NIOSH Extramural Award Final Report Summary

Title: Hazard Surveillance in the Defense Nuclear Industry
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Abstract:
The overall goal of this research is to develop an integrated theory, approach, and methodology to exposure assessment and hazard surveillance, which emphasizes characterization of exposure to complex mixtures of chemical toxicants and biomechanical problems as well as single agents. The research has relevance to identification and characterization of problems associated with decommissioning and decontamination of Department of Energy sites, application to the defense nuclear industry and other high-risk industrial locations. This research represents collaboration between the University of California at Los Angeles and Berkeley, Lawrence Livermore and Los Alamos National Laboratories. The specific aims of the overall research can be subdivided into subsections.

1. Exposure assessment and hazard surveillance: To identify appropriate statistical tools for characterizing multiple chemical agents; to explore toxicologic and epidemiologic implications of multivariate exposure characterization; to measure task-specific exposures with real time instrumentation and integrated sampling; to develop models of exposure based on task specific data; to test these models with integrated sampling, and to refine the models based on the results.
2. Modeling pollutant concentration between source and worker: Improve our understanding of small scale (0 to 2 m) dispersion of contaminants with the ultimate goal of predicting personal exposure based on the minimum number of area concentration measurements. To provide a tool for efficient screening of a large number of work sites for potential inhalation hazards.
3. Application of biologic monitoring and biomarkers of exposure for exposure assessment and hazard surveillance: To make use of biologic monitoring, biomarkers of exposure, and toxicokinetic modeling to better estimate internal and target tissue dose from exposure to single and multiple chemical agents and evaluated interactive effects associated with toxicokinetic interaction.
4. Integrated task and postural analysis for ergonomic exposure analysis: To develop, pilot test, and validated an integrated task and postural analysis for ergonomics exposure assessment.

5. Evaluation of current exposure and medical surveillance programs at Los Alamos and Lawrence Livermore National Laboratories: To evaluated the medical and exposure surveillance programs at LANL, identify discrepancies between health and safety "needs" and established monitoring programs, and develop an integrated surveillance system that efficiently combines hazardous-exposure, biological, and health-outcome monitoring of the worker population.
6. Assessing risks from exposure to multiple physical and chemical agents: To develop and implement a risk based framework and methodology that permits estimation of the incidence of adverse health impact predicted from environmental/biological exposure and enables development of surveillance programs and intervention strategies to prevent adverse consequences of exposures.

Publications

Chen WG, McKone TE: Chronic Health Risks from Aggregate Exposures to Ionizing Radiation and Chemicals: Scientific Basis for an Assessment Framework. Risk Analysis, 21, pp 25-42, 2001

Wu JD, Milton DK, Hammond SK, Spear RC: Hierarchical Cluster Analysis Applied to Workers' Exposures in Fiberglass Insulation Manufacturing. Ann. Occup. Hyg., 43, pp 43-55, 1999

6. Assessing risks from exposure to multiple physical and chemical agents
To develop and implement a risk based framework and methodology that permits estimation of the incidence of adverse health impact predicted from environmental/biological exposure and enables development of surveillance programs and intervention strategies to prevent adverse consequences of exposures.

Two approaches will be used to organize this final report. For the most part we shall address each specific aim listed above and we shall include manuscripts as appendices. These manuscripts may be published papers, papers under review or in final preparation. Some sections will also have text where a specific manuscript has not yet been prepared.

1. **Exposure assessment and hazard surveillance**

- **to identify appropriate statistical tools for characterizing multiple chemical agents;**
- **to explore toxicologic and epidemiologic implications of multivariate exposure characterization;**
- **to measure task-specific exposures with real time instrumentation and integrated sampling;**
- **to develop models of exposure based on task specific data;**
- **to test these models with integrated sampling, and to refine the models based on the results.**

Significance of work performed under the NIOSH-DOE Project at Berkeley

There are two significant results from our work, one the identification and specification of the nature of a major unresolved challenge for risk assessment relating to multiple agent exposure and the second significant progress on an approach to the characterization and study of this class of occupational exposures. In particular:

- In our studies of approaches to assessing the cumulative health risks from aggregate exposures to ionizing radiation and chemicals, we found in the literature essentially no guidance for conducting risk assessment for two agents with different mechanisms of action (i.e., energy deposition from ionizing radiation versus DNA interactions with chemicals) but similar biological endpoints (i.e., chromosomal aberrations, mutations, and cancer). Our analysis reveals that this is due to the absence of both the basic science and an appropriate evaluation framework for the combined effects of mixed-agent exposures. This makes it difficult to determine whether there is truly no interaction or somehow the interaction is masked by the scale of effect observation or inappropriate dose-response assumptions. An evaluation framework is proposed.

- In a new approach to characterizing multiple agent exposure, we have developed a computer simulation approach that utilizes both qualitative and quantitiative workplace data for the purpose of both forecasting the complex multivariate nature of these exposures as well as to plan efficient and cost effective exposure measurement and surveillance programs. We feel that this approach circumvents inherently costly approaches based on measurements alone.

Appendix A contains the papers derived from the research associated with this specific aim. The papers are listed here and the numbers in the parentheses following the citation denotes the specific aims to which the work relates.

To date one manuscript describing the work under the grant has been published in a refereed journal, one is in press, two are in the process of being submitted and one is in preparation. The papers are:

Hierarchical Cluster Analysis Applied to Workers' Exposures in Fiberglass Insulation Manufacturing, J.D. Wu, D.K. Milton, S.K. Hammond and R.C. Spear, Ann. Occup. Hyg., 43, pp 43-55, 1999. (1, 2)

Chronic Health Risks from Aggregate Exposures to Ionizing Radiation and Chemicals: Scientific Basis for an Assessment Framework, W.G. Chen and T.E. McKone, Risk Analysis, 21,pp 25-42, 2001. (2)

Integration and Exploration of Task-Based Exposure Data: Part I: Design of an Exposure Simulator, J.D. Wu, N. Shang, S.K. Hammond, and R.C. Spear, In review, Am. Ind. Hyg, Assn. J., 2000 (4)

Integration and Exploration of Task-Based Exposure Data: Part II: Simulation of Solvent Exposures in Raft Manufacturing, J.D. Wu, K. Vork, and R.C. Spear, In review, Am. Ind. Hyg, Assn. J., 2000 (3, 4)

Evaluating the Attributes of Incomplete Data on Workers' Exposure to Benzene; A CART Analysis, W.G. Chen, S.K. Hammond and T.E. Mckone, In preparation (2)

To date four papers have been presented at national meetings on the outcome of work sponsored under the grant:

Simulation of Occupational Exposures to Mixtures, J.D. Wu, Intl. Soc. of Exp. Anal. Annual Meeting, Research Triangle Park, November 1997;

Risks for Workers Exposed to Mixed Physical and Chemical Agents: Benzene and Radiation Case Study, W.G. Chen, T.E. McKone and R.C. Spear, Soc. for Risk Anal. Annual Meeting, Washington D.C. December 1997.

Biological Monitoring to Assess the Health Risks for Workers Exposed to Mixtures of Chemical and Physical Agents, W.G. Chen, T.E. McKone and R.C. Spear, Am. Ind. Hyg. Conf. And Exposition, Atlanta, GA, May 1998

Exploring the Use of Simulation as a Tool for Workplace Exposure Assessment, Shao-wen Liaw, Katharine Hammond, Robert C. Spear, Jyun-De Wu, and Mark Nicas, Am. Ind. Hyg. Conf. And Exposition, St. Louis, MO, May 1999

We are pleased with our accomplishments in meeting four of these five aims. We made a number of attempts to locate a DOE site in which decommissioning and deconstruction work

was underway and where we could pursue the fifth aim of testing our modeling and methods development work, but without success. At one time or another we had conversations with individuals from Los Alamos, Livermore, and Savannah River, with some discussion regarding Hanford, but for one reason or another things never worked out. Indeed, we had hoped to work at a DOE site in our earlier work relating to aim 3, but were frustrated in that as well and chose to work in a commercial plant.

2. Modeling pollutant concentration between source and worker
Improve our understanding of small scale (0 to 2 m) dispersion of contaminants with the ultimate goal of predicting personal exposure based on the minimum number of area concentration measurements. To provide a tool for efficient screening of a large number of work sites for potential inhalation hazards.

William Hinds and Bart Ashley

Near-Field Dispersion Modeling
Abstract:

This component of the project addresses the development of a screening tool for hazard surveillance in the defense nuclear industry, including decommissioning and decontamination. The approach evaluated here is the development of an indoor, nearfield dispersion model for estimation of exposure concentration based on source strength and worker location for typical indoor spaces. This report presents the results of a literature search, the use of a three-axis sonic anemometer to characterize turbulent mixing, and estimate eddy diffusion coefficients, and the development of experimental designs and models to estimate to near-field (0 to 3 meters) concentrations within indoor environments. The results of the literature search indicate that there has been little research conducted into the development of near-field dispersion models. Related topics were identified that may prove useful during the development and validation of a near-field dispersion model. These papers cover a wide range of applications that include massbalance models to estimate contaminant concentrations arising from industrial emissions, numerical analysis methods to characterize contaminant dispersions, and topics relating to the design of experiments to characterize air motion within an enclosed space. The results of the use of the three-axis sonic anemometer indicate that is useful technique to characterize turbulent mixing, and estimate eddy diffusion coefficients within the indoor environment. The use of three-axis sonic anemometry data coupled with a serial correlation data analysis program has proven successful at differentiating indoor environments with different levels of turbulent mixing, (e.g. wind tunnel at 1 m/s versus normal laboratory environment). The three axis sonic anemometer data coupled with a defined angle range data analysis program estimated eddy diffusion coefficients for different levels of turbulent intensity. Several experimental designs have been developed which utilize three-axis sonic anemometry data to estimate near-field concentrations. Experimental measurement of concentration as a function of distance from a point source allowed the development of a simple power function model to predict concentration up to 1 m from a source in a laboratory setting.

Specific Aims

The specific aim for this component (modeling concentration between the source and the worker) of the study, as stated in the proposal, is: "To improve our understanding of small scale (0 to 2 m) dispersion of contaminants with the ultimate goal of predicting personal exposure based on the minimum number of area concentration measurements. To provide a tool for efficient screening of a large number of work sites for potential inhalation hazards.

Background And Significance

Background: Hazard surveillance includes the assessment of the occurrence and distribution of the hazards associated with exposure to chemical agents as a means of preventing adverse outcomes. Because of the high potential for chemical exposure at DOE sites, including decommissioning and decontamination, there is a need for screening tools that can identify those exposure situations that have high potential for overexposure to chemicals. This component of the project addresses one such screening tool, the estimation of exposure concentration, based on source strength and the physical location of the worker relative to the source, through the use of indoor, near-field dispersion models.

Currently there is limited understanding regarding how the average concentration of an airborne contaminant changes with distance from the generating source in the near-field (0-3 meters) of an indoor environment. The development of a near-field dispersion model that is capable of estimating airborne concentrations at different locations from a generating source would be valuable in identifying and estimating workers at risk of inhalation exposure in the workplace. Near-field dispersion models could serve as a screening tool to evaluate a large number of workplaces for potential inhalation hazards. The most useful near-field dispersion model would be able to predict potential personal inhalation exposures based on a minimum number of area concentration measurements. The results of the near-field dispersion estimates would allow for maximizing the value of limited air sampling resources to identify changes in workplace exposures or processes that may result in higher exposures to workers.

The type of input data required for near-field models depends on whether the generating source is continuous or intermittent. To utilize a near-field model for a continuously generating source the exposure geometry, air velocity distribution and source strength or average room concentration of the contaminant must be determined. The average room concentration for a reasonable well-mixed room can be determined readily by measuring the average concentration of the contaminant over a period of time at the exhaust duct for the room (Hinds, 1995). Assessment of the exposure geometry requires analysis of the location of the generating source relative to the worker and requirements of the task being performed by the worker. Characterization of air velocity in the room is a more involved task that requires measuring the air velocities at different locations within the room and a determination of the amount of mixing that takes place as a result of the air motion. For intermittent generation sources the near-field model would require knowing the amount of contaminant generated per unit time or the amount of contaminant generated per unit operation and their duration and the exposure geometry and air motion characteristics.

The accuracy of a near-field dispersion model for an indoor environment is dependent upon the characteristics of the air motion within that space. Currently the air motion characteristics of an

indoor environment are incorporated into a mixing factor. The mixing factor is used to estimate the effect of incomplete mixing upon the potential contaminant concentration. In effect the K factor is a general purpose safety factor which is used to account for, among other things, the fact that mixing within an indoor environment is not complete. The K factor is based on several considerations: 1) the efficiency of mixing and distribution of replacement air introduced into the room or space being ventilated; 2) the toxicity of the airborne contaminant; 3) other factors such as duration of the process, operational cycle, location of workers relative to sources of contaminants, location and. number of generation points within the workplace, seasonal changes in the amount of natural ventilation and possible reduction in the operating effectiveness of mechanical air moving devices (ACGIH, 1995). After evaluating these considerations the K factor value selected generally ranges from 1 to 10. A potential problem with the use of the K factor arises from the fact that many of the criteria that are used to estimate the K factor are based on professional judgment. Because professional judgment is widely variable a more quantitative estimate of the amount of mixing is desired. The use of a three-axis anemometer provides a quantitative measurement of the amount of mixing that occurs within an indoor environment.

A. Literature Search

A literature search was completed for topics relating to near-field dispersion models. The Results of the literature search indicate that there has been little research into the development and evaluation of near-field dispersion models. During the course of the literature search several related research topics were identified that may prove useful in the development of near-field dispersion models. The majority of the literature that relates to the study of contaminant dispersion can be grouped into one of three categories: (1) model development and analysis based on emission rates, (2) room mixing research, and (3) data analysis methodologies. The relevant publications for each category are discussed below.

Model Development

Brief, R.S., et. al., published a paper entitled "Development of exposure control strategy for process equipment.," which discussed the need to evaluate near-field dispersion models in order to estimate the expected emissions resulting from process equipment failures, such as leaking seals and gaskets in the petroleum industry. The near-field dispersion equation described in their paper needs to be validated in a controlled laboratory environment to determine its accuracy in predicting contaminant concentrations in the near-field.

Six papers have been published by Wadden, R.A., and co workers, which describe methods to quantify indoor contaminant concentrations in terms of emission factors and emission rates. Emission rates are the mass of pollutant released per unit time and the emission factor is the mass of pollutant released per unit of source activity. The benefit of generalizing contaminant concentrations in terms of emission rates and emission factors is that the results can be applied to other spaces or environments that contain the same type of pollution sources and processes (Wadden, et. al., 1994). Emission rates are determined by measuring the area concentration patterns surrounding each pollutant generating device while production is taking place. An emission rate is calculated from a mass-balance model which relates measures

of source activity, process conditions and equipment geometry. Several papers relating to the development of emission factors are briefly described below.

Wadden, et. al. (1994) discussed the determination of emission rates for ethyl alcohol resulting from a candy glazing operation. The emission rates were translated into emission factors by relating them to observations of source activities. The investigators used a combination of mass balance modeling approach and on-site field testing to determine that the ethanol emission rate was between 38.4 kg/hour and 49.6 kg/hour. The emission factor was determined to be between 291 grams ethanol/batch of candy to 500 g/batch. The investigators reported that the calculated emission data was in agreement with the estimate of 446 g/batch based on the glaze mixture composition and the amount of glaze mixture added to each batch of candy. An one-compartment completely mixed space mass-balance model was used to transform the area concentration measurements to emission rates.

Wadden, et. al. (1991) used two different mass-balance models to estimate emission factors for trichloroethylene degreasing operations. The authors used a Multi-Point Eddy Diffusion mass-balance model to determine the emission rates when one pollution source is the major contributor to concentrations in the surrounding space. This model assumes a hemispherical space and neglects surface deposition effects (Wadden et. al., 1991). The authors used a Completely Mixed mass balance model in the case where many sources make significant contributions to area concentrations. The Completely Mixed mass balance model was used under the assumption that the workplace approaches the conditions of a completely mixed container. This model also neglects the effects of reaction and surface deposition.

Conroy, et. al. (1995) described the use of the Multi-Point Eddy Diffusion model to determine the emission rates and emission factors for a chromium plating operation. The authors reported a emission factor of 2.6×10^{-4} mg chromium/A•hr.

Wadden, et. al. (1995) utilized a Completely Mixed Space Mass Balance model (Analytical Approach) described previously and the Experimental Mass Balance Approach to determine the emission rates and emission factors for volatile organic compounds in a sheet-fed offset printing operation. The authors reported a correlation coefficient, $r^2=0.55$ between measured and predicted concentrations for the Experimental Mass Balance equation and a correlation coefficient, $r^2=0.46$ between measured and predicted concentrations for the Completely Mixed Space Mass Balance equation.

Liao, et. al. (c.a. 1995) utilized a Chemical Mass Balance model to estimate copper and chromium emissions in an electroplating shop. The authors used a weighted-least squares technique to solve the equation for source coefficients. The weights used were the reciprocal of the measured concentration squared ($1 /conc^2$). The authors reported that the Chemical Mass Balance model explained 100% of the variations in copper concentrations measured at three locations in the copper plating shop, with contributions of 95-98% attributable to the plating line. The CBM model explained 100% of the variations in chromium concentrations measured at four locations in the plating shop.

Scheff, et. al. (1992) compared a Box model and an Advective-Diffusion model to translate area concentration measurements to emission rates for open-top vapor degreasers. Freon concentrations were measured using both charcoal-tube/gas chromatographic and Tedlar bag/infrared absorption methods. The box model assumes that rapid mixing occurs throughout the box, the system is steady state and the air velocity and pollutant emission rates are constant. The authors reported that the average emission predicted by the box model for the six hours with bag and charcoal-tube data was 74 g/hour, which was close to the 95 g/hour predicted by the advection-diffusion model for the same hours. The correlation between the two models for these six hours was 0.91.

Jaycock, et. al. (1995) evaluated the use of a ,simplified concentration model which requires only source rate and saturated vapor concentration as experimentally derived inputs. Airborne exposure models were developed to account for volatilizing sources and adsorbing surface sinks of isothiazone biocide volatilized from treated wood into glass chambers and real-world environments. The authors reported that the model provided acceptable accuracy in that it overestimated measured room concentrations in two tests to within a factor of 2-4. The investigators also found significantly lower equilibrium concentrations in a real room environment compared to a glass test chamber.

Keil, (1998) completed field studies to develop and evaluate toluene emission factors for a parts-washing operation. The author used a mass-balance model that divided the airspace into near and far fields of exposure concentrations. Near field concentration measurements were taken at 0.3 meters and 0.6 meters from the source. Far field concentrations were measured at a 12 and 24 meters from the source and represented the average room concentration. The author reported good correlation between the predicted toluene concentration estimates and the measured concentrations (slope=1.1, r^2=0.66, $p<0.05$).

Drivas, et. al. (1996) proposed a simplified analytical indoor model that describes the concentrations as a function of position and time in a room following a short-term release of particles and gases. The indoor dispersion model considers point-source dispersion with wall reflection and general room concentration decay due to deposition and room ventilation. The authors reported excellent agreement between model predictions and experimental results conducted by other researchers.

Room Mixing

Gonzales, et. al. (1974) investigated the relationship between fixed aerosol samplers and breathing zone air sampling measurements. The authors utilized dioctylphthalate as a tracer aerosol and measured aerosol concentration throughout the work area, with different ventilation rates. The results indicated that aerosol concentrations in the worker's breathing zone was up to 250 times greater than concentrations which might be indicated by typical fixed room-air samplers located adjacent to the glove box. General ventilation conditions (room air changes per hour and air direction relative to the leak flow) were varied. Under all ventilating conditions the highest aerosol concentrations were close to and in front of the leak source.

Nicas, M. (1996) investigated the potential error in exposure estimates that may occur due to use of the well-mixed room model. The author reported that use of the well-mixed room model may

lead to an underestimation error of worker exposure intensity, up to 40%. The author presents a methodology for estimating ventilation efficacy using realtime measurements at the worker location and room exhaust, coupled with room exhaust rate.

Rigsbee and Perkins (c.a. 1995) investigated the variability in the K factor values based upon worker location and turbulence intensities. The authors reported that based on the results of experimental data the ACGIH recommended K factor may only be applicable to a few workplace locations. This study also reported that as the generation rate increases and the exhaust flow rate increases, the average K factor and K factor variance increases, and K factor variances are lowest in turbulent conditions.

Data Analysis Methodologies

Madsen, et. al. (1996) investigated the relationship between several aerosol parameters (particle diameter, density, and initial velocity) and their influence on aerosol dispersion and capture by a local exhaust. A numerical model based on the Langrangian method was used to estimate the motion of a spherical, rigid particle in a fluid flow system. Computational fluid dynamic software (EOL) was used for the numerical simulations. The authors reported that numerical method used in this study is capable of handling the complex system of contaminant sources in the industrial environment.

Nabar, et. al. (1996) investigated the use of computational fluid dynamics (CFD) to evaluate experimental and simulated air mixing for dilution ventilation of confined spaces. Using residence time distributions (RTD), two air mixing parameters were defined and computed for physical experiments and computer simulations. The authors concluded that CFD simulations of ventilation experiments using commercially available software were reasonably consistent with physical experiments and RTD-based mixing parameters were useful in evaluating air mixing in physical experiments and computer simulations.

Rodes, et. al. (1995) investigated experimental considerations for conducting controlled studies of the dispersion of contaminants near a mannequin. In addition the authors identified the need to develop realistic respiratory systems in the mannequins.

Literature Search Discussion:

Based on the results of the literature search the use of anemometry data to describe turbulent mixing is a technique which has not previously been applied. Many of the papers that were identified during the course of the literature search are useful in developing experimental methods to describe air motion in indoor environments.

Of particular interest are the papers which describe the use of computational fluid dynamics, which can be used as a comparison against experimentally obtained results. The use of computational fluid dynamic methods appears to be a sophisticated technique that may yield valuable insight when properly applied.

The mass-balance models presented by the research group at the University of Illinois at Chicago may prove to be useful if eddy diffusion data can be estimated from the anemometer data to describe the air motion within a room. Presently these models utilize very limited air motion data

to characterize the contaminant dispersion resulting from industrial processes such as chrome plating and sheet-fed printing. The attempt to quantify the mixing factors by Rigsbee and Perkins, and the work of Nicas are useful papers which may provide some helpful experimental methods which may be used in the future during further research on turbulent mixing and the role of eddy diffusion in contaminant dispersion.

Keil, (1998) has proposed a near field dispersion model that accounts for the concentration differences between the near and far fields. This method uses a mass balance model that defines a near field transport coefficient for concentrations from 0.3 to 0.6 meters from the source and a far field coefficient for concentrations from 12 to 24 meters from the source. This model would be improved by adding more comprehensive data regarding the air velocity distributions from the 3-axis sonic anemometer characterizations of air velocity distributions. The anemometer data allows for the calculation of the mean velocity, duration of coherent segments, and the net displacement for all segments. This information could then be utilized to determine eddy diffusion coefficients for both near and far fields.

B. Development of theoretical dispersion models

Three-Axis Sonic Anemometer:
An Applied Technologies 3-Axis Sonic Anemometer, Model SAT-211/3Vx was used to determine the efficacy of using anemometry data to describe air motion characteristics of different indoor environments. The sonic anemometer is capable of measuring wind velocities in three orthogonal axes (u, v and w) and sonic temperature. The sonic temperature is used to calculate the speed of sound at the temperature of operation. In the current data collection set-up the a and v axes are horizontally aligned and the w axis is vertically aligned. The sonic anemometer is composed of a probe array, whose sonic transducers are separated by 15 cm (Applied Technologies, 1995). Sonic pulses are generated at the transducers and are received by opposing transducers. By measuring the transit times, t, and t2 in opposite directions on the same sound path (axis), Vd (velocity parallel o the sound path) can be determined.

Serial Correlation Method
The data from the anemometer are collected using a 586 100 MHz laptop computer equipped with Pro Com Plus data collection software. The collected data are analyzed using a Turbobasic computer program which calculates the rms velocities (m/s) with standard deviations, along the three axes for the sample period. After the rms velocities are calculated the data analysis program computes 500 serial correlations. Serial correlations are used to determine how the wind speed of a particular axis at time t, relates to the speed at t2, t3, t,, , where n is equal to 500. The 500 serial correlations are based on the first 50 seconds of the data run. Anemometer measurements are collected at a point within a room or other indoor space and the rms air velocity along each of the three axes determined. A characteristic turbulent signature is obtained from these data by calculating serial correlation coefficients for lag times of 0.1 to 50 seconds. The serial correlations determine how a velocity measurement at t, relates to subsequent measurement at t2, t3, t4, t, where tX - t, is the lag time and x is equal to 2, 3, 4 n. The decay of correlation with lag time reflects the decay of temporal and spatial coherence of the instantaneous air motion and is the key to estimating eddy diffusion coefficients from anemometry data. This method has proven successful at differentiating indoor environments with different levels of turbulent mixing.

Defined Angle Range Method

The current data analysis program is designed to determine the velocity vector (speed and direction) of the air motion within the environment being measured, every tenth of a second. The program first removes the systematic velocity components to leave only the random velocity component, for each of the three axes. The program then analyzes the set of random velocities to determine the direction and average velocity for each period in which the velocity is reasonably constant in direction. Each such period is considered a coherent velocity segment and its attributes stored. The range of angles to be included in a segment is under control of the investigator. When this analysis is complete the mean velocity, duration, and the net displacement for all segments are calculated. These data are used to estimate eddy diffusion coefficients by the equation given below.

Eddy Diffusion Coefficients Analogous to Molecular Diffusion

Based on the results of the 3-axis anemometry measurements within the wind tunnel and laboratory environments it appears that the use of anemometry data to describe the air motion characteristics of indoor spaces is a promising technique. The 3-axis anemometer data coupled with the serial correlation data analysis program provides a means of differentiating the amount of turbulent mixing that is present in an indoor environment. In addition the delay time which corresponds to a serial correlation value can also be used to estimate the eddy diffusion coefficient at the point of measurement. If eddy diffusion is characterized in terms similar to molecular diffusion the eddy diffusion coefficient can be estimated by the following process. Hinds (1999) gives molecular diffusion coefficient as,

$D = 1/3\ \bar{c}\ \lambda$ where: D = molecular diffusion
$\bar{c}$ = mean thermal velocity
λ = mean free path

By analogy to molecular diffusion and neglecting any systematic velocity component:

$D_e = k(\bar{u}')\ L_e$ where: D_e =eddy diffusion
k = empirically derived constant
$\bar{u}'$ = rms velocity in the u-axis
L_e = distance air parcel travels during time t, see below
$L_e = (\bar{u}')(t_c)$
where t_c is the time over which displacement is correlated (e.g. delay time for a correlation of 0.5)

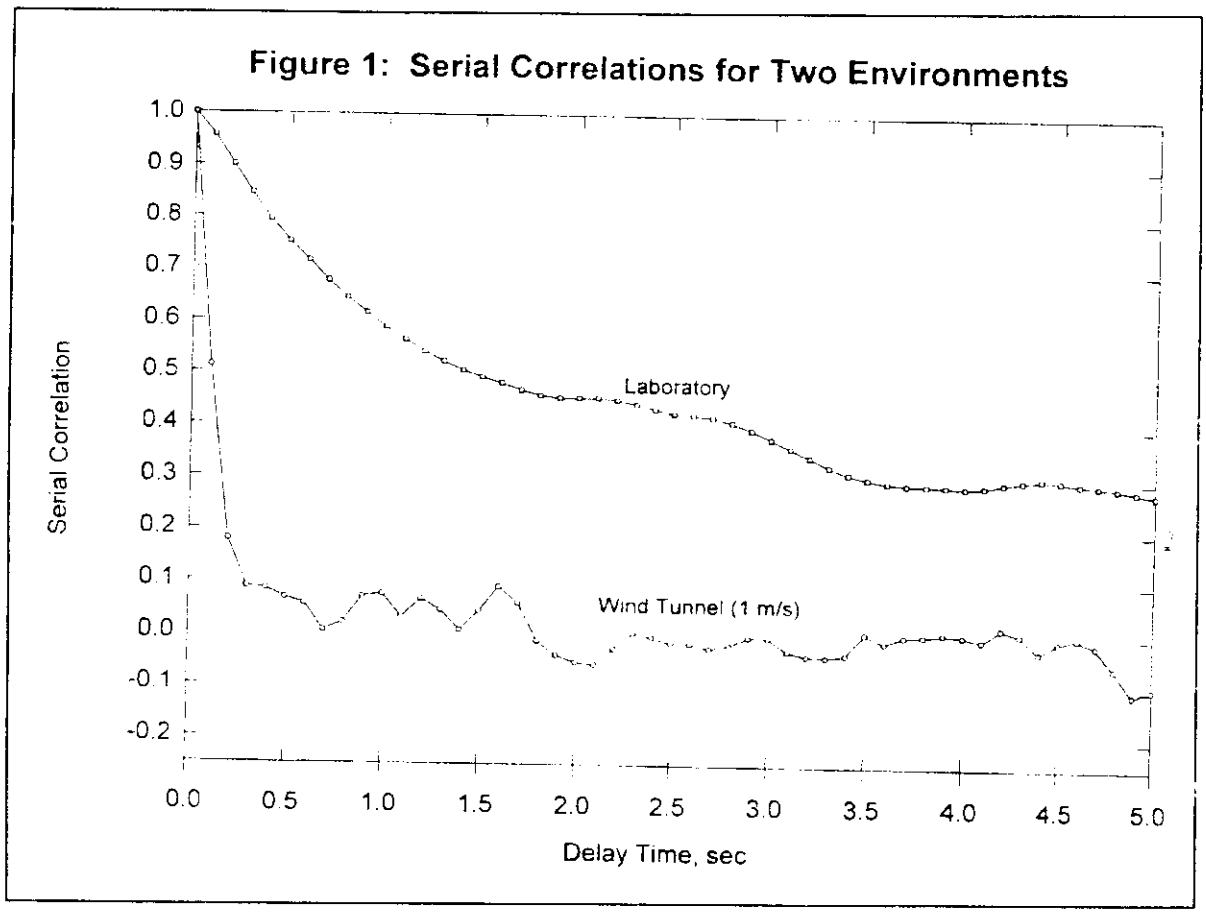
$D_e = k\ (\bar{u}')(\bar{u}')\ t_c = k(\bar{u}')^2\ t_c = k[(m/s)(m/s)(s)] = m^2/s$

Characterization of laboratory and work stations in terms of distribution of air velocities.

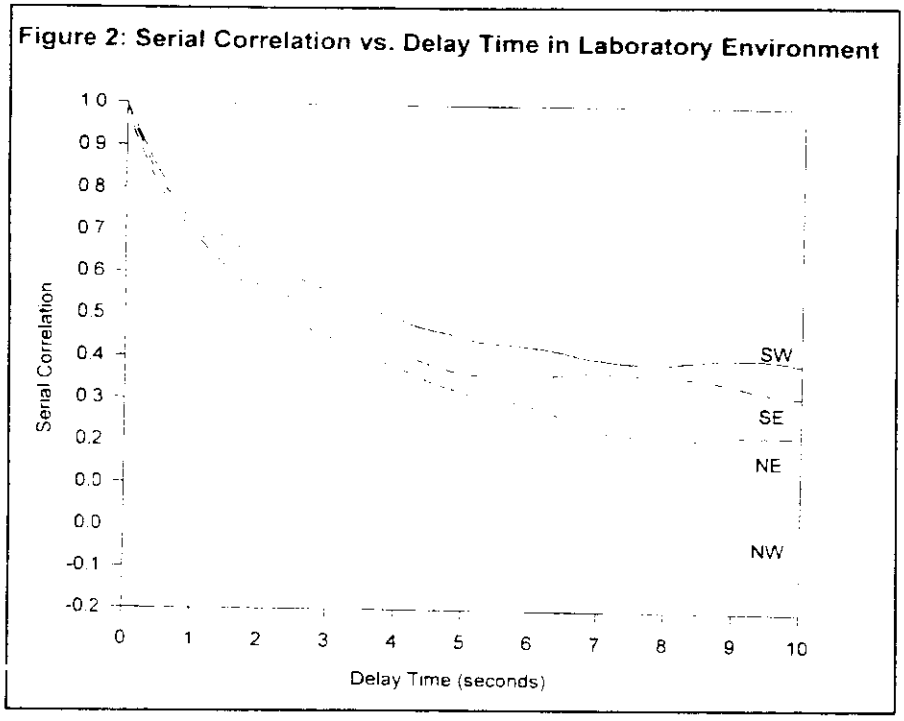
Experimental Results:

Using the serial correlation method described previously the sonic anemometer was able to distinguish between the laboratory and wind tunnel environments.

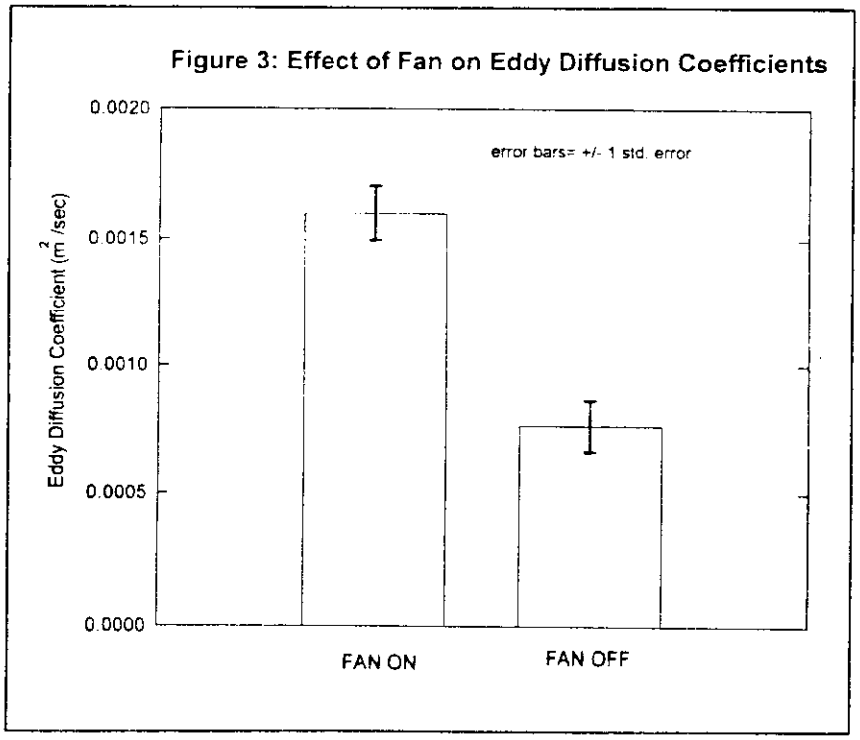
Using the serial correlation data analysis program the three axis sonic anemometer was used to characterize two indoor environments with different turbulent mixing characteristics as shown in Figure 1. The anemometer was placed within a wind tunnel with a 1 meter per second horizontal velocity and in a laboratory. As expected the results show that the correlation of the velocity component lasts for a longer period of time in the less turbulent laboratory environment than in the wind tunnel. In the wind tunnel environment the serial correlation drops to 0.5 in less than 0.25 seconds. It takes approximately 1.5 seconds for the serial correlation to reach 0.5 in the less turbulent laboratory environment.



A subsequent investigation into differentiating indoor environments was to characterize the turbulent mixing characteristics at four locations with the sonic anemometer within a laboratory environment. The serial correlation data analysis was then used to determine any differences in the turbulent mixing characteristics at four different locations within the laboratory. As shown in Figure 2 there are differences in turbulent mixing characteristics within the laboratory. The time required to reach a serial correlation of 0.5 ranged from approximately 2.0 seconds to 4.0 seconds. The longer delay times to reach a 0.5 serial correlation indicate a lower turbulent intensity and slower mixing.



Using the three axis sonic anemometer and the Defined Angle Range data analysis method eddy diffusion coefficients were calculated within the laboratory. An air- circulating fan was placed within the laboratory to create greater turbulent intensity. As shown in Figure 3 the eddy diffusion coefficients calculated with the fan operating are significantly larger than those calculated with the fan off.



Validation and improvement of local dispersion models in the laboratory

- Computer Data Analysis

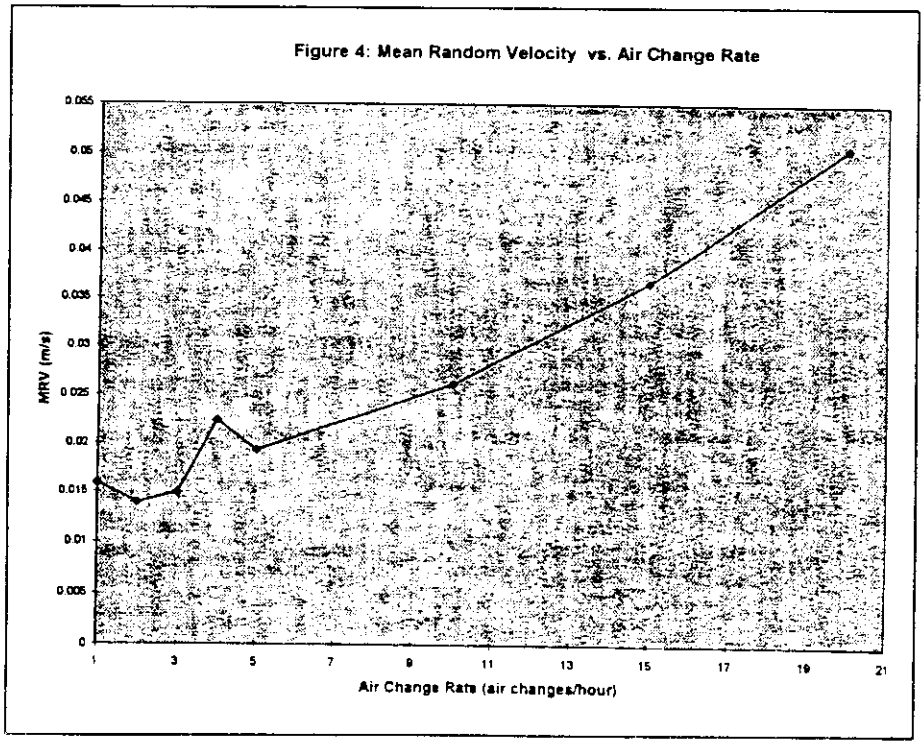
Characterization of laboratory work stations using the three-axis sonic anemometer has been completed in a mechanically ventilated laboratory. The sonic anemometer records the air velocity in 0.1 second intervals, along each of the three axes. A computer program is then used to reduce the data, calculate the systematic and rms velocities in each axis. The computer program then checks the data for transmission errors, allows removal of background anemometry data, and permits selection of any portion of the full data file.

- Rochester Chamber Results

Our attempts to establish known eddy diffusion coefficients in a modified 0.4 m3 Rochester exposure animal exposure chamber were unsuccessful due to the formation of convection cells in the chamber. This gave nonuniform mixing in the chamber so it could not be used to validate our prediction based on anemometry data, using the defined angle range method described previously, not be used to determine the turbulent intensity within the chamber. Several experimental results were verified with the Rochester chamber despite the nonuniform mixing.

Experimental Results:

Despite the formation of convection cells within the Rochester chamber, it was possible to confirm that as the air change rate increased the mean random velocity calculated by the defined angle range method data analysis program increased as well. This was expected and seen in Figure 4.



E. Acetone Generation System

A steady-state acetone generation system was developed to generate known concentrations of acetone vapor shown in Figure 5. The acetone vapor is generated by passing a compressed air stream at known flow rate through two flasks, in series, filled with liquid acetone. This stream is then mixed with a dilution air stream to achieve the desired concentration of acetone vapor. The acetone vapor exits the generating system via a porous 2.5 cm diameter ball. After exiting each flask the vapor stream flows through a 6-mm ID copper tube approximately 4 m long. This allows thermal equilibrium between the vapor stream and the ambient environment to within 1 K. A temperature probe is used to measure the temperature of the acetone vapor prior to leaving the generating system. The concentration of acetone vapor is then measured at different locations from the 2.5 cm ball, using a PE Potomac 2020 Photo ionization Air Monitor (PAM). Monitoring of the concentration of acetone vapors is achieved by mounting the PAM on a laboratory ring stand which can be placed at locations surrounding the ball. Measurements were made over the range of 0.1 to 1,700 p.m., a range found to be linear within five percent by calibration.

Uniformity of acetone vapors leaving the system through the 2.5 cm ball was confirmed by monitoring the concentration of acetone vapors at 6 positions on the surface of the ball. The concentration values obtained indicate that the acetone is released in a 360 degree sphere, which is then acted upon by the prevailing air motions within the laboratory. A smoke tube was added to the system during these investigations to provide visual confirmation of the concentration data obtained from the PAM. Data characterizing concentration decay as a function of the distance from the source were obtained by three dimensional concentration measurements in the vicinity of the porous ball (0 to 1 m) acetone emitter in a laboratory environment.

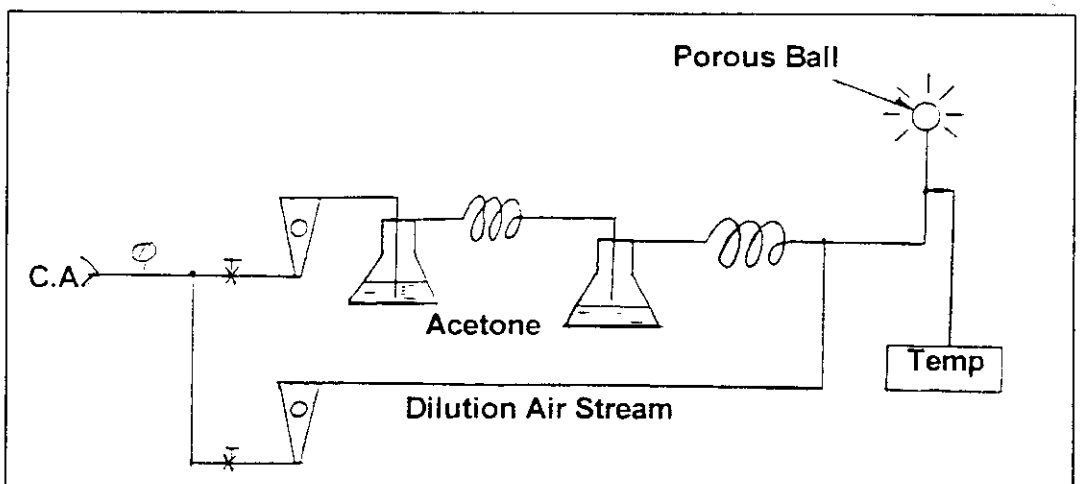


Figure 5. Schematic Diagram of Acetone Generator

Density of the acetone vapor

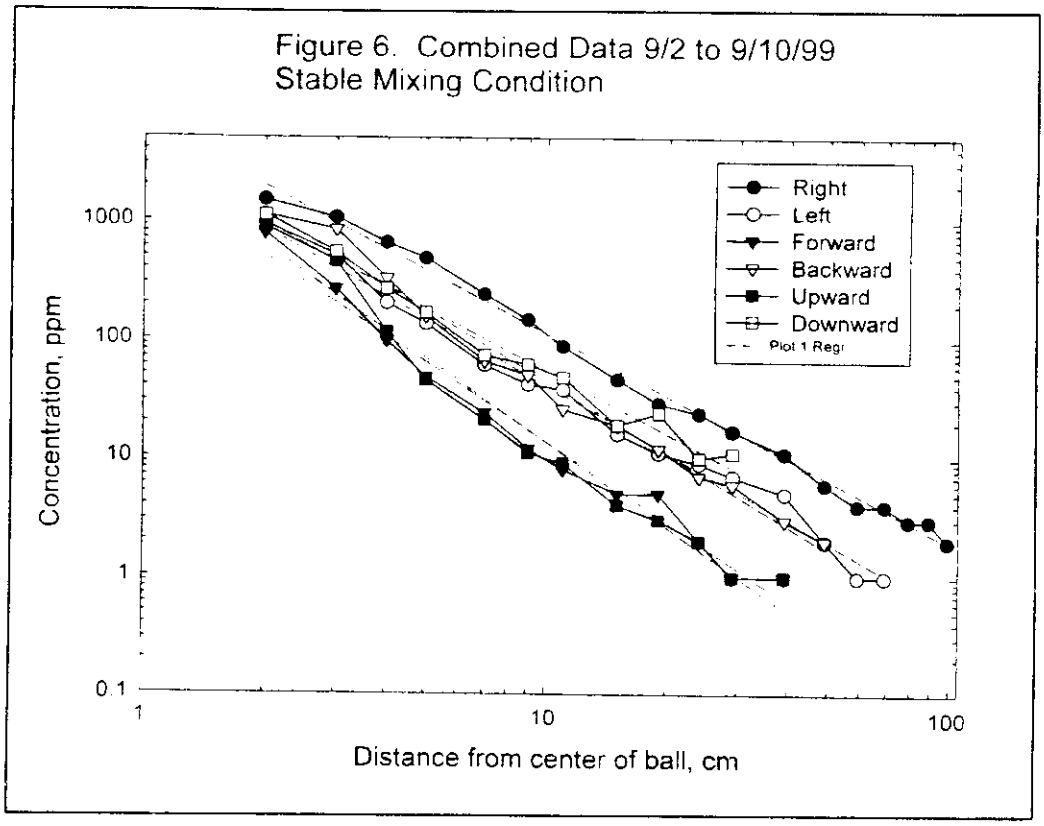
The density of the acetone vapor generated was calculated based on the flow rates of acetone vapor and dilution air stream and temperature of the stream prior to leaving the ball. The density of the acetone vapor was compared to vapor-free air for a range of possible laboratory temperatures. This information is required to eliminate the possibility that buoyancy effects may be affecting the transport of the acetone vapor. A smoke tube was added to the vapor generating system for these investigations. The qualitative results from the smoke tube runs indicate that the relative buoyancy of the acetone vapor leaving the system can be predicted based on flow rates and the temperature of the airstream. This was confirmed qualitatively with the smoke tubes.

The porous ball was located 35 cm above a standard lab bench and 28 cm back from the front edge of lab bench. The bench is 56 cm deep from the front edge to the open shelves at the back. The shelves extended 1 m above the bench. Concentration measurements were made along three octagonal axes (six directions away from the ball). Average concentration measurements were made over five-minute intervals at 18 positions from 2 to 99 cm from the center of the ball in all six directions. The directions are labeled as one would when facing the lab bench, right, left, forward (towards the edge), backwards (away from the edge), and upwards and downwards.

Two levels of mixing were evaluated. One was termed "stable", representing a laboratory environment with no human activity. The only air motion was that due to room ventilation from overhead supply and exhaust ducts. This is the environment characterized by line NE in Figure 2. The second mixing stability condition is typical of moderate activity in a laboratory setting, It was simulated by using the same conditions as for the stable condition described above, but with the addition of a person walking by every 30 seconds. The person walked at 2 m/s as close to the counter edge as possible, and alternated directions. Her shoulder was approximately 33 cm from the ball.

Seven replications of the stable condition were made and four replications were made for the moderately active condition. Measurements were made out to 0.99 m in all directions, except where there was a physical constraint or concentrations were not detectable by the PAM, 0.1 p.m.

Results for the stable condition along all six directions are shown on a log-log plot, Figure 6. Also shown is the least squares best fit line for each direction. The decay with distance from the center the ball shows a good fit to a straight line on the log-log graph. The slope for the lines shown ranged from -1.77 to -2.38 with an average value of - 2.02 and a standard deviation of 0.25. The lines show of best fit show a 20-fold range in concentration at a distance of 0.5 m, with the right direction having the largest concentration at any given distance. The upward direction and the forward direction showed the steepest slope, indicating the most rapid decrease in concentration with distance. The other directions show intermediate decay rates.



These results are consistent with two factors that affect decay of concentration with distance, prevailing air motion and openness or absence of reflecting services. The concept of prevailing air motion direction requires elaboration. It is not the usual direction of the wind, where wind is going in one direction with fluctuations in the magnitude of the wind velocity. Rather, it is air motion that goes in all directions, first one-way and then the other, but goes more often in the direction of the prevailing air motion direction. Here the fluctuations are much greater than the average velocity in contradistinction to the usual situation with wind.

Reflecting surfaces such as the lab bench restrict the size of eddies and reflect concentrations at the surface, that is they do not allow concentration to decay into the surface. Both the prevailing direction and reflecting surfaces have the effect of reducing the rate of decay with distance from the source. In this setup the prevailing air motion direction due to room ventilation was to the right along the bench.

Figures 7a to 7c show decay of concentration for both directions along each of the axes. Also shown are error bars (t one standard deviation). Although there is scatter in the points there is no indication that concentration is not following a straight line on the loglog plot of concentration vs distance from the center of the ball.

Figure 7a Combined Data 9/2 to 9/10/99

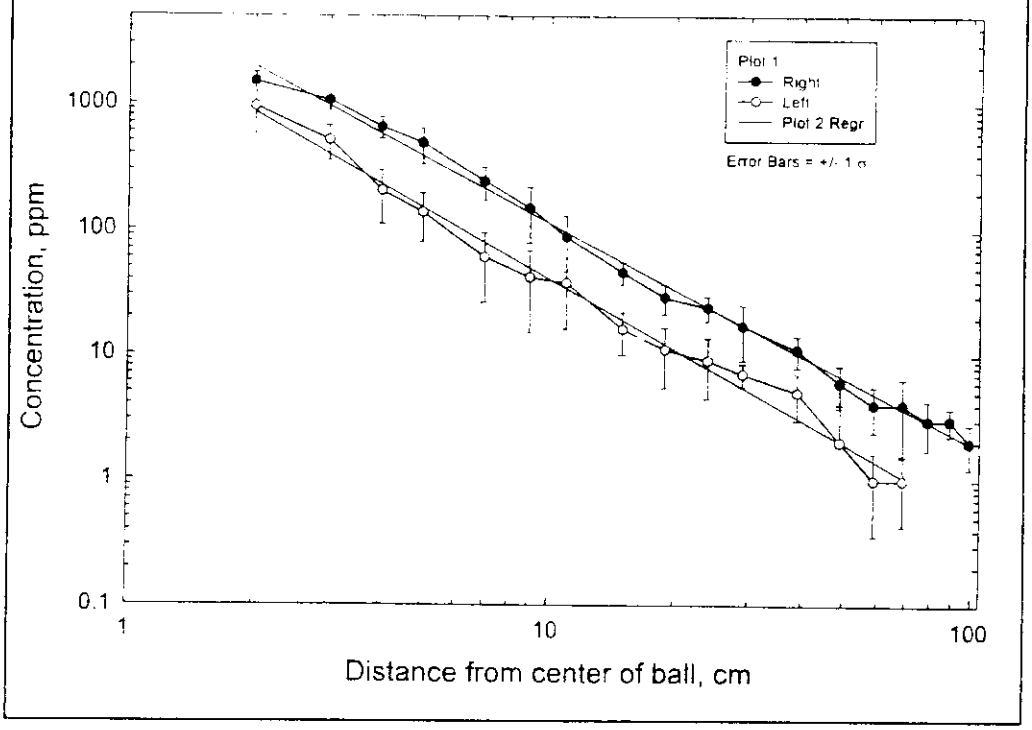
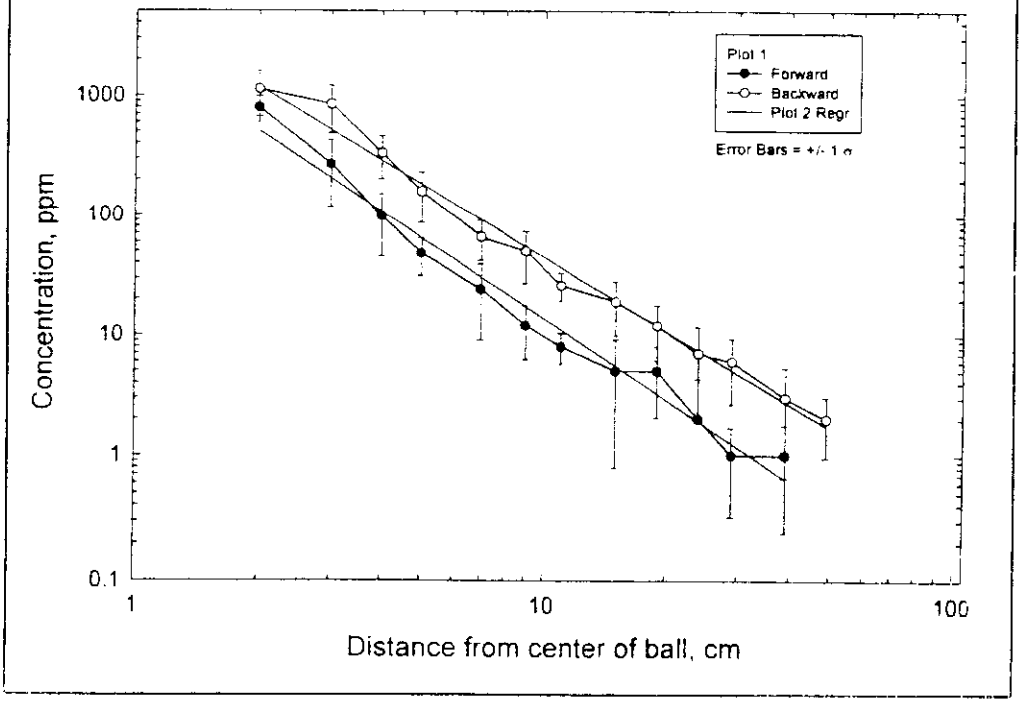


Figure 7b Combined Data 9/2 to 9/10/99



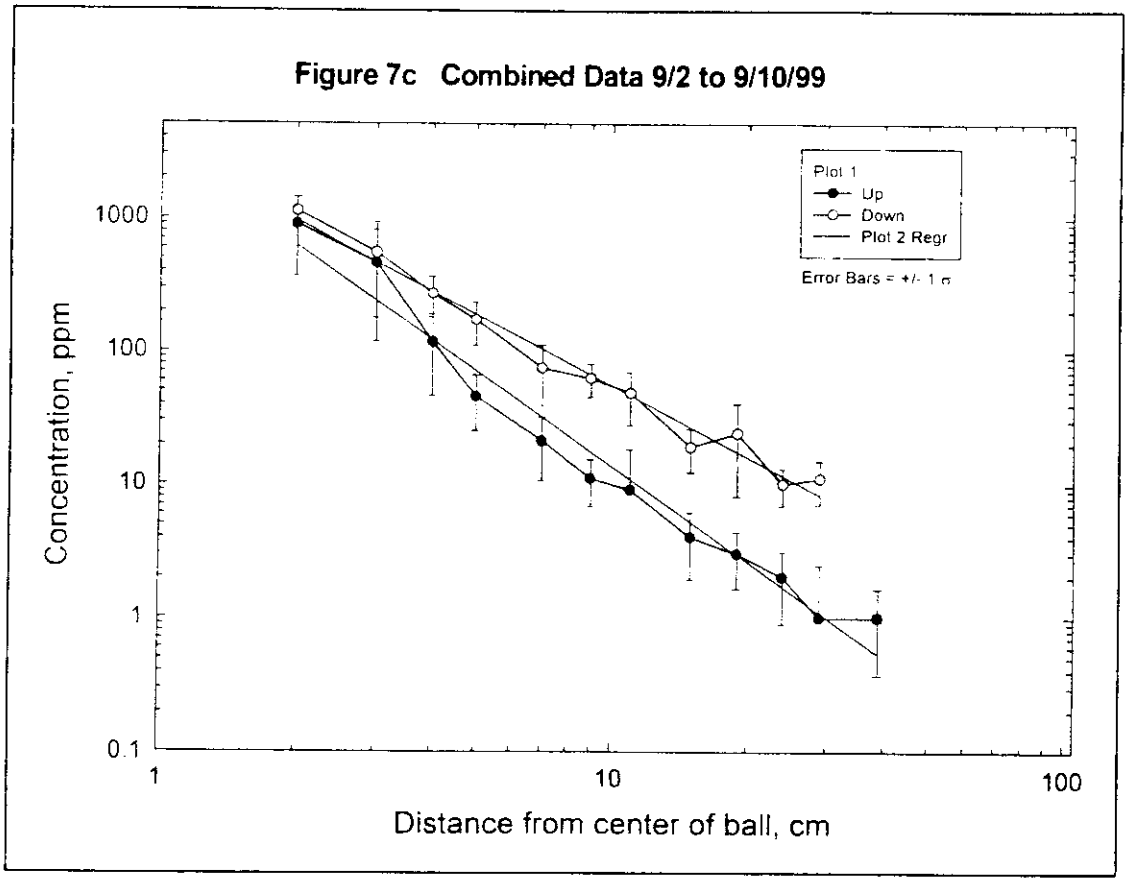
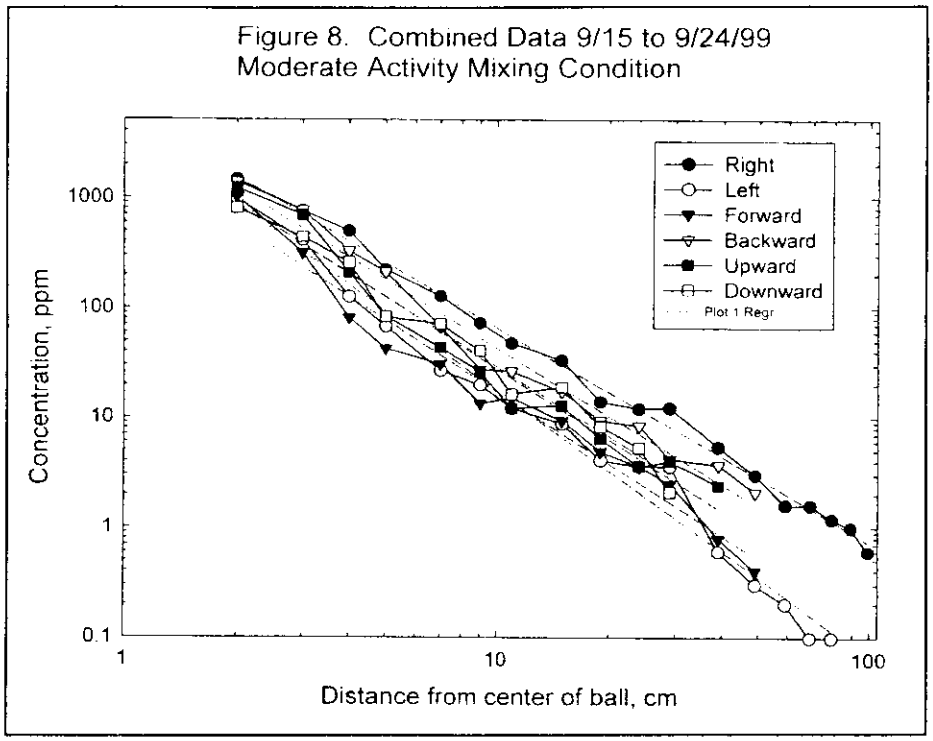


Figure 8 shows results for the six directions for the moderate activity condition. The pattern is similar, but the lines are closer together indicating better mixing and that there is less of an effect due to direction or reflection from surfaces. There is a bit more scatter for this condition because the data shown are based on only four replications instead of the seven replications for the stable condition. As with the stable condition, the right direction shows the slowest decay and the forward and upward directions show more rapid decay with distance. The slopes of the decay curves range from -1.96 to -2.43 with an average of -2.16 and a standard deviation of 0.17. Thus, the range of slopes is narrower and the slopes are slightly steeper; consistent with better mixing. There is a tenfold range in concentration at 0.5 meters for this stability condition.



These results can be summarized in the form of a model to predict concentration within one meter from a point source in a laboratory environment. The straight lines shown in the figures 6 to 8 can be represented as power functions giving the decay of concentration with distance from a point source. Thus, for the stable condition, concentration at a distance x from a point source C(x) can be expressed by

$$C(x)=Ax^{b}=3{,}714x^{-2.02} \qquad (1)$$

where x is in centimeters. For x in meters Equation (1) becomes

$$C(x)=0.339x^{-2.02} \qquad (2)$$

For the moderate activity condition the single best equation is

$$C(x)=4{,}298x^{-2.16} \qquad (3)$$

for x in cm.

While the data shown here are limited, the following is our best estimate of the effect of the two factors that appear to influence the rate of decay. For the stable condition the exponent in equation 1, b = - 2.02 can be replaced by,

$$b=-1.89+d \qquad (4)$$

where:

d = - 0.42 if no reflecting surfaces are present in the direction of interest.

d = + 0.12 if direction is in the prevailing air motion direction and a reflecting surface, such as a counter, is nearby in that direction.
d = 0 for all other conditions.

For the moderate activity condition the exponent becomes

$$b=-2.28+d \qquad (5)$$

where:

d = + 0.32 if the direction is in the prevailing wind direction and a reflecting surface, such as a lab bench, is nearby in that direction.
d = 0 for all other conditions.

Despite the limitations of these tests they do provide a detailed concentration decay model, not previously available, for concentration decay for distances of 0.01 to 1.0 m from a point source in a laboratory setting.

Recommendations:
The results outlined here are very promising, consequently we recommend that these ideas be developed further so that they can be put into practice. Specific needs are: (1) to evaluate concentration decay over a wider range of conditions so that the physical factor that affect decay rate can be identified; (2) to establish correlations between anemometry measured eddy diffusion coefficients and concentration decay rates; (3) to develop methods to predict decay rate and concentration at a location from anemometry data and source strength; and (4) to perform a field evaluation of these methods for well-defined single source exposure situations.

Conclusion:
There is very little published data on the transport of airborne contaminants within the indoor environment. The development of a near-field dispersion model to predict contaminant concentrations would provide an efficient screening tool for indoor work environments.

The use of the three-axis sonic anemometer is a promising technique in the development of a near-field dispersion model. The 3-axis anemometer data can be used in characterizing the turbulent mixing characteristics of indoor environments with varying levels of turbulent intensity. Through the use of computer data analysis programs, such as the serial correlation and defined angle range programs, this anemometer data can be used to estimate eddy diffusion coefficients. Eddy diffusion coefficients can then be coupled with mass-balance models to give more accurate predictions of contaminant concentrations within the near field (0 to 3 meters) of the indoor environment.

The data analysis programs previously described has proven successful at estimating eddy diffusion coefficients in a variety of turbulent conditions. The eddy diffusion coefficients calculated based on the anemometer data can be compared with those calculated by iteratively solving mass-balance concentration equations. Predicted concentration profiles obtained from mass-balance models can be compared against actual measured concentrations to determine which eddy diffusion coefficients provide the most accurate concentration estimates.

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3. Application of biologic monitoring and biomarkers of exposure for exposure assessment and hazard surveillance

To make use of biologic monitoring, biomarkers of exposure, and toxicokinetic modeling to better estimate internal and target tissue dose from exposure to single and multiple chemical agents and evaluate interactive effects associated with toxicokinetic interaction.

The published, accepted, or submitted papers or reports for this section are found in Appendix B. There are seven documents. The titles are as follows:

Toxicokinetic interaction of 2,5-hexanedione and methyl ethyl ketone, R.C. Yu, D. Hattis, and J.R. Froines, Submitted to Archives of Toxicology

Progress report: Metabolic interaction between styrene and butadiene, A. Cho, D. Schmitz and J.R. Froines

Short term non-invasive biomarkers for the processes of producing long term lung damage—evaluation of the feasibility of candidate measurement systems; S. Eylam, D. Hattis, and J. R. Froines.

Optimized portable cordless vacuum method for sampling dry, hard surfaces for dusts, S.Y. Kim, S. Que Hee, and J.Froines, Appl. Occup. Environ. Hyg., 15: 503-511.

Surface sampling for a pesticide in dust and small spills of a solid dye, S. Que Hee, Y. Shen, and J.C. Tso, Accepted, Appl. Occup. Environ. Hyg.

Effect of dust on the viability of Vibrio fischeri in the microtox test, Ecotoxicol. Environ. Safety, Submitted, Ecotox. Environ. Safety

Microtox test as a validation method for surface sampling of bacteria in dust, K. Park, Masters thesis, Department of Environmental Health Sciences, UCLA School of Public Health

Dr. John R. Froines and colleagues

Rong Chun Yu, Dale Hattis, Elliot M. Landaw, John R. Froines

Toxicokinetic Interaction of 2,5-Hexanedione and Methyl Ethyl Ketone

Abstract
Co-exposure to methyl ethyl ketone (MEK) potentiates the neurotoxicity of n-hexane in humans as well as in animals. This effect is associated with increased persistence of 2,5-hexanedione (2,5-HD) in blood, probably due to inhibition of 2,5-HD metabolism by MEK. There is no previous quantitative toxicokinetic model describing this interaction. In this study we constructed a toxicokinetic model to depict the inhibition of 2,5-HD metabolism by MEK. Experimental data of 2,5-HD blood concentrations in rats from a published study were used to estimate model parameters. Three different inhibition mechanisms: competitive, uncompetitive, and noncompetitive, were evaluated. Extrapolation from high to low doses was made to assess the effects of MEK on 2,5-HD metabolism beyond the experimental conditions. We found the developed model successfully described the toxicokinetic behavior of 2,5-HD that was inhibited by MEK. The competitive inhibition was regarded as the most probable mechanism ($V_{max,HD}$ = 7.6 mg/hr, K_m = 32.2 mg/L, and $K_{i,MEK}$ = 65.5 mg/L) to co-exposure to MEK. The biological half-life of 2,5-HD appeared to be a linear combination of the apparent Michaelis-Menten constant, 2,5-HD and MEK concentrations in the blood of rats. The AUC of 2,5-HD in rats was a nonlinear function of 2,5-HD and MEK concentrations in the blood. This study highlights the importance of the effect of metabolic deactivation of 2,5-HD by MEK, illustrating the advantage of toxicokinetic modeling to investigate chemical interactions associated with exposure to multiple chemical agents.

Keywords: chemical mixture, toxicokinetic interaction, competitive inhibition, 2,5-hexanedione, methyl ethyl ketone, biological half-life, AUC

Abbreviations: 2,5-HD: 2,5-hexanedione, MEK: methyl ethyl ketone, MnBK: methyl n-butyl ketone, AUC: area under the serum concentration-time curve

Symbols: V_{max}: rate of maximum reaction of Michaelis-Menten kinetics (mg/hr); K_m: apparent constant of Michaelis-Menten kinetics (mg/L); $V_{max,HD}$: rate of maximum reaction of 2,5-HD metabolism (mg/hr); $K_{m,HD}$: apparent Michaelis-Menten constant of 2,5-HD metabolism (mg/L); $V_{max,MEK}$: rate of maximum reaction of MEK metabolism (mg/hr); $K_{m,MEK}$: apparent constant of MEK metabolism (mg/L); $K_{i,MEK}$: constant of 2,5-HD inhibition by MEK (mg/L); $K_{i,HD}$: constant of MEK inhibition by 2,5-HD (mg/L); M_{HD}: mass of 2,5-HD in the blood (mg); M_{MEK}: mass of MEK in the blood (mg); C_{HD}: blood concentration of 2,5-HD (mg/L); $C_{HD}(0)$: initial blood concentration of 2,5-HD (mg/L) after administration of 2,5-HD; C_{MEK}: blood concentration of MEK (mg/L); V_{HD}: volume of distribution of 2,5-HD (L or mL), equivalent to blood; V_{MEK}:

volume of distribution of MEK (L or mL), equivalent to blood; BW^{rat}: body weight of rats (kg); BW^{human}: body weight of humans (kg); MW_{HD}: molecular weight of 2,5-HD (mg/mol)

Introduction

Humans are often exposed to multiple chemical agents in environmental and occupational settings. There is limited research on chemical interactions that result in enhancement or attenuation of toxicological outcomes. These interactions can be broadly divided into toxicokinetic or toxicodynamic phases. As seen in Figure 1 (*1-4*), toxicokinetic interactions may occur during the process of absorption, metabolic activation and deactivation, and elimination. Toxicodynamic interactions take place during cellular and molecular processes of binding to macromolecules or cellular membranes, repair and disassociation of macromolecular adducts, damage, and recovery or regeneration of target cells. The focus of this research was to investigate the toxicokinetic basis of metabolic interactions that were responsible for the enhancement of potential health effects due to co-exposure to 2,5-hexanedione (2,5-HD) and methyl ethyl ketone (MEK).

n-Hexane is known to cause neurotoxicity among occupationally exposed individuals and there are numerous reports of polyneuropathies associated with "glue sniffing" of hexane containing solvents and glues (*5-7*). The neurotoxic effect is characterized by loss of sensation and distal reflexes in the feet and hands after prolonged exposure to n-hexane. The current knowledge to explain this pathogenesis is formation of pyrrole from a hexane metabolite 2,5-HD, and subsequent reaction of the oxidized pyrrole with protein nucleophiles (*7*). In addition to central-peripheral distal axonopathy, experimental animals exposed to 2,5-HD exhibit testicular atrophy (*8,9*), possibly resulting from the apoptosis of the germ cells (*10,11*).

Metabolism of n-hexane involves complex pathways (Figure 2). n-Hexane is first metabolized by hepatic mixed-function oxidase system to form 2-hexanol. The latter is metabolized further to either methyl n-butyl ketone (MnBK) or 2,5-hexanediol and then to 5-hydroxy-2-hexanone. Oxidation of 5-hydroxy-2-hexanone leads to the formation of 2,5-HD (*12*). 2,5-HD may be excreted from urine in free form or undergoes further metabolism to produce 4,5-dihydroxy-2-hexanone that is excreted from urine as the glucuronide (*13*). 2,5-HD is considered the ultimate toxic metabolite and the neurotoxic potency of n-hexane is strongly correlated with the area under the serum concentration-time curve (AUC) of 2,5-HD, not the applied dose of n-hexane (*12,14*).

MEK is often used as a solvent in the manufacturing of colorless synthetic resins, artificial leather, rubbers, lacquers, varnishes, and glues. It is usually found in mixtures with other solvents, including n-hexane (*15*). Neurotoxicity increases as a result of co-exposure to MEK and either n-hexane, MnBK, or 2,5-HD in human studies, in vivo, and in vitro studies. The enhanced effects of MEK on n-hexane-induced neurotoxicity are unequivocal. MEK metabolism potentially interferes with n-hexane metabolism and has implications for toxicokinetic interaction. MEK is first metabolized to either 2-butanol, a minor process, or 3-hydroxy-2-butanone, a microsomal ω-1 oxidation and the limiting step of MEK metabolism. The latter metabolite is further metabolized to 2,3-butanediol (*16,17*). Because of similarities between the biotransformation of MEK and n-hexane it is plausible there may be competition between the two pathways.

Co-exposure to MEK may affect more than one step in the metabolism of n-hexane. Previous experiments suggest the concentration of 2,5-HD in blood decreases more slowly when rats are simultaneously exposed to MnBK and MEK (*18*), n-hexane and MEK (*19*), as well as 2,5-HD and MEK (*20*). AUC of 2,5-HD in blood increases in greater values in rats treated with 2,5-HD and MEK than those treated with 2,5-HD alone (*14*). The clearance of 2,5-HD in urine increases by 6-fold in animals co-administered both MnBK and MEK (*21*), but decreases in animals co-exposed to n-hexane and MEK (*22-24*). Krishnan and his colleagues (25) suggest hexane is an example of exposure to a single chemical that results in concomitant exposure to many chemicals because of the complex biotransformation processes. Co-exposure to MEK further complicates these interactions. Currently, there is no information in the literature describing these complex interactions. To investigate such complexity in a manageable manner it is necessary to seek simplifying assumptions to limit the study to a workable scope.

The objective of this study was to evaluate the efficacy of toxicokinetic modeling to investigate toxicological interaction between 2,5-HD and MEK. A toxicokinetic interaction model was developed and its kinetic parameters were estimated. The underlying toxicokinetic mechanisms of interaction: competitive, noncompetitive, or uncompetitive inhibition, were evaluated. The model was extrapolated from high to low doses to examine the effect of co-exposure to MEK on 2,5-HD AUC.

Materials and Methods

Materials

Published data of Yasui et al. (*20*) that investigates the blood concentration of 2,5-HD as influenced by co-exposure to 2,5-HD and MEK were selected to estimate model parameters. The authors subcutaneously injected 2.6 mmol/kg 2,5-HD alone or 2.6 mmol/kg 2,5-HD plus MEK at 2.6 mmol/kg and 13 mmol/kg to male Wistar rats. Following treatment for 0.5, 1, 2, 4, 8, and 16 hr, they sacrificed the animals and determined the blood concentrations of 2,5-HD.

A Model of Toxicokinetic Interaction between 2,5-HD and MEK

We addressed three issues on the toxicokinetic behavior of 2,5-HD as influenced by MEK: metabolism of 2,5-HD, metabolism of MEK, and the toxicokinetic interaction between 2,5-HD and MEK. A single saturable pathway was used to describe the deactivation and elimination of 2,5-HD in blood (Figure 3). 2,5-HD was assumed to be instantaneously available to the systemic circulation in rats administered 2,5-HD via subcutaneous injection. After the treatment, the mass balance governing 2,5-HD in the blood was

$$\frac{dM_{HD}}{dt} = -\frac{V_{max,HD} \cdot C_{HD}}{K_{m,HD} + C_{HD}}, \qquad (1)$$

where M_{HD} = mass of 2,5-HD in the blood (mg); $C_{HD} = M_{HD}/V_{HD}$, concentration of 2,5-HD in the blood (mg/L); V_{HD} = volume of distribution of 2,5-HD (L), equivalent to blood; $V_{max,HD}$ = maximum elimination rate of 2,5-HD (mg/hr); and $K_{m,HD}$ = apparent Michaelis constant for 2,5-HD metabolism (mg/L).

Similarly, a single compartment model was used to model the metabolism of MEK in blood (Figure 3), with parameters $V_{max,MEK}$ and $K_{m,MEK}$. Following the treatment of MEK, the mass balance governing MEK in the blood was

$$\frac{dM_{MEK}}{dt} = -\frac{V_{max,MEK} \cdot C_{MEK}}{K_{m,MEK} + C_{MEK}}, \quad (2)$$

where M_{MEK} = mass of MEK in the blood (mg); $C_{MEK} = M_{MEK}/V_{MEK}$, concentration of MEK in the blood (mg/L); V_{MEK} = volume of distribution of MEK (L), equivalent to blood; $V_{max,MEK}$ = maximum elimination rate of MEK (mg/hr); and $K_{m,MEK}$ = apparent Michaelis constant for MEK metabolism (mg/L).

Figure 3 depicts the toxicokinetic model of the interaction between 2,5-HD and MEK. The interaction may occur by one of three inhibition modes: competitive, uncompetitive, or noncompetitive. Competitive inhibition occurs when an inhibitor competes directly with a normal substrate for the same binding sites available on the enzyme. If MEK competitively inhibits 2,5-HD metabolism, Equation (1) can be modified (*26*), as follows,

$$\frac{dM_{HD}}{dt} = -\frac{V_{max,HD} \cdot C_{HD}}{K_{m,HD} \cdot \left[1 + C_{MEK}/K_{i,MEK}\right] + C_{HD}}, \quad (3)$$

where $K_{i,MEK}$ (mg/L) in Equation (3) refers to the constant for competitive inhibition of 2,5-HD metabolism by MEK.

Uncompetitive inhibition results from an inhibitor binding to the enzyme-substrate complex to produce an inactive enzyme-substrate-inhibitor complex. The presence of the inhibitor molecule not only affects formation of the final product but also influences production of the enzyme-substrate complex. If the interaction of 2,5-HD and MEK occurred under this mechanism, Equation (1) can be modified (*26*), as follows

$$\frac{dM_{HD}}{dt} = -\frac{C_{HD} \cdot \left[\frac{V_{max,HD}}{1 + C_{MEK}/K_{i,MEK}}\right]}{C_{HD} + \left[\frac{K_{m,HD}}{1 + C_{MEK}/K_{i,MEK}}\right]}, \quad (4)$$

where $K_{i,MEK}$ (mg/L) in Equation (4) refers to the constant for uncompetitive inhibition of 2,5-HD metabolism by MEK.

In noncompetitive inhibition, an inhibitor binds not only to the free enzyme to form an enzyme-inhibitor complex but also to the enzyme-substrate complex to generate an inactive enzyme-substrate-inhibitor complex. If the interaction of 2,5-HD and MEK operated in this mode, Equation (1) can be modified (*26*), as follows

$$\frac{dM_{HD}}{dt} = -\frac{C_{HD}\cdot\left[\dfrac{V_{max,HD}}{1+C_{MEK}/K_{i,MEK}}\right]}{K_{m,HD}+C_{HD}}, \tag{5}$$

where $K_{i,MEK}$ (mg/L) in Equation (5) refers to the constant for noncompetitive inhibition of 2,5-HD metabolism by MEK.

Influence of Metabolic Inhibition on Biological Half-Life of Chemical Mixtures

The biological half-life of 2,5-HD ($T_{½,HD}$) as inhibited by MEK, by definition (*27*), can be described as:

$$T_{1/2,HD} = \frac{0.693\cdot V_{HD}}{\dfrac{dM_{HD}}{dt}/C_{HD}}, \tag{6}$$

where V_{HD}, is volume distribution of 2,5-HD and the denominator $\left(\dfrac{dM_{HD}}{dt}/C_{HD}\right)$ is the clearance (*27*) of 2,5-HD. In the case of MEK competitively inhibiting 2,5-HD metabolism, substitution of Equation (3), without the negative sign for elimination, into Equation (6) and rearrangement of the latter equation give

$$T_{1/2,HD} = \frac{0.693\cdot V_{HD}}{V_{max,HD}}\left(K_{m,HD}+C_{HD}+\frac{K_{m,HD}}{K_{i,MEK}}\cdot C_{MEK}\right). \tag{7}$$

In the cases of MEK uncompetitively and noncompetitively inhibiting 2,5-HD metabolism, the $T_{½,HD}$ can be written in Equations (8) and (9), respectively, as follows:

$$T_{1/2,HD} = \frac{0.693\cdot V_{HD}}{V_{max,HD}}\left(K_{m,HD}+C_{HD}+\frac{C_{HD}}{K_{i,MEK}}\cdot C_{MEK}\right), \tag{8}$$

and

$$T_{1/2,HD} = \frac{0.693\cdot V_{HD}}{V_{max,HD}}\left(K_{m,HD}+C_{HD}+\frac{K_{m,HD}+C_{HD}}{K_{i,MEK}}\cdot C_{MEK}\right). \tag{9}$$

Estimation of Model Parameters

Hira et al. (*28*) report the values of $V_{max,MEK}$ and $K_{m,MEK}$ for humans, 30 μmol/min (=130 mg/hr) and 2 μM (=0.14 mg/L), respectively. In this study, we assumed the value of $K_{m,MEK}$ of rats was

the same as that in Liira's study, i.e., 0.14 mg/L. Based on the principle described by Gargas et al. (*29*), the value of $V_{max,MEK}$ was extrapolated from humans to rats, as follows

$$V_{max,MEK}^{rat} = V_{max,MEK}^{human} \times \left(\frac{BW^{rat}}{BW^{human}} \right)^{0.7} = 130\ mg/hr \times \left(\frac{0.272\ kg}{71\ kg} \right)^{0.7} = 2.64\ mg/hr,$$

where body weight (BW) of rats and humans were based on data reported by Yasui et al. (*20*) and Liira et al. (*28*), respectively. Table 1 shows the equivalent volume of distribution of MEK to blood (V_{MEK} = 280 ml, blood equivalent), which was estimated by physical volume of the major physiological compartments (including fat, liver, slow-perfusion tissue group, rich-perfusion tissue group, and liver) of rats and the tissue/blood partition coefficients of MEK in these compartments.

The AR program in BMDP (30) was used to fit experimental data and estimate kinetic parameters. The program is a derivative-free nonlinear regression procedure that estimates the parameters for a wide variety of nonlinear functions by the method of least squares (or equivalent to the method of maximum likelihood) using a pseudo-Gauss-Newton iterative algorithm. It simultaneously estimated 4 unknown model parameters: $V_{max,HD}$, $K_{m,HD}$, V_{HD}, and $K_{i,MEK}$, where V_{HD} was calculated as follows:

$$V_{HD} = \frac{BW^{rat} \times Dose \times MW_{HD}}{C_{HD}(0)} \times 1000, \qquad (10)$$

In Equation (10), BW^{rat} = 0.272 kg (*20*), Dose = 2.6 mmol/kg (*20*), MW_{HD} = 114 mg/mmol, and $C_{HD}(0)$, the initial blood concentration of 2,5-HD (mg/L). The AR program run three separate models that assumed the toxicokinetic interaction between 2,5-HD and MEK were competitive, un-competitive, and non-competitive inhibition, respectively. It reported the estimates and asymptotic standard errors of kinetic parameters.

Model Simulations

Simulation studies were carried out to extrapolate the effect of co-exposure to MEK on the AUC of 2,5-HD from high to low doses. We run a total of 186 simulations, including any combinations of six 2,5-HD regimens (0.1, 0.3, 0.6, 1.2, 2.0 and 3.0 mmol/kg) and thirty-one MEK regimens (0 to 3.0 mmol/kg, with an increment of 0.1 mmol/kg). In each simulation experiment, the AUC of 2,5-HD was estimated by the trapezoidal rule, an approximate numeric integration algorithm (*27*).

Results

The developed model, which assumed MEK competitively inhibited 2,5-HD metabolism, successfully fit the experimental data of Yasui et al. (*20*) (Figure 4). The estimates of the kinetic parameters were $V_{max,HD}$ = 7.6 mg/hr, $K_{m,HD}$ = 32.2 mg/L, V_{HD} = 264 mL, and $K_{i,MEK}$ = 65.5 mg/L. It not only predicted the behavior of 2,5-HD in the blood of the rats exposed to 2.6 mmol/kg 2,5-HD alone (the line labeled with "HD" in Figure 4) reasonably well but also those exposed to 2.6 mmol/kg 2,5-HD plus 2.6 mmol/kg MEK (the line labeled with "HD+MEK") and 2.6 mmol/kg 2,5-HD plus 13 mmol/kg MEK (the line labeled with "HD+5MEK"). This

toxicokinetic model showed the persistence of 2,5-HD in rats was MEK dependent; the higher MEK dose the more profound the persistence of 2,5-HD.

Figures 5 and 6 show the uncompetitive and noncompetitive inhibition models also fit reasonably well to the experimental data. The goodness-of-fit was not substantially different from that of the competitive model (Figure 4). In comparison with the kinetic parameters, the values of V_{HD}, $V_{max,HD}$, and $K_{m,HD}$ were not substantially different (Table 2). The mean squared error (MSE) obtained from the competitive model (MSE=0.008124) was smaller than, but not substantially different from, those of the uncompetitive (MSE=0.008747) and noncompetitive (MSE=0.008424) models. The inhibition constant for competitive inhibition model ($K_{i,MEK}$ = 65.5 ±8.5 mg/L) was significantly lower than those of noncompetitive inhibition (403±86.5 mg/L) and uncompetitive inhibition (440±77.1 mg/L).

The developed toxicokinetic model was used to extrapolate the behavior of 2,5-HD in rats as influenced by MEK from high to low doses. Figure 7 depicts the AUC of 2,5-HD as a function of the co-exposed dose of MEK when rats are co-administered 2,5-HD at 0.1, 0.3, 0.6, 1.2, 2.0, and 3.0 mmol/kg. The figure shows the $\log_{10}$(AUC) approximately linearly increases with increase in MEK dose ranging from 0 to 3 mmol/kg. This corresponds to an increase in AUC by a factor of approximate 10^{β} per unit dose of MEK, where β is the slope of the linear relationship.

Table 3 summarizes the slopes of the linear relationships and the corresponding values of 10^{β} at various doses of 2,5-HD. The slopes decreased from 0.399 to 0.09 [$\log_{10}$(AUC) per mmol/kg MEK] with increase in 2,5-HD doses from 0.1 to 3.0 mmol/kg. For every increment of 1 mmol/kg MEK, the AUC approximately increases 2.5 fold in the rat co-administered 0.1 mmol/kg 2,5-HD. In comparison, the AUC approximately increases only 1.23 fold at 3.0 mmol/kg 2,5-HD (Table 3).

Equations (7)-(9) show $T_{½,HD}$ is a linear combination of $K_{m,HD}$, C_{HD} and C_{MEK}. Figure 8 depicts the $T_{½,HD}$ as a function of blood MEK concentration at various blood concentrations of 2,5-HD at 10, 75, 150, 250 and 350 mg/L. At 10 mg/L 2,5-HD, approximately equal to the initial 2,5-HD concentration of the rats subcutaneously injected with 0.1 mmol/kg, the $T_{½,HD}$ was 1 hr when there was no inhibition by MEK. In comparison, at 350 mg/L, approximately equal to the initial 2,5-HD concentration of the rats exposed to 3 mmol/kg, the half-life was 9.2 hr. When rats were co-administered with MEK, $T_{½,HD}$ increased 1.18 hr for every increment of 100 mg/L blood concentration of MEK.

Discussion

The objective of this study was to use toxicokinetic modeling to investigate the mechanistic basis for the interaction of MEK with 2,5-HD. The model focused upon the metabolism and elimination of 2,5-HD, without considering the complex interactions of intermediary metabolites (Figures 1 and 2). The agreement between model predictions and experimental data suggested reasonable model specification and parameter estimation, as well as the probable mechanism of metabolic inhibition between 2,5-HD and MEK. The model, although not physiologically based, captured the essential feature of 2,5-HD metabolism and its interaction with MEK and demonstrated the utility of toxicokinetic modeling in investigating metabolic interactions

between chemical mixtures. It highlighted the significance of inhibiting deactivation of a toxic metabolite on its persistence in the body.

Metabolic inhibition was the mechanism likely responsible for increased persistence of 2,5-HD in rats co-exposed to 2,5-HD and MEK. Ralston et al. (*14*) found concomitant administration of MEK reduced blood 2,5-HD clearance and increased the AUC of 2,5-HD. Yasui et al. (*20*) demonstrated that blood 2,5-HD concentration in rats increased significantly in the co-exposure groups and that the increase was MEK dose-dependent. These experiments unequivocally demonstrated that increased persistence of 2,5-HD was due to MEK. However, the kinetic mechanism had not been elucidated by these two studies. We employed a toxicokinetic model to test the hypothesis that inhibition of metabolism was the likely mechanism for the increased persistence of 2,5-HD in animals co-exposed to 2,5-HD and MEK. Our results suggest a single Michaelis-Menten kinetic pathway of the elimination satisfactorily describes 2,5-HD metabolism in the rat.

The method of goodness-of-fit did not clearly identify the mode of metabolic inhibition in this study although it has been used in other studies to elucidate the inhibitory mechanism of metabolic interactions between chemicals (*37,43-47*). In most cases, there is clear evidence that a particular inhibition mechanism provides better goodness-of-fit than the others in toxicokinetic modeling. However, as shown in Figures 4-6, it was difficult to discern which inhibition mechanism provided the best fit by comparing the goodness-of-fit in the figures. The mean squared error for the competitive inhibition model was slightly smaller than those for the uncompetitive and the noncompetitive models (Table 2). But, the differences could not be statistically tested because these three models were not nested. These results suggest that the goodness-of-fit of toxicokinetic modeling to experimental data may not always result in clearly differentiating mechanisms of inhibition mechanism in metabolic interaction studies.

In the case of 2,5-HD inhibition by MEK, the competitive mode might have operated more efficiently than uncompetitive and noncompetitive modes. From the perspective of toxicokinetic modeling, the major difference among these three inhibition modes was the inhibition constant $K_{i,MEK}$ (Table 2). Fitting the experimental data of Yasui et al. (*20*) to the competitive mode gave the smallest estimate of the inhibition constant (65.5 mg/L), compared to those of the uncompetitive (403 mg/L) and the noncompetitive modes (440 mg/L). These results were consistent with those found in the study of Thrall et al. (*45*), in which the inhibition constants of toluene and trichloroethylene in the competitive mode were reported to be smaller than those of the uncompetitive and noncompetitive modes. The authors argued that competitive inhibition was the most plausible type of metabolic interaction between these two solvents because the inhibition constants are closest to the corresponding Michaelis-Menten constants. We considered competitive inhibition to be operating more efficiently than uncompetitive and noncompetitive modes because of a much lower inhibition constant for competitive inhibition.

Extrapolation of the behavior of 2,5-HD AUC in the blood from high to low doses of 2,5-HD and MEK was the major application for the toxicokinetic model developed in this study. The experimental data of Yasui et al. (*20*) focused on the behavior of 2,5-HD as influenced by MEK in high dose scenarios. The concave nature the of 2,5-HD concentration profiles highlights the saturation of 2,5-HD metabolism (HD lines in Figures 4-6) and increased persistence of 2,5-HD

by co-administration of MEK (HD+MEK and HD+5MEK lines). Since 2,5-HD AUC has been shown to correlate with the neurotoxic potency of n-hexane (*12,14*), it was extrapolated from high doses, more often used in toxicological experiments, to low dose regions, relevant at the exposure levels found in the environment or even the workplace. Figure 7 depicts the predicted 2,5-HD AUC in rats subcutaneously co-administered with 2,5-HD and MEK at a variety of dose ranges. Although our study applied toxicokinetic modeling to shed new light upon the impact of MEK on 2,5-HD metabolism, we did not take into consideration of species extrapolation (rats to humans), route differences (subcutaneous injection to inhalation), and exposure regimen (acute to chronic) for the developed toxicokinetic model. Further studies are needed to address these issues.

The 2,5-HD $T_{½}$ is quantitatively related to the kinetic parameters of metabolic inhibition. Yasui et al. (*20*) showed MEK is capable of prolonging the 2,5-HD $T_{½}$ in the blood, which was thought to relate to the potentiation of 2,5-HD neurotoxicity by MEK. Zhao et al. (50) showed MEK (as well as acetone and toluene) decreased the elimination rate constants of 2,5-HD (or equivalent to prolong 2,5-HD $T_{½}$) in co-administration groups. These empirical observations consistently suggest a qualitative relationship between 2,5-HD $T_{½}$ and the concentrations of 2,5-HD and MEK. However, there have been no quantitative relationships documented in the literature. In this study, we showed that 2,5-HD $T_{½}$ following co-administration of 2,5-HD and MEK was a linear combination of the apparent Michaelis-Menten constant, the blood concentrations of 2,5-HD and MEK, and depended on the nature of the metabolic inhibition [Equations (7)-(9)]. The limiting conditions of these relationships are discussed below:

1. At low concentrations of 2,5-HD ($C_{HD} \rightarrow 0$) and no MEK inhibition ($C_{MEK} = 0$), $T_{½,HD}$ in Equations (7)-(9) is reduced to a constant

$$T_{1/2,HD} = \frac{0.693 \cdot V_{HD} \cdot K_{m,HD}}{V_{max,HD}},$$

a limiting condition of linear kinetics of metabolism.

2. When C_{HD} concentrations are relatively high and no MEK inhibition occurs ($C_{MEK} = 0$). Equations (7)-(9) can be reduced to

$$T_{1/2,HD} = \frac{0.693 \cdot V_{HD}}{V_{max,HD}}(K_{m,HD} + C_{HD}),$$

a nonlinear kinetic condition of 2,5-HD metabolism that is consistent with the derivation by Gibaldi and Perrier (*27*).

3. When the blood concentration of MEK is high $T_{½,HD}$ is also a function of the MEK concentration that is multiplied by a factor $\left(\frac{K_{m,HD}}{K_{i,MEK}}\right)$, $\left(\frac{C_{HD}}{K_{i,MEK}}\right)$, or $\left(\frac{K_{m,HD} + C_{HD}}{K_{i,MEK}}\right)$ for

competitive, uncompetitive, or noncompetitive inhibition, respectively. These factors suggest that competitive inhibition operates more effectively in prolonging $T_{½,HD}$ than uncompetitive and noncompetitive inhibition modes because the inhibition constant of former mode is 6 fold lower (Table 2).

Although 2,5-HD and MEK can theoretically interact with each other (Figure 3), inhibition of MEK metabolism by 2,5-HD is relatively unimportant. We conducted a sensitivity analysis to examine the impact of the competitive inhibition constant of MEK by 2,5-HD ($K_{i,HD}$) on other kinetic parameters and mean squared errors. Our analyses found the models including $K_{i,HD}$ above 600 mg/L did not improve the goodness-of-fit at all, in comparison to the model including $K_{i,HD}$ at 600 mg/L. Under the latter condition, other kinetic constants were estimated to be: V_{HD} = 264.5 mL, $V_{max,HD}$ = 7.6 mg/hr, $K_{m,HD}$ = 32.2 mg/L, and $K_{i,MEK}$ = 66.0 mg/L. These values were very close to those estimated when $K_{i,HD}$ was not included in the model (V_{HD} = 264.3 mL, $V_{max,HD}$ = 7.6 mg/hr, $K_{m,HD}$ = 32.2 mg/L, and $K_{i,MEK}$ = 65.5 mg/L, see Table 2). The mean squared errors between these two conditions differed by only 1.5%. In contrast, when $K_{i,HD}$ was less than 600 mg/L, the kinetic parameters gradually departed from the values shown in Table 2. The mean squared errors also increased substantially, suggesting poor goodness-of-fit under the conditions of $K_{i,HD}$ < 600 mg/L. Because the $K_{i,HD}$ did not improve the fit, we eventually eliminated $K_{i,HD}$ from the model to make it simpler without loss of descriptive power.

Metabolic interactions occurring in the process of n-hexane activation to 2,5-HD could affect the metabolism of n-hexane in the body. Andersen and Clewell (*31*) considered multiple competitive interactions between hexane, MnBK and 2,5-HD. They used PBPK modeling to describe the kinetics of the three compounds and suggested at high concentrations or continuous exposure the formation of 2,5-HD may be inhibited in part because 2,5-HD and MnBK might inhibit hexane metabolism. In a study of Iwata et al. (*23*), rats inhaled n-hexane plus MEK at 1000 ppm for 8 hrs. Forty-eight hour urinary excretion of 2-hexanol decreased in the animals exposed to n-hexane and MEK, suggesting production of 2-hexanol was inhibited by MEK. In another study (*24*), rats were exposed to hexane and MEK at 500 ppm 8 hrs a day for 33 weeks. The authors demonstrated a similar decrease in 2-hexanol elimination. The studies of Shibata et al. (*22,32*) showed that 2-hexanol level in serum and its elimination in urine decreased in animals exposed to mixture of hexane and MEK, compared to those exposed to hexane only. In a study of human volunteers exposed to a mixture of hexane and MEK (*33*), the serum concentration of 2,5-HD decreased and the time to reach maximum concentration of 2,5-HD increased. Previous studies show hydroxylation of hexane is inhibited by the presence of MEK as a result of competition for the CYP2E1 enzyme that is required for biotransformation of hexane (*34*) and MEK (*35,36*). In brief, many steps in the activation of n-hexane to 2,5-HD, as outlined in Figure 2, can be affected by co-exposure to MEK. Our study could not address this issue.

There is no compelling evidence to indicate that MEK affects the toxicity of hexane or MnBK at the toxicodynamic level. In one relevant report (*14*), rats were treated with 2.2 mmol/kg/day 2,5-[^{14}C]HD alone and in combination with 2.2 mmol/kg/day MEK for 3 weeks. Total ^{14}C activity was examined in peripheral nerve crude homogenate, neurofilament-enriched fraction of peripheral nerve, spinal cord crude homogenate, and neurofilment-enriched fraction of spinal cord. The binding of radiolabeled 2,5-HD to the nervous tissues and their corresponding neurofilament-enriched fractions in rats exposed to 2,5-HD plus MEK were not consistently

different from that in animals exposed to 2,5-HD alone. These results indicate that enhancement of n-hexane-induced neurotoxicity by MEK does not result from increased binding of 2,5-HD to neurofilament at toxicodynamic level.

We conclude that the persistence of 2,5-HD in the blood of experimental animals probably results from competitive inhibition of 2,5-HD metabolism by MEK. The estimated values of kinetic parameters provide a quantitative basis for description of 2,5-HD deactivation metabolic process in the presence of MEK. The biological half-life of 2,5-HD appears to be a linear combination of the apparent Michaelis-Menten constant, 2,5-HD and MEK concentrations in the blood of rats. The AUC of 2,5-HD in rats is a nonlinear function of 2,5-HD and MEK concentrations in the blood. This study demonstrates the utility of toxicokinetic modeling in the investigation of metabolic interactions between chemical mixtures.

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Table 1. Estimation of Distribution Volume of MEK

Compartment	Volume[a], ml	Tissue/Blood[b]	Volume, ml (blood equiv.)
Fat	19.0	0.88	17
Liver	10.9	0.98	11
SPG	204.0	1.16	237
RPG	13.6	1.12[c]	15
Total			280

[a]Fat, liver, SPG (slow-perfusion tissue group), and RPG (rich-perfusion tissue group) consist of 4, 7, 5, and 75% of tissue volume, respectively (*41*).
[b]Tissue/blood partition coefficients were estimated by Perbellini et al. (*42*) for humans.
[c]This value was an average of tissue/blood partition coefficients of kidney, brain, and heart reported by Perbellini et al. (*42*).

Table 2. Parameters used to Fit Models of Different Inhibition Mechanisms

	Mode of Inhibition		
	Competitive	Uncompetitive	Noncompetitive
V_{HD} (mL)	264.3 ± 6.9[a]	275 ± 7.0	273.9 ± 6.9
$V_{max,HD}$ (mg/hr)	7.6 ± 0.7	6.7 ± 0.6	6.9 ± 0.6
$K_{m,HD}$ (mg/L)	32.2 ± 8.7	20.7 ± 7.0	22.8 ± 7.7
$K_{i,MEK}$ (mg/L)	65.5 ± 8.5	403.0 ± 86.5	440.2 ± 77.1
Mean Squared Error	0.008124	0.008747	0.008428

[a] Mean ± (asymptotic) standard errors.

Table 3. The Slope (β) of the Linear Relationship between $\log_{10}$(AUC) and MEK and 10^{β} at various Doses of 2,5-HD

2,5-HD Dose (mmol/kg)	β [$\log_{10}$(AUC) per MEK dose]	10^{β}
0.1	0.399	2.50
0.3	0.340	2.19
0.6	0.278	1.90
1.2	0.198	1.58
2.0	0.136	1.37
3.0	0.090	1.23

Captions of Figures

Figure 1. Paradigm of potential toxicological interactions of chemical mixtures [adapted from (*1*)-(*4*)].

Figure 2. Metabolism of n-hexane and its metabolites [adapted from (*12*) and (*13*)].

Figure 3. A diagram presentation of a metabolic interaction model of 2,5-HD and MEK. The big compartment represents the overall model that consists of two distinct compound-specific compartments: 2,5-HD and MEK. Elimination of the compound is characterized by Michaelis-Menten kinetic parameters $V_{max,HD}$ and $K_{m,HD}$ for 2,5-HD, and $V_{max,MEK}$ and $K_{m,MEK}$ for MEK. The inhibition of 2,5-HD by MEK ($K_{i,MEK}$) and that of MEK by 2,5-HD ($K_{i,HD}$) were employed to capture mutual inhibition between the two compounds.

Figure 4. Time course of 2,5-HD concentration in the serum of male Wistar rats subcutaneously injected with 2.6 mmol/kg 2,5-HD (HD), 2.6 mmol/kg 2,5-HD plus 2.6 mmol/kg MEK (HD+MEK) and 13 mmol/kg MEK (HD+5MEK). The solid lines are predictions of the competitive inhibition model (see Equation (3) for model specification and Table 2 for model parameters). The symbols represent experimental data from Yasui et al. (*20*).

Figure 5. Time course of 2,5-HD concentration in the serum of male Wistar rats subcutaneously injected with 2.6 mmol/kg 2,5-HD (HD), 2.6 mmol/kg 2,5-HD plus 2.6 mmol/kg MEK (HD+MEK) and 13 mmol/kg MEK (HD+5MEK). The solid lines are predictions of the uncompetitive inhibition model (see Equation (4) for model specification and Table 2 for model parameters). The symbols represent experimental data from Yasui et al. (*20*).

Figure 6. Time course of 2,5-HD concentration in the serum of male Wistar rats subcutaneously injected with 2.6 mmol/kg 2,5-HD (HD), 2.6 mmol/kg 2,5-HD plus 2.6 mmol/kg MEK (HD+MEK) and 13 mmol/kg MEK (HD+5MEK). The solid lines are predictions of the noncompetitive model (see Equation (5) for model specification and Table 2 for model parameters). The symbols represent experimental data from Yasui et al. (*20*).

Figure 7. Effects of co-exposure to MEK on 2,5-HD AUC at various doses of 2,5-HD. The competitive inhibition model (see Equation (3) for model specification and Table 2 for model parameters) predicts the values of 2,5-HD AUC, based on the assumption that rats were subcutaneously administered with 2,5-HD and MEK.

Figure 8. Effects of co-exposure to MEK on the biological half-life of 2,5-HD at various blood concentrations of 2,5-HD. The latter is a linear combination [see Equations (7)] of apparent Michaelis-Menten constant, the concentrations of 2,5-HD and MEK in blood.

