GUIDE TO THE CARE AND USE OF EXPERIMENTAL ANIMALS

Volume 1 1993

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Citing certain devices or manufacturers is not to be perceived as the endorsement of the Canadian Council on Animal Care (CCAC) of one particular product over another.

In keeping with the CCAC policy of revising statements and guidelines as needed, users of this *Guide* are encouraged to forward any comments to the Secretariat.

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DEDICATION

The Canadian Council on Animal Care
dedicates this *2nd Edition of*
Volume 1 of its Guide to the Care
and Use of Experimental Animals
to CCAC's founder and executive director
until his retirement in 1992, Dr. Harry Rowsell.
His vision and devotion to the cause of experimental
animal welfare have set an example which many
seek to emulate, but few achieve.

PREFACE

In 1961, the Committee on Animal Care of the Canadian Federation of Biological Societies (CFBS) prepared a one-page placard outlining "Guiding Principles for the Care of Experimental Animals". These principles were quickly approved by most national scientific associations and, despite their brevity, addressed essentially the same basic principles of animal care embodied in this 2nd Edition of Volume 1 of the Canadian Council on Animal Care (CCAC) *Guide to the Care and Use of Experimental Animals*.

The hundreds of pages of information contained in the current two volumes of the *Guide* represent steps in the evolution of efforts by the CCAC to provide the means by which the use of animals in research, teaching and testing in Canada can be performed in accord with basic principles of humane treatment.

The CCAC is deeply indebted to the many veterinarians, animal care employees, humane society members, administrators, scientists, and others who have willingly contributed time and expertise to its programs and projects. This edition of Volume 1 of the *Guide* is only one example of the many CCAC-directed activities that owe their existence and success to the tremendous generosity and good will of these Canadians. Their broad participation in animal welfare is one of the most important, and least-recognized, merits of the non-legislated, participatory, peer review system employed in Canada.

April, 1993

Donald P.J. Boisvert, MD, PhD
Executive Director
Canadian Council on Animal Care

FOREWORD

Since its inception in 1968, the Canadian Council on Animal Care (CCAC) has brought about enhanced animal care and use through education, voluntary compliance, and codes of ethics. The Council's unique flexibility allows it to readily respond to the concerns of both the scientific community and the general public, as exemplified by numerous amendments to CCAC's "living documents," such as its *Ethics of Animal Investigation*, which appear elsewhere in this *Guide*.

In line with increasing concerns for enrichment of the animal's environment (see Social and Behavioural Requirements of Experimental Animals), in addition to optimal physical standards, CCAC is placing increased emphasis on performance standards: it is of primary importance that the animal is comfortable and well-adjusted.

Local institutional Animal Care Committees (ACC) or Animal Research Ethics Boards (AREB) were introduced by CCAC in 1968, and are now embodied in American legislation. These committees serve as the "conscience" of the institution in order to ensure ethical concerns are addressed in the protocols for and conduct of the research being undertaken. As with its documentation, CCAC's assessment program and suggested terms of reference for ACCs continue to be subject to considerable change as experience is gained and new technology becomes available. Most of these changes have come in response to concerns expressed by the scientific community, although some have been influenced by concerns expressed by animal protection organizations.

Contemporary animal care programs address the comfort, health, safety and security of animals. At least to date, the numbers of animals needed has steadily declined, at least partially because of the scientific community's development of alternative techniques. Specific Pathogen Free (SPF) rodents, rabbits, etc., have been introduced. Microbiological and genetic monitoring have reduced animal disease, and thus diminished animal suffering.

The following, as included in the Foreword of Volume 1 of this *Guide* (1980) bears repeating:

> "*The increasing use of cell cultures, microbial systems, computer simulation and other replacement techniques provides clear evidence of the scientific community's commitment to implementing the Russell-Burch tenet of 'reduction, replacement, and refinement' in the use of experimental animals. However, such methods are, of necessity, complementary to animal experimentation and are initially dependent on animal-based research. The applicability of such techniques depends on validation utilizing animal systems, and on clinical studies. Confirmation of the data frequently requires the investigator to 'return to the whole animal'*."

In conclusion, it is not CCAC's responsibility to act as an advocate for the many contributions made through the use of animals in research. Its mandate is to develop programs to enhance animal care and to make changes as required, based on sound expertise and input. However, the Council maintains the right to advocate the benefits of its voluntary control program. It is incumbent upon each institution to promulgate this program by supporting the decisions of its ACC and the researcher who has received the ACC's ethical approval for his/her studies.

Progress has been, and will continue to be made when the scientific community and those in the general public concerned with the welfare of animals join together to seek the middle ground. Through responsible and learned discussion, without acrimony, extravagant zeal for a cause, or polarized view, agreements will be made which will benefit the animals we utilize in research, teaching, and mandatory testing.

CCAC guidelines are not all-encompassing or "etched in stone". Their application requires good judgement and common sense, based on training and experience. CCAC's program encourages the

development of consensus amongst those using the guidelines and those required to oversee their application.

General reference works and publications dealing with the care and use of experimental animals, if not available in institutional libraries or facility reading rooms, may often be borrowed for limited periods from the CCAC Secretariat library without charge, except for mailing costs.

Harry C. Rowsell, OC, DVM, PhD
Executive Director (1968-92)
Canadian Council on Animal Care

ACKNOWLEDGEMENTS

The Secretariat would like to thank members of the Canadian Council on Animal Care (CCAC) for their careful perusal of and input into the material contained in the chapter on Social and Behavioural Requirements of Experimental Animals (SBREA). Directors of Animal Care Services, Chairpersons of Animal Care Committees (ACC), and members of the scientific community in Canada, the U.S. and abroad are also thanked for their suggestions.

We are particularly grateful to the following for comments regarding:

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Dr. Irwin Bernstein, University of Georgia
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Dr. Peter Gerone, Delta Regional Primate Research Center
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Cats or Dogs:

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Animals Used in Agricultural Research:

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Dr. Helene Guttman, U.S. Department of Agriculture

Dr. Ruth Newberry, Agriculture Canada
Dr. Suzanne Robert, Agriculture Canada
Dr. Joseph Stookey, University of Saskatchewan
Dr. Tarjei Tennessen, Nova Scotia Agricultural College
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I. RESPONSIBILITY FOR THE CARE AND USE OF EXPERIMENTAL ANIMALS

All experimental care and use of animals in this country is subject to the requirements of the Canadian Council on Animal Care (CCAC), a national, peer review organization founded in Ottawa in 1968. Its mandate is straightforward and concise:

> *"to work for the improvement of animal care and use on a Canada-wide basis".*

A. NATIONAL LEVEL

1. Evolution of the Canadian Council on Animal Care

The 1950s and 1960s were a period of phenomenal growth in Canadian research and graduate teaching, particularly in biomedical sciences. Expression of public concern over the use of animals in research also increased during this period, as did the awareness of the scientific community that this constituted a sensitive area, and raised ethical questions, not the least of which was responsibility for animal care and use.

In 1963, the Medical Research Council (MRC) decided that the matter warranted further study, and the following year it requested that the National Research Council (NRC) establish a committee to investigate the care and use of experimental animals in Canada. The report of the Special Committee on the Care of Experimental Animals (1966) recommended the creation of a voluntary control program exercised by scientists in each institution, subject to peer judgement and committed to implement the guiding principles of an independent advisory body.

A feasibility study of these proposals was undertaken (Rowsell, 1967) and, as a result, all universities and government departments where animals were used agreed to support the formation of a Canadian council on animal care (Anon., 1967). The CCAC was established in 1968 as a standing committee of the Association of Universities and Colleges of Canada (AUCC). Comprising 12 member organizations, including the Canadian Federation of Humane Societies (CFHS), its terms of reference included the making of recommendations for improvements in:

a) the procurement and production of experimental animals;
b) the facilities and care of experimental animals;
c) the control over experiments involving animals.

At its inaugural meeting, held January 30, 1968, the CCAC adopted as its objective: "to develop guiding principles for the care of experimental animals in Canada, and to work for their effective application." A Resources Panel and an Animal Care Review Panel were created to assist the secretariat in achieving its objective. The functions of the original resources panel have, in recent years, been assumed by specific committees responsible for the development and implementation of national policies on laboratory animal resources.

Prior to CCAC's establishment, the only guidelines which had been defined for the care and use of experimental animals in Canada had been the Canadian Federation of Biological Societies' (CFBS) one-page *Guiding Principles for the Care of Experimental Animals* (1961). Within its first year, the Council published comprehensive guidelines entitled *Care of Experimental Animals: A Guide for Canada.* It demanded use of non-sentient methods wherever possible, and noted that "when an animal must be used there is an obligation to:

a) provide humane care and treatment;
b) minimize pain and discomfort;
c) avoid unnecessary use."

2. The Contemporary Council

Co-funded by this country's two major granting agencies, the MRC and the Natural Sciences and Engineering Research Council (NSERC), the CCAC comprises 20 member organizations, whose representatives include scientists, educators, and representatives of industry and the animal welfare movement. They include:

1. Agriculture Canada (AC)
2. Association of Canadian Faculties of Dentistry (ACFD)
3. Association of Canadian Medical Colleges (ACMC)
4. Association of Universities and Colleges of Canada (AUCC)
5. Canadian Association for Laboratory Animal Medicine (CALAM)
6. Canadian Association for Laboratory Animal Science (CALAS)
7. Canadian Federation of Humane Societies (CFHS)
8. Committee of Chairpersons of Departments of Psychology (CCDP)
9. Canadian Society of Zoologists (CSZ)
10. Confederation of Canadian Faculties of Agriculture and Veterinary Medicine (CCFAVM)
11. Department of National Defence (DND)
12. Environment Canada (EC)
13. Fisheries and Oceans Canada (FOC)
14. Health and Welfare Canada (HWC)
15. Canadian Heart and Stroke Foundation of Canada (HSFC)
16. Medical Research Council (MRC)
17. National Cancer Institute (NCI)
18. National Research Council (NRC)
19. Natural Sciences and Engineering Research Council (NSERC)
20. Pharmaceutical Manufacturers' Association of Canada (PMAC)

CCAC assessment panels evaluate animal care and use in Canadian universities and community colleges, government laboratories, and commercial laboratories. The effects of the CCAC program have included improvement in both housing and management practices. As a result, although the research community is growing, the numbers of experimental animals has, to date, steadily declined; however, they may increase in future because of increased use of transgenic animals and genetic manipulation. This has already occurred in the U.K. (Anon., 1992d).

The concerns of the Council in such areas as alternatives, animal biotechnology, facilities standards, immunological procedures, invertebrates and wild vertebrates are reflected in its committees which comprise experts from a wide variety of biological disciplines and the animal welfare community.

3. CCAC's Assessment Program

The CCAC carries out its national responsibility for animal care through education in the form of workshops, publications, presentations, etc., and its assessment program, which focuses on animal care and use, and the evaluation of the effectiveness of local Animal Care Committees (ACC). These institutional committees are responsible for assuring ethical animal use and compliance with CCAC guidelines at the local level, and must evaluate the ethical aspects of proposed research before the study may commence. (*CCAC Recommended Terms of Reference and Guidelines for Animal Care Committees* are found later in this chapter.) Assessments are based on Volumes 1 and 2 of this *Guide* and CCAC position papers. In-depth assessments are normally scheduled approximately every three years. In addition, a number of Special or Unannounced Visits are conducted if a panel and/or the Council feels that

the conditions at an institution so warrant, or upon request by the institution.

a) The Panel

The scientific members of an assessment panel are chosen by CCAC from an inventory of individuals with experience and special knowledge of various aspects of animal experimentation and care; they are selected, as much as possible, with reference to the predominant thrust of the research at the institution to be assessed. As well, each panel includes a CFHS or other animal welfare appointee, usually drawn from the geographic area of the institution to be visited. All panellists serve voluntarily and without remuneration except expenses. The average complement of a panel is three scientists and one animal welfare representative, with the Director or Associate Director of Assessments serving as an *ex-officio* member. It is sometimes necessary in the larger institutions to increase the size of the panel and divide it into sub-groups. In some of the larger institutions, assessments may be carried out on a faculty or departmental basis.

b) Preparation for the Site Visit

Prior to each assessment visit, the Council requests and receives from the institution current information pertaining to:

i) administrative organization;
ii) animal care personnel;
iii) space allocation and location(s) for animal housing and use;
iv) animal use.

The institution also provides an animal use summary indicating the species and numbers of animals being used, and an indication of the research or teaching project in which they are involved. This permits the panel to give notice of specific projects members may wish to examine in depth, including direct discussion with the principal investigator. Statistics on animal use retained by CCAC are now updated annually through the use of the Animal Research Protocol Management System (ARPMS) computer program developed and provided to each institution by CCAC.

c) The Assessment

Most assessments begin with a meeting with the ACC and senior administrative personnel of the institution. At this time, the institution's animal care program is reviewed, and its response to the previous assessment panel's recommendations discussed. Other topics might include changes to facilities, changes in personnel, occupational health and safety programs, major changes in animal use, the "state-of-the-art" of animal care, and the importance of environmental enrichment.

A considerable amount of time is spent with the institutional ACC reviewing terms of reference, minutes from meetings and discussing the protocol approval process to ensure the committee is functioning effectively.

All areas which house or hold animals are visited, as are all areas in which procedures on animals are performed, e.g., surgical suites and laboratory testing areas. During these visits, many individual investigators are interviewed, and specific procedures or techniques may be observed. The panel is usually accompanied on the site visit by a member(s) of the ACC, and pertinent departmental officials.

Following the site visit, a meeting with the ACC and senior officials is reconvened. This is particularly important if the panel has major or serious concerns about any aspect of the animal care and use; if so, the panel will request immediate appropriate action if a particular situation so warrants. A general outline of the panel's observations and Major or Serious recommendations, which will be forthcoming in an in-

depth report, is usually provided at this time.

Deficiencies which are of a Major nature, i.e., where an animal's health is at risk, must be addressed immediately. Failure to take action would further jeopardize animal welfare and could place an institution in a status of Non-compliance.

Institutions are given only short periods of time (e.g., three months) to respond to recommendations of a Serious nature. Regular recommendations, which most commonly concern housekeeping or facility maintenance, must be implemented at least by the time of the next in-depth assessment.

d) Relevant Reports

A subsequent in-depth report and recommendations, prepared by the panel, are aimed at helping the institution to improve its animal care practices and/or facilities to a standard in keeping with this *Guide*. These reports are circulated to the members of the CCAC prior to being forwarded to the senior administrative official of the institution. The report and its contents are considered confidential; however, the institutional official(s) may distribute copies to whom they wish. If they wish to release the report to the public, the CCAC should have prior notification. Occasionally, a panel member may have concerns which do not reflect the view of the majority of the panel. In such cases, this member has the right to submit a minority report.

In response to the panel's report, institutions are asked to submit to the CCAC within six months a report describing the methods it proposes employing in order to implement the report's recommendations. Should this implementation report be considered unsatisfactory by the Council, the CCAC may instruct its secretariat to determine the reasons for non-compliance and to take such further actions as deemed necessary. For example, the CCAC notifies the MRC and NSERC of any institution found to be in non-compliance with CCAC standards, and which has not responded satisfactorily within the time given to correct the situation. A statement issued in March, 1985, notes:

"On receipt of a statement on non-compliance, and after reviewing the full evidence, NSERC and MRC reserve the right, either separately or together, to bring their concerns to the appropriate authorities in the research institution concerned and, if they deem it necessary, to implement such financial or other sanctions as may be in the power of either Council. Such sanctions may be taken whether or not the non-compliance concerns research funded by either Council and might include the freezing or withdrawal of research funds for any or all research programs funded by either or both Research Councils in the institution."

e) Summary

CCAC's programs have been well received, and enjoy the support and co-operation of all institutions involved, including private sector companies and government departments that are not dependent on funds from granting agencies. It has also resulted in an increased level of awareness and sensitivity to the ethics of animal experimentation amongst scientists and investigators. The Council continues to encourage the use of alternatives to animal use wherever possible, as exemplified by Russell and Burch's "3R" tenet of Replacement, Reduction and Refinement (Russell and Burch, 1959; Smythe, 1978).

4. CCAC Position Statements

In addition to the *Guide*, (Volumes 1 and 2) the CCAC develops and publishes position statements on a number of matters. Such position statements are subject to periodic revision. These include *Ethics of Animal Investigation, CCAC Guidelines on Acceptable Immunological Procedures*, and *Categories of Invasiveness in Animal Experiments*, which may be found in the Appendices. The *Social and Behavioural Requirements of Experimental Animals* (SBREA) document may be found elsewhere in this *Guide*. As noted, these position statements, along with the *Guide*, form the basis on which CCAC assessments are

made.

5. Legislation Governing Experimental Animals

Animal welfare is governed in Canada under Section 446 of the Criminal Code. Legislation pertaining specifically to experimental animals exists in two provincial acts: Animals for Research Act (Ontario) and Universities Act (Alberta). There are numerous other laws that impact on animals kept for research, teaching and testing.

a) Federal Legislation

The Criminal Code of Canada, Section 446, Cruelty to Animals, forbids "causing unnecessary suffering." The century-old (1892) Code states that: "Everyone commits an offence who wilfully causes or, being the owner, wilfully permits to be caused unnecessary pain, suffering or injury to an animal or bird...."

The Law Reform Commission of Canada (LRCC), after 15 years' preparation (Anon., 1988a), and considerable public input, proposed widespread amendments to the Criminal Code in a report entitled*Report 31 Recodifying Criminal Law* in 1988 (LRCC, 1987; Anon., 1988b). The proposed changes relating to animals centred on treating animals as sentient beings, and not merely chattel put here for humans' use, a position the Canadian Veterinary Medical Association (CVMA), among others, considered merited support (Olfert, Finley, Laniel *et al.* 1989). Chapter 20, Title IV, Crimes Against the Natural Order, Crimes Against Animals, focused on three areas: cruelty to animals, organizing sporting events and animal neglect. It noted, for example, that "Everyone commits a crime who unnecessarily causes injury or serious physical pain to an animal." Scientific research is exempt "unless the risk of injury or serious physical pain is disproportionate to the benefit expected from the research." Although tabled in Canada's House of Commons May 19, 1988 (Anon., 1988c), no action has been taken to date to implement the report.

In 1989, the CFHS proposed federal legislation governing experimental animals, which included economic sanctions and more power for non-user members of institutional ACCs (Anon., 1989). The CFHS proposal was later refused by the then Minister of National Health and Welfare, The Hon. Perrin Beatty, who considered it would be more expensive than CCAC's program, and "would not improve the already excellent level of care given to animals in Canadian laboratories."

The federal **Health of Animals Act, C-66 (June, 1990, rev. March, 1992); 38-39 Elizabeth II, Chapter 21** (replacing the Animal Disease and Protection Act and Part III of the Livestock and Livestock Products Act) is aimed at protecting Canadian livestock from contagious diseases such as tuberculosis and brucellosis, and keeping out foreign diseases. Under Regulations, the Act states that "the Governor in Council may make regulations for the purpose of protecting human and animal health...including regulations...

i) for the humane treatment of animals and generally governing the care, handling and disposition of animals;

ii) governing the manner in which animals are transported within, into or out of Canada; and

iii) providing for the treatment or disposal of animals that are not cared for, handled or transported in a humane manner."

Agriculture Canada has published Codes of Practice for pigs, veal calves, poultry, dairy cattle, beef cattle, ranched fox and mink (Agriculture Canada, 1771/E, 1984; 1821/E, 1988; 1757/E, 1989; 1853/E, 1990; 1870/E, 1992; 1831/E, 1989; 1819/E, 1988). In addition, a revision of the Recommended Code of

Practice for the Care and Handling of Farm Animals--Pigs (1898/E) is now in press. The Codes represent the current industry standards and, for the most part, are the minimum CCAC requires for research institutions undertaking agricultural research. Researchers and others working with agricultural animals must be fully conversant with these Codes.

The federal **Food and Drug Act and Regulations** (August 5, 1982), cover use of animals in the testing of new drugs and vaccines, and for toxins in foods. Health and Welfare Canada's Bureau of Biologics is responsible for the monitoring of biologics, the virulence and efficacy of which can usually be tested only in the living animal.

Very little testing of cosmetics and consumer chemicals is conducted in Canada due to the fact that almost none of these items are developed here (Gilman, 1980). That said, cosmetics are the responsibility of the Drugs Directorate, through the Bureau of Non-prescription Drugs. Safety evidence is required of the manufacturer and could involve *in vivo* (animal) testing in the case of new chemicals and chemical combinations.

Responsibility for food safety/nutritional quality lies with the Bureau of Microbiological Hazards, Chemical Safety, and Nutrition. The identification of specific microorganisms and toxins in food requires some specific animal tests (e.g., tests are conducted in mice for paralytic shellfish toxins and botulism).

b) Provincial Legislation

i) Saskatchewan

Under the Veterinarians Act of 1987 of the Province of Saskatchewan (Chapter V-5.1) "a person using an animal in research at a university," and employing procedures in studies approved by an ACC which includes a veterinarian, is exempt from the Act's provision that only a member of the Saskatchewan Veterinary Medical Association "shall engage...in the practice of veterinary medicine."

ii) Alberta

Alberta, in 1966, passed its **Universities Act**, (Section 50, "Dog Control and Procurement"; Regs. 341-366). This legislation forbade research institutions from purchasing dogs for research purposes, but made it incumbent upon municipal pounds to make all unclaimed dogs available to faculties of medicine on request. In 1972, Alberta Regulation 33-72 was expanded to cover the treatment of **animals**. Conditions under which they were to be transported, maintained, used and disposed of were specified. This included mandatory exercise for caged dogs. It required use of anesthesia, analgesia, and standards of post-surgical treatment; the demand that those using animals be qualified; and safeguards to permit owners' retrieval of their animals from an isolation/quarantine facility (Secord, 1974).

iii) Ontario

The use of experimental animals in Ontario is governed by its **Animals for Research Act** (Revised Statutes of Ontario, 1980, Chapter 22 as amended by 1989, Chapter 72,s6 and Regulations 16,17,18,19. Revised Regulations of Ontario, 1980, March 1990). Before its passage after much controversy in May, 1971, the then Minister of Agriculture and Food, The Hon. William Stewart, stated February 19, 1969 in the Provincial Legislature, that: "By controlling the source of animals and by making animals more readily available to research facilities, undesirable practices such as the theft of dogs--dognapping--and the operations of unscrupulous dealers will be curbed..." (Stewart, 1969a). On June 17, 1969, again in the Provincial Legislature, Mr. Stewart said that: "When this legislation is enacted, the Government of Ontario will embody in these Regulations the principles relating to the care of experimental animals as spelled out by the Canadian Council on Animal Care (CCAC)..." (Stewart, 1969b); however, this was never done.

This Act, which is administered by the Ontario Ministry of Agriculture and Food (OMAF), requires annual registration of all research facilities in the province. It includes clauses requiring anesthetics and analgesics in order to prevent animal suffering and unnecessary pain, local ACCs which include a veterinarian and authority to require that research projects be modified; regulated minimum standards for housing, procedures and care, inspection of research premises and a minimum redemption period of 72 hours (which municipalities may extend). Under the Act, after this period, the pound may sell unclaimed animals for pets, hunting and for use in registered research establishments.

Under the Animals for Research Act, the **Ontario Society for the Prevention of Cruelty to Animals Act, 1955** "does not apply in respect of animals in the possession of the operator of a registered research facility (RSO 1980, c.22, s.19)."

In 1985, **Bill 21**, a Private Member's Bill proposed by MLA Ed Philip, would have allowed municipalities the choice of whether or not to provide unwanted stray animals to research establishments. In 1986, according to the Ontario Public Trustee's Office, the Coalition Against Pound Seizure, "a project funded by the Toronto Humane Society" spent more than $200,000 fighting the Animals for Research Act (McAndrew, 1987). Bill 21 was opposed by veterinary societies (Sanderson, 1986), and the Ontario Humane Society, which suggested that its members disassociate themselves from the coalition. Researchers and academics argued that work crucial to saving human lives was threatened because of pressure from such groups (Wilson, 1986).

Although Bill 21 passed 2nd Reading in 1985, it was not put forth for 3rd (and final) Reading (Anon., 1987; Comeau, 1987; Sheppard, 1987; Wilson, 1986).

In 1988, MLA C.J. "Bud" Wildman introduced **Bill 190**, which sought to amend the Animals for Research Act by prohibiting use of animals in non-medical testing. Hearings were held to debate it in 1989 (Anon., 1989; Harvey, 1990) and it received 2nd Reading, but not the necessary 3rd Reading. However, meetings in 1991/92 between Ontario's Minister of Agriculture and Food, The Hon. Elmer Buchanan, and various groups have resulted in his decision to "increase public input into the research process as well as restrict the usage of animals in testing."

iv) Quebec

Following charges that 2,000 stray dogs were picked up off suburban Montreal streets and sold in the U. S. for use in research (CP, 1985), the Montreal-based Canadian SPCA conducted an investigation which found that 3,000-5,000 dogs and 2,000 to 3,000 cats were being shipped each year from Quebec, primarily because research establishments in the Northeastern U.S. had passed laws against the sale of animals from municipal pounds for research (Duquette, 1986). The CSPCA subsequently developed and proposed legislation governing animal use (Duquette, 1986).

More recently, a provincial Table de concertation (Working Group) on Cruelty to Animals examined animal welfare in the Province of Quebec, and reviewed animal welfare legislation proposed by the CSPCA (Anon., 1991a). The Table's (1992) report requested that CCAC ensure strict adherence to its guidelines in Quebec by increasing the number of assessments, and unannounced visits in particular, ensure effective functioning of ACCs, pay particular attention to the qualifications of researchers and animal care attendants, ensure management and monitoring are adequate, strongly encourage adherence to Russell and Burch's "3R" tenet of Replacement, Reduction and Refinement (Russell and Burch, 1959) and ensure ACCs include representatives from the community, including known animal welfare societies.

The recommendations also asked Quebec ministries involved in animal use to ensure CCAC standards were applied in all institutions receiving provincial funding, and that CCAC periodically supply the Ministry of Agriculture, Fisheries and Food with a list of the institutions assessed, so that the Ministry could request copies of assessment reports.

c) Legislation in the United Kingdom

The U.K.'s **Animals (Scientific Procedures) Act 1986,** which replaced the century-old Cruelty to Animals Act 1876, gives the Home Secretary the responsibility for judging the scientific merit of the research he authorizes, "for which he will be answerable to parliament" (Hollands, 1986). Section 5(4) states that "In determining whether and on what terms to grant a project licence, the Secretary of State shall weigh the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the program to be specified in the licence." This clause was included at the insistence of an alliance comprising the British Veterinary Association (BVA), the Committee for the Reform of Animal Experimentation (CRAE), and the Fund for the Replacement of Animals in Medical Experiments (FRAME) (Smith, 1988).

Commenting on this requirement, Balls (1989) states: "What then will follow is the crucial weighing of the balance, but how certain will we need to be that a particular program of work will lead to the relief or avoidance of significant human (or animal) suffering before we allow undeniably painful procedures to be applied to laboratory animals? One part of the answer should be to apply a more rigorous and more considerate and humane analysis in the future than in the past, where (to agree with Les Brown) (Brown, 1988) supposed benefits couched in generalities such as the 'increase of knowledge' have all too often been enough. Also, let us in future be more willing to give the animal the benefit of the doubt--for who can have the foresight to satisfactorily and convincingly quantify the extent to which the rate of progress in medical research would really suffer as a consequence?"

The Act also calls for a statutory 21-member Animal Procedures Committee (APC) which has wide powers to advise the Home Secretary, who controls the overall severity permitted. It also dictates that all research animals (with some exceptions (e.g., farm animals and animals taken from the wild) be obtained from registered suppliers (Balls, 1986). The APC was to provide the mechanism for ethical debates. However, member Judith Hampson, former Animal Experiments Officer with the RSPCA, has stated that the committee deals only with a few cases referred to it by the Home Office and "has failed to deal adequately with issues of particular concern to the public...and there is very little public accountability." She called for formation of institutional ethics committees with community representation (Hampson, 1992).

The Animals (Scientific Procedures) Act was widely debated and criticized, both before and after passage (Balls, 1990; Aldhous, 1990; McKie, 1986; Fisher, 1990), and was even described by animal activists as "the vivisectors' guide to pain" (Churchward, 1986). Others subsequently questioned whether the Act had failed (Balls, 1990; Anon., 1990) when experiments carried out in an inadequately anesthetized rabbit by an elderly neurophysiologist at the National Institute for Medical Research (NIMR) were exposed by an antivivisectionist group (Anon., 1990) using videotaped evidence. The organization later claimed that "the Home Secretary failed to weigh adequately the likely benefit of the research against the adverse effects on the animals involved."

The scientist and his assistant relinquished their licences and a subsequent Medical Research Council (MRC) enquiry reported that the requirements of the Act had, indeed, been broken (Anon., 1991b). Many felt, however, that the case was an isolated incident, and the British Veterinary Association (BVA) came to the conclusion that, overall, the Act was working well, although improvements are needed in administration and operation (Anon., 1991c).

d) Legislation in the United States

In the U.S., two primary federal laws govern animal use. The **Health Research Extension Act (or NIH Authorization Act)** passed in 1985 requires establishment of Institutional Animal Care Committees (IACCs) and IACC inspections (Traystman, 1990). The **Animal Welfare Act** (1985) includes in its requirements provision of adequate veterinary care with appropriate use of anesthetic, analgesic, tranquillizing drugs, or euthanasia, consideration by the principal investigator of alternatives to any procedure likely to produce pain and distress, and establishment of ACCs, which must include a veterinarian.

The Department of Agriculture, Animal and Plant Health Inspection Service, March 15, 1989 published Animal Welfare; Proposed Rules in the *Federal Register* (pgs. 33447-33531) to amend the Animal Welfare Act (7 U.S.C. 2131-2157), which itself was an amendment to the Farm Bill (1985). The 1985 amendments had required standards for pre- and post-surgical care, inspection of research facilities, requirement for ACCs, establishment of an information service at the National Agricultural Library, annual training sessions for laboratory personnel and increased penalties for facilities violating the animal welfare standards (Schwindaman, 1990).

After much public input and debate (Meyers, 1990), Final Rules for Part One (definitions) and Part Two (regulations) were published August 31, 1989 in the *Federal Register* (pgs. 36112-36163). Although these Final Rules approved the governing of small animals, Part Three proposed "additional standards... directed primarily toward the exercise of dogs, the psychological well-being of primates and standards for research facilities" (Schwindaman, 1990).

Eventually, the Final Regulations, issued February 15, 1991, and published in the *Federal Register* (pgs. 6426-6505) were "performance based," i.e., the health of the animals would be of greater importance than details of their housing (Anon., 1991c). By August 14, 1991, institutions had to produce written plans for providing exercise for dogs and improving the psychological well-being of non-human primates (NHP) (Myers, 1991). However, a U.S. judge has invalidated the regulations because they did not set minimum standards (Mervis, 1993).

As the result of a lawsuit launched by the Humane Society of the United States (HSUS) and the Animal Legal Defence Fund (ALDF), experimental mice, rats and fish will now be covered by the legislation in the U.S. January 8, 1992, the U.S. district court in Washington, D.C. ruled that the U.S. Department of Agriculture (USDA) had violated the federal Animal Welfare Act by denying basic protection to America's 15 million mice, rats and birds used annually in research (Anon., 1992a). However, the government has appealed this decision (Mervis, 1993). Institutional Animal Care and Use Committees (IACUC) (Orlans, Simmonds and Dodds, 1987), must now review all research proposals involving these species, as the CCAC has required since its inception.

A compilation of U.S. state laws concerning research animal use has recently been published (NABR, 1991), as well as animal-related legislation introduced in the 102nd Congress (Anon., 1992b). August 26, 1992, the U.S. also passed the "Animal Enterprise Protection Act of 1992" (Public Law 102-346) which made it a Federal offence to enter a research laboratory without authorization and "steal, destroy or make unauthorized use of research animals, equipment or data" (Heflin, 1992; Anon., 1992c).

6. Pre-University Use of Animals

Before the establishment of CCAC guidelines, pre-university use of experimental animals was governed by a one-page document prepared by the CFBS. The Federation required compliance with its Guiding Principles, and said that "all experiments employing animals must be carried out under the supervision of a qualified teacher."

At the present time, animal use in the school is subject to the requirements of legislation such as the Health of Animals Act (Bill C-66), the Criminal Code of Canada, Section 446, Cruelty to Animals, and provincial legislation, where such exists. Primary responsibility for animal use at the pre-university level now lies, however, with the Youth Science Foundation (YSF) (904-151 Slater St., Ottawa, Ontario Canada K1P 5H3), which requires compliance with the CCAC guidelines in the conduct of biological research.

The YSF, amongst its responsibilities, regulates animal experimentation in Science Fairs. All research intended for Science Fairs must be screened by a committee cognizant of current requirements; if none is available, the YSF may be contacted. Science Fair Regulations permit use of lower forms of life (bacteria, fungi, protozoa, insects, plants and invertebrate animals). Vertebrate animals (birds, fish, mammals,

reptiles, amphibians) "are not to be used in any active experiments which may be deleterious to the health, comfort or physical integrity of the animal..." Observation of wild animals, animals in zoological parks, farm animals and pets is permitted.

It should be noted that, before any such projects are undertaken, adequate arrangements should be made for the care of the animal while in the classroom, and its subsequent disposition, which may involve euthanasia.

Some school boards, such as the Peel Board of Education, have produced their own guidelines (Henshall, Scott and Scott, 1986). The Ontario Egg Producers' Marketing Board (7195 Millcreek Dr., Mississauga, Ontario Canada L5N 4H1) has also published A Teacher's Guide to Hatching Eggs in the Classroom (1990). An American leaders' manual for avian embryology, entitled Beginning of Life, is also available (Publ. #408-029. Virginia Co-operative Extension Service; Clinton, V. Turner, Administrator, 1890 Extension Program, Virginia State University, Petersburg, VA).

Guidelines have also been published in the U.S. (NABT, 1990; Orlans, 1977; McGiffin and Brownley, 1980; ILAR, 1989), and in the U.K. by the RSPCA (1985) (Causeway, Horsham, West Sussex RH12 1HG, U.K.).

B. LOCAL LEVEL

1. The Institutional Animal Care Committee

It is essential that the necessity for and the benefits of effective control in the care and use of experimental animals be recognized. Regardless of whether this control is "voluntary" or legislated, each institution has a commitment to be cognizant of the nature of all experiments involving animals in their establishments and to ensure their propriety. This responsibility is best met by an effective local ACC, reporting to the appropriate senior administrative officer of the institution. The local ACC should be responsible for formulating and implementing policy on all matters concerning the general care and use of animals as outlined below.

Terms of Reference for Animal Care Committees* (Revised in 2006)

1. Membership | 2. Authority | 3. Responsibility | 4. Meetings | 5. General

The Canadian Council on Animal Care (CCAC) requires that institutions conducting animal based research, teaching or testing establish an animal care committee (ACC), and that it be functionally active. Each committee's operation must be governed by formal Terms of Reference that include the following Terms, but need not be limited to them. The ACC's Terms of Reference must be tailored to reflect and refer to the institution's animal care and use program, including the members of the program and the institution's policies, practices and procedures.

Most institutions have a single ACC. A few large institutions choose to have more than one ACC: this is acceptable, as long as the ACC system is well structured to avoid potential conflicts of interest, and has an institutional ACC that oversees the work of the ACCs for the various units and establishes policies and procedures to ensure sound general standards in order to meet CCAC guidelines throughout the institution. The elements covered in this policy statement may be divided out between the various ACCs of an institution, as long as all elements are covered in an appropriate and structured fashion and are defined in suitable terms of reference for each committee.

ACCs may also choose to form subcommittees to work on specific areas such as protocol review or development of standard operating procedures (SOPs). Protocol review subcommittees should include at least one scientist, one veterinarian, one community representative, one institutional member who does not use animals, one technical staff representative and the ACC coordinator.

Institutional ACCs should be responsible directly to the senior administrator responsible for animal care and use for the institution (president, vice president, rector, CEO, etc.), and this link should be specified in writing. ACCs for faculties or other divisions of the institution should have representation on the institutional committee and report directly to it, in addition to the reporting line(s) to any other senior administrators. The CCAC policy statement on: senior administrators of animal care and use programs (in preparation)

should be consulted for details on the roles and responsibilities of the institution and its senior administrators.

The institution must work with the ACC to ensure that all animal users and caregivers are informed of and comply with institutional animal care and use policies and procedures.

The institution must be supportive of the committee's work. This includes appointing an ACC coordinator, who may work part-time for the ACC in the case of smaller institutions, whereas larger institutions will need one or more employee(s) to accomplish this work. The ACC coordinator must support the ACC by ensuring that animal use protocols are well managed, that committee minutes and reports are promptly produced and distributed, that all exchanges between the ACC and animal users are well documented and filed in a timely manner, and that animal users and ACC members are provided with necessary information.

The institution must also ensure that ACC members are provided with training opportunities to understand their work and role: these must include at least a formal orientation session, to introduce new ACC members to the institution's animal care and use program and its members, policies and procedures, as well as to the animal facilities and to CCAC guidelines and policies. Material on the CCAC website (and other relevant websites), such as the Modules on the Core Topics of the Laboratory Animal/Teaching Stream of the CCAC Recommended Syllabus, can be introduced as possible resources. Ongoing opportunities to better understand animal care and use in science should also be provided, such as time spent with animal care givers and users, access to relevant journals and materials, and meetings/workshops related to animal care and use, including the CCAC National Workshop.

The institution and its senior administrators must also ensure that the ACC is well respected within the institution, and that all ACC members and the ACC Chair are valued and recognized.

* May also be referred to as:

  * institutional animal care and use committees (IACUC)
  * animal research ethics boards (AREB)
  * Ethics Committees

and by other names, as long as their function is clear and they operate according to Terms of Reference based on this document.

1. Membership

ACC members should be appointed for terms of no less than two years and no more than four years, renewable only up to a maximum of eight consecutive years of service. This maximum should not be exceeded, except in the case of very small institutions (i.e. those that have 3 or fewer animal users). This does not apply to ACC members who must be part of the ACC because of their role within the institution (ex officio members): the ACC Coordinator, the veterinarian(s) and the animal facility manager. The complement of the committees will vary and should be determined by the needs of each institution, but should include:

a. scientists and/or teachers experienced in animal care and use, who may or may not be actively using animals during their term on the ACC; there should be a minimum of two such members, and representation of all the major animal-using divisions of the institution must be ensured;

b. a veterinarian, normally experienced in experimental animal care and use;

c. an institutional member whose normal activities, past or present, do not depend on or involve animal use for research, teaching or testing;

d. at least one, and preferably two or more, person(s) representing community interests and concerns, who has (have) had no affiliation with the institution, and who has (have) not been involved in animal use for research, teaching or testing; community representation must be ensured for all ACC activities throughout the year;

e. technical staff representation (either an animal care, an animal facility or an animal research technician) if there is (are) (a) technical staff member(s) actively involved in animal care and/or use within the institution;

f. student representation (graduate and/or undergraduate), in the case of institutions that have programs where students use animals; and

g. the ACC coordinator (the institutional employee who provides support to the ACC).

The senior administrator to whom the committee reports must not be a member of the ACC, but there can be a representative of the senior administration on the committee.

The person with overall responsibility for the animal facilities, whether a veterinarian, a scientist or a technical staff member, must be included on the ACC. In large institutions with several facilities, consideration can be given to having individual facility managers included on the ACC on a rotating basis.

ACCs benefit from having occupational health and safety and biosafety representatives (if this is not the case, other ways must be found of ensuring close links), and ACCs can also benefit from the presence of biostatisticians, ethicists and those responsible for public relations.

Every ACC must have a chair who should not be directly involved in the management of the institutional animal facilities, nor be a clinical veterinarian for the institution, nor be an animal health or veterinary personnel member charged with ensuring compliance with CCAC guidelines, nor be involved in the preparation of a significant number of the protocols to be reviewed by the committee, in order to avoid potential conflicts of interest. Provision should be made to co-opt other persons to the ACC as the need arises. A reasonable quorum, such as a majority of the members, should be established for ACC meetings, and the quorum should include community and veterinary representation. Meetings should be scheduled at times that are convenient for all members, including community representatives.

2. Authority

The ACC must have the authority, on behalf of the senior administrator responsible for animal care and use for the institution, to:

a. Stop any objectionable procedure if it considers that unnecessary distress or pain is being experienced by an animal;

b. Stop immediately any use of animals which deviates from the approved use, any non approved procedure, or any procedure causing unforeseen pain or distress to animals; and

c. Have an animal killed humanely if pain or distress caused to the animal is not part of the approved protocol and cannot be alleviated.

The Chair of the ACC and the veterinarian(s) must have access at all times to all areas where animals are or may be held or used.

Each institution must establish procedures for post-approval monitoring of animal use protocols, and must define the roles and responsibilities of the members of the animal care and use program in the monitoring process. The institutional ACC is the body responsible for determining and working to correct breaches of compliance with approved animal use protocols and SOPs. Breaches of compliance that cannot be corrected by the ACC working with the concerned animal users and veterinary/animal care staff must be referred to the senior administration, which must inform all members of the animal care and use program about sanctions that will be taken by the administration in the event of serious breaches of compliance.

As the ACC is generally not present when animal use protocols are being undertaken, the committee must work with the members of the veterinary and animal care staff to ensure compliance with its decisions and with the conditions set out in approved protocols. The veterinary and animal care staff must work in a collegial manner with animal users and attempt to correct deficiencies collaboratively. Where there are persistent breaches of compliance or threats to the health and safety of personnel or animals, these must be reported back to the Chair of the ACC, and the Chair and ACC must promptly address these issues,

through communications with the animal user(s), meetings and site visits, and eventually communications with the senior administrator, as necessary.

The ACC must also delegate to the veterinarian(s) the authority to treat, remove from a study or euthanize, if necessary, an animal according to the veterinarian's professional judgment. The veterinarian must attempt to contact the animal user whose animal is in poor condition before beginning any treatment that has not previously been agreed upon, and must also attempt to contact the ACC Chair, but the veterinarian must have the authority to proceed with any necessary emergency measures, whether or not the animal user and ACC Chair are available. A written report should be sent by the veterinarian to the animal user and to the ACC following any such event.

The veterinarian and ACC may also choose to delegate certain responsibilities to one or more senior animal care staff member(s).

3. Responsibility

It is the responsibility of the ACC to:

a. Ensure that no research or testing project or teaching program (including field studies) involving animals be commenced without prior ACC approval of a written use protocol; further to this, that no animals be acquired or used before such approval. This includes internally funded projects;

b. Ensure that no animals be held for display or breeding purposes, or for eventual use in research, teaching or testing projects, without prior ACC approval of a written animal use protocol, except where current CCAC guidelines provide for exemptions. The ACC should also be aware of other animal-based activities, such as commercial or recreational activities, within the institution, and should work with the persons responsible for these activities to ensure that animal care and use is undertaken according to appropriate procedures;

c. Require all animal users to complete an animal use protocol form and ensure that the information therein includes the following points, clearly presented in a form that all members of the ACC can readily understand (supplemental information can be found in the CCAC guidelines on: animal use protocol review, 1997). To facilitate the work of both protocol authors and ACC members, appropriate SOPs should be referred to as much as possible. Approved protocols and SOPs should be readily available in the areas where animal-based work is taking place.

    i. project title and descriptive procedural keywords or brief description of the procedures to be conducted on animals, as defined in the CCAC Animal Use Data Form;

    ii. principal investigators/teachers, and all personnel (post-doctoral fellows, research staff, graduate and undergraduate students) who will handle animals, along with their training and qualifications with respect to animal handling (see point 3m) iii)); in the case of undergraduate students, who may have very little training, close supervision is required;

    iii. departmental affiliation;

    iv. proposed start date, proposed end date (if the study is to take place over more than one year, the work and numbers of animals for the first year only should be approved, and further work can then be approved in yearly protocol renewal(s) or new protocols - see Section 3g) on protocol renewals);

    v. for research or testing projects, funding source(s) and status of funding approval;

    vi. for research projects, an indication of whether the project has received peer review for scientific merit;

    vii. for teaching programs, a course number and an indication of whether the course has been re-viewed with respect to the pedagogical merit of using live animals; institutional or departmental curriculum committees can be called upon to provide a review of pedagogical merit to the ACC; a specific appendix or separate protocol form can be used to better capture information relevant to

the ethical review of teaching programs (see Section 12 of the CCAC guidelines on: animal use protocol review);

viii. for testing projects, an indication that the testing has been planned according to the most current regulatory requirements, using guidelines acceptable to the regulatory agency(ies) and which meet the requirements of the CCAC policy statement on: ethics of animal investigation; that the planned animal use not exceed the requirements of the regulatory authorities - if it does, justification for the additional animal use must be provided;

ix. lay summary;

x. an indication of the use of biohazardous, infectious, biological, chemical or radioactive agents in animal-based projects; and, if so, an indication of institutional approval of this use;

xi. category(ies) of invasiveness as defined in the CCAC policy statement on: categories of invasiveness in

xii. information with regard to the Three Rs (replacement, reduction and refinement alternatives) of animal use, to include:

xii.1. a description of why sentient animals must be used for the project, of how the applicant arrived at this conclusion (e.g., searches of databases on alternatives), and of possible replacement alternatives (non-animal methods, cell/tissue culture, computer simulations, audio-visual teaching methods, the replacement of sentient animals with animals of lower sentiency, etc.) and justification if these are not to be employed;

xii.2. justification of the species and numbers of animals to be used over the course of the year, to emphasize reduction of animal use within an appropriate experimental design, while ensuring that sufficient numbers of animals will be used to fulfill requirements for statistical significance/scientific validity in the case of research projects, or for acceptance of regulatory tests;

xii.3. a description of all of the refinements to be employed to protect and enhance animal health and welfare, which may include:

xii. 3.1 anesthesia and analgesia, including dosages and methods of use, for all invasive protocols; strong scientific justification must be provided for not using anesthesia or analgesia in the case of invasive protocols;

xii. 3.2 other medical treatments as appropriate, as indicated through veterinary consultations;

xii.3.3 housing and husbandry methods, and environmental enrichment as a means to refine animal care; any limitations on environmental enrichment from that normally offered to animals in the institution, based on CCAC guidance, must be justified to the ACC;

xii.3.4 refinements to the procedures to be employed on the animals;

xii.3.5 refinements to the length of time that animals will be held/used;

xii.3.6 any other possible refinements;

xiii. a clear description detailing the procedures that are carried out on the animals (referring to appropriate SOPs as much as possible); the use of graphic representations is encouraged;

xiv. a description of the endpoint(s) of the experimentation, selected according to the CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing, 1998 (refer to institutional SOPs, if available and relevant); the person(s) responsible for monitoring the animals and applying endpoints should be identified, and the schedule for monitoring animals and any relevant checklists of signs and symptoms to be used

when evaluating the animals should be included; all protocols, even non-invasive ones, must identify endpoints, to ensure that any animals requiring treatment are treated and that animals are not simply kept indefinitely; relevant information for identifying and applying endpoints must be readily available, preferably posted, in the area where the animal-based work is taking place;

xv. a description of capture, restraint, transportation and/or housing of animals used in field studies, as well as any other information pertinent to field studies, such as capture of non target species, ecological impacts and potential injuries or mortality during capture or transportation, if relevant; wildlife studies should be addressed in either a separate section or appendix of the protocol form, or can have their own protocol form, especially where a significant number of wildlife studies are undertaken (see the suggested wildlife protocol form in Appendix B of the 2003 CCAC guidelines on: the care and use of wildlife);

xvi. the method of euthanasia, if used; justification for any physical euthanasia methods, or for any methods that deviate from those described in the most recent CCAC guidance on euthanasia;

xvii. a description of the fate of the animals if they are not to be euthanized, including the length of time that they are to be held;

xviii. any other information considered important or necessary and pertinent, including information or results derived from any relevant previous protocols; the description and use of previous relevant results is particularly important to ensure that methodologies are not simply re-used without learning from any animal welfare problems that were encountered in the past, that the protocol continues to have relevant goals and methodology, and that appropriate refinements to protect and enhance animal welfare are sought and implemented;

d. Ensure that each research project has been found to have scientific merit through independent peer review before approving the project; if the review is not carried out by an external, peer review agency, the institution should require that it be obtained according to the CCAC policy statement on: the importance of independent peer review of the scientific merit of animal based research projects, 2000. The institution must implement a mechanism through which non-peer-reviewed projects are reviewed for their scientific merit either by calling upon the expertise of individual independent peers or by making use of scientific committees or advisory boards;

e. Review and assess all animal use protocols, with particular emphasis on the CCAC policy statement on: ethics of animal investigation and CCAC guidelines on: animal use protocol review as well as on all other relevant CCAC guidelines and policy statements and, where necessary, require further supportive information from the investigator/teacher or meet with the investigator/teacher to ensure that all members of the committee understand the procedures to be used on the animal. Information exchanges and ACC discussions with protocol authors can be very useful, but protocol authors and members of their teams must always clearly remove themselves from ACC decision-making on their own protocols.

The committee must also ensure that all procedures comply with CCAC guidelines, and, if at variance with those guidelines, require justification for the variance on scientific grounds. ACCs should both discuss protocols and make decisions on them during full committee meetings, rather than through individual reviews, and should attempt to reach decisions by consensus. Electronic tools are widely used for protocol management purposes and to facilitate and expedite the submission and review of protocols. This is encouraged as long as ACCs or protocol review subcommittees continue to meet in person for protocol discussions and final approvals.

An ACC may delegate the responsibility of interim approvals to an interim approval subcommittee, which must include at least one scientific member, one veterinarian and one community representative, one of which should preferably be the chair of the ACC. However, such interim approvals should only be used infrequently, and the interim review process, including exchanges between the ACC and protocol authors, must be documented and must then be subject to discussion and final approval at a full meeting of the committee. The ACC should define its own protocol review process, with or without (a) protocol review subcommittee(s), in its Terms of Reference. This process should include or refer to clear instructions to protocol authors, to ensure that all animal users in the

institution understand how the ACC works, when it meets, how to fill out and submit a protocol form and what to expect after submission of the form;

f. Ensure that animal users update their protocols with any modifications they intend to make, and approve any modifications to a protocol before they are implemented. Minor modifications (e.g., 1 or 2 animal users added or removed, a small number of animals added, etc.), as defined by the ACC, can be approved by the Chair of the ACC or a delegate.

   For any major changes to a protocol, require that a new one be submitted. ACCs should define, in writing, their own criteria as to what constitutes a major change to a protocol (e.g., a considerable increase of the number of animals required vs. the number in the original protocol, a change of species, use of more invasive or more frequent procedures, use of entirely new procedures, or other criteria).

   Ensure that animal users report any unanticipated problems or complications, as well as on the steps they have taken to address the problem(s), to the ACC;

g. Review all protocols annually, i.e., within a year of commencement of the project; annual renewals should be approved by at least a scientist, a veterinarian and a community representative and should be brought to the attention of the full ACC for its information. Institutions may choose to use a shorter protocol renewal form, but no matter what form is used, all protocol renewals must emphasize:

   i. the number of animals used in the preceding year;

   ii. the number of animals needed for the year to come, with a justification;

   iii. a brief progress report, describing any complications encountered relative to animal use (unpredicted outcomes, and any animal pain, distress or mortality), any amendments to the original protocol, and any progress made with respect to the Three Rs of replacement, reduction and refinement of animal use;

   iv. a brief report on the adequacy of the endpoints for the protocol, and on any complications encountered or refinements made relative to protecting animals from pain, distress or mortality; and

   v. any other changes from the original protocol.

   Require the submission of a new protocol after a maximum of three consecutive renewals;

h. Document all ACC discussions and decisions in the committee minutes and on attachments to the protocol forms;

i. Define an institutional appeal mechanism that can be used by the author of a protocol in the event that animal use is not approved by the ACC. This mechanism should include appropriate expertise and ensure a separate, fair and impartial process. The CCAC may be called upon for information purposes; however, appeals cannot be directed to the CCAC;

j. Ensure that all ACC members and animal users have the opportunity to become familiar with the CCAC Guide and CCAC policy statement on: ethics of animal investigation and all other CCAC guidelines and policy statements, federal, provincial or municipal statutes that may apply, as well as institutional requirements;

k. Ensure appropriate care of animals in all stages of their life and in all experimental situations. Veterinary care must be available. Formal arrangements must be made to obtain the services of a veterinarian, at least on a consultative basis, if they are not readily available within the institution. These formal arrangements must be based on the elements contained in the CALAM/ACMAL Standards of Veterinary Care of the Canadian Association for Laboratory Animal Medicine (2004),

which define the roles and responsibilities of veterinarians involved in scientific animal care and use programs;

l. Establish procedures, commensurate with current veterinary standards, to ensure that:

   i. unnecessary pain or distress is avoided, and animal stress and injuries are avoided, whether during transfers of animals or in their normal quarters;

   ii. anesthesia and analgesia are properly and effectively used; the only exception to this may be when agents must be withheld as a scientifically justified requirement of the study, and that this has been approved by the ACC. Painful studies requiring exemption from the use of either anesthetics or analgesia must be subject to particular scrutiny, not only prior to approval, but also during the experiment;

   iii. appropriate post-operative care is provided;

   iv. all due consideration is given to animal welfare, including environmental enrichment;

   Ensure that policies to provide for a system of animal care that will meet the needs of the institution are established and implemented, and include:

   i. the requirement that all animal care and animal experimentation are conducted according to CCAC guidelines and policies, and to any federal, provincial and institutional regulations that may be in effect;

   ii. ensuring adequate animal care and management of the animal facilities, in particular by verifying that there is a person clearly designated to be in charge of animal care and management of the animal facilities, who should be a member of the ACC (see Section 1), and who should keep the other ACC members updated on the activities within the animal facilities;

   iii. the training and qualifications of animal users and animal care personnel; veterinarians and animal care staff must receive continuing education in their field, and animal users (scientists/study directors, post-doctoral fellows, graduate students and research technicians) must receive appropriate training according to the CCAC guidelines on: institutional animal user training, 1999, either within the institution or through the programs of other institutions;

   iv. an occupational health and safety program for those involved in animal care and use, in collaboration with the institutional authorities on occupational health and safety, that will appropriately protect all those who may be affected by animal-based work, according to CCAC guidelines (see Chapter VIII of Volume 1 (2nd Edn, 1993) of the CCAC Guide or the most recent CCAC guidance on occupational health and safety);

   v. standards of husbandry, facilities and equipment;

   vi. standard operating procedures for all activities and procedures that involve animals, including animal care and facility management SOPs (typically produced by the veterinary and animal care staff), and animal use SOPs (typically produced by animal users, in collaboration with veterinary/animal care staff as needed); the ACC should receive all SOPs and ensure that all necessary SOPs are produced and regularly reviewed (see also Section 5a)iii));

   vii. procedures for euthanasia;

n. Encourage the use of pilot studies with few animals when new approaches, methods or products are being tried, before approving new, large scale protocols. Ensure that animal users report on the results of any pilot studies, no matter whether they wish to pursue the study immediately or not, in order to preserve important data on various approaches to animal-based studies, whether they work well or not; and

o. In the case of projects involving proprietary or patentable research or testing, ensure that as much information as possible is provided to the ACC in terms of what effects to expect on animal health

and welfare, and insist on close monitoring of animals in order to respect the elements outlined in 3l).

4. Meetings

Animal care committees should meet at least twice per year (most institutions in Canada have programs which will require more frequent meetings) and as often as necessary to fulfil their Terms of Reference and be satisfied that all animal use within their jurisdiction is in compliance with institutional, municipal, federal and provincial regulations, and CCAC guidelines. Minutes detailing ACC discussions, decisions and modifications to protocols must be produced for each meeting, and must be forwarded to the senior administrator responsible for animal care and use.

In addition, the ACC should regularly visit all animal care facilities and areas in which animals are used, in order to better understand the work being conducted within the institution, to meet with those working in the animal facilities and animal use areas and discuss their needs, to monitor animal-based work according to approved protocols and SOPs, to assess any weaknesses in the facilities (ageing facilities, overcrowding, insufficient staffing and any other concerns) and to forward any recommendations or commendations to the person(s) responsible for the facilities and for animal use.

Visits of the animal facilities should be conducted at least once a year, and should be documented through the ACC minutes or written reports. Those responsible for the animal facilities should respond to any ACC recommendations in writing, and site visit reports should always be followed up on jointly by the senior administration and the ACC. For small institutions, the full ACC may tour the facilities as a group; for larger institutions, visits to animal care facilities and areas in which animals are used may be divided between the various members of the committee. No matter what the process employed, each member of the ACC should participate in some of the facility visit(s) on an annual basis.

More frequent ACC site visits should be made as necessary to follow up on any protocols that have raised significant concern during the protocol review process, or where problems have been encountered with a protocol being carried out in practice or with other aspects of animal facility operations; these visits may be carried out by the Chair of the ACC or delegate, accompanied or not by other members or animal care staff.

5. General

The animal care committee:

a. Must regularly review (at least every three years):

   i. its Terms of Reference to meet new CCAC guidelines or policies and changing needs within the institution, the scientific community, the animal welfare community and society as a whole, and expand its Terms of Reference to meet the requirements of each institution;

   ii. the security of the animals and research facilities;

   iii. standard operating procedures and institutional animal care and use policies; SOP review may be delegated to ACC members with the appropriate expertise, but SOPs should be accessible to all ACC members, and the full ACC should review all SOPs that involve procedures that may result in deleterious effects to animal health or welfare; and

   iv. policies and procedures for monitoring animal care and experimental procedures within the institution, including the identification of the persons responsible for monitoring animal health and welfare, and the procedures carried out by the ACC to conduct monitoring;

b. Must maintain liaison with the CCAC Secretariat, and inform the Secretariat of any changes to their program: to the senior administrator responsible for animal care and use, the chairperson of the ACC, or the veterinary or senior animal care personnel;

c. Must submit complete and accurate animal use information in the CCAC Animal Use Data Form (AUDF) format for all protocols annually (animal use information for each calendar year must be submitted by March 31 of the following year) and also in pre-assessment documentation;

d. Must develop a crisis management program for the animal facilities and for the animal care and use program, in conjunction with any general institutional crisis management plan(s). This program must detail plans in the event of power outages (short and prolonged), work stoppages, fires, natural disasters, large chemical spills and other similar crises, and must include a communications plan for addressing public and media inquiries on concerns related to animal use;

e. Should, from time to time, sponsor seminars or workshops on the use of animals in science and the ethics of animal experimentation, and encourage as many animal users, animal caregivers, students, ACC members and other interested parties to attend as possible;

f. Should try to achieve and maintain a high profile within the institution and in the community in order to demonstrate the institution's efforts in promoting animal welfare and to allay some of the public concerns regarding animal experimentation; and

g. Should be open to developing and maintaining communication with animal welfare organizations.

March 2006

(previous revision: March 2000)

This policy statement supersedes all previous CCAC policies/guidelines on terms of reference for animal care committees.

2. The Veterinarian

The availability of professional assistance from a veterinarian with interest and experience in laboratory animal science is of prime importance in achieving and maintaining optimal conditions of laboratory animal care. In addition to the formal practice of laboratory animal medicine, the veterinarian should be a major contributor to the development of each institution's animal care policies and procedures.

"Adequate veterinary care," as defined by the Canadian Association for Laboratory Animal Medicine (CALAM), [based originally on guidelines prepared by the American College of Laboratory Animal Medicine (ACLAM)], is the basis upon which CCAC policy is being developed. It includes: the establishment of Standard Operating Procedures (SOP) for health monitoring and disease control of laboratory animals;

the prevention of zoonoses; and the responsibility for ensuring that proper precautions are followed for containment and disease control in specialized colonies such as transgenic animals, Severe Combined Immune Deficiency (SCID) animals, biohazard studies, and the personnel working with them.

Veterinarians associated with facilities involved in the practice and evaluation of livestock intensive management production methods face an especially challenging situation which includes ensuring that the subject of stress associated with restricted space, as it may relate to animal behaviour and well-being, is treated objectively (Mench, Mayer and Krulisch, 1992; Spira, 1986) (see also Social and Behavioural Requirements of Experimental Animals).

a) General Responsibilities

i) Disease prevention

The veterinarian's duties will include responsibility for a disease prevention program for all animals maintained within the institution, treatment of ill or injured animals, maintenance of appropriate health records, provision of advice on anesthesia and analgesia regimes, antibiotics, anxiolytics, and other therapeutic agents for humane animal care. The veterinarian should ensure that only approved euthanasia procedures are conducted and that these are properly carried out (see also Euthanasia). The veterinarian should also be available for consultation and provide assistance on technical and surgical procedures.

ii) Education

It will often prove valuable in educational institutions if the veterinarian is involved in the teaching of surgical principles and other aspects of experimental animal care and handling in courses for senior undergraduates, graduate students, technicians, and investigators.

iii) Quarantine/conditioning

Serological testing is important in determining and monitoring the epidemiological characteristics of colony infections (Richter, Lehner and Henrickson, 1984). In addition to determining if an animal is infected with a disease that may pose a threat to the colony, the quarantine period also serves as a conditioning period.

A veterinarian or a technician with appropriate training and experience should examine all animals on their arrival for overt signs of disease which may have been exacerbated by stress caused by travel (Love, 1980; Reinhardt, 1992). Depending on the species, source, etc., the quarantine/conditioning period should include routine treatment for external and internal parasites (Owen, 1992), grooming, cleaning, and providing the animal with clean food and water (see also Laboratory Animal Care; General Practices).

The veterinarian is responsible for ensuring that animals appearing ill on arrival or suspected of having been exposed to infection are placed in isolation, examined and treated. If this is not economically feasible, animals should be humanely euthanized.

Small rodents obtained from reliable sources generally require only a physical examination on arrival. This need not, on a routine basis, necessarily involve the veterinarian directly; however, s/he should be advised of any health or other problem involving the well-being of the shipment noted by either the technician or researchers involved in receipt of the animals.

Information on method of transportation, the supplier's quality control profile, and the animal's former environment should be available and, if not provided, should be requested of each supplier.

Dogs, cats, non-human primates, and large domestic animals obtained from random sources will usually be accompanied by only very limited information on their genetic and medical history. Examination and quarantine procedures for these animals must be stringent; specific diagnostic testing and immunization procedures should be established and carried out following institutional SOPs for each specific species.

Federal government quarantine regulations, where applicable, will vary with the species, the source, and the condition of the animal. These regulations may be obtained from the nearest district veterinary officer, Food Production and Inspection Branch, Agriculture Canada.

3. Animal Care Personnel

We are morally responsible for any living thing that we cause to be dependent upon us, including animals used in research, teaching and testing. Exemplary standards of humane care and treatment must be exercised by each person associated with captive animals. Satisfactory buildings and equipment are, of course, necessary; however, of even greater importance is the common sense and concern of all levels of personnel involved in the care and use of experimental animals. Only the general responsibilities of such persons can be outlined here; detail will vary with the institution and programs involved.

In every institution utilizing animals, a competent professional staff member must be clearly designated with overall responsibility for care of experimental animals.

a) Chief or Director

The Chief or Director of animal care in large institutions is responsible for administration of the animal care facility and should be directly responsible to a senior administrative official; and should also be an *ex-officio* member of the ACC. This individual should be qualified in an appropriate scientific discipline, possess considerable experience with a variety of species, understand the requirements of research and be a competent administrator.

This individual should be responsible for establishing or promoting participation in educational programs which will, at the technical level, improve the quality and efficiency of animal care and, at the professional level, assist in the proper training of prospective investigators in the use of laboratory animals.

The responsibility to ensure that animals used for research, teaching and testing are of a high quality, appropriate to the requirements of the investigator or teacher should also rest with this individual.

In small institutions, the duties of "Director of Animal Care" may be assigned on a part-time basis.

b) The Scientist-Teacher

The scientist-teacher should have knowledge of the characteristics, care and handling of the species being utilized, and be committed to comply with the guidelines for care and ethical use of animals as contained in this *Guide*.

The primary responsibility for the prevention of pain and discomfort in the experimental situation lies with the investigator.

c) Animal Care Staff

It is the responsibility of the institution, through its ACC, to assure that its technicians have the

opportunity to become as well-qualified as possible. As in any field, opportunity for continuing education should be provided, and all personnel should be encouraged to participate.

Support staff are in a prime position to ensure both high quality animal care and the success of an experiment through their diligence and daily observation of their charges. It is noteworthy that distress in animals is not limited to the experimental situation, but may result from improper housing and handling. Animals respond in a positive manner to gentleness and considerate attention from their attendants.

Staff working with experimental animals may be involved either in their daily maintenance or in the performance of primary experimental procedures or a combination of these roles.

It is important that these persons be skilled and conscientious, as the well-being of the animal and the success of the experiment on a day-to-day basis, are in their hands. In addition to in-house training, formal courses in animal health and animal care technology are now offered at 15 community colleges across Canada. Organizations exist which provide training programs for the animal technician, leading to certification in his/her field; e.g., Canadian Association for Laboratory Animal Science (CALAS). A co-operative venture between employers and a community college has recently been undertaken (Benn and McLaughlin, 1992).

C. REFERENCES

AGRICULTURE CANADA. Publication 1771/E. Recommended code of practice for the care and handling of pigs. Communications Branch, Agriculture Canada, Ottawa, Ont. K1A 0C7. 1984.

IBID. Publication 1898/E. Recommended code of practice for the care and handling of farm animals--pigs. In Press.

IBID. Publication 1819/E. Recommended code of practice for the care and handling of mink. 1988.

IBID. Publication 1821/E. Recommended code of practice for the care and handling of special fed veal calves. 1988.

IBID. Publication 1757/E. Recommended code of practice for the care and handling of poultry from hatchery to processing plant. 1989.

IBID. Publication 1831/E. Recommended code of practice for the care and handling of ranched fox. 1989.

IBID. Publication 1853/E. Recommended code of practice for the care and handling of dairy cattle. 1990.

IBID. Publication 1870/E. Recommended code of practice for the care and handling of farm animals--beef cattle. 1992.

ALDHOUS, P. Lax enforcement of animal rules alleged. Nature 1990; 345: 190.

ANIMAL WELFARE ACT. 7 U.S.C., 2131-2157. Rev. 1985.

ANON. Animal care council outlined. Toronto Globe and Mail August 1, 1967.

ANON. Pound law to the boneyard. Toronto Star 1987 January 23.

ANON. (editorial) A Criminal Code fit for our time. Toronto Star May 21, 1988a: D2.

ANON. It's time to reform the Criminal Code. Ottawa Citizen 1988b May 21: B2.

ANON. Take Zundel's 'false news' crime off the books, law commission urges. The Montreal Gazette May 20, 1988c.

ANON. CFHS President announces campaign for lab animal legislation. CFHS (Canadian Federation of Humane Societies) Animal Welfare in Focus 1989 Fall: 1.

ANON. Special investigation--Professor Wilhelm Feldberg, CBE, FRS. Advocates for Animals Quarterly, 79th Annual Report, year ending Dec. 31, 1990: 21-32.

ANON. Quebec considers research animal law. CCAC (Canadian Council on Animal Care) Resource 1991a; 15(2): 3.

ANON. MRC reports on the Feldberg case. Vet. Rec. 1991b; 128(6): 120.

ANON. More monkey business. Nature 1991c; 349: January 3: 5.

ANON. The day the mice roared. Animal Activist Alert 1992a; 10(1): 1.

ANON. Legislation introduced in 102nd Congress. Washington Vet. News 1992b; 16(1): 4-5.

ANON. Animal Enterprise Protection Act is law. NABR (National Association for Biomedical Research) Update 1992c; 13(16): 1.

ANON. Decline in animal experiments halted. Vet. Rec. 1992d; 131(5): 86.

BALLS, M. Animals (Scientific Procedures) Act 1986: The Animal Procedures Committee. ATLA (Alternatives To Live Animals) 1986; 14: 6-13.

BALLS, M. (editorial) Animal experimentation: the weighing of benefit and suffering. ATLA (Alternatives To Live Animals) 1989; 16: 212.

BALLS, M. (editorial) Has the Animals (Scientific Procedures) Act 1986 failed? ATLA (Alternatives To Live Animals) 1990; 17(4): 284.

BENN, D.M. and McLAUGHLIN, S.M. Training programs for personnel working with laboratory animals. In: Animal care committees: Role and responsibilities. Ottawa, Ont.: Canadian Council on Animal Care, 1992: 43-47.

BROWN, L. Cruelty to animals--the moral debt. Basingstoke: McMillan Publications, Ltd., 1988.

CANADIAN PRESS. Quebec companies supply stray pets for research in U.S. Toronto Globe and Mail 1985 July 31.

CHURCHWARD, P. The vivisectors' guide to pain. Liberator June/July 1986: 11.

COMEAU, P. Bill would keep pets from labs. Toronto Sun 1987 January 28.

DUQUETTE, M. Quebec's pound animals. Canadian SPCA Animag 1986 Fall: 10-11.

FISHER, C. Stepping up the pace. Liberator Autumn 1990: 20-21.

GILMAN, J.P.W. Safety testing of toxic substances. A survey. Ottawa, Ont.: Canadian Federation of Humane Societies, 1980: x.

HAMPSON, J. The secret world of animal experiments. New Scientist April 11, 1992: 24-27.

HARVEY, I. Researchers, activists in battle over animals. Sunday Sun March 25, 1990: 36.

HEALTH RESEARCH EXTENSION ACT OF 1985, Public Law 99-158, November 20, 1985, "Animals in Research."

HEFLIN, H. S.544. Animal Welfare Information Center Newsl. 1992; 3(3): 1, 7-8.

HENSHALL, P., SCOTT, J.M. and SCOTT, J.L., eds. The use and care of animals in the classroom. Cooksville, Canada: Peel Board of Education, 1986.

HOLLANDS, C. The Animals (Scientific Procedures) Act 1986. Lancet July 5, 1986: 32-33.

INSTITUTE OF LABORATORY ANIMAL RESOURCES. Principles and guidelines for the use of animals in precollege education. ILAR News 1989; 31(3): A2-A3.

LAW REFORM COMMISSION OF CANADA. Report 31. Recodifying criminal law. Ottawa, Ont.: Law Reform Commission, 1987: 97-99.

LOVE, J. Primate diseases. In: Johnston, C.E., ed. Proc. Animal diseases workshop. Halifax, November 15, 1980. Halifax: Atlantic Provinces Inter-University Committee on the Sciences, 1980: 87-94.

McANDREW, B. Humane society spent $200,000 fighting law. Toronto Star 1987 May 21.

McGIFFEN, H. and BROWNLEY, N., eds. Animals in education. The use of animals in highschool biology classes and science fairs. Washington, DC: Institute for the Study of Animal Problems, 1980.

McKIE, D. The Animals (Scientific Procedures) Act 1986. Lancet March 1, 1986: 513.

MENCH, J.A., MAYER, S.J. and KRULISCH, L. The well-being of agricultural animals in biomedical research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992.

MERVIS, J. U.S. judge throws out laboratory rules for dogs, primates. Nature 1993, 362(6415): 5.

MYERS, C. U.S. eases proposed regulations on care of laboratory animals; Researchers are relieved, but welfare groups are critical. Chronicle of Higher Education 1990 September 5: A20.

MYERS, C. Universities must draw up written plans for their treatment of research animals. Chronicle of

Higher Education 1991 April 17: A21, A24.

NATIONAL ASSOCIATION FOR BIOMEDICAL RESEARCH. State laws concerning the use of animals in research. Washington, DC: NABR, 1991.

NATIONAL ASSOCIATION OF BIOLOGY TEACHERS. The responsible use of animals in biology classrooms. Including alternatives to dissection. NABT, 19-11250 Roger Bacon Drive, Reston, VA, 22090; 1990.

OLFERT, E.D., FINLEY, G.G., LANIEL, A.-M., LONGAIR, J.A. and ROWSELL, H.C. The proposed new cruelty to animals law-Implications for veterinarians. Can. Vet. J. 1989; 30: 115-116.

ORLANS, F.B. Animal care from protozoa to small mammals. Menlo Park, CA; Don Mills, Ont.: Addison-Wesley Publishing Co., 1977.

ORLANS, F.B., SIMMONDS, R.C. and DODDS, W.J., eds. Effective animal care and use committees. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1987.

OWEN, D.G. Parasites of laboratory animals. Laboratory handbooks No. 12. (Published for Laboratory Animals Ltd.) London: Royal Society of Medicine Services Ltd., 1992.

REINHARDT, V. Transport-cage training of caged rhesus macaques. Anim. Tech. 1992; 43(1): 57-61.

RICHTER, C.B., LEHNER, N.D.M. and HENRICKSON, R.V. Primates. In: Fox, J.G., Cohen, B.J. and Loew, F. M., eds. Laboratory Animal Medicine. Orlando, San Diego, San Francisco, New York, London, Toronto, Montreal, Sydney, Tokyo, Sao Paulo: Academic Press, 1984: 298-321.

ROWSELL, H.C. Report of a feasibility survey on the establishment of a Canadian Council on Animal Care. Ottawa, Ont.: Association of Universities and Colleges of Canada, 1967.

ROYAL SOCIETY FOR THE PREVENTION OF CRUELTY TO ANIMALS. Animals in schools. 2nd Ed. West Sussex: RSPCA, 1985.

RUSSELL, W.M.S. and BURCH, R.L. The principles of humane experimental technique. Springfield: Charles C. Thomas, 1959.

SANDERSON, T.A.H. (letters) CVMA issues statement on use of pound animals in medical research. Can. Vet. J. 1986; 27(5): A7-A8.

SCHWINDAMAN, D.F. Regulatory requirements for exercise of dogs. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 3-7.

SECORD, D.C. The Alberta research animal legislation. Can. Vet. J. 1974; 15(3); 92-94.

SHEPPARD, R. Little hope to save pound pups. Toronto Globe and Mail 1987 January 23.

SMITH, M. (editorial) The weighing of benefit and suffering. FRAME (Fund for the Replacement of Animals in Medical Experiments) News 1988; 20: 1-2.

SMYTHE, D.H. Alternatives to animal experiments. London: Scolar Press-Royal Defence Society, 1978.

SPECIAL COMMITTEE ON THE CARE OF EXPERIMENTAL ANIMALS. Report. Ottawa, Ont.: National Research Council of Canada, 1966.

SPIRA, H. Here's what you can do to make a difference. Factory farming. Animal Rights Coalition Co-ordinator's Report '86: September 1986: 4. (Animal Rights International, New York.)

STEWART, W.A. Debates in the Province of Ontario Legislature, February 19, 1969a: 1352.

STEWART, W.A. Debates in the Province of Ontario Legislature, June 17, 1969b: 5722.

TRAYSTMAN, R.J. (commentary) The goal of animal welfare, animal "rights," and antivivisectionist groups in the United States. J. Neurosurg. Anesthesiol. 1990; 2(3): 153-158.

WILSON, D. Researchers irked by move on strays. Toronto Globe and Mail July 9, 1986.

II. LABORATORY ANIMAL FACILITIES

A. INTRODUCTION

A laboratory animal facility must facilitate research by minimizing undesirable experimental variables while providing for the physiological, social and behavioural requirements of the animal. Different research projects and/or different species of animals often require differing facilities and environments. To accommodate such needs, an animal facility must have separate areas for carrying out different functions, specialized rooms and equipment, and closely controlled environments.

Animal facilities providing the appropriate environment are expensive to build. It is, therefore, imperative that every effort be made to ensure that any proposed new facility is programmed, designed, and built to meet the size and scope of current animal use, and yet to be versatile enough to allow flexibility in the years to come.

A number of alternative design approaches to achieve any given functional need are available. For example, the *Handbook of Facilities Planning, Volume 2: Laboratory Animal Facilities* (Ruys, 1991), is a useful reference for the planning phase. Other references and assistance may be obtained from the Canadian Council on Animal Care (CCAC). It is strongly recommended that the CCAC be involved at an early stage in the planning phase and that plans be evaluated by the Council before the start of construction.

B. LOCATION

Animal facilities should be located so as to minimize public access or through-traffic, as well as the movement of animals, cages, waste, etc., through public corridors and elevators. The facilities should be readily accessible by animal users yet easily secured. Direct access to the outside for deliveries and disposal is desirable. Facilities located on higher floors should be accessed by a minimum of two elevators, one for clean and one for dirty materials, unless appropriate measures are taken to clean and sanitize a single elevator following the transport of dirty materials. For very small and/or satellite facilities, alternative precautions to minimize contamination may be acceptable.

