

Working Time Society consensus statements: Circadian time structure impacts vulnerability to xenobiotics—relevance to industrial toxicology and nonstandard work schedules

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Abstract: The circadian time structure (CTS) has long been the subject of research in occupational medicine, but not to industrial toxicology, including methods of setting threshold limit values (TLVs) and employee biological monitoring. Numerous animal and human investigations document vulnerability to chemical, contagion, and other xenobiotics varies according to the circadian time of encounter. Permanent and rotating nightshift personnel are exposed to industrial contaminants in the same or higher concentration as dayshift personnel, and because of incomplete CTS adjustment to night work, contact with contaminants occurs during a different biological time than day workers. Thus, the amount of protection afforded by certain TLVs, especially for employees of high-risk settings who work night and other nonstandard shift schedules, might be inadequate. The CTS seems additionally germane to procedures of employee biological monitoring in that high-amplitude 24 h rhythms in biomarkers indicative of xenobiotic exposure may result in misjudgment of health risks when data are not gathered in sufficient frequency over time and properly interpreted. Biological reference values time-qualified for their rhythmic variation, currently of interest to laboratory medicine practice, are seemingly important to industrial medicine as circadian time and work-shift specific biological exposure indices to improve surveillance of personnel, particularly those working nonstandard shift schedules.

Key words: Circadian rhythms, Chronotoxicology, Threshold limit values, Biological monitoring, Biological exposure limits, Rotating shift work, Permanent night work, Nonstandard work schedules

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Consensus Statements

We propose three key consensus statements, founded on new applications of circadian rhythm research, that are of potentially high relevance to the health and wellbeing of permanent night and rotating shift workers. Recommendations that follow each key statement derive from numerous around-the-clock laboratory animal and human investigations that infer shift-dependent differences in vulnerability to workplace xenobiotics and also previously trialed innovative comprehensive methods of employee biological monitoring that entail serial waking-time self-assessments coupled with improved data interpretation. Since these are novel considerations in occupational medicine, some recommendations are research oriented.

- 1) Permanent and rotating nightshift personnel are subjected to industrial contaminants in the same or higher concentrations as dayshift personnel. Numerous (more than 125) laboratory animal and human research investigations demonstrate that the adverse effects of chemical, contagion, physical, and other xenobiotic exposures can differ extensively according to the time of the encounter. Threshold limit values are often established on the basis of homeostatic theory, i.e., the unsubstantiated assumption of constancy of biological processes and functions, and databases of acute adverse events that lack qualification for biological time or work shift of occurrence. Adjustment of the circadian time structure of normally diurnally active humans when suddenly altered by night work is slow and often incomplete. Thus, exposure of permanent night and rotating shift workers to industrial contaminants is likely to occur during a different circadian time compared to dayshift workers. Therefore, the extent of protection afforded by some threshold limit values, particularly for employees of high-risk industrial and medical settings, might be inappropriate when working nights or other nonstandard schedules. Thus, we recommend:
 - a. Workplace xenobiotic and other biological stressors be researched for circadian differences in acute and chronic adverse effects, with findings utilized to create time-weighted average, short-term, and ceiling threshold limit values optimally protective for both day and night workers.
 - b. Databases inventory acute adverse health events according to hours of work on the given shift as well as clock hour, more preferably circadian time of the affected employee, to ensure protective threshold limit values, not only for day but permanent night and other nonstandard shift work personnel.
 - c. Novel approaches entailing transcriptomics and metabolomics, which are currently used in biological rhythm research to determine circadian time through single time-of-day sampling of blood, breath, and hair follicle or tissue, be researched for applications to industrial toxicology to identify the circadian time of acute adverse reaction to workplace xenobiotic and other biological stressors.
- 2) Human beings by nature are a diurnal species. Under ordinary conditions of life, the circadian time structure is organized to support a routine of activity during the natural light of day and sleep during the natural darkness of night. Nonstandard permanent and rotating night work schedules require the circadian time structure be reorganized to the unnatural routine of nocturnal activity and diurnal sleep. Biological adjustment to such major alteration of the normal activity/sleep pattern is neither instantaneous nor harmonious and results in a transient state of circadian disruption, i.e., mismatch between the staging of 24 h rhythms of bodily processes and functions and cyclic environmental demands and challenges and/or alteration of the period of circadian rhythms from 24.0 h. Research involving non-human models infers circadian disruption, itself, can increase the vulnerability to chemical and other xenobiotic stressors. Additionally, animal and human experiments suggest circadian disruption can be a potential adverse effect of xenobiotics. Thus, we recommend:
 - a. Research be initiated to determine if the circadian disruption caused by working nonstandard permanent and rotating night schedules poses elevated risk for acute adverse reactions to workroom xenobiotic and hazards and, accordingly, whether it needs to be an additional criterion of consideration for establishing or adjusting short-term, time-weighted, and ceiling threshold limit values.
 - b. Research be initiated to determine whether disturbance of the circadian time structure—alteration from

normal of the period, staging, and/or amplitude of individual circadian rhythms or the overall circadian time structure—can be an acute and/or chronic adverse effect of workplace exposures to xenobiotics and other biological stressors and thus an additional criterion of consideration in establishing threshold limit values.

