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# Environmental and Equipment Monitoring

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I. INTRODUCTION

Since publication of the first edition of this chapter in 1983, many advances have been made in mouse biology (Malakoff 2000). The increased use of inbred and genetically altered mice, often with compromised immune systems, has resulted in the need for stringent environmental conditions and management practices (Marshall 2000). This in turn has increased the need to thoroughly monitor the environment of mice and the equipment used for their care.

As used in this chapter the term “environment” refers not only to those physical, chemical, and microbial factors that influence the well-being of the mouse, but also those aspects of the physical plant, equipment, supplies, and employee activity that have an impact on the mouse's well-being. The impingement of these factors on the mouse is depicted in Fig. 12-1.

Environmental quality of animal facilities has been reviewed by Ruys (1991), Besch (1980), McSheehy (1976), and the Institute of Laboratory Animal Resources (ILAR 1978a). Further, environment, housing, and management requirements for laboratory animals are discussed in the Guide for the Care and Use of Laboratory Animals (NRC 1996).

II. MANAGEMENT

The Health Research Extension Act of 1985 under Public Law 99-158, applicable to all organizations receiving funds from the United States Public Health Service, directed the National Institutes of Health (NIH) to establish guidelines for the proper care of animals to be used in biomedical and behavioral research. From this Act the Office of Laboratory Animal Welfare (OLAW) was created. In turn, OLAW developed the requirement for an Assurance Statement to be filed by each organization receiving PHS funds. The Assurance Statement requires the presence of a management program for all vertebrate species. The most recent edition of the Guide for the Care and Use of Laboratory Animals (NRC 1996) is the key reference and standard for establishing an animal care and management program.

Environmental and equipment monitoring are viewed here as management tools used to control the quality of mice produced or housed in the facility. Their purpose is to help safeguard the health of the animals and personnel. As Fig. 12-1 illustrates, a quality control program is the basis of a protective curtain enveloping the animals. Direct health surveillance of the mouse

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Fig. 12-1  The mouse’s response to experimental manipulations is the product of the interaction of its genome with the numerous environmental factors that may or may not be present. Environmental quality control strives to define those factors impinging on the mouse. Management practices ultimately dictate to what the mouse will be exposed. (Modified from Serrano 1971b and Lindsey et al. 1978.)
is the subject of Chapter 11 in Volume 3 and is not discussed here. However, it should be clearly understood that no health surveillance program is a disease prevention program. Likewise, environmental and equipment monitoring systems do not control the environment or equipment located within the animal facility; they only define and identify the situation. These systems are incorporated within the facility to monitor, record events, and notify selected individuals of an alarm condition requiring immediate attention. The ability to record temperature, humidity, light levels, ammonia, CO₂, and O₂ at the animal cage level can provide valuable data for both research and management of the animals.

If the institution’s administrative officers, animal care staff, and users of the animals are sensitive to the importance the quality of the environment plays in maintaining the health of the mice and the integrity of the experimental protocol, the chances for a healthy and stabilized colony are greatly enhanced. If the size of the facility warrants it, consideration should be given to including maintenance and engineering support as an integral part of the animal facility staff responsible to the facility director. This aids in ensuring a high level of preventive maintenance and sensitivity to the need for immediate response to problems in the physical plant. Maintenance staff and outside repair personnel need to be trained in how to work within the animal facility, so as not to compromise the health and safety of both themselves and the animals.

Planned periodic inspection and preventive maintenance of buildings and equipment are necessary to avoid unexpected interruptions of service or contamination of colonies. Heating, ventilating, and air conditioning (HVAC) equipment, autoclaves, and cage washing equipment require constant attention. Stocks of commonly replaced parts and supplies need to be maintained. Provisions need to be made in advance for the rapid replacement of costly but critical items that may not be stocked on the premises. A history of quality performance, reliable operation, availability of service, and ease of obtaining replacement parts should be factors in making purchases of major equipment.

Use of computer-based maintenance programs to track maintenance performed and schedule routine maintenance are encouraged. This provides a reliable and accessible record.

Security against unauthorized entry into the animal facility is no longer an option. Institutions must emphasize security and establish standard operating procedures for operating animal facilities in a safe and secure manner. It is essential that all staff and users be thoroughly trained regarding the security policies and procedures. Refresher training sessions should be regularly scheduled. Inadvertent or deliberate exposure of animals to toxic or infectious agents by visitors, staff, or intruders may lead to major losses of research effort (Broad 1979). Theft of equipment or animals compromises a facility’s ability to meet the needs of investigators. In a facility with space shared by many investigators who must enter and leave at all hours, security can be difficult. Ideally, only personnel and visitors requiring access to the vivarium and its animals will be admitted. Admittance of maintenance staff and outside repair personnel should be carefully coordinated with the vivarium staff and reviewed for the potential of introducing disease or compromising on going research. It is reasonable to provide training to the maintenance staff and outside repair personnel, to the extent necessary, so that they understand the basics of research with animals, the care given, security requirements, and the need for the rules of conduct while in the vivarium. The need for accompaniment by a vivarium staff member should be assessed on a case by case basis.

Unless necessary, it is best for vendor representatives and other invited visitors to meet with the vivarium staff outside the vivarium. Sales personnel often visit many different facilities on a given day. They may unintentionally serve as a vector of disease.

Entry into the vivarium must be controlled. Ideally, all persons should pass through a central monitoring point at time of entry. This monitoring point may be a public reception area staffed by a member of the animal facility or an entry area controlled by unique electronic identification cards. Electronic key cards have the advantage of being monitored during use and, if lost or stolen, deactivated. Twenty-four hours a day monitoring of all access points with video recording equipment and archiving of activity is desirable. Conventionally keyed locks are frequently used but do not provide a record of who used the key or whose key was used. Standard metal keys are successful only as long as access to keys is controlled. Frequent changes of lock cylinders are time consuming, expensive, and troublesome for staff and users alike. For tracking and accounting purposes, each key should be uniquely identified. Keys should be the kind that make it difficult to obtain duplicates outside of the organization. Stamping each key “Do Not Duplicate” may be only a minor deterrent to duplication. Keys must be accounted for and returned when no longer needed or when employment ends. Withholding the final paycheck in the absence of returning all keys should be considered policy. Security against unauthorized intrusion or use of a facility is expensive to implement, entails a degree of inconvenience for users, and requires continuing education. However, lack of security can lead to significant losses in time, research effort, physical assets, and financial resources. In addition, breaches of security in animal facilities are destructive to the morale of those using and caring for the animals.

III. ENVIRONMENTAL FACTORS

A. Heating, Ventilating, and Air Conditioning

HVAC are among the most critical environmental factors in a vivarium. A discussion of environmental factors must differentiate between the room (macro) environment and the cage.
(micro) environment. The introduction of the isolation cage by Kraft (1958) proved to be effective in protecting mice from infection with the rotavirus causing epidemic diarrhea of infant mice. Subsequently, static microisolation cage systems, as originally developed by Sedlacek et al. (1981) were widely used during the 1970s and 1980s. These systems were instrumental in the development and use of specific pathogen-free rodents in research (Corning and Lipman 1991; Lipman 1992). The effect of the filter on the cage environment became evident with the reduction of air changes within the cage compared to the room research (Coming and Lipman 1991; Lipman 1992). The effect of the filter on the cage environment became evident with the reduction of air changes within the cage compared to the room research (Coming and Lipman 1991; Lipman 1992).

Murakami 1971; Serrano 1971a; Simmons 1976; Weihe 1965). With the added labor of changing cages, in some cases twice per week, including sterilizing cages and water bottles and using sterile techniques within a Class 100 workstation, labor cost more than doubled. The need for improved ventilation within these isolation cages as well as the ability to reduce the change interval presented a challenge.

One of the first introductions of individually ventilated caging (IVC) occurred at the Jackson Laboratory, under the direction of Dr. Ed Les, in collaboration with Mr. William Thomas, B. S. of Thoren Caging Systems, and was made available to the laboratory animal industry in 1978 (Lipman 1999). Their focus was to improve intracage ventilation and to increase housing capacity. By the early 1990s, IVCs gained widespread popularity with a wide selection of cage sizes, rack configurations, and air flow designs available. The advantage of intracage ventilation with conditioned air that is high-efficiency particulate air (HEPA) filtered proved to be an effective method of microenvironmental control. As compared to the static cage environment, there was a marked improvement with respect to temperature, humidity, carbon dioxide, and ammonia levels (Lipman et al. 1992).

Individually ventilated rodent cages are now the dominant cage design in new research facilities worldwide. More advanced building designs include the direct integration of the rack exhaust and supply directly into the building HVAC system. With each IVC manufacturer requiring varying volumes of exhaust air, supply air, and static pressures, the proper sizing of the HVAC system becomes extremely important. The importance of air balancing, commissioning, and monitoring once the facility is on-line is critical (Lipman 1993).

There are four primary configurations for integrating the ventilated racks into the building (Deitrich, 1996).

1. Racks with associated supply and exhaust units are IVCs that include the manufacturer’s HEPA filtered supply blower and exhaust blower mounted to the rack. Conditioned air is taken from the room through the rack supply blower and extracted from the cage through a HEPA exhaust blower back into the room.

2. Racks with associated supply and building exhaust units are IVCs that include the manufacturer’s HEPA filtered supply blower mounted to the rack. The rack’s exhaust directly connected to the building’s exhaust duct, thus eliminating the need for a rack exhaust blower.

3. Racks with room associated supply and building exhaust include several IVCs that are connected to a central HEPA filtered supply unit located in the room or above the ceiling, thus eliminating a supply blower for each rack. Racks are individually connected to the building exhaust.

4. Racks with building supply and building exhaust are IVCs that are connected to the building HVAC supply and exhaust. This is the most advanced design. It requires a dedicated central supply for the IVCs which must provide HEPA filtration and take into consideration intracage temperature and humidity.

Room air exchange rates, temperature, and relative humidity directly effect the intracage environment (Perkins and Lipman 1996). Building automation systems (BAS) are designed to monitor and control the HVAC system and to provide alarms for immediate response. In addition to the BAS, some facilities may elect to use local monitoring systems located at the room, rack, and/or cage level that provide alarm notification and data 24 h a day. A response to an electrical outage or fan failure that is supporting either static or ventilated cages requires immediate response to avoid severe stress or death of the animals. Monitoring at the room and cage level can provide the added advantage of accurate data collection with regard to variations in the animal’s environment that may have an impact on the research results.

Each of these building integration designs stress the importance of environmental monitoring and control as well as the maintenance and certification of blowers and air filtration systems. In addition to monitoring environmental conditions, animal room air flow and differential pressures should also be considered with respect to the corridor, animal room, and cage. Although modern HVAC designs and IVC systems have the ability to contain or exclude airborne microorganisms, their successful operation remains the responsibility of those managing the vivarium.

Although most new housing for mice generally uses one of the IVC systems described previously, many older facilities and some newer small vivariums continue to use static microisolation cages or open cages. Management of HVAC factors in this environment requires careful attention to husbandry to minimize build up of toxic and noxious substances in static microisolation cages and exposure of mice in open cages to infectious agents. At the same time, open cages expose people to high levels of mouse allergens. Requirements for monitoring the animal room environment in which static microisolation cages and open cages are used are not significantly different from requirements for animal rooms housing IVCs.

The air exchange rate in animal rooms needs to be determined initially and whenever the air handling system is altered or rebalanced. At a minimum, the room air needs to be monitored.
environmental conditions to detect contamination or verify abatement of a hazard. Random and undirected microbiologic sampling of air is not warranted. It is important that the level of activity and other conditions in the room be constant between and during sampling periods, otherwise counts will have little meaning. High-volume air sampling can be useful in epidemiologic studies. For example, McGarrity and Dion (1978) recovered polyoma virus from the air of a mouse room while cages were being changed. Usually, monitoring of air for its microbial content is done by one of three methods: (1) settling plates (Petri dishes) exposed to the air for a stated period of time, usually 1 h; (2), high-volume air impact samplers, that is, Andersen Cascaded Sieve Sampler (Andersen Samplers, Inc., Smyrna, GA); and (3) liquid impingers. Settle plates are inexpensive and can give a qualitative picture of the organisms (bacteria and fungi) present if they grow on the culture media used. Do not use settle plates to quantify the concentration of airborne fungal spores (Streifel referenced in CDC 2003). Methods 2 and 3 will also allow for recovery of some viruses, provide quantitative answers and have the advantage of sampling, a large volume of air; however, the equipment is more expensive and requires more training to use it properly. The routine monitoring of air for its microbial content should not be part of a quality control program. Examining the animals will undoubtedly provide more useful information.