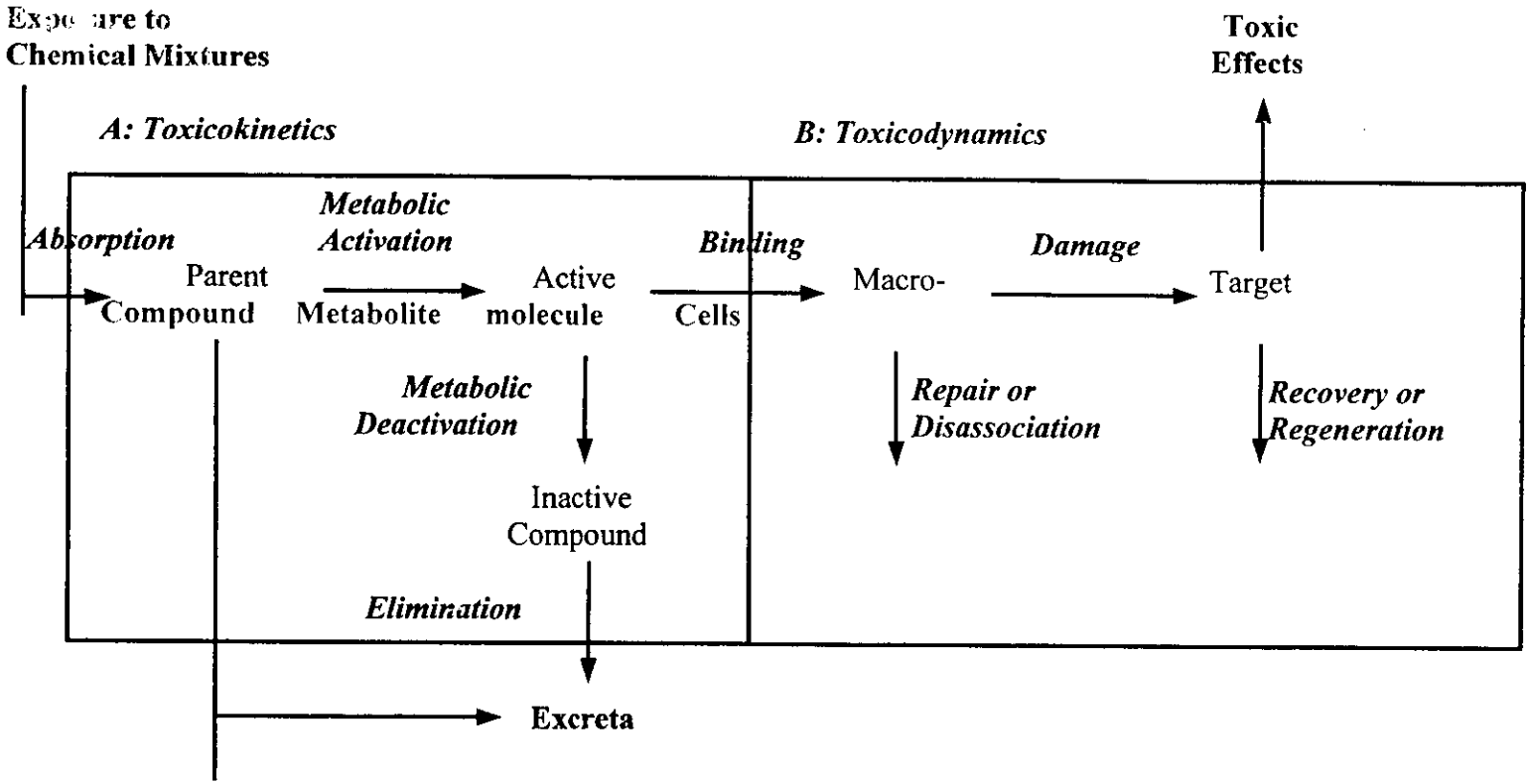
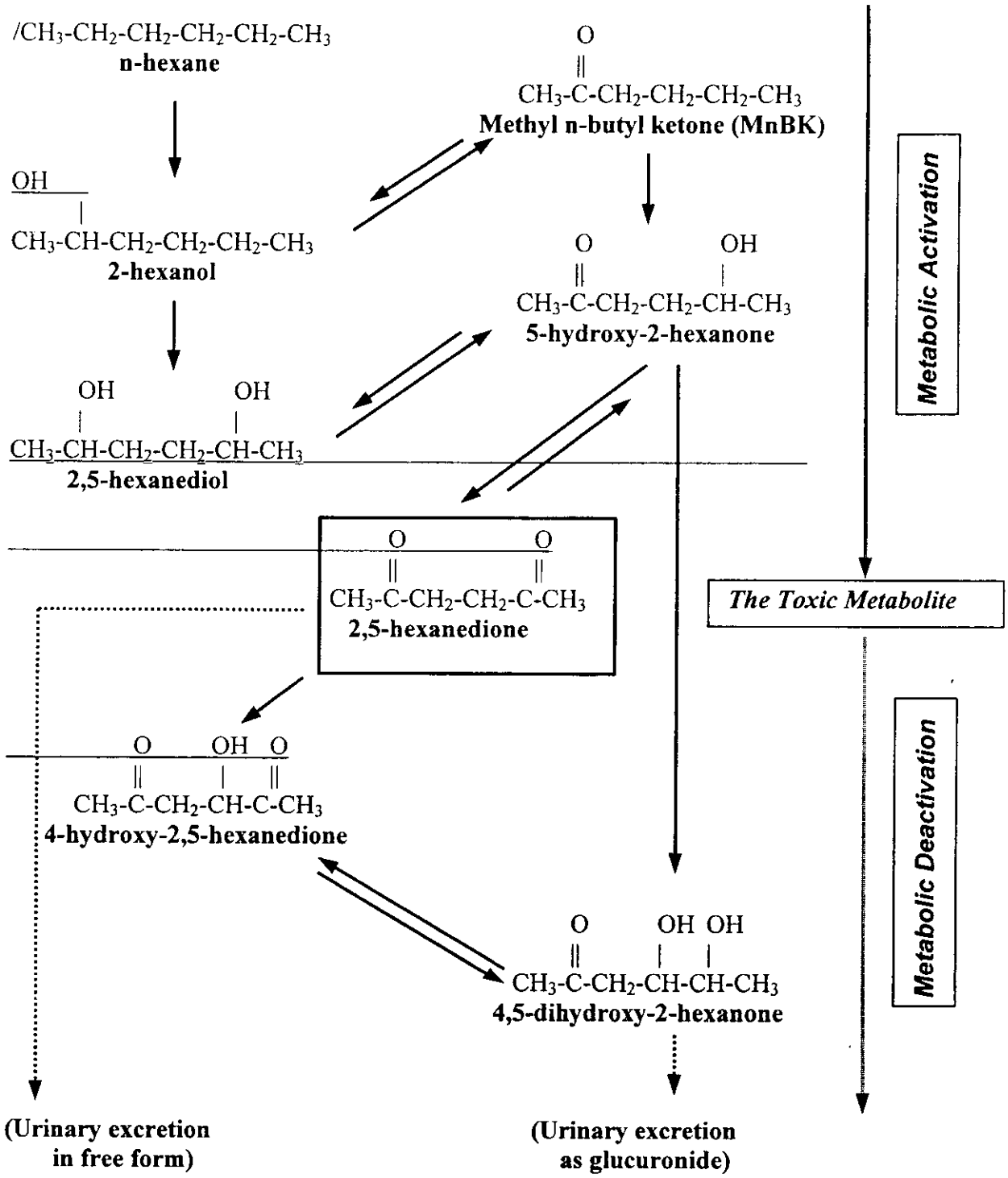

Exposure to
Chemical Mixtures
Toxic
Effects
A: Toxicokinetics
B: Toxicodynamics
Absorption
Metabolic
Activation
Binding
Damage
Parent
Compound
Metabolite
Active
molecule
Cells
Macro-
Target
Metabolic
Deactivation
Repair or
Disassociation
Recovery or
Regeneration
Inactive
Compound
Elimination
Excreta

Figure 1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OH
CH3-CH-CH2-CH2-CH2-CH3
2-hexanol
OH OH
CH3-CH-CH2-CH2-CH-CH3
2,5-hexanediol
O
CH3-C-CH2-CH2-CH2-CH3
Methyl n-butyl ketone (MnBK)
O OH
CH3-C-CH2-CH2-CH-CH3
5-hydroxy-2-hexanone
O O
CH3-C-CH2-CH2-C-CH3
2,5-hexanedione
O OH O
CH3-C-CH2-CH-C-CH3
4-hydroxy-2,5-hexanedione
O OH OH
CH3-C-CH2-CH-CH-CH3
4,5-dihydroxy-2-hexanone
Metabolic Activation
The Toxic Metabolite
Metabolic Deactivation
(Urinary excretion in free form)
(Urinary excretion as glucuronide)

Figure 2

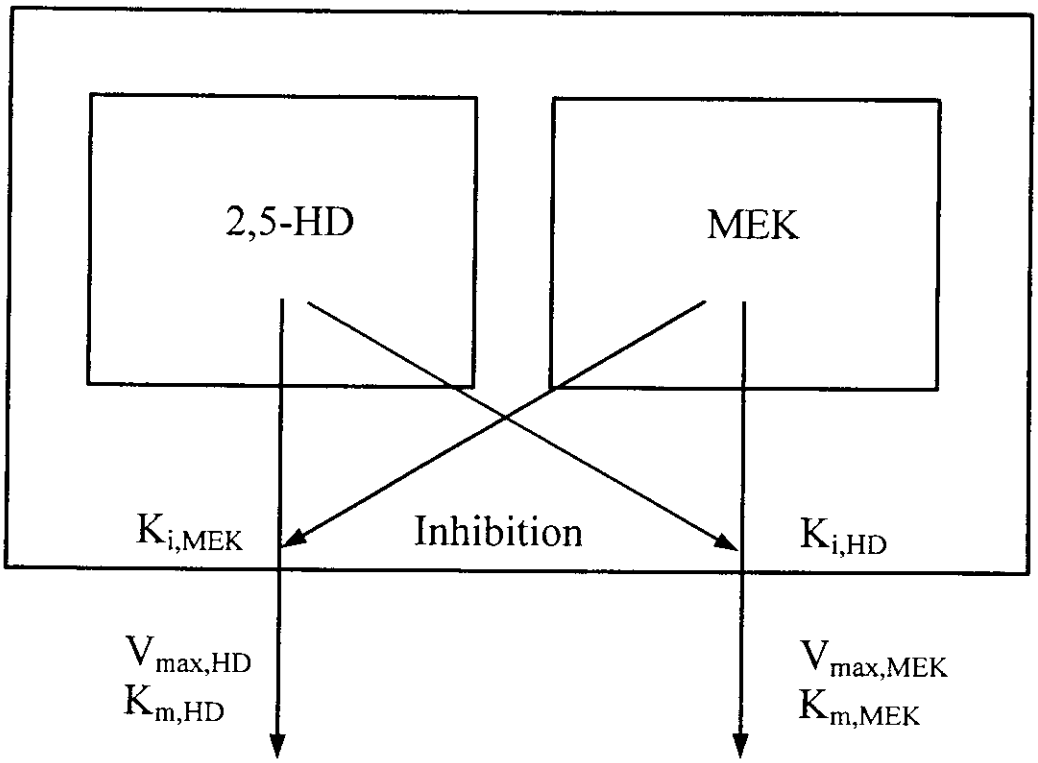


Figure 3

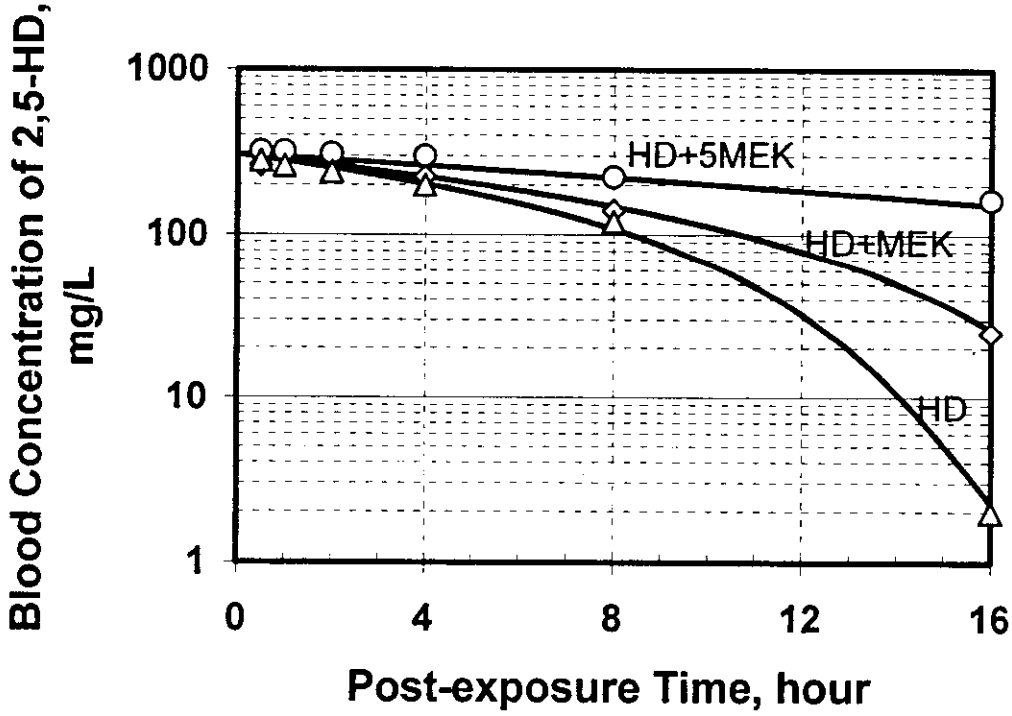


Figure 4

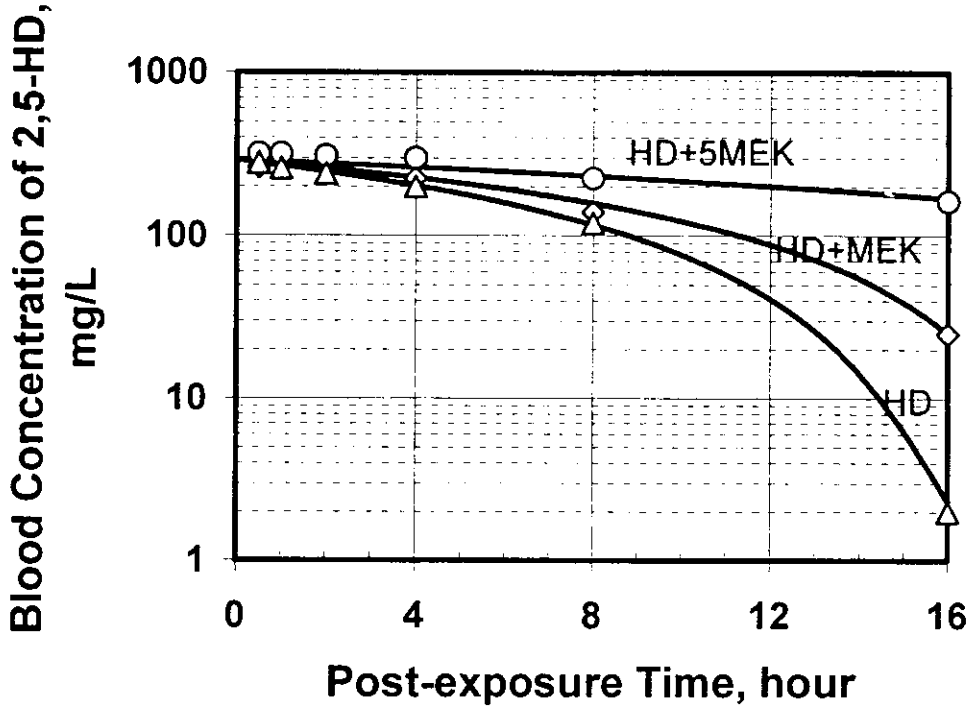


Figure 5

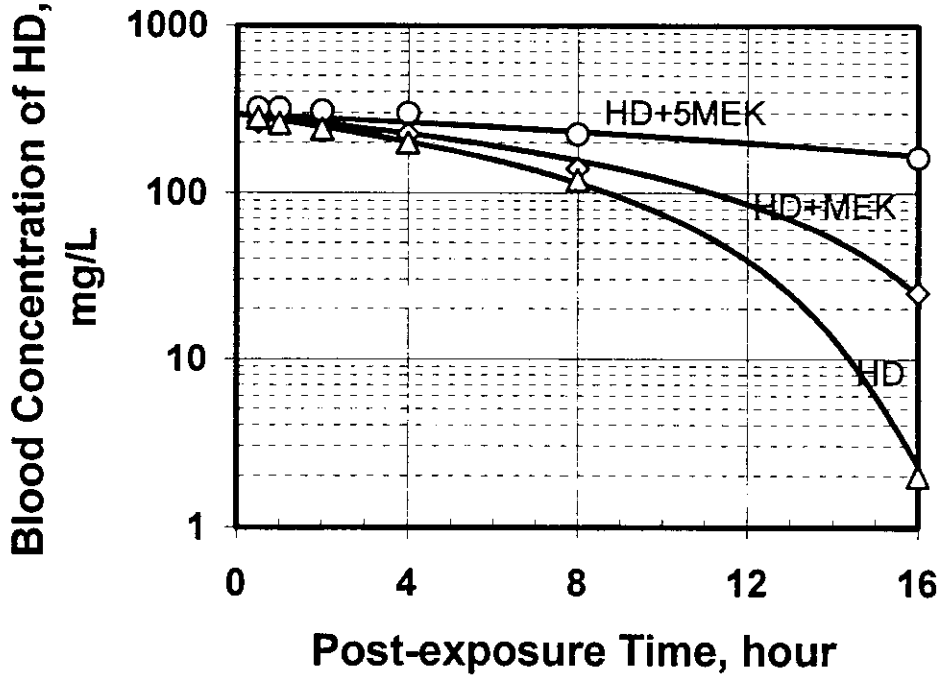


Figure 6

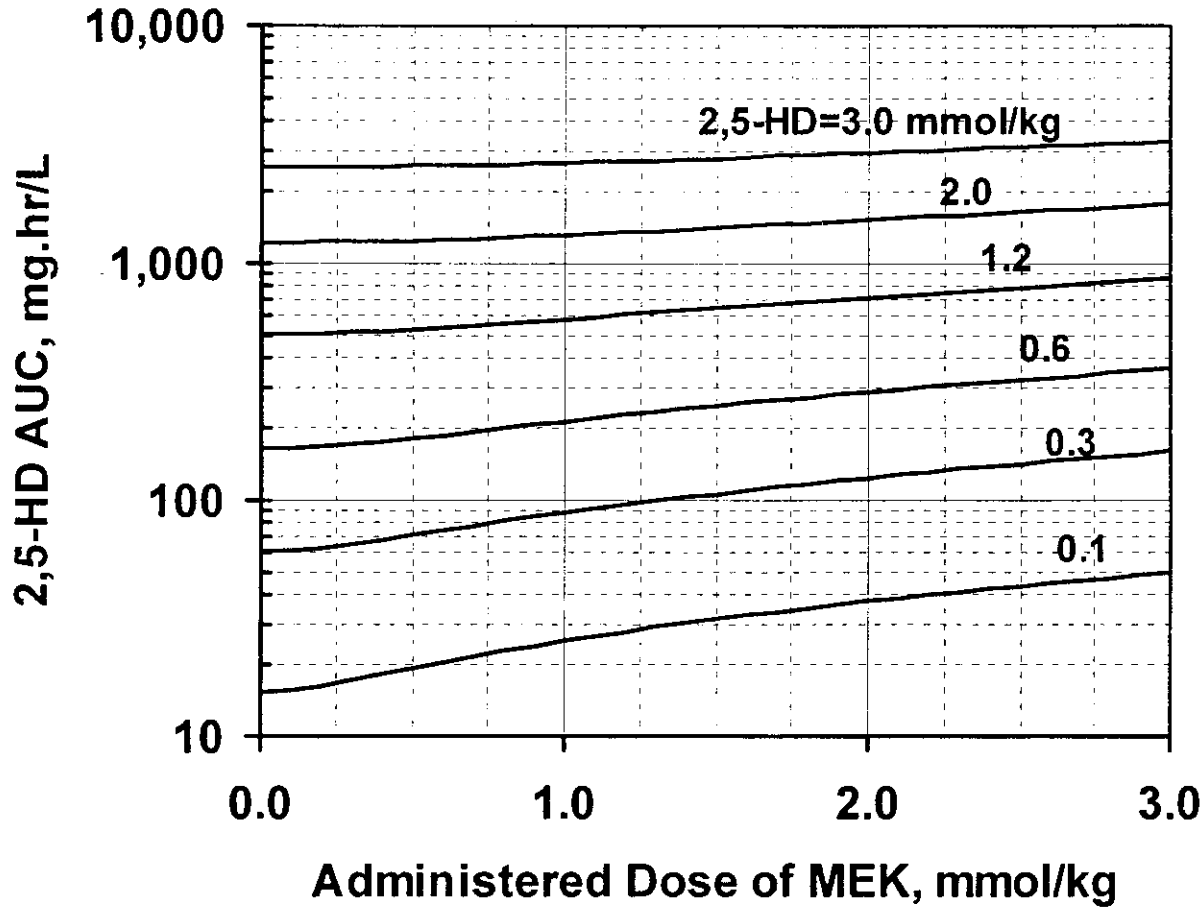

10,000
1,000
100
10
2,5-HD AUC, mg.hr/L
2,5-HD=3.0 mmol/kg
2.0
1.2
0.6
0.3
0.1
0.0
1.0
2.0
3.0
Administered Dose of MEK, mmol/kg

Figure 7

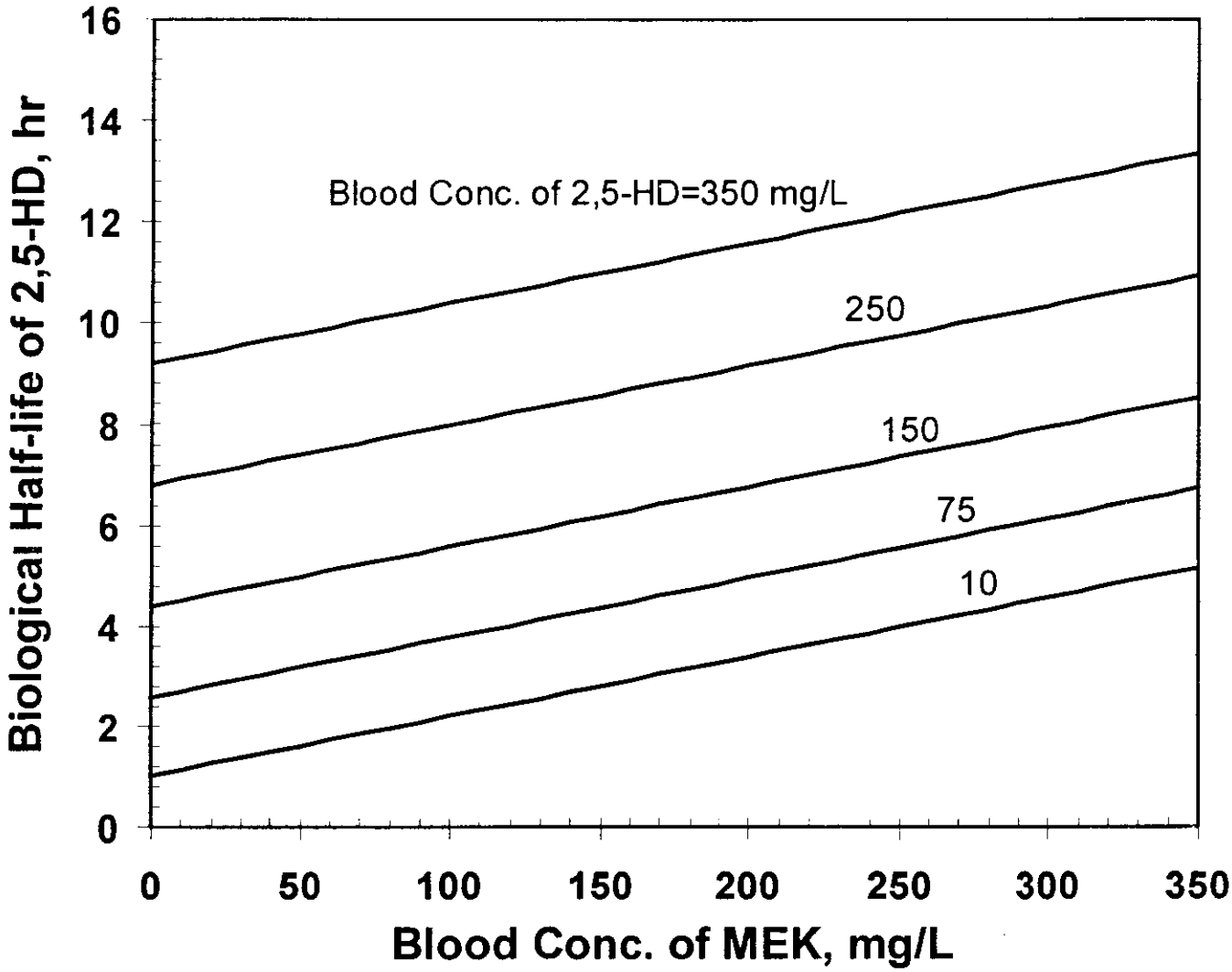

Biological Half-life of 2,5-HD, hr
16
14
12
10
8
6
4
2
0
Blood Conc. of 2,5-HD=350 mg/L
250
150
75
10
0
50
100
150
200
250
300
350
Blood Conc. of MEK, mg/L

Figure 8

Arthur Cho, Deborah Schmitz, and John R. Froines

Metabolic interaction between styrene and butadiene

Abstract

Butadiene (BD) and styrene (ST) are protoxins, i.e., compounds that are metabolically activated to toxic epoxides in a variety of tissues. In some industrial conditions, workers can be exposed to both compounds simultaneously so the nature of the metabolic interaction between the two compounds is clearly important from an occupational health perspective. Pharmacokinetic studies of the interaction between ST and BD in mice and rats showed that although ST inhibits the metabolism of BD, BD had no effect on ST metabolism. Since the toxicity of both of these compounds is dependent on metabolism, the interaction has been interpreted to reflect a "protective effect" by ST on BD toxicity by some. The compounds are converted to their toxic epoxides by enzymes of the cytochrome P450 (CYP) superfamily in what is commonly thought to be the rate limiting step in their overall metabolic transformation. To evaluate the interactions of the two compounds with CYP, we have studied epoxide formation from BD in the presence of ST and epoxide formation from ST in the presence of BD in rat liver microsomes. We considered two possible mechanisms, an irreversible one, in which the olefins could be converted to compounds capable of forming covalent bonds with the enzyme and a second possibility, that of competitive inhibition. Irreversible inhibition could be a protective action, i.e., by blocking the formation of a toxic epoxide. The results indicated that irreversible inhibition did not occur and that BD was a competitive inhibitor of ST epoxidation. These observations, made at subcellular levels, differ from the results of the *in vivo* studies, leading us to conclude that interaction at the CYP level is not the basis for the observed *in vivo* interaction. Other investigators have recently suggested that the *in vivo* interaction may involve transporters so that competition for access to the metabolic enzymes may be responsible. These subcellular studies did not address this possibility and attempts to study the interaction at the cellular level with hepatocytes were unsuccessful.

Introduction

Butadiene (BD) and styrene (ST) are protoxins, i.e., compounds that are metabolically activated to toxic epoxides in a variety of tissues (Gadberry et al 1996). Both compounds are converted to their toxic epoxides by enzymes of the cytochrome P450 (CYP) superfamily in what is commonly thought to be the slow step in their overall metabolic transformation(Nakajima et al 1993; Nakajima et al 1994; Nedelcheva 1996; Nieusma et al 1998; Vaz et al 1998). The epoxides are then hydrolyzed to the corresponding diols by the enzyme, epoxide hydrolase (Gadberry et al 1996; Kemper & Elfarra 1996; Krause et al 1997; Mendrala et al 1993), and subsequently undergo conjugation reactions before they are eliminated. In some industrial conditions, workers can be exposed to both compounds simultaneously and the nature of the metabolic interaction between the two compounds is clearly important from an occupational health perspective. Pharmacokinetic studies of the interaction between ST and BD in mice (Leavens & Bond 1996; Leavens et al 1996) and rats [(Filser et al 1993; Laib et al 1992) showed that although ST inhibits the metabolism of BD, BD had no effect on ST metabolism. Since the toxicity of both of these compounds is dependent on metabolism, the interaction has been interpreted to reflect a "protective effect" by ST on BD toxicity by some (Laib et al 1992).

The interaction of these compounds with cytochromes P450 can occur by several different mechanisms.

1. As unsaturated compounds have been shown to be irreversible inhibitors of CYP enzymes (e.g.,(Ortiz de Montellano 1986) , each could irreversibly inhibit CYP to block formation of the epoxide. In this case, formation of the toxic epoxides would be reduced and a protective effect could result.
2. If both compounds were oxidized by the same CYP enzyme, they would be competitive substrates and, depending on the values of their respective Michaelis' constants (Km), one could inhibit the epoxidation of the other.
3. A third possibility is that ST and BD epoxidation is catalyzed by different CYP enzymes so there would be no interaction on epoxide formation. This is an explanation for the inability of ST to inhibit BD metabolism by more than about 50% (Leavens & Bond 1996). In this case, interaction could take place on epoxide hydrolase, allowing the accumulation of the epoxide with lower affinity, or higher Km value.
4. Alternatively, the interaction could take place on a transporter rather than an enzyme. Laib et al., (Laib et al 1992) have suggested that the first order metabolism of BD and ST is limited by the capacity of transport processes. If competition for a common transporter is the basis for the interaction, the concentration dependency could be very complex. There are inward and outward transporters in different tissues so that interaction could take place to either increase or decrease the net inward movement of these compounds. For example, the ABC transporter, P-glycoprotein, removes lipid soluble compounds from cell membranes by outward transport. Inhibition of this transporter could actually increase the level of intracellular substrate.

To evaluate these possible interaction mechanisms at the level of cytochrome 450 enzymology, the kinetics of epoxide formation by ST and BD were determined in liver microsomes from naïve rats. The results thus far indicate that BD inhibits ST epoxidation but ST has no effect on BD epoxidation.

Materials and methods.

Metabolism of Styrene in Rat Hepatocytes

Primary culture incubations were conducted in 24 mL glass vials coated with collagen type 1 and sealed with Teflon-faced septa. Hepatocytes were isolated from ~200-250g Sprague-Dawley rats (Harlan) and plated at a density of 1×10^6 cells in Williams E Media containing fetal bovine serum and Streptomycin-Penicillin and allowed to grow overnight. The following day, the medium was removed and styrene, dissolved in Williams E Media, was added to initiate the metabolism. Aliquots of 100 µL of media was removed at specified time points and added to extraction tubes containing 1.5 mL hexane and internal standards, trans-ß-methylstyrene and (1R,2R)-(1)-1-phenylpropylene oxide. Samples were mixed on a vortex and centrifuged at 2000 rpm for 10 min. The hexane layer was transferred to 2mL vials. The vials were capped and stored at 4° C until analysis.

Metabolism of Styrene in Rat Hepatic Microsomes

Rat microsomal incubations with styrene were conducted in 24mL glass vials sealed with Teflon-faced septa. The incubation vials were set on ice and styrene in 0.4% acetone, 0.1M Hepes buffer, pH 7.6, deionized water, rat hepatic microsomes (1 mg-1.9mg), and 1 mM cyclohexene oxide (an epoxide hydrolase inhibitor) were added to a final volume of 2 mL. The incubation mixture was equilibrated for 10 min. at 37°C and the reaction initiated by the addition of a NADPH-generating system consisting of 0.5 mM $NADP^+$, 8 mM glucose 6-phosphate, 1 unit of glucose 6-phosphate dehydrogenase and $MgCl_2$ (to a final concentration of 5 mM). Aliquots of 100 µL were removed via a gas-tight syringe extracted and with 1.5 mL of hexane containing trans-ß-methylstyrene and (1R,2R)-(1)-1-phenylpropylene oxide as internal standards. Samples were mixed on a vortex mixer and centrifuged at 2000 rpm for 10 min. The hexane layer was transferred to 2mL vials. The vials were capped and stored at 4° C until analysis.

Metabolism of Butadiene

Rat microsomal incubations with butadiene were conducted in 24mL glass vials sealed with Teflon-faced septa. The incubation vials were set on ice and 0.1M Hepes buffer, pH 7.6, deionized water and rat hepatic microsomes (1-1.8mg) in a final volume of 2 mL. Then, butadiene was added to the mixture via a gas-tight syringe and the mixture was equilibrated for 10 min. at 37°C. The reaction was initiated by the addition of a NADPH-generating system consisting of 0.5 mM NADP, 8 mM glucose 6-phosphate, 1 unit of glucose 6-phosphate dehydrogenase and $MgCl_2$ (final concentration, 5 mM). A syringe was used to remove aliquots of 0.25-0.5 mL of the incubation mixture. The aliquots were extracted with 1mL of methylene chloride containing 1, 2 epoxyhexane as internal standard. Samples were mixed on a vortex and centrifuged at 2000 rpm for 10 minutes. The aqueous layer was removed by aspiration and the methylene chloride layer was transferred to 2 mL vials.

GC/MS analysis of Styrene and Styrene Oxide

A H-P 5971A Mass Selective Detector connected to an HP model 5890 GC was used for the determination of styrene oxide. The GC/MS was fitted with a retention gap (5m, 053 mm) connected with a quartz deactivated column connector to a HP-5MS capillary column (0.25 mm i.d., 0.25 µM film thickness, 30 m length). Initial column temperature was 35°C for 0.5 min then ramped to 120 °C at a rate of 70 °C/min. Retention times for styrene, trans-ß-methylstyrene, styrene oxide and (1R,2R)-(1)-1-phenylpropylene oxide were 5.10, 7.50, 8.76 and 10.86 min respectively. The fragments monitored were m/z 104.2.0 for styrene, m/z 117.2 for trans-ß-methylstyrene and m/z 89.0 for styrene oxide and (1R,2R)-(1)-1-phenylpropylene oxide .

GC/MS analysis of butadiene monoxide

A H-P 5971A Mass Selective Detector connected to an HP model 5890 GC equipped with a cool-on-column injector was used for the determination of butadiene monoxide. The GC/MS was fitted with a retention gap (5m, 0.53 mm) connected with a quartz deactivated column connector to a HP-5MS capillary column (0.25 mm i.d., 0.25 µM film thickness, 30 m) . Initial column temperature was 35°C for 4.0 min then ramped to 100 °C at a rate of 40 °C/min. Retention times for butadiene monoxide and 1,2 epoxyhexane were 3.93 min. and 6.06 min respectively. The fragments monitored were *m/z* 39.0 for butadiene monoxide and *m/z* 71.1 for 1, 2 epoxyhexane.

Results

Hepatocyte studies

Initially, an attempt was made to utilize hepatocyte cultures as the source of tissue interaction. Hepatocytes contain the entire array of metabolic enzymes as well as membrane transporters so they would be a useful preparation in which to study details of the interactions between the two compounds.

In the initial experiments, ST was dissolved in the culture medium and incubated for 0-90 min in hepatocytes that had been cultured for 24 hours. Analysis of the medium for ST, indicated that its disappearance was linear for about 60 minutes over a concentration range between 50 and 100 μM. Accordingly, BD was added and ST disappearaance again determined. However, when BD (1000 ppm; 16 μM) was added to the hepatocytes, the cells became detached from the vials and did not survive, indicating that BD, under these conditions, was toxic to the cells. For this reason, the hepatocyte approach was abandoned in favor of a microsomal preparation. Since the slow step in the metabolic activation of ST and BD was likely to be the CYP based reaction, microsomal preparations would permit the interaction on this enzyme system.

Partition properties of BD

The gaseous nature of BD at room temperature, required an approach to determining the concentration in the incubation media based on the head space concentration. Accordingly, the partition properties of BD between head space and medium in microsomal suspensions were determined. After equilibration of BD with the microsomal preparation, head gas samples were collected from incubation vials containing 2, 5 and 10 mL of microsomal preparation after a 15 minute incubation. There was no change in concentration in the head gas, i.e., it was independent of the volume of the microsomal mixture, indicating that the aqueous concentration was equal to the head gas concentration over the range of BD used in these studies.
Based on an apparent partition of one, the concentration in the aqueous phase could be calculated from the head space concentration with the assumption of ideal gas bahavior.

Thus:

1 ppm = 1 μg/L; 1000 ppm = 1 μg /L = 1 μL/mL
Incubation vial = 24 mL; BD @ 1000 ppm = 24 μL/24mL

From ideal gas considerations:

1 mmole = 25.4 mL at 37°; 1 mL = 39.4 μmoles
1 μL = 39.4 nmoles

24 μL * 39.4 nmols/μL = 945.6 nmoles

Assuming equal distribution (Kp = 1) betweeen gas/liquid,
1000 ppm = 945.6 nmoles/24 mL = 39.4 nmoles/mL = 39.4 μM.

Then,

BD added (μL/24 mL)	ppm	microM
	1000	39.4
	5000	197
	12500	492
	50000	1970

Effect of cyclohexane epoxide (CHO) on ST to styrene epoxide (SO) formation.

We used CHO to inhibit the epoxide hydrolase present in the microsomal preparation to limit the reactions studied to epoxidation, independent of epoxide hydrolase (EH) action. CHO at 1 mM, the concentration used to inhibit EH, had no effect on SO or BMO formation from 100 μM ST and BD, respectively, the highest concentrations used.

Styrene oxide (SO) formation

The kinetics of SO formation was determined in the presence of 1 mM CHO in 15 minute incubations at ST concentrations of 5, 10, 50, 100, 200 and 500 μM. The results, determined by nonlinear regression analysis, gave a maximal velocity of 9.24 ± 2.0 nmole/min per mg microsomal protein and the concentration for half maximal velocity was 887.6 ± 278.2 μM. The Km values are much higher than those reported (Mendrala et al 1993) but those investigators used higher concentrations of acetone as a solvent for ST. There is also the possibility of multiple CYP enzymes catalyzing the reaction but the data are too limited to statistically evaluate this possibility.

Additional experiments examining the effects of a 30 minute preexposure to ST on subsequent SO formation indicated that there was no inactivation of CYP by ST.

Butadiene mono epoxide (BMO) formation

The kinetic parameters for BMO formation in liver microsomal preparations were determined at substrate concentrations between 3.9 and 197 μM. Under these conditions, the maximal velocity was found to be 3.07 ± 0.30 nmol/min*mg, and the Km, 43.00 ± 11.19 μM. The lower Km observed for BD compared to ST indicated that BD should inhibit ST metabolism at μM concentrations.

Interaction between BD and ST

The effect of varying BD concentration (98-1970 μM) on SO formation from ST at 50 μM is shown in figure 1. The data fit a competitive inhibition model, as shown in the figure with Km and Ki values of 1.62 and 7.5 μM, respectively. Thus, at these BD inhibits ST conversion to SO by a competitive process, consistent with the notion of a common enzyme, presumably a cytochrome P450 enzyme, catalyzing the epoxidation of both compounds.

However, the effect ST on BD metabolism was not consistent with this model. The effect of varying ST on BO formation at a BD concentration of 197 μM is shown in figure 2. Under these conditions, no consistent pattern of inhibition was observed.

Discussion

The interaction between ST and BD has been proposed to occur at an enzymatic and a transporter level. There are multiple transporters of xenobiotics on cell membranes that may contribute to the kinetics of different substrates. Neutral lipophilic organic compounds are common substrates for the Mdrt, or muliti drug resistant transporter (Zegers & Hoekstra 1998). These transporters move substrates from the cell membrane to extracellular space so they prevent entry into the cell. Inhibition of the transporter therefore allows greater entry into the cell by the substrate by diffusion. The transporter is present in hepatocyte membranes and could participate in xenobiotic movement. Attempts to assess the interaction at a transporter level in vitro were unsuccessful because of the toxicity of BD to the cells. We were unable to culture the cells in the presence of BD.

We then examined the interaction at the microsomal level. This preparation examines the metabolic interaction between the two compounds, and in contrast to the observations made in in vivo studies, BD inhibited SO formation but ST had no effect on BO formation. The discrepancy between the two sets of studies, may lie in the participation of transporters in the rate limiting step of BD and ST biotransformation. Furthermore, preincubation experiments indicated that neither ST no BD exhibited inactivation of CYP under the conditions used. Thus, it is unlikely that activation of either ST or BD to a CYP inhibitor takes place under the conditions of these experiments.

In vivo studies show that ST inhibits the metabolism of BD to BO [(Filser et al 1993; Laib et al 1992), but BD has no effect on SO formation. If the interaction is competitive, the competition should be mutual, i.e., either one should inhibit the other. The lack of this mutual effect suggests that a more complex interaction is occurring and that additional studies are necessary to elucidate mechanisms.

We conclude, however, that:

1. Neither ST nor BD is converted to irreversible inhibitors of their metabolizing enzyme in vitro.
2. BD appears to act as a competitive inhibitor of SO formation but ST does not affect BO formation. The interaction at the enzyme level is clearly more complex and may involve multiple cytochrome P450 enzymes. The concentration range of ST used in the metabolism covered the range found in the plasma of rats exposed to 100 μmoles/kg. Under these conditions (ST at 50 μM) BD inhibited ST epoxidation by 50% at a BD concentration of 100 μM.
3. The interaction at the transporter level should be studied in cells under conditions that minimize the direct effect of BD on cell adhesion. .

Figures

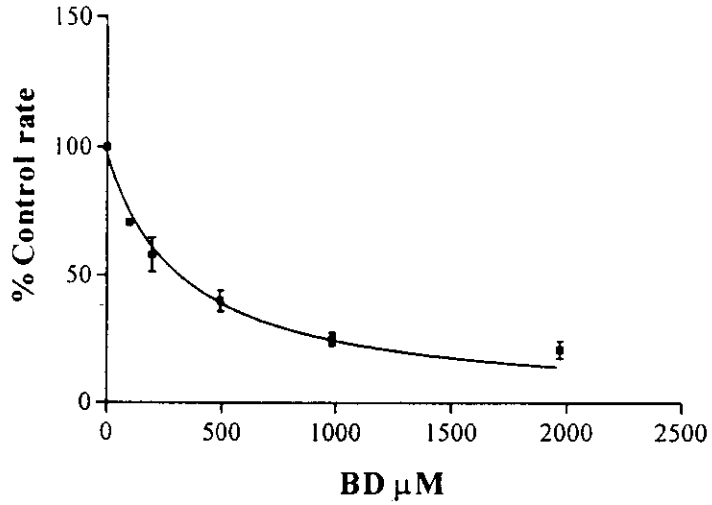
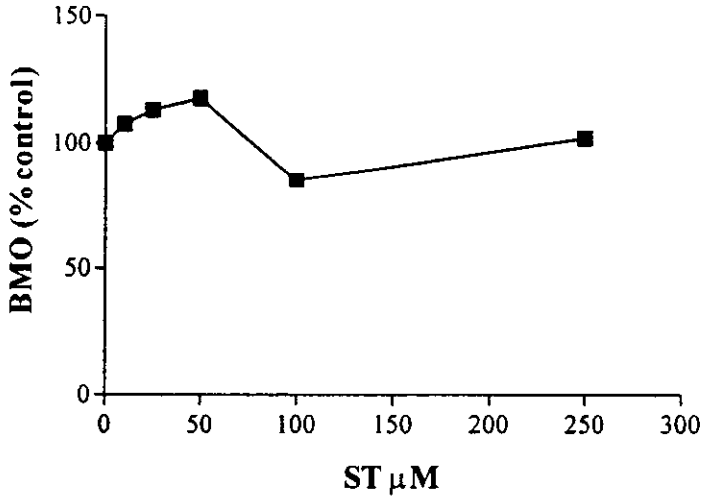


Figure 1: The effect of varying BD on SO formation from ST at 50 μM

SO concentration was determined after 15-minute incubation of ST in the presence of the indicated concentrations of BD. The data are expressed as % control (0.69 nmol SO formed /minute*mg microsomal protein). The curve represents a fit to a competitive inhibition model with the values of Km as 1.16 and Ki as 7.5 μM.

Figure 2. Effect of ST on BO formation from BD at 197 μM.



BO formation was determined in the presence of the indicated concentrations of ST. The results are expressed as percent of control activity which was 0.39 nmols/min*mg protein.

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Shachar Eylam, Dale Hattis, and John Froines

Short Term Non-Invasive Biomarkers for the Processes Producing Long Term Lung Damage--Evaluation of the Feasibility of Candidate Measurement Systems

Abstract

This report analyzes the potential for a few different potential biomarkers to serve as short term measures of ongoing chronic lung damage processes resulting from occupational exposures. We focus primarily on non-invasive measurements of ethane and pentane in exhaled air, and the urinary excretion of the elastin degradation products desmosine and isodesmosine. Excretion of the alkanes is a putative measure of oxidative processes in the lung resulting from macrophages activated by particulate exposures. Desmosine and isodesmosine are putative markers for the proteolytic processes that have been established as part of the pathogenesis of emphysema and similar conditions. For ethane we develop a physiologically-based pharmacokinetic model that can be used to interpret alveolar ethane measurements in terms of production--subtracting out the contribution of ethane stored in the body from ambient air exposures, adjusting for differences in body fat composition. A similar model for pentane is in process.

Both the ethane and desmosine/isodesmosine measurements have promise and appear feasible. Three basic suggestions are made to help assure the collection of high quality data for workers exposed to suspected lung-damaging agents:

1) For both ethane and pentane, there should be a small series of measurements of pharmacokinetic parameters both in vitro (e.g., ethane blood/air, liver/air, and related partition coefficients) and in vivo (based on measured exposures) to confirm the pharmacokinetic modeling proposed here.

2) Improved ethane (and/or pentane) ambient air measurements need to be made covering the day or two prior to breath measurements for at least a sample of study subjects.

3) Characterization of individual rates of excretion of desmosine and isodesmosine may benefit from a small series of repeated samples, spaced apart by at least a couple of weeks. Group differences, however, may well be detected by cross-sectional samples in which only a single measurement is made per person.

Introduction

By definition, chronic cumulative diseases take a long time to develop into clinically recognizable cases of impairment or illness. And therefore "clinically recognizable cases"--the usual starting point for epidemiological work--are generally years and even decades removed from opportunities for direct measurement of causally relevant exposures. It would greatly facilitate both epidemiological research and (in the long run) the targeting of possible risk control measures, if measurements could be made of the ongoing cumulative damage processes as the damage is occurring.

The potential applications of such short term measurements in epidemiology are particularly significant. Causal components of complex mixtures could be identified; effects of technical changes in the composition of the complex mixtures could be evaluated, and dose response

relationships could be analyzed in ways that are just not possible if one must wait decades to observe the end effects of the pathological processes.

However, appropriate design of research in this area must acknowledge and attempt to minimize some drawbacks and hazards. In the near term, the definition of biomarkers of putative chronic cumulative processes will need to be based on our best current (albeit incomplete) understanding of the mechanisms involved. Ideally the biomarker should be highly correlated with one or more of the steps leading from external exposure to a hazardous material through the chronic accumulation of damage. Each biomarker will therefore reflect a qualitative hypothesis about the relationship between a particular kind of measurement and the cumulative pathology (see below). But there is no easy or rapid way, in the short run, to empirically verify that the measurement does in fact reflect a process that is on the causal sequence leading to pathology, or to assess the quantitative significance of particular observations for long term risk. This kind of testing of the qualitative hypotheses and measurement of long term risk implications is likely to require a long term prospective study of people exhibiting various levels of the biomarker over a defined period.* Before completion of that kind of work, it will not be possible to definitively interpret biomarker results in terms of individual risks. This fact must be made clear to all participants, particularly the subjects of the research.

The work reported here assembles information needed to design initial experimental and field trials of a few promising types of putative biomarkers of lung damaging processes:

- Exhalation of ethane and possibly pentane in the breath. These simple alkanes are the products of oxidation of omega-3 and omega-6 fatty acids, respectively. The primary hypothesis is that they would be a measure of relatively high levels of macrophage activity likely to result from excessive particulate loading in the deep lung, as has been observed in autopsy and bronchoalveolar lavage studies in coal miners (Rom, 1990). Auburtin et al. have recently reported relatively high levels of ethane exhalation in active underground coal miners relative to retired miners regardless of the presence of already-developed coal workers' pneumoconiosis in some of the retired miners (Table 1). (Much less impressive elevations were not seen for pentane in these worker groups--data not shown.) Lapp and Castranova (1993) have reviewed current theories on the involvement of macrophage products in the pathogenesis of both coal workers' pneumoconiosis and silicosis. In brief, phagocytically active macrophages are important both in the direct secretion of oxidants and proteolytic enzymes, and in the secretion of mediators that recruit polymorphonuclear leukocytes into the airspaces, and stimulate the release of oxidants and proteases from those cells. According to Begin et al. (1989), "...the initial early lung lesion in all mineral dust pneumoconioses is a fibrosing macrophagic alveolitis." Incidental release of oxidants can particularly be expected to result from futile attempts by macrophages to digest particles such as asbestos and silica (Archer, 1979). An additional hypothesis that also supports the potential relevance of alkane exhalation arises from observations that coal dust itself

* In the interim, another type of methodology that might produce tentative risk-related information is a case control study comparing workers of similar age and exposure who have and have not developed one of the pneumoconioses in question. For an example application to the release of TNF from blood monocytes by coal dust, see Borm et al. (1990). The difficulty with this approach is that altough it can provide some tentative indications of an association between a biomarker and a disease, it can be difficult to tell whether an elevated level of the biomarker is (1) a predictor of early effect or susceptiblity, or (2) a later consequence of the disease process.

appears able to generate hydroxyl radicals from hydrogen peroxide and induce lipid preoccupation in vitro (Dalal et al., 1995). This activity appears to be related to the surface iron content of the coal particles. If it were found that this is reflected in *in vivo* lipid peroxidation it could provide a specific physical/chemical measurement that might ultimately be useful in targeting control measures. Concentrations of stable coal borne free radicals are associated with the severity of coal workers' pneumoconiosis in lung tissue samples from deceased coal miners (Dalal et al., 1991). Related mechanisms have been documented for silica particles (Ghio et al., 1990) and asbestos Kamp et al. (1992).

- Urinary excretion of breakdown products of elastin (desmosine and isodesmosine) and collagen (hydroxyproline). These biomarkers are hypothesized to reflect excessive amounts of proteolytic activity in the lung. Excessive proteolysis has previously been associated with elevated risk of emphysema in humans by observations of the effects of genetic variants of alpha-1 antitrypsin--a protease inhibitor that is important in protecting lung tissue against neutrophil elastase (Evans and Pryor, 1994; Gadek, 1992; Tockman et al., 1993).* Inactive forms of this protease inhibitor cause several fold elevations in susceptibility to emphysema among smokers (Kalsheker, 1994; Shimizu and Mizunuma, 1991). More recently, work in rats has shown that crystalline silica, but not the relatively benign particulate titanium dioxide, induces a sustained elevated release of desmosine and hydroxyproline into bronchoalveolar lavage fluid, apparently in parallel with the presence of increased numbers of polymorphonuclear lymphocytes (Li et al., 1996). In addition to the possible action of silica in stimulating cellular release of proteolytic enzymes, freshly ground silica can apparently directly inactivate alpha-1 antitrypsin via an oxidation-dependent mechanism (Zay et al., 1995).

- A few other biomarkers that have been proposed based on measurements in plasma (Porcher et al., 1993). The most promising of these as measures of current exposure and possible contributions to long term pathological processes are leukocyte elastase (HLE), plasma neutral metalloendopeptidase elastase type (NMEP), and fibronectin (FN). All three biomarkers have been observed to be increased in coal miners as compared to control workers without mine dust exposure. HLE and NMEP are possible measures of elastase proteolytic activity, and thus reflect the same causal hypothesis as the urinary desmosine/isodesmosine measurements discussed in the previous bullet. [Most recently, a 5-7 fold increase in NMEP in active coal miners has been confirmed in further work (Soleihac et al., 1996)]. Fibronectin is reportedly involved in the recruitment and proliferation of fibroblasts during firbrogenesis (Ruoshlati, 1988).

* Neutrophil elastase is the basis of a well known experimental model of emphasema on tracheal instillation in animals (Mauderly et al., 1989). In humans, smoking has been shown to increase the local retention of externally-supplied neutrophils in the lung (MacNee et al., 1989).

Table 1
Measurements of Ethane in the Breath of Active and Retired Coal Minters--Data of Auburtin et al.

A. Results Stated In Terms of Ethane Production Rate (pmole/min)

	Gmean current dust exposure ($\mu g/m^3$		Ethane production rate (pmol/min)	
		N	Geom mean	GSD
Surface workers	**0.31**			
Smokers		15	147	1.9
Nonsmokers		15	57.9	2.6
Total		30	92.3	2.5
Underground miners	**1.61**			
Smokers		15	307	1.7
Nonsmokers		17	469	7.3
Total		32	384	4.4
Retired miners (- pneum)	**0**	38	58.5	2.5
Retired miners (+ pneum)	**0**	25	44.1	3

B. Results Stated In Terms of Alveolar Air Ethane Concentration (pmol/liter)

	Gmean current dust exposure ($\mu g/m^3$)		Ethane concentration (pmol/liter)	
		N	Geom mean	GSD
Surface workers	**0.31**			
Smokers		15	209	2.1
Nonsmokers		15	104	2.2
Total		30	147	2.3
Underground miners	**1.61**			
Smokers		15	437	1.8
Nonsmokers		17	614	7.3
Total		32	523	4.4
Retired miners (- pneum)	**0**	38	90	2.3
Retired miners (+ pneum)	**0**	25	90.1	2.4

Section 2 below will first give a basic technical description of the three types of assays, and generally assesses their feasibility. For the urinary measurements of desmosine and isodesmosine, this will include our limited information about the dynamics of change following an unusual high-elastin meal. Section 3 will then describe a preliminary physiologically-based pharmacokinetic model for ethane that assess the needs for different types of washout times to avoid confusion between internally-generated ethane and ethane absorbed from the external air.

Initial adaptation of this modeling framework to pentane will also be discussed. Finally, the concluding section will make recommendations for the design of preliminary laboratory and field studies to develop these biomarkers.

Basic technical description and feasibility of measurements in exposed workers

Breath Alkane Measurements

There are now nearly two decades of published reports of breath pentane and ethane output as measures of lipid peroxidation in vivo (Wade and van Rij, 1985; Morita et al., 1986; Dilard et al, 1978). More recently, measurements of breath pentane have been controversial (Springfield and Levitt, 1994). There have been very wide differences (reportedly over 1000-fold) among different laboratories in reported background pentane levels (our own summary is reproduced in Table 2). Some of the differences probably arose because of the similar retention times of pentane and isoprene in many gas chromatography system. Isoprene is evidently present normally in expired air in about 20-fold larger concentrations than pentane (Kohlmuller and Kochen, 1993), and it migrates at a similar rate in many commonly used gas chromatography systems, making it likely that some past researchers have recorded appreciable amounts of isoprene as pentane. This appears especially likely for researchers who have reported the greatest outputs of pentane (e.g., Zarling and coworkers). In experiments with authentic reference materials, pretreatment with aqueous potassium permanganate removed all the isoprene but did not alter pentane concentrations. When the same potassium permanganate treatment was applied to alveolar breath samples it was possible to greatly reduce the previously apparent "pentane" peak leading to the conclusion that this material was in fact isoprene (Springfield and Levitt, 1994).

A final concern raised by Springfield and Levitt is that past researchers have used different and generally inadequate periods of time breathing low-hydrocarbon air for "washout" of stored pentane from fat tissue. In experiments in rats, they measured the rate of decline in expired pentane after 20-hour exposures to high levels (2,000-4,000 ppm) of pentane in external air.

For normal rats (7% fat), they report recovery of about 64 μmoles/kg body weight of pentane under these conditions--including 24 μmoles/kg body weight in a relatively slowly-exchanging compartment (half life of about 2.8 hours) that they identify as likely to be fat. For an obese strain of rats (Zucker--50% fat tissue) a total of about 630 μmoles/kg body weight were stored and later recovered, including 520 μmoles/kg in a slow exchanging compartment with a half life of 8.5 hours (Table 3). The rather dramatic conclusion of this paper is an expression of doubt that endogenously generated pentane has ever been accurately measured in people.

This issue should eventually be addressed by the development of a full PBPK model for pentane. It should be noted here, however, that the ratio of recoverable stored pentane per body weight per ppm of external exposure in these rat experiments gives us a starting point for reasoning about how much pentane could potentially be stored and re-released in humans exposed to normal ambient background pentane. This background is reported to be of the order of 2.4-7.6 ppb in urban outdoor air--corresponding to 110-340 pmol/liter. This is somewhat more than the average of about 134 pmol/liter reported by Kohlmüller and Kochen (1993) or the potentially somewhat higher concentrations in the environment of mine workers (for which data will need to be

gathered). Another implication of these findings is that regardless of the pharmacokinetic modeling that is possible based on existing data, there should at least be some preliminary uptake and re- release experiments with ethane and pentane in humans with concurrent estimation of body fat via skinfold thickness measurments.

Kohlmüller and Kochen (1993) report finding an appropriate method to be used in humans in order to isolate ethane and pentane; it is based on cryofocusing (a gas trapping system that focuses the organic volatile components of the air using a helically formed glass-lined tubing immersed in liquid nitrogen at one end to avoid occlusion by CO_2 and water) in combination with gas chromatography and is adaptable to mass spectrometry. The column they recommend for separation of *n*-pentane and isoprene is a Poraplot U column from Chrompack (Kohlmüller and Kochen, 1993). Unfortunately, while these authors are exquisitely attuned to the precautions needed for accurate analytical chemistry in measuring pentane, they do not appear to have taken the same care on the biological/pharmacokinetic side. They do not report using a "washout" period with low-hydrocarbon air to reduce the contribution of pentane stored in the body from prior exogenous exposures. And although their results for each person reflect 3-8 replicate chemical measurements, they do not appear to have collected replicate samples to assess the stability of the pentane exhalation measurements within study subjects. Finally, at least with the relatively small volumes of expired gas they analyze, their system is apparently not sensitive enough to measure ethane levels.

Table 2
Overview of Past Measurements of Ethane and Pentane in Exhaled Air

Reference	Condition	Ethane	Pentane
Refat '91	in children with vitamin E deficiency	Control: 31 ± 12 pmol/kg/min Patients: 78 ± 10 pmol/kg/min	
Habib '95	in adults	Background (air): 6.64 ± 0.33 pmol/L Nonsmokers: 0.59 ± 0.18 pmol/min/kg Smokers: 2.9 ± 0.52 pmol/min/kg Ex-smokers: 1.55 ± 0.36 pmol/min/kg	
Pincemail '90	exercise		At rest: 4.13 ± 2.14 pmol/L After exercise: 17.1 ± 7.73 pmol/L w/propranolol: At rest: 1.76 ± 0.77 pmol/L Exercise: 5.93 ± 5.76 pmol/L
Wade '85	in healthy adults	95.1 ± 19 pmol/h/kg (~1.6 pmol/min/kg)	

Zarling '92	in healthy adults	Results: 0.41 ± 0.32 nmol/L Normal: 0.8 ± 0.39 nmol/L	Results: 4.3 ± 1.7 nmol/L Normal: 3.7 ± 1.2 nmol/L
Seabra '91	in control subject	$2x10^{-9}$ moles/kg/min	$1.5x10^{-9}$ moles/kg/min
Kohlmüler '93	in control subject		0.022 — 0.377 nmol/L (isoprene: 0.27—3.13 nmol/L)
Massias '93	in control subject	4.83 ± 3.0 nmol/L	3.16 ± 2.05 nmol/L
Van Gossum '92	in adults		Nonsmokers: 5.82 ± 0.46 pmol/kg/min Smokers: 12.8 ± 1.43 pmol/kg/min
Euler '96	in adults		Nonsmokers: 0.23 ± 0.3 nmol/L Smokers: 0.17 ± 0.03 nmol/L Ambient air: 0.05 ± 0.01 nmol/L Mainstream smoke: ND (<0.02 nmol/L)

Table 3
Metabolism vs. Uptake Of Environmental Pentane In The Rat (Data of Springfield and Levitt, 1994)

Types of rats	Uptake of pentane over 20 hrs (pmoles/kg)	Pentane Recovery (pmol/kg) Lungs	Slowly equilibrating tissues (fat)	Rapidly equilibrating tissues
Normal	900	0.62	24 ± 2	39 ± 4
Zucker (obese)	1020	0.57	520 ± 70	110 ± 20

Mean ± SEM

Clearly, however, before any field measurements of pentane are undertaken, there will need to be a reasonably extensive series of preliminary analytical chemistry and human experimental pharmacokinetic observations using controlled exposures and washout periods in order to establish the fundamental validity of the measurement system.

It should be noted that the objections expressed by Springfield and Levitt are directed exclusively at pentane measurements. Ethane, being much lighter, is likely to be stored in fat to a much lesser extent relative to the amount exhaled. Ethane also has the advantage of being much less readily metabolized, leading to reduced complications with interindividual variability in metabolic elimination. For these reasons Habib and coworkers have chosen to use exhaled

ethane as the focus of their studies in cigarette smokers and controls with and without antioxidant supplementation (Habib et al., 1995; Do et al., 1996).

Habib et al. use a relatively short 2-4 min. period of washout with low hydrocarbon air (6.64 ± 0.33 pmol/liter of ethane) before collecting timed 2-minute samples of expired air. (In all cases this background concentration of ethane is subtracted from the measured values.) After collection and mixing, approximately 12 liters of the contents of the collection bag are passed through duplicate cold traps of freshly prepared activated charcoal immersed in frozen ethylene glycol at -70 °C (so that each cold trap gets about 6 liters of expired air). The remaining volume of expired air is then measured with a spirometer. The charcoal from each trap was placed in a test tube with an open screw top, and heated for 3 min. at 250 °C with agitation. 5 ml samples of the headspace from these tubes were then injected into a gas chromatography system (2 meter glass column packed with Carbosphere 60/80 at 220 °C and eluted with helium at 25 ml/minute. The retention time of ethane under these conditions was 4.74 min. The subjects were requested not to eat or smoke for at least an eight-hour period prior to the measurements.

Our analysis of data extracted from the figures shown in Habib et al. (1995) indicates a short-term half-life for ethane exhalation following the smoking of a single cigarette by smokers is about 0.53 hrs (32 min) while the corresponding value for nonsmokers is 0.146 hrs (about 9 min). [Caveat, this estimate of the rate constant is likely to be too low because the calculations ignored the large variation for values at Time 0 (cigarette smoking time) for both smokers and nonsmokers (mean pmol/min-kg ± SEM: 24.5 ± 5.33 and 12.9 ± 4.1, respectively), and therefore the half life may be shorter than indicated.]

Overall, this initial paper indicates greater baseline excretion of ethane in smokers (2.90 ± .52 pmol/min-kg than either nonsmokers (1.11 ± 0.26; p = .0064) or ex-smokers (1.55 ± .36 pmol/min-kg; p = .044). This initial result is therefore promising--suggesting that current smoking is associated with excess ethane exhalation that could reflect some ongoing excess of oxidative activity in the smokers (assuming, for the moment, that residual stored ethane from 8+ hour past smoking is not contributing significantly to the observations). Lognormal Z-Score plots of these data as extracted from figures in the manuscript are shown in Figure 1. The data indicate rather more variability in the measurements for nonsmokers (steeper slope) probably because of a combination of less precise measurements at the lower levels of excretion (particularly in the one isolated point to the extreme left in the nonsmoker plot), and greater difficulty in reading the nonsmoker points from the graph in the paper.

These initial results have been followed up in a subsequent paper testing the effects of antioxidant supplementation on ethane exhalation in 10 smokers and a more limited group of 3 non-smokers (Do et al., 1996). In brief, Do et al. (1996) find that supplementation with a combination of the antioxidants beta-carotene, alpha tocopherol, and vitamin C modestly but significantly reduces ethane exhalation among the smokers (from 4.06 ± 1.49 [SD] pmol/kg-min to 2.90 ± 1.29 pmol/kg-min). (Lognormal probability plots of these data are shown in Figure 2.) There was no comparable finding in the very small group of non-smokers. Moreover after the supplementation, there was a significant positive relationship between ethane exhalation and the ongoing smoking rate in packs per day. Finally, there was a curious finding that the degree of reduction of ethane exhalation levels with antioxidant supplementation was greatest in those

smokers who had the worst lung function as measured by % Predicted FEV. Such a result, suggesting a greater benefit of supplementation associated with putatively greater accumulated damage from past smoking is almost too good to be true, especially considering the very limited sample size on which it is based. Overall, the preliminary Do et al. (1986) findings tend to support the notion that ethane exhalation, as they measure it, is related to oxidative processes that are in turn related to smoking.

In conclusion, measurement of ethane in expired air shows some initial promise as a measure of oxidative processes that might be occurring at larger than normal rates in both smokers and coal miners. However, each of the sets of data has its difficulties that need to be worked out in further preliminary studies. The Aubertin data quoted in Table 1 on their face appear to provide support for both the effects of cigarette smoke and coal dust on ethane exhalation--both qualitatively and quantitatively. However there was no description of a washout procedure in the brief report of this study, and data have some internal difficulties. If one divides the reported ethane production rates (pmole/min) by the reported air concentrations (pmole/liter), the implied alveolar ventilation rates range from about 0.5 to 0.75 liters per minute--about ten fold too low. This reduces confidence in the accuracy of the absolute values reported. Probably it would be prudent to confer with the Auburtin group to resolve this problem before making comparisons with the Do et al findings. It would also be advisable to contact and possibly visit the Habib/Do group (in Arizona) to learn of any further work that may now be in press, and to get information about any experimental assay details that are not fully described in the paper.

Figure 1

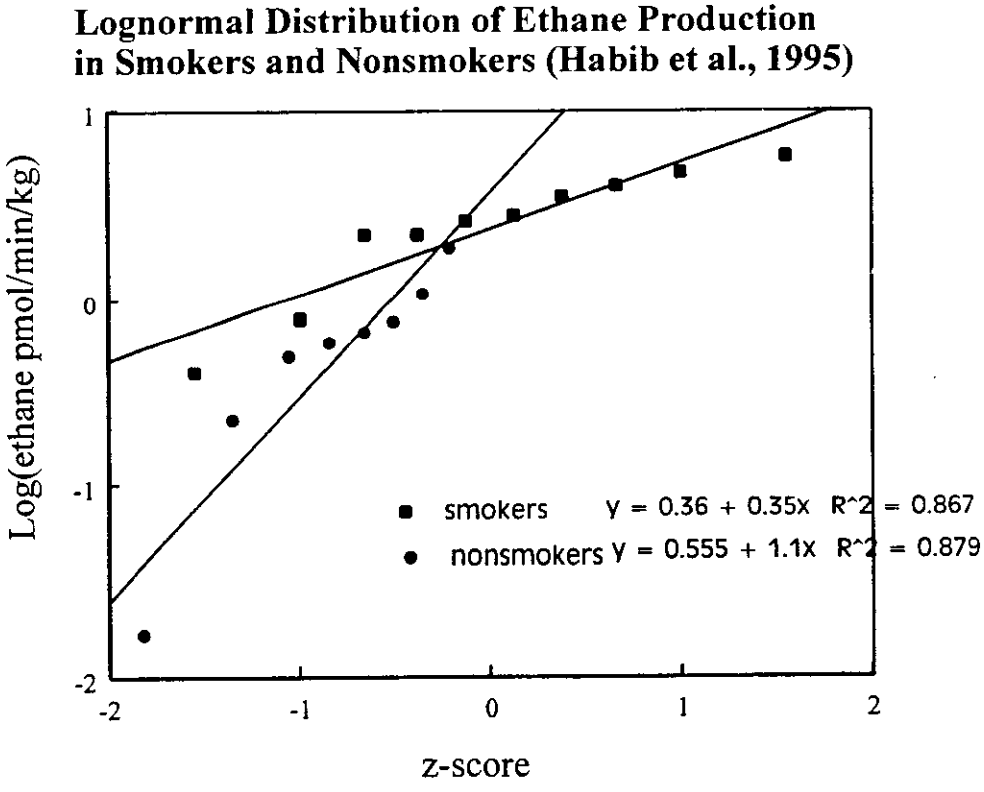
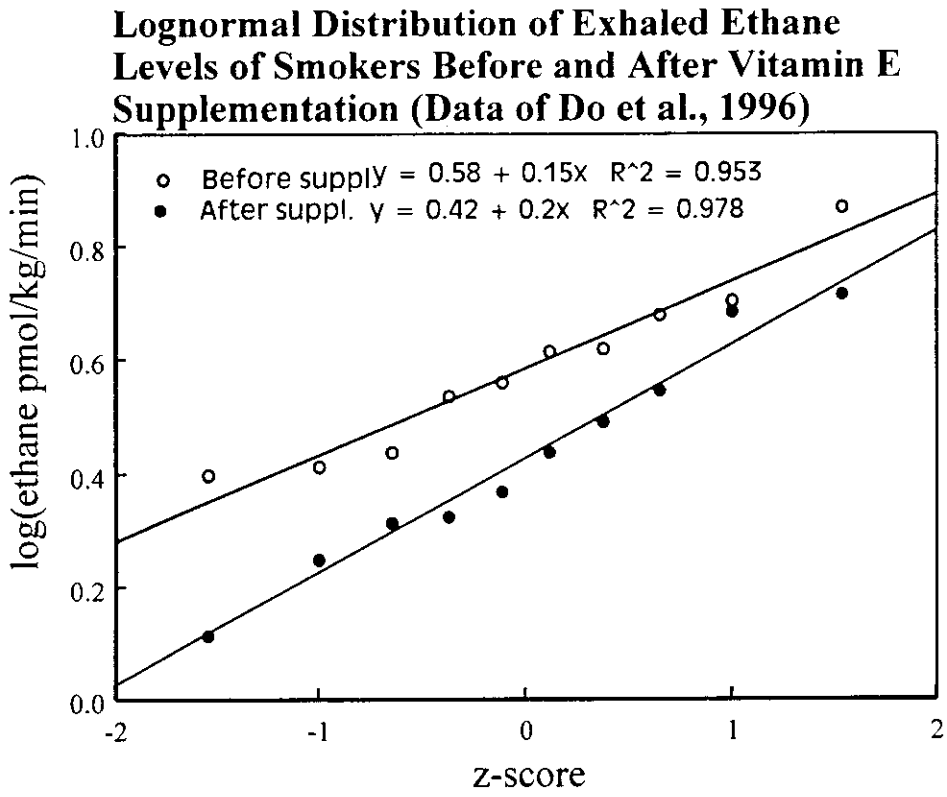


Figure 2



A final possibility that merits exploration is the possibility of single-breath measurements of alkane exhalation by a portable field mass spectrometry system described by Karla Thrall of the Battelle Pacific Northwest National Laboratory* (Richland Washington) at a recent DOE-sponsored conference on biomarkers (Thrall and Kenny, 1997).

Urinary Measurements of Elastin and Collagen Breakdown Products

The single most helpful papers for guiding the design of specific measurement procedures for urinary desmosine and isodesmosine are Cumiskey et al. (1995), and Stone et al. (1994, 1995). Both groups first collect 24-hour urine samples. (There does not seem to be any reason in principle why shorter collection periods or spot samples could not be used, with creatinine correction, but sampling variability might well be increased with less than 24 hour samples. The analytical procedure was routinely done on 24 ml volumes of urine). Then there is a hydrolysis step with strong acid (6 M HCl), followed by removal of interfering compounds with fibrous cellulose powder columns, and finally HPLC separation and quantification by absorption of ultraviolet light at characteristic frequencies. The Cumiskey et al. paper provides a very detailed protocol. The limit of detection is reportedly about 30 pmol--equivalent to about 2 pmol/ml in unhydrolyzed urine.

* Telephone: 509-375-6702.

Table 4 shows the basic results of Cumiskey et al. in which a total of six urine samples from three subjects were analyzed on two different days. The comparison of the measurements on the two different days gives us a measure of the experimental reproducibility (coefficient of variation = 16-21%; the comparison of average results for each subjects gives an approximate measure of the interindividual variability [coefficient of variation = 14-32%; or, in logarithmic terms log(GSD) = .064-.13)]. In the light of the observed day-to-day variability in the assay, the authors advise that several days of sampling should be used to characterize elastase activity in individual people.

Table 4
Inter-Assay Variability in Desmosine and Isodesmosine Concentration Measurements in Urine Samples from Healthy Subjects--Data of Cumiskey et al. (1995)
(concentrations in pmol/mg creatinine)

A. Individual Sample Data

subject	sample	IDES-day 1	IDES-day 2	DES-day 1	DES-day 2	IDES+DES-day 1	IDES+DES-day 2
11	A	7.06	7.06	10.43	7.34	17.49	14.40
11	B	6.99	8.00	7.58	9.55	14.57	17.55
12	C	10.27	9.67	7.92	8.12	18.19	17.79
12	D	12.74	14.03	10.12	12.08	22.86	26.11
13	E	4.52	6.10	5.06	6.47	9.58	12.57
13	F	7.61	8.08	8.52	8.88	16.13	16.96

B. Summary Data for all Measurements for Each Subject

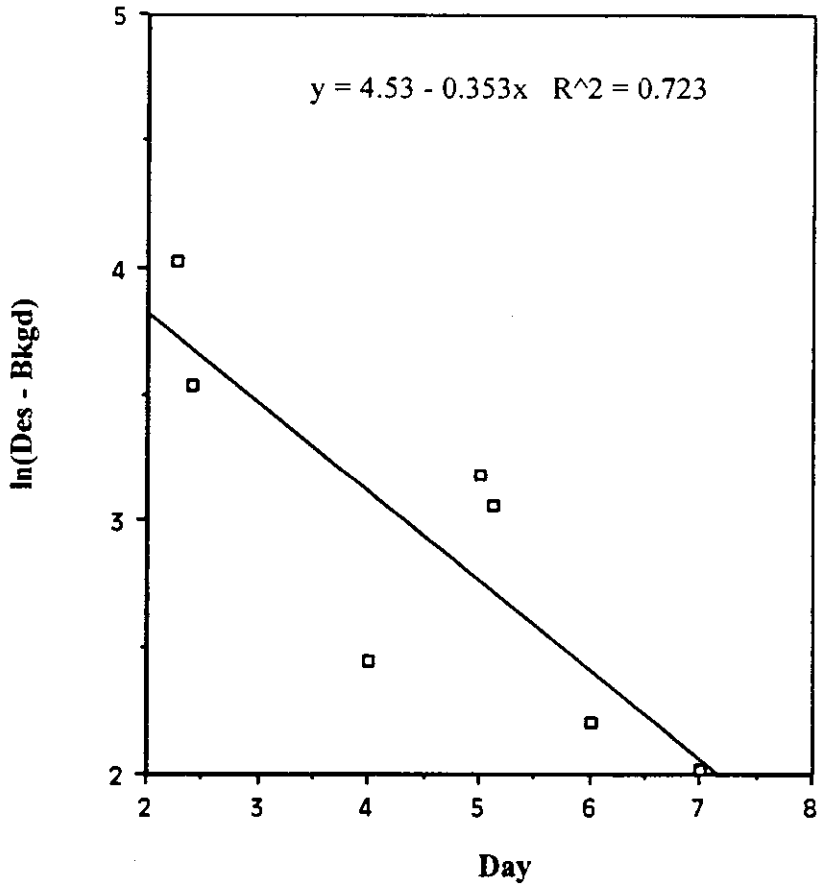
Subject	Mean IDES	Std error IDES	Mean DES	Std error IDES	Mean DES + IDES	Std error IDES
11	7.28	0.24	8.73	0.75	16.00	0.88
12	11.68	1.03	9.56	0.98	21.24	1.99
13	6.58	0.81	7.23	0.90	13.81	1.70
Interindividual Coefficient of Variation (%)	32		14		22	
Interindividual Log(GSD)	0.134		0.064		0.097	

Another reason for sampling on multiple occasions is that the elimination of desmosine from the system appears to be relatively slow. Stone et al. (1994) followed the excretion of desmosine following the ingestion of a special elastin-rich meal (300 g of calf ligamentum nuchae, containing 100 g of elastin, including 1.5 grams of desmosine and 1.2 g of isodesmosine. With a 1-2 day delay (presumably for absorption), this caused 8-10 fold increases in daily excretion of

the two elastin derivatives per g of creatinine. (By contrast a 1 lb meal of ground beef led to only about a 28% increase, after correction for creatinine, which was not statistically significant). After reaching a peak, there was a prolonged period of fall in desmosine excretion, apparently with a half-life of about 2 days (Figure 3). Stone et al. (1994) suggest a prolonged process of absorption in this experiment in part on the basis of earlier measurements of an elimination half-life of about 3 hours from the systemic circulation (Pai et al., 1991). On the other hand, Stone et al. (1995) have successfully used spot urine samples in 22 never-smokers, 13 smokers, and 21 patients with Chronic Obstructive Pulmonary Disease to characterize group differences. The latter two groups had approximately a 50% increase in desmosine and isodesmosine excretion compared to the never-smokers. Based on an approximate 74-year half-life of lung parenchymal elastin, they estimate that in never-smokers normal degradation of lung elastin accounts for about 19% of urinary desmosine. On this basis they estimated that their observations of excess desmosine excretion in smokers would be consistent with approximately a three-fold increase in

Figure 3

Log-Linear Plot of the Decline of Excess Excretion of Urinary Desomosine Following a High-Elastin Meal [Combined Data for Subjects 4 and 10 of Stone et al. (1994) After Subtraction of Background]



the rate of degradation of lung elastin. The results for COPD patients were interpreted as indicating a somewhat larger increase in baseline lung elastin turnover in that group.

In conclusion some surveys of urinary desmosine and isodesmosine excretion seem possible based on spot urine samples of workers with current or past high occupational particulate exposures.

Pharmacokinetics/pharmacodynamics of changes in measured alkane exhalation rates
In this section we address the issue of tissue storage and re-release of ethane and pentane. We do this by constructing physiologically-based pharmacokinetic models (as best we can from the available data) and simulating the time course of ethane excretion following an abrupt change from ambient hydrocarbon levels to those present in relatively low hydrocarbon air. (Later work could apply this approach to pentane). Given this, we ask:

- Are procedures such as those of Habib et al. (1995) and Do et al. (1996) sufficient to ensure that there is negligible interference from stored ethane (or pentane)?
- What "washout" periods are advisable?
- Under what circumstances of background ethane/pentane exposure is it likely to be necessary or helpful to measure body fat content to aid in separating out stored/rereleased hydrocarbons from hydrocarbons that are generated by ongoing oxidative processes?

Breath Alkane Pharmacokinetic Measurements Useful for Model Calibration
By far the most useful information for pharmacokinetic modeling of ethane and pentane is contained in some early papers by Wade and Van Rij (1985) and Van Rij and Wade (1987). The former paper has measurements of the "solubility coefficients" of ethane and pentane in various tissues based on an apparently conventional vial headspace equilibration technique (Table 5). We interpret these as tissue/air partition coefficients.

Table 5

Tissue/Air Partition Coefficient Measurements (Mean ± SD; N = 4) Reported by Wade and Van Rij (1985)

Gas	Fat	Muscle	Viscera
Ethane	2.7 ± 0.2	< 0.05	< 0.05
Propane	7.3 ± 0.5	0.4 ± 0.1	0.8 ± 0.2
Butane	17.0 ± 2.9	1.9 ± 0.4	2.0 ± 0.5
Pentane	37.5 ± 5.2	3.0 ± 0.6	5.8 ± 1.2

From these coefficients and unstated assumptions about body composition, these authors calculate overall body tissue/air coefficients of 0.44 for ethane and 8.4 for pentane. These numbers are similar to values calculated earlier by Filser et al. (1983) for rats--(0.61 for ethane and 5.46 for Pentane).

For pentane, we can compare the Wade and Van Rij measurements with a more extensive set of measurements made by Perbellini et al. (1985) in tissues from two men who had died sudden

deaths (Table 6). It can be seen that the fat/air values for pentane compare very well. There is, however considerable divergence for muscle/air. Muscle partition coefficients are notoriously difficult to measure because the fibrous nature of the tissue may impede equilibration. Less understandable is the apparent divergence for "Viscera" (corresponding to the "Vessel Rich Group in conventional Physiologically Based Pharmacokinetic Modeling). If we average the liver/air, kidney/air, and brain/air values of Perbellini, we obtain a value of 1.63-- which, like the muscle value, is less than a third of the corresponding tissue/air partition coefficient for "viscera" given by Wade and Van Rij. (1985). The Perbellini et al. observations indicate tissue/blood partition coefficients of 104, 5.53, 4.3, and 1.83 for fat, liver, VRG, and muscle, respectively.

Table 6

Tissue/Air and Blood/Air Partition Coefficient Measurements (Mean ± SD) for Pentane Reported by Perbellini et al. (1985)

Oil	47 ± 2.3
Blood	0.38 ± 0.08
Liver	2.1 ± 0.9
Kidney	0.6 ± 0.3
Brain	2.2 ± 0.5
Fat	39.6 ± 2.6
Muscle	0.7 ± 0.4
Heart	0.2 ± 0.4
Lung	0.5 ± 0.03

Beyond the information on partition coefficients, The Wade and van Rij papers report results from experiments in which six healthy subjects were attached to a rebreathing circuit containing a 14 liter spirometer after a preliminary "washout" period of 1.5-2 hours during which subjects were exposed to air containing very low levels of hydrocarbons (<1 pmol/liter). Rates of production of ethane and pentane were measured in this system over a two-hour period. In later studies (Van Rij and Wade, 1987) the rebreathing system was initially charged with a known high hydrocarbon concentration 41-45 nmol/liter), and the fall in ethane and pentane levels was followed over time. Experiments in this system with and without inhibition of metabolism (by dithiocarb; dosage not specified) allowed the authors to make estimates of metabolic rates. The authors' summary estimates of the relevant pharmacokinetic parameters in these papers are shown in Table 7.

Development of a Physiologically Based Pharmacokinetic Model for Ethane

Model Structure and Exogenous Parameter

We implemented a standard PBPK model structure in the Stella dynamic modeling language (Hattis, 1991). "Compartments" were specified as the Liver, Fat, Muscle Group ("poorly perfused" in other work), and the Vessel Rich Group ("richly perfused" in other work). As is conventional, full equilibration was assumed between each tissue and the blood flowing through

each tissue, and between alveolar air and arterial blood. To increase speed, the lung was not included as a storage compartment, but exchange was modeled as a dynamic equilibrium between alveolar air and arterial blood. The Stella model diagram is given in Figure 4; equations for the ethane model are summarized in Table 8. Human tissue volumes and flow rates are taken from our earlier work (Hattis, 1991; Hattis et al., 1986).

Table 7
Key Pharmacokinetic Summary Observations of Wade and Van Rij (1985) and Van Rij and Wade (1987) Used for the Development of Our PBPK Models

Parameter	Ethane	Pentane
Half-life with metabolism intact	3.6 hours (87 paper) 4.1 hours (85 paper)	45 min (87 paper) 52 min (85 paper)
Half life after inhibition of metabolism	6.2 hours	2 hours
Production Rate (pmoles/kg BW-hr ± SD)	95.1 ± 19.0	not given
Concentration in rebreathing system after 2 hours, with metabolism intact (pmole/liter)	370	120 ± 50
Concentration in rebreathing system after 2 hours, with inhibition of metabolism (pmole/liter)	622	1,600

Fitting Values of Key Unknown Parameters

For ethane, the key parameter that is missing from the available data is the blood/air partition coefficient. To estimate this, and corresponding values for tissue/blood coefficients, we set up a structure in which the model parameters for all of the tissue/blood partition coefficients depended on a specific trial value for the blood/air coefficient (see diagram and equations--we tentatively treated the "< .05" estimates of tissue/air for viscera and muscle as equal to .05). By running a small series of simulations,[1] we found that a blood/air partition coefficient of 0.105

[1] For these simulations, the model first was allowed to approach dynamic equilibrium with 1000 minutes of running under baseline conditions (external air concentration = 374 pmoles/liter; production of ethane = 111 pmoles/min). Then external exposure, ethane production and metabolism were turned off, and the half life for the decline in the ethane exhalation rate was measured in 20 minute periods beginning at t = 20 minutes after the change:

Time after shutoff of metabolism, production, and external exposure (min)	Elimination half life (hrs) measured over 20 min periods beginning at the times indicated
20	1.0
40	5.5
60	6.3
80	6.4
100	6.3

Figure 4

Ethane Model Diagram

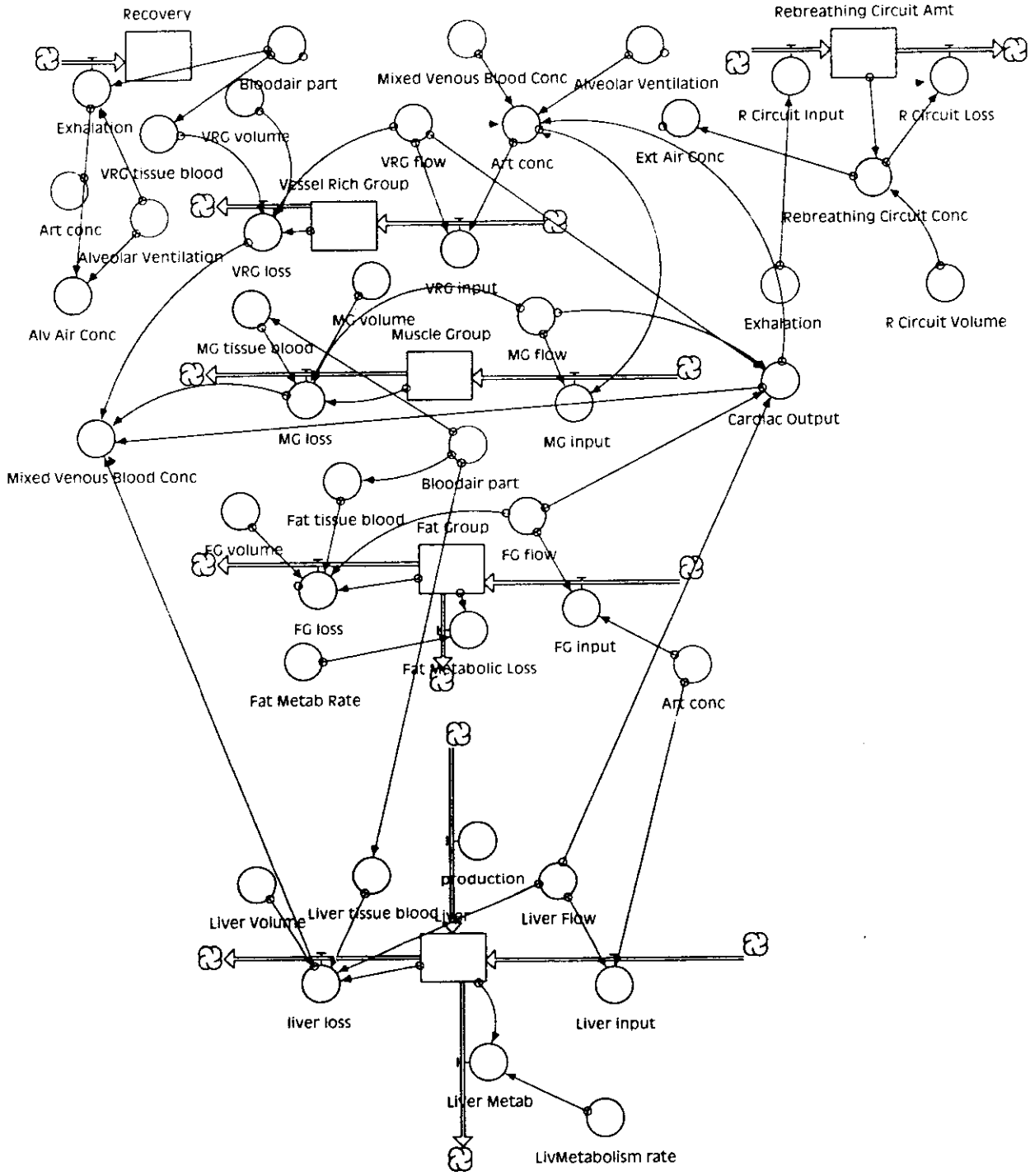


Table 8
Ethane Model Equations

Equations Describing the Inputs and Outputs from Compartments (Boxes in the Model Diagram):

Fat_Group(t) = Fat_Group(t - dt) + (FG_input - FG_loss) * dt
INIT Fat_Group = 20000

FG_input = Art_conc*FG_flow
FG_loss = Fat_Group*FG_flow/(FG_volume*Fat_tissue_blood){Place right hand side of equation here...}

Liver(t) = Liver(t - dt) + (Liver_input + production - liver_loss - Liver_Metab) * dt
INIT Liver = 60.34{Place initial value here...}

Liver_input = Art_conc*Liver_Flow{moles/min}
production = 80 {80 pmol/min estimated for an 80 kg man acording to Habib data; 111 pmol/min would be predicted from Wade and Van Rij central estimate of 95.1 ± 19 pmol/hr-kg for a 70 kg modeled person }
liver_loss = Liver*Liver_Flow/(Liver_Volume*Liver_tissue_blood){ moles/min}
Liver_Metab = Liver*LivMetabolism_rate {Place right hand side of equation here...}

Muscle_Group(t) = Muscle_Group(t - dt) + (MG_input - MG_loss) * dt
INIT Muscle_Group = 655{Place initial value here...}

MG_input = Art_conc*MG_flow{ Place right hand side of equation here...}
MG_loss = Muscle_Group*MG_flow/(MG_tissue_blood*MG_volume) {Moles/min}

Rebreathing_Circuit_Amt(t) = Rebreathing_Circuit_Amt(t - dt) + (R_Circuit_Input - R_Circuit_Loss) * dt
INIT Rebreathing_Circuit_Amt = 0{pmoles}

R_Circuit_Input = if (time<1090) then 0 else Exhalation {pmoles/min}
R_Circuit_Loss = if (Time<1090) then 0 else Alveolar_Ventilation*Rebreathing_Circuit_Conc

Recovery(t) = Recovery(t - dt) + (Exhalation) * dt
INIT Recovery = 0

Exhalation = Alveolar_Ventilation*Art_conc/Bloodair_part{moles/min}

Vessel_Rich_Group(t) = Vessel_Rich_Group(t - dt) + (VRG_input - VRG_loss) * dt
INIT Vessel_Rich_Group = 66.92{Place initial value here...}

VRG_input = Art_conc*VRG_flow{moles/min}

VRG_loss =
Vessel_Rich_Group*VRG_flow/(VRG_volume*VRG_tissue_blood){moles/min}

Equations for Parameters (Circles in the Model Diagram)

Alveolar_Ventilation = 7 {liters/min}

Alv_Air_Conc = Exhalation/Alveolar_Ventilation{pmol/liter}

Art_conc = Bloodair_part*(Cardiac_Output*Mixed_Venous_Blood_Conc+Ext_Air_Conc*
Alveolar_Ventilation)/(Cardiac_Output*Bloodair_part+Alveolar_Ventilation) {pmoles/liter}

Bloodair_part = .105

Cardiac_Output = FG_flow+Liver_Flow+MG_flow+VRG_flow{liters/min}

Ext_Air_Conc = If (Time<1000) then 374 else 0*Rebreathing_Circuit_Conc
{Rebreathing_Circuit_Conc 374 pmoles/liter atmospheric background? 6.64 pmol/l in
hydrocarbon free air according to Habib data}

Fat_tissue_blood = 2.7/Bloodair_part

FG_flow = .34{liters/min}

FG_volume = 15.024 {liters}

Liver_Flow = 1.34{liters/min}

Liver_tissue_blood = .05/Bloodair_part

Liver_Volume = 2.476{liters}

LivMetabolism_rate = 0.3

MG_flow = 1.5{liters/min}

MG_tissue_blood = .05/Bloodair_part

MG_volume = 34.756 {liters}

Mixed_Venous_Blood_Conc =
(FG_loss+liver_loss+MG_loss+VRG_loss)/Cardiac_Output{moles/liter}

Rebreathing_Circuit_Conc = Rebreathing_Circuit_Amt/R_Circuit_Volume{pmoles/liter}

R_Circuit_Volume = 14{liters}

VRG_flow = 3.38 {liters/min}

VRG_tissue_blood = .05/Bloodair_part}

VRG_volume = 3.551{liters}

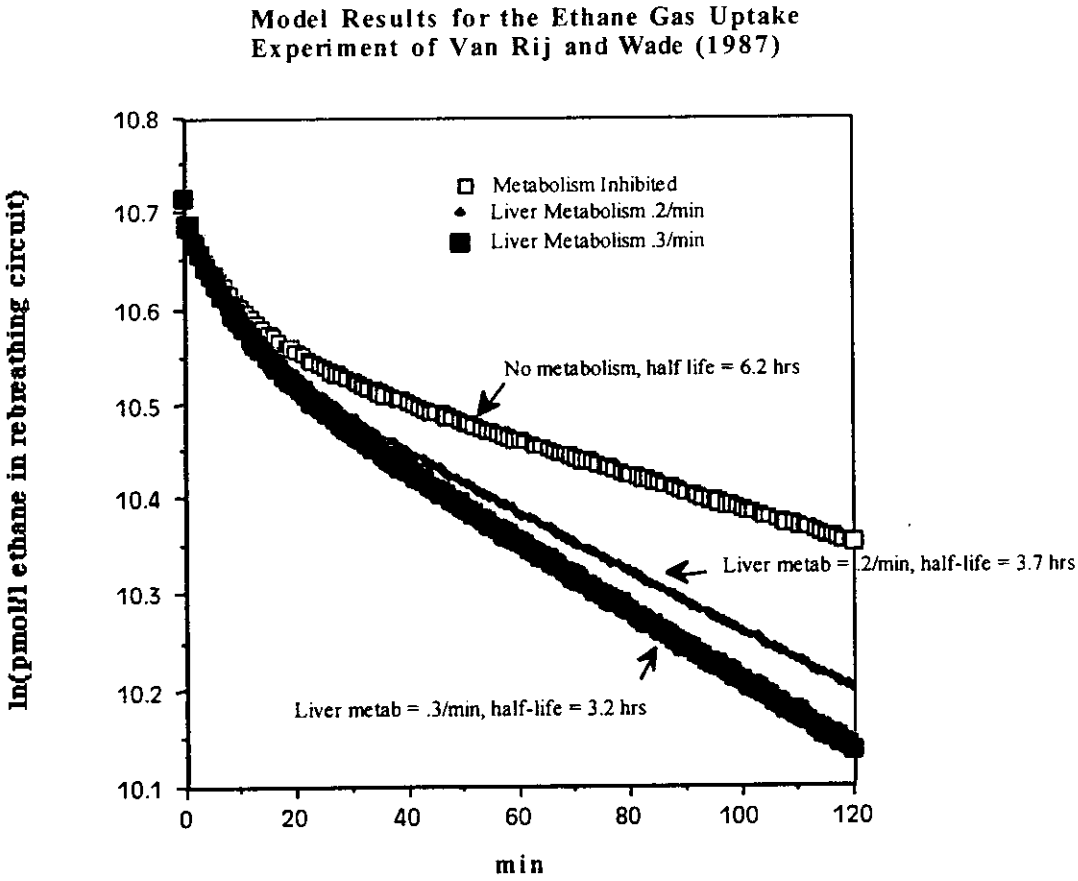
would yield a model in which the rebreathing circuit concentration would decline with an elimination half-life without metabolism corresponding to the observed value of 6.2 hours. This resulted in the following estimates for the various tissue/blood partition coefficients:

Tissue	Tissue/blood partition coefficient
Fat	25.7
Muscle, Liver, VRG	0.48

Based on this, we then tuned the liver metabolism rate to .2 to .3/min (giving half lives of 3.7 and 3.2 hours, respectively in comparison to the target value of 3. 6 hours). Plots of these results in a form comparable to Figure 1 of the Van Rij and Wade(1987) paper's are shown in Figure 5. The overall pattern of the model decline is similar to the original data.

We can also compare the model expectations for the accumulation of ethane in the rebreathing system with the actual observations. To do so, however, we need to confront the one of the central questions we posed at the beginning of this section--how much is the ethane "production" measured in these experiments likely to include ethane absorbed from the external environment, stored in the fat, and then released slowly into the expired air?

Figure 5



This clearly requires some information or an assumption about how much ambient ethane the Wade and Van Rij (and Habib/Do) subjects were exposed to over the day or two prior to the measurements. Unfortunately neither group provides this information. Failing this, we have drawn on data of Shah and Heyerdahl (1988) as quoted in the on-line Hazardous Substances Data Base. Based on 571 measurements, this source reports an urban average ethane concentration of 9.15 ppb by volume, which translates into 374 pmoles/liter at 25° C.

Table 9 shows the basic results of exercising the model to make comparisons with the Van Rij and Wade (1987) observations of the end concentration of ethane in their rebreathing system after (1) a 90 minute period of "washout" with air containing no ethane and then (2) a 120 minute period of connection with the rebreathing system (in which the "external air concentration" in the model is set equal to the continually increasing rebreathing system concentration). The Van Rij and Wade (1987) observations are given in the last column; the model "predictions" for the end concentration in the rebreathing system are given in the second to the last column for various combinations of the parameters shown in the earlier columns.

The first two lines of Table 9 reflect our base assumptions--the ethane production rate reported by the authors, and the typical urban external air concentration we have imported from other data. It can be seen that under these base conditions, the model predicts ethane concentrations in the rebreathing system that are somewhat higher (by 28-58%) than those observed

Table 9
Model Comparisons with the End Concentrations of Ethane in the Wade and Van Rij Rebreathing System Experiments

External Air Conc. (pmoles/l)	Liver Metabolism Rate (per min)	Ethane Production Rate (pmol/min)	Fat Group Flow (l/min)	Fat Group Storage Prior to Washout (nmoles)	Modeled End Conc. in Rebreathing System (pmoles/l)	Reported End Conc. in Rebreathing System (pmoles/l)
374	0.3	111	0.34	15.6	584	370
374	0	111	0.34	15.8	799	622
374	0.3	111	0	15.6	582	370
374	0	111	0	15.8	819	622
100	0.3	111	0.34	4.5	534	370
100	0	111	0.34	4.7	744	622
100	0.3	80	0.34	4.4	390	370
100	0	80	0.34	4.5	541	622
374	**0.3**	**80**	**0.34**	**15.5**	**440**	**370**
374	**0**	**80**	**0.34**	**15.6**	**597**	**622**

The third and fourth lines indicate that the problem is not with excessive release of stored ethane from the fat. Even though the predicted fat store is substantial (third column--over 15 nmoles = 15,000 pmoles), cutting off the blood flow to and from the fat compartment does not materially change the net output reflected in the end-rebreathing concentration. The opposite signs of the effect of cutting off blood flow in the two cases reflect the fact that in the normal case (when blood is flowing through the fat) the fat plays two different roles in the context of the rebreathing system--at early times after the connection is made, the fat makes a net positive contribution to ethane output; however toward the end of the two hour period the fat is actually absorbing more of the generated ethane than it is releasing when the external air concentration rises above the 300+ pmole/liter air concentration with which it is equilibrated. The intrinsic half-life for the release of ethane from the fat compartment is $\frac{\ln(2)}{\text{fat flow}/(\text{fat volume} * \text{fat tissue/blood})} = 13$ hours so it is not possible to eliminate the ethane from the fat compartment with any feasible washout procedure. The fat contribution can be estimated however, based on estimates of the background air concentration and simple measurements of body composition. Later we will exercise the model to develop preliminary formulae for this correction as a function of washout time.

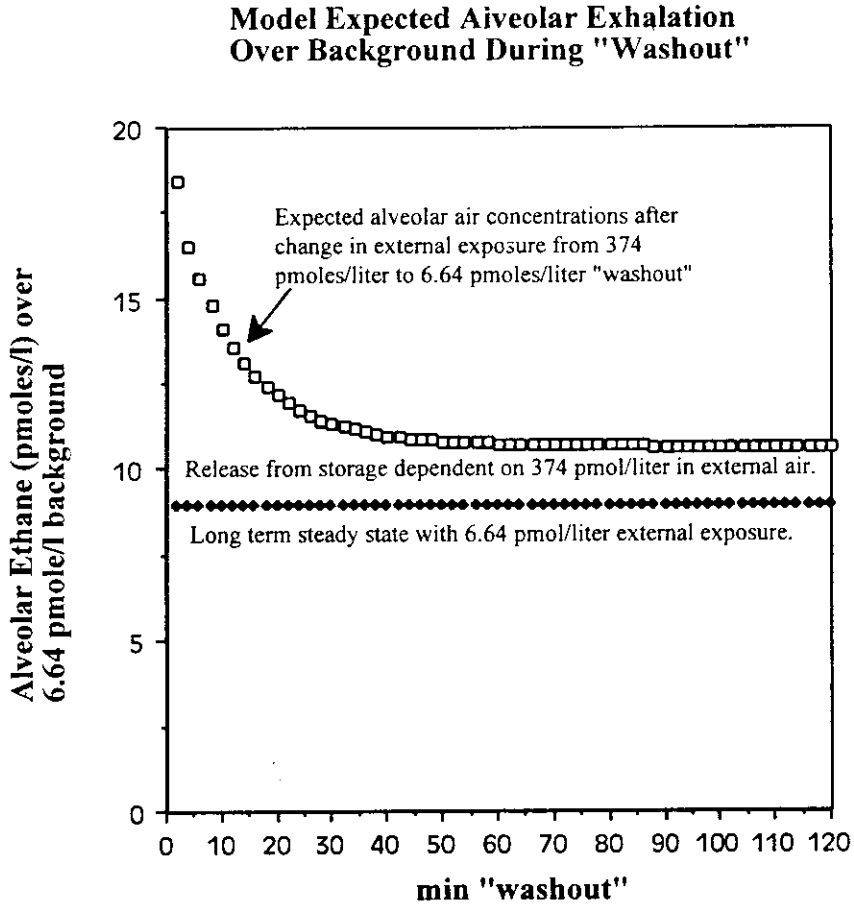
The fifth and sixth lines of Table 9 show what happens to the model projections when the external air concentration assumed for pre-equilibration is lowered to 100 pmoles/liter. It can be seen that this leads to a major reduction in the accumulated fat store, but not a large enough reduction in the air output to the rebreathing system to reconcile the results with the observations of Van Rij and Wade (1987). The differential is due to the fact that in part because of the 90 minute washout period, the rate of production of ethane has a much stronger influence on the output to the rebreathing system than the release from the fat stores [compare with the results in the seventh through tenth lines of the table, which reflect a modest (28%) reduction in the production from 111 pmole/minute (equivalent to Van Rij and Wade's estimate of 95.1 pmoles/kg-hr) to 80 pmole/minute]. The final (9th and 10th) lines of the table reflect our best judgment of the set of parameters that is most compatible with all our available information. This form of the model will be used to make tentative inferences about the effects of various washout periods, background ethane exposures, and body fat content on alveolar ethane concentration measurements as done by Habib et al. (1995).

Use of the Ethane Model to Assess the Influence of Various Factors on Ethane Exhalation, and Interpretation of Ethane Measurements In Terms of Internal Ethane Production

Figure 6 shows the results of using our model to simulate the fall of alveolar ethane concentrations during "washout" as specified by Habib et al. (1995), assuming prior equilibration with 374 pmoles/liter in external air and 80 pmoles/minute true production. It can be seen that a few minutes of washout as described by these authors cannot be expected to be sufficient to achieve good stability in the excretion of ethane originating from "background" sources. 40-90 minutes of "washout" seems desirable. After attainment of a full steady state, the model predicts an excess exhalation of 8.98 pmoles/liter in alveolar air over and above the 6.64 pmole/liter background in the low hydrocarbon air used by Habib et al. Because the model assumption is for an alveolar exhalation rate of 7 liters per minute, this means a net excreted ethane production of 62.9 pmoles/minute. (This is less than the 80 pmoles/min production rate built in to the model because some of the ethane produced is metabolized before it has a chance to be exhaled.) Table

10 shows the decline in excess exhalation of ethane from body stores over this background at various times after the start of "washout".

Figure 6



The data in Table 10 allow a relatively simple correction to be made to observed ethane alveolar exhalation data for prior steady state exposures to ethane in external air. Because the model is completely linear, the expected excesses due to stored ethane form external air exposures other than 374 pmoles/liter should simply be adjusted upward or downward in proportion to the actual average external background exposure level for the previous day or so.

It is also of interest, of course, to assess how this correction should be changed in relation to body build/fat content. There is some ambiguity in exactly how one should adjust the model for a larger fat compartment. One can, of course, simply increase the fat volume parameter in the model, but it is not completely clear whether or how one should make corresponding adjustments in the blood flow rate to the fat tissue. For our preliminary purposes here we have elected to bound the problem with two extreme cases: (1) an assumption that the fat flow increases in parallel with the fat volume, keeping the flow/volume ratio (and hence the half life of loss from

the fat tissue) constant; and (2) an assumption that fat flow is unchanged even as fat volume increases. Using both assumptions we tested the effect of a doubling of fat flow.

Table 10
Expected Decline of "Excess" Alveolar Exhalation from Body Stores During Various Periods of Washout

Minutes after start of washout	Excess alveolar conc over steady state resulting from stored ethane	pmoles/min excess excretion dependent on release of stored ethane
2	9.46	66.22
4	7.55	52.85
10	5.16	36.12
20	3.19	22.33
30	2.36	16.52
40	2.02	14.14
50	1.86	13.02
60	1.79	12.53
70	1.75	12.25
80	1.73	12.11
90	1.71	11.97
100	1.69	11.83

Table 11
Effect of Doubling The Size of the Fat Group (From About 15 liters to 30 liters) on the Expected Decline of "Excess" Alveolar Exhalation from Body Stores During Various Periods of Washout

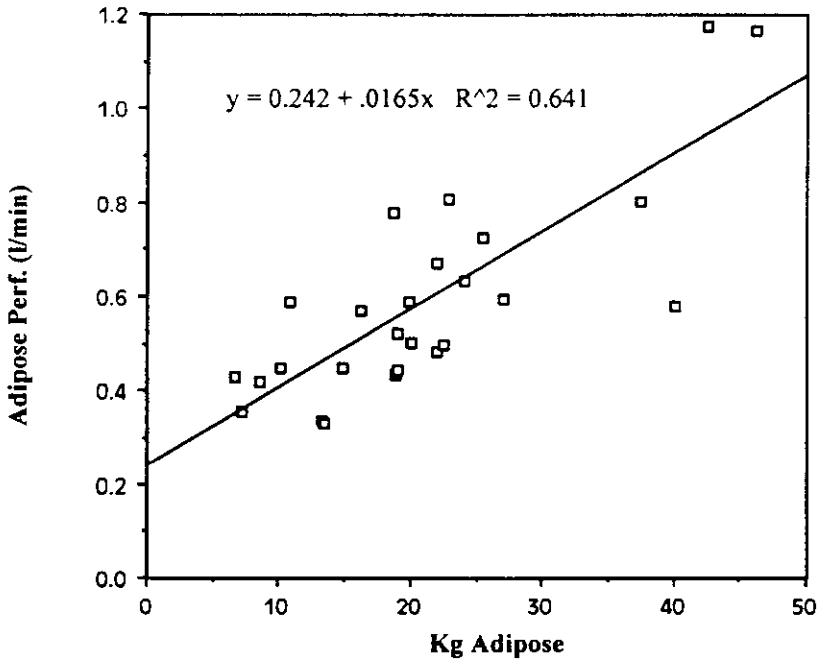
	Double FG Volume and Flow		Double FG Volume Only	
Minutes after start of washout	Excess alveolar conc over steady state resulting from stored ethane	pmoles/min excess excretion dependent on release of stored ethane	Excess alveolar conc over steady state resulting from stored ethane	pmoles/min excess excretion dependent on release of stored ethane
2	11.19	78.33	9.45	66.15
4	9.3	65.1	7.55	52.85
10	6.93	48.51	5.17	36.19
20	4.95	34.65	3.2	22.4
30	4.12	28.84	2.38	16.66
40	3.76	26.32	2.04	14.28
50	3.6	25.2	1.9	13.3
60	3.51	24.57	1.83	12.81
70	3.46	24.22	1.8	12.6
80	3.42	23.94	1.79	12.53
90	3.39	23.73	1.77	12.39
100	3.36	23.52	1.76	12.32

The results are shown in Table 11 in parallel to the format of Table 10. Comparing the results in the two tables, it can be seen that the effect of simply doubling the fat volume (Case #2) is minimal. On the other hand, if the fat group flow is also doubled, then the increment to excess alveolar concentration over steady state at long times after the start of washout is also approximately doubled.

Some guidance on which of these is closer to the truth can be gleaned from recent studies of Pierce et al. (1996) for toluene. In an excellent series of clinical pharmacokinetic experiments on 26 people with a defined exposure and extensive post-exposure follow-up, Pierce et al. developed individual estimates of several key pharmacokinetic parameters, including the blood flow through the fat tissue. The relationship between estimated fat blood flow and measured adipose tissue volume is shown in Figure 7. These data indicate that fat blood flow does tend to increase with the size of the fat compartment, but not quite proportionally as assumed in our "case 2". The relationship shown in Figure 7 allows a better basis for developing the needed correction factors for the excretion of stored ethane.

Figure 7

Observed Relationship Between Adipose Tissue Volume and Adipose Perfusion in Humans Exposed to Toluene--Data of Pierce et al (1996)



In conclusion, it does seem that it is feasible to measure ethane production in humans with the aid of alveolar air samples taken ideally with a substantial (40-90 minute) period of washout. The accuracy of such measurements can be enhanced by pharmacokinetic model-based corrections, taking into account (1) body fat content and (2) measurements or estimates of the concentration of ethane in ambient air breathed in by the subject in the day prior to the breath measurements.

Development of a Physiologically-Based Pharmacokinetic Model for Pentane
Work is currently under way to apply the same analytical approach to development of a PBPK model for pentane. Initially, we have chosen to use the complete set of blood/air and tissue blood partition coefficients given by Perbellini et al. (1983) (see Table 6, above). The pentane production rate is being tuned to produce the rebreathing system concentration seen by Van Rij and Wade (1987) with inhibition of metabolism (1600 pmoles/liter). Then the metabolism rate will be tuned to reduce output sufficiently to reduce the model "predicted" rebreathing system concentration to the 120 pmoles/liter reported by Van Rij and Wade(1987).

Even before completion of this modeling it is clear that fat storage of pentane taken in from the ambient air is likely to be a significant factor that will need to be taken into account in interpreting any pentane breath measurements in terms of internal pentane production. Given the estimated fat/blood partition coefficient for pentane of 104, the indicated half-life for loss of pentane from fat is expected to be about 53 hours--about four times longer than the corresponding figure for ethane. Measurements of pentane air levels in urban areas range widely and may be complicated by the same sort of measurement difficulties (e.g., interference from isoprene) as have caused such confusion with respect to breath samples. Initial modeling is being done based on the 134 pmoles/liter reported by Kohlmuller and Kochen (1993).

Conclusions--recommended procedures for study of these parameters in populations exposed to putative lung-damaging agents, and in ex-workers previously exposed.

What screening/control questions need to be asked in order to adequately control for confounders?

For alkane exhalation it is clearly necessary to make measurements or estimates of body fat content, and to ascertain whether the subject is a smoker. It may also be helpful, but probably not absolutely necessary to measure vitamin E levels in serum.

For urinary desmosine/isodesmosine measurements there should be some consideration to asking the subject about dietary habits--and in particular the consumption of meats with a high content of cartilage. The findings of relatively modest (statistically not significant) excess desmosine/isodesmosine urinary output suggest that these measurements will not usually be seriously affected by meat-eating habits, but some further inquiry in this regard seems prudent. Whether spot urine samples are used or 24-hour collections are made, the results must be corrected for creatinine excretion. This may have the effect of reducing whatever meat-consumption effect there may be on these parameters.

Finally, smoking and occupational history information should also be collected for studies of all biomarkers of putative lung damaging agents.

Based on an analysis of the pharmacokinetic/pharmacodynamic information, what experimental precautions need to be taken (e.g., washout, repeated measures) in order to produce high quality data?

Three basic suggestions have emerged from the prior analysis:

1) For both ethane and pentane, there needs to be a small series of measurements of pharmacokinetic parameters both in vitro (e.g., ethane blood/air, liver/air, and related partition coefficients) and in vivo (based on measured exposures) to confirm the pharmacokinetic modeling outlined earlier. These models will be an important part of assuring that breath observations can be corrected for the release of alkanes stored as the result of exposures to the same materials in ambient air.

2) Improved ethane (and/or pentane) ambient air measurements need to be made covering the day or two prior to breath measurements for at least a sample of study subjects.

3) Characterization of individual rates of excretion of desmosine and isodesmosine may benefit from a small series of repeated samples, spaced apart by at least a couple of weeks. Group differences, however, may well be detected by cross-sectional samples in which only a single measurement is made per person.

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Dr. Shane Que Hee and colleagues

1. Three papers have been published/accepted (1,2) or submitted (3). One has been published from the Master of Science thesis of Soo Young Kim (1), and another accepted from the work of doctoral students Yang Shen and Ju-Chien Tso (2). The third has been submitted from the Master of Science thesis of Keummi Park (3). (Appendix B)

The major achievement was the optimization (efficiency >80%; imprecision <10%) of the cordless microvacuum method for sampling dust of <180 μm particle size that contains xenobiotics. The latter included metallic species like metal oxides (1,4), a formulation of the reference pesticide chlorpyrifos (2) and Rhodamine 6G dye (2)) and a freeze-dried microbe (*Photobacterium phosphoreum*) (3). The optimized method utilized a flow rate of 4.0 L/min and multiple sampling passes of the sampling probe over a known area defined by a sampling

template containing >15 mg/100 cm^2 up to at least 170 mg/100 cm^2. The sampling technique for total dust was based on a microsampling technique originally reported by Que Hee et al in 1985 (4) for available dust in lead abatement and lead surface sampling activities.

The initial study (1) investigated the effect of particle size, surface coverage, surface type, flow rate, number of sampling passes, sampling technique, sampling face velocity, and degree of training on soil sampling efficiency from relatively flat hard surfaces, using the low flow technique (4) at 1.5 L/min as the starting point. The sampling apparatus was the same as the portable personal sampling pump/filter cassette combination that is standard for air sampling of aerosols by industrial hygienists, with the addition of a 5.0 cm x 0.6 cm ID Tygon sampling probe whose sampling end was cut at a 45° angle. Minimum soil holdup on the sampling apparatus surfaces allowed low soil surface coverages to be sampled efficiently. Soil was used since the Santa Ana wind from the desert causes much infiltration of Los Angeles homes, and soil is imported into homes by shoes. Efficient sampling (efficiency ≥87%; coefficient of variation (CV) <5%) of dry, loose soil on a 100 cm^2 template was achieved between coverages of 10 mg/100 cm^2 and 170 mg/100 cm^2 using at least 3 sampling passes for particle sizes between 63 μm and 180 μm. The technique was efficient because the face velocity developed at the surface was as high as 1083 ft/min for a 1 mm^2 surface-to-probe space.

The second paper (2) is the accepted paper demonstrating that spilled Rhodamine 6G dye and a NIST dust and a NIST soil impregnated with a chlorpyrifos formulation at its expected maximum coverage will also be sampled efficiently and precisely by the technique developed in Appendix B. Rhodamine 6G is used at Los Alamos National Laboratory as a fluorescent dye in tunable dye lasers. Powder is often spilled preparing the solutions, and a dry deposit occurs after a spill of solution. Spilled powder tends to cake since it is slightly hygroscopic, and thus is difficult to sample since it tends to cling to the surface. The flow rate had to be ≥3.0 L/min to provide quantitative sampling recovery on the first sampling pass. Below 2.0 L/min, all of the sample resided in the sampling probe and did not reach the filter cassette at all. As the flow rate increased more sample was retained on the filter. The weight of the entire sampling ensemble requires measurement, not the filter portion alone. The mass collected on the filter is only likely to be representative of the spill at the highest sampling flow rate of 4.0 L/min.

Chlorpyrifos, an organophosphorothioate pesticide, has been measured at concentrations up to 1300 mg/kg dust, and surface coverages up to 990 μg/m^2. The EPA in 2000 banned application of chlorpyrifos within dwellings where children could be exposed. The hypothesis relative to sampling efficiency was that the pesticide of highest concentration in dust was most likely to decrease the sampling collection efficiency of uncontaminated dust using the optimized 3-pass methodology at 4.0 L/min. Thus the experiment involved comparison of coated and uncoated dust. NIST SRM 2711 Montana Soil of <74 μm particle size, and SRM 1649a Urban Dust of <125 μm particle size were coated with Lorsban 2E formulation. The latter consisted of 40.7% chlorpyrifos, and 59.3% inert ingredients, mostly alkylbenzenes, as demonstrated through GC/MS analysis of the formulation. 31.94 mg in acetonitrile was coated onto 10 g of dust or soil to simulate the highest measured dust concentration of 1300 mg/kg. Loadings of 10-20 mg of impregnated soil and dust at 1300 mg/kg concentration were investigated relative to uncoated dust and soil on the 100 cm^2 template surface using the 3-sampling pass technique developed in Appendix B. The lower surface coverage range for efficiencies >79% and imprecisions <16%

CV was 15-20 mg/100 cm^2 for both coated and uncoated dust or soil, with lower efficiencies being evident for coated soil and uncoated dust. The lower efficiencies for coated soil were caused by enhanced particle agglomeration and increased stickiness; this cannot explain the contrary results for coated dust. For the latter, the initial particle size was bigger than for the soil, and particle sizes between 63 μm to 180 μm cause no difference in sampling efficiency.

The third paper currently under review (3) presents the research associated with sampling spills of the freeze-dried bacterium, *Photobacterium phosphoreum*. The major finding relative to sampling spilled powder was that since freeze-dried bacteria was a very deliquescent powder the cassette entry port inner diameter had to be increased to accommodate large chunks to achieve sampling efficiences >80%. Once the material becomes too damp, swabs with handles are the best way to sample/clean up spills. During this investigation, the Microtox test of viability based on bioluminescence was compared with a commercial colorimetric test to assess the effect of dust on bacterial viability. The addition of 20 mg of NIST 1649a urban dust per mL caused complete inhibition of the Microtox test as did 5 mg of dust for the colorimetric test. Complete desiccation caused bacterial death in both tests. The major problem with the colorimetric test using the naked eye was its insensitivity; sensitivity was improved twofold by developing a spectrophotometric technique at 508 nm, and this rivalled the sensitivity of the Microtox test. Microtox test results were only completely reliable for homogeneous solutions that passed a 0.45 μm filter.

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American Industrial Hygiene Conference And Exposition (Aihce) Presentations

1. SY Kim, SS Que Hee, JR Froines. Optimized portable cordless vacuum method for sampling dry, hard surfaces. AIHCE, May 17-23, 1997, Dallas, TX. Abstract 144.
2. Y Shen, SS Que Hee, JR Froines. A surface sampling portable cordless vacuum method for Rhodamine 6G. AIHCE, May 20-25, 2000. Orlando, FL. Abstract 340.
3. JC Tso, SS Que Hee, JR Froines. Surface sampling for soil impregnated with chloropyrifos formulation. AIHCE, Orlando, FL, 2000. Abstract 328.

Master Of Science Theses

1. Soo Young Kim. Optimized portable cordless vacuum method for sampling dry, hard surfaces for dusts, 1997.
2. Keummi Park. Microtox test as a validation method for surface sampling of bacteria in dust, 1999

4. Integrated task and postural analysis for ergonomic exposure analysis. To develop, pilot test,and validate an integrated task and postural analysis for ergonomics exposure assessment.

Wen Chen V. Liu, Ph.D., CIH, CSP

Hazard Surveillance in the Defense/Nuclear Industry - Occupational Ergonomics Component - Overview

The Occupational Ergonomics component of this research project contains five subprojects. The first two subprojects focused on the development of exposure and response surveillance tools to be used through Internet as a means for ergonomic hazard surveillance in the office work environment using visual display terminals. The third and the fourth studies focused on the evaluation of ergonomic hazards in a defense/nuclear facility that utilizes glovebox and Hypalon glovebox gloves. The fifth subproject focused on the development of a real-time heat stress-heat strain personal monitor for workers wearing total encapsulated protective suits, as heat stress has been indicated as one of the major hazards encountered by many workers in the defense / nuclear industry are involved in the decommission and decontamination activities. The title, significant findings, usefulness of the findings, and dissemination of the findings of each of the subproject are briefly summarized as follows.

