



Title	Interaction of probiotics and mycotoxins: benefits to human health
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Citation	The 6th Asian Conference on Food and Nutrition Safety, Singapore, 26-28 November 2012.
Issued Date	2012
URL	http://hdl.handle.net/10722/183978
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Interaction of Probiotics and Mycotoxins - Benefits to Human Health

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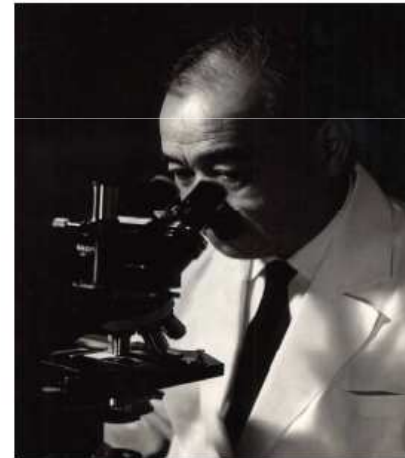
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<http://www.ilsa.org/SEA/Region/Pages/ViewEventDetails.aspx?WebId=4D540914-E936-40E4-89EB-0B73BA3D76C1&ListId=478BE3C5-581B-49A2-A280-8E00CCB26F9C&ItemID=34>

Probiotics

- Definition

Living microorganisms when administered in adequate amounts confer health benefits on the host



Dr. Minori Shirota
First developed *L. casei* Shirota
as a commercial product in 1935

Probiotics – Daily example

1. Fermented Milk Products



Milk



Lassi



Smoothie



Frozen yogurt



Ice cream



Cheese



Valio Gefilus® 20 years of probiotic dairy products in Europe

Probiotics - Daily example

2. Food & Drinks



Candy & granola bars



Mint candies



Cookies



Cereal



Infant formula

3. Pharmaceutical products



Probiotics supplement

In form of:

Tablets, Capsules, Granules
Single or Cocktail of species

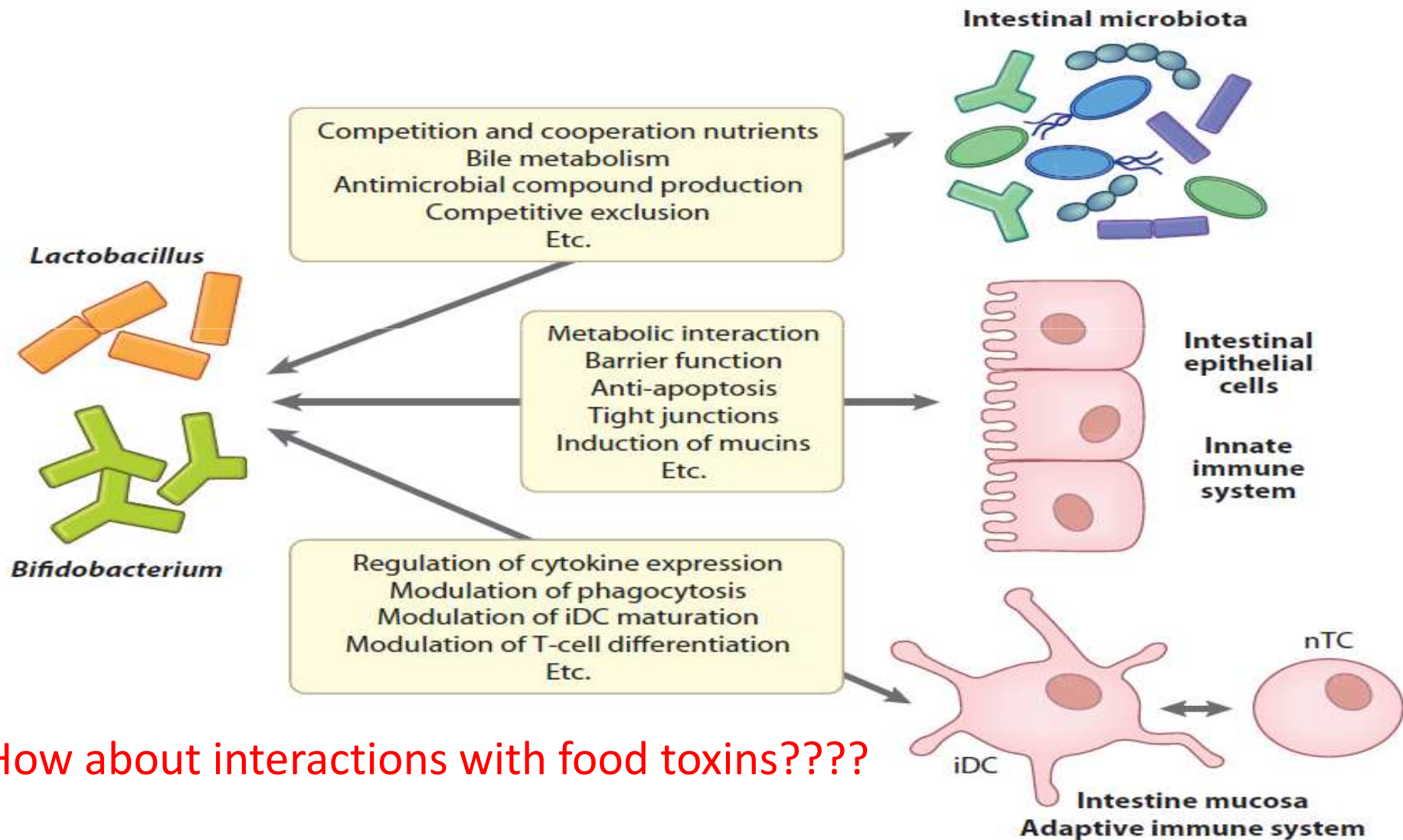
Encapsulation help protect probiotics from:

- Air & moisture (esp. anaerobic species)
- Stomach acid

Dosage:

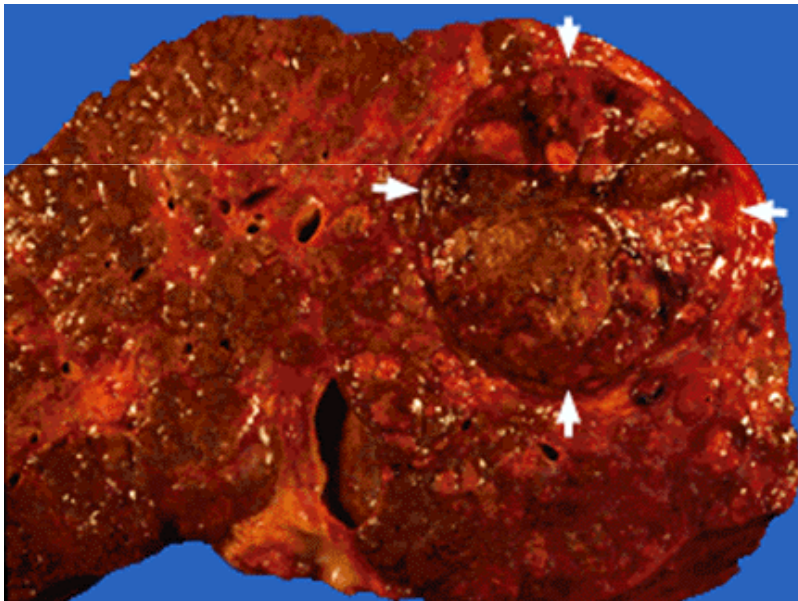
$10^9 - 10^{10}$ bacteria / day / adult

Probiotics have several mechanisms of action that may contribute to human health



How about interactions with food toxins????

Hepatocellular Carcinoma (HCC)



- Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer in the world with an estimating 473,000 new cases annually, accounting for 5.4% of all human cancer cases.
- Late presentation, typically males aged 66 with chronic liver disease.
- Median survival of 6 months from time of diagnosis.
- Surgery is the only potentially curable form of treatment.