B. Light

Color temperature (wavelength) and intensity (lux, lumens/m² or fc) of light affect mice (Robison et al. 1982). When establishing lighting conditions for mouse rooms, the intensity should be stabilized unless experimental conditions require otherwise. Current guidelines recommend lower light intensity in animal rooms than previous reports (ILAR 1978b; NRC 1996). Unless the mice are extra-sensitive to the effects of the light, levels of approximately 325 lux (30 ft-candle) about 1 meter above the floor are suggested (NRC 1996). Although well-lighted rooms are usually kept cleaner, light levels previously recommended, 75–125 ft-candles (807–1345 lumens/m²) (ILAR 1978b) are known to damage vision severely in albino mice (Robison and Kuwabara 1976; Robison et al. 1982). Similar damage occurs in rats and has been reviewed by Bellhorn (1980). If mice require intact photoreceptors for the success of a study, attention to light intensity is critical. It needs to be emphasized that a large number of commonly used mouse strains and stocks are blind for genetic reasons (see Robison et al. 1982).

Regardless of the room light level maintained, its intensity in the cage is what is critical. Care must be taken that mice, by virtue of their cage’s position on the rack or in the room relative to the proximity of the light fixtures, do not receive excessive light. Greenman et al. (1982) reported significant increases in retinal atrophy among mice in cages on the top
two selves. In addition, the light-dark cycle and the color temperature should be observed. For normal physiologic functions, the light should be cyclical. Many facilities use 12 h light-12 h dark without apparent problems. The estrous cycle tends to lengthen when light should be cyclical. Many facilities use 12 h light-12 h dark without apparent problems. The estrous cycle tends to lengthen and persist in continuous light (Campbell et al. 1976; Ziemann and Kitell 1980).

Consistency of the light-dark cycle is best ensured by the use of automatic timers; however, timers need to be monitored for mechanical or electrical failure. Also, personnel may alter them when working after regular hours and fail to return them to the automatic mode. Cunningham and Doss (1979) describe an easily made recording device to monitor animal room lights. There are inexpensive commercially available data loggers that will record the light-dark cycle. Some models are available that combine recording of temperature, relative humidity, and the light-dark cycle. Data are easily retrieved for review and archiving (HOBO, www.onsetcomp.com). A general discussion of the effect of light on animals is presented by Weihe (1976).

Color temperature of the light may be important in some cases. Most modern animal facilities use light from cool-white or warm-white fluorescent tubes. Except for special experimental requirements, these choices appear to be adequate. Fluorescent tubes are available in a number of types, each having its own spectral characteristics. The influence of the light's spectrum is emphasized by the work of Kittel and Ziemann (1979) who demonstrated that the estrous cycle of mice kept in red light was longer than when mice were kept in blue, green, or yellow light. Also, the length of the estrous phase increased as the light's wavelength increased. Chignell et al. (1981) reported a delay in the first litter from mice maintained under cool white or pink fluorescent lights compared to those maintained under daylight-simulating fluorescent lights. Saltarelli and Coppola (1979) reported significant differences in weights of the pituitary, adrenals, kidneys, and prostate of male and the adrenals, thyroid, and pineal gland in female Hae(ICR) mice reared for 30 days under various fluorescent lamps (pink, blue, black uv, cool white, and full spectrum).

The use of light-emitting diodes (LEDs) for area lighting is gaining interest due to their efficiency and lack of heat. Hecke et al. (1999) compared the effect of LED and cool white fluorescent light of equal intensity on suppression of pineal gland melatonin in male Sprague Dawley rats. Both light sources similarly suppressed melatonin. Retinal function and morphology were similar in the two groups. They concluded that LED light does not cause retinal damage. Effects of these same LED lights were not studied in mice.

C. Noise

Mice have been studied extensively for the effects of noise on the intact animal (Willott 2001). Turner et al. (2005) recently published an extensive review of the nonauditory effects of noise on laboratory animals.

Fletcher (1976) defined noise as "any sound which is undesirable because it interferes with speech and hearing, or is intense enough to damage hearing, or is otherwise annoying." Anthony (1963) refers to noise as "any unwanted or undesirable sound." Sound is measured by its frequency or oscillations per second and by its intensity or pressure level. Frequency is designated in hertz (Hz) and intensity in decibels (dB). Both humans and animals are more directly responsive to increases in the intensity of a sound than they are to changes in frequency. Humans are most aware of sounds in the 20–20,000 Hz (0.02–20 kHz) range (Turner et al. 2005). Peak response in mice is between 10 and 20 kHz with hearing responses recorded up to 110 kHz (reviewed by Brown and Pye 1975). Ultrasound production by mice is recognized with the range of sound extending from 33 to 140 kHz (Sewell 1967). Holy and Guo (2005) reported ultrasonic vocalizations (30–110 kHz) of a rapid series of chirp-like syllables by B6D2F1 male mice presented urine of either sex of BALB/c mice. Their conclusion was that the vocalizations represent songs, with different mice singing recognizably different songs.

High noise levels in animal facilities are, to some extent, unavoidable. This is especially true in multispecies facilities and in large single-species facilities using considerable amounts of mechanical equipment. Recognized problems with high noise levels usually stem from complaints by people working in the area or from inspections by health safety officers. Physiologic effects of noise on the mice are rarely considered (Pfaff 1974). However, considerable data exist pointing to adverse biologic effects of noise on rodents (Anthony 1963; Gamble 1982; Iturrian and Fink 1968; Kimmel et al. 1976; Seyfried 1979, 1982; Welch and Welch 1970; Zakem and Alliston 1974). Fletcher (1976) and Peterson (1980) reviewed the effects of noise on laboratory animals. Physiologic effects of noise are considered to be primarily mediated through the anterior pituitary acting on the adrenal gland, and the effects observed are those associated with increased levels of corticosteroids and epinephrine (Anthony 1955; Anthony and Ackerman 1955, 1957; Anthony et al. 1959). Eosinopenia occurs but is transitory unless noise exposure is prolonged. Also, the degree of eosinopenia is greater in audiogenic seizure-prone mice. There was a transitory hypertrophy of the fasciculate zone of the cortex of the adrenal glands 5–8 h following exposure to sound. These studies were done at a sound-pressure level of approximately 110 dB in a 10–20 kHz band for periods of 15 or 45 minutes a day over a 30-day period. Busnel and Molin (1978) studied the effects of noise coupled with mechanical development. Their studies utilized a recording of Paris subway sounds at a level of 105 ± 5 dB, which was not noxious for the auditory organs and an oscillating shaker for 4 and 2 h a day, respectively. Increased numbers of resorptions and birth defects and low birth weight were found in sound and vibration exposed groups of hearing mice. Unfortunately, a vibration-only control group was not included in the study. Miline and Kochak (1951), studying rabbits and
guinea pigs, felt that mechanical vibration exerted more of a stress than noise alone (cited in Anthony and Ackerman 1955). Zoric (1959) reported degenerative changes in the reproductive organs of male mice repeatedly exposed to the noise of an electric bell for 8 h each day. Interference with normal fetal development has been reported following exposure of pregnant mice to excessive noise (Ward et al. 1970).

Average noise levels or occasional loud noises may have no adverse effects on most strains of mice; however, one should be aware of the exquisite sensitivity of certain strains of mice to audiogenic seizures (Fuller and Sjursen 1967; Ralls 1967; Schreiber 1978). Susceptibility to seizures decreases rapidly with age. The use of a handheld whistle to assess animal hearing has been described (Lenhardt 1979).

The sound or noise level in animal facilities varies considerably, both from site to site and with time at a given site. Sound in animal facilities is generated by a variety of sources, not the least of which are the animals themselves. Common sources of increased noise levels are feeding and cleaning operations within animal rooms and cage washers with the associated handling of cages. Movement of cages, racks, and mechanical vehicles provide additional sources of noise. Coupled with this high noise level may be mechanical vibrations that are transmitted to animal rooms. Horns, buzzers, and alarm bells serve as sources of intense noise. Consideration should be given to the use of silent alarms where possible. Clough and Fasham (1975) described a fire alarm audible to people but not to rats, and Gamble (1976) indicated this alarm was inaudible to mice.

Placement of autoclaves near animal rooms may create a problem due to the alarm, which usually signals the end of the cycle. Likewise, alarms on refrigeration equipment placed near animals may pose a problem. Acoustically and mechanically induced vibrations can result from construction with the use of air hammers and drills. Seizures associated with fur clipping, tail tattooing, and fire alarms in FVB mice or mice with an FVB background has been reported. Neuronal necrosis was reported in the cerebral cortex, hippocampus, and thalamus (Goelz et al. 1998). The use of radios in animal rooms is a common practice, and the sound level as well as the frequency distribution varies considerably. The effect of high-frequency sound from electronic medical devices, radios, and television sets on animals remains to be fully elucidated (Pfaff and Stecker 1976; Turner et al. 2005). The sound and vibration of air-conditioning equipment may be transmitted through ducts to animal rooms and provide an increased sound level. Canines and primates generate significant noise levels, especially while people are in the room. Housing these species distant from mice is highly desirable, not only for reasons of noise but to prevent transmission of disease, that is, salmonellosis, to the mice.

Control of noise in animal facilities may be a problem. Ideal design features for the control of noise, for example, acoustical ceilings, acoustical panels, and insulation, are frequently counterproductive to good vermin control and sanitation; however, thoughtful planning with attention to choice of materials; finishes; equipment; and placement of activities, species, and traffic flow can go a long way to minimizing noise problems.

It is difficult to ascertain if any equipment or activities associated with the operation of the vivarium induce sound related changes in mice. It is suggested that unless the sound frequency is relatively high (4–32 kHz), mice probably will not hear the sound and will not be affected. White noise of significant energy within this frequency range is of concern. As a precaution, it is recommended that areas housing mice be surveyed every few years, or when a significant change in the physical or mechanical structure is made, to determine that noise levels above 4 kHz are less than 70 dB SPL. Measurements should be made with a high-quality sound meter and the linear scale (not the A weighted scale) should be used (personal communication with Dr. Rick Davis, National Institute of Occupational Safety and Health 2004).

IV. EQUIPMENT AND SYSTEMS

A. Cage Washing and Cage Washers

Methods for cage cleaning vary among facilities and depend in part on the number and type of cages to be cleaned and the presence or absence of injurious agents, such as carcinogens, radioactive substances, or infectious organisms. Ament (1971) has described three types of cage washers: (1) each phase of the cycle uses fresh water, (2) water from the final rinse is used for the next cycle’s prewash, and (3) wash solution is recirculated. Although reusing the detergent containing wash solution saves hot water, detergent, and energy, it is seldom done in rack or cabinet washers today due to the possibility of contaminating the next load. It is standard practice in most situations to save the final rinse and use it for the prewash phase of the next cycle, especially if several cycles are being run consecutively. The objective of washing should be to render the cages and associated equipment (1) physically clean and (2) free of potentially harmful substances including viable vegetative bacteria, carcinogens, and radioactive substances. Even with a properly operating modern cage washer, soil removal may be incomplete. Sansone and Fox (1977) and Fox and Helfrich-Smith (1980), using sodium fluorescein to simulate a carcinogen, demonstrated the deposition of nanogram quantities of fluorescein on “washed” cages when the final rinse from the previous load was used for the prewash cycle in the following load. When dealing with cages exposed to toxic substances, testing washing performance, for example, trace residues, is warranted. In tunnel washers the detergent solutions are used for extended periods and often changed only daily or less often. Detergent solutions become loaded with debris from the cages and redeposit it on the cages. All debris may not be removed in
the final rinse, although no viable vegetative microorganisms remain. In this situation, it is important that the machine operators pay close attention to the cages as they exit the cage washer.

A cage wash quality assurance program should monitor the following: (1) time of exposure to and temperature of the wash and final rinse solutions, (2) the pressure in and function of the spray heads, (3) detergent concentration, (4) soil removal, (5) presence of viable vegetative microorganisms, and (6) if required, presence of chemical and radioactive contamination. If items (1)–(3) are monitored and maintained according to the manufacturer’s specifications and washed equipment is visually inspected by the machine operator, few problems with residues of viable microorganisms will occur.

Performance recommendations applied to cage washers are taken from studies done on and standards developed for commercial dishwashers under the auspices of the National Sanitation Foundation (NSF) (1964, 1977) (reviewed by Brown undated). These studies utilizing *Mycobacterium phlei*, an organism more heat resistant than *M. tuberculosis*, determined the minimum water temperature requirements which will produce, with an adequate margin of safety and the most economy, the required cumulative heat factor for sanitization in a commercial multiple tank, conveyor spray-type dishwashing machine meeting the minimum mechanical operating requirements of NSF Standard No. 3 (NSF 1977). Critical to this work is the use of the concept of cumulative heat factor (CHF) originally developed by Fuchs (1951). The work of Fuchs in establishing the concept of CHF was reviewed in the first edition (Small 1983) and subsequently by Wardrip et al. (1994).

