5. Summary of Data Reported and Evaluation

5.1 Exposure data

Hepatitis B virus (HBV) is a small DNA virus made up of an outer envelope, bearing hepatitis B surface antigen (HBsAg), and an internal nucleocapsid. The nucleocapsid contains the hepatitis B core antigen (HBcAg), DNA polymerase/reverse transcriptase and the viral DNA genome. The viral genome is a circular, partially double-stranded DNA molecule about 3.2 kilobases long. It has four open reading frames, which encode for the different viral antigens, including hepatitis B e antigen (HBeAg) and hepatitis B x antigen (HBxAg), and replicates asymmetrically by reverse transcription of an RNA intermediate. Naturally occurring HBV mutants have been identified, but their pathobiological significance has not been defined. HBV belongs to a group of hepatotropic DNA viruses (hepadnaviruses) which include the hepatitis viruses of the woodchuck (*Marmota monax*), Beechey ground squirrel (*Spermophilus beecheyi*) and domestic duck (*Anas domesticus*). These viruses are highly species specific; they infect primarily hepatocytes.

Current serological methods of detection are highly sensitive and specific and are based on the detection of viral antigens, antibodies to viral antigens and viral DNA. The presence of HBsAg or HBV DNA indicates current HBV infection. The presence of HBeAg indicates a high level of viral replication. Seroconversion to anti-HBe is usually associated with reductions in replication and in disease activity. The presence of immunoglobulin M class anti-HBc indicates acute HBV infection; the immunoglobulin G class anti-HBc appears after acute HBV infection and persists during chronic HBV infection.

Transmission of infection in areas of high prevalence is predominantly between children; mother-to-child (perinatal) transmission plays a particularly important role in Asia. The modes of transmission in childhood are unclear. In areas of intermediate and low endemicity, the pattern of perinatal, childhood and adult infection is mixed. In adults, sexual transmission is a major mode of transmission, although intravenous use of drugs plays an important role in

some populations. In many cases in areas of low endemicity, the mode of transmission is unknown.

The course and clinical manifestations of HBV infection are highly variable and depend on age at infection, gender, the immune competence of the host and, possibly, viral factors. Infection perinatally and in early childhood is the major risk factor for chronicity, which frequently leads to progressive liver disease and cirrhosis.

The prevalence of chronic HBV infection varies markedly around the world. High rates of infection, defined as prevalences $\geq 8\%$, occur in China, Southeast Asia, the Pacific Basin, sub-Saharan Africa and the Amazon Basin. In western Europe, North America, Australia and New Zealand, the prevalences of chronic infection are low ($< 2\%$), and infection occurs predominantly in adults. Intermediate prevalences of infection, between 2 and 7%, occur elsewhere in the world.

The incidence of infection is reduced by vaccination with plasma-derived or recombinant vaccines, which are highly immunogenic and confer long-lasting protection against acute hepatitis and chronic infection. The efficacy of vaccines against chronic infection is in excess of 85% in regions where child and adult infection predominate and greater than 70% in regions where perinatal infection plays an important role. The efficacy of vaccination in preventing perinatal infection is improved by the addition of hepatitis B immunoglobulin administration soon after birth.

5.2 Human carcinogenicity data

In 15 cohort studies, carrier status for HBV was determined by the presence of HBsAg in serum. In all studies, the risk for hepatocellular carcinoma increased in association with HBsAg seropositivity, with estimates of relative risk ranging from 5.3 to 148.

Many case–control studies have been reported on the association between hepatocellular carcinoma and chronic infection with HBV, as determined by HBsAg seropositivity. Most of the studies were conducted in Asia and in Africa, but some have been reported from Europe and North America. The studies were of variable quality, but the majority showed a strong association, with relative risks between 5 and 30.

Potential confounding by aflatoxin, infection with hepatitis C virus, cigarette smoking and alcohol drinking appears to have been excluded in studies in which those factors were evaluated.

Serological patterns of HBV markers other than HBsAg, such as anti-HBc and anti-HBs, have been examined in many studies, but variability in methods of determination and reporting of results precluded evaluation of their association with hepatocellular carcinoma.

In general, cohort studies have not reported increased risks for cancers other than hepatocellular carcinoma. No consistent evidence of increased risk was found in case–control studies of other cancers (including cholangiocarcinoma of the liver).