Risk factors for HCC

- Viral
 - *Chronic hepatitis B
 - *Chronic hepatitis C
- Preexisting liver disease
 - * Cirrhosis
 - Metabolic liver disease
 - Alcohol abuse
 - * Adenoma
- Environmental
 - *Aflatoxin
 - *Contraceptives and androgens

The aflatoxins

- Turkey "X" Disease
 - Fungal infection by *Aspergillus flavus* and *Aspergillus parasiticus*
 - Primary contamination
 - High energy content foods e.g. grain, nut and soy products
 - Secondary contamination
 - Dairy products, meat & eggs



Commodities in which aflatoxins have been detected

Flour

Corn meal

Peanut

Meat pies

Milk

Cottonseed

Cassava

Brazil nuts

Oilseeds

Pumpkin seeds

Cocoa

Cheese

Sausage

Bread

Macaroni

Copra

Cooked meat

Pistachio nuts

Rice

Soy

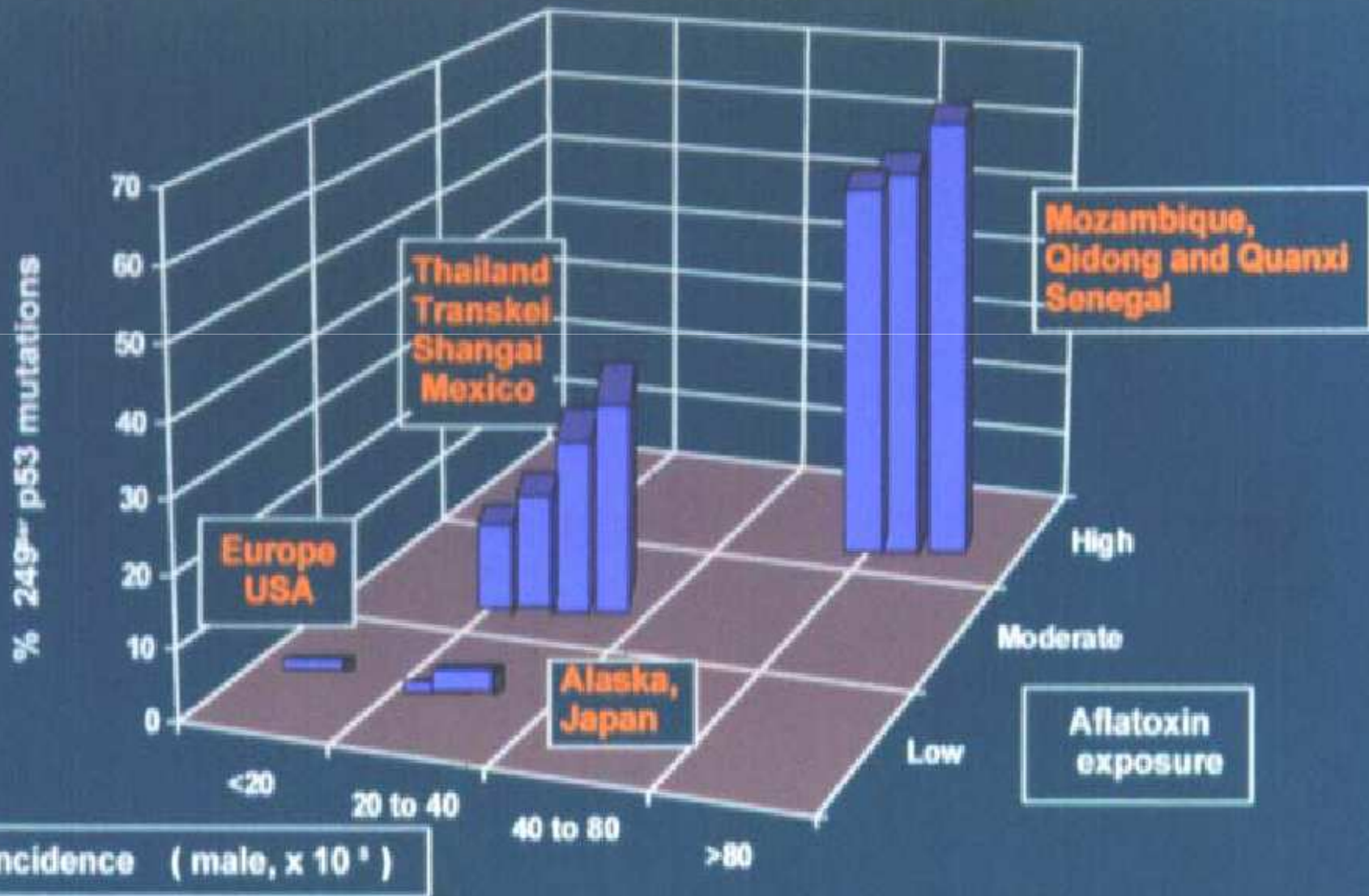
TOXIC EFFECTS OF AFLATOXIN

- Human

- Aflatoxin B₁ is highly mutagenic, probably through mechanisms of epoxidation resulting in covalent binding to DNA.
- A specific mutation at the third base of codon 249 of the tumor-suppressor gene p53 has been described in HCC tissue and significantly associated with exposure to aflatoxin B₁.

Prevalence of 249^{Ser} p53 mutation – Aflatoxin Exposure Incidence of Hepatocellular Carcinoma

(Total number of cases: ~ 1000)



