Use of an Improved Method for Analysis of Urinary Aflatoxin M1 in a Survey of Mainland China and Taiwan

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Abstract
An improved monoclonal antibody immunoaffinity chromatography/high-pressure liquid chromatography/fluorescence detection method was developed to measure aflatoxin (AF) exposure by quantifying AFM1 in human and rat urine samples.

Analysis of different amounts of various AF metabolites showed that the immunoaffinity resin was highly selective for aflatoxin B1 (AFB1), AFB2, and AFM1. Recovery of added AFs increased with the amount of immunoaffinity resin and was virtually complete within the range of 0.01–10 ng of AFM1, by using 7 ml of resin. The detection limit of this method is 0.5 pg/ml urine.

Rats dosed with tritiated AFB1 excreted in their urine tritiated AFM1, among other AF metabolites, as indicated by chemical derivative confirmation and chromatography with authentic AFM1 and agreement of radioactivity and fluorescence quantitation. A linear dose-response relationship was found over the range of 0.05–50 μg/kg of body weight/day. Two humans dosed with 1.0 μg of pure AFB1 excreted 6–7% of the dose as urinary AFM1 over 5–7 days.

Pooled urine samples from 30 men from each of 69 rural counties in mainland China and 16 survey areas in Taiwan, with two villages per county or area, were analyzed with this improved method (170 villages total). The correlation coefficient of urinary excretion of AFM1 compared between villages within all 85 survey areas was 0.50 (P < 0.001). Sixty-five % of the samples contained detectable concentrations of AFM1, with an average excretion of 3.1 ng/12 h. Assuming an excretion rate of 2–6%, this AFM1 excretion corresponds to a very low average daily AF consumption of 0.1–0.3 μg/day (possible range, 0–11 μg/day). Patterns of urinary excretion of AFM1 were similar in mainland China and Taiwan.

Introduction
AFs2 are a group of toxic secondary metabolites produced by the fungus Aspergillus flavus/parasiticus, which may contaminate a variety of human and animal food crops (1). The most common and most biologically active of these fungal metabolites is AFB1 (2). Upon ingestion and absorption of AFB1, a series of metabolites is produced, primarily in the liver. These include AFepoxide, AFM1, AFB1, AFQ1, AFB2, and aflatoxicol (3).

AF has been declared a human carcinogen by an IARC working group (4). In contrast to several earlier ecological studies (5–9), our comprehensive ecological study of 48 counties in rural China reported no relationship (r = −0.17) between AF exposure and a wide range of primary liver cancer mortality rates (10).

This conclusion was challenged (11, 12) on grounds that the methodology (13) to analyze the urine samples for oxidized AFB1 metabolites as a means of assessing AF exposure was inadequate. Because of this criticism, we developed a much more sensitive and specific method using monoclonal immunoaffinity column resin and HPLC to measure AFM1 in a more recent collection of urine samples from 16 survey sites of Taiwan and 69 rural counties in mainland China (including samples from the original 48 counties reported earlier).

Materials and Methods
Experimental Design of Mainland China Ecological Study. The study design for the cross-sectional ecological survey performed in 1983 was described by Chen et al. (14). The 1989 study was essentially identical to the 1983 study, with a few important differences. Only those details relevant to the data presented in this report are described here. The original 65 survey counties were chosen in 1983 by a multistage sampling procedure in order to obtain a wide geographical scatter and to encompass the full range of mortality rates for seven of the more common cancers in China. These counties were located in 25 provinces and autonomous regions and widely dispersed throughout the mostly rural regions of mainland China. In 1989, an extra four counties from the more remote regions of China were added to the study (two from Xinjiang Autonomous Region and two from Ningxia Autonomous Region), bringing the total to 69 counties. The additional counties contained high percentages of national minority groups and extended the breadth of dietary practices for the entire survey. In 1983 two xiangs (subcounties) were randomly selected within each county, and within each xiang, one village was randomly chosen, yielding a total of 130 villages. Survey xiangs were restricted to those within 4 h travel time of the county laboratory. Within each of the villages, 25 households were randomly selected, and within each household, one adult male was randomly selected.

