Final Performance Report

The Utility of Biomarkers as Exposure and Dose Measures

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Abstract

This study examined variation in the levels of biological measures of exposure to workplace contaminants. Although intra- and inter-individual differences have been characterized for a large number of airborne exposures across occupational groups, similar variation had not been investigated extensively for biological measures. A primary objective of this study was to compile a database of repeated biological measures so that the within- and between-worker sources of variability could be partitioned for as wide a range of biomarkers as possible. Following a review of the world's published literature, biological monitoring data were abstracted from 52 studies that examined workers' exposures to metals, solvents, polycyclic aromatic hydrocarbons, and pesticides. Forty-four percent of the studies also reported personal sampling results, which were compiled as well. Over 4,000 measurements collected on 577 workers in 55 workplaces are contained in the biological database, which represents a wide range of biomarkers collected in blood, urine, and exhaled air. In addition, two relatively large databases of monitoring data collected on workers at a Swedish chloralkali plant from 1988-1997 and at eight reinforced plastics plants in the Emilia-Romagna region of Italy during 1985-1999 were compiled. At the chloralkali plant, 325 air mercury measurements, 847 blood mercury measurements, and 1,165 urinary mercury measurements had been collected and included in the database. For the styrene database, a total of 1,714 measurements of mandelic acid and phenyyglyoxalic acid were abstracted from laboratory reports and compiled. Occupational groups of workers were classified on the basis of a common work environment (i.e., on the basis of plant or facility). When sufficient data were available, workers were further classified by job title or primary work tasks performed.

To evaluate sources of variation in the biological monitoring data, random- and mixed-effects models were applied. Based on our assessment, there was generally more variation among workers employed at the same plant than across shifts. Owing to the effects of intra-individual variation, we confirmed that estimating workers' exposures from relatively few measurements could attenuate measures of effect should the monitoring data be used in an epidemiologic study to evaluate workers' exposures. Given the physiological and kinetic parameters that influence body burdens of contaminants, statistical methods that incorporate the serial dependence among measures were applied, where possible. Notwithstanding the advantages that biomarkers offer in assessing exposure, the use of biological indices of exposure places an additional burden on an exposure assessment strategy since data may be serially correlated (as evidenced in our study), which could result in biased estimates of the variance components if autocorrelation is undetected or ignored in the statistical analyses.

In the application of random- and mixed-effects models, which provide invaluable information that can be used in the control of hazards in the workplace and in the design of studies to evaluate health effects associated with occupational exposures, it is advantageous to pool information across different groups of workers. Yet, the underlying assumption that the degree of variation over time and among workers is the same for all groups has yet to be fully investigated. In our study of four groups of workers exposed to inorganic mercury at a chloralkali plant, there was no evidence of significant heterogeneity in the levels of variation over time or between workers for air mercury levels. For the biological monitoring data, however, our findings indicate that groups did not share common levels of variability and that it was not appropriate to pool the data and obtain single estimates of the within- and between-worker variance components. Our results suggest that additional studies are warranted to evaluate whether it is reasonable to assume that the degree to which exposures vary over time and among workers is the same across occupational groups who share common work environments.

Significant Findings

Study 1: Assessment of Variability in Biomonitoring Data Using a Large Database of Biological Measures of Exposure (Symanski and Greeson, submitted, 2001)

Following a review of the world's published literature, biological monitoring data were abstracted from 52 studies that examined workers' exposures to metals, solvents, polycyclic aromatic hydrocarbons, and pesticides. In total, over 4,000 biological measurements collected on 577 workers in 55 workplaces were compiled, which represents a wide range of biomarkers collected in blood, urine, and exhaled air. The majority of the biological monitoring data arose in the manufacturing sector (86%) and originated in workplaces in Western Europe (36%), Scandinavia (27%), or the United States (26%). Since studies reporting the biological measurements did not always include personal sampling results, the air-monitoring database contains fewer measurements (1,847) collected on a smaller number of workers (192). Altogether, the database contains 6,174 measurements collected on 577 workers.

In evaluating variability in the biological monitoring data, nearly two-thirds of the groups (73/121 data sets) exhibited more variation in exposure among workers than variation from day-to-day. In comparing the heterogeneity among workers employed at the same plant, we found that 26 percent of the groups were homogeneous, with a two-fold range or less in 95% of the workers' mean exposure levels. However, an almost equal percentage of groups exhibited rather heterogeneous exposures among workers with 20-fold or higher differences in 95% of the workers' mean exposure levels. Notably, more than 100-fold differences in exposures among workers were observed in five percent of the groups. For biological contaminants with half-lives of seven days or longer, 70% of the data sets were characterized by more variation among workers than across shifts compared to 52% of the sets for biomarkers with half-lives of less than seven days.

Although only a small number of data sets (n=25) were suitable for analysis of autocorrelation, the majority (72%) of the biological monitoring data were serially correlated ($p < 0.05$). Among these data sets, point estimates of the autocorrelation parameter ranged from 0.25 to 0.99, which suggests moderate to substantial levels of serial correlation. In comparing the results of the random-effects model with compound symmetry to the model with an autocorrelated error structure, the differences suggest that ignoring serial correlation underestimates the within-worker variance, but overestimates the between-worker variance.

