

Role of Aflatoxin Exposure and Infection with Hepatitis B Virus in the Etiology of Hepatocellular Carcinoma

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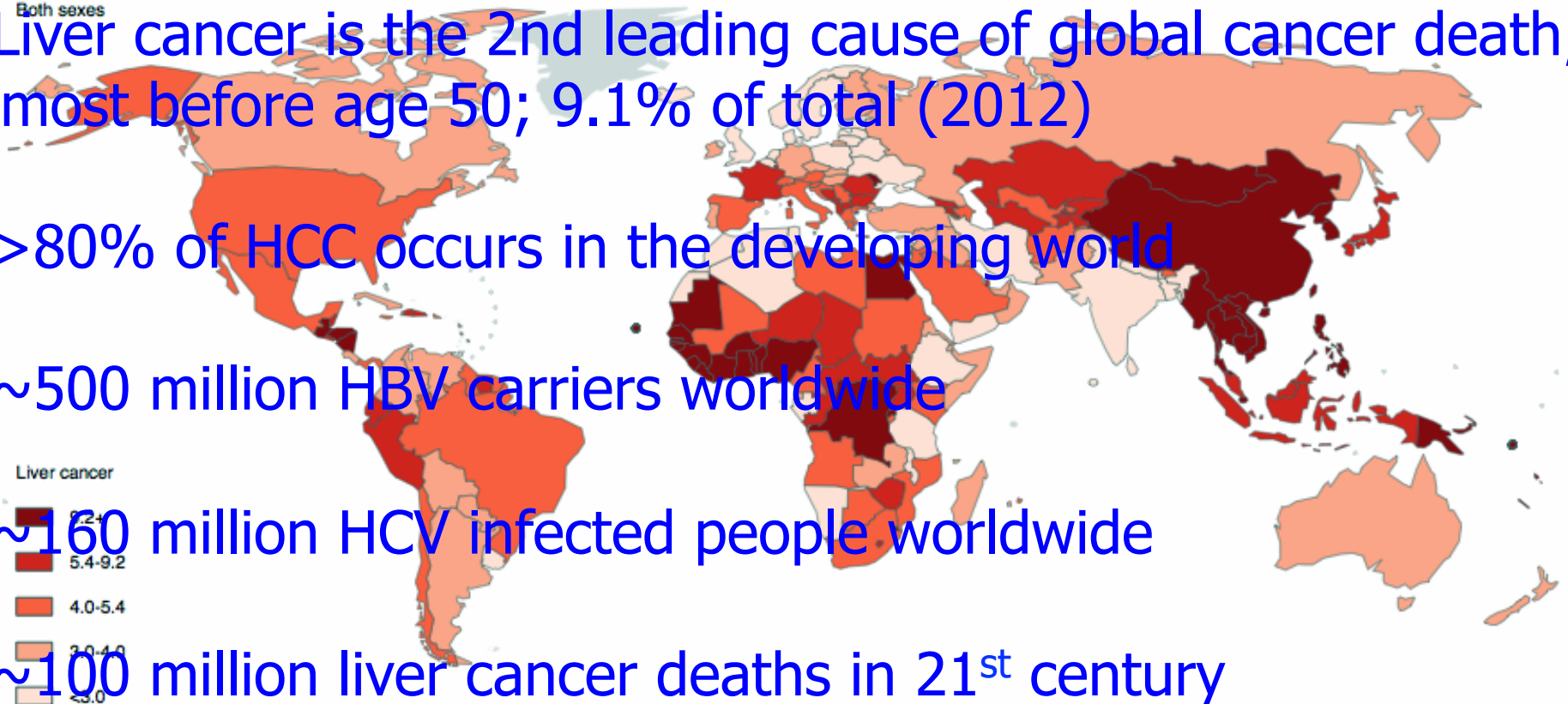
Johns Hopkins Bloomberg School of Public Health



Liver Cancer Mortality

Mortality ASR

Both sexes



International Agency for Research on Cancer



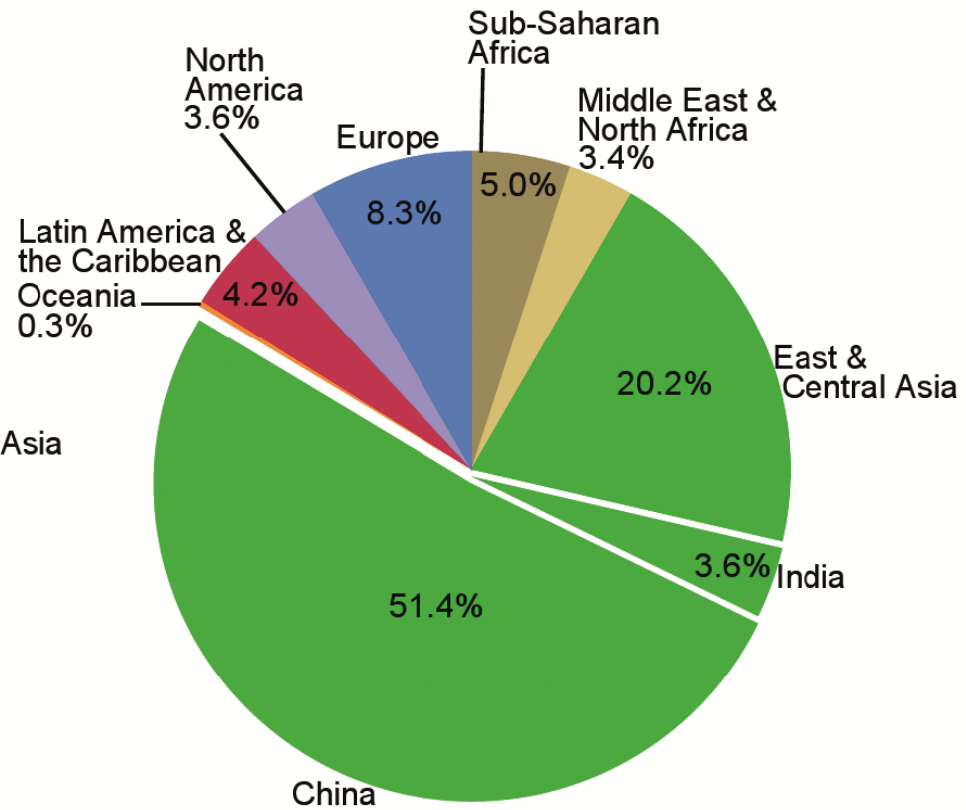
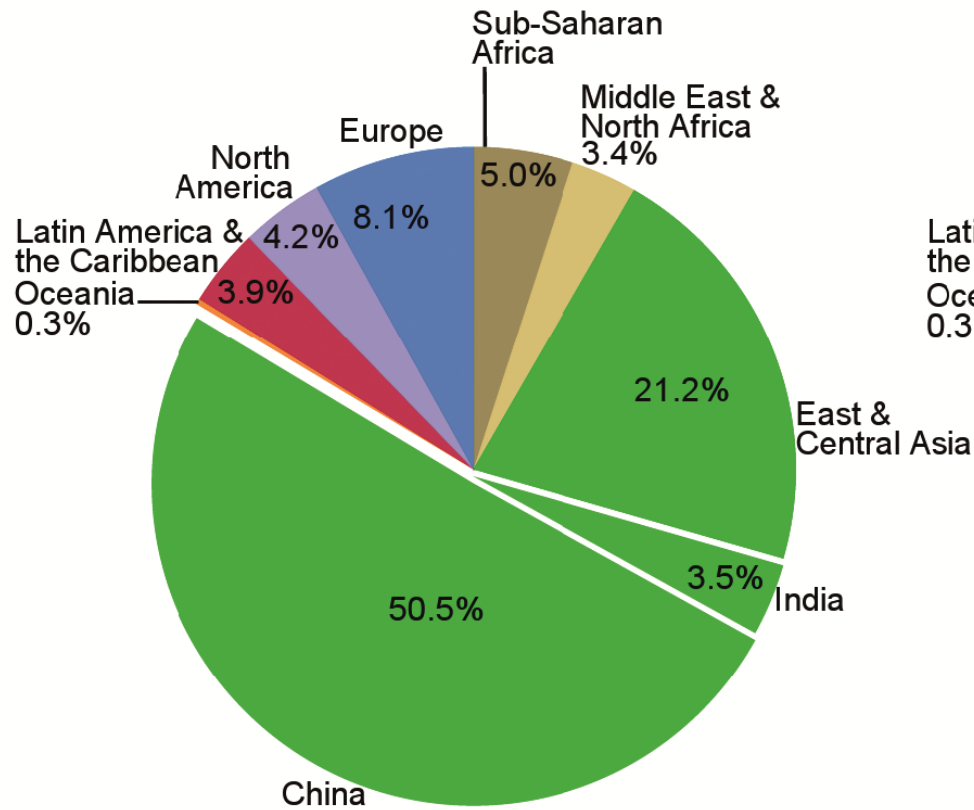
Source: GLOBOCAN 2012 (IARC)

- Liver cancer is the 2nd leading cause of global cancer death; most before age 50; 9.1% of total (2012)
- >80% of HCC occurs in the developing world
- ~500 million HBV carriers worldwide
- ~160 million HCV infected people worldwide
- ~100 million liver cancer deaths in 21st century
- etiology of 90-95% of liver cancer now known, but exposures are changing, e.g. fatty liver disease

