Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6672–6676, October 1985
Medical Sciences

Detection of benzo[*a*]pyrene diol epoxide–DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers

(synchronous fluorescence spectrophotometry/ultrasensitive enzyme radioimmunoassay/biochemical epidemiology/carcinogenesis)

CURTIS C. HARRIS*†, KIRSI VAHAKANGAS*, MARK J. NEWMAN‡, GLENNWOOD E. TRIVERS*, ABULKALAM SHAMSUDDIN*§, NUNTIA SINOPOLI*¶, DEAN L. MANN*, AND WILLIAM E. WRIGHT‖

*Laboratory of Human Carcinogenesis, Division of Cancer Etiology, National Cancer Institute, Bethesda, MD 20205; ‡Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814; and ‖Department of Preventive Medicine, University of Southern California School of Medicine, Los Angeles, CA 90033

Communicated by Gerald N. Wogan, May 22, 1985

ABSTRACT **Coke oven workers are exposed to high levels of carcinogenic polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene (B[*a*]P), and are at increased risk of lung cancer. Since B[*a*]P is enzymatically activated to 7β,8α-dihydroxy(9α,10α)epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (B[*a*]PDE) that forms adducts with DNA, the presence of these adducts was measured in DNA from peripheral blood lymphocytes by synchronous fluorescence spectrophotometry and enzyme radioimmunoassay. Approximately two-thirds of the workers had detectable levels of B[*a*]PDE–DNA adducts. Antibodies to the DNA adducts were also found in the serum of 27% of the workers. B[*a*]PDE–DNA adducts were not detectable in lymphocytes and antibodies to the adducts were not detected in sera from a control group of nonsmoking laboratory workers. DNA adducts and/or antibodies to the adducts indicate exposure to B[*a*]P and its metabolic activation to the carcinogenic metabolite that covalently binds to and damages DNA. Detection of adducts and antibodies to them may also be useful as internal dosimeters of the pathobiological effective doses of chemical carcinogens.**

Benzo[*a*]pyrene (B[*a*]P) is a ubiquitous chemical carcinogen found in tobacco smoke, atmospheric pollution due to burning of fossil fuels, and a variety of foods (1). B[*a*]P can also be used as an indicator of general exposure to other carcinogenic polycyclic aromatic hydrocarbons (PAH). B[*a*]P is a procarcinogen that requires metabolic activation, which results in its putative ultimate carcinogenic metabolite, 7β,8α-dihydroxy(9α,10α)epoxy7,8,9,10-tetrahydrobenzo[*a*]-pyrene (B[*a*]PDE) (2). The predominant DNA adducts formed from this compound have been studied in experimental animals and in cultured human tissues and cells (3, 4) and are highly variable, probably due to differences in metabolic enzymes. DNA adduct levels are also dependent on DNA repair rates. Although rates for excision DNA repair vary severalfold among people (5), the interindividual variation in the DNA repair rates of these B[*a*]PDE–DNA adducts in humans is not known. Therefore, the amount of B[*a*]PDE–DNA adducts measured at any time point is dependent on many factors, including exposure to B[*a*]P, its absorption and transport, the metabolic balance between activation and deactivation of B[*a*]P, and, finally, the capacity of the cells to repair DNA adducts.

Because the major B[*a*]PDE–DNA adduct formed in cultured human tissues and cells incubated with B[*a*]P is identical to that found in cultured tissues from experimental animal species in which B[*a*]P is known to be carcinogenic, we have initiated studies to determine if such adducts could be detected in DNA from cells from people environmentally exposed to B[*a*]P. Our approach has been to utilize immunological and physical methods that can detect B[*a*]PDE–DNA adducts at the level of one adduct in 10 million or more DNA bases (6–9). Knowing also that carcinogen–DNA adducts are antigenic when injected into mice, rats, and rabbits (10, 11) and that DNA-modifying drugs such as procainamide and hydralazine can elicit antibodies to DNA in humans (12–14), we examined the serum from these individuals for antibodies to an epitope(s) on B[*a*]PDE–DNA. Coke oven workers are exposed to substantial amounts of B[*a*]P in their work atmosphere (7–10 $\mu g/m^3$) (15) and are at increased risk of lung cancer (16). We selected this occupational population to determine if B[*a*]PDE–DNA adducts could be found in the DNA of their peripheral blood lymphocytes and if B[*a*]P elicited an immune response and thus serum antibodies to these adducts.