The abbreviations used are: AF, aflatoxin; DMSO, dimethylsulfoxide; HPLC, high-performance liquid chromatography; TEAF, triethylammonium formate.

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2 The abbreviations used are: AF, aflatoxin; DMSO, dimethylsulfoxide; HPLC, high-performance liquid chromatography; TEAF, triethylammonium formate.
selected from an official registry of residences. From a random one-half of the households, one male, age 35-64 years, was then invited to participate in the study. If the selected subject was absent or declined to participate (<1% of those initially identified), then another subject in a neighboring household was selected. The same individuals participating in the 1983 survey were invited to participate in the 1989 survey. If the subjects were no longer available (by reason of death, illness, translocation, unwillingness to participate, or falling outside the age group of the study), then new participants were randomly selected. Five additional households per village were randomly selected for inclusion so that from each county 60 male subjects participated, yielding a total of 4140 subjects (69 counties, 30 males from each of 2 villages per county). This study was approved by the Cornell University Committee on Human Subjects.

Experimental Design of 1989 Taiwan Ecological Study. A stratified multistage random sampling procedure was used to select 16 survey areas in Taiwan. The sampling procedure was based on the level of urbanization, ethnicity, crude cancer mortality rates, population size, and geographical location. These survey areas included coastal, inland, mountain, and plains areas. In each survey area, two xiangs, in each xiang, two villages, and in each village, 33 males, ages 35-64, were chosen randomly. If the number of subjects was not enough to meet the intended number, then additional subjects in neighboring households were selected. The total number of participating male subjects was 1056 (16 survey areas, 33 males from each of two xiangs). When added to the survey subjects of mainland China, a total of 5196 adult males were included, compared to 3250 adult males in the 1983 survey, most of whom participated in both surveys.

Human Urine Collection and Storage. Each male was invited to donate a urine sample. In 1983 this was a 4 h collection after an oral dose, for other purposes, of riboflavin and ascorbic acid. In 1989, an overnight 12 h urine sample was collected from 7 p.m. to 7 a.m. A 5% aliquot of each sample was combined into a pool for each xiang, then was stored at -20°C. Within 1 week the samples were defrosted and urine specimens were frozen complete 24 h urine sample, two men were administered 1.0 .tg AFM, and were stored at -80°C. The AFs were eluted with 14 ml of 80% DMSO in water, and then evaporated under nitrogen in a 50°C water bath. After the eluate was evaporated to 0.3-0.5 ml, 0.3 ml of 0.1 mol/liter HCl was added, mixed, and cooled to room temperature, and then 0.5 ml of 1 mol/liter ammonium formate, pH 4.5, was added. The volume was adjusted to 10 ml with water, and the sample was applied to a 7 ml monoclonal immunofluorometric column and allowed to elute under gravity. For the subsequent elutions, a small aquarium pump was used to apply slight pressure to the top of the column. The column was washed with two 14-ml volumes of PBS, pH 7.4, and then 14 ml of water. The AFs were eluted with 14 ml of 70% DMSO in water; this was followed with two more washes of 14 ml PBS. The DMSO fraction and the two following PBS washes were combined and diluted to 84 ml with water to dilute out the DMSO. This sample was applied to a conditioned Sep-Pak cartridge, prepared as described above, to remove DMSO. The AFs were eluted with 8 ml of 80% methanol, collected in a glass conical 15 ml-centrifuge tube, and then evaporated under nitrogen in a 50°C water bath to 0.1 ml. To this solution 100 .&l of 0.1 mol/liter HCl was added, which was then mixed and adjusted to pH 3.0 with 200 .&l of 1.0 mol/liter ammonium formate, pH 4.5. The sample was then adjusted to 0.5 ml with 0.01 mol/liter TEAF and submitted to HPLC analysis.

