Macromolecule Adducts as Biomarkers of Exposure to Environmental Mutagens in Human Populations

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A cancer epidemiologist recently said that "adduct measurement has so far been of little use to epidemiological research." This remark gives us a starting point for the discussion of the purposes of measuring macromolecule adducts that originate from electrophilic compounds or metabolites in humans and animals. Historically, methods for adduct monitoring were developed as a means of determining target doses that, combined with measurements of genotoxic potencies, could be used for risk assessment. With mass spectrometric methods, adducts can be quantified at levels that are thousands of times lower than those in which the cancer incidence associated with this exposure is detectable in disease–epidemiological studies. Furthermore, mass spectrometric techniques permit identification of the chemical structure of the adduct, particularly in the case of hemoglobin adducts. Adduct measurement therefore constitutes not only a means of risk estimation but it may be used as a complement of disease epidemiology in situations in which, for statistical reasons, the risk is too low to be detectable—which does not signify that the risk is acceptably low. It also gives a possibility of identification of the dangerous components in mixed exposures and of the relevant reactive intermediates in cases of complex metabolism. — Environ Health Perspect 104(Suppl 3):423–428 (1996)

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Introduction

The rationale of the use of biomarkers has often been misconceived. In particular, this concerns macromolecule adducts from genotoxic chemical agents, the most sensitive biomarkers originally suggested to be used for risk assessment. The measurement of adducts, particularly DNA adducts, is now very widespread. The results of these adduct studies are mostly limited to demonstrated differences between exposed and unexposed groups, with little contribution to quantitative risk assessment.

In this review, we will highlight the basic arguments for adduct measurement rather than procedures and results in the hope of stimulating future debate and development—a lot remains to be developed. In particular, the quantitative aspects of dose, dosimetry, and risk are emphasized, among other things, to show the usefulness as a subsidiary tool of adduct monitoring in epidemiological research and cancer risk assessment. We will show that disease–epidemiology has a detection level that is too high, often by orders of magnitude, to permit detection of cancer risks within the whole range of unacceptability. However, this gap can be bridged by the measurement of macromolecule adducts due to possibilities of relating adduct levels to exposure doses, *in vivo* doses, and risks. The establishment of these relationships is based on studies of dose response at low doses, calculation of doses from observed adduct levels, and determination of relative genotoxic potencies with low-LET (linear energy transfer) radiation or ethylene oxide as reference standards.

Linearity of Dose Response at Low Doses?

A central question in the risk philosophy concerns dose–response relationships, particularly the existence of no-effect thresholds. The genotoxic factor most intensely studied in this respect is ionizing radiation; it was indicated in the mid-1930s that the frequency of mutations induced by X rays in *Drosophila* depended linearly on the dose, without any indication of a threshold (*1*). Later, similar conclusions were drawn for genotoxic chemicals. Although it had been suggested earlier that changes in the genetic material played a role in the development of a tumor (*2*), it was not until the mid-1960s that it was more generally accepted that tumor induction, too, follows linear dose–response curves without thresholds (*3*). This issue is, however, still under debate. Proponents of the existence of thresholds refer to the multistep nature of carcinogenesis (*4*) and to the unequivocal appearance of thresholds in certain cases of experimentally induced tumors [for radiogenic cancer, see (*5*)].

This uncertainty, or contradiction, is settled by the demonstration of a (certain) proportionality between the incidence increments due to exposure and the background incidences of the different tumor types in the animal strain studied. This multiplicative character of the dose response was most convincingly shown by Storer et al. (*6*) in their systematic comparison of radiogenic tumors in mouse strains with different spectra of background tumors, but it is also valid for mutagenic chemicals such as ethylene oxide and butadiene [Ehrenberg et al., in preparation; (*7*)]

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Abbreviations used: LET, linear energy transfer; D, dose; C, concentration; Mhr, (mol/kg)×hr; *s*, Swain-Scott substrate constant; *n*, nucleophilic strength; EO, ethylene oxide; Hb, hemoglobin; SA, serum albumin; DEB, diepoxybutane; DDREF, dose and dose-rate effectiveness factor.