Study 2: Inter- and Intra-Individual Sources of Variation in Levels of Urinary Styrene Metabolites (Symanski et al., 2001a)

Given the paucity of studies that have examined variability in biological measures of exposure to workplace contaminants, we quantified the intra- and inter-individual sources of variation in urinary levels of mandelic acid (MA) and phenylglyoxylic acid (PGA) among workers exposed to styrene. A secondary objective of this study was to examine effects of job task and the timing of sampling during the workweek on the variation in workers' urinary styrene metabolite levels. A total of 1,714 measurements of mandelic acid and phenylglyoxalic acid collected on 331 workers from eight reinforced-plastics plants were compiled from laboratory reports. Six plants were involved in the production of silos, containers, or tanks, one in the production of carousel parts, and one in the production of polyester resins. There was wide variability across plants as to the period of sampling and the number of years during which measurements were collected, with some plants contributing data for just a few years compared to others where biological monitoring had been conducted for 10 years or longer. Altogether, the data spanned from 1985 through 1999. The majority of the workforce was male except at one plant.

Separate analyses were conducted by plant for the pre- and post-shift urine samples, for raw concentrations of MA, PGA, and the sum of both metabolites, and for concentrations of these metabolites expressed in units of mg/l and mg/g creatinine. In general, PGA levels were characterized by less total variation than levels of MA. With relatively few exceptions, comparisons for each metabolite suggest that the concentrations expressed as a function of creatinine yielded smaller estimates of the intra-individual source of variation. With one exception, the inter-individual variance was generally higher than the intra-individual variance for post-shift urine samples in all of the plants. In contrast, the results for styrene metabolite levels measured in pre-shift urine samples suggest greater variation, on average, across work-shifts than among workers at the same plant.

In evaluating the effects of work tasks on the variation in urinary metabolite levels in three plants, laminators had significantly higher exposure levels ($p < 0.05$) than workers in assembly, finishing, maintenance or managerial positions at the plant ('non-laminators') irrespective of the metabolite analyzed (MA, PGA, or MA+PGA) or the time of sampling (pre- or post-shift). Exposures for workers whose tasks were unknown were also generally higher compared to non-laminators, but were not significantly different in most cases ($p > 0.05$). The influence of work tasks on the variation in metabolite levels was further examined by comparing estimates of the inter-individual variance component obtained from the one-way random-effects model to the mixed-effects model with a fixed effect added for job classification (Bryk and Raudenbush, 1992). The percent reduction in the inter-individual variance component ranged from 8 to 100%. While this measure represents the fraction of the variation among workers that is explained by job classification, careful interpretation is warranted when the point estimate of the inter-individual variance component is small. In that instance, the fixed effect may explain a significant proportion of very little variation. As expected, there were trivial differences in the estimates of the intra-individual variance component under both models.

Two of the plants had sufficient data collected throughout the week to examine effects related to the timing of sampling on levels of MA, PGA, and the sum of both metabolites. We found that levels of the sum of both metabolites were higher ($p < 0.05$) for samples collected on Wednesday or later in the week compared to samples collected on Mondays or Tuesdays. When analyses were conducted for levels of MA and PGA separately, similar patterns emerged with statistically significant differences ($p < 0.05$) detected in all but one case.

Study 3: Variability in Airborne and Biological Measures of Exposure to Mercury in the Chloralkali Industry: Implications for Epidemiologic Studies (Symanski et al., 2000)

In this study, we examined the intra- and inter-individual sources of variation in exposure to mercury vapor as measured in air, blood, and urine among four groups of workers during 1990-97 at a Swedish chloralkali plant. In total, 282 air mercury measurements, 646 blood mercury measurements, and 955 urinary mercury measurements were compiled from laboratory reports. For airborne mercury, the proportion of the total variability attributable to the intra-individual source of variation differed among groups. Cell hall maintenance workers (cell cleaners, basement flushers, and mercury-pump repairmen) and shift workers were characterized by extreme day-to-day variability; little variation was detected between workers. In contrast, there appeared to be as much or greater variation among individuals compared to variation across shifts in both the cell hall production workers (cell hall foremen, voltage regulators of the mercury cells, and cell switchers) and non-cell hall workers (instrument technicians, mechanical workshop workers and foremen, staff electricians, operating engineers, plastic workshop workers, and laboratory workers). There was greater variation between, rather than within, workers for the biomonitoring data when all workers were combined whereas equivocal results were obtained when each occupational group was analyzed one at a time.

To assess the influence of measurement error in air or biological levels of mercury, we constructed a hypothetical scenario in which estimates of average levels of the log-transformed mercury values in air, blood, or urine for each worker were used to examine the relation with a continuous health outcome using a simple linear regression model. Under the standard assumptions that underlie simple (unweighted) linear regression analysis, the observed slope coefficient (under expectation) is smaller than the true coefficient (Thomas et al., 1993). The extent of the attenuation is a function of the magnitude of the intra- and inter-individual sources of variation in exposure and of the number of repeated measurements collected on each individual. For airborne, blood, or urinary measures, estimates of the number of repeated measurements per worker needed to minimize the attenuation of an observed slope coefficient to 90%, 75%, and 60% of its true value were calculated. When workers were evaluated together irrespective of occupational category, the sampling requirements were reduced for mercury measured in blood or urine compared to those in air. Across occupational groups, the sampling demands varied and in some instances sizeable differences were noted.

