Detectable Levels of Serum Aflatoxin B<sub>1</sub>-Albumin Adducts in the United Kingdom Population: Implications for Aflatoxin-B<sub>1</sub> Exposure in the United Kingdom<sup>1</sup>

The Jack Birch Unit for Environmental Carcinogenesis, Department of Biology, University of York, Heslington, York YO1 5DD, United Kingdom

Abstract
This study aimed to estimate aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) exposure in the United Kingdom population by measuring levels of serum AFB<sub>1</sub>-albumin (alb), using immunoassay and high-performance liquid chromatography (HPLC) with fluorescence detection. A self-questionnaire on dietary habits from 104 volunteers (47 men and 57 women) in York was completed, and blood samples were collected. Serum alb was extracted, and AFB<sub>1</sub>-lysine (lys), the digest product of AFB<sub>1</sub>-alb, was isolated and measured. A sensitive ELISA (detection limit, ~1.4 pg of AFB<sub>1</sub>-lys) was developed. A good correlation was found between calibration of ELISA results and scintillation counting, for rats dosed with [3H]AFB<sub>1</sub> (r = 0.972; P < 0.001). This ELISA was subsequently used to analyze human serum alb. For United Kingdom human sera, the mean adduct levels were 29.3 ± 14.8 pg AFB<sub>1</sub>-lys equivalents (eq) mg albumin (males) and 26.9 ± 14.4 pg AFB<sub>1</sub>-lys eq/mg alb (females).

Confirmation of the ELISA data was sought using reversed-phase HPLC with fluorescence detection. HPLC chromatograms of digested York serum alb were compared to digested serum alb for humans from Qidong County, People’s Republic of China, and from AFB<sub>1</sub>-dosed rats. These all gave similar HPLC profiles. Each sample contained fluorescent material that coeluted with and just before the AFB<sub>1</sub>-lys standard. Fluorescent fractions were found to be inhibitory in a separate anti-AFB<sub>1</sub>-lys ELISA, indicating that these earlier fluorescent peaks contained AFB<sub>1</sub> residues.

Our results suggest that measurable internal AFB<sub>1</sub> exposure may be occurring in some United Kingdom individuals, albeit at lower levels than those seen for areas with high AFB<sub>1</sub> exposure. The source of this exposure may reflect the known difficulties in accurately monitoring regulated imported foodstuffs and/or the lack of regulations on other potentially contaminated imports. However, no positive correlations were found between our AFB<sub>1</sub>-lys measurements and any dietary questionnaire information. Animal studies, as well as human studies, have been important in developing exposure and internal adduct relationships in humans. Based on this literature, our AFB<sub>1</sub>-alb data indicate a mean daily exposure of 3 μg of AFB<sub>1</sub> and a mean internal dose in liver DNA of 5.9 adducts/10<sup>7</sup> nucleotides. We believe this may be an overestimate of the AFB<sub>1</sub> exposure level in the United Kingdom, and further studies are needed to accurately relate external dose and internal AFB<sub>1</sub> biomarkers in humans.

Introduction
AFB<sub>1</sub>, a human class 1 carcinogen (1), is a potential contaminant of a wide range of human foodstuffs (2). Current United Kingdom regulations (3) regarding the mandatory analysis of AFB<sub>1</sub> in imported foodstuffs are restricted to nuts, nut products, dried figs, and dried fig products and do not include all potentially contaminated foodstuffs (4). Bulk food surveillance is not an effective way to control human exposure to the aflatoxins because contamination can occur in isolated food pockets (5).

Accurate and sensitive biological markers (biomarkers) of carcinogen exposure are now used to estimate cancer risks (6). Biomarkers can be metabolites, DNA adducts, or protein adducts (7, 8). Knowledge of the metabolic fate of AFB<sub>1</sub> has led to the use of several biomarkers in blood, urine, feces, and tissues that can be used to monitor AFB<sub>1</sub> exposure. AFB<sub>1</sub> is among the few compounds for which the relationship between biomarkers and exposure has been extensively studied (9).

