Using Molecular Epidemiology in Assessing Exposure for Risk Assessment

P.A. SCHULTE[a] AND M. WATERS

National Institute for Occupational Safety and Health (NIOSH), Education and Information Division, Robert A. Taft Laboratories, Cincinnati, Ohio, USA

ABSTRACT: Quantitative estimation of health risks depends on exposure characterization, the nature of the dose response relationships, and the toxicity of the agents involved. The greatest uncertainties in risk assessment almost always arise from sparse or inadequate exposure data, inadequate understanding of exposure mechanisms, and insufficient understanding of the exposure-dose-response pathway. Additional sources of uncertainty arise when mixed or multiple exposures are implicated in the disease pathway, and as a result of variability in both exposures and responses within and between individuals. Here we consider the role of exposure assessment in the risk assessment process, the use of biological markers or molecular epidemiology to contribute to improvements in exposure assessment for risk assessment, and uncertainties associated with the use of biological markers.

INTRODUCTION

The classic risk assessment paradigm includes hazard identification, dose-response assessment, exposure assessment, and risk characterization.[4] Using molecular epidemiology for exposure measurement may contribute in various ways to these stages in the risk assessment process. The hazard identification stage involves the determination of any threat to human health any an agent might pose. Here there is a need to link an exposure with an outcome. In the exposure and dose-response assessment stages there is need to understand the specific effects that result from different exposures, particularly lower exposures. In the exposure assessment stage, the extent of exposure depends strongly on the agent and environment. This stage builds on the specific source-path-receiver model used during hazard identification. The source-path-receiver model is the common thread in linking source chemicals, the pathway of movement in the environment, and the route or routes of exposure of various receptors—in this case individuals or groups of individuals.[3] Critical issues in exposure assessment include characterization of the magnitude, frequency, and duration of exposure; the basis for the assessment; and the identification of highly exposed subgroups. The risk characterization stage eventually results in identification of *acceptable* and *unacceptable* levels of exposures. This requires an explanation of any assumptions and models used, together with discussion of uncertainty.

[a]Address for correspondence: Paul A. Schulte, Ph.D., National Institute for Occupational Safety and Health (NIOSH), Education and Information Division, Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, OH 45226, USA. 513/533-8481 (voice); 513/533-8588 (fax).
e-mail:pas4@cdc/gov

What role can molecular epidemiology play in the process of evaluating exposure and attendant uncertainties? This is the question that will be addressed in this paper. Molecular epidemiology is a term used for the incorporation of molecular, cellular, and physiologic biological markers (biomarkers) as dependent and independent variables in epidemiologic explorations of relationships between markers with either health outcomes or other markers within populations.[5] However, some commentators inappropriately apply these terms to a range of endeavors that use molecular measurements on people, rather than limiting the term to studies of the distribution of determinants of health effects within populations. In this discussion, we also consider using molecular and other biomarkers to serve as indicators of exposure. There is a growing body of literature to suggest that these exposure biomarkers can supplement traditional exposure assessment methods, and thus possibly make a contribution to the risk assessment process by reducing uncertainty in exposure assessment.[5–10]

HAZARD IDENTIFICATION

The role of molecular biomarkers and molecular epidemiology in hazard identification can be illustrated by the following examples. To determine whether a xenobiotic is hazardous or not, biomarkers may be used to increase the accuracy of approaches based on less sensitive measures of exposure; for example, the use of job titles as exposure proxies. Similarly, in situations where exposures occur that are variable or intermittent, and the effect of exposure is integrated, cumulative exposure biomarkers might be useful. A molecular epidemiology approach may clarify the exposure-outcome relationship better than classical methods, as a result of reduced exposure measurement error. For example, the role of aflatoxin exposure and liver cancer was not clear when studied by using a dietary questionnaire to assess intake of foods, that were potentially contaminated with aflatoxin. However, a strong association was observed by means of urinary biomarkers (metabolites and DNA adducts) of aflatoxin exposure[6,11] (see TABLE 1). In this example the molecular biomarker is useful because it is quite specific. It provides a better indicator of exposure than can be inferred from use of a questionnaire, since respondents are not aware of how much aflatoxin they consume. One might think that direct measurement of the aflatoxin component of all foodstuffs ingested, and measurement of amounts of food intake per day, could also lead to a better measure of exposure than the questionnaire surrogate. In the case of aflatoxin, this is probably not true due to the difficulty of measuring food intake, the possible variability of aflatoxin levels within food, the difficulty of extracting aflatoxin from foods, and analytical detection limits for such methods. However, for some other agents, external direct measures of exposure may be feasible, as cost effective as biological measures, and also provide improved estimates over such surrogates as questionnaire data. Qualitative tests may be used to determine whether external exposure or an exposure biomarker would offer the best predictor for disease.[12] One test is to determine if the biomarker is more highly correlated with the disease than external exposure. A second conditional test is to determine if, given the same level of exposure, those with higher levels of the biomarkers are more likely to develop the disease.

