



Diet, nutrition, physical activity and **liver cancer**

2015

In partnership with

Contents

About World Cancer Research Fund International	1
Executive Summary	3
1. Summary of panel judgements	7
2. Trends, incidence and survival	8
3. Pathogenesis	9
4. Other established causes	11
5. Interpretation of the evidence	11
5.1 General	11
5.2 Specific	12
6. Methodology	12
6.1 Mechanistic evidence	13
7. Evidence and judgements	14
7.1 Aflatoxins	14
7.2 Fish	18
7.3 Coffee	19
7.4 Alcoholic drinks	22
7.5 Physical activity	27
7.6 Body fatness	28
7.7 Other	32
8. Comparison with the Second Expert Report	33
9. Conclusions	33
Acknowledgements	34
Abbreviations	36
Glossary	37
References	41
Appendix – Criteria for grading evidence	46
Our Recommendations for Cancer Prevention	49

WORLD CANCER RESEARCH FUND INTERNATIONAL

OUR VISION

We want to live in a world where no one develops a preventable cancer.

OUR MISSION

We champion the latest and most authoritative scientific research from around the world on cancer prevention and survival through diet, weight and physical activity, so that we can help people make informed choices to reduce their cancer risk.

As a network, we influence policy at the highest level and are trusted advisors to governments and to other official bodies from around the world.

OUR NETWORK

World Cancer Research Fund International is a not-for-profit organisation that leads and unifies a network of cancer charities with a global reach, dedicated to the prevention of cancer through diet, weight and physical activity.

The World Cancer Research Fund network of charities is based in Europe, the Americas and Asia, giving us a global voice to inform people about cancer prevention.



OUR CONTINUOUS UPDATE PROJECT (CUP)

World Cancer Research Fund International's Continuous Update Project (CUP) analyses global cancer prevention and survival research linked to diet, nutrition, physical activity and weight. Among experts worldwide it is a trusted, authoritative scientific resource, which underpins current guidelines and policy for cancer prevention.

The CUP is produced in partnership with the American Institute for Cancer Research, World Cancer Research Fund UK, World Cancer Research Fund NL and World Cancer Research Fund HK.

The findings from the CUP are used to update our Recommendations for Cancer Prevention, which were originally published in '*Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective*' (our Second Expert Report) [1]. These ensure that everyone – from policymakers and health professionals to members of the public – has access to the most up-to-date information on how to reduce the risk of developing the disease.

As part of the CUP, scientific research from around the world is collated and added to a database of epidemiological studies on an ongoing basis and systematically reviewed by a team at Imperial College London. An independent panel of world-renowned experts then evaluate and interpret the evidence to make conclusions based on the body of scientific evidence. Their conclusions form the basis for reviewing and, where necessary, revising our Recommendations for Cancer Prevention (see inside back cover/page 49).

A review of the Recommendations for Cancer Prevention is expected to be published in 2017, once an analysis of all of the cancers being assessed has been conducted. So far, new CUP reports have been published with updated evidence on breast, colorectal, pancreatic, endometrial, ovarian and prostate cancers. In addition, our first CUP report on breast cancer survivors was published in October 2014.

This CUP report on liver cancer updates the liver cancer section of the Second Expert Report (section 7.8) and is based on the findings of the CUP Liver Cancer Systematic Literature Review (SLR) and the CUP Expert Panel discussion in June 2014. For further details, please see the full Continuous Update Project Liver Cancer SLR 2014 (wcrf.org/sites/default/files/Liver-Cancer-SLR-2014.pdf).

HOW TO CITE THIS REPORT

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EXECUTIVE SUMMARY

Background and context

The latest statistics reveal that cancer is now not only a leading cause of death worldwide, but that liver cancer is one of the deadliest forms. Indeed, liver cancer is the second most common cause of death from cancer worldwide, accounting for 746,000 deaths globally in 2012 [1].

One of the reasons for the poor survival rates is that liver cancer symptoms do not manifest in the early stages of the disease, which means that the cancer is generally advanced by the time it is diagnosed. In Europe the average survival rate for people five years after diagnosis is approximately 12 per cent [2].

In addition, the number of new cases is also on the increase. World Health Organization statistics show that 626,162 new cases of liver cancer were diagnosed in 2002, but by 2012 the figure had risen to 782,451. This figure is projected to increase by 70 per cent to 1,341,344 cases by 2035 [1].

Statistics on liver cancer show that the disease is more common in men than women, and that 83 per cent of liver cancer cases occur in less developed countries, with the highest incidence rates in Asia and Africa. On average, the risk of developing liver cancer increases with age and is highest in people over the age of 75, although it can develop at a younger age in people in Asia and Africa - typically around the age of 40.

In addition to the findings in this report, other established causes of liver cancer include:

1. Disease:

- ◆ Cirrhosis of the liver.

2. Medication:

- ◆ Long term use of oral contraceptives containing high doses of oestrogen and progesterone.

3. Infection:

- ◆ Chronic viral hepatitis.

4. Smoking:

- ◆ Smoking increases the risk of liver cancer generally, but there is a further increase in risk among smokers who also have the hepatitis B or hepatitis C virus infection and also among smokers who consume large amounts of alcohol.

In this latest report from our Continuous Update Project - the world's largest source of scientific research on cancer prevention and survivorship through diet, weight and physical activity - we analyse worldwide research on how certain lifestyle factors affect the risk of developing liver cancer. This includes new studies as well as studies published in our 2007 Second Expert Report, *'Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective'* [3].

How the research was conducted

The global scientific research on diet, weight, physical activity and the risk of liver cancer was systematically gathered and analysed, and then the results were independently assessed by a panel of leading international scientists in order to draw conclusions about which of these factors increase or decrease the risk of developing the disease.

The research included in this report largely focuses on the main type of liver cancer, hepatocellular carcinoma, which accounts for 90 per cent of all liver cancers [4].

More research has been conducted in this area since our 2007 Second Expert Report [3]. In total, this new report analyses 34 studies from around the world; this comprises over eight million (8,153,000) men and women and 24,600 cases of liver cancer.

To ensure consistency, the methodology for the Continuous Update Project (CUP) remains largely unchanged from that used for our 2007 Second Expert Report [3].

Findings

Strong evidence

- ◆ There is strong evidence that being overweight or obese is a cause of liver cancer. Being overweight or obese was assessed by body mass index (BMI).
- ◆ There is strong evidence that consuming approximately three or more alcoholic drinks a day is a cause of liver cancer.
- ◆ There is strong evidence that consuming foods contaminated by aflatoxins (toxins produced by certain fungi) is a cause of liver cancer. (Aflatoxins are produced by inappropriate storage of food and are generally an issue related to foods from warmer regions of the world. Foods that may be affected by aflatoxins include cereals, spices, peanuts, pistachios, Brazil nuts, chillies, black pepper, dried fruit and figs).
- ◆ There is strong evidence that drinking coffee is linked to a decreased risk of liver cancer.

Limited evidence

- ◆ There is limited evidence that higher consumption of fish decreases the risk of liver cancer.
- ◆ There is limited evidence that physical activity decreases the risk of liver cancer.

Findings that have changed since our 2007 Second Expert Report

The findings on being overweight or obese, coffee, fish and physical activity in this report are new; those for alcoholic drinks were strengthened and for aflatoxins remain unchanged from our 2007 Second Expert Report [3].

Recommendations

To reduce the risk of developing liver cancer:

1. Maintain a healthy weight.
2. If consumed at all, limit alcohol to a maximum of 2 drinks a day for men and 1 drink a day for women.

This advice forms part of our existing Cancer Prevention Recommendations (available at wcrf.org). Our Cancer Prevention Recommendations are for preventing cancer in general and include eating a healthy diet, being physically active and maintaining a healthy weight.

References

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- 2 De Angelis R, Sant M, Coleman MP, et al. Cancer Survival in Europe 1999-2007 by country and age: results of Eurocare-5 – a population-based study. *Lancet Oncol* 2014; 15: 23-34.
- 3 World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC: AICR, 2007 (Second Expert Report). Available from www.wcrf.org
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DIET, NUTRITION, PHYSICAL ACTIVITY AND LIVER CANCER

		DECREASES RISK	INCREASES RISK
STRONG EVIDENCE	Convincing		Aflatoxins ¹ Alcoholic drinks ² Body fatness ³
	Probable	Coffee	
LIMITED EVIDENCE	Limited - suggestive	Fish Physical activity ⁴	
	Limited - no conclusion	Cereals (grains) and their products, non-starchy vegetables, fruits, peanuts (groundnuts), meat and poultry, salted fish, tea, green tea, glycaemic index, calcium and vitamin D supplements, vitamin C, water source, low fat diet	
STRONG EVIDENCE	Substantial effect on risk unlikely		

1. Foods that may be contaminated with aflatoxins include cereals (grains), as well as pulses (legumes), seeds, nuts and some vegetables and fruits.
2. Based on evidence for alcohol intakes above around 45 grams per day (about 3 drinks a day). No conclusion was possible for intakes below 45 grams per day. There is insufficient evidence to conclude that there is any difference in effect between men and women. Alcohol consumption is graded by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) [2].
3. Body fatness is marked by body mass index (BMI).
4. Physical activity of all types.

1. Summary of panel judgements

Overall the Panel notes the strength of the evidence that aflatoxins, body fatness and alcoholic drinks are causes of liver cancer, and that coffee protects against liver cancer.

The Continuous Update Project (CUP) Panel judges as follows:

- ◆ **Aflatoxins:** Higher exposure to aflatoxins and consumption of aflatoxin-contaminated foods are convincing causes of liver cancer.
- ◆ **Alcoholic drinks:** Consumption of alcoholic drinks is a convincing cause of liver cancer. This is based on evidence for alcohol intakes above about 45 grams per day (around 3 drinks a day).
- ◆ **Body fatness:** Greater body fatness (marked by BMI) is a convincing cause of liver cancer.
- ◆ **Coffee:** Higher consumption of coffee probably protects against liver cancer.
- ◆ **Fish:** The evidence suggesting that a higher consumption of fish decreases the risk of liver cancer is limited.
- ◆ **Physical activity:** The evidence suggesting that higher levels of physical activity decrease the risk of liver cancer is limited.