Once ingested, AFB<sub>1</sub> is metabolized by the cytochrome P-450 system, primarily in the liver (10). The resulting reactive AFB<sub>1</sub>-8,9-epoxide binds to DNA, forming AFB<sub>1</sub>-guanine adducts (11, 12). The persistent AFB<sub>1</sub>-formamidopyrimidine DNA adduct may be a useful biomarker of AFB<sub>1</sub> exposure but is limited by availability of tissue (13). A proportion of AFB<sub>1</sub>-guanine adducts released from DNA or RNA by repair, turnover, or chemical hydrolysis are excreted in the urine (14). Metabolism of AFB<sub>1</sub> can also lead to metabolites such as AFB<sub>1</sub>-8,9-dihydrodiol (17). The major AFB<sub>1</sub>-ad-

Received 1/27/97; revised 2/29/98; accepted 2/11/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The funding for this research was provided by the Ministry of Agriculture, Fisheries and Food (United Kingdom).

2 To whom requests for reprints should be addressed. Present address: Molecular Epidemiology Unit, 3rd floor, Algenon Firth Building, University of Leeds, Leeds LS2 9JT, United Kingdom.

3 The abbreviations used are: AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; AFM<sub>1</sub>, aflatoxin M<sub>1</sub>; alb, albumin; lys, lysine; HPLC, high-performance liquid chromatography; Tw, Tween 20; eq, equivalent(s); IAC, immunoaffinity chromatography.
duced protein found in the blood of AFB₁-dosed rats is alb; this binding occurs specifically at lys residues (17). It has been shown that formation of AFB₁-alb occurs in a dose-dependent manner, with an accumulation to steady-state levels that correlate with AFB₁-DNA adduct levels in internal organs. Hence, the measurement of AFB₁-alb adducts in the blood may be used as a surrogate dose monitor for target organ DNA adducts (18).

Rat alb has been shown to bind 1-3% of a dose of AFB₁ (19-22). Humans are reported to form AFB₁-alb adducts in the blood and DNA in a manner and rate similar to those observed in the rat (23). Therefore, because human blood alb has a half-life of approximately 20 days, AFB₁-alb adduct detection should provide a useful biomarker of cumulative AFB₁ exposure (20). Therefore, because human blood alb has a half-life of approximately 20 days, AFB₁-alb adduct detection should provide a useful biomarker of cumulative AFB₁ exposure (20).

AFB₁ biomarkers have been extensively validated using animal studies. For example, the dose-dependent excretion of AFB₁-glutamine and AFB₁ in urine has been shown in rats acutely dosed with AFB₁ (24), and the usefulness of DNA and protein adducts in chronic dosing studies has been demonstrated (20, 25). These biomarkers have now been examined in humans and appear to be useful in biomonitoring studies (14, 19, 26). It is widely considered that AFB₁-DNA and AFB₁-alb are among the most suitable biomarkers for the examination of human population exposure. They provide a measure of the amount of AFB₁ activated to AFB₁-8,9-epoxide that is available to bind to macromolecular targets with deleterious consequences (27).

Previous preliminary studies from our laboratory reported measurable levels of AFB₁-DNA adducts in autopsy tissue collected from United Kingdom individuals (28). In this study, we sought verification of this reported AFB₁ exposure by measuring human serum AFB₁-alb levels. To do this, we have established a sensitive ELISA method for monitoring human exposure. Confirmation of our ELISA results was sought using HPLC with fluorescence detection and ELISA of individual HPLC fractions. ELISA and HPLC data from AFB₁-dosed rats and from humans in known AFB₁-contaminated areas of the world, were compared to those from the York study.

Materials and Methods
H₂O₂ (30%, v/v), PBS tables, tetramethylbenzidine, Tw, NaH₂PO₄ and Na₂HPO₄ (SigmaUltra for HPLC) were supplied by Sigma Chemical Co. Poole, Dorset, United Kingdom. All solvents were HPLC grade, supplied by Aldrich Chemical Co. (Gillingham, Dorset, United Kingdom.) Other reagents (Analar grade) were supplied by Merck Ltd. (Lutterworth, Leicester, United Kingdom).

AFB₁, Rat Dosing Study. Male Wistar rats (Harlan Olac Ltd., Bicester, Oxon, United Kingdom), weighing 200-250 g, were housed in wire mesh floor cages (two per cage) and fed expanded QC diet and water ad libitum. Two animals per dose were i.p. injected with 0, 10, 50, or 200 μg/kg body weight [³H]AFB₁ (28 mCi/mmol; Moravek Chemicals, Brea, CA). Twenty-four h after dosing, bleeds were obtained from each animal. Bleeds were collected into serum vacutainers (Sarstedt Ltd., Leicester, United Kingdom), and serum was allowed to separate overnight at 4°C.

