Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1504050

Duzce Med J, 2024;26(S1):19-23
Düzce Tıp Fak Derg, 2024;26(S1):19-23

Animal Experiments and Laboratory Safety within the Scope of Occupational Health and Biosafety

İş Sağlığı ve Biyogüvenlik Kapsamında Hayvan Deneyleri Çalışmaları ve Laboratuvar Güvenliği

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Received / Geliş Tarihi : 14.05.2024
Accepted / Kabul Tarihi : 14.06.2024
Available Online / Çevrimiçi Yayın Tarihi : 24.06.2024

ABSTRACT

Occupational health and biosecurity terms include scientific approaches regarding ethical values, reliable methods, and a series of precautions to be taken against risks and hazards for employees and the living material being studied. Research and development activities carried out within the scope of biotechnology involve many risks under laboratory conditions, which are their own physical area. Many precautions and practices have been defined to analyze these risks and take precautions before they occur, and laboratory guides have been created in line with these definitions. During studies carried out with experimental animals under laboratory conditions, safe working rules may be violated due to physical conditions, equipment and materials, treatments applied to the subjects, negligence, or faulty practices caused by the researcher or expert. Biosafety in animal experiments consists of a set of ethical conditions and practices declared in the guidelines of specialized laboratories at their own level, protecting employees, subjects, and life outside the laboratory. In this review, possible risks and dangers that may arise at different biosafety levels and the precautions and practices that can be taken against them are evaluated. In this way, it was aimed to contribute to the development of laboratory and biosafety criteria in animal experiments.
Keywords: Occupational health and safety; biosecurity; experimental animals.

ÖZ

İş sağlığı ve biyogüvenlik terimleri çalışanların ve üzerinde çalışma yapılan canlı materyalin etik değerler, güvenilir yöntemler, risk ve tehlikelere karşı alınacak bir dizi önlemlere dair bilimsel yaklaşımları içermektedir. Biyoteknoloji kapsamında gerçekleştirilen araştırma ve geliştirme faaliyetleri, kendi fiziki alanı olan laboratuvar koşullarında pek çok riski bünyesinde barındırmaktadır. Bu risklerin analiz edilerek, oluşmadan önlem alınmasına yönelik birçok önlem ve uygulama tanımlanmış ve bu tanımlar doğrultusunda laboratuvar rehberleri oluşturulmuştur. Laboratuvar koşullarında deney hayvanları ile yapılan çalışmalar sırasında fiziki koşullar, ekipman ve materyaller, deneklere uygulanan tedaviler, ihmaller veya araştırmacı veya uzmandan kaynaklanan hatalı uygulamalar nedeniyle güvenli çalışma kuralları ihlal edilebilmektedir. Hayvan deneyleri çalışmalarında biyogüvenlik ise kendi seviyesinde özelleşmiş laboratuvarların kılavuzlarında bildirilen, çalışanları, denekleri ve laboratuvar dışındaki yaşamı koruma altına alan bir dizi etik şart ve uygulamalarından ibarettir. Bu derlemede genel olarak farklı biyogüvenlik seviyelerinde ortaya çıkabilecek olası risk ve tehlikeler ile bunlara karşı alınabilecek önlem ve uygulamalar değerlendirilmiştir. Bu sayede, hayvan deneyleri çalışmalarında laboratuvar ve biyogüvenlik kriterlerinin geliştirilmesine katkı sağlaması amaçlanmıştır.
Anahtar kelimeler: İş sağlığı ve güvenliği; biyogüvenlik; deney hayvanları.

INTRODUCTION

As a social living being, humans have been carrying out many activities to meet their individual and community needs since their existence on earth. These activities reveal scientific and technological specializations according to the needs of the period, and this creates the basis for job and employee differentiation according to the power and technical capacities of individuals. However, these resulting specializations and differentiations have caused tragic work accidents, occupational diseases, poisoning, and deaths since the early periods of humanity.

The concept of occupational health and safety (OHS) comes from Hippocrates (460-370 BC), who detected lead poisoning in workers: "The cost of obtaining precious metals such as gold and silver; all substances are poison". There is no substance that is not poisonous. It emerged until Paracelsus (1493-1541) with his words "The appropriate dose reveals the difference between poison and medicine", and until Ramazzini (1), the founder of OHS, with his approach "Ask patients about their profession". Following the Industrial Revolution, there were historical cases such as diseases seen in workers, anatomical and physiological damages, necrotic wounds caused by chemical contamination, chimney sweep child workers, and London fires (2-4). These cases and the legal processes experienced during the period paved the way for the formation of today's occupational and worker health rules and organizations in the fields of health and work on an international scale World Health Organization (WHO), International Labor Organization (ILO) (5-7).

