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Biologic Markers in Risk Assessment for Environmental Carcinogens

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The potential of biologic markers to provide more timely and precise risk assessments for environmental carcinogens is viewed against the current state-of-the-art in biological monitoring/molecular epidemiology. Biologic markers such as carcinogen-DNA adducts and oncogene activation are currently considered valid qualitative indicators of potential risk, but for most chemical exposures research is needed to establish their validity as quantitative predictors of cancer risk. Biologic markers have, however, already provided valuable insights into the magnitude of interindividual variation in response to carcinogenic exposures, with major implications for risk assessment.

Introduction

Cancer prevention remains an unfulfilled goal because of the limited tools available to make timely and meaningful risk estimates for environmental carcinogens. Traditionally, these estimates have been based on dose-response data derived from controlled experiments in genetically homogeneous laboratory animals. Generally, response at the relevant low level of exposure is extrapolated from results observed at doses several orders of magnitude higher. In rare cases, dose-response data from epidemiologic studies (usually from the occupational in both instances, setting) are used. However, the dose is the amount of carcinogen administered or the external exposure rather than the actual target dose, and the response is the tumor yield. In addition, even the most conservative risk extrapolation models make the simplifying assumption that the human population is homogeneous in its biologic response to carcinogens.

Biologic markers have the potential to improve risk assessment in a number of specific areas. First, markers can provide quantitative estimates of the biologically effective dose of the carcinogen to the target tissue. Calibration of molecular dose in humans to that in laboratory animals for whom tumor evidence is known should greatly enhance extrapolation of risks between species. Second, markers of preclinical biologic response (established to be predictive of cancer risk) can provide an earlier occurring, more sensitive outcome than tumor incidence. Thus, markers can allow epidemiology to escape the tyranny of cancer latency to become a more timely contributor to risk assessment and cancer prevention. Third, markers can provide information on interindividual variation in response to a carcinogenic exposure, thereby filling a major void in risk assessment. Fourth, biologic markers can help identify the mechanisms by which carcinogens exert their effect, thus enabling more effective prevention strategies.

The field of biologic markers is still, however, at an early stage of development. An impressive number of studies have related increases in biologic markers such as carcinogen-DNA adducts, genetic alterations, and oncogene activation to specific occupational or lifestyle exposures, demonstrating the ability of markers to flag carcinogenic hazards (qualitative risks). Much less research effort has been aimed at evaluating dose-response relationships between exposure and molecular dose or effect. In most cases, peripheral blood cells have been assayed as a surrogate for target tissue. In addition, much work remains to establish the value of biologic markers as quantitative predictors of carcinogenic risk. We can point to only one case of a well-developed quantitative risk assessment based upon biomonitoring data (*1-3*). Although considerable progress has been made using biologic markers that reflect a genotoxic end point, there is a dearth of methods for biomonitoring individuals with exposure to carcinogens (such as dioxin and the chlorinated organics) that do not interact efficiently with the genetic material.

The purpose of this paper is twofold. First, we will highlight recent progress in the validation of biologic markers to better assess their potential in risk assessment. The common denominator in our review will be carcinogen-DNA or carcinogen protein adducts (as a measure of biologically effective

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Table 1. Biological markers in smokers and nonsmokers (*14*).

Marker	Mean (SE) Smokers	Nonsmokers
4-ARP-Hb, pg/g	154.5 (11.3)*	32.2 (2.9)
n	19	18
SCE, average/metaphase	10.8 (0.603)†	8.1 (0.47)
n	11	10
Cotinine, ng/mL	419.2 (47.4)*	0.3 (0.032)
n	10	10

*$p = 0.0001$.
†$p = 0.002$.

dose) in conjunction with a complementary marker of biologic effect (oncogene activation). Second, we will review available information regarding the magnitude of interindividual variation in molecular or cellular biologic response to carcinogens. This information will serve as the basis for estimating the degree to which failure to incorporate interindividual variation into quantitative risk assessment may lead to underestimation of risk.

