National Toxicology Program

Peer Review of Draft Report on Carcinogens Monograph on Night Shift Work and Light at Night

October 5, 2018

National Institute of Environmental Health Sciences Research Triangle Park, NC

Draft Peer-Review Report

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Contents

I. Attendees*

Peer-Review Panel

Laura Beane Freeman (Chair), National Cancer Institute
Massimo Bracci, Università Politecnica delle Marche
Loning Fu, Baylor College of Medicine
Steven Hill, Tulane University School of Medicine
Francis Lévi, Warwick University Medical School
Florence Menegaux, Institut National de la Santé et de la Recherche Médicale
Marie-Élise Parent, Institut National de la Recherche Scientifique–
Institut Armand-Frappier Research Centre
Eva Schernhammer, Harvard T. H. Chan School of Public Health

National Toxicology Program Board of Scientific Counselors Liaison

Daniel Kass, Vital Strategies (by webcast)

Other Federal Agency Staff

Christina Lawson, National Institute for Occupational Safety and Health

Other Technical Advisors

Mariana Figueiro, Rensselaer Polytechnic Institute

National Institute of Environmental Health Sciences Staff

Brian Berridge
Windy Boyd
John Bucher
Virginia Guidry
Ramesh Kovi
Gloria Jahnke
Ruth Lunn
Barry McIntyre
Suril Mehta
Georgia Roberts
Andrew Rooney
Sheena Scruggs
Nigel Walker
Amy Wang
Mary Wolfe

Report on Carcinogens Contract Support Staff

Stanley Atwood, ILS
Kate Helmick, ICF
Susan Dakin, Independent Consultant
Andrew Ewens, ILS
Sanford Garner, ILS
Lara Handler, ILS
Jeanne Luh, ICF
Alton Peters, ILS
Pamela Schwingl, ILS
Kelly Shipkowski, ICF
River Williams, ICF

*The meeting was webcast. Individuals who viewed the webcast are not listed except as noted.

Public Attendees

Judy Hess, Shell Oil Company
Martin Moore-Ede, Circadian
Mark Rea, Rensselaer Polytechnic Institute

II. Introductions and Welcome

The National Toxicology Program (NTP) peer-review panel for the *Draft Report on Carcinogens Monograph on Night Shift Work and Light at Night* convened on October 5, 2018 in Rodbell Auditorium, Rall Building, National Institute for Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Laura Beane Freeman served as chair. Mr. Daniel Kass attended by webcast as the NTP Board of Scientific Counselors (BSC) liaison. Representing NTP were Dr. Brian Berridge, Associate Director, NTP; Dr. Mary Wolfe, Director, NTP Office of Liaison, Policy, and Review; Dr. Ruth Lunn, Director, Office of the Report on Carcinogens (ORoC); Dr. Gloria Jahnke, ORoC; Mr. Suril Mehta, ORoC; and Dr. John Bucher, Senior Scientist, NTP. Dr. Wolfe served as the Designated Federal Official.

Dr. Beane Freeman called the meeting to order at 8:30 a.m., welcomed everyone to the meeting, and asked all attendees to introduce themselves. Dr. Berridge welcomed the Panel and thanked them for their service. Dr. Wolfe read the conflict of interest policy statement and briefed the attendees on meeting logistics. Dr. Beane Freeman informed the Panel and the audience of the format for the peer review.

III. Draft RoC Monograph: Objectives and Methods

Dr. Lunn presented background information about the Report on Carcinogens (RoC) and the process and methods used to prepare the draft RoC monograph. She noted that the RoC is congressionally mandated and identifies substances that pose a cancer hazard for U.S. residents. It is prepared for the Secretary of Health and Human Services (HHS) by NTP and is cumulative, including substance profiles for newly listed substances and for all substances listed in previous reports.

Dr. Lunn outlined the four-part formal process for preparing the RoC: (1) selection of substances for evaluation, (2) preparation of draft RoC monographs, (3) peer review and finalization of the monographs, and (4) approval of the substance profiles by the HHS Secretary and release of the RoC. The process incorporates opportunities for public comment, scientific input, and peer review of the scientific information.

Dr. Lunn outlined the steps of the process that had been completed for selection and evaluation of night shift work and light at night. Light at night (LAN) was nominated for review by several individuals, who referenced the International Agency for Research on Cancer evaluation, which concluded that shift work involving circadian disruption is probably carcinogenic to humans. ORoC presented a draft concept document explaining the rationale and proposed approach for the RoC review to the NTP BSC in June 2013. ORoC convened a workshop in March 2016 to obtain scientific input on topics important for informing the literature-based hazard assessments. The workshop recommended that night shift work and LAN be framed as “modern lighting practices.” ORoC then developed a protocol for preparation of the draft monograph.

ORoC proposes that night shift work and LAN can result in circadian disruption, causing biological effects characteristic of recognized carcinogens and thus resulting in cancer. Dr. Lunn emphasized the importance of defining the two exposure scenarios as they relate to cancer in ways that are supported by the science.

Dr. Lunn outlined the framework for the evaluation and types of evidence, which included human epidemiology studies and supporting studies in experimental animals, studies of exposure and biomarkers of circadian disruption, studies of exposure and biological effects (e.g., key characteristics of carcinogens), and studies of circadian disruption and biological effects or cancer. She described the ORoC systematic review process for reaching cancer hazard conclusions. Dr. Lunn reviewed the RoC criteria for listing a substance as *known to be a human carcinogen* or *reasonably anticipated to be a human carcinogen*. She emphasized that "sufficient evidence" can come not only from human cancer studies, but also from human mechanistic studies, and that the listing recommendations are based on scientific judgment considering all relevant information. She briefly discussed the legislative requirement for past or present exposure of a significant number of people living in the United States, noting that it is a scientific judgment.

Dr. Lunn said the draft monograph would be revised based on NTP's review of the peer-review comments, with consideration of public comments. The revised monograph, the peer-review report, and NTP's response to the peer-review report would be provided to the NTP BSC at a public meeting, after which the monograph would be finalized.

The charge to the Panel was as follows:

- Comment on whether the Draft RoC Monograph on Night Shift Work and Light at Night is technically correct, clearly stated, and objectively presented.
- Provide an opinion on whether a significant number of U.S. residents (1) work or formerly worked night shifts or (2) are or were in the past exposed to light at night.

The Panel was asked to vote on the following questions:

- Whether the scientific evidence supports NTP's conclusions on the level-of-evidence for carcinogenicity from cancer studies in humans.
- Whether the scientific evidence supports NTP's preliminary policy decisions on the RoC listing status of (1) persistent night shift work that causes circadian disruption and (2) certain lighting conditions that cause circadian disruption.

IV. Public Comments

IV.A. Written Public Comments

No written public comments on the draft monograph were received.

IV.B. Oral Public Comments

Oral comments were presented by Dr. Martin Moore-Ede, a former Harvard Medical School professor who conducted research on identification of the circadian clock in the human brain, circadian regulation of neuroendocrine function, and the end results of circadian disruption in animal models and shift work. Dr. Moore-Ede is Chief Executive Officer of Circadian®, which

is involved in shift work health, performance, and safety issues, and of Circadian ZircLight, which is developing light-emitting diode(LED) chips that do not emit blue light. He called the RoC draft monograph a major step forward — well-researched, definitive, outstanding, and thorough. He suggested that the monograph more precisely define the conditions that increase the risk of circadian disruption, and that it encourage the development of responsible and practical policies to mitigate the risk.

Dr. Moore-Ede noted that lighting during night shift work is necessary to allow performance of tasks that require visual discrimination, to maintain safety, and to enhance alertness and performance. The Illuminating Engineering Society has set standards of 200 to 500 lux for high-contrast visual tasks and 500 to 1,000 lux for medium-contrast tasks, significantly higher than the 100 to 200 lux cited in the monograph as typical for fluorescent illumination of offices. In addition, energy-efficiency standards are driving the adoption of conventional blue-pump LEDs, which emit 10% to 20% of their spectral content in the circadian-disruptive blue area. Workplace illumination standards of 200 to 1,000 table-top lux translate to typical LED blue corneal irradiance (at 400 to 490 nm, which accounts for 90% of the circadian stimulus) of 4 to 29 $\mu w/cm^2$. Human studies have established that the threshold for circadian disruptive effects from blue corneal irradiance lies between 2 and 7.2 $\mu w/cm^2$; further research is needed to more precisely establish the threshold.

