Assessment of the Prerequisites for the Establishment of an Animal Facility at the School of Medicine, University of Namibia: Comparison of the current situation with International Standards and Requirements, and with discussion of Alternative Options

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Abstract

The School of Medicine (SoM) of the University of Namibia (UNAM) intends to establish an animal house for teaching and experimental research within its facilities. A number of rooms are dedicated for this purpose and partially equipped. This review investigates the feasibility of the project in the light of international standards for animal experimental settings, the prerequisites for the physical establishment, the technical installations, compliance to globally valid regulations of animal welfare, and the probability of getting reliable research outcomes from the given background. The identified current situation within the SoM is subsequently compared to the internationally accepted benchmarks for such institutions, and the principal features of the commonly followed guidelines are briefly listed. A selection of alternative methods to animal experiments is succinctly described and set in the context of the departments of SoM. However, the examination of the physical housing, technical equipment, required human resources and available infrastructure led to the conclusion that the intended use cannot be recommended since the relevant criteria are not met. Instead it would

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be advisable to construct a separate animal house based on recognized internationally valid standards. In the meantime, less costly non-animal state of the art methods could be introduced.

Keywords: Animal experiments, animal house, animal welfare, alternative methods to animal experiments, Namibia.

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1 Introduction

The focus of this review is directed at the requirements for an animal house, which is the housing for laboratory animals for use in research, its management and the procedures for the conduct of animal experiments, and research at the Schools of Medicine (SoM) and Pharmacy (SoP) of the University of Namibia. The design of the animal facility rooms at SoM were assessed for the suitability, equipment, and the technical infrastructure of the building had been taken into account. The results were compared with the international standard settings and guidelines for the management of laboratory animals. This is important, as non-compliance to these standards will impact negatively on the validity and recognition of research results emanating from the proposed animal facility on an international scientific platform.

2 Laboratory animals and ethics

Laboratory animals are animals used in research, teaching, testing or generally for scientific experiments. They are distinguished from other animals by their intended use and because they possess specialized anatomic, genetic, physiologic or metabolic conditions that differ from other members of the same species. In practice, the term is generally applied to those animals with a defined health and genetic status and usually purpose-bred for their intended uses in research (International Association of Colleges of Laboratory Animal Medicine).

Research, tests, trials and experimentations are carried out e.g. by universities, medical and pharmacy schools, pharmaceutical companies or other industries (e.g., cosmetic and chemical industry), and/or commercial facilities that offer these services to industrial clients. The selection, living conditions and handling of these laboratory animals should comply with international standards in order to deliver comparable and comprehensible results. The structural conditions of housing and environmental management of animal husbandry should be the first issues to get solved in order to establish the desirable environment for scientific research.

There are several ethical and legal concerns to consider in the establishment of an animal facility. Guidelines and policies in this matter have been developed at international level, and regulatory bodies that survey institutions working with laboratory animals are available to assist in conformity to international standards. Any researcher who wants to come up with research results based on or including animal experiments has to show evidence of the adherence to and fulfillment of these internationally valid standards, or else such research will risk not being accepted internationally.

The principles of the internationally available regulatory framework are briefly highlighted and compared with the current situation in Namibia. A short overview is given of alternative methods to animal experiments, so that research objectives can be addressed and experiments conducted if the requirements to run an animal house are not yet met.

3 Methods

An examination and inspection of the facilities at SoM and their technical equipment was conducted, the dimensions of the rooms taken, the infrastructure of the building explored, and a list of results and implicated drawbacks elaborated. For the theoretical background of the exploratory review a number of research engines were used including: Pubmed, Science Direct, and Google Scholar. These web sites were searched in June 2014. Search terms used were: animal house, animal welfare, animal testing, alternatives to animal testing. Limitations were then specified, for example, "in Africa". Articles or links from journals, magazines and periodicals of relevance were located, and publications identified from reference lists also included. Websites of several animal welfare organizations were sought out as well as those of associations working on alternative methods to animal testing like 'Doctors against animal experiments' and 'PETA' (People for the Ethical Treatments of Animals). Personal communication was sought with the Namibian State Veterinary Office, the Veterinary Council, and the Central Veterinary Laboratory.

3.1 Regulatory Framework

Before looking closer at the operational standards of the laboratory animal environment, some other facts should be looked at: For decades there has been a worldwide shift in the developed countries towards the idea of animal welfare, animal protection and animal rights that had led to the founding of numerous institutions, foundations, associations and organizations (ATLA; Balls 2007; Balls 2010).