The Panel judgements for liver cancer are shown in the matrix on page 6.

2. Trends, incidence and survival

The liver is the body's largest internal organ. It processes and stores nutrients and produces cholesterol and proteins such as albumin, clotting factors and the lipoproteins that carry cholesterol. It also secretes bile and performs many metabolic functions, including detoxification of several classes of carcinogens.

Different types of tumour occur in the liver, and each has potentially different causes and natural history. The most common type of liver cancer is hepatocellular carcinoma (hepatoma or HCC), accounting for 90 per cent of all liver cancers [3]. It starts in the main liver cells, the hepatocytes, and has various subtypes. Another type, cholangiocarcinomas, starts in the small bile ducts within the liver and accounts for far fewer primary liver cancers. Other types of liver cancer, including hepatoblastoma and angiosarcoma, are even less common.

Liver cancer is the sixth most common cancer worldwide, with 782,000 new cases diagnosed in 2012 [4]. It is the second most common cause of death from cancer and is more common in men than women. The risk increases with age, with most cases diagnosed over the age of 75 [4]. However, in people living in less developed countries in Asia and Africa compared with those in more developed countries worldwide, the disease can develop at a younger age (typically around the age of 40) [4,5]. About 83 per cent of liver cancer cases occur in less developed countries, with the highest incidence of liver cancer in Asia and Africa and the lowest incidence in Europe and in Latin America and the Caribbean. The age-standardised rate of this cancer is more than six times higher in Eastern Asia than in Northern Europe [4].

The early stages of liver cancer do not usually produce symptoms, so the disease is generally advanced when it is diagnosed. Survival rates are poor: for example, in European adults diagnosed with liver cancer between 2000 and 2007, the mean age-standardised survival rate at five years was approximately 12 per cent [6]. For further information see **box 1**.



Box 1 Cancer incidence and survival

The cancer incidence rates and figures given here are those reported by cancer registries, now established in many countries. These registries record cases of cancer that have been diagnosed. However, many cases of cancer are not identified or recorded: some countries do not have cancer registries; regions of some countries have few or no records; records in countries suffering war or other disruption are bound to be incomplete; and some people with cancer do not consult a physician. Altogether, this means that the actual incidence of cancer is most probably higher than the figures given here.

The information on cancer survival shown here and elsewhere is usually global averages. Survival rates are generally higher in high-income countries and other parts of the world where there are established services for screening and early detection of cancer as well as well-established treatment facilities.

Survival is often a function of the stage at which a cancer is detected and diagnosed. The symptoms of some cancers, such as liver cancer, are often evident only at a late stage, which accounts for the relatively low survival rates.

3. Pathogenesis

Patients with cirrhosis (scarring of the liver due to previous damage) have the highest risk of developing hepatocellular carcinoma: approximately 90–95 per cent of people who develop hepatocellular carcinoma have underlying cirrhosis [7]. So any cause of cirrhosis, either viral or chemical (see **box 2**), is likely to increase cancer risk. The liver is also a common site for metastasis of tumours originating in other organs.

Box 2 Hepatitis viruses

Hepatitis B and hepatitis C viruses are causes of liver cancer. The former appears to act directly by damaging cells and their DNA (deoxyribonucleic acid). The latter shows an indirect effect, mediated by cirrhosis. For both, there is potential for nutritional status to have an effect at several stages: susceptibility to and duration of infection, liver damage, DNA damage and cancer progression [8].

It is estimated that two billion people worldwide are infected with hepatitis B virus [9]. It is mostly spread through contact with blood and sexual transmission. It is often acquired at birth or in childhood and is endemic in areas of Africa and Asia.

Approximately one million people die each year from hepatitis B-related chronic liver disease, including liver cirrhosis and hepatocellular carcinoma. Chronic hepatitis B virus carriers have a 100-fold greater chance of developing liver cancer than non-carriers, and the virus is responsible for 50–90 per cent of hepatocellular carcinoma in high-risk areas [9]. Liver cancer in hepatitis B virus carriers is not necessarily connected with cirrhosis: up to 40 per cent of associated liver cancer cases are non-cirrhotic. Hepatitis B virus carries its genetic code as DNA rather than RNA. Viral DNA can insert itself into liver cells and alter their DNA. Those infected in adulthood have a lower risk of this cancer than those infected in childhood because there is less time for the virus to cause inflammation. Vaccination against hepatitis B virus has been shown to reduce the prevalence of liver cancer [9].

It is estimated that just over 2 per cent of the world's population are infected with hepatitis C virus [9], and it is more prevalent in high-income countries. A high proportion of these infections become chronic, of which 15–27 per cent develop into cirrhosis [9]. Of those, around 1–4 per cent develop into liver cancer each year. Interruption of the sequence of chronic hepatitis developing into cirrhosis prevents liver cancer. Also, there is an interaction between hepatitis C virus infection, liver cancer risk and consumption of alcoholic drinks [10]. There is no vaccine against hepatitis C. It is mostly spread through contaminated blood.

As for cancers at most sites, accumulated sequential changes (see Second Expert Report section 2.5), specifically in mature hepatocytes, lead to the development of dysplastic nodules; over the course of around five years, 30 per cent may develop into tumours [11]. Hepatocellular carcinoma cells show numerous genetic changes, perhaps accumulated during cellular proliferation, which is part of the normal liver repair process [12]. The hepatitis B virus related type appears to be more genetically unstable than others [13, 14] and acts by directly damaging cells and their DNA, whereas hepatitis C virus shows more of an indirect effect, mediated by cirrhosis (see **box 2**).

4. Other established causes

(Also see Second Expert Report, sections 2.4 and 7.1.3.1)

Other diseases

Cirrhosis of the liver increases the risk of liver cancer, and so can be seen as a cause of this cancer [7].

Infection and infestation

Chronic viral hepatitis is a cause of liver cancer (see **box 2**). Infestation of liver flukes is a cause of cholangiocarcinoma [15].

Medication

Long term use of oral contraceptives containing high doses of oestrogen and progesterone increase the risk of this cancer [16].

Smoking

Smoking increases the risk of liver cancer. In smokers who also have hepatitis B or hepatitis C virus infection, the risk is increased further, and those who smoke as well as consume large amounts of alcohol may also be at increased risk compared with those who do not smoke or drink [15, 17].

5. Interpretation of the evidence

5.1 General

For general considerations that may affect interpretation of the evidence, see sections 3.3 and 3.5, and boxes 3.1, 3.2, 3.6 and 3.7 in the Second Expert Report.

‘Relative risk’ (RR) is used in this report to denote ratio measures of effect, including ‘risk ratios’, ‘rate ratios’, ‘hazard ratios’ and ‘odds ratios’.

5.2 Specific

Considerations specific to cancer of the liver include:

Classification

Most of the data is on hepatocellular carcinoma, the most well characterised (and most common) form of liver cancer. However, different outcomes are reported for unspecified primary liver cancer, compared with hepatocellular carcinoma and cholangiocarcinoma. This suggests different causation and so therefore may be a cause of heterogeneity among the study results.

Confounding

Smoking and hepatitis B and C viruses are possible confounders or effect modifiers. Most studies adjust for smoking, but only a few high quality studies adjust for hepatitis B and C viruses. Studies identified on patients with hepatic cirrhosis (including only patients with cirrhosis), hepatitis B or C viruses, alcoholism or history of alcohol abuse were not included in the Liver Cancer SLR 2014.

6. Methodology

To ensure consistency, the methodology for reviewing the epidemiological evidence in the CUP remains largely unchanged from that used previously for the Second Expert Report [1]. However, based upon the experience of conducting the systematic literature reviews (SLRs) for the Second Expert Report, some modifications to the methodology were made. The literature search was restricted to Medline and included only randomised controlled trials, cohort and case-control studies. Due to their methodological limitations, case-control studies, although identified, were not included in the Liver Cancer SLR 2014, unlike the 2005 SLR for the Second Expert Report.

Where possible for this update, meta-analyses for incidence and mortality were conducted separately. However, analyses combining studies on liver cancer incidence and mortality were also conducted to explore whether the outcome can explain any heterogeneity. Separate meta-analyses were also conducted for men and women, and by geographical location, where possible.

Studies reporting mean difference as a measure of association were not included in the Liver Cancer SLR 2014, as relative risks estimated from the mean differences are not adjusted for possible confounders, and thus not comparable to adjusted relative risks from other studies.



Non-linear meta-analysis was applied when the data suggested that the dose-response curve is non-linear and when analysis detected that a threshold of exposure might be of interest. Details about the non-linear meta-analyses can be found in the Liver Cancer SLR 2014.

The Liver Cancer SLR 2014 included studies published up to 31 March 2013. For more information on methodology, see the full Liver Cancer SLR 2014 at wcrf.org/sites/default/files/Liver-Cancer-SLR-2014.pdf.

6.1 Mechanistic evidence

Where relevant, mechanistic reviews conducted for the Second Expert Report are included in this report (more details can be found in chapters 2, 4 and 6 of the Second Expert Report). These reviews have not been updated, but in future will be updated as part of a systematic literature review for the CUP of the mechanistic evidence (see below). A brief summary of possible mechanisms for aflatoxins, fish, coffee, alcoholic drinks, physical activity and body fatness is given. Where an exposure presented in this report was previously judged as 'limited – no conclusion' or was not discussed for the Second Expert Report, there was no formal review of the mechanisms. Plausible mechanisms identified by CUP Panel members and published reviews are included in this report.

Work is under way to develop a method for systematically reviewing human, animal and other experimental studies, and in future this method will be used to conduct reviews of mechanisms for all cancer sites (see www.wcrf.org for further information). A full review of the mechanistic evidence for liver cancer will form part of this larger review.

7. Evidence and judgements

The following sections summarise the evidence identified by the CUP in the Liver Cancer SLR 2014, a comparison with the findings from the Second Expert Report, and the Panel's conclusions. They also include a brief description of potential mechanisms for each exposure.