Study 4: Heterogeneity in Sources of Exposure Variability among Groups of Workers Exposed to Inorganic Mercury (Symanski et al., 2001b)

Although statistical methods that combine exposure data collected on workers from different occupational groups are more efficient, the underlying assumption that the degree of variation over time and among workers is the same for all groups has yet to be fully investigated. Given the utility of different modeling approaches when assessing exposures, we investigated assumptions of homogeneity of variance within and between workers using both random- and mixed-effects models. In evaluating sources of variation in four groups of workers exposed to inorganic mercury (Hg) at a chloralkali plant, there was no evidence of significant heterogeneity in the levels of variation over time or between workers for air Hg levels. For the biological monitoring data, however, our findings indicate that groups did not share common levels of variability and that it was not appropriate to pool the data and obtain single estimates of the within- and between-worker variance components. Classification of job group as a random or fixed effect had no effect on the results and yielded the same conclusions when the models were compared.

In this study, trends in exposure levels were evaluated in both the air and biological monitoring data. While trends in exposure were not detected in either the air or blood Hg data, urinary mercury levels declined slightly ($p < 0.05$) at a rate of approximately 3% per year.

Given that exposures vary both within and between workers in an occupational group, the 'exceedance' (i.e., the probability that a single measurement obtained from a randomly-selected worker in the i-*th* group on a randomly-selected day exceeds an occupational exposure limit) can be expressed as a function of the mean and variance components of the underlying distribution of the natural log-transformed exposures for the i-*th* job group (Rappaport *et al.*, 1999). Similarly, the probability that the conditional mean exposure for a randomly selected worker exceeds an occupational exposure limit (i.e., the probability of overexposure) can be expressed as a function of the mean and variance components of the underlying distribution. The exceedance and the probability of overexposure were estimated based on the parameter estimates that were generated under various models with different assumptions regarding the variance components for air, blood, and urinary mercury. Threshold limit values and biological exposure indices (ACGIH, 1999) of 25 $\mu g/m^3$, 75 nmol/L and 35 µg/g creatinine were chosen as the exposure limits for air, blood, and urinary mercury, respectively. The likelihood that a single randomly-collected air measurement would exceed 25 $\mu g/m^3$ ranged considerably across the four groups with the highest values observed for maintenance workers (~ 40%) who are involved in activities that give rise to extremely variable exposures. For this same group of workers, two- and four-fold differences in the exceedance probabilitites for blood and urinary Hg, respectively, were observed across models. As with the exceedance probabilities, there were differences in some cases when comparing the results for the estimated probabilities of overexposure obtained from different models.

Usefulness of findings

Study 1: Assessment of Variability in Biomonitoring Data Using a Large Database of Biological Measures of Exposure (Symanski and Greeson, submitted, 2001)

The database compiled for this study is unique in that it represents a wide range of repeated biological measures of exposure to workplace contaminants in a broad cross-section of industries worldwide. As such, the database provided an opportunity to quantify the within- and between-worker sources of variation in workers' exposures as assessed by biological monitoring. Our findings indicate that, in general, there was more variation among workers at the same plant than across shifts. In contrast, a majority of studies that have examined variability in airborne measures of exposure reported that the variation within workers was greater than that between workers. Differences in the classification schemes used to group workers may explain, in part, the equivocal findings. Previous studies which examined airborne contaminants and that grouped workers by either job title (Heederik et al, 1991; Milton et al., 1996) or production area (Nieuwenhuijsen et al., 1995) across facilities and those that established groupings by both job title (or work area) and location (Kromhout et al., 1993; Woskie et al., 1994; Kumagai et al., 1996; Milton et al., 1996; Symanski et al., 1996) reported more variation within than between workers. If workers who share the same job (whether at the same location or not) have more similar exposures than workers at the same location but with different jobs, the former classification would likely result in a larger within- than between-worker source of variation, as reported in the aforementioned studies, and vice versa for the latter classification, as reported in the current study. Nonetheless, when the grouping of workers was conducted at the plant level in investigations that evaluated air-monitoring data, a larger between-worker variance component relative to the within-worker variance was reported in some (Kromhout et al., 1987; Kromhout and Heederik, 1995; Rappaport et al., 1995), but not all (Milton et al., 1996; Peretz et al., 1997; Symanski et al., 2000; Rappaport, 1991) studies.

Relying on estimates of the within- and between-worker variance components, we found that a majority of groups were heterogeneous, with approximately three-fourths of the data exhibiting 2-fold differences or greater among 95% of the workers' mean exposure levels. The lack of homogeneity in exposure in a considerable proportion of groups is consistent with results reported previously for the large database of airborne exposure measurements (Kromhout et al., 1993). Moreover, our findings support the view that observational approaches that rely on job titles or on the tasks that workers perform may not *necessarily* establish groups of workers with similar exposures (Rappaport et al., 1993; Burstyn and Kromhout, 2000) and that quantitative methods are necessary to evaluate the degree of homogeneity of exposures irrespective of whether the assessment relies on personal or biological monitoring.

Our results indicate that biological measures, particularly those with long half-lives, may be superior to airborne measures since, in general, they are characterized by greater variability among workers than across shifts over time. Notwithstanding the advantages that exposure measures offer which are characterized by relatively little variation over time, the use of biological indices of exposure places an additional burden on the sampling strategy (and on the selection of the statistical model applied) since data may be serially correlated as evidenced in our study. Should autocorrelation go undetected or be ignored in the statistical analyses, the potential for biased estimates of the variance components raises the possibility of making important errors of inference about the relative magnitude of the sources of variation in exposure and thereby could hinder our ability to accurately assess exposure.