Dr. Moore-Ede suggested that the monograph should (1) recognize and encourage new industry and regulatory standards for workplace lighting that minimize circadian disruption while promoting alertness and safety, such as ANSI RP-755 for petrochemical workplaces, (2) identify undesirable or impractical responses to the identified health risks, such as banning shift work or reducing lighting intensity, and (3) encourage development and adoption of effective lighting solutions, such as light sources that provide blue-rich light during the day but remove it at night or eyewear that selectively removes circadian-disruptive blue light at night, while providing evidence for those solutions.

V. Peer Review of the Draft RoC Monograph on Night Shift Work and Light at Night

V.A. Cancer Hazard Evaluation

V.A.1 Introduction and Exposure (Section 1) and Studies of Circadian Disruption (Section 2)

V.A.1.1 Presentation on Introduction and Exposure (Section 1) and Studies of Circadian Disruption (Section 2)

Dr. Lunn presented an introduction to the characteristics of modern lighting practices, focusing on two exposure scenarios: electric LAN and shift work at night. U.S. residents are exposed to electric LAN from outdoor light, light in the home before or during sleep, and the use of self-luminous electronics. Since the introduction of electric light, technological advances have increased the proportions of short (blue) wavelengths in modern lighting. Dr. Lunn described night shift work as a complex exposure scenario that involves extreme exposure to LAN, sleep disruption, altered meal timing, reduced exposure to sunlight resulting in reduced production of vitamin D, and stress and altered behaviors. Night shift work has been defined as working at

least 3 hours between midnight and 5:00 a.m. on either permanent or rotating shifts. According to the National Institute for Occupational Safety and Health (NIOSH), over 10 million adults in the United States frequently work night shifts. Shift work is most common among men, minorities, and lower socioeconomic groups and is most prevalent in the protective services, transportation, healthcare, and production and manufacturing sectors.

Dr. Lunn presented a high-level overview of circadian regulation, whereby the light-dark cycle synchronizes the circadian system to the 24-hour day. External light cues are transmitted to the master clock in the brain, which synchronizes molecular clocks in the peripheral tissues to regulate cyclical body functions. Key roles are played by melatonin and clock genes. Environmental exposures that disrupt the circadian system include night shift work, exposure to LAN, sleep disturbances, transmeridian travel, and social jet lag.

Dr. Lunn summarized studies of circadian disruption biomarkers. The effectiveness of LAN in inducing melatonin suppression depends on light wavelength and intensity, exposure duration and timing, daytime light exposure, and individuals' susceptibility. Melatonin suppression in night shift workers is increased with persistent night shift work. The strongest evidence that LAN and night shift work alter clock gene expression is from animal studies, supported by human studies.

NTP concludes that a significant number of U.S. residents are exposed to electric LAN or frequently work night shifts.

V.A.1.2 Peer-Review Comments on Introduction and Exposure (Section 1)

Circadian Regulation and Disruption (Section 1.1) and Light at Night (Section 1.2)

Dr. Francis Lévi, first reviewer, said he was impressed by the quality and balance of the draft monograph. He suggested that the introduction emphasize and more clearly describe the multiple redundancies of the circadian system, which includes the suprachiasmatic nuclei (SCN), an array of physiological rhythms, and genetic clocks in virtually all cells. Although the melatonin hypothesis has been a driving force for research on the effects of shift work, this concept has been broadened by the circadian disruption hypothesis, which integrates the melatonin hypothesis.

Dr. Lévi suggested placing more emphasis on the critical roles of body temperature and glucocorticoid hormone (cortisol) rhythms in coordinating and resetting peripheral clocks, which control many pathways whose deregulation can lead to cancer. For example, he noted that simulated chronic jet lag caused cancer even in melatonin-deficient mice, and that pinealectomized human patients showed few symptoms of circadian disruption. The introduction could also address variability in the function of the circadian system, such as inter-subject and sex differences in endogenous circadian periods and function; not all shift workers develop circadian disruption (or cancer). Dr. Lévi did not agree that "melatonin … plays a vital chronobiological role by directing the temporal organization of almost all organs" (p. 3), though it does play an important role in regulation of the circadian timing system and as a biomarker of that system's function. He stated that the percentage of the transcriptome controlled by the molecular circadian clock is probably over 50%, based on data from non-human primates and from humans postmortem tissue samples; this should be highlighted in the monograph.

Dr. Lévi observed some apparent confusion over the term "circadian disruption." The lack of coordination among rhythms is important, but so is the complete suppression of some rhythms. The term "external desynchronization" was coined to describe the situation (equivalent to social jet lag) in which the system is working, but is not optimally coordinated with the external day-night cycle, and the term "internal desynchronization" was coined to describe the situation in which circadian rhythms are present but have lost their mutual physiological relationships. The term "circadian disruption" was coined to describe the complete suppression of some rhythms; this is the condition most likely to result in carcinogenicity.

Dr. Lunn thanked Dr. Lévi for his comments and said that they would be considered when preparing the final document. Dr. Bucher mentioned the redundancies of circadian disruption and asked Dr. Lévi if he felt that the glucocorticoid and other effects detracted from the association between shift work or LAN and cancer. Dr. Lévi responded that he thought these effects supported that association.

Shift Work (Section 1.3) and Transmeridian Travel and Social Jet Lag (Section 1.4)

Dr. Massimo Bracci, first reviewer, found the characteristics of shift work to be well-summarized in Table 1-3 and the text to be clear and technically accurate; however, the monograph needs to provide an unambiguous definition of night shift work. The descriptions of exposure to shift work and of the characteristics of and exposure to transmeridian travel and social jet lag were clear and technically accurate. Dr. Bracci suggested several editorial revisions (provided in his written comments) to make Tables 1-3 and 1-4 clearer and easier to interpret.

Dr. Lunn noted that sometimes the definition of night shift work varies between references; however, she agreed that the definition of night shift work should be 12 AM to 5 AM (sometimes 6 AM was used) and noted that this definition would be used consistently through out the monograph. She agreed that some definitions were clearer in the profile relative to the document and said this was something that could be fixed.

V.A.1.3 Panel Discussion on Introduction and Exposure (Section 1)

Dr. Steven Hill noted that the statement in Section 1.1.1(p. 3) that ROR-alphas and RZR-betas are melatonin receptors was incorrect; although these receptors can be affected by melatonin, it does not bind to them. Dr. Hill noted that the reference from which that statement came from had been withdrawn, and provided information on a published erratum.

Dr. Marie-Élise Parent agreed with Dr. Bracci on the need for a consistent definition of night shift work. She suggested the Objectives and Scope section of the monograph should note that the premise for definition of night shift work as 12 AM to 5 AM was that it involves circadian disruption. Section 1.1.3 (p. 5) suggests that although some topics are mentioned in subsequent sections of the monograph, they should also be mentioned in Section 1 including chronotypes and their role in adaptation of workers to shift work, and Section 1.3.2 (p. 14)should mention lack of sleep and limited exposure to daylight as other potential exposures of night shift workers. In the summary (Section 1.5. p. 16), production and manufacturing should be added to the list of industries with the highest prevalence of night work.

Stanley Atwood asked Dr. Hill for clarification on the influence of melatonin on RORs. Dr. Hill said that although melatonin is not a ligand for these receptors, it can influence them through

multiple mechanisms, some involving phosphorylation and inactivation of kinases that regulate these receptors.

V.A.1.4 Peer-Review Comments and Panel Discussion on Studies of Circadian Disruption (Section 2)

Biomarkers and Characteristics of Circadian Disruption (Section 2.1)

Dr. Lévi, first reviewer, found the section to be clearly organized. In Section 2.1.1, he suggested adding a statement that "experimental studies show that melatonin can modulate expression of circadian oscillator genes in some central and peripheral tissues." In Section 2.1.2, a more balanced discussion is needed on mRNA expression of clock genes in leukocytes and mononuclear cells, as well as in total blood. Clock gene expression patterns have been assessed in various blood compartments and in a few tissues from healthy human volunteers, as well as in 64 tissues from a non-human primate and in postmortem human tissues. The potential for clock timing assessment based on transcriptome determination in a single tissue sample could also be mentioned, because it is an area of very active research; it may be possible in the near future to identify how the molecular clock is working in a given tissue, providing a useful biomarker.