Wardrip et al. (2000) also verified the bacterial killing effects of the wash time versus wash temperatures. When evaluating dishwashers, the temperature used to develop the CHF is that measured at the dish (or cage) surface. Thermal death of organisms is primarily due to the effects of the temperature transmitted from the water impinging on them rather than from the temperature effects transmitted from the object being washed. The CHF is based on the 30 minutes (1800 sec) at 62°C (143°F) required for pasteurization of milk, which, in turn, is based on the killing of *M. tuberculosis*. In the case of *M. phlei* the 1800 sec required at 62°C (143°F) is reduced to 80 seconds at 68°C (154°F) and only 15 seconds at 72°C (161°F). The CHF takes into account the total time an object is subjected to water at temperatures above 62°C. This temperature relationship is made use of in designing dishwashers and cage washers. In the tunnel washer, initial exposure is to the wash solution at a minimum of 66°C (150°F). In most batch cage washers there is a prerinse utilizing the final rinse from the previous cycle prior to its being discarded. This prerinse has the advantage of reducing the soil load in the detergent solution and raising the temperature of the washed items. The relatively low temperature of the detergent (wash) solution precludes “baking” proteinaceous waste on the equipment. Also, some detergents specify they are to be used at temperatures between 60°C and 71°C (140°F–160°F). Detergents may be increasingly corrosive at higher temperatures and they may break down.

The current edition of the Guide (NRC 1996) accepts the concept of the CHF and the fact that sanitization of animal care equipment can be achieved by using a final rinse of less than 82°C (180°F) if the time of exposure is adequate (Wardrip et al. 2000). The temperature of the final rinse should not exceed 91°C (196°F), as above this temperature, cavitation may occur in water pumps due to the formation of steam pockets. This results in less effective spray patterns.

Other factors that are important in the correct operation of a cage washer are pump pressure, placement of spray nozzles, volume of water flowing over a cage, and the time of exposure to each phase of the cycle. As the water leaves the spray nozzle, it forms droplets, decreases in velocity, and cools. The higher the pressure, the smaller the droplets and, subsequently, increased heat loss. Temperature and velocity decrease as the distance from the nozzle increases; therefore, the geometric configuration of the nozzles and their distance from the object being washed is very important. Because of these factors, measurement of water temperature in the tank or at the nozzle does not accurately reflect the temperature of the water striking a cage surface. The recommended final rinse temperature of 82°C (180°F) as measured in the tank takes this temperature drop into account.

Improper loading of cage washers may result in equipment not being effectively sanitized. Because there are no published industry standards for cage washers, it behooves the user to take the time to understand how the equipment works and to establish that the equipment is meeting his or her needs.

Temperature and pressure gauges should be observed at frequent intervals. Modern cage washers are equipped to monitor the time and temperature of each portion of the cage wash cycle and print out these data. These data should be constantly reviewed and archived. These real-time monitors and recorders have mostly replaced spot checks for temperature. In the absence of continuous temperature monitoring, spot checks for maximum temperature during one or more cycles daily are warranted. Physical indicators are available which indicate peak temperature during the cycle. However, they do not reflect the CHF. A glass tube containing a steel ball held in a wax pellet that melts at 82°C (Zwarum and Weisbroth 1979) is often used. The tube is attached to equipment being washed and examined at the end of the cycle for melting of the wax and dropping of the ball. Heat-sensitive indicator strips designed to turn colors when one or more specific temperatures are reached are also used. Tunnel washers should be constantly monitored for temperature. If wash and rinse temperatures are maintained within acceptable ranges and washed equipment is observed to be physically clean, few if any failures of sanitization will be encountered. However, tunnel washers, because they recirculate the detergent solution which continuously accumulates dirt and waste from the cages, deposit debris laden water on the cages before the rinse cycles. If the rinse cycles are deficient,
cages may be free of viable vegetative microbes, but still contaminated with a residue from the debris.

Periodic culturing of washed equipment for vegetative bacteria as it exits the cage washer is of value in validating cage washer operation if you can define how it assists management in making decisions. However, with continuous monitoring of the temperature for each cycle in batch washers and continuous monitoring of the temperatures in tunnel washers, the need for culturing washed equipment is greatly reduced. Any of three culture methods can be used (Branson 1972), the swab method, the swab-rinse technique, or the agar contact plates (RODAC) (Baltimore Biological Laboratory, Becton Dickinson, Inc.) described by Hall and Hartnett (1964).

A new method for evaluating the efficacy of cage washers to clean and sanitize equipment is the detection of residual microbial adenosine triphosphate (ATP) on the cage surface by the technique of ATP bioluminescence. This method was developed for assessing sanitation in the food processing industry. Major advantages over culture methods are time (about one minute) and ability to measure total ATP, representing both dead and live microbes (Ayicicek et al. 2006; Chittock et al. 1998; Davidson et al. 1999; Oulahal-Lagsir et al. 2000). This method is not a substitute for culturing surfaces when sterility or the total absence of live vegetative organisms is the desired end result. However, this method actually monitors cleaning (Schneider 2004). The ATP bioluminescence assay can be used to guide change out time for detergent solutions in tunnel washers (and other washers using recirculating detergent solutions).

Filter screens and spray nozzles need to be monitored and cleaned as required. Clogged filter screens result in reduced pump pressure and ineffective spray patterns. Further, debris from dirty screens may lead to plugged spray nozzles, thereby resulting in deficient cleaning.

Water quality varies considerably among locations, and selection of a suitable detergent and the proper concentration may require some effort. Mineral content of the water, soil load, equipment to be cleaned, and the detergent dispensing system are factors to be considered. Detergent concentration is monitored by a conductivity cell that determines electrical resistance based on a certain concentration in a given water sample. The desired conductivity will vary with the product. In most cases in which a recirculating wash solution is used, an automatic dispenser with an in-line conductivity cell is used. Automatic dispensers may also be used on batch washers. As the detergent solution is diluted by the addition of fresh water, the resistance increases, activating a valve causing the release of additional detergent. When the ionic strength of the wash solution reaches the level preset in the dispenser, the valve closes. For this system to operate properly the conductivity probe in the wash tank must be protected from physical damage and he kept free of mineral deposits. Depending on the amount of use, weekly inspection with periodic cleaning in a descaling solution is suggested. Many detergent manufacturers will furnish equipment to measure detergent concentration. Also, most companies will periodically inspect and service without charge the dispensing equipment they install. This service does not relieve the facilities manager from the responsibility for understanding the equipment and monitoring its operation. Although most manufacturers aim for a 0.2–0.25% use-dilution of detergent, this can vary with the quality of water available. In addition to added cost, excess detergent may damage equipment or result in detergent residues. Determination of the minimal concentration of detergent required will be a matter of trial and error. The main considerations are complete cleaning without damage to the cage and absence of detergent residue following the final rinse. Using detergents formulated for the material being washed is extremely important. Each container of detergent should be verified by its label that it is the intended product. Personnel responsible for operating the cage wash equipment should be trained to recognize the physical nature of approved detergent containers, presence of seals on outlets, and the colors of the products if distinctive. The accidental washing of mouse cages made of high temperature polycarbonate in a solution of a quaternary ammonium disinfectant resulted in severe damage to the cages and leaching of bisphenol A from the plastic. Bisphenol A has estrogenic activity. (Howdeshell et al. 2003; Hunt et al. 2003; Koehler et al. 2003). Also, see Lipman, Chapter 9, this volume.

Failure of the final rinse can lead to serious problems with alkali residues on equipment. Dehydration from failure to drink or chemical burns of the mice may occur. Also, detergent residues may add contaminants to the diet. As detergents are usually quite alkaline, rinse water at a pH above that of the tap water used suggests detergent residue, pH indicator paper, or indicator solutions may be used as a quick check of rinse water pH. Rinsing the surface of equipment or the interior of sipper tubes and water bottles with a small quantity of distilled water of known pH and measuring the pH electronically is even better.

B. Cages and Racks

Plastic cages need to be examined for cracks and damaged corners through which mice can escape. Metal cages and feeders need to be examined for sharp or damaged edges, cracked welds, and corrosion. Automatic water manifolds need to be examined for integrity of welds and connections and function of the valves. Casters should have grease fittings and be lubricated on a regular schedule using lubricants designed for such service. Caretakers should be instructed to observe the condition of equipment when changing cages. Cage washer operators should examine equipment before it is returned to service. Defective equipment should be removed from service and either repaired or discarded.

Cage filters need to be examined for fit and freedom from holes. At regular intervals they should be washed to remove accumulations of dust and then autoclaved. Molded filters tend
to distort when autoclaved; however, this can be minimized by
loosely stacking the filters and autoclaving for not more than
15 min at 120°C. Similarly, sheet filters used on racks can be
autoclaved successfully if sharp creases are avoided during
autoclaving, and if they are flattened while still warm.

C. Water and Watering Systems

1. History

The United States Congress, through Public Law 93–523,
charged the Environmental Protection Agency (EPA) with
establishing federal standards for protection of the public from
all harmful contaminants and established a joint federal-state
system for ensuring compliance with these standards and for
protecting underground sources of drinking water (Safe
Drinking Water Committee 1977). From this charge resulted the
1977 report of the National Academy of Sciences' Safe
Drinking Water Committee. Since the 1977 report, several addi-
tional highly focused reports have been issued. The law was
amended in 1986 and 1996 and its provisions are contained in
the United States Code of Federal Regulations 40 CFR141-149.

2. Water Treatment

a. Acidification Hydrochloric acid is most often used.
Enough acid is added to give a pH of 2.0–3.0 (usually 2.4–2.8).

McPherson (1963) used the equivalent of 6.0 ml of 1.0 N HCl
(8.5%) per liter of tap water. The pH was maintained over a
7-day period, at which time the water bottles were changed.
However, water supplies vary considerably and each user
should determine the exact amount of acid required to obtain
the desired pH. The pH should be checked with a calibrated pH
meter as pH test papers are unreliable in solutions of low ionic
strength. If an in-line acidifying system is used, a pH meter
should be incorporated and connected to an alarm system in
case the pH falls outside the acceptable range. If the alarm is
located in the animal area, it is better to use a flashing light than
a bell as an indicator of failure. It needs to be recognized that
hydrochloric acid-treated water will not eliminate \textit{P. aeruginosa}
from infected mice nor necessarily prevent transfer of organisms
from infected to noninfected cagemates by the fecal oral route.
However, the elimination of the spread of \textit{P. aeruginosa}
and other bacteria through the water can be expected.

In addition to hydrochloric acid, sulfuric acid (Hall \textit{et al.}
1980) and several organic acids, including peroacetic (Juhr
\textit{et al.} 1977, 1978), have been used to control microorganisms in
drinking water. Identified negative effects of the use of acidi-
\textit{fied water include reduced water intake and slower weight
 gains (Hall \textit{et al.} 1980; McPherson 1963) and possible loss of
tooth enamel (Karle \textit{et al.} 1980; Toco and Erichsen 1969).

b. Chlorination The hyperchlorination (8–20 ppm) of
rodent drinking water to control \textit{P. aeruginosa} and other gram-
negative microorganisms has been practiced for many years
(Beck 1963; Homberger 1993; McDougal \textit{et al.} 1967;
McPherson 1963; Woodward 1963). The antimicrobial activity
of sodium hypochlorite (household bleach) is most active in an
approximate pH range of 5–6.5 (slightly acid). The addition of
hydrochloric acid or other acids to lower the pH of hyperchlo-
rinated drinking water to approximately 2.8 cannot be
recommended. Concentrations of free chlorine in water
decrease from outgassing or combining with organic matter in
the water. McPherson (1963) recorded drops in free chlorine
from 12 ppm to 0.25 ppm in water bottles after 72 h, with
bacterial contamination nearly equal that of tap water without
the addition of chlorine. In contrast, Homberger \textit{et al.} (1993)
demonstrated a decline in free chlorine from 6 ppm to 2 ppm in
water bottles over a 1-week period. A possible reason for this
difference may have been the number of mice per cage and
their microbial burden. McPherson's study, done in the early
1960s, used round glass jars as mouse breeding cages in a pro-
duction colony, whereas Homberger \textit{et al.} used polycarbonate
cages holding only two mice each with well-defined microbial
burdens. Either chlorine gas or sodium hypochlorite may be
used as a source of chlorine. Chlorine is a highly toxic and reactive element in the free state and extreme caution must be exercised when handling it. If using sodium hypochlorite as a source of chlorine, dilute concentrated solutions in clean containers. Unless the chlorine content is determined by the user, only stock solutions less than 6 months old and stored below 21°C should be used. Household bleach containing 5.25% sodium hypochlorite, diluted 1 ml per gallon of tap water gives approximately 13–14 ppm free chlorine. When purchasing sodium hypochlorite solutions, especially as household bleach, check the label for percentage of sodium hypochlorite and the absence of any other chemicals such as fragrances, detergents, or cleaning “enhancers.” It is best to verify with the manufacturer the suitability of the specific product for your intended use.