In summary, this study represents the first comprehensive evaluation of the within- and between-worker sources of variability in biological monitoring data. In general, our results suggest that biological measures of exposure are characterized by more variability among workers employed at the same facility as compared to variability from day-to-day. However, the relative magnitude of the within- and between-worker variance components varied considerably across groups in that variability in some data sets was attributable almost entirely to differences among workers and, in other sets, the variability was largely due to random fluctuations from one time period to the next. Such findings underscore the importance of quantifying the within- and between-worker sources of variation in exposure to workplace contaminants, especially given the implications of variability when designing sampling strategies, the results of which can be used either for the control of hazards in the workplace or for epidemiologic studies of occupational cohorts.

Study 2: Inter- and Intra-Individual Sources of Variation in Levels of Urinary Styrene Metabolites (Symanski et al., 2001a)

Information on intra- and inter-individual sources of variation in biomarkers of exposure is generally lacking in the literature and this was the first study, to our knowledge, that quantified the sources of variability of urinary levels of mandelic acid (MA) and phenylglyoxalic acid (PGA). In our investigation, we found less variability in levels of PGA than MA, and for concentrations expressed in units of milligrams per gram creatinine. Since the smoothing of variability in airborne exposures depends upon the contaminant's half-life in the body (Rappaport 1985), our results are consistent with the underlying kinetics of both compounds because of the slower rates of elimination for PGA compared to MA (Bond 1989). The greater variation of styrene metabolite levels expressed in units of milligrams per liter as compared to milligrams per gram creatinine is likely due to fluctuations in urinary flow rate in spot samples and confirms a similar finding reported in an investigation of biomonitoring data collected on choralkali-plant workers (Symanski et al., 2000). While expressing metabolite levels in terms of urinary creatinine concentration typically decreased the intra-individual source of variation, it also tended to increase the inter-individual source of variation and thereby increased the total variation in some cases. These results are consistent with Alessio et al. (1985) who reported differences in urinary creatinine levels among individuals and may explain, in part, studies that reported no statistical advantage in evaluating metabolite levels as a function of creatinine (Sollenberg et al., 1988; Imbriani et al., 1990). On the other hand, expressing metabolite levels in terms of creatinine concentration normalizes data not only for urinary flow, but also for body mass and sex. Although this may introduce an additional source of measurement error and also increase inter-individual variation, it is fully justified on pathophysiologic grounds. Since the primary aim of biological monitoring (of exposure) is to obtain estimates of internal dose, some components of variation (e.g., errors in sampling and analysis) should be minimized, whereas others, such as interindividual differences in uptake, metabolism, and excretion, must be taken into account to properly interpret the biomonitoring data for assessing associated health risks (Mutti 1999).

In addition to variation from day-to-day, the intra-day variation in external levels is transmitted to variation in biological levels of the contaminant or its metabolite for compounds with relatively short half-lives. As such, post-shift samples are likely to be more sensitive to peaks in exposures experienced during the day, especially in the latter portion of the work shift. We found, however, that the magnitude of the intra-individual source of variation was generally less for measurements collected at the end, rather than at the beginning, of the workday. Although the greater variation in pre-shift samples may offer partial explanations for the weaker correlation between styrene exposure and urinary metabolite levels observed in morning samples as compared to end-shift samples (Ong et al., 1994), such results have not been consistent in all studies (De Rosa et al., 1988). Since the pre- and post-shift measurements in our study were not collected on consecutive days, it is also possible that the differences that we observed were due to dissimilarities in the tasks that workers performed from one sampling period to another. Moreover, there are likely other factors unrelated to the external exposure that contribute to the intra-individual variation in pre-shift urine samples, perhaps due to the greater analytical variability associated with the very low levels sometimes recorded in such samples. However, more work is warranted before a satisfactory explanation can be provided.

Measurement error operates to bias regression coefficients towards zero when a simple linear model adequately describes an exposure-response relation (Thomas et al., 1993). When making comparisons, we found that pre-shift urine samples were less efficient than post-shift urine samples. This finding opens the question whether samples collected prior to the beginning of a work shift are the most reliable measure of styrene uptake as previously suggested (Droz and Guillemin,1983; Bartolucci et al., 1986; Guillemin and Berode, 1988; Pekari et al., 1993). Although our data indicate that a single measurement may be unreliable as a measure of long-term exposure in epidemiologic studies, the bias in an observed regression coefficient would be greatly diminished if a second or third urine sample were collected. The expected benefits, however, lessen thereafter with increasing sample size.

While there were little differences in the total variation of MA+PGA levels (mg/g creatinine) in two of the plants involved in our study, a similar sampling strategy (in terms of the number of measurements collected on each worker) would produce inconsistent regression results should such data be used to evaluate workers' exposures at both plants. The inconsistency would arise because of differences in the relative magnitude of the intra- to inter-individual sources of variation between the two groups of workers, thereby making it difficult to compare the regression results obtained for each group and suggesting that important errors of inference can be made as a result of measurement error. This result underscores the importance of not only quantifying the total variation in biomarker levels, which is routinely done in most studies, but also quantifying the sources of variation between and within workers.

To explore the possibility that measurements collected closer together may have been more highly correlated than those farther apart in time, we evaluated serial correlation in data collected at one plant whose workers often provided multiple post-shift urine samples over the course of several days. Moderate to substantial levels of serial correlation were detected. Our findings also suggested that ignoring serial correlation results in an underestimation of the intra-individual variance component, which is a well-known consequence of positively autocorrelated processes (Diggle 1990), and an overestimation of the inter-individual variance component. While these differences were modest, the potential for serial correlation in biological monitoring data should be evaluated to avoid problems associated with mis-specified models and possible errors of inference.