Populations at risk of aflatoxin exposure

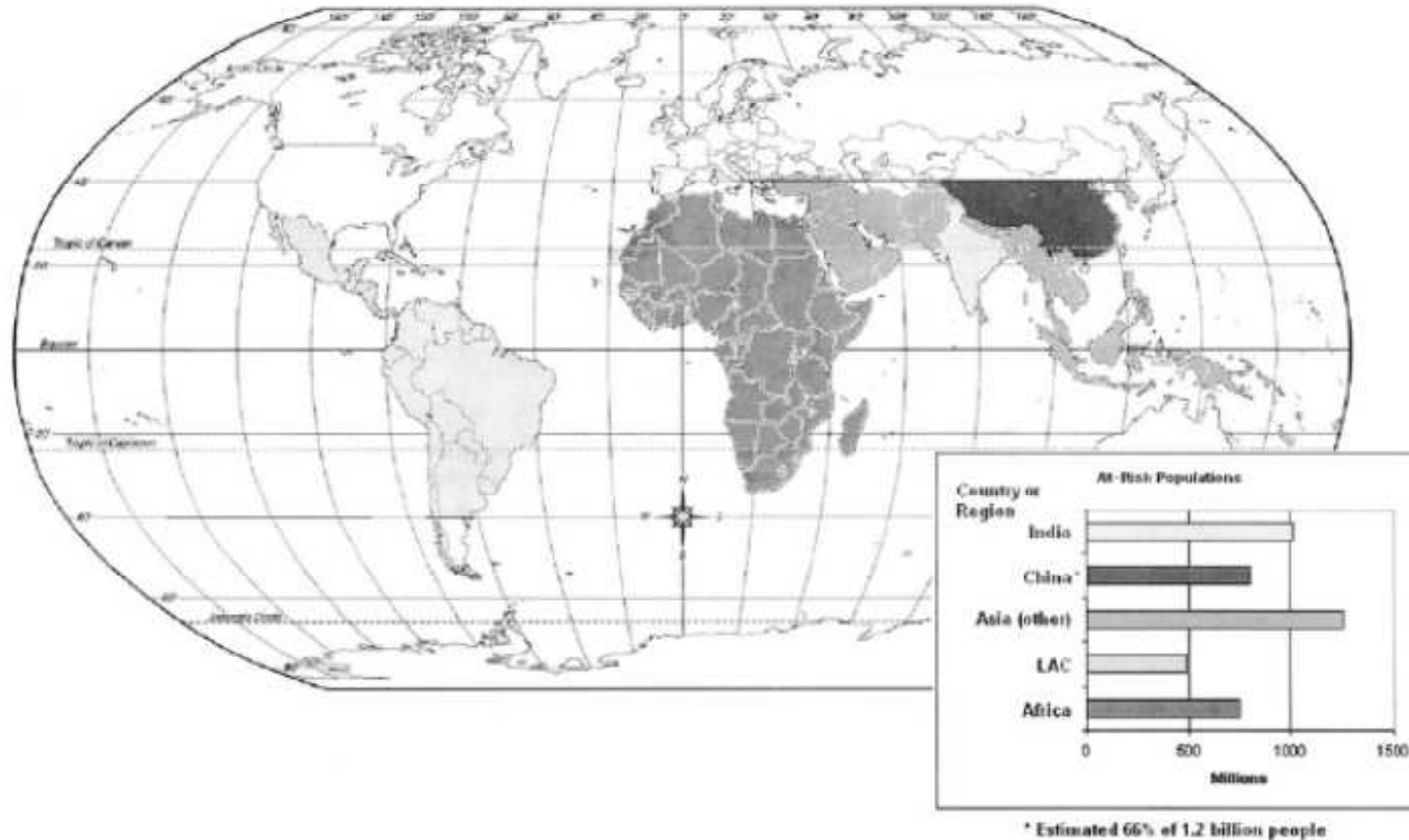
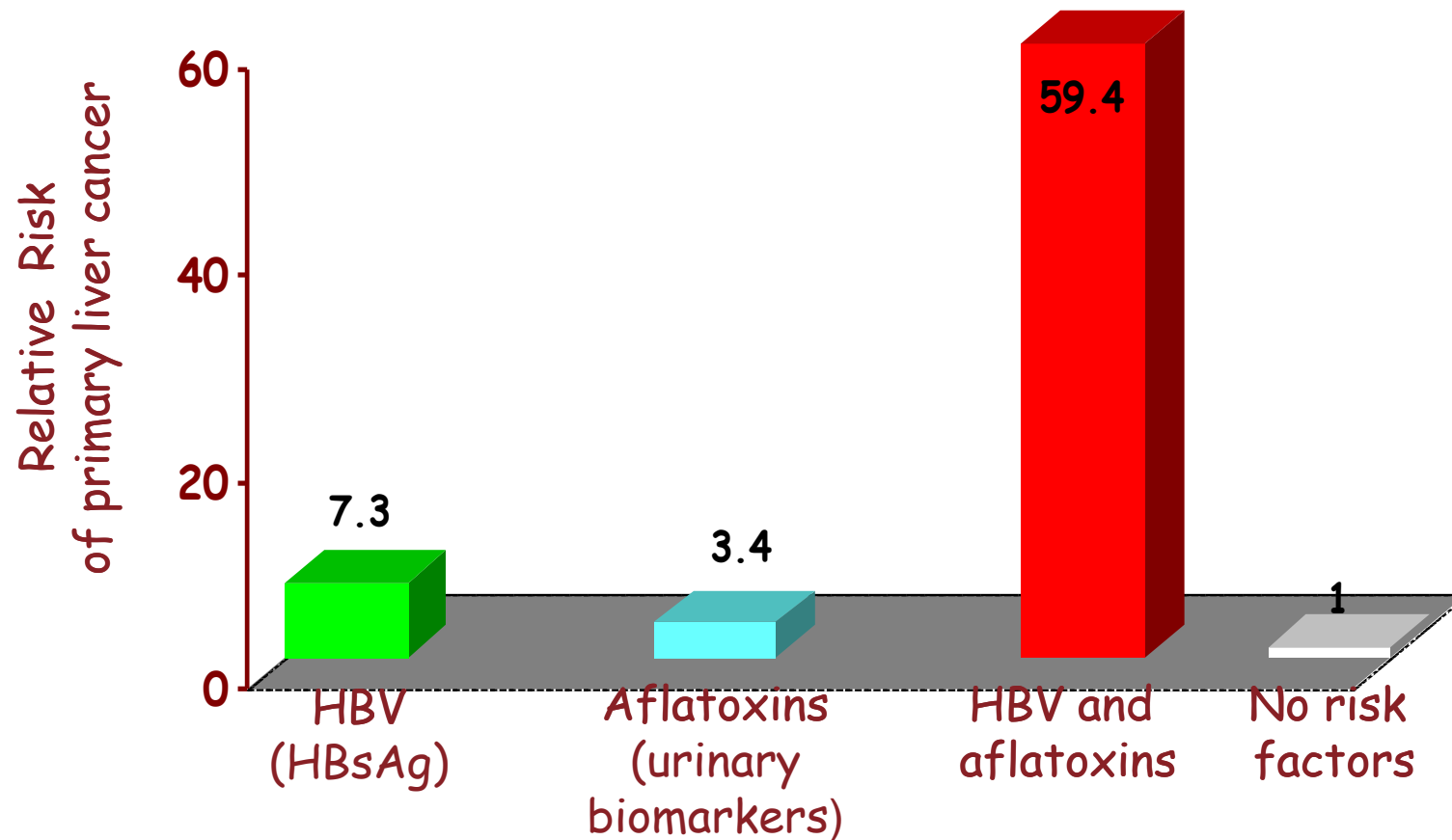


FIGURE 3. Areas and populations at risk of chronic exposure to uncontrolled aflatoxin. LAC, Latin America and the Caribbean.

Chronic hepatitis B together with exposure to dietary aflatoxins increases the risk of liver cancer



Adapted from Qian et al, 1994

Available options for solving the problem

Once food is contaminated with toxins, there are only two options

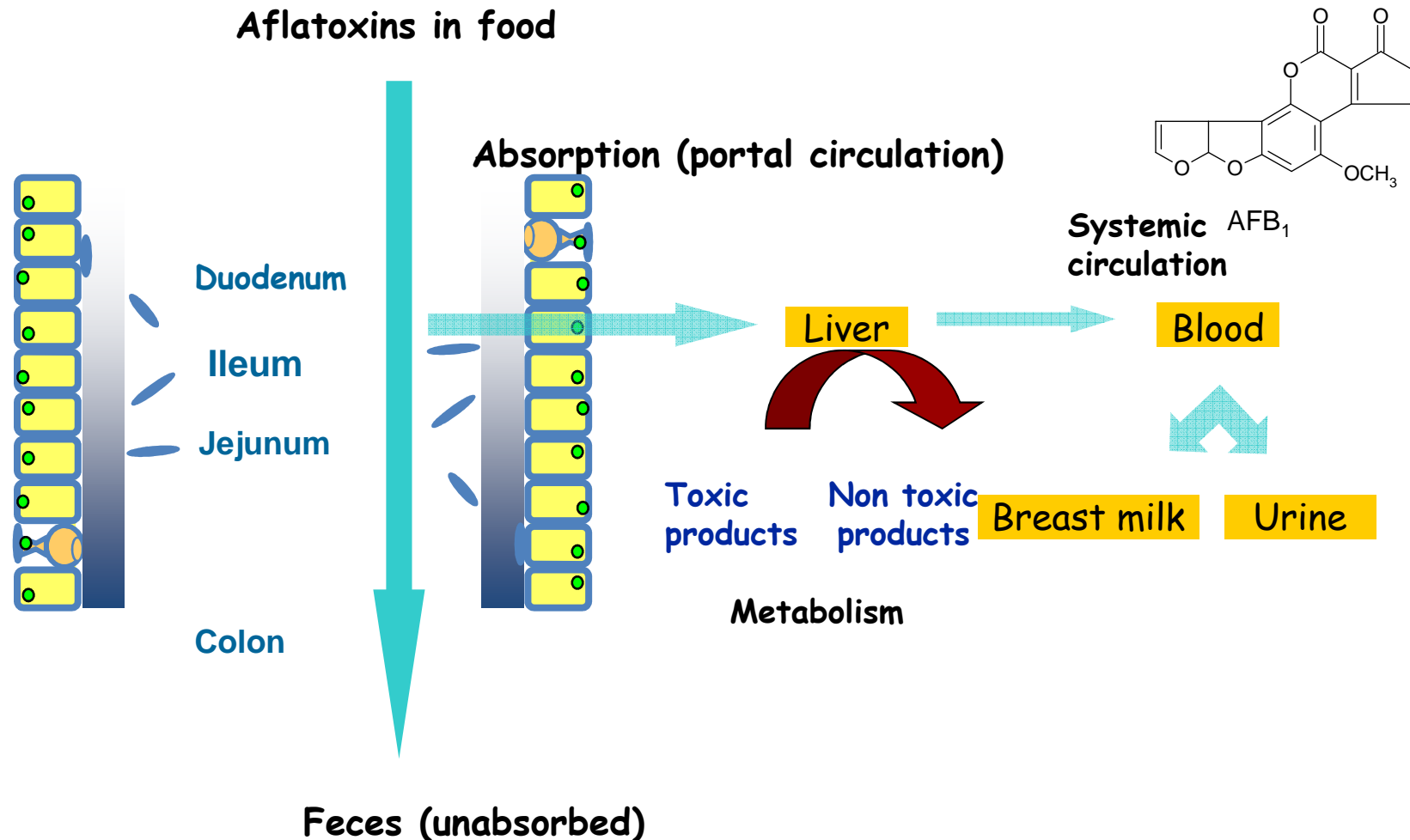
if the food is to be used:

- **the toxin can be removed**
- **the toxin can be degraded into less toxic or non-toxic compounds**

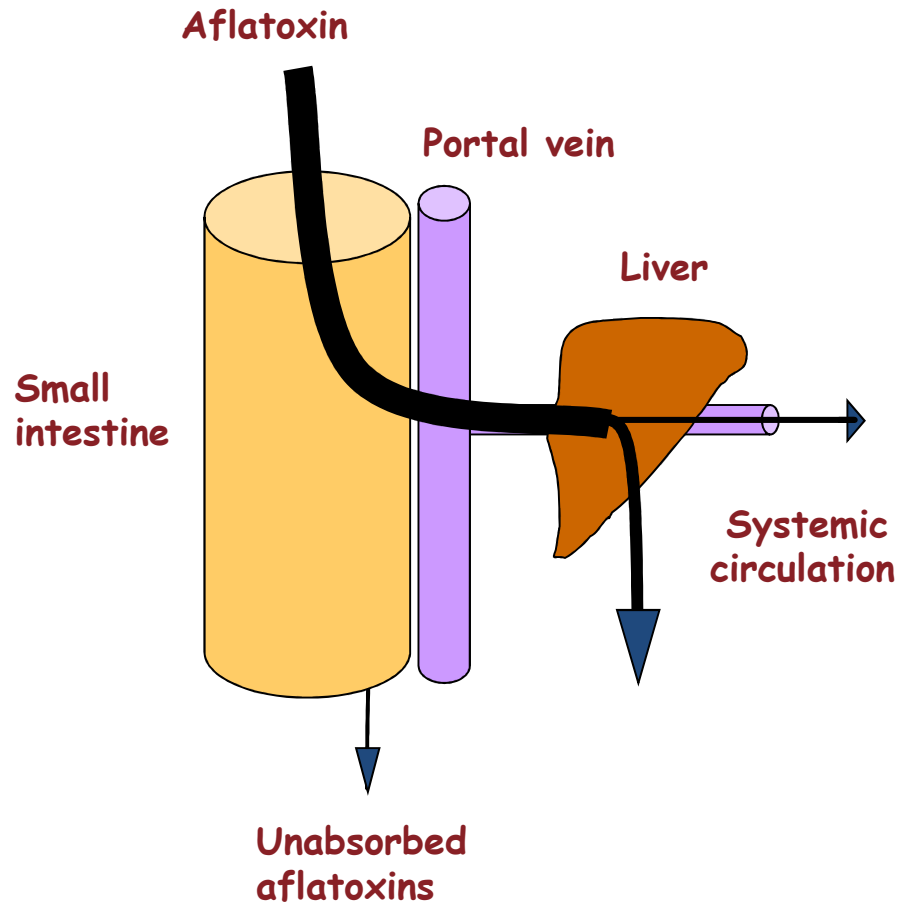
Control measures

- **Physical control (e.g. UV radiation, electronic sorting)**
 - suitable for very limited products
- **Chemical control (e.g. ammoniation)**
 - health effects are not fully studied
- **Monitoring AF levels and rejection of produce**
 - extremely costly option

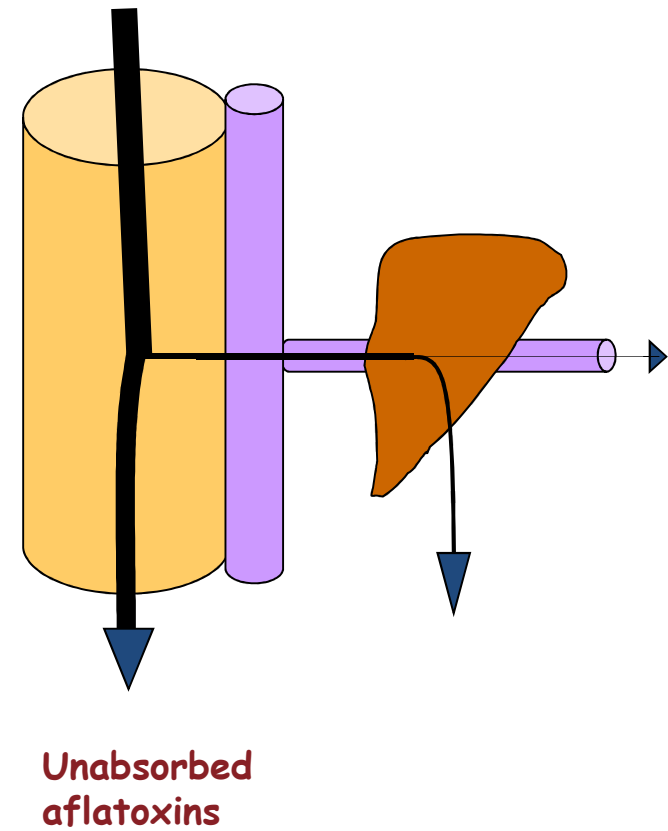
Strategies for intervention at individual level