Today, OHS has begun to find a place for itself in every field by specializing in various branches of science in parallel with developments in technique and technology. Among the most prominent of these fields is biotechnology. "Biosafety" rules have been established to ensure the safety of researchers and technical personnel working in this field, as well as to keep the risk and stress factors of experimental animals used in research under control. In this context, OHS is a systematic and scientific study carried out to protect against conditions that may harm health arising from various reasons during the execution of work in the workplace. Biosecurity is defined as control principles, technologies, and practices applied to prevent exposure to biological agents for any reason or their uncontrolled release (8). Within the framework of both definitions, the field of study of the term biosecurity refers to all measures aimed at detecting the possible risks that the biological agents, living materials, and applied techniques used in modern biotechnology applications may pose to humans, animals, and other living and inanimate environments, and solving or controlling the problem at its source.

Research and development activities carried out within the scope of biotechnology involve many risks under laboratory conditions, which are their own physical area. Many precautions and practices have been defined to analyze these risks and take precautions before they occur, and laboratory guides have been created in line with these definitions.

This review aims to evaluate possible risks and dangers that may arise at different biosafety levels and the precautions and practices that can be taken against them with the purpose of contributing to the development of laboratory and biosafety criteria in animal experiments.

BIOSAFETY LEVELS AND RISK GROUPS

WHO (9) and US Centers for Disease Control and Prevention (CDC) animal biosafety level (ABSL) guide was created and possible risks were grouped under four groups.

Risk Group 1

In this group, there is no individual or social risk or the probability of occurrence is very low. This level of microorganism is unlikely to cause human or animal diseases.

Risk Group 2

In this group, there is a moderate individual risk and a low social risk. A pathogen that can cause human or animal disease but is unlikely to pose a serious hazard to laboratory workers, the public, livestock, or the environment. Laboratory exposures can cause serious infection, but effective treatment and preventive measures are available and the risk of spreading the infection is limited.

Risk Group 3

In this group, there is high individual risk and low social risk. A pathogen that usually causes serious human or animal disease but does not normally spread can be transmitted from one infected person to another. Effective treatment and preventive measures are available.

Risk Group 4

There is a high individual and social risk in this group. An easily cleared pathogen that usually causes serious human or animal disease is transferred directly or indirectly from one person to another. Effective treatment and preventive measures are often not available (Table 1).

Table 1. Characteristics of animal facilities and laboratory safety practices (9)

Risk Group	Biosafety Level	Laboratory Practices and Safety Equipment
1	ABSL-1	Restricted entry, protective clothing, and gloves
2	ABSL-2	In addition to ABSL-1 applications, danger warning signs should be used. Class 1 or 2 BSC should be used for activities that generate aerosols. Waste and cages should be decontaminated before washing.
3	ABSL-3	In addition to ABSL-2 applications, controlled entry. Special protective clothing must be worn for BSCs and all activities.
4	ABSL-4	In addition to ABSL-3 applications, full limited entry. Change of clothes before entering. Class 3 BSCs or positive pressure suits. You must take a shower upon exit. Decontamination of all waste before removal from the facility.

ABSL: animal biosafety level, BSC: biological safety cabinet

PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) includes wearable equipment worn by personnel to provide an additional barrier between personnel and biological agents under investigation, reducing the risk of possible exposure (10-13). Laboratory coats, gloves, various laboratory masks, caps, shoe covers, etc. materials are among the basic PPE. Basic protective equipment varies and specializes according to the job and risk definitions of the employees, laboratory safety levels, and the biological material to be used in the experiment (14,15).

It is an enclosed, ventilated work area designed to provide protection to the user, laboratory environment, and/or work materials during aerosol hazard activities. Containment is achieved by separating the work from the main area of the laboratory and/or by using controlled, directional airflow mechanisms. Exhaust air is passed through a high-efficiency particulate air filter (HEPA) before being recirculated to the laboratory or building's heating, ventilation, and air conditioning system. There are different types (Type I, II, and III) of biological safety cabinets (BSCs) that provide different levels of protection.

BSC Type I

They are open-fronted cabinets with inward airflow, designed to protect the user and the environment from infectious aerosols generated during operation. In the cabin that uses room air, the air passing through a HEPA filter is released back into the environment.

BSC Type II

These cabinets, which have a more complex structure than BSC Type I cabinets, are open at the front and are in contact with the room air. However, differently, the air taken into the cabin first passes through the HEPA filter, and the air in the cabin is released into the environment by passing through a second HEPA filter before being released to the outside. This cabin, which is used especially in cell culture studies, is important in terms of protecting the health of employees and the environment. It can be affected by environmental factors such as its orientation in the room in which it is located, air flow in the room, and pressure changes.

BSC Type III

Unlike other cabins, the front is closed and there is a separation between the user and the research environment and material. The researcher works with arm-length gloves made of rubber integrated into the system. Airflow is provided by a special exhaust system outside the cabin. This system keeps the interior of the cabin under negative pressure compared to the surrounding area. The air taken from the HEPA filter passes into the working environment and passes through a second HEPA filter before being released (8,16,17).