MATERIALS AND METHODS

Donors. Coke oven workers from a single plant volunteered for the study. The mean (±SD) age of the 41 participants was 45.0 ± 9.6 years and their ages ranged from 28 to 61 years. The minimal duration of work on the coke ovens was 5 years, and the maximal duration was 30 years. The mean (±SD) number of years worked at the coke ovens was 17.8 ± 4.2 years and the median was 16.8 years. The participants were questioned about their smoking habits, work, diet, and medication, using a standardized questionnaire administered by interview. Cigarette smoking was defined as consumption of >20 packs of cigarettes in a lifetime. Approximately 50 ml of blood was obtained from the 41 participants, and the uncentrifuged whole blood was shipped in plastic containers by an overnight express carrier to the National Cancer Institute for analysis. Work histories for the participants' employment at the coke ovens were available from the personnel office of the company. Industrial hygiene measurements of benzene-soluble particulates collected from the work area atmosphere for each coke oven job were also provided by the company. These measurements, done by

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Abbreviations: PAH, polycyclic aromatic hydrocarbon(s); B[*a*]P, benzo[*a*]pyrene; B[*a*]PDE, 7β,8α-dihydroxy(9α,10α)epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; USERIA, ultrasensitive enzyme radioimmunoassay; SFS, synchronous fluorescence spectrophotometry.

†To whom reprint requests should be addressed.
§Present address: Department of Pathology, University of Maryland School of Medicine, Baltimore, MD 21201.
¶Present address: Istituto di Patologia Generale II-III, Cattedra Università degli Studi Roma, La Sapienza, Viale Regina Elena 324, Rome, 00161 Italy.

using methods specified by the Occupational Safety and Health Administration standard for exposure to coke oven emissions (17), were used in the study to represent relative exposure to PAH.

DNA was purified from peripheral blood mononuclear cells that were frozen at −70°C in Hepes buffer after isolation from peripheral blood using lymphocyte separation medium (Litton Bionetics). The cell suspension was thawed, diluted 1:1 with a buffer containing NaCl (100 mM), Tris·HCl (50 mM, pH 8.0), $NaDodSO_4$ (1%), and EDTA (10 mM), and the DNA was isolated as described by Vahakangas *et al.* (9). The serum for antibody studies from these individuals was frozen at −70°C until used.

ELISA for Detection of Antibody to B[*a*]PDE–DNA Adducts. *Noncompetitive ELISA.* Human serum samples were tested for the presence of antibodies against B[*a*]PDE–DNA by ELISA. The ELISA used is similar to a published method (6) and is described briefly here. The B[*a*]PDE-modified DNA and unmodified DNA were used as the standard antigens. These were attached to polyvinylchloride microtiter plates (Costar, Cambridge, MA) by drying at 37°C for 12 hr at the concentration of 20 ng per well in 50 μl of 3 M NaCl/0.3 M sodium citrate, pH 7.0. Plates coated with the unmodified DNA or without DNA (NaCl/sodium citrate buffer alone) were used as controls. The plates without DNA were used to establish background binding levels of the test serum and the plates coated with unmodified DNA were used to distinguish antibodies that bound to DNA from those that bound to B[*a*]PDE-modified DNA. Plates were coated with B[*a*]PDE-modified or unmodified bovine serum albumin by incubating 100 ng of these test antigens in 0.06 M carbonate/bicarbonate buffer (pH 9.6) for 12 hr. These test agents were used in an attempt to identify antibodies against B[*a*]PDE alone. Affinity-purified goat anti-human immunoglobulin reagents (Cappel Laboratories, Cochranville, PA) were biotinylated as described (18) and stored at 1 mg/ml at 4°C.