TABLE 1. Comparison between exposure data derived from questionnaires and data obtained by using biomarkers[a]

A. Relative risks based on dietary intake of aflatoxin		
Dietary aflatoxin B_1 exposure (μg/yr)	Relative risk	95% Confidence interval
<71	1.0	
71–113	1.6	0.8, 3.1
113+	0.9	0.4, 1.9
B. Relative risks based on urinary biomarkers of aflatoxin		
Biomarker	Relative risk (present/absent)	95% Confidence interval
metabolites or adducts	5.0	2.1, 11.8
aflatoxin M_1 with adduct	16.1	3.6, 72.5

[a]Study of aflatoxin and risk of liver cancer in Shanghai, China from 1986 to 1992 (55 cases).
SOURCE: Qian *et al.*, 1994; adapted from Howe, 1998.

The basic rationale for using biomarkers is that, in some cases, they can provide a more accurate method for assessing exposure and, ultimately, risk (see FIGURE 1). This is accomplished by reducing exposure misclassification by markers for biologically effective dose, and also by incorporation of exposure and host factors (e.g., metabolic capabilities) to the same marker. Although use of biomarkers can reduce misclassification, it is also possible that measurement error in the biomarker may contribute to bias in the measure of association.[7,9] Such errors can be evaluated and their impact adjusted for, but on balance they are better avoided or, at least, minimized by good laboratory and epidemiologic practices.

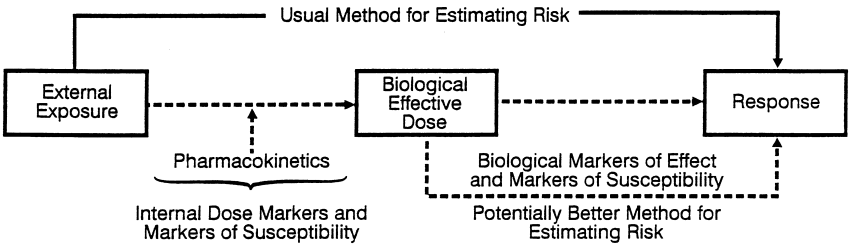

FIGURE 1. Rationale for using biomarkers to assess risk. (Adapted from numerous presentations at EPA and FDA in the 1980s.)

DOSE RESPONSE

In risk assessment the ascertainment of a dose-response relationship is crucial in ultimately determining the shape of the curve and for selecting a no-observed-adverse-effect level (NOAEL). Biomarkers for exposure can be used as indicators for dose, which can then be assessed against classical measures of morbidity or mortality. Another use of biomarkers is as outcome measures that correlate with exposure. In this case exposure markers are not what are needed; rather, the need is for effect markers. Effect markers are markers that relate to or predict disease. A marker that has been validated to predict disease can be applied to a surrogate for disease. For example, specific types of chromosomal aberrations that appear to predict cancer risk on a group basis can be used as the outcome variables in a dose-response analysis, as in the case of ionizing radiation exposure.[13]

Before a biomarker is useful in risk assessment it needs to be validated, that is, the relationship between the biomarker and what it represents needs to be established. Biomarkers can depict exposure, effect, or susceptibility. The process of biomarker validation of has been described, and it includes a laboratory phase and a population phase.[14] Molecular epidemiology is practiced in the field phase.

