

**Comparison of Aflatoxin-Induced Hepatocellular Carcinoma in the United States and  
China**

by

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University of Pittsburgh, 2022

## **Abstract**

According to the WHO, liver cancer is the third leading cause of cancer death globally and primarily affects developing countries. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and can be caused by aflatoxins. More than 53 percent of HCC patients worldwide come from China, while in the United States, hepatocellular carcinoma is a relatively rare cancer. This essay focuses on aflatoxin-induced HCC, analyzing the extent of aflatoxin contamination, the incidence of HCC, the leading causes, and the relevant laws and regulations of the two countries.

China is one of the world's largest importers of food and animal feed, and the United States is the world's top food exporter. Therefore, the public health and food safety issues caused by aflatoxins have received significant attention in both countries. In addition, there are differences between the two countries regarding food production and handling environments, legislative aspects, and responses to public health issues.

Comparing the leading causes of HCC in the two countries, it was found that the incidence of HCC the HBV-infected population were higher in Asia due to ethnic differences and different types of hepatitis viruses prevalent in the two countries. Nevertheless, comparing the incidence and significant causes of HCC among Japanese, Asians living in the U.S., and identical twins, environmental factors were more influential than genes. Therefore, aflatoxins are the main HCC risk factor of concern herein.

Both countries have room for improvement in food quality regulation, work exposure regulation, popular science and publicity, and HCC prevention. In the discussion section, recommendations are made to the relevant governing departments of the two countries.

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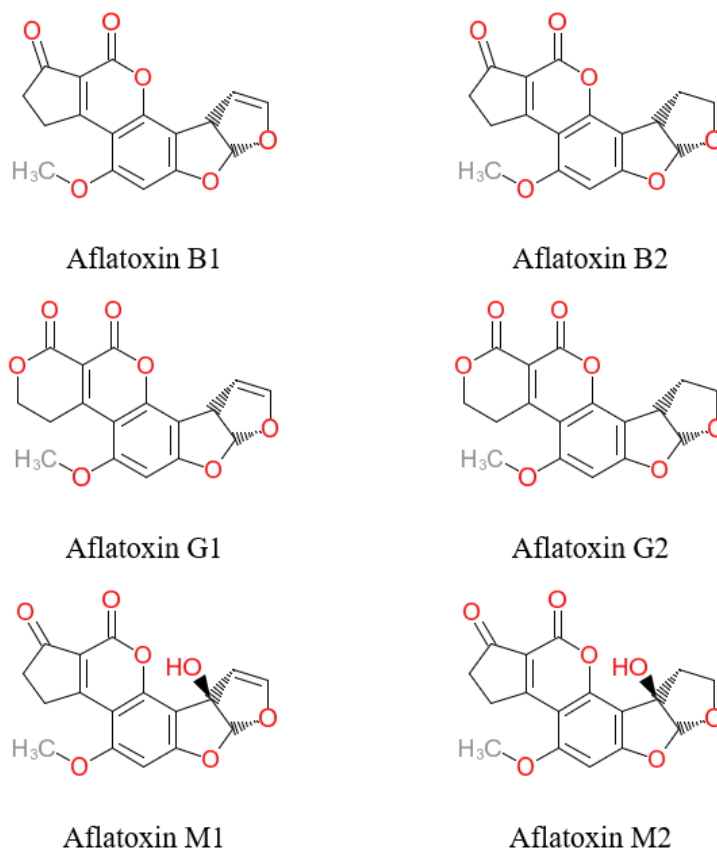
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## 1.0 Introduction

In moldy, toxic animal feed, aflatoxins were discovered in the United Kingdom in about 1960 when 100,000 turkey poults died of unknown turkey "X" disease.<sup>1</sup> In follow-up studies, scientists have successfully separated more than 20 types of aflatoxins from some molds (*Aspergillus flavus* and *A. parasiticus*). Nowadays, there are three main series of aflatoxins that are considered to be closely related to human food safety, which are the B-series (AFB1 and AFB2), the G-series (AFG1 and AFG2), and the M-series (AFM1) (Figure 1).<sup>2</sup>



**Figure 1. Major Aflatoxins**

(Source: <https://doi.org/10.1111/1541-4337.12734>, assessed June 17, 2022)

Acute exposure to aflatoxins can cause nausea, vomiting, and convulsions. Long-term exposure to a certain level of aflatoxins can cause chronic diseases. Typical effects include liver cirrhosis and hepatocellular carcinoma.<sup>2</sup> The aflatoxin B1, the most common, toxic, and carcinogenic aflatoxin, is an important contributor to hepatocellular carcinoma (HCC).<sup>3</sup> AFG1 also has carcinogenic potency. However, AFB2 and AFG2 are less toxic, but they can be metabolically oxidized to AFB1 and AFG1 in vivo.<sup>2</sup> AFM1 is the principal hydroxylated metabolite of AFB1, which has reported carcinogenic effects.<sup>4</sup> Therefore, aflatoxicosis is a public health threat that cannot be ignored. Nowadays, aflatoxin contamination is more prevalent in developing countries, primarily in Africa and Asia. China is an example of a country with a severe aflatoxin contamination problem. The incidence of HCC in China is also very high, and it is the eighth highest incidence of liver cancer in the world.<sup>5</sup>

Liver cancer is the sixth leading cancer in new cases and was the third leading cause of cancer deaths worldwide in 2020, according to the Global Cancer Observatory.<sup>6</sup> Hepatocellular carcinoma is the most common type of primary liver cancer. At the same time, it is one of the chronic effects of exposure to aflatoxin. Unlike other types of liver cancer, HCC develops very slowly in the early stages, so it is not easily detected. Many patients are at an advanced stage when HCC is first discovered.<sup>7</sup> HCC spreads very rapidly in advanced stages, resulting in high mortality and low five-year survival rates for HCC compared to other cancers. It is worth noting that the overall survival of HCC patients in different countries is also very different. For example, the median survival of HCC patients in the United States is 33 months, while the median survival of HCC patients in China is 23 months.<sup>7</sup> It may be related to the differences in the number and scope of surveillance programs in the two countries and the average medical conditions. As an important risk factor leading to HCC, aflatoxins may also promote the development of HCC, which leads to

a decrease in the survival rate of patients and an increase in mortality. According to risk assessors, up to 28.2% of liver cancer cases worldwide each year are associated with aflatoxin exposure.<sup>8</sup>

There are also specific risk groups for HCC. For example, a synergistic effect of aflatoxin and hepatitis B virus (HBV) promotes the development of HCC.<sup>3</sup> So, patients with hepatitis B are at greater risk of exposure to aflatoxin than the normal population. People who work in some food factories or feed processing plants are at higher risk of aflatoxin exposure and more chance of developing HCC.