Subprojects:

I. Computer Usage and Upper Extremity Musculoskeletal Discomfort in an Engineering Firm of the Aerospace/Defense Industry - A Survey through Intranet.

Significant Findings

This study represents the first electronic survey through Intranet that evaluated the association between self-perceived computer usage and self-reported musculoskeletal discomfort of the upper extremity. In addition, the population studied included engineers in an Aerospace/Defense Industry, a profession that has not been studied in terms of the prevalence of self-reported musculoskeletal discomfort. Furthermore, the survey was conducted electronically the company's Intranet. Nine hundred and ninety-seven employees (277 females and 717 males) of the company responded to the survey in two weeks. The results of logistic regression analysis indicated that gender, job tenure, and hours of computer usage were three factors significantly associated with the prevalence of musculoskeletal discomfort in the upper extremity.

Usefulness of the Findings

This study demonstrated that Intranet and Internet is an expedient and useful tool for the surveillance ergonomic hazards in office work environment.

Dissemination of the Findings

An abstract of the results of the study was presented at the 1999 American Industrial Hygiene Conference and Exhibition. A manuscript based on the study is being reviewed for consideration for publication in a peer-reviewed journal (Applied Occupational and Environmental Hygiene).

II. A Software-Based Tool for Exposure Assessment of VDT Work through Internet.

Significant Findings

An innovative software-based exposure surveillance tool was developed in this subproject to objectively quantify office workers' usage of computer input devices. This software-based tool would replace subjective estimate of exposure to biomechanical factors associated with the development of computer input device-related musculoskeletal disorders/discomfort.

Usefulness of the Findings

In this project we created a software-based exposure surveillance tool that can objectively quantify office workers' usage of computer input devices. The results of the study were also incorporated in another research proposal funded by the NIOSH under the NORA initiative for field evaluation.

Dissemination of the Findings

An abstract of the results of the study was presented at the 1999 American Industrial Hygiene Conference and Exhibition. A manuscript based on the study is being revised for consideration for publication in a peer-reviewed journal (American Industrial Hygiene Journal).

III. Hazards in Glovebox Operations in a Defense/Nuclear Facility - Through Ergonomic Task Analysis.

Significant Findings

Two ergonomic task analyses were conducted for two glovebox operations in a Defense/Nuclear Facility. The first ergonomic task analysis was conducted for an operation that casts metal inside a glovebox. The specific aim of this analysis was to identify opportunities for continuous improvement in terms of reducing musculoskeletal load. The ergonomic task analysis, consisting of task analysis, postural analysis, and static strength modeling, was applied to two on-site simulations and to actual casting operation recorded on videotape. The results of the postural analysis indicate that the work environment and the tasks involved in casting operation do occasionally place the operators in awkward posture. As a result, the working conditions should be modified sometime soon. The results of static strength modeling show that several tasks involving lifting heavy molds are not designed for the majority of the general population. These tasks could be modified by rearranging the

internal layout of the glovebox and by installing auxiliary material handling devices in the glovebox.

The second ergonomic task analysis was conducted of an operation that refines metal in specific forms. This particular glovebox operation has experienced high frequency of abnormal glove breaches. The specific aim of the analysis was to identify improvements that could reduce the incidence of glovebox glove failures. Document reviews, individual interviews, small group meetings, and on-site observations were conducted during the investigation. The results indicate that there are many micro and macro ergonomic improvements that could be made to increase the reliability of the glovebox gloves and the comfort of the workers.

Usefulness of the Findings

This case study demonstrated the utility of a systemic approach that addresses both macroergonomic and microergonomic issues in improving the health and safety of workers in a Defense / Nuclear facility. A portion of the study was also used to demonstrate the Defense / Nuclear facility's effort and achievement under the Price-Anderson Act regulating the facility.

Dissemination of the Findings

A case study was presented by a collaborator of the study at the 1998 American Industrial Hygiene Conference and Exhibition. A manuscript is being revised for submission for consideration of publication in a peer-reviewed journal (Industrial Health).

IV. Validation of an Experimental Device for the Measurement of Kinetic Friction.

Significant Findings

An experimental device was developed in this project for the measurement of kinetic coefficient of friction. In addition, the kinetic coefficient of friction (μ_k) of fingertip was measured for six Chinese subjects on textured and non-textured surfaces in this exploratory study. The results indicate that μ_k of the fingertip skin varies with the magnitude of normal force and the surface texture of the test plates. The μ_k decreased as the exerted normal force increased. The highest value of μ_k was 2.05 for males and 2.26 for females with 1 N normal force on a test plate of 100% contact area. When the load increased to 10 N, μ_k decreased to 1.09 for males and 1.11 for females on the same test plate. The μ_k increased as the contact area increased. For males, there was a 39% and 64% increase, respectively in μ_k as the contact area increased from 50% to 75% and 100%. For female, there were, respectively, a 41% and a 77% increase in μ_k as the contact area increased from 50% to 75% and 100%.

Usefulness of the Findings

Friction is a common mechanical stressor upon human skin. High friction may produce erosions and blisters to human palmar. Friction also affects our ability to manipulate and

grasp objects with the hand. Friction between the surface of an object and fingers is also needed for an individual to adjust the amount of force applied in manipulating objects. Low friction objects tend to slip out of the hand resulting in an increase in the potential for injury. Low friction objects therefore require greater grasp forces than objects with high friction. Prolonged, excessive grip forces applied to prevent slippage may cause injuries to tendons and tissues. The experimental device developed in the study has been adopted by a defense / nuclear facility with a robot arm for testing the wearability of glovebox gloves. The results of the study will be used to develop guidelines of glovebox glove design.

Dissemination of the Findings

An abstract of the results of the study was presented at the 1999 American Industrial Hygiene Conference and Exhibition. A manuscript based on the results of the study is being revised for consideration for publication in a peer-reviewed journal (Applied Ergonomics).

V. A Real-Time Personal Heat Stress & Heat Strain Monitor in Protective Suit.

Significant Findings

The goal of this study was to develop a real-time personal monitor capable of evaluating heat stress and heat strain encountered by workers wearing encapsulating protective clothing. This monitor simultaneously characterized the climatic condition of the microenvironment and the physiological responses of the worker in protective clothing. Specifically, the study: (1) integrated temperature and humidity sensors to continuously characterize the microenvironment in a protective suit; (2) integrated heart rate sensors and body temperature sensors to characterize the physiological response of the person wearing a protective suit; and (3) tested the utility of two wireless transmitters, 0.9 GHz and 2.4 GHz, to transmit the heat stress and heat strain signals.

Usefulness of the Findings

This study represents an innovative integration of information technology and sensor technology for hazard surveillance. In addition, this study has direct application to personal exposure surveillance of workers wearing protective suits and utility for the design of protective clothing.

Dissemination of the Findings

An abstract of the preliminary results of the study has been submitted for presentation at the 2000 American Industrial Hygiene Conference and Exhibition. Manuscript is being revised according to reviewers' comments for publication in a peer-reviewed journal (Applied Occupational and Environmental Hygiene Journal).

Wen Chen V. Liu, Ph.D., CIH, CSP and Craig Conlon, MD

Computer Usage and Upper Extremity Musculoskeletal Discomfort in an Engineering Firm of the Aerospace/Defense Industry - A Survey through Intranet – Subproject I

Abstract

This paper presents the results of a survey that evaluated the association between computer usage and musculoskeletal discomfort of the upper extremity in an engineering firm. The survey was conducted electronically by the firm's ergonomic team through the company's Intranet. Nine hundred and ninety-seven employees (277 females and 717 males) of the company responded to the survey in two weeks. Job functions of the respondents included management (10%), administration (13%), engineering (54%), and others (23%). On the average, the respondents spent more than five hours a day on their computers. Female employees appeared to work with the computers more than male employees do with the computer, 6 hours/day vs. 4.9 hours/day. Forty-seven percent of the respondents reported that they had experienced upper extremity musculoskeletal discomfort 1 month before and at the time of the survey. However, respondents in administrative and "other" job functions seemed to be more likely to experience musculoskeletal discomfort than respondents in engineering and management positions did. Experience of musculoskeletal discomfort in the upper extremity was evaluated using a logistic regression model. The results of logistic regression analysis indicated that gender, job tenure, and hours of computer usage were three factors significantly associated with the prevalence of musculoskeletal discomfort in the upper extremity.

Keywords: Musculoskeletal Discomfort, Engineering Firm, Intranet

Introduction

The use of personal computer in the office environment has been linked to the development of musculoskeletal discomfort/disorders among workers. Bergqvist (1984) and Hunting et al. (1983) demonstrated that visual display terminal (VDT) operators are prone to report musculoskeletal discomfort in the shoulder and in the neck. Kukkonen et al. (1983) have also shown the data entry operators are likely to experience musculoskeletal discomfort in the neck. These studies all focused on workers in jobs characterized by continuous, intensive keying. Other types of office workers, such as engineers and managers, have thus far received relatively little attention in terms of their usage of computers and their experience of musculoskeletal discomfort in the upper extremity.

In this paper, we present the results of a pilot study conducted by an ergonomics team of an engineering firm. This engineering firm has been in operation for more than thirty-five years. At the time of the survey more than four thousand employees were employed. The company had 4000+ personal computers in operation. Some of the employees experienced carpal tunnel syndrome and other musculoskeletal discomfort/disorders believed to be associated with the use of VDT workstation, based on internal medical findings. Together with the firm's Safety and Health staffs, the employees formed an ergonomic team to identify and solve musculoskeletal discomfort some employees were experiencing.

Being an engineering firm with more than two thousand engineers, the ergonomic team had a tremendous amount of resources in terms of computer programming abilities, email and Intranet connectivity. In addition, the management is committed to provide the human resources necessary in initiating a study to identify the prevalence of the musculoskeletal discomfort/disorders problem associated with computer usage. Having received some reference materials regarding the design of a self-reported exposure assessment and discomfort survey, the ergonomic team developed a dynamic webpage and conducted a pilot study.

This pilot study, because of its exploratory nature, had a simple objective. That was to get an estimate of the computer usage among employees and of the prevalence of musculoskeletal discomfort in upper extremity.

Methods

The methods the ergonomics team used to accomplish the above goals was to conduct an exposure and discomfort survey through Intranet. The engineer developed a questionnaire using the HyperText Markup Language. Emails were then sent to managers in the company soliciting the managers' cooperation in forwarding the messages to their groups of employees, including the professionals, clerical staffs, technicians, etc. The emails forwarded to the employees contained a link to the survey webpage on their Intranet. If the employee chose to respond, by a single click on the link, he/she gets onto the questionnaire webpage. Within a two-week period, nine hundred and ninety-seven employees of the company responded to ergonomics team's email request.

Excluding three respondents who did not provide information regarding their gender, there were 277 females and 717 males. Mean age of the male respondents 44.5 (SD=10.4 year) is not significantly greater than the mean age of female respondents, 41.4 year (SD=10.5 year). Mean tenure of the respondents was 9.3 year and the SD was 8.1 year. Male respondents had a mean tenure of 9.8 year (SD=8.5 year), higher than the mean tenure of 9.3 year (SD=8.1 year) of the female respondents ($p < 0.01$). Male respondents were also taller and heavier than female respondents were. For male respondents, the mean stature was 178.7 cm (SD=7.3 cm) and for female respondents the mean stature was 163.6 cm (SD=7.2 cm). For male respondents the mean body weight was 85.2 kg (SD=15.4 kg) and for female respondents the mean body weight was 66.1 kg (SD=14.7 kg).

Questionnaire

The self-administered questionnaire had two sections. The first section collected basic demographic data such as age, height, weight, job tenure, and gender were also collected using the questionnaire.

The second section contained 12 questions. Two questions asked for the number of hours of computer usage and the percentage of which using keyboard, mouse and watching screen. Two questions asked for the frequency of taking breaks away from computer work. One question was regarding the perceived work demand in keying and using mouse. Two questions asked for the dominant hand for writing and for using the mouse. One question asked whether the respondent wore glasses, especially the bifocal or multifocal. One question was on whether the respondent had had his/her workstation evaluated for ergonomic problems. Two questions were asked

regarding the specific fingers used in keying and in clicking mouse buttons. One final question asked whether the respondent had experienced any musculoskeletal symptoms in the upper extremity that lasted more than two consecutive days while using the computer. "Musculoskeletal symptoms" were defined as pain/discomfort in neck, shoulder, elbow, forearm, hand; numbness or tingling in arm or hand; and loss of strength.

Data Analysis

Descriptive statistics, chi-square analysis and logistic regression analysis were conducted. Logistic regression was conducted to model the association between the risk factors and the prevalence of upper extremity discomfort observed. Odds ratios were estimated as the antilog of the regression coefficients (Kleinbaum et al. 1982; Hosmer and Lemeshow 1989). A 95% confidence interval was calculated as the antilog of the standard error coefficients multiplied by 1.96 and -1.96. All statistical analysis was conducted with software package SPSS on a personal computer (SPSS 1994). Trend test based on logistic regression was also conducted (Rothman 1986).

Results

Job Function

As shown in Figure 1, 54% of the respondents were engineers and 16% of that were females. 13% of the respondents were administrative staffs and 65% of that were females. 10% of the respondents were managers and 12% of that were females. Respondents with other job titles constituted 23% of the respondents and 40% of which were females. Job titles in the "others" category included: accountants, editors, and drafters.

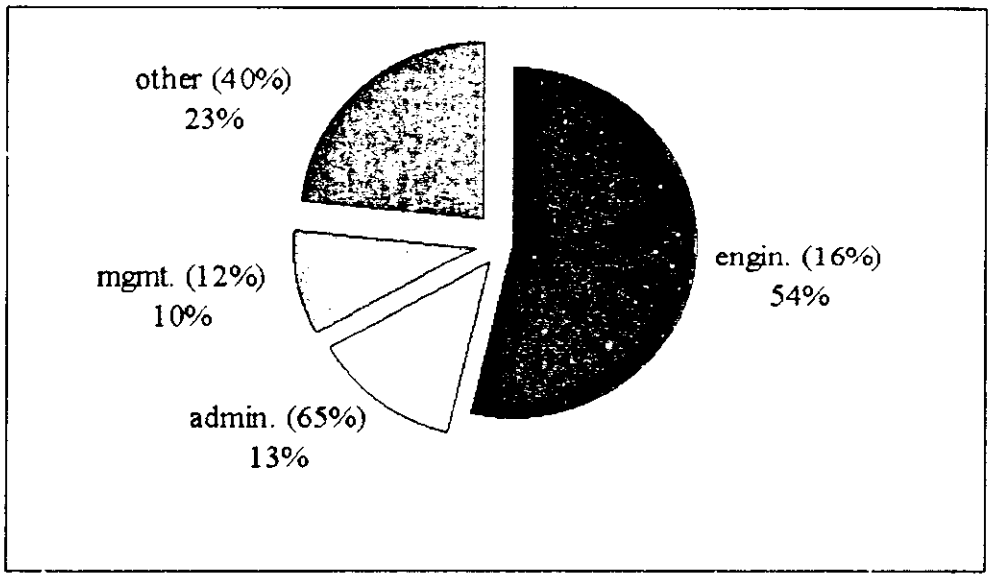

Figure 1. Percentage of participants in four job categories. (% female in each job category)

Computer Usage

Figure 2 graphs self-reported hours of computer usage. Overall, the employees spend 5.2 hours/day on computer. However, female employees seemed to spend more hours each day

(mean: 6 hours, SD: 1.7 hours) on computer than male employees did (mean: 4.9 hours, SD: 1.9 hours). The gender difference in the hours of computer usage was statistically significant ($t=8.65$, $p < 0.01$).

Computer usage in this engineering firm also depends on the job functions. As expected, computer usage by the managers (mean: 3.7 hours/day, SD=1.43 hours) was considerably lower than the computer usage (mean: 5.4~5.5 hours/day, SD=1.7~1.9 hours) by respondents of other job functions. The difference in computer usage among respondents of management, engineering, administration, and others was statistically significant ($F=23.6$, $p < 0.01$).

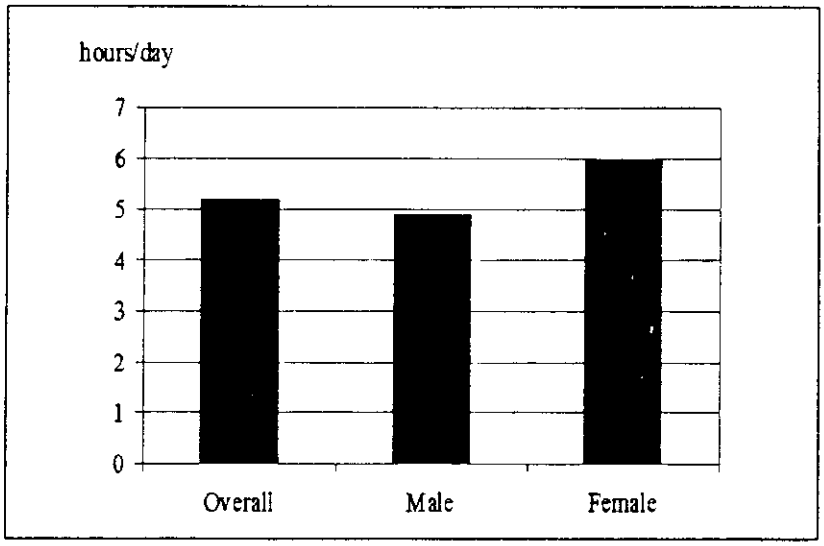

Figure 2. Self-Reported Computer Usage

Prevalence of discomfort by job function.

Figure 3 shows the prevalence of musculoskeletal discomfort in the upper extremity among employees in four job functions. 43% of the engineers and 41% of managers that responded to the questionnaire reported having experienced upper extremities musculoskeletal discomfort, while 56% of the administrative respondents and 52% of the respondents in the job category "Others" reported having experienced musculoskeletal discomfort. Chi-square analysis showed that there was a significant difference in the prevalence of upper extremity discomfort among respondents of these four job functions (Chi-square = 11.0, $p < 0.05$).

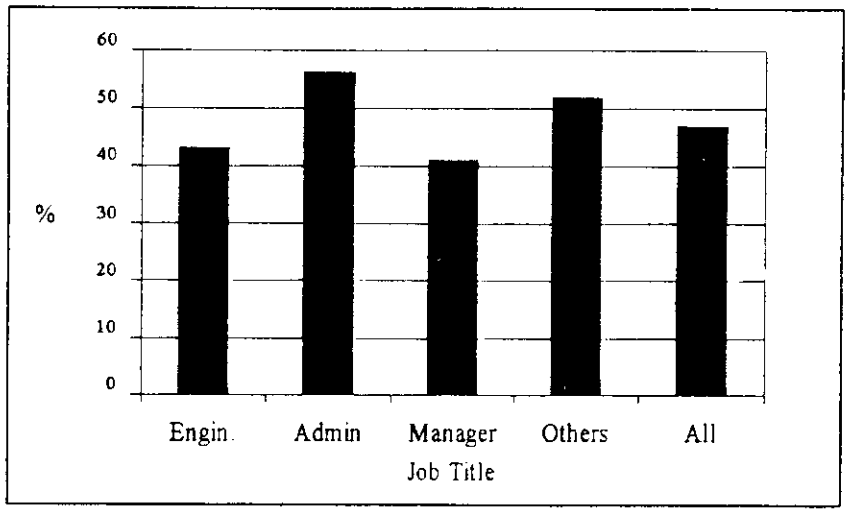

Figure 3. Prevalence of Self-Reported Upper Extremity Discomfort by Job Title

Prevalence of discomfort by tenure group.

Figure 4 shows the prevalence of discomfort by different tenure groups. It seemed that the prevalence of musculoskeletal discomfort increased as the job tenure increased. Overall, the prevalence of discomfort increased from 36.8% for the "less than and equal to 1 year" tenure group to 54.2% in the "> 15 year" tenure group. Chi-square analysis indicated that the prevalence of upper extremity discomfort among tenure groups were statistically significantly different (Chi-square = 16.6, $p < 0.01$). A similar trend was found for both male and female respondents. However, statistically significant difference in the prevalence of discomfort among job tenure groups was found only with the female respondents (Chi-square = 14.6, $p < 0.01$). Across all tenure groups, the prevalence of discomfort was always higher among the female groups than among the male group.

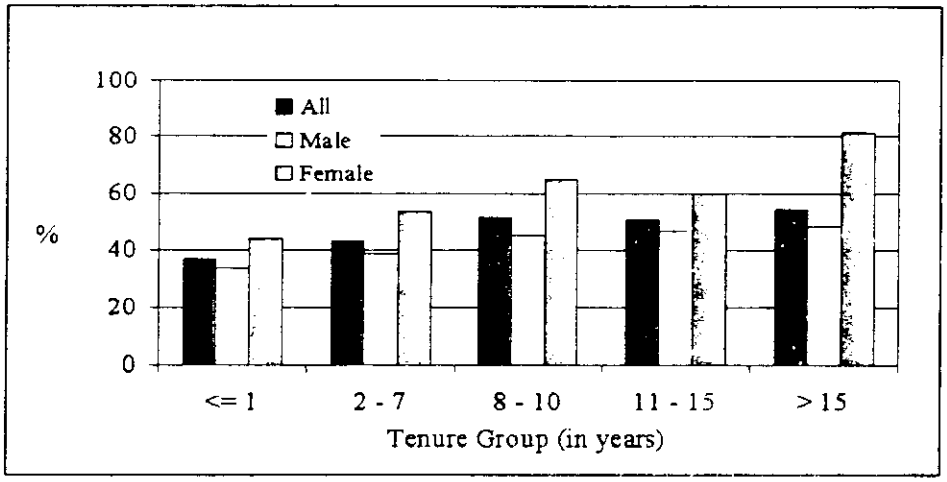

Figure 4. Prevalence of Self-Reported Upper Extremity Discomfort by Tenure

Prevalence of discomfort by the number of hours of computer usage

Figure 5 shows the relationship between the prevalence of discomfort and the number of hours per day spent on the computer. It seemed that as the number of hour of computer usage increased the prevalence of discomfort increased (Chi-square = 92.2, $p < 0.01$). Overall, the prevalence of discomfort increased from 20% for the "less than and equal to 2 hours/day" group to 66.7% for the "greater than 8 hours/day" group. Similar trends were observed in the male and the female respondents.

It is also interesting to note that if one defines "intensive keyboarding" as "spending more than 4 hours/day on the computer," there seemed to be a large increase in the prevalence of discomfort from the "non-intensive keyboarding group" to the "intensive keyboarding group." This was observed for both male and female respondents.

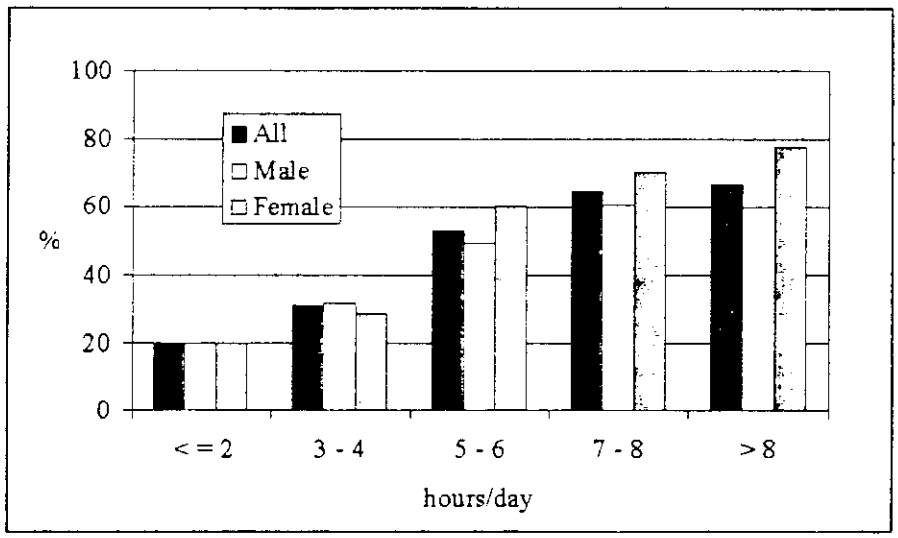

Figure 5. Prevalence of Self-Reported Upper Extremity Discomfort by Computer Usage

Prevalence of discomfort by age group

Figure 6 shows the prevalence of upper extremity discomfort among five age groups. Chi-square analysis showed that age was a significant factor (Chi-square = 16.7 year, $p < 0.01$). From group I (<= 35 years) to group II (36-40 years), there was an increase in the prevalence of discomfort. This was especially obvious for the female respondents. As the age increased from group II (36-40 years) to group III (41-46 years), there was a decrease in the prevalence of musculoskeletal discomfort. The prevalence of upper extremity discomfort started to level off after group III. For male respondents, the age difference in prevalence of discomfort was not statistically significant (Chi-square = 6.1, $p > 0.05$), for female respondent the difference was statistically significant (Chi-square = 26.9, $p < 0.01$). Across all age groups, the prevalence of discomfort among the female respondents was always higher than that among the male respondents.

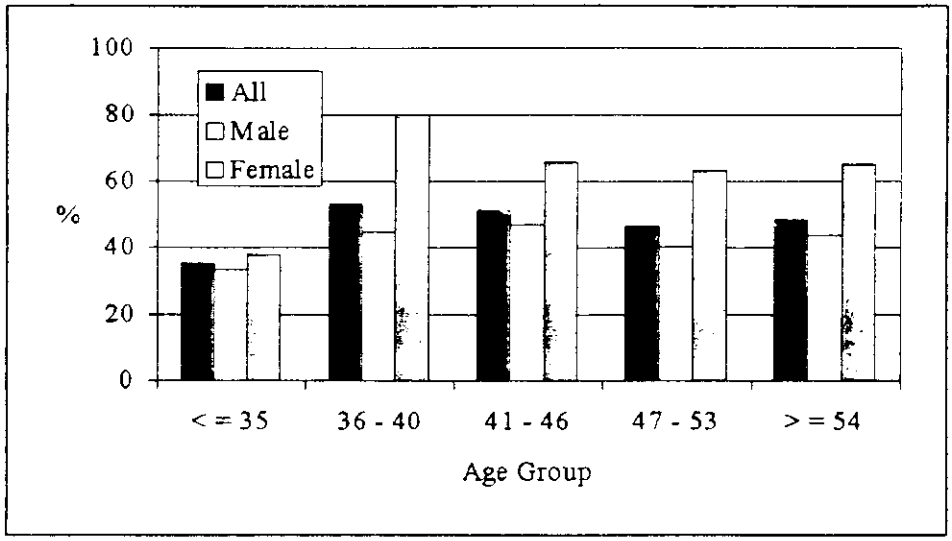

Figure 6. Prevalence of Self-Reported Upper Extremity Discomfort by Age Group

Prevalence of discomfort among respondents wearing bifocal glasses

Figure 7 shows that upper extremities discomfort tends to be slightly more prevalent among respondents with bifocals than those without bifocals. However, this is only true for the female respondents. For the male respondents, the prevalence of upper extremity discomfort was about the same for both groups, with and without bifocals.

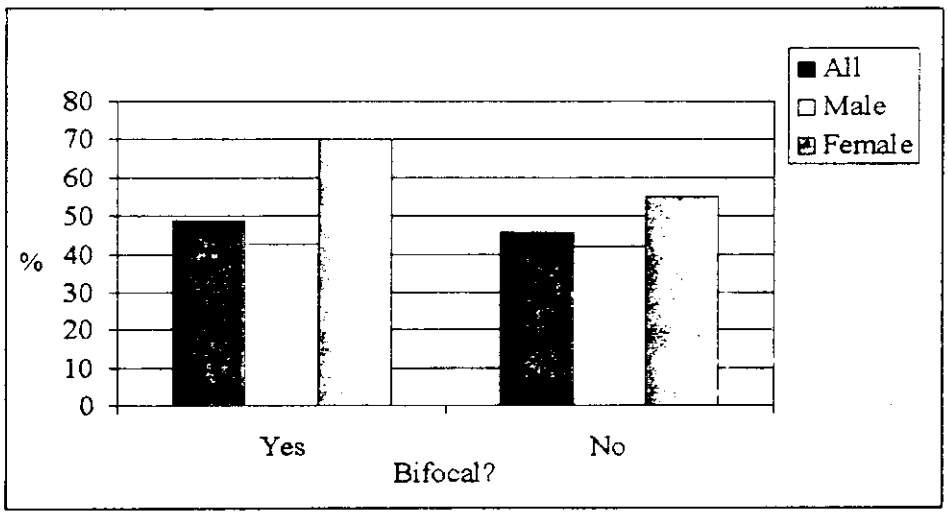

Figure 7. Prevalence of Self-Reported Upper Extremity Discomfort by Bifocal

Prevalence of upper extremity discomfort and job function

Figure 8 gives the prevalence of upper extremity discomfort and job function. Engineers and managers seemed to have slightly lower prevalence, 41% and 43% respectively, than administrative personnel and personnel in other job functions, 56% and 52%. This trend was [illegible]ous among female respondents than among male respondents.

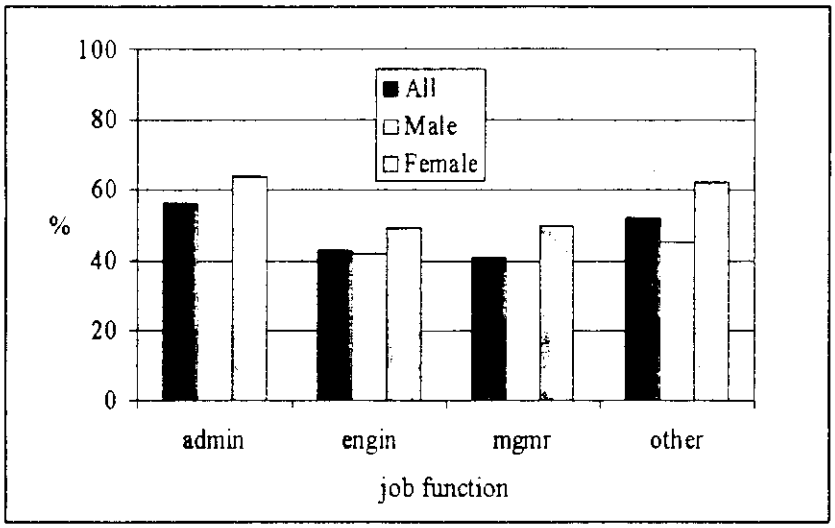

Figure 8. Prevalence of Self-Reported Upper Extremity Discomfort by Job Function

Perceived Work Demand in Keying and Using Mouse

Respondents gave self-perceived work demands in keying and using mouse as "continuously," "intermittently," and "rarely." 70% of the respondents regarded their computer work as requiring them to key or use the mouse "intermittently." 15% of the respondents perceived their computer work as requiring them to use the keyboard and mouse "continuously" and another 15% of the respondents regarded the demand was "rarely." 28.6% of the "rarely" group, 46.5% of the "intermittently" group, and 64.9% of the "continuously" group reported musculoskeletal discomfort of the upper extremity. Chi-square analysis gave a Chi-square of 39.0 ($p < 0.01$) indicating a statistically significant association between the self-perceived work demand in keying and using mouse with the self-reported experience of upper extremity discomfort.

Frequency of taking breaks away from computer work

Respondents were classified into four groups based on the self-reported frequency of taking breaks, i.e., "> 4/hr," "2-3/hr," "1/hr," and "< 1/hr." As shown in Figure 9, 31.4% of the "> 4/hr" group (n=172), 38.5% of the "2-3/hr" group (n=288), 53.5% of the "1/hr" group (n= 254), and 59% of the "< 1/hr" group (n=273), reported musculoskeletal discomfort of the upper extremity. A Chi-square analysis crude analysis yielded a Chi-square value of 45.2 ($p < 0.01$). It appeared that respondents that took fewer breaks were more likely to report upper extremity discomfort than the respondents that took more breaks did.

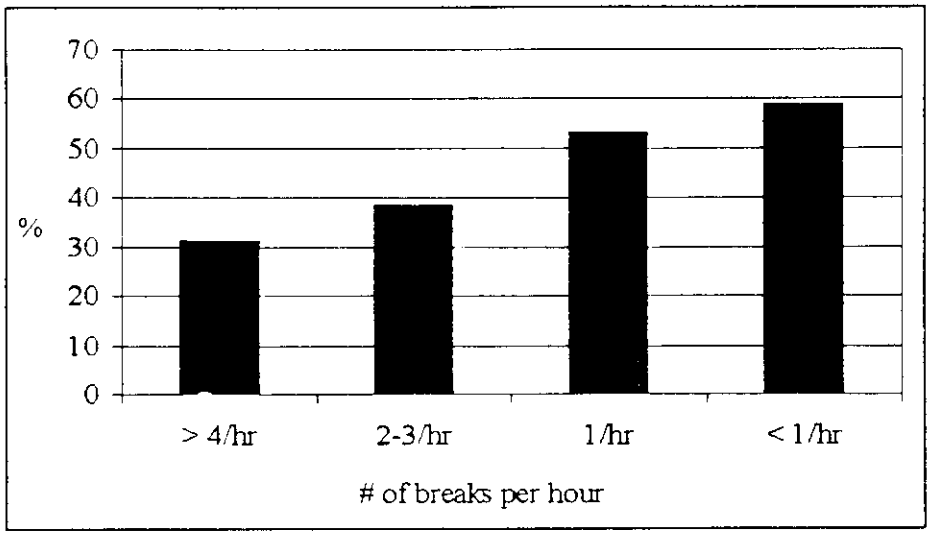

Figure 9. Prevalence of Self-Reported Upper Extremity Discomfort by break pattern

Logistic Regression

Using a logistic regression model, we evaluated the association of the prevalence of self-reported upper extremity discomfort with age, gender, tenure, hours of computer usage, use of bifocal, and job function. Age of the respondent was treated as a continuous variable in the model, Gender, and groups formed based on job tenure, hours of computer usage, job function, frequency of breaks, and perceived computer work demand, were treated as categorical variables. The results indicated that gender was significantly associated with the prevalence of self-reported upper extremity discomfort. Female respondents were more likely to report musculoskeletal discomfort of the upper extremity than male respondent did. The odds ratio was 1.4 ($p < 0.05$) with a 95% confidence interval (95%CI) of 2.06 and 1.03.

Logistic regression analysis also showed that computer usage in hours/day was significantly associated with the prevalence of self-reported upper extremity discomfort. Using Group I (<= 2 hours/day) as the reference group, the odds ratios ranged from 1.88 (95%CI: 1, 3.54) for group II, 4.15 (95%CI: 2.21, 7.8) for group III, 6.22 (3.17, 12.2) for group IV, to 7.63 (95%CI: 2.62, 22.21) for group V, respectively. All were statistically significant at the 0.05 level. Trend test gave a slope of 1.39 and its standard error of 0.36. The 95%CI is (0.68, 2.09), indicating a positive trend in the odds ratio with increasing exposure.

Logistic regression analysis also showed that job tenure was significantly associated with the prevalence of upper extremity musculoskeletal discomfort. In this analysis tenure group I (< = 1 year job tenure) was used as the reference group. The odds ratios for groups III to V were 1.84 (95%CI: 1.21, 2.78), 1.68 (95%CI: 1.07, 2.65) and 2.13 (95%CI: 1.31, 3.44), respectively, all significant at the 0.05 level. There was no significant increase in the prevalence of upper extremity discomfort as the job tenure increased from group I to Group II. Trend test yielded a slope of 0.29 with a 95%CI of 0.14 and 0.45, indicating a positive trend in the odds ratios with increasing job tenure.

Frequency of taking breaks from computer work also was shown by the logistic regression analysis to be a factor significantly associated with the prevalence of upper extremity discomfort. Respondents that took either one break/hours or less than one break/hour were more than twice likely to experience musculoskeletal discomfort of the upper extremity than those respondents that took more than 4 breaks/hour did. The odds ratio for the "less than 1 break/hour" group was 2.2 (95%CI: 1.42, 3.42) and the odds ratio for the "1 break/hour" was 2.1 (95%CI: 1.38, 3.28). Trend test yielded a slope of 0.3 with a standard error of 0.1. The 95%CI of the slope was (0.1, 0.5), indicating a positive trend in the odds ratio with less frequent breaks.

Discussions

This study showed that 47% of the respondents in this particular engineering firm experienced musculoskeletal discomfort of the upper extremity. This result is consistent with the prevalence of musculoskeletal discomfort/disorders of the upper extremity reported in the literature. For example, Arras (1994) found a prevalence of 30.5% of neck/shoulder discomfort among VDT users. Bergqvist et al. (1995a, 1995b) in studies of office workers found that the prevalence of neck/shoulder discomfort was around 60%. In a study of 3,000 workers in editorial, circulation, classified advertising and accounting departments, Bernard et al. (1994) reported a prevalence of neck discomfort of 26%. Among female data entry workers, the prevalence of neck discomfort found by Kukkonen et al. (1983) was 47%. Ryan and Bampton (1988) reported similar prevalence of neck/shoulder discomfort, 44%, in their study of data processing operators.

This study also found that the number of hours of computer usage was significantly associated with the prevalence of self-reported musculoskeletal discomfort of the upper extremity in the engineering firm. As the number of hours of computer usage increased the prevalence of upper extremity increased. This finding is similar to that reported by Rossignol et al. (1987). In their study of 191 workers involved in computer and data processing, the prevalence of neck discomfort increased 39% for "0.5 –3 hours" of VDT use to 61% for "7 or more hours" of VDT use. Similarly, Yu and Yong (1996) found with 151 VDT users in a Hong Kong bank that frequent VDT users were more likely to report neck discomfort than infrequent VDT users.

The study showed that respondents who took fewer breaks were more likely than respondents who took more breaks to report musculoskeletal discomfort of the upper extremity. This suggests that computer workers should take more breaks from computer work to prevent musculoskeletal discomfort, as recommended by other researchers and by many professional ergonomists. (Henning et al., 1989, 1993, 1997) However, as there were still more than 30% of the respondents in the group that took "at least 4 breaks/hour" felt upper extremity discomfort, more frequent breaks seem to be needed.

Because of the exploratory nature of the pilot study, several less than desirable features of the study need to be noted. First, non-specific body joint discomfort was used as the endpoint. Discomfort in the neck, shoulders, elbows, wrists and hands were all grouped together. Back discomfort was not included in the phrasing of the question. Second only self-reported hours of computer usage and self-perceived musculoskeletal discomfort were included in the study. As the experience of musculoskeletal discomfort might have affected the self-reported number of hours of computer usage, a more objective means of quantifying the use of computer need to be designed.

Third, the apparent response rate, 22%, seemed low, based on the number of the employees, 4,576, at the time of the survey. However, the actual response rate could be higher since the ergonomic team only distributed the initial invitation through departmental managers. There is a possibility that a portion of the managers did not pass on the invitation to their employees. Subsequently, the employees of the department would not have the chance to respond. The again points to the importance and need for a planning a survey through Internet, despite of the fact that this study demonstrated the feasibility and efficiency of using Intranet as a means of conducting discomfort survey.

Conclusions

Musculoskeletal discomfort of upper extremity was shown in this study to be common among employees in this particular high tech engineering firm. Four factors associated with the prevalence of self-perceived upper extremity musculoskeletal discomfort are gender, number of hours of computer usage, frequency of taking breaks, and job tenure. The study also demonstrated that the feasibility and efficiency in using Intranet as a means for discomfort survey.

Acknowledgment

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A Software-Based Tool for Exposure Assessment of VDT Work through Internet - Subproject II

Abstract

There is a need for an objective exposure assessment tool to evaluate the association between task demands and musculoskeletal discomfort/disorders commonly experienced by workers that use visual display terminals (VDT). This paper (1) describes the process involved in the development of a software-based tool designed for exposure surveillance of VDT work through Intranet and Internet; and (2) presents the results of laboratory validation and preliminary feedback from a pilot field evaluation. The software-based tool consists three components. The first component intercepts the signals from the keyboard and the mouse. The second component, based on the assumption of a touch typist and the keyboard signals intercepted, increases the number of counts of nine counters designated to fingers of the two hands. Every second the program writes the resultant counts of all the counters into a data log file. The third component of the program uploads the log file to a central network server at the end of the work shift for data collection and further analysis. Laboratory simulations gave satisfactory results in tracking the signals from keyboard and mouse, finger assignment, and file transfer from local computer workstation to central server through Intranet and Internet.

Keywords: Exposure Surveillance, VDT, Computer, Mouse, Keyboard

Introduction

Musculoskeletal discomfort and pain in the shoulders, neck, back, and hand/wrist are common among visual display terminal (VDT) workers.[1-7] Hagberg and Wegman showed that use of VDT for more than 20 hours per week was associated with excessive risk for certain musculoskeletal endpoints.[8] Bergqvist reported that increased keyboard use increased the risk of hand/wrist problems.[9] Foglman and Brogmus, based on the workers' compensation data, found that musculoskeletal disorders associated with computer mouse use appears to be a growing problem and deserves more research.[10]

However, field evaluation of the association between VDT work and musculoskeletal complaints is in general hindered by a lack of objective measurement of exposure. Self-reported VDT work duration tends to be the measure of the physical demands posed by VDT in most studies. Self-reported exposure is prone to bias. And work duration as a surrogate of exposure only gives a partial picture of the physical demands associated with VDT work, as intermittent short break and variation of office activities may reduce the total physical demands of VDT work.[11, 12]

An objective exposure assessment tool is needed to improve the quality of exposure estimate in epidemiological study and to facilitate health surveillance in VDT work. This ideal tool should have at least the following characteristics. First, it should be reasonably accurate in providing an estimate of not only the total duration of VDT work but also the pattern of the intermittent breaks. Second, it should be relatively in expensive and easily installed in many VDT workstations so that exposure could be estimated for as many VDT operators as possible. Third, it should require minimal, if any, operator training and involvement in using the surveillance

tool. Fourth, it should not affect the productivity of VDT operators. Fifth, it should be relatively easy for data collection and analysis so that feedback to the VDT operators could be provided relatively quickly. As keyboard and mouse represent two major input devices in PC-based VDT work and as Intranet and Internet are becoming more prevalent in modern office environment, an exposure surveillance tool satisfying the above criteria may be developed.

This paper describes the development of a software-based exposure assessment tool and presents the preliminary results of evaluation in the laboratory. This software-based exposure assessment tool is designed for VDT workstation using Microsoft Windows based system. The tool tracks and categorizes the signals second by second from the keyboard and the mouse. The resultant counts of keystrokes and mouse-clicks are saved in a log file in the local computer then uploaded to a central server for data processing. A unique feature of the software-based tool is that all data collection is conducted through Intranet and Internet.

Methods

Program Development

As many personal computers nowadays operate under Windows based operating systems, the software-based exposure assessment tool was developed for Windows based system. The Windows-based operating system handles keyboard and mouse signals and sends these signals as messages to the software program that is active at the time of data input. This active application program could be a word processing program, a spreadsheet program, or a web browser. This event-handling property of the Windows operating system is the basis for the development of the software-based exposure assessment tool described in the present paper.

The software-based exposure assessment tool consists three components. The first component captures the signals from the keyboard and mouse. The second component increases the count of a counter assigned for a specific finger, assuming that a touch typist enters the keyboard input. The third component uploads individual exposure record from multiple VDT workstations to a central server. Figure 1 outlines the programming logic in a flow chart.

Component I. Tracking Keyboard and Mouse Signals at Individual VDT Workstation

The first component was developed using an Object Link & Embedding (OLE) program in Visual Basic 4. This OLE program intercepts signals from keyboard and mouse; gives the necessary Window messages, including those messages specifically for keyboard and mouse signals; and returns an identification number of the message intercepted. Based on the identification number returned, the keyboard and mouse signals were identified and recorded. In addition to tracking the signals from the keyboard and the mouse, this OLE program also allowed us to track the active window, i.e., the active application software, for which the keyboard or mouse signals were intended. For example, if the VDT user was using a word processing program and entered several keys, not only the keyboard signals will be tracked but also the name of the word processing program and the file name of the document could be tracked.

"Keyboard Input"

A typical keyboard has 101 keys. According to their function and where they located on the keyboard, they could be grouped as alphanumerical keys, function keys, cursor movement keys, and numerical pad. There are 58 keys on the section for alphanumerical keypad, though some of the newer keyboards have additional keys, such as "turbo" and "Windows Start" keys. There are twelve function keys and one 'Escape" key on the top row of the keyboard. Three system keys: "Print Screen", "Scroll Lock" and "Pause", are also located on the top row. Six keys for "insert," "delete," "home," "end," "PageUp," and "PageDown," are located in the middle section of right-hand side of the keyboard. Below them are four cursor keys. Seventeen keys are located on the numerical pad.

These keys can also be differentiated as printable keys such as the "a" and "1", and non-printable keys such as the function keys "Escape" and "F1" and cursor movement keys "PageUp" and "PageDown". The printable keys are identified by comparing them directly with the characters represented by the ASCII code. The non-printable keys are identified using their individual virtual key code provided by the Windows message.

Mouse Input

The OLE program installed within the software-based tool also allows us to track the status of mouse movement, and the up and down states of each of the two buttons (left and right) of the mouse. Single click and double clicks can also be differentiated with the software-based tool.

Component II. Finger Assignment based on a Touch-Typist Assumption

A total of nine counters were created to store the keystroke counts. For a touch typist, eight of the nine counters stores the keystrokes made by the four fingers of the left and the right hands. For example, cumulative counts of keystrokes made on "q, a, z, !, Q, A, Z," are stored in one counter created for the left little finger, while that for "2, @, w, W, s, S, x, X" are kept in another counter designated for the left ring finger. The cumulative count of keystrokes made on the "Space" key is kept in the ninth counter designated as "thumbs," since the space-bar on the keyboard can be triggered by either the right or the left thumb. The "Shift", "Ctrl", and "Alt" keys are three sets of keys that can be identified but cannot be differentiated into left or right since they share the same function but located at opposite sides.

Component III. Multiple VDTs Exposure Assessment – File Transfer to a Central Server

This component of this software-based tool was developed using a File Transfer Program (FTP). This component facilitates collecting data from multiple VDT workstations. This component initiates a windows-based program at the end of the data gathering session at individual VDT and calls a file transfer program included in operating systems such as the Windows 95™ or Windows NT™, which then uploads the resultant exposure file to a central server for analysis.

The process of transferring the log file is automatically completed through a script file generated by the software-based exposure assessment tool. The script file includes date and time of exposure data collection, file name, user's login name, and a password, if required by the server's security.

Laboratory Validation

Laboratory validation of the software-based tool was done at the UCLA Occupational Safety/Ergonomics Laboratory. The software-based exposure assessment tool was installed in computers equipped with CPUs made by Intel, Cyrix, and AMD. All computers had at least 16 MB and with connection to UCLA Intranet. Input device used includes a 104-key AT keyboard and a Microsoft mouse or an MS compatible serial mouse. Operating systems tested include Windows95, Windows98, and WindowsNT. The software-based tool was tested with a word-processing software package and a spreadsheet software package.

Counting the Keystrokes - All Keys without Finger Assignment

A virtual keyboard template as shown in Figure 2 was developed for testing the software-based tool. The virtual template has counters arranged in four sections. Sections, I through III, are for keys on a typical keyboard. Each counter of the virtual keyboard template increased by one each time its corresponding key was pressed. We tested each key on a typical AT keyboard by pressing them one by one to verify the increment of each counter. The virtual keyboard template was developed using Visual Basic 4.

Printable Keys without Finger Assignment – Tests with Application Software

During the later phase of software development, a similar, but simplified, test was conducted using a word processing software package and a web browser. This test was done without the finger assignment. A simple sentence, "The quick brown fox jumps over the lazy dog," with all the 26 printable characters included was entered 15 times on a computer equipped with a AMD K6-2 350 MHz CPU and a Windows98 operating system.

Test with Rollover and Chord Techniques without Finger Assignment

Rollover, i.e., pressing the second key while the first key was still down, was tested. Two keys were entered without correction as fast as possible into a text document with a word processing package.

Chord technique, i.e., entering multiple keys simultaneously, in data entry was also tested. Specifically, the software-based tool was tested with 2-key and 3-key chord technique.

A stopwatch was used to time the duration of data entry in the two tests. Number of characters in the document were manually counted and compared with the counts tracked by the software-based tool without finger assignment.

Printable Keys with Finger Assignment

Once a signal from the keyboard is identified, the assignment is a simple process of looking up the specific key in a table and increasing by one the specific counter designated for the finger. A look-up table based on the assumption of a touch-typist is shown in Table I. For printable keys, the software-based tool was tested in a word processing software by tracking the keys with finger assignment. Three lines of printable characters were entered during three trials. The first line included the upper cases and lower cases of 26 alphabetic characters, space keys, and a period. The second line included 9 numbers, and special keys such as "! @ # $ % ^ & * ("and a period. The third line included the rest of special keys, i.e., "` - = [ ] \ ; ' , . / ~ _ + { } | : < > ?" and one period. Cumulative counts of the finger counters provided by the software-based tool was then

compared to the predetermined number of finger counts assuming the text was entered without error. These tests were conducted on three computers with three different Central Processor Units (CPUs) such as Pentium 100 MHz and Pentium 133 MHz. Each test paragraph was entered 15 times on each computer.

Tracking the Mouse

The virtual keyboard template of Figure 2 also shows a section for the mouse signals (Section IV). There are 7 counters, five of which are for the single and double clicks of the left and right buttons and the mouse movement. These five signals are tracked with the current version of the software-based tool. The other two counters designated for single and double clicks of middle button of the mouse are for future expansion of the software-based tool.

Single click and double clicks were tested by clicking the buttons of the mouse and by observing the increment of the corresponding counter. The mouse movement was tested with the virtual keyboard template, a word processing software package and a spreadsheet software package.

Mouse Movement

Test of the software-based tool in tracking mouse movement was conducted with virtual template, word-processing software, and spreadsheet software. Two serial mice, one Microsoft and one Microsoft compatible, were tested with a computer equipped with an AMD K6-350 MHz CPU and Windows98 operating system.

Four tests were conducted with each mouse by moving the mouse in four predetermined directions. The experimental set-up, as shown in Figure 3, consists a mouse pointer and a computer monitor on which a rectangle (30 cm x 16 cm) with eight markers was placed. The markers on the screen were numbered clockwise as 1 to 8. The distance between #1 marker and #3 marker was 30 cm measured on the screen. The distance between #1 and #7 markers was 16 cm. Marker #2 was place at the midpoint between #1 and #3 while #4 marker was placed between #3 and #5 markers.

During the first test, the mouse was moved so that the cursor moved twice from #1 marker to and from the #5 marker. In the second test, cursor was moved from #8 marker to and from #4 markers twice. Similarly, the third test was conducted so that the cursor moved twice from #7 to and from #3 twice. In the final test, the mouse was moved so that the cursor moved back and forth from #2 marker to #6 marker. Count increment of the "mouse move" counter was recorded during each test. Mouse movement during each trial was videotaped with a camcorder (Panasonic, OmniMovie) placed perpendicularly to the tabletop. Permanent timecode was also placed on each frame of the videotape using a timecode generator (Horita II, TRG-5). A marker was placed on the mouse to track the position of the mouse.

Validation of the File Transfer Program

File transfer from the local computer to the server is initiated automatically when the software-based software tool is terminated. Files created during these trials were programmed to upload to an FTP server. Two validation tests were conducted. One was conducted by uploading the data files created by the software-based tool from two offices to a server at the micro-computing laboratory managed by the UCLA School of Public Health. The second test was conducted by

uploading data files from three computers in the occupational ergonomics laboratory to another computer running an FTP server.

Preliminary Field Test

The software-based exposure assessment tool was pilot tested at an engineering firm by its ergonomics team and information technology group. This engineering firm uses WindowsNT servers and workstations. A computer equipped with an AMD-K6 166 MHz CPU, 64-MB memory and Windows98 operating system was installed on-site as the FTP server. Test runs were conducted to validate the functionality of the software-based tool.

Statistical Analysis

Correlation and regression analysis was done using a personal computer with Statistical Package for Social Science (SPSS).[13]

Results

Printable and Non-Printable Keys

Errors in counting due to programming errors were corrected during the initial phase of the software development process. Each key on the keyboard was tracked without error in the final test.

Test of Finger Assignment

Having adjusted for few errors in data entry during the test, the software-based tool assigned each key of the test phrases correctly.

Test with Rollover and Chord Techniques

In rollover tests, 432 keystrokes and 437 keystrokes were made in 1.09 second and 59 second, respectively, in two tests. On the average, it took about 135 to 160 millisecond to make one single key during the first and second trials, respectively. No difference was found between the count of characters entered in the document and the number of counts tracked by the software-based tool.

In 2-key chord tests, the average duration to complete one set of keys entry varied from 67 millisecond to 120 millisecond. There was no difference between the counts tracked by the software-based tool and that registered in the word document.

In 3-key chord tests, the software-based tool gave zero count and the word processing software registered no character.

Mouse Single Click and Double Clicks

Results of mouse click tests showed no difference between the count increments tracked by the software-based tool and the number of single and double clicks entered. The software-based tool tracked mouse clicks of the left and right buttons without error.

Mouse Movement

Mouse-move counter increased as the mouse was moved. There was high correlation between the mouse-move count increments and the amount of time during which the mouse moved; despite of the fact that two types of mouse and three software were tested. The R-squared values ranged from 0.96 to 0.98. The slope of the regression line relating count increment and the amount of time that the mouse was moved ranged from 0.038 to 0.042 with a stand error of 0.001.

Format of the Output File

Table II shows a sample of the data log file generated by one version of the software-based tool. The first line gives the date and time at which the software-based tool was initiated. The second line gives the names of the eighteen counters tracked by the specific version of the software-based tool. Starting from the third line gives the second-by-second cumulative counts of the keystrokes and mouse activities. There are twenty columns in each line. The first column gives the time in cumulative second for which the data were collected. The second column gives the total number of keystrokes made till the end of the current second. Starting from the third column are cumulative counts tracked for four fingers of the left hand, thumbs, and four fingers of the right hand. Following the counts for fingers is a counter giving the counts of total keystrokes made on the twelve Function Keys. The next two columns give the number of keystrokes made on the cursor movement keys (including insert, delete, PageUp, PageDown, left, right, etc.) and the keys of the numerical pad. The next three columns give the number of counts tracked for mouse clicks (single and double) of the left button and mouse movement. The last column of the file gives the software and the file name when the keyboard and the mouse were tracked.

In this sample, a total of 9,191 keystrokes were entered in 86 minutes. Fifty-two percent of the keystrokes were done by the left hand and forty-eight percent of the keystrokes were done by the right hand, as the person who entered this set of data was a touch typist. Function keys were not used during the 86-minute interval. However, miscellaneous keys were used 552 times and number keys from the numerical pad were entered 29 times. The left button of the mouse was single clicked 161 times and was double-clicked 15 times. Mouse was moved for slightly more than four minutes. The active application software used during this 86-minute interval was Microsoft Word. Figure 4 gives three lines representing the cumulative counts of the keystrokes made by all fingers and by the left and right hands separately during the 86-minute interval. Figure 5 shows the cumulative counts of the mouse usage during the same 86-minute session.

File transfer to server in laboratory and in field

Data files created by the software-based tool were successfully uploaded to the designated servers in laboratory tests at UCLA and in preliminary tests in an engineering firm.

Discussion

In this study we have not only developed a software-based tool for assessing objectively the duration and usage of data input devices involved in the VDT work, but also characterized its functionality in laboratory tests. This software-based tool tracked signals from the keyboard and the mouse accurately and assigned finger usage correctly based on the assumption of a touch typist except for the three-chord technique.

The software-based tool is inexpensive, since it is software-based and does not require the use of any additional hardware not already installed in the personal computer workstation.

No special training is required to install and use the software-based tool. Installation of the software-based tool is similar to installing any other software. Once installed and initiated, the software-based tool sits quietly in the Windows background collecting data.

Impact on computer productivity seems to be minimal. Our laboratory tests showed that the software-based tool has no apparent negative impact on productivity if the personal computer system is equipped with a 166 MHz or faster CPU, 32 or more MB of RAM and a Windows95 operating system or later. The computer should have at least 8 megabyte of hard disk space in addition to what is needed for Windows operating system, since the resultant data log file for an 8-hour work shift will be about 8 megabyte. As we have only tested the software-based tool with a word-processing and spreadsheet software, further field tests with other application software are needed.

Data collection is easy, as the keystrokes and mouse clicks are stored in an ASCII file ready to upload. For workplace where there is no network, one only needs to compress the file to a diskette. For workplace with network, the data log file from each personal computer workstation is transferred to an FTP server automatically. In both cases, the ASCII file is ready for conversion for data analysis with other software such as a SPSS, SAS, or a spreadsheet program.

Preliminary field tests of installation, usage, and FTP confirmed the results found in our laboratory. The ergonomics team found the software user-friendly and not posing excessive hardware requirement and computer resources. However, the ergonomics team did provide two valuable suggestions for improvement. First, for security reason, the software-based tool is not permitted to track neither the name of the active application software nor the name of the file that a VDT user is working on. Second, assumption of touch-typist is not realistic. More than 30% of the VDT users in the company are not touch typist. The software-based tool has subsequently been modified to accommodate the need of this company.

It should also be noted that the software-based exposure assessment tool applies only to Microsoft Windows-based work environment. Though many VDT operators nowadays work under Microsoft Windows operating system, there are still many VDT operators work with Unix or Macintosh based systems. Similar software-based tool should be developed for these non-Windows VDT users.

Though the software-based tool satisfies our criteria for providing an objective estimate of physical demand of VDT work, the tool could be further improved in several ways. First, the software-based tool does not register signals entered with a 3-key chord technique. This means that usage of 3-key macro in application software will not be tracked. Though we believe keystrokes missed by the software through this mechanism would be low in reality, work is currently underway to find ways to track 3-key chord. Second, the software-based tool in its current form does not differentiate between left and right for "Ctrl", "Shift" and "Alt" keys, since Windows95 operating system does not provide separate left or right messages for these keys.

However, more recent operating systems such as Windows98 and WindowsNT do provide separate left and right messages for these three keys. If there is a need to differentiate these keys in the future, the software-based tool could be refined to provide such a measure.

Only serial mouse has been tested so far with the software-based tool. There are other types of mice, such as Universal Serial Bus (USB) based mouse, and other types of input device, such as digitizing tablet, on the market. The response of these input devices should be tested.

Conclusion

A software-based tool for objective exposure assessment in VDT work was developed and characterized. This tool tracks and categorizes the signals second by second from the keyboard and the mouse. The software can help computer users acknowledge how many keystrokes from each finger during the certain period of keystroke activities and the amount of mouse click status. It also monitors computer usage patterns over time and trend to collect the repetitive keyboard and mouse usage.

The quantification of keyboard and mouse-related activities will provide an objective exposure estimate needed for the establishment of an exposure-response relationship between musculoskeletal discomfort and disorders and VDT work. The software-based tool will form a foundation for future epidemiologic studies of VDT-related musculoskeletal discomfort/disorders.

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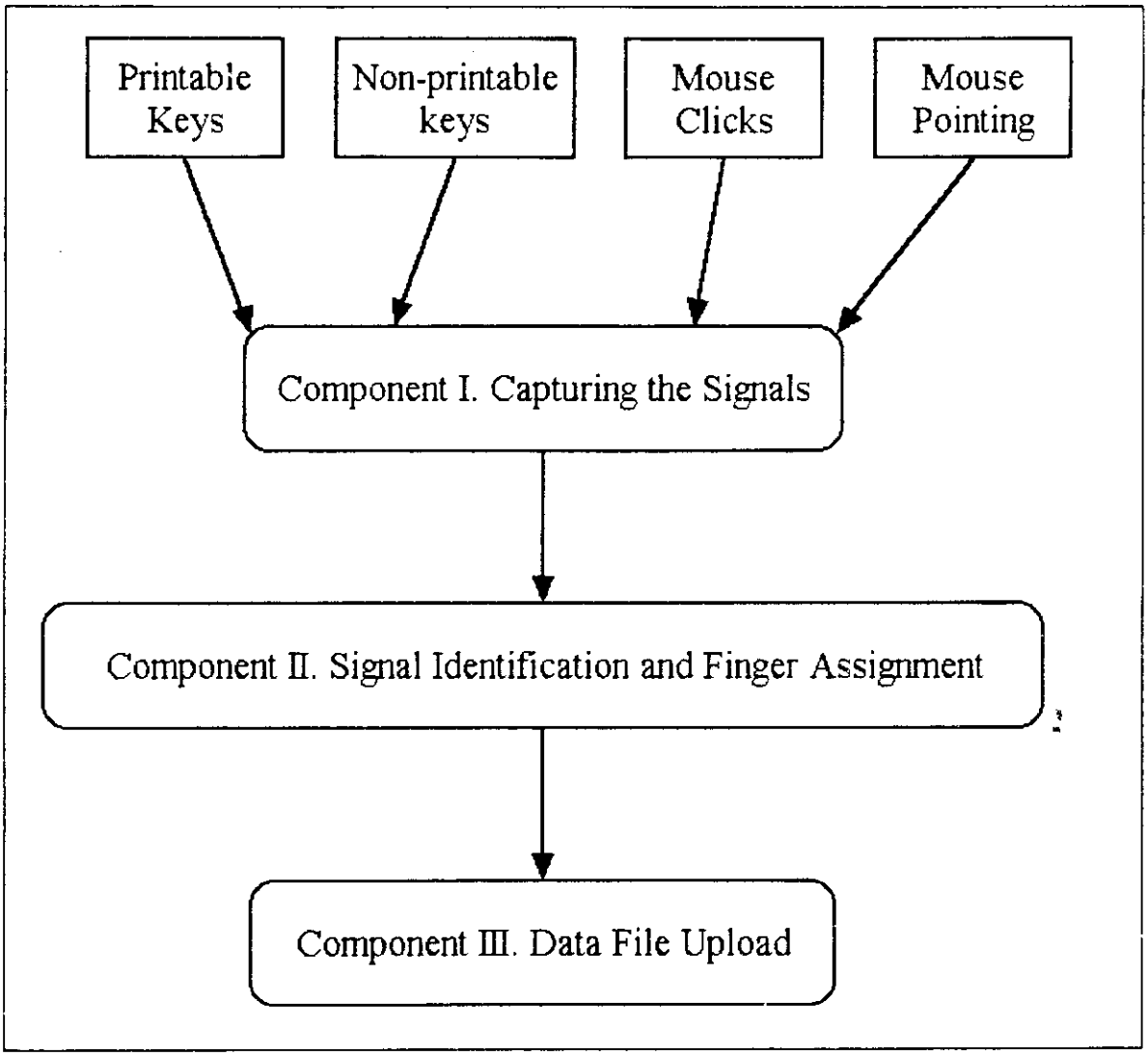
Figure 1. Program Components



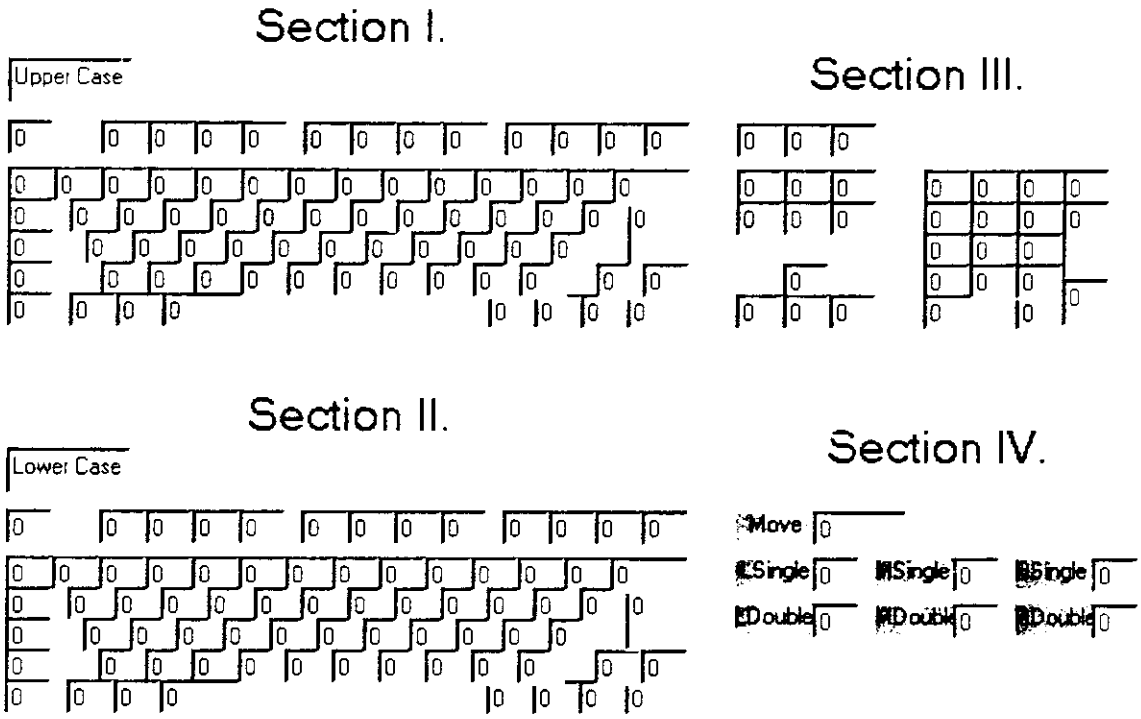


Figure 2. Virtual Keyboard

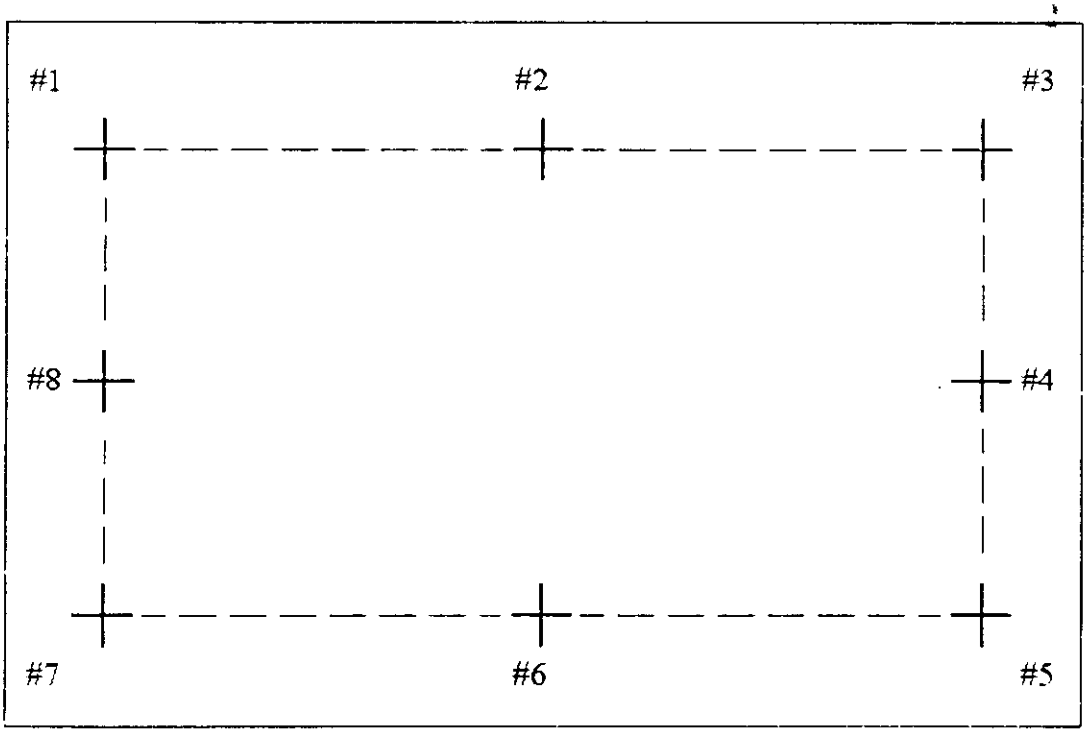


Figure 3. Experimental setup to validate the tracking of mouse-movement with the software developed in the present study.

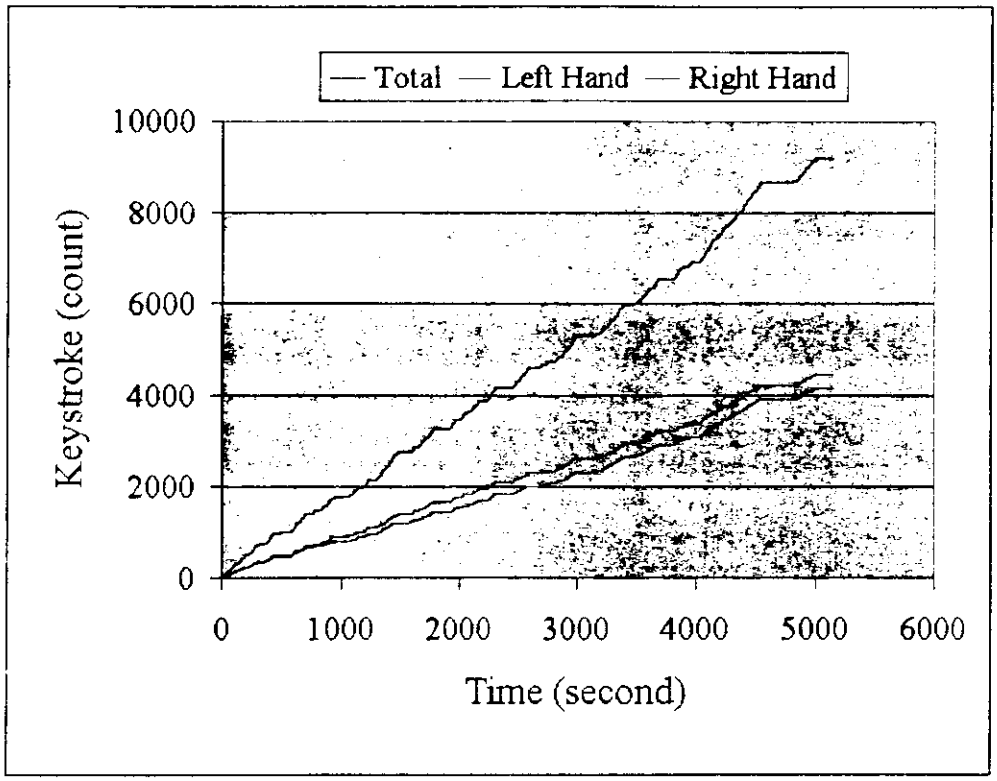
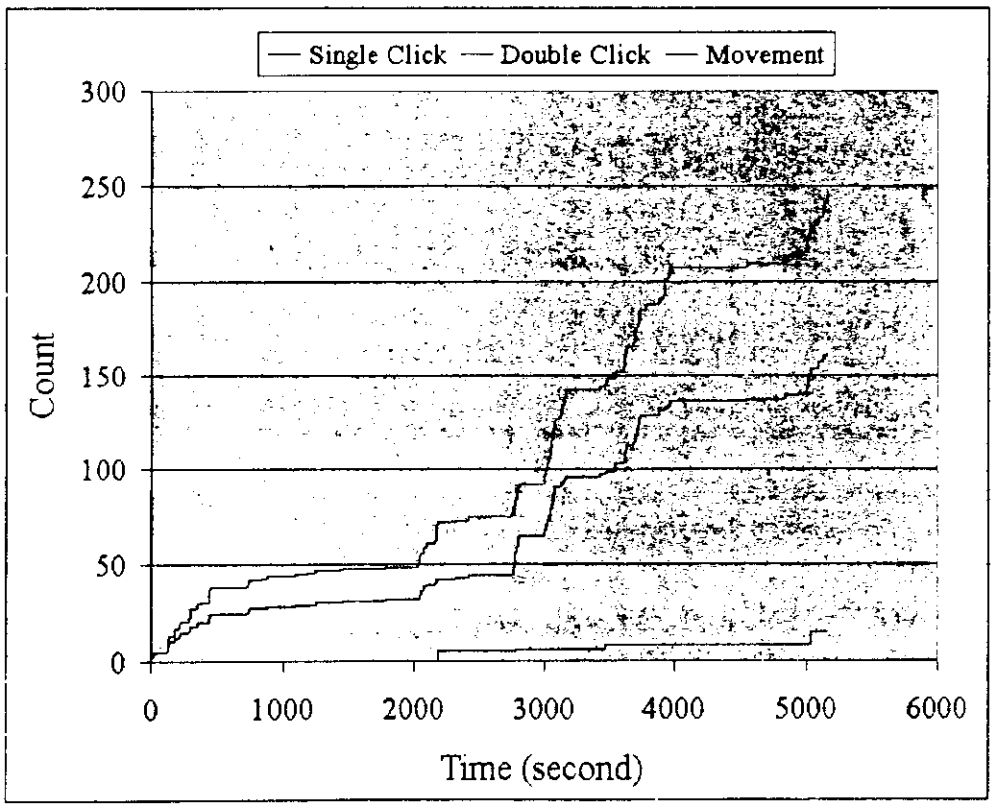


Figure 4. Sample of cumulative counts recorded with the tracking software.



Sample of cumulative count of mouse usage recorded with the tracking software.

Table I. Touch-typist look-up table for finger assignment

Finger	Keys				
Left Little	Esc	~	!	Tab	Q
	Caps	A	Shift(Left)	Z	Ctrl(Left)
	Alt(Left)				
Left Ring	F1	F2	@	W	S
	X				
Left Middle	F3	#	$	E	D
	C				
Left Index	F4	%	^	R	T
	F	G	V	B	
Thumb	Space	Num 0			
Right Index	F5	F6	&	Y	U
	H	J	N	M	Print Scrn
	Insert	Delete	Arrow Left	Num Lock	Num 7
	Num 4	Num 1			
Right Middle	F7	*	(	I	K
	<	Scroll Lock	Home	End	Arrow Up
	Arrow Down	Num /	Num 8	Num 5	Num 2
Right Ring	F8	)	O	L	>
	Pause	Page Up	Page Down	Arrow Right	Num *
	Num -	Num 9	Num 6	Num 3	Num .
	Alt (Right)				
Right Little	F9	F10	F11	F12	_
	+	|	Back Space	P	{
	}	Enter	:	"	?
	Shift(Right)	Num +	Num Enter	Ctrl (Right)	

Table II. Sample data log file

6/27/98		13:38:07												
T(s)	TK	LL	LR	LM	LI	TH	RI	RM	RR	RL	Func.	Misc.	Num.	LMSC
1	0	0	0	0	0	0	0	0	0	0	0	0	0	2
:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
5158	9191	889	449	1181	1318	1233	1079	606	888	967	0	552	29	161

*T(s): cumulative time in second
TK: cumulative count of keystrokes
LL: cumulative count of keystrokes – Left little finger
LR: cumulative count of keystrokes – Left ring finger
LM: cumulative count of keystrokes – Left middle finger
LI: cumulative count of keystrokes – Left index finger
TH: cumulative count of keystrokes – Thumbs (space bar)
RI: cumulative count of keystrokes – Right index finger
RM: cumulative count of keystrokes – Right middle finger
RR: cumulative count of keystrokes – Right ring finger
RL: cumulative count of keystrokes – Right little finger
Func.: cumulative count of keystrokes – Function keys
Misc.: cumulative count of keystrokes – Miscellaneous keys (PageUp, PageDown, etc.)
Num.: cumulative count of keystrokes – Numerical Pad
LMSC: cumulative count of mouse clicks – single click of left mouse button
LMDC: cumulative count of mouse clicks – double click of left mouse button
MMC: mouse-move duration in second

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Hazards in Glovebox Operations in a Defense/Nuclear Facility - Through Ergonomic Task Analysis - Subproject III

Abstract

Two ergonomic task analyses were conducted of two glovebox operations in a Defense/Nuclear Facility. The primary goal of the ergonomic task analyses was to identify factors that might have contributed to the development of musculoskeletal discomfort/disorders among glovebox workers and to the relatively high incidence of glovebox glove failure. The ergonomic job analysis consisted of task analysis through on-site observations and of interviews with experienced glovebox workers.

The first ergonomic task analysis was conducted of an operation that casts metal inside a glovebox. The specific aim of this analysis was to identify opportunities for continuous improvement in terms of reducing musculoskeletal load. The ergonomic task analysis, consisting of task analysis, postural analysis, and static strength modeling, was applied to two on-site simulations and to actual casting operation recorded on videotape.

The results of the postural analysis indicate that the work environment and the tasks involved in casting operation do occasionally place the operators in awkward posture. As a result, the working conditions should be modified sometime soon. The results of static strength modeling show that several tasks involving lifting heavy molds are not designed for the majority of the general population. These tasks could be modified by rearranging the internal layout of the glovebox and by installing auxiliary material handling devices in the glovebox.

The second ergonomic task analysis was conducted of an operation that refines metal in specific forms. This particular glovebox operation has experienced high frequency of abnormal glove breaches. The specific aim of the analysis was to identify improvements that could reduce the incidence of glovebox glove failures. Document reviews, individual interviews, small group meetings, and on-site observations were conducted during the investigation. The results indicate that there are many micro and macro ergonomic improvements that could be made to increase the reliability of the glovebox gloves and the comfort of the workers.

Background

Glovebox, as an enclosure with gloves with confinement to or from the atmosphere using low differential pressure, is utilized as the first and primary defense to protect the safety and health of workers in many operations handling highly hazardous materials. Guidelines to ensure the integrity, in terms of design and fabrication, of the glovebox has been available for many years (AGS, 1998). However, the design of operations to be performed in the glovebox has received relatively little attention, especially in terms of the ergonomics. As a result, misuse and false reliance may give rise to other types of hazards, such as musculoskeletal discomfort/disorders, or may reduce the degree of protection offered by a "properly designed and fabricated" glovebox.

Anecdotal data in a facility that utilizes glovebox extensively have shown that musculoskeletal discomfort/disorders are common among glovebox workers. In addition, the facility has

experienced frequent glove changes due to both normal glove wear and abnormal glove breaches. This paper reports the results of two ergonomic evaluations of glovebox operations in this particular facility. The goals of these ergonomic evaluations were to identify factors that may have contributed to musculoskeletal discomfort/disorders among glovebox workers and to the relatively high incidence of glovebox glove failure.

Two ergonomic task analyses were conducted of two glovebox operations in a Defense/Nuclear Facility. The goals of the ergonomic task analyses were to identify factors that may have contributed to musculoskeletal discomfort/disorders among glovebox workers and to the relatively high incidence of glovebox glove failure. The ergonomics job analysis consisted of task analysis through on-site observation and interview.

The first ergonomic task analysis was conducted of an operation (Operation I) that casts metal inside a glovebox. The objective of the analysis was to identify opportunities for improvement in terms of reducing musculoskeletal load associated with glove box operation. The concern for musculoskeletal discomfort and disorders arose from the results of an in-house survey that showed that 57% of the glovebox operators experienced back disorders and more than 24% of the operators experienced neck and shoulder discomfort. As this evaluation was primarily concerned with physical demands aspect of human-machine interface, it is considered as a micro-ergonomics analysis (Hendrick, 1991).

The second ergonomic task analysis was conducted of an operation (Operation II) that has experienced high frequency of abnormal glove breaches. The objective of the analysis was to identify opportunities for improvement in terms of reducing an increasing incidence of failure of Hypalon gloves utilized in glovebox operations. As the foci of this ergonomic evaluation were not only on interface between human and hardware but also on formal rules and procedures, and information and decision support systems, the second ergonomic evaluation considered both micro and macro-ergonomic issues, as described by Hendrick (1991, 1995).

Lead-loaded Hypalon gloves (North Safety Product), 30 mil in thickness, were used as a primary protective device to protect the glovebox operators from radiation in this Defense / Nuclear facility. At the time of the analysis, there was more than thirty glovebox operations in this facility and more than 4000 pairs of glovebox gloves are in use each day at this facility.

Glove changes due to abnormal breach poses risk of radiation exposure. Premature change of gloves increases not only the amount of radioactive waste but also the risk of radiation exposure. The Defense / Nuclear facility has developed a computer database to schedule glovebox glove changes. The schedule of glove changes based on a rating of the tasks performed in a specific glovebox, the frequency of usage, and the application of a survival analysis.

In a period of approximately two years, more than 1,400 pairs of gloves have been replaced and 5.64% of the replacement was caused by glove failure. During the same period, operation II had 90 pairs of glove replaced and 22.2% of the replacement was due to glove failure. Abnormal glove failure is defined at this facility as failure due to puncture, tear, cut, chemical, and heat. Overall, glove changes due to abnormal wear accounted for only 6.2%.

Methods

Operation I.

The ergonomic job analysis of Operation I was completed in three steps. First, the metal casting operation recorded on videotape was broken down into distinct tasks. A time code was added to the videotape using a timecode generator (Horita, CA). The duration of each task was estimated using the timecode between each distinct task. The duration estimate was verified by experienced casting operators to ensure the task being analyzed was representative of the task typically performed in the operation. The weight and dimension of casting apparatus such as molds and crucible were measured on site.

The second step consists of collecting information on the posture typically adopted by glovebox operators while performing the casting operation. This step was conducted in two on-site simulations. Each simulation included all the tasks in the casting operation except for the melting process since the latter involves only visual monitoring by the operator. One operator with seven years of experience simulated the tasks for a period of 17 minutes and the other operator in training simulated the tasks for 14 minutes.

The Ovako Working Posture Analysis System (OWAS) (Karhu, et al., 1977) protocol was used to classify operators' postures at the neck, arms, trunk, and legs every minute. With the OWAS analysis, one could classify the specific operation into one of the four possible "Action" categories, i.e., none (No Action Required), soon (Remedial Action Soon), very soon (Remedial Action Required Very Soon), and immediately (Remedial Action Required Immediately). The classification of the "Action" category is based on the percentage of duration that an operator spends in a particular posture category.

The third step consists of estimating the percentage of population with sufficient strength in three lifting tasks typically performed in the glovebox casting operation. The estimation was based on computer modeling using the 3D Static Strength Prediction Program (University of Michigan, 1994). Weight and dimensions of objects being handled and the operator's posture were inputs of each computer simulation. Three tasks were simulated. Task I involved the lifting of an empty mold, 11 kg, with one extended arm from the right side of the operator. This scenario represents transferring an empty mold from the compartment between two glove boxes. In task II, a mold with the metal rod inside, a total weight of 17 kg, was lifted with the left arm from the left side of the glove box. The scenario simulates a task retrieving a full mold from the furnace area in the glovebox. Task III involved the lifting of a full mold with both hands in front of the operator's chest. This scenario represents a task in positioning the mold for disassembly.

Operation II.

The methods used in the analysis of the operations include document review, on-site observation, individual interviews, and small group meetings. The focus of this portion of the study was on macroergonomic issues that might be improved to reduce the frequency of glove breach. Macroergonomic approach was adopted in this project because microergonomic approach can sometimes achieve marginal success without resolving simultaneously macroergonomic issues. The macroergonomic issues addressed in this analysis were characteristics of the workforce, information sharing and transfer, and decision-making.

At the time of the analysis there were four staffs and six technicians in Operation II. There was one female technician. The mean age of the staffs and technician was about 40 years. Job tenure ranged from 2 to 10 years. The operations performed in Operation II included ingot-casting, metal extraction, electrorefining, oxide reduction, and salt extraction.

Results and Discussion

Operation I.

Task Analysis

Casting metal rods, in general, consists of sixteen distinct tasks, and usually takes about 45 minutes to complete. These sixteen tasks, as shown in Figure 1, fall into three categories. The first category includes the preparation of a mold and a crucible, loading metal scraps, and setting up the casting apparatus. This preparatory stage takes about 17 minutes to complete, accounting for 39% of the time for metal rod casting. The second category involves melting metal scrap in the crucible and casting the molten metal into the mold. It takes about 20 minutes, 46% of the 45-minute casting time, to complete the melt-and-cast process. The third category includes tasks associated with dismantling the casting apparatus and retrieving four metal rods from the mold. It takes about 15 minutes, 15% of the casting duration, to dismantle the casting apparatus and to retrieve the rods. Tasks in the first and the third categories require direct manipulation by the operator while tasks during melting and casting need only visual monitoring.

Tools used in metal casting are general maintenance tools, such as a hammer, a flat-head screwdriver, a scraper, a pair of tweezers, and a crescent wrench. Though most tools were of lightweight, several tasks did involve the use of extreme force. For example, cleaning residues from the crucible required the operator hammering or scraping with a pointed rod with high force.

The metal casting operation also required transferring objects of various weights, shapes, and dimensions from one end of the glove box to the other end. An empty mold weighs 11 kg and is in the form of a block with 11.25", 4.75", and 3.25" on each side. The mold weighs up to 17 kg after casting. The crucible used was a 3-kg hollow cylinder and 9" in length and 4" in diameter. It weighs 9 kg when it is filled with metal scrap. Constrained by the glove ports and the shape of the mold, the operator was required to grasp the mold with a pinch grip and an extended arm.

Postural Analysis

The postures adopted by an experienced glove box operator in a simulation were the basis of postural analysis of this report. Since operator's posture was not observed during the melt-and-cast process, neutral posture for twenty minutes, a typical duration of the process was assumed.

The results of posture analysis showed that tasks performed inside the glovebox did induce the operator to adopt awkward postures of the neck, arms, trunk, and legs. The operator's neck was in a bent forward and bent backward posture for 23% and 16% of the 45-minute casting operation. The operator's back was in a bent and twisted position for 8% of the time. The operator stood for 11% of the time with both knees bent. The operator worked for 26% of the [illegible] with both arms above the shoulder. These results place the metal casting operation in an

OWAS "Action" category as requiring action sometime in the future. One should note that the OWAS protocol gives as prioritizing tool four action categories, i.e., none, soon (action in the future), very soon, and immediate.

However, one should be cautioned in interpreting this set of data. The postural analysis was based on simulated activities without considering the weight of the metal. The posture involved and the duration of materials handling may be different from that in a real working situation. In addition, assumption of neutral posture during the melt-and-cast operation was made in calculating the percentage of time spent in adopting specific posture. This assumption should be verified through work sampling when operators are actually performing specific casting tasks.

Static Strength Modeling

The results of static strength modeling showed that metal casting operation was not designed optimally to suit the capability of the general male population. In general, one should design a task so that at least 99% of male population and at least 75% of the female population have the strength necessary to do the task. However, based on the results of the computer modeling for task I, only 68% of the male population have a strength sufficient to lift the empty mold, 11 kg, with an extended right arm. Elbow and hip were the other two body joints not satisfying general ergonomic design guideline. For task II, the results show that only 7% of the male population has the shoulder strength and only 67% of the male population has the elbow strength to transfer the full mold with one extended arm. Torso, hip, knee, and ankle are joints not satisfying the design guideline. Task III is more easily performed than tasks I and II in terms of more than 95% of the male population capable of doing the task. However, those joints not satisfying design guideline include shoulder, torso, hip, knee, and ankle.

The computer modeling was done without considering the type of grip and the effect of wearing gloves. Lifting with pinch grip, a very poor coupling between the hands and the object, would require the exertion of additional force to lift the mold. This will result in a lower percentage of the population capable of performing the task. Wearing gloves may have a similar effect.

Specific Micro-Ergonomics Recommendations

Operation I. Micro-Ergonomics

There are many potential modifications that one could consider in metal casting operation to reduce the number of lifting and duration required for material handling. The following recommendations were provided to facility's management and operator as ideas for discussion. Nonetheless, these recommendations do not represent a comprehensive list of options. The feasibility, effectiveness, and acceptability of each option should be carefully evaluated by glovebox operators before full implementation.

1. Lower the bucket on the rope system in trunk lines to the same height as the transfer compartment and support the bucket with casters.

2. Place rollers on the transport compartment. The roller could be activated by a small motor.

3. Add a pallet carousel in the glove box next to the transfer compartment and make the top surface of the pallet carousel at the same level as the rollers to be place in the transfer compartment. The pallet carousel could reduce the amount of lifting required by turning the pallet. A 30-inch diameter will fit in the glove box (36" x 32") and is commercially available.

4. Design handle into the mold to facilitate grasping. Avoid using a pinch grip for holding heavy objects such as a mold. Use handles to reduce the distance for reaching the object.

5. Add a tool stand on a 'lazy Susan' and place it on top of the pallet. A tool stand would help organize the tools used in the glove box and reduce the reach distance for retrieving the tools.

6. Use air or electricity powered tools to clean the crucible. A Dremel tool with grinding bits of proper shape should be evaluated.

7. Change the mechanism for removing the cover of the mold shield. Lifting the cover with retractable air cylinder is the current practice. Currently, the operator has to adopt a posture with an extended neck and with the left arm high above the shoulder. The same air cylinder could be used to retract the cover to the glove box on the left hand side that has plenty of unused space. A threaded rod could also be used.

8. Redesign the glass shield into two sections. The shield is light in weight. However, it is not easy to align the glass shield between the crucible and the induction coils, because of the size of the glass shield and of the air cylinders near the ceiling. A smaller section could be reduced the effort in positioning the glass shield. A second section could first be assembled with cover fitted with sensor and stirrer, and then place on top the first section of glass shield already in the desired position.

9. Install an automated adjustable platform to replace the two platforms and the step stool. Though an automated adjustable platform is expensive, it has three advantages. First, it provides accommodation to operators of different statures. Second, it allows easy access to the glove ports at the upper level. Third, it does not present the same hazards as the platforms and step stool do. Sliding the platform away creates a hazard in bumping into the utility lines underneath the glove box. Standing on the step stool to manipulate an object inside the glove box presents a fall hazard.

Operation II. Micro-Ergonomics Issues

Need to integrate ergonomics in current glovebox task design

Glovebox operations, because of its physical design and its requirement of wearing long-sleeve thick gloves, needs more in-depth, detailed consideration of basic principles of ergonomics to optimize human performance capability. Because of the current design of the glovebox task, glovebox operators have to work in a condition that reduces range of motion and dexterity. As a result, operators have to compensate by exerting more force, adopting more awkward posture, and repeating the same manual movements more frequently to get the "job" done. It is no

wonder that glovebox operators appear to be a highly selective group in terms of body build and physical strength. One worker mentioned that it was necessary to build up especially the upper body strength to do the job. These factors, i.e., repetition, additional force, and awkward posture, may increase not only the risks for the operators to develop musculoskeletal discomfort/disorders and the rate of deterioration of glove and increase the chance of breaching the glove.

It is imperative that the management of this Defense / Nuclear facility design the job and select the equipment and tools that will compensate for limitations posed by the glovebox and gloves. The goal of the ergonomic design is to create a man-machine interface with which all glovebox workers could perform the job with less force, less time, and less contact area between glove and objects. However, the current work conditions prove to be the contrary. The management failed in recognizing the need for better tools.

The tools utilized in both Operations I and II, were the usual household maintenance tools. For example, some of the "tools" in Operation II were just steel rods. To make the condition even worse, some of the tools were used in ways for which the tools were not designed. For example, a worker used the pointed end of a file to either knock or scrap impurity off a funnel, while holding the file in his hand. The glove was not only in contact with the sharp edge of the file, but also was being filed by the rough surface of the file. The former condition was a potential cause for cut and the latter condition could be a cause for abrasion, wear and tear. In addition, there was no handle on the file. The pointed end of the file may be a cause for puncture. Other tools, such as the wire brush and the screwdrivers, all had pointed end.

It is recommended that management and the operators hold regular sessions to evaluate the use of each and every hand tool used inside the glovebox and the tool's potential in breaching the gloves. Ways to modify tools or new designs of special tools could be defined through such a brainstorming session. One has to remember that the operators are the true "Experts" in the operation since they have been working in the condition for many years and understand the difficulty involved in utilizing specific tools to accomplish certain tasks. Without management's support and encouragement, they just learn to adapt without thinking there could be a better way. It is the management's duty to provide such supporting atmosphere. Management is nothing more than motivating people.

The equipment in Operation II was also a source of ergonomic concern. Take electrorefining as an example. The workers were required to tighten and loosen six hex cap screws on the flange head of the furnace. The hex cap screw had a width of 0.5 inch, a height of about one-eighth inch, and a thread length of about three-quarter inch. The worker had to align the flange head and turned the screws one by one with a pinch grip. Turning a screw with bare hand was already a task requiring precise thumb and index finger movement and high dexterity. A thick glove would only make it difficult and require more force and time. In addition, the hex cap screw had six pointed angles and sharp edges. High force and sharp edges could be factors contributing to the breaching of gloves. The final tightening of the screws required the use of either an adjustable crescent wrench or a ratchet box wrench. The ratchet box wrench should function much efficiently than the crescent wrench. One should "consult" the workers in terms of reasons for choosing one or the other. Both wrenches still had some potential to stress the gloves.

Operation II was in the right direction in terms of changing from the hex cap screws to a bolt with a T-shape handle. The T-shape handle should be made bigger so that the force would not be concentrated at the ends of the handles and compress the glove.

The stirring rod of the furnace was also a feature requiring some attention. The rod was positioned using several Teflon spacers and a knurled copper ring. The workers would have to tighten it with hand. The knurled ring had ridges very similar to the texture being tested in another study performed by the authors. Obvious wear on the gloves was observed even after 36 hours of testing.

Retrieving the round bottom well-type crucible from the furnace was another task of concern. The worker relied on the friction between eight fingers and the internal surface of crucible to pull the heavy crucible up and out of the furnace. This was not only an awkward lifting job but also a potential contributing factor to the wear and tear of the gloves.

Use of Work-Study

A detailed work-study should be conducted at Operation II to quantify the frequency, force, surface texture, and contact area when Hypalon gloves were used. This type of the study would provide the data necessary to determine the types of stress that a glove has to endure while in service.

The work-study would also be useful in providing information necessary in developing a realistic specification of glovebox gloves, and a protocol for glove testing. At the time of the analysis, this Defense / Nuclear facility initiated the evaluation of the tensile strength of the Hypalon gloves. This endeavor is a good start but needs to be expanded to include more realistic testing conditions.

For example, it is necessary to determine the maximum force that the Hypalon gloves in its current design could endure without being punctured not only for a new glove but also for gloves that have been in service for different period of time. In addition, whether there will be some defects that could be visually identified by glovebox operators under the normal and abnormal conditions should be ascertained. At the time of the analysis, operators were required to visually inspect the gloves through leaded glass windows while wearing the safety glasses. The leaded glass was slightly yellowish tinted and might have been slightly covered on the inside with dust and the glove being inspected was usually covered with dust. The human factors issue in such case can not be ignored especially when the average age of the workforce is around 40 years.

Other testing conditions should be included so that the mechanisms of breaching a glove could all be considered. At the time of the analysis, in Operation II, puncture, cut, tear, and heat were the four main mechanisms to breach a glove, though the work activities and tools that were involved had not been well documented. The tensile strength test being performed would clearly not be relevant if the breaching of glove was due to primarily thermal stress.

Operation II. – Macro Ergonomics Issues

Systematic Approach to Identify Causes of Glove Breach

At the time of the study, the management team seemed to be preoccupied with the idea that high incidence of abnormal glove breach was due to defective glove from the vendor and the aging, i.e., limited shelf lives, of the Hypalon gloves. The management team documented that a defective batch, e.g., with blister, cracking, and discoloration, was received at the facility. However, this was based on only one batch of gloves. The facility needs to establish a program that requires the warehouse or stockroom to sample and inspect the gloves upon receiving each shipment. An alternate would be having the operators examine the gloves when the gloves are signed off at the stockroom.

The effect of aging on glove's service life was only a hypothesis. There were no data to support such a hypothesis. The Hypalon glove is supposedly designed with a feature that minimizes the effect of oxidation. In terms of storage, the manufacturer (North, Inc.) of the Hypalon gloves, recommended laying them on a flat surface and keeping them away from the sunshine. However, once installed on to the glovebox, the glove would most likely hang from the ring (on an active glovebox) or being tied into a knot on a non-active glovebox. On an active box, manual material handling would certainly put the gloves to more stress, probably in regions where the glove has most contact with the objects being handled. This Defense / Nuclear facility should consider a study that evaluates the effect of factors, such as the age, duration in service, frequency of usage, type of manual operation, and temperature, on the reliability of the gloves. The existing glove exchange program could serve as a starting point to link glove failure with task information and manual material handling while wearing gloves.

There may be other factors that have contributed to both the normal wear and the abnormal breach of the glovebox gloves. At the time of the study, the facility relied on the use of survival analysis to schedule the change of the gloves for "normal wear." Though statistical approach, i.e., using survival analysis, seems to be a good method to prevent glove breach and the resultant radiation exposure, it inevitably assumes that the glovebox task is incapable of being modified. The management should take a systematic, proactive approach in applying ergonomic task analysis to glovebox task and identify ways to redesign glovebox task and tools.

Management Information System – A Better Link among Existing Databases

A chart of existing databases at this facility should be created to identify how each database should be linked and how the information could be used to improve the process and to reduce the glove failure. For example, the radiation incidence report available in the facility was not linked with the glove exchange database.

It seems that data on glove usage were being gathered. However, the data collected have not been optimally used. The results of the analysis seem to indicate that this particular Defense / Nuclear facility was not collecting and wisely utilizing the information needed to plan their activities for the prevention of glove breach. At the time of the study, this particular Defense / Nuclear facility did not have a management information system to provide such information.

Communication between Labor and Management

Better communication includes not only verbal and written communication in terms of sharing information and soliciting suggestions, but also a behavioral communication. It is only when a manager puts on his/her coverall, goes through the same manual procedures, exercising the same precautions, and gets a hands-on feel of the difficulty involved in the operation, will the operators feel the sincere concern of the manager and develop trust in the management.

At the time of the study, there seemed to be some doubt as to the intent of the ergonomic assessment work group. Operation II supervisor felt that some workers might perceive the objective of the current study in reducing the incidence of glove breaching as faultfinding. A review of radiation occurrence reports also leads to the suspicion that current incident investigation practice in this defense/nuclear facility could give the operators the impression of faultfinding.

In addition, automation may be an issue of concerns among workers. It is common among many industries that workers become weary about their job security when automation is one of the options being considered by the management. If operators are not asked to participate in decision-making and if the management's intention is not clearly communicated, all other endeavors to improve the work environment, processes, and tools, may well be considered as preparatory steps toward automation. As a result, workers are less likely to provide assistance and receptive to changes.

Rationalization in System Planning

The management team of the facility could benefit from exercising "Rationalization" in system planning. Rationalization is a concept in industrial engineering and industrial management. The purpose of rationalization is to develop and execute an action plan that will result in a simpler, more reliable, more efficient and easier to maintain process, in this case, the glovebox operation at Operation II. This requires a careful examination and reevaluation of the goal of the process and the means to achieve the specific goal. The following two examples identified in the study demonstrate how rationalization could assist in the system planning in this Defense/Nuclear facility.

The first example relates to a new glovebox, housing a big chisel-like breaker and that was being installed in Operation II. The "Breaker" machine would be used to break a metal ring in half for storage because of the limited size of the vault. However, the metal ring took Operation II operators a lot of time and efforts to produce. One should at least consider either modifying the configuration of the metal ring, under the constraint of criticality, or changing the size of the vault for storage. Planning and executing an efficient process, i.e., less contact time, fewer steps, and less use of gloves, is probably the key to success not only in manufacturing and in the prevention of glove breach.

The second example involves the use of a paint scraper to clean salt residues surrounding a chlorine scrubber and on the tabletop of a glovebox. Due to an overflow of chlorine and untimely housekeeping, salt residue formed inside the glovebox. Certainly, one could reduce the potential of chlorine overflow by installing a flow-limiting valve in line, a primary engineering control. Wiping off the salt solution immediately after spills with a rag is probably much safer

than using the paint scraper. Once dried, the salt residues formed and adhered to the tabletop rendering difficult to clean. Operator may have to use a sharp object, in this case a paint scraper, to clean the surfaces. It is quite natural for a worker to hold his left hand in front of the scraping action to keep the loose residue in one place. There is a danger of puncturing the left-hand glove if there would be a slip of the right hand with the scraper. Gripping a sharp object in the right hand forcefully for the scraping action increases the potential for cut, tear, and wear of the glove. Timely housekeeping is a part of production activities most people tend to overlook. However, in terms of glove breach, timely housekeeping in the glovebox operation may have a special meaning, despite the fact that current housekeeping in glovebox is much and much better than 10 years ago.

On-line Process Control

During the data collection process through individual interviews and group discussion, the management emphasized many times that retrieving a metal ingot from a crucible could be very difficult and required frequent manual material handling. However, on-site observation revealed that a smooth operation in retrieving the ingot was achievable. The supervisor and the operators gave a 50%-50% chance of having a bad run versus a smooth run. For a process that is important to national security, a 50%-50% chance should not be acceptable. The management and operators should join force in experimenting with the process parameters to identify the causative factors leading to a bad run. Experimental design, using methods similar to the Taguchi method, should be developed and implemented for continuous improvement of the process.

Conclusion

This study demonstrates the utility of a micro- and a macro-ergonomic task analysis for the identification of hazards in a Defense / Nuclear facility. The results of the study showed that the operators involved in glovebox operations were exposed to ergonomic stressors due to the design of glovebox tasks. The study also showed that there were macro ergonomic issues that need to be addressed in order for micro ergonomic issues to be resolved. The safety and health personnel at the facility need to include ergonomic task analysis in their routine hazard surveillance. The management and the workforce should be educated of the importance of ergonomics in continuous improvement of the production process.

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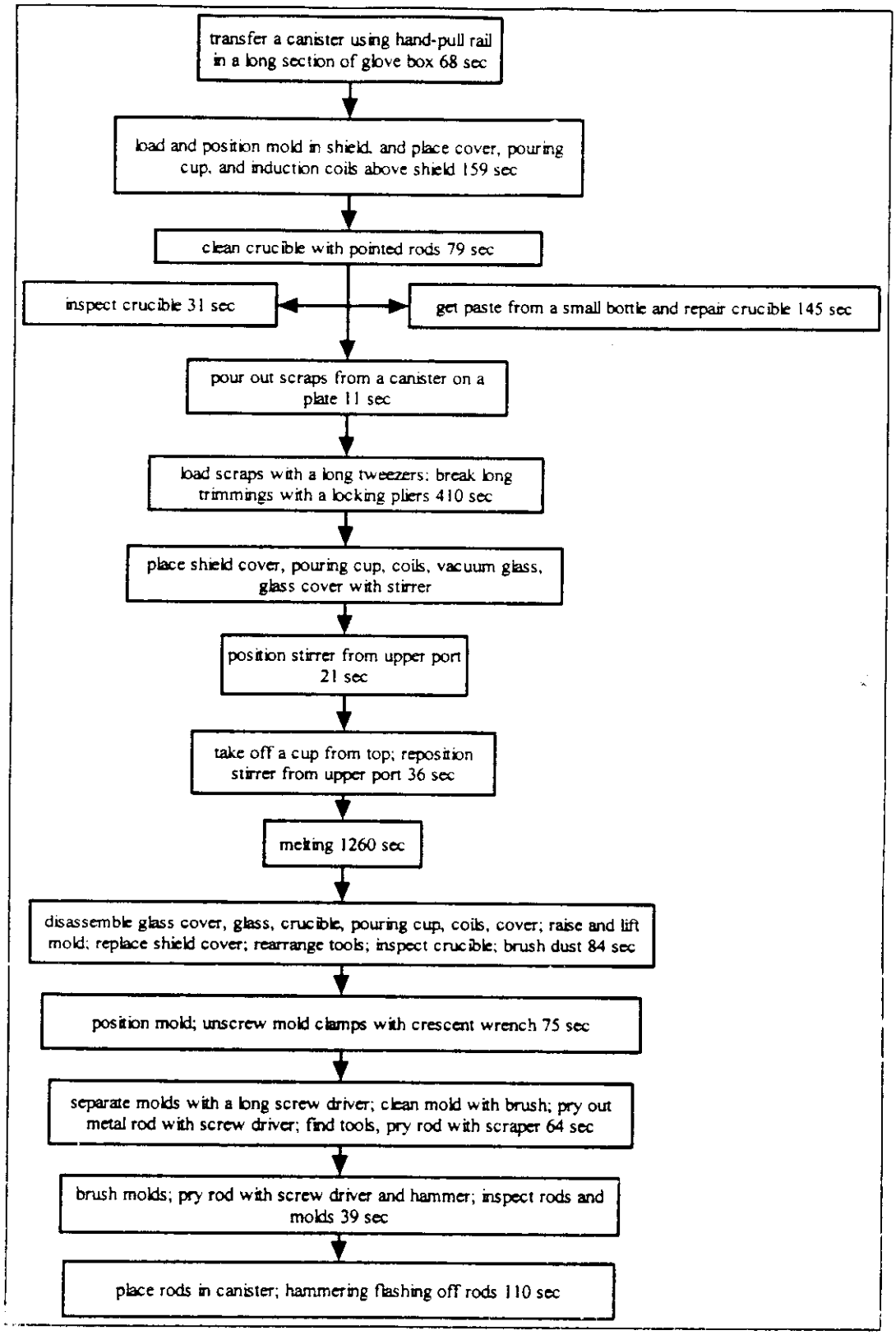
Figure 1. Task flow of casting operation in a glovebox



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Validation of an Experimental Device for the Measurement of Kinetic Friction - Subproject IV

Abstract

An experimental device was developed in this project for the measurement of kinetic coefficient of friction. In addition, the kinetic coefficient of friction (μ_k) of fingertip was measured for six Chinese subjects on textured and non-textured surfaces in this exploratory study. The results indicate that μ_k of the fingertip skin varies with the magnitude of normal force and the surface texture of the test plates. The μ_k decreased as the exerted normal force increased. The highest value of μ_k was 2.05 for males and 2.26 for females with 1 N normal force on a test plate of 100% contact area. When the load increased to 10 N, μ_k decreased to 1.09 for males and 1.11 for females on the same test plate. The μ_k increased as the contact area increased. For males, there was a 39% and 64% increase, respectively in μ_k as the contact area increased from 50% to 75% and 100%. For female, there were, respectively, a 41% and a 77% increase in μ_k as the contact area increased from 50% to 75% and 100%.

Keywords: Kinetic Coefficient Friction; Texture; Chinese.

Introduction

Friction is a common mechanical stressor upon human skin. High friction may produce erosions and blisters to human palmar skin (Knapik et al. 1995). Friction also affects our ability to manipulate and grasp objects with the hand. Friction between the surface of an object and fingers is also needed for an individual to adjust the amount of force applied in manipulating objects (Cadoret and Smith 1996). Low friction objects tend to slip out of the hand resulting in an increase in the potential for injury. Low friction objects therefore require greater grasp forces than objects with high friction. Prolonged, excessive grip forces applied to prevent slippage may cause injuries to tendons and tissues (Putz-Anderson 1988). Armstrong (1985) pointed out the importance of proper friction in the design of hand tools. Frederick and Armstrong (1995) studied the effect of friction and load on pinch force in a simple hand transfer task and suggested that the use of tool handle friction enhancements might reduce required pinch force for objects requiring upwards of 50% or more of maximum pinch strength.

Frictional properties between two surfaces is characterized by the coefficient of friction (μ), a dimensionless ratio of the friction force (F_f) between two bodies to the normal contact force (F_n) pressing the two bodies together (ATSM 1993). There are two types of coefficients, static coefficient of friction (μ_s) and kinetic coefficient of friction (μ_k). Static coefficient of friction, μ_s, is defined as the ratio of frictional force immediately before movement occurs to the normal contact force, while kinetic coefficient, μ_k, is defined as the ratio of frictional force, under conditions of macroscopic relative motion between two bodies, to the normal contact force. Both static and kinetic coefficients of friction are important in the design of hand tools and other manually manipulated objects. The static coefficient of friction of index fingertip has been found to vary with test materials in many studies. For example, when tested with silk, suede, and sandpaper, Johansson et al. (1984) found the static coefficients of index finger and thumb to range from 0.22 to 2.0, while Westling et al. (1984) reported a μ_s ranging from 0.35 to 1.21.

Smith et al. (1997) reported static coefficients of friction of 1.5 to 1.8 for pinch grip by thumb and index finger against smooth and etched polyamide plastic. However, using the same type of materials, i.e., smooth and etched polyamide plastic, Cadoret et al. (1996) reported static coefficients of friction for barehand ranged from 1.1 to 2.0. On a surface made of aluminum, the static coefficients of friction of finger were found to range from 0.15 to 0.6.

Kinetic coefficients of friction of fingertip have only been reported in a few studies. Tested against rubber surface, the kinetic coefficients of friction of finger skin were found to range from 0.25 to 0.75 (Roberts and Brackley 1990). On textured polycarbonate plates, Bobjer et al. (1993) reported kinetic coefficients of friction varied from 0.56 to 2.22, using a specially designed instrument. Since Bobjer et al. (1993) studied only Caucasian male subjects; there is a need to measure the kinetic coefficients of friction in females and in populations of other ethnicity. It is also of importance to learn whether there is a gender difference in μ_k, since skin compliance and gender seem to be correlated (Woodward 1993). Woodward (1993) defined two measures of skin compliance, a two-point compliance and gap compliance, and found that the hands of males were less compliant than the hands of females. Thus, it would be of interest to learn whether there is a significant gender difference in the kinetic coefficients of friction of fingertip on textured and non-textured surfaces.

In this paper, we report the measurement of kinetic coefficient of friction of the index fingertip against surfaces made of polycarbonate plate. In addition, this study evaluated the effects of gender, magnitude of normal force and surface texture on the kinetic coefficient of friction.

Methods

Instrumentation

To estimate the coefficient of kinetic friction between the skin of fingertip and hand tool surface, a specially designed friction-measuring device was fabricated at the UCLA Occupational Ergonomics Laboratory. This device, as shown in Figure 1, consists of three polycarbonate boards, four friction-less ball bearings, and three strain gauges. The top and bottom boards were positioned horizontally and separated by four ball bearings. The third board was mounted vertically on the bottom board and perpendicular to the top board. This board served as an anchor for the strain gauge.

The top board has a dimension of 24 cm by 14 cm with a thickness of 0.5 cm and a cut-out area of 13.5 cm x 3.5 cm in the center that allows the placement of test plates with specific texture. The top board was supported by the four friction-less ball bearings that sit on the bottom board. The bottom board was clamped onto a table and provided the stability of the testing device. The ball bearings were used to provide free movement of the top board in the horizontal direction.