Blocking/reducing absorption of AFB₁ from the small intestine



Aflatoxin + blocker

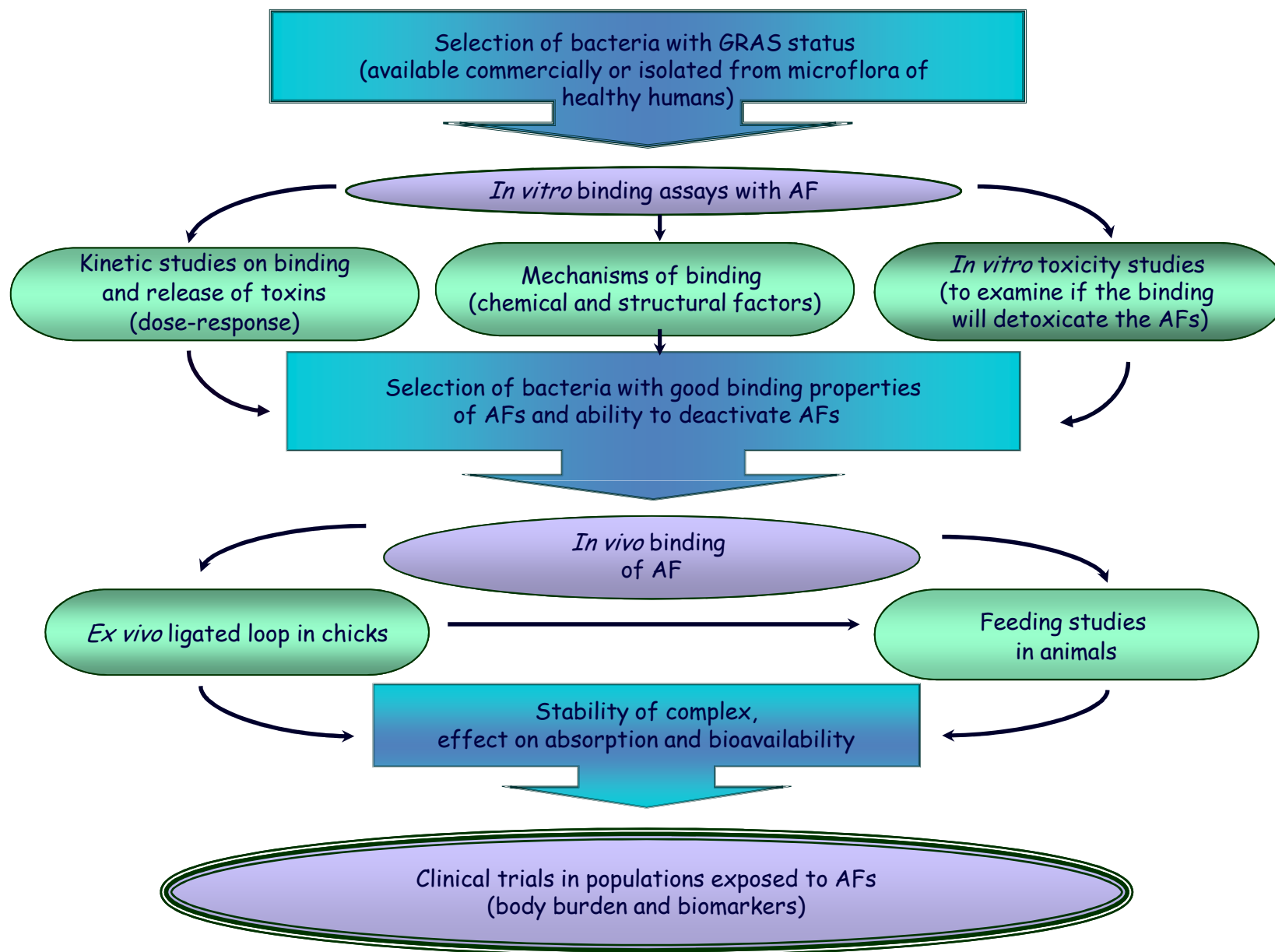


Requirements for dietary tools of blocking/ reducing aflatoxin absorption in humans

- Part of normal human diet
- Long history of safe use
- Able to bind a range of harmful compounds including aflatoxins
- Binding takes place immediately and is stable under GIT conditions
- No effect on absorption of micro and macro nutrients
- Inexpensive and practical for food enrichments

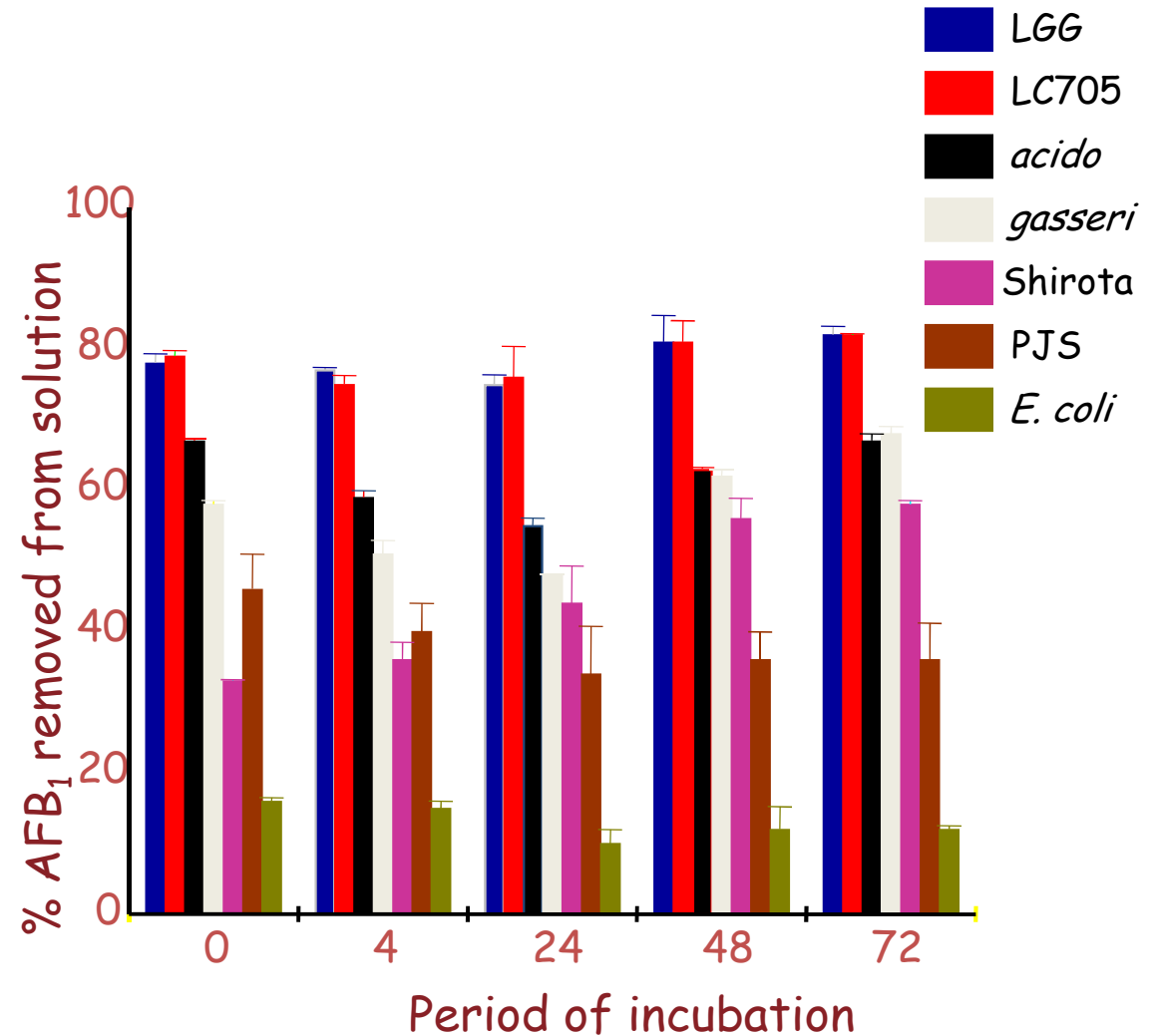
Lactic acid bacteria (LAB)

- LAB involved in the production of fermented foods
 - o one quarter of our diet
 - o characterised by safe history
 - o extended shelf life compared to raw materials
- LAB has some health effects
 - o growth inhibition of food spoiling bacteria
 - o production of antimicrobial compounds
 - o probiotic effects as live organisms in food



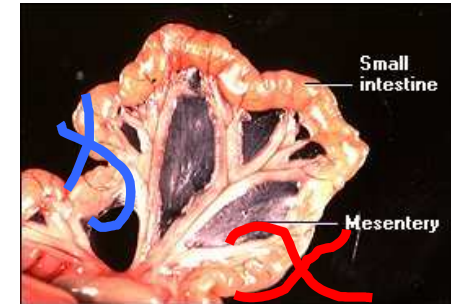
Aflatoxin is bound by probiotic bacteria - *in vitro* evidence