Collection of Human Blood Samples. Blood samples were collected from 104 individuals aged 18-65 years, following advertising for volunteers within the University of York. Informed consent was obtained regarding the nature and purpose of the study. All volunteers agreed that they would have no right of access to any individual datum obtained from the study and that blood samples would be used for no other purpose. Questionnaires were completed by all persons taking part in the study concerning health status, recent travel outside of Europe, and dietary habits (amounts and types of nuts/nut products, dried fruit (particularly figs and dates) spices, cereals, and beers), including vegan or vegetarian status. Volunteers were healthy and of mixed ethnicity (although they were predominantly Caucasian), and there were no volunteers with a known high risk of liver cancer. Serum was isolated from whole blood as described previously and stored at -70°C until analysis. In addition, several alb samples from Kenya (kindly donated by Prof. C. Wild, University of Leeds, Leeds, United Kingdom) and Qidong County, People's Republic of China (kindly donated by Prof. Zongtang Sun, Cancer Institute, Chinese Academy of Medical Science, Beijing, People's Republic of China) were used.

Extraction and Purification of AFB₁ Serum alb Adducts. alb was extracted from rat and human sera according to Chapat and Wild (29), using HPLC-grade solvents. alb was quantified using the Coomassie Protein Kit (Pierce and Warinner UK Ltd., Chester, United Kingdom), according to the manufacturer's instructions.

Methods for the isolation of AFB₁ residues from alb were modified from Chapat and Wild (29). Briefly, 2 mg of alb were digested with 610 μg of pronase (Calbiochem-Novabiochem, Ltd., Nottingham, United Kingdom) in 800 μl of 0.02 M phosphate buffer for 16 h at 37°C with shaking (250 rpm). Samples were stored on ice for 1 h. Pronase and undigested alb were precipitated with 2 volumes of acetone (−20°C) on ice for 2 h. Samples were centrifuged (2000 × g, 15 min, 0°C), the supernatants were retained, and the pellet was washed with 1 ml of cold acetone. The pellet was centrifuged (as above), and the two supernatants were combined. These were dried in vacuo and dissolved in 10 ml of water.

Sep-Pak cartridges (Waters Ltd., Watford, United Kingdom) were used to purify digest material according to manufacturer’s instructions, except that the numbers of passages of test material were altered to investigate AFB₁-lys recovery. AFB₁-lys was synthesized according to the method of Chapat and Wild (29). AFB₁-lys standards (0, 2.86, 11.4, and 45.7 pg) were purified by one passage and repeated passages (8). Percentage recoveries for the two processes were then determined using ELISA. Samples were dried in vacuo and dissolved in 0.5 ml of PBS for quantitation by ELISA.

AFB₁-lys ELISA. Competitive ELISAs were carried out using 7.13-1830 pg/ml AFB₁-lys standards and a monoclonal anti-AFB₁-BSA antibody, 6e9 (Biocode Ltd., York, United Kingdom). All incubations were carried out in the dark for 2 h, and between each antibody incubation, the plates were washed five times with PBS containing 0.1% Tw. Negative controls were also included to verify that nonspecific binding of the various antibodies did not confound the assay. Immulon 4 microtiter plates (Dynatech Laboratories Ltd., Billinghurst, United Kingdom) were coated with 2.5 ng of AFB₁-ovalbumin conjugate (Biocode Ltd., York, United Kingdom) in 0.05 M NaH₂CO₃-Na₂HCO₃ buffer, pH 9.6 (4 h in the dark).

HPLC was carried out using an analytical Dynamax-300A (5 μm) 25 cm × 4.6 mm reversed-phase C18 HPLC column (Waters Ltd., Watford, United Kingdom). Solvents were filtered using 0.2 μm nylon membrane filters (Whatman Instruments Ltd., Maidstone, Kent, United Kingdom) and degassed with helium. Solvent flow rate was 0.75 ml/min, with a 40-100% (v/v) methanol in 0.02 M sodium phosphate buffer (pH 7.2) 20-min linear gradient. Fluorescence detection parameters were set to excitation at 399 nm and emission at 457 nm.
ELISA inhibitory material (750 pg) from the test samples was injected onto the HPLC column, both with and without coinjection with AFB₁-lys standard.

