5. Summary of Data Reported

5.1 Exposure data

Several types of shift systems exist, described according to several main characteristics: permanent or rotating, continuous (all days of the week are covered) or discontinuous (interruption at the weekend or on Sunday), or with or without night work. Other important organizational factors that may have an impact on health are: length of the shift cycle, duration of shifts, number of alternating workers/crews, start and finish time of the duty periods, speed and direction (clockwise or anticlockwise) of shift rotation, number and position of rest days and regularity/irregularity of the shift schedules.

The amount of night work in any shift period is the most important factor to be considered in the disruption of biological functions. The amount of sleep of the shiftworker decreases both in terms of quantity and quality, both on night shifts (due to circadian and environmental reasons) and on early-morning shifts.

Shiftwork, that includes night work, is estimated to involve about 15–20% of the total working population, although reliable and comparable statistics are not available in most countries. In Europe, large differences have been recorded among countries (from 6.4 to 30%), and between self-employed (5.7%) and employees (19.8%); in the USA, the average prevalence of shiftwork that includes night work is 14.8% (16.7% in men and 12.4% in women). Shiftwork is most prevalent among workers in the health care, transportation, communication, leisure and hospitality sectors (above 30%), and in the service, mining and industrial manufacturing sectors (20–30%). The prevalence of shiftwork is more common in work schedules of younger workers but decreases with the age of the workers, from more than 20% in the youngest decades of life to approximately 10% after 55 years of age.

At the time of writing, there is no known biomarker of exposure for shiftwork. However, because of the importance of melatonin in the relation to the activity in the circadian rhythm, levels of melatonin could be useful biomarkers of circadian disruption. Melatonin can be measured in the blood, saliva, or urine. The measurement of melatonin concentration in plasma at regular intervals (e.g. hourly) can identify the onset, offset and duration of melatonin secretion, the time at which peak secretion occurred and the total amount of melatonin secreted. Salivary melatonin concentration is a good alternative measure as it is highly correlated with serum concentrations. The primary urinary metabolite of melatonin, 6-sulfatoxymelatonin, may also be a useful biomarker.

The disruptive effects of night work on the biological functions and social life have been recognized by some regulators both at the national and international levels, i.e. the International Labour Organization, the European Union, and the Federal Aviation Administration.

5.2 Human carcinogenicity data

Female breast cancer

Eight studies from various geographic regions have been designed to assess the relationship between breast cancer and shiftwork that involves night work. Six of these eight studies, including two prospective cohort studies in nurses, have consistently pointed towards a modestly increased risk of breast cancer among long-term employees who performed night shiftwork, defined in different ways. Most studies reported this increased risk after controlling for potential confounders. Two of the eight studies, one of which appeared to be hampered by important limitations in design, were not supportive of an association between shiftwork and breast cancer. There were a relatively limited number of studies (most focused on a single profession, i.e. nurses), some potential for confounding by unknown risk factors, and inconsistent and inaccurate exposure assessments of shiftwork, which may have biased the results towards the null.

Another occupational group of shiftworkers is flight cabin crew personnel, who also experience circadian disruption due to the crossing of time zones. The incidence of breast cancer has been studied in eight cohorts of female flight attendants, all but one consistently reported an increased risk for breast cancer which was greater after a longer duration of employment. Limitations of these studies included the potential for detection bias among female cabin crew due to a higher prevalence of breast cancer screening in this occupational group, proxy measures of exposure used in dose–response relationships, and potential confounding by reproductive factors and cosmic radiation.

The Working Group concluded that the evidence for an association with breast cancer and shiftwork that involves night work was consistent in the studies that were specifically designed to address this question. The studies of cabin crews provided additional support.

Other cancers

Few studies have investigated the association between shiftwork and cancers at other organ sites. Increased risks of cancers of the prostate, colon, and endometrium have been reported. The earliest studies of airline pilots also showed a markedly elevated incidence of prostate cancer compared with national reference levels, but limitations of these studies included the potential for detection bias due to a higher prevelance of screening for prostate cancer in this occupational group.

5.3 Animal carcinogenicity data

Animal models have been used extensively to test the impact of the circadian system (central circadian pacemaker in the suprachiasmatic nuclei and the pineal gland/melatonin-generating system) and its disruption (i.e. phase shifts, light during the dark period, melatonin suppression) on tumour development and growth at all stages of oncogenesis.

Two studies examined the impact of continuous high-intensity light versus low-intensity light on tumour development in mice. One study demonstrated clear increases in the incidence of lung adenocarcinomas, leukaemias and lymphomas combined. The second study showed an increase in the incidence of and mortality from mammary tumours in one substrain that had normal vision, and no increase in a substrain of the same strain that had retinal degeneration due to genetic predisposition. A third study showed no effects.

All of the remaining experimental studies used initiation–promotion protocols or tumour growth models following the transplantation of syngeneic tumour fragments, cells, or human cancer xenografts. The species used in these studies included both sexes of rats, mice and hamsters, all of which yielded positive results in at least one study. The types of rodent model systems studied included mammary adenocarcinoma/fibroadenoma, cancers of the peripheral nervous system and kidney, hepatocarcinoma, pancreatic adenocarcinoma, colon adenocarcinoma, prostate adenocarcinoma, squamous-cell carcinoma and fibrosarcoma, osteosarcoma and carcinosarcoma, melanoma, neuroblastoma, and undifferentiated neoplasms.