- 3) Current methods for the conduct of biological monitoring and interpretation of derived data against daytime-derived reference biological exposure indices are founded on homeostatic theory, which incorrectly assumes biological constancy. Consequently, biological monitoring is typically restricted to single or before and after work shift assessments. Many biomarkers of exposure and biological exposure indices of reference are governed by circadian processes and exhibit predictable day/night variation, sometimes of considerable magnitude, suggesting the utility of so-called biological-time or shift-qualified reference values for greater precision of data interpretation. Thus, we recommend:
 - a. Biological monitoring be more extensively integrated into high-risk permanent and rotating night shift settings.
 - b. Biological monitoring, particularly in high-risk settings, entail employee serial self-assessments of biomarkers indicative of health status throughout the waking span, i.e., both at and off work. This is exemplified by surveillance programs consisting of: (i) serial self-measurement of peak flow rate (measure of airway patency and ease of respiration) by personnel of certain work environments using small, easy-to-use, inexpensive instrumentation to detect early signs of detrimental effects on the airways and lungs, and (ii) serial self-collections of body fluids, such as urine and/or saliva, by employees routinely exposed to dangerous chemical and other contaminants to better detect risk for acute and/or chronic adverse effects;
 - c. Research to assess the need for biological-time or shift-qualified reference values to improve interpretation of data derived from biological monitoring when high peak-to-trough 24 h variation is characteristic of reference biological exposure indices.
 - d. More extensive research be conducted to develop wearable small-scale, inexpensive, worker-acceptable biosensors/technology for convenient real-time biological monitoring, including for sampling blood or other bodily fluids, especially for personnel of high risk occupational settings engaged in nonstandard work schedules.
 - e. Companies responsibly manage personal information when collecting, storing, and processing biological monitored and other employee data. In Europe this policy now falls under the General Data Protection Regulation; <https://www.eugdpr.org/>. Data are increasingly gathered by internet, mobile phones, sensors, radio-frequency identification tags, and information systems, enabling management to build comprehensive personal biometric and psychometric profiles. The greater the amount of information collected and processed, the more complete the 'fingerprint' of the employee and the greater the difficulty of maintaining anonymity and security. Personal data should not be used or processed in ways incompatible with approved purposes. Workers need to be clearly informed about how their employer transmits, stores, processes, and protects their personal information, and they must know their rights, such as the right to withdraw consent for use of their biological monitored or other data without prejudice or penalty.

Consensus statements review expert panel: Arne LOWDEN¹(Chair), Claire CARUSO², Francesco PORTALUPPI³

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Full consensus among panel members on all statements.

Introduction

This consensus paper focuses on the pertinence of the human circadian time structure (CTS) to industrial toxicology, specifically threshold limit values and biological monitoring. It is one of several consensus papers developed by the Working Time Society, commissioned by the International Commission on Occupational Health⁽¹⁾, published in this journal issue. Each describes the current state of knowledge, identifies health and safety risks, and offers recommendations for effective interventions and future research through a series of key consensus statements developed utilizing procedures outlined in Wong *et al.*⁽¹⁾. Collectively, the reports provide guidance to a broad international audience of researchers, industry members, labor representatives, policy makers, workers, and other interested stakeholders on managing fatigue and ensuring health and safety of those routinely engaged in nonstandard work schedules.

Background

The impact of the CTS and its disruption by permanent night and rotating shift schedules on worker fatigue, performance, accidents, and health is well documented^(2–18). Its relevance to industrial toxicology, i.e., development of threshold limit values (TLVs) and conduct of biological monitoring (BM), for optimal protection of the health and wellbeing not only of day but night and other nonstandard shift workers, however, is far less appreciated. This is the situation even though more than 125 investigations substantiate the acute adverse effects of a wide array of chemical, biological, and other xenobiotic stressors often vary extensively according to the time of exposure with regard to the staging of responsive circadian rhythms, and the several published position papers that corroborate their relevance to industrial toxicology^(17, 19–22). Of additional relevance are recent findings reported by Jay *et al.*⁽²³⁾ revealing permanent and rotating nightshift personnel are subjected to workroom contaminants in the same or higher concentration than dayshift personnel. Collective knowledge of circadian rhythms in vulnerability plus between-shift differences in exposure to harmful substances infers the nightshift may pose greater risk for adverse effects than the dayshift.

Threshold limit values

The American Conference of Governmental Industrial Hygienists defines a TLV as the level of a chemical, physical, or biological agent to which nearly all workers may be

repeatedly exposed, day after day, without adverse health effects. Three types of TLVs regulate industrial exposures—time-weighted average ones applicable to typical 8 h/d, 40 h/wk exposures; short-term exposure limit ones applicable to brief (≤ 15 min) encounters of ≤ 4 times/d with ≥ 60 min between each; and ceiling limit, i.e., maximum allowed exposures ones—and industrial hygienists or other trained specialists are responsible for ensuring compliance⁽²⁴⁾. A major theoretical construct underlying TLVs is homeostasis, i.e., the unsubstantiated assumption of constancy of biological functions and processes, implying the time or shift during the 24 h when workplace contaminants are experienced is inconsequential. The well corroborated concept of chronobiology—rhythmicity of biological functions and processes—in contrast, implies the opposite perspective, i.e., the time during the 24 h when exposure occurs can be a critical determinant of vulnerability and risk for adverse health effects⁽²⁴⁾.

Circadian Time-Keeping

Human and other life forms are organized both spatially as cells, tissues, organs, and systems and also temporally as endogenous biological rhythms of different period [τ , τ], i.e., ultradian ($\tau < 20$ h), circadian ($\tau \approx 24$ h), and infradian ($\tau > 28$ h)⁽²⁴⁾. This consensus report focuses on the totality of the body's circadian rhythms, which we term the circadian time structure (CTS), as the basis of the possible differential vulnerability of night versus day workers to contaminate xenobiotic chemical, contagion, and other potentially harmful stressors.

