this evaluation are listed in Table 2 of this report. In most cases, the occupational exposure limits are the same from each reference. In those cases where there is a difference, the NIOSH recommended standard or the most stringent value is the criteria used for the purposes of this evaluation. Table 2 also lists the major health effects or sites of action of those chemcals.

These criteria are intended to represent the maximum airborne concentrations of substances to which most workers may be exposed for eight hours a day, 40 hours per week (or other durations where indicated) without adverse health effects. The time-weighted average (TWA) exposure refers to the average concentration during a normal 8-hour workday. The Short-Term Exposure Limit (STEL) is the maximum allowable concentration, or ceiling, to which workers can be exposed during a period of up to 15 minutes, provided that no more than four excursions per day are permitted, with at least 60 minutes between excursion periods. Because of wide variation in individual susceptibility, a small percentage of workers may

experience discomfort from some substances at concentrations at or below the recommended criteria.[5] A smaller percentage may be more seriously affected by aggravation of a pre-existing condition or by a hypersensitivity reaction.

These currently available criteria as discussed above cover only a small number of potentially hazardous chemicals. Much of the data used to establish these criteria are based only on animal studies or exposures to healthy male workers. Little information is available on how to apply this data to the special circumstances of the pregnant worker and her fetus or to the possible adverse affects on the spouse of the male worker. The NIOSH Registry of Toxic Effects of Chemical Substances (RTECS) identifies many substances which may be harmful and provides information on potential carcinogenesis, mutagenesis, and teratogenesis. However, there is little or no information about the effects of mixtures or combinations of substances or the effects these mixtures would have on reproduction.

At the present time there are no established exposure criteria for dimethyl sulfoxide, 2-mercaptoethanol, or o-phenylphenol. A brief review of the toxicity for these compounds and the compounds listed on Table 2 are presented below.

DIMETHYL SULFOXIDE or DMSO has a low order of oral toxicity, with oral LD 50's ranging from 23.3 g/kg for rats to above 4 g/kg for primates.[7] Rhesus monkeys given intravenous injections of a 40% DMSO solution at a dose of 3 g/kg for 9 days showed no significant changes in blood chemistry, hematology, urine, and ocular, neurological, and cardiovascular systems.[8] The lethal airborne concentration for rats is reported to be 1600 mg/M^3 for a four hour exposure. DMSO can irritate the skin causing redness, itching and sometimes scaling with occasional allergic dermatitis in sensitive individuals. DMSO is readily absorbed through the skin. Human volunteers have reported nausea, vomiting, cramps, chills, and drowsiness following skin absorption.[9] Humans exposed to DMSO experience a garlic-like odor on the breath, believed to be caused by the formation of dimethyl sulfide. Dimethyl sulfide and dimethyl sulfone are the major metabolites of DMSO produced in rats.[7] DMSO is also reported to increase the permeability of the skin to other toxic chemicals. Based on animal studies, DMSO does have teratogenic potential. For example, DMSO has been found to alter the permeability of cell membranes and other membranes in chick embryos, producing swellings and blisters from the resulting osmolar imbalance.[10] DMSO has been classified as a mutagen and teratogen in the NIOSH RTECS resulting from the reproductive, developmental, and mutagenic effects observed in animal studies and cellular test systems reported in the literature.

2-MERCAPTOETHANOL is classified by the NIOSH RTECS as a primary irritant and a mutagen. The oral LD 50 for rats was reported to be 300 mg/kg. 2-ME has also caused eye and skin irritation in

studies with rabbits. Only one study, by Humangentik, reporting mutagenic effects on human leucocyte somatic cells at 100uL/L, was cited in RTECS. No threshold limit value has been established for this compound by the ACGIH.

SODIUM AZIDE (NaN_3) hydrolyzes to form hydrazoic acid (HN_3), the vapor of which may be present when NaN_3 solutions are prepared or handled. Sodium azide is rapidly absorbed from the gastrointestinal tract, from an injection site, from the skin, and from the respiratory tract.[7] The acute effects in humans from exposure to HN_3 vapor are eye irritations, bronchitis, headache, a fall in blood pressure, and weakness and collapse. Similar hypotensive effects have been produced in laboratory animals using NaN_3. Studies of accidental exposures to azides have compared its toxicity to sodium cyanide. Azide has been shown to form strong complexes with the blood hemoglobin, blocking oxygen transport.[11] Sodium azide has been reported to induce mutations in bacteria and barley seeds and has affected the genetic material of soybeans.[7] The ACGIH TLV's for HN_3 and NaN_3 at 0.1 ppm and 0.3 ppm respectively, are recommended to provide a reasonable factor of safety for prevention of discomfort and headache, and to protect against significant lowering of blood pressure.[11]