Two strain gauges were mounted on the edges of the central hollow area of the top plate were used to support the test plate and measure the normal contact force during the test. An additional strain gauge was placed between one side of the top plate and the vertical plate. This strain gauge was used to measure the frictional force. Two types of strain gauges (Omega LCL005 and LCL816) were used in this study. The LCL005 has a maximum capacity of 5-lb force (22.3 N) and the LCL816 has a maximum capacity of 2-lb force (8.9 N). Three LCL005 strain gauges

were used for measurement when a normal force of 10 N was exerted. Three LCL816 gauges were used in measurement with 1 and 5 N normal force.

Figure 2 shows a block diagram of the experimental setup for data collection. Two power supplies (Omega model PSS-5A and PST-4130) provided 5 Vdc to the strain gauges. An amplifier (Omega model OCT-01) amplified the analog signal from the strain gauges with a gain of 100. A 16-channel data acquisition board (DAS 1601) and a 486-DX computer were used to collect, process, and display the force measurements. Force measurement was taken at a sampling frequency of 10 Hz during the 3-second sampling duration for each test.

Four test plates made of polycarbonate were fabricated by UCLA Machine Shop. Each plate had a dimension of 13.5 cm x 3.5 cm with a thickness of 0.5 cm and specific surface characteristics. The surface texture of test plates, as adapted from the study by Bobjer et al. (1993), was defined in unit of distance between the ridges and grooves (peaks and valleys). The depth of all grooves was 0.5 mm. As shown in Figure 3, test plate I has a ridge and a groove width of 1.5 and 0.5 mm, respectively. The widths of ridges and grooves of test plate II were both 1mm. Test plate III has a ridge width of 0.5 mm and a groove width of 1.5 mm, i.e., an inverted image of test plate I. Test plate IV has a non-textured surface without ridges and grooves.

Subjects

Three Asian males (age: 31 - 43 years) and three Asian females (age: 25 – 42 years) participated in this study. All these subjects were right handed. Maximum tip pinch strength and palmar pinch strength were measured for each subject using Jamar pinch strength meter.

Protocol

All measurements of kinetic coefficients were taken with dry bare hands. Prior to the start of each session subjects washed their hands with soap and water and used paper towel to clean their hands and maintain dry barehanded condition. The test plates were also wiped and cleaned to maintain a dry and clean condition. The temperature in the laboratory was about 24 °C and the relative humidity was about 44%.

Three normal forces of 1, 5, and 10 N were used for both male and female subjects. Each force-texture combination was presented in random order to the subjects. Several trials were performed before each test, to familiarize the subjects with the procedures and the target force level. Each subject was asked to press the index finger of his/her dominant hand on the test plate and then pull the finger toward himself/herself. Each subject controlled exerted force according to a digital readout displaying the magnitude of the normal force continuously on the screen of a computer monitor. The subjects were asked to keep the normal force within ±10% of the target normal contact force.

Figure 4 shows the data collected during a typical run for the force measurements, normal contact force and frictional force. In this example, 1 N normal force was the target force level. The kinetic coefficient of friction was calculated by taking the ratio of frictional force to normal contact force for each data point. As shown, the exerted normal forces for five data points (from 1.6 to 2.0 seconds) were all within ±10% of the target normal contact force 1 N. Hence, the

mean value of kinetic coefficient of friction for this particular example was calculated to be 0.85 by averaging five values of kinetic coefficient of friction from 1.6 to 2.0 seconds.

Monitoring the sliding velocity

During each test, the position of each subject's test finger (index finger) on the test plate was recorded on videotape. The sliding velocity of the finger was later calculated through digitization using a two-dimensional Motion Analysis System (Peak Performance, Inc.). Mean sliding velocity was obtained by averaging five instant velocity measurements taken at the instant of time when the μ_k was determined.

Measurement of contact surface area

The surface contact area was measured by pressing each of the force levels of 1, 5, and 10 N onto a millimeter graph paper placed on top of test plate IV (plain, non-textured surface). The index fingertip was dyed with red ink for a clear impression on the millimeter graph paper. Surface pressure was calculated as ratio of normal force to the surface contact area between the finger pad and the plain non-textured surface.

Statistical Analysis

Data were retained for analysis only if the exerted force was within ±10% of the target normal contact force. If the exerted force was not within the 10% of the desired range, new trials were repeated. For each valid datum, a μ_k was calculated by taking the ratio of frictional force to normal contact force. Mean μ_k was determined by taking the average of five estimates of the μ_k. Descriptive statistics and analysis of variance with repeated measures were performed using the Statistical Package for Social Science (SPSS 1994). The mean and standard deviation of the kinetic coefficient of friction was calculated. In the analysis of variance, the independent variables in this study were gender, texture and normal force level. The dependent variable was the estimates of kinetic coefficient of friction. Analysis of variance with repeated measures was used to evaluate the main effects of gender, textures, normal forces, and their interactions on the estimated coefficient of dynamic friction. The Bonferroni's t-test with $\alpha = 0.05$ (Morrison 1983) was used to evaluate the differences in the μ_k's between male and female subjects in this study.

Results

Kinetic coefficient of friction

Table 1 lists the mean and standard deviation of kinetic coefficients of friction for male and female volunteers of this study. The mean kinetic coefficient varies depending on the level of normal force and the type of texture. For males, the mean kinetic coefficient ranged from 0.73 (Test plate III, 10 N) to 2.05 (Test plate IV, 1 N), while for females, the mean kinetic coefficient ranged from 0.77 (Test plate II, 10 N) to 2.13 (Test plate IV, 1 N). The standard deviation of the kinetic coefficient ranged from 0.02 to 0.89 for male subjects and from 0.11 to 0.58 for female subjects, respectively. There are some minor differences in the mean kinetic coefficients of friction between male and female subjects. The largest absolute difference in kinetic coefficients of friction between the male and females subjects was 0.72 (Test Plate IV-5 N) and the smallest absolute difference was 0.02 (Test Plate I - 10 N). However, the gender difference in the measured kinetic coefficient of friction was not statistically significant, $F = 2.01$ and $p > 0.05$.

As there was no significant gender difference, we pool the male and female data together. Figure 5 plots the overall mean kinetic coefficients of friction of the six subjects under twelve test conditions, i.e., three levels of normal force and four types of texture. The results also suggest that both the texture of the surface and the exerted normal force affect the measured kinetic coefficients of friction. Under all three levels of normal force, test plate IV always yielded the largest kinetic coefficients of friction followed by test plates I, II, and III. The overall mean kinetic coefficient of friction ranged from 0.78 (10 N force on test plate III) to 2.09 (1 N force on test plate IV). The difference in the mean kinetic coefficient of friction due to texture of the test plates is statistically significant ($F = 31.54$, $p < 0.05$). However, there is essentially no difference in the mean kinetic coefficients of friction between test plates II and III for all three levels of force.

The results also indicate that there is a decreasing trend in the mean kinetic coefficients of friction as the exerted normal force increases. On the average, as the force increased from 1 N to 10 N, the mean kinetic coefficient of friction decreased from 1.42 to 0.94, a difference which is statistically significant ($F=6.83$, $p < .05$).

There was also a significant interaction effect due to texture and force level ($F = 3.29$, $p < .05$). The differences in the mean kinetic coefficients of friction among test plates were much greater for the 1 N force condition than for the other two force conditions. For the 1 N force condition, the biggest absolute difference in the mean kinetic coefficients of friction between test plates was 1.16, while for the 10 N force condition, the difference between plates in mean kinetic coefficients of friction was 0.39.

Finger Contact Area and Pressure

The gender difference in tip pinch strength was statistically significant, $t = -2.87$, $p < 0.05$. While the tip pinch strength of male subjects had were 73.6 N (SD: 11.5 N), the mean tip pinch strength of female subjects was 49.1 N (SD: 9.3 N). There was also significant gender difference in the palmar pinch strength, $t = -7.98$, $p < .05$. The palmar pinch strength of male subjects had a mean of 111.4 Newton and a standard deviation of 8.0 Newton. The palmar pinch strength of female subjects had a mean of 71.5 Newton and a standard deviation of 3.2 Newton.

Females of this study seemed to have finger contact areas larger than that of male subjects. For females, the nominal finger contact area while exerting 1 N normal force had a mean of 98 mm^2 and a SD of 39 mm^2. As the normal force increased to 5 N and 10 N, the mean nominal finger contact areas increased to 155 mm^2 (SD: 29 mm^2) and 226 mm^2 (SD 47 mm^2), respectively. For males, the nominal finger contact area while exerting 1 N normal force had a mean of 61 mm^2 and a SD of 24 mm^2. The mean nominal finger contact areas increased to 111 mm^2 (SD: 33 mm^2) and 175 mm^2 (SD: 69 mm^2), respectively, as the exerted normal force increased to 5 N and 10 N. The apparent gender differences in the nominal finger contact areas at these three normal force levels, however, were not statistically significant.

As a result, females in general experienced approximate a 3-fold increase (from 11.5 kPa (SD: 5.1 kPa) to 32.9 kPa (SD:5.8 kPa)) in the finger contact pressure as the exerted normal force increased from 1 N to 5 N. A ten-fold increase in the normal force, however, resulted in only a 4-fold increase to 45.7 kPa (SD: 10.9 kPa) in the contact pressure. Compared to female subjects,

male subjects experienced less increase in the finger contact pressure. With 1 N normal force, the mean finger contact pressure was 18.3 kPa (SD: 8.0 kPa), while at 5 N normal force, the mean finger contact pressure was 48.2 kPa (SD: 16.1 kPa), an increase of about 2.5 times. With 10 N normal force, the mean finger contact pressure was about 66.2 kPa (SD: 33.9 kPa), an increase of about 3.5 times from that with 1 N normal force.

Overall, a 5-fold increase in normal force from 1 N to 5 N resulted in an increase of 3.5 times in finger contact pressure from 14.9 kPa (SD: 7.1 kPa) to 40.6 kPa (SD: 13.7 kPa). A 10-fold increase in normal force from 1 N to 10 N resulted in an increase of about 3.8 times in crease in the finger contact pressure to 55.9 kPa (SD: 25.2 kPa).

Discussion

No gender difference was found in the present study of the fingertip kinetic coefficients of friction on textured surfaces. Our finding of no significant gender effect seems to be consistent with the finding by Kennis (1994) in a study of fabric-to-skin friction. The results suggest that skin compliance may not be a significant determinant of kinetic friction of fingertip. Skin compliance measurements for males in Woodward's study were significantly lower than for females (mean two-point compliance: female = 2380.97 micron; male = 2088.14 micron; mean gap compliance: female = 1901.33 micron, male = 1528.21 micron). We did not measure in this study skin compliance in the way that Woodward (1993) did in his study. However, the results that the finger contact area of female subjects seems larger than that of the male subjects seemed to suggest that females' fingertips are in general more compliant than that of males. Nonetheless, the effect of such difference on the mean kinetic coefficients of friction seems minimal.

It is of interest to compare the normal finger contact areas of the Chinese subjects of the present study with that of the fourteen Caucasian male subjects in Bobjer et al's study of 1993. Bobjer et al. (1993) found the mean normal finger contact areas at 1 N and 10 N normal force were about 175 mm^2 and 250 mm^2, respectively. They were slightly greater than the 98 mm^2 and 226 mm^2 for the female subjects of this study, but much greater than 61 mm^2 and 175 mm^2, for the male subjects at 1 N and 10 N normal force. As the sample sizes of both studies were relatively small, it is premature to say that there was any definitively ethnic difference between Caucasian and Chinese in finger contact area or in finger compliance.

However, there seems to be no significant difference in the kinetic coefficients of friction reported in the study by Bobjer et al. (1993) and that found in the present study. For the Chinese male and female subjects of the present study, the kinetic coefficients of friction on textured surfaces were found to range from 0.79 to 2.09. The test plates in our study were directly adapted from four of the five test plates used by Bobjer et al. (1993). Thus, the results of the present study could be directly compared to that by Bobjer et al. (1993). As shown in Table 2, under similar test conditions, the mean kinetic coefficients of friction in the study by Bobjer et al. (1993) ranged from 0.74 to 2.22. There were minor differences in the kinetic coefficients of friction between these two studies. However, none of the differences were statistically significant based on the Bonferroni's t-test (Morrison 1983) at an alpha level of 0.05. Thus, ethnicity of the subjects did not appear to be a factor in the kinetic coefficients of friction.

Effect of surface texture and normal force on kinetic coefficient of friction

A negative relationship between the kinetic coefficients of friction and the exerted normal force was found in the present study. However, as seen in Figure 5, the negative correlation was more prominent when the surface of the test plate is either smooth (test plate IV) or with 75% of the area covered with ridge (test plate I) than when there is less ridge area. This is consistent with what others (Buchholz et al. 1988; Bobjer et al. 1993) have found regarding the frictional characteristics of human palmar skin: the human palmar skin does not follow the Amonton's laws of friction. Comaish and Bottoms (1971) measured the coefficients of static and dynamic friction between clean dry skin and sheet or knitted materials. They reported that skin obeys Amonton's laws of friction over a limited load range and this deviation may occur because skin is subject to viscoelastic rather than purely plastic deformation. As Bobjer et al. (1993) pointed out the frictional force of human palmar skin depends on the size of surface areas in contact with the skin and is not directly proportional to the normal force.

Human skin may have similar characteristics as the polymeric materials and thus behaves differently from the classical concepts of friction (Bobjer et al. 1993). As such, the kinetic coefficient of friction may vary not only with the surface contact pressure, but also with the sliding speed (Yamaguchi, 1990). In their study Bobjer et al (1993) controlled the speed of the finger across the test plates in the range 4.5 ~ 5.5 cm/s. In the present study, the sliding speed of the finger across the test plates was not controlled, but found to be in the range 0.6 - 3.8 cm/s. Nonetheless, the difference in finger velocity across the surface did not seem to affect the results, as evidenced by the insignificant difference in the kinetic coefficients of friction of the two studies.

There are limitations of the study. First, small sample size was used in this exploratory study. Only six subjects were studied. Second, none of the subjects had much experience in industrial activities requiring intensive use of hand tools. More studies of larger sample size with diverse industrial experience need to be conducted to identify relevant skin characteristics. Third, the textured and non-textured surfaces made of polycarbonate represent only a small fraction of the hand tool surface utilized in industrial environment. Other types of material such as aluminum and other types of textured surface should be used to characterize the frictional properties of palmar skin and hand tool surface and estimate hand force exertion in manual work. In addition to flat surface, the influence of curvature surface instead of flat surface on coefficient of dynamic friction should be further investigated. The effect of these factors on the coefficient of dynamic friction should be further evaluated to characterize the frictional properties between human palmar skin and hand tool surface.

Conclusions

This exploratory study characterized the frictional properties of the index fingertips of six Chinese, three males and three females, using a simple device. Three textured surfaces and one non-textured surface made of polycarbonate materials were used in this study to simulate the hand tool surface. Despite the simplicity in the design of the device, the kinetic coefficients determined in the study is similar to what other researchers have found. The kinetic coefficients of friction of index fingertip depend on surface texture and magnitude of the normal force exerted. There was no significant gender difference in the kinetic coefficients of friction.

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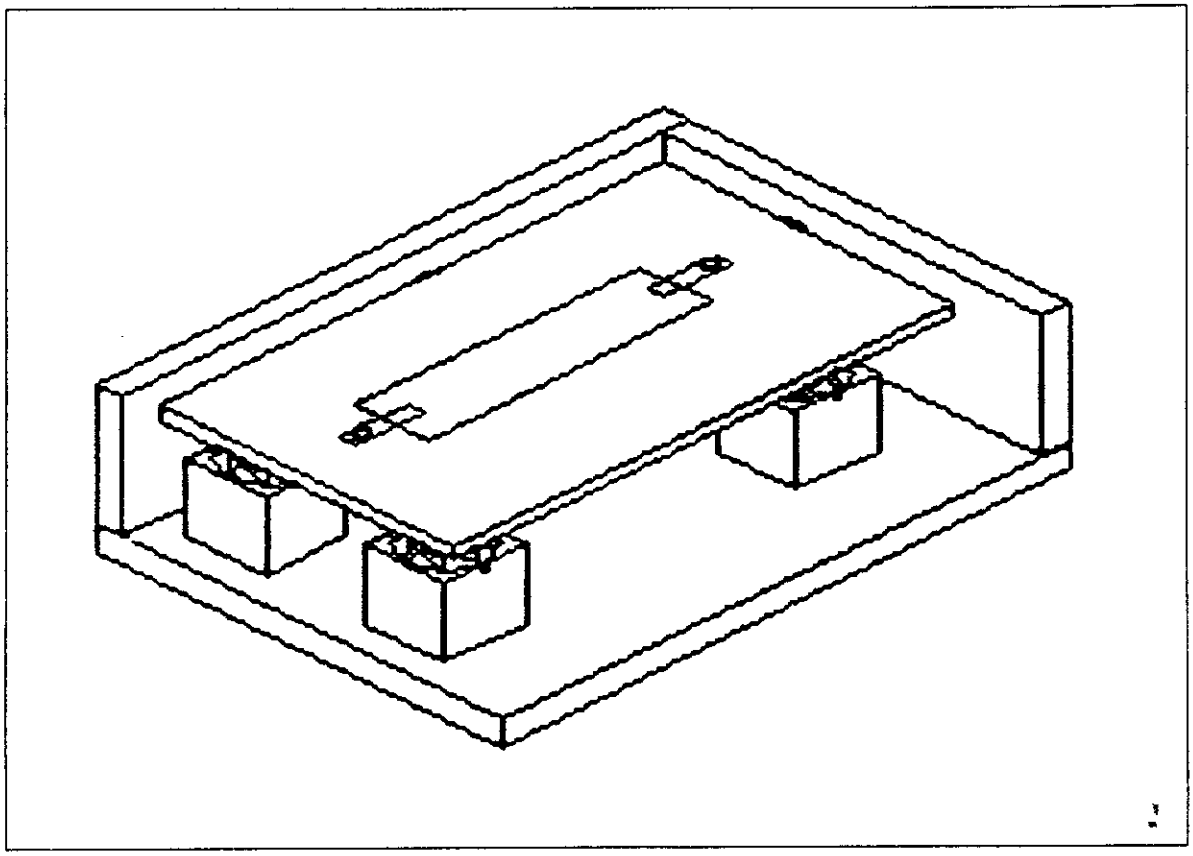

Figure 1. Experimental device for measuring the kinetic coefficient of friction

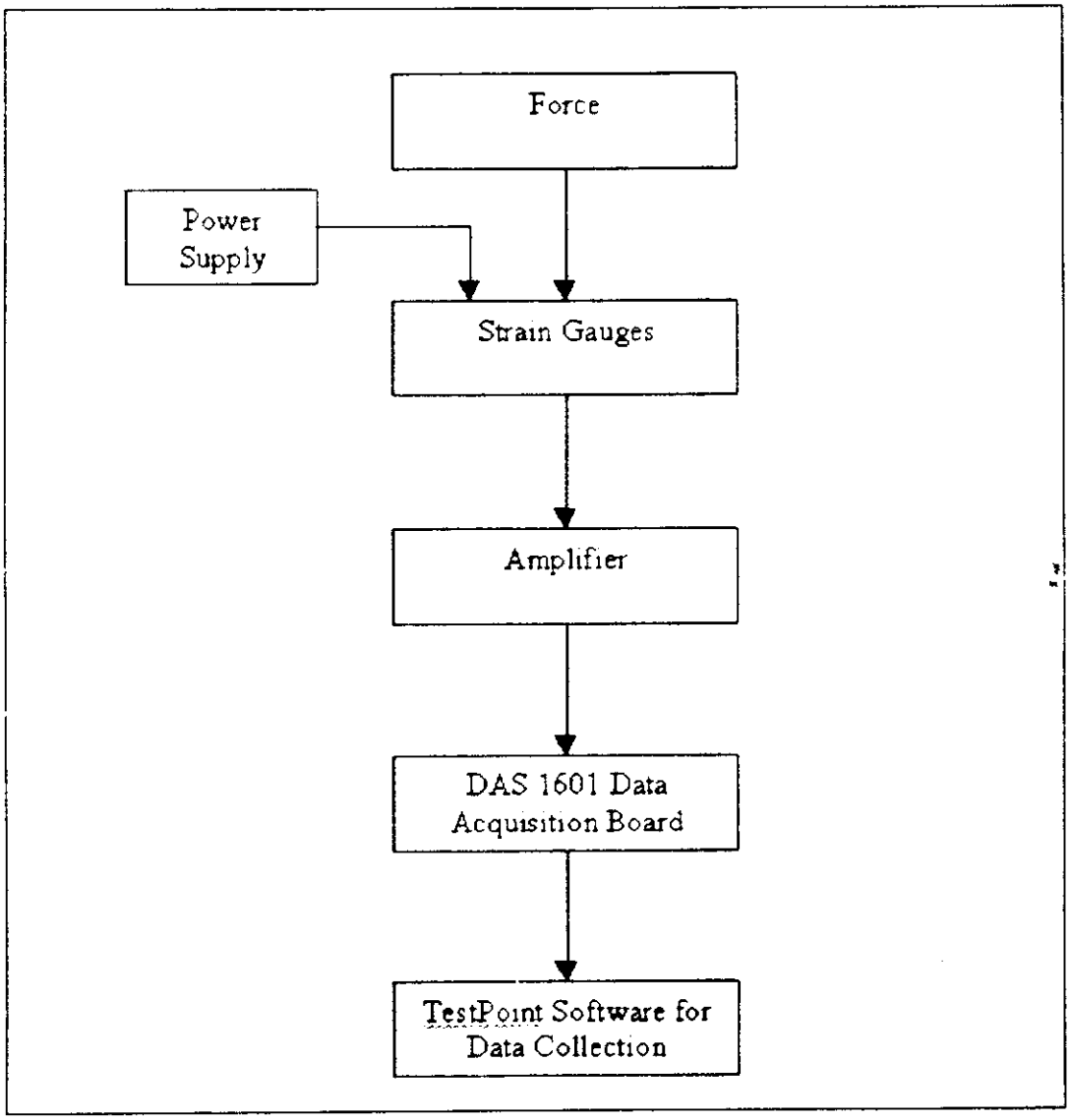

Figure 2. Block diagram of data collection system

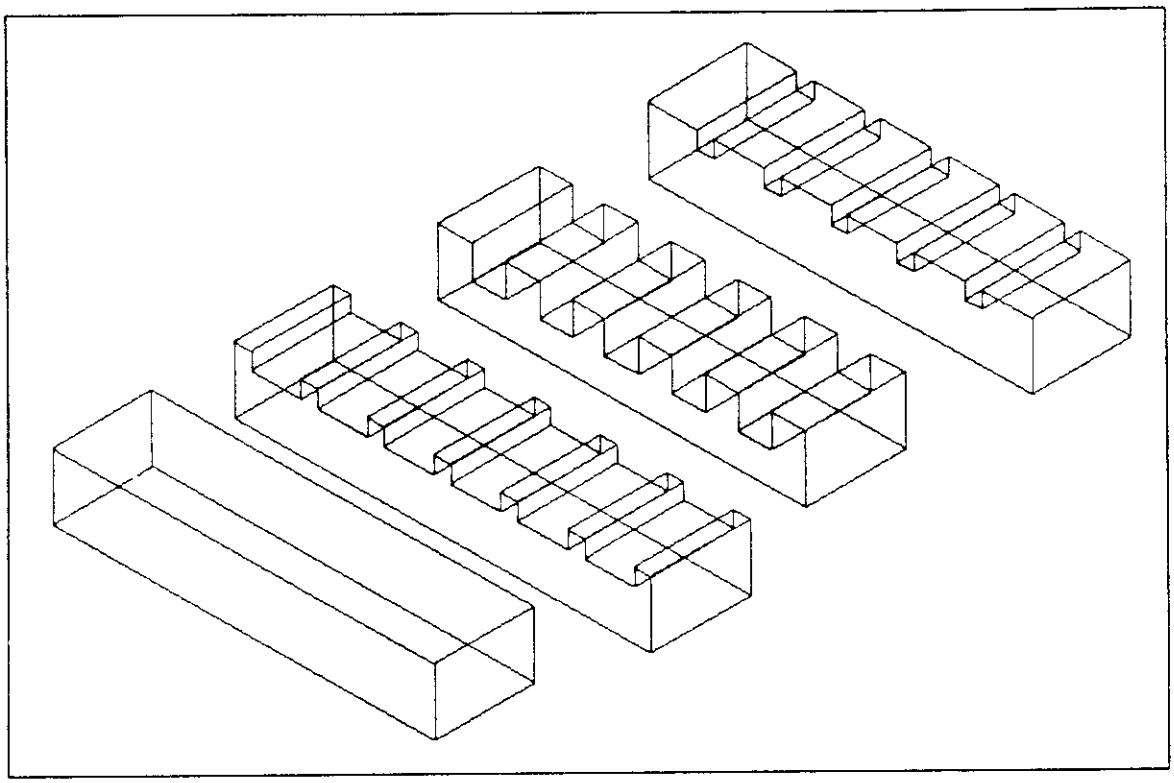

Figure 3. Configuration of textured and non-textured test plates

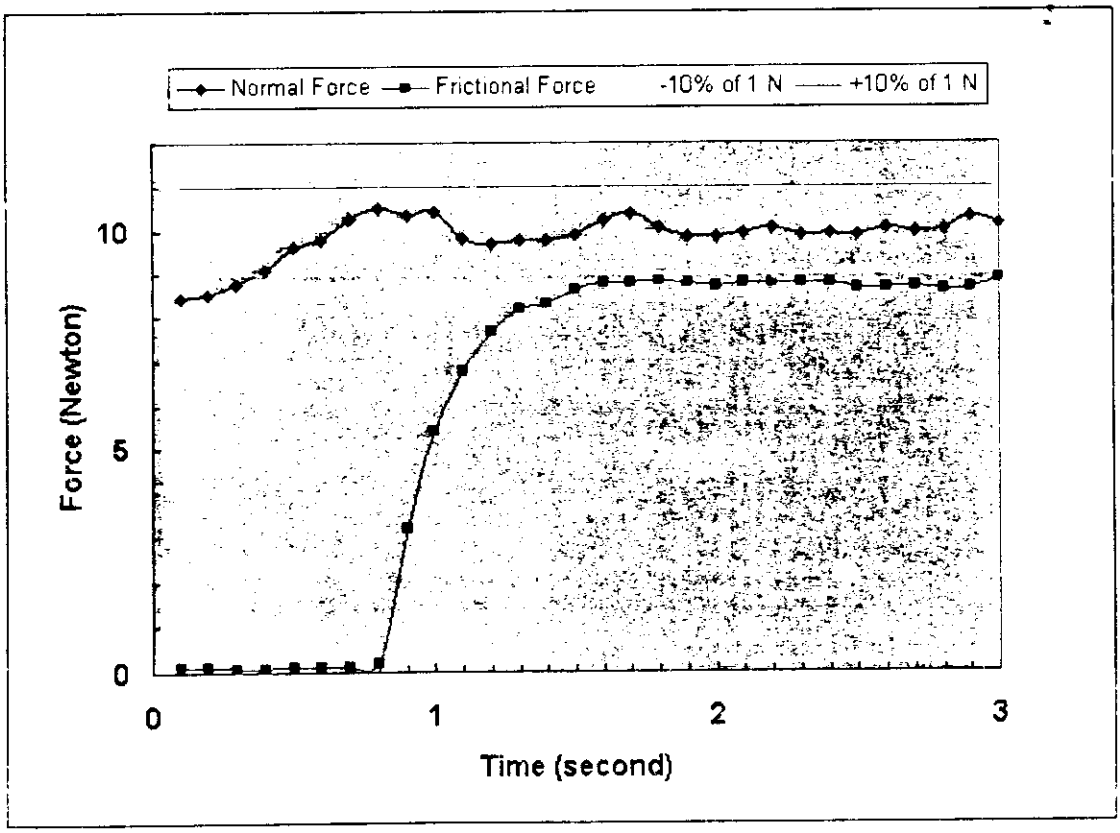

Figure 4. Sample of a typical measurement session

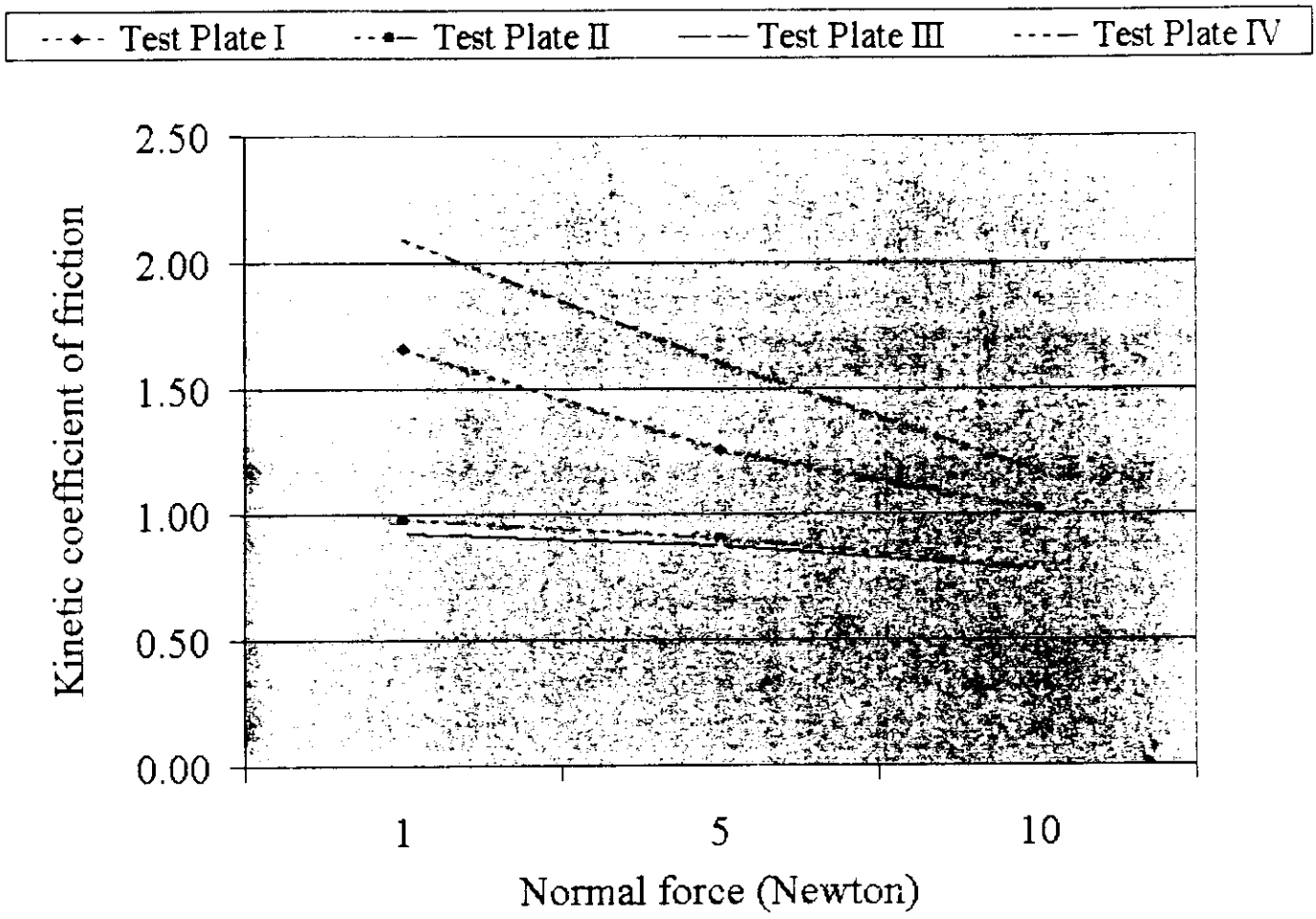

Figure 5. Overall mean kinetic coefficients of friction vs. texture and normal force

Table 1. Mean and standard deviation of kinetic coefficient of friction

Test Plate (% ridge area)	Normal Force (N)	Male Mean (Standard Deviation)	Female Mean (Standard Deviation)	Overall Mean (Standard Deviation)
Test plate I (75%)	1	1.61 (0.46)	1.70 (0.50)	1.66 (0.43)
	5	1.15 (0.40)	1.36 (0.12)	1.25 (0.29)
	10	1.03 (0.09)	1.01 (0.35)	1.02 (0.20)
Test plate II (50%)	1	0.96 (0.08)	1.00 (0.21)	0.98 (0.15)
	5	0.86 (0.02)	0.95 (0.20)	0.91 (0.14)
	10	0.81 (0.04)	0.77 (0.11)	0.79 (0.07)
Test plate III (25%)	1	0.82 (0.06)	1.04 (0.39)	0.93 (0.28)
	5	0.81 (0.05)	0.94 (0.20)	0.87 (0.15)
	10	0.73 (0.07)	0.82 (0.07)	0.78 (0.08)
Test plate IV (100%)	1	2.05 (0.89)	2.13 (0.58)	2.09 (0.67)
	5	1.25 (0.09)	1.97 (0.47)	1.61 (0.49)
	10	1.09 (0.12)	1.24 (0.08)	1.17 (0.12)

Table 2. Kinetic coefficients of friction: Present study vs. Bobjer et al. (1993)

Test Plate (% ridge area)	Normal Force (N)	Present Study Mean (Standard Deviation)	Bobjer et al. (1993) Mean (Standard Deviation)
I (75%)	1	1.66 (0.43)	1.44 (0.68)
	10	1.02 (0.20)	0.85 (0.19)
II (50%)	1	0.98 (0.15)	0.75 (0.30)
	10	0.79 (0.07)	0.74 (0.17)
III (25%)	1	0.93 (0.28)	0.79 (0.11)
	10	0.78 (0.08)	0.76 (0.03)
IV (100%)	1	2.09 (0.67)	2.22 (1.12)
	10	1.17 (0.12)	1.01 (0.35)

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A Real-Time Personal Heat Stress & Heat Strain Monitor in Protective Suit

Abstract

The goal of this study is to develop a real-time personal monitor capable of evaluating heat stress and heat strain encountered by workers wearing encapsulating protective clothing. This monitor simultaneously characterizes the climatic conditions of the microenvironment and the physiological responses of the worker in protective clothing. Specifically, the study (1) integrated temperature and humidity sensors to continuously characterize the microenvironment in a protective suit; (2) integrated heart rate sensors and body temperature sensors to characterize the physiological response of the person wearing a protective suit; and (3) tested the utility of two wireless transmitters, 0.9 GHz and 2.4 GHz, to transmit the heat stress and heat strain signals.

This study represents an innovative integration of information technology and sensor technology for hazard surveillance. In addition, this study has direct application to personal exposure surveillance of workers wearing protective suits and utility for the design of protective clothing.

Background

Heat stress is a well-recognized safety and health hazard especially for workers that have to wear protective clothing in their work (NIOSH, 1985, 1986). However, the exposure surveillance of heat stress for workers wearing protective suits is still limited to a general assessment of the marcoenvironment (Morris, 1995). An underestimate of the heat stress based on this general approach may result, and subsequently increase the worker's risk for head-induced illnesses and injuries. An overestimation of the heat stress and the resultant control measures may lead to a loss of productivity. It is more meaningful to evaluate the heat stress based on measurements taken in the microenvironment in the protective suit.

Workers may also differ in their bodily response, heat strain, to the heat stress. This difference in heat tolerance may be due to a variety of factors, such as acclimatization, age, and physical condition on a particular day. Identifying heat-intolerant individuals and providing real-time monitoring have been long recognized as important issues in need of research and development (NIOSH, 1986; Bernard and Kenney, 1994; Reneau and Bishop, 1996). Currently, there are at least three heat-strain monitors available commercially. The Questemp II (Quest Technologies, Inc.) measures only the tympanic membrane temperature, while the HS-3800 (Metrosonics, Inc.) measures the both heart rate and skin temperature. Another telemetric system (VitalSense by Mini-Miter Co., Inc.), capable of monitoring body temperature, heart rate, and activities, tends to be expensive and has not received wide acceptance in the field. These three monitoring instruments or systems may be useful for heat strain evaluation.

However, none of these commercially available personal monitors provides measurements of the microenvironment in the protective suit. Therefore, it is impossible to provide the data necessary for the establishment of a field-based relationship between heat stress and heat strain in the protective suit. There is a definite need for a real-time, personal monitor to evaluate heat stress-heat strain for worker in a protective suit.

It is the goal of this study to develop a real-time personal monitor capable of evaluating heat stress and heat strain encountered by workers wearing encapsulating protective clothing. The monitor consisting of 5 sensors and two transmitters will be a valuable tool for the purpose of exposure surveillance and biological monitoring. In addition, the monitor will allow the collection of field data necessary not only for addressing the research needs identified by the National Institute for Occupational Safety and Health (NIOSH, 1986) but also for potential design modification of protective suits.

The real-time personal monitor developed in this study is capable of evaluating heat stress and heat strain encountered by workers wearing encapsulating protective clothing. This monitor simultaneously characterizes the climatic conditions of the microenvironment and the physiological responses of the worker in the protective clothing.

Specifically, the study (1) integrated temperature and humidity sensors to continuously characterize the microenvironment in a protective suit; (2) integrate heart rate sensors and body temperature sensors to characterize the physiological response of the person wearing a protective suit; and (3) tested the utility of two wireless transmitters, 0.9 GHz and 2.4 GHz, to transmit the heat stress and heat strain signals.

Methods

As shown in figure 1, the conceptual design of the heat stress and heat strain monitor consists of two sets of sensors, transmitters and receivers. The first set of sensors and transmitters is for the measurement of physiological responses and consists of two thermistor probes (Sensor Scientific, Inc.), one for chest skin temperature and one for tympanic membrane temperature, and one heart rate monitor (Polar, Inc.). The signals from these three sensors are transmitted through transmitter #I to receiver #I.

The second set of sensors and transmitters is for heat stress surveillance based on the measurement of the climatic condition inside the protective suit. This set of sensors and transmitter is composed of one thermistor for temperature and one sensor for relative humidity (Vernier Software, Inc.) and a transmitter. Signals from both sensors are transmitted through transmitter #2 to receiver #2. Methods for real-time data collection depend on the type of wireless transmitters.

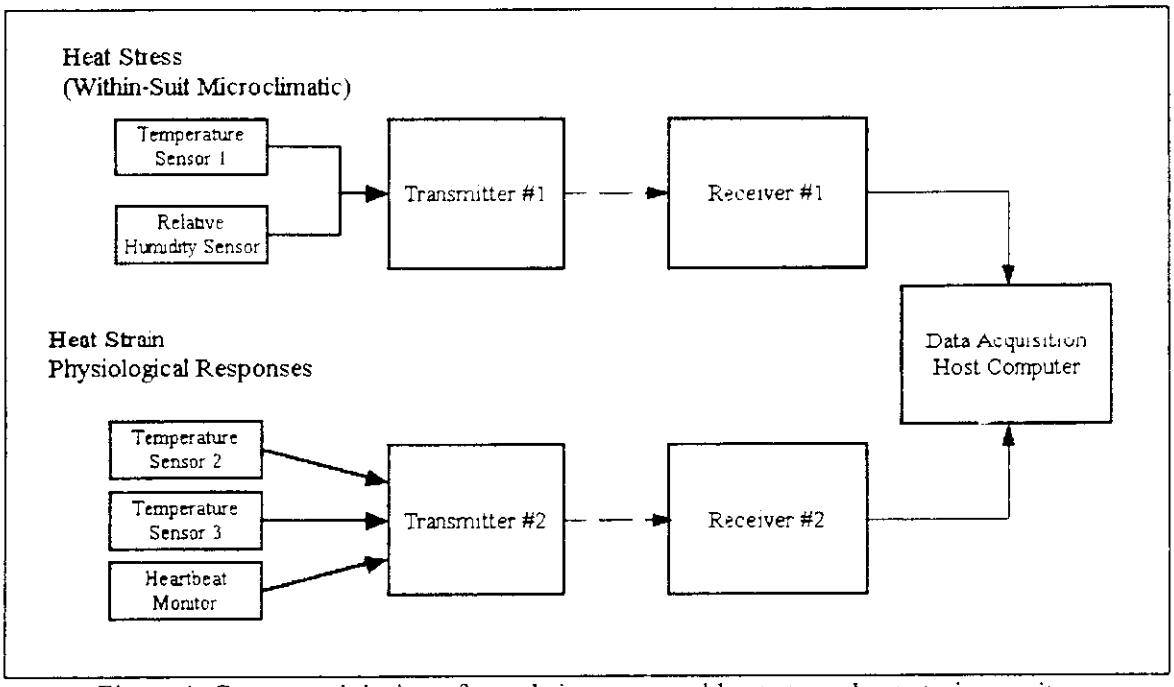

Figure 1. Conceptual design of a real-time personal heat stress-heat strain monitor

Temperature Measurement

Thermistor probes were used to measure the temperature of the air inside the protective suit and of the human body probe.

Humidity Measurement

Relative humidity (RH) was measured using a sensor purchased from the Vernier, Inc. As this RH sensor is sensitive to light and electrostatic discharge, the sensor was encased in a box specially built in the laboratory. The RH sensor responds to the humidity change by changing its output voltage with a range from 1.023 V to 3.821 V for 0% and 100% RH, respectively. The RH reading from the sensor was verified using a sealed chamber containing either desiccant or water to control the humidity inside at 0% and 100% RH. Another digital humidity sensor (Humidiguide 5566) used as a reference sensor was also placed in the chamber.

As the RH sensor has a response time of more than 60 minutes, it was necessary to fit the housing of the sensor with a miniature, 5-volt DC brushless fan to create sufficient air movements. The resultant housing for the RH sensor was of a dimension of 1.5" x 1.5" x 1.5". Using the 5V micro-fan in a non-transparent box, the response time came down to less than three minutes in the extreme situation. However, the response time was less than a minute for a typical environmental condition, i.e., from 100% RH to about 50% RH.

The 0.9 GHz transmitter has a maximum input value of 1.5 V and the RH sensor gives a maximum output signal of 3.82 V. Therefore, additional resistors, 2-M ohms and 1-M ohms, were placed between the RH sensor output and the 0.9 GHz transmitter to reduce the input signal to the 0.9 GHz transmitter. These resistors were selected to match the internal impedance of the

RH sensor. With this configuration, three levels of RH, i.e., 0%, 53% and 100%, were tested to establish a calibration line.

Heart Rate Measurement

The heart rate (HR) monitor (Polar, Inc.) is composed of two components, a transmitter belt and a receiver. The transmitter detects each heartbeat through two electrodes with ECG accuracy and transmits the heart rate information to the receiver with the help of a low frequency electromagnetic field. For each heartbeat detected, the HR monitor's receiver receives the transmission, and passes a 3V pulse. The reception range between the HR monitor's transmitter belt and its receiver should be less than 3 feet, as recommend by the manufacturer.
In this study, the receiver was placed by the forehead and was within 1-foot distance of the transmitter belt. The reading of the HR monitor was compared to manual counting of the pulse at the common carotid artery of the neck for a minute.

Transmitter Test

Two types of transmitters were tested in this study. One transmitter (MicroStrain, Inc.) transmits digital signals at a frequency of 0.9 GHz, and the other transmitter (RF-Link, Inc.) transmits analog signal at 2.4 GHz. The 0.9 GHz transmitter is powered by a 9-volt battery and is capable of transmitting five sets of digital signals. The 2.4 GHz transmitter is powered by a 12-volt battery and is capable of transmitting simultaneously two sets of analog signals with switching among four channels.

Both transmitters require receivers. The 0.9 GHz transmitter uses a receiver that transmits real-time digital signals through an RS-232 communication port to a personal computer. Transmission of the data is controlled by an MS-DOS (Microsoft Disk Operating System) software provided by the vendor.

The 2.4 GHz uses a receiver that transmits the analog signals and therefore requires an additional analog/digital data acquisition board for data collection. In the present study, the 2.4 GHz receiver was connected to a data acquisition system consisting of an A/D converter (DAS 1601, Keithley, Inc.) controlled by an application program written in-house with TestPoint software package (ADAC Corp.).

The sampling rate of the 0.9 GHz transmitter was set at 100 Hz, while the sampling rate of the 2.4 GHz transmitter is only limited by the A/D acquisition board and the number of channels (up to16 channels) used. A computer equipped with a Pentium 100-MHz processor was used for data collection.

Statistical Analysis

Correlation and regression analyses were conducted to evaluate the correlation between two sets of measures of a specific parameter, e.g., thermistor probe's resistance readings vs. temperature reading of the mercury thermometer, and to establish a calibration line. Analysis of variance was used to evaluate the differences between thermistors. All statistical analysis was conducted using a personal computer with statistical analysis procedure in the Microsoft Excel spreadsheet.

Results

Temperature Sensor

Temperature Sensor Using the 0.9 GHz transmitter

Figure 2 shows the thermistor probes' readings collected through the 0.9 GHz transmitter. The transmitted digital signal is represented as an averaged digital reading. The R-squared value of the regression line was greater than 0.999 ($p < .001$). The regression line for calibration was determined to be: Temperature (°F) = 0.002825 (Digital Reading) + 10.69617 to be: Temperature (°F) = 0.002825 (Digital Reading) + 10.69617

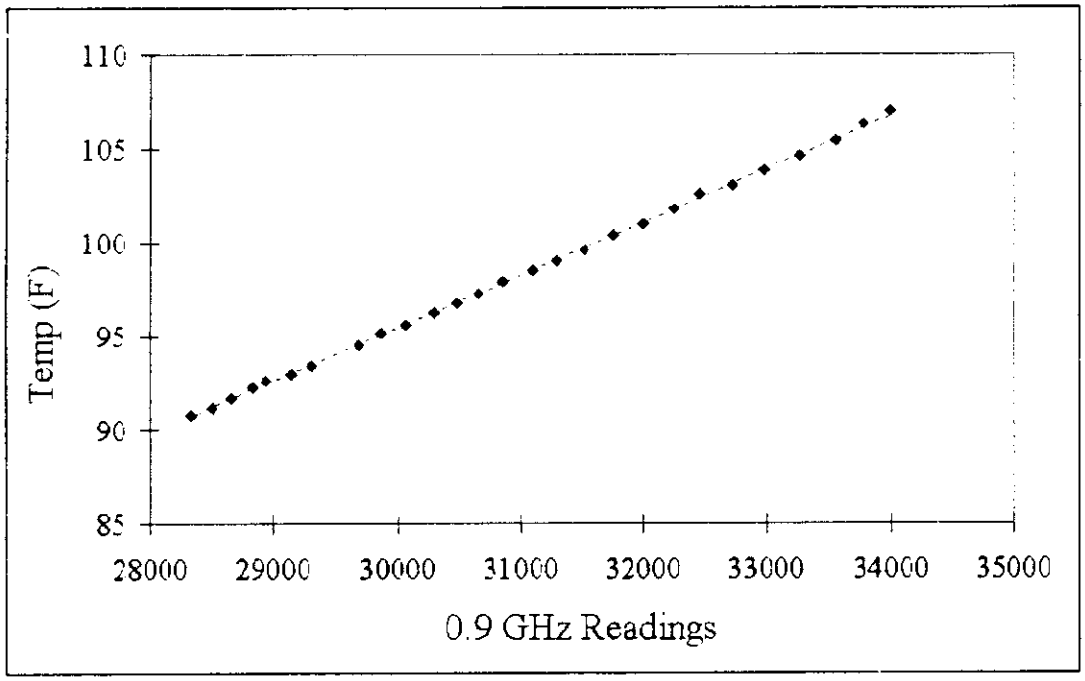

Figure 2. Thermistor probe temperature readings transmitted through a 0.9 GHz transmitter.

Multiple-Channel Temperature Measurement Using a Whetstone Bridge

Additional tests were conducted to verify the responses of three thermistors used in the present study. This was done to determine the variation among thermistors. Three thermistors were simultaneously inserted into a beaker full of water at temperature ranging from 100.2 °F to 104.3 °F. Consistent results were found with these three thermistor probes. There was no statistically significant difference among the thermistors.

Temperature Measurement of Multiple Thermistors Using the 0.9 GHz Transmitter

There were differences, not statistically significant, among the thermistor probes if the data were transmitted through a Whetstone bridge and the 0.9 GHz transmitter.

Relative Humidity Sensor

RH Measurement using the 0.9 GHz Transmitter

Figure 3 shows the RH calibration data. The R^2 value of the correlation and regression analysis was 0.998 ($p < 0.05$). The regression equation for RH is as follows:

Relative Humidity (%) = 0.00723 (0.9GHz Transmitter reading) - 276.7

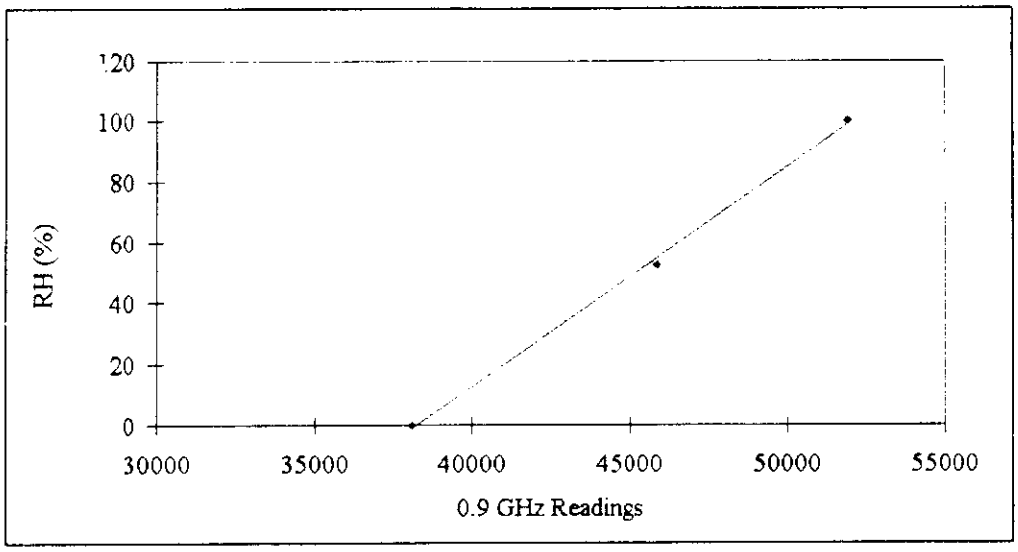

Figure 3. Relative humidity calibration with signals transmitted through a 0.9 GHz transmitter.

RH Measurement Using 2.4 GHz Transmitter

The 2.4 GHz transmitter in its current configuration can only takes AC peak-to-peak signal of 1 V. Since the RH sensor output signal is a DC signal varying between 1.2 V to 3.8 VDC, it can not be used with the 2.4 GHz transmitter.

Heart Rate Monitor

Heart Rate Measurement Using Analog/Digital Converter

Figure 4 shows the heart rate data collected with the HR monitor using an A/D converter and that obtained with manual pulse counting. The heart rate measured ranged from 76 beats/min to 123 beats/min. The correlation between the two was, an R^2 value of 0.999 ($p < .001$).

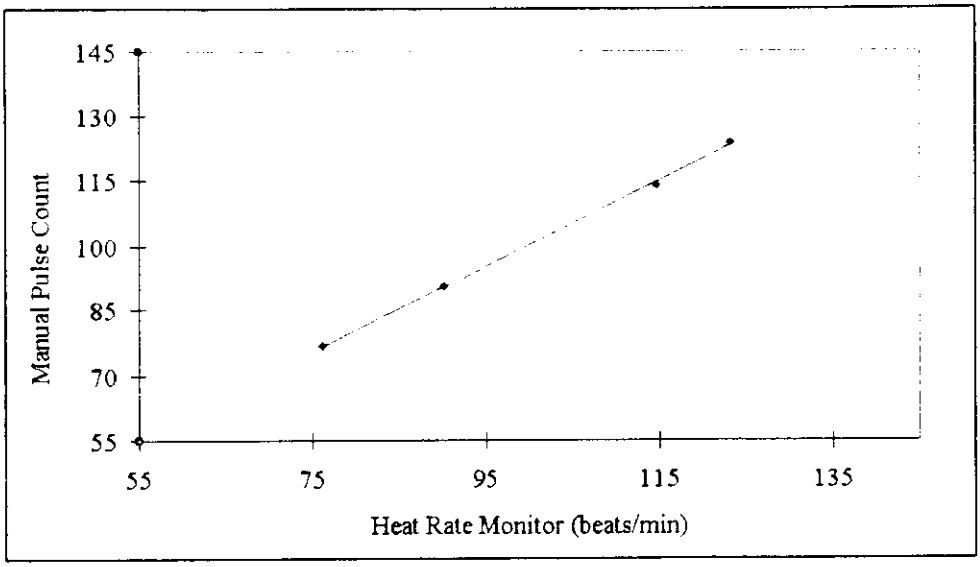

Figure 4. Manual pulse count vs. heartbeat monitor count

Heart Rate Measurement Using the 0.9 GHz Transmitter

The results of the study indicate that the HR monitor signal can be used directly with 0.9 GHz transmitter. Figure 5 shows a sample of the data collected and the correlation coefficient was greater than 0.99.

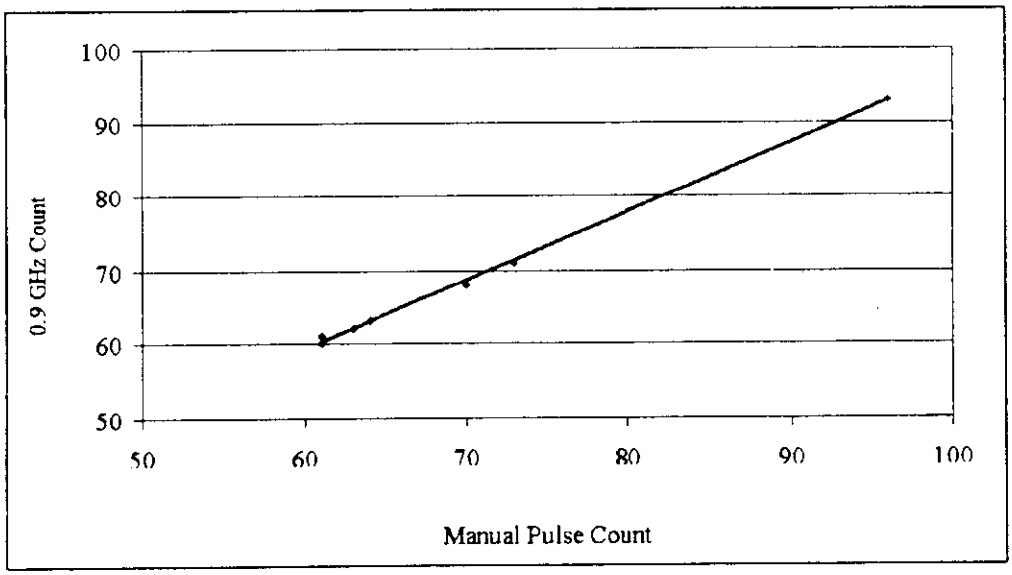

Figure 5. Manual heartbeat count vs. heartbeat monitor count transmitted through a 0.9 GHz transmitter.

Heart Rate Measurement Using 2.4 GHz Transmitter

Heart rate ranging from 62 to 120 beats/minute was tested with the heart rate monitor. Compared to the pulse sensed with fingers, occasionally there were only one or two miscounts, as shown in Figure 6.

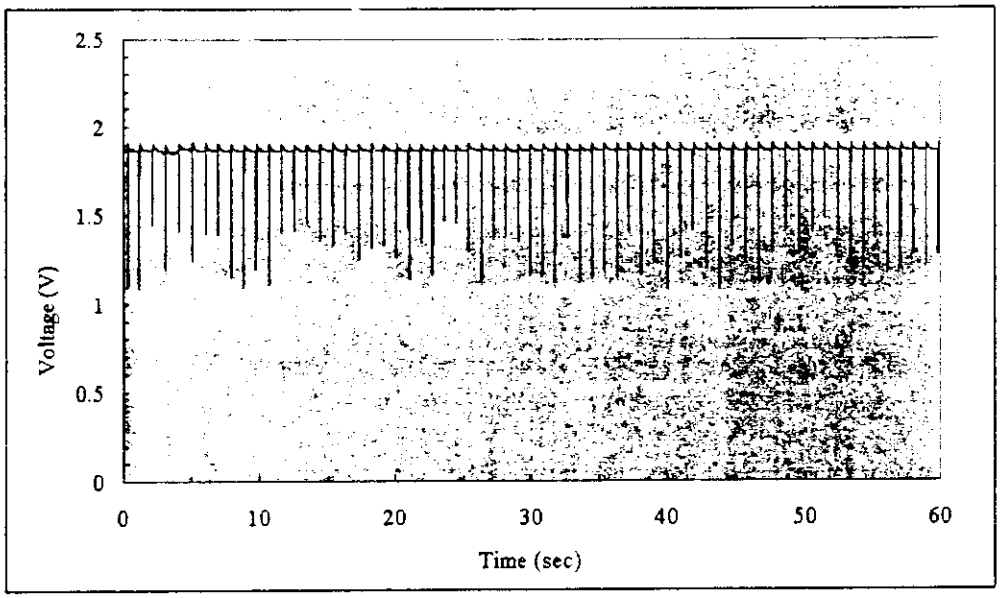

Figure 6. Heartbeat signals received from a 2.4 GHz transmitter.

Discussion

This study evaluated the feasibility of integrating thermistor temperature probes, a relative humidity sensor, and a heart rate monitor, with wireless transmitters for the surveillance of both heat stress and heat strain. The results showed that while some of the sensors, such as the thermistor temperature probes, functioned satisfactorily and could be used without modification for the surveillance of heat stress and heat strain, others required additional modification for the purpose of hazard surveillance.

For example, the thermistor probes used in the present study were calibrated in the laboratory between 32 °C and 42 °C. High correlation, $R^2 > 0.99$, was found between the temperature measured with a mercury thermometer and the derived temperature reading based on the resistance change of the thermistor. Inter-day and inter-thermistor variability has also been evaluated and found to be minimal. The results also showed that heartbeat monitor also performed as expected. Each heartbeat at a rate from 60 beats/minute to 120 beats/minutes was clearly discernible during laboratory tests.

As for relative humidity measurement, the sensor needs modification to shorten the response time. The response time of the sensor in its original design is too long. However, the response time of the sensor was reduced to less than a minute by adding a small, brushless fan for increased air circulation. The modified sensor-fan design performed satisfactorily in the controlled laboratory settings. Calibration line at 20 °C for three relative humidity levels, $R^2 >$ [illegible], was established. Concordance with another digital humidity sensor gave satisfactory results.

The test results of two wireless transmitters, operating at 0.9 GHz and 2.4 GHz, respectively, showed that each transmitter has its advantages and disadvantages. While the 0.9 GHz transmitter can easily transmit signal from the thermistor probes and heartbeat monitor, it can not take the signal directly from the humidity sensor. As for the 2.4 GHz, while it can transmit the heartbeat signals in its current configuration, it does need an additional transducer to transmit the signal from the relative humidity sensor. In this study, we have built a simple oscillator circuit that allows the transmission of relative humidity sensor's signal.

Based on the results of the present study, the design of the real-time personal monitor for heat stress and heat strain would consist of three thermistor temperature probes, a relative humidity sensor, a heart rate monitor, one 0.9 GHz and one 2.4 GHz wireless transmitters. The 0.9 GHz transmitter will be used to transmit signals from the three thermistor probes. One thermistor probe will be used for air temperature and the other two thermistor probes will be used for body temperature, measured either at skin surface of different location or at the mouth or in the middle ear.

More tests and developmental work should be conducted to optimize the design of the real-time personal heat stress - heat strain monitor. One consideration is the cost. While the 2.4 GHz transmitter costs less than $200 a pair, the 0.9 GHz transmitter and receiver set costs more than $1,500. To reduce the cost of the real-time heat stress - heat strain monitor, it is preferable to use the 2.4 GHz transmitter. However, it would require the conversion of DC signals from the temperature sensor to AC signal and a remote, automated channel switch. The is due to the fact that the 2.4 GHz transmitter/receiver in its current package only accepts AC signals and allows the transmission of two sets of signals on one channel at a time, despite of the four channels available. Our laboratory is working on building an inverting amplifier so that all sensors could be connected directly to the 2.4 GHz transmitter and receiver.

Conclusion

This study demonstrated the feasibility of integrating sensor technology and wireless transmission technology for the heat stress and heat strain surveillance in the defense / nuclear industry. This real-time heat stress - heat strain monitor is not only useful as a research tool to elucidate the relationship between heat stress (within-suit microclimate) and heat strain but also of great utility for exposure surveillance in the field.

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5. Evaluation of current exposure and medical surveillance programs at Los Alamos and Lawrence Livermore National Laboratories

To evaluate the medical and exposure surveillance programs at LANL, identify discrepancies between health and safety "needs" and established monitoring programs, and develop an integrated surveillance system that efficiently combines hazardous-exposure, biological, and health-outcome monitoring of the worker population.

Abstract
We assessed whether medical and industrial hygiene exposure monitoring information routinely collected at nuclear facilities such as the Los Alamos National Laboratory (LANL) are useful screening tools to help predict long-term health outcomes and would allow to conduct epidemiologic research to assess health effects in current and former nuclear workers from exposure to mixtures of chemicals and radiation.

First, we found that medical records did not systematically collect job location, job history, and job title information. A pilot study of machinists revealed that no other records existed or could be made available to us that would have allowed us to link medical test results to information from industrial hygiene area sampling data and/or other exposure-related data bases maintained by the Environmental Health and Safety department.

Second, we linked three large LANL databases: 1) noise sampling data; 2) hearing conservation data, and 3) audiometric testing data in order to examine the effectiveness of the hearing conservation program (HCP). We found that due to the inherent lack of worker location and job history data it was impossible to assign available noise level measurements for work areas to individual workers. We analyzed data for a small group of workers for whom individual noise measurements existed and saw a clear relationship with duration of exposure, noise levels, and 'intermittent' noise. If these databases were maintained in a manner that would allow to link outcome (audiometry) data for workers to area noise measurements, they would be of great use for future and continued evaluations of the HCP and hearing loss prevention measures.

Finally, we used data collected by the occupational physician of the Fernald nuclear facility and documented in CEDR to evaluate the effect of combined exposure to chemicals and radiation on cancer mortality in nuclear workers. We combined an extensive chemical exposure data set with mortality files and radiation data provided in CEDR and investigated whether and to what extent the different and multiple chemical exposures contributed to the mortality experience of Fernald

workers. The results of these analyses have been documented in two publications attached to this report.

Introduction

The goal of the epidemiologic component of this 'hazard surveillance in the nuclear industry' project was to assess whether medical information routinely collected for medical surveillance and exposure information collected during industrial hygiene exposure monitoring at nuclear facilities such as the Los Alamos National Laboratory (LANL) could be used to:

(1) assess whether routinely collected medical data allows us to predict long-term health outcomes, i.e. can be used as a screening tool;
(2) evaluate the effectiveness of medical surveillance programs with regard to prevention of chronic occupational diseases;
(3) whether data from both medical and industrial hygiene surveillance could be used to aid epidemiologic research and help us assess long-term health effects in current and former nuclear workers due to exposure to mixtures of chemicals and radiation.

Dr. Ritz and her graduate students visited LANL several times during the course of this project and established collaboration with members of the medical group, specifically with Drs. Williams, Smith, and Wiggs. The main goal of these visits was to gain an overview over past and current medical surveillance strategies employed at the laboratory, to identify information that would allow us to assess LANL's medical surveillance needs, and to determine how epidemiologic methods could be employed to evaluate program effectiveness and gaps. Specifically, we expected that epidemiologic tools should be able to help improve the effectiveness of medical surveillance and aid efforts to prevent chronic diseases of occupational origin. Originally, we had proposed to target the following diseases: cancers, musculo-skeletal diseases, and neurologic/ neuropsychiatric disorders. However, in the course of the project it became necessary to shift our focus to the outcome 'hearing loss', a chronic sensori-neuronal disorder. The reason was that no data was available at LANL that would have allowed us to collect information about the occurrence of the former diseases/disorders among LANL workers in a systematic manner i.e. beyond collecting singular case reports (see also our pilot project results below).

In order to evaluated whether medical data routinely collected at the LANL facility might be useful for the detection of early symptoms of occupationally related diseases and, thus, be potentially useful as screening devices, workers needed to be systematically followed to assess longer-term health outcomes. Problems preventing us from conducting long-term follow-up of employees at LANL - even within small defined subsets of workers - are described exemplary in our pilot case study of a cluster of machinists diagnosed with elevated liver-enzymes in the late 1980s (see below). In addition, since LANL medical records did not systematically collect job location, job history, and job title information we were unable to link medical test results to information from industrial hygiene area sampling data and/or other exposure-related databases maintained by the Environmental Health and Safety (EHS-2) department. We were also unable to find a database providing worker location information that would have allowed us to link medical records to industrial hygiene area sampling for chemicals.

Thus, after completion of the LANL machinist pilot project (see below) we decided to proceed with the following two projects to achieve goals 2) and 3) formulated above. We:

(1) evaluated the effectiveness of the hearing loss prevention program at LANL, since for this chronic sensori-neuronal disorder we were able to obtain systematic outcome information for LANL workers;
(2) used data collected and documented by the occupational physician of the Fernald nuclear facility to evaluated the effect of combined exposure to chemicals and radiation on cancer mortality in nuclear workers.

In the following we will first describe and make some recommendations concerning the LANL medical and industrial hygiene surveillance programs. Second, we will report the results of our machinist pilot project, followed by a brief report of results from our Fernald nuclear worker mortality study (publications are included into this report). Last, we will present our evaluation of the LANL hearing loss prevention program.

Medical Surveillance at LANL

The medical surveillance at the LANL focuses on surveillance required by regulations and certification programs such as human reliability tests required for firefighters and for workers using respirator equipment as well as 'fitness for duty' exams. LANL-UC employees are invited to medical examinations in 1-, 2-, and 4-year intervals, depending on age and membership in 62 surveillance or certification categories (see attached list 1, attachment). Depending on which surveillance category a worker qualifies for, exams include any of 26 routine medical tests (see list 2, attachment). The only chemical for which biological monitoring data is routinely collected is blood lead.

The medical staff performs between 5,000 and 6,000 exams annually. Every LANL employee is invited for new-hire, work-termination, and possibly some periodic exams. While the new-hire exam is required by laboratory policy, termination exams are required only for a subgroup of employees, and periodic exams can only be offered when the medical department has the capacity to perform medical exams apart from those required. Employees are free to deny the offer of non-required exams. Members of the medical department felt that the acceptance rate for periodic exams decreased due an increased participation of the occupational medical facility in drug- and alcohol-screening programs which created some reluctance to accept non-required medical benefits.

Employees who return from sick leave after more than five days are required to be examined by occupational medicine staff before they are allowed to return to work. Shorter absences are not documented in the files of the occupational medicine department. Medical histories and test results are recorded in a standardized manner and retained almost exclusively in hardcopy format in employee's files. For some medical test procedures, such as audiometry tests, computerized data are available.

Furthermore, the medical department also offers human reliability exams and drug testing and new-hire and termination exams to employees of the two main subcontractors at LANL (1500 craft employees and about 500 professionals). An arrangement has been made to report all on-

the-job injuries and illnesses of subcontractor employees to the occupational medicine department as well.

Recommendations
We strongly recommend that all medical test results are stored with a personal identifier and are available in computerized format.

Industrial Hygiene Surveillance at LANL
It is the task of the industrial hygiene/health and safety group at LANL to identify and refer employees for inclusion in any of the medical surveillance programs. The UCLA team learned that in the early 1990s LANL began to integrate the medical and industrial-hygiene systems. Some of these efforts were required by the Laboratory's UC contract as well as by DOE-orders (DOE 5480.8A, 5480.10, 5480.4, 5483.1A) and enhanced by a peer-review process involving all three UC Laboratories (Berkeley, Lawrence Livermore, LANL). They were initiated in 1993 and focused on a medical and industrial hygiene interface to integrate data across the two systems, i.e., across the medical and exposure surveillance databases.

The industrial hygiene group at LANL created databases to be integrated into a "health-hazard-assessment" (HHA) system. The HHA system is based on operation code data, an assessment of the types of chemicals used, the expected dose (which might depend on vapor pressure and particle size etc.), the duration and frequency of exposure, toxicity, and an evaluation of whether protective control measures are non-existent, effective or non-effective. Also, different from other systems previously used this system systematically collects personal identifiers for all workers involved in operations and processes evaluated. A final score based on all of the collected IH-data guides the development or improvement of protective controls and/or medical surveillance activities.

The content of the HHA system is based on general criteria previously established by IH to evaluate work place hazards. Nevertheless, previously field notes have been kept as individual documents. Since they were not sufficiently standardized, they provided only a fragmented view of hazards in the workplace. Furthermore, these documents were not available to medical-care providers and, thus, could not be used as a guide to potential health hazards encountered by employees in the work place.

The main databases (first established in 1993) that contributing to health hazard assessment at LANL are:

- An automated chemical inventory system (all chemical substances for which Material Safety Data Sheets (MSDS) exist). This database is linked to procurement and allows the industrial hygienist to know which chemicals are bought and used at the facility. A 1991 baseline inventory of chemicals is regularly beingupdated. The system is further fed by annual reconciliation updates that tracks movement of containers once each year and chemical disposal records. This system tracks about 250,000 containers (95% of all chemical containers at the facility), of which 170,000 are in active use.
- A second database contains information on carcinogens only. This database identifies individual employees who use each carcinogen and the processes in which they are used. The

list of chemicals is periodically reviewed and changed according to changes in the use of chemicals at the facility.

- An on-line database of MSDS information obtained from a commercial vendor.
- An operation code database that identifies tasks and processes with a hazard component and a list of employees involved in each task or process. This database relies on industrial hygiene-field work and facility walk through to identify the necessary information. This database will include exposure information about personnel hired by subcontractors as well.
- OSHA 200 log information on workplace injuries and illness (excluding detailed medical information).
- An occupational exposures sampling databases containing information on location, substance, test result, and possibly personal identifiers of the sampled employees.

Recommendations

This computerized HHA system when available to medical-care providers will aid medical surveillance efforts at LANL by providing an instant guide to potential health hazards in the work place. If the information is not only available for active employees, but stored it could aid epidemiologic exposure assessment efforts in an unprecedented way by providing personal exposure information for workers over long periods of time. Unfortunately, the system was not yet functional when we conducted this hazard surveillance project and thus could not be evaluated.

Pilot project: a case study of machinists at LANL

The limited budget available for the epidemiologic evaluation of medical surveillance as part of this hazard surveillance project precluded extended data-collection efforts at the facility. Thus, during our first visits to the LANL facility, we decided that it was necessary to conduct a pilot epidemiologic evaluation project that would allow us to identify all data resources available – possibly in computerized format - to track past and current workers at risk of chronic health problems, and to develop a plan for evaluating the success of established routine medical-surveillance procedures.

At one of the first meetings with the medical staff at LANL, we identified two areas that needed attention: 1) potential chronic neurotoxic effects due to solvent use in machining operations; and 2) potential musculoskeletal disorders caused by the insufficient ergonomic design of glove boxes used at the facility. We agreed to concentrate on the medical and epidemiologic surveillance of neurotoxic effects, since Dr. Liu (UCLA) was responsible for the ergonomic analysis of glove-box work as part of this grant.

The question of long-term neurotoxic effects from machining operations was raised first when a former machinist was diagnosed with an organic brain syndrome. The occupational physician who examined this worker recalled the case of another machinist who several years prior to this event had complained of chronic sleep disturbances. Furthermore, the physician recalled that a group of machinists showed elevated values for several liver enzymes during routine medical exams in the late 1980's. Some machinists who had tested positive were re-tested for liver enzymes elevations 6 weeks after the first positive result, and others were also examined by an outside gastro-enterologist. However, the occupational medicine department was unable to conduct a systematic investigation into the causes of this cluster of workers with abnormal liver

function because at that time it occurred the machining department was undergoing major changes and many machinists were transferred to other departments. Three cases of chemically related hepatotoxicity were diagnosed in this group.

The increased liver enzymes may have been precursors of chronic neurotoxic effects induced by solvent use. In addition, occupational medicine noted that most of the older machinists were wearing hearing aids, although they had been included in a hearing-conservation program for more than a decade. The hearing loss experienced by machinists might be influenced by neurotoxic changes in addition to excess noise encountered in the work place. Thus, after identifying machinists who were employed in the mid- to late 1980's at LANL, we intended to analyze audiometric data available for these employees. Specifically we wanted to determine whether a pattern of chemically related hearing loss existed among machinists, pure noise-related hearing impairment should show a pattern of loss for higher frequencies first. Also, it was of interest whether there was an association between hearing loss and reported increases in liver enzymes and between hearing loss and certain job locations or solvent use. Furthermore, we intended to invite all former and current members of the machining department to take part in neuropsychological testing at LANL. All test results taken together, could have helped us to identify whether machinists at LANL have unusually high rates of cognitive abnormalities and whether the prevalence of these abnormalities are related to hearing loss pattern, liver function, and solvent use.

Yet, in order to test this hypothesis we needed to identify all members of the former machining department in which this cluster occurred. Unfortunately, this task was less than straightforward. A major problem was that work locations for LANL employees were not available in any routine records system maintained by LANL. Location codes on personnel records are used for administrative purposes and do not represent the work place in most cases. Furthermore, job titles are often non-descriptive of the actual work performed by the individual; i.e., a machinist might be called a technical staff member in the personnel files. The computerized files of the LANL epidemiologic group -- containing adequate job title information and radiation exposure data -- were not updated after 1977 due to funding constraints. In the absence of a better alternative, we queried this database and were able to identify about 1000 machinists according to job titles recorded at first or last employment at LANL. Out of these machinists, however, only 59 were found to be still actively employed at LANL (mean length of employment 24.8 years).

The current personnel filing system contains computerized records starting in 1991 and identified only machinists active in 1991 and still active as of the time of our query (1996). This record system identified 184 additional machinists, who had been first employed after 1979. Of these, 118 were still actively employed as of 1997 (mean employment duration of 14.1 years). Specifically, we don't know how many machinists we missed due to the gaps in the personnel data system at LANL. We encountered large gaps for the period 1978-1990 and potential further for 1991-1997, which could only be filled if it was possible to find and computerizing archived paper records. Yet, our efforts to retrieve archived data have proven very frustrating and inefficient. Someone would have to look through hardcopy, scanned, and/or microfiched records or/or a computer consultant would be required to bring some old electronic data on-line and do searches to find this information, this effort was outside the scope of our budget

Trying to restrict ourselves to the data collected until 1977, we evaluated the potential for contacting former employees by checking the local phone book for matching names. We were able to find matching individuals for about 12% of the 80 names checked. Again, to validly assemble a cohort or nested case-control group of current and mostly former machinists we would need to use an expensive state or national tracing system not feasible within the funding limits of this project.

Significant Findings: Pilot Study of Machinists

We have to conclude that an epidemiologic evaluation of medical surveillance at LANL is impossible without an extensive and expensive record retrieval and abstraction effort. Any study of work-related factors associated with chronic disease outcomes will encounter this problem. From this pilot project we concluded that currently it is impossible to identify groups of workers exposed to chemical agents in a systematic way from routine records kept at the LANL.

Usefulness of Findings: Pilot Study of Machinists

If these databases were maintained in a manner that would allow to link outcome data for workers to exposure measurements, they would be of great use for future worker health studies.

Cancer mortality from chemicals and radiation in Fernald uranium workers

Two data sets provided by the Comprehensive Epidemiology Data Resource (CEDR) referring to the same uranium processing workers employed at the Fernald facility allowed us to evaluate the contribution of chemical mixtures and radiation exposures to cancer mortality in a nuclear worker cohort. Dr. Jerome Wilson, the occupational physician responsible for this work force in the end 1970 and 1980s, conducted a study of respiratory morbidity at the Fernald facility for which he collected information on a number of chemicals such as solvents, kerosene, and cutting fluids used between 1950 and 1983. Facility industrial hygienists rated each job title and location and created a two or three category score for level of exposure to each of the chemicals in use. In addition, the length and timing of employment in a job and location has been recorded and was used to create duration measures of exposure and lag exposure. We combined this extensive chemical data set with the mortality files and the radiation data provided in CEDR and investigated whether and to what extent the different and multiple chemical exposures contributed to the mortality experience of Fernald workers. The results of these analyses have been documented in two recent publications attached to this report (Ritz 1999 a and b). (Appendix C)

Significant Findings:

Results indicated that Fernald workers exposed to ionizing radiation experienced an increase in mortality from total cancer (per 100 mSv external dose rate ratio (RR) = 1.92; 95% confidence interval (CI) 1.11, 3.32), radiosensitive solid cancer (RR = 2.00; 95% CI 1.02, 3.94), and lung cancer (RR=2.77; 95% CI 1.29, 5.95). Effects were strongest when exposure had occurred at older ages (>40 years). In addition, we observed an increase in lung-cancer mortality for workers exposed to >200 mSv of internal (alpha-) radiation (RR=1.92; 95% CI 0.53, 6.96).

Our results furthermore suggest that workers who were exposed to TCE experienced an increase in mortality from cancers of the liver. Cutting fluid exposure was found to be strongly associated with laryngeal cancers and, furthermore, with brain, hemato- and

lymphopoietic system, and bladder and kidney cancer mortality. Finally, kerosene exposure increased the rate of death from several digestive tract cancers (esophageal, stomach, pancreatic, colon and rectum cancers) and from prostate cancer. Effect estimates for these cancers increased with duration and level of exposure and were stronger when exposure was lagged.

Usefulness of Findings:
Our results demonstrate the importance of a long follow-up time when studying solid cancers, the potential for bias due to worker selection associated with concomitant chemical exposures, problems of exposure measurement, confounding, and effect modification due to age at exposure and the limits of pooled analysis of uranium-processing workers that can only partially address these issues.

Evaluation of the Hearing Conservation Program at LANL

Introduction
Noise-induced occupational hearing loss continues to be one of the most frequent work-related diseases [1,2] and a clear link exists between noise exposure and hearing loss. [1,3,4] Occupational noise-induced hearing loss is a slowly developing sensorineural loss of hearing over a long period of time, usually ten or more years, as the result of exposure to continuous or intermittent loud noise. [5] It is almost always bilateral and the earliest damage occurs at the range of 3-6 kHz. This injury is irreversible and cannot be medically repaired. Hearing loss maybe exacerbated by synergistic effects of other exposures, such as vibrations or organic solvents. [3,5] In order to protect employees from this potential hazard, OSHA requires that employers shall administer a continuing, effective Hearing Conservation Program (HCP) whenever worker noise exposure exceeds the 8-hour time weighted average sound level of 85 dB measured on the A scale, or, equivalently, a dose of fifty percent. [6]

The goal of our project was to assess the effectiveness of the Hearing Conservation Program (HPC) implemented by the Los Alamos National Laboratories (LANL) and examine whether data stored at the facility would allow us to examine the contributions of noise and organic solvent exposure to hearing loss. A large quantity of audiometric data has accumulated in the LANL archives, but the potential risk of occupational noise or solvent induced hearing loss in the LANL worker population has never been systematically assessed nor has the effectiveness of the hearing loss prevention strategies implemented throughout the last decades ever been formally evaluated. The purpose of our research was to provide such an evaluation relying on data available at the facility in computerized and/or archived format. We received access to three databases: 1) noise sampling data; 2) hearing conservation data, and 3) audiometric testing data. A description of the complete LANL Hearing Conservation Program and the content of each of these databases can be found in Appendix 1 of this report.

We combined these data bases and examined (1) whether and to what extent it is possible to link case and non-case records to work locations and actual noise measurement performed by ESH-5 (Environmental Health and Safety) and (2) whether and which work-related risk factors for

hearing loss can be examined epidemiologically after linking information from medical and industrial hygiene records.

Description of the LANL Hearing Conservation Program

The Hearing Conservation Program (HCP) has been designed to protect employees against hearing loss. LANL utilizes Air Force Regulation (AFR) 161-35 as recommended by DOE-Albuquerque [7]. Industrial Hygiene and Medical departments developed the LANL- HCP based on AFR 161-35 and Occupational Safety and Health Act (OSHA) 29 CFR 1910.95, Occupational Noise Exposure. Impact/impulse noise is being controlled based upon the threshold limit values of the American Conference of Governmental Industrial Hygienists.

The HCP applies to all LANL employees who are exposed to noise levels at or above fifty percent of the Occupational Exposure Limit (OEL), that is at 85 dB or more of A-weighted sound pressure level for eight hours in any 24-hour period or an equivalent exposure at higher levels for shorter times according to AFR 161-35. Employees, supervisors or health and safety personnel may request to enroll an employee in the HCP. Machine shop employees are automatically entered into the HCP regardless of the noise exposure. Supervisors, employees or health and safety personnel may request to remove an employee from the HCP. Usually, the reasons for removal are a change into a job without noise exposure or retirement. According to the LANL-HCP description, an employee is removed from the HCP whenever noise exposures above the action level no longer occur (action level is an 8-hour time weighted average of 80 dBA or a noise dose of 50% of the permissible OEL).

The purpose of the Hearing Conservation Program is to identify and characterize high noise areas, conduct measurements, and implement controls to reduce employee exposures to workplace noise. The HCP components include:

- A monitoring program designed to identify potentially high noise levels in the workplace
- Identification and notification of employees routinely exposed to hazardous noise
- Recommendation of engineering controls to reduce workplace noise and hearing protection for employees in potentially high noise areas
- Employee training (initial and annual)
- Audiometric testing and physician review
- Evaluation, investigation and reporting of suspected noise-induced hearing loss

LANL HCP includes all components recommended by NIOSH, except one. There is no program effectiveness evaluation component included in the LANL HCP.

Noise monitoring program

Description

Noise exposure monitoring is conducted via three noise surveys.

1. Walk around survey. A general survey is conducted to determine the locations and boundaries of hazardous noise areas. It is usually done with a Type 2 sound level meter and the results are used to plan a work shift sampling strategy.

2. Noise control survey. In this survey, Type 1 sound level meters are used with an octave band analyzer to obtain data that might to aid in selecting noise control methods.
3. Work shift sampling. This survey utilizes sample employees representative of each area in which over-exposure to noise may occur. Personal dosimeters are used to assess individual noise exposure.

Walk around and noise control surveys are considered area measurements and work shift sampling procedures are considered personal dosimetry measurements.

According to LANL HCP description, the records of noise measurement are being kept for a period consistent with DOE Order 1324.2A and will include as a minimum:

- Number, type and location of noise sources
- Number and identification of personnel in the work area and their daily exposure and duration (dosimetry is preferred)
- Type, model, ESH-5 number of test equipment and calibration data
- Location, date and time of noise measurement
- Noise levels measured and hazard radius
- Name of the person who made the survey

Concerns for HCP evaluation
We found that record keeping over the last decades was inconsistent leading to missing information. Approximately 75% of all records in the noise-sampling database (see description below) are missing actual noise measurement at each of the frequencies (0.125-8kHz). Sixty six percent of records are missing a verbal description of the exposure. HCP worker population is heterogeneous with regards to noise exposure, with some workers being exposed only to continuos noise, others to intermittent or impulse. Reportedly, some shop workers have "gray area" exposure, and may not qualify for enrollment into the HCP, yet may be exposed to occasional noise exceeding OEL. In addition, frequently workers are added to the HCP by the request of line management before the area can be evaluated. For example, a group might decide they need a small local carpentry shop. They organize the shop and then indicate which people are going to use the shop and request these employees to be put into the HCP. Thus, workers are placed and taken out of HCP continuously, sometimes without proper exposure identification.

Engineering controls and personal hearing protection

Description
Engineering controls are the first step at LANL in controlling excess noise exposure. These include isolating high noise machinery into separate rooms with audio isolation. If a particular location experiences noise level of 90dBA or above, a noise hazard warning sign is posted at the entrance. Administrative controls include worker shift rotations.

Hearing protection must be provided by line management for workers exposed to noise levels at or above action level. Hearing protection must be worn by employees exposed to noise levels at or above OEL. Employees may select their hearing protection devices from among several types which provide adequate protection.

Concerns for HCP evaluation
Records of hearing protection use do not exist. Employees store hearing protection devices at their work sites and apparently there is no uniform control to ensure compliance with the hearing protection requirements. Moreover, 99.8% of records in the audiometry database (see description below) are missing hearing protection information.

Employee training

Description
Initial and annual training is required for all employees exposed to noise at or above action level. The training program includes information on:

- The effects of noise on hearing
- The purpose and use of hearing protection devices, the advantages and disadvantages of each
- Instructions in the selection, fitting, use and care of hearing protection
- The purpose of audiometric testing and an explanation of the test procedures

The training class lasts for about an hour plus time for questions and a self-assessment test. A LANL group developed a test that can be sent to employees every other year. The test is a booklet including all of the above training and a self-test to send in after completion. The worker will need to receive an 80% or better score to pass. If the worker does not pass, he must attend the training class.

Concerns for HCP evaluation
No data exists about compliance with this HCP component.

Audiometric testing

Description
Audiometric testing has been performed at LANL since 1978. The recruitment of workers for testing prior to 1986, however, was not clearly defined. In 1986, a hearing conservation program has been adopted enrolling workers in a routine audiometric testing program at entry into a work area of high noise exposure (>85 decibel per 8 hr average). An employee is being assigned to the hearing conservation program by their supervisor when the work area is determined to fall under the criteria for high noise (established and monitored by ESH-5).

The audiometric testing program is performed and administered by ESH-2, the Medical program. ESH-5, Industrial Hygiene, identifies personnel who are exposed to noise at or above the action level and provides this information to ESH-2. Test results are maintained in the worker's medical record. Reportedly, no medical records are destroyed, even after termination of employment. Baseline audiograms must be performed within 6 months of an employee's first exposure and annual re-testing is required.

If an annual audiometry shows a threshold shift of 10 dB at any frequency compared to the baseline audiometry, a re-test is ordered after 48 hours of quiet. If a threshold shift still persists after the quiet period, a confirmation test is scheduled in 30 days. If the problem is not resolved

the worker is referred to an audiologist and on-site further action is taken to investigate whether the hearing loss may be occupational noise-induced.

Audiometry is done using two MAICO 800 Automatic Computer Audiometers, which are being calibrated every day. Both machines are serviced every year by a special technician.

Concerns for HCP evaluation

Audiometric testing is performed on all LANL workers, including contractors (construction, guards) and county firefighters. It is not possible to differentiate between these workers given the information in the audiometry database. Furthermore, contract workers often are hired repeatedly for numerous separate projects by LANL. They are required to have audiometric testing at the day of hire; thus, multiple audiometric tests for the same person might be obtained within short periods of time. However, contract workers are not annually re-tested or systematically followed-up.

Audiometric testing is performed throughout the day. This may lead to underestimation of hearing loss if a baseline test was administered at the end of the day or to an overestimation of hearing loss if the baseline was administered in the morning, but subsequent testing was done at a different time. Also, workers ordered for a re-test after a 48-hour quiet period may forget about this requirement. Finally, there might be major problems with the accuracy of any audiometric reading because employees may not understand instructions, fail to respond properly, or are unable to concentrate during the test.

Program evaluation

Current HCP description does not include a program evaluation component. Reportedly, several audits have been conducted 1986-1991, one possibly by DOE-Albuquerque; however, we do not have any information pertaining to that. In addition, currently LANL HCP is being revised to use the ACGIH standards regarding hazardous noise exposure.

Specific Findings: Evaluation of HCP effectiveness

Our objective was to use epidemiologic tools to assess the effectiveness of LANL-HCP by observing changes in hearing threshold levels ('Standard Threshold Shifts' (STS)) dependent on membership in the HCP- program and documented noise exposure levels. In order to achieve this, we had to link audiometric test results with noise exposure data collected for individual workers and in area samples. Since our endpoints of interest were hearing level changes, only those workers with at least two audiometric tests reported in the audiometry database could be included in our evaluation effort. We did not employ any restrictions based on age or prior hearing loss. The first available audiogram was selected to provide a baseline value for hearing capacity and all subsequent audiograms were compared to the first one, thus, all threshold shifts presented in this report refer to differences between the first and last available audiogram.

Audiometry data exists for 19,875 workers, but only for 12,130 workers at least two tests are available. The noise-sampling database contains 5,481 records of noise sampling, 295 are noise measures for individual workers the rest area measurements; and the hearing conservation data refers to 676 individual workers. Thus noise-sampling database provided us with exposure data,

but the majority of samples were area noise measurements and only a small number (about 5%) were personal measures. We found that most area noise measurements had been performed in Technical Area (TA) 3 which houses various shops including the machine shop and in TA 53. Unfortunately, we found that it was impossible to link measured workplace exposure levels to individual workers because the audiometric database does not contain worker location information, and no database exists or was made available to us that contains worker location and, thus, would allow us to match existing area noise measurements to individual workers. In fact, we were able to match only 155 individuals across all three available databases, and for no more than 106 of these workers personal noise measurements were available. This small group will be the basis for our analyses and we will refer to this group in the following as 'workers with noise measurements' (WWNM).

In Tables 1-3 we show the proportion of workers at LANL who experienced hearing loss in at least one ear according to different definitions and for two groups, i.e. workers with both audiometric and noise level measures (WWNM) and workers for whom we have audiometric data only. The OSHA definition - defining a change in hearing threshold for an average of 10dB or more at 2,3,4 kHz in either ear relative to a baseline audiogram [6]- clearly provides the most sensitive definition. According to the OSHA criteria, about one third of all workers for whom we have noise measurements experienced a loss in at least one ear while such a threshold shift was less prevalent among all workers ever tested audiometrically, about a quarter of them exhibited a shift. The observed crude difference between these two groups of workers persisted independent of which hearing loss definition was employed. Thus, the comparison might suggest that workers for whom industrial hygienists at LANL measured noise were not only a more highly exposed group but also experienced subsequent hearing loss at a higher rate.

For our preliminary comparisons of noise exposed with non-exposed workers we chose the OSHA standard threshold shift definition but relied on a threshold shift in both rather than either ear. We based this decision on our observation that audiometric data provided in the computerized database was not systematically corrected for measurement errors due to the failure of a worker to respond properly during audiometric testing. Thus, we believe that a shift in both ears can be considered a more reliable outcome. All comparisons presented are based on the difference between the first and last available audiometric test result. The follow-up period for audiometry among workers with noise measurements ranged from 0.6 to 20.6 years with a mean of 12.2 years. The earliest threshold shift increases for both ears were observed after a minimum 5-year difference between first and last audiometric test, but mostly shifts were observed after 15-20 years of follow-up and a minimum age of 40 years at the time of the last test. Thus, as expected, the proportion of workers who developed hearing loss increased as more time elapsed between the first and last available audiometry test result and as the population grew older.

Among the 106 noise monitored workers 52% had reportedly been exposed to noise levels of 85 dBA or more. Occupational hearing loss is expected to be bilateral and 24 workers (22.6%) in the WWNL group developed bilateral hearing loss (Table 5). Furthermore, 57 WWNL workers (53.8%) experienced hearing loss according to the OSHA criteria in at least one ear. Comparing the 106 exposed WWNL workers (>=85dBA) with those unexposed (<85dBA) we estimated a 2.8-fold increase in the risk for hearing loss (crude OR=2.81; 95% confidence limits 1.5, 7.5;

age-adjusted OR= 2.63; 95%CI 0.93, 7.43). As shown in Table 6, the risk of hearing loss steadily increased with an increase in dbA-level measured. Also, the risk increased by about 16% with every year that past between the first and last audiometric examination (table 6), that is after age-adjustment, we observed a two-fold increase for workers to experience a threshold shift with a 10 year interval and a 4-fold risk increase with a 15 and more year interval between the two audiometric examination. Finally, the largest effect, i.e., a 9-fold risk for hearing loss was experienced if the noise was recorded was being 'intermittent'. Most of the workers experiencing hearing losses due to intermittent noises worked in TA 40 or 3 and the noise was mostly due to the use of saws (see also Table 7).

Discussion

Our ability to evaluate LANL HCP effectiveness was limited mostly by a lack of worker location and job history data that would have allowed us to attribute noise level measurements for work areas to individual workers. Thus, our results are based on a quite small and possibly selective group of workers for whom individual noise measurements existed. Hearing loss is most likely work-related if an employee has a history of long-term exposure to noise levels sufficient to cause a pattern of hearing loss evident in audiometry tests. [3] Thus, for these 106 workers we had to assume that the noise levels measured and reported in the noise sampling database represented their actual exposure over an extended period of time. While it is critical to know the duration of noise exposure, we were only able to equate duration of exposure to the time elapsed between the first and the last available audiometry assuming that workers were consistently exposed at the levels reported at one time in the noise sampling database. It is obvious that this difference might not adequately reflect exposure duration. Nevertheless, we not only found a relation with increasing time between two audiometric tests but also with intensity (dbA) of exposure measured with personal monitoring devices.

There is no information on race or sex of individuals, however, reportedly, all employees are male. According to Environmental Safety and Health (ESH-5) management, a high percentage of workers are exposed to excessive noise outside of the workplace (e.g., recreational activities such as hunting and power tool use - chain saws), but information about such potential confounding factors was not available to us. Finally, workers might experience multiple exposures at their workplace, such as ototoxic solvents in addition to noise, but we were unable to obtain any chemical exposure data.

Conclusions

The major limitation of our evaluation of the LANL Hearing Conservation Program was our inability to link workers' audiometric data to area noise level measurements. Yet, for the small group of workers for whom noise measurements were available we saw a clear relationship with presumed duration of exposure, noise levels, and the noise type 'intermittent' mainly attributed to the use of saws in TA 40. We believe that the three databases provided to us could be of great use for further evaluation and help to implement appropriate prevention measures if these databases were maintained in a manner that would allow to link outcome data (audiometry data) for all workers to area noise measurements which represent 95% of all measurements taken at the facility.

In order to accomplish these goals in the future we thus recommend:

1. To create a noise distribution map for the facility and workplaces based on the noise monitoring program data collected by ESH-5 in order to identify technical areas (TA) with high rates of workers who experienced a STS.
2. To obtain historical lists of workers for each TA to allow a linkage of audiometry data with location and exposure data
3. To abstract archival database records to get a historic roster of employees enrolled in HCP.
4. Identify workers enrolled and not enrolled in HCP and compare the proportion of hearing loss experienced in each group given noise exposure levels and compare whether HCP effectiveness varied by technical area.
5. Conduct a match case-control study, i.e. match cases of hearing loss to non-cases enrolled in the audiometric monitoring program and matched according to year of baseline audiometric testing and age. For these cases and controls, compare the average noise levels encountered over the years at the facility and, in addition, retrieve information about protective equipment and exposure to solvents and metals to examine whether combined exposures increase the risk of developing hearing loss.
6. Evaluate the influence of bias due to the fact that exit exams are not mandatory and there might be a number of workers not reporting for these exams.

Usefulness of Findings: Evaluation of HCP effectiveness

Our results demonstrate that if routinely collected data were maintained at LANL in a manner that would allow to link outcome (audiometry) data for workers to area noise measurements, they would be of great use for future continued evaluations of the HCP and hearing loss prevention measures.

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Table 1. Frequency of 'standard threshold shift' (STS) for the **left ear** between the first and last available audiometry for workers with noise measurements (WWNM_group) and all workers with at least 2 audiometry measurements (audiometry group), by various standards (0 = no shift, 1 = shift present)

	WNL_group, 106 workers			Audiometry group, 12,130 workers		
	Shift	Frequency	Percent	Shift	Frequency	Percent
OSHA						
Average increase **10** dB at **2, 3, 4** kHz	0	72	67.9	0	9186	75.7
	1	34	32.1	1	2944	24.3
NIOSH, current						
Increase of **15** dB at **.5, 1, 2, 3, 4, 6, 8, (10)**kHz	0	93	87.7	0	11229	92.6
	1	13	12.3	1	901	7.4
NIOSH, 1972						
Increase of **25** dB at **1, 2, 3,** kHz	0	103	97.2	0	11937	98.4
	1	3	2.8	1	193	1.6
Mild hearing loss, consensus						
Increase of **40** dB at **1, 2, 3,** kHz	0	105	99.1	0	12084	99.6
	1	1	0.9	1	46	0.4
Mild hearing loss, consensus						
Increase of **40** dB at **2, 3, 4** kHz	0	104	98.1	0	12056	99.4
	1	2	1.9	1	74	0.6

Table 2. Frequency of 'standard threshold shift' (STS) for the **right ear** between the first and last available audiometry for workers with noise measurements (WWNM_group) and all workers with at least 2 audiometry measurements (audiometry group), by various standards (0 = no shift, 1 = shift present)

	WNL_group, 106 workers			Audiometry group, 12,130 workers		
	Shift	Frequency	Percent	Shift	Frequency	Percent
OSHA						
Average increase **10** dB at **2, 3, 4** kHz	0	59	55.7	0	9044	74.6
	1	47	44.3	1	3086	25.4
NIOSH, current						
Increase of **15** dB at **.5, 1, 2, 3, 4, 6, 8,(10)** kHz	0	87	82.1	0	11182	92.2
	1	19	17.9	1	948	7.8
NIOSH, 1972						

Increase of **25** dB at **1, 2, 3,** kHz	0 1	98 8	92.5 7.5	0 1	11933 197	98.4 1.6
Mild hearing loss, consensus Increase of **40** dB at **1, 2, 3,** kHz	0 1	105 1	99.1 0.9	0 1	12068 62	99.5 0.5
Mild hearing loss, consensus Increase of **40** dB at **2, 3, 4** kHz	0 1	103 3	97.2 2.8	0 1	12046 84	99.3 0.7

Table 3. Frequency of 'standard threshold shift' (STS) for **both ears** between the first and last available audiometry for workers with noise measurements (WWNM_group) and all workers with at least 2 audiometry measurements (audiometry group), by various standards (0 = no shift, 1 = shift present)

	WWNL_group, 106 workers			Audiometry group, 12,130 workers		
	Shift	Frequency	Percent	Shift	Frequency	Percent
OSHA Average increase **10** dB at **2, 3, 4** kHz	0 1	82 24	77.4 22.6	0 1	10268 1862	84.6 15.4
NIOSH, current Increase of **15** dB at **.5, 1, 2, 3, 4, 6, 8, (10)** kHz	0 1	96 10	90.6 9.4	0 1	11663 467	96.2 3.8
NIOSH, 1972 Increase of **25** dB at **1, 2, 3,** kHz	0 1	104 2	98.1 1.9	0 1	12038 92	99.2 0.8
Mild hearing loss, consensus Increase of **40** dB at **1, 2, 3,** kHz	0 1	106 0	100.0 0.0	0 1	12101 29	99.8 0.2
Mild hearing loss, consensus Increase of **40** dB at **2, 3, 4** kHz	0 1	105 1	99.1 0.9	0 1	12095 35	99.7 0.3

Table 4. Left, right, worst and both ears threshold shift of 10dB or more by exposure status (Exposure: 0 = unexposed, <85 dBA, 1 = exposed, 85+ dBA; Outcome: 0 = no shift, 1 = shift present)

Left ear

Exposed	Threshold shift 0	1	Total
0	39	12	51
	76.47	23.53	
1	33	22	55
	60.00	40.00	
Total	72	34	106

Right ear

Exposed	Threshold shift 0	1	Total
0	32	19	51
	62.75	37.25	
1	27	28	55
	49.09	50.91	
Total	59	47	106

Worst Ear

Exposed	Threshold shift 0	1	Total
0	27	24	51
	52.94	47.06	
1	22	33	55
	40.00	60.00	
Total	49	57	106

Both ears

Exposed	Threshold shift 0	1	Total
0	44	7	51
	86.27	13.73	
1	38	17	55
	69.09	30.91	
Total	82	24	106

Table 5. Left, right, and both ears threshold shift of 10dB or more by age group at the last available audiometry (0 = no shift, 1 = shift present)

LEFT EAR

Threshold shift

Age group

	0	1	Total
<30	4	1	5
	80.00	20.00	
30-39	20	3	23
	86.96	13.04	
40-49	27	12	39
	69.23	30.77	
50-59	15	12	27
	55.56	44.44	
60+	6	6	12
	50.00	50.00	
Total	72	34	106

RIGHT EAR

Threshold shift

Age group

	0	1	Total
<30	5	0	5
	100.00	0.00	
30-39	21	2	23
	91.30	8.70	
40-49	22	17	39
	56.41	43.59	
50-59	8	19	27
	29.63	70.37	
60+	3	9	12
	25.00	75.00	
Total	59	47	106

BOTH EARS

Threshold shift

Age group

	0	1	Total
<30	5	0	5
	100.00	0.00	
30-39	23	0	23
	100.00	0.00	
40-49	30	9	39
	76.92	23.08	
50-59	17	10	27
	62.96	37.04	
60+	7	5	12
	58.33	41.67	
Total	82	24	106

Table 6. Effect estimates for factors associated with a threshold shift of 10dB between first and last audiometric exam in both ears; results from logistic regression models (WWNM-group, N=106)

	Number of workers without STS	Number of workers with STS	Odds ratio (95% CI)
Age at last audiometric exam (in years)			1.11 (1.04, 1.19)
Years between the first and last audiometric exam (in years)			1.16 (1.01, 1.33)
dbA			
▪ <80	14	2	1.00
▪ 80-84	30	5	2.41 (0.32, 18.2)
▪ 85-89	28	8	2.87 (0.44, 18.8)
▪ ≥90	10	9	11.60 (1.54, 87.7)
Type of noise			
▪ steady	24	3	0.73 (0.16, 3.37)
▪ intermittent	4	7	9.03 (1.97, 41.3)
▪ impulse	3	1	1.67 (0.13, 21.4)
▪ mixed	5	3	2.78 (0.44, 17.6)

Table 7. Distribution of workers (WWNM-group, N=106) with and without a threshold shift of 10dB in both ears between first and last audiometric exam by technical area (TA)

Technical Area	Number of workers without STS	Number of workers with STS
unknown	0	1
3	35	7
9	2	0
16	4	0
35	7	1
39	1	0
40	6	7
41	1	1
43	2	0
46	4	0
50	1	0
53	13	5
55	5	0
59	1	2

Appendix 1
DATA SOURCES AVAILABLE FOR HCP EVALUATION

In all databases, workers are identified by their LANL identification number, a Z#.

Description

1. Noise sampling database.

Reportedly, this database contains information on LANL workers regardless of HCP enrollment status. Data records started in 1960, with regular measurements starting in 1970. This database includes noise exposure measurements, both personal dosimetry and area; location of measurement (Technical Area (TA), building and room number); description of exposure; date of sampling; duration of sampling; work group.
We have 5481 records in the noise sampling database, corresponding to 295 individual Z#.

2. Hearing conservation database.

Reportedly, this database contains information on people enrolled in the HCP. The records start in 1985. Database includes location (TA and building); LANL organization; nature of exposure; measurement date; noise level; date a person was added to the database; date deleted from the database; training date; deletion reason; HCP enrollment status (ever vs. never).
We have 1380 records in the noise database, corresponding to 676 individual Z#.

3. Audiometry database.

This database contains audiometry data on all workers at LANL, regardless of HCP enrollment status and including contractor employees (JCI - construction, PTLA - guards, and county firefighters). Regular records start in 1963. Database includes date of audiometric exam; audiometric measurements for left and right ear at 0.5, 1, 2, 3, 4, 6, 8, 10 kHz; interval until recheck; ear damage (e.g., tympanic membrane rupture); threshold shift.
We have 61054 records in the audiometry database, corresponding to 19875 individual Z#.

4. Birth year file.

This file just contains worker identification number and year of birth.
We have 30062 records in the birth year file, corresponding to 29917 individual Z#.

Concerns for HCP evaluation

The audiometry database does not contain information on worker location and start of employment. It appears that it is not possible to establish duration of noise exposure for each worker other than by looking at the time elapsed between the first and the last recorded audiometry. There is no single source of work history or worker location information contained in any of the databases. None of the available databases provides HCP enrollment status.

5. To develop and implement a risk-based framework and methodology that permits estimation of the incidence of adverse health impact predicted from environmental/biological exposure and enables development of surveillance programs and intervention strategies to prevent adverse consequences of exposure.

Research Activities

6. Assessing Risks from Exposures to Multiple Physical and Chemical Agents

Exposure to ionizing radiation in combination with chemicals is an important problem in many developed countries and is likely to be an issue in developing countries. Not only a growing concern at many hazardous waste sites, where various radioactive and toxic chemical wastes are buried, aggregate exposures to these two classes of hazardous agents are also common in the military, in the defense nuclear industry, and in many research laboratories. Moreover, in both the medical research and medical service sectors of modern economies, mixed exposure to ionizing radiation and certain chemicals can frequently occur. Mixed exposure to ionizing radiation and chemicals is also expected to be an important issue for many of the emerging research and technology industries in developed countries.

Very little quantitative analysis is currently available on the cumulative effects of exposure to multiple hazardous agents that have either similar or different mechanisms of action. Over the past several years, efforts have been made to develop the methodologies for risk assessment of chemical mixtures, but mixed exposures to two or more dissimilar agents such as radiation and one or more chemical agents have not yet been addressed in any substantive way. To address this issue, we carried out a review and evaluation of the current understanding of the health risks arising from mixed exposures to ionizing radiation and specific chemicals. We compiled information on how radiation/chemical exposures, when evaluated in aggregation, were linked to chronic health endpoints such as cancer and intermediate health outcomes such as chromosomal aberrations. We also consider the extent to which the current practices are consistent with the scientific understanding of the health risks associated with mixed-agent exposures. From this we identified research needs for assessing the cumulative health risks from aggregate exposures to ionizing radiation and chemicals. Our evaluation indicates that essentially no guidance has been provided for conducting risk assessment for two agents with different mechanisms of action (i.e., energy deposition from ionizing radiation versus DNA interactions with chemicals) but similar biological endpoints (i.e., chromosomal aberrations, mutations, and cancer). Our analysis reveals the problems caused by the absence of both the basic science and an appropriate evaluation framework for the combined effects of mixed-agent exposures. This makes it difficult to determine whether there is truly no interaction or somehow the interaction is masked by the scale of effect observation or inappropriate dose-response assumptions.

This effort resulted in a proposed framework for measuring and evaluating radiation/chemical exposures. This framework was applied to workers at the U.S. Department of Energy Savannah River Site in South Carolina where exposures to both benzene and ionizing radiation have been measured. The key findings and recommendations from this study are the following:

1) The environmental health sciences community needs an evaluation framework that makes possible consideration potential interactions between chemical agents and ionizing radiation.
2) The limited power of epidemiological studies may be inadequate to uncover the potential synergisms that would be important from a policy perspective.
3) Carefully designed studies of chromosomal aberrations may have the potential to reveal the synergisms caused by mixed exposure to genetoxic agents.

4) The environmental health community needs standardized procedures for characterizing the risks of mixed-agent exposures.
5) Uncertainties in extrapolation from experimental data to human risks must be properly characterized.
6) Risk assessment guidance must be explicit on procedures to address the combined effects of mixed-agent exposures.
7) There is an absence of case studies on the health effects of mixed-agent exposures.

Waste Incinerators as Case Study of Failure to Address Worker Exposure

Waste incineration has emerged over the last century as a viable strategy for (a) reducing the volume of municipal waste, (b) for reducing substantially the volume of chemical and biological hazardous wastes, (c) for destroying medically contaminated hospital waste, and (d) for producing energy. Whether waste incineration poses a health risk to occupational and residential populations has been the subject of continuous scientific debate. In November 1999, the National Research Council released a report titled "Waste Incineration and Public Health" that addressed pollutant emissions, exposures and health risks from waste incineration. We carried out a study to provide some background both on the health issues that have emerged for waste incineration and to discuss some of the issues raised in the NRC report. This work was published in the journal *Environmental Science and Technology*. In this report, we identified three areas in which the limitations and uncertainty in the data impact health effects assessments. First, there is very little emissions data for any event other that normal operation. Second, we still lack data needed to characterize intermedia transfers of emitted chemicals from ambient air to food webs and to indoor environments. Third, we note that the existing framework used to assess human exposures and health effects from incinerators has focused on local populations but excluded both workers and the larger regional populations.

Workers at incinerators are and understudied and important population for exposures to multiple chemical agents. Workers come into close contact with not only the stack emissions, but also with toxic pollutants captured in the air pollution control equipment, including electrostatic precipitators and bag houses. These must be cleaned out periodically, and high concentrations of dioxin and various metals have been measured in the air during these operations. Both personal and area sampling of workers cleaning out electrostatic precipitators at municipal incinerators demonstrated exposures greatly in excess of recommended limits for dioxins and metals (arsenic, lead, cadmium and aluminum). Elevated levels of dioxin and lead have been reported in the blood of municipal incineration workers. Higher concentrations of hydroxypyrene in the urine of municipal incineration workers indicate exposure to higher levels of polycyclic aromatic hydrocarbons; similarly, higher levels of urinary mutagens have been reported among refuse incinerator workers.

Research Products

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APPENDIX A

Hierarchical Cluster Analysis Applied to Workers' Exposures in Fiberglass Insulation Manufacturing, J.D. Wu, D.K. Milton, S.K. Hammond and R.C. Spear, Ann. Occup. Hyg., 43:43-55, 1999.

Chronic Health Risks from Aggregate Exposures to Ionizing Radiation and Chemicals: Scientific Basis for an Assessment Framework, W.G. Chen and T.E. McKone, Risk Analysis, 21:25-42, 2001.

Integration and Exploration of Task-Based Exposure Data: Part I: Design of an Exposure Simulator, J.D. Wu, N. Shang, S.K. Hammond, and R.C. Spear, In review, Am. Ind. Hyg, Assn. J., 2000.

Integration and Exploration of Task-Based Exposure Data: Part II: Simulation of Solvent Exposures in Raft Manufacturing, J.D. Wu, K. Vork, and R.C. Spear, In review, Am. Ind. Hyg, Assn. J., 2000.

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Exploring the Use of Simulation as a Tool for Workplace Exposure Assessment, Shao-wen Liaw, Katharine Hammond, Robert C. Spear, Jyun-De Wu, and Mark Nicas, Am. Ind. Hyg. Conf. And Exposition, St. Louis, MO, May 1999.

NIOSH Extramural Award Final Report Summary

Title:	Hazard Surveillance in the Defense Nuclear Industry
Investigator:	John R. Froines, Ph.D.
Affiliation:	University of California
City & State:	Los Angeles, CA
Telephone:	(310) 206-6141
Award Number:	5 R01 CC912034-03
Start & End Date:	9/30/1995–9/29/1999
Total Project Cost:	$1,191,563
Program Area:	NORA
Key Words:	

Abstract:
The overall goal of this research is to develop an integrated theory, approach, and methodology to exposure assessment and hazard surveillance, which emphasizes characterization of exposure to complex mixtures of chemical toxicants and biomechanical problems as well as single agents. The research has relevance to identification and characterization of problems associated with decommissioning and decontamination of Department of Energy sites, application to the defense nuclear industry and other high-risk industrial locations. This research represents collaboration between the University of California at Los Angeles and Berkeley, Lawrence Livermore and Los Alamos National Laboratories. The specific aims of the overall research can be subdivided into subsections.

