Modeling Repeated Measurement Data for Occupational Exposure Assessment and Epidemiology

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Modellering van herhaalde meetgegevens voor blootstellingskarakterisering in de werkomgeving en epidemiologie (met een samenvatting in het Nederlands)

by
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In memory of my father Yaakov Shpiro

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INTRODUCTION

PURPOSES AND DESIGNS OF REPEATED MEASUREMENTS

Longitudinal repeated measurements, in which the same variable of interest is measured on each subject on several different times, occur frequently in the assessment of occupational exposure to toxic chemicals, as part of occupational hygiene surveys or monitoring programmes.[1-9] Such designs are suited to study the association between exposure levels and exposure determinants both varying along time and to assess variance components of exposure variability in "exposure groups". In this thesis exposure levels on a continuous scale will be dealt with.

In epidemiological studies with repeated measurements designs, the aim is mostly to evaluate the associations of exposure with other covariates and health outcomes, both changing over time, or to investigate changes over time within a subject's health status.

Repeated measurements in epidemiology

Longitudinal study designs with repeated measurements of a covariate (e.g. a time related risk factor) and a health outcome are appealing in epidemiology since they offer the opportunity to study the temporal order of events, to observe individual patterns of change and to measure exposure and other covariates prospectively. The temporal order is essential to assess causality, making longitudinal designs superior to cross-sectional ones. Differences in time related variables, e.g. age-related changes within and between individuals can be estimated with less bias caused by selective follow up (= different times of follow up) and cohort effects.

Furthermore, longitudinal designs have greater power and precision than cross-sectional studies because between individual variability does not contribute to the variability of the individual change. In cross-sectional studies part of the explanatory variables have to be estimated retrospectively, and are thus more likely to be biased.[10]

In some epidemiological studies the risk factor (e.g. exposure) may be measured repeatedly while the health outcome is measured only once.

The usual health outcomes are mortality or morbidity indexes, such as: acute and chronic symptoms, chronic respiratory diseases, respiratory infections and pulmonary function (PF) in respiratory epidemiology.[11] Those health outcomes can be measured on a binary scale (e.g. yes-have the disease/no-don't have the disease), counts (e.g. number of symptoms) or on a continuous scale (e.g. PF measurements).

Repeated measurements in occupational hygiene

Repeated shift-long exposure measurement data sets are gathered at workplaces as part of:[12-15]

(i) Compliance strategies where exposure levels have to be compared with health based exposure limits (such as TLVs- Threshold Limit values or OELs- Occupational Exposure Limits);

(ii) Surveillance strategies to monitor exposure patterns over time, and;

(iii) Epidemiological studies in which exposure data has to be associated with biological markers or specific health occurrences.

Hygiene surveys performed to meet those short- (i) and long-term (ii,iii) objectives involve repeated sampling of selected exposed persons. They should be carried out with an appropriate technique and procedure, and the inherent limitations should be fully understood. The repeated surveys should be performed with the same air monitoring strategy along the whole period (air sampling technique, analysis, selection of workers etc.).[15] As a whole, strategies for performing repeated measures along time should take into account two dimensions : how to sample workers and how to sample the measuring days.

Occupational health legislation is aimed at protecting the individual worker. Within this framework, hygiene monitoring should provide a tool for assessing the exposure of every worker. Due to limitations of money and time, monitoring strategies are department-oriented (using production department, store etc. as sampling unit), or focus at the job level (using fuel fillers, laboratory workers etc. as sampling unit). Typical workers within these sampling units, called exposure-groups (or industrial hygiene groups) are usually monitored.[12-15]

Usually personal measurements, measurements in the breathing zone of the worker, are taken within exposure-groups. For epidemiological studies and surveillance programmes (not including the "worst case" oriented) the same exposure-groups oriented strategy usually holds.

Nowadays it is known that exposure levels can vary considerably, between 10 and 4000 fold, from day to day for the same worker. This fact strengthens the need for repeated measurements designs for a valid exposure assessment.[16] Such surveys designs range from repeated measurements on consecutive or randomly selected days within a single year[2,4,6,9,17-25], to studies which include historical measurements collected periodically over periods ranging from 2 to 30 years.[1,3,5,7,8,18] The time points of measuring are mostly not identical across subjects as well as the time-interval between adjacent measures of the same worker. Consequently, the number of repetitions per subject is usually not the same, leading to unbalanced designs.

Collection of ancillary information during the measurement process such as: work-temperature, task, outdoor/indoor working etc., is an indispensable requisite since many of these factors are exposure determinants which are associated with elevated or reduced exposure levels. The information on exposure determinants can be gathered to evaluate relationships between determinants and exposure for exposure control purposes (identification), and for evaluation of exposure response relationships in an epidemiological study. For the latter, the exposure may be estimated (predicted) on the basis of information on the determinants and those predicted exposures may further be used in exposure response modeling.[26] Workers performing the same job will show considerable differences in average exposure levels (between-worker variability) , but will also experience different exposure levels from day-to-day (within-worker variability). Assessing exposure variance components, between-workers and within-worker, is an inherent part of evaluating exposure distributions and estimating probabilities of exposure above standards, within exposure-groups and over time.

Therefore, whatever the purpose, statistical modeling of relationships between exposure and determinants of exposure as well as statistical estimation of exposure variance components are key elements in modern exposure assessment strategies.

HISTORICAL REVIEW
OF STATISTICAL ANALYSIS FOR REPEATED MEASUREMENTS DESIGNS

Since the early fifties, several aspects of statistical modeling of exposure data have been recognized and implemented in the concepts regarding statistical treatment of repeated exposure data. The developments in statistical methods used to analyze repeated measures data, of a continuous exposure variable, are described below, and range from the use of simple summary measures to more complex multiple regression type modeling as follows:

1. Using simple summary measures: The multiple measurements per subject or a group are reduced to one summary measure such as overall mean. In 1952 Oldman and Roach[15] performed stratified sampling of workers and simply calculated mean exposure over all workers in each stratum and applied this mean to each worker in the stratum. They implicitly introduced, the concept of the homogeneous exposure group. Ashford in 1958 extended this approach to epidemiological purposes in the framework of the large scale British Coal Workers Surveys,[15] using repeated exposure measurements. He described a statistical approach to allocate the sampling effort on the basis of the time spent in a particular occupational title. The exposure was calculated as the product of the exposure measured for a certain occupational group and the time exposed. An underlying assumption was that the exposure of an individual worker is supposed to be indistinguishable from the shift average of the total group. In 1979, a zoning method was proposed by Corn & Esmen[13] for sub-grouping workers based on their job and similarities in their environment. Then geometric mean and geometric standard deviations were calculated for each zone to represent the individual's exposure, assuming lognormality. Again, issues concerning repeated measurement designs were not explicitly dealt with. This approach ignores the existence of variability between workers in the "homogeneous" exposure group and there is a non-exhaustive use of all the information available. Similar observations can be made for exposure compliance strategies in which the repeated structure of the data is usually ignored. The well known manual for estimating the probability of exposure over a certain exposure limit, written in the late seventies by NIOSH,[13] mentions repeated measurement

designs. However, the manual does not explicitly state that repeated measurements should come from the same individual. The approaches described allowed measurements to be taken from different workers and the same worker for one series of measurement, assuming the individual as an interchangable variable. Again, this implies that also for compliance strategies between worker variability issues were not recognized and exposure categories were implicitly or explicitly considered homogeneous.

2. Regression analysis: As a next step, regression models were used to estimate the mean exposure based on exposure determinants, as well as estimating the relative effect of each exposure determinant. One of the first examples of this approach is the use of regression modeling in evaluating determinants of asbestos exposure in the study by Dement et al. in 1983.[3] More recent examples where regression models were used to analyze repeated measurements can be found in the review by Burstyn and Teschke.[27] The advantage was that relatively simple models with a limited number of variables could describe associations present in larger datasets. This approach treated the observations of the same subject as independent and ignored potential correlations between repeated observations. The assumption of independence between observations is misleading and may cause biased estimators. Several researchers that used this approach were aware of the disavantages at the time.[27] However, no convenient alternatives were easily available for routine data analysis and the disadvantages were taken for granted.

3. One way random effects model analysis of variance (ANOVA) with worker as a random effect was used to estimate the within- and between-worker variability which are systematic changes in exposure between days and between workers.[28-30] This approach was first applied to evalute the homogeneity of exposure grouping schemes.[26,31] The introduction of this approach marked the evaluation of homogeneity of exposure groups and eventually efficient grouping schemes based on optimizing within-individual and within and between group variability. At the same time, epidemiologists were interested in evaluation of the within-individual and between-

individual variance components since the ratio of these two determines the attenuation of exposure response relationships.[29]

4. In general the random effects ANOVA approach accounts for a specific correlation structure between measurements (CS-Compound Symmetry). Since repeated exposure measurements consist of short time series with a non-fixed time interval only few studies examined autocorrelation issues explicitly.[30,32-34,] In these studies there were only anecdotal suggestion for significant AR(1) (Auto Regressive of first order) autocorrelation, a correlation that reduces consequently with time. In another comprehensive study 25% of the data-sets coming from several sources were found to show evidence of auto correlation.[32] Even though the one way ANOVA method takes into account the correlation between repeated measures of the same subject , it is unefficient since one does not use all available information regarding exposure determinants which actually affect exposure levels and consequently the variance components.

5. A two step process was applied consisting of a separate regression analysis and a one way random effects ANOVA.[2,24,26,27,35,36] A modification of this approach has been applied in the situation where the dependent variable was not the mean exposure, but the variances of exposure-groups.[37] Researchers applied this approach to evaluate their exposure data, including repeated measurements for both purposes. In this approach all available information was used but random effects were estimated separately from fixed effects, using models which were most likely not optimal for either type of analysis.[38] In addition, by applying multiple testing statistical type I error might increase.

THE MIXED EFFECTS MODEL

The mixed effects model is a generalization of the standard linear model (a regression model) , that enables the analysis of data generated from several sources of variation instead of just one.[39-42] The unique aspect of the mixed model is the inclusion of both fixed and random effects associated with the independent variables. A random effect is an effect whose values are drawn from a normally distributed random process with mean zero and common variance. Effects are defined as random when the levels are randomly selected from a large population of possible levels. Inferences are made using only a few levels but can be generalized across the whole population of random effects levels. Otherwise effects are defined as fixed.

The mixed effects model in which the effect of the worker is assumed to be a random effect, can easily deal with repeated measurement designs.[40,41] Subjects are assumed random because they are selected from a larger population for which we want to generalize the findings. Since measurements done on the same subject may be correlated, this correlation has to be taken into account in the modeling. The assumptions concerning dependency among the repeated outcomes can have different forms leading to specific covariance structures. The mixed model supports several covariance structures of the observations. As in the ANOVA model, a simple covariance structure can have a Compound Symmetry structure where the correlation between repeated measurements is assumed to be equal between any 2 measurements. However, more complex structures, such as first order Auto-Regressive covariance structures can be assumed as well.

The number of repetitions per subject is similar (balanced designs) and time points are identical across subjects, only in specific circumstances. More commonly the case, the number of repetitions per subject is different (unbalanced designs) and deviant sampling schemes are most frequently used. The study designs range from repeated surveys on consecutive or randomly selected days within a single year[2,4,6,9,17-25] to studies which include historical measurements collected over periods ranging from 2 to 30 years.[1,3,5,7,8,18]

The time points can either be identical across subjects or not. The time-interval between the repeated observations can vary across repetitions. The mixed model can easily deal with these different sampling schemes.

For the analysis of occupational hygiene data with the purpose of exposure assessment this means that the mixed effects model has the advantage over all other described methods that it can estimate fixed effects associated with different jobs and other covariates (e.g. exposure determinants), and simultaneously estimate the within- and between-worker variance components associated with the random effects. It is a more efficient extension of the two-step approach using simple regression and ANOVA modeling.[38] Despite the advantages of the mixed model, it is finding wide spread application only since the very recent years. The mixed models for exposure assessment were used only lately in studies regarding exposure to different pollutants , published by only a few research groups in 1996 and between 1999-2002.[38,43-53] Apart from the advantages for estimating effects from determinants of exposure, some other application are expected to be explored in the near future because of the specific properties of the mixed models. First, complex study designs with repeated measurements over time and space (multiple samples at the same time at the same individual, multi-level structures etc.) can be analyzed efficiently. Secondly, the mixed model can be used to predict exposures on the basis of measurement of proxies of exposure (surrogate variables) in epidemiological studies which may be used in exposure-response analysis. Thirdly, the mixed model can be used to design exposure assessment strategies, especially with regard to allocation of the sampling effort over time and workers, or groups of workers.

GOALS OF THE THESIS

The main objective of this thesis is to study applications of the mixed model to evaluate potential benefits from using mixed effects models for occupational exposure assessment and epidemiological studies by analysing several data sets from surveys with repeated exposure measurements. Evaluation of the relative contributions of particular fixed characteristics affecting the exposure level (the response variable) was assessed while controlling for the random effects as well as the evaluation of the variance components of exposure levels mainly for grouping strategy. In addition, an improved estimator of the between-worker variability is evaluated in specific conditions. Finally, an estimation of

workers' exposure (a surrogate variable), based on an exposure-exposure determinants mixed effects model, was assessed and used further to avoid possible attenuation and to obtain more detailed exposure data than obtained through classical grouping strategies while exploring the shape of a relationship with a health response.

STRUCTURE OF THE THESIS

Chapter 1 provides a general introduction to the thesis. Chapter 2 deals with a specific experimental design, a reliability study, consisting of general estimation of variance components of exposure over a year in random factories. The scheme of the design consisted of a nested design, 6-13 workers within 6 factories, and each 2 using the same contaminant. In each factory 10 repeated hygiene surveys were performed within 1 year, at random intervals of 3-7 weeks. Thus a nested random effects ANOVA model was fitted to the unbalanced design assuming equal correlation between any pair of measurements, a Compound Symmetry (CS) dependence structure (part I) as well as a mixed effects model which account for both fixed and random effects (part II). In chapter 3 the benefits of using mixed effects models for occupational exposure assessment are presented. Two existing data sets with repeated exposure measurements and auxiliary information on work characteristics were re-analyzed. Mixed effects models were applied with and without work characteristics effects and resulting estimates of the within- and between-worker variance components were compared. In addition, the significance of the effects of exposure determinants are compared between common models (regression) and valid ones (mixed effects). In chapter 4 the association between airborne benzene exposure in fuel distribution facilities and task and time-related factors is described, based on a longitudinal database collected over 8 years in repeated hygiene-monitoring surveys in Israel. By using the mixed-effects model, hazardous conditions associated with high exposure levels were identified and this resulted in recommendations for better hazard control and a routine sampling scheme. In chapter 5 an improved estimator of the between-subject variability in exposure concentrations is presented. This estimate was proposed in order to be able to deal with negative estimates of variance components The phenomenon of obtaining a negative

estimate has its origin in the nature of occupational exposure data since we are dealing with relatively small between worker exposure variability and large day-to-day exposure variability. It has implications on further estimation of the probability that a worker's mean exposure exceeds the occupational exposure standard, where the worker's mean exposure is relevant to the risk of chronic adverse health effects.

In chapter 6 we present a study in 270 Dutch bakers. In phase I, we present different exposure-exposure determinant models for (i) a classical grouping strategy leading to various industrial hygiene groups, and for (ii) estimating exposure levels for work profiles. In phase II we present the process of investigating the shape of relationship (linear or not) between either actual or estimated exposure (from phase I) with bakers' sensitization. Firstly, we fitted a semi-parametric generalized additive model and secondly we applied a parametric model – a quadratic logistic model based on previous results, to associate exposure and sensitization. The final model allowed us to present with high certainty, by atopic status, the probability of sensitization by exposure.

Finally, in chapter 7 the main findings are being discussed. Although the emphasis of the thesis is on application of mixed modeling to exposure data in occupational hygiene, an example of application of mixed modeling to repeated heath outcome data can be found in the Appendix. The appendix contains results from a study on lung function change along time, in children living around a power-plant.

REFERENCES

1. Amandusd HE, Wheeler R, Jankovic J, et al. The morbidity and mortality of vermiculitr miners and millers exposed to tremolte-actinolite: Part I. Exposure estimates. Am J Ind Med 1987;11:1-14.
2. Burstyn I, Teschke K, Kennedy SM. Exposure levels and determinants of inhalable dust exposure in bakeries. Ann Occup Hyg 1997;41:609-624.
3. Dement JM, Harris RL, Symons MJ, Shy CM. Exposures and mortality among chrysotile asbestos workers. Part I: Exposure estimates. Am J Ind Med 1983; 4:399-419.
4. Demer FR, Rosen JC, Finman TJ. Carbon monoxide exposure during the use of propane-powered floor burnishers. App Occup Env Hyg 1996;11:1087-1091.
5. Eisen EA, Smith TJ, Wegman DH et al. Estimation of long term dust exposures in the Vermont granite sheds. Am Ind Hyg Assoc J 1984;45:89-94.
6. Greenspan CA, zmoure-Erason R, Wegman DH et al. Occupational Hygiene characterization of a highway construction project: a pilot study. App Occup Env Hyg 1995;10:50-58.
7. Hallock MF, Smoth TJ, Woskie SR, Hammond SK. Estimation of historical exposures to machining fluids in the automative industry. Am J Ind Med 1994;26:621-634.

8. Hornung RW, AL Greife, LT Stayner et al. Statistical model for prediction of retrospective exposure to ethylene oxide in an occupational mortality study. Am J Ind Med 1994;25:825-36.
9. Kalliolokoski P. Estimating long-term exposure levels in process-type industries using production rates. Am Ind Ind Assoc J 1990;51:310-312.
10. Rijcken B, , Schouter JP Weiss ST,Ware JH. SERS/ATS workshop on longitudinal analysis of pulmonary function data, Barcelona, September 1995. Eur Respir J. 1997 ;10:758-63.
11. Samet JM, Speizer FE. Assessment of health effects in epidemiologic studies of air pollution. Environ Health Perspect. 1993;101 Suppl 4:149-54.
12. BOHS-British Occupational Hygiene Society: Technical Guide No. 11: Sampling Strategies for Airborne Contaminants in the Workplace. H & H Scientific Consultant Ltd, UK, 1993.
13. NIOSH- National Institute for Occupational Safety and Health. Occupational Exposure Sampling strategy Manual, Publication No. 77-173. NIOSH Chincinnati, USA, 1977.
14. HSE-Health and Safety Executive. Monitoring Strategies for Toxic Substances. Guidance Note, Environmental Hygiene 42. HSE, Leeds, UK, 1989.
15. Boleij J, Buringh E, Heederik D, Kromhout H. Occupational hygiene of chemical and biological agents. Elsevier, Amsterdam, The Netherlands 1995.
16. Patty's Industrial Hygiene. Fifth Edition, Volume 1, 2000 John Wiley & Sons. Inc. (Chapter eighteen, SM Rappaport).
17. Kromhout H, Swuste P, Boleij JSM. Empirical modeling of chemical exposure in the rubber manufacturing industry. Ann Occup Hyg 1994;38:3-22.
18. Materna BL. Occupational exposure to perchloroethylene in the dry cleaning industry. Am Ind Hyg Assoc J 1985;46:268-273.
19. McDevit JJ, Lees PSJ, McDiarmid MA. Exposure of hospital pharmacists and nurses to antineoplastic agents. J Occup Med 1985; 46:268-273.
20. Preller L, Heederik D, Kromhout H et al. Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modeling. Ann Occup Hyg 1995;39:545-557.
21. Rocskay AZ, Robins TG, Echeverria D, et al. Estimation of cummulative exposures to naptha at an automobile fuel-injector manufacturing plant. Am Ind Hyg Assoc J 1993;54:480-7.
22. Smid T, Heederik D, Mensink G et al. Exposure to dust, endotoxin and fungi in the animal feed industry. Am Ind Hyg Assoc J 1992;53:362-368.
23. Teschke K, Hertzman C, Morrison B. Level and distribution of employee exposures to total and respirable wood dust in two Canadian sawmills. Am Ind Hyg Assoc J 1994;55:245-250.
24. Teschke K, Marion SA, van Zuylen MJ et al. Maintenance of stellite and tungsten carbide saw tips: determinants of exposure of cobalt and chromium Am. Ind Hyg Assoc J 1995;53:661-669.
25. Woskie SR, Smith TJ, Hammond K, et al. Estimation of the direct exhaust exposures of railroad workers: I. Current exposures. Am J Ind Med 1988;13:381-394.
26. Preller, L, Kromhout H, Heederik D, Tielen M. Modelling long-term average exposure in occupational exposure-response analysis. Scand J Work, Environ, Health 1995;21:504-512.
27. Burstyn I, Teschke K. Studying the determinants of Exposure: A review of methods. Am Ind Hyg Assoc J 1999;60:57-72.
28. Heederik D, Boleij JSM, Kromhout H, Smid T. Use and analysis of exposure data for epidemiological purposes. Applied Industrial and Environ Hyg 1991;6:458-464.
29. Heederik D, Kromhout H, Burema J. Assessment of long-term exposures to toxic substances in air. Ann Occup Hyg 1991;35:671-674.
30. Symanski E, Rappaport SM. An investigation of the dependence of exposure variability on the interval between measurements Ann Occup Hyg 1994;38:361-372.

31. Rappaport SM, Selvin S. A method for evaluating the mean exposure from a lognormal distribution. Am Ind Hyg Assoc J. 1987;48:374-9.
32. Kromhout H, Oostendorp Y, Heederik D, Boleij JS. Agreement between qualitative exposure estimates and quantitative exposure measurements. Am J Ind Med 1987;12:551-62.
33. Francis M, Selvin S, Spear R, Rappaport S. The effect of autocorrelation on the estimation of workers' daily exposures Am Ind Hyg Assoc J 1989;50:37-43.
34. Lagorio S, Iavarone I, Iacovella N, Proietto IR, Fuselli S, Baldassarri LT, Carere A. Variability of benzene exposure among filling station attendants. Occup Hyg 1998;415-30.
35. Kumagai S, Matsunaga I, Kusaka Y. Autocorrelation of short-term and daily average exposure levels in workplaces. Am Ind Hyg Assoc J 1993;54:341-350.
36. Woskie SR, Smith TJ, Hammond K, et al. Factors affecting worker exposures to metal working fluids during automotive component manufacturing Appl Occup Environ Hyg 1994;19:612-621.
37. Kromhout H, Symanski E, Rappaport SM. A comprehensive evaluation of within- and between-worker components of occupational exposure to chemical agents. Ann Occup Hyg 1993;37:253-70.
38. Symanski E, Kupper LL, Kromhout H, Rappaport SM. An investigation of systematic changes in occupational exposure. Am Ind Hyg Assoc J. 1996;57:724-35.
39. Diggle PJ, Liang KY, Zeger SL, Analysis of longitudinal data, Oxford University Press, New-york, USA 1994.
40. Everitt BS. The Analysis of Repeated Measures: A practical review with examples. The Statistician 1995;44:113-135.
41. Lindskey JK. Models for repeated measurements. Oxford, Clarendon Press, 1993.
42. Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS system for mixed models. Cary, NC: SAS Inc., USA 1996.
43. Nylander-French LA. An investigation of factors contributing to styrene and styrene-7,8-oxide exposures in the reingforced-plastics industry. Ann Occup Hyg 1999;43:99-105.
44. Rappaport SM, Weaver M, Taylor D, Kupper L, Susi P. Application of mixed models to assess exposures monitored by construction workers during hot processes. Ann Occup Hyg 1999;43:457-69.
45. Burstyn I, Kromhout H, Kauppinen T, Heikkila P, Buffeta P. Statistical modeling of the determinants of historical exposure to bitumen and polycyclic aromatic hyiocarbons among paving workers. Ann Occup Hyg 2000;44:43-56.
46. Vermeulen R, de Hartong J, Swusteg P, Kromhout H. Trends in exposure to inhalable particulate and dermal contamination in the rubber manufacturing industry: Effectiveness of control measures implemented over a nine-year period. Ann Occup Hyg 2000;44:343-354.
47. Symanski E, Chang CC, Chan W. Long-term trends in exposure to Nickel aerosols Am Ind Hyg Assoc J 2000;61:324-33.
48. Tielemans E, Kupper LL, Kromhout H, Heederik D, Houba R. Individual-based and group-based occupational exposure assessment; some equations to evaluate different strategies. Ann Occup Hyg 1998;42:115-9.
49. Park D, Teschke K, Bartlet K. A model for predicting endotoxin concentrations in metalworking fluid sumps in small machine shops. Ann Occup Hy. 2001;45:569-76.
50. Symanski E, Bergamaschi E, Mutti A. Inter- and intra-individual sources of variation in levels of urinary styrene metabolites. Int Arch Occup Environ Health. 2001;74:336-44.
51. Hines CJ, Deddens JA. Determinants of chlorpyrifos exposures and urinary 3,5,6-trichloro-2-pyridinol levels among termiticide applicators. Ann Occup Hyg 2001;45:309-21.
52. van Tongeren MJ, Gardiner K. Determinants of inhalable dust exposure in the European carbon black manufacturing industry. Appl Occup Environ Hyg 2001;16:237-45.

53. Symanski E, Chan W, Chang CC. Mixed-effects models for the evaluation of long-term trends in exposure levels with an example from the nickel industry. Ann Occup Hyg 2001;45:71-81.

CHAPTER 2:
THE VARIABILITY OF EXPOSURE OVER TIME:
A PROSPECTIVE LONGITUDINAL STUDY

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Ann Occup Hyg 1997;41:485-500.

PART I : APPLICATION OF RANDOM EFFECTS MODELS

ABSTRACT

Four hundred and forty personal air measurements were carried out on 54 workers, employed in the main processes in six different factories (6-13 in each). Potential exposure was to lead, benzene and dust. Ten randomly repeated hygiene surveys were carried out over one year. In order to estimate the magnitude of the variability in workers' exposure over time, its sources, the variance between workers and the variance within a worker, a nested unbalanced analysis-of-variance model was fitted to the logged data. Of the total exposure variance, the within variance of a worker's exposure over time was 51% (GSD=3.1) and the between workers, factories and air contaminants variance was 49%. The exposure variance between all of the workers was mainly due to variance between workers within the same factory (67%). Outdoor locations, mobility of the worker and mobility of the sources of exposure result in a positive influence on both the variance between (26%, ANOVA) and the variance of a worker over time (39%, Regression). These variables are therefore important in the sampling strategy of workers' exposure. For valid compliance testing and assessment of workers' exposure the mean and the within- and between-variance of the workers' exposure over time should be considered. The exposure should be measured several times a year randomly in order to prevent workers misclassification. To assess yearly exposure a GSD=3.1 can be used to calculate confidence limits for the arithmetic mean of worker's exposure measurements, in circumstances similar to those in this study.

INTRODUCTION

The phenomenon of exposure variability over time in the workplace has become a subject of increasing attention of late.[1-7] This variability is related mainly to changes in production cycle, to the degree of ventilation (seasonal variation etc.), to location characteristics and to work practices. It should affect the basic elements of the exposure assessment strategy in three areas: hazard control surveys, compliance tests and exposure-response relationships in epidemiological studies. Exposure variability is usually expressed by the geometric standard deviation (GSD), which is a function of the variance of the log transformed exposure levels. The total exposure variance can, therefore, be divided into two main components: the variance between- and the variance within-worker. The between-worker variance component within the same factory is essential for effective hazard control. The latter, the within-worker variance component, expresses the exposure variance in the same worker over a long period. In Israel, as in other countries, occupational health legislation is aimed at protecting the individual worker; thus, hygiene monitoring should provide a tool for assessing exposure of every worker.

In routine hygiene surveys, measurements are generally made between one to a maximum of four times per year and not necessarily on the same worker. There is a difficulty, therefore, in evaluating the between- and within-worker variance components simply on the basis of routine surveys. In order to further explore the variability between- and within-worker, there is a need for a specially designed study based on multiple repeated measurements taken over an extended period of time. Moreover, on the basis of a one-time exposure survey or successive surveys carried out over a short period, inferences are made for the long-term exposure. This practice may cause bias since a single survey may not represent the annual exposure distribution, and successive surveys may result in autocorrelated exposure results.[8] The findings of a specifically designed study can provide an estimation of long-term variance that can be used for calculating a confidence interval around the individual mean exposure. This interval estimate is more valid for assessing long-term exposure than a point estimate, such as one measurement, as it gives an indication of the precision of the estimate.[9] Due to budget as well as logistic restraints the

estimation of long-term variability has mostly been based on retrospective data in specific job-groups.

This study is a prospective one aimed at exploring long-term hygiene exposure variations based on a specific experimental design assessing the yearly exposure distribution of a cohort of workers by repeated exposure measurements, carried out at random intervals. The studied units were both individual worker and factory-group. In addition location characteristics were taken into account. The three specific aims of the study were to estimate the magnitude of exposure variability over-time including its components-the variances between- and within-worker, to explore the causes for the between-worker variance, and to model location variables affecting the within-worker variance.

METHODS

Subjects

The sample consisted of 54 workers employed in six factories, 6-13 workers in each; two car-battery factories with exposure to lead, two fuel distribution installations (not gas stations) with exposure to benzene, one machine-tools factory with exposure to hard metal dust and one power station with exposure to coal dust. The factories working with these air contaminants are obliged by law to perform periodic hygiene monitoring surveys. Each of the six factories met the following a priori characteristics: the factory would not be closed in the near future, a wide range of exposure-levels was expected (based on previous measurements), the workers and processes had not changed recently. Only workers engaged in the main production processes during the morning shift were randomly selected for monitoring. The workers employed in the factories involved in this study were not assigned to homogenous work groups since almost each job title and location was unique. The workplaces selected were publicly or privately owned; four were medium size (10-40 workers) and 2 had more than 100 workers. They are situated throughout Israel. The processes were diverse as well as the ventilation conditions. Figure 2.1 shows the distribution of location characteristics of the 54 workers studied.

Fig 2.1 Distribution of workstation characteristics of 54 workers

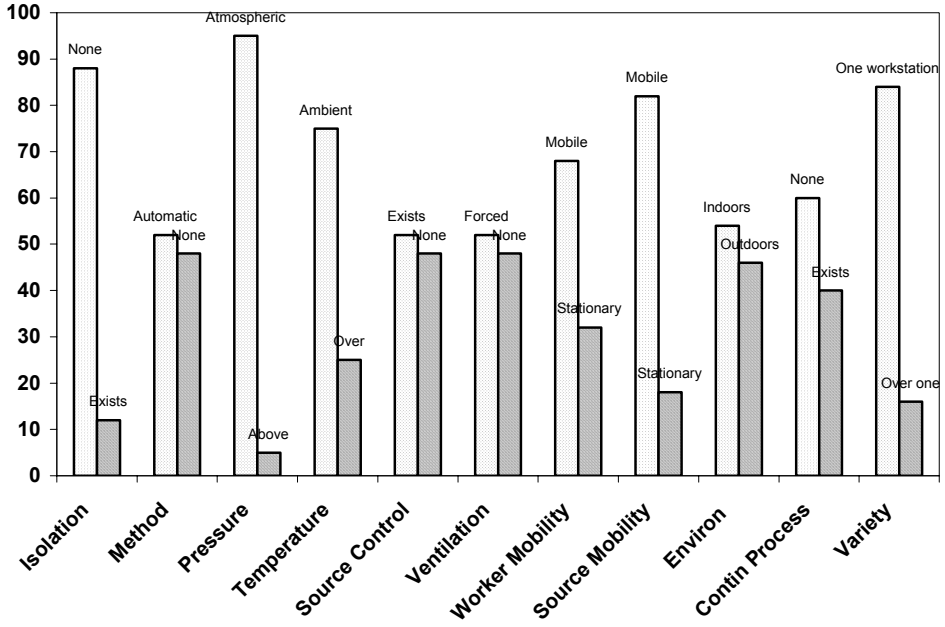


Table 2.1 Location variables

Location variable	Description	
Isolation	0-None	1-Exist
Method	0-None auto	1-Automatic
Pressure	0-Atmospheric	1-Above atmospheric
Temperature	0-Ambient	1-Over ambient
Source control	0-None	1-Exist
Ventilation	0-Natural	1-Forced
Worker mobility[a]	0-Stationary	1-Mobile
Source mobility	0-Stationary	1-Mobile
Environment	0-Indoors	1-Outdoors
Continuous process	0-Exist	1-None
Variety of locations[b]	0-One	1-Over one

NOTES:
[a] Mobility around a single work station
[b] Mobility between different workstations

Measuring protocol

In each factory we performed 10 repeated hygiene surveys within one year, at random intervals of 3-6 weeks. This was done in order to cover the seasonal changes in the weather and all the phases of the production cycle. Some of the workers were missing during some surveys due to illnesses, vacations etc. but each worker had at least 6 measurements. The same hygienist carried out all of the surveys.

Sampling and analysis were carried out by internationally accepted procedures.[10,11] The samples were personal breathing zone samples carried out in one shift, over 6-8 hours. In addition to internal quality controls, validity of the analyses was verified by participating in external quality assurance schemes from the UK[12] and the USA.[13] Surveys were carried out after prior notification to the factory.

Statistical analysis

In order to bring exposures expressed in various scales to a common scale each measurement is expressed as a fraction of the TLV-TWA according to Israeli Regulations (lead-0.1 mg/m^3, benzene-0.6 ppm, hard metals-0.05 mg/m^3, coal dust-4 mg/m^3). Goodness of fit of the logged exposure measurements, obtained from each worker, to the normal distribution were evaluated with the w-s test[14] at a significant level of 0.05. Furthermore, estimation of the arithmetic mean (AM) exposure of each worker was calculated as the direct average of the observed values due to the number ,6-10, of repeated measurements. In addition the geometric standard deviation (GSD) was calculated for each worker. Analysis of variance (ANOVA): components of the total variance in exposure were estimated by employing the random-effect ANOVA model from Proc Nested of the SAS System Software.[15] The unbalanced data were derived from a nested structured sample and were fitted to the following ANOVA Model:[16]

$$Y_{ijkl} = \log(X_{ijkl}) = \mu_y + \alpha_i + \beta_{ij} + \gamma_{ijk} + \varepsilon_{ijkl} \qquad (i=l,...,3);(j=l,2); \qquad (k=l,...,13);(l=l,...,10)$$

where,

X_{ijkl} = the exposure level in the l -th repetition within the k-th worker within the j-th factory within the i-th air contaminant.

μ_y = mean of Y_{ijkl}

α_i = the random effect of the i-th air contaminant.

β_{ij} = the random effect of the j-th factory, within the i-th air contaminant.

γ_{ijk} = the random effect of the k-th worker, within the j-th factory, within the i-th air contaminant.

ε_{ijkl} = the random effect of the l -th repetition, within the k-th worker, within the j-th factory, within the i-th air contaminant.

It is assumed that: X_{ijkl} - has a log-normal distribution. α_i, β_{ij}, γ_{ijk} and ε_{ijkl} are normally distributed with 0 means and variances $\sigma^2_\alpha, \sigma^2_\beta, \sigma^2_\gamma, \sigma^2_e$ respectively. The random effects α_i, β_{ij}, γ_{ijk} and ε_{ijkl} are assumed to be mutually independent.

The total variance σ^2_t is divided to the variance between σ^2_b and the variance within σ^2_w. In the above model the variance between is the sum of the variances $\sigma^2_\alpha, \sigma^2_\beta, \sigma^2_\gamma$ and the variance within is σ^2_e. It is important to distinguish between $\sigma^2_\gamma, \sigma^2_b$. The first one, σ^2_γ, is the variance between workers controlled for the factory and the air contaminant or in other words, the variance between workers within factory within air contaminant. The latter, σ^2_b, is the variance between workers uncontrolled for the factory and the air contaminant or in other words, the variance between workers, factories and air contaminants. The estimations of the variances σ^2_t, σ^2_b and σ^2_w are s^2_t, s^2_b and s^2_w accordingly. The corresponding geometric standard deviations are:

GSD-T= $\exp(s_t)$ GSD-W= $\exp(s_w)$ GSD-B=$\exp(s_b)$

The above model is a three way nested random effects model . One-way ond two-way random effects models were also used. The one way random effects model , with the worker as the random effect, was fitted to the data in factory groups. This model has been used by other researchers.[3,5] The two way nested random effects model, with factory and worker within factory as the random effects, was fitted to the data in the air contaminant groups.