PHENOLS of the type found in disinfecting detergents such as Amphyl have a low order of oral toxicity in rats e.g. 2.7 g/kg for o-phenylphenol and 1.8 g/kg for p-tert-amylphenol. These compounds are potentially irritating to the skin and eyes as determined from studies with laboratory rabbits[12]. However tests of 0.5% o-phenylphenol solutions in sesame oil and 0.1% aqueous solutions of its sodium salt failed to cause either primary skin irritation or skin sensitization among 200 adult human subjects.[7] There is evidence to suggest however that infants and perhaps the fetus of a female exposed to phenolic detergents may be at greater risk. Use of phenolic detergents in hospital nurseries has been suggested as the causal agent in at least three episodes of neonatal hyperbilirubinema[1,2]. Whether exposure to phenolic detergents would affect the risk of spontaneous abortions is at this time purely speculative. Although o-phenylphenol is classified in the RTECS as a mutagen, both negative and positive findings have been reported from animals and microbial studies.[12,13] There were no reports of mutagenic effects for p-tert-amylphenol.

B. Laboratory Hood/Ventilation Criteria

According to the NIOSH Recommended Industrial Ventilation Guidelines Manual,[15] the hood applications and minimum exhaust velocity requirements for laboratory hoods based on contaminant class are as follows:

Contaminant Class	Face Velocity	
	Minimum	Average
I - Substances with exposure limits of 100 ppm and above. (e.g. ethanol)	50 fpm	100 fpm
II - Substances with exposure limits of 1 ppm and above (up to 100 ppm) (e.g. phenol)	75 fpm	100 fpm
III - Substances with exposure limits below 1 ppm; also radio-isotopes, carcinogens, and cancer suspect agents. (e.g. benzene)	125 fpm	150 fpm

Note: fpm = feet per minute

VI. RESULTS

A. Epidemiology Study

Five miscarriages occurred in seven pregnancies over an 8-month period; from life-table projections only 1.3 would be expected ($p = .01$). The relative risk was 3.9, with 95% confidence limits of 1.25 and 9.01.

Number of Age-Adjusted Expected Spontaneous Abortions Compared with Observed

Maternal Age (Years)	Number Pregnancies	P*	No. Spontaneous Abortions	
			Expected	Observed
20-24	1	.137	.137	1
25-29	2	.138	.276	1
30-34	1	.138	.138	1
35+	3	.248	.744	2
Totals	7	--	1.295	5

P = 0.01 (Poisson)

*Age-specific probability of first-trimester spontaneous abortion.

Adapted from Harlap et al., 1980 (ref. 14)

Cytogenetic analysis showed no abnormality in the one product of conception examined.

Analysis of the seven pregnancies by date of conception and by date of termination shows that in all five pregnancies resulting in adverse outcomes, conception occurred after January 1981. Three pregnancies in 1980 ended in live births. Two of the women who had 1980 pregnancies suffered spontaneous abortions in 1981.

The seven pregnant women are highly educated, fully employed, and above the mean maternal age at birth for the United States (Table 3). They had no exposure to X-rays and only minimal use of alcohol, medications, and cigarettes. They had no prior problems in becoming pregnant; only one woman had a prior miscarriage, and two women had a family history of miscarriage. All five women who aborted had pets reported to be healthy. Three of these five women reported having had stressful events during pregnancy.

The number of spontaneous first-trimester abortions which occurred in employees or spouses of employees in the Immunology Branch during 1981 was significantly higher than expected.[14] Although there was a clustering of events in 1981, the lack of pregnancies before that year makes this increase difficult to evaluate. In all five of the cases in 1981 the women conceived after January. No common associations are known to have occurred at or after this time to account for this finding.