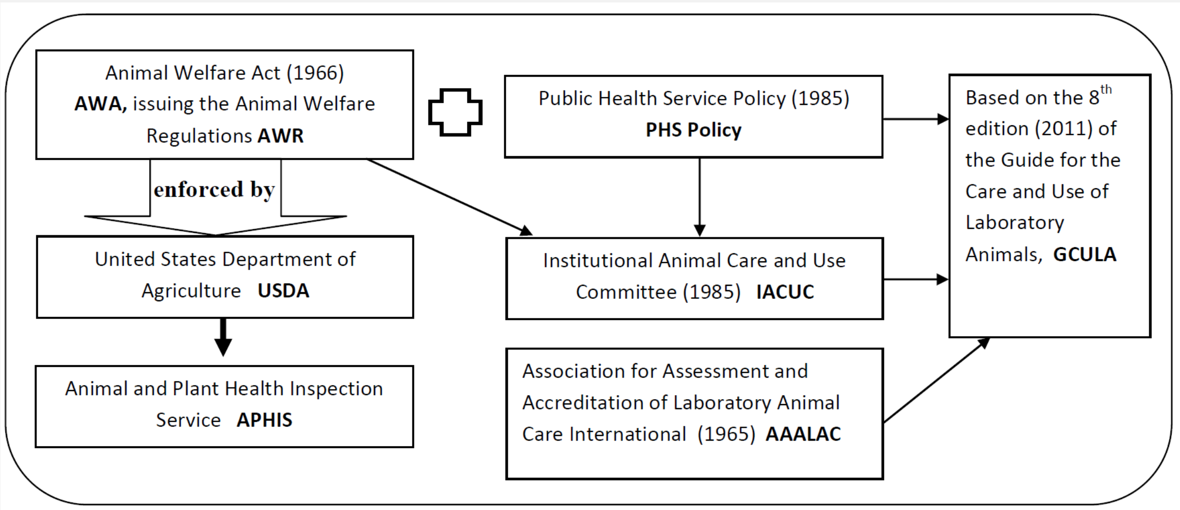

Figure 1: Overview of regulatory institutions in the USA. (Comparable structures exist e.g. for Canada, New Zealand, European Union, India, Japan.

Guidelines and policies in this matter have been created, and a strict survey of institutions working with laboratory animals were established and organized. As a consequence a number of regulations, policies and prescriptions are in force (Fig. 1). Most laboratories must nowadays adhere to animal welfare regulations, keep research protocols and prove, regularly, their compliance with policies. Therefore they are subjected to unannounced inspections. The system of policies, regulations, survey and guidelines is instituted as a self-reporting mechanism along with routine controls. Failure to meet requirements can result in fines, revoked registration and disqualification of research outcome (Regulatory Requirements for Laboratory Animals, 2013).

Any researcher who wants to come up with research results based on or including animal experiments has to prove the adherence to, and, fulfillment of internationally valid standards regarding husbandry of laboratory animals, the handling during experimentation, laboratory equipment and veterinary survey. Otherwise, such research will risk not been accepted by the international platform of scientists.

A detailed framework of standards of animal health and welfare has been developed by the World Organisation for Animal Health (OIE), the 'OIE Terrestrial Health Code' (OIE. Terrestrial Animal Health Code, 2011). OIE is the intergovernmental organization responsible for improving animal health worldwide and was created in 1924 by an international agreement. The member states commit themselves to the guidelines supplied by OIE. The laws in force dealing with animal welfare and protection in several member states root in this code, and the regulations that give detailed instructions base on the recommendations provided by it. An example therefore is the 'Guide for the Care and Use of Laboratory Animals' (GCULA) released in 2011 by US government authorities and being the leading framework of instructions worldwide (Guide for the Care and Use of Laboratory Animals, 2011).

The Animal Welfare Regulations (AWR), issued by the Animal Welfare Act (AWA), are enforced by subdivisions of the US States Department of Agriculture (USDA). Since 1985, both AWA and Public Health Service Policy amendments have required the appointment of specific institutional animal care committees (IACUCs) that oversee the care and use of laboratory animals within research facilities.

Research protocols have to be approved by the IACUCs, unannounced inspections are conducted by the Animal and Plant Health Inspection Service (APHIS). The common guidelines for these bodies are given by the Guide for the Care and use of Laboratory Animals (GCULA).

This guide is also the basis of the program accreditation offered by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). As of 2013 more than 890 companies, universities, hospitals and research institutions in 37 countries worldwide have voluntarily earned AAALAC accreditation (Association for Assessment and Accreditation of Laboratory Animal Care 2014). This shows clearly that the GCULA is being accepted as a framework for the international standards of laboratory animal welfare. Comparable frameworks and standards corresponding more or less to this guide are in use in Europe, Canada, India, New Zealand, Japan and other parts of the globe (Górska, 2000; Guidelines for use of Laboratory Animals 2001; Kagiyama and Nomura, 2003).