- Certain strains of lactobacilli are capable of binding up to 80% of AFB₁ *in vitro* (El-Nezami *et al*, 1996, 1998a,b,c), Fusarium toxins (El-Nezami *et al*, 2002a,b, 2004), PhIP and Trp-P-1 (Haskard *et al*, 2001)
- AFB₁ is predominantly bound to a carbohydrate moiety on the surface of the bacteria (Haskard *et al*, 2002)
- The complex formed between the bacteria and AFB₁ is stable under different conditions (Haskard *et al*, 2002, Lee *et al*, 2003)



El-Nezami *et al*, Food Chem Toxicol 1998

Ex vivo study in chicks

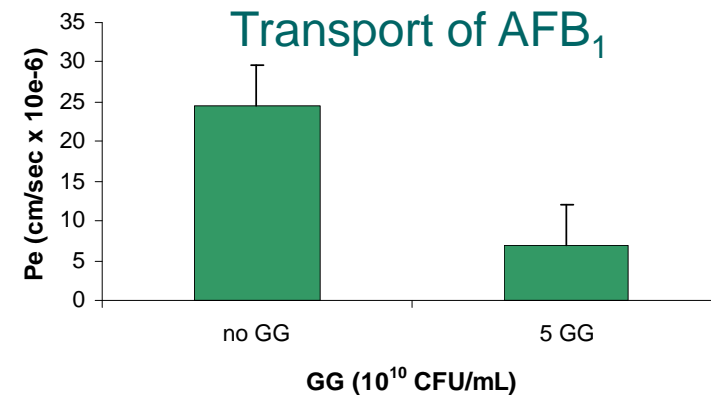
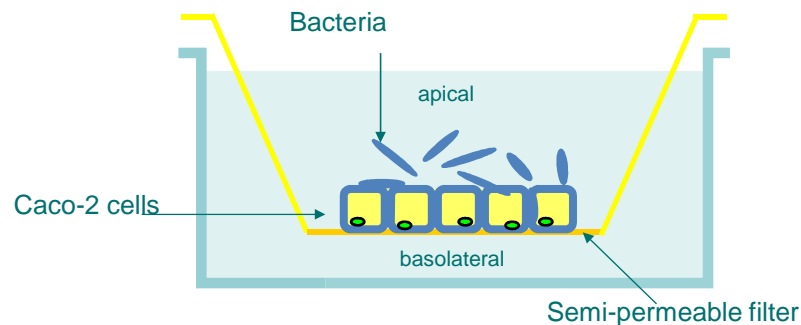


The concentration of AFB₁ ± SD extracted from

Group	Duodenal tissue ^b		Soluble fraction ^c of luminal fluid		Insoluble fraction of luminal fluid	
	1 min	60 min	1 min	60 min	1 min	60 min
AFB ₁ only	0.27 ± 0.09	ND	1.04 ± 0.36	0.05±0.01	ND	ND
LBGG+AFB ₁	0.07 ± 0.05	ND	0.48 ± 0.15	ND	0.76±0.04	1.38±0.16
LC705+AFB ₁	0.17 ± 0.11	ND	0.58 ± 0.10	0.08 ± 0.06	0.54±0.10	1.07±0.12
PJS+AFB ₁	0.10 ± 0.05	ND	0.67 ± 0.13	0.13 ± 0.02	0.55±0.11	1.24±0.06

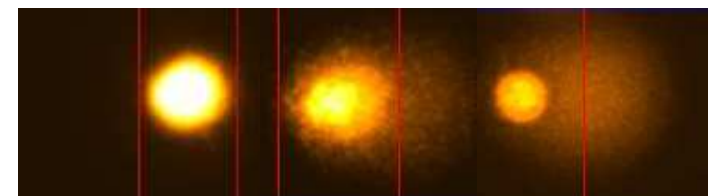
El-Nezami et al. (2000)): Journal of Food Protection. , JGratz S. et al. (2005): Journal of Food Protection.

Intestinal AFB₁ transport and toxicity

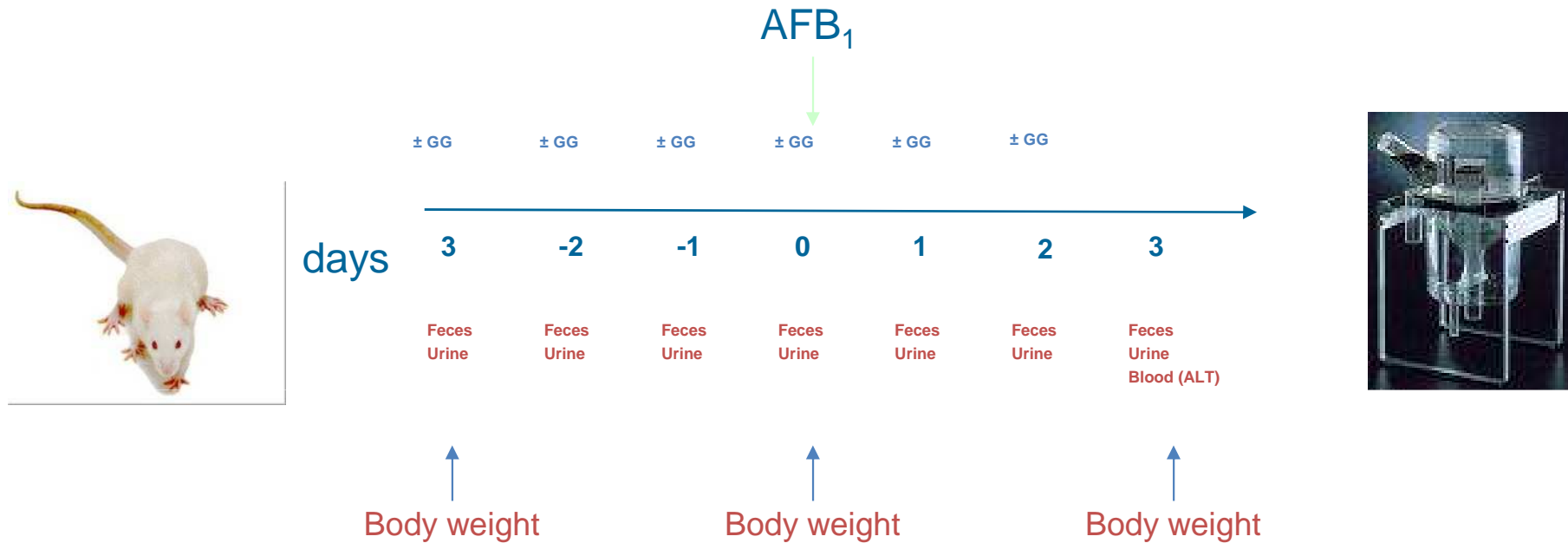


- Transport of AFB₁ through monolayer was reduced by GG
- AFB₁ induced TER (membrane integrity) reduction was attenuated
- AFB₁ induced DNA damage was attenuated

Gratz S., et al. (2007) Applied and Environmental Microbiology.