The CTS of mammals, including humans, is orchestrated by a central brain time-keeping axis composed of clock genes of the suprachiasmatic nuclei (SCN) located within the hypothalamus plus the pineal gland through the synthesis and circulation of the hormone melatonin only during the darkness of night. Collectively, the SCN and pineal gland coordinate the τ and staging, such as the peak and trough times, of the multitude of autonomous peripheral biological clocks that govern cellular and organ 24 h rhythms^(24, 25). Under usual circumstances, the CTS is organized by external time cues, i.e., natural 24 h cyclic phenomena, which in totality constitute the environmental time structure (ETS)⁽²⁵⁾. The most important time cues for ancestral humanoids was natural sunrise and sunset, but for humans today, it is mainly the onset and offset times of artificial electric light sources^(18, 25, 26). These natural and artificial light cues are sensed by the photopigment melanopsin contained within intrinsically photoreceptive

ganglion cells of the retinae and conveyed via the retino-hypothalamic neural tract to the master brain clock, the SCN^{27, 28)}. Additional temporal cues, like time (s) of nutrient consumption, are also of importance, especially to peripheral cellular, tissue, and organ clocks^{24, 26)}. The τ and staging of the numerous endogenous circadian rhythms that compose the CTS under normal conditions of life are internally coordinated and synchronized relative to the τ and staging of cyclic 24 h phenomena that comprise the ETS. Thus, biological processes of human beings are organized in time to support optimal efficiency of metabolic, cognitive, and physical processes during the usual diurnal activity span and repair and restorative processes during the usual nocturnal sleep span²⁴⁾. Throughout evolution the well-integrated and highly organized CTS, precisely entrained to natural ambient light/dark and other cyclic environmental signals, conferred functional and survival advantage through differential biological and cognitive readiness during the 24 h to cope with usual predictable-in-time extraneous challenges^{24, 29)}.

Biomarkers of Circadian Time

Throughout the years a variety of biomarkers have been used to determine circadian time^{17, 30, 31)}; however, the peak time or other features of the body temperature and hormone melatonin circadian rhythms are perhaps the most popular ones^{32–34)}. Early research studies enlisted the cooperation of shift workers to self-record the clock time of sleep onset and offset plus oral temperature taken at frequent intervals during the wake span while working night and dayshifts and also during off days³⁵⁾. In many such studies, urine voids or saliva samples were also self-collected to determine melatonin concentration. Customarily, such data were analyzed by special time series, so-called Cosinor, analyses to objectively determine the peak or other characteristics of the respective biomarkers of circadian time^{17, 25, 31)}, and in the case of melatonin the dim light melatonin onset time³⁴⁾. These biomarkers and the methods utilized to obtain them, while appropriate for research investigation, are impractical for routine application in occupational medicine, because of likely noncompliance of workers to self-collect body fluids at designated times of the day and night plus high cost to industry to analyze such samples for melatonin or other constituents indicative of circadian time. New transcriptome and metabolome approaches coupled with interpretative algorithms enable determination of circadian time by once-a-day sampling of blood, breath, and hair follicle and other

tissue^{36–43)}. Such methods are currently being explored in forensic medicine to estimate circadian time from analysis of single blood spots⁴⁴⁾, and seemingly it is worthy of research for future application to occupational medicine and industrial toxicology.

Impact of Nonstandard Work Schedules on Circadian Time-Keeping

The staging of individual rhythms of the CTS accommodate both to natural and unnatural alteration of the ETS, respectively, seasonal variation in daily photoperiod duration and rapid transmeridian displacement by aircraft or working permanent night and rotating shift schedules^{29, 31, 35)}. Indeed, nonstandard work schedules that entail artificial light at night exposure and abnormal meal timings disrupt the normal phase relationships between individual 24 h rhythms and/or perturb the circadian τ from 24.0 h^{26, 35, 45)}. Accommodation of the CTS to major alteration of the normal diurnal activity in light/nocturnal rest in darkness routine, for example, necessitated by nonstandard work schedules, is neither instantaneous nor harmonious^{30, 46, 47)}. For some persons, adjustment may be arduous or even impossible due to age, genetic, medical, or other factors, resulting in persistent circadian rhythm disruption with associated symptoms of disordered sleep, digestive system complaints, altered mood, and marked and persistent fatigue^{14, 29, 48)}.

Circadian Rhythms in Disposition of Xenobiotics

Uptake of chemical and other xenobiotic substances by the skin, lungs, and gastrointestinal tract and their distribution, metabolism, and elimination are governed by circadian processes²⁴⁾. Their absorption by skin is modulated by 24 h rhythms of tissue hydration, acid-base balance, porosity, blood flow, immunology, sebum formation, and keratinocyte, melanocyte, fibroblast, and basal cell processes^{24, 49, 50)}. Deposition of gasses, particulates, and other matter via the alveolar tissue of the lungs depends on endogenous 24 h periodicities of respiratory rate, tidal and minute volume, lung diffusion capacity, plus alveolar blood flow and cellular processes^{24, 51–53)}. Ingestion of chemical and solid matter by the gastrointestinal tract depends on nyctohemeral rhythms of gastric pH, enzyme concentration and activity, motility/transit time, blood flow, bacterial biome, active and passive transport phenomena, and local cellular processes^{24, 54)}. The metabolism, detoxification,

and elimination of chemical substances also are governed by circadian rhythms of Phase I (e.g., P450 cytochromes, aldehyde dehydrogenases, and carboxylesterases), Phase II (e.g., glucuronosyltransferases, sulfotransferases, and glutathione S-transferases conjugations), and Phase III (uptake and efflux transporter) processes^{24, 54}). These and other CTS-regulated activities are of proven relevance to clinical medicine²⁴), and laboratory animal and human investigations clearly demonstrate they are the basis for the observed 24 h variation in vulnerability to a great variety of chemical, biological, and other xenobiotic stressors of relevance to industrial toxicology practice.