3.1.1 Regulatory Framework in Namibia

However the situation looks different in Namibia. The awareness, sensitivity, knowledge and conception about animal protection, ethics, and the conception of the idea that animals are sentient beings are quite dissimilar in Africa (Masiga and Munyua, 2005). On the other hand a comprehensive Animal Health Act promulgated in 2011 is in force in Namibia, containing detailed regulations, subsections and indications about the handling of animals, their products, import, export, diseases, quarantine and related issues. The Act contains inclusive provisions about quarantine stations and quarantine areas, the notifiable diseases and parasitoses are included in the annexure (Animal Health Act, 2011). The Bergvlug Research and Quarantine Farm, east of Windhoek, owned by the Government and surveyed by the Central Veterinary Laboratory (CVL) falls under this Act. Problem orientated veterinary research is undertaken if required.

There is no law in Namibia that regulates animal testing or animal experiments, but, a member of the OIE, Namibian institutions are requested to respect the guidelines anchored in the 'Terrestrial Animal Health Code'. Nonetheless since 1962 an Animals Protection Act is in force in Namibia (Animals Protection Act 71 of 1962; Namibia). This Act, however, mainly consolidates and amends the laws relating to the prevention of cruelty against animals generally. It refers chiefly to the husbandry of farm- and companion animals, transport conditions of animals, the handling and hunting of wild animals. It addresses also the removal of mistreated animals from the owners and the imposition of fines. Yet no indications are contained dealing with the conditions of keeping, handling of and working with laboratory animals.

In other words, a comparable regulatory network for laboratory animal care does not exist in Namibia. Therefore it is strongly recommended that each institution intending to include animal testing in its scientific agenda should first strive to establish a proper animal care program, preferably in accordance with the GCULA, even so there is currently no law in force that would dictate this. Otherwise any research outcome based on animal tests would risk not been accepted internationally. Additionally, it can be applied for the accreditation at AAALAC.

In most countries, the procedures of animal experiments should be consistent with the requirements of Good Laboratory Practice (GLP), as given by the Food and Drug Administration (FDA), a federal agency of the US government (Guidance for Industry. Good Laboratory Practices, Questions and Answers, 1981-2007). Good laboratory practices have been universally recognized as rules that govern the conduct of non-clinical safety studies. The GLP goal is to ensure the quality, integrity, and reliability of the data developed from such studies (Food and Drug Administration, CFR, 2013).

As this might be a long way to go for an African country where these structures are not yet well in progress and largely dependent on governmental and federal cooperation, it might be advisable to seek for alternative laboratory methods that replace animal experiments.

3.2 Standards and requirements for animal facilities

The focus of this paper is directed on the aspects of the requirements for an animal house, its management and the conduct of animal experiments in the context of the research and scientific work program at the Schools of Medicine and Pharmacy of the University of Namibia (UNAM). Therefore it is looked at the available rooms, equipment, and technical infrastructure of the buildings of the School of Medicine that were chosen to become an 'animal house'. This is compared to the standard settings and guidelines for the management of laboratory animals of the above mentioned US institutions.

The balance between ethical and science-based practice is indicated by the GCULA, setting this way a benchmark for internationally comparable standards. Numerous parameters are listed there, as for instance for the structural conditions and design of animal facilities and laboratories, appropriate animal housing and caging, management of technical and electronic installations, equipment and cleaning space. Reliable results of experiments on laboratory animals are largely dependent on the standardization of the factors affecting physiological reactions of these animals, and on the broad idea of the well-being of laboratory animals (Górska, 2000).

For the practical aspect it can be summarized that animal houses for laboratory animals are facilities containing animal headquarters, storage and cleaning rooms. Laboratories might be integrated or situated separately.

International standards encompass:

1. Animal welfare regulations
2. Regulatory requirements for laboratory animal health conditions
3. Requirements for animal facilities
4. Requirements for laboratory work (GLP)

3.3 Guide of the care and use of laboratory animals

The following details of the short overview are essentially taken from the 'Guide of the care and use of laboratory animals':

3.3.1 Physical conditions in animal houses, micro- and macro-environment

Microenvironment describes the immediate physical environment surrounding the animal (i.e., the environment in the primary enclosure such as the cage, pen, or stall), whereas the term macroenvironment applies to the environment of the secondary enclosure (e.g., a room, a barn, or an outdoor habitat). Although both are related, the microenvironment can be notably different and affected by several factors, including the design of the primary enclosure (cage structure, seize, material) and macroenvironmental conditions such as room climate, light, ventilation and humidity. Temperature, humidity and concentration of gases (intracage ammonia, CO_2) and particulate matter are often higher in the microenvironment.