As a cancer with high mortality, HCC has been paid attention to worldwide. As a result, countries have adopted the popularization of HBV/HCV vaccination to prevent the occurrence of HCC. However, the incidence of HCC in China is still at a high level after the widespread popularization of the HBV vaccine. Therefore, this essay aims to examine the reasons for the difference in aflatoxin exposure and the incidence of HCC apparent between the United States and China to inform policy aimed at reducing aflatoxin-induced HCC incidence in China.

## **2.0 Backgrounds**

### **2.1 Aflatoxins**

Aflatoxins are widely distributed around the world and can contaminate various crops and agricultural by-products. The human exposure routes of aflatoxins are diverse and the net affect on public health may be varied.

#### **2.1.1 Source of Aflatoxin**

Aflatoxins are natural contaminants widely present in grains, vegetables, fruits, dairy products, and feedstuffs. Aflatoxins B1 etc. are toxic metabolites produced by *Aspergillus* species under high temperature and humidity conditions.<sup>9</sup> Aflatoxins M1 and M2 are present in milk excreted by ruminants following ingestion of feed contaminated with aflatoxin B1 and B2.<sup>10</sup> Fermented food is also an important source of aflatoxins.<sup>11</sup> According to some studies from China, Thailand, and Korea, fermented foods contain particularly high levels of aflatoxins.<sup>12-14</sup> In many parts of Asia people like to eat fermented foods and condiments, such as bean paste, soy sauce, and fermented dark tea. Since the fermentation process requires a warm and humid environment, conducive to the growth of *Aspergillus*, if the raw materials are contaminated with aflatoxin, the final product will have further elevated aflatoxin content.

### **2.1.2 Global Geographical Distribution of Aflatoxin Contaminants**

The optimum temperature range for *Aspergillus* growth and aflatoxin development is 80 – 110 degrees Fahrenheit with a relative humidity of 62 – 99%.<sup>15</sup> Therefore, the detection rate of aflatoxin in food and feed in tropical and subtropical regions is exceptionally high.<sup>16</sup> In some countries in Africa, East Asia, and Southeast Asia, the climate is tropical and subtropical, warm and rainy, which is the most suitable environment for the growth of *Aspergillus*.

Due to global warming, aflatoxin-contaminated areas have gradually spread from tropical and subtropical to temperate regions. For example, in temperate countries such as Italy and Portugal, studies have predicted that climate change will increase aflatoxin pollution in the countries, which will gradually become a major food safety problem in Europe.<sup>17-18</sup>

China and the United States also have subtropical climates in some regions. For example, Guangxi and Zhejiang provinces in southern China have subtropical climates. Moreover, the two provinces have oceanic and monsoonal climates, respectively, with sufficient rainfall. Both places are areas with severe aflatoxin contamination.<sup>19-20</sup> The central and southern United States is also a high aflatoxin risk area. Most of these regions have monsoon subtropical, Mediterranean, or tropical climates.<sup>21</sup>

In addition to climatic factors, economic factors also affect the geographic distribution of aflatoxin contamination. Aflatoxin crop contamination is more common in developing countries, such as some countries in Africa. For example, in Kenya, the incidence of aflatoxins in maize ranges from 25% to 98%.<sup>22</sup> According to surveys conducted between 2006-2009, only 17% of the country's production is suitable for human consumption.<sup>22</sup> Due to the poor economy and backward technological conditions, these countries install improved technology to lower the aflatoxin content of their crops. In addition, the lack of local food supervision may also cause contaminated

food to enter circulation. Although developed countries also have aflatoxin contamination problems, due to strict supervision, the content of aflatoxin in the food circulating in the market is not high, and it does not significantly impact public health.

### **2.1.3 Routes of Exposure**

Aflatoxins are mainly found in agricultural products, such as peanuts, beans, rice, and processed foods, such as milk and condiments. So, the primary route of exposure to aflatoxins is ingestion by consuming contaminated food or the dairy production of animals that have consumed contaminated feed.

There is also an occupational health risk due to aflatoxins. Previous epidemiological studies have found that farmers and other agricultural workers may be exposed to aflatoxins by inhaled dust while handling contaminated crops or feed.<sup>23</sup> In an experiment in India, 32.6% of food workers' bronchoalveolar lavage (BAL) showed the presence of aflatoxins. In addition, workers exposed to grain processing had significantly higher levels of aflatoxins than workers in other occupations.<sup>23</sup> Eye contact and skin absorption may also occur if people are exposed to high enough concentrations of aflatoxins, but the efficiency of eye contact and skin absorption is normally very low. However, long-term exposure to high aflatoxin concentrations on the eye or skin will also cause chronic diseases.

## **2.2 Mechanisms of Action of Aflatoxins in the Induction of Hepatocellular Carcinoma**

Aflatoxin B1 is the most effective hepatocarcinogen among aflatoxins,<sup>24</sup> confirmed in animal experiments. When people ingest AFB1, cytochrome P-450 is involved in the metabolic activation of AFB1 to AFB1-8,9-epoxide.<sup>25</sup> This reactive intermediate can covalently bind to DNA in urine to form an AFB1-guanine adduct.<sup>3</sup> The AFB1-guanine adduct can cause specific missense mutations in key genes,<sup>26</sup> leading to liver tumor formation. Residual AFB1 in the liver also negatively affects the p53 tumor suppressor gene and protein Rb pathway in hepatocellular carcinoma.<sup>27</sup> AFB1 can cause a specific missense mutation at codon 249, exon7 in p53, leading to the substitution of arginine for serine.<sup>28</sup> Scientists use the specific missense mutation in p53 as a marker for the presence of AFB1 in the body, representing the aflatoxin-induced HCC in individuals.

In rodent experiments, AFB1, AFB2, and AFG1 were shown to induce DNA damage in bacteria and covalently bind to DNA in rodent cells.<sup>29</sup> However, there is insufficient evidence for the carcinogenicity of AFG2. In addition, AFM1 has also been shown to induce mutations in mammalian cells that are oncogenic.<sup>30</sup> Therefore, when considering aflatoxin-induced cancer, not only the most common and toxic aflatoxin B1 in the market but also several common types are also considered.