For information on the criteria for grading the epidemiological evidence see the **Appendix** in this report. References to studies added as part of the CUP have been included; for details of references to other studies from the Second Expert Report [1], see the Liver Cancer SLR 2014.

7.1 Aflatoxins

(Also see Liver Cancer SLR 2014: Section 4.2.2.2.2)

The CUP identified one new publication from a nested case-control study included in the 2005 SLR [18]. This study showed that aflatoxin B₁ exposure increased risk of liver cancer: a statistically significant increased risk was observed for those with aflatoxin B₁ adducts and urinary aflatoxin B₁ metabolite levels above the mean, compared to those with levels below the mean (see **table 1** (Liver Cancer SLR 2014 table 29)).

Eight other papers from four nested case-control and cohort studies identified in the 2005 SLR reported an increased risk with elevated levels of any biomarker of exposure, most of which were statistically significant (see **table 1** (Liver Cancer SLR 2014 table 29)). A variety of measures were used to collect the data, so meta-analyses were not possible.

Table 1: Summary of nested case-control and cohort studies - aflatoxins
(any biomarker of exposure)

STUDY DESCRIPTION	PUBLICATION	NO. CASES/ CONTROLS	RR (95% CI)	CONTRAST
Community-based Cancer Screening Cohort, Taiwan	Wu 2009 [18]	241 HCC 1052 controls	1.54 (1.01–2.36)	AFB ₁ -albumin adducts above vs. below mean (59.8 fmol/mg)
			1.76 (1.18–2.58)	Urinary AFB ₁ above vs. below mean (55.2 fmol/mL)
	Sun 2001 [19]	HBsAg carriers 75 HCC 140 controls	2.0 (1.1–3.7)	AFB ₁ -albumin adducts detectable vs. non-detectable
	Wang 1996 [20]	56 HCC 220 controls	1.6 (0.4–5.5)	Serum level aflatoxin-albumin detectable vs. non-detectable
			3.8 (1.1–12.8)	Urinary levels of aflatoxin high vs. low
Shanghai Cohort Study, China	Yuan 2006 [21]	213 HCC 1087 controls	3.25 (1.63–6.48)	Urinary aflatoxin biomarker positive vs. negative
	Qian 1994 [22]	55 HCC 267 controls	5.0 (2.1–11.8)	Any urinary aflatoxin biomarker vs. none
	Ross 1992 [23]	22 HCC 110 controls	2.4 (1.0–5.9)	Any urinary aflatoxin biomarker vs. none
Qidong Cohort, China	Sun 1999 [24]	22 HCC 149 controls	3.3 (1.2–8.7)	Urinary AFM ₁ detectable (above 3.6 ng/L) vs. non-detectable
Cohort Gov. Clinics, Taiwan	Yu 1997 [25]	HBsAg carriers 21 HCC 63 controls	12.0 (1.2–117.4)	Both markers (urinary AFM ₁ and AFB ₁ -N7-guanine adducts) vs. none
	Chen 1996 [26]	HBsAg carriers 32 HCC 73 controls	3.8 (1.0–14.5)	AFB ₁ -albumin adducts high vs. undetectable

Abbreviations: AFB₁, aflatoxin B₁; AFM₁, aflatoxin M₁; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma

An ecological study (which are not included as part of the CUP) showed that a fall in the exposure to aflatoxins was associated with a significant decrease in mortality from liver cancer. A reduction of aflatoxin exposure from 100 per cent to 23 per cent of samples positive for aflatoxin–albumin adducts resulted in an estimated population attributable benefit of 65 per cent for reduction in the rate of primary liver cancer. Because of the strong synergy between aflatoxin and hepatitis B virus, only 17 per cent of the population-attributable benefit was estimated to be due to the reduction of aflatoxin among those without infection [27].

Published meta-analyses

Several reviews examining aflatoxin exposure and liver cancer risk have been published. The most recent published meta-analysis identified in the Liver Cancer SLR 2014 [28] included case-control and nested case-control studies from China, Taiwan and sub-Saharan Africa. For the nine studies reporting on the general population (adjusted for HBsAg positive), there was a statistically significant increased risk (RR 4.75 (95% CI 2.78–8.11)).

Mechanisms

*Note: This is adapted from sections 2.4.2.6 and 4.1.5.4, and box 4.1.4 of the Second Expert Report. In future, an updated review of mechanisms for this exposure will form part of a larger review of mechanisms (see **section 6.1** in this report).*

Aflatoxin B₁, (AFB₁), a product of the *Aspergillus* fungus and a common contaminant of cereals (grains) and peanuts, is known to be genotoxic and is formed in the liver [29]. The product of AFB₁ metabolism causes damage to DNA, including G:C to T:A transversion. Glutathione S-transferases (GSTs) can repair this damage, with varying efficiency between isoenzymes. Studies have shown that aflatoxins can damage the p53 gene, which is an important regulator of the cell cycle [24]. Damage to p53 can lead to increased proliferation of abnormal cells and formation of cancers.

The synergistic effect of hepatitis B virus infection and aflatoxin exposure might be explained by the virus increasing the production of cytochrome P450 enzymes that produce the genotoxic metabolite of aflatoxin. There may also be a number of other interactions between the two carcinogens, including integration of hepatitis B virus X gene and its consequences, as well as interference with nucleotide excision repair, activation of p21waf1/cip1, generation of DNA mutations and altered methylation of genes [30]. However, the potency of AFB₁ in different species is strongly influenced by other biotransformation enzymes as well. This is best documented for GSTs, of which a specific isoform in mice (GST mYc) very efficiently removes these adducts, and has been suggested to largely account for the observed interspecies difference (1000-fold) between rats (who are sensitive) and mice (who are resistant). Overall, protection against AFB₁-induced hepatocellular carcinoma is demonstrated by the induction of (specific) GSTs and/or the inhibition of CYP1A2 [29].

The synergy observed in epidemiological studies between hepatitis B virus infection and AFB₁ exposure has been experimentally addressed in various animal model systems ranging from tree shrews (rodent species sensitive to hepatitis B virus infection) to rats and genetically engineered mice. As a result, the following routes mainly resulting in an increase in mutation rate are proposed:

- ◆ Hepatitis B virus infection induces CYP1A2, resulting in increased levels of the proximate carcinogen AFB₁ exo-8,9,-epoxide.
- ◆ Hepatitis B virus X protein (HBx) expression correlates with a 24 per cent increase in LacZ (bacterial enzyme β-galactosidase) gene mutations and a doubling of accompanying G:C to T:A transversions.
- ◆ HBx inhibits nucleotide excision repair, resulting in the persistence of existing adducts, and leads to an increase in mutation rate after replication.
- ◆ HBx acts as a tumour promoter in diethylnitrosamine (DEN)-induced murine liver tumours.
- ◆ Hepatocyte necrosis/apoptosis and compensatory regeneration results in an oxyradical overload due to reactive oxygen and nitrogen species formation, resulting in increased mutation rates.

In summary, the overall effect of aflatoxin exposure is mainly modified by biotransformation enzymes and the presence of viral oncoproteins through mechanisms not completely understood, but with the levels of persistent AFB₁ dG adducts as a major player.

CUP Panel's conclusion:

The overall evidence for a relationship between aflatoxins and liver cancer was consistent. No meta-analysis was conducted, but all of the studies identified in the Liver Cancer SLR 2014 reported results in a positive direction, most of which were statistically significant. Results were also consistent with recent reviews published on aflatoxins and liver cancer. The Panel noted that although the main areas affected by higher aflatoxin exposure are Africa and Asia, it is a global issue of public health relevance. The CUP Panel concluded:

Higher exposure to aflatoxins and consumption of aflatoxin-contaminated foods are convincing causes of liver cancer.

7.2 Fish

(Also see *Liver Cancer SLR 2014: Section 2.5.2*)

The CUP identified four new or updated studies (four publications) [31-34], giving a total of six studies (seven publications) (see *Liver Cancer SLR 2014* table 20 for a full list of references). Three studies (three estimates) reporting on liver cancer incidence, and one study (with separate estimates for men and women) reporting on liver cancer mortality, reported non-significant inverse associations when comparing the highest versus the lowest categories of intake (see *Liver Cancer SLR 2014* figure 14).

Four of the six studies were included in the dose-response meta-analysis ($n = 1,812$), which showed a statistically significant 6 per cent decreased risk per 20 grams per day (RR 0.94 (95% CI 0.89–0.99)) (see *Liver Cancer SLR 2014* figure 15). Moderate to high heterogeneity was observed ($I^2 = 53%$).

Only two studies could control for hepatitis B and C virus infection status [33, 34], and in these studies, the inverse association with fish intake was stronger than in the other studies.

Two studies were not included in any of the CUP analyses due to insufficient data [35, 36].

In contrast to the *Liver Cancer SLR 2014*, the 2005 SLR showed no clear association between fish consumption and liver cancer. No dose-response meta-analysis was conducted for the 2005 SLR. The *Liver Cancer SLR 2014* included more studies and cases of liver cancer.

Mechanisms

*Note: In the future, a full review of mechanisms for this exposure will form part of a larger review of mechanisms (see **section 6.1** in this report).*

In general, but also for human hepatocellular carcinoma, the epidemiological data on associations between fish consumption and cancer risk are not consistent. Fish consumption may act as a surrogate marker for n-3 fatty acid intake. Increasing evidence from animal and *in vitro* studies indicates that n-3 fatty acids, especially the long-chain polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as present in fatty fish and fish oils, inhibit carcinogenesis [37]. This is also supported in one of the most frequently applied rodent models of hepatocarcinogenesis, the DEN-PB (diethylnitrosamine-phenobarbital) treated rat. Most of the more recent data indicate a protective effect of a variety of fish oils, especially with regard to the formation of pre-neoplastic stages including foci and nodules [38-40].