The random-effects ANOVA model was also used to find causes for the variance between workers. Location variables were included in the model as effects, instead of the worker effect, (Proc Varcomp, SAS[15]). The extent of their weights among the total exposure

variance were considered to indicate their importance in consisting the variance between workers.

Multiple regression model: the yearly exposure-distribution of each worker can be characterized by a mean and a variance. These parameters of the whole cohort are scattered (each set has its own distribution). In order to explain the diversion of the exposure variance of a worker over time, we built a multiple regression model. The dependent variable was the SD of the logged data, which reflects the individual variance over time. Usually variances distribute chi-squared, however the SD of the logged data was found to have a normal distribution. The independent variables were dichotomous variables characterizing location (Table 2.1).

RESULTS

Distribution of exposures

Each worker in the cohort (total -54 workers) has a set of 6-10 measurements, describing the yearly distribution of his exposure. Table 2.2 shows the percent of sets fitting the normal distribution with or without either square-root transformation or natural log transformation.

Table 2.2 Goodness of fit tests to a normal distribution: percentage of sets accepted as a good fit according to W-S test

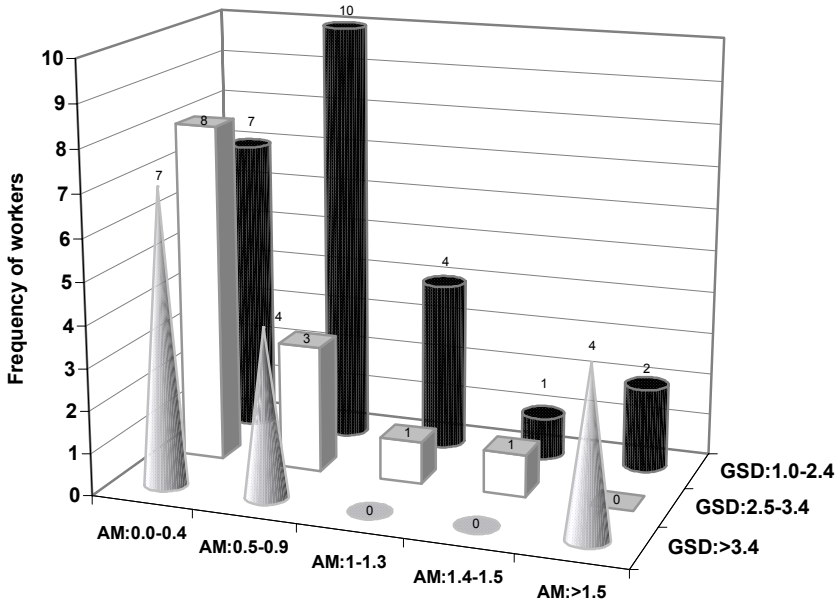
Data-type	α=0.05, P>0.95	α=0.10[a], P>0.90
Raw data	47%	37%
Transformed to square root	73%	61%
Transformed to natural log (ln)	92%	82%

NOTES: A set= worker's yearly measurements; no. of sets=54
[a] more severe criterion.

From these results it is clear that transformation to natural log resulted in the best fit to normality. Thus exposure-levels within each individual can be assumed to be log-normally

distributed, which is a common distribution for hygiene exposures. [5,8,17-19] The joint distribution (in categories) of AM and GSD, in the whole cohort, is shown in Fig. 2.2.

Fig 2.2 Yearly exposure distribution of the 54 workers by categories of arithmetic means (AM) and geometric standard deviations (GSD); exposure expressed as TLV fraction

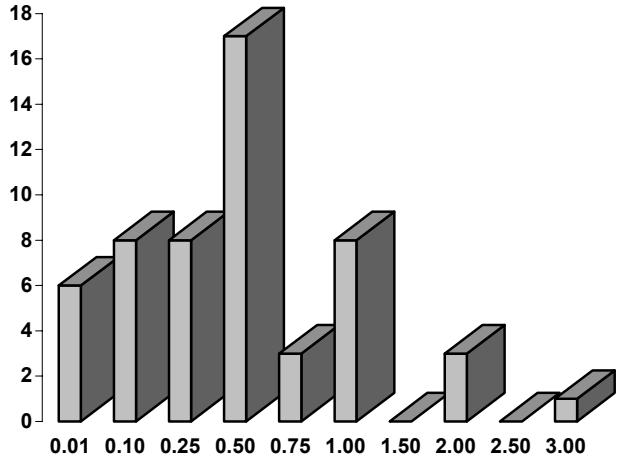


Exposure variability- components

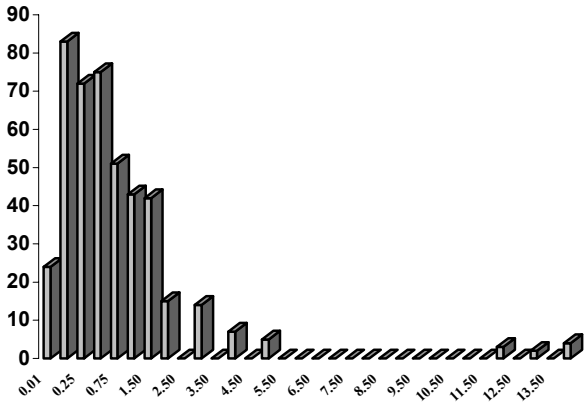
The total and between distributions of the exposure are shown in Fig. 2.3a and 2.3b.

Fig 2.3 (a) Distribution of 440 exposure measurements of 54 workers, 6-10 measurements per worker, within a year. (b) Distribution of yearly median exposures of 54 workers.

(a)



(b)



We used the nested ANOVA random effects model, shown in Equation (1) to estimate the variance components between- and within and their weights (Table 2.3).

Table 2.3 Analysis of exposure variance between workers and factories and within a worker in groups exposed to the various air contaminants

Air contaminant exposure group	Main component	Sub component	DF	VAR	GSD	% of variance among total variance
Lead	Between	Factory	1	0.25	1.6	15
		Worker[a]	17	0.27	1.7	17
	Within (= Error)		141	1.10	2.9	68
	Total		159	1.62	3.6	100
Benzene	Between	Factory	1	0.00	1.0	0
		Worker	14	2.05	4.2	52
	Within (= Error)		117	1.91	4.0	48
	Total		132	3.96	7.3	100
Dust	Between	Factory	1	1.23	3.0	51
		Worker	17	0.35	1.8	15
	Within (= Error)		128	0.83	2.5	34
	Total		146	2.41	4.7	100

NOTES: A nested model. 440 measurements on 54 workers.
DF= Degrees of freedom ; VAR=The variance of the log transformed data
[a] The effect of the worker within the factory

The between component for each air contaminant consists of the nested random effect of the worker within the factory and the random effect of the factory. The exposure variance components of each factory can be seen in Table 2.4.

Table 2.4 Analysis of exposure variance between and within worker according to air contaminant and factory level.

Air Contaminant	Exposure Group	Number of Workers	n	GSD-T	GSD-B (% of Total Variance)	GSD-W (% of Total Variance)
Lead	Factory 1	10	78	2.4	1.6	2.1
					(31)	(69)
	Factory 2	9	82	4.4	1.8	3.9
					(15)	(85)
Benzene	Factory 3	10	54	5.7	3.5	3.3
					(52)	(48)
	Factory 4	6	79	8.4	4.6	4.4
					(51)	(49)
Dust	Factory 5	13	106	2.8	2.0	2.2
					(42)	(58)
	Factory 6	6	41	5.1	1.0	5.1
					(0)	(100)

NOTES: n = no. of Measurements; GSD-T=Geometric Standard Deviation, Total GSD-B=Geometric Standard Deviation, Between-Workers; GSD-W=Geometric Standard Deviation, Within-Worker; % of Total Variance=Percentage of Between or Within Variance of the Total Variance

It can be seen from Table 2.3 that the variance of the random effect of the worker within the factory, in each air contaminant, was different and greater than zero. For each contaminant, this means that the random effects of the workers within factory were not the same. It can be concluded, therefore, that in the population there were differences in median exposures (in the population, median=GM) among the workers within each factory. In addition as the weights of the random effects of the factory in each contaminant were: 15% for lead, 0% for benzene and 51% for dust, it was concluded that there was no difference in the median exposure level between the two benzene factories, there was a slight difference in the median exposure between the two lead factories and that there was a

large difference between the two dust factories. Looking into the weights of the within-variances in each air contaminant each of them was over a third of the total variance. An overview of the whole cohort is shown in the Table 2.5.

Table 2.5 Analysis of exposure variance between workers, factories and air contaminants and within a worker

Main Component	Sub Component	DF	VAR	GSD	% of variance among total variance
Between	Air contaminant	2	0.00	1.0	0
	Factory[a]	3	0.39	1.9	16
	Worker[b]	48	0.82	2.5	33
	Total	53	1.21	3.0	49
Within (= Error)		386	1.29	3.1	51
Total		439	2.47	4.8	100

NOTES: A nested model. 440 measurements on 54 workers
DF=Degrees of freedom
VAR=The variance of the log transformed data
[a] The effect of the factory within the air contaminant
[b] The effect of the worker within the factory within the air contaminant

The table relates to four components of the the total variance, the weights of each sum up to 100% of the total variance. The variance within has the greatest weight, 51%. The second component is the variance between workers within the same factory within specific air contaminant and accounts for 33%. The third component weighs 16%, the variance between factories within the same air contaminant. There is no variance between the median exposures of the air contaminants (weight=0%).

Factors affecting the within- and between-variance

To model the relationship between the within-worker variance and factors in the individual workers locations we used the regression model.

Table 2.6 Relationship between worker's exposure variance over-time[a] and location characteristics -linear regression models

Work Station characteristics	R^2	P Fitness	P[b] Intercept	P[b] Environ	P[b] Worker M	P[b] Source M	P[b÷] Variety
All work station characteristics (11 characteristics)	.45	.0035	NS	.0108	.0688	NS	NS
Environ	.33	.0001	.0001	.0001	---	---	---
Environ,Worker M.	.36	.0001	.0001	.0010	NS	---	---
Environ, Source M.	.38	.0001	.0001	.0001	---	NS	---
Environ, Variety	.37	.0001	.0001	.0001	---	---	.0909
Environ, Worker M., Source M.	.40	.0001	.0001	.0006	.0569	.0897	---
Environ, Worker M., Variety	.40	.0001	.0001	.0024	.1177	---	.0711
Environ, Source M., Variety	.38	.0001	.0001	.0001	---	NS	.0993
Environ, Worker M., Source M., Variety	.43	.0001	.0001	.0016	.0421	.0901	.0719

NOTES: Environ= Environment: outdoors/indoors; Worker M= Worker Mobility: stationary/mobile; Source M= Source Mobility: stationary/mobile; Variety= Variety of locations: one/two+

[a] GSD of the logged data

[b] Significance of the coefficient in the regression model

The dependent variable was the SD of the logged data. From all possible regressions the best models characterizing the relationship (according to R^2 and *P*-values of the coefficients) are shown in Table 2.6.

Four variables in the individual worker location were significantly related to the within variance: environment, mobility of worker, mobility of source and variety. The environment alone had the most significant relation with the within variance (R^2=0.33). The environment (P=0.0006) together with the mobility of the worker (P=0.0569) and the

mobility of the source (P=0.0897) raised the correlation by 7% (R^2=0.40). The variety of work locations of the individual worker and the mobility of the worker raised also the correlation in the model but the coefficients were less significant.

In order to find causes for the variance between workers we investigated certain random effects of worker-location which replaced the worker effect, in two ANOVA models. The first model included the random effects of air contaminant, factory, mobility of worker, mobility of source, and environment. These effects should explain the variance between workers as they replaced the worker effect. It was found that the factory accounted for 13% of the total variance, the mobility of source 17%, and the environment, 5%. The random effects of the air contaminant and worker's mobility had 0% weight. Thus the variance between workers can be attributed to the mobility of the source, the environment and the factory. In the second model only three random effects were included: mobility of the worker, mobility of the source, and environment. Similar weights for the random effects of source mobility and environment were found (18%+8%= weight of 26%).

DISCUSSION

Recently it has been suggested that assessment should be based on an ongoing process since exposure varies with time.[5,17,18] By collecting exposure data over a period, which fully covers varying production patterns and external environmental conditions, the statistical depictors (mean and standard deviation) should have more validity in representing the true exposure distribution over time. Very few systematic studies have been carried out on this subject. One was based on on 780 workers with 3321 measurements[6] and another one used this data with additional sources, resulting in a study involving ~1600 workers with ~14000 measurements.[3] Both of them were retrospective. Another study was carried out over consecutive days and was based on 4 repeated measurements for each job group.[20] Another study that concentrated only on flour dust exposure performed three repeated measurements over a period of one week only.[21]

Our research was a prospective one carried out over a period of one year in each factory. Over this period ten surveys were performed at random intervals with approximately 3-7

weeks between each survey. Our protocol was aimed at removing any serial correlation of consecutive measurements (autocorrelation). Such autocorrelation may be caused by similarities in the production and ventilation conditions, which may occur when carrying out the surveys day after day. This prospective study was able to control for confounding variables, such as those related to the circumstances of production, the strategy of sampling, and variation between hygienists.[22]

Our nested sampling design (sampling workers within factories within air contaminants) enabled us to look simultaneously into sub-components responsible for the overall variability in exposure. The above nested model has four random effects: air contaminant, factory, worker and error. This model can be extended to include also the random effect of a homogenuous /homomorphic group, in which case the model will have five random effects: air contaminant, factory, group, worker and error. In such a case the between workers variation is controlled for three factors, the air contaminant, the factory and the group.

The unbalanced nested ANOVA model assumes conditional independent repeated measurements. Empirically, we found an almost constant simple correlation (R^2=~0.3) between repeated measurements. However, we chose to use this model since it takes into account both the nested and the unbalanced situation, which is the case in our study. In addition the use of a random effect model allows us to make comparisons with other studies that have used the same model. Other models cannot handle a nested design or omit some of the measurement results in order to convert an unbalanced design to a balanced one. This omission could give rise to the loss of important exposure information. In addition, for the ANOVA model we assumed homoscedacity, a uniform within variance, which is a common assumption, especially after having carried out a log-transformation of the data. Due to the robustness of the model this assumption is reasonable. Empirically the variances of the individual workers over time were diverse. Hence, when the study group is large enough it is more valid, statistically, to divide it into homogeneous sub-groups according to the long term variances of the individual workers.

In looking for the combination of variables which together are responsible for the single worker variance over time we used a multiple regression model. This kind of investigation

into variables related to the between- and within-worker variances can contribute to a subject, which has health related and legal implications.

Total variance

As the exposure data were found to fit a log-normal distribution, the total exposure variance is characterized by GSD-T (geometric standard deviation-total). For the whole cohort the GSD-T was 4.8, for the lead workers GSD-T was 3.6, for the benzene workers GSD-T was 7.3 and for the dust workers GSD-T was 4.7. Two studies which relied on homogenous job-title groups[3,20] reported much lower values, 2.4 and 2.5 respectively, the former relating to various chemicals and the latter to sodium borate dust only. A comparison between our data which consisted of sampling workers within factories in a nested sample with 10 randomly repeated measurements within a year and the above studies[3,20] may not be reasonable due to the difference in the study design. They used job-title exposure groups and small number of repeated measurements or short measuring period, repeated on four consecutive days.

A notion expressed[17] that exposure to dust tends to vary more than other agents was not substantiated by our data, but was, however, consistent with the data of other studies.[3,20] In contrast to the above mentioned studies based on homogenous job-title groups, our sample represented workers in the main processes within factory within air contaminant. In order to carry out an overall exposure assessment in factories it is recommended to sample workers randomly within homogenous job-title etc. groups.[17,18] Nowadays it is realized that such homogenous groups based on an observational strategy are not truely homogenous.[7] The majority of the factories examined (~70%) employed less than 40 workers and the subgroups according to job-titles consisted of only 1-3 workers. From our previous experience of these and other similar factories a wide range of between worker exposure levels was expected. Therfore it was not reasonable to define homogeneous groups according to exposure levels and hence our studied unit was the individual worker. The factory and subgroup size is typical of the situation in Israel (and perhaps also in other countries) and is too small for analysis of variance related to job title groups.

Main variance components

The variance of occupational exposure in each group of workers, consists of two main components : the variance within a worker, and the variance between workers, factories and air contaminants. During our analysis of the variance components we found that the within variance was half (51%) of the total variance of the long-term exposure of the whole cohort. The other half (49%) of the total variance was due to the variances between workers, factories and air contaminant. Other studies mentioned above reported higher weights of within variances compared to the between ones. This observation may be mainly due to their homogenous job-title subgroups.[3,20]

As the workers in our study represented different processes in factories we expected a priori that the sum of the weights of the variation between workers, factories and air contaminants would be greater than the weight of the variance-within. However, as mentioned above, this was not the case; we found an almost even split contribution of the within- and between- variances. The within-variance should, therefore, be given high attention when conducting exposure assessment. Usually in hygiene surveys for diagnostic and compliance tests this issue is ignored.

As regards the magnitude of the variance components, the first component, the GSD within workers, was found to be 3.1, which is a higher value than that suggested in other countries e.g. Holland[2] 2.7 , Great Britain[17] 1.8. The reasons for our higher variance component may be related to our different study design, different work situations and work practices and different meteorological conditions.

Our findings on the magnitudes and weights of the two variance components have practical implications. Regarding the control of hazards, when the within variance is high, efforts should be addressed to reduce the exposure variability within locations; the within variability gives information on the relative importance of periods of high exposure in the long-term exposure. There is a need to control the source of exposure, to improve local ventilation and to ensure that standard operating procedures are written and are being applied.

Regarding compliance testing, when focusing on a single measurement and hence ignoring the within and between variances, the long-term exposure picture is missing. This might cause over-exposed workers to be classified as non-exposed and vice versa. In situations in

which regulations require periodic monitoring, or when a workplace requests a follow-up survey, the sampling strategy should be based on two dimensions-the magnitude of the within worker variance as well as the between worker variance, since both of them are high. In the subsequent surveys, therefore, measurements should take into account these factors including repeat measurements on the same workers.

For evaluating exposure-response relationships, when the variance is not taken into account the relationship can be attenuated artificially.[23]

Factors affecting the variance components

In our nested design sample, the total variance consisted of the following sub-components:

(a) Variance between different agents;

(b) Variance between different factories within the same agent;

(c) Variance between different workers within the same factory within the same agent;

(d) Variance between different times of measuring within the same worker within the same factory within the same agent.

The first three sub-components accumulate to the variance between component and the last one is the within variance component over time. According to the nested and unbalanced analysis of variance model we found that the weight of the sub-component (c), the variance between workers within the same factory within the same agent, was the highest (67%) amongst the first 3 sub-components, indicating a diversity in median-exposure levels among workers in the same factory. This sub-component reflects the differences between location characteristics and individual work habits/practices ("dirty" or "clean" workers for example) in the same factory. A further investigation of the location characteristics, revealed that environment (indoors/outdoors) and the mobility of source (stationary/mobile), were partly responsible for this variability. Within a particular factory, conclusions cannot be drawn on group exposures on the basis of one or a few individual measurements. In many situations, group medical examinations are carried out on the basis of these individual measurements. In some cases this may result in a waste of valuable resources. In other cases it may be decided not to carry out group medical examinations and at some future date this could affect the health of the worker.

The weight of sub-component (b), the variance between factories within the same agent, was 37% of the first 3 sub-components, meaning a medium-diversity (on a TLV scale) in median-exposures between these factories. This reflects the differences between the structure, geographical location etc. of the factories within the same agent. Unexpectedly, the weight of sub-component (a) was 0%, meaning no-difference in median exposures (on TLV scales) among the three agents. The findings that the variance between agents was zero and that the variance between workers within the same factory was high, suggest that the strategy of homogenous group exposure according to air contaminant should be considered alongside other exposure assessment strategies. This finding should be a subject for further investigation.

Sub-component (d), the variance between measurement times of the year within the same worker within the same factory within the same agent was the highest weight 51% among the four sub-components, showing that it has a major importance in exposure variability. As previously mentioned this is an important factor in planning an exposure measuring strategy.

By a multiple regression model it was found that the amount of variability in a worker's exposure level over time is related to the environment of the location (R^2=0.33,*P*=0.0001). These finding were similar to those of another study.[3] In outdoor locations, the environmental variability was higher than indoors. This variability was raised for mobile workers or mobile sources of exposure than for non-mobile ones (R^2=0.40, *P*=0.0001). However, as the time factor has a significant effect on the exposure levels, long-term worker exposure should be assessed by an interval estimate relating to the exposure variability over time instead of a point estimate based upon one or two measurements. Recently it has been suggested[5] that the arithmetic mean is more relevant to long term risk assessment and thus the confidence interval should be a confidence interval for arithmetic means of data originating from a log-normal distribution.[24] This is laborious to compute and therefore approximations were suggested.[9] Within the last year, a confidence interval has been suggested for the arithmetic mean of a group of workers exposed over a long period of time.[25] In general, the number of repeated measurements is less than five and exposure variability is high, as was found in this study. Thus in these situations one should use the exact confidence limit instead of the approximation.[9] One should take into

consideration that the confidence interval assumes independence between repeated measurements which is not always the case. In addition, the large variation causes a very large confidence interval, especially when the confidence level is high. For example, if AM=0.5TLV, GSD=3.0, with five repeated measurements and a confidence level of 95% , the exact confidence interval is then (0.2-25 TLV). Therefore it is extremely desirable to increase the number of repeated measurements. In any case, there is a need to find technical solutions that will allow continuous measurements to be carried out over long periods relatively cheaplyin order to have a valid assessment of long term exposure to hazardous chemicals that affect the health of the worker.[5]

CONCLUSIONS AND RECOMMENDATIONS

The exposure variance both within- and between-worker are high. For a valid assessment of exposure, a single measurement/survey of hygiene-exposure is insufficient. Rather, the level of exposure should be measured repeatedly over a year, with intervals of several weeks.

The present method of compliance testing should be replaced with one in which the mean and standard deviation of the exposure over time should be taken into consideration The traditional approach is based on a dichotomy where a worker's exposure level can only be above or below the TLV in each single measurement. Due to the worker's high exposure variance over time, workers might be classified as overexposed when this is not the case and vice versa. In addition, in order to present the exposure distribution in a factory it is essential that the strategy of workers' sampling should rely upon statistical sampling methods to account for both the variance within- and between-worker, since both of them are high.

For both sampling of workers for exposure assessment in individual surveys and over time, one should take account of those working in different environments (indoor, outdoor) and mobilities (the source and the worker) in order to cover a large range of workers' exposure distribution. In similar circumstances as described in this study, a primary estimator of the environmental variability in exposure level over time can be: GSD=3.1. In the absence of

more specific information, this value can be used to calculate the confidence limits of the mean exposure findings when measuring exposure levels of workers. Grouping of workers according to the agent may not be relevant in identifying homogenous exposure groups.

There is a need to study the construction of the correlation matrix between randomly repeated measurements. In order to improve the statistical analysis required in hygiene exposure assessment, the consistency of various statistical models using the same data with various matrix covariance/correlation constructions should be studied.

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REFERENCES

1. Burdorf A. Shifting concepts in assessment of occupational exposures. Ann Occup Hyg 1993;37:447-450.
2. Buringh E, Lanting R. Exposure variability in the workplace: its implication for the assessment of compliance. Am Ind Hyg Assoc J 1991;52:6-13.
3. Kromhout H, Symanski E, Rappaport SM. A Comprehensive evaluation of within- and between- worker components of occupational exposure to chemical agents. Ann Occup Hyg 1993;37:253-270.
4. Nicas M, Simmons BP, Spear RC. Environmental versus analytical variability in exposure measurements. Am Ind Hyg Assoc J 1991;52:553-557.
5. Rappaport SM. Assessment of long-term exposure to toxic substances in air. Ann Occup Hyg 1991a;35:61-121 .
6. Rappaport SM. Selection of the measures of exposure for epidemiology studies. Appl Occup Environ Hyg 1991b;6:48-457.
7. Rappaport SM, Kromhout, H, Symanski E. Variation of exposure between workers in homogenous exposure groups. Am Ind Hyg Assoc J 1993;54:654-662.
8. Francis M, Selvin S, Spear R, Rappaport SM. The effect of autocorrelation on the estimation of workers' daily exposures. Am Ind Hyg Assoc J 1989;50:37-43.
9. Armstrong BG. Confidence intervals for arithmetic means of lognormally distributed exposures. Am Ind Hyg Assoc J 1992; 53:481-485.

10. NIOSH Occupational Exposure Sampling Strategy Manual, Publication No. 77-173. US Department of Health, Education and Welfare, National Institute for Occupational Safety and Health, Chincinnati, Ohio, 1977.
11. NIOSH Manual of Analytical Methods, 3 ed. US Department of Health, Education and Welfare, National Institute for Occupational Safety and Health, Cincinnati, Ohio, 1985.
12. HSE Workplace Analysis Scheme for Proficiency. Health and Safety Executive, Sheffield, UK, 1995.
13. NIOSH Proficiency Analytical Testing ProgrAM US Department of Health, Education and Welfare, National Institute for Occupational Safety and Health, Cincinnati, Ohio, 1995.
14. Shapiro SS, Wilk MB. An analysis of variance test for normality. Bimetrica 1965;53:591-611.
15. SAS (1989) SAS User's Guide 6 ed. SAS Institute , North Carolina.
16. Snedecor GW, Cochran WG. Statistical Methods. Iowa State University Press, Iowa, 1967.
17. BOHS Sampling Strategies for Airborne Contaminants in the Workplace. Technical Guide No.11. H and H Scientific Consultants Ltd, British Occupational Hygiene Society, Leeds, UK, 1993.
18. Boleij J, Buringh E, Heederik D, Kromhout H. Occupational Hygiene of Chemical and Biological Agents. Elsevier, Amsterdam, The Netherlands, 1995.
19. Oldham P. The Natyre of the Variability of Dust Concentrations at the Coal Face. Br J Ind Med 1953;10:227-234.
20. Woskie SA, Shen P, Eisen EA, et al. The real time dust exposures of sodium borate workers: examination of exposure variability. Am Ind Hyg Assoc J 1994;55: 207-217.
21. Nieuwenhuijsen MJ, Lowson D, Venebles KM, Newman Taylor AJ. Flour dust exposure variability in flour mills and bakeries. Ann. Occup. Hyg 1995;39:299-305.
22. Olsen E, Laursen B. Bias and Random Errors in Historical Data of Exposure to Organic Solvents. Am Ind Hyg Assoc J 1991;52:204-211.
23. Preller L, Kromhout H, Heederik D, Tielen M. Modelling Long-Term Average Exposure - An Application in Occupational Exposure-Response Analysis. Wageningen University, Wageningen, The Netherlands, 1995.
24. Crown EL, Shimizu K. Lognormal Distributions: Theory and Applications. Marcel Dekker Inc, NY, USA, 1988.
25. Rappaport SM, Lyles RH, Kupper LL. An exposure assessment strategy accounting for within- and between worker sources of variability. Ann Occup Hyg 1995;9:469-495.

PART II: APPLICATION OF MIXED EFFECTS MODELS

INTRODUCTION

In our year-long longitudinal exposure study we used a one way random-effects model,[1] as most researchers[2-7] do to estimate variance components, ignoring the work characteristics. Since work characteristics usually are fixed effects, random effects models are not the appropriate statistical tool to handle them.

The mixed-effects model can model the influence of both fixed and random work-environment characteristics, on the observed exposure levels and estimate the within- and between-worker variance components controlled for work characteristics and their standard errors. In addition, mixed-effects models are able to identify and deal with the presence of time trends in the concentrations of air pollutants.

In this appendix we illustrate the benefits of using the mixed effects models for our data in comparison to a random effects model.

METHODS

Eleven characteristics of the working conditions were recorded in each workstation in our study. Four of these variables appeared to affect exposure levels in a statistically significant manner: work method (automated/non-automated), temperature (ambient/above-ambient), mobility of the worker (stationary/mobile) and mobility of the source (stationary/mobile). The influence of these four work characteristics on the estimated variance components was evaluated by applying the mixed-effects model for unbalanced data. A one way random-effects model was used as a reference.

Existence of a time-trend was also examined, by dividing the 10 measurements sessions into 2 periods: the first five measurements and the last five measurements.

We assumed a compound symmetry structure of the correlation matrix, as there was no reason to assume a more complex structure and we also assumed independence across subjects.

The mixed-effects model for unbalanced data is specified by the following expressions:

$$y_{ij} = \beta_0 + \beta_1 X_{ij1} + \ldots\ldots\ldots + \beta_p X_{ijp} + b_i + \varepsilon ij$$

For i=1,.....,k (workers) and j = 1,.....,n_i (repetitions of the i'th worker)

Where:

Y_{ij} = log-transformed exposure levels (devided by the TLV)

β_0 = overall (fixed) group mean; mean of Y_{ij}

$\beta_1, \ldots\ldots\ldots, \beta_p$ = fixed effects (= covariates)

$x_{ij1}, \ldots\ldots\ldots\ldots, x_{ijp}$ = values of the effects for the i'th worker on the j'th day

b_i = i'th worker random effect

It is furthermore assumed that:

$b_i \sim N(0, \sigma_b^2)$, b_i's are all independent ; $\varepsilon_{ij} \sim N(0, \sigma_w^2)$, ε_{ij}'s are all independent

$\sigma^2 = \sigma_b^2 + \sigma_w^2$; σ_b^2 = variance between-workers; σ_w^2 = variance within-workers

$\rho = \sigma_b^2 / \sigma$;

$$corr(y_{ij,} y_{il}) = \begin{cases} 1 & j = l \\ \rho & j \neq l \end{cases} \qquad cov(y_{ij,} y_{il}) = \begin{cases} \sigma^2 & j = l \\ \rho\sigma^2 & j \neq l \end{cases}$$ compound symmetry structure

For every i , i= 1,.....,k and for every j,l , j,l = 1,.....,n_i

(the y_{ij}'s of the same worker are correlated, those of different workers are uncorrelated)

To model the influence of work characteristics on the exposure levels they were considered as fixed effects in the above model :

β_1 = method effect

β_2 = temperature effect

β_3 = worker mobility effect

β_4 = source mobility effect

x_{ij1} = method value (0-automated/1-none) for the i-th worker on the j-th day

x_{ij2} = temperature value (0-ambient/1-above) for the i-th worker on the j-th day

x_{ij3} = worker mobility (0-stationary/1-mobile) for the i-th worker on the j-th day

x_{ij4} = source mobility (0-stationary/1-mobile) for the i-th worker on the j-th day

To identify time trends in the above model a fixed time effect was added to the model ,

β_5 = period

x_{ij5} = period value (0-first period /1-second period), for the i'th worker on the j-th day

The one-way random-effects model used is a specific case of the mixed-effects model with an equation that includes only the workers' random effects.

Both models were analysed by using PROC MIXED from the SAS System Software Version 6.1.[8] Variance components were estimated by the Restricted Maximum Likelihood (REML) method. The exposure concentrations were assumed to be log-normally distributed.[9-11]

RESULTS

The estimated variance components controlled for the 4 work characteristics that resulted from applying the mixed effects model are presented in table 2.7, as well as the one way random-effects model variances estimators, which are uncontrolled for work characteristics.

From the table it can be seen that the within-worker variability in exposure concentrations (s^2ww) is significantly greater than zero in all factories with both models. The within-worker variance component remained unchanged when the time-independent covariates were added in the mixed-effects model.

However, a distinct reduction was observed for the between-worker variance component (s^2bw) in four factories (1, 2, 4, and 6). In factory 4, the working method alone reduced the between worker variability in exposure concentrations by 85% (from 2.30 to 0.35). In factory 6 the limited amount of between-worker variability present was completely explained by differences in working method. In factory 2 it was mainly the temperature that reduced the between worker variability by 59% (from 0.29 to 0.12). In factory 1, mobility of the source was found to have a significant effect, resulting in a reduction of 100% of the between-worker variability in exposure concentrations (from 0.23 to 0.00). In factory 5 none of these covariates had a significant effect on the exposure levels and therefore on the between-worker variability.

No statistically significant time-trend was found for each of the factories. The estimators of the variance components of the exposures remained unchanged in the mixed-effects model when period was taken into account.

When comparing the estimated variance components in the two models with (mixed model) and without (random effects model) work characteristics, in general as expected variance components were lower in the mixed model. Specifically, the work characteristics affected the between-worker variance estimators consistently (59-100%).

Table 2.7 One-way random-effects and mixed-effects models for exposures in six factories

				Random-effects model		Mixed-effects model				
Factory	n	k	variance	est (se)	p	est (se)	p	Covariates[a]	β[b]	p[c]
1	78	9	s^2bw	0.23 (0.14)	ns	0 (.)[d]	.	Intercept	-1.56	.02
battery			s^2ww	0.54 (0.09)	.0001	0.53 (0.09)	.0001	c1	-0.41	ns
lead								c2	0.21	ns
								c3	-0.21	ns
								c4	1.49	<.01
2	82	10	s^2bw	0.29 (0.23)	ns	0.12 (0.21)	ns	Intercept	-1.93	<.01
battery			s^2ww	1.64 (0.27)	.0001	1.65 (0.27)	.0001	c1	-0.94	.11
lead								c2	1.31	.01
								c3	0.24	ns
								c4	0.38	ns
3	54	6	s^2bw	1.56 (1.09)	ns	1.75 (1.92)	ns	Intercept	-3.01	ns
fuel			s^2ww	1.46 (0.30)	.0001	1.46 (0.30)	.0001	c1	-0.14	ns
benzene								c2	2.89	ns
								c3	0.00	nr
								c4	1.11	ns
4	79	10	s^2bw	2.30 (1.22)	.0587	0.35 (0.32)	ns	Intercept	0.25	ns
fuel			s^2ww	2.21 (0.38)	.0001	2.21 (0.38)	.0001	c1	-2.78	<.01
benzene								c2	0.00	nr
								c3	0.00	nr
								c4	0.00	nr
5	106	13	s^2bw	0.45 (0.21)	.0372	0.54 (0.29)	.0649	Intercept	-0.49	ns
tools			s^2ww	0.64 (0.09)	.0001	0.64 (0.09)	.0001	c1	-0.27	ns
cobalt								c2	-0.46	ns
								c3	0.42	ns
								c4	0.00	nr
6	41	6	s^2bw	0.07 (0.16)	ns	0 (.)	.	Intercept	-2.38	.03
power			s^2ww	1.33 (0.32)	.0001	1.30 (0.30)	.0001	c1	0.75	.07
coal								c2	0.00	nr
								c3	0.00	nr
								c4	-0.23	ns

NOTES: n = no. of measurements ; k = no. of workers; est = estimator; se = standard error ; s^2bw = between- worker variance component; s^2ww = within-worker variance component

[a] covariates: c1 = method: automated (0) / non-automated (1); c2 = temperature: ambient (0) / above-ambient (1);c3 = worker mobility: stationary (0)/mobile (1);c4 = source mobility:stationary (0)/mobile (1)

[b] coefficients for fixed effect related to log-transformed standardised (TLV fractions) concentrations;

[c] p-value: ns = non-significant, p>0.11 ; nr = non-relevant (covariate is constant in all workers or is correlated with other covariates);

[d] cannot be estimated due to numerical limitations

DISCUSSION

The re-analyses with the mixed model resulted in criteria for uniformly exposed groups of workers. Grouping workers into uniformly exposed sub-groups is an inherent part of exposure assessment[9-11] and there is so far only limited experience with optimisation of these strategies.[9,12,13]

In four out of six factories, three factors 'working method', 'temperature' and 'source mobility', were found to significantly affect exposure. They reduced the between-worker variance component by 59%-100 in the different factories. In this example, *a priori* grouping based on job titles was not meaningful since almost each worker had a different task. However, *a posteriori* grouping by 'working method' (automated/non-automated), 'temperature' (ambient/over-ambient), and 'source mobility' (mobile/not-mobile) based on the results of the mixed model, will result in more uniformly exposed groups of workers.

In contrast, the available information on work characteristics did not allow for explanation of within-worker variability in exposure concentrations, which was quite high in most factories, since these characteristics were constant for the same worker during the one-year follow-up period.

