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The Use of Biological Markers in Risk Assessment

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Abstract. The use of biologic markers (such as DNA adducts) as more proximate measures of effective dose has many potential advantages for risk assessment. We can hope for:

- Better modeling of dose-response relationships, by avoiding the attribution of high dose pharmacokinetic nonlinearities to the fundamental multiple mutation process of carcinogenesis.
- Better interspecies projection of doses and risks.
- Improved evaluation of past doses in epidemiologic studies.
- Insights into the magnitude and significance of human interindividual variability.
- Possibly some identification of previously unrecognized genetic hazards.
- In the very long run, the quantification of rates of post-initiation stages in tumor development by comparative measurements of the prevalence of adducts, premalignant foci/clones and tumors as a function of age in different tissues.

Realizing these advantages will require statistically oriented professionals in risk assessment to gain familiarity with more complex, simulation-type modeling approaches with multiple points of comparison between theory and experiment—rather than the straightforward curve-fitting that has built the field to this point. It will also require some precautions to avoid mistakes and misuse of these new kinds of data and related theory.

Key words and phrases: Risk assessment, delivered dose, molecular epidemiology, perchloroethylene, dose response, interspecies projection.

1. INTRODUCTION

In recent years there has been considerable discussion of opportunities to improve the state of the art of carcinogenesis risk assessment by making use of more detailed information on the biologic processes involved—including the dynamics of absorption, elimination, metabolic activation and inactivation and DNA reaction and repair (Anderson, Hoel and Kaplan, 1980; Hattis and Smith, 1987; Gehring, 1979; Starr and Buck, 1984). The use of biologic markers, such as DNA adduct formation, as more proximate indicators of delivered dosage than external exposure levels is part of this process (Hattis, 1986). Below I will explore a number of the potential benefits and the pitfalls of this type of analysis. My major focus, however, will be on the use of biologic markers for two specific purposes:

- Elucidating the shapes of dose-response relationships at low doses.
- Interspecies projection of carcinogenic risks.

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2. ELUCIDATING BASIC MECHANISMS AND DOSE RESPONSE RELATIONSHIPS

Opportunities and Potential Benefits

We are now much closer than we were a decade ago to understanding the fundamental mechanisms involved in carcinogenic transformation. For some time it has been clear that tumors arise as a result of a series of changes or rearrangements of information

coded in DNA within single cells (Fialkow, 1977; Knudson, 1977, 1973; Cleaver and Bootsma, 1975; Vogel and Motulsky, 1979; McCann, Choi, Yamasaki and Ames, 1975; Hattis, 1982). These changes are often induced by electrophilic metabolites of the parent compounds to which organisms are exposed (Miller and Miller, 1981). With the identification of "oncogenes," we are now getting some detailed molecular characterization of what some of the resulting DNA changes are (Fischinger and DeVita, 1984; Yunis, 1983; Hoel, 1985; Modali and Yang, 1986).

It has also been apparent for some time that it will not be possible to make much further headway in elucidating the shapes of carcinogenesis dose-response relationships at low doses simply by increasing the numbers of animals studied in conventional animal bioassays. A wide variety of different mathematical models with dramatically different consequences for low dose risk can always be found that fit the observations about equally well (Whittemore, 1983; Maugh, 1978). Low dose risk projections are therefore inevitably much more determined by the choice of model than by the available data (Whittemore, 1980; Guess, Crump and Peto, 1977) if what is meant by "the data" is restricted to observations of the incidence of ultimate adverse effects in small groups of animals.

Clearly, to make real progress in the modeling of carcinogenic risks we must use our knowledge of the fundamental processess involved to break open the black box between external exposure levels and the ultimate production of tumors. The use of intermediate parameters ("markers") to characterize the dose-response characteristics of small segments of the causal pathway to carcinogenesis has considerable potential to improve our dose-response modeling for the process as a whole. Such markers can include both those that may be directly along the causal pathway, such as DNA adduct formation—and putative correlates such as hemoglobin adduct formation, which can be good indicators of the concentration X time product of active intermediates in the systemic circulation. (Of course, in using correlates, it is important that they are in fact better correlates of at least a portion of the actual carcinogenic process than external exposure—and it will often be difficult to be sure that this is the case in actual risk assessment situations.)