In vivo protective effects of probiotics against AFB₁ toxicity



AFB₁ (1.5 mg/kg bw, single dose on day 0)

GG (5×10¹⁰CFU, daily for 6 days)



Rat results

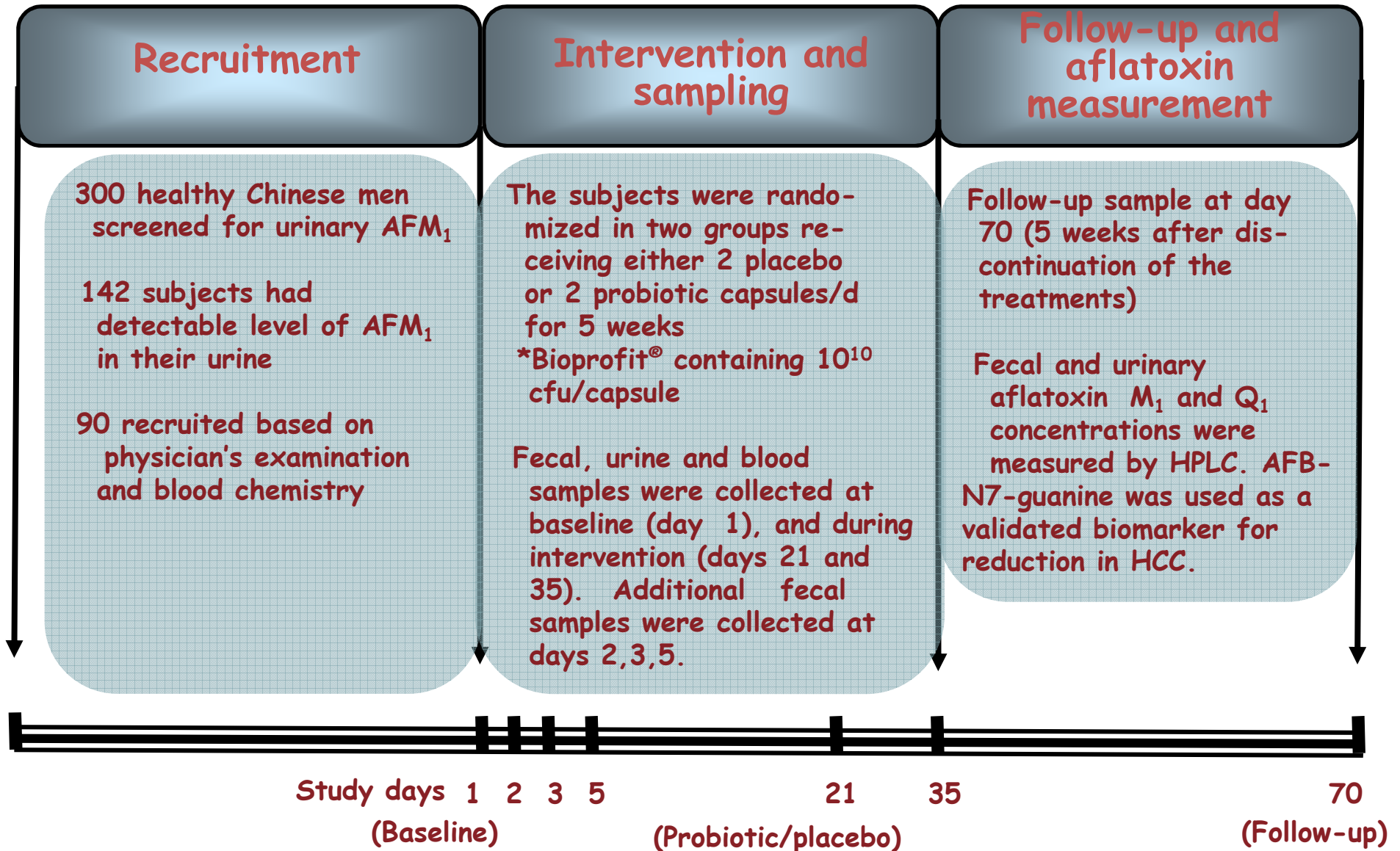
GG administration:

- Increased fecal AFB₁ by 122%
- Increased fecal AFM₁ by 152%
- Decreased plasma AFB₁-albumin by 29%
- Decreased change in liver function (ALT) by 54%
- Prevented body weight loss

WHY CHINA?

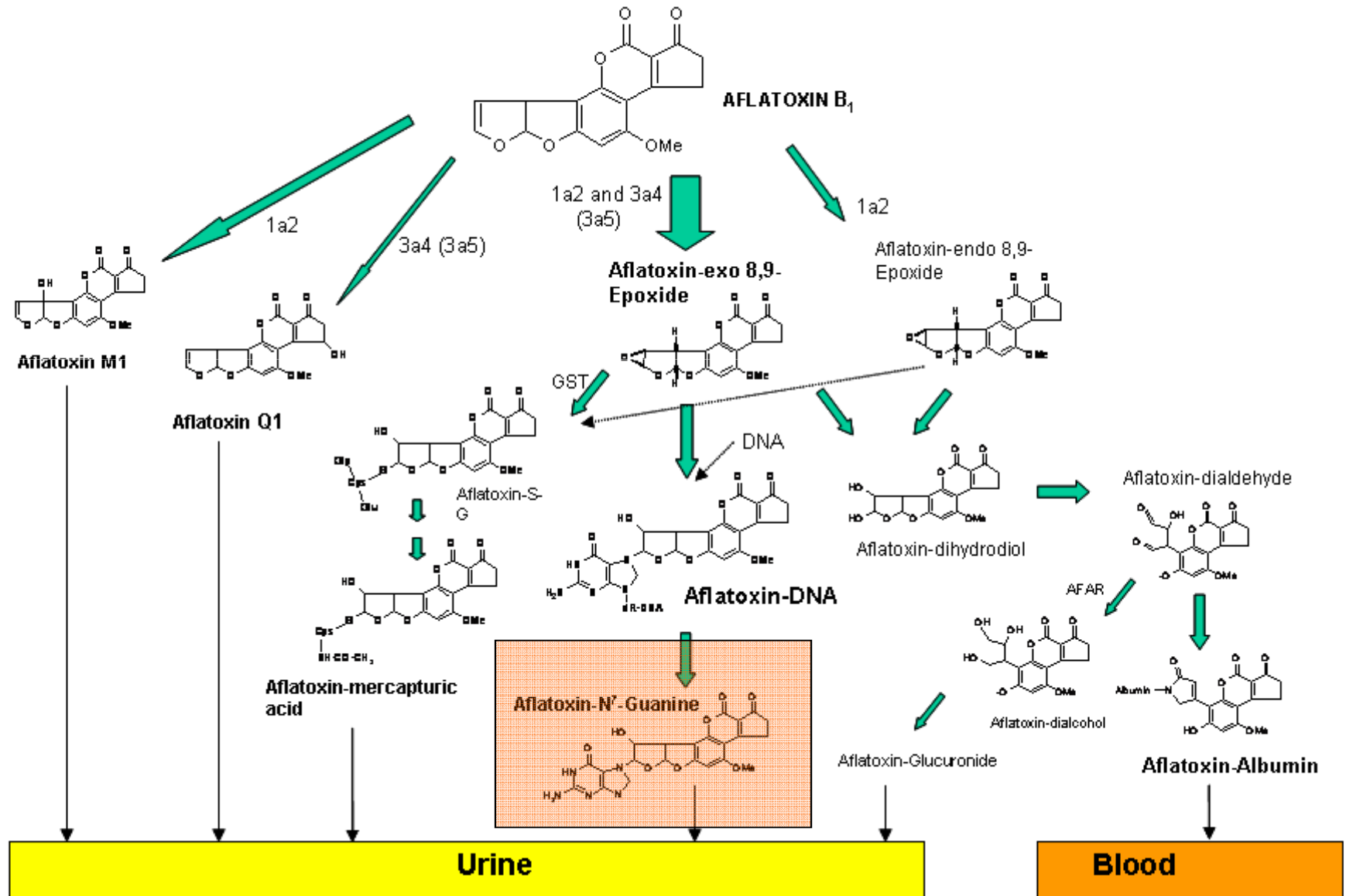
- Primary liver cancer (PLC) is one of the most common cancers in China.
- There more than 250,000 new cases diagnosed yearly with liver cancer in China.
- The mortality rates both in rural and urban areas are 25 and 21 per 100 000, respectively, in the EU 3 per 100,000.
- The main 3 factors for the development of liver cancer are prevalent in China. Aflatoxins are consistent contaminants of the food supply in China, HBV and HCV are endemics in China.
- 500,000,000 individual infected with HBV
(250,000,000 in China)
- 170,000,000 individuals infected with HCV
(10,000,000 in China)
- 1,000,000 individuals dies annually because of complication associated with HBV, similar figure also expected for HCV
(250,000 in China)