1. Exposure assessment and hazard surveillance: To identify appropriate statistical tools for characterizing multiple chemical agents; to explore toxicologic and epidemiologic implications of multivariate exposure characterization; to measure task-specific exposures with real time instrumentation and integrated sampling; to develop models of exposure based on task specific data; to test these models with integrated sampling, and to refine the models based on the results.
2. Modeling pollutant concentration between source and worker: Improve our understanding of small scale (0 to 2 m) dispersion of contaminants with the ultimate goal of predicting personal exposure based on the minimum number of area concentration measurements. To provide a tool for efficient screening of a large number of work sites for potential inhalation hazards.
3. Application of biologic monitoring and biomarkers of exposure for exposure assessment and hazard surveillance: To make use of biologic monitoring, biomarkers of exposure, and toxicokinetic modeling to better estimate internal and target tissue dose from exposure to single and multiple chemical agents and evaluated interactive effects associated with toxicokinetic interaction.
4. Integrated task and postural analysis for ergonomic exposure analysis: To develop, pilot test, and validated an integrated task and postural analysis for ergonomics exposure assessment.
5. Evaluation of current exposure and medical surveillance programs at Los Alamos and Lawrence Livermore National Laboratories: To evaluated the medical and exposure surveillance programs at LANL, identify discrepancies between health and safety "needs" and established monitoring programs, and develop an integrated surveillance

system that efficiently combines hazardous-exposure, biological, and health-outcome monitoring of the worker population.
6. Assessing risks from exposure to multiple physical and chemical agents: To develop and implement a risk based framework and methodology that permits estimation of the incidence of adverse health impact predicted from environmental/biological exposure and enables development of surveillance programs and intervention strategies to prevent adverse consequences of exposures.

Publications

Chen WG, McKone TE: Chronic Health Risks from Aggregate Exposures to Ionizing Radiation and Chemicals: Scientific Basis for an Assessment Framework. Risk Analysis, 21, pp 25-42, 2001

Wu JD, Shang N, Hammond SK, Spear RC: Integration and Exploration of Task-Based Exposure Data: Part I: Design of an Exposure Simulator, Am. Ind. Hyg. Assn. J., 2000

Wu JD, Vork K, Spear RC: Integration and Exploration of Task-Based Exposure Data: Part II: Simulation of Solvent Exposures in Raft Manufacturing. Am. Ind. Hyg. Assn. J., 2000

Wu JD, Milton DK, Hammond SK, Spear RC: Hierarchical Cluster Analysis Applied to Workers' Exposures in Fiberglass Insulation Manufacturing. Ann. Occup. Hyg., 43, pp 43-55, 1999

Pergamon

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Hierarchical Cluster Analysis Applied to Workers' Exposures in Fiberglass Insulation Manufacturing

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The objectives of this study were to explore the application of cluster analysis to the characterization of multiple exposures in industrial hygiene practice and to compare exposure groupings based on the result from cluster analysis with that based on non-measurement-based approaches commonly used in epidemiology. Cluster analysis was performed for 37 workers simultaneously exposed to three agents (endotoxin, phenolic compounds and formaldehyde) in fiberglass insulation manufacturing. Different clustering algorithms, including complete-linkage (or farthest-neighbor), single-linkage (or nearest-neighbor), group-average and model-based clustering approaches, were used to construct the tree structures from which clusters can be formed. Differences were observed between the exposure clusters constructed by these different clustering algorithms. When contrasting the exposure classification based on tree structures with that based on non-measurement-based information, the results indicate that the exposure clusters identified from the tree structures had little in common with the classification results from either the traditional exposure zone or the work group classification approach. In terms of the defining homogeneous exposure groups or from the standpoint of health risk, some toxicological normalization in the components of the exposure vector appears to be required in order to form meaningful exposure groupings from cluster analysis. Finally, it remains important to see if the lack of correspondence between exposure groups based on epidemiological classification and measurement data is a peculiarity of the data or a more general problem in multivariate exposure analysis. © 1999 British Occupational Hygiene Society. Published by Elsevier Science Ltd.

Keywords: glass fibre; cluster analysis; exposure groups; occupational groups; epidemiology

INTRODUCTION

Hines *et al.* (1995) used hierarchical cluster analysis in exploring the concurrent exposure of female workers in the semiconductor fabrication industry to a number of chemical and physical agents in the context of an epidemiological investigation of spontaneous abortions. We have been further investigating the application of cluster analysis to the characterization of multiple exposures in other aspects of industrial hygiene practice. This work has been motivated by the fact that all workers are exposed to multiple hazards during a typical workday and the technology to measure these exposures is increasingly available. It is not clear, however, how best to summarize this type of multivariate data for use in exposure monitoring and surveillance. It is of some interest, for example, to speculate on the multivariate equivalent of the homogeneously exposed group or, from a different perspective, to consider the multivariate analog of the random effects model (Rappaport *et al.*, 1995) in exposure characterization.

In epidemiological studies, exposure assessments often are performed by classifying study subjects into discrete exposure categories. When dealing with exposures to multiple agents, the exposure classification problem becomes much more complex because of the increase in dimensionality. Although multiple regression or logistic regression models are often applied to explore exposure-relationships, a

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well-defined outcome (or dependent) variable is required before using these models. However, the construction of objective exposure classes does not require any information about health outcomes. Examples of multivariate statistical methods which can meet the purposes of exposure assessment include principal component analysis and hierarchical cluster analysis. For example, Simmons and Spear (1993) utilized principal components techniques to characterize workers' exposures to a variety of solvents in a printing plant. Sahl *et al.* (1994) also used the method to examine the intercorrelation between different summary measures of 60 Hz magnetic field exposure among utility workers. Recently, Bye (1996) emphasized the advantages of the technique for the management of large data sets and the application to the generation of new hypotheses for investigations of complex systems. While principal components analysis and its variants have been applied in various exposure-related applications, this has not been the case for cluster analysis. Hence, the purpose of this study was to explore, via cluster analysis, exposure patterns among workers exposed to three agents (airborne endotoxin, phenolic compounds and formaldehyde) in fiberglass insulation manufacturing, and to characterize exposures based on these patterns.

There are many variants of cluster analysis and one may generally expect differences in the final results of such an analysis depending on which particular procedure is used. Hence, one of the principal objectives in this study was to gain some sense of the magnitude of these differences when common clustering procedures were applied to typical exposure data. As will be seen, we will contrast exposure groupings for the fiberglass workers based on cluster analysis with non-measurement-based strategies commonly used in epidemiology. In making these comparisons, it is important to be confident that any differences observed between the two approaches are not artifacts of the statistical procedures used in identifying exposure clusters.

CLUSTER ANALYSIS

Everitt (1994) has given a clear definition of cluster analysis, '*Cluster analysis is a generic term used for a large number of techniques which attempt to determine whether or not a data set contains distinct groups, and, if so, to find the groups.*' That is, cluster analysis is a good statistical tool of searching for objects with similar attributes in a data set. The following paragraphs describe the general aspects of the approach with respect to the selections of distance metrics, clustering algorithms, or criteria for determining the number of clusters and for validating a tree structure.

In general, cluster analysis regards each object as a point in a multi-dimensional space defined by the values of each of its attributes. The distance between two objects is measured to determine the similarity of the objects in terms of each of its attributes. Therefore, the choice of a distance metric is the initial step of cluster analysis. There are a variety of distance metrics available, but Euclidean distance is the most common and intuitive and was used throughout this study.

Because differences in units and in the magnitude of the variance in each of the individual attributes may influence the computation of distance metrics, variable standardization is important for cluster analysis. Various standardization methods have been proposed. Milligan and Cooper (1988) conducted a Monte Carlo study to compare the performance of seven different variable (attribute) standardization methods in recovering known clusters of synthetic data. They found that the standardization approach which divides each variable by its range exhibited consistently superior recovery of the structures under different error conditions, separation distances, clustering algorithms, and coverage levels. In contrast to Milligan and Cooper's study, Schaffer and Green (1996) evaluated the variable standardization methods by using real data sets and external validation. They too discovered that no standardization did as well or better than the range standardization. Although the range standardization is considered to be a good method for variable standardization in cluster analysis, it is not possible to conclude that it is always the most effective approach.

After a distance metric is selected and the variables are standardized, the next step is the determination of a clustering algorithm. Since the purpose of cluster analysis is to combine objects into groups or clusters, some rules or methods are required to determine how to form these groups. Clustering algorithms are the rules or procedures used for this purpose. In general, the issue is to decide when two objects are sufficiently similar to form a cluster and then to decide whether other objects should be added to this nucleus, to another, or to start a new cluster. Some of the popular algorithms are the centroid method, the single-linkage (or nearest-neighbor) method, the complete-linkage (or farthest-neighbor) method, the average-linkage method and the Ward's method. Complete-linkage, single-linkage, average-linkage and model-based clustering algorithms are the methods available in S-plus (Venables and Ripley, 1994) and were used in this study.

The detailed explanations of these algorithms are well described in Everitt's book (Everitt, 1993) and Banfield and Raftery's paper (Banfield and Raftery, 1993). Brief descriptions of these algorithms are given here to provide an understanding of how they work. The centroid method calculates the distance between two clusters based on the weighted centers of the clusters; two clusters with the smallest distance are grouped and a new centroid is computed. In the single-linkage method, the distance between two clusters is defined as the minimum of the distances between all possible pairs of objects in the two clusters. In the complete-linkage method, the distance between two clusters is represented by the maximum of the dis-

tances between all pairs of objects in the clusters. The average-linkage uses the mean distance from all objects in one cluster to all objects in another; two clusters with the smallest mean distance are then merged to form a new cluster. Ward's method does not compute distances between clusters, but instead forms clusters by maximizing within-clusters homogeneity where the within-cluster sum of squares is used as the measure of homogeneity. Unlike the algorithms previously described, the model-based clustering algorithm allows one to choose cluster features *a priori*, i.e., shape, size and orientation; this is achieved by reparameterization of the covariance matrix and utilizes information resulting from eigenvalue decomposition (Banfield and Raftery, 1993).

It has been pointed out that different clustering algorithms may produce different shaped and sized clusters. As described by Everitt (1993), single linkage clustering is good for finding elliptical clusters; both centroid and Ward's methods have a tendency to obtain spherical clusters. Therefore, it has been a concern that the orientation, size and shape, inherently existing in a data set determine whether or not a particular clustering algorithm gives useful results. However, these characteristics are not known *a priori*. Although this concern originated from a statistical perspective, it also has important implications for industrial hygiene and toxicology. For example, if the size of a cluster is 'large', within-cluster variability is 'large'. Thus, it may not be reasonable to claim that the members of the cluster share homogeneous exposures because the exposure can vary significantly in both magnitude and composition. In summary, however, a cluster is comprised of a set of sample points (in this study each point defined by an endotoxin-phenolic-formaldehyde level) which are 'close' together as defined by the application of clustering algorithms to the measured Euclidean distances between the points.

For hierarchical clustering algorithms, the number of clusters at each step is one less (or more) than the previous one. A dendrogram or hierarchical tree is the graphical presentation of various steps of the hierarchical clustering process. Normally, the vertical axis of a hierarchical tree indicates the Euclidean distance or level of dissimilarity where two objects or clusters merge to form a larger cluster. The tree shown in Fig. 1 is a typical hierarchical tree. Cutting a hierarchical tree horizontally creates a clustering. The horizontal axis of a hierarchical tree identifies the objects being classified. Objects connected by lines represent clusters which are nested together. The tree clearly shows the Euclidean distance between clusters and the numbers of clusters at each merging stage. In order to form clusters from a hierarchical tree, a threshold on the Euclidean distance or dissimilarity value needs to be specified. Hence, a method of determining the number of clusters in a data set is needed to form objective and representative exposure clusters or patterns.

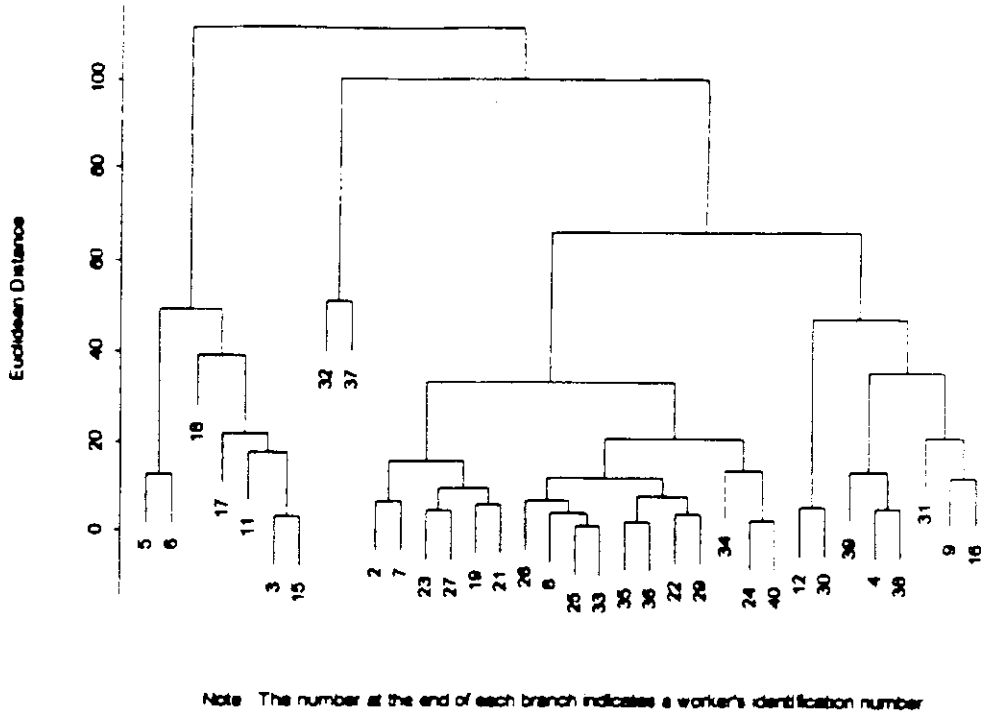
There are different approaches to determining the number of clusters. These include the variance ratio criterion (VRC) (Calinski and Harabasz, 1974), the point serial correlation coefficient for called MH index) (Jain and Dubes, 1988), and the approximate weight of evidence (AWE) approach (Bunnefld and Raftery, 1993). Milligan and Cooper (1985) conducted a Monte Carlo study which evaluated 30 procedures for determining the number of clusters in data sets with different numbers of non-overlapping clusters. The detailed discussion of how these approaches perform is not within the scope of this paper. For the purpose of illustration, however, the VRC approach is presented to show the determination of the number of clusters in the fiberglass data set. This method determines the number of clusters by comparing the ratio of between cluster sum-of-squares to within cluster sum-of-squares.

The final important issue in cluster analysis is the determination of the validity of a tree structure. That is, how does one conclude that a tree structure obtained in a particular cluster analysis was not produced by chance? Methods have been proposed for accomplishing this task (Jain and Dubes, 1988) but their discussion is also beyond the scope of this paper although the Rand index (Hubert and Arabie, 1985) was applied to assess the validity of the tree structure of the fiberglass data set.

THE DATA SET

The data set used in this study was collected in an epidemiological study of peak expiratory flow change among workers exposed to endotoxin, phenolic resin and formaldehyde in a fiberglass wool manufacturing plant (Milton *et al.*, 1996). Worker exposures were measured by taking personal air samples and recording time-activity worklogs. Sampling and analysis techniques for these samples were discussed in detail in Walters (1993). A total of 393 half-shift (4-hour) personal air samples were collected from 37 workers in four work groups for 5–6 days each; two measurements were taken per day for almost all workers. The four work groups include two production groups (A and D) and two maintenance groups (M and N). These groups were selected largely for sampling convenience and are reported here since they correspond roughly to traditional classifications based on job titles. The prior expectation would be that the two production groups would be similar to one another, as would the maintenance groups, but that production exposures would differ from maintenance exposures. Ten to twelve personal measurements were taken for most workers.

In a previous study Walters (1993) used job titles and observations of work tasks and areas to classify these workers into four exposure zones (B, F, O and X for basement, forehearth, curing ovens and maintenance, respectively). Detailed descriptions of the assignment of the exposure zones were given in a later study (Milton *et al.*, 1996). In summary, the workers



Note: The number at the end of each branch indicates a worker's identification number

Fig. 1. Hierarchical tree based on workers' mean exposures using complete-linkage and non-standardized data.

in the exposure zones B, O and F mainly stayed in their work areas to conduct their work tasks. Therefore, they were considered to be 'fixed location workers.' The workers in the exposure zone X were considered to be 'mobile workers' because they moved around different work areas. Although the workers in this exposure group were not entirely maintenance workers, they shared a common characteristic of spending most of their time in low-exposure areas.

Statistical analyses were performed by using S-plus for Windows version 3.2. Summary statistics were calculated including the mean, variance, coefficient of variation and correlation coefficients. Quantile-quantile plots were generated to examine the distributions of workers' exposures. Among 380 valid measurements, no value was under the detection limit for endotoxin. There were a large (59%) and small (12%) proportions of the measurements under the detection limits of phenolic compounds and formaldehyde, respectively. The half-shift exposures of these workers were approximately lognormal when examining the quantile-quantile plots after removal of the values under the detection limits. Measurements below the detection limits were replaced by the values of half the detection limits for the cluster analysis. Maximum likelihood methods were used in estimating the means and variances in the summary statistics as described below.

RESULTS

Descriptive statistics

Exposure means and standard deviations of these three agents and four work groups are shown in Table 1. Because the high proportion of exposure measurements under the detection limit for phenolic compounds probably resulted in unreliable estimates of exposure means and standard deviations, a maximum likelihood estimation (MLE) algorithm was used under S-plus to estimate these means and standard deviations. The basic assumptions underlying the MLE algorithm is that the values under the detection limit follow the same distribution as those above the detection limit. Here, the distribution of the values above the detection limit is assumed to be lognormal. Although the MLE was used to estimate the means and standard deviations of the work groups, the group mean exposures were not subsequently used in cluster analysis. As expected, the median exposure of groups A and D were similar to each other and higher than groups M and N. Three univariate analyses of variance (ANOVA) were conducted on the logs of the individual concentration data to explore differences in exposure by zone. The model used for the ANOVA was a fixed-effects model. The results illustrate that there was a significant difference between the median exposures for different exposure zones for each agent (Tables 2 and 3).

However, analysis of consecutive four-hour measurements suggests that some degree of autocorrelation was present in the formaldehyde data in particular. Hence, the independence assumption underlying the ANOVA significance tests was compromised. The F values were sufficiently large and the strength of the autocorrelation sufficiently modest, however, to support the conclusion that a difference in median exposure exists between zones. The Pearson

Table 1. Mean, median and standard deviation of workers exposure measurements by work groups

Agents	Work groups	Number of samples	Mean (μg m⁻³)	Median (μg m⁻³)	Range (μg m⁻³)	Standard deviation (μg m⁻³)
Endotoxin						
	A	91	0.0323	0.0091	0.0003–0.8170	0.1090
	D	104	0.0467	0.0129	0.0003–1.9860	0.1983
	M	82	0.0202	0.0033	0.0006–0.7642	0.0859
	N	87	0.0164	0.0023	0.0002–0.2929	0.0394
	Total	364				
Phenolic Compounds	A	*91(35)	**50.10[66.85]	35.40	5.57–281.98	**58.48 [204.15]
	D	104(47)	38.23[46.32]	21.80	5.24–302.36	47.50 [127.92]
	M	82(71)	16.08[13.92]	7.13	5.78–135.65	24.97 [59.49]
	N	87(66)	***37.30[NA]	7.54	4.60–605.89	90.93 [NA]
	Total	364(219)				
Formaldehyde						
	A	91(13)	**64.60[115.21]	36.37	1.12–314.97	**68.21 [469.63]
	D	104(3)	75.65[95.25]	66.74	1.05–344.80	61.24 [164.61]
	M	82(15)	24.91[32.94]	12.44	1.29–148.25	33.57 [104.62]
	N	87(11)	29.08[36.06]	15.86	1.09–183.97	33.15 [84.01]
	Total	364(42)				

*The number in the parenthesis is the number of measurements under the detection limit.
**The number in the brackets is the maximum likelihood estimate.
***The maximum likelihood estimate is not available.

Table 2. Mean, median and standard deviation of workers exposure measurements by exposure zones

Agents	Exposure zones	Number of samples	Mean (μg m⁻³)	Median (μg m⁻³)	Range (μg m⁻³)	Standard deviation (μg m⁻³)
Endotoxin						
	B	77	0.08	0.02	0.0006–1.9860	0.24
	F	69	0.03	0.02	0.0006–0.3871	0.05
	O	34	0.01	0.01	0.0009–0.0857	0.02
	X	188	0.02	0.00	0.0002–0.7642	0.06
	Total	368				
Phenolic Compounds						
	B	77	59.91	31.46	5.68–302.36	70.90
	F	69	48.24	43.15	5.24–147.35	34.17
	O	34	20.85	8.21	5.53–125.54	27.45
	X	188	24.38	7.39	4.60–605.89	64.67
	Total	368				
Formaldehyde						
	B	77	74.33	49.96	1.13–314.97	67.24
	F	69	76.43	75.92	1.05–217.12	57.52
	O	34	74.54	48.70	1.85–344.80	73.07
	X	188	25.57	13.66	1.09–183.97	33.13
	Total	368				

product-moment correlation between the individual exposures was low when all exposure zones were combined. In general, a medium correlation (between 0.30 and 0.45) existed between exposures to endotoxin and phenolic compounds and between exposures to phenolic compounds and formaldehyde in exposure zones B, F and X (Table 4). However, a high correlation (0.709) between exposures to endotoxin and phenolic compounds was observed in exposure zone O (Table 4). It was not clear what caused the observed high exposure correlation. Because the correlation between the agent exposures was generally not high, the adjustment for the intercorrelations between the variables was not performed before calculating the Euclidean distance for cluster analysis.

Preliminary analyses

In order to apply cluster analysis to the multivariate data with repeated measurements, mean values of worker exposures to each agent were used in most analyses. A data matrix consisted of 37 rows (workers) and 3 columns (agents) was thereby created. By viewing each worker's mean exposure as a point in a three-dimensional (three-agent) space, the distance between

Table 3 Analysis of Variance of Exposure Zones for Log Agent Exposures

	DF	Sum of square	Mean square	F value	Pr > F
Log (endotoxin)					
Exposure zones	3	233.1*	77.72	39.37	0.0001
Residuals	364	718.60	1.97*	–	
Log (phenolic compounds)					
Exposure zones	3	101.49	33.83	33.67	0.0001
Residuals	364	365.72	1.00	–	–
Log (formaldehyde)					
Exposure zones	3	144.71	48.24	26.34	0.0001
Residuals	364	666.53	1.83	–	–

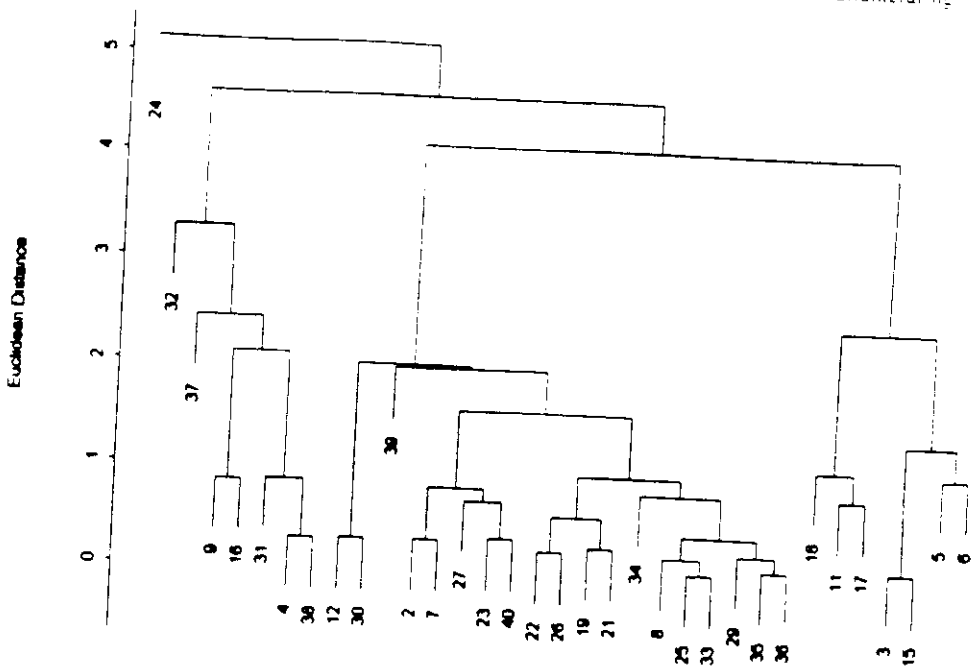
Table 4 Correlation matrix of agent exposures

All exposure zones combined	Endotoxin	Phenolic compounds	Formaldehyde
Endotoxin	1.000	0.286	0.156
Phenolic compounds		1.000	0.330
Formaldehyde			1.000
Exposure Zone B			
Endotoxin	1.000	0.329	0.149
Phenolic compounds		1.000	0.424
Formaldehyde			1.000
Exposure zone F			
Endotoxin	1.000	0.263	−0.059
Phenolic Compound		1.000	0.351
Formaldehyde			1.000
Exposure zone O			
Endotoxin	1.000	0.709	0.020
Phenolic compounds		1.000	0.155
Formaldehyde			1.000
Exposure zone X			
Endotoxin	1.000	0.253	0.238
Phenolic compounds		1.000	0.240
Formaldehyde			1.000

each pair of workers was calculated. Thus, a distance or similarity matrix was obtained. By applying clustering algorithms to the distance matrix, a hierarchical tree was constructed from which the similarity of workers' exposures can be assessed. Examining the hierarchical trees of the unstandardized and standardized (z-standardization) arithmetic mean exposures based on Euclidean distance and three different clustering algorithms: complete-linkage, group-average and single-linkage, showed three workers (#1, 13 and 14) had very different exposures from all others. These three points formed long branches which did not join the main tree until very late stages. Hence, the algorithms tended to identify clusters comprised of only one worker. Therefore, we chose to treat these workers as having unique exposures and they were deleted from the following analyses. Figure 1 is the hierarchical tree of the unstandardized mean exposures based on Euclidean distance and the complete-linkage (or farthest-neighbor) clustering algorithm with the three outliers deleted. The numbers at the end of each node are the identification numbers of the workers. Examining the hierarchical tree, we can see the 34 workers can be classified into three, four or five exposure clusters (or groups) by cutting the tree at Euclidean distances of about 65, 55 or 50.

Effects of standardization

Because the difference in the magnitude of exposure and in variability between the three agents are likely to influence clustering, the data were standardized to mean zero and variance one (z-standardization) and analyzed by the complete-linkage algorithm. The hierarchical tree obtained from the standardized data is showed in Fig. 2. Comparing this tree with that obtained from the unstandardized data with the same clustering algorithm (Fig. 1), it can be seen that some



Note: The number at the end of each branch indicates a worker's identification number

Fig. 2. Hierarchical tree based on workers' mean exposures using complete-linkage and z-standardization.

workers changed clusters. This difference is largely due to the fact that endotoxin exposures in the unstandardized data were small compared with those of other two variables, hence that exposure component did not play an important role in the unstandardized clustering. After standardization, the differences of endotoxin exposures among these workers made a contribution to the exposure clustering.

Effects of clustering methods

The influence of clustering algorithms on the formation of a tree structure has been discussed extensively (Everitt, 1993; Jain and Dubes, 1988). Here, this issue was explored by the production of tree structures using complete-linkage, group-average and single-linkage clustering algorithms applied to standardized data. Because this data set is three dimensional the use of scatter plots supplements the tree diagrams in displaying the effects of different clustering procedures. Figure 3a is one such plot which illustrates the clusters found by the complete-linkage algorithm when four exposure clusters were chosen (i.e., the tree was cut at a distance of 3.5 in Fig. 2). Figure 3b and 3c are the plots from group-average and single-linkage clustering algorithms, respectively, when four clusters were specified. When comparing the latter two plots with that produced by complete-linkage (Fig. 3a), there are minor differences between the group-average and complete-linkage clustering algorithms, but, very significant differences between the single-linkage and complete-linkage clustering algorithms. The exposure clusters produced by the single-linkage algorithm contain three single-member clusters which illustrate the danger of specifying the number of clusters *a priori*.

Finally, the model-based clustering algorithm was applied to the data set and asked to identify ellipsoidal clusters. Figure 3d shows the three-dimension exposure cluster plot produced by this algorithm. Comparing these exposure clusters with that from complete-linkage clustering algorithm, we can see significant changes on the members of clusters where there are high exposures to three agents. Therefore, the specification of the cluster shape had an impact on the outcome.

From these results it is clear that different clustering algorithms produce different results when applied to typical exposure data as they do in other statistical applications. The next issue was to investigate whether any of these statistically-defined clusters correlated with the epidemiological classifications of exposure based on work area or work group.

Clusters versus epidemiological classification

When Fig. 2 was labeled according to the exposure zones, it is easily seen that workers in the same exposure zones were classified into different exposure clusters [Fig. 4(A)]. Again, when the exposure clusters were labeled by the work groups A, D, M and N [Fig. 4(B)], one cluster was comprised of only workers from the production work groups (A and D) and another cluster had about two-thirds of the members from the maintenance work groups (M and N). However, 6 out of 16 workers in the production work groups were classified into the exposure clusters of the maintenance

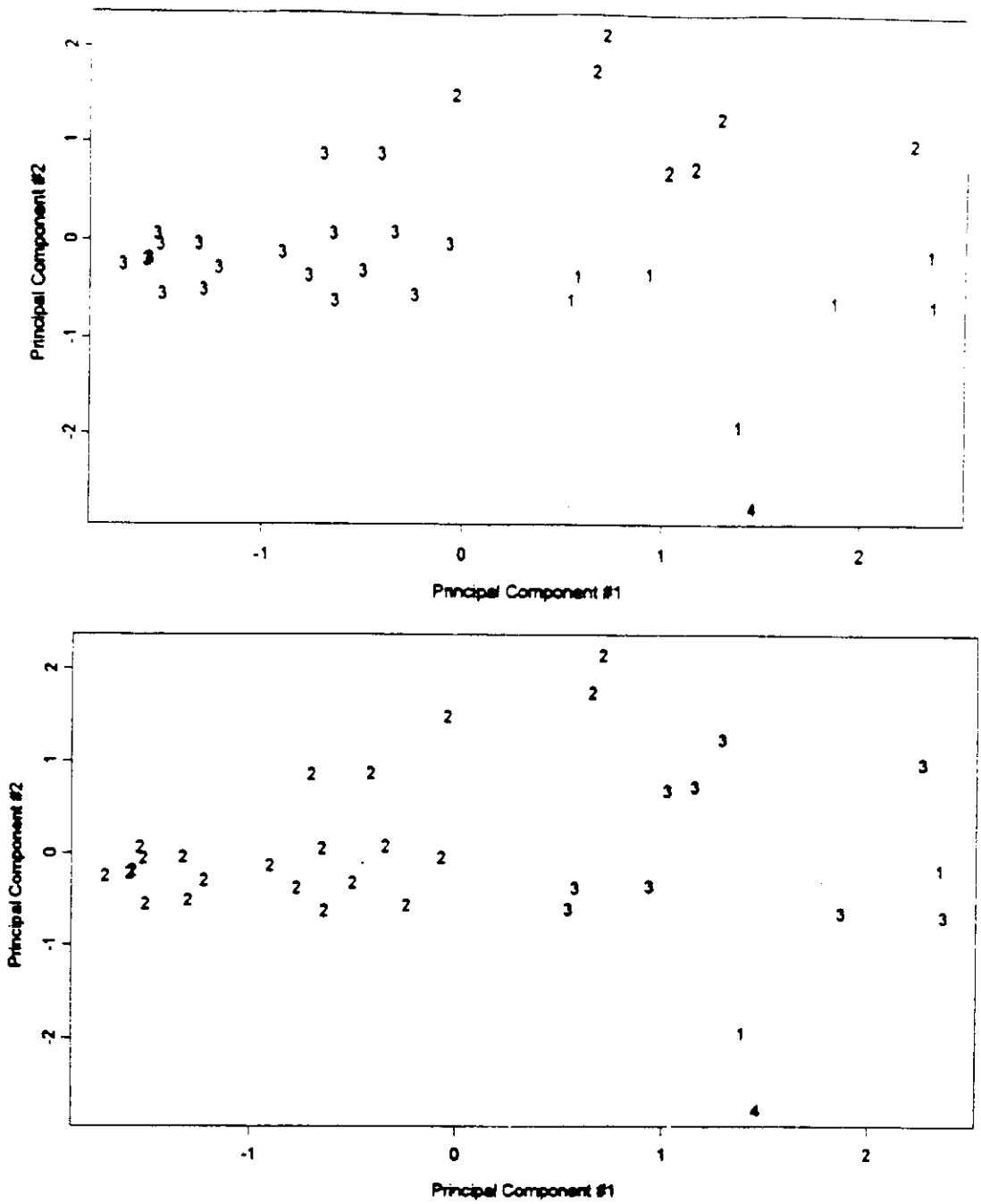


Fig. 3. (a) Principal component plot of workers' standardized mean exposures based on cutting the tree into four clusters (complete-linkage). (b) Principal component plot of workers' standardized mean exposures based on cutting the tree into four clusters (group-average).

work groups and another cluster, formed at high Euclidean distance, consisted of about equal number of workers from both the production and maintenance work groups. All these results illustrate that the exposure classification based on actual measurements differed from that based on subjective criteria. Although the small numbers of workers in some of the exposure zones and clusters made the determination of a representative exposure cluster of an exposure zone difficult, it was evident that the exposure clusters identified from the standardized data set had little relationship with the classification results from either the exposure zone or the work group classification approach. While we cannot allege that one exposure classification approach is superior to another in all settings, most experts in exposure assessment prefer methods based on field measurements versus those based on expert opinion (Kromhout *et al.*, 1992; Post *et al.*, 1991).

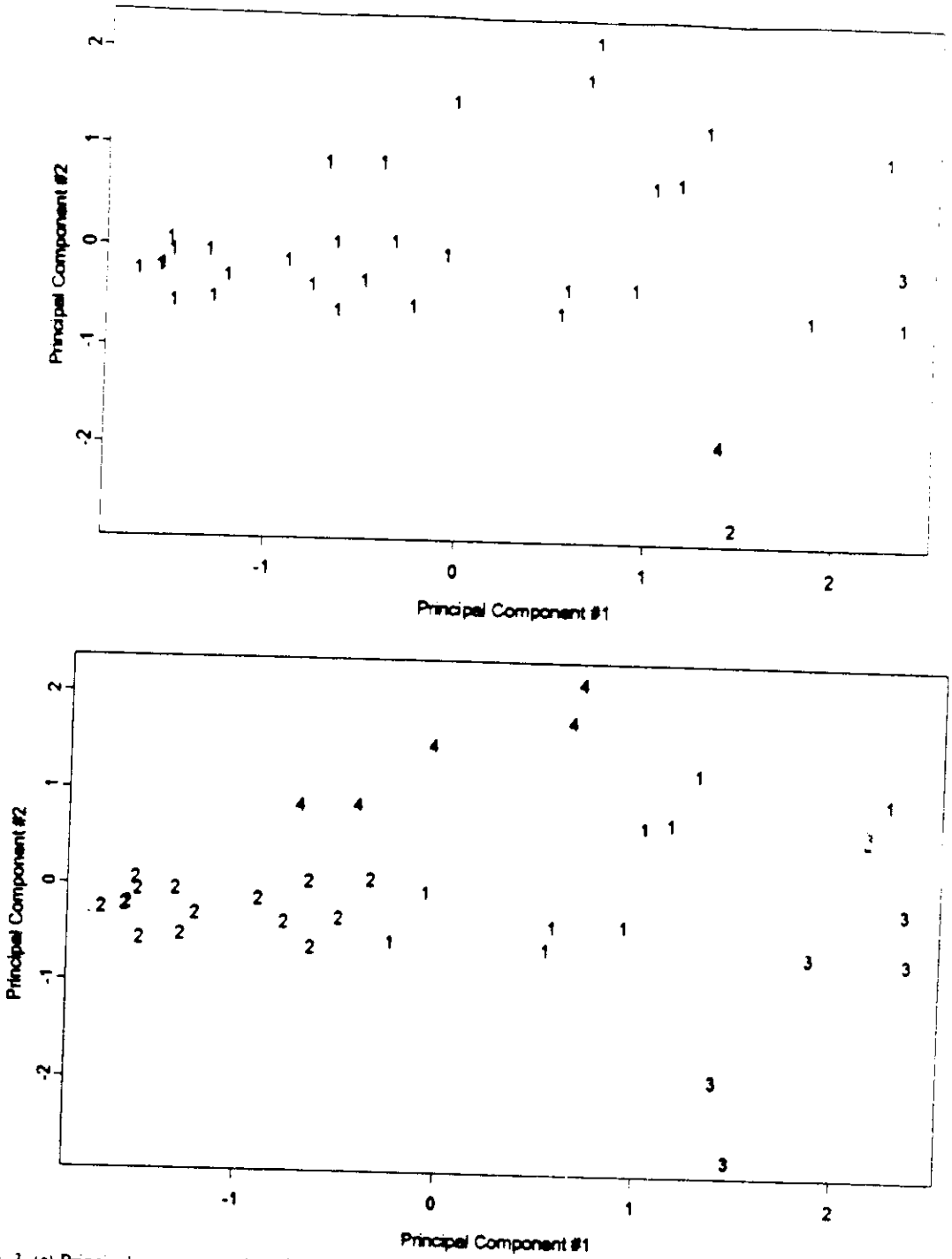


Fig. 3. (c) Principal component plot of workers' standardized mean exposures based on cutting the tree into four clusters (single-linkage). (d) Principal component plot of workers' standardized mean exposures based on cutting the tree into four clusters (model-based).

It is interesting to compare the difference of between-worker geometric standard deviations (GSD_b) of the marginal distributions of each of the three agents between the groupings based on the exposure zone and cluster analysis approaches. Table 5 shows the GSD_b of these two classification approaches. As can be seen, the cluster analysis based on the complete linkage approach tends to give a smaller GSD_b than does the exposure zone approach. While these differences in GSD_b are not large in this case, it must be recalled that the membership in the cluster is quite different than that based on exposure

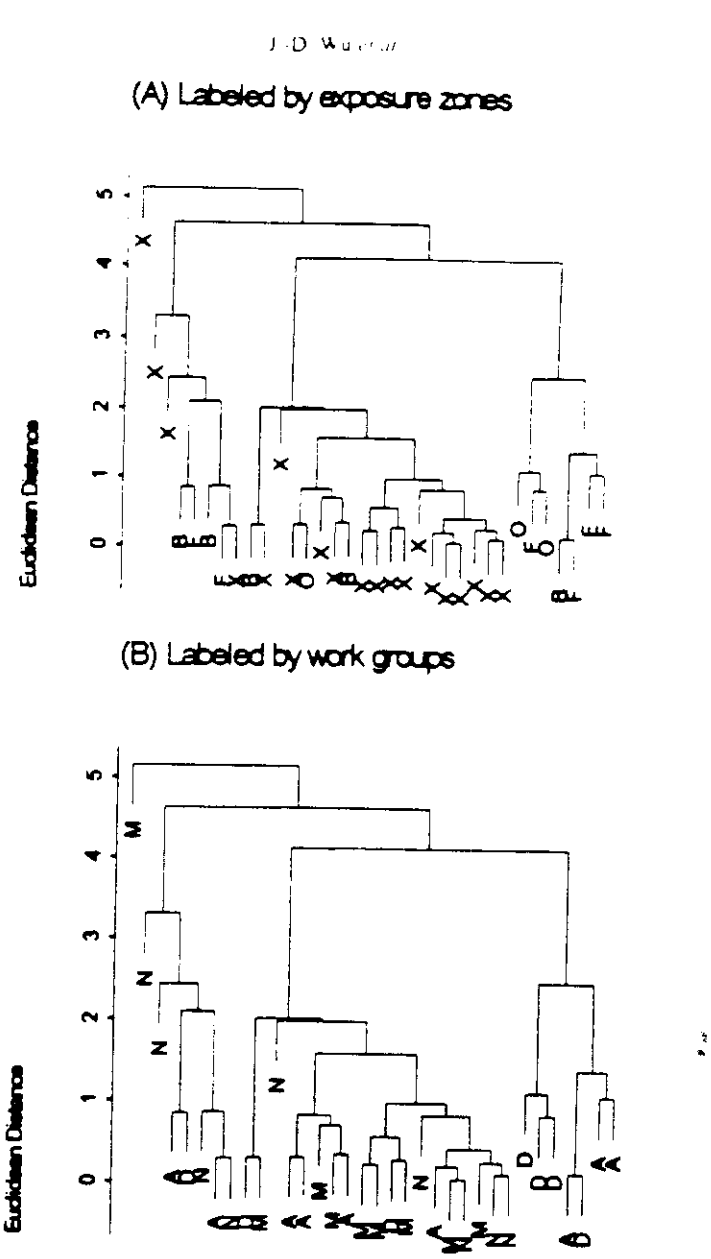


Fig. 4. Hierarchical tree based on workers' mean exposures using complete-linkage and z-standardization.

Table 5. Comparison of gEometric Mean and between-worker geometric standard deviation (GSD_b) between exposure groupings based on cluster analysis and exposure zone approach

Clusters	Number of workers	Cluster analysis: Endotoxin GM	Endotoxin GSD_b	Phenolic compounds GM	Phenolic compounds GSD_b	Formaldehyde tGM	Formaldehyde GSD_b
#1	7	0.035	1.415	52.272	1.496	40.597	1.481
#2	7	0.016	1.583	35.233	1.543	86.125	1.147
#3	19	0.007	1.886	14.121	1.754	26.428	1.544
#4	1	NA	NA	NA	NA	NA	NA

Number of Zones	Number of workers	Exposure zones: Endotoxin GM	Endotoxin GSD_b	Phenolic compounds GM	Phenolic compounds GSD_b	Formaldehyde GM	Formaldehyde GSD_b
F	6	0.023	1.801	46.539	1.281	72.042	1.289
B	5	0.024	1.657	30.666	1.550	49.889	1.452
X	20	0.009	2.63	17.147	2.31	24.692	1.522
O	3	0.012	1.302	20.475	1.188	70.808	1.528

zones. Also, the difference of GMs between groups was larger for the cluster analysis than the traditionally constituted exposure zones.

Determination of number of exposure clusters

Although our results to this point suggest that statistically-based definitions of clusters may not be particularly helpful in analyzing exposure data, there is one additional aspect of cluster methodology that might provide further insight. This relates to the number of clusters that exist in a data set. In all of the foregoing analyses the number of clusters was chosen to match the epidemiological classification and based on the original tree structure of Fig. 2. In exploring a more organized approach to defining the number of clusters appropriate to the data set, the tree structure produced by using the complete-linkage clustering algorithm was used. Figure 5 is the result of applying the VRC (variance ratio criterion) approach mentioned earlier. According to the criterion described by Calinski and Harabasz (1974), we interpret this figure to infer that the most plausible number of clusters is four because the curve flattens at that point. Because the VRC approach was originally based on the single-linkage clustering algorithm, the tree structure produced by using the single-linkage clustering algorithm was also tested by using the VRC approach. The result (not shown here) also indicated that clusters were not well separated and that it was difficult to determine the number of clusters of the tree structure.

The Rand index (Hubert and Arabie, 1985) was used to assess the validity of the tree structure. Briefly, the basic question is, given the fiberglass exposure data, how likely is it that a given number of clusters would be determined by a particular clustering algorithm if there was no underlying structure to the data? In this case, the complete-linkage algorithm was used in a bootstrap scheme to estimate the probability that 3, 4 or 5 clusters would be determined by chance. The bootstrap approach estimated the number of clusters in the data using calculations based on the hypothesis that there was no underlying structure in the data. That is, we calculated the probability of observing certain structures in the data by chance. The result of this bootstrap approach was that, for this data set, the probability of observing 3 clusters by chance was 0.55, of 4 clusters 0.04, and of 5 clusters 0.02. The 3-cluster structure identified in the data was also frequently observed in the random samples but the 4- and 5-cluster structures were not. Hence, a result yielding either 4 or 5 clusters is likely to represent the data structure well, since such a result is unlikely to be observed by chance.

The lack of consistency in the number of clusters of the different determination approaches suggests that there is little separation between the clusters. However, the result is based on a relative small data set for multivariate statistical analysis. The unstable result may limit our ability to make conclusions but it also motivates more consideration of multivariate statistical approachs to classifying multiple occupational exposures and forming homogeneous exposure groups.

DISCUSSION AND CONCLUSIONS

To the extent that a cluster is regarded as identifying an exposure group, it is clear that different clustering

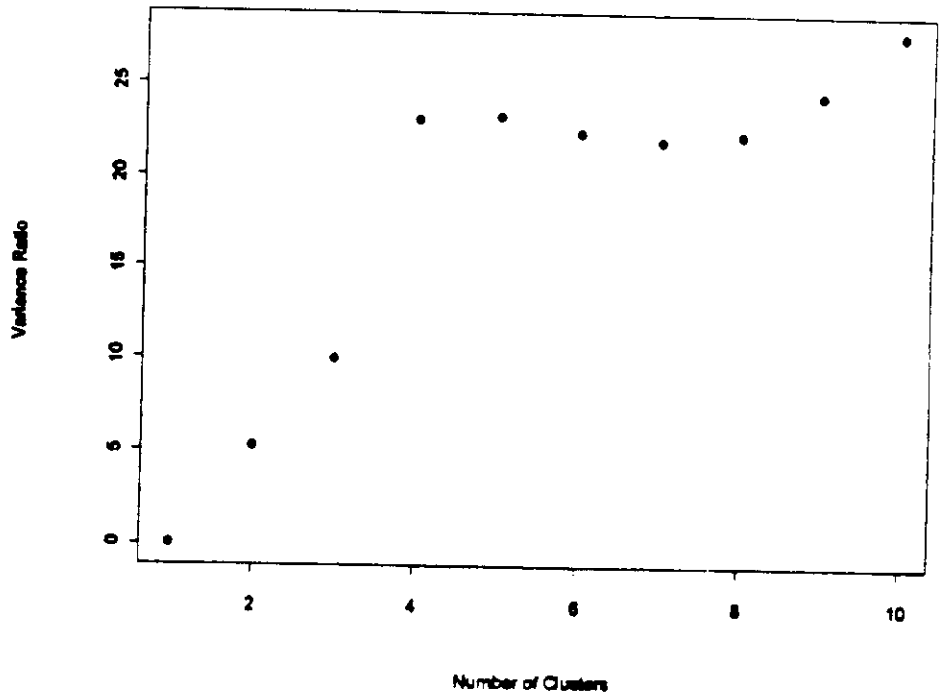


Fig. 5. Relationship between variance ratio (BGSS WGSS) and number of clusters.

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Chronic Health Risks from Aggregate Exposures to Ionizing Radiation and Chemicals: Scientific Basis for an Assessment Framework

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Very little quantitative analysis is currently available on the cumulative effects of exposure to multiple hazardous agents that have either similar or different mechanisms of action. Over the past several years, efforts have been made to develop the methodologies for risk assessment of chemical mixtures, but mixed exposures to two or more dissimilar agents such as radiation and one or more chemical agents have not yet been addressed in any substantive way. This article reviews the current understanding of the health risks arising from mixed exposures to ionizing radiation and specific chemicals. Specifically discussed is how mixed radiation/chemical exposures, when evaluated in aggregation, were linked to chronic health endpoints such as cancer and intermediate health outcomes such as chromosomal aberrations. Also considered is the extent to which the current practices are consistent with the scientific understanding of the health risks associated with mixed-agent exposures. From this the discussion moves to the research needs for assessing the cumulative health risks from aggregate exposures to ionizing radiation and chemicals. The evaluation indicates that essentially no guidance has been provided for conducting risk assessment for two agents with different mechanisms of action (i.e., energy deposition from ionizing radiation versus DNA interactions with chemicals) but similar biological endpoints (i.e., chromosomal aberrations, mutations, and cancer). The literature review also reveals the problems caused by the absence of both the basic science and an appropriate evaluation framework for the combined effects of mixed-agent exposures. This makes it difficult to determine whether there is truly no interaction or somehow the interaction is masked by the scale of effect observation or inappropriate dose-response assumptions.

KEY WORDS: Risk assessment; multiple-agent exposure; ionizing radiation; chemical

1. INTRODUCTION

Modern technologies that consume, produce, release, and dispose of multiple agents have given rise to growing concerns about the cumulative risks of aggregate exposures to physically, chemically, and biologically hazardous agents. Historically, national and international regulatory agencies have conducted risk assessments and set standards primarily for individual hazardous substances. Recognizing this approach may not be adequately protective, some agencies, such as the U.S. Environmental Protection Agency (EPA), have published general guidelines for risk assessment of chemical mixtures.[1] In the past several years, efforts have been made to develop methodologies for risk assessment of chemical mixtures.[2-4] However, aggregate exposures to two or more dis-

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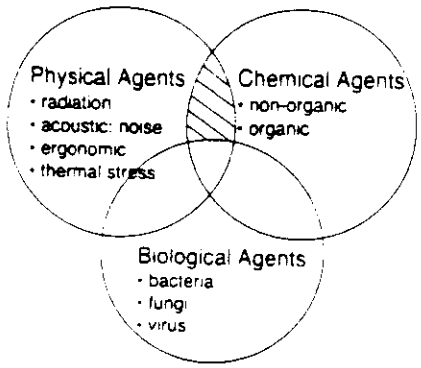

Fig. 1. This figure illustrates how exposures can be aggregated within a particular toxic agent class (i.e., physical agents, chemical agents, biological agents) or across one or more agent classes. The hatched area illustrates the focus of this article on aggregate exposures to a physical agent (ionizing radiation) in combination with a chemical agent.

similar agents—agents from different agent classes—such as radiation and one or more chemical agents, have not yet been addressed in any substantive way (see Fig. 1). Some of the health effects that have been identified for different combinations of multiple-agent exposures are summarized in Table I. For convenience, the term "mixed-agent exposures" is used here as an equivalent term for exposures to two or more dissimilar agents (also see the glossary in the Appendix).

Exposure to ionizing radiation in combination with chemicals is an important problem in many developed countries and is likely to be an issue in developing countries. Not only a growing concern at many hazardous waste sites where various radioactive and toxic chemical wastes are buried, aggregate exposures to these two classes of hazardous agents are also common in the military,[5] in the defense nuclear industry, and in many research laboratories. Moreover, in both the medical research and medical service sectors of modern economies, mixed exposure to ionizing radiation and certain chemicals can frequently occur. Mixed exposure to ionizing radiation and chemicals is also expected to be an important issue for many of the emerging research and technology industries in developed countries.

Historically, the approaches for setting environmental and occupational standards for ionizing radiation and for chemicals were developed by different groups of scientists, with little reference to each other.[6] Therefore, when there are aggregate exposures to ionizing radiation and chemicals from the same source—for example, drinking water—the exposures are seldom considered in a way that would enable cumulative health risks to be assessed. For instance, the World Health Organization (WHO) guidelines for drinking-water quality[7,8] suggest that total radiation dose from all radionuclides is the relevant quantity to be considered; for chemicals, risk addition may be appropriate when several chemical carcinogens are present. Nevertheless, there is no guidance or standard approach for assessing the cumulative risks from the aggregate exposures to both agent types. Exposure standards are usually set for one agent as if the other did not exist, even when the endpoint of concern is the same for both. For agents regulated by different agencies, even the additive approach is rarely applied to assess the cumulative risks associated with mixed-agent exposures. Moreover, there are nontechnical aspects of the differences in approaches to standard setting between radiation and chemicals. Tran, Locke, and Burke[9] argue that much of the distinction in standard setting for these agent classes is due to differences in history and "culture." More credence should also be given to the difficulty of the "boundary" question once the one-compound-at-a-time approach has been abandoned. The problems with defining acceptable levels of risk may become very difficult without a clear rationale for drawing system or operational boundaries around multiple sources of risks.

In an occupational setting, when there are potential synergisms or antagonisms, it does not make sense to only regulate one risk at a time because failure to consider the total health risks from aggregate exposures to multiple agents would significantly impact the cost and efficiency of risk management. In addition, population subgroups with higher risks are unlikely to be identified if only one risk at a time is regulated. Therefore, it is important to consider the total health risks posed by aggregate exposures to the agent(s) of concern over time, and over different exposure routes and pathways. In the following discussions, the term "cumulative health risks" is used to refer to the total health risks from the aggregate exposures of concern. Understanding the cumulative health risks associated with mixed-agent exposure would also inform decisions about what risks to manage in the workplace or the environment.

In this article, the focus and evaluation are on the current understanding of the health risks arising from aggregate exposures to ionizing radiation and chemicals (especially chemical carcinogens). Of particular interest is how mixed-agent exposures (i.e., ionizing radiation and chemicals), when evaluated in aggregation, are linked to both chronic health endpoints such as cancer and common markers of likely

Table I. Examples of the Potential Health Effects Due to Multiple-Agent Exposures

Types of mixtures	Health effects
Chemical mixtures	
Asbestos and smoking	Synergistic increase in the induction of lung cancer was found in asbestos workers with long-term smoking.[42]
Environmental endocrine disrupters	Mixture of two weakly estrogenic chemicals could be 160 to 1,600 times more potent than the individual chemicals in hER-mediated transactivation.[103]
Solvent mixtures	Human central nervous system dysfunction[104,105]
Combined exposure of physical agents	
Ionizing radiation and UV	Interaction between UV radiation and alpha radiation may be involved in the observed increase in skin cancer in uranium miners.[106]
	It is suggested that skin cancer in the irradiated human population is due to the interaction of UV radiation and X rays.[107]
Combined exposure of biological agents	
Pathogenic microorganisms in sewage sludge	Communities that rely on groundwater for domestic use can become exposed to significant levels of pathogens, leading to a potential disease outbreak.[108]
Chemical agents combined with physical agents	
Chemical agents and noise	Significant interaction effect on hearing losses was found in workers with combined exposure to noise and solvents.[109,110]
Tobacco smoke and ionizing radiation	Uranium miners who have smoked 20 pack-years of cigarettes had lung cancer mortality rates per unit of cumulative radiation exposure that are about five times those of the nonsmoking miners.[31]
	The analysis of lung cancer mortality data for uranium miners indicated a synergistic effect associated with combined radon exposure and tobacco smoke.[21,36]
Chemical agents and ionizing radiation	The risk of secondary acute leukemia is significantly greater in cases treated with combined chemotherapy and radiotherapy than with radiotherapy alone.[24,27,28]
	Retinoids have been shown to inhibit X ray–induced transformation of cells *in vitro*.[66]
	Many aspects of radioprotectors and sensitizers have been extensively studied.[53,111]
	The combined effect of phorbal ester (TPA) with either X rays or fission neutrons was synergistic *in vitro*.[64]
	The combined cytogenetic effect of benzene with radiation in cultured human lymphocytes could be synergistic exclusively in dicentrics and rings, but additive for the other types of aberrations.[72]
Hormones and ionizing radiation	Certain hormones increase the expression of radiation-initiated cells in specific tissues.[112]
	Synergism between both estrogens and diethylstibestrol (DES) and radiation has been demonstrated.[47,49,50]
Chemical agents combined with biological agents	
Aflatoxin and hepatitis B virus	There was a strong synergistic interaction between serological markers of chronic hepatitis B infection and aflatoxin exposure in liver cancer risk.[113]
Chemical agents and biological agents indoors	Sick-building syndrome (SBS).[114]
Physical agents combined with biological agents	
UV and fungal infection	It is demonstrated that death from systemic infection of mice with *C. albicans* can be accelerated by exposing them to UV radiation at a critical time before infection.[115]
UV and viral infection	UV exposure resulted in an immunosuppressive effect that is manifest in some instances in increased morbidity or an increased rate of herpes simplex virus (HSV) recrudescence.[116]

Note: hER = human estrogen receptor; UV = ultraviolet.

health outcomes such as chromosomal aberrations. Also explored is the extent to which the current practices in the risk assessment community are consistent with the scientific understanding of the health effects from mixed exposure to these two dissimilar agents. The discussion then proceeds to the research needs for assessing the cumulative health risks of mixed exposure to ionizing radiation and chemicals.

2. CURRENT UNDERSTANDING OF THE POTENTIAL HEALTH EFFECTS THAT RESULT FROM AGGREGATE EXPOSURES TO IONIZING RADIATION AND CHEMICALS

The overview of published studies on potential adverse health effects focuses on a chronic health end-

point, cancer, as well as markers of intermediate health outcomes such as chromosomal aberration (CA), which has been shown in the literature to have potential as a reliable quantitative indicator for future cancer risks.[10-14] The association between cancer risks and the CA frequency in lymphocytes was recently revealed by the preliminary results of two cohort studies carried out in Europe. These two ongoing studies reported a statistically significant linear trend in CA strata with regard to subsequent cancer risk.[11-13]

In the following sections, the epidemiological and experimental evidence of potential interaction caused by mixed exposure to ionizing radiation and chemicals is described. These effects are reviewed according to the experimental systems (human populations, animal, cell culture, etc.) and according to the effects observed. Table II provides a summary of the biological effects that have been reported for exposures to ionizing radiation in combination with chemicals in different experimental systems: human populations, animal populations, *in vivo* systems, *in vitro* systems, and so forth. In this table, the effects are summarized by the types of interaction observed—synergism, additive effects, and antagonism (see the Appendix for definitions of these terms).

The terminology and methods used to characterize the combined effects of two or more agents have been poorly standardized, with some blurring of concepts derived from toxicology, biostatistics, and epidemiology. As Blot and Day[15] point out, interaction is a statistical concept referring to a departure from additivity for a particular model (which may include multiplicative models after transformation on a log scale). In contrast, the terms "synergism" and "antagonism" have often been regarded as public health concepts reflecting the situation in which the effects resulting from combined exposure to two or more agents are respectively greater or smaller than the sum of the effects from exposure to each agent alone. Thus, in the epidemiological context, synergism could be viewed as a particular type of interaction—an interaction based on a null model in which excess relative risks are additive.[15] Rothman[16] argues that "the existence of synergism or antagonism, the causal counterparts of statistical interaction, is neither arbitrary nor dependent on one's viewpoint or scale of observation" (p. 385). The interaction assessment, however, depends on the ability to measure the effects in a meaningful way, and sometimes transformation of the scale of observation can incorrectly indicate the presence of interaction.[16] Therefore, the term "synergism" and the term "interaction" may not necessarily be interchangeable. This situation may well be explained by the studies on the combined exposure to cigarette smoking and asbestos. Data from several published studies[17] reveal that the relative risk of lung cancer following combined exposure to these two agents can be approximated by the product of the relative risks of smoking and of asbestos exposure, which is consistent with a multiplicative model without interaction. Alternatively, the same data are also consistent with an additive excess relative risk model with a positive interaction term. If the multiplicative model is accepted as the underlying null model, then one would conclude that there was no interaction between smoking and asbestos. In the public health context, however, cigarette smoking and asbestos exposure can be said to be strongly synergistic, which means that the number of lung cancer cases resulting from both cigarette smoking and asbestos exposures are greater than the sum of the lung cancer cases resulting from one agent exposure alone.

2.1. Human Experience—The Effects of Mixed Exposure to Ionizing Radiation and Chemicals

For mixed exposure to ionizing radiation and chemicals, the epidemiological evidence concerning the potential interaction of human carcinogens (mostly involving long-term cigarette smoking) has been extensively reviewed.[18-21] Of particular interest in terms of carcinogenesis is whether additive or multiplicative risk models (see the Appendix) better describe the exposure–effect relationships. Nevertheless, a quantitative risk assessment methodology has not yet been well developed. The significant human studies are summarized below and listed in Table II.

2.1.1. Cancer

To date, studies of radiotherapy in combination with chemotherapy and studies of smoking and radon exposure are the only human studies relating mixed-agent exposures to cancer. These studies provide evidence for chemical/ionizing radiation interaction in humans and are summarized below.

Surviving patients with Hodgkin's disease have been followed to determine the risk of secondary cancer in relation to treatment. These studies raise the question of whether the treatment should be modified so that the risk of secondary cancer can be reduced. A number of studies indicate that the risk of secondary acute leukemia is significantly higher in patients treated with combined radiotherapy and chemother-

Table II. Effects and Types of Interaction Reported to Result from Mixed Exposures to Ionizing Radiation in Combination with Chemicals

Interaction	Agents exposed	Experimental systems or populations	Endpoints observed	References
Synergism	Benzene and ionizing radiation	Human peripheral lymphocytes	Chromosomal aberrations	72
	Smoking and radon exposure	Uranium miners	Lung cancer	18, 21, 31, 36
	Pocarbazine and X rays	(BALB/c × DBA/2)F_1 mice	Lung tumors	48
	Myleran and ionizing radiation	Mice	Thymic lymphoma	45
	DMBA and ionizing radiation	BALB/c mice	Mammary tumors	51
	Phorbal ester (TPA) and ionizing radiation	C3H $10T_{\frac{1}{2}}$ cells	Malignant transformation	64
	CCl_4 and neutron irradiation	Mice	Liver tumors	46
	DES/estrogen and ionizing radiation	Rats	Radiation-induced breast cancer	47, 49, 50
	Cortisone and X rays	C3H $10T_{\frac{1}{2}}$cells	Oncogenic transformation	62
	Isoniazid and X rays	Bone marrow cells of Chinese hamster	Chromosomal aberrations	57
	Cadmium acetate and ionizing radiation	Rats	Biochemical changes in lung	59
	Paraquat and ionizing radiation	Rats	Biochemical changes in lung	60
	Lead chloride and ionizing radiation	*In vitro* embryonic cells	Increase of micronuclei	65
Additive effects	Smoking and ionizing radiation	Atomic bomb survivors	Lung cancer	32
	Chemotherapeutic agents and ionizing radiation	Peripheral lymphocytes from cancer patients	Chromosomal aberrations	39, 40
	Thiophosphamide and ionizing radiation	Human lymphocytes *in vitro*	Chromosomal aberrations	70
	Mitomycin-C and γ rays	Human lymphocytes *in vitro*	Chromosomal aberrations and SCEs	71
	Urethane and X rays	Rats	Tumors	52
	17-β estradiol and X rays	C3H $10T_{\frac{1}{2}}$cells	Oncogenic transformation	63
Antagonism	Radioprotectors and ionizing radiation	BALB/c mice	Lung tumors	53
	Retinoids and X rays	Rodent cell culture *in vitro*	Radiation-induced oncogenic transformation	61, 66
	Selenium and ionizing radiation	Rodent cell culture *in vitro*	Radiation-induced cell transformation	117

Note: DMBA = 7,12-dimethylbbanzzantracnene; DES = diethylstibestrol; SCEs = sister chromatid exchanges.

apy than with radiotherapy alone,[22-28] but the data have not been evaluated in a way that provides information about how combined exposures interact. In one retrospective analysis of multicenter trial data, the risks of secondary leukemia after chemotherapy alone and combined chemotherapy and radiotherapy were not significantly different.[29] A case-control study carried out in the Netherlands reported a more-than-multiplicative interaction between the carcinogenic effects of smoking and radiotherapy.[30]

The association of combined smoking and radon exposures with lung cancer risk has been discussed extensively in the literature.[18,21,31-36] The uranium miner study reported by Whittemore and McMillan[31] suggests a strong synergistic effect between radon and tobacco smoking in the induction of lung cancer, and the interaction of smoking and radiation exposure was found to be multiplicative. In a 1988 study,[18] the BEIR (Biological Effects of Ionizing Radiation) IV Committee of the U.S. National Research Council (NRC) reported that the findings of epidemiological studies on uranium miners, particularly the large study by Whittemore and McMillan,[31] were consistent with a multiplicative relative risk model for the combined effects of cigarette use and radon exposure. A quantitative model for this interaction was not, however, provided by BEIR IV. Moolgavkar, Luebeck, Krewski, and Zielinski[33] analyzed the lung cancer mortality data from the Colorado Plateau miners cohort by applying the two-mutation clonal expansion model of carcinogenesis; it was the first attempt to apply a biologically based model to describe cancer mortality rates among the Colorado miners. Taking into account the patterns of exposure to radon and cigarette smoking experienced by individuals in the cohort, the age-specific relative risks associated with combined exposure to radon and cigarette smoking were found to be more than additive (superadditive), but less than multiplicative (submultiplicative). When synergism is based on a null model of additive excess relative risk (or additive incidence rate difference), their data seemed to indicate a synergistic effect between radon exposure and cigarette smoking. However, since the authors found no suggestion of any interaction between radon and cigarette smoke on the cellular level, they did not provide a direct answer to the question of synergism. The latest update from the Colorado Plateau uranium miners cohort also indicated a synergistic effect associated with the combined exposure to radon and cigarette smoking.[36] In the more recent BEIR VI Committee report on risks from radon exposure,[21] the analysis of miner studies indicated a synergistic effect associated with the combined exposure to these two agents. Based on the approach developed by Lubin and Steindorf,[35] the BEIR VI lung cancer risk model for radon exposure was adjusted to explicitly account for smoking status. In contrast to the studies of radon, no significant synergistic effect was found between radiation and tobacco smoke exposures in atomic bomb survivors who died of lung cancer.[32] The differences between uranium miners and atomic bomb survivors come to mind as potential explanations for this discrepancy, which include different exposure timing and patterns between these two populations and the dose distributions in the lung; that is, concurrent long-term mixed-agent exposure for uranium miners versus transient radiation exposure in combination with long-term cigarette use for atomic bomb survivors who died of lung cancer.

2.1.2. Cytogenetics (Chromosomal Aberrations)

The data obtained from studies on human organisms are of great interest in understanding the cytogenetic effects of ionizing radiation and chemicals. Evidence for how interactions of ionizing radiation and chemicals impact CAs is provided by studies of radiotherapy in combination with chemotherapy and studies of workers occupationally exposed to mixed ionizing radiation and chemicals.

The peripheral blood lymphocytes of cancer patients treated with radiotherapy in combination with chemotherapy provide an opportunity to study the combined effect on CAs.[37,38] Two studies reported additive effects of aberrations from combined radiotherapy and chemotherapy.[39,40] Most of the studies of induced chromosome aberrations in cancer patients, however, focused more on the clinical and biological aspects of the observations rather than understanding the potential interaction between radiotherapy and chemotherapy or the potential interaction between radiotherapy and tobacco smoking.

Although there have been very few studies addressing the health outcomes of workers occupationally exposed to ionizing radiation in combination with chemicals, there have been studies of the impact of combined exposures on CAs. Brandom, McGavran, Bistline, and Bloom[41] analyzed the frequency of CAs and sister chromatid exchanges (SCEs) in plutonium workers with different levels of internal and external irradiation, and occupational exposure to single or multiple chemicals (e.g., benzene, trichloroethylene). Their results could only demonstrate a

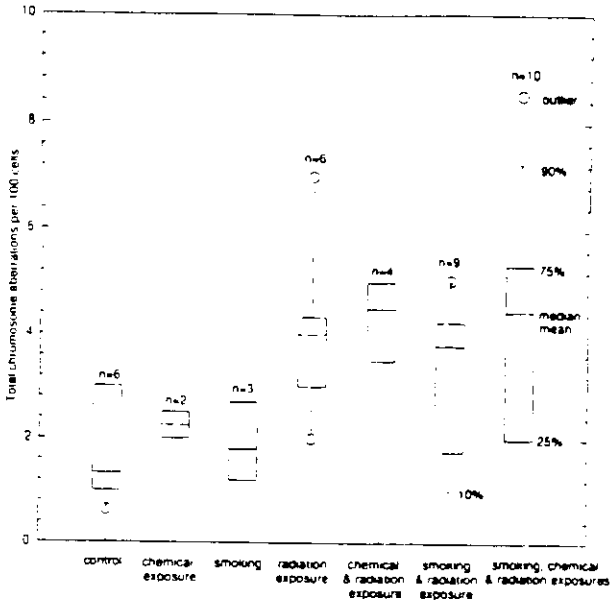

Fig. 2. The cytogenetic effects of Chernobyl cleanup workers as a result of different types of exposure. Chemical exposures include occupational exposures to gasoline and oil products, synthetic dyes, solvents, and so forth. Data from Lazutka and Dedonyte.[42]

significant increase in CA frequencies in cells of workers who had an intake of plutonium greater than 740 Bacquerels (Bq). The potential interaction of mixed-agent exposures was not discussed in this study.

In Lithuania, a cytogenetic examination of the workers involved in cleaning up radiation from the Chernobyl release revealed that the increases of CA frequencies were strongly associated with the levels of radiation exposure and that a slight increase in CA frequencies was found to be associated with occupational exposure to various chemicals and smoking.[42] The authors did not provide any quantitative measures for the chemical exposures, however, and no significant interaction was reported between chemical and radiation exposures. The results of this study are illustrated in Fig. 2.

Gundy[43] observed a two- to sixfold increase of CA frequencies in workers exposed to low-level ionizing radiation, that is, below the internationally accepted dose limit. The author also reported that workers occupationally exposed to chemical mutagens such as vinyl chloride and organic solvents like benzene and toluene have two to four times higher CA frequencies than the controls; but the potential interaction between chemical and radiation exposures was not addressed in those subgroups with mixed-agent exposures.

2.1.3. Other Health Endpoints

In another series of studies on the combined effects of chemotherapy and radiotherapy, lesions such as pulmonary fibrosis that may occur in the treatment of malignant neoplasms were the health endpoints considered.[44] However, the potential interaction has never been quantitatively characterized for mixed-agent exposures on these noncancer endpoints.

2.2. Animal Data on the Combined Effects of Ionizing Radiation and Chemicals

The combined effects of ionizing radiation and chemicals have been reported in a number of animal studies. Several of these studies are summarized in Table II. In most of these experimental studies the combined effects have been evaluated in terms of carcinogenicity and cytogenicity.

2.2.1. Cancer

The combined action of ionizing radiation and various chemicals has been found to result in synergism in the induction of cancer in rats and mice.[45-51] In one past study, urethane was found to have an additive effect in tumor induction in rats when combined with X-ray exposure.[52] Still other studies have demonstrated the antagonistic effect between ionizing radiation and a group of chemicals that were used as radioprotectors.[53]

For the case of radon/smoking, animal experiments on the combined exposure to cigarette smoke and radon progeny have not yielded strong evidence of synergism,[54,55] and the findings of synergism are inconsistent and sometimes dependent on the sequence of exposures.[54,55] In contrast, most of the human studies on mixed radon/smoking exposures focus the efforts on the long-term health effects, and seem to be fairly consistent in showing the synergism as a result of the interaction between these two agents.

2.2.2. Cytogenetics (In Vivo Studies)

Typically, mutations such as CAs at a specific tissue site are used to study the health effects of combined radiation/chemical exposures *in vivo*. This makes it possible to estimate the effectiveness of mutagen interactions and the modification of their effects by

another mutagen.[56] For example, Miltenburger and Korte[57] reported that the chromosomal damage in bone marrow cells of Chinese hamsters was significantly enhanced after 48-hr treatment of the animals with isoniazid combined with X rays, where the interaction was found to be synergistic. Other studies have observed CA changes qualitatively but the interaction has not been quantitatively assessed—see, for example, Hong, Alfieri, Kim, and Kim.[58]

2.2.3. *Other Health Endpoints*

In addition to the use of mutation markers (e.g., CAs), attempts have been made using several other biochemical markers, such as enzyme activities, to identify explanatory attributes for the effects of mixed exposure to ionizing radiation and chemicals.[59,60] Most of these studies focused on *in vivo* biochemical changes caused by mixed-agent exposures, but the observations were not directly related to any specific disease endpoints. To date, these nonmutation effect markers have rarely been demonstrated in epidemiological studies to be associated with human cancer risks or other disease endpoints.

2.3. Supporting *In Vitro* Evidence: Cytogenetic Damage Due to the Combined Effects of Ionizing Radiation and Chemicals

A number of investigations of the combined mutagenic effects of ionizing radiation and chemicals have been carried out *in vitro*. These studies are summarized in Table II. Most of these studies evaluated the combined effects on mammalian cells in terms of mutagenicity and carcinogenicity,[61–64] cytogenicity,[65,66] and teratogenicity.[67,68] Most of these studies have been carried out in cultured lymphocytes and embryonic cells.

The combined effect of X rays and thiophosphamide, a known chemotherapeutic agent, has been extensively studied. Korotkikh and Tarasov[69] observed an increased cytogenetic damage by the combined action of X rays and thiophosphamide on fibroblast cultures of human embryos. Unfortunately, the authors did not specify if there is a synergistic increase in the CA levels. It was not possible to classify the interactions as synergistic or additive without having the original experimental data. Bochkov, Yakovenko, and Voskoboiynik[70] also studied the influence of the timing and order of mutagen treatments on the combined effects of radiation and thiophosphamide in lymphocytes; these authors concluded that the additivity model could explain the combined effects for the cases of weak interaction. Another study by Iijima and Morimoto[71] reported that the combined effects of mitomycin-C (a chemotherapeutic agent) and γ rays are additive based on CAs and SCEs as endpoints.

A synergistic increase of micronuclei was found *in vitro* as a result of the combined exposure to lead chloride and ionizing radiation, accompanied by an inhibition of embryonic development.[65] An earlier study by Morimoto[72] indicated that the combined cytogenetic effects of benzene with γ radiation appeared to be synergistic exclusively in dicentrics and rings, but almost additive in other types of aberration. It has also been shown that benzene at higher concentrations may inhibit the repair of radiation-induced chromosome breaks.[73] In addition, the major metabolite of benzene, phenol, can also significantly inhibit rejoining of radiation-induced chromosome breaks at very low concentrations.[74]

Natarajan, Obe, and Dulout[75] have observed that the combined effect of radiation and caffeine depends on multiple factors, such as the cell cycle phase when radiation is applied, length of the time interval after caffeine treatment when radiation is applied, and interindividual variability of lymphocyte culture of donors from different groups. For assessing cytogenetic effects of radiation in combination with chemicals, a further complicating issue is suggested in this work by the fact that these effects may vary at different stages of the cell cycle.

2.4. Summary Findings on Cumulative Effects of Mixed-Agent Exposures

In both residential and occupational settings, human populations are rarely exposed to a single agent, but to various concentrations of mixtures. Based on the present review of studies on combined effects of ionizing radiation and chemicals, there is insufficient evidence to conclude which type of interaction is more likely to occur. However, there is an indication of potential interactions (especially the synergism observed in several human and animal studies, as well as supporting *in vitro* studies), which warrants further studies in this arena. In addition, the absence of relevant human and animal data makes it difficult to construct a two-agent dose-response function that can be used to develop risk models for combined exposures to ionizing radiation and chemicals.

Ideally, it is desirable to classify certain groups of chemicals as having the potential of synergistic response when combined with ionizing radiation, and others as having the potential of antagonistic response when combined with ionizing radiation. The data currently available in the literature, however, do not provide the opportunity to reanalyze the data from those past studies. As a result, it is almost impossible at this point to conclude that synergism is more likely to occur for certain groups of chemicals and antagonism for others. This reveals the problems caused by the absence of a systematic evaluation framework for the combined effects of toxic agents from different agent classes. Without an appropriate evaluation framework, one cannot pose questions about how combined exposures interact, or to organize and design studies to answer these questions. Moreover, based on the studies carried out in workers exposed to low levels of mixed-agent exposures, it is observed that both exposure measurements and the health effects quantified by various dose-response assumptions are important factors if one wants to identify or quantify the potential interactions in either an epidemiological or toxicological study, the standard approach for assessing human health risk. Such information will provide a foundation for evaluating the conflicting and negative epidemiological evidence reviewed in the above.

The cytogenetic studies reviewed above did provide evidence of potential interaction in the induction of chromosomal damage from combined exposures to ionizing radiation and specific genotoxic chemicals. For cases where biomarkers of response are defensible predictors of cancer risk, it may be plausible using intermediate biomarkers such as CAs to characterize the dose–response relationships of combined exposures to ionizing radiation and certain genotoxic chemicals. Nevertheless, it should be noted that the interpretation and comparison of CA studies are sometimes difficult. This difficulty comes about in large part because the experimental protocols differ with regard to treatment procedures, exposure time frame, cell stage at the moment of or shortly after exposure, and experimental systems used, that is, human or animal populations, *in vivo* or *in vitro* systems, and so forth. Once this difficulty can be overcome, it would be of great interest to use CAs or other potential surrogate markers as a more reliable explanatory variable to explore the dose–response relationship for mixed-agent exposures. Markers such as CAs have a lower threshold and provide an outcome measure much sooner after exposure than a disease outcome such as cancer. If biomarkers such as CAs do indeed correlate with later cancer incidence then their use should significantly improve the resolution of the dose–response relationship.

3. CURRENT AND PROPOSED APPROACHES FOR RISK ASSESSMENTS OF MULTIPLE-AGENT EXPOSURES

The risk assessment paradigm set by the NRC in 1983[76] and updated in 1994[77] has been widely used in the process of health risk assessment. The NRC paradigm, however, is directed primarily at single-agent exposures. In the past decade, there has been considerable effort made to develop and refine methods for risk assessment of chemical mixtures.[2] In contrast, there has been almost no focus on development of risk assessment methods for the cumulative effects from exposures to two or more dissimilar agents, such as ionizing radiation in combination with chemicals. Nevertheless, the current risk assessment approaches for multiple-agent exposure (such as those for chemical mixtures) may provide some common ground from which to develop an appropriate framework for evaluating the health risks from aggregate exposures to ionizing radiation and chemicals. To evaluate the extent to which the current practices in the risk assessment community are adequate to incorporate the scientific understanding of the health risks associated with mixed-agent exposures, presented below is a review of the approaches for regulating mixture exposures as well as the risk assessment methods used for multiple-agent exposures.

3.1. Current Approaches for Regulating Exposure to Mixtures

In regulating workers' exposures to mixtures, the approach used by the American Conference of Governmental Industrial Hygienists (ACGIH) is to consider the combined effects when two or more hazardous agents acting upon the same organ system are present.[78] When the components in a mixture have similar toxicological effects, the threshold limit value (TLV) of the mixtures

$$\frac{C_1}{TLV_1} + \frac{C_2}{TLV_2} + \cdots + \frac{C_n}{TLV_n}$$

should not exceed unity, where C_i indicates the observed atmospheric concentration (time weighting not specified at this point) and TLV_i the corresponding threshold limit. When the main effects of the dif-

ferent hazardous agents are not additive but are independent, as when purely local effects on different organs of the body are produced by the various components of the mixture, then the TLV is exceeded only when one member of the series itself has a value exceeding unity, for example,

$$\frac{C_1}{TLV_1} \geq 1, \text{ or } \frac{C_2}{TLV_2} \geq 1.$$

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) recently defined the application of MAK values (maximum concentration at the workplace) to mixtures of substances in its List of MAK and BAT Values.[79] MAK values for mixtures are only established after specific toxicological evaluation of the mixtures of concern. In practice, there are a few cases in which a common MAK value for the sum of all components was provided, such as for mixtures of isomers or mixtures of related compounds, in which the components of the mixture show comparable toxicological effects. For mixtures containing components with genotoxic and carcinogenic potential, the MAK Commission considered it inappropriate to establish a safe threshold; this approach is still under discussion. Currently, carcinogenic mixtures are categorized according to either the classification of the carcinogens included in the mixture or the carcinogenicity of the mixture. For components with the same target organ and mode of action or with interfering metabolism, synergistic effects must be expected and the respective exposure limits must be lowered.[80] Nevertheless, if there is evidence that the components act independently, the exposure limits of the individual compounds are not modified.

3.2. The U.S. EPA Risk Assessment Guidance for Chemical Mixtures

In 1986, the U.S. EPA issued risk assessment guidelines for chemical mixtures.[1] The main objective of these guidelines was to develop a general approach for evaluating data on the chronic and subchronic effects of chemical mixtures. The framework developed by the U.S. EPA described three approaches that can be used to conduct a quantitative risk assessment for the potential health effects associated with exposure to chemical mixtures. Which of these approaches applies depends on the availability of data on the mixture and on the components in the mixture, that is: (1) data available on the actual mixture of concern, (2) data available on similar mixtures, and (3) data available only on mixture components.[1]

For these situations the U.S. EPA provides guidance as follows. When data are available on the health impacts of the mixture of concern or similar mixtures, these data should be used in formulating the risk models. When data are not available on the actual mixture or similar mixture of concern, data from risk assessments of individual components are then used to estimate the risk of the mixture of concern by applying a dose additivity model for systemic toxicants and a response additivity model for carcinogens, if no interactions occur.[1] The dose additivity model assumes that the components in the mixture have the same mode of action and elicit the same health effects. Currently, the two most accepted dose additivity approaches are the hazard index (HI) approach and the toxicity equivalency factor (TEF) approach. The response additivity model is primarily used in cancer risk assessment of chemical mixtures; it is assumed that the components in the mixture act independently on the same target site but by different mechanisms of action, thus the toxicological responses to each component in the mixture are summed.[1,2]

In recent years, a major advance in chemical mixture risk assessment was the newly developed interaction-based method outlined by Mumtaz and Durkin.[81] This method uses binary interaction data to modify the dose-additive hazard index as follows:

$$HI_I = HI_{ADD} \times UF_I^{WOE_s}, \qquad (1)$$

where HI_I is the interactive HI based on the approach developed by Mumtaz and Durkin,[81] HI_{ADD} is the noninteractive HI based on dose addition; UF_I is the uncertainty factor for interactions, and WOE_s is the scaled binary weight-of-evidence score. This new procedure reflects the strength and consistency of the evidence provided by available interaction studies as well as the amounts of each component in the mixture. The weaknesses in this new approach include (1) little guidance is provided on selecting the uncertainty factor for interaction, (2) the procedures for determining the binary weight-of-evidence score are fairly complex, (3) the magnitude of the interaction is not included, (4) no systematic procedure is given to determine the relative weights applied to various categories of information, and (5) the interaction information is only presented by the multiplicative factor $UF_I^{WOE_s}$, which is applied to the entire additive HI (HI_{ADD}).

A new U.S. EPA guidance document, *Guidance for Conducting Health Risk Assessment of Chemical Mixtures*,[4] as a supplement to the original *Guidelines*

of 1986,[1] is currently under external scientific peer review. This new guidance provides more specific details on the nature of the desired information and the procedures for data analyses. It also reflects recent scientific advances in the area of chemical mixture risk assessment, including methods for using whole-mixture data on a toxicologically similar mixture, methods for incorporating information on toxicological interactions into an HI—modified from the original method developed by Mumtaz and Durkin,[81] procedures for including carcinogen interactions in mixture risk characterization, and generalized procedures for mixtures involving classes of similar chemicals.

The dose additivity and response additivity assumptions widely used in chemical mixture risk assessment may result in substantial errors in risk characterization if synergistic or antagonistic interactions occur. For example, when the interactions depend on the dosing sequence, the results by using the dose additivity approach will be the same for any dosing regiment (concurrent, sequential, sequential with delay, etc.) but the response could vary significantly among the dose regiments. Although the U.S. EPA[1] discussed several mathematical models and the measurement of combined action in general, no guidance was provided in their risk assessment guidelines for chemical mixtures on how to assess the potential synergism of multiple-agent exposures. Presumably the dose additivity approaches used for chemical mixtures may not work for the combined exposure to ionizing radiation and chemicals since these two agents simply do not have similar mechanisms of action.

3.3. Methods Considered by the NRC and Others

3.3.1. NRC Model Proposed to Estimate the Risks of a Mixture of Carcinogens

In 1988, the NRC Committee on Methods for the *In Vivo* Testing of Complex Mixtures[19] developed a generalized additive model to estimate the risks of a mixture of carcinogens regardless of the model used to obtain the risk estimates for the individual components in the mixture. To date, this generalized model has only been calibrated by Reif,[82] who applied this approach to examine the individual and combined effects of tobacco smoking and uranium exposure. The model was proven satisfactory in predicting the risk of lung cancer in individuals exposed to both cigarette smoke and uranium. However, more data sets are still needed so that the model can be adequately validated before its widespread applicability can be evaluated.

3.3.2. NRC Model for Assessing the Risk from Exposure to a Mixture of Compounds Acting Independently on the Target Sites

According to the NRC's *Science and Judgement in Risk Assessment*,[77] the risk from exposure to a mixture of compounds that act independently on the target sites can be estimated as follows:

$$P = 1 - \prod_{i=1}^{m} \prod_{j=1}^{n} (1 - P_{ij}). \qquad (2)$$

In this expression P is the probability of any toxic effect associated with a set of multiple-agent exposures, and P_{ij} is the probability of toxic endpoint j for agent i. This equation states that the combined probability of any effect is equal to 1 minus the probability of not experiencing any effect among a number of agents, which basically follows the statistical law of independent events. More specifically, when a low-dose, nonthreshold model is assumed for each independent event probability, P_{ij}, Equation (2) becomes

$$P = 1 - \exp\left(-\sum_{i=1}^{m} \sum_{j=1}^{n} q_{ij} D_{ij}\right), \qquad (3)$$

where D_{ij} refers to the effective dose rate and q_{ij} (the linear coefficient in dose) is the parameter characterizing the potency of compound i for inducing toxic endpoint j.[77] For very small values of P ($<< 1$) relevant to environmental regulatory concern, P can be approximated by

$$P \approx \sum_{i=1}^{m} \sum_{j=1}^{n} q_{ij} D_{ij}. \qquad (4)$$

The key limitation of this model is that, because it is based on the assumption that all agents act independently, it is of little value in exploring anything other than additive impacts. Thus, it cannot be used to explore potential interactions.

3.3.3. BEIR VI Lung Cancer Risk Model for Radon Exposure and Smoking Status

The generalized additive model developed by the NRC in 1988[19] as well as the lung cancer risk model recently developed by the NRC BEIR VI Committee[21] are the only ones that have been applied to deal with aggregate exposures to two dissimilar agents. Currently, no standard methods are yet in place in regulatory agencies to incorporate mixed-agent interactions.

and no biologically based mathematical models have been developed that could serve as a default method.

In most of the studies that explore the combined effects of radon exposure and cigarette smoking, the exposures from smoking are not assessed quantitatively. Hence, even though the NRC BEIR VI Committee formally addressed smoking status in the committee's lung cancer risk model,[21] the committee found the data on smoking still too sparse to develop an explicit mathematical model for estimating the combined effects of smoking and radon exposure. Thus, instead of using a quantitative measure of cigarette smoking, semiquantitative measures (e.g., "never-smokers," "ever-smokers") were used to assess smoking status and the contribution of smoking status to relative risk. In spite of the models in BEIR VI,[21] which use quantitative measures for one agent, no single mathematical expression has yet provided an accurate and unified representation of mixed-agent risk estimates.

3.3.4. Risk Assessment Methods Considered by Others

Chen, Gaylor, and Kodell[83] proposed a formal statistical method for estimating the combined risk of multiple-agent exposure based on single-agent experiments. Although this method can be used to estimate the risks for more than one agent, it is based on the assumption that the overall risk for mixtures is additive. In the past few years, efforts have also been made to develop mechanistic models of carcinogenic and noncarcinogenic processes to be applied in chemical mixture risk assessment. Kodell, Krewski, and Zielinski[84] have reported that the two-stage clonal expansion model of carcinogenesis can be applied to mixtures of compounds that may affect the same or different stages of the carcinogenic process. Cohen and Ellwein[85] have also developed a mechanistic model in a form that can be applied to the mixtures of chemical carcinogens. These mechanistic models may be able to handle chemicals and ionizing radiation, but this issue has not yet been addressed in the risk assessment community.

4. DISCUSSIONS AND RECOMMENDATIONS ON THE RESEARCH NEEDS FOR ASSESSING THE HEALTH RISKS OF MIXED-AGENT EXPOSURES

The goal of this article is to review and evaluate the current understanding of the health risks arising from combined exposures to ionizing radiation and chemicals (especially chemical carcinogens). In this section, a summary of key findings and recommendations that derive from this effort is provided.

4.1. Are Synergisms More Likely to Occur for Mixed Exposures to Ionizing Radiation and Chemicals?

Even though there is insufficient evidence to conclude which type of interaction is more likely to occur for the combined effects of ionizing radiation and chemicals, the current literature review indicates there are potential nonadditive interactions being observed, in particular, synergistic effects. This warrants further study. The literature review also revealed that very few studies have been conducted in a systematic manner to evaluate the cumulative health impacts of mixed-agent exposures. This makes it difficult to judge whether there is truly no interaction or rather that somehow the interaction is masked by the scale of observation or by inappropriate dose-response assumptions. We believe that an evaluation framework that makes possible consideration of such potential interactions would provide an incentive for more study.

4.2. The Limited Power of Epidemiological Studies May Be Inadequate to Uncover the Potential Synergisms That Would be Important from a Policy Perspective

To evaluate the health impact of mixed-agent exposures, the important questions to ask are whether and how these combined exposures interact. If they do interact it must be determined whether they act additively, synergistically, or antagonistically on the disease outcome in human populations. To answer these questions requires scientific studies that can measure these potential interactions. It is important to recognize that without the explicit use of models the problem is simply intractable. There are far too many pairs of compounds (much less triplets, etc.) to go about this empirically. Some potentially generalizable principles must be put forward, tested, and accepted/rejected, and models are a useful starting point for this process.

Based on what is known of past and present levels of mixed radiation/chemical exposures in human populations, it appears to be difficult to obtain from cancer incidence data statistically robust measures of interactions in populations exposed to low levels of ionizing radiation and chemicals. Under the theory of generalized linear modeling, one commonly sees that

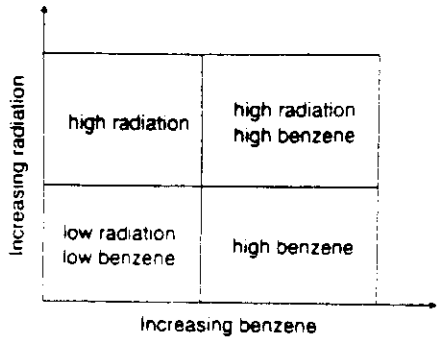

Fig. 3. An example for the design of epidemiological visibility assessment: mixed exposures to ionizing radiation and benzene.

the interaction is "epidemiologically visible" (see glossary in the Appendix) when the coefficients of exposure products are significantly different from zero. This means that the interaction can be observed through an epidemiological investigation only when the statistical variation in the health effects is at the same scale as the variation in the combined logarithms of exposure measures. When there are mixed exposures to ionizing radiation and chemicals that have the same disease endpoint—such as ionizing radiation and benzene both having leukemia as a disease endpoint—it would be of great interest to determine the characteristics of the dose–response relationships that would make the interaction epidemiologically visible (see Fig. 3 as an example). Such information will be useful to establish the dose-response functions for mixed-agent exposures and can also provide a foundation for evaluating the conflicting and negative epidemiological evidence such as the studies reviewed in Section 2.

4.3. Carefully Designed Studies of CAs May Have the Potential to Reveal the Synergisms Caused by Mixed Exposure to Genetoxic Agents

The value of CAs as a biomarker of intermediate health outcomes has been confirmed by a large number of biological monitoring studies that have been carried out in populations exposed to genotoxic agents.[86] CA frequency in peripheral blood lymphocytes is a sensitive cytogenetic assay for detecting both exposure and risk from mutagens and carcinogens;[87] it has also been used for dose assessment in radiation exposures[88-92] and in occupational exposures to several chemicals.[93] However, as described in Section 2.1, the combined cytogenetic effects of radiation and chemicals have not yet been studied in a systematic way to elucidate the nature of biological interactions associated with these two agents.

The proposed guidelines for carcinogen risk assessment[94] have noted that the precursor effect assessment may provide insight for the likely shape of the dose-response curve for tumor incidence below the range of tumor observation. Recent analyses by Mendelsohn[95] suggested that CA is a potential risk marker for radiation-induced leukemia. It is found that the dose-response curve of CA frequency in atomic bomb survivors overlies the dose-response curve of the radiation-induced leukemia data, indicating a strong association between CA frequencies and leukemia risks. Sorsa, Wilbourn, and Vainio[10] also suggest that structural CAs *in vivo* have advantages over other cytogenetic endpoints in predicting potential human cancer risk. The preliminary results of two ongoing cohort studies have recently revealed the existence of an association between cancer risks and the CA frequency in lymphocytes.[11-13] Since ionizing radiation and many genotoxic chemical carcinogens have chromosomal damages as intermediate health outcomes and also have cancer as a disease endpoint, it is of interest to evaluate to what extent nontumor markers like CAs can provide information about the dose–response relationships of mixed-agent exposures.

4.4. Need of Standardized Procedures for Characterizing the Risks of Mixed-Agent Exposures

An important issue that has not been explored in the environmental health science community for stochastic endpoints (i.e., cancer and heritable genetic effects) is whether it is possible to define a tissue-specific measure of dose response with equivalent units for chemical and radioactive agents. Thus, there is an important research need to develop methods for synthesizing scientific information on the damage resulting from aggregate exposures to ionizing radiation and chemicals. Controlled studies carried out in experimental systems on cumulative effects of radiation and chemicals will be useful for dose-response assessment to develop models predicting either health outcomes or early health effects. In particular, such studies should be designed to support the development of a two-agent dose-response surface model. This requires systematic experiments to investigate the underlying dose–response relationship of ionizing radiation in various combinations with chemicals. Another important need is for experimental systems

to facilitate exploration of the extent to which concurrent exposure or the timing of sequential exposures makes a difference in any expected interaction effects. Several past studies, as summarized in Section 2 (see 2.1 and 2.2 for examples), have revealed the possibility that exposure timing could play a role in the induction of the disease endpoints.

4.5. Proper Characterization of the Uncertainties in Extrapolation from Experimental Data to Human Risks

For dose-response assessment, the problem of relating the levels of dose applied *in vitro* to human exposure is one of the major blocks to incorporating experimental data into a risk assessment framework, that is, how do we convert the dosing into a useful metric for comparison? We could not identify standard methods for converting *in vitro* data to *in vivo* doses. To fill this gap, there is a need for work on physiologically based pharmacokinetic (PB-PK) modeling. It should give some idea of how to convert the dose levels used in a tissue culture experiment back to a plausible human exposure. Moreover, as the interaction effects may result from events taking place at the site of toxic action or during the processes of absorption, distribution, metabolism, excretion, or repair, the application of PB-PK modeling becomes an important tool for evaluating the weight of evidence for interactions. Some initial efforts in PB-PK modeling have been made for simple and complex chemical mixtures.[96]

4.6. Need of Risk Assessment Guidance That Is More Explicit in Addressing the Combined Effects of Mixed-Agent Exposures

Better understanding of the combined effects of low-level exposures to multiple agents is an important challenge for occupational and environmental health scientists in this century. To date there has been little scientific assessment of exposures that overlap in time. Very little has been done with agents from different agent classes. Essentially no risk assessment guidance has been provided for two agents with different mechanisms of action (e.g., energy deposition on DNA from ionizing radiation versus DNA interactions with chemicals) but similar biological effects (CAs, mutations, and cancer). In particular, little guidance has been provided on how to assess the potential for interaction (especially for synergism) among these agents. Moreover, in situations where there are aggregate exposures to agents managed through different agencies and regulatory guidelines, even the additive model is rarely applied. An evaluation framework is needed to define studies that can measure potential interactions and provide the opportunity to characterize the health risks associated with aggregate exposures to ionizing radiation and chemicals.

For combined exposure to two or more carcinogens, it should be noted that the U.S. EPA has not addressed any aspect of the mixture issue in the 1986 *Guidelines for Carcinogen Risk Assessment*[97] and its proposed revision.[94] The 1996 Food Quality Protection Act (FQPA)[98] requires the U.S. EPA to consider the cumulative effects of pesticide residues and other substances that have a common mechanism of action in setting allowable levels of pesticides on a food crop. The risk assessment approaches for mixed pesticide residue exposures, however, are still under development. Recently, the newly published guidelines for ecological risk assessment[99] and neurotoxicity risk assessment[100] both address the mixture issue in general terms but not for human receptors.

4.7. Need of Case Studies on the Health Effects of Mixed-Agent Exposures

To carry out a mixed-agent risk assessment, it is necessary not only to collect detailed aggregate exposure data on both ionizing radiation and chemicals, but also to develop methods for synthesizing the information on the health impacts resulting from mixed-agent exposures. We believe that case studies addressing these issues will provide the opportunity to examine how the findings of synergism would impact a risk-based framework for protecting humans from mixed-agent exposures. These case studies could also be used to identify information gaps and future research needs. It is believed this work will also lead to important evaluations and recommendations for current occupational and environmental exposure standards.

APPENDIX: GLOSSARY OF TERMS

Terms used in this article are defined as follows

Additive effects: When the effects of the combined action are equal to the sum of the effects of the two or more agents acting alone, the resulting situation is called "additive effects."[102]

Agent classes: Refers to three classes of agents—physical agents, chemical agents, and biological agents.

Aggregate exposures: For the agents of concern, this refers to the exposures that are combined among

the agents over time, and over different exposure routes and pathways.

Antagonism: When the combined action results in an effect that is less than expected from the sum of the effects of the two or more agents acting alone, the situation is termed "antagonism."

Cumulative health risks: Refers to the total health risks posed by aggregate exposures to the agent(s) of concern over time, and over different exposure routes and pathways.

Epidemiological visibility: When the response (rate, incidence, risk, or probability) of effects from the exposure of concern is significantly different from the response of effects without the exposure of concern, and when such difference can be observed through an epidemiological investigation, then the response of effects from the exposure of concern is defined as "epidemiologically visible." Under the theory of generalized linear modeling, one commonly sees that the interaction is epidemiologically visible when the coefficients of exposure products are significantly different from zero, which means that interaction is statistically present on the scale used in the model and can be observed through an epidemiological investigation.

Mixed-agent exposures: An equivalent term for exposures to two or more dissimilar agents, which is distinct from exposures to mixtures of compounds in the same agent class such as chemical mixtures.

Multiplicative/additive risk models: Multiplicative relative risk models are based on an assumption that the relative risk resulting from the exposure to two risk factors is the product of the relative risks from the two factors taken separately, which can be given by $RR(z_1, z_2) = R_1(z_1) \times R_2(z_2)$, where $R_1(z_1) = RR(z_1, 0)$ and $R_2(z_2) = RR(0, z_2)$ are relative risk functions for each agent separately. An additive relative risk model is similarly described by $RR(z_1, z_2) - 1 = [R_1(z_1) - 1] + [R_2(z_2) - 1]$. At a specified $z = (z_1, z_2)$, relative risk models can be classified as supermultiplicative, multiplicative, or submultiplicative according to whether $RR(z)$ exceeds, equals, or is less than $R_1(z_2) \times R_2(z_2)$. Similarly, the relative risk may be superadditive, additive, or subadditive according to whether $RR(z)$ exceeds, equals, or is less than $1 + [R_1(z_1) - 1] + [R_2(z_2) - 1]$.

Synergism: Synergism occurs when the effect of two or more agents acting together exceeds the sum of their effects when acting alone. In the epidemiological context, where the effects are usually measured as disease risks, synergism (or positive interaction) is observed when the risk attributable to the combined exposure exceeds the sum of the risks attributable to each exposure separately.[16] Blot and Day argue that synergism could also be viewed as "a particular type of interaction—an interaction based on a null model in which excess relative risks are additive" (p. 99).[15] Similar to that suggested by Blot and Day, Rothman, Greenland, and Walker[101] argue that, in the public health context, synergy and antagonism should ordinarily be interpreted as departures from additivity of incidence rate differences, while in the context of individual decision making, synergism should ordinarily be interpreted as a departure from additivity of risk differences.

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Integration and Exploration of Task-Based Multivariate Exposure Data: Part I: Design of an Exposure Simulator

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Abstract

While there is a long tradition in industrial hygiene practice of characterizing worker exposures by direct measurements in the workplace, there is the potential for improved exposure assessments by incorporating qualitative aspects of the structure of the work. This need is particularly acute in situations where exposure is to multiple agents within a workday because of the added cost and complexity of collecting sufficient 8-hour TWA measurements to adequately characterize exposure variability. We have attacked this issue by constructing a computer-based exposure simulator which produces multivariate 8-hour TWA estimates for homogeneous exposure groups based on the task structure of the work, the specification of airborne chemicals to which the groups are exposed, task-based exposure distributions, task time estimates, arid assuptions regarding the correlation and autocorrelation structure of short-term exposures. The correlation structure of the short-term estimates is required in order to capture the character of exposures arising from the use of products comprised of more than one chemical or from the sequential use of several chemicals in a task. This correlation structure, the need to simulate the autocorrelation of short-term exposures, and the log-normal character of workplace exposure distributions pose challenges in the generation of multivariate time series with the desired properties. An approximate solution is presented and its properties demonstrated. An application of the simulator to the characterization of solvent exposures in raft manufacturing is presented in a companion paper.

Introduction

There is a long tradition in industrial hygiene practice of characterizing worker exposures by direct measurements in the workplace. Whether these measurements were made using fixed position samplers or personal samplers, measurement has been preferred in the occupational setting to a model-based approaches where the spatial and temporal emission characteristics of the source and subsequent air mixing patterns are used to characterize exposures. While model-based approaches are common in community air pollution studies and occasionally in indoor air or workplace studies (Nicas, 1996), the preference for the measurement-based approach in industrial hygiene has led in recent years to a statistical perspective on workplace exposure assessment. Often the focus has often been on the probability of compliance with standards (Selvin et al., 1987; Lyles and Kupper, 1996; Tornero-Velez, et al., 1997) or with understanding the sources of variability in the measured data (Kromhout et al., 1993; Symanski and Rappaport, 1994). However, because industrial hygienists tend to have considerable information on the nature and activity of workers and the materials they are working with, it is attractive to integrate this information with measurement data into exposure assessments in some structured way. This suggests a modeling approach that is a hybrid between the statistical models and models based on physical and chemical principles typical of engineering analyses.

There has been some effort to impose *a priori* structure on workplace exposure data, largely from the perspective of minimizing resources devoted to making the measurements. The exposure zoning concept of Corn and Esman (1979) was based on the premise that professional judgment could identify groups of workers who were similarly exposed, thereby assuming away the need to characterize differences in exposure between them and reducing the measurement burden. This homogeneous exposure group (HEG) assumption has been shown not to be a common property of measured data sets (Kromhout et al, 1993) which has led some to prefer more elaborate statistical models (Rappaport et al. 1995). Nevertheless, the HEG the notion persists and

we shall adopt it below, albeit as an initial step on the road to a more realistic construct. However, the fundamental notion of Corn and Esman, that a measurement strategy can be informed by qualitative data about the nature and structure of work, is patently sensible. Our attempt here is to include such qualitative data in an explicit way.

As will be seen, a fundamental complication of our approach to the problem arises because of our interest in simultaneous exposures to multiple airborne chemicals. Initially we attempted to extend the statistical approach to exposure characterization to the case where multiple agents are involved. We found that the increase in dimensionality, coupled with the nature and very limited extent of field data, tends to preclude all but exploratory analyses of these data. Hence, there was considerable motivation for us to consider using more of the data available to the hygienist than is commonly done when approaching the problem from a strictly statistical perspective. Moreover, with increasing interest in multiple exposures, we decided to attack the more complicated multivariate problem from the outset.

Because the most common index of the risk of exposure is based on eight-hour TWA concentrations, we chose to address the specific problem of forecasting the distribution of the eight-hour TWA values based on the task structure of the work, task-specific chemical usage, and estimates of short-term exposure distributions. To integrate the various elements which determine the eight-hour TWA distributions, we chose to implement our approach through an exposure simulation program. The simulator is task-based as outlined by Nicas and Spear (1993). In essence, a workday is comprised of a specific number of tasks, each of which occupies a given fraction of the workday and, in each of which, a particular set of chemicals are used. In our current version of the simulator the basic exposure descriptor is the distribution of 15-minute TWA values, so tasks are comprised of specified numbers of 15-minute segments in a workday.

The principal complexity introduced into the simulator by designing it to accommodate multiple chemical agents is the possibility of a correlation structure in the short-term exposures. This might be due to what we term *product-based* exposures. That is, it is

common that particular products, for example positive photo-resists in semi-conductor chip fabrication, are comprised of several chemicals. Hence, exposure to one implies a simultaneous exposure to the others (Hines et al., 1995). Alternatively, the task may involve several chemicals used simultaneously or sequentially within the 15-minute period which may also result in correlated exposures. Hence, to accommodate these possibilities, the simulator has been designed with a capability of simulating correlated exposures.

In simulation exercises of the sort we will describe in detail below, it is important to be clear from the beginning that the input data is subject to various forms of uncertainty. For some elements the uncertainty is modest, and for others, considerable. Hence the output of the simulations must be interpreted as simply the integrated result of the best input information we have at the time of the analysis. Whatever the specific use of the simulator, we regard it as a tool for exploring preliminary and approximate information rather than an adjunct to the analysis of comprehensive data sets.

Structure of the Simulator, Inputs and Outputs

In addition to acknowledging the task-based structure of work and the possibility of correlation between short term exposures, the simulator is also structured to reflect the following properties of workplace exposures:

- Short-term exposures are generally assumed to be log-normally distributed (Esmen and Hammad, 1977; Kumagai and Matsunaga, 1994, 1995a and b; Kumagai et al. 1997;)
- Short-term exposures occurring sequentially are autocorrelated (Francis et al., 1989; Kumagai et al. 1993)
- Exposures are classified as near or far-field depending on the position of the worker with relation to the source.

Hence, the input data to the simulator are of two sorts, those descriptors relating to the task structure of the workday and chemical usage by task, and those parameters describing the short-term exposure distributions as well as their correlation and autocorrelation. The issue of autocorrelation arises from the fact that the underlying nature of exposure data is most naturally described as time series data (Spear, et al., 1986) where airborne concentrations, for example, cannot change instantaneously. Hence, concentrations close together in time are more highly correlated than those distant in time. There is also the suggestion in some community air pollution studies that the autocorrelation structure of exposures may be an independent risk factor relating to health outcomes (Pope and Dockery, 1992).

Specifically, the input variables to the simulator are:

- the number of tasks in the workday and the time spent at each;
- the chemicals used in each task which generate airborne exposures and a specification of their correlation structure, if any;
- an estimate of the mean and variance of each 15-minute TWA concentration of each chemical used, by task, together with an estimate of an autocorrelation parameter. It is assumed that the autocorrelation function is approximately an exponentially decaying function of the interval between 15-minute segments of the time series.
- for correlated agents, an estimate of the correlation matrix which describes the correlation of each pair of chemicals in the product in each 15-minute period.
- the ratio of far to near field exposures. It is assumed that this is a general characteristic of the workspace and constant for all components of the exposure in a given task.

Given the foregoing input information, the simulator generates an eight-hour TWA value for each individual in the workgroup and for each chemical used in the workplace:

$$C_8 = \sum_{h=1}^{k} \sum_{i=1}^{n_h} \frac{C_i}{32} \qquad (1)$$

where C_i is the 15-minute TWA concentration of a single chemical in the i-th task, n_h is the number of 15-minute periods during which the i-th task is conducted over the day, and k is the total number of tasks. The simulator generates random samples of the C_i with the statistical properties outlined above and repeatedly calculates values of the vector of eight-hour concentrations $\mathbf{C_8}$. The dimension of this vector equals the total number of chemical agents used in all tasks. These values provide the basis for the calculation of whatever statistical characteristics of the multivariate distribution of eight-hour TWA values are of interest.

As presently configured, the simulator does not distinguish between individuals in a workgroup. Hence, for a given task structure, task times, chemical inventory, and short-term exposure distributions, the distribution of $\mathbf{C_8}$ is for a homogeneous exposure group. Equivalently, the distribution is that for a single worker over different workdays, assuming no correlation between one workday's exposure and the next.

The complexity of the simulator arises from the generation of the 15-minute values C_i. First, consider the issue of autocorrelation. If the workday is structured so that the all n_h 15-minute periods during which a particular task is conducted are continuous in time, then the C_i values should be generated with the appropriate autocorrelation structure. Alternatively, if the task is conducted during periods interspersed throughout the day, then it is more appropriate to set the autocorrelation to zero thereby defining the interspersed exposures in this task to be independent. The second complex issue in generating the C_i relates to the possibility of correlated exposures. If, for example, a task involves the use of a product which generates airborne exposures with more than one chemical component, then we assume that the C_i for each component of the mixture are not independent, but correlated. We now turn to the issue of generating log-normally distributed numbers that will produce the desired exposure structure.

Random Number Generation

Male (1982) showed how a log-normally distributed time series with a specified autocorrelation function could be generated from a normally distributed random variable. Male employed a first-order autoregressive process of the form:

$$X_t = \alpha X_{t-1} + Z_t \qquad (2)$$

where $\sigma_x^2 = \sigma_z^2/(1 - \alpha^2)$, and the autocorrelation function can be shown to be $\rho(k) = \alpha^k$ for k greater or equal to zero. If a new variable is now defined, $Y_t = \exp(X_t)$, then the mean and variance of X are related to the mean and variance of Y by:

$$\mu_x = \ln(\mu_y) - \sigma_x^2/2 \quad \text{and} \quad \sigma_x^2 = \ln(1 + \sigma_y^2/\mu_y^2) \qquad (3)$$

Similarly, the relation between the autocorrelation functions is given by:

$$\rho_x(k) = \frac{\ln(1 + \rho_y(k)\frac{\sigma_y^2}{\mu_y^2})}{\ln(1 + \frac{\sigma_y^2}{\mu_y^2})} \qquad (4)$$

The derivation of equations 3 and 4 is given in the Appendix.

Since, the process described by equation 2 has an autocorrelation function given by $\rho_x(k) = \alpha^k$, and this function adequately describes what we know of that observed in workplaces, we desire that $\rho_y(k)$ possesses similar characteristics. It can be shown that σ_y^2/μ_y^2 depends only on GSD_y. Clearly, for highly autocorrelated processes, eg. $\rho_x \equiv 1$, $\rho_x \equiv \rho_y$ regardless of the value of GSD_y. If GSD_y is small, say 1.25, it is also the case that $\rho_x \equiv \rho_y$. If $GSD_y = 3.0$ which is high for 15 minute distributions, then a ρ_x

of 0.8 corresponds to a ρ_y of 0.7. But, a ρ_x of –0.8 yields a ρ_y of -0.27 due to the compression of the logarithmic transformation. Negative values of the autocorrelation function arise from oscillatory processes which have not been reported in workplace air monitoring applications. Hence, in most industrial hygiene applications we suggest that the autocorrelation structure is adequately represented by the first order autoregressive model of equation 2 with positive α. Therefore, for our purposes, ρ_y is positive and approximately equal to ρ_x .