One key fact should be recognized from the beginning about pharmacokinetic modeling. Nonlinearities may be produced at high doses by the saturation of enzymes, the saturation of active transport processes, the depletion of cellular reactants for electrophilic agents or changes in cell division rates to make up for cell killing due to overt toxicity. However, all of these nonlinearities must necessarily disappear as one approaches very low dose rates (Hattis, 1982). The slope of the line relating ultimate DNA lesions in replicating cells to external dose may well be very different at low doses than at high doses, but it must be linear.

The basic reason for this is that at low doses the rates of the transport and transformation processes that lead to DNA damage and repair directly depend on the number of collisions between molecules of an "input" chemical (or activated intermediate or DNA adduct) and a resident cellular reactant (or hole in a membrane or repair enzyme molecule). At low doses the number of resident cellular reactant molecules does not change appreciably as a function of the concentration of the "input." Therefore the number of relevant collisions and the rates of the reactions and side reactions in the causal sequence at low dosage must be direct linear functions of the amounts of input chemical and its activated derivatives. Some finite fraction of the ultimate DNA lesions must escape repair before the next cell replication so long as the cells affected have a non-zero turnover rate, there are a finite number of repair enzyme molecules, and the repair molecules operate at a finite rate.

Because of sample size limitations, animal carcinogenesis bioassays must be done within a limited range of relatively high dose levels. Typically the difference between the minimum detectable response and a response that effectively saturates the system or causes interference through overt toxicity can be only one or two orders of magnitude (often even less). Over this high dose range near levels where the agent produces overt toxic effects, enzyme saturation and other forms of pharmacokinetic nonlinearities are most likely. If in dose-response modeling for risk assessment we do not separate out the nonlinearities of pharmacokinetic origin from whatever nonlinearities may arise from the multiple mutation mechanism that is central to carcinogenesis, our ordinary curve-fitting procedures will implicitly attribute the pharmacokinetic nonlinearities to the fundamental carcinogenic process (Hoel, Kaplan and Anderson, 1983). The resulting errors are particularly serious if one wishes to produce best point estimates of carcinogenic risk in addition to upper confidence limits.

The use of biologic markers promises to greatly expand the range of dosage over which estimates of the early parts of the causal sequence leading to carcinogenesis can be made. In the exploration of the potential of hemoglobin adducts as dosimeters, it is apparent that often linear dose-response relationships can be observed over a 10^4 range of dosage (Green, Skipper, Turesky and Tannenbaum, 1984; Neumann, 1980; Pereira and Chang, 1982, 1981), although some nonlinearities are observed with some compounds (Bailey, Connors, Farmer, Gorf and Rickard, 1981). The recent development of DNA post-labeling with ^{32}P and enzyme-linked immunoassay systems now allows the detection of extremely small quantities of

ordinary unlabeled DNA adducts produced by the exposures of daily life, without requiring special experimental exposure of subjects to radiolabeled material (Randerath, Randerath, Agrawal, Gupta, Schurdak and Reddy, 1985; Perera, Poirier, Yuspa, Nakayama, Jaretzki, Curnen, Knowles and Weinstein, 1982; Gupta, 1982; Hsu, Poirier, Yuspa, Yolken and Harris, 1980; Santella, Lin, Cleveland and Weinstein, 1984; Groopman, Haugen, Goodrich, Wogan and Harris, 1982). Adduct frequencies as low as 10^{-8} per nucleotide have been measured in recent work with aromatic carcinogens (Reddy, Gupta, Randerath and Randerath, 1984; Lu, Disher, Reddy and Randerath, 1986). A critical additional point is that the measurement of DNA or hemoglobin adducts or pharmacokinetic parameters (Andersen, Gargas, Jones and Jenkins, 1980; Andersen, 1982; Bolt, Filser and Buchter, 1981) does not take 2 years to yield results in animals or decades in people.