Morbidity and Mortality for Liver Cancer

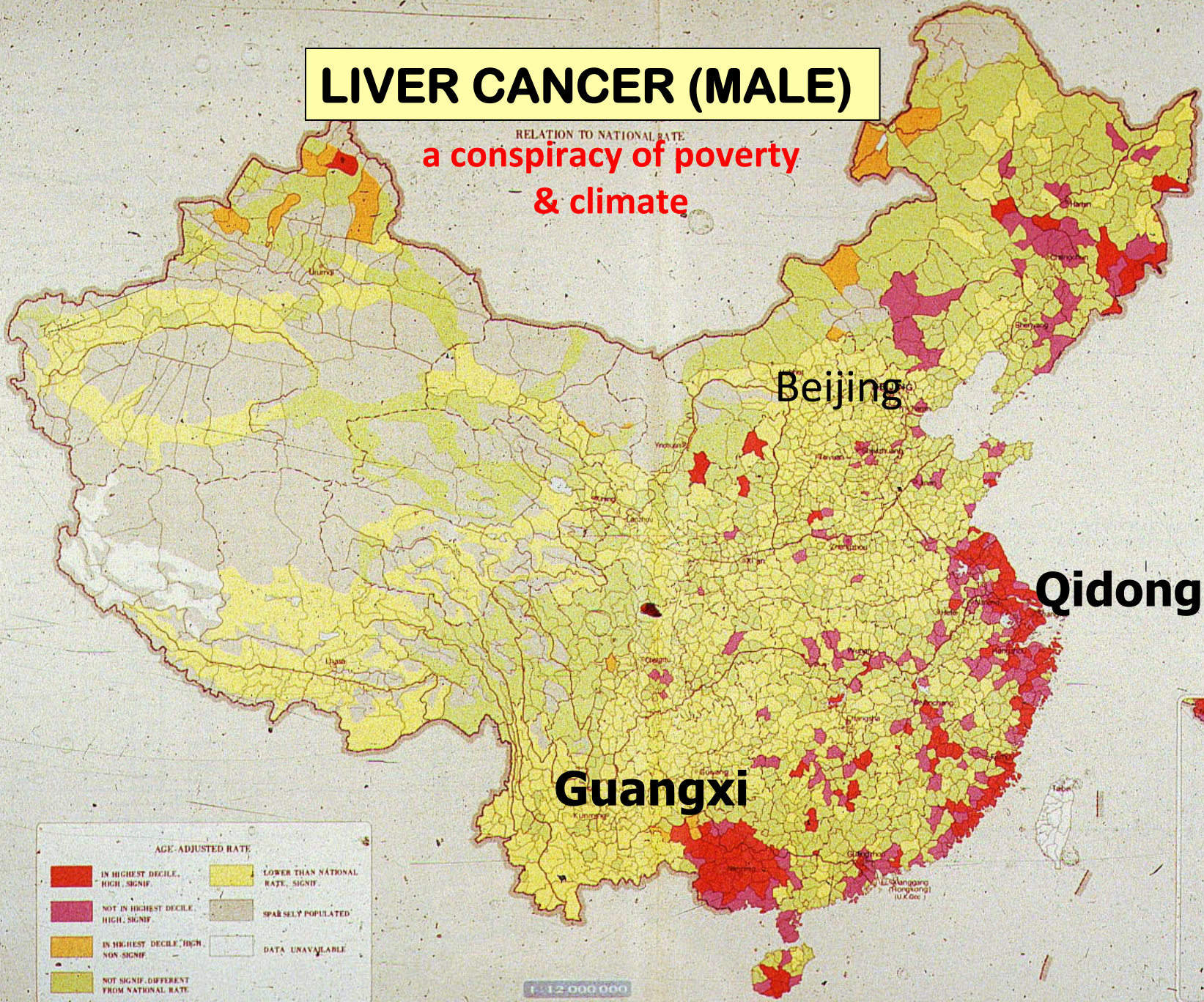
Incidence: 782 000 estimated new cases

Mortality: 746 000 estimated deaths



LIVER CANCER (MALE)

RELATION TO NATIONAL RATE
**a conspiracy of poverty
& climate**



AGE-ADJUSTED RATE	
■	IN HIGHEST DECILE, HIGH SIGNIF.
■	NOT IN HIGHEST DECILE, HIGH SIGNIF.
■	IN HIGHEST DECILE, HIGH, NON SIGNIF.
■	LOWER THAN NATIONAL RATE, SIGNIF.
■	SPARSELY POPULATED
■	DATA UNAVAILABLE
■	NOT SIGNIF. DIFFERENT FROM NATIONAL RATE

1:12 000 000

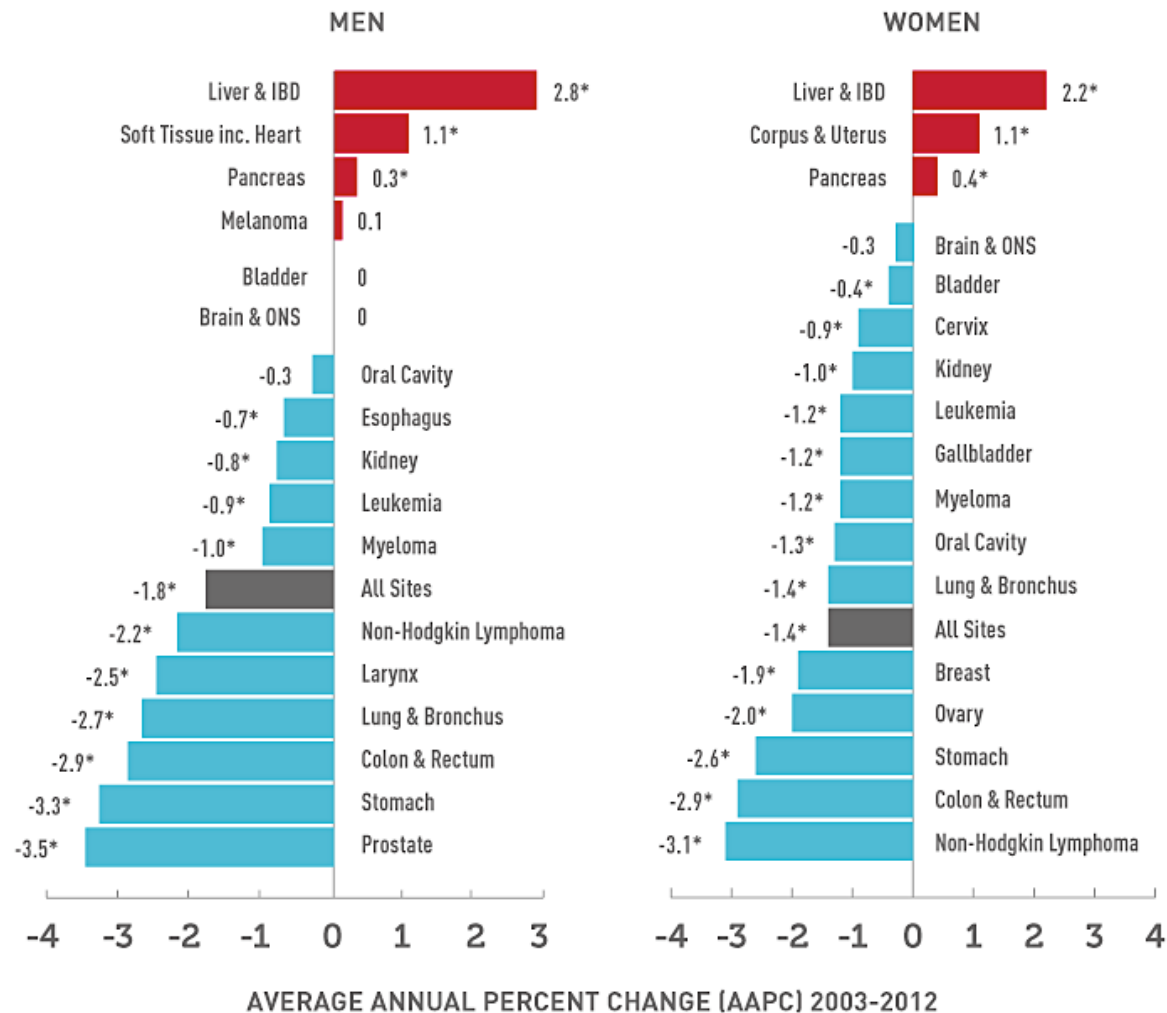


SOUTH CHINA SEA ISLANDS
1:24 000 000

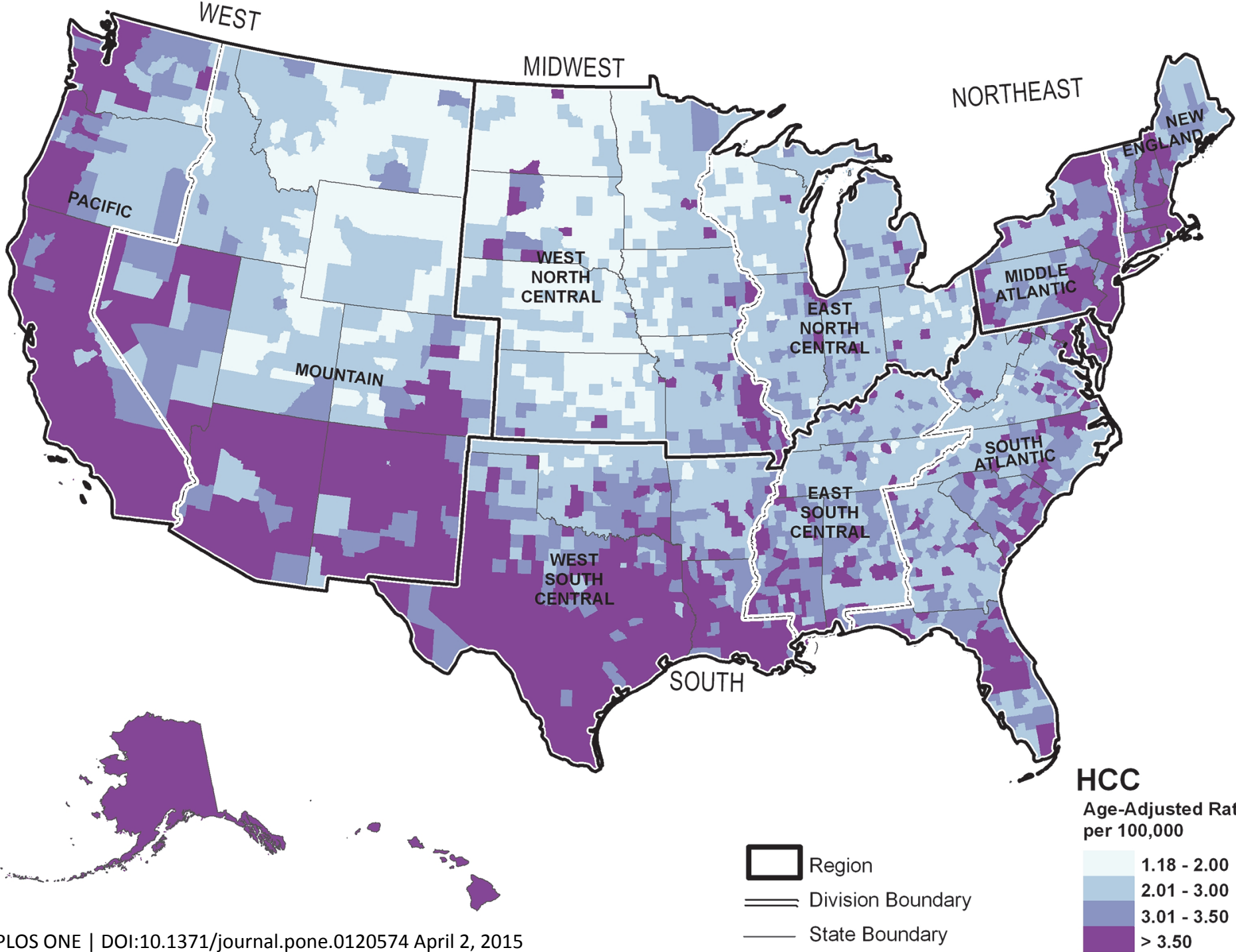
Etiology of HCC is multifactorial and varies with geographic region

- **Hepatitis B Virus**
 - Major cause of viral hepatitis in Africa and Asia
- **Hepatitis C Virus**
 - Major cause of viral hepatitis in Japan, Europe and SE Asia
 - Emerging major issue in US
- **Environmental Factors**
 - Aflatoxin in foods
 - Alcoholic beverages
 - Microcystins (algal toxins) in drinking water
- **Emerging Factors**
 - NAFLD, NASH T2D

NATIONAL CANCER INSTITUTE 10-YEAR MORTALITY TRENDS



* AAPC is significantly different from zero ($p < .05$).