3.3.2 Temperature and humidity

Since the maintenance of body temperature within normal circadian variation is necessary for animal well-being, animals should be housed within temperature and humidity ranges appropriate for the species. The species dependent thermoneutral zone (TNZ) would be the optimal range. Within the TNZ thermoregulation occurs without the need to increase metabolic heat production or activate evaporative heat loss mechanisms. TNZ is defined by the upper and lower critical temperatures for a given species (UCTs and LCTs). In general, the true thermodynamic temperature in animal rooms, the dry-bulb temperature, should be set below the animals' LCT to avoid heat stress. Nesting material will provide the adequate resource for thermoregulation and constitutes an important part in simulating an animal's natural milieu. Factors that contribute to variation in temperature and humidity include housing design, construction material, use of filter tops, ventilation, enrichment devices such as shelters and nesting materials, frequency of bedding changes, number, age, type, and size of animals per cage.

The acceptable range of relative humidity is considered to be 30% to 70% for most mammalian species. In climates where it is difficult to provide a sufficient level of environmental relative humidity, animals should be closely monitored for negative effects.

3.3.3 Ventilation and air quality

Appropriate air quality, sufficient oxygen and a stable environment are essential factors for the animal's well-being. This is effectuated by proper ventilation, which is preferably combined with a potent air filter system to consecutively remove airborne pathogens, allergens and thermal loads. Provision of 10 to 15 fresh air changes per hour in animal housing rooms is an acceptable guideline. Modern heating, ventilation, and air conditioning (HVAC) systems allow ventilation rates to be set in accordance with other room variables. It is also possible to install individually ventilated cages (IVCs) that either directly ventilate the micro-environment using filtered room air or are ventilated independently of the room. As these systems require a stable supply of electricity, a generator in case of electricity failure is indispensable.

The use of recycled air to ventilate animal rooms may entail risks such as the spread of airborne pathogens. Exhaust air recycled into HVAC systems that serve multiple rooms presents a risk of cross contamination. The air from animal rooms may contain a relatively high burden of ammonia and other gases, dust, allergenic particles e.g., from bedding, and microorganisms. Therefore the exhaust air should principally be filtered at minimum with 85-95% to avoid pollution, and filter efficiency, loading and integrity should regularly be assessed.

3.3.4 Illumination

Light can affect the physiology, behavior and well-being of animals. Badly chosen light levels may act as so called photostressor and affect negatively the animals as well as the research results. This includes inappropriate photoperiod, photointensity, spectral quality of light, or simply said, light intensity, wavelength and the duration of the animal's exposure to light. The light programme must relate to the circadian rhythm typical for the animal species. For example, rodents possess photosensitive retinal ganglion cells that are distinct from cones and rods. These ganglion cells are important for the neuroendocrine, circadian, and neurobehavioral regulation and can respond to light wavelength that differ from other photoreceptors. This might influence the type of lighting selected for certain types of research. Generally speaking, lighting should be diffused throughout an animal holding area so that sufficient illumination for the animal's well-being is provided while on the other hand permitting good house-keeping practices.

Photoperiod is also a critical regulator of the reproductive behavior in many animal species. Therefore inadvertent light exposure during the dark cycle should be avoided. Most commonly used laboratory rodents are nocturnal, for that reason their biological rhythm must be taken in account. Suitable light levels for the care of rodents are given by about 325 lux measured 1 m above the floor. It is advisable to install an automated time-controlled lighting system in order to guarantee a regular diurnal cycle.

3.3.5 Noise and vibration

Noise generated by animals and animal care activities is an inevitable side effect in the operation of an animal house. The effects thereof will depend on the sound susceptibility of the different animal species, and the intensity, frequency and vibration potential of the sound. Inaudible sound frequencies are perceivable by many animal species: For example, rodents are very sensitive to ultrasound and vibration. The potential effects of equipment (such as ventilation systems, radios or sound generators, electronic devices), materials and working methods that produce noise in the hearing range of nearby animals, can thus become an uncontrolled variable for research experiments and should therefore be avoided.

Exposure to sound frequencies, intensities and vibration beyond the comfort zone of a given species can cause biochemical and reproductive changes in laboratory animals, and therefore may constitute a risk factor for the research outcome. Cage systems with moving components, such as ventilated caging system blowers, may create vibrations that could affect the animals housed within. Excessive vibration can negatively influence the body functions of the laboratory animal and compromise expected results.

Finally, human and animal areas need to be separate in order to minimize disturbances to both, human and animals.

3.3.6 Microenvironment - primary enclosure, cages

The primary enclosure of all animals should provide sufficient space for movement, resting, feeding and shelter and meet the animal's physiological, physical, and behavioral needs. In regard to laboratory rodents this would be the cages. Animals should have adequate bedding substrate, nesting material, structures for resting, sleeping and movement. Settings that fail to meet these requirements may cause abnormal development, dysfunction or behavioral disorders, and consequently compromise research conditions and results.

Another aspect is the space allocation. This will depend on the species and purpose, e.g., breeding animals require more space, especially when newborn animals will be raised together. There is no ideal formula for calculating the animal space needs based only on body size or weight. At a minimum, animals must have enough space to express their normal posture and some movement without touching the enclosure walls, and easily have access to food and water. There must also be room enough for resting places away from spots soiled by urine and feces. Table 1 (adapted from the 'Guide of the care and use of laboratory animals'), shows the recommendations for the minimum space of some laboratory animals.