In summary, in this study, the comparison between the variance components estimators in the nested models; the mixed model which accounted for the work characteristics and the random effects model which did not account for the work characteristics enabled us to evaluate the extend of the combined "contribution" of those specific work characteristics to the variance between workers within an exposure group. Consequently, more refined homogeneous exposure-groups can be declared for further exposure assessment surveys and epidemiological dose-response studies.

REFERENCES

1. Peretz C, Goldberg P, Kahan E, Grady S, Goren A. The variability of exposure over time: a prospective longitudinal study. Ann Occup Hyg 1997;41:4:485-500.
2. Burdorf A, Lillienberg L, Brisman J. Characterization of exposure to inhalable flour dust in Swedish bakeries. Ann occup Hyg 1994;38:67-78.
3. Preller L, Kromhout H, Heederik D, Tielen M. Modelling long-term average exposure in occupational exposure-response analysis. Scand J Work, Environ, Health 1995;21:504-12.

4. Nieuwenhuijsen, MJ, Lowson D, Venable KM , Newman Taylor AJ. Flour dust exposure variability in flour mills and bakeries. Ann Occup Hyg 1995;39:299-305.
5. Kumagai S, Kusaka Y, Goto S. Cobalt exposure level and variability in the hard metal industry of Japan. Am Ind Hyg Assoc J 1996;57:365-9.
6. Woskie SA, Shen P, Eisen EA, et al.. The real time dust exposures of sodium borate workers: examination of exposure variability. Am Ind Hyg Assoc J 1994;55:207-17.
7. Symanski E, Kupper LL, Kromhout H, Rappaport SM. An Investigation of systematic changes in occupational exposure. Am Ind Hyg Assoc J 1996;57:724-35.
8. SAS. SAS/ STAT software, changes and enhancements. SAS Institute , USA, North Carolina, 1996.
9. Boleij J, Buringh E, Heederik D, Kromhout H. Occupational hygiene of chemical and biological agents, Amsterdam, The Netherlands, Elsevier, 1995.
10. Rappaport SM, Smith TH. Exposure assessment for epidemiology and hazard control. ACGIH, Michigan, USA, Lewis Publishers Inc. 1991.
11. Rappaport SM, Kromhout H, Symanski E. Variation of exposure between workers in homogenous exposure groups. Am Ind Hyg Assoc J 1993;54:654-62.
12. Kromhout H, Loomis DP, Kleckner RC, Savitz DA. Sensitivity of the relation between cumulative magnetic field exposure and brain cancer mortality to choice of monitoring data grouping scheme. Epidemiology 1997;8:442-5.
13. Seixas, NS, Sheppard L. Maximizing accuracy and precision using individual and grouped exposure assessments. Scand J Work, Environ, Health 1996;22:94-101.

CHAPTER 3:
APPLICATION OF MIXED-EFFECTS MODELS FOR EXPOSURE ASSESSMENT

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ABSTRACT

The benefits of using linear mixed effects models for occupational exposure assessment were studied by re-analysing three data sets from two published surveys with repeated exposure measurements. The relative contributions of particular characteristics affecting exposure levels were assessed as in a multiple regression model, while controlling for the correlation between repeated measurements. While one-way ANOVA allows one only to estimate unconditioned variance components, a mixed model enables estimation of between- and within-worker variance components of exposure levels while accounting for the fixed effects of work characteristics. Consequently, we can identify the work characteristics affecting each variance component. Mixed models were applied to the data sets with repeated measurements and auxiliary information on work characteristics. The between-worker variance components were reduced by 35%, 66% and 80% respectively in the three data sets when work characteristics were taken into account. The within-worker (day-to-day) variability was reduced only in the pig farmer data set, by 25%, when accounting for work activities. In addition, coefficients of work characteristics from the mixed model were compared with coefficients resulting from originally published multiple linear regression models. In the rubber manufacturing data, the coefficients of the mixed model showed similar relative importance, but were generally smaller than the coefficients from regression models. However, in the pig-farm data, only the coefficients of work activities were somewhat reduced. The mixed model is a helpful tool for estimating factors affecting exposure and suitable variance components. Identifying the factors in the

working environment that affect the between-worker variability facilitates *a posteriori* grouping of workers into more uniformly exposed groups. Identifying the factors that affect the within-worker variance is helpful for hazard control and in designing efficient sampling schemes with reference to time schedule.

INTRODUCTION

Ideally, exposure assessment of air pollutants at the workplace should be based on repeated measurements on randomly selected days of a randomly selected number of workers from *a priori* defined occupational groups.[1-5] Usually measurements done on the same worker are correlated. Since exposure varies both within and between workers in a given exposure group,[1,2,4,6,7] these variance components should be taken into account in exposure assessment and for more effective hazard-control, as well as in compliance testing and evaluation of exposure-response relationships.[8-11]

Understanding the factors in the work environment that affect mean exposure levels enables the estimation of the between- and within-worker variance components conditioned on these factors. The identification of uniformly exposed groups of workers is essential for valid compliance testing and exposure-response evaluation. Identification of the factors in the work environment that are related to the between-worker variance component enables sub-grouping of workers into more uniformly exposed groups. An understanding of the factors affecting within-worker variance assists in the identification of conditions in the work environment that cause varying concentrations from day to day. This is a prerequisite for better protection of the individual worker from hazardous exposures.

So far, in studies with repeated measurements designs, most researchers have used either a) a one-way random-effects model to estimate variance components, ignoring work characteristics[12-19] and/or b) multiple linear regression to model the effect of work characteristics on observed exposure levels, ignoring the correlation between repeated observations from the same worker.[14-15] Mixed-effects models for unbalanced data simultaneously estimate both the effects and the variance components in a more efficient way.[20-22] For exposure groups those models can describe the influence of fixed and random

work environment characteristics on the observed exposure levels, and estimate the within- and between-worker variance components controlled for work characteristics and other determinants of exposure. Recently, mixed effects models were used by several researchers for different purposes.[19, 23-25] A time trend can be introduced into these models as a fixed effect. In this paper we present the benefits of using mixed effects models for unbalanced data to estimate variance components while controlling for work characteristics. In addition, we present the coefficients of the work characteristics that affect exposure levels, controlled for the correlation between repeated measurements. Finally, we will show which work characteristics affect between- and within-worker variability in exposure concentrations.

For our analysis, we used three existing data sets with repeated personal exposure measurements as described previously in two published papers.[14,15,18] A common feature of the data sets was that they were from systematic surveys. Auxiliary information on the work environment and activities was collected during the measurements. The data sets stemmed from two industry-wide surveys among workers from the rubber manufacturing industry and one survey among pig farmers in the Netherlands. Detailed information on these studies and the results from the one-way random-effects models and from the multiple linear regression models can be found in the above-mentioned papers.[14,15,18]

METHODS

Study design, data collection and previous statistical analysis

First Example: Industry-wide survey of the rubber manufacturing industry

This study of the rubber manufacturing industry was performed in the Netherlands, to examine relationships between working conditions and chemical exposures. Personal exposures to airborne particulate, rubber fumes and solvents, as well as dermal contaminants, were measured in a representative sample of 10 factories producing an array of different rubber products. For each plant, the measurements and observations took four days (Tuesday-Friday). Auxiliary data on tasks performed, use of personal protection devices, ventilation characteristics and process characteristics were collected through interviews of sampled workers. Workers were selected, stratified by production function

and by the job done, and surveyed on randomly chosen days during the course of the four-day measurement period.

Multiple regression models were applied to evaluate the relationships between the collected auxiliary data and exposure levels , for two groups of workers: 234 workers with 620 measurements exposed to inhalable particulate, and a sub-group of 36 workers with 59 measurements exposed to rubber fumes (measured as the cyclohexane-soluble fraction of the inhalable particulate). Details of the study and the modeling can be found elsewhere.[18]

Second Example: Survey on Pig Farmers' Exposure to Inhalable Endotoxin

In a study among 98 pig farmers from the south of the Netherlands, exposure to inhalable dust and endotoxin was monitored by personal sampling. Exposure was measured during one work shift on a randomly chosen day of the week; one day during the summer of 1991 and one day during the winter of 1992. Outdoor temperature was obtained from a monitoring station in the south of the Netherlands. Task activity patterns and farm characteristics were also recorded. Activities, which were represented by time spent in each activity, were based on daily averages during 12-14 days For the purpose of this paper, only the exposure data on endotoxin will be used. Multiple linear regression analysis were applied to evaluate the relationship between farm characteristics, activities and outdoor temperature and log-transformed endotoxin concentrations. One-way random-effects model was applied to estimate variance components. Details of the study and the modeling can be found elsewhere.[14,15]

Sources of exposure variability

We postulated that the variability of the exposure levels in an industrial hygiene group of workers arises from 4 sources:

1. Systematic between worker variation: Systematic differences in factors that define the work conditions of different workers. These factors are mostly spatially related (e.g. local ventilation), varying among workers but constant in time for each worker. Sometimes these factors are both temporal and spatial (e.g. process temperature), meaning that the levels differ among workers (the mean value) and within the same worker along time.

2. Random between worker variation: Differences among workers beyond what can be explained by specific factors. This additional variation may be associated with factors that are not measured due to time/money limitations, inability to measure (e.g. workers' habits) or lack of awareness.
3. Systematic within worker variation: Systematic differences in factors that define the work conditions of the same worker over time. These factors are temporal (e.g. burden, activities) and may be common to all workers in the IH group. Time itself is one possible systematic, within worker, factor (e.g. season, year). Usually these within worker changes are related to the cycle of work, the production, seasonality etc.
4. Random within worker variation: Differences among measurements on the same worker at different time points beyond what can be explained by specified factors. This additional variation may be associated with further within-worker factors (e.g. changes of habits of a worker) that are not measured due to time/money limitations, inability to measure or lack of awareness (e.g. measures taken by different hygienists, measurement errors).

Thus, within the same exposure group along time, the usual partition of the total exposure variance into 2 components: between workers and within workers[1,2,4,6,7] can be refined when work characteristics are taken into account. 1. Systematic variation (sources 1,3) accounts for differences in work characteristics. These differences can be included as explanatory variables in the model whose effects can be estimated. 2. Random variation (sources 2,4), which is partitued into 2 components: a. Between worker variation, "conditioned" on the effects of the observed work characteristics (source 2). This variance component reflects additional variation among workers beyond differences due to work factors. b. Within worker variation (source 4), which reflects additional variation at the within-worker level. The total random variance when work characteristics are taken into account is a conditional variance, so its value is less than the total variance when those factors are not taken into account, and is the sum of the conditioned random between worker variance and the random within worker variance.

Mixed effects models

A mixed effects model is a generalisation of the standard linear model (a multiple regression model) that enables the analysis of data generated from several sources of variation instead of just one.[26] It associates one continuous dependent variable (a response, an outcome,) with several explanatory variables (categorical or continuous). The unique aspect of the mixed effects model is the inclusion of both fixed and random factors. Fixed effects provide estimates of the average responses in the group, like in a common regression model, while random effects (e.g. subjects' effects) account for the natural heterogeneity in the responses of different individuals and allow estimation of responses for each individual in the study. Since measurements done on the same subject are correlated, this correlation must be taken into account in modelling. The dependence among the repeated responses can be of different types leading to specific covariance/correlation structures. The model allows the assumption of several covariance structures and enables estimation of the effects as well as variance parameters. The number of observations per subject can be either the same (a balanced design) or different (an unbalanced design). The time points can be either identical across subjects or not. The time-interval between repeated observations can vary across repetitions.[21]

Current application of the mixed effects model

The application of this model for identifying the determinants of exposure and assessing variance components is presented in this paper using two examples: one from the rubber manufacturing industry, and another from pig farming. In both examples, the dependent variable was the exposure level of a pollutant and the explanatory variables were workplace characteristics. These fixed effects were either time-dependent (e.g. outdoor temperature) or fixed along time (e.g. stable flooring). The individual worker's effect was taken as a random effect. We looked at two sources of random variance: the random variance between workers and the random variance among repeated measurements within workers. In the first example we had three repetitions, and in the second example we had only two repetitions per worker. We assumed that any two repeated measurements of the same worker have equal correlation irrespective of the time interval between them; a compound symmetry covariance structure. This is the covariance structure assumed in classical

repeated measurements ANOVA. Furthermore, we assumed that the variance between workers is equal across the fixed factors of work characteristics (defined as homogeneity) and that the effects as well as the variance within workers are equal across all workers in the same group. The residuals were assumed to be independent of each other. Exposure concentrations were assumed to be log-normally distributed.[1,2,4,7] Mixed models were applied with and without the fixed effects (without- is equivalent to the random effects model), to determine the impact of the fixed effects upon the variance component associated with the random effects. The estimated variance components of both models were compared.

PROC MIXED from SAS System Software Version 6.1[26] was used for the analysis. The procedure enables simultaneous estimation of the effects, their standard error and significance tests, as well as variance components and their confidence limits. Variance components were estimated using the Restricted Maximum Likelihood (REML) method. Nested models were compared by likelihood ratio test.

The mixed-effects model for unbalanced data is specified by the following expressions:

$$y_{ij} = \beta_0 + \beta_1 X_{ij1} + \ldots + \beta_p X_{ijp} + b_1 z_1 + \ldots b_k z_k + \varepsilon_{ij}$$

For i=1,...,k (workers) and j = 1,...,n_i (repetitions of the i'th worker)

Where:

Y_{ij} = log-transformed exposure level

β_0 = an overall intercept for the group that corresponds to mean background exposure (log-transformed) when all factors equal zero

$\beta_1, \ldots, \beta_p$ = fixed effects

$x_{ij1}, \ldots, x_{ijp}$ = values of the variables for the i'th worker on the j'th day

$b_1, \ldots, b_k$ = workers' random effects

b_i = i'th worker random effect, which corresponds to the discrepancy between his intercept and the group intercept β_0

$Z_1, \ldots, Z_k$ = workers' indicators (0/1)

It is furthermore assumed that:

$b_i \sim N(0, \sigma_b^2)$, b_i's are all independent ; $\varepsilon_{ij} \sim N(0, \sigma_w^2)$, ε_{ij}'s are all independent,

b_i's and ε_{ij}'s are all independent of each other

$\sigma^2 = \sigma_b^2 + \sigma_w^2$; $\sigma_b^2 =$ variance between-workers; $\sigma_w^2 =$ variance within-workers.

$\rho = \sigma_b^2 / \sigma^2$; $\rho =$ correlation between any two repeated measures of the same worker

$$corr(y_{ij,}y_{il}) = \begin{cases} 1 & j = l \\ \rho & j \neq l \end{cases} \qquad cov(y_{ij,}y_{il}) = \begin{cases} \sigma^2 & j = l \\ \rho\sigma^2 & j \neq l \end{cases}$$ compound symmetry structure

For every i, i= 1,...,k and for every j,l, j,l = 1,...,n_i

(All y_{ij} of the same worker are correlated, and those of different workers are uncorrelated)

To model the influence of work characteristics on the exposure levels they were considered as fixed effects in the above model, for example:

β_1 = process pressure effect

β_2 = process temperature effect

β_3 = local exhaust ventilation effect

x_{ij1} = process pressure value for the *i*-th worker on the *j*-th day

x_{ij2} = process temperature value for the *i*-th worker on the *j*-th day

x_{ij3} = local exhaust ventilation indicator (0-non present/1-present) for the *i*-th worker on the *j*-th day

To identify time trends in the above model a time term was added to the model:

β_4 = period effect

x_{ij4} = period indicator (0-first period /1-second period), for the *i*-th worker on the *j*-th day

RESULTS

First Example: Industry-wide survey of the rubber manufacturing industry

Results of the application of both the random-effects model (the model without the fixed effects) and the mixed-effects model (the model with the fixed effects) were compared in the 2 data sets with exposure to inhalable particulate and rubber fumes (see Table 3.1, 3.2). The effect of the day of the week on the exposure level was tested separately. Table 3.1 shows that for workers exposed to inhalable particulate, the factors affected the between-worker component of variance (s^2_{bw}) considerably (35% reduction from 1.30 to 0.84), but did not alter the within-worker component of variance (s^2_{ww}). In the two models s^2_{bw} is different from zero ($p<.05$) and the models were significantly different ($p=.0023$).

Table 3.1 Variance components estimates for the one-way random-effects and mixed-effects models for exposure to inhalable particulate among workers in the rubber manufacturing industry (n=620, k=234, l=10)

Variance	Random-effects model est (CI)	Mixed-effects model est (CI)
s^2_{bw}	1.30 (1.07-1.59)	0.84 (.68-1.06)
s^2_{ww}	0.29 (.25-.34)	0.30 (.26-.34)

NOTES: n = no. of measurements; k = no. of workers; l = no. of factories;
s^2_{bw} = between-worker variance component; s^2_{ww} = within-worker variance component;
est= estimator; CI= 95% Confidence Interval

Table 3.2 Variance components estimates for the one-way random-effects and mixed-effects models for exposure to rubber fumes among workers in the rubber manufacturing industry (n=59, k=36, l=7)

Variance	Random-effects model est (CI)	Mixed-effects model Full model est (CI)
s^2_{bw}	0.53 (.31-1.17)	0.18 (.08-.70)
s^2_{ww}	0.32 (.20-.60)	0.30 (.19-.55)
	Mixed-effects model including local ventilation	Mixed-effects model including process' temperature and pressure
s^2_{bw}	0.49 (.28-1.08)	0.26 (.13-.83)
s^2_{ww}	0.31 (.20-.58)	0.32 (.20-.59)

NOTES: n = no. of measurements; k = no. of workers; l = no. of factories;
s^2_{bw} = between-worker variance component; s^2_{ww} = within-worker variance component;
est= estimator; CI= 95% Confidence Interval

Table 3.3 Coefficients for binary (0/1)[a] factors affecting exposure to inhalable particulate among workers in the rubber manufacturing industry in the mixed effects model and multiple linear regression model (n=620, k=234, l=10)

	Mixed-effects model		Multiple linear regression	
	β	P	β	p
Intercept	-0.05		-0.17	
Punching powdered products	3.41	<.01	3.79	<.01
Tube inspection	3.47	<.01	3.34	<.01
Packing powdered products	2.67	<.01	2.82	<.01
Jointing	2.41	<.01	2.71	<.01
utoclave	0.63	.01	1.66	<.01
Weighing	1.26	<.01	1.43	<.01
Heating mill	0.26	ns	1.10	<.01
Repair buffing	0.71	.02	0.82	<.01
Bench fitting	0.51	.01	0.78	<.01
Open mill	0.20	ns	0.61	.03
Internal mill	0.31	ns	0.52	.03
Packing	0.51	.01	0.48	.02
Cleaning	0.15	ns	0.37	<.01
Transport	0.15	ns	0.34	.02
Rubber cutting	-0.18	ns	-0.24	bs
Inspection	-0.29	ns	-0.29	ns
Breakdown work	-0.51	<.01	-0.38	.05
Punching	-0.20	ns	-0.46	ns
Unrolling	-0.49	bs	-0.60	.05
Calendering	-0.13	ns	-0.64	<.01
Assembling machine	-0.69	ns	-0.68	bs
Manual assembling	-0.41	.04	-0.73	<.01
Loading-unloading	-0.43	ns	-0.80	.05
Weighing products	-0.71	.04	-0.93	.02
Extruding-slicing	0.05	ns	-0.97	.03
Lead extrusion	-0.24	ns	-1.00	.05
UHF curing	-0.48	ns	-1.05	<.01
Braiding machine	-1.22	bs	-1.21	.02
Laboratory work	-0.87	.03	-1.28	<.01
Autoclave LEV	-0.69	.03	-1.39	<.01
General trimming	-1.56	.03	-1.94	<.01

NOTES: n = no. of measurements; k = no. of workers; l = no. of factories; bs .05<p≤.10; ns p>.10

[a] binary variable: 0= non-present, 1=present

The difference between the models suggests no systematic changes in work tasks and production characteristics for individual workers during a week. The main difference between the mixed-effects model and the original multiple regression model[18] assuming independence between repeated measurements, was that fewer exposure-affecting factors were statistically significant or borderline statistically significant ($p<0.10$) (17 versus 28 factors, see Table 3.3). However, the coefficients had the same sign for all factors except one task ("Extruding-Slicing"), whose coefficient was nearly 0 in the mixed-effects model. The tasks "Punching Powdered Products", "Packing Powdered Products", "Tube Inspection" and "Jointing" affected exposure to inhalable particulate most dramatically, according to both the mixed-effects model and the original multiple regression model.

For workers exposed to rubber fumes (Table 3.2), the same phenomenon for the components of variance was observed. The three factors, one pure spatial between workers factor (local ventilation) and the rest (process- temperature and pressure) both spatial and temporal factors, affected the between-worker component of variance (66% reduction from 0.53 to 0.18). The within-worker component of variance (s^2_{ww}) was not affected. In the 2 models s^2_{bw} is different from zero ($p<.05$) and the models were not significantly different. The coefficients of the factors were almost identical when compared to the original multiple linear regression (Table 3.4).

Table 3.4 Coefficients for factors affecting exposure to rubber fumes among workers in the rubber manufacturing industry in the mixed-effects model and multiple linear regression model (n=59, k=36, l=7)

	Mixed-effects model		Multiple linear regression	
	β	p	β	p
Intercept	4.94		4.97	
Process-Temperature[a]	0.0056	.04	0.0049	.05
Process-Pressure[b]	0.0038	<.01	0.0042	<.01
Local Exhaust Ventilation[c]	-0.69	<.01	-0.68	<.01

NOTES: n = no. of measurements; k = no. of workers; l = no. of factories
[a] per 1 °C;
[b] per 1 bar;
[c] binary variable: 0=non-present,1=present

The effect of 'day of the week' (Tuesday-Friday) on the mean exposure and on the components of variance was non-significant for both exposure modelling (to inhalable particulate and rubber fumes), suggesting no systematic differences in exposure over the course of a week.

Second Example: Survey on Pig Farmers' Exposure to Inhalable Endotoxin

Table 3.5 presents the results of both the original one-way random-effects model and a mixed-effects model with 21 fixed effects for both farm characteristics and activities. Furthermore, results from three additional mixed models with only farm characteristics, only activities and outdoor temperature, and only a season term, respectively were elaborated and are presented in the same table.

From the random effects model (Table 3.5) it was clear that the within-worker variance component had a greater weight than the between-workers variance component (85% versus 15% of the total exposure variance). The between-worker variance component was low 0.11. Table 3.5 shows the extent of reduction in both the within- and between-worker variance components by including all the statistically significant factors from the original multiple regression model. The within-worker variance component was reduced by 0.16 (25%) and the 2 models are significantly different ($p<.0001$). The between-worker variance component was reduced by 0.09 (80%) and while in the random effects model s^2_{bw} is different from zero ($p<.05$) in the mixed model with the farm characteristics $s^2_{bw} \sim 0$.

In Table 3.5 we see that farm characteristics appeared to be solely responsible for the reduction in the between-worker variance component ($s^2_{bw}=0.01$). The model with outdoor temperature and farmers' activities pure within worker factors had no effect on the between-worker variance component ($s^2_{bw}=0.12$), while it reduced the within-worker variance component by 25%.

Table 3.5 Variance components estimates for the one-way random-effects and mixed-effects models (full model and three sub-hierarchical models) for exposure to endotoxins among pig farmers (n=348 , k=198)

Variance	Random-effects model est (CI)	Mixed-effects model Full model est (CI)
s^2_{bw}	0.11 (.05-.45)	0.02 ([a])
s^2_{ww}	0.64 (.52-.80)	0.48 (.04-.60)

Variance	Mixed-effects model including farm characteristics est (CI)	Mixed-effects model including activities and temperature est (CI)	Mixed-effects model including season effect est (CI)
s^2_{bw}	0.01 ([a])	0.12 (.06-.33)	0.14 (.07-.39)
s^2_{ww}	0.64 (.52-.80)	0.48 (.39-.61)	0.59 (.48-.74)

NOTES: n = no. of measurements; k = no. of workers; l = no. of factories
s^2_{bw} = between-worker variance component; s^2_{ww} = within-worker variance component;
est= estimator; CI= 95% Confidence Interval ;
[a] cannot be computed

This clearly shows that different work environment characteristics contributed independently to the variance components. Farm characteristics, which are almost constant over the time period studied (one year), were responsible for differences in average endotoxins concentration between farmers, while changes from day to day in temperature and activities performed, led to temporal variability in exposure concentrations. The season factor was found to have a minor influence on the within- worker variance component.

The two models (the random effects model and the mixed effects model with season effect) were found to be significantly different ($p<.0001$). When compared to the coefficients of the original multiple linear regression model[14] the estimated coefficients from the Unbalanced Mixed Effects Model were almost exactly the same for the farm characteristics, but somewhat smaller for the activities (Table 3.6). Neither the relative order nor the p-values changed.

Table 3.6 Coefficients for factors affecting exposure to endotoxines among pig farmers, in the mixed-effects model and multiple linear regression model (n=348, k=198)

	Mixed-effects model		Multiple linear regression	
	β	p	β	p
Intercept	4.44		4.44	
Temperature				
Outdoor temperature (per 10%)[a]	-0.35	<.01	-0.35	<.01
Farm characteristics				
Feeding				
manual dosage dry feeding (1/0)[b]	-0.38	<.01	-0.37	<.01
pig starter (1/0)	0.35	.03	0.35	.03
automated dry feeding (per 10%)	-0.06	.02	-0.06	.02
Flooring				
Convex floor (1/0)	-0.22	.02	-0.22	.01
Fully slatted floor (per 10%)	0.08	<.01	0.08	<.01
Fully slatted floor with piglet mat (per 10%)	-0.08	<.01	-0.08	<.01
Synthetic grid (per 10%)	-0.13	.04	-0.13	.03
Concrete and metal grid (per 10%)	-0.15	<.01	-0.15	<.01
Floor heating (per 10%)	0.07	<.01	0.07	<.01
Floor heating with delta heating tubes (per 10%)	0.10	.04	0.10	.03
Other				
Overall very dusty	0.12	.02	0.12	.01
Air exhaust via pit (1/0)	-0.33	<.01	-0.33	<.01
Activities (per 10 minutes)[c]				
Feeding	0.04	<.01	0.03	<.01
Controlling	0.03	.03	0.02	.02
Re-penning	0.04	.05	0.02	.05
Floor sweeping	0.08	<.01	0.06	.01
Iron injection	0.09	.03	0.09	.03
Castrating	0.07	.03	0.05	.03
Teeth cutting	0.25	<.01	0.17	.01
Ear tagging	0.22	<.01	0.14	<.01

NOTES: n = no. of measurements; k = no. of workers

[a] per 10% of total time spent in pig farming;

[b] binary variable: 0= non-present, 1=present

[c] per 10 minutes spent on a task

DISCUSSION

The above examples illustrate the major contribution of the mixed-effects model in unbalanced designs to the investigation of exposure variance components and exposure affecting factors. In contrast with the one-way random-effects model, the mixed effects model deals with both fixed and random effects**.** It estimates the between- and within-worker variance while adjusting for fixed effects. Simultaneously, it assesses the linear relationships between the determinants of exposure (usually fixed factors) and exposure levels. Common multiple linear regression can be correctly applied when each worker has only a single measurement.

With repeated measurements of each worker, some dependence amongst repeated measurements will exist and the correlation between values for a given person must be taken into account when estimating the relationship between the determinants and exposure levels.[21,22] The mixed effects model is capable of taking this dependence into account in the modeling process.

In this study we used mixed effects models to understand the relationship between specific work environment characteristics and between- and within-worker exposure variance components. Identification of specific work characteristics, which affect the between-worker variance component, will enable development of criteria for defining uniformly exposed groups of workers.[1,5,10,19] Grouping workers into sub-groups is an inherent part of the work of an industrial hygienist in exposure surveys, as well as in compliance tests and epidemiological studies.[1,5,6] Despite the widespread use of grouping strategies, there is so far only limited experience with optimisation of these strategies.[5,27,28]

The analyses with the mixed effects models form the basis for the creation of uniformly exposed groups of workers. Reliance on observational factors such as a job title, which may lead to non-uniformly exposed groups, seems no longer necessary.[5] For instance, classifying curing workers in rubber manufacturing based on the determinant process-temperature and -pressure will lead to more uniform and distinctly different rubber fume exposure groups. It is further recommended that if the IH group cannot be split into sub-homogeneous groups due to a small number of workers, the testing of overexposure in IH groups, as suggested by Lyles et al,[11] which relate to both within and between variance

components, should be refined to account for the effects of work characteristics in these groups. As we have shown here, these effects can be estimated with mixed effects models. Also, in this study, specific factors were identified which mainly influenced variability in exposure levels from day to day (within-worker). Hazard control should focus on these factors.

The two examples presented in this paper describe different work situations and measurement strategies. In the first example, one to three randomly chosen measurements were performed within a week on two groups of rubber workers in The Netherlands.[18] In the second example, one to two measurements were collected in two different seasons of a particular year among Dutch pig farmers.[14,15] Different work characteristics were documented in each examples. In the first example, for exposure to particulate, a reduction of 25% of the between-worker variance component was the effect of 17 factors affecting exposure. Classification of rubber workers into uniformly exposed groups will have to rely on those identified factors. In the second example, exposure to endotoxin among pig-farmers, the reduction of 82% in between-worker variance was mainly an effect of the inclusion of 12 farm characteristics. Consequently, classification of pig farmers into uniformly exposed groups will have to rely on those farm characteristics.

In the second example (pig farmers), there was a clear distinction between characteristics influencing each of the variance components. Eight time-dependent activities, as well as outdoor temperature, all pure within worker factors, reduced the within person (day-to-day) variability by 25%. From diary information collected among the pig farmers, it appeared that some of the activities followed a distinct temporal pattern, with some activities taking place only on particular days of the week.14 The farm characteristics, pure between worker factors, were responsible for reducing the between-worker variance component to zero.

In the mixed model, when the between-worker variance component is close to zero, the coefficients for the fixed effects would be very similar to those from a multiple regression model, assuming independence between repeated observations. In the model for the pig farmers, this was indeed the case (between-worker component 0.02). For the rubber workers' exposure to inhalable particulate the opposite was true a very large between-worker variance component, led to changes in the coefficients. In addition, fewer

statistically significant exposure-affecting factors were found in the mixed model, since the significance tests of the coefficients in the mixed model are different.

In all the examples, we assumed that any two repeated measurements of the same worker had equal correlation, irrespective of the time interval between them; a compound symmetry covariance structure. This, since we had only two repetitions per worker in the second example and three in the first example. We also assumed independence across subjects. In general, the residuals can be analysed to check for departures from the independence assumption. However, such analyses will be relevant only when there are a reasonable number of observations within workers, say at least 8-10. Other models with different dependence structure may be applied when the time course of the exposure level of each person is of primary interest, that is, when the correlation itself has scientific relevance. The unbalanced mixed-effects model is a specific case of the generalised linear models. These models were developed over the last decade and were recently introduced in common statistical packages such as SAS (Proc Mixed), BMDP (5V) and S-Plus.[26,29,30] The computerised procedures enable simultaneous estimation of parameters, approximate standard errors and significance tests that have not been available before. One should take into account the fact that in models for unbalanced data, the estimators are proxies since the Maximum Likelihood Estimates have approximately normal distribution. Balanced data (the same number of repeated measurements for each worker) is preferable whenever possible, in order to obtain more accurate estimators.

However, the mixed effects model enables estimation of variance components of exposure levels that have been adjusted for workplace factors in order to improve the assessment of exposure. This statistical method can be used to improve future sampling strategies through the grouping of workers into more uniformly exposed groups, and the identification of specific workplace conditions that should be controlled.

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REFERENCES

1. Rappaport SM. Assessment of Long-Term Exposure To Toxic Substances In Air. Ann Occup Hyg 1991; 35, 61-121.
2. Rappaport SM, Smith TH. Exposure Assessment for Epidemiology and Hazard Control. ACGIH, Michigan, Lewis Publishers Inc..1991.
3. Burdorf, A. Shifting Concepts in Assessment of Occupational Exposures. Ann Occup Hyg 1993; 37, 447-450.
4. Kromhout H, Symanski E, Rappaport SM. A Comprehensive Evaluation of Within- and Between-Worker Components of Occupational Exposure to Chemical Agents. Ann Occup Hyg 1993;37, 253-270.
5. Boleij J, Buringh E, Heederik D, Kromhout H. Occupational Hygiene of Chemical and Biological Agents, Amsterdam, Elsevier 1995.
6. Rappaport SM, KromhoutH, Symanski E. Variation of Exposure Between workers in Homogenous Exposure Groups. American Industrial Hygiene Association Journal .1993; 54, 654-662.
7. Peretz C, Goldberg P. Kahan E, Grady S, Goren A. The Variability of Exposure over Time: A Prospective Longitudinal Study. Ann Occup Hyg .1997;41, 485-500.
8. Buringh E, Lanting R. Exposure Variability in the Workplace Its Implication for the Assessment of Compliance. American Industrial Hygiene Association Journal 1991; 52, 6-13.
9. Heederik D, Boleij JSM, Kromhout H. Use and Analysis of Exposure Monitoring Data in Occupational Epidemiology. An Example of an Epidemiological Study in the Dutch Animal Food Industry. Appl. Occupational Environ. Hygiene 1991;5, 458-464.
10. Rappaport SM., Lyles RH, Kupper LL. An Exposure Assessment Strategy Accounting for Within- and Between Worker Sources of Variability. Ann Occup Hyg 1995;39, 469-495.
11. Lyles RH, Kupper LL, Rappaport SM. A Lognormal Distribution-Based Exposure Assessment Method for Unbalanced Data. Ann Occup Hyg 1997;41, 63-76.
12. Burdorf A, Lillienberg L, Brisman J. Characterization of Exposure to Inhalable Flour Dust in Swedish Bakeries. Ann Occup Hyg 1994;38, 67-78.
13. Woskie SA., Shen P, Eisen EA, Finkel MH, Smith TJ, Smith R, Wegman DH. The Real Time Dust Exposures of Sodium Borate Workers: Examination of Exposure Variability. American Industrial Hygiene Association Journal 1994; 55, 207-217.
14. Preller L, Kromhout H, Heederik D, Tielen M. Modelling Long-Term Average Exposure in Occupational Exposure-Response Analysis. Scandinavian Journal of Work, Environment and Health 1995; 21, 504-512.

15. Preller, L., Heederik, D. , Kromhout, H. and Tielen, M. .1995; Determinants of Dust and Endotoxin Exposure of Pig Farmers: Development of a Control Strategy Using Empirical Modelling. Ann Occup Hyg 39, 545-557.
16. Nieuwenhuijsen MJ, Lowson D, Venable KM, Newman Taylor AJ. Flour Dust Exposure Variability in Flour Mills And Bakeries. Ann Occup Hyg 1995; 39, 299-305.
17. Kumagai S, Kusaka Y, Goto S. Cobalt Exposure Level and Variability in The Hard Metal Industry of Japan. American Industrial Hygiene Association Journal 1996;57, 365-369.
18. Kromhout, H, Swuste, P. and. Boleij, J. S. M. .1994; Empirical Modelling of Chemical Exposure in The Rubber Manufacturing Industry. Ann Occup Hyg 38, 3-22.
19. Symanski E, Kupper, L. L., Kromhout, H. and Rappaport, S. M. .1996;. An Investigation Of Systematic Changes In Occupational Exposure. American Industrial Hygiene Association Journal 57, 724-735.
20. Searle, S. R Mixed Models and Unbalanced Data: Wherefrom, Whereat And Whereto Commun. Statistics. A-Theory And Methods .1988; 17, 935-968.
21. Lindskey, J. K. Models for Repeated Measurements. Oxford, Clarendon Press,1993.
22. Burton, P., Gurrin, L. and Sly, P. Extending the Simple Linear Regression Model to Account for Correlated Responses: An Introduction to Generalized Estimating Equation and Multi-Level Mixed Modelling. Statistics in Medicine .1998; 17, 1261-91.
23. Nylander-French, L.A., Kupper, L. L. and Rappaport, S. M. An investigation of Factors Contributing to Styrene and Styrene-7,8-oxide Exposures in the Reinforced-Plastics Industry. Ann Occup Hyg .1999; 43, 99-109.
24. Rappaport, S. M., Weaver, M., Taylor, D., Kupper, L. and Susi, P.; Application of Mixed Models to Assess Exposures Monitored by Construction Workers during Hot Processesr. Ann Occup Hyg .1999;43, 457-69.
25. Burstyn, I., Kromhout, H., Kauppinen, T., Heikkila, P., and Bofefetta, P. Statistical Modelling of the Determinants of Historical Exposure to Bitumen and Polycyclic Aromatic Hydrocarbons Among Paving Workers Annals of Occupational Hygiene.2000; 44, 43-56.
26. SAS, SAS/ STAT Software Changes and Enhancements ,Carl, North Carolina, SAS Institute.1996; .
27. Kromhout, H., Loomis, D. P., Kleckner, R. C. and Savitz, D. A.; Sensitivity of The Relation between Cumulative Magnetic Field Exposure and Brain Cancer Mortality to Choice of Monitoring Data Grouping Scheme. Epidemiology .1997;8, 442-445.
28. Seixas NS, Sheppard L. Maximizing Accuracy and Precision Using Individual and Grouped Exposure Assessments. Scandinavian Journal of Work, Environment and Health. 1996; 22, 94-101.
29. BMDP, Statistical Software, Vol 2, Berkeley, University Of California 1990.
30. S-Plus, Data Analysis Products Division, Seattle, WA: Math-Soft. 1997.