Recent Research on Macromolecular Adducts and Oncogene Activation

There is a strong biologic rationale for selecting macromolecular adducts and oncogene activation. Adducts result from covalent interaction of electrophilic carcinogens with DNA. If unrepaired, DNA adducts can lead to gene mutation and initiation of cancer. Protein adducts can serve as an easily obtained surrogate for those formed with DNA. Various methods are available to measure adducts, including immunoassays, spectrometry, and postlabeling (*4*). Carcinogen-DNA binding is considered to be a necessary but not sufficient event in chemical carcinogenesis (*5–11*).

Activated oncogenes have been identified in human tumors of all types and in common premalignant lesions, suggesting that oncogenes represent a sufficiently early step in the carcinogenic process to allow for clinical intervention. Moreover, activated oncogenes have been linked experimentally and in human studies to exposure to carcinogens including polycyclic aromatic hydrocarbons (PAHs) (*12*). Using monoclonal antibodies and modified Western blotting techniques, it is now possible to detect oncogene protein products in tumor tissue and in urine or serum as surrogates for target tissue (*13*).

Because the design of biomonitoring studies so strongly dictates the interpretation of data, we will review examples of cross-sectional, longitudinal serial sampling, case-control, and nested case-control studies using these and related biomarkers. The preponderance of research on macromolecular adducts has been cross-sectional in nature, aimed at elucidating the relationship between carcinogen-DNA or carcinogen-protein adducts and estimated exposure to carcinogens such as cigarette smoke, diet, industrial pollution, and combination chemotherapy.

Cigarette smoke is a classical complex exposure that has been the subject of much molecular analysis. For example, in a study of 22 cigarette smoking and 24 nonsmoking volunteers (Table 1), a battery of markers was evaluated in repeat peripheral blood samples drawn several days apart (*14*). Environmental histories were obtained by questionnaire. 4-Aminobiphenyl-hemoglobin (4-ABP-Hb) adducts measured by GC/MS were found to be more specific to cigarette smoke than PAH-DNA adducts measured by (ELISA) immunoassay. Unlike PAH-DNA adducts for which there was a high and variable background, 4-ABP-Hb levels were significantly different ($p = 0.0001$) in smokers and nonsmokers. A repeat blood sample (2 days later) gave comparable results for 4-ABP-Hb: 139.0 (7.9) pg/g for the smokers versus 36.2 (5.02) for the nonsmokers. The frequency of sister chromatid exchange (SCE) was also significantly elevated in the smokers and was positively correlated with 4-ABP-Hb. This finding suggests that aromatic amines contribute significantly to the integrated genotoxic effect of cigarette smoke.

Ethylene oxide (EO) is another genotoxic constituent of cigarette smoke. Recently, samples from a subset of the original group were analyzed for ethylene oxide-hemoglobin (EO-Hb) adducts by the procedure of Tornqvist et al. (*15*). The levels of EO-Hb (hydroxyethyl valine) adducts were significantly higher in smokers ($n = 13$) than nonsmokers ($n = 7$) (*16*). Ethylene oxide and 4-aminobiphenyl adducts were highly correlated ($r = 0.83$) in the smokers and nonsmokers combined and in the smokers as a group ($r = 0.50, p < 0.0005$).

Table 2. DNA adducts in white blood cells of smokers and nonsmokers.

Assay	Population (*n*)	Range, μmole/mole[a]	% Positive	Mean positive μmole/mole[b]	Reference
PAH-DNA, ELISA	Smokers (22)	0.054–0.17 (3×)	41	0.096	(*14*)
	Nonsmokers (24)	0.035–0.14 (4×)	33	0.08	
PAH-DNA, USERIA	Smokers (32)	<0.032–0.29 (> 9×)	9	0.192	(*43*)
	Nonsmokers (49)	<0.032–0.13 (> 4×)	4	0.096	
DNA adducts, ^{32}P	Smokers (4)	<0.002–0.014 (> 7×)	100	0.0065	(*44*)
	Nonsmokers (6)	0.004–0.01 (2.5×)	100	0.0065	

[a]Values are μmole adduct per mole DNA. ELISA, enzyme-linked immunosorbent assay; USERIA, ultrasensitive enzymatic radioimmunoassay; ^{32}P, ^{32}P-postlabeling. Values in parentheses show the range of adducts seen across all samples, e.g., 3-fold.
[b]The value given is the mean for positive samples; i.e., those with detectable levels of adducts.