Table 1: The recommendations for the minimum space of some laboratory animals.

Animals	Weight	Area/animal (cm^2)	Height (cm)	Corresponding length of cage floor (cm/animal)
Rats in groups	Up to 200 g	≈110	≈ 18	≈10.5
	up to 400 g	≈260	≈ 18	≈16.2
Guinea pigs	up to 350 g	≈387	≈ 18	≈19.8
	≥ 350 g	≈650	≈ 18	≈25.5
Rabbits	≤ 2 kg	≈0.14 m^2	≈ 40.5	≈0.38 m/animal
	up to 5.4 kg	≈ 0.37 m^2	≈ 40.5	≈0.61 m/animal
	≥ 5.4 kg	≥ 0.46 m^2	≈ 40.5	≈0.68 m/animal

Several species-specific aspects must be taken into account for the group composition: Age, sex, breeding requirements, social organization and behavior, and general health status. For rodents and some other species specialized housing systems are available, so-called isolation-type cages (IVCs). These provide their own microenvironment by closing tightly and being ventilated by an extra system connected to the cages. It goes without saying that a back-up generator must then be available, because in the case of any power failure the animals would pitifully suffocate. This caging system also requires specialized cleaning, disinfecting, and sterilization regimens.

3.3.7 Bedding and nesting material

These are controllable factors that can influence experimental data. Bedding is used to absorb moisture, dilute and bind the animal's excreta, and reduce the growth of microorganisms. Some materials can also decrease the buildup of intracage ammonia. There is no one suitable bedding type for all species. Rodents show a clear preference for specific materials and build better nests with appropriate substances. Bedding materials are for example paper cuts, cotton, hay, straw, softwood shavings, and chips. The latter might not be recommended for some research protocols since they can affect the metabolism. Other wood shavings may contain aromatic hydrocarbons that are cytotoxic. Whatever the source of the material may be, it must be compatible with laboratory disinfection methods, because all bedding substances can be contaminated with microorganisms, toxins, vermin, and fungi. Appropriate treatment methods are for example kiln drying (a type of thermally insulated oven), or autoclaving. Since bedding can absorb moisture during autoclaving, correct drying time and storage conditions must be adapted. In case that sterile bedding is wished, gamma-irradiated materials should be used.

3.3.8 Sanitation

Sanitation encompasses bedding change, cleaning and disinfection of cages, removal of excrement, dirt and debris and cleaning of the macroenvironment. Cleaning and disinfection of cages, cage rack, and equipment like feeders and watering devices should be done regularly. The schedule will depend on husbandry practice and cage material, but a weekly interval is strongly indicated. For the sanitation of cages mechanical washers are recommended, effective disinfection can be achieved with wash and rinse water at 80°C, detergents and chemical disinfectants. It is indispensable to remove first the debris and soiled bedding from the cages, and to rinse and clean them manually before they can put in an automated cleaning system. Whether the sanitation is automated or manual, regular evaluation and assessment of the effectiveness are strongly advised.

3.3.9 Waste disposal

Biologic and hazardous waste should be disposed safely. If possible, licensed commercial waste disposal companies should be used. If on-site incineration takes place, it should comply with all state and local regulations. A dedicated waste storage area should be available, waste containers must be tight and equipped with tight-fitting lids. For cold storage of certain disposal matter an extra refrigerator that is labeled as such, must be used. Hazardous waste must be rendered safe by sterilization, containment, or other appropriate methods before they can be removed.

3.3.10 Pest control

Pest control programs should be implemented to prevent the infestation of vermin and other pests. This is essential in an animal environment, since the smell of the animals themselves as well as the stored animal feed will constantly attract parasites or pests.

3.3.11 Emergency, weekend and holiday care

Qualified personnel should care for laboratory animals every day, a specialized veterinarian should be available, and emergency veterinary care must be granted after work hours, on weekends, and on holidays.

3.3.12 Functional areas

The development of a practical, functional and efficient animal house and laboratory unit should be guided and advised by professional judgment. Space is required for:

- Animal housing, care, and sanitation
- Receipt, quarantine, separation of animals
- Separation of species or isolation of individual projects if necessary
- Storage (feed, bedding, pharmaceuticals, equipment, spare parts, supplies, cleaning material etc.)
- Laboratory
- Cleaning area: Washing, sterilization of equipment and supplies
- Space for storing wastes
- Space for administrative and supervisory personnel

- Showers, sink, lockers, toilet, and break areas for personnel
- Areas for maintenance and repair of specialized animal housing systems and equipment

3.3.13 Construction guidelines

If an animal house facility is part of another building, it should be separated by structural modifications. Double-door entry vestibules are desirable to avoid contamination of both, the animal room and the personnel space. As mentioned above, stable environmental climate conditions are best provided by an automated heating, ventilation, and air conditioning system (HVAC). Positive and negative air pressure can be regulated by this in order to control airborne contamination and odors. Relative humidity should generally be maintained within a range of 30-70% throughout the year.