CHAPTER 4:
EXPOSURE TO BENZENE IN FUEL DISTRIBUTION INSTALLATIONS: MONITORING AND PREVENTION

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ABSTRACT

An association between airborne benzene exposure and task and timing factors was examined, applying a mixed effects model on a cohort of 258 workers randomly chosen, in 7 fuel distribution facilities. During 8 years, 692 repeated personal measurements were performed. Filler-task, warm month, Tuesday, credit-day and period (1992-1996) were significantly associated with higher exposure to benzene. After controlling for the period effect, task-type was found to highly affect the between worker variance and thus, two homogeneous exposure groups - fillers and non-fillers are adequate for exposure grouping strategy. The timing factors were found to affect the high within worker variance (>2 than between worker variance) after controlling for the task and period effects. To better represent long-term exposure, the sampling strategy should be stratified by warm/non warm months and measurement days should be selected randomly.

INTRODUCTION

Long-term low level exposures to benzene (less than 10 ppm) have been recently found to be probably associated with acute myeloid leukemia.[1] The major occupational sources for benzene exposure, -through the inhalation of gasoline vapors-, in the fuel supply industry ,are oil refineries, distribution facilities and service stations. Exposure levels of benzene among attendants in service stations are generally lower than the existing Threshold Limit Value (TLV), 1 ppm, and are primarily affected by the type of fuel, the work practice, the work load and weather conditions.[2-6] However, the time weighted average exposure levels reported for distribution facilities and refineries are higher, 0.003-8.9 ppm.[7-8] They are probably affected by additional factors, especially the task and the filling method.[5,7-12] Assessing long term exposure in occupational groups is essential for exposure-response evaluation.[13-15] Assessing the determinants of exposure is essential for hazard control and for effective use of control measures.[13,16-18] However, due to study limitations, the exposure assessment in those studies was not satisfactory.[1,10] In many situations, chronic exposures have been inferred on the basis of a one-time exposure survey or successive surveys carried out over a short period.[19] Since exposure varies within the same worker over time, [13,19-24] even to a very high degree, [5,19,22] this practice may cause bias since a single survey as well as successive surveys - which may result in autocorrelated exposures[25] - may not represent long term exposure. For a valid exposure assessment, monitoring designs should be based on repeated measurements within the same worker.[4,19,23]

Occupational labor inspection regulations for benzene in Israel stipulate that: (i) Airborne benzene monitoring should be carried out four times a year (every 3 months) in fuel distribution facilities, and the exposure results should be kept for 20 years (ii) A Threshold Limit Value (TLV-TWA) of 0.6 ppm and an Action Level (AL) of 0.3 ppm were established (iii) Biological monitoring and medical surveillance along time. However, only a few studies regarding exposure assessment to chemicals at workplaces, based on multiple non successive repeated measurements over time, have been published should be conducted every 6 months among workers exposed above the Action Level and among all workers in the production of benzene or in filling tankers or storage tanks.

The existence of a systematic and accessible long-term exposure data-base for all fuel distribution facilities in Israel, enables the investigation of benzene monitoring for both exposure assessment and exposure determinants, in this industry. Thus, the specific aims of the present study were to evaluate the association between work task and exposure and between timing factors - day, month, year and exposure. Moreover, since it is known that exposure in occupational groups varies between and within-workers, these variance components were estimated in different conditions.

METHODS

Study subjects

The study cohort included 258 workers from 7 fuel distribution facilities in Israel, whose environmental monitoring was carried out systematically during 8 years (1989-1996). In each monitoring survey the workers selected to represent the main processes in each facility. The major tasks were: fuel-filling (top loading), maintenance, gate-guarding, workshop activities, and laboratory work. All except one were men and besides two laboratory, the workers were all blue-color workers. The number of fillers in each facility varied between 3-33 and comprised 55% of the study cohort.

Environmental monitoring

In each installation 2-4 hygiene surveys were performed each year. Altogether, 18-32 surveys were performed at each facility during the whole period, giving a total of 151 surveys. The sampling strategy employed was monitoring a representative number of workers in all work-areas with potential exposure. The sampling scheme varied from one survey to another depending on different selection of workers by different hygienists who performed the surveys. The majority of the samples were personal breathing zone samples in which air was drawn through a commercial charcoal tube (SKC226-01). Benzene analysis was carried out by gas chromatography. In addition to internal quality controls, validation was verified by participation in external quality assurance programs of

Proficiency Analytical Testing (PAT) and Workplace Analytical Scheme for Proficiency (WASP).

Repeated measurements

Results of 692 full shift personal measurements, time weighted average, were included in the study. Of these, 64% were performed on fuel-fillers. The repetitions varied between 1-12 measurements per worker along the 8 years. Fifty five percent of the workers had two or more repeated measurements; 16% had over 5 repetitions.

Data source

Data were obtained from the occupational hygiene unit of the Institute for Occupational Health of Tel Aviv University. Each observation included the following variables: exposure concentration, date (day, month, year), measuring type (personal/fixed points), department and workstation identification - usually including subject name. Due to non-uniformity in the department name and workstation identification even within the same facility, a rearrangement was done for these two variables with a suitable coding.

Statistical analysis

Univariate analysis: descriptive statistics, such as geometric means and standard deviations, maximum likelihood arithmetic means and proportions, were used to describe benzene exposure by different characteristics. Multivariate analysis: to associate between exposure (dependent variable) and its determinants (independent variables) a multiple model, the mixed effects model for unbalanced data was used.[26-29] The dependent variable was the log-transformed values of benzene concentrations since these concentrations were assumed to have a log-normal distribution.[15,19-22] The five dichotomous independent variables were: work-task (filler, non-filler), month (warm, non-warm), day of the week (Tuesday, other), credit-day (yes, no) and period (1989-1992, 1993-1996). *

* Credit day is an account day and the day after it:1,2,10,11,15,16,20,21,30,31 of each month. The days on which the gasoline stations prefer to receive supply due to financial considerations

Details regarding the model are presented in the Appendix. To investigate variance components, three hierarchical mixed effects models were applied. The first included only the background exposure (=the intercept), while the second included the task and period effects and the background exposure. The third included all 5 exposure effects, the independent variables and the background exposure.

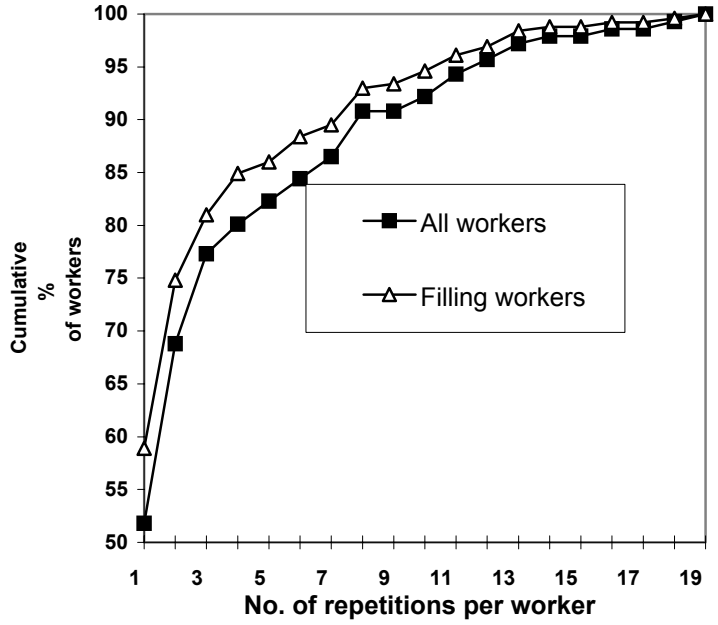
The application of the mixed effects models was carried out using PROC MIXED from the SAS System Software,[30] and the estimation of the variance components was done by using the Restricted Maximum Likelihood (REML) method.

RESULTS

Data description

The accumulated percentage of workers by number of repeated measurements per worker is shown in Fig 4.1, both for the fillers and for the whole group.

Fig 4.1. Distribution of workers by number of repeated measurements per worker (n=692)



Twenty percent of the workers had more than 3 repeated measurements and among the fillers twenty percent had more than 4 repeated measurements. The analysis is based on 692 measurements performed on 258 workers, along 8 years of follow-up. Table 4.1 presents univariate statistics of benzene distribution by specific characteristics (exposure determinants), assuming independence between repeated measurements within a worker.

Table 4.1 Benzene exposure (ppm) by specific characteristic among gasoline distribution workers

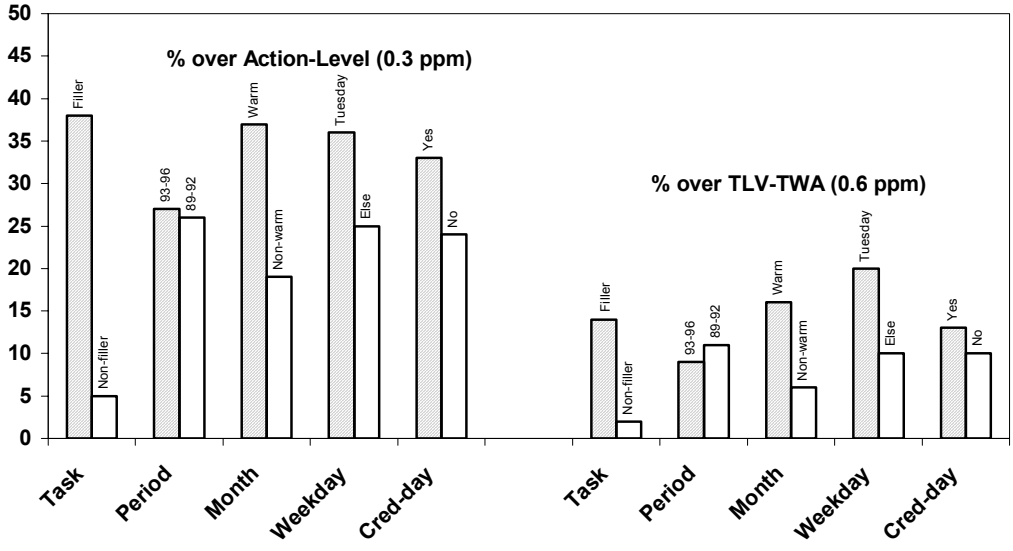
Characteristic		k	n	AM	GM	GSD	P-value
Task	Filler	141	445	.38	.18	3.35	
	Non-filler	117	247	.07	.03	3.25	<.0001
Month	Warm[a]	196	411	.43	.14	4.44	
	Non-warm	144	281	.20	.08	3.97	<.0001
Week-day	Tuesday	77	105	.36	.12	4.31	
	Other	228	687	.27	.10	4.22	BS(.1021)
Credit-day	Yes[b]	121	202	.35	.13	4.14	
	No	205	490	.26	.09	4.26	.0048
Period	1989-1992	110	268	.29	.10	4.39	
	1993-1996	182	424	.28	.10	4.18	NS
Total		258	692	.29	.10	4.26	

NOTES: k = no. of workers; n = no. of measurements;
AM = Maximum likelihood Arithmetic Mean; GM = Geometric Mean;
GSD = Geometric Standard Deviation; BS =.05<P-value<=.10; NS = P-value>.10
[a] Warm months: Jun., Jul., Aug., Sept.
[b] Days of the month when it is financially advantageous to receive supply

Significant higher geometric mean exposures can be observed amongst fillers, on warm months and on credit-days. There was a borderline significant difference in the geometric mean exposure on Tuesdays in comparison to all other days. A non-significant difference between the geometric mean exposures in the 2 periods, 1989-1992 and 1993-1996 could be demonstrated.

Compliance may be regarded with respect to the percentage of results exceeding exposure standards. The percentage of results exceeding the Israeli TLV-TWA and AL, by specific characteristics, are presented in Fig 4.2.

Fig 4.2. Percentage of measurements (n=692) that exceeded standards, by specific characteristics



A difference in the level of overexposure, in relation to the TLV-TWA, between the categories of 4 characteristics (task, month, week-day, credit-day) can be seen. These differences are even larger regarding exposure over the AL. There is almost no difference in percentage of overexposure between the 2 periods

Exposure factors relating to benzene levels in the air

In Table 4.2, the modeling of exposure determinants by the multiple mixed effects model is presented. As can be seen from this table, all the 5 effects were significantly associated with exposure. The coefficients of the model represent the contribution of the factors to the mean exposure expressed as log-transformed benzene concentrations, when a value of 1 was assigned to task (meaning fillers), month (meaning a warm month), week-day

(meaning Tuesday), credit-day (meaning a credit day), period (meaning 1989-1992). The background geometric mean exposure was found to be 0.03 ppm (Table 4.2, intercept).

Table 4.2 Factors affecting benzene exposure (log-transformed benzene concentrations in ppm) according to a mixed effects model (based on 258 workers and 692 measurements)

Factor	Coefficient	(SE)	P-value	Exponent of Coefficient
Intercept	-3.62	(.10)	.0001	0.03
Task	1.71	(.11)	.0001	5.52
Month	0.49	(.09)	.0001	1.63
Week-day	0.19	(.09)	.0492	1.21
Credit-day	0.24	(.10)	.0148	1.27
Period	0.27	(.10)	.0048	1.31

NOTE: SE=Standard Error

In order to calculate the estimated geometric mean exposure in different conditions, the exponent of coefficients (= factors) should be multiplied by 0.03 and by each other. Task was found to be the most important factor associated with exposure. Geometric mean exposure to benzene was 5.5 times higher among fillers than among non-fillers. It is noteworthy that the results for fillers are related to top loading. Each of the other factors - month, week-day and credit-day, significantly increased the geometric mean exposure by a factor less than two. The simultaneous existence of all of the factors, increased the exposure 2.5 fold (1.63*1.21*1.27).

In addition, a significant period effect was found since the geometric mean exposure in 1993-1996 was 1.3 times higher than that in 1989-1992. Thus, the period effect should be controlled for the association between exposure and task and date determinants. It should be noted that in the univariate analysis (Table 4.1), the p-value of the period characteristic was non-significant, while in the multiple analysis (Table 4.2) it was found significant.

When the mean exposure is above exposure standards, such as TLV-TWA and AL, remedial action should be taken and control devices should be applied to reduce exposure. Table 4.3 shows the estimated geometric mean (GM) exposures of fillers and non-fillers in different conditions controlled for period effect.

Table 4.3 Estimated Geometric Mean (GM) exposure to benzene (ppm) according to the mixed effects model in 8 out of 32 possible conditions (based on 258 workers and 692 measurements)

Period	Task	Condition	GM Exposure
1993-1996	Filler	Warm month, Tuesday, credit day	0.41
		Warm month, not Tuesday, non-credit day	0.28
		Not warm month, not Tuesday, non-credit day	0.17
	Non-filler	Warm month, Tuesday, credit day	0.07
1989-1992	Filler	Warm month, Tuesday, credit day	0.31
		Warm month, not Tuesday, non-credit day	0.21
		Not warm month, not Tuesday, non-credit day	0.13
	Non-filler	Warm month, Tuesday, credit-day	0.05

The highest 3 GM exposures of fillers were 0.41 ppm, 0.31 ppm and 0.28 ppm. With an action level of 0.3 ppm in Israel, these conditions indicate potential hazardous situations that should be controlled in order to reduce exposure. In contrast, even in the most extreme conditions of high potential exposure, non-fillers were subject to very low mean exposures, of 0.07 ppm and 0.05 ppm, in the different periods.

Variance components of benzene concentrations

In Table 4.4, the estimators of variance components of the benzene concentrations are shown in geometric standard deviations. The geometric standard deviations between workers and within workers, in the 8 years of follow up, are presented in 3 hierarchical mixed effects models.

Table 4.4 Within- and between-worker variability in 3 models relating various factors to exposure (based on 258 workers and 692 measurements)

	MODEL 1 (factors=none)		MODEL 2 (factors=task, period)		MODEL 3 (factors=task, month, week-day, credit day, period)	
Component	Estimate	P-value	Estimate	P-value	Estimate	P-value
GSD- b	2.34	.0001	1.34	.0729	1.37	.0305
GSD- w	3.26	.0001	3.21	.0001	3.07	.0001

NOTES: GSD-b/w=Geometric Standard Deviation, between/within-worker;
P-value, estimate not equal one

The first model, a one way random effects model, included only the background exposure. The second model included in addition the task and period factors, and the third model included all the 5 factors affecting the exposure in addition to the background exposure. In all the models the geometric standard deviation components were at least borderline significantly different from 1 (namely the variances were different from zero).

In Models 2 and 3, the GSD-w were found to be 3.21 and 3.07 respectively, more than twice higher then the GSD-b which were 1.34 and 1.37 respectively. The work-task was found to be the major predictor of exposure and was responsible for a 43% reduction of the GSD-b ((2.34-1.34)/1.34*100) based on comparing a model without exposure determinants (Model 1) to a model including task and controlling for the period effect (Model 2). However the month, credit-day and day of the week were responsible for a reduction of 6% of the GSD-w ((3.26-3.07)/3.26*100), controlling for task and period when comparing model 3 to model 1. Since the variance components may be different in the different task groups[13,22,33] the variance components were estimated separately for fillers and non-fillers (Table 4.5). The estimations, controlled for other exposure-factors, seemed to be of the same order of magnitude in both task groups.

Table 4.5 Within- and between-worker variability in 2 task-group; controlling for month, week-day, credit-day

	Task	
	Fillers	Non-fillers
No. of workers	141	117
No. of measurements	445	247
GSD- b	1.39	1.25
GSD- w	3.07	3.13

NOTES: GSD-b/w= Geometric Standard Deviation, between/within-worker

DISCUSSION

The aim of this comprehensive follow-up study was to assess exposure to benzene in the fuel distribution industry in Israel and to investigate exposure determinants. The study included environmental monitoring data gathered over eight years of follow-up. The analysis was based on accumulated data compiled for administrative purposes on an ongoing basis. Even though exposure registries could be a useful tool in public health response to occupational exposure, such evaluations are rarely done.

Since this is a historical-prospective study, the exposure determinants examined were limited to those mentioned in the data-source. The study might be slightly biased due to non uniform schemes of workers monitoring. However, there is no reason to assume any specific bias and consequently the study results may be generalized.

In a mixed effects model for an unbalanced repeated measurements design, five determinants, task and four timing factors (month, week-day, credit-day, period) were tested for their effects on benzene exposure. A strong task-type effect was found. Fuel-filling workers, on average, experienced exposure levels 5 times higher than all other types of workers. Controlling for the four timing determinants, the predicted average exposure of the fillers was 0.41 ppm in their presence (namely on warm months, Tuesdays, credit-days, second period) as opposed to 0.13 ppm in their absence. Exposure standards aim to prevent health impairment of workers. Exposure of 0.41 ppm is higher than the action

level of 0.3 ppm, thus, a sincere effort should be made in all the facilities to reduce fillers exposure.

An averaged benzene concentration of 0.17 ppm (=0.55 mg/m^3) was found in an Italian study regarding 111 filling station attendants in Rome, which were monitored 6.3 times (on average) during one year.[4] The exposure of the non-fillers found in our study is of the same order of magnitude as that of benzene attendants .

In this study warm months, and to a lesser extent, credit-days, Tuesdays and the later period were associated with increased exposures to benzene. Warm months in Israel (June-September) are characterized by high temperatures, on average 24°c ±0.5 (opposed to 18°c ±3.5 during the rest of the year), which cause higher fuel evaporation and consequently higher exposures. The average exposure level obtained on warm months was 1.6 times higher than on non-warm months. Credit-days indicate higher activity, since it is financially advantageous for the gasoline stations to receive supply on the account day and the following day. Exposure on credit-days was 1.3 times higher than on non credit-days. An attempt was made to associate the day of the week and the level of exposure, controlling for all the other exposure determinants. A higher average exposure was found on Tuesdays, indicating a work peak, compared to the beginning and the end of the week. According to a subjective estimate of workers in the facilities, the work-load has been uniformly distributed during the week, except for the higher work load on credit-days. Since we did find an effect of the day of the week this effect should be further investigated.

During the 8 years of the follow up, there were no changes in the sampling and analysis methods or in work processes (including job-tasks and equipment) . The percent of benzene in the fuel ranges from 1.5%-2.5%, the variation being due to differences between refineries and to market demands. However, it is known that measurements taken over periods longer than 5 years appear to exhibit systematic changes in exposure results. [31] In order to control for a period effect, the 8 years of the follow up were divided into two periods, of four years each. A period effect was included in the multiple mixed effects model and indeed was found to be significant. The average exposure of the period 1993-1996 was 1.3 times higher than that of the period 1989-1992. This may be due to changes

in work load. Hence, the period effect should be controlled for in the investigation of the exposure and its determinants over a long period of follow-up.

In order to reduce the exposure to benzene, it is essential that hygienists, occupational physicians, employers and workers understand the main factors contributing to higher exposure. Extreme conditions should therefore be better controlled in order to avoid risky situations. Such information can also assist hygienists in planning a representative environmental sampling strategy and thus better assess occupational exposure for both hazard control and epidemiological studies - of exposure-response relationships. Based on our findings, warm months and credit-days should be considered as part of the sampling strategy. Today the sampling surveys are performed on fixed months in each facility and thus may not represent higher exposures on warm months. This procedure should be changed to a stratified one by warm and non-warm months. In addition, selecting measurement days at random should maximize the likelihood that they are representing different work load conditions.

Modeling exposure and its determinants has always been performed by regression models.[16] In repeated measurements designs, this means ignoring the dependence between the measurements , a procedure which may entail biased models for assessing exposure. The mixed effects model used in this study enables handling unbalanced repeated measurements and modeling exposure in a more efficient way.

Estimating exposure variance components between and within workers in homogeneous exposure groups is essential for exposure assessment.[13,15,22,32-34] Several investigators have ignored the contribution of exposure determinants and used a one way analysis of variance model to estimate variance components in task groups, thus limiting the possibility of understanding the factors affecting within and between worker variance.[22,23,33] In a study regarding benzene exposure in petroleum refining, 19 task groups were defined and had varying GSD-b and GSD-w values (1.0-9.29). However, the period effect was not controlled for as has recently been suggested by Symanski et al.,[31] nor were other timing effects controlled.

Since in this study, task was found to highly influence the between worker variance, two homogeneous exposure groups- fillers and non-fillers were found to be adequate for exposure grouping strategy in this industry. The geometric standard deviation within-

workers was found to be more than twice higher than that between workers, after controlling for the task and time effects, meaning a wide range of exposure levels for each worker during a time period. Control of factors which affect the within-worker variance of exposure such as: month, credit-day and day of the week may assist in reducing exposure levels. Other work characteristics such as worker-mobility, source mobility, ventilation, personal hygiene etc. may affect the within-variance exposure.[19,22]

In conclusion, the mixed effects model used in this study enables a better assessment of occupational exposure and a better assessment of the relative contribution of exposure determinants. Consequently, more effective prevention of hazardous exposure and an improvement in sampling strategy can be established. This study shows that an administrative exposure registry can have a strong interactive role in channeling relevant risk information to the primary care provider.

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APPENDIX

The mixed effects model:

The mixed effects model can be specified by the following expression:

$Yij=\beta 0+ \beta 1Xij1+ \ldots\ldots+ \beta pXijp+ b1Z1+.....+bkZk+\varepsilon ij$

i=1,.....,k (workers) j=1,.....,ni (repetitions of the i'th worker)

Yij= log-transformed values of benzene concentrations

$\beta 0$ = overall (fixed) group mean; mean of Yij

$\beta 1,\ldots\ldots\ldots , \beta p$ = fixed effects

Xij1, ….....,Xijp= values for the i'th worker on the j'th repetition for each effect

b1,.....,bk= workers' random effects

Z1,.....,Zk= workers' indicators (0/1)

The concentrations of the benzene were assumed to have a log-normal distribution.

$\varepsilon ij\sim N(0, \sigma^2 e)$ independent within a worker ; $bi\sim N(0, \sigma^2 b)$

εij's and bi's are all independent.

$E(Yij)= \mu i$ for specific i; for every j=1,.....,ni

$$var(Yij)=\begin{cases} \sigma^2 & i=j \\ \rho\sigma^2 & i\neq j \end{cases}$$ compound symmetry structure

$\rho=corr(\varepsilon il, \varepsilon im)$ for every i=1,.....k; l,m=1,.....,ni $l\neq m$

Exposure determinants were considered as fixed effects in the above model, as follows:

$\beta 0$ = background exposure (=intercept)

$\beta 1$ = task effect

$\beta 2$ = month effect

$\beta 3$ = day of the week effect

$\beta 4$ = credit-day effect

$\beta 5$ = period effect

Xij1= task indicator (0/1) for the i'th worker on the j'th repetition

0- non-filler, 1-filler.

Xij2= month indicator (0/1) for the i'th worker on the j'th repetition

0-non-warm months, 1-warm months (Jun,Jul,Aug,Sep).

Xij3= day of the week indicator (0/1) for the i'th worker on the j'th repetition

0- any day except Tuesday, 1-Tuesday.

Xij4= credit-day indicator (0/1) for the i'th worker on the j'th repetition

0- any day except credit-day 1-specific dates each month: 1,2,10,11,15,16,20,21,30,31.

Xij5= period indicator (0/1) for the i'th worker on the j'th repetition

0-1989-1992, 1-1993-1996.

REFERENCES

1. Savitz DS, Anderews KW. Review of epidemiologic and evidence on benzene and lymphatic and hematopoietic cancers. Am J Ind Med 1997;31:287-95.
2. Foo Swee Ceng. Benzene pollution from gasoline usage. Sci Total Environ 1991:103;19-26.
3. Fung KK, Wright BJ. Monitoring of benzene in ambient air with organic vapor badges. J of Air Pollution Control Assoc 1986:36; 819-21.
4. Lagorio S, Forastiere F, Iavarone N, et al. Exposure assessment in a historical cohort of filling stations attendants. Int J of Epidemiology 1993;s51-s56.
5. Rappaport SM, Selvin S. Exposure hydrocarbon components of gasoline in the petroleum industry. Appl Ind Hyg 1987;2:148-54.
6. Spear RC, Selvin S (personal communication) Benzene exposure in the petroleum refining industry. Appl Ind Hyg 1987;4:155-63.
7. Armstrong TW, Pearlman ED, Schnatter AR, et al. Retrospective benzene and total hydrocarbon exposure assessment for a petroleum marketing and distribution worker epidemiology study. Am Ind Hyg Assoc J 1996; 57:333-43.
8. Lewis SJ, Bell GM, Cordingley N, et al. Retrospective estimation of exposure to benzene in a leukaemia case-control study of petroleum marketing and distribution workers in the United Kingdom .Occup Environ Med 1997;54:167-75.
9. Irving WS, Grumbles TG. Benzene exposures during gasoline loading at bulk marketing terminals Am Ind Hyg Assoc J 1979;40:468-73.
10. Rushton L, Thar WE Retrospective exposure assessment for benzene; issues, methods and recommendations from an international workshop on petroleum marketing and distribution worker studies. Occupational Hygine 1996;5:295-305.
11. Verma DK, Julian JA, Bebee G, et al. Hydrocarbon exposures at petroleum bulk terminal and agencies. Am Ind Hyg Assoc J 1992;53:645-56.
12. Halder CA, Van-Gorp GS, Hatoum NS, et al. Gasoline vapor exposures. Part I. Characterization of workplace exposures Part II. Evaluation of the nephrotoxicity of the major c4/c5 hydrocarbon components. Am Ind Hyg Assoc J 1986;47:164-75.

13. Boleij J, Buringh E, Heederik D, et al. Occupational hygiene of chemical and biological agents. Elsevier, Amsterdam, The Netherlands, 1995.
14. Preller L, Kromhout H, Heederik D, et al. Modelling long-term average exposure - in occupational exposure-response analysis. Scand J Work, Environ, Health 1995;21:504-12
15. Rappaport SM. Assessment of long-term exposure to toxic substances in air. Ann Occup Hyg 1991;35:61-121.
16. Burstein I, Teschke K. Methodological considerations in studies of determinants of exposure. Am Ind Hyg Assoc J (accepted for publication).
17. Heederik D, Boleij JSM, Kromhout H, et al. Use and analysis of exposure monitoring data in occupational epidemiology: an example of an epidemiological study in the Dutch animal food industry. Appl Occup Environ Hyg 1991;5:458-64.
18. Kromhout H, Swuste P, Boleij JSM. Empirical modeling of chemical exposure in the rubber manufacturing industry. Ann Occup Hyg 1994;38:3-22.
19. Peretz C, Goldberg P, Kahan E, et al. The variability of exposure over time: a prospective longitudinal study. Ann Occup Hyg 1997;41:4:485-500.
20. Burdorf A. Shifting Concepts in Assessment of Occupational Exposures. Ann Occup Hyg 1993;37:447-50.
21. Dewell P. Technical Handbook Series No. 1: Some Application of Statistics in Occupational Hygiene. BOHS, British Occupational Hygiene Society Science Reviews Ltd with H & H Scientific Consultants, Leeds, UK, 1989.
22. Kromhout H, Symanski E, Rappaport SM. A comprehensive evaluation of within- and between- worker components of occupational exposure to chemical agents. Ann Occup Hyg 1993;37:253-70.
23. Nieuwenhuijsen MJ, Lowson D, Venable KM, et al. Flour dust exposure variability in flour mills and bakeries. Ann Occup Hyg 1995;39:299-305.
24. Francis M, Selvin S, Spear R, et al. The effect of autocorrelation on the estimation of worker's daily exposures. Am Ind Hyg Assoc J 1989;50:37-43.
25. Kumagai S, Kusaka Y, Goto S. Cobalt exposure level and variability in the hard metal industry of Japan. Am Ind Hyg Assoc J 1996;57:365-69.
26. Breslow NE, Clayton DG. Appoximate inference in generlaized linear models. J of American Statistical Association 1993; 88:9-25.
27. Lindskey JK. Models for repeated measurements Clarendon Press, Oxford, GB, 1993.
28. Searle SR. Mixed models and unbalanced data: wherefrom, whereat and whereto. Communication in Statistics-Theory and Methods 1988;17-24:935-968.
29. Searle SR, Casella G , et al. Variance Components , John Wily and Sons , New York USA, 1992.
30. SAS: SAS/ STAT software, changes and enhancements. SAS Institute , North Carolina USA, 1996.
31. Symanski E, Kupper LL, Kromhout H, et al. An investigation of systematic changes in occupational exposure. Am Ind Hyg Assoc J 1996;57:724-35.
32. Rappaport SM, Lyles RH, Kupper LL.An exposure assessment strategy accounting for within- and between worker sources of variability. Ann Occup Hyg 1995;39:469-95.
33. Rappaport SM, Kromhout H, Symanski E. Variation of exposure between workers in homogenous exposure groups. Am Ind Hyg Assoc J 1993;54:654-62.

CHAPTER 5:
IMPROVED NON-NEGATIVE ESTIMATION OF VARIANCE COMPONENTS FOR EXPOSURE ASSESSMENT

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ABSTRACT

Hygiene surveys of pollutants exposure data can be analyzed by an analysis of variance (ANOVA) model with a random worker effect. Typically workers, are classified into homogeneous exposure groups, so it is very common to obtain a zero or negative ANOVA estimate of the between-worker variance (σ_B^2). Negative estimates are not sensible and also pose problems for estimating the probability (θ) that in a job-group, a randomly selected worker's mean exposure exceeds the occupational exposure standard. Therefore, it was suggested by Rappaport et al to replace a non-positive estimate with an approximate one-sided 60% upper confidence bound. This article develops an alternative estimator, based on the upper tolerance interval suggested by Wang and Iyer. We compared the performance of the two methods using real data and simulations with respect to estimating both the between-worker variance and the probability of overexposure in balanced designs. We found that the method of Rappaport et al. has three main disadvantages: (i) the estimated σ_B^2 remains negative for some data sets; (ii) the estimator performs poorly in estimating σ_B^2 and θ with 2 repeated measures per worker and when true σ_B^2 is quite small, which are quite common situations when studying exposure; (iii) the estimator can be extremely sensitive to small changes in the data. Our alternative estimator offers a solution to these problems.

INTRODUCTION

Recently, analysis of variance (ANOVA) random effects models have been applied to data sets consisting of repeated measurements of pollutants within factories in order to identify random determinants of exposure and estimate within- and between-worker variance components. The within-worker variance in these studies reflects day-to-day variations in the levels of exposure to pollutants, which often vary greatly. Between-worker variance, on the other hand, is often rather small due to the use of homogeneous exposure groups. Thus the variance ratio λ ($=\sigma_B^2/\sigma_w^2$) may be quite small. As a result, when analyzing data using ANOVA random effects models, it is very common to obtain a zero or negative estimate of the between-worker variance. In many applications, it is common practice to report such negative values as zeros.

The occurrence of negative or zero between-worker ANOVA variance estimates causes a number of problems. First, zero between-worker variance appears to be an unrealistic result since it implies that all workers have the same mean exposure. This contradicts common industrial hygiene experience. Furthermore, in exposure assessment in epidemiological studies and for hazard control, the probability θ of overexposure is often of more interest than the variance components themselves. This is the probability that in a job group, a randomly selected worker's mean exposure exceeds the occupational exposure standard, where the worker's mean exposure is relevant to the risk of chronic adverse health effects.[1] The probability of overexposure depends on both σ_B^2 and σ_W^2. Common practice is to adopt a "plug in" approach in which σ_B^2 and σ_W^2 are estimated and their estimates are inserted into the formula for θ. This approach is impossible to employ when the estimate of σ_B^2 is zero or negative. Finally, the variance ratio should have implications for planning future sampling design. Small variance ratios imply that it may be advantageous to sample fewer individuals but at more time points.

The estimation of the probability of overexposure (point estimator) becomes meaningless when a zero or negative between-worker variance estimate appears. Therefore, it was suggested by Rappaport et al.[1] to replace a negative or zero estimator with an approximate one-sided 60% upper bound, as derived from formulas of Willaims and cited in Searle et

al.[2] This practice is based on empirical evidence that such a procedure has minimal impact on significance levels and statistical power. This proposal does have some drawbacks. Many negative ANOVA estimates are not adjusted to positive values and the estimator is very sensitive to small changes in the data.

This article develops an alternative- the bias corrected variance component estimator- based on the upper tolerance interval suggested by Wang and Iyer,[3] to deal with the problem of negative variance component estimates. We compare the performance of the two methods using real data and simulations, focusing on the estimation of probabilities of overexposure (beyond standards) in balanced designs.

ANOVA method

We briefly review the ANOVA, or least squares (LS), method for estimating variance components in a balanced one-way random effects model.

We denote:

k = number of subjects in a group,

n = number of repeated measurements obtained from each subject in the group.

MSW=SSW/(k(n-1)) ; MSB=SSB/(k-1); F=MSB/MSW

The estimators of the between-subject (σ_B^2) and within-subject (σ_W^2) variance components are: $\hat{\sigma}_B^2 = [MSB - MSW\,] / n$; $\hat{\sigma}_w^2 = MSW$ For more details, see Searle et al.[2]

An example from real data: lead exposure

Nineteen workers at two Car Battery Producers in Israel were repeatedly measured to study their annual exposure to lead. They were randomly selected- 9 workers in the first factory and 10 in the second- to represent those exposed to the main processes (details can be found elsewhere[4]). Ten hygiene surveys, with intervals of 3-7 weeks, were performed in each factory over the course of a year. Due to missing data (absence of worker,s etc.) each worker had 6-10 repeated measures. We have taken the first six measures of each worker, and estimated the variance components σ_B^2 and σ_W^2 at each factory. According to Israel's regulations for factories with exposure to lead, it is mandatory to conduct two hygiene surveys each year.