B. Initial Environmental Findings

General ventilation in the IB laboratory was considered excellent. All laboratory facilities in this building are ventilated using a "one-pass" system which provides 100% outdoor air (no air is recirculated) at all times. One of three laboratory "fume hoods" did not meet NIOSH guidelines for Class III substances (e.g. carcinogens, radioisotopes).[15] This hood was used for radioisotope work with I_{125} and had a face velocity of less than half the NIOSH recommended flow rate. However, radiation surveys conducted by the CDC Office of Bio-safety on June 18, 1981 and November 11, 1981 detected only normal background radiation in all areas where I_{125} or I_{125}-labeled proteins were handled.

Laboratory Hood Face Velocity
(hood window sash fully open)

Location	Average Face Velocity (feet per minute)	Recommended Velocity (feet per minute)
Room 1212	275	100
Room 1226	112	100
Room 1211	70	150

The primary chemical storage area had shelves which were open on the sides and backs allowing bottles of chemicals to be easily knocked off and broken. Some incompatible chemicals were stored near each other instead of being separated. New shelving was provided by CDC following our initial survey.

The results of the RTECS search for 83 chemicals used by the IB laboratory are presented in Table 1. No information was available on 14 chemicals. Reports on 39 chemicals showed mutagenicity, teratogenicity, or embryotoxicity. Inhaled vapors of five of

these suspect chemicals: acetic acid, benzene, formaldehyde, and toluene, and ethylene glycol methyl ether (methyl cellosolve), are reported in RTECS or other sources (see Table 3) to be mutagenic or teratogenic in laboratory animal studies. All five women who aborted were potentially exposed to 3 of these 39 suspect chemicals: sodium chloride, hydrochloric acid, and sodium azide. Four were potentially exposed to 5 more of these 39 chemicals: methyl alcohol, ethyl alcohol, streptomycin, 2-mercapto-ethanol, and dimethyl sulfoxide (DMSO).

C. Follow-up Environmental/Biological Monitoring

1. Air Sampling Results

No vapors of 2-ME were detected in personal exposure samples collected during the follow-up NIOSH survey. Only a trace amount of 2-ME, just above the analytical detection limit, was found inside the laboratory hood were a 1 molar (apx. 0.7%) solution of 2-ME was being mixed with cell media. Only 0.05 ppm was detected in an area sample located in the immediate vicinity of the acrylamide gel electrofeeze procedure. No airborne azides were detected in any of the samples taken during the preparation and use of the sodium azide solutions. The results from all samples are presented in Table 4.

2. Urine Sample Results

As presented in Table 5, none of the 8 urine samples taken from IB personnel contained detectable amounts (limit of detection = 4 ug/mL) of DMSO. However, all 8 samples were found to contain $DMSO_2$ at an average level of 4.6 ug/mL with a standard deviation of 2.1 ug/mL (46%). Five control samples taken from Arthur D. Little Inc. personnel who were not exposed to DMSO also contained $DMSO_2$ at an average concentration of 4.9 ug/mL with a standard deviation of +/- 2.9 ug/mL (59%). These results were found to be consistent with the literature which indicates that normal human urine contains an average of 6.2 mg $DMSO_2$/24 hours/person which is approximately equivalent to 4.4 ug/mL for a person with a normal urine output of 1400 mL per day.16

Urinary phenol levels for IB personnel who had used stronger than recommended solutions of phenol containing Amphyl detergent ranged from 4.2 to 7.2 ug/mL. This is within the normal background range for humans (11.56 +/- 10.86 ug/mL). The highest level detected, 15.6 ug/mL, was one of two control (non-exposed) samples (Table 5).

VII DISCUSSION

Spontaneous abortion is a frequent event during the first or second trimester of pregnancy. The percentage of abortions detected depends upon when the pregnancy is recognized. Estimates are that up to 78% of all fertilized ova do not survive to full

term.[17] One prospective study reported a 43% fetal loss after implantation[18]; in 78% of these instances the loss was unrecognized clinically. These early pregnancies were detected only by sensitive pregnancy testing before the first missed menstrual period. Of pregnancies which are clinically recognized at 5 weeks of gestation, 15%-25% result in spontaneous abortion during the first or second trimester.[14,19-22] Of the fetuses aborted at less than 16 weeks' gestation, at least 50% are chromosomally abnormal.[23] About 50% of the chromosomally normal aborted fetuses are morphologically abnormal, leaving about 25% that are apparently normal. The timing and type of insult determine the kind of spontaneous abortion which occurs. An increase in the number of spontaneous abortions in any of these groups may not result in an increase in the overall rate of spontaneous abortions.