Dr. Lunn asked Dr. Lévi to clarify his request for further discussion of clock gene expression. Dr. Lévi said that he meant clock gene expression observable in human tissues, in relation to biomarker testing. Dr. Parent asked for clarification of apparently contradictory statements about when melatonin production peaks (pp. 17 and 19). Dr. Lunn clarified that production begins to increase in early evening and reaches a peak in the middle of the night.

Light at Night and Circadian Disruption Biomarkers (Section 2.2)

Dr. Lévi, first reviewer, found the section to be clearly written and accurate. In Section 2.2.1 (p. 22), he suggested adding information on the determination of chronotype, which is classically assessed via a self-administered questionnaire that identifies morning types, evening types, and intermediate types, the last category accounting for the vast majority of people. Most commonly used are the Horne-Östberg and Munich questionnaires.

With respect to the introduction to Section 2.2.2 (p. 26), Dr. Lévi noted that experimental studies of circadian disruption in rodents have used both chronic jet lag protocols and constant light exposure, and that SCN ablation and clock-gene-mutant mice have been used as "host" models of circadian disruption despite exposure to light-dark synchronization. Many chronic jet lag or other circadian disruption experiments have used mouse strains with atypical melatonin patterns and low peak melatonin levels; despite the ability of these mice to adapt to shifts in schedule faster than melatonin-proficient mice, they show susceptibility to development or accelerated progression of cancer.

Dr. Hill, second reviewer, found the section to be well-written overall. He commented that using only melatonin levels as a biomarker was problematic, as individuals vary in their expression of melatonin receptors; in humans, these receptors are associated with breast cancer survival and response to therapy. He suggested that the melatonin receptors MT1 and MT2 be used as biomarkers in animal studies. Dr. Lunn asked whether any studies in humans exposed to LAN or shift workers had looked at these receptors; Dr. Hill said they had not and identified this as a research gap.

Dr. Parent wondered whether research had been done on individual sensitivities to LAN, specifically the need for a completely dark environment in order to sleep. She noted the importance of the statement in Section 2.2.2 (p. 27) that "…the totality of the daily light environment includes complementary exposures that contribute to circadian disruption…." In other words, all sources of exposure to daylight are important. She wondered whether it would be worth noting that this had not in fact been addressed in epidemiology studies; the lack of full information about light exposure could be important in interpretation of the findings. Dr. Lunn asked whether any studies had addressed total light exposure. Dr. Mariana Figueiro confirmed that few field studies have collected total light exposure; she agreed that the lack of this information was an important point.

Shift Work and Circadian Disruption Biomarkers (Section 2.3)

Dr. Bracci, first reviewer, found Section 2.3.1 to be well written, clear, and accurate. On page 31, line 5, he suggested that the meaning of the phrase "type of shift worker" be clarified, and on line 23, he suggested either deleting the words "which was most pronounced among men who worked the most nights in the railroad industry" or better explaining the comparison. Dr. Bracci had a number of minor corrections and editorial comments to Table 2-3 (and in some cases the corresponding text), which he would provide in writing.

Dr. Hill, second reviewer, referenced Dr. Lévi's comments on Section 2.2.2 and emphasized that in discussing studies of melatonin effects in animals, it is important to specify whether mouse strains were melatonin-deficient or -proficient; this information might be provided in a table. Dr. Bucher asked if this was lineage-specific and could be inferred; Dr. Hill said yes, and suggested including a table with information on different mouse strains and their melatonin production.

Dr. Fu, third reviewer, noted that some of the inconsistencies across the results of human studies resulted from their having only measured one or two biomarkers, rather than measuring uncoupling of the biomarkers. The uncoupling of diverse physiological processes (which indicates circadian disruption) was not really measured in the human epidemiological studies. Although the control subjects were not shift workers or were not exposed to LAN, other lifestyle factors could have activated a stress-response pathway, confounding the results. In her written comments, Dr. Fu provided additional references to animal studies, including more information on the effects of simulated shift work or jet lag on clock gene expression (Table 2-5).

V.A.1.5 Exposure of a Significant Number of U.S. Residents to Light at Night

The Panel unanimously concurred with the statement that a significant number of U.S. residents either (1) work or formerly worked night shifts or (2)are or were in the past exposed to light at night.

The meeting was recessed at 10:30 a.m. and reconvened at 10:45 a.m.

V.A.2 Human Breast Cancer Studies (Section 3)

V.A.2.1 Presentation on Human Breast Cancer Studies (Section 3)

Dr. Pamela Schwingl presented an overview of the key information on breast cancer studies in humans. It has been suggested that the high and rising incidence of breast cancer may be

associated with the increasing prevalence of a 24/7 culture. Because breast cancer survival is high, the cancer hazard evaluation included only studies of incidence, not mortality.

The cancer hazard evaluation of night shift work included 9 cohort studies and 12 case-control studies, 13 of which were considered to have moderate to high utility and 8 had low utility for informing the evaluation; most of the concerns of the low utility studies had to do with potential exposure misclassification (especially non-differential) or low sensitivity to detect a true effect. Of the 21 studies, 17 provided evidence of an association of night shift work and breast cancer risk. Excess risk of breast cancer among ever-nightshift workers was observed primarily in the high- and moderate-utility studies, while most of the low-utility studies reported relative risks near unity. Breast cancer risk was associated with long duration and high frequency of night shift work, either cumulative (a combination of duration and intensity) or by frequency type (nights or hours per week or month). In 4 studies, risk increased with a combination of high frequency and long duration of night shift work. Night shift work was associated with increased risk of receptor-positive breast cancer, and breast cancer risk was increased by long-duration, high-frequency night shift work at younger ages (i.e., among premenopausal women).

Strengths of the evidence included the large database of informative studies, the consistency of findings for persistent night shift work across occupations and populations, the unlikelihood of confounding by lifestyle factors, and the stronger association with receptor-positive cancer subtypes. Limitations included the low sensitivity of most of the cohort studies, the possibility of differential recall bias in the case-control studies, the lack of data on potential confounding by occupational co-exposures, and the finding of null results in 2 of the informative studies. NTP's preliminary level-of-evidence conclusion was that there is limited evidence that persistent night shift work causes breast cancer in humans. The evidence was considered strong but not sufficient. "Persistent" night shift work was defined as long-term, frequent, and starting in young adulthood.

V.A.2.2 Peer-Review Comments and Panel Discussion on Introduction and Overview of Breast Cancer Epidemiology (Section 3.1)

Dr. Eva Schernhammer, first reviewer, said she was very impressed with the work that had been done, which was extremely thorough and of high quality. She noted that information on trends in breast cancer incidence over time could be extended further back. She suggested adding discussion of whether circadian disruption acts as a tumor initiator or promoter and adding a brief discussion of studies in blind women. Dr. Schwingl agreed that the issue of promotion was interesting, and said that more information could be added to the report. Dr. Lunn noted that studies on melatonin and blind women are described in the mechanistic section of the report, and that they had tried to integrate the concept of promotion in the last sections of the document.

V.A.2.3 Peer-Review Comments and Panel Discussion on Night Shift Work (Section 3.2)

Dr. Florence Menegaux, first reviewer, agreed with Dr. Schernhammer about the quality of the draft monograph. She noted inconsistencies among Sections 3 and 7 and the substance profile in characterizing the utility of Vistisen *et al.* (2017), which she considered to be of low utility. She suggested that Table 3-3 should distinguish among types of questionnaires (self-administered or in-person or telephonic interview). The definition of night shift work should include discussion of permanent vs. rotating shift work. With respect to the statement that "the proportion of women

exposed to night work in these populations also varied considerably…" (p. 55), she said it was important to distinguish between population-based studies and studies of specific occupational groups.

Dr. Schernhammer, second reviewer, suggested that although no study had examined the effects of night shift work after a diagnosis of breast cancer, this topic should at least be mentioned. She cautioned against lumping together the Nurses' Health Studies, given the differences in their methods for assessing shift work. In Table 3-1, she suggested adding information on the average duration of follow-up of cohort studies and the numbers of breast cancer cases. She suggested adding in complete follow-up as a study-quality criterion. Dr. Schernhammer noted that 3 studies found about the same risk for *in situ* and invasive breast cancer. As left-truncation bias is less of an issue for cancer *in situ*, the similar findings for invasive cancer may suggest that left-truncation bias may not be as great as concern as we thought or at least provides some information to evaluate this type of bias. She suggested identifying which occupational co-exposures are known risk factors for breast cancer. She emphasized the importance of physical exercise as a potential confounding factor, along with smoking (possibly as an effect modifier). She did not fully agree with discarding body mass index (BMI) as a potential confounder simply because it is on the cancer causal pathway; for example, in one study, women starting night shift work had a higher BMI than women who did not go into night shift work.