This study design has the advantage of simplicity and feasibility. However, had the differences between the two groups (smokers and nonsmokers) been smaller, they might have been missed because of swamping by interindividual variability. For example, a 32% coefficient of variation was seen in levels of 4-ABP-Hb among the smokers, all of whom smoked between 1 and 2 (mean, 1.4 ± 0.4) packs per day.

In this study the coefficient of variation for samples with detectable levels of PAH-DNA adducts was 38% in the smokers compared to 40% in the nonsmokers. In Table 2, the results for PAH-DNA are compared with adduct data by immunoassay and postlabeling on smokers and nonsmokers from two other laboratories. Across all studies, the range in binding levels was 3-fold to greater than 9-fold among smokers, compared to 2.5-fold to greater than 7-fold among nonsmokers. This high and variable background in DNA adducts has been consistently seen in human studies, except for those involving administration of certain drugs.

In contrast to the smoker/nonsmoker study, a cross-sectional study of PAH-DNA in long-term employees in an iron foundry in Finland showed a clear dose-response relationship between estimated source-specific exposure to PAH and levels of PAH-DNA adducts ($p = 0.0001$) (*17*). Thirty-five workers were classified into three categories according to their estimated exposure to benzo(*a*)pyrene (BaP) as a representative PAH: high exposure (8-hr time-weighted average > 0.02 $\mu g/m^3$); medium exposure (0.2–0.05 $\mu g/m^3$); or low exposure (< 0.5 $\mu g/m^3$). Controls were 10 workers with no occupational exposure to PAHs. Here, also, significant interindividual variation was seen in addduct levels in the exposed workers. For example, in 13 foundry workers with comparable medium exposure (0.05–0.2 $\mu g/m^3$ BaP) there was a 20-fold range of PAH-DNA adducts (0.03–0.7 μmole/mole); for the low exposure group ($n = 18$), a 29-fold range of PAH-DNA adducts (0.01–0.28 μmole/mole) was observed. These results are consistent with data for exposed workers in other occupational biomonitoring studies. As shown in Table 3, the levels of PAH-DNA adducts in coke oven and foundry workers in three other studies showed a similar range (>3–137×) as did DNA adducts by the postlabeling method (< 50×).

Recently, we have investigated whether levels of PAH-DNA adducts (as a marker of biologically effective dose) correlated with activation of oncogenes (as a marker of biologic effect) since various PAHs have been shown experimentally to activate the *ras* oncogene (*18,19*). Therefore, sera from foundry workers and controls were assayed for oncogene protein products (*ras, fes, myc, myb, sis, B-EGF, int-1, myb, src, and mos*) as previously described (*20*).

The method was able to determine whether the oncogene was overexpressed but not whether it had undergone a point mutation. A fivefold increase in protein expression was point considered a positive result. As shown in Table 4, one individual in the medium exposure group showed elevated levels of the *ras* oncogene product in one of three serum samples tested. Repeat serum samples from two other individuals in the medium and high exposure groups had significantly elevated levels of the *fes* oncogene protein product. Samples from the 10 unexposed controls were uniformly negative. As will be discussed, both *fes* and *ras* serum proteins have been detected in lung cancer patients and cigarette smokers. Since foundry workers are also at elevated risk of lung cancer (*21*), these results suggest that oncogene activation may prove to be a useful early marker for respiratory cancer related to PAH exposure. Obviously, this clue must be followed up by further studies.

As with all cross-sectional studies, which provide a snapshot at a particular point in time, this research cannot establish temporal or causal relationships between exposure, adduct formation, and oncogene activation. For this reason, longitudinal studies in individuals whose exposure changes significantly over time are preferable in terms of establishing the relationship between exposure and biologic markers.

