AFLATOXINS (Group 1)

A. Evidence for carcinogenicity to humans (*sufficient*)

A positive correlation between estimated aflatoxin intake or level of aflatoxin contamination of market food samples and cooked food and incidence of hepatocellular cancer was observed in early studies in Uganda, Swaziland, Thailand and Kenya[1-4]. Similar correlations between aflatoxin intake and hepatocellular cancer incidence and mortality have been reported from Mozambique and China, where there is considerable geographical variation in the occurrence of this cancer[5-8]. Summary analysis of the data obtained from studies conducted in different regions of Africa and Asia where hepatocellular cancer incidence or mortality and aflatoxin intake were measured revealed a highly significant correlation between these variables[5].

In south-eastern USA, in an area with a high average daily intake of aflatoxin B_1 (13-197 ng/kg bw), a 10% excess (6% for the 30-49 age group) in hepatocellular cancer incidence was observed compared to 'northern' and 'western' areas with low aflatoxin B_1 intake (0.2-0.3 ng/kg bw)[9].

A case-control study in the Philippines, where mean aflatoxin contamination levels in dietary items were established and individual levels of aflatoxin consumption were determined retrospectively, demonstrated an increased, dose-related relative risk of developing hepatocellular cancer in persons with higher ingestion of aflatoxin. Relative risks of developing hepatocellular cancer by category of overall mean load of aflatoxin in the diet, e.g., very heavy *versus* light and moderately heavy *versus* light, were 17.0 and 13.9 (both significant at the 0.05 level). The effect of aflatoxin on relative risk was increased by alcohol consumption, and heavy aflatoxin/heavy alcohol consumption gave a relative risk of 35.0[10].

In a case-control study conducted in Hong Kong where 107 hepatocellular cancer patients and 107 controls were studied, the relative risk of hepatocellular cancer was not related to dietary intake of corn or beans, which are the chief sources of aflatoxin contamination in Hong Kong. The relative risk was increased (2.2), but not significantly, for consumption of 'other grains', including wheat, barley and oats[11]; however, among 878 market food samples, only 22 contained aflatoxins[12].

One major difficulty in interpreting these studies is potential confounding due to hepatitis virus B infection, which is endemic in many areas where the relationship between aflatoxin intake and hepatocellular carcinoma has been examined. However, in three recent studies, both factors have been taken into account. In China, both dietary and urinary levels of aflatoxins were found to be related to hepatocellular cancer incidence. Levels of aflatoxin M_1 as high as 35 ng were found in urine in high-incidence areas, whereas levels of <2 ng were observed in low-risk areas. Serological surveys did not show corresponding differences in the prevalence of the hepatitis B virus-carrier state[13]. In another area of China, the mortality rate for hepatocellular cancer was 9.9 times higher in villages with high aflatoxin contamination of foodstuffs than in villages with lightly contaminated foods. In this area, aflatoxin contamination of foods appeared to be a risk factor over and above hepatitis B infection[14]. In Swaziland, in a study based on surveys of levels of aflatoxin intake across four broad geographic regions, liver cancer incidence was strongly associated with estimated levels of aflatoxin. In a multivariate analysis involving ten smaller subregions, aflatoxin exposure emerged as a more important determinant of the variation in liver cancer incidence than the prevalence of hepatitis B infection. This analysis was based on 52 cases spread over ten subareas, and estimations of aflatoxin intake and prevalence of hepatitis B infection were based on surveys conducted among the general population and on samples from blood donors, respectively. Imprecision in measuring intake of aflatoxin or in establishing hepatitis B infection, or the presence of unmeasured confounders, seem unlikely to account for the five-fold differences in hepatocellular cancer incidence seen in association with aflatoxin intake[15].

Additional evidence for a causative association between aflatoxin exposure and human cancer was found in a retrospective cohort study of 71 workers in a plant in the Netherlands where oil was extracted from linseeds and from peanuts. In the aflatoxin-exposed group, the observed mortality over the entire 18-year study period was higher than expected for all cancers (standardized mortality ratio [SMR], 250; 95% confidence interval, 140-400) and

for the first six years of observation (SMR, 438; 180-870). An increase in mortality from respiratory cancer in the exposed group was also evident (SMR, 253; 100-500)[16].

A few case reports of cancer other than hepatocellular in aflatoxin-exposed workers have been published[1,17-19].