Chronotoxicology

Chronotoxicology is the study of biological time-dependent differences in the adverse effects of xenobiotic substances^{17, 24, 55, 56}). Hundreds of animal and human research studies corroborate the CTS mediates the responses to and consequences of a wide variety of toxic challenges. Investigations involving other species, such as insects, fish, and plants, also document prominent disparity in vulnerability according to the circadian time of exposure to insecticides and pesticides— β -cyfluthrin, chlorfenapyr, dichlorodiphenyltrichloroethane, dursban, malathion, permethrin, and trichlorfon—and herbicides—glyphosate, glufosinate, fomesafen, and chlorimuron ethyl^{24, 57–65})—thus demonstrating the ubiquity across species of the phenomenon of chronotoxicity.

The concept of chronotoxicity also includes circadian disruption as an adverse effect of challenging exposures. Animal^{66–69}) and human^{4, 18, 25, 26, 70–74}) research demonstrates xenobiotics can induce alteration of molecular clock gene behavior giving rise to abnormalities of the amplitude, phase, and/or period (τ) of 24 h rhythms. Moreover, some investigations indicate circadian disruption, itself, can increase vulnerability to chemical and other challenges and thus pose enhanced risk for compromised health and wellbeing, even oncogenesis^{75, 76}).

The current knowledge of the CTS-dependent reactions to and adverse outcomes of exposures to contagion, physical (noise, temperature, etc.), gaseous, chemical, metal, irritant, allergenic, carcinogenic, and teratogenic agents is summarized in the following sections. Because the literature is so vast, we often cite a recent review paper by some of the authors²⁴) to respect journal guidelines concerning reference number.

Contagions

The immune system exhibits strong circadian organization⁷⁷). Thus, it is not surprising that both laboratory animal experiments and human vaccination trials demonstrate prominent circadian time-dependent differences in the acute response to and consequence (morbidity and/or mortality in rodent investigations) of bacterial and viral contagions, such as *Bacillus Calmette-Guérin*, *Brucella*, *E. coli* endotoxin, coxsackie B3, *Staphylococcus aureus* pneumococcal, *Salmonella Typhimurium*, vesicular stomatitis, herpes, influenza, and malaria/*plasmodium berghei* challenges^{17, 24, 77–81}). Moreover, case study reports link sudden nocturnal death of young workers from exposure to *Staphylococcus aureus* and other toxins of super-antigen properties that trigger strong inflammatory response⁸²).

Physical agents

Loud noise-provoked seizure and death of laboratory rodents plus high ambient temperature-induced physiological responses and limits of tolerance of humans are CTS-dependent^{17, 24}). Moreover, adverse effects and mortality of animals caused by ionizing and ultraviolet-B radiation are also governed by circadian time^{24, 83–89}).

Gases

Human research documents day/night difference in carboxyhemoglobin elimination, and animal experiments validate circadian-time disparity in vulnerability to oxygen-induced seizures, ozone-provoked injury of alveolar and terminal bronchiole tissue of the airways, and carbon monoxide lethality^{24, 90}). CTS-related variation in animal and/or human responses to and adverse effects of low oxygen partial pressure (hypoxia of elevated altitude), rapid hyperbaric decompression, and central nervous system toxicity of hyperbaric oxygen is also reported^{24, 67, 91}).

Chemicals

Investigations reveal differences according to circadian time in methyl isocyanate tolerance of humans and alcohol-provoked hypothermia of humans and rodents and also alcohol-induced death of laboratory animals²⁴). Additionally, the adverse effects of a wide array of chemical xenobiotics, including death of animals from a so-called 50% lethality dose—dose expected to cause 50% mortality of the test population—show circadian dependency. This is illustrated by studies entailing nicotine, nikethamide, strychnine, potassium cyanide, urethane [ethyl carbamate], and cholinesterase inhibitors—the pesticides of soman [*O*-Pinacolyl methylphosphono-fluoridate], chlorpyrifos,

dichlorvos, malathion, parathion, phenitrothion, and trichlorometaphos-3—plus herbicides paraquat and glufosinate, plant insecticide dinitro-ortho-cresol, and bacteriostat florfenicol^{17, 24, 92–95}). Additionally, abnormalities of sperm structure, total sperm count, and serum testosterone concentration of male rodents caused by the pyrethroid insecticide fenvalerate vary in extent relative to the biological time of exposure²⁴). CTS dependency is also displayed for neurobehavioral deficits induced by trichloroethylene; hyperthermia caused by aconitum napellus; hepatotoxicity provoked by bromobenzene, carbon tetrachloride, chloroform, dichloroethylene, trichloroethylene, and styrene [ethenylbenzene]; and nephrotoxicity produced by carbon tetrachloride^{24, 93, 96–99}).

Metals

Laboratory animal studies substantiate substantial circadian differences in risk for renal injury and death from several metals, including cadmium, mercury, lithium, lead, nickel, chromium, copper, and zinc^{24, 93, 100–107}).

Irritants/allergens

Circadian rhythms modulate the intensity of irritant and immune reactions to various substances, e.g., delayed and recall immune system responses of rodent skin to oxazolone and of human skin to sodium lauryl sulfate and lapyrium chloride²⁴). Furthermore, they influence the magnitude of the erythema and wheal (induration) responses of human skin to the capillary dilator histamine and antigens of house dust, grass, ragweed, and penicillin²⁴). The scale of the airways inflammatory and constricting reactions of both laboratory animals and humans to inhaled ozone, house dust, histamine, methacholine, and acetylcholine is additionally CTS-dependent²⁴).