The use of chlorine gas is usually restricted to large-scale operations in which the flow of chlorinated water is greater than 5 gallons per minute. Organic matter in water lines, bottles, and sipper tubes will combine with and decrease the level of free chlorine. Therefore, determinations of chlorine levels made from the mixing or holding tank may not accurately reflect chlorine levels in water consumed by the mice. Several methods for determining residual chlorine levels are available (American Water Works Association 2005).

Increased water consumption has been observed in mice given water containing 12 ppm chlorine (McPherson 1963). Decreased in-vitro activity of peritoneal macrophages from mice receiving 24–30 ppm chlorine but not 12–16 ppm chlorine in the drinking water has been reported (Fidler 1977). Blabaum and Nichols (1956) observed no adverse effects in weanling mice given drinking water containing 100 ppm (males) or 200 ppm (males and females) for 33 and 50 days, respectively. Weight gains and water consumption were considered comparable to the control mice, and no gross or histologic lesions were observed. Reproductive performance improved in C3H/HeJ and C57BL/6J mice given water containing hydrochloric acid (pH 2.5) and 10 ppm chlorine (Les 1968).

Haist et al. (2004) reported that city water (Ann Arbor, MI) passed through a 5-μm filter and offered to individually caged mice in water bottles remained free of coliform bacteria for up to 14 days.

3. Watering Systems

Mice need to have available a reliable source of potable water. Interruption of the supply resulting in deprivation and dehydration may have serious consequences (Brown et al. 1974).

a. Bottles and Sipper Tubes Bottles need to be examined for cleanliness and chips and cracks. Defective bottles should be discarded. Many bottles today are made of plastic, usually polycarbonate or polysulfone. The problems of glass bottles with their weight and attendant dangers of cuts from glass plus the aggravation and lost time make plastic an excellent choice. However, caution must be exercised in washing plastic (see section on cage washing) and interaction between the plastic and any test substance added to the drinking water must be understood. The sipper tube is probably the most difficult piece of portable animal care equipment to clean. Stoppers and sipper tubes need to be examined for cleanliness and condition. Microorganisms form a biofilm on the inner wall of sipper tubes and on the stopper that is difficult to remove by conventional washing techniques. Often, sipper tubes and stoppers are soaked or agitated in a detergent solution or a detergent solution containing chlorine. Failure to rinse the solution from the tube lumen can result in highly alkaline “soapy” water when next used. This can result in dehydration and even chemical irritation or burns in the mice. The presence of foam in freshly filled bottles should alert one to the possibility of detergent residue (See section on Cage Washing for test methods). Sipper tubes and stoppers can be sterilized in the autoclave. Autoclaving, however, does not physically clean the tubes and stoppers. Further, centrally generated steam frequently contains one or more corrosion inhibiting chemicals that may affect the mice. As an alternative to sipper tubes, bottles are available with a hole drilled through the neck. The mice lick the water through the hole.

Gelled water packaged in an inert plastic pouch can be purchased prepackaged ready to use. This is a sterile product made with food grade ingredients. It is also available with fenbendazole added for treatment of pinworms and other susceptible endoparasites. As an alternative to using bottles, equipment for installation in the vivarium is available to fill disposable plastic pouches with water and insert a disposable drinking valve. In both cases, the plastic pouches are designed to fit in the bottle holder of drop lids and replace glass or plastic bottles. Gelled water pouches are an excellent choice for a water source when mice are shipped by commercial carriers or otherwise are not housed in standard housing. The ability to stockpile ready to use water for use during an emergency is an attractive option. Another application is containment and quarantine areas where disposability is required. Gelled water pouches are also used when mice, usually for medical reasons, have difficulty reaching the standard sipper tube or valve.

b. Automatic Watering Systems Automatic watering systems are designed to supply a constant supply of potable water and at the same time save labor. Although capable of doing both, they cannot be totally ignored by the animal caretaker. Valves have been greatly improved during the past 20 years; however, they do need to be checked frequently, if not daily, to ensure that mice are receiving water and that there are no leaks.

There are two approaches for distribution of water to the rack manifold, each having their own unique advantages: recirculating and flush systems. The recirculating systems take advantage of continuous flow of water through the corridor, room, and as an option, through the racks 24 h a day. This design maintains a constant pressure of 3 to 5 psi, ensuring proper drinking valve operation and eliminating airlocks in the system. Pressure reducing stations are not required with
this design. These systems combine reverse osmosis filtration with high intensity ultraviolet light for sterilization. If desired, this processed water may be also be acidified. The continuous flow and exposure of the water to high intensity ultraviolet light inactivates bacteria and viruses and aids in blocking the development of a biofilm (Wallbank and Lebtag 1985).

A second approach for distribution of water to rack manifolds is a flush system that provides daily flushing of the room distribution and the attached racks to the drain controlled by a microprocessor unit that communicates with the pressure control stations. This design incorporates reverse osmosis filtration with chlorination or acidification. Multiple pressure regulating stations are present and electrically operated valves are used to increase pressure during the manifold flush cycle. Water is discharged to a sink/drain. Following the flush period of a few minutes, pressure reverts to 3 to 5 psi. These systems may have on-line rack flush controlled by a rack mounted electrical valve. This allows for daily flushing of the manifold while the distribution system is being flushed. The size of the flush system RO unit supply must be determined based on the large volumes of water that will be flushed to the drain daily and the cost of the RO water. Sanitizing this system requires filling and flushing the pipes, tubing, valves, and manifolds with a biocide, usually sodium hypochlorite at 20–50 ppm for 30–60 minutes. Both approaches use devices to monitor and record the daily operation of the equipment.

The pressure should be noted on the gauge downstream of the pressure reducer. Correct pressure for mice is approximately 3 to 5 psi; however, this may vary somewhat with the design of the valve. As the line filter becomes clogged, it may be necessary to increase the pressure of the water supply. The filter serves to keep out particles which may interfere with valve function. Its role is not to provide bacteria-free water. In fact, filters frequently serve as a nidus for large numbers of microorganisms, and they should be examined at regular intervals and replaced as required. The problem of bacteria growing through filters has been discussed (Thunert 1975).

When racks with automatic watering manifolds are washed, the water lines need to be drained and decontaminated. Several decontamination methods have been used, including flushing with potable 71°–82°C water or steam, filling the manifold with 20–50 ppm sodium hypochlorite for 30–60 minutes, followed by flushing with potable water. If the rack is not washed in a rack washer, in addition to the in-place sanitizing of the manifold, the valves should be individually sprayed with the sodium hypochlorite solution, allowed to sit for 2–5 min, and then rinsed with water. Costello et al. (1998) reported steam autoclaving was necessary to rid automatic watering manifolds of bacteria. After flushing racks with water at 180°F for 20–30 minutes, 34% remained culture positive; however, flushing did eliminate gram-negative bacteria.

Although daily preparation of fresh hypochlorite solution ensures sufficient chlorine, Rutala et al. (1998) demonstrated that 5.25% Na hypochlorite solutions (standard household bleach) diluted 1:50 (~1050 ppm) or 1:100 (~525 ppm) and stored in translucent containers at room temperature for 30 days retained 40% to 50% of the original activity. For 1:50 and 1:5 dilutions stored in closed brown containers 97% to 100% activity remained after 30 days. In the same paper Rutala, using the AOAC Use-Dilution Method assay, reported 100 ppm as the lowest concentration of chlorine that reliably inactivated S. aureus, S. choleraeuis, and P. aeruginosa.

Purification by reverse osmosis, deionization, ultrafiltration, ultraviolet radiation, and their combinations produces very pure water; however, if offered from a water bottle, the water may become seeded with microorganisms when the mice regurgitate saliva and food particles into the sipper tube. The addition of chloride or acid, that is, hydrochloric acid, may be required if growth of microorganisms in the water bottle is to be controlled. There are conflicting reports regarding bacterial growth in water bottles. Recent reports suggest that mice using water bottle do not heavily contaminate the water (Haist et al. 2004; Homberger et al. 1993) and older reports suggest rapid contamination (Beck 1963; McPherson 1963).

4. Water Monitoring

In most laboratories, examination of water, if it is done at all, is restricted to measuring the pH or chlorine concentration and checking for bacteria, especially P. aeruginosa. The use of Wensinck’s glycerol broth as described by Wensinck et al. (1957), Flynn (1963b), and McPherson (1963) is a very practical technique that lends itself to mass screening of water samples.

One to 2 ml of water are added to a culture tube containing 7–10 ml of glycerol broth. The tube is incubated at 37°C for 3–7 days and examined for the appearance of a blue-green color, which indicates the production of pyocyanin thus indicating the presence of P. aeruginosa. The test can be read after 3 days, although negative tubes should be incubated for 7 days before being considered free of P. aeruginosa. Vigorous shaking will intensify the color if it is present, and extraction of the broth with 1–2 ml of chloroform will concentrate and make visible small amounts of pigment not otherwise seen. This method will not identify none pyocyanin producing strains of P. aeruginosa or species of Pseudomonas.

The formula for Wensinck’s glycerol broth is given in the following tabulation:

| Ingredient* | Amount (g) |
|-------------|------------|
| Glycerine (w/v) | 10 |
| Protease peptone (Difco) | 15 |
| Potassium phosphate, dibasic | 0.4 |
| Magnesium sulfate 7 H₂O | 20 |
| Ferrous sulfate | 0.01 |
| Distilled water | 1000.00 ml |

*Adjust pH to 7.5 before sterilization at 121°C for 5 minutes; filter, then sterilize a second time at 110°C for 10 minutes. Modified from Wensinck et al. (1957).
D. Laminar Flow Equipment

Racks, cages, rooms, bedding disposal equipment, and workstations, incorporating the principle of controlled unidirectional airflow, frequently referred to as laminar flow or mass airflow, have received considerable attention (Beall et al. 1971; McGarrity and Coriell 1973; Rake 1979; van der Waaij and Andreas 1971). A fan circulates air through a coarse (roughing) filter followed by a HEPA filter that is rated to retain 99.97% of particles 0.3 μm or larger. Critical to the effective use of HEPA-filtered air is the maintenance of the filters. On receipt from the manufacturer, equipment needs to be checked for leaks through and around the filters. Certification of the integrity of HEPA filters is quite involved and is usually done by members of an organization’s safety group. Once a portable unit is certified, movement of the unit from area to area should be avoided as integrity of the filters may be disturbed. As the filters accumulate dirt, the velocity of the air coming from the filter (face velocity) will drop. Frequent replacement or cleaning of the roughing filters will greatly prolong the life of HEPA filters and the blower motor. Many units have gauges indicating the face velocity and a control to regulate the blower motor speed. The face velocity should be adjusted to meet the recommendations of the manufacturer. For most applications, a face velocity of between 50 and 90 ft/min is maintained.

E. Autoclaves

Steam sterilization utilizes saturated steam (free of air) at an elevated temperature and pressure. As the pressure is raised the temperature rises. Steam is capable of inflicting serious injuries or even fatal burns. No one should operate an autoclave, regardless of size, without receiving proper training. Autoclaving of liquids requires special attention as the liquid is super heated at the end of the sterilization cycle and under pressure. Glass containers must be of heat-resistant glass and approved for use in autoclaves. The pressure of the liquid in its container and the pressure within the autoclave chamber must be slowly equalized with the room pressure prior to opening the chamber door. Otherwise, the fluid will boil out of its container on opening the door. Most autoclaves in use today have a liquid cycle, which automatically and slowly reduces pressure within the chamber; however, caution should be observed when opening the door. It is suggested that one stand to the side of the door when opening it and do so slowly, in case steam or fluids escape. Containers of liquids should be allowed to cool prior to being removed from the autoclave chamber.

Typical time-temperature relationships for sterilization with steam are as follows (Gould 2004): 15 minutes at 121°C (15 psig); 10 minutes at 126°C (20 psig); and 3 minutes at 134°C (30 psig). These times relate only to the period the object being sterilized is at the stated temperature. Time must be allowed for steam to saturate the chamber, penetrate the object, and the temperature of the object to reach sterilizing temperature. The size of the autoclave’s chamber, characteristics of the items to be sterilized, heat transfer characteristics of containers, and the physical size of the total load all play a role in determining the time of the sterilization cycle (Rutala et al. 1982).