Antigen-coated test plates were prepared for the assay by multiple washing steps using distilled water to remove salt crystals followed by a 30-min room temperature incubation of all wells with 150 μl of a 0.05 M phosphate-buffered saline containing 10% normal goat serum (phosphate-buffered saline/NGS) to block protein binding sites on the solid phase. Human serum was tested in triplicate using four $\log_5$ serial dilutions, with all dilutions being made in the phosphate-buffered saline/NGS solution. Test serum (50 μl per well) was incubated for 60 min with the antigen-containing plates and then was washed five times with phosphate-buffered saline. The binding of human immunoglobulin was detected with the biotinylated goat anti-human immunoglobulin reagents and the avidin-biotin horseradish peroxidase system (ABC Vectastain kit, Vector Laboratories, Burlingame, CA) as described by the manufacturer. The enzyme reaction was developed by the addition of 100 μl of the substrate solution [0.05 M citrate buffer (pH 4.0), 1 mg of *o*-phenylenediamine per ml, and 0.5 μl of 30% H_2O_2 per ml] to each of the wells. The enzymatic reaction was stopped after a 20-min room temperature incubation by the addition of 50 μl of 2.0 M H_2SO_4. Plates were read with an automatic ELISA reader (Dynatech, Alexandria, VA) and the absorbance at 490 nm was recorded. An anti-human immunoglobulin reagent that reacted with all isotypes of human serum immunoglobulin was used for initial testing. Each ELISA included the testing of a serum on B[*a*]P-modified and unmodified DNAs as well as on a plate without any type of bound DNA. The modified and unmodified bovine serum albumins were used similarly.

Antigen competition ELISA. Human serum that was found to contain antibodies that reacted against the B[*a*]PDE-modified DNA was tested for specificity of reactions by using a competitive ELISA. In this assay the binding of serum antibodies to solid-phase antigen (B[*a*]PDE–DNA) underwent competition by preincubation of the serum with different test antigens (B[*a*]PDE-modified or unmodified DNA) prior to testing in the ELISA. Human serum was diluted to a point at which binding was 80–90% maximal. The diluted serum was mixed with varying amounts of modified or unmodified DNA (400, 200, and 100 ng/ml) and incubated at room temperature for 60 min. These serum–DNA mixtures were then tested as described for the noncompetitive ELISA against both the modified and unmodified DNA. The results were expressed as "percent inhibition," which was determined by using the levels of binding of each serum without any competing antigen as the level of maximal binding and the background binding of each serum as the level of minimal antibody binding.

Assays for B[*a*]PDE–DNA Adducts. *Synchronous fluorescence spectrophotometry (SFS).* The DNA solution was adjusted to 0.1 M HCl and heated at 90°C for 3 hr to hydrolyze the DNA adduct to putative B[*a*]P tetrols as described (9). The samples were assayed by a Perkin–Elmer fluorescence spectrophotometer 650-40 with a Perkin-Elmer 3600 data station. All measurements were done with a constant wavelength difference of 34 nm ($\Delta\lambda$ = 34 nm) between excitation and emission during the scanning. By this system, the B[*a*]PDE–DNA and hydrolysis products of B[*a*]P tetrols give specific emission peaks at 382 and 379 nm consecutively (9).

Ultrasensitive enzyme radioimmunoassay (USERIA). The USERIA (19) was also used to detect B[*a*]PDE–DNA adducts. This assay method utilized a rabbit antiserum against B[*a*]PDE–DNA and was performed in a manner similar to that described previously (6, 20). Briefly, polyvinyl microtiter plates were precoated with 0.2 ng of B[*a*]PDE–DNA in 10× concentrated phosphate-buffered saline (GIBCO) and stored at −20°C. Prior to adding the competition mixtures, the plates were washed free of salt and treated for 1 hr with 2% horse serum. For the test, 10 μg (or less) of each DNA sample was adjusted to 210 μl with buffer, heated to 90°C for 15 min, and then cooled in ice water to obtain single-stranded DNA. An equal volume of a 1:300,000 dilution of rabbit antiserum to B[*a*]PDE–DNA in 2% horse serum was added to each sample and to tubes containing serially diluted B[*a*]PDE–DNA prepared in solutions of unmodified DNA at 40 or 20 μg/ml for the standard curve on each plate. One hundred microliters of each antigen/antibody solution was added to a triplicate set of microtiter wells containing B[*a*]PDE–DNA as the solid-phase competitor and to one well containing unmodified DNA for the solid-phase specificity control. The plates were incubated for 90 min at 37°C and then washed, and alkaline phosphatase-conjugated goat anti-rabbit IgG [$F(ab)_2$ (Cappel), 1:500 in 1% horse serum] was added for an additional hour at 37°C. The plate was washed, and 100 μl of the substrate [20 pmol of *p*-nitro[^{3}H]phenyl phosphate (New England Nuclear) and 80 pmol of unlabeled substrate per well in 20 mM diethanolamine buffer (pH 9.6) containing 10 mM $MgCl_2$] was added, and the plate was incubated for 4 hr at 37°C. Finally, 20 μl from each well was diluted in 2 ml of buffer and mixed with 4 ml of Econofluor 2, separating hydrolyzed *p*-nitro[^{3}H]phenol in the organic phase to measure the radioactivity for the final calculations of the inhibition of the immunoreactions when compared to the uninhibited controls.