Difficulties and Pitfalls

To be sure, the appropriate use of biologic markers as more proximate indicators of dose is not without its pitfalls. In particular, it must be recognized that the production and repair of adducts is a dynamic process (see, for example, the results of Birnboim, Fisher and Sawyer, 1986; DiGiovanni, 1986). Unless the experimental data are produced under conditions of long-term continuous dosage, the high dose nonlinearities that the biologic markers are intended to provide information about are likely to change with the duration of dosing and time after dosing. Single measurements at single time points after dosing may therefore prove misleading. Dynamic models will often be necessary to appropriately interpret such data, and this will complicate the job of risk assessors.

I can illustrate this from my own recent work on perchloroethylene (Hattis, Tuler, Finkelstein and Luo, 1986). I needed to derive metabolic parameters (V_{max} and K_m) from data on observed metabolic patterns in experimental animals that were given the chemical either (a) by gavage or (b) by inhalation for 6 hrs. In each case the experimenters had only measured the ultimate disposition of administered perchloroethylene 48–72 hr after dosing (Pegg, Zempel, Braun and Watanabe (1979); Schumann, Quast and Watanabe, 1980; Mitoma, Steeger, Jackson, Wheeler, Rogers and Milman, 1985). Interpreting the gavage data turned out to be complicated because there was no information available on the rate at which the administered material was absorbed from the stomach. Other things being equal, more rapid absorption would produce greater saturation of the metabolizing enzymes and more opportunity for the perchloroethylene to be exhaled before metabolic activation. The inhalation data posed a different but equally serious problem for interpretation.

Because the animals were not put into metabolism cages for collection of exhaled air and excreta until after the end of the 6 hr of exposure, some unmeasured amount of the metabolized perchloroethylene was likely to have been lost in urine, etc. passed during the 6 hr. These difficulties did not turn out to be fatal for our study. With some sensitivity analysis we were able to make reasonably robust estimates and to characterize the added uncertainty in our estimates attributable to the interpretive problems. I sincerely hope, however, that the experimental community can be encouraged to produce a few more data points at different durations of dosing or times after dosing for the next time I have to characterize a dynamic process.

There are other pitfalls that operate more at a science policy level. The greater complexity of risk assessments based on dynamic models—involving the use of biologic markers whose connection with causal processes often cannot be established with great confidence—necessarily gives the analyst greater responsibility for making discretionary technical judgments that are likely to affect the final results. Analysts must take even greater care than usual to disclose their important assumptions and explore the effects of reasonably likely alternative interpretive assumptions and modeling procedures on their final conclusions.

For example, one of the more famous cases of the use of a measure of delivered dose to reassess dose-response relationships is the analysis of formaldehyde risks by Starr and Buck (1984) of the Chemical Industry Institute of Toxicology (CIIT). As their indicator of "delivered dose" these authors used measurements of DNA-protein cross-links in the nasal epithelium of rats after only two 6-hr inhalation exposures. (It would have been much better if these measurements had been made in animals that were chronically exposed, like the animals in the carcinogenesis bioassay.) Because their delivered dose indicator was essentially linear between 6 ppm and 15 ppm it did not help explain any of the steep upward-turning nonlinearity seen in the tumor response in this dose region. At the same time, the delivered dose indicator provided evidence of additional nonlinearity in the region below 6 ppm. The effect of the added nonlinearity in delivered dose as a function of external dose below 6 ppm was to reduce estimates of low dose risk.

When CIIT personnel presented these results at the Society for Risk Analysis meeting in 1985, they also presented data on the effects of chronic formaldehyde exposure on cell proliferation in the nasal mucosa. In contrast to their earlier results with short-term

exposures, it appeared that animals chronically exposed to the highest doses used in their experiments showed much more persistent cell division than animals chronically exposed to lower doses.