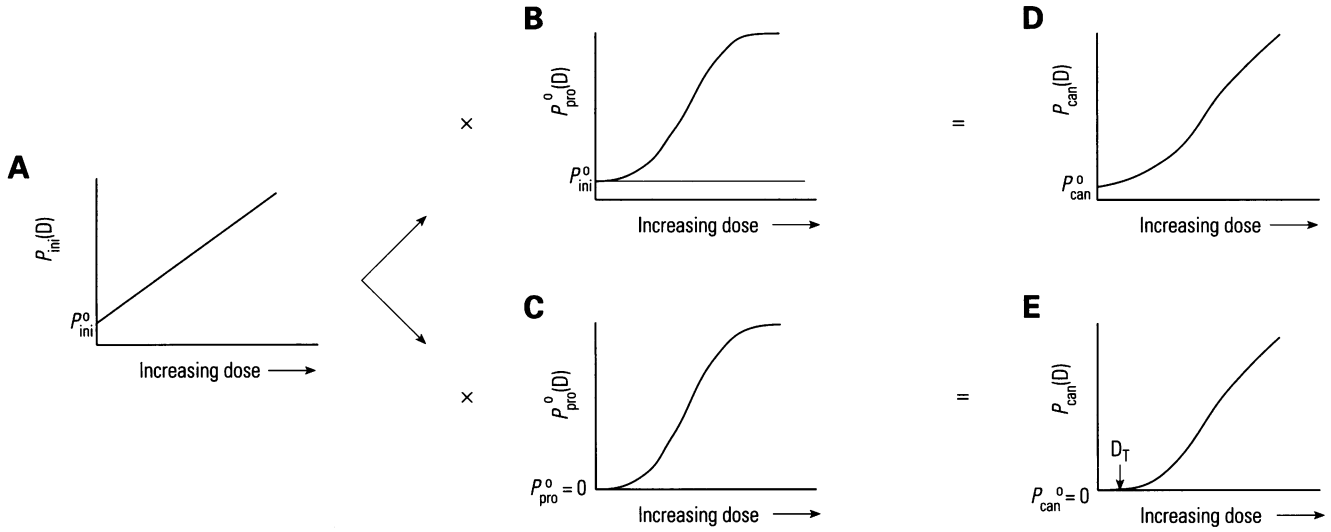

Figure 1. Dose–response relationship as a function of the dependence on interaction of mutation (initiation) and promotion according to $P_{ini} \times P_{pro} = P_{can}$. *A*, P_{ini} is shown as a linear function of dose; *B* and *C* show the two alternatives for P_{pro}: presence (*B*) or absence (*C*) of a background promotive condition, both with increased promotion above a threshold dose. The resulting increase in cancer incidence, $P_{can}(D)$ will be linear at low doses if $P^{o}_{pro} > 0$ (*D*) but will appear with a threshold (D_T) when $P^{o}_{pro} = 0$ (*E*).

Accordingly, a multiplicative model is now preferred for the description of dose–response relationships (*8*)

$$P(D) = (1 + \beta D) \times P^{o}, \qquad [1]$$

where D is the dose, β is a risk coefficient, and P and P^{o} are the estimated and the background probabilities, respectively.

In agreement with this picture, tumors of types that do not occur in a studied strain are not (or are seldom) inducible at low doses (*9*). These tumors do appear, however, at doses above a threshold, an effect ascribed to a promoter action of a complete carcinogen, i.e., a carcinogen with both initiating and promoting properties. Promoting properties are caused, for example, by reparative growth (wound healing) in tissue damaged at high doses. If exposure to the genotoxic agent (e.g., benzo[*a*]pyrene) is followed by treatment with an efficient promoter [e.g., 12-*O*-tetradecanoylphorbol-13-acetate (TPA)], the thresholded dose–response curve for treatment with the genotoxic agent alone becomes linearized (*10*).

The assumption of a role of mutation in carcinogenesis has received strong support by recent progress in biochemical genetics, which has shown that a number (~6) of mutations in oncogenes and, particularly, tumor suppressor genes are required for the change of a normal cell to a malignant cell (*11*). For statistical reasons it is expected that, at the low doses usually received by members of the public, a specific genotoxic factor is rarely able to cause more than one of the mutations required. This would be in line with a (linear) incidence increment due to exposure at current levels.

This model is summarized in Figure 1. In agreement with this model, human cancer incidence increments are compatible with a linear dependence on radiation dose (*8*).

It may be practical to subdivide doses (and corresponding dose rates) into very low, low, intermediate, and high (Figure 2). At low doses (dose rates), the dose dependence of the response is approximately linear. In the intermediate region, a rise in effectiveness due to increasing saturation of repair or detoxification is often observed; at high doses cell killing leads in many cases to a decrease of the response.