Nested case-control was also conducted in Qidong, China, to assess the role of aflatoxins in the HCC epidemic.<sup>31</sup> It is the longest observed cohort study globally, up to 25 years, and has a low attrition rate. The researchers used mutations in p53 tumor suppressor gene (TP53) to indicate aflatoxin exposure. Mutations in the p53 gene were found in about half of HCC cases in Qidong and were present in both surgically resected tissue and plasma samples. In addition, the

concentration of serum AFB1-albumin adduct was also proportional to the risk of HCC, confirming that exposure to AFB1 is one of the important causes of HCC in Qidong.

### **2.2.1 Quantitative Carcinogenesis and Dosimetry for Aflatoxin**

In order to fully demonstrate the induction risk of aflatoxin on liver cancer, the scientists analyzed the concentration of hepatic aflatoxin B1-DNA adduct (HADA) and the risk of liver cancer in rats and trout. The results showed that there is a linear and quantitative correlation between the concentration of HADA and the risk of HCC.<sup>32</sup> Based on the results of animal experiments, scientists also estimated the lowest practical human safe dose of aflatoxin, which is about 0.264 ng/kg/day.<sup>32</sup>

## **2.3 Hepatocellular Carcinoma**

### **2.3.1 Causes of Hepatocellular Carcinoma**

Hepatocellular carcinoma (HCC) is a multifactorial disease. Many risk factors can cause or promote the occurrence of HCC.<sup>33</sup> The risk factors associated with HCC include chronic HBV and HCV infection, HBV/HCV or alcohol-induced cirrhosis, nonalcoholic fatty liver disease (NAFLD), aflatoxins, chronic alcoholic liver disease (ALD), obesity, and diabetes.<sup>33-34</sup> HBV/HCV hepatitis is the most common cause, accounting for about 80% of the total HCC.<sup>34</sup>

The major risk factors for HCC in developing countries are chronic HBV infection and dietary exposure to aflatoxin B1.<sup>35</sup> Some developing countries were popularizing the HBV vaccine

and improving aflatoxin regulations. So, the incidence of HCC is decreasing yearly in these countries, such as in China.

Major risk factors for HCC in developed countries are chronic HCV infection, excessive alcohol consumption, obesity, and NAFLD.<sup>33</sup> The increase in obesity and NAFLD has increased the HCC incident rate in the United States.

It is worth noting that chronic HBV-induced HCC is more likely to occur in Asians because HBV genotypes vary by region. For example, the A genotype is generally in Northern Europe and North America. On the other hand, the B and C genotypes are common in Asia. In addition, genotypes B and C are mainly in chronic HBV patients, and genotype A is common in acute HBV patients. Therefore, Asians are more susceptible to HBV genotypes B and C, which cause chronic HBV, further leads leading to hepatocellular carcinoma.<sup>36</sup> It also leads to different incidences of HCC in different regions. While many factors can contribute to HCC, researchers can distinguish whether aflatoxins play a role in inducing HCC by genetic mutations in p53.

### **2.3.2 Symptoms of Hepatocellular Carcinoma**

Hepatocellular carcinoma is divided into different stages according to tumor size, number, and location. There are two main staging systems to guide HCC therapy. One is based on the American Joint Committee on Cancer tumor/lymph node/metastasis classification system. It is one of the most common tumors' staging methods and is based on the primary tumor, lymph node spread, and whether it has metastasized to tissues further away in the body. Table 1 provides the specific details of the TNM classification.<sup>34</sup>

**Table 1. TNM Classification for Hepatocellular Carcinoma<sup>34</sup>**

Stage	T (Primary Tumor)		N (Regional lymph nodes)		M (Distance Metastasis)	
0	TX	Primary tumor cannot be assessed	NX	Regional lymph nodes cannot be assessed	M0	No distant metastasis
	T0	No evidence of primary tumor				
I	T1	Solitary tumor < 2 cm, or >2 cm without vascular invasion	N0	No regional lymph node metastasis	M0	No distant metastasis
	T1a	Solitary tumor < 2 cm				
	T1b	Solitary tumor >2 cm without vascular invasion				
II	T2	Solitary tumor >2 cm with vascular invasion; or multiple tumors, none >5 cm			M1	Distant metastasis
IIIA	T3a	Multiple tumors, at least one of which is >5 cm				
IIIB	T4	Single tumor or tumors of any size involving a major branch of the portal vein or hepatic vein, or tumor(s) with direct invasion of adjacent organs other than the gallbladder or with perforation of visceral peritoneum				
IVA	Any T	Single tumor or multiple tumors of any size	N1	Regional lymph node metastasis	M0	No distant metastasis
IVB	Any T		Any N	May or may not have spread to nearby lymph nodes	M1	Distant metastasis

The other staging system is Barcelona-Clinic Liver Cancer staging system (BCLC). Table 2 shows the BCLC system which is better for determining prognosis.<sup>34</sup> The BCLC staging system helps standardize the stage of a patient's condition, allowing doctors to formulate treatment plans based on cancer background. The BCLC staging system has been included in treatment guidelines in the United States but has not been used in Asia.

**Table 2. Barcelona-Clinic Liver Staging System for Hepatocellular Carcinoma<sup>34</sup>**

Stage	Performance Status	Tumor Stage	Okuda Stage	Liver function	Treatment Guideline
A: Early HCC					
A1	0	Single, <5 cm	I	No portal hypertension, normal bilirubin	Curative Therapies (i.e., liver transplantation, surgical resection, radiofrequency ablation)
A2				Portal hypertension, normal bilirubin	
A3				Portal hypertension, elevated bilirubin	
A4		3 tumors, <3 cm	I-II	Child-Pugh A-B	
B: Intermediate HCC					
B	0	Large, multinodular	I-II	Child-Pugh A-B	Palliative treatment (i.e., transcatheter arterial chemoembolization and sorafenib)
C: Advanced HCC					
C	1-2	Vascular invasion or extrahepatic spread	I-II	Child-Pugh A-B	Same as stage B
D: End-Stage HCC					
D	3-4	Any	I-II	Child-Pugh C	Supportive care and palliation

Hepatocellular carcinoma is cancer that starts in the hepatocyte. If the tumor is very early stage (stage 0) or early stage (stage A),<sup>34</sup> the tumor can be cured by surgery or liver transplantation, and the survival time is longer than in the advanced stage. Although there are not necessarily symptoms of most HCC in the early stage, some show light symptoms, including mild or moderate pain in the upper abdomen, bloating, satiety, fatigue, weight loss, and so on.<sup>33</sup> However, since there are few symptoms in the early stage of HCC, or the symptoms are similar to cirrhotic nodules

and dysplasia,<sup>33</sup> and they are difficult to distinguish on imaging and histopathology, it is challenging to detect HCC at this stage.