The model systems used to study the role of the central circadian function and its disruption on cancer development and/or growth encompassed the exposure of animals to chronic alterations in the light–dark environment (i.e. constant bright light, constant darkness, altered light–dark schedules, intermittent light during darkness, single light pulse during darkness). Other model systems used more focused experimental manipulations that included phase-shifting central circadian activity only (i.e. exposure to experimental chronic jet lag), suppression or ablation of the nocturnal circadian melatonin signal (i.e. pinealectomy or exposure to dim light during darkness), ablation of the central circadian activity and of melatonin production (i.e. induction of lesions in suprachiasmatic nuclei), clock gene mutations (i.e. mPer2 knockouts) and the impact of carcinogen administration at different circadian times on tumorigenesis. A specialized model system evaluated the acute proliferative activity of tissue-isolated melatonin-receptor-positive murine or human tumours perfused *in situ* with different physiological levels of melatonin from natural diurnal blood changes and artificial manipulation.

The major patterns of light–dark environments that have an impact on cancer development and/or growth (i.e. stimulation) are constant light exposure (two positive of three studies, five positive of six initiation–promotion studies, five positive of five tumour-growth studies), dim light during darkness (five positive of five studies), experimental chronic jet lag (two positive of two studies), and circadian timing of carcinogens (four positive of four studies). Two conditions that produced no clear effects or even slowed tumour growth were light pulses during the dark period (two of two studies), and constant darkness (two of two studies). Mechanistically oriented animal studies specifically aimed at investigating the role of the pineal gland (i.e. pinealectomy-induced stimulation of cancer development and/or growth) and the nocturnal melatonin profile (i.e. inhibition of cancer proliferative activity) also had a major impact on cancer (18 positive of 26 studies). Furthermore, a limited number of studies on suprachiasmatic nuclei or clock genes yielded important results with respect to increased tumorigenesis (two positive of three studies).

5.4 Other relevant data

The evidence that relates laboratory investigations and mechanistic considerations to shiftwork-induced carcinogenesis can be divided into two basic fields: disturbance of the circadian system due to light at night with alteration of the sleep–activity pattern leading to potential melatonin suppression and circadian gene alterations; and sleep deprivation that results from the need to sleep when it is not readily possible and misaligned with the surrounding active daytime social environment.

The disturbance of the circadian system is studied at the level of the molecular circadian oscillation. Genes that are responsible for maintaining circadian rhythms have been identified, and may function as transcriptional factors and regulate expression of genes in cancer-related pathways, such as cell cycle, DNA repair, and apoptosis. Animal studies have shown that knockout of the circadian *Period* gene, *Per2*, promotes tumour development. Some evidence in humans links genetic polymorphisms in circadian genes to breast cancer and non-Hodgkin lymphoma, and functional loss of the *PERIOD* genes has been observed in various human tumours. Exposure to artificial light during the night has been demonstrated to disrupt circadian gene expression in mice and humans, which in turn, may alter circadian-regulated biological pathways. Because of their possible roles in tumorigenesis, the light-mediated dysfunction of circadian genes may provide a possible mechanism for the putative carcinogenic effect of light that may or may not involve melatonin.

The light-induced alteration of the circadian system is in part linked to the suppression of melatonin, which is secreted by the pineal gland and acts throughout the organism as a time signal. The suppression of melatonin leads to changes in the gonadotropic axis that specifically involves estrogens and androgens in experimental animals, and may be stimulatory or inhibitory depending on the species and the situation. In humans treated with low pharmacological doses of melatonin, few melatonin-induced changes were documented except a stimulation of prolactin. In animal studies, melatonin in the target sites interferes with the metabolism of estrogen through several metabolic pathways. No data clearly link nocturnal blood levels of endogenous melatonin with endogenous production of estradiol in women.

The decrease in endogenous melatonin may lead to diminished free radical scavenging that may induce local tissue damage, the extent and importance of which is not entirely clear at present.

Melatonin is a direct and indirect immunostimulant; its suppression leads to a state of immunodeficiency that is aggravated by the pronounced effects of sleep deprivation upon the immune system. Prolactin, a strong immunostimulant, is decreased during sleep deprivation.

Direct inhibitory effects of melatonin on tumour cell proliferation have been shown in several animal models not only at pharmacological but also at physiological concentrations.

Sleep deprivation is a common feature in most forms of shiftwork that involves night and/or early morning hours. Changes in the immune system have been shown to occur in

partial (early or late night) sleep deprivation and comprise changes in the cytokine pattern that favours the Th2 group of cytokines and decreases Th1 cytokines (e.g. interferon γ) which act in cellular immune defence and in immune surveillance to counteract tumour growth. In the majority of studies of sleep deprivation, suppression of natural-killer-cell activity has been shown, and this also leads to a decrease in anti-tumour surveillance.

The evidence in support of shiftwork-induced carcinogenesis thus links events at the cellular level that affect cell proliferation and endocrine changes with hormonal constellations that promote endocrine-dependent cancers with defects in the immune surveillance that enhance tumour development and growth.

None of these changes stands in isolation; they are all linked to the disruption of the circadian system of shiftworkers and, in combination, may alter the risk of cancer through both tumour induction and promotion.

The experimental data from animal studies in several inter-related physiological systems are strongly suggestive of a causal link between circadian disruption and all its consequences and the development of malignant tumours. Human studies are suggestive of physiological effects that are possibly relevant to carcinogenesis.