Urinary AFM, HPLC Analysis. The HPLC system was a gradient model 334 from Beckman Instruments (Fullerton, CA) with a model FL-750BX fluorescence detector (McPherson Division of Schoeffel Instruments Corporation, Acton, MA). The excitation wavelength was 365 nm with an interference UT-1 filter. The emission filter was a long-band pass filter. The column was an Ultramex 5 .m C18 250 X 4.6 mm column (Phenomenex, Inc., Torrance, CA) and was heated with a column temperature controller (Timberline, Inc., Boulder, CO), a model SP 4290 integrator (Spectra Physics, San Jose, CA), was attached. The mobile phase was 13% ethanol and 3% methanol in TEAF, and the flow rate was 0.6 ml/min. A typical system pressure of 50-70 bar was observed. A 0.2 μm precolumn filter (Upchurch Scientific, Oak Harbor, WA) was changed daily. Authentic AF standards were obtained from Sigma Chemical Co. (St. Louis, MO). AFN7G was a gift from Dr. John Groopman (Johns Hopkins University, Baltimore, MD).

For each human urine sample, two subsamples of 0.25 ml each were prepared for HPLC analysis. One subsample was run as is, and a second was run after adding an amount of AFM, standard approximately equal to that which appeared in the first chromatogram. The retention time of AFM, was about 12 min. Calculations were made by comparing the integrated peak areas for AFM, external standards with peak areas for the sample AFM, areas. Excretion of AFM, per 12 h was calculated by multiplying by the appropriate volume of the 12 h urine pools.

Rat Experimental Protocol. Male Fisher-344 rats were purchased at 6 weeks of age from Charles River Breeding Laboratories, Inc. (Wilmington, MA) and housed singly in wire-bottomed metabolic cages in a temperature- and humidity-controlled environment with a 12-h-light/12-h-dark photoperiod. Food and water were available ad libitum. Animals
were fed AIN-76A diet (17), and at 7 weeks of age had attained body weights between 160 and 180 g. They were then randomly assigned into seven groups of five animals per group. Six groups were dosed daily Monday through Friday by oral gavage for 2 weeks and then killed. One group was dosed with AFB2, AFG1, and AFG2 for 1 week each on Monday through Friday, and after 3 weeks they were killed. The dose for each of these AFs was 50 μg/kg of body weight/day. 3H-AFB1 (Moravek Biochemicals, Brea, CA) and unlabeled AFB2, AFG1, and AFG2 were administered in tricaprylin oil. Among the six groups dosed for 2 weeks, a control group of rats was treated with only tricaprylin oil, whereas the other five were treated with 3H-AFB1 at dosages of 0.005, 0.05, 0.5, 2, and 5 μg/kg of body weight/day.

Rat Urine Collection. Quantitative collection of urine samples was accomplished by washing down the metabolic cage surfaces with 10 ml of water each day. The average daily sample contained approximately 10 ml of water and 10 ml of urine. Three-day urine samples from 9:00 a.m. Wednesday morning through 9:00 a.m. Saturday morning of each week were pooled and kept frozen at −80°C. Generally, 1 ml of pooled urine was adjusted to pH 4 with 0.1 mol/liter HCl. The volume was adjusted to 10 ml by adding water and analyzed for urinary AFM1, as described above. Radioactivity was quantitated with Liquiscint scintillation fluid (National Diagnostics, Somerville, NJ) in a model LS 7000 liquid scintillation counter (Beckman Instruments).