Carcinogens/teratogens

Laboratory animal research validates the critical importance of circadian time in determining the vulnerability of the skin, brain, and gastrointestinal tissues to tumor induction by the chemical carcinogens methylnitrosourea, beta-propiolactone, and 7,12-dimethylbenz[a]anthracene, and also Ehrlich ascites tumor cells^{17, 24, 78, 108–111}). Furthermore, prominent circadian-stage discrepancy is demonstrated in laboratory animals for the cytotoxicity and genotoxicity of the immunosuppressant mycophenolate mofetil and alkylating agent nitrosourea^{24, 112, 113}). Finally, circadian time disparity in risk for fetal teratogenesis during gestation is demonstrated through laboratory animal investigations entailing alcohol, dexamethasone, hydroxy-

urea, 5-fluorouracil, cyclophosphamide, and Th-R [N-mustard]^{24, 114}).

Magnitude of circadian time-dependent toxicity

The extent of variation during the 24 h in vulnerability to the diverse xenobiotic stressors is not trivial. For instance, in laboratory animals the lethality of a dose of potassium cyanide expected to produce 50% mortality is actually 100% when administered at the commencement of the nocturnal activity span, while 12 h later at the commencement of the rest span it is *only* 20%²⁴). Fatality of laboratory animals to a single whole body x-irradiation dose of 550r when timed during the middle of the nocturnal activity span is 100%, while when timed near the end of the diurnal rest span it is 0%²⁴). Finally, exposure of gravid animals to the classical teratogen hydroxyurea at the commencement of the nocturnal activity span is safe and does not cause any malformations, while exposure of animals of the same gestational stage but at a different circadian time 12 h later that corresponds to the end of the activity span causes numerous abnormalities^{24, 114}).

Industrial Toxicology and Threshold Limit Values

Modern concepts of industrial toxicology, especially concerning threshold limit values, are credited to Theodore Hatch, who in the 1960s envisioned adverse effects of chemical exposures in terms of two—impairment and disability—risk scales, with impairment perceived as a precursor of disability, i.e., reversible and irreversible pathology, and death in the extreme¹¹⁵). Hatch proposed occupational exposures be limited in intensity to the capability of inherited regulatory homeostatic and adaptive compensatory coping mechanisms, the latter exemplified by induction of liver enzymes with repeated moderate chemical exposures, improved efficiency of heat loss with repeated exposures to hot environments, and increased oxygen carrying capacity plus enhanced pulmonary, cardiac, and metabolic efficiency with repeated exposures to low oxygen partial pressure at high altitude^{116–120}). Hatch's perspective, which is consistent with the concept and goals of preventive medicine, constitutes the theoretical basis for the promulgation of workplace TLVs.

Figure 1 is a modification of Hatch's Fig. 2 of his 1968 Archives of Environmental Health publication¹¹⁵). It displays risk for worker disability, i.e., pathology (left vertical axis), over a hypothetical range of xenobiotic exposures of different intensities relative to risk for physi-

ologic impairment (horizontal axis). The upper limits of tolerance to such stressors, based on the functional capabilities of inherited homeostatic and compensatory (labeled 'Comp') mechanisms, are depicted at the mid-center and right center portions of the lower horizontal axis by two emboldened short vertical hatched lines. The curve extending from near the left lower corner to upper right corner symbolizes in a kind of dose-effect manner the potential magnitude of disability according to the extent of biological impairment that might be induced by chemical or other workroom contaminants. Exposures that do not exceed biological tolerance, i.e., capabilities of inherited homeostatic and compensatory mechanisms, do not cause impairment or disability, i.e., are no threat to health, and are compatible with the tenets of preventative medicine. Exposures that exceed the upper limit of biological tolerance, depicted by the gray tone shading at the far right of the figure, have pathological consequences that necessitate curative medical intervention, and which in the extreme might culminate in death.

Half a century ago there was little knowledge of circadian rhythms and their relevance to industrial toxicology, particularly potential between-shift differences in susceptibility to xenobiotic stresses. We added shadings of arbitrary width to the above-described boundaries of involved homeostatic and compensatory processes to denote, in accordance with the findings of the above-reviewed animal and human literature, CTS-dependent differences in biological state that can give rise to work-shift discrepancy in risk for impairment and disability. This updated figure thus incorporates circadian differences in risk/vulnerability of employees relative to the protection afforded by TLVs, not only for those working standard day but also nonstandard permanent night and rotating shift schedules for whom CTS adjustment is likely to be incomplete. It is noteworthy Hatch also advocated TLVs take into consideration age-dependent and inter-individual disparity in vulnerability, including that due to diminished health status arising from previously experienced work-induced disability¹¹⁵.

Circadian Rhythms and Methods of Biological Monitoring

Biological monitoring (BM) is a common procedure in modern medicine exemplified by around-the-clock electrocardiology and ambulatory blood pressure patient studies for heart disease and hypertension, wrist actigraphy for appraisal of activity and sleep disorders, daytime serial peak expiratory flow rate [PEFR] self-assessments for determi-