EXPOSURE ASSESSMENT FOR RISK ASSESSMENT

The exposure assessment component of risk assessment includes the consideration of such issues as representativeness of exposure measurements for a population, differences in exposures within and between individuals, individual differences in uptake and biotransformation, identification of factors that control or modify exposures, exposure estimation methods that are applicable in the absence of direct measurements, and identification of the most relevant dose metric for the agent under consideration. Biomarkers are most broadly defined to include markers for exposure, intermediate endpoints, host susceptibility to disease, and early clinical effects. The use of biomarkers in assessing exposure for risk assessment is usually limited to the first two of these, but increasingly may include the consideration of susceptibility factors in conjunction with exposure factors, for example, the presence of a specific genetic polymorphism for a metabolic enzyme.[15]

Quite often epidemiologic studies employ exposure surrogates rather than direct measurement of exposure. For environmental studies, surrogates might include geographic location, such as residence for a drinking water or air pollution study, age of housing in studies of lead-based paint exposures, or proximity of residence to electrical power lines. When direct measurements are not available or are limited, occupational studies use surrogates such as job title, job group, years worked at a plant, pounds of pesticide applied per week, and tasks performed.[16,17] The use of quantified direct measurements of personal exposure can lower uncertainty in the risk assessment process considerably in comparison with the use of such exposure surrogates.

EXPOSURE BIOMARKERS

The validation of biomarkers for exposure requires equal attention in assessing both the exposure and the biomarkers so that a fair comparison can be made. However, the relationship between the biomarkers and exposure varies due to host factors, as the biomarkers are further removed from the exposure depending on the number of steps in the absorption, metabolism, and clearance pathways between uptake and the specific biomarker. This applies to any form of exposure, due to intervening host factors that vary between individuals, such as breathing rate and capacity, activation, detoxification, elimination, and DNA repair. A high correlation between exposure and marker may not always be observed and an exposure-response relationship may vary between people. Therefore, it is important to identify and adjust for factors that can influence an exposure-response relationship. For example, to validate hydroxy-ethyl hemoglobin adducts as exposure biomarkers for ethylene oxide at low dose, we adjusted for age, smoking, and education in a linear regression model.[18] It may also be useful to adjust for genetic effect modifying factors.

EFFECT BIOMARKERS

Biomarkers for intermediate effects, that is, effects that are observed between exposure and disease, can be validated in case-control studies and cohort studies.[6,19,20] Once validated, these markers can serve as surrogates for disease, albeit on a probabilistic basis since generally not all people with a given biomarker will develop the disease, but the groups with high levels will generally be at greatest risk. This has been observed for both chromosomal aberrations and cancer.[21]

Other potentially useful effect markers are found in the spectra of mutations, such as in p53, since these show a unique fingerprint with a given exposure.[22,23] Most of the epidemiology studies of workers involving p53 mutations, although purporting to identify a predominant mutational spectrum, do not include one that occurred in more than 50% of the cases with purported common exposures.[22,23] This may be due to exposure misclassification but also may be due the failure to account for other important pathways not involving p53.

SUSCEPTIBILITY BIOMARKERS

Perhaps the greatest potential contributions of molecular epidemiology to risk assessment and risk management can be found in the inclusion of inherited susceptibility biomarkers.[15] Susceptibility biomarkers can influence exposure effects and are, therefore, important to consider in risk assessments. These biomarkers, offer both promises and perils for individual and population risk estimation. The promises are for a more refined assessment of risk through the identification of gene-gene and gene-environment interactions, and also for focusing prevention and control programs on high risk individuals. The perils include ethical and social issues, including stigmatization, discrimination, and the misconception that removing a susceptible person from the exposure scenario without reducing exposure opportunities reduces

risk whereas this may not be so, on a comparative basis.[25] There are also issues in using susceptibility markers as effect modifiers in epidemiology studies. These include misclassification of a genotype due to various technical flaws, such as the failure to recognize a variant that contributes to the genotype.[26]

One of the most widely known examples of how a susceptibility biomarker, combined with an exposure measure, can give information about risk assessment is the metabolic polymorphism for N-acetyltransferase in the case of bladder cancer where arylamine-exposed slow acetylators have a much higher risk of bladder cancer than fast acetylators.[27–29]

Calabrese demonstrated that genetically-determined biochemical differences between people for a range of phenotypes could exceed 10-fold.[30] In a pioneering study, Bois *et al.,* by modeling of DNA adducts in the bladder of people exposed to 4-aminobiphenyl, illustrated that the adduct levels of the most susceptible individuals are 10,000 times higher than those for the least susceptible and that the 5th and 95th percentiles differ by a factor of 160.[29] Therefore, accounting for genetic variability may have important implications for risk assessment.