C. MECHANICAL SERVICES

Heating, ventilation and air conditioning systems for animal facilities are usually quite sophisticated and costly (see also The Environment). Placement of these systems should allow servicing to proceed with a minimum of disturbance to the animals and the work patterns in the facilities. This may be accomplished by placing mechanical services on a separate service floor immediately above the animal facilities so that maintenance does not require entry into the animal facilities. It is, however, more common to locate the mechanical systems in the ceiling space between floors. In this location all access to the mechanical systems should be from the corridors, and not from the animal rooms or restricted zones such as biohazard areas.

D. DESIGN

The size of animal rooms should be based on the species to be maintained, and a multiple of the size of pens, cages or cage racks to be contained, allowing for adequate ventilation and servicing. Animal rooms should be designed for ease of sanitation and hence should have a minimum of built-in equipment. In

many cases, a single, small sink for hand washing may be all that is required. The placement of the animal rooms and ancillary rooms will depend on the species, experimental use and microbial quality. The design should facilitate traffic flow from cleaner to dirtier areas. Rooms requiring frequent access by investigators should be located near the entry to the facilities to minimize traffic.

E. MAJOR FUNCTIONAL DIVISIONS

The design of an experimental animal facility should take into consideration the needs of the experimental animal and the requirements and convenience of the scientists and technical staff. Good animal care facilities must provide for several separate functions and sometimes highly specialized areas (Clough, 1986; Home Office, 1986). Animal holding rooms should be separate from experimental rooms. Important aspects of good design are provision for efficient and effective sanitation, efficient work traffic patterns and orderly expansion. The following identifies the major functional areas in an ideal animal facility.

1. Animal Reception Area

The reception area should be situated so that animals entering it do not pass through holding or experimental areas. Similarly, waste material should not pass through the receiving area. It should provide sufficient space for the uncrating and initial examination of animals as well as for holding them under appropriate environmental conditions until they are relocated either in the conditioning area or one of the animal rooms.

2. Conditioning Rooms

Conditioning rooms are ones in which animals may be maintained for detailed health examination, observation and conditioning in preparation for experimentation. The availability of a proper conditioning room(s) is particularly important where random-source animals are being acquired (e.g., some dogs, cats, non-human primates, and animals from the wild). Under certain circumstances, where space permits, it may be possible and even desirable to immediately house animals in an experimental room, provided the animals are derived from a single source and contact with other animals can be avoided.

3. Holding Rooms

Separate animal holding rooms should be available for each species, from each source, and for each investigator's project. Consequently, it is usually better to have many small rooms rather than a few large ones. Exceptions can be made where investigators are using the same species from the same source for different projects (e.g., antibody production in rabbits). Mixing should be limited to compatible social and health status groups within a single species. Where mixing of species is necessary, some degree of isolation may be achieved by specialized room design, equipment and/or cage selection. Cross-contamination can be minimized when controlled airflow cubicles, portable laminar airflow units, and various forms of isolator cages are used. The use of radioisotopes, infectious agents and highly toxic substances requires special holding rooms. Rooms suitable for special purposes may also be required (e. g., breeding colonies, controlled environmental studies and for the housing of both farm and wild animals).

It is important, when designing holding rooms to consider possible future uses of these facilities. Where animal use has been consistent over the years, it may be acceptable to design all animal rooms for specific species use. However, in many facilities, animal use fluctuates considerably, making flexibility extremely important. A flexible holding room is one which meets the acceptable requirements for housing different species.

4. Quarantine / Isolation Rooms

Within the facility, but apart from the conditioning area, quarantine/isolation rooms may be required to separate sick animals or those animals returned to the facility after use in an investigator's laboratory.

5. Experimental and Treatment Facilities

Experimental manipulations should not be carried out in animal holding rooms, unless mandated by experimental design or containment needs and approved by the Animal Care Committee (ACC). Separate facilities should be available to allow for surgery, euthanasia, etc.; however, not all need to be located within the animal facilities. The animal holding rooms should therefore be located as conveniently as possible to the research and teaching laboratories.

Animal facilities may include rooms for some or all of the following: pre-surgical preparation, surgery, post-operative recovery (see also Standards for Experimental Animal Surgery), radiology, necropsy, diagnostic services, special diet preparation, dispensary, etc. The design and organization of special facilities will depend on their intended scope and use; however, even very modest facilities will usually need to provide a special area or procedures room for minor surgery and/or treatments, and a separate necropsy room.

Separate diagnostic areas for laboratory animal diseases may not be feasible for smaller institutions. In such cases, arrangements for provision of such services should be made.

6. Support Facilities

a) Facilities for Washing and Sterilizing Equipment

Facilities for washing and sterilizing equipment and supplies should be designed for this purpose and be located so as to minimize disturbance to animals, staff, and neighbours. Ventilation should be sufficient to prevent odours, excess heat and steam from affecting the rest of the facility. Sinks for hand-washing and for cleaning specialized pieces of equipment are useful. Large, deep sinks are useful. Autoclaves and other special equipment may be located in this area. Ideally, the wash-up area should provide for the separation of clean and dirty equipment. If spray washing of either cages or racks is to be used, provision of a walled-off bay with hot and cold water and disinfectant dispenser is recommended.

b) Waste Disposal

The waste disposal area should provide for proper storage of animal material, excrement, soiled bedding, etc. Waste awaiting collection should be placed in a dedicated refrigerated container or cold room. Waste stored outside the facility should be in secure covered containers. Facilities must comply with local bylaws governing waste storage and disposal. Toxic, infectious or radioactive waste handling must comply with institutional, federal (HWC/MRC, 1990) and other regulations (see also Occupational Health and Safety).

c) Food and Bedding Storage

Small quantities of food and bedding may be stored in an animal room in suitable, covered containers. Separate cool (<15C), dry, vermin-proof facilities should be available for the storage of food to minimize spoilage and contamination. Food for farm animals, such as hay, may contain vermin and should be isolated from the food and bedding of other laboratory animals.

d) Equipment Storage

Lack of sufficient storage space is one of the most frequent and more serious deficiencies encountered in

facility design. Equipment storage should not be permitted in halls, corridors, or in rooms housing animals. Even clean equipment designated for use in an animal room should not be brought into the room until required. Areas used for storing clean equipment should be separate from those where soiled equipment is received. For the average facility, 11% storage space (of net space) has been estimated as adequate. This proportion will need to be increased up to 20% or more in facilities handling multiple species under differing barrier conditions.

7. Personnel, Office and Reception Areas

These functional areas may or may not be combined. It is preferable to have these adjoining, but not within the animal facilities. Enough office space is required to accommodate all administrative staff, occasionally technicians, and the extensive records that must be maintained.

8. Facilities for Personnel

Personnel facilities should encourage high standards of personal hygiene by providing staff with easily accessible changing rooms with lockers, showers, sinks and toilets. Depending on the design of the facility, these may have to be replicated in different zones. Suitable protective clothing should be supplied (see also Occupational Health and Safety).

Facilities should be provided for staff rest periods, lunch, and for meetings. It is preferable that these areas are adjoining, but not within the animal holding areas. An information centre for staff (which could include books, journals, newsletters, catalogues, and other related materials) would be helpful.

F. SECURITY

Access to experimental animal facilities must be restricted in order to assure consistent environmental control and to minimize interferences which might alter experimental results. Entry and exit should be limited and the facilities maintained secure at all times. Access should be allowed only to those who have a recognized need to enter. Where a large number of investigators are using the same facilities, it is often advisable to have individual room locks. Electronic access control systems are available.

G. CONSTRUCTION GUIDELINES FOR ANIMAL ROOMS

1. Floors and Drains

Floors should be seamless, durable, non-slippery, impervious to water and easy to disinfect. They should be coved to the walls to eliminate any sharp corners. They should slope towards any floor drain(s) and the proper level of this slope should be verified in all new construction. Recommended minimum pitch of sloped floors is 2.1 cm/m (0.25"/ft). Special attention should be given to ensuring that this critical component of floor construction is properly carried out.

It is recommended that drains be provided with a flush mechanism so that a clean water seal (i.e., clean water in the trap) can be maintained. However, care should be taken to assure that the location of the flush mechanism does not interfere with cage or pen placement. Drains should be provided with an adequate cover and pitch basket trap. Drain and sewage lines should be at least 10.5 cm (4") in diameter. Where dog excreta is washed down the drain, the diameter should be 15.0 cm (6"). Floor drains used for waste disposal should be placed at the end of the main drainage line. Drains should be checked regularly to ensure proper functioning, an effective water seal, and absence of insects. When not in use, they should be capped and sealed.

Floor drains may not be required in rooms designed solely to house small species. Instead wet vacuum devices which permit sweeping and mopping with disinfectants or a cleaning compound may be used.

2. Walls and Ceilings

Walls should be of an impervious material, free of cracks, damage resistant, and easily cleaned and disinfected. With these kinds of surfaces, noise reduction is difficult. Walls need not be as resistant as the floor, provided that they are protected by a cove or bumper guard. Pipe and service sleeves should be adequately closed off and sealed so as to exclude vermin.

Ceilings within all rooms should be seamless and free of cracks, with ceiling-wall joints well sealed. In some corridors, it may be necessary to use ceiling tiles in order to allow access to the mechanical systems. These tiles should be of a type which can be easily sanitized and which prevent the entry of vermin into the ceiling space.

3. Doors

Animal room doors should be designed and built to exclude vermin. Self-closing, metal or metal-covered doors with obscurable viewing windows and kick plates are preferred. A replaceable sweeper pad should be installed along the bottom if clearance exceeds 0.32 cm (1/8"). Recommended minimum door sizes are 107 cm (42") wide and 213 cm (84") high to allow free passage of equipment.

4. Windows

Exterior windows interfere with temperature control due to radiation and conduction which may jeopardize animal health and research results. They also interfere with photoperiod control. If windows are already present these should be designed or altered to minimize the above effects and to maximize cleanliness.

5. Corridors

Corridors should be strategically located to facilitate traffic flow for the desired work patterns. It may be more efficient to divide the animal facilities into zones with single corridors than to use a double corridor (clean/dirty; supply/return) system.

Design standards for corridor floors, walls, drains, coving, bumper guards, etc., should duplicate those for animal rooms. Traffic corridors should be at least 1.82 m (6') wide. Other corridors should be wide enough to allow free movement of personnel and equipment. Walls should be free of any projecting fixtures up to a height of 213 cm (84"); alternatively, these fixtures should be adequately protected with guards. Exposed corners should be protected with steel plates or similar durable material. All guards and fixtures should be sealed to exclude vermin. Corridors leading to noisy areas should have double doors or similar noise-trapping devices.

6. Services

Service lines should be located on the floor above the animal facilities or in the ceiling space above the corridors so as to eliminate in-room maintenance. Separate hot and cold water lines should be supplied to each animal room for hand washing, cleaning, and automatic watering. Every animal room should have at least one electrical outlet; these should be water, insect and explosion-proof. Switches and thermostats should be similarly designed. An emergency power source should be available in case of a power failure.

H. CAGING

The size of caging chosen to house each species should be appropriate for that species (see Appendix I).

Cages and pens must not only confine the animals securely, but also ensure their comfort and safety by permitting normal postural and behavioural adjustments, and provide for environmental enrichment. Animals which are social by nature should not be singly housed unless this is a necessary requirement of the research protocol, and approved by the ACC (see also Social and Behavioural Requirements of Experimental Animals).

Cages must provide for adequate ventilation, satisfactory viewing and easy access to the animal. Food and water delivery systems should be designed and located so as to allow the animal ready access, but prevent contamination with excrement. Cage design should facilitate cleaning and disinfection.

The intensity of light perceived by the animal, the level of noise to which it is exposed, the ventilation and temperature of its microenvironment are affected by cage design and material. Considerable care should be used when choosing the appropriate caging for a particular species and usage. Caging for animals other than the conventional laboratory species requires special consideration.

Unless contra-indicated by the nature of the research (e.g., nutritional studies) solid bottom cages should be chosen (over suspended wire caging) for rodents and guinea pigs in that they permit creation of microenvironments and facilitate provision of environmental enrichment (see also Social and Behavioural Requirements of Experimental Animals).

1. Shoebox Cages

The shoebox cages used mainly for small rodents are particularly suited for breeding purposes. They are usually made of plastics such as polycarbonate, polystyrene, and polypropylene. Polycarbonate is clear, autoclavable, and resistant to most disinfectants. Polystyrene and polypropylene do not withstand high temperatures well. Polypropylene cages are translucent and offer animals more privacy, which may be beneficial for some breeds or wild species. However, opaque cages should not be placed on shelves above eye level since the animals within cannot be readily observed.

A contact bedding (e.g., woodchip, ground corncob, etc.) is used in the bottom of shoebox cages, allowing an animal to form its own microenvironment. These cages are considered comfortable for the animal, and the cage of choice for breeding. However, animals in these cages are in contact with their own excreta and airflow is restricted. Therefore, it is important to clean the cages frequently. Filter caps restrict the airflow even more if cages are not individually ventilated. Faster buildup of ammonia, carbon dioxide and moisture necessitates more frequent cleaning (up to three times per week may be required). Shoebox cages can be fitted with wire grid floors for certain projects which require that there be no contact with excreta.

2. Larger Solid Bottom Caging

Large plastic tubs have been used quite successfully for group housing guinea pigs and rabbits. These tubs must be strong enough to support the weight of the animals contained, have rounded corners to facilitate cleaning and be resistant to disinfectants. These are used with a contact bedding.

3. Suspended Cages

Suspended cages may be top or front opening. Most top opening suspended cages use the rack shelves as the top for the cage. The top opening cages are used primarily for smaller rodents, whereas the front opening cages are better suited to guinea pigs, cats, dogs, rabbits and non-human primates (NHP).

Most suspended cages have a floor of wire mesh, steel rod, perforated metal or plastic, above a collection tray or solid floor. It is extremely important that the size of the floor perforations be appropriate for the species housed. They should be large enough to permit excreta to freely pass through, but small enough to prevent foot and leg injuries. The gauge of wire should support the animal's weight without sagging. Floors should be designed so the animal's feet can grip during movement, so as to minimize slipping. Wire mesh floors are not suitable for guinea pigs nor for use in rodent littering cages.

In suspended cages, animals are not in contact with their own excreta and the cages are usually well-ventilated. The pans or trays can be cleaned more frequently than the cage, resulting in less disturbance of animals. The animals, however, do not have the opportunity to form their own microenvironment, and so control of the room environment becomes more critical.

It is recommended that these cages be fabricated from stainless steel or other woven metal alloys, corrosive resistant plastic and/or in the case of some front opening cages, fibreglass. Fibreglass is strong, warm-feeling, and more sound-resistant than other materials, making it especially suitable for post-operative care. NHP and cats should be supplied with one or more resting boards or perches at different levels. A squeeze device built into the cage facilitates restraint of NHP.

4. Other Cages

Many cages are designed to meet specific requirements. Examples include metabolism cages, mechanical exercise cages, gang cages, transfer cages, restraint cages and walk-in cages (used for housing groups of animals).

Additional information on housing large domestic animals and fowl may be found in Agriculture Canada's *Canadian Farm Building Handbook* (Agriculture Canada, 1988) (see also Farm Animal Facilities and Environment) as well as Social and Behavioural Requirements of Experimental Animals in this volume. Guidelines for the use of agricultural animals have also been published in the U.S. (Curtis, 1988).

For information on cages for wild animals, contact the Secretary-Treasurer of the Canadian Association of Zoological Parks and Aquariums, c/o Metro Toronto Zoological Society, P.O. Box 280, West Hill, Ontario M1E 4R5.

Information on shipping crates and transport cages for a wide range of domestic, wild and laboratory animals may be obtained from the most recent volume of *Live Animals Regulations* (1992) of the International Air Transport Association (IATA), 2000 Peel Street, Montreal, Quebec H3A 2R4.

All cage types must take into consideration the well-being of the animal(s) during its confinement.

I. REFERENCES

AGRICULTURE CANADA. Publication 1822/E. Canadian farm building handbook. Communications Branch, Agriculture Canada, Ottawa, Ont., K1A 0C7. 1988.

CLOUGH, G. The animal house: Design, equipment and environmental control. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. New York, NY: Churchill Livingstone Inc. 1986: 108-158.

CURTIS, S.E., ed. Guide for the care and use of agricultural animals in agricultural research and teaching, 1988. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural

Research and Teaching, 309 West Clark Street, Champaign, IL 61820.

HEALTH AND WELFARE CANADA/MEDICAL RESEARCH COUNCIL. Laboratory biosafety guidelines. Ottawa, Ont.: Office of Biosafety, Laboratory Centre for Disease Control, Health and Welfare Canada, 1990.

HOME OFFICE. Code of practice for the housing and care of animals used in scientific procedures. Act Eliz. II 1986 c.14 Section 21, Animals (Scientific Procedures) Act. London: Her Majesty's Stationary Office 1986: 4-8.

INTERNATIONAL AIR TRANSPORT ASSOCIATION. Live Animals Regulations. 19th Ed. Montreal, Quebec: IATA, 1992.

RUYS, T., ed. Handbook of facilities planning. Laboratory animal facilities. New York, NY: Van Nostrand Reinhold, 1991: 2.

III. THE ENVIRONMENT

There are many physical, chemical, and biological factors which may influence experimental animals and thus modify the results of the investigations (Melby, 1983; Small, 1983). The experimental results obtained are, in principle, only valid for the conditions under which they were obtained and only useful for comparison if all the relevant information concerning experimental conditions is made available.

Among the environmental factors which should be recorded for possible inclusion in scientific reports are: temperature (C and range), relative humidity (% and range) and whether or not these are regulated; air exchanges/hour, proportion of fresh and recirculated air, and gas or particle concentrations in the air; lighting (natural and/or artificial, photoperiod, and intensity); water type, quality, and pretreatment; bedding type, quality, and pretreatment; housing density; housing equipment; and physical measures to protect microbiological status. The microbiological status of the animal should be reported [conventional, Specific Pathogen Free (SPF) for stated pathogens, or gnotobiotic with microorganisms specified] (WCBCLA, 1985).

A. CLIMATE CONTROL

Environmental requirements vary with the species and the experimental protocol. Environmental parameters are usually measured at the level of the room. More important, however, is the microenvironment established at the cage level, since the conditions between the two may differ dramatically (Woods, 1980; Corning and Lipman, 1992). A summary of some environmental parameters for individual species is given in Appendix I.

The design of the animal facility should permit adjustment of environmental controls to meet the needs of the species and the experimental protocol. Ideally, each animal room would be controlled independently. In facilities not originally constructed with this capability, this ideal could be approached through proper management and the installation of ancillary automatic light timers, rheostats, thermostatically controlled exhaust fans, humidifiers, and air conditioning units.

1. Temperature

Published data on optimal temperatures for housing laboratory animals are variable (CCAC, 1984; Clough, 1984; NRC, 1985). All these were considered when the guidelines of the Council of Europe were formulated (European Convention, 1986).

It is essential that emergency equipment be available to maintain environmental temperatures, particularly in rooms housing small laboratory animals, fish, and non-human primates (NHP).

In special cases, for example, when housing very young or hairless animals, higher room temperatures than those indicated may in Appendix I be required.

Animal room temperatures should be monitored daily, preferably by continuous recording. A less-costly alternative is the use of a maximum/minimum thermometer which is examined and re-set daily; however, this does not indicate how long the room was held at a particular temperature, knowledge of which is extremely important (McSheehy, 1983). If the experimental protocol or management practices require that an animal be housed at temperatures outside the recommended range, an adequate time should be given for adaptation to occur (Baker, Lindsey and Weisbroth, 1979). The temperature of the microenvironment should also be monitored. Factors affecting temperature in the cage include the type of cage and bedding or nesting material, the use of filter covers, age, sex, strain, species, and housing density (Woods, 1980; Corning, 1992).

Environmental temperatures and variability can affect animal research and testing, influencing an animal's response to drugs, susceptibility to infectious disease, fertility, production, feed and water intake, growth curves, and hematologic parameters (Baker, Lindsey and Weisbroth, 1979; Lindsey, 1978; Yamauchi, 1981). Occasionally, optimal temperature for the research animal is not the most comfortable for personnel; however, human preferences should not compromise the experimental requirements or the health and comfort of the animal.

2. Humidity

Most laboratory animals prefer a relative humidity around 50%, but can tolerate a range of 40-70% as long as it remains relatively constant and the temperature range is appropriate (Clough, 1987). Discomfort results when humidity levels adversely affect the animal's ability to maintain thermal homeostasis. In facilities where humidity is difficult to control within an acceptable range, dehumidification or humidification devices may need to be installed.

Humidity levels can affect experimental results by influencing temperature regulation, animal performance, and disease susceptibility.

3. Ventilation

Ventilation influences temperature, humidity, and gaseous and particulate contaminants in the animal cage and holding room. The design of the building ventilation system should permit the maintenance of these parameters within acceptable limits. The actual ventilation rate required varies with age, sex, species, stocking density, frequency of cleaning, quality of incoming air, ambient temperature and humidity, and construction of primary and secondary enclosures, among other factors. Draft-free air exchanges in the range of 15-20 per hour are commonly recommended for rooms containing small laboratory animals under conventional housing conditions (Clough, 1984). Achieving these rates does not guarantee adequate ventilation at the cage level, particularly if filter-tops are used (Keller, White, Sneller *et al.* 1989). Laminar flow units and rooms provide good ventilation with an unidirectional airflow having few eddy currents. These systems may effectively isolate cages within them, controlling the spread of odours and airborne pathogens (Phillips and Runkle, 1973; McGarrity and Coriell, 1976).

Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures are used in clean areas relative to dirty or biohazardous ones, in order to minimize contamination (Hessler and Moreland, 1984). In facilities where containment or exclusion of airborne microorganisms depends in part on differentials in air pressure, inclined manometers or magnehelic gauges can be used to measure the difference between the high and low pressure areas in millimetres of water. Generally, 2.5-5.0 mm (0.1-0.2 in.) differential is maintained (Small, 1983).

The design of the ventilation system should take energy conservation into account (Besch, 1980). Although total air exchange systems are preferable, they are not always economical, especially in regions experiencing temperature extremes. Recirculating air systems must be equipped with effective filters (and scrubbers, if necessary) to avoid the spread of disease and to remove particulate and gaseous contaminants (e.g., NH_3) (Hessler, 1984).

4. Lighting

The three characteristics of light which can influence laboratory animals are intensity, quality, and photoperiod. The lighting should provide good visibility and uniform, glare-free illumination. Previous recommendations of 807-1345 lux (75-125 fc) at 76 cm from the floor have been shown to cause retinal degeneration in albino rats (Belhorn, 1980; NRC, 1985; Semple-Rowland and Dawson, 1987). The recommended level of 323 lux (30 fc) approximately 1.0 m above the floor has proved sufficient for the performance of routine animal care duties and does not cause rodent phototoxic retinopathy (Belhorn,

1980). A level of approximately 200 lux does not appear to cause retinal damage and has been shown to be adequate for reproduction and normal social behaviour among most rodents (Weihe, 1976). At this level, an additional light source on a separate switch is needed to enhance illumination during caretaking activities.

The intensity experienced by animals housed close to the source may differ markedly from that experienced by those farther away, because light intensity is inversely proportional to the square of the distance from its source. Additionally, light intensity within a cage is dependent upon cage type and construction, position of the cage on the rack, and type of rack, and may vary markedly from front to back (McSheehy, 1983). Light intensity can influence aggressiveness and the incidence of cannibalism in rodents (Weihe, 1976; Fall, 1974). Gradual changes between dark and light periods allow time for behavioural adjustment and the expression of crepuscular behaviours. Fish and amphibians may take thirty minutes to make intra-ocular adjustments to changing light intensities (Allen, 1980).

There are few studies on the effect of light quality, or spectrum, on laboratory animals. It has been stated that animal room illumination should duplicate the characteristics of sunlight as closely as possible. There is some disagreement over the necessity for this in every case (Belhorn, 1980; Small, 1983). Among laboratory rodents, a light spectrum that differs markedly from sunlight may reduce breeding efficiency, cause behavioural abnormalities, and enhance spontaneous tumour development (Weihe, 1976). High levels of ultraviolet (UV) light can induce cataracts in laboratory mice (Belhorn, 1980). The wavelength to which guppies are exposed influences fecundity and affects development and sex ratios in offspring (Mulder, 1971). Exposure to UV light may cause epithelial damage in some species exposed to photosensitizing agents. Electromagnetic waves outside the visible spectrum may influence behaviour and activity of laboratory rats (Mulder, 1971). Light tubes which imitate the spectrum of sunlight are commercially available.

Photoperiod is probably the most influential of light characteristics on laboratory animals. Photoperiod influences the circadian rhythms seen in biochemical, physiological, and behavioural aspects of the animal - patterns stimulated and synchronized through the neuroendocrine pathway. The circadian cycle can affect the animal's response to drugs or resistance to inoculated infectious organisms (McSheehy, 1983). The light/dark ratio can affect reproductive performance and sexual maturity. It is suggested that, if a change occurs in an animal's photoperiod, no experiments be conducted with that animal for at least a week (Davis, 1978). If the light phase is interrupted by dark, there are few significant effects; however, if the reverse occurs, endogenous rhythms can be significantly skewed (Davis, 1978). This is one reason why automatic timers should control light cycles in all animal rooms. Timer function should be monitored or hooked into an alarm system. Additionally, any windows in an animal room should be occludable.

Differences in light, temperature, and airflow between locations on a cage rack can affect experimental results and should be minimized by either rotating cages through different positions on a rack, or by assigning animals to cages based on a table of random numbers.

B. OTHER ENVIRONMENTAL FACTORS

1. Noise

The effects of noise on laboratory animals are related to its intensity, frequency, rapidity of onset, duration and characteristics of the animal (species, strain, noise exposure history). Species differ in their auditory sensitivity and susceptibility to noise-induced hearing loss. Prolonged exposure to high levels of noise can cause auditory lesions in animals. Although a maximum background noise of 85 db has been recommended (Baker, 1979), adverse changes have occurred in rats exposed to intermittent noise at 83 db (Gerber, Anderson and Van Dyne, 1966). Exposure to uniform stimulus patterns may lead more readily to hearing loss, whereas exposure to irregular patterns may be more likely to cause disorders due to repeated activation of the neuroendocrine system (Peterson, 1980).

Intense noise can cause alterations in gastrointestinal, immunological, reproductive, nervous, and cardiovascular systems, as well as changes in development, hormone levels, adrenal structure, blood cell counts, metabolism, organ weights, food intake, and behaviour (Agnes, Sartorelli, Abdi *et al*. 1990; Bailey, Stephens and Delaney, 1986; Fletcher, 1976; Kraicer, Beraud and Lywood, 1977; Nayfield and Besch, 1981; Pfaff, 1974; Gerber and Anderson, 1967). Sudden intense sound can elicit startle responses and can precipitate epileptiform seizures in several species and strains of laboratory animals (Iturrian, 1971; Pfaff, 1974). Ultrasound emissions can cause behavioural disturbances in a variety of species (Algers, 1984). Although firm criteria for noise tolerance have not been established for laboratory animals as for humans (Falk, 1973; Welch and Welch, 1970), unnecessary and excessive noise may be assumed to be an important experimental variable and a possible health hazard.

Noise can be controlled in an animal facility through proper facility design and construction, thoughtful selection of equipment, and good management practices. Naturally noisy animals should be located where they minimally disturb quiet, noise-sensitive species. Fire alarms which operate at low frequencies are audible to humans, but do not disturb mice and rats. Telephones should not be placed in animal rooms. Many noise sources in an animal facility emit ultrasound (Sales, Wilson, Spencer *et al*. 1988). These include running taps and squeaking chairs. Efforts should be made to identify and correct or shield these sources.

Noise can also disturb or harm animal care staff, researchers, and other nearby personnel. It may be necessary to provide ear protectors in some areas such as dog, pig, or monkey rooms, or the cage-washing facility.

2. Chemicals

Chemicals in the environment can adversely affect the laboratory animal in a variety of ways. Inherently toxic compounds or toxic metabolites can have local and/or systemic effects on virtually every system. Although most chemicals found in animal facilities exert their major effect by altering hepatic microsomal enzyme activity, immune function, or behaviour, allergens, mutagens, teratogens, and carcinogens have also been detected. Their ultimate effect is modulated by the interplay between chemical factors (concentration; physicochemical properties; duration, frequency, and route of exposure; interaction with other agents) and host factors (species, age, sex, strain, nutritional status, immune function, disease status) (Baker, Lindsey and Weisbroth, 1979).

Chemicals arrive in the microenvironment through air, water, food, bedding, and contact surfaces. Common air pollutants include dust and bedding particles, ammonia, disinfectants, pheromones, organic solvents, volatile anesthetics, insecticides, and perfumes or deodorants.

The most common air contaminant in animal facilities is ammonia (NH_3) resulting from the decomposition of nitrogenous waste. Ammonia causes irritation of the respiratory epithelium and increases susceptibility of rodents to respiratory mycoplasmosis (Broderson, Lindsey and Crawford, 1976; Lindsey, Connor and Baker, 1978). Sub-clinical pathological changes in the respiratory tract due to ammonia complicate inhalation toxicity studies in laboratory rodents (Gamble, 1976). In humans, 25 ppm is the level below which there are no harmful effects from an 8 hr/day, 5 day/week exposure [American Conference of Government and Industrial Hygienists Threshold Limit Value (TLV)]. The human odour detection threshold for ammonia is 8 ppm. In comparison, the TLV is 17 mg/m^3.

The animal's microenvironment must be checked as well as the room, because conditions often differ significantly between the two (Corning and Lipman, 1992). Ammonia levels build up when production components (species, sex, housing density, bedding) exceed elimination components (cage design, air exchange, frequency of cleaning) (Serrano, 1971). Filter covers, which reduce air exchange at the cage level, can rapidly lead to detrimental concentrations of NH_3. Controlling NH_3 within safe levels requires constant attention to stocking density and to frequency of cage cleaning.

Perfume and deodorants should never be used to mask ammonia or other animal odours in lieu of proper husbandry. These substances may be harmful to the animals (Baker, Lindsey and Weisbroth, 1979; Pakes, Lu and Meunier, 1984). Volatile anesthetics should be used only with proper scavenging equipment.

Chemicals can enter the animal's environment through the water. Other than checking for bacterial contaminants, water quality is rarely monitored except for aquatic animals. Chlorinated municipal water sources are commonly used. Over 700 organic compounds have been isolated from such sources - 90% are natural decomposition products. These may react with chlorine to produce chloroform (Pakes, Lu and Meunier, 1984). Inorganic solutes, particularly copper (from copper pipe) and chlorine are especially hazardous to aquatic organisms.

Food may be contaminated with heavy metals (e.g., lead, arsenic, cadmium, nickel, mercury), naturally occurring toxins (e.g., mycotoxins, ergot alkaloids, pyrrolizidine alkaloids, estrogenic compounds), agricultural chemicals (e.g., herbicides, pesticides, fertilizers), and additives (e.g., antibiotics, colouring, preservatives, flavourings, unintentionally incorporated drugs) (Baker, Lindsey and Weisbroth, 1979; Pakes, Lu and Meunier, 1984; Silverman and Adams, 1983).

Chemicals found on contact surfaces include cleaning agents such as soaps, wetting agents, detergents, solvents, and disinfectants (Burek and Schwetz, 1980). Unless otherwise specified as safe according to the manufacturer's instructions, these substances should be thoroughly rinsed from surfaces which will contact animals. The efficacy of the rinse cycle of the cage-washer should be checked periodically.

Bedding materials, particularly wood products, may introduce naturally occurring volatile oils, herbicides, pesticides, and preservatives into the animal's microenvironment. Other possible contaminants include PCB's and antibiotics (Silverman and Adams, 1983). Volatile hydrocarbons in cedar and pine shavings can induce hepatic microsomal enzymes (Weisbroth, 1979).

3. Bedding

The choice of bedding materials and cage flooring profoundly affects the microenvironment of small rodents. In most circumstances, contact bedding is recommended. Most species should be provided with solid flooring and bedding prior to parturition. Some desirable characteristics of contact bedding are listed below.

Bedding material should always be taken into consideration in designing an experiment and should be uniform throughout the study because of its influence on behavioural and physiological responses and on toxicity and carcinogenesis studies.

DESIRABLE CRITERIA FOR RODENT CONTACT BEDDING (Kraft, 1980)

Moisture absorbent	Unlikely to be chewed or mouthed
Dust free	Non-toxic
Unable to support bacterial growth	Non-malodorous
Inedible	Nestable
Non-staining	Disposable by incineration
Non-traumatic	Readily available
Ammonia binding	Relatively inexpensive
Sterilizable	Fire resistant
Deleterious products not formed as a result of sterilization	Remains chemically stable during use
Easily stored	Manifests batch to batch uniformity
Non-desiccating to the animal	Optimizes normal animal behaviour
Uncontaminated	Non-deleterious to cage-washers
Non-nutritious	Non-injurious and non-hazardous to personnel
Non-palatable	

Unsterilized materials are a possible source for the introduction of disease into rodent colonies. Wild rodents enjoy nesting in packages of bedding, and cats will defecate in loose bedding (Newman and Kowalski, 1973). Recommended bedding materials for each species are discussed in Volume 2 of this *Guide*.

4. Population Density and Space Limitations

Population density and group size influence the physiological and psychological state of the animal and can profoundly affect experimental responses (Baer, 1971; Clough, 1976). Productivity, growth, and behaviour of laboratory mice may be seriously altered by variations in floor space alone. Infant growth and survival, as well as maternal behaviour, may be adversely affected by excessive floor space. Infant mortality in large cages can occur from failure of females to nurse their young due to inhibition of mammary development. Nest-building behaviour in rats is adversely affected in densely populated pens, leading to an increasing tendency to ignore the pups and to infant death. Housing density can affect efficiency of feed utilization and the incidence of skin lesions (Les, 1968, 1972).

Isolation stress may result in increases in nervousness, aggression, susceptibility to convulsions and certain drugs, metabolism, and adrenocortical activity (Balazs and Dairman, 1967; Hatch, Weiberg, Zawidzka *et al*. 1965; Moore, 1968). As much as possible, housing type and densities should be kept uniform throughout a study. Further details on appropriate housing (see also Laboratory Animal

Facilities). Individual species requirements are discussed in Volume 2 of this *Guide* (see also Social and Behavioural Requirements of Experimental Animals). Recommended housing densities are listed in Appendix I.

C. MICROBIOLOGICAL CONTROL

The effects that microbiological agents can have on experimental results and the health of laboratory animals have been widely documented (Baker, Lindsey and Weisbroth, 1979; Lindsey, Connor and Baker, 1978; Pakes, Lu and Meunier, 1984). Control of the microbiological status of the experimental animal and its environment is necessary for valid scientific results and animal well-being. The sources of microbial contamination include vermin, experimentally infected and spontaneously ill laboratory animals or their tissues or tumours, air, food, water, bedding, ancillary equipment, and personnel. Good facility management practices and constant surveillance are necessary to minimize the introduction of unwanted microbes. Insect and rodent vermin should be strictly controlled or excluded from the facility (Small, 1983).

Whenever possible, the health status of all animals should be ascertained before the animal is brought into the facility. Animals having an unknown health status should be quarantined and tested before being admitted to the facility (Loew and Fox, 1983). Additionally, all tumour and cell lines should be tested before being introduced (Small, 1984). Research on contagious diseases must be carried out in appropriate containment facilities (see 3. below).

The laboratory animal veterinarian should be consulted about regular monitoring of the health status of animals within a facility, as it is important to verify the microbiological standing for publication of experimental results and to minimize cross-contamination between areas (Baker, Lindsey and Weisbroth, 1979). The use of sentinel animals is one proven, sensitive, and practical component of an animal health surveillance program (Loew and Fox, 1983). Health monitoring programs should consider the source and species of animal, husbandry practices, the nature of research carried out in the facility, and the association of personnel with laboratory animals in other locations. The efficacy of cage and equipment sanitation should be tested periodically by culturing for microorganisms, as well as by checking physical indicators (Baker, Lindsey and Weisbroth 1979; Small, 1983). Feed, water, and bedding should also be sampled and cultured periodically. The frequency and intensity of microbiological monitoring programs will be dependent upon husbandry practices, the level of confidence desired, associated risk factors, and economics, in addition to the factors mentioned above (Small, 1984).

Personnel must be instructed in the precautions they must take to avoid introducing diseases into the facility. The specific precautions will vary between areas and facilities, depending upon the nature of the facility, the status of the animals, and the type of research being conducted. The co-operation of all staff working with animals, in both caretaking and experimental activities, is essential to maintain facility and scientific standards.

1. Conventional Facilities

A conventional room or facility is one which is not especially designed for isolation procedures. An isolation unit could operate conventionally if isolation management practices are not employed. The following practices reduce the probability of contamination in a conventional facility:

- Personnel should wear clean clothing and outer protective garments in animal rooms.

- Personnel should wash their hands upon entering and leaving a room.

- There should be no movement of personnel and equipment between rooms which house animals of different

microbial status without proper precautions.

- Animals entering shared facilities, such as laboratories, surgery, irradiators, etc., should not be returned to the holding room unless the shared room and equipment therein have been disinfected between groups of animals.

- Cleaning and sanitation practices as outlined in Laboratory Animal Care should be followed.

2. Barrier Facilities

Gnotobiotes, SPF breeding colonies, aging study colonies, and immunodeficient or immunosuppressed animals require a higher level of control of the microbial environment than practised in conventional housing (Hessler and Moreland, 1984). Barrier housing prevents infectious agents from entering and infecting animals inside the barrier. Barriers can be established at the room level as in large-scale commercial production of disease-free rodents; around groups of cages as for gnotobiotes or breeding colony nuclei in free-standing flexible film isolators; or at the level of the individual cage as in microisolation cages.

Closed barrier systems employ variations of the following principles:

- The room, isolator, or isolation cage is sterilized chemically or physically prior to entry of animals, supplies, or equipment.

- Animals enter through ports from isolators or transport containers which prevent contamination.

- All other materials, supplies, and equipment are sterilized before entering the barrier.

- Effective entry and exit systems include pass-through autoclaves, sterilized double-door transfer chambers, or germicidal dunk tanks.

- Exits from large barriers may be through airlocks with powerful exhaust coming from the inside of the unit.

- Personnel must shower, dress in sterile garments, and don head covers, masks and Gloves before entering a large barrier.

- The interior of smaller isolators is accessed through rubber or neoprene gloves sealed to the isolation unit.

- Incoming air is filtered with high-efficiency particulate air (HEPA) filters and air pressures are carefully balanced to consistently prevent backflow into the barrier.

- Water is sterilized through filtration, UV light, acidification, or autoclaving.

- Feed and bedding are autoclaved or irradiated before entering the barrier. Special enriched diets must be

used if the feed is to be autoclaved (Hessler and Moreland, 1984).

Microisolation cages are generally used to protect animals in otherwise conventional rooms. With laminar flow cage-changing stations and special management procedures (sterilization of feed, bedding, water, etc.), highly disease-susceptible animals such as thymus deficient and Severe Combined Immune Deficiency (SCID) mice may be successfully maintained in a conventional room. Rigorous microbiological monitoring is necessary to maintain and verify the health status of animals kept in barrier-sustained systems.

3. Biohazard Containment

Containment is required for animals exposed to known infectious microorganisms. Required containment and management procedures vary with the biohazard classification of the microorganism, based on the degree of risk to man and other animals (HWC/MRC, 1990). Personnel may be required to shower before leaving the containment unit. All cages and materials are sterilized upon leaving the area. Air pressures are balanced so that the highest pressure is outside the containment area. Air exiting the facility is diluted with clean air, filtered, or incinerated. Because it is hazardous to staff and animals, UV light is not generally recommended for routine disinfection of laboratory air. The infectious disease unit should be segregated as much as possible from the rest of the animal facility. Specific requirements will differ with the degree of risk. Depending on the hazard, containment of small groups of animals may be accomplished with flexible film isolators or microisolation cages. The use of laminar airflow racks to prevent cross-contamination between cages should be carefully evaluated as the transfer of certain pathogens may be enhanced in some instances (Clough, 1973). Infectious disease units should be disinfected immediately following use. Recommendations for control of biohazards can be found in *Laboratory Biosafety Guidelines* (HWC/MRC, 1990) and elsewhere (Barkley and Richardson, 1984). Biological safety cabinets approved for the appropriate biohazard level must be used for experimental manipulations. These cabinets must be inspected and tested annually by trained personnel (HWC/MRC, 1990).

Persons working in infectious disease units should be protected with a comprehensive occupational health and safety program.

D. CHEMICAL AND RADIOISOTOPE UNITS

In Canada, laboratory use of radioisotopes is regulated by the (federal) Atomic Energy Control Board (AECB), in accordance with the Atomic Energy Control Regulations. The AECB issues licences to the institution for the possession of radioactive material. When radioisotopes are used in animals experimentally, Standard Operating Procedures (SOPs) to ensure that related hazards are minimized should be defined and enforced; these SOPs are considered by the AECB when it issues the Radiation Licence. As well, the AECB recommends that the institution's Radiation Safety Officer sit on the Occupational Health and Safety Committee in an *ex-officio* capacity.

The Workplace Hazardous Materials Information System (WHMIS) is regulated by federal and provincial health and safety authorities. It legislates labelling requirements, availability of Material Safety Data Sheets (MSDS), and training programs required for personnel to work safely with certain hazardous materials.

The chemical and radiation hazard area should be separated from other animal housing and work areas. The hazardous area must be clearly posted and entry restricted to necessary personnel. Contaminated cages should not be transported through corridors. Safe transport equipment and procedures should be developed if necessary. Laminar flow cage-changing stations are recommended to protect the staff from aerosolized contaminants (Hessler and Moreland, 1984) (see also Occupational Health and Safety).

E. REFERENCES

AGNES, F., SARTORELLI, P., ABDI, B.H. and LOCATELLI, A. Effect of transport loading or noise on blood biochemical variables in calves. Am. J. Vet. Res. 1990; 51: 1679-1681.

ALGERS, B. A note on behavioural responses of farm animals to ultrasound. Appl. Anim. Behav. Sci. 1984; 12: 387-391.

ALLEN, D.M. A device providing gradual transitions between light and dark periods in the animal room. Lab. Anim. Sci. 1980; 30: 252-254.

BAER, H. Long-term isolation stress and its effects on drug response in rodents. Lab. Anim. Sci. 1971; 21: 341-349.

BAILEY, K.J., STEPHENS, D.B. and DELANEY, C.E. Observations on the effects of vibration and noise on plasma ACTH and zinc levels, pregnancy and respiration rate in the guinea pig. Lab. Anim. 1986; 20: 101-108.

BAKER, H.J., LINDSEY, J.R. and WEISBROTH, S.H. Housing to control research variables. In: Baker, H.J., Lindsey, J.R. and Weisbroth, S.H., eds. The laboratory rat. Toronto, Ont.: Academic Press, 1979(1): 169-192.

BALAZS, T. and DAIRMAN, W. Comparison of microsomal drug-metabolizing enzyme systems in grouped and individually caged rats. Toxicol. Appl. Pharmacol. 1967; 10: 409-410.

BARKLEY, W.E. and RICHARDSON, J.H. Control of biohazards associated with the use of experimental animals. In: Fox, J.G., Cohen, B.J. and Loew, F.M., eds. Laboratory animal medicine. Toronto, Ont.: Academic Press, 1984: 595-602.

BELHORN, R.W. Lighting in the animal environment. Lab. Anim. Sci. 1980; 30: 440-450.

BESCH, E.L. Environmental quality within animal facilities. Lab. Anim. Sci. 1980; 30: 385-406.

BRODERSON, J.R., LINDSEY, J.R. and CRAWFORD, J.E. The role of environmental ammonia in respiratory mycoplasmosis of rats. Am. J. Path. 1976; 85: 115-127.

BUREK, J.D. and SCHWETZ, B.A. Considerations in the selection and use of chemicals within the animal facility. Lab. Anim. Sci. 1980; 30: 414-421.

CANADIAN COUNCIL ON ANIMAL CARE. Guide to the care and use of experimental animals. Vol. 2, Ottawa, Ont.: CCAC, 1984.

CLOUGH, G., HILL, A. and BLACKMORE, D.K. Evaluation of a filter rack for laboratory rodents. Lab. Anim. 1973; 7: 149-159.

CLOUGH, G. The immediate environment of the laboratory animal. In: McSheehy, T., ed. Control of the animal house environment. Laboratory animal handbook 7. Buckden Huntington, Cambs., U.K.: Laboratory Animals Ltd., 1976: 77-94.

CLOUGH, G. Environmental factors in relation to the comfort and well-being of laboratory rats and mice.

Proc. of LASA-UFAW (Universities Federation for Animal Welfare) Symposium "Standards in Laboratory Animal Management" UFAW, 1984(1): 60-64.

CLOUGH, G. The animal house: design, equipment and environmental control. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987.

CORNING, B.F. and LIPMAN, N.S. A comparison of rodent caging systems based on microenvironmental parameters. Lab. Anim. Sci. 1992; 41: 498-503.

DAVIS, D.E. Social behavior in a laboratory environment. In: National Research Council Institute of Laboratory Animal Resources. Laboratory animal housing. Washington, DC: National Academy of Sciences, 1978: 44-64.

EUROPEAN CONVENTION FOR THE PROTECTION OF VERTEBRATE ANIMALS USED FOR EXPERIMENTAL AND OTHER SCIENTIFIC PURPOSES. Appendix A: Guidelines on accommodation and care. Report No. 123, HMSO, London 1986.

FALK, S.A. and WOODS, N.F. Hospital noise levels and potential health hazards. New Eng. J. Med. 1973; 289: 774-781.

FALL, M.W. The use of red light for handling wild rats. Lab. Anim. Sci. 1974; 24: 686-687.

FLETCHER, J.L. Influence of noise on animals. In: McSheehy, T., ed. Control of the animal house environment. Laboratory animal handbook 7. Huntingdon, U.K.: Laboratory Animals Ltd., 1976.

GAMBLE, M.R. and COUGH, G. Ammonia buildup in animal boxes and its effect on rat tracheal epithelium. Lab. Anim. 1976; 10: 93-104.

GERBER, W.F., ANDERSON, T.A. and VAN DYNE, V. Physiologic response of the albino rat to chronic noise stress. Arch. Environ. Health 1966; 12: 751-754.

GERBER, W.F. and ANDERSON, T.A. Cardiac hypertrophy due to chance audiogenic stress in the rat, *Rattus norvegicus* and rabbit, *Lepus cuniculus*. Comp. Biochem. Physiol. 1967; 21: 273-277.

HATCH, A.M., WIBERG, G.S., ZAWIDZKA, Z., CANN, M., AIRTH, J.M. and GRICE, H.C. Isolation syndrome in the rat. Toxicol. Appl. Pharmacol. 1965; 7: 737-745.

HEALTH AND WELFARE CANADA/MEDICAL RESEARCH COUNCIL. Laboratory biosafety guidelines. Ottawa, Ont.: Office of Biosafety, Laboratory Centre for Disease Control, Health and Welfare Canada, 1990.

HESSLER, J.R. and MORELAND, A.F. Design and management of animal facilities. In: Fox, J.G., Cohen, B. J. and Loew, F.M., eds. Laboratory animal medicine. Toronto, Ont.: Academic Press, 1984: 505-526.

ITURRIAN, W.B. Effect of noise in the animal house on experimental seizures and growth in weanling mice. In: National Academy of Sciences. Defining the laboratory animal. Washington, DC: National Academy of Sciences, 1971.

KELLER, L.S.F., WHITE, W.J., SNIDER, M.T. and LANG, C.M. An evaluation of intracage ventilation in three animal caging systems. Lab. Anim. Sci. 1989; 39: 237-242.

KRAFT, L.M. The manufacture, shipping, and receiving and quality control of rodent bedding materials. Lab. Anim. Sci. 1980; 30: 366-376.

KRAICER, J., BERAUD, G. and LYWOOD, D.W. Pars intermedia ACTH and MSH content: effect of adrenalectomy, gonadectomy and a neurotropic (noise) stress. Neuroendocrinol. 1977; 23: 352-367.

LES, E.P. Cage population density and efficiency of feed utilization in inbred mice. Lab. Anim. Care 1968; 18: 305-313.

LES, E.P. A disease related to cage population density, tail lesions and C3H/HeJ mice. Lab. Anim. Sci. 1972; 22: 56-60.

LINDSEY, J.R., CONNER, M.W. and BAKER, H.J. Physical, chemical, and microbial factors affecting biologic response. In: National Research Council/Institute of Laboratory Animal Resources. Laboratory animal housing. Washington, DC: National Academy of Sciences, 1978: 44-64.

LOEW, F.M. and FOX, J.G. Animal health surveillance and health delivery systems. In: Foster, H.L., Small, J.D. and Fox, J.G., eds. The mouse in biomedical research. III. Normative biology, immunology, and husbandry. Toronto, Ont.: Academic Press, 1983: 83-100.

MCGARRITY, G.J. and CORIELL, L.L. Maintenance of axenic mice in open cages in mass airflow. Lab. Anim. Sci. 1976; 26: 746-750.

MCSHEEHY, T. Overview of the state-of-the-art in environmental monitoring. In: Melby, E.C. Jr. and Balk, M.W., eds. The importance of laboratory animal genetics, health, and the environment in biomedical research. Toronto, Ont.: Academic Press, 1983; 161-182.

MELBY, E.C. JR. and BALK, M.W., eds. The importance of laboratory animal genetics, health, and the environment in biomedical research. Toronto, Ont.: Academic Press, 1983.

MOORE, K.E. Studies with chronically isolated rats: tissue levels and urinary excretion of catecholamines and plasma levels of corticosterone. Can. J. Physiol. Pharmacol. 1968; 46: 553-558.

MULDER, J.B. Animal behaviour and electromagnetic energy waves. Lab. Anim. Sci. 1971; 21: 389-393.

NATIONAL RESEARCH COUNCIL. Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences. Guide for the care and use of laboratory animals. NIH Publication No. 85-23. Bethesda, MD: United States Department of Health and Human Services, Public Health Service, National Institutes of Health, 1985.

NAYFIELD, K.C. and BESCH, E.L. Comparative responses of rabbits and rats to elevated noise. Lab. Anim. Sci. 1981; 31: 386-390.

NEWMAN, E. and KOWALSKI, J.J. Fresh sawdust bedding - a possible source of Klebsiella organisms. Am. J. Vet. Res. 1973; 34: 979-980.

PAKES, S.P., LU, Y.S. and MEUNIER, P.C. Factors that complicate animal research. In: Fox, J.G., Cohen, B. J. and Loew, F.M., eds. Laboratory animal medicine. Toronto, Ont.: Academic Press, 1984: 649-665.

PETERSON, E.A. Noise and laboratory animals. Lab. Anim. Sci. 1980; 30: 422-439.

PFAFF, J. Noise as an environmental problem in the animal house. Lab. Anim. 1974; 8: 347-354.

PHILLIPS, G.B. and RUNKLE, R.S. Biomedical applications of laminar airflow. Cleveland, OH: CRC Press, 1973.

SALES, G.D., WILSON, K.J., SPENCER, K.E.V. and MILLIGAN, S.R. Environmental ultrasound in laboratories and animal houses: a possible cause for concern in the welfare and use of laboratory animals. Lab. Anim. 1988; 22: 363-375.

SEMPLE-ROWLAND, S.L. and DAWSON, W.W. Retinal cyclic light damage threshold for albino rats. Lab. Anim. Sci. 1987; 37: 289-298.

SERRANO, L.J. Carbon dioxide and ammonia in mouse cages: effects of cage covers, population, and activity. Lab. Anim. Sci. 1971; 21: 75-85.

SILVERMAN, J. and ADAMS, J.D. N-nitrosamines in laboratory animal feed and bedding. Lab. Anim. Sci. 1983; 33: 161-164.

SMALL, J.D. Environmental and equipment monitoring. In: Foster, H.L., Small, J.D. and Fox, J.G., eds. The mouse in biomedical research. III. Normative biology, immunology, and husbandry. Toronto, Ont.: Academic Press, 1983: 83-100.

SMALL, J.D. Rodent and lagomorph health surveillance - quality assurance. In: Fox, J.G., Cohen, B.J. and Loew, F.M., eds. Laboratory animal medicine. Toronto, Ont.: Academic Press, 1984: 709-723.

WEIHE, W.H. The effect of light on animals. In: McSheehy, T., ed. Control of the animal house environment. Laboratory animal handbook 7. Buckden, Huntingdon, Cambs., U.K.: Laboratory Animals Ltd., 1976: 63-76.

WEISBROTH, S.H. Chemical contamination of lab animal beddings: problems and recommendations. Lab. Animal Nov.-Dec. 1979: 24-34.

WELCH, B.L. and WELCH, A.S., eds. Physiological effects of noise. New York, NY: Plenum Press, 1970.

WOODS, J.E. The animal enclosure - a microenvironment. Lab. Anim. Sci. 1980; 30: 407-413.

WORKING COMMITTEE FOR THE BIOLOGICAL CHARACTERIZATION OF LABORATORY ANIMALS/GV-SOLAS. Guidelines for specification of animals and husbandry methods when reporting the results of animal experiments. Lab. Anim. 1985; 19: 106-108.

YAMAUCHI, C., FUJITA, S., OBARA, T. and UEDA, T. Effects of room temperature on reproduction, body and organ weights, food and water intake, and hematology in rats. Lab. Anim. Sci. 1981; 31: 251-258.

IV. FARM ANIMAL FACILITIES AND ENVIRONMENT

These guidelines are intended for farm animals used in agricultural research and teaching. Where agricultural animal species serve as models for humans in biomedical research projects and teaching demonstrations, they are to be kept in similar facilities compatible with each animal's normal requirements and under conditions that will minimize stress, bearing in mind the conditions required for non-agricultural species used in similar experiments.

When farm animals are brought to the laboratory, consideration must be given to the transition from the ambient outdoor conditions (e.g., cold weather, photoperiod), so that the animals are given as smooth a transition period as possible. Bringing animals in from the cold will result in physiological changes (e.g., hyperventilation in sheep) which will also be reflected in changes in their dietary requirements. Husbandry procedures such as shearing sheep, the trimming of hoofs, may also be of benefit to the animals at this time. The time required for the animals to adapt to the laboratory's environment will vary.

The transition back to outdoor farm conditions following laboratory confinement also requires careful planning, not only with respect to the ambient climate, but also with respect to the regrouping of the animals.

Comprehensive guidelines for environmental enrichment, as well as for housing large animals in metabolism crates, are found in the chapter on Social and Behavioural Requirements of Experimental Animals.

The use of metabolism cages or crates necessarily reduces the animal's social and behavioural activities. This practice should not, therefore, be used merely for the purpose of convenient restraint, but should be reserved for approved metabolic studies. **Animals so housed should be under close and expert observation throughout the period of the study** (see also Social and Behavioural Requirements of Experimental Animals).

A. FACILITIES

Acceptable baseline information on facilities and housing for farm animals for production purposes may be found in the National Research Council (NRC) *Canadian Farm Building Code* (NRC, 1990). Similarly, the various recommended Codes of Practice for livestock and poultry published by Agriculture Canada (Agriculture Canada, 1771/E, 1984; 1821/E, 1988; 1757/E, 1989; 1853/E, 1990; 1870/E, 1991) are also useful references. In addition, a revision of the Recommended Code of Practice for the Care and Handling of Farm Animals--Pigs (1898/E) is now in press.

Where a new facility or extensive remodelling of existing housing is contemplated, the plans should be discussed with agricultural engineering experts (provincial departments of agriculture and regional agricultural colleges). Detailed information is available in the most recent edition of the *Canadian Farm Building Code* (NRC, 1990), and the *Canadian Farm Building Handbook* (Agriculture Canada, 1988).

The American *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Curtis, 1988), contains useful information. The Scientists Center for Animal Welfare (SCAW) has also published a volume on farm animal well-being (Mench, Mayer and Krulisch, 1992).

A number of articles on Farm Animal Housing were compiled as a special feature by the *Veterinary Record* (Wathes, Jones and Webster, 1983; Linklater and Watson, 1983; Sainsbury, 1983). The British Veterinary Association's (BVA) Animal Welfare Foundation has published guidelines for the detection and relief of

pain in a number of species of farm animals (Edwards, 1985; Gentle, 1985; Oldham, 1985; Silver, 1985). It has also published guidelines on transportation of farm species (Gibson and Paterson, 1986).

Facility design and the nature of the primary enclosures used for the housing of farm animals have a major impact on their welfare. The conditions for production-oriented agricultural research must often be simulated and sometimes intensified in commercial applications of intensive husbandry practices in food animal production (Fraser, 1975). With others, however, attempting to impose close confinement can introduce a severe stress and "skew" research results.

Probably the most important factor in the provision of appropriate animal care for farm animals is the attitude and concern for animal well-being of the animal attendants and herdsmen.

Domestication is a continuing process, and much of today's livestock and poultry production involves animals of genetic strains that were selected for growth or reproduction in various environments under varying degrees of control (Siegel, 1984).

Currently, no precise objective measures exist which can be employed to evaluate the stress level of livestock production systems. Due to problems inherent in biochemical monitoring, the physiological parameters of stress cannot be completely relied upon (Freeman, 1971). The suggestion that high productivity does not constitute a reliable indication of a lack of stress may, in some special instance, be correct. However, wide acceptance of the negative correlation between stress and productivity has proven most useful and beneficial in that its acceptance by agricultural producers has given rise to continuing efforts to upgrade environmental conditions (Mann and Harvey, 1971; Wilson, 1971; Agriculture Canada, 1988). The *Report of the Technical Committee to Enquire into the Welfare of Animals kept under Intensive Livestock Husbandry Systems* in Britain concluded that no one factor can be considered conclusive in assessing well-being, and the fact that farm animals are producing normally should be taken as no more than a guide in this regard (Brambell, 1965). The well-being of farm animals probably will be assessed best by an integrated system of indicators in four categories: 1) reproductive and productive performance; 2) pathological and immunological traits; 3) physiological and biochemical characteristics; and 4) behavioural patterns (Curtis, 1988; Duncan, 1981; Curtis, 1982; Smidt, 1983).

Cages and pens should not only serve to confine the animal, but must also ensure its comfort and safety by permitting normal postural and behavioural adjustments. Adequate ventilation, ready access to food and water, and satisfactory viewing of the confined animal are also mandatory. The Brambell Report in dealing with the implications of modern technology on animal welfare, summarizes this concept by suggesting that regardless of the system of management, five basic freedoms should be respected for all farm animals; the freedom to get up, lie down, groom normally, turn around and stretch its limbs (Brambell, 1965). Criticism that these criteria are not always fully met, and that intensive livestock systems restrict living space and in some cases drastically reduce freedom of movement, is often justified. The equivocal point is, to what extent the potential stress of confinement is counterbalanced by such things as the period of the imposed stress, injury prevention and improved disease control.

If slatted or partially slatted floors are used, the slat width and spacing will vary with the species, but should be such as to provide adequate support and minimize the risk of injury while permitting free drainage of excrement (Smith and Robertson, 1971). Slat material should be durable. The possibility that toxic gases may develop from the liquid manure disposal system must always receive consideration, as these may prove dangerous to both livestock and personnel.

Solid floor surfaces for farm animals should be finished with materials and finishes that will minimize slippage and thus the probability of injury and bruising. Epoxy resin floors if properly keyed have been recommended for swine. The use of heavy rubber matting (rubber cow mats) may prove useful in farrowing crates and for tethered animals, as well as for stanchion-tied cattle. The arrangements for tethering animals in relation to each other and to service areas within a facility may have a considerable influence on the well-being, health and production of the animals. For example, sows in tie stalls will generally thrive better if they can see each other and are fed simultaneously.

B. SPECIFIC ENVIRONMENTAL CONSIDERATIONS

Consideration of the facilities and environmental requirements of cattle and poultry have been dealt with in more depth than have those for other classes of livestock. These two species are used as type species in order to exemplify many of the principles common to the environmental requirements of other species of farm mammals and birds.

1. Cattle

Conditions of housing for beef and dairy cattle suitable to northern hemisphere conditions are well described in several Canadian and U.S. publications (NRC, 1990; Agriculture Canada, 1988; Curtis, 1988; MWPS, 1987). **It should be remembered that the importance of good and intelligent management will increase proportionately with the intensity of the animal production systems employed.**

i) Temperature

Cattle are tolerant of a wide range of ambient temperatures provided they are healthy, well-fed, and not exposed to extremes of solar radiation, humidity or high wind speeds (Webster, 1983). Undesirable conditions of sanitation, mud, disease, parasites and various insect pests reduce cattle tolerance to extremes of temperature.

Newborn calves are more vulnerable to extremes and fluctuations of temperature than are older animals, with fluctuations tending to be more critical than the absolute temperature. For dairy cows and calves maintained in closed housing systems, the optimum temperature is one close to 20ºC with an acceptable range between 10 and 25ºC (Sainsbury and Sainsbury, 1988).

Cattle maintained in free-stall and other open housing systems frequently choose to stand in areas where the temperature is near or below 0ºC. Cattle maintained in cold environments require more total feed which can readily meet the extra maintenance requirement of about 1% per each 1ºC reduction in effective environmental temperature. Under these conditions, productivity is not lowered and cattle do not appear to be uncomfortable.

When still air temperatures climb above 25ºC, feed intake and performance of heavily fed cattle begin to be affected and they may become physiologically stressed. Tolerance to heat and to cold vary with genotype. In general, beef cattle appear to be more winter hardy than dairy cattle. In fact, the lower critical temperature of intensively fed and housed dairy cattle is probably only about -7ºC, while in beef cattle it can be -20ºC. Windbreaks in windy areas and overhead shelter in geographical areas subject to cold rains, sleet and wet snow are highly desirable regardless of breed or type of cattle.

ii) Ventilation and humidity

The objective of a ventilation system is to provide the air exchange required to maintain environmental temperature and humidity within the desired ranges and to remove methane and carbon dioxide expelled from the rumen and lungs of cattle, ammonia from the decomposition of feces and urine, dust from feed and bedding, and airborne microorganisms.

In winter, removal of water vapour is a prime need in order to avoid condensation within the building. Adequate insulation and, in some special cases, supplemental heat (e.g., calf housing in certain locations) will also aid in maintenance of dry premises. Cold weather ventilation rates should be sufficient to maintain relative humidity below 80% and above 40% (Curtis, 1983). During cold weather, ventilation in

housing for neonatal animals should maintain acceptable air quality without chilling the animals. In summer, ventilation aids in keeping ambient temperature below the upper critical level of 25ºC. Ideally, the ventilation rate should be high enough to prevent indoor temperatures from exceeding outdoor by more than 3ºC when the atmospheric temperature is above 25ºC (Curtis, 1988).

A proper ventilation system should move the right amount of air for moisture and pollutant removal in winter and for heat, moisture and pollutant removal in summer. The system should provide a relatively uniform temperature, as this is more important than the absolute temperature. Similarly, the airflow that is established through the building should be even, so that neither drafts nor dead air pockets are created.

Open housing systems should be built so as to permit extra air movement in the summer and minimum drafts in winter. Air current patterns are also of importance in winter in relation to snow accumulation. Cattle should be able to feed, rest and exercise with minimum exposure to cold wind and low temperature precipitation.

The indoor relative humidity range recommended in the *Canadian Farm Building Code* is 25 to 75%. Levels of 50 to 55% humidity may be considered ideal and to provide a minimum of influence on the physiological effects of other environmental parameters such as temperature and ventilation. The comfort zone of animals is reduced (or narrower) at both high and low temperatures under conditions of high humidity. High humidity in closed housing at low temperatures leads to condensation; the resulting dampness enhances the risk of disease transmission.

iii) Odours

Odours result from rumination by cattle, from feces and urine, from silage, spoiled feeds, etc. Odours may taint the milk and, if highly repugnant to herdsmen, may also result in the delivery of poorer animal care. Odours often indicate the presence of gases which can be harmful to cattle and to man. This is especially true with modern liquid manure systems in which hydrogen sulphide (H_2S), ammonia (NH_3) and methane (CH_4) are produced. High concentrations of H_2S are lethal, and low to moderate levels of H_2S and NH_3 are implicated in reduced animal health and performance. High CH_4 concentrations are explosive and at lower levels CH_4 is a simple asphyxiant. CO_2 results primarily from rumen fermentation and exhalation by cattle. Except in extreme cases of poorly ventilated housing, coupled with liquid manure agitation, CO_2 accumulation is not considered to be injurious to humans or animals. Occupational health standards for gases are shown in Table 1.

TABLE 1 OCCUPATIONAL HEALTH STANDARDS FOR GASES

Gas	TLV[a] ppm	Excursion factor[b]	TWA limit[c] ppm
H_2S	10	2	20
NH_3	25	1.5	37.5
CO_2	5000	1.25	6250

[a] TLV (threshold limit value) represents conditions under which it is believed that nearly all workers may be repeatedly exposed for
an eight-hour day and 40 hour work week without adverse effect.

[b] Excursion factor defines the magnitude of the permissible excursion about the TLV.

[c] TWA (time-weighted average) limit defines the maximum concentration permitted for a short exposure period.
TLV x Excursion Factor = TWA limit.

iv) Lighting

Light intensity must be adequate to maintain a high level of husbandry. For instance, an intensity of 538 lux (50 fc) is desirable in the area of the udder in a milking parlour so that the operator can properly care for the cow's udder. Two hundred and fifteen (215) lux (20 fc) are quite adequate in most general housing situations with cattle. While approximately equal hours of light and dark have generally been considered acceptable, some evidence suggests that longer hours of light increases feed intake and cattle performance.

v) Bedding

Bedding materials used in stalls and pens are chosen on the basis of availability, cost and suitability, as well as the need. The housing system, and in particular the manure disposal system, will largely dictate the bedding material if any, and how much is appropriate. Straw or other appropriate materials are commonly used with or without rubber matting, and on concrete, sand or wood bases. Comfort and cleanliness of animals is dependent not only on amount and type of bedding, but also on animal stocking density, type of shelter, temperature and humidity levels. In open housing under cold conditions, loose straw is very helpful in minimizing heat loss from cattle, and a straw-manure base sufficient to allow for fermentation can provide additional heat. Under conditions in which cattle consume significant amounts of bedding, freedom from toxic compounds in the bedding is critical.

vi) Population density

Conditions for housing of beef and dairy cattle are well described in the *Canadian Farm Building Handbook* (Agriculture Canada, 1988). Space requirements vary depending on size and type of animal, type of shelter, whether tied or loose, numbers of animals per group, and level of management. **As more intensive animal agriculture is practised, quality of management must improve accordingly.**

2. Sheep

General information on the facility requirements and environmental conditions suitable to the raising and maintenance of sheep in Canada is available in a number of books and monographs (Agriculture Canada, 1988; NRC, 1990; Curtis, 1988; Ensminger and Parker, 1986).

i) Temperature

The comfort zone for the various classes of sheep has been reported as follows: ewes and rams 7-24^{o}C; feeder lambs 5-21^{o}C; newly born lambs - until dry 24-27^{o}C, which may be provided by heat lamps (Ensminger and Parker, 1986). While these are stated to be the comfort zones, sheep will not suffer in temperatures below -18^{o}C if they are in fleece and the humidity is low.

ii) Ventilation

The requirements vary widely due to geographic location. During the winter, the recommended ventilating capacity of buildings housing sheep is 0.6-0.7 m^3/min for each ewe and 0.3 m^3/min for each lamb. In summer the ventilating system should provide 1.1-1.4 m^3/min per ewe and 0.65 m^3/min per lamb (Ensminger and Parker, 1986).

Preferred relative humidity is considered to be around 60%; however, a range from 50% to 75% is acceptable (Ensminger and Parker, 1986).

iii) Lighting

There are no specific light requirements cited for sheep. Where windows equate to 3 to 5% or more of the floor area, these will provide sufficient natural light. Photoperiod may need to be regulated for the purpose of controlling the onset of estrus.

Although natural light is normally sufficient for sheep in most situations, supplemental lighting should be provided during lambing periods.

iv) Bedding

Straw is the most common bedding material used. Some modern units use a liquid manure system with floors of expanded metal, wire or slats and no bedding. Providing that space allocation is correct, these systems are acceptable.

v) Population density

Table 2 taken from the 1988 edition of the *Canadian Farm Building Handbook* (Agriculture Canada, 1988) gives the accepted detail for sheep accommodation. The space allocation cited **may** be considered generous from the viewpoint of practical commercial sheep raising, **but acceptable for research and teaching animals.**

Generally the number of animals per pen should not greatly exceed 100 pregnant ewes or 50 ewes with lambs or 500 feeder lambs.

TABLE 2 ACCOMMODATION FOR SHEEP

ACCOMMODATION	EWES AND RAMS	FEEDER LAMBS
Feed lot (m^2/head) hard surfaced soil[1]	1.4 6.5	0.6 2.8
Open-front shed floor area (m^2) Pregnant ewe Dry ewe Ceiling height minimum (m)	1.4 0.93 2.7	0.6 2.7
Slotted floors (m^2/head)[2] % floor area slotted slot width (mm) slat width	0.65 100 19 50-75	0.4 100 16 50-75
Lambing pen, not slotted (m, minimum) Claiming pen only Lambing and claiming pen	1.2 x 1.2 1.2 x 1.5	

Feed rack, length per head (mm) Group feeding Self-feeding Height at throat (mm) Small breeds Large breeds	400 150 300 375	300 100 250 300
Feed storage Hay (kg/day per head) Small breeds Large breeds Grain (kg/day per head) (maintenance) (finishing)	1.4 2.3 0.15	0.9 0.23 0.45-1.13
Bedding storage (kg/day per head)	0.34	0.11
Water surface area (m^2/40 head)	0.1	0.1

[1] Soil-surfaced feed lots should be used only where annual precipitation is less than 500 mm. A paved strip next to the feed bunk should be at least 1.8 m wide, or as wide as the tractor used for cleaning. The strip should slope 1:25 away from the feed bunk.

[2] An alternative to slotted floors, for ewes, rams or lambs is 25 x 50 mm, 4 mm-gauge expanded and lattened metal mesh.
Expanded metal mesh floors may be covered with a solid panel to retain bedding for lambing.

3. Swine

Detailed information and guidelines for swine housing may be found in the *Canadian Farm Building Handbook* (Agriculture Canada, 1988). The Veterinary Infectious Disease Organization has published three booklets on *Farrowing Barn Design and Management*, *Swine Nursery Design*, and *Feeder Barn Design and Management*, designed to provide swine producers with current information on modern building design and operation (VIDO, 1986, 1987).

The internal surfaces of all swine houses and equipment contained therein should be constructed of smooth, non-porous materials which can be readily and effectively cleaned and disinfected. Pen dividers and feeders should be free of sharp edges or projections which might cause injury to the animals. Passageway and pen floors should be effectively drained. All floors, whether solid, slatted, or wire mesh, should provide adequate footing and be non-injurious to the pigs.

It is not feasible to state specific values for such environmental parameters as temperature, humidity and ventilation that are meaningful for all classes of swine in all possible research and teaching situations. The precise requirements will vary considerably with age, type of housing, density of population, etc., and the ranges cited in most instances refer to the upper and lower limits of generally accepted comfort zones.

i) Temperature

With the possible exception of the neonatal and nursing piglets, swine are extremely adaptable and comfortable over a wide range of climatic conditions, if they are provided with the proper facilities to conserve or dissipate body heat. Pole barns or outside huts can be comfortable even in extremely cold weather, if the unit has a sufficient population and is provided with adequate and appropriate bedding for the pigs to create a comfortable microenvironment. Animals with access to outside runs or paddocks in hot weather should have a shaded, preferably damp, area so they can stretch out on the ground and

dissipate body heat by conduction. Total confinement, on concrete or slats, may interfere with conductive heat transfer so the environmental support systems must be adequate to maintain a satisfactory comfort zone through all seasons.

For adults and most growing pigs (>30 kg) the comfort zone range is about 15-25ºC (Curtis, 1988). The farrowing facility presents a special concern because the environmental requirements for the sow, and the newborn piglet are drastically different. For the comfort of the sow a temperature of 15-26ºC should be maintained, whilst the creep area should be dry, draft-free and provide a temperature of from 26-32ºC at all times for the newborn piglets (Curtis, 1988).

ii) Ventilation and humidity

Adult and growing pigs will thrive at a relative humidity within the range of 40-80% (Curtis, 1988). Ventilation rates in winter should be sufficient to control moisture. In summer the airflow rates required to remove heat produced by the animals are 15-20 X higher than the rates required for moisture control (VIDO, 1987). Metal bars or wire mesh partitions between individual pens are preferable to solid structures as they facilitate air movement at the level of the animal.

iii) Lighting

Photoperiod has a definite effect on the age at which sexual maturity is achieved and may also influence growth rate and feed efficiency (Maybry, Jones and Seerley, 1983), although Berger, Mahone, Svoboda *et al*. (1980) suggest that no particular photoperiod is necessary for growing pigs. From the viewpoint of good animal care, the light intensity should be such that animals in all areas of the facility can be observed clearly at all times.

iv) Noise and odours

Some noise and odour will inevitably be present in any practical swine unit. Odours may be minimized by regular efficient cleaning and adequate ventilation. Noise levels can be held down by ensuring that mechanical equipment operates relatively quietly and by minimizing procedures which disturb the animals.

v) Bedding

Where swine are held for relatively short periods in small units, straw or other appropriate materials may be used. In large units, with automated cleaning and manure handling, it is customary to house pigs without bedding. Where pigs are maintained in pole barns or outside huts, deep straw bedding should be provided.

vi) Population density

Young pigs up to 10 or 12 weeks of age get along very well, and substantial numbers can be kept together in a single pen (Curtis, 1988). As the animals grow older, aggressive behaviour develops and fighting or bullying will occur, particularly in larger groups. Generally, group size should be kept to ten or less for mature sows and animals in the late growing phase (Sainsbury and Sainsbury, 1988). With electronic feeding stations, larger groups may be more appropriate. Whenever groups of sows are first established, some fighting will take place for the first few days. The belligerent interactions between individuals within the group should subside as the social hierarchy is established; however, it is difficult to add new animals to previously established groups. If the grouped pigs are limit-fed, it is essential that sufficient feeder space be provided so that all animals can eat at the same time.

When adult pigs are confined to stalls, the stall should always be long enough to allow the pig to lie fully relaxed without its head or nose touching the feeder or front of stall. The stall must also be wide enough to allow the animal to lie fully relaxed on its side with its feet and legs extended. A stall width of 0.65 m will usually satisfy this requirement.

Although not recommended by the Canadian Council on Animal Care (CCAC), if a tie stall is to be used, extreme care must be taken with regard to the design of the collar or belt utilized and the tethered pig(s) must be closely monitored when first restrained. If any abrasions occur in the region of the collar or belt the animal must be released immediately. **As a general rule, the tie system should not be used unless the animal has been acclimatized to it at an early age.** Where slatted or partially slatted floors are used, care must be exercised to insure that slot width is such that no portion of the pig's hoof or leg will pass through. Particular attention must be paid to the floor structure when dealing with newborn piglets. Tables 3 and 4 are pen floor space allowances for growing pigs and replacement gilts and sows which will be included in a forthcoming Recommended Code of Practice for the Care and Handling of Farm Animals--Pigs (Agriculture Canada, 1898/E in press).

TABLE 3 RECOMMENDED PEN FLOOR SPACE ALLOWANCES FOR GROWING PIGS BASED ON BODY WEIGHT$^{.667}$

Body Weight		**Fully Slatted (0.035*BW$^{.667}$)++**		**Partial Slats (0.039*BW$^{.667}$)**		**Solid Bedded (0.045*BW$^{.667}$)**	
kg	**(lbs)**	**m^2**	**(ft^2)**	**m^2**	**(ft^2)**	**m^2**	**(ft^2)**
10	22	0.16	(1.7)	0.18	(1.9)	0.21	(2.2)
20	44	0.26	(2.8)	0.29	(3.1)	0.33	(3.5)
50	110	0.48	(5.2)	0.53	(5.7)	0.61	(6.6)
75	165	0.62	(6.7)	0.70	(7.5)	0.80	(8.6)
90	198	0.70	(7.5)	0.78	(8.4)	0.91	(9.7)
100	220	0.76	(8.2)	0.85	(9.1)	0.97	(10.4)
110	242	0.81	(8.7)	0.90	(9.7)	1.03	(11.1)

++For calculations; body weight (BW) is in kg, area in m^2.