The mean and standard deviation of each triplicate set were determined and the corrected means (minus control DNA values) were used to calculate the percent inhibition. The variation among triplicates was <20%. Cases judged as positive by USERIA produced a percent inhibition within the linear portion of the standard curve—i.e., between 20–25% and 80–85% inhibition.

RESULTS

B[*a*]PDE–DNA Adducts. *SFS.* Putative B[*a*]PDE–DNA adducts were found as a peak at 378–380 nm of emission. Examples of emission spectra observed in testing DNA from two cases and comparison with a spectrum from B[*a*]PDE-modified calf thymus DNA are shown in Fig. 1. The two patterns exemplified here are the sharp peak characteristic of the calf thymus DNA adduct and the broad peak. In most of the cases, the peaks were of the broad type rather than the sharp peak produced by hydrolyzed authentic B[*a*]PDE–DNA. Thirty-one of 41 samples from different individuals showed these patterns. The pattern in the 10 negative samples was similar to that seen with calf thymus DNA. SFS analysis is quantitative as well as qualitative. However, the broad peak does not allow specific quantitation; therefore, the results are assigned as either positive or negative.

USERIA. Sufficient DNA was available in only 27 of the 41 cases to measure B[*a*]PDE–DNA adducts by USERIA. The standard curves were established with reference B[*a*]PDE–DNA diluted in unmodified DNA (20 μg or 10 μg per microtiter well). The 50% inhibition ranged from 8 to 16 fmol and a minimal detectable level ranged from 0.06 to 0.23 fmol of B[*a*]PDE per μg of DNA per well—i.e., a minimal detectability level of 1 mol of B[*a*]PDE in 2×10^7 mol of DNA.

Eighteen of the 27 samples tested (64%) were positive (Table 1), showing amounts that ranged from 0.4 to 34.3 fmol of B[*a*]P per μg of DNA. Nonsmokers and former smokers had approximately the same percentage of positive values and no significant difference in the levels was detected. Current smokers had a slightly higher proportion of positive values (9/12, 75%) than nonsmokers and former smokers combined (9/15, 60%). Among the cases in jobs with the highest exposure to benzene-soluble particulates (Larry car operator, door cleaner, lid tender, etc.), 6 of 7 cases (86%) were positive by USERIA.

Antibodies Toward B[*a*]PDE–DNA. Sera from the same individuals whose lymphocyte DNAs were studied for B[*a*]PDE–DNA adducts were studied for antibodies of reactions that suggested that these sera contained specific antibodies to B[*a*]PDE–DNA (Table 1).

The ELISA binding curves established by dilution of the serum are of three types (Fig. 2): (*i*) 3 of the 11 positive sera produced antibody binding patterns similar to that shown for serum 18 in that the specific antibody titers to B[*a*]PDE–DNA were low—i.e., 25–125—and no antibody reactivity to unmodified DNA was observed; (*ii*) the binding patterns of 7 of 11 sera were similar to that shown for serum 28 and the titers were higher—i.e., 625 to ≥3125; and (*iii*) the remaining positive serum, no. 26, reacted against both B[*a*]PDE–DNA and unmodified DNA.