Given that the occupational group may distinguish workers based upon what work is performed and where tasks are carried out, it serves as a surrogate for various determinants of exposure and can easily be evaluated in mixed models to assess the combined effects of 'job' upon exposure (Rappaport et al. 1999). In this study, we classified job titles or the primary work tasks of individuals into three broad occupational groups (laminators, non-laminators, and unknown) to investigate the effect of styrene exposure on levels of urinary metabolites. Consistent with other studies (Galassi et al. 1993), our findings indicate that there are significant differences based on work tasks, which explained moderate to substantial portions of the variation in styrene metabolite levels among workers. Our data also confirm previous findings (Sollenberg et al 1988; Imbriani et al. 1990; Marhuenda et al. 1997) of higher styrene metabolite levels towards the end of the workweek. Thus, information about the timing of sampling should be ascertained when evaluating differences in metabolite levels from one time period to another or between different groups of workers to insure that valid comparisons are made.

While the information provided in this study may prove useful to other investigators when designing prospective sampling strategies to evaluate exposures to styrene in the reinforced-plastics industry, estimates of the variance components may be unique for different worker populations because the degree of variability in biological measures of exposure depends on the magnitude of variation in external levels of the contaminant, physiologic differences among the workers under investigation, and on sampling and assay variability. Thus, we encourage other investigators to carry out studies to quantify intra- and inter-individual differences in biomarker levels among exposed workers. In instances when routine biological measurements do not provide suitable data for analysis, biological-monitoring strategies should be developed to collect repeated measurements on representative workers so that the sources of variation in exposure can be assessed. Such an assessment would provide useful information to optimize the design of prospective studies and allow for the collection of sufficient data to reliably estimate workers' exposures when evaluating health risks associated with occupational contaminants.

Study 3: Variability in Airborne and Biological Measures of Exposure to Mercury in the Chloralkali Industry: Implications for Epidemiologic Studies (Symanski et al., 2000)

Effects related to intra-individual variation have long been recognized in the statistical and epidemiologic literature (Cochran, 1968; Liu et al., 1978). However, the quantification of the inter- and intra-individual sources of exposure variability in the occupational arena has focused primarily on airborne contaminant levels (Kromhout et al., 1993;Rappaport et al., 1993; Kumagai et al., 1996; Lagorio et al., 1997) and this study represents one of the few investigations that examined sources of variation in both airborne and biological monitoring data collected on the same group of workers. In assessing airborne mercury levels, our finding that a substantial percentage of the variability was due to day-to-day variation is in agreement with an investigation of variability in airborne contaminants across a broad cross-section of workplaces worldwide (Kromhout et al., 1993). Our results also confirm that fluctuations of daily airborne mercury levels are smoothed in both the body burdens of mercury in blood, and to a greater extent, to that in urine. Given that the damping of variability in airborne exposures is highly dependent upon the contaminant's half-life in the body (Rappaport, 1985), these results are consistent with the underlying kinetics of mercury in blood and in urine, with slow elimination phases of several weeks and two to three months (Sällsten et al., 1993; Sällsten et al., 1994; Roels et al., 1991), respectively.

The proportion of the intra-individual variability to the total variance generally decreased in levels of mercury in blood or urine when compared to airborne mercury levels. A notable exception was the group of shift workers for which the percentage of variation attributable to intra-individual variability was higher in biological levels (especially in blood mercury) compared to airborne levels. In this group, the geometric mean levels of blood mercury (reflecting both inorganic and organic mercury exposure) was 18 nmol/l, which is only slightly higher than that found in the general Swedish population (Barregård, 1993; Langworth et al., 1991). It is likely that the greater intra-individual variation relative to the total variability in blood mercury levels in shift workers is due to fluctuations in exposures from non-occupational sources (primarily from contaminated fish and amalgam fillings) (Langworth et al., 1991), which play a bigger role in influencing body burdens of contaminants when workplace exposures are low.

Relying on quantitative estimates of the intra- and inter-individual sources of variation in exposure to mercury as measured in the air, blood, and urine among workers at a Swedish chloralkali plant, we also evaluated effects on regression results should such data be used to examine long-term health effects associated with mercury exposure. Our results suggest that the underestimation of the regression coefficient can be substantial when limited numbers of measurements are collected (although the benefits of collecting additional measurements diminish with increasing sample size). While requisite sample sizes are not the only factor to consider when evaluating exposure measures, estimating the distribution of measurement errors and quantifying differences among measures provides invaluable information that can be used to plan future investigations..

Based on kinetic considerations alone, urinary mercury may be deemed a superior measure relative to blood mercury since exposures are integrated over longer periods (Roels et al., 1991). Yet, our results for the entire group of chloralkali plant workers indicate that similar numbers of measurements would be required should blood or uncorrected urinary mercury be used to estimate individual workers' mean levels in a regression analysis. Since variations in urinary flow rate (e.g., due to variable water intake) increases the variability in urinary mercury concentrations in spot samples (Droz et al., 1991), creatinine-corrected urinary mercury produced less variable results and, thus, yielded the expected benefits when compared to mercury in blood. Nevertheless, in situations when the primary aim of biological monitoring is to detect temporary peak exposures, rather than assess the long-term body burden of mercury, mercury in blood would be a superior measure, owing to the damping of such peaks in urinary levels.