Dr. Parent, third reviewer, suggested that the tables of cohort studies should include the numbers of new cases accrued during follow-up. She noted that Koppes *et al.* (2014) (p. 56, last line) did specify which hours were worked. She suggested that comparing women working 3 or more vs. 1 or more nights per month was not meaningful, because these open-ended exposure categories overlapped. She suggested changing "data on lifetime exposure were not available" (p. 58, line 4) to "data on exposure in jobs not in the textile industry were not available." She raised the question of how much weight to give studies lacking exposure information for other jobs. She suggested adding physical activity as a potential confounder (p. 59), noting that it is usually poorly measured.Dr. Parent suggested that night shift workers might have less opportunity than day shift workers for other types of exposure to LAN outside the sleep issues (such as night time activities). Dr. Schwingl agreed that complete information on exposure to LAN was not available.

Dr. Parent suggested citing a new case-control study which found that exposure to night shift work increased the risk of breast cancer; the reference was provided in her written comments. She suggested that the questions used to assess exposure (especially information on work schedules) generally were not reported explicitly enough. The process of collecting these metrics is error-prone, and not enough importance is given in the individual studies to capturing and coding the information correctly. She noted that frequency of exposure was defined as the cumulative number of night shifts (p. 76); however, "cumulative exposure" refers to frequency times duration. She suggested moving the information on cumulative exposure to the section on combined measures of duration and frequency. Her written comments provided additional editorial corrections and references.

Dr. Schwingl clarified that Vistisen *et al.* (2017) was judged to be of moderate utility. She confirmed the availability of the additional data requested by the peer reviewers; some of this information was provided in Appendix B to the monograph. Dr. Lunn said that ORoC had plans to possibly make all data downloaded in the literature review available in an Excel file, which

could be used for further analyses. Dr. Schwingl said that the concerns about questionnaire quality probably would result in non-differential misclassification of exposure. Dr. Lunn asked Dr. Parent whether the level of physical activity on the job would be likely to differ between day workers and night shift workers; Dr. Parent said she would be more concerned about variation at the population level.

Dr. Lévi asked about the importance of differences in how long after the initiation of night shift work the studies began following women. Dr. Schwingl noted that studies which had started assessing women's exposures relatively late (i.e., were subject to left truncation) were considered less informative. The latency period for breast cancer is expected to be perhaps 13 to 15 years, and cancer was more commonly observed in women with longer durations of night shift work. Dr. Lévi noted that night shift work was associated with ER and PR positivity, which are favorable prognostic factors, but also with HER2 positivity, which is an unfavorable prognostic factor. Dr. Lévi noted that co-exposure to environmental carcinogens, including hormones, could be considered not only as a potential confounding factor, but also as a potential synergistic factor with night shift work, because of increased sensitivity due to circadian disruption.

Dr. Lunn asked Dr. Christina Lawson whether NIOSH had plans to look at co-exposures and shift work; Dr. Lawson said that NIOSH was contemplating future research on gaps identified in the draft monograph. Dr. Parent commented that generally not enough care was taken in measuring the potentially confounding variables for which the studies purportedly adjusted.

V.A.2.4 Discussion of the Preliminary Level-of-Evidence Conclusion on Night Shift Work

Dr. Schernhammer moved to modify the preliminary level-of-evidence conclusion that there is limited evidence that persistent night shift work causes breast cancer in humans. She proposed that the conclusion be revised to: sufficient evidence that persistent night shift work causes breast cancer in humans. The motion was seconded by Dr. Lévi.

Dr. Parent said she was less inclined to find the evidence sufficient, because exposure to LAN from sources other than night shift work was not known. Dr. Hill asked for clarification as to whether "carcinogenicity" in the context of the level-of-evidence conclusion referred to initiation or promotion. Dr. Bucher said that the mechanism was not taken into consideration, and that carcinogenicity was defined as the "totality of cancer". Dr. Lunn clarified that the criterion was increased risk in humans, regardless of the importance of co-factors. Dr. Schernhammer said she did not think that exposure to other sources of LAN affected the results for night shift work, but she suggested that breast cancer and other sources of LAN be discussed before the Panel voted on the level-of-evidence conclusion for night shift work. Dr. Beane Freeman deferred the vote until after the discussion of breast cancer and LAN.

V.A.2.5 Presentation on Light at Night (Section 3.3) and Transmeridian Travel (Section 3.4)

Dr. Schwingl presented an overview of the key information on human breast cancer and environmental light at night (outdoor or indoor) and transmeridian travel.

Outdoor Light at Night. The evaluation included 5 studies that provided individual-level exposure and outcome data, which collectively showed small increases in breast cancer risk with high LAN exposure. Two studies found excess risk among premenopausal but not

postmenopausal women. NTP's preliminary level-of-evidence conclusion was that there is limited evidence that outdoor light at night causes breast cancer in humans. Consistently elevated risks at higher LAN levels were found in moderate- and high-utility studies; however, light measured in satellite-imagery studies may not be an appropriate surrogate for exposure to light that causes circadian disruption.

Indoor Light at Night. Ten studies of moderate and low utility assessed light in the sleeping area during sleep. Increased risk of breast cancer was found mainly for high self-reported exposure in the moderate-utility studies. NTP concluded that the data available from studies in humans are inadequate to evaluate the relationship between human breast cancer and exposure to indoor light at night. Limitations of the studies included inconsistent results across studies using the same exposure metric, wide variation in the exposure metrics used and uncertainty about their meaning, potential misclassification of self-reported exposure, and lack of specificity of the exposure metrics.

Transmeridian Travel. Female flight attendants showed evidence of increased breast cancer risk in 5low- or moderate-utility cohort studies. NTP's preliminary level-of-evidence conclusions were that the data available from studies in humans are inadequate to evaluate the relationship between breast cancer and transmeridian travel. The number of informative studies was small, exposure assessment was challenging, and co-exposures, such as exposure to cosmic radiation, were highly correlated with numbers of time zones crossed.

V.A.2.6 Peer-Review Comments and Panel Discussion on Light at Night (Section 3.3) and Transmeridian Travel (Section 3.4)

Dr. Menegaux, first reviewer, said the monograph did not fully address the question of "whether satellite imagery data is an appropriate surrogate for exposure to light that causes circadian disruption"(p. 89). Although positive associations were observed in 3 studies using such data, it was unclear how the reported exposure levels (in microwatts per cubic centimeter) related to the exposure levels in the experimental studies of circadian disruption discussed in Section 2 (measured as lux).

Dr. Schernhammer, second reviewer, noted that in the studies of outdoor LAN, the exposure of interest was actually indoor exposure to light coming from outdoors, the levels of which were unknown. Studies of outdoor light exposure were flawed by imprecise estimates of light levels at specific locations, the lack of information on the extent to which outdoor light could reach people indoors, and the lack of information on whether people had their eyes closed or open. Although the studies of indoor LAN might have somewhat more value, the results of those studies were inconsistent. Given that the mechanistic data did not support a strong direct effect of exposure to LAN, she suggested deemphasizing studies of outdoor and indoor LAN.
Dr. Schernhammer found the discussion of transmeridian travel to be clear and a good summary.

Dr. Figueiro clarified that there is no direct conversion from measurements of microwatts per cubic centimeter (irradiance) to lux (which characterizes the response of the visual system), because it would depend on the spectral power distribution of the light source. Dr. Lunn noted that in the evaluation, NTP struggled with some of the issues raised by Dr. Schernhammer, particularly whether the studies could be used as a proxy for human behavior, but did consider the epidemiological studies of LAN to support the mechanistic studies of LAN and breast cancer.

Dr. Schernhammer considered the relationship between breast cancer and exposure to outdoor LAN to be less clear than suggested by the draft monograph.