B. Evidence for carcinogenicity to animals (*sufficient*)

Aflatoxins produce liver tumours in mice, rats, fish, ducks, marmosets, tree shrews and monkeys after administration by several routes, including the mouth. In rats, cancers of the colon and kidney were also seen[1]. Recent studies have extended these findings. In hamsters, aflatoxin B_1 produced cholangiocellular but not hepatocellular tumours[20]. In mice, aflatoxin B_1 administered orally or intraperitoneally resulted in an increased incidence of lung adenomas[1,21]. All rats fed 5 mg/kg of diet aflatoxin B_1 for six weeks developed hepatocellular carcinomas[22]; neoplastic hepatic nodules were produced in rats by oral administration of a single dose of 5 mg/kg bw aflatoxin B_1[23]; rats fed peanut oil containing 5-7 μg/kg aflatoxin B_1 developed parenchymal liver damage but no liver-cell tumour[24]. Aflatoxin B_1 can induce liver tumours in monkeys[25,26]; osteogenic sarcoma, adenocarcinoma of the gall-bladder or bile duct and carcinomas of the pancreas were also observed[26]. Aflatoxin B_1 also induced liver tumours in the subhuman primate tree shrew, *Tupaia glis*[27]. Intraperitoneal administration of aflatoxin B_1 to pregnant rats induced liver and other tumours in the mothers and in the progeny[28]. Aflatoxin M_1, a hydroxy metabolite of aflatoxin B_1, produced fewer hepatocellular carcinomas following its oral administration to rats than aflatoxin B_1 given at the same dose level and by the same route[29].

C. Other relevant data

In one study, aflatoxin B_1-DNA adducts were excreted in human urine. No data were available on the genetic and related effects of aflatoxins B_2, G_1, G_2 or M_1 in humans[30].

Aflatoxin B_1 has been tested extensively for genetic effects in a wide variety of tests *in vivo* and *in vitro*, giving consistently positive results. It induced chromosomal aberrations, micronuclei, sister chromatid exchanges, unscheduled DNA synthesis and DNA strand breaks, and bound covalently to DNA in cells of rodents treated *in vivo*; it was reported to be weakly active in a dominant-lethal mutation assay in mice. In human cells *in vitro*, it induced chromosomal aberrations, micronuclei, sister chromatid exchanges and unscheduled DNA synthesis and bound covalently to DNA. It induced cell transformation in several test systems, and induced chromosomal aberrations, sister chromatid exchanges, mutation, unscheduled DNA synthesis and DNA strand breaks in rodent cells *in vitro*. It induced sex-linked recessive lethal mutations and somatic mutation and recombination in *Drosophila*. In fungi, aflatoxin B_1 was mutagenic and induced gene conversion and mitotic recombination. It was mutagenic and induced DNA damage in bacteria and bound covalently to isolated DNA[30].

Aflatoxin B_2 bound covalently to DNA in hepatocytes of rats treated *in vivo*. It transformed Syrian hamster embryo cells and induced sister chromatid exchanges in Chinese hamster cells *in vitro* and induced unscheduled DNA synthesis in rat hepatocytes,

but not in human fibroblasts, *in vitro*. It was not mutagenic to fungi in the absence of a metabolic system and did not induce gene conversion or mitotic recombination in yeast. Aflatoxin B_2 induced mutation but not DNA damage in bacteria[30].

Aflatoxin G_1 induced chromosomal aberrations in bone-marrow cells of Chinese hamsters treated *in vivo* and bound to DNA in kidney and liver cells of treated rats. It induced unscheduled DNA synthesis in human fibroblasts and rat hepatocytes *in vitro* and caused chromosomal aberrations and sister chromatid exchanges in Chinese hamster cells *in vitro*. It induced mutation in *Neurospora crassa* but neither mutation nor gene conversion in *Saccharomyces cerevisiae*. Aflatoxin G_1 induced mutation and DNA damage in bacteria and bound covalently to isolated DNA[30].

Aflatoxin G_2 did not induce unscheduled DNA synthesis in human fibroblasts *in vitro*. It induced sister chromatid exchanges in Chinese hamster cells and unscheduled DNA synthesis in rat and hamster hepatocytes *in vitro*. It did not induce mutation in cultured rodent cells or in fungi in the absence of a metabolic system. Aflatoxin G_2 gave conflicting results for mutation in bacteria and did not cause DNA damage[30].

Aflatoxin M_1 induced unscheduled DNA synthesis in rat hepatocytes *in vitro* and was mutagenic to bacteria[30].

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