A variety of mechanisms have been suggested by which n-3 PUFAs may influence the risk of liver cancer [37, 41]. The most prevalent hypothesis is that n-3 PUFAs exert a protective effect by the inhibition of eicosanoid production from n-6 fatty acid precursors, especially arachidonic acid. Other mechanisms include altering gene expression and related signal transduction, for example by acting as a ligand for nuclear hormone receptors like the peroxisome proliferator-activated receptors, or by modulating the



expression of other inflammation-related genes like NF- κ B and tumour-necrosis factor alpha (TNF- α). Finally, an increase in the production of reactive oxygen and nitrogen species (oxyradical load) and the alteration of oestrogen metabolism are also possible mechanisms.

CUP Panel's conclusion:

The evidence for fish consumption was limited but generally consistent. The dose-response meta-analysis showed a significant decreased risk of liver cancer per 20 grams per day intake; however, this only included four studies, and moderate to high heterogeneity was observed. The CUP Panel concluded:

The evidence suggesting that a higher consumption of fish decreases the risk of liver cancer is limited.

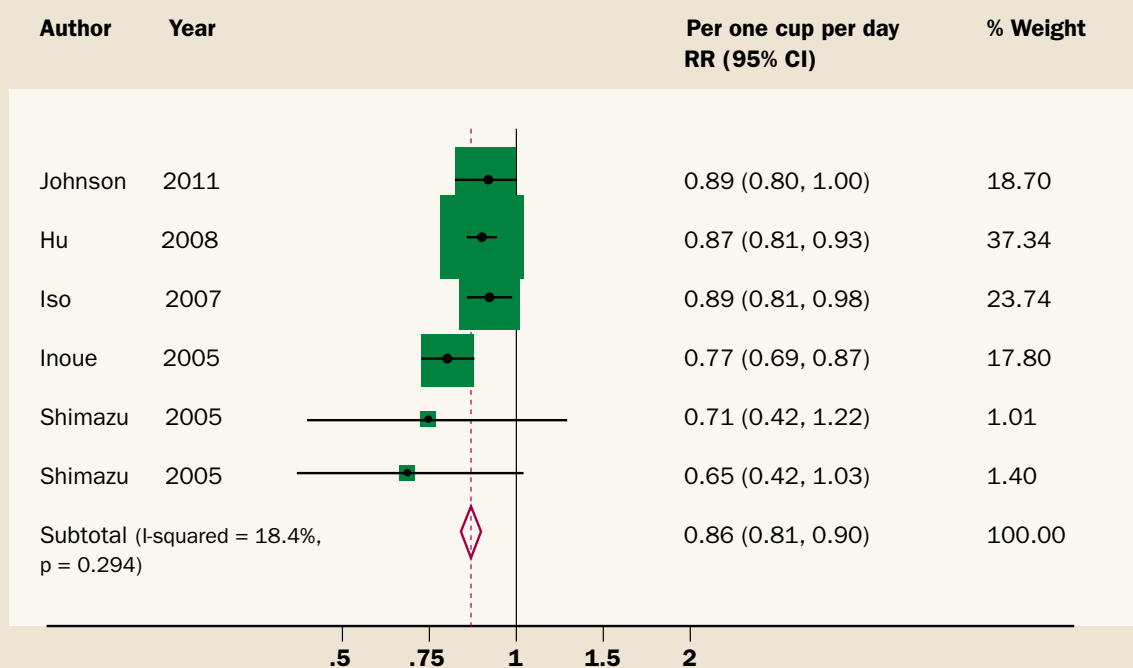
7.3 Coffee

(Also see *Liver Cancer SLR 2014: Section 3.6.1*)

The CUP identified six new or updated studies (seven publications) [42-48], giving a total of eight studies (11 publications) (see *Liver Cancer SLR 2014* table 24 for a full list of references). Of seven studies (10 estimates) reporting on liver cancer incidence, six reported an inverse association, two of which were significant in men but not women, and one study reported a non-significant positive association in men and a non-significant inverse association in women, when comparing the highest versus the lowest categories of intake (see *Liver Cancer SLR 2014* figure 18). One study (two estimates) reporting on liver cancer mortality reported an inverse association, which was significant in men but not women.

Six of eight studies were included in the dose-response meta-analysis ($n = 1,582$), which showed a statistically significant 14 per cent decreased risk per one cup per day (RR 0.86 (95% CI 0.81–0.90)) (see **figure 1** (*Liver Cancer SLR 2014* figure 19)). Low heterogeneity was observed ($I^2 = 18\%$).

Figure 1: Dose-response meta-analysis of coffee and liver cancer, per one cup per day



When stratified by sex, the dose-response meta-analysis showed a decreased risk per one cup per day, which was statistically significant in men but not women (see **table 2** and Liver Cancer SLR 2014 figure 22).

Table 2: Summary of CUP 2014 stratified dose-response meta-analysis – coffee

ANALYSIS	INCREMENT	RR (95% CI)	I ²	NO. STUDIES	NO. CASES
MEN	Per one cup/day	0.84 (0.78-0.90)	21%	3	766
WOMEN	Per one cup/day	0.91 (0.83-1.01)	0%	3	377

The Liver Cancer SLR 2014 findings for coffee were consistent with the results from the 2005 SLR, in which all cohort studies showed a decreased risk with higher levels of coffee consumption. No dose-response meta-analysis was conducted in the 2005 SLR. The Liver Cancer SLR 2014 included more studies and more than double the number of cases of liver cancer.

Published meta-analyses

The results from three published meta-analyses on coffee and liver cancer were identified in the Liver Cancer SLR 2014 [49-51]. One of the most recent published meta-analyses [50] included eight cohort studies and reported a statistically significant decreased risk per one cup per day (RR 0.83 (95% CI 0.78–0.88); $n = 1,448$). The other recent meta-analysis [51] included seven cohort studies and reported a significant decreased risk when comparing the highest volume coffee drinkers with those who never or almost never drink coffee (RR 0.48 (95% CI 0.38–0.62); $n = 1,309$; $I^2 = 0\%$). The third meta-analysis [49] included four cohort studies and also reported a statistically significant decreased risk per two cups per day (RR 0.56 (95% CI 0.46–0.69); $n = 709$; $I^2 = 0\%$).

Mechanisms

*Note: In the future, a full review of mechanisms for this exposure will form part of a larger review of mechanisms (see **section 6.1** in this report).*

Mechanisms that support a protective effect of coffee on liver cancer relate largely to studies in animals, although some human studies contribute to the evidence. Compounds in coffee have been shown to induce the endogenous defence system, for example UDP-glucuronosyltransferase (a Phase II enzyme), which mitigates the effects of toxins including aflatoxin B₁. Such effects may be mediated by the transcription factor NrF2 (nuclear factor erythroid-2-like 2 factor), which controls the production of these proteins involved in detoxification, antioxidant defence and protein degradation [52].

Induced DNA repair capacity by constituents in coffee may also exert chemopreventive effects [52]. There is evidence from small intervention studies that coffee consumption reduces DNA damage in blood cells and prevents *ex vivo*-induced DNA damage in healthy volunteers. *In vitro* studies have demonstrated that certain compounds (kahweol and cafestol) reduce genotoxicity by 50 per cent in human-derived hepatoma cells via an induction of Phase II enzymes [53].

Both coffee and coffee extracts have also been shown to reduce the expression of genes involved in inflammation, and the effects appear to be most pronounced in the liver [52]. For example, in several rat models of hepatic injury, disease progression has been shown to be inhibited, and induction of inflammatory markers, such as interleukin-6, TNF- α , interferon- γ and tumour growth factor β , is inhibited by the administration of coffee. Coffee has also been shown to inhibit the transcription factor NF- κ B (nuclear factor kappa B) (involved in immune and inflammatory processes and over-expressed in many cancers) in monocytes *in vitro* and *in vivo* in transgenic reporter mice [54]. However, evidence for its effects is not completely consistent [52].

Evidence from clinical trials in patients with chronic hepatitis C has shown that coffee may also induce apoptosis [55]. Specific components of coffee identified include caffeine, cafestol and kahweol [52].

Type 2 diabetes has also been associated with an increased risk of hepatocellular carcinoma [56]. Specific compounds in coffee may exert protective effects on this type of cancer by improving insulin sensitivity and reducing the risk of diabetes [57].

Anti-angiogenic activity in *in vitro* systems may also be affected by coffee [52]. The formation of new blood vessels, angiogenesis, is necessary to support growing tumours with oxygen and nutrients. An essential feature of tumour angiogenesis is the induction of vascular endothelial growth factor and interleukin-8, and tumour angiogenesis can be induced by lack of oxygen that triggers the expression of the hypoxia-inducible factor 1 α .

CUP Panel's conclusion:

The evidence for coffee was generally consistent, and the dose-response meta-analysis showed a significant decreased risk of liver cancer per one cup per day. This was consistent with findings from three published meta-analyses. When stratified by sex, the association was significant for men but not for women. No threshold was identified, and there was no evidence regarding specific components of coffee that were attributable to the decreased risk. The CUP Panel concluded:

Higher consumption of coffee probably protects against liver cancer.

7.4 Alcoholic drinks

(Also see *Liver Cancer SLR 2014: Section 5.4*)

The Panel is aware that alcohol is a cause of cirrhosis, which predisposes to liver cancer. Studies on patients with hepatic cirrhosis (including only patients with cirrhosis), hepatitis B, hepatitis C, alcoholism or history of alcohol abuse were not included (see sections 4 and 5.2 in this report).