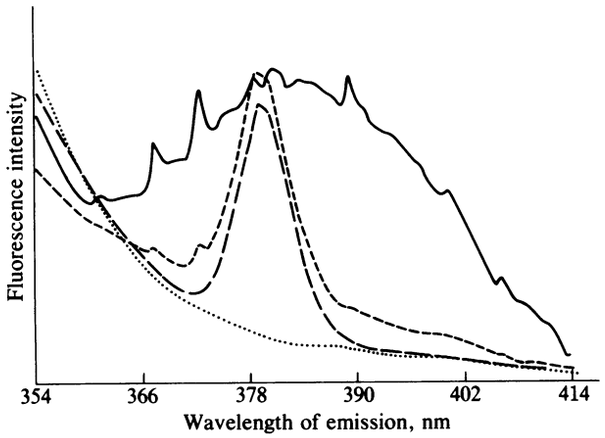

FIG. 1. SFS spectra of calf thymus DNA (····), B[*a*]PDE–DNA (––), and peripheral blood lymphocyte DNA from two coke oven workers. ---, Case 11; —, case 1.

Table 1. Presence of putative B[*a*]PDE–DNA adducts and antibodies in coke oven workers according to tobacco smoking history

Sample no.	Putative B[*a*]PDE–DNA adducts		
	SFS†	USERIA, fmol/μg of DNA	Antibody titer‡
Nonsmoker*			
3	×	1.2§	–
7	–	4.3	625
9	–	1.1§	–
24	–	1.4§	25
26	+	NT	>3125
35	×	<0.06	–
37	×	4.4	–
Former smoker			
1	×	<0.06	–
2	–	0.9	–
5	+	NT	–
10	×	0.4	–
13	–	6.1	–
14	+	NT	125
16	×	NT	–
17	+	NT	–
19	–	<0.06	–
21	–	NT	–
28	×	<0.06	>3125
29	×	<0.06	–
38	×	1.0	–
41	–	<0.06	–
Current smoker			
4	+	NT	–
6	–	<0.06	–
8	–	NT	–
11	+	26.5	–
12	+	NT	–
15	×	<0.06	–
18	×	NT	125
20	×	NT	–
22	×	9.8	–
23	×	0.9	–
25	+	34.3	625
27	×	23.8	–
30	×	4.3	>3125
31	×	<0.06	–
36	+	NT	–
39	+	NT	–
40	×	NT	625
42	×	2.7	>3125
43	×	2.3	–
44	×	2.0	>3125

NT, not tested; insufficient DNA remaining in unhydrolyzed aliquot after SFS to analyze by USERIA.
*Never smoked.
†+, Sharp emission peak at 379 and 382 nm; ×, broad emission peak at 379 and 382 nm; and –, no peak at those wavelengths.
‡When a number is not listed, antibody to B[*a*]PDE–DNA was not detected.
§Mean; each assay was done in triplicate and the variation was generally <10%.

Specificity of the antibodies or B[*a*]PDE–DNA in the 11 positive sera was confirmed by the use of antigen competition ELISA. Examples of three of the antigen competition binding curves with B[*a*]PDE–DNA, the 2,3-oxide of aflatoxin B_1 (aflatoxin B_1–DNA) as a control for modification of DNA by

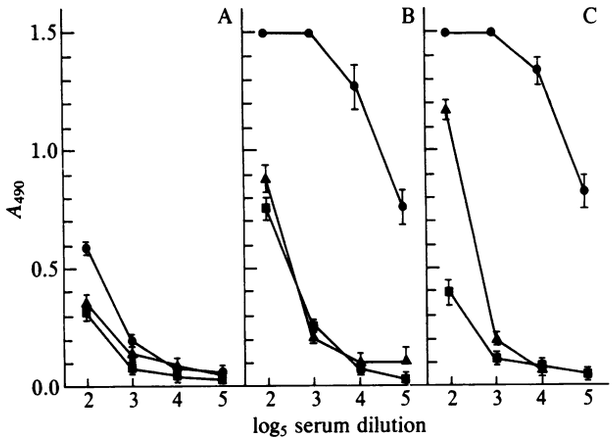

FIG. 2. Binding curves of three human sera on B[*a*]PDE–DNA (●), on control DNA (▲), and on plates without DNA (■). (*A*) Serum 18. (*B*) Serum 28. (*C*) Serum 26. Data points represent the mean ± SEM of triplicate noncompetitive ELISA absorbance values. The sera were tested by using four $\log_5$ dilutions.

a chemical, and unmodified DNA are shown in Fig. 3. The binding of antibodies from positive serum was inhibited specifically by B[*a*]PDE–DNA for all of the positive samples; aflatoxin B_1–DNA and unmodified DNA had no significant effect except for serum 26. This pattern of inhibition for this serum shows that it contains antibodies to unmodified DNA as well as to B[*a*]PDE–DNA.