An emergency power supply like a generator is indispensable for regions where electricity cut or power failure may occur more often. If the critical services cannot be maintained, the health of the animals and the outcome of research projects will be at risk.

3.4 Alternative methods to animal experiments

A worldwide tendency of developing alternatives to animal testing can be noticed since in 1959 the two British researchers Russell and Burch promoted the concept of the three Rs in their book 'The principles of humane experimental technique' (Russel and Burch 1959). The Rs stand for the principles of 'Refinement, Reduction, Replacement' of experiments on animals wherever possible (Balls 2007; Balls 2010). As a result, a decrease in the number of animal experiments can be seen in Europe, and a noticeable trend towards technology based and other methods is observed worldwide in the developed countries (Harrison et al., 2006; Spielmann et al. 2007; Balls 2010; ATLA; Doctors Against Animal Experiments; PETA).

The methods briefly described below base chiefly on these references. Alternatives were looked for partly because of ethical reasons, such as described in the 'three Rs concept' by Russel and Burch, and the authors mentioned above. Further reasons were the costs involved in animal testing (Humane Society International 2009; Knight 2011), and increased criticism about the applicability of the results to humans (Pound et al. 2004; Perel et al. 2007; Bracken 2008). So the world's most forward-thinking scientists moved ahead to develop and use methods for studying diseases and testing products that replace animals and are relevant to human health. Currently there exist two major alternatives to the animal testing *in vivo*: The *in vitro* cell culture techniques and the *in silico* computer simulation. The options of a suitable training in these methods will need to be assessed in a different report.

Cell cultures are used in many fields: Skin testing of corrosion, irritation and phototoxicity, organ cells culture for example for hepatoxicity tests, or cells to create a model of the human immune system. Two common *in vitro* skin tests employing reconstituted human epidermis models are EpiSkin, a human epidermis reconstructed on collagen based on tissue-engineering (EpiSkin; EpiDerm) which is a three-dimensional, human-cell-derived skin model that replicates the traits of normal human skin.

Generally said, a variety of cell-based tests and tissue models can be used to assess the safety of drugs, chemicals, cosmetics, and consumer products. A wide range of computer simulations have been developed by researchers, including very sophisticated models that imitate human biology and the progression of developing disease. This encompasses for instance models of asthma, plaque build-up and cardiovascular risk, human metabolism generally, toxicity evaluation, brain activity and traumata simulation. Some of these models can accurately predict the ways that new drugs will react in the human body and replace the use of animals in exploratory research and many standard drug tests (Martonen et al., 2003). Other computer-based techniques are Quantitative Structure-Activity Relationships (QSARs) that can replace animal tests by calculating probabilities of a substance's likelihood of being hazardous, based on its similarity to existing substances and the knowledge of human biology. In the developed countries companies and governments are increasingly using QSAR techniques instead of animal testing of chemicals. QSARs are actively promoted and internationally funded for example by PETA (PETA, funding non-animal methods, n.d.).

A relatively new method is the 'organ on a chip' technology, which is essentially a microchip lined by human cells (Wyss Institute). The procedures combine microfabrication techniques with modern tissue engineering: Microchips recapitulate the microarchitecture and functions of living organs, such as the lung, heart, and intestine. The results are capable of mimicking the complicated mechanical and biochemical behaviors of organs. Models of 'lung on a chip' and 'human gut on a chip' are already on the market. The chips can also be used instead of animals in disease research, drug testing, and toxicity testing. They have been shown to replicate human physiology, diseases, and drug responses more accurately because traditional animal models do not accurately mimic human physiology. These chips were processed by certain companies into devices that other researchers can purchase and use in order to replace animal tests (HuREL® Corporation).

Another option for the study of drug metabolism is the use of microbes as a model for the mammalian metabolism of xenobiotics. This concept is well established since the early seventies of the last century. This method offers a number of advantages over the use of animals in metabolism studies because of its ease of setup and manipulation, higher yield and diversity of metabolite production, and lower cost of production (Abourashed, E.A. et al., 1999.). The fungal model using fungi like *Cunninghamella elegans* is one of the best established (Wackett and Gibson, 1982). Since it is able to degrade xenobiotics, it can be used e.g. to investigate the metabolism of phenols, flavones and polycyclic aromatic hydrocarbons, the degradation of herbicides, for the biotransformation in enzyme technique or the economical bioconversion of the natural antimalarial drug artemisin into semi-synthetic derivatives (Parshikov et al., 2004). *Cunninghamella elegans* and related strains are easily cultivated on conventional culture media.