**ELISA Analysis of HPLC Fractions.** For each HPLC run, 0.5-min fractions were collected. Each fraction was dried in vacuo and then reconstituted in 200 µl of PBS for ELISA. M107, a mouse anti-aflatoxin-BSA monoclonal antibody (an in-house monoclonal antibody produced during the study) was used as an alternative to 6e9 in the ELISA. Assay conditions were identical, except M107 was diluted 1:2 × 10² in PBS, Tw, and FCS.

**Results**

**6e9 Anti-AFB₁-lys ELISA.** The coefficient of variation of the AFB₁-lys competitive ELISA within runs was <0.1, and between runs, it was <0.2, indicating that the assay was reproducible. The detection limit was ~1.4 pg of AFB₁-lys. Investigation of the recovery of AFB₁-lys from Sep-Pak cartridges, used to purify the rat and human alb digests, revealed that losses of adduct were reduced by passage of the samples three times through the cartridge before elution. By this method, recoveries were increased from 61.1 ± 56.2 to 85.3 ± 9.2%. AFB₁-lys was used to generate the standard curve in ELISA, and results are expressed as pg AFB₁-lys eq/mg alb. For the ELISA of all digested alb samples, the sensitivity of the assay was restricted to the linear portion of standard curves. Positive results were only designated for samples giving greater than 20% and less than 80% inhibition in competitive ELISA.

**Comparison of AFB₁-lys Adduct Levels, from [³H]AFB₁-dosed Rats, by Competitive ELISA and Scintillation Counting.** The amounts of AFB₁-lys, obtained from alb digests, for rats dosed with [³H]AFB₁ at several concentrations, were measured by ELISA and scintillation counting (Table 1). There was a good statistical correlation between the ELISA and the scintillation counting data ($r = 0.972; P < 0.001$).

**ELISA Analysis of Human Serum alb from York, Kenya, and Qidong County, People’s Republic of China.** The amount of AFB₁-lys eq pg/mg of albumin from human blood samples for individuals from York, Kenya, and Qidong County were determined (Fig. 1). All samples (except the Kenyan samples) were analyzed in triplicate on at least two occasions. A cross-section of coded samples from Kenya were supplied by Prof. C. Wild (University of Leeds, Leeds, United Kingdom) to validate our methodology. Analysis of the Kenyan alb samples was also carried out independently by Wild’s group (IARC, Lyon, France). Comparison of the two sets of data indicated a good statistical correlation ($r = 0.952$). The York blood samples generally had low levels of AFB₁-alb, ~45% in the range of 15–45 pg AFB₁-lys eq/mg albumin. The Qidong County and York samples showed some overlap; however, only ~4% of the York samples had >55 pg of AFB₁-lys eq/mg albumin, compared to ~45% of the Qidong County samples. The mean values from York were 29.3 ± 14.8 pg AFB₁-lys eq/mg alb (males) and 26.9 ± 14.4 pg AFB₁-lys eq/mg alb (females). For each human sample, a coefficient of variation was determined. The coefficient of variation for the human samples in competitive ELISA were <0.1 within assays and a mean of 0.53 between assays.

**Verification of AFB₁-alb Digest ELISA Data by HPLC Fluorescence Analysis.** Standard AFB₁-lys had a retention time of approximately 8 min on the gradient system used and a fluorescence detection limit of 22.9 pg. For each test sample, 750 pg AFB₁-lys eq were injected. The injected test samples produced chromatograms with several fluorescent peaks followed by a minor peak (approximately 5% of the total integrated area) at 8 min. This minor peak coeluted with standard AFB₁-lys.

HPLC fluorescence analysis of alb digests from dosed rats and human samples revealed similar profiles, with a large number of fluorescent peaks eluting prior to the retention time of AFB₁-lys. To determine if any of these peaks contained AFB₁ residues, 30-s HPLC fractions were collected, dried, and measured by ELISA. These samples had been obtained by immunoaffinity purification of alb digest using immobilized 6e9 antibody; therefore, an alternative antibody was required for the ELISA of fractions. This would increase the probability that ELISA inhibition was due to AFB₁ residues. The antibody (mouse monoclonal antibody, M107) used for this purpose was raised in-house against AFB₁-alb-ovalbumin. M107 had a slightly higher IC₅₀ with AFB₁-lys, AFB₂, aflatoxin B₂, aflatoxin G₁, aflatoxin G₂ and AFM₁ (data not shown) and, thus, produced a less sensitive assay. For AFB₁-lys, the limit of detection in the M107 ELISA was ~3.2 pg.