In most instances of spontaneous abortion the exact cause is not known. Various factors are known or alleged to increase the risk of spontaneous abortions, but these factors account for only a small proportion of the total. Fever, influenza, genital infections with Mycoplasma or herpes simplex, high-dose irradiation, and medications (goitrogens and folic acid antagonists) have reportedly increased the risk in exposed women. Maternal age over 35 years is associated with an almost twofold risk.[14] Spontaneous and induced abortions are associated with an increased risk of spontaneous abortions in later pregnancies,[14,24-26] although some studies suggest that induced abortion does not increase future risk.[27]. Maternal alcohol consumption[28,29] and cigarette smoking[30] are generally accepted as increased risk factors for spontaneous abortions, but one study showed that women with unwanted pregnancies tended to smoke more, and this explained almost all of the asssociation between smoking and spontaneous abortion.[31]

Increased exposure to known risk factors did not account for this cluster of spontaneous abortions. The women had a higher prevalence of spontaneous abortion than would be expected for their age. Other known or suspected causes such as alcohol, tobacco, X-rays, induced abortion, or prior miscarriage were not present in a majority of cases. Specific hypotheses were not tested because of the small number of cases and potential controls.

Occupational exposures causing spontaneous abortions are poorly documented with the possible exception of maternal exposure to anesthetic gases.[32]. Many reports have shown possible links between maternal occupational exposures and spontaneous abortions. The list of agents includes industrial and hospital laboratory chemicals[33-35] and solder fumes in the metal industry,[36] but the evidence is not strong for any of them. Paternal exposure to anesthetic gases,[37] chloroprene,[38] and vinyl chloride monomer[39] is reported to increase the risk of spontaneous abortion in spouses, but the evidence is weak. Other potential risk factors such as stress and physical exertion[40] are difficult to study, and their role in spontaneous abortion is undefined.

There is no indication that exposures to the chemicals used in the IB laboratory were sufficient to cause or increase the risk of spontaneous abortions among female personnel or spouses of male personnel. Of the chemicals used by most of the women studied, no significant exposures were detected. The one poorly operating laboratory hood was not used by any of the women who miscarried after the IB lab was moved to its current location. NIOSH found no history of laboratory contamination from the small amount of radioisotopes used by IB personnel. The improper storage of chemicals was also not a factor because no accidental spills or exposures had resulted. There was no clear evidence that the physical environment contributed to this cluster of spontaneous abortions.

The NIOSH RTECS search identified many substances which are potentitally mutagenic, teratogenic, and embryotoxic, based mostly on tests with laboratory animals or microbial test systems. How these reported effects might relate to human exposure is largely unknown. Sometimes extremely large doses are needed to produce adverse effects in animals. There is little or no information about the effects of mixtures or combinations of substances or the effects these mixtures would have on reproduction. Such combinations could be encountered at work, at home, or from use of medications. In this study, the five women who had spontaneously aborted had minimal or no occupational exposure to most of the chemicals identified by the NIOSH RTECS search.

VIII. CONCLUSIONS

This cluster of spontaneous abortions was an unexpected event for which we could not identify a definite causal agent or associated risk factor. The cluster may represent an "expected-unexpected" event, since one would expect that out of the many groups of people working together, a small number would have a cluster of some event, such as spontaneous abortion. However, based on the limitations of current knowledge with respect to reproductive effects of environmental exposures to chemical or biological substances or combinations of substances, we cannot exclude the possibility that employees' exposures in the laboratory increased their risk for spontaneous abortions.

IX. RECOMMENDATIONS

1. All laboratory procedures should be performed under laboratory hoods or biological hoods whenever possible. All hoods should provide for exhaust face velocities as recommended by NIOSH guidelines. Simply lowering the window sash to increase flow rates is not recommended.

2. IB personnel should develop techniques which will minimize direct contact with any chemical or biological agent. Personnel should realize that most solvents and many organic compounds will quickly penetrate laytex gloves.

3. CDC should continue to observe current and future pregancies of employees and spouses of employees in the IB laboratory.

4. CDC should establish a surveillance system on reproductive health for all CDC employees and their spouses to determine if laboratory employees or other job catagories have an increased risk of miscarriage.