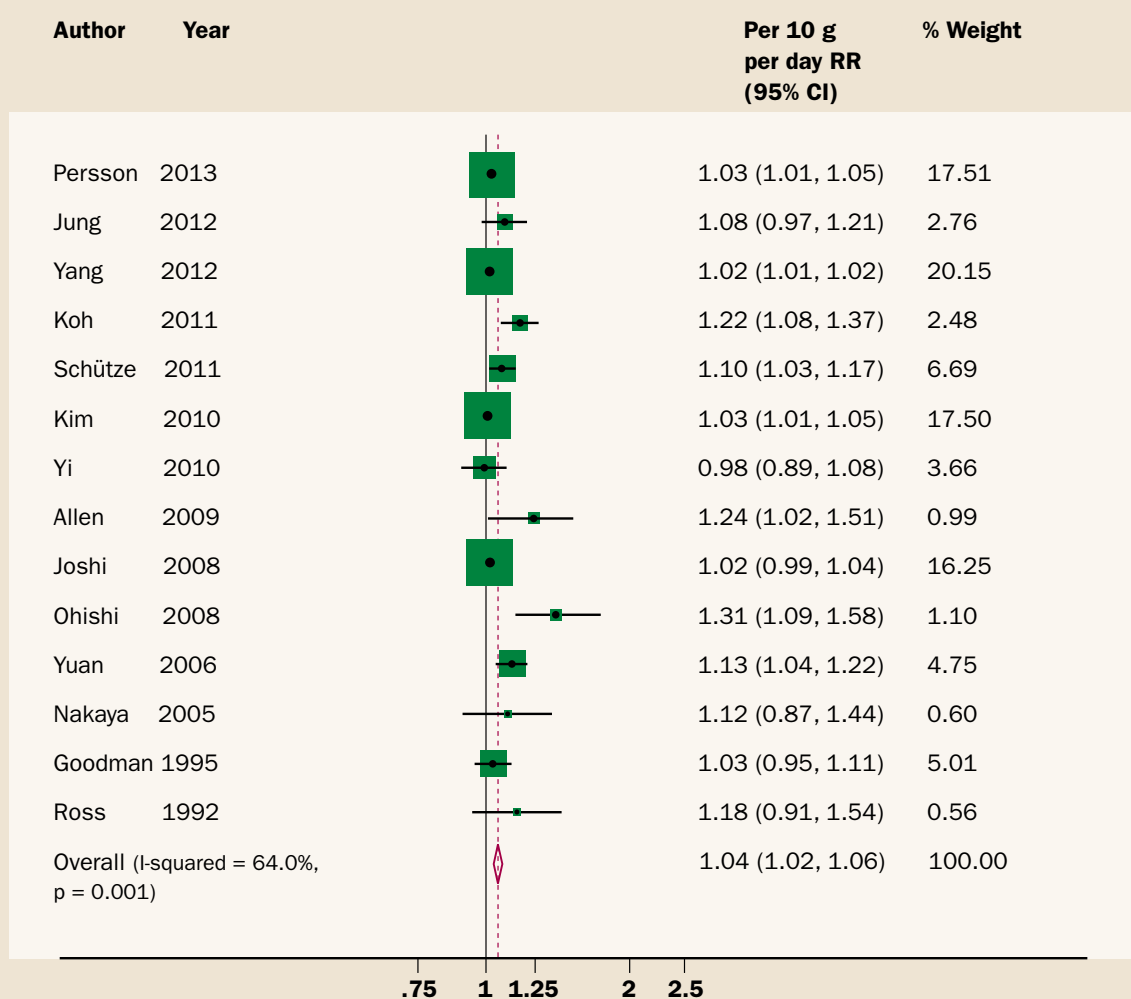
Alcohol (as ethanol)

The CUP identified 13 new or updated studies (14 publications) [21, 45, 48, 58-68], giving a total of 19 studies (30 publications) on liver cancer (see *Liver Cancer SLR 2014* table 41 for a full list of references). Of 11 studies (13 estimates) reporting on liver cancer incidence, 10 studies reported a positive association, of which seven were statistically significant, and one study reported a non-significant inverse association when comparing the highest and the lowest categories of consumption (see *Liver Cancer SLR 2014* figure 34). Of six studies (seven estimates) reporting on liver cancer mortality, five studies (six estimates) reported a positive association, two of which were statistically significant and one only significant in men but not women, and the other study reported a non-significant inverse association.



Fourteen of 19 studies on liver cancer were included in the dose-response meta-analysis ($n = 5,650$), which showed a statistically significant increased risk of 4 per cent per 10 grams of alcohol per day (RR 1.04 (95% CI 1.02–1.06)) (see **figure 2** (Liver Cancer SLR 2014 figure 36)). High heterogeneity was observed ($I^2 = 64\%$), which appeared to be mainly due to the size of the effect. There was evidence of funnel plot asymmetry with Egger’s test ($p = 0.001$) (see Liver Cancer SLR 2014 figure 38).

Figure 2: Dose-response meta-analysis of alcohol intake and liver cancer, per 10 g per day



When stratified by outcome, a dose-response meta-analysis showed a statistically significant increased risk per 10 grams per day for both liver cancer incidence and mortality, with a greater effect observed for liver cancer incidence. When stratified by sex, there was a statistically significant increased risk per 10 grams per day in both men and women. Finally, when stratified by geographic location, dose-response meta-analyses showed an increased risk per 10 grams of alcohol per day in both North American and European (combined), and Asian studies, but this was statistically significant only for Asian studies (for which there was a much larger number of studies and cases) (see **table 3** and Liver Cancer SLR 2014 figures 37, 38 and 41).

Table 3: Summary of CUP 2014 stratified dose-response meta-analyses – alcohol

ANALYSIS	INCREMENT	RR (95% CI)	I ²	NO. STUDIES	NO. CASES
Incidence	Per 10 g/day	1.12 (1.05-1.18)	69%	9	1,738
Mortality	Per 10 g/day	1.02 (1.01-1.03)	0%	5	3,912
Men	Per 10 g/day	1.03 (1.01-1.05)	51%	8	4,132
Women	Per 10 g/day	1.19 (1.04-1.35)	12%	4	637
North America & Europe	Per 10 g/day	1.08 (1.00-1.16)	74%	3	930
Asia	Per 10 g/day	1.04 (1.02-1.07)	63%	11	4,720

The exclusion of former drinkers may have attenuated the association of alcohol and liver cancer in some studies. The dose-response relationship was derived from categorical data in which the reference category used was 'never drinkers' in five out of the 14 studies included in the dose-response meta-analysis. Former drinkers were not included in the dose-response analysis in these studies.

In a meta-analysis of four studies that reported a risk estimate for former alcohol drinkers versus never drinkers [63, 66, 69, 70], a significant positive association was observed (RR 2.58 (95% CI 1.76–3.77)) (see Liver Cancer SLR 2014 figure 43).

One study was not included in any of the CUP analyses due to reporting insufficient data [71].

The Liver Cancer SLR 2014 findings were consistent with the dose-response meta-analysis from the 2005 SLR, which included six studies and showed a significant positive association per 10 grams per day (RR 1.10 (95% CI 1.02–1.17); n = 400). The effect observed in the Liver Cancer SLR 2014 was smaller (mainly because it excluded studies of people who were carriers of or infected with hepatitis, which tend to show a greater effect) but included more studies and more cases of liver cancer.

Published pooled analyses and meta-analyses

One published pooled analysis [72] and one meta-analysis [73] on alcohol and liver cancer were identified in the Liver Cancer SLR 2014. The pooled analysis of four Japanese studies reported a positive effect per 10 grams of alcohol per day, which is consistent with the Liver Cancer SLR 2014, but this was statistically significant only in men. When the studies identified in the Liver Cancer SLR 2014 (but not in the pooled analysis) were combined with the results of the pooled analysis of Japanese cohort studies, a statistically significant 4 per cent increased risk per 10 grams of alcohol



Table 4: Summary of CUP 2014 meta-analyses and published pooled analysis – alcohol

ANALYSIS	INCREMENT	RR (95% CI)	I ²	NO. STUDIES	NO. CASES	FACTORS ADJUSTED FOR
CUP Liver Cancer SLR 2014	Per 10 g/day	1.04 (1.02-1.06)	64%	14	5,650	
Pooled analysis of Japanese cohort studies [72]	Per 10 g/day (men)	1.02 (1.004-1.04)	-	4	605	Geographical location, age, history of diabetes, smoking and coffee intake
	Per 10 g/day (women)	1.11 (0.96-1.29)	-	4	199	
Liver Cancer SLR 2014 additional analysis: pooled analysis of Japanese cohort studies [72] combined with studies from the CUP*	Per 10 g/day	1.04 (1.02-1.06)	0%	17	6,372	

*The Miyagi Cohort [74] was the only study in the pooled analysis of Japanese cohort studies that was also included in the Liver Cancer SLR 2014.

per day was observed, the same as reported in the CUP dose-response meta-analysis. The published meta-analysis of seven cohort studies reported no association when comparing the highest and the lowest categories of intake (RR 1.00 (95% CI: 0.85–1.18)). Results from the Liver Cancer SLR 2014 and the pooled analysis are presented in **table 4**.

Mechanisms

*Note: This is adapted from sections 4.8.5.1 and 7.8.5.3 of the Second Expert Report. In the future, an updated review of mechanisms for this exposure will form part of a larger review of mechanisms (see **section 6.1** in this report).*

Chronic excessive alcohol consumption is known to cause significant acute liver damage resulting in hepatic fibrosis and eventual cirrhosis. The majority of liver cancer cases have underlying cirrhosis (see section 3 in this report) and the effect of alcohol on liver cancer is likely to be largely mediated through cirrhosis as an intermediate state.

The mechanisms through which ethanol exerts its damaging effects on the liver are still not clearly understood. In general, a distinction is made between direct genotoxic effects and tumour-promoting effects.

Alcohol consumption is graded by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) [2]. The mechanisms proposed for the carcinogenic effects of high alcohol intake are concentrated on four different mechanisms:

- ◆ Carcinogenicity of ethanol and acetaldehyde, demonstrated in experimental animals [2].
- ◆ Interaction with folate within the complex story of one-carbon metabolism (resulting in alterations in the normal methylation process and/or imbalances in the steady state level of DNA precursors and/or chromosome changes) [67].
- ◆ Modulation of the activity of detoxifying enzymes (e.g., P450 family members like CYP2E1) for carcinogens.
- ◆ Its ability, as a solvent, to facilitate enhanced penetration of carcinogens.

A functional polymorphism in the alcohol dehydrogenase gene (ADH1C) leads to enhanced production of acetaldehyde formation in the liver, and in studies of moderate to high alcohol intake, ADH1C*1 allele frequency and rate of homozygosity was found to be significantly associated with increased risk for liver cancer, as well as some other cancers [75].