SAFE WORKING WITH EXPERIMENTAL ANIMALS

Laboratory animals live under the influence of environmental factors such as temperature, humidity, airflow, ventilation degree, suspended dust particles in the air, and noise under the environmental conditions they live in. In addition, experimental animals are under the influence of many stress factors, such as breeding, transportation, and care. In order to obtain reliable and sustainable results, genetically and microbiologically defined pedigrees are needed, as well as having a good laboratory animal laboratory. Moreover, infectious diseases in experimental animals must be kept under control and safe conditions must be provided for researchers and other employees (18-22).

Biological risk assessment is a key factor in safe laboratory work. Risk assessment requires careful decision-making and is an important responsibility for managers of microbiology and biomedical laboratories and principal investigators. Institutional structures such as Institutional Biosafety Committees or equivalent units, animal care and use committees, biological safety specialists, occupational health personnel, and laboratory animal veterinarians share responsibility. When assessing the risk, previous incidents should be taken into consideration, and criteria regarding trust conditions should be determined. Biosafety guides that include risk management should be created under the responsibility of the laboratory manager in line with the determined criteria. Creating these guidelines alone is not sufficient; it is of great importance to implement them and transmit them as a biosafety culture among working generations.

The main risks that may arise when safe conditions are partially or completely eliminated are classified as follows (21,23-25):

- Direct skin, eye, or mucosal membrane exposure to an agent
- Bites from a contaminated laboratory sharps instrument or infected animals and arthropod vectors
- Ingestion of a liquid suspension of an infectious agent or exposure from contaminated hand-to-mouth
- Inhalation of infectious aerosols

The dangers arising from these phenomena, called risks, can be grouped under five main headings (21,26,27):

- **Biting and scratching:** Acting outside the rules of working with experimental animals or dangers arising from an unforeseen reason.
- **Allergenic effect:** Body fluids and secretions of laboratory workers' experimental animals, hair, feathers, hides, etc. their sensitivity to the material.
- **Hazards specific to the experiments:** Piercing-cutting, contamination-toxic, etc. used in the experiments. Hazards arising from the material.
- **Natural hazards:** These are the dangers arising from the violation of rules and carelessness of employees / falling, ergonomic deficiencies, noise, etc.
- **Zoonoses:** Diseases that can pass between humans and animals/hantavirus, Salmonella, etc.

SAFE WORKING WITH ARTHROPODS

As with vertebrates, the biosecurity level of the animal facility where research on arthropods is conducted is determined by taking into account the biosecurity element and risk groups subject to research. However, additional precautions can be taken for some arthropods that have the ability to fly. The basic precautions that must be taken for safe work in laboratories where arthropod experiments are carried out can be listed as follows (12,25,28-30):

- Separate chambers should be provided for infected and non-infected invertebrates.
- The rooms must have sufficient technology to seal the environment against fumigation.
- Insecticide sprays should be available.

- "Cooling" facilities should be available to reduce invertebrate activity when necessary.
- Access should be provided through a room entrance equipped with insect traps and arthropod-proof curtains on the doors.
- All ventilation ducts and openable windows must have arthropod-proof surfaces.
- Waste catchers in sinks and weirs should not be allowed to dry out.
- All waste should be purified by autoclaving, as some invertebrates may be resistant to disinfectants.
- The numbers of larval and adult forms of flying, crawling, and jumping arthropods should be checked.
- Live storage containers used for ticks and mites should be placed in oil trays.
- Infected or potentially infected arthropods and flying insects should be kept in double-mesh cages.
- Infected or potentially infected arthropods should be handled in biological safety cabinets or isolators.
- Infected or potentially infected arthropods may be restricted in their movement on the arthropod cooling tray.

CONCLUSION

During studies carried out with experimental animals under laboratory conditions, safe working rules may be violated due to physical conditions, equipment and materials, treatments applied to the subjects, negligence, or faulty practices caused by the researcher or expert. "Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees" was published in the Official Gazette dated 15 February 2014 and numbered 28914, in order to prevent these negligence and violations, to establish the basis of practices based on ethical principles on employee safety and laboratory animals, and to determine their standards. In this regulation, the central ethics committee for animal experiments and local ethics committees for animal experiments were defined, criteria for safe working with laboratory animals were determined, and the way was opened for organizing certified training through laboratory animal use courses. Correct studies are to be applied to experimental animals in courses carried out in accredited laboratories that have permission to work with laboratory animals.

In the courses held in accredited laboratories that have permission to work with experimental animals, the correct working methods to be applied to laboratory animals (gavage, disease models, etc.), as well as the risks and dangers that these methods may carry within the framework of OHS rules, are evaluated.

The data obtained from all these studies constitute the infrastructure of biosafety laboratory manuals according to their levels. However, this infrastructure is not stable and is open to rapid changes under the influence of modern techniques and scientific schools. For this reason, it is inevitable that more advanced occupational health and biosafety criteria and practices, which can develop and change rapidly in the light of science and are specialized in different fields, will be needed in the near future.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: MU; Design: MU; Data Collection/Processing: MU; Analysis/Interpretation: MU; Literature Review: MU; Drafting/Writing: MU; Critical Review: MU.

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