Steam autoclaves are highly reliable if they are used properly and maintained. Still, they must be monitored to ensure that they are operating as designed. People should be given training in both the principles of sterilization and autoclave safety before being assigned responsibility for operating autoclaves. Each sterilizer should be operated according to the manufacturer’s instructions. The highest temperature that is reached during sterilization and the length of time that this temperature was maintained should be recorded and checked for adequacy; this check is the most important means of ensuring sterility. Checks of steam sterilization performance should be carried out at least weekly. A recently developed rapid readout biologic indicator (BI) for measuring the success of steam sterilizer cycles has been described and is commercially available. It is based on the fluorescent detection of a Geobacillus (Bacillus) stearothermophilus (an organism whose spores are particularly heat resistant, thus ensuring a wide margin of safety) spore-associated enzyme and provides results within 3 h compared to 24–48 h for conventional BI tests using the same organism (McCormick et al. 2003, Schneider 2004; Vesley et al. 1995). The significant improvement is the shortened time required to validate the success of the sterilization cycle. A single positive spore test does not necessarily indicate that objects processed in the same sterilizer are not sterile. It does require that the sterilizer be rechecked for proper temperature, pressure, and use and that the test be repeated.

Steam sterilization of pelleted rodent diets and bedding present a particular challenge, and even with the use of a presterilization high vacuum cycle, failures may occur. Placement of thermocouples or wireless data loggers measuring temperature and pressure in the center of the load followed by culture of samples for thermophilic anaerobes is recommended. Data loggers are small self-contained microelectronic devices capable of repeatedly recording temperature, humidity, or pressure or a combination of these parameters during the sterilization cycle. On completion of the cycle, the data logger(s) are connected to a computer and the data are transferred for analysis. Data loggers are less cumbersome, more reliable, and less time consuming to use than thermocouples. Also, data loggers can be easily used in autoclaves not equipped with a gland, which is usually required for the use of thermocouples (www.mesalabs.com/datatrace/pdfs/ValidationfolISO17025Poster.pdf). Use of thermocouples or data loggers with food has the added advantage of monitoring the load for excessive temperature, which may result in subsequent loss of nutrient activity. The use of chemical-based steam sterilization indicators may give an erroneous sense of security and should

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not be relied on to check the efficacy of steam autoclaves (Lee et al. 1979).

Ethylene oxide (EtO) is widely used in specially designed autoclaves or chambers to sterilize heat- and moisture-sensitive instruments and materials. Compared to steam sterilization, it is a more complex, lengthy, and expensive process. EtO finds few uses in animal facilities outside of sterilizing heat-sensitive instruments and items for surgical implantation. EtO is highly flammable and explosive when mixed with air. Prior to December 1995 EO for sterilization was mixed with a chlorofluorocarbon. From 1996 onward the FDA has approved the use of 100% EtO and EtO stabilized with carbon dioxide or hydrochlorofluorocarbon (Rutala and Weber 2001). Before sterilization, objects need to be cleaned and wrapped. EtO sterilizing chambers of various sizes are available that use 100% EtO in single-use containers. A negative aspect of using EtO is the long exposure time required for sterilization followed by a period of “degassing” or exposure to room air. Chemically sensitive indicators should be used with each package to show that it has been exposed to the gas sterilization process. Gas sterilizers should be checked with commercial preparations of spores of Bacillus subtilis (an EtO resistant spore). If an EtO sterilizer is used infrequently, it should be checked with each load. Use of EtO is highly regulated by the National Institute for Occupational Safety and Health (NIOSH). Time weighted average (TWA) exposures for 100% EtO in single-use containers. A negative aspect of using EtO is the long exposure time required for sterilization followed by a period of “degassing” or exposure to room air. Chemically sensitive indicators should be used with each package to show that it has been exposed to the gas sterilization process. Gas sterilizers should be checked with commercial preparations of spores of Bacillus subtilis (an EtO resistant spore). If an EtO sterilizer is used infrequently, it should be checked with each load. Use of EtO is highly regulated by the National Institute for Occupational Safety and Health (NIOSH). Time weighted average (TWA) exposures for 8-h workday and for 15 minutes are 1 ppm and 5 ppm, respectively. The Environmental Protection Agency (EPA) considers EtO to be a probable human carcinogen and has ranked it in EPA's Group B1. Exposure of personnel to EtO must be monitored and records retained for 30 years (ATSDR 2003; CFR 29: Part 1926.1147; EPA (2006) Air Toxics Website-EtO). Due to its toxicity, all exhaust from EtO sterilizers and aerators for EtO sterilization must be safely vented. All items processed by EtO sterilization need special aeration according to manufacturer’s recommendations to remove toxic residues of gas.

EtO should not be used to sterilize food or bedding as it reacts with moisture present to form toxic ethylene glycol and possibly other compounds (Allen et al. 1962). Increased numbers of tumors were observed in female mice and male mice were infertile and died with thoracic and abdominal hemorrhages following accidental exposure to ethylene oxide-treated corn cobs for 150 days (Reyniers et al. 1964). The use of EtO in medical facilities has been reviewed (Joslyn 2001).

V. FEED AND BEDDING

Rodent diets and bedding attract wild and escaped rodents as well as other animals and insect pests. Concern for quality maintenance of feed and bedding begins with understanding how the products are manufactured and transported to your facility. Some manufacturers ship directly to the end user. Others ship to distributors who store the products in their own warehouse or facility prior to delivering them to the end user. Factors such as shipment by a manufacturer's dedicated truck versus a commercial common carrier needs to be considered. A second concern is the condition of the dealer's storage facility. When possible, inspection of dealer’s facilities on a regular, but unannounced, basis is suggested. The dealer should have a quality control program in place that includes good record keeping, a professionally supervised pest management program, and monitoring of temperature and humidity. Records should be available for your examination. In some instances this service will be provided by the vendor's pest management program contractor.

Areas where animal diets and bedding are stored need to be monitored and inspected frequently for the presence of birds, insects and rodent contamination, spilled feed, and damaged bags. It is recommended that bags be examined on arrival and that damaged bags not be accepted. Some facilities use autoclavable diets and autoclave bedding. Others use irradiated diets. This provides significant protection from the introduction of disease via the diet or a contaminated bag of bedding. Bags of diet and bedding that have a plastic inner lining can be lightly sprayed with a dilute sodium hypochlorite or chlorine dioxide solution and whipped down. When receiving large numbers of bags, they can be passed through an enclosed tunnel in which they are misted with chlorine dioxide.

Provisions for continuous monitoring and recording of temperature and humidity in the storage area are recommended. Secure storage with provisions for storage off the floor on metal or plastic dunnage racks, shelves, or carts needs to be provided. Storage should be away from the walls so that the space between the wall and the racks is visible and can be cleaned. Racks and shelves of wood construction should not be used. Ideally, space will be adequate to allow for easy access, movement, inventory, and cleaning.

Processed feed, even pelleted feed that is subjected to steam in the pelleting process, can contain viable fertilized ova of stored grain insects that may hatch. Alternatively, feed may become contaminated with insects or grain mites following manufacture. Once an area becomes infested with insects, it must be emptied of feed and decontaminated before being reused. The use of insecticides in a feed storage area cannot be recommended as they may contaminate the feed. Instead, the use of glue boards and sticky traps (for monitoring purposes) coupled with a high level of sanitation with frequent cleaning and inspection is recommended. Although it is ideal to store feed at 15°C or less, this usually is not possible except for small quantities of experimental diets. However, care should be taken that the temperature in feed storage areas does not exceed 21°C (NRC 1996). Further, good management practices dictate that feed be utilized within 180 days of manufacture, not days of receipt (NRC 1996). Fullerton et al.
(1982) compared the effects of storage conditions on the nutritional quality of a semipurified diet and a natural ingredient diet. Levels of thiamine, vitamin A, peroxide values, and mold and bacteria counts were followed for 6 months. The natural ingredient diet remained satisfactory under all storage conditions, including a sample stored at temperatures varying between 23° and 30°C. The semipurified diet showed significant deterioration at 20°C but not at 4°C or below. These limited studies suggest that natural ingredient diets may be stored for longer periods at room temperature than has been previously thought. In a limited study Oller et al. (1985) found no changes in mice receiving an autoclaved diet stored for 130 days. Autoclaved diets often become too hard for mice to chew. Thigpen et al. (1993) describe the use of a device for measuring the hardness of pelleted rodent diets.

All bags of feed and bedding should come marked with the date of manufacture and the batch number. Uncoded (open dating) is now generally used. If you purchase feed or bedding from a manufacturer who uses a code, ask for the information necessary to read the code. When writing contracts for feed and bedding, make open dating a requirement. Quality assurance of rodent diets has been discussed (Newberne and Fox 1980).

Depending on the type and quality of bedding purchased, the microbiologic status can vary considerably. Most reputable manufacturers of bedding today are able to supply data describing the general microbial content and chemical profile of their beddings. Many bedding products are natural plant materials, processed into a particular size and form. Unless they are protected from contamination, heat treated or steam sterilized, and properly bagged, they are may contain high counts of microorganisms. These microbes are not necessarily pathogens. An outbreak of murine leptospirosis was associated with the use of unprocessed soft wood sawdust, which most likely became contaminated by wild mice (Stoennner et al. 1958). In another case, unheated wood chip bedding contaminated with Aspergillus fumigatus altered the immune response of mice exposed to the bedding and was associated with the widespread contamination of several vivarium facilities (Mayeux et al. 1995). Unless it is known to be free of pathogens, bedding is best stored separate from feed. Different bedding products have various levels of dust content generated during the manufacturing process. Dust can be a problem for both the mice and the people who must work with the bedding. Bedding dust is especially a problem for athymic mice which are prone to developing conjunctivitis due to dust. Thigpen et al. (1989) developed a standard method for measuring the size of bedding particles and the dust content. Bedding storage areas should receive the same considerations as feed storage areas except that the temperature need not be as tightly controlled. It is helpful if the nature of the source(s) and processing of the bedding used is understood. Consultation with suppliers is helpful. Kraft (1980) reviewed rodent bedding materials.

VI. SANITATION

A. Background

The science of preventing, monitoring for, controlling, and managing environmental-based infectious disease outbreaks in human health care facilities has developed into a professional specialty. Government (Centers for Disease Control and Prevention) and professional organizations (Association for Professionals in Infection Control and Epidemiology, Inc, APIC) have developed guidelines, published conference proceedings, and provided training and continuing education on the subject. When it comes to maintaining a clean and disease-free vivarium, managers of animal facilities share many goals with and face many of the same problems as their counterparts in human health care facilities. The body of research in the broad field of infection control applied to human health care facilities and the resulting guidelines serve as an excellent platform for adapting and developing similar programs for animal facilities.

Guidelines for Environmental Infection Control in Health Care Facilities (2003) from the CDC is a four-part document available on-line at www.cdc.gov/ncidod/dhqp/glenvironinfection.html. It is authored by members of the CDC and the Health Infection Control Practices Advisory Committee (HICPAC). See Sehulster et al. (2004) for the full print version. Part II contains all of the recommendations with pertinent references. In addition to being part of the total report, it was published separately in MMWR 52(No. RR-10):1-44. It too is available on-line at www.cdc.gov/mmwr/pdf/rr/rr5210.

A comprehensive sanitation program based on science and need is essential for the proper maintenance of an animal facility. The presence of large numbers of animals with the accompanying quantities of feed and bedding and resulting waste creates an opportunity to host colonies of wild and feral rodents and insects. At the same time, many pesticides, cleaning agents and deodorizers have the potential to interfere with experimental studies by altering the animal’s metabolism (Cinti et al. 1976; Jori et al. 1969; Lang and Vesell 1976; Vesell et al. 1976). Therefore, careful consideration needs to be given to the selection and use of chemical-based products in animal facilities.

Although not within the purview of this chapter, it is worth noting that good design takes into consideration the daily operation and maintenance of a facility. Time spent during the design stage to consider sanitation, waste management, and pest control issues will pay dividends later in efficiency and reduced operating costs.

B. Disinfectants and Their Use

Disinfectants are classified under Federal law as antimicrobial pesticides and are regulated by the EPA as provided for in
the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (www.epa.gov/pesticides/regulating/laws EPA 2006). (Sanders 2004)

Spaulding (1968) first introduced the concept of associating risk of infection in patients with the selection of a chemical disinfectant or sterilant. He assigned items three levels of risk: critical, semi-critical, and noncritical. Critical refers to items that are introduced into the body, for example, surgically or via a blood vessel and come in contact with sterile tissues. Such items must be sterile. Semi-critical refers to those items that contact mucous membranes or nonintact skin. Semi-critical items are often sterile; however, bacterial spores may be present in low numbers. Noncritical refers to items that contact intact skin, but not mucous membranes or sterile tissues. As this scheme of assigning risk for infection to the selection of sterilants and disinfectants is applied to animal facility management, the similarities between human health facilities and vivariums become evident.

Definitions (Favero and Bond 2001)

**Sterilization:** Physical or chemical procedure to destroy all microorganisms, including large numbers of resistant spores.