With regard to the tumour-promoting effects of alcohol, research on the mechanisms of alcohol-induced hepatitis and consequently liver fibrosis is focusing in particular on inflammation [76, 77], but also on inflammation-dependent and inflammation-independent alterations in apoptosis. Special attention has been paid to the innate immune response [78] although other parts of the immune system, including T cells, may also play a role [79]. Alcohol consumption, even at moderate levels, is associated with increases in levels of circulating hepatitis C virus RNA in carriers [10]. Hepatitis C virus infection is highly prevalent among alcoholics with chronic liver disease and appears to accelerate the course of alcoholic liver disease.

Higher alcohol consumption is also positively associated with general adiposity and to a greater extent with central adiposity [80]. Obesity is a risk factor for non-alcoholic steatohepatitis (NASH), which may progress to cirrhosis and therefore an increased risk of developing liver cancer. NASH is the most severe form of non-alcoholic fatty liver disease (NAFLD), the hallmark of which is hepatic steatosis characterised by the accumulation of intracytoplasmic lipid within hepatocytes in the form of triglycerides. In contrast to simple steatosis, the more severe NASH form is characterised by inflammation with the presence of steatosis, hepatocellular ballooning and fibrosis. The low-grade systemic inflammation associated with obesity is believed to contribute to metabolic deregulation (peripheral and hepatic insulin resistance) and the progression of NAFLD to NASH, fibrosis, cirrhosis and finally hepatocellular carcinoma.

CUP Panel's conclusion:

The overall evidence was consistent with a positive dose-response relationship for alcohol and liver cancer, and this association was still apparent when stratified by outcome, sex and geographical location. There was evidence of high heterogeneity, but this appeared to be mainly due to the size of the effect. The results were consistent

with findings from the 2005 SLR, but with more studies and cases, and consistent with findings from a published pooled analysis. There was ample evidence suggestive of a non-linear relationship with a statistically significant effect above about 45 grams per day. No conclusion was possible for intakes below 45 grams per day. There was insufficient evidence to conclude that there is any difference in effect between men and women. There is also evidence of plausible mechanisms operating in humans. Alcohol is a known cause of cirrhosis and a known carcinogen. The CUP Panel concluded:

Consumption of alcoholic drinks is a convincing cause of liver cancer. This is based on evidence for alcohol intakes above about 45 grams per day (around 3 drinks a day).

7.5 Physical activity

(Also see *Liver Cancer SLR 2014: Sections 6, 6.1, 6.1.1.2, 6.1.1.4 and 6.1.3*)

The evidence for total physical activity, leisure-time physical activity, walking and vigorous physical activity is presented below and followed by an overall conclusion that incorporates all of these exposures.

The CUP identified four new studies (four publications) [81-84]. The results reported by the individual studies are summarised below. No meta-analysis was conducted in the Liver Cancer SLR 2014. No studies were identified in the 2005 SLR.

Total physical activity

One cohort study in Japanese men and women [82] observed a non-significant decreased risk of liver cancer when comparing the highest and lowest levels of activity (RR 0.54 (95% CI 0.23–1.29); $n = 64$).

Leisure-time physical activity

Two cohort studies were identified [81, 83]. The most recent study [83] reported a statistically significant decreased risk of liver cancer when comparing higher levels of activity with lower levels of activity (RR 0.88 (95% CI 0.81–0.95); $n = 169$). The other study reported a non-significant decreased risk of liver cancer mortality in both men and women when comparing the highest levels of activity with the lowest levels of activity (RR 0.88 (95% CI 0.64–1.21) and RR 0.64 (95% CI 0.37–1.11) for men and women respectively) [81].

Walking

One cohort study in Japanese men and women [81] reported a statistically significant decreased risk of liver cancer mortality in both men and women when comparing the highest and the lowest levels of walking per day (RR 0.70 (95% CI: 0.54–0.91); $n = 377$ and RR 0.54 (95% CI 0.37–0.78); $n = 143$ for men and women respectively).

Vigorous physical activity

One cohort study [84] reported a statistically significant decreased risk of liver cancer when comparing vigorous physical activity five or more times per week with no activity (RR 0.56 (95% CI 0.41–0.78); $n = 415$).

Mechanisms

*Note: In the future, a full review of mechanisms for this exposure will form part of a larger review of mechanisms (see **section 6.1** in this report).*

Physical activity may reduce risk of liver cancer through its beneficial effect on insulin sensitivity and body fatness. Regular physical activity helps to achieve and maintain a healthy body weight and improves glucose utilisation, independent of the effect of weight loss on insulin sensitivity [84]. Regular physical activity may also protect against liver cancer by reducing chronic inflammation; some studies suggest that this is mediated through weight reduction. It may also decrease the risk for liver cancer through a mechanism involving reducing oxidative stress, which is associated with inducing liver cancer.

CUP Panel's conclusion:

The evidence was generally consistent and all studies reported a decreased risk of liver cancer with higher levels of physical activity; however, because different types of activity were measured and a variety of measures were used to collect the data, no meta-analyses could be conducted. The CUP Panel concluded:

The evidence suggesting that higher levels of physical activity decrease the risk of liver cancer is limited.

7.6 Body fatness

(Also see Liver Cancer SLR 2014: Section 8.1.1)

The Panel interpreted body mass index (BMI) as a measure of body fatness. The Panel is aware that this anthropometric measure is imperfect and does not distinguish between lean mass and fat mass.

Body mass index

The CUP identified 14 new or updated studies (18 publications) [45, 48, 58, 59, 85-98], giving a total of 15 studies (22 publications) on liver cancer (see Liver Cancer SLR 2014 table 55 for a full list of references). Of 11 studies (13 estimates) reporting on liver cancer incidence, nine reported a positive association when comparing the highest and the lowest categories, of which six were statistically significant; one reported a significant positive association in men and a non-significant positive association in women; and one reported a positive association in men and an inverse association in women, both of which were not significant (see Liver Cancer SLR 2014 figure 52).



Figure 3: Dose-response meta-analysis of BMI and liver cancer, per 5 kg/m²



All three studies (five estimates) on liver cancer mortality reported positive associations when comparing the highest and the lowest categories, one of which was statistically significant in men but not women.

Twelve of 15 studies on liver cancer were included in the dose-response meta-analysis ($n = 14,311$), which showed a statistically significant increased risk of 30 per cent per 5 kg/m² (RR 1.30 (95% CI 1.16-1.46)) (see **figure 3** (Liver Cancer SLR 2014 figure 53)). High heterogeneity was observed ($I^2 = 78\%$), which appeared to be mainly due to the size of the effect. There was evidence of non-linearity ($p < 0.0001$), with a steeper increase in risk at higher BMI levels (see Liver Cancer SLR 2014 figures 59 and 60, and table 56).

When stratified by outcome, a dose-response meta-analysis showed an increased risk per 5 kg/m² for both liver cancer incidence and mortality, but this was significant only for incidence. When stratified by sex, there was a statistically significant increased risk per 5 kg/m² for both men and women. Finally, when stratified by geographical location, dose-response meta-analyses showed a statistically significant increased risk per 5 kg/m² in both European and Asian studies, with a stronger association in European studies (see **table 5** and Liver Cancer SLR 2014 figures 54, 55 and 56).

Table 5: Summary of CUP 2014 stratified dose-response meta-analyses – BMI

ANALYSIS	INCREMENT	RR (95% CI)	I²	NO. STUDIES	NO. CASES
Incidence	Per 5 kg/m ²	1.43 (1.19-1.70)	84%	8	11,530
Mortality	Per 5 kg/m ²	1.13 (1.00-1.28)	43%	4	2,543
Men	Per 5 kg/m ²	1.21 (1.02-1.44)	84%	8	11,180
Women	Per 5 kg/m ²	1.21 (1.10-1.33)	11%	4	2,337
Europe	Per 5 kg/m ²	1.59 (1.35-1.87)	42%	4	588
Asia	Per 5 kg/m ²	1.18 (1.04-1.34)	60%	7	12,520

The Liver Cancer SLR 2014 showed a significant positive dose-response relationship between greater BMI and liver cancer, which strengthened the limited findings from the 2005 SLR in which all cohort studies showed an increased risk of liver cancer with increased BMI except in one group of African-American men (no dose-response meta-analysis was conducted in the 2005 SLR). The Liver Cancer SLR 2014 included more than twice as many studies and many more cases of liver cancer.

Published pooled analyses and meta-analyses

The results from four published pooled analyses [99-102] and five meta-analyses [103-106] on BMI and liver cancer were identified in the Liver Cancer SLR 2014. All published pooled analyses and meta-analyses reported positive associations for continuous and highest versus lowest estimates, consistent with the Liver Cancer SLR 2014, but not all were statistically significant. The CUP included more liver cancer cases than any of the published pooled analyses. Results from the published pooled analyses are presented in **table 6**.

Table 6: Summary of CUP 2014 meta-analysis and published pooled analyses – BMI

ANALYSIS	INCREMENT/ CONTRAST	RR (95% CI)	I ²	NO. STUDIES	NO. CASES	FACTORS ADJUSTED FOR
CUP Liver Cancer SLR 2014	Per 5 kg/m ²	1.30 (1.16-1.46)	78%	12	14,311	
Asia-Pacific Cohort Studies Collaboration [99]	≥25 vs. 18.5–22.9 kg/m ²	1.27 (0.93-1.74)	-	44	420 deaths	Age, sex, study, alcohol, blood pressure, smoking, serum cholesterol and diabetes
Prospective Studies Collaboration [100]	Per 5 kg/m ²	1.47 (1.26-1.71)	-	57	422 deaths	Study, baseline age and smoking
Asia-Pacific Cohort Studies Collaboration [101]	30–60 vs. 18.5–24.9 kg/m ²	1.10 (0.63-1.91)	-	39	774	Age, smoking
	Per 5 kg/m ²	1.11 (0.63-1.91)	-			
European cohorts [102]	HvL quintile (median) BMI 31.3 vs. 20.7 kg/m ²)	1.92 (1.23-2.96)	-	7		Age, smoking status and BMI, stratified by birth years, sex and sub- cohorts, and corrected for regression dilution ratio

Mechanisms

*Note: This is adapted from sections 6.1.3.1 and 7.8.5.4 of the Second Expert Report. In the future, an updated review of mechanisms for this exposure will form part of a larger review of mechanisms (see **section 6.1** in this report).*

Body fatness directly affects levels of many circulating hormones, such as insulin, insulin-like growth factors and oestrogens [107], creating an environment that encourages carcinogenesis and discourages apoptosis. It stimulates the body's inflammatory response, which may contribute to the initiation and progression of several cancers. Body fatness is strongly associated with increased risk of type 2 diabetes [108], which is itself associated with increased risk of hepatocellular carcinoma [56].