HPLC fluorescence chromatograms and data obtained from ELISA of the HPLC fractions were overlaid for each test sample (Fig. 2). In each case, the data overlapped. The total amounts of fluorescent material obtained (area under the chromatogram) were calculated by comparison to AFB₁-lys standards for each test. The total AFB₁-lys eq from the ELISA of HPLC fractions for each test were also calculated. These values were expressed as a percentage of AFB₁-lys eq, measured by 6e9 ELISA, prior to HPLC (Table 2). For HPLC fluorescence detection, just over 70% of the amount of material obtained by the 6e9 ELISA was detected for all test samples. For ELISA of HPLC fractions, approximately 50% of the ELISA injected material was detected. Assumptions used for this quantitation are discussed below.

**Discussion**

Regulations regarding the mandatory analysis of AFB₁ in foodstuffs imported into the United Kingdom are restricted to nuts, nut products, dried figs, and dried fig products. However, other foodstuffs in the United Kingdom may be potentially contaminated, e.g., spices, breakfast cereals, corn and commeal, dairy products, and other processed foods (30–33). An earlier study from our laboratory reported the detection of AFB₁-DNA adducts in humans from the United Kingdom (4). The adduct levels reported in that study were not confirmed in subsequent studies in our laboratory. Problems in the development of a highly specific and sensitive AFB₁ DNA immunoassay seemed to restrict the monitoring of aflatoxin exposure via DNA adducts. Nonspecific binding of antibody and control DNA occurred in several of the assays developed. In addition, postmortem samples from elderly persons were our source of material.
Eating habits, including quantity and variety, of these persons would not reflect those in the general population. Therefore, alternative methods to measure aflatoxin exposure were sought.

Monitoring human serum albumin samples provides a useful, noninvasive measure of AFB1 exposure because AFB1-alb correlates with liver AFB1-DNA adduct levels (20), and AFB1 does not appreciably bind to any other macromolecule in the blood (17). To estimate internal exposure to AFB1, we have measured AFB1-alb adduct levels from volunteer subjects primarily by ELISA. With the use of monoclonal antibodies, ELISA provides one of the most sensitive methods for the detection small molecules. A rat dosing study was carried out using [14C]AFB1. Serum alb was extracted and digested, and AFB1 residues isolated. By comparing scintillation counting data with that from the ELISA, the reliability of the assay was established (Table 1). We confirmed our ELISA results for York human serum alb using a physicochemical method and by comparison to serum alb from AFB1-dosed rats and human serum alb samples from areas where AFB1 exposure is known to occur.

A highly sensitive AFB1-lys ELISA (detection limit, < 1.4 pg) was developed. The good statistical correlation (r = 0.972; P < 0.001) between ELISA and scintillation counting for AFB1-dosed rat provides strong evidence that our ELISA methodology was suitable for measuring AFB1-alb adduct levels. When comparing the analysis of a set of Kenyan samples by two separate groups (IARC and York), similar levels of adducts were observed (r = 0.952). Although the lower adducted samples were not in complete agreement (our results were generally slightly higher), this may be explained by the improved recovery of AFB1-lys standard observed at low levels during our isolation procedure.

ELISA of human serum samples from 104 individuals indicated that 95% contained AFB1-alb, albeit at lower levels than those from high-risk areas. The mean values for York samples were 29.3 ± 14.8 pg AFB1-lys eq/mg albumin (males) and 26.9 ± 14.4 pg AFB1-lys eq/mg albumin (females). Although no negative control serum was used for human studies, the rat study indicated that no AFB1 dose were no adducts detectable by 6e9 competitive ELISA.

To confirm our ELISA data, HPLC analysis was carried out on samples of digested human alb. AFB1-lys standard coeluted with the minor fluorescent peak at approximately 8 min, indicating the presence of AFB1-lys in the digested serum albumin. In addition, fluorescent material eluted from the column, forming several peaks, immediately prior to the AFB1-lys standard. The profiles of these peaks were similar for AFB1-dosed rat alb digests and human (York and Qidong County) alb digests.