Symptoms of advanced HCC include abdominal pain, fatigue, diarrhea, and anorexia. If metastasis occurs, it will be accompanied by symptoms of other tumors, such as bone pain in bone metastases and dyspnea in lung metastases.<sup>33</sup> The survival rate for advanced HCC is low, with a five-year survival rate of only 19.5%. Therefore, early detection of HCC is important, but complex.

### **2.3.3 Geographical Distribution of Hepatocellular Carcinoma Worldwide**

The highest incidence of HCC globally is in Asia and Africa,<sup>33</sup> coincident with the regions having high aflatoxin contamination. The new liver cancer cases reported in China in 2018 accounted for 55.41% of the global reports.<sup>35</sup> In recent years, China has vigorously promoted hepatitis B vaccination, changed its dietary structure, and reduced the intake of corn as a staple food, dramatically reducing the amount of aflatoxin ingested by humans. As a result, the number of HCC cases has decreased year by year. However, China is still a region with high incidence of HCC.

In countries with a high incidence of HCC, there are also significant geographical differences in HCC. For example, in China, where HCC is associated with socioeconomic status (SES), the incidence of HCC is significantly higher in regions with lower incomes.<sup>37</sup> Geographical differences in HCC risk by SES also exist in the United States, with low community SES and immigration status likely contributing to higher HCC risk in these populations.<sup>38</sup>

### **3.0 Synergistic Effect of Hepatitis B Virus (HBV) and Aflatoxin B1 (AFB1) in Causing Hepatocellular Carcinoma (HCC)**

Chronic hepatitis B virus (HBV) infection is a major risk factor for hepatocellular carcinoma. In addition, the presence of aflatoxins can increase the risk of HCC. Two prospective cohort studies were conducted in Shanghai and Taiwan to demonstrate the synergy of the two factors. The two studies recruited 18,224 and 6,487 residents for screening surveys and regular follow-up examinations (up to 12 years).<sup>3</sup> All experiments measured AFB1 exposure levels by examining serum AFB1-albumin adducts in serum or AFB1 metabolites in urine. Among HBsAg-negative individuals, people with high AFB1 exposure had four times increased risk of HCC, and among HBsAg-positive individuals, people with high AFB1 exposure had 111 times increased risk of HCC. The result shows the significant combined effect of AFB1 and HBV on HCC.

In addition to cohort experiments in the population, the researchers also conducted animal experiments to confirm the synergistic effect of AFB1 and HBV in hepatocellular carcinoma. The subjects were mice, woodchucks, and ducks. Investigations in these types of animals confirmed that animals infected with hepatitis virus had a higher incidence of hepatocellular carcinoma after exposure to AFB1 for 6 to 26 months than controls exposed to AFB1.<sup>39</sup> In addition, after exposure to AFB1, liver cells developed more rapidly and abnormally.

Therefore, although the hepatitis B virus is recognized as the main factor leading to hepatocellular carcinoma, the synergistic effect of aflatoxin and HBC still cannot be ignored. The synergistic impact significantly increases the risk of HCC.

## **4.0 Comparison Between China and the United States**

### **4.1 Aflatoxins Contamination Conditions in China**

China is located on the Pacific coast of Southeast Asia, with a latitude range of 18-52 degrees. Most of the southern part has a humid subtropical climate, which is the most suitable environment for the growth of *Aspergillus*. As a result, aflatoxin contamination is most serious in cities in provinces in southern China, such as Zhejiang, Guangdong, and Guangxi Province.<sup>20-21, 40</sup>

Zhejiang Province has tested aflatoxin B1 concentration in various local foods, including peanut oil, nuts, nut products, grain, grain products, and condiments.<sup>20</sup> Among the 792 samples, 27.1% were detected with aflatoxins, among which peanut oil was the most contaminated. As high as 82.5% of peanut oil were aflatoxins positive, 70% were AFB1 positive, and 0.8% exceeded the Chinese standard. In addition, the AFB1 content of 9.9% of nuts exceeds the regulatory limit in China by 131 times. 0.7% of the condiment samples exceeded the Chinese limit.

In Guangdong Province, rice, wheat products, corns, vegetable oils (including homemade peanut oil), nuts, and tea were tested for AFB1.<sup>40</sup> Most of the food's AFB1 levels were below one ppb; only the mean of the AFB1 level of homemade peanut oil was 38.74 ppb, and the median reached 141.4 ppb, which is seven times above the standard.

With one of the high incidences of liver cancer in China, Guangxi Province has inspected peanut oil in bulk, packaged (factory processing), and other vegetable oils.<sup>21</sup> Among the 146 vegetable oil samples, the AFB1 excess rate was 31.51%. On the other hand, the excess rate of peanut oil reached 78.08%, with an average content of 30.80 ppb. Among them, the average AFB1

of the packaged peanut oil is 6.33 ppb, which is within the national standard, while the average AFB1 of the bulk peanut oil is 41.5 ppb, which is more than twice the Chinese standard.

All three studies assessed dietary exposure risk to AFB1, and the three provinces used a margin of exposure (MOE) based on BMDL<sub>10</sub> to estimate human exposure levels. A lower MOE indicates a higher potential risk of exposure to the substance. In general, MOEs greater than 10,000 are considered safe.<sup>20</sup> The MOEs of peanut oil, rice, and corn in Zhejiang are lower than 10,000.<sup>20</sup> It is worth noting that although the concentration of AFB1 detected in rice in the samples was not high, it showed a greater risk of exposure due to the large intake of rice as the leading staple food. The result of Guangdong's research shows that the MOE of suburban residents is lower than that of urban residents, and the MOE values are all below 10,000.<sup>40</sup> Therefore, it indicates that all the Guangdong residents are at risk of AFB1 exposure, and suburban residents are at greater risk of exposure. It may be related to more homemade peanut oil consumption and poorer food storage conditions of suburban residents. In Guangxi, where the pollution situation is the most serious, the MOE value is as low as 12 – 96,<sup>21</sup> and the tested bulk and packaged peanut oil and vegetable oil are at high risk of exposure.