When these genetic polymorphic pathways represent major routes of elimination it is important that this fact be included in risk assessments.[31] Determination of the population prevalence of alleles for metabolic polymorphisms involves molecular epidemiologic approaches and this is even more true when assessing gene-environment interactions.

LOW LEVELS OF EXPOSURE

The target area in the risk assessment process is usually the risk at low levels of exposure. This is where there is a lack of data and where projections of effects and risks are most needed. The capability exists to measure extremely low levels of exposure in many contemporary situations but not necessarily in historical cases. Futhermore, it is particularly important, in the case of low exposure levels, to understand the linkages between markers of exposure, metabolites or conjugation products, subclinical effects and frank disease, and the role of mediating or buffering factors on these markers.

When using biomarkers to assess exposure at low levels it is important to account for all sources of exposure. A dose marker generally integrates all routes and sources of exposure. Thus, for example, in the study of hemoglobin adducts and ethylene oxide, we had to account for smoking as a source of hydroxy-ethyl hemoglobin adducts, and also to account for endogenous sources.[18]

ADVANTAGES OF BIOMARKERS IN ASSESSING EXPOSURE FOR RISK ASSESSMENT

One advantage to using a biomarker for exposure instead of an environmental measure is that it accounts for individual differences in uptake. These differences may arise due either to actual differences in environmental exposure levels that are not easily identified among groups of people, or to differences in uptake and biotransformation between individuals. For example, since air concentrations of

chemicals are known to vary widely in many indoor and outdoor environments, individuals presumed to have the same exposure potential, may actually have different exposures. If this environmental variability is not characterized, as is often the case due to the cost associated with a greater number of environmental measurements, the contribution of this source of variability to uncertainty in assessing levels of exposure remains unknown. Uptake and metabolism of xenobiotic chemical agents may also differ widely between individuals. The kinetics and efficiency associated with each step in the relevant pathway from absorption across a dermal, inhalation, or ingestion barrier to dose at the target organ and hence to measurable response in a molecule or tissue, may vary widely between individuals and again lead to uncertainty in the estimated exposure. Measuring the biologically effective dose at the target tissue or receptor for each individual, when this is possible, minimizes these sources of uncertainty.

Biomarkers also obviate the need to estimate the effects of exposure-modifying external factors, such as the use of personal protective equipment or personal hygiene, on environmental exposure levels. Typically, environmental characterization of exposures does not incorporate these factors directly, and thus their efficacy must be estimated. This estimation process is often not validated and, therefore, makes an unknown contribution to the risk assessment process.

When multiple agents may lead to changes in the same biomarker level, the use of a biomarker may be more useful than an external measure of exposure, since the biomarker is a summary measure of exposure to all agents that are biologically processed in a similar manner. For example, trichloroacetic acid is a measurable intermediate in the biotransformation of trichloroethane, trichloroethylene, and tetrachloroethylene exposures.

When variations of biomarker levels within and between individuals are less than the variability of environmental measurements, biomarkers offer the advantage of requiring fewer measurements to estimate exposures than external measures. This dampening of variability has been demonstrated for blood lead levels.[32]

LIMITATIONS OF BIOMARKERS IN ASSESSING EXPOSURE FOR RISK ASSESSMENT

Biomarkers are most useful in assessing exposures when there is a high correlation between environmental measures of individual exposures and the biomarker, and when the variability of the biomarker is less than that of environmental exposure measures.[33,34] The data requirements to demonstrate this are not trivial, however.