Dr. Parent agreed with Dr. Schernhammer's characterization of outdoor LAN exposure studies; she suggested adding under Key Issues (p. 98) that the actual exposure levels in studies of outdoor LAN were not known. In the breast cancer hazard assessment of environmental LAN, she emphasized the importance of considering the quality of the exposure assessments. In the preliminary level-of-evidence conclusion (p. 104), she suggested adding a statement that none of the studies evaluated overall LAN exposure from all potential sources that could disrupt circadian rhythms, and that the partial assessment of overall exposure could be problematic in evaluating breast cancer risk. Dr. Parent also noted that indoor exposure to LAN outside of the sleeping area was not considered; she wondered whether there was literature on this topic. Dr. Schwingl noted that with respect to LAN, there is no truly unexposed group, and that exposure will always be somewhat underestimated in the epidemiological studies, but that this would result in underestimation of the effects of LAN exposure. Dr. Lévi noted that the motivation for having LAN in the home was a potential confounding factor that had not been addressed.

V.A.2.7 Vote on the Preliminary Level-of-Evidence Conclusion on Night Shift Work

Returning to the motion on the table, the Panel agreed (4 yes, 3 no, 0 abstentions) that there is sufficient evidence that persistent night shift work causes breast cancer in humans, with "persistent" defined as long-term, frequent, and starting in young adulthood. Drs. Menegaux, Bracci, and Parent voted no. Dr. Menegaux voted no because she considered the evidence to be strong, but not sufficient, due to inconsistency in the definition of night shift work across studies and uncertainty due to the complexity of the exposure scenario. Dr. Bracci voted no because of the complexity of the exposure, the existence of several confounding factors, the heterogeneity of night shift work, and the lack of clarity across studies in the definition of night shift work; he considered the evidence to be strong, but not quite sufficient. Dr. Parent voted no because of uncertainty about other sources of exposure to LAN in the "unexposed" groups in studies of night shift workers and insufficient overall coverage of exposure that could lead to circadian disruption.

The meeting was recessed at 12:37 p.m. and reconvened at 1:10 p.m.

V.A.2.8 Vote on the Preliminary Level-of-Evidence Conclusion on Outdoor LAN

Dr. Schernhammer moved to modify the preliminary level-of-evidence conclusion that there is limited evidence that outdoor light at night causes breast cancer in humans. She proposed that the conclusion be revised to state that: data available from studies in humans are inadequate to evaluate the relationship between outdoor light at night and breast cancer. The motion was seconded by Dr. Parent. Dr. Schernhammer summarized the limitations of the studies, which (as discussed above, in Section V.A.2.6) resulted in uncertainties about the actual levels of exposure to outdoor LAN. Dr. Lévi noted that neither the exposure nor the response of the circadian system to the exposure were known. The Panel concurred that the findings of an increased risk of breast cancer among individuals with high exposure to LAN were based on a limited number of studies and substantial uncertainty about exposure and circadian response. The motion passed unanimously (7 yes, 0 no, 0 abstentions).

V.A.2.9 Vote on the Preliminary Level-of-Evidence Conclusion on Indoor LAN

Dr. Schernhammer moved to accept the preliminary level-of-evidence conclusion that data available from studies in humans are inadequate to evaluate the relationship between exposure to indoor light at night and breast cancer. Dr. Hill seconded the motion. Dr. Parent moved to amend the rationale for the preliminary level-of-evidence conclusion to: data available from studies in humans are inadequate to evaluate the relationship between human breast cancer and exposure to indoor light at night because of inconsistent findings across the studies and incomplete exposure assessment. Dr. Schernhammer seconded the motion, which passed unanimously (7 yes, 0 no, 0 abstentions).

V.A.2.10 Vote on the Preliminary Level-of-Evidence Conclusion on Transmeridian Travel

Dr. Parent moved to accept the preliminary level-of-evidence conclusion that data available from studies in humans are inadequate to evaluate the relationship between transmeridian travel exposure and breast cancer. Dr. Schernhammer seconded the motion. Dr. Bracci moved to amend the rationale for the preliminary level-of-evidence conclusion to: data available from studies in humans are inadequate to evaluate the relationship between human breast cancer and transmeridian travel because of the small number of informative studies, potential confounding, and inadequate exposure assessment. Dr. Hill seconded the motion, which passed unanimously (7 yes, 0 no, 0 abstentions).

V.A.3 Other Human Cancer Studies (Section 4)

V.A.3.1 Presentation on Prostate Cancer (Section 4.1)

Suril Mehta presented an overview of the key information on prostate cancer and night shift work. Because of the high survival rate, mortality studies were not included. The evaluation included 10 cohort or case-control studies, 5 of which were judged to be of high to moderate utility. Evidence for an association of night shift work with prostate cancer was seen in 7 studies. Of the high- to moderate-utility studies, 4 of 5 found an association with ever having worked night shifts, and all 5 found an association with longer duration of shift work. NTP's preliminary level-of-evidence conclusion was that there is limited evidence that persistent night shift work causes prostate cancer in humans. Strengths of the evidence included consistency of the findings across studies and increased risk with long duration of night shift work. A few studies reported higher risks for more aggressive types of prostate cancer. Limitations included the small number of informative studies, variation in the exposure metrics assessed, and potential misclassification of shift work status in the lower-quality studies, which may have biased the findings towards the null.

V.A.3.2 Peer-Review Comments and Panel Discussion on Prostate Cancer (Section 4.1)

Dr. Parent, first reviewer, suggested that the issue of prostate cancer severity should receive more attention and be discussed in the introduction to the section. The reason for differences in the aggressiveness of prostate cancers is unknown, but recent evidence suggests that low- and high-grade prostate cancer are different entities and may have different etiologies and risk-factor profiles. It should be noted that Wendeu-Foyet *et al.* (2018)(p. 112) also saw an increased risk associated with the longest duration of exposure among subjects with aggressive cancers. There

is consistent evidence that BMI is associated with aggressive prostate cancer; it may be a confounding factor. More attention should be paid to screening practices, as differences in practices among workplaces could be a source of bias. Occupational exposures may not be important potential confounders.

Dr. Schernhammer, second reviewer, said the review was very well done. With respect to the issue of tumor aggressiveness, she suggested that it could be important to consider the mortality studies. She agreed with the definition of "persistent" exposure as considering frequency and duration of night shift work. Dr. Menegaux, third reviewer, said the section was very well done. She agreed with Dr. Parent's point about different types of prostate cancer based on aggressiveness.

Mr. Mehta clarified that mortality studies were excluded not *per se*, but because of limitations such as inadequate exposure assessment or poorly characterized shift work. Dr. Hill asked whether any of the studies had included analyses of diabetes, given its link with metabolic syndrome and melatonin. Dr. Parent agreed that this was a valid point, but also mentioned the inverse relationship between type II diabetes and prostate cancer. Dr. Lévi asked whether other concurrent diseases and medications had been looked at. Mr. Mehta did not think that diabetes or other diseases had been considered in the prostate cancer studies. Dr. Lunn suggested that such information might be available from studies that looked at multiple outcomes. Dr. Menegaux mentioned going back and looking at case-control studies, and noted nonsteroidal anti-inflammatory drugs as being potentially associated with prostate cancer.

V.A.3.3 Vote on the Preliminary Level-of-Evidence Conclusion for Prostate Cancer

Dr. Schernhammer motioned to accept the preliminary level-of-evidence conclusion that there is limited evidence from studies in humans that persistent night shift work causes prostate cancer. Dr. Parent requested an amendment, and Dr. Schernhammer withdrew her motion. Dr. Parent moved to revise the rationale for the preliminary level-of-evidence conclusion to state that the evidence was limited by the small database of useful studies, poor characterization of night shift work exposure across studies, and the fact that few studies looked at disease aggressiveness or the role of screening. Dr. Schernhammer seconded the motion, which passed unanimously (7 yes, 0 no, 0 abstentions).

V.A.3.4 Presentation on Colorectal, Hormonal, and Lung Cancer, Other Types of Cancer, and Exposures Other Than Night Shift Work (Sections 4.2 through 4.6)

Mr. Mehta presented an overview of the key information on other types of human cancer and exposures other than night shift work.

Colorectal Cancer. The evaluation of colorectal cancer and night shift work included 5 cohort or case-control studies, 4 of which showed evidence of increased colorectal cancer risk. NTP's preliminary level-of-evidence conclusion was that the data available from studies in humans are inadequate to evaluate the relationship between night shift work and colorectal cancer. Although higher-quality studies showed an increased risk, the results were inconsistent for long-duration exposures, and the evidence was limited by the small number of informative studies, potential confounding bias, and potential misclassification of shift work status, which may have biased the findings towards the null.