An alternative model setup for the investigation of biological system functions is given by the utilization of the small nematode *Caenorhabditis elegans*. This multicellular organism is utilized to address fundamental questions in developmental biology, neurobiology and behavioral biology. One of the key strengths of this model system is that the genome has been entirely identified and sequenced which makes it an ideal organism for the study of gene regulation and function (Worm Book, n.d.). About 35% of *C. elegans* genes have human homologues so that biological information gathered from *C. elegans* may be directly applicable to more complex organisms, such as humans. The worms are also used in toxicology (Maxwell et al., 2008) and cancer research (Saito and van den Heuvel, 2002), drug screening and antimicrobial testing (Ewbank and Zugasti 2011; Man-Wah et al., 1999). The about 1 mm long nematodes are easy to handle in the laboratory, being cultivated on solid support or in liquid, in Petri dishes, tubes or well plates. *C. elegans* can be exposed to toxic substances by injection, feeding or soaking. The nematode therefore represents an excellent complement to *in vitro* or cell-based systems and *in vivo* vertebrate models.

3.5 The facilities at the School of Medicine

Three sets of two connected cellar rooms are available, located in the Life Science I Building, Anatomy Section. These facilities have been assessed with respect to the dimensions, the technical equipment and infrastructure, lighting, air flow and pressure gradient, humidity, continuous power supply, capacities for animal hygiene including safe waste disposal, space for personnel and risk of contamination. Technical advice and explanation was given by the responsible technician of this section (Broekman 2014, personal communication).

4 Results

The dimensions of the three sets of cellar rooms are:
1 Set: $8.9m^2$ and $18.3m^2$
2 Set: $6.5m^2$ and $15.5m^2$ (L-shaped)
3 Set: $6.8m^2$ and $18.3m^2$

The smaller entry rooms are all equipped as follows:
1 small water basin for cold water
1 double plug
2 neon tubes, no switch

The bigger rooms are all equipped as follows:
1 surrounding plastic board with 11 plug-stations
2 neon tubes, 1 timer (4 neon tubes in L-shaped room)
1 water tap for cold water at about 50 cm distance from the floor
1 drain in the floor at even level, no slope

Cages are installed in all three of the bigger rooms:

Set 1: 1 cage rack with 66 Isocage mice cage systems, which obviously are supposed to possess their own integrated filter systems;

- The inherent problem of power failure and the non-availability of a generator have already been addressed, including the issue of specialized handling of these systems in regard of cleaning, disinfection, sterilization. In case of electricity failure the most critical service of air supply is not maintained, and the animals would pitifully die from suffocation.

Set 2 and 3: Contain 4×6 rat cages, also as an Isocage isolation system, and one cage system for rabbits (2×3 open system cages);

Two of the bigger rooms are each equipped with a Biosafety changing station (Tecniplast) which suggests the intended simultaneous use of the rooms as laboratory as well as animal housing.

- It was already discussed that the combined use of laboratory and animal housing rooms is obsolete and must not be encouraged.
- The presence of the cage systems currently suggests an intended use for the keeping of mice, rats and rabbits.
- The space measured in the sets 1,2 and 3 is too small for the utilization of these cage systems to keep laboratory animals in accordance to the GCULA (p. 55-58).
- The room dimensions and the technical equipment do not provide for an adequate provision of 10 to 15 fresh air changes per hour in animal housings (GCULA p. 46).

There is a small room available (not being part of the sets), equipped with an automated cage washing system and an autoclave.

- However, there are no storage places for the drying of cages and bedding, neither is there a separate room for the manual pre-cleaning and washing of cages and used equipment, and the waste disposal.

Personnel for the animal care:
As stated by the GCULA, the animal holding unit should be looked after permanently, meaning 24/7. A weekend, after work hours, and holiday service must be established and ensured. At least one technician should be specially trained in laboratory animal health care and husbandry. The veterinarian should be trained either, and be on service for these animals only. This would require the employment of new staff with the implications of rooms for personnel, showers, and gear.

5 Discussion

In their current set up, the animal house facilities do not satisfy most the due conditions as listed under 3.2: **Standards and requirements for animal houses**' of this paper, and described in detail by the GCULA, and the OIE. Terrestrial Animal Health Code (2011, Chapter 7.8).

Catalog of Reasons:

- The dimensions of the rooms are too small for the intended use of the cage systems listed above; No double-door entry vestibules which are desirable to avoid contamination of both, the animal room and the personnel space;
- Drains are included in each animal room, but no slope; so after flushing the floor, not all the water can run immediately into the drain pipe, and excess water containing potentially contaminating material will spill over.
  - There is no cover for the drains: Drains not being in use for a longer period need to be capped and sealed to prevent backflow of sewer gases, vermin, and contaminants.
- Ceiling extraction grids are built in, to which the air flow is directed being attracted by an electric extraction motor on the roof of the building (working like a big fan).