The relevant time frame must be identified when using biomarkers for exposure assessment. This requires an understanding of the uptake and absorption, distribution, biotransformation, repair and clearance processes, and of the target receptors. Long-lived xenobiotics or their metabolic products may accumulate in a tissue compartment and provide a measure of long-term exposure. Examples include PCBs and DDT in adipose tissue. Similarly, certain types of chromosomal aberrations due to ionizing radiation are persistent and cumulative; they are measured years after exposure.[35] The kinetics of uptake, biotransformation, distribution, and clearance must be understood in order to identify the relevant time period for biomarker collection and to interpret biomarker data. The time between exposure and sample collection is

of greater importance in cases where repair or clearance processes dominate, such as n-hexane in alveolar air, or radiation exposure and certain types of DNA damage.

When variations in biomarker levels exceed variations in environmental exposure levels, due to interindividual differences in uptake, biotransformation, distribution of chemicals, or in efficiency of repair processes, biomarker data may be less useful than environmental levels in assessing exposure. In the case of styrene exposures and sister chromatid exchanges, the increased variability of SCEs between workers compared with the variability of full-shift personal exposures, indicates that the external measures require fewer samples to assess the exposure distributions.[32]

Specificity is a desirable characteristic for a biomarker. In the simplest case the biomarker is unique to the exposure. For example, metals in blood or urine, or a unique chemical-hemoglobin, or a chemical-DNA adduct in the case of electrophilic carcinogens, are unique to the causative agent just as the TB-tubercule is to tuberculosis infection. More commonly, multiple agents may produce the biomarker, and the possibility of confounding exposures, or a mixed exposure pattern, must be accounted for. The protein adduct of benzo(a)pyrene may be indicative of either smoking or foundry work exposures, among others. In the case of chromosomal aberrations, the effects of benzene, arsenic, or ionizing radiation on elevated levels of long-lived chromosomal aberrations may be confounded with concomitant exposures to cigarette smoke, or to dietary factors such as the use of diet sweeteners.[36]

Background levels of biomarkers in unexposed groups must be available in order to properly interpret biomarker levels in exposed individuals. Often, such background data are not available and then characterization of the baseline level distribution must be a component of the study.

FUTURE APPROACHES

On the horizon for use in epidemiology studies and risk assessments are high throughput DNA chip technologies to study gene functions and expression under various conditions.[37] These technologies will allow for evaluation of temporal and spatial patterns of gene expression under various exposure conditions. It will be possible to determine which genes are up- and which are down-regulated in response to exposures. The difficulties in using DNA chips include issues of data reduction, multiple comparisons, complex interaction assessments, and interpretation of results. Ideally, if these problems can be solved, it may be possible for the first time in history to evaluate changes in expression of many genes of an individual at the same time.[37] Cross-species comparison could be enhanced. This technology could lead to better characterization and understanding of disease processes and exposure-disease relationships, which would be of great assistance to risk assessors.

CONCLUSION

Molecular epidemiology approaches may be useful in assessing exposure in the various steps in risk assessment. These approaches, however, should be viewed only as another tool available for researchers and risk assessors, not as a replacement for

traditional measures such as environmental exposure assessment, job exposure matrices, questionnaires, and record reviews.

Molecular epidemiology data implies an understanding of the mechanism, and incorporation of mechanistic data into risk assessment methods is currently a laudable goal. However, risk assessments and regulations should not wait the development of mechanistic data,[1] nor should uncertainty about mechanism be used to block public health action. Conversely, if there is sufficient uncertainty about whether an agent truly causes disease, then imposing regulations may lead to inappropriate utilization of resources. This resource issue needs to be taken into account, but it should not be the deciding factor.

We have discussed how biological markers may have a use in risk assessment. We have not addressed the ethical, legal, or social implications of this use; they have been discussed elsewhere.[38] Generally, biomarkers for exposure generate fewer of these issues than biomarkers for effect or susceptibility, because an acquired effect indicating disease risk may, in some ways, be considered similar to an inherited one. Nevertheless, the distinction between these categories is subjective and is often blurry. Care needs to be taken to guard against stigmatization, discrimination, and loss of opportunity that can result from use of biomarkers in research or practice.