The predominant immunoglobulin isotype of the anti-B[*a*]PDE–DNA antibodies was determined to be IgG. Two of the 11 positive sera also contained B[*a*]PDE–DNA-reactive antibodies of the IgM class (cases 25 and 28). Antibody binding to B[*a*]PDE–bovine serum albumin could not be detected, suggesting that the antibodies present in the reactive serum recognize only B[*a*]PDE as it is presented on DNA.

Cases with detectable levels of antibodies were compared to the other cases according to the number of years worked at the coke ovens. There was no statistically significant difference between the groups (17.2 ± 7.7 years, antibody positive, vs. 18.1 ± 8.0 years, antibody negative). Three of the 11 cases with detectable antibodies had jobs with highest exposure to benzene-soluble particulates (27%) and 6 of the remaining 30 individuals (20%) had these jobs at the time of the study.

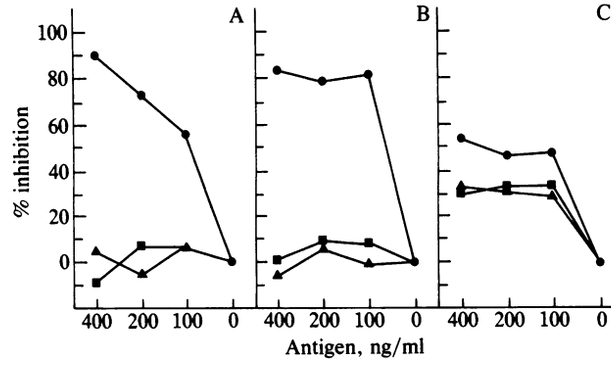

FIG. 3. Percent inhibition curves of three human sera as determined by competitive ELISA. (*A*) Serum 18. (*B*) Serum 28. (*C*) Serum 26. Binding curves represent antibody reactivity as tested on B[*a*]PDE–DNA with antigen competition. ●, B[*a*]PDE–DNA; ■, aflatoxin B_1–DNA; ▲, control DNA.

DISCUSSION

Detection of B[*a*]PDE–DNA adducts and/or antibodies to the adducts indicates exposure to B[*a*]P, its metabolic activation to its ultimate carcinogenic metabolite that reacts with DNA, and immune response to the B[*a*]PDE–DNA adducts. Because all of these individuals have been exposed to substantial amounts of B[*a*]P, the presence and varying titers of antibody in 28% of the cases may be more dependent on interindividual differences in metabolism of B[*a*]P, DNA repair rates, and/or immune responsiveness to the adducts than on variation in dose of B[*a*]P. In those cases in which antibodies to B[*a*]PDE–DNA adducts were detected, the time of the initial antigenic stimulus could not be predicted, but considering the potential longevity of immunological memory that can be recalled by reexposure to antigen, the initial antigenic stimulus could have occurred many years ago. In fact, we propose that antibodies to carcinogen–DNA adducts may be indicators of past exposure to specific environmental carcinogens and thus be useful in epidemiological studies. The persistence of these antibody titers needs to be addressed by a serial sample study of antibody-positive individuals leaving a high-exposure environment to a relatively low-exposure one. It should also be emphasized that the "fine specificity" of these serum antibodies remains undetermined. Our results demonstrated a preferential reactivity of serum antibodies to B[*a*]PDE–DNA when compared to unmodified DNA or to aflatoxin B_1–DNA. The possibility that these antibodies could cross-react with other adducts that are chemically closely related to B[*a*]PDE cannot be excluded.

The observation that B[*a*]PDE–DNA adducts were not found in every case probably reflects the variation in B[*a*]P exposure and an individual's metabolic balance between activation and deactivation as well as DNA repair capacity. Adduct levels may also be present in some subjects and below the detection limit of the assays; in 11 cases tested by USERIA only small amounts of DNA were available for the assays. Compared to serum antibodies, the half-life of B[*a*]PDE–DNA adducts may be considerably shorter. The persistence of antibody titers and adducts could be measured following cessation of exposure, such as discontinuation of employment as a coke oven worker and/or of tobacco smoking. In animal studies, the persistence of B[*a*]PDE–DNA adducts *in vivo* has been 1–2 weeks (21, 22). The precise contribution of the various sources of B[*a*]P exposure—e.g., tobacco smoke, coke oven, and diet—to detectable levels of either adducts or antibodies to adducts remains to be studied.