Confirmatory Test for Urinary AFM1. A chemical derivative confirmatory test for AFM1 was performed on several human and rat urine samples to confirm the identity of AFM1 by this new method. This test was originally developed to identify AFB1 in food (18), although later it was used to identify AFM1 in human urine (19). In the present study, 10 suspected AFM1-positive urine samples from mainland China with different levels of AFM1, and 1 rat urine sample from each of the AFB1-dosed groups were selected for AFM1 confirmation. When the urine was ready for HPLC injection, it was adjusted from 0.50 to 0.75 mL with 0.01 mol/liter TEAF. A 0.25 ml subsample was evaporated just to dryness in a 50°C water bath under nitrogen to remove residual water. Ethanol (0.25 ml) was added, and the vial was placed in a 50°C water bath for 30 min to evaporate the ethanol under nitrogen. Concentrated formic acid (0.2 ml) and 1 drop of thionyl chloride were added to the residue. The mixture was allowed to stand at room temperature for 5 min and then was evaporated to dryness under the same conditions as described above. The residue was dissolved in 0.25 ml 0.01 mol/liter TEAF and injected onto the HPLC column. Authentic AFM1 standard dissolved in ethanol also was treated with formic acid and thionyl chloride in the same manner as the test samples for HPLC analysis.

Statistical Methods. Minitab statistical software, Release 8 (Minitab Inc., State College, PA) was used to assess simple statistic parameters.

Results
Development of the AFM1 Method. This newly modified method was tested initially by examining the recovery of various AFs added to water (Table 1). Using the 4-ml immunoadfinity column recommended in the original method of Groopman et al. (15), only AFB1, AFB2, and AFM1 bound well to the resin and were eluted by DMSO. Recoveries of AFG1 and AFP1 in the DMSO fraction were poor and only reached 25%. Other AF metabolites, including AFN7G, were completely eluted before application of the DMSO. Recovery of added AFM1, using three different column volumes, is shown in Table 2. In 4 ml of resin, percentage of recovery decreased when decreasing amounts of resin were used. In contrast, recovery improved when increasing amounts of resin were used. Recovery of AFM1 reached 96% when using 7 ml of resin and as little as 10 pg of AFM1 per 10-ml urine sample. Subsequently, 10 ml of urine and 7 ml of resin were used in all analyses, unless otherwise stated.

HPLC chromatogram of 10 pg AFM1 standard is shown in Fig. 1. Although this very small quantity of pure AFM1 standard was clearly measurable, urine samples, in contrast, contained considerable amounts of interfering fluorescent material. To minimize the background fluorescence, the urine sample volume was reduced from the original 25 to 10 ml. With these conditions, the detection limit of this method was about 2.5 pg of AFM1 per injection, or about 0.5 pg/ml of urine. Among every 20 human subjects, one human control urine sample spiked with 10 pg AFM1 was analyzed. A total of 10 spiked samples showed a mean recovery of 96% (87–109%). In addition, 12 human samples from China containing varying
Urinary Aflatoxin dose between 0.05-50 pg/kg of body weight/day and the logarithm of urinary AFM was correlated (r = 0.96), with a coefficient of variation of about 10%.

**Rat Urinary Excretion of AFM**. No potentially interfering peaks were detected in the urine samples of the control animals or in the urine samples from animals dosed with AFB2, AFG1, or AFG2. About 10% of the administered radioactivity was excreted in the urine for each of the [3H]AFB1-dosed groups. Between 37 and 68% of the urinary radioactivity eluted in the void and wash volumes, reflecting the nonretention of many of the AFB1 metabolites by the resin (see Table 1).

AFM1 was detected in the urine samples of all AFB1-dosed rats except those in the lowest dosage group. Approximately 1-2% of dietary AFB1 was excreted as urinary AFM1. There was a linear relationship between the logarithm of the dose between 0.05-50 μg/kg of body weight/day and the logarithm of urinary AFM1 in ng/day (r = 0.91; P < 0.001). The suspected AFM1 peak of the rat urine samples was chromatographically identical to the suspected AFM1 in the human urine samples and to the AFM1 standard. Moreover, added AFM1 standard cochromatographed as one peak with the suspected AFM1. And finally, quantitation of AFM1 either by fluorescence detection or by radiotracer content in the collected peak was also in good agreement.