Recently, we carried out a pilot serial sampling study of "smokenders," obtaining repeat blood samples from 21 active smokers upon enrollment into the smoking cessation program and then 21 days and 3 to 6 months after enrollment (*22*). Roughly half of the individuals quit smoking entirely within 3 weeks (stoppers) and the other half either reduced smoking or continued to smoke at the same level (nonstoppers). SCEs were significantly decreased in the stoppers (but not the nonstoppers) at least 3 to 6 months after quitting, while levels of 4-ABP-Hb and cotinine fell sharply within 21 days. However, PAH-DNA adducts were not significantly affected by smoking cessation,

Table 3. PAH-DNA in white blood cells of workers.[a]

Assay	Population (*n*)	Range, μmole/mole[b]	% Positive	Mean positive μmole/mole[c]	Reference
PAH-DNA, USERIA	Coke oven (20)	<0.032–0.13 (> 4×)	35	0.064 (0.022)	(*45*)
PAH-DNA, ELISA	Coke oven (27)	<0.032–10.9 (> 3.4×)	66	2.27 (1.51)	(*40*)
PAH-DNA, USERIA	Coke oven (38)	<0.032–4.38 (>137×)	34	0.51 (0.18)	(*41*)
PAH-DNA, ELISA	Foundry (35)	<0.01–0.90 (> 90×)	86	0.20 (0.17)	(*11*)
DNA adducts, ^{32}P	Foundry[d] (25)	<0.002–0.1 (> 50×)	100	0.018	(*46*)

[a]Values are μmole adduct per mole DNA. ELISA, enzyme-linked immunosorbent assay; USERIA, ultrasensitive enzymatic radioimmunoassay.
[b]Values in parentheses show the range of adducts seen across all samples, e.g., 4-fold.
[c]The value given is the mean for positive samples; i.e., those with deductible levels of adducts. Values in parentheses are means for all samples assayed.
[d]Medium and high exposed.

Table 4. PAH-DNA adducts and serum oncogene protein expression in foundry workers and unexposed controls (12).

Ambient exposure, μg BaP/m³	Subject number	Cigarettes/day	PAH-DNA adduct levels, fmole/μg[a]			Serum oncogene proteins[a]					
						fes			*ras*		
			Post vacation	Work 1	Work 2	Post vacation	Work 1	Work 2	Post vacation	Work 1	Work 2
>0.2 (high exposure)	1	0	0.11	0.8	NA	−	−		−	−	
	2	20	0.13	2.0	NA	+	+		−	−	
	3	0	NA[b]	2.8	NA	−	−		−	−	
0.05-0.2 (medium exposure)	4	15	0.13	0.36	NA	−	−		−	−	
	5	20-25	NA	0.32	0.32	−	−	−	−	+	−
	6	15	NA	0.8	0.5	−	−	−	−	−	−
	7	0	0.48	1.28	NA	+	+		−	−	
	8	20	ND[b]	0.4	NA	−	−		−	−	
0.05 (low exposure)	9	20	ND	0.38							
	10		ND	1.52							
	11		ND	0.32							
	12		0.08	1.20							
0 (unexposed controls)	9	15		0.08			−			−	
	10	10		ND			−			−	
	11	0		0.14			−			−	
	12	0		ND			−			−	
	13	0		ND			−			−	
	14	0		0.3			−			−	
	15	0		ND			−			−	
	16	10		ND			−			−	
	17	15		0.1			−			−	
	18	0		0.1			−			−	

[a]Post vacation samples were drawn after a 1 month vacation; work 1 samples for workers (but not for controls) were drawn 6 weeks after returning to work; work 2 samples were drawn more than 2 months after returning to work.
[b]NA, not available; ND, nondetectable.

possibly because of their lack of specificity for cigarette smoke constituents and/or the short follow-up period.