TABLE 4 RECOMMENDED PEN FLOOR SPACE ALLOWANCES FOR REPLACEMENT GILTS AND SOWS

Body Weight		**Partial Slats (0.054*BW$^{.667}$)++**		**Solid Bedded (0.059*BW$^{.667}$)**	
kg	**(lb)**	**m^2**	**(ft^2)**	**m^2**	**(ft^2)**
100-150	(220-330)	1.5	(16)	1.7	(18)
150-200	(330-440)	1.8	(19)	2.0	(22)
200-250	(440-550)	2.1	(23)	2.3	(25)
>250	(>550)	2.3	(25)	2.6	(28)

++For calculations; body weight (BW) is in kg, area in m^2.

4. Horses

The basic conditions for housing and maintaining a proper environment for horses are outlined in the *Consortium Guide* (Curtis, 1988) and also in the *Horse Housing and Equipment Handbooks* (MWPS, 1986). The *Canadian Farm Building Handbook* (Agriculture Canada, 1988) deals specifically with housing for riding horses.

A bright, airy stable with access to an exercise paddock is desirable in housing horses in order to maintain top condition, muscle tone and health. The housing area should allow for adequate space within its alleyways to permit the safe movement of horses and attendants.

Stall construction should preferably be of hardwood at least 3.75 cm thick. Doors and partitions should be metal faced, particularly along their top edges, to discourage "cribbing." There should be no protuberances which might be injurious to the animals. Stall walls should be of sufficient height to prevent interference with adjacent animals. It is important that doors be of adequate width (1.25 m) and height (2.25 m) to permit the easy movement of horses, without risk of injury. Ceilings and overhead supporting beams should also be of sufficient height, preferably 3 m, to permit the horse to assume a normal posture and to guard against possible head injury.

Floors should have durable, non-slip surfaces. Roughened concrete is satisfactory; wood flooring in the standing stall is often used; packed earth may, in some instances, be acceptable in box stalls. Under institutional and laboratory conditions, a room or area separate from the stable area should be provided to perform special procedures (e.g., the collection of large quantities of blood, etc.).

i) Temperature

Horses can tolerate low temperatures provided there is adequate shelter from extremes of wind, rain and snow. Similarly, quite high temperatures can be tolerated provided adequate shade is available to the free-ranging animal and that appropriate ventilation and humidity are provided in the stable. An abundant supply of fresh, potable water should be available at all times, and is particularly important in hot weather. When housed in a dry draft-free environment, horses can also tolerate a wide range of environmental temperatures (-7 to 29ºC). However, the optimum appears to lie between 10 and 15ºC (Ensminger, 1969). The relative humidity in horse quarters should range between 50-80% (Curtis, 1988).

ii) Ventilation

Ventilation rate capacity should be at least 0.7 m^3/min. per 450 kg of horse at temperatures of -18 to -7ºC and 2.8 m^3/min. per 450 kg of horse at temperatures of -1 to 10ºC (MWPS, 1987). The capacity will need to be increased during hot weather.

iii) Lighting

The level of lighting in a horse barn should allow for adequate examination of animals and bedding. Total darkness should be avoided. A light source should be present at night. Illumination of at least 200 lux is recommended for alleys, handling and feeding areas (Currence and McFate, 1984). One 100 W incandescent lamp per 8 m of floor or each box stall will produce the required light level (MWPS, 1987).

iv) Bedding

Sufficient bedding should be provided in the form of straw, wood shavings or other suitable material. Adequate floor drainage must be assured in both the box and standing stall to guard against foot problems and unnecessary soiling of the animal.

Manure and soiled bedding should be removed daily to keep horses clean and dry, and the environment free of dust and odours (Curtis, 1988). The animal should not have access to the manure storage area due to the high risk of parasite infestation from this source.

Regular grooming is strongly advocated for members of this species, particularly if housed in tie stalls

with limited freedom of movement.

v) Population density

Ideally juvenile and mature horses should be housed in individual box stalls of at least 3.5 m x 3.5 m, with access to an exercise paddock of 10.0 m x 27.50 m or larger. Horses may be quite satisfactorily maintained in standing stalls providing there is good separation between each animal and regular access to an exercise paddock. Subsequent to weaning, it is desirable that each animal have a separate stall. In the exercise paddock, separation by age and compatibility should be maintained.

5. Poultry

It is not feasible in the space of a few pages to deal in detail with the housing, feeding and management of poultry. More detailed information may be found in the literature (Curtis, 1988; Agriculture Canada, 1988; Moreng and Evans, 1985; North and Bell, 1990).

a) Chicks

In experimental studies, chicks are either brooded and reared in floor pens or in batteries. Buildings that house such facilities should be designed and operated in such a manner as to provide maximum comfort for the birds and minimum risk of disease transmission.

It is customary for such buildings to have concrete floors equipped with floor drains and walls of **sealed** concrete block or **sealed** plywood to facilitate easy cleaning and disinfection. Insulation of walls and ceilings is essential as is adequate ventilation.

i) Temperature

Initial brooding temperature should be 35ºC as measured on a level with the backs of the chicks. As the birds age, the brooding temperature should be reduced at the rate of about 2.5ºC per week. By the time the birds are 5-6 weeks old, the house temperature should be down to 18 to 21ºC (Curtis, 1988). A thermometer alone, however, is a poor tool for ensuring chick comfort. The chicks themselves should also be the indicators (North and Bell, 1990).

ii) Ventilation

All poultry buildings must be adequately ventilated either naturally or by forced air. In most installations, the minimum ventilation rate in summer should be about 12 air changes per hour. Such ventilation rates are usually adequate to keep ammonia levels in the buildings down to acceptable levels. Levels of ammonia in poultry houses should not exceed 25 ppm. Higher levels are likely to prove detrimental to the birds and uncomfortable for attendants.

iii) Lighting

Lighting systems vary widely; however, artificial light controlled by a time clock should always be employed. Chicks brooded in batteries are usually subjected to about 35 lux (3.5 fc) of white light on a continuous basis for the first four days after hatching. Broiler chicks brooded in floor pens should receive 35 lux of illumination at the floor for the first 48 hours after hatching, with a light cycle lasting 23 hours, the dark cycle one hour. A 23-hour program is preferable to 24 hours of light, because it acquaints the flock with periods of darkness. This is very bright illumination; however, it is essential that chicks learn to drink and eat as soon as possible. After the first two days, light intensity should be reduced to about 10 lux (1 fc) at floor level (North and Bell, 1990). Replacement stock are generally lighted like broiler chicks

until about six weeks of age when a restricted lighting schedule is introduced. Such schedules, over a period of time, reduce the hours of light to about eight hours per day.

iv) Bedding

Many different types of litter have been used successfully in floor pens. Preference in most parts of Canada is for wheat straw or wood shavings. In general, visual cleanliness of litter is considered of less importance than dryness; spillage of water must be minimized by using suitable waterers. A sufficient number of air changes must be provided in the building to remove the moisture-laden air from the pens; at the same time, care must be taken not to reduce the moisture level to the point where a dust problem is created.

v) Food and water

A good supply of fresh, clean water must be provided and maintained at all times. Feeders of many different types (troughs, hanging feeders and mechanical feeders) have all been used successfully. Where practical, they should be provided with reels or grills to prevent wastage and fouling of the feed by the birds. Feeders should be of a type and size suitable for the age (size) of the birds. Sufficient feeder space must be provided to permit all of the birds to eat at one time.

vi) Population density

Some guidelines for space requirements for chickens will be found in Table 5. The recommended allowances should be considered as "rules of thumb" rather than as absolute minimum allowances.

TABLE 5 GUIDELINES FOR MINIMUM SPACE REQUIREMENTS FOR POULTRY

		Floor area / bird cm^2
Brooding and Growing Period		
Floor pen housing	0-6 weeks	64[a]-1116[b]
	>6 weeks	742[a]-2786[b]
Cage housing[c]	Leghorn-type/Medium-size	
	0-6 weeks	97/155
	6-12 weeks	194/310
	12-20 weeks	290/348
Laying Period		
Floor pen housing on litter		1625
Leghorn-type		1858
Medium-size		387
Cage housing		452
Leghorn-type		
Medium-size		
Breeding flocks - males and females		1393[a]-2786[d]
Floor pen housing on litter		
Feeder space - Length/bird cm.		10
Nests - per 100 layers		25

Adapted from Curtis (1988) and North and Bell (1990)
[a] Mini-Leghorn pullets

[b] Meat-type cockerels
[c] Cages should allow birds to stand erect
[d] Meat-type

b) Laying and Breeding Hens

In general, the type of building required for housing laying and breeding chickens is similar to that required for floor brooding of chickens; however, the internal design and equipment required are different. Houses for layers and breeders maintained on floors are usually equipped with dropping pits or dropping boards, nests, appropriate troughs or hanging feeders, and automatic waterers.

In order to prevent cannibalism, the birds are often beak-trimmed with an electric beak trimmer. Heavily beak-trimmed birds may suffer a severe setback in growth and in subsequent performance in the laying house. Excessive trimming should, therefore, always be avoided both on humane and economic grounds.

i) Ventilation

Ventilation rates required in summer and winter in houses for laying and breeding chickens are fairly similar to those required in houses for rearing replacement stock. In winter, the system must remove moisture build up whilst maintaining an optimum house temperature between 18 and 24ºC. In the summer, the ventilation system should maintain the house temperature below 27ºC. At temperatures above 27ºC laying pullets begin to suffer and performance diminishes (North and Bell, 1990).

ii) Lighting

Artificial light, controlled by a time clock, must be provided to layers for optimal production. It is usual to provide 14 hours of white light per day at a light intensity of 10 lux (1 fc) at the feeders and waterers (North and Bell, 1990).

iii) Bedding

The floors are usually covered with litter of straw or shavings. Occasionally, such houses have floors of 2.5 cm x 5 cm mesh, heavy gauge, electrically welded wire which obviates the need for litter.

Houses suitable for maintaining layers in cages usually have single or multiple cages arranged in rows about 76 cm apart. Stair-step or single-deck type cages, with mechanical cleaning arrangements underneath are perhaps the most popular cages, since removal of droppings is made easier with such cages than with multiple-deck cages.

iv) Food and water

Feeds appropriate to the stage and level of production are readily available commercially. Food may be supplied in mash or pelleted form. Laying or breeding rations usually contain about 16% protein. Water is generally supplied by automatic waterers. Both trough and large cup waterers have been found satisfactory for layers in floor pens while trough, drip and small cup waterers have been found suitable for layers in batteries.

v) Population density

Some guidelines for space requirements for hens will be found in Table 5.

c) Commercial vs. Experimental Conditions

The housing, feeding and management of chickens have been treated above as in commercial practice. The principal difference between the commercial situation and the experimental situation is that in the latter, many different treatments and replications are involved which often may necessitate the use of many small floor pens or many small battery groups. These must be group- or individually-fed with individual or group data being collected from each. Production-oriented agricultural research, in order to be relevant, will most often require an approximation of good commercial management and housing practices.

Where chickens are utilized as a bioassay tool in biomedical and behavioural research, the environmental conditions given above for chicks and mature birds should be considered as constituting minimal acceptable standards. When chickens must be introduced into a laboratory animal facility in which poultry are not usually accommodated, it is necessary to provide appropriate caging. In these circumstances, advice should be sought from someone knowledgeable in poultry science and husbandry as well as laboratory animal science to assure that such considerations as sufficient head room, feeder space and proper flooring are satisfactorily met. Care should also be taken to ensure that feeders and waterers (particularly where fountains and open cups are used) are located so as to avoid becoming fouled with feces or clogged with bedding.

C. PEST CONTROL

Programs should be in place to control infestation by vermin (flies, mosquitoes, fleas, lice, ticks, rodents, skunks and birds). The most effective control is by preventing entry by the appropriate screening of openings and sealing cracks, maintaining the integrity of all surfaces, and eliminating vermin breeding sites. Pesticides should only be used judiciously and when necessary and where the risk to animals and the experimental process is minimal.

Cats are sometimes used for rodent and bird control and if so they should receive appropriate veterinary care including complete inoculation against the common feline diseases, including rabies.

D. REFERENCES

AGRICULTURE CANADA. Publication 1771/E. Recommended code of practice for the care and handling of pigs. Communications Branch, Agriculture Canada, Ottawa, Ont., K1A 0C7. 1984.

IBID. Publication 1898/E. Recommended code of practice for the care and handling of farm animals--pigs. In Press.

IBID. Publication 1821/E. Recommended code of practice for the care and handling of special fed veal calves. 1988.

IBID. Publication 1757/E. Recommended code of practice for the care and handling of poultry from hatchery to processing plant. 1989.

IBID. Publication 1853/E. Recommended code of practice for the care and handling of dairy cattle. 1990.

IBID. Publication 1870/E. Recommended code of practice for the care and handling of farm animals--beef cattle. 1991.

AGRICULTURE CANADA. Publication 1822/E. Canadian farm building handbook. Communications Branch, Agriculture Canada, Ottawa, Ont., K1A 0C7. 1988.

BERGER, T., MAHONE, J.P., SVOBODA, G.S., METZ, K.W. and CLEGG, E.D. Sexual maturation in boars and growth of swine exposed to extended photoperiod during decreasing natural photoperiod. J. Anim. Sci. 1980; 51: 672-678.

BRAMBELL, F.W.R. CHR. Report of the Technical Committee to Enquire into the Welfare of Animals Kept Under Intensive Livestock Husbandry Systems. Her Majesty's Stationery Office, London, England 1965.

CURTIS, S.E. Measurement of stress in animals. In: Woods, W.R., ed. Proc. Symp. Manage. Food Producing Anim. Purdue Univ., West Lafayette, IA, 1982; 1: 1-10.

CURTIS, S.E. Environmental management in animal agriculture. Ames, IA: Iowa State Univ. Press, 1983.

CURTIS, S.E., ed. Guide for the care and use of agricultural animals in agricultural research and teaching. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (309 West Clark Street, Champaign, IL 61820) 1988.

CURRENCE, H.D. and MCFATE, K.L. Agricultural wiring handbook. 7th Ed. Publ. 8401, Columbia, MO: Natl. Food and Energy Counc., 1984.

DUNCAN, I.J.H. Animal rights - animal welfare: A scientist's assessment. Poult. Sci. 1981; 60: 489-499.

EDWARDS, G.B. Clinical assessment of pain, distress and discomfort in ruminants. In: Gibson T.E. and Paterson, D.A., eds. The detection and relief of pain in animals. London: British Veterinary Association Animal Welfare Foundation, 1985: 85-88.

ENSMINGER, M.E. Horses and Horsemanship. 4th Ed. Danville, IL. The Interstate Printers and Publishers Inc., 1969.

ENSMINGER, M.E. and PARKER, R.O. Sheep and goat science. 5th Ed. Danville, IL. The Interstate Printers and Publishers Inc., 1986.

FRASER, A.F. Behaviour of livestock under intensive conditions of husbandry. Appl. Anim. Ethol. 1975; 1: 111-112.

FREEMAN, B.M. Stress and the domestic fowl; a physiological appraisal. World Poultry Sci. J. 1971; 27: 263-275.

GENTLE, M. Measurements of pain, distress and discomfort in poultry and other birds. In: Gibson T.E. and Paterson, D.A., eds. The detection and relief of pain in animals. London: British Veterinary Association Animal Welfare Foundation, 1985: 92-95.

GIBSON, T.E. and PATERSON, D.A., eds. The welfare of animals in transit. London: British Veterinary Association Animal Welfare Foundation, 1986.

LINKLATER, K.A. and WATSON, G.A.L. Sheep housing and health. Vet. Rec. 1983; 113(24): 560-564.

MAYBRY, J.W., JONES, R.D. and SEERLEY, R.W. A comparison of an 8-versus 16-hour photoperiod during

lactation on suckling frequency of the baby pig and maternal performance of the sow. J. Anim. Sci. 1983; 57: 292-295.

MANN, C.M. and HARVEY, P.N. Cage size and stocking density for laying hens. World Poultry Sci. J. 1971; 27: 350-356.

MENCH, J.A., MAYER, S.J. and KRULISCH, L. eds. The well-being of agricultural animals in biomedical research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992.

MIDWEST PLAN SERVICE. Horse housing and equipment handbook. 7th Ed. Ames, IA: MidWest Plan Service, Iowa State University, 1986.

IBID. Structures and environment handbook. 11th Ed. Ames, IA: MidWest Plan Service, Iowa State University, 1987.

MORENG, R.E. and EVENS, J.S. Poultry science and production. Reston, VA. Reston Publishing Company, Inc., 1985.

NATIONAL RESEARCH COUNCIL. Canadian farm building code. 7th Ed. Ottawa, Ont.: National Research Council, 1990.

NORTH, M.O. and BELL, D.D. Commercial chicken production manual. 4th Ed. New York, NY: Van Nostrand Reinhold, 1990.

OLDHAM, J.G. Clinical measurement of pain, distress and discomfort in pigs. In: Gibson T.E. and Paterson, D.A., eds. The detection and relief of pain in animals. London: British Veterinary Association Animal Welfare Foundation, 1985: 89-91.

SAINSBURY, D.W.B. Poultry housing and disease. Vet. Rec. 1983; 113(24): 565-568.

SAINSBURY, D.W.B. and SAINSBURY, P. Livestock health and housing. 3rd Ed. Toronto, Ont.: Baillière Tindall, 1988.

SIEGEL, P.B. The role of behaviour in poultry production: A review of research. Appl. Anim. Ethol. 1984; 11: 299-316.

SILVER, I.A. Horses. In: Gibson T.E. and Paterson, D.A., eds. The detection and relief of pain in animals. London: British Veterinary Association Animal Welfare Foundation, 1985: 82-84.

SMIDT, D. Advantages and problems of using integrated systems of indicators as compared to single traits. In: Smidt, D., ed. Indicators relevant to farm animal welfare. Boston, MA: Martinus Nijhoff, 1983: 201-207.

SMITH, W.K. and ROBERTSON, A.M. Observations on injuries to sows confined in part slatted stalls. Vet. Rec. 1971; 89: 531-533.

VETERINARY INFECTIOUS DISEASE ORGANIZATION. Farrowing barn design and management. VIDO (124 Veterinary Road, Saskatoon, Saskatchewan S7N 0W0), 1986.

IBID. Swine nursery design; feeder barn design and management 1987.

WATHES, C.M., JONES, C.D.R. and WEBSTER, A.J.F. Ventilation, air hygiene and animal health. Vet. Rec. 1983; 113(24): 554-559.

WEBSTER, A.J.F. Environmental stress and the physiology, performance and health of ruminants. J. Anim. Sci. 1983; 57: 1584-1593.

WILSON, W.O. Evaluation of stressor agents in domestic animals. J. Anim. Sci. 1971; 32: 578-583.

V. LABORATORY ANIMAL CARE

A. INTRODUCTION

a) Animal Care and Handling

All animal facilities should have in place Standard Operating Procedures (SOP) for animal care. Assistance in this regard may be found in a manual and computer program developed by Olson, Morck, and Nabrotzky (1992).

All animals must be observed at least once daily.

Animals may have to be handled when being put into new cages or removed for various experimental purposes. Most domestic and laboratory animals need no restraint for such routine handling, and will respond to gentleness. Under normal conditions, all standard laboratory animals, except non-human primates (NHP), can be handled without the use of gloves or other restraining aids. In all cases, only the minimal amount of force necessary should be employed. Manipulation of the type and intensity of light used often proves useful in handling small wild mammals and birds (Fall, 1974).

Successful handling requires the ability to recognize the animal's state of mind, which may include bewilderment, apprehension and, in some cases, discomfort or pain. Proper training is important to provide consistency in handling which usually results in more manageable animals.

Whenever possible, the use of cumbersome protective garments, such as gloves, should be avoided, as they often prevent the handler from developing the proper sense of touch and cause the animal discomfort. However, with wild and semi-domesticated species (mink, monkeys, etc.) use of protective gauntlets and restraining equipment is usually necessary. The use of specialized cage squeeze mechanisms followed by tranquillization is often advocated for handling the larger NHP. Transfer devices, pole tether devices and training through reward may be used to effect routine cage changes. Transfer devices have also been designed and utilized for a variety of small wild rodent species (Caudill and Gaddis, 1973).

The handling of individual species is addressed in Volume 2 of the *Guide*. In addition, Agriculture Canada has published Codes of Practice for handling many species of farm animals (Agriculture Canada, 1771/E, 1984; 1821/E, 1988; 1757/E, 1989; 1853/E, 1990; 1870/E, 1991). In addition, a revision of the Recommended Code of Practice for the Care and Handling of Farm Animals--Pigs (1898/E) is now in press.

B. GENERAL PRACTICES

1. Reception

The portal through which animals and containers enter a facility is important in the overall prevention of disease. The examination of newly arrived animals should have several aims: evaluating the condition and health of the animals; preventing cross-contamination of animals from different sources; and ensuring that the order has been accurately filled. The health status of the animals at their source and the possibility of cross-contamination during transport are important considerations. Cross-contamination is always a greater risk if the animals are not shipped in a vehicle dedicated to the transport of animals, coming from a single source. However, this risk can be decreased by the use of filtered shipping crates.

Each new shipment of animals should be received, uncrated and examined by trained personnel and

placed in clean cages in a designated reception area separate from the animal holding room(s). The reception area should be cleaned and disinfected after each new shipment. Shipping containers should not enter the main facility unless properly decontaminated and should be properly disposed of, or thoroughly cleaned and disinfected if they are to be reused. Incoming animals should be identified and their arrival appropriately recorded.

Animals that appear unhealthy, or which have been in any way debilitated in transit, should be separated from the remainder and held in an appropriate location for observation and treatment. Where this is not feasible, such animals should be euthanized without delay.

2. Conditioning/Quarantine

The level of conditioning required will depend on the differences in microbial status between the resident animals and the incoming animals. Rodents are commonly purposebred for the laboratory and can be received from the supplier in a defined state, with a known health, nutritional and, to a varying extent, genetic background. Similarly other purposebred species obtained from reputable sources will have complete health profiles and will have received specified prophylactic treatment. Such animals may not normally require further quarantine for confirmation of their health status; however, a holding period of several days will give the animals the opportunity to adjust to their new surroundings. **A minimum adjustment period of two days is required after shipping for immune function, corticosterone levels and other physiological parameters to stabilize** (Small, 1984; Toth and January, 1990).

Legally procured strays or donated animals, acquired feral animals, such as NHP, and animals from other random sources should be subjected to a period of conditioning following their reception. Conditioning requires that the animal be held for a varying period of time (1-6 weeks) in separate quarters. The length of the conditioning period will depend on the species, the health status of the animals, the reliability of the supplier, and whether an active process of screening animals for the presence of or exposure to infectious agents is undertaken. During this period, thorough physical examinations should be undertaken. Further examination will depend on species and intended use.

The conditioning period should be of sufficient duration to permit the proper evaluation of the suitability of the animal(s) for their intended use, and testing for contagious, zoonotic and other diseases that may be of concern. This can include serological screening for antibodies to viruses and other pathogens, the examination for ecto- and endo-parasites, mycoplasma, and pathogenic bacteria. If contamination during transport is a possibility, an appropriate period to allow expression of disease or antibody production should be allowed. Sufficient time must also be allowed for appropriate treatment or vaccination against diseases that tend to be endemic in the species being conditioned.

During the conditioning/quarantine period, the animals should be held in facilities separated from other animals with no crossover of personnel, equipment, supplies or ventilation (Frost and Hamm Jr., 1990), unless effective measures are taken to prevent cross-contamination.

3. Holding (Maintenance)

Only one species should be housed in a conventional animal room unless maintained in isolator caging, racks or cabinets. Shipments of the same species, acquired from different suppliers, should also be separated according to health status if space permits, or housed in isolator caging. Where the mixing of species and/or stocks from different sources is unavoidable, every effort should be made to place together those that are behaviourally compatible, have similar environmental requirements, and a low probability of cross-infection. NHP should not be housed with any other species.

Animals should be held in enclosures adequate for that species as described in the individual sections of Volume 2 of this *Guide*.

4. Identification and Records

Cage or group identification may be used for small laboratory animals if individual identification is not an experimental prerequisite. Individual identification can be by ear tagging, ear notching, tattooing, tail marking, subcutaneous microchip implant or other species appropriate method (Hayden, 1974; Ball, Argentieri, Krause *et al*. 1991; Iwaki, Matsuo and Kast, 1989; Castor and Zaldivar, 1973). Dye marking on the hair provides for short-term identification. Larger laboratory animals should always be individually identified by tattoo, neck band, individual tag, or a subcutaneous identity tag.

The Canadian Council on Animal Care (CCAC) opposes the use of toe clipping as a method of identification for the short-term learning experience of field studies. Where it is necessary to provide permanent individual identification between litter members of newborn rodents, toe clipping may be necessary. If, for any reason, this procedure has to be undertaken on other than neonatal animals, either a local or general anesthetic should be administered (Stonehouse, 1978).

The importance of keeping complete and thorough records on all experimental animals cannot be overemphasized. The following information should be recorded for each animal: **arrival date, sex, estimated age and weight, breed and type, colour and markings and any physical abnormalities or other identifying features** (ILAR, 1984). **The name of the project or investigator and protocol number to which it is allocated should be noted, as well as that of the supplier and eventual disposition.** Animal records should be kept for a period of one year after the final disposition of the animal. The cages in which animals are housed, prior to or during an experiment, should be clearly marked indicating the sex and number of contained animals, the investigator responsible for them and such special instructions as may be pertinent to their care. Records, especially when used in conjunction with data processing equipment, can facilitate facility management (Wasserman, Blumrick and Liddell, 1982; Rieger and Beriault, 1983).

Use of room cards/boards on the doors of animal rooms indicating the species, investigator(s) whose animals are being held and any special notations that may be pertinent is a good practice.

People donating animals to research facilities are required to sign a statement that they are the legal owner. This document should include identification of the animal by the criteria previously noted, and should specifically transfer ownership and disposal of the animal to the institution. Animals (such as dogs, for which a system of national registry exists) should always be checked for the presence of identifying markings.

C. CARE OF THE ANIMAL

1. Food

All experimental animals should receive palatable, wholesome and nutritionally adequate food according to the requirements of the species, unless the study requires otherwise. In certain experiments where small quantities of chemical residues may influence results, certified diets with documented analysis of contaminant pesticides, herbicides, etc., are available from commercial manufacturers of laboratory animal diets.

a) Food Storage

Whenever possible, pasteurized or sterilized diets obtained from reputable suppliers should be used. Proper storage of foods is necessary to minimize the possibility of contamination, deterioration or spoilage. Dry laboratory animal diets should be used within six months of the milling date when stored in cool, dry, well-ventilated quarters. Irradiated diets kept under the same conditions have approximately double the shelf life. Primate and guinea pig diets should be used within three months of the milling date,

unless vitamin C is supplemented. To avoid problems from age deterioration, the date of milling of each shipment should be obtained from the supplier (this is usually marked in code on the bags). Bags should then be marked, put on plastic or metal pallets or racks to keep them off the floor, and stored so that the oldest will be used first. Stale shipments should not be accepted. Shelf life will be appreciably enhanced if the storage area is maintained at a temperature of <16C (60.8F) (Weihe, 1987). Canned foods can be safely stored for long periods. Clean green vegetables suitable for human consumption may enhance the diet; however, vegetable discards may prove sources of infection and should be avoided.

Food used in microbe-controlled environments is often autoclaved. Autoclaving decreases the concentrations of some vitamins and antioxidants (Maerki, Rossbach and Leuenberger, 1989). However, autoclavable diets are available which contain higher concentrations of heat-labile ingredients to compensate for the losses induced by heat-sterilization. Shelf life may be decreased, but need not be if the process is handled properly (Oller, Greenman and Suber, 1985). Gamma irradiation is also used for diet sterilization (Halls and Tallentire, 1978).

Diet in large quantities should not be stored in animal holding rooms. Small quantities, sufficient for one or two days may be kept in the room in covered, vermin-proof containers.

b) Special Considerations

All animals tend to reduce their food intake when sick. Animals with a high metabolic rate, e.g., small rodents and those requiring fairly frequent feedings of high protein diets (e.g., the cat), can become debilitated very rapidly. In cases of anorexia in these species, oral intubation and force feeding as well as intravenous therapy (cat) should be instituted without undue delay. Restricted feeding for maintenance of adult animals is commonly practised for some species and strains, such as rabbits. Animals on restricted food or fluid intake for experimental purposes should be closely monitored for weight loss, signs of dehydration, signs of stress and deterioration in health (McIntosh and Staley, 1989). It should be noted that food and water restriction may have a marked effect on the response of animals to toxic substances and other experimental variables (Damon, Eidson, Hobbs *et al*. 1986). For some species, particularly NHP, providing a variety of foods can be useful as a form of environmental enrichment.

Generally, food should not be scattered over the bottom of the cage, where it may be contaminated or wasted. Exceptions to this include provision of food to newly hatched birds and abnormal (handicapped) animals, such as mice with muscular dystrophy.

2. Water

Drinking water should be available to animals at all times, unless contra-indicated by the experimental protocol. Tap water, even if from municipal water systems, is not sterile and quickly becomes contaminated with even more bacteria after the bottle is placed on the cage (Tober-Meyer and Bieniek, 1981). Monitoring water quality is an important aspect of any research program, as water contamination and chemical composition can affect the health of animals and the results of animal experiments.

Methods available to remove both microbial and chemical contamination include acidification, chlorination, reverse osmosis, ultrafiltration and ultraviolet (UV) light (Newell, 1980). Some of these methods may alter immune function (Herman, White and Lang, 1982; Fidler, 1977) and growth rates in experimental animals (Hall, White and Lang, 1980; Tober-Meyer, Bieniek and Kupke, 1981). Regardless of whether or not the water supply is treated, all water dispensing equipment should be thoroughly sanitized according to institutional SOPs, and periodically monitored for bacterial contaminants.

A watering method unlikely to spread disease or contaminate the water supply should be chosen. Water bottles should be transparent so as to permit ready observation of cleanliness and water level; of a material that will withstand sterilization, and of a wide mouth design to facilitate cleaning. Water bottles should always be replaced with clean, freshly filled ones, rather than by refilling the ones in use. Animals

housed under freezing conditions may require heated water bowls.

Automatic watering devices are economical to operate, but if not properly designed, are difficult to disinfect properly and may lead to cross-contamination (Malatesta and Schwartz, 1985). Recirculating systems eliminate stagnation of water and help prevent buildup of microorganisms. The correct pressure in the drinking valves prevents backflow of water into the lines when animals drink from or play with the valve. Malfunction of automatic watering systems can lead to drowning or drought; consequently, the system must be routinely and thoroughly checked. Some animals need to be taught to use automatic watering devices. Automatic watering devices are not recommended for guinea pigs, unless they are habituated to them.

Most fish have a low tolerance for both copper ions and chlorine. Their water supply, therefore, should either be dechlorinated or obtained from an untreated source, and should not be brought into the aquarium through copper piping.

3. Exercise

Experts disagree about the need for exercise in laboratory animals. A judgement in such cases must thus be made by the laboratory animal veterinarian in consultation with the investigator. Although many adult animals do not seem to have a motivation to exercise *per se*, in the process of satisfying their behavioural needs, they do get exercise (Fox, 1990). Exercise requirements for animals should reflect species, age and environment. Research information on the requirements of each species for exercise is limited and varied, but continually increasing. Young animals of most species involve themselves in much more play and exercise activity than adults. For some species, exercise may not be required in adult animals for physiological health (Weihe, 1987; Clark, 1990; Campbell, 1990). Several studies suggest that there are no beneficial effects on behaviour, health or in enhancement of voluntary activity in the laboratory-bred beagle from increasing the cage dimensions beyond the standard 76 cm x 76 cm x 76 cm (30" x 30" x 30") size, provision of half-hour daily exercise, or from 1.22 m x 3.05 m (4' x 10') floor pen housing (Newton, 1972; Hite, Hanson, Bohidar *et al*. 1977). Judgment should be based on the animal's breed, temperament, physical condition, the conditions under which it has previously been kept and the length of time it is to be confined. Animal cages must, however, always be large enough to allow the **innate normal behavioural and postural adjustments** (see Appendix I). There are many varied methods and programs of exercise which are successfully used in dogs (Eckstein, Moran, Gomez *et al*. 1987; Clark, 1990; Hughes and Campbell, 1990), including walking programs using outside volunteers. Caged rats spontaneously exercise by playing with cage mates and during feeding (Weihe, 1987) (see also Social and Behavioural Requirements of Experimental Animals).

D. CARE OF THE FACILITY

1. Cleaning and Sanitation

Employees must be aware of proper cleaning and disinfecting procedures and their importance in disease prevention (Small, 1984; Harrison and Mahnke, 1991; Van Houton and Hayre, 1991). All cages, pens, racks, aquaria, accessory equipment, etc., must be thoroughly cleaned and disinfected before reuse. Most of these items should be subject to regular (usually weekly) cleaning during use. **As a general rule, laboratory animals should be moved to freshly cleaned cages at least once a week.** Cleaning practices need to be modified according to the species and housing system for domestic animals, fowl, reptiles and aquatic animals. The effectiveness of detergents, disinfectants and facility cleaning programs should be monitored and constant (Thibert, 1980).

The ability to clean and sanitize a facility is greatly influenced by facility design and construction materials. The objective of a sanitation program is to reduce the microbial contamination or "bioburden" to a level that reduces the possibility of any cross-contamination (Harrison and Mahnke, 1991). Proper sanitation will not compensate for the transfer of infection by personnel. Cleaning and sanitation merely

complement proper procedures which minimize contamination (Thibert, 1980). Activities such as pressure spraying and dumping bedding can aerosolize microorganisms allowing cross-contamination if animals are present (Frost and Hamm Jr., 1990). Opening doors can alter the airflow in a facility, enhancing the possibility of transfer of contaminants (Keene and Sansone, 1984). Moveable equipment can transmit organisms between areas. Therefore, such equipment should be dedicated to a particular room or area.

Procedure rooms using animals from different sources are a potential source of cross-contamination. Proper disinfection of surfaces should be ensured after use.

Bedding in animal cages or pens should be changed as often as necessary to keep the animal clean, dry, and relatively odour-free and ammonia levels in the cage at appropriate levels. In rats, this is 25 ppm (Schoeb, Davidson and Lindsey, 1982). Smaller laboratory animals require one to three changes per week, depending on such variables as the sizes of the animals, population density and type of caging and whether or not litters are being produced. Larger species such as dogs, cats and NHP usually require at least a daily change.

Food containers should be easily cleaned and disinfected.

Animal cages are most efficiently cleaned and sanitized with mechanical washing equipment operating at 83C (180F) or higher, for a minimum of ten minutes. Cages should be carefully rinsed to remove all traces of washing and disinfecting agents, as exposure to these may adversely affect both the animal and the experimental results. All automatic washing equipment should be subjected to regular maintenance to assure proper performance. Where an automatic cagewasher is not available, use of a spray washer and disinfectant are preferable to the dip tank and rinsing method. It should be noted that sodium hypochlorite and iodophores are effective on most animal viruses; however, disinfectants should be chosen according to the spectrum of viruses and organisms required to be killed and the possibility of deactivation by the local environment. There are references available to aid in identification of the appropriate disinfectants (Block, 1983; Harrison and Mahnke, 1991; Orcutt, 1991). Chlorine dioxide sterilants/disinfectants have become more recently available and are often used in facilities maintaining SPF or immunosuppressed animals because of their rapid broad spectrum activity, even in the presence of an organic load (Frost and Hamm Jr., 1990).

All chemicals should be used properly, according to label directions. Detergents, disinfectants and pesticides may cause changes in the experimental animal by inducing or inhibiting cellular enzyme activity (Burek and Schwetz, 1980). This should be a consideration when conducting experiments which may be adversely affected.

2. Waste Disposal

Dead animals, animal tissues and excreta, bedding, unused food, etc., should be collected in leak-proof metal or plastic containers with leak-proof, disposable liners and tight lids. Liners are essential for animal tissues, carcasses, and radioactive or toxic waste. Infectious waste should, ideally, be incinerated on the site. If the waste is to leave the facility it should be sterilized (autoclaved) before removal. Gamma irradiation is a relatively recent method of disinfection of waste products which may come into more prominent use (Garcia, Brooks, Stewart *et al*. 1987).

Waste which cannot be rapidly disposed of should be stored in a cold storage area provided for that purpose. Such areas must be vermin-free, easily cleaned and disinfected as well as being physically separated from other storage facilities. The waste storage area should be located so that wastes need not be carried through other rooms of the facility.

Dead animals should be removed from cages as soon as they are noticed. The laboratory animal veterinarian who should have been immediately informed of sick animals, should also be informed of dead ones. Dead animals should be properly identified, placed in disposable plastic bags and taken to the

postmortem area immediately upon discovery. In the postmortem area they should be held under refrigeration for necropsy or for disposal in accordance with the investigator's instructions. National guidelines as well as local and provincial laws control waste disposal practices that could endanger public health (HWC/MRC, 1990). Saskatchewan, Alberta and New Brunswick regulate livestock management and manure disposal, and Ontario has a suggested Code of Practice on the same subject. (Copies of these may be obtained from provincial departments of Agriculture.)

Considerable forethought and extensive consultation is advisable before installing an incineration facility for the disposal of pathological waste.

3. Vermin Control

A properly constructed building should be vermin-proof, but may not be free from vermin. Vermin enter on food, bedding, people and animals. Insects and arthropods thus introduced, may act as the intermediate hosts of certain parasites and may also mechanically transmit bacterial and other pathogens (Hughes, Kassim, Gregory *et al*. 1989). Wild rodents may transmit a wide variety of bacteria, viruses, and parasites to caged members of closely related species (Levine and Lage, 1984). New facilities should be checked critically for vermin before any animals are moved in.

Vermin should also be controlled in already-infested older buildings. A control program will include the proper training of personnel, good waste disposal, sealing or eliminating breeding sites, extermination through pesticides or trapping, and the recovery of all escaped and/or wild animals. It is important that pesticides be applied only under professional supervision. Many pesticides are dangerous to humans, and may adversely affect the experimental animal and the research (Bell, Farrell and Padgett, 1975). Any control program that is initiated must extend throughout all areas of the facility, with special attention to food and bedding storage. The practice of using a free-roaming cat for the control of wild and escaped rodents is not acceptable except in farm animal facilities, and only under close management.

If insect colonies are kept in or near an animal care facility, there must be regular monitoring of the facility against infestation from escapees. Such insect colonies should be kept behind a screened enclosure or inside an escape-proof container. The use of insecticides must also be compatible with these insect colonies.

4. Holiday and Emergency Care

a) Weekend and Holiday Care of Laboratory Animals is Essential

It should be recognized that changes in personnel and feeding and cleaning schedules, as can occur during these periods, are known to be stressful to routine-oriented animals (Beaver, 1981).

b) Animal Care is a Continuous and Daily Responsibility

This point should be emphasized in job descriptions for animal care personnel and in union contracts. Basic animal care should be categorized as an **"essential service"** and a clause to this effect should be included in all collective agreements, and should not be subject to interruption through strike action. Staff must be provided for weekends and holidays, and skilled assistance must be available in the event of an emergency.

The names and telephone numbers of staff responsible for the animals should be given to security personnel. Some institutions may also choose to have contact telephone numbers posted prominently in the facility. In either case, directions for contacting responsible animal care staff must be made available in the facility. All the animal care staff should be informed of their responsibilities in emergency situations.

The CCAC suggests use of the following:

Essential Services Provision

To be inserted near the Strikes and Lockouts clause:

Clause

"*Designation of Employees to Care for Research Animals*

The parties agree that proper care* of all research animals** will be maintained by the members of the bargaining unit in the event of a strike or lockout in the course of this Agreement or its continuance.

At least seven days before the commencement of a strike or lockout, the employer will designate and identify a number of employees which it deems sufficient to provide for continuous proper care of the animals during the strike or lockout. A list of the names will be delivered to the Union and the parties agree to meet with a view to executing a formal agreement with respect to the employees affected. Should the parties be unable to reach agreement on the persons to be designated, the matter will be referred to the CCAC, for final and binding resolution by the Council.

All persons so designated will be paid their regular salary during the period of designation.

Due regard will be had for previously arranged vacations and other matters and as far as possible the designated duties will be dispersed among all appropriate employees equally. No other duties will be assigned to these designated employees.

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* Proper care implies provision of appropriate temperatures, humidity, light cycles, ventilation, food, water and cleaning as well as
exercise and nursing care where appropriate.

** Research animals means any live non-human vertebrate or invertebrate utilized in research, teaching and testing."

E. REFERENCES

AGRICULTURE CANADA. Publication 1771/E. Recommended code of practice for care and handling of pigs. Communications Branch, Agriculture Canada, Ottawa, Ont., K1A 0C7. 1984.

IBID. Publication 1898/E. Recommended code of practice for the care and handling of farm animals--pigs. In Press.

IBID. Publication 1821/E. Recommended code of practice for the care and handling of special fed veal calves. 1988.

IBID. Publication 1757/E. Recommended code of practice for the care and handling of poultry from hatchery to processing plant. 1989.

IBID. Publication 1853/E. Recommended code of practice for the care and handling of dairy cattle. 1990.

IBID. Publication 1870/E. Recommended code of practice for the care and handling of farm animals--beef cattle. 1991.

BALL, D.J., ARGENTIERI, G., KRAUSE, R. *et al.* Evaluation of a microchip implant system used for animal identification in rats. Lab. Anim. Sci. 1991; 41(2): 185-186.

BEAVER, B.V. Behavioural considerations for laboratory dogs and cats. Compend. Cont. Ed. 1981; 2: 212-215.

BELL, T.G., FARRELL, R.K. and PADGETT, G.A. Ataxia, depression and dermatitis associated with the use of Dichlorvos impregnated collars in the laboratory cat. J. Am. Vet. Med. Assoc. 1975; 167: 579-586.

BLOCK, S.S., ed. Disinfection, sterilization and preservation. 2nd Ed. Philadelphia, PA: Lea and Febiger, 1983.

BUREK, J.D. and SCHWETZ, B.A. Considerations in the selection and use of chemicals within the animal facility. Lab. Anim. Sci. 1980; 30: 414-419.

CAMPBELL, S.A. Effects of exercise programs on serum biochemical stress indicators in purpose-bred beagle dogs. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 77-80.

CASTOR, G.B. and ZALDIVAR, R.A. Tattooing rabbits' ears for identification. Lab. Anim. Sci. 1973; 23: 279-281.

CAUDILL, C.J. and GADDIS, S.E. A safe and efficient handling device for wild rodents. Lab. Anim. Sci. 1973; 23: 685-686.

CLARK, J.D. Research studies in exercise and behaviour of dogs. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 61-64.

DAMON, E.G., EIDSON, A.F., HOBBS, C.H. and HAHN, F.F. Effect of acclimation to caging on nephrotoxic response of rats to uranium. Lab. Anim. Sci. 1986; 36: 24-27.

ECKSTEIN, E.C., MORAN, D., GOMEZ, E., POMERANZ, M.L., BLOCK, N.L. and KLINE, J. A method for tethering dogs in a run. Lab. Anim. Sci. 1987; 37: 234-235.

FALL, M.W. Use of red light for handling wild rats. Lab. Anim. Sci. 1974; 24: 686-687.

FIDLER, I.J. Depression of macrophages in mice drinking hyperchlorinated water. Nature 1977; 270: 735-736.

FOX, M.W. Canine behavior. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 21-28.

FROST, W.W. and HAMM, T.E. Jr. Prevention and control of animal disease. In: Rollin, B.E., ed. The experimental animal in biomedical research. Boca Raton, FL: CRC Press, 1990; 1: 133-152.

GARCIA, M.M., BROOKS, B.W., STEWART, R.B., DION, W., TRUDEL, J.R.J. and OUWEWKERK, T. Evaluation of gamma radiation levels for reducing pathogenic bacteria and fungi in animal sewage and laboratory effluents. Can. J. Vet. Res. 1987; 51: 285-289.

HALL, J.E., WHITE, W.J. and LANG, C.M. Acidification of drinking water: its effects on selected biological phenomena in male mice. Lab. Anim. Sci. 1980; 30: 643-651.

HALLS, N.A. and TALLENTIRE, A. Effects of processing and gamma irradiation on the microbiological contaminants of a laboratory animal diet. Lab. Anim. 1978; 12: 5-10.

HARRISON, S.K. and MAHNKE, C. Selection and use of disinfectants and sterilants. AALAS (Am. Assoc. Lab. Anim. Sci.) Bull. 1991; 30(2): 10-14.

HAYDEN, P. (letters to the editor) Identification methods for rodents. Lab. Anim. Sci. 1974; 24: 428.

HEALTH AND WELFARE CANADA/MEDICAL RESEARCH COUNCIL. Cat. No. MR 21-1/1990/E. Laboratory biosafety guidelines. Ottawa, Ont.: Office of Biosafety, Laboratory Centre for Disease Control, Health and Welfare Canada, 1990.

HERMAN, L.M., WHITE, W.J. and LANG, C.M. Prolonged exposure to acid, chlorine or tetracycline in the drinking water: effects on delayed-type hypersensitivity, hemagglutination titers and reticuloendothelial clearance rates in mice. Lab. Anim. Sci. 1982; 30: 603-608.

HITE, M., HANSON, H.M., BOHIDAR, N.R., CONTI, P.A. and MATTIS, P.A. Effects of cage size on patterns of activity and health of beagle dogs. Lab. Anim. Sci. 1977; 27: 60-64.

HUGHES, D.E., KASSIM, O.O., GREGORY, J., STUPART, M., AUSTIN, L. and DUFFIELD, R. Spectrum of bacterial pathogens transmitted by Pharaoh's ants. Lab. Anim. Sci. 1989; 39: 167-168.

HUGHES, H.C. and CAMPBELL, S. Effects of primary enclosure size and human contact. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 66-73.

INSTITUTE FOR LABORATORY ANIMAL RESOURCES (Committee on Laboratory Animal Records, 1984). Washington, DC: ILAR/National Academy of Sciences, 1990.

IWAKI, S., MATSUO, A. and KAST, A. Identification of newborn rats by tattooing. Lab. Anim. 1989; 23: 361-364.

KEENE, J.H. and SANSONE, E.B. Airborne transfer of contaminants in ventilated spaces. Lab. Anim. Sci. 1984; 34: 453-457.

LEVINE, J.F. and LAGE, A.L. House mouse mites infesting laboratory rodents. Lab. Anim. Sci. 1984; 34: 393-394.

MALATESTA, P.F. and SCHWARTZ, L.H. Use of a chemical tracer to evaluate water movement through two automatic watering rack manifolds during flushing. Lab. Anim. Sci. 1985; 35: 89-91.

MAERKI, U., ROSSBACH, W. and LEUENBERGER, J. Consistency of laboratory animal food following incubation prior to autoclaving. Lab. Anim. 1989; 23: 319-323.

MCINTOSH, J. and STALEY, E.C. (moderators) Limitations of food and water deprivation. In: Guttman, H. N., Mench, J.A. and Simmonds, R.C., eds. Science and animals: addressing contemporary issues. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1989.

NEWELL, G.W. The quality, treatment and monitoring of water for laboratory rodents. Lab. Anim. Sci. 1980; 30: 377-383.

NEWTON, W. An evaluation of the effects of various degrees of long-term confinement on adult beagle dogs. Lab. Anim. Sci. 1972; 22: 860-864.

OLLER, W.L., GREENMAN, D.L. and SUBER, R. Quality changes in animal feed resulting from extended storage. Lab. Anim. Sci. 1985; 35: 646-650.

OLSON, M.E., MORCK, D.W. and NABROTZKY, V.C.A. Manual of standard operating procedures for animal facilities, 1992. Available from: CALAS/ACTAL National Office, Bioscience Animal Service, M524 Biological Sciences Building, University of Alberta, Edmonton, Alberta, T6G 2E9 CANADA.

ORCUTT, R.P. Evaluation of disinfectants and sterilants. AALAS (Am. Assoc. Lab. Anim. Sci.) Bull. 1991; 30(2): 15-17.

RIEGER, D. and BERIAULT, R. A microcomputer based program for the storage and retrieval of experimental animal information. Lab. Anim. Sci. 1983; 33: 390-394.

SCHOEB, T.R., DAVIDSON, M.K. and LINDSEY, J.R. Intracage ammonia promotes growth of *Mycoplasma pulmonis* in the respiratory tracts of rats. Infect. Immun. 1982; 38: 212-217.

SMALL, D.J. Rodent and lagomorph health surveillance - quality assurance. In: Fox, J.G., Cohen, B.J. and Loew, F.M., eds. Laboratory animal medicine. Toronto, Ont.: Academic Press, 1984: 709-723.

STONEHOUSE, B., ed. Animal markings: Recognition marking of animals in research. London and Basingstock: McMillan Press Ltd., 1978.

THIBERT, P. Control of microbial contamination in the use of laboratory rodents. Lab. Anim. Sci. 1980; 30: 339-349.

TOBER-MEYER, B.K. and BIENIEK, H.J. Studies on the hygiene of drinking water for laboratory animals. 1. The effect of various treatments on bacterial contamination. Lab. Anim. 1981; 15: 107-110.

TOBER-MEYER, B.K., BIENIEK, H.J. and KUPKE, I.R. Studies on the hygiene of drinking water for laboratory animals. 2. Clinical and biochemical studies in rats and rabbits during long-term provision of acidified drinking water. Lab. Anim. 1981; 15: 111-117.

TOTH, L.A. and JANUARY, B. Physiological stabilization of rabbits after shipping. Lab. Anim. Sci. 1990; 40: 384-387.

VAN HOUTON, J. and HAYRE, M.D. Disinfectants and sterilants: their chemistry, use and evaluation. AALAS (Am. Assoc. Lab. Anim. Sci.) Bull. 1991; 30(3): 24-27.

WASSERMAN, R.S., BLUMRICK, K. and LIDDELL, R.W. III. An automated interactive animal colony management system. Lab. Anim. Sci. 1982; 32: 550-552.

WEIHE, M.H. The laboratory rat. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. New York, NY: Churchill Livingstone, Inc. 1987: 309-330.

VI. SOCIAL AND BEHAVIOURAL REQUIREMENTS OF EXPERIMENTAL ANIMALS

A. INTRODUCTION

In the past, emphasis has been directed towards providing adequate caging for experimental animals in order to contain them hygienically, to facilitate husbandry, and minimize (husbandry) variables. However, increasing importance is now being placed on reducing the animal's stress, and improving its social and behavioural well-being. Provision of varied environmental enrichment may or may not result in increased cost of operation; however, it is considered that there is often immediate benefit to the animal and ultimately to the researcher and the research.

This chapter contains general principles rather than specifics. It is not infallible. Nor should it be taken literally at the expense of the animal (for example, it may be found that an otherwise desirable environment or social grouping is not suitable for an individual animal). This chapter, including a CCAC-approved policy statement, will receive continuing review, with changes made as needed. The ability to treat animals in the way we would wish requires sensitive and conscientious applications of knowledge. Critical, rigorous, scientific endeavour is required of us all in order to reach this goal. Management and housing situations that fulfil the animal's behavioural requirements should be interpreted as providing an ideal toward which we should aim.

1. What is Animal Well-being or Welfare?

Animal welfare is described by Broom (1986) as an animal's "state as regards its attempts to cope with its environment". Blood and Studdert (1988) define it as "maintaining appropriate standards of accommodation, feeding and general care, the prevention and treatment of disease..." The American Veterinary Medical Association (AVMA) enlarges on this to include "all aspects of animal well-being, including proper housing, management, nutrition, disease prevention and treatment, responsible care, humane handling, and, when necessary, humane euthanasia" (Anon., 1990).

Fraser (1989) notes that animal well-being encompasses "both the physical and psychological. These normally coexist. Physical well-being is manifested by a state of clinical health. Psychological well-being is reflected, in turn, in behavioural well-being. The latter is evident in the presence of normal behaviour and the absence of substantially abnormal behaviour."

The World Veterinary Association (WVA) states that animal ethology "puts the emphasis on knowledge which is scientifically based. Its aim is to clarify: a) needs that can be filled; and b) harm that can be avoided..." (WVA, 1989).

Hurnik (1988) defines animal well-being as "a state or condition of physical and psychological harmony between the organism and its surroundings." However, it is agreed that animal welfare is not a single phenomenon, and that on one definition will satisfy everyone (Moberg, 1992; Baxter, 1993; Duncan and Dawkins, 1983).

The Royal Society for the Prevention of Cruelty to Animals (RSPCA) has recently recognized the need to draw attention to stress in experimental animals and the necessity for alleviating this condition whenever it is associated with suffering (RSPCA, 1992). An annotated bibliography on animal welfare has recently been prepared (Murphy, Rowan and Smeby, 1991).

One must always remember, as well, Hollands's sensitive definition written more than a decade ago: "This then would be my definition of animal welfare: dignity-according to animals the natural dignity

which is due them as living, sentient creatures" (Hollands, 1980).

2. Environmental Enrichment

Environmental enrichment is defined by Beaver (1989) as "additions to an animal's environment with which it can interact."

As a general rule, most experimental animals are social animals and benefit from the company of conspecifics or humans. As well, predictability in interactions usually enhances the animal's well-being, while the opposite results from frequent regrouping and restabilizing.

It should be remembered that an animal's experiences during the developmental phases determine social behaviour. Therefore, conditions of animal holding in a breeding facility will impact on the animal's future well-being.

The social needs of animals used in research, teaching, or testing, should be given equal consideration with environmental factors such as lighting, heating, ventilation and containment (caging). Particularly in the case of singly housed animals, daily observation provides an alternative form of social contact for the animal and commonly facilitates handling in that the animal becomes accustomed to the human presence.

A more complex environment, use of artificial appliances, and better use of existing space enhance stimulation. Simply increasing the number of square inches or centimetres available to an animal does not promote better space utilization (Line, 1987; Fajzi, Reinhardt and Smith, 1989); however, the amount of space should be appropriate to the species. In group housed animals, the size of the social group in relation to its available space should be regularly appraised.

3. Group Formation

When animals are introduced to each other and pairs or groups are established, there is an initial period during which they work out their social relationships (dominance ranks, etc.). There may be aggressive interactions; however, when conditions are right, the social organization will stabilize. Once the hierarchy has been established the interactions are subtle, and based more on avoidance or ritualized threat than overt aggressive action. If their daily routine is disrupted, if resources such as food or resting spaces are limited, or the animals are poorly grouped, the hierarchy becomes disestablished and the number of aggressive interactions increases. The animal's well-being is threatened when:

a) space is insufficient for maintaining behaviourally adequate distance;

b) feeding or resting space for all individuals is insufficient; or when feeding and resting cannot be accomplished concurrently;

c) regrouping is performed so frequently that animals must repeatedly undergo the stabilization process; and

d) group sizes are inappropriate for the species.

The above statement challenges intense confinement practices which prohibit animals from engaging in their normal social-behavioural activities.

In addition to sufficient primary space for resting, animals also need what could be called secondary

space, for freedom of movement at their own will. An important exception may occur at the time of parturition, when most individual animals should be given their own quarters.

Most animals should not be housed singly unless required by medical condition, aggression, or the dictates of the study. Singly housed animals should have some degree of social contact with others of their own kind. For most species, at the very least there should be potential visual contact. Olfactory and auditory contact with other animals is also usually desirable.

Protocols which involve single housing must describe proposed measures for meeting the social requirements of the isolated animal (e.g., where appropriate, increased positive human contact). Investigators must justify any deviations from the CCAC guidelines before an Animal Care Committee (ACC) and receive its approval, before any study can begin. All protocols must be reviewed at least annually by the ACC.

4. Position Statement

"**SOCIAL AND BEHAVIOURAL REQUIREMENTS OF EXPERIMENTAL ANIMALS (SBREA)**

Well-being in animals has two components: physical and behavioural. Physical well-being is manifested by a state of clinical health. Behavioural well-being is manifested by behaviour considered to be normal for that species and strain, together with the absence of significantly abnormal behaviour. Behavioural well-being is considered to reflect psychological well-being, and to that extent, the terms are considered to be synonymous in our usage.

In the interest of well-being, a social environment is desired for each animal which will allow basic social contacts and positive social relationships. Social behaviour assists animals to cope with circumstances of confinement. Caging, whether for single animals, pairs, or groups, should be enriched appropriately for the species.

It is necessary to recognize affiliations which commonly occur within and between species. Chronic isolation as a method of accommodation, should not normally occur. However, in exceptional circumstances, and with clear scientific and biological justification, some animals may be better kept alone. Positive interactions with human-beings are important in some species, and particularly in conditions of social isolation. Some individuals seem more readily accepted by animals than others; this concept should be used to maximize the benefits of these affiliations.

February, 1990"

B. ANIMALS USED IN AGRICULTURAL RESEARCH

1. Introduction

The report of the Brambell Commission in 1965, described the farm animal's "Five Freedoms" as the ability to easily "turn around, groom itself, get up, lie down, and stretch its limbs" (Brambell, 1965). In 1989, the WVA adopted its own Five Freedoms, which applied to all species and which were based on those of Britain's Farm Animal Welfare Council (FAWC) (Webster, 1987). The FAWC has recently revised these Five Freedoms, which define ideal states and which now include:

a) Freedom from hunger and thirst;
b) Freedom from discomfort;
c) Freedom from pain, injury and disease;
d) Freedom to express normal behaviour;
e) Freedom from fear and distress (Seamer, 1993).

Carpenter (1980) suggested enough freedom to perform natural physical movement, including daily routines of natural activities; facilities for comfort activities such as rest, sleep, and body care; adequate food and water to maintain full health; social contact with other animals of their own kind; opportunity for exploration and play, especially in young animals; and satisfaction of minimal spatial and territorial requirements.

The necessary scientific knowledge upon which to base a comprehensive set of guidelines which would fully protect the welfare of agricultural animals is not yet available (Expert Committee on Farm Animal Welfare and Behaviour, 1987). Nonetheless, the CCAC believes that there is sufficient information to set out some general principles that can be updated and expanded as

Codes of Practice for Canada for pigs, veal calves, poultry, dairy cattle and beef cattle (Agriculture Canada, 1771/E, 1984; 1821/E, 1988; 1757/E, 1989; 1853/E, 1990; 1870/E, 1992) represent the basic industry standards and, for the most part, are the minimum CCAC requires for research institutions undertaking agricultural research. In addition, a revision of the Recommended Code of Practice for the Care and Handling of Farm Animals--Pigs (1898/E) is now in press. Researchers and others working with agricultural animals must be fully conversant with these codes.

The Health of Animals Act (Government of Canada, 1990), which replaced the Animal Disease and Protection Act, states that the "Governor-in-Council may make regulations:

a) for the humane treatment of animals and generally:

i) governing the care, handling and disposition of animals;

ii) governing the manner in which animals are transported within, into or out of Canada; and

iii) providing for the treatment or disposal of animals that are not cared for, handled or transported in a humane manner."

Curtis (1992) suggests that there is a double standard in place regarding current farm practices and the procedures used with agricultural animals in biomedical research. This is a dilemma not uncommonly faced by ACCs. Another dilemma occurs because agricultural animals are used in food and fibre-related research, as well as biomedical research, and it is often difficult to clearly label such studies as being only agricultural or only biomedical (Stricklin, Purcell and Mench, 1992).

In attempting to develop guidelines, thought, observation, and concern for the animal must be uppermost in our minds for, as Hurnik (1988) states: "There is a need to exercise extreme care to avoid the emotional tendency to rely exclusively on characteristics which may be of concern to humans, but that are not necessarily central to the overall quality of animal life." Spedding (1988) also warns that "perhaps the biggest danger is that a dissatisfied public may demand changes that eliminate what is disliked, but result in either no improvement or even a reduction in the welfare of the animals involved."

In that we must "work to ensure the animal's welfare in life and at the point of death" (Webster, 1987), we might note a recent example of change resulting from human perception of what constitutes humaneness, when Great Britain passed Slaughter of Animals (Humane Conditions) Regulations 1990 (effective July 5, 1992), requiring head restraint during slaughter of cattle. Unfortunately, after the fact, research showed, by measuring cortisol levels, that the restraint process was far more stressful than allowing the animal to stand free when the captive bolt was fired (Ewbank, Parker and Mason, 1992).

2. Animal Stress

Suffering, loosely defined as experiencing a wide range of unpleasant emotional states such as pain, fear, anxiety, frustration and perhaps boredom, can be a major threat to an animal's welfare. Poor animal welfare may be apparent in changes in an animal's behaviour, physiology, health status, reproduction or growth. Many clinical conditions in animals first become apparent to observers through a set of behavioural indicators (Fraser, 1984/85). Depressed animals show a depletion of the behavioural repertoire characteristic of the normal animal (Fraser, 1984/85, 1988).

The three ways in which an animal responds to a stressful situation include changes in behaviour, activation of the autonomic nervous system, and activation of the neuroendocrine system. Because of its rapid and specific responses to many stressors, the autonomic nervous system has been an appealing candidate for the diagnosis of stress by measuring heart rate, respiration, and the secretion of catecholamines. Many investigators accept the increased secretion of glucocorticoids as proof of stress occurring. It has been demonstrated that stress associated with transportation, restraint or handling diminishes immune function in a number of livestock species (Grandin, 1992; Kelley, Osborne, Evermann *et al*. 1981; Coppinger, Minton, Reddy *et al.* 1990).

However, Moberg (1985) states that monitoring these autonomic nervous system and endocrine system responses outside the laboratory has not been practical, and thus they have proven to be of little use in defining stress and well-being in domestic animals. Duncan (1992) notes that sophisticated biological systems have evolved to help animals cope with stress, and that, while it is impossible for us to shield agricultural animals from all stress, the key to protecting their well-being is to minimize the biological costs of unavoidable stress, and to recognize the need for research directed at stress.

The idea that animals have certain "behavioural needs" has received considerable attention. For example, Baxter (1983) has argued that all psychological processes which affect animal welfare must have a desired state or set point (or set band covering a range of values) at which the animal will strive to remain, or to which it wishes to return. Deviations from this set point will cause a reduction in the animal's well-being. However, Hughes and Duncan (1988) reviewed the literature in this area and showed that, in certain cases, an argument could be made for animals needing to be able to perform particular behaviours, even if the goal of the behaviour has already been provided to the animal.

A behavioural need will appear in the cases of behaviour motivated mainly by internal factors or by a complex interaction between internal and external factors where the performance of the pattern is constrained. For example, a hen provided with a replica of a nest is still motivated to perform normal nest building behaviours (Hughes, Duncan and Brown, 1989).

3. Housing and Husbandry

Three major factors influence welfare in animal agriculture: a) housing; b) stockmanship; and, c) management (Hurnik, 1988). Hughes and Duncan (1988) suggest that, in order to fully protect welfare, a housing system would have to allow the performance of certain behaviour patterns in addition to providing all the environmental needs of the animals. However, which behaviour patterns are essential has yet to be elucidated, and research is being undertaken to answer this question (e.g., Dawkins, 1990).

In the U.S., the American Association for the Accreditation of Laboratory Animal Care (AAALAC)(1991), takes the position that, in accredited facilities, housing and care for farm animals should meet the standards that prevail on a high quality, well-managed farm.

In maintaining farm animals in the laboratory, it is important to be aware of their behaviour in a farm situation. A prominent feature in farm animal behaviour is the active way in which individuals associate with each other and form social groups (Mench, Stricklin and Purcell, 1992; Fraser and Broom, 1990; Grafen, 1990). Isolation from conspecifics is a profound source of stress (Grandin, 1992; Gross and Siegel, 1981), as social animals obtain both physical and physiologic comfort from each other (Friend and Dellmeier, 1988; Van Putten, 1988).

Proper handling and restraint of farm animals in a laboratory environment are key elements to their success as research animals. Prior familiarizing of experimental animals to handlers and experimental procedures can be of considerable benefit in the laboratory, says Panepinto (1992), the developer of the Panepinto sling used to restrain miniature swine and sheep. Stroking the pig, as opposed to stressing it, has been shown to have a beneficial effect on reproduction (Panepinto, 1992; Anon., 1992).

Animals raised in a barren, non-stimulating environment may display stereotypies such as bar biting, cribbing or tongue rolling in horses (Van Putten, 1988; Fraser and Broom, 1990; Fraser, 1992). Environmental enrichment will often reduce unwanted excitability and help prevent abnormal behaviour (Grandin, 1992). For example, in housing domestic poultry used in experimentation, nest boxes and perches (high and low) should be provided. Aquatic fowl should have access to a swimming facility or some form of wetting in clean water.

Animals should be housed singly only when the experimental procedure requires this; e.g., in metabolism studies, some infectious disease studies or nutritional research.

When animals are introduced to each other and a group is established, there is an initial period during which the animals often fight in an effort to work out their social relationships (McGlone and Curtis, 1985; Fraser and Rushen, 1987; Fraser and Broom, 1990; Mench, Stricklin and Purcell, 1992). Subsequently, dominance-subordinate relationships continue; however, interactions are subtle, based more on avoidance or ritualized threat. Animals should be regrouped as infrequently as possible, so that they need not repeatedly endure the stabilization process (Fraser and Broom, 1990; Kenny and Tarrant, 1982).

In housing domestic species, Mench, Stricklin and Purcell (1992) suggest that there should be enough space for maintaining some minimum separation from each other, equal access to feed and water, and the ability to engage in significant behaviours and make normal postural adjustments. However, it has been demonstrated that when animals are group housed, the area available to a particular animal does not consist just of its individual space, but instead the entire area in which the animal is enclosed. Therefore, the space requirements per animal are greater when one houses one or few individuals (Stricklin, Purcell and Mench, 1992).

The animal should also receive, on a regular and substantial basis, attention from an attendant trained to deal with the species (Kilgour and Dalton, 1984). It is noted that the domestication of various species has depended on their capacity for social affinity with humans (Gross and Siegel, 1982; Gonyou, 1991). Substantial periods of observation must be maintained by the personnel responsible for the well-being of these animals (e.g., animal attendants, veterinarians) and adequate provision made in the work-time schedule to permit regular appraisal of the balance between social group and space.

A stockperson's quality will depend on his or her ethical sensitivity, familiarity with the animals, skill in interpreting behavioural symptoms indicating deprivation, suffering and morbidity, and the care shown in handling animals. The stockperson's skill in carrying out particular tasks such as castration, injecting animals, clipping teeth, etc., is also very important. Objective studies are now being carried out to identify the characteristics of good stockmen (Seabrook, 1984, 1987; Hemsworth, Barnett, Coleman *et al.* 1989). The quality of management refers to such things as decisions about operation of ventilation systems, provision of food and water, provision for emergencies, provision of sanitation and prophylactic measures, and choice of techniques and procedures for castration, dehorning, giving injections, etc.

The quality of a housing and husbandry system can affect welfare in many different ways. It can, of course, act in a direct physical way by causing injury, by reducing health, or by providing climatic conditions which are far from optimal. It may also reduce welfare by affecting the behaviour, or the physiological and immune systems of the animals.

Duncan (1981, 1983) has proposed the following classification for physical and social effects of husbandry systems.

a) Physical Effects

Aspects of the physical environment provided may reduce welfare by altering the animal's behaviour by: 1) blocking or frustrating the performance of a particular activity; 2) failing to provide the specific releasing stimuli necessary for eliciting certain behavioural patterns; and 3) providing too high a level of general stimulation. For example, if the environment is too complex or keeps changing in a way that the animals cannot predict, the animals may then become fearful and anxious. Alternatively, if the environment is too barren and monotonous, the level of general stimulation may be too low, leading to boredom. While it is not easy to measure fear and boredom in a scientific way, we must not assume that animals do not experience these emotions.

b) Social Effects

Husbandry systems can also influence the behaviour and welfare of animals by altering and controlling the animals' social environment. All of the common agricultural species are gregarious, which means that an inadequate social environment can be expected to reduce welfare. Compared to what might be considered as "normal" or "natural," many husbandry systems often deviate in the following ways: 1) the parent-offspring bond may be disrupted or prevented from forming; 2) young animals may be weaned too early; 3) animals may be kept in groups that are too large or small; 4) animals may be kept at too high a density; 5) animals may be kept in single-age or single-sex groups; 6) group membership may be disrupted; and 7) animals may be isolated to some extent.

4. General Principles

In order to improve and enhance the animal's environment, the CCAC encourages research institutions:

a) to experiment with group housing for such animals as lactating sows (after a few days in farrowing crates), calves, dairy cows and sheep. It is recognized that group housing may lead to increased aggression or bullying among animals, increased chance of disease transmission, and increased difficulty in detecting health problems of individual animals. *However, unless the welfare or safety of an animal is in danger, these facts should not be used to reject group housing*. The level of animal management will probably have to increase and the training required for stockpersons will have to change. Evidence is accumulating that new piggeries should not have individual stalls for gestating gilts or sows unless required for experimental purposes (Barnett, Winfield, Cronin *et al.* 1985; Barnett and Hemsworth, 1991; Becker, Ford, Christenson *et al.* 1985; Cronin, Van Tartwijk, Van Der Hel *et al.* 1986; Schouten, Rushen and De Passillé, 1991; Von Borell and Ladewig, 1989).

b) to practise environmental enrichment such as providing toys for pigs, "teats" for veal calves, small orifice nipples and frequent feedings for artificially raised lambs, and greater opportunities for animals to perform normal food searching and foraging behaviours.

c) to provide the means for increased social contact and to allow the animals to perform a wider range of behaviours.

d) to increase the age at weaning. For example, piglets weaned at three weeks have an increased incidence of belly nosing (an abnormal behaviour); the recommended minimum age for weaning is therefore four weeks.

e) to shorten periods of isolation and restraint and to use them only when it is absolutely necessary, and not merely for the convenience of the experimenter. Individually penned animals should be allowed to maintain visual contact with at least one other animal while standing or lying in the pen, unless the isolation is required for experimental purposes and has been approved by the ACC. For pigs, in particular, maintaining olfactory contact may be as important as visual contact. In sheep, the head region is the

primary focal point used to recognize each other and should therefore be the least obstructed region from the perspective of neighbouring sheep trying to maintain visual contact.

The Expert Committee on Farm Animal Welfare and Behaviour (1987) suggests that government agencies and universities should re-order their priorities so that research on farm animal welfare and behaviour will have a level of staffing and support typical of that supplied to other disciplines such as nutrition, reproductive physiology, genetics, and food products.

The CCAC recognizes the importance of education in improving animal well-being and welfare. Ideally, all students of animal production and veterinary medicine should receive instruction in farm animal behaviour, animal welfare, and the ethics of livestock production.

C. ANIMALS (LARGE) HELD IN METABOLISM CAGES

The use of metabolism cages or crates necessarily reduces the animal's social and behavioural activities. This procedure should not, therefore, be used merely for the purpose of convenient restraint, but should be reserved for approved metabolic studies. Animals so housed should be under close and expert observation throughout the period of the study.

1. Conditioning

A seven to 10 day conditioning period in a floor pen, to "acclimatize" the animal to a new diet (if this is necessary), and before its placement in the associated metabolism crate, is required, followed by three to four days' adjustment to the crate.

2. Size of Metabolism Crates

Enough space must be provided for the animal to rise and lie down normally. Some animals (e.g., calves and sheep) swing their weight forward when rising; therefore, the required length of crates should be greater than the simple length of the animal. Width of the crates must be sufficient to allow sternal recumbency.

Other postures for example shown by sheep, as well as serving to make the animal comfortable, also have a thermoregulatory function. If the dimensions of a metabolism crate do not permit such postures (e.g., lateral recumbency), then proper temperature and other environmental control becomes a responsibility of the research.

3. Contact with Other Animals

Many animals are highly social. An isolated animal is often not normal behaviourally, nor possibly metabolically. To minimize stress, crates should be designed and positioned so that there is good visual, auditory, and olfactory contact with conspecifics.

4. Pre-, During and Post-Experiment Checks

A physical and behavioural assessment of the animal should be done before, during, and after an experiment. Animal care personnel should observe the animal before and after eating in order to ascertain, for example, if intake has decreased.

5. Observing Changes in Behaviour

Strict attention should be paid to observing changes in behaviour, which can indicate a degree of stress or anxiety, or fear stereotypies (e.g., increased drinking in sheep). Noting such changes is important to good science as well as good animal care.

6. Duration of Confinement

Enforced immobility has a negative effect on bones, joints, and muscles. For this reason, animals should be released for periodic exercise or released from the metabolism crates for at least three hours per seven days.

Any period of study exceeding 21 days in a metabolism crate must be justified to the ACC by the investigator, on grounds of experimental design and scientific merit. However, the total period in the crate should not exceed 30 days.

7. Exceptional Circumstances

In exceptional circumstances (e.g., catheterization studies), it may not be possible to implement the recommended weekly exercise requirement. In such cases, variances to these guidelines require justification by the investigator, and review and approval of the institutional ACC.

D. CATS

1. Introduction

Various authors have proposed schemes to assist those who are attempting to enhance the welfare of research animals. Beaver (1989) proposes five basic methods which can be used to alter an animal's environment so as to permit the animal to live and produce to its full potential. These include behavioural enrichment, social peers, artificial appliances, food gathering activities, and control of the environment.

Spinelli (1989) in commenting on Beaver's five methods, disagrees with her definition of enrichment. However, he notes that there are a variety of strategies that will be useful singly or in combination to promote the psychological well-being of laboratory animals. Spinelli contends that environmental enrichment and an animal's psychological well-being "may be one of the most important areas of study in laboratory animal science over the next few years."

Beaver's five areas will be interpreted in light of recognized behaviour systems for cats (*Felis catus*). Such systems represent species-typical behaviours which are co-ordinated to serve a specific function that has adaptive value (Catcott, 1975). As such, they must all be integrated to a greater or lesser extent in any model which seeks to optimally meet the needs of, and avoid harm to, the animals in our care. These systems include:

Social Behaviour	Sexual Behaviour
Feeding Behaviour	Parental - Offspring Behaviour
Eliminative Behaviour	Comfort Behaviour
Play Behaviour	Resting - Locomotory Behaviour
Exploratory Behaviour	Agonistic Behaviour

Species-typical behaviours which occur in the domestic environment are co-ordinated to serve a specific function that has an adaptive value and therefore should not be a redirected response to some external stressor. For example, spraying is a normal behaviour in the wild environment, but is a sign of conflict behaviour in domestic animals kept in close quarters. An awareness of normal behaviour patterns in the

species is essential in order for care-givers to identify and address abnormal behaviour. Both are extensively addressed by Hart and Pedersen (1991).

In the absence of scientific data to indicate a better management scheme, there is an underlying presumption that mimicking the wild habitat should be highly desirable for captive animals in general. Generally, this is accomplished in a modified fashion for most species, eliminating, for example, such features as predators which the species might encounter in the wild (Beaver, 1989). However, even in this latter regard, Markowitz and Laforse (1987) have discussed artificial prey as behavioural enrichment.

2. Behavioural Enrichment

Behavioural enrichment should, in general terms, foster and promote a full and extensive repertoire of normal behaviour (ethogram) for the domestic animals, whilst preventing the development of abnormal behaviour. The provision of physical stimuli and target objects that will promote the expression of species-typical behaviours should be incorporated into any plan for behavioural enrichment.

It may be possible to assess program success by the extent to which it prevents development of behavioural abnormalities and promotes normal behaviour, or minimizes the expression of or eliminates pre-existing abnormalities that an individual or group displayed prior to enrichment attempts.

The home range of domestic cats varies tremendously based on population density, need (hunger), desire (hunting, mating), and such natural and man-made barriers as rivers, fences, etc. While domestic cats living in rural areas may range over many tens of acres on a daily basis, as urban crowding intensifies, the territory commonly shrinks to a home range of one-fifth of an acre each or less (Morris, 1986).

Although cats have been portrayed as asocial loners (Leyhausen, 1990; Beaver, 1981), some authors now question this contention, as well as how much the social nature is being changed by selective breeding (Morris, 1986; Liberg and Sandell, 1990; Hurni and Rossbach, 1989). Even now, most cats are not highly social because they still need individual space and privacy. However, compatible individuals may share their first order home (house, room or even chair) as well as their home range (backyard, neighbourhood, or acres of farmland) (Morris, 1986; Leyhausen, 1990; Macdonald and Moehlman, 1982).

3. Social Peers

Insofar as "solitary confinement" is considered an unnatural condition for most species (Beaver, 1989), it is necessary to examine the role of conspecifics in promoting well-being and behavioural enrichment in domestic cats. A wealth of anecdotal information suggests that pairs and stable groups are successful alternatives to single cage housing for cats or other species. However, finding scientific data to demonstrate this and other social functions important to behavioural well-being in cats is a challenge.

Beaver (1981) reports that, although the socialization process is not well understood in cats, the critical window for their socialization may end as early as nine weeks of age. Cats weaned at an early age and raised in isolation later displayed excessive undirected activity, disorganized behaviour and fear of novel situations (Seitz, 1959).