To determine if all fluorescent peaks from the HPLC contained AFB1 residues, HPLC fractions were collected every 30 s, and ELISA of fractions was carried out. The use of the same antibody in both ELISA of HPLC fractions and IAC may confound results. For this reason, it was prudent to use a distinct antibody for these two processes. The ELISA of HPLC fractions indicated that the fluorescent peaks contained AFB1 residues. This was not unexpected because incomplete digestion of AFB1-alb, generating several fluorescent peaks that elute earlier than AFB1-lys from reversed-phase HPLC, has been reported previously (34). AFB1-lys standards and AFB1-lys connected with alb digest eluted at the time indicated in Fig. 2.

It was important to have quantitative comparisons of the data for the 6e9 ELISA, the HPLC, and the ELISA of HPLC fractions. For each test sample, 750 pg AFB1-lys eq (as determined by 6e9 ELISA) was IAC-purified and injected onto HPLC. The amounts of AFB1-lys eq determined by HPLC fluorescence and ELISA of HPLC fractions were ~ 70 and 50%, respectively, of the amount of material obtained by 6e9 ELISA. There may be some loss of material during the IAC of AFB1-alb digests carried out prior to injection on HPLC. Loss of some AFB1 residues during IAC purification of AFB1-alb digests has been reported previously (34, 35).

The amounts of adduct detected by both fluorescence and ELISA of HPLC fractions correlate reasonably well with the original 6e9 ELISA data. Although incomplete digestion products containing AFB1 residues will be fluorescent and inhibitory in ELISA, the exact nature of these adducts has not been established. Therefore, the absolute amount of non-AFB1-lys fluorescence and ELISA inhibition remains uncertain. AFB1 has been shown to bind to albumin specifically at lys residues (17). Therefore, incomplete digestion products of AFB1-alb will be short peptides linked to AFB1 via lysine. The fluorescent and ELISA properties of these residues are likely to be similar to those of AFB1-lys.

The major finding from these studies is the implicated levels of internal AFB1 exposure and the estimated dose in target organ DNA. There appears to be a clear linear relationship between AFB1 intake and AFB1-alb adducts in rats (20) and in humans (19, 36). The average exposure of AFB1 for persons in York based on AFB1-alb levels would be ~3 μg/day [based on 3% of ingested AFB1 being bound to serum albumin (19), a 30-fold accumulation of AFB1-alb (37), and total pe-
Reversed-phase HPLC with fluorescence detection was carried out for digested alb from an AFB₁-dosed rat (a), individuals from York (b), and individuals from Qidong County (c). Fractions were collected every 30 s of the HPLC run. Each of these was analyzed using an ELISA distinct from the original 6e9 ELISA. These are overlaid. Fluorescence detection (line graph; left) was measured in mV. The ELISA of HPLC fractions (bar chart; right) was measured in aflatoxin-lys eq (fmol). For ELISA of fractions, the AFB₁,lys eq calculated for a blank (phosphate buffer) injection at each time point was subtracted from the value for each test sample. Arrow, AFB₁-lys standard coeluted.

Peripheral blood albumin = 96 g). On the basis of this level of AFB₁-abl, the expected mean internal dose at liver DNA would be 5.9 adducts/10⁷ nucleotides (20). Our value for AFB₁ exposure was lower than those estimated for parts of China (southern Guangxi), where up to 120 µg/day exposure has been reported (38), but was relatively high when compared to some African countries with exposure between 0.2 and 12 µg/day.

No positive correlation was found between AFB₁-alb adduct levels and any of the responses from the dietary questionnaire. There was difficulty in obtaining reliable details of relative amounts and types of foodstuffs consumed by people. Many people simply replied often or sometimes to specific food questions. The sample population used in this study was also fairly small.

The relationship between AFB₁-alb adduct levels and exposure was recently estimated for humans (23). The value was 1.56 pg AFB₁-lys eq/mg albumin per µg AFB₁/kg body weight following acute exposure. This value was based on ~25% recovery of AFB₁ residues and 1% of AFB₁ being bound to peripheral blood alb. There are many stages prior...
Serum Aflatoxin B1-Albumin in the United Kingdom

It is also represented as a percentage of the ELISA estimated material. AFB1-lys eq (750 pg: based on original ELISA) was injected for each test. This exposed to AFB1 were found to contain measurable levels of immunoassay in which AFB1 adducts may be lost. We mined AFB1-lys eq (pg) \times \frac{\text{ELISA of HPLC fractions-determined AFB1-lys eq (pg)}}{6 \times 10^9 \text{ELISA-determined AFB1-lys eq (pg)}} \times 100.