Based on these results, we are beginning a more definitive study of smokenders who will be followed for 2 years after quitting to evaluate persistence of DNA adducts, hemoglobin adducts, and oncogene protein products. We will also be evaluating several potential genetic/metabolic markers of susceptibility including arylhydrocarbon hydroxylase (AHH) activity and glutathione-*S*-transferase activity. This approach will allow a detailed evaluation of the persistence of biologic markers in human peripheral blood cells.

Another natural experiment was provided by the plant-wide annual vacation taken by the Finnish foundry workers during the month of July. As shown in Table 4, white blood cell DNA from individuals was assayed for PAH-DNA adducts after the workers returned from their 4-week vacation (sample 1) and then 6 weeks following their return to work (sample 2). The levels of adducts were significantly higher ($p = 0.002$, Wilcoxon sign rank test) in sample 2, showing a clear biologic effect of exposure.

Another longitudinal, serial sampling study has evaluated biologic markers in patients treated with *cis*platinum (*cis*DDP)-based chemotherapy (23) (Table 5). Peripheral blood samples were collected just prior to and following the first, second, and last cycles of chemotherapy. In subjects with a baseline and at least two post-treatment samples, levels of SCE, plasma protein binding, and hemoglobin binding measured by atomic absorption spectrometry (AAS) were significantly increased following treatment ($p < 0.01$). This finding was consistent with prior studies of DNA and prtein adducts and SCE in *cis*DDP-treated patients (24–26).

Since *cis*DDP-based chemotherapy is standardized to body

Table 5. Levels of biomarkers in *cis*platinum-treated patients by time point of sample collection (23).[a]

	Baseline[b] (0)	Post cycle 1[c] (99.23 ± 13.3.24)	Pre cycle 2[b] (105.45 ± 28.41)	Post cycle 2[d] (197.00 ± 35.82)	Post final cycle* (351.9 ± 60)
SCE	10.8 ± 3.01 (12)	17.39 ± 6.52* (12)	17.45 ± 5.03* (12)	—	—
Plasma protein bg, μg/g	0.47 ± 1.07 (10)	27.5 ± 9.34* (10)	2.37 ± 3.34 (2)	33.37 ± 11.07* (10)	33.49 ± 9.77* (10)
Hemoglobin bg, μg/g	0.01 ± 0.04 (11)	2.08 ± 1.04* (11)		2.97 ± 1.25* (11)	2.89 ± 1.04 (4)

[a]Values in parentheses are *n*; values in brackets are the average cumulative dose of *cis*platinum (mg/m²) received prior to that sample collection. Subjects with baseline and at least two post-treatment samples (SCE or protein bg).
[b]Samples drawn within 24 hr prior to treatment.
[c]SCE sample drawn 2 weeks after treatment; protein sample drawn 12–24 hr post-treatment.
[d]SCE and/or protein sample drawn 12–24 hr post-treatment.
*$p < 0.01$, Wilcoxon and *t*-tests.

Table 6. Adducts in *cis*platinum-treated patients.

Assay[a]	Tissue	*n*	Range	Reference
DNA adducts, ELISA	Peripheral blood lymphocytes	45	0– > 0.066 μmole/mole	(*24*)
DNA adducts, LC, ELISA	Peripheral blood lymphocytes	6	0.2–1.4 μmole/mole	(*25*)
DNA adducts, immunocytochemistry	Buccal mucosa	9	0– > 150 nuclear density	(*48*)
Protein adducts, AAS	Hemoglobin	32	0.2–4 ng/mg (20×)[b]	(*26*)
Protein adducts, AAS	Hemoglobin	18	0–3.49 ng/mg	(*23*)

[a]ELISA enzyme-linked immunoassay; LC, liquid chromatograph; AAS, atomic absorption spectrophotometry.
[b]Twentyfold variability or range of adducts.

weight or surface area of patients, it provides a good model for examining interindividual variability. *cis*DDP reacts directly with DNA, unlike the aromatic hydrocarbons which require metabolic activation; therefore, interindividual differences among patients would be due to uptake and retention of the drug and repair of adducts. Table 6 provides the results of our recent study in the context of other reports on *cis*DDP-treated patients. For 18 subjects given one cycle of chemotherapy, the mean level of hemoglobin adducts was 1.90 ± 0.99 (coefficient of variation = 52%). The range of hemoglobin adducts in samples with detectable levels was 0.35 to 3.49 μg/g or about 10-fold. One individual showed no measurable adduct formation after treatment. Among all four studies in Table 6, the range of DNA adduct formation was at least 10- to 20-fold.