This is compatible with what one might expect theoretically (Hattis, Mitchell, McCleary-Jones and Gorelick, 1981)—that the most appropriate measure of "delivered dose" is the number of DNA lesions present at the time of cell division. (Cell division is the time when DNA lesions can give rise to permanent changes in the coded information.) A better index of "delivered dose" might then be the relative amount of DNA-protein cross-links times the cell replication rate. From the audience, I pointed out that if they were to use this measure of delivered dose they would explain some of the high dose nonlinearity observed in the animal bioassays. Less of the nonlinearity would therefore be attributed to the central carcinogenic process, and estimates of low dose risks would be correspondingly increased—contrary to their major conclusion. I continue to hope that some time in the future the CIIT authors will produce a revised analysis incorporating this alternative definition of "delivered dose," and advertise its implications for low dose risks as widely as they have advertised the results of the original analysis.

3. INTERSPECIES PROJECTION OF DOSES AND RISKS

The interspecies projection problem is one of the most fascinating puzzles in modern biology. We know that among mammals there is something like a 50-fold variation in lifespan and a 10,000-fold variation in size, leading to a combined 500,000-fold difference in the raw opportunity for spontaneous carcinogenic transformation per cell per unit of time (Hart and Turturro, 1981). What improvements in defense mechanisms do we relatively large, long-lived mammals have that prevent us from having a greater incidence of cancer than we do?

Of the major current problems in risk assessment, this is perhaps the one where the use of biologic markers can make the most important contributions. Recently allometric techniques for systematically comparing parameters among species have been productively applied to problems in comparative pharmacology (Boxenbaum, 1982). Such systematization will need to be applied to the now somewhat isolated work on parameters such as uptake, metabolic activation, deactivation and excretion (Young and Kadlubar, 1982; Weisburger, 1981; Weisberger and Fiala, 1981; King and Weber, 1981; Autrup and Stoner, 1982; Autrup, Wefald, Jeffrey, Tate, Schwartz, Trump and Harris, 1980; Autrup, Grafstrom, Christensen and Kieler, 1981) DNA reaction and repair (Hart and Setlow, 1974; Teo and Karran, 1982; Ishikawa and Takayama, 1982) and subsequent steps in the carcinogenic process. Early modeling efforts appear to have achieved some success in interspecies prediction in some cases (Ramsey and Andersen, 1984) although the appropriate modeling of these processes in humans is likely to remain a challenging and hazardous enterprise for quite some time.

As a practical example of how the use of an intermediate variable such as metabolism can alter the interspecies projection of carcinogenic risks, I will present my own latest results for perchloroethylene (Hattis, Tuler, Finkelstein and Luo, 1986).

Perchloroethylene is a relatively simple molecule whose uptake and metabolism has been relatively well studied in both rodents and people. It is a member of a well studied family of compounds, including vinyl chloride, that are at least transiently converted to epoxides and other active intermediates that then can react with DNA and other cellular nucleophiles. Like vinyl chloride (Maltoni, 1977), in a number of rodent bioassay experiments there appears to be a plateauing of the tumor response to perchloroethylene at high doses (National Toxicology Program, 1985; National Cancer Institute, 1977). There is also good evidence from a number of metabolism studies in rodents that the enzymes responsible for metabolism of perchloroethylene approach saturation in about the same region of dosage that was used for the carcinogenesis bioassays (Pegg, Zempel, Braun and Watanabe, 1979; Schumann, Quast and Watanabe, 1980; Mitoma, Steeger, Jackson, Wheeler, Rogers and Milman, 1985). Given this, it is reasonable to postulate that the carcinogenic activity of perchloroethylene is mediated by some initial metabolic step. We were fortunate to have available data that could be used to independently estimate the metabolism of perchloroethylene in all three species of interest—mice, rats and humans, and to base our projections of carcinogenic risk on the expected amounts of metabolism in each species under anticipated conditions of exposure. To assess the metabolism in the three species, we built dynamic simulation models, roughly patterned after those of Fiserova-Bergerova (1983), but, for humans, incorporating realistic diurnal changes in breathing rates and blood flows to different body compartments as a function of expected activity levels. Because the human metabolic parameters were based on data from actual human occupational exposures (Ohtsuki, Sato, Koizumi, Kumai and Ikeda, 1983; Ikeda et al., 1972), no interspecies projection of these parameters was necessary.