5.3 Animal carcinogenicity data

Hepatitis B virus

Studies over the past two decades have shown that chimpanzees can be infected with HBV and can become carriers, exhibiting mild hepatitis. Progressive liver disease, including

hepatocellular carcinoma, is not known to develop in HBV-infected chimpanzees, although reporting of long-term studies of infected animals is sparse and inadequate. A single report suggested that Asian macaques are susceptible to HBV infection and to progressive liver lesions; a possible hepatocellular carcinoma developed in an HBV-infected macaque.

In three studies in transgenic mice on the expression of integrated HBV genes (pre-S, S and/or X genes) in hepatocytes, increased numbers of liver tumours were associated with a high level of expression of the large surface antigen and X proteins. The relevance of the finding that hepatocellular carcinomas are produced in these transgenic mice for evaluating the carcinogenicity of HBV is unclear.

Other hepadnaviruses

Woodchucks are susceptible to infection with the related hepadnaviruses, woodchuck and ground squirrel hepatitis viruses (WHV and GSHV), both of which lead to chronic hepatitis but not to cirrhosis. In one study of naturally infected, captive adults, one study of experimentally infected adults and newborns and one study of experimentally infected newborns, infection with WHV was associated with development of hepatocellular carcinoma in up to 85% of woodchucks with chronic infection. Uninfected animals did not develop hepatocellular carcinoma. Newborn woodchucks experimentally infected with GSHV also developed hepatocellular carcinoma. Beechey ground squirrels are susceptible to infection with GSHV, with the development of mild chronic hepatitis but not cirrhosis. In one study of Beechey ground squirrels captured in the wild, 11/24 (45%) animals naturally infected with GSHV developed hepatocellular carcinoma, while 2/26 (8%) uninfected animals developed the tumour. One study showed that captive Richardson ground squirrels may be infected with a similar but poorly characterized hepadnavirus, but the association of viral infection and hepatocellular carcinoma in this species has not been firmly demonstrated. Domestic ducks are susceptible to infection with a hepadnavirus, duck hepatitis B virus (DHBV). Hepatocellular carcinoma has been observed in free-ranging ducks infected with DHBV, but in three studies of experimentally infected animals and one study of congenitally infected ducks, no increase in the incidence of hepatocellular carcinoma was observed.

5.4 Other relevant data

The mechanisms whereby HBV may induce hepatocellular carcinoma are uncertain. HBV does not contain a known oncogene. HBV DNA is integrated into host DNA in the great majority of hepatocellular carcinomas in HBV carriers, and chromosomal translocations associated with integrated HBV sequences have been reported. In only three cases of hepatocellular carcinoma have HBV DNA sequences been shown to be integrated into any known host gene. This molecular event is, however, common in woodchucks: in about 50% of hepatocellular carcinomas arising in animals infected chronically with WHV, viral DNA sequences were integrated in or adjacent to c-*myc* or N-*myc* genes. In humans, sequences of the S and X genes of HBV are almost always present in integrated HBV DNA, and X gene protein has been shown to *trans*-activate both HBV and cellular genes. There is

no well documented evidence for overexpression of known oncogenes as a result of HBV DNA integration in human hepatocellular carcinoma. Deletions on multiple chromosomes and mutations of the *p53* tumour suppressor gene occur in hepatocellular carcinoma, but no pattern of these changes has been found to be specific to hepatocellular carcinomas arising in chronically HBV-infected humans.

The great majority of hepatocellular carcinomas that arise in association with chronic HBV infection occur in conjunction with cirrhosis or chronic hepatitis. Chronic HBV infection is generally established in early childhood, and several decades of chronic hepatitis usually precede development of the cancer. Studies of HBV integration have demonstrated that many regenerative nodules in cirrhotic liver have independent clonal origins; clonal regeneration reflects the extensive cell turnover that renders host DNA more susceptible to mutagenesis.

5.5 Evaluation[1]

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with hepatitis B virus.

There is *inadequate evidence* in experimental animals for the carcinogenicity of hepatitis B virus. Some hepadnaviruses closely related to hepatitis B virus produce hepatocellular carcinoma in susceptible species.

Overall evaluation

Chronic infection with hepatitis B virus *is carcinogenic to humans (Group 1).*