**Disinfection:** Eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms on inanimate objects.

**Decontamination:** Process or treatment that renders a medical device, instrument, or environmental surface safe to handle. Decontamination procedures can range from sterilization or disinfection to cleaning with soap and water.

**Antisepsis:** A germicide applied to the living skin or tissue for the purpose of inhibiting or destroying microorganisms.

High-level disinfectants: Demonstrate activity against spores (glutaraldehyde, formaldehyde, hydrogen peroxide, chlorine dioxide, peracetic acid). Note: Formaldehyde is now considered a carcinogen and should no longer be used as a disinfectant (ATSDR 1999).

**Intermediate disinfectants:** Not necessarily able to kill spores but will kill *Mycobacterium tuberculosis var bovis* (70–90% ethanol or isopropyl alcohol, sodium hypochlorite, 500 ppm, some phenolic and iodine preparations).

**Low-level disinfectants:** Those disinfectants that cannot be relied on to destroy *Mycobacterium tuberculosis var bovis*, bacterial spores, all fungi or small nonlipid viruses (quaternary ammonium, certain iodophors and phenolics, varies with concentration and time).

In application, the comparison to surgical intervention and vascular access in humans and animals is direct and obvious; however, there are some significant differences in the use of higher level disinfectants in animal facilities.

Mice in a state-of-the-art vivarium housed in individually ventilated cages, breathing HEPA filtered air, drinking filtered or acidified or hyperchlorinated water, eating sterilized or pasteurized diet, and housed on sterilized bedding usually never have contact with other mice outside of their home cage. They live in a virtual “Petri dish.” Studies in human health care facilities have identified noncritical environmental surfaces as sources of bacterial and viral organisms for patient and staff infections (Rutala and Weber 2004a). Many mice received from vendors today are disease free and have a limited microbial flora in the gastrointestinal tract. In many situations the intent is to continue to limit the mouse’s contact with extraneous microorganisms. To meet this goal, a high level of surface disinfection is used to maintain the animal rooms, cages, and equipment associated with changing cages. The concern is that any item or surface contacting the mouse or its cage may serve as a source of adventitious microorganisms. The use of high-level disinfectants for routine disinfection of environmental surfaces in the mouse room runs counter to the *Guidelines* (CDC 2003). However, this use is done with the understanding that mice may become exposed to adventitious microorganisms present on environmental surfaces when outside their cage. This exposure becomes even more of a threat if the mice involved have a compromised immune system. Modern vivariums for mice are often operated on a basis of zero tolerance for the presence many infectious agents.

Selection of the class of disinfectant-detergent agent should be the result of professional judgment. The guidelines for routine cleaning and disinfection of floors and other smooth hard surfaces are as follows: Select EPA registered disinfectants, and use them in accordance with the manufacturer’s instructions (CDC 2003). All EPA registered disinfectants are assigned a registration number and have passed basic tests to support claims for efficacy against specific organisms. In-house testing of disinfectants using the various official tests is not recommended (Mallison 1977). A difference between disinfectant-detergents for the human health care environment and the vivarium environment is that items for use in research animal facilities should not contain perfumes and odiferous essential oils that can affect an animal’s metabolism. Regardless of the disinfectant-detergent selected, “elbow grease” is an important ingredient in controlling microbial contamination. Cleaning must always precede high-level disinfection and sterilization (Rutala and Weber 2004b). Although this statement was made with regard to medical devices, it also applies to many environmental surfaces in a mouse vivarium housing disease-free mice with a limited microbial flora. A product compatible with local tap water, not overwhelmed by organic soil, and used properly should give good results (Mallison 1975). Ability to clean is not assessed by the EPA tests; however, adequate detergency should be a major factor in selecting registered disinfectant-detergents. A physically clean surface is a critical aspect of ensuring decontamination. It may be necessary to preclean a severely soiled surface with a heavy duty detergent prior to use of a disinfectant-detergent. When surfaces are known to be contaminated with pathogens, inactivation of pathogens may take precedence over cleaning. It may be necessary to repeat the decontamination process followed by additional cleaning.
People applying disinfectants must be trained in their safe application. Routine mopping of floors is best done using a two-bucket system; the first containing diluted disinfectant, the second clear rinse water. A dry, freshly laundered mop head should be used each time. Disinfectant is applied for 10 minutes or as the manufacturer indicates, before it is picked up. Enough disinfectant solution must be applied to keep the surface wet for the allotted time. Each time before the mop is returned to the bucket of disinfectant, it is rinsed in the clear water. The rinse water should be changed as it becomes dirty.

The use of disinfectants in an animal facility should be monitored to ensure correct use. Mice can be affected through direct contact with (touch or ingestion) disinfectants (Serrano 1972) or inhalation of the volatile chemicals they contain (Conney and Burns 1972; Jori et al. 1969; Kulkarni and Hodgson 1980; Vesell et al. 1976). Disinfectants are commonly purchased in drums and dispensed into smaller containers. To prevent possible injury to the animals and people as well as the waste of material, the use dilution of the disinfectant must be monitored. This is most frequently done by dispensing premeasured packages of disinfectants soluble in water, use of dispensing pumps, or dedicated measuring cups.

Note: Users of disinfectants need to be aware that the evaluation of disinfectants for efficacy is currently a very active area of research. Many new test methods have been described, and a great deal of international cooperation among several organizations has taken place (Sattar and Springthorpe 2004). Confidence in the long-time standard AOAC Use-Dilution test has been questioned following a collaborative study among 18 experienced laboratories. These laboratories had difficulty reproducing the manufacturer’s bactericidal label claims. There was great variability in the test results among laboratories testing identical products (Cole et al. 1988; Rutala and Cole 1987).

The chemical bases for most disinfectant-detergent combinations presently sold for the purpose of routine sanitization and cleaning in animal facilities include substituted phenols (phenolics), quaternary ammonium compounds (quats), and iodophors. Each has advantages and disadvantages. Solutions of all three of these disinfectants, under some conditions, are permisive to the survival or growth of gram-negative bacteria (Rutala and Cole 1984). The substituted phenols exhibit activity against most vegetative forms of bacteria (including Mycobacterium spp.), fungi, and some viruses. They maintain this activity in the presence of moderate soil loads and can be formulated to have excellent detergency. Disadvantages are their toxic nature and the resistance of certain phenolics to biodegradation (Voets et al. 1976). Skin contact should be avoided as phenolics may depigment skin (Kahn 1970). Mixed with other chemicals, they may form toxic lethal compounds (Lynch et al. 1975). A laboratory study has suggested that the rotation of phenolic disinfectants—one alkaline, one acid—improved efficacy over time against P. aeruginosa adherent to stainless steel (Conner and Eckman 1993).

This observation may have been influenced by the number of organisms present in the test situation. Under field conditions, emergence of microbial populations resistant to disinfectants does not appear to be a problem. Rotation of disinfectants to prevent selection for resistance is not necessary provided environmental monitoring data show a consistent microbial profile (Kopis 2001). However, in the pharmaceutical industry at least, rotation of disinfectants used in aseptic processing areas is almost universally practiced (Denny and Marslk 1997). In a study comparing hospital isolates of antibiotic resistant bacteria to stock cultures of sensitive bacteria only in 1 of 20 comparative trials did antibiotic resistant bacteria exhibit increased resistance to a disinfectant. The authors concluded that the development of antibiotic resistance does not appear to correlate with increased resistance to disinfectants (Rutala et al. 1997). The question of germicide use and development of bacterial resistance or tolerance to germicides and antibiotics has been reviewed (Favero 2004; Russell 2004).

Quats are relatively mild cationic chemicals active against many bacteria and fungi and enveloped viruses. They are not sporicidal and most are not tuberculocidal (Best et al. 1990; Rutala et al. 1991). Quats are formulated in many ways with various detergents and are the most widely used general purpose disinfectant-detergent combination for hard surfaces. Quats have considerable detergent activity in their own right; however, they are inactivated by natural soaps and other anionic substances. They do not tolerate soil well and are less effective against gram-negative organisms than against gram-positive organisms.

The iodophors are iodine complexed with an organic molecule, usually polyvinylpyrrolidone as a carrier and combined with a detergent at an acid pH. Iodophors are also formulated for application to human and animal skin and mucous membranes. Do not substitute environmental disinfectant-detergent formulations of iodophors for formulations designed for use on skin. Detergent-disinfectant formulations contain acids, usually phosphoric or hydrochloric and detergents. Activity is measured in terms of available iodine and generally 75–150 ppm is used for routine sanitation. They are active against Mycobacterium sp. provided the recommended dilution is maintained, and the temperature and contact time requirements are followed. Iodophors are quickly inactivated by heavy soil loads. They have the advantage that the amber-brown color indicates active iodine and the usefulness of the solution can be monitored. Staining of painted surfaces and fabrics occurs at the higher concentrations. This may be permanent or temporary. Some metals are corroded by iodine, and prolonged contact should be avoided. Because of its volatility, iodophors should not be used in water over approximately 40°C. During an outbreak of mousepox, a decontamination protocol using an iodophor disinfectant was developed for use in animal rooms that could not be decontaminated with formaldehyde gas (Small and New 1981).
When it becomes necessary to decontaminate an animal facility due to a disease outbreak or as part of a scheduled maintenance, routinely used disinfectant-detergents may not suffice. In the case of a disease outbreak, it is essential to know the causative infectious agent, its vulnerabilities to disinfectants, and characteristics. Decontamination is only part of a plan to deal with a disease outbreak. Use of a high level sterilant or disinfectant with sterilization of environmental surfaces may not be necessary or possible in all cases.

In the past, formaldehyde gas, usually generated by heating solid paraformaldehyde in an electric frying pan was the standard method for high-level decontamination of animal facilities (Grossgebauer 1975; Songer et al. 1972; Taylor et al. 1969). Although very effective and relatively inexpensive, formaldehyde has been listed as a carcinogen (www.atsdr.cdc.gov) and should be avoided if possible (Rutala 1990). Formaldehyde has been largely replaced by vaporized hydrogen peroxide (VHP) and gaseous chlorine dioxide when fumigation is required. Often, depending on the microorganism involved, a less active disinfectant will suffice and be more convenient to use.

VHP is an antimicrobial pesticide registered by the EPA to kill bacterial spores on environmental surfaces in an enclosed area. It is used in commercial, institutional, and industrial settings to decontaminate or sterilize sealed enclosures such as isolators, workstations, pass-through rooms, medical and diagnostic devices, and for other biologic safety applications (EPA 2003). VPH is a low-temperature sterilant created by a machine, a “generator” (www.steris.com). It has been demonstrated to be effective in providing high-level disinfection and sterilization at the room level. Rooms can be equipped with ports to which the generator is docked while the VHP is pumped into the room (Block 2001; EPA 2003; Jacobs and Lin 2001; Steris M1941EN-www.steris.com).

Chlorine dioxide is an antimicrobial pesticide recognized for its disinfectant properties since the early 1900s. Chlorine dioxide boils at 11°C and is a gas at room temperature. At 25°C chlorine dioxide gas has a solubility of 3.01 g/L. In its gaseous phase, chlorine dioxide is stable in concentrations of less than 10% in air at atmospheric pressure. Unstable in light and in concentrations (gaseous phase) greater than 10% at atmospheric pressure, it is easily detonated by sunlight or heat (Merck Index 2001). Chlorine dioxide cannot be stored in compressed form. It is used as an environmental disinfectant primarily as a gas dissolved in water. Antimicrobial activity is unaffected in the pH range of 6–10. This is markedly different from chlorine (Knapp and Battisti 2001).

Chlorine dioxide was first registered by the EPA in 1967 in its liquid form as a disinfectant and sanitizer. In 1988, chlorine dioxide gas was registered as a sterilant. Chlorine dioxide kills microorganisms by disrupting transport of nutrients across the cell wall. It can be generated in a gas or liquid form and smells like chlorine bleach. Chlorine dioxide should not be confused with chlorine gas. They are two distinct chemicals that react differently and produce-by-products that have little in common (EPA 2006a). Chlorine dioxide can be produced as a gas from a generator in much the same manner as VHP (www.clordisys.com). Liquid chlorine dioxide sprayed as a mist on all reachable hard surfaces is used to decontaminate rodent facilities. Currently, chlorine dioxide solutions are often used in animal rooms to decontaminate working surfaces and the surface of equipment when manipulating mice outside of their cage and a high level of disinfection is desired. Unlike sodium hypochlorite solutions, chlorine dioxide is not corrosive and not inactivated by organic matter.

Peroxymonosulfate, a powerful oxidizing agent with a broad spectrum of antimicrobial activity, including spores, has found considerable use in disinfecting hard surfaces (Block 2001). Peroxymonosulfate (Virkon-S) was studied for use against Salmonella spp. and Staphylococcus aureus on hard surfaces in a veterinary teaching hospital (Dunowska et al. 2005; Patterson et al. 2005). In laboratory study, peroxymonosulfate (as well as chlorine dioxide) inactivated feline herpesvirus, feline calicivirus, and feline parvovirus (Eleraky et al. 2002).