Our results are summarized in part A of Table 1. Part B of Table 1 shows the results of more naive

TABLE 1

Comparison of the results of pharmacokinetic versus conventional multistage model assessment of the carcinogenic risk of 1 year occupational exposures to perchloroethylene

Probability per Individual of Developing at Least One Additional Cancer		
A. Risks based on pharmacokinetic modeling of metabolite production		
Exposure level (8 hr/day, 5 days/week)	Best estimate[a]	Plausible upper limit[a]
1 ppm	1.5×10^{-5}	2.8×10^{-4}
10 ppm	1.4×10^{-4}	2.5×10^{-3}
100 ppm	1.0×10^{-3}	.023

B. Risks assuming that rodents and people are equally sensitive to perchloroethylene on a ppm lifetime exposure basis

	Maximum likelihood estimates		
Exposure level (8 hr/day, 5 days/week)	From rat data (average, both sexes) on leukemia	From mouse data (males only) on hepatocellular carcinoma	Upper 95% confidence limit from male mice on hepatocellular carcinomas and adenomas
1 ppm	2.6×10^{-5}	6.9×10^{-5}	1.6×10^{-4}
10 ppm	2.6×10^{-4}	6.9×10^{-4}	1.6×10^{-3}
100 ppm	2.6×10^{-3}	6.9×10^{-3}	.016

[a] Please see the accompanying text for a listing of the several sets of assumptions underlying each of these estimates.

calculations based on a simple assumption that rodents and people are equally sensitive to perchloroethylene on a ppm (fraction of lifetime exposure) basis. A formal listing of the major assumptions underlying the results in part A of Table 1 can provide a sense of the intricacy of this kind of analysis.

The "best estimates" are based on:

- A model of human metabolite formation based on the observations of Ohtsuki, Sato, Koizumi, Kumai and Ikeda (1983).
- Maximum likelihood estimates of leukemia risk for male and female rats from inhalation exposure to perchloroethylene in relation to metabolite formation. Metabolite formation was estimated from models of rat metabolism derived from data of Pegg, Zempel, Braun and Watanabe (1979) and Mitoma, Steeger, Jackson, Wheeler, Rogers and Milman (1985) (see pages 137–146).
- Maximum likelihood estimates of the risk of hepatocellular carcinoma for male mice, using data combined from inhalation and gavage exposures of perchloroethylene, in relation to metabolite formation. Metabolite formation was estimated from models of mouse metabolism derived from data of Schumann, Quast and Watanabe (1980).
- The "metabolic energy density" rule for interspecies projection of risks—assuming equal risk for an equal amount of daily lifetime average metabolite formation per unit of body weight to the ¾ power (Boxenbaum, 1982).

Our "plausible upper limit" estimates in part A of the table are based on:

- (Below 10 ppm) a model of human metabolite formation based on the observations of Ikeda et al. (1972).
- (At 10 ppm and above) the sum of estimated metabolism plus loss via the route of perchloroethylene disappearance we have called "skin loss" for lack of a better term.
- 95% upper confidence limit estimates of the risk of total liver tumors in male mice, in relation to metabolite formation estimated from the data of Schumann, Quast and Watanabe (1980).
- The "surface area" rule for interspecies projection of risks—assuming equal risk for an equal amount of daily lifetime average metabolite formation per unit of body weight to the ⅔ power.

Both sets of calculations implicitly assume a primary genetic mode of action for carcinogenesis by perchloroethylene's metabolites—an assumption that will no doubt be controversial in some quarters (Schumann, Quast and Watanabe, 1980).