In order to realise the sensitivity of the σ_B^2 estimator we have created new data sets, each including just two repeated measures out of the six. In total we had 15 sets of data with two repetitions for each factory. The exposure level was taken as a log transformation of the TLV* fraction (= log (concentration/ TLV).[4] The TLV-TWA standard for occupational lead exposure according to Israel's Regulations is 0.1 mg/m^3 .

Table 5.1a shows summary measures of the estimators in each factory, in comparison to the original estimators (="accurate") based on six repetitions. It can be seen that a negative σ_B^2 estimate resulted from 40% of the series in the first factory (with true λ=.17), and from 20% of the series, in the second factory (with true λ=.09) . In addition the ANOVA estimators for λ were quite poor. This reinforces the importance of performing more than 2 repeated surveys per year. In practice, though, many surveys are limited to two measurements as mentioned above for lead exposure. So the example also highlights the need for statistical methods that can cope with small samples. Table 5.1b shows summary measures of the estimators if four repeated surveys were performed in each factory. One can see the improvement in the estimation when doubling the number of repeated measurements per subject. The MSE [=$(\text{mean}\,\hat{\sigma}_B^2 - \sigma_B^2)^2 + \text{var}\,\hat{\sigma}_B^2$] is reduced by about 75% in the two factories.

* TLV = threshold limit value; a health-based concentration to which nearly all workers may be exposed without adverse effect.
TLV-TWO = threshold limit value, with respect to 8-h time-weighted average, that should not be exceeded during any part of the day.

Table 5.1 Summary measures of ANOVA (LS) estimators, mean, SD (min, max) on semi-simulated data sets

	Series	No. of series	$\hat{\sigma}_B^2$	$\hat{\lambda}$ $(=\hat{\sigma}_B^2/\hat{\sigma}_w^2)$
(a) n^a=2				
Factory 1	k^b=9	Accurate	.09	.17 (=.09/.53)
	Total	15	.10,.23 (-.23, .46)	.35,.57 (-.33,1.35)
	Positive	9	.26,.14 (.00, .46)	.72,.42 (.01,1.35)
	Negative	6	-.14,.08 (-.23,-.04)	-.20,.11 (-.33,-.05)
Factory 2	k^b=10	Accurate	.11	.09 (=.11/1.29)
	Total	15	.13,.37 (-.59, .65)	.24,.36 (-.27, .88)
	Positive	12	.29,.20 (.03, .65)	.36,.30 (.03, .88)
	Negative	3	-.48,.10 (-.59,-.42)	-.23,.04 (-.27,-.20)
(b) n^a=4				
Factory 1	k^b=9	Accurate	.09	.17 (=.09/.53)
	Total	15	.09,.08 (-.03, .24)	.19,.18 (-.06, .58)
	Positive	12	.12,.07 (.03, .24)	.25,.16 (.06, .58)
	Negative	3	-.02,.01(-.03,-.01)	-.04,.02 (-.06,-.02)
Factory 2	k^b=10	Accurate	.11	.09 (=.11/1.29)
	Total	15	.12,.12 (-.09,.29)	.11,.12 (-.05, .38)
	Positive	12	.16,.09 (.00, .29)	.14,.10 (.00, .38)
	Negative	3	-.05,.03 (-.09,-.02)	-.03,.02 (-.05,-.01)

NOTES:
[a] n= no. of repetitions;
[b] k= no. of workers

ESTIMATING θ IN THE PRESENCE OF A NEGATIVE ANOVA ESTIMATE OF σ_B^2

Overexposure

For hazard control, the probability θ of overexposure is very important. We present here the basic equations for overexposure as derived by Rappaport et al.[1] They followed the common assumption that the exposure x_{ij} of worker *i* on day *j* follows a lognormal distribution with:

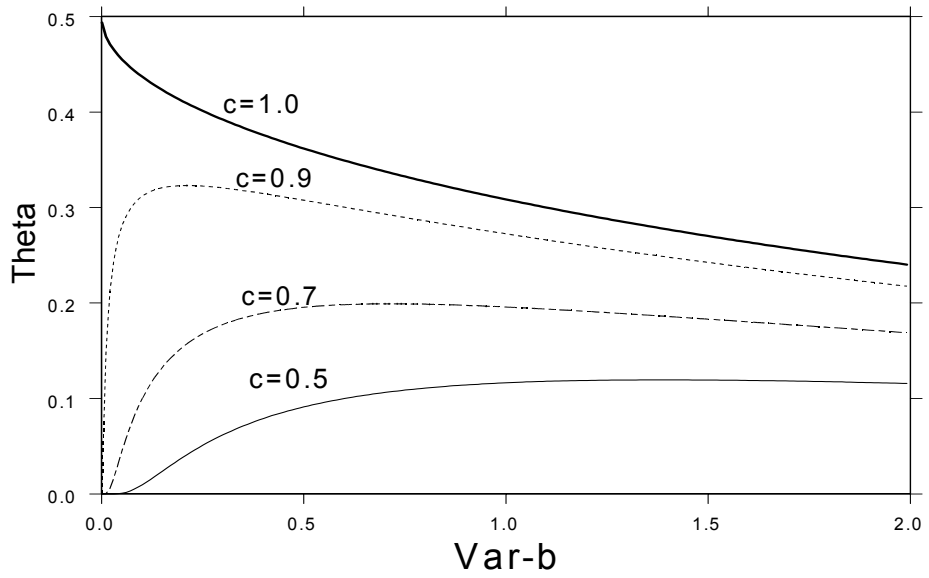
$$y_{ij} = \ln(x_{ij}) = \mu_y + \alpha_i + \varepsilon_{ij}$$

where μ_y is the mean of the overall logged exposure distribution in the group, α_i is a random effect for the *i*th worker and ε_{ij} is the within-worker random error. This model is applied to homogeneous work groups consisting of workers who perform similar tasks and therefore should have similar exposures. A worker is considered overexposed if his mean value μ_{xi} (conditional on α_i) exceeds a Standard limit (S). The probability θ that a randomly selected person from a work group is overexposed is thus:

$$\theta = p\{\mu_{xi} > S\} = p\{Z > \frac{\ln(S) - \mu_y - 0.5\sigma_w^2}{\sigma_B} = Z_{1-\theta}\} \qquad \textit{equation (1)}$$

The relationship between σ_B^2 and θ for different values of C, C= μ_x /S. (for σ_W^2=.5) is presented in Fig 5.1. It can be seen that when 0.5 ≤C≤1.0, θ has a maximum and then decreases, with little sensitivity to σ_B^2. Therefore, as θ is calculated based on an estimate of σ_B^2, the estimate of θ is quite stable for σ_B^2 values which are slightly larger than zero. There is a problem in the estimation of θ when σ_B^2 is near zero, because θ is sensitive to σ_B^2 in that region and because the ANOVA estimate of σ_B^2 estimators may be negative. Since nowadays there is an emphasis on making the exposure groups as homogeneous as possible, we may be faced with applications that have small values of λ (=σ_B^2/σ_w^2).

Figure 5.1 Relationship of the between-workers variance (Var-b) and the probability of overexposure (Theta) for different values of c (=μ_x /Standard) for the group of workers (σ_W^2=.50).



Rappaport et al. method

Rappaport et al.[1] recognized the problems of negative between-worker variance component estimates for estimating overexposure probabilities and for testing for compliance to standards. They proposed the following alternative estimator.

Use the ANOVA estimate if $\hat{\sigma}_B^2$ is positive. Otherwise, substitute $\hat{\sigma}_{B,.1-\alpha}^2$ for $\hat{\sigma}_B^2$ where $\hat{\sigma}_{B,.1-\alpha}^2$ is an approximate 100(1-α)% upper confidence bound for σ_B^2, namely

P ($\sigma_B^2 < \hat{\sigma}_{B,.1-\alpha}^2$) $\approx$ 1-α. The upper bound derived by Williams [8] and sited in Searle et al.[2] , is

$$\hat{\sigma}_{B,1-\alpha}^2 = \frac{(k-1)(MSB - F_L MSW)}{n\chi^2_{k-1,L}}$$

where

$P\{F^{(k-1)}_{k(n-1)} \le F_L)\} = 1-\alpha/2$ $\quad P\{\chi^2_{k-1} \le \chi^2_{k-1,L})\} = 1-\alpha/2$, Where $F^{(k-1)}_{k(n-1)}$ and χ^2_{k-1} represent random variables distributed as *F* with (k-1) numerator and k(n-1) denominator degrees of freedom and χ^2 with (k-1) degrees of freedom, respectively. Rappaport et al.[1] suggested using a 60% confidence bound. In a subsequent article, Lyles et al.[5] used the same basic approach but with a 95% rather than a 60%, approximate upper confidence bound for a negative $\hat{\sigma}^2_B$ ANOVA estimate. Although the latter article dealt only with hypothesis testing, the 95% upper bound could also be used in estimating θ.

Some drawbacks of the Rappaport et al. estimator

We note here two problems with the between-worker variance component estimator proposed by Rappaport et al. First, the adjustment made to negative ANOVA estimates is often insufficient to produce a positive estimate. We illustrate this feature later in a simulation study.

Second,the fact that Rappaport et al.'s estimator only corrects negative ANOVA estimates makes it very sensitive to small changes in the data. According to Rappaport et al. when $\lambda(\sigma^2_B/\sigma^2_w) \approx 0.1\text{-}0.2$, negative estimated values of σ^2_B could be observed as much as 30-40% of the time when k=10 and $2 \le n \le 4$. This probability can be reduced by increasing the sample size; however, in reality, many occupational hygiene groups are of this order of magnitude, having 20-40 repeated measurements.[6]

We illustrate the sensitivity of Rappaport et al.'s estimator with simple example using simulated data with k=10, n=2 , σ^2_B=.1 and σ^2_W=1. First, a random set was generated and gradually, eight slight changes were made to create eight further sets, each with the same worker averages but with increasingly larger within worker residuals.

Table 5.2 presents the variance components estimates according to ANOVA and Rappaport et al.'s method with the 60% confidence bound.

From step 7 on, the ANOVA estimator $\hat{\sigma}^2_B$ ANOVA, was negative. The Rappaport et al. estimate for σ^2_B makes a sudden jump from very small to very large values at step 7 and thus is quite sensitive to small changes in the study data. A slight increase in the within-

workers mean square could change a positive ANOVA estimate to a negative one, thus sharply increasing Rappaport et al.'s estimate. This change could lead to a much larger estimate of θ. Since the error term in exposure measurements is already known to vary greatly over time (contributing to the within worker variability), measuring the same exposure group at different times can easily produce negative $\hat{\sigma}_B^2$ ANOVA estimates.

Table 5.2 Sensitivity of σ_B^2, σ_W^2 estimators to small changes in values of a set of data

Set	Percent inflation of residuals	$\hat{\sigma}_{w}^2$_ANOVA	$\hat{\sigma}_{\boldsymbol{B}}^2$_ANOVA	$\hat{\sigma}_{\boldsymbol{B}\_1}^2$[a]
1	random set	.72	.23	.23
2	5	.80	.20	.20
3	10	.87	.16	.16
4	15	.96	.12	.12
5	20	1.04	.07	.07
6	25	1.13	.03	.03
7	30	1.22	-.02	3.47
8	35	1.32	-.06	3.45
9	40	1.42	-.11	3.43

NOTES: n=2; k=10, original: σ_B^2=.1 , σ_W^2=1

[a] $\hat{\sigma}_{\boldsymbol{B}\_1}^2$ – according to Rappaport et al.'s method, based on upper bound of 60%

Bias-adjusted variance component estimation (BAVCE)

We suggest an alternative estimate to overcome some of the limitations of the estimator proposed by Rappaport et al. Our method, which we call BAVCE, is based on the upper tolerance interval suggested by Wang and Iyer.[3]

It takes account of the fact that an upper confidence bound will typically be biased high and multiplies by a factor that attempts to adjust for this bias. The estimator is defined as follows:

$\hat{\sigma}_B^2 = \omega^2 (MSB - F_L MSW) / n$

where

$P\{ F_{k(n-1)}^{(k-1)} \leq F_L)\} = \eta$ for $\eta = (1-\gamma)/n$,

γ is the confidence level (which we have taken to be .95),

$\omega^2 = \tilde{\phi} / \left[1 - (1 - \tilde{\phi}) F_L \right]$ and $\tilde{\phi} = \max(0, 1 - F_L MSW / MSB)$.

The BAVCE estimator, like the others (Rappaport et al.[1] ,Lyles et al.[5]) reduces the frequency of negative or zero estimates by subtracting less than the full value of MSW from MSB. However, their use of an upper confidence bound as an estimator almost guarantees an overestimate of σ_B^2. The factor ω^2 in the BAVCE attempts to correct the upward bias. To see how the bias correction works, we present an approximation to the expected value of $\hat{\sigma}_B^2$ by assuming that $\phi = 1 - [\sigma_W^2 / (n\sigma_B^2 + \sigma_W^2)]$ is known. Then

$$\omega^2 = \frac{n\sigma_B^2 / (n\sigma_B^2 + \sigma_W^2)}{1 - F_L \sigma_W^2 / (n\sigma_B^2 + \sigma_W^2)} = \frac{n\sigma_B^2}{n\sigma_B^2 + (1 - F_L)\sigma_W^2}$$

and

$$E\{\hat{\sigma}_B^2\} = \omega^2 E\{MSB - F_L MSW\} / n = \omega^2 [n\sigma_B^2 + (1 - F_L)\sigma_W^2] / n$$

$$= \frac{n\sigma_B^2}{n\sigma_B^2 + (1 - F_L)\sigma_W^2} [n\sigma_B^2 + (1 - F_L)\sigma_W^2] / n = \sigma_B^2.$$

The bias correction is implemented by using a "plug-in" estimator of ϕ in which the observed mean squares replace their expected values.

Comparison of estimators on simulated data

Simulated Data

Simulations were run to compare the different estimators of σ_B^2 and θ. The estimators of σ_B^2 were the ANOVA estimator, the estimator of Rappoport et al. with a 60% bound (method 1) and with a 95% bound (method 1A), and the BAVCE proposed here (method

2). The estimators of θ were generated by plugging the estimators of σ_B^2 along with the ANOVA estimator of σ_W^2 and the sample average into the equation (1) in Section 4. The simulations covered three different practical settings defined by the number of repetitions (n) and the number of subjects (k):

(i). 1000 data sets for k=10, n=2 (20000 observations);

(ii). 1000 data sets for k=10, n=3 (30000 observations); and

(iii). 1000 data sets for k=10, n=4 (40000 observations).

In addition, we examined several different values of σ_B^2. The within-subject variance σ_W^2 was held constant at 1 in all the simulations. Original values for θ for n=2,3,4 were computed from equation (1). When the least squares estimate of the between-workers variance component σ_B^2 was negative, method 1 modified it to a larger value. The method 2 estimator increased all the σ_B^2 estimates, not just the negative ones.

Comparison of estimators

Tables 5.3 and 5.4 present the estimators based on the simulated data for n=2 , 3 and 4, when original σ_B^2=.2 (Table 5.3) or σ_B^2=.05 (Table 5.4) , which are representative of the results that we found for all the values of σ_B^2.

Tables 5.3a and 5.4a relate to the estimators when negative ANOVA estimates were found. Tables 5.3b and 5.4b relate to the estimators when positive ANOVA estimates were found. As was found previously, the ANOVA estimator of σ_B^2 was often negative for the cases we studied. In Table 5.3a (σ_B^2=.2, λ=.2), we can see that more than 40% of the data sets for n=2,3,4 resulted in a negative σ_B^2 ANOVA estimate and in Table 5.4a (σ_B^2=.05, λ=.05) we can see that the percentage was higher, over 50%.

A serious problem with Rappaport et al.'s method is that many negative estimates of σ_B^2 remained negative. The problem is especially acute with the 60% confidence bound. Even with σ_B^2=.20 and four replications per subject, almost 30% of the negative ANOVA estimates remained negative with this method. Using their method with a 95% confidence bound reduces the problem but did not eliminate it, with 7-10% of the negative ANOVA

estimates remaining negative. Our method was much more successful in this regard. Negative estimates are automatically adjusted to 0 and these occurred in less than 4% of the cases with negative ANOVA estimates in all the settings we examined.

In conclusion, there is an estimation problem using method 1 when n=2 or 3 and σ_B^2 / σ_W^2 is less than 0.20.

Table 5.3 Comparison of estimation methods based on simulations of 1000 sets for ten workers with 2,3,4 repetitions

a. Results for negative LS estimators of σ_B^2

Repetitions (no. of series)		$\hat{\sigma}_B^2$ (original=.20)				$\hat{\theta}$ (original=.34)	
		ls_method	method_1	method_1a	method_2	method_1	method_2
2[a]							
(473)	mean, SD	-.23,.19	.06,.23	.51,.37	.16,.11	.32, .03	.31, .06
	min, max	-1.13,.00	.24, 6.14	-.55, 1.83	.00, .58	.00, .34	.00, .34
3[b]							
(478)	mean, SD	-.13, .10	.05, .13	.32, .23	.11, .07	.31, .05	.32, .05
	min, max	-.61, .00	-.47, .43	-.33, 1.21	.00, .41	.04,.34	.00, .34
4[c]							
(413)	mean, SD	-.09, .07	.04, .10	.24, .16	.09, .05	.30, .06	.31, .06
	min, max	-.41, .00	-.30, .26	-.19, .67	.00, .24	.00, .34	.00, .34

b. Results for positive LS estimators of σ_B^2

Repetitions (no. of series)		$\hat{\sigma}_B^2$ (original=.20)		$\hat{\theta}$ (original=.34)	
		method_1 (ls_method)	method_2	method_1	method_2
2					
(527)	mean, SD	.27, .21	.49, .21	.31, .05	.32, .02
	min, max	.00, 1.14	.11, 1.30	.00, .34	.25, .34
3					
(522)	mean, SD	.17, .14	.34, .14	.31, .06	.33, .01
	min, max	.00, .76	.10, .93	.00, .34	.28, .34
4					
(587)	mean, SD	.14, .11	.28, .11	.30, .06	.33, .01
	min, max	.00, .53	.08, .63	.00,.34	.31,.34

NOTES: ls_method = least squares method; method_1= Rappaport's et al methods based on upper bound of 60%; method_1a= Rappaprt's et al methods based on upper bound of 95%; method_2 = our method, based on a modified upper bound

[a] 298/473 positive according to method_1; 437/473 positive according to method_1a; 463/473 positive according to method_2

[b] 318/478 positive according to method_1; 445/478 positive according to method_1a; 462/478 positive according to method_2

[c] 293/413 positive according to method_1; 386/413 positive according to method_1a; 408/413 positive according to method_2

Table 5.4 Comparison of estimation methods based on simulations of 1000 sets for 10 workers with 2,3,4 Repetitions.

a. Results for negative LS estimators of σ_B^2

Repetitions (no. of series)		$\hat{\sigma}_B^2$ (original=.05)			$\hat{\theta}$ (original=.31)		
		ls_method	method_1	method_1a	method_2	method_1	method_2
2[a]							
(505)	mean, SD	-.25,.19	.04,.24	.47, .37	.15,.11	.32, .05	.31, .06
	min, max	-1.11,.00	-.87, .72	-.52, 1.82	.00, .59	.00, .34	.00, .34
3[b]							
(538)	mean, SD	-.14, .10	.03, .14	.29, .22	.10, .07	.31, .06	.31, .06
	min, max	-.62, .00	-.48, .40	-.33,.1.15	.00, .39	.00,.34	.00, .34
4[c]							
(510)	mean, SD	-.09, .07	.03, .10	.22, .15	.08, .05	.30, .07	.31, .06
	min, max	-.39, .00	-.28, .24	-.18, .63	.00, .22	.00, .34	.00, .34

b. Results for positive LS estimators of σ_B^2

Repetitions (no. of series)		$\hat{\sigma}_B^2$ (original=.05)		$\hat{\theta}$ (original=.31)	
		method_1 (ls_method)	method_2	method_1	method_2
2					
(495)	mean, SD	.24, .20	.46, .20	.31, .05	.32, .02
	min, max	.00, 1.05	.10, 1.16	.00, .34	.27, .34
3					
(462)	mean, SD	.15, .12	.32, .12	.31, .05	.33, .01
	min, max	.00, .83	.11, .93	.00, .34	.28, .34
4					
(490)	mean, SD	.12, .10	.25, .10	.30, .07	.33, .01
	min, max	.00, .50	.08, .60	.00,.34	.31,.34

NOTES: ls_method = least squares method; method_1= Rappaport's et al method based on upper bound of 60%, method_1a= Rappaprt's et al method based on upper bound of 95%, method_2 = our method, based on a modified upper bound

[a] 303/505 positive according to method_1; 462/505 positive according to method_1a; 485/505 positive according to method_2

[b] 336/538 positive according to method_1; 485/538 positive according to method_1a; 521/538 positive according to method_2

[c] 326/510 positive according to method_1; 469/510 positive according to method_1a; 501/510 positive according to method_2

AN EXAMPLE

Survey on pig farmers' exposure to inhalable endotoxins

In a study of 200 pig farmers from the south of the Netherlands, exposure to inhalable dust and endotoxins was monitored by personal sampling. Exposure was measured during one work shift on a randomly chosen day of the week, one day during the summer of 1991 and one day during the winter of 1992. Outdoor temperature was obtained from a monitoring station in the south of the Netherlands. Task activity patterns on the day of measurement and farm characteristics were also recorded.[7]. For the purpose of this paper, only the exposure data on endotoxins will be used on 153 farmers out of the 200 who had two measurements (the rest had some failure in the measuring process for one measurement). For the whole study population (n=153), the following estimates were calculated and they were considered to be the accurate parameters for the pig farmers': σ_B^2=.13, σ_W^2=.64, θ=.32, μ_y=7.81. We have taken the standard to be 8.29 (Standard=log(4000ng/m^3) =8.29) for this example.

We compared the different estimators of σ_B^2 by generating 100 sub-samples . Each farmer was included /excluded from a particular sample by drawing a binomial random variable with probability 0.1 for inclusion. For the 100 sub-samples, mean±SD. of the μ_y values= 7.81±0.15. The same parameters were estimated while σ_B^2 was estimated by the different methods (see table 5.5).In this example, only about 20% of the series resulted in negative ANOVA estimates. Thus , one might expect that our method, which always corrects $\hat{\sigma}_B^2$, might be less successful. Nonetheless, for θ, our estimate performed better than that of Rappaport et al., with a smaller SD especially for the samples with positive ANOVA estimate. For $\hat{\sigma}_B^2$, Rappaport et al.'s estimate over the 100 samples seemed to perform better than our method. This conclusion differs from our previous conclusion regarding the simulated data due to the different sample sizes. Here, on average, 15 subjects were included in each sample while in our previous samples, we had only 10 subjects per each sample. The Rappaport et al.'s estimator of σ_B^2 was more accurate with a 60% bound than with a 95% bound.

Table 5.5 Parameter estimation according to the different methods (mean ± SD)

	k	n	$\hat{\sigma}_w^2$	$\hat{\sigma}_B^2$				$\hat{\theta}$	
			ls_method	ls_method	method_1	method_1a	method_2	method_1	method_2
All farmers	153	2	.64	.13 (λ=0.20)					
Subsamples Negative $\hat{\sigma}_B^2$_ls (21 series)	8-20	2	.73±.14	-.09±.06	.09[b]±11	.34±.21	.11±.07	.29[b]±.21	.27±.18
Subsamples positive $\hat{\sigma}_B^2$_ls (79 series)	5-25	2	.59±.15	.19±.15	.19±.15	.19±.15	.34±.15	.32±.17	.32±.09

NOTES: ls_method = least squares method, method_1= Rappaport's et al method based on upper bound of 60%, method_1a= Rappaprt's et al method based on upper bound of 95%, method_2 = our method, based on a modified upper bound

[a] Randonuni function- returns a number from the uniform distribution on the interval (0,1). The function was applied 100 times on the original data set , each time with another seed. In each time, observations with a random number less than 0.1 were included in the new sub-sample.

[b] Four negative values for $\hat{\sigma}_B^2$ according to method_1a consequently 4 missing values for $\hat{\theta}$ according to method_1

DISCUSSION

The use of Rappaport et al.'s approach for assessing compliance for hazard control is a new application. It has inherent statistical considerations and takes into account the variance components of the hazardous exposure based on real-life data sets and should be recommended for use. However since it is a new tool, caution and further study are needed. In exposure data sets, ANOVA estimators for between-variance components are quite often negative (see sensitivity analysis).

The common practice of changing such negative values to zeros prevents the application of popular plug-in estimators in compliance assessment and it also appears to be an unrealistic result since it implies that all workers have the same mean exposure.

The modified variance component estimator for negative values proposed by Rappaport et al.[1] and by Lyles et al.[5] has three main disadvantages:

1. It remains negative for some data sets.
2. It performs poorly in estimating σ_B^2 and also θ when n=2 and when original σ_B^2 is quite small and 0.5*standard$\leq\mu_x\leq$1.0*standard, which are quite common situations when studying exposure.
3. Discontinuous behavior: small changes in the data set can make the ANOVA estimator negative, resulting in the use of the modification, which may cause a large change in the conclusions of a study.

In this paper we have proposed an alternative variance component estimator, the BAVCE, to cope with the problem of negative and zero between-worker ANOVA estimates. Our modification seems to react better than the estimator of Rappaport et al. as can be seen in the tables. Thus, from our simulations and the simulated subsets of data.

We think that further thought should be given to analysis of data from unbalanced designs, which are common in real-life exposure data sets due to absence of workers and changes in work practices. Here exposure was measured in industry and agriculture. The same ideas can be applied to environmental exposure within the community.

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REFERENCES

1. Rappaport SM, Lyles RH, Kupper LL. An exposure -assessment strategy accounting for within-and between-worker sources of variability. Ann Occup Hyg 1995;39:469-95.
2. Searle SR, Casella G, McCulloch CE. Variance Components, John Wiley & Sons, New York, 1992.
3. Wang CM, Iyer HK. Tolerance intervals for the distribution of true values in the presence of measurement errors. Technometrics 1994;36:162-70.
4. Peretz C, Goldberg P, Kahan E, Grady S, Goren A. The variability of exposure over time: a prospective longitudinal study. Ann Occup Hyg 1997;41:485-500.
5. Lyles RH, Kupper LL, Rappaport SM.Assessing regulatory compliance via the balanced one-way random effects ANOVA model. Journal of Agricultural, Biological and Environmental Statistics 1997;2:64-86.
6. Kromhout H, Symanski E, Rappaport SM. A comprehensive evaluation of within- and between-worker components of occupational exposure to chemical agents. Ann Occup Hyg 1993;37:253-70.
7. Preller L, Kromhout H, Heederik D, Tielen M. Modelling long-term average exposure in occupational exposure-response analysis. Scand J Work, Environ, Health 1995;21:504-12.
8. Williams JS. A confidence interval for variance components. Biometrica, 1962;49:278-81.

CHAPTER 6:
EXPOSURE ASSESSMENT TO WHEAT FLOUR AND SHAPE OF THE RELATIONSHIP FOR SPECIFIC SENSITIZATION

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ABSTRACT

Objective: We aimed to assess dust exposure among different wheat exposed workers and to study their risk for sensitization. Methods: About 520 measures of inhalable dust and wheat allergens were performed among 270 individuals from four sectors of industry. Data on sensitization to wheat and common allergens (atopy) was also available. Using mixed effects models, we estimated exposure levels according to sector, job title and tasks. The shape of the relationship between sensitization and exposure was studied using a 2-stage modeling approach: a semi-parametric generalized additive model and consequently a parametric logistic model. To avoid possible risk estimate attenuation, we modeled variance weighted estimate (VWE) of exposure in addition to the actual exposure. Results: The effect of exposure to both inhalable dust and to wheat allergens on sensitization was found to be a quadratic relationship. The probability of sensitization increased with exposure up to ~2.7 mg/m^3 for inhalable dust and ~25.7μgEQ/m^3 for wheat allergens. The risk decreased at higher exposures (possibly a healthy worker effect or tolerance) (OR=.84,.94 p=.0121 and p=.0731 for dust and wheat respectively). Atopy and sector of industry modified the sensitization risk significantly in all the analyses (OR for atopy was ~6, p<.0001; OR in the industrialized bakeries was ~4.2 times higher than in flour mills). Using VWE exposures corrected for bias resulted in almost the same point risk estimates. Conclusions: The exposure-response relationship may be non-linear and requires exploration. Our findings also do not indicate a threshold for occupational exposure standards, alternatively, other approaches such as benchmarking seem warranted.

INTRODUCTION

Recently, there has been growing interest in risk assessment for allergens by several countries and professional bodies (e.g., the American Conference of Governmental Industrial Hygienists, ACGIH,[1,2] and the Dutch Expert Committee for Occupational Standards, DECOS.[3] Based on these risk assessments, health-based exposure standards were proposed for diminishing the risk for becoming sensitized against wheat flour allergens. Such proposals were made by both ACGIH and DECOS.[1-3] Other organizations, such as the Health and Safety Executive (HSE) from the United Kingdom and the Nordic Expert Group, favored a different approach of which the rationale was described earlier.[4,5] The standard setting process is complicated because only a few studies have dealt in any detail with exposure among bakers and sensitization, mainly because these data were not available until recently.[6-9] Moreover, the shape of the relationship was not studied in depth in most earlier studies: it was either based on simple categorization of the exposure or on job titles (e.g., bread versus cake bakers), or assumed to be linear. Risk assessments have as a result been fairly simple, straightforward and based on a subjective or intuitive interpretation of the data. The shape of exposure-response relationships for sensitization agents has received specific attention in more recent studies in the indoor environment, and the results were suggestive of a bell-shaped curve for some allergens and linear for others.[10] The current paper is therefore based on new data for an occupational allergen collected in four sectors of industry, thereby including workers with higher levels of exposure to both inhalable dust and wheat flour, than in earlier studies. Earlier analyses of this data set ignored the fact that task information can be used and allows more powerful modeling approaches. Advanced exposure modeling is necessary to obtain a refined assessment of exposure estimates that allows subsequent evaluation of the derived exposure- response shape.

Therefore, in this paper, exposure determinant modeling was performed to evaluate estimated exposure levels to inhalable dust and wheat allergens based on measurements among occupational wheat exposed populations. In addition, for risk assessment purposes, a refined non-linear exposure-sensitization relationship was explored with advanced statistical tools.

METHODS

Subjects

The data originated from a survey among Dutch bakers including a medical and a hygiene part, carried out between August 2000 and July 2001. We limited our study to those 270 workers who were included in both the hygiene and medical survey, belonging to 83 flour-processing enterprises, from four industrial-sectors.

Occupational factors

Occupational factors included industry-sector, job-title within a sector and tasks-performed within a job-title, referred to as exposure determinants. We investigated four sectors: traditional bakeries, industrialized bakeries, flour mills and bakery-ingredient industries. Each sector can be described by several jobs (see appendix for details). About thirty jobs within sectors were observed. In addition about 80 tasks were distinguished.

Dust and allergen exposure assessment

Personal inhalable dust samples were collected in the workers breathing zone during full-shift periods of 6-8 hours using PAS6 sampling heads at a flow rate of 2 L/min. Dust levels (mg/m^3) were measured by weighing in a preconditioned weighing room before and after the measurements. Wheat allergens were recovered from the filters by extraction using a buffer solution (PBS) and the wheat allergen concentrations were measured in the extract by inhibition immuno-assay, using a pool of human IgG_4 polyclonal antibodies, as described earlier and expressed in $\mu gEQ/m^3$.[7] For the 270 workers 335 personal inhalable dust samples were collected and out of them 298 wheat allergen exposure measurements were performed.

Repeated exposure measures

Of the 270 bakers, 208 (77%) had one exposure measurement, 59 (22%) had 2 repeated measures and 3 (1%) had 3 repeated measures. Repeated measures were taken within a 2–6 weeks period on randomly selected workers in the four sectors. This procedure was

performed for further assessing the day-to-day variability of exposure in a subset of workers.

Health outcomes

Wheat specific sensitization: Venous blood samples were analyzed for the presence of specific IgE antibodies against wheat flour allergens using the Pharmaca Diagnostics Uni CAP assay. Individuals with levels of class 1 or higher were considered positive.

Atopy: Sera were also analyzed for the presence of IgE against common allergens: house dust mite and grasses. Individuals were considered atopic if any of the common allergens had a level of class 1 or higher.

Statistical analyses

Statistical analyses were performed using SAS software.[12,13] Exposure values below the detection limit were replaced by two thirds of this limit. Some extremely high exposure levels were replaced by the 98 percentile: 100 mg/m^3 for inhalable dust and 400$\mu gEQ/m^3$ for wheat allergens level. Exposure distributions for both inhalable dust and wheat allergens were found to be log-normal,[7] and therefore the levels were natural log-transformed for the statistical analysis.

Exposure assessment

Linear mixed effects models, which account for the correlation between repeated measures were used for estimation (SAS- Proc mixed) of exposure to inhalable dust and wheat allergens.

a. We used a model with log exposure level as the dependent variable, and sector of industry, job-title and 80 tasks as covariates, to identify tasks associate with exposure. We then included those tasks found to have an effect at least borderline statistical significance ($p<=.10$) on the level of either inhalable dust or wheat allergen exposure.

b. For estimating variance components between (var-b) and within (var-w) workers we used different approaches. Four mixed models were applied with log exposure levels as the dependent variable. The four models included 1) no covariates, 2) sector of industry, 3) sector of industry and job title (nested within sector of industry), 4) sector of industry, job-

title and "effective tasks" (nested within job title). In addition, we assessed the goodness of fit of the model with the three covariates in comparison to models with one or two covariates using the likelihood ratio test.

c. Estimated (=predicted) mean exposure level in each job title within a sector was estimated using the models above. We also estimated the var-b and var-w of the estimated exposures. For further analysis, for each worker with repeated measurements, we averaged the estimated values.

Modeling exposure-response relationship

The relationship between sensitization (defined as a binary health outcome) and exposure to inhalable dust and wheat allergens accounting for atopic status and sector was firstly evaluated by calculating sensitization prevalence among categories of exposure, sector of industry and atopic status (SAS-Proc Freq/Univariate).

To explore the shape of the relationship between sensitization and exposure:

a. We fitted a semi-parametric generalized additive model. We adjusted for the following parametric effects: atopy (no/yes) and sector of industry (4 categories) as the linear predictors of the parametric part of the model and log-concentration as an additive predictor as the non-parametric part of the model. The term for the additive predictor was fitted using a spline as a smoother. The model is a generalized model since the probability distribution of the dependent variable sensitization (binary no/yes), is binomial and the relationship with the predictors is through a non-linear link function (logit= p/(1-p)). The degrees of freedom for the additive predictor were selected by a generalized cross-validation method that indicates the degree of the polynomial that represents the data (SAS-Proc GAM). We used scatter-plots (not shown) to present the relationship of logged concentration and their partial prediction by the smoother for dust and wheat exposure.

b. After inspection of the semi-parametric curve and interpretation of the cross-validation results we choose a parametric model – a generalized linear model (with distribution=binomial and link=logit) where the dependent variable was the logit of sensitization and the independent variable were: atopy, sector of industry, linear and quadratic terms of logged concentration. Odds ratios as well as confidence limits of exposure, atopic status and sector of industry for sensitization were estimated for this

logistic model (SAS- Proc Genmod) Goodness of fit of model was evaluated according to the deviance.

The semi-parametric model (step a) and the parametric model (step b) were applied to the estimates from the four different exposure assessment approaches: measured exposure , estimated exposure based on the industrial-sector of industry and job title (2 covariates), estimated exposure based on sector of industry, job-title and tasks performed (3 covariates) and on a Variance-Weighted Estimator (VWE) of measured exposure and estimated exposure (see appendix b). The VWE is a modification of the approach proposed by Seixas et al.[19] in which the model predicted exposure and the actual measurements are combined. It reduces negative effects of grouping strategies (described in relation to Berkson error).