## **4.2 Aflatoxin Contamination Conditions in the USA**

Aflatoxins have been a recurring contaminant problem in corn in the southern and mid-southern United States, costing more than \$2 billion in damage each year.<sup>41</sup> Undoubtedly, this is related to the warm and humid local climate. The southern part of the United States mainly has a subtropical climate. The central and southern parts include subtropical and temperate climates, both of which are suitable for the growth of *Aspergillus*. However, aflatoxin poisoning is not a

significant concern in the US because contaminated crops are recalled and destroyed due to the regulations.<sup>41</sup> The United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) have established strict regulations for the testing and permissible levels of aflatoxins.

To further confirm the effectiveness of regulations against aflatoxins, the National Center for Health Statistics conducted a National Health and Nutrition Examination Survey. The concentration of aflatoxin-albumin adducts, aflatoxin B1-DNA adduct, or AFB1-guanine adduct can reflect the degree of exposure to aflatoxin. It turned out that only about 1% of adults showed evidence of exposure to AFB1, a level well below that in countries that lack surveillance programs. It demonstrates that aflatoxin exposure does not pose a high public health risk in the US population and, in practice, can be managed to remain low.

### **4.3 Legislation of Aflatoxin Levels**

In order to control aflatoxin levels in food, both China and the United States have established standards for crop or dairy products that are susceptible to contamination. Table 3 summarizes the aflatoxin content limits for grains, beans, nuts, oils, condiments, and dairy products in China and the United States. As can be seen from the table, the standards set by China are only for aflatoxins B1 and M1, and the standards for most crops and foods are below 20 ppb.<sup>42</sup> However, in addition to targeting AFB1, the United States also restricts the content of total aflatoxins, and the legal limit is lower than that set by China. For processed agricultural products, the aflatoxin limit in the United States is more stringent. For example, the AFB1 content of vegetable oil products are below 2.0 ppb,<sup>43</sup> while the Chinese standard for this is below 10.0 ppb.<sup>42</sup>

**Table 3. Aflatoxins Limits in Foods**

<b>Food Category</b>	<b>Limit (ug/kg = ppb)</b>	<b>Limit (ppb)</b>
	China <sup>*1</sup>	America <sup>*2</sup>
<b>Grains and grain products</b>		
Corn, corn flour (grits, flake) and corn products	20 (Aflatoxin B1)	15 (B1)
Paddy rice, brown rice, rice	10 (B1)	20 (Total Aflatoxins, Sum of aflatoxin B1, B2, G1 and G2)
Wheat, barley, other grains	5.0 (B1)	
Wheat flour, cereal, other husked grains	5.0 (B1)	
<b>Beans and bean products</b>		
Fermented bean products	5.0 (B1)	5.0 (B1) 10 (Total Aflatoxins)
<b>Nuts and seeds</b>		
Peanut and its products	20 (B1)	8 (B1) <sup>*3</sup>
Pistachio		15 (Total Aflatoxins) <sup>*3</sup>
<b>Fat and its products</b>		
Vegetable oil and fat (except for peanut oil, corn oil)	10 (B1) 20 (B1)	2.0 (B1) <sup>*3</sup> 4.0 (Total Aflatoxins) <sup>*3</sup>
Peanut oil, corn oil		
<b>Condiment</b>		
Soy sauce, vinegar, fermented paste (using grains as the major materials)	5.0 (B1)	4.8 (B1) <sup>*4</sup> 15 (Total Aflatoxins) <sup>*4</sup>
<b>Dairy Products</b>		
Formula foods for infants and young children	0.5 (B1, in powdered product basis) 0.5 (M1)	0.5 (M1)
<b>Animal feeds</b>	30 (B1)	20 (B1) <sup>*3</sup>

<sup>\*1</sup>: China's Maximum Levels for Mycotoxins in Foods set the standard.<sup>44</sup>

<sup>\*2</sup>: Food and Drug Administration (FDA) standard; otherwise, will note out.

<sup>\*3</sup>: Data from the Peanut Aflatoxin Program Requirements.<sup>45</sup>

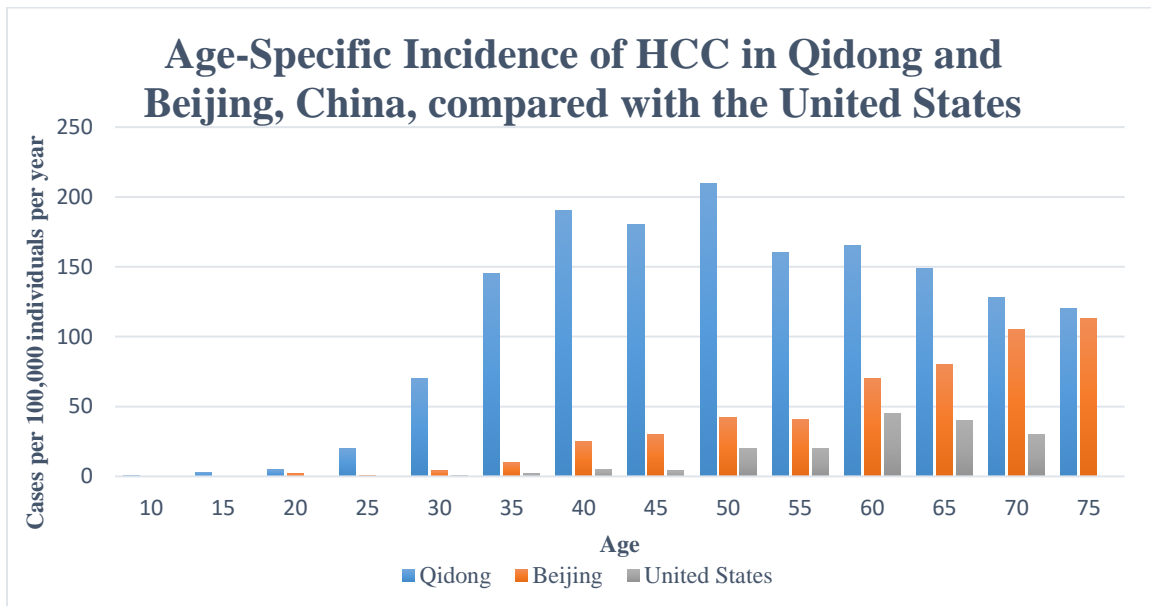
<sup>\*4</sup> Data from Quality Index of Starter Culture Soy Sauce Samples.<sup>46</sup>

Neither country has laws on occupational exposure limits to aflatoxins.<sup>42</sup> However, according to the OSHA Personal Protective Equipment Standard (29 CFR 1910.132), when potentially exposed to a hazard, workers are required to wear PPE<sup>43</sup> to avoid skin contact, eye contact, or inhalation of aflatoxins. NIOSH-approved negative pressure, air-filtering respirator is also required when potentially exposed to aflatoxins.<sup>43</sup> China does not yet have recommendations related to occupational exposure to aflatoxins; only dietary exposure is considered.