We have recently begun a follow-up study that will assess a full battery of markers: *cis*DDP-DNA and *cis*DDP hemoglobin adducts, SCE and micronuclei, chromosomal aberrations, gene mutation (HPRT and GPA) and oncogene activation in peripheral blood cells and/or semen.

To summarize, the advantage of longitudinal serial sampling studies such as those described is that the problem of interindividual variation is mitigated by having each individual serve as his or her own control. However, such research opportunities are rare, and, although they address the temporal relationship between exposure and biomarkers, they leave unanswered the question of whether biomarkers are related to cancer risk.

The first step in understanding the role of various biomarkers as risk factors for cancer has been taken in several modified case-control studies. These have a practical advantage over prospective studies in terms of feasibility and can provide circumstantial evidence of an association between biomarkers and cancer risk. However, as with cross-sectional studies, case-control studies cannot establish the temporal sequence for adduct formation, gene mutation, oncogene activation, and cancer. The retrospective approach also suffers because the relevant exposure period for most cancer induction is years to decades in the past, and biologic markers formed at that critical time are likely to be lost or diluted out. Only in situations where exposure has been continuous and unchanged and where metabolic processes have not altered over time, can biological measurements taken in the present be informative.

We have recently evaluated 81 cases with primary carcinoma of the lung and 67 controls for levels of PAH-DNA adducts and SCEs in order to examine whether, when lifetime exposure to PAHs and other mutagens/carcinogens was comparable, cases had higher levels of biomarkers than control (*23*). This would suggest that the ability to efficiently activate and bind carcinogens may have been a risk factor in their disease. SCEs in lymphocytes did not differ between cases and controls. As in the smokers/nonsmokers study, PAH-DNA adducts in white blood cell DNA were not specific to cigarette smoking but apparently reflected the numerous background sources of PAH such as diet and ambient air. Among current smokers, lung cancer cases had significantly higher levels of PAH-DNA adducts ($p = 0.01$, Wilcoxon rank sum test). This case-control difference was entirely contributed by blood samples collected during the months of July through October. This seasonal effect is consistent with the observation of seasonal variation AHH activity in human lympocytes (*27*). Genetically regulated metabolism of PAHs by the AHH microsomal enzyme system has been linked (in some but not all studies undertaken) to lung cancer risk (*28,29*).

As in the foundry workers study, Western blotting techniques were used to screen sera of 18 lung cancer cases and controls for peptide sequences of nine different proteins including *ras*, *fes* and *myc*. As shown in Table 7, 18 cases, all current or former smokers, were positive for at least one oncogene protein, in contrast to 2 of the 20 healthy controls (both current smokers). In 15 of these cases, all but one of which were nonsquamous cell cancers, *ras* gene protein products were identified. Eleven of 18 cases had activated *fes* compared to 0 of the controls. All of the lung cancer patients were former or current smokers. These results are consistent with prior studies in that PAHs are known to activate the *ras* gene, and a high prevalance of activated *ras* has been seen in nonsmall cell carcinomas and in tumors from cigarette smokers. High levels of *fes* expression have also been detected in human lung tumors (*12*).

PAH-DNA adducts and SCEs were measured in white blood cell DNA from these 18 patients. Three of the 18 individuals had detectable levels of PAH-DNA adducts in white blood cell DNA. All 3 were positive for the *ras* oncoprotein; however, 11 of the *ras*-positive cases did not have detectable levels of adducts. Thus, there was no apparent correlation between adducts and oncogene activation, possibly because of the small number of subjects studied.