RESULTS

Exposure- Descriptive statistics

Table 6.1 presents summary statistics on exposure within the various sectors, ignoring the dependence between repeated measures. The highest mean exposure level, for both inhalable dust and wheat allergens, was observed in the flour mills (geometric means: 2.72 mg/m^3 and 9.41$\mu gEQ/m^3$, respectively) while the mean exposure level was the lowest, both to inhalable dust and wheat allergens, in industrialized bakeries (geometric means 1.03 mg/m^3 and 2.14 $\mu gEQ/m^3$, respectively).

Table 6.1 Summary statistics regarding exposure to inhalable dust (mg/m^3) and wheat allergens ($\mu gEQ/m^3$) within sectors

Sector	k	Inhalable dust		Wheat allergens	
		GM (GSD)	n	GM (GSD)	n
Traditional bakeries	70	1.71 (2.99)	80	8.63 (7.45)	65
Industrialized bakeries	72	1.03 (3.72)	91	2.14 (14.07)	83
Flour mills	73	2.72 (4.07)	94	9.41 (9.32)	85
Enzyme processing	55	1.16 (4.42)	70	2.66 (11.03)	65
Total	270	1.56 (3.98)	335	4.64 (11.38)	298

NOTES: k=no. of workers; n= no. of measurements

Variance components between- and within-workers using different approaches with respect to exposure determinants

Actual exposure measurements: Different approaches were evaluated with respect to the three exposure determinants, sector of industry, job title and nature of tasks. Table 6.2 presents the variance components between- and within-workers obtained from models with and without exposure determinants. The grouping strategy based on sector of industry and job title led to a reduction of the variance between workers by 55% for dust and by 50% for wheat. Adding task information, however, reduced the variance between workers even more, by 78% for dust and by 56% for wheat. Thus, industrial sector, job-title and specific tasks could explain the variance in exposure between mean exposures of different bakers over time. The tasks also had an influence on the day-to-day variability of exposure to wheat (i.e., the variance within workers was reduced from 1.88 to 1.62).

Estimated exposure values: The model with sector of industry and job title fitted significantly better than the model with industry sector alone. The 3-covariate model with the sector of industry, job title and tasks demonstrated a superior fit in comparison to the other models for both exposure to inhalable dust and wheat allergens ($p<.0001$ for all, based on likelihood ratio tests). We therefore calculated two exposure proxies for each worker based on his sector of industry and job title or sector of industry, job title and tasks performed. If a worker had different tasks or jobs during the 2-3 repeated measures, he would have 2-3 different exposure estimates. Variance components (between- and within-

workers) based on estimated exposure levels were found to be significantly lower ($p<.01$) than those based on actual exposure measurements (table 6.2). As expected, estimated exposures were more precise and thus may correlate better with health responses.9

Modeling exposure -response relationship

Table 6.3 presents the prevalence of sensitization among the various categories of exposure (quartiles), sector of industry and atopic status. We fitted a semi-parametric generalized additive model to discover the appropriate shape of the relationship between sensitization and exposure, and the results of this model for exposure to dust and wheat allergens are given in table 6.4 and Appendix c. The effect of exposure using a smoothing spline was at least borderline significant for inhalable dust and wheat allergens ($p=0.0462$ and $p=0.0941$, respectively) (table 6.4). The relationship between log-concentration and sensitization seemed to be quadratic from the scatter-plot of logged concentration and their partial prediction by the smoother. Consequently, we applied a parametric model (i.e., a generalized linear model with binominal distribution and logit link), where the dependent variable was the logit of sensitization and the independent variables were atopy, sector of industry, and the linear and quadratic terms of log concentration. The modeling was also applied for estimated exposure levels, and the same quadratic relationship was found with the semi-parametric model (not shown).

Table 6.2 Variance components and 95% confidence intervals (CI) for different approaches with respect to exposure determinants (exposure to inhalable in dust mg/m^3 and to wheat allergens in μgEQ/m^3)

	Inhalable dust			Wheat allergens		
Exposure determinants	Var-b (95%CI)	Var-w (95%CI)	Reduction of var-b	Var-b (95%CI)	Var-w (95%CI)	Reduction of var-b
a. Using measured exposure, with different exposure determinants						
None	1.16	.74	-	4.00	1.88	-
	(.86,1.64)	(.54,1.07)		(3.03,5.51)	(1.30,2.96)	
Sector	1.03	.75	11%[a]	3.61	1.89	10%
	(.75,1.49)	(.55,1.08)	(λ^b=.61)	(2.69,5.09)	(1.31,2.98)	(λ=.68)
Sector+job	.52	.80	55%	2.00	2.00	50%
	(.29,1.17)	(.57,1.22)	(λ=.58)	(1.28,3.59)	(1.37,3.18)	(λ=.66)
Sector+job+task	.25	.78	78%	1.75	1.62	56%
	(.10,1.57)	(.55,1.20)	(λ=.39)	(1.08,3.31)	(1.06,2.79)	(λ=.50)
b. Using estimated exposures based of different exposure determinants						
Sector+job	.56	.12	52%	1.93	.35	52%
	(.46, .70)	(.09, .17)		(1.59,2.39)	(.26, .51)	
Sector+job+task	.63	.35	46%	2.12	1.03	47%
	(.49, .85)	(.26, .49)		(1.65,2.82)	(.76,1.47)	

NOTES: Exposure was expressed in logged concentrations
Var-b: variance between workers; var-w: variance within-worker; estimation was based on mixed effects models.

[a] (1.16-1.03)*100/1.16=11%

[b] λ= var-b/(var-b + var-w) known as reliability ratio

Table 6.3 Prevalence (%) of sensitization among categories of: exposure to inhalable dust and wheat allergens (inhalable dust mg/m^3 , wheat allergens μgEQ/m^3), sector and atopy (k=no. of workers)

Factor	Categories		k	Sensitization (%)
Exposure	[.01- .59)		54	18.5
To inhalable dust	[.59- 1.14)		54	33.3
	[1.14- 2.06)		54	29.6
	[2.06- 4.70)		54	27.7
	[4.70-100.00)		54	20.4
Exposure	[.03-.34)		54	10.6
to wheat allergens	[.34-4.84)		54	25.5
	[4.84-15.18)		54	34.0
	[15.18-47.39)		54	29.8
	[47.39-400)		54	27.1
Atopy	No		193	37.1
	Yes		77	50.6
Sector	Traditional bakeries		70	37.1
	Industrialized bakeries		72	34.7
	Enzyme processing		55	16.4
	Flour mills		73	13.7
Sector & Atopy	Traditional bakeries	No	42	19.0
		Yes	28	64.3
	Industrialized bakeries	No	52	26.9
		Yes	20	55.0
	Enzyme processing	No	45	8.9
		Yes	10	50.3
	Flour mills	No	54	9.3
		Yes	19	26.3
Total			270	25.9

Table 6.4 Effects of exposure (inhalable dust mg/m^3 , wheat allergens μgEQ/m^3), sector and atopy on sensitization – using a semi-parametric generalized additive model with a smoothing spline

		Inhalable Dust			Wheat allergens		
		estimate	Se	p	estimate	Se	p
Regression model	Int.	-2.62	.18	<.0001	-2.86	.23	<.0001
(parametric part)	Traditional bakeries	1.22	.34	.0004	1.25	.39	.0015
	Industrializd bakeries	1.45	.38	.0002	1.48	.36	<.0001
	Enzyme processing	.56	.54	NS	.71	.53	NS
	Flour mills#	0	.		0	-	-
	Atophy	1.75	.33	<.0001	1.69	.36	<.0001
	Linear[a] log conc.	.11	.17	NS	.15	.09	.0824
Smoothing model (non-parametric part)	Spline log conc.	DF =~2[b]		.0462	DF =~2[b]		.0941

NOTES: log conc.= log transformed concentration ; NS=(p>.10) ; #reference group
[a] linear term of log concentration
[b] Degrees of Freedom (DF) were selected by a generalized cross-validation method

Table 6.5 and fig. 6.1 show the results of the quadratic logistic regression model for the whole group. The results in table 6.5a and fig. 6.1(i) are based on measured exposure levels and those in table 6.5b and fig. 6.1(ii) on the estimated ones. Table 6.5 presents the odds ratios (ORs), confidence limits (CIs) and significance levels (p) for exposure, atopic status and sector of industry on sensitization risk. Fig. 6.1 depicts the quadratic relationship between exposure and the probability of sensitizing in terms of the different sectors among atopic and non-atopic individuals.

Table 6.5 Effects of exposure to inhalable dust ($mg/m^{3)}$, wheat allergens ($\mu gEQ/m^3$) and atopy on sensitization- using a quadratic logistic regression model (k=270): Odds ratios and 95% confidence intervals

Based on:

a. Actual exposure with sector as a covariate

		Inhalable dust			Wheat allergens		
		Odds ratio	95% LRCI	p[b]	Odds ratio	95% LRCI	p
Log-conc		1.41	1.03 - 2.01	.0329	1.31	1.05- 1.67	.0142
Log-conc^2		.84	.72 - .96	.0087	.96	.90- 1.02	.1539
Atopy		6.12	3.26 -11.79	<.0001	5.49	2.87-10.75	<.0001
Sector	Traditional bakeries	3.28	1.36 - 8.34		3.46	1.42- 8.86	
	Industrialized bakeries	4.40	1.80 -11.54		4.57	1.85- 12.06	
	Enzyme processing	1.78	.61- 5.28		2.00	.68- 5.84	
	Flour mills[a]	1.00		.0049	1.00		.0040

b. Estimated exposure according to sector, job title and tasks

Log-conc	1.24	.83- 1.98	.3300	1.50	1.10 - 2.18	.0185
Log-conc^2	.62	.43- .83	.0039	.87	.79 - .95	.0037
Atopy	5.90	3.23-11.00	<.0001	5.90	3.23 -10.99	<.0001

c. Estimated exposure according to sector and job title

Log-conc	1.10	.71-1.82	.6800	1.33	.99-1.93	.0865
Log-conc^2	.71	.49-.95	.0039	.91	.81-1.00	.0651
Atopy	5.78	3.18-10.68	<.0001	5.66	3.13-10.42	<.0001

NOTES: 95% LRCI= 95% likelihood ratio confidence interval;
Log-conc =linear term of log transformed concentration; Log-conc^2 =quadratic term of log-conc

[a] Reference category

[b] p based on LR statistics, comparing models with and without the effect

d. Calibrated exposure (a weighted average of actual exposure and estimated exposure according to sector, job title and tasks)

		Inhalable dust			Wheat allergens		
		Odds ratio	95% LRCI	p	Odds ratio	95% LRCI	p
Log-conc		1.44	1.01 - 2.15	.0467	1.42	1.09 - 1.89	.0074
Log-conc^2		.77	.60 - .93	.0042	.93	.85 - 1.00	.0555
Atopy		6.19	3.29 -11.95	<.0001	5.56	2.91 -10.91	<.0001
Sector	Traditional bakeries	2.99	1.24- 7.62		3.31	1.37 - 8.46	
	Industrialized bakeries	4.23	1.72 -11.11		4.54	1.84 - 11.98	
	Enzyme processing	1.67	.56 - 4.92		1.90	.65 - 5.58	
	Flour mills	1.00		.0074	1.00		.0044

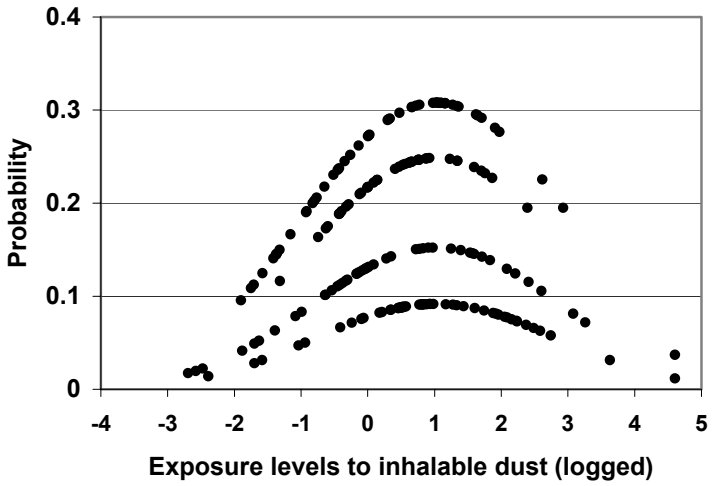
The probability of sensitization increased with greater exposure until it reached ~2.7 mg/m^3 for inhalable dust and ~25.7 μgEQ/m^3 for wheat allergens. It later decreased, indicating possibly either a healthy worker effect or development of tolerance. Likewise, the ORs for the logged concentrations were at least borderline significant 1.41 (p=0.0329) for inhalable dust and 1.31 (p=0.0142) for wheat allergens (table 6.5a). For the squared logged concentrations, the ORs were at least borderline significant .84 (p=0.0087) for inhalable dust and .96 (p=0.1539) for wheat allergens.

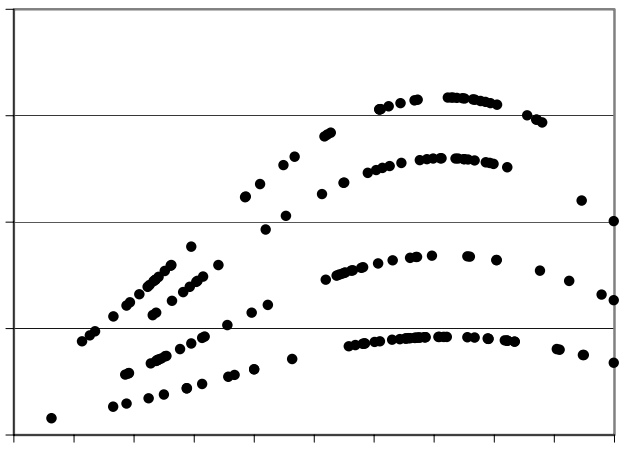
Atopy was a highly significant risk factor for sensitization, with an OR of ~6 (p<0.0001) in models with both exposures (6.12 and 5.49 for inhalable dust and wheat allergens, respectively). Overall, the probability for sensitization was much higher among atopic individuals compared to non-atopic ones. As for the sectors; the ORs for the industrialized bakeries were about 4.5 times higher than both the flour mills and the bakery-ingredients industry for both exposures (fig. 6.1(i)).

Figure 6.1 Probability for sensitization (Probability) as a function of exposure to inhalable dust (logged mg/m^3) and wheat allergens (logged μgEQ/m^3); Among non-atopic (n=193) and atopic workers (n=77) of four sectors: industrialized bakeries, traditional bakeries, bakery-ingredient suppliers and flour mills (from top to bottom).

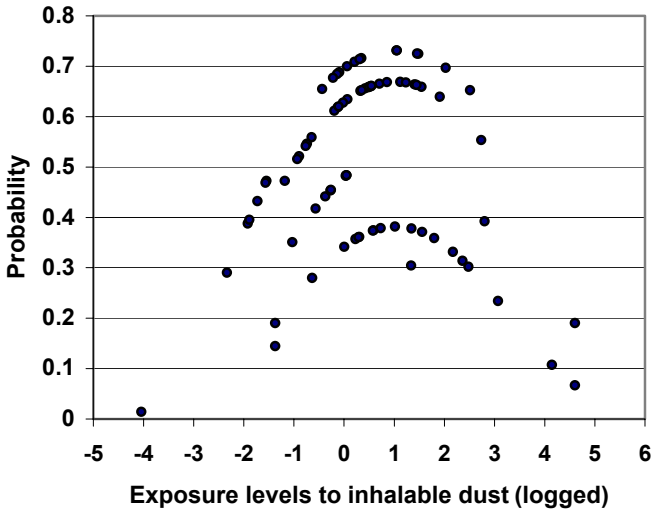
(i) Based on actual measured exposure

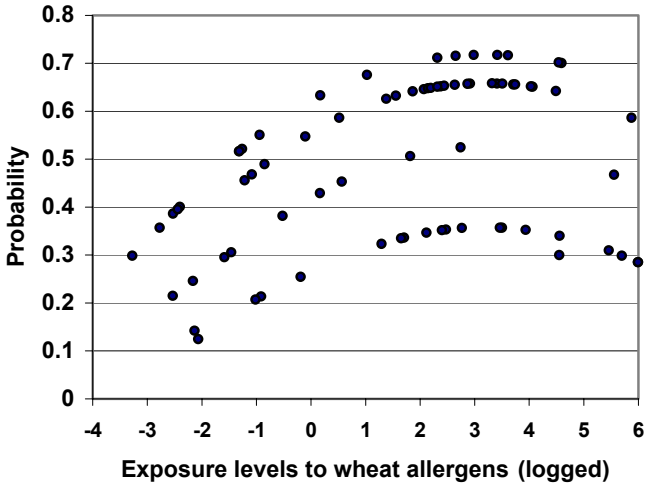
a) Non-atopic workers





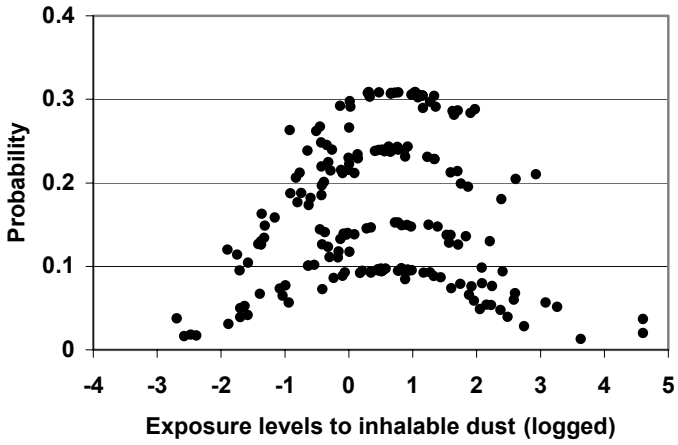
b) Atopic workers

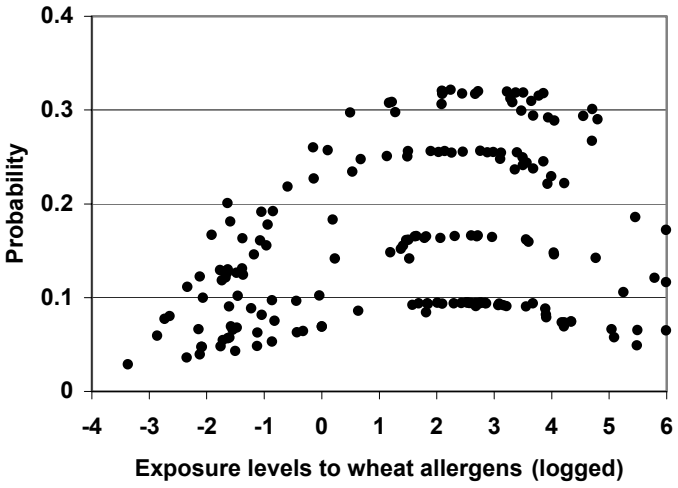




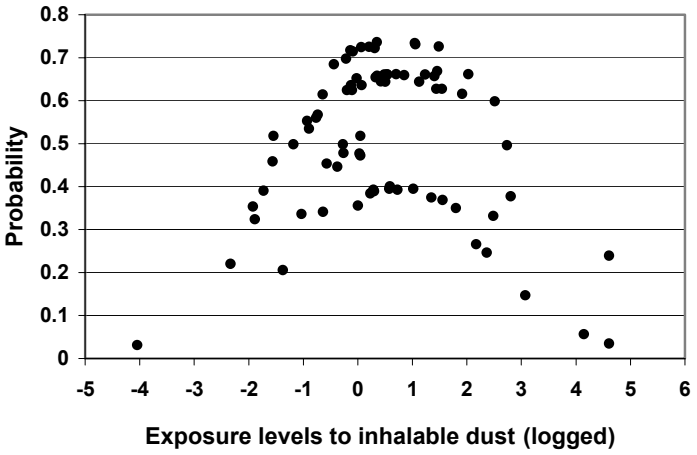
(ii) Based on VWE

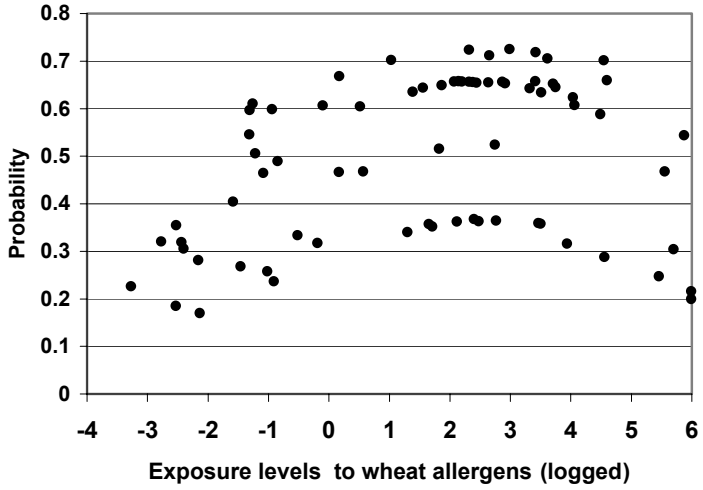
(a) Non-atopic workers





(b) Atopic workers





There were no significant differences in the ORs between traditional and industrialized bakeries nor between bakery-ingredients industry and flour mills.

Risks based on estimated exposures according to two and three covariates (table 6.5c compared to table 6.5b) were not markedly different; as expected, the risk for the linear exposure term was somewhat lower. In a previous analysis using exposure-exposure determinants modeling, the model with three covariates fitted the data better than did the model with two covariates ($p<.001$). Consequently, the risk estimators shown in table 6.5b are more accurate from a statistical point of view than those shown in table 6.5c.

Table 6.5d and fig. 6.1(ii) present the risks based on "VWE exposure" which is a weighted average of the actual exposure and exposure estimator according to the three covariates of sector of industry, job title, and tasks. The point risk estimators were quite similar to those based on actual exposure, but they were more accurate. They had almost the same precision and, in exposure to wheat allergens, the quadratic term which was not significant in model a ($p=0.1539$) turned out to be much more significant in model d ($p=0.0555$).

We assumed that for each industry-sector the exposure-response relationship is the same. When modeling exposure-response relationship separately for each sector there was some evidence of a positive linear relationship in 2 sectors in high exposures. However for risk assessment purposes, only the left hand part of the curve regarding low exposures is relevant.

DISCUSSION

The aims of the current study were to assess exposure to inhalable dust and wheat allergens among bakers within different sectors and jobs and to evaluate the shape of its relationship with specific sensitization for risk assessment purposes. We discuss the results in three sections. The first one deals with exposure assessment; the second and third ones deal with risk assessment, specifically with exposure response relationship modeling and implications for an occupational health standard.

Exposure assessment

The current study population is composed of bakers from four industry-sectors, two of which have hardly been studied earlier, namely, flour mills and bakery-ingredients industry. These two sectors were found to have higher significant exposure to both inhalable dust and wheat allergens ($p<.001$) compared to the traditional and industrialized bakers. Certain jobs, particularly weighing, filling of bags and "dumping of additives" in the bakery-ingredients industry, contributed to high levels of exposure. We found grouping by sector of industry and job title resulted in homogeneity, expressed as a relatively low exposure variance between workers within a given job category.[14] Accounting for tasks resulted in a lower variance between workers and a better fit of the model that associated exposure with its determinants. Our estimated exposures based on grouping strategy by sector of industry and job title, were found to be more precise than the individually measured ones. Moreover, those estimated-exposure values accounting also for the performed tasks were more valid and more informative for exposure to both inhalable dust and wheat allergens.

Exposure-response relationship

We aimed to study the shape of the exposure sensitization relationship since there are too few available epidemiological data with a quantitative exposure-response investigation among bakers for risk assessment purposes. Those studies, in which a monotonic exposure-–response relationship was tested for, had two main disadvantages.[15] First, they imposed an *a priori* choice of cut-off. This may result in bias and even raise concern that investigators may select cut-off values that produce a desired result. Second, they made a restrictive assumption of a parametric model of linear shape on the logit scale and applied a linear logistic model. This restrictive assumption was not tested, even though the conclusions from these tests depend on its validity. Only one study considered the shape of the relationship without the above-mentioned restrictive assumption by using a flexible GAM method.[7] Any GAM model has the disadvantage that even if it successfully fits the data, it is difficult to obtain estimates of the variability of the parameters/risk factors in order to assess their significances (non-robustness/non-stability). Therefore, parametric models are preferable over non-parametric ones due to the inferential statistics. We used

smoothing as intermediate step, as exploratory tool, leading to the desired parametric model. The smoothing analysis showed that the non-linear relationship can best be approximated by a quadratic one. Consequently, we could assume the parametric form of the model as being a quadratic logistic regression. We used two exposure assessment approaches, based on either actual exposure measurements or on estimated exposures. Both approaches yielded a significant quadratic dose-response relationship between exposure to inhalable dust and wheat allergens and sensitization ($p<0.05$ in comparison to a model without exposure terms). Based on actual measured exposure levels, the relationship was found to be monotonic up to a value of ~2.7 $\mu g/m^3$ for inhalable dust and ~25.7 $\mu gEQ/m^3$ for wheat allergens, namely, the higher the exposure the higher the risk. The decline at higher exposures may indicate some kind of tolerance in a specific group of workers, as was suggested to play a role in exposure of children to domestic allergens,[3,16] or a healthy worker effect.[7] This phenomenon especially with regard to the biological mechanism requires further exploration.

Previous studies that dealt with an exposure-response relationship had a smaller exposure range and presumably assumed a monotonic relationship, so that this phenomenon had not been observed before among atopic workers except in part in one earlier study.[7] Because of the cross-sectional nature of the current study, we could not evaluate this any further.

Atopy was found to be a significant modifier in our exposure-response relationship. It is a known risk factor for sensitization,[7,11] and atopic individuals had a 6-time higher risk than non-atopic ones, accounting for the exposure level and the sector of industry. It was found to be a weaker modifier (a factor of 2 in PR) in the Heederik and Houba study,[7] perhaps due to their lower exposure levels or modeling procedure. The industry sector was found to be a significant factor in the exposure-response relationship for both inhalable dust and wheat allergens. Industrialized bakeries had a 4 times higher risk to become sensitized at the same exposure level than workers in flour mills, and a twice as high risk as workers in the bakery-ingredients industry. So, the sector of industry might serve as a proxy for the kind of dust-mixture to which workers were exposed as well as for the local environment (e.g., temperature, the degree of using ventilation devices) or may be individual characteristics (other than age, gender or cigarette smoking[21]), which contributed to differences between industries.

The difference in prevalence in atopy between the industries seems a key explanatory variable. A remarkable difference in the prevalence of atopy among the four industrial sectors was observed (i.e., 22%, 35%, 38%, 67% in bakery ingredients industry, flour mills, industrialized and traditional bakeries, respectively). This may point to a strong selection effect (healthy worker effect), but the exact reason for the difference in atopy prevalence between industries remains obscure.

We have assumed that the shape of the relationship between sensitization and exposure is the same within each industry, which can be questioned. However, regardless of the assumption about the shape of exposure response curve, up to intermediate exposure levels, the curve is monotonically increasing. Only at very high exposure levels, the curve flattens or even decreases. However, one should be aware that such high levels are not encountered by a substantial number of workers and do not occur regularly. Especially for risk assessment purposes, this part of the curve, at the high exposure levels, is of less relevance.

Previous studies involving linear exposure-response relationships found that there was attenuation when using individual exposure data instead of exposure-group means.[14,15,17] In our study, quadratic logistic regression with the estimated exposure instead of the actual one led to almost the same point estimators of the risk factors. Point estimates using the estimated exposure based on three exposure determinants (sector of industry, job title, tasks) were in part higher than those based on two exposure determinants (i.e., sector of industry and job title). As in our previous study,[18] the random within-worker variability of exposure is highly reduced when tasks that vary from day to day are accounted for. Based on actual measured exposure, the risk estimators for wheat allergens were found to be marginally attenuated in comparison to those estimated according to 3 exposure determinants. The new 4-stage approach for assessing exposure combines actual and estimated exposure to avoid bias in both point and range estimation of the risk for an illness. The idea of a combined estimate was offered earlier,[19] but modified.

Implications of this study for an occupational health standard

The development of an exposure-response curve based on human health effects is important for promulgating occupational standards.[20] However, in previous studies involving linear exposure-response relationships, the use of individual exposure data instead of exposure-group means was associated with attenuation. Our approach tried to overcome this problem and therefore its conclusions seem to be more valid. Our curves do not suggest a true zero response (= no zero probability for sensitization), known as the "no observed effect level" (NOEL) in toxicology. This is in line with earlier findings,[7] — although very few studies have thus far attempted to evaluate the shape of the relationship between wheat exposure and health outcomes. Therefore, a true threshold dose level (i.e., the dose with which a zero response is associated) cannot be defined: instead, only a dose associated with a preset increase of risk (benchmarking) can be calculated.

REFERENCES

1. American Conference of Governmental Industrial Hygienists (1980). In Documentation of the Threshold Limit Values. Cincinnati, Ohio, USA, pp. 374-375.
2. American Conference of Governmental Industrial Hygienists (1999). In Documentation on Flour Dust. Cincinnati, Ohio, USA.
3. Heederik D, Thorne PS, Doekes G. Health-based exposure occupational exposure limits for high molecular weight sensitizers: How long is the road we must travel? Ann Occup Hyg 2002;46:175-185.
4. Tikkainen U, Louhelainen K, Nordman H. The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals, 120. Flour dust. Arbete Och Hälsa 1996.
5. Brisman J. The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. 111. Industrial Enzymes. Arbete Och Hälsa, 1994; 28.
6. Cullinan P, Lowson D, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. Work related symptoms, sensitisation, and estimated exposure in workers not previously exposed to flour. Occup Environ Med 1994; 51: 579-583.
7. Heederik D, Houba R. An exploratory quantitative risk assessment for high molecular weight sensitization: wheat flour. Ann Occup Hyg 2001;45:175-185.
8. Houba R, Heederik D, Doekes G, van Run PEM. Exposure-sensitization relationship for α-amylase allergens in the baking industry. Am J Respir Crit Care Med 1996;154: 130-136.
9. Musk AW, Venables KM, Crook B, Nunn AJ, Hawkins R, Crook GD, Graneek BJ, Tee RD, Farrer N, Johnson DA, Gordon DJ, Darbyshire JH, Newman Taylor AJ. Respiratory symptoms, lung function, and sensitisation to flour in a British bakery. Br J Ind Med 1989;46:636-642.
10. Platts-Mills TAE, Vaughan J, Squillace S, Woodfolk JA, Sporik R. Sensitization, asthma and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet 2001; 357: 752-6.

11. Cullinan P, Lowson D, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venable KM, McDonald JC, Newman Taylor AJ. Work related symptoms, sensitization and estimated exposure in workers not previously exposed to flour Occup Eniron Med 1994;51:579-83.
12. SAS/STAT User's Guide, Version 8.
13. SAS/STAT Software: Changes and Enhancements, Release 8.1
14. Heederik D, Attfield M. Characterization of dust exposure for the study of chronic occupational lung disease- a comparison of different exposure assessment strategies. Am J Epid, 2000; 151:982-990.
15. Leuraud K, Benichou J. A comparison of several methods to test for the existence of a monotonic dose-response relationship in clinical and epidemiological studies Statist Med 2001;20:3335-3351.
16. Platts-Mills TAE, Perzanowski M,Woodfolk JA, Lundback B, Relevance of early or current pet ownership to the prevalence of allergic disease. Clin Exp All nn Occup Hyg 2002;32:335-338.
17. Nieuwenhuijsen MJ, exposure assessment in occupational epidemiology: measuring present exposures with an example of a study of occupational asthma, exposure inworkers not Occup Int Arch Occup Eniron Health 1997;70:295-308.
18. Peretz C, Goren A, Smid T, Kromhout H. Application of mixed effects models for exposure assessment. Ann Occup Hyg 2002;46:69-77.
19. Seixas NS, Sheppard L, Maximizing accuracy and precision using individual and grouped exposure assessments. Scand J Work Environ Health 1996; 22:94-101.
20. Seeley MR, Tonner-Navarro LE, Beck BD, Deskin R, Feron VJ, Gunnar J, Bolt H. Procedures for health risk assessment in Europe. Regulation Toxicology and Pharmacology 2001;34:153-169.
21. Cullinan P, Cook A, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. Allergen and dust exposure as determinants of work-related symptoms and sensitization in a cohort of flour-exposed workers; a case-control analysis. Ann Occup Hyg 2001;45(2):97-103.
22. Smith TA, Parker G, Hussain T. Respiratory symptoms and wheat flour exposure: a study of flour millers. Occup Med 2000 ;50(1):25-29.

Appendix a: Job titles

Industry	Job title
Traditional bakeries	Bread baker
	Confectioner
	Mixed baker (both bread and confectionery)
	Oven worker
Industrialized bakeries	Bread baker
	Doughmaker
	Control baker/quality assurance
	Cleaning worker
	Confectioner
	Oven worker
	Slicers, packers and transport workers
	Warehouse worker (additives)
	Production manager
	Maintenance worker
Flour mills	Wheat (grain handler)
	Operator (allround)
	Operator silo
	Operator flour mill
	Operator wheat cleaning
	Worker involved in filling of bags
	Manager
	Cleaning worker
	Lorry/truck driver
	Analyst
	Maintenance worker
	Mixer additives
	Control baker/quality control
	Warehouse worker (additives, e.g. transport)
Enzyme processing industry	Weighing
	Filling of bags
	Dumping of additives
	Operator all round
	Stacking of filled bags
	Warehouse worker (additives, e.g. transport)
	Control baker/quality control
	Office worker
	Operator (almond paste, fats)
	Maintenance worker

Appendix b: The establishment of the VWE exposure

v = exposure level on a one day basis, subject to high day-to-day variability (within-worker variability) ;
x = average exposure level over a period.