Whether biological monitoring offers advantages when compared to airborne monitoring depends upon kinetic factors, as well as upon the relative magnitude of the inter- and intra-individual sources of variation in each exposure measure. It is interesting to note that Rappaport et al. (1995) found that airborne measures of styrene yielded the least biased measure when compared to measurements of styrene in exhaled air among boat-manufacturing workers whereas one of the biological measures of exposure performed the most efficiently in our study. In any case, our investigation demonstrates that quantitative information about intra- and inter-individual sources of variation in exposure can be used to select optimal exposure measures when evaluating health risks associated with workplace or environmental contaminants.

Study 4: Heterogeneity in Sources of Exposure Variability among Groups of Workers Exposed to Inorganic Mercury (Symanski et al., 2001b)

The application of random- and mixed-effects models to evaluate sources of variation in exposure to workplace contaminants is growing in the occupational arena. One of the distinctive characteristics of random-effects models is that they accommodate the correlation among measurements collected on the same individual or in the same location (Symanski et al., 2001c). Mixed-effects models provide an additional advantage because they can be used to evaluate determinants of exposure (e.g., effects due to type of work, ventilation controls, or changes in the process), while incorporating the covariation among certain measurements.

In the application of random- or mixed-effects models to data collected on workers from several occupational groups, it is statistically advantageous to pool information across groups because more precise estimates of the variance components are obtained, which in turn lead to smaller standard errors associated with the fixed effects (Sullivan et al., 1999). Decisions to pool data should be based, in part, upon whether it is reasonable to expect that the degree of variation among measurements is similar across groups. While qualitative evaluations regarding likely differences in the magnitude of variability between and within workers across groups represent a useful first step, the statistical methods applied in this study provided a more rigorous approach in making such an evaluation. For the biological exposure indices, our findings indicate that groups did not share common levels of variability. Thus, it would not be appropriate to pool the urinary or blood mercury data to generate single estimates of the within- and between-worker variances. Based on these findings, studies should be conducted to determine whether it is reasonable to assume common variances and covariances among measurements collected on different occupational groups before applying models that make assumptions about homogeneity in the degree of variation within and between all workers.

Valuable information is contained in the variance components, which can be used to evaluate the utility of different grouping schemes, assess the bias in measures of effect in health effects studies, and estimate probabilities that exposures exceed occupational exposure limits. However, important errors can be made when assumptions regarding homogeneity in the degree of variation within and between workers across groups are not met. To illustrate effects related to the particular specification of the variance-covariance structure in a mixed-model, we calculated the probabilities that workers were exposed at levels exceeding occupational exposure limits using the results obtained from models that assumed common or distinct within- and between-worker variances. While exposures are generally below acceptable levels for all groups except for maintenance workers, we detected moderate to large differences in the exceedance and overexposure probabilities across models in some cases. Such differences are of little consequence when the probabilities are low (<< 5%), but could become more important when values begin to fall within the range of unacceptable levels. For airborne mercury, it is interesting to note that the estimated probabilities of overexposure compared to the probabilities of exceedance were lower for shift workers, production workers, and non-cell hall workers, but considerably higher for maintenance workers. These results confirm previous findings that the probability that a randomly-collected measurement exceeds an exposure limit compared to the probability that the mean exposure for a randomly-selected worker exceeds that same limit may not be equal, and that the exceedance probability is not necessarily higher than the probability of overexposure (Tornero-Velez et al., 1997).

Focusing on maintenance workers, important questions are raised regarding the equivocal conclusions that would be drawn based on the three exposure indices. For air mercury, the probabilities of exceedance (which ranged from 38 to 42% depending on the model that was applied) and overexposure (which ranged from 53 to 75%) were considerably higher than the corresponding values for blood or urine mercury. Given that air measurements were commonly collected in 2- or 3-day campaigns, it is possible that worst-case exposures were targeted and that the air-monitoring data are not representative of the full-range of exposures experienced by workers. The possible lack of representative data point, once again, to the limited utility of biased data in making meaningful statements about exposure (Symanski et al., 1998). In comparison to the air-monitoring program, however, the blood and urinary samples were collected routinely on nearly the entire workforce over the 10-year period. Yet, the exceedance and overexposure probabilities in maintenance workers were two to four times greater for urine mercury than blood mercury. These differences could be partly explained by the fact that the average ratio between urine mercury and blood mercury in reality is higher than the ratio between limit values given by the ACGIH (1999) (35 μg/g creatinine and 75 nmol/L, respectively). In establishing the biological exposure indices (BEI) for inorganic mercury in urine and blood, the ACGIH BEI committee stated that the recommended level for urinary mercury did not include a safety factor and that no significant health effects had been observed at a level of 75 nmol/L for blood mercury (BEI Committee, 1990). Moreover, they noted that urine to blood mercury ratios varied considerably across studies. Nevertheless, our results open the question as to which measure might be more suitable to evaluate whether exposure levels fall within an acceptable range. Owing to kinetic differences, peak airborne exposures are dampened in urinary mercury but more easily detected using blood mercury. A comparatively higher 'limit of exceedance' for blood mercury versus urine mercury could therefore be interpreted in light of the emphasis placed on average rather than peak exposures, in line with differences between shift-long and short-term exposure limits for air contaminants.