In general the involvement of insulin-like growth factor metabolism, inflammation, adipogenesis and its influence on lipid metabolism, steroid hormones and mTOR signalling are under intense investigation at the basic level as well as in relation to cancer.

Obesity is a risk factor for non-alcoholic steatohepatitis (NASH), which may progress to cirrhosis and therefore an increased risk of developing liver cancer [80]. NASH is the most severe form of NAFLD, the hallmark of which is hepatic steatosis characterised by the accumulation of cytoplasmic triacylglycerols within hepatocytes. In contrast to simple steatosis, the more severe NASH form is characterised by inflammation with the presence of steatosis, hepatocellular ballooning and fibrosis. The low-grade systemic inflammation associated with obesity is believed to contribute to metabolic deregulation (peripheral and hepatic insulin resistance), and the progression of NAFLD to NASH, fibrosis and finally hepatocellular carcinoma [80].

CUP Panel's conclusion:

The evidence for BMI and liver cancer was generally consistent and the dose-response relationship showed a statistically significant positive association. This association was still apparent when stratified by sex and geographical location. Results from several published pooled analyses and meta-analyses were also consistent with the Liver Cancer SLR 2014 in the direction of the effect, although not all showed findings that were statistically significant. Non-linear analysis showed a steeper increase in risk at higher BMI levels. There is also evidence of plausible mechanisms operating in humans. The CUP Panel concluded:

Greater body fatness (marked by BMI) is a convincing cause of liver cancer.

7.7 Other

Other exposures were evaluated. However, data were either of too low quality or too inconsistent, or the number of studies too few to allow conclusions to be reached. The list of exposures judged as 'Limited – no conclusion' is summarised in the matrix on page 6.

The evidence for fruits, previously judged as 'limited – suggestive' in the Second Expert Report was less consistent, and the Panel could not draw any conclusions from the updated evidence (see Liver Cancer SLR 2014 section 2.2.2).

Evidence for the following exposures, previously judged as 'limited – no conclusion' in the Second Expert Report, remains unchanged after updating the analyses with new data identified in the Liver Cancer SLR 2014: cereals (grains) and their products, non-starchy vegetables, peanuts (groundnuts), salted fish, water source (for example, river, reservoir) and tea.

In addition, evidence for the following new exposures, for which no judgement was made in the Second Expert Report, is too limited to draw any conclusions: meat and poultry, green tea, glycaemic index, calcium and vitamin D supplements, vitamin C and low fat diet.



8. Comparison with the Second Expert Report

Overall the evidence from the additional cohort studies identified by the CUP was consistent with that reviewed as part of the Second Expert Report. Much of the new evidence was related to body fatness, which has substantially strengthened the ‘limited – suggestive’ conclusion from the Second Expert Report, and also to alcoholic drinks, for which the conclusion was upgraded from probable in the Second Expert Report to convincing. There was also new evidence that coffee probably decreases the risk of liver cancer, for which no conclusions were possible in the Second Expert Report.

9. Conclusions

The CUP Panel concluded:

- ◆ **Aflatoxins: Higher exposure to aflatoxins and consumption of aflatoxin-contaminated foods are convincing causes of liver cancer.**
- ◆ **Alcoholic drinks: Consumption of alcoholic drinks is a convincing cause of liver cancer. This is based on evidence for alcohol intakes above about 45 grams per day (around 3 drinks a day).**
- ◆ **Body fatness: Greater body fatness (marked by BMI) is a convincing cause of liver cancer.**
- ◆ **Coffee: Higher consumption of coffee probably protects against liver cancer.**
- ◆ **Fish: The evidence suggesting that a higher consumption of fish decreases the risk of liver cancer is limited.**
- ◆ **Physical activity: The evidence suggesting that higher levels of physical activity decrease the risk of liver cancer is limited.**

The CUP database is being continually updated for all cancers. The Recommendations for Cancer Prevention will be reviewed in 2017 when the Panel has reviewed the conclusions for the other cancers.

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Abbreviations

AFB₁	Aflatoxin B ₁
AFM₁	Aflatoxin M ₁
AICR	American Institute for Cancer Research
BMI	Body mass index
CI	Confidence interval
CUP	Continuous Update Project
DEN	Diethylnitrosamine
DNA	Deoxyribonucleic acid
HBsAG	Hepatitis B surface antigen
HCC	Hepatocellular carcinoma
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
No.	Number
PUFA	Polyunsaturated fatty acids
RR	Relative risk
SLR	Systematic literature review
TNF-α	Tumour necrosis factor alpha
WCRF	World Cancer Research Fund
<i>n</i>	Number of cases



Glossary

Adjustment

A statistical tool for taking into account the effect of known confounders.

Aflatoxins

Naturally occurring mycotoxins that are produced by many species of *Aspergillus*, a fungus, most notably *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are toxic and carcinogenic to animals, including humans.

Anthropometric measures

Measures of body dimensions.

Bias

In epidemiology, deviation of an observed result from the true value in a particular direction (systematic error) due to factors pertaining to the observer or to study design or analysis. See also selection bias.

Bile

A greenish-yellow fluid secreted by the liver and stored in the gallbladder. Bile plays an important role in the intestinal absorption of fats. Bile contains cholesterol, bile salts and waste products such as bilirubin.

Body mass index (BMI)

Body weight expressed in kilograms divided by the square of height expressed in metres ($BMI = \text{kg}/\text{m}^2$). It provides an indirect measure of body fatness. Also called Quetelet's Index.

Carcinogen

Any substance or agent capable of causing cancer.

Carcinoma

Malignant tumour derived from epithelial cells, usually with the ability to spread into the surrounding tissue (invasion) and produce secondary tumours (metastases).

Case-control study

An epidemiological study in which the participants are chosen based on their disease or condition (cases) or lack of it (controls) to test whether past or recent history of an exposure such as smoking, genetic profile, alcohol consumption or dietary intake is associated with the risk of disease.

Cholangiocarcinoma

A malignant tumour in the ducts that carry bile from the liver to the small intestine.

Cirrhosis

A condition in which normal liver tissue is replaced by scar tissue (fibrosis), with nodules of liver regenerative tissue.

Cohort study

A study of a (usually large) group of people whose characteristics are recorded at recruitment (and sometimes later), followed up for a period of time during which outcomes of interest are noted. Differences in the frequency of outcomes (such as disease) within the cohort are calculated in relation to different levels of exposure to factors of interest, for example smoking, alcohol consumption, diet and exercise. Differences in the likelihood of a particular outcome are presented as the relative risk comparing one level of exposure to another.

Confidence interval (CI)

A measure of the uncertainty in an estimate, usually reported as 95 per cent confidence interval (CI), which is the range of values within which there is a 95 per cent chance that the true value lies. For example, the effect of smoking on the relative risk of lung cancer in one study may be expressed as 10 (95% CI 5–15). This means that in this particular analysis, the point estimate of the relative risk was calculated as 10, and that there is a 95 per cent chance that the true value lies between 5 and 15.

Confounder

A variable, within a specific epidemiological study, that is associated with both an exposure and the disease but is not in the causal pathway from the exposure to the disease. If not adjusted for, this factor may distort the apparent exposure–disease relationship. An example is that smoking is related both to coffee drinking and to risk of lung cancer and thus, unless accounted for (controlled) in studies, might make coffee drinking appear falsely as a possible cause of lung cancer.

Confounding factor (see confounder)

Deoxyribonucleic acid (DNA)

The double-stranded, helical molecular chain found within the nucleus of each cell, which carries the genetic information.

Dose-response

A term derived from pharmacology that describes the degree to which an effect changes with the level of an exposure, for instance the intake of a drug or food (see Second Expert Report box 3.2).

Egger's test

A statistical test for small study effects such as publication bias.

Exposure

A factor to which an individual may be exposed to varying degrees, such as intake of a food, level or type of physical activity, or aspect of body composition.

Fatty acid

A carboxylic acid with a carbon chain of varying length, which may be saturated (no double bonds) or unsaturated (one or more double bonds). Three fatty acids attached to a glycerol backbone make up a triglyceride, the usual form of fat in food and adipose tissue.

Hepatitis

Inflammation of the liver, which can occur as the result of a viral infection or autoimmune disease or because the liver is exposed to harmful substances.

Hepatocellular carcinoma

Primary malignant tumour of the liver.

Hepatocytes

The main cells of the liver.

Heterogeneity

A measure of difference between the results of different studies addressing a similar question in meta-analysis. The degree of heterogeneity may be calculated statistically, for example using the I^2 test.

Hormone

A substance secreted by specialised cells that affects the structure and/or function of other cells or tissues in another part of the body.

Immune response

The production of antibodies or specialised cells in response to foreign proteins or other substances.

Incidence rates

The number of new cases of a condition appearing during a specified period of time expressed relative to the size of the population, for example 60 new cases of breast cancer per 100,000 women per year.

Inflammation

The immunologic response of tissues to injury or infection. Inflammation is characterised by accumulation of white blood cells that produce several bioactive chemicals, causing redness, pain and swelling.

Insulin

A protein hormone secreted by the pancreas that promotes the uptake and utilisation of glucose, particularly in the liver and muscles. Inadequate secretion of, or tissue response to, insulin leads to diabetes mellitus.