The basis of the calibration is the replacement of v by E(x/v), written here as $\mu_{x/v}$. In our quadratic logistic model we will replace v and v^2 by the estimates of $\mu_{x/v}$ and $\mu_{x^2/v}$

(a) estimation of $\mu_{x/v}$

In general:
$v = x + \delta$ (δ = "measurement error" due to day-to-day variability, independent of x, with E(δ)=0). This implies that

$x = \alpha + \beta v + \varepsilon$ (ε = random error) ***(equation 1)***

(v and x have means: μ_v, μ_x and variances: σ_v^2 σ_x^2 ,respectively, and covariance σ_{xv}^2

δ and ε have zero means and variances : $\sigma_\delta^2, \sigma_\varepsilon^2$,respectively)

where:

$$\beta = \frac{\sigma_{xv}}{\sigma_v^2} = \frac{\sigma_x^2}{\sigma_x^2 + \sigma_\delta^2}$$

$\alpha = \mu_x - \beta\mu_v = (1-\beta)\mu_x$ (since $\mu_x = \mu_v$)

hence:

$x = (1-\beta)\mu_x + \beta v + \varepsilon$

$\mu_{x/v} = (1-\beta)\mu_x + \beta w$ ***(equation 2)***

For the i-th worker:

z_i = exposure estimator based on exposure determinants of worker i (stage 1)

s_b^2, s_w^2 =estimated variance components of the z_i the same for all workers (stage 2)
n_i = no. of repeated measures of worker i (=1,2,3)
$\bar{v}_i$ = mean actual measured exposure for worker i, based on 1-3 repetitions.

then:

$\hat{\mu}_{x_i} = z_i$; $\hat{\beta} = \lambda_i = \dfrac{s_b^2}{s_b^2 + s_w^2/n_i}$;

$\hat{\mu}_{x_i/v_i} = (1-\lambda_i)zi + \lambda_i \bar{v}_i$, this is the VWE exposure for the *i*-th worker

(b) estimation of $\mu_{x^2/v}$

In general:

$\mu_{x^2/v} = \sigma^2_{x/v} + [\mu_{x/v}]^2 = \sigma^2_{\varepsilon} + [\mu_{x/v}]^2$ (from equation 1)

$\sigma^2_x = \beta^2\sigma^2_v + \sigma^2_{\varepsilon} \Rightarrow \sigma^2_{\varepsilon} = \sigma^2_x - \beta^2\sigma^2_v$

hence:

$\mu_{x^2/w} = \sigma^2_{\varepsilon} - \beta^2\sigma^2_v + [(1-\beta)\mu_x + \beta v]^2$ (from equation 2)

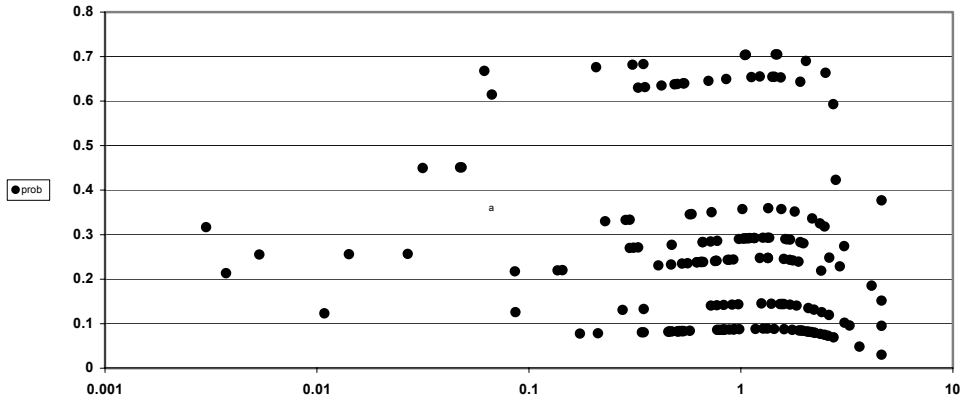
For the *i*-th worker:

$\hat{\mu}_{x^2/w} = s_b^2 - \lambda_i^2(s_b^2 + s_w^2/n_i) + [(1-\lambda_i)z_i + \lambda_i\bar{v}_i]^2$

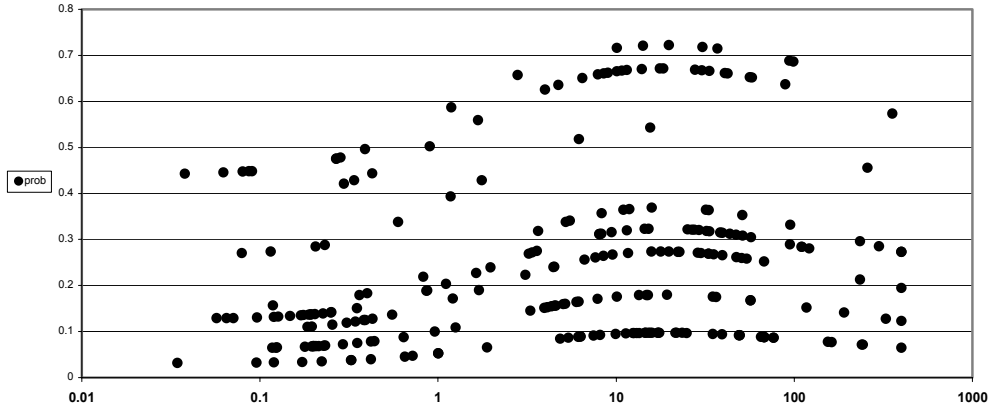
Appendix c: GAM modeling

Probability for sensitization (Probability) as a function of exposure to inhalable dust (logged mg/m^3) and wheat allergens (logged µgEQ/m^3); Among non-atopic (n=193) and atopic workers (n=77) of four sectors: industrialized bakeries, traditional bakeries, bakery-ingredient suppliers and flour mills. (from top to bottom)

a. Inhalable dust



b. Wheat allergens



CHAPTER 7:
GENERAL DISCUSSION

The mixed effects model is a generalization of the standard linear regression model that enables studying effects of covariates on an outcome when the data is generated from several sources of variation, instead of just one.[1-7] This model is relevant for example, when we study the effects of exposure determinants on exposure level where repeated measures have been taken for a group of workers. Then, both between- and within-subjects variability in exposure are present, exhibiting two sources of variation.

The main objective of this thesis is to evaluate and explore possibilities of using mixed effects models for occupational exposure assessment. General benefits of mixed models are discussed below, with respect to both statistical and hygiene aspects. Several exposure data sets from surveys with repeated measurements were explored and studied in this thesis with different study designs and with a range of different determinants of exposure. In all studies, we explored variability in exposure to air pollutants, a common denominator in this thesis.

The major benefits are the ability to estimate both the variance components of exposure (and their derivatives) and the unbiased regression coefficients for determinants of exposure, simultaneously in one model. Other benefits include modeling relationships between exposure and health effects, with improved approaches to deal with biases that occur as a result of measurement error. Classical hygiene aspects covered are grouping strategies, hazard control and over-exposure assessment.

Correlation between repeated exposure measures

Longitudinal hygiene surveys include measurements collected over periods ranging from 1 week to 30 years with different number of repeated measurements per subject and usually the personal measurements of the same subject are correlated. The mixed model method is capable of handling unbalanced data and estimating the correlation between repeated measures for a worker, accounting for other covariates such as job title and time period whereas multiple regression models use only balanced designs and consequently lose

information. Our data sets consisted of 2-10 repeated measurements per worker, which took place on 3-4 successive days or over a period of one year. In addition, exposure groups of varying sizes were present in the data, from 6-12 workers to about 600. This illustrates the extremely unbalanced nature, typical of data sets in occupational hygiene.

Amongst all methods for longitudinal repeated measures modeling, only the mixed model enables the assumption of different correlation-structures. Assuming a constant correlation between any two repeated measures of a worker, in about 50% of our data sets we found a relatively non-ignorable correlation, greater than 0.50. The correlation value can be easily derived from the variance components evaluated in recent studies of exposure-groups,[8-12] (within to between variance component ratio of more than 1:1 is equivalent to a correlation value of .50 or more) but only a few of them also accounted for systematic effects[10] and therefore a detailed comparison between our studies and other studies cannot be made. The correlation is important as an indicator of the (in-) variability in exposure and has further implications for determining the exact role of exposure determinants, which will be explored later on.

Covariance structure/ variance components

It is known nowadays that exposure levels can vary greatly from day-to-day within the same worker as well as between workers performing the same job. This variability of exposure can be due to a wide range of sources, known and unknown to the hygienist, and statistically it is reflected with different covariance structures depending on the information available. The mixed model can handle different covariance structures (not only one structure as the repeated measures ANOVA model) and further to the random effects model can estimate variance components accounting for the fixed effects.

In the data sets of this thesis (Chapters 3-6) two random variance components, between- and within-workers, were considered. Those components have been mostly estimated so far, through a one way random effects model,[7-9] but with the mixed modeling they could be adjusted for the fixed effect exposure determinants,[10-12,14] under the assumption of a compound symmetry (CS) covariance structure. CS means an equal correlation between repeated measures irrespective of the time interval between them. It is also possible that a factor would have an effect on the variance of the response. Therefore, we investigated

heterogeneity of the variance among benzene workers with respect to their job-task (fillers/non-fillers) and found that the pooled between- and within-variances led to almost the same values of the variance components when fillers and non-fillers were allowed to have distinct variance components. The same was found among groups of workers exposed to inorganic mercury,[12] but previous results from a study among construction workers indicated that the between-worker variance could not be pooled among jobs.[14] Weaver et al.[15] attempted to generalize these issues on a larger scale, in 39 data sets each containing multiple groups. With different between worker variance components they advocated testing the assumption of homogeneous within-worker variance components, using a likelihood ratio test, and found that it was often valid to pool the data across groups to estimate a common within-worker variance component. This has implications for statistical parsimony.

When the number of observations in each group is small, like in common hygiene studies, estimation of a reduced number of parameters is preferable. Sometimes the random effects in the model structure can be affected by the fact that measurements that took place close in time are more highly correlated then those further apart in time,[16] e.g. a first order autoregressive- AR(1) covariance structure (see appendix for application). Only one study compared results of using AR(1) and CS covariance structures on the same exposure data set. At face value, parameter estimators were similar.[11] An unstructured covariance may also be considered when there are few repeated measures for the same worker (this is not yet done). Therefore the mixed model offers the hygienist information regarding the significant random sources of the variability, as well as the magnitude of the contribution of each specific source.

Modeling mean exposure: assessing unbiased effects of exposure determinants

Collection of exposure determinants during the measurement process such as: work temperature, task, outdoor/ indoor working is part of each hygiene survey since those factors are associated with elevated or reduces exposure levels. Therefore the estimation of their relative contribution to the mean exposure is of interest to the industrial hygienist. Modeling mean exposures with a mixed effects model corrects for bias in evaluating the determinants of exposure. This bias may occur when ignoring the dependence between

repeated measurements,[18] as in regression models which until very recently have been used for "mean modeling".[1-4] This benefit of the mixed model is illustrated with examples of two sets of exposure data (Chapter 3), that were analyzed by both models. A large between-worker variance component, as was estimated through a mixed model for the rubber workers' exposure to inhalable particulates, led to changes in the regression coefficients, the standard errors and in the tests of statistical significance. With the between-worker variance component close to zero (almost no correlation), e.g. in the pig farmers data, the coefficients for the fixed effects in the mixed model were almost the same as those from a regression model. To conclude, as the dependence between the repeated measurements increases, the standard regression estimates become less statistically efficient. This has implications when interpreting exposure data and when choosing a parsimonious predictive model for assessing a long-term exposure. For hazard control, it is essential that conditions that contribute to higher exposure are known. By focusing upon those conditions (e.g. work done on a very warm and busy day- benzene data) it is possible to better control exposures.

Time- and non-time related covariates/ exposure determinants

The variability of exposure has both systematic and random sources, which should be evaluated while studying the temporal pattern of exposure. The mixed model enables us to distinguish between systematic and random fluctuations of exposure along time. The systematic variability may be evaluated by the incorporation of time dependent covariates (those that have different values along time) to the model such as worker's activities (e.g. pig farmers data), and periodic variables (e.g. calendar date, weekday- benzene data). The systematic trends can be divided into short-term changes (e.g. in activities) or long-term trends (e.g. 4-years periodical changes in benzene use).

The mixed model enables evaluation of the relative contribution of time-independent exposure determinants, while simultaneous adjusting for the time-related ones. Symanski et al.,[10] found that for periods extending beyond a year, systematic changes in exposure were more likely to occur and that ignoring such changes can bias the assessment of exposure, based on more than 500 exposure groups. Common repeated ANOVA models assume that the exposure is stationary, i.e., that the true mean of exposure (as well as the variance and

auto-covariance) does not change over time. The mixed models can accommodate non-stationary situations by controlling for the time effects (like calendar year in the benzene data). Knowledge on systematic changes enables better control of exposure, although random fluctuations are indicative of more unmeasured exposure determinants, which should be identified.

Bias correction for exposure-health response relationships

Exposure response modeling is of vital importance in occupational epidemiology. In general, the high day-to-day variation (within-worker variance) reduces the power of a study to detect an association between exposure and disease (or any health outcome) and can introduce bias into the estimate of the risk of disease due to exposure, usually referred to as "attenuation" [22-25]. Bias reduction for exposure-health response modeling can be facilitated through the use of mixed models.

To avoid this bias (to control for worker's day to day variation in exposure) usually worker's observed exposure levels are replaced by the mean of their exposure-group. [25-26]

In this thesis an alternative approach has been explored, in which instead of the observed exposure, the mixed model predicted exposure is used in the exposure response analysis. Two distinct scenarios were applied; one where mixed model predicted exposure values were used in the exposure response analysis. A mean exposure value estimated by the mixed effect model based on exposure determinants which is an improvement compared to "exposure-group's" mean exposure; another where mixed model predicted exposure values were combined with individual observations. A suggestion to combine mean group exposure values with actual observed individual exposure measurements was made earlier by Seixas et al.[25] The combination of the actual observed values with the predicted values weighed by variance components is done according to James-Stein.[27] A modification of this principle has been applied in this thesis (Appendix, Chapter 6).

Identification of uniform exposure groups (grouping strategy)

Grouping of workers is an inherent part of the work of an industrial hygienist in exposure surveys as well as in compliance tests and epidemiological studies.[19-21] Despite the widespread use of grouping strategies, there is only limited experience with optimisation of

these strategies. The mixed models assist in grouping workers into uniformly exposed groups further than on the basis of observed characteristics such as job-title or location. By applying nested mixed models (Chapter 3), we could identify specific work characteristics (both time and non-time related) that are associated with the between-worker variance components e.g. process temperature and pressure or farm-flooring characteristics. The time-independent factors (e.g. farm characteristics) may help in future surveys for a priori grouping. The time-related factors like process-temperature, together with the time-independent factors should play a role in a posteriori sub-grouping of workers (see Chapter 6).

Identification of hazardous time-related conditions for hazard control and sampling

Hazard control should focus on time related conditions namely on conditions with high exposures that occur not on a regular basis and can put the worker at risk. By applying nested mixed models we can understand the relationship between specific work-environment characteristics and the within-worker/day-to-day exposure variance component. Variables responsible for variance in exposure levels from day-to-day were found to be certain activities, month (indicating outdoor temperature and relative humidity and wind speed), week day and credit day (indicating work-burden).

Such information can also assist hygienists and researchers in planning a representative environmental sampling strategy and thus better assess occupational exposure for both hazard control and epidemiological studies of exposure-response relationships. For example, in the fuel-installations (Chapter 4), selecting measuring days at random should maximize the likelihood that they are representative of different workload conditions during warm and cold months.

Assessment of over-exposure

Occupational exposure limits/standards aim to prevent health impairment in workers and even their offspring. Thus, exposure above the standards, or over-exposure, should be evaluated and controlled. Variance components of exposure, estimated by the mixed models, should be included in the assessment of over-exposure. Evaluation of over-exposure includes estimation of the probability that in a group, a randomly selected

worker's mean exposure exceeds the occupational exposure standards.[5] Rappaport et al. and Lyles et al.,[5,6] were the first to propose a statistical strategy which accounts for variance-between and within workers,[11] when evaluating over-exposure and Symanski et al.[12] applied this on exposure job-groups with different assumptions regarding homogeneity in the between- and within-worker variance components. This statistical strategy may be extended to situations where workers are not classified into homogeneous job groups because it is meaningless or impossible (e.g. in small workshops where each worker is doing another job-task). When additional information with respect to tasks etc. is available, more refined variance components, adjusted for the tasks, based on a suitable mixed effects model, can be estimated for the heterogeneous group (see estimators in Chapters 2, 4, 6) and incorporated into the statistical strategy to evaluate over-exposure together with the predicted means. Thus a more exhaustive use of the hygiene information available can lead to a more valid evaluation of over exposure, which has direct implication for workers health.

ASSUMPTIONS AND LIMITATIONS

The use of empirical data for modeling generally, and specifically in this thesis, involves assumptions, which should not be neglected when interpreting results:

Random samples: Statistical inferences rely on random sampling. However, in the studies in this thesis the selection of workers was dependent on accessibility to factories, which may have introduced some bias. In addition, workers were selected among those who were present during the survey days.

Missing values: Missing values may not have been missing at random. Specific workers may have been chosen to have repeated measures not in a systematic scheme. This could have caused bias in the modeling.

Modeling: One should carefully check the assumptions of the mixed effects model as well as the covariance structure before application; this is not always straightforward. When modeling, one should avoid over-parameterization. In addition, the stability of the coefficients is still questionable in small groups. Asymptotic standard errors are derived

and the larger the data sets, the “better” the standard errors to assess the statistical significances of covariates. In mixed models “Maximum Likelihood" estimates of variances are biased because they fail to account for the estimation of the fixed parameters, and so “Restricted Maximum Likelihood” is generally advocated. No standard statistical methodology is available for comparing variance models that are not nested.

CONCLUSIONS AND FUTURE PERSPECTIVE

The small sample size of “exposure groups” and the natural dependence between measurements may have implications on the statistical inference while evaluating exposure data sets. Given the advantages of the mixed effects modeling for pooling short dependent data sets nested within groups, one may assess exposure and exposure-response relationship, of the whole set of data with different variance sources accounting for different covariates. Therefore, additional applications of the mixed effects models should be performed in the future for different occupational health type of data as well as for complicated study designs such as: hierarchical (multi-level) and double repeated either in time or space.

Analysis of data sets with repeated measures both in time and space (e.g. different locations on the body, different areas) or within nested groups (e.g. workers within occupational groups, groups within a factory, factories within an industry, industries within a region, etc.) should be considered as well.

Repeated measures can be derived also from biological monitoring or from qualitative exposure assessment (self assessed or performed by expert evaluations by nominal and ordinal scales) with binary outcomes. Consequently, the association between repeated qualitative exposure and quantitative repeated outcomes should be modeled.

Biomarkers may provide more sensitive, specific, quantitative or reproducible proxies of study endpoints than traditional approaches, and therefore may in theory improve both study efficiency and validity. Consequently exposure-response mixed effects modeling will be needed for valid risk assessment where exposures and endpoints are repeated.

Hopefully, these models will be more widely applied and contribute to improve hazard control and exposure-response modeling.

REFERENCES

1. Verbeke G, Molenberghs G. Linear Mixed Models for Longitudinal Data, New York, Springer-Verlag, 2001.
2. Liang KY, Zeger SL. Longitudinal Data Analysis Using Generalized Linear Models. Biometrika 1986;73:13-22.
3. Lindskey JK. Models for Repeated Measurements. Oxford, Clarendon Press, 1993.
4. Searle SR. Mixed Models and Unbalanced Data: Wherefrom, Whereat And Whereto Commun. Stat. A-Theory And Methods 1988;17:935-968.
5. Rappaport SM, Lyles RH , Kupper LL. An exposure assessment strategy accounting for within- and between-worker source of variability. Ann Occup Hyg 1997;41:485-500.
6. Lyles RH, Kupper LL, Rappaport SM. Assessing regularity compliance via the balanced one-way random effects ANOVA model. J of Agricultural, Biological and Environmental Statistics 1997; 2:64-86.
7. Heederik D, Boleij JSM, Kromhout H, Smid T. Use and analysis of exposure monitoring data in occupational epidemiology: an example of an epidemiological study in the Dutch animal food industry. App Occup Envir Hyg 1991;6:458-64.
8. Kromhout H, Symanski E, Rappaport SM. A Comprehensive Evaluation of within- and between-worker components of occupational exposure to chemical agents. Ann Occup Hyg 1993;37:253-270.
9. Kromhout H, Heederik D. Occupational epidemiology in the rubber industry: Implications of exposure variability Am J Ind Med 1995;27:171-85.
10. Symanski E, Kupper LL, Kromhout H, Rappaport SM. An investigation of systematic changes in occupational exposure. Am Ind Hyg Assoc J 1996;57:724-745.
11. Symanski E. Chan W, Chang CC. Mixed-effects models for the evaluation of long-term trends in exposure levels with an example from the nickel industry. Ann Occup Hyg 2001;45:71-81.
12. Symanski E. Sallsten G, Chan W, Barregard L Heterogeneity in sources of exposure variability among groups of workers exposed to inorganic mercury. Ann Occup Hyg 2001;45:677-87.
13. Burstyn I, Heederik D., Bartlett K, Doekes G, Houba R, Teschke K, Kennedy KM, Wheat antigen content of inhalable dust in bakeries: modeling and an inter-study comparison. Appl Occup Environ Hyg 1999;14:791-798.
14. Rappaport SM, Weaver M, Taylor D, Kupper L, Susi P.Application of mixed models to assess exposures monitored by construction workers during hot processes. Ann Occup Hyg 1999;43:457-69.
15. Weaver MA, Kupper LL, Taylor D, Kromhout H, Susi P, Rappaport SM. Simultaneous assessment of occupational exposures from multiple worker groups. Ann Occup Hyg 2001;45:525-542.
16. Symanski E, Rappaport SM. An investigation of the dependence of exposure

variability on the interval between measurements. Ann Occup Hyg 1994;38:361-372.
17. Wolfinger, RD, Kass, RE. Non-Conjugate Bayesian analysis of variance component models. Biometrics 2000; 56, 768-774.
18. Burstyn I, Teschke K. Studying the determinants of exposure: A review of Methods. Am Ind Hyg Assoc J 1999;60:57-72.
19. Boleij J, Buringh E, Heederik D, Kromhout H. Occupational hygiene of chemical and biological agents, Amsterdam, Elsevier, 1995.
20. Rappaport SM, Smith TH. Exposure assessment for epidemiology and hazard control. ACGIH, Michigan, Lewis Publishers Inc., 1991.
21. Rappaport SM, Kromhout H, Symanski E. variation of exposure between wokers in homogenous exposure groups. Am. Ind. Hyg. Assoc. J. 1993;54:654-662.
22. Heederik, D, Attfield M. Characterization of dust exposure for the study of chronic occupational lung disease- a comparison of different exposure assessment strategies. Am J Epid 2000;151:982-990.
23. Nieuwenhuijsen MJ. Exposure assessment in occupational epidemiology: measuring present exposures with an example of a study of occupational asthma. Int Arch Environ Health 1997:70:295-308.
24. Heederik, D., Kromhout H, Braum W. The influence of random exposure estimation error on the exposure response relationship when grouping into homogeneous exposure categories. Occup Hyg 1996;3:229-41.
25. Seixas NS, Sheppard L. Maximizing accuracy and precision using individual and grouped exposure assessments. Scand J Work Environ Health 1996; 22:94-101.
26. Tielemans E, Kupper LL, Kromhout H, Heederik D, Houba R. Individual-based and group-based occupational exposure assessment strategies: some equations to evaluate different strategies. Ann Occup Hyg 1998;42:115-9.
27. James W, Stein C. Estimation with quadratic loss. 1961. Berkely (CA), University of California press. Proceedings of the fourth Berkely symposium on mathematical statistics and probability.

APPENDIX:
PREDICTORS OF CHANGES IN LUNG FUNCTION IN A LONGITUDINAL STUDY OF ASTHMATIC AND NON-ASTHMATIC CHILDREN IN ISRAEL

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ABSTRACT

Background: The study was aimed to understand the growth of lung function (LF) over the course of one year, among asthmatic and non-asthmatic schoolchildren. It was based on a repeated measurements design in order to estimate LF predictors and to evaluate LF variability within a child. Methods: A panel of 79 asthmatic and 79 matched non-asthmatic schoolchildren (9 and 12 years old) were followed up. During each of the season height and weight were measured and 10 repeated lung function tests were scheduled for each child. The parents completed health questionnaire. Statistical mixed models were applied to estimate longitudinal predictors such as changes in height and weight within a child and cross-sectional predictors among children (e.g. initial height, age). The model allowed us to estimate variability around all individual prediction curves as well as a within child variability estimator for the LF parameters. Results: In total 3780 LF measurements were performed. Controlled for gender , grade and asthma, a 1 cm growth within the same child increased his FVC and FEV1 by about 11 ml (longitudinal effect) while a 1 cm difference across children, increased FVC and FEV1 by about 30 ml (cross-sectional effect). However, longitudinal and cross-sectional weight effects were the same. In addition, obesity reduce FVC and FEV1 significantly. Seasonal time trend, indicating a child's lung growth within a season, was found to be significant only in fall. Gender was found to be a significant modifier of LF in subgroups of grade and asthma status. The variability of a child's LF outcomes over the study-time was quite high. On average the adjusted within

child standard deviation, in a year, was estimated to be about 250 ml for FVC and FEV1 and 650 ml/s for FEF50. FEF50 variability was found to be significantly associated with having the symptom of coughing apart from cold.
Conclusions: For a better validity and insight of LF prediction curve of a group, the evaluation should be based on repeated measurements per child; and the modeling procedure should take into account the within variability of the child's LF.

INTRODUCTION

Many studies in recent years have collected daily measurements of peak expiratory flow in children to examine daily correlates of those measurements and determinants of variability.[1-3] Those studies have typically collected data for one +to three months. On a different time scale, other studies have collected annual measurements of lung function using spirometers.[4-7] These studies have helped us understand the growth of children's lung function and the determinants of that growth. We have focused on an intermediate time scale that is less well studied, by obtaining frequent measures of Lung Function (LF) in school children during one year. This allows us to look at how LF grows over the course of the year, including effects of the child's height and weight growth and of seasonal patterns. Because asthmatic children may behave differently we have chosen a matched sample of asthmatic and non-asthmatic children, living in the Hadera-area in Israel (a typical location along the Mediterranean). Thus, this study was aimed to model the effect of seasonal changes in height and weight and within season growth on LF values of FVC, FEV1 and FEF50.

A second goal of this study was to examine predictors of the variability of lung function over time. Increased variability in lung function may be an important predictor of respiratory health. For example, peak flow variability has been shown to be an important predictor of asthmatic status,[7a] and is correlated with baseline level of lung function, among other determinants. Less is known about the determinants of variability in FEV1 and other spirometric measures over the course of a year. Therefore, a second goal was to evaluate the variability of LF of each child over the year after controlling for the time-dependent

effects and for being asthmatic, grade and gender. Since we used a matched asthmatics-non asthmatics data set, we also studied the importance of each of the three LF parameters in predicting the likelihood of being asthmatic, after control for respiratory symptoms , ETS , parents' asthma and atopy of either child or the parents.

METHODS

Subjects

During 1996 we have examined 3740 school-children in the Hadera area , Israel, using a cross-sectional study design. Out of this sample we selected a cohort of 79 children who were diagnosed by a physician as having asthma or frequent wheezes ("asthmatic"), based on parents' reporting. A second cohort of 79 non-asthmatic children, selected from the same study population, were matched on gender and class, school and residential-area. Among the asthmatics, a quarter reported being under regular medical follow up and one third reported that their asthma was diagnosed under the age of 1 year. The children lived in three residential areas in Hadera, for at least 7 years: 55 in the town of Hadera, 50 in a suburb of Hadera and 53 in a nearby village. In grade 3 (9 years old) , 91 children and in grade 6 (12 years old), 67 children were followed up. In total there were 68 girls and 90 boys in the study.

Lung Function tests and repeated measurements design

Lung function tests consisted of forced vital capacity (FVC), forced expiratory volume in first second (FEV1), FEV1/FVC, peak expiratory flow (PEF), forced expiratory flow in 50% volume (FEF50), and forced expiratory flow in 75% volume (FEF75). LF tests were carried out by a trained technician using a Minato AS-500 portable spirometer (ATS approved). The expiratory maneuver was carried out with the subject standing and was repeated at least three times until two similar tests (agreed within 5%) were achieved. The best test (highest FVC+FEV1) was chosen. The lung function testing was done in the children's schools between May 1997 and March 1998, within 3 seasons: spring, autumn and winter (during the summer, the children were on vacation). In each season, ten

repeated tests were scheduled for each child spaced 5-7 days apart. All but 12 children were tested in each season. Nine out of those 12 were tested only in the spring. The median number of repeated measurements was 26 for asthmatic children and 25 for non-asthmatic children. All the participants were weighed (kg) and their height measured (cm) before carrying out the expiratory maneuver in the first test of each season (3 times during the year).

Questionnaire

A health questionnaire, to be completed by the child's parents, was adapted from that recommended by the American Thoracic Society and National Heart Lung and Blood Institute.[8] The questionnaire included data on the child's previous diseases and respiratory symptoms and parent's respiratory diseases, as well as siblings' health condition, some socio-economic indicators as well as smoking in the child's home (ETS- environmental tobacco smoke). The parents had to fill out the questionnaire twice: in the spring and fall. The concordance between the questionnaires was quite high; out of 20 dichotomous questions, only two among the asthmatics and four among the non-asthmatics showed a non-significant Kendall correlation. Importantly, for the questions regarding having symptoms, the correlation was higher among the asthmatics than among the non-asthmatics. For this analysis the fall questionnaire was used.

Variables relating to time, height and weight

In our data, eight variables were available to assess the association of baseline height and weight (cross-sectional effects), change in height and weight between seasons, and trend within season on lung function (longitudinal effects). Time was considered a variable to allow us to control for growth in lung function within season, since height and weight were only obtained once per season. In addition, we have defined indicator variables for very tall and very heavy children and included them in our models to assure that our slopes for height and weight are not unduly influenced by outlier values for those measurements.

Statistical analysis

Lung Function

We used mixed effects models,[9] to examine the effect of changes in height, in weight and in time, on each of the 3 LF values: FVC, FEV1, FEF50, controlling for being asthmatic, grade, gender and initial height and weight. Because children's growth rates may not be equal in each season we fitted separate time trends within each season. The LF values were assessed to have a normal distribution and all the variables were considered as fixed effects in the above models. We assumed an AR(1) (first order autoregressive) correlation between repeated measures, meaning that the correlation between the residuals of LF in each child declined with the time between measurements, which is a common assumption in this kind of time series.[6,10] The mixed model can handle unbalanced repeated measurements designs and accounts for the variance of the LF parameter within a subject. The modeling was done by using PROC MIXED of SAS.[11]. In addition to the linear terms of height and weight, non-linear terms: height2, height3, weight2 and weight3 were tested for inclusion in the mixed models as predictor variables. We also fitted generalized additive models for the data, using the GAM function in SPLUS.[12] The GAM models allowed us to fit non-parametric smooth function of the predictor variables height and weight, and test the significance of the improvement in model fit, compared to linear height and weight terms.

LF Variability

As mentioned above the mixed model enabled us to model for each child his specific prediction curve, from which residuals could be calculated (residual = observed LF value minus predicted LF value according to the prediction curve). The SD of these residuals indicates the variability of this child's LF outcomes over the year, after controlling for the time-dependent effects, grade, and gender. We have calculated this value for each child. Afterwards we applied a multiple linear regression to model the relationship between these SD's and certain effects that we hypothesized might affect lung function variability. These include: an area effect, respiratory symptoms, ETS, parents' asthma, and atopy of either the child or the parents. All regressions included control for gender and grade. SD was assumed to have a normal distribution and the modeling was done by using the PROC GLM of SAS.[13]

Comparison between Asthmatics and Non-Asthmatics

To study the difference in LF results between asthmatics and non-asthmatics, we have applied three logistic models, one for each of the LF parameters (FVC, FEV1, FEF50). In these models, each child was represented by his last measurement. The dependent variable was a dichotomous one, being asthmatic (value=1) or non-asthmatic (value=0) and the independent variables were the LF parameters and some factors we examined as predictors of SD. The modeling was done by using PROC GENMOD of SAS.[11]

RESULTS

Two sets of variables were available: time invariant variables such as initial height and weight, gender and grade, which are commonly used in cross-sectional studies. In addition we had time variant variables: changes in height and weight across seasons, as well a linear time trend term within each season to capture within seasonal growth. These variables are discussed in turn.

Prediction of LF

Asthma, gender and grade

Table A.1 presents mean and SD, of the three LF parameters: FVC, FEV1 ,FEF50 in sub-groups of grade and gender for both asthmatic and non-asthmatic subjects, without accounting for the subject's within variability (correlation between repeated measurements). While applying the mixed models controlling for effects relating to height, weight and time we have found that the interaction effect of grade and gender was significant. Hence, table A.2 presents the coefficients (se) of each level of grade and gender, in the model, when arbitrarily selected girls in the 6th grade were the reference group. The coefficient presents the difference in mean LF between each level and the reference group after controlling for being asthmatic, change in height and weight, and time (growth) effects. It can be seen that in the 3rd grade boys, had higher FVC and FEV1 values than girls, both in asthmatics and non-asthmatics (by ~100ml, ~150ml

respectively). However in the 6th grade , the gender affect differed between asthmatics and non-asthmatics . Among the non-asthmatics, girls had higher values than boys (by ~150ml in FEV1, ~350ml/s in FEF50) while among the asthmatics, girls had much lower values than boys (by ~240ml in FVC and FEV1, ~400ml/s in FEF50). Moreover in the 6th grade, asthmatic boys had the same values as non-asthmatics boys in FVC and FEV1, while asthmatics girls had significantly lower values than non-asthmatic girls (- 290ml, -420ml, -970ml/s in FEV1, PEF, FEF50 respectively). For FEF50, even in the 3rd grade asthmatics had lower values than non-asthmatics, for both boys and girls.

Table A.1 Mean±sd of lung function outcomes and initial height and weight in subgroups of grade and gender in asthmatic and non-asthmatic children

Grade	Gender	k	FVC ml (n)	FEV1 ml (n)	FEF50 ml/s (n)	Height cm	Weight kg
Asthmatics							
3	M	22	2033±289 (551)	1776±267 (534)	2277±532 (551)	135.2±5.8	32.2±5.6
	F	24	1822±321 (599)	1622±314 (579)	2320±761 (599)	133.9±6.4	32.7±6.2
6	M	23	2582±402 (519)	2289±382 (501)	3015±706 (519)	149.5±7.3	41.0±8.5
	F	10	2291±429 (251)	2000±406 (241)	2550±728 (251)	149.5±7.2	38.8±6.7
Non-Asthmatics							
3	M	21	2010±323 (507)	1824±269 (489)	2618±483 (507)	134.5±6.0	30.5±6.9
	F	24	1817±303 (569)	1663±269 (547)	2644±716 (568)	132.9±7.5	30.7±7.5
6	M	24	2635±358 (545)	2365±350 (526)	3264±786 (545)	151.6±7.1	40.5±6.4
	F	10	2797±413 (231)	2606±393 (222)	3793±746 (230)	151.9±5.9	44.6±9.8

NOTES: k = no. of children; n = no. of measurements

Height and weight

Table A.1 presents mean and SD of initial height and weight in sub-groups of grade and gender for both asthmatic and non-asthmatic subjects. Table A.3 presents the coefficients (se) of height and weight effects after controlling for being asthmatic , grade, gender, change in height and weight, and time (growth) effects. A difference of 1 cm in baseline height among children was associated with a change of about 30 ml in FVC and FEV1, and an increase of about 30 ml/s in FEF50. The effect of a difference in 1 kg in weight among children varied by age. In 3rd grade children, the effect was small and not significant. In the 6th grade children, it was associated with about 15 ml increase in FVC, a 12 ml increase in FEV1 and a 16 ml/s increase in FEF50. Obese children had lower values for LF than would otherwise be expected.

Table A.2 Effects (se) of grade and gender on lung function stratified by asthmatic status

Grade	Gender	FVC ml	FEV1 ml	FEF50 ml/s
Non-Asthmatics				
3	M	556.7 (110.2)	640.6 (114.4)	1178.2 (334.0)
	F	448.6 (110.6)	564.2 (114.9)	1352.8 (335.0)
6	M	235.2 (42.6)	265.2 (44.3)	596.1 (130.4)
	F	293.0 (51.6)	417.7 (53.7)	967.3 (157.8)
Asthmatics				
3	M	577.7 (111.4)	600.9 (115.7)	890.9 (337.4)
	F	409.0 (112.9)	482.7 (117.5)	994.2 (343.2)
6	M	236.7 (43.3)	246.8 (44.8)	408.8 (131.6)
	F[a]	0.0	0.0	0.0

NOTES: After controlling for initial height and weight, changes in height and weight, growth within season and autocorrelation, using a mixed effects model

[a] Reference category

Table A.3 Effects (se) of initial height and weight on lung function

	FVC ml	FEV1 ml	FEF50 ml/s
Height at 1st measurement (cm)	29.7 (1.9)	27.3 (2.0)	29.7 (5.8)
Weight at 1st measurement (kg)			
For grade 3	3.8 (2.5)	- 0.2 (2.6)	- 6.8 (7.5)
For grade 6	14.9 (2.2)	12.2 (2.2)	15.6 (6.5)
Tall	71.9 (41.1)	ns	ns
Heavy	-146.2 (76.5)	- 91.6 (74.0)	ns

NOTES: After controlling for being asthmatic, gender, grade, growth within season, and autocorrelation using a mixed effects model; ns=non-significant

Changes in height, weight and time

Table A.4 presents the coefficients (se) of specific time-varying predictors, controlling for time invariant effects (being asthmatic, gender, grade, height and weight at first measurement). We found that a continuous time trend within season was only significant for fall where all three LF measures showed significant growth. This was not the case in spring and winter. However, the change in height and weight from season to season was associated with increase in both FVC and FEV1, even after control for season.