**Confirmatory Tests for AFM1 in Human Urine**. Selected human urine samples prepared for HPLC analysis were each divided into three subsamples. The first untreated subsample was chromatographed, and a peak with a retention time similar to authentic AFM1 standard was observed (Fig. 2). In the second subsample, added AFM1 standard cochromatographed with the suspected AFM1 peak (Fig. 3). The suspected AFM1 peak of the third subsample was selectively derivatized by thionyl chloride treatment (Fig. 4). When this procedure was performed on the radiolabeled rat samples, not only did the fluorescent suspected AFM1 peak disappear, but most (about 93%) of the suspected AFM1 radioactivity was not recovered at the expected retention time. The results of these various confirmatory tests provided evidence that the suspected AFM1 peak of the urine samples was the same compound as the standard AFM1.

**Excretion of AFM1 by Dosed Humans**. Two adult human males were administered 1.0 μg AFB1, and urine was collected over the next 10 days until no further AFM1 was detected. One subject excreted 5.6% of the initial AFB1 as AFM1 over a period of 5 days, whereas the second subject excreted 6.6% of the AFB1 dose as AFM1 over a period of 7 days (Table 3). Maximum excretion occurred during the second day after dosing. Total body clearance half-lives were approximately 0.9 days. Urine samples collected before and after this period contained no AFM1.

**Analysis of AFM1 in Human Urine**. A total of 138 urine samples from mainland China and 32 from Taiwan representing pools for each village were analyzed (Fig. 5). Eighty-eight pooled samples (64%) from mainland China and 21 (66%) from Taiwan showed measurable levels of urinary AFM1. The mean and highest levels of AFM1 excretion rates in mainland China were 3.2 and 108 ng/12 h, respectively, whereas the mean and highest levels in Taiwan were 2.7 and 17 ng/12 h, respectively. Urinary AFM1 excretion rates were not significantly different between mainland China and Taiwan.

With the exclusion of the single highest outlier value, the correlation between urinary AFM1 measurements from the two xiangs within each of the 69 counties in rural China was r = 0.50 (P < 0.001), thus indicating considerable homogeneity of AF exposure within each survey site and providing validation of the AF exposure procedures. However, AF exposure in 1989 (as urinary AFM1) was not correlated with AF exposure in 1983 (as urinary oxidized AF metabolites) (r = -0.04), thus reflecting either a considerable variability of AF exposure over time or the modification in methodology.

**Discussion**

The immunoaffinity column method did not perform as well as previously indicated (15, 16, 20). Not all metabolites were retained on the resin; some (AFN7G and AFQ1) were eluted either in the void volume or with the PBS and water eluants (Table 1). However, AF binding patterns, as indicated by elution order, still reflected the sequence of binding constants.
observed for the "2B11" antibody originally used in the preparation of the column (16). AFB1, AFM1, and AFB2 bound well and were eluted quantitatively with DMSO. The difference in AFN7G binding between this and previous studies suggests that slight changes in procedure or in batches of resin material may be responsible for major alterations in performance of the immunoaffinity column procedure.