In addition to the difference in the limits of aflatoxin concentrations, the two countries also have differences in handling food safety issues. Strict regulation of the quality of crop raw materials in the US costs hundreds of thousands of dollars per year,<sup>43</sup> but simultaneously ensures that Americans are exposed to extremely low levels of aflatoxins. Due to the difficulty of comprehensive coverage in rural areas, in addition to sampling, the US has adopted strategies such as good agricultural practices (GAP) and the promotion of new processing technologies to reduce the concentration of aflatoxins in foods. There are also testing requirements for agricultural raw materials in China. However, due to the low industrialization and standardization of agricultural and sideline products in China, many products are sold without aflatoxin testing. At the same time, China's penalties for food safety issues are relatively light; for example, in July 2020, it was found that a food company in Shanxi Province left the finished product with excessive pollutants and did not inspect the finished product. As a result, the court only imposed a fine of 5,000-yuan (\$745).<sup>47</sup> It is a light penalty for a company.

#### 4.4 Leading Causes and Development Trends of Hepatocellular Carcinoma

In China, the primary causes of HCC are HBV and aflatoxins.<sup>33</sup> In a study conducted in Qidong, a high-incidence area of HCC in China, it was found that 50% of HCC patients showed a G to T mutation in codon 249 of the p53 gene,<sup>48</sup> which is a tumor suppressor gene mutation caused by aflatoxin exposure. With the popularization of HBV in China, the incidence of HCC in China has declined. However, the incidence of HCC is still relatively high in areas with high aflatoxin exposure. Figure 2 compares the number of HCC cases at different ages in Qidong and Beijing, China.<sup>48</sup> They have the same HBV infection rate, but Qidong is in a high-risk area for aflatoxins. It can be seen from the figure that the HCC incidence in people aged 35-60 in Qidong is much higher than that in Beijing. The total incidence is also much higher than that in Beijing.



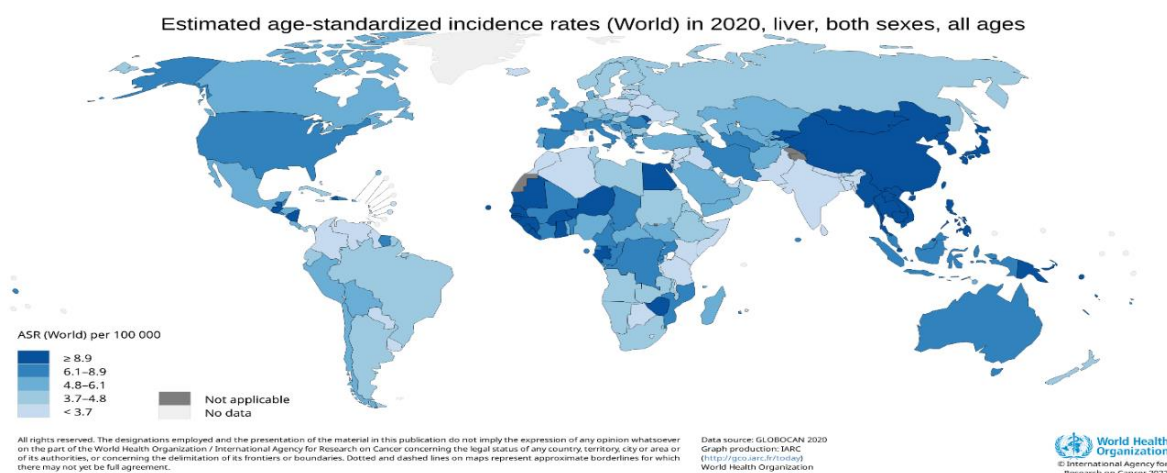
**Figure 2. Age-specific Incidence of HCC in Qidong and Beijing, China, Compared with the United States**

(Source: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6528445/>, assessed August 10, 2022)

Comparing Beijing and the United States, the total HCC incidence in Beijing is higher than in the United States. The average age of onset is earlier, which may be due to many aspects, such as the higher HBV infection rate and exposure to aflatoxins in Beijing, and genetically chronic HBV is more susceptible in Asian races. The leading causes of HCC in the United States are HCV, obesity, and NAFLD. In recent years,<sup>33</sup> due to the increasing incidence of NAFLD, NAFLD has gradually become a common cause of HCC in North America. NAFLD is often associated with hepatocellular carcinoma.<sup>34</sup> Also, the incidence of non-viral hepatocellular carcinoma has increased simultaneously.<sup>48</sup>

#### 4.4.1 HCC Incidence Differences Due to Race

According to the Global Cancer Observatory, the overall incidence of liver cancer in Asians is higher than in Europe and the Americas (Figure 3).<sup>49</sup> Therefore, differences in the incidence of HCC due to race differences should also be considered.