HBV surface antigen in serum identifies men at high risk

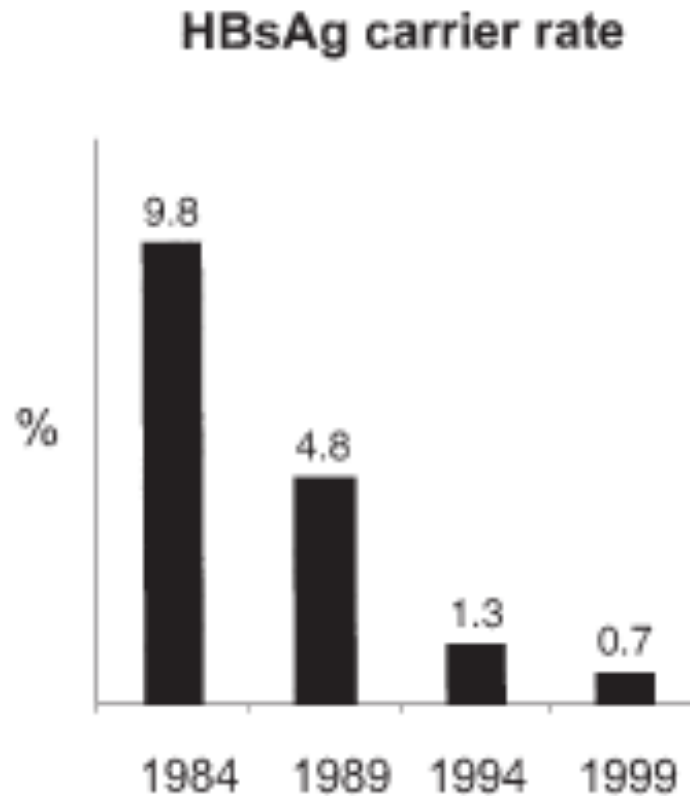
Prospective study in 22,707 Chinese men in Taiwan

HBsAg Status	Relative Risk for Liver Cancer
HBV Antigen Negative	1.0
HBV Antigen Positive	98.4

- **Demonstrates the power of an immunologic biomarker**

Immunization Against HBV

Prevention of Chronic HBV and HCC In Children by HBV Vaccination in Taiwan



Ni et al. Ann Int Med 2001; 135: 796.

Chang et al. NEJM 1997; 336: 1857

Decreased Incidence of Hepatocellular Carcinoma in Hepatitis B Vaccinees: A 20-Year Follow-up Study

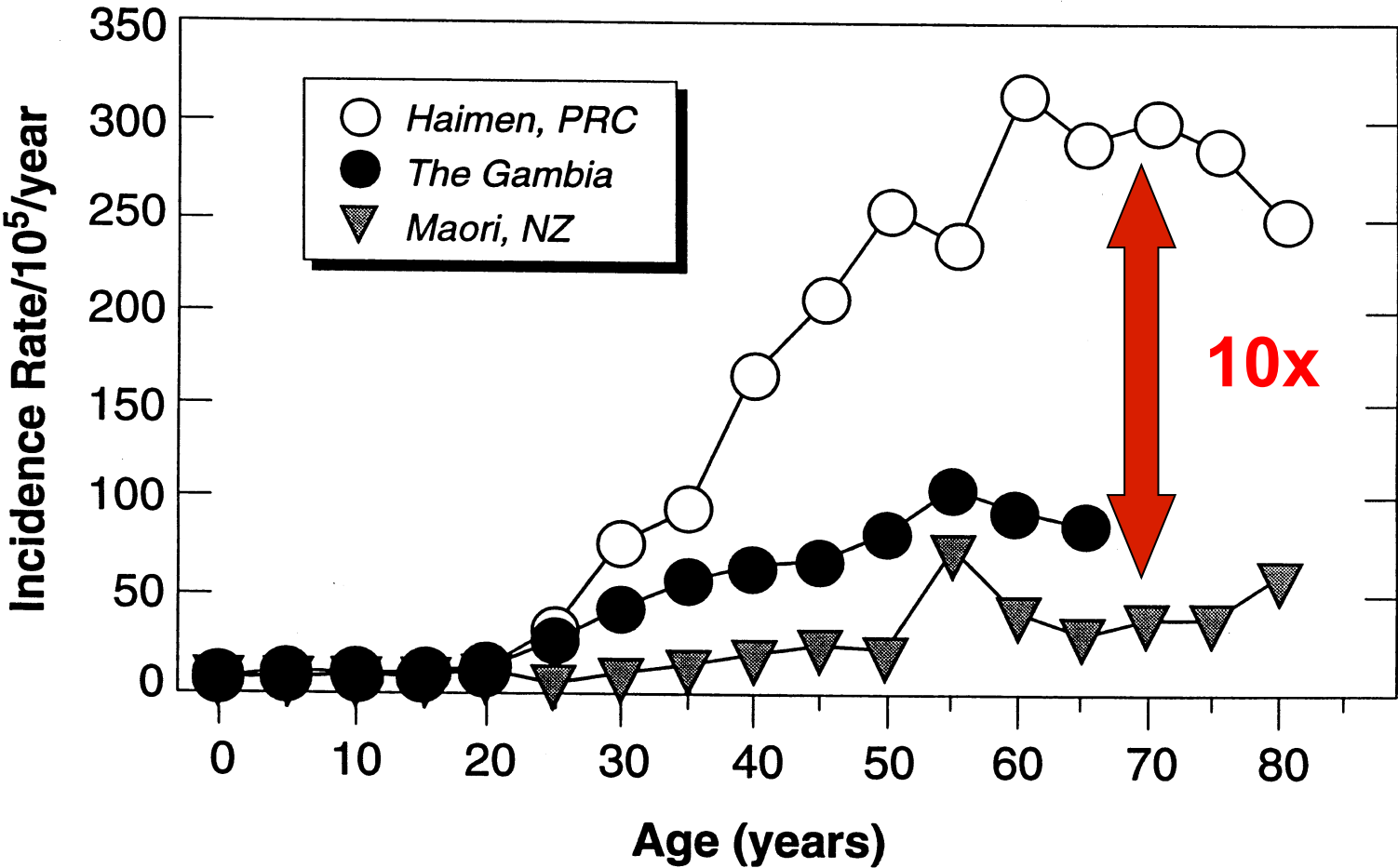
Table 2. Incidence rates of hepatocellular carcinoma (HCC) diagnosed in 1983–2004 among children who were 6–9, 10–14, or 15–19 years old and born before or after the launch of the Taiwanese universal hepatitis B virus (HBV) vaccination program in July 1, 1984*

Age at diagnosis, y	Birth year	Person-years	No. of HCCs	HCC		
				Incidence rate (per 100 000 person-years)	Rate ratio (95% CI)	<i>P</i>
6–9	July 1973 to June 1979	7 028 287	36	0.51	1 (referent)	
	July 1979 to June 1984	8 012 261	38	0.47	0.93 (0.59 to 1.46)	.74
	July 1984 to June 1998	17 010 463†	26†	0.15	0.30 (0.18 to 0.42)	<.001
10–14	July 1968 to June 1979	17 025 965	102	0.60	1 (referent)	
	July 1979 to June 1984	9 982 652	50	0.50	0.84 (0.60 to 1.17)	.29
	July 1984 to June 1994	14 395 987†	28†	0.19	0.32 (0.21 to 0.49)	<.001
15–19	July 1963 to June 1979	26 506 175	138	0.52	1 (referent)	
	July 1979 to June 1984	9 941 064	80	0.80	1.55 (1.17 to 2.04)	.001
	July 1984 to June 1989	6 302 890†	10†	0.16	0.30 (0.16 to 0.58)	<.001

* Rate ratios with 95% CIs and *P* values were estimated by Poisson regression analysis. All statistical tests were two-sided. CI = confidence interval.

† Only 64 children with HCC were aged 6–19 years and born after the initiation of HBV vaccination program.

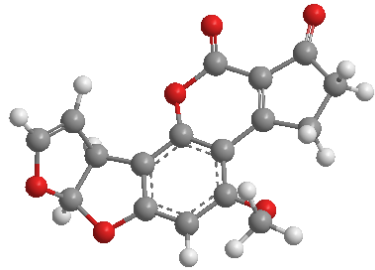
Geographic variation of HCC incidence in male HBV carriers suggests environmental risk factors



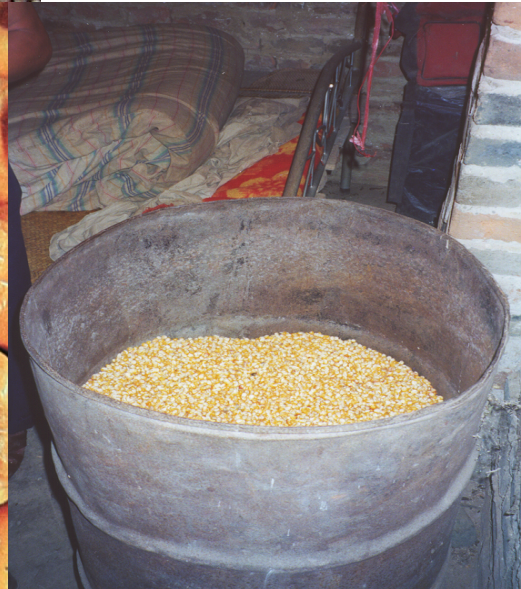
T. London (1994)

Observational epidemiology provided early evidence that aflatoxin is a risk factor for HCC

- Frequent contaminant of human diets in developing countries
- Estimated intake correlates positively with HCC incidence