X. AUTHORSHIP AND ACKNOWLEDGEMENTS

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	Staff Toxicology Branch Clinical Chemistry Div. Center for Envir. Health

XI. DISTRIBUTION AND AVAILABILITY

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Copies of this report have been sent to:

1. Chief, Immunology Branch, CID, CDC
2. Chief, Birth Defects Branch, CDD, CIH, CDC
3. U.S. Department of Labor, OSHA, Region IV
4. TEC/FAP, OSHA, Region IV
5. NIOSH Region IV
6. Appropriate Agencies of The State of Georgia

For the purpose of informing the approximately 21 "affected employees", the employer will promptly "post" this report for a period of thirty (30) calendar days in a prominent place(s) near where the affected employees work.

XII. REFERENCES

1. Wysowski DK, Flynt JW Jr., Goldfield M, Altman R, Davis AT. Epidemic neonatal hyperbilirubinema and use of phenolic disinfectant detergent. Pediatrics; 61(2):156-170, 1978.

2. Doan HM, Keith L, Shennan AT. Phenol and neonatal jaundice. Pediatrics; 64(3):324-325, 1979.

3. Choudhary G. Gas-liquid chromatographic determination of 2-mercaptoethanol. Journal of Chromatography; 200:211-215, 1980.

4. Ogata M, Fujii T. Quantitative determination of urinary dimethyl sulfoxide and dimethyl sulfone by the gas chromatograph equipped with a flame photometric detector. Industrial Health; 17(2):73-78, 1979.

5. American Conference of Governmental Industrial Hygienists (ACGIH). Threshold limit values for chemical substances and physical agents in the workroom environment with intended changes for 1981. Cincinnati, Ohio: ACGIH, 1981.

6. Occupational Safety and Health Administration (OSHA). OSHA safety and health standards. 29 CFR 1910.1000. Occupational Safety and Health Administration, revised 1980.

7. Patty FA. Patty's industrial hygiene and toxicology. Vol 2A--toxicology, 3rd revised ed. New York: John Wiley & Sons, 1981.

8. de la Torre JC, Surgeon JW, Drnest T, Wollmann R. Subacute toxicity of intravenous dimethyl sulfoxide in rhesus monkeys. J Toxicology Environmental Health; 7(1):49-57, 1981.

9. Stecher PG, Windholz M, Leahy D, eds. The Merck Index: an encyclopedia of chemicals and drugs. 8th ed. Rahway, N.J.: Merck & Company, Inc., 1968.

10. Doull J, Klaassen C, Amdur MO, eds. Casarett and Doull's toxicology: the basic science of poisons. 2nd ed. New York: Macmillan Publishing Company, Inc., 1980.

11. American Conference of Industrial Hygienists. Documentation of the threshold limit values. 4th. ed., with supplemental documentation for 1981. Cincinnati, Ohio: ACGIH, 1980.

12. Lewis RJ, Rodger T, eds. Registry of toxic effects of chemical substances: from National Library of Medicine Data Base. National Institute for Occupational Safety and Health, 1982.

13. Toxicology Data Bank: from National Library of Medicine Data Base.

14. Harlap S, Shiono PH, Ramcharan S. A life table of spontaneous abortions and the effects of age, parity, and other variables. In: Porter IH, Hook EB, eds. Human embryonic and fetal death. New York: Academic Press, 1980;145-58.

15. The National Institute for Occupational Safety and Health. Recommended industrial ventilation guidelines. Cincinnati, Ohio: National Institute for Occupational Safety and Health, 1976. (DHEW (NIOSH) Publication No. 76-162).

16. Williams KIH, Burstein S, Layne D. Arch., Biochemical Biophys.; 113: 251, 1966

17. Roberts CJ, Lowe CR. Where have all the conceptions gone? Lancet 1975;1:498-9.

18. Miller JF, Williamson E, Glue J, Gordon YB, Grudzinskas JG, Sykes A. Fetal loss after implantation: a prospective study. Lancet 1980;2:554-6.

19. French FE, Bierman JM. Probabilities of fetal mortality. Public Health Rep 1962;77:835-45.

20. Shapiro S, Jones EW, Densen PM. A life table of pregnancy terminations and correlates of fetal loss. Milbank Memorial Fund Quarterly 1962;40:9-45.

21. Warburton D, Fraser FC. Spontaneous abortion risks in man: data from reproductive histories collected in a medical genetics unit. Hum Genet 1964;16:1-25.

22. Wilcox AJ, Treloar AE, Sandler DP. Spontaneous abortion over time: comparing occurrence in two cohorts of women a generation apart. Am J Epidemiol 1981;114:548-53.