Hormonal Cancers. The database was inadequate to evaluate night shift work and female hormonal cancers. Increased risks were seen for ovarian cancer in 2 studies and endometrial cancer in 1 study, though not consistently with longer duration of exposure. The evidence was limited by the small number of studies, poor characterization of night shift work, and low to moderate study sensitivity.

Lung Cancer. The database was inadequate to evaluate night shift work and lung cancer. The results were inconsistent for ever having worked a night shift and for duration of exposure. The evidence was limited by the small number of studies, which were of moderate or low utility; potential confounding by smoking; a possible healthy worker survivor effect; and variable characterization of night shift work.

Other Types of Cancer. Additional types of cancer were not included in the evaluation of night shift work because the study databases were inadequate. Elevated risks were reported in studies of skin tumors, leukemia/lymphoma, and cancer of the stomach and pancreas.

Exposures Other Than Night Shift Work. Exposures other than night shift work were not included in the evaluation because the study databases were inadequate. One study found an increased risk of prostate cancer with exposure to indoor and outdoor blue LAN, and one study found an increased incidence of multiple cancers in airline crew members.

V.A.3.5 Peer-Review Comments and Panel Discussion on Colorectal, Hormonal, and Lung Cancer, Other Types of Cancer, and Exposures Other Than Night Shift Work (Sections 4.2 through 4.6)

Dr. Menegaux, first reviewer, questioned the statement that gender did not affect the risk of colorectal cancer (p. 124). Although only 1 of the 5 informative studies looked at both men and women, it found an interaction by gender for ever- vs. never-night shift work, though not for duration. Dr. Parent, second reviewer, said the evaluation was very well done, despite the limited data.

V.A.3.6 Votes on the Preliminary Level-of-Evidence Conclusions for Colorectal Cancer, Hormonal Cancers, and Lung Cancer

Dr. Parent moved that the Panel accept NTP's preliminary level-of-evidence conclusion that the data available from studies in humans are inadequate to evaluate the relationship between persistent night shift work and colorectal cancer, because of potential exposure misclassification, the small database of informative studies, and potential confounding. Dr. Schernhammer seconded the motion, which passed unanimously (7 yes, 0 no, 0 abstentions).

Dr. Schernhammer moved that the Panel accept NTP's preliminary level-of-evidence conclusion that the data available from studies in humans are inadequate to evaluate the relationship between persistent night shift work and female hormonal cancers, because of potential exposure misclassification and the small database of informative studies. Dr. Parent seconded the motion, which passed unanimously (7 yes, 0 no, 0 abstentions).

Dr. Parent moved that the Panel accept NTP's preliminary level-of-evidence conclusion that the data available from studies in humans are inadequate to evaluate the relationship between persistent night shift work and lung cancer, because of potential exposure misclassification, the small database of informative studies, and potential confounding. Dr. Schernhammer seconded the motion, which passed unanimously (7 yes, 0 no, 0 abstentions).

V.A.4 Cancer Studies in Experimental Animals (Section 5)

V.A.4.1 Presentation on Cancer Studies in Experimental Animals (Section 5)

Dr. Gloria Jahnke presented an overview of the key information on cancer studies in experimental animals. She noted that the rodent models of simulated shift work, chronic jet lag, and LAN exposure were intended primarily to provide mechanistic evidence. Because studies were not classic cancer studies and cancer incidences were not usually reported, NTP drew no level-of-evidence conclusions; however, the database was adequate for evaluating effects on tumor growth.

Compared to animals on a 12-hour light and 12-hour dark schedule, simulated shift work or chronic jet lag increased tumor multiplicity, tumor burden, or decreased latency in initiation-promotion studies, and increased tumor growth, tumor multiplicity, and metastasis of tumor implants or injected tumor cells. The tissue sites included liver, mammary gland, lung, pancreas, bone, liver, and plasma cells. Simulated shift work decreased the latency of spontaneous mammary gland tumors in a genetic model and chronic jet lag increased the incidence of spontaneous liver tumors. Exposure in various LAN models (e.g., constant bright light, dim or intermittent LAN) in rats and mice decreased the latency and increased the incidence of mammary-gland tumors with co-exposure to tumor initiators, increased the growth of breast xenografts compared to rodents receiving 12hours of light and 12hour of dark. Exposure to constant light increased the multiplicity of spontaneous mammary-gland tumors in a transgenic model. Over 25 studies found increased growth of many other types of tumors in LAN models. NTP found compelling evidence that animal models of simulated shift work or LAN exposure showed enhanced tumor growth and decreased tumor latency.

V.A.4.2 Peer-Review Comments and Panel Discussion on Cancer Studies in Experimental Animals (Section 5)

Dr. Fu, first reviewer, stated that the section was comprehensive and included most of the available data. She noted that the introduction to the section focused on studies of melatonin in animal models. She emphasized the differences between rodents and humans in the role of melatonin and suggested that the introduction should mention studies of other clock output pathways in tumorigenesis. Evidence is accumulating to show that other pathways of circadian deregulation are involved in human cancer risk, including the sympathetic nervous system (SNS). There is very strong evidence that in humans, night shiftwork causes SNS dysfunction that is directly linked to sleep disruption, metabolic syndrome, immune suppression, and oncogenesis. Melatonin secretion in vertebrates is regulated by the SNS, via beta-1 adrenergic signaling, and melatonin deregulation is secondary to SNS circadian output pathway deregulation. In the summary (Section 5.3, last paragraph), Dr. Fu suggested that discussion be added of whether circadian disruption was actually observed in the studies of spontaneous tumors in mice. If the simulated shift work did not induce peripheral clock disruption, then it would not be expected to affect tumor incidence.

Dr. Hill, second reviewer, commented that chronic and intermittent LAN are very different exposures; it is important to know their effects on the melatonin profile. Interpretation of these studies also depends on whether they used melatonin-proficient or -deficient mouse strains. Bright and dim LAN also are very different exposures; bright light should disrupt the central clock, but there is evidence that dim light did not (based on the absence of changes in water

intake or feeding behavior), though it did suppress nighttime melatonin production. He noted that the mechanisms of tumor initiation by DMBA and NMU are very different; DMBA induces a prolactin-responsive tumor, whereas NMU induces a *Ras* mutation. He suggested adding some discussion of these issues. Dr. Hill agreed that the animal studies provided strong evidence that LAN, simulated shift work, and simulated chronic jet lag could cause circadian disruption. However, he agreed with Dr. Fu that the studies focused heavily on melatonin and that more information was needed on circadian disruption; he said he would provide additional references.

Dr. Bracci said that the second sentence of the summary (Section 5.3, p. 152), concerning exposure of shift workers to constant dim light during daylight hours, was unclear. Dr. Lévi questioned the relevance of rodent models of shift work to human exposure. Dr. Hill noted that while the pattern of melatonin production is very similar in rodents and humans, their metabolism is very different. Dr. Lévi emphasized that night shift work is a complex exposure involving other factors besides LAN that cannot be captured in rodent models. He considered the simulated shift work models to be simply versions of chronic jet lag models.

The meeting was recessed at 2:35 p.m. and reconvened at 2:42 p.m.

V.A.5 Mechanistic and Other Relevant Data (Section 6)

V.A.5.1 Presentation on Mechanistic and Other Relevant Data (Section 6)

Stanley Atwood presented an overview of the key information on mechanistic and other relevant data. The melatonin hypothesis proposed that exposure to LAN decreased nighttime melatonin production, leading to increased circulating estrogen levels, increased turnover of epithelial stem cells, and increased breast cancer risk. Studies in shift workers provided some evidence for increased breast cancer risk with lower melatonin levels. Cancer studies of rats exposed to LAN found an exposure-related decrease in endogenous melatonin levels; co-exposure of exogenous melatonin or perfusion of breast xenografts with nocturnal melatonin from blood from women without exposure to LAN suppressed LAN-promoted breast tumor growth. In contrast, perfusion of breast xenografts with melatonin-depleted blood from women exposed to LAN had no effect on LAN-induced tumors. In addition to its anti-estrogenic effects, melatonin has been shown to suppress tumors via a number of other pathways, most of which are mediated by the melatonin receptor.