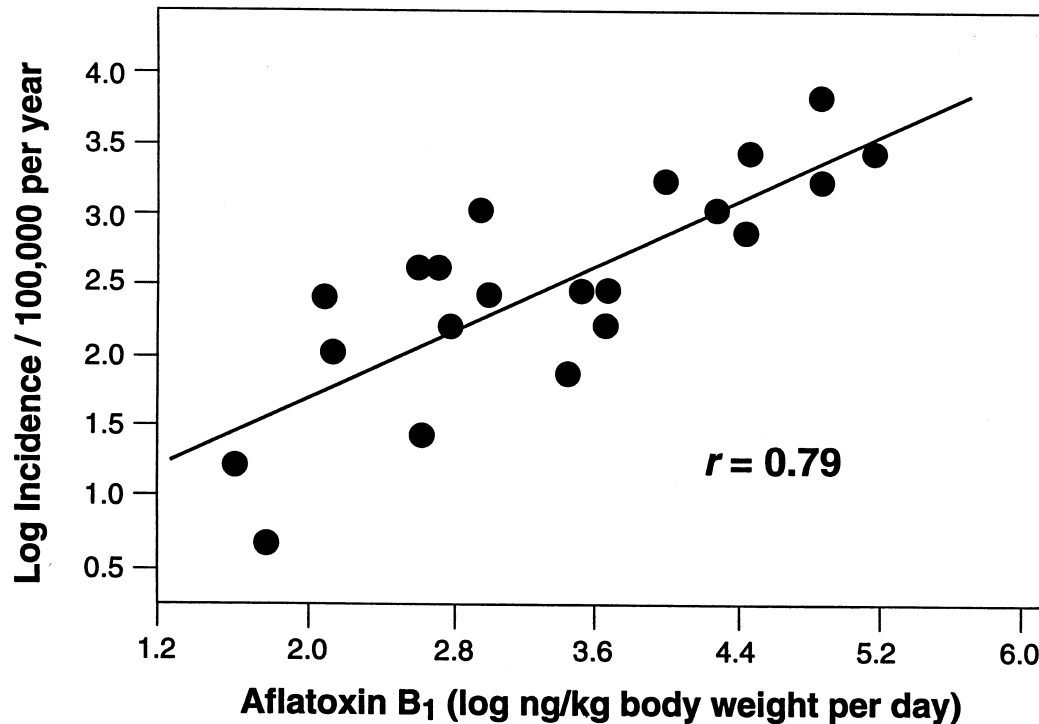
Corn Peanuts Soy Sauce



Incidence of liver cancer in men is associated with aflatoxin ingestion

Aflatoxin intake quantified by analysis of food as eaten, HCC incidence from registry data

Thailand, 1972; Kenya, 1973; Swaziland, 1976, 1987; Mozambique and Transkei, 1985



COHORT STUDY OF LIVER CANCER IN CHINA: Viral-Chemical Interactions

- 18,244 urine and blood samples collected from healthy men age 45-65
- 50 liver cancer cases and 247 controls
- Urinary aflatoxin biomarkers measured in blinded samples
- HBV status determined for each subject

BIOMARKERS: HBsAg AND URINARY AFLATOXINS	RELATIVE RISK FOR LIVER CANCER
NO BIOMARKERS DETECTED	1.0
HBV (YES) AFLATOXIN (NO)	7.3
HBV (NO) AFLATOXIN (YES)	3.4
HBV (YES) AFLATOXIN (YES)	59.4

Lancet 339: 943-946, 1992
and *C.E.B.P.* 3: 3-11, 1994

“Geographic Pathology”

江苏省肝癌分布图
(1973-1975)

Mortality from Liver Cancer by Township: Jiangsu Province

< 1 per 10^5 /yr



> 50 per 10^5 /yr

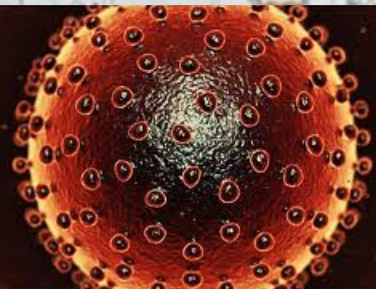
OR = 7.3

OR = 3.4

25-fold change
in HCC rate
in 200 km

Shanghai

BOTH = 59.4

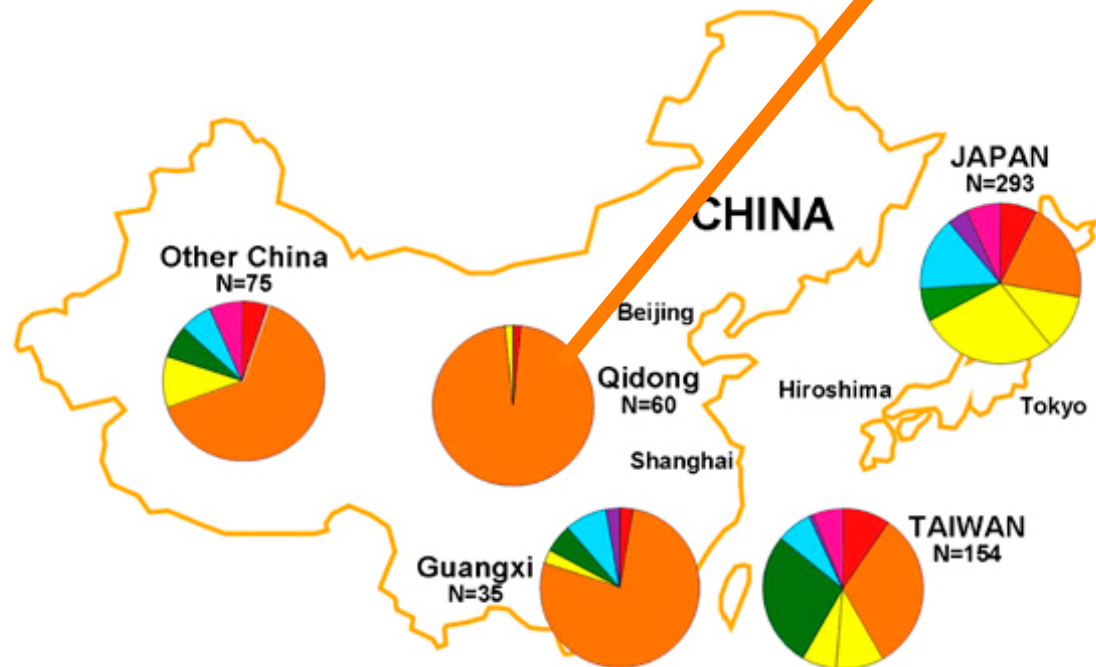


HEPATITIS B

AFLATOXIN B1

p53 Mutations in HCC

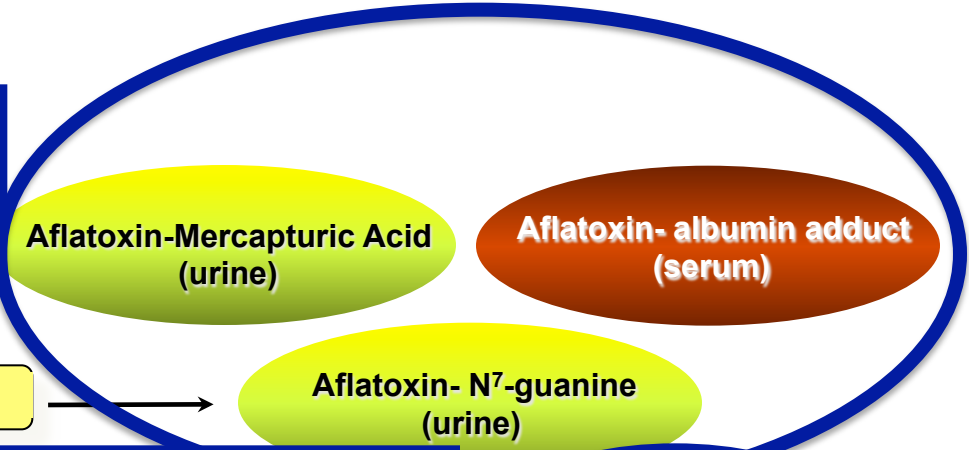
- codon 249 p53 mutations are detectable in DNA in human plasma and liver samples
- strong mechanistic studies link aflatoxin DNA adduct formation with this p53 mutation in codon 249



HBV DNA double mutation (1762^T/1764^A) and risk of HCC among HBsAg positive subjects at baseline

HBV 1762 ^T /1764 ^A mutation in serum	No. of cancer patients	No. of control subjects	Age-adjusted OR (95% CI)
Mutant status			
Negative	27	76	1.00
Positive	22	21	3.10 (1.45-6.59)

Short-lived biomarkers that track exposure to aflatoxin



Promutagenic DNA Lesions

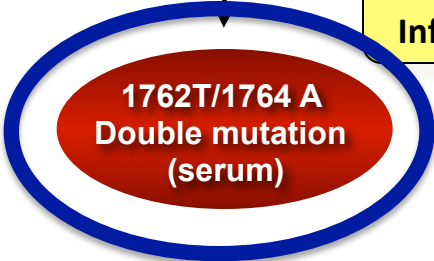
Long-lived biomarker detected at least 5 years prior to HCC diagnosis