It should be stressed that, in all the cases of mutation frequencies and cancer incidences discussed so far, the causative doses, although low in the subdivision above, have been relatively high compared with current doses judged to be of concern to human health. For statistical reasons it is mostly impossible to obtain information about the true shape of the dose–response curves at very low doses and dose rates (region I in Figure 2). At doses leading to about one potentially mutagenic hit per cell (which might be defined as the upper limit of a virtually low dose), the concentration of repair enzymes will be higher than that of the substrate (repairable DNA damage). It is therefore expected that at very low doses, repair will be more efficient than if ordinary Michaelis-Menten kinetics were applied (*13*). However, in experiments specially designed to study dose response in this region, particularly with respect to the possible magnitude of a no-effect threshold, data agree either with linearity down to dose zero (curve a in Figure 2) or with a superlinear hump at the lowest doses (curve b in Figure 2) (*12,14*). This hump is explainable in terms of inducibility of error-free repair, with the effect that the lowest doses hit the material in an uninduced and therefore more sensitive condition. The low-dose limbs of these humps indicate the mutagenic effectiveness

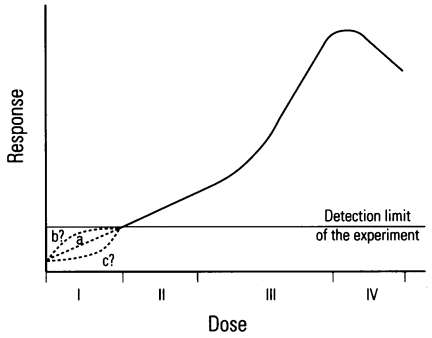

Figure 2. Dose–response relationships at low (region II), intermediate (III), and high doses (IV). At very low doses (I), information on the dose response cannot be obtained for statistical reasons. For this dose region, three alternative curve shapes have been indicated: a, linear; b, superlinear hump; c, tendency to no-effect threshold. Modified from Ehrenberg et al. (*12*).

to be a factor of 2 to 3 times higher than in the linear part of the curve at low to intermediate doses observed under usual experimental conditions. This factor may, for the time being, be considered an uncertainty that is not very large compared with other uncertainties in cancer risk estimation.

From kinetic points of view, dose rates (doses per unit of time) are low if there is no (measurable) saturation of repair and detoxification, i.e., with linear dose response for one-hit effects. At intermediate dose rates, such saturation leads to convex dose–response curves (*12*). If at very low dose rates, time intervals between doses—in some cases single hits—leading to induction of repair are longer than the persistence of the induced condition, dose–response curves could again show superlinear humps (*12*).

It can be concluded that frequencies of mutation and cancer induced by DNA-damaging (genotoxic) agents depend linearly on the dose at low doses. It is indicated that this rule is valid also at the very lowest doses. In principle, this means that any dose increment of a genotoxic agent leads to a corresponding risk increment.

Reaction-kinetic Aspects

Miller and Miller (*15*) showed that cancer initiators (and mutagens) are, or are metabolized to, electrophilically reactive agents that give rise to macromolecule adducts *in vivo*. Alkylating agents have been tentatively characterized in terms of the reaction-kinetic parameters of the Swain-Scott (*16*) free energy relationship

$$\log(k_n/k_{H_2O}) = s \times n, \qquad [2]$$

where k_n and k_{H_2O} are the second-order rate constants for reaction with an atom of nucleophilicity n or with water ($n = 0$), respectively, and s is the substrate or selectivity constant. (In this relationship the values of n for different compounds were determined from the reactivities toward methyl bromide, setting $s = 1$ for this compound.) The reaction rate k_n at any value n can be calculated from the first-order rate constant (k') for hydrolysis ($k' = k_{H_2O} \times 55.5$, where 55.5 is the molarity of water) and the value of s:

$$k_n = k_{H_2O} \times 10^{(s \times n)}. \qquad [3]$$

When Equation 3 was applied to monofunctional alkylating agents, it was found that the potency for eliciting forward mutation in *Escherichia coli* Sd-4 was approximately proportional to k_n where $n = 2$, if the compounds were compared at equal dose (*17*). Dose (D) has then to be defined as the time integral of concentration (C):

$$D = \int_t C(t)\,dt. \qquad [4]$$

Thus, defined dose will have the dimension concentration × time, e.g., millimole per kilogram × hr or millimolar-hr (mMhr) (*12*). The correlation of mutagenic potency with k_n (n=2) then implies a key role of the cumulative frequency of alkylations at $n = 2$, a value that would correspond to guanine-O^6 in DNA.