Continued socialization with conspecifics is essential to the well-being of developing kittens. Blackshaw (1985a) notes that: "Kittens reared in the absence of other cats from the 7th week on - and so deprived of the possibility of social play - later showed poor control of attack and escape behaviours, sexual and parental encounters."

Similar findings regarding social deprivation have been reported for other species including calves (Broom and Leaver, 1978), rodents (Rosenzweig and Bennett, 1977), and dogs (Scott and Fuller, 1965).

Cats would be best adapted to the research environment when raised and socialized to research facilities and human handlers before seven weeks of age. Continued, regular human contact is also important (Beaver, 1989; Karsh and Turner, 1990) in order to maintain the human socialization. Beaver (1981) notes that excessive handling can be stressful to the unsocialized or asocial animal.

Animal caretaker styles can also affect the behaviour of animals (Beaver, 1981, 1989; Hurni and Rossbach, 1989; Fox, 1986). It is considered that calm, gentle, consistent keepers reduce the stress in a population.

Visual stimuli can also improve behavioural well-being.

In most animals, changes in routine should be avoided or minimized as much as possible. For example, even the introduction of a new technician can change liver enzymes in chimpanzees (Moor-Jankowski and Mahoney, 1989). Hemsworth and Barnett (1987) report that inconsistent behaviour has the effect of increasing pigs' fear of humans.

4. Enrichment Devices (Artificial Appliances)

Having recognized the need for adequately sized, appropriate caging that is clean, safe, secure, and suitably bedded, consideration is now being given to provision of species-appropriate activities through introduction of complexity within the cage space. Enrichment devices would include, for example, toys, scratch posts, climbing apparatus, PVC culverts for privacy and play, etc. Activity can be encouraged by hanging an object that can be swatted or watched, or by providing an object that will roll when batted (Beaver, 1981).

The concept of novelty is an important one when considering play-articles for cats. It is widely reported anecdotally that continued exposure to an item reduces the play value to the extent that the cat quickly becomes indifferent to the article, only to show renewed interest after a brief period of removal.

One must also consider the age of the cats. Kittens require a *variety* of articles with which to play. Play behaviour in kittens occupies almost 10% of total time and is considered to assist in the acquisition of information and skills includes an opportunity to learn the communicative value (message and meaning) of displays, particularly "graded displays" (Blackshaw, 1985a).

5. Food Gathering Activities

Food gathering activities can be manipulated to foster environmental and behavioural enrichment. Unfortunately, although much has been written about the food gathering activities of non-human primates (NHP), there is a relative dearth of literature for domestic cats. Until recently, it was assumed that if an animal had adequate supplies of nutritious food and clean water, its "feeding" needs were met. Clearly, this approach denies the complex range of behaviour exhibited by cats as part of a predator's feeding behaviour system, which includes, but is not limited to searching, chasing, catching, killing and consuming prey (food). Four of these five behaviours are redundant for animals provided with ample nutritious food, and they are deprived of the opportunity to express such behaviours.

The diet of feral cats includes small rodents, birds, etc., which have partially digested vegetable material in their intestinal tracts. The cravings many cats have to consume small amounts of grass, house plants, etc., may reflect a craving for plant material in a more natural form than as commercial pet food (Leyhausen, 1990; Beaver, 1981; Blackshaw, 1985b; Beaver, 1980). This need could be met in a variety of ways (e.g., by providing small amounts of fresh grass or other safe plant, or cooked vegetables which can be ingested without causing gastric irritation).

A cat's appetite may be affected by lighting and noise level, by the presence or absence of people, by the

type and cleanliness of the container, and by the presence or absence of other cats (Scott, 1975).

Preference tests have shown that cats prefer their food at 30C (86F) (McKeown and Luescher, in press). Although it may not be possible or necessary to incorporate this documented preference in all feeding regimes, such knowledge forms the basis for enhanced care for individuals undergoing unusual stresses, (e.g., partial anorexia in the immediate post-operative period), or when introducing new individuals to a group, etc.

Most cats generally do not like to eat from narrow, deep bowls; however, some will only drink by dipping a paw in such a bowl and licking the foot. If an animal will not eat from a container on the floor, the bowl may be placed on the perch.

Some cats prefer fresh, clean water that has been allowed to stand for a time to permit the dissipation of chemical odours that result from water treatment. Other cats refuse to drink unless from a running source, such as a dripping faucet. Many cats are loath to eat or drink from any container which is contaminated with odour or saliva from another cat. Obviously these factors and countless others are significant in providing the optimal housing for cats under a variety of caging conditions.

Idiosyncrasies of behaviour which reflect the fastidious nature of felines should be considered in any effort to provide for their social and behavioural needs. For example, texture is important to cats: the texture of their food can affect appetite; the texture of resting and sleeping locations can determine preferences in this regard.

Many cats will not use a litter box that has been soiled by another cat. Indeed, many cats even have preferences for certain textures or types of litter and will not use others. Even the location of the food, water, and litter containers relative to each other, resting areas, doors, etc., can have a significant impact on the well-being of cats.

6. Control of the Environment

Assumptions are widely held that an animal's well-being is enhanced by affording some degree of control over its environment (Line, 1987). Whether this is expressed in the negative language of "reducing stress" or the positive language of "environmental enrichment" we continue to seek ways to allow animals to express their individual needs or desires. Radio broadcasts played during working hours make cats less frightened by sudden noises and more easily accustomed to strange human voices (Hurni and Rossbach, 1989).

The existence of a range of temperatures within the enclosures allows for an individual to seek preferred resting spots. Provision of safe indoor/outdoor enclosures permits further freedom of choice and therefore some degree of control. Choice of texture, height, temperature, and degree of enclosure are examples of a few methods of environmental enrichment for animals. Cats are noted for their enjoyment of a warm, sunny sleeping location.

Height (as provided by perches, for example) is also a desirable factor for many cats when seeking a resting spot (Beaver, 1989; Blackshaw, 1985a). In group housing, access to the individual's preferred vertical niche, with sufficient elevated resting places for all cats, will enhance the enrichment program.

Some prefer a dark, secluded spot for rest (Beaver, 1981); others may choose to sleep closely by another member of the group. Anecdotally, it is noted that some cats prefer to sleep on (sanitizeable) fleeces and soft blankets.

Many anecdotal observations regarding cats have been well documented in other species. For example, Chamove and Anderson (1989) report that such arboreal species of monkeys as the callitrichids rarely

come to ground in the wild. Furthermore, they state that in captivity these monkeys almost never visit a bare floor (1% of the time); however, the time spent on the ground increases ten-fold if contact bedding such as a leaf-like substrate is used. Obviously, since the floor may encompass as much as 40% of the total usable surface area and more than 60% of the horizontal surface area, addressing the issue of texture preference can provide a potent tool for environmental enrichment for many species.

Both predictability and controllability are important variables to reduce stress. Therefore, when controllability cannot be provided, allowing the animal some degree of predictability is one coping strategy which should enhance well-being (Beaver, 1989).

The concept of predictability must be interpreted in light of species-typical behaviours. For example, while some species such as higher primates may respond positively to changes in time and content of their daily feedings (Line, 1987), other species can experience unnecessary distress from alterations to their routine. Changes in routine which may, for example, occur during weekend feeding and cleaning schedules, are known to be stressful in routine-oriented animals (Beaver, 1981).

7. Housing

The least stressful housing environment for research cats will usually be gang housing, especially if there are numerous perches on which individuals can rest (Beaver, 1989). When establishing pairs or small groups, compatibility can first be determined by observing which animals sit near each other.

Group pens or cages should be provided with adequate, usable, vertical space (e.g., shelves, or a tree-like structure with platforms). If kittens are present, access to higher levels can be provided through the use of a slanting board or similar object. Group housed cats prefer warmed floor areas on which to sleep (McKeown, Pers. Comm., 1990).

The housing should provide an area for eating and for elimination. In the latter regard, provision of a number of litter boxes can reduce the possibility of refusal by individual animals to use a particular box.

Aggression can occur between adjacent, individually housed animals. Provision of a dark, secluded hide-out (e.g., box) can allow a retreat from this type of stressful environment (Beaver, 1981). [This also proved beneficial in NHP which were provided with a privacy panel (Reinhardt, 1990).]

If animals must be singly housed for an experiment, then where possible and appropriate, they should be group housed with their original conspecifics between studies. Females are considered more appropriate for long-term holding, as they are generally more amiable towards one another and can be kept in groups after several days of limited exposure (Hurni and Rossbach, 1989).

If possible, all animals not in large pens should be exercised daily, unless contra-indicated for health or experimental protocol reasons.

Some authors (e.g., Hurni and Rossbach [1989]), suggest that intact males be housed separately by four to six months unless they remain with litter mates and no stranger is introduced. However, Taylor (Pers. Comm., 1990) reports long-term success with several colonies of intact males housed in groups of 6-14. New individuals, carefully introduced, caused minimal disruption and in more than two years of observation only three to four episodes of aggression greater than ritual display were noted. None resulted in serious injury; however, in each of those cases it was only necessary to remove one individual to restore harmony.

Asocial individuals should also be singly housed, as the biggest stress to these cats is other cats. In addition, intact male cats not kept in a breeding harem, cats recuperating from surgery, and research animals being conditioned to a cage, are usually singly caged; however, cats can form bonds. This can be

illustrated sometimes by the manifestation of separation anxiety (McKeown, Pers. Comm., 1990).

8. Maternal Behaviour

The duration of gestation in the cat ranges from 60 to 68 days, with an average of 65 to 66 days. During approximately the last third of pregnancy, obvious behavioural changes occur, although some queens have already been showing increased docility. Along with a rapid weight gain, due primarily to fetal growth, there is an accompanying increase in appetite, decrease in activity, and decrease in agility. Distention of the mammae may also occur.

In the week immediately preceding parturition, the queen will seek a dark, dry area where she can remain relatively undisturbed. A nesting box fills this need. During this period before delivery, the queen usually spends an increasing amount of time in self-grooming, particularly of her mammary and perineal areas. She may also become more irritable or defensive, possibly as a result of the extreme stress associated with this time of pregnancy.

As parturition becomes imminent, the female becomes increasingly restless, digs at the floor or nesting material, and assumes a defecation posture without defecating. There may be calling vocalizations, especially by Siamese cats, and a few queens become excessively anxious and even hysterical (Fox, 1974).

Each of the four phases of parturition is highly variable, although their order holds true for the majority of births. The initiation of each new phase is usually marked by an abrupt behavioural change, from contractions causing genitoabdominal licking to placental delivery resulting in the consumption of the placenta (Beaver, 1980).

Hurni and Rossbach (1989) suggest that queens in group housing be provided with a cage to which they be confined overnight and during mid-day; this would be made available from just before parturition until four to six weeks after. By letting them out for a few hours in the forenoon and afternoon into the stock pen, the female remains socialized to the community and the stress of changes in the population hierarchy is reduced.

9. Random-Source vs. Purposebred Animals

Since it is considered advantageous to house research animals in a social environment, the housing of animals which are genotypically sociable is a distinct advantage. Cats' tendency to socialize well is a trait which is carried genetically by the male (McKeown, Pers. Comm., 1990). Proper selection for genotypically social cats is therefore possible in purposebred populations. As well, kittens growing up with social conspecifics become more social than those raised with non-social conspecifics (Schar, 1983). By culling animals which exhibit undesirable traits even after adequate socialization (Ringler and Peter, 1984), the population can be further selected to include more animals which are behaviourally adapted to the research environment.

For certain types of studies, use of purpose-bred cats provides advantages which enhance the quality and validity of the research. These include a known health status, and control over the animal's age, genetic factors, and environment. This allows the production and use of a much more uniform population of known status. Losses are less, results are more valid, and therefore fewer animals need be used. In addition, there are many advantages to the cats kept in the research environment, in terms of their social and behavioural well-being.

E. DOGS

1. Introduction

Dogs (*Canis familiaris*) have been human-beings' companions for over 12,000 years (MacArthur, 1987). In the laboratory, this potential for developing a close relationship with people may be realized through appropriate socialization of the animal at an early age. As well, most breeds of dogs used in research, teaching and testing are naturally gregarious and seek the companionship of other dogs (MacArthur, 1987; Beaver, 1981). This tendency is also seen in packs of feral or wild dogs travelling together (Dunbar, 1979). Therefore, unless contra-indicated by the protocol, medical condition, or the animal's aggressiveness, dogs should be paired or group housed with conspecifics in cages or runs, with space adequate for active normal behaviour. If this is not possible, dogs should be released at regular intervals into space adequate to permit this normal species-typical behaviour.

Social rearing of puppies is the most effective means of ensuring compatible conspecific behaviour as adults (Fox, 1972). Moreover, dogs that have been handled as puppies show greater resistance to stress and greater disease tolerance than those which are not handled (Fox, 1975).

The appropriate maintenance of dogs will be discussed under breed differences, criteria for assessing well-being, housing, socialization to people, and enrichments.

2. Breed Differences

The differences in size between Newfoundlands and Chihuahuas represent some of the extremes seen among the many breeds of dogs. These differences include not only morphology, but also temperament (e.g., terriers vs. labrador retrievers), conformation (e.g., beagles vs. greyhounds), urea metabolism (dalmatians), development of behaviour patterns (MacArthur, 1987) and other important considerations. Although every dog belongs to the single species (*Canis familiaris*), each breed has specific behavioural and social needs.

Interbreed morphological differences become important in selection of proper cage size (even though they have the same body weight, long, lean dogs are likely to require different cage sizes than short, stocky dogs). The decision to group house will depend to some degree upon breed differences. Much can be learned appropriate to the well-being of dogs from a basic knowledge of breed-typical behaviours; however, attention to the uniqueness of each individual animal is the only way in which well-being can be assured.

In addition to understanding breed differences, an understanding of intrabreed differences, which are the natural outcome of environmental and genetic factors, is also of great value. Litter mates, even when reared under the same conditions, may behave entirely differently.

3. Criteria for Assessing Well-being

Evaluation of animal well-being includes both engineering (environmental) standards (e.g., minimum cage size, temperature, light cycles, etc.) and performance or outcome measures (McCarthy, 1989) standards (the dogs' general state of health and their compatibility in social groups and with people).

The well-being of dogs is dependent on a number of factors which include: training and dedication of the scientific, animal care, and veterinary staff; a facility in compliance with this *Guide*; observations of the animal's physical health (does it appear healthy, alert, active?); observation of the dog's behaviour; pair or group housing of compatible animals; and socialization to people.

a) Clinical Observations

i) Eyes

Clarity and expressiveness of the eyes is a good indicator of general health. This should not be confused with non-eye contact, sometimes demonstrated by dogs raised as subordinate to people.

ii) Posture

Ill or distressed dogs may appear lethargic or cower in the rear of the cage or kennel. Abnormal gait or the carrying of a limb is suggestive of a localized trauma or infection.

iii) Hair coat

Ill or chronically distressed dogs will often manifest a rough, unkempt hair coat. Self-grooming may be absent.

iv) Stool

Presence of diarrhea, or stool with mucus, blood, or helminths (worm-like endoparasites) should be questioned.

v) Appetite

Inappetence or too-rapid ingestion of food should be questioned; sudden changes in weight, drinking or eating behaviour should also be questioned and investigated.

b) Behaviour

i) General

One should look for evidence of how well the dogs are adapted to the environment. Whether singly or socially housed, they should not normally exhibit highly repetitive or atypical behaviours. Dogs generally perceive the cage or kennel as home territory and, when the threat is not too great (e.g., the door remains closed), they may bark in defence of the territory. Opening the kennel door may elicit very different behaviours; solicitation from human-bonded animals and fear from unsocialized ones. These differences may not be seen with the door closed; however, judgement about a fear-response to strange individuals must be made cautiously, for a certain degree of inquisitiveness or anxiety over the presence of unknown persons is normal.

ii) Toward cagemates

Compatible cagemates should demonstrate equal desire for attention when the cage is approached by familiar people. Overly dominant individuals (and dogs overly socialized to people and undersocialized to dogs) (Beaver, 1981) will prevent subordinate individuals from being touched by the person, which, at times, may lead to aggression that continues as long as the person remains at the cage door.

iii) Toward people

Dogs that bark excessively, remain in the rear of the cage, refuse to come to the cage door even for familiar technicians, or demonstrate aggressive tendencies when approached, are likely not well-socialized to people. Unsocialized dogs are fearful of people, may become "fear-biters," are difficult to catch and restrain, and may have physiological variability incompatible with some scientific studies. These

manifestations are *de facto* evidence of distress and poor well-being. Such dogs are poor candidates for chronic studies.

iv) Maternal

Toward the end of gestation, the bitch will begin to seek seclusion, a safe, warm, dark and quiet place, and it is advisable to make such provisions by placing a whelping box in some corner that she will accept. She should be shown her whelping quarters early on in her pregnancy and be given amble time to become accustomed to them (Fox, 1972). Where possible, during the birth of the pups any kind of handling or interference should be avoided.

The behaviour of the pregnant bitch changes toward the end of gestation. She is generally more restless and appears uncomfortable. She may show nesting behaviour and start to tear up newspapers and to scratch at the floor of the whelping box. Often the bitch will go off her food, and some occasionally vomit during the few days immediately prior to parturition. It is not unusual for the bitch to pant a great deal and to regularly look apprehensively at her hindquarters (Dunbar, 1979).

Nest boxes for whelping bitches, provided with bedding materials and heat sources, keep homiothermic newborn pups warm and dry.

4. Housing

Housing should facilitate social group formation, human interaction, comfort, and sanitation. The use of modular cages or runs that can be converted to accommodate either pairs or groups of dogs is desirable.

Hite, Hanson, Conti *et al*. (1977) and Hughes, Campbell and Kenney (1989) discuss the effects of cage size on beagles (the most commonly used purposebred dog). Caging should permit ready access by personnel and permit visual, olfactory, and auditory contact with other dogs.

Resting boards made of non-conductive, non-permeable materials should be provided to permit animals to escape the floor, especially when temperature control and wetting may be a problem.

i) Social housing

Social housing is desirable for most breeds of dogs. Centuries of interaction with people and other dogs have developed species-typical behavioural patterns, which must be understood in order to evaluate and provide for their well-being (Beaver, 1981). Some breeds of dogs (e.g., hounds) are highly social; others such as terriers are not (Beaver, 1981). Single caging for most social breeds may be stressful.

Movement of an individual(s) away from compatible animals can be disruptive. Dogs that are removed from a social group (by virtue of health, protocol, or aggression) should remain in the same room, as close to the same social group as possible, and should be returned to the group as soon as possible. When the group is stable, positions within the room should not be changed without cause.

ii) Single housing

For those animals comfortably adapted to solitary living, introducing cagemates may induce distress. In these circumstances, exceptions to social housing may be appropriate, especially where human companionship is provided and they are in visual and auditory contact with other dogs.

If dogs must be housed singly, they should be in visual, auditory and olfactory contact with others in the

room. It is likely that multiple social groups exist within such a room, with the most stable groups consisting of individuals immediately adjacent to or across from each other.

It should be remembered that dominance can be expressed across the aisle, so that an animal removed from a cagemate because of dominance aggression should not be placed directly across the aisle from its original cagemate, but moved to a location away from the overly dominant individual.

5. Socialization to People

Of all the common laboratory species, dogs are the most highly domesticated and adapted to live in intimate association with people. Socialization creates an attachment and trust of people, which assists in the development of coping strategies that serve to bridge periods of adaptation to new procedures and environments, thereby reducing stress and experimental variability.

Without early exposure to people (i.e., socialization), dogs rapidly become fearful of humans and manifest fear and distress in a variety of physiological and behavioural ways (e.g., "fear biting") (Beaver, 1981), all of which are incompatible with their well-being and can influence the reliability of research data derived from them.

The dog's ability to cope when a person enters the scene, or its environment changes, is a key criterion to well-being (Dunbar, 1979). Coping connotes the ability of the dog to adapt to stresses with minimal behavioural or physiological alteration (Archer, 1979).

Therefore, all dogs used in a facility, for whatever purpose, should be socialized to people, (either in the facility or by the supplier), or serious consideration be given to their euthanasia or use in acute non-survival studies. Socialization (handling by people) should take place when pups are between 6-10 weeks of age (Wolfle, 1989a, 1989b; MacArthur, 1987; Fox, 1975). A number of other investigators believe the socialization period should extend to at least 12 weeks (Pfaffenberger, 1963; Bateson, 1987; Vanderlip, Vanderlip and Myles, 1985a, 1985b; Scott and Fuller, 1965). Fox (1968, 1990) contends that puppies deprived of human contact until after 10 weeks of age will be very difficult to handle later in life.

Adult dogs that demonstrate lack of socialization should not remain in the facility any longer than it takes to determine that the behaviour is unlikely to respond to remedial socialization, which, in any event is time and energy consuming and not at all sure of success (Dunbar, 1979). Such dogs should be either euthanized or used immediately in an acute, non-survival study. Socialization should be considered a critical part of every breeding program, and when animals are purchased from a supplier, socialization should be written into contract specifications.

Human/dog interactions will ensure continuation of the benefits gained from socialization. Quantification of contact during the socialization period, in terms of specific frequencies or durations, is less important than the quality of the interaction. Puppies are susceptible to attachment to humans or other animals. Thus, *repetitive interaction* with people during this period is more important than the exact nature, frequency, or duration of the interaction.

Wolfle (1990) described the socialization of large numbers of foxhound puppies with only five minutes per puppy per week. However, it should be noted that this was a complex, rich, bi-weekly socialization procedure where littermates were treated as a group and thus each pup benefitted from the interaction of people with the littermates. Nevertheless, it is clear that the amount of "hands-on" time required to socialize large numbers of puppies does not seem to be critical, and should be possible to accomplish with existing staff in most facilities.

Through observation, it should be established whether each dog in a social group is behaving normally (Beaver, 1981). By having a variety of people participate in the socialization of each dog and by reinforcing their socialization as adults, the problem of over-attachment to an individual (person) can be

avoided.

Housekeeping routines should include recognition of each dog as the technician works about the room. Moments taken to speak to and pet the dogs will be repaid through reduction in the dogs' anxiety and physiological variability (Wolfle, 1990, 1985, 1989a, 1989b). The effects of animal caretaker styles may affect the animal (Fox, 1986) and thus experimental results.

6. Enrichment Devices (Artificial Appliances)

"Enrichments," often in the form of toys or other appliances, are frequently given to dogs to produce a desired change in behaviour. For example, abnormal or persistent grooming may be moderated by giving the dog rawhide or other treats on which to gnaw; however, this should be done only with the knowledge of the facility manager and investigator. Beaver (1989) notes that dogs respond well to running through mazes as a means of environmental enrichment.

Music has long been used to reduce stress in many laboratory animal facilities (Line, Clarke, Ellman *et al*. 1987) and dairy barns (Ewbank, 1968), (perhaps because of its initial stress-reducing effect on the attendant). However, few definitive data exist to recommend its use for dogs. If used, the volume should be placed at conversational levels. Levels exceeding 85 db for a sustained period may cause auditory damage. It should also be remembered that many laboratory animals, including dogs, are able to hear frequencies above what humans can hear (Dunbar, 1979). If violin music, for example, is played at high volume, dogs may be in acute discomfort. Conversational (talk show) radio sound may accustom the animal to the human voice.

7. Exercise

Exercise for dogs has recently been mandated in American law which requires "that research facilities shall establish, in consultation with the attending veterinarian, written procedures and systems for exercise of dogs..." (USDA, 1989).

Dr. Dale Schwindaman, Assistant Deputy Minister for Regulatory Enforcement, Animal and Plant Health Inspection Services, U.S. Department of Agriculture, has stated that, in looking at exercise and socialization requirements, it may turn out that social contact with other dogs or with humans in the case of singly housed animals is more important than exercise. He reports that it has been proposed that, in addition to housing in compatible groups, the ability to see and hear other dogs will be required. Singly housed animals would receive positive physical contact with humans. Any exceptions to the requirement for exercise and socialization would have to be approved by the Institutional Animal Care and Use Committee (IACUC). It has also been proposed that animals held in (space that is) less than what is required for permanent housing as mandated by the *Guide for the Care and Use of Laboratory Animals* (USDHHS, 1985) would have to be released for exercise for at least thirty minutes daily (Schwindaman, 1990).

Scientific data have indicated that cage size had no significant effects on hematologic or serum biochemical values of purposebred beagles; that the dogs had little inclination to exercise when released alone into an exercise area, unless humans were present in the room; and that even a moderate exercise program had no demonstrable effect on biochemical parameters such as hematology, clinical chemistry or indicators of stress (Campbell, Hughes, Griffen *et al*. 1988; Hughes, Campbell, and Kenney, 1989; Campbell, 1990).

Studies demonstrated that on the average, dogs spend only 0.5 to 1.5 hours daily in any type of activity, regardless of the housing system. Most of the dog's activity takes place during the morning hours when there is the greatest amount of human activity in the area. Providing increased human contact will improve the handling and behavioural characteristics of the dog, but not its activity, because dogs that do not have enhanced human contact may move around the cage in an effort to attract attention (Hughes

and Campbell, 1990). These authors contend that they have shown that "dogs are basically lazy. They do not like to exercise and have no particular inclination to run about an area." Fox (1986) reports that dogs that are well-fed and content do not exercise routinely.

Although, unlike the U.S., no legal requirements for the exercise of dogs exist in Canada, the concept of exercise, and perhaps more importantly communal housing and socialization of the animal, both with conspecifics and humans, is considered of great importance by the CCAC. Institutions are being asked to furnish documentation of ACC approval for any dog housed individually. Increasingly, the provision of environmental enrichment, in its various forms, will be strongly recommended by the CCAC.

In conclusion, it should be remembered that, as Erwin (1985) advised, reactions of animals to any type of environmental enrichment should be monitored to determine whether the desired outcome is achieved.

Beaver (1989) reminds us that studies have not determined the amount of activity that is actually beneficial to any species. Neither has it been shown whether stereotypic behaviour is beneficial or harmful (Fox, 1986). Much knowledge of animal behaviour remains to be garnered and established in order to produce an environment that will enhance the dog's well-being.

F. NON-HUMAN PRIMATES

1. Introduction

When animals are used, efforts must be made to provide a physical and social environment conducive to their well-being. As well, social structure makes many experimental animals sensitive to the ill effects of inappropriate housing conditions. In Canada, only four species of non-human primates (NHP) are currently used in research, teaching and testing: rhesus monkeys (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), African green monkeys (*Cercopithecus aethiops*) and squirrel monkeys (*Saimiri sciureus*). Common and scientific names of a number of species are included as Addendum 1.

In focusing on NHP, emphasis must be placed on enhancing their social and behavioural well-being. As Markowitz and Line (1989) point out: "It is clearly possible to find methods by which environmental enrichment can be combined with a research protocol to enhance both."

Even though the animal may appear healthy, researchers "cannot be content with defending the status quo," says Line (1987). He challenges investigators to seek practical ways to expand opportunities for primates to display normal behaviour, "especially those housed singly". As Volume 2 (1984) of this *Guide* noted: "Any primate housed alone will probably suffer from social deprivation, the stress from which may distort processes, both physiological and behavioural." It is important, therefore, to provide the company of compatible conspecifics or other NHP species, and, if this is impossible, increased human company.

There is a growing body of scientific data on space/cage size appropriate for NHP. While enclosure size is an important variable, the primary emphasis should be on providing laboratory animals with the option for species-appropriate activities (Bayne, 1989; Bayne and McCully, 1989; Line, 1987; Bantin and Saunders, 1989; Fajzi, Reinhardt and Smith, 1989; Chamove, 1989; Markowitz and Spinelli, 1986; Segal, 1989a). Wilson (1982) found that in captive gorilla and orangutans, enclosure size had no effect on the level of activity. She suggested that objects within the environment were more important than the size or complexity of the enclosure. Primates maintained in the absence of external stimuli tend to display locomotion far more frequently than other categories of behaviour such as facial expressions, play, and inquisitive behaviour (Martinic, 1990). Chamove (1989) notes that many successful enrichment techniques act in a way similar to that of increasing physical space. Snowdon, Savage and McConnell (1984) note the adverse effects of too-small caging on reproduction, and the widely accepted fact that small cages increase the incidence of stereotyped movements and other non-locomotory abnormal behaviour.

Animals that are not housed properly and treated humanely "yield data that are clearly confounded with distress" (Markowitz and Spinelli, 1986), i.e., may yield unreliable data due to the effects of behavioural stress (Levine, 1985) and introduce unwanted variables (Morton and Griffiths, 1985). It is important, therefore, that those using NHP should first acquaint themselves with the animal's distinctive characteristics and needs. Differences within and between species make the task difficult (Snowdon, 1990). Wolfle (1990) suggests that the researcher consult the psychological literature about animal cognition and perception. "The best tool of all for providing well-being begins with routine frequent observation of every animal," he concludes.

2. Interpretation of the Behavioural and Morphological Postures

Primate users sometimes misinterpret the meaning of the behavioural or morphological signals of NHP, as well as the effect of certain human behaviours on NHP. Inadequate animal husbandry practices are likely to increase the level of stress during cleaning, feeding and handling (Fox, 1986; Line, Morgan, Markowitz *et al*. 1989), and increase the risk of injuries to both humans and animals. Some of these misinterpreted signals are described below.

a) The Stare

The stare usually expresses an aggressive mood in NHP (e.g., rhesus). Threats are always initiated and accompanied by a stare, which usually precedes attack. This behaviour typically elicits one of the following responses by the recipient: a threat (in increasing order of intensity are staring back, staring with the mouth open, and grunting), an attack (lunging, hitting, biting), or a submissive reaction (avoiding to look, leaving, displaying a fear grimace). Primate users should keep in mind that when looking intensely at a monkey, they are threatening it and announcing an imminent attack.

b) The Fear Grimace

The fear grimace resembles an exaggerated smile; the mouth corners are fully retracted, showing all the teeth. This expression may be accompanied by a high-pitched, loud vocalization (Van Hoof, 1963, 1967). The fear grimace, or bared-teeth display is a ritualized signal of submissiveness emitted unidirectionally by subordinate to dominant individuals.

Thus, the fear grimace does not convey a playful mood or an aggressive motivation. The fear grimace is often inadvertently elicited by primate handlers when they move towards a monkey, while looking at it. The best way not to elicit a fear grimace is to avoid staring at the monkey, and to approach it indirectly.

c) Lip-Smacking or Teeth Chattering

In the many species in which it occurs (e.g., stump-tails), the teeth chattering face indicates a tendency to flee, the lip-smacking face a stronger sense of social attraction (Van Hoof, 1963). These are greeting gestures that express an affiliative mood, and probably include a submissive component, depending on the context.

d) Grooming

Removal of particles of dirt and ectoparasites in NHP is undertaken to establish, maintain or restore positive social bonds by expressing a state of non-aggression and by reducing tension. The cleaning function of social grooming is only secondary in importance. Grooming may pacify another animal, but is also used to maintain social bonds, as in mothers grooming infants or juveniles, and between members of a mated pair.

Grooming also serves to appease dominant individuals and prevent aggression, to provide contact-comfort (consolation) to the victims of attacks, to reconcile with an opponent after a fight, or to reassure subordinates. For example, grooming is directed by males to females in courtship, and is an important component of co-operative partnerships, such as coalitions and alliances.

e) Sexual Swelling

In many species, females in estrus manifest a reddening and/or swelling of the perineum. This signals their sexual receptivity to males. The extent of this swelling is highly variable among species. Sexual swellings are sometimes misinterpreted as injuries or symptoms of a pathologic condition. Blaffer-Hrdy and Whitten (1987) present comparative data on cycle length, duration of menstrual flow, visual signals, and the behaviour of males as well as females in estrus, for all species.

3. Distinctive Characteristics

a) Locomotion

In contrast with most other experimental animals, which are primarily terrestrial, NHP are characterized by a number of major morphological and behavioural adaptations to a three-dimensional arboreal life. These adaptations are stereoscopic vision, manipulative skills and specific modes of locomotion (climbing, leaping, etc.). Most primates show vertical flight reactions (Burt and Plant, 1990). For each species there is a defined behavioural repertoire, and for each species, preferred vertical limits in the wild should be considered.

b) Social Life

Most primate species, including the majority of those used in laboratories, are highly social (Boccia, 1989) and live in complex social groups; however, such social groups are not necessarily permanent. Species which are primarily solitary include some lemurs and orangutans (Jolly, 1985). The three major categories of societies are the family, the one male/multifemale group, and the multimale/multifemale group. Most laboratory primates belong to the third category.

Many studies have shown that NHP recognize individually every member of their group and that they establish long-term bonds, extending over years or a lifetime, with many of their kin and non-kin. Such relationships are bilateral and multidimensional, involving play, contact-comfort, grooming, sexual activity, protection, support during conflicts, etc.

Because of the social bonding that takes place in most species, social isolation is likely to affect individual animals. Studies have indicated that the effects of social isolation differ among rhesus, crab-eating macaques and stump-tailed macaques, with rhesus most severely affected (Sackett, Ruppenthal, Fahrenbruch *et al*. 1981).

The major contributions regarding social deprivation were made by Harlow in the 1960s. Animals raised in total social isolation were characterized as withdrawn, personally bizarre, and aberrant in social, sexual and exploratory behaviour (Harlow and Harlow, 1965). Goosen (1981) has discussed the isolation of rhesus, noting that individually housed monkeys have little opportunity to develop coping strategies, and may exhibit bizarre behaviour patterns (Novak and Suomi, 1988).

c) Cognitive Abilities

Intelligence is reflected in many of the NHP behaviours. For example, lion-tailed macaques, chimpanzees and capuchin monkeys are known to manufacture probing tools, and a number of species use "tools" to facilitate food acquisition such as cracking nuts (Beck, 1980). Research has also indicated that lion-tailed

macaques manipulate objects, and use objects to serve as ladders, to create perches, and to apply leverage (Westergaard, 1988). *Macaca nemestrina* learn through the observation of others and pass on social traditions (Cole, 1963). They excel in the use and manipulation of third parties by forming coalitions and competing for the strongest allies, through the utilization of affiliative strategies. They are capable of some forms of deception (Smuts, Cheney, Seyfarth *et al*. 1987).

d) Emotions

Physical and emotional stress cause the release of various hormones, one of the main groups of which are the adrenal steroids, particularly cortisol (Moberg, 1985).

If it is accepted that humans and apes are related through evolution, it is considered that the African apes (the gorilla and two chimpanzee species) are humans' closest kin (Martin, 1988). NHP exhibit many external manifestations of emotions such as facial expressions, vocalizations, postures, gestures, and reactions, similar to those of humans.

Many of the ways in which NHP exhibit emotional responses, for example, separation and external threat, appear similar to ways in which humans react to comparable situations. Moreover, many of the abnormal behaviours displayed by captive NHP are similar to the behaviour patterns of institutionalized humans (Passingham, 1982).

4. Assessing Social and Behavioural Well-being

Psychological well-being can be defined as "a state of harmony, both physical and psychological of an animal with itself and with its environment" (Coelho and Carey, 1990). Dresser (1988) points out that an animal's well-being "...connotes not only the absence of pain and distress. It implies as well that an individual's physiological, security and behavioural needs are fulfilled."

Although we cannot measure psychological well-being in NHP, the following criteria serve as indications that such a state exists: a) good physical health; b) no signs of pain, distress or discomfort; and c) no abnormal behaviours.

Moberg (1985) proposes that an animal's well-being is compromised only when it is stressed by events in its environment, and suggests that researchers should look for changes in the animal's immune competence, reproductive function or growth and development: "The existence of pre-pathological states in these systems would indicate the animal's well-being was threatened."

a) State of Physical Health

Poor health and physical injuries are not compatible with psychological or physical well-being. Physical health should be routinely assessed by a qualified veterinarian.

Some of the most obvious external signs that can be monitored are the condition of the coat and skin, the appearance of the eyes, and, if the size of cage permits, the gait pattern.

Examples of abnormalities include unresponsiveness and hypersubmissiveness, hair pulling-and-eating (Reinhardt, Reinhardt and Houser, 1986), and may include the crouching posture.

b) Absence of Signs of Pain, Distress and Discomfort

Although NHP express fear through high-pitched screams, when experiencing pain they are unlikely to

emit loud vocalizations. Instead, they display a hunched or crouched posture, an abnormal or slow gait. They stop self-grooming and avoid conspecifics. They may moan, refuse to eat and drink, and often attract the attention of conspecifics (Hinde and Rowell, 1962) (see Control of Animal Pain).

c) Absence of Abnormal Behaviours

Laboratory primates may exhibit abnormal behavioural disorders in both a barren cage or in a compatible group (Reinhardt, Reinhardt and Houser, 1986). However, Reinhardt (1990b) reports that most behave in normal ways even in an impoverished environment.

Every species of NHP is characterized by a specific behavioural repertoire. Ethograms or descriptive lists of species-typical behaviours, have been published for many species (Van Hoof, 1967; Bertrand, 1969; Fedigan, 1976; Skinner and Lockard, 1979; O'Keefe and Lefshitz, 1985; Walsh, Bramblett and Alford, 1982; Erwin and Deni, 1979). A vast body of information on the social behaviour of NHP living in the wild or in large outdoor enclosures is also available. For geographical distribution, ecology, diet, reproduction and social behaviour of a representative sample of taxonomic subgroup of NHP see Smuts, Cheney, Seyfarth *et al*. (1987).

Despite the existence of a fair degree of interspecific and intergroup variation, it is possible to identify general categories of abnormal behaviours observed in captive species of NHP. Examples are summarized below. More details and a description of idiosyncratic variants may be found in the literature (Goosen, 1981; Walsh, Bramblett and Alford, 1982; Erwin and Deni, 1979).

d) Examples of Abnormal Behaviour Patterns

i) Bizarre postures and behaviours

Self-biting, self-clasping, self-grasping, hair pulling-and-eating, feces spreading, face or eye poking, penis sucking, and "floating limb" accompanied by attack of the limb.

ii) Stereotypical behaviours

Pacing, "saluting," head tossing or weaving, walking or bouncing in place, somersaulting, rocking, and cage charging.

iii) Appetitive disorders

Coprophagia (ingestion of animal's own feces), urine drinking, hyperphagia (excessive over-eating), and polydipsia (excessive, long-term thirst).

iv) Abnormal levels of activity

Inactivity, depression.

v) Abnormal social behaviours

Maternal neglect of infants, maternal over-protectiveness of infants, high levels of fearfulness and over-dependence of infants, inappropriate sexual behaviour, hyperaggressiveness, hypersubmissiveness, and avoidance of social interactions.

5. Ways of Promoting Social and Behavioural Well-being

There are a number of ways to alter or improve an animal's environment. Beaver (1989), for example, suggests five basic means: behavioural enrichment (by creating an environment similar to the wild habitat), social peers, artificial appliances, food gathering activities, and control of non-food items. Some of these will be described below.

a) Social Peers

The best psychological enrichment is social enrichment (Crockett, 1990). Providing opportunities for social interactions is by far the best way to help NHP cope with the two main categories of problems associated with captivity: boredom (understimulation) and fear. Social interactions appear to provide the richest source of stimulation and the best source of emotional security. Segal (1989b), in editing a new publication on NHP noted that several authors independently reached the conclusion that "the single most important thing one can do to enrich the life of a captive primate is to provide it with a companion animal." Moreover, it is believed that more social interaction may take place in an environmentally enriched milieu (Martinic, 1990).

b) Housing

i) Single housing

The reasons commonly cited for housing primates singly are reviewed, and contested, by Reinhardt (1990a); these include wounding, disease transmission, dominance hierarchies, social distress, and undernourishment of a lower-ranking partner. Reinhardt concludes that chimpanzees, orangutans, rhesus monkeys and stump-tailed macaques have been resocialized without undue risks or disadvantages: "There is no reason to suspect that other primate species are less suitable for careful resocialization programs." He warns, however, that such programs must be tested for each species before social housing is implemented. Fritz (1989) reported that resocializing singly caged chimpanzees caused neither wounding nor death.

Single housing is strenuously discouraged, except in those situations when it is necessary for the experimental protocol, in the case of aggression, or to prevent or contain disease. When single housing is required for an experimental protocol, the institutional ACC must be very diligent in assuring this aspect is necessary to achieve the experimental objectives. **ACCs should require the investigator to submit scientific justification for environmental impoverishment, e.g., as a result of regulations or the demands of the study protocol.**

Singly housed animals have exhibited depressed heart rates and elevated blood pressure similar to the elevated blood pressure noted in humans diagnosed as being depressed (Coelho and Carey, 1990).

If single caging must be used, every effort should be made to enrich it (Reinhardt, Houser, Eisele *et al*. 1987; Bayne, Mainzer, Dexter *et al*. 1991), although strategies for so doing are apparently still very limited (Chamove, 1989). If possible, NHP should be given the opportunity to take part in species-typical activities. In the case of singly housed NHP, the role of the animal care technician takes on added importance (Chamove, 1989; Wolfle, 1990). Familiarity with the handler, surrounding and procedure can significantly reduce anxiety. Positive reinforcement, using rewards such as food, encourage animals to accept manipulations without apprehension.

In order to preclude the need for single housing to facilitate sampling of body fluids and chronic monitoring of physiological parameters, a system of social tethering has been developed (Coelho and Carey, 1990).

ii) Pair housing

Novak and Suomi (1988) claim that pair-housed primates enjoy a state of physical health that is usually superior to that of many free-ranging monkeys. Pairing of 700 captive cynomolgus breeding females (and subsequent offspring) was successfully pioneered in 1983 in the Ottawa colony of the Health Protection Branch of Health and Welfare Canada (McWilliam, 1989). Although pair housing is not advocated as universally beneficial (Crockett, 1990; Rupenthal and Walker, 1989), the benefits appear to outweigh the risks (Crockett, 1990).

Most all age-sex combinations of pair housing are possible. Reinhardt (1987, 1988, 1991) and Reinhardt, Houser, Eisele *et al*. (1988) have successfully paired rhesus unrelated adult females, unrelated adult males, and adults of both sexes with infants.

Pairing provides social stimulation and makes it possible to avoid some of the problems associated with larger groups (Erwin, 1979; Crockett, 1990). It elicits most species-typical social interactions for the sexes and ages concerned, except for the multi-animal interactions.

In an interesting innovation, Reinhardt (1990c) in a recently controlled study provided isosexually paired (male-male, female-female) adult rhesus monkeys with privacy panels. It was found that they spent more time in close proximity and more time grooming each other and huddling, while the incidence of agonistic conflicts was significantly reduced.

Monkeys that are paired should be compatible. Compatibility can be defined as an affiliative relationship in which such interactions as grooming occur and in which both members appear relaxed. This only occurs after a dominance relationship has been established. Reinhardt (1987) suggests compatibility is shown when neither animal exhibits signs of depression, and neither has inflicted serious injury on the other. Before being paired, prospective cagemates should be allowed to become familiar with each other in adjacent cages permitting visual and auditory communication. Dominance signals, such as staring, open-mouth threat, displacement, or fear grimaces, may be displayed in this situation or soon after the monkeys have been put together.

The pair may be formed in a third cage in order to avoid aggression (Erwin, 1979) which to some species such as gibbons may be related to territoriality. They should be monitored regularly for signs of incompatibility, such as injuries, avoidance of contact, or inappetence. Once pairs have been established, they should not be disrupted unless dictated by an experimental protocol.

iii) Group housing

Larger groups usually offer a much richer social environment and should be favoured over pairs when groups will remain relatively stable. However, it should be noted that chimpanzees associate in temporary parties, unlike stable groups of most large primates (Nishida and Hiraiwa-Hasegawa, 1987). For troop-living primates such as rhesus, the best way to promote their well-being in the laboratory may be to rear them with partners or in social groups (Novak and Drewsen, 1989). When groups are being formed, observers must adjust group composition so the units show minimal aggression (Wolff and Ruppert, 1991).

There are drawbacks to group housing which should be considered, however. Increased social interaction may result in disease transmission as well as the risk of injury and death (Beaver, 1989; Line, Clarke and Markowitz, 1989; Novak and Suomi, 1988; Wolverton, Ator, Beardsley *et al*. 1989; Line, 1987). Snowdon (1990) notes that the various species have different responses to group housing. Group formation may be stressful; Sapolsky (1989) contends that it takes up to 12 to 15 months for animals' stress markers to return to normal levels; however, Reinhardt, Cowley, Scheffler *et al.* (1990) disputes this as regards to rhesus. Erwin (1979) notes that "fighting is a fairly common occurrence in primate groups even in natural settings, but trauma due to aggression is an especially pressing problem in captive groups of macaques

and baboons."

NHP are prompt to form coalitions through which they establish their dominance ranks and compete for food and sexual partners. Removing a monkey from its group may disrupt the existing network of alliances and induce rank changes, which may be associated with vicious fighting, resulting in injuries (Kaplan, Manning and Zucker, 1980; Reinhardt, Reinhardt, Eisele *et al*. 1987). Animals that are to be reintroduced should be kept away from the group for as short a time period as possible.

c) Social Interaction with Humans

It is suggested that there be as much interaction as possible between the NHP and the investigator or technician (Hearn and Dixson, 1984; Bayne, 1989). The interaction, however, must not involve handling other than what is necessary for the maintenance of the animal or for investigational procedures. The necessary precautions are described in Volume 2 of this *Guide* (CCAC, 1984).

Direct physical contact between humans and NHP should be evaluated from facility to facility. In many instances it should be kept to an absolute minimum for example, because of the need to break the human/animal bond when staff changes occur or when an animal must be euthanized, as well as the hazards posed by zoonotic diseases. Some of the most significant diseases are *Herpesvirus simiae* infection (B-Virus) and infectious hemorrhagic fever viruses. Also, many NHP have extreme physical strength in relationship to body size, and can inflict serious injury on personnel. Furthermore, humans can transmit infectious diseases to primates, e.g., measles, tuberculosis.

Forced physical contact between humans and NHP can be extremely stressful to the monkey. Moor-Jankowski and Mahoney (1989) have reported that even the introduction of a new technician can change the NHP's liver enzymes to the point that it can compromise a study. Many animals react to the presence of a human observer with anti-predator behaviour such as mobbing and alarm calling (Caine, 1989), and threat behaviour toward observers (Wolff and Ruppert, 1991). Talking to the monkeys, combined with the physical presence of the human, will accustom the NHP to the human presence, and may thus reduce stress. Burt and Plant (1990) suggest that use of mesh cage fronts are preferable to the barred variety aiding interaction between animals and staff.

d) Diet Supplementation and Food Gathering Activities

The core diet of the NHP should be a complete, well-balanced commercial diet, or alternatively a diet of equal quality prepared in the diet kitchen of the institution. This diet should be supplemented to suit the nutritional requirements of the primate species being used (Jones, 1972).

Supplementation and innovative ways of presenting the food to the monkey are effective ways of enhancing well-being, particularly of those in individual caging. Examples of items used for supplementation are raisins, fruit, chicken scratch feed, and Prima-Treats (Addendum 2). Fresh branch clippings may be used for supplementation, providing toxic plants are avoided and the dust and pesticides are washed off the branches.

The diet supplement can also be provided as food puzzles (e.g., Kong Toys) containing frozen juice, peanut butter or raisins (Addendum 2). Seeds, etc., can be hidden in deep litter. These methods of supplementation require the monkey to search and/or work for the food (Anderson and Chamove, 1984). This challenge will simulate the foraging activity which the NHP pursue in their natural habitat, and has been found to reduce stereotypies and increase exploratory behaviour (Anderson and Chamove, 1984; Boccia, 1989).

e) Exercise

The majority of NHP in their natural environment move widely and regularly within their established home ranges. Except for owl monkeys and many prosimians, primates are diurnal, spending large portions of the day foraging for food or participating in grooming and other social activities. For this reason, NHP housed in standard cages over long periods of time, whether they are held singly or in pairs, appear to benefit from species-appropriate activities reminiscent of those in the wild (Hearn and Dixson, 1984; Chamove, 1989; Burt and Plant, 1990).

It has not been demonstrated that simply enlarging the amount of available space will improve the well-being of the animal (Novak and Suomi, 1988; Fajzi, Reinhardt and Smith, 1989; Novak and Meyer, 1988). Indeed, an increase in aggression has been associated with an increase in space for some captive primates (Novak and Meyer, 1988).

Exercise cages for NHP were introduced over a decade ago (Tolan, Malone and Rogers, 1980). However, it is considered that environmental complexity, rather than size alone should be increased (Line, 1987; Line, Clark and Markowitz, 1989; Bryant, Rupniak and Iversen, 1988). Wolff and Ruppert (1991), reporting on an exercise program involving rhesus, cynomolgus and capuchins, note that the majority of animals interacted in a positive fashion. Constant observation prevented fights and thus minimized injury. Much of the aggressive behaviour was non-physical (e.g., vocalizing or teeth baring rather than biting).

The NHP entering an exercise area for the first few occasions will usually exhibit a fear response (Wolff and Ruppert, 1991). However, exercise can be stimulated by synchronizing it with feeding. Deep litter in which food can be hidden can also be used in the exercise cage and will stimulate the monkeys to forage. To instill a feeling of security, freedom of movement between the exercise cage and the home cage is preferable.

If more than one monkey is exercised at the same time, the animals should be cage mates. However, it is sometimes possible that singly housed or paired monkeys can be exercised in larger groups if they have been confined in the same room, and are exercised together on a regular basis. These animals must, however, first be tested for compatibility.

f) Physical Enrichment of the Cage Environment

It is important that the animal be given as much control (or even the perception of control), as possible over its environment (Line, 1987). Guidelines for minimum cage size have been established by the CCAC (see Appendix I). Life in cages can be enriched and activity promoted by the installation of devices such as small branches (O'Neill, 1989), toys (Line, Clarke and Markowitz, 1989), perches (Crockett, 1990), swings (Bayne, Suomi and Brown, 1989) and food puzzles (Beaver, 1989; Chamove and Anderson, 1989). Such enrichment is particularly important to the singly caged animal (Fajzi, Reinhardt and Smith, 1989), where devices which encourage foraging appear most successful (Crockett, 1990; Bayne, Mainzer, Dexter *et al*. 1991). Jerome and Szostak (1987) contend that foraging devices are used more frequently than play objects by baboons. Climbing is an especially good exercise. Bryant, Rupniak and Iversen (1988) contend that enrichment of the home cage could benefit the animals more than exposure to a playpen routine. Enrichment devices and their suppliers are listed in Addendum 2.

Wolfle (1990) notes that choice tests permit the animal to indicate that one environment or toy is preferred over another. Other tests measure the frequency of use of new space or new "toys." It has been suggested that toys be rotated in order to minimize understimulation (McWilliam, 1989).

Because of the importance of vision to the NHP, particularly *M. nemestrina*, (Cole, 1963), cages should be positioned so that the monkeys can see animals of like species. Solid-sided caging prevents visual contact. If physical contact is possible, there must be assurance that the animals are compatible.

There is a diversity of opinion with respect to the use of audiovisual devices (radio, video, television) as a means of enhancing the well-being of NHP. They appear to be of most benefit if the monkey can turn the

equipment on and off at will (Beaver, 1989; Line, Clarke, Ellman *et al*. 1987). In some situations, audio may serve as a contentment device for the primate; however, it is possible for some sounds to be irritating and stressful.

Visual means of enrichment may be stressful to the animal if the monkey perceives the picture to be threatening. This may be circumvented by the preparation of tapes or videos especially prepared for primate entertainment. It has been reported anecdotally that monkeys are particulary fascinated by visuals depicting their natural environment, or animals that are found in their natural habitat. NHP are fascinated by videos of themselves (Chapais, Pers. Comm., 1990).

6. Disposition

Following completion of the study, consideration should be given to further use of NHP utilized in non-invasive research, in an effort to minimize the numbers of animals used. However, monkeys used in invasive or stressful projects should not be subjected to further stressful procedures or conditions and should be humanely killed according to CCAC euthanasia guidelines found elsewhere in this *Guide*. Maximum utilization of NHP tissues, histological specimens, etc., is encouraged.

Following completion of research, retention of an animal in the laboratory, on the assumption that it may be required for future studies, is rarely justifiable.

7. Summary

In considering all the factors related to well-being of NHP, we should remember that "because well-being is subject to past experiences, present circumstances, and future expectations, it is a dynamic and changing phenomenon" (Wolfle, 1990).

G. RODENTS AND RABBITS

1. Introduction

Over the course of the past 25 years, standards have been established which are considered to represent optimum housing requirements for laboratory animals. Many of the improvements in housing and management standards were primarily designed to reduce variables and enhance the reproducibility of experimental results (Lang and Vessell, 1976). However, during the past decade, increased emphasis has been placed on the behavioural and social needs of animals kept in the laboratory setting. Most investigations into enhancement of the animal's environment have concentrated on the "higher mammals," particularly NHP. This document's conclusions have been based on contemporary concerns.

Laboratory rodents and rabbits are frequently perceived to have relatively few requirements other than basic housing, husbandry, and dietary needs. Thus, control of the environment has frequently been the primary consideration, with little (or no) emphasis on other areas.

"Well-being" is frequently difficult to assess objectively in these species. Weight gains, general behaviour, and adrenal weights are examples of criteria which have been used in studies of this type (Chamove, 1989).

Housing conditions should be evaluated carefully for each species, and consideration given, wherever possible, to innovative group housing in species such as guinea pigs and rabbits.

Qualified investigators should be encouraged to do additional objective, controlled studies on the

environmental needs and preferences of rodents and rabbits in the laboratory setting.

Awareness of normal behavioural patterns in each species is essential. For example, coprophagy (reingestion of feces) is a normal function in several species, including rabbits and rats (Smelser, 1985; Newton, 1978). Rats normally ingest 35-65% of their feces on a daily basis. Deprived of this opportunity, 15-25% reductions in weight gains have been observed (Newton, 1978).

Beaver (1989) suggests that five aspects in particular may contribute to environmental enrichment: behavioural enrichment, social peers, artificial appliances, food gathering activities, and control of the environment. Some of these are discussed below.

2. Behavioural Enrichment and Social Peers

Social interaction with peers is recognized to be a desirable, if not an essential aspect of laboratory animal well-being.

a) Mice

Mice thrive when housed in groups of two or more per cage. In one study, evidence of "stress" was minimal in mice housed at four per cage, compared with groups of two or eight per cage (Peng, Lang and Drozdowicz, 1989). A high incidence of stress-related tail lesions has been observed in cages housing up to 40 mice which were placed together after weaning. The problem was resolved when the groups were reduced to five per cage (Les, 1972).

As another example, female C3H/He mice in an intensive breeding program, and housed under conditions of severe social stress, had an incidence of spontaneous mammary tumours considerably different from counterparts kept under ideal conditions. At 400 days of age, approximately 90% of animals maintained under adverse conditions had mammary tumours, while the incidence of tumours was around 10% in females housed and mated under optimum conditions (Riley, 1975).

Compatibility is a critical consideration. It may be impossible to house male mice together after puberty, particularly those of more aggressive strains.

b) Rats

Rats are frequently housed singly for certain types of studies; however, it is desirable that two or more compatible rats be housed together in an appropriate cage. Post-puberal males are usually compatible, particularly if they have been together since an early age. It has been shown that even groups of highly standardized male rats exhibit a high level of variability of behavioural patterns (Gärtner, Ziesniss, Karstens *et al*. 1991).

c) Guinea Pigs

Guinea pigs live in groups of five to ten individuals in the wild (Sutherland and Festing, 1987) and thrive under group housing, although it is unlikely that two or more sexually mature males will live together without incident unless they have been together since birth. In their natural environment, guinea pigs exhibit a strong herd or family orientation, and this should be maintained in the laboratory setting, if at all possible. The one boar per harem arrangement is the recommended procedure in breeding colonies. Guinea pigs should not be housed singly; however, if this is necessary, Sutherland and Festing (1987) recommend a minimum of 700 cm^2. Vocalization appears to play an important part in guinea pig social behaviour, and they call for attention from human caretakers (Sutherland and Festing, 1987).

d) Hamsters

Adult hamsters are frequently caged separately because of their tendency to fight, with the exception of the female during the time she is ready to mate. However, they have been housed together under certain circumstances, particularly if they have been weaned and raised together since birth (Hobbs, 1987); nonetheless, the European hamster becomes more aggressive as it grows older. Hamsters spend more time in social proximity if they have had prior group housing experience. Singly housed hamsters show more agonistic behaviour with conspecifics, and lower weight gains than group-housed animals. Early housing experience can profoundly affect later social preferences and behaviour.

e) Gerbils

Most gerbil species are gregarious and live in large groups (Norris, 1987). Therefore, they should be housed in pairs or in larger groups wherever possible. If animals are placed together before they reach puberty, fighting should not be a problem. Gerbils usually mate for life; thus, it is advisable that single pairs be kept together throughout life. Most are normally docile, although aggression may be noted after pairing for mating.

Mature mongolian gerbils of either sex may show a characteristically severe form of epileptiform seizure (Norris, 1987).

f) Rabbits

In their natural habitat in the wild, rabbits of the genus *Oryctolagus* are social animals, frequently living in warrens of up to 100 or more rabbits of various ages. In the laboratory, convention has dictated that sexually mature animals be housed singly: a) to avoid fighting injuries; and b) to prevent ovulation and subsequent pseudopregnancy due to physical interaction in mature does. Male rabbits, if penned together, become increasingly aggressive from about 90 days (Adams, 1987). However, group housing for adult rabbits has been under study, both in the laboratory setting, and in commercial rabbitries (Stauffacher, 1992; Love, 1988; Anon., 1989a).

Group housing in larger enclosures has provided animals with the opportunity to live a more natural lifestyle, including ample opportunity for adequate exercise, mutual grooming, and general improved well-being (Love, 1988; Boyd, 1988). Breeding colonies have been established, using the group housing approach (Anon., 1989b). In some facilities, compatible rabbits are allowed to exercise in a designated floor area several times per week.

3. Enrichment Devices (Artificial Appliances)

a) Mice

Mice have used empty plastic water bottles placed in the cage for a "urinal," and an additional bottle for nesting and as a "bolt hole". It was concluded that provision of the bottle was beneficial in several respects, including improved sanitation, and an opportunity to establish their own optimal environment in the nesting bottles (Boyd, 1988). However, in one study, the addition of objects such as flower pots and bricks has been reported to increase aggression among male mice (Ayling, 1989), presumably because of territorial instincts.

b) Rabbits

The use of resting boards has been shown to have a calming effect on rabbits, which use them to hide beneath (Anon., 1989a), and the use of tubing as "bolt holes" has been suggested.

c) Hamsters

Hobbs (1987) states that wheel-running has not been shown to be beneficial; however, a raised top to the cage provides opportunity for climbing and exercise.

d) Gerbils

It has been recommended that gerbils be supplied with appliances such as PVC plastic pipe since they are burrowers in their natural habitat, and retain this behavioural pattern, as well as food hoarding, in the laboratory (Norris, 1987). It is likely that other small rodents would also benefit from these additions.

4. Caging and Bedding

i) Floor space per animal is an important consideration, and requirements for individual species have been identified in Appendix I [the U.S. and the U.K. have developed standards as well (USDHHS, 1985; UFAW, 1987)]. Although ample floor space per animal is essential, there is some evidence that the actual needs for group-housed guinea pigs, for example, may be less than current guidelines indicate (White, Balk and Lang, 1989).

ii) Solid bottom cages are strongly recommended for housing rodents, particularly for long-term studies. Solid floors with appropriate bedding are particularly critical for breeding rodents (Weihe,1987). Wire bottom cages, although less labour intensive to use, are far removed from the natural environment.

iii) Bedding is also an important consideration. For example, gerbils are active burrowers, and prefer bedding that can be used for digging and tunnel making, an important activity in this species. There have been studies of bedding preferences in small rodents (Iturrian and Fink, 1968). Straw bedding has been recommended for rabbits (Adams, 1987) who also notes that breeding females kept in metal cages must be provided with nest-boxes some days before parturition. In metal caging, Adams (1987) found that 16 mm mesh, 2 mm gauge wire was satisfactory in preventing sore hocks.

a) Rats

It is now recognized that rats like to run, stand on their hind legs, and jump (Weihe, 1987); unfortunately, presently available caging does not permit this. Weihe (1987) recommends addition to the caging of paper, wood, pellets or grain as a means of environmental enrichment. He also suggests caging of solid plastic with a wire mesh lid, and criticizes use of wire mesh-floored cages. Rectangular cages are more satisfactory than square caging, with 20 cm high cages suggested (Weihe, 1987).

b) Mice

In one study conducted in mice, vertical dividers were placed in cages, and the animals' performance and well-being compared with that of animals housed in conventional cages. Mice preferred the complex cages, and appeared to be "less emotional" than were the mice kept in regular cages. It was concluded that the divided cage represented a more natural housing arrangement, and that its use would lead to healthier animals (Chamove, 1989).

c) Gerbils

Any cage suitable for rats and golden hamsters is satisfactory for gerbils. As gerbils often stand erect on their hind legs, the cages should have a solid bottom, with floor to lid height at least 15 cm. A monogamous breeding pair requires a floor area of about 700-900 cm^2 and gerbils caged in large groups

need about 100 cm^2 floor area per animal (Norris, 1987).

d) Rabbits

Adams (1987) suggests that, for laboratory purposes, rooms designed to accommodate units of 50-60 rabbits are best. If metal caging is used, 16 mm mesh, 2 mm gauge wire is satisfactory in order to preclude sore hocks.

These are often built with portable sides with or without a roof or raised grid floor. They may be used to house a variety of species such as cats, dogs and NHP. Rabbits and guinea pigs have also been successfully housed in floor pens. In Swiss studies, near-to-nature surroundings for rabbits have been replaced by manageable artificial substitutes (Stauffacher, 1992).

e) Guinea Pigs

In guinea pigs, easily sanitized boxes with an end opening, placed in the floor pens, have proven to be an unqualified success. These boxes serve as a place to hide and as a secure place for farrowing, and provide some variety in the environment (White, Balk and Lang, 1989).

5. Food Gathering

Good quality legumes or appropriate vegetables (e.g., carrots, cabbage, etc.) are useful supplements to commercially available diets for **guinea pigs, rabbits and gerbils.** Seed mixtures are recommended additions for species such as **gerbils and hamsters** although Norris (1987) warns that gerbils will eat sunflower seeds and exclude other seeds. He also suggests feeding seed mix to young animals on the cage floor. Nutritious food items of this type will provide a pleasant diversion, as well supply additional nutrients to these species. However, quality control of such materials is essential since there is the potential for biological or chemical contamination. This practice could be contra-indicated in animals in nutritional or toxicology studies.

Rabbits are said to prefer pelleted commercial feed rather than meal, and to have a higher requirement for fibre than other species (Adams, 1987).

6. Control of the Environment

Ambient temperature, humidity, air changes, frequency of cage cleaning, light-dark cycles, noise and daily routines, are examples of environmental conditions that affect the welfare of animals in a research facility (Clough, 1982; Gamble, 1982; Riley, 1975; Peterson, 1980; McSheehy, 1983; Everitt, McLaughlin and Helper, 1987; Besch, 1980; Gärtner, Büttner, Döhler *et al*. 1980; Anon., 1989b).

Significant variations in certain blood constituents were observed in rats subjected to various handling and experimental procedures. On the other hand, the presence of a familiar animal attendant in the room in the absence of manipulations had minimal influence on the blood characteristics under study (Gärtner, Büttner, Döhler *et al*. 1980). Sudden changes in humidity may adversely affect rabbits (Anon., 1989b).

In laboratory rodents and rabbits, stimuli and conditions have been identified which may have adverse effects on psychological well-being and general health. **Animal rooms may not be used for performing any experimental procedures requiring manipulation, particularly those likely to evoke fear and/or vocalization.**

a) Noise

Levels of 50-70 DBA or higher are considered likely to be detrimental to the hearing of rodents and rabbits. Adverse effects have included audiogenic seizures in young mice (Bevan, 1955; Gamble, 1982), and reduced fertility in mice and rats (Newton, 1978).

b) Lighting

Light intensity can influence rodent activity, maternal behaviour, and various other aspects of reproductive physiology (Clough, 1982). Reproductive disorders have been identified in mice and rats housed under inappropriate light/dark cycles, or in the absence of such a cycle (Newton, 1978). In albino rats, continuous exposure to light levels of greater than 700 Lux can cause severe retinal degeneration over a period of time (Everitt, McLaughlin and Helper, 1978; Clough, 1982; Semple-Rowland and Dawson, 1987), and there are other reports of light-associated retinal damage in albino rats (McSheehy, 1983). Data on acceptable light levels for laboratory rodents are available (ILAR, 1977).

H. WILDLIFE HELD IN THE LABORATORY

Wildlife species which are threatened, endangered or Convention on International Trade in Endangered Species of flora and fauna (CITES)-listed must be conserved, and every effort should be made to replace these animals after study, either through reintroduction to the environment of origin, or placement in captive breeding-release projects.

Researchers planning to use large numbers of animals should, where feasible, breed replacement stock rather than continuing to remove animals from the wild.

The chapter on wild vertebrates in Volume 2 of this *Guide* (CCAC, 1984) is comprehensive and wide-ranging, and should constitute the primary source of information and guidance, along with the *Categories of Invasiveness* and *Ethics of Animal Investigation* document found elsewhere in this Volume.

As noted in Volume 2, such animals should only be brought into an institution after the investigator proposing to use them has demonstrated adequate knowledge of the animals' social and behavioural requirements or those of a closely related species. Those who will be responsible for such animals must also be able to provide for appropriate management and housing before the animals are introduced into the laboratory.

A number of recent, excellent publications are listed under Additional Reading.

I. REFERENCES

ADAMS, C.E. The laboratory rabbit. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 415-435.

AGRICULTURE CANADA. Publication 1771/E. Recommended code of practice for the care and handling of pigs. Communications Branch, Agriculture Canada, Ottawa, Ont., K1A 0C7. 1984.

IBID. Publication 1898/E. Recommended code of practice for the care and handling of farm animals--pigs. In Press.

IBID. Publication 1821/E. Recommended code of practice for the care and handling of special fed veal calves. 1988.

IBID. Publication 1757/E. Recommended code of practice for the care and handling of poultry from hatchery to processing plant. 1989.

IBID. Publication 1853/E. Recommended code of practice for the care and handling of dairy cattle. 1990.

IBID. Publication 1870/E. Recommended code of practice for the care and handling of farm animals--beef cattle. 1992.

AMERICAN ASSOCIATION FOR ACCREDITATION OF LABORATORY ANIMAL CARE. AAALAC Accreditation Program. Bethesda, MD: AAALAC, 1991.

ANDERSON, J.R. and CHAMOVE, A.S. Allowing captive primates to forage. In: Standards in laboratory animal management. Potters Bar, England: UFAW (Universities Federation for Animal Welfare), 1984: 253-256.

ANON. Communal housing makes for happy rabbits. CCAC (Canadian Council on Animal Care) Resource 1989a; 13(2): 4.

ANON. Environmental norms. Rabbit J. 1989b; (May/August) 20: 10-12.

ANON. Animal Welfare Committee looks at animal rights. J. Amer. Vet. Med. Assoc. 1990; 196(1): 17.

ANON. Pigs respond to the gentle touch. Vet. Rec. 1992; 130(18): 387.

ARCHER, J. Behavioural aspects of fear in animals and man. In: Sluckin, W., ed. Fear in animals and man. Princeton, NJ: Van Nostrand Reinhold, 1979.

AYLING, S. Laboratory mice: The effects of differences in holding conditions on aggression. UFAW (Universities Federation for Animal Welfare) Report 1989; 12.

BANTIN, G.C. and SAUNDERS, P.D. Animal caging: Is big necessarily better? Anim. Technol. 1989; 40: 45-54.

BARNETT, J.L. and HEMSWORTH, P.H. The effects of individual and group housing on sexual behaviour and pregnancy in pigs. Anim. Reprod. Sci. 1991; 25: 265-273.

BARNETT, J.L., WINFIELD, C.G., CRONIN, G.M., HEMSWORTH, P.H. and DEWAR, A.M. The effect of individual and group housing on behavioural and physiological responses related to the welfare of pregnant pigs. Appl. Anim. Behav. Sci., 1985; 14: 149-161.

BATESON, P. Imprinting as a process of competitive exclusion. In: Rauschecker, R. and Marler, P., eds. Imprinting and cortical plasticity. New York, NY: J. Wiley & Sons, Ltd., 1987.

BAXTER, M.R. Ethology in environmental design for animal production. Appl. Anim. Ethol. 1983; 9: 207-220.

BAYNE, K. Resolving issues of psychological well-being and management of laboratory non-human primates. In: Segal, E.F., ed. Housing, care and psychological well-being of captive and laboratory primates. Park Ridge, NJ: Noyes Publications, 1989: 27-38.

BAYNE, K.A.L. and MCCULLY, C. The effect of cage size on the behaviour of individually-housed rhesus monkeys. Lab Animal, October 1989: 25-28.

BAYNE, K.A.L., SUOMI, S. and BROWN, B. A new monkey swing. Lab. Prim. Newsl. 1989; 28(4): 16-17.

BAYNE, K.A.L., MAINZER, H., DEXTER, S., CAMPBELL, G., YAMADA, F. and SUOMI, S., eds. The reduction of abnormal behaviours in individually housed rhesus monkeys (*Macaca mulatta*) with a foraging/ grooming board. Am. J. Primatol. 1991; 23(1): 23-35.

BEAVER, B.V. Veterinary aspects of feline behavior. St. Louis, MI: Mosby, C.V. 1980.

BEAVER, B.V. Environmental enrichment for laboratory animals. ILAR (Institute for Laboratory Animal Resources) News 1989; 31(2): 5-11.

BEAVER, B.V. Behavioral considerations for laboratory dogs and cats. Cont. Ed. Art. No. 4; 2(4) July/ August 1981: 212-215.

BECK, B.B. Animal tool behaviour: The use and manufacture of tools by animals. New York, NY: Garland, 1980.

BECKER, B.A., FORD, J.J., CHRISTENSON, R.K., MANAK, R.C., HAHN, G.L. and DESHAZAR, J.A. Cortisol response of gilts in tether stalls. J. Anim. Sci. 1985; 60: 264-270.

BERTRAND, M. The behavioural repertoire of the stump-tail macaque. Basel: Karger, S., 1969.

BESCH, E.L. Environmental quality within animal facilities. Lab. Anim. Sci. 1980; 30: 385-406.

BEVAN, W. Sound-precipitated convulsions 1947-1954. Psychol. Bull. 1955; 52(6): 473-504.

BLACKSHAW, J.K. Human and animal inter-relationships. Normal behaviour pattern of cats. I. Aust. Vet. Pract. 1985a; 15(4): 159-162.

BLACKSHAW, J.K. Human and animal inter-relationships: Behavioural problems of cats. II. Aust. Vet. Pract. 1985b; 15(4): 164-168.

BLAFFER-HRDY, S. and WHITTEN, P.L. Patterning of sexual activity. In: Smuts, B.B., Cheney, D.L., Seyfarth, R.M., Wrangham, R.W. and Struhsaker, T.T., eds. Primate societies. Chicago, IL: University of Chicago Press, 1987: 370-384.

BLOOD, D.C. and STUDDERT, V.P. Ballière's comprehensive veterinary dictionary. London: Ballière Tindall, 1988: 265.

BOCCIA, M.L. Preliminary report on the use of a natural foraging task to reduce aggression and stereotypes in socially housed pigtail macaques. Lab. Primate Newsl. 1989; 28: 3-4.

BOYD, J. Mice. Humane Innov. Altern. 1988; 2: 49-50.

BRAMBELL, F.W.R. (Chairman). Report of the Technical Committee to Enquire into the Welfare of Animals

Kept Under Intensive Husbandry Systems. London: Her Majesty's Stationary Office, 1965.

BROOM, D.M. and LEAVER, J.D. Effects of group-rearing or partial isolation on later social behaviour of calves. Anim. Behav. 1978; 26: 1255-1263.

BROOM, D.M. Indicators of poor welfare. Brit. Vet. J. 1986; 142: 524.

BRYANT, C., RUPNIAK, N. and IVERSEN, S. Effects of different environmental enrichment devices on cage stereotypes and autoaggression in captive cynomolgus monkeys. J. Med. Primatol. 1988; 17: 257-269.

BURT, D.A. and PLANT, M. Observations on a caging system for housing stump-tailed macaques. Anim. Technol. 1990; 41(3): 175-179.

CAINE, N.G. Unrecognized anti-predator behaviour can bias observational data. Anim. Behav. 1989; 39 (1): 195-196.

CAMPBELL, S.A., HUGHES, H.C., GRIFFEN, H.E. and LANDI, M.S. Some effects of limited exercise on purpose-bred Beagles. Am. J. Vet. Res. 1988; 49(8): 1298-1301.

CAMPBELL, S.A. Effects of exercise programs on serum biochemical stress indicators in purpose-bred beagle dogs. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 77-80.

CANADIAN COUNCIL ON ANIMAL CARE. Non-human primates. In: Guide to the care and use of experimental animals. Vol. 2. CCAC, Ottawa, Ont. 1984: 163-173.

CARPENTER, E. Animals and ethics. London: Watkins, 1980.

CATCOTT, E.J., ed. Feline medicine and surgery, 2nd Ed. Santa Barbara, CA; American Veterinary Publications, Inc. 1975.

CHAMOVE, A.S. Cage design reduces emotionality in mice. Lab. Anim. 1989; 23: 215-219.

CHAMOVE, A.S. Environmental enrichment: Review. Anim. Technol. 1989; 40(3): 155-174.

CHAMOVE, A.S. and ANDERSON, J.R. Examining environmental enrichment. In: Segal, E.F., ed. Housing, care and psychological well-being of captive and laboratory primates. Park Ridge, NJ: Noyes Publications, 1989: 183-202.

CLOUGH, G. Environmental effects on animals used in biomedical research. Biol. Rev. 1982; 57: 487-523.

COELHO, A.M. and CAREY, K.D. A social tethering system for non-human primates used in laboratory research. Lab. Anim. Sci. 1990; 40(4): 388-394.

COLE, J. *Macaca nemestrina* studied in captivity. In: Napier, J. and Barnicot, N.A., eds. The primates. London: Zoological Society of London, 1963: 105-114.

COPPINGER, T.R., MINTON, J.E., REDDY, P.G. and BLECHA, F. Repeated application of stressor reduces cell mediated immunological function in lambs. J. Anim. Sci. 1990; 68 (Suppl. 1): 77-78 (Abstract).

CROCKETT, C. Psychological well-being and enrichment workshop held at Primate Centers' Directors' meeting. Lab. Prim. Newsl. 1990; 29(3): 3-6.

CRONIN, G.M., VAN TARTWIJK, J.M.F.M., VAN DER HEL, W. and VERSTEGEN, M.W.A. The influence of degree of adaptation to tether housing by sows in relation to behaviour and energy metabolism. Anim. Prod. 1986; 42: 257-268.

CURTIS, S.E. (moderator) Animal Care and Use Committee workshop. In: Mench, J.A., Mayer, S.J. and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Proc. SCAW (Scientists Center for Animal Welfare)--sponsored conference, Agricultural Animals in Research. Bethesda, MD: Scientists Center for Animal Welfare, 1992: 38-48.

DAWKINS, M. From an animal's point of view: motivation, fitness and animal welfare. Behav. Brain Sci. 1990; 13: 1-61.

DRESSER, R. Assessing harm and justification in animal research: Federation policy opens the laboratory door. Rutgers Law Review 1988; 40: 723-795.

DUNBAR, I. Dog behavior. Why dogs do what they do. Neptune, NJ: T.F.H. Publications, Inc., 1979.

DUNCAN, I.J.H. Animal behaviour and welfare. In: Clark, J.A., ed. Environmental aspects of housing for animal production. London: Butterworths, 1981: 455-470.

DUNCAN, I.J.H. and DAWKINS, M.S. The problem of assessing "well-being" and "suffering" in farm animals. In: Schmidt, D., ed. Indicators relevant to farm animal welfare. The Hague: Martinus Nijhoff, 1983: 13-24.

DUNCAN, I.J.H. Behavioral assessment of welfare. In: Mench, J.A., Mayer, S.J., and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Proc. SCAW (Scientists Center for Animal Welfare)--sponsored conference, Agricultural Animals in Research. Bethesda, MD: Scientists Center for Animal Welfare, 1992: 62-68.

DUNCAN, I.J.H. Assessing the effect of housing on welfare. In: Baxter, M.R., MacCormack, J.A.C. and Baxter, S.H., eds. Farm animal housing and welfare. The Hague: Martinus Nijhoff, 1983: 27-35.