Genomic Insertion

X-Gene Mutations

Selective Clonal Expansion and p53 Allelic Deletions



Chronic Inf

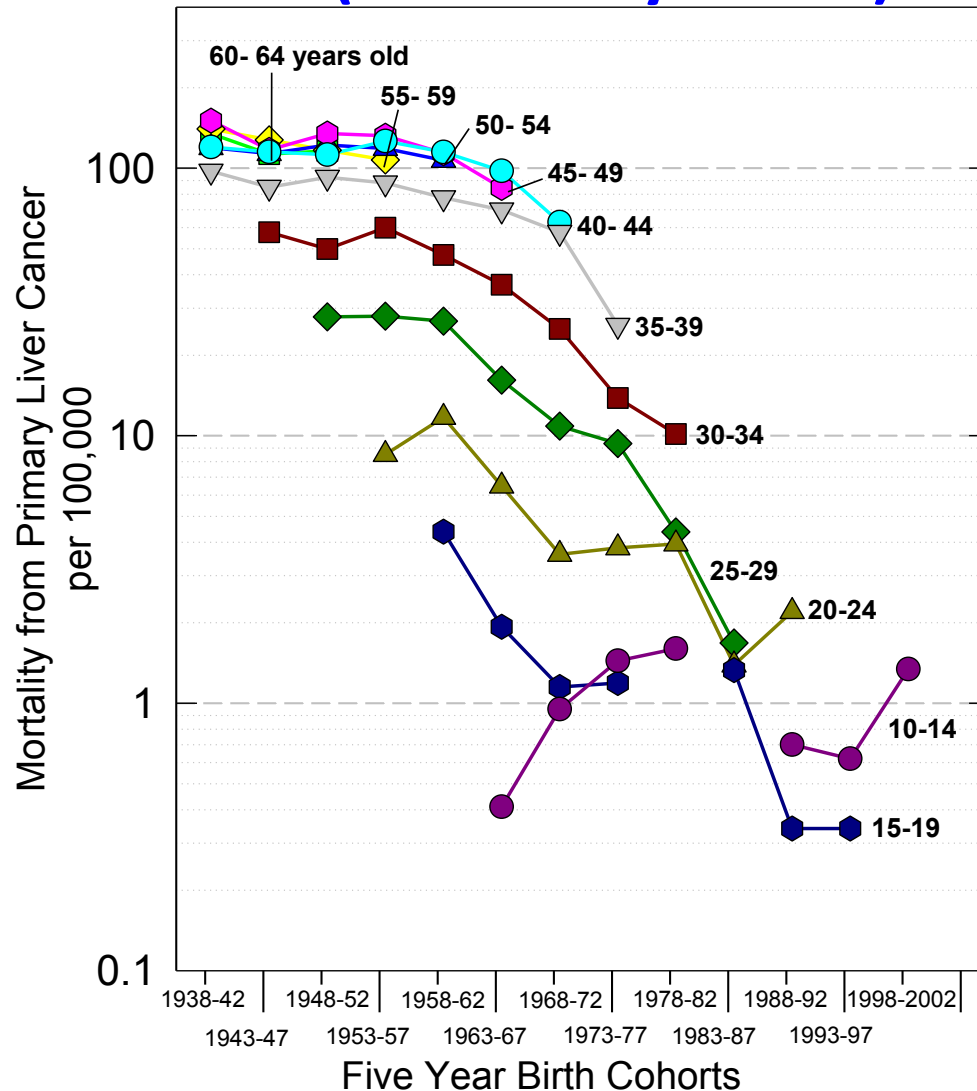
Chronic Hepatitis

Mutation detected in plasma up to 20 years prior to HCC diagnosis

Hepatocellular Carcinoma

Prevention and Economic Development: Gaining insights in etiology

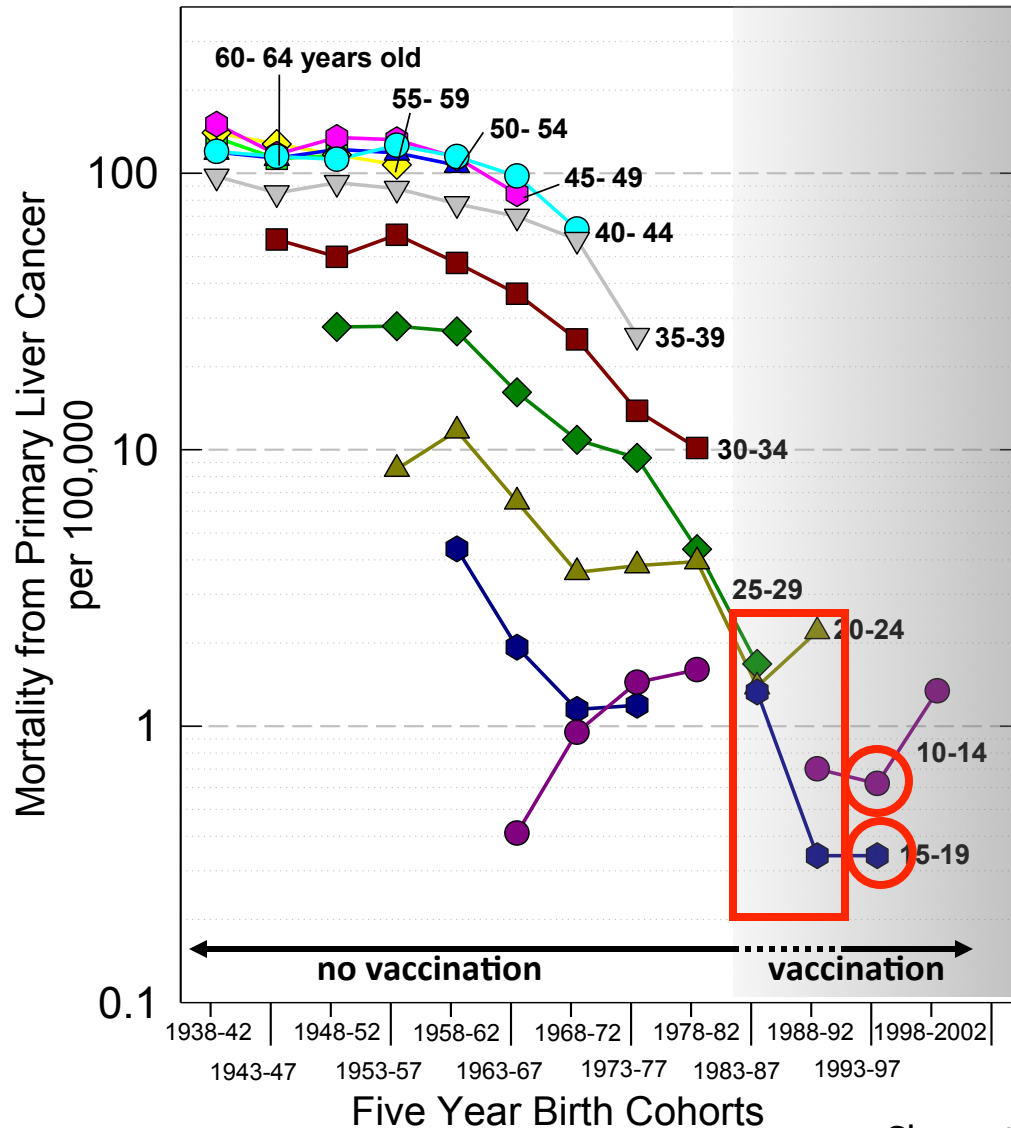
There is a Dramatic Drop in Age-Specific Mortality Rates from Liver Cancer in the Younger Birth Cohorts (after early 1960s)



What is driving the decline in liver cancer In the younger birth cohorts?

- HBV vaccination?
- Reduced aflatoxin exposures?
- Other factors?
 - drinking water

Most of the decline in liver cancer has occurred in birth cohorts never vaccinated against HBV

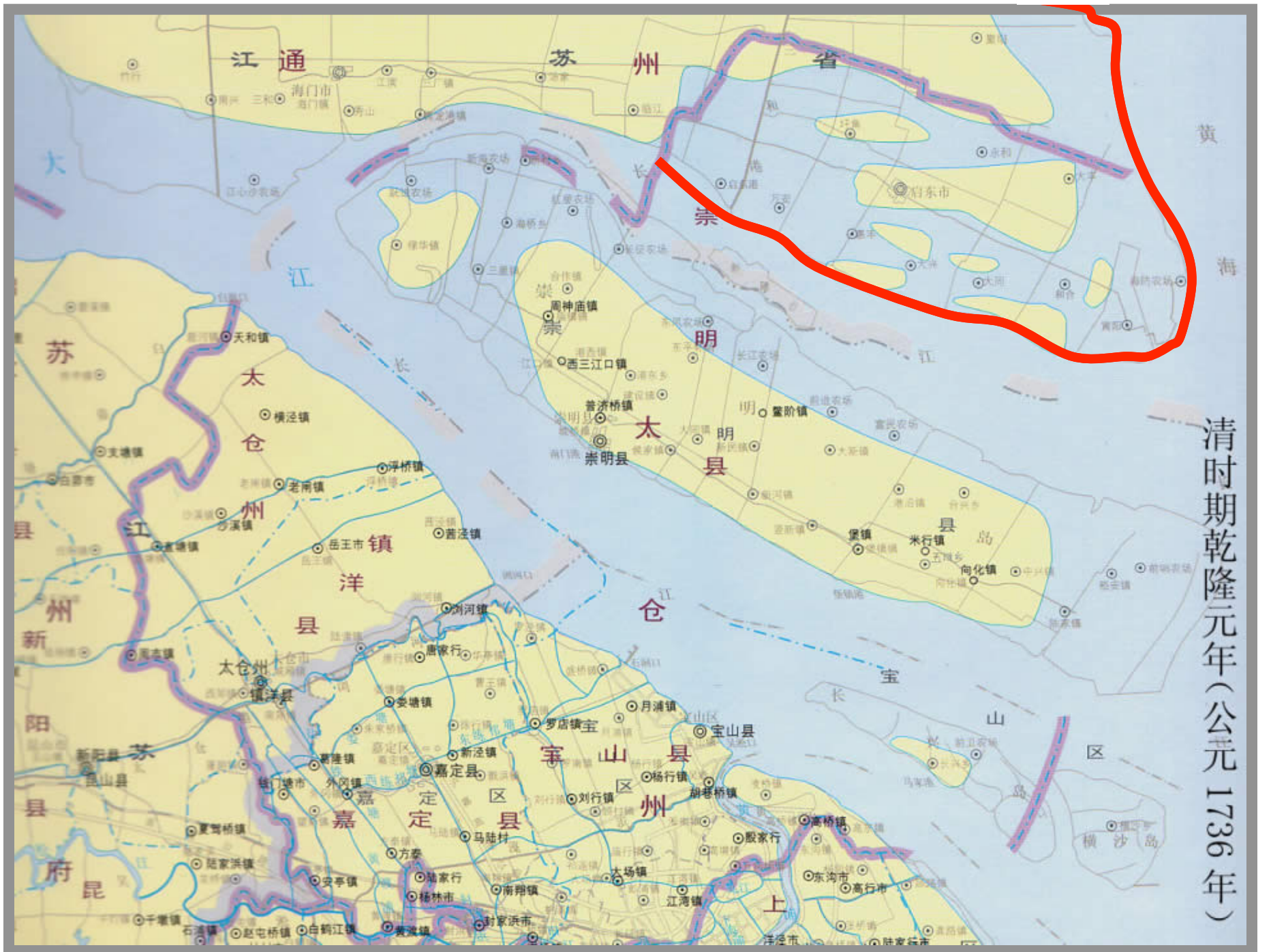


○ likely vaccinated in 2002 as 5-10 year olds

perhaps 25% vaccinated

If it's not HBV, what about aflatoxin?