The largest single contributor to the spread between our "best estimate" and "plausible upper limit" calculations is the uncertainty in the rate of human metabolism of perchloroethylene. This uncertainty arose in part from the fact that our two sources of direct human metabolism data suggested low dose metabolism rates that differed from one another by 5-fold. Another part of the uncertainty arose from a detailed comparison of the perchloroethylene breath concentrations predicted by our model with those

observed in groups of human subjects by Stewart, Hake, Forster, Lebrun, Peterson and Wu (1974). Basically their observations showed somewhat lower perchloroethylene breath concentrations than were predicted by our physiologic pharmacokinetic model. Because the difference could be an uncharacterized route of metabolism, our "plausible upper limit" calculation includes this material as metabolized.

One perspective on the results in Table 1 is that humans are expected to be somewhat less sensitive to perchloroethylene than the rodents that were the subjects of the NTP inhalation bioassays. If we had assumed that humans were equally sensitive as rats and mice on a ppm for ppm basis, and we used the same basic tumor data sets, we would have produced the risk estimates in part B of Table 1. It can be seen that the "best estimate" risks in part A of the table are somewhat lower than the "maximum likelihood estimates" calculated directly from the rodent data and shown in part B of the table. However, our "plausible upper limit" estimates in part A of the table are somewhat higher than the risks calculated from the "upper 95% confidence limit" dose-response relationships in part B. The increase in the distance between "best estimate" and "plausible upper limit" in the more complex analysis in part A of the table primarily resulted from our uncertainty in human metabolism rates. Thus our modeling has made us aware of a source of uncertainty that would not have been captured in the more standard type of projection represented in part B of the table. This "new" source of uncertainty, however, could be reduced by relatively short-term studies of actual metabolic rates in exposed working populations.

4. EVALUATION OF PAST DOSES IN EPIDEMIOLOGIC STUDIES

The problem of reconstructing past exposure levels frequently presents itself both in risk assessments and in epidemiologic studies. Often it is only possible for epidemiologists to characterize groups as having "high," "medium" or "low" exposure, and this seriously limits the usefulness of the observations in quantitatively projecting human risks for other populations. If the assay of Perera, Poirier, Yuspa, Nakayama, Jaretzki, Curnen, Knowles and Weinstein, (1982) had been available for applicaiton to the study of the lungs of deceased coke oven workers (Redmond, Wieand, Rockette, Sass and Weinberg, 1981), or if adducts could have been measured in the DNA of bladder epithelium of workers previously exposed to benzidine, we might today have a much firmer basis to reconstruct exposure and effective dose in those populations. Recently Haugen, Becher, Benestad, Vahakangas, Trivers, Newman and Harris (1986) have successfully measured benzo(a)pyrene adducts in coke oven workers. Ongoing case control studies could also greatly benefit by being able to quantitatively assess past exposures to putative causal agents, instead of being limited to such simple observations as "occupations involving exposure to wood dust were 2.5 times more frequent in nasal cancer patients than in controls." On the other hand, it should be stressed that this use of biologic markers will require the same kind of dynamic modeling as is needed for appropriate use in determining dose-response relationships.

5. UNDERSTANDING THE MAGNITUDE AND SIGNIFICANCE OF HUMAN INTERINDIVIDUAL VARIABILITY

When careful calibrating measurements of external dose are made, or when relevant processes are assessed in vitro (e.g., Harris, Autrup, Stoner, Trump, Hillman, Schafer and Jeffrey, 1979; Autrup, Barrett, Jackson, Jesudason, Stoner, Phelps, Trump and Harris, 1978; Lambert, Ringborg and Skoog, 1979), the use of biologic markers of delivered dose offers considerable promise for providing quantitative information on the degree of interindividual variability for processes along the causal pathway for carcinogenesis. Even animal data may contribute in some cases to our understanding of likely human interindividual differences. For example, Lu, Disher and Randerath (1986) have measured DNA-adduct formation for three different carcinogens in pregnant and nonpregnant animals. The DNA binding of one of the carcinogens, safrole, was increased 2.3–3.5-fold in the liver and kidney of pregnant animals.