Malignant

The capacity of a tumour to spread to surrounding tissue or to other sites in the body.

Meta-analysis

The process of using statistical methods to combine the results of different studies.

Metastasis

The spread of malignant cancer cells to distant locations around the body from the original site.

Nested case-control study

A case-control study in which cases and controls are drawn from the population of a cohort study, often used for studies of prospectively collected information or biological samples.

Odds ratio (OR)

A measure of the risk of an outcome such as cancer, associated with an exposure of interest, used in case-control studies, approximately equivalent to the relative risk (RR).

p53

A protein central to regulation of cell growth. Mutations of the p53 gene are important causes of cancer (see Second Expert Report box 2.2).

Pathogenesis

The origin and development of disease. The mechanisms by which causal factors increase the risk of disease.

Physical activity

Any movement using skeletal muscles.

Pooled analysis (see pooling)**Pooling**

In epidemiology, a type of study in which original individual-level data from two or more original studies are obtained, combined and analysed.

Publication bias

A bias in the overall balance of evidence in the published literature due to selective publication. Not all studies carried out are published, and those that are may differ from those that are not. Publication bias can be tested, for example, with either Begg's or Egger's tests.

Randomised controlled trial (RCT)

A study in which a comparison is made between one intervention (often a treatment or prevention strategy) and another (control). Sometimes the control group receives an inactive agent (a placebo). Groups are randomised to one intervention or the other, so that any difference in outcome between the two groups can be ascribed with confidence to the intervention. Usually neither investigators nor subjects know to which condition they have been randomised; this is called 'double-blinding'.

Reactive oxygen species

Oxygen-containing radical species or reactive ions that oxidise DNA (remove electrons), for example, hydroxyl radical (OH⁻), hydrogen peroxide (H₂O₂) or superoxide radical (O₂⁻).

Relative risk (RR)

The ratio of the rate of disease or death among people exposed to a factor compared to the rate among the unexposed, usually used in cohort studies.

Ribonucleic acid (RNA)

The molecule created by RNA polymerase from DNA (transcription) that carries the genetic message to ribosomes (translation), where proteins are made.

Selection bias

Bias arising from the procedures used to select study participants and from factors influencing participation.

Statistical significance

The probability that any observed result might not have occurred by chance. In most epidemiologic work, a study result whose probability is less than 5 per cent ($p < 0.05$) is considered sufficiently unlikely to have occurred by chance to justify the designation 'statistically significant' (see confidence interval).

Systematic literature review (SLR)

A means of compiling and assessing published evidence that addresses a scientific question with a predefined protocol and transparent methods.



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Appendix - Criteria for grading evidence

(Taken from Chapter 3 of the Second Expert Report)

This appendix lists the criteria agreed by the Panel that were necessary to support the judgements shown in the matrices. The grades shown here are ‘convincing’, ‘probable’, ‘limited – suggestive’, ‘limited – no conclusion’ and ‘substantial effect on risk unlikely’. In effect, the criteria define these terms.

CONVINCING (STRONG EVIDENCE)

These criteria are for evidence strong enough to support a judgement of a convincing causal relationship, which justifies goals and recommendations designed to reduce the incidence of cancer. A convincing relationship should be robust enough to be highly unlikely to be modified in the foreseeable future as new evidence accumulates.

All of the following were generally required:

- ◆ Evidence from more than one study type.
- ◆ Evidence from at least two independent cohort studies.
- ◆ No substantial unexplained heterogeneity within or between study types or in different populations relating to the presence or absence of an association, or direction of effect.
- ◆ Good quality studies to exclude with confidence the possibility that the observed association results from random or systematic error, including confounding, measurement error and selection bias.
- ◆ Presence of a plausible biological gradient (‘dose-response’) in the association. Such a gradient need not be linear or even in the same direction across the different levels of exposure, so long as this can be explained plausibly.
- ◆ Strong and plausible experimental evidence, from either human studies or relevant animal models, that typical human exposures can lead to relevant cancer outcomes.

PROBABLE (STRONG EVIDENCE)

These criteria are for evidence strong enough to support a judgement of a probable causal relationship, which would generally justify goals and recommendations designed to reduce the incidence of cancer.

All the following were generally required:

- ◆ Evidence from at least two independent cohort studies or at least five case control studies.
- ◆ No substantial unexplained heterogeneity between or within study types in the presence or absence of an association or direction of effect.
- ◆ Good quality studies to exclude with confidence the possibility that the observed association results from random or systematic error, including confounding, measurement error and selection bias.
- ◆ Evidence for biological plausibility.

LIMITED — SUGGESTIVE

These criteria are for evidence that is too limited to permit a probable or convincing causal judgement but is suggestive of a direction of effect. The evidence may have methodological flaws, or be limited in amount, but shows a generally consistent direction of effect. This judgement almost always does not justify recommendations designed to reduce the incidence of cancer. Any exceptions require special explicit justification.

All the following were generally required:

- ◆ Evidence from at least two independent cohort studies or at least five case control studies.
- ◆ The direction of effect is generally consistent, though some unexplained heterogeneity may be present.
- ◆ Evidence for biological plausibility.

LIMITED — NO CONCLUSION

Evidence is so limited that no firm conclusion can be made. This category represents an entry level and is intended to allow any exposure for which there are sufficient data to warrant Panel consideration, but where insufficient evidence exists to permit a more definitive grading. This does not necessarily mean a limited quantity of evidence. A body of evidence for a particular exposure might be graded ‘limited – no conclusion’ for a number of reasons. The evidence might be limited by the amount of evidence in terms of the number of studies available, by inconsistency of direction of effect, by poor quality of studies (for example, lack of adjustment for known confounders) or by any combination of these factors.

When an exposure is graded ‘limited – no conclusion’, this does not necessarily indicate that the Panel has judged that there is evidence of no relationship. With further good quality research, any exposure graded in this way might in the future be shown to increase or decrease the risk of cancer. Where there is sufficient evidence to give confidence that an exposure is unlikely to have an effect on cancer risk, this exposure will be judged ‘substantial effect on risk unlikely’.

There are also many exposures for which there is such limited evidence that no judgement is possible. In these cases, evidence is recorded in the full CUP SLRs on the World Cancer Research Fund International website (www.wcrf.org). However, such evidence is usually not included in the summaries.

SUBSTANTIAL EFFECT ON RISK UNLIKELY (STRONG EVIDENCE)

Evidence is strong enough to support a judgement that a particular food, nutrition or physical activity exposure is unlikely to have a substantial causal relation to a cancer outcome. The evidence should be robust enough to be unlikely to be modified in the foreseeable future as new evidence accumulates.

All of the following were generally required:

- ◆ Evidence from more than one study type.
- ◆ Evidence from at least two independent cohort studies.
- ◆ Summary estimate of effect close to 1.0 for comparison of high and low exposure categories.

- ◆ No substantial unexplained heterogeneity within or between study types or in different populations.
- ◆ Good quality studies to exclude with confidence the possibility that the absence of an observed association results from random or systematic error, including inadequate power, imprecision or error in exposure measurement, inadequate range of exposure, confounding and selection bias.
- ◆ Absence of a demonstrable biological gradient ('dose-response').
- ◆ Absence of strong and plausible experimental evidence, either from human studies or relevant animal models, that typical human exposures lead to relevant cancer outcomes.

Factors that might misleadingly imply an absence of effect include imprecision of the exposure assessment, an insufficient range of exposure in the study population and inadequate statistical power. Defects in these and other study design attributes might lead to a false conclusion of no effect.

The presence of a plausible, relevant biological mechanism does not necessarily rule out a judgement of 'substantial effect on risk unlikely'. But the presence of robust evidence from appropriate animal models or in humans that a specific mechanism exists, or that typical exposures can lead to cancer outcomes, argues against such a judgement.

Because of the uncertainty inherent in concluding that an exposure has no effect on risk, the criteria used to judge an exposure 'substantial effect on risk unlikely' are roughly equivalent to the criteria used with at least a 'probable' level of confidence. Conclusions of 'substantial effect on risk unlikely' with a lower confidence than this would not be helpful and could overlap with judgements of 'limited – suggestive' or 'limited – no conclusion'.

SPECIAL UPGRADING FACTORS

These are factors that form part of the assessment of the evidence that, when present, can upgrade the judgement reached. So an exposure that might be deemed a 'limited – suggestive' causal factor in the absence, say, of a biological gradient, might be upgraded to 'probable' in its presence. The application of these factors (listed below) requires judgement, and the way in which these judgements affect the final conclusion in the matrix are stated.

- ◆ Presence of a plausible biological gradient ('dose-response') in the association. Such a gradient need not be linear or even in the same direction across the different levels of exposure, so long as it can be explained plausibly.
- ◆ A particularly large summary effect size (an odds ratio or relative risk of 2.0 or more, depending on the unit of exposure) after appropriate control for confounders.
- ◆ Evidence from randomised trials in humans.
- ◆ Evidence from appropriately controlled experiments demonstrating one or more plausible and specific mechanisms actually operating in humans.
- ◆ Robust and reproducible evidence from experimental studies in appropriate animal models showing that typical human exposures can lead to relevant cancer outcomes.

Our Recommendations for Cancer Prevention

Body fatness

Be as lean as possible without becoming underweight

Physical activity

Be physically active for at least 30 minutes every day

Foods and drinks that promote weight gain

Limit consumption of energy-dense foods

Plant foods

Eat more of a variety of vegetables, fruits, wholegrains and pulses such as beans

Animal foods

Limit consumption of red meats (such as beef, pork and lamb), and avoid processed meats

Alcoholic drinks

If consumed at all, limit alcohol to a maximum of 2 drinks a day for men and 1 drink a day for women

Preservation, processing, preparation

Limit consumption of salt, and avoid mouldy grains and cereals

Dietary supplements

Don't use supplements to protect against cancer

Breastfeeding

It is best for mothers to breastfeed exclusively for up to six months and then add other liquids and foods

Cancer survivors

After treatment, cancer survivors should follow the recommendations for cancer prevention



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