Compounds or ultimate alkylating species that are positively charged were found to be about 5 (range, 3–10) times more effectively genotoxic than predicted by the Swain-Scott principle. This concerns isopropyl methanesulfonate, which reacts according to S_N1 (nucleophilic substitution, first order) via the carbocation $(CH_3)_2CH^+$, and *N*-methyl-*N*-nitrosourea, which reacts via the diazonium ion $CH_3N_2^+$ (*18*). The rate of reaction of these positively charged species toward the polyanion DNA is enhanced due to electrostatic attraction. Ongoing work indicates that the model can be improved by allowing for influences of charge of the reactants.

Deviations that are different from the proportionality of genotoxic potency with k_n where $n = 2$ are represented by bifunctional agents such as diepoxybutane (DEB, one of the alkylating metabolites of butadiene) (*19*). In mammalian cells DEB is about 100 times more effective than expected from this proportionality rule. This is due to the formation of cross-links, partly involving the more reactive guanine-*N*-7 (*20*). This rule for relative potency has been shown for the effects of a large number of alkylators in various materials including mammalian cells, plant seeds, and yeast.

The experience from kinetic studies leads to the conclusion that the relative genotoxic potency of alkylating agents or metabolites can be assessed from a determination of hydrolysis rate (k_{H_2O}) and of s, if necessary, with correction for charge and functionality.

Comparisons of Genotoxic Potencies with Reference Standards

To convert the genotoxic potency values based on reaction-kinetic parameters to values of absolute risk, two additional pieces of information are required: a comparison of these genotoxic potency values with that of a reference standard with known dose–response (dose–risk) relationship for cancer in human populations and access to methods to determine dose in target tissues (target dose), defined according to Equation 4 above (*12*).

At the onset of this work in the late 1960s and in the 1970s, the only environmental factor for which somewhat reliable (and continuously improving) data for cancer risk were available was low-LET ionizing radiation (X rays and γ radiation). By the use of radiation as a reference standard, allowances are made for the influence of the background incidence, $P°$, in Equation 1.

In the choice of γ radiation as a reference standard for the relative genotoxic potencies of chemical carcinogens, it was assumed that induced mutations would show the same interference with promotive and modifying factors, i.e., would have the same probability of leading to a tumor regardless of the nature of the causative mutagen. A tentative determination of radiation dose equivalents of chemical doses somewhat surprisingly showed approximately the same value in various test systems (*21*). For instance, a chemical dose (of a monofunctional agent) giving rise to a degree of alkylation equal to about 1×10^{-7} at n=2 was shown to induce the same mutation frequency as 1 rad (0.01 Gy) γ radiation. (Recent recalculations indicate 1.3×10^{-7} as a more correct value; see Figure 3.)

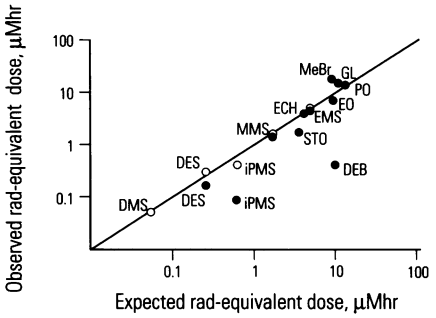

Figure 3. Observed and expected rad-equivalent doses for mutation. Abbreviations: DEB, diepoxybutane; DES, diethyl sulfate; DMS, dimethyl sulfate; ECH, epichlorohydrin; EMS, ethyl methanesulfonate; EO, ethylene oxide; MeBr, methyl bromide; iPMS, isopropyl methanesulfonate; PL, β-propiolactone; PO, propylene oxide; STO, styrene oxide. The rad-equivalent doses were expected to correspond to 1.3×10^{-7} alkyls per unit at n=2. ●, *E. coli* Sd-4; ○, *HPRT* mutations in CHO cells. Modified from Vogel et al. (*18*).

Ethylene oxide (EO) has long been used as a model compound in the development of methods to estimate cancer risks. For this compound, the rad equivalent of chemical dose of 80 (range 40–160) rad/mMhr has long been used as a mean value for various biological systems (*22*). A validation of this value leads to 40 (range, 20–80) rad/mMhr as a more correct value (Ehrenberg et al., in preparation; (*23*)].