Table A.4 Effects (se) of changes in height, weight and within season growth on lung function

	FVC ml	FEV1 ml	FEF50 ml/s
Change in weight from 1st measurement (kg)	10.2 (4.0)	7.9 (3.9)	ns
Change in height from 1st measurement (cm)	11.2 (5.2)	12.4 (4.2)	ns
Time from 1st measurement			
For spring	-0.6 (0.5)	-0.8 (0.5)	-2.8 (1.2)

	For winter	-0.3 (0.4)	-0.4 (0.4)	-0.9 (1.0)
For fall		1.5 (0.4)	1.4 (0.4)	1.8 (0.9)

NOTES: After controlling for time invariant effects (being asthmatic, gender, grade and initial height and weight) season and autocorrelation, using a mixed effects model; ns=non-significant

Repeated LF measurements: correlation and variance within a child

Fig A.1 presents the repeated LF values of a non asthmatic 6th grade boy, along the studied year. His specific mean and sd of 25 LF outcomes were: for FVC 2362±246 (ml), for FEV1 2077±226 (ml) and for FEF50 2819±444 (ml/s). For each LF parameter, the SD indicates quite a high variability.

Fig A.1 Repeated pulmonary function outcomes during a year (n = 25) of a non-asthmatic boy of the 6th grade (12 years)

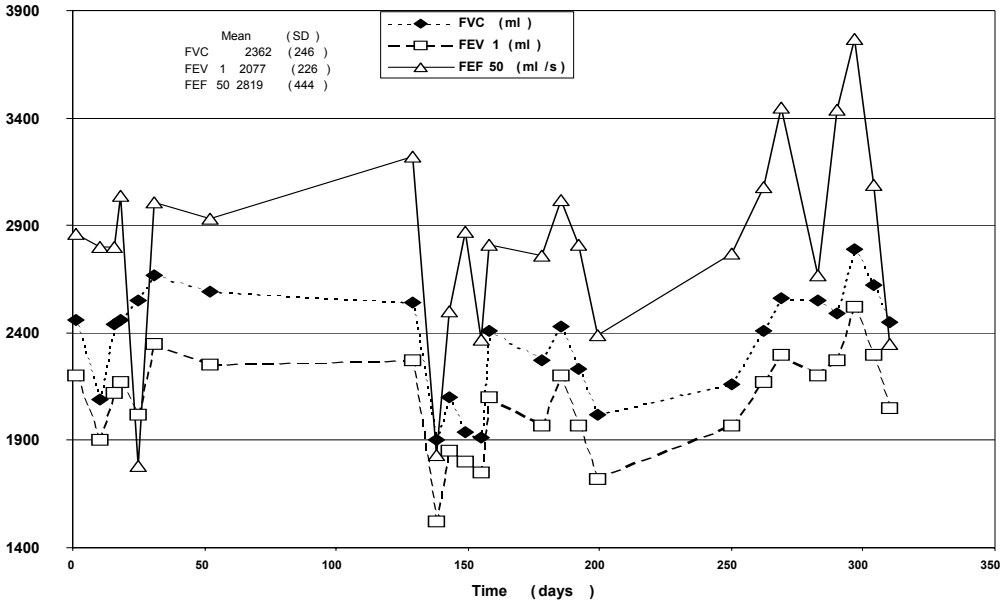

Table A.5 presents the estimated correlation between consecutive measurements of lung function measurements. The longitudinal correlation for FEF50 was as high as for FEV1. These correlations declined with distance in time between the repeated measurements, justifying the use of an autoregressive covariance structure . Since we found a high variability in each child's repeated measurements, Table A.5 also presents the estimated mean of the residuals variance for a child - the within-variance component. Thus, after

control for the variables mentioned above (being asthmatic, gender, grade, height and weight at 1st measurement, changes in height, weight and time), this variance was greater for FEF50 than for FEV1. That is, our model explains less of the variance for this flow measure. This is because of our inability to find cross-sectional predictors that explain each child FEF50 as well as we can explain their FEV1. Longitudinally, however, FEF50 tracks as well as FEV1 over our study period.

Predictors of LF Variability

We applied a multiple regression to model the relationship between the individuals' SD of LF residuals and the following effects: residential-area, having respiratory symptoms, ETS, parents' asthma and atopy of either child or parents. After controlling for gender and grade, few of these effects were found to be significant. Only coughing apart from cold was found to be a significant predictor of FEF50 variability.

Table A.5 Correlation between consecutive measurements and within-child variance of repeated lung function measurements

	FVC ml	FEV1 ml	FEF50 ml/s
Correlation	0.74	0.77	0.83
Variance-within child	62728	61176	417525
(SD)	(250.5)	(247.3)	(646.2)

Comparison between Asthmatics and Non-Asthmatics

Table A.6 presents the difference in LF parameters between asthmatics and non-asthmatics, accounting for three known risk factors of asthma.

Table A.6 Odds ratio[a] (95%CI) of lung function (LF) outcomes and significant risk factors for having asthma

	Model 1 FVC ml	Model 2 FEV1 ml	Model 3 FEF50 ml/s
LF parametera	0.92[b]	1.292	1.91
	(.56-1.50)	(.80-2.12)	(1.18-1.91)
Coughing without a cold	4.45	4.17	3.67
	(1.41-17.47)	(1.32-16.29)	(1.14-14.59)
Wheezing with a cold	4.61	4.71	5.06
	(1.98-11.65)	(2.02-11.93)	(2.10-13.39)
Atopy (home-dust, plants, pets)	2.53	2.47	2.47
	(1.06-6.32)	(1.04-6.10)	(1.02-6.24)

NOTES: According to logistic regression

[a] Odds ratio and CI for one interquartile change in LF parameter (69 ml for FVC, 60 ml for FEV1, 103 ml/s for FEF50)

[b] non-significant factor

Model 1 relates to FVC, model 2 to FEV1 and Model 3 to FEF50. Coughing without a cold, wheezing apart from cold and atopy of the child were substantial risk factors for being asthmatic (OR=4.45 CI=1.41-17.47, OR=4.61 CI=1.98-11.65 , OR=2.53 CI=1.06-6.32, respectively for model 1). Of the 3 LF parameters, only FEF50 was significantly different between the 2 groups, asthmatics and non-asthmatics, after controlling for these symptom variables. An interquartile range decrease (103m/l) in FEF50 was associated with an odds ratio of 1.97 (CI=1.18-3.25) for asthma.

DISCUSSION

We have produced a model of lung function development in asthmatic and non-asthmatic children based on seasonal changes in height and weight, and time within season (as proxy for within season growth). Our model (for the whole group) takes into account the within-child LF variability, based on his repeated LF measurements. This is in contrast to other models that refer to one summary measure for a child (e.g. his LF mean value) while estimating the prediction curve for the whole group. Thus, a lot of important and available information is not included in the model.

Controlling for the time variant variables, we found a gender difference in lung function parameters in our young subjects (3rd grade , aged 9 years), with boys having larger values for their height and weight than girls. In healthy subjects this difference disappeared by grade 6 (age=12) since girls lung function is usually grows more rapidly than boys lung function at this age. This is in line with other studies regarding preadolescent and adolescent school children,[4, 14, 15]

In the asthmatic 6th grade children, some of the gender difference persisted. This may reflect a slight retardation of the beginning of a growth spurt in asthmatic girls. Both initial-height and -weight were found in our models to be significant predictors of higher lung function. Our models included an obesity indicator as well as an indicator for being very tall. Usually weight is considered to be a much less important predictor than height.[4,5,15] However its impact on LF is not always linear and thus can be reduced in models that only include linear weight term as obesity may reduce lung function.

Our models found a significant positive effect of weight along with a negative effect of the obesity dummy variable. Schoenberg et al,[16] already in 1978, showed that "FVC in adolescents initially increases with increasing weight, "muscullarity" effect and then decreases "obesity effect" ".

Nonlinear terms for height (e.g. $height^2$, $height^3$) were non-significant as predictors of any of the lung function parameters, in our data. To confirm these findings we fitted generalized additive models for the data using the GAM function in SPLUS. GAM models allow us to fit non-parametric smooth function of predictor variables, such as height, and test the significance of the improvement in model fit compared to a linear height term. We

found that the deviation from linearity was not significant. These results contrast our model with that of Dockery and coworkers,[4] who assumed FEV1 was proportional to height squared. Seasonal changes in height, in weight and within season growth were found to significantly effect LF. After control for gender, grade and asthma, a 1 cm growth within the same child increased his FVC and FEV1 by about 11 ml. (both in 3trd and 6th grade). A 1kg increase in weight among 6th grade children, increased FVC and FEV1 by about 13 ml. This is in addition to the finding that 1 cm difference among children at baseline, increased FVC and FEV1 by about 30 ml and a 1kg difference among 6th grade children, increased FVC and FEV1 by about 13 ml. The cross-sectional and longitudinal effects of weight are identical. The longitudinal effect of 1 cm increase in height is less than the cross-sectional effect, suggesting the cross-sectional results are capturing effects that are not strictly sized. These findings regarding difference across children are in line with the Israeli-specific prediction equations built by Hellmann and Goren based on a cross-sectional study.[17] We also found that being obese reduces FVC and FEV1 significantly. Time trend was considered to be another lung function predictor. Recent studies using peak flow diaries have shown that a continuous time trend variable is important in models of peak flow in children,[6, 10] and has been taken as representing the growth of the child's lungs within the period of the study. However, we have found that this time trend was only significant in fall and not in spring and winter. Partly, this reflects our use of seasonal changes in height and weight as predictors. It is possible that growth of child lung function is not uniform, with greater growth in fall. We found a 15 ml increase on average in FVC and FEV1 per 10 days growth in fall.

In all, our prediction model for lung function included gender, grade, being asthmatic, initial height and -weight, being heavy or very tall ,seasonal change in height and weight, and within season time trend. A logarithmic transformation of LF values was not found to improve the prediction model as was found by others.[4, 6, 14] Our statistical approach used a mixed effects model, which is a regression model, that takes into account dependence between repeated measurements within the same child (variability within a child). Common regression models assume independence between repetitions of the same subject, which is not usually the case in repeated measures. The use of the mixed effects model is relatively new in the analysis of lung function data. Pope et al.,[10] present autoregressive

covariance structure among the methods for analysing lung health data. We have used such a structure AR(1) , rather than a fixed within subject covariance structure (compound symmetry), assumed by Gerard et al.[18] to evaluate the dependence between the repetitions. We have found that the correlation between consecutive measurements of lung function 3-5 days apart was estimated to be .75, .72, .83 for FVC, FEV1, FEF50 respectively. However this correlation declines, as the distance in days between the repeated measurements becomes larger. In our data, the correlation between the first measurement and the 10th , declines to zero meaning that within three months, the deviation in a child's lung function from that predicted by our longitudinal model was no longer related to past deviations. The variability of a child's LF outcomes over the study-time was quite high. On average , the within child standard deviation during a year, was estimated to be about 250 ml for FVC and FEV1 and 650 ml/s for FEF50.

Coefficient of variation (CV) was used by others as a measure of reproducibility[19]. This measure was based on very few repetitions and as mentioned by Hoek et al., the number of repetitions and their interval have a significant effect on this measure.[20] In addition the CV did not account for the covariates. In contrast, our variability measure accounted for the covariates: gender, baseline height and weight, seasonal changes of height and weight, and time trend and was based on about 25 repetitions per child during a year. Since in our study the variability was found to be quite high, we have tried to investigate the relationship between the day to day variability of LF (i.e. variability within a child) and the effects of residential-area, being asthmatic, having respiratory symptoms, ETS, parents' asthma and atopy.

Our statistical approach was a more refined one than that was used by Timonen et al.[21] It has the advantage that the variability of the observed LF outcomes were referred to the predicted values of each child (accounting for his height and weight change, time trend, gender, grade and primary height and weight). However we did not find any specific effect that may be correlated with the variability of FVC and FEV1. This may be related to the richness of our initial model regarding the LF predictors or to the sample size.

Of the three LF parameters: FVC, FEV1 and FEF50, only FEF50 was found to be significantly different between asthmatic and non-asthmatic children. This supports the suggestion that FEF50 can be a sensitive index of airway obstruction since it is effort

independent, and thus avoids the learning effect which can cause spurious variation in FVC and FEV1.[21,22] We confirmed the well established association between asthma and wheezing with a cold, atopy of the child (plants, home-dust, pets) and coughing apart from cold. We did not find any association between asthma and environmental tobacco smoke, parental asthma or allergies of the subject to food.

To conclude, for a better validity and insight of LF prediction curve of a group, the evaluation should be based on repeated measurements within a child; and the modeling procedure should take into account the within variability of the child's LF. Hence, both longitudinal effects measured by time related variables (e.g. changes in height, weight and time) and cross-sectional effects, measured by non-time related variables (e.g. gender, baseline height and weight) can be evaluated simultaneously.

REFERENCES

1. Frischer T, Meinert R, Urbanek R, Kuehr J. Variability of peak expiratory flow rate in children: short and long term reproducibility. Thorax 1995; 50:35-39.
2. Neas LM, Dockery DW, Burge H, Koutrakis P, Speizer FE. Fungus spores, air pollutants, and other determinants of peak expiratory flow rate in children. Am J Epidemiol 1996; 143:707-807.
3. Strachan DP. Repeatability of ventilatory function measurements in a population survey of 7 year old children. Thorax 1989; 44:474-479.
4. Dockery DW, Berky CS, Ware JH, Speizer FE, Ferris BG. Distribution of forced vital capacity and forced expiratory volume in one second in children 6 to 11 years of age. Am Rev Respir Dis 1983; 128:405-412.
5. Dockery DW, Speizer FE, Stram DO, Ware JH, Spengler JD, Ferris BG. Effects of inhalable particles on respiratory health of children. Am Rev Respir Dis 1989; 139:587-594.
6. Hoek G, Brunekreef B. Effects of low-level winter air pollution concentrations on respiratory health of Dutch children. Environmental Research 1994; 64:136-15
7. Schwartz J, Katz SA, Fegley RW, Tockman MS. Sex and race differences in the development of pulmonary function. Am Rev Respir Dis 1988;138:1415-1421. 7a. Scadding JG. Definition and clinical categorisation. In: Weiss EB, Segal MS, eds. Bronchial asthma: mechanisms and therapeutics. Boston: Little, Brown, 1976:19-30.
8. Colley JRT, Brasser LJ. Chronic Respiratory diseases in children in relation to air pollution. Report on a WHO study, EURO reports and studies 28, Copenhagen 1980.
9. Little RC, Milliken GA, Stroup WW, Wolfinger RD. SAS System for Mixed Models, Cary, NC: SAS Institute Inc., 1996.
10. Pope CA, Schwartz J. Time series for the analysis of lung health data. Am J Respir Crit Care Med 1996;154:S229-S233.
11. SAS SAS/STAT Changes and enhancements, SAS Institute, Cary NC, USA, 1996.
12. Stat Sci. S-PLUS Guide to Statistical and Mathematical Analysis. Seattle, USA, 1995.
13. SAS SAS/STAT User's guide, version 6, SAS Institute, Cary NC, USA, 1989.

14. Gold DR, Wypij D, Wang X, Speizer FE, Pugh M, Ware JH, Ferris BG, DW Dockery. Gender- and race-specific effects of asthma and wheeze on level and growth of lung-function in children in six US cities. J Respir Crit Care Med 1994; 149:1198-1208.
15. Wang X, Dockery DW, Wypij D, Fay ME, Ferris BG JR. Pulmonary function between 6 and 18 years of age. Pediatr Pulmonol 1993;15:75-78.
16. Schoenberg JB, Beck GJ, Bouhuys A. Growth and decay of pulmonary function in healthy blacks and whites. Respir Physio 1978;33:367-393.
17. Hellmann S, Goren A. The necessity of bulding population specific prediction equations for clinical assessment of pulmonary function tests. Eur J Pediatr 1999;158:519-522.
18. Hoek G, Wypij D, Brunekreef B. Self reporting versus parental reporting of acute respiratory symptoms of children and their relation to pulmonary function and air pollution. Int J Epidemiology 1999;28:293-299.
19. Quanjer H, Stocks J, Polgar G, Wise M, Karlberg J, Borsboom G. Compilation of reference values for lung function measurements in children. Eur Respir J 1989;2 :184s-261s.
20. Hoek G, Brunekreef B. Time trends in repeated spirometry in children. Eur Respir J 1992;5:553-559.
21. Timonen KL, Nielsen J, Schwartz J, Gotti A, Vondra V, Gratziou C, Giaver P, Roemer V, Brunekreef B. Chronic respiratory symptoms, skin test results, and lung function as predictors of peak flow variability. Am J Respir Crit Care Med 1997; 156:776-782.
22. Klein RB, Fritz GK, Yeung A, McQuaid EL, Mansell A. Spirometric patterns in childhood asthma: peak flow compared with other indices. Pediatr Pulmonol 1995; 20:372-379.

SUMMARY:

Repeated measurements designs, in which the same variable of interest is measured on each subject on several different times, occur frequently in the assessment of exposure to toxic chemicals. Chapter 1- the introduction, provides an historical review of classical statistical analysis for repeated measurements designs from using simple summary measures, with a non-exhaustive use of the known exposure distribution, through regression analysis which ignores the potential correlation between repeated measures or a one way random effects model which ignores exposure determinants. The mixed effects model, of which the application started at the late nineties in environmental hygiene, enables to estimate simultaneously in one model both the variance components of exposure (between- and within-subject) and the unbiased regression coefficients for determinants of occupational exposure.

The mixed effects model is a generalization of the standard linear model (a regression model), that enables the analysis of data with several sources of variation instead of just one. The possibilities of using mixed effects models for occupational exposure assessment are evaluated and explored in this thesis. The thesis deals with the effects of exposure determinants on mean exposure to several materials while accounting for and estimating the exposure variance components. It deals with "strange" negative variance components and with the application of mixed effects modeling for exposure response evaluation.

In Chapter 2 the focus is on the variance structure: since exposure varies both between and within-workers, "general" measures of these variance components of exposure, accounting for air pollution, factory and workers effects in a working population were estimated. The cohort consisted of Israeli workers (Chapter 2, part I) chosen in a nested-design. The geometric standard deviations representing variation between workers (after adjustment for air pollutant and factory) and within workers were 3.1 and 3.0, respectively. These values may be used, as rough estimates of exposure variability to get an interval estimate of mean exposure, instead of a point estimate and for planning future statistical sampling. Modeling mean exposure by its determinants while accounting for the variance components was illustrated with 3 cohorts: the above-mentioned cohort and cohorts of Dutch rubber

manufacturing workers and pig farmers (Chapter 2, part II and Chapter 3). Exposure determinants (e.g. specific work characteristics) reduced the random between-worker variance estimators between 59-100% (Chapter 3). Interestingly, the random within-worker variability was reduced only in the pig farmer data set, by 25%, when accounting for specific work activities that varied over time.

If the correlation between repeated measures is not dealt with appropriately, or more generally, the different variance components of exposure are not accounted for, then the effects of exposure determinants on mean exposure may be biased. Therefore, we compared results of linear regression and mixed models (Chapter 3). In the rubber manufacturing data, the coefficients of the mixed model showed similar relative importance as those of the regression model, but were generally smaller. The main difference was that fewer factors affecting exposure to inhalable particulates were statistically significant in the mixed, rather than in linear regression models, due to the high correlation found between repeated measurements (r=0.82).

Among the benzene-workers (Chapter 4) both time related factors and a non-time related factor (e.g. job task) were found to affect the mean exposure significantly, accounting for the correlation between repeated measures. The random between workers variance was highly affected by the job task. Time related factors (warm month, “credit day”-an over loaded day, day of the week), were found to be responsible for the high random within-worker (day-to-day) variance, which was more than two times higher than the between-worker variance.

Grouping strategies in occupational health and epidemiology are used to create so called homogeneous exposure groups. Hence, the between-worker variance is often small. Besides, the within-worker variance that reflects day-to-day variations in exposure often varies greatly. Consequently, in simulated data based on real exposure data (Chapter 4) we found that it is very common to obtain a zero or negative ANOVA estimate of the between-worker variance. This is not reasonable in real life and also poses problems for estimating the probability that, in a group, a randomly selected worker's mean exposure exceeds the occupational exposure standard. We evaluated an approach proposed earlier to use an upper confidence bound when the estimate is negative and found that this method has three main disadvantages: the estimator remains negative for some data sets, the estimator

performs poorly with only two repeated measures per worker (common, and even recommended practice), and the method can be extremely sensitive to small changes in the data. Our alternative estimator which incorporates the "plugging in" of an estimator in which the observed mean squares replace the expected values offers a solution to these problems. It gave a bias-adjusted confidence bound for all data sets.

Exposure assessment plays an important role in a valid exposure-response evaluation in epidemiology. In a study among Dutch bakers (Chapter 6) we used mixed modeling in two procedures: firstly to estimate exposure based on specific exposure determinants and secondly for exposure-response relationship where the estimated variance components were used as a scaling factor to avoid possible risk estimate attenuation. The shape of the relationship between sensitization and exposure, was found to be a quadratic function, based on mean exposures estimated by different strategies for exposure assessment (with respect to sector of industry, job title and tasks). The probability of sensitization increased with exposure and at higher exposures decreased, possibly as a result of a healthy worker effect. In all analyses, atopy and industry-sector affected risk significantly. The use of a regression calibration method which accounts for both within worker variability and predicted exposures (based on exposure determinants) to improve validity and accuracy of the odds ratios estimators, sharpened them slightly. An application of mixed modeling on repeated lung function measures can be found in the appendix, aiming to explore the lung function change over the course of a year among asthmatic and non-asthmatic children living around a power plant.

Finally, the major issues that have not been dealt explicitly within the individual chapters regarding benefits of using mixed models, are discussed in Chapter 7, broken down into major statistical and occupational hygiene aspects. The statistical aspects includes the ability to estimate simultaneously in one model both the variance components of exposure between- and within-workers (and their derivatives), and the unbiased regression coefficients for determinants of exposure. As well as in modeling relationships between exposure and health effects when exposure measure has some variability and may attenuate the risk estimators, regarded as “measurement error modeling” in statistics. Hygiene aspects include those that are relevant to grouping strategies, hazard control and over-exposure assessment.

SAMENVATTING:

Studies op basis van herhaalde metingen, waarbij de te meten variabele bij ieder individu herhaald is gemeten op meerdere momenten, worden veel toegepast om de blootstelling aan toxische stoffen te karakteriseren. Hoofdstuk 1 – de inleiding, geeft een historisch overzicht van de statistische analyse technieken die gebruikt zijn voor de analyse van herhaalde metingen. De technieken lopen uiteen van toepassing van simpele maten zoals het gemiddelde, waarbij informatie over de verdeling van de gegevens genegeerd wordt, regressie analyse waarbij de correlatie tussen de herhaalde metingen niet wordt verdisconteerd, tot een "one way random effects ANOVA" model voor herhaalde metingen waarin de determinanten van de blootstelling niet worden meegenomen. Het "mixed effect model" dat vanaf de late jaren negentig van de vorige eeuw wordt toegepast in de milieuhygiëne, maakt het mogelijk om simultaan in een model de variantie-componenten van de intra- en inter-individuele variatie te bepalen alsmede de regressiecoëfficiënten voor de determinanten van de uitkomstvariabele, de blootstelling aan het agens.

Het 'mixed effect' model is een generalisatie van het klassieke lineaire (regressie) model dat het mogelijk maakt de invloed van meerdere variatiebronnen tegelijkertijd te bestuderen, in plaats van één. Dit proefschrift beschrijft een verkenning en een evaluatie van de mogelijkheden van toepassing van "mixed" modellen voor blootstellingkarakterisering. Dit proefschrift behandelt de effecten van blootstellingdeterminanten op de gemiddelde blootstelling aan verschillende agentia terwijl rekening wordt gehouden met de blootstelling variantie-componenten structuur. Het proefschrift behandelt 'vreemde' negatieve variantie componenten en de toepassing van het 'mixed effect' model voor blootstelling-respons modellering.

In hoofdstuk 2 wordt ingegaan op de modellering van de variantie structuur: omdat de blootstelling intra- en inter-individuele variatie vertoont, worden de variantiecomponenten van intra- en inter-individuele variatie geschat, rekening houdend met determinanten van de blootstelling zoals bedrijf, functie, taak etc. De analyse is toegepast op een cohort Israëlische werknemers met blootstelling aan lood, benzeen en stof (Hoofdstuk 2, deel 1), die in een zogenaamde "nested-design" waren opgenomen. De geometrische standaard

deviaties voor de inter- en intra-individuele variatie (na correctie voor agens en bedrijf) waren respectievelijk 3,1en 3,0. Deze waarden kunnen als globale schatting worden gehanteerd voor toekomstig op te zetten meetstrategieën.

Het modelleren van de gemiddelde blootstelling, terwijl rekening wordt gehouden met de variantie-componenten, wordt geïllustreerd aan de hand van drie verschillende studies. Het eerder genoemde cohort, populaties van rubber werkers met blootstelling aan stof en rubberdampen, en varkenshouders met blootstelling aan endotoxine (Hoofdstuk 2, deel 2 en Hoofdstuk 3). Determinanten van blootstelling (zoals specifieke werkplekkenmerken) reduceerden de inter-individuele variatie in endotoxine blootstelling met 59-100% in de studie onder varkenshouders. Interessant is dat de intra-individuele variatie afnam met 25% als de over de tijd veranderende taken in het model werden meegenomen.

Als geen rekening wordt gehouden met de correlatie tussen herhaalde metingen, of algemener, als geen rekening wordt gehouden met variantie-componenten, dan kan vertekening (bias) optreden in de schattingen van de effecten van determinanten op de blootstelling. Daarom zijn resultaten van een klassieke lineaire regressie analyse vergeleken met de resultaten van een "mixed" analyse (Hoofdstuk 3). Uit de analyse van de gegevens van de rubberindustrie bleken de coëfficiënten geschat op basis van het 'mixed' een zelfde rangorde te vertonen voor wat betreft de grootte van de coëfficiënt als schattingen op basis van de klassieke regressieanalyse. Echter, de coëfficiënten geschat op basis van het 'mixed' model waren kleiner dan die afkomstig van de klassieke regressie analyse. Het belangrijkste verschil was dat minder determinanten significant bijdroegen aan de verklaring van de inhaleerbaar stofconcentratie in geval van de 'mixed' modellen in vergelijking met de klassieke regressie, door de sterke correlatie (r=0,82) tussen de herhaalde metingen.

Onder aan benzeen blootgestelde werknemers (Hoofdstuk 4) bleken tijdgerelateerde en niet-tijdgerelateerde determinanten de gemiddelde blootstelling te bepalen als rekening werd gehouden met de variantie-componenten. De willekeurige intra-individuele variatie in blootstelling bleek sterk samen te hangen met de uitgevoerde taken. Tijdgerelateerde variabelen (warme maand, betaaldag, een zwaar belaste dag, dag van de week) bepaalden ook in hoge mate de intra-individuele variatie (dag-tot-dag variatie), welke tweemaal zo groot was als de inter-individuele variatie.

Groeperen van werknemers in homogene blootstellinggroepen is een veel gehanteerde strategie in arbeidsepidemiologisch onderzoek. In de meeste situaties is de inter-individuele variatie in blootstelling in een groep relatief gering. De dag-tot-dag variatie in blootstelling is veelal groot. Als gevolg hiervan is het mogelijk dat ANOVA modellen een negatieve inter-individuele variantie opleveren. Dit is in werkelijkheid onmogelijk en levert daarom problemen op bij het schatten van de kans dat in een groep werknemers voor een willekeurig geselecteerde werknemer de blootstelling een grenswaarde overschrijdt. Dit is nader bestudeerd in gesimuleerde gegevens, die wel op werkelijke situaties geënt zijn en dus realistisch zijn. Een methode is geëvalueerd die uitgaat van een bovengrens van het betrouwbaarheidsinterval wanneer de geschatte variantie negatief is. Deze methode bleek drie principiële nadelen te hebben: in sommige gevallen blijft de schatting van de variantie negatief, de schatter gedraagt zich niet optimaal bij slechts twee herhaalde metingen, en de methode is gevoelig voor kleine veranderingen in de beschikbare gegevens. Een alternatieve schatter die deze problemen oplost wordt voorgesteld en heeft als kenmerk dat de geobserveerde gemiddelde kleinste kwadraten worden vervangen door de verwachte waarde. Deze schatter leverde een gecorrigeerd betrouwbaarheidsinterval op voor alle gegevensbestanden.

Karakterisering van de blootstelling speelt een belangrijke rol bij valide blootstelling-respons analyse in de epidemiologie. In een studie onder bakkers werden ‘mixed’ modellen gebruikt voor twee doeleinden; allereerst om de determinanten van de blootstelling te identificeren en ten tweede om blootstelling te schatten op basis van de determinanten waarbij de variantiecomponenten als een “kalibratie factor” werden gebruikt om zogenaamde onderschatting van de blootstelling respons relatie (“attenuatie”) te vermijden. De vorm van de blootstelling respons relatie bleek zich te gedragen als een kwadratische functie, uitgaande van de gemiddelde blootstelling per bakker, geschat op basis van verschillende determinanten (industrie, functie en taken). De kans op sensibilisatie nam toe met toenemende blootstelling en nam weer af bij hogere blootstelling niveaus, mogelijk als gevolg van het zogenaamde “healthy worker effect” of de ontwikkeling van tolerantie. In alle analyses bleken atopie en branche het risico sterk te beïnvloeden. Gebruik van de regressie kalibratie methode op basis van zowel de intra-individuele als inter-individuele

variatie en voorspelde blootstelling op basis van determinanten leidde tot een verbetering in de validiteit en nauwkeurigheid van de schattingen van de Odds Ratio's.

Een toepassing van 'mixed' modellen op herhaalde eindpunt metingen (longfunctie) kan worden gevonden in de Appendix. Deze studie had tot doel de longfunctieverandering over een jaar te exploreren bij astmatische en niet astmatische kinderen rond een elektriciteitcentrale.

Uiteindelijk worden een aantal belangrijke statistische en arbeidshygiënische thema's, die betrekking hebben op het gebruik van 'mixed' modellen, die in eerdere hoofdstukken niet aan bod zijn gekomen in de discussie besproken (Hoofdstuk 7). De statistische aspecten hebben betrekking op het simultaan kunnen schatten van de niet vertekende bijdrage van determinanten van blootstelling (regressiecoëfficiënten) en de intra-en interindividuele variatie in blootstelling. De variatie-componenten spelen ook een rol in de analyse van blootstelling-respons relaties, wanneer de blootstelling in enige mate varieert en als gevolg daarvan onderschatting van de relatie op kan treden ("attenuatie"). De arbeidshygiënische aspecten omvatten groepeerstrategieën en het vaststellen van blootstelling boven een bepaalde grenswaarde. Toekomstige ontwikkelingen in het gebruik van "mixed" modellen worden ook kort besproken.

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CURRICULUM VITAE:

Chava Peretz (Shpiro) was born on October 4, 1952 in Tel-Aviv Israel. From 1972 to 1976, she studied at Tel-Aviv University, where she obtained a BSc degree in Mathematics (Statistical track). After graduation she worked as an applied statistician in Sheba Hospital, Israel, in the Epidemiology Department, involved in data-analysis in medical research, focusing on epidemiological studies.

In 1987 she joined the Epidemiology unit in the Occupational Health Institute in Tel-Aviv University, Sackler School of Medicine. She was responsible for hygiene data-analysis, made independent studies, cooperated with other researchers and supervised Master students in Occupational Health (MOccH) in statistical data analysis. In 1994 she completed her MOccH degree from Tel-Aviv University, Sackler School of Medicine. She attended several advanced statistical courses in the Statistical Department, at the Sackler Mathematical School, Tel Aviv University, and three advanced epidemiological-statistical courses in the Netherlands (Erasmus and Wageningen Universities).

In 1998 she joined the Epidemiology unit in the Environmental Research Institute in Tel-Aviv University, Sackler School of Medicine, as a researcher, for 3 years. At present she is a lecturer and statistical supervisor in the Health professions (Nursing and Physical Therapy) departments in Tel Aviv University, Sackler School of Medicine. She actively participated in workshops and conferences, both local and international, promoting the subject of occupational and environmental exposure assessment, focusing on statistical issues. She was twice awarded for her original studies, in international conferences. Chava is married to Ilan and they have three children: two sons, Nevo and Omri and one daughter named Shahaf.

List of publications

Published

1. Seligsohn U, Zivelin A, Peretz C, Modan M. Detection of hemophilia a carriers by replicate factor VIII activity and antigenicity determination. Br J Hematol 1979;42:433-439.
2. Yaar I, Ron E, Modan B, Modan M, Peretz H. Long-term effects of small doses of X-radiation applied in childhood , as manifested in adult visually evoked responses. Trans Am Neurol Assoc 1979;104:264-268.
3. Ben-Bassat I, Many A, Modan M, Peretz C, Ramot B. serum immunoglobulins in chronic lymphatic leukemia. Am J Med Sci 1979;278:4-9.
4. Yaar I, Ron E, Peretz H, Modan, B. Long-term cerebral effects of small doses of X-irradiation in childhood as manifested in adult visual evoked responses. Ann Neurol. 1980;8:261-8.
5. Peretz C. Industrial hygiene surveys in factories: needs versus actual service.
   a. Work, Welfare and National Insurance 1991; 2:77-78 (In Hebrew),
   b. Occupational Health 1991;3: 16-19 (In Hebrew)
6. Peretz C, Goldberg P, Derazne E, Kahan E. Assessment of the extent of implementation of the recommendations made in occupational. hygiene survey reports. Ann Occup Hyg 1992; 36:229-238.
7. Derazne E, Kahan E, Peretz C, Hassidim Y. Responses of occupational physicians and laboratory staff to reports of high blood lead levels. Israel Jornal of Medical Science 1992;28:556-60.
8. Goldberg P, Peretz C. The effectiveness of regulations regarding periodic monitoring occupational hygiene: Public Health Review 1995;23:59-71.
9. Kahan E, Peretz C, Rybski M. Determination of consumer satisfaction. A basic step for quality improvement of an occupational hygiene service. Occupational Medicine 1995;45:193-8.
10. Peretz C, Goldberg P, Kahan E, Grady S, Goren A. The variability of exposure over time: a prospective longitudinal study. Ann Occup Hyg 1997;41:485-500.
11. Kahan E, Lemesh C, Pines A, Mehoudar O, Peretz C, Ribski M. Workers' right-to-know legislation: does it work? Occup Med 1999;49:11-5.
12. Peretz C, Froom P, Pardo A, Goren A. Exposure to benzene in fuel distribution installations: monitoring and prevention. Arch Environ Health 2000;55:4439-46.
13. Dvir Z, Prushansky T, Peretz C. Maximal vs. feight active cervical motion in healthy subjects: the coefficient of variation as an indicator of sincerety of effort. Spine 2001;26:1680-8.
14. Peretz C, Steinberg DM. Improved non-negative estimation of variance components for exposure assessment J Expos Anal Environ Epidem 2001;11:414-21.
15. Peretz C, Goren A, Smid T, Kromhout H. Application of mixed-effects models for exposure assessment. Ann Occup Hyg 2002 Jan;46(1):69-77.
16. Wouters IM, Hilhorst SKM, Kleppe PK, Doekes G, Douwes J, Peretz C, Heederik D. Upper airway inflammation and respiratory symptoms in domestic waste collectors. Occup Environ Med 2002 Feb;59(2):106-12.
17. Dvir Z, Werner V, Peretz C. The effect of measurement protocol on active cervical motion in healthy subjects. Physiother Res Int. 2002;7(3):136-45.
18. Dvir Z, Steinfeld-Cohen Y, Peretz C. Identification of feigned shoulder flexion weakness in normal subjects. Am J Phys Med Rehabil. 2002 Mar;81(3):187-93.
19. Bronner G, Peretz C, Ehrenfeld M. Sexual harassment of nurses and nursing students. J Adv Nurs. 2003 Jun;42:637-644.

20. Sadetzki S, Calderon-Margalit R, Peretz C, Novikov I, Barchana M, Papa MZ. Second primary breast and thyroid cancers (Israel) Cancer Causes and Control. 2003 14:367-375.

Submitted for publication

1. Peretz C, Goren A, D. Heederik, Schwartz J. Predictors of changes in pulmonary function in a longitudinal study of asthmatic and non-asthmatic children.
2. Peretz C, de Pater N, de Monchy J, Oostenbrink J, Heederik D. Assessment of exposure to wheat flour and the shape of its relationship with specific sensitization.
3. Yahalom G, Simon ES, Thorne R, Peretz C, Giladi N. Hand rhythmic tapping and timing in Parkinson's disease.