One of the more unfortunate features of current procedures for carcinogenesis risk assessment is that there is no treatment of human interindividual variability. Implicitly, all people are assumed to have the same intrinsic sensitivity. This is unfortunate because uniformity is clearly a polar assumption. In recent modeling work (Hattis, Erdreich and DiMauro, 1986b) I have found that if people's sensitivities are distributed log normally, an assumption of uniform sensitivity will tend to understate carcinogenic risks at low doses. The magnitude of the understatement is greatest for the lowest risk levels and for relatively steeply upward turning dose-response relationships (e.g., where risk is proportionate to $(\text{dose})^2$ or $(\text{dose})^3$ rather than dose itself).

6. IDENTIFICATION AND PRELIMINARY QUANTIFICATION OF PREVIOUSLY UNRECOGNIZED GENETIC HAZARDS

One recent example of the use of a biologic marker to recognize a risk that was not entirely appreciated

before is the determination by Tornqvist, Osterman-Golkar, Kautiainen, Jensen, Farmer and Ehrenberg (1986) of tissue doses of ethylene oxide in cigarette smokers, based on hemoglobin adduct levels. The internal dose of ethylene oxide evidently results partly from the presence of ethene as a major gaseous constituent of cigarette smoke. Ethene is then oxidized to ethylene oxide by metabolizing enzymes. Some small amounts of ethylene oxide are also present preformed in cigarette smoke. Based on the hemoglobin adduct levels and the inferred dosage of ethylene oxide, they estimated that ethene/ethylene oxide might contribute on the order of 15% of the carcinogenic activity in cigarette smoke. This kind of inference process may be quite useful in assessing the relative risks of other components of complex mixtures.

7. QUANTIFICATION OF RATES OF POST-INITIATION STAGES IN TUMOR DEVELOPMENT BY COMPARATIVE MEASUREMENTS OF THE PREVALENCE OF PREMALIGNANT FOCI/CLONES AS A FUNCTION OF AGE IN DIFFERENT TISSUES

In the longer term, I would hope for the development of biologic markers along two additional lines to plug gaps in our quantitative knowledge of the carcinogenic sequence:

- The recent use of radioactively tagged antibody to tumor antigens to locate microscopic tumor metastases suggests that similar antibodies to generic kinds of tumor antigens could be used on autopsy tissue specimens to survey the incidence of very small benign or malignant tumors or altered foci in populations (e.g., in the liver). In this way it might be possible to ascertain the quantitative importance of "immune surveillance" mechanisms in controlling tumors at an early stage, and the possible importance of age- or exposure-related changes in immune surveillance in influencing the risk of clinically detectable tumors.
- rDNA probes and related cytogenetic studies could be used to measure the incidence in tissues of the DNA modifications and rearrangements thought to represent the early stages of carcinogenesis. If we had good assays for cells that had undergone what were known to be the initial stages in the carcinogenic process, cancer epidemiology could be made both much more sensitive (because cells, rather than people would be the units of analysis) and capable of being done much earlier after the onset of exposure (perhaps months, rather than decades).

In short, by changing the unit of analysis from the whole organism to the cellular or molecular level, molecular epidemiologic measurements and corresponding measurements in animal systems can be of considerable help in the elaboration of dose-time-response models for risk assessment.

8. CONCLUSION

In closing I should observe that the use of biologic markers will certainly make the lives of risk assessors more difficult and risk assessments more costly. So much for a "cost effectiveness" criterion. Moreover, for this extra difficulty and cost, there may often be no improvement in what passes for estimates of the uncertainty of risk assessments. If my experience with perchloroethylene is any guide, the reported confidence limits may in fact widen with the inclusion of more sources of uncertainty. Finally, by increasing the complexity of risk assessments, one makes them even less comprehensible by the nonexpert audience and expands the potential opportunities for corrupt manipulation of the results. In all, the only positive thing that can be said for the promise of making use of sophisticated biologic markers and pharmacokinetic modeling in risk assessments must still be said in a small voice. These tools, used with great care, might help us get a little closer to the truth about the interactions of our complex living systems with our complex world.

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