In light of the previous demonstration that urinary AFM1 is highly correlated with AFB intake (15, 19–21), we proceeded to explore and to adopt four major changes in the methodology earlier used in studies in Guangxi (20), The Gambia (15), and Shanghai (22). These changes included the following: First, the chief analyte of interest was changed from AFN7G to AFM1. In addition to the poor recovery of AFN7G from the immunoaffinity column, AFM1 was also chosen because of its later retention on the HPLC column, thus minimizing the interference resulting from the earlier eluting peaks; Second, the earlier HPLC method relied on a mobile phase gradient. This modified method, which was less encumbered with early peak interference, employed isocratic elution, which typically yields more reproducible peak areas and shorter analysis times; Third, the immunoaffinity column volume was increased from 4 to 7 ml, thus obtaining reproducible recovery of the extremely low levels of AFM1 usually found in urine samples; and fourth, AFM1, which is more fluorescent than AFN7G and which is present at about twice the concentration of AFN7G in human urine (15, 20), was analyzed by a specialized McPherson model FL-750BX fluorescence detector, which uses a 200-W xenon-mercury lamp that had a strong emission peak at about the 365 nm absorption maximum that is characteristic of AF metabolites. The matching of this emission peak and the absorption maximum of AFs contributed to the enhancement by more than 100-fold of the detection limit of this method when pure standards between this and earlier methods were compared (22). In studies validating the use of AFN7G as a biomarker of AFB intake (15, 19, 20), daily AFM1 excretion also exhibited a relatively strong and highly significant correlation (r = 0.55–0.66) with AFB intake, although this correlation was somewhat less than that for AFN7G. All of these considerations and modifications served to enhance the sensitivity of the present method to assess AF exposure, particularly at low doses.

The detection limit of the method used for studies validating AFN7G and AFM1 excretion as biomarkers of AFB intake in Africa and China was approximately 40 pg/ml urine for both metabolites (15, 20, 22). However, Weaver and Groopman (23) recently developed still-newer methods for urinary AFN7G based on immunoaffinity column sample preparation.
and either synchronous fluorescence spectroscopy or HPLC quantitation. For HPLC quantitation, the AFN\textsuperscript{7}G-containing fraction was collected from one HPLC run, then subjected to acid hydrolysis and subsequent quantitation as AFB\textsubscript{1}-diol by another HPLC run. The detection limit of this method for pure AFN\textsuperscript{7}G standard was about 1.5 pg/ml. Our method, when comparing standard solutions of AF in water, exhibited a detection limit of 0.2 pg/ml. In another recent method also using an immunoaffinity column and HPLC but using postcolumn derivatization, the detection limit was 6.8 pg/ml (24).

Our study appears to be the first in which humans were intentionally dosed with pure AFB\textsubscript{1}. The enhanced sensitivity of this new method allowed for very small doses of AFB\textsubscript{1} to be consumed. The quantity consumed is equivalent to the amount of AFB\textsubscript{1} found in a sandwich made with peanut butter containing the maximum legal limit of AFB\textsubscript{1} in the United States (20 ppb). The two adult male subjects (Z. C. and M. R.) who consumed AFB\textsubscript{1} gave similar urinary excretion patterns. The percentage of the original dose of AFB\textsubscript{1} found in the urine was 6–7% over a period of 5–7 days, which is higher than the 1–4% earlier estimated by us and others (15, 19, 21) for shorter collection periods. Randomly collected urine samples from these same two subjects showed no evidence of AFB\textsubscript{1} exposure.

The purpose of the rat experiment was to confirm the biological source, the chemical identity, and the quantitation of AFM\textsubscript{1} in urine. Based on the lack of interfering peaks from AFB\textsubscript{2}, AFG\textsubscript{1}, or AFG\textsubscript{2} and the dose-response relationship between AFB\textsubscript{1} dose and AFM\textsubscript{1} excretion, we are confident that the source of the suspected AFM\textsubscript{1} peak is derived from the dose of AFB\textsubscript{1}, and is not from other AF metabolites. The percentage of administered radiolabel derived from the ingested AFB\textsubscript{1} found in the urine, the percentage of urinary AF that binds to the affinity column, and the percentage of dosed AFB\textsubscript{1} excreted as urinary AFM\textsubscript{1} all confirm similar percentages found in earlier studies (13, 16). Fluorescence detection and radioactivity measurements provided similar estimates of AFM\textsubscript{1} concentrations; moreover, formic acid-thionyl chloride derivatization of the peak provided additional confirmation of its identity.