As noted above, case-control studies have the serious limitation that if exposure or pharmacokinetic processes have altered between the time of cancer induction and diagnosis, current levels of macromolecular adduct levels may bear little relationship to cancer risk. Thus, the nested case-control is the optimal design for evaluating the relationship between biologic markers and cancer risk, provided that the marker is stable in samples

Table 7. DNA adducts and serum oncogene protein in lung cancer patients (12).[a]

Patient	Age	Sex	Smoking history, pack-years	Tumor type	Lymphocyte adducts (fmole/μg)	Oncogene								
						ras	*fes*	*myb*	*int*	*sis*	*myc*	*mos*	*src*	*EGF*
1	75	F	S(NA)	A	−	++	−	+	+	−	−	−	−	−
2	71	F	S(90)	A(S)	−	++	++	−	−	−	−	−	−	−
3	67	F	S(31)	A	−	++	−	−	−	−	−	−	−	−
4	42	M	S(20)	L	−	++	−	−	−	−	−	−	−	−
5	67	F	S(40)	A	−	++	−	−	−	−	−	−	−	−
6	82	M	S(55)	A	+(0.45)	++	−	−	−	−	−	−	−	−
7	49	M	S(63)	S	−	++	−	−	−	−	−	−	−	−
8	65	M	S(40)	L	+(0.16)	++	++	−	−	+	−	−	−	−
9	68	F	S(62)	A	−	++	++	+	−	−	−	−	−	−
10	62	M	S(61)	S	−	−	++	−	−	−	−	−	−	−
11	69	F	S(40)	A	−	−	++	−	−	−	−	−	−	−
12	73	F	S(84)	S	−	−	−	−	+	−	−	−	−	−
13	67	F	S(105)	A	−	++	++	−	−	−	−	−	−	−
14	67	F	S(13)	L	−	++	++	−	−	−	−	−	−	−
15	60	F	S(20)	L	−	++	−	−	−	−	−	−	−	−
16	63	M	S(84)	A	−	++	++	−	−	−	−	−	−	−
17	69	M	S(50)	A	+(0.13)	++	++	−	−	−	−	−	−	−
18	62	M	S(30)	A	−	++	++	−	−	−	−	−	−	−
Controls														
19	58	M	NS			−								
29	25	M	NS			−								
21	30	M	ES			−								
22	31	M	CS			+	(All others negative)							
23	52	M	ES			+								
24	39	M	CS			−								
25	29	M	NS			−								
26	34	M	CS			−								
27	26	M	NS			−								
28	35	M	CS			−								
29	35	M	NS			−								
30	52	M	CS			−								
31	36	M	NS			−								
32	56	M	NS			−								
33	56	M	NS			−								
34	32	M	NS			−								
35	48	M	CS			−								
36	44	M	NS			−								

[a](++) stongly positive; (+) positive; (−) negative; NA, not available; NS, nonsmoker; ES, exsmoker; CS, current smoker; S, smoker; A, adeno; AS, adenosquamous; L = large cell; S = squamous.

collected at the outset of a prospective study. In this approach, the cohort is followed, and when cancer cases appear among the cohort, their stored biological samples are analyzed and compared with those from one or more matched controls. The relative risk (i.e., the measure of association between the incidence rates of cancer and the biomarkers being evaluated) can then be calculated. Examples include a recent study of serum retinol and retinol-binding protein and lung cancer (*30*) and an ongoing study of hepatitis B chronic carriers involving retrospective measurement of aflatoxin B_1 adducts on albumin and other markers once incident hepatocellular carcinoma cases are identified (*31*).

Adducts in Quantitative Risk Assessment and Mechanistic Studies

Biological dosimetry data in humans and experimental animals have been used to a limited extent to predict human risk. As mentioned, the best-developed example is that of EtO. Briefly, this was calculated as follows. The tissue dose (the actual concentration of the reactive electrophile within the tissue) of EtO was assumed to be directly proportional to the extent of both DNA and protein binding in humans, as had been demonstrated in mice exposed to EtO. The *in vivo* tissue dose (mole/hr) of EtO that produced the same mutagenic response in experimental systems as 1 rad of low-LET radiation in the low dose-response region was calculated and termed the "rad equivalent." Measurements of EtO-hemoglobin binding in sterilizer plant workers were then used to estimate their tissue dose of EtO in terms of rad equivalents. This rad equivalent measure allowed a calculation of the excess risk of leukemia in the workers (*1,32–34*). The resultant prediction of increased risk of cancer in this cohort was subsequently borne out by epidemiological studies (*3*).