Analyses of cancer mortality data from the Hiroshima and Nagasaki populations and from patients irradiated with therapeutic doses support a cancer mortality risk of about 8 to 10% per Gy (1 Gy = 100 rad) at high doses and high dose rates, the lower figure considering exposure at ages 18 to 65. From other experimental data, it has been concluded that the mutagenic and carcinogenic effectiveness is lower by a factor of 2 to 10 at low dose rates or low doses (*8,24*). Current radiation protection recommendations (*24*) are based on a prudently assumed dose and dose–rate effectiveness factor (DDREF) of 2; the estimated risk is 4 to 5%/Gy at low doses and low dose rates (*24*). If experimental data for influences of dose rate on the induction of mutation and cancer are taken into consideration, a more likely value for DDREF is 3 to 4 (*8*). We have therefore considered a reasonable figure for the risk of cancer death to be 3×10^{-2}/Gy (3×10^{-4}/rad). Although the DDREF for EO is still uncertain, it may be assumed that one mMhr of EO is associated with a cancer mortality risk of 12×10^{-3}. [For deviations of this value from other estimates, see Törnqvist et al. (*25*)]. This value for EO may be used as a reference standard for estimations of cancer risks of other alkylators. The expression of potencies in terms of EO equivalents might present an alternative for those who find the direct comparison of chemical and radiation risks to be unacceptable.

From the radiation dose equivalents of genotoxic chemicals and the relatively well-characterized risk coefficents for radiogenic cancer, the chemical cancer risks may be estimated, provided human target doses could be measured.

Measurement of *in Vivo* Dose (Target Dose)

The International Commission on Radiological Protection (*26*) has suggested that the limit of acceptability of the annual cancer mortality risk to individuals of the public due to radiation exposure from man-made sources is in the range of one per million to one per hundred thousand. This value was taken at first as an indicator of the sensitivity required for methods to be used in chemical dosimetry. With the above risk of 3×10^{-4}/rad, the above range for acceptability corresponds to radiation doses in the range 3×10^{-3} to 3×10^{-2} rad/year. With the above radiation-dose equivalence (~40 rad/mMhr) of the standard compound EO, this corresponds to about 10^{-7}—about 10^{-6} Mhr/year at continuous or intermittent exposure.

Without any known exception, electrophiles react with nucleophilic centers in both nucleic acids and proteins. In blood, the most easily available tissue, hemoglobin (Hb) and serum albumin (SA) are available in gram quantities in a 10 ml sample, whereas leukocyte DNA amounts to about 1 mg. Other than the advantages to high sensitivity of large amounts of the monitor compound, the use of the proteins for dosimetric purposes has been preferred because of the absence of repair (which for DNA adducts varies between cell types and adducts) and because of better possibilities of chemical identification of adducts.

It should be stressed that measurement of the level of adducts to Hb, SA, or leukocyte DNA generate a value for the dose in the blood (D_{blood}) with the same need for a determination of the relationship between D_{blood} and the doses in different target organs. At present, these ratios have to be estimated from acute-exposure studies in animal models. For EO, the doses in blood and in vessel-rich organs of the mouse were found to be approximately the same (*27,28*).

The requirement for sensitivity discussed above was set as a goal in the development of a new method based on the determination of adducts to N-terminal valines in Hb by a modified Edman peptide-sequencing method. One major reaction site in Hb for many simple alkylating agents is the N-terminal valines in the four globin chains. With the new method, the *N*-alkyl Edman method, this goal regarding sensitivity has been reached (*29,30*). Under chronic or intermittent exposure, the adducts are accumulated over the life span of the erythrocytes (18 weeks in humans); the steady-state adduct level obtained corresponds to the cumulative level in one-half of the erythrocyte life span. The dose received during 1 year could thus be estimated from the dose received during 9 weeks on the basis of the steady-state adduct level. Doses of chemicals calculated by the rad-equivalence approach, which correspond to the risk in the range 10^{-6} to 10^{-5}/year, could thus be expressed in a steady-state adduct level. The adduct level to N-terminal valine in Hb from EO corresponding to this risk, and the large number of compounds with similar values of s will be 1 to 10 pmol/g globin. At present, analysis of 50 mg globin permits determination of 0.1 to 1 pmol/g globin, a value that can be reduced further by increasing the amount of globin per analysis. It is of interest to note that the measurement of DNA adducts by the ^{32}P postlabeling technique currently reaches approximately the same sensitivity, one adduct per 10^{10} nucleotides, corresponding to about 0.3 pmol/g DNA.

It has been shown for a number of compounds that the possibilities of measuring small adduct increments in individuals are reduced by the presence of identical background adducts, to a large extent endogenous in origin (*31*).

Other than using adduct measurement for exposure monitoring, i.e., as a qualitative indicator of raised exposure or for qualitative and quantitative studies of metabolic pathways, a methodology is thus available permitting measured adduct levels to be translated to cancer risks (or, if the detection level is not reached, the upper limits of possible risks).