AF contamination of foods (1) observed over time has long been known to be exceptionally variable. Thus, it would be expected that urinary excretion of AF metabolites by individuals consuming those foods also would be unusually variable, although somewhat less so. With a biological half-life of about 1 day, the urinary excretion of biomarkers such as AFM\textsubscript{1} could reflect AFB\textsubscript{1} intake averaged over 1–3 days. In the ecological study described herein, 170 pooled urine samples, each comprising 30 men, were analyzed, thus yielding approximately 30–90 man-days of integrated exposure for each data point, or approximately 5,100–15,300 total man-days of exposure for the entire survey. This represents substantial improvement in sampling stability over previous studies, although seasonal and yearly changes in AF exposure still remain underrepresented. It is difficult to predict how these results would compare to surveys performed in other seasons or years. The sensitivity of the method reported herein allows for the detection of 1 subject out of 30 in a pool consuming about 1 &g of AFB\textsubscript{1} once during the 3 days prior to urine sampling and pooling. Thus, the combination of sampling stability and analytical method sensitivity greatly improves the ability to assess AF exposure.

In the survey in mainland China and Taiwan, the urinary excretion of AFM\textsubscript{1} was very low, with one-third of the samples below the detection limit. Assuming that the 12 h collection represents one-half of the daily excretion and that 2–5% of AFB\textsubscript{1} consumed in the diet is excreted as AFM\textsubscript{1}, the average intake of AFB\textsubscript{1} in mainland China and Taiwan was about 0.1–0.3 &g/day; the highest estimated level of intake was about 11 &g/day. Most of these intakes are considerably below those from high-primary liver cancer areas such as The Gambia, Guangxi, and the Philippines (15, 19, 20), where average in-
takes have been reported to range from 12 to 77 μg/day. They are also below previous reports for Taiwan of total urinary aflatoxin excretion (25).

Because these low estimates of AFB1 intake were puzzling, we reanalyzed a collection of samples blind about 1 year later to confirm our earlier analyses. Correspondence between replicates was excellent. As noted earlier, validation of this method also was examined by several other analytical procedures, including assessment of AFM1 excretion by rats and humans. A possible explanation of these low levels is deterioration of the AFM1 during the 4 years between collection and analysis. No direct evidence is available on this point. Wild et al. (26) contend that AF-albumin adducts in sera are stable when kept frozen at -70°C for as long as 15 years. Our own experience with the samples was that no decay occurred over 1 year between the first and second testing of a subset of samples. Thus, we are confident that these estimates of AF intake are reliable. It also should be noted that this estimate of relatively low AF intake corresponds to the findings of two other reports from China (27, 28), both of which used an ELISA method to directly measure AFB1 intake and which gave mean intakes of 0.1 and 3.4 μg/day for subjects residing in Fusui county. In a study of aflatoxin exposure in The Gambia, Wild et al. (29) found an average daily intake of 1.4 μg/day. In a survey of chemical contaminants of food in four areas of China, the average intake of AFB1 was 0.15 μg/day (30). In our survey county, corn consumption, a common source of AFB-contaminated food, decreased about 40% between 1983 and 1989, according to preliminary dietary intake data from our ecological survey. We have no further explanation for the relatively high estimates of AFB1 intake from other studies except to suggest that the populations under investigation may have been biased toward very high intake areas. Although the intakes from this survey are unusually low, the highly significant correlation between villages within the same counties supports the geographic diversity of AFB exposure.

In conclusion, a reliable, sensitive, selective, reproducible method for measuring urinary AFB1 has been developed. Animal studies confirm the source, identity, and quantitation of the AFB1. The excretion rate from two intentionally dosed humans is 5–6%. AFB1 excretion across mainland China and Taiwan was very low (3.1 ng/12 h), with good agreement between villages from the same county and similar results from mainland China and Taiwan. AFB1 intake was calculated to average 0.1–0.3 μg/day (possible range, 0–11 μg/day).

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