ERWIN, J. Aggression in captive macaques: interaction of social and spatial factors. In: Erwin, J., Maples, T.L. and Mitchell, G., eds. Captivity and behaviour: Primates in breeding colonies, laboratories and zoos. New York, NY: Van Nostrand Reinhold, 1979: 139.

ERWIN, J. and DENI, R. Strangers in a strange land: Abnormal behaviors or abnormal environments. In: Erwin, J., Maple, T.L. and Mitchell, G., eds. Captivity and behaviour. Primates in breeding colonies, laboratories and zoos. New York, NY: Van Nostrand Reinhold, 1979: 1-28.

ERWIN, J. Environments for captive propagation of primates. Interaction of social and physical factors. In: Benirschke, K., ed. Primates: the road to self-sustaining populations. New York, NY: Springer Verlag, 1985: 299-305.

EVERITT, J.I., MCLAUGHLIN, S.A. and HELPER, L.C. Diagnostic exercise: eye lesions in rats. Lab. Anim. Sci. 1987; 37: 202-203.

EWBANK, R. The behaviour of animals in restraint. In: Fox, M.W., ed. Abnormal behaviour in animals.

Philadelphia, London, Toronto: W.B. Saunders, 1968: 159-178.

EWBANK, R., PARKER, M.J. and MASON, C.W. Reactions of cattle to head-restraint at stunning: A practical dilemma. Animal Welfare 1992; 1: 55-63. Published by UFAW (Universities Federation for Animal Welfare), South Mimms, Potters Bar, Herts, Essex.

EXPERT COMMITTEE ON FARM ANIMAL WELFARE AND BEHAVIOUR. Farm animal welfare and behaviour in Canada. Report. Ottawa, Ont.: Agriculture Canada, 1987.

FAJZI, K., REINHARDT, V. and SMITH, M.D. A review of environmental enrichment strategies for singly caged non-human primates. Lab Animal 1989; 18(2): 23-35.

FEDIGAN, L. A study of roles in the Arashiyama West troop of Japanese macaques (*Macaca fuscata*). Contributions to Primatology, Vol. 9. Basel: Karger, S. 1976.

FOX, M.W. Socialization, environmental factors, and abnormal behavioural development in animals. In: Fox, M.W., ed. Abnormal behaviour in animals. Philadelphia, London, Toronto: W.B. Saunders, 1968.

FOX, M.W. Understanding your dog. New York, NY: Coward, McGann and Geoghegan, Inc., 1972.

FOX, M.W. Understanding your cat. New York, NY: Coward, McGann and Geoghegan, Inc., 1974.

FOX, M.W. Evolution of social behavior in canids. In: Fox, M.W., ed. The wild canids. New York, NY, Van Nostrand Reinhold, 1975.

FOX, M.W. Laboratory animal husbandry: Ethology, welfare and experimental variables. Albany, NY: State University Press, 1986.

FOX, M.W. Canine behavior. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 21-31.

FRASER, A.F. The behaviour of suffering in animals. Appl. Anim. Behav. Sci. 1984/85; 13: 1-6.

FRASER, A.F. Animal suffering: The appraisal and control of depression and distress in livestock. Appl. Anim. Behav. Sci. 1988; 20: 127-133.

FRASER, A.F. (letters) Welfare and well-being. Vet. Rec. 1989; 125(12): 332.

FRASER, A.F. The behavior of the horse. Tucson, AZ: CAB Int'l., 1992.

FRASER, A.F. and BROOM, D.M. Farm animal behaviour and welfare, 3rd Ed. London, Toronto, Philadelphia: Ballière Tindall, 1990.

FRASER, D. and RUSHEN, J. Aggressive behaviour. Vet. Clin. North Am. Food Anim. Pract. 1987; 3(2): 285.

FRIEND, T.H. and DELLMEIER, G.R. Common practices and problems related to artificially rearing calves: An ethological analysis. Appl. Anim. Behav. Sci. 1988; 20: 47-62.

FRITZ, J. Resocialization of captive chimpanzees: an amelioration procedure. Amer. J. Primatol. Suppl. 1989; 1: 79-86.

GAMBLE, M.R. Sound and its significance for laboratory animals. Biol. Rev. 1982; 57: 395-421.

GÄRTNER, K., ZIESNISS, K., KARSTENS, A. and MUHL, G.I. Differences in personality of isogenic rats living under highly standardized conditions shown by behavioural patterns. Lab. Zhyvotnye 1991; 1(3): 34-44.

GÄRTNER, K., BÜTTNER, D., DÖHLER, R., FRIEDEL, J., LINDENA, J. and TRAUTSCHOLD, I. Stress response of rats to handling and experimental procedures. Lab. Anim. 1980; 14: 267-274.

GONYOU, H.W. The interaction of humans with food animals. In: Appleby, M.C., Horrell, R.I., Petherick, J. C. and Rutter, S.M., eds. Applied animal behaviour: Past, present and future. South Mimms, Potter's Bar, Herts, U.K.: UFAW (Universities Federation for Animal Welfare), 1991: 31.

GOOSEN, C. Abnormal behaviour patterns in rhesus monkeys: symptoms of mental disease? Biol. Psych. 1981; 16: 697-716.

GOVERNMENT OF CANADA. Health of Animals Act, June, 1990, 38-39. Eliz. II, Chapter 21: 387-417.

GRAFEN, A. Do animals recognize kin? Anim. Behav. 1990; 39: 42-54.

GRANDIN, T. Handling and transport of agricultural animals used in research. In: Mench, J.A., Mayer, S. J., and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Proc. SCAW (Scientists Center for Animal Welfare)--sponsored conference, Agricultural Animals in Research. Bethesda, MD: Scientists Center for Animal Welfare, 1992: 74-84.

GROSS, W.B. and SIEGEL, P.B. Long-term exposure of chickens to three levels of social stress. Avian Dis. 1981; 25: 312.

GROSS, W.B. and SIEGEL, P.B. Socialization as a factor in resistance to infection, feed efficiency and response to antigen in chickens. Am. J. Vet. Res. 1982; 43: 20010-20012.

HARLOW, H.F. and HARLOW, M.K. The effectional systems. In: Schrier, A.M., Harlow, H.F. and Stollnitz, F., eds. Behaviour of non-human primates, Vol. 2. New York, NY: Academic Press, 1965: 287-334.

HART, B.L. and PEDERSEN, N.C. Behavior. In: Pedersen, N.C., ed. Feline husbandry. Goleta, CA: American Veterinary Publications, 1991: 289-323.

HEARN, J.P. and DIXSON, A.F. Assessment of comfort and well-being in New World primates. In: Standards of laboratory management. Potters Bar: UFAW (Universities Federation for Animal Welfare), 1984: 206-216.

HEMSWORTH, P.H. and BARNETT, J.L. Human interactions. Vet. Clin. North Am. (3,2, Farm animal behaviour) 1987: 339-356.

HEMSWORTH, P.H., BARNETT, J.L., COLEMAN, G.J. and HANSEN, C. A study of the relationships between the attitudinal and behavioural profiles of stockpersons and the level of fear of humans and reproductive performance of commercial pigs. Appl. Anim. Behav. Sci. 1989; 23: 301-314.

HINDE, R.A. and ROWELL, T.E. Communication by posture and facial expressions in the rhesus monkey (*Macaca mulatta*). Proc. Zool. Soc. of London 1962; 138: 1-21.

HITE, M., HANSON, H.L.M., CONTI, N.R. and MATTIS, P.A. Effects of cage size on patterns of activity and health of beagle dogs. Lab. Anim. Sci. 1977; 27: 60-64.

HOBBS, K.R. Hamsters. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 377-392.

HOLLANDS, C. Compassion is the bugler. The struggle for animal rights. Edinburgh: Macdonald Publishers, 1980.

HUGHES, B.O. and DUNCAN, I.J.H. The notion of ethological "need," models of motivation and animal welfare. Anim. Behav. 1988; 36: 1969-1707.

HUGHES, B.O., DUNCAN, I.J.H. and BROWN, M.F. The performance of nest building by domestic hens: is it more important than the construction of a nest? Anim. Behav. 1989; 37: 210-214.

HURNI, H. and ROSSBACH, W. The laboratory cat. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 476-492.

HUGHES, H.C., CAMPBELL, S. and KENNEY, C. The effects of cage size and pair housing on exercise of beagle dogs. Lab. Anim. Sci. 1989; 39(4): 302-305.

HUGHES, H.C. and CAMPBELL, S. Effects of primary enclosure size and human contact. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 66-73.

HURNIK, J.F. Welfare of farm animals. Appl. Anim. Behav. Sci. 1988; 20: 105-117.

INSTITUTE OF LABORATORY ANIMAL RESOURCES. Laboratory animal management: rodents. Washington, DC: National Academy of Sciences, 1977.

ITURRIAN, W.B. and FINK, G.B. Comparison of bedding material, habitat preference of pregnant mice, and reproductive performance. Lab. Anim. Care 1968; 18: 160-164.

JEROME, C.P. and SZOSTAK, L. Environmental enrichment for adult female baboons (*Papio anubis*). Lab. Anim. Sci. 1987; 37: 508-509.

JOLLY, A. Social group, breeding group, foraging group. Ecol. 1985: 116-119.

JONES, C. Natural diets of wild primates. In: T-W-Finnes, R.N., ed. Pathology of simian primates. New York, NY: S. Karger, 1972.

KAPLAN, J.R., MANNING, P. and ZUCKER, E. Reduction of mortality due to fighting in a colony of rhesus monkeys (*Macaca mulatta*). Lab. Anim. Sci. 1980; 30(3): 565-570.

KARSH, E.B. and TURNER, D.C. The human-cat relationship. In: Turner, D.C. and Bateson, P., eds. The

domestic cat. The biology of its behaviour. Cambridge: Cambridge University Press, 1990: 159-177.

KELLEY, K.W., OSBORNE, C.A., EVERMANN, J.F., PARISH, S.M. and HINRICHS, D.J., eds. Whole blood leucocytes vs. separated mononuclear cell blastogenesis in calves, time dependent changes after shipping. Can. J. Comp. Med. 1981; 45: 249-258.

KENNY, F.J. and TARRANT, P.V. Behaviour of cattle during transport and penning before slaughter. In: Moss, R., ed. Transport of animals intended for breeding, production and slaughter. Curr. Top. Vet. Med. Anim. Sci. 1982; 18: 87-102. The Hague: Martinus Nijhoff.

KILGOUR, R. and DALTON, C. Livestock behaviour: a practical guide. London: Granada, 1984.

LANG, C.M. and VESSELL, E.S. Environmental and genetic factors affecting laboratory animals: impact on biomedical research. Fed. Proc. 1976; 35: 1123-1165.

LES, E.P. A disease related to cage population density: tail lesions of C3H/HeJ mice. Lab. Anim. Sci. 1972; 22: 56-60.

LEVINE, S. A definition of stress? In: Moberg, G.P., ed. Animal stress. Bethesda, MD: American Physiological Society, 1985: 51-69.

LEYHAUSEN, P. The tame and the wild--another Just-So story? In: Turner, D.C. and Bateson, P., eds. The domestic cat. The biology of its behaviour. Cambridge: Cambridge University Press, 1990: 57-66.

LIBERG, O. and SANDELL, M. Spatial organization and reproductive tactics in the domestic cat and other felids. In: Turner, D.C. and Bateson, P., eds. The domestic cat. The biology of its behaviour. Cambridge: Cambridge University Press, 1990: 83-98.

LINE, S.W. Environmental enrichment for laboratory primates. J. Amer. Vet. Med. Assoc. 1987; 190: 854-859.

LINE, S.W., CLARKE, A.S., ELLMAN, G. and MARKOWITZ, H. Behavioral and hormonal responses of rhesus monkeys to an environmental enrichment apparatus. Amer. Vet. Soc. Anim. Behav. Newsl. 1987; 10: 6-7.

LINE, S.W., CLARKE, A.S. and MARKOWITZ, H. Adult female rhesus macaque responses to novel objects. Lab Animal 1989; May/June: 33-40.

LINE, S.W., MORGAN, K., MARKOWITZ, H. and STRONG, S. Heart rate and activity of rhesus monkeys in response to routine events. Lab. Prim. Newsl. 1989; 28(2): 1-4.

LOVE, J.A. Housing for rabbits. Humane Innov. Altern. Anim. Exper. 1988; 2: 47-48. Saranac Lake, NY: Currier Press (Psychologists for the Ethical Treatment of Animals), 1988.

MACARTHUR, J.A. The dog. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 456-475.

MACDONALD, D.W. and MOEHLMAN, P.D. Co-operation, altruism, and restraint in the reproduction of carnivores. In: Bateson, P.P.G. and Klopfer, P., eds. Perspectives in ethology. London: Plenum Press, 1982.

MARKOWITZ, H. and SPINELLI, J. Environmental engineering for primates. In: Benirschke, K., ed. Primates. The road to self-sustaining populations. New York, NY: Springer-Verlag, 1986: 489-498.

MARKOWITZ, H. and LAFORSE, S. Artificial prey as behavioural enrichment devices for felines. Appl. Anim. Behav. Sci., 1987; 18(1): 31-44.

MARKOWITZ, H. and LINE, S. Primate research models and environmental enrichment. In: Segal, E.F., ed. Housing care and psychological well-being of captive and laboratory primates. Park Ridge, NJ: Noyes Publications, 1989: 203-212.

MARTIN, L. Which ape is man's closest kin? The Sciences March/April 1988: 52-58.

MARTIN, P. The time and energy costs of play behaviour in the cat. Z. Tierpsychol. 1984; 64: 298.

MARTINIC, G. A short report on the construction of an ethogram from observations made on four *Macaca fascilularis* monkeys. Anim. Technol. 1990; 41(3): 217-222.

MCCARTHY, C. Public Health Service Policy. In: National Institutes of Health, Office for Protection from Research Risks/Office of Animal Care and Use. Animal care and use: Policy issues in the 1990s. Bethesda, MD: NIH, 1989: 5-11.

MCGLONE, J.J. and CURTIS, S.E. Behaviour and performance of weanling pigs in pens equipped with hide areas. J. Anim. Sci. 1985; 60: 20-24.

MCKEOWN, D. and LUESCHER, A. Handbook of feline behaviour. Ontario Veterinary College, University of Guelph. In Press.

MCSHEEHY, T. An overview of the state-of-the-art of environmental monitoring. In: Melby, E.C. and Balk, M.W., eds. The importance of laboratory animal genetics, health, and the environment in biomedical research. New York, Toronto: Academic Press, 1983: 161-182.

MCWILLIAM, A.A. Important reports on HPB monkey colony due soon. CCAC (Canadian Council on Animal Care) Resource 1989; 14(1): 1,4.

MENCH, J.A., STRICKLIN, W.R. and PURCELL, D. Social and spacing behaviour. In: Mench, J.A., Mayer, S. J. and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Proc. SCAW (Scientists Center for Animal Welfare)--sponsored conference, Agricultural Animals in Research. Bethesda, MD: Scientists Center for Animal Welfare, 1992: 69-73.

MOBERG, G.P. Biological response to stress: key to assessment of animal well-being? In: Moberg, G.P., ed. Animal stress. Bethesda, MD: Amer. Physiol. Soc., 1985: 27-49.

MOBERG, G.P. Stress: Diagnosis, cost and management. In: Mench, J.A., Mayer, S.J. and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Proc. SCAW conf. "Agricultural Animals in Research." Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992: 58-61.

MOOR-JANKOWSKI, J. and MAHONEY, C.J. Chimpanzees in captivity: humane handling and breeding within the confines imposed by biomedical research and testing. J. Med. Primatol. 1989; 18: 1-26.

MORRIS, D. Catwatching. The essential guide to cat behaviour. London: Cape, 1986.

MORTON, D.B. and GRIFFITHS, P.H.M. Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. Vet. Rec. 1985; 116(16): 431-436.

MURPHY, R.A., ROWAN, A.N. and SMEBY, R.R., eds. Annotated bibliography on laboratory animal welfare. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1991.

NEWTON, W.M. Environmental impact on laboratory animals. Advance Vet. Sci. Comp. Med. 1978; 22: 1-28.

NISHIDA, T. and HIRAIWA-HASEGAWA, M. Chimpanzees and bonobos: Co-operative relationships among males. In: Smuts, B.B., Cheney, D.L., Seyfarth, R.M., Wrangham, R.W. and Struhsaker, T.T., eds. Primate societies. Chicago, IL: University of Chicago Press, 1987: 165.

NORRIS, M.L. Gerbils. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 360-376.

NOVAK, M.A. and MEYER, J.S. What we don't know about lab animals. Lab. Primate Newsl. 1988; 27(2): 16-17.

NOVAK, M.A. and SUOMI, S.J. Psychological well-being of primates in captivity. Amer. Psychol. 1988; 43 (10): 765-773.

NOVAK, M.A. and DREWSEN, K.H. Enriching the lives of captive primates. Issues and problems. In: Segal, E.F., ed. Housing, care and psychological well-being of captive and laboratory primates. Park Ridge, NJ: Noyes Publications, 1989: 161-182.

O'KEEFE, R.T. and LEFSHITZ, K. A behavioural profile for stump-tail macaques (*Macaca arctoides*). Primates 1985; 26: 143-160.

O'NEILL, P. A room with a view for captive primates: issues, goals, related research and strategies. In: Segal, E.F., ed. Housing, care and psychological well-being of captive and laboratory primates. Park Ridge, NJ: Noyes Publications, 1989: 135-160.

PANEPINTO, L.M. The minimum-stress physical restraint of swine and sheep in the laboratory. In: Mench, J.A., Mayer, S.J. and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Proc. SCAW (Scientists Center for Animal Welfare)--sponsored conference Agricultural Animals in Research. Bethesda, MD: Scientists Center for Animal Welfare, 1992: 85-87.

PASSINGHAM, R.E. The human primate. San Francisco, CA: W.H. Freeman & Co., 1982.

PENG, X., LANG, C.M., DROZDOWICZ, C.D. and OHLSSON-WILHELM, B.M. Effect of cage population density on plasma corticosterone and peripheral lymphocyte populations of laboratory mice. Lab. Anim. 1989; 23: 302-306.

PETERSON, E.A. Noise and laboratory animals. Lab. Anim. Sci. 1980; 30(2), Part II: 422-439.

PFAFFENBERGER, C. New knowledge of dog behaviour. New York, NY: Howell, 1963.

REINHARDT, V., REINHARDT, A. and HOUSER, D. Hair pulling-and-eating in captive rhesus monkeys. Folia Primatol. 1986; 47: 158-164.

REINHARDT, V. Advantages of housing rhesus monkeys in compatible pairs. SCAW (Scientists Center for Animal Welfare) Newsl. 1987; 9(3): 3,5-6.

REINHARDT, V., HOUSER, D., EISELE, S. and CHAMPOUX, M. Social enrichment of the environment with infants for singly-caged adult rhesus monkeys. Zoo Biology 1987; 6: 365-371.

REINHARDT, V., REINHARDT, A., EISELE, S., HOUSER, D. and WOLF, J. Control of excessive aggressive disturbance in a heterogenous troop of rhesus monkeys. Appl. Anim. Behav. Sci. 1987; 18: 371-377.

REINHARDT, V., HOUSER, D., EISELE, S., COWLEY, D. and VERTEIN, R. Behavioral responses of unrelated rhesus monkey females paired for the purpose of environmental enrichment. Am. J. Primatol. 1988; 14: 135-140.

REINHARDT, V. Preliminary comments on pairing unfamiliar adult male rhesus monkeys for the purpose of environmental enrichment. Lab. Prim. Newsl. 1988; 27: 1-3.

REINHARDT, V. Social enrichment for laboratory primates: A critical review. Lab. Prim. Newsl. 1990a; 29 (3): 7-11.

REINHARDT, V. Evaluating the effectiveness of environmental enrichment. Lab. Prim. Newsl. 1990b; 29 (1): 15.

REINHARDT, V. Privacy panel for isosexual pairs of caged rhesus monkeys. Am. J. Primatol. 1990c; 20: 225-226 (abstract).

REINHARDT, V., COWLEY, D., SCHEFFLER, J. and VERTEIN, R. Living continuously with a compatible companion is not a distressing experience for rhesus monkeys. Lab. Prim. Newsl. 1990; 29(2): 16-17.

REINHARDT, V. Agonistic behaviour responses of socially experienced unfamiliar adult male monkeys (*Macaca mulatta*) to pairing. Lab. Prim. Newsl. 1991; 30(1): 5-7.

RILEY, V. Mouse mammary tumours: alteration of incidence as apparent function of stress. Science 1975; 189: 465-467.

RINGLER, D.H. and PETER, G.K. Dogs and cats as laboratory animals. In: Fox, G.J., ed. New York, NY: Academic Press, 1984: 241-271.

ROSENZWEIG, M.R. and BENNETT, E.L. Effects of environmental enrichment or impoverishment on learning and on brain values in rodents. In: Oliveerio, A., ed. Genetics, environment and intelligence. New York, NY: Elsevier, 1977: 163-196.

ROYAL SOCIETY FOR THE PREVENTION OF CRUELTY TO ANIMALS. The assessment of stress in laboratory animals. Causeway, Horsham, West Sussex, U.K.: RSPCA, 1992.

RUPPENTHAL, G.C. and WALKER, C.G. Behavioral development of "together-together" reared pigtailed monkeys. Am. J. Primatol. Suppl. 1989; 18: 164.

SACKETT, G.P., RUPPENTHAL, G.C., FAHRENBRUCH, C.E. and HOLM, R.A. Social isolation rearing effects in monkeys vary with genotype. Devel. Psychol. 1981; 17(3): 313-318.

SAPOLSKY, R. Physiological perspectives on non-human primate well-being. SCAW (Scientists Center for Animal Welfare) Newsl. 1989; 11(3): 4-8.

SCHAR, R. Influence of man on life and social behaviour of farm cats. Poster, Int'l. Symp. on the Human/ Pet Relationship, Vienna, 1983.

SCHOUTEN, W., RUSHEN, J. and DE PASSILLÉ, A.M.B. Stereotypic behaviour and heart rate in pigs. Physiol. Behav. 1991; 48: 91-96.

SCHWINDAMAN, D.F. Regulatory requirements for exercise of dogs. In: Mench, J.A. and Krulisch, L. eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 3-7.

SCOTT, J.P. and FULLER, J.L. Genetics and the social behavior of the dog. Chicago, IL: University of Chicago Press, 1965.

SCOTT, P.P. Nutrition and disease. In: Catcott, E.J., ed. Feline medicine and surgery. 2nd Ed. Santa Barbara, CA: American Veterinary Publications, Inc., 1975: 131-144.

SEABROOK, M.F. The role of the stockman in livestock productivity and management. In: Seabrook, M.F., ed. The role of the stockman in livestock production and management. Brussels: Commission of the European Communities Report EUR 10982 EN, 1987: 35-51.

SEABROOK, M.F. The psychological interaction between the stockman and his animals and its influence on performance of pigs and dairy cows. Vet. Rec. 1984; 115: 85-87.

SEAMER, J.H. Farm animal welfare in Britain. SCAW (Scientists Center for Animal Welfare) Newsl. 1993; 14(4): 13-14.

SEGAL, E.F. (commentary) ILAR (Institute for Laboratory Animal Resources) News 1989a; 31(2): 11-12.

SEGAL, E.F., ed. The housing, care, and psychological well-being of captive and laboratory primates. Park Ridge, NJ: Noyes Publications, 1989b.

SEITZ, P.F.D. Infantile experience and adult behaviour in animal subjects. II. Age of separation from the mother and adult behaviour in the cat. Psychosom. Med. 1959; 21: 353-378.

SEMPLE-ROWLAND, S.L. and DAWSON, W.W. Retinal cyclic light damage threshold for albino rats. Lab. Anim. Sci. 1987; 37(3): 289-298.

SKINNER, S.W. and LOCKARD, J.S. An ethogram of the lion-tailed macaque (*Macaca silenus*) in captivity. Appl. Anim. Ethol. 1979; 5: 241-253.

SMELSER, J.F. Rabbits: a practical guide for the veterinary technician. Vet. Tech. 1985; 6(3): 121-129.

SMUTS, B.B., CHENEY, D.L., SEYFARTH, R.M., WRANGHAM, R.W. and STRUHSAKER, T.T., eds. Primate societies. Chicago, IL: University of Chicago Press, 1987.

SNOWD0N, C.T. Variables among species and individual differences within species. In: Mench, J.A. and Krulisch, L., eds. Well-being of non-human primates in research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 26-31.

SNOWDON, C.T., SAVAGE, A. and MCCONNELL, P.B. A breeding colony of cotton-top tamarins (*Saguinus oedipus*). Lab. Anim. Sci. 1984; 35: 477-480.

SPEDDING, C.R.W. Animal welfare and the BSAP. Speech delivered at the Winter meeting of the British Society of Animal Production, Scarborough, U.K. 1985. As quoted in CURTIS, S.E. Animals in food production--American issues. Appl. Anim. Behav. Sci. 1988; 20: 151-157.

SPINELLI, J.S. (commentary) Environmental enrichment for laboratory animals. ILAR (Institute for Laboratory Animal Resources) News 1989; 31(2): 12-13.

STAUFFACHER, M. Group housing and enrichment cages for breeding, fattening and laboratory rabbits. UFAW (Universities Federation for Animal Welfare) Animal Welfare 1992; 1: 105-125.

STRICKLIN, W.R., PURCELL, D. and MENCH, J.A. Farm animals in agricultural and biomedical research. In: Mench, J.A., Mayer, S.J. and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Proc. SCAW (Scientists Center for Animal Welfare)--sponsored conference, Agricultural Animals in Research. Bethesda, MD: Scientists Center for Animal Welfare, 1992: 1-4.

SUTHERLAND, S.D. and FESTING, M.F.W. The guinea pig. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 393-410.

TOLAN, J.C., MALONE, D.R. and ROGERS, C.M. An exercise cage for monkeys. Lab. Prim. Newsl. 1980; 19 (1): 3-5.

U.S. DEPARTMENT OF AGRICULTURE. Federal Register. Part III. 54(49) March 15, 1989: 10887.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, PUBLIC HEALTH SERVICE/NATIONAL INSTITUTES OF HEALTH. Guide for the care and use of laboratory animals. 1985: 21.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES. Guide for the care and use of laboratory animals. Bethesda, MD: NIH, 1985.

U.S. FED. REG. July 16, 1990; 55(136): 28877-29000.

UNIVERSITIES FEDERATION FOR ANIMAL WELFARE. Guidelines on the care of laboratory animals and their use for scientific purposes: 1. Housing and care. UFAW, 1987. (8 Hamilton Close, South Mimms, Potters Bar, Herts, EN6 3QD.)

VAN HOOF, J.A. Facial expressions in higher primates. In: The primates. London: Zoological Society of London, 1963: 103-104.

VAN HOOF, J.A. The facial displays of the catarrhine monkeys and apes. In: Morris, D., ed. Primate ethology. London: Weidenfeld and Nicolson, 1967.

VAN PUTTEN, G. Farming beyond the ability for pigs to adapt. Appl. Anim. Behav. Sci. 1988; 20: 63-71.

VANDERLIP, S.L., VANDERLIP, J.E. and MYLES, S. A socializing program for laboratory-raised canines. Part 1. Lab Animal 1985; 14(1): 33-36.

VANDERLIP, S.L., VANDERLIP, J.E. and MYLES, S. A socializing program for laboratory-raised canines. Part 2: the puppy socialization schedule. Lab Animal 1985; 14(2): 27-36.

VON BORELL, E. and LADEWIG, J. Altered adrenocortical response to acute stressors or ACTH(1-24) in intensively housed pigs. Domest. Anim. Endocrin. 1989; 6: 299-309.

WALSH, S., BRAMBLETT, C.A. and ALFORD, P.L. A vocabulary of abnormal behaviours in restrictively reared chimpanzees. Am. J. Primatol. 1982; 3: 315-319.

WEBSTER, A.J.F. Meat and right: farming as if the animal mattered. Can. Vet. J. 1987; 28(8): 462-465.

WEIHE, W.H. The laboratory rat. In: Poole, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 309-330.

WESTERGAARD, G.C. Lion-tailed macaques (*Macaca silenus*) manufacture and use tools. J. Comp. Psychol. 1988; 102(2): 152-159.

WHITE, W.J., BALK, M.W. and LANG, C.M. Use of cage space by guinea pigs. Lab. Anim. 1989; 23: 208-214.

WILSON, S.F. Environmental influences on the activity of captive apes. Zoo. Biol. 1982; 1: 201-209.

WOLFF, A. and RUPPERT, G. A practical assessment of a non-human primate exercise program. Lab Animal 1991; 20(2): 36-38.

WOLFLE, T.L. Laboratory animal technicians: their role in stress reduction and human-companion animal bonding. In: Symposium on the human-animal bond. Quackenbush, J. and Voith, V.L., eds. Vet. Clin. of North Amer. Sm. Anim. Pract. 1985; 15(2): 449-454.

WOLFLE, T.L. Dog socialization. In: National Institutes of Health. Animal care and use: policy issues in the 1990s. Bethesda, MD: NIH, 1989a.

WOLFLE, T.L. The behavior of people around animals. In: Stark, D., ed. Behavior and well-being of laboratory animals. Monograph Series I, American Association for Laboratory Animal Science: Cordova, TN, 1989b: 1-2.

WOLFLE, T.L. Policy, program and people: The three p's to well-being. In: Mench, J.A. and Krulisch, L., eds. Canine research environment. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 41-47.

WOLFLE, T.L. Non-human primate well-being: an issue of science or politics? In: Mench, J.A. and Krulisch, L., eds. Well-being of non-human primates in research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990: 64-71.

WOOLVERTON, W.L., ATOR, N.A., BEARDSLEY, P.M. and CARROLL, M.E. Effects of environmental conditions on the psychological well-being of primates: a review of the literature. Life Sci. 1989; 44: 901-917.

WORLD VETERINARY ASSOCIATION. WVA policy statement on animal welfare, well-being and ethology. ILAR (Institute for Laboratory Animal Resources) News 1989; 31(4): 29-30.

Additional Reading

AMERICAN FISHERIES SOCIETY, AMERICAN INSTITUTE OF FISHERIES RESEARCH BIOLOGISTS, AMERICAN SOCIETY OF ICHTHYOLOGISTS AND HERPETOLOGISTS. Guidelines for use of fishes in field research. 1987.

AMERICAN MEDICAL ASSOCIATION. Animals in research. JAMA 1989; 261(245): 3602-3606.

AMERICAN ORNITHOLOGISTS UNION, COOPER ORNITHOLOGICAL SOCIETY, WILSON ORNITHOLOGICAL SOCIETY, Report of Ad Hoc Committee on the Use of Wild Birds in Research. AUK 1988; 105 (Suppl. 1): 1A-41A.

AMERICAN SOCIETY OF ICHTHYOLOGISTS AND HERPETOLOGISTS, THE HERPETOLOGISTS LEAGUE, AND SOCIETY FOR THE STUDY OF AMPHIBIANS AND REPTILES. Guidelines for use of live amphibians and reptiles in field research. 1987.

AMERICAN SOCIETY OF MAMMALOGISTS' AD HOC COMMITTEE ON ACCEPTABLE FIELD METHODS IN MAMMALOGY. Acceptable field methods in mammalogy. 1987.

ANDERSON, A.C. Outdoor kennel for dogs at the school of veterinary medicine, University of California, Davis. J. Amer. Vet. Med. Assoc. 1960; 137: 129-135.

ANDERSON, J.R. and CHAMOVE, A.S. Self-aggressive behaviour in monkeys. Curr. Psychol. Rev. 1981; 1: 139-158.

ANDERSON, J.R. and CHAMOVE, A.S. Early social experience and responses to visual social stimuli in young monkeys. Curr. Psychol. Res. and Rev. 1984; 3: 32-45.

ANIMAL WELFARE ACT, 7 U.S.C. 2131, et seq. (Public Law 89-544, 1966, as amended.) Implementing regulations are published in the Code of Federal Regulations (CFR), Title 9, Subchapter A, Parts 1, 2, 3 and 4 and are administered by the U.S. Department of Agriculture.

ANON. Cat care, management and feeding. Lab. Anim. Digest 1967; 3(1): 3-6.

ANON. Should your pet travel by air? Consumer Reports 1973; 38(3): 200-201.

ANON. Correcting house-soiling problems in cats. Mod. Vet. Pract. 1985; 66(1): 53-54.

ANON. Welfare and behavioural problems in domestic animals. Austral. Vet. J. 1985; 62(6): 199-200.

ANON. Reasons for liking and choosing a cat as a pet. Austral. Vet. J. 1988; 65(10): 332-333.

ARCHER, J. Animals under stress. London: Edward Arnold, 1979.

ARDREY, R. The territorial imperative. New York, NY: Dell Publishing Co. 1966.

BAKER, H.J., LINDSAY, J.R. and WEISBROTH, S.H., eds. The laboratory rat. Vol. 1. Biology and diseases. New York, London, Toronto, Sydney, San Francisco: Academic Press, 1979.

BARNETT, J.L. The physiological concept of stress is useful for assessing welfare. Austral. Vet. J. 1987; 64 (6): 195-196.

BAXTER, M.R. Needs - behavioural or psychological? Appl. Anim. Behav. Sci. 1988; 19(3-4): 5-12.

BELZUNG, C. and ANDERSON, J.R. Social rank and responses to feeding competition in rhesus monkeys. Behav. Proc. 1986; 12: 307-316.

BERKSON, G., MASON, W. and SAXON, S. Situation and stimulus effects on stereotyped behaviour of chimpanzees. J. Comp. Physiol. Psychol. 1963; 56: 786-792.

BESCH, E.L., KADONO, H. and BRIGMON, R.L. Body temperature changes in dogs exposed to varying effective temperatures. Lab. Anim. Sci. 1984; 34(2): 177-180.

BITO, L.Z. Animal restrainers. Lab. Anim. Care 1969; 19(2): 244-246.

BLACKSHAW, J.K. Human and animal inter-relationships...: Normal cat behaviour. Austral. Vet. Pract. 1986; 16(1): 19-22.

BLACKSHAW, J.K. Abnormal behaviour in cats. Austral. Vet. J. 1988; 65(12): 395-396.

BLOOMSMITH, M.A. Feeding enrichment for captive great apes. In: Segal, E.F., ed. Housing, care and psychological wellbeing of captive and laboratory primates. Park Ridge, NJ: Noyes Publications, 1989: 336-356.

BLOOMSTRAND, M., ALFORD, P.L. and MAPLE, T.L. An analysis of feeding enrichment for captive chimpanzee. Paper presented at meeting American Society of Primatologists, Madison, WI. 1987.

BLOOMSTRAND, M., RIDDLE, K., ALFORD, P. and MAPLE, T.L. Objective evaluation of a behavioural enrichment device for captive chimpanzees (*Pan troglodytes*). Zoo Biol. 1986; 5: 293-300.

BOVARD, R. Social stimulation and the response to stress. Psychol. Rev. 1959; 58: 267-287.

CAMPBELL, W.E. Correcting house-soiling problems in cats. Clin. Insight 1988; 3(11): 528.

CHAMOVE, A.S. Non-genetic induction of acquired levels of aggression. J. Abnor. Psychol. 1980; 89: 469-488.

CHAMOVE, A.S. Establishment of a breeding colony of stump-tailed monkeys (*Macaca arctoides*). Lab. Anim. 1981; 15: 251-259.

CHAMOVE, A.S. Role of vision in social interaction in monkeys. Child Dev. 1984; 55: 1394-1411.

CHAMOVE, A.S. Long-term learning deficits of mentally retarded monkeys. Am. J. Ment. Defic. 1984; 88:

352-368.

CHAMOVE, A.S. Exercise improves behaviour: A rationale for occupational therapy. Brit. J. Occup. Therapy 1986; 49: 83-86.

CHAMOVE, A.S. and ROHRHUBER, B. Moving callitrichid monkeys from cages to outside areas. Zoo Biol. 1989; 8: 151-163.

CHAMOVE, A.S. and ANDERSON, J.R. Woodchip litter in macaque groups. J. Inst. Anim. Technol. 1979; 30: 69-74.

CHAMOVE, A.S. and BOWMAN, R.E. Rhesus plasma cortisol response at four dominance positions. Aggress. Behav. 1978; 4: 43-55.

CHAMOVE, A.S. and ANDERSON, J.R. Self-aggression, stereotypy and self-injurious behaviour in man and monkeys. Curr. Psychol. Rev. 1981; 1: 245-256.

CHAMOVE, A.S., ANDERSON, J.R. and NASH, V.J. Social and environmental influences on self-aggression in monkeys. Primates 1984; 25: 319-325.

CHAMOVE, A.S., BAYART, F., NASH, V.J. and ANDERSON, J.R. Dominance, physiology and self-aggression in monkeys. Aggress. Behav. 1985; 11: 17-26.

CHAMOVE, A.S., HOSEY, J. and SCHAETZEL, P. Visitors excite primates in zoos. Zoo Biol. 1988; 7: 359-369.

CHAMOVE, A.S., ANDERSON, J.R., MORGAN-JONES, S.C. and JONES, S.P. Deep woodchip litter: hygiene, feeding, and behavioural enhancement in eight primate species. Int. J. Stud. Anim. Prob. 1982; 3: 308-318.

CHAPMAN, C. The influence of habitat on behaviour in a group of St. Kitt's green monkeys. J. Zool., London (A) 1985; 206: 311-320.

CHAURAND, J.P. (Anxiety in Cats) (French). Pointe veterinaire 1987; 19(108): 497-502.

CHEEKE, P.R. Rabbit feeding and nutrition. New York, Toronto: Academic Press, 1987.

CLARK, M.M. Effects of rearing environment on adrenal weights, sexual development, and behaviour in gerbils. An examination of Richter's domestication hypothesis. J. Comp. Physiol. Psychol. 1980; 94: 857-863.

CLUTTON-BROCK, T.H. and HARVEY, P.H. Species differences in feeding and ranging behaviour in primates. In: Clutton-Brock, T.H., ed. Primate ecology. London: Academic Press, 1977.

de WAAL, F.B.M., VAN HOOF, J.A. and NETTO, W. An ethological analysis of types of agonistic interaction in a captive group of Java monkeys. Primates 1976; 17: 257-290.

DIAMOND, J.M. Why cats have nine lives. Nature 1988; 332(6165): 586-587.

DRAPER, W.A. and BERNSTEIN, I.S. Stereotyped behaviour and cage size. Percept. Mot. Skills 1963; 16:

231.

ELTON, R.H. Baboon behaviour under crowded conditions. In: Erwin, J., Maple, T.L. and Mitchell, G., eds. Captivity and behaviour of primates in breeding colonies, laboratories and zoos. New York, NY: Van Nostrand Reinhold, 1979: 125-138.

ERWIN, J., ANDERSON, B., ERWIN, N., LEWIS, L. and FLYNN, D. Aggression in captive groups of pigtail monkeys. Effects of provision of cover. Percept. Mot. Skills 1976; 42: 219-224.

EVANS, S. Captive management of marmosets and tamarins. In: Standards in laboratory animal management. Potters Bar, England: UFAW (Universities Federation for Animal Welfare), 1984: 250-252.

EWBANK, R. Use and abuse of the term 'stress' in husbandry and welfare. Vet. Rec. 1973; 30: 709-710.

FEISTNER, A.T.C. and CHAMOVE, A.S. High motivation toward food increases food-sharing in cotton top tamarins. Develop. Psychobiol. 1986; 19: 439-452.

FENTRESS, J.C. and RYON, J. A long-term study of distributed pup feeding in captive wolves. In: Harrington, F.H. and Paquet, P.C., eds. Wolves of the world: perspectives of behavior, ecology and conservation. Park Ridge, NJ: Noyes Publications, 1982: 238-261.

FERRARI, A. (Anxiety in cats, a clinical disorder?) (Italian). Obiettivi e documenti veterinari 1988; 9(9): 15-17.

FINLAY, T., JAMES, L.R. and MAPLE, T.L. Peoples' perceptions of animals: the influence of the zoo environment. Environ. Behav. 1988; 20: 508-528.

FOSTER, H.L., SMALL, J.D. and FOX, J.G., eds. The mouse in biomedical research. III. History, genetics and wild mice. New York, London, Toronto, Sydney, San Francisco: Academic Press, 1982.

IBID. Vol. II. Diseases.

IBID. Vol. III. Normative biology, immunology and husbandry (1983).

IBID. Vol. IV. Experimental biology and oncology.

FOUTS, R.S., ABSHIRE, M.L., BODAMER, M. and FOUTS, D.H. Signs of enrichment: toward the psychological well-being of chimpanzees. In: Segal, E.F., ed. Housing, care and psychological well-being of captive and laboratory primates. Philadelphia, PA: Noyes Publications, 1989: 376-388.

FOX, M.W. Environmental factors influencing stereotyped...behaviour. Lab. Anim. Care 1965; 15(5): 363-370.

FOX, M.W. Canine pediatrics: Development, neonatal and congenital diseases. Springfield, IL: Charles C. Thomas, 1966.

FOX, M.W. The dog: Its domestication and behavior. New York, NY: Garland, 1978.

GAGNON, A.C. (Behaviour of cats towards plants) (French). Recueil de med. vet. 1987; 163(10): 889-892.

GARBER, P.A. Locomotor behaviour and feeding ecology of the Panamanian tamarin (*Saguinus oedipus geoffroyi*, Callitrichidae Primates). Inter. J. Primatol. 1980; 1: 185-201.

GARBER, P.A. and SUSSMAN, R.W. Ecological distinctions between sympatric species of *Saguinus*. Amer. J. Phys. Anthropol. 1984; 65: 135-146.

GARBER, P.A. Influence of group size on dietary and foraging patterns in *Saguinus maystax* and *Saguinus fuscicollis* in Amazon Peru. Primate Rep. 1986; 14: 12.

GILBERT, S.G. and WRENSHALL, E. Environmental enrichment for monkeys used in behavioural toxicology studies. In: Segal, E.F., ed. Housing, care and psychological well-being of captive and laboratory primates. Philadelphia, PA: Noyes Publications, 1989: 244-254.

GLATSTON, A.R., GELLVOET-SOETEMAN, E., HORA-PECEK, E. and VAN HOOFF, J.A.R.A.M. The influence of the zoo environment on social behaviour of groups of cotton-topped tamarins, *Saguinus oedipus*. Zoo Biol. 1984; 3: 241-253.

GOOSEN, C., FRANSEN, S. and GOMMERS, M.V.D. Social aspects of abnormal locomotion stereotypy. Primate Rep. 1986; 14: 166.

GOOSEN, C., VAN DER GULDEN, W., ROZEMOND, H. *et al*. Recommendations for the housing of macaque monkeys. Lab. Anim. 1984; 18: 99-102.

GOULD, F. and BRES, M. Regurgitation and reingestion in captive gorillas. Description and intervention. Zoo Biol. 1986; 5: 241-250.

HARKNESS, J.E. and WAGNER, J.E. The biology and medicine of rodents and rabbits. 3rd Ed. Philadelphia, PA: Lea and Febiger, 1989.

HARLOW, H.F. Learning and satiation of responses in intrinsically motivated complex puzzle performance by monkeys. J. Compar. Physiol. Psychol. 1950; 43: 289-294.

HARLOW, H.F. Learning to love. San Francisco, CA: Albion, 1971.

HARRISON, M.J.S. Age and sex differences in the diet and feeding strategies of the green monkey (*Cercopithecus sabaeus*). Anim. Behav. 1983; 31: 969-977.

HART, B.L. I. Solving feline behavioural problems. Tijdschrift voor diergeneeskunde 1989; 114 (Suppl. 1): 64s-70s.

HOME OFFICE. Animals (Scientific Procedures) Act 1986. Code of practice for the housing and care of animals used in scientific procedures. London: Her Majesty's Stationary Office; February 7, 1989.

HOSEY, G.R. and DRUCK, P.L. The influence of zoo visitors on the behaviour of captive primates. Appl. Anim. Behav. Sci. 1987; 18: 19-29.

HUGHES, B.O. Behavioural wisdom and preference tests. Appl. Anim. Ethol. 1977; 3: 391-392.

HUGHES, B.O. and DUNCAN, I.J.H. Behavioural needs: can they be explained in terms of motivational

models? Appl. Anim. Behav. Sci. 1988; 19(3-4): 352-355.

KALIN, N.H., CARNES, M., BARKSDALE, C.M., SHELTON, S.E., STEWART, R.D. and RISCH, S.C. Effects of acute behavioural stress on plasma and cerebrospinal fluid ACTH and B-endorphin in rhesus monkeys. Neuroendocrinol. 1985; 40: 97-101.

KAPLAN, J.R. Psychological stress and behaviour in nonhuman primates. In: Mitchell, G. and Erwin, J., eds. Comparative primate biology, Vol. 2, part A: Behaviour, conservation, and ecology. New York, NY: Alan R. Liss, 1986.

KAUFMAN, C. and ROSENBLUM, L.A. A behavioural taxonomy for *Macaca nemestrina* and *Macaca radiata* based on longitudinal observations of family groups in the laboratory. Primates 1966; 7: 205-258.

KING, J.E. and NORWOOD, V.R. Free environment rooms as alternative housing for squirrel monkeys. The psychological well-being of primates. In: Segal, E.F., ed. Housing, care and psychological well-being of captive and laboratory primates. Philadelphia, PA: Noyes Publications, 1989: 102-114.

KINZEY, W.G. Feeding, travel distance and group size in *Callicebus torquatus*. Primate Rep. 1986; 14: 11.

KIRKWOOD, J.K. and DOW, S.M. Feeding primates in captivity. Nutritional and behavioural considerations. Primate Rep. 14: 42.

LINE, S.W., MORGAN, K.N., MARKOWITZ, H., ROBERTS, J.A. and RIDDELL, M. Behavioural responses of female long-tailed macaques to pair formation. Lab. Prim. Newsl. In press.

LOVERIDGE, G.G. The establishment of a barriered respiratory disease-free cat breeding colony. Anim. Technol. 1984; 35(2): 83-92.

LYNCH, J.J. Psychophysiology and development of social attachment. J. Nerv. Ment. Dis. 1979; 151: 231-244.

MACKENZIE, M.M, MCGREW, W.C. and CHAMOVE, A.S. Social preferences in stump-tailed macaques (*Macaca arctoides*). Effects of companionship, kinship, and rearing. Develop. Psychol. 1985; 18: 115-123.

MAPLE, T.L. Great apes in captivity. The good, the bad, and the ugly. In: Erwin, J., Maple, T.L. and Mitchell, G., eds. Captivity and behavior of primates in breeding colonies, laboratories and zoos. New York, NY: Van Nostrand Reinhold, 1979: 239-272.

MARKOWITZ, H. Environmental enrichment and behavioral engineering for captive primates. In: Erwin, J., Maple, T.L. and Mitchell, G., eds. Captivity and behaviour of primates in breeding colonies, laboratories and zoos. New York, NY: Van Nostrand Reinhold, 1979: 217-238.

MARKOWITZ, H. Behavioral enrichment in the zoo. New York, NY: Van Nostrand Reinhold, 1982.

MARRIOT, B. Social influence on activity based energy intake and expenditure in free-ranging rhesus monkeys. Primate Rep. 1986; 14: 151.

MARTIN, P. An experimental study of weaning in the domestic cat. Behav. 1986; 99(3-4): 221-249.

MAY, R.M. Control of feline delinquency. Nature 1988; 332(6163): 392-393.

MCFARLAND, M.J. Food competition and foraging group size in the black spider monkey, *Ateles paniscus*. Primate Rep. 1986; 14: 12.

MCGREW, W.C. Social and cognitive capabilities of nonhuman primates: Lessons from wild to captivity. Inter. J. Stud. Anim. Prob. 1981; 2: 138-149.

MCGREW, W.C., BRENNAN, J.A. and RUSSELL, J. An artificial "gum tree" for marmosets (*Callithrix j. jacchus*). Zoo Biol. 1986; 5: 45-50.

MCKENZIE, S.M., CHAMOVE, A.S. and FEISTNER, A.T.C. Floor coverings and hanging screens alter arboreal monkey behaviour. Zoo Biol. 1986; 5: 27-39.

MCKEOWN, D. and LUESCHER, A. The handbook of canine behaviour. Ontario Veterinary College, University of Guelph. In Press.

MELNICK, D.J. and PEARL, M.C. Cercopthecines in multimale groups: Genetic diversity and population structure. In: Smuts, B.B., Cheney, D.L., Seyfarth, R.M., Wrangham, R.W. and Struhsaker, T.T., eds. Primate societies. Chicago, IL: University of Chicago Press, 1987.

MENDL, M. The effects of litter-size variation on the development of play behaviour in the domestic cat: litters of one and two. Anim. Behav. 1988; 36(1): 20-34.

MICHENER, G.R. Ethical issues in the use of wild animals in behavioural and ecological research. In: Driscoll, J.W., ed. Animal care and use in behavioural research: regulations, issues and applications. AWIC/USDA National Agricultural Library, 1989: 1-6.

MILITZER, K., ed. Ways of assessing the welfare of laboratory, zoo and domestic animals (German). Berlin: Verlag Paul Parey, 1986.

MILLAR, S.K., EVANS, S. and CHAMOVE, A.S. Oldest offspring contact novel soonest in callitrichid families. Behav. Biol. 1988; 13: 82-96.

MINEKA, S., GUNNAR, M. and CHAMPOUX, M. Control and early socio-emotional development: Infant rhesus monkeys reared in controllable versus uncontrollable environments. Child Develop. 1986; 57: 1241-1256.

MITCHELL, G., MAPLE, T.L. and ERWIN, J. Development of social attachment potential in captive rhesus monkeys. In: Erwin, J., Maple, T.L. and Mitchell, G., eds. Captivity and behavior of primates in breeding colonies, laboratories and zoos. New York, NY: Van Nostrand Reinhold, 1979: 59-124.

MOBERG, G.P. Influence of stress on reproduction: measure of well-being. In: Animal stress. Bethesda, MD: Amer. Physiol. Soc., 1985: 245-267.

MOODIE, E.M. and CHAMOVE, A.S. Brief excitement beneficial for captive tamarins? Zoo Biol. In press.

MORAN, G., ed. Zoo animal behaviour. Appl. Anim. Behav. Sci. 1987; 18(1): 1-118.

NASH, V.J. Tool use by captive chimpanzees at an artificial termite mound. Zoo Biol. 1982; 1: 211-221.

NATIONAL INSTITUTES OF HEALTH. Guide for the care and use of laboratory animals. Bethesda, MD: NIH, 1985.

NEAMAND, J., SWEENY, W.T., CREAMER, A.A. and CONTI, P.A. Cage activity in the laboratory beagle: a preliminary study to evaluate a method of comparing cage size to physical activity. Lab. Anim. Sci. 1975; 25(2): 180-183.

NEURINGER, A.J. Animals respond for food reward with free food present. Sciences, New York, NY 1970; 166: 399-401.

NEYMAN, P.F. Ecology and social organization of the cotton-top tamarin. PhD dissertation. Berkeley, CA: University of California, 1980.

OATES, J.F. Food distribution and foraging behaviour. In: Primate societies. Smuts, B.B., Cheney, D.I., Seyfarth, R.M., Wrangham, R.W. and Struhsaker, T.T., ed. Chicago, IL: University of Chicago Press, 1986.

OHKAWA, N. and HIDAKA, T. Communal nursing in the domestic cat, *Felis catus*. J. Ethol. 1987; 5(2): 173-183.

OLM, D.D. and HOUPT, K.A. Feline house-soiling problems. Appl. Anim. Behav. Sci. 1988; 20(3): 335-345.

PARKER, S.T. and GIBSON, K.R. A development model for the evolution of language and intelligence in early hominids. Behav. Brain Sci. 1979; 2: 367-408.

PAULK, H.H., DIENSKE, H. and RIBBENS, L.G. Abnormal behaviour in relation to cage size in rhesus monkey. J. Abnor. Psychol. 1977; 86: 87-92.

PHILLIPS, D.P., DANILCHUK, W., RYON, J. and FENTRESS, J.C. Food-caching in timber wolves, and the question of rules of action syntax. Behav. Brain Res. 1990; 38: 1-6.

PUBLIC HEALTH SERVICE POLICY ON HUMANE CARE AND USE OF LABORATORY ANIMALS 1986. Implements Public Law 99-158 the Health Research Extension Act of 1985. Available from the Office for the Protection from Research Risks (OPRR), National Institutes of Health, Building 31, Room 4B09, Bethesda, MD 20902.

REGAL, D.M., BOTH, R., TELLER, D.Y. and SACKETT, G.P. Visual acuity and visual responsiveness in dark-reared monkeys (*Macaca nemestrina*). Vision Res. 1987; 16: 530-532.

REICHART, E. (Zootechny of the cat, a laboratory animal) (French). Stal (sciences et techniques de l'animal de laboratoire) 1988; 13(4): 263-294.

REINHARDT, V., REINHARDT, A. and HOUSER, W.D. Partner directed hair-pulling-and-eating in rhesus (*Macaca mulatta*). Primate Rep. 1986; 14: 166.

REINHARDT, V. Environmental enrichment program for caged stump-tailed macaques (*Macaca arctoides*). Lab. Prim. Newsl. 1990; 29(2): 10-11.

REINHARDT, V. Improved installation method for branches as cage enrichment. Lab. Prim. Newsl. 1987; 26: 1.

REINHARDT, V., HOUSER, W.D., COWLEY, D. and CHAMPOUX, M. Preliminary comments on environmental enrichment with branches for individually caged rhesus monkey. Lab. Prim. Newsl. 1987; 26: 1-3.

REINHARDT, V., COWLEY, D., EISELE, S., VERTEIN, R. and HOUSER, D. Preliminary comments on pairing unfamiliar adult female rhesus monkeys for the purpose of environmental enrichment. Lab. Prim. Newsl. 1987; 26: 5-8.

RENNER, M.J. and ROSENZWEIG, M.R. Enriched and impoverished environments. London: Springer-Verlag, 1987.

RENQUIST, D.M. and JUDGE, F.J. Use of nylon balls as behavioural modifiers for caged primates. Lab. Prim. Newsl. 1984; 24: 4.

RICHARD, A.F. Primates in nature. New York, NY: W.H. Freeman & Co., 1985.

RILEY, V. and SPACKMAN, D. Housing stress. Lab Animal 1977; 6: 16-21.

ROBINSON, P. and COX, H.W. Reproduction performance in a cat colony. Lab. Anim. 1970; 4: 99-112.

ROSENBLUM, L.A. and SMILEY, J. Therapeutic effects of an imposed foraging task in disturbed monkeys. J. Child Psychol. Psych. 1984; 25: 485-497.

RYON, J.C. Aspects of dominance behaviour in groups of sibling coyote/red wolf hybrids. Behav. and Neur. Biol. 1979; 25: 69-78.

RYON, J., FENTRESS, J.C., HARRINGTON, F.H. and BRAGDON, S. Scent rubbing in wolves (*Canis lupus*): the effect of novelty. Can. J. Zool. 1985; 64: 573-577.

SACKETT, G.P. Monkeys reared in isolation with pictures as visual input. Evidence for an innate releasing mechanism. Sci. 1966; 154: 1468-1470.

SARAH, J.F. and BERMAN, E. Outdoor feline colony. Lab. Anim. Care 1967; 17(1): 81-92.

SCHAR, R. Influence of man on social behaviour of farm cats (abst). Experentia. 1985; 41(9): 1226.

SCHARMANN, W. Fear and the reduction of fear in animal experiments (German). Tierarztliche Umschau 1988; 43(6): 383-384.

SCIENTISTS CENTER FOR ANIMAL WELFARE. Field research guidelines. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1988.

SCOTT, J.P. Critical periods in the development of social behaviour in puppies. Psychosom. Med. 1958; 20 (1): 42-54.

SIGG, H. and WEIHE, W.H. Activity and resting behaviour in the dog as indicators of well-being. Zeitschrift für versuchstierkunde 1986; 28(5): 215-216.

SNOWDON, C.T. The criteria for successful captive propagation of endangered primates. Zoo Biol. Suppl. 1989; 1: 149-161.

THOMPSON, W.R. and HERON, W. The effects of early restriction on activity in dogs. Can. J. Psychol. 1952; 8: 17-31.

TRIPP, J.K. Increasing activity in captive orangutans. Provision of manipulable and edible materials. Zoo Biol. 1985; 4: 225-234.

TURNER, D.C. Cat behaviour and man/cat interaction (French). Animalis familiaris 1988; 3(2): 16-20.

TURNQUIST, J.E. Gang-caged versus free-ranging rhesus monkeys. A comparison of body proportion and passive joint mobility. Primate Rep. 1986; 14: 171.

UNIVERSITIES FEDERATION FOR ANIMAL WELFARE. Laboratory animal welfare research: rodents. Proc. UFAW Symp. UFAW, 1989. (8 Hamilton Close, South Mimms, Potters Bar, Herts, EN6 3QD.)

VAN HOOSIER, G.L. and MCPHERSON, C.W. Laboratory hamsters. New York, Toronto: Academic Press, 1987.

VANDERLIP, J.E. and VANDERLIP, S.L. Establishing a breeding colony using random-source dogs. Lab Animal 1983; 12(4): 35-43.

VERGA, M. (Behavioural characteristics and abnormal behaviour of the cat) (Italian). Obievetti e documenti veterinari 1989; 10(4): 41-42.

VISALBERGHI, E. and ANTINUCCI, F. Tool use in the exploitation of food resources in *Cebus apella*. In: Primate ecology and conservation, Vol. 2. Else, J.C. and Lee, P.C., eds. London: Cambridge University Press, 1986.

VOIT, V.L. and BORCHELT, P.L. Elimination of behavioural problems in cats. Vet. Techn. 1986; 7(5): 206-208.

WASER, P. Interactions among primate species. In: Primate societies. Smuts, B.B, Cheney, D.L., Seyfarth, R.M., Wrangham, R.W. and Struhsaker, T.T., eds. Chicago, IL: University of Chicago Press, 1986.

WEBSTER, A.J.F. Animal housing as perceived by the animal. Vet. Ann. 1989; 29: 1-8.

WEISBROTH, S.H., FLATT, R.E. and KRAUS, A.L., eds. The biology of the laboratory rabbit. New York, London, Toronto: Academic Press, 1974.

WESTERGAARD, G.C. and FRAGAZY, D.M. Effects of manipulatable objects on the activity of captive capuchin monkeys (*Cebus apella*). Zoo Biol. 1985; 4: 317-327.

WOLFLE, T.L. Control of stress using non-drug approaches. J. Am. Vet. Med. Assoc. 1987; 191(10): 1219-1221.

WOOLPY, J.H. Socialization of wolves. In: Masserman, J.H., ed. Animal and human. New York, NY: Grune and Stratton, 1968.

ADDENDUM 1

COMMON AND SCIENTIFIC NAMES OF NON-HUMAN PRIMATES

Common Name	**Scientific Name**
African Green monkey	*Cercopithecus aethiops*
Assamese macaque	*Macaca assamensis*
Baboon	*Papio spp.*
Bush baby	*Galago spp.*
Capuchin monkey	*Cebus apella*
Chimpanzee	*Pan troglodytes*
Common marmoset	*Callithrix jacchus*
Cynomolgus macaque	*Macaca fascicularis (M. irus)*
Gibbon	*Hylobates spp.*
Japanese macaque	*Macaca fuscata*
Lion-tail macaque	*Macaca silenus*
Owl monkey	*Aotus trivirgatus*
Rhesus monkey	*Macaca mulatta*
Squirrel monkey	*Saimiri sciureus*
Spider monkey	*Ateles spp.*
Stump-tailed macaque	*Macaca arctoides (M. speciosa)*
Tamarin	*Saguinus spp.*
Pig-tail macaque	*Macaca nemestrina*

ADDENDUM 2

SOURCES OF ENRICHMENT DEVICES

Puzzle feeders and treats Kong Toys Prima-hedrons (perch and swing toys)	Primate Products 1755 East Bayshore Road, Suite 28A Redwood City, CA 94063 Telephone: 415-368-0663 Fax: 415-368-0665
Nyla Balls (Wolf size are used for Cynomolgus monkeys)	H & L Pet Supplies 27 Kingston Crescent Kitchener, Ontario N2B 2T6 Telephone: 519-743-8954 Central Sales 60 Eastern Avenue Brampton, Ontario L6W 1X8 Telephone: 1-800-387-2522 Rolf C. Hagen Inc. 3225 Sartelon Ville St. Laurent, Quebec H4R 1E8 Telephone: 514-332-0914
Fruit-punch flavoured fructose pellets (for environmental enrichment treats)	P.J. Noyes Company Inc. Whitefield Road P.O. Box 381 Lancaster, NH 03584 Telephone: 603-788-4952
Fleece foraging/grooming board Foraging crumbles "mixed size" Turf foraging board Foraging bites Prima-burgers Prima-gel Prima-treats Marmoset diets	BIO-SERV® P.O. Box 450 Frenchtown, NJ 08825 Telephone: 908-996-2155 Outside USA: 908-996-4123
Prima-treats Prima-Foraging/Grooming boards Kong Toys Nyla Balls and Nyla Toys Boomer balls	Kaplan Laboratory Animal Supplies and Services 4960 Almaden Exp., Suite 233 San Jose, CA 95118 Telephone: 408-266-1235

VII. SPECIAL PRACTICES

A. ANIMAL ACQUISITION

1. Procurement

All animals must be legally acquired. Animals received as donations for research should have been legally released (see Laboratory Animal Care, Identification and Records). It is almost always preferable to obtain standard laboratory species from an established breeder or licensed supplier. Several provinces have regulations governing the procurement of dogs (see also Responsibility for the Care and Use of Experimental Animals). In Ontario, laboratory animal supply facilities are licensed and inspected. All commercial producers of laboratory animals, regardless of whether or not they come under provincial legislation, are expected to provide housing facilities and to follow practices similar to those outlined in this *Guide*.

It is in the best interests of the user and the supplier to co-operate in the elimination of any undesirable condition affecting the health and quality of the animal. The institution (i.e., the receiver) should inform the supplier of any undesirable conditions observed in the stock received. The supplier should, if requested, provide detailed information on health status monitoring, breeding, and husbandry practices followed.

The acquisition of animals should be dependent upon the prior approval of the project by the institutional Animal Care Committee (ACC). Acquisition procedures should be in place to ensure that the institution will have an up-to-date and ongoing inventory of all animal experiments for which it may be accountable, and to allow for the prior preparation of appropriate space and such other arrangements as may be necessary for the reception of the incoming animals.

2. Transportation

a) Introduction

Depending upon the species and size of the animal, the modes of transportation can be by land, sea or air. For most laboratory species, the most common method is either by ground transportation, over relatively short distances, or by air for longer distances. The objective of any method of travel is to ensure the safety, security, and comfort of the animal in the container, and to take it to its destination as quickly and as safely as possible.

Although many agencies are concerned with animal transportation *per se*, those dealing with the transportation of experimental animals are few in number. The Animal Transportation Association (formerly known as the Animal Air Transportation Association) (AATA) is involved in making improvements to all modes of transportation. Publications are available on the subject of animal transportation. The Canadian Federation of Humane Societies (CFHS) (1988), for example, conducted a survey of surface livestock transportation in Canada. A recent British Veterinary Association (BVA) symposium has examined the welfare of animals in transit (Gibson, Paterson and Conville, 1986), as has a symposium of the World Association for Transport Animal Welfare and Studies (WATAWS) (Laing, 1991).

b) Regulations--Containers and Transportation

The International Air Transport Association (IATA) annually produces the *IATA Live Animal Regulations*, which includes information concerning the documentation, the containers and other requirements for humane transportation of live animals (IATA, 1992). Some 81 containers are described under the

headings of species, design and construction, preparations for dispatch, feeding guide, general care and loading. While the container information is specific for air transportation, the requirements for the containers are applicable to all modes of transport, for they ensure safety, comfort, and security for the animals (Rowsell, 1992).

In order to ensure accurate technical content, the IATA *Regulations* are prepared in consultation with representatives from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the Office International des Epizooties (OIE). For over 10 years, the Canadian Council on Animal Care (CCAC), the International Council for Laboratory Animal Science (ICLAS), as well as the Eurogroup for Animal Welfare, have maintained close liaison with the IATA Live Animals Board, addressing primarily the issues concerned with transportation of experimental animals.

Legislation in Canada addressing transportation includes the (federal) Health of Animals Act (C-66, June, 1990, rev. March, 1992; 38-39 Elizabeth II, Chapter 21), Ontario's Animals for Research Act (Revised Statutes of Ontario, 1980, Chapter 22 as amended by 1989, Chapter 72,s6 and Regulations 16,17,18,19), and, in Alberta, the Universities Act (Section 50, "Dog Control and Procurement"; Regs. 341-366). In 1972, Alberta Regulation 33-72 was expanded to cover the treatment of **animals** (Rowsell, 1974). As well, there is specific legislation governing the transportation of animals in provinces and specific bylaws in municipalities.

The transportation of animals from the United States into Canada is affected by the U.S. Department of Agriculture's Animal Welfare Act (1966) by specifying minimum and maximum temperatures to which animals in transit may be exposed. The U.S. Department of the Interior, U.S. Fish and Wildlife Service, is responsible for the enforcement of the importation of wild mammals and birds; as well, the U.S. Department of the Interior is responsible for the enforcement of the Marine Mammal Protection Act.

c) Transportation Stress

One of the least controllable variables in animal experimentation is the effect on the research caused by moving animals from one area to another (Landi, Kreider, Lang *et al*. 1982, 1985; Aguila, Pakes, Lai *et al*. 1988; Bean-Knudsen and Wagner, 1987, Reinhardt, 1992). This might involve great distances between countries, or minor distances, such as within the animal facility itself. Even moving animals within the animal room affects the stress indices, which are more greatly influenced if the individual doing the transporting is a stranger (Gärtner, Büttner, Döhler *et al*. 1980).

The stressors in animal transportation include inexperienced handlers, the amount of time spent in preparation, in transit, and on arrival at the destination, and the state of the mode of transport, e.g., rough roads, rough rail beds, rough seas and air turbulence. Of importance are the comfort and suitability of the container, sufficient time spent for adaptation to the container prior to transport, and the temperature and ventilation of both the container and the ambient temperature of the environment and the various temperature zones through which the animal may pass. It is essential to make appropriate pre-arrangements concerning the transport of the animal in order to minimize the length of time spent in transit.

Transportation stresses can be minimized by avoiding long distance shipping, slow modes of transport and, as mentioned, by acclimatizing the animals to the containers and, if possible, to the mode of transport. Kiley-Worthington (1990), in studies involving the transportation of animals in circuses and zoos, showed that unhandled, naive animals (i.e., those that are wild or have not been domesticated or are not used to confinement), will probably suffer the most. Although there is a great deal of concern about circus animals, show dogs, or competitive horses subject to regular transportation, this researcher reported that there was "no evidence suggesting that the transporting of circus animals is necessarily or even unusually distressing or traumatic for the animal, although it is for naive livestock."

The requirement for training those involved in the transportation of animals is often neglected. Although it is well established that inexperienced handlers can affect the animals significantly, little has been done

to ensure proper training of individuals engaged in animal transportation. In 1978, the CCAC produced an audio-slide presentation on "Humane Transportation of Live Animals" which was made available to all airlines and any of the other agencies engaged in the transportation of animals (Fletch, 1978; Rowsell, 1990).

The principles enunciated in the CCAC-produced training program continue to be applicable today. It notes, for example, that personnel engaged in the transportation of animals, including those employed by the animal facility, require knowledge of the various types of animals, the differences between species of birds and mammals, as well as consideration of the invertebrates. They must also be cognizant of the container requirements, and specification requirements for labelling and marking, and for completion of the proper documentation, which includes any licensing requirements, both for export and the country of designation. They should be knowledgeable of shippers' responsibilities as well as those of the individuals receiving the animals. They should realize the importance of making advance arrangements concerning the shipment and transport of animals.

The responsibility for knowing about the safe and humane transport of animals includes, as well, those who, in the conduct of their duties, are exposed to the animals or have responsibility for the animals they may be carrying, i.e., in the truck, on-board ship or in the aircraft. The institution receiving the animals should be prepared for accepting the animals by providing proper facilities and appropriate handling by trained, experienced personnel.

d) Animal Handling

The animal in a laboratory setting becomes conditioned to the environmental conditions such as temperature, humidity, air changes, noises, the habits of those working within the animal room, and animal or human pheremones (substances secreted and released by animals for detection and response by another of the same species). All of the foregoing may change when the animals are transported to the facility or moved within it (Slatnetz, Fratta, Crouse *et al.* 1957; Baker, Lindsey and Weisbroth, 1979; Gibson, Paterson and Conville, 1986; Aguila, Pakes, Lai *et al.* 1988; Rowsell, 1988). Unfortunately, many of these changes have not been measured precisely (Yousef, 1988). As well, questions have been raised as to the suitability of certain tests used to measure the stress induced by environmental changes. Behavioural changes such as increased agitation, difficulty in handling, reluctance to eat or to drink, bristling of the coat, hiding, and abnormalities in behaviour may go largely unnoticed, but may cause variations in experimental data. Therefore, it is essential that the animal be allowed to equilibrate to a new environment. Because the period for equilibration of each animal and between species varies, knowledge of the species and of the individual animals is essential.

When the animal arrives at its destination, the institution must ensure that it is then brought to the institution in a safe and humane manner. Air conditioned vehicles specifically designed for this purpose are essential in order to reduce stressors that may have increased during the transport period. Acclimatization to the environment and a stabilization of the animal, physiologically and behaviourally, are essential prerequisites before the animal is used. Landi, Kreider, Lang *et al.* (1982) demonstrated that, following air transportation of rodents, a two-week period was required for blood and stressor parameters to return to normal. The adherence to the principles of humane transportation and handling throughout the transport period and on arrival at the institution should help ensure that, when animals are used in research, teaching, or testing, the results are meaningful and scientifically valid.

3. Breeding

A review of modern methods of breeding laboratory animals is beyond the scope of this *Guide*. However, it is axiomatic that all types of animals involved in a breeding program for animal production research purposes will require the best of care and that accurate breeding records must be kept (Box, 1976). It is essential that breeders and researchers who propose to breed animals for specific research purposes acquire detailed information on the anatomy, behaviour and physiology of reproduction relating to the stocks concerned (Altman and Dittmer, 1972; Greep, 1974; Hafez, 1970; Crawford, 1990). Blaffer-Hrdy and Whitten (1987) present comparative data for non-human primates (NHP) on cycle length, duration of

menstrual flow, visual signals, and the behaviour of males as well as females in estrus, for all species. Breeders should refer to the sections of this *Guide* dealing with management, caging and special housing of the various species.

If an animal model of human disease is to be produced, it is mandatory that the scientist or breeder fully appreciates the basic physiological and pathological processes involved in the defect. The research facility is generally the best location for this type of breeding program. However, under some circumstances it may be preferable to contract for supply of a particular mutant strain, either with a reliable commercial enterprise or by arrangement with some other scientist who is already breeding and utilizing the same model (ILAR, 1979). The breeding of any laboratory animal must be done in accordance with the accepted genetic standards and genetic nomenclature (Festing, Kondo, Poiley *et al.* 1972; ILAR, 1979; Lyon, 1981; Lyon and Searle, 1989), subjects which are beyond the scope of this *Guide*.

The decision to establish a breeding colony program in a research institution is one that the investigator and the institutional ACC should always study carefully in terms of the nature of the project. Unless breeding is an integral, even essential, part of the research or teaching exercise, thorough evaluation ought always to be undertaken of: a) the ultimate real cost (to the institution) of animals bred within the research facility; b) the occupancy of valuable and costly space which will no longer be available for other research; and c) the ultimate numbers of animals that will have to be produced versus the actual numbers that will be utilized. Small in-house breeding programs almost always involve the need to dispose of animals superfluous to the needs of the project, and the maintenance of excess breeders in order to try to cope with fluctuating demands.

In commercial livestock production, selection may be based on ancestry, individual performance or progeny or combinations of these factors. However, in small laboratory species, emphasis has been placed on maintaining genetic purity (Festing, Kondo, Poiley *et al*. 1972). In recent years, questions raised about the genetic homogeneity of some strains has led to the genetic testing of animals. The larger suppliers of small laboratory animals provide genetic control for their stocks and strains, and may provide genetic testing services to investigators using other strains.

The genetic nature of an animal population can be changed in three ways: by selection, through manipulation of the breeding system, or by altering the genome through the introduction of alien genes (DeTolla, 1991). Traditionally, the rapidity with which genetic change in a population is achieved will depend in part on the sex of the selected animals; the female has less effect on the maximal selection differential than the male, as the latter can produce many more progeny. The decision as to which animals are to be bred will depend on numerous criteria directly related to the purpose of the breeding exercise.

4. Breeding Transgenic Animals

The time required to produce large numbers of transgenic animals will depend on the reproductive capacity of the animal. Transgenic animals are now considered a standard biomedical research tool (Saffer, 1992) and are increasingly being used as animal models for human diseases (Merlino, 1991), gene therapy, the study of virus-induced disease, the physiology of foreign gene expression (Palmiter and Brinster, 1986; Geistfeld, 1991), probes into complex systems (Hanahan, 1989), and as models for genetic toxicology studies (Myhr and Brusick, 1991). Their use is increasing, and indeed, has been cited as a major cause in the first rise in animal use in the U.K. for many years (Anon., 1992).

Management of a transgenic mouse colony has recently been described by Geistfeld (1991), and a procedures manual has been published by Hogan, Constantini and Lacey (1986). As well, there are numerous research reports (Brinster, Chen, Trumbauer *et al.* 1985; Bishop and Smith, 1989; Depamphilis, Herman, Martinez-salas *et al.* 1988; Gordon, Scangos, Plotkin *et al.* 1980; Jaenisch, 1976, 1988) describing transgenic animal use. Britain's Health and Safety Executive published *Guidelines on Work with Transgenic Animals* (1989) (Baynards House, 1 Chapstow Place, London, W2) which must be followed when creating, breeding or handling transgenic animals in the U.K. (Connor, 1989).

In describing management of a transgenic mouse colony Geistfeld (1991) suggests that Caesarian-derived pups be used in order to eliminate pathogens. Various breeding systems are feasible; usually a 2:1 to 3:1 harem mating system is effective, although there are no hard and fast rules.

Some of the problems associated with the breeding of transgenic mice have been outlined by Donnelly and Walsh-Mullen (1991). These include contamination of the media used in collecting eggs and blastocysts for microinjection, because of which the surrogate dam fails to deliver. They note that introducing foreign genes may cause deleterious insertions that prove lethal to the animal or that compromise reproduction.

The CCAC recently established a Committee on Animal Biotechnology (CABT), which has as its mandate: "to develop ongoing guidelines for embryo manipulation, fetal research, and transgenic animals". *The CABT considers it acceptable for transgenic/embryo manipulation research to produce animals which do not have a negative impact on the animal's well-being or on the environment, and which have a positive, scientifically justifiable endpoint. Should transgenic technology result in a new species or strain of animal, research techniques must be developed to adequately test the impact of such animals, in the view of the Committee. Some of Canada's institutions have prepared their own guidelines for research involving transgenics.*

5. Animal Models with Special Needs

Animal models of human disease are used to study the causes and therapeutic and preventive methods for human disease, as well as to develop new drugs (Nomura, Katsuki, Yokoyama *et al.* 1987). Models are available for many diseases and conditions, e.g., hemophilia (Moake, 1988) atherosclerosis (Reddick, Read, Brinkhous *et al*. 1990; Farrell, Saunders, Freeman *et al*. 1986), Pasteurellosis (Morck, Costerton, Bolingbroke *et al*. 1990), intestinal disease (Pfeiffer, 1985), hepatic degeneration (Hultgren, Stevens and Hardy, 1986), enteric diseases such as *Campylobacter jejuni* (Fox, Ackerman, Taylor *et al*. 1987), cardiomyopathy (Wagner, Reynolds, Weisman *et al*. 1986) and neurological disease (Barnes, 1986). Animal models for advancing understanding of diseases such as hypertension, gastrointestinal tract and cardiovascular disease were discussed at a symposium of the British Laboratory Animals Veterinary Association (BLAVA) (Anon., 1986). Another meeting addressed the challenges facing researchers into the human immunodeficiency virus (HIV) and the need for animal models in this regard (Groopman, 1991).