Although the results with EtO are generally encouraging, this may be a somewhat special case because EtO is a stable electrophile that distributes evenly between tissues, in contrast to substances such as vinyl chloride and dimethylnitrosamine, which require metabolism and thus produce less stable intermediates (*34*). At present, we rarely have comparative data on adducts in humans and laboratory animals with chronic exposure. While this situation should improve rapidly for individual

substances, human exposures frequently involve complex mixtures that are rarely tested in laboratory animals. In addition, human studies are generally carried out in worker populations, smokers, chemotherapy patients, or accidental situations where exposures are far higher than those encountered by the general population. Thus, risk assessment will still necessitate low-dose extrapolation with its underlying assumptions.

An elegant juxtaposition of results from a conventional case-control study of bladder cancer and from biomonitoring of individuals who were representative of the same resource population has provided additional circumstantial evidence that adduct formation is causally related to cancer risk. The data have also provided insights into the mechanisms of bladder cancer causation. In the case-control study, the risk of bladder cancer in Turin, Italy, was 2.5 times higher among smokers of black tobacco than among smokers of blond tobacco (*35*).

A subsequent study of volunteers from Turin showed correspondingly higher levels of 4-ABP-Hb adducts in the blood of black tobacco smokers compared to smokers of blond tobacco (*36*). The differences in adduct levels were approximately proportional to the relative risk of each group. Urinary mutagenicity was also twice as high in black tobacco smokers as in blond tobacco smokers (*37*). These findings were not surprising since aromatic amines, including 4-ABP, are more concentrated in black than blond tobacco smoke. Given the different composition of black and blond tobacco smoke, it is possible that the two types of tobacco affect different stages in the multistage carcinogenic process. Indeed, in smokers of black tobacco, there was a gradient of risk with early exposure; and smokers who had quit never showed a drop to baseline levels of bladder cancer risk. This suggests both an early and late effect of black tobacco smoke, in contrast to blond tobacco smoke which appears to contain late-stage carcinogens (as evidenced by the dramatic drop in risk for blond tobacco smokers following smoking cessation). Here the epidemiologic data bearing upon mechanism and stage of carcinogenic action were supported by a relevant biologic marker, 4-ABP-Hb adducts.

Need to Account for Interindividual Variation in Risk Assessment

The observed variation in macromolecular binding between individuals with comparable environmental or clinical exposure to carcinogens is consistent with data on interindividual differences in carcinogen activation, detoxification, and DNA repair (*38,39*). Although not well characterized, variation in cellular processes (such as cell replication rates, age, diet, and immune status) related to promotion of carcinogenesis is also likely to be significant.

As biologic markers become more precise and reproducible, they can be useful in accurately characterizing the population at risk in terms of variation in sensitivity to carcinogenic exposures. (Of course, intraindividual variability and assay variability must be well characterized and separated out from interindividual variation.) Unfortunately, conventional representation of results of biomonitoring and molecular epidemiologic studies in terms of the mean and standard deviation and coefficient of variation (as we have been guilty of in this paper) does not accurately portray the extent of variability within a population. A useful approach to estimating population variability based on small data sets is based on probit analysis, which allows expression of values as measures independent of sample size. For example, using the observed range of PAH-DNA values for 35 foundry workers (*11*) as an intermediate between the variation seen in two other occupational studies (*40,41*), one can estimate that 95% of a standard population (±2 SD) would have 60-fold variability, and 99.7% (±3 SD) would have a 460-fold variation in adduct formation.

The failure of current procedures for carcinogenic risk assessment to explicitly account for human interindividual variation may result in a significant understatement of carcinogenic risks at low doses, especially for the most sensitive individuals, but also for the population as a whole (*42*).

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