Since the log-normal parameters will generally be the input parameters, equations 3 and 4 yield the parameters of an x-variable which is the process generated by the simulator according to equation 2 and then exponentiated to yield the time series of 15-minute TWA values of the air concentrations. For each chemical agent without short-term correlation, the simulator uses the foregoing approach, treating each chemical exposure within a task independently. However, if the exposures are assumed to be correlated, a multivariate approach is required.

Extending the general approach of Male to the multivariate case, we use a multivariate normal process of the form:

$$\mathbf{x}_{k+1} - \mu_x = \mathbf{P}(\mathbf{x}_k - \mu_x) + \mathbf{Q}\mathbf{u}_k \qquad (5)$$

where $\mathbf{u}_k$ is a vector of independent N(0,1) variables, $\mathbf{P}$ a square matrix of dimension n x n, and $\mathbf{Q}$ an n x r rectangular matrix where $1 \leq r \leq n$ (Takahashi, et al., 1970) . The eigenvalues of $\mathbf{P}$ must lie within the unit circle in order for the process to be stationary. Hence, the $\mathbf{x}_k$ are normally distributed, random variables with mean vector μ_x whose covariance matrix is determined by multiplying equation 2 by its transpose to yield:

$$\mathbf{X} = \mathbf{P}\mathbf{X}\mathbf{P}^T + \mathbf{Q}\mathbf{U}\mathbf{Q}^T \qquad (6)$$

where $\mathbf{X} = E(\mathbf{x}_k\mathbf{x}_k^T)$ and $\mathbf{P}^T$ is the transpose of $\mathbf{P}$. The multivariate equivalent of the autocovariance function is given by $\Gamma = E(\mathbf{x}_{k+m}\mathbf{x}_k^T) = \mathbf{P}^m\mathbf{X}$.

As in the univariate case, we generate a normally distributed time series, $\mathbf{x}_k$, which, when exponentiated, results in a log-normal series, $\mathbf{y}_k$, with the desired properties. Since the input data refer to the log-normal variables, it is necessary to calculate the normal parameters needed to specify **P** and **Q**. The transformation equations given in equation 3 allows the calculation of the means and variances, and needed in equations 5 to 6, which will result in normally distributed time series $\mathbf{x}_k$ that will yield the desired series, $\mathbf{y}_k$.

Given the normal parameters, the next step is to specify **P** and **Q** to produce exposure series consistent with our design specifications. The specified input parameters define the correlation matrix **X** and the mean values of each of the chemicals. However, the multivariate situation is somewhat more complicated with respect to the autocorrelation structure. As noted above, we regard an exponentially decaying function of the general form $\rho(k) = \alpha^k$ with $0 \le \alpha \le 1$ as an adequate description for our purposes since we may certainly expect the autocorrelation function to be exponentially bounded as long as a reasonably well-functioning general ventilation system is in use. Hence, we wish to impose a similar condition on the product-based exposures which requires that the diagonal elements of the matrix $\Gamma = E(\mathbf{x}_{k+m}\mathbf{x}_k^T) = \mathbf{P}^m\mathbf{X}$ are approximately equal to ρ_x^m unless there is a reason to believe that some components of the mixture will disappear or persist in air at a significantly different rate than others. To achieve this we impose the additional condition on the **P** matrix that $\text{diag}(\mathbf{PX}) = \text{diag}(\mathbf{RX})$ where **R** is a diagonal matrix with diagonal elements equal to ρ_x which can all be equal or differ by compound. As we shall show, this condition seems to produce the desired time series remarkably well.

Implicit in equation 5 is a cross correlation structure between the elements of $\mathbf{x}_k$ in different 15-minute time intervals. For example, to what degree does the current concentration of chemical 1 correlate with the concentration of chemical 2 forty-five minutes from now? (Again using a community air pollution example, the morning concentration of hydrocarbons in Los Angeles air is strongly cross correlated with the ozone concentration in the afternoon.) The matrix **X** describes the degree of that

correlation within any 15 minute interval, but $\mathbf{P}^m\mathbf{X}$, for m > 0, defines these correlations between intervals. However, the study of this cross-correlation structure has not, as far as we know, been the subject of study in the industrial hygiene literature. Hence we chose not to impose any cross-correlation conditions beyond the specification of the covariance matrix, **X**. However, the fact that the eigenvalues of **P** lie within the unit circle insures that the elements of $\mathbf{P}^m\mathbf{X}$ approach zero as m gets large which is consistent with physical expectations. For example, it is highly unlikely that 15-minute exposures to chemical 1 at 8:00AM will have any correlation with exposures to chemical 2 at 4:30 PM.

The specification of **P** and **Q** begins, then, with the need to meet the conditions imposed by equations 6 and the diagonal condition that diag(**PX**) = diag(**RX**) . In addition, there are a set of inequality constraints that must be satisfied in order that **P** be a stable matrix. For any given **P** and **Q** this can be checked in various ways (Takahashi, et al., 1970) .

Because **X** is symmetric, equation 6 generates n(n + 1)/2 equations and the diagonal condition an additional n equations. **P** contains n^2 elements and **Q** rn elements. Even for r =1 there are more unknowns than equations, so there is no unique solution. Hence, there are many values of the elements of **P** and **Q** which will satisfy our conditions. Since the equations are nonlinear, we chose Broyden's variant of the Newton-Raphson method (Press et al., 1992) to find a solution which minimizes a sum of squares criterion function. A solution is deemed acceptable when the criterion function is less than 10^{-6}. The values of **P** and **Q** thus determined are then inserted in equation 5, the time series x generated, and each x-value exponentiated to yield the y-values, the 15-minute TWA's for the product-based agents.

Testing the Multivariate Random Number Generator

The first test of the product-based exposure simulator assumed a product of three chemical components and used the following input data:

$$X = \begin{bmatrix} 46.6 & 91.3 & 418. \\ 91.3 & 231. & 877. \\ 418 & 877 & 5460 \end{bmatrix}$$

$$\mu_y = \quad (7.1 \quad 21.1 \quad 99.9) \qquad (7)$$

$$\text{diag}(\mathbf{R}) = \quad (0.65 \;\; 0.65 \quad 0.65)$$

The values of the cross-correlations implicit in **X** are 0.90 between chemical 1 and 2, and 0.80 between 1 and 3, and between 2 and 3.

A set of random initial guesses for the values of **P** and **Q** were selected from within the range (-1, 1) to start the iteration process. In some cases, these initial values led to local minima and the routine could not converge to an acceptable solution. In these cases a new random starting point was chosen and the process restarted. One thousand different but acceptable realizations of the time series described by the above parameters were generated. Each was of length 500.

Figure 1 shows a time plot from a typical realization of the three series in which the cross-correlation structure is apparent, for example, the large simultaneous peak just prior to day 400. Figure 2 shows a histogram of the values of y_k, without regard to their sequence in time, which displays their log-normal character. Table 1 shows the characteristics of the 1000 replications. Clearly, the mean values of each of the input parameters estimated from the 1000 observations is quite close to the design value given in equations (7) above. The variability about the mean is most easily seen from the coefficient of variation, expressed at the bottom of the table in percent which shows that only the variance of the three times series shows considerable variation from run to run as is commonly the case.

To verify our qualitative conclusions about the relation of the autocorrelation of the x-series and it relation to that of the y-series, as well as to verify the effect of the condition

that diag(**PX**) = diag(**RX**), we conducted a second set of numerical experiments with the same μ_y as above but with the other parameters as given in Table 2. The GSDs include the variances of the y-series used above, but the other values were used to assess the effect of varying the ratio of σ_y^2/μ_y^2 on the autocorrelation of the y-variables as described in equation 4. Recall that $GSD_y = \exp[\ln(1 + \sigma_y^2/\mu_y^2)]^{1/2}$

For each set of GSD_y values 400 sets of time series were generated each with different solutions for **P** and **Q**. Each series was 200 time steps in length (200 15-minute intervals). Figure 3 shows the mean value of the first 15 values of the autocorrelation function for each GSD_y. As can be confirmed by checking values of the corresponding x-series autocorrelation, values, $\rho_x(k) = \alpha^k$, the effect of the variation of the GSD_y on the mean autocorrelation function of the y-series is very modest. Recall that the x-series values are equal to the diagonal elements of the matrix **R** which are the inputs to the simulation. Figure 4 shows the corresponding effect on the standard deviation of values of the autocorrelation function as the GSD_y varies. Here we do see a general tendency for more variability in the autocorrelation function as the GSD_y increases. Although the variability in the autocorrelation function begins to decrease at fairly low values of time lag, k, it does not decrease nearly as fast as the mean values in Figure 3 which implies that the relative variability increases with the time lag.

Constructing the Exposure Series Over the Workday

Recall that the eight-hour work day is comprised of h tasks each of which is carried out over n_h fifteen-minute periods. Hence the final step in the construction of the daily exposure series is to assemble the appropriate number of pieces of each of the h separate time series together to comprise as many days of exposure as required.

All of the foregoing pertains to near field exposures experienced by workers in the conduct of the specific tasks. However, it is clearly possible, and often the case, that one worker can be exposed, generally at lower concentrations, to the chemicals being used by a second worker. In the present version of the simulator this was handled simply by

worker can be exposed, generally at lower concentrations, to the chemicals being used a second worker. In the present version of the simulator this was handled simply by assuming a single ratio of near- to far-field exposures for all tasks and applying this ratio to the mean exposure for all chemicals in all other tasks except that in which a worker is currently engaged during each 15-minute period. This assumes that all tasks are always being carried out simultaneously which may not be the case. Recalling that this version of the simulator pertains only to homogeneously exposed group, this is but one of several issues that can be addressed in the future as the general issue of between worker variability is incorporated.

Discussion

While the general concept of using a simulation approach to the integration of qualitative and quantitative data on workplace exposures is straightforward and appealing, in the case of multiple chemicals there are complexities, largely due to the correlation structure that we postulate for short-term chemical exposures. Our attempt to extend the general approach of Male to the multivariate case seems to have been successful, at least given the paucity of field information on the auto- and cross-correlation structure of workplace exposures.

While the random number generation procedure meets our design requirements on the average, it is possible that there are individual realizations that are far from the average. It is possible to impose on the simulator further conditions to select a subset of realizations that more closely meet special criteria. For example, if it is important to constrain one of the variances more closely to the design value, a variance estimate can be calculated for each realization and those rejected that depart too far from the design value.

In Part II of this series, we will apply the simulator to a particular exposure assessment task in order to explore the strengths and weaknesses of the present version and to identify issues to be addressed in an improved version.

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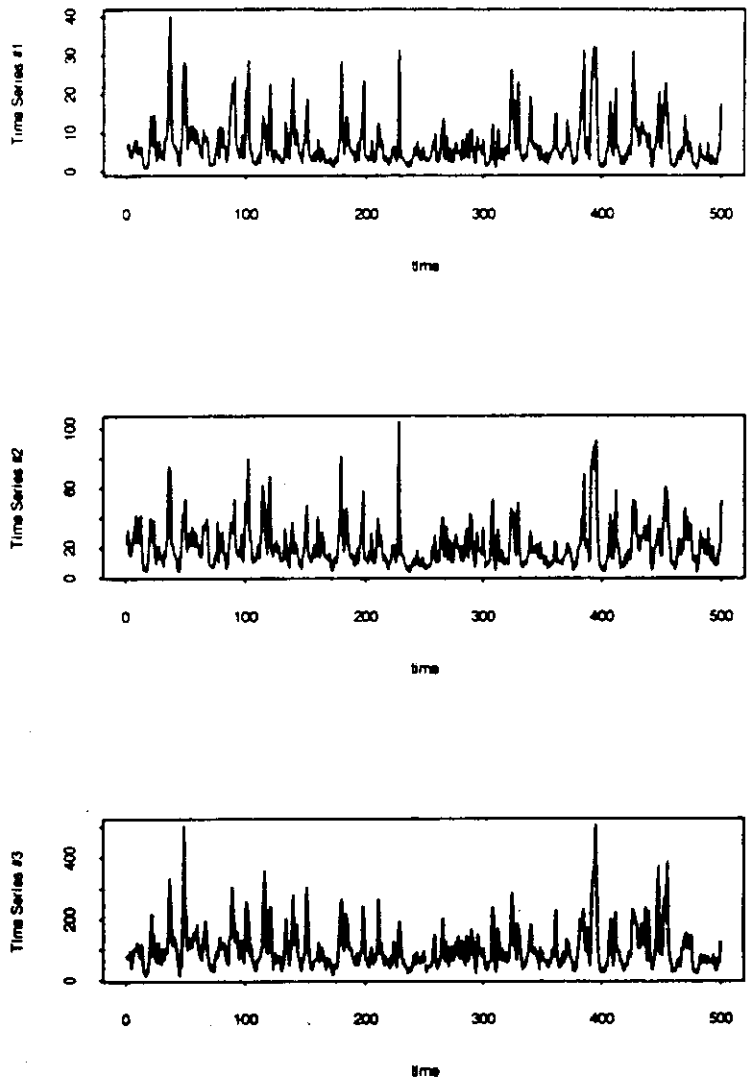


Figure 1. Three Simulated Time Series

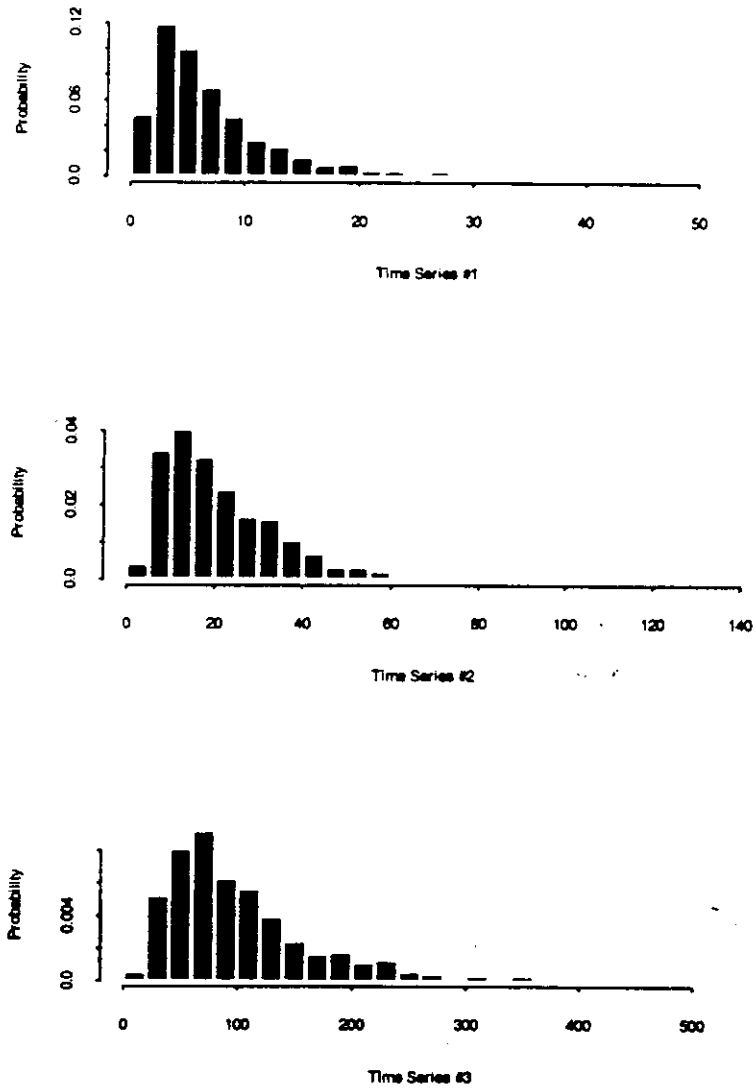


Figure 2. Histograms of Three Simulated Time Series

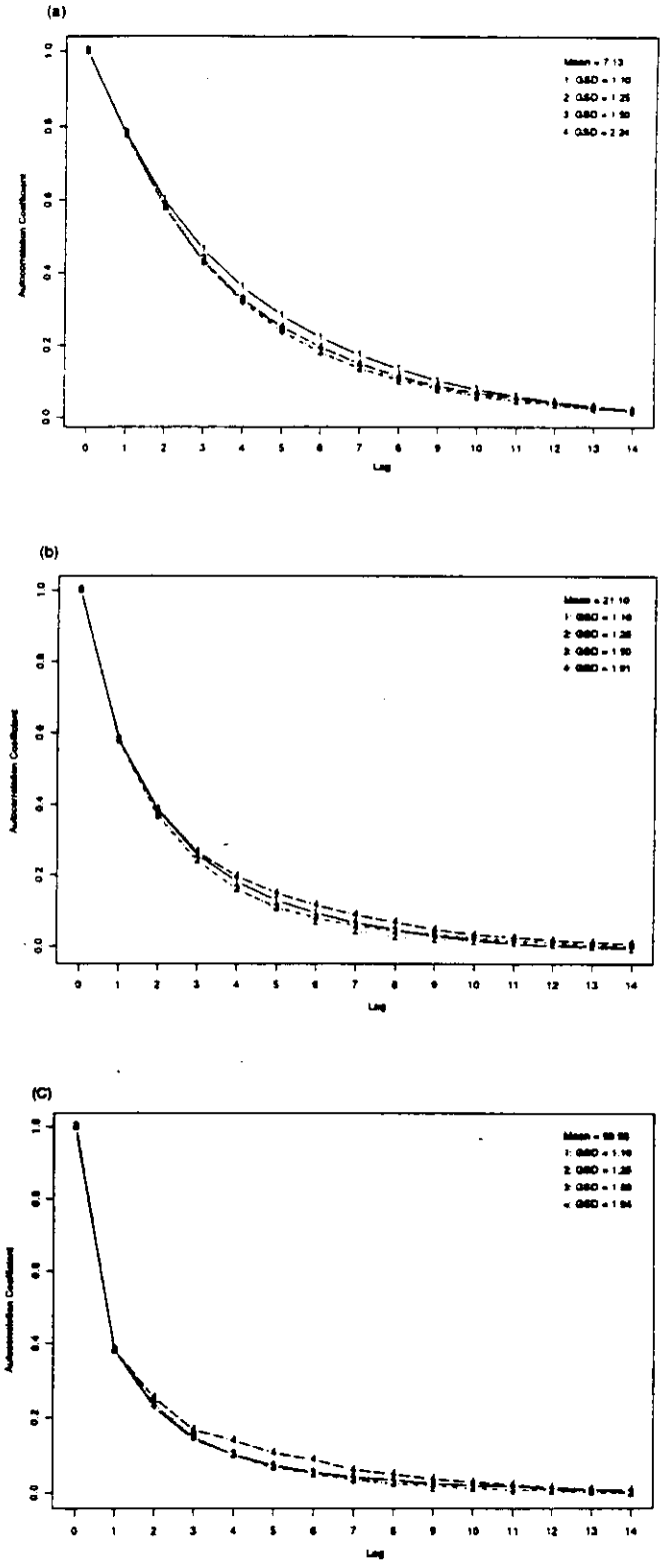


Figure 3. Autocorrelation functions of time series with different variance.

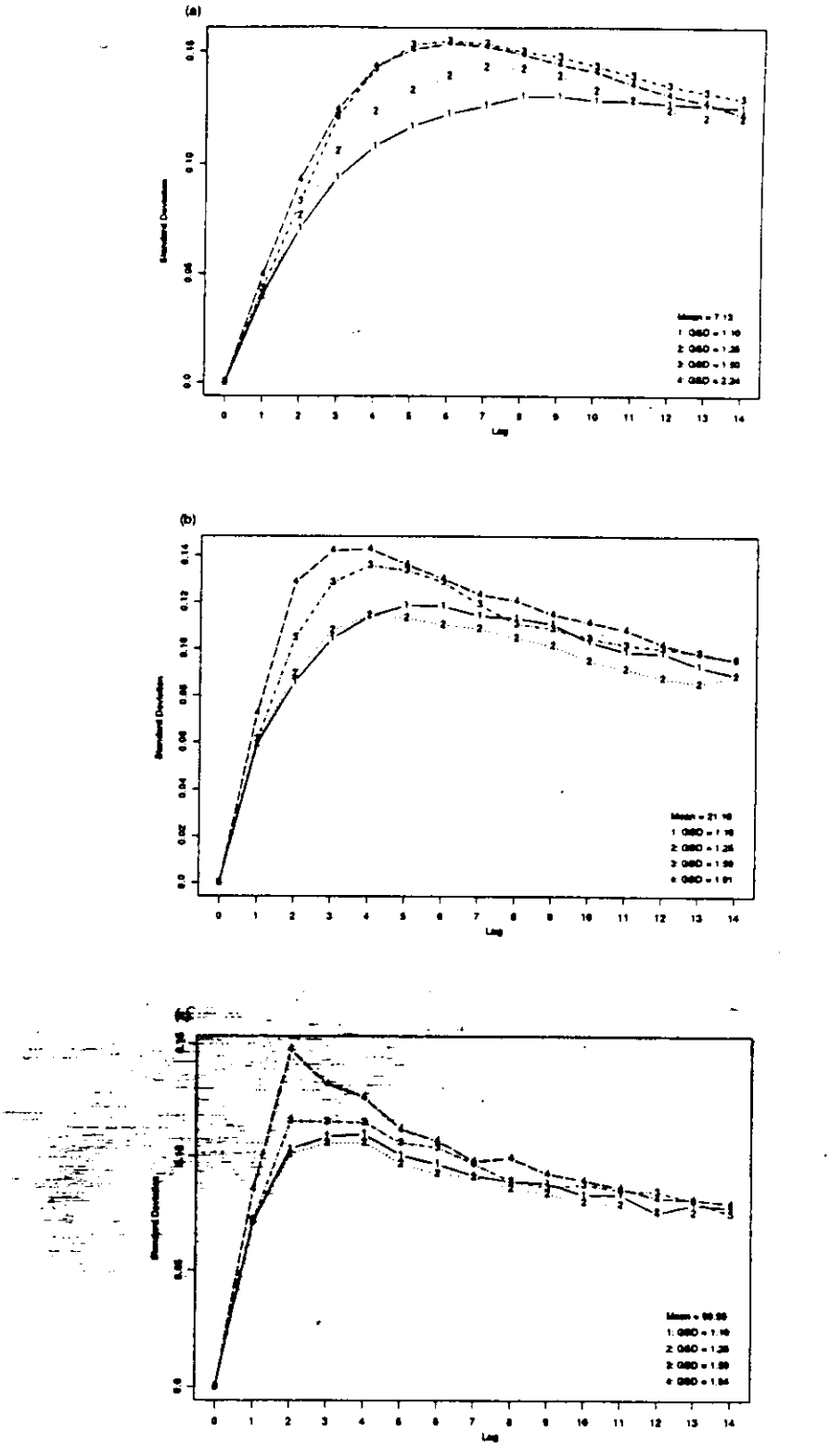


Figure 4. Standard deviations of autocorrelation coefficients of time series with different variance.

Appendix I. Statistical Derivations for the Parametric Relationships between Normal and Lognormal Time Series

Theorem 1.

If x is a normal distribution with mean μ and standard deviation σ, then

$$E(e^{cx}) = e^{cx+c^2\frac{\sigma^2}{2}},$$

where c is a constant.

Let $y=exp(x)$. Therefore, y is a lognormal distribution with mean μ' and standard deviation σ'. The mean and variance of y can be expressed by x as the following:

$$\mu' = E(y) = E(e^x) = e^{\mu+\frac{\sigma^2}{2}} \tag{1}$$

$$E(y^2) = e^{2\mu+2^2\cdot\frac{\sigma^2}{2}} = e^{2\mu+2\sigma^2} \tag{2}$$

$$\begin{aligned}\sigma'^2 = Var(y) &= E[(y-E(y))^2] = E(y^2) - E(y)^2 \\ &= (e^{2\mu+2\sigma^2}) - (e^{2\mu+\sigma^2}) = (e^{2\mu+\sigma^2})\cdot(e^{\sigma^2}-1)\end{aligned} \tag{3}$$

From Equations (1) and (3), we can obtain:

$$\mu = \ln(\mu') - \frac{\sigma^2}{2} \tag{4}$$

$$\sigma^2 = \ln(1+\frac{\sigma'^2}{\mu'^2}) \tag{5}$$

Theorem 2.

If x_1 and x_2 are bivariate normal distribution with means, μ_1 and μ_2, standard deviations, σ_1 and σ_2, and correlation ρ. That is,

$$\begin{pmatrix} x_1 \\ x_2 \end{pmatrix} \sim N\left(\begin{pmatrix} x_1 \\ x_2 \end{pmatrix}\begin{pmatrix} \sigma_1^2, \rho\sigma_1\sigma_2 \\ \rho\sigma_1\sigma_2, \sigma_2^2 \end{pmatrix}\right)$$

According to the properties of conditional distribution, then

$$(x_1 \mid x_2) \sim N(\mu_1 + \rho \frac{\sigma_1}{\sigma_2}(x_2 - \mu_2),\ \sigma_1{}^2 \cdot (1-\rho^2)).$$

Therefore,

$$\begin{aligned} E(e^{x_1+x_2}) &= E(e^{x_1}) \cdot E_{x_2}(e^{x_1} \mid x_2) \\ &= E(e^{x_2} \cdot e^{\mu_1+\rho\cdot(\frac{\sigma_1}{\sigma_2})\cdot(x_2-\mu_2)+\frac{1}{2}\cdot\sigma_1^2\cdot(1-\rho^2)}) \\ &= (e^{\mu_1-\rho\cdot(\frac{\sigma_1}{\sigma_2})\cdot\mu_2+\frac{1}{2}\cdot\sigma_1^2\cdot(1-\rho^2)}) \cdot E(e^{(1+\rho\cdot(\frac{\sigma_1}{\sigma}))\cdot x_2}) \\ &= (e^{\mu_1-\rho\cdot(\frac{\sigma_1}{\sigma_2})\cdot\mu_2+\frac{1}{2}\cdot\sigma_1^2\cdot(1-\rho^2)}) \cdot (e^{(1+\rho\cdot(\frac{\sigma_1}{\sigma_2}))\cdot\mu_2+(1+\rho\cdot(\frac{\sigma_1}{\sigma_2}))^2\cdot(\frac{\sigma_2^2}{2})}) \\ &= e^{\mu_1+\mu_2+\frac{1}{2}(\sigma_1^2+\sigma_2^2+2\rho\sigma_1\sigma_2)} \end{aligned} \tag{6}$$

The correlation between the $\exp(x_1)$ and $\exp(x_2)$ is:

$$\begin{aligned} corr(e^{x_1}, e^{x_2}) = \rho' &= \frac{E(e^{x_1+x_2}) - E(e^{x_1}) \cdot E(e^{x_2})}{E(e^{2x_1})^{1/2} \cdot E(e^{2x_2})^{1/2}} \\ &= \frac{(e^{\mu_1+\mu_2+\frac{1}{2}(\sigma_1^2+\sigma_2^2)}) \cdot (e^{\rho\sigma_1\sigma_2} - 1)}{(e^{\mu_1+\frac{\sigma_1^2}{2}}) \cdot (e^{\mu_2+\frac{\sigma_2^2}{2}}) \cdot (e^{\sigma_1^2}-1)^{1/2} \cdot (e^{\sigma_2^2}-1)^{1/2}} \\ &= \frac{e^{\rho\sigma_1\sigma_2} - 1}{(e^{\sigma_1^2}-1)^{1/2} \cdot (e^{\sigma_2^2}-1)^{1/2}} \end{aligned} \tag{7}$$

From Equation (5),

$$e^{\sigma_1^2} - 1 = \frac{\sigma_1'^2}{\mu_1'^2}$$

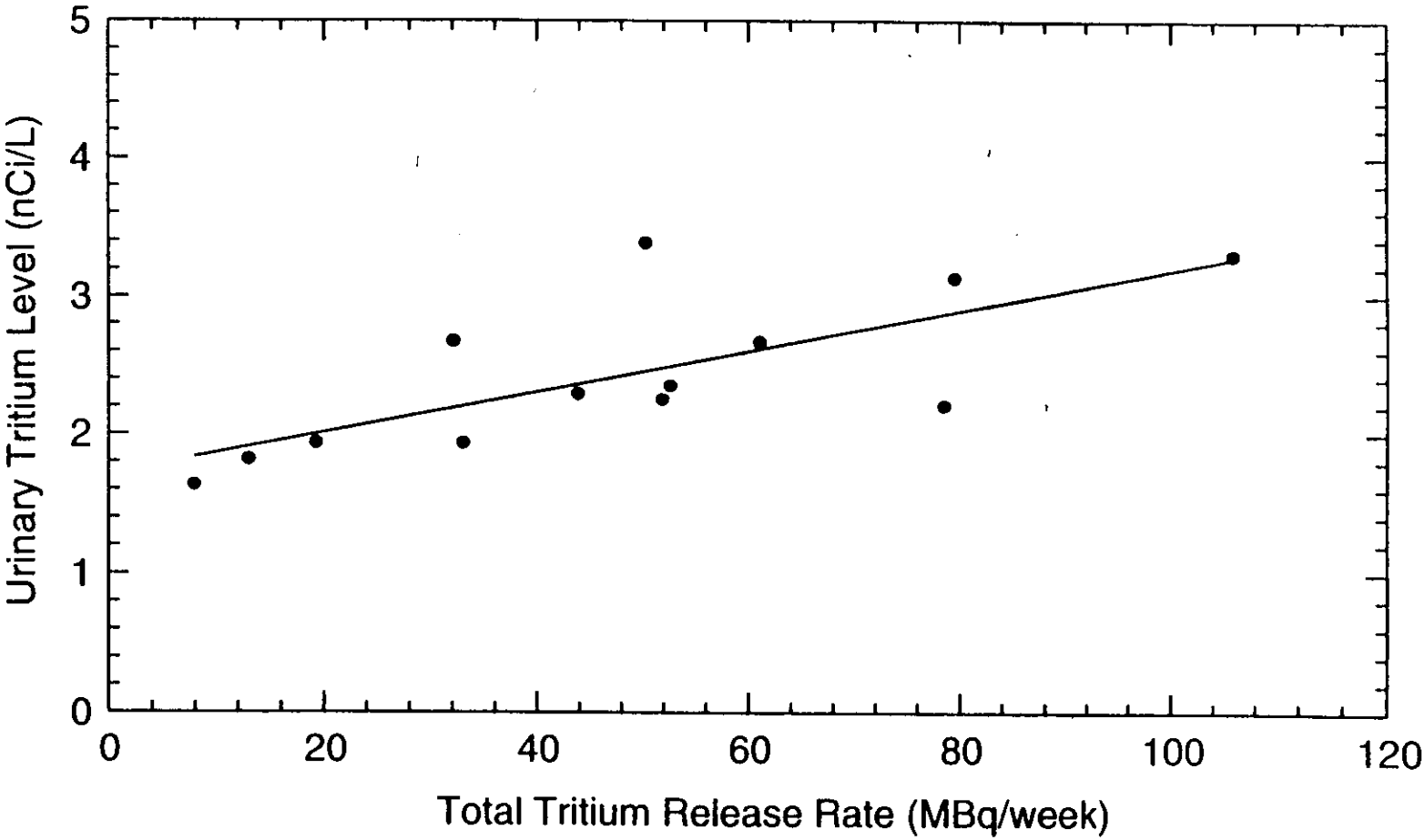
$$e^{\sigma_2^2} - 1 = \frac{\sigma_2'^2}{\mu_2'^2}$$

PRELIMINARY RESULTS

- **Literature review and framework development**
- **Examination on the feasibility of urinary tritium as exposure marker**

Data source: levels of occupational exposure in the National Tritium Labeling Facility (NTLF) at LBNL and in other nearby buildings.

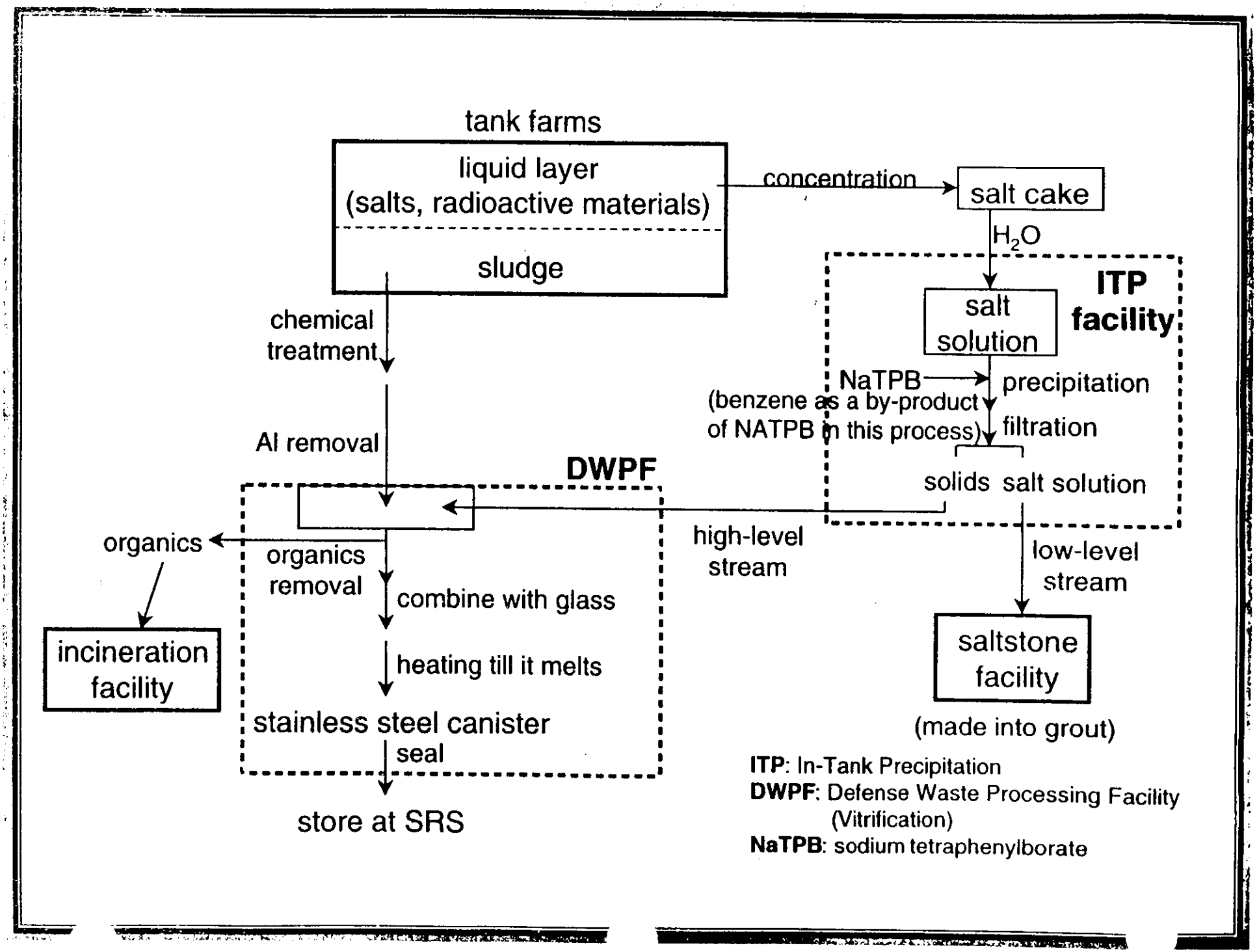
* Multivariate regression analysis:
  - Stack T emission is a strong predictor to estimate the urinary T levels measured in workers at NTLF
  - Up to 80% of the total variance can be explained by the best-fit model
  - Other predictive variables: windspeed and wind direction



Distributions of weekly tritium concentrations in urine samples from Building 75 compared to three-week average tritium releases from the LBNL tritium labeling facility during a three-month period

PRELIMINARY RESULTS (cont.)

- **Review and selection of field site**
  - Savannah River Site (SRS)
    In-Tank Precipitation Facility (ITP)
    Defense Waste Processing Facility (DWPF)
  - Epidemiological findings
    Excess leukemia risk


tank farms
liquid layer
(salts, radioactive materials)
sludge
concentration
salt cake
H_2O
ITP
facility
salt
solution
NaTPB
precipitation
(benzene as a by-product
of NATPB in this process)
filtration
solids
salt solution
chemical
treatment
Al removal
DWPF
organics
organics
removal
combine with glass
heating till it melts
stainless steel canister
seal
store at SRS
high-level
stream
low-level
stream
incineration
facility
saltstone
facility
(made into grout)
ITP: In-Tank Precipitation
DWPF: Defense Waste Processing Facility
(Vitrification)
NaTPB: sodium tetraphenylborate

INTRODUCTION

- Overview
- Background
- Biological basis

APPROACH

- Benzene and radiation case study
- Risk assessment framework

PRELIMINARY RESULTS

- Literature review and framework development
- Examination on the feasibility of urinary tritium as exposure marker
- Review and selection of field site

SUMMARY/DISCUSSION

Table 1. Evaluation of Dispersion of Three Time Series Based on 1000 Samples

	Time Series #1	Time Series #2	Time Series #3
Mean			
Mean	7.121	21.076	99.839
Variance	45.895	230.066	5421.309
Autocorrelation	0.646	0.647	0.646
*Cross-correlation	0.882	0.830	0.789
Standard Deviation			
Mean	0.378	0.719	3.938
Variance	11.909	38.426	1010.694
Autocorrelation	0.033	0.027	0.028
Cross-correlation	0.022	0.018	0.021
CV(%)			
Mean	5.3	3.4	3.9
Variance	25.9	16.7	18.6
Autocorrelation	5.1	4.2	4.3
Cross-correlation	2.5	2.2	2.7

* The values shown in columns #1, 2 and 3 are the cross-correlations between time series #1 and 2, time series #1 and 3, and time series #2 and 3.

Table 2. Designed Parameters for Evaluating Influence of Variance on Autocorrelation Coefficients

	Time Series #1	Time Series #2	Time Series #3
Mean	7.13	21.10	99.93
Variance	GSD = 2.24	GSD = 1.91	GSD = 1.94
	1.50	1.50	1.50
	1.25	1.25	1.25
	1.10	1.10	1.10
Autocorrelation	0.80	0.60	0.40
Cross-correlation	$\begin{pmatrix} 1.00, 0.80, 0.70 \\ 0.80, 1.00, 0.60 \\ 0.70, 0.60, 1.00 \end{pmatrix}$		

SUMMARY/DISCUSSION

Risk Assessment for Mixed Exposures

- Develop and critically evaluate the risk assessment framework
- Identify information gaps and the future research needs

Developing a Tiered Approach for Mixed Radiation/Benzene Exposure

- Source/receptor relationships
- Receptor/dose estimates
- Markers of exposure/dose
- Markers of early health effects

ABSTRACT FOR THE PRESENTATION DELIVERED AT THE 1998 AMERICAN INDUSTRIAL HYGIENE CONFERENCE & EXPOSITION (1998 AIHCE)
ATLANTA, GEORGIA; MAY 1998

BIOLOGICAL MONITORING TO ASSESS THE HEALTH RISKS FOR WORKERS EXPOSED TO MIXTURES OF PHYSICAL AND CHEMICAL AGENTS

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Limited scientific data is available on the combined effects of toxic agents that have either similar or different mechanisms of action. The goal of this study is to use biological monitoring to construct and critically evaluate a framework for assessing risks arising from exposure to physical and chemical agents in various combinations. We consider two agents widely used at U.S. Department of Energy (DOE) facilities, tritium--a source of ionizing radiation, and benzene--chemically toxic and a known human carcinogen. Both ionizing radiation and benzene appear to have similar manifestations of genetic damage within living cells. A 1976 Japanese study indicated that the combined cytogenetic effect of benzene with radiation in cultured human lymphocytes could be synergistic, but follow-up studies are lacking. We present here early results of a pilot study in which we apply the steps of the standard risk assessment paradigm to mixed physical/chemical agent exposures and use markers of exposure and dose at each step. This study provides the opportunity to examine how the findings of synergism in the Japanese study, if correct, would impact a risk-based framework for protecting workers from mixed agent exposures in the workplace. Specifically we examine the feasibility of using tritium levels and benzene metabolites in urine as markers of occupational exposures at DOE facilities. At one DOE facility multiple regression analysis was used to evaluate the correlation between urinary tritium levels of workers in various buildings and tritium release rate, wind speed, wind direction, precipitation and other parameters. Among parameters considered, correlation was strongest between urine levels and three-week average of the tritium source strength prior to the sampling date. We also report here on the process used to select and assess a DOE site with combined radiation and benzene exposures in the occupational environment.

Therefore,

$$\rho = \frac{\ln(1+\rho' \cdot (e^{\sigma_1^2}-1)^{1/2} \cdot (e^{\sigma_2^2}-1)^{1/2})}{\sigma_1 \sigma_2}$$

$$= \frac{\ln(1+\rho' \cdot \frac{\sigma_1'}{\mu_1'} \cdot \frac{\sigma_2'}{\mu_2'})}{\sigma_1 \sigma_2} \qquad (8)$$

The relationship in Equation (8) is not only valid for the correlation between two variables of a bivariate distribution, but also for the autocorrelation of a time series. For a stationary time series, the mean and variance do not change over time. That is,

$$\mu_1 = \mu_2 = \mu$$
$$\sigma_1^2 = \sigma_2^2 = \sigma^2$$

Therefore, the autocorrelation (r and r') can be expressed as the following:

$$r = \frac{\ln(1+r' \cdot \frac{\sigma'}{\mu'} \cdot \frac{\sigma'}{\mu'})}{\sigma^2} = \frac{\ln(1+r' \cdot \frac{\sigma'^2}{\mu'^2})}{\sigma^2} \qquad (9)$$

Integration and Exploration of Task-Based Multivariate Exposure Data: Part II: Simulation of Solvent Exposures in Raft Manufacturing

Jyun-De Wu, Kathleen Vork and Robert C. Spear

ABSTRACT

In Part I of this series the structure and mathematical details of an exposure simulator were presented for the study of multiple exposures to airborne chemicals in the occupational environment. Here, the simulator is applied to estimating the 8-hour TWA exposures to four solvents used in the manufacture of inflatable rafts. A preliminary study was conducted of a small manufacturing facility with the object of characterizing the tasks involving solvent exposures and collecting short-term exposures measures associated with each of these tasks. These data were used to estimate the various inputs required by the simulator. Where the field data were inadequate to estimate input parameters, information from the literature was used. The simulator was then used to estimate the 8-hour exposure distributions of three fictitious groups of workers who were postulated to work in an enlarged plant. The specific question related to the number of shift-long samples needed to adequately characterize the multivariate exposure of the three homogeneously exposed groups in the new plant. Cluster analysis was used to analyze the simulated 8-hour exposures. With the variability in exposure inherent in the tasks conducted by the three groups, it was found that six days of exposure data per worker was sufficient to associate each worker with the correct work group with low misclassification error. While this first exercise of the exposure simulator showed it to be a useful means of integrating qualitative and quantitative types of exposure-related data, including within-group exposure differences is a high priority for future improvements.

INTRODUCTION

In Part I of this series the structure and mathematical details of an exposure simulator were presented for the study of multiple exposures to airborne chemicals in the occupational environment (Wu et al, 2000). In brief, the simulator is based on short-term exposure distributions associated with the various tasks comprising a day-long job and it includes the ability to impose a correlation structure on exposures to multiple agents. Short-term exposures are assumed to be lognormally distributed and autocorrelated when they occur sequentially. There is also a provision to include both near and far-field exposures based on assumptions concerning the position of the worker with relation to the source. Thus, the essential inputs to the simulation include the mean and variance of the task-based short-term exposure distributions, their autocorrelation and cross-correlation, the ratio of far to near field exposure, as well as descriptors of the nature of the work including the numbers of tasks in the workday, the time spent at each , and the agents used in each task.

The distribution of shift-long time-weighted average (TWA) exposures for a group of workers is the output of the simulation because it is these values which are constrained by PELs or other occupational health standards for chronic or delayed toxic agents. In addition, it is the 8-hour TWA value which is often used for epidemiological classification of exposure groups when such data are available. In this paper we report the application of the simulator to exposure classification in the context of solvent exposures in a plant manufacturing inflatable rafts. The various input data for the simulator are based on a preliminary survey of an actual rafting plant supplemented by data from the literature where necessary. We then postulate a fictitious exposure scenario based on the premise of expanded production and focus on three task-defined groups of workers. The question relates to a future study of chronic solvent exposure in which we postulate a need to estimate group differences in 8-hour TWA exposures. Specifically, we ask how many days would one need to collect 8-hour measurements from workers in the new facility to reliably determine the differences in their exposures from a multivariate perspective. Clearly, we assume that the task-based exposures in the new facility

will be similar to those in the existing plant, although we will assume the work is structured somewhat differently.

PRELIMINARY SURVEY OF THE RAFTING PLANT

The plant surveyed is that of an inflatable raft manufacturer with five employees who produce one to three boats per day. This output varies greatly by month and season however, the time of the survey was during the peak season, so shifts generally last 12 hrs/day for 6 days/wk. This company occupies a large open area on the ground floor of a large warehouse building. Hundred lb. rolls of nylon-rubber material are stored inside of the building along with containers of adhesives, lacquer, and pigments. There is a computer controlled cutting table and assembly tables placed in a circle in the center of the building. The general ventilation inlet is located on the second floor. The building is leaky and ambient wind velocity and direction can greatly affect indoor air flow patterns. A galvanized steel enclosed spray booth equipped with a cross-draft local exhaust ventilation system is located to one side of the main work area. Inside, it has a sprinkler system, negative air filters in the door inlet, and paint arresters at the exhaust outlet and plenum where fan is located. The fan, runs at 10,000 cubic feet per minute and the negative pressure system is monitored via an external manometer.

Materials are cut out on an automatic cutting table. A rolling cutter equipped with a guard plate rotates around the table. Cut pieces are prepared by manually rolling adhesive made of liquid neoprene and toluene onto the edges of each cut sheet and around holes cut for inserting valves. Workers manually glue one panel of neoprene material onto another until the entire raft hull is assembled. These assembled raft pieces are left to air dry overnight. Two workers coat with methyl ethyl ketone adhesive an approximately 55 ft^2 area of inflated floor in woven nylon fabric material made with poly vinyl chloride. They adhere the floor onto a layer of neoprene material that is glued onto the raft hull. Later, workers inflate the assembled raft hulls and attach D-rings, handles, and a rubber bumper strip around the outside perimeter of the raft. The inflated raft hull and center pieces are sprayed with urethane mixed with a pigment the spray booth. The negative pressure system remains on to dry the coated raft. Cured rafts are carried

out of the booth and placed on the floor in a separate area. Two logos are silk screened onto the raft. Finished rafts are carried to the building storage area and packed for shipping. Rafts weigh approximately 120 lb. fully assembled. Waste products include neoprene scrap, dried urethane coating (2 gal/day) and cured rubber, all disposed of in dumpsters. Liquid plastic and paint over spray is filtered and exhausted through the roof of the warehouse building.

The initial survey of the plant was conducted over a period of 5 days and included the identification of tasks described above and the chemical exposures associated with each. For purposes of the subsequent simulation the workers were classified into three groups which were cleaning, gluing and assembling. Some chemicals were used in all tasks and some were unique to particular tasks. For example, the gluing task involves the use of urethane adhesive and toluene; the cleaning task is done with toluene; the assembling task involves the use of toluene and glue. Exposures to four main agents, methyl ethyl ketone (MEK), toluene, hexane and ethyl acetate, occurs in these tasks. Because the work environment was in a single closed space, it is possible for workers in different work areas to receive far-field exposures to all the agents. Both near- and far-field exposures were considered important for assessing the workers' total exposures.

Forty-five personal measurements and twelve area measurements to these four agents were measured during the field investigation. The area measurements were used in the estimation of far-field exposures and were collected at a distance 15 feet from workers at the same time the personal samples were being collected. All measurements were short-term (15 minutes) samples collected on charcoal tubes using personal pumps operating at 1 lpm. Chemical analysis was conducted by a commercial laboratory using NIOSH methods. The mean and variance of these measurements were calculated for each agent in each work task. The results are given in Table 1 and show marked differences in the exposure distribution between tasks. Generally, these differences corresponded with what would be expected from the different amounts and frequencies of agents used in the tasks.

Table 2 presents the data relating to the near- versus far-field exposure ratios. Substantial differences exist in these ratios for agents in different work groups. There is tendency for high exposure ratios to occur when the near-field exposures are low. This observation illustrates that the personal samples reflect the sum of near- and far- field exposures. As a consequence, the MEK and hexane exposure ratios observed in the assembly work group and the ethyl acetate exposure ratio of the cleaning work group were regarded as reasonable estimates of the near/far exposure ratios when actual near field exposures were significant. In general, we assumed that exposures below 5 ppm were far-field.

Table 3 shows the agent-specific correlation data for each task in which there were sufficient values for all four solvents above detection limits. Task F involved near-field exposure only to toluene, so no correlations were estimated. Insufficient data were available to estimate the correlation matrix for task A and the estimate shown in the table were separately derived as noted below. Tasks C and E, cleaning and hull assembly, suggest particularly strong correlation structures.

THE EXPOSURE SCENARIO

As noted above, the fictitious scenario we explored was based on the premise that the plant is to expand and that the same tasks will be involved in the new setting, but will be carried out by three work groups comprised of about 10 workers each. When the new plant begins operation, a study is to be initiated of their solvent exposure with a the objective of determining possible health effects. The immediate question is to design a study to determine the differences in their exposure which will in turn determine if this new plant promises to be a good study site. Recall that the current version of the simulator addresses only homogeneously exposed groups, so each of these three work groups is so defined. We simulate the situation in which an 8-hour time-weighted average (TWA) measurement is taken for each worker on each day of data collection and analyzed for all four chemical agents. We ask how many days of data collection will be necessary to allow a clear separation of the exposures into the three groups. If the group exposure data is statistically separable and with little misclassification, we assume that

whatever exposure estimates one wishes to calculate from the data will give a good picture of the group-based differences. We used cluster analysis as a means of separating the groups, but alternative statistical approaches are possible.

Clearly, a simulator is not necessary to estimate the expected values of the near-field eight-hour TWA exposures since these values are directly calculable from the task means and the task times. Hence, even in the fictitious example we are more interested in capturing the variability inherent in the exposures with the objective of setting data collection requirements that will, in the future field study, produce reliable estimates of the actual mean values of the near-field and the far-field exposures. Looking toward the future, however, approximating the mean exposures will not be as easily done when task times are allowed to vary and the homogeneous exposure structure is replaced with a more realistic construct.

METHODS

Parameterizing the Simulator

Figure 1 shows the structure of the simulator and indicates the required input data. As noted above, we are dealing with 3 work groups who each carry out three separate tasks involving exposure to four agents in at least one of the tasks. The number of hours per day devoted to each task is very loosely based on the survey data and set to 2 hours per day for tasks B, C, E, F, H, and I, and 4 hours per day for tasks A, D, and G.

The mean and variance of the personal exposures for each agent in each task, Table 4, are based on the measured values listed in Table 1 with the solely far-field exposures removed. The other differences between Tables 1 and 4 are adjustments to the variances due to the need for a positive definite covariance matrix in order for the estimation procedure for the P and Q matrices of the random exposure generator to converge. That such adjustments are needed is not surprising due to the small sample sizes on which these estimates are based, but future effort is required to develop a means of carrying out such adjustments in a standard way.

The input cross-correlation between the agents in a task, Table 5, was based on the observed cross-correlation data shown in Table 3. However, in task A some exposures were below

detection limits making it impossible to directly calculate the cross-correlation matrix. Hence, all exposures occurring in the cleaning work group were pooled to approximate the task A correlation estimates. As noted above, the task F near-field exposure was assumed to be to a single agent. Regarding the far-field exposures, we postulated that, in the new facility, the general ventilation would be improved. Therefore, in the simulation, a fixed far/near-field exposure ratio, 0.1, was used for all tasks.

Because the measured short-term exposures were not sequential in time, it is not possible to estimate from the survey data the autocorrelation of the workers' exposures. We assumed the autocorrelation of the workers' short-term exposures was similar to that observed by Kumagai and Matsunaga (1993, 1994, 1995a and 1995b) in their exposure studies of organic solvent exposures. They found autocorrelation coefficients for 7.5, 15, 30 and 60-minute TWA exposure series to be 0.42, 0.32, 0.20 and 0.01, respectively. Moreover, they determined that autocorrelation decreased as averaging time increased in a manner well fitted by an exponential function as assumed in the structure of the simulator. In addition, in the examination of inter-relationship between short-term (7.5-minute) TWA exposures to two organic solvents, the correlation coefficients between these two exposure concentrations showed high (0.71 ~ 0.98), medium (0.59) and low (-0.32 ~ 0.27) values corresponding to the chemicals mixed in a fixed ratio, in various ratios and used in a separate process, respectively (Kumagai and Matsunaga, 1995b). Based on these results, we assumed the autocorrelation of the workers' short-term exposures in the simulation to be 0.30 for all tasks. Utilizing all of the foregoing input data resulted in the P and Q matrices shown in Table 6 which were then used to iteratively generate exposure values.

The Simulation Experiment

As noted above, the question to be addressed concerned estimating the number of days of 8-hour TWA measurements necessary to determine a clear and unambiguous multivariate description of the differential exposure of the groups. The similarity metric chosen for the cluster analysis used for this purpose was Euclidean distance and the clustering algorithm used

was complete-linkage (Ref). The members of the groups formed from the cluster analysis were then compared with their actual assignments. Thus, the misclassification rate of the exposure-based clustering for a particular tree structure was determined. A single simulation run was conducted which generated 8-hour TWA exposures for all thirty workers to the four agents for each of 30 workdays. In all cases, the simulated daily exposures were kept in the original format without standardization for the cluster analyses.

RESULTS

To illustrate the clustering concept, consider an impractical design in which we collect an 8-hour TWA measurement on each worker for each of 30 days and average each worker's daily values. Clearly, this should give a very good estimate of the long-term mean exposures sustained by each of the groups in a stationary environment. Figure 2 is an example of the tree structure determined from the simulated 30-day mean values. In this figure, the numbers shown at the base of the tree indicates the identification number of each of the 30 workers. The numbers, 1 to 10, 11 to 20 and 21 to 30, belong to the cleaning, gluing and assembly work groups, respectively. Ideally, the workers of each work group should form a cluster without misclassification and, in this extreme case, they do.

On the other end of the spectrum, consider the same procedure, but based on a single day's exposure data for each worker. Figure 3 shows such a case. Cutting the tree at a distance of about 175 produces three groups, but one of them only has 3 members and another 20 members. To determine the misclassification rate, let us cut the tree at the level where the three largest clusters have a minimum number of misclassifications. That is, we count as misclassifications all members of a cluster who are not part of the majority as well as all members of the smaller clusters. In Figure 3, a cut at a distance of 100 produces five clusters and 9 misclassifications as opposed to the 11 obtained with the cut at 175. Nine is the minimum number of misclassifications for this tree. Applying this procedure to each of the 30 daily TWA exposures produced no perfect classifications and a worst case of 10 misclassifications. The median

number misclassified was 6. Even if one considered less than or equal to 4 workers misclassified to be acceptable, there is only a 25% chance of obtaining such a result.

Since a single day's exposure cannot provide an acceptable exposure classification for the work groups in this case, how many days should be measured and averaged to determine an acceptable classification? To answer this question we chose to estimate the probability of a perfect classification. To accomplish this, a random sample of 200 sets of two-day means was generated and the number of perfect classifications determined. This process was repeated to generate three-, four-,..., and ten-day mean exposures. Thus, 9 sets of different day-mean exposures were calculated.

Cluster analysis was applied to each day-mean exposure set. Three clusters were produced from each tree structure. Because perfect exposure grouping was desired, the tree cutting scheme in this evaluation differed from that used in the single-day analysis. The tree cutting scheme was forced to produce three clusters from each tree structure, but the level of Euclidean distance used to cut each tree structure into three exposure clusters was not a fixed value. Table 7 presents the misclassification rate of these 9 sets of day-mean exposure. As expected, the perfect classification rate increases as the number of averaging days increases. Two-day mean exposures result in about 20% perfect classification, five-day mean exposures provides about an 80% chance of perfect classification, and eight-day mean exposures almost completely recover the true groupings.

DISCUSSION

As we have pointed out elsewhere, differences in exposure in the multivariate case take on a new meaning in that it is necessary to consider both differences in intensity of exposure and in its composition (Wu et al., 1999). In the foregoing example, we determined the number of 8-hour measurements necessary to separate statistically the exposures of the three homogeneously exposed groups , but the toxicological implications of these differences is another matter entirely. The difficulty can be seen by inspecting the complex patterns of the

30- day average exposures of the three groups as shown in Table 8. While there is a developing literature on the assessment of health risks from mixed chemical exposures (2 or 3 refs), the issue is far from resolved. Even from the more limited perspective of exposure assessment, the sensible definition of a group is murky when the variation in exposure within a group is introduced. However, it seems likely that the univariate ideas relating to within-group and between group-variance will carry over to the multivariate case.

While we acknowledge that our first application of the simulator is principally suggestive of interesting future applications, we feel that there is considerable potential in utilizing the task-based structure of the simulation for better understanding of the sensitivity of the 8-hour exposures to the various task-related variables. OSHA took such an approach in the 1993 interim final lead standard where they adjoined a PBPK model to task-based exposure estimates to determine the sensitivity of blood lead to variations in the exposure pattern (ref). We can foresee the use of the simulator in this context in which the effects of both task-based controls and administrative controls on shift-long exposures can be estimated.

Finally, we see the next stage of development of the simulator as being the introduction of within work group variability in exposure. The work of adding between-worker variance into the simulator is not conceptually difficult. There are at least two different elements of this variability that come immediately to mind, variations in task-time assignments and/or variability in the short-term exposure distributions. Since the variability in the short-term distributions is not likely to be informed by preliminary data of the sort obtained in the rafting survey given the need for multiple samples per person, it may be that there will always be a greater degree of uncertainty in this element of the process. In any case, the simulation idea does appear to offer a means of integrating qualitative and quantitative data relating to workplace exposure in an explicit and useful way.

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Table 1. Field Data: Means and Variances of 15-minute Exposures by Task

	MEK		Toluene		Hexane		Ethyl Acetate	
	mean (ppm)	variance (ppm^2)	mean (ppm)	variance (ppm^2)	mean (ppm)	variance (ppm^2)	mean (ppm)	variance (ppm^2)
Cleaning								
Task A. Mixing paint	11.2	11.5	56.3	2880.	9.7	177.	47.2	375.
Task B. Spraying paint	7.9	46.4	91.7	3829.	12.8	73.	24.1	264.
Task C. Cleaning	2.2	11.2	132.9	13369.	2.0	3.66	12.9	133.
Gluing								
Task D. Applying neoprene adhesive	4.1	14.5	229.	3854.	78.7	2442.	2.1	1.47
Task E. Hull assembly	1.9	3.3	147.	4177.	20.4	147.	1.5	0.35
Task F. Glue cleanup	0.3	3.1	20.6	0.85	3.6	0.08	0.38	0.07
Assembly								
Task G. Applying PVC adhesive	215.	21555.	86.2	2458.	28.1	1368.	3.2	4.5
Task H. Floor assembly	110.	14285.	111.2	3135.	29.2	908.	3.3	4.0
Task I. Floor installation	34.5	2292.	211.7	10608.	33.4	43.1	3.1	0.3

Table 2. Field Data: Near- and Far-field Exposures

Agents	Work Groups		
	Gluing	Cleaning	Assembling
MEK			
Near-field mean exposure (ppm)	2.9	7.0	121.
Far-field mean exposure (ppm)	1.3	3.1	22.0
Ratio	0.44	0.4	0.2
Toluene			
Near-field mean exposure (ppm)	176.	96.5	123.
Far-field mean exposure (ppm)	87.5	59.3	34.4
Ratio	0.5	0.6	0.3
Hexane			
Near-field mean exposure (ppm)	48.3	9.3	29.7
Far-field mean exposure (ppm)	17.1	7.7	5.6
Ratio	0.4	0.8	0.2
Ethyl Acetate			
Near-field mean exposure (ppm)	1.7	25.2	3.3
Far-field mean exposure (ppm)	1.0	5.7	1.2
Ratio	0.6	0.2	0.4

Table 3. Field Data: Exposure Correlation Matrices by Task

Task A	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.17	0.54	0.77
Toluene	-	1.00	0.36	0.04
Hexane	-	-	1.00	0.26
Ethyl Acetate	-	-	-	1.00

Task B	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.38	0.38	0.67
Toluene	-	1.00	0.88	0.09
Hexane	-	-	1.00	-0.21
Ethyl Acetate	-	-	-	1.00

Task C	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.88	0.92	0.99
Toluene	-	1.00	0.62	0.81
Hexane	-	-	1.00	0.96
Ethyl Acetate	-	-	-	1.00

Task D	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.49	0.74	0.97
Toluene	-	1.00	0.35	0.64
Hexane	-	-	1.00	0.62
Ethyl Acetate	-	-	-	1.00

Task E	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.82	0.83	0.62
Toluene	-	1.00	0.97	0.82
Hexane	-	-	1.00	0.90
Ethyl Acetate	-	-	-	1.00

Task F	MEK	Toluene	Hexane	Ethyl Acetate
MEK	-	-	-	-
Toluene	-	-	-	-
Hexane	-	-	-	-
Ethyl Acetate	-	-	-	-

Task G	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.67	-0.66	0.90
Toluene	-	1.00	0.11	0.70
Hexane	-	-	1.00	-0.53
Ethyl Acetate	-	-	-	1.00

Task H	MEK	Toluene	Hexane	Ethyl Acetate

MEK	1.00	-0.30	-0.37	0.16
Toluene	-	1.00	0.71	0.33
Hexane	-	-	1.00	0.46
Ethyl Acetate	-	-	-	1.00

Task I	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	-0.80	-0.67	-0.48
Toluene	-	1.00	0.98	0.91
Hexane	-	-	1.00	0.97
Ethyl Acetate	-	-	-	1.00

Table 4. Input Means and Variances for Simulation

	MEK		Toluene		Hexane		Ethyl Acetate	
	Mean (ppm)	Variance (ppm^2)	Mean (ppm)	Variance (ppm^2)	Mean (ppm)	Variance (ppm^2)	Mean (ppm)	Variance (ppm^2)
Cleaning								
Task A	11.2	356. (3.19)	56.3	2880. (2.24)	9.7	178. (2.804)	47.2	4063. (2.77)
Task B	7.9	46. (2.11)	91.7	6172. (2.10)	14.0	328. (2.86)	24.1	1275. (2.94)
Task C	-	-	133.	13370. (2.12)	-	-	12.9	133. (2.15)
Gluing								
Task D	-	-	229.0	74447. (2.56)	78.7	6847. (2.37)	-	-
Task E	-	-	147.6	28665. (2.50)	20.4	846. (2.87)	-	-
Task F	-	-	20.7	628. (2.59)	-	-	-	-
Assembly								
Task G	215.2	165311. (3.43)	86.18	13544. (2.77)	28.1	1368. (2.73)	-	-
Task H	110.0	14286. (2.42)	111.16	10163. (2.17)	29.2	908. (2.34)	-	-
Task I	34.5	2292. (2.82)	211.67	70171. (2.64)	33.4	3082. (3.16)	-	-

* Geometric standard deviation (GSD).

** The cells without values are the mean exposures less than 5 ppm. It is assumed that the solvents are not used in these tasks.

Table 5. Cross-correlation Matrices for Rafting Simulation

Work Task A	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.17	0.54	0.77
Toluene	-	1.00	0.36	0.04
Hexane	-	-	1.00	0.26
Ethyl Acetate	-	-	-	1.00
Work Task B	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.45	0.30	0.55
Toluene	-	1.00	0.80	0.05
Hexane	-	-	1.00	-0.15
Ethyl Acetate	-	-	-	1.00
Work Task C	MEK	Toluene	Hexane	Ethyl Acetate
MEK	-	-	-	-
Toluene	-	1.00	-	0.81
Hexane	-	-	-	-
Ethyl Acetate	-	-	-	1.00
Work Task D	MEK	Toluene	Hexane	Ethyl Acetate
MEK	-	-	-	-
Toluene	-	1.00	0.35	-
Hexane	-	-	1.00	-
Ethyl Acetate	-	-	-	-
Work Task E	MEK	Toluene	Hexane	Ethyl Acetate
MEK	-	-	-	-
Toluene	-	1.00	0.97	-
Hexane	-	-	1.00	-
Ethyl Acetate	-	-	-	-
Work Task F	MEK	Toluene	Hexane	Ethyl Acetate
MEK	-	-	-	-
Toluene	-	-	-	-
Hexane	-	-	-	-
Ethyl Acetate	-	-	-	-
Work Task G	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	0.50	-0.21	-
Toluene	-	1.00	0.11	-
Hexane	-	-	1.00	-
Ethyl Acetate	-	-	-	-
Work Task H	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	-0.30	-0.37	-
Toluene	-	1.00	0.71	-
Hexane	-	-	1.00	-
Ethyl Acetate	-	-	-	-
Work Task I	MEK	Toluene	Hexane	Ethyl Acetate
MEK	1.00	-0.30	-0.30	-
Toluene	-	1.00	0.95	-
Hexane	-	-	1.00	-
Ethyl Acetate	-	-	-	-

Table 6. P and Q Matrices Used in Simulation

	P matrix				Q matrix			
Task A.	0.360254	-0.285504	-0.112260	0.288178	-0.420946	0.104382	-0.548181	0.666469
Mixing Paint	0.525343	0.185193	-0.009551	-0.033343	0.288206	0.192917	-0.320399	-0.166926
	0.767851	0.295291	-0.319385	0.131341	-0.137886	-0.244350	-0.051613	0.438873
	-0.049361	-0.239829	-0.323278	0.602975	-0.476172	0.347532	-0.395463	0.602975
Task B.	0.542656	0.606962	-0.224260	-0.394037	0.244562	-0.219136	0.472562	-0.027099
Spraying Paint	0.230832	0.554062	-0.247752	-0.244711	-0.123815	0.344117	0.553638	-0.182789
	-0.111095	0.361317	0.099036	-0.330426	-0.445543	0.287053	0.632613	-0.154978
	1.015184	0.784779	-0.691342	-0.273168	0.808263	-0.182065	0.293576	0.082687
Task C	0.325838	0.042969			0.525689	0.460942		
Cleaning	-0.034400	0.394506			0.703728	0.114476		
Task D	0.211216	0.462512			-0.769341	0.148303		
Applying Neoprene Adhesive	-0.293769	0.528138			-0.438020	-0.617735		
Task E	0.777858	-0.336920			-0.782050	0.303874		
Hull Assembly	0.634015	-0.113100			-0.807116	0.494802		
Task F	NA				NA			
Glue Cleanup								
Task G	0.272422	0.335888	-0.051031		0.146256	-0.987385	-0.354223	
Applying PVC	0.399874	-0.007974	0.647787		0.487123	-0.374241	-0.620999	
Adhesive	0.399929	-0.433680	0.787587		0.366551	0.787800	-0.251554	
Task H	0.300120	0.790247	-0.645701		0.163120	0.562567	0.404871	
Floor Assembly	-0.268387	0.149623	0.071950		0.471899	-0.458242	-0.184306	
	0.153576	-0.304983	0.714272		0.297666	-0.391680	-0.589929	
Task I	0.310762	2.057736	-1.876311		-0.340461	-0.272826	0.612316	
Floor Installation	0.088084	-1.692749	1.806607		0.501225	0.182450	-0.509687	
	0.068833	0.128900	0.315524		0.728796	0.034255	-0.570356	

Table 7. Perfect Classification Rate of Different Day-Mean Exposures

# of Day	2	3	4	5	6	7	8	9	10
No. of Samples	200	200	200	200	200	200	200	200	200
No. of Samples Perfectly Classified	43	108	137	165	182	183	192	197	197
Perfect Classification Rate	0.22	0.54	0.69	0.83	0.91	0.92	0.96	0.99	0.99

Table 8. 30-Day Group Mean Exposure

	MEK	Toluene	Hexane	Ethyl Acetate
Cleaning	8.27	83.3	9.29	31.7
Gluing	20.8	158	43.9	3.66
Assembly	143.	124.	29.9	4.45

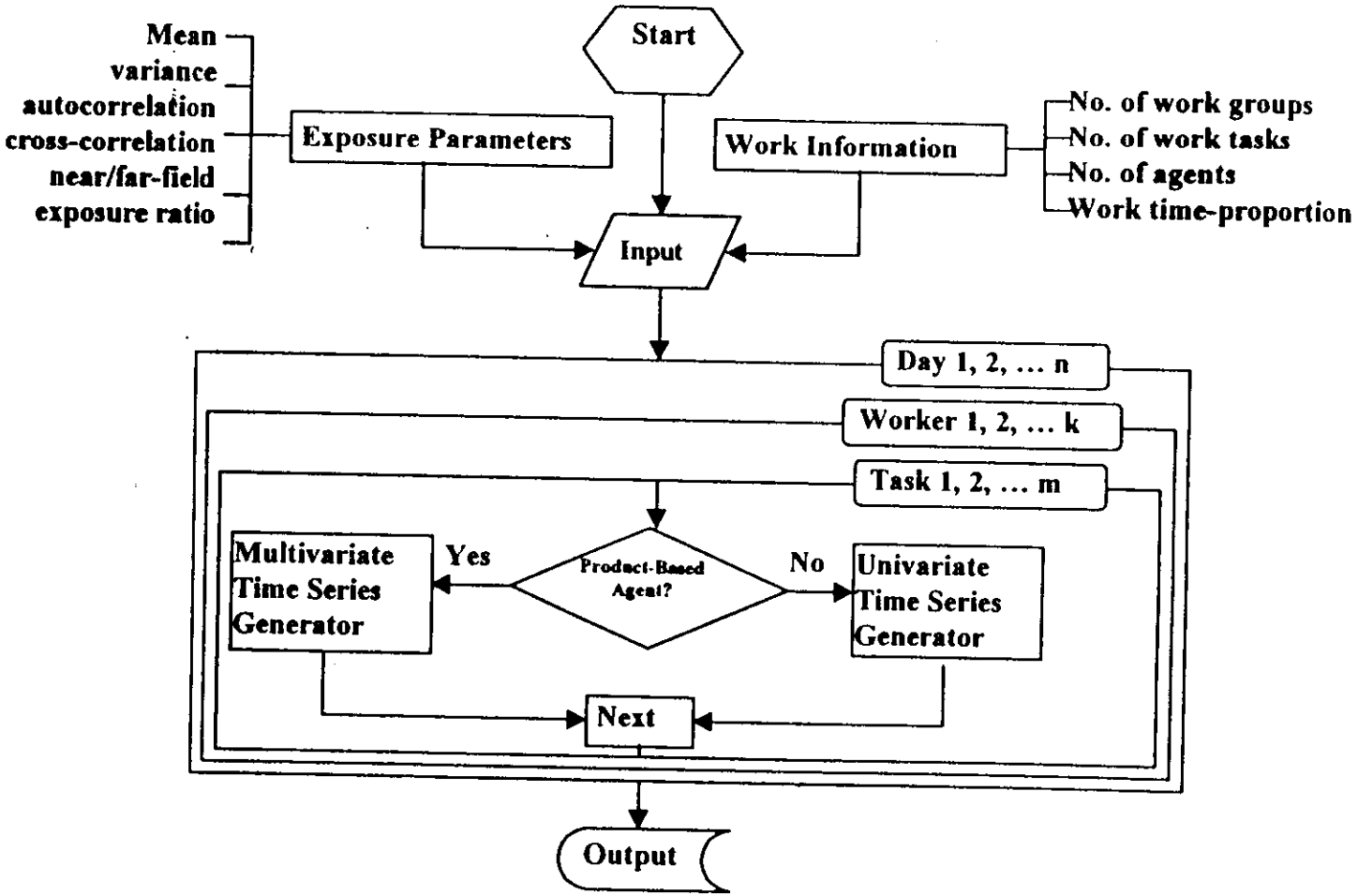

Figure 1. Structure of the computer simulator for occupational multiple exposures

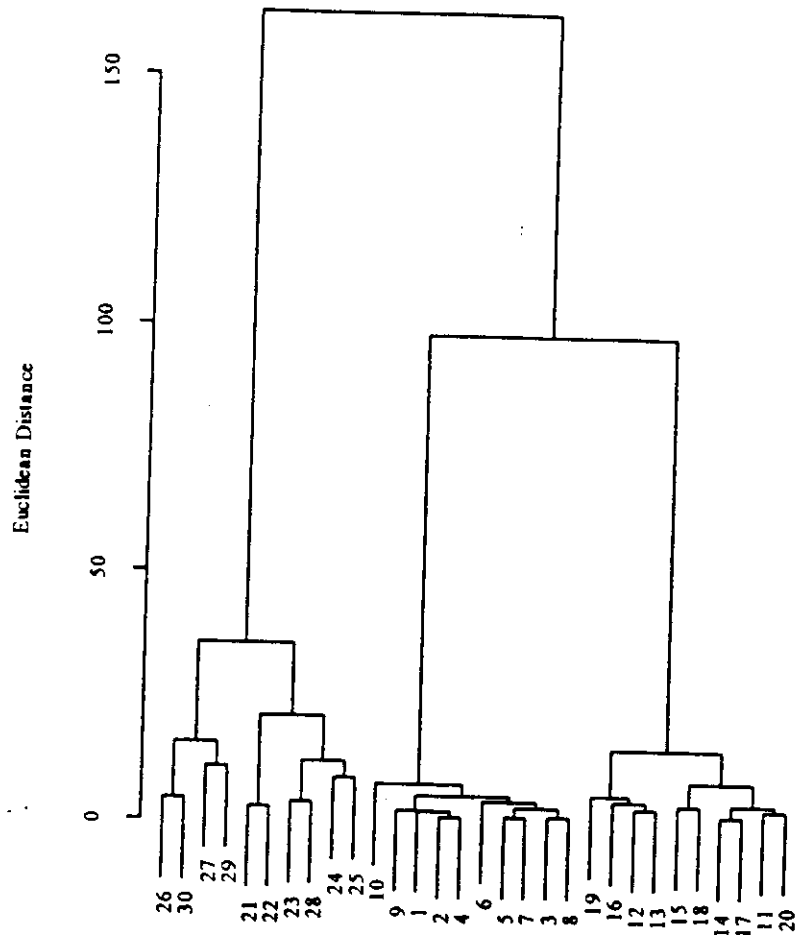

Figure 2. Hierarchical Tree Based on 30-day Mean Exposure

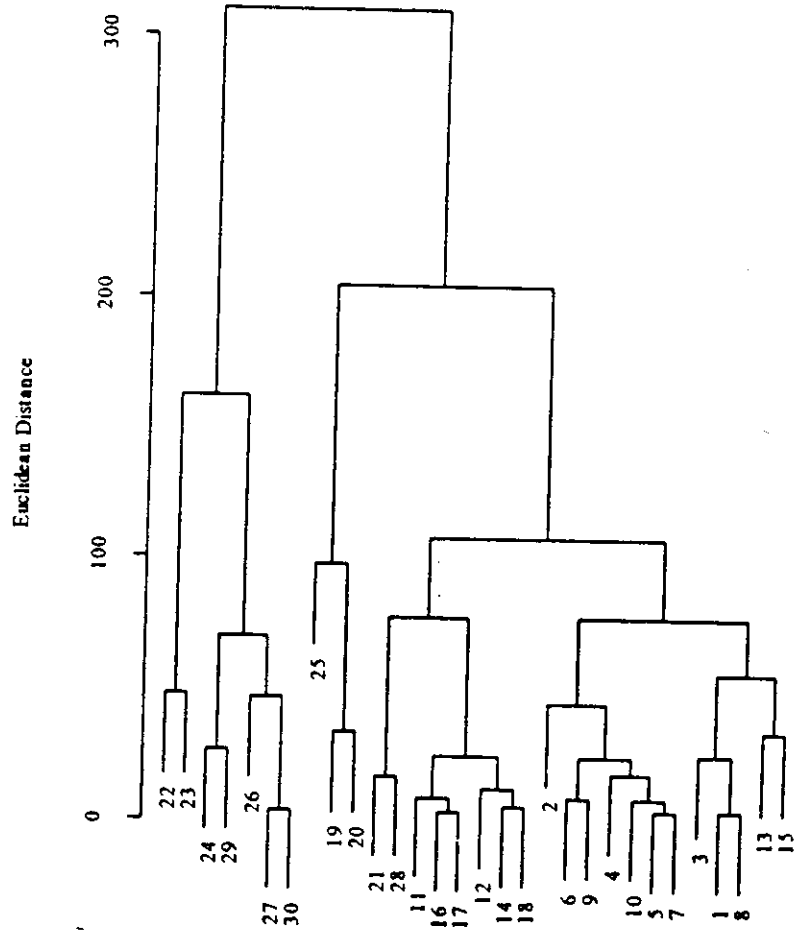

Figure 3. Hierarchical Tree of a Single Day Exposure ~~with More Than Three Clusters~~

Evaluating the Attributes of Incomplete Data on Workers' Exposures to Benzene: A CART Analysis

W.G. Chen, S.K. Hammond, T.E. McKone

In the U.S. Department of Energy (DOE) production facilities and several National Laboratories, radioactive and toxic chemicals are used or managed in many activities. Exposures to benzene and ionizing radiation have each been associated with increased risk of cancer in human population. How combined exposures to these two agents impact the cancer risk has not been characterized or quantified. We selected the Savannah River Site (SRS) to carry out a case study on mixed-agent exposures since at least two SRS facilities have been identified to have workers exposed to ionizing radiation in combination with benzene. Because the radiation dose database is not electronically or otherwise linked with any other database, such as medical or industrial hygiene data, the first stage of this work involves retrieving the benzene exposure data available at SRS in order to identify the workers we are interested in. The purpose of this paper is to demonstrate the use of classification and regression tree (CART) analysis to evaluate the sparse information available from the SRS about benzene exposures that were characteristic of the experience of exposed workers on site. 526 personal exposure samples collected from 1991 to 1998 were retrieved from SRS industrial hygiene database along with the relevant job information such as job titles, work locations, personal protection equipment used, control applied, etc. Additional job description and time activity pattern information was obtained from supplementary questionnaire surveys filled out by the SRS industrial hygienists. A CART analysis was applied to the combined information to determine what kind of job characteristics resulted in higher levels of benzene exposures. The results indicate that job title is the most important determining factor for higher benzene exposures. We also developed preliminary exposure distribution for each node in the tree diagram by using the regression tree analysis. Our results illustrate the unique benefit of CART analysis in maximizing the use of sparse data available on site.

Abstract Form
For the International Society of Exposure Analysis (ISEA) Annual Meeting
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Abstract Title: Simulation of Occupational Mixture Exposure to Multiple Airborne Agents

Several investigators have recently explored the factors contributing to the variability of workplace exposures to single airborne agents from day-to-day and between individuals in a work group. When attempting to extend this exploration to exposures where multiple agents are involved, the increase in dimensionality, coupled with the nature and extend of field data, tends to preclude all but exploratory analyses of the sources of variability in exposure. Attacking this problem from a different direction, we have constructed an exposure simulator which is based on short-term exposure distributions associated with the various tasks that comprise a day-long job. The inputs to the simulation include the number of chemicals associated with each task, the means and variances of their short-term exposure distributions, task duration as a proportion of the workday, the autocorrelation structure of the short-term exposures, the cross-correlation of exposures within a task, and a rough index of the near- and far-field exposures associated with each task in the workplace. The output of the simulator is the time-weighted average (TWA) exposure for the complete set of chemicals over extended periods, typically an eight-hour workday. For example, the distributions of eight-hour exposures can be estimated for a single worker or for groups engaged in the same tasks. The simulator thereby allows the evaluation of the sensitivity of the integrated exposures to changes in any of the input parameters as well as explorations of the performance of various multivariate statistical techniques that might be used in the classification of exposures based on limited field data.

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Signature: Jyun-De Wu Date: 5/19/97

Simulation of Occupational Mixture Exposure to Multiple Airborne Agents

Jyun-De Wu and Robert C. Spear

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Abstract

Several investigators have recently explored the factors contributing to the variability of workplace exposures to single airborne agents from day-to-day and between individuals in a work group. When attempting to extend this exploration to exposures where multiple agents are involved, the increase in dimensionality, coupled with the nature and extend of field data, tends to preclude all but exploratory analyses of the sources of variability in exposure. Attacking this problem from a different direction, we have constructed an exposure simulator which is based on short-term exposure distributions associated with the various tasks that comprise a day-long job. The inputs to the simulation include the number of chemicals associated with each task, the means and variances of their short-term exposure distributions, task duration as a proportion of the workday, the autocorrelation structure of the short-term exposures, the cross-correlation of exposures within a task, and a rough index of the near- and far-field exposures associated with each task in the workplace. The output of the simulator is the time-weighted average (TWA) exposure for the complete set of chemicals over extended periods, typically an eight-hour workday. For example, the distributions of eight-hour exposures can be estimated for a single worker or for groups engaged in the same tasks. The simulator thereby allows the evaluation of the sensitivity of the integrated exposures to changes in any of the input parameters as well as explorations of the performance of various multivariate statistical techniques that might be used in the classification of exposures based on limited field data.

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* We would like to present it in platform sessions.

ABSTRACT FOR THE PRESENTATION DELIVERED AT
THE 1997 ANNUAL MEETING OF THE SOCIETY FOR RISK ANALYSIS (SRA)
WASHINGTON, D.C.; DECEMBER 1997

RISKS FOR WORKERS EXPOSED TO MIXED PHYSICAL AND CHEMICAL AGENTS: BENZENE AND RADIATION CASE STUDY. W.G. Chen, T.E. McKone, R.C. Spear; School of Public Health, 140 Warren Hall, #7360, University of California, Berkeley, CA 94720-7360.

Very little scientific data is currently available on the combined action of toxic agents that have either similar or different mechanisms of action. We consider here two agents widely used at U.S. Department of Energy (DOE) facilities, tritium—a source of ionizing radiation, and benzene—a chemically toxic agent and known human carcinogen. As human carcinogens, both ionizing radiation and benzene appear to have similar manifestations of genetic damage within living cells. Although numerous risk assessments have been conducted to estimate the risks associated with radiation and benzene, separately; no quantitative approach has yet been developed to assess the risks of occupational exposure to these agents in combination. Two decades ago, a Japanese study indicated that the combined cytogenetic effect of benzene with radiation in cultured human leukocytes could be synergistic. However, there have been very few studies revisiting this issue. For occupational risks arising from mixed agent exposures at DOE facilities we are exploring the extent to which multiple-agent (i.e. physical/chemical) exposures can be linked to both common health endpoints and common markers of likely health outcomes. We present here a case study in which we will follow and analyze for benzene exposure in combination with radiation exposure the framework for estimating the risks associated with health impacts due to multiple-agent exposures. In this analysis, we follow the steps of a typical risk assessment, but using markers of exposure and dose at each step. This case study provides the opportunity to examine how the findings of synergism in the Japanese study, if correct, would impact a risk-based framework for protecting workers from mixed agent exposures in the working environment.

RISKS FOR WORKERS EXPOSED TO MIXED PHYSICAL AND CHEMICAL AGENTS: BENZENE AND RADIATION CASE STUDY

W.G. Chen, T.E. McKone, R.C. Spear

Division of Environmental Health Sciences
School of Public Health
University of California at Berkeley

INTRODUCTION

APPROACH

PRELIMINARY RESULTS

SUMMARY/DISCUSSION

MOTIVATION

Why mixtures?

Humans are frequently exposed to multiple agents either simultaneously or in sequence

What's lacking?

- combined effects of multiple agents
- workplace standards for mixtures
- integrating mixed exposures over:

  multiple agents
  exposure routes
  time-exposure patterns
  agents having the same target tissue
  agents having additive or synergistic impacts
  ...

GOALS

Objective

To construct and critically evaluate a framework for assessing risks arising from exposure to physical and chemical agents in various combinations

Principal Focus

The risks arising from mixed occupational exposures at U.S. DOE facilities

BACKGROUND

- Risk assessment paradigm: NAS/NRC (1983; 1994)
- U.S. EPA (1986)
  Guidelines for risk assessment of chemical mixtures
- Dose additivity approaches for systemic toxicants
  Response additivity approaches for carcinogens
- Risk of a mixture of compounds that act independently on target sites (NRC, 1994)

$$P = 1 - \prod_{i=1}^{m} \prod_{j=1}^{n} (1 - P_{ij})$$

BIOLOGICAL BASIS

- **Epidemiological Evidence: human experience with mixed-agent exposures**

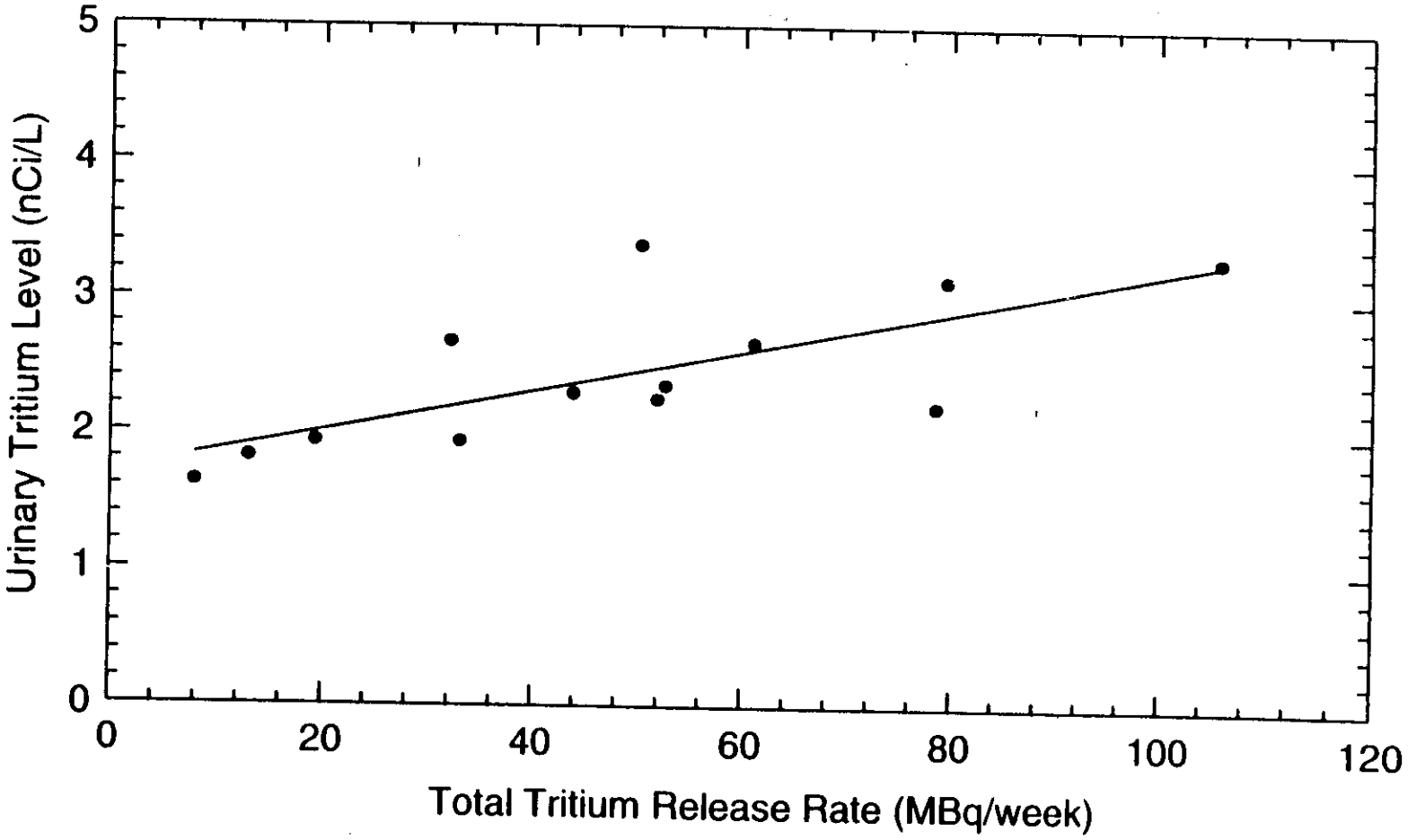
- **Evidence from Cytogenetic Studies: chromosome aberrations resulting from the combined effects of radiation and chemicals**

PRELIMINARY RESULTS

- **Literature review and framework development**
- **Examination on the feasibility of urinary tritium as exposure marker**

Data source: levels of occupational exposure in the National Tritium Labeling Facility (NTLF) at LBNL and in other nearby buildings.

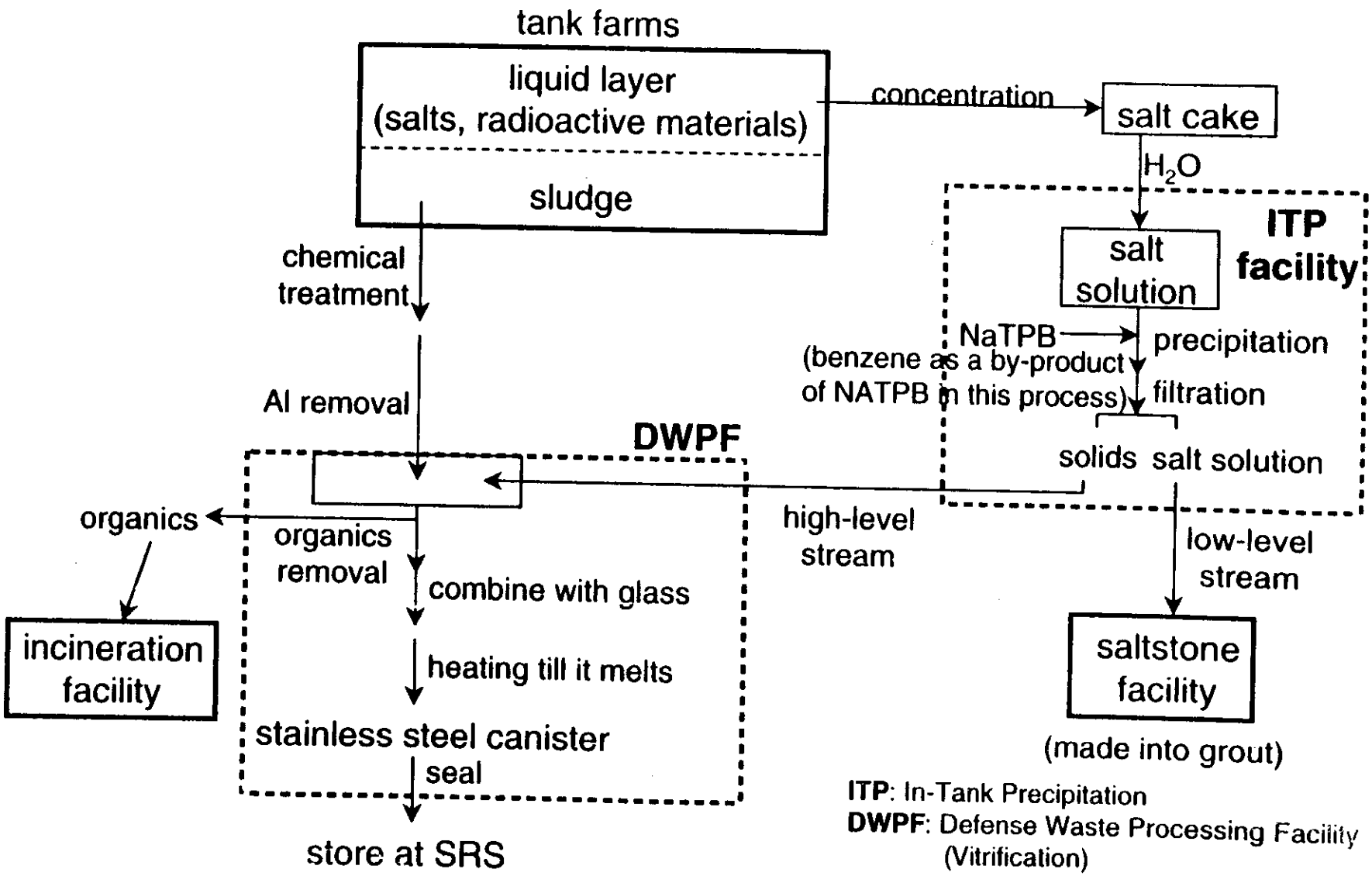
* Multivariate regression analysis:
  - Stack T emission is a strong predictor to estimate the urinary T levels measured in workers at NTLF
  - Up to 80% of the total variance can be explained by the best-fit model
  - Other predictive variables: windspeed and wind direction



Distributions of weekly tritium concentrations in urine samples from Building 75 compared to three-week average tritium releases from the LBNL tritium labeling facility during a three-month period

PRELIMINARY RESULTS (cont.)

- **Review and selection of field site**
  - Savannah River Site (SRS)
    In-Tank Precipitation Facility (ITP)
    Defense Waste Processing Facility (DWPF)
  - Epidemiological findings
    Excess leukemia risk


tank farms
liquid layer
(salts, radioactive materials)
sludge
concentration
salt cake
H_2O
ITP
facility
salt
solution
NaTPB
precipitation
(benzene as a by-product
of NATPB in this process)
filtration
solids
salt solution
chemical
treatment
Al removal
DWPF
organics
organics
removal
combine with glass
heating till it melts
stainless steel canister
seal
store at SRS
high-level
stream
low-level
stream
incineration
facility
saltstone
facility
(made into grout)
ITP: In-Tank Precipitation
DWPF: Defense Waste Processing Facility
(Vitrification)
NaTPB: sodium tetraphenylborate

INTRODUCTION

- Overview
- Background
- Biological basis

APPROACH

- Benzene and radiation case study
- Risk assessment framework

PRELIMINARY RESULTS

- Literature review and framework development
- Examination on the feasibility of urinary tritium as exposure marker
- Review and selection of field site

SUMMARY/DISCUSSION

SUMMARY/DISCUSSION

Risk Assessment for Mixed Exposures

- Develop and critically evaluate the risk assessment framework
- Identify information gaps and the future research needs

Developing a Tiered Approach for Mixed Radiation/Benzene Exposure

- Source/receptor relationships
- Receptor/dose estimates
- Markers of exposure/dose
- Markers of early health effects

ABSTRACT FOR THE PRESENTATION DELIVERED AT THE 1998 AMERICAN INDUSTRIAL HYGIENE CONFERENCE & EXPOSITION (1998 AIHCE)
ATLANTA, GEORGIA; MAY 1998

BIOLOGICAL MONITORING TO ASSESS THE HEALTH RISKS FOR WORKERS EXPOSED TO MIXTURES OF PHYSICAL AND CHEMICAL AGENTS

T.E. McKone, W.G. Chen, R.C. Spear
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140 Warren Hall
Division of Environmental Health Sciences
School of Public Health
University of California
Berkeley, CA 94720-7360

Limited scientific data is available on the combined effects of toxic agents that have either similar or different mechanisms of action. The goal of this study is to use biological monitoring to construct and critically evaluate a framework for assessing risks arising from exposure to physical and chemical agents in various combinations. We consider two agents widely used at U.S. Department of Energy (DOE) facilities, tritium--a source of ionizing radiation, and benzene--chemically toxic and a known human carcinogen. Both ionizing radiation and benzene appear to have similar manifestations of genetic damage within living cells. A 1976 Japanese study indicated that the combined cytogenetic effect of benzene with radiation in cultured human lymphocytes could be synergistic, but follow-up studies are lacking. We present here early results of a pilot study in which we apply the steps of the standard risk assessment paradigm to mixed physical/chemical agent exposures and use markers of exposure and dose at each step. This study provides the opportunity to examine how the findings of synergism in the Japanese study, if correct, would impact a risk-based framework for protecting workers from mixed agent exposures in the workplace. Specifically we examine the feasibility of using tritium levels and benzene metabolites in urine as markers of occupational exposures at DOE facilities. At one DOE facility multiple regression analysis was used to evaluate the correlation between urinary tritium levels of workers in various buildings and tritium release rate, wind speed, wind direction, precipitation and other parameters. Among parameters considered, correlation was strongest between urine levels and three-week average of the tritium source strength prior to the sampling date. We also report here on the process used to select and assess a DOE site with combined radiation and benzene exposures in the occupational environment.

Biological Monitoring to Assess the Health Risks for Workers Exposed to Mixtures of Physical and Chemical Agents

W.G. Chen, T.E. McKone, R.C. Spear

Division of Environmental Health Sciences
School of Public Health
University of California at Berkeley

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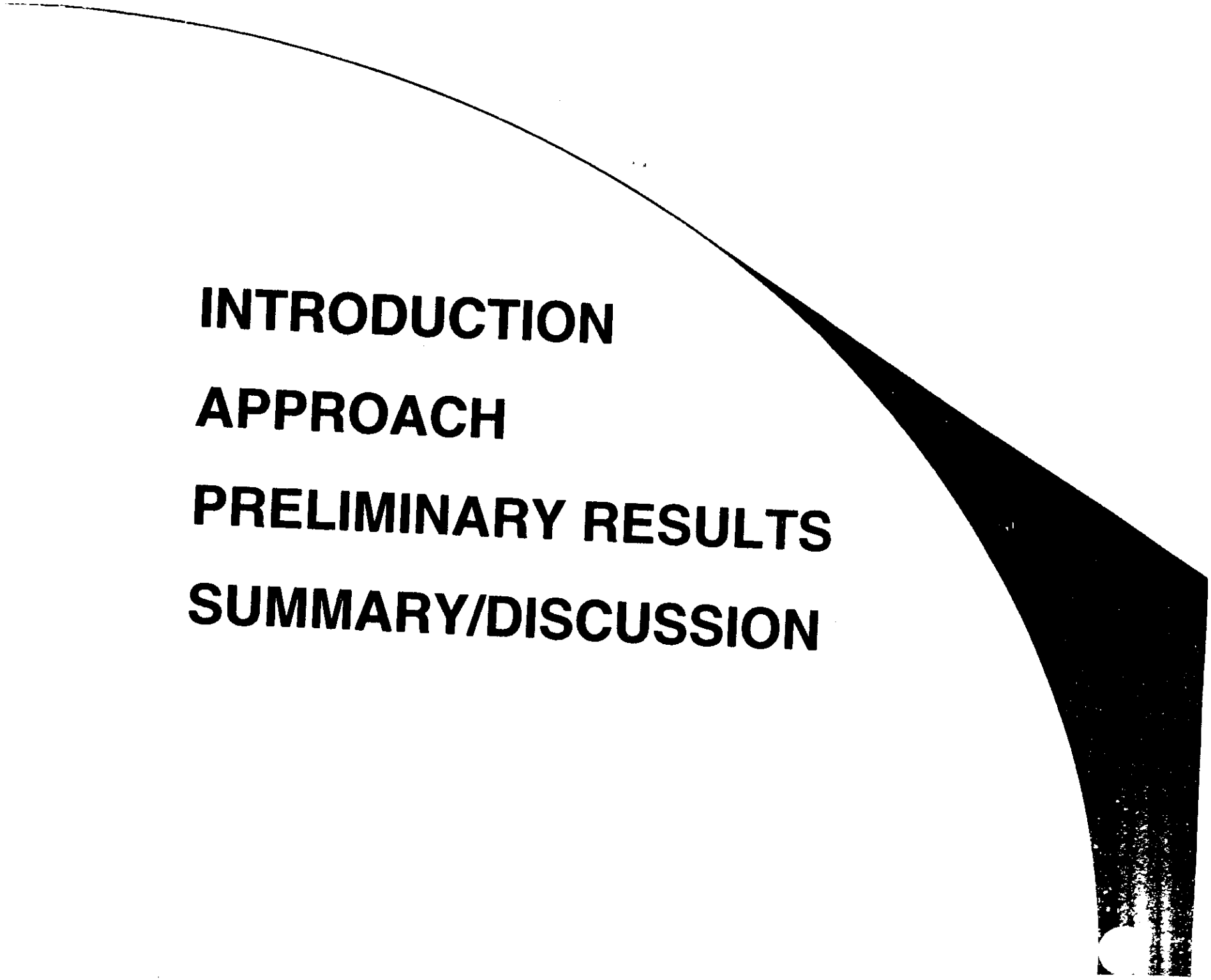


MOTIVATION

Why Mixtures?

Humans are frequently exposed to multiple agents either simultaneously or in sequence

What's Lacking?

- combined effects of multiple agents
- workplace standards for mixtures
- integrating mixed exposures over:

  multiple agents
  exposure routes
  time-exposure patterns
  agents having the same target tissue
  agents having additive or synergistic impacts
  ...

BACKGROUND

- **NAS/NRC (1983; 1994)**

  Risk assessment paradigm

- **U.S. EPA (1986)**

  Guidelines for risk assessment of chemical mixtures

- **Absence of Guidelines for Mixed Agents**

GOALS

Objective

Using biological monitoring to construct and critically evaluate a risk assessment framework for mixed exposures to physical and chemical agents

Principal Focus

The risks arising from mixed occupational exposures at the U.S. DOE facilities

BIOLOGICAL BASIS

Epidemiological Evidence: Human Experience with Mixed Agents

- Chemotherapy in combination with radiotherapy
  Increased risk of a secondary acute leukemia
- Tobacco smoke
  — Uranium miners — Atomic bomb survivors
- Workers occupationally exposed to radiation in combination with chemicals

BIOLOGICAL BASIS (cont.)

Evidence from Cytogenetic Studies: Chromosome Aberrations

Patients treated with radiation in combination with chemotherapy

Experiments using human cell culture

- Radiation and alkylating agents
- Radiation and benzene (Morimoto, 1976)

BIOLOGICAL BASIS (cont.)

The Morimoto Study

- Combined cytogenetic effect of benzene with radiation:
  synergistic in dicentrics and rings
  additive for other types of aberrations
- Benzene at higher concentrations may inhibit repair of radiation-induced chromosome breaks
- Phenol could significantly inhibit rejoining of radiation-induced chromosome breaks at very low concentrations

INTRODUCTION

- Motivation/Goal
- Background
- Biological Basis

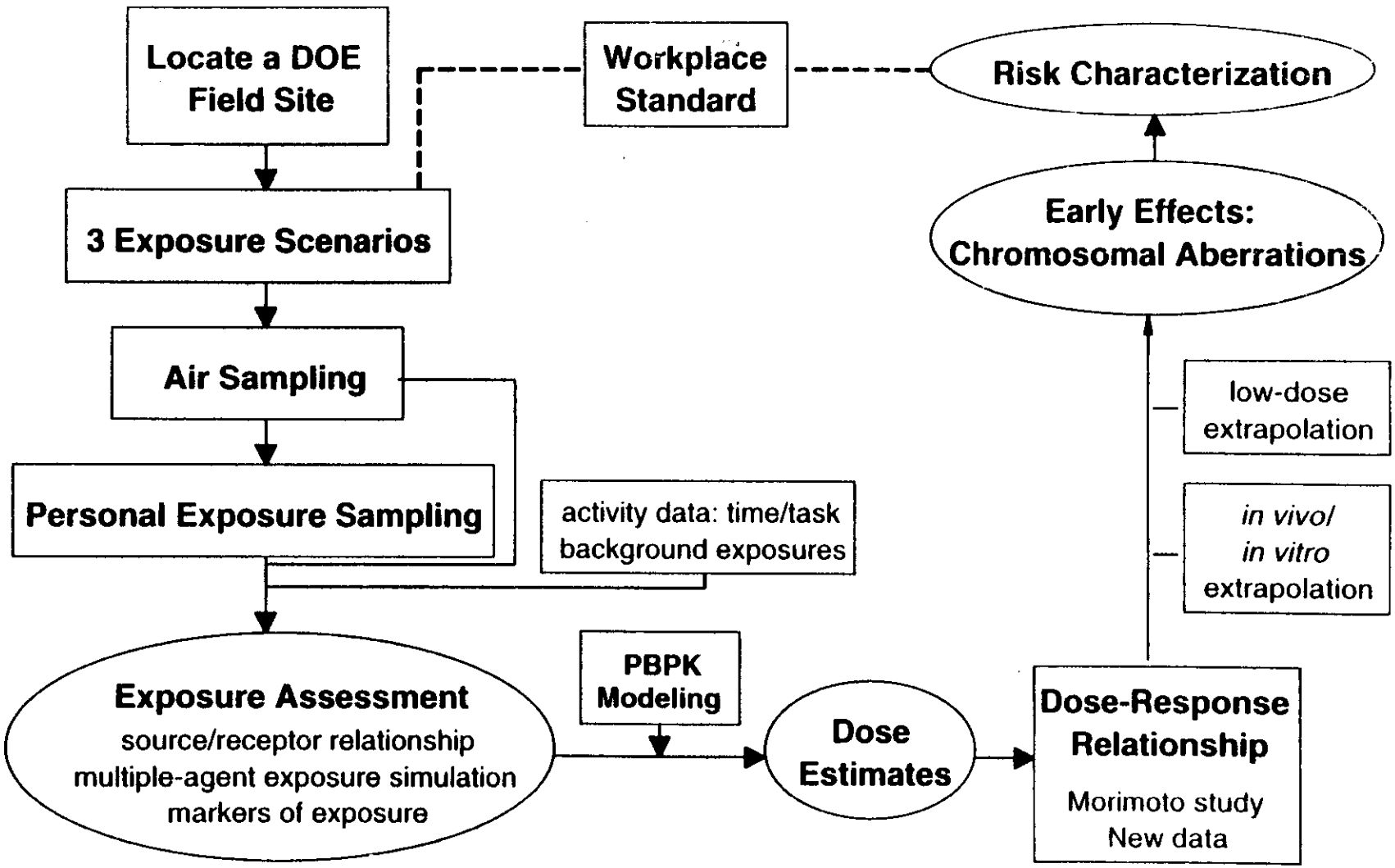
APPROACH

PRELIMINARY RESULTS

SUMMARY/DISCUSSION

APPROACH

- Case Study: Benzene and Radiation (Tritium)
- Selecting a Mixed-Exposure Population
- Using Biological Monitoring to Construct and Evaluate the Risk Assessment Framework



Proposed framework for assessing risks arising from occupational exposure to benzene in combination with radiation

INTRODUCTION

- Motivation/Goal
- Background
- Biological Basis

APPROACH

- Benzene and Radiation Case Study
- Risk Assessment Framework

PRELIMINARY RESULTS

SUMMARY/DISCUSSION

PRELIMINARY RESULTS

- Literature Review and Framework Development
- Examination on the Feasibility of Urinary Tritium as Exposure Marker
- Review and Selection of Field Site
- Chromosome Aberrations as Risk Marker

PRELIMINARY RESULTS (cont.)