The general applicability of this methodology, primarily to low molecular weight alkylating agents or metabolites, is supported by results of ongoing studies which indicate that tumorigenic dose 50 (TD_{50}) values for experimental cancer (*32*) can be rather accurately predicted by the key reaction kinetic parameters (k_{H_2O}, s) and the blood dose per amount absorbed [Ehrenberg et al., in preparation; (*18*)].

In principle, cancer risk increments (P_i) from exposures to chemicals i may be estimated from

$$P_i = Q_i \times k_{std} \times D_i, \qquad [5]$$

where Q_i is the relative genotoxic potency compared with a standard with risk coefficient k_{std} per unit of dose and D_i is the target dose of chemical i. k_{std} is equal to $\beta_{std} \times P^\circ$ in Equation 1. The risk estimation procedure, with ethylene oxide and ethene as examples, has been reviewed (*7,33*).

Sensitive methods are thus available for the measurement of *in vivo* doses of genotoxic chemicals in humans. Cancer risks may be estimated by a relative-potency method, which expresses the doses as radiation dose equivalents (rad-equivalents) or ethylene oxide dose equivalents.

Epidemiological Aspects

Cancer is a primary cause in approximately 20% of deaths in western populations. In exposed groups of the size that is usually available in disease–epidemiological studies, it is rarely possible to detect risk factors leading to relative risks less than 1.5 to 2 (*34*), which corresponds with risk increments of 10 to 20% (for total cancer mortality). For an accurate assessment of the magnitude of studied risks, these risks have to be even higher. If we decide that individual risks higher than 10^{-6} to 10^{-5} annually (i.e., 7×10^{-5} to 7×10^{-4} or about 0.01–0.1% of the deaths in a 70-year lifetime) should be avoided, it is evident that disease–epidemiological studies are about 1,000 times too insensitive to detect and properly assess risk factors that do not occur in large excess in specifically exposed populations. A consequence of reliance upon data for disease or death from cancer may, due to this low resolving power, be that many factors associated with important nonacceptable risks are liable to escape detection if high exposures do not occur. In particular, this concerns weak carcinogens that, if widely distributed, are associated with considerable collective risks.

This insensitivity gap (see Figure 4) can be bridged by the introduction of macromolecule adduct measurement as an auxiliary tool in epidemiological studies. The above discussion of the linearity of dose–response relationships shows that an observed raised adduct level can, in principle, be directly translated to a risk increment. An advantage of this approach, besides its high resolving power, is its ability to identify the chemical structure of the adducts, with clues to the nature of the causative environmental factor (of particular importance in cases of mixed exposure or in the presence of confounders). Furthermore,

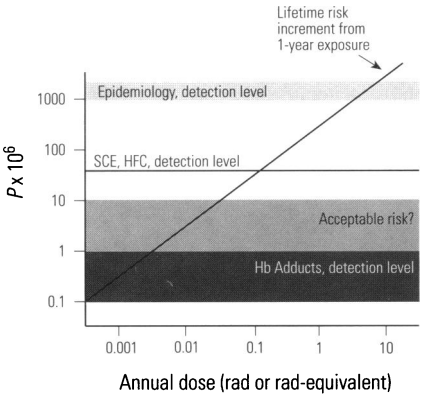

Figure 4. Detection levels of cancer epidemiology, SCE, HFC (sister chromatid exchanges, high frequency cells), and hemoglobin adducts, compared with acceptable risk of cancer death.

adduct monitoring is a way to overcome the long latency times from exposure to full expression of disease, often several decades. In fact, since P° in Equation 1 and P°_{can} in Figure 1D refer not only to tumor localization but also to the influence of age, some two-thirds of the total cancer incidence increment in western populations is expected to occur above age 65, according to Swedish cancer statistics (*35*).

In Figure 4 the resolving power of the most sensitive cytogenetic end point, SCE high-frequency cells (*36*), is also indicated. This and other genetic biomarkers are generally too insensitive, but they play important roles as early hazard indicators and in the clarification of action mechanisms.