Sodium hypochlorite (Na hypochlorite) sold as household bleach (approximately 5.25–6%) has been used for many years as an economical and potent source of chlorine for purposes of disinfecting hard surfaces (see review by Rutala and Weber 1997). Na hypochlorite, depending on its concentration is recognized as a broad-spectrum disinfectant with activity against bacteria (including mycobacteria and spores), viruses, and fungi. Depending on its dilution, it has been used to decontaminate water, environmental surfaces, and equipment. As Dakin’s solution (usually approximately 0.5% Na hypochlorite), it was first used about 1915 to irrigate and decontaminate wounds in people and animals. In laboratory animal facilities, diluted household bleach has been used for spot decontamination. Solutions of 1000 ppm Na hypochlorite killed >99.9% of human coronavirus and human parainfluenza virus (Sattar 1989 cited in Rutala and Weber 1997). Rutala et al. (1998) demonstrated that dilute solutions of household bleach (1:50 and 1:100) retained 40% to 50% of their chlorine over a 30-day period in translucent polyethylene containers stored in light at 20°C and 97–100% for 1:50 and 1:5 dilutions stored in closed brown containers. Na hypochlorite’s corrosiveness and its neutralization by organic matter are significant disadvantages. Also, Na hypochlorite reacts with ammonium hydroxide and acids to produce noxious toxic gases. However, Na hypochlorite remains a widely used and very valuable disinfectant.

Alcohol, either isopropyl or ethyl (62–91%) is a very effective and useful spot disinfectant on hard surfaces. It is very rapid in action against bacteria, including mycobacteria, fungi, and some viruses but not spores. Because of toxic fumes and flammability concerns, alcohol should only be used to disinfect very small areas (Ali et al. 2001).

For in-depth resources on disinfectants, sterilization, and infection control in health care facilities, see Block (2001), Wenzel (2004), and Mayhall (2004).
C. Microbiologic Monitoring

The Guidelines state: “The scientific basis to support the random, undirected, microbiologic sampling of air, water, and environmental surfaces in health care facilities is lacking” (Schulster 2004). However, that said, there are times and reasons for monitoring. The Guidelines go on to provide guidance. When indicated, conduct microbiologic sampling as part of an epidemiologic investigation or during assessment of hazardous environmental conditions to detect contamination or verify abatement of a hazard. Further in part, limit microbiologic sampling for quality assurance purposes to biologic monitoring of sterilization processes. (Garner and Favero 1986).

The acquisition of infections by disease-free mice on entering or while in an animal facility is not unlike people acquiring an infection while hospitalized or while working in a health care facility. Such infections are referred to as nosocomial infections. The infective microorganisms may originate either from endogenous sources, as indigenous commensal flora carried by the patient (mouse), or from exogenous sources, as recent acquisitions from animate or inanimate objects within the hospital (animal facility) (modified from American Hospital Association 1974). Direct or indirect contact with mice originating from different sources or that have acquired infectious agents since leaving their colony of origin is the most common means for disease-free mice to become infected. In addition, feed, bedding, cages, and experimental apparatus with which the mouse comes in contact are all potential vectors of undesirable microorganisms.

The epidemiology of nosocomial infections in humans and the recommendations for their control is instructive for those who must develop environmental monitoring and animal health monitoring schemes for laboratory animal facilities and colonies. The following is abstracted from the American Hospital Association’s (1974) “Statement on Microbiological Sampling in the Hospital.”

Although the American Hospital Association no longer issues this publication on microbiological sampling in the hospital, it is felt that this statement from 1974 is so clearly written, to the point, and germane to the management of mouse colonies, that it deserves retention in this 2nd edition of the *Mouse in Biomedical Research*. The focal point of hospital infection control must necessarily be the patient; both the patient who already has an infection and the patient who does not have an infection but is at risk of acquiring one. In either instance, measures must be directed toward preventing spread of infection from any source to non-infected personnel or patients. It is from this point of view that microbiologic sampling must be considered and its value judged.

“Routine environmental” microbiologic sampling programs are those programs conducted on a regularly scheduled basis. They include sampling of air, surfaces, linens, fomites, and so forth in patient care areas, surgical suites, and nurseries irrespective of specific nosocomial infection problems.

The Committee on Infections within Hospitals is of the opinion that “routine” microbiologic sampling of the hospital environment, done with no specific epidemiologic goal in mind, is unnecessary and economically unjustifiable. Unfortunately, in many hospitals, environmental sampling programs appear to have taken the place of infection surveillance programs. As a result, in some hospitals the infection committee and hospital administration have acquired much uninterpretable and often irrelevant data about the levels of microbial contamination on floors, walls, and linens and in the air, but little or no knowledge of the frequency of occurrence of hospital-acquired infection.

Microbiologic sampling of the hospital environment must, therefore, always be a means to an end, never an end in itself.

The most useful role of microbiologic sampling lies in the investigation of specific problems within the hospital, and here it should be considered a necessary adjunct to the infection control program.

The Committee on Infections within Hospitals recognizes the necessity for carrying out a certain number of routine sampling procedures as quality control checks of sterilization procedures.

In summary, microbiologic sampling procedures, if carried out when indicated in the investigation of specific epidemiologic problems, can be extremely helpful in the control of nosocomial infections. Similarly, quality control of disinfection and sterilization procedures is justified on a planned basis, particularly when new methods are introduced. Much research remains to be done in order to define possible reservoirs within the hospital of many organisms associated with nosocomial infection. “Routine” microbiologic sampling of the hospital environment, however, not only has provided data that are impossible to interpret but also has contributed little to hospital infection control.

The Committee on Infections within Hospitals concludes that “routine” environmental sampling is unnecessary and wasteful.

Although routine environmental monitoring is not indicated for animal facilities, monitoring of decontamination procedures following an outbreak of disease or prior to stocking rooms with defined flora animals is a prudent practice. The swab method and contact agar plates as mentioned previously for use in assessing cage washer effectiveness are the two most frequently used means of checking surfaces for adequate decontamination. When sampling flat surfaces by the swab technique, using a heavy-gauge metal or plastic template with a standard size opening, for example, 5 cm × 10 cm, provides a degree of repeatability. Between uses the template is cleaned with alcohol and allowed to dry. Samples are collected 24 h following decontamination, during which time the surface has dried (and the room has been vacant). The swab is moistened with lecithin broth from a tube containing 9 ml, wrung out, and vigorously rubbed over the designated area in both directions. The swab is broken off in the tube of broth. After shaking to vigorously rub over the designated area in both directions. The swab is broken off in the tube of broth. After shaking to
prior to plating assists in recovering those few organisms that may be present. By standardizing the previous procedure and using a calibrated loop to streak the plates (or make pour plates), a degree of quantitation can be achieved and results can be compared over time. Methods for the microbiological sampling of surfaces have been reviewed (Bond and Schulster, 2004; Favero et al., 1968).

VII. MONITORING FOR PESTS

Animal facilities, with supplies of food, water, bedding, and shelter, plus large amounts of waste material, lend themselves to harboring vermin. Surveillance for pests is an integral part of environmental monitoring in an animal facility. By developing an integrated pest management (IPM) program that identifies potential and existing pest problems and acceptable strategies for dealing with them, the number of pests present can be greatly reduced. Importantly, the number and amount of pesticides used can be minimized (Greene and Breisch 2002; Kramer 2004; Williams 2005). An IPM program is focused on three issues: prevention, monitoring, and control, with the emphasis on prevention and monitoring. Control, with the minimum amount of toxic pesticides, is the smallest element of the IPM program. The IPM program should extend beyond the animal facility to include the landscape features and especially the interface of the buildings with the ground.

Managers of animal facilities need to be aware of conditions and pest management programs in place at facilities of vendors, especially those supplying food and bedding. It is reasonable to request that feed and bedding dealers inform you about their pest management program and make available pest management reports pertaining to their facilities. Periodic visits of these dealers with a walk through inspection of their facilities should be a condition of doing business with them. Movement of wild rodents, other mammals, birds, and insects into an animal facility is a constant threat to the health of the experimental animals (as well as to the health of the staff). The level of infestation with pests is an excellent index of the overall management and sanitation of an animal facility.

A vivarium staff knowledgeable about IPM is a prevention-oriented staff. A thorough understanding of the biology of the pests likely to be encountered is essential to preventing them from gaining entrance. Knowledge of pesticides and their effects on animals and humans is critical; otherwise, efforts may be wasted, experiments jeopardized, and people endangered by the incorrect selection and application of chemicals. Senior management must be responsible for approving all use of pesticides within and around the vivarium. Many publications are available that provide information on pests and their control (Ebling 1978; Grad 1980; Mallis 2004). In addition, anyone with responsibility for the use of pesticides needs to be familiar with applicable federal and state laws. The EPA regulates the use of pesticides under the authority of two federal statutes: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (www.epa.gov/pesticides/regulating/laws.htm) (EPA 2006). FIFRA provides the basis for the regulation, sale, distribution, and use of pesticides in the United States. It also authorizes EPA to review and register pesticides for specific uses. The FFDCA authorizes EPA to set maximum residue levels, or tolerances, for pesticides used in or on foods or animal feed (Diederich 2004).

Based on the complexity of the current rules, regulations, and laws pertaining to the acquisition and legal use of pesticides, it is recommended that research facilities contract for the services of an experienced pest management professional as a consultant. If the facility or institution is large enough, perhaps a full-time pest management professional can be supported. Selection of this person must be done with care as he or she will become a member of the vivarium management team and his or her decisions will affect research. Ideally, the person will have experience working with and in research facilities, be knowledgeable about pest management strategies for vivariums, and understand the needs of the vivarium staff and scientists, working with them to accommodate those needs. He or she should also be familiar with current strategies and issues in pest management and be knowledgeable of applicable federal, state, and local laws. As part of the selection process, interviews of prospective candidates with the candidate making a presentation to the vivarium staff on pest control in research facilities should be considered. Management should determine that all required professional licenses, insurance, and bonds are in place and that presented credentials are accurate. Because of the importance proper pest management plays in the operation of a modern vivarium, it may be to the advantage of the vivarium manager if he or she assumes responsibility for the institution's IPM program.

Before a pest management program is initiated, a basic decision needs to be made, namely, is the program's intent to reduce the level of infestation to zero or will a certain low level of infestation be tolerated, and if so, what levels of each pest. In modern rodent vivariums the emphasis of an IPM program should be exclusion of pests with zero tolerance for their presence. Management practices should support this position. Facility design should take into account exclusion with a zero tolerance for pests. To this end, it is recommended that budgets for new construction or remodeled space include funds to treat buildings during construction to exclude pests (Tarshis 1964). Total elimination of pests from facilities with established populations is not impossible; however, it is much more difficult and costly than implementing a preventive program during construction.
A. Prevention Programs

An IPM program for a rodent vivarium is primarily focused on invertebrate pests and wild and feral rodents. Keys to prevention are (1) a continuously monitored sanitation program; (2) examination of incoming supplies, especially packaging material, decontamination of outer wrapping prior to entry if necessary; (3) understand the behavior of pests most likely to invade the vivarium and block them (know the enemy!); (4) make pest prevention and monitoring part of everyone’s job, train the staff and vivarium users alike; (5) continually monitor and maintain the physical integrity of the facilities and surrounding grounds; (6) especially monitor entry points, including the loading docks and outside trash containers; (7) respond rapidly and effectively to discovery of pests; and (8) fully document and retain records of IPM program activities, especially occurrence of pests and actions taken.

Monitoring for the presence of pests in animal facilities is important. A surveillance program for escaped laboratory rodents and wild rodents in animal facilities generally makes use of traps in each animal room and in service areas, including the loading docks and cage wash facilities. Traps can be of the common snap type designed to kill or a live trap capable of catching several mice. Also, glue boards and other adhesive-based traps can be used. They have the advantage of being effective in trapping insects as well as rodents. These traps are placed for the purpose of monitoring, not eliminating or controlling, a pest population, should one be present. For humane reasons as well as for monitoring purposes, traps should be examined daily. Packs of gelled water can be added to enclosed live traps to provide a water source. This becomes important when traps cannot be examined every day (Caviness et al. 2004). Trapped rodents should form part of the population examined in the vivarium’s disease monitoring program. Records of mice caught in animal rooms can be useful indicators of possible disease problems and failures in the physical plant and breaks in the management program. Trapped mice and rats should be handled with the thought that they may be carrying zoonotic diseases. A high percentage of wild rats and mice carry Leptospira spp., and mice may carry lymphocytic choriomeningitis virus. In addition, they may also carry a number of other pathogenic bacteria and parasites. By requiring a daily record of mice caught and their coat colors, attention is focused on the problem of cage and room security. Something is wrong if other than albino mice are trapped in a room housing only albino strains. Infestations with rats and mice can be detected by looking for droppings, rub marks, urine, tracks, gnawing marks, nests, and runs and by noticing their odors. Bait stations commonly used outside of and in surrounding buildings need to be monitored for activity. Burrows need to be noted, investigated for activity, depopulated, and sealed (Corrigan 2004; Howard and Marsh 1976; Marsh and Howard 1977; Pratt et al. 1976). Amazingly, adult mice can penetrate openings only 10 mm wide (Rowe 1981) and, in general, wild mice are very athletic, being able to jump 30.5 cm vertically from a flat surface, jump from a height of 2.5 m without injury, run up rough vertical surfaces, run along wires and ropes, and swim if necessary (Marsh and Howard 1977).