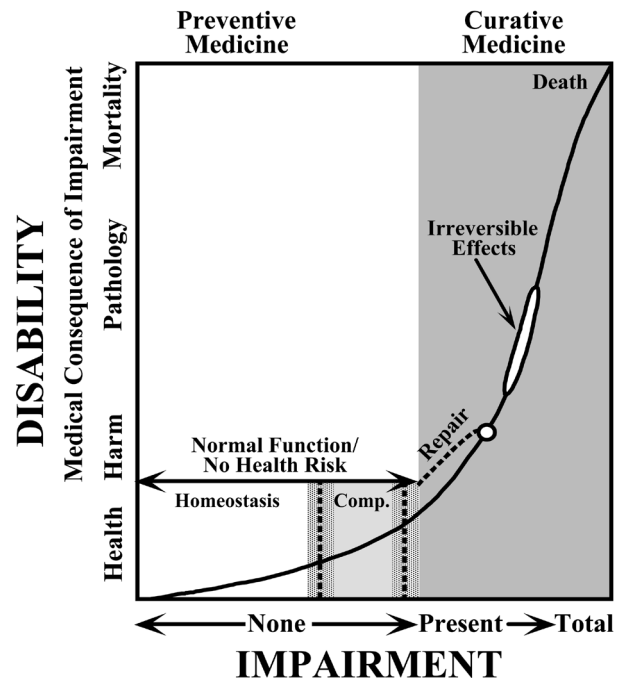


Fig. 1. Theodore Hatch's scales of biological impairment and functional disability as the conceptual basis for the promulgation of TLVs¹¹⁵. Impairment due to excessive industrial exposures is viewed as a precursor of disability—reversible and irreversible pathology, and death in the extreme. Curve extending from bottom left corner to upper right corner depicts the potential level of disability relative to the severity of biological impairment stemming from xenobiotic exposures. Exposures not exceeding the capability of inherited regulatory homeostatic and compensatory mechanisms (labeled 'Comp'), whose respective upper limits are denoted by two short vertical hatched lines at the mid-center and right-center portions of the lower horizontal axis, are unlikely to provoke impairment or disability for the vast majority of workers. This perspective is consistent with the tenets of preventive medicine. Exposures beyond the biological limits of tolerance that increase the risk for biological impairment and disability—pathology (gray shading of the far right-hand portion of figure)—entail curative medical interventions. Accordingly, TLVs that respect normal homeostatic and compensatory processes avert impairment and disability. New to Hatch's original 1968 figure is the depiction by stippled shading of arbitrary width of the predictable-in-time 24 h variation of the limits of involved inherited homeostatic and compensatory coping mechanisms; the shading thus symbolizes the potential magnitude of the day/night difference in the vulnerability of shift-workers to xenobiotic and other workspace stressors. Figure is a modification of both Hatch¹¹⁵ and Smolensky *et al*²⁴.

nation of airways health of asthma sufferers, and collection of urine voids and blood samples for routine diagnostics and dose-titration of medications. Since the 1960s, BM in the form of autorhythmometry—serial self-measurement of biological and behavioral variables and/or self-collection

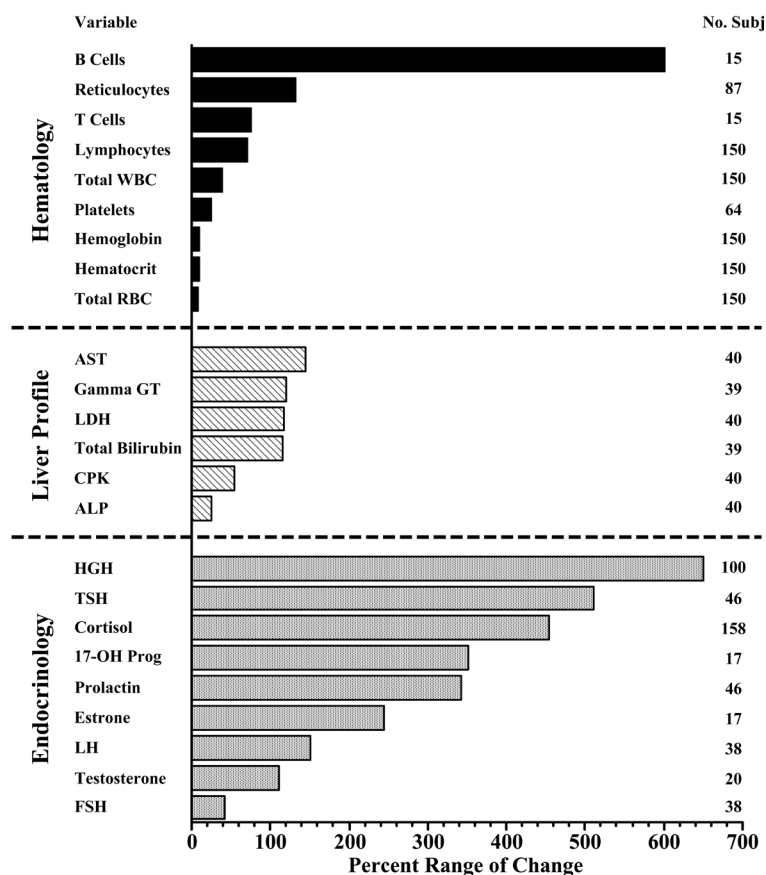


Fig. 2. Illustrated rationale of the necessity of BTQRVs as BEIs. Figure created using databases of several different studies in which blood samples were collected at 1 to 4 h intervals throughout a single 24 h span from working-age healthy men and women adhering to a routine of diurnal activity and nocturnal sleep and consuming breakfast, lunch, and dinner meals at usual times^{122–126}. Length of the individual horizontal vectors represents the total amount of the 24 h variation displayed in blood samples by selected hematology, liver, and hormone variables. Average magnitude of the day/night variation per variable across subjects is calculated as the difference between the highest and lowest value divided by the 24 h mean of all values multiplied by 100 and expressed in %. Temporal variation in hematological variables is depicted in the top panel; for hemoglobin, hematocrit, and total RBC (total red blood cells) it is $\leq 10\%$ and negligible, while for total WBC (total white blood cells) and platelet blood cells it is moderate, and for total lymphocytes, T and B cell lymphocytes, and reticulocytes it is high, $\geq 100\%$. Middle panel displays mainly liver function variables; AST (aspartate aminotransferase, also known as serum glutamate oxaloacetate transaminase [SGOT]), Gamma GT (gamma glutamyl transferase), LDH (lactate dehydrogenase), CPK (creatine phosphokinase isoenzymes—more indicative of heart, muscle, and brain than liver tissue status), total bilirubin, and ALP (alkaline phosphatase) exhibit extensive, $\geq 100\%$, 24 h variation. Bottom endocrinology panel depicts the range of 24 h disparity in the serum concentration of reproductive and other hormones. Most display great, $\geq 100\%$, day/night variation; this is particularly evident for HGH (human growth hormone), TST (thyroid stimulating hormone), cortisol, 17-OH Prog (17-OH progesterone), prolactin, estrone (an estrogen), and testosterone, although not for LH (luteinizing hormone).

of sequential urine, saliva, or other body fluids at frequent intervals during the wake or entire 24 h span for several consecutive days—has been extensively utilized to explore the chronobiology of human health and disease^{24, 121}.