Although these basic principles seem to be valid, further developments are needed. This concerns, for example, dosimetry and risk estimation of polycyclic aromatic hydrocarbons, aromatic amines, and carbonyl compounds such as unsaturated aldehydes and techniques offered by recent developments of tandem mass spectrometry to determine adducts from *a priori* unknown electrophiles. In such work, increased resolving power is expected to be gained by analysis of samples from individuals deficient in detoxification functions (*37*). The value of including in epidemiological studies the genetic characterization in this respect of participating individuals should therefore be pointed out. In addition, dose monitoring should take into consideration the induction status, i.e., the phenotypic expression, of bioactivating and detoxifying enzymes. For retrospective dosimetry, as well as for the planning of measures for risk reduction, it is also essential to improve methods for exposure assessment.

It should be emphasized that nongenotoxic carcinogens have to be monitored by methods different from, but combinable with, those discussed here. These agents present other problems because, in general, their biological effects follow dose–response relationships with no-effect thresholds.

Cooperation between disease epidemiologists and biochemical epidemiologists is important to both groups. Adduct measurement may be used in disease–epidemiological investigations to identify genotoxic agents, to estimate their *in vivo* doses, and to estimate risks by application of the rad equivalence or ethylene oxide equivalence. Both risk figures and dose estimates are useful for the design and dimensioning, as well as for the interpretation of results of disease–epidemiological studies. Disease–epidemiological studies are required for verification, adjustment, and further development of dosimetry-based methods for risk estimation. By and large, biochemical epidemiology could assist cancer epidemiology to overcome some of its limitations (*38*).

REFERENCES

1. Timoféeff-Ressovsky NW. Experimentelle Mutationsforschung in der Vererbungslehre. Dresden, Germany: Th. Steinkopff, 1937.
2. Boveri T. Zur Frage der Entstehung maligner Tumoren. Jena, Germany: Fischer, 1914.
3. United Nations Scientific Committee on the Effects of Atomic Radiation. Report to the General Assembly. New York:United Nations, 1964.
4. Walinder G. Has Radiation Protection Become a Health Hazard?, Nyköping, Sweden:The Swedish Nuclear Training & Safety Center, 1995.
5. United Nations Scientific Committee on the Effects of Atomic Radiation. Ionizing Radiation: Levels and Effects. Vol 2: Effects. Report to the General Assembly. New York:United Nations, 1972.
6. Storer JB, Mitchell TJ, Fry RJM. Extrapolation of the relative risk of radiogenic neoplasms across mouse strains and to man. Radiat Res 114:331–353 (1988).
7. Törnqvist M, Ehrenberg L. Risk assessment of urban air pollution. Pharmacogenetics 2:297–303 (1992).
8. United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and Effects of Ionizing Radiation. Report to the General Assembly. New York:United Nations, 1993.
9. Littlefield NA, Farmer JH, Gaylor DW, Sheldon WG. Effects of dose and time in a long-term, low-dose carcinogenic study. J Environ Pathol Toxicol 3(3):17–34 (1980).
10. Burns F, Alberg R, Altshuler B, Morris E. Approach to risk assessment for genotoxic carcinogens based on data from the mouse skin initiation–promotion model. Environ Health Perspect 50:309–320 (1983).