REFERENCES

1. BECKING, G.C. 1995 Use of mechanistic information in risk assessment for toxic chemicals. Toxicol. Lett. **77**: 15–24.
2. MCCLELLAN, R.O. 1995. Risk assessment and biological mechanisms: lessons learned, future applications. Toxicology **102:** 239–258.
3. NELSON, D.I. 1997. Risk assessment in the workplace. *In* The Occupational Environment—Its Evaluation and Control, S. DiNardi, Ed.: 328–359. American Industrial Hygiene Association Press.
4. US NATIONAL RESEARCH COUNCIL (NRC). 1983.. Risk assessment in the Federal Government: Managing the Process. National Academy Press, Washington, D.C.
5. SCHULTE, P.A. & F.P. Perera. 1993. Molecular epidemiology: Principles and practices. Academic Press, San Diego.
6. HOWE, G.R. 1998. Practical uses of biomarkers in population studies. *In* Biomarkers: Medical and Workplace Applications. M.L. Mendelsohn, L.C. Mohr & J.P. Peeters, Eds.: 41–49. John Henry Press, Washington, DC.
7. WHITE, E. 1997. Effects of biomarker measurement error on epidemiological studies. *In* Application of Biomarkers in Cancer Epidemiology. P. Toniolo *et al.,* Eds.: 73–93. IARC Scientific Publication No. 142, Lyon.
8. HATTIS, D. & K. SILVER. 1993. Use of biomarkers in Risk assessment. *In* Molecular Epidemiology: Principles and Practices. P.A. Schulte & F.P. Perera, Eds.: 257–273. Academic Press, San Diego.
9. SARACCI, R. 1997. Comparing measurements if biomarkers with other measurements of exposure. *In* Application of Biomarkers in Cancer Epidemiology. P. Toniolo *et al.,* Eds.: 303–312. IARC Scientific Publication No 142, Lyon.
10. TIMBRELL, J.A. 1998. Biomarkers in toxicology. Toxicology **129:** 1–12.
11. QIAN, G.S., R.K. ROSS, M.C. YU, J.M. YUAN, Y.T. GAO, B.E. HENDERSON, G.N. WOGAN & J.D. GROOPMAN. 1994. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol. Biomarkers Prev. **3:** 3–10.
12. STEENLAND, K., J. TUCKER, & A. SALVAN. 1993. Problems in assessing the relative predictive value of internal markers versus external exposure in chronic disease epidemiology. Cancer Epidemiol. Biomarkers Prev. **2:** 487–491.