It has also been the principle means of investigating the speed of adjustment of the CTS of workers to rotating and permanent nightshift schedules and travelers to rapid time-zone displacement by jet aircraft^{30, 31, 35, 45}.

BM has also long been incorporated into occupational medicine to assess worker exposures and health status, although without consideration of the implications posed by the CTS in its conduct. Formal endorsement in the United States of BM of workers dates to the early 1970s; although, it was not until the early 1980s that the American Conference of Governmental Industrial Hygienists created a committee to recommend normative reference BEIs to aid interpretation of values²⁴. Typically, bodily fluids and/or biological measures are collected before and after work shifts, with data evaluated relative to biological exposure indices (BEIs), generally informed by no-observed-adverse-effect, lowest-observed-adverse-effect, and/or benchmark dose values that lack work-shift and clock-time specification²⁴.

BEIs, like TLVs, are promulgated without consideration of possible CTS influences. Some blood, saliva, and urine constituents show such small 24 h variation they do not necessitate special referencing for circadian time of sampling. However, judgment of the normality or abnormality of BM-derived variables that exhibit large predictable-in-time difference during the 24 h is likely to benefit from reference values qualified for biological time. Such biological time-qualified reference values (BTQRVs) have been proven valuable in clinical medicine to assess normality of determined blood constituents known to display high-amplitude circadian variation^{122–126}, and they seem relevant to occupational medicine and industrial toxicology to improve interpretation of BM data, particularly those obtained at atypical times from night workers²⁴.

Rationale for the need of BTQRVs as BEIs for certain biomarkers is illustrated in Fig. 2. It presents by the length of individual horizontal vectors the total extent of the day/night variation of several hematology, liver, and hormone variables of blood samples. The figure is created using databases of several studies in which blood samples were collected at 1 to 4 h intervals throughout a single 24 h span from working-age healthy men and women adhering to a routine of diurnal activity and nocturnal sleep and consuming breakfast, lunch, and dinner meals at usual times^{122–126}. The length of the horizontal vector represents the average magnitude of the 24 h variation of the given variable across all studied subjects, calculated as the highest value minus the lowest value divided by the 24 h mean of all values multiplied by 100 and expressed in %. The number of subjects represented per variable is listed in the right-hand column of the figure. The amount of temporal variation in the hematological variables is shown in the top panel; that of

hemoglobulin, hematocrit, and total RBC (total red blood cells) is negligible, $\leq 10\%$, that of total WBC (total white blood cells) and platelet blood cells is moderate, and that of total lymphocytes, T and B cell lymphocytes, and reticulocytes is high, $\geq 100\%$. The middle panel depicts primarily liver function variables; AST (aspartate aminotransferase, also known as serum glutamate oxaloacetate transaminase [SGOT]), Gamma GT (gamma glutamyl transferase), LDH (lactate dehydrogenase), CPK (creatine phosphokinase isoenzymes that are more indicative of heart, muscle, and brain than liver tissue status), total bilirubin, and ALP (alkaline phosphatase) exhibit extensive, $\geq 100\%$, 24 h variation. The bottom endocrinology panel depicts the extent of the 24 h disparity in the serum concentration of reproductive and other hormones. Most display great, $\geq 100\%$, temporal variation; this is particularly evident for HGH (human growth hormone), TST (thyroid stimulating hormone), cortisol, 17-OH Prog (17-OH progesterone), prolactin, estrone (an estrogen), and testosterone, although not LH (luteinizing hormone). It is obvious that many biomarkers of health and wellbeing are characterized by substantial 24 h variation, thereby implicating the need of BTQRVs to properly interpret whether values obtained through employee BM are normal or abnormal according to the circadian time of biomarker sampling. In many occupational settings, 8 h or 12 h shift-specific BTQRVs may be more easily applicable than clock-hour or circadian-time ones. Biological variables additionally exhibit weekly and annual rhythms, and in young women menstrual rhythms^{24, 122–128}; however, the relevance of BTQRVs for these time domains to better interpret BM-derived values awaits appraisal.

A common method of BM in some occupational settings comprises serial measurements conducted at regular intervals during work and off hours. For instance, in the United Kingdom, BM of workers routinely exposed to chemicals, irritants, and allergens that can adversely affect the airways may entail repeated self-assessments throughout the waking span of peak expiratory flow rate (PEFR), a measure of the patency of the airways and ease of breathing, using an inexpensive, simple-to-use, personal light-weight, hand-held peak flow meter. Published normative reference values take into account ethnicity, age, sex, height, and weight²⁴; however, like all other BEIs they are regarded as if static, i.e., without circadian variation. The peak-to-trough circadian variation (rhythm amplitude) in PEFR values of diurnally active healthy young adults is small, equal only to 3–5% of the overall 24 h mean. Typically PEFR is highest during the middle

and late afternoon—9–12 h after commencement of the daytime activity period—and lowest between the middle and latter span of nighttime sleep²⁴⁾. The PEFR 24 h mean of individuals with obstructive or reactive airways disease is significantly lower than normative age and sex reference values, and the circadian amplitude is larger, generally 20–25%, but sometimes as much as 40–50%, of the 24 h mean. The more severe the airways insult and pathology, the lower the PEFR 24 h mean and greater the PEFR circadian amplitude. Additionally, the time of day of best airway function may be significantly advanced or delayed from the normal reference time²⁴⁾.