In addition, LAN or night shift work has been shown to result in deregulation of the core clock genes, which control the expression of perhaps more than half of the genome. Desynchronization of the central clock, SNS, and peripheral clock axis resulted in disruption of cell signaling, loss of cell cycle control, and altered metabolism, leading to increased cell proliferation, decreased apoptosis, and decreased tumor suppression and DNA repair. Mice with clock gene mutations showed increased tumor incidence and accelerated tumor growth.

Night shift workers and rodents exposed to LAN or simulated jet lag or shift work exhibited a number of biological changes characteristic of those caused by recognized carcinogens, including epigenetic alterations, decreased DNA repair, increased genomic instability, oxidative damage, and inflammation, or immune suppression, and altered cellular metabolism. The relative contributions of other factors to the carcinogenicity of night shift work exposure — vitamin D deficiency resulting from insufficient exposure to sunlight, sleep disruption, and altered meal timing — are uncertain; plausible mechanisms have been identified, but more studies are needed.

Dr. Schernhammer questioned whether melatonin levels had been assessed in night shift workers, as these workers are usually excluded from melatonin studies. Mr. Atwood said he and Dr. Schwingl would confirm. Dr. Lévi said it was important to distinguish between effects of melatonin observed at physiological vs. pharmacological levels. Mr. Atwood said some of the studies he discussed included exposure to physiological levels of melatonin.

V.A.5.2 Peer-Review Comments and Panel Discussion on Mechanistic and Other Relevant Data (Section 6)

General Comments and Overview of Breast Cancer Carcinogenicity (Section 6.1)

Dr. Hill, first reviewer, said the section was generally well written, but that the peer-review discussions had raised some issues that should be included. He agreed with Dr. Lévi's point that rodents are not exposed to "shift work," but rather to LAN and not other components of shift work. Dr. Schernhammer generally agreed with Drs. Hill and Lévi about characterizing rodent models as involving exposure to LAN, rather than as simulated shift work. However, she noted exposure to LAN does not capture the real issue, which is the change in lighting schedule. Mr. Atwood noted that some rodent studies of simulated shift work did employ forced activities and feeding schedules. Dr. Lunn agreed that rodent models of simulated shift work (as used in the literature) are not the same as shift work in humans and the definition of the model does not affect the overall conclusions. Drs. Schernhammer and Hill suggested the animal model could be referred to as "shift work simulated" or "shift work proxy." Dr. Lunn noted that the term simulated shift work model was used in the monograph.

Dr. Hill noted that the rat and mouse xenograft models differed in that the tumors were hypoxic in mice (as with most human breast tumors) but not in rats, which has implications for the mechanism involving the peripheral clock. He suggested adding some discussion of the role of post-translational modification of proteins and kinases in the circadian system, in particular as related to melatonin and to the role of glycogen synthase kinase 3 beta in regulating the molecular clock and downstream processes.

Dr. Lévi cautioned against using the term "biological effects" as shorthand for characteristic effects caused by carcinogens, as circadian disruption is itself a biological effect. He also emphasized the need to recognize that melatonin not only is produced in response to darkness, but also has its own endogenous rhythm. Dr. Hill suggested emphasizing that the discussion of mechanistic data specifically addressed pineal melatonin production, not melatonin from other sources.

Proposed Mechanisms (Section 6.2)

Dr. Hillsaid the section 6.2.1 (Melatonin Hypothesis) was well written. He suggested replacing the reference to Cos *et al.* (1998)on inhibition of the invasiveness of MCF-7 breast cancer cells with a stronger reference (provided in his written comments), as MCF-7 cells are poorly invasive. Dr. Bracci, second reviewer, found the section to be clear and accurate. He emphasized the importance of the concept of total light exposure; he said it should be introduced earlier in the monograph and that its definition should be made consistent throughout. He said the evidence was well-summarized and synthesized, and that Table 6-1 was effective.

Dr. Bracci, found the section, Section 6.2.2 (Circadian Disruption Theory) to be clear. He suggested that Figure 6-2 be revised to take into consideration Figure 5 of Takahashi *et al.* (2008), and noted that the first paragraph on page 168, lines 5 and 6, should refer to five clock genes, including *PER3*. He suggested consistency throughout the monograph in the order in which LAN and night shift work were mentioned.

Dr. Fu suggested that the introduction to this section should include more evidence from human studies, especially concerning the role of the SNS. She said it was important to mention that circadian disruption in humans does not depend on gene mutation; it can suppress circadian gene expression by causing neuroendocrine dysfunction. She commented that Section 6 was too focused on the melatonin hypothesis and suggested that it be reorganized around circadian neuroendocrine dysfunction, of which changes in melatonin are a consequence. Dr. Fu provided extensive written comments illustrating the types of revisions she suggested, with additional references. She noted that Figure 6-2 was actually a proposed model of cell growth regulation by the molecular clock, not the circadian clock; a model of the circadian clock would start with light entering the eye.

Dr. Lévi emphasized that circadian disruption is not an extension of the melatonin hypothesis; rather, it incorporates the melatonin hypothesis. He suggested that the introduction to this section describe the levels at which the carcinogenic process is affected by circadian disruption, from initiation to promotion to progression. Rather than saying that little is known about the mechanistic link from shift work to LAN to circadian disruption to cancer, he suggested emphasizing that the carcinogenic effects of circadian disruption were consistently demonstrated in different models involving chronic jet lag or clock gene mutation, via upregulation of *c-Myc* and downregulation of p53. He agreed with Dr. Hill's comment about the use of MCF-7 breast cancer cells, as they do not have a well-established molecular clock. He suggested emphasizing the coupling of the molecular clock with the cell-division cycle at the single-cell level in normal cells; this is an important mechanism. He suggested possibly adding a discussion of the importance of circadian disruption as a negative prognostic factor for human cancer patients, as this relates to the effect of circadian disruption on tumor progression.

In response to Dr. Bucher's question on the need for reorganization of the proposed mechanisms, Dr. Hill emphasized the need to move from causes to outputs, for example, from light regulating the central clock to regulation of its outputs. Dr. Lévi agreed with the concept of organizing the section around the organization of the circadian timing system, moving from light reception to the peripheral clocks. He emphasized that as most cancers occur in peripheral tissues, the role of peripheral clocks is critical, and that melatonin does not play a major role at that level. Dr. Bracci suggested that the section's summary should follow the same organization as its introduction. Dr. Bucher suggested that this could be accomplished by revising the introductory paragraph.

LAN and Shift Work Studies: Biological Effects Related to Cancer (Section 6.3) and Other Mechanisms Associated with LAN and Shift Work (Section 6.4)

Dr. Bracci, first reviewer, said that in the first paragraph of Section 6.3.1, "all DNA repair pathways" should be changed to "several DNA repair pathways" and that the reference to Vaskova *et al.* (2011) should be replaced with the references provided in his written comments. In the last sentence of the first paragraph on page 169, the identity of the controls should be specified. In the reference to work by Bhatti *et al.* (2016, 2017), the difference in urinary

clearance of 8-OH-dG between night shift and day shift workers was not statistically significant; the mention of day shift workers should be removed. In the last sentence of the same paragraph, "is frequently disrupted" should be changed to "could frequently be disrupted." In the second-to-last sentence of the first paragraph on page 181, the circadian mutant mouse models referenced should be better specified. In the second-to-last line on page 181, the reference to Papantoniou *et al.* (2015c) was incorrect. Dr. Bracci suggested additional editorial changes in his written comments and said he would provide written corrections to Table 6-2.

V.A.6 Evidence Integration and Preliminary Listing Recommendations (Section 7)

Dr. Lunn described NTP's integration of evidence from Sections 1 through 6 of the draft monograph to reach preliminary listing recommendations for night shift work and light at night.

V.A.6.1 Presentation on Evidence Integration and the Preliminary Listing Recommendation for Night Shift Work (Section 7.2)

Dr. Lunn summarized NTP's conclusions from the evaluation of night shift work. Conclusions were reached by integrating the data from (1) studies in humans and experimental animals on night shift work and biomarkers of circadian disruption (Section 2), cancer (Sections 3 to 5) and key characteristics of cancer (Section 6) as well as (2) studies in humans, animals and cells on circadian disruption and key characteristics of carcinogens and cancer (Section 6). These data were presented in a series of evidence-based tables and a figure. NTP concluded that there is sufficient evidence for the carcinogenicity of persistent night shift work that causes circadian disruption from studies in humans, based on the collective body of evidence from cancer epidemiological studies and mechanistic studies in humans and experimental animals. Specifically, human epidemiological studies showed that persistent night shift work increases the risk of female breast cancer; animal and *in vitro* mechanistic studies provided evidence that circadian disruption plays a role in carcinogenicity; and human mechanistic studies provided evidence that night shift work causes circadian disruption and biological carcinogenic effects similar to those observed in animal cancer models. There is limited evidence that night shift work increases the risk of prostate cancer.