List of Publications

1. Symanski E, Sällsten G, Barregård L. Variability in airborne and biological measures of exposure to mercury in the chloralkali industry: Implications for epidemiologic studies. *Environ Health Perspect* 108:569-573 (2000).

2. Symanski E, Bergamaschi E, Mutti A. Inter- and intra-individual sources of variation in levels of urinary styrene metabolites, *Int Arch Environ Occup Health.* 74:336-344 (2001a).

3. Symanski E, Sällsten G, Chan W, Barregård L. Heterogeneity in sources of exposure variability among groups of workers exposed to inorganic mercury, *Ann occup Hyg* 45:677-687 (2001b).

4. Symanski E and Greeson NMH. Assessment of Variability in Biomonitoring Data Using a Large Database of Biological Measures of Exposure, *Am Ind Hyg Assoc J*, submitted, 2001.

Explanation of How Publications Relate to the Aims of the Project

Specific Aim #1 - Develop a database of repeated biological measures of exposure. If environmental monitoring (personal sampling) had also been conducted, compile airborne measurements as well.

In all four studies, biological monitoring data were compiled. In study #1 (Symanski and Greeson, submitted), over 4,000 biological measurements collected on 577 workers in 55 workplaces were compiled, which represents a wide range of biomarkers collected in blood, urine, and exhaled air. In this study, personal sampling data were compiled when reported in the published literature. The air-monitoring database contains 1,847 measurements collected on 192 workers. In study #2 (Symanski et al., 2001a), a total of 1,714 measurements of mandelic acid and phenylglyoxalic acid collected on 331 workers from eight reinforced-plastics plants were compiled. Personal samples were not routinely collected at any of the reinforced plastics plants. Both air and biological monitoring had been conducted at the chloralkali plant under investigation in studies #3 and #4. In study #3 (Symanski et al., 2000), 282 air mercury measurements, 646 blood mercury measurements, and 955 urinary mercury measurements were compiled. In study #4 (Symanski et al., 2001b), the period of investigation was extended over a longer time interval and additional data were added to the database to include a total of 325 airborne mercury measurements, 847 blood mercury measurements and 1,165 urinary mercury measurements.

Specific Aim #2 - Characterize the intra- and inter-individual variation of biological measures taking into account serial correlation.

In all four studies, the intra- and inter-individual sources of variation in the biological monitoring data were evaluated. In study #1, two different patterns of correlation between measurements collected on the same individual were considered. In the first model, it was assumed that the correlation between measurements collected on the same worker was the same irrespective of the time interval separating them (i.e., a compound symmetric error structure was assumed). In a second model, a first-order autoregressive error structure [AR(1)] was assumed, which models the correlation between measurements collected on the same individual as a function of the interval separating them. In an AR(1) model, the correlation function decays exponentially as the interval between measurements increases. To identify data that were possibly serially correlated, the average interval between all pairs of measurements collected on the same worker was calculated for each data set. A random-effects model with an AR(1) error structure was then applied to data sets which met the following criteria: 1) biological monitoring of five or more workers had occurred on a minimum of five occasions and 2) the average interval between measurements (computed first by individual and then across all workers in the group) was less than the estimated half-life for the biomarker. The results were reported in the manuscript describing this study, which has been submitted for publication.

In study #2, there was likely to be little serial correlation among repeated measurements because the majority of the data were separated by intervals of months or longer relative to the short elimination half-lives of less than 24 hours for both mandelic acid (MA) and phenylglyoxalic acid (PGA). However, in one of the plants (the plant involved in the manufacture of silos and containers) workers often provided multiple post-shift urine samples over the course of several days. Thus, it was possible that measurements that were collected closer together were more highly correlated than those farther apart in time. To explore this possibility, we applied a mixed-effects model with an AR(1) error structure. The results from the model with an AR(1) error structure for MA, PGA, and the sum of both metabolites were presented in the manuscript describing this study (Symanski et al., 2001a).

In the study investigating inorganic mercury exposures at the chloralkali plant (study #3), we computed the interval between measurements for each individual and found that only 10 percent of the urinary data were less than four months apart. Likewise, only one percent of the blood measurements were collected at intervals of one month or less. Thus, the biological monitoring data were unlikely to be autocorrelated and we chose to apply the more parsimonious model when evaluating the intra- and inter-individual sources of variation in exposure (Symanski et al., 2000). In study #4 (Symanski et al., 2001b), which also relied on the air and biological monitoring data that had been collected on workers at the chloralkali plant, we made the same assumption that the covariance between measurements collected on the same worker was the same regardless of the interval separating them.

Specific Aim #3 - Evaluate the assumption of stationarity in the underlying body burden distribution. Account for the presence of trends, if detected, when evaluating sources of variation in biological measures of exposure.

In study #1 (Symanski and Greeson, submitted, 2001), stationarity in the mean exposure levels over the period during which workers were monitored was assessed by visually inspecting the plots of the concentration values versus time for sets of data collected over three or more time points. In addition, the original studies were reviewed to determine whether evidence was provided that exposure levels had changed over time. Based on both assessments, the majority of the data appeared to be stationary. In 17 cases where exposure levels may have changed over time, such changes could not be formally evaluated in the models that were applied because of insufficient data.