Animal models of some conditions or diseases have special needs beyond those of normal, healthy laboratory animals. These special needs must be recognized and accommodated when such animal models are going to be used in research. It should be the responsibility of the principal investigator to take into consideration the special needs of the animals before embarking on the research project. These special needs will no doubt impact on the research budget in terms of additional animal care time, materials, and equipment. ACC reviews of research proposals should include an assessment of these extra considerations for the animals.

The principle that encompasses this responsibility to attend to the special needs of animal models could be stated as follows: that any pain, suffering, distress, or deficits in function that negatively affect the animal's well-being, not scientifically "necessary" for the study, should be alleviated or minimized. Cost or convenience should not deter from this. Further, as soon as the study is done, the animal suffering should be terminated (Olfert, 1992).

6. Identification of the Sexes

Generally, sexes are kept separated subsequent to weaning, except for breeding purposes or because of experimental design requirements. Unwanted matings amongst stock animals should not occur, and under experimental conditions may compromise the experimental results.

The sexing of animals may be difficult in the newborn or in species with which one is unfamiliar. In addition to the genital organs themselves, secondary sex characteristics can be used in some cases (Valle, 1990). Detailed descriptions of the techniques/observations used to sex the various common laboratory animal species can be found in the species chapters of the CCAC *Guide* Volume 2, or in other general books on species used as laboratory animals (Poole, 1987). Descriptions of the techniques used to determine the sex of some of the less common species are also available (Goin and Goin, 1971; Frye, 1991; Marcus, 1981).

B. RESTRAINT AND MANIPULATIONS

1. Physical Restraint

a) Introduction

It is necessary to restrain most animals for even the simplest of procedures (e.g., to take the animal's temperature). When short-term chemical restraint (e.g., anesthetic, tranquillizer, etc.) is not possible and/or is not compatible with the experimental requirements, some form of physical/mechanical restraint may have to be used.

It is well known that the quality of the restraint will influence the animal's response (Hemsworth, Barnett and Hansen, 1986). With laboratory rodents, Lee (1992) contends "there is no superior restraint than an able technician aide's hands." It has been shown that an experienced technician picking up a rat disturbs the animal less than a totally inexperienced technician who is afraid of being bitten (Barclay, Herbert and Poole, 1988). Immobilization stress is known to have an effect on rat performance (Grilly and Gowans, 1986). Paré and Glavin (1986) have reviewed restraint-induced stress.

Animals become accustomed to repeated, gentle, short-term restraint which is not accompanied by stressful or painful procedures. Longer-term restraint is accompanied by evidence of stress unless the animal has undergone a prolonged period of acclimatization to the experimental situation, and is not subjected to any painful procedures while it is being restrained (Golub and Anderson, 1986; Rushen, 1986). The level of distress varies from animal to animal in the same situation, and may affect experimental results.

Two factors which have a strong influence on the degree of stress experienced by a restrained animal are the isolation from conspecifics (during restraint), and the degree of immobilization. These two factors should be considered if the experimental procedure requires that the animal be restrained. Visual, auditory and olfactory contact with conspecifics may suffice to reduce stress levels. Sheep, for example, should not be out of sight and sound of other sheep, even if they are able to move around in the isolation pen (see also Social and Behavioural Requirements of Experimental Animals).

In all situations in which prolonged physical restraint is required, the use of proposed restraining methods must first have been justified and received approval through peer review and the institutional ACC, using the guidelines established by the CCAC. Such reviews should provide assurance that the technology is either lacking or its use is not warranted for obtaining the required measurements in the unrestrained animal. The restrained animal will always require special consideration, care and surveillance (see also *Social and Behavioural Requirements of Experimental Animals*; *Categories of Invasiveness* statement; *Ethics of Animal Investigation* document, found elsewhere in this *Guide*).

b) General Guidelines for Care of Restrained Animals

i) Restraint procedures should only be invoked after all other less stressful procedures have been rejected as alternatives.

ii) Supervision of animals in restraining devices should only be assigned to fully qualified and experienced personnel.

iii) The principal investigator has the responsibility to ensure that all members of the research team, particularly those responsible for day-to-day animal care, are fully aware of the rationale for the restraint procedures and for the complications for the animal which may occur as a result of the restraint.

iv) Consultation should be sought with those experienced in the restraint procedures to be invoked, prior to its initial use, to ensure that minimal restraint is used to accomplish the experimental goals.

v) Physiologic, biochemical and hormonal changes occur in any restrained animal (Gärtner, Büttner, Döhler *et al.* 1980; Toth and January, 1990; Moberg, 1992; Bush, Custer, Smeller *et al.* 1977; Mayer and Bowman, 1972; Markowitz and Spinelli, 1986), and investigators should consider how these effects will influence their proposed experiments.

c) Special Surveillance

The following comments are generally applicable to all restrained animals; however, they refer especially to restraint in non-human primates (NHP):

i) Regardless of duration of restraint, special attention must constantly be paid to the possible development of associated ill effects.

ii) A minimum of twice daily, careful physical inspection of each restrained animal is mandatory.

iii) Inspection should include not only physical examination, but also evaluation of general behaviour. Food and water consumption, as well as the animal's weight if possible should be recorded daily. When any unusual manifestations develop, or physiological parameters show undue variance, immediate therapeutic measures must be taken.

d) Restraint Devices

Devices developed for short-term laboratory animal restraint include a plastic cage for restraint of opossum (Thomason and Russell, 1986), an acute restraint device for rhesus monkeys described as "a practical and inexpensive alternative to the standard primate chair" (Robbins, Zwick, Leedy *et al*. 1986), and a full-body retraining device for small animals that permits the short-term recording of physiologic data (Yagiela and Bilger, 1986). The use of slings, pioneered for miniature swine by Panepinto (Panepinto, Phillips, Norden *et al*. 1983) has now been extended to rats and rabbits (Kumar, Wong, Johnson *et al*. 1979) and other species. Commercial sources of slings for rats and rabbits are available (e. g., Harvard Bioscience, Ealing Scientific Ltd., 6010 Vanden Abeele St., St. Laurent, Quebec Canada H4S 1R9), and for livestock species and dogs (Munk's Livestock Sling Mfg. Inc., 1143 W. Marches Pt. Rd., Anacortes, WA 98221 USA).

Efforts are being made to provide less restrictive restraint systems that facilitate movement and permit sampling or protect the instrumentation or equipment (Houghton, 1985; Anderson and Houghton, 1983; Dalton, 1985; Munson, 1974). Examples include; an intravenous catheter system for long-term (at least six to eight weeks) parenteral nutrition of unrestrained rats (Brenner, Muller, Walter *et al*. 1985), a backpack system for mini-pump infusions in marmosets (Ruiz de Elvira and Abbott, 1986), and a tethering system for intravenous and intragastric drug administration in the baboon (Lukas, Griffiths, Bradford *et al*. 1982).

Scientists are continuously seeking new methods which will lessen the stress involved in the collection of

samples such as bile (Rath and Hutchison, 1989; Kanz, Vanoye-Trevino and Molsen, 1989), and blood (Lawhorn, 1988). It should be re-emphasized that efforts to reduce the stress of monitoring often involve the training and socialization of the animal to the procedure (Vanderlip, Vanderlip and Myles, 1985a, 1985b).

The advent of vascular access ports has made it possible to take repeated blood samples from, or administer drugs to, a wide variety of animals with the minimum of restraint (Houghton, 1985). These ports consist of an intravascular catheter attached to a reservoir with a diaphragm (Dalton, 1985; Harvey-Clark, 1990). The appropriate blood vessel is cannulated and the reservoir is located subcutaneously at a suitable location. With the use of a topical, penetrating analgesic cream, access to the port can be achieved painlessly. Samples must be taken using sterile technique, in order to avoid contamination of the vascular access port. These ports are usually maintained patent by filling them with a heparin solution, and may remain functional for many months or years.

Another example of a less restrictive restraint system that facilitates animal movement, yet permits sampling or protection of instrumentation or equipment is the "Jacket and Swivel Tethering System" developed by Chatham (1985). It allows much greater movement and permit simultaneous fluid sampling, drug infusion, and electronic measuring and monitoring. The tethered animal can be maintained comfortably and humanely for months, while fully instrumented. The light weight and flexibility of the tether, combined with the low friction of the swivel, inhibits very little of the animal's normal cage activity. (Alice King Chatham Medical Arts, 5043 Oaknoll Ave., Los Angeles, CA 90043; Harvard Bioscience, Ealing Scientific Ltd., 6010 Vanden Abeele St., St. Laurent, Quebec Canada H4S 1R9.)

The "Pole and Collar" system (Anderson and Houghton, 1983) provides a standardizable, efficient means of training monkeys to calmly leave their cages, enter restraint devices for as long as the procedures require, and return to their cages without the need for chemical restraint or forced manual submission (Houghton, 1985).

Biotelemetry systems are increasingly being used to transmit biological information from animals to a remote location. A variety of biotelemetry systems are available, from backpack systems to those that are totally implantable (Halpryn, 1985).

2. Implantation, Cannulation and Sampling

Chronic studies involving the implantation of electrodes, cannulae and catheters will require that the animal be anesthetized at the time of implantation, and restrained awake when sampling is undertaken.

Electrode implantations must be undertaken by, or under the direct supervision of personnel experienced in the techniques involved, and must utilize proper surgical and anesthetic procedures (Meyer and Meyer, 1971; NIH, 1991) (see also Standards for Experimental Surgery and Anesthesia). Detailed descriptions of stereotaxic surgical techniques may be found in several sources (Hart, 1969; Pellegrino and Cushman, 1971; Skinner, 1971; Singh and Avery, 1975).

3. Bleeding

Guidelines for blood removal from laboratory mammals and birds have recently been published in Great Britain (BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement, 1993). Efforts should constantly be made to refine scientific techniques so as to reduce the volume of the blood sample. In small animals such as mice, volume and frequency are of particular importance. If the animal's welfare is threatened by the volume of the sample required, either more animals should be used, or compensatory blood transfusion considered.

Rather than multiple sampling carried out by repeated needle punctures, a butterfly needle or a

percutaneous (over the needle) cannula taped in position, should be utilized.

In removing volumes greater than 0.1 ml, as large a bore as possible should be used in order to ensure rapid blood withdrawal without collapsing the vein, with the constraint of avoiding hematomata formation. Before taking a sample, it is important to accurately locate the vein and dilate it by gentle obstruction or warming. If general body warming is used, the animal must be constantly observed to prevent hyperthermia, as evidenced by more rapid breathing, panting or salivating. The use of xylene (xylol, dimethylbenzene) as a dilator is not recommended as it causes skin rashes and is easily misused.

Common bleeding sites may be found in Appendix VIII of this *Guide*.

4. Motivation Procedures

In the conduct of behavioural studies, the use of positive reinforcement (e.g., reward in the form of a preferred food) is preferable to use of aversive stimulation (Lea, 1979). CCAC's *Ethics of Animal Investigation* (found elsewhere in this *Guide*) also notes that investigators, ACCs and Review Committees are advised to "be especially cautious in evaluating...electric shock as negative reinforcement." Elsewhere in this *Guide* under the Use of Animals in Psychology, it is noted:

"Similarly, when researchers use electric shock as a means of producing stress or motivating animals to escape or avoid, they are fully recognizant that electric shock does not normally occur in nature. They do assume, however, that this particular, easily controlled aversive event can serve as a model for analogue of other unpleasant events that do occur naturally and affect behaviour...."

Experimenters are urged to use the least aversive shock intensity x duration x frequency combination that is compatible with the goals of the research (Olfert, 1992). Particular values of these parameters will thus vary with species, and with the goal of the research. In many instances, there are well-developed procedures for determining the appropriate value of shock, based upon behavioural criteria. For example, "titration" procedures allow the minimum value of shock that will maintain a given behaviour to be determined for each individual animal. In all cases, it behooves the investigator to base the selection of shock values upon behavioural criteria, so as to use the least aversive shock that will permit collection of orderly data and the success of the research. For example, the arbitrary selection of a "low", a "medium" and a "high" intensity, without careful consideration of the behavioural effects of these values and the interaction of the elicited behaviours with the desired behavioural outcomes, may cause undue suffering and waste of research animals, and affect the research.

When shock is being used in combination with other stressors, the investigator should be aware of the possibility of summation of negative effects.

C. REFERENCES

AGUILA, H.N., PAKES, S.P., LAI, W.C. and LU, Y.-S. The effect of transportation stress on the splenic natural killer cell activity in C57BL/6J mice. Lab. Anim. Sci. 1988; 38(2): 148-151.

ALTMAN, P.L. and DITTMER, D.S. Biology data book, Vol. 1. Bethesda, MD: Federation of American Societies for Experimental Biology, 1972.

ANDERSON, J.H. and HOUGHTON, P. The pole and collar system. A technique for training and handling non-human primates. Lab Animal 1983; 12(6): 47-49.

ANIMAL WELFARE ACT OF 1966 (Public Law 89-544, (a) as amended by the Animal Welfare Act of 1970 (Public Law 91-579); the Animal Welfare Act of 1976 (Public Law 94-279); (b) as amended by the

Improved Standards for Laboratory Animals Act of December 1985 (Public Law 99-198) (Deputy Administrator, U.S. Department of Agriculture, APHIS-VS, Federal Bldg., 6505 Belcrest Road, Hyattsville, MD 20782, USA).

ANON. Animal models advance understanding of diseases. Vet. Rec. 1986: 118(18).

ANON. Decline in animal experiments halted. Vet. Rec. 1992; 131(5): 86.

BAKER, H.J., LINDSEY, J.R. and WEISBROTH, S.H. Housing to control research variables. In: Baker, H.J., Lindsey, J.R. and Weisbroth, S.H., eds. The laboratory rat. Vol. 1. New York, NY: Academic Press, 1979: 169-172.

BARCLAY, R.J., HERBERT, W.J. and POOLE, T.B. The disturbance index: a behavioural method of assessing the severity of common laboratory procedures on rodents. Potters Bar, South Mimms, Herts, U. K.: Universities Federation for Animal Welfare, 1988.

BARNES, D.M. Bird chimeras may be models for certain neurological diseases. Science 1986; 232: 930-932.

BEAN-KNUDSEN, D.E. and WAGNER, J.E. Effects of shipping stress on clinicopathologic indicators in F344/N rats. Am. J. Vet. Res. 1987; 48(2): 306-308.

BISHOP, J.O. and SMITH, P. Mechanism of chromosomal integration of micro-injected DNA. Mol. Biol. Med. 1989; 6: 283-298.

BLAFFER-HRDY, S. and WHITTEN, P.L. Patterning of sexual activity. In: Smuts, B.B., Cheney, D.L., Seyfarth, R.M., Wrangham, R.W. and Struhsaker, T.T., eds. Primate societies. Chicago, IL: University of Chicago Press, 1987: 370-384.

BOX, P.G. Criteria for producing high quality animals for research. Lab. Anim. Sci. 1976; 26: 334.

BRENNER, U., MULLER, J.M., WALTER, M. and KELLER, H.W. A catheter system for long-term intravenous infusion in unrestrained rats. Lab. Anim. 1985; 19: 192-194.

BRINSTER, R.L., CHEN, H.Y., TRUMBAUER, M.E., YAGLE, M.K. and PALMITER, R.D. Factors affecting the efficiency of introducing foreign DNA into mice by micro-injecting eggs. Proc. Natl. Acad. Sci. 1985; 82: 4438-4442.

BRITISH VETERINARY ASSOCIATION/FUND FOR THE REPLACEMENT OF ANIMALS IN MEDICAL EXPERIMENTS/ROYAL SOCIETY FOR THE PREVENTION OF CRUELTY TO ANIMALS/UNIVERSITIES FEDERATION FOR ANIMAL WELFARE JOINT WORKING GROUP ON REFINEMENT. Removal of blood from laboratory animals and birds. Lab. Anim. 1993; 27: 1-22.

BUSH, M., CUSTER, R., SMELLER, J. and BUSH, L.M. Physiological measures of non-human primates during physical restraint and chemical immobilization. J. Amer. Vet. Med. Assoc. 1977; 171: 866.

CANADIAN FEDERATION OF HUMANE SOCIETIES. Surface livestock transportation in Canada. A survey. Nepean: CFHS, 1988. (CFHS, 102-30 Concourse Gate, Nepean, Ontario K2E 7V7.)

CHATHAM, A.K. Jacket and swivel tethering systems. Lab Animal 1985; 14(8): 29, 31-33.

CONNOR, S. A shepherd for transgenic animals. New Scientist 1989; January 21: 25.

CRAWFORD, R.D., ed. Poultry breeding and genetics. Amsterdam: Elsevier, 1990.

DALTON, M.J. The vascular port. Lab Animal 1985; 15(5): 21-23, 29-30.

DEPAMPHILIS, M.L., HERMAN, S.A., MARTINEZ-SALAS, E., CHALIFOUR, L.E., WIRAK, D.O., CUPO, D.Y. and MIRANDA, M. Micro-injecting DNA into mouse ova to study DNA replications and gene expression and to produce transgenic animals. BioTechniques 1988; 6: 662-680.

DETOLLA, L. Overview of transgenic systems. AALAS (Amer. Assoc. Lab. Anim. Sci.) Bull. 1991; 30(1): 9-12.

DONNELLY, T.M. and WALSH-MULLEN, A. Problems that limit or complicate breeding transgenic mice. Lab Animal 1991; 20(3): 34-35.

FARRELL, A.P., SAUNDERS, R.L., FREEMAN, H.C. and MOMMSEN, T.P. Arteriosclerosis in atlantic salmon: Effects of dietary cholesterol and maturation. Arteriosclerosis 1986; 6(4): 453-461.

FESTING, M., KONDO, K., POILEY, S.M. and SPEIGEL, A. International nomenclature for outbred stocks of laboratory animals. ICLAS (Intern. Coun. Lab. Anim. Sci.) Bull. 1972; 30.

FLETCH, A. Humane air transportation of live animals (Canadian Council on Animal Care training package). In: Proc. 4th Animal Air Transportation Association International Meeting. Toronto, Ont.: AATA, 1978: 55-66.

FOX, J.G., ACKERMAN, J.I., TAYLOR, N., CLAPS, M. and MURPHY, J.C. *Campylobacter jejuni* infection in the ferret: An animal model of human campylobacteriosis. Am. J. Vet. Res. 1987; 48(1): 85-90.

FRYE, F.L. Biomedical and surgical aspects of captive reptile husbandry. 2nd Ed. Malabar, FL, 1991.

GÄRTNER, K., BÜTTNER, D., DÖHLER, K., FRIEDEL, R., LINDENA, J. and TRAUTSHOLD, I. Stress response of rats to handling and experimental procedures. Lab. Anim. 1980; 14: 267-274.

GEISTFELD, J.G. Transgenic mouse colony management. Lab Animal 1991; 20(1): 21-29.

GIBSON, T.E., PATERSON, D.A. and CONVILLE, G. The welfare of animals in transit. Proc. The Animal Welfare Foundation, 3rd Symposium. London: British Veterinary Association Animal Welfare Foundation, 1986. (7 Mansfield St., London, England W1M 0AT.)

GOIN, C.J. and GOIN, O.B. In: Introduction to herpetology. 2nd Ed. 1971: 115, 96.

GOLUB, M.S. and ANDERSON, J.H. Adaptation of pregnant rhesus monkeys to short-term chair restraint. Lab. Anim. Sci. 1986; 36(5): 507-511.

GORDON, J.W., SCANGOS, G.A., PLOTKIN, D.J., BARBOSA, J.A. and RUDDLE, F.H. Genetic transformation of mouse embryos by micro-injection of purified DNA. Proc. Natl. Acad. Sci. 1980; 77: 7380-7384.

GREEP, R.O., ed. Reproductive physiology. In: MTP Int. Rev. Sci. Physiol., series 1,8. Baltimore, MD:

University Park Press, 1974.

GRILLY, D.M. and GOWANS, G.C. Comparison of the effects of morphine and immobilization stress on discrimination performance of rats. Behav. Neurosci. 1986; 100(4): 512-524.

GROOPMAN, J.E. Of mice, monkeys and men. Nature 1991; 349: 568-569.

HAFEZ, E.S.E., ed. Reproduction and breeding techniques for laboratory animals. Philadelphia, PA: Lea and Febiger, 1970.

HALPRYN, B. Biotelemetry monitoring systems. Lab Animal 1985; 14(6): 21-27, 29, 31.

HANAHAN, D. Transgenic mice as probes into complex systems. Science 1989; 246: 1265-1275.

HART, B.L. Experimental neuropsychology: A laboratory manual. San Francisco, CA: W.H. Freeman & Co., 1969.

HARVEY-CLARK, C. Clinical and research use of implantable vascular access ports in avian species. In: Proc. of the 1990 annual conference of the Association of Avian Veterinarians, 1990: 191.

HEMSWORTH, P.H., BARNETT, J.L. and HANSEN, C. The influence of handling by humans on the behaviour, reproduction and corticosteroids of male and female pigs. Appl. Anim. Behav. Sci. 1986; 15: 303-314.

HOGAN, B., CONSTANTINI, F. and LACEY, E. Manipulating the mouse embryo: A laboratory manual. Cold Spring Harbor, NY, 1986.

HOUGHTON, P. Animal handling systems. Current trends in animal restraint. Lab Animal 1985; 14(5): 20.

HULTGREN, B.D., STEVENS, J.B. and HARDY, R.M. Inherited, chronic, progressive hepatic degeneration in Bedlington Terriers with increased liver copper concentrations: Clinical and pathologic observations and comparison with other copper-associated liver diseases. Am. J. Vet. Res. 1986; 47(2): 365-377.

INSTITUTE OF LABORATORY ANIMAL RESOURCES. Animals for research. 10th Ed. Washington, DC: National Academy of Sciences, 1979.

INTERNATIONAL AIR TRANSPORT ASSOCIATION. Live Animals Regulations, 19th Ed. Montreal: IATA, 1992. (2000 Peel Street, Montreal, Quebec H3A 2R4.)

JAENISCH, R. Germ line integration and Mendelian transmission of the exogenous Moloney Leukemia Virus. Proc. Natl. Acad. Sci. 1976; 73: 1260-1264.

JAENISCH, R. Transgenic animals. Science 1988; 240: 1468-1473.

KANZ, M.F., VANOYE-TREVINO, C. and MOSLEN, M.T. The use of a silastic shield to protect an externalized biliary cannula. Lab. Anim. 1989; 23: 36-38.

KILEY-WORTHINGTON, M. Animals in circuses and zoos. Chiron's world? Basildon, Essex, U.K.: Little Eco-Farms Pub., 1990: 41-44. (29 Delhi Road, Pitsea, Basildon, Essex SS13 2EH, U.K..)

KUMAR, A., WONG, D.A., JOHNSON, R.G., HERBERT, M.A. and SALTER, R.B. The restraint of rabbits in a special sling. Lab. Anim. Sci. 1979; 29(4): 512-515.

LAING, J.A., ed. Prevention, management and repair of trauma in transport animals. Oxford, U.K.: World Association for Transport Animal Welfare and Studies, 1991.

LANDI, M.S., KREIDER, J.W., LANG, C.M. and BULLOCK, L.P. Effects of shipping on the immune functions in mice. Am. J. Vet. Res. 1982; 43: 1654-1657.

LANDI, M., KRIEDER, J.W., LANG, C.M. and BULLOCK, L.P. Effect of shipping on the immune functions of mice. In: Archibald, J., Ditchfield, J. and Rowsell, H.C., eds. The contribution of laboratory animal science to the welfare of man and animals. Stuttgart, New York: Gustav Fischer Verlag, 1985: 11-18.

LAWHORN, B. A new approach for obtaining blood samples from pigs. J. Amer. Vet. Med. Assoc. 1988; 192(6): 781-782.

LEA, S.E.G. Alternatives to the use of painful stimuli in physiological psychology and the study of animal behaviour. ATLA (Alternatives To Laboratory Animals) Abstracts 1979; 7(1): 20.

LEE, P. (correspondence) Lab. Anim. Sci. 1992; 42(6): 433.

LUKAS, S.E, GRIFFITHS, R.R., BRADFORD, L.D., BRADY, J.V. and DALEY, L. A tethering system for intravenous and intragastric drug administration in the baboon. Pharmacol. Biochem. Behav. 1982; 17: 823-829.

LYON, M.F. Ch. 3 Nomenclature. In: Foster, H.L., Small, J.D. and Fox, J.G., eds. The mouse in biomedical research, Volume I. History, genetics and wild mice. New York, NY: Academic Press, 1981.

LYON, M.F. and SEARLE, A.G., eds. Genetic variants and strains of the laboratory mouse. 2nd Ed. Oxford: Oxford University Press, 1989.

MARCUS, L.C. Veterinary biology and medicine of captive amphibians and reptiles. Philadelphia, PA: Lea and Febiger, 1981.

MARKOWITZ, H. and SPINELLI, J. Environmental engineering for primates. In: Benirschke, K., ed. Primates. The road to self-sustaining populations. New York, NY: Springer-Verlag, 1986: 489-498.

MAYER, J.S. and BOWMAN, R.E. Rearing, experience, stress and adrenocorticosteroids in the rhesus monkey. Physiol. Behav. 1972; 88: 339.

MERLINO, G.T. Transgenic animals in biomedical research. FASEB J. 1991; 5: 2996-3001.

MEYER, P.M. and MEYER, R.D. Neurosurgical procedures with special reference to aspiration lesions. In: Meyer, R.D., ed. Methods in psychobiology, Vol. 1. New York, NY: Academic Press, 1971.

MOAKE, J.L. (editorial) Von Willebrand factor and the pathophysiology of thrombotic thrombocytopenia: From human studies to a new animal model. Lab. Invest. 1988; 59(4): 415-417.

MOBERG. G.P. Stress: diagnosis, cost and management. In: Mench, J.A., Mayer, S.J. and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Bethesda, MD: SCAW

(Scientists Center for Animal Welfare), 1992: 58-61. (4805 St. Elmo Ave., Bethesda, MD 20814 USA.)

MORCK, D.W., COSTERTON, J.W., BOLINGBROKE, D.O., CERI, H., BOYD, N.D. and OLSON, M.E. A guinea pig model of bovine pneumonic pasteurellosis. Can. J. Vet. Res. 1990; 54: 139-145.

MUNSON, E.S. Arterial cannulation in awake restrained monkeys. Lab. Anim. Sci. 1974; 24: 793.

MYHR, B. and BRUSICK, D. A transgenic mouse model for genetic toxicology studies. Lab Animal 1991; 20 (1): 31-35.

NATIONAL INSTITUTES OF HEALTH. Preparation and maintenance of higher animals during neuroscience experiments. Report of a National Institutes of Health workshop, 1991.

NOMURA, T., KATSUKI, M., YOKOYAMA, M. and TAJIMA, Y. Future perspectives in the development of new animal models. In: Animal models: assessing the scope of their use in biomedical research. Alan R. Liss, Inc., 1987: 337-353. (Canadian distributor, J. Wiley & Sons Ltd., 22 Worcester Rd., Rexdale, Ont. M9W 1L1.)

OLFERT, E.D. Ethics of animal models of neurological diseases. In: Boulton, A.A., Baker, G.B. and Butterworth R.F., eds. Neuromethods: animal models of neurological disease. I. Cliston, NJ: Humana Press, 1992: 1-28.

PALMITER, R.D. and BRINSTER, R.L. Germ-line transformation of mice. Ann. Rev. Genet. 1986; 20: 465-499.

PANEPINTO, L.M., PHILLIPS, R.W., NORDEN, S., PRYOR, P.C. and COX, R. A comfortable, minimum stress method of restraint for Yucatan miniature swine. Lab. Anim. Sci. 1983; 33(1): 95-97.

PARÉ, W.P. and GLAVIN, G.B. Restraint stress in biomedical research: A review. Neurosci. Biobehav. Rev. 1986; 10: 339-370.

PELLEGRINO, L.J. and CUSHMAN, A.J. Use of stereotaxic technique. In: Meyer, R.D., ed. Methods in psychobiology. New York, NY: Academic Press, 1971.

PFEIFFER, C.J., ed. Animal models for intestinal disease. Boca Raton, FL: CRC Press, 1985: 135-145.

POOLE, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987.

RATH, L. and HUTCHISON, M. A new method of bile duct cannulation allowing bile collection and re-infusion in the conscious rat. Lab. Anim. 1989; 23: 163-168.

REDDICK, R.L., READ, J.S., BRINKHOUS, K.M., BELLINGER, D., NICHOLS, T. and GRIGGS, T.R. Coronary atherosclerosis in the pig. Induced plaque injury and platelet response. Arteriosclerosis 1990; 10(4): 541-550.

REINHARDT, V. Transport-cage training of caged rhesus macaques. Anim. Tech. 1992; 43(1): 57-61.

ROBBINS, D.O., ZWICK, H., LEEDY, M. and STEARNS, G. Acute restraint device for rhesus monkeys. Lab. Anim. Sci. 1986; 36(1): 68-70.

ROWSELL, H.C. Legislation regulations pertaining to laboratory animals-Canada. In: Melby, E.C. Jr. and Altman, N.H., eds. CRC handbook of laboratory animal science. Vol. 1. Cleveland, OH: CRC Press, 1974: 11-17.

ROWSELL, H.C. Perspectives of the welfare of animals during transportation. In: Erichsen, S., Coates, M. E. and Chatikavanij, P., eds. Proc. IX International Council for Laboratory Animal Science international symposium on laboratory animal science. Bangkok, Thailand: ICLAS, 1988: 313-320.

ROWSELL, H.C. Current perspectives on the problems related to the transport of live animals. Proc. Animal Transportation Assoc. 16th Annual Conference. Dallas, TX: AATA, 1990: 19-24.

ROWSELL, H.C. Transportation of animals. A global animal welfare issue. Live Animal Trade and Transport Magazine 1992 March: 37-41.

RUIZ DE ELVIRA, M.-C. and ABBOTT, D.H. A backpack system for long-term osmotic mini-pump infusions into unrestrained marmoset monkeys. Lab. Anim. 1986; 20: 329-334.

RUSHEN, J. Aversion of sheep to electro-immobilization and physical restraint. Appl. Anim. Behav. Sci. 1986; 15: 315-324.

SAFFER, J. Transgenic mice in biomedical research. Lab Animal 1992; 21(3): 30-38.

SINGH, D. and AVERY, D.D. Physiological techniques in behavioural research. Belmont, CA: Wadsworth Publishing Co., 1975.

SKINNER, J.E. Neuroscience: A laboratory manual. Philadelphia, PA: W.B. Saunders Co., 1971.

SLATNETZ, G., FRATTA, I., CROUSE, G. and JONES, S. Stress and transportation of animals. In: Proc. Animal Care Panel 1957; 7: 278-289.

TOTH, L.A. and JANUARY, B. Physiological stabilization of rabbits after shipping. Lab. Anim. Sci. 1990; 40 (4): 384.

THOMASON, J.J. and RUSSELL, A.P. A plastic cage for restraint of the opossum (*Didelphis virginiana*). Lab. Anim. Sci. 1986; 36(5): 547-549.

VALLE, F.P. Sex-typing newborn rats: An improved procedure with agouti strains. Behav. Res. Meth. Instru. 1990; 2: 205.

VANDERLIP, S.L., VANDERLIP, J.E. and MYLES, S. A socializing program for laboratory raised canines. Part 1. Lab Animal 1985a; 14(1): 33-36.

VANDERLIP, S.L., VANDERLIP, J.E. and MYLES, S. A socializing program for laboratory-raised canines. Part 2: The puppy socialization schedule. Lab Animal 1985b; 14(2): 27-36.

WAGNER, J.A., REYNOLDS, I.J., WEISMAN, H.F., DUDECK, P., WEISFELDT, M.L. and SNYDER, S.H. Calcium antagonist receptors in cardiomyopathic hamster: Selective increases in heart, muscle, brain. Science 1986; 232: 515-518.

YAGIELA, J.A. and BILGER, P.A.L. A custom restraining device for small animals. Lab. Anim. Sci. 1986; 36 (3): 303-305.

YOUSEF, M.K. Animal stress and strain: definition and measurements. Appl. Anim. Behav. Sci. 1988; 20: 119-126.

VIII. HEALTH AND SAFETY IN THE WORKPLACE

Those working with experimental animals risk exposure to physical hazards (e.g., heat, noise, radiation), chemical hazards (e.g., disinfectants, cleaning solutions), as well as intestinal parasites, enteric bacteria, pathogenic organisms, and animal bites (Soave and Brand, 1991). As well, those working with swine in confinement buildings may suffer future chronic and irreversible lung damage, according to Donham and Leininger (1984). Those working with non-human primates (NHP) must take special precautions. Guidance for these individuals may be found in Volume 2 of this *Guide*.

A. REGULATORY REQUIREMENTS

As is the case with other laboratories, the animal care facility should have an Occupational Health and Safety program. All persons using the facility should also be familiar with the requirements of relevant federal, provincial and municipal legislation. This would include, for example, the federal Health of Animals Act (38-39 Elizabeth II, Chapter 21, pgs. 387-421), which replaced the Animal Disease and Protection Act and which governs control of animal diseases and toxic substances. Those working with animals should also be cognizant of institutional and/or facility safety program (see also Volume 2 of this *Guide* [CCAC, 1984a]).

The Workplace Hazardous Materials Information System (WHMIS), which resulted from federal and provincial co-operation, was instituted in 1988. Federal government laboratories are governed by federal WHMIS and the Canada Labour Code. The following publications are available free of charge from Labour Canada: *The Employer and WHMIS; Introduction to the WHMIS Program; Exercise WHMIS in the Workplace,* and a relevant poster.

Elsewhere, provincially enacted Health and Safety legislation specifies the accountability of owners and directors and the rights and responsibilities of employers, supervisors and workers in the workplace. The right to refuse unsafe work is a part of the Occupational Health and Safety (OHS) Act. WHMIS regulations are also a section of this legislation and require that each employer provide safe working conditions and that employees be informed about all hazards they will face in the course of their duties. Employees are also given the right to withdraw from the workplace if faced with an unsafe condition. All hazardous substances, including microorganisms, must be labelled in a specified manner, and a Material Safety Data Sheet (MSDS) must be available to accompany each hazardous substance. Each province has adapted these federal government guidelines for its own purposes. WHMIS material may be obtained from provincial Ministries of Labour.

All personnel working with animals must understand how to handle the species involved, both for their own safety and health, and for that of the animals. Training for this should be provided.

B. BIOLOGICAL HAZARDS

Guidelines for working with biohazards (e.g., bacteria, viruses, parasites, fungi and other infectious agents), are provided in the Health and Welfare Canada/Medical Research Council *Laboratory Biosafety Guidelines* (HWC/MRC, 1990). The guidelines include such items as biohazard containment, laboratory design, personal hygiene and safety facilities, and can be used to provide training for employees as mandated by WHMIS.

The biosafety guidelines apply to all research carried out or supported by the federal government and have been adopted by many industries.

Standard Operating Procedures (SOP) based on the guidelines, aimed at minimizing risks to humans in biohazard risk areas, should be developed and enforced.

Personal cleanliness is an important barrier to infection and washing of hands after handling any animal will reduce the risk of disease spread and self-infection. All employees working with animals, as well as visitors to the facility, should wear protective clothing, minimally a lab coat.

All contaminated material must be decontaminated before disposal. Necropsy of animals infected with highly infectious agents should be carried out in certified and tested biological safety cabinets. Necropsy material for disposal should be sealed in plastic bags, properly labelled and incinerated. The necropsy room should be properly equipped to provide adequate refrigeration and hand-washing facilities.

C. ZOONOSES

Those infections that are "secondarily transmitted from animals to humans" are referred to as zoonoses (Schnurrenberger and Hubbert, 1981; August and Loar, 1987; Acha and Szyfres, 1989) and can seriously affect research (Hamm, 1986; Bhatt, Jacoby, Morse *et al.* 1986; ILAR/NRC, 1991).

While most infectious agents show a considerable degree of species specificity, they also may, from time to time, vary widely in virulence and in their capacity to break through species barriers. Thus, infections that have not commonly been considered to be zoonotic hazards may sporadically affect susceptible persons or animals. Persons potentially at higher risk are those who suffer from defective immune systems and those who are under severe stress or who have non-overt clinical disease. Numerous pathogenic microorganisms, such as those responsible for tuberculosis, brucellosis, rabies, etc., which are normally perpetuated by direct transmission from one or more species of vertebrate animals, are also readily transmissible to humans.

Transmission of infections from animals to humans can generally be avoided through proper veterinary care and adherence to SOPs for control of transmission. However, when animals are obtained from areas in which zoonotic diseases are known to exist, e.g., in NHP acquired from the wild (Houghton, 1986) special attention is required.

Work involving exposure to hazardous microorganisms might require prior immunization of the staff, if a vaccine is available. It is recommended, for example, that all personnel handling random-source dogs and cats, including dealers and handlers, should receive routine rabies vaccination (see also Special Animal Colonies, Infectious Disease Units).

Serological testing and banking of reference serum samples from all personnel working in the animal facility is advisable. This is of particular importance where NHP are being handled and/or agents infectious to humans are being used.

Caution should be exercised in assigning women of childbearing status to animal care duties that might expose them to potential or known teratogens. For example *Toxoplasma gondii*, a protozoan that infects most species of warm-blooded animals, including humans, is spread primarily by oocysts shed in cat feces. These oocysts sporulate in two to four days and may survive for more than a year (Fraser and Mays, 1986). Human toxoplasmosis can result in spontaneous abortion, prematurity, stillbirth or congenital defects (Schnurrenberger and Hubbert, 1981).

The life cycle of the causative organisms implicated in a number of indirect zoonoses may involve transmission through one or more other vertebrate and/or invertebrate intermediate hosts before affecting humans (for example, in taeniasis, tularemia, and vesicular stomatitis). Amongst invertebrate vectors of zoonotic disease, the biting insects are the main offenders. A list of some of the diseases transmitted to humans from animals is included in Appendix VII.

The role of cold-blooded vertebrates in the epidemiology of zoonoses should not be overlooked. In particular, turtles infected with salmonella may constitute a human health hazard in the student laboratory as well as in the animal facility (Sherris, 1990).

D. PROCEDURES FOR WORKING WITH NON-HUMAN PRIMATES

This topic has also been discussed in Volume 2 of this *Guide* (CCAC, 1984b).

All animals must be regarded as potential sources of zoonoses, although the risk of this occurring will vary widely with the class, species, and source of the animal involved. In general, the more closely related phylogenetically a species is to humans, (Anon., 1987a, 1987b; FRAME, 1987; Rice, 1987a, 1987b), the greater the likelihood of zoonoses. It is for this reason that special precautions must be followed for NHP (Love, 1980; Wong and Gardell, 1982; Richter, Lehner and Henrickson, 1984; Else, 1988).

Each institution that maintains a NHP facility is responsible for providing the proper veterinary and human medical services to safeguard the health and safety of both personnel and animals. International guidelines have been prepared for those working with NHP (FRAME/CREA, 1987; Kaplan, 1987; Anon., 1989; MRC, 1985).

Outbreaks of viral diseases, e.g., Callitrichid Hepatitis Virus (Anderson, 1991) recently "rocked the primatologist's world," and current procedures for the rapid diagnosis of primate viral diseases include serology, virus isolation, direct visualization using electronmicroscopy or immunofluorescence, and detection of viral components (Kalter and Herberling, 1990).

The most reasonable and effective approach in reducing occupational infection risks is to develop and follow SOPs that preclude or minimize overt occupational exposure among personnel working with NHP or biological samples therefrom. SOPs should be established for an Occupational Health and Safety Program for personnel which would include serological screening and vaccination, use of protective clothing, containment, stress on personal hygiene, procedures for accidents including bite wounds and/or other exposure to potential risk, and for quarantine and quality control procedures for confined animals.

Guidelines have been prepared for prevention of *Herpes Simiae* (B-Virus) infection by a B-Virus Working Group which convened at the Centers for Disease Control (Anon., 1987c; Kaplan, Balk, Brock *et al.* 1987; Schulhof, 1990). *Herpes Simiae* is fatal in humans (Kalter and Herberling, 1989).

Similarly, because of the expanding use of Simian Immunodeficiency Virus (SIV), which is closely related to the Human Immunodeficiency Virus (HIV), guidelines in this regard have been prepared as well (Anon., 1989). Standard serological procedures to identify SIV antibody are used in laboratories conducting research with the virus, and the National Institutes of Health (NIH) and World Health Organization (WHO) have expanded their diagnostic services (Kalter, 1987).

In addition to NHP from the wild, those within the colony may also carry indigenous latent infections (Baulu, Everard and Everard, 1987; Dance, King, Aucken *et al*. 1992). It is important to establish rigid quarantine and quality control procedures for the animal colony and to better define and be more aware of the potential risks. The Ebola-like virus outbreak in the U.S. in 1989 exemplified such risks (Anon., 1990; Anderson, 1990a, 1990b; Dalgard, Hardy, Pearson *et al*. 1992).

Adherence to the following list of precautions is recommended:

a) all NHP must be considered as potentially carrying a disease transmissible to humans;

b) NHP, or anything that has been in direct contact with them, should not come in contact with the skin;

c) protective clothing, including coveralls, boot covers, surgical caps, masks and gloves should be worn when working with NHP, and removed when leaving the NHP quarters;

d) smoking and bringing food and drink into NHP rooms are strictly forbidden;

e) facilities for washing hands must be made available and used by all personnel immediately upon leaving NHP rooms;

f) personnel with sores, cuts, and other lacerations should not come in contact with NHP. However, if this is unavoidable, then the lesion must be adequately covered prior to and during any activity in a room containing NHP, and dressings must be changed immediately upon leaving. These dressings and any other disposable items so exposed must be treated as biological hazardous waste;

g) all cuts, bites, scratches, or needle punctures acquired while working with or in proximity of NHP must be reported to the medical authority designated by the institution. SOPs for all wounds so encountered should be developed and followed accordingly. Immediate treatment must ensure that the wound is made to bleed freely and thoroughly scrubbed and cleansed with soap and water. A flushing of the wound area with a provodine iodine solution is recommended. In the event that sterility has been breached (e.g., tearing or puncture of a surgical glove) the hands must be re-scrubbed before leaving the room and re-gloved before continuing the procedure.

Following injury by a NHP, the animal concerned must immediately be immobilized and examined for excessive salivation and for lesions of the oral cavity which may be characteristic of Herpes (B-Virus). SOPs must be followed for dealing with this type of accident. Procedures for sampling for Herpes B of the animal and of the injured person must be followed. The results of the examination must be communicated to the previously designated medical authorities, along with information on the species of NHP, length of time in the colony, and contacts with other species;

h) special precautions should be taken whenever conducting necropsies on NHP that have died during the conditioning period; necropsy procedures should include the wearing of protective clothing, surgical caps, and masks, gowns and surgical gloves. The use of biosafety cabinets for conducting all necropsy of NHP tissue is
recommended;

i) because of the possible danger of contacting Hepatitis A, it is recommended that personnel working with newly imported chimpanzees receive hyper-immune serum globulin prophylactically. Animals should be tested for human hepatitis antigens and, if positive, strictly quarantined;

j) all personnel having contact with NHP must be free of tuberculosis and should receive a tuberculin skin test not less than once yearly and X-ray examination as prescribed. It should be noted that it has recently been reported that misleading positive tuberculin reactions were caused in squirrel monkeys that had received Freund's Complete Adjuvant (FCA) (Pierce and Dukelow, 1988);

k) protective leather gauntlets should be worn when handling conscious NHP. Several varieties are available commercially;

l) all laundry that has been in direct contact with NHP or their excreta should be autoclaved prior to being sent out for washing.

E. ALLERGIES

Allergies to laboratory animals are a significant occupational health concern for people regularly working with the common laboratory animal species (Aoyama, Ueda, Manda *et al*. 1992; Olson, 1986; Bland, Levine, Wilson *et al*. 1986; Botham, Davies and Teasdale, 1987; Kibby, Powell and Cromer, 1989; Lutsky, 1987; Slovak and Hill, 1987; Venables, Tee, Hawkings *et al*. 1988). Laboratory animal allergy (LAA) is an immediate-type hypersensitivity reaction, IgE-mediated, which develops upon exposure to a laboratory animal, its fur or dander, its urine, saliva, serum or other body tissues. Typical symptoms range from mild (e.g., upper respiratory signs such as sneezing, itchy and/or runny nose and eyes, and skin reactions such as red, raised and itchy wheals after contact with animals, their tissues or their excreta), to severe [e.g., wheezing, shortness of breath, and a feeling of chest tightness (asthma)]. Persons experiencing such symptoms should be advised to contact their physician for diagnosis and treatment.

Measures which can reduce the degree of exposure to laboratory animal allergens include:

a) use of protective gear such as gloves, face masks, gowns, shoe covers, etc., worn only in animal rooms;

b) regular hand-washing, and showering after work;

c) use of improved filtration in animal room ventilation systems, and the use of special filtered caging systems; and

d) educational programs for employees identifying high risk (e.g., high allergen load) areas and tasks, and strict use of preventive measures, as set out by the institution's SOPs.

Institutions are encouraged to include a consideration of LAA in their Occupational Health and Safety programs. As noted above, identifying high risk areas and tasks (Eggleston, Newill, Ansari *et al*. 1989; Gordon, Tee, Lowson *et al*. 1992; Swanson, Campbell, O'Hallaren *et al*. 1990), and the use of SOPs in these areas, along with education of staff, are useful in reducing the severity of the problem (Botham, Davies and Teasdale, 1987). Procedures for monitoring exposure, health-monitoring of staff at risk, and for dealing with staff who become allergic should also be considered (Botham, Davies and Teasdale, 1987; Lutsky, 1987; Newill, Evans and Khoury, 1986).

F. PHYSICAL INJURIES AND CHEMICAL HAZARDS

Physical injuries related to the handling of animals may be kept to a minimum by ensuring that:

a) all staff are trained and experienced in handling the species with which they work, and that they know the particular hazards associated with each species;

b) all staff are familiar with the hazards of the experiment, and are provided with (and use) a proper working area, protective clothing and equipment;

c) a mechanism is in place in every unit to deal with animal-inflicted injury, and for referral for any further medical treatment if this is required.

Responsibility for ensuring that first aid kit(s) are available and always properly stocked must be clearly identified. The location of the first aid kit(s) should be prominently marked and all personnel using the facility should be made aware of these locations.

Injuries from chemicals can be avoided by treating all chemicals with care, by knowing their properties and adhering to the accepted safety practices for handling that type of product. WHMIS, legislative and institutional requirements must be met.

Care should always be taken in handling such common chemicals as industrial detergents used in cage washers, cleaning agents, and powerful disinfectants. These substances should be stored separate from animal feed and bedding materials. Volatile liquids used as anesthetics or for euthanasia, and other toxic and volatile materials, should be stored in well-ventilated fume hoods or cabinets designed for that purpose.

G. RADIATION AND ULTRAVIOLET LIGHT

Radioactive materials present special hazards. All persons working with these materials should know the properties of each, and be familiar with the appropriate safe handling techniques. The possession of radioactive materials is authorized by Radioisotope Licences issued by the (federal) Atomic Energy Control Board (AECB) to the institutions. The Radiation Safety Program is administered by a Radiation Safety Officer, who the AECB recommends sit as an *ex-officio* member of the institution's Occupational Health and Safety Committee. Use of X-rays is governed by Occupational Health and Safety Acts under provincial Ministries of Labour.

Isotope-treated animals may pass radioactive material in their excrement, which should therefore be disposed of in an approved manner, as must the animal itself after death. Complete records should be kept through to the final disposition of these animals.

The eye and skin are critical areas for exposure to ultraviolet (UV) light. The eye, in particular, can be seriously injured. Staff should not be exposed to UV rays; however, if they must be, they should be warned of the hazards and provided with "wraparound" safety glasses. As well, the source of illumination should be suitably marked. The maximum intensities tolerated by sensitive faces for a seven-hour day, range from 0.1 to 0.5 milliwatt per square foot.

H. REFERENCES

ACHA, P.N. and SZYFRES, B. Zoonoses and communicable diseases common to man and animals. 2nd Ed. Washington, DC: Pan American Health Organization/World Health Organization, 1989.

ANDERSON, G.C. Monkey imports may be curtailed in U.S.. Nature March 22, 1990a: 280.

ANDERSON. G.C. U.S. shuts down monkey trade. Nature March 29, 1990b: 369.

ANDERSON, G.C. Emerging virus threat. Nature May 9, 1991: 89.

ANON. My close cousin the chimpanzee. Science, Research News 1987a; 238: 273-275.

ANON. B-Virus infection in humans. Lab. Primate Newsl. 1987b; 26(3): 2-4.

ANON. The use of non-human primates in laboratories. The Lancet Jan. 31, 1987c: 286.

ANON. Guidelines to prevent Simian Immunodeficiency Virus infection in laboratory workers and animal handlers. Lab. Primate Newsl. 1989; 28(1): 17-21.

ANON. Concurrent Ebola and SHF in imported primates. Lab. Primate Newsl. 1990; 29(1): 1-2.

AOYAMA, K. UEDA, A., MANDA, F. *et al*. Allergy to laboratory animals: an epidemiological study. Brit. J. Indust. Med. 1992; 49: 41-47.

AUGUST, J.R. and LOAR, A.S. Zoonotic diseases. Vet. Clin. North Amer. Sm. Anim. Pract. 17(1), 1987. Philadelphia, London, Toronto, Mexico City, Rio de Janeiro, Sydney, Tokyo, Hong Kong: W.B. Saunders Co.

BAULU, J., EVERARD, C.O.R. and EVERARD, J.D. Leptospires in vervet monkeys (*Cercopithecus aethiops sabaeus*) on Barbados. J. Wildl. Dis. 1987; 23(1): 63-68.

BHATT, P.N., JACOBY, R.O., MORSE, H.C. III *et al*. Viral and mycoplasmal infections of laboratory rodents. Effects on biomedical research. Orlando, San Diego, New York, Austin, Boston, London, Sydney, Tokyo, Toronto: Academic Press, 1986.

BLAND, S.M., LEVINE, M.S., WILSON, P.D. *et al*. Occupational allergy to laboratory animals: an epidemiological study. J. Occup. Med. 1986; 28(11): 1151-1157.

BOTHAM, P.A., DAVIES, G.E. and TEASDALE, E.L. Allergy to laboratory animals: a prospective study of its incidence and of the influence of atopy on its development. Brit. J. Indust. Med. 1987; 44: 627-632.

CANADIAN COUNCIL ON ANIMAL CARE. Guide to the care and use of experimental animals, Volume 2. Ottawa, Ont.: CCAC, 1984a: 170-172.

CANADIAN COUNCIL ON ANIMAL CARE. Non-human primates. In: Guide to the care and use of experimental animals. Vol. 2. Ottawa, Ont.: CCAC, 1984b: 163-173.

DALGARD, D.W., HARDY, R.J., PEARSON, S.L., PUCAK, G.J., QUANDER, R.V., ZACK, P.M., PETERS, C.J. *et al.* Combined simian hemorrhagic fever and ebola virus infection in cynomolgus monkeys. Lab. Anim. Sci. 1992; 42(2): 152-157.

DANCE, D.A.B., KING, C., AUCKEN, H., KNOTT, C.D., WEST, P.G. and PITT, T.L. An outbreak of melioidosis in imported primates in Britain. Vet. Rec. 1992; 130(24): 525-529.

DONHAM, K.J and LEININGER, J.R. Animal studies of potential chronic lung disease of workers in swine confinement buildings. Amer. J. Vet. Res. 1984; 45(5): 926-931.

EGGLESTON, P.A., NEWILL, C.A., ANSARI, A.A. *et al*. Task-related variation in airborne concentrations of laboratory animal allergens: Studies with rat n1. J. Allergy Clin. Immunol. 1989; 84: 347-352.

ELSE, J.G. IPS international guidelines for the acquisition, care and breeding of non-human primates. International Primat. Soc. 1988: 1-36.

FRASER, C.M. and MAYS, A. The Merck veterinary manual. 6th Ed. Rahway, NJ: Merck and Co., 1986.

FUND FOR THE REPLACEMENT OF ANIMALS IN MEDICAL EXPERIMENTS/ COMMITTEE FOR THE REFORM OF ANIMAL EXPERIMENTATION. The use of non-human primates as laboratory animals in Great Britain. Nottingham: FRAME, 1987. (FRAME, Eastgate House, 34 Stoney St., Nottingham, NG1 1NB, U.K.)

GORDON, S., TEE, R.D., LOWSON, D. *et al*. Reduction of airborne allergenic proteins from laboratory rats. Brit. J. Indust. Med. 1992; 49: 416-422.

HAMM, T.E. Jr., ed. Complications of viral and mycoplasmal infections in rodents to toxicology research and testing. Washington, New York, London: Hemisphere Pub. Corp., 1986.

HEALTH AND WELFARE CANADA/MEDICAL RESEARCH COUNCIL OF CANADA. Laboratory biosafety guidelines. Cat. No. MR 21-1/1990E. Ottawa, Ont.: Supply and Services Canada, 1990.

HOUGHTON, P. Collecting feral cynomolgus macaques. Lab Animal 1986; 15(5): 19,20,22.

INSTITUTE OF LABORATORY ANIMAL RESOURCES/NATIONAL RESEARCH COUNCIL. Infectious diseases of mice and rats. Washington, DC: National Academy Press, 1991.

KALTER, S.S. Simian AIDS testing available. Lab Primate Newsl. 1987; 26(1): 4.

KALTER, S.S. and HERBERLING, R.L. B-virus infection of primates in perspective. Lab Animal 1989: 31-34.

KALTER, S.S. and HERBERLING, R.L. Current procedures for the rapid diagnosis of primate viral diseases. Lab Animal 1990: 39-47.

KAPLAN, J.E., BALK, M., BROCK, B. *et al*. (correspondence) Guidelines for prevention of *Herpesvirus Simiae* (B-virus) infection in monkey handlers. Lab. Anim. Sci. 1987; 37(6): 709-712.

KIBBY, T., POWELL, G. and CROMER, J. Allergy to laboratory animals: A prospective and cross-sectional study. J. Occup. Med. 1989; 31(10): 842-846.

LUTSKY, I. A worldwide survey of management practices in laboratory animal allergy. Ann. Allergy 1987; 58: 243-247.

MEDICAL RESEARCH COUNCIL. The management of simians in relation to infectious hazards to staff. Council's Simian Virus Committee, 1985: 1-17.

NEWILL, C.A., EVANS, R. and KHOURY, M.J. Preemployment screening for allergy to laboratory animals: epidemiological evaluation of its potential usefulness. J. Occup. Med. 1986; 28(11): 1158-1164.

OLFERT, E.D. Allergy to laboratory animals. An occupational disease. Lab Animal 1986; 15(5): 24-31.

PIERCE, D.L. and DUKELOW, W.R. Misleading positive tuberculin reactions in a squirrel/monkey colony. Lab. Anim. Sci. 1988; 38(6): 729-730.

RICE, D.C. Primate research: relevance to human learning and development. Dev. Pharmacol. Ther. 1987a; 10: 314-327.

RICE, D.C. Methodological approaches to primate behavioural toxicological testing. Neurotoxicol. Teratol. 1987b; 9: 161-169.

SCHNURRENBERGER, P.R. and HUBBERT, W.T. An outline of zoonoses. Ames, IA: Iowa State University

Press, 1981.

SCHULHOF, J. Group sets precedents for monkey bite treatment. Lab Animal 1990; 19(2): 11.

SLOVAK, A.J.M. and HILL, R.N. Does atopy have any predictive value for laboratory animal allergy? A comparison of different concepts of atopy. Brit. J. Indust. Med. 1987; 44: 129-132.

SOAVE, O. and BRAND, C.D. Employer responsibility for employee health in the animal environment. Lab Animal 1991; 20(2): 41-44.

SWANSON, M.C., CAMPBELL, A.R., O'HALLAREN, M.T. *et al*. Role of ventilation, air filtration, and allergen production rate in determining concentrations of rat allergens in the air of animal quarters. An. Rev. Resp. Dis. 1990; 141: 1578-1581.

VENABLES, K.M., TEE, R.D., HAWKINGS, E.R. *et al*. Laboratory animal allergy in a pharmaceutical company. Brit. J. Indust. Med. 1988; 45: 660-666.

WONG, J.H. and GARDELL, C. Conditioning program for non-human primates. CALAS (Can. Assoc. Lab. Anim. Sci.) Newsl. 1982; 14: 93.

IX. STANDARDS FOR EXPERIMENTAL ANIMAL SURGERY

A. INTRODUCTION

Distress resulting from inappropriate or inadequately performed surgical technique or post-operative care constitutes "unnecessary pain". Adequate knowledge of topics such as animal physiology, pharmacology and anatomy is essential for the success of any research program involving the use of experimental animals, especially where surgical techniques are required. Good surgical techniques, appropriate anesthesia, proper instrumentation and competent pre- and post-operative care are all essential to the welfare of the experimental animal and the success of the surgical component of the research project, as are correctly designed surgical facilities.

All persons performing surgical techniques should have demonstrated ability in the surgical procedures required. In this respect, it is essential that institutions provide the opportunity for basic training and practice in required procedures before experimental surgery is conducted. Cadaver practice and non-survival trials can help train investigators. Adequate training and practice will help minimize anesthetic and surgical time and contribute to faster recovery of the animal.

Medical training does not include training in the husbandry, medicine or surgery of laboratory animals. It cannot be assumed, therefore, that prior human surgical experience will result in good experimental animal surgery because there are significant differences in both anesthesia and surgical technique. The guidelines of the Academy of Surgical Research (ASR, 1989) should be consulted regarding the training necessary for the various groups of professionals. In large experimental surgery programs, a key member of the team should be an experienced veterinary surgeon. The primary objective is always responsible use of the experimental animal. It is important that all personnel involved in acute or chronic surgery treat the animals humanely and with dignity at all times. It is the responsibility of the principal investigator to ensure that proper procedures and precautions are observed. The following standards have been developed as a guide to this end.

B. FACILITIES FOR SURVIVAL SURGERY

The physical environment in which surgery is performed may vary from a specially designed, sophisticated surgery suite to a small, specifically designated area of a laboratory. What is required will depend on the surgical procedure and whether or not the animal is to be recovered from anesthesia. Definitions for major and minor surgery are included in the Glossary.

The suite in which aseptic surgery is performed should consist of the following separate areas:

a) animal preparation area;

b) human preparation/scrub area;

c) operating room(s);

d) recovery area adequate for intensive care and post-operative support of animals;

e) support areas which would include areas for storing instruments, packs, supplies and for washing and sterilizing instruments.

Only items used on a regular basis (e.g., anesthetic machines, suture materials, stainless steel (s/s) kick buckets, s/s instrument tables) should be stored in the operating room (OR). Ancillary equipment such as electrosurgery units, respirators, and electrocardiogram (ECG) monitors should be easily sanitized, portable, and stored in the support area if not used regularly.

It is strongly suggested that surgical facilities be within or adjacent to the animal facility. The surgical facility should be located away from the general facility/institutional traffic. Access to the area should be restricted to essential support staff.

The interior surfaces of the surgical facility should be impervious to moisture and easily cleaned. Floor drains and high pressure hoses may be necessary in facilities used for large domestic animals. The ventilation system of the OR should provide a net positive pressure with respect to the surrounding facilities. The surgery should be supplied with non-recirculated air. Incoming air should be as sterile as possible by means of filtration or some other appropriate system. The operating room floor should be skid-proof. Electrical outlets should be covered and located at least 1.5 m above the floor. The lighting in the OR must be adequate for both surgery and clean-up. Surgical lights, either free standing or mounted on walls or ceiling, are essential. They are equipped with sterilizable handles so that the surgeon can adjust them. Piped-in gas services eliminate the safety hazard of exposed pressurized tanks. Ideally services for oxygen (and nitrous oxide) and suction should be present in the surgery, animal preparation area and in animal intensive care/recovery. All these areas should also be equipped with a system for scavenging anesthetic gases. Surgery tables should be durable, impervious to moisture and easily cleaned. Stainless steel and plastic are ideal materials for this (Bennett, Brown and Schofield, 1990).

Ideally **ALL** recovery surgery should be performed in a suite especially designed for this purpose. However, it is recognized that minor (minimally invasive) surgery in small rodents of the suborder *Myomorpha* (rats and mice) is often performed in laboratories. In this case, an area in the laboratory should be set aside and used only for surgery, and should be out of the main laboratory traffic. It should be uncluttered, easily cleaned, well-lit, and should have facilities for evacuating/scavenging anesthetic gases if they are in use. **At no time should surgery be performed in an animal housing room.** Major (invasive) surgery in rodents, including stereotaxic surgery, should be performed in a dedicated surgery room.

Intensive care/post-operative recovery should be located adjacent to the OR and close to the persons responsible for post-operative monitoring. This area should be easily sanitized and contain cages/pens of the appropriate size for the species being used. Cages may vary from a sophisticated commercial unit that provides oxygen and heat, to a standard cage in which hypothermia is prevented by increasing the environmental temperature, e.g., by use of circulating hot water blankets, heat lamps or hot water bottles. The type of monitoring equipment found in this room will depend on the surgery performed. However, the means to monitor the animal's cardiovascular system, respiratory system and core temperature should be available. An emergency kit and crash cart should be readily available. Large domestic species (e.g., ruminants or pigs) may be recovered in their individual stalls. The stalls should be clean, warm, dry and well-bedded. If bedding is not in use, animals should not be recovered on the stall floor, but on rubber mats or raised platforms. Since the stalls are likely to be at some distance from the surgery suite, it is important that there be frequent and careful post-operative monitoring. The recovery room should contain an area for record keeping.

C. PRE-OPERATIVE PLANNING AND ANIMAL PREPARATION

All persons involved in an experimental surgery program should be identified to ensure that they are properly trained in the principles and practice of aseptic technique, proper instrument use, tissue handling, closure and suturing techniques, anesthesia and analgesia.

The primary investigator must develop a written protocol for the operative procedure in which possible complications or special maintenance requirements arising from the procedure are anticipated. The protocol should clearly identify the responsibilities of all persons involved in the project; support staff,

animal care staff, research technicians and investigators. Adequate staff must be available for proper care of each animal during the peri-operative period. For some projects, the surgical facility may need to be staffed on a 24 hour basis.

It is recommended that pre-operative care, operative technique and post-operative care practices be developed in consultation with a veterinarian. A laboratory animal veterinarian must be consulted to ensure that there is **adequate veterinary care for the animal,** including appropriate anesthesia and analgesia.

Only healthy, disease-free animals should be used in an experimental surgery program. Specific Pathogen Free (SPF) rodents and rabbits are available commercially. Random-source animals must undergo a conditioning period as recommended by the laboratory animal veterinarian.

A period of **acclimatization,** in which the animal can adjust to new environments, special housing, tethers, slings, other forms of restraint or frequent handling, is very important. This will greatly decrease the amount of distress or disorientation experienced by the animal and ensure the validity of experimental results.

Surgical records should be kept for all experimental animals. The degree of detail recorded will vary with the procedure and the species. The amount of information recorded for a calf undergoing heart transplantation will be very different from that recorded for a group of rats undergoing adrenalectomy, for example.

Each species has a different **fasting time before surgery**. Food is usually withheld for 12 hours before surgery in dogs, cats, ferrets, non-human primates (NHP) and pigs (Flecknell, 1987). Water should be withheld only for two to three hours (if at all) before the actual surgery so that dehydration does not result. Fasting ruminants for 24 to 48 hours prior to surgery helps to reduce the incidence of rumenal tympany (bloat) (Flecknell, 1987). It is unnecessary to withhold food and water from rodents and rabbits except in special circumstances such as surgery of the lower bowel.

If fasting is required, it can be done overnight in large rodents, or for up to 24 hours in rabbits, as they retain their food longer. Mice or other small rodents with similarly high metabolic rates should not be fasted for more than three or four hours (see also Anesthesia).

D. SURGICAL PROCEDURES AND INTRA-OPERATIVE NURSING CARE

Methods for restraining animals for injections or the collection of body fluids are described in Volume 2 of this *Guide* (CCAC, 1984). Table 1 provides a summary of the injection sites, needle sizes and volumes to be introduced for the smaller common laboratory species.

All species undergoing surgery should receive a similar level of care and attention. Recovery surgery in all species of animals should be performed using aseptic technique. Instruments should be sterile. Objects introduced into the animal, such as telemetry implants, osmotic minipumps, vascular access ports, cannulae and any other biomedical devices, must be sterile. Suitable preparation of the surgeon will include wearing a scrub suit, performing a surgical scrub, wearing a cap, mask, sterile gown and sterile surgical gloves. For minor recovery surgery in rodents, a minimum of a clean lab coat, hand scrub, mask and sterile surgical gloves is required of the surgeon.

Surgery in field conditions should be performed in as clean an environment as possible, with sterile instruments, sterile surgical gloves and aseptic technique.

Every effort must be made to minimize infection. The rat may exhibit increased resistance to post-

surgical infection compared to other rodents; however, this should not be an excuse for less-than-adequate sterilization of implants, cannulae, etc., or for non-sterile technique. Routine use of antibiotics is inappropriate.

Those performing "multiple run" surgeries, in which a large number of rodents are undergoing the same procedure, should also use aseptic technique. Several sets of sterile instruments will be required. Instruments, if used more than once, should be kept in a germicidal solution between animals.

General publications are available that describe in detail the pre-surgical preparation of the animal and the incision site, the preparation and sterilization of instrument packs, drapes, fluids, etc., and the draping of the animal. For surgeries that are frequently performed in veterinary practice (e.g., rumenotomies, thoracotomies, castrations), clinical approaches may be used. For experimental surgery, guides to approaches for each body system are available (Gay, 1986a, 1986b, 1989; Swindle and Adams, 1988).

TABLE 1 FOR EACH SPECIES, SITE OF INJECTION, MAXIMUM NORMALLY ACCEPTED VOLUME AND NEEDLE SIZE*

Species	Subcutaneous	Intramuscular	Intraperitoneal	Intravenous
MOUSE	Scruff, 2-3 ml, <20 G	Quadriceps/posterior thigh, 0.05 ml, <23 G	2-3 ml, <21 G	Lateral tail vein, 0.2 ml, <25 G
RAT	Scruff, back, 5-10 ml, <20 G	Quadriceps/posterior thigh, 0.3 ml, <21 G	5-10 ml, <21 G	Lateral tail vein, sublingual vein, penile vein (jugular vein, femoral vein--cut down), 0.5 ml, <23 G
HAMSTER	Scruff, 3-4 ml, <20 G	Quadriceps/posterior thigh, 0.1 ml, <23 G	3-4 ml, <21 G	Femoral or jugular vein (cut down), 0.3 ml, <25 G
GUINEA PIG	Scruff, back, 5-10 ml, <20 G	Quadriceps/posterior thigh, 0.3 ml, <21 G	10-15 ml, <21 G	Ear vein, saphenous vein, dorsal penile vein, 0.5 ml, <23 G
RABBIT	Scruff, flank, 30-50 ml, <20 G	Quadriceps/posterior thigh, lumbar muscles, 0.5-1.0 ml, <20 G	50-100 ml, <20 G	Marginal ear vein, 1-5 ml, (slowly) <21 G
CAT	Scruff, back, 50-100 ml, <20 G	Quadriceps/posterior thigh, 1.0 ml, <20 G	50-100 ml, <20 G	Cephalic vein, 2-5 ml, (slowly), <23 G
DOG	Scruff, back, 100-200 ml, <20 G	Quadriceps/posterior thigh, 2-5.0 ml, <20 G	200-500 ml, <20 G	Cephalic vein, 10-15 ml, (slowly), <21 G
BIRD (domestic fowl)	--	Pectoral muscles, 1-2 ml, <21 G	Midline, halfway between cloaca and sternum, 10-15 ml, <21 G	Brachial (wing) vein, 2-3 ml, <21 G

* In intravenous administration for infusion, the amount of fluid replacement may exceed recommended maximum volumes, particularly in dogs and cats.

Reference

TUFFERY, A.A., ed. Laboratory animals: An introduction for new experimenters. J. Wiley & Sons Ltd. 1987

When selecting a surgical approach, it is important that the surgeon consider the anatomy and normal body posture of the animal. This is especially important in ruminants. In this way, the least painful approach or the one promoting a speedy recovery can be chosen. The surgeon should also be familiar with the behaviour of the animal species being used, so that the appropriate closure technique can be used.

During surgery, it is important that the physiological condition of the animal be monitored and kept stable. The degree of monitoring will depend on the equipment available. Basic monitoring of the cardiovascular system, respiratory system and core temperature requires very little equipment. These observations should be recorded in the animal's surgery record. It is essential that the animal be clinically examined at least twice per day in the immediate post-operative period.

Attention should be paid to the fluid requirements of the animal. Careful attention should be paid to hemostasis during surgery, to avoid hypovolemic shock, especially in small animals. Prolonged surgical procedures or those in which there will be significant blood loss require intravenous electrolyte replacement and/or blood transfusion.

The animal should be positioned on the table so as to avoid compromising cardiovascular or respiratory function and pressure point tissue necrosis. It should be protected from hypothermia and firmly, but carefully restrained in the operative position.

The use of a single animal in multiple survival surgeries is strongly discouraged. Multiple major surgery protocols must be approved by the institution's Animal Care Committee (ACC), and allowed only if for scientific reasons. **Multiple major surgeries on a single animal are not to be performed in order to save money**. A second major surgery may be performed if it is non-survival.

Minor procedures such as biopsies may be performed more than once. However, it is important that animals recover completely between procedures.

The subject of anesthesia is covered elsewhere in this *Guide*; however, the following points should be noted by experimental surgeons:

a) all surgical procedures are to be carried out under anesthesia;

b) those doing surgery have an obligation to be aware of the efficiency of the anesthetic technique being used;

c) it is the responsibility of the surgeon and anesthetist to ensure that this animal is spared discomfort during the entire peri-operative period. This includes the period during the induction of anesthesia, for the entire surgical period and for the post-surgical recovery period.

d) in no case is it acceptable to use muscle paralytics without appropriate anesthetics. No ACC should approve the use of a "paralysed-awake animal" (see also *Ethics of Animal Investigation*) in a surgical or other procedure which might involve pain or distress.

E. POST-OPERATIVE RECOVERY AND SUPPORT

Recovery from anesthesia can be hazardous and requires frequent, perhaps continuous monitoring. Depending on the anesthetic regime, recovery may take from a few minutes to several hours. Qualified staff must be available to monitor the animal throughout the entire recovery period. In the case of recovering neonatal rodents, care must be taken to prevent maternal cannibalism. **Under no circumstances should any animal be allowed to recover unattended.**

A number of nursing activities will be required during the **immediate post-operative period,** e.g., removal of endotracheal tube if used, maintenance or removal of intravenous lines, frequent turning of the animal to avoid bruising and vascular and respiratory problems, and recording of physiological parameters. All these should take place in a designated area suitable for intensive care.

When normal eating and drinking behaviour has resumed, and physiological parameters have been stabilized or are within expected limits, the animal may be removed from intensive care to more standardized husbandry. However, the animal must continue to be monitored carefully; the wound will need attention, sutures need to be removed, catheters flushed, etc. Depending on the model created, **long-term post-operative care** may involve special diets, daily medication, physiotherapy or some other form of specialized treatment. All animals must be monitored for signs of post-surgical infection or other complications.

The goal of the surgery team must be to minimize any pain or distress. The degree of post-operative pain will vary; however, in all cases, every attempt must be made to relieve pain with appropriate use of analgesics and good nursing care. **Investigators must consult with a veterinarian to set up an analgesic regime for ALL species of animals used.** The type of analgesic, the dose and duration of treatment will depend on the species and temperament of the animal and the type of surgery it has undergone. Most analgesics in use are relatively short acting and require administration every few hours. **It is the responsibility of the investigator to make sure that the necessary staff are available to administer analgesics as prescribed.** The laboratory animal veterinarian will have the necessary expertise to advise on the newer analgesics and methods of administration.

All personnel in the project should be familiar with the animal's behaviour and posture when normal and when in pain.

a) Responsibility for Surgical Standards

i) The responsibility for the animal in each surgical case lies with the person doing the surgery who, in turn, should be accountable to the institutional ACC for his/her adherence to these standards and for demonstrating an acceptable level of expertise.

ii) The responsibility for supervision of the experimental animal surgical facility should be clearly defined.

iii) Where supportive treatment is required (analgesics, tranquillizers, antibiotics, etc.), the surgical investigator must institute suitable treatment in consultation with a veterinarian.

iv) If the animal, as a result of the experimental manipulation, is in distress that cannot be relieved, authorized personnel, e.g., the laboratory animal veterinarian, should be contacted immediately and procedures instituted for euthanasia.

F. REFERENCES

ACADEMY OF SURGICAL RESEARCH. Guidelines for training in surgical research in animals. J. Investig. Surg. 1989; 2: 263-268.

BENNETT, B.T., BROWN, M.J. and SCHOFIELD, J.C. Essentials for animal research: a primer for research personnel. Beltsville, MD: National Agricultural Library 1990.

CANADIAN COUNCIL ON ANIMAL CARE. Guide to the care and use of experimental animals. Vol. 2. Ottawa, Ont.: CCAC, 1984.

FLECKNELL, P.A. Laboratory animal anesthesia. London: Academic Press, 1987.

GAY, W.I., ed. Methods of animal experimentation. Research surgery and care of the research animal--Part A: patient care, vascular access, and telemetry. New York, NY: Academic Press, 1986a; VII.

GAY, W.I., ed. Methods of animal experimentation. Research surgery and care of the research animal--Part B: surgical approaches to the organ systems. New York, NY: Academic Press, 1986b; VII.

GAY, W.I., ed. Methods of animal experimentation. Research surgery and care of the research animal--Part C: surgical approaches to the organ systems. New York, NY: Academic Press, 1989; VII.

SWINDLE, M.M. and ADAMS, R.J., eds. Experimental surgery and physiology: induced animal models of human disease. Baltimore, MD: Williams and Wilkins, 1988.

Additional Reading

BINNINGTON, A.G. and COCKSHUTT, J.R. Decision-making in small animal soft tissue surgery. Toronto, Ont.: B.C. Decker Inc. 1988.

BOJRAB, M.J., ed. Current techniques in small animal surgery. 3rd Ed. Philadelphia, PA: Lea and Febiger, 1990.

CUNLIFFE-BEAMER, T.L. Surgical techniques. In: Guttman, H.N., ed. Guidelines for the well-being of rodents in research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1990.

DOUGHERTY, R.W. Experimental surgery in farm animals. 1st Ed. Ames, IA: Iowa State University Press, 1981.

FLECKNELL, P.A. The relief of pain in laboratory animals. Lab. Anim. 1984; 18: 147-160.

GOURLEY, I.M. and VASSEUR, P.B., eds. General small animal surgery. Philadelphia, PA: J.B. Lippincott Company, 1985.

KNECHT, C.D., ALLEN, A.R., WILLIAMS, D.J. and JOHNSON, J.H. Fundamental techniques in veterinary surgery. 3rd Ed. Philadelphia, PA: W.B. Saunders Company, 1987.

LANE, D.R., ed. Jones's animal nursing. 4th Ed. New York, NY: Pergamon Press, 1985.

MORTON, D.B. and GRIFFITHS, P.H.M. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet. Rec. 1985; 116: 431-436.

OEHME, F.W., ed. Textbook of large animal surgery. 2nd Ed. Baltimore, MD: Williams and Wilkins, 1988.

PIERMATTEI, D.L. An atlas of surgical approaches to the bones of the dog and cat. 2nd Ed. Philadelphia, PA: Saunders, 1979.

PRATT, P.W., ed. Medical nursing for animal health technicians. 1st Ed. Goleta, CA: American Veterinary Publications, Inc. 1985.

PRICE, C., ed. Practical veterinary nursing. Cheltenham, Eng.: BSAVA Publications, 1985.

ROMATOWSKI, J. Prevention and control of surgical wound infection. J. Am. Vet. Med. Assoc., 1989; 194: 107-114.

SLATTER, D.H., ed. Textbook of small animal surgery. Philadelphia, PA: W.B. Saunders Company, 1985.

SWINDLE, M.M. Basic surgical exercises using swine. New York, NY: Praeger Publishers, 1983.

TRACY, D.L., ed. Small animal surgical nursing. St. Louis, MO: C.V. Mosby Company, 1983.

TUFFERY, A.A., ed. Laboratory animals: An introduction for new experimenters. Chichester, Eng.: J. Wiley & Sons Ltd., 1987.

WAYNFORTH, H.B. Experimental and surgical technique in the rat. London: Academic Press, 1980.