The presence of invertebrate pests is a frequent problem in animal facilities. Cockroaches and flies present most of the problems, although stored-grain insects and grain mites may also present problems. The cockroaches include about 7500 described and undescribed species (Roth and Willis 1960); however, the species of major concern in animal facilities in the United States is the German cockroach Blattella germanica (L.). Other domestic species found in the United States, the brown-banded cockroach Supella longipalpa (L.), the oriental cockroach Blatta orientalis (L.), and the American cockroach Periplaneta americana (L.), are less numerous in buildings. The given names are misleading as all species that have adapted to living with humans are considered to have originated in north or tropical Africa (Ebeling 1978).

Monitoring programs for the presence of invertebrate pests in rodent facilities primarily makes use of sticky traps and visual inspection for cockroaches. Flying insects, usually flies, are also monitored visually. Light traps (LTs) placed indoors near entrances can be useful for both monitoring and controlling flying insects, especially flies (Gilbert 1984; Harris 2006; Kolbe 2004; Lillie and Goddard 1987; Pickens 1989). LTs make use of blacklight (BL), blacklight blue (BLB), or black-light green (BLG) fluorescent bulbs in combination with an electrical grid that kills the insect or a sticky panel that traps it. The bulbs produce light in the ultraviolet A (UVA) band, 320–400 nm, with a peak near 365 nm. Most flying insects are phototropic, with their greatest response to light in the UVA band. However, different insects have preferences for specific wavelengths within this band. This allows one to set up a LT with tubes most attractive to the insects of interest. Pickens (1989) showed that combinations of BL and BLB bulbs did not attract more house flies, Musca domestica L., or stable flies, Stomoxys calcitrans L., than were attracted by BL bulbs used alone. BLB bulbs are more effective in attracting insects of the orders Lepidoptera and Coleoptera. BLG bulbs emit UVA and visible green light. This bulb has been shown to attract more flies than a standard BL bulb (Harris 2006). All UVA emitting bulbs have a limited effective life of about 7000 h. At this point UVA emissions are reduced to 50% or less of original output. Bulbs must be replaced every 10–12 months for the traps to remain effective. It is a good idea to synchronize replacement of the bulbs with the peak of the insect season. Do not use UVB (tanning) or UVC (germicidal) bulbs. They are not effective in attracting insects and can cause injuries.

Several different types and designs of traps exist. Some use glue boards rather than electrical grids. If placing an electrical LT in new construction or remodeled space, remember to include a 115-volt switched outlet for each LT.
Placement of traps is as important as is maintenance. In a vivarium, LTs serve useful dual purposes, as monitors and as a means of control; however, they should not be needed in or adjacent to the animal rooms. Flies generally move close to the floor, and LTs are most effective if placed vertically and within 5 feet of the floor (Pickens et al. 1969). Incorrectly placed, they may attract flies into a facility. LTs should not be seen from outside entrances as they will draw insects into the building. Study of the references and consultation with pest management experts prior to specifying or ordering LTs is recommended. Correct selection and placement are important to derive maximum benefit. Identification of insects caught in LTs can yield valuable information relating to their origin.

Glue boards present for trapping mice or rats will also trap invertebrates. Glue traps designed for catching crawling insects are also available. Again, identifying what is caught is important. Pyrethrum sprays have been used for years as flushing agents to force cockroaches out of hiding (Barcay 2004). This can assist in determining population densities. However, use of chemical sprays in a research facility is to be avoided. Pheromone traps are used for monitoring for several stored grain pests (Barcay 2004; Mueller 2004). A pheromone from the saliva of the German cockroach, *B. germanica* is used in a commercial trap (Mueller 2004). A recent advance is the isolation, characterization, and synthesis of the sex pheromone of *B. germanica* (Nojima 2005). If it becomes possible to synthesize this pheromone at a commercial level, this discovery will revolutionize the control of the German cockroach.

Many bacteria, fungi, and viruses have been demonstrated to be harbored either naturally or experimentally by cockroaches (Barcay 2004; Roth and Willis 1957; Zurek and Schal 2004). *P. americana* given mouse encephalomyelitis virus shed virulent virus for at least 7 days (Syverton and Fischer 1950), and four strains of human poliomyelitis virus were isolated from three species of cockroaches from premises of paralytic poliomyelitis patients (Syverton et al. 1952).

Cockroaches frequently enter facilities in feed and packaging material. Corrugated cardboard is a particularly good means for introducing roaches. Sticky traps are frequently used to monitor for cockroaches (Lavendar and Stark 1980).

Control of cockroaches depends on a high level of sanitation, denial of food and harborage, and, where required, selective use of pesticides. The use of nonvolatile blatticides as baits rather than the repeated application of volatile agents reduces the amount of pesticide used. Further, nonvolatile blatticides target specific populations of pests and reduce the chance that mice may be affected (Burden 1980). Boric acid (Ebeling 1978; Ebeling et al. 1966, 1967, 1968a, 1968b; Gore and Schal 2004; Slater et al. 1979) has been used with considerable success as has silica aerogel (Tarshe 1964). Several insecticides exert a high degree of repellency against the German cockroach, and although there may be considerable initial mortality, surviving roaches will avoid treated areas (Burden 1975; Ebeling 1978; Ebeling et al. 1966). Boric acid is readily accepted by the German cockroach, and poisoning occurs through both ingestion and by contact. Gore and Schal (2004) and Gore et al. (2004) studied the efficacy of borates plus sugar in the laboratory and in hog barns. They recorded a 90% kill of *B. germanica* in hog barns in 15 days using a 0.5–2.0% aqueous solution of boric acid with any of several different sugars at molar concentrations of 0.05–1.0. Silica aerogel can be repellent; however, if cockroaches cannot escape contact with the dust, it can be highly effective. Placement of silica aerogel in electrical raceways and other dry, closed spaces during and after construction where the substance will not be disturbed has proved effective in eliminating cockroaches.

A new group of blatticides are the insect growth regulators (IGR). These chemicals act like the growth hormones of insects or block development of chitin, the major building block of the cockroach’s exoskeleton. The juvenile hormone–like chemicals hydpropene and pyriproxyfen interfere with the cockroach’s ability to molt and develop into a sexually mature and normally appearing adult. These analogs of juvenile hormones work slowly by eliminating future generations of cockroaches. Inhibitors of chitin development, represented by the benzoylphenyl urea (BPU) group of chemicals, block development of the cockroach embryo. However, BPs also affect adults. Used in baits, these chemicals have gained popularity and great acceptance for control of the German cockroach in buildings as people need not come in contact with the product or the cockroaches that feed on it (Barcay 2004). IGRs are chemicals. Although their use in proximity to research animals appears to be safe, caution needs to be exercised and more research is warranted before they can be recommended for use within animal facilities.

Like cockroaches, the common domestic filth flies have numerous opportunities to establish themselves and breed around animal facilities. These flies include the common house fly (*M. domestica*) and related members of the Family Muscidae as well as members of the families Anthomyiidae, Calliphoridae, Drosophilidae, and Sarcophagidae. Flies found in animal facilities should be identified to aid in developing the proper response. Large flies may travel considerable distance and breed some distance from the vivarium. Small species usually breed within the facilities or near by. For help in identifying flies and other insect pests the use of illustrated keys is recommended (CDC 1969). These keys are out of print, but available from the CDC on a CD (www.cdc.gov/dpdx/HTML/CDCproducts..htm). Photographs and drawings of flies and other pests are found in Mallis 2004.

Spread of disease by flies is based on both experimental studies and on epidemiologic studies of disease (Kolbe 2004). More than 100 species of pathogenic microorganisms have been isolated from or associated with flies (Ebeling 1978). Flies have been shown experimentally to transmit hog cholera virus (Morgan and Miller 1976). Alam and Zurek (2004) isolated *Escherichia coli* O157:H57 from *M. domestica* in association with cattle. Zurek et al. (2001) inoculated *M. domestica* with *Yersinia pseudotuberculosis* and detected organisms for up to 36 h in the digestive tract.
Although not usually done within animal facilities, fly infestations can be quantified. Techniques include fly cards, sticky strips, grids, or traps (R.S. Patterson, personal communication, Insects Affecting Man and Animals Research Laboratory, ARSE Administration, United States Department of Agriculture, Gainesville, FL) (Dodge 1960):

1. Fly card: A white 3 × 5 inch file card is placed in a holder on the wall. The number of fly specks are counted in a 24-h period or any time interval as long as it is constant and the cards are placed where the flies normally rest. (Note: The house fly is attracted to sunlight.)
2. Sticky strips or panels: Commercial fly strips or panels coated with "TACK TRAP" (Animal Repellents, Inc., Griffin, GA).
   The number of flies captured after 24 h can be counted.
3. Electric grids: These can be used in windows and the dead flies counted after a 24-h period.
4. Cone trap: Usually used outside buildings.

Further information on the study of fly populations and monitoring and collecting techniques will be found in Kolbe (2004), Morgan and Pickens (1978), Murvosh and Thaggard (1966), and Pickens et al. (1972).

The key to controlling house flies is a high-level sanitation program and prevention of entry into animal areas by keeping doors and windows closed or using tight-fitting screens. Installation of air doors between two interior rooms is recommended (Kolbe 2004) as are LTs (see previous discussion). In both cases, professional assistance is suggested for selection and installation. Depending on the temperature, the house fly completes its life cycle in as few as 6 days (Metcalf quoted in Kolbe 2004) or 8 days (Ebeling 1978), and most other filth flies have a longer life cycle; therefore, if mouse cages are changed at least once a week and all waste is removed from the room and promptly disposed of, the animal room should not be a source of new flies. Wet mops not washed but left to dry can be a source of small flies. Filth flies lay their eggs in animal feces, garbage, lawn clippings, and rotting food. Therefore, close monitoring of waste removal and disposal by incineration or deep burial becomes essential to a successful fly control program. Migration of flies from nearby areas can be a problem. Although not strong fliers, houseflies can travel 1 or 2 miles and have been known to travel 28 miles (Ebeling 1978; Kolbe 2004).

Despite good sanitary practices, flies sometimes gain entrance to animal facilities. Their elimination, especially in animal rooms is important for effective disease control. Sticky strips are helpful in reducing the adult fly population in closed areas such as in animal rooms. A newer chemical means of controlling flies is the use of chemosterilants (Jurd et al. 1979). These methods offer the advantage of not coming in contact with the experimental animals; however, their effectiveness drops if flies are migrating into the area in large numbers. Space spraying, with its attendant problems of animal exposure to chemicals (and possibly people) is still used but rarely.

The most common agents are synergized pyrethrum, allethin, or the synthetic pyrethroid, resmethrin. Again, sanitation is the key to fly control not chemical pesticides, which may interfere with normal physiologic activity of the mice.

Both arthropod and rodent pests develop resistance to pesticides (Ebeling 1978); therefore, monitoring for pesticide effectiveness is an important aspect of any pest management program.

An outline for a total pest management program is beyond the scope of this chapter. However, the animal facility director needs to be thoroughly familiar with the IPM program in his or her facility and pest management in general. The program can be carried out by intramural staff or it can be contracted to a licensed pest management professional. In either case, the director of the animal facility must play a significant role in developing the program and he or she must monitor it carefully.

Currently in its 9th edition, the Handbook of Pest Control, by Mallis is the standard reference in the field of pest control. It is recommended as a general reference on pest management.

VIII. CONCLUSION

In its distilled form, much of environmental and equipment monitoring is common sense coupled with a constant awareness that an animal care program is a dynamic organization dependent on the interaction of animals and people with the physical facility. If the animals are to remain healthy and the physical plant is to continue to function properly, there is little room for error. The errors will arise not from the animals or the equipment but from the people who are responsible for developing a management policy and style that maintains the health of the animals and the integrity of the physical plant, yet at the same time makes it a pleasant and functional place for both mice and people to accomplish their intended tasks.

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