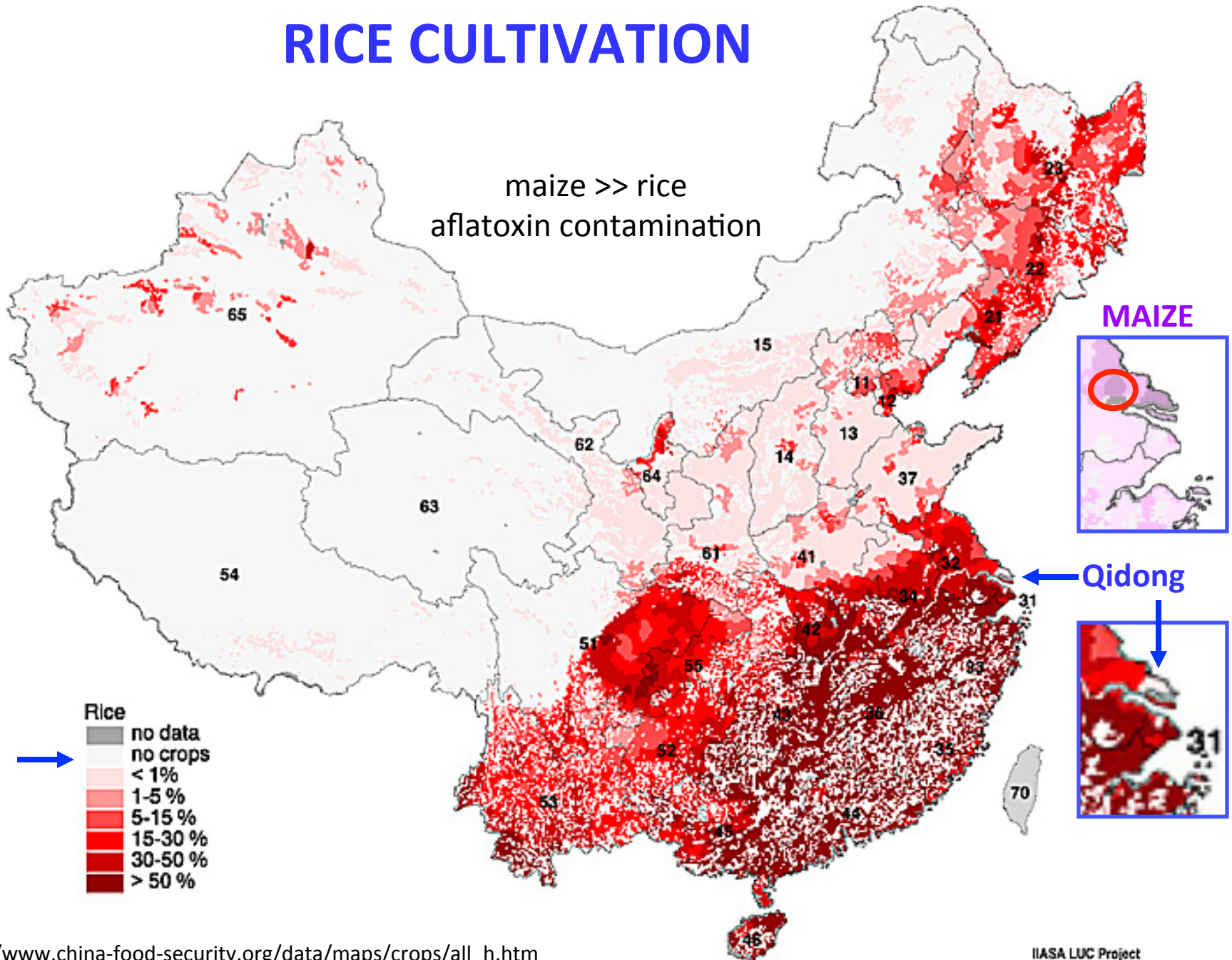
Aflatoxin is a significant contaminant of maize and peanuts, but not rice.



清时期乾隆元年(公元1736年)

RICE CULTIVATION

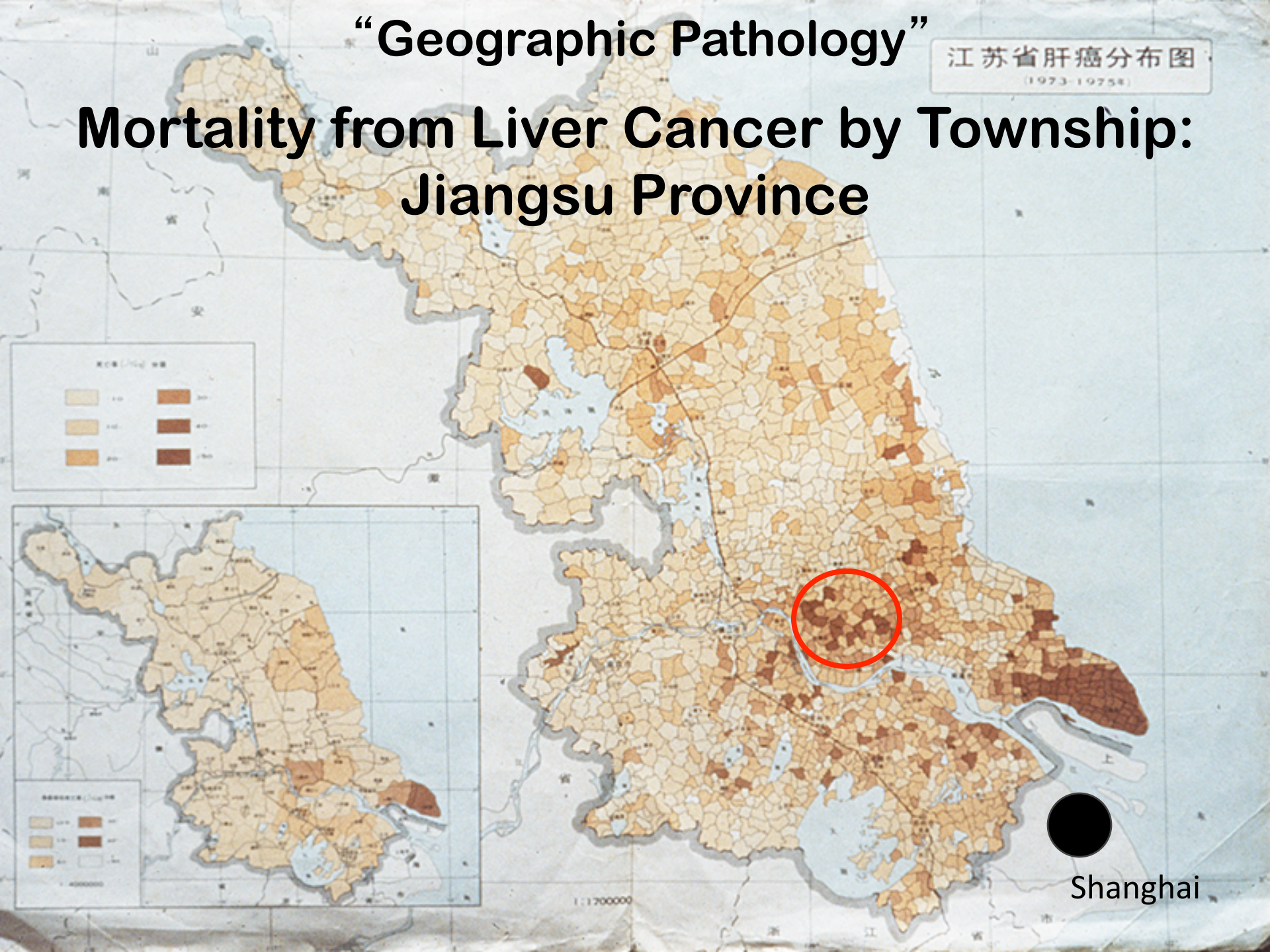
maize >> rice
aflatoxin contamination



“Geographic Pathology”

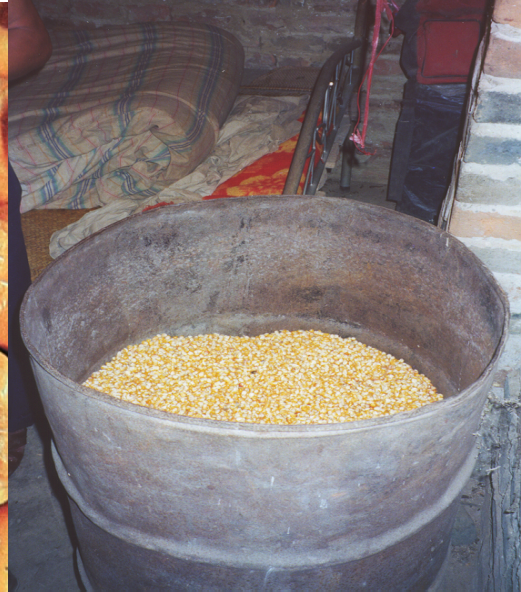
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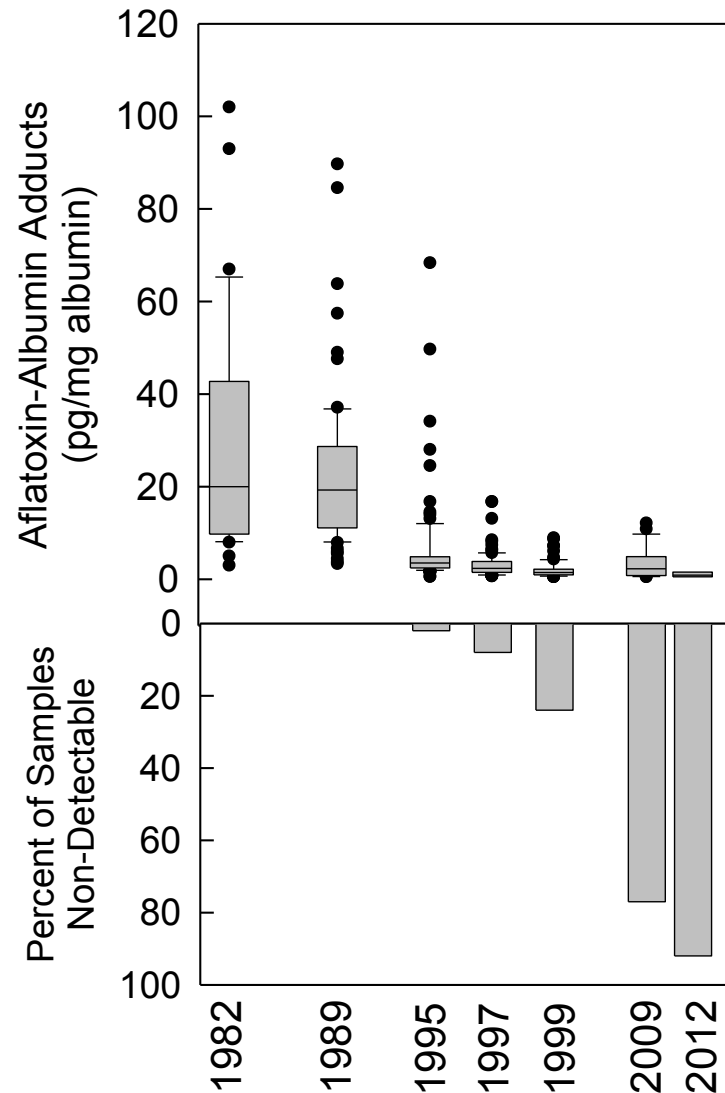