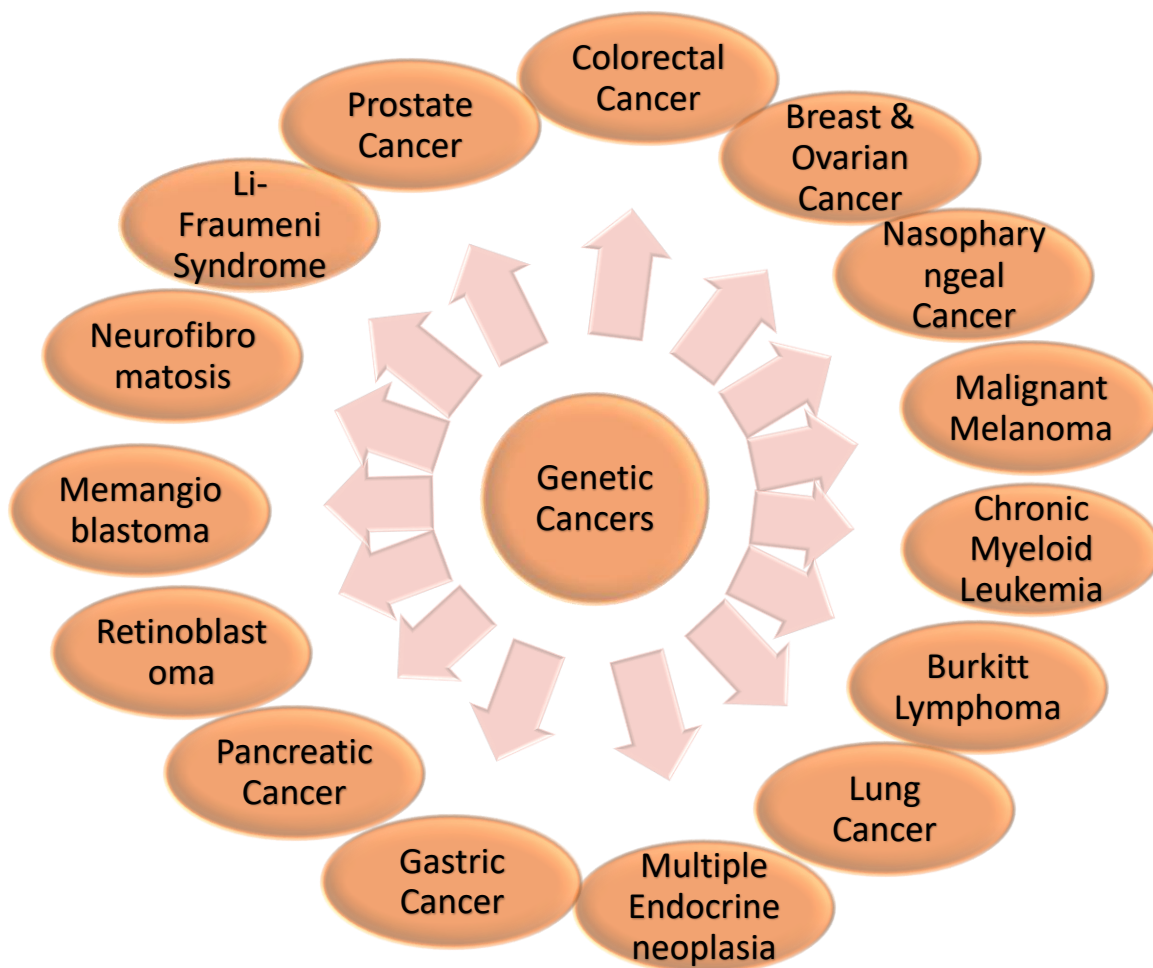


**Figure 3. Estimated Age-standardized Incidence Rates in 2020, Liver, Both Sexes, All Ages**

(Source: [https://seer.cancer.gov/archive/csr/1975\\_2018/](https://seer.cancer.gov/archive/csr/1975_2018/), assessed June 17, 2022)

By comparing the incidence of HCC between native Chinese residents and Chinese immigrants to the United States, it can be found that the incidence of HCC in the Chinese population in the United States is lower than that in the native Chinese population.<sup>35</sup> At the same time, by comparing the incidence of HCC within Asia, the incidence in developed countries such as Japan and Korea, is significantly lower than that in developing countries such as Laos, Cambodia, and Thailand.<sup>50</sup> Therefore, it is speculated that while race differences influence the incidence of HCC, the degree of race effect is not as large as that of lifestyle, environmental factors, and economic conditions.

Research on monozygotic twins has also shown that lifestyle and environmental factors in cancer development are more important than genetics. In a study of the influence of genetic and environmental factors on cancer in Sweden, Denmark, and Finland, 44,788 twins were surveyed and followed up.<sup>51</sup> The overall response rate was approximately 87%. Experiments have shown that the concordance rate of twins is usually less than 0.1. Inherited gene defects cause only 5-10% of cancers (Figure 4), and most cancers are caused by mutations resulting from interactions with the environment.



**Figure 4. Genes Associated with Risk of Different Cancers**

(Source: <https://www.researchgate.net/publication/5225070>, assessed August 10, 2022)

#### **4.5 Prevention and Surveillance of Hepatocellular Carcinoma**

Because HCC is often detected at an advanced stage, advanced HCC is cancer with a poor prognosis.<sup>7</sup> Currently, prevention strategies for HCC mainly focus on primary and secondary prevention. The target population of primary prevention is mainly the general population. Specific primary prevention processes include preventing or reducing risk factors such as HBV, HCV,

chronic alcoholic liver disease (ALD), NAFLD, and aflatoxins.<sup>52</sup> Secondary prevention is mainly for patients in the early stage of HCC, including timely screening and disease development prevention.

#### **4.5.1 Primary Prevention**

China's current primary prevention focuses on two risk factors: HBV and aflatoxin. The processes include

- Popularizing the HBV vaccine<sup>36</sup>
- Setting aflatoxin limit standards for packaged food and animal feed<sup>39</sup>
- Changing the structure of staple foods in some areas with a high incidence of aflatoxin and HCC.<sup>56</sup>

Primary prevention in the US also includes popularizing the HBV vaccine and setting standards for aflatoxins in food and animal feed.<sup>45-46</sup> However, the major contributors to HCC in the United States also include HCV, NAFLD, and obesity. Because there is currently no vaccine against HCV, the US uses syringe services programs (SSPs) to minimize HCV infection, promoting the safe disposal of used needles and syringes to avoid blood-borne diseases. Experiments show that SSP is a safe, effective, and cost-effective way.<sup>53</sup> However, due to the influence of opioids, HCV transmission rates are still high.

In order to reduce HBV-induced HCC, China and the US have incorporated the HBV vaccine into routine immunization services. Since China included the HBV vaccine in childhood immunization programs in 2002, the hepatitis B vaccination rate of newborns in China has increased from 22% in 1992 to more than 90%.<sup>54</sup> However, since the effect of the HBV vaccine

on HCC needs to be observed for years to be discovered, controlling for other influencing factors may result in a faster response.

In summary, due to the different national characteristics of the two countries, China mainly needs to pay attention to controlling food contamination caused by aflatoxin. In contrast, the United States needs to focus on controlling hepatitis C transmission and obesity rates to reduce NAFLD.