The USERIA and SFS assay measure different endpoints and achieve their high sensitivity by different methods. USERIA utilizes antibodies to carcinogen–DNA adducts prepared by immunization of experimental animals with carcinogen-modified DNA and the immunological reaction is amplified in the solid-phase immunoassay by an immunoglobulin-conjugated enzyme that catalyzes a radioactively labeled substrate to its products at a rapid rate—e.g., 10^5 molecules per minute (6, 19). SFS measures a physical property of a carcinogen–DNA adduct—i.e., its fluorescence—and PAH, such as B[*a*]P, are highly fluorescent. In contrast to the enzyme immunoassays in which the epitope recognized by the antibody may require a sterically intact carcinogen–DNA adduct, the level of detectability of a carcinogen in the fluorimetric assay can be increased by disruption of the adduct and removing the DNA that quenches the fluorescence of the carcinogen. For example, level of detectability of B[*a*]PDE in DNA can be increased 20- to 30-fold by hydrolysis to release the B[*a*]P tetrols (7, 9). Although the spectra of PAH obtained from SFS are highly specific (7, 9), it is possible that a similar spectrum can be produced from moieties released by acid hydrolysis from

non-B[*a*]PDE chemicals that have adducted DNA. In addition, DNA adducts of non-B[*a*]PDE chemicals may share the same epitope(s) recognized by the polyclonal rabbit antiserum to B[*a*]PDE–DNA and cross-react in the USERIA. Although data obtained from a single type of assay may yield falsely positive results, positive results obtained by both SFS and USERIA strongly suggest the presence of the B[*a*]PDE moiety in the DNA sample and hence the existence of B[*a*]PDE–DNA adducts.

In this study, 67% (18 of 27) of a sample of coke oven workers had detectable B[*a*]PDE–DNA adducts by USERIA and 76% (31 of 41) had emission peaks in the area of B[*a*]P tetrols by SFS. In a previous study (20), several other occupational groups with potential exposure to B[*a*]P were studied. By using USERIA, roofers had detectable B[*a*]PDE–DNA adducts in 7 of 28 cases (25%) and foundry workers had detectable B[*a*]PDE–DNA adducts in 7 of 20 cases (35%); B[*a*]PDE–DNA adducts were not detectable in lymphocytes from a control group of donors. In addition, we did not detect B[*a*]PDE–DNA adducts by USERIA or SFS in peripheral blood lymphocytes or antibodies to the adducts in serum from 9 laboratory workers who did not smoke tobacco (unpublished results). Perera *et al.* (23) have found B[*a*]PDE–DNA adducts in 4 of 19 lung samples from lung cancer patients, but adducts were not detected in 13 lung samples from noncancer patients. Therefore, the proportion of cases with detectable adducts and the mean level of adducts are substantially higher among the coke oven workers, who also have among the highest exposure to B[*a*]P of any industrial setting.

Note Added in Proof. As noted in the text, antisera (human or rabbit) reacting with B[*a*]PDE–DNA adducts may also recognize other chemical–DNA adducts due to their polyclonal nature or to recognition of a shared epitope by the adducts (or both). We have recently found that human as well as rabbit antisera cross-react with DNA modified by chrysene, a carcinogenic PAH. The stereochemical structures of B[*a*]PDE–guanosine and (anti)chrysene-1,2-diol-3,4-oxide–guanosine are nearly identical.

We thank David A. Kandel, Corporate Manager of Industrial Hygiene, Kaiser Steel Corp., Fontana, CA, for his assistance and facilitation of this study and the workers who kindly volunteered so that this study could be done. The rabbit B[*a*]PDE–DNA antiserum was generously provided by Dr. M. Poirier, National Cancer Institute, Bethesda, MD. The technical aid of Marilyn Rowe, Ivory Baker, and Debbie Culbreth and the secretarial assistance of Michele V. McGlynn is appreciated. This work was partially supported by Grant SIG-2 to the University of Southern California from the American Cancer Society.

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