"Persistent night shift work" is defined as frequent and long-term night shift work, especially beginning at an early age. In general, female night shift workers found to be at increased risk for breast cancer were those who started working before age 30 and worked night shifts at least 3 times a week and for 10 or more years; however, the exact conditions that put an individual at increased risk may depend on the specific combination of these metrics or other factors. "Night shift work" is defined as working at least 3 hours between midnight and 5:00 a.m., and is a complex exposure condition that includes exposure to LAN, disrupted sleep, altered meal timing, and other behavioral changes.

Peer-Review Comments on Evidence Integration and the Preliminary Listing Recommendation for Night Shift Work (Section 7.2)

Dr. Schernhammer asked for clarification of the definition of "frequent and long-term night shift work"; both she and Dr. Menegaux questioned the use of a specific criterion, such as at least3 times per week for at least 10 years. Dr. Schernhammer thought that 3 times a week was rather high. Dr. Lunn noted the difficulty of providing a definition of frequency and duration, given that the conditions resulting in increased risk may vary. She stated it was based on the pooled case-

control study and the purpose was to contextualize what long-term and frequent night shift work mean to the public; however, the exact conditions are not part of the conclusions. Dr. Menegaux agreed with it was important to provide context for communication purposes.

Dr. Parent commented that the evidence on recency of exposure and on progression seemed to be overlooked. Dr. Bracci suggested that in the last paragraph on page 189, the concept of "the exact conditions" of night shift should be explained with the same wording as in the second bullet point of Section 7.4 (p. 196). Dr. Lévi stated he enjoyed the section and suggested revisions to Figure 7-1, including clarifying that circadian disruption is a biological effect. He stated that circadian disruption involves not only the molecular clock, but also alteration of the signals that coordinate circadian clock genes in peripheral tissues and rhythms of cortisol, the SNS, and body temperature. The figure should also show the interconnection between circadian disruption and melatonin.

V.A.6.2 Vote on the Preliminary Listing Recommendation for Night Shift Work

Dr. Hill moved that the Panel accept NTP's preliminary listing recommendation that persistent night shift work that causes circadian disruption should be listed in the Report on Carcinogens as *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans. Dr. Schernhammer seconded the motion. Dr. Bucher explained that the vote should be based solely on the evidence for carcinogenicity, not on the consequences of the listing, as the Report on Carcinogens is not a regulatory document. The motion was approved (6 yes, 0 no, 1 abstention). Dr. Menegaux abstained because she did not consider the evidence from human studies to be sufficient evidence of a direct, causal relationship. The panel briefly discussed amending NTP's rationale for the listing. Dr. Hill moved that the Panel accept NTP's explanation for the listing as is, and Dr. Lévi seconded the motion, which passed (6 yes, 0 no, 1 abstention). Dr. Menegaux abstained because she had abstained from the vote to approve the preliminary listing recommendation.

V.A.6.3 Presentation on Evidence Integration and the Preliminary Listing Recommendation for Light at Night (Section 7.3)

Dr. Lunn summarized NTP's conclusions from the evaluation of exposure to LAN, which were reached using a similar approach as night shift work. NTP concluded that there is strong evidence that LAN acts through mechanisms that are likely to cause cancer in humans, based on toxicological and mechanistic data indicating that exposure to LAN causes melatonin suppression and other types of circadian disruption, which lead to the proliferation and growth of breast or mammary-gland cancer in experimental animals. There is also strong evidence that LAN causes biological effects characteristic of those caused by recognized carcinogens. There is limited evidence that LAN increases the risk of breast cancer in humans, based on the results of epidemiological studies and observations that it causes melatonin suppression.

The preliminary listing recommendation is for exposure to "certain lighting conditions that cause circadian disruption," which are defined as (1) exposure to excessive LAN having the characteristics most likely to cause circadian disruption, including shorter wavelength, longer duration, exposure to electric light during the biological night, and higher intensity or levels, and (2) insufficient daylight exposure, based on experimental animal studies showing that blue light exposure during the day positively affected circadian regulation and decreased tumor growth,

and on evidence that nighttime sensitivity of humans to LAN was influenced by exposure to light during the day.

Peer-Review Comments on Evidence Integration and the Preliminary Listing Recommendation for Light at Night (Section 7.3)

Dr. Schernhammer noted that animal models do not mimic human exposure to outdoor LAN but may be more relevant to exposure to indoor light. Dr. Lunn clarified that if outdoor light exposure conditions did not meet the criteria for causing circadian disruption, then these conditions would not be considered carcinogenic.

Dr. Parent questioned whether the role of insufficient daylight exposure in carcinogenicity is understood. Dr. Hill noted that exposure to blue light in humans during the day can reset the circadian clock. His lab has done experiments in melatonin-proficient rodents that show that daytime exposure to blue-enriched light increased nighttime melatonin levels 5- to 6-fold over those following daytime exposure to standard cool fluorescent light. Not being exposed to blue light during the day decreased nighttime melatonin levels. Tumor growth suppression was also greater in animals exposed to daytime blue light followed by LAN than in animals exposed to standard fluorescent light followed by LAN. Dr. Parent asked whether insufficient exposure to daylight referred to light from natural or artificial sources; Dr. Lunn said it could be either.

Dr. Schernhammer asked whether the evidence from animals was based on simulated shift work models. Dr. Lunn clarified that for the LAN evaluation, the simulated shift work models were not used as they were evaluating the LAN animal models that were discussed by Dr. Jahnke. Dr. Schernhammer asked for clarification between the LAN and simulated shift work models. Dr. Hill provided some examples of LAN models, e.g., dim light and bursts of light did not simulate the conditions of night shift work. Dr. Schernhammer suggested modifying the language of NTP's preliminary listing recommendations by replacing "LAN" with "certain lighting conditions" throughout, to make it clear that the listing was not based on exposure to outdoor LAN that did not cause circadian disruption and to avoid the question of the differences in physiological night between humans and rodents.

Vote on the Preliminary Listing Recommendation for Light at Night

Dr. Schernhammer moved to modify the preliminary listing recommendation to: certain lighting conditions that cause circadian disruption should be listed in the Report on Carcinogens as *reasonably anticipated to be a human carcinogen* based on strong evidence that certain lighting conditions act through mechanisms that are likely to cause cancer in humans. Dr. Bracci seconded the motion, which was approved (6 yes, 1 no, 0 abstentions). Dr. Lévi voted no, because he questioned the relevance of rodent models to mechanisms in humans, because of the lack of evidence from epidemiological studies, and because of uncertainties about the exposure required to produce chronic effects such as cancer.

The meeting was recessed at 5:17 p.m. and reconvened at 5:22 p.m.

V.B. Draft RoC Substance Profiles

Dr. Lunn summarized the purpose and contents of the draft substance profiles. Due to time constraints, the panel did not discuss the draft substance profiles.

VI. Closing Remarks on the Draft RoC Monograph

Dr. Bucher thanked the Panel members for their valuable comments. Dr. Beane Freeman thanked the ORoC and contractor staff for their excellent job in summarizing the literature. Dr. Hill reiterated how impressed he was with the draft monograph, which was comprehensive and well done. He said he considered it to be an important document that would help move the field forward and alert people to real health issues. Dr. Lévi said he was impressed with how the draft monograph captured the critical issues related to this important problem; the monograph was generally well organized and provided a wealth of information on an unusual and complex topic. He said the information was presented clearly and accurately. Dr. Menegaux agreed.
Dr. Schernhammer praised the tremendous amount of brilliant work that went into the draft monograph, which provided a service to the research community and the public and would have future implications. Dr. Bracci agreed that the draft monograph was a good synthesis of the body of knowledge and would have a large impact.

The meeting was adjourned at 5:30 p.m.

VII. Approval of the Peer Review Report by the Chair of the Peer Review Panel

This peer review report has been read and approved by the chair of the October 5, 2018, NTP Report on Carcinogens Monograph Peer Review Panel.

Laura Beane Freeman, Ph.D.

Peer Review Panel Chair

Date: 11/27/2018