The PEFR circadian rhythm is well known to most pulmonary medicine and some occupational health physicians. Nonetheless, BM of employees exposed to respiratory irritants and allergens typically is restricted to one or two PEFR measurements—either only after or both before and after the work shift—to assess airways health. Frequent self-assessment of PEFR during work and remaining wake span has been utilized as a more comprehensive method of BM for employees exposed to polyvinylchloride and toluene diisocyanate aerosols as well as grain and cork dusts. Adverse effects of such exposures are manifested both by a lower than predicted PEFR 24 h mean and higher than normal between-measurement variation²⁴⁾. Even more comprehensive BM has involved serial PEFR self-measurements done throughout the waking span of consecutive work *and* off days. Using this approach, Randem *et al.*¹²⁹⁾ found solder workers routinely exposed to fumes of pine resin sap, i.e., colophony, display relative to normative reference values a lower PEFR 24 h mean, larger circadian amplitude of variation, and somewhat altered clock time of best airways flow rates on work compared to non-work weekend and vacation days.

The findings of Randem *et al.*¹²⁹⁾ are in line with those reported almost six decades ago by McKerrow *et al.*¹³⁰⁾, who noted employees toiling in dusty cotton mills are prone to chest tightness and breathing difficulty on Mondays following off-work weekends and vacations. These symptoms, which are indicative of the lung disease byssinosis, progressively worsen during the work shift. In the early stages of this occupational illness, symptoms are limited to the first workday, Monday, of each work week, but in advanced stages they additionally manifest Tuesdays or even every work and off day. The BM by McKerrow *et al.*¹³⁰⁾ of cotton workers involved serial PEFR self-assessments done throughout the waking span of the daytime work shift, finding the PEFR 24 h mean to be markedly depressed by 35–40% and the PEFR

circadian amplitude enhanced by 2–3-fold relative to the respective normative reference values. Furthermore, the peak time of the PEFR circadian rhythm (time of day of greatest airflow rate that denotes best airway function) was greatly advanced—by 9 to 12 h to the early morning at 06:30 h—from afternoon/early evening as expected for diurnally active workers. This later alteration is indicative of the progressive increase during the daytime work shift of breathing distress produced by cotton dust exposure. Thus, a proven BM procedure for early detection of occupationally induced adverse effects and pathology of the airways is frequent serial self-assessment during work *and* off time of PEFR using an inexpensive personal peak flow meter. Early indicators of acute adverse effects of workplace contaminants on the airways include alteration of not just one but as many as three parameters of the PEFR circadian rhythm—the 24 h mean, amplitude, and peak time.

Serial self-collection of urine voids is an additional means of biologically monitoring chemical and other xenobiotic exposures, e.g., to chemical sanitizing agents. Chlorine-based disinfectants are routinely utilized by cleaning personnel to control microbial contamination and minimize risk for infection. Such chemicals enter the body by inhalation or other means during work and are metabolized by liver enzymes into harmful, i.e., cancer-inducing trihalomethane, byproducts. Gängler *et al.*¹³¹⁾ collected consecutive urine voids from human volunteers working with chlorine disinfectants at different times of the day and night, finding the time of day of their use determined the urinary concentration of the surrogate biomarker 4-hydroxynonenal and detrimental trihalomethane metabolites. Urinary biomarker concentrations were low following morning-time exposures but considerably higher following afternoon and nighttimes ones, substantiating significant circadian rhythm-dependent differences in the body burden of the harmful byproducts of the chlorine disinfectants. The time-of-day differences were not due to corresponding disparities in environmental exposures; rather, they resulted from circadian rhythm-dependent differences in the activity of the liver enzyme CYP2E1 that metabolizes chlorine chemicals. Circadian rhythms in benzene metabolism and biomarkers also have been identified by Watts¹³²⁾ that additionally demonstrate the need for TQFVs as BEIs according to the time of urine sampling.

Conclusions

Personnel of nonstandard work schedules, especially those engaged in permanent and rotating nightshift work,

may be exposed to harmful xenobiotic contaminants in the same or even greater concentrations than dayshift workers. Moreover, night workers are likely to be exposed to workroom contaminants during a different circadian time that may be deterministic of higher vulnerability than day workers. Establishment of threshold limit values and methods of biological monitoring, including interpretation of derived values against reference biological exposure indices, are based solely on homeostatic theory, the unsubstantiated assumption of biological constancy, and databases of adverse events that lack qualification for biological time or work shift. Rarely, if ever, has the vulnerability of personnel to industrial contaminants and other stressors been explored according to work shift or circadian time. The goal of this consensus paper is to call attention to the extensive literature pertaining to laboratory animal and human investigations that document the importance of the circadian time structure to industrial toxicology. The collective findings of the many investigations reviewed herein entailing contagion, physical, gaseous, chemical, metal, irritant, allergenic, carcinogenic, and teratogenic xenobiotics suggest circadian factors ought to be an additional important criterion used to decide threshold limit values, particularly to protect personnel working nonstandard work schedules. Furthermore, realization that biomarkers of adverse workplace xenobiotic exposures may be governed by high-amplitude circadian rhythms should serve to better inform the methods of biological monitoring and data interpretation, not only for night but also day workers.

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