23. Stein Z, Susser M, Warburton D, Wittes J, Kline J. Spontaneous abortion as a screening device: the effect of fetal survival on the incidence of birth defects. Am J Epidemiol 1975;102:275-90.

24. Harlap S, Shiono PH, Ramcharan S, Berendes H, Pellegrin F. A prospective study of spontaneous fetal losses after induced abortions. N Engl J Med 1979;301:677-81.

25. Levin AA, Schoenbaum SC, Monson RR, Stubblefield PG, Ryan KJ. Association of induced abortion with subsequent pregnancy loss. JAMA 1980;243:2495-9.

26. World Health Organization Task Force on Sequelae of Abortion. Gestation, birth-weight and spontaneous abortion in pregnancy after induced abortion. Lancet 1979;1:142-145.

27. Kline J, Stein Z, Susser M, Warburton D. Induced abortion and spontaneous abortion: no connection? Am J Epidemiol 1978;107:290-8.

28. Harlap S, Shiono PH. Alcohol, smoking, and incidence of spontaneous abortions in the first and second trimester. Lancet 1980;2:173-6.

29. Kline J, Shrout P, Stein Z, Susser M, Warburton D. Drinking during pregnancy and spontaneous abortion. Lancet 1980;2:176-80.

30. Himmelberger DU, Brown BW, Cohen EN. Cigarette smoking during pregnancy and the occurrence of spontaneous abortion and congenital abnormality. Am J Epidemiol 1978;108:470-9.

31. Kullander S, Kallen B. A prospective study of smoking and pregnancy. Acta Obstet Gynec Scand 1971;50:83-94.

32. Cohen EN, Bellville JW, Brown BW. Anesthesia, pregnancy and miscarriage: a study of operating room nurses and anaesthetics. Anesthesiology 1971;35:343-7.

33. Hansson E, Jansa S, Wande H, Kallen B, Ostlund E. Pregnancy outcome for women working in laboratories in some of the pharmaceutical industries in Sweden. Scand J Work Environ Health 1980;6:131-4.

34. Hemminki K, Franssila E, Vainio H. Spontaneous abortions among female chemical workers in Finland. Int Arch Occup Environ Health 1980;45:123-6.

35. Strandberg M, Sandeack K, Axelson O, Sundell L. Spontaneous abortions among women in hospital laboratory (letter). Lancet 1978;1:384-5.

36. Hemminki K, Niemi M-L, Koskinen K, Vainio H. Spontaneous abortions among women employed in the metal industry in Finland. Int Arch Occup Environ Health 1980;47:53-60.

37. Askrog V, Harvald B. Teratogen effekt af inhalations anaestetika. Nord Med 1970;16:498-500.

38. Sanotsky IV. Aspects of the toxicology of chloroprene: immediate and long-term effects. Environ Health Perspect 1976;17:85-93.

39. Infante PF, Wagoner JK, McMichael AJ, Waxweiler RJ, Falk H. Genetic risks of vinyl chloride. Lancet 1976;1:734-5.

40. Goldman AS. Critical periods of prenatal toxic insults. In: Schwarz RH, Yaffe SJ, eds. Drug and chemical risks to the fetus and newborn. New York: Alan R. Liss, 1980:9-31.

Mutagenic, Teratogenic, and Embryotoxic Effects of Chemicals Used by the Immuniology Branch Laboratory as Classified by the NIOSH Registry of Toxic Effects of Chemical Substances (RTECS)

HETA 82-089

Chemical Name	Mutagen	Teratogen	Embryotoxic	Cases using*	
(Common Chemicals)				females	males
sodium chloride		x	x	3	2
sodium phosphate				2	2
ammonium sulfate				1	1
ammonium carbonate				0	0
barbital	x			0	0
boric acid	x			1	1
EDTA	x	x	x	1	2
glycine				2	2
THAM				0	2
sucrose		x		0	1
trichloracetic acid	x			1	1
urea	x			0	0
(acids, bases, solvents)					
hydrochloric acid	x			3	2
sodium hydroxide				3	1
acetic acid (1)	x			1	2
pyridine	x			0	1
xylene		x		1	0
acetone				1	2
methyl alcohol		x		2	2
ethyl alcohol	x	x	x	2	2
formamide		x	x	0	1
ethylene glycol	x			2	1
ethylene glycol methyl ether (5)				1	1
sulfuric acid				1	1
benzene (2)	x	x	x	0	0
formaldehyde (3)	x	x		1	1
(less common chemicals)					
ammonium sulfide				0	0
aluminum sulfate				0	0
copper sulfate	x	x		1	1
sodium tartrate				1	1
dextran				2	2
glutaraldehyde	x			2	0
hexadecyltrimethyl amm. brom.*					
silver chloride*					
PEG	x	x	x	2	1
phenol	x			2	0
potassium iodide	x	x		0	0
sodium iodide				0	0
potassium oxylate*					
sodium azide	x			3	2