13. JOKSIC, G., & V. SPASOJEVIC-TISMA. 1998. Chromosome analysis of lymphocytes from radiation workers in the tritium-applying industry. Int. Arch. Occup. Environ. Health **71**: 213–220.
14. PERERA, F.P & L.A. MOONEY. 1993. The role of molecular epidemiology in cancer prevention. *In* Cancer Prevention. V.T. DeVita, S. Hellman & S.A. Rosenberg, Eds.: 1–15. J.B. Lippincott, Philadelphia.
15. YANG, Q. & M.J. KHOURY. 1997. Evolving methods in genetic epidemiology: III. Gene-environment interaction in epidemiologic research. Epidemiologic Reviews **19:** 33–43.
16. GOLDBERG, M. & D. HEMON. 1993. Occupational epidemiology and assessment of exposure. Int. J. Epidemiol. **22**(Suppl. 2): s5–s9.
17. STEWART, P.A., A. BLAIR, M. DOSEMICI *et al*. 1991. Collection of exposure data for retrospective occupational epidemiologic studies. Appl. Occup. Environ. Hygiene **6:** 280–289.
18. SCHULTE, P.A., M. BOENIGER, J.T. WALKER, S. SCHOBER, S.E. PERERA, D.K. GULATI, J.P. WOJCIECHOWSKI, A. GARZA, R. FROELICH, G. STRAUSS, W.E. HALPERIN, R. HERRICK & J. GRIFFITH. 1992. Biological markers in hospital workers exposed to low levels of ethylene oxide. Mutat. Res. **278:** 237–151.
19. MUNOZ, A. & S.J. GANGE. 1998. Methodological issues for biomarkers and intermediate outcomes in cohort studies. Epidemiologic Rev. **20:** 29–42.
20. ROTHMAN, N., W.F. STEWART & P.A.SCHULTE. 1995 Incorporating biomarkers into cancer epidemiology: a matrix of biomarker and study design categories. Cancer Epidemiol. Biomarkers Prev. **4:** 301–311.
21. HAGMAR, L., L. BROGGER, I.L. HANSTEEN, S. HEIM, B. HOGSTEDT, L. KNUDSEN, B. LAMBERT, K. LINNAINMAA, F. MITELMAN, I. NORDENSON, C. REUTERWALL, S. SALOMAA, S. SKERFVING & M. SORSA. 1994. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic Study Group on the Health Risk of Chromosome Damage. Cancer Res. **54:** 2919–2922.
22. NIGRO, J.M. & S.J. BAKER. 1989. Mutations in the p53 gene occur in diverse human tumour types. Nature **342:** 705–708.
23. HERNANDEZ-BOUSSARD, T.M. & P. HAINART. 1998. A specific spectrum of p53 mutations in lung cancer from smokers: review of mutations compiled in the IARC p 53 database. Env. Health Perspect. **106:** 385–391.
24. GREENBLATT, M., W. BENNETT, M. HOLLSTEIN & C. HARRIS. 1994. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathegenesis. Cancer Res. **54:** 4855–4878.
25. VINEIS, P. & P.A. SCHULTE. 1995. Scientific and ethical aspects of genetic screening of workers: the case of the N-acetyltranferase phenotype. J. Clin. Epidemiol. **48:** 189–197.
26. ROTHMAN, N., W.F. STEWART, N.E. CAPORASO & R.B. HAYES. 1993. Misclassification of genetic susceptibility biomarkers: implication for case-control studies and cross population comparisons. Cancer Epidemiol. Biomarkers Prev. **2:** 299–303.
27. CARTWRIGHT, R.A., R.W. GLASHAN, H.J. ROGERS *et al*. 1982. Role of N-acetyltransferase phenotype in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. Lancet **11**: 842–846.
28. TAYLOR, J.A., D.M. UMBACH, E. STEPHENS *et al*. 1998. The role of N-acetylation polymorphisms in smoking–associated bladder cancer: evidence of a gene–gene-exposure three-way interaction. Cancer Res. **58:** 3403–3610.
29. BOIS, F.Y., G. KROWECH & L. ZEISE. 1995. Modeling human interindividual variability in metabolism and risk: The example of 4-aminobiphenyl. Risk Analysis **15:** 205–213.

30. CALABRESE, E.J. 1997 Role of genetic factors in environmentally induced toxic response: historical consideration, present status and future directions. University of Massachusetts, Amherst.
31. RENWICK, A.G. & N.R. LAZARUS. 1998. Human variability and noncancer risk assessment-an analysis of the default uncertainty factor. Reg. Tox. Pharmacol. **27:** 3–20.
32. RAPPAPORT, S.M. 1985. Smoothing of exposure variability at the receptor: implications for health standards. Ann. Occup. Hyg. **29:** 201–214.
33. RAPPAPORT, S.M., E. SYMANSKI, J.W. YAGER & L.L. KUPPER. 1995. The relationship between environmental monitoring and biologic markers in exposure assessment. Env Health Perspectives **103**(Suppl. 3): 49–53.
34. BRUNEKREEF, B., D. NOY & P. CLAUSING. 1987. Variability of exposure measurements in environmental epidemiology. Am. J. Epidemiology **125:** 892–898.
35. TUCKER, J.D., E.J. TAWN, D. HOLDSWORTH, S. MORRIS *et al.* 1997 Biological dosimetry of radiation workers at the Sellafield nuclear facility. Radiation Res. **148:** 216–26.
36. RAMSEY, M.J., D.H. MOORE, J.F. BRINER *et al.* 1995. The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosomal painting. Mutation Research **338:** 95–106.
37. WOYCHIK, R.R., M.L. KLEBIG, M.J. JUSTICE, T.R. MAGNUSON & E.D. AVNER. 1998. Functional genomics in the post-genome era. Mut. Res. **400:** 3–14.
38. SCHULTE, P.A., D. HUNTER, N. ROTHMAN. 1997. Ethical and social issues in the use of biomarkers in epidemiological research. *In* Application of Biomarkers in Cancer Epidemiology. P. Toniolo, P. Boffetta, D.E.G. Shuker *et al.,* Eds.: 313–318. IARC Scientific Publications No. 142, Lyon.