- That way an undesirable airflow is generated, detrimental for any animal housing located within these areas, as well as for laboratory personnel in case the rooms are used as laboratories;
- Furthermore, detrimental germs can be widely spread to the environment.

- The noise generated by this air draught will have a negative effect on animals housing in such a room, in addition to the sounds and clatter caused by the animals themselves;
    - Constant and enhanced noise and vibration levels may cause stress on animals, and lead to changes in body-functions. This may negatively affect the expected results from experiments conducted with needlessly stressed animals.
- No air-filter system is in place, which is a must for any laboratory animal holding area in order to avoid environmental pollution.
- Neither is there an air pressure system that would maintain a negative air pressure in the animal room and a positive one in the other rooms, with the aim to avoid the spread of allergens and microorganisms:
    - The airflow will move downhill a pressure gradient from the side of higher to the one of lower pressure, rather carrying the air from outside into the animal room, reducing that way cross contamination between soiled and clean equipment.
- No system is in place to regulate or provide constant humidity and/or temperature:
    - The ambient temperature should range within the parameters of upper and lower critical temperature of a given animal species (called thermoneutral zone). Within this zone an animal can regulate the body temperature without any need to produce heat by metabolic reactions, or to activate the sweat glands in order to reach heat loss.
    - To a lesser extent, the relative humidity needs also to be monitored: a range of 30% to 70% is considered to be acceptable for most mammalian species. This parameter plays a much greater role for animals kept in isolator-cages which provide a closed environment.
    - Extreme or abrupt changes and fluctuations in temperature and humidity may have negative effects on the animal well-being, and may subsequently influence negatively the research outcomes.
- No back-up generator does exist that would maintain the most critical service of air supply in case of electricity failure. Animals kept in ventilated cage systems would die from suffocation;

- Each unit is equipped with a Biosafety changing station which suggests the simultaneous use of the space as laboratory as well as animal housing. It was already discussed that the combined use of laboratory and animal housing rooms is obsolete and must not be encouraged:
  - The GCULA (p. 134) states: 'Animals should be housed in facilities dedicated to or assigned for that purpose, not in laboratories merely for convenience. If animals must be maintained in a laboratory to satisfy the scientific aims of a protocol, that space should be appropriate to house and care for the animals and its use limited to the period during which it is required'.
- A small room is available, equipped with an automated cage washing system and an autoclave. However there are no storage places for the drying of cages and bedding;
- Neither is there a separate room for the manual pre-cleaning and washing of cages and used equipment and the waste disposal;
- No space for staff rooms, showers and gear;
- Specially trained personnel is currently not employed, an animal holding unit should be looked after permanently;

Therefore the SoM should construct a stand-alone animal facility that must conform to the standard requirements and research ethics with respect to the use of animals. A physically centralized unit as described in the GCULA (p. 134 ff) will comprise support, care, laboratory, and study sites as well as a small veterinary facility, being located in the next neighborhood to the animal housing area. Centralization may reduce transport time between cages and laboratory site, and therefore contribute to the reduction of stress and risk factors of infection for the animals. Staff and animals can also be easier monitored. Operating costs can be reduced through centralization, and equipment, personnel and support services more efficiently used.

However, such a costly and demanding project can only be realized after a thorough planning involving all the relevant stakeholders. It is recommended that in the meantime the more cost effective alternative methods to animal experiments should be taken into consideration, particularly under the aspect of meeting an important global trend and being consistent to international research standards.

Existing resources should be reconsidered and addressed: Laboratory areas can be re-evaluated and vacant space used for the setting up of an alternative working group, available laboratory technicians could be trained in substitute methods to animal experiments. The easy manipulability of the above described nematode *C. elegans* combined with automation technologies could be explored as well in the departments of microbiology, biochemistry and physiology as in the School of Pharmacy. The important matter of drug metabolism could be investigated by making use of the capacities of the fungal model of Cunninghamella species.

6 Conclusion

The facilities that have been assessed at the UNAM School of Medicine do not satisfy the due standards in regard of space available, safety for personnel and animals, hygiene, technical equipment and infrastructure as well as trained human resources. Therefore it is recommended that the SoM should rather construct a stand-alone animal house as a centralized unit that must conform to the standard requirements and research ethics with respect to the use of animals. In the meantime the more cost effective alternative methods to animal experiments should be considered and the available resources being in place exploited, particularly the operating laboratories on hand and the trained personnel. The Namibian Veterinary Council recommends the use of more modern research methods like illustrated in this paper (Marais 2014, personal communication).

Examples of feasible laboratory approaches are described that can replace animal experiments, such as *in vitro* cell and tissue culture, a fungal model for the study of drug metabolism, an alternative model setup for the investigation of biological system functions by a small nematode, and several in silico computer-based techniques.-

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