- **Examination on the Feasibility of Urinary Tritium as Exposure Marker**

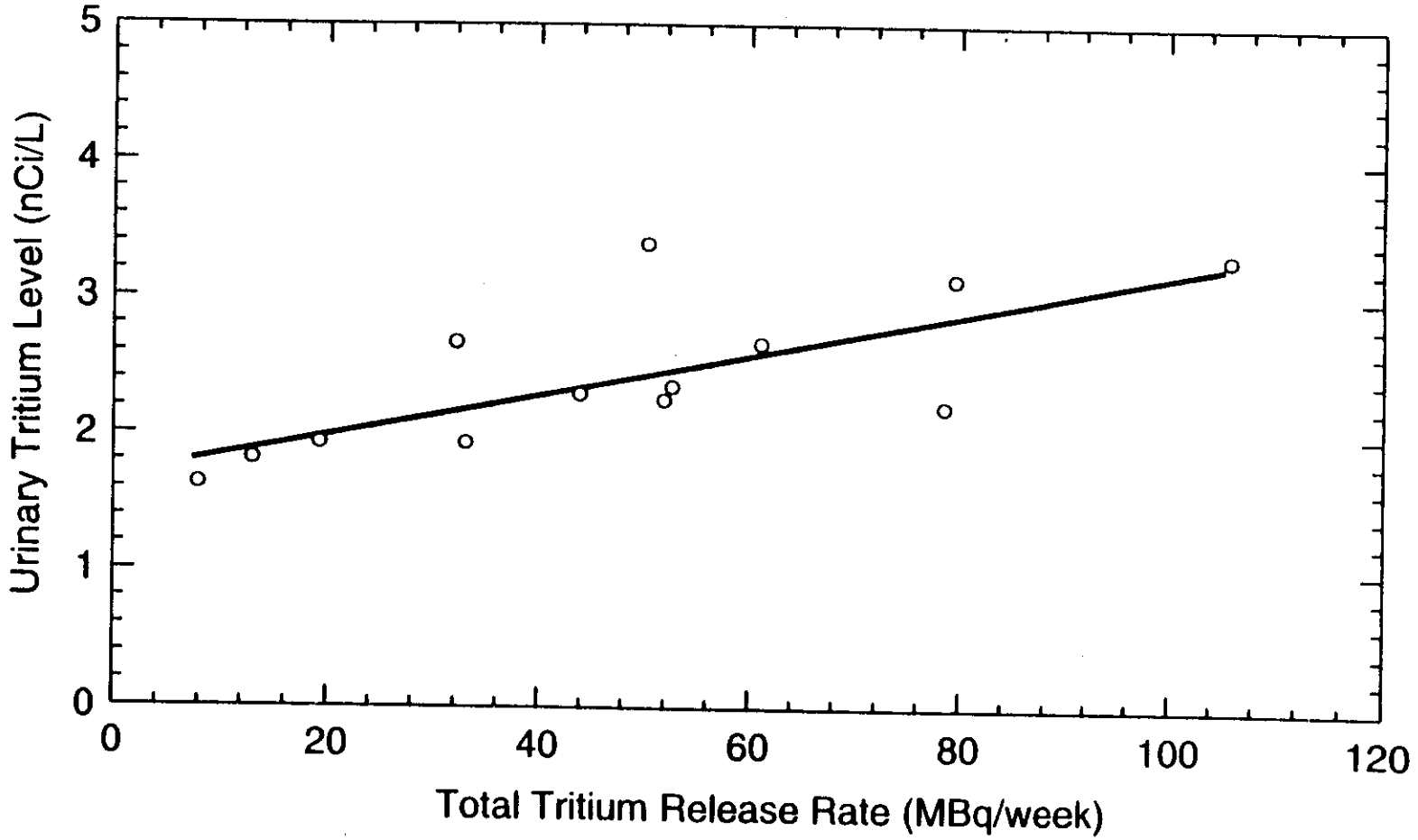
Data source: levels of occupational exposure at the NTLF-LBNL and other nearby buildings

* Multivariate regression analysis:

  – Strongest correlation between urinary T levels and three-week average of the T emission prior to the urine sampling date

  – Up to 80% of the total variance can be explained by the best-fit model

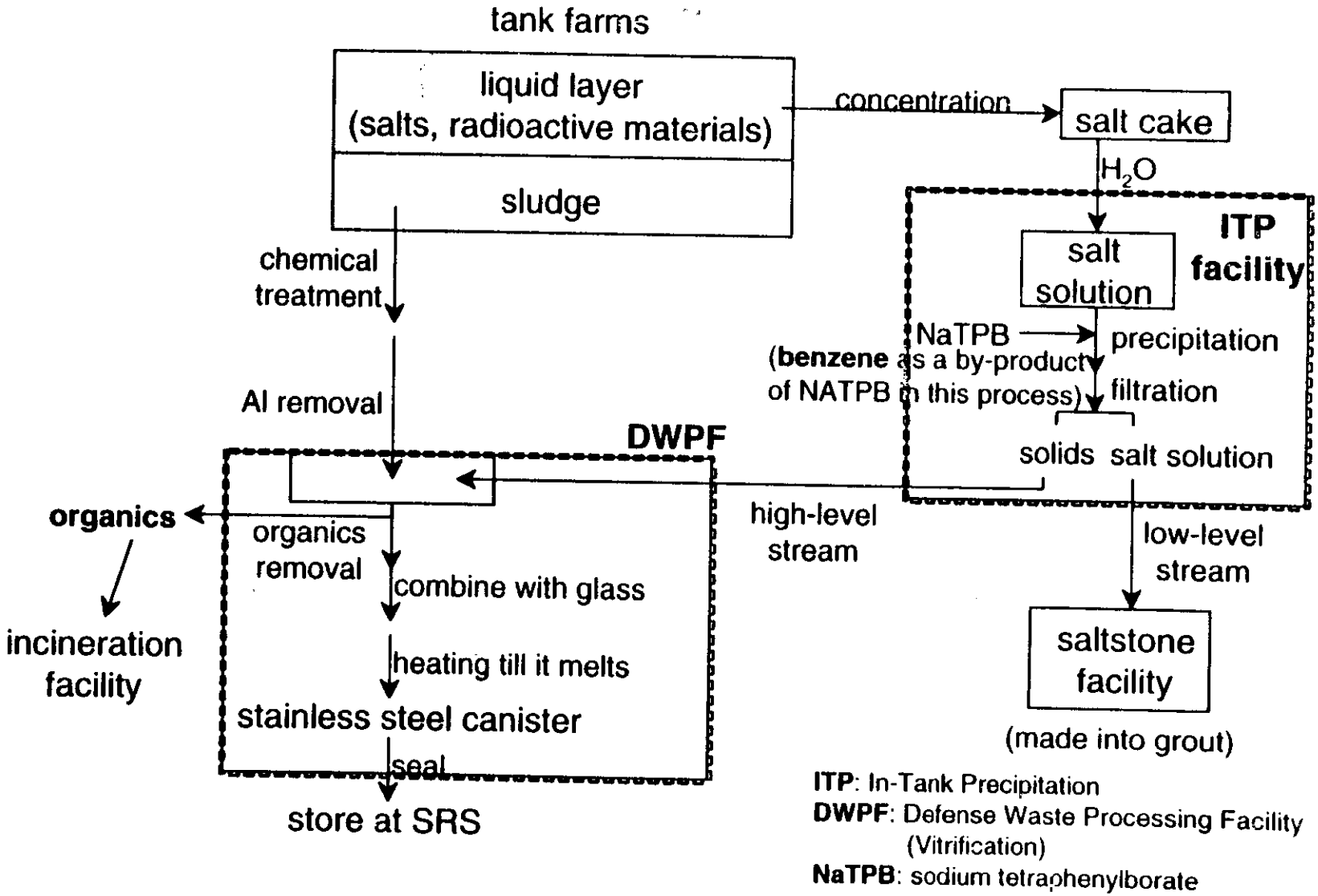
  – Other predictive variables: windspeed and wind direction



The distribution of weekly tritium concentrations in urine samples from Building 75 compared to three-week average tritium releases from the LBNL tritium labeling facility during a three-month period

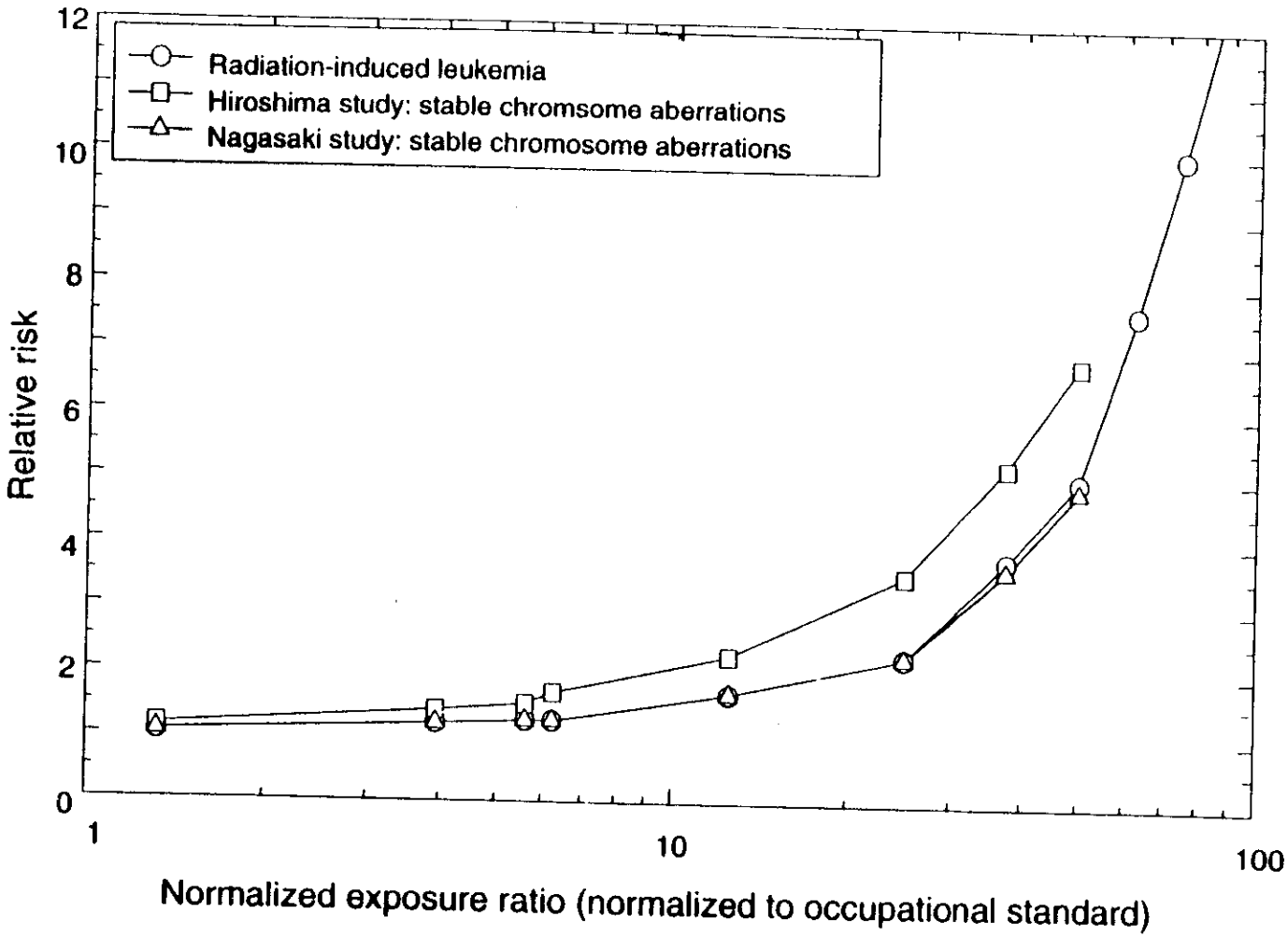
PRELIMINARY RESULTS (cont.)

- **Review and Selection of Field Site**
  - Savannah River Site (SRS)
    In-Tank Precipitation Facility (ITP)
    Defense Waste Processing Facility (DWPF)
  - Epidemiological Findings
    Excess leukemia risk
    (Cragle et al., 1988 & 1995)

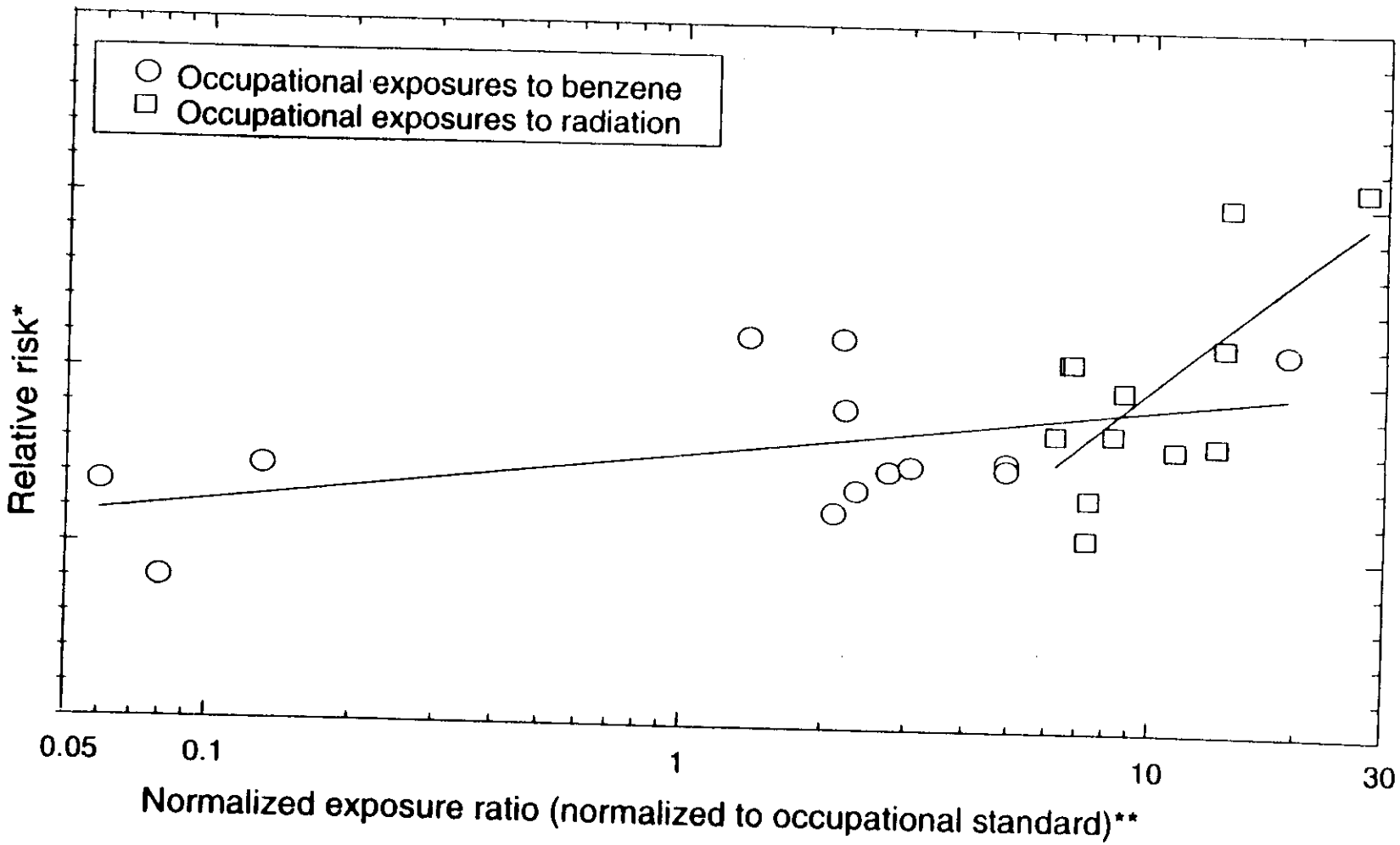

tank farms
liquid layer
(salts, radioactive materials)
sludge
concentration
salt cake
H_2O
ITP
facility
salt
solution
NaTPB
precipitation
(benzene as a by-product
of NATPB in this process)
filtration
solids
salt solution
chemical
treatment
Al removal
DWPF
organics
organics
removal
combine with glass
heating till it melts
stainless steel canister
seal
store at SRS
high-level
stream
low-level
stream
incineration
facility
saltstone
facility
(made into grout)
ITP: In-Tank Precipitation
DWPF: Defense Waste Processing Facility
(Vitrification)
NaTPB: sodium tetraphenylborate

PRELIMINARY RESULTS (cont.)

- **Chromosome Aberrations as Risk Marker**
  - Radiation-induced chromosome aberration vs. leukemia data (Mendelsohn 1995)
  - CA is predictive of future cancer risk (Hagmar et al. 1994; Bonassi et al. 1995)
  - Preliminary Analysis



The relationship between relative risks and normalized exposure ratios for radiation-induced chromosome aberration and leukemia data (data source: Mendelsohn, 1995)



The cytogenetic effects caused by occupational exposures to benzene and ionizing radiation. (* Relative risks are estimated as the ratio of chromosome aberration frequency in exposed to control. **The x axis is the normalized exposure ratio which is normalized to the current occupational standard for each agent, 20 mSv for ionizing radiation and 1 ppm for benzen[illegible])

INTRODUCTION

- Motivation/Goal
- Background
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APPROACH

- Benzene and Radiation Case Study
- Risk Assessment Framework

PRELIMINARY RESULTS

- Literature Review and Framework Development
- Urinary Tritium as Exposure Marker
- Review and Selection of Field Site
- Chromosome Aberrations as Risk Marker

SUMMARY/DISCUSSION

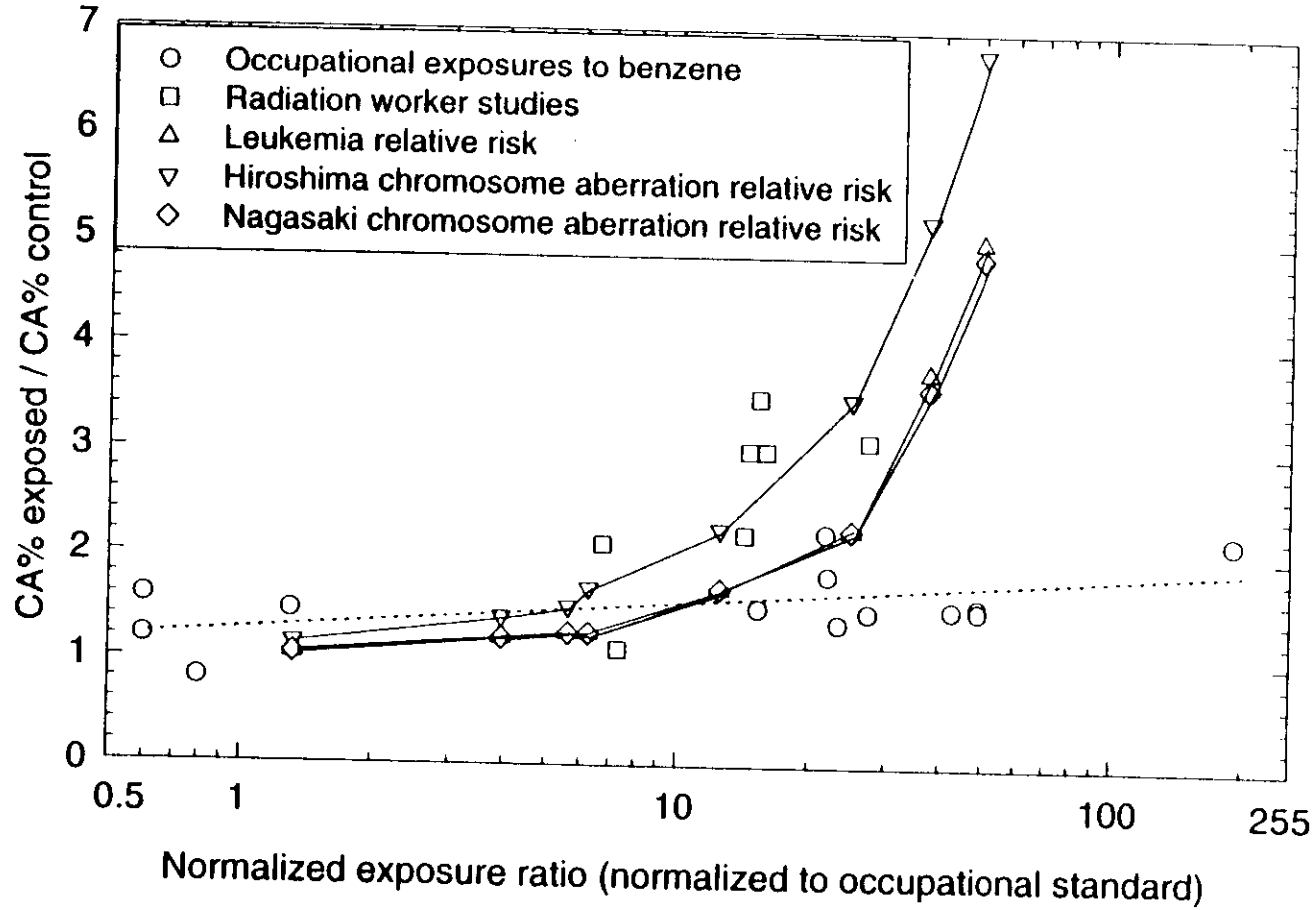
SUMMARY/DISCUSSION

Risk Assessment for Mixed Exposures

- Develop and critically evaluate the risk assessment framework
- Identify information gaps and the future research needs

Developing a Tiered Approach for Mixed Radiation/Benzene Exposure

- Source/receptor relationships
- Receptor/dose estimates
- Markers of exposure/dose
- Markers of early health effects



The cytogenetic effects caused by occupational exposures to benzene and radiation. (The x axis is the normalized exposure ratio which is normalized to the current occupational standard for each agent, 20mSv for ionized radiation and 0.1ppm for benzene.)

EXPLORING THE USE OF SIMULATION AS A TOOL FOR WORKPLACE EXPOSURE ASSESMENT

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(1) Abstract

Data relevant to occupational exposures comes in many forms ranging from chemical inventories to measured exposures over various periods or tasks. We have been investigating the use of computer simulation techniques as a means of integrating these diverse data. A first generation simulator, developed previously, was used in these studies. In general, the simulator output is in the form of exposure distributions for various workers or work groups.

In this paper, we demonstrate the use of the exposure simulator to a hypothetical screen-printing rkplace, where chemical usage, tasks and worker information, and environmental factors are gathered from real screen-printing studios. Based on the collected information, we utilize mathematical models to obtain inputs for this simulator. Data sets are generated to simulate workers' exposures under different work conditions. We explored the statistical issues and technical problems encountered during the process of simulator operation. The results suggested that incorporating between-worker variability, improving the methods of inserting input parameters, and a more user-friendly computer interface will be the next priorities in improving the simulator.

By combing the use of mathematical models and the modified simulator, the approach developed in this study can be used as a useful tool for workplace exposure assessments to guide exposure surveys in the field. The simulation results can serve as guidelines to design sampling strategies, identify high risk workers, or to decide where control devices should be provided. This method may provide guidance in the design of work environments.

(2) Introduction

(Column 1)

Problem of Traditional IH Workplace Exposure Assessment Methods:

- High Cost (Sampling, Laboratory Analysis or Direct-reading Instrument), especially for multiple-agent exposures.
- ... to workplaces where real problem exist.

- Sample Representative (insufficient sample size, non-random sampling)
  The difference between the samples and the actual exposure distribution is not usually considered in the traditional methods.
  or
- High proportions of measurement values under the limit of detection.

(Column 2)

Goal of Conducting This Study:

- Applying mathematical model and computer simulation as a tool to obtain workers' exposure information.
- Exploring a method to obtain preliminary workplace exposure information to overcome the disadvantages of the traditional methods of workplace exposure assessment. A good method will have the following advantages:
  - Quick-and-clean operation
  - Low cost
  - Could be used to for exposure assessment for multiple agents.
  - Instead of a snapshot measurement for one worker in the workplace, "exposure distributions" and overall exposure patterns for workers in different job tasks could be obtained.
- Exploring other potential functions and uses of this approach.

(Column 3)

The "Simulator"

The Simulator was developed by Jyun-De Wu (Doctoral dissertation, 1997).

- Original purpose of developing this program was to generate data sets to simulate multiple exposure (or mixtures) in occupational settings that can be used for multivariate exposure assessments (e.g. cluster analysis).
- One important feature of the simulator is its ability to integrate different kinds of exposure data and work information (quantitative and qualitative) to generate data sets reflecting the important characteristics of real workplace exposures.
- Due to the mixture characteristics, and the time and spatial variability of multiple chemical agents, multivariate statistical methods were built into the simulator to characterize the exposure distributions.

However, one should keep in mind that the computer simulation does not replace the traditional field measurement data. It just integrates different types of information to estimate possible ranges of exposure that might be expected and the explore how these exposure depend on job structures, environmental factors, and chemical uses, etc.

(Column 4)

Operation of the Simulator

The Simulator is written in C++ language. Chemical usage, job/task distribution, exposure variability, and environmental factors are collected in the input files (work information and exposure parameters) for the operation of simulation.

- Inputs to the Simulator
  - *Means and variances of short-term (15-minute) exposure distribution.
  - Autocorrelation
  - Cross-correlation
  - Far-field / near-field exposure ratio
  - Task-time proportion

  * In the simulation, all short-term exposure distributions are assumed to be lognormal. Mean and variances of near-field short-term exposures are the principal inputs for the simulator. These values could be obtained from measurements data or from published literature. In this study, mathematical models were applied to calculate the short-term means from limited exposure information obtained from the field.

- The output of the simulator is 8-hour time-weighted average (TWA) exposure of all workers in the workplace

(Add Fig 2-1 to show how simulator works)

(Column 5)

Estimation of Near-field Shot-term Exposure

Two-Box Model

- Near-field vs Far-field
- Diagram:

Mass balance equations:

- Near-field: $V_N \cdot d\,C_N(t) = G(t) \cdot dt + \beta \cdot C_F(t) \cdot dt - \beta \cdot C_N(t) \cdot dt$
- Far-field: $V_F \cdot d\,C_F(t) = \beta \cdot C_N(t) \cdot dt - (Q + \beta) \cdot C_F(t) \cdot dt$

Where: G(t): generation rate function of the chemical agent (mg/min)
β: interzonal airflow rate (m^3/min) between near-field and far-field
Q: ventilation rate (m^3/min) of the room (near-field + far-field)

- TWA concentration:

$$C\,i\,t_1 - t_2 = \frac{1}{t_2 - t_1} \cdot \int_{t_1}^{t_2} C(t) \cdot dt$$

- Assuming equilibrium (steady state) concentrations for both near-field and far-field are both reached instantaneously, short-term exposure concentrations can be obtained with the following equations (Nicas, 1996):
  - $C_{N,SS} = G / B + G / Q$ ➔ **to be used as input short-term exposure mean (E(x))**
  - $C_{F,SS} = G / Q$

Estimate the Variance of exposure intensity

It is assumed that all short-term exposure distributions are lognormal. With the lognormal property of exposure distribution, and with assigned GSD from literature, variances can be calculated with the following equations:

- $GM(x) = E(x) / \exp(0.5 \times (\ln GSD(x))^2)$
- $Var(x) = (GM(X)^2) \times \{\exp(\ln GSD(X))^2\} \times \{\exp(\ln GSD(X))^2 - 1\}$

Where E(x) is the short-term exposure mean obtained from previous calculation.
GSD(x) is the geometric standard deviation, which is assigned to the exposure environment
Var(x) is the variance of the short-term exposure

(3) Study Design and Method

(Column 1)

Study Design

- Originally we intended to collect real field measurements (both short-term 15-min and 8-hour TWA) and validate the computer simulation program. However, due to difficulty of getting accesses to workplaces where probably significant exposures exist, and due to pilot samples collected were either well below exposure limits or under limits of detection, we decided to change the approach to obtain exposure information.

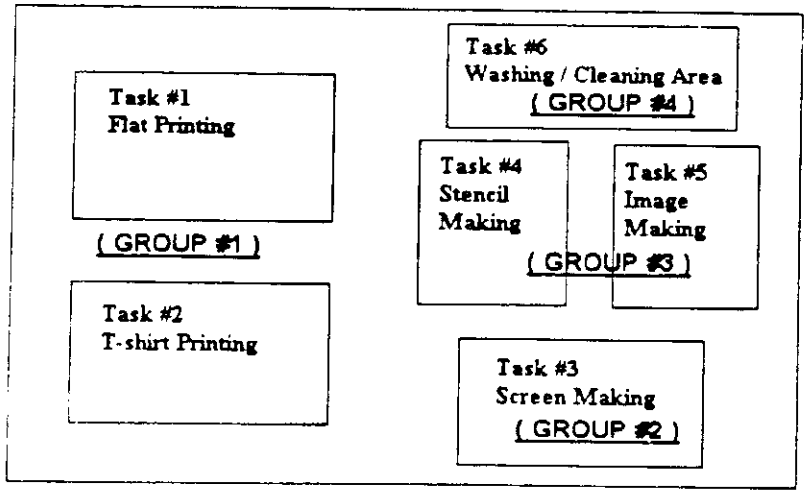
- We selected traditional Screen-printing industry as an example for the application of our simulation. Because:
  - Large amount of solvents is used in screen-printing, and some of them are very toxic.
  - In these workplaces, chemical usage and exposure are usually less well-controlled, and fewer protective devices are used.
  - Art-related workers are less informed of and less sensitive to health-related issues, and sometimes are more inclined to use their original work practices, which may lead to higher exposures to some agents.

(Column 2)

Construction of Hypothetical Workplace

- Under the worst-case scenario and based on the information collected from the field, we hypothetically constructed a workplace with the feature of a typical printing workplace, where most of the tasks are assumed continuously performed 8 hours a day, and 40 hours a week.
  (Diagram Fig. 4-1 Printing Studio Layout)

Fig 4-1 Layout of the Hypothetical Workplace



- In this hypothetical workplace, we assumed there are 8 workers working on 6 different tasks. 4 work groups are identified in this workplace: Group #1: Printing, Group #2: Screen making, Group #3: Image and stencil making, and Group #4: Cleaning / Washing Area. Workers' proportion of task time performing in each task are summarized in the following Table:

Table 4-1. Proportion of Task Time Performing in Each Task

Work Group	Worker	Work Time Proportions for Each Task
1	1, 2	50% Task #1, 50% Task #2
2	3, 4	100% Task #3
3	5, 6	50% Task #4, 50% Task #5
4	7, 8	100% Task #6

- Based on the chemical usage information collected in the real printing studios, target chemicals selected in our hypothetical workplace are: mineral spirit, methylene chloride, and ethyl acetate.

Table 4-7. Exposure Limits of the Target Chemicals in the Hypothetical Workplace

Agent	Exposure Limit	Mineral Spirit	Methylene Chloride	Ethyl Acetate
OSHA	PEL	2900	90	1440
NIOSH	REL	350	LFC (Ca)[2]	1440
ACGIH	TLV	550	180 (A3)[3]	1440 (A4)[3]

Note:

1. Unit: mg / m^3
2. NIOSH listed Methylene Chloride as potential occupational carcinogen. Occupational exposure is recommended to be limited to Lowest Feasible Concentrations (LFC).
3. ACGIH categorized Methylene Chloride as A3 – Animal Carcinogen, and Ethyl Acetate as A4 – Not Classifiable as a Human Carcinogen.

(4) Result

Column 1)

Examples of Simulation

- 30-day Simulations were run for 27 scenarios for the hypothetical workplace. Each scenario is characterized by a near-field radius (r = 1, 1.5 or 2 m), workplace airspeed (v = 0.1, 0.3, and 0.6 m/s), and exposure variability (GSD = 1.5, 2.5, and 3.5). The following are results of example simulations:

- Exposure scenario:
  - Exposure variability: GSD = 3.5

Mineral Spirits

Radius of near-field: 1 meter			
Wind speed	0.1 m / s	0.3 m / s	0.6 m / s
E [C] (mg/m^3)	820	290	130
Var [C]	360	170	60
Pr [C > PEL]	0%	0%	0%
Pr [C > REL]	98%	28%	0%
Radius of near-field: 1.5 meter			
Wind speed	0.1 m / s	0.3 m / s	0.6 m / s
E [C] (mg/m^3)	350	130	60
Var [C]	170	60	20
Pr [C > PEL]	0%	0%	0%
Pr [C > REL]	37%	0%	0%
Radius of near-field: 2 meter			
nd speed	0.1 m / s	0.3 m / s	0.6 m / s
C] (mg/m^3)	220	70	34
Var [C]	120	30	15
Pr [C > PEL]	0%	0%	0%
Pr [C > REL]	15%	0%	0%

Methylene Chloride

Radius of near-field: 1 meter			
wind speed	0.1 m / s	0.3 m / s	0.6 m / s
E [C] (mg/m^3)	410	130	70
Var [C]	180	60	30
Pr [C > PEL]	100%	82%	17%
Pr [C > REL]	-	-	-
Radius of near-field: 1 meter			
wind speed	0.1 m / s	0.3 m / s	0.6 m / s
E [C] (mg/m^3)	200	55	35
Var [C]	100	25	20
Pr [C > PEL]	95%	7%	3%
Pr [C > REL]	-	-	-
Radius of near-field: 1 meter			
wind speed	0.1 m / s	0.3 m / s	0.6 m / s
E [C] (mg/m^3)	120	35	20
Var [C]	50	15	15
C > PEL]	65%	2%	2%
> REL]	-	-	-

re E[C] and Var[C] represent mean and variances of 30-day simulated 8-hour TWA
Pr[C>PEL/REL] are the probability of the concentrations exceeding the PEL/REL in any one-day result

(Column 2)

Conclusion on Workers' Exposure in Hypothetical Workplace

- If the workplaces is with relatively high air speed, or the radius of near-field is larger or equal to 1.5 meter (workers have higher mobility), airborne exposures to all agents will generally be below the current PELs.

- Near-field exposure to methylene chloride should be the major concern for the workers in this workplace.

- The mineral spirit exposure of workers in cleaning areas should be reduced.

(Column 3)

Discussion on Simulated Results

- Exposure data of all workers of each group to each agents are generated. These results provide information of exposure distributions for each scenario, and a relative exposure relationship with a workplace.

- Theoretically, if all short-term exposure information are given, all 8-hour mean exposures and variances can be calculated by:

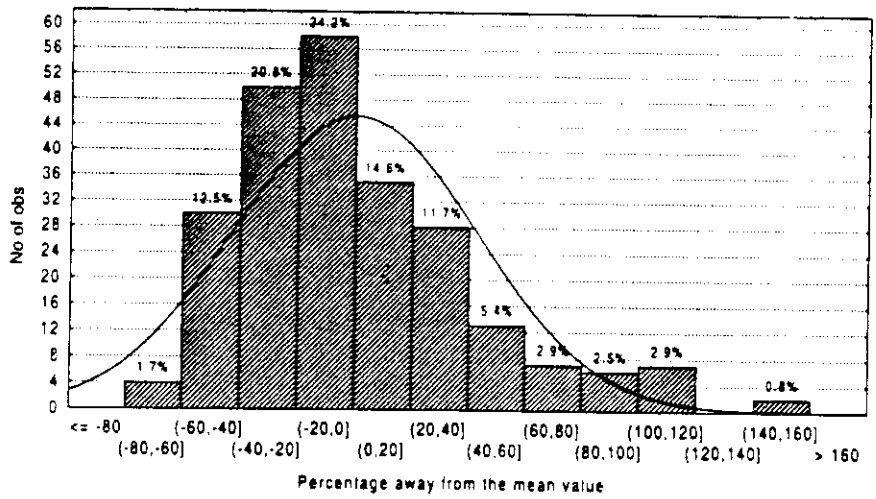
$$E[c_{8-hr}] = \sum_{h=1}^{k} Wh \times U_{15/h}$$

$$Var[C_{8-hr}] = \sum Wh^2 \times \frac{\sigma^2_{15/h}}{n_h}[1 + \frac{2}{n_h}\sum_{i=1}^{n_h-1}(n_h - i)\cdot e^{-\lambda u}]$$

- However, doing the calculation for each worker to construct daily exposure distributions for each of the agents is rather complicated and often not feasible. The simulator provides a way to generate data sets including all these information. The data generated for the hypothetical workplace are close to the results calculated from the above two equations, which means the simulation is a useful tool for obtaining estimations of means, variances, and distributions of occupational exposures.

- In the output of the simulator, log-normality in the distributions of each agent in all simulation results are observed. Larger dispersion of the distributions for larger short-term GSD are also observed, as expected.

Fig 4-3.2 Dispersion of the Exposure Data (Methylene Chloride)
(Simulation for r=1m, v=0.1 m/s, and GSD=3.5)



(Column 4)

Limitation and Problem Using the Simulator

- Between-worker variability in the exposure distribution is not incorporated.
- All parameters assigned to the simulation are fixed.
- Input parameter settings for workers who have no near-field exposure.
- Statistical issues related to built-in program
- A need for a more user-friendly computer interface.

(5) Conclusion

(Column 1)

The approach developed in this study depicts a different way to obtain exposure information without making real measurement. If the program is modified and to be further validated, this approach could be a useful and potentially powerful tool for workplace preliminary exposure assessment. Data sets can be generated to reflect the properties and characteristics of exposure distributions for all workers in the workplaces. Instead of getting information from a snapshot measurement, simulator provides an overall exposure pattern from exposure distributions. However, one should keep in mind that simulator is developed to integrate different types of information to estimate possible ranges of exposures to be expected. The simulations could also give inaccurate estimation if the input parameters are not accurately collected or assigned.

(Column 2)

Potential Applications of Computer Simulations in Industrial Hygiene

- A guide to direct exposure surveys and exposure reduction in the field.
- A preliminary tool to collect workplace exposure information when only limited access and sources can be obtained.
- A guide to decide where or how new control devices should be installed.
- A tool to predict workplace exposure before the workplaces are actually built.

(Column 3)

Future Work and Research Direction

- Validation of the functions of mathematical models and the simulator.
- Improving the estimation of the short-term exposure with mathematical models.
- Modify current study design and application to other industries.
- Modification for the simulator
  - Providing a more user-friendly computer interface
  - Improving the function that data are generated from short-term exposure distributions, but not fixed short-term exposure mean values
  - Solving the statistical issues inherent in current simulator programming
  - Including the ability to assign changeable exposure parameter in the simulations.
  - Incorporate automated short-term exposure calculations to obtain accurate exposure information when no field measurements are available

APPENDIX B

Optimized Portable Cordless Vacuum Method for Sampling Dry, Hard Surfaces for Dusts, S.Y. Kim, S. Que Hee, and J.Froines, Appl. Occup. Environ. Hyg., 15:503-511.

Surface Sampling for a Pesticide in Dust and Small Spills of a Solid Dye, S. Que Hee, Y. Shen, and J.C. Tso, Accepted, Appl. Occup. Environ. Hyg.

Effect of Dust on the Viability of Vibrio Fischeri in the Microtox Test, Submitted, Ecotox. Environ. Safety.

Applied Occupational and Environmental Hygiene
Volume 15(6): 503–511, 2000

1047-322X/00 $12.00 + .00

Optimized Portable Cordless Vacuum Method for Sampling Dry, Hard Surfaces for Dusts

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Surface sampling in industrial/environmental hygiene is a growing field that needs validated standardized methods. There are few standard methods, one being the American Society for Testing and Materials (ASTM) method involving a portable, cordless air sampling pump. In the present work, the ASTM technique was modified to increase efficiency and versatility. A soil sample was first dried and sieved. Known weights of different sieved sizes (125 μm–180 μm, 90 μm–125 μm, and 63 μm–90μm) were then sampled at an average flow rate of 4.0 ± 0.2 L/min from a template of inner dimensions 10 cm by 10 cm on two different surfaces (rough and smooth). Five consecutive sampling passes were performed. For the smooth surface, the first pass efficiency for the largest particles were 45% ± 45% (CV = 100%), and 75% ± 20% (CV = 27%) for the smallest particles. After three passes, the efficiency independent of particle size exceeded 83 percent with a CV better than 11 percent. After five passes, the efficiency exceeded at least 85 percent with about the same precision as for three passes. The rough surface allowed higher efficiencies for the first two sampling passes. Three to five passes are recommended to achieve acceptable efficiencies for the surface loose dust/soil range 100 μg/cm² to 1,500 μg/cm².

Keywords Lead, Surface Sampling, Dust, Dust Organics, Dust Metals, Surface Soil

Interest in surface dust sampling has been stimulated by research in evaluating metal,[1–25] organic chemical,[26–39] organic dust (biological),[40–52] and radioactive[9,35,53–56] exposures, but especially to children.[1–3,5–7,9–20,23–27,31,34,39,42–49] Among the metals, lead has been of prime interest, especially lead paint exposures to children,[1–3,5–7,9–20,23–25] lead abatement workers,[1,16,17] and potential nonferrous foundry exposures from dusts on surfaces.[4] Among the organics, exposure and surface pesticide residues after spraying is well studied.[27–30,32,33,35,37–39] For the biological vectors, studies on exposures to surface allergens[42–49] and microorganisms[40,41,50–52] are currently popular. Some studies on nonspecific dusts have also appeared,[5,57–61] mostly relating to sampling[5,57–59,61] or risk assessment.[5,18,39,56,60,61] All of the hazardous agents may be resuspended into the air by people, vehicles, pets, cleaning, equipment, abatement activities, ventilation, and wind to create inhalation threats.[11,14,17,41–56]

Out of all of these studies, very few investigators have provided efficiency data for sampling surface dusts. The Occupational Safety and Health Administration (OSHA) wipe technique efficiency for foundry dusts on work surfaces was reported in 1984 to vary between 43 to 90 percent, depending on pressure exerted, whether wet or dry (wet wiping was more efficient), and number of wipes (two wipes were more efficient than one).

A cordless vacuum method was reported by the present author in 1985[5] for sampling bioavailable contaminated dust from dry, hard surfaces using personal sampling pumps commonly used in industrial hygiene for air sampling. In addition, hand-wiping and hand-rinsing techniques were evaluated. The efficiency of the cordless method for five passes for known amounts of deposited surface dust of about 50 mg varied between 29 percent to 81 percent (arithmetic mean ± standard deviation 57 ± 14%, coefficient of variation CV = 25%, number n = 12) at a flow rate of 2.5 L/min using a stainless steel sampling probe for several different surfaces.[5] In contrast, for one sampling pass, the respective efficiencies were 21 percent to 59 percent (43 ± 14, CV = 33%, n = 12). The corresponding one-pass recoveries in terms of mass recovered after five passes were 44 percent to 93 percent (74 ± 14, CV = 19%, n = 12). A similar Tygon sampling probe at 2.0 L/min showed[5] a one-pass recovery between 42 percent to 89 percent (63 ± 11, CV = 17%, n = 7). The surface sampling efficiency for the Tygon probe was 70.0 ± 7.1 percent for particles <177 μm of an actual leaded house dust comprising about 81–82 percent of the leaded fraction and of dust weight. The collection efficiency decreased above this diameter; the overall efficiency for the whole dust was about 62 percent, this not being statistically different from the absolute efficiency

for the stainless steel sampler of 57 ± 14 percent. For a 20 L/min vacuum, the first pass recovery was 76 ± 12 percent, CV = 16%, n = 7 relative to the 63 ± 11, CV = 17%, n = 7 for the 2 L/min technique, not statistically different at $p \leq 0.05$ relative to imprecision (*F*-test) or recovery (*Student t* test). Thus, surface vacuum techniques, although they were not very efficient, were at least relatively precise (<20% CV).

The sampling techniques with several different handwipes varied in efficiency and precision also.[5] Efficiencies for five handwipings to recover 50 mg varied between 16 percent to 132 percent (79 ± 35%, CV = 44%, n = 11) for adults and children, with the range for one wiping being from 1.3 to 87 percent (mean 41 ± 26, CV = 61%, n = 11). The handwiping technique was not as precise as the surface vacuum technique, but was as efficient at $p \leq 0.05$. Hand rinsings also varied between 10 percent to 74 percent for five rinsings relative to 10 percent to 45 percent for one rinsing. A combination wipe/rinse method gave an efficiency of about 84 percent. These results explained why epidemiological studies up to that time did not show correlations between hand lead and blood lead for imprecise, inefficient handwiping/handrinsing techniques, but did between surface dust lead and blood lead for precise but moderately inefficient vacuum techniques. The sensitivity and precision of sampling and analytical techniques for lead were less important for when lead dust or soil levels were high and a large sample was taken for analysis (for example, >100 mg), as for epidemiological studies on populations living around lead smelters,[1-3] smelter or brass foundry workers, or on children that ate pica. Even if lead concentration was high, a low dust loading on hands usually caused sensitivity and precision problems.

The Environmental Protection Agency (EPA) in 1986 published a model that involved a reference child being exposed by mouthing activity to average daily doses of dust of 50 mg of average lead concentration 0.3 mg/g within households, 40 mg from street dust of average lead concentration 0.090 mg/g, and 10 mg of average lead concentration 0.150 mg/g at schools.[65]

If the hand lead sampling technique was also imprecise and dust loading on hands was low, statistical comparisons of exposed and "unexposed" populations often led to statistically nonsignificant results in studies before 1985.[65] The availability of the dust deposit for human exposure was also postulated as an important variable,[5] surface carpet dust being more important in exposing children than deep carpet dust, so that recovery of all dust from carpets was not so important to ascertain average lead exposure concentration and for the available dust exposure to children. The method was potentially usable for other dusts containing nonvolatile pollutants.

The method was the basis for ASTM Method PS 46-96 of 1996 designed for use in lead abatement activities.[16] In a 1985 report,[66] the estimated contribution of surface dust lead using the 1985 surface sampling technique[5] to ln blood lead of 57 children of age 18 months in the Cincinnati Prospective Lead [illegible] by structural equations analysis had a regression weight about the same contribution as hand lead, and with 3.33 times more contribution of surface dust lead to hand lead than to blood lead. The regression weights varied with child age, a surrogate for time of exposure and crawling.

The other studies that reported efficiencies of sampling surface dusts were: 41 percent–48 percent for sampling bacteria on human skin in 1990[40]; 84–90 percent for the herbicide glyphosate on monkey skin in 1991[30]; 50–55 percent for wet wipe sampling of surface dusts of 71 metals in 1992[6]; 24 percent for a wipe method on carpet, and >100 percent on linoleum and wood for lead dust in 1997[18]; 95 percent for a high vacuum method for carpet and 54 percent–61 percent for linoleum and wood in 1997[18]; and for the ASTM PS 46-96 method of unspecified number of sampling passes, 31 percent for carpet and 47–67 percent for linoleum and wood in 1997.[18]

The collection efficiencies of the vacuum sampling technique for dusts depend on several factors: particle size, dust loading, surface type, capture velocities, and ergonomic characteristics of the optimized sampling technique. The present study examines these variables in more depth to increase the cumulative absolute efficiency of the cordless vacuum method for hard surfaces for loose dusts.

MATERIALS AND METHODS

The protocols and apparatus of the ASTM method[16] were used as reference. This included calibration of air sampling pumps, testing for leaks, and the sampling templates used. The apparatus is the Tygon tube sampler variant[5] discussed above. All glassware was cleaned and made metal-free by scrubbing manually with brush and detergent, rinsing in distilled water, soaking overnight in 10 percent (v/v) nitric acid, rinsing copiously with distilled water, drying in a dustless oven, and then storing in transparent plastic bags contained in drawers.

Selection and Preparation of Soil for Sample Collection

A large proportion of household dust is soil brought in on shoes or feet or from windborne material.[13,15] Therefore, dust collection methods should be able to cope with soil particles that are part of house dusts. In the workplace, deposited lead metal fume dust is often associated with soil particles from coring materials and sands, in addition to when soil floors are present in unpaved workshops. In the Los Angeles area, the semidesert climatic conditions cause facile home infiltration of desert soils, especially during when hot dusty desert winds blow from the east, termed the "Santa Ana" condition. In areas with more rain, as investigated before by the present investigator in the Cincinnati area[5] and by the many other investigators on the east coast of the United States, the extensive long-distance wind transport of soil is negligible unless dust bowl conditions develop. Another major source of dust in urban Los Angeles homes is the fallout from automobile exhaust and its multiple suspensions and dispersions by traffic, but a reference sample of that dust was not available for this study.

A 1-kg sample of surface soil was taken from the U.S. desert section of the UCLA Botanical Gardens in a clean, wide-mouth Pyrex jar equipped with a Teflon-lined screw cap. The soil was collected with a trowel from several inches below the surface pebbles. The sample in the laboratory was broken up, and then dried in a dustless oven at 130°C to constant weight in an acid-clean pyrex tray. The dust was then sieved completely (minisieve, Fisher Scientific, Tustin, CA) through a 710 μm sieve (25 mesh), and then subsieved into <63 μm (230 mesh), 63–90 μm (230–170 mesh), 90–125 μm (170–120 mesh), and 125–180 μm (120–80 mesh). The particle size <149 μm (<100 mesh) simulates clean room dust.[62] Each sieved fraction was stored individually in a closed, clean, wide-mouth bottle with a Teflon-lined screw cap.

Sampling Apparatus

A 10-cm-square template was used to define the sampling area (wipe sample test kit, SKC West, Fullerton, CA). A three-piece polystyrene matched weight filter cassette with 0.8 μm 37-mm diameter mixed cellulose ester filters (SKC West) was weighed before and after sampling. The sampling probe was of 5-cm-long Tygon tubing with an inner diameter of 6.0 ± 0.5 mm. One end of the probe was cut at a 45 degree angle.[5] The other end was connected to the sampling cassette. The cassette exit was connected by 6.35 mm i.d. Tygon tubing to a portable battery-operated vacuum pump (SKC Aircheck Sampler Model 224-PCXR4). Calibration with a M-5 MiniBuck calibrator (Buck Scientific, East Norwalk, CT) occurred with the sampling assembly in-line.

The inner surface margin of the sampling template was taped down with masking tape with about a tape margin of 1–2 mm covering the inner surface to be sampled. Two types of surfaces were used, rough and smooth, both made of polyethylene (dissecting board, Fisher Scientific). A hand-held inclinometer (Lev-O-Gage, Edmund Scientific, Barrington, NJ) was utilized to measure the angles of the probe at optimal sampling conditions. A calibrated model 1440-4 model 5415 II hot-wire anemometer (Kurz, Monterey, CA) measured face velocity. Surface hardness was assessed with a portable tensiometer (type D Durometer Model 307L, Davis Instruments, Baltimore, MD).

Methods

Each dust of different size and of known weight was poured slowly through the appropriate sieve which was moved six inches above and all over the template sampling surface. The pump flow rate (4.0 ± 0.2 L/min) was checked before and after each experiment. The pump was stabilized for five minutes before calibrating and sampling.

The dust was collected by slowly sliding the probe along the inner taped border of the template, and then in a concentric rectangular pattern inwards to the center. Every 30 seconds or about 50 percent of a single complete sampling pass and at the end, the probe was inverted and tapped to loosen any dust sticking to the tubing to facilitate dust collection on the filter. The probe was held in a manner to provide the most efficient sampling. This included tilting, bending, and combinations. The index finger placed on the probe was used to manipulate probe position, direction, and downward pressure.

Five separate sampling passes were done for each experiment. The total weight of the dust sampled cumulatively after each of one through five passes was measured by subtracting the original weight of the cassette from the total weight. The sampling efficiency was then calculated from the weight sampled divided by that applied. The particle size ranges 63–90 μm, 90–125 μm, 125–180 μm, and mixed size dust (63–180 μm of equal proportion by weight of the three size fractions) were evaluated. Different dust weights were applied ranging from ~10 mg to ~160 mg. Experiments were done in triplicate. The loading capability of the filter to cause decrease in flow rate was also determined. Probe weight changes were noted for each experiment, as well as the weight of the probe-filter cassette assembly.

Optimal sampling angles of the probe indicated by the inclinometer were observed. Times for sampling, and the times to achieve consistent results and constant CV were also determined.

The dependence of probe face velocity on area was assessed by covering the open end of the probe with transparent tape to allow the area not blocked to be determined by outline onto graph paper. A supporting spatula ensured the tape did not bend during the experiment.

Surface hardness measurements were done with the sampling surfaces on a flat surface with measurements done 1 cm away from the template edge and at least 6 mm apart. At least five measurements were done.

RESULTS

Cumulative Efficiency of Surface Sampling for Various Soil Size Ranges and Surface Type

The influence of particle size and type of surface on mean collection efficiencies of applied weights of 125–160 mg is given in Table I. At least three sampling passes were required for a sampling efficiency exceeding 80 percent for both surfaces independent of particle size and surface type. The rough surface promoted more efficient and precise sampling than the smooth one for the first two sampling sweeps. The two smaller particle sizes were sampled more efficiently and precisely than the larger size during the first two sweeps for both surfaces. However, both variables did not affect sampling efficiencies after the second sweep, where sampling was both accurate and precise. The actual efficiency for sampling a 1:1:1 mixture of the three size fractions for about the same surface loading and sampling flow rate was not significantly different at $p \leq 0.05$ from the overall calculated average for five sampling passes.

The particles adsorbed by a new Tygon probe never exceeded 0.7 mg. This sets a conservative method collected mass detection limit of about three mg using a S/N Student t criterion of 4.6 at 4 degrees of freedom (ν) at $p \leq 0.01$ (2.58 at $\nu = \infty$), and a practical similar lower quantitation limit of about 12 mg using

TABLE I

Sampling mean cumulative efficiency dependence on particle size range and surface for triplicate experiments using applied soil masses and a flow rate of 4.0 L/min

Surface	Applied mass (SD) (mg)	Diameter range (μm)	Cumulative efficiency (SD) in % for sampling pass				
			1	2	3	4	5
Rough	151.1 (6.1)	63–90	88.1 (8.1)	91.9 (4.8)	93.2 (3.7)	93.3 (3.7)	93.8 (3.2)
	144 (13)	90–125	89.8 (8.7)	94.7 (3.2)	96.2 (2.1)	96.2 (2.1)	96.4 (1.8)
	149 (17)	125–180	68 (35)	81 (28)	89 (16)	94.7 (6.3)	95.4 (5.0)
Mean (SD)	148.0 (3.6)	63–180	82 (12)	89.2 (7.2)	92.8 (3.6)	94.7 (1.5)	95.2 (1.3)
CV	2.4		14	8.1	3.9	1.6	1.4
	123 (19)	63–180^					97.33 (0.65)
		(1:1:1)					
Smooth	134 (13)	63–90	75 (20)	82.4 (8.7)	83.4 (8.0)	84.2 (7.6)	84.7 (7.3)
	136.8 (9.8)	90–125	72 (22)	86.3 (9.7)	87.3 (9.3)	87.9 (9.2)	88.4 (8.4)
	128.7 (7.3)	125–180	45 (45)	68 (25)	85.8 (9.4)	88.7 (8.3)	90.6 (9.6)
Mean (SD)	133.2 (4.1)	63–180^	64 (17)	78.9 (9.6)	85.5 (2.0)	86.9 (2.4)	87.9 (3.0)
CV	3.1		27	12	2.3	2.8	3.4

SD, standard deviation; CV, coefficient of variation.
^The range.

TABLE II

Influence of rough surface loading for specific particle size ranges on the five-pass sampling efficiency at 4.0 L/min flow rate

Particle size range (μm)	Loading (mg)	Efficiency (%)	Mean (%)	SD (%)	CV (%)
63–90	9.3	85.0			
	23.8	88.8			
	27.1	87.4			
	146.6	97.4			
	148.7	91.7			
	158.0	92.2			
	9.3–158	85–97.4	90.4	4.4	4.8
90–125	10.3	92.0			
	26.4	102.7			
	29.9	93.7			
	130	96.2			
	148.4	98.2			
	155.5	94.7			
	10.3–155.5	92.0–102.7	96.3	3.8	4.0
125–180	10.3	94.0			
	22.3	94.5			
	27.6	91.4			
	136.2	89.7			
	142.7	89.7			
	168.8	98.5			
	10.3–168.8	89.7–98.5	93.0	3.4	3.7
Grand ranges	9.3–168.8	85–103	90.4–96.3	3.4–4.4	3.7–4.8
Overall grand means			93.2	4.4	4.7

SD, standard deviation; CV, coefficient of variation.
The overall mean, its standard deviation, and its CV are for n = 18.

an equivalent S/N criterion of 10 at $\nu = \infty$ as recommended by the U.S. Environmental Protection Agency (EPA).[63] Probe masses ≤0.1 mg were found in 27 out of 39 sampling runs when the probe was presaturated with dust. The method detection limit is then about 0.4 mg and the practical lower quantitation limit is about 2 mg.

Influence of Surface Loading on Sampling Efficiency

Table II shows the effects of surface loading and particle size on the five-pass sampling efficiency at the same flow rate for the rough surface. As expected from Table I, there is no dependence on particle size for the five-sweep technique, the mean efficiencies for each size fraction not being statistically different at $p \leq 0.05$. In addition, there is no significant difference at $p \leq 0.05$ either, for surface loading in the range 9.3–169 mg. The overall mean efficiency of 93.2 percent has an associated range of 85–103 percent and a CV of 4.7 percent. The efficiency near 10 mg loading (90.3 ± 4.7) percent is still independent of particle size investigated. In comparison, the sampling efficiency above 100 mg loading independent of particle size was (94.3 ± 3.5) percent, not statistically different at $p \leq 0.05$.

Other Characterizations

Table III gives the observed data for sampling time, pump-related variables (flow rate and face velocity), probe angle during sampling, and hardness characterization of the two surfaces. The average time for sampling decreased from >60 s initially to 48 s after about 20 sampling trials. The interrun CV also decreased to 10 percent. The decrease in pump flow rate from initial to final calibration for each set of five passes was not significantly different at $p \leq 0.05$, even for high loadings.

The dependence of face velocity on open probe area shows that the face velocity increases linearly from 40 mm^2 to 8 mm^2 (Velocity = −7.06 area + 510 for r = −0.999 for velocity in ft/min units and area in mm^2), but increases exponentially below 8 mm^2 due to turbulent flow. Difficulties in measurement were apparent below and at 1 mm^2 because of anemometer probe size relative to size of the sampling aperture. The face velocity at 1 mm^2 was 1083 ft/min (330 m/min), a high enough capture velocity to lift loose surface dust or soil particles. It is about double the velocity predicted from the linear regression equation.

The angle at which the probe had to be held relative to the surface to be sampled depended on particle diameter deposited. The large particle size required angles of 30–35 degrees, with bending of the probe from its inclination to increase capture velocity inside the probe to remove any stuck particles. The smallest particles needed steeper angles (30–45 degrees) for successful sampling, resulting in more probe tilting. The intermediate particle size required both bending and tilting. Probe inversion with tapping was also necessary from time to time to promote collection on the filter.

TABLE III

Variables (pass time, face velocity, flow rate stability, particle size dependence on probe angle for efficient sampling, and surface hardness) measured to further characterize the optimized cordless sampling method at flow rate 4.0 L/min

Variable	Observations	
Sampling time for one pass	47, 52, 48, 46 s with mean ± SD of 48.3 ± 2.6 s	
Face velocity at probe entrance	Area (mm^2)	Face velocity (ft/min) (SD for n = 3)
	35	262(10)
	20	369(10)
	15	405(10)
	8	452(10)
	4	571(20)
	3	869(20)
	1	1083(30)
Decrease in flow rate from beginning to end of sampling	Load sampled (mg)^	Relative flow decrease (%)
	101.2	−3.7
	129.1	−0.67
	138.3	−0.48
Probe angle and particle size	Particle size range (μm)	Probe angle (degrees from horizontal)
	63–90	30–45
	90–125	35–40
	125–180	30–35
Surface hardness	Surface type	Hardness (load pound)
	Smooth	7.08(0.46)
	Rough	6.64(0.19)

The bracketed numbers are standard deviations (SD).
^125–180 μm diameter.

There was no statistical difference at $p \leq 0.05$ in surface hardness for the two polyethylene surfaces.

DISCUSSION

Several modifications of the ASTM method[16] based on the cordless vacuum technique[5] resulted in enhanced reproducible sampling efficiencies exceeding the NIOSH minimum of 75 percent and imprecision less than 10 percent, and a greater understanding of the limitations of the method to sample loose surface dusts/soils at coverages between 0.1 mg/cm^2 to 1.5 mg/cm^2.

The method innovations included: tapping and inversion of the probe, taping the inner margin of the sampling template, saturation of the inner surface of the probe, sampling from the inner walls of the template to the center, increasing the flow rate to about 4.0 L/min, and weighing the entire sampling train.

Vigorous tapping of the probe in the inverted position at the halfway mark of each sampling pass helped to give efficiencies above 90 percent for rough surfaces and above 80 percent for smooth surfaces, and also allowed more dust to be collected on the filter for later analytical chemical analysis. Probe local bending increased the local capture velocity inside the probe to assist transfer to the filter, and to loosen wall material. Tilting varied the face velocity at the sampling surface. The method is ineffective without movement on very smooth surfaces. This accounts for why rough surfaces are sampled more efficiently than smooth, flat surfaces. Rough surfaces allow makeup air to move surface particles whereas probe movement is necessary for very smooth surfaces.

Another modification was taping the inside edge of the template to the surface with no more than 1–2 mm of sampling surface margin covered with tape. A 2-mm margin produces 4 percent error, and a 1-mm margin causes 2 percent error. Taping minimized sample loss from soil/dust being pushed underneath the template inner edge, and prevented movement of the template during sampling to ensure the sampled area remained constant and did not change inadvertently. A sampling motion inwards was inherently better than one from the center to the edge.

Preliminary presaturation of the probe to constant weight by surface sampling of particles assured higher sampling efficiencies at low coverages below 10 mg to a practical lower limit of about 2 mg. In its field use for areas of low dust coverage yielding <10 mg of total collected dust, it is therefore recommended to presaturate the probe in the general area to be sampled using a sensitive portable electronic or mechanical balance to indicate probe presaturation and to provide the initial probe presampling weight. The alternative is to sample multiple 100 cm^2 areas until sufficient dust/soil is collected to make the mass held by the probe to be small (<10%) relative to the total mass collected in the cassette and maximizing that collected on the filter for subsequent analysis. Thus, the whole sampling train (probe plus cassette) needs to be weighed rather than just the filter because [illegible] values at low collection masses are affected, whereas [illegible]ncentration collected on the filter is not, if this [illegible] the least quantifiable limit.

The technique must also be "in the fingers" and "in the wrist" as shown by an acceptable CV in performance-based tests with known amounts of applied dusts and soils in laboratory practice runs, before field sampling can be done. An initially untrained operator required about 20 cassettes with a five-pass technique/cassette to lower the interrun CV to below 10 percent, and to optimize sampling times.

The increased flow rate allowed higher capture velocities to be generated on the surface, resulting also in higher collection efficiencies for the key size fraction. The effects of particle size, surface coverage, operator training, flow rate, and operator technique become less important if three to five sweeps are performed rather than just one or two. Natural dusts will have a spectrum of dust sizes. The sampling of mixtures of particle sizes appears as efficient or more so, than predicted by the size fraction studies of Tables I and II.

From previous work,[5] the technique at 2 L/min is inefficient at removal of all dust from deep pile carpet, greasy surfaces, and for the larger particle sizes >177 μm of loose dust on smooth surfaces. However, the most powerful vacuum cleaners and shampooing do not remove all the dust from plush carpets,[13,15] and even exhaustive scrubbing is often necessary to remove greasy deposits. Deep carpet dust is unavailable for exposure unless dispersed into the air by some agency, for example, carpet shaking, frequent walking, or children playing boisterously on the carpet. Dust on the surface of greasy deposits is also not readily bioavailable. It is also expected that a technique that employs a 16 L/min sampler as utilized for the Baltimore repair and maintenance study[12,19,23] should be more efficient at sampling larger particles than one that utilizes 2 to 4 L/min, though this depends on the aerodynamics at the sampled surface and exactly how the technique is put into practice. Such details are hardly ever provided in published papers, thus making comparisons difficult.

The cordless vacuum sampling technique[5] was originally developed to gauge the availability of surface dusts to the hands of children relative to their hand-to-mouth or toy-to-mouth behavior. The reasoning was as follows: children at play are often on all fours on a surface, whether hard or otherwise. The dust (mostly <149 μm[62] on the surface, comprising about 76% of the surface dust[5]) is partially retained on the hand. The highest hand dust retention occurred[5] for deposited loose dust sizes 149–246 μm with about fourfold lower adherence for 44–149 μm and 246–392 μm dusts, and 1.6 times lower adherence for the <44 μm fraction. The efficiency was independent of particle size below 180 μm, which comprised about 81 percent of the weight of reference house dust and about 82 percent of the lead. This fraction included the most available, hand-retained fraction of dust.[5]

The modified technique should be more efficient for larger particle sizes also, but these are a minor fraction of house dust and of complex retention characteristics.[5] The increase in flow rate to 4 L/min and the other modifications have resulted in a modified technique that is more efficient (the average efficiency for the size fraction 63 μm to 177 μm was increased from

70 ± 13% to 93.2 ± 4.4%). The modifications still retain the cordless pump advantage, but take advantage of technological advantages not available when the technique was first developed in the early 1980s. How the more efficient technique will relate to blood lead remains to be tested.

The apparent inconsistencies[12] of the 1985 method[5] may be related to availability of the dust being sampled rather than total dust, to defective or different comparison statistical designs, to the number of dust samples taken (for example, 36/house for 205 children in reference 12), inadequate or noncomparable training in use of sampling techniques (all training must be performance-based, in our opinion, as emphasized above), and to the wide variabilities of field dust coverage, xenobiotic concentration, and particle sizes, as also demonstrated by use of lognormal statistics in reference 12 to describe surface sampling parameters so that a random number selection design for side-by-side sampling area and method may not be adequate. This last factor is best controlled in laboratory studies. Nevertheless, the data on laboratory recovery of applied leaded dust on linoleum and wood surfaces for the 16 L/min versus the 2 L/min methods in reference 19 show no statistical differences in the means at $p \leq 0.05$ with significant differences, as expected, for carpet. These results were the same as for the studies with the 20 L/min and 2.0 L/min samplers reported in reference 5 using applied weights of dusts and soils. In field studies, the only way to account for side-by-side surface deposit variability and surface variation is what was done in reference 5, that is, resample the same surface already sampled by the rival technique. This was not done in reference 12 or 19.

Another problem for study-to-study comparisons involving blood lead and environmental parameters is that many studies involve low blood leads (as for reference 12 children where only six or 3% had blood leads ≥20 μg/dL with a population mean of 7.7 ± 5.1 μg/dL) and so make interpretation of any correlations difficult relative to others (as for the Cincinnati Prospective Lead Study[66,67] where at least 27 children in 1985 had blood leads ≥20 μg/dL with an arithmetic mean of 20 ± 10 μg/dL between 15–24 months of age for n = 57 for children having blood lead ≥20 μg/dL at least once) or when blood leads are close to background blood leads.

Blood lead values from different age groups often may be compared, and it is known that the contributions of surface and hand lead vary with house activity of the child. With regard to the latter, the steep increase in blood lead for the Cincinnati Prospective Lead Study children occurred between 4 to 15 months of age, followed by a gradual decrease.[67] The regression weight contributions to ln blood lead for surface dust lead stayed fairly constant (0.16–0.23) between 12 to 21 months, and those for hand lead (0.09 to 0.16) between months 9 to 18. However, only hand lead at month 18 was a significant contributor at $p \leq 0.05$. In reference 12, all the correlations of blood lead [illegible] environmental sampling alternatives appear based on the [illegible]tion of children between 12–30 months of age, an averaging procedure that will miss some age-related correlations. Also, it should be kept in mind that correlations do not necessarily signify cause and effect.

An activity analysis often shows that a child usually mouths only selected parts of the hand or toy. Thus, not all the dust that is on the hand will frequently be actually bioaccessible (ingested) to be bioavailable (absorbed). It is exceedingly difficult to simulate the bioavailability of pollutants coingested with the dust. Sampling methods that remove all dust from a surface[9,12,13,18,19] provide maximum answers of pollution potential and allow the best chance to detect xenobiotic concentrations, but correlations with blood lead may be misleading because the available dust fraction adheres to the hand, and then only part of the adhered dust may be ingested. A surface sampling technique that mimics the characteristics of the available dust, the hand retention characteristics of the dust, and the favored hand mouthing area of the child is clearly still desirable, just as air particulate samplers that mimic inhalability or respirability of particulates are advantageous.[64] Most of the existing alternative sampling techniques including the present modified technique strive for efficiency rather than to mimic the exposure situation of the child simply because adequate analytical sensitivity is still a major problem, though this is now less of a factor with the advent of inductively coupled plasma/mass spectrometry which, however, is expensive.

The application of the present technique to sample surface dusts and soils containing metals, nonvolatile organics like many pesticides, organic (biological) dusts, and radioactive compounds reviewed in the introduction is obvious.

CONCLUSIONS

The cordless vacuum method for sampling surface dusts on hard surfaces has been optimized relative to sampling efficiency, number of sampling passes, manipulation of the sampling probe, flow rate, and surface loading. The technique utilizes pumps that are currently used for air sampling for particulates and for some gases and vapors by industrial and environmental hygienists. At least 20 cassettes with five sampling passes per cassette in the laboratory are necessary to provide acceptable speed, precision, and accuracy for training purposes before field sampling will be adequate.

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SURFACE SAMPLING FOR

A PESTICIDE IN DUST AND SMALL SPILLS

OF A SOLID DYE

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ABSTRACT

The aim was to determine whether a published sampling technique for loose soil on hard surfaces achieved acceptable efficiency at surface dust/soil coverages of 10-20 mg/100 cm^2. The sampler was a cordless personal sampling pump operated at 4.0 L/min connected to a cassette containing a filter, the cassette being also connected to a Tygon sampling probe that was moved manually on the surface to be sampled. Rhodamine 6G dye dust was evaluated at 10 mg/100 cm^2 coverage at flow rates of 1.0-4.0 L/min. Two NIST standard reference materials (SRMs) were used for pesticide experiments: SRM 2711 Montana Soil, and SRM 1649a Urban Dust. The efficiencies for these SRMs were determined uncoated, and coated with 1300 µg chlorpyrifos/g as a Lorsban 4E emulsifiable concentrate formulation. The 3-pass technique sampled both the pesticide-coated and uncoated dust and soil at >79% efficiency and at better than 16% coefficient of variation (CV) above 15 mg collected mass. Sampling at 20 mg/100 cm^2 coverage lowered the CV to ≤7.1%. The dye was sampled with similar efficiency ≥1.5 L/min, but the CV for the 3 pass technique was <10 % at 1.5-2.0 L/min, and <20% at 1.5-4.0 L/min. The efficiency for a 5-pass technique for the dye exceeded 90% at ≥1.5 L/min with CVs <10% at 1.5, 2.0, 2.5, and 3.5 L/min. The weight change of the sampling probe and the cassette must be measured for accurate determination of low surface coverages rather than just the weight change of the filter alone. The method allows use of personal sampling equipment for aerosols to measure low coverages of loose dust, soil, and chemical solids on hard surfaces.

Keywords: pesticide; surface coverage; chlorpyrifos; spill; dust; soil

Introduction

Most pure low-volatile pesticides are solid at 25 °C and 760 mm Hg (1). Such pesticides include the major chemical classes of organophosphates, organocarbamates, organochlorines, and inorganics (1). Since application by using the pure solid pesticide is hazardous, difficult, and uneconomic, commercial pesticides are most often applied over large areas as aerosol sprays using water carrier at the appropriate dilution. The pesticide formulation therefore often has a surfactant to promote pesticide water solubility and a petroleum fraction to modulate droplet size and surface coverage (1). Such pesticide formulations are called emulsifiable concentrates. The formulation components without the pesticide are the "inert components". Thus a field and workplace application of a pesticide usually involves a complex mixture of chemicals besides the pesticide active ingredient. After field application in such diverse areas as farms, silos, orchards, parks, gardens, restaurants, golf courses, washrooms, retail stores, and offices, the volatile water carrier evaporates to leave an oily surface film which in soils and in dusty workplaces adsorbs to the outside of dust or soil particles. These particles can then be resuspended by wind, air blasts, and turbulent eddies from moving vehicles, pets, and people to cause exposure by inhalation, oral absorption, and skin exposure.

Many studies have monitored soil pesticide residues (2-12). The half lives of low-volatile pesticides are often short in the ambient soil environment because of biodegradation

(especially in moist nutrient-rich soils), photodegradation by sunlight, hydrolysis, and water leaching. Conditions on dry, hard surfaces out of sunlight are much more conducive to adsorbed species on dusts being persistent. Most research on such situations has focussed on measuring risk to children via pesticides in room dust (5,8,12-27) rather than to adults, but some worker studies have been done (29-32).

One of the authors in 1985 developed a general method to sample dust available to children (33), focusing on lead in dust in a particle size range characteristic of clean room dust (<149 μm). The method consisted of repetitive sampling of a surface area (defined within a template) by a portable cordless pump connected to a cassette with a 0.8 μm mixed cellulose ester filter, and with a 5-cm Tygon or stainless steel sampling appendage operated at 2.5 L/min. The method eventually became ASTM Method PS 46-96 (34). The objective was not to measure total dust on the surface, but rather the dust that was available to children. To assess potential dust resuspendability in air, the total amount of dust in defined particle size ranges must be known. This aim was accomplished for loose soil by using a higher flow rate of 4.0 L/min, obtaining a better understanding of the ergonomics of the sampling process, while still retaining the cordless advantage (35). Soil particle sizes of 63-180 μm were sampled with efficiencies between 83-85% (coefficient of variation CV=11%). The technique performed better for a rough surface than a smooth one of the same surface material for the first two passes, but the type of

surface made no statistical difference at $p \leq 0.05$ by the end of the third pass. The method was validated over the soil coverage range 100-1,500 $\mu g/cm^2$ or 10-150 mg/100 cm^2 using a 100-cm^2 template. The sampling of polydisperse surface soil particles was more efficient than expected from the efficiencies for monodisperse particles (35).

The new technique has not been demonstrated to apply for all chemical species of interest, nor for dust, or for small spills of solid chemicals. The present work demonstrates how the new technique performed for a model pesticide adsorbed on soil and dust, and for small spills of a model dye.

Methods

Rationale for the Selected Pesticide. The organic pesticide that has been detected in dusts inside homes in 25% of the situations sampled is the chlorinated organophosphate chlorpyrifos (Dursban; Lorsban) (22). The maximum literature levels are: a concentration of 1,300 $\mu g/g$ in soil or dust (22); a surface dust/soil coverage of 6.4 $\mu g/m^2$ (5); and a surface deposition pad coverage of 990 $\mu g/m^2$ (26). Chlorpyrifos has been used for structural, crack, crevice, and outdoor turf and perimeter treatments for the control of pests in the urban environment (26). The compound has been the focus of attempts to assess if wipe sampling can provide estimates of skin exposures (36-38). Chlorpyrifos is O,O-diethyl-3,5,6-trichloro-2-pyridyl phosphorothioate, and was first patented in 1966 by

the Dow Chemical Company (39). Its melting point is 43 °C, and at 25 °C it has a vapor pressure of 2.5 mPa and a water solubility of 2 mg/L (40). It is relatively persistent in soils with a $t_{0.5}$ of 60 to 120 days (40). The 1999 air threshold limit value (TLV) is 0.2 mg/m^3, with a "skin" notation (41). The pure chemical is not genotoxic, teratogenic, or mutagenic unlike some of its formulations and technical grades, which may contain the more genotoxic sulfotepp and trichloropyridinol impurities. On June 8 2000, EPA banned uses in areas where children could be exposed.

Use of a liquid formulation in spraying rather than a pure solid pesticide is expected to cause a difference in sampling parameters due to greater stickiness and tackiness of the contaminated soil and dust relative to without pesticide formulation. The part of the formulation that persists after volatilization of the water carrier and the lower boiling fraction of the formulation is the pesticide plus the surfactant in a thin film of the high boiling compounds of the petroleum fraction. The sampling situation which would cause the lowest sampling efficiency would be just after spraying since the dust would be damp. However, most people do not come willingly into freshly sprayed rooms or fields because of odors and wet surfaces, and are generally not exposed to this situation.

Thus the practical situation that would be the worst sampling situation relative to exposure is after a reentry interval, when a site is reoccupied the next morning after

overnight spraying operations (as is common for restaurants), or when odors cease. This situation was simulated by loading the dust or soil at the highest surface coverage and/or concentration observed so far in field studies, that is, 990 $\mu g/m^2$ (26) or 1300 $\mu g/g$ (22). The 990 $\mu g/m^2$ coverage corresponds to 990 $\mu g/g$ assuming 10 mg is sampled from 100 cm^2. Thus, the concentration maximum of 1300 $\mu g/g$ is the worst case scenario that might influence practical sampling after reentry. This loading concentration was therefore selected for investigation with a Lorsban 4E formulation containing 40.7% (w/w) active ingredient (38), as obtained from Dow Elanco.

Thus 10 g samples of each soil and dust were coated uniformly by first solubilizing 32 mg of the formulation in 20 mL of acetonitrile, adding the solution to the soil or dust in a round-bottom flask with a ground glass joint, and then evaporating the solvent by rotary evaporation. The solid residue was held in a vacuum desiccator until constant weight (2-3 days). Gas chromatography/mass spectrometry (GC/MS) of the Lorsban 4E formulation was done for quality control purposes using conditions published elsewhere (38).

Rationale for Solid Pure Chemical to be Used. Many dry solid chemicals are often spilled in small amounts, for example, drugs in pharmacies; chemicals in laboratories; waste materials by technicans; powders by workers; and food ingredients in the home. The solid organic chemical chosen was to be as nontoxic as possible for health and safety

reasons. Thus pesticides, drugs, and irritant chemicals were not considered. Rhodamine 6G or Basic red 1 dye (99 %w/w) was chosen since it is often used in tunable dye lasers, for example for laser eye and other surgery (42, 43), for fluorescent labeling purposes in biochemistry (44), as a fluorescent tracer in environmental studies (45), and has also been used to dye silk, cotton, wool, leather, plastic, paper, and bast fiber (45). This xanthene dye, 2-(6-ethylamino)-3-(ethylimino)-2,7-dimethyl-3H-xanthen-9-yl)benzoic acid ethyl ester monohydrochloride, is moderately soluble in water, and has a low vapor pressure since it is a salt. Even so it has measurable volatilization at 200 °C (45). There are no workplace guidelines. The chemical is in the EPA inventory under TSCA. There is no evidence that it is carcinogenic in two-year studies in mice of either gender, but there is some equivocal evidence in rats of either gender relative to site specific tumor induction in the adrenal gland medulla (pheochromocytomas) at the highest dose of 250 ppm in the diet (45). The chemical tends to cling to surfaces since it is slightly deliquescent, and it is hard to remove from hands once they are contaminated. There was a need to develop a sampling/cleaning method that involved minimum skin exposure.

Rationale for Dusts and Soils Selected. The previous optimization study (35) utilized fractions of sieved local soil for method development purposes. Transfer of technology is best done with standard reference materials (SRMs), for example from the National Institute for Standards and Technology NIST(46).

NIST SRM 2711 is Montana II till soil of particle size <74 μm of about 1.5-2.2% water and about 2 % carbon content with 24 elements defined (47). SRM 1649a is an Urban Dust atmospheric particulate of particle size <125 μm. The volume distribution has an arithmetic mean particle diameter in μm of 34.6±0.4, and the median is 24 μm. It has water content of 1.23±0.07%, and a carbon content of 16±5%. It is certified at the ng/g level for 22 polyaromatic hydrocarbons (PAHs), 35 polychlorinated biphenyl (PCB) congeners, and 8 chlorinated pesticides. Reference values for another 22 PAHs, another chlorinated pesticide, 32 inorganic constituents, total organic carbon, and carbon composition are also provided (48).

These SRMs were sieved to check particle sizes (Labline Instruments Minisieves, Catalog No. 4610), and also resieved after coating with the chlorpyrifos formulation since particle agglomeration was expected.

Sampling Method. The components of the surface sampling apparatus were: a portable, cordless pump of 4.0 L/min flow rate (SKC Airchek Model 224) connected by 6.4 mm ID Tygon tubing to a 37-mm polystyrene sampling cassette with a mixed cellulose ester filter of 0.80 μm pore size or a Teflon filter of 5.0 μm pore size. The entrance port of the cassette had a sampling probe of Tygon tubing of 6.4 mm I.D. x 5.0 cm length with the sampling end cut at a 45 ° angle (35). A 10 cm x 10 cm sampling template (SKC West,

Fullerton CA) was taped with masking tape to the sampling surface at the inner margin of the template. The sample was applied over the surface by pouring it through a sieve of size 180 μm that was moved slowly about 3 cm above the template surface. Although the 100-cm^2 smooth surface was not covered uniformly, the applied mass was accurately known and was varied between 10 mg to 20 mg. A smooth surface used previously (35) was utilized as it produced the lowest dust recoveries (35).

The sampling technique involved up to 5 passes over the sampling area proceeding from the template inner margin into the center as described elsewhere (35). The sampling probe was tilted as appropriate, with regular inversion accompanied by finger tapping to ensure no blockages and facilitate dust collection on the filter. Weights were determined on a four-decimal place balance by subtracting cassette/sampling probe weight before sampling from that after sampling, as well as weighing each component part of the sampling train before and after sampling. All experiments were done at least in triplicate.

Results

Chlorpyrifos

The results for the surface sampling of coated and uncoated SRM 2711 and SRM 1649a at 4.0 L/min for up to three sampling passes are provided in Table 1.

The uncoated SRM 2711 soil at 10.5 mg/100 cm^2 coverage was sampled at better than 90 % efficiency and CV 5.3% by even one sampling pass. The coated soil showed collection efficiencies for 3 sampling passes of >80 % at ≥15 mg/100 cm^2 for coated soil, whereas 10 mg/100 cm^2 uncoated soil and 20 mg/100 cm^2 of coated soil were sampled at >90 % efficiency. At 9.9 mg/100 cm^2 coated soil, the 3-pass efficiency was 59% with CV 27%, being inefficient and imprecise. The proportion of coated soil in the sampling probe and in the cassette was about 1:1 at about 10 mg/100 cm^2 . The cassette always contained >50% beyond 15 mg/100 cm^2 for the coated soil.

The sampling efficiencies of the 3-pass technique for 20 mg/100 cm^2 and 15 mg/100 cm^2 SRM 1649a dust were not statistically different from each other at $p \leq 0.05$ within the coated and uncoated classifications. Both classifications also had CVs <11% at these coverages. However, the coated dust was sampled significantly more efficiently than uncoated dust at $p \leq 0.05$ at all coverages, though sampling efficiencies still exceeded 70% at all coverages whether the dust was coated or not. Coating the dust improved recoveries at lower coverages, the reverse of the findings for soil. The uncoated particles passed completely through a 230 mesh sieve (<62 μm). Only 95% of the coated particles came through the 230 mesh sieve, but all passed through a 170 mesh sieve (<84 μm). Thus the small increase in particle size caused by coating may help increase sampling efficiency, a phenomenon also observed for sampling sieved local soil (35).

Dust and soil, coated or uncoated, were sampled by the 3-pass technique with an efficiency above 79% at 15 mg/100 cm^2 or above with CV ≤16%. Thus 15 mg/100 cm^2 coverage rather than 10 mg/100 cm^2 is nearer the least quantifiable limit (CV of 10.6%, preferred inaccuracy of ±10%, and minimum inaccuracy of ±25% according to NIOSH (51)) for these coated and uncoated dusts and soils.

Sampling of Rhodamine 6G

Since this system was so different relative to coated and uncoated dusts and soils, a complete flow rate dependence study was performed at 10 mg/100 cm^2 (Table 2).

Evaluation of different flow rates using 5-sampling passes over a surface coverage of 10 mg/cm^2 showed that flow rates ≥1.5 L/min were necessary for sampling efficiencies of >90%. The corresponding efficiencies for three sampling passes were not statistically significant from those for five passes at $p \leq 0.05$. For flow rates ≤2 L/min, all the collected sample resided in the sampling probe. Even at 4.0 L/min flow rate, a substantial proportion (about 20%) of the sampled mass did not reach the filter cassette.

Residual color showed that a small amount of sample (generally <1%) still remained on the surface. This residue could be removed by wet swabs (water solvent) using at least three fresh swabs per single sampling pass for a total of 3 sampling passes. The dye on

each swab was desorbed by ultrasonication in 1 mL of water. Absorbances were read at 528 nm in a 1-cm Suprasil cuvet. The linear range was 1.0-10 µg dye/mL. The measured molar absorptivity was 82,727±200 M^{-1} cm^{-1} (literature, >81,400 M^{-1} cm^{-1} (49)).

Discussion

There are no previous reports on sampling efficiencies for spilled Rhodamine 6G dye, or for pesticide-containing dusts on hard surfaces, for the cordless vacuum technique. The results for the dye in Table 2 showed that the whole cassette and sampling probe rather than just the material on the filter had to be weighed before and after sampling at 10 mg/100 cm^2 coverage to determine the surface coverage accurately. Thus while the mass collected on the filter could be analyzed to determine analyte concentration in the dust/dirt, the surface coverage could not be assessed accurately from weights collected on the filter or cassette, even when the proportion collected there increased with increasing flow rate. The use of filter mass changes alone leads to a bias towards low coverages. The slight deliquescence of Rhodamine 6G is probably responsible for the greater imprecision observed for the 5-pass sampling technique versus the 3-pass technique, and observed for high flow relative to the lower flow rates for 1.5-4.0 L/min.

All uncoated and coated particles used in the present study had sizes well below the 180 µm upper diameter limit of the optimized method validated with local soil (35). In the

latter study, the mass of soil retained in an unsaturated probe was consistently about 0.7 mg. This implied that 12 mg or more should be collected to define the method's least quantifiable limit. The present data are in good agreement. Use of a presaturated probe lowers this limit, but then cross contamination may occur. We recommend that a wide enough defined area be sampled to ensure masses ≥15 mg are collected with this technique to have a representative filter mass for analysis and to provide accurate data at low surface coverages. Use of a cordless balance is indicated in the field if available. In addition, if analyte specific concentrations are also desired, a mixed cellulose ester filter is recommended for collection of inorganics and metal compounds since these filters facilitate nitric acid digestions (33), and a Teflon filter should be used for organics since this filter material is inert and suitable for liquid/solid extractions.

Lewis et al (52) showed that the pesticides bendiocarb, carbaryl, chlordane, chlorpyrifos, DDT, diazinon, dichlorvos, heptachlor, methyoxychlor, permethrin, *o*-phenylphenol, and propoxur as well as 10 PAHs were enriched in the fraction <106 μm relative to the 106-2000 μm fraction of carpet dust. About 60% of the carpet dust mass was of size <150 μm. Since carpet dust is a major reservoir of house dust on hard surfaces, the focus on particle sizes <180 μm is also justified in the present study.

The prior methods that have been utilized for hard surfaces to sample dust and soils

containing pesticides have been of much greater flow rate or vacuum such as a vacuum cleaner (8, 19, 22, 25). These methods depend on a source of electrical power. The major aim is to clean the surface at high dust surface coverages. Such cleaners are difficult to use in low coverage situations since there is much solid holdup in the nonbag sampling path of vacuum cleaners, and a large proportion of sampled surface solids may not be collected in the collection bag for weighing. Clearly more work is required to define the low loading collection efficiency cutoffs for vacuum cleaners on hard and carpeted surfaces. The HVS-3 vacuum system used in many recent studies for carpet and floor dust loadings (8,22,25) has been evaluated in the ASTM D5483-93 method for carpet (50). The sampling efficiency on hard floors for low dust coverages is unknown. Another type of noncordless vacuum cleaner to sample inorganic dusts in carpets that employs a nozzle with teeth has also been reported (53-55).

The present cordless technique does not require a local source of power, though requirements include effective chargers, battery packs, an accurate mass balance, pre- and post-sampling pump calibration, and a performance-based validation of the sampling technique of any human operator (35). Nevertheless, the present technique is more flexible than techniques dependent on plug-in power sources, and can perform acceptably in situations of low dust coverage, a situation where vacuum cleaners may not be suited for dual use as cleaners and to measure low surface coverage. Low dust coverages can be

measured by the present cordless vacuum technique because the area of exposure to dust in the dust collection path is minimal. A stainless steel sample sampling probe as used originally in 1985 (33) may further minimize sampling probe holdup at low dust coverage but at more expense. In addition, the same pumps used to take personal aerosol samples can be used for surface dust monitoring: industrial hygienists are already familiar with pump use and calibration. The application of the optimized method for lead and other inorganic chemical species relative to other methods is discussed elsewhere (35). Further studies to increase confidence in its general applicability would be helpful.

Conclusions

At least 15 mg of sample must be collected with use of the appropriate filter (mixed cellulose ester of pore size 0.8 μm for inorganics and a 5.0 μm Teflon filter for organics) and by 3-5 sampling passes. The probe and cassette must be preweighed and these components reweighed after sampling to obtain accurate low surface coverages of 10-20 mg/100 cm^2. Use of a cordless field balance is recommended. Sufficient mass must be accumulated on the filter to allow the filter analysis to be representative of the sample.

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Table 1. Efficiency of Sampling Coated and Uncoated Montana Soil (SRM 2711) and Urban Dust (SRM 1649a) using Different Surface Loadings over the 100-cm² Smooth Surface and a Three-Pass Sampling Technique at 4.0 L/min.

SRM	Coated	Loading[a]	Cumulative Efficiency[b] for Pass		
			1	2	3
2711	-	10.5(0.6)	94(5.0)	92(11)	93(10)
	+	9.9(0.5)			59(16)
		15.3(0.4)	83(9)	85(7)	80(13)
		16.8(0.3)	82(1)	80(2)	79(4)
		19.60(0.6)	91(5)	90(5)	90(5)
1649a	-	10.2(0.6)	72(9)	72(3)	70(4)
		15.3(1.0)	73(8)	76(5)	79(9)
		20.5(1.1)	77(6)	78(6)	79(5)
	+	10.2(1.1)	91(3)	92(2)	93(3)
		15.0(0.5)	94(5)	97(4)	94(4)
		21.4(0.3)	86(5)	86(5)	84(6)

The quantities in parentheses are standard deviations of triplicate measurements

[a], mg/100 cm²; [b], in percent

Table 2. Dependence of Flow Rate and Number of Sampling Passes for 10 mg of Rhodamine 6G Deposited on a Smooth 100 cm^2 Surface.

Flow Rate	Cumulative Efficiency for Pass					In-Probe
(L/min)	1	2	3	4	5	(% Recovered)
1.0	58(11)	70(17)	70(21)	69(25)	64(25)	102(3)
1.5	82(9)	92(9)	91(9)	96(13)	93(9)	106(3)
2.0	79(10)	94(8)	93(5)	98(15)	94(8)	98(4)
2.5	68(14)	78(8)	85(13)	86(12)	90(5)	80(5)
3.0	96(10)	117(18)	117(23)	112(28)	118(27)	60(7)
3.5	96(20)	101(14)	102(11)	99(6)	102(1)	32(10)
4.0	89(13)	100(10)	100(15)	98(20)	96(21)	20(5)

The quantities in parentheses are standard deviations of triplicate measurements

EFFECT OF DUST ON THE VIABILITY OF

VIBRIO FISCHERI IN THE MICROTOX TEST

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ABSTRACT

The standard Microtox test involving the bioluminescent bacterium *Vibrio fischeri* is a much used ecotoxicological bioassay whose EC_{50} values have been correlated to acute toxicity parameters of vertebrates, to irritancy measures, and to cytotoxicity indices. The aims were to explore the dependence of light output on viable cell number, the effects of dust on the bioluminescence and cell viability, how the viability of the cells is affected after spills, and how spills can be sampled. The linear dynamic range of the light emitting bacterium was first defined in terms of volume of reconstituted bacteria in growth media. The effects of dust were then explored on this range by the method of standard additions of 5 mg, 10 mg, and 20 mg of Standard Reference Material Urban Dust 1649a to simulate dust samples collected by a cordless vacuum technique involving a filter cassette. The number of viable cells using the Total Microbe Hunter kit based on a tetrazolium salt, and the effects of dust on the viability test were also assessed. A spectrophotometric modification of the Total Microbe Hunter test using a wavelength of 508 nm was developed that was as sensitive as the Microtox test, and was twice as sensitive as the naked eye test for viability. A mass of 20 mg dust totally inhibited the Microtox test as did 5 mg the viability test. Mechanical shock involved with spilling and sampling bacterial reagent on hard surfaces killed the luminescent bacteria and inhibited luminescence. The optimum filter cassette for Microtox reagent collection was a 25-mm 1.00-μm PTFE filter in a 25-mm Delrin holder operated at 4.0 L/min, with a Tygon sampling probe.

INTRODUCTION

The Microtox test that uses the luminescent marine bacterium *Vibrio fischeri* (formerly *Photobacterium phosphoreum*) is now regarded in Canada, France, Germany, Spain, Sweden, and The Netherlands as a standard ecotoxicological bioassay that is also a reference alternative test of acute toxicity, cytotoxicity, and irritation (Ronnpagel *et al.*, 1995; Boyd *et al.*, 1997; Pardos *et al.*, 1999; Tchounwou and Reed, 1999; Domart-Coulon *et al.*, 2000). In the United States, it is involved in American Society for Testing and Materials (ASTM) method D-5660 (ASTM, 1996), and in the *Standard Methods for the Examination of Water and Wastewater* as Part 8050 (Clesceri *et al.*, 1998a). It is sensitive to heavy metals, organics, and their mixtures (Kaiser and Devillers, 1994; Kong et al., 1995). The original test is supplemented by chronic, genotoxic, and acute solid-phase bioassays, using the same instrumentation (Azur Inc., 2000).

The major application of the Microtox test has been to aqueous media and to detect substances that are sufficiently water soluble or toxic to lower luminescence relative to an unexposed positive control blank at a specific time, usually by defining the effective concentration that decreases light emission by 50%(EC_{50}). The bacterium is standardized by storing and transporting it as a freeze-dried powder that is reconstituted into a saline growth medium at 15 °C at the time of the analysis. Our research group has published on the variables that affect the test (Chou and Que Hee, 1992, 1993; Chen, 1995), and on some applications (Chou and Que Hee, 1992, 1993, 1994a,b; Chen, 1995; Que Hee, 1997). The present study explored the dependence of light output on viable cell number, the effects of dust on the Microtox test and cell viability, how the viability of the cells is affected after spills, and how spills can be sampled.

MATERIALS AND METHODS

Reagents

The following were from Azur International, Carlsbad, CA: Microtox bacterial rea-gent stored at -20 °C, Microtox diluent, and Microtox reconstitution solution. Total Microbe Hunter to measure bacteria viability contained <5% w/w 2,3,5-triphenyltetrazolium chloride in tryptic soy broth (Crescent Chemical Company, Hauppauge, New York). Standard Reference Material (SRM) 1649a for Urban Dust was from the National Institute for Standards and Technology, Gaithersburg, MD. Phenol to assure Microtox data was from Fisher Scientific, Tustin, CA.

Apparatus

The Microtox Model 500 and data collection/data system was from Microbics, Carlsbad, CA (Microbics Corp., 1992). A Hewlett-Packard 8451A diode array spectrophotometer (Palo Alto, CA) and 1-cm Suprasil cells were used for ultraviolet/visible spectrophotometry for viability measurements. A 10 cm x 10 cm plexiglas template (SKC Inc, Eighty Four, PA) defined the sampling area for spilled materials. Sampling cassettes utilized were: three piece clear polystyrene sampling cassette with preweighed 37-mm 0.80-µm mixed cellulose ester filter (Omega Specialty Instrument Co, Chelmsford, MA); 25-mm 0.45-µm Teflon membrane filter in a polypropylene Swinnex-25 holder (Gelman Sciences from Fisher Scientific, Tustin, CA); and 25-mm 1.00-µm PTFE filter in a 25-mm Delrin holder (Gelman Sciences from Fisher Scientific. Tustin, CA). The sampling probe to the filter holder was a 3.5 cm long 0.60 cm inner diameter (ID) tubing with the sampling end cut at a 45° angle with a scalpel. The cordless portable sampling pump that provided the collection vacuum was an Airchek Model 224-PCXR4 from

SKC Inc. Pump calibrations before and after each sampling were performed with a mini Buck Model M-5 calibrator (A.P. Buck, Orlando, FL). An inclinometer (Edmund Scientific Co., Lev-O-Gage) measured the angle to the sampling surface and the sampling probe.

A refrigerated water bath allowed preequilibration of samples at 15 °C. A calibrated 10-μL gas chromatography syringe facilitated accurate dispensing of volumes between 0.1 to 10 μL.

Microtox Bioassay

The techniques for Microtox assays and quality control/assurance with phenol are described elsewhere (Chou and Que Hee, 1992, 1993). Instrument equilibration time was set at 30 min; the equilibration time for reconstitution and diluent solutions was 5 min at 15 °C; and the equilibration time for the reconstituted bacterial reagent was 15 min at 15 °C. Additions were always followed by mixing through filling and dispensing the solution with a pipettor. Light levels were read relative to the positive control blank at 0, 5, 15, and 30 min after the target solution was mixed with the diluted solution containing 10.0 μL reconstituted bacteria.

To assess the basic sensitivity of the procedure, reconstituted Microtox reagent was added in triplicate from the original 1.0 mL reconstituted solution at 15 °C in aliquots of 10, 7, 5, 3, 1, 0.7, 0.5, 0.3, 0.1, and 0 μL with the Microtox diluent volume adjusted correspondingly so as to maintain constant total volume as for the standard 10 μL method.

The same procedure in triplicate was repeated with 5-, 10-, and 20- mg of SRM 1649a mixed in

with the lyophilized bacterial reagent for 30 sec by manual shaking before addition of 1 mL of Microtox reconstitution solution, except that centrifugation at 3400 rpm (900g) for 2 min occurred before the 15-min stabilization period before dispensing 10-μL aliquots for analysis.

Assessment of Viability of Vibrio fischeri

A 4-mL volume of reconstituted Microtox reagent (4 vials) was added to 4 mL of Microtox diluent in the sampling cup of the Total Microbe Hunter, and then mixed with a 1-mL pipettor by filling and dispensing five times before stabilizing at 15 °C for 15 min. A Total Microbe Hunter ampoule was then inserted into one of the slots in the bottom of the sampling cup, and the ampoule allowed to fill by breaking the tip. The ampoule powder was mixed by hand rotation as directed by the kit directions until all the powder dissolved. The solution was placed in an incubator at 35.0±0.2 °C. The color was monitored every 5 min and the time noted when an orange color was evident, and then the ampoule removed. The number of cells was then read from the Total Microbe Hunter plot of cell number versus time to change color. Similar procedures were used for dilutions of 3x, 4x, 5x, 7.5x, and 10x the original cell number, all done in triplicate. Ultraviolet/visible scanning from 190 to 820 nm of the final colored solution relative to its original solution occurred in a 1-cm Suprasil cell. The maximum wavelengths were identified with a view to enhance sensitivity relative to the naked eye.

To assess the influence of SRM Urban Dust on the viability test, 5, 10, and 20 mg of dust were evaluated. The specific weight of dust was added to each of 3 vials containing the freeze-dried bacteria, mixed by manual shaking for 30 s, then 1.0 mL of reconsitution solution added to each,

followed by mixing. The contents of these 3 vials were then added to 6.0 mL of diluent in the sampling cup of the Total Microbe Hunter, and mixed again and allowed to stabilize at 15 °C for 15 min. The Total Microbe Hunter ampoule was then inserted, filled, its contents mixed, and then incubated at 35 °C as indicated above. Color changes were monitored every 5 min for 8 hours at the various dust masses and cell numbers examined above in triplicate. Absorbance measurements were also performed at maximum and other specific wavelengths.

Surface Sampling for Microtox Reagent Spills

The sampling method was based on a previously published technique developed by one of the present authors (Kim *et al.*, 2000), modified from an earlier one (Que Hee *et al.*, 1985).

The technique consists of applying a known weight of a solid over a hard smooth surface defined by a template 10 cm x 10 cm square, and then vacuuming up the particles using multiple sampling passes. A portable cordless pump is connected to a filter sampling cassette by Tygon tubing of 0.60 cm inner diameter (ID). The sampling cassette also has a Tygon tubing sampling probe 3.5 cm long and 0.60 cm ID with the sampling end cut at an angle of 45°. The sampling probe was held with the thumb and index finger at a 45° angle and a flow rate of 4.0 L/min. The difference of the mass of the probe and cassette after sampling relative to that before is the mass collected, and is compared with the mass applied to define sampling efficiency per sampling pass or over a number of sampling passes (Kim *et al.*, 2000). One sample pass is from along the inner edge of the template area to its center in patterns of squares of progressively decreasing area.

The weighed Microtox reagent bottle was opened, and the solid quickly poured onto the surface of the template. The empty bottle was reweighed to define the mass applied by difference. The particles were crushed quickly with the bottom of the Microtox reagent bottle. The solid was dispersed physically with a spatula. The optimum geometry of the collection cassette was evaluated from the following choices: a three piece clear polystyrene sampling cassette with preweighed 37-mm 0.80-μm mixed cellulose ester filter; 0.45-μm Teflon membrane filter in a polypropylene Swinnex-25 holder; and a 25-mm 1.00-μm PTFE filter in a 25-mm Delrin holder. After the final weighing, 1.0 mL of reconstitution solution was applied slowly along the inner walls of the filter cassette, and the solution shaken for 15 s. The solution was then transferred with a Pasteur pipet to a clean Microtox cuvet, and equilibrated for 15 min in the Model 500 at 15 °C before a 10.0-μL aliquot was taken for evaluation as described above. The contents of a new Microtox reagent vial in 1.0 mL of reconstitution solution served as positive control.

RESULTS

Dependence of Light Intensity in the Microtox Test on Inoculation Volume and Time

As expected, light intensity relative to positive control at zero time decreased with increasing time after sample inoculation, and with decreasing inoculation volume at a specific time. For the latter, the inoculation volume linear dynamic range was 1-10 μL for 5, 15, and 30 min. The slopes at 15 and 30 min were 72.7±1.9% and 51.506±0.071% of those at 5 min, the value for the latter being 6.97±0.14 light intensity units relative to positive control at zero time/μL inoculation volume. The zero time value is 10 light intensity units/μL inoculation volume. The least quantifiable limit (LQL) for <10% coefficient of variation (CV) was about 0.70 μL inoculation

volume. The detection limit for signal/noise of 3.0 was <0.1 μL. Thus reliable readings of luminescence were possible for a cell number down to 0.10 that used for inoculation in the standard assay, with a detection limit of about 0.01 that cell number.

Effect of Dust on Microtox Luminescence

When the bacterial reagent was mortared and pestled in the absence of soil, no luminescence was detected. Thus mortar and pestle could not be used to mix dust and bacteria. Therefore the addition was done by adding the dust sample to the bacteria reagent, and manually shaking the bacteria and dust within the bottle. No detectable luminescence was observed after adding 20 mg dust, but light emission occurred after the 5-mg and 10-mg additions. Addition of 5 mg dust over the inoculation volume range 0.1-7.0 μL at 5, 15, and 30 min caused sensitivity to decrease to 46%, 60%, and 80% of the corresponding values without dust, respectively. Similarly, addition of 10 mg decreased the luminescence to 18%, 26%, and 36%, respectively. For both 5- and 10 mg- additions there was no dependence on reading time, the respective arithmetic mean slopes and standard deviations being 3.05± 0.18 (CV=5.9%) and 1.293± 0.023 (CV=1.8%) light units/μL inoculation volume, respectively. This behavior was also observed in the absence of dust at low light intensities for inoculation volumes between 0.1 to 0.5 μL, where CV values also varied between 5.7 to 80%. However, both the 5 and 10 mg dust additions produced luminescence above the LQL of the no dust response curve. CV values for individual inoculation volumes varied between 17 to 43%, supportive of a dust matrix effect.

Viability and the Microtox Test, and the Influence of Dust

The first task was to determine the original number of viable bacteria in the Microtox Reagent. The Total Microbe Hunter color change chart data of log of colony forming units(cfu)/mL (y) versus color change time (t) was:

$$\log y = -0.5968\, t + 8.3417 \quad r^2=0.9982 \quad p \le 0.05 \qquad (1)$$

The times to change color for different dilutions of the Microtox reagent are provided in Table 1 for the Total Microbe Hunter test. The CV for 2-fold dilution is too high (45%), with acceptable precision (<10% CV) at greater dilutions up to 5-fold. Beyond 5-fold dilutions, the viability test is too insensitive. The grand mean and standard deviation for the original cell number in the Microtox Reagent was (3.68 ±0.21) x 10^8 cfu, CV=5.6% (Table 1). Thus the Total Microbe Hunter Test detected only between 0.74 x 10^8 to 3.68 x 10^8 cells/mL of *Vibrio fischeri*.

Spectrophotometry showed that the Total Microbe Hunter blank had maxima at about 240 and 290 nm. After the color change, the maxima beyond 300 nm were 370 and 508 nm. Table 1 also presents the absorbance data at these absorption maxima and at 490 nm on the short wavelength side of the long wavelength band. Only the 508 nm wavelength is suitable for spectrophotometric assay for cell number since its absorbances decreased consistently with increasing dilution. The spectrophotometric method is more sensitive and convenient than the color change time method because absorbance values at 508 nm still changed at 7.5- and 10-fold dilution. Thus spectrophotometry at 508 nm is about double the sensitivity of the naked eye.

For 5-mg dust additions of the 3-fold diluted Microtox Reagent, the dust precipitated after 30-min incubation at 35 °C in the viability test. However, there was no color change even after 8 hours of incubation. Thus dust is a negative interference in the Total Microbe Hunter test.

Sampling Microtox Reagent Spills

The Microtox reagent consists of 4% (w/w) *Vibrio fischeri*, 3% sodium chloride, 92% skim milk solids, and 1% water according to Azur Environmental (2000). When the reagent was weighed directly on a balance, its weight continually increased, and the original powder became sticky and pebbly. The bacterial reagent was deliquescent.

The standard sampling technique for dry solids on hard smooth surfaces (Kim *et al.*, 2000) did not work because the 3-mm inlet of the filter cassette was blocked by a wet Microtox reagent pebble that caused uncontrolled variation in sampling flow rate. The cassette was also too large to allow 1.0 mL of reconstitution solution to dissolve all the solid on the filter surface, but 2.0 mL did. The inlet diameter did not allow a pipet tip to be inserted to allow the usual mixing. After 2 mL reconstitution solution was placed inside the cassette and then manual shaking, Microtox analysis of a 20-μL aliquot led to no light emission.

Obstruction also occurred for the 4-mm inlet of the 0.45-μm Teflon filter in its 25-mm filter cassette. The inlet diameter still did not allow the usual mixing. The cassette volume was too small to contain 1.0 mL of reconstitution solution.

A 1.0-μm 25-mm diameter Teflon filter in a 25-mm Delrin filter cassette of inlet ID 10 mm successfully collected the pebbles, permitted mixing by the standard technique after adding 1 mL of reconstitution solution, and allowed the taking of a 10-μL aliquot by the standard pipet for inoculation. The measured sampling efficiency for the filter cassette on a mass basis was at least 81% at 4.0 L/min flow rate with the rest of the collected mass on the sampling probe walls. There was a large imprecision in recovered mass for triplicates because of the deliquescent nature of the Microtox reagent. On Microtox testing, the light intensity for each triplicate was below the LQL of the positive control, but decreased with incubation time as a valid Microtox test should. In addition, the CV for the light intensity at 5, 15, and 30 min varied between 0.67% to 4.5%, also acceptable behavior. Thus, sufficient bacteria were recovered in the cassette to be detected in the Microtox test, but the viability of the cells was still affected.

DISCUSSION

The viable cell number dependence of the Microtox test was examined with a view to sampling low numbers of luminescent and nonluminescent bacteria in dust samples. While the intrinsic sensitivity to viable bacteria ranged down to about an order of magnitude below the original cell number for both the standard Microtox test and spectrophotometric analysis at 508 nm for the Total Microbe Hunter test, the color change test dependent on the naked eye for the Total Microbe Hunter test was only half as sensitive. The actual detection limit of the Microtox test was equivalent to about $<4 \times 10^4$ viable cells. The LQL was about 3.7×10^5 viable cells compared with 3.7×10^6 viable cells used in the standard test. The Microtox manual provides a figure of about 10^8 cfu in the storage vial (Microbics Corp, 1992). The present investigation determined

the number to be actually (3.68±0.21) x 10^8 cfu.

The Total Microbe Hunter test utilizes a tetrazolium salt that is reduced to a colored formazan product when electrons are accepted from oxidized substrates or appropriate reducing agent cofactors like reduced nicotinamides and flavins produced during growth and division, but which are not produced by dead cells (Vistica *et al.*, 1991; Bitton *et al.*, 1992). Johnson *et al.* (1985) incorporated tetrazolium dye reduction into portable kits that shortened the time of analysis for viable gram negative bacteria from 15-18 hours for the standard laboratory test to 4-6 hours, with 93% correlation. Both methods are still much shorter than 3-4 days required for the standard agar plate dilution/colony counting bioassay for bacteria in aqueous media (Clesceri *et al.*, 1998b) or collected on a filter (Clesceri *et al.*, 1998c). Hjertstedt *et al.* (1998) showed that sporulating *Candida albicans* yeast contributed to the tetrazolium bioassay response, but did not form colonies. This is not a factor in bacterial bioassays. A factor in the insensitivity of the Total Microbe Hunter test might be the short lifetime of *Vibrio fischeri*, even at 15 °C.

Mechanical shock intrinsic to the sampling process lowered the viability of the luminescent bacteria of the Microtox test, whether from its original storage bottle or on a surface. Since only viable bacteria luminesce in the Microtox test, bioluminescence diminuition could be interpreted as chemical toxicity, if the standard reconstitution technique is not followed exactly for the case of the original storage bottle. Such effects might also be observed for dropped or roughly-handled vials, and may account for some of the known intershipment variability.

While no luminescence was detected for the 20-mg addition, 5-mg and 10-mg dust additions did allow Microtox test responses, but with low light emission. Masking or luminescence absorption by the dust akin to a color interference may be occurring. No matter the cause, the addition of a dust matrix even at as low a mass as 5-mg causes an apparent chemical toxicity effect that must be accounted for when using the standard Microtox test for turbid liquids, sediments, sludges, or solid samples. The recommendation for turbid liquid samples is to centrifuge and then filter the supernatant through 0.45-μm and then 0.20-μm filters and dilute appropriately before adding the reconstitution solution containing bacteria. A high apparent background soil toxicity in the standard Microtox test has also been reported recently (Cassells *et al.*, 2000).

Much recent work has occurred with the new Microtox solid-state bioassay (Bitton *et al.*, 1992; Kong *et al.*, 1995; Ronnpagel *et al.*, 1995; Kwan and Dutka, 1995; Wendt *et al.*, 1996; Cheung *et al.*, 1997; Harkey and Pradhan, 1998; Lee *et al.*, 1999; Dorn and Salanitro, 2000), although the modified test still appears to lack sensitivity for xenobiotics in soils, sludges, and sediments. More satisfactory results are still obtained for xenobiotics in extractions from soils, sludges, and sediments by the standard Microtox test (Bitton *et al.*, 1992; Kong *et al.*, 1995; Karuppiah *et al.*, 1997; Sunahara *et al.*, 1998,1999; Cassells *et al.*, 2000; Cook *et al.*, 2000).

The surface sampling results showed that *Vibrio fischeri* is a delicate bacterium that lost viability quickly on a hard smooth surface. The sampling of the Microtox reagent itself had to be done as quickly as possible because of its deliquescence, with subsequent stickiness and pebbling.

Very few methods to sample bacteria on hard surfaces have been reported. Eginton *et al.*, (1995) using a tetrazolium salt based method and colony counting showed that 15 sampling passes had to be done to obtain a negative tetrazolium salt test for transfer of gram negative bacteria deposited on hard smooth tiles to agar plates. The authors attributed their results to the strength of the attachment of the bacteria to the tile surface, but decreasing bacteria viability may also have been a factor in light of the results of the present investigation. Bacteria on human skin have been sampled with a pad and rinsing techniques (Hambraeus *et al.*, 1990). Bacteria on citrus fruit surfaces were removed better by washing and waxing rather than by washing alone (Pao and Brown, 1998). Scrubbing allowed removal of bacteria from the skin of horses (Hague *et al.*, 1997). Real-time monitoring of *E. coli* adhering to beef carcase surfaces was accomplished by inserting a *lux* reporter gene into the applied *E. coli*, and monitoring the surface bioluminescence to assess surface adherence and decontamination in real time (Siragusa *et al.*, 1999).

Linking tetrazolium salt data with the data from other tests focusses on the potential infectiveness of the microorganisms involved on the surface or in the substrate. Gram-negative bacteria endotoxin is toxic whether the bacteria are alive or dead, and a marker like 3-hydroxymyristic acid (Kirschner *et al.*, 1985; Parenteral Drug Association, 1990) or the *Limulus* amoebocyte lysate test (Parenteral Drug Association, 1990; Hurley, 1995) to detect toxic endotoxin liposaccharides are essential adjuncts to screening tests for viable bacteria to achieve public health and environmental safety.

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APPENDIX C

Cancer Mortality Among Workers Exposed to Chemicals During Uranium Processing, B. Ritz, 41:556-566, JOEM.

Radiation Exposure and Cancer Mortality in Uranium Processing Workers, B. Ritz, 10:531-538, 1999, Epidemiology.

Cancer Mortality Among Workers Exposed to Chemicals During Uranium Processing

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Data provided by the Comprehensive Epidemiology Data Resource allowed us to study patterns of cancer mortality as experienced by 3814 uranium-processing workers employed at the Fernald Feed Materials Production Center in Fernald, Ohio. Using risk-set analyses for cohorts, we estimated the effects of exposure to trichloroethylene, cutting fluids, and kerosene on cancer mortality. Our results suggest that workers who were exposed to trichloroethylene experienced an increase in mortality from cancers of the liver. Cutting-fluid exposure was found to be strongly associated with laryngeal cancers and, furthermore, with brain, hemato- and lymphopoietic system, bladder, and kidney cancer mortality. Kerosene exposure increased the rate of death from several digestive-tract cancers (esophageal, stomach, pancreatic, colon, and rectal cancers) and from prostate cancer. Effect estimates for these cancers increased with duration and level of exposure and were stronger when exposure was lagged.