11. McCormick J, Maher VM. Analysis of the multistep process of carcinogenesis using human fibroblasts. Risk Anal 14:257–263 (1994).
12. Ehrenberg L, Moustacchi E, Osterman-Golkar S. Dosimetry of genotoxic agents and dose-response relationships of their effects. Mutat Res 123:121–182 (1983).
13. Ehrenberg L. The problem of cancer risks at low dose rates of ionizing radiation and chemicals. Bull Natl Comm Radiol Protection Ukraine (in press).
14. Granath F. Statistical problems in estimating a threshold in a dose-response model. In: Statistical Methods in Toxicology, Lecture Notes in Medical Informatics, Vol 43 (Hothorn L, ed). Berlin:Springer-Verlag, 1981;79–85.
15. Miller EC, Miller JA. The mutagenicity of chemical carcinogens: correlations, problems and interpretations. In: Chemical Mutagens, Vol 1 (Hollaender A, ed). New York:Plenum, 1971;83–119.
16. Swain CG, Scott CB. Quantitative correlation of relative rates. Comparison of hydroxide ion with other nucleophilic reagents toward alkyl halides, esters, epoxides and acyl halides. J Am Chem Soc 75:141–147 (1953).
17. Turtóczky I, Ehrenberg L. Reaction rates and biological action of alkylating agents. Preliminary report on bactericidal and mutagenic action in *E. coli*. Mutat Res 8:229–238 (1969).
18. Vogel EW et al. DNA damage and repair in mutagenesis and carcinogenesis: Implications of structure-activity relationships for cross-species extrapolation. Mutat Res (in press).
19. Ehrenberg L, Hussain S. Genetic toxicity of some important epoxides. Mutat Res 86:1–113 (1981).
20. Brookes P, Lawley PD. The alkylation of guanosine and guanylic acid. J Chem Soc 1961:3923–3928 (1961).
21. Ehrenberg L. Risk assessment of ethylene oxide and other compounds. In: Assessing Chemical Mutagens: The Risk to Humans Banbury Report No. 1. (McElheny VK, Abrahamson E, eds). Cold Spring Harbor, NY:Cold Spring Harbor Laboratory, 1979;73–88.
22. Kolman A, Segerbäck D, Osterman-Golkar S. Estimation of the cancer risk of genotoxic chemicals by the rad-equivalence approach. In: Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (Bartsch H, Hemminki K, O'Neill IK, eds). IARC Scientific Publications No. 89. Lyon: International Agency for Research on Cancer, 1988; 258–264.
23. Segerbäck D, Rönnbäck C, Bierke P, Granath F, Ehrenberg L. Comparative two-stage cancer tests of ethylene oxide, *N*-(2-hydroxyethyl)-*N*-nitrosourea and X-rays. Mutat Res 307:387–393 (1994).
24. ICRP. 1990 Recommendations of the International Commission on Radiological Protection. ICRP Publication 60, Annals of the ICRP, Vol 21, no 1–3. Oxford:Pergamon Press, 1991.
25. Törnqvist M, Segerbäck D, Ehrenberg L. The "rad-equivalence approach" for assessment and evaluation of cancer risks, exemplified by studies of ethylene oxide and ethene. In: Human Carcinogen Exposure, Biomonitoring and Risk Assessment (Garner RC, Farmer PB, Steel GT, Wright AS, eds). Oxford: IRL Press, 1991;141–155.
26. ICRP. Recommendations of the International Commission on Radiological Protection. ICRP Publication 26, Annals of the ICRP, Vol 1, no. 3. Oxford:Pergamon Press, 1977.
27. Segerbäck D. Alkylation of DNA and hemoglobin in the mouse following exposure to ethene and ethene oxide. Chem Biol Interact 45:139–151 (1983).
28. Segerbäck D. *In vivo* Dosimetry of Some Alkylating Agents as a Basis for Risk Estimation. PhD thesis, Stockholm University, Stockholm, Sweden, 1985.
29. Törnqvist M, Mowrer J, Jensen S, Ehrenberg L. Monitoring of environmental cancer initiators through hemoglobin adducts by a modified degradation method. Anal Biochem 154:255–266 (1986).
30. Törnqvist M. Epoxide adducts to N-terminal valine of hemoglobin. In: Methods in Enzymology, Vol 231, Hemoglobins (Everse J, Vandegriff KD, Winslow RM, eds). San Diego, CA: Academic Press, 1994;650–657.
31. Törnqvist M, Kautiainen A. Adducted proteins for identification of endogenous electrophiles. Environ Health Perspect 99:39–44 (1993).
32. Gold LS, Sawyer CB, Magaw R, Backman GM, de Veciana M, Levinson R, Hooper NK, Havender WR, Bernstein L, Peto R, Pike MC, Ames BN. A carcinogenic potency database of the standardized results of animal bioassays. Environ Health Perspect 58:9–319 (1984).
33. Törnqvist M, Hindsø Landin H. Hemoglobin adducts for *in vivo* monitoring and cancer risk estimation. J Occup Environ Med 37:1077–1085 (1995).
34. Breslow NE, Day NE, eds. Statistical Methods in Cancer Research, Vol 1. IARC Scientific Publications No. 32. Lyon: International Agency for Research on Cancer, 1980;14–40.
35. The Swedish National Board of Health and Welfare. Cancer Incidence in Sweden 1992. Stockholm:Official Statistics of Sweden, 1995.
36. Tates AD, Grummt T, Törnqvist M, Farmer PB, van Dam FJ, van Mossel H, Schoemaker HM, Osterman-Golkar S, Uebel Ch, Tang YS, Zwinderman AH, Natarajan AT, Ehrenberg L. Biological and chemical monitoring of occupational exposure to ethylene oxide. Mutat Res 250:483–497 (1991).
37. Törnqvist M. Search for unknown adducts: increase of sensitivity through preselection by biochemical parameters. In: Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (Bartsch H, Hemminki K, O'Neill IK, eds). IARC Scientific Publications No. 89. Lyon: International Agency for Research on Cancer, 1988;378–383.
38. Taubes G. Epidemiology faces its limits. Science 269:164–169 (1995).