AFB1-alb values and AFB1 exposure, e.g., tamed in Ref. 23. Our calculations are in good agreement with the value obtained in Ref. 23.

There are several anomalies with the relationship between AFB1-alb values and AFB1 exposure, e.g., control human serum albumin “obtained from persons unlikely to have been exposed to AFB1,” were found to contain measurable levels of adducts, \( \sim 50 \pm 25 \) pg AFB1-lys eq/mg albumin (34). This would represent a daily exposures between 4 and 6 \( \mu \)g of AFB1 per day. A relationship between AFB1-alb and AFB1 exposure was recently reported for the F344 rat (23). However, discrepancies exist for studies in rats, e.g., a study on F344 rats chronically dosed with 200 \( \mu \)g/kg AFB1 daily (35). The AFB1-alb value at steady states should have been approximately 300 pg AFB1-lys eq/mg albumin. The reported values were 400 pmol AFB1-lys eq/mg albumin (or \( >100,000 \) pg AFB1-lys eq/mg albumin).

The above examples have used immunoassay to obtain AFB1-alb values. It is possible that nonspecific inhibition in immunoassay may be effecting the results. Nonspecific inhibition was determined using albumin from a Boston donor for a study measuring AFB1-alb values for residents of Guangxi Province, People’s Republic of China (19). This value was subtracted from the values obtained for Guangxi Province samples. Although there was a highly significant association between AFB1 intake and AFB1-alb values, the regression line did not pass through the origin, i.e., no dose, no adducts. At no dose, the level of AFB1-alb adducts was \( \sim 45 \) pg AFB1-lys eq/mg of alb. This would reflect a level of AFB1 consumption of \( \sim 5 \) \( \mu \)g per day. It was suggested that this may be a nonspecific response in the assay. Considering alb from a Boston donor had been used a negative control, this was surprising.

It would appear that, on a population basis, there is a good relationship between AFB1 consumption and AFB1-alb adduct level. However, there seems to be no absolute certainty that measurement of a quantity of adduct relates to a fixed level of AFB1 intake at the individual level. Correlation of estimated dietary intake of aflatoxin and measurement of AFB1-alb was carried out for persons in the People’s Republic of China (36). In this study, AFB1 exposure estimates of \( \sim 100 \) \( \mu \)g/day lead to AFB1-alb levels, ranging from 0 to 200 pg AFB1-lys eq/mg albumin. This type of variation makes it difficult to relate AFB1-alb adduct levels to AFB1 exposure at the individual level. Obtaining quantitative data on AFB1 dietary intake for individuals over extended periods even when plate food was analyzed may create difficulties with these types of correlation (39). Differences in the quantitative disposition of AFB1 metabolites related to ethnicity, age, sex, nutrition, or disease status of individuals may also hinder AFB1 exposure correlations.

Levels of AFB1-alb found in populations chronically exposed to AFB1 are related to the half-life of alb (19, 20). Much of the data on alb turnover in humans are based on limited experiments in humans injected with radiolabeled albumin (40). However, alb turnover is more variable for persons with diseases such as cirrhosis or hepatitis (41). Temporal patterns of aflatoxin-alb adducts in residents of Daxin, Qidong County, People’s Republic of China, have been determined using fluorescence detection for AFB1-lys. The profiles of digest material from AFB1-dosed rat and human York and human Qidong County alb using HPLC were all virtually identical, including a minor peak that coeluted with synthetic AFB1-lys standard. A separate ELISA of HPLC fractions suggested that these fluorescent peaks were AFB1 derived. Quantitative estimations of AFB1-lys eq were in good agreement by all three methods. Although no extremely high levels of AFB1 exposure were detected, the detection of some AFB1-alb adducts in the blood suggests that exposure to AFB1 has occurred, and there may be an associated health risk.