X. CONTROL OF ANIMAL PAIN IN RESEARCH, TEACHING AND TESTING

A. INTRODUCTION

"Many of the advances made in our knowledge of the basic mechanisms of pain and advances in pain therapy would have been impossible without experiments in animals, which have yielded enormous benefits for both humans and animals. The knowledge gained has resulted in more effective methods of pain control in both humans and animals, has brought about a decrease in suffering, and has thus improved the quality of peoples' lives" (Bonica, 1992).

The assessment and management of pain and suffering is a challenge that must be faced if animals are to be treated ethically and humanely (Fosse, 1991). A landmark publication on animal pain was Dawkins's (1980) *Animal Suffering: The Science of Animal Welfare*. Recent valuable additions include *Animal Pain: Ethical and Scientific Perspectives* (Kuchel, Rose and Burrell, 1990), *Animal Pain* (Short and Van Poznak, 1992), and a handbook, *Recognition and Alleviation of Pain and Distress in Laboratory Animals*, prepared by the Committee on Pain and Distress in Laboratory Animals of the Institute of Laboratory Animal Resources (ILAR), which discusses stressors in the laboratory and the animal behaviours they cause, the physiology of pain and distress, drug dosages and euthanasia (ILAR, 1992).

The first symposium on animal pain was presented in 1982 by the Federation of American Societies for Experimental Biology (FASEB) (Kitchell, Erickson, Carstens *et al.* 1983). It was quickly followed by other publications, symposia, and guidelines related to pain relief in animals (Zimmerman, 1983; RSPCA, 1983; Wall and Melzack, 1984; Flecknell, 1984; Gibson and Paterson, 1985; Morton and Griffiths, 1985; AVTRW, 1986; Frenk, Cannon, Lewis *et al*. 1986; AVMA, 1987; Beynen, Baumans, Bertens *et al.* 1987; Rowan, 1988; Anon., 1990; Balls, 1989, 1990; Arena and Richardson, 1990; Dawkins, 1990; Goyd, 1990; LASA, 1990; Bateson, 1991; Moberg, 1992).

Sackman (1991) has prepared a review article on control of pain in cats and dogs.

B. WHAT IS ANIMAL PAIN?

In addition to ethical concerns, poor health, pain or distress in animals interject unwanted variables into research that can greatly interfere with interpretation of the studies (Montgomery, 1990). Pain research often requires the production of the same sensations and behaviour in animals that ethical guidelines say must be eliminated (Amyx, 1990). Wall (1992) suggests that instead of agonizing over an undefinable concept of pain, we simply study the animal's efforts to stabilize its internal environment and then aid it, or at least not intrude on those efforts without good reason.

The question of distress in animals and how to define and measure it is still quite perplexing (Olfert, 1992; Lewis, 1942; Brown, 1988; Molony, 1985).

Reduction or alleviation of stress or pain is considered by Flecknell (1987) as a refinement in animal care, as part of Russell and Burch's (1959) "3Rs" of refinement, reduction and replacement (Smyth, 1978; Rowsell and McWilliam, 1986). Poor anesthetic techniques, for example, can adversely affect research and may produce unnecessary pain (Flecknell, 1987). Animal suffering includes stress, distress, discomfort, and deprivation, (Smith, 1988) as well as anxiety and fear. Freedom from discomfort and freedom from pain, injury or disease are two of the animal's Five Freedoms, as promulgated by the U.K.'s Farm Animal Welfare Council (FAWC) (Seamer, 1993).

In the absence of evidence to the contrary, it may be assumed that any stimuli or experience which produces pain and discomfort in humans, also does so in animals (LASA, 1990; RSPCA, 1983), as first promulgated by the Littlewood Committee in 1965. Amyx (1990) suggests that when Animal Care Committees (ACC) are reviewing protocols which involve aversive stimuli, members test the stimulus on themselves.

Discomfort may not be sufficient to manifest observable pain. However, it is important to be able to assess discomfort, because this provides the first steps towards avoiding it.

Attempts to define what constitutes stress have been made for some time, with little agreement (Levine, 1985). However, it was recently defined by ILAR (1992) as "the effect produced by external (i.e., physical or environmental) events or internal (i.e., physiologic or psychologic) factors, referred to as stressors, which induce an alteration in an animal's biologic equilibrium." The presence or absence of stress appears to be the only acceptable indicator of animal well-being (Duncan, 1992).

In addition to stress in the research setting, the stress of animal transportation, even for short distances, has been demonstrated in laboratory animals (Gärtner, Büttner, Döhler *et al*. 1980; Clark, Mason and Moberg, 1988; Toth and January, 1990) and farm animals (Fraser and Broom, 1990).

Sherrington (1947) originally defined a noxious stimulus as one which was actually or potentially damaging to the skin, to which Lineberry (1981) added production of escape behaviour in animals. The receptors specifically responsive to noxious stimuli are termed "nociceptors" (Kitchell, Erickson, Carstens *et al*. 1983). However, Wall (1992) states that there is evidence that the Central Nervous System (CNS) can extract information relevant to pain from afferents other than specific nociceptors.

The strongest intensity of noxious stimulation that a human-being will permit is called the "pain tolerance threshold" (Kitchell, Erickson, Carstens *et al*. 1983). Bateson (1991) notes that the subjective experiences of an animal, if it has any, may be totally different from humans, reflecting its different way of life and the different ways in which its body works. For example, most clinical veterinary neurologists are amazed by the high pain thresholds of some dogs (Kitchell, Erickson, Carstens *et al*. 1983).

In pain research, the vast majority of animals used are rodents, specifically rats (Amyx, 1990). Silverman (1991) notes, however, that "pain detection in rodents is not easy. Slight behavioural changes, vocalizations, abnormal use of body parts may signal pain, but we may not be able to evaluate its magnitude." In rodents, important indicators are recumbency and changes in the hair coat and brightness of the eyes (Montgomery, 1990).

Criteria for assessing morbid and moribund conditions in oncologic and toxicologic research include impaired activity, change in temperament, restlessness, decreased feed or water intake, abnormal vocalization, abnormal posture, self-mutilation and changes in bowel or urinary activity (Montgomery, 1990).

One of the characteristics of pain or distress in animals is a change in behaviour and reflex attributes (Amyx, 1990). Animal care personnel and research investigators must be familiar with the normal behavioural characteristics of the experimental animal, for the success or failure of the study can depend on the expertise of the technician observing the animals to minimize pain and distress (Montgomery, 1990; Bateson, 1991). Moreover, familiarity with the handler, surroundings and procedures can reduce anxiety in the animal, as can positive re-enforcement (LASA, 1990).

C. GUIDELINES

The first code of laboratory procedures regarding animals in North America was formulated by Walter B. Cannon in 1909, and was adopted and enforced in the laboratories of American medical schools, and later

served as the basis of American Physiological Society's (APS) *Guiding Principles in the Care and Use of Animals* (Cecil and Samuels, 1987).

In both Britain and The Netherlands, suffering is categorized as mild, moderate or substantial in severity (Smith, 1988).

A working party of Britain's Laboratory Animal Science Association (BLASA) has discussed the assessment and control of pain in experimental animals (LASA, 1990). Barclay (1988) has developed a disturbance index for rodents. The Laboratory Animal Science Association's (LASA) Working Party on Assessment and Control of Severity has developed a Severity Index (SI). This has been applied to such areas as administration of substances, collection of tissues and body fluids, surgical techniques and restraint. The SI is reached by means of assigning scores based on consciousness, anesthesia, preparation (preparatory manipulation), restraint (ranging from brief manual restraint to whole body restraint), duration, tissue sensitivity, organ risk and mortality. As well, the consequences in terms of pain, distress and deprivation are evaluated. Procedures are judged on a scale of up to 34 points. Examples ranged from two points for intravenous perfusion in an anesthetized animal, to 24 points for parabiosis (reversible suspension of obvious vital activities or anatomical and physiological union of two organisms) (LASA, 1990).

It is LASA's contention, based on Maslow (1970) and Curtis (1985) that interference with the basic physiological functions or needs presents a greater risk to well-being or survival than interference with behavioural requirements (LASA, 1990). The Canadian Council on Animal Care (CCAC) believes, however, that at least as regards non-human primates, "measures to safeguard psychological stability should take equal precedence to those concerning physical health" (CCAC, 1984).

This *Guide* includes the *Categories of Invasiveness in Animal Experiments* which were originally based on those of the Washington-based Scientists Center for Animal Welfare's (SCAW)*Categories of Biomedical Experiments Based on Increasing Ethical Concerns for Non-Human Species* (Orlans, Simmonds and Dodds, 1987). The *Categories of Invasiveness* document has since been amended nine times. In the management of animal pain, see also the CCAC statement on *Ethics of Animal Investigation* which is found elsewhere in this *Guide*.

D. THE ROLE OF THE VETERINARIAN IN REDUCING PAIN

Veterinary training and expertise play a vital role in fulfilling an institution's responsibilities to prevent and minimize pain and suffering in all animals used for research, teaching and testing (Gorham, 1991; Rowsell, 1992). The Canadian Association for Laboratory Animal Medicine (CALAM) in 1990 adopted a document--*Adequate Veterinary Care*, which the CCAC considers a basis for its own policy on this topic. The document covers the prevention and relief of animal pain.

The contribution of trained animal technicians has already been noted. The Canadian Association for Laboratory Animal Science (CALAS) sets standards, examines and registers laboratory animal technicians in Canada.

E. SIGNS OF PAIN AND DISTRESS*

There are numerous stereotypical responses to stress or pain stimuli in animals, particularly in mammals. Nevertheless, species differences do exist. Recognition of changes in behaviour and physical appearance in the species under study will allow early identification of an animal experiencing pain or distress. Some species specific observations are presented in this section.

Non-human Primates (NHP)

Monkeys often show remarkably little reaction to surgical procedures or to traumatic injury. Obvious signs of pain are not readily seen. Loud and persistent vocalization, for example, commonly signifies only alarm or anger. The animal in pain may be huddled in a crouching posture with a "sad" facial expression and glassy eyes, or it may sit hunched with its head forward and its arms across its body. It may avoid its companions and may stop grooming itself. A monkey in pain may also attract increased attention from its cage mates, which can vary from social grooming to attack. Acute abdominal pain may be shown by facial contortions, clenching of the teeth, restlessness, and shaking accompanied by grunts and moans. Food and water intake is usually diminished or absent.

Key Signs: hunched position, failure to groom, refusal of food or water, dejected appearance.

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* With acknowledgement to: Association of Veterinary Teachers and Research Workers. *Guidelines for the recognition and assessment of pain in animals*. Potters Bar, Herts: Universities Federation for Animal Welfare (UFAW), 1989; Laboratory Animal Science Association Working Party. The assessment and control of the severity of scientific procedures on laboratory animals. Lab. Anim. 1990; 24: 97-130; Hansen, B., Hardie, E. and Young, M. Recognition of acute pain and distress in the dog. Humane Innovations and Alternatives to Animal Experimentation 1990; 4: 170-173; Morton, D.B. and Griffiths, P. H.M. Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. Vet. Rec. 1985; 116(16): 431-436.

Dogs

Dogs in pain generally appear quieter, less alert, and withdrawn, with stiff body movements and an unwillingness to move. In severe pain, the dog may lie still or adopt an abnormal posture in order to minimize its discomfort. In less severe states, it may appear restless and the immediate response to acute, but low intensity pain may be an increased alertness. There may be inappetence, shivering, and increased respirations with panting. Spontaneous barking is unlikely; the dog is more likely to whimper or howl, especially if unattended, and may growl without apparent provocation. A dog may lick or scratch at painful areas of its body. When handled, it may be abnormally apprehensive or aggressive. The animal exhibits anxious glances; it seeks cold surfaces. Its tail is often between its legs.

Penile protrusion and frequent urination may also be noted.

Key Signs: inappetence, bites at pain regions, abnormally apprehensive.

Cats

Cats in pain are generally quiet, with an apprehensive facial expression; the forehead may appear creased. There may be crying or yowling and the cat may growl and hiss if approached or made to move. There is inappetence and a tendency to hide or to separate from other cats. The posture becomes stiff and abnormal, varying with the site of the pain. A cat with head pain may keep its head tilted. If the pain is generalized in the thorax and abdomen, the cat may be crouched or hunched. With thoracic pain alone, the head, neck, and body may be extended. In abdominal or back pain, the cat may lie in lateral recumbency with its back arched. If the animal is standing or walking, the back is arched and the gait stilted. Incessant licking is sometimes also associated with localized pain. Pain in one limb usually results in limping or holding up of the affected limb.

A cat in severe pain may show demented behaviour and make desperate attempts to escape. If a painful area is touched or palpated, there may be an instant and violent reaction. There may be panting, with an increased pulse rate and pupillary dilatation. A cat in chronic pain may have an ungroomed appearance and show a marked change from its normal behaviour. The animal exhibits tucked in limbs, hunched head

and neck, and utters a distinctive cry or hissing and spitting sound. Its ears are flattened. It shows fear of being handled and may cringe.

Key Signs: stiff posture, demented behaviour, lack of grooming, hunched head and neck, inappetence.

Mice

After procedures which cause pain, mice may increase their sleeping times. Reduced food and water intake, with resultant weight loss, dehydration and wasting of the muscles on the back may be observed. Piloerection (erection of hair) and a hunched appearance indicate pain or distress. The animal fails to groom, but scratches more frequently. Sick mice are often isolated from the remainder of the group. Aggressive vocalization is observed in the early stages, decreasing where pain or stress reduces the ability to move and respond.

The eyes appear sunken, and ocular and nasal discharge may be noted as the animal's condition worsens. The respiration rate increases and breathing may be forced or laboured. Defecation/urination are immediate reactions to stress in the mouse, and increase or decrease as stress continues. The movement of vibrissae (muscle hairs) becomes less evident as pain or stress continues. Affected mice become more timid and apprehensive; however, as pain or stress increases, they may become aggressive, with a tendency to bite. The animal may attempt to bite the source of pain or affected area, and may self-mutilate the affected part.

Writhing movements are noted when the pain is abdominal. There is gradual assumption of a hunched, 'sleeping posture' away from any light source. Where limbs or feet are affected, sudden running movements are exhibited as an escape mechanism; there is increasing difficulty in maintaining posture. The mouse may show unsteady gait, difficulty in moving in straight line, and circling movements where balance is affected. A rolling gait is often noted with developing ascites.

As its condition worsens, the animal becomes quiet and unresponsive, separates from the group and eventually becomes unaware of its surroundings. Hypothermia is observed with increasing deterioration in condition; the animal feels 'cold' to the touch.

Key Signs: withdrawal, biting response, piloerection, hunched back, sunken eyes and abdomen, dehydration, weight loss.

Rats

Rats are generally docile and less aggressive than mice towards members of their own species and humans. Acute pain or distress is usually accompanied by constant vocalization and struggling. Rats will often lick or guard a painful area. Increased scratching can indicate chronic pain. A rat in pain will often sit crouched with its head turned into its abdomen. Sleeping periods will be disturbed and increase if pain or distress are present. An elevated respiratory rate associated with sneezing occurs where the respiratory system is affected. Increasing piloerection (staring coat) is noted, along with an increasingly untidy appearance as the animal fails to groom itself. There may be some hair loss. The animal ceases to eat and drink normally. There is poor skin tone, and evidence of muscle wasting along the back--indicative of dehydration and weight loss.

During repeated painful or distressing procedures, animals may become more aggressive and resist handling, which will increase with increasing pain or distress. The eyelids rapidly assume a half-closed or almost-closed position. The eyes may appear sunken, and ocular discharge is common, often progressing to red-coloured hematoporphyrin exudate which may encircle the eye. Nasal discharge, if present, may

be red-coloured as well (Harkness and Ridgway, 1980).

Constipation or diarrhea may occur depending on the organ system(s) affected. Urination decreases with reduced water intake; however, frequency may increase where urinary infection or hormonal disturbance is present. Animals in pain initially show increased awareness/aggressive responses and a tendency to bite, but eventually become depressed and unresponsive. Exploratory behaviour lessens. Aversive behaviour is shown towards other animals. There is possible self-mutilation of affected parts in later stages. Abdominal contraction and stilted movements may occur if abdominal pain is present. There may be increasing pain associated with locomotion. Lameness in one of the limbs or simply careful gait may be noted. A "waddling" gait occurs where abdominal enlargement take place as a result of intestinal obstruction or ascites. Circling often occurs where balance is disturbed.

Initially, the rat exhibits increased angry or aggressive vocalization, especially on handling. There is a gradual reduction in vocal response as the pain or stress continues, and movement ceases unless a sudden painful stimulus is experienced. Hypothermia indicates significant deterioration in the animal's condition. A pale appearance indicates anemia or blood loss.

Key Signs: vocalization, struggling, licking/guarding, weight loss, piloerection, hunched position, hypothermia.

Guinea Pigs

Guinea pigs are alert, but timid and apprehensive animals which will try to avoid capture and restraint. Rarely is there any aggression towards humans. Any sign of acceptance indicates the animal is unwell. Loud vocalization will accompany even minor and transient pain. Guinea pigs often appear sleepy when in pain. Initially, there is an increased level of response to painful or stressful stimuli. However, this gradually subsides and the animal becomes unresponsive. It gradually appears more apprehensive. The eyes may be sunken and dull. The respiratory rate increases as a painful or stressful stimulus increases or continues; where the respiratory system is affected, respirations become increasingly forced and laboured. Often loss of weight occurs as well as hair loss, scaly skin, and dehydration. Where the gastrointestinal tract is affected there may be evidence of diarrhea. There is a tendency to 'barbering' under dietary stress with failure to eat or drink. Group aggression may occur and damage to the skin of the back may result from fighting. There is excessive salivation where abnormal teeth cause eating difficulties, a tendency to an arched back where abdominal pain is present, and failure of the "righting" reflex in seriously ill animals. There may be pain associated with locomotion, lameness, and careful gait due to sore feet in older animals.

Key Signs: withdrawal, vocalization, failure to resist restraint, staring coat, unresponsive.

Mongolian Gerbils

Gerbils are highly active, nervous animals and usually attempt to avoid restraint. Signs of pain and distress are difficult to assess, as gerbils apparently object to any interference. There is an increased level of response under painful or stressful stimuli. Ocular discharge is common. Under stressful conditions, the eyelids may be half closed, with dry matting of the eyelids. The increased respiratory rate associated with lung involvement is difficult to assess by eye. Loss of coat condition occurs. Loss of hair from the tail may be seen in overcrowded animals. Facial lesions and sores may result from excessive burrowing in the corners of the cage.

Dehydration is rarely seen, since the gerbil's normal metabolism enables full utilization of the water content of the diet. Only small quantities of urine are voided under normal conditions. Feces are normally firm, dry pellets. Constipation is rare. Diarrhea, if it occurs, may quickly lead to death from fluid loss.

Gerbils are normally extremely active and nervous. Under severe stress, there may be temporary collapse and apparent shock syndrome; however, the animals recover, given time. Changes in exploratory behaviour and increased aggressive response may occur. A hunching up and arching of the back may be observed, especially with abdominal involvement. Abnormal gait is associated with locomotion or abdominal involvement.

Key Signs: hunched appearance, weight loss, shock syndrome.

Syrian (golden) Hamsters

Under normal conditions, hamsters will sleep for long periods during the day, and little activity will be seen. They often appear aggressive towards their cage mates and emit loud screeching noises, disproportionate to the degree of interference, when handled. This response increases under painful or stressful stimuli. Ocular discharge is commonly associated with stress. An increased respiratory rate is associated with lung involvement. Loss of coat condition is seen where the diet is deficient in Vitamin E and short chain fatty acids. Loss of body condition occurs with decreased food and water intake. Constipation is unusual in the hamster. Diarrhea, when it occurs, is profuse and liquid, staining the perineal region. Increasing depression takes place when the animal is left undisturbed. Daytime sleep periods may be extended and increasing lassitude may be seen except when the animal is being handled. Exploratory behaviour is reduced. A hunched appearance is noted, as is an unwillingness to move, especially where abdominal organs are involved. Lateral recumbency can indicate that the animal is moribund. Normal gait is affected when pain is associated with locomotion. Stilted movements are sometimes associated with abdominal involvement, e.g., ascites following cirrhosis of the liver.

Key Signs: weight loss, hunched appearance, increased aggression or depression, extended sleep periods.

Rabbits

The rabbit presents significant difficulties in recognition of pain and distress, as it often quietly accepts apparently painful or distressing procedures; this may relate to its feral behaviour where concealment is important to survival. Even healthy rabbits may not move frequently or indulge in exploratory behaviour. Pain is usually characterized by a reduction in food and water intake (and thus weight loss and dehydration) and limited movement. Although rabbits frequently become ill and distressed without showing much apparent loss of condition, careful examination will reveal a loss of muscle mass on the lower back. Ocular discharge is a common response to stress in the rabbit, with protrusion of the nictitating membrane.

Under continued pain or stress, rabbits assume a 'sleepy' appearance. The animal exhibits increased depression, progressive unawareness and lack of response. The animal will often face the back of cage, away from light. An increased respiratory rate is associated with either apprehension or lung involvement. There is fecal staining of the coat. Night time pellet production may be interrupted. Constipation and diarrhea are common responses to pain or stress. Excessive self-grooming may precipitate hair balls in the stomach. Where foot soreness is involved, weight may be thrown forward or backward to reduce discomfort. Body stretching and lying flat are common indications of abdominal discomfort. Pain may be associated with locomotion, especially with sore feet.

Key Signs: reduced eating and drinking, faces towards back of cage, limited movement, apparent photosensitivity.

Horses

Periods of restlessness are noted in horses experiencing pain or distress. Food is held in the mouth uneaten. The horse exhibits an anxious appearance with dilated pupils and glassy eyes; increased respiration and pulse rate with flared nostrils; profuse sweating and a rigid stance. In prolonged pain, behaviour may change from restlessness to depression with the head lowered. In pain associated with skeletal damage, limbs may be held in unusual positions and there is a reluctance to move, with the head and neck "fixed." There may be a pain-induced tachycardia.

In abdominal pain, a horse may look at, bite or kick its abdomen; it may get up and lie down frequently; walk in circles; or roll. When near collapse, the horse may stand very quietly, rigid and unmoving, but with signs of deteriorating circulatory status such as mucosal cyanosis and prolonged capillary filling time. Horses in pain generally show a reluctance to be handled.

Key Signs: anxious appearance, restlessness, biting at site of pain, depression, fixed position.

Cattle

Cattle in pain often appear dull and depressed, with the head held low and showing little interest in their surroundings. There is inappetence, weight loss and, in milking cows, a sudden drop in milk yield. Severe pain often results in rapid shallow respirations. On being handled, they may react violently or adopt a rigid posture designed to immobilize the painful region. Grunting and grinding of the teeth may be heard. Acute pain may be associated with bellowing. Generally, signs of abdominal pain are similar to those seen in the horse, but are less marked. Rigid posture may lead to a lack of grooming due to an unwillingness to turn the neck. In acute abdominal conditions, such as intestinal strangulation, the animal adopts a characteristic stance with one hind foot placed directly in front of the other. Localized pain may be indicated by persistent licking of an area of skin, or kicking at the offending area.

Key Signs: dull, depressed, inappetence, grunting, grinding of the teeth, rigid posture.

Sheep and Goats

In general, signs of pain in these species are similar to those in cattle. Changes in posture and movement are apparent, and a change in facial expression may be indicative of pain or distress. There is a general reluctance to move. Goats are more likely than cattle to vocalize in response to pain. Grinding of the teeth and grunting are also heard. Sheep, in particular, tolerate severe injury without overt signs of pain or distress. Following procedures such as castration and tail docking, lambs may show signs of discomfort such as standing up and lying down repeatedly, tail wagging, occasional bleating, neck extension, dorsal lip curling, kicking, rolling and hyperventilation.

Key Signs: rigid posture and reluctance to move.

Pigs

Pigs in pain may show changes in gait and posture. They normally squeal and attempt to escape when handled; however, these reactions may be accentuated when the animal is in pain. Adult pigs may become aggressive. Squealing is also characteristic when painful areas are palpated. Handling of chronic lesions may not elicit signs of pain. Pigs will often be unwilling to move and may hide in bedding if possible.

Key Signs: vocalization and the lack of normal social behaviour may be helpful indicators of a

pig in pain.

Birds

Birds in pain may show escape reactions with vocalization and excessive movement. Head movements increase in extent and frequency. There may be an increase in heart and respiratory rates. Prolonged pain will result in inappetence and inactivity with a drooping, miserable appearance. The eyes may be partially closed, the wings held flat against the body, and the neck retracted. When handled, the escape reaction may be replaced by a state of tonic immobility. Birds with limb pain will avoid use of the affected limb and will "guard" it from extension.

Key Signs: escape reactions, atonic immobility, inappetence, avoidance of use of pain site.

Reptiles

Acute pain in reptiles may be characterized by flinching and muscle contractions. There may be aversive movements away from the unpleasant stimulus, and attempts to bite. More chronic and persistent pain may be associated with anorexia, lethargy and weight loss, although it is difficult to associate any of these signs of lack of well-being specifically with pain.

Key Signs: flinching and muscle contractions, weight loss, anorexia.

Fishes

It is difficult to determine the nature of the response to pain in fish. Although they exhibit a pronounced response to injuries or to contact with irritants, their response to chronic stimuli may be small or absent. Fish with severe wounds which would cause immobility in a mammal, will often appear to behave completely normally, even resuming feeding. Fish will react to noxious stimuli, such as that administered by a hypodermic needle, by strong muscular movements. When exposed to a noxious environment, such as a strong acid, they show abnormal swimming behaviour with attempts to jump from the water, their colouring becomes darker and their opercular movements become more rapid. Such effects are indicative of some degree of distress; however, it is not possible to describe these unequivocally as signs of pain.

F. ANALGESIC AGENTS

The appropriate use of analgesics during or after a painful procedure is an integral part of a protocol plan. The following general information, as well as details of administration and dosages by species, are given in several references (Sawyer, 1985; Sackman, 1991; Flecknell, 1984) in addition to the anesthetic textbooks listed in the additional reading.

The opioids (morphine-like drugs) are the most widely used analgesic agents. Opioids act by binding to specific receptors. The main classes of receptors are m.

1. Opioid Agonists

Opioids produce potent hypnotic and analgesic effects including significant depression of the cardiovascular and respiratory systems and an alteration in the thermoregulatory mechanism. The euphoria and addiction associated with opioids in human is not a problem in animals when the drugs are

used properly. Some opioids induce vomiting in dogs and NHP and rapid intravenous injection may occasionally result in an excitatory phase in most species. In farm animals, as well as in the cat and mouse, the effects of opioids are less predictable, and undesired excitement may occur. Avoidance of the excitement phase in species with an enhanced sensitivity to opioids can often be achieved by the use of very low dosages (Green, 1982).

Opioids used in the veterinary medicine include morphine, meperidine, fentanyl, oxymorphone, etorphine (M99) and carfentanil. They are pure or relatively pure m agonists and are all good analgesics.

a) Morphine is most frequently used clinically for the control of post-operative pain in dogs and NHP, providing up to four hours of pain relief. In dogs, its use is complicated by undesirable gastrointestinal effects. Intravenous bolus administration in dogs may cause histamine release which may contribute to morphine's hypotensive action (Hall and Clarke, 1991). As a pre-medicant, morphine's stimulatory effect on the vagus nerve may induce bradycardia, unless atropine is given in advance. Profound respiratory depression occurs rapidly and is dose-related. Intracranial and intra-ocular pressure are increased (Sackman, 1991).

b) Meperidine has effects similar to morphine and is the drug of choice for premedication in the dog, as very little gastrointestinal stimulation is induced. However, severe hypotension may occur after intravenous use. As analgesia lasts only one to two hours, it is not recommended for alleviating post-surgical pain. This drug has also proven useful as a post-operative sedative for NHP and horses.

c) Fentanyl is a very potent short-acting opioid. It is combined with droperidol to make a neuroleptanalgesic that provides profound analgesia. New synthetic compounds of fentanyl include alfentanil, which has an ultrashort half-life, and sufentanyl with a half-life shorter than fentanyl, but with fewer peripheral side effects (Flecknell, 1984).

d) Oxymorphone is more potent than morphine and produces more sedation in the dog than morphine or meperidine. Post-surgical pain relief lasts two to six hours. Cardiovascular stability is much greater than with the other opioids. It is frequently combined with diazepam or acepromazine for anesthesia and analgesia in old or sick animals. An anticholinergic should be given to prevent severe bradycardia.

e) Etorphine (M99) is an extremely potent morphine derivative with a high tendency to produce initial excitement followed by depression. Because of its potency, it has been widely used in dart guns for the capture and immobilization of zoo animals and wild game (Fowler, 1986; Green, 1982). The drug has also been used successfully in certain cold blooded animals (Fowler, 1986; Green, 1982). It is extremely dangerous to humans; therefore, diprenorphine (M5050) must be available for immediate reversal should accidental human exposure occur (Fowler, 1986).

f) Carfentanil is currently preferred to etorphine by many zoo veterinarians because of its higher potency, which allows administration by swabbing or spraying of the buccal or nasal mucosa. It can be reversed by cyprenorphine (M285) or diprenorphine (M5050) (Lumb and Jones, 1984) and naltrexone. Carfentanil can be fatal in humans if accidentally injected (partial immobilizing dose).

2. Opioid Agonist/Antagonists

The search for analgesic agents with fewer side effects than pure mμ agonists led to the development of partial mμ agonists and kappa agonists such as butorphanol and buprenorphine. This group of drugs may also be used to reverse the depressant effects of an opioid while preserving the analgesic qualities.

a) Butorphanol is a synthetic analgesic with five times the potency of morphine. A degree of sedation occurs and the respiratory depression has a ceiling effect that does not increase with higher doses. Cardiovascular effects are minimal and it is a poor opioid antagonist (Dyson, 1990). Analgesia lasts two to five hours following

subcutaneous injection, and may be accompanied by some dysphoria.

b) Buprenorphine is a long-acting analgesic that antagonizes the depressant effects of the opioid agonists, while still maintaining long-term post-operative analgesia of 8-12 hours in many species (Flecknell, 1984).

c) Pentazocine lactate is a poor analgesic with a very short duration (approximate half-life in the dog of 22 minutes). It has minimal cardiovascular effects and is a mild respiratory depressant.

d) Nalbuphine is slightly less potent than morphine, with a wide safety margin and minimal cardiovascular and respiratory depression. Analgesia lasts three to eight hours. It has also been used as an opioid antagonist to reverse sedation and respiratory depression of opioids while maintaining analgesia (O'Hair, Dodd, Phillips *et al*. 1988).

3. Opioid Antagonists

Naloxone hydrochloride, an effective antagonist, is available to reverse the effects of opioids (this includes the analgesia). It has no agonist properties and does not produce respiratory or cardiovascular depression. It has an antagonistic effect for one to four hours, and can be used to reverse the effects of any of the opioid agonist/antagonist group. Nalorphine or diprenorphine must be available when using etorphine, in case of accidental human administration (Lumb and Jones, 1984). Naltrexone is a long-acting derivative of naloxone. At present, its use in veterinary medicine is limited; however, should a pure long-acting antagonist be required, it could prove useful (Hall and Clarke, 1991).

4. Non-steroidal Anti-inflammatory Drugs (NSAIDS)

These agents produce analgesia by reducing inflammation and thus peripheral sensitization. They have little, if any, central analgesic action. Side effects are interference with platelet and renal function and gastric ulceration. Cats metabolize these agents slowly and must be dosed infrequently to prevent toxicity. Those commonly used in dogs are the carboxylic acid group (aspirin, naproxen, meclofenamic acid, flunixin) and the enolic acids (phenylbutazone, dipyrone, and piroxicam). In cats aspirin, phenylbutazone and dipyrone are frequently used at low dosages (Sawyer, 1985).

a) Aspirin relieves pain associated with peripheral inflammation, but is ineffective for visceral pain. In cats, it should be given only every 48 to 72 hours.

b) Naproxen is used when aspirin does not relieve the pain, and the once daily administration is convenient. As is the case with aspirin, gastric ulcers are listed as a side effect.

c) Meclofenamic acid is popular for treating musculoskeletal pain that is refractory to aspirin. It has 1.5 times the potency of phenylbutazone.

d) Flunixin is reported to have greater analgesic properties than phenylbutazone, meperidine or codeine, and is used for osteoarthritic pain. Its use in large animals is well established. Its use in small animals is expanding. In dogs, it has the potential to produce serious gastrointestinal effects (bleeding) if a maximum of three doses is
exceeded (Hall and Clarke, 1991).

e) Phenylbutazone relieves musculoskeletal pain, but has been associated with blood dyscrasias, gastrointestinal disturbances, nephropathies and hepatitis.

f) Dipyrone is an analgesic, antipyretic, anti-inflammatory that also may cause blood dyscrasias with

prolonged use.

g) Piroxicam is popular in human medicine for its once daily dosage and very effective relief of osteoarthritic pain. Toxicity is similar to that of other non-steroidal anti-inflammatory drugs (Sackman, 1991).

5. Analgesia provided by Local Anesthetics

As an alternative to systemically administered agents, analgesia can be provided by use of local anesthetics. Bupivacaine, a long-acting local anesthetic, is preferred for post-operative analgesia (Flecknell, 1992). It can either be injected around specific nerve trunks which supply the surgical site, or infiltrated into the muscular and subcutaneous tissue layers during closure of a surgical incision. Use of local anesthesia to infiltrate the surgical wound is a simple technique that can provide 4-12 hours analgesia.

Selective blocking of the intercostal nerves two to three intercostal spaces on either side of a thoracotomy incision has recently been recommended for relief of pain following thoracotomy in dogs. Bupivicaine hydrochloride is used before incision closure, and provides four to five hours of analgesia. The respiratory pattern of dogs recovering from thoracotomies is not significantly changed. This provides a distinct

advantage over opioid analgesics which may cause significant respiratory depression. This technique, while providing relief of pain associated with the surgical incision, does not elevate visceral (intrathoracic) pain (Sackman, 1991).

6. Neuroleptanalgesics

Neuroleptanalgesia is a state of sedation and analgesia produced by the combined use of a tranquillizer (neuroleptic) and an opioid. Minor surgery can be performed; however, the patient remains rousable and responds to certain stimuli. Moderate respiratory depression occurs, and muscle relaxation tends to be poor, but can be counteracted by combination of the neuroleptanalgesic with a benzodiazepine (Flecknell, 1987). The most commonly used preparation is Innovar-Vet, (droperidol 20 mg/ml and fentanyl 0.4 mg/ml), which should not be confused with Innovar, prepared for human use (droperidol 2.5 mg/ml and fentanyl 0.0005 mg/ml).

Innovar-Vet has been used extensively in the dog and is reported useful in many other species. It has a wide margin of safety, is well tolerated by animals in poor physical condition, and is partially reversible with naloxone. Significant bradycardia may be avoided by prior use of atropine. Its use is contra-indicated in the cat, cow, horse and sheep due to CNS stimulation. Animals remain responsive to auditory stimuli, and aggressiveness during recovery and other disposition changes lasting several days have been reported in the dog (Lumb and Jones, 1984).

A variety of other opioids and tranquillizers can be combined to produce neuroleptanalgesia; among these, mixtures of morphine/promazine and etorphine/acepromazine have proven useful in a variety of animals (Flecknell, 1987). Meperidine/acepromazine (Lumb and Jones, 1984) and oxymorphone/acepromazine (Short, 1987) have also been used for the dog and cat.

G. AREAS FOR FUTURE STUDY

Farm animal welfare, transgenic animals, amphibians, reptiles and invertebrates all constitute areas of increasing concern.

The welfare of domestic animals is of great importance, and it is claimed by Spira (1986) that, because of their numbers, 95% of all animal suffering takes place in intensive management ("factory farming") practices; thus, every 1% reduction in their suffering will accomplish more than all other protection campaigns for other species of animals put together. Animal behaviour and applied animal ethology are increasingly becoming areas of great importance (Maxie, 1987; Fraser, 1988; Fraser and Broom, 1990; McKeown and Luescher, 1988; Duncan, 1992).

Another area that will become of increasing concern is the production of transgenic animals (Jaenisch, 1988; Baker, 1988; Ewing, 1990; Cross, 1990; McLaren, 1990; Page, 1990) and the possible pain and distress that may be caused them. Their uses in research have recently been discussed (Saffer, 1992; Merlino, 1991) as well as management of their colonies (Geistfeld, 1991). The CCAC, foreseeing the need for guidelines on animal biotechnology, has recently established a committee comprising knowledgeable scientists, and representatives of industry and animal welfare, to develop such guidelines to include embryo manipulation, fetal research and transgenic animals. Agriculture Canada is also considering a proposed framework for regulating the production and use of transgenic animals (Sethi, 1992).

Only recently has the analgesia, anesthesia and euthanasia of amphibians, reptiles and fish been addressed (UFAW, 1989; Johnson, 1992; Iwama, 1992; Davis, 1992). Evaluation of pain and stress has been discussed in reptiles (Lance, 1992), cold-blooded vertebrates (Arena and Richardson, 1990; Fiorito, 1986), and birds (Gentle, 1992). It has been contended that fish can experience pain and fear to a degree which can be compared with human reactions (Anon., 1988).

In addition to studies involving vertebrates, the use of invertebrates in research is governed in Canada by CCAC's *Categories of Invasiveness* found elsewhere in this *Guide*. It states that cephalopods and some other higher invertebrates have nervous systems as well developed as some vertebrates and therefore may warrant inclusion in the Categories under B, C, D and E. In Great Britain, only one species of cephalopod, the common octopus (*Octopus vulgaris*), is only now being brought under the Animals (Scientific Procedures) Act (Anon., 1993). Handling, anesthesia and surgery of cephalopods are outlined in a recent Universities Federation for Animal Welfare publication (Boyle, 1991).

Other areas of concern to the scientific community and ACCs include, for example, the effects of blood loss (McGuill, 1989) and the use of Freund's Complete Adjuvant (FCA) (Broderson, 1989). An expert committee of CCAC has been established especially to examine this latter issue and investigate replacements for FCA. The *CCAC Guidelines on Acceptable Immunological Procedures* appear elsewhere in this *Guide*, and are revised as new knowledge becomes available.

In the development of research in the future, the scientist should consider the importance of refinement and should address those studies which are known to cause the most pain and suffering (Rowsell, 1992).

We have a considerable distance to go before animals need no longer be used. However, as Medawar (1972) notes: "We must grapple with the paradox that nothing but research on an animal will provide us with the knowledge that will make it possible for us, one day, to dispense with the use of them altogether." And to paraphrase Wall (1984): "So long as one animal remains in pain and we cannot help, our knowledge of pain remains inadequate."

H. REFERENCES

AMERICAN VETERINARY MEDICAL ASSOCIATION. Proc. Colloquium on "Recognition and Alleviation of Animal Pain and Distress." J. Am. Vet. Med. Assoc. 1987; 191(10): 1184-1298.

AMYX, H.L. Guidelines for studies in pain of rodents. SCAW (Scientists Center for Animal Welfare) Newsl. 1990; 12(2): 6-8.

ANON. Pain and fear in fish: fish do experience pain. Anim. Inter. 1988; 27: 9.

ANON. Proc. animal pain conference. ACCART (Australian Council for the Care of Animals in Research and Teaching) Newsl. 1990; 3(1): 11.

ANON. 1986 Act extended to the octopus. Royal Defence Soc. News 1993: 3.

ARENA, P.C. and RICHARDSON, K.C. The relief of pain in cold-blooded vertebrates. ACCART (Australian Council for the Care of Animals in Research and Teaching) Newsl. 1990; 3(1): 1.

ASSOCIATION OF VETERINARY TEACHERS AND RESEARCH WORKERS (Working Party). Guidelines for the recognition and assessment of pain in animals. Vet. Rec. 1986; 118(12): 334-338.

BAKER, H.J. (correspondence). Lab. Anim. Sci. 1988; 38(6): 661.

BALLS, M. (editorial) Animal experimentation: the weighing of benefit and suffering. ATLA (Alternatives To Live Animals) 1989; 16: 212.

BALLS, M. (editorial) Has the Animals (Scientific Procedures) Act 1986 failed? ATLA (Alternatives To Live Animals) 1990; 17: 284.

BARCLAY, R.J., HERBERT, W.J. and POOLE, T.B., eds. Disturbance index method for assessing severity of procedures on rodents. Herts, U.K.: Universities Federation for Animal Welfare, 1988.

BATESON, P. Assessment of pain in animals. Anim. Behav. 1991; 42: 827-839.

BEYNAN, A.C., BAUMANS, V., BERTENS, A.P.M.G., HAVENAAR, R., HESP, A.P.M. and VANZUTPHEN, L.F.M. Assessment of discomfort in gallstone-bearing mice: a practical example of problems encountered in an attempt to recognize discomfort in laboratory animals. Lab. Anim. 1987; 21: 35-42.

BONICA, J.J. Pain research and therapy: History, current status and future goals. In: Short, C.E. and Van Poznak, A., eds. Animal pain. New York, Edinburgh, London, Melbourne, Tokyo: Churchill Livingstone, 1992: 1-29.

BOYLE, P.R., ed. The UFAW handbook on the care and management of cephalopods in the laboratory. Potters Bar, U.K.: Universities Federation for Animal Welfare, 1991.

BRODERSON, J.R. A retrospective review of lesions associated with the use of Freund's Adjuvant. Lab. Anim. Sci. 1989; 39(5): 400-405.

BROWN, L. Cruelty to animals--the moral debt. Basingstoke: MacMillan Publications Ltd., 1988.

CANADIAN COUNCIL ON ANIMAL CARE. Guide to the care and use of experimental animals, Vol. 2. Ottawa, Ont.: CCAC, 1984.

CECIL, H.C. and SAMUELS, W.M. Use and care of laboratory animals. In: History of the American Physiological Society 1887-1987. Bethesda, MD: American Physiological Society, 1987: 391.

CLARK, A.S., MASON, W.A. and MOBERG, G.P. Interspecific contrasts in responses of macaques to transport cage training. Lab. Anim. Sci. 1988; 38(3): 305-309.

CROSS, B. Protecting all interests? (Book Review) Nature 1990; 343: 223-224.

CURTIS, S.E. What constitutes animal well-being. In: Moberg, G.P., ed. Animal stress. Bethesda, MD: Amer. Physiol. Soc., 1985: 1-14.

DAVIS, K.B. Stress management in aquaculture. In: Scheaffer, D.O., Kleinow, K.M. and Krulisch, L. The care and use of amphibians, reptiles and fish in research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992: 116-121.

DAWKINS, M.S. Animal suffering. The science of animal welfare. London: Chapman and Hall 1980.

DAWKINS, M.S. From an animal's point of view: motivation, fitness and animal welfare. Behav. Brain Sci. 1990; 13: 1.

DUNCAN, I.J.H. Behavioral assessment of welfare. In: Mench, J.A., Mayer, S.J. and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Proc. SCAW (Scientists Center for Animal Welfare)--sponsored conference, "Agricultural Animals in Research." Bethesda, MD: Scientists Center for Animal Welfare, 1992: 62-68.

DYSON, D.H. Update on butorphanol tartrate: use in small animals. Can. Vet. J. 1990; 31: 120-121.

EWING, T. Superpigs go to market. Nature 1990; May 31; 345: 377.

FIORITO, G. (letters) Is there "pain" in invertebrates? Behav. Proc. 1986; 12: 383.

FLECKNELL, P.A. The relief of pain in laboratory animals. Lab. Anim. 1984; 18: 147-160.

FLECKNELL, P.A. Laboratory animal anesthesia. An introduction for research workers and technicians. New York, London, Toronto: Academic Press, 1987.

FLECKNELL, P.A. Anesthesia of laboratory animals. Short course, University of Guelph, Ont., 1992.

FOSSE, R.T. Pain, pain recognition and treatment in laboratory animals. Lab. Zhyvotnye 1991; 1(3): 81-83.

FOWLER, M.E. Zoo & wild animal medicine. Toronto, Ont.: W.B. Saunders Co., 1986.

FRASER, A.F. Animal suffering: the appraisal and control of depression and distress in livestock. Appl. Anim. Behav. Sci. 1988; 20: 127-133.

FRASER, A.F. and BROOM, D.M. Farm animal behaviour and welfare, 3rd Ed. London, Philadelphia, Toronto: Ballière Tindall, 1990.

FRENK, H., CANNON, J.T., LEWIS, J.W. and LIEBESKIND, J.C. Neural and neurochemical mechanisms of pain exhibition: In: Sternbach, R.A., ed. Psychology of pain. 2nd Ed. New York, NY: Raven Press, 1986.

GÄRTNER, K., BÜTTNER, D., DÖHLER, K. and FRIEDEL, R. Stress response of rats to handling and experimental problems. Lab. Anim. 1980; 14(3): 267-278.

GEISTFELD, J.G. Transgenic mouse colony management. Lab Animal 1991; 20(1): 21-29.

GENTLE, M.J. Pain in birds. Animal Welfare 1992; 1: 235-247.

GIBSON, T.E. and PATERSON, D.A., eds. Proc. Brit. Vet. Assoc. Animal Welfare Foundation symp. "The Detection and Relief of Pain in Animals." BVA Animal Welfare Foundation, April 1985.

GORHAM, M.E. Veterinarians need greater involvement in animal welfare issues, says Tufts' Loew. DVM January 1991: 15.

GOYD, J.S. Pain control symposium attracts international audience. J. Am. Vet. Med. Assoc. 1990; 197 (5): 562.

GREEN, C.J. Laboratory animal handbook 8. Animal anesthesia. London: Laboratory Animals Ltd., 1982.

HALL, L.W. and CLARKE, K.W. Veterinary anesthesia. 9th Ed. Toronto, Ont.: Baillière Tindall, 1991.

HARKNESS, J.E. and RIDGWAY, M.D. Chromodacryorrhea in laboratory rats (*Rattus norvegicus*): etiologic considerations. Lab. Anim. Sci. 1980; 30: 841-844.

INSTITUTE FOR LABORATORY ANIMAL RESOURCES, COMMISSION ON LIFE SCIENCES, NATIONAL RESEARCH COUNCIL. Recognition and alleviation of pain and distress in laboratory animals. Washington, DC: National Academy Press, 1992.

IWAMA, G.K. Anesthesia, analgesia and euthanasia in fish. In: Scheaffer, D.O., Kleinow, K.M. and Krulisch, L. The care and use of amphibians, reptiles and fish in research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992: 167-174.

JAENISCH, R. Transgenic animals. Science 1988; 240: 1468-1473.

JOHNSON, J.H. Anesthesia, analgesia and euthanasia in reptiles and amphibians. In: Scheaffer, D.O., Kleinow, K.M. and Krulisch, L. The care and use of amphibians, reptiles and fish in research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992: 49-52.

KITCHELL, R.L., ERICKSON, H.H., CARSTENS, E. and DAVID, L.E., eds. Animal pain: perception and alleviation. Proc. symp. Am. Fed. Soc. Exp. Biol., New Orleans, April 20-21, 1982. Bethesda, MD: American Physiological Society, 1983.

KUCHEL, T.R., ROSE, M. and BURRELL, J., eds. Animal pain: ethical and scientific perspectives. Proc. conf. held at Barossa Valley, Adelaide, Australia, April, 1990. ACCART, 1992. (Australian Council on the Care of Animals in Research and Teaching, P.O. Box 19, Glen Osmond, SA, 5064, Australia.)

LABORATORY ANIMAL SCIENCE ASSOCIATION (Working Party). The assessment and control of the severity of scientific procedures on laboratory animals. Lab. Anim. 1990; 24: 97-130.

LANCE, V.A. Evaluating pain and stress in reptiles. In: Scheaffer, D.O., Kleinow, K.M. and Krulisch, L. The care and use of amphibians, reptiles and fish in research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992: 101-106.

LEVINE, S. A definition of stress? In: Moberg, G.P., ed. Animal stress. Bethesda, MD: American Physiological Society, 1985: 51-69.

LEWIS, T. Pain. London: MacMillan Press, 1942.

LINEBERRY, C.G. Laboratory animals in pain research. In: W.I. Gay, ed. Methods of animal experimentation. Vol. 6. New York, NY: Academic Press, 1981: 237-311.

LUMB, W.V. and JONES, E.W. Veterinary anesthesia. Philadelphia, PA: Lea and Febiger, 1984.

MASLOW, A.H. Motivation and personality. New York, NY: Harper and Row, 1970.

MAXIE, G. (editorial) The continuing evolution of veterinary education. Can. Vet. J. 1987; 28(10): 627-628.

MCGUILL, M.W. and ROWAN, A.N. Biological effects of blood loss: implications for sampling volumes and techniques. ILAR (Institute for Laboratory Animal Resources) News 1989; 31(4): 5-18.

MCKEOWN, D. and LUESCHER, A. A case for companion animals behaviour in veterinary practice. Can. Vet. J. 1988; 29(1): 74-75.

MCLAREN, A. Of MIS and the mouse. Nature 1990, May 10; 345: 111.

MEDAWAR, P. The hope of progress. London: Methuen, 1972.

MERLINO, G.T. Transgenic animals in biomedical research. FASEB (Federation of American Societies for Experimental Biology) J. 1991; 5: 2996-3001.

MOBERG, G.P. Stress: Diagnosis, cost and management. In: Mench, J.A., Mayer, S.J. and Krulisch, L., eds. The well-being of agricultural animals in biomedical and agricultural research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992: 58-61.

MOLONY, V. Procedures painful in humans are painful in animals. True or false? In: Gibson, T.E. and Paterson, D.A., eds. Proc. British Veterinary Association Animal Welfare Foundation symposium on "The Detection and Relief of Pain in Animals" April 16, 1985: 7-14.

MONTGOMERY, C.A. Oncologic and toxicologic research: alleviation and control of pain and distress in laboratory animals. Cancer Bull. 1990; 42(4): 230-237.

MORTON, D.B. and GRIFFITHS, P.H.M. Guidelines on the recognition of pain, distress, and discomfort in experimental animals and an hypothesis for assessment. Vet. Rec. 1985; 116(16): 431-436.

O'HAIR, K.C., DODD, K.T., PHILLIPS, Y.Y. and BEATTIE, R.J. Cardiopulmonary effects of nalbuphine hydrochloride and butorphanol tartrate in sheep. Lab. Anim. Sci. 1988; 38: 58-61.

OLFERT, E.D. Ethics of animal models of neurological diseases. In: Boulton, A., Baker, G. and Butterworth, R., eds. Animal models of neurological disease. 1. Cliston, NJ: Humana Press, 1992: 1-28.

ORLANS, F.B., SIMMONDS, R.C. and DODDS, W.J., eds. Effective animal care and use committees. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1987.

PAGE, S. Building a better cow through cloning. Ottawa Citizen, July 29, 1990.

ROWAN, A.N. Animal anxiety and animal suffering. Appl. Anim. Behav. Sci. 1988; 20: 135-142.

ROWSELL, H.C. The future of control of pain in animals used in teaching and research. In: Short, C.E. and Van Poznak, A., eds. Animal pain. New York, Edinburgh, London, Melbourne, Tokyo: Churchill Livingstone, 1992: 525-537.

ROWSELL, H.C. and MCWILLIAM, A.A. The search for alternatives: the Canadian initiative. ATLA (Alternatives To Live Animals) 1986; 13(3): 208-211.

ROYAL SOCIETY FOR THE PREVENTION OF CRUELTY TO ANIMALS: Pain and suffering in experimental animals in the United Kingdom. London: RSPCA, 1983.

RUSSELL, W.M.S. and BURCH, R.L. The principles of humane experimental technique. Springfield: Charles C. Thomas, 1959.

SACKMAN, J.E. Pain. Part II. Control of pain in animals. Comp. Sm. Anim. Cont. Ed. Article No. 1, 1991; 13(2): 181-193.

SAFFER, J.D. Transgenic mice in biomedical research. Lab Animal 1992; 21(3): 30-38.

SAWYER, D. Use of narcotics and analgesics for pain control. Proc. AAHA 52nd Annual Meeting 1985: 7-11.

SEAMER, J.H. Farm animal welfare in Britain. SCAW (Scientists Center for Animal Welfare) Newsl. 1993; 14(4): 13-14.

SETHI, M.S. Proposed framework for regulating the production and use of transgenic animals. Ottawa, Ont.: Veterinary Biologics and Biotechnology, Animal Health Division, Agriculture Canada, 1992.

SHERRINGTON, C. The integrative action of the nervous system. New Haven, CT: Yale University Press, 1947.

SHORT, C.E., ed. Principles and practice of veterinary anesthesia. Baltimore, MD: Williams and Wilkins, 1987.

SHORT, C.E. and VAN POZNAK, A., eds. Animal pain. New York, NY: Churchill Livingstone, 1992.

SILVERMAN, J. How much is enough? Lab Animal 1991; 20(2): 20.

SMITH, M. (editorial) The weighing of benefit and suffering. FRAME (Fund for the Replacement of Animals in Medical Experiments) News 1988; 20: 1-2.

SMYTH, D.H. Alternatives to animal experiments. London: Scolar Press-Royal Defence Society, 1978.

SPIRA, H. Here's what you can do to make a difference. Factory farming. Animal Rights Coalition Co-ordinator's Report '86: September 1986: 4. (Animal Rights International, New York.)

TOTH, L.A. and JANUARY, B. Physiological stabilization of rabbits after shipping. Lab. Anim. Sci. 1990; 40 (4): 384-387.

UNIVERSITIES FEDERATION FOR ANIMAL WELFARE/WORLD SOCIETY FOR THE PROTECTION OF ANIMALS. Euthanasia of amphibians and reptiles. Herts, U.K.: UFAW, 1989.

WALL, P.D. Introduction. In: Wall, P.D. and Melzack, R., eds. Textbook of pain. Edinburgh, London, Melbourne and New York: Churchill Livingstone, 1984.

WALL, P.D. and MELZACK, R., eds. Textbook of pain. Edinburgh, London, Melbourne, and New York: Churchill Livingstone, 1984.

WALL, P.D. Defining pain in animals. In: Short, C.E. and Van Poznak, A., eds. Animal pain. New York, Edinburgh, London, Melbourne, Tokyo: Churchill Livingstone, 1992: 63-79.

ZIMMERMAN, M. (Guest editorial) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16: 109-110.

XI. ANESTHESIA

This chapter provides guidance and information on anesthesia and relief of pain in experimental animals. It is not meant to be comprehensive, and non-veterinary users should consult with a veterinary anesthesiologist or laboratory animal veterinarian when such drugs are to be administered. Information on common dosages and means of administration of analgesic, tranquillizing and anesthetic agents are given in the Appendices. **The agents described in this chapter are all prescription and/or controlled drugs. Non-veterinary users may obtain prescription drugs from a licensed veterinarian, and should contact the Bureau of Dangerous Drugs, Health and Welfare Canada regarding the use of controlled drugs in research.**

Methods for assessing the depth of anesthesia vary with the species and the drug, and are discussed in Green (1982). Specific details are available in the textbooks and review articles listed in the references.

A. MANAGEMENT OF ANESTHESIA

1. General

Sedatives, analgesics, and general anesthetic agents must be utilized for the control of pain and distress unless contrary to the achievement of the objectives of the study. **In the latter case, approval of the institutional Animal Care Committee (ACC) is mandatory.**

Anesthetic agents frequently affect the cardiovascular, respiratory and thermoregulatory mechanisms, in addition to the central nervous system (CNS). Every effort should therefore be made to maintain the circulation, respiratory function and the body temperature of the anesthetized subject within normal physiological limits (Parker and Adams, 1978). Endotracheal intubation ensures that the airway remains patent and free from obstruction.

Hypothermia may occur during exposure to anesthetic gases and during intra-abdominal surgery, particularly in small animals. This may result in death or a greatly prolonged recovery from the anesthetic. The degree of hypothermia may be reduced by placing the animal on a circulating warm water blanket or other device that assists in conserving body heat (Muir and Hubbell, 1989; Lumb and Jones, 1984; Flecknell, 1987).

2. Handling the Patient

The animal should always be handled gently and calmly in order to minimize struggling and fright. Prolonged excitement will disturb the circulatory and metabolic state of the patient and induce a degree of shock. Furthermore, attempts to anesthetize a struggling animal present physical problems in addition to enhancing the likelihood of an abnormal response to the anesthetic agents. These points are particularly important when restraining and anesthetizing wild animals (Fowler, 1986).

3. Fasting

Cats, dogs, non-human primates (NHP), ferrets and pigs should receive no food during the 8-12 hours prior to induction of anesthesia in order to minimize the risk of vomiting during induction or recovery from anesthesia (Flecknell, 1987). Very small or immature mammals should be subjected to a much shorter fast, usually from two to four hours, due to their higher metabolic rate. Withholding food from ruminants for 12-24 hours may help reduce the incidence of ruminal tympany (bloat); however, reduction of the volume of digesta in the rumen requires much longer periods of starvation (36-72 hours). Water should be withheld for 12 hours before surgery to prevent gorging and increase in the volume of rumen

contents. Pre-anesthetic fasting of small rodents or rabbits is unnecessary since they do not vomit during induction (Flecknell, 1987). Guinea pigs should be fasted 6-12 hours before anesthesia to allow time to clear their mouths of the food bolus commonly carried at the base of the tongue. Small birds often are not fasted at all, in order to maintain energy during the stress of the procedure (Muir and Hubbell, 1989; NRC [U.S.], 1977). Fasting pregnant animals of all species, particularly ruminants, can produce severe metabolic disturbances. Other than ruminants, every animal should be provided with drinking water until approximately one hour before induction of anesthesia (Flecknell, 1987).

4. Anticholinergics

Anticholinergics block parasympathetic stimulation to the cardiopulmonary system and reduce salivary secretion. They are used in combination with sedatives and analgesics as pre-medication to general anesthesia. Anticholinergics are no longer routinely administered to each animal undergoing anesthesia. They are administered selectively, after a pre-anesthetic clinical examination of the animal, and according to the determined needs of the individual patient, the anticipated response to the anesthetic medication, and the tendency to develop bradycardia or excessive salivation (Short, 1987).

a) Atropine is the most commonly used anticholinergic agent; however, routine administration is controversial due to the high incidence of associated cardiac dysrhythmias (premature ventricular contractions and sinus tachycardia) (Lumb and Jones, 1984; Flecknell, 1987). It is most commonly recommended for use in NHP, pigs, guinea pigs and chinchillas in order to decrease airway secretions, but should not be given if a marked tachycardia is already present (Green, 1982).

b) Glycopyrrolate is a quaternary ammonium anticholinergic. Although its mechanism of action is similar to that of atropine, its effects last longer. Glycopyrrolate seems to be less likely than atropine to produce sinus tachycardia (Paddleford, 1988). It does not penetrate the CNS because of its difficulty in crossing the blood-brain barrier. It is also less likely than atropine to cross the placental barrier, indicating that it is a selective peripheral anticholinergic agent (Short, 1987).

B. TRANQUILLIZERS AND SEDATIVES

Tranquillizers produce a calming effect without sedation (Green, 1982). They have no analgesic properties, and even at the high doses that cause ataxia (failure of muscular co-ordination) and depression, animals are easily aroused. Tranquillizers are useful over a wide range of species, often in combination with other drugs, to lessen the dose of a general anesthetic and produce a smoother induction and recovery. Sedatives are used to produce drowsiness and reduce fear and apprehension (Flecknell, 1987).

The psychological state of the animal prior to administration of tranquillizers may markedly affect the degree of sedation achieved. Animals that are vicious, intractable and in a state of excitement may not become manageable except with very high (incapacitating) doses.

a) Phenothiazines (promazine, acepromazine) produce sedation and reduce the dose of drugs needed for general anesthesia, but also cause moderate hypotension and hypothermia (Lumb and Jones, 1984; Flecknell, 1987).

b) Benzodiazepines (diazepam, midazolam) produce variable sedation depending on the species (Lumb and Jones, 1984; Flecknell, 1987; Green, 1982). They are good muscle relaxants and have no marked undesirable side effects. Diazepam cannot be mixed with other water soluble agents, while midazolam can (Flecknell, 1987).

c) Butyrophenones (azaperone, droperidol) have similar effects as phenothiazines, but are more potent and cause less hypotension (Lumb and Jones, 1984; Flecknell, 1987; Green, 1982). Droperidol is

used in combination with an opioid to produce neuroleptanalgesia (Flecknell, 1987).

d) alpha-2-adrenergic agonists (xylazine, detomidine, medetomidine)

i) Xylazine (Rompun) is a sedative and analgesic that acts as a CNS depressant and induces muscle relaxation by inhibiting the transmission of impulses in the CNS. Its major use in laboratory animal anesthesia is in combination with ketamine to produce surgical anesthesia. This combination has been used in dogs, cats, NHP, large farm animals and wild animals (Olson and McCabe, 1986; Lumb and Jones, 1984). It causes respiratory depression and a bradycardia which may progress to heart block (Flecknell, 1987). It also increases the susceptibility of the myocardium to circulating catecholamines during halothane anesthesia (Short, 1987). Vomiting may occur in dogs and cats, and gas accumulation due to gastrointestinal atony (lack of normal tone or strength) may be a problem in both large dogs and ruminants (Lumb and Jones, 1984). Xylazine produces profound physiological changes and its safe use requires knowledge of these effects which are often species specific. Yohimbine and 4-aminopyridine reverse most of the effects of xylazine without relapse in many species (Jernigan, Wilson, Booth *et al*. 1988), with the exception of NHP (Lynch and Line, 1985).

ii) Detomidine is marketed for use in horses, and has the same cardiovascular effects (bradycardia and hypotension) as xylazine, but is more potent and has a longer-acting effect.

iii) Medetomidine is being evaluated for use in dogs and cats, and has cardiovascular effects similalr to xylazine. A medetomidine/ketamine combination in cats has the advantage over xylazine/ketamine in that a lower dose of ketamine is needed, the duration of action is longer and the analgesia better (Verstegen, Fargetton, Donnay *et al*. 1990).

C. GENERAL ANESTHETICS

1. Dissociative Anesthetics

Dissociative anesthetics produce a state of chemical restraint and anesthesia characterized by muscle rigidity and dissociation of the mind from the external environment. The eyes remain open, necessitating use of protective ointment. Various reflexes, including the blinking reflex and laryngeal reflex, remain intact, and adequate respiration is normally maintained. An increase in heart rate, blood pressure and intracranial pressure frequently occurs. Thus, their use is contra-indicated in head injuries or intra-ocular surgery. While the use of dissociative anesthetic agents is most common with NHP and cats, they have also been used in most other mammalian species as well as birds and reptiles (Jones, 1977). Combination with a tranquillizer is recommended in most species to enhance analgesia and reduce muscle tone (Flecknell, 1987; Green, 1982).

a) Ketamine hydrochloride is the most commonly used member of this group. Depth of anesthesia is dose related. Side effects include excessive salivation which may be controlled with atropine (Flecknell, 1987), a tendency toward convulsions, and a recovery characterized by excitement, disorientation, and hallucinations which may be controlled by tranquillizers and barbiturates (Lumb and Jones, 1984). In all cases, a smooth recovery will be facilitated if the patient is left undisturbed in a quiet, darkened environment.

b) Tiletamine is similar to ketamine, but is longer lasting and more potent; therefore, a smaller dose volume is needed. It is most commonly sold in combination with the tranquillizer zolazepam (Telazol), which improves muscle relaxation, CNS depression, and emergence from anesthesia. It also prevents tiletamine seizures. Cats may take 12-36 hours to be clinically "normal" following tiletamine anesthesia. Tiletamine/zolazepam has proven successful in rats and gerbils, but not in mice or hamsters (Hrapkiewicz, Stein and Smiler, 1989). Tiletamine causes nephrotoxicity in rabbits (Brammer, Doerning, Chrisp *et al*. 1991; Doerning, Brammer, Chrisp *et al*. 1992).

2. Barbiturates

Barbiturates differ from tranquillizers and opioids in that increasing the dose progressively increases the depth of depression until a state of general anesthesia is reached. They are poor analgesics. Their primary use is in the induction and/or maintenance of general anesthesia. Barbiturates are potent respiratory depressants and their effects on the cardiovascular system are variable. At intermediate dosages, excitement is sometimes induced (Green, 1982).

The barbiturates are grouped according to duration of action into long acting (e.g., phenobarbital), short- or intermediate-acting (e.g., pentobarbital) and ultrashort-acting (e.g., thiopental, thiamylal, methohexital) (McLaughlin, 1988). The short- and ultrashort-acting drugs are commonly used for anesthesia. Anesthetic duration varies widely with species; however, in general, short/intermediate barbiturates produce approximately 2-3 hours of anesthesia and ultrashort barbiturates range from 10 to 20 minutes (McLaughlin, 1988).

Variation in dose response and duration of effect of barbiturates is extreme within and between species (Olson, 1986a; Green, 1982; McLaughlin, 1988). The following are examples of the variation found with pentobarbital (intermediate) anesthesia:

i) cats frequently having a considerably prolonged sleeping time (McLaughlin, 1988);

ii) mice on hardwood bedding take almost twice as long to recover as mice on softwood bedding, and male mice sleep longer than female mice (McLaughlin, 1988);

iii) the anesthesia produced in adult horses and cattle is of relatively short duration; however, the recovery period is long and difficult (Lumb and Jones, 1984).

Whenever possible, barbiturates should be administered intravenously, slowly, to effect. Administration by other routes is far less satisfactory, as dosage is more difficult to judge and the anesthetic effects are less predictable. Any of the barbiturates can cause skin sloughing if perivascular injection accidently occurs (McLaughlin, 1988).

Although barbiturates are commonly used, they are often poor choices for general anesthesia due to poor analgesia, profound cardiovascular effects, high mortality and numerous external factors that can affect dose response and sleeping time. Adequate anesthesia can be obtained by combining a barbiturate with a tranquillizer, sedative or an opioid (Olson, 1986a; Lumb and Jones, 1984; McLaughlin, 1988).

3. Chloralose

Chloralose may be used for **non-survival experiments** requiring prolonged anesthesia and **minimal surgical interference** (Flecknell, 1987; Holzgrefe, Everitt and Wright, 1987). There is disagreement about whether chloralose is a true anesthetic agent or a hypnotic with little analgesic action. It is used primarily for physiological studies to preserve the vagal and central baroreceptor reflexes or in acute cardiovascular studies to preserve myocardial function. While chloralose is generally considered to have no application in survival studies or in clinical veterinary medicine (Lumb and Jones, 1984), one recent study used chloralose repeatedly over a long time period in puppies without any signs of toxicity (Grad, Witten, Quan *et al*. 1988).

4. Urethane (Urethan, Ethyl Carbamate)

Urethane produces long periods of anesthesia, has a wide safety margin and little effect on normal blood pressure and respiration. It produces sufficient analgesia to allow surgical manipulations (Flecknell,

1987). **However, the drug should be handled with extreme care as it is considered to be cytotoxic, carcinogenic and immunosuppressive.** It also causes profound changes in gastrointestinal function and is stimulatory to the hypothalamus and pituitary (Olson, 1985). **Animals should not be allowed to recover following urethane anesthesia.**

5. Saffan

Saffan is a combination of two steroids, alphaxalone and alphadolone dissolved in a surfactant (vehicle), Cremaphor EL, to solubilise it. It is administered intravenously or intramuscularly, although the latter route gives more unpredictable results. Muscle relaxation is good, and recovery rapid. It is rapidly metabolized and is an excellent agent for long-term maintenance (Flecknell, 1987). It has been used for the cat, pig, large farm animals, small NHP, rodents, birds and exotics (Lumb and Jones, 1984; Flecknell, 1987; Green, 1982). It is not recommended in the dog due to the associated massive histamine release caused by the Cremaphor EL vehicle that often occurs (Flecknell, 1987). Saffan must not be used with barbiturates (Flecknell, 1987).

6. Tribomoethanol (Avertin)

The use of Avertin is controversial because of the wide variation in results between laboratories. Although no longer available in Canada, it may be introduced in a different formulation. Purchased as a powder, it must be dissolved in amylene hydrate and then diluted with distilled water at 40C immediately prior to use. Great care must be taken to use only fresh solutions as it decomposes very rapidly in light or temperatures above 40C, producing byproducts that are severe tissue irritants. In rodents, it is given intraperitoneally (Green, 1982), resulting in good muscle relaxation and moderate respiratory and cardiovascular depression (Flecknell, 1987; Green, 1982); however, post-operative fatalities are often high due to peritoneal adhesions. Even if a freshly prepared solution is used, mortality is often high after administration of a second anesthetic at a later date (Green, 1982; Norris and Turner, 1983).

7. Non-specific Injectable Anesthetic Antagonists

Several agents have the ability to reverse many of the effects of non-opioid injectable anesthetics through non-specific antagonistic properties.

a) Yohimbine blocks central alpha-2-adrenoreceptors, and partially antagonizes barbiturates, xylazine, ketamine, benzodiazepines and phenothiazines (Fowler, 1986; Lumb and Jones, 1984).

b) 4-aminopyridine (4-AP) partially antagonizes xylazine, ketamine and barbiturates. Yohimbine and 4-AP are often combined for a more effective reversal (Lumb and Jones, 1984).

c) Doxapram is a respiratory stimulant and not a reversal agent *per se*; however, it has been used to partially antagonize the respiratory depression produced by barbiturate anesthesia in dogs (Hatch, Jernigan, Wilson *et al*. 1986).

8. Inhalant Anesthetics

Inhalant anesthetics have the advantage of requiring minimal detoxification by the body, as they are exhaled through the lungs, and the level of anesthesia can be easily and rapidly controlled. However, their use requires specialized equipment for administration, and constant monitoring of the patient (Stimpfel and Gershey, 1991). Some are explosive or inflammable, or tissue irritants. Chronic exposure to some agents is hazardous to the health of the operating room personnel (Lumb and Jones, 1984).

The speed of induction and recovery depend on the solubility of the anesthetic in blood. Highly soluble

anesthetics (methoxyflurane) are slow to reach an equilibrium in the blood; therefore, induction and recovery are prolonged. Insoluble anesthetics (halothane) reach an equilibrium rapidly, making manipulation of anesthetic depth easier, but also more hazardous due to the potential for rapid overdose (Flecknell, 1987).

The use of inhalation anesthesia requires the following equipment:

i) a vaporizer for the volatile anesthetics;

ii) a source of carrier gas (usually oxygen or air);

iii) a breathing system from which the anesthetic mixture is inhaled;

iv) a mask or endotracheal tube for connecting the breathing system to the patient (Sedgwick and Jahn, 1980; Gilroy, 1981). Exceptions are discussed with the individual agents. Numerous simple systems have been devised and reported in the laboratory animal literature for use in small laboratory animals (Dudley, Soma, Barnes *et al*. 1975; Skartvedt and Lyon, 1972; Rich, Grimm, Wong *et al*. 1990; Olson, 1986b; Levy, Zwies and Duffy, 1980; Mulder and Hauser, 1984).

Unnecessary exposure of personnel to gases from volatile anesthetics must be avoided by use of appropriate scavenger systems (Muir and Hubbell, 1989). Several reports have suggested a health risk associated with prolonged and repeated exposure to low concentrations of halothane (hepatocellular toxicity), methoxyflurane (renal toxicity), nitrous oxide (neurologic disease and pernicious anemia) and to the chronic ingestion of chloroform (renal and hepatic tumours in rodents) (Rettig, 1987; Stimpfel and Gershey, 1991). Expired gases should be vented to the exterior or adsorbed onto activated charcoal (Mitchell, 1976).

a) Ether-based Volatile Agents

i) Diethyl ether is a highly volatile agent of relatively low potency and wide range of safety. Ether produces good muscle relaxation and analgesia; however, it is very irritating to mucous membranes. The vapours are highly explosive, necessitating extreme caution in its use and storage. **Due to the risk of explosion, the use of ether is discouraged as excellent alternatives are now available** (Flecknell, 1987; Stimpfel and Gershey, 1991).

ii) Methoxyflurane (Metofane) is a highly soluble, potent ether-based anesthetic. Because of its low volatility, it may be used safely for induction with anesthetic chambers, and nose cone maintenance. Methoxyflurane produces some respiratory and cardiovascular depression, but less than halothane at comparable depths of
anesthesia. Myocardial sensitization occurs, but is not as severe as with halothane. Muscle relaxation and analgesia are good, and it is neither irritating nor explosive in anesthetic concentrations. In animals, methoxyflurane anesthesia for less than one hour is not usually associated with hepatorenal toxicity, especially if periods of hypoxia and/or hypercapnia are avoided (Stimpfel and Gershey, 1991).

iii) Enflurane provides rapid induction and emergence from anesthesia. It provides moderate levels of analgesia and muscle relaxation, the latter decreasing as anesthetic concentrations increase. It produces profound depression of respiratory functions and myocardial performance (Short, 1987). It is largely eliminated via the lungs. Unlike halothane, very little of the drug is metabolized by the liver. This may offer some experimental advantages; otherwise, there is little to choose between enflurane and halothane in terms of efficacy (Flecknell, 1987). Enflurane is expensive and requires a special vaporizer.

iv) Isoflurane is less potent than halothane or methoxyflurane. It is relatively insoluble which leads to fast inductions and recoveries. It may be used in halothane vaporizers that have been recalibrated. It

produces a slightly more severe respiratory depression than does halothane, but slightly less depression of the cardiovascular system (Flecknell, 1987). There is very little myocardial sensitization to catecholamines; in fact, isoflurane has the greatest margin of safety with the cardiovascular system of all the inhalant anesthetics. Isoflurane produces better muscle relaxation than halothane, but has poorer analgesic properties. It undergoes even less biotransformation than enflurane and is almost completely eliminated in exhaled air (Flecknell, 1987). Isoflurane has a pungent odour which may cause breath holding during induction. It has no known toxicities, but it is expensive (Raper, Barker, Burwen *et al*. 1987).

b) Halogenated Hydrocarbons

i) Halothane, a halogenated hydrocarbon, is highly potent and volatile. It should be used only with a finely calibrated precision vaporizer. It produces dose-dependent depression of the cardiopulmonary system and hypotension (Flecknell, 1987). There is direct myocardial depression and sensitization to circulating catecholamines. The analgesia offered by halothane is reasonable, as is muscle relaxation. The vapours are neither explosive nor irritating, but can be hepatotoxic to man (Lumb and Jones, 1984).

c) Other Agents

i) Nitrous oxide has very low anesthetic potency. Induction of a state of general anesthesia or even unconsciousness is not possible in most animal species (Flecknell, 1987; Mahmoudi, Cole and Shapiro, 1989). As it exerts minimal effects on the cardiopulmonary system, it can be used to reduce the required concentration of other agents and so reduce the degree of depression at a particular depth of anesthesia (Flecknell, 1987). It has some analgesic properties in animals; however, the potency is less than half that experienced in humans (Short, 1987). Following cessation of nitrous oxide administration, 100% oxygen must be administered to the
animal to prevent hypoxia caused by the rapid diffusion of the gas from the body (Flecknell, 1987; Short, 1987). **Because it presents numerous occupational hazards, nitrous oxide should be scavenged. I**f a carrier gas is required 100% oxygen is effective and non-toxic as well as being vital to life (Stimpfel and Gershey, 1991).

D. MUSCLE RELAXANTS

1. Glyceryl Guiacolate

Glyceryl guiacolate (guaifenesin) is a centrally acting muscle relaxant, with its action on the internuncial neurons of the spinal cord. As the drug has little effect on the diaphragm, it produces muscle relaxation without respiratory paralysis. A state of sedation and hypnosis is produced; however, the degree of analgesia is in dispute. Guaifenesin is most often used as part of induction technique in large farm animals. It is useful in combination with thiobarbiturate for short surgical procedures and for intubation prior to the administration of an inhalant anesthetic (Lumb and Jones, 1984). Guaifenesin has been added to ketamine and xylazine to produce effective anesthesia in ponies, dogs and pigs with minimal cardiovascular and respiratory depression. This same combination has also been used in a continuous infusion for long term anesthesia in cats (Brown, McCarthy and Bennett, 1991).

2. Neuromuscular Blocking Agents

Succinylcholine (a depolarizing agent), curare, pancuronium, gallamine, atacurium and vecuronium (non-depolarizing agents) are neuromuscular blocking agents which act peripherally at the neuromuscular junctions. Anticholinesterases such as neostigmine, pyridostigmine and edrophonium are antagonistic to the non-depolarizing agents, but ineffective against the depolarizing agents (Lumb and Jones, 1984). Neuromuscular blocking agents are used as adjuncts to general anesthetics where profound muscle relaxation is desired.

These agents produce motor paralysis only. There is no sedation or analgesia. Their use on conscious animals is prohibited (see also *Ethics of Animal Investigation*).