#### **4.5.2 Secondary Prevention**

The main secondary prevention measure for HCC is screening. Periodic alpha-fetoprotein (AFP) testing is recommended for specific risk groups in the United States, according to guidelines for HCC screening, testing, diagnosis, and treatment issued by the American Association for the Study of Liver Diseases (AASLD).<sup>55</sup> AFP is the most widely used serum marker for auxiliary diagnosis of liver cancer. AASLD suggested risk groups include cirrhotic patients and some non-cirrhotic hepatitis B carriers.<sup>55</sup> Meanwhile, the National Comprehensive Cancer Network (NCCN) recommends ultrasonography (US) every six months for patients with specific causes of cirrhosis, including HBV/HCV, alcohol, hereditary hemochromatosis, and nonalcoholic fatty liver disease.<sup>55</sup>

A trial of AFP screening for HCC has also been conducted in China, with risk groups defined as those with hepatitis, hepatitis B virus carriers, history of blood transfusion, or family history of HCC.<sup>4</sup> The screening program, which has been in place in select cities for 20 years, has been shown to diagnose HCC early and treat it symptomatically to improve patient survival.<sup>53</sup>

Chemoprophylaxis is also a secondary preventive measure in the experimental phase.<sup>56</sup> Some in vitro and animal experiments have demonstrated that certain drugs can indirectly reduce the incidence of HCC. For example, in mouse models, the antiplatelet effect of aspirin can inhibit

platelet aggregation and reduce the incidence of HCC;<sup>56</sup> low dose erlotinib can reduce liver fibrosis and prevent HCC.<sup>57</sup> Nevertheless, there is currently not enough clinical evidence to support the widespread application of this approach.

## 5.0 Discussion

Aflatoxin contamination is a public health problem faced by many developing countries, especially in Asia and Africa. Aflatoxin-induced gene mutations have been shown to lead to hepatocellular carcinoma in both animal and human experiments. However, in developing countries like China, the control of aflatoxin contamination has not received enough attention. Due to their eating habits, Chinese people eat more foods that are susceptible to growing aflatoxins, such as rice, corn, and peanut oil; also, as the staple food, the daily intake is large. Although China has established standards for such foods, it only regulates the concentration of AFB1 without other types of aflatoxins. The standards for AFB1 are also relatively loose.<sup>44-46</sup> Regarding packaged foods on the market, there are clear regulations on aflatoxin content for foods processed in factories.<sup>44</sup> However, the penalties for illegally selling unqualified products, factories, and companies that deliberately missed inspections are too lenient,<sup>47</sup> resulting in manufacturers not paying enough attention to food safety incidents such as aflatoxin contamination. At the same time, due to the large market share of bulk food, a large part of crops is listed and traded without testing. The occupational exposure to aflatoxins is not considered.<sup>47</sup> For example, farmers inhale aflatoxins during grain production, harvesting, and drying, or workers in food processing plants are exposed to *A. flavus* during processing contaminated food.<sup>5</sup> Together, these factors contribute to the high incidence of aflatoxin-related hepatocellular carcinoma in China.

For developed countries, such as the United States, which have strict market testing standards and policies, aflatoxin contamination will mainly lead to economic losses rather than public health problems such as HCC. The United States has carried out farmer training in awareness of preventing aflatoxin contamination.<sup>44</sup> It has enabled more agricultural practitioners

to understand the importance of preventing aflatoxin contamination. Also, the program reduces the occurrence of aflatoxin-contaminated food entering the market and endangering human health from the source. So, more noteworthy is the increasing incidence of HCC due to the increasing incidence of NAFLD in the USA. There are no studies or regulations on occupational exposure in the United States, and no workplace exposure limits are set. However, the study of BAL fluid from workers in India's food processing plants<sup>23</sup> shows that occupational exposure to aflatoxin cannot be ignored. Therefore, the current OSHA and NIOSH recommendations<sup>43</sup> can be translated into law requiring that workers in establishments that may be exposed to aflatoxin-contaminated products must wear PPE and fine particulate respirators to work and replace them regularly.

## **6.0 Conclusion: Future Trends and Suggestions for Attention**

With the effects of global warming, rising temperatures and increasingly extreme weather will lead to northern regions becoming suitable places for aflatoxins to grow. As a result, aflatoxin contamination, which originally mainly existed in the United States and southern and central-southern China, will gradually expand to the north. So, it may lead to more people at risk of aflatoxin exposure, and the resulting economic losses may also increase. Therefore, setting stricter and more scientific agricultural product preservation standards should be considered, such as testing the ventilation rate, temperature control, and mold contamination level of each granary to ensure that a large number of aflatoxins will not be produced due to poor preservation after harvesting.

Legislation on food safety also needs to be paid attention to. For example, strict sampling and testing should be carried out before listing. The supervision of bulk food should also be strengthened, and penalties should be increased to make businesses pay attention to the problem of aflatoxin contamination.

China also needs to conduct widespread scientific publicity on the carcinogenicity of aflatoxins. The target population includes consumers, bulk and retail food producers, and merchants.

Due to the high incidence of HCC in China, screening for HCC can be extended to the whole country and strive to detect hepatocellular carcinoma as early as possible and treat it in time. Moreover, considering the cost issue, the price of AFP is lower, and regular AFP detection can be promoted temporarily.

Since obesity rates are increasing in both the US and China, NAFLD will be not only a significant factor in HCC in the US but also one of the main factors in the occurrence of HCC in China. Therefore, government departments and communities in both countries need to focus on advocating healthy lifestyles and diets to reduce obesity rates and gradually reduce the incidence of NAFLD.

The high lethality and growing incidence of hepatocellular carcinoma are a world concern. This paper argues that aflatoxin is an inescapable factor in inducing HCC by introducing the carcinogenic mechanism of aflatoxins and the synergistic effect of AFB1 and HBV. Moreover, as global warming intensifies, aflatoxin pollution will become an important public health problem in more places. Comparing China and the US in various aspects, the differences in the two countries' efforts to control aflatoxin contamination, food safety, and popularize the harm of aflatoxins have led to differences in the incidence of HCC. In terms of preventive measures for HCC, the focus of the two countries is different, but both countries need to establish complete primary prevention. For example, China needs to improve food safety monitoring and, like retail and bulk agricultural product sellers, learn about the harm of aflatoxin, and reduce it as much as possible. Aflatoxin levels in commercially available foods. The US needs to strengthen the prevention of needle-transmitted HCV and minimize obesity rates to reduce HCV-induced HCC and NAFLD-induced HCC. At the same time, for the secondary prevention of HCC, it is necessary to vigorously promote HCC screening and strive to allow more people at risk of HCC to detect HCC at an early stage and receive timely treatment.

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n\_group=0&ages\_group%5B%5D=17&nb\_items=10&group\_cancer=1&include\_nmsc=0&include\_nmsc\_other=0&projection=natural-earth&color\_palette=default&map\_scale=quantile&map\_nb\_colors=5&continent=0&show\_ranking=0&rotate=%255B10%252C0%255D.

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