Aflatoxin Exposure in Qidong

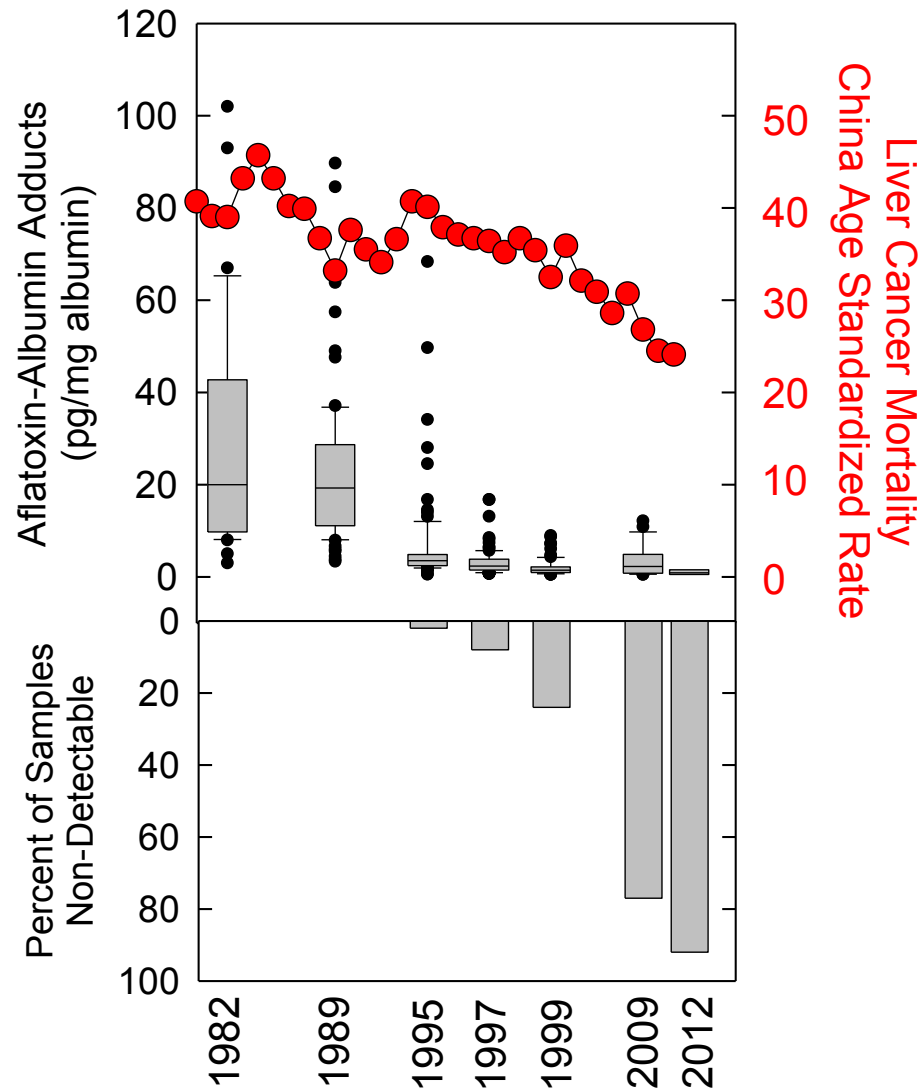
- 1970s
- Qidong farmland unsuitable for growing rice; maize is major dietary staple as import of rice is very restricted due to central planning policies
 - average annual per capita maize consumption ranged from 82 – 124 kg: consistent contamination of corn with aflatoxin
- 1980s
- Deng Xiaoping: shift from planned economy to market-oriented economy: in 1985 dietary staple quickly shifted from maize to rice



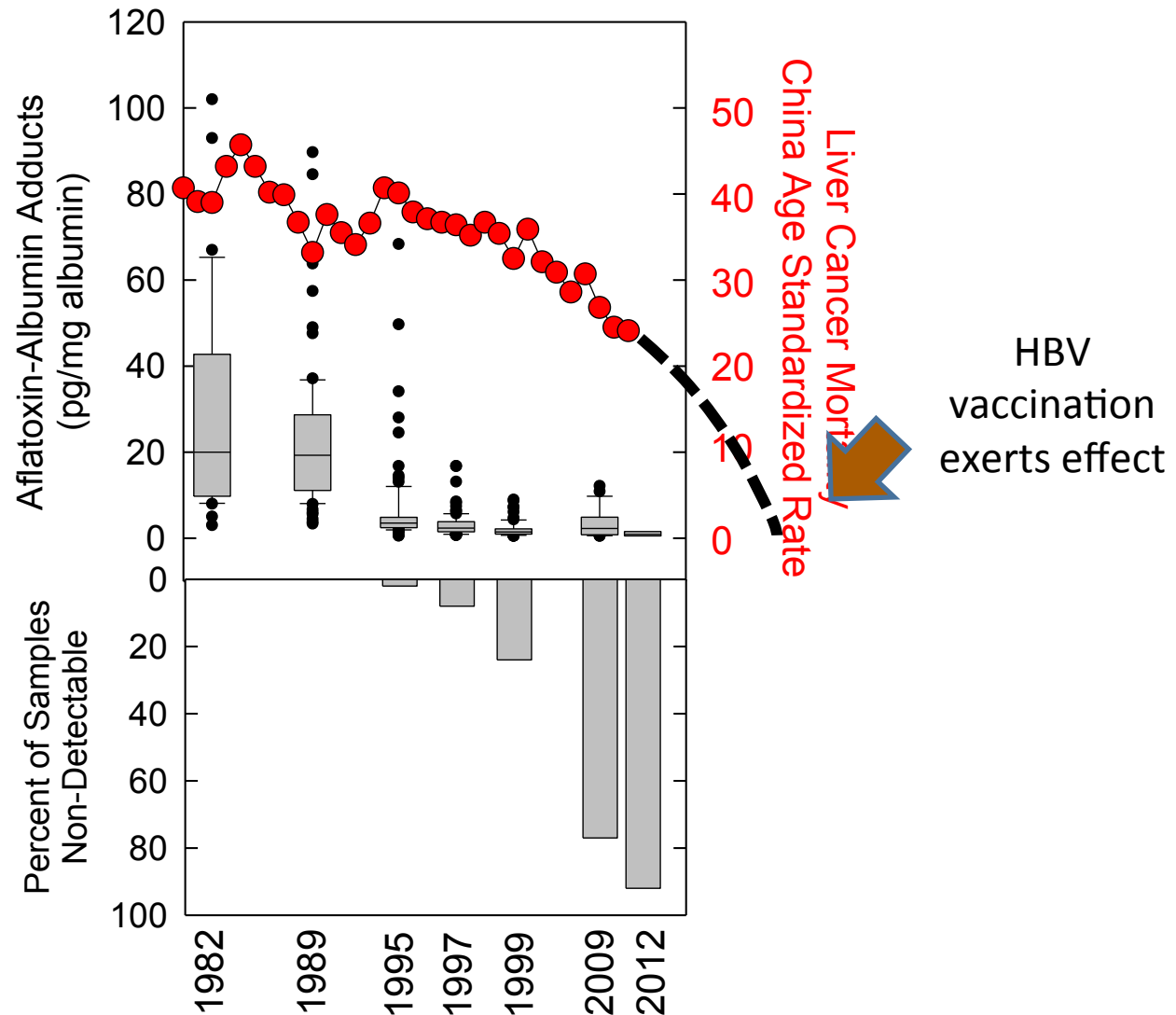
Declining Aflatoxin Exposure in Rural China from 1980s to Present



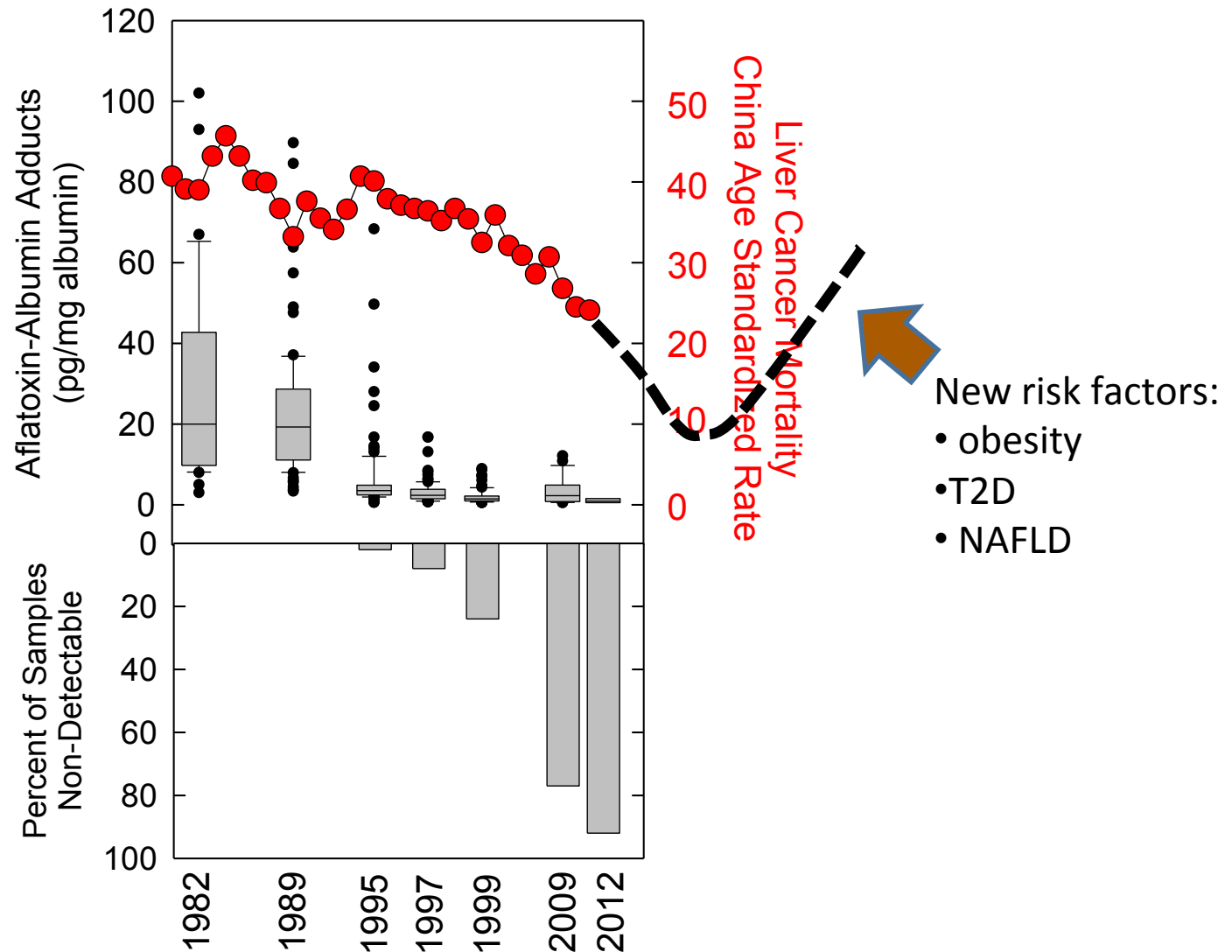
Declining Liver Cancer in Rural China from 1990s to Present