Using AFB1-lys as a biomarker serves to estimate AFB1 intake and the effective dose at the critical target. There is clearly considerable evidence from the studies in rats relating AFB1 intake, AFB1 binding at liver DNA, and surrogate dose monitors (blood and urine adducts). For alb adducts, there appears to be a linear relationship between AFB1 intake and AFB1-alb adducts. Despite this linear relationship, there are some noteworthy deviations in human studies, as outlined above. It would, thus, appear that absolute predictions from AFB1 biomarkers of AFB1 exposure should be done cautiously. AFB1-alb measurements serve as useful dose monitors in parts of the world where there is high AFB1 contamination. AFB1-alb levels are the most accessible and useful means of monitoring the efforts to reduce AFB1 exposure (or alter AFB1 metabolism) at critical sites such as liver DNA. Levels of exposure to AFB1, extrapolated from our AFB1-lbs determinations, would suggest that United Kingdom regulations for AFB1 contamination may need to be investigated. Three \( \mu \)g/day may be an overestimate of the true level of exposure, but until more reliable human data exist, we must rely on the correlations already established for our estimates.

Conclusions. The results from scintillation counting of albumin from dosed rats were in good agreement with ELISA data in our study. The same ELISA was used to quantify alb adducts in human serum samples from the York area. HPLC was carried out using fluorescence detection for AFB1-lys. The profiles of digest material from AFB1-dosed rat and human York and human Qidong County alb using HPLC were all virtually identical, including a minor peak that coeluted with synthetic AFB1-lys standard. A separate ELISA of HPLC fractions suggested that these fluorescent peaks were AFB1 derived. Quantitative estimations of AFB1-lys eq were in good agreement by all three methods. Although no extremely high levels of AFB1 exposure were detected, the detection of some AFB1-alb adducts in the blood suggests that exposure to AFB1 has occurred, and there may be an associated health risk.

Using AFB1-lys as a biomarker serves to estimate AFB1 intake and the effective dose at the critical target. There is clearly considerable evidence from the studies in rats relating AFB1 intake, AFB1 binding at liver DNA, and surrogate dose monitors (blood and urine adducts). For alb adducts, there appears to be a linear relationship between AFB1 intake and AFB1-alb adducts. Despite this linear relationship, there are some noteworthy deviations in human studies, as outlined above. It would, thus, appear that absolute predictions from AFB1 biomarkers of AFB1 exposure should be done cautiously. AFB1-alb measurements serve as useful dose monitors in parts of the world where there is high AFB1 contamination. AFB1-alb levels are the most accessible and useful means of monitoring the efforts to reduce AFB1 exposure (or alter AFB1 metabolism) at critical sites such as liver DNA. Levels of exposure to AFB1, extrapolated from our AFB1-lbs determinations, would suggest that United Kingdom regulations for AFB1 contamination may need to be investigated. Three \( \mu \)g/day may be an overestimate of the true level of exposure, but until more reliable human data exist, we must rely on the correlations already established for our estimates.

Acknowledgments
We thank Prof. C. Wild (University of Leeds, Leeds, United Kingdom) and Prof. Zongtang Sun (Chinese Academy of Medical Science, Beijing, People’s Republic of China) for supplying alb samples from Kenya and China, respectively.

Table 2 Calculation of AFB1-lys eq/pg for HPLC fluorescence and ELISA fractions

<table>
<thead>
<tr>
<th>Test sample</th>
<th>HPLC fluorescence analyzed</th>
<th>ELISA of HPLC fractions analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total AFB1-lys eq (pg)</td>
<td>% of ELISA of estimated material</td>
</tr>
<tr>
<td>York</td>
<td>549</td>
<td>73.4</td>
</tr>
<tr>
<td>Qidong</td>
<td>555</td>
<td>74.0</td>
</tr>
<tr>
<td>County</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>537</td>
<td>71.6</td>
</tr>
</tbody>
</table>

a Fluorescence-determined AFB1-lys eq (pg) \times 10^9 ELISA-determined AFB1-lys eq (pg) \times 100.

\[ \text{ELISA of HPLC fractions-determined AFB1-lys eq (pg)} \times \frac{\text{ELISA of HPLC fractions-determined AFB1-lys eq (pg)}}{6 \times 10^9 \text{ELISA-determined AFB1-lys eq (pg)}} \times 100. \]
References


Detectable levels of serum aflatoxin B1-albumin adducts in the United Kingdom population: implications for aflatoxin-B1 exposure in the United Kingdom.

P C Turner, K H Dingley, J Coxhead, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/7/5/441

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.