* Not listed in RTECS

Chemical Name	Mutagen	Teratogen	Embryotoxic	Cases Using*	
(less common chemicals cont.)					
sodium thiocyanate				0	1
d-sorbitol				0	0
tannic acid				0	0
palmityl chloride*					
pristane				2	0
sodium chromate	x			1	1
diethanolamine				1	0
sodium deoxycholate				0	0
PMA	x			1	0
L-cysteine	x			0	1
lanthanium chloride				0	0
imidazole	x			0	0
3-amino-9-ethyl-carbazole				0	0
agar				1	2
DE + CM cellulose*					
mineral oil				3	0
Freund°s*					
immersion oil*					
p nitrophenyl phosphate*					
3-amino-9 ethylcarbazole*					
sodium periodate				0	0
sodium metabisulfite				0	0
sodium nitrite	x	x		1	0
trinitrophenol*					
toluene (4)	x	x	x	0	0
(metabolic Inhibitors)					
colchicine	x	x	x	0	0
cytochalasin B				0	0
mitomycin-C	x	x	x	0	1
actinomycin-D	x	x	x	0	1
(tissue culture chemicals)					
RPMI*					
MEM*					
penicillin				2	2
streptomycin	x	x	x	2	2
amphotercin-B*					
2-mercaptoethanol	x			2	2
HEPES*					
DMSO	x	x	x	2	2
gentamicin				0	0
(other chemicals)					
benzidine	x			1	0
acrylamide, n,n°-methylenebis-				0	1
tetramethylenediamine (TEMED)	x			0	1
ammonium persulfate				0	1
sodium dodecylsulfate (SDS)	x			1	1

* Not listed in RTECS

Mutagenic/Teratogenic Effects Reported in Animals
Exposed Through Inhalation of Vapor

NOTES: (1) Acetic acid caused mutations in insects exposed to 1000 ppm for 24 hours.

(2) Reproductive embryotoxic effects have been observed in mice exposed to benzene concentrations as low as 50 ppm for 24 hours on the 7th-14th days of pregnancy.

(3) DNA damage has been observed in rats exposed intermittently to a formaldehyde concentration of 0.03 ppm for 8 weeks. Reproductive newborn effects have been reported for rats exposed to formaldehyde at 0.01 ppm for 24 hours on the 15th day of pregnancy and from the 1st-22nd day of pregnancy.

(4) Mutagenic effects have been observed in rats exposed intermittently to toluene at 162 ppm for 16 weeks. Reproductive developmental effects have been observed in rats exposed to toluene concentrations as low as 266 ppm for 24 hours on the 7th-14th day of pregnancy.

The following Studies Were Not Listed in RTECS

(5) Testicular atrophy has been induced in laboratory mice, given orally, high doses of ethylene glycol methyl ether (EGM). Significant decreases in testes weights were observed in mice given at least 250 mg/kg of EGM. (Jap. J, Ind. Health, 21:29-35, 1979)

Antifertility effects and sperm abnormalities were observed in mice exposed to EGM at 500 ppm, 7hr./day for 5 days. (presented at the European Environmental Mutagen Society 9/14-19/80, Athens, Greece)

TABLE 2

SUMMARY OF EXPOSURE LIMITS* and HEALTH EFFECTS
for SUBSTANCES MEASURED at the Immunology Branch Laboratory
Centers for Disease Control
Atlanta, Georgia
April 12-15, 1982