In study #2 (Symanski et al., 2001a), one of the plants (the plant involved in the manufacture of silos and containers) reported a decrease in production levels from 1992 onwards. Thus, a mixed-effects model was applied to investigate systematic changes in metabolite levels between the two monitoring periods (1986-91 and 1992-99). No changes in the workplace were reported at the other facilities during the period over which monitoring data had been collected and inspection of the time plots revealed no discernible trends or shifts in metabolite levels. Thus, we reported on the results from the mixed model for the plant noted above and on the results from the one-way random-effects model (which assumed that the mean exposure level remained constant over time) for data collected at the other seven plants.

In study #3 (Symanski et al., 2000), temporal effects were examined by visually inspecting graphs of the annual mean levels for the airborne and biological monitoring data collected over the period 1990-97. While the airborne mercury levels appeared to fluctuate erratically above and below the mean value for the entire period, a shift in exposure levels in 1994 was apparent for the biological monitoring data that was likely due to a change in laboratory for the biological samples that occurred in June of 1994. Thus, a systematic change in the urinary and blood mercury levels was evaluated when sources of exposure variability were examined.

In study #4 (Symanski et al., 2001b), scatter plots of the annual mean levels of the natural logarithms of the data were inspected. While no trends were apparent in the air monitoring data, there was a downward shift in urinary and blood mercury levels in 1994 as noted in Symanski et al., 2000. Because this downward shift in biological levels made it difficult to discern whether trends were present over the entire monitoring period, time trends were formally evaluated in the mixed-models that were applied. While trends in exposure were not detected in either the air or blood Hg data, urinary mercury levels declined slightly ($p < 0.05$) at a rate of approximately 3% per year.

Specific Aim #4 - Assess the impact of intra- and inter-individual variation on the design of epidemiological studies using biomarkers as measures of exposure. Make comparisons, where possible, to the use of airborne measures of exposure.

To assess the influence of measurement error in biological measures of exposure, a hypothetical scenario was constructed in studies #2 and #3 (Symanski et al., 2001a; Symanski et al., 2001b) whereby it was assumed that estimates of average levels of the log-transformed biological measurements for each worker would be used to examine the relation with a continuous health outcome measure. It was further assumed that there were no other explanatory variables to consider as covariates in the linear model; as such, a simple linear regression model could be applied to examine the exposure-response relation. In the face of measurement error, the observed slope coefficient (under expectation) is smaller than the true coefficient and is a function of the magnitude of the within- and between worker variance components and the number of repeated measurements collected on each individual. Thus, it is possible to estimate samples sizes that would be necessary to minimize the attenuation of an observed slope coefficient to specified levels if information about the sources of variation is known or can be estimated. In study #2 (Symanski et al., 2001a), an evaluation of the estimated bias in an observed regression coefficient as a function of the number of measurements collected on each worker was made using the sum of both urinary metabolites of styrene in pre-shift samples as the exposure measure in all eight plants. In study #3 (Symanski et al., 2000), comparisons were made among all three measures of exposure (airborne, blood, and urinary mercury measurements). In general, our data indicate that a single measurement would be unreliable as a measure of long-term exposure in epidemiological studies and that the bias in an observed regression coefficient would be greatly diminished as the number of measurements collected on each worker increased. At the chloralkali plant, creatinine-corrected urinary mercury performed the most efficiently when compared to either airborne mercury or blood mercury.

Specific Aim #5 (revised) - Evaluate heterogeneity in the intra- inter-individual sources of variation in exposure across groups of workers employed at the same plant. Illustrate effects related to proper specification of the variance-covariance structure when random- or mixed-effects models are applied.

Preliminary analyses suggested that there was insufficient data to carry out specific aim #5 as originally proposed (in large part because of issues related to the timing of the personal and biological monitoring, small sample sizes, and the appropriateness of evaluating exposure-biomarker relations when the half-life of the biomarker is relatively long). Thus, a decision was made to take advantage of the large database that had been compiled in study #3 (Symanski et al., 2000) and evaluate the validity of exposure assessment strategies that combine data across groups of workers who perform similar job tasks in common locations. Critical to this approach have been assumptions that the degree of variation over time and among workers is the same for all groups. Yet, in only one investigation had this issue been examined, which was restricted to an assessment of air monitoring data collected on four groups of construction workers (Rappaport et al., 1999). Thus, we undertook study #4 to explicitly evaluate whether the within- and between-worker sources of variation were common among groups of workers exposed to inorganic mercury at a chloralkali plant. Our assessment was conducted using both air and biological monitoring data and relied on different models that specified job group as either a random or fixed effect. For air mercury levels, there was no evidence of significant heterogeneity in the levels of variation over time or between workers. For the biological monitoring data, however, our findings indicated that groups did not share common levels of variability and that it was not appropriate to pool the data and obtain single estimates of the within- and between-worker variance components. Classification of job group as a random or fixed effect yielded the same conclusion when the models were compared. To illustrate effects related to the proper specification of a model, the likelihood of exceeding certain levels (which is a function of the parameters of the underlying distribution of the natural log-transformed exposures) was evaluated using the results obtained from the different models. Although the probability that workers' mean exposures exceeded occupational exposure limits for air, urine and blood mercury was generally low (< 10%) for all groups except maintenance workers, the estimated values sometimes varied depending upon the particular model that was applied. While quantification of the intra- and inter-individual sources of variation in exposure provide valuable information that can be used to evaluate compliance with occupational exposure limits [or to assess the degree of bias that may be introduced in measures of effect when health-effects studies are carried out (Symanski et al., 2000; Symanski et al., 2001a)], such information is of limited utility if inappropriate models are applied.

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