Probiotic intervention in China

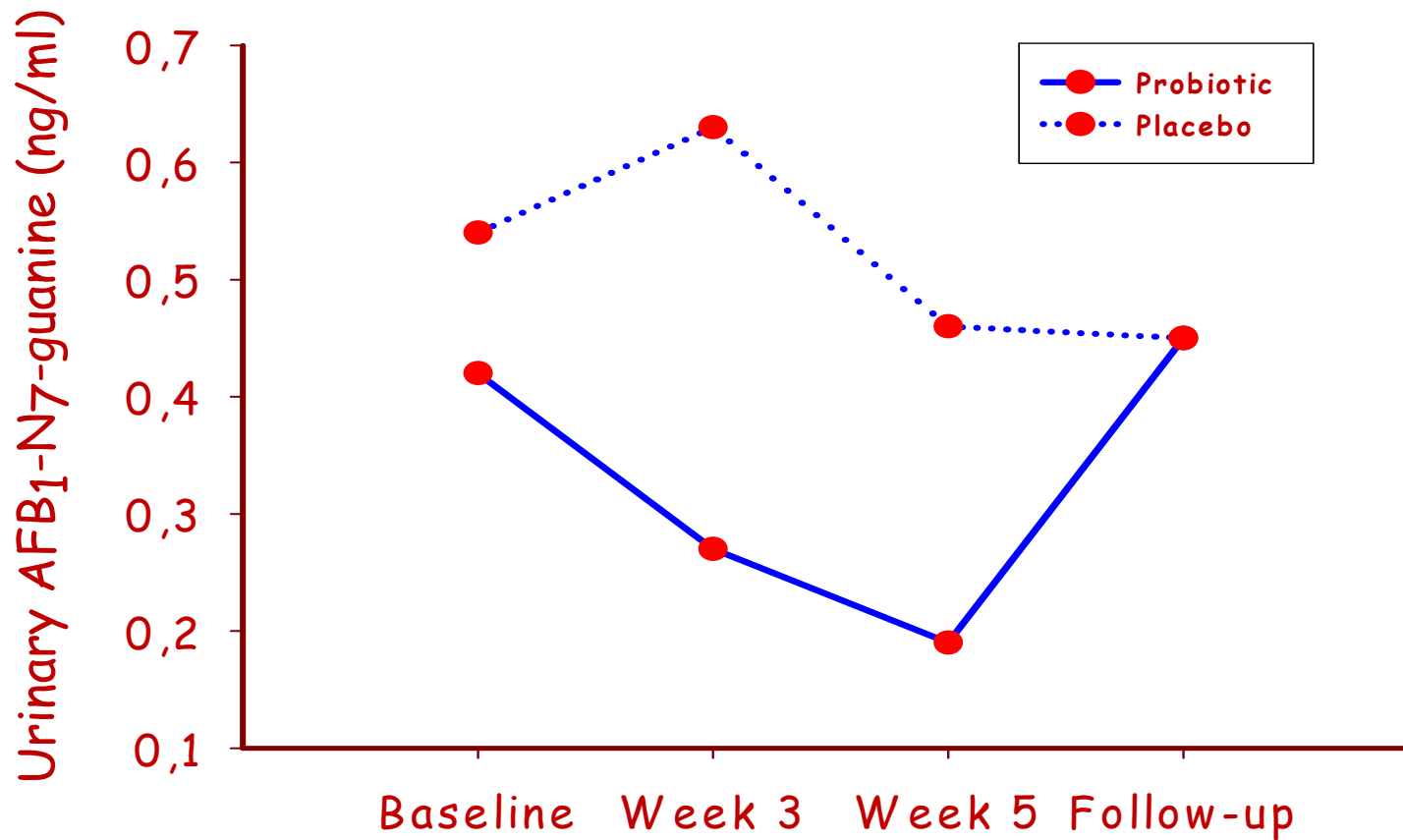




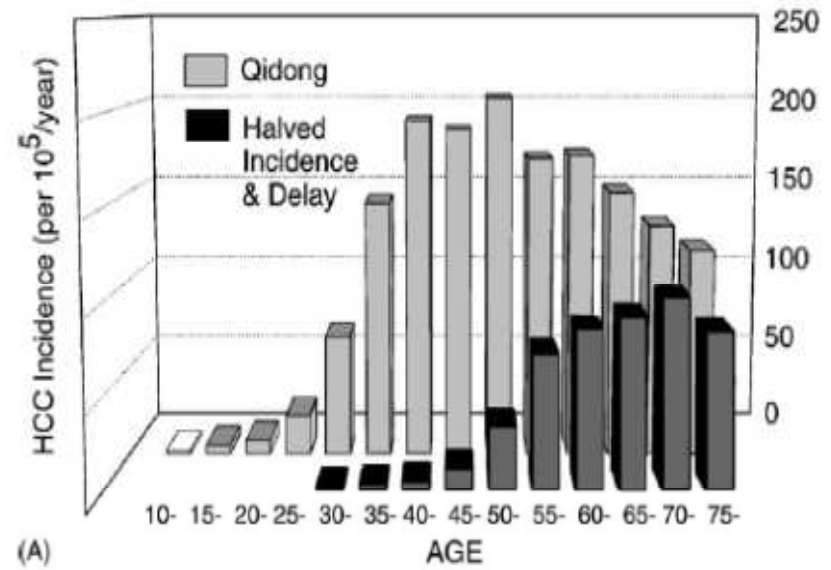
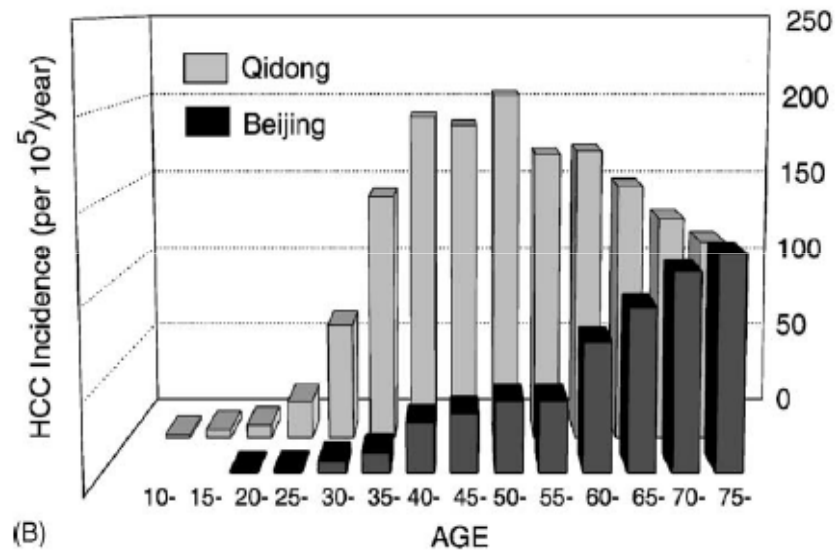
Principle Metabolites of Aflatoxin B₁ and Potential Biomarkers



Probiotic supplementation reduces the urinary excretion of AFB₁-N⁷-guanine, a biomarker of biologically effective dose of exposure to AFB₁



What our findings mean?



Egner et al, Mutation Res 523-524:209-216, 2003