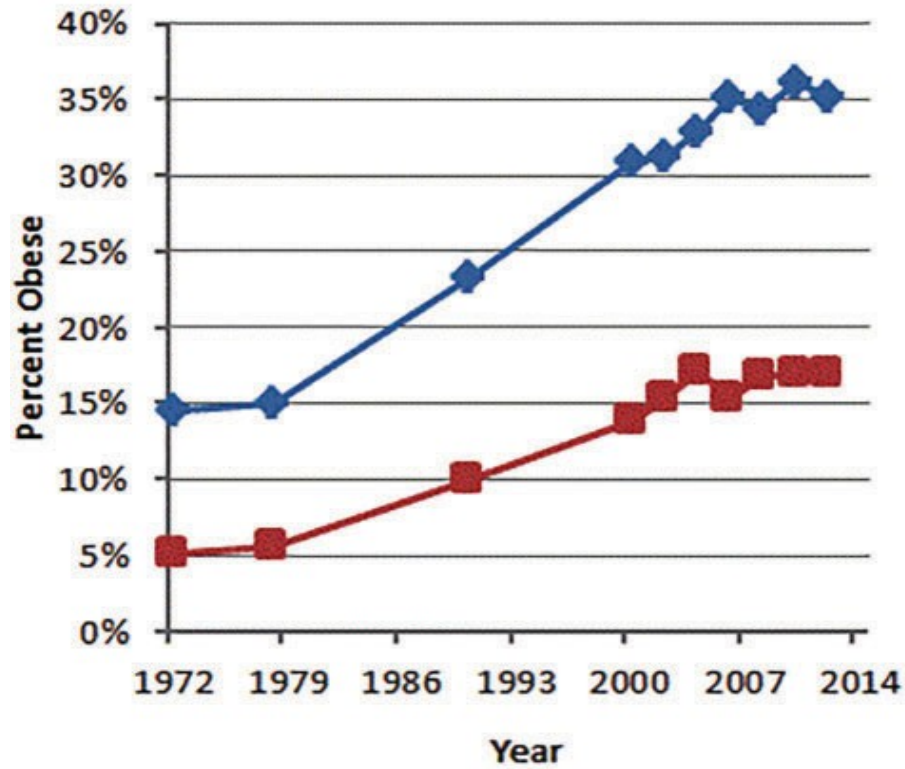
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Declining Liver Cancer in Rural China from 1990s to Present



Obesity Trends in the United States

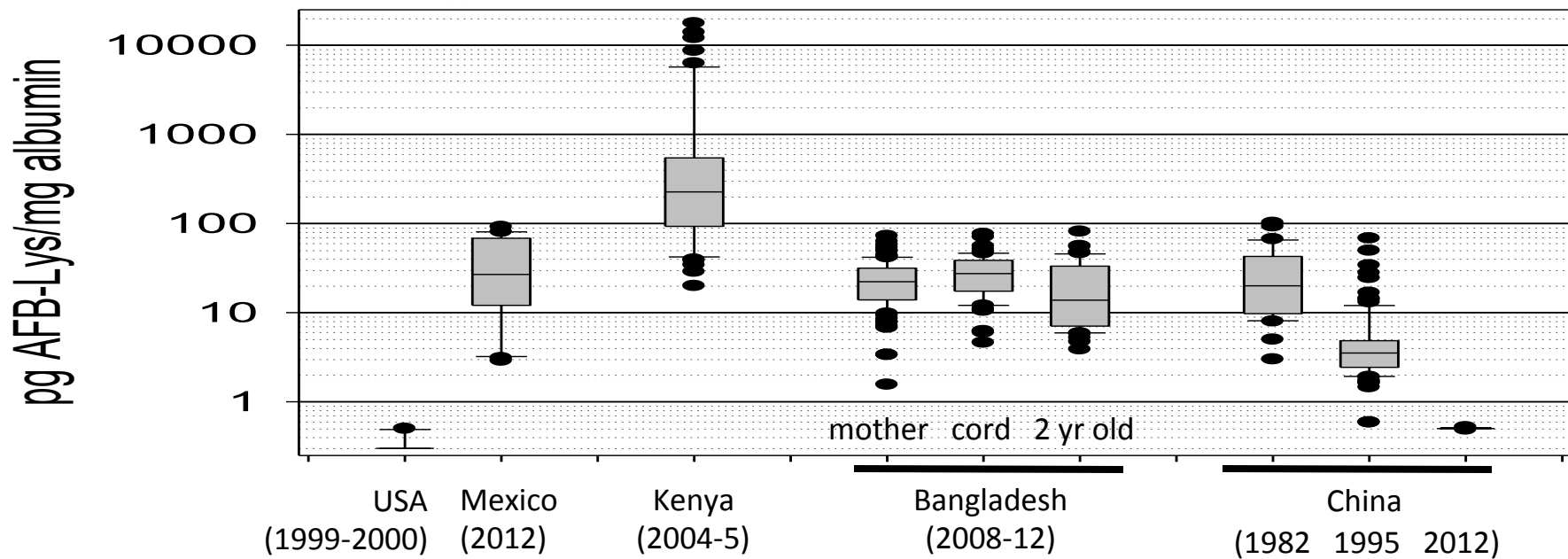
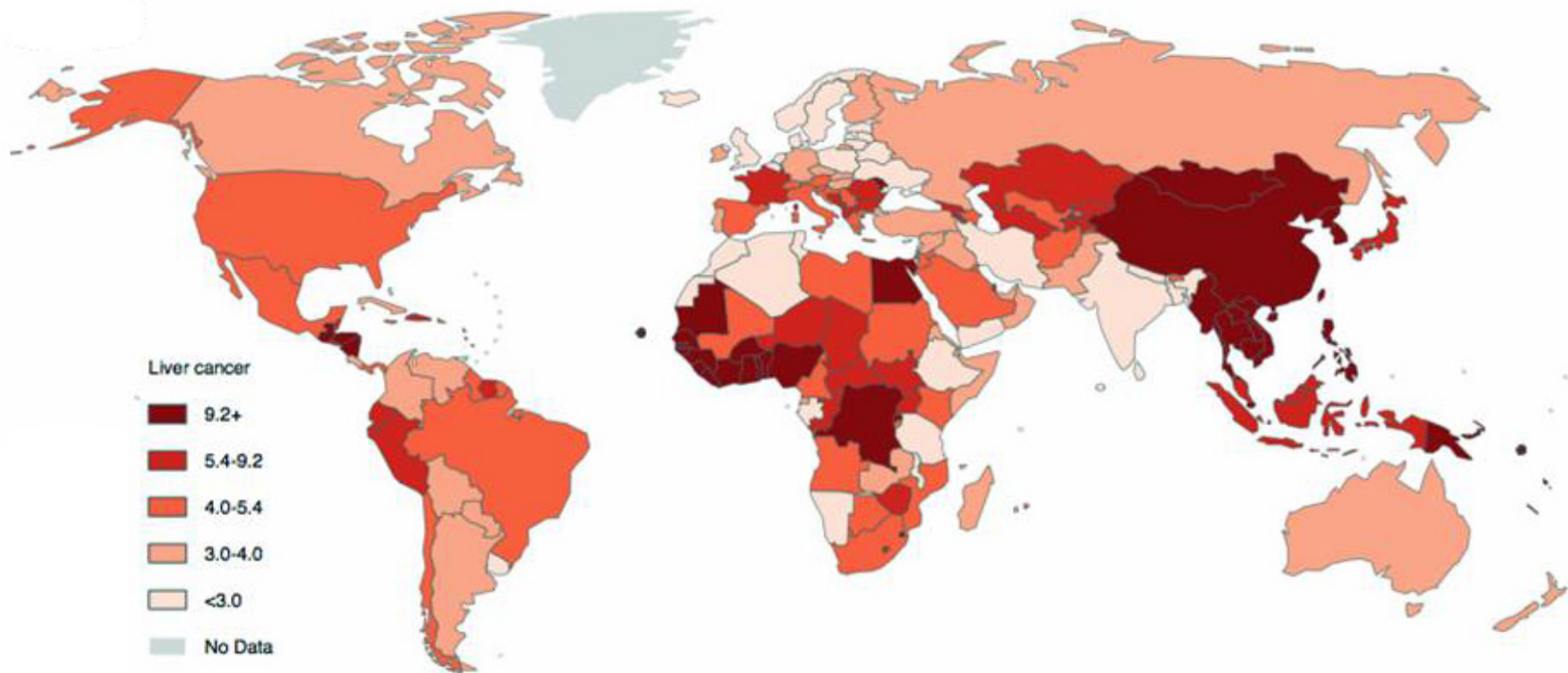


Adults
20-74 years of age

Children/Youth
2-19 years of age

Source: Centers for Disease Control and Prevention

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STRATEGIES FOR PREVENTION OF HEPATOCELLULAR CARCINOMA

PRIMARY

- Immunization with HBV vaccine
- Reduced aflatoxin consumption:
 - improve food storage; biocontrol
 - changes in dietary staples

SECONDARY

- Chemopreventive interventions:
e.g., oltipraz, broccoli sprouts,
chlorophyllin, green tea

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