HETA 82-089

SUBSTANCE	OSHA PEL**	ACGIH TLV***	NIOSH RECOMMENDATION	HEALTH EFFECTS CONSIDERED	REFERENCE
Dimethyl sulfoxide (DMSO)	---	---	---	Eye & skin irritation, alters permeability of cell membranes, possible mutagen & teratogen	9,10
Sodium azide	---	0.3 mg/M^3 (ceiling)	*	Acute effects: eye irritation, headache,	11,7
Hydrazoic acid	---	0.1 ppm (ceiling)	---	bronchitis, fall in blood pressure, weakness, dizziness, faintness; possible mutagen	
2-mercaptoethanol	---	---	---	Eye and skin irritation	12
Phenols					
o-phenylphenol	---	---	---	Eye & upper respiratory	12
p-tert-amylphenol	---	---	---	irritation	

* NIOSH Current Intelligence Bulletin No. 13, August 16, 1976 - Notified users of blood cell counters of possible explosive hazard from azide salts in lead and copper drain pipes.

Table 3

Immunology Branch Laboratory
Centers for Disease Control

Atlanta, Georgia
HETA 82-089

Characteristics of Pregancies 1981*

	Miscarriages (n=5)	Full-Term Pregnancies (n=2)
Age range--years	21-37	25-35
Mean	30.4	30.0
Previous pregnancies	4	2
Mean	0.8	1.0
Previous abortions+	1	0
Family history of miscarriage	2	0
Some college education	5	2
Employed	5	1
Stress during pregnancy	3	1
Exposure to X-rays	0	1
Pets	5	0
Alcohol (>1 drink/wk during pregnancy)	2	0
Cigarettes	1	0
Coffee	1	2
Tea	3	2
Vitamins	3	2
Other medications	1	1
Drugs	0	0

*Excludes pregnancies at time of investigation.
+Excludes induced abortions because of confidentiality.

TABLE 4

Immunology Branch Laboratory
Centers for Disease Control
Atlanta, Georgia
HE 82-089

Environmental Air Sampling Results

Location/Job Description	Type of Sample	Sampling Time	Airborne Concentration 2-mercaptoethanol
Hybridomal Lab, media prep	personal	10:27am-11:22am	N.D.
Media prep, under hood	area	10:25am-11:22am	N.D.
Prep. acrylamide gel	personal	9:50am-1:54pm	N.D.
" " "	area	9:52am-1:54pm	0.05 ppm
In hood where 2-ME handled	area	9:54am-11:22am	Trace

N.D. = Not Detected
Trace = Detected, but below limits of quantitation
Limit of Detection = 10 micrograms per sample

Location/Job Description	Type of Sample	Sampling Time	Airborne Concentration Sodium Azide
Near scale, weighing azides	area	8:15am-10:53am	N.D.
Preparing azide solution	personal	8:15am-8:30am	N.D.
" " "	personal	8:29am-8:42am	N.D.
Near vortex equip used with 1% azide solution	area	8:27am-10:45am	N.D.

N.D. = Not Detected
Limit of Detection = 0.5 micrograms per sample

TABLE 5

Immunology Branch Laboratory
Centers for Disease Control
Atlanta, Georgia
HE 82-089

Biological Monitoring Results of
Urine Specimens Collected April 14, 1982

Urine Sample	Concentration in ug/mL Dimethyl Sulfone (duplicate analysis)		Dimethyl Sulfoxide
Pre DMSO use - 8:30am	2.0	2.0	N.D.
During use (media prep.) - 11:15am	2.8	2.8	N.D.
Post DMSO use - 1:15pm	3.4	3.4	N.D.
Pre DMSO use - 7:45am	7.6	6.9	N.D.
During use (media prep.) - 12:00pm	7.6	2.4	N.D.
Post DMSO use - 2:00pm	6.3	5.6	N.D.
IB Controls (no-exposure) (4/12/82)	7.3	5.4	N.D.
" "	4.1	3.2	N.D.
Detection Limits	1.0		4.0
ADL Control Samples (no-exposure)			
Male	8.0		
Male	5.5		
Female	1.5		
Male	7.4		
Female	2.3		
Normal Levels (Ref. ?)	4.4		

Urine Sample	Concentration in ug/mL Urinary Phenol
Pre Amphyl use - 8:30am	7.2
Post Amphyl use - 2 hrs after	6.2
Post Amphyl use - 4 hrs after	4.8
Pre Amphyl use - 9:00am	4.0
Post Amphyl use - 2 hrs after	4.8
Post Amphyl use - 4 hrs after	6.2
Control (no exposure) - 8:30am	4.4
Control (no exposure) - 7:45am	15.6
Normal levels (background mean)	11.56 ± 10.86

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