The US Department of Energy recently assembled the Comprehensive Epidemiology Data Resource (CEDR).[1] These epidemiologic data were collected at multiple nuclear facilities over the past 30 years and have been made available to the research community at large for the first time. Combining several independently collected data files included in CEDR provided us with the opportunity to examine the influence of chemical exposures in the work environment of the Fernald Feed Materials Production Center (FFMPC) in Fernald, Ohio, on cancer mortality. Workers at the FFMPC processed uranium-ore concentrate and uranium of low-enrichment grade into fabricated uranium metal products and, to a much lesser extent, produced thorium metal. Operations began in late 1951 and halted in July 1989. The uranium-processing work conducted at this facility involved the use of large amounts of nonradioactive industrial chemicals, many of which are potent respiratory irritants (hydrofluoric acid, ammonia, nitric and sulfuric acid, tributyl phosphate) or suspected carcinogens (trichloroethylene [TCE] and cutting fluids). Large-scale chemical operations consisted of dissolving ore concentrates in nitric acids to produce uranyl nitrate solution, which then was purified via solvent extraction, concentrated through evaporation, and thermally denitrated to uranium trioxide. Uranium trioxide was converted to uranium tetrafluoride and reduced to metal. Other chemical processes at the facility required fur-

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nacing or wet chemical-hydrometallurgical processing.

In the early 1980s, the widespread use of chemicals prompted Wilson[2] to conduct an investigation of respiratory morbidity in FFMPC workers. The Wilson study provided historical measures of chemical use that, in combination with mortality data collected for all workers monitored for radiation exposure, allowed us to examine whether cancer mortality was associated with exposure to these chemicals while also allowing us to control for the effects of radiation exposures. In this report, we will focus our analyses on the effects of TCE, cutting fluids, and a combination of kerosene exposures with carbon (graphite) and other solvents. All of these chemicals were used in large quantities at the facility, and either these chemicals are suspected chemical carcinogens or carcinogens were likely to have been formed in the processes in which they were used. The Fernald cohort is characterized by an extremely long follow-up and by the fact that its workers were monitored for exposure to both internal and external radiation. This article is solely based on the data provided by the CEDR, and the description of this study's methods relies on the CEDR documentation[1] and the article by Wilson.[2]

Materials and Methods

Study Population

The study population consists of 3814 white male employees identified from company rosters and personnel records who were first hired at the facility between January 1, 1951, and December 31, 1972, were continuously employed for at least 3 months, and were monitored for radiation. For all workers, vital status was first ascertained on January 1, 1980. Thus follow-up began on January 1, 1951, or date of hire, whichever was later, and ended either on date of death or on December 31, 1989, whichever date came earlier. Mortality data for FFMPC workers are only available in the CEDR database for those workers who were radiation-monitored. Thus we had to exclude 287 workers for whom chemical-exposure data existed but who could not be matched to the records provided in the radiation files. Approximately 88% of all cohort members were hired at the facility before 1960. Employment at the facility peaked in 1956 and slowly decreased until all operations halted in July 1989. Vital-status searches were conducted using two record systems: the Social Security Administration, for the period before 1979, and the National Death Index, for the period 1979 to 1989. Workers not known to be alive and not identified by either system as dead were assumed to be alive at the end of follow-up. Death-certificate information was available for a total of 1045 workers who died during the follow-up period.

Exposure Assessment

In the late 1970s and early 1980s, plant experts—including industrial hygienists, a plant foreman, and an engineer—historically determined the likelihood of chemical exposure for each job title and plant area. All experts included in this rating procedure had been at the company for at least 20 years and were supervised by the medical director during this task. For the period 1952 to 1977, these experts classified workers into four main categories of chemical exposure, from none (level 0) to heavy (level 3). For the following analyses, measures of intensity (exposure level) and duration (exposure in years) were derived from the job-exposure matrix created by the plant expert to describe exposure to TCE, cutting fluids, and kerosene.

Approximately 62% of all workers held only one job title during employment at the Fernald facility, one quarter held two titles, and only 5% changed titles four times or more. Almost 45% of employees worked in one physical location of the plant, whereas one fifth moved between locations more than five times. Only 15% of the workforce was employed at the plant for less than 1 year, 31% between 1 and 5 years, and over half (54%) for more than 5 years (ie, this cohort constitutes a fairly stable workforce).

External radiation exposure was reported as annual deep doses derived from film badge dosimeters. Internal radiation exposure was reported as annual lung doses, based on individual urine bioassays and area sampling. Exposure to internal radiation-emitting particles at Fernald originated from airborne long-lived radioactive materials such as uranium, thorium, and radium compounds. Most of the exposures, however, were due to U-235 isotopes varying from depleted to slightly enriched. Workers were placed on an internal-exposure monitoring program for uranium when industrial hygiene surveys indicated high levels of radioactive substances in the work environment. The soluble and insoluble uranium and thorium compounds used at the facility may also have exhibited some chemical toxicity, primarily targeting kidneys and lungs. Although no independent measures for the chemical toxicity of these radioactive compounds exist, estimates of internal radiation dose might reflect chemical toxicity to some extent.

Smoking history was available on medical records for a small subsample of workers (approximately 20%) employed on or after January 1, 1968, when the company introduced pulmonary-function testing. Because information about smoking history was only available for approximately one fifth of all subjects, we were not able to adjust for smoking in our analyses. However, we examined the smoking distributions by chemical exposure and determined whether such exposure was related to smoking prevalence. The records also provided information on salary status (hourly vs salaried; Table 1).

TABLE 1
Characteristics of the All-Male Fernald Cohort

Number of employees	3,814
Average follow-up time, years	31.5
Average age at entry into cohort, years	30.5
Number of person-years of follow-up	120,237
Number of deaths	1,045
Total mortality rate, per 10^5/year	869
Total cancer mortality rate, per 10^5/year	273
Pay type	
Salaried	1,224
Hourly/union	2,590

Statistical Analyses

We used two different analytical approaches: (1) external comparisons of our monitored workers with the general US population and—for all-causes and total cancer mortality—with the NIOSH-CORPS cohort,[3] and (2) internal comparisons among monitored workers, according to level and duration of chemical exposure (dose-response analyses).

In external comparisons, the Monson[4] program was used to estimate standardized mortality ratios (SMRs; observed/expected deaths) for the monitored study population. Expected numbers of deaths were estimated from the mortality rates of the US white male population or NIOSH-CORPS data, stratified by age (5-year categories) and calendar year (5-year intervals). Estimation of 95% confidence intervals for the SMRs was based on a formula derived by Byar and recommended by Breslow and Day.[5]

For internal comparisons of workers exposed to chemicals at different levels, we examined cancers by site, concentrating on organ sites for which effects have previously been reported in the literature. The small numbers of many specific cancers [illegible] necessary to group some [illegible] in order to achieve a minimum of ten cases. Thus, for most analyses, [illegible] with few cases were combined, based on the assumption that any carcinogenic effect of exposures should be similar at these sites because of anatomical proximity, tissue similarity, similarity of exposure routes, or similarity of diagnostic categories. The single cancers and cancer groups we examined were as follows: (1) all hemato- and lymphopoietic cancers (International Classification of Diseases, 9th revision [ICD-9][6] codes 200 to 208) and, in addition, lymphomas (excluding Hodgkin's lymphomas) and leukemias separately; (2) esophagus and stomach cancers (ICD-9 codes 150 and 151); (3) liver and biliary tract cancers (ICD-9 codes 155 and 156); (4) pancreatic cancers (ICD-9 code 157); (5) colon and rectal cancers (ICD-9 codes 153 and 154); (6) bladder and kidney cancers (ICD-9 codes 188 and 189); (7) prostate (ICD-9 code 185); (8) brain (ICD-9 codes 191 and 192), and (9) lung cancers (ICD-9 code 162). We also conducted separate analyses for two rare cancers for which we observed fewer than ten cases (ie, eight cancers of the liver and biliary tract [ICD-9 codes 156 and 157] and five cancers of the larynx [ICD-9 code 161]), because these cancers have consistently been linked to two of the chemicals in previous studies, in which liver cancers were found to be associated with TCE exposure[7] and laryngeal cancers with cutting fluid exposures.[8]

For internal comparisons, we utilized the risk-set approach for cohort analysis described by Breslow and Day,[5] a method basically equivalent to Cox proportional hazards modeling. When using risk sets, conditional logistic regression is used to compare individuals who have died of cancer with individuals still at risk of dying from cancer ("survivors"). We constructed risk sets of deaths and survivors by matching to each cancer death all cohort members who were still alive at the time of the index subject's death and who were within 3 years of the index case's age. This age-range specification helped us avoid constructing risk sets with fewer than five non-cases, ie, we based our analyses on risk sets that included between five and 830 survivors for each cancer death.

We used multiplicative rather than additive models because the limited size of our data set did not allow us to adequately distinguish between alternative models. We evaluated cumulative exposure duration (in years) as a set of binary variables (minimum of 2 and 5 years of exposure), as three categories of duration (<2 years, 2 to 10 years, >10 years of exposure), and as a continuous variable (in years). To allow for a period of cancer induction/latency after exposure and, additionally, to reduce possible selection bias, cumulative exposure duration was lagged.[9] Lagging entailed limiting the exposure duration for each individual in a risk set to the duration at the level achieved 15 years prior to the index death.

Results of the conditional logistic regression analyses were used to estimate rate ratios and 95% confidence intervals (95% CIs) for chemical effects. Individual exposure duration and other time-related variables, such as time since first hired, were treated as time-dependent. All models were adjusted for the same confounders: pay status, time since first hired, and cumulative time-dependent external- and internal-radiation doses (continuous). Time since first hired was used to control for the selective loss of less healthy workers.[10] Because of the small number of exposed cases, the number of variables that could be included in many of our models without causing convergence problems was limited. This limitation in numbers and the fact that some chemical exposures only occurred concomitantly (such as moderate kerosene, carbon, and other solvent exposures) meant that, for the most part, we only included one chemical at a time in our models (see also below). In addition, whenever possible, we report

TABLE 2
Exposure to Chemicals, by Exposure Level, for Fernald Workers

Exposure Level	No. of Workers Exposed to		
	Trichloroethylene (TCE)	Cutting Fluids	Kerosene
1—Light	2,792*	1,792†	1,691‡
2—Moderate	179	82§	905‖
3—Heavy	0	405¶	0

* Approximately half of the workers were also exposed to cutting fluids.
† All workers were exposed to TCE and most were exposed to kerosene and carbon at level 1.
‡ All workers were exposed to cutting fluids, TCE, carbon, and all respiratory irritants at level 1.
§ All workers are also exposed to TCE at level 2.
‖ All workers were exposed to carbon at level 2 but to no other chemicals.
¶ Some workers were exposed to TCE at levels 1 but to no other chemicals.

results for models in which we adjusted for other chemicals.

Results

The Fernald cohort of white male workers is characterized by a long follow-up period (average, 31.5 years) and a high percentage of hourly employees (68%) (Table 1). During the study period, 26.5% of all cohort members died (1045 total deaths; Table 1). Of these deaths, 328 were due to cancer, yielding a total cancer-mortality rate of 273 per 10^5/year (Table 1).

Table 2 displays the exposure distribution of the three chemicals included in our internal comparisons of cohort members. Most notably, low-level exposure to all of the three chemicals of interest occurred in a large number of workers simultaneously (1691; 44%). In addition to being exposed to these three chemicals these 1691 workers were also exposed to all of the respiratory irritants used at FFMPC. Low-level chemical exposure to cutting fluids and kerosene thus almost always represents exposure to a combination of all of the chemicals used at the facility. Moderate exposure (level 2) occurred either as a combination-type exposure or as a singular exposure, depending on the jobs and plant areas; moderate cutting fluid exposure always occurred in combination with moderate TCE exposure. The job titles for which such mixed exposure was documented were set-up workers, riggers, and degreasers, whereas electricians (n = 92) were exposed to TCE at moderate levels only. No exposure to TCE was rated as heavy (level 3). Heavy cutting fluids exposure (level 3) was attributed to machining tool operators and laboratory machinists, some of whom also experienced low-level TCE exposure. Moderate kerosene exposure was experienced by chemists and chemical operators (n = 905) all of whom were also exposed to moderate levels of carbon and all respiratory irritants. Thus, at the highest level at which kerosene exposure occurred (level 2), any effects on cancer outcome represent the combined effects of kerosene and other chemicals (carbon and solvents), but none of these workers was at the same time exposed to TCE or to cutting fluids.

The overall mortality rate was lower among Fernald workers than among US white males (SMR = 0.84, 95% CI, 0.79 to 0.90); however, the rate of deaths from all malignant neoplasms was slightly increased (SMR = 1.10; 95% CI, 0.99 to 1.23; Table 3). Similarly, when we compared this uranium-worker cohort with NIOSH-CORPS workers, we found that the SMR for all causes was still lower (SMR = 0.81; 95% CI, 0.76 to 0.86) but the cancer mortality rate was even higher (SMR = 1.24; 95% CI, 1.11 to 1.38) among the uranium workers. Thus the Fernald cohort is generally healthier than other worker cohorts, especially with respect to cardiovascular mortality, but exhibits a 24% increase in mortality due to cancers. Compared with the US population, the SMRs for the Fernald cohort were greater than one for cancers of all digestive system organs, the prostate, and the lymphopoietic system. Yet none of the CIs in these external comparisons excluded the null value.

TCE exposure was strongly associated with liver cancers (exposure duration, >5 years; 15-year lag rate ratio [RR] = 12.1; 95% CI, 1.03 to 144); Table 4). Furthermore, TCE exposure seemed to be associated with brain cancers in the analyses of single chemicals (Table 4); however, this effect completely disappeared when cutting-fluid exposure was added to the model. As shown in Table 5, cutting-fluid exposure at any level was associated with hemato- and lymphopoietic system, brain, bladder, and kidney cancer mortality, but the estimates for high-exposure levels were based on very small numbers of exposed cases. Cutting fluids were, furthermore, very strongly related to the rare cancers of the larynx (exposure duration, >5 years; 15-year lag RR = 236; 95% CI, 9.93 to 5630); Table 5).

Kerosene exposure at moderate levels (level 2) and of long duration (>5 years) increased the rate of prostate cancers, especially after adjustment for TCE exposure (for 15-year lag RR = 4.50; 95% CI, 0.98 to 20.8; Table 6). Furthermore, when we adjusted for the other two chemicals in the models, kerosene exposure at moderate levels (level 2) was strongly associated with esophageal and stomach cancers (exposure duration, >2 years; and 15-year lag RR = 9.22; 95% CI, 2.34 to 36.3; and exposure duration, >5 years; 15-year lag RR = 12.4; 95% CI, 2.53 to 60.8, respectively). Also, the results displayed in Table 6 suggest a

TABLE 3
Observed (Obs) and Expected (Exp) Numbers of Deaths for White Male Subjects for the Fernald Cohort and Estimated Standardized Mortality Ratios (SMRs) and 95% Confidence Intervals (CIs): Comparison With the US Population, by Cause of Death

Causes of Death*	Obs	Exp	SMR	95% CI
All causes (ICDA-8 001–998)	1045	1238.40	0.84	0.79–0.90
All malignant neoplasms	328	297.38	1.10	0.99–1.23
Cancers				
Buccal cavity and pharynx (ICDA-8 140–149)	9	8.75	1.05	0.48–1.99
Digestive organs and peritoneum (ICDA-8 150–159)	85	73.37	1.16	0.93–1.43
Esophagus (ICDA-8 150)	9	7.32	1.23	0.56–2.33
Stomach (ICDA-8 151)	15	10.97	1.37	0.76–2.26
Large intestine (ICDA-8 153)	26	26.07	1.00	0.65–1.46
Rectum (ICDA-8 154)	7	6.56	1.07	0.43–2.20
Liver (ICDA-8 155–156)	8	4.83	1.66	0.71–3.26
Pancreas (ICDA-8 157)	18	15.05	1.20	0.71–1.89
Respiratory system (ICDA-8 160–163)	120	113.92	1.05	0.87–1.26
Larynx (ICDA-8 161)	5	4.15	1.20	0.39–2.81
Lung—primary and secondary (ICDA-8 162)	112	108.72	1.03	0.85–1.24
Bone (ICDA-8 170)	0	0.97	0.00	0.00–3.78
Skin (ICDA-8 172–173)	4	6.28	0.64	0.17–1.63
Prostate (ICDA-8 185)	24	17.14	1.40	0.90–2.08
Testis (ICDA-8 186–187)	1	1.45	0.69	0.01–3.85
Bladder (ICDA-8 188)	8	6.83	1.17	0.50–2.31
Kidney (ICDA-8 189)	5	7.72	0.65	0.21–1.51
Brain and other central nervous system (ICDA-8 191–192)	12	9.43	1.27	0.66–2.22
Thyroid (ICDA-8 193)	0	0.53	0.00	0.00–6.90
Lymphosarcoma and reticulosarcoma (ICDA-8 200)	8	4.69	1.71	0.73–3.36
Hodgkin's disease (ICDA-8 201)	6	2.88	2.09	0.76–4.54
Leukemia and aleukemia (ICDA-8 204–207)	12	10.96	1.09	0.56–1.91
Lymphatic tissue (ICDA-8 202–203, 208)	10	9.72	1.03	0.49–1.89
Lymphopoietic cancer (ICDA-8 200–208)	37	28.85	1.28	0.90–1.77
Cancer residual†	23	22.11	1.04	
Other causes				
Benign neoplasms (ICDA-8 210)	1	3.39	0.29	0.00–1.64
Diseases of blood and blood-forming organs (ICDA-8 280–289)	2	2.60	0.77	0.09–2.78
All diseases of circulatory system (ICDA-8 390–458)	450	577.89	0.78	0.71–0.85
Arteriosclerotic heart disease, including CHD (ICDA-8 410–414)	332	417.67	0.79	0.71–0.89
All vascular lesions of CNS (ICDA-8 430–438)	47	58.50	0.80	0.59–1.07
All respiratory diseases (ICDA-8 460–519)	53	78.35	0.68	0.51–0.88
Emphysema (ICDA-8 492)	3	14.28	0.21	0.04–0.61
All diseases of digestive system (ICDA-8 520–577)	48	62.94	0.76	0.56–1.01
Cirrhosis of liver (ICDA-8 571)	33	36.07	0.91	0.63–1.28
All diseases of the genitourinary system (ICDA-8 580–629)	3	14.00	0.21	0.04–0.63
All external causes of death (ICDA-8 800–998)	110	122.89	0.90	0.74–1.08
Suicide (ICDA-8 950–959)	24	30.47	0.79	0.50–1.17
Total residual‡	15	2.61	5.76	

*ICDA, *International Classification of Diseases, Adapted 8th Revision;* CHD, coronary heart disease; CNS, central nervous system.
† Cancers of unspecified site.
‡ Including undetermined causes of death and missing causes of deaths due to missing death certificates.

twofold increase in the mortality rate from colon and rectal cancers; however, the 95% CI included the null value. Similarly, a two- to threefold increase in mortality was observed [illegible] digestive tract cancers: pancreatic cancers (low exposure, >5 years; 15-year lag RR = 1.33; 95% CI, 0.31 to 5.66; and moderate exposure, >5 years; 15-year lag RR = 2.78; 95% CI, 0.51 to 15.2) and oropharyngeal tract cancers (ICD-9 codes 140 to 149) (low exposure, >2 years; 15-year lag RR = 1.85; 95% CI, 0.37 to 9.36; and moderate exposure, >2 years, 15-year lag RR = 2.87; 95% CI, 0.43 to 19.2), but the confidence intervals for the estimates included the null value. When we combined the cancers at all digestive-tract sites, we estimated a consistent two- to threefold increased mortality rate for moderate-level kerosene exposure of more than 5 years (low exposure: 15-year lag RR = 1.80; 95% CI, 1.05 to 3.10; and moderate exposure: 15-year lag RR = 2.71; 95% CI, 1.37 to 5.34). These estimates for kerosene exposure remained stable when we

TABLE 4
Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Effects of TCE Exposure (Both Levels in Model) and Cancer Mortality, by Cancer Site and Level, 0 and 15 Years' Lag for Exposures: Results From Conditional Logistic Regression Analyses Matched on Time and Age (±3 Years)*

Exposure Duration, by Cancer Site	TCE Light (level 1)						TCE Moderate (level 2)					
	Lag Zero			Lag 15 Years			Lag Zero			Lag 15 Years		
	Case *n*	RR	95% CI	Case *n*	RR	95% CI	Case *n*	RR	95% CI	Case *n*	RR	95% CI
Hemato- and lymphopoietic cancers (*n* = 37)												
>2 Years	18	1.35	0.68–2.69	15	1.45	0.68–3.06	1	0.98	0.13–7.41	1	1.17	0.15–9.00
>5 Years	15	1.85	0.87–3.95	12	1.79	0.78–4.08	0	—		0	—	
Esophagus and stomach cancers (*n* = 24)												
>2 Years	15	2.21	0.91–5.33	12	2.61	0.99–6.88	0	—		0	—	
>5 Years	8	1.03	0.40–2.63	5	1.02	0.32–3.21	0	—		0	—	
Liver cancers (*n* = 8)												
>2 Years	3	0.93	0.19–4.53	3	1.16	0.24–5.60	1	4.97	0.48–51.1	1	5.53	0.54–56.9
>5 Years	3	1.90	0.35–10.3	3	2.86	0.48–17.3	1	8.82	0.79–98.6	1	12.1	1.03–144
Prostate cancers (*n* = 24)												
>2 Years	10	0.78	0.33–1.85	10	0.91	0.38–2.18	1	1.35	0.17–10.4	1	1.44	0.19–11.4
>5 Years	8	0.83	0.33–2.09	8	1.04	0.40–2.70	1	1.58	0.20–12.5	1	1.96	0.25–15.6
Brain cancers (*n* = 12)												
>2 Years	6	1.81	0.49–6.71	4	2.29	0.42–12.5	1	3.26	0.37–28.9	1	6.94	0.66–73.1
>5 Years	3	1.32	0.28–6.17	3	5.41	0.87–33.9	1	4.52	0.49–41.5	1	14.4	1.24–167

* Adjusted for time since first hired, pay type (salaried/hourly), external and internal radiation dose (continuous and lagged), and same chemical at a different level.

adjusted for the other chemicals in the model.

For all associations reported above, the effects were generally stronger at higher levels of exposure and increased with duration of exposure. Effect size furthermore increased when a 15-year lag was utilized.

Internal comparisons showed no or no consistent effect for any of the examined chemicals on cancers of the lung after we controlled for internal and external radiation doses and/or for other chemicals (Table 7).

Discussion

A previous study that examined cancer mortality due to internal and external radiation among Fernald workers found that mortality from lung and some other radiosensitive [illegible] cancers was increased among workers who had experienced exposures to high levels of alpha and gamma radiation.[10a] The analyses presented here were conducted to examine whether chemical exposures occurring during uranium processing contributed to cancer mortality in workers when the radiation effects on cancer were taken into account. We concentrated our analyses on three potentially carcinogenic exposures: that is, TCE, cutting fluids, and kerosene, in combination with a mixture of several solvents and carbon.

According to the CEDR, approximately 60% of all Fernald workers were exposed to cutting fluids and/or the kerosene-solvent mixture at any level, and almost 80% were exposed to TCE. Moderate levels of exposure to TCE occurred among 5% of all workers, 11% of workers were exposed to high levels of cutting fluids (level 3), and 24% of all workers were exposed to the kerosene-solvent mixture at moderate levels (level 2). Although fewer workers were categorized as having had moderate or high levels of exposure to chemicals, it is important to note that the categories "light" to "heavy" reflect an ordinary rating scale used by plant experts to attribute the likelihood of exposure to workers according to job titles and plant areas and are not based on actual measurements from industrial hygiene surveys.

We found a strong effect of TCE exposure on liver and biliary tract cancers in this cohort. According to a recent review article,[8] TCE exposure has been most consistently linked to three types of cancer: liver and biliary tract cancers, non-Hodgkin's lymphoma, and kidney cancers. Thus, although our liver-cancer results are based on a small number of cases (eight cases, four of whom were exposed to TCE), they are consistent with previous study results. Furthermore, all previous studies examining the association between TCE and liver/biliary tract cancers

TABLE 5

Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Effects of Cutting-Fluid Exposure (Both Levels in Model) and Cancer Mortality, by Cancer Site and Level, 0 and 15 Years' Lag for Exposures: Results From Conditional Logistic Regression Analyses Matched on Time and Age (±3 Years)*

Exposure Duration, by Cancer Site	Cutting Fluid, Light and Moderate Exposure (levels 1 and 2)						Cutting Fluid, Heavy Exposure (level 3)					
	Lag Zero			Lag 15 Years			Lag Zero			Lag 15 Years		
	Case *n*	RR	95% CI	Case *n*	RR	95% CI	Case *n*	RR	95% CI	Case *n*	RR	95% CI
HLL† (*n* = 37)												
>2 Years	16	1.97	0.96–4.08	14	2.18	1.00–4.76	4	2.94	0.96–9.01	3	2.87	0.80–10.3
>5 Years	15	3.26	1.48–7.20	12	2.95	1.25–6.95	3	4.76	1.28–17.7	2	4.14	0.87–19.8
Laryngeal cancer (*n* = 5)												
>2 Years	3	4.49	0.52–38.6	2	2.02	0.24–16.9	2	36.1	3.57–365	2	23.2	2.68–201
>5 Years	2	4.31	0.40–46.4	2	8.05	0.52–125	2	190	9.57–3758	2	236	9.93–5630
Esophagus and stomach cancers (*n* = 24)												
>2 Years	11	1.67	0.69–4.03	10	2.36	0.90–6.19	1	1.17	0.15–9.33	0	—	
>5 Years	6	0.86	0.32–2.34	4	0.97	0.29–3.24	0	—		0	—	
Pancreatic cancers (*n* = 18)												
>2 Years	9	1.90	0.70–5.11	7	1.81	0.62–5.33	1	1.09	0.14–8.69	0	—	
>5 Years	7	1.62	0.58–4.48	3	0.83	0.22–3.22	0	—		0	—	
Bladder and kidney cancers (*n* = 13)												
>2 Years	5	1.36	0.40–4.69	4	1.03	0.29–3.72	3	5.31	1.26–22.3	3	5.67	1.33–24.2
>5 Years	4	1.29	0.35–4.73	3	1.02	0.25–4.14	2	4.93	0.95–25.6	2	6.33	1.15–34.7
Prostate cancers (*n* = 24)												
>2 Years	7	0.53	0.21–1.33	7	0.59	0.23–1.49	0	—		0	—	
>5 Years	6	0.58	0.22–1.55	6	0.71	0.26–1.94	0	—		0	—	
Brain cancers (*n* = 12)												
>2 Years	7	4.70	1.26–17.5	5	8.45	1.50–47.6	1	3.18	0.33–30.5	1	6.52	0.54–79.4
>5 Years	4	3.11	0.70–13.6	4	12.5	1.80–87.4	0	—		0	—	

* Adjusted for time since first hired, pay type (salaried/hourly), external and internal radiation dose (continuous and lagged), and same chemical at a different level.

† HLL, hemato- and lymphopoietic cancers.

also based their conclusions on as few as two to six exposed cases ineach cohort studied. We did not observe an effect for kidney cancers, which, overall, was a rare occurrence in this cohort (n = 5). An effect for all hemato- and lymphopoietic system cancers was suggested for cases with long durations (>10 years) of TCE exposure; however, this excess was not solely attributable to non-Hodgkin's lymphomas, and the positive associations for TCE exposure disappeared when we adjusted our analysis for cutting-fluid exposures. The slightly increased rate of brain cancers observed at moderate levels of TCE exposure was based on only one case, with the job title "degreaser," who was exposed to a combination of cutting fluids and TCE, and the effect disappeared completely after adjustment for cutting-fluid exposure.

Cutting fluids comprise three classes of fluids: straight oils, soluble, and synthetic fluids, each of which is a complex and variable mixture of many substances, some of which are known or suspected carcinogens or co-carcinogens.[11] Furthermore, certain processes, such as high-speed grinding, may alter the composition of the fluids and their carcinogenic properties. The carcinogens contained to some degree in most cutting fluids prior to 1980 are polycyclic aromatic hydrocarbons and nitrosamines.[12,13] Because no information concerning the types of cutting fluids used in the operations at Fernald throughout the 30-year study period was documented, it was impossible to differentiate exposures with respect to the type of fluid. However, when we examined the overall effect of cutting-fluid exposure at different levels and duration, we found that exposure at this uranium-processing facility was associated with increased rates of hemato- and lymphopoietic system cancers, brain, bladder, and kidney cancers and was also very strongly related to the generally rare cancers of the larynx.

Machining or cutting fluids have previously been linked to excess mortality from laryngeal cancers,[11,12,14,15] and several researchers observed an association between bladder and urinary tract cancers and work as a machinist.[12,16,17] Thus the

TABLE 6
Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Effects of Kerosene Exposure (Levels 1 and 2 in Model) and Cancer Mortality, by Cancer Site and Level, 0 and 15 Years' Lag for Exposures: Results From Conditional Logistic Regression Analyses Matched on Time and Age (±3 Years)*

Exposure Duration, by Cancer Site	Kerosene, Light Exposure (level 1)						Kerosene, Moderate Exposure (level 2)					
	Lag Zero			Lag 15 Years			Lag Zero			Lag 15 Years		
	Case *n*	RR	95% CI	Case *n*	RR	95% CI	Case *n*	RR	95% CI	Case *n*	RR	95% CI
Esophagus and stomach cancers (*n* = 24)												
>2 Years	10	1.98	0.77–5.09	9	3.46	1.22–9.80	5	3.00	0.81–11.2	5	7.71	2.04–29.1
>5 Years	5	0.96	0.32–2.94	3	1.26	0.31–5.15	4	2.86	0.60–13.6	4	10.7	2.26–50.7
Colon and rectum cancers (*n* = 33)												
>2 Years	10	1.13	0.49–2.60	9	1.20	0.50–2.91	8	1.80	0.64–5.06	7	2.11	0.75–5.97
>5 Years	9	1.26	0.52–3.01	7	1.40	0.52–3.74	5	1.13	0.31–4.18	4	1.91	0.50–7.27
Bladder and kidney cancers (*n* = 13)												
>2 Years	3	0.63	0.16–2.54	3	0.71	0.18–2.89	3	2.27	0.47–11.0	3	1.13	0.23–5.47
>5 Years	3	0.87	0.21–3.55	2	0.61	0.12–3.10	2	1.63	0.24–11.1	2	0.91	0.13–6.32
Prostate cancers (*n* = 24)												
>2 Years	7	0.76	0.29–2.02	7	0.88	0.33–2.36	6	2.00	0.54–7.34	6	2.44	0.69–2.36
>5 Years	6	0.92	0.32–2.63	6	1.10	0.37–3.23	6	3.69	0.91–15.0	5	3.40	0.78–14.8

* Adjusted for time since first hired, pay type (salaried/hourly), external and internal radiation dose (continuous and lagged), and same chemical at different level.

findings for laryngeal and bladder cancers in the study presented here are supported by previous research investigating the effects of cutting fluid. The association between cutting fluids and brain cancer mortality suggests a possible contamination of fluids with nitrosamines, which have previously been linked to cancers of these organ systems.[18] However, the observed increase in mortality from blood and lymph system cancers in workers exposed to cutting fluids is not easily explainable by one of the more prevalent carcinogenic cutting-oil contaminants. Other comparable work environments for which increased rates of blood and lymph system cancers among workers have been reported are petroleum and chemical plants.[19,20]

Heavy cutting-fluid exposure at Fernald was attributed solely to workers with two job titles: machine tool operators, who used lathes and grinders for the machining of uranium metal pieces, and laboratory technicians, who performed test machining of uranium metal. No other chemical exposures at more than light levels were reported for these workers. Low and moderate cutting-fluid exposure occurred in toolmakers, welders, millwrights, helpers, degreasers, and some other jobs, in combination with exposure to many other chemicals at low or moderate levels.

Cutting fluids have previously been found to increase the rates of digestive-tract cancers such as cancers of the stomach,[17,21,22] rectum,[12,22] and pancreas.[12,21] Surprisingly, we did not find an association between digestive-tract cancers and cutting fluids in this cohort as expected in the case of nitrosamine contamination of cutting fluids; rather, all digestive-tract cancers in this cohort were associated with kerosene/solvent-mixture exposures. Kerosene was used in the chemical refining process of uranyl nitrate that involved the use of an organic solvent mixture which included tributyl phosphates and kerosene. The group of workers who experienced exposures to the kerosene/solvent mixture and to most respiratory irritants used at the facility and, in addition, to carbon at moderate levels were called chemical operators. However, this group of moderately exposed workers was exposed to neither TCE nor to cutting fluids. Thus the effects observed for prostate and digestive-tract cancers in this cohort can be attributed to the combination of kerosene, carbon, and other non-TCE solvent exposures. The absence of lung cancer effects for this chemical mixture suggests that exposure might have involved the inhalation of aerosolized particles of nonrespirable size that were trapped in the upper respiratory tract and consequently swallowed. This reasoning is also supported by the fact that kerosene/solvent mixture exposures increased the risk of cancers along the entire gastrointestinal tract, ie, from the oral cavity to the rectum.

The results of our external comparisons suggest that the Fernald cohort of uranium-processing workers is healthier than the white male US population, and thus exhibits the well-known "healthy worker effect." A deficit is also apparent when other worker NIOSH-cohorts[3] replace the US as the reference population in the calculation of expected mortality rates. This deficit is primarily due to

TABLE 7

Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Effects of Chemical Exposure Cancer Mortality, by Cancer Site and Level, 0 Years Lag for Exposures: Results From Conditional Logistic Regression Analyses Matched on Time and Age (±3 Years)*

	Lung Cancers (n = 112)		Hemato- and Lymphopoietic Cancers (n = 37)		Esophagus and Stomach Cancers (n = 24)		Colon and Rectum (n = 33)		Pancreatic Cancers (n = 18)		Brain Cancers (n = 12)		Prostate Cancer (n = 24)		Bladder and Kidney Cancers (n = 13)	
Exposure (in years)	Case n	RR (95% CI)	Case n	RR (95% CI)	Case n	RR (95% CI)	Case n	RR (95% CI)	Case n	RR (95% CI)	Case n	RR (95% CI)	Case n	RR (95% CI)	Case n	RR (95% CI)
TCE (level 1)																
<2	68	1	19	1	9	1	21	1	8	1	6	1	14	1	6	1
2–10	22	0.72 (0.44–1.16)	8	0.98 (0.42–2.26)	9	2.34 (0.91–6.02)	5	0.54 (0.20–1.46)	6	2.11 (0.79–5.66)	5	1.80 (0.49–6.67)	4	0.68 (0.22–2.08)	5	1.94 (0.59–6.44)
>10	22	0.77 (0.47–1.29)	10	2.17 (0.88–5.33)	6	2.09 (0.66–6.67)	7	0.85 (0.33–2.16)	2	0.57 (0.12–2.85)	1	0.94 (0.09–9.36)	6	0.82 (0.29–2.32)	2	0.76 (0.14–4.00)
TCE (level 2)																
<2	110	1	36	1	24	1	33	1	17	1	11	1	23	1	13	1
2–10	2	4.19 (0.51–34.2)	1	1.67 (0.22–12.4)	0	—	0	—	1	3.54 (0.45–27.9)	1	4.20 (0.51–34.2)	0	—	0	—
>10	0	—	0	—	0	—	0	—	0	—	0	—	1	2.15 (0.28–16.6)	0	—
Cutting fluids (levels 1, 2)																
>2	80	1	21	1	13	1	22	1	9	1	5	1	17	1	8	1
2–10	14	0.61 (0.34–1.08)	6	1.11 (0.44–2.84)	7	1.85 (0.71–4.83)	4	0.62 (0.21–1.83)	7	2.83 (1.02–7.88)	6	4.85 (1.33–17.7)	2	0.38 (0.09–1.65)	3	1.32 (0.34–5.17)
>10	18	0.75 (0.44–1.29)	10	3.31 (1.32–8.28)	4	1.27 (0.37–4.35)	7	1.18 (0.46–3.01)	2	0.76 (0.16–3.84)	1	2.00 (0.19–21.1)	5	0.68 (0.23–2.02)	2	0.80 (0.15–4.07)
Cutting fluids (level 3)																
<2	105	1	33	1	23	1	31	1	17	1	11	1	24	1	10	1
2–10	4	0.85 (0.31–2.34)	2	1.60 (0.37–6.92)	1	1.49 (0.19–11.5)	1	0.84 (0.11–6.32)	1	1.23 (0.16–9.46)	1	2.08 (0.23–18.4)	0	—	2	4.56 (0.94–22.1)
>10	3	1.45 (0.45–4.64)	2	5.72 (1.18–27.8)	0	—	1	1.37 (0.25–15.4)	0	—	0	—	0	—	1	5.70 (0.63–52.0)
Kerosene (level 1)																
<2	84	1	23	1	14	1	23	1	9	1	6	1	17	1	10	1
2–10	13	0.67 (0.37–1.22)	5	1.02 (0.37–2.78)	6	1.77 (0.65–4.87)	4	0.81 (0.27–2.41)	7	3.62 (1.29–10.2)	5	5.38 (1.44–20.2)	2	0.46 (0.10–2.02)	1	0.43 (0.05–3.53)
>10	15	0.73 (0.41–1.30)	9	3.07 (1.22–7.71)	4	1.37 (0.40–4.67)	6	1.18 (0.44–3.16)	2	0.97 (0.20–4.78)	1	2.44 (0.23–26.0)	5	0.82 (0.26–2.43)	2	0.77 (0.15–3.85)
Kerosene (level 2)																
<2	86	1	33	1	19	1	25	1	14	1	12	1	18	1	10	1
2–10	8	0.87 (0.42–1.83)	3	0.97 (0.28–3.37)	2	1.85 (0.39–8.78)	4	2.01 (0.66–6.11)	2	1.40 (0.30–6.59)	0	—	1	1.02 (0.13–8.18)	2	1.83 (0.38–8.79)
>10	18	1.35 (0.65–2.79)	1	0.24 (0.02–2.52)	3	3.66 (0.59–22.8)	4	1.33 (0.31–5.65)	2	1.14 (0.15–8.66)	0	—	5	4.29 (0.89–20.5)	1	0.52 (0.04–7.64)

* Adjusted for time since first hired, pay type (salaried/hourly), external radiation dose (continuous), and internal radiation dose (continuous). Note that no other chemical exposures were included in these models.

TABLE 8
Smoking Prevalence for a Subgroup of 757 Fernald White Male Workers, by Chemical Exposure Level*

	TCE			
Exposure Level	No. (%) Smokers	No. (%) Ex-Smokers	No. (%) Non-Smokers	Total (%)
0	75 (43.4)	37 (21.4)	61 (35.3)	173 (100)
1	236 (43.7)	135 (25.0)	169 (31.3)	540 (100)
2	24 (54.6)	11 (25.0)	9 (20.5)	44 (100)
Total	335 (44.3)	183 (24.2)	239 (31.6)	757 (100)
	Cutting Fluids			
Exposure Level	No. (%) Smokers	No. (%) Ex-Smokers	No. (%) Non-Smokers	Total (%)
0	103 (41.2)	56 (22.4)	91 (36.4)	250 (100)
1	162 (42.1)	98 (25.5)	125 (32.5)	385 (100)
2	20 (66.7)	5 (16.7)	5 (16.7)	30 (100)
3	50 (54.4)	24 (26.1)	18 (19.6)	92 (100)
Total	335 (44.3)	183 (24.2)	239 (31.6)	757 (100)
	Kerosene			
Exposure Level	No. (%) Smokers	No. (%) Ex-Smokers	No. (%) Non-Smokers	Total (%)
0	85 (49.4)	39 (22.7)	48 (27.9)	172 (100)
1	149 (40.8)	96 (26.3)	120 (32.9)	365 (100)
2	101 (45.9)	48 (21.8)	71 (32.3)	220 (100)
Total	335 (44.3)	183 (24.2)	239 (31.6)	757 (100)

* In 1965, 51.3% of the US white male population over the age of 20 were cigarette smokers.[23]

reduced rates of death from cardiovascular diseases. Fernald workers, however, died at higher rates from many cancers, when compared with either reference population. Furthermore, they were less likely to smoke than the US population (according to the Surgeon General,[23] during the 1960s, 51.3% of the white male US population over the age of 20 were cigarette smokers), which might partially explain the observed reduction in cardiovascular disease mortality. Because the Fernald cohort is, in general, healthier and engages in behavior that is less likely to cause many cancers, the observation of increased cancer rates points to causative agents in the work environment.

As mentioned previously, exposure to respiratory irritants was very common at this facility, prompting Wilson to conduct an exposure assessment and to examine nonmalignant respiratory morbidity.[2] He was, however, unable to find any association between the chemicals studied (ammonia, carbon, cutting fluids, kerosene, lime, nitric acid, sodium hydroxide, tributyl phosphate, TCE) and the occurrence of nonmalignant respiratory diseases. As listed in Table 3, significantly fewer Fernald workers died of respiratory diseases or emphysema, compared with the US population. Possible explanations for the lack of an increase in respiratory morbidity for workers heavily exposed to respiratory irritants could be either that the entire cohort represents a highly selected group of healthy workers or that the most heavily exposed workers were the healthiest. A selection of healthier workers into exposed jobs could also lead to a bias toward the null for the internal comparisons of cancer mortality performed in this study.

Table 8 shows that there are no clear patterns of association between smoking and general levels of chemical exposure, making it unlikely that smoking is an explanation for the observed relationships between chemicals and cancer mortality. Yet the comparisons in Table 8 represent a rather crude picture, because we did not base our conclusions for chemical-exposure effects on a simple comparison of exposure levels but rather on time-dependent and lagged-duration measures. If the effects observed for chemicals were really due to differences in smoking, one would expect to see an association with lung cancers, the most strongly smoking-related cancer. We found no association of any chemical exposure in this cohort with lung-cancer mortality, after adjustment for radiation effects.

Because chemical-exposure data in the CEDR was only available for the period 1952 to 1977, we lack exposure information for the remaining 12 years of the plant's operation (1978 to 1989). Yet this lack of data could be compared with a lack of postemployment exposure history. The resulting exposure misclassification would most likely be nondifferential with respect to case status and thus expected to bias our results toward the null.

Our results for the two rare cancers of the liver and larynx are quite strong and are corroborated by results from previous studies that linked TCE exposure to liver cancers and cutting-fluids exposure with laryngeal cancers. Furthermore, cutting-fluid exposure in this cohort was associated with brain cancers, hemato- and lymphopoietic cancers, and bladder and kidney cancers, whereas kerosene exposures increased the chances of death from all digestive-tract cancers and from prostate cancers. Causal inference is strengthened by the fact that the effect estimates for all reported associations increased with duration and level of exposure, were observed to be stronger when exposure was lagged, are biologically plausible, and/or have previously been observed among workers who experienced similar chemical exposures.

Acknowledgment

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Fat Cities

. . .Stay away from New Orleans; Columbus, Ohio; and Milwaukee. These cities have the highest rate of overweight men and, not surprisingly, the largest number of fast-food burger chains, *Men's Fitness* magazine reports. Not for nothing is Milwaukee's major league baseball team named the Brewers: Wisconsin has the highest per capita consumption of alcohol of any state.

—From Schogol M. Food Watch. *Philadelphia Inquirer*, January 6, 1999, p F2.

Radiation Exposure and Cancer Mortality in Uranium Processing Workers

Beate Ritz[1,2]

Data from the Comprehensive Epidemiology Data Resource (CEDR) allowed me to study patterns of cancer mortality in a cohort of 4,014 uranium-processing workers. Employing risk-set analysis for cohort data, I estimated the effects of external (gamma) and internal (alpha) radiation on cancer mortality. My results indicate that Fernald workers exposed to ionizing radiation experienced an increase in mortality from total cancer (per 100 mSv external dose rate ratio (RR) = 1.92; 95% confidence interval (CI) = 1.11–3.32), radiosensitive solid cancer (RR = 2.00; 95% CI = 1.02–3.94), and lung cancer (RR = 2.77; 95% CI = 1.29–5.95). Effects were strongest when exposure had occurred at older ages (>40 years). In addition, I observed an increase in lung-cancer mortality for workers exposed to ≥200 mSv of internal (alpha) radiation (RR = 1.92; 95% CI = 0.53–6.96). Furthermore, my results demonstrate the importance of a long follow-up time when studying solid cancers, the potential for bias due to worker selection associated with concomitant chemical exposures, problems of exposure measurement, confounding, and effect modification due to age at exposure. Owing to lack of data, a previous pooled analysis of uranium-processing workers could only partially address these issues. (Epidemiology 1999;10:531–538)

Keywords: exposure age, cancer, chemicals, ionizing radiation, occupational cohort study, radionuclides, selection bias.

For decades, scientists have been debating to what extent chronic, low-dose exposure to ionizing radiation might cause cancer in nuclear workers.[1] Results from some nuclear-worker studies have raised the possibility that risk estimates for cancer extrapolated from A-bomb-survivor data might underestimate the carcinogenic effect of external, low-dose radiation exposure,[2] while other studies suggest that such radiation has caused no cancer other than leukemias.[3] In any event, the effect size is expected to be small, and random fluctuation is an issue that needs to be addressed. One increasingly common approach to this problem has been to pool data from several worker studies, thus increasing statistical power and limiting random fluctuation.[3,4]

The studies of nuclear cohorts to date have varied with respect to dose rates, exposure lag, and duration of follow-up. In addition, errors in measuring exposures or outcomes, healthy worker selection biases, residual confounding due to unmeasured factors such as smoking and chemical exposures, and different distributions of effect modifiers have likely contributed to inconsistencies across studies. Because data regarding such factors are variably available from study to study, pooled estimates of effects averaged across studies are necessarily limited by the assumption that the combined worker cohorts do not differ substantially. Heterogeneity across study populations, however, is not merely a nuisance obstructing efforts to derive average radiation-effect estimates. Rather, examining the special features of each nuclear-worker cohort may reveal information obscured by a common estimator.[5]

Here I evaluate such special features in a cohort of nuclear workers employed at the Fernald Feed Materials Production Center (FFMPC) in Ohio. Compared with other nuclear workforces monitored for external radiation exposures, the Fernald cohort is small (N = 4,014); yet it is one of the largest monitored for both external and internal exposures and has the advantage of an extremely long follow-up time (mean of 30.9 years). Fernald workers were primarily engaged in processing uranium-ore concentrate and uranium of low-grade enrichment into fabricated uranium metal products, resulting in exposure to radiation and some potentially carcinogenic chemicals.[6]

Selected members of the Fernald cohort were included in an earlier case-control study pooling lung-cancer cases from four uranium-processing facilities.[4] More extensive data for Fernald have recently become available through the Comprehensive Epidemiology Data Resource (CEDR), an archive supported by the Department of Energy to provide de-identified data from

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previous studies of nuclear workers. Using the CEDR data base[6] exclusively, I will: (1) expand analysis to include mortality from cancers other than the lung, (2) add 7 years of follow-up, (3) evaluate the potential for biases due to concomitant chemical exposures and worker-selection processes, (4) examine whether age at exposure influences the effect of external radiation dose, and (5) estimate the effect of internal combined with external radiation exposures.

Methods

Study Population/Outcome Assessment

The study population comprises 4,014 white male workers employed between 1951, when Fernald opened, and 1989, when operations halted. About 85% of cohort members were hired before 1960. The facility continuously monitored workers for radiation exposures, and its epidemiologic surveillance program collected information on vital status. Vital status searches, conducted through January 1, 1990, relied upon two record systems: Social Security Administration (SSA) files for the period before 1979 and the National Death Index (NDI) for the period 1979–1989. I assumed that workers were alive at the end of follow-up if not identified as dead by CEDR. Death certificate information was available for 99% of 1,064 deceased workers.

Exposure Assessment

External radiation exposure was reported as annual penetrating doses, derived from film-badge measurements, and internal radiation exposure as annual lung doses, based on a combination of individual urine bioassays and environmental area sampling.

Exposure to internal radiation emitters involved airborne long-lived radioactive materials such as uranium, thorium, and radium compounds. Most of the exposures were due to U^{235}, varying from depleted to slightly enriched (less than 1% of U^{235}), and characterized mainly as insoluble compounds; thorium was used in small amounts. Workers stationed in areas in which industrial hygiene surveys indicated significant potential for internal radiation exposures were routinely monitored for uranium content in urine, and additional samples were taken when a significant intake of radioactive material was suspected. For estimation of annual individual doses, the data derived from urinalyses were complemented by measurements of uranium dust collected in area air filters.

Estimating lung doses from urine measures for radionuclides requires formulation of an uptake/retention/excretion model based on the biological half-life and chemical/physical properties of the radioactive particles.[7,8] The description of exposure assessment provided by CEDR[6] and Wilson[9] give no detail about either the model used to estimate lung-dose equivalents from urine measures or the relative contributions of individual urinalyses vs area air samples to the overall lung doses.

Nevertheless, it is known that urinalyses are primarily an indicator of the amount of soluble uranium that has been incorporated through the lungs and transported by the blood to the kidneys for excretion. Insoluble uranium compounds, however, are most likely either retained in the lungs, deposited in tracheobronchial or other thoracic lymph nodes, or swallowed.[10] Urinary bioassay monitoring at Fernald, thus, may not adequately reflect the amount of internal radiation dose for insoluble radioactive particles or those of non-respirable size. Environmental sampling, on the other hand, while sensitive to all particle types, obscures differences in individual exposures among workers in the same area. Therefore, the reported doses for internal exposures can be considered no more than crude indicators of relative levels of exposure among Fernald workers.

Covariate Information

Processing of uranium involved exposure to a large number of non-radioactive but potentially carcinogenic chemical compounds, including solvents and cutting fluids. The CEDR provided sufficient data to allow us to control for exposure to trichloroethylene (TCE) and cutting fluids for 3,814 (95%) of our cohort. Plant experts had ranked job titles and work sites according to their potential for chemical exposure, and workers were classified into four exposure categories, from "none" to "heavy," for each chemical.[9] We considered the 5% of the cohort for which chemical data were unavailable as unexposed.

The CEDR reported smoking histories for workers who were continuously employed for at least 3 months between November 1967 and March 1973 (17% of our cohort). Although this sample was too limited to allow us to adjust for smoking in our analyses, it did provide enough information to assess the relation between radiation dose and smoking behavior.

CEDR data on salary status provided us with a surrogate measure of socio-economic status.

Statistical Analysis

For external comparisons with the general U.S. white male population, we used the life-table program developed by Monson[11] to estimate standardized mortality ratios (SMRs = observed/expected deaths) and Byar's formula to derive confidence limits.[12]

For dose-response analyses, there were rarely sufficient deaths to conduct informative analyses by specific cancer site. Accordingly, for external radiation assessments, we restricted the outcomes examined to deaths from all cancers, from lung cancer (International Classification of Diseases, 9th revision (ICD-9)[13] 162), and from two *a priori* groups of cancers classified as radiation sensitive by BEIR V criteria.[1] The first group comprised hematopoietic and lymphopoietic cancers (ICD-9 200–208), excluding chronic lymphatic leukemias (CLL); the second combined all solid cancers identified as radiosensitive, including cancers of the lung (ICD-9 162), esophagus

brain (ICD-9 191–192), and urinary-tract system (ICD-9 188–189). Bone and thyroid cancers were not observed. I excluded one, extremely rare, male breast cancer because I could not access the original data to verify the diagnosis.

In estimating the effects of internal alpha radiation, I focused on those organ systems through which radioactive particles pass from intake to excretion. Of primary interest was the respiratory system (ICD-9 160–162) as the entry organ for uranium dusts.[14] The naso-oropharyngeal regions, esophagus, and stomach—grouped together as "upper gastrointestinal tract" (ICD-9 140–151)—may also be irradiated, mainly by larger, non-respirable-sized and insoluble particles.[15] Lower gastrointestinal-tract organs such as colon and rectum (ICD-9 153–154) might also become exposed, although to a more limited extent. In addition, transport systems, specifically the hematopoietic and lymphopoietic systems (ICD-9 200–208, excluding CLL) are likely to be affected, as are exit organs of the urinary tract (ICD-9 188–189)[15]; moreover, uranium can have toxic effects on the kidneys. Radioactive materials can be stored in the liver and bones; however, I observed no bone and only three primary liver cancers, too few to allow meaningful dose-response analysis.

For all dose-response comparisons, I employed the risk-set approach for cohort analysis described by Breslow and Day,[12] providing estimates equivalent to those obtained from a Cox-proportional hazard model.[15] I constructed risk sets by matching to each cancer death all cohort members who were still alive at the calendar time of the index subject's death (on average 3,300 survivors per death). For some analyses, risk sets were also matched on age at death of the case (±2.5 years).

I modeled cumulative radiation dose both as a set of categorical and as continuous variables (in mSv). Using cutpoints established in previous studies, I categorized dose equivalents for external radiation into <10 mSv, 10-<20 mSv, 20-<100 mSv, and ≥100 mSv. I chose categories of <10 mSv, 10-<50 mSv, 50-<100 mSv, 100-<200 mSv, and ≥200 mSv for internal dose because most workers received higher doses and the exposure distribution was less skewed (see below). To allow for a period of cancer induction/latency after exposure to radiation, and to reduce possible selection bias,[16] I lagged cumulative doses by 0, 10, and 15 years limiting cumulative dose to the level achieved x years before the index death. I treated radiation doses and all time-varying factors as time dependent.

I used results of conditional logistic regression analyses to estimate rate ratios (RR) and 95% confidence intervals (CI). All models adjusted for the same factors chosen according to the Greenland[17] criterion: pay type (salaried vs hourly), time since first monitoring, age at risk (continuous), and internal or external radiation dose (continuous). Some models, in addition, included exposure to TCE and/or cutting fluids defined

TABLE 1. Characteristics of the White Male Fernald Cohort

Number of employees	4,014
Average follow-up time (years)	30.9
Average age at entry into cohort (years)	30.4
Number of person-years	124,177
Number of deaths	1,064
Total mortality rate (per 10^5/yr)	857
Total cancer mortality rate (per 10^5/yr)	267
Pay category	
Salaried	1,311
Hourly/union	2,703

Radiation Dose (mSv)	External	%	Internal	%
0–<10	2,764	68.9	1,111	27.7
10–<50	886	22.1	1,573	39.2
50–<100	259	6.4	999	24.9
100–<200	97	2.4	309	7.7
≥200	8	0.2	22	0.5
Total	4,014		4,014	

Results

A long follow-up period (average 30.9 years) and a high percentage of hourly employees (67.3%) characterize the Fernald cohort. Internal exposure from radionuclides was responsible for the bulk of the radiation doses recorded, with only 27.7% of the workers registering a cumulative dose less than 10 mSv. In contrast, most monitored workers (68.9%) received cumulative external radiation doses of less than 10 mSv, only 2.6% had doses in excess of 100 mSv, and none exceeded 300 mSv (Table 1).

Mortality rates from all causes were lower among Fernald workers than among U.S. white males; the deficit was mostly attributable to a low cardiovascular-disease mortality rate (SMR = 0.78; 95% CI = 0.71–0.86; Table 2). Rates for all malignant neoplasms, however, were slightly increased in workers (SMR = 1.09; 95% CI = 0.98–1.22; Table 2). SMRs were also slightly increased for cancers of the prostate, brain, bladder, the hematopoietic and lymphopoietic systems, and most of the digestive system.

External radiation doses 100 mSv and above increased mortality from all cancers, all radiosensitive solid cancers, and lung cancers, but the small number of deaths in this dose category limited the precision of my estimates (Table 3). Models treating external dose as continuous variable demonstrated dose-related increases in mortality for all of these cancers, especially after lagging exposures by more than 10 years and adjusting for internal dose, cutting fluids, and TCE. Findings for hematopoietic and lymphopoietic cancers were based on too few cases to be conclusive.

The effects of external radiation doses were greatest among workers exposed after the age of 40 (Table 4). Thus, while mortality increased only marginally with exposure before age 40, later exposures, when lagged 15 years, increased mortality by two- to threefold per 100

TABLE 2. Observed (OBS), and Expected (EXP) Numbers of Deaths for White Male Subjects and Estimated Standardized Mortality Ratio (SMR): Comparison with the U.S. Population, by Cause of Death*

Causes of Death	OBS No.	EXP No.	SMR	95% CI
All causes (ICDA 001–998)	1064	1264.7	0.84	0.79–0.89
All cancers (ICDA 140–229)	332	303.6	1.09	0.98–1.22
Cancer sites				
Buccal cavity and pharynx (ICDA 140–149)	9	8.75	1.03	0.47–1.95
Digestive organs and peritoneum (ICDA 150–159)	87	74.86	1.16	0.93–1.43
Esophagus (ICDA 150)	9	7.48	1.20	0.55–2.28
Stomach (ICDA 151)	15	11.18	1.34	0.75–2.21
Large intestines (ICDA 153)	28	26.60	1.05	0.70–1.52
Rectum (ICDA 154)	7	6.68	1.05	0.42–2.16
Liver (ICDA 155–156)	8	4.93	1.62	0.70–3.20
Pancreas (ICDA 157)	18	15.53	1.17	0.69–1.85
Respiratory system (ICDA 160–163)	120	116.33	1.03	0.86–1.23
Larynx (ICDA 161)	5	4.24	1.18	0.38–2.76
Lung-primary and secondary (ICDA 162)	112	111.03	1.01	0.83–1.21
Bone (ICDA 170)	0	0.99	0.00	0.00–3.70
Skin (ICDA 172–173)	4	6.45	0.62	0.17–1.59
Prostate (ICDA 185)	25	17.42	1.44	0.93–2.12
Testis (ICDA 186–187)	1	1.46	0.67	0.01–3.74
Bladder (ICDA 188)	8	6.95	1.15	0.50–2.27
Kidney (ICDA 189)	5	7.89	0.63	0.20–1.46
Eye (ICDA 190)	0	0.21	0.00	0.00–17.30
Brain and other central nervous systems (ICDA 191–192)	12	9.66	1.24	0.64–2.17
Thyroid (ICDA 193)	0	0.54	0.00	0.00–6.76
Lymphosarcoma and reticulosarcoma (ICDA 200)	8	4.79	1.67	0.72–3.29
Hodgkin's disease (ICDA 201)	6	2.95	2.04	0.74–4.43
Leukemia and aleukemia (ICDA 204–207)	13	11.21	1.16	0.62–1.98
Lymphatic tissue (ICDA 202–203, 208)	10	9.94	1.01	0.48–1.85
Lymphopoietic cancer (ICDA 200–208)	38	29.50	1.29	0.91–1.77
Cancer residual†	23	22.59	1.02	
Other causes				
Benign neoplasms (ICDA 210)	1	3.47	0.29	0.00–1.60
Diseases of blood and blood-forming organs (ICDA 280–89)	2	2.65	0.75	0.08–2.72
All diseases of circulatory system (ICDA 390–458)	460	588.83	0.78	0.71–0.86
Arteriosclerotic heart disease, including CHD (ICDA 410–14)	339	425.62	0.80	0.71–0.89
All vascular lesions of CNS (ICDA 430–438)	48	59.49	0.81	0.59–1.07
All respiratory diseases (ICDA 460–519)	53	79.78	0.66	0.50–0.87
Emphysema (ICDA 492)	3	14.52	0.21	0.04–0.60
All diseases of digestive system (ICDA 520–577)	49	64.33	0.76	0.56–1.01
Cirrhosis of liver (ICDA 571)	34	36.92	0.92	0.64–1.29
All diseases of genito-urinary system (ICDA 580–629)	3	14.25	0.21	0.04–1.29
All external causes of death (ICDA 800–998)	112	126.92	0.88	0.73–1.06
Suicide (ICDA 950–959)	24	31.46	0.76	0.49–1.14
Total residual‡	15	2.68	5.60	

* According to ICDA-8.
† Cancers of unspecified site.
‡ Including undetermined causes of death and missing causes of death due to missing death certificates.

Internal radiation doses ≥200 mSv, lagged by 15 years, appeared to increase by twofold the rate of mortality from respiratory cancers (all cases were lung cancers) (Table 5). Also in models with a 15-year lag, there were slight increases in mortality from bladder and kidney cancers at doses of 50 to <200 mSv and from upper gastrointestinal-tract cancers at doses of 10 to <100 mSv, with no case occurring at the highest dose levels. I found no indication of any effect of internal radiation on cancers of the lower gastrointestinal tract or the hematopoietic and lymphopoietic system. In all of the internal exposure analyses, the 95% CIs were wide (Table 5). The effects did not change after adjustment for cutting fluid and TCE exposures (not shown).

An analysis of the combined effects of external and internal radiation on lung-cancer mortality showed a strong effect of internal doses at levels ≥200 mSv when external radiation doses exceeded 50 mSv (Table 6). radiation doses in excess of 50 mSv with lower doses of internal radiation.

Discussion

These results suggest that in this cohort of uranium workers, the rate of death from all cancers, total radiosensitive solid cancers, and lung cancers increased with increasing external (gamma) radiation dose. The effects were most pronounced when workers were exposed at older ages (>40 years) and when radiation doses were lagged by more than 10 years. Lung-cancer mortality also appeared to be elevated in workers exposed to more than 200 mSv of internal alpha radiation. The estimates for the other possible effects of alpha radiation were too imprecise for firm inference.

In an earlier case-control study that pooled 787 lung-cancer deaths from four uranium-processing facilities, including 51 cases from Fernald, Dupree *et al*[1] found an

TABLE 3. Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Effect of Cumulative External Radiation Dose on Cancer Mortality, by Cancer Type and by Exposure Lag, Controlling for Various Covariates: Results of Conditional Logistic Regression Analyses of Risk Sets

External Radiation Dose Categories (in mSv)*	All Cancers			All Radiosensitive Cancers†			Lung Cancers‡			Hematopoietic and Lymphopoietic Cancers§		
	Case N	RR	95% CI	Case N	RR	95% CI	Case N	RR	95% CI	Case N	RR	95% CI
<10	206	1.00		117	1.00		64	1.00		27	1.00	
10–<20	26	0.87	0.57–1.34	10	0.52	0.27–1.00	5	0.43	0.17–1.10	1	0.36	0.05–2.73
20–<100	88	1.19	0.88–1.62	52	1.02	0.69–1.51	35	1.10	0.68–1.79	8	1.37	0.50–3.77
≥100	12	1.43	0.74–2.76	10	1.56	0.74–3.28	8	1.78	0.75–4.24	1	2.08	0.22–20.0
Total Case N	332			189			112			37		
Continuous dose (100 mSv increments)												
0-Year lag												
Without internal dose		1.29	0.91–1.83		1.49	0.96–2.27		1.72	1.05–2.81		0.94	0.26–3.41
Internal dose continuous		1.55	1.04–2.32		1.56	0.95–2.56		1.60	0.90–2.85		1.86	0.43–8.02
Internal dose categorical¶		1.83	1.23–2.71		1.90	1.17–3.07		2.13	1.21–3.76		2.09	0.49–8.83
Internal dose (cat.) plus chemical exposure		1.88	1.27–2.79		1.94	1.19–3.14		2.02	1.13–3.62		2.28	0.53–9.73
10-Year lag												
Internal dose continuous		1.49	0.93–2.40		1.60	0.90–2.83		1.70	0.88–3.29		1.92	0.36–10.2
Internal dose categorical¶		1.75	1.10–2.79		1.86	1.05–3.29		2.28	1.17–4.41		1.90	0.37–9.87
Internal dose (cat.) plus chemical exposure		1.79	1.12–2.86		1.88	1.06–3.32		2.13	1.08–4.18		2.13	0.40–11.2
15-Year lag												
Internal dose continuous		1.66	0.96–2.89		1.80	0.92–3.52		2.21	1.04–4.69		2.08	0.31–13.9
Internal dose categorical¶		1.88	1.09–3.24		1.99	1.02–3.90		2.91	1.37–6.18		1.97	0.30–13.0
Internal dose (cat.) plus chemical exposure		1.92	1.11–3.32		2.00	1.02–3.94		2.77	1.29–5.95		2.25	0.33–15.2

* Adjusted for age at failure time, time since first monitored, pay type (salaried/hourly), internal dose, employing a 0-year lag for external dose.
† ICD-9 150, 151, 153, 162, 188, 189, 191, 192.
‡ ICD-9 162. Note: lung cancers are a subgroup of the radiosensitive cancers.
§ ICD-9 200–208, excluding chronic lymphatic leukemia.
‖ Referent category.
¶ Same categories as presented in Table 5.

those workers exposed to >50 mSv of external radiation and hired (hence exposed) after the age of 45 years. These authors found no effect for internal doses less than 250 mSv, but a slight increase in mortality at higher doses (OR = 2.05; 95% CI = 0.20–20.70). All results were based on too few cases to be conclusive, however.

The present study's focus on the Fernald workforce alone was made possible in part by the greater statistical efficiency of complete-cohort analysis compared with the 1:1 matched case-control design, as well as by the additional cases (for example, 61 new lung-cancer deaths) accumulated during the extra 7 years of follow-

TABLE 4. Adjusted Rate Ratio (RR) Estmates (and 95% CI) for the Effect of 100 mSv Cumulative External Radiation, by Age at Exposure and Cancer Sites: Results from Conditional Logistic Regression Models for Continuous Cumulative Doses within Age Categories, for 0- and 15-Year Lags, Matched for Calendar Time and Age at Failure Time (±2.5 years)*

Outcome	No. of Cancer Deaths	Total†		Age at Exposure			
				15–39		>40	
		RR	95% CI	RR	95% CI	RR	95% CI
All cancers	332						
0-Year lag		1.51	1.01–2.27	1.03	0.42–2.57	1.37	0.92–2.04
15-Year lag		1.68	0.97–2.91	1.05	0.37–2.99	1.90	1.03–3.50
Radiosensitive solid cancers‡	189						
0-Year lag		1.47	0.90–2.41	1.63	0.57–4.64	1.41	0.86–2.33
15-Year lag		1.74	0.89–3.40	1.59	0.49–5.13	2.07	0.96–4.50
Lung cancers§	112						
0-Year lag		1.46	0.82–2.59	1.63	0.44–6.01	1.62	0.91–2.86
15-Year lag		1.97	0.93–4.16	1.24	0.27–5.65	2.88	1.29–6.44
Hematopoietic and lymphopoietic cancers‖	37						
0-Year lag		1.88	0.40–8.17	0.16	0.00–16.7	1.28	0.31–5.17
15-Year lag		2.02	0.29–14.0	0.02	0.00–59.5	2.55	0.42–15.4

* Adjusted for pay type (salaried/hourly), internal radiation dose, time since first monitored.
† This is the estimate for cumulative external dose (continuous) for all ages at exposure.
‡ ICD-9 150, 151, 153, 162, 188, 189, 191, 192.
§ ICD-9 162.

TABLE 5. Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Effect of Cumulative Internal Radiation Dose on Cancer Mortality, by Cancer Sites, for 0- and 15-Year Lags for Exposure: Results of Conditional Logistic Regression Analyses*

Internal Exposure (mSv)	Respiratory Tract Cancers†			Upper gastrointestinal tract cancers‡			Lower gastrointestinal tract cancers§			Bladder and kidney cancers‖			Hematopoietic and Lymphopoietic Cancers¶		
	Cases N	RR	95% CI	Cases N	RR	95% CI	Cases N	RR	95% CI	Cases N	RR	95% CI	Cases N	RR	95% CI
0-Year lag															
<10**	23	1.00		7	1.00		9	1.00		2	1.00		7	1.00	
10–<50	37	0.66	0.39–1.13	12	0.85	0.32–2.24	12	0.54	0.23–1.30	3	0.81	0.12–5.40	19	1.60	0.61–4.19
50–<100	41	0.63	0.35–1.12	12	0.97	0.33–2.90	11	0.37	0.14–0.99	6	1.91	0.28–13.2	10	0.92	0.28–3.06
100–<200	14	0.46	0.20–1.03	2	0.44	0.07–2.79	2	0.14	0.02–0.79	2	1.83	0.16–21.6	1	0.21	0.02–2.32
≥200	3	1.18	0.32–4.41	0			1	0.81	0.08–8.20	0			0		
	118			33			35			13			37		
15-Year lag															
<10**	39	1.00		10	1.00		10	1.00		2	1.00		14	1.00	
10–<50	36	0.51	0.31–0.86	15	1.65	0.57–4.82	17	1.18	0.45–3.12	4	1.04	0.16–6.75	14	0.96	0.37–2.52
50–<100	31	0.51	0.28–0.95	8	1.68	0.43–6.52	5	0.51	0.14–1.93	5	2.12	0.27–16.4	8	0.98	0.29–3.33
100–<200	9	0.48	0.20–1.19	0			3	1.28	0.25–6.43	2	3.96	0.35–44.6	1	0.56	0.06–5.40
≥200	3	1.92	0.53–6.96	0			0			0			0		
	118			33			35			13			37		

* Adjusted for age at failure time, time since first monitored, pay type (salaried/hourly), external radiation dose (continuous).
† ICD-9 160–162.
‡ ICD-9 140–151.
§ ICD-9 152–154.
‖ ICD-9 188–189.
¶ ICD-9 200–208, excluding chronic lymphatic leukemias.
** Referent category.

up. Thus, the increase in lung-cancer mortality associated with internal doses exceeding 200 mSv was based entirely on new Fernald cases occurring after 1982. This finding underscores the importance of the long follow-up times that Pierce *et al*[18] have suggested may be necessary for detecting effects of radiation on solid tumors, such as lung cancers, that normally develop late in life. Because lung-cancer incidence increases markedly after age 60, and Fernald workers were on average 30 years old at entry into the cohort, a 31-year follow-up allowed the average worker to reach the age of increased risk.

My access to cohort-wide data on external radiation exposures that were largely unavailable to Dupree *et al* allowed me to refine estimates of internal-dose effects by adjusting for external radiation doses. In addition, I observed dose-response effects of external radiation exposure on mortality not only from lung cancer, but also from all radiosensitive cancers and cancer as a whole. The demonstration that these effects were mostly due to exposures received after age 40 confirmed Dupree *et al*'s preliminary observation of an increase in lung-cancer mortality among workers exposed to over 50 mSv of external radiation and hired after the age of 45.

Although the largest pooled-cohort study to date found no effect of external radiation on cancers other than leukemias,[3] a number of single-cohort investigations have observed radiation-related increases in lung cancer. Among Oak Ridge Y-12 uranium-processing workers,[19] a trend of increasing lung-cancer mortality with increasing gamma radiation dose was most pronounced for those who also received more than 50 mSv

TABLE 6. Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Combined Effects of Cumulative Internal and External Radiation Dose on Lung Cancer Mortality, by Dose Level Assuming a 0- and 15-Year Lag for Both Exposures: Results from a Conditional Logistic Regression Analyses*

External dose (mSv)	Internal Dose (mSv) <100			Internal Dose (mSv) ≥100–<200			Internal Dose (mSv) ≥200		
	No. of Cancer Deaths	RR	95% CI	No. of Cancer Deaths	RR	95% CI	No. of Cancer Deaths	RR	95% CI
<50									
0-Year lag	84	1.00†		6	0.72	0.31–1.67	0		
	90	1.00†		6	1.24	0.53–2.89	0		
≥50									
15-Year lag	8	1.36	0.65–2.83	5	1.36	0.54–3.40	1	7.68	1.06–55.7
	9	2.03	1.00–4.14	2	1.28	0.31–5.32	2	18.0	4.32–74.9
0-Year lag	3	2.28	0.71–7.31	3	1.42	0.44–4.54	2	5.87	1.42–24.2
15-Year lag	1	1.53	0.21–1.17	1	2.01	0.28–14.7	1	17.7	2.36–133

* Adjusted for age at failure time, pay type (salaried/hourly), time since first monitored.

TABLE 7. Smoking Prevalence for Fernald Workers Who Were Employed on or after January 1, 1968, First Hired Between January 1, 1952 and December 31, 1972, by Cumulative Radiation Dose Level (mSv)

Radiation Dose Level (mSv)	Smokers		Ex-smokers		Nonsmokers		Total	
	N	%	N	%	N	%		%
External radiation								
<50	232	42.6	139	25.6	173	31.8	544	100
50–<100	69	50.0	29	21.0	40	29.0	138	100
≥100	34	45.3	15	20.0	26	34.7	75	100
Internal radiation								
<50	71	39.9	40	22.5	67	37.6	178	100
50–<100	180	44.9	99	24.7	122	30.4	401	100
≥100	84	47.2	44	24.7	50	28.1	178	100

of alpha radiation, much as the greatest effects at Fernald were associated with high doses of external and internal radiation in combination. In Rocketdyne workers, Ritz *et al*[20] found an increase in lung-cancer mortality with doses of external radiation above 200 mSv, but not with internal exposures—a difference that may reflect the much lower internal-dose levels at Rocketdyne than at either Oak Ridge or Fernald. In a cohort of British nuclear workers, Fraser *et al*[21] observed a slightly increased risk of lung-cancer mortality among those exposed to any radionuclide, compared with those with no internal radiation exposure. Finally, both U.S.[22] and British[23] studies of nuclear workers have linked alpha radiation exposure from plutonium and thorium to lung-cancer mortality.

Leukemias have been consistently linked to external radiation exposure.[3] The Fernald data suggested an increased risk of hematopoietic/lymphopoietic cancer at external doses ≥100 mSv; however, this result was based on only one lymphoma in the highest dose category, and the evidence associating external radiation with lymphomas is equivocal.[1,24]

The influence of age at exposure on the radiation effects at Fernald may have become apparent only because of the extended follow-up. My colleagues and I have previously described a similar pattern of greater mortality from lung cancer, all radiosensitive cancers, and total cancers among workers exposed at older ages to external radiation in the Rocketdyne cohort, which also had a long period of follow-up.[25] Studies of the Hanford[26,27] and Oak Ridge[2,28] nuclear cohorts have reported comparable effects of exposure age on overall cancer mortality.

As mentioned in Methods, internal-radiation dose measures, based on relatively inexact methods of estimation, are subject to considerably more uncertainty than those for external exposures. The type of measurement error at any facility depends heavily on monitoring practices and the types of radionuclides for which estimates are derived; in general, assessments of internal-dose measures probably suffer from exposure misclassification to a much greater degree than do external-dose analyses. Therefore, although estimates of internal doses are expressed in mSv, it may be more appropriate to consider them only as a relative ranking of internal exposure within a cohort, not suitable for comparison with either external-dose measures in the same cohort or with internal-dose levels reported at other facilities. Thus, the failure to detect effects of alpha radiation on the respiratory system at dose levels <200 mSv might reflect the properties of the mostly insoluble uranium of low enrichment grade processed at Fernald, but could also be a consequence of large (nondifferential) exposure misclassification at lower exposure levels, biasing my results toward the null.

Worker-selection bias may have contributed to some underestimation of radiation effects in the Fernald cohort. Uranium processing at the facility involved the use of large amounts of chemicals known to be respiratory irritants, including tributyl phosphate, ammonium hydroxide, sulfuric acid, and hydrogen fluoride, which were the focus of an earlier investigation of respiratory disease at Fernald.[9] I found that exposure to these chemicals was highly correlated with radiation dose; the 905 workers highly exposed to these chemicals received almost double the average radiation dose (internal and external) and worked on average 2 years longer than coworkers. This observation suggests that workers healthier and less responsive to the toxic and irritating effects of these substances may have selectively filled jobs entailing exposure to lung irritants and radiation. Although I do not know the extent to which baseline lung-cancer risk might be affected by such selection forces, these forces may well result in a net healthy worker effect for those workers exposed to radiation at the highest levels, which could bias all radiation results toward the null.

The sample of Fernald workers for whom I had smoking data had proportionately fewer smokers than the adult U.S. white male population[29] (Table 7), which may account for the cohort's reduced SMRs for cardiovascular disease, emphysema, and all respiratory diseases. Data from the sample suggested that workers exposed to higher levels of internal radiation might have been slightly more likely to smoke (Table 7), but the difference was not large enough to be likely to result in confounding.[30] I was unable to adjust for smoking in my analyses, but I may have adjusted indirectly for smoking behavior by adjusting for pay-type in the dose-response analyses, because, compared with hourly employees, salaried workers smoked less (32.8% *vs* 48.2% smokers) and had lower mortality rates from lung cancers (RR = 0.53; 95% CI = 0.32–0.90) and from all other causes

lated cancers together (RR = 0.54; 95% CI = 0.25–1.13 for oropharyngeal, laryngeal, esophageal, pancreatic, and bladder cancers combined). Matching on age at death and calendar time in these analyses might have also helped to match for smoking habits specific to different birth cohorts.

The results from this small cohort of radiation workers underscore the importance of conducting a comprehensive analysis of all data available for each worker population included in a pooled study to explore and understand the importance of issues related to length of follow-up, selection bias, potential confounding and worker selection associated with chemical exposures, problems of exposure measurement, and effect modification due to age at exposure. A previously conducted pooled analysis of uranium-processing workers could only partially address these issues, since the relevant data either did not exist or were available for only small subsets of all subjects included in the analyses.

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Radiation Exposure and Cancer Mortality in Uranium Processing Workers

Beate Ritz[1,2]

Data from the Comprehensive Epidemiology Data Resource (CEDR) allowed me to study patterns of cancer mortality in a cohort of 4,014 uranium-processing workers. Employing risk-set analysis for cohort data, I estimated the effects of external (gamma) and internal (alpha) radiation on cancer mortality. My results indicate that Fernald workers exposed to ionizing radiation experienced an increase in mortality from total cancer (per 100 mSv external dose rate ratio (RR) = 1.92; 95% confidence interval (CI) = 1.11–3.32), radiosensitive solid cancer (RR = 2.00; 95% CI = 1.02–3.94), and lung cancer (RR = 2.77; 95% CI = 1.29–5.95). Effects were strongest when exposure had occurred at older ages (>40 years). In addition, I observed an increase in lung-cancer mortality for workers exposed to ≥200 mSv of internal (alpha) radiation (RR = 1.92; 95% CI = 0.53–6.96). Furthermore, my results demonstrate the importance of a long follow-up time when studying solid cancers, the potential for bias due to worker selection associated with concomitant chemical exposures, problems of exposure measurement, confounding, and effect modification due to age at exposure. Owing to lack of data, a previous pooled analysis of uranium-processing workers could only partially address these issues. (Epidemiology 1999;10:531–538)

Keywords: exposure age, cancer, chemicals, ionizing radiation, occupational cohort study, radionuclides, selection bias.

For decades, scientists have been debating to what extent chronic, low-dose exposure to ionizing radiation might cause cancer in nuclear workers.[1] Results from some nuclear-worker studies have raised the possibility that risk estimates for cancer extrapolated from A-bomb-survivor data might underestimate the carcinogenic effect of external, low-dose radiation exposure,[2] while other studies suggest that such radiation has caused no cancer other than leukemias.[3] In any event, the effect size is expected to be small, and random fluctuation is an issue that needs to be addressed. One increasingly common approach to this problem has been to pool data from several worker studies, thus increasing statistical power and limiting random fluctuation.[3,4]

The studies of nuclear cohorts to date have varied with respect to dose rates, exposure lag, and duration of follow-up. In addition, errors in measuring exposures or outcomes, healthy worker selection biases, residual confounding due to unmeasured factors such as smoking and chemical exposures, and different distributions of effect modifiers have likely contributed to inconsistencies across studies. Because data regarding such factors are variably available from study to study, pooled estimates of effects averaged across studies are necessarily limited by the assumption that the combined worker cohorts do not differ substantially. Heterogeneity across study populations, however, is not merely a nuisance obstructing efforts to derive average radiation-effect estimates. Rather, examining the special features of each nuclear-worker cohort may reveal information obscured by a common estimator.[5]

Here I evaluate such special features in a cohort of nuclear workers employed at the Fernald Feed Materials Production Center (FFMPC) in Ohio. Compared with other nuclear workforces monitored for external radiation exposures, the Fernald cohort is small (N = 4,014); yet it is one of the largest monitored for both external and internal exposures and has the advantage of an extremely long follow-up time (mean of 30.9 years). Fernald workers were primarily engaged in processing uranium-ore concentrate and uranium of low-grade enrichment into fabricated uranium metal products, resulting in exposure to radiation and some potentially carcinogenic chemicals.[6]

Selected members of the Fernald cohort were included in an earlier case-control study pooling lung-cancer cases from four uranium-processing facilities.[4] More extensive data for Fernald have recently become available through the Comprehensive Epidemiology Data Resource (CEDR), an archive supported by the Department of Energy to provide de-identified data from

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previous studies of nuclear workers. Using the CEDR data base[6] exclusively, I will: (1) expand analysis to include mortality from cancers other than the lung, (2) add 7 years of follow-up, (3) evaluate the potential for biases due to concomitant chemical exposures and worker-selection processes, (4) examine whether age at exposure influences the effect of external radiation dose, and (5) estimate the effect of internal combined with external radiation exposures.

Methods

Study Population/Outcome Assessment

The study population comprises 4,014 white male workers employed between 1951, when Fernald opened, and 1989, when operations halted. About 85% of cohort members were hired before 1960. The facility continuously monitored workers for radiation exposures, and its epidemiologic surveillance program collected information on vital status. Vital status searches, conducted through January 1, 1990, relied upon two record systems: Social Security Administration (SSA) files for the period before 1979 and the National Death Index (NDI) for the period 1979–1989. I assumed that workers were alive at the end of follow-up if not identified as dead by CEDR. Death certificate information was available for 99% of 1,064 deceased workers.

Exposure Assessment

External radiation exposure was reported as annual penetrating doses, derived from film-badge measurements, and internal radiation exposure as annual lung doses, based on a combination of individual urine bioassays and environmental area sampling.

Exposure to internal radiation emitters involved airborne long-lived radioactive materials such as uranium, thorium, and radium compounds. Most of the exposures were due to U^{235}, varying from depleted to slightly enriched (less than 1% of U^{235}), and characterized mainly as insoluble compounds; thorium was used in small amounts. Workers stationed in areas in which industrial hygiene surveys indicated significant potential for internal radiation exposures were routinely monitored for uranium content in urine, and additional samples were taken when a significant intake of radioactive material was suspected. For estimation of annual individual doses, the data derived from urinalyses were complemented by measurements of uranium dust collected in area air filters.

Estimating lung doses from urine measures for radionuclides requires formulation of an uptake/retention/excretion model based on the biological half-life and chemical/physical properties of the radioactive particles.[7,8] The description of exposure assessment provided by CEDR[6] and Wilson[9] give no detail about either the model used to estimate lung-dose equivalents from urine measures or the relative contributions of individual urinalyses *vs* area air samples to the overall lung doses reported in CEDR.

Nevertheless, it is known that urinalyses are primarily an indicator of the amount of soluble uranium that has been incorporated through the lungs and transported by the blood to the kidneys for excretion. Insoluble uranium compounds, however, are most likely either retained in the lungs, deposited in tracheobronchial or other thoracic lymph nodes, or swallowed.[10] Urinary bioassay monitoring at Fernald, thus, may not adequately reflect the amount of internal radiation dose for insoluble radioactive particles or those of non-respirable size. Environmental sampling, on the other hand, while sensitive to all particle types, obscures differences in individual exposures among workers in the same area. Therefore, the reported doses for internal exposures can be considered no more than crude indicators of relative levels of exposure among Fernald workers.

Covariate Information

Processing of uranium involved exposure to a large number of non-radioactive but potentially carcinogenic chemical compounds, including solvents and cutting fluids. The CEDR provided sufficient data to allow us to control for exposure to trichloroethylene (TCE) and cutting fluids for 3,814 (95%) of our cohort. Plant experts had ranked job titles and work sites according to their potential for chemical exposure, and workers were classified into four exposure categories, from "none" to "heavy," for each chemical.[9] We considered the 5% of the cohort for which chemical data were unavailable as unexposed.

The CEDR reported smoking histories for workers who were continuously employed for at least 3 months between November 1967 and March 1973 (17% of our cohort). Although this sample was too limited to allow us to adjust for smoking in our analyses, it did provide enough information to assess the relation between radiation dose and smoking behavior.

CEDR data on salary status provided us with a surrogate measure of socio-economic status.

Statistical Analysis

For external comparisons with the general U.S. white male population, we used the life-table program developed by Monson[11] to estimate standardized mortality ratios (SMRs = observed/expected deaths) and Byar's formula to derive confidence limits.[12]

For dose-response analyses, there were rarely sufficient deaths to conduct informative analyses by specific cancer site. Accordingly, for external radiation assessments, we restricted the outcomes examined to deaths from all cancers, from lung cancer (International Classification of Diseases, 9th revision (ICD-9)[13] 162), and from two *a priori* groups of cancers classified as radiation sensitive by BEIR V criteria.[1] The first group comprised hematopoietic and lymphopoietic cancers (ICD-9 200–208), excluding chronic lymphatic leukemias (CLL); the second combined all solid cancers identified as radiosensitive, including cancers of the lung (ICD-9 162), esophagus (ICD-9 150), stomach (ICD-9 151), colon (ICD-9 153),

brain (ICD-9 191–192), and urinary-tract system (ICD-9 188–189). Bone and thyroid cancers were not observed. I excluded one, extremely rare, male breast cancer because I could not access the original data to verify the diagnosis.

In estimating the effects of internal alpha radiation, I focused on those organ systems through which radioactive particles pass from intake to excretion. Of primary interest was the respiratory system (ICD-9 160–162) as the entry organ for uranium dusts.[14] The naso-oropharyngeal regions, esophagus, and stomach—grouped together as "upper gastrointestinal tract" (ICD-9 140–151)—may also be irradiated, mainly by larger, non-respirable-sized and insoluble particles.[15] Lower gastrointestinal-tract organs such as colon and rectum (ICD-9 153–154) might also become exposed, although to a more limited extent. In addition, transport systems, specifically the hematopoietic and lymphopoietic systems (ICD-9 200–208, excluding CLL) are likely to be affected, as are exit organs of the urinary tract (ICD-9 188–189)[15]; moreover, uranium can have toxic effects on the kidneys. Radioactive materials can be stored in the liver and bones; however, I observed no bone and only three primary liver cancers, too few to allow meaningful dose-response analysis.

For all dose-response comparisons, I employed the risk-set approach for cohort analysis described by Breslow and Day,[12] providing estimates equivalent to those obtained from a Cox-proportional hazard model.[13] I constructed risk sets by matching to each cancer death all cohort members who were still alive at the calendar time of the index subject's death (on average 3,300 survivors per death). For some analyses, risk sets were also matched on age at death of the case (±2.5 years).

I modeled cumulative radiation dose both as a set of categorical and as continuous variables (in mSv). Using cutpoints established in previous studies, I categorized dose equivalents for external radiation into <10 mSv, 10-<20 mSv, 20-<100 mSv, and ≥100 mSv. I chose categories of <10 mSv, 10-<50 mSv, 50-<100 mSv, 100-<200 mSv, and ≥200 mSv for internal dose because most workers received higher doses and the exposure distribution was less skewed (see below). To allow for a period of cancer induction/latency after exposure to radiation, and to reduce possible selection bias,[16] I lagged cumulative doses by 0, 10, and 15 years limiting cumulative dose to the level achieved x years before the index death. I treated radiation doses and all time-varying factors as time dependent.

I used results of conditional logistic regression analyses to estimate rate ratios (RR) and 95% confidence intervals (95% CI). All models adjusted for the same confounders chosen according to the Greenland[17] criteria: pay type (salaried vs hourly), time since first monitoring, age at risk (continuous), and internal or external radiation dose (continuous). Some models, in addition, included exposure to TCE and/or cutting fluids defined as "highest exposure received for at least 2 years."

TABLE 1. Characteristics of the White Male Fernald Cohort

Number of employees	4,014
Average follow-up time (years)	30.9
Average age at entry into cohort (years)	30.4
Number of person-years	124,177
Number of deaths	1,064
Total mortality rate (per 10^5/yr)	857
Total cancer mortality rate (per 10^5/yr)	267
Pay category	
Salaried	1,311
Hourly/union	2,703

Radiation Dose (mSv)	External	%	Internal	%
0-<10	2,764	68.9	1,111	27.7
10-<50	886	22.1	1,573	39.2
50-<100	259	6.4	999	24.9
100-<200	97	2.4	309	7.7
≥200	8	0.2	22	0.5
Total	4,014		4,014	

Results

A long follow-up period (average 30.9 years) and a high percentage of hourly employees (67.3%) characterize the Fernald cohort. Internal exposure from radionuclides was responsible for the bulk of the radiation doses recorded, with only 27.7% of the workers registering a cumulative dose less than 10 mSv. In contrast, most monitored workers (68.9%) received cumulative external radiation doses of less than 10 mSv, only 2.6% had doses in excess of 100 mSv, and none exceeded 300 mSv (Table 1).

Mortality rates from all causes were lower among Fernald workers than among U.S. white males; the deficit was mostly attributable to a low cardiovascular-disease mortality rate (SMR = 0.78; 95% CI = 0.71–0.86; Table 2). Rates for all malignant neoplasms, however, were slightly increased in workers (SMR = 1.09; 95% CI = 0.98–1.22; Table 2). SMRs were also slightly increased for cancers of the prostate, brain, bladder, the hematopoietic and lymphopoietic systems, and most of the digestive system.

External radiation doses 100 mSv and above increased mortality from all cancers, all radiosensitive solid cancers, and lung cancers, but the small number of deaths in this dose category limited the precision of my estimates (Table 3). Models treating external dose as continuous variable demonstrated dose-related increases in mortality for all of these cancers, especially after lagging exposures by more than 10 years and adjusting for internal dose, cutting fluids, and TCE. Findings for hematopoietic and lymphopoietic cancers were based on too few cases to be conclusive.

The effects of external radiation doses were greatest among workers exposed after the age of 40 (Table 4). Thus, while mortality increased only marginally with exposure before age 40, later exposures, when lagged 15 years, increased mortality by two- to threefold per 100 mSv for all cancers, all radiosensitive solid cancers, and lung cancers separately.

TABLE 2. Observed (OBS), and Expected (EXP) Numbers of Deaths for White Male Subjects and Estimated Standardized Mortality Ratio (SMR): Comparison with the U.S. Population, by Cause of Death*

Causes of Death	OBS No.	EXP No.	SMR	95% CI
All causes (ICDA 001–998)	1064	1264.7	0.84	0.79–0.89
All cancers (ICDA 140–229)	332	303.6	1.09	0.98–1.22
Cancer sites				
Buccal cavity and pharynx (ICDA 140–149)	9	8.75	1.03	0.47–1.95
Digestive organs and peritoneum (ICDA 150–159)	87	74.86	1.16	0.93–1.43
Esophagus (ICDA 150)	9	7.48	1.20	0.55–2.28
Stomach (ICDA 151)	15	11.18	1.34	0.75–2.21
Large intestines (ICDA 153)	28	26.60	1.05	0.70–1.52
Rectum (ICDA 154)	7	6.68	1.05	0.42–2.16
Liver (ICDA 155–156)	8	4.93	1.62	0.70–3.20
Pancreas (ICDA 157)	18	15.53	1.17	0.69–1.85
Respiratory system (ICDA 160–163)	120	116.33	1.03	0.86–1.23
Larynx (ICDA 161)	5	4.24	1.18	0.38–2.76
Lung-primary and secondary (ICDA 162)	112	111.03	1.01	0.83–1.21
Bone (ICDA 170)	0	0.99	0.00	0.00–3.70
Skin (ICDA 172–173)	4	6.45	0.62	0.17–1.59
Prostate (ICDA 185)	25	17.42	1.44	0.93–2.12
Testis (ICDA 186–187)	1	1.46	0.67	0.01–3.74
Bladder (ICDA 188)	8	6.95	1.15	0.50–2.27
Kidney (ICDA 189)	5	7.89	0.63	0.20–1.46
Eye (ICDA 190)	0	0.21	0.00	0.00–17.30
Brain and other central nervous systems (ICDA 191–192)	12	9.66	1.24	0.64–2.17
Thyroid (ICDA 193)	0	0.54	0.00	0.00–6.76
Lymphosarcoma and reticulosarcoma (ICDA 200)	8	4.79	1.67	0.72–3.29
Hodgkin's disease (ICDA 201)	6	2.95	2.04	0.74–4.43
Leukemia and aleukemia (ICDA 204–207)	13	11.21	1.16	0.62–1.98
Lymphatic tissue (ICDA 202–203, 208)	10	9.94	1.01	0.48–1.85
Lymphopoietic cancer (ICDA 200–208)	38	29.50	1.29	0.91–1.77
Cancer residual†	23	22.59	1.02	
Other causes				
Benign neoplasms (ICDA 210)	1	3.47	0.29	0.00–1.60
Diseases of blood and blood-forming organs (ICDA 280–89)	2	2.65	0.75	0.08–2.72
All diseases of circulatory system (ICDA 390–458)	460	588.83	0.78	0.71–0.86
Arteriosclerotic heart disease, including CHD (ICDA 410–14)	339	425.62	0.80	0.71–0.89
All vascular lesions of CNS (ICDA 430–438)	48	59.49	0.81	0.59–1.07
All respiratory diseases (ICDA 460–519)	53	79.78	0.66	0.50–0.87
Emphysema (ICDA 492)	3	14.52	0.21	0.04–0.60
All diseases of digestive system (ICDA 520–577)	49	64.33	0.76	0.56–1.01
Cirrhosis of liver (ICDA 571)	34	36.92	0.92	0.64–1.29
All diseases of genito-urinary system (ICDA 580–629)	3	14.25	0.21	0.04–1.29
All external causes of death (ICDA 800–998)	112	126.92	0.88	0.73–1.06
Suicide (ICDA 950–959)	24	31.46	0.76	0.49–1.14
Total residual‡	15	2.68	5.60	

* According to ICDA-8.
† Cancer, of unspecified site.
‡ Including undetermined causes of death and missing causes of death due to missing death certificates.

Internal radiation doses ≥200 mSv, lagged by 15 years, appeared to increase by twofold the rate of mortality from respiratory cancers (all cases were lung cancers) (Table 5). Also in models with a 15-year lag, there were slight increases in mortality from bladder and kidney cancers at doses of 50 to <200 mSv and from upper gastrointestinal-tract cancers at doses of 10 to <100 mSv, with no case occurring at the highest dose levels. I found no indication of any effect of internal radiation on cancers of the lower gastrointestinal tract or the hematopoietic and lymphopoietic system. In all of the internal-exposure analyses, the 95% CIs were wide (Table 5), and effects did not change after adjustment for cutting fluid and TCE exposures (not shown).

An analysis of the combined effects of external and internal radiation on lung-cancer mortality showed a strong effect of internal doses at levels ≥200 mSv when external radiation doses exceeded 50 mSv (Table 6). Effects were somewhat smaller and unstable for external radiation doses in excess of 50 mSv with lower doses of internal radiation.

Discussion

These results suggest that in this cohort of uranium workers, the rate of death from all cancers, total radiosensitive solid cancers, and lung cancers increased with increasing external (gamma) radiation dose. The effects were most pronounced when workers were exposed at older ages (>40 years) and when radiation doses were lagged by more than 10 years. Lung-cancer mortality also appeared to be elevated in workers exposed to more than 200 mSv of internal alpha radiation. The estimates for the other possible effects of alpha radiation were too imprecise for firm inference.

In an earlier case-control study that pooled 787 lung-cancer deaths from four uranium-processing facilities, including 51 cases from Fernald, Dupree *et al*[4] found an up to twofold increase in lung-cancer mortality among

TABLE 3. Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Effect of Cumulative External Radiation Dose on Cancer Mortality, by Cancer Type and by Exposure Lag, Controlling for Various Covariates: Results of Conditional Logistic Regression Analyses of Risk Sets

External Radiation Dose Categories (in mSv)*	All Cancers			All Radiosensitive Cancers†			Lung Cancers‡			Hematopoietic and Lymphopoietic Cancers§		
	Case N	RR	95% CI	Case N	RR	95% CI	Case N	RR	95% CI	Case N	RR	95% CI
<10	206	1.00		117	1.00		64	1.00		27	1.00	
10–<20	26	0.87	0.57–1.34	10	0.52	0.27–1.00	5	0.43	0.17–1.10	1	0.36	0.05–2.73
20–<100	88	1.19	0.88–1.62	52	1.02	0.69–1.51	35	1.10	0.68–1.79	8	1.37	0.50–3.77
≥100	12	1.43	0.74–2.76	10	1.56	0.74–3.28	8	1.78	0.75–4.24	1	2.08	0.22–20.0
Total Case N	332			189			112			37		
Continuous dose (100 mSv increments)												
0-Year lag												
Without internal dose		1.29	0.91–1.83		1.49	0.96–2.27		1.72	1.05–2.81		0.94	0.26–3.41
Internal dose continuous		1.55	1.04–2.32		1.56	0.95–2.56		1.60	0.90–2.85		1.86	0.43–8.02
Internal dose categorical¶		1.83	1.23–2.71		1.90	1.17–3.07		2.13	1.21–3.76		2.09	0.49–8.83
Internal dose (cat.) plus chemical exposure		1.88	1.27–2.79		1.94	1.19–3.14		2.02	1.13–3.62		2.28	0.53–9.73
10-Year lag												
Internal dose continuous		1.49	0.93–2.40		1.60	0.90–2.83		1.70	0.88–3.29		1.92	0.36–10.2
Internal dose categorical¶		1.75	1.10–2.79		1.86	1.05–3.29		2.28	1.17–4.41		1.90	0.37–9.87
Internal dose (cat.) plus chemical exposure		1.79	1.12–2.86		1.88	1.06–3.32		2.13	1.08–4.18		2.13	0.40–11.2
15-Year lag												
Internal dose continuous		1.66	0.96–2.89		1.80	0.92–3.52		2.21	1.04–4.69		2.08	0.31–13.9
Internal dose categorical¶		1.88	1.09–3.24		1.99	1.02–3.90		2.91	1.37–6.18		1.97	0.30–13.0
Internal dose (cat.) plus chemical exposure		1.92	1.11–3.32		2.00	1.02–3.94		2.77	1.29–5.95		2.25	0.33–15.2

* Adjusted for age at failure time, time since first monitored, pay type (salaried/hourly), internal dose, employing a 0-year lag for external dose.
† ICD-9 150, 151, 153, 162, 188, 189, 191, 192.
‡ ICD-9 162. Note: lung cancers are a subgroup of the radiosensitive cancers.
§ ICD-9 200–208, excluding chronic lymphatic leukemia.
‖ Referent category.
¶ Same categories as presented in Table 5.

those workers exposed to >50 mSv of external radiation and hired (hence exposed) after the age of 45 years. These authors found no effect for internal doses less than 250 mSv, but a slight increase in mortality at higher doses (OR = 2.05; 95% CI = 0.20–20.70). All results were based on too few cases to be conclusive, however.

The present study's focus on the Fernald workforce alone was made possible in part by the greater statistical efficiency of complete-cohort analysis compared with the 1:1 matched case-control design, as well as by the additional cases (for example, 61 new lung-cancer deaths) accumulated during the extra 7 years of follow-

TABLE 4. Adjusted Rate Ratio (RR) Estmates (and 95% CI) for the Effect of 100 mSv Cumulative External Radiation, by Age at Exposure and Cancer Sites: Results from Conditional Logistic Regression Models for Continuous Cumulative Doses within Age Categories, for 0- and 15-Year Lags, Matched for Calendar Time and Age at Failure Time (±2.5 years)*

Outcome	No. of Cancer Deaths	Total†		Age at Exposure			
				15–39		>40	
		RR	95% CI	RR	95% CI	RR	95% CI
All cancers	332						
0-Year lag		1.51	1.01–2.27	1.03	0.42–2.57	1.37	0.92–2.04
15-Year lag		1.68	0.97–2.91	1.05	0.37–2.99	1.90	1.03–3.50
Radiosensitive solid cancers‡	189						
0-Year lag		1.47	0.90–2.41	1.63	0.57–4.64	1.41	0.86–2.33
15-Year lag		1.74	0.89–3.40	1.59	0.49–5.13	2.07	0.96–4.50
Lung cancers§	112						
0-Year lag		1.46	0.82–2.59	1.63	0.44–6.01	1.62	0.91–2.86
15-Year lag		1.97	0.93–4.16	1.24	0.27–5.65	2.88	1.29–6.44
Hematopoietic and lymphopoietic cancers‖	37						
0-Year lag		1.88	0.40–8.17	0.16	0.00–16.7	1.28	0.31–5.17
15-Year lag		2.02	0.29–14.0	0.02	0.00–59.5	2.55	0.42–15.4

* Adjusted for pay type (salaried/hourly), internal radiation dose, time since first monitored.
† Note: This is the estimate for cumulative external dose (continuous) for all ages at exposure.
‡ ICD-9 150, 151, 153, 162, 188, 189, 191, 192.
§ ICD-9 162.
‖ ICD-9 200–208 excluding chronic lymphatic leukemias.

TABLE 5. Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Effect of Cumulative Internal Radiation Dose on Cancer Mortality, by Cancer Sites, for 0- and 15-Year Lags for Exposure: Results of Conditional Logistic Regression Analyses*

	Respiratory Tract Cancers†			Upper gastrointestinal tract cancers‡			Lower gastrointestinal tract cancers§			Bladder and kidney cancers‖			Hematopoietic and Lymphopoietic Cancers¶		
Internal Exposure (mSv)	Cases N	RR	95% CI	Cases N	RR	95% CI	Cases N	RR	95% CI	Cases N	RR	95% CI	Cases N	RR	95% CI
0-Year lag															
<10**	23	1.00		7	1.00		9	1.00		2	1.00		7	1.00	
10–<50	37	0.66	0.39–1.13	12	0.85	0.32–2.24	12	0.54	0.23–1.30	3	0.81	0.12–5.40	19	1.60	0.61–4.19
50–<100	41	0.63	0.35–1.12	12	0.97	0.33–2.90	11	0.37	0.14–0.99	6	1.91	0.28–13.2	10	0.92	0.28–3.06
100–<200	14	0.46	0.20–1.03	2	0.44	0.07–2.79	2	0.14	0.02–0.79	2	1.83	0.16–21.6	1	0.21	0.02–2.32
≥200	3	1.18	0.32–4.41	0			1	0.81	0.08–8.20	0			0		
	118			33			35			13			37		
15-Year lag															
<10**	39	1.00		10	1.00		10	1.00		2	1.00		14	1.00	
10–<50	36	0.51	0.31–0.86	15	1.65	0.57–4.82	17	1.18	0.45–3.12	4	1.04	0.16–6.75	14	0.96	0.37–2.52
50–<100	31	0.51	0.28–0.95	8	1.68	0.43–6.52	5	0.51	0.14–1.93	5	2.12	0.27–16.4	8	0.98	0.29–3.33
100–<200	9	0.48	0.20–1.19	0			3	1.28	0.25–6.43	2	3.96	0.35–44.6	1	0.56	0.06–5.40
≥200	3	1.92	0.53–6.96	0			0			0			0		
	118			33			35			13			37		

* Adjusted for age at failure time, time since first monitored, pay type (salaried/hourly), external radiation dose (continuous).
† ICD-9 160–162.
‡ ICD-9 140–151.
§ ICD-9 152–154.
‖ ICD-9 188–189.
¶ ICD-9 200–208, excluding chronic lymphatic leukemias.
** Referent category.

up. Thus, the increase in lung-cancer mortality associated with internal doses exceeding 200 mSv was based entirely on new Fernald cases occurring after 1982. This finding underscores the importance of the long follow-up times that Pierce *et al*[18] have suggested may be necessary for detecting effects of radiation on solid tumors, such as lung cancers, that normally develop late in life. Because lung-cancer incidence increases markedly after age 60, and Fernald workers were on average 30 years old at entry into the cohort, a 31-year follow-up allowed the average worker to reach the age of increased risk.

My access to cohort-wide data on external radiation exposures that were largely unavailable to Dupree *et al* allowed me to refine estimates of internal-dose effects by adjusting for external radiation doses. In addition, I observed dose-response effects of external radiation exposure on mortality not only from lung cancer, but also from all radiosensitive cancers and cancer as a whole. The demonstration that these effects were mostly due to exposures received after age 40 confirmed Dupree *et al*'s preliminary observation of an increase in lung-cancer mortality among workers exposed to over 50 mSv of external radiation and hired after the age of 45.

Although the largest pooled-cohort study to date found no effect of external radiation on cancers other than leukemias,[3] a number of single-cohort investigations have observed radiation-related increases in lung cancer. Among Oak Ridge Y-12 uranium-processing workers,[19] a trend of increasing lung-cancer mortality with increasing gamma radiation dose was most pronounced for those who also received more than 50 mSv

TABLE 6. Adjusted Rate Ratio (RR) Estimates (and 95% CI) for the Combined Effects of Cumulative Internal and External Radiation Dose on Lung Cancer Mortality, by Dose Level Assuming a 0- and 15-Year Lag for Both Exposures: Results from a Conditional Logistic Regression Analyses*

	Internal Dose (mSv)								
	<100			≥100–<200			≥200		
External dose (mSv)	No. of Cancer Deaths	RR	95% CI	No. of Cancer Deaths	RR	95% CI	No. of Cancer Deaths	RR	95% CI
<50									
0-Year lag	84	1.00†		6	0.72	0.31–1.67	0		
15-Year lag	90	1.00†		6	1.24	0.53–2.89	0		
50–<100									
0-Year lag	8	1.36	0.65–2.83	5	1.36	0.54–3.40	1	7.68	1.06–55.7
15-Year lag	9	2.03	1.00–4.14	2	1.28	0.31–5.32	2	18.0	4.32–74.9
≥100									
0-Year lag	3	2.28	0.71–7.31	3	1.42	0.44–4.54	2	5.87	1.42–24.2
15-Year lag	1	1.53	0.21–1.17	1	2.01	0.28–14.7	1	17.7	2.36–133

* Adjusted for age at failure time, pay type (salaried/hourly), time since first monitored.
† Referent category.

TABLE 7. Smoking Prevalence for Fernald Workers Who Were Employed on or after January 1, 1968, First Hired Between January 1, 1952 and December 31, 1972, by Cumulative Radiation Dose Level (mSv)

Radiation Dose Level (mSv)	Smokers		Ex-smokers		Nonsmokers			
	N	%	N	%	N	%	Total	%
External radiation								
<50	232	42.6	139	25.6	173	31.8	544	100
50–<100	69	50.0	29	21.0	40	29.0	138	100
≥100	34	45.3	15	20.0	26	34.7	75	100
Internal radiation								
<50	71	39.9	40	22.5	67	37.6	178	100
50–<100	180	44.9	99	24.7	122	30.4	401	100
≥100	84	47.2	44	24.7	50	28.1	178	100

of alpha radiation, much as the greatest effects at Fernald were associated with high doses of external and internal radiation in combination. In Rocketdyne workers, Ritz *et al*[20] found an increase in lung-cancer mortality with doses of external radiation above 200 mSv, but not with internal exposures—a difference that may reflect the much lower internal-dose levels at Rocketdyne than at either Oak Ridge or Fernald. In a cohort of British nuclear workers, Fraser *et al*[21] observed a slightly increased risk of lung-cancer mortality among those exposed to any radionuclide, compared with those with no internal radiation exposure. Finally, both U.S.[22] and British[23] studies of nuclear workers have linked alpha radiation exposure from plutonium and thorium to lung-cancer mortality.

Leukemias have been consistently linked to external radiation exposure.[3] The Fernald data suggested an increased risk of hematopoietic/lymphopoietic cancer at external doses ≥100 mSv; however, this result was based on only one lymphoma in the highest dose category, and the evidence associating external radiation with lymphomas is equivocal.[1,24]

The influence of age at exposure on the radiation effects at Fernald may have become apparent only because of the extended follow-up. My colleagues and I have previously described a similar pattern of greater mortality from lung cancer, all radiosensitive cancers, and total cancers among workers exposed at older ages to external radiation in the Rocketdyne cohort, which also had a long period of follow-up.[25] Studies of the Hanford[26,27] and Oak Ridge[2,28] nuclear cohorts have reported comparable effects of exposure age on overall cancer mortality.

As mentioned in Methods, internal-radiation dose measures, based on relatively inexact methods of estimation, are subject to considerably more uncertainty than those for external exposures. The type of measurement error at any facility depends heavily on monitoring practices and the types of radionuclides for which estimates are derived; in general, assessments of internal-[illegible] probably suffer from exposure misclassification to a much greater degree than do external-dose analyses. Therefore, although estimates of internal doses are expressed in mSv, it may be more appropriate to interpret them only as a relative ranking of internal exposure within a cohort, not suitable for comparison with either external-dose measures in the same cohort or with internal-dose levels reported at other facilities. Thus, the failure to detect effects of alpha radiation on the respiratory system at dose levels <200 mSv might reflect the properties of the mostly insoluble uranium of low enrichment grade processed at Fernald, but could also be a consequence of large (nondifferential) exposure misclassification at lower exposure levels, biasing my results toward the null.

Worker-selection bias may have contributed to some underestimation of radiation effects in the Fernald cohort. Uranium processing at the facility involved the use of large amounts of chemicals known to be respiratory irritants, including tributyl phosphate, ammonium hydroxide, sulfuric acid, and hydrogen fluoride, which were the focus of an earlier investigation of respiratory disease at Fernald.[9] I found that exposure to these chemicals was highly correlated with radiation dose; the 905 workers highly exposed to these chemicals received almost double the average radiation dose (internal and external) and worked on average 2 years longer than coworkers. This observation suggests that workers healthier and less responsive to the toxic and irritating effects of these substances may have selectively filled jobs entailing exposure to lung irritants and radiation. Although I do not know the extent to which baseline lung-cancer risk might be affected by such selection forces, these forces may well result in a net healthy worker effect for those workers exposed to radiation at the highest levels, which could bias all radiation results toward the null.

The sample of Fernald workers for whom I had smoking data had proportionately fewer smokers than the adult U.S. white male population[29] (Table 7), which may account for the cohort's reduced SMRs for cardiovascular disease, emphysema, and all respiratory diseases. Data from the sample suggested that workers exposed to higher levels of internal radiation might have been slightly more likely to smoke (Table 7), but the difference was not large enough to be likely to result in confounding.[30] I was unable to adjust for smoking in my analyses, but I may have adjusted indirectly for smoking behavior by adjusting for pay-type in the dose-response analyses, because, compared with hourly employees, salaried workers smoked less (32.8% vs 48.2% smokers) and had lower mortality rates from lung cancers (RR = 0.53; 95% CI = 0.32–0.90) and from all other smoking re-

lated cancers together (RR = 0.54; 95% CI = 0.25–1.13 for oropharyngeal, laryngeal, esophageal, pancreatic, and bladder cancers combined). Matching on age at death and calendar time in these analyses might have also helped to match for smoking habits specific to different birth cohorts.

The results from this small cohort of radiation workers underscore the importance of conducting a comprehensive analysis of all data available for each worker population included in a pooled study to explore and understand the importance of issues related to length of follow-up, selection bias, potential confounding and worker selection associated with chemical exposures, problems of exposure measurement, and effect modification due to age at exposure. A previously conducted pooled analysis of uranium-processing workers could only partially address these issues, since the relevant data either did not exist or were available for only small subsets of all subjects included in the analyses.

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