The use of neuromuscular blocking agents abolishes some of the signs used to judge the depth of anesthesia. Autonomic functions remain intact with the newer agents (atacurium, vercuronium); therefore, increases in heart rate and arterial blood pressure may indicate the perception of pain. Animals must be artificially ventilated as the respiratory muscles are paralyzed. Should neuromuscular blocking agents be a component of an anesthetic protocol, it is extremely important that proper equipment and personnel with experience in the use of these agents be available.

E. LOCAL AND REGIONAL ANESTHETICS

Local anesthetics such as lidocaine, procaine, bupivacaine and tetracaine may be used to block the nerve supply to a limited area for the performance of minor or rapid procedures. Local anesthesia is also frequently used as an adjunct to various sedative and hypnotic agents in more prolonged and invasive procedures, such as caesarian section. Local anesthetic agents may be used for the regional infiltration of a surgical site, field blocking, nerve blocks, and for epidural and spinal anesthesia (Green, 1982; Elmore, 1981; Kero, Thomasson and Soppi, 1981; Gray and McDonell, 1986). Veterinary assistance should be sought in the initial use of the last three procedures (Lumb and Jones, 1984; Gray and McDonell, 1986). A combination of lignocaine/prilocaine has also been used topically for pain-free venipuncture in some laboratory animals (Flecknell, Liles and Williamson, 1990).

F. ANIMAL HYPNOSIS (Tonic Immobility)

A state of hypnosis or tonic immobility can be readily induced in a variety of animals including rabbits, birds, small rodents and reptiles (Prestrude and Crawford, 1970; Danneman, White, Marshall *et al*. 1988). It is characterized by a lack of spontaneous movement or overt response to external stimuli for up to several minutes, and is usually exhibited under stressful or fearful conditions. There is evidence that animals remain aware of external events and hypnosis can be interrupted by mild tactile or auditory stimuli. It is usually induced by placing the animal on its back and gently extending the neck and hind legs to place traction on the spine. Recent work indicates that some degree of analgesia is produced with hypnosis; however, individual animal susceptibility to hypnosis varies greatly and in consequence **hypnosis cannot be recommended as a suitable alternative to appropriate analgesics when painful procedures are to be performed** (Danneman, White, Marshall *et al*. 1988).

G. SPECIES CONSIDERATIONS

a) Canine

General anesthesia: sedation, followed by intravenous induction with an ultrashort-acting barbiturate, intubation and maintenance with an inhalant anesthetic. Alternatively, intermediate or long-acting barbiturates may be used but are poor analgesics and can result in profound respiratory and cardiovascular depression (Flecknell, 1987; Green, 1982). Minor surgical procedures can be carried out using neuroleptoanalgesics, xylazine combinations and diazepam combinations (Green, 1982).

b) Feline

General anesthesia: sedation, induction using an injectable agent, intubation and maintenance with an inhalant anesthetic (Green, 1982). The larynx should be sprayed with a local anesthetic such as 2% lidocaine (without epinephrine) prior to intubation (Flecknell, 1987). Mask induction with an inhalant anesthetic is also well tolerated if the cat is sedated previously and handled expertly. Ketamine and

ketamine combinations have proven very useful for restraint and minor surgical procedures (Flecknell, 1987; Ingwersen, Allen, Dyson *et al*. 1988). Saffan or xylazine also produce sedation and anesthesia for minor surgical procedures (Flecknell, 1987; Green, 1982).

c) Ferrets

Administration of intravenous drugs can be difficult in the awake ferret; therefore, alternate routes are usually used. Intramuscular ketamine and ketamine combinations are useful (Muir and Hubbell, 1989; Moreland and Glaser, 1985), as are fentanyl/ droperidol and intravenous Saffan (Flecknell, 1987; Green, 1982). For induction with inhalation anesthetics, a special induction chamber is usually used, with maintenance by mask or intubation (Poole, 1987; Moody, Bowman and Lang, 1985).

d) Rabbits

Neuroleptanalgesics and ketamine combinations with xylazine, acepromazine or azaperone have been used successfully (Muir and Hubbell, 1989; Olson, 1986a; Flecknell, 1987; Lipman, Marini and Erdman, 1990). Ketamine alone does not produce adequate anesthesia or analgesia (Lumb and Jones, 1984; Flecknell, 1987). The degree of analgesia produced by Saffan is generally low. At the higher dose rates needed to produce medium or deep surgical anesthesia, there may be sudden apnea followed by cardiac arrest (Flecknell, 1987). A technique of continuous intravenous infusion of ketamine and xylazine has been reported to maintain a light anesthetic plane for up to 4 hours, although hypoxemia and hypotension are marked (Wyatt, Scott and Richardson, 1989). Inhalant anesthetics and mask induction are readily tolerated (Peeters, Gil, Teske *et al*. 1988). Endotracheal intubation in the rabbit is relatively difficult for anatomical reasons. Barbiturates alone are not recommended in rabbits, as the dose required to produce surgical anesthesia is very close to the lethal dose. Respiratory arrest frequently occurs before the onset of surgical anesthesia. They may be used, if combined with a sedative or tranquillizer (Olson, 1986a; Peeters, Gil, Teske *et al*. 1988). If atropine is used it must be at high dose levels to counteract the presence of serum atropinase (Muir and Hubbell, 1989).

e) Small laboratory rodents (rats, mice, guinea pigs, gerbils, hamsters and wild rodents)

Withholding food and water is unnecessary prior to anesthesia, since vomiting normally does not occur (Flecknell, 1987). Anesthetic agents used include barbiturates, ketamine, ketamine combinations (Muir and Hubbell, 1989; Flecknell, 1987; Wixson, 1987a, 1987b), neuroleptoanalgesics (Muir and Hubbell, 1989; Green, 1982; Parkes, 1987; Olson, 1986a), tiletamine/zolazepam (Muir and Hubbell, 1989) and Saffan (Green, 1982). Ketamine alone produces severe respiratory depression at doses high enough for surgical anesthesia in small rodents (Flecknell, 1987). Intramuscular ketamine/xylazine causes muscle necrosis in Syrian hamsters and is not recommended in that species (Gaertner, Boschert and Schoeb, 1987). The same problem has been noted with fentanyl/droperidol in guinea pigs (Holmes, 1984). Ketamine combinations and pentobarbital are poor anesthetics in the gerbil, but fentanyl/metomidate (Flecknell, John, Mitchell *et al*. 1983) and tiletamine/zolazepam have proven effective (Hrapkiewicz, Stein and Smiler, 1989). Barbiturates are still in common use, but are very poor analgesics, and often cause high mortality, especially when given intraperitoneally or when full-strength commercial solutions are used intravenously (dilution is recommended). When combined with a sedative, tranquillizer or an opioid, adequate anesthesia results (Olson, 1986a).

Induction of anesthesia with an inhalational agent is best accomplished with an induction chamber. Anesthesia may be maintained with a face mask. Endotracheal intubation is difficult in small rodents and requires purpose-made laryngoscopes (Flecknell, 1987).

The safe administration of general anesthesia to the guinea pig is notoriously difficult, since they often maintain their pedal reflex and make squirming movements even when deeply anesthetized (Holmes, 1984). Their response to many injectable anesthetics is very variable. Post-anesthetic complications such as respiratory infections, digestive disturbances, and generalized depression, are seen (Flecknell, 1987). Spinal anesthesia offers a useful alternative (Green, 1982).

Very brief procedures (e.g., orbital blood sampling) may be performed on rodents by using a 50:50 mixture of carbon dioxide and oxygen, if the animal is removed from the gas chamber as soon as the pedal reflex has disappeared (Green, 1982; Fenwick and Blackshaw, 1989).

Hypothermia may be used to anesthetize neonatal mice and rats (1-2 days old). The pup is placed in an ice water slush for 20-30 minutes (Green, 1982).

f) Non-human Primates

Ketamine and its combinations are most often used for restraint, particularly where rapid recovery is desired. Neuroleptanalgesics have also been used, and Saffan is useful for small species such as marmosets. The NHP can be intubated and inhalation anesthesia administered using techniques similar to those used for the human (Flecknell, 1987; Sainsbury, Eaton and Cooper, 1989).

g) Horses

Both induction and recovery from anesthesia may be associated with excitement. Due to their size and strength, special facilities are required for induction and recovery in horses. Veterinary consultation should be sought. Xylazine and acepromazine are most commonly used as pre-anesthetics, followed by an induction agent (thiamylal sodium, guaifenesin, etc.) and inhalation anesthesia (Muir and Hubbell, 1989; Green, 1982).

h) Ruminants

Many surgical procedures can be performed under local or regional anesthesia (Muir and Hubbell, 1989; Green, 1982; Gray and McDonell, 1986). The greatest problems with sedation and general anesthesia are regurgitation, hypoventilation and bloat. The use of atropine in ruminants is controversial, as it induces bloat and increases the viscosity of the saliva, while not decreasing the quantity. Xylazine is given at one-tenth the dose of horses, and usually results in recumbency. It is probably best to administer xylazine by slow intravenous injection in sheep and goats as results produced by intramuscular administration are unpredictable. It should be noted that some goats appear particularly sensitive to xylazine (Hall and Clarke, 1991). Bloat is often a problem following xylazine administration, and abortion can be induced in the last trimester (Muir and Hubbell, 1989). Xylazine/ketamine with or without guaifenesin can be combined for shorter surgical procedures (Coulson, Januszkiewicz, Dodd *et al*. 1989). Other recommended injectables for sheep are Saffan and ketamine/diazepam (Flecknell, 1987).

Although thiopental is useful for induction, pentobarbital is not recommended, especially in goats, due to respiratory depression. Animals less than three months old metabolize barbiturates very poorly (Muir and Hubbell, 1989).

Mask induction with an inhalant anesthetic such as halothane or isoflurane is particularly useful with the smaller species. Sheep should always be intubated to prevent aspiration if regurgitation occurs. Intubation is accomplished with the use of a laryngoscope in small ruminants and by direct palpation of the larynx in large ones. A lidocaine spray should be used on sheep vocal cords prior to intubation to prevent laryngospasm (Flecknell, 1987).

i) Swine

Pigs should be fasted 12 hours before surgery to prevent vomiting; however, water can be offered until the pre-anesthetic is given (Muir and Hubbell, 1989). As respiratory depression is a frequent sequela to general anesthetics in pigs, reversible drugs such as xylazine or opioids are recommended (Green, 1982; Muir and Hubbell, 1989). Epidural anesthesia is also commonly used (Muir and Hubbell, 1989). Ketamine

in combination with xylazine, diazepam, acepromazine or fentanyl/droperidol have produced good results as general anesthetics (Muir and Hubbell, 1989; Green, 1982; Swindle, 1985), as have other injectable anesthetic such as Saffan (Flecknell, 1987) and tiletamine/zolazepam (Muir and Hubbell, 1989; Bauck, 1984; Cantor, Brunson and Reibold, 1981). Barbiturates are generally used only in combination with a sedative (Muir and Hubbell, 1989). Azaperone produces sedation, but has no analgesic effect (Flecknell, 1987).

Inhalation agents are well tolerated and mask induction can be carried out on small pigs with ease (Becker, 1986). Intubation of the trachea is difficult for anatomical reasons (Lumb and Jones, 1984; Flecknell,1987; Green, 1982), and a lidocaine spray on the vocal cords is used to prevent laryngospasm (Green, 1982).

Malignant hyperthermia has been observed in response to inhalation anesthetics (especially halothane), depolarizing muscle relaxants and stress in swine. A predisposition to hypothermia is probably inherited (Basrur, Bouvet and McDonell, 1988), and is commonest in the Landrace and Poland China breeds. Dantrolene is effective therapy for malignant hyperthermia (Muir and Hubbell, 1989).

j) Avian

Hypothermia is a frequent problem in general anesthesia, especially for small birds. Small birds are also prone to handling shock, and small friable vessels make intravenous injection difficult (Green, 1982). Ketamine is an effective pre-anesthetic, and ketamine/xylazine (Muir and Hubbell, 1989) or ketamine/ diazepam (Fowler, 1986) are two of the safest injectable anesthetics. Tiletamine/zolazepam is an alternative to ketamine/xylazine (Muir and Hubbell, 1989; Green, 1982). Diazepam combined with chloropent (chloral hydrate, sodium pentobarbital, magnesium sulfate) provides surgical anesthesia for 60-90 minutes in the domestic fowl (Christensen, Fosse, Halverson *et al*. 1987). Saffan has been used in a variety of avian species (Lumb and Jones, 1984). However, it should only be administered intravenously, and even then used with great caution due to associated cardiac arrhythmias (Green, 1982; Short, 1987).

Inhalant anesthesia with mask induction can be used fairly safely and effectively; however, because of the efficiency of the avian respiratory system, changes in anesthetic depth tend to occur very rapidly, especially in small birds (Muir and Hubbell, 1989; Lumb and Jones, 1984; Green, 1982). Resuscitation is complicated due to accumulation in the air sacs (Fowler, 1986; Ludders, Mitchell and Schaefer, 1988). Inhalants cannot be used for thoracic procedures because the gas leaks through the opened air sacs (Christensen, Fosse, Halverson *et al*. 1987), and positive pressure ventilation is necessary for abdominal procedures due to an incomplete diaphragm. Restraint must allow free movement of the sternum for respiration. Isoflurane is the safest inhalation anesthetic, followed by halothane (Muir and Hubbell, 1989).

k) Cold Blooded Animals

Agents commonly used include tiletamine/zolazepam, ketamine, Saffan, tricaine methanesulfonate (MS-222) and inhalant anesthetics. Dosage varies widely between species. Absorption and excretion of injectable anesthetics are directly related to environmental temperature.

Fish should be fasted 24-48 hours to prevent vomiting (Green, 1982). They are commonly anesthetized by immersion or use of a recirculation system that passes an anesthetic solution over the gills. Tricaine methanesulfonate (MS-222) (Brown, 1987), and benzocaine (Green, 1982) are recommended, although numerous other anesthetics including carbon dioxide, ether, chloral hydrate, halothane and Saffan have also been used (Muir and Hubbell, 1989; Lumb and Jones, 1984; Green, 1982). Benzocaine is as effective as MS-222, equally safe for personnel and much less expensive (Green, 1982). Exposure of benzocaine to direct sunlight causes breakdown and releases highly toxic chlorine (Poole, 1987).

Reptiles and amphibians can be effectively anesthetized with local anesthetics, immersion in a solution

containing an anesthetic agent, injectable or inhalation anesthetics (Muir and Hubbell, 1989). Hypothermia should only be used for restraint in non-painful procedures, as it is not known whether or not analgesia is induced. Secondary tissue damage also results from the practice. Hypothermia is not a suitable anesthetic for major surgery (Muir and Hubbell, 1989). Amphibians can be anesthetized by immersion in MS-222, which provides excellent muscle relaxation and analgesia (Muir and Hubbell, 1989; Green, 1982). Preferred injectable anesthetics for reptiles include ketamine and tiletamine/zolazepam, although Saffan and etorphine have also been used successfully (Muir and Hubbell, 1989; Fowler, 1986).

Inhalation anesthesia is induced by soaking a cotton ball with a volatile anesthetic and placing it with the animal in a box or bag, or using an induction chamber or face mask (Muir and Hubbell, 1989). Halothane, isoflurane and methoxyflurane are preferred to ether (Muir and Hubbell, 1989). Reptiles are relatively easy to intubate, as the larynx is readily visualized. Their slow respiratory rates and ability to breath-hold constitute complicating factors (Muir and Hubbell, 1989). Inhalants are not recommended for turtles (Green, 1982).

Johnson (1992) warns that in administering anesthetics to amphibians and reptiles, one must consider the structure of the reptilian respiratory system. Respiratory movements are different in snakes, which have one lung, crocodiles which have diaphragms, and lizards which have pleuroperitoneal cavities. He suggests that, because their respiratory movements may be weak, if a volatile anesthetic is used, one may have to assist respiration because they have a poor way of expelling air. Johnson also notes that, if anesthesia is to be done for a long period of time, amphibians must be kept moist; as they are all poikilotherms, keeping them at their preferred optimum temperature zone will have an effect on the absorption and excretion of the anesthetic.

I) Invertebrates

Volk (1986) discusses methods of evaluating anesthetic depth in various invertebrates and includes a complete list of anesthetics.

H. REFERENCES

BASRUR, P.K., BOUVET, A. and MCDONELL, W.N. Open canalicular system of platelets in porcine stress syndrome. Can. J. Vet. Res. 1988; 52: 380-385.

BAUCK, S.W. An evaluation of a combination of injectable anesthetic agents for use in pigs. Can. Vet. J. 1984; 25: 162-165.

BECKER, M. Anesthesia in Gottingen miniature swine used for experimental surgery. Lab. Anim. Sci. 1986; 36: 417-419.

BRAMMER, D.W., DOERNING, B.J., CHRISP, C.E. and RUSH, H.E. Anesthetic and nephrotoxic effects of Telazol in New Zealand white rabbits. Lab. Anim. Sci. 1991; 41: 432-435.

BROWN, L.A. Recirculation anaesthesia for laboratory fish. Lab. Anim. 1987; 21: 210-215.

BROWN, M.J., MCCARTHY, T.J. and BENNETT, B.T. Long-term anesthesia using a continuous infusion of guaifenesin, ketamine and xylazine in cats. Lab. Anim. Sci. 1991; 41: 46-50.

CANTOR, G.H., BRUNSON, D.B. and REIBOLD, T.W. A comparison of four short-acting anesthetic combinations for swine. VM SAC 1981; 76: 715-720.

CHRISTENSEN, J., FOSSE, R.T., HALVERSEN, O.J. and MORILD. I. Comparison of various anesthetic regimens in the domestic fowl. Am. J. Vet. Res. 1987; 48: 1649-1657.

COULSON, N.M., JANUSZKIEWICZ, A.J., DODD, K.T. and RIPPLE, G.R. The cardiorespiratory effects of diazepam-ketamine and xylazine-ketamine anesthetic combination in sheep. Lab. Anim. Sci. 1989; 39: 591-597.

DANNEMAN, P.J., WHITE, W.J., MARSHALL, W.K. and LANG, C.M. An evaluation of analgesia associated with the immobility response in laboratory rabbits. Lab. Anim. Sci. 1988; 38: 51-57.

DOERNING, B.J., BRAMMER, D.W., CHRISP, C.E. and RUSH, H.E. Nephrotoxicity of tiletamine in New Zealand white rabbits. Lab. Anim. Sci. 1992; 42: 267-269.

DUDLEY, W.R., SOMA, L.R., BARNES, C., SMITH, T.C. and MARSHALL, B.E. An apparatus for anesthetizing small laboratory animals. Lab. Anim. Sci. 1975; 25: 481-482.

ELMORE, R.G. Food-animal regional anesthesia. Porcine blocks: lumbosacral (epidural). VM SAC 1981; 76: 387-388.

FENWICK, D.C. and BLACKSHAW, J.K. Carbon dioxide as a short-term restraint anesthetic in rats with subclinical respiratory disease. Lab. Anim. 1989; 23: 220-228.

FLECKNELL, P.A., JOHN, M., MITCHELL, M. and SHUREY, C. Injectable anesthetic techniques in 2 species of gerbils (*Meriones libycus* and *Meriones unguiculatus*). Lab. Anim. 1983; 17: 118-122.

FLECKNELL, P.A. Laboratory animal anesthesia. Toronto, Ont.: Academic Press, 1987.

FLECKNELL, P.A., LILES, J. and WILLIAMSON, H. The use of lignocaine-prilocaine local anesthetic cream for pain-free venipuncture in laboratory animals. Lab. Anim. 1990; 24: 142-146.

FOWLER, M.E. Zoo & wild animal medicine. Toronto, Ont.: W.B. Saunders Co., 1986.

GAERTNER, D.J., BOSCHERT, K.R. and SCHOEB, T.R. Muscle necrosis in Syrian hamsters resulting from intramuscular injections of ketamine and xylazine. Lab. Anim. Sci. 1987; 37: 80-83.

GILROY, B.A. Endotracheal intubation of rabbits and rodents. J. Am. Vet. Med. Assoc. 1981; 179: 1295.

GRAD, R., WITTEN, M.L., QUAN, S.F., MCKELVIE, D.H. and LEMEN, R.J. Intravenous chloralose is a safe anesthetic for longitudinal use in beagle puppies. Lab. Anim. Sci. 1988; 38: 422-425.

GRAY, P.R. and MCDONELL, W.N. Anesthesia in goats and sheep Part I. Local analgesia. Compend. Contin. Ed. Pract. Vet. 1986; 8: S33-S39.

GREEN, C.J. Laboratory animal handbook 8. Animal anesthesia. London: Laboratory Animals Ltd., 1982.

HALL, L.W. and CLARKE, K.W. Veterinary anesthesia. 9th Ed. Toronto, Ont.: Baillière Tindall, 1991.

HATCH, R.C., JERNIGAN, A.D., WILSON, R.C., LIPHAM, L.B., BOOTH, N.H., CLARK J.D. and BROWN, J. Prompt arousal from fentanyl-droperidol-pentobarbital anesthesia in dogs: a preliminary study. Can. J. Vet. Res. 1986; 50: 251-258.

HOLMES, D.D. Clinical laboratory animal medicine. Ames, IA: Iowa State University Press, 1984.

HOLZGREFE, H.H., EVERITT, J.M. and WRIGHT, E.M. Alpha-chloralose as a canine anesthetic. Lab. Anim. Sci. 1987; 37: 587-595.

HRAPKIEWICZ, K.L., STEIN, S. and SMILER, K.L. A new anesthetic agent for use in the gerbil. Lab. Anim. Sci. 1989; 39: 338-341.

INGWERSEN, W., ALLEN, D.G., DYSON, D.M., PASCOE, P.J. and O'GRADY, M.R. Cardiopulmonary effects of a ketamine hydrochloride/acepromazine combination in healthy cats. Can. J. Vet. Res. 1988; 52: 1-4.

JERNIGAN, A.D., WILSON, R.C., BOOTH, N.H., HATCH, R.C. and AKBARI, A. Comparative pharmacokinetics of yohimbine in steers, horses and dogs. Can. J. Vet. Res. 1988; 52: 172-176.

JOHNSON, J.H. Anesthesia, analgesia and euthanasia in reptiles and amphibians. In: Schaeffer, D.O., Kleinow, K.M. and Krulisch, L., eds. The care and use of amphibians, reptiles and fish in research. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1992.

JONES, D.M. The sedation and anesthesia of birds and reptiles. Vet. Rec. 1977; 101: 340-342.

KERO, P., THOMASSON, B. and SOPPI, A. Spinal anesthesia in the rabbit. Lab. Anim. 1981; 15: 347-348.

LEVY, D.E., ZWIES, A. and DUFFY, T.E. A mask for delivery of inhalation gases to small laboratory animals. Lab. Anim. Sci. 1980; 30: 868-870.

LIPMAN, N.S., MARINI, R.P. and ERDMAN, S.E. A comparison of ketamine/xylazine and ketamine/xylazine/acepromazine anesthesia in the rabbit. Lab. Anim. Sci. 1990; 40: 395-398.

LUDDERS, J.N., MITCHELL, G.S. and SCHAEFER, S.L. Minimum anesthetic dose and cardiopulmonary dose response for halothane in chickens. Am. J. Vet. Res. 1988; 49: 929-932.

LUMB, W.V. and JONES, E.W. Veterinary anesthesia. Philadelphia, PA: Lea and Febiger, 1984.

LYNCH, S. and LINE, S. Failure of yohimbine to reverse ketamine anesthesia in rhesus monkeys. Lab. Anim. Sci. 1985; 35: 417-418.

MAHMOUDI, N.N., COLE, D.J. and SHAPIRO, H.M. Insufficient anesthetic potency of nitrous oxide in the rat. Anesthesiol. 1989; 70: 345-349.

MCLAUGHLIN. S. Anesthesia-Barbiturates. Part I and II. CALAS (Can. Assoc. Lab. Anim. Sci.) Newsl. 1988; 20(5): 104-110.

MITCHELL, B. (correspondence) Health hazards in operating theatre environments. Vet. Rec. 1976; 98: 326.

MOODY, K.D., BOWMAN, T.A. and LANG, C.M. Laboratory management of the ferret for biomedical research. Lab. Anim. Sci. 1985; 35: 272-279.

MORELAND, A.F. and GLASER, C. Evaluation of ketamine, ketamine-xylazine and ketamine-diazepam anesthesia in the ferret. Lab. Anim. Sci. 1985; 35: 287-290.

MUIR, W.W. and HUBBELL, J.A.E. Handbook of veterinary anesthesia. Toronto, Ont.: C.V. Mosby Co., 1989.

MULDER, J.B. and HAUSER, J.J. A closed anesthetic system for small laboratory animals. Lab. Anim. Sci. 1984; 34: 77-78.

NATIONAL RESEARCH COUNCIL (U.S). Laboratory animal management: Wild birds. Washington, DC: National Research Council, National Academy of Science, 1977.

NORRIS, M.L. and TURNER, W.D. An evaluation of tribomoethanol (TBE) as an anesthetic agent in the Mongolian gerbil (*Meriones unguiculatus*). Lab. Anim. 1983; 17: 324-329.

OLSON, M.E. Problems associated with urethane anesthesia. CALAS (Can. Assoc. Lab. Anim. Sci.) Newsl. 1985; 17: 115-119.

OLSON, M.E. Injectable anesthetics in rodents and rabbits. CALAS (Can. Assoc. Lab. Anim. Sci.) Newsl. 1986a; 18: 120-122.

OLSON, M.E. A simple anesthetic chamber. Lab. Anim. Sci. 1986b; 36: 703.

OLSON, M.E. and MCCABE, K. Anesthesia in the Richardson's ground squirrel: Comparison of ketamine, ketamine and xylazine, droperidol and fentanyl, and sodium pentobarbital. J. Am. Vet. Med. Assoc. 1986; 189: 1035-1037.

PADDLEFORD, R.R., ed. Manual of small animal anesthesia. New York, NY: Churchill Livingstone, 1988.

PARKER, J.L. and ADAMS, H.R. The influence of chemical restraining agents on cardiovascular function. Lab. Anim. Sci. 1978; 28: 575.

PARKES, M.J. Anaesthesia in the pregnant guinea pig. Vet. Rec. 1987; 121: 512-514.

PEETERS, M.E., GIL, D., TESKE, E., EYZENBACH, W., V.D. BROM, W.E., LUMEIJ, J.T. and DE VRIES, H.W. Four methods for general anesthesia in the rabbit: A comparative study. Lab. Anim. 1988; 22: 355-360.

POOLE, T., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. Harlow, Essex: Longman Scientific and Technical, 1987.

PRESTRUDE, A.M. and CRAWFORD, F.T. Tonic immobility in the lizard, iguana. Anim. Behav. 1970; 18: 391-395.

RAPER, S.E., BARKER, M.E., BURWEN, S.J. and JONES, A. Isoflurane as an anesthetic for experimental animal surgery. Anat. Rec. 1987; 218: 116-122.

RETTIG, B. An inexpensive anesthetic gas-scavenging device that any technician can make. Vet. Techn. 1987; 8: 27-32.

RICH, S., GRIMM, C., WONG, K. and CESAR, L. Gas anesthesia setup for methoxyflurane use in small

rodents. ILAR (Institute for Laboratory Animal Resources) News 1990; 32: 17.

SAINSBURY, A.W., EATON, B.D. and COOPER, J.E. Restraint and anesthesia of primates. Vet. Rec. 1989; 125: 640-644.

SEDGWICK, C. and JAHN, S. Techniques for endotracheal intubation and inhalation anesthesia for laboratory animals. Calif. Vet. 1980; 34: 27-31.

SHORT, C.E., ed. Principles and practice of veterinary anesthesia. Baltimore, MD: Williams and Wilkins, 1987.

SKARTVEDT, S.M. and LYON, N.C. A simple apparatus for inducing and maintaining halothane anesthesia of the rabbit. Lab. Anim. Sci. 1972; 22: 922-924.

STIMPFEL, T.M. and GERSHEY, E.L. Selecting anesthetic agents for human safety and animal recovery surgery. FASEB (Federation of American Societies for Experimental Biology) J. 1991; 5: 2099-2104.

SWINDLE, M.M. Anesthesia in swine. Charles River Techn. Bull. 3(3). The Charles River Laboratories Inc., 251 Ballardvale St., Wilmington, MA 01887 1985.

VERSTEGEN, J., FARGETTON, X., DONNAY, I. and ECTORS, F. Comparison of the clinical utility of medetomidine/ketamine and xylazine/ketamine combinations for the ovariectomy of cats. Vet. Rec. 1990; 127: 424-426.

VOLK, L. Anesthesia in invertebrates. CALAS (Can. Assoc. Lab. Animal Sci.) Newsl. 1986; 18: 28-33.

WIXSON, S.K., WHITE, W.J., HUGHES, H.C., LANG, C.M. and MARSHALL, W.K. The effects of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam anesthesia in adult male rats. Lab. Anim. Sci. 1987a; 37: 726-730.

WIXSON, S.K., WHITE, W.J., HUGHES, H.C., LANG, C.M. and MARSHALL, W.K. The effects of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam on noxious stimulus perception in adult male rats. Lab. Anim. Sci. 1987b; 37: 731-735.

WYATT, J.D., SCOTT, R.A.N. and RICHARDSON, M.E. The effects of prolonged ketamine-xylazine intravenous infusion on arterial blood Ph, blood gases, mean arterial blood pressure, heart and respiratory rates, rectal temperature and reflexes in the rabbit. Lab. Anim. Sci. 1989; 39: 411-416.

XII. EUTHANASIA

A. INTRODUCTION

The term "euthanasia," is derived from the Greek terms "eu" for "good" and "thanatos" for "death" or an easy death (Bennett, Brown, Schofield *et al*. 1990). However, the term "euthanize" will be used in this Chapter, rather than the more accurate "euthanatize." Whichever term is used, the method must be "humane": that is, it must be painless, must minimize fear and anxiety, be reliable, reproducible, irreversible, simple, safe and rapid. If possible, it should also be esthetically acceptable for the person carrying out the procedure, as well as for any observer.

In the 1950s, the term "euthanasia" was rarely heard; euphemisms included "sacrifice," "destroy," "put down," or "put to sleep" (Zweighaft, 1990). However, rarely were these terms prefixed with the word "humane"; nor was it considered necessary. Humane Slaughter Acts were not promulgated and applied until the late 1950s and early 1960s in Canada or the USA. Even with the advent of legislation in this regard, many of the meat-producing species, e.g., fowl, were not included under the Regulations.

In the use of animals in research, teaching, and testing it is essential that the scientific community take on the mantle of responsibility for applying scientific judgement and new knowledge to ensure that, when the life of an animal is taken, it is assured of a "good death." Even with non-consumptive or non-invasive studies using animals, there are occasions when it is necessary to euthanize the animal (e.g., return of a wild animal to a hostile habitat) (see also *Categories of Invasiveness* found elsewhere in this *Guide*).

In the first edition of this volume of the *Guide*, the Chapter on euthanasia states: "The most important criterion of acceptance of a euthanasia method as humane is that it have an initial depressive action on the Central Nervous System (CNS) to ensure immediate insensitivity to pain." Although the principle of this criterion remains sound, to the original wording should be added "to produce rapid unconsciousness and thus assurance of insensitivity to pain; this must be followed by cardiac and respiratory arrest."

It is important that Russell and Burch's (1959) "Three R" principle noted elsewhere in this *Guide*, be equally applied to methods of euthanasia. Refinement of procedures is an often-neglected area and one which should be addressed in order to ensure that the criteria for a humane death are in place.

The application of these euthanasia guidelines requires the use of professional judgement, technical competence, coupled with an understanding of the animal, its behaviour and its physiology, as well as an understanding of the environmental and ecological impact, the sensitivities of other personnel and the concerns of the general public.

B. CRITERIA FOR A HUMANE DEATH

The person applying the method of euthanasia is a most important factor in ensuring that an animal's death is humane. Regardless of whether the procedure is applied to an individual animal or to a group, it must always attempt to meet the following criteria:

a) death without signs of panic, pain or distress;

b) minimum time to loss of consciousness, i.e., shortest lag time;

c) reliability and reproducibility;

d) safety for personnel involved;

e) minimal undesirable physiological and psychological effects on the animal;

f) compatibility with the requirement and the purpose of the scientific study;

g) minimal or no emotional effects on the observer and the operator;

h) minimal environmental or ecological impact;

i) simple, inexpensive mechanical equipment which is relatively maintenance free; and;

j) a location remote and separate from the animal rooms.

It is often difficult to recognize evidence of stress when animals are euthanized in the presence of other animals. Recent information on pheromones provides evidence that animals can communicate with one another through various types of signals. In certain experiments with rats, stress induced by experimental treatment may give rise to the production of signals that affect non-treated animals housed nearby (Duncan and Petherick, 1991; Beynen, 1992; Short and Van Poznak, 1992).

C. PAIN AND STRESS

The control of animal pain is discussed elsewhere in this *Guide* (see Control of Animal Pain in Research, Teaching and Testing) and should be reviewed by those conducting euthanasia procedures. The literature is growing regarding animal pain (Dawkins, 1980, 1990; Bateson, 1991; Flecknell, 1984; Wall, 1992; Fosse, 1991; Rowsell, 1992). It is sufficient to note that, over the past 25 years, there has been a revolution in our understanding of pain mechanisms which was generated by experimental work on animals. We cannot get inside the head of the animal; thus, an important part of assessing animal pain is empathy coupled with the ethical concerns.

Briefly, it is believed that, for pain to be experienced, the cerebral cortex and sub-cortical structures must be functional. If the cerebral cortex or sub-cortical structures are rendered non-functional by any method, such as hypoxia, pharmacological depression, electric shock or concussion, then the feeling of pain is inhibited. Unfortunately, we have no critical means of determining the adequacy of anesthesia and the assessment of its depth in animals, whereas a range of different measures have been used for determining the adequacy of anesthesia in humans (Whelan and Flecknell, 1992). The crude criteria available to judge unconsciousness in an animal include, for example, the absence of a blinking reflex, toe pinch reflex and tail reflex. Rarely is an electroencephalogram (EEG) available showing complete flattening of the EEG as an indication of brain death. This is the criterion adopted by the American Academy of Neurology as acceptable for establishing brain death in young children after other clinical criteria, such as deep coma, failure to breathe spontaneously, and the absence of reflexes occur (Anon., 1987).

In assessing death, it is important to observe that heart action has stopped, thus ensuring a cessation of blood delivered to the brain, as well as the cessation of respiration. No animal should be considered dead until reflex movement as well as cardiac and respiratory movements, have ceased.

If an animal has been given a curare-like preparation, the absence of reflexes should not be used to indicate unconsciousness and thus insensitivity to pain.

D. MECHANISMS FOR CAUSING DEATH

Death can ensue when the brain is affected by hypoxia, direct or indirect, when there is direct depression of neurons essential for living physiological functions, or there is physical disruption of brain activity to produce unconsciousness.

a) In **hypoxia**, death must be considered painless and free of stress only when unconsciousness precedes the loss of muscle activity (paralysis). **Paralyzing agents (e.g., *curariform agents* such as curare, succinylcholine, gallamine, nicotine sulphate, magnesium or potassium salts and other neuromuscular blocking agents) *must never be used alone for killing animals*** (Rowsell, 1990). Following unconsciousness due to brain hypoxia, some animals exhibit a degree of reflex motor activity.

b) Direct depression of neurons: the depression of neurons of the brain which produces unconsciousness and then death, is sometimes associated with vocalization and muscle activity. Death is produced by hypoxemia, direct depression of the respiratory centres in the CNS, or cardiac arrest.

c) Physical disruption of brain activity produces immediate unconsciousness; however, there may be marked physical muscular activity due to the depolarization of the nerve cells. While the movement is esthetically unpleasant, it is not a manifestation of pain or distress: the animal feels nothing.

E. METHODS USED FOR EUTHANASIA

1. Physical

Physical methods of euthanasia include stunning, cervical dislocation, electrocution, pithing, decapitation, shooting, maceration, microwave radiation, and exsanguination.

In the laboratory, physical methods are normally restricted to those animals which are easily handled, such as small rodents, poultry, large domestic animals, and some amphibians and reptiles. If the research protocol requires a physical method of euthanasia because other methods could invalidate the scientific study, the use of such methods must be justified by the scientist and approved by the Animal Care Committee (ACC). Prior sedation or tranquillization should take place whenever possible.

Decisions to use physical methods for euthanasia must be based on professional judgement and be undertaken only by experienced individuals. Acquiring (or re-acquiring) the skills to use physical methods of euthanasia may be accomplished by practising the techniques on dead animals, preferably those recently killed, and be subject to close scrutiny by those experienced in the methodology.

a) Stunning is sometimes used with small laboratory rodents. The blow must be delivered to the central skull bones with sufficient force to produce massive cerebral hemorrhage and thus immediate depression of the CNS, producing rapid unconsciousness. This technique should not be undertaken in the presence of casual observers or the uninformed, for it is esthetically unpleasant. However, when properly applied, the animal is immediately rendered unconscious and thus insensitive to pain. Subsequent to stunning, the animal's major blood vessels should be cut, and the chest and the heart opened.

b) Cervical dislocation is suitable for poultry, mice, immature rats or rabbits, or similar small species. The technique consists of separation of the skull and the brain from the spinal cord by pressure applied posterior to the base of the skull (Clifford, 1984). When the separation of the cord occurs, CNS stimulation of respiration and heart beat is interrupted, leading to death. The supply of blood to the brain continues to nourish it because the carotid arteries and jugular veins are intact; however, the blood will rapidly be depleted of oxygen and there will be an increase in carbon dioxide after respiration ceases,

leading to brain disfunction.

Studies have demonstrated that the EEG flattens and the blinking reflex disappears immediately after the spinal cord separates, thus indicating that the animal is not sensitive to pain (Allred and Berntson, 1986; Rowsell, 1990; Derr, 1991). In addition, the severed spinal cord does not deliver a painful stimuli from areas posterior to the separation; thus, with the separation of the spinal cord from the brain, painful stimuli cannot be perceived. However, significant muscular movements may take place.

The *Report of the AVMA Panel on Euthanasia* (AVMA, 1993) differs from its 1986 predecessor in that it notes that data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation (Vanderwolf, Buzsaki, Cain *et al*. 1988). In addition to mice, it lists as suitable subjects immature rats weighing less than 200 g, rabbits weighing less than one kg, poultry and other small birds. It also notes that: "In heavier rats and rabbits, the greater muscle mass in the cervical region makes manual cervical dislocation physically more difficult; accordingly, it should be performed only with mechanical dislocators or by individuals who have demonstrated proficiency in euthanatizing heavier animals." The need for proper training is stressed.

c) Decapitation with guillotine is used primarily to euthanize rodents and small rabbits. Used alone, it provides a means of ensuring that tissues and body fluids are chemically uncontaminated as well as providing a means of obtaining an anatomically intact brain and brain tissues for further study.

After consulting the literature (Vanderwolf, Buzsaki, Cain *et al*. 1988; Derr, 1991; Mikeska and Klemm, 1975), the Canadian Council on Animal Care (CCAC) concurs with the *Report of the AVMA Panel on Euthanasia* (AVMA, 1993) that pre-sedation before decapitation or cervical dislocation is not necessary. However, **the use of cervical dislocation and decapitation with guillotine as euthanasia methods must be scientifically defended by the investigator and approved by the ACC.** Well-designed and easily operated guillotines are available from commercial sources. Guillotines should not be used by personnel who have not been properly trained in the methodology and how to properly handle the animal. Decapitation as a means of euthanizing amphibia and reptiles is not recommended (AVMA, 1986; Cooper, Ewbank, Platt *et al.* 1989).

d) Pithing, which is used to euthanize frogs and turtles by destroying the brain after the frog has been anesthetized, requires considerable skill. A sharp, pointed probe is inserted through the skin between the skull and the atlas. It is then pushed forward through the foramen magna into the cranial cavity, using a twisting motion. The technique should be attempted only after acquiring knowledge of anatomy using skeletons, and after a period of training including practice on dead animals. This method can cause pain and suffering if the proper regions of the brain are not completely destroyed.

e) Shooting by penetrating captive bolt pistol has been used primarily for pre-slaughter stunning of food animals. To a lesser extent, it is used in emergency situations such as roadside injuries or other similar events where no other method, or the expertise necessary to apply it, is available.

Captive bolt pistols are also used in emergency situations in slaughter of horses. However, because of their behavioural/physical response of rearing on their hind legs and falling backwards, unless proper restraints are applied, there is a danger to the operator. It is essential that the captive bolt pistol be withdrawn from the animal's head instantaneously. An extension handle is attached to the pistol to allow the operator to put one or both hands in front of the horse's eyes, thus reducing head tossing. This specially designed captive bolt pistol for killing horses requires knowledge of the placement needed in order to penetrate the deep structures of the brain (Watts, 1976).

Britains' Royal SPCA, following the use of the captive bolt pistol in the abattoir, developed a smaller, hand-held model for emergency euthanasia of injured dogs and cats which was used by some SPCAs and humane societies (UFAW, 1968). In Canada, however, it was considered esthetically repugnant by the general public and therefore its use was limited to those occasions when the injured animal could be removed from public view (UFAW, 1988).

More recently, a captive bolt pistol was developed for killing rabbits and goats (Accles and Shelvoke Ltd., Aston, Birmingham, Eng. B64QD). With this device, the clinical evidence, including the loss of corneal reflex and organized EEG activity, suggested that loss of consciousness and cerebral death occurred almost immediately (Dennis, Dong, Weisbrod *et al*. 1988).

Only commercially produced penetrating or captive bolt pistols should be used. Proper utilization is essential. Considerable technical competence and precise knowledge of the animal's anatomy are necessary; therefore, this method should be used only by an experienced operator. Exsanguination must follow unconsciousness.

f) Percussion stunning is carried out by means of a non-penetrating form of captive bolt stunning device with mushroom-shaped tip. The effects on brain function will depend on the speed of the bolt on impact, its proper positioning, and the thickness of the cranial bone. Unfortunately, the minimum speeds necessary for effective percussions have not yet been determined. The value of this method is that it can be used to replace some ritual slaughtering methods. Percussion stunning has been used as an alternative method to the penetrating captive bolt by some Canadian abattoirs. Unfortunately, the instruments are more subject to breakdown and malfunctioning than the standard penetrating captive bolt. More recently, a percussion-type instrument has been developed for use in animal control, particularly for dogs. Its feasibility and its humaneness as a technique need to be established in the field operations.

Captive bolt pistols, including percussion stunning devices, require permits for their possession in Canada.

g) Shooting is an effective means of humanely destroying animals in the field. ***Only experts should carry out this procedure*.** The subject must be shot at close range and the bullet must strike the brain so as to render the animal immediately insensitive to pain; 12-bore or 20-bore shotguns, or 22-calibre rifles or revolvers may be employed, depending upon the species and size of the animal to be killed. Whether or not prior sedation should be used is debatable (Ebedes, 1988). The use of shooting by firearms in a laboratory setting is prohibited. In such settings, there are always other available methods of euthanasia and individuals with appropriate expertise.

There are advantages of shooting as a euthanasia method in the field, for unconsciousness is instantaneous if the bullet destroys a significant portion of the brain involving particularly the vital centres. In the field, this may be the only possible method to render the animal immediately unconscious and to produce death. The need for expertise of the marksman cannot be overemphasized. For use in emergency situations, *Guidelines for Euthanasia of Domestic Animals by Firearms* have been prepared by the Animal Welfare Committee of the Canadian Veterinary Medical Association (CVMA) (Longair, Finley, Laniel *et al*. 1991).

h) Electrocution, used mainly to kill domestic animals (Eikelenboom, 1983) is rarely used in the laboratory setting. If electric shock is used, it must be delivered in two phases: the first electric shock passes through the brain, stunning the animal; the second, delivered a fraction of a second later, produces a fibrillation of the heart, killing the animal. Violent extension of the extremities, part of the seizure induced by electrocution, is esthetically unpleasant.

i) Microwave radiation is a relatively new technique employed mainly by neuroscientists who wish to maintain the anatomic, enzymatic and physiological chemical composition of the animal's brain in an unaltered state (Stavinoha, 1983; Ikarashi, Maruyama and Stavinoha, 1984). Microwave radiation must be delivered specifically to the brain; therefore, *standard household microwave ovens must not be used* (Stavinoha, Frazer and Modak, 1977); **only instruments which have been designed specifically for this purpose and have the appropriate power and microwave distribution may be utilized.**

j) High altitude decompression is considered unacceptable by the CCAC. At one time, it was used by

some animal control agencies and humane societies for killing unwanted dogs and cats, but is now not recommended by them (White, 1984). When animals have upper respiratory problems or gastrointestinal upsets, the gases in these areas, as well as in the sinuses, expand and cannot be vented, thus producing significant pain and distress (White, 1984).

k) Exsanguination (depriving an animal of blood) *is only acceptable as a euthanasia procedure if the animal is first rendered unconscious* by physical means such as stunning, or pharmacological means, e.g., injection of an anesthetic (Gregory and Wotton, 1984). The use of this method must be scientifically justified and approved by an ACC, who must have established the technical competence of the person who will conduct the technique.

l) Maceration requires the application of severe restrictions on the size and age of the animals that may be subjected to this process (Ewbank, 1987). For example, it is used to kill newborn mice, and in some poultry operations for killing surplus one-day-old male chicks, where it has been deemed acceptable by Agriculture Canada. Equipment has been specifically designed for this purpose and, although esthetically unpleasant to consider as well as to observe, the method produces instantaneous unconsciousness and death when used properly.

m) High pressure water jet has recently been proposed for stunning slaughter pigs (Schatzmann, Leuenberger, Fuchs *et al*. 1991). However, its use has not been reviewed by other experts qualified to evaluate humane slaughter techniques.

2. Non-inhalant Pharmacologic Agents

The majority of injectable drugs used as anesthetic agents are acceptable for euthanasia if an adequate overdose is given. The preferred route is intravenous (IV), and should be accompanied by adequate restraint, making the animal as comfortable as possible with minimum distress or anxiety. Pre-sedation or tranquillization may be necessary for wild, feral or fearful animals not accustomed to restraint.

If the animal is too small to receive intravenous injections, or if anatomically suitable veins are not visible or apparent, e.g., in small rodents and guinea pigs, the intraperitoneal (IP) injection of a non-irritating overdose of a pharmacological agent is acceptable.

With most of the available injectable anesthetic agents, the amount to be injected is too large to use the intramuscular, subcutaneous, intrathoracic, intrapulmonary, and intrathecal sites. Administration by a route other than intravenous in most cases results in a delayed onset of the anesthetic effect of the drug. Under these circumstances, it is essential that the animal be placed in a cage or enclosure to ensure prevention of injuries through stumbling and or falling, and in order to make the animal more comfortable and facilitate onset of the overdose of anesthetic.

a) Barbituric acid derivatives (barbiturates) used as anesthetics are effective in producing euthanasia when given as an overdose. The pharmacological action of these drugs is to depress the CNS, starting with the cerebral cortex, and progressing through the stages of anesthesia to produce unconsciousness. With an overdose, deep anesthesia develops, followed by apnea as the respiratory centre is depressed, followed by cardiac arrest and death. Some of the combination barbiturate derivatives have a cardiotoxic effect; however, this is of no consequence because the animal dies before such an effect histologically manifests itself.

Barbituric acids are controlled substances under regulation by the Bureau of Dangerous Drugs, Health and Welfare Canada. As controlled substances, they must be stored in locked cabinets and a written log maintained of date and amount used and the purpose of use. Euthanasia agents containing barbituric acid derivatives are often coloured to make them clearly identifiable. The amount used for euthanasia should be in accordance with the manufacturer's direction. The abuse or misuse, either accidental or deliberate, of such substances creates a significant risk and associated legal liability. Those possessing such

combinations for euthanasia must provide adequate security.

b) T-61 is manufactured by Hoechst-Roussel Canada Ltd. (4045 Cote Vertu, Montreal, Quebec, H4R 2E8). It contains a local anesthetic (tetracaine Hcl), a strong hypnotic agent which depresses the CNS causing unconsciousness (brain death), as well as a curariform drug which has a paralytic effect on the respiratory centre and a relaxing effect on skeletal muscles (Rowsell, 1979). A recent study demonstrated that induction of muscle paralysis and unconsciousness occur simultaneously (Hellebrekers, Baumans, Bertens *et al.* 1990). These authors concluded that the muscular activity and vocal response seen in some dogs was not a conscious response.

T-61 should be administered intravenously at the dose and administration rate directed by the manufacturer. If instructions are not followed, it is possible for T-61 to produce an excitatory phase and vocalization. It is not registered or restricted by the Bureau of Dangerous Drugs and may be used by non-medical, technical personnel. However, it must be ordered by a veterinarian and be shipped directly to the veterinary clinic involved (Clarke, 1990). Its availability in other countries has been affected because of criticism levelled primarily as a result of failure to follow the manufacturer's direction for the dosage and the rate of injection. Although this is not a restricted drug, the same guidelines concerning its safekeeping apply as to any of the barbituric acid derivatives and anesthetics, for its use has been abused (Smith and Lewis, 1989).

c) Chloral hydrate is a dissociative anesthetic and produces no loss of the corneal or blinking reflex. Difficulties include slow onset of action, restraint difficulties, and the amount that must be administered. Death is due to hypoxemia caused by progressive depression of the respiratory centre. It may be preceded by gasping, muscle spasms, and vocalization, causing difficulties in comfortably restraining the animal being euthanized. Therefore, **while it should not be used for dogs and cats,** it is acceptable for intravenous use in large animals and is an effective euthanasia agent for poultry.

A combination of chloral hydrate, magnesium sulphate, and sodium pentobarbital, administered intravenously to large domestic animals as an overdose, is an acceptable method of euthanasia.

d) Ketamine hydrochloride, also a dissociative anesthetic, cannot be recommended for euthanasia because it is difficult to assess what constitutes an overdose.

e) Magnesium sulphate alone is a neuromuscular blocking agent (Hevner and de Johng, 1973); however, it does not depress the CNS. Administration of magnesium sulphate should only be used in combination with barbituric acid derivatives and administered only by the IV route. The IP route is not acceptable because of the irritating nature of a saturated solution.

3. Inhalant Anesthetics

An overdose of inhalant anesthetics such as ether, halothane, methoxyflurane, isoflurane, and enflurane fulfils the principle of a humane death. Their use, however, poses a risk to human subjects who may be exposed to their vapours; thus, they are considered an occupational hazard (see Anesthesia, as well as Occupational Health and Safety). Chambers are available commercially to expose animals to such anesthetic gases in order to either produce anesthesia or, by overexposure, to produce euthanasia. Scavenging systems to remove excess gases are readily adapted to such enclosures. Additionally, anesthetic masks can be prepared to fit even small rodents. The vapours are inhaled until respiration ceases. The animal is then checked to ensure that it is dead.

The soaking of gauze with inhalant anesthetics and then placing it in a container with the animal(s) to be euthanized may be used only if there are no other methods of delivery of the anesthetic gases. The fact that inhalant anesthetics are liquid makes it essential that animals be exposed to vapours only, as the liquid form is a topical irritant. The delivery system should provide sufficient oxygen with the anesthetic vapour to ensure unconsciousness precedes hypoxia.

a) In the past, **chloroform and ether** have been commonly used as anesthetics or, when exposure to their vapours is of sufficient concentration and duration, to produce euthanasia. However, **chloroform is no longer recommended because of its carcinogenic, hepatotoxic and nephrotoxic potential.** *Ether is a flammable and explosive agent*, and should never be used in the presence of flame or where electrical equipment is not protected and shock resistant.

b) Nitrous oxide is of value as an agent for euthanasia only in combination with other volatile inhalant anesthetics. It is a combustible agent, but is non-flammable and non-explosive. With the exception of ether, most inhalant anesthetic agents are expensive and require special anesthetic delivery mechanisms; thus, their use as euthanasia agents is limited to species where venipuncture is found to be too difficult or impossible. Additionally, use of inhalant anesthetics for euthanasia of large animals is expensive because of the amounts that must be used.

4. Non-anesthetic Gases

Non-anesthetic gases include **carbon monoxide, carbon dioxide, nitrogen, argon** and **cyanide.**

a) Carbon monoxide, even in low concentrations, can harm other animals and humans exposed to its fumes. A colourless, odourless gas, it is difficult to detect. Carbon monoxide obtained from the exhaust of a gasoline combustion engine contains impurities and thus can produce irritation and discomfort. Therefore, delivery of an irritant-free carbon monoxide is mandatory if this is the chosen method. Carbon monoxide is rarely used now to destroy unwanted dogs and cats by animal control agencies and humane societies; however, it continues to be used for some of the fur-bearing species. *In the laboratory, because of the safety problems associated with its delivery, carbon monoxide is not recommended as a euthanasia agent* (Chalifoux and Dallaire, 1983).

b) Nitrogen and argon are inert gases which are both colourless and odourless, non-flammable and non-explosive. They are considered as having a minimal impact on the environment or the atmosphere. Both are used in a closed chamber through a process called "flushing" in which the passage of these gases reduces oxygen levels to a maximum of 1.5%. At such low levels of oxygen, the animal will collapse, and death will be produced by hypoxemia. However, dogs, cats, and rabbits may vocalize at the 1.5% level of oxygen, as well as show increased muscular activity and struggling. Nitrogen and argon do not produce a narcosis prior to the onset of hypoxia which in itself will lead to unconsciousness followed by death resulting from paralysis of the respiratory centre in the anoxic brain. Carbon dioxide, on the other hand, can induce a narcosis because of its physiological effect on the CNS (Herin, Hall and Fitch, 1978).

Quine, Buckingham and Strunin (1988) found that use of acepromazine as a tranquillizer before placing dogs in a nitrogen euthanasia flushing chamber produced longer survival times. However, it was not recorded whether or not these treated dogs showed the same amount of hyperventilation prior to or following the loss of consciousness; yelping, gasping, convulsions and muscular tremors usually accompany this process. Chalifoux and Dallaire (1983), however, concluded that premedication with tranquillizers improved the humaneness of carbon monoxide euthanasia.

c) Argon gas is more dense than air and thus tends to remain in the lower layers; however, both nitrogen and argon have no analgesic or anesthetic properties.

d) Carbon dioxide (CO_2) is frequently used to kill rodents and birds in the laboratory. Although it is a component of room air, pure CO_2 is heavier than air and practically odourless. It concentrates in the lower portion of the euthanasia chamber, and rats and other burrowing animals will tend to keep their noses in the lower zone containing adequate concentrations of the gas.

Its use as a euthanasia agent is dependent (for its humaneness) on whether or not it is in sufficient

concentration to produce a narcosis. Maintenance at the correct level requires some manipulation to physiologically achieve, but will produce a narcosis and if oxygen levels are not increased, will lead to death. Carbon dioxide also stimulates the respiratory centre in the brain and in low concentrations of up to 10% of inspired gas is considered a potent respiratory stimulant causing a tenfold increase in the ventilation rate and a feeling of profound respiratory distress. The stimulation of the respiratory centre produces hyperventilation, thus critically affecting the onset of CO_2 narcosis. At approximately 40%, the CO_2 induces anesthesia which is slow in onset and accompanied by involuntary excitement. Eventually, there is apnea, a fall in blood pressure, and death (Ontario Ministry of Agriculture and Food Memo to Pound Operators and Veterinarians in Ontario, August 12, 1987). Britt (1986) found that the slow induction of narcosis is preferable, as using a precharged chamber, or passing CO_2 into the chamber too rapidly, can produce obvious signs of distress in the animals. However, he concluded that "neither (slow nor swift) method was found to be stress-free, so no recommendation can be a counsel of excellence."

McArthur (1976) reported on a method for delivering a carbon dioxide euthanasia of small animals, including cats, puppies, mice, rats, gerbils, guinea pigs and hamsters. In this study, unconsciousness was produced without distress to the animals, and occurred within a minute when levels of oxygen were maintained at 31 to 33%, with CO_2 ranging from 56 to 63%. Unconsciousness was used to describe animals that had collapsed and showed complete relaxation. Deep surgical anesthesia was produced by leaving the animals in this environment for a three-minute exposure period. Once deep surgical anesthesia was reached, the oxygen supply was turned off and CO_2 filled the chamber.

Carbon dioxide may be purchased in cylinders or in the solid state, as dry ice. It is relatively inexpensive, non-inflammable, non-explosive and essentially non-hazardous. It presents no danger to operators or attendants when used with properly designed equipment in an adequately ventilated space.

The essential criteria for producing a CO_2 narcosis is to maintain the oxygen level close to, or slightly below normal air levels, and increase the percentage of carbon dioxide in the air. It is important to understand that newborn animals, having existed in an environment with low oxygen levels prior to parturition, require higher levels of carbon dioxide in order to produce a humane death. Therefore, newborn animals should not be removed from chambers charged with carbon dioxide for approximately one-half hour after all movement has ceased.

Carbon dioxide does not accumulate in tissues; thus, there is no residue in food-producing animals. Neither does it appear to cause any distortion of the cells, which appear normal under microscopic examination.

Dogs, cats and other larger animals with an investigative behaviour will often extend their heads above the zone of effective CO_2 concentrations and thus be exposed to levels that excite rather than depress the CNS; thus, some will hyperventilate, struggle, stagger, and fall. The important element is to maintain an even distribution of CO_2 in the euthanasia chamber; this requires additional equipment.

Carbon dioxide has proven to be non-effective in killing diving mammals that have adapted to a relatively anerobic (oxygen-free) environment; 100% CO_2 is required to kill mink. Carbon dioxide is also used for pre-slaughter stunning in swine (Gregory, Moss and Leeson, 1987).

e) Potassium cyanide is a very potent paralyzing agent of the respiratory centre. It is one of the most rapidly acting poisons. Death appears to be almost instantaneous and irreversible through the production of rapid anoxia with CNS depression. However, *death by exposure to cyanide gas is not considered humane because convulsions or seizures occur prior to death*. **As well, because of the extreme danger associated with its use, cyanide is not recommended as a method in the laboratory.**

F. SPECIFIC SPECIES

In addition to the euthanasia methods for common laboratory species noted above, the following apply to:

1. Amphibians, Fishes and Reptiles

The most commonly used method for euthanizing amphibians, fish, and reptiles is by stunning, using the methodology previously outlined for vertebrates and terrestrial mammals. This can be followed by decapitation or crushing of the skull.

Sodium pentobarbital and barbituric acid derivatives may be used intravenously or introduced directly into the abdominal or pleuroperitoneal cavity of most cold blooded animals, if their anatomy permits. Tricaine methanesulfonate (MS-222) may be administered by a variety of routes to induce euthanasia. For aquatic mammals, as well as amphibians and fish, this material may be placed in the water. Alternatively, in the case of large fish, a gill cover is placed over the gills and concentrated MS-222 is flushed over the gills. Benzocaine hydrochloride is a compound similar to MS-222 and may be used as a bath or in a recirculation system for the euthanasia of fish.

It is worth noting that many experts in the study of amphibians and reptiles approve of the use of cold for the anesthesia of these animals. Subsequent freezing or decapitation is not considered to be painful; unfortunately, there is no evidence that cooling to 4ºC will lessen the pain threshold (Cooper, Ewbank, Platt *et al*. 1989). Cooper (1986) states that hypothermia would have to be instantaneous if it were to be painless.

Inhalant anesthetic agents, such as halothane, methoxyflorane, etc., may be used to kill reptiles and amphibia either in a chamber or by a suitably adapted face mask. Special attention must be paid to ensure they are dead following application of the method.

Carbon dioxide is not suitable for all species and concentrations must be maintained at a high level. Many semi-aquatic and terrestrial mammals, as well as reptiles and amphibia, are accustomed to living without oxygen and have a tolerance for hypoxia; thus, they have exaggerated anaerobic capabilities (Hochachka, 1980) (see also Chapter 22, Wild Vertebrates in the Field and in Laboratory in Vol. 2 of this *Guide* [CCAC, 1984]).

Decapitation as a means of killing amphibians and reptiles is unacceptable because of the differences in their physiology (Cooper, Ewbank, Platt *et al*. 1989; AVMA, 1986).

2. Domestic Animals Killed for Food

Although this chapter is concerned primarily with experimental animals, there are times when farm animals in production studies or, in some cases, in biomedical studies, will ultimately be killed for their food value. Although we do not apply the term "euthanasia" to the killing of such animals, the principles enunciated in the foregoing and in other documents apply: it is essential that humane treatment of animals be provided. In North America, the term "humane slaughter" is commonly used; however, in the United Kingdom, the term used is "pre-slaughter stunning" (Cockram and Corley, 1991; Ewbank, Parker and Mason, 1992; Knowles and Warriss, 1992). Pre-slaughter handling, the amount of time the animal is brought to the area to be killed and when it is stunned, and the proper design of restraint equipment affect the level of stress during handling, stunning and slaughter (Grandin, 1992a; 1992b). A comparison has been made between dislocation and percussion in chickens (Gregory and Wotton, 1990).

All killing of food animals must comply with federal legislation (Agriculture Canada's Meat Inspection Act S.C., 1985, C17 [May 1985 Part 2, Item 39, for 851078S14]; the federal Health of Animals Act [C-66

June, 1990, rev. March 1992]; 38-39 Elizabeth II Chapter 21]). As well, slaughter is discussed in Agriculture Canada Codes of Practice for the various livestock species (Agriculture Canada, 1771/E, 1984; 1821/E, 1988b; 1757/E, 1989b; 1833/E, 1990; 1870/E, 1992) as well as mink and foxes (Agriculture Canada, 1819/E, 1988a and 1831/E, 1989a) and provincial legislation (see Legislation). Provincial legislation on humane slaughter, where it exists, as well as municipal and local bylaws must also be followed.

3. Fur-bearing Animals

Mink, foxes, chinchillas, nutria and opossums are often maintained in research facilities. The methods listed above can be applied to the killing of these species. Ranched mink are commonly killed by the use of carbon monoxide, carbon dioxide and nitrogen (Hansen, Creutzberg and Simmonson, 1991) or electrical stunning followed by cervical dislocation.

G. TISSUE EFFECTS OF EUTHANASIA METHODS

Tissue effects of euthanasia methods may be either direct or indirect. They may affect only components in the intravascular compartment or they may affect fixed tissues, thus influencing histological or electronmicroscopic findings. In the majority of cases, death is so rapid that even electronmicroscopic changes are non-existent or minuscule. In most instances, the concern of the investigator that demonstrable histocytotoxic changes may occur is unfounded.

1. Direct Effects

In general, the direct effects of the euthanizing method are subtle or lacking, particularly with the non-inhalant pharmacological agents. The changes produced by methods which cause anoxia depend upon the rapidity of the induction of the anoxic state, and occur as the result of changes in blood gases. For example, pulmonary congestion and edema may be observed on gross observation in the anoxic state; however, the degree is dependent upon rapidity of death.

Lamellar bodies in Purkinje cells of the cerebellum have been observed in some hypoxic anesthetized dogs subjected to rapid decompression (Bowman, Cooke and Carry, 1969). The knowledge of the sequential series of morphological and biochemical events leading to hypoxic injury to neurons and glial cells has not been adequately studied (Kim, 1975). There is nothing to suggest that hypoxic changes produced by carbon dioxide make tissues unsuitable for routine examination of the respiratory tract (Fawell, Thompson and Cooke, 1972).

Barbiturates ionize on injection into the intravascular compartment. The degree of ionization will depend upon the dissociation constant of the drug and the Ph of the blood. Cell penetration can occur only with the undissociated drug. After cell penetration takes place, dissociation again occurs and the binding of the drug to intracellular organelles will take place. **Tissue changes due to cellular penetration and intracellular binding of barbiturates have not been described.** At the same time as the barbiturates bind with plasma proteins forming an equilibrium of bound and unbound drug in circulating blood, splenic dilation with sequestration of red blood cells produce an enlarged and grossly blue-black spleen (Lumb, 1974).

2. Indirect Effects

The major indirect effects are due to tissue hypoxia brought on by the death of the animal. Thus, it is important that tissues for histological and electronmicroscopy examination be prepared as rapidly as possible following unconsciousness and death of the animal.

The tissue requirements for oxygen vary widely. The damages to the neurons of the CNS occur most rapidly, but are dependent upon the degree of tissue hypoxia and time elapsed after death before tissue preparation. Hypoxia changes to neurons require electronmicroscopic examination.

In those tissues whose requirements for oxygen are not as great as the neurons (e.g., osteocytes, chondrocytes of bone and cartilage, or other less oxygen-sensitive tissues) changes may be difficult to detect even with electronmicroscopy.

Proper handling of the animal prior to death, with immediate processing of tissues after death, are important to obtain optimal electronmicrographs with minimal changes.

H. EFFECT ON OBSERVERS

We must recognize that a significant number of people experience emotional uneasiness, physical and psychological discomfort or distress when animals are killed, even if a humane method is applied. For example, the 1987 Malouf Commission *Report on Seals and Sealing* stated that, although the method used for killing seals (striking the animal's head with a hakapik) was proven to be humane by numerous veterinary pathologists, the general public considered the act repugnant. For over a decade, the whitecoat seal on the ice on the Gulf of St. Lawrence or at the Front of St. Anthony, Newfoundland, was the only animal that the public had seen being killed in media presentations.

Inexperienced observers might misinterpret any movement, vocalization, or reflex reactions as indicators of pain and distress. Therefore, it is preferable that euthanasia methods, in addition to providing a humane death, minimize or eliminate such involuntary movements.

Over the past several years, recognition has been given to the effect of euthanasia on those required to carry it out (e.g., humane society workers, laboratory animal veterinarians, animal caretakers and others whose jobs involve euthanasia) (Rollin, 1986; Rollin and Kesel, 1990; Grier and Colvin, 1990). Arluke (1992) stated that, understandably, uneasiness was particularly noticeable among newcomers; with seasoned workers, it was most common among animal caretakers; it occurred among technicians, but was relatively rare among veterinarians and scientists. Owens, Davis and Smith (1981) have noted that those who must euthanize animals have developed ways and means of dealing with their emotions by avoiding unnecessary contact with the animals or by believing that, by being killed, the animal is being spared additional suffering.

Workers can (and will) accept that, when an animal is showing pain and distress that cannot be relieved, it must be killed. In this, their attitudes differ little from those of an owner of a companion animal who must face similar decisions. Like the pet owner, the employee can feel grief; therefore, it is important that strong communications be established with all staff members, with a willingness to listen and to provide support for those who may be feeling distressed or disturbed. It is important that such "feelings" not be suppressed in the research setting. Employees should be aware that, regardless of the purpose of the experimental study, at an established time and date, the animal must be humanely destroyed. Seminars or workshops to help staff cope with animal death may be useful.

I. EUTHANASIA STATEMENTS--OTHER AGENCIES

As noted, the *Report of the AVMA Panel on Euthanasia* has recently been published (AVMA, 1993). Euthanasia has been discussed by the U.S. National Research Council (NRC, 1991). Reference to euthanasia may also be found in the U.K.'s Universities Federation for Animal Welfare (UFAW) *Report on Euthanasia of Unwanted, Injured or Diseased Animals for Education or Scientific Purposes* (UFAW, 1986), its *Humane Slaughter of Animals for Food* (UFAW, 1987) and Britain's Animals (Scientific Procedures) Act, 1986 (Balls, 1986; McKie, 1986; Fisher, 1990). The Australian Council for the Care of Animals in

Research and Teaching has prepared a bibliography which includes a number of references to euthanasia (ACCART, 1991). Attention is also drawn to *Field Research Guidelines* (Orlans, 1988) which lists a number of guidelines (ASM, 1987; AOU, 1988; ASIH, HL, SSAR, 1987; ASIH, AFS, AIFR, 1987; Zwart, deVries and Cooper, 1989).

J. REFERENCES

AGRICULTURE CANADA. Publication 1771/E. Recommended code of practice for the care and handling of pigs. Communications Branch, Agriculture Canada, Ottawa, Ont., K1A 0C7. 1984: 28-29.

IBID. Publication 1898/E. Recommended code of practice for the care and handling of farm animals--pigs. In Press.

IBID. Publication 1819/E. Recommended code of practice for the care and handling of mink. 1988a: 16-18.

IBID. Publication 1821/E. Recommended code of practice for the care and handling of special fed veal calves. 1988b: 33-35.

IBID. Publication 1831/E. Recommended code of practice for the care and handling of ranched fox. 1989a: 17-18.

IBID. Publication 1957/E. Recommended code of practice for the care and handling of poultry from hatchery to processing plant. 1989b: 15.

IBID. Publication 1853/E. Recommended code of practice for the care and handling of dairy cattle. 1990: 36-37.

IBID. Publication 1870/E. Recommended code of practice for the care and handling of farm animals--beef cattle. 1992: 35.

ALLRED, J.B. and BERNTSON, G.G. Is euthanasia of rats by decapitation inhumane? J. Nutr. 1986; 116: 1859-1861.

AMERICAN ORNITHOLOGISTS' UNION. Report of Committee on use of wild birds in research. AUK 1988; 105 (1 suppl.): 1A-41A.

AMERICAN SOCIETY OF ICHTHYOLOGISTS AND HERPETOLOGISTS, THE HERPETOLOGISTS' LEAGUE, and THE SOCIETY FOR THE STUDY OF AMPHIBIANS AND REPTILES. Guidelines for the use of live amphibians and reptiles in field research. J. Herpetol. 1987; 4 (suppl.): 1-14.

AMERICAN SOCIETY OF ICHTHYOLOGISTS AND HERPETOLOGISTS, AMERICAN FISHERIES SOCIETY, AMERICAN INSTITUTE OF FISHERIES RESEARCH BIOLOGISTS. Guidelines for the use of fishes in field research. Copeia (suppl.) 1987: 1-12.

AMERICAN SOCIETY OF MAMMALOGISTS. Acceptable field methods in mammalogy; preliminary guidelines approved by the American Society of Mammalogy. J. Mammal. 1987; 68(4) (suppl.): 1-18.

AMERICAN VETERINARY MEDICAL ASSOCIATION. 1986 Report of the AVMA Panel on Euthanasia. J. Am. Vet. Med. Assoc. 1986; 188(3): 252-268.

AMERICAN VETERINARY MEDICAL ASSOCIATION. 1993 Report of the AVMA Panel on Euthanasia. J. Am. Vet. Med. Assoc. 1993; 202(2): 229-249.

ANON. EEG used to establish brain death in children. Medical Post 1987 September 15: 38 (as cited in Neurology, June, 1987).

ARLUKE, A. Trapped in a guilt cage. How do scientist and technicians avoid getting close to the animals they work with? Research in the U.S. reveals strategies that help them keep their distance. New Scientist 1992 April 4: 33-35.

AUSTRALIAN COUNCIL FOR THE CARE OF ANIMALS IN RESEARCH AND TEACHING. The care and use of animals for scientific purposes. A selected bibliography. Canberra, Aust.: ACCART, 1991: 34-36. (c/o Australian Vice-Chancellor's Committee, GPO Box 1142, Canberra, Australia ACT 2601.)

BALLS, M. Animals (Scientific Procedures) Act 1986: The Animal Procedures Committee. ATLA (Alternatives To Live Animals) 1986; 14: 6-13.

BATESON, P. Assessment of pain in animals. Anim. Behav. 1991; 42: 827-839.

BENNETT, B.T., BROWN, M.J. and SCHOFIELD, J.C. Euthanasia. In: Bennett, B.T., Brown, M.J. and Schofield, J.C., eds. Essentials for animal research: A primer for research personnel. Beltsville, MD: National Agriculture Library, 1990: 89-99.

BEYNEN, A.C. Communication between rats of experiment-induced stress and its impact on experimental results. Animal Welfare 1992; 1: 153-159.

BOWMAN, R.W., COOKE, J.P. and CARRY, H.W. Electronmicroscopy of canine cerebellar Purkinje cells after rapid decompression to a near-vacuum. Aerospace Med. 1969; 40: 869.

BRITT, D.P. The humaneness of carbon dioxide as an agent of euthanasia of laboratory rodents. In: Euthanasia of unwanted, injured, or diseased animals or for educational or scientific purposes. Herts, U. K.: Universities Federation for Animal Welfare, 1986: 19-31. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

CANADIAN COUNCIL ON ANIMAL CARE. Wild vertebrates in the field and in the laboratory. In: Guide to the care and use of experimental animals. Vol. 2. Ottawa, Ont.: CCAC, 1984: 191-208.

CANADIAN VETERINARY MEDICAL ASSOCIATION. Position statement on euthanasia. Directory of Canadian veterinarians. Ottawa, Ont.: CVMA, 1988: B7.

CHALIFOUX, A. and DALLAIRE, A. Physiologic and behavioural evaluation of CO_2 euthanasia of adult dogs. Amer. J. Vet. Res. 1983; 44: 2412-2417.

CLARKE, R. (letters) Purchase of T-61 euthanasia fluid by humane societies. College of Veterinarians of Ontario Update 1990; August/September: 6.

CLIFFORD, D.H. Preanesthesia, anesthesia, analgesia and euthanasia. In: Fox, J.G., Cohen, B.J. and Loew, F.M., eds. Laboratory animal medicine. New York, NY: Academic Press, 1984: 528-563.

COCKRAM, M.S. and CORLEY, K.T.T. Effect of pre-slaughter handling on behaviour and blood composition of beef cattle. Brit. Vet. J. 1991; 147: 444-454.

COOPER, J.E. Euthanasia of captive reptiles and amphibians: report of the UFAW/WSPA Working Party. In: Euthanasia of unwanted, injured, or diseased animals or for educational or scientific purposes. Herts, U.K.: Universities Federation for Animal Welfare, 1986: 34-38. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

COOPER, J.E., EWBANK, R., PLATT, C. and WARWICK, C. Euthanasia of amphibians and reptiles. Herts, U. K.: Universities Federation for Animal Welfare; London, U.K.: World Society for the Protection of Animals, 1989.

DAWKINS, M.S. Animal suffering. The science of animal welfare. London: Chapman and Hall, 1980.

DAWKINS, M.S. From an animal's point of view: motivation, fitness and animal welfare. Behav. Brain Sci. 1990; 13: 1.

DENNIS, M.B., DONG, W.K., WEISBROD, K.A. and ELCHLEPP, C.A. Use of captive bolt as a method of euthanasia in larger laboratory animal species. Lab. Anim. Sci. 1988; 38(4): 459-462.

DERR, R.F. Pain perception in decapitated rat brain. Life Sci. 1991; 49(19): 1399-1402.

DUNCAN, I.J.H. and PETHERICK, J. The implications of cognitive processes for animal welfare. J. Anim. Sci. 1991; 69: 5017-5022.

EBEDES, H. The sedation of wild animals for translocation. In: Proc. 14th International Conference, Animal Air Transportation Association. Amsterdam, The Netherlands: AATA, 1988: 141-151.

EIKELENBOOM, G., ed. Stunning of animals for slaughter. Boston, MA, The Hague: Nijhoff Publishers, 1983.

EWBANK, R. Euthanasia of day old chicks; Carbon dioxide and carbon dioxide/air mixtures. In: Universities Federation for Animal Welfare. Euthanasia of unwanted, injured and diseased animals for educational or scientific purposes. Herts, U.K.: Universities Federation for Animal Welfare 1987: 11-14. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

EWBANK, R., PARKER, M.J. and MASON, C.W. Reactions of cattle to head restraint at stunning. Animal Welfare 1992; 1(1): 55-63.

FAWELL, J.K., THOMSON, C. and COOKE, L. Respiratory artifact produced by carbon dioxide and pentobarbitone sodium euthanasia in rats. Lab. Anim. 1972; 6: 321.

FISHER, C. Stepping up the pace. Liberator 1990 Autumn: 20-21.

FLECKNELL, P.A. The relief of pain in laboratory animals. Lab. Anim. 1984; 18: 147-160.

FOSSE, R.T. Pain, pain recognition and treatment in laboratory animals. Lab. Zhyvotnye 1991; 1(3): 81-83.

GRANDIN, T. Observations of cattle restraining devices for stunning and slaughtering. Animal Welfare

1992a; 1(2): 85-90.

GRANDIN, T. (letters) Some thoughts about cattle restraint. Animal Welfare 1992b; 1(3): 230-231.

GREGORY, N.G. and WOTTON, S.B. Timed loss of brain responsiveness following exsanguination in calves. Res. Vet. Sci. 1984; 37: 141-143.

GREGORY, N.G. and WOTTON, S.B. Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. Vet. Rec. 1990; 126(23): 570-571.

GREGORY, N.G., MOSS, B.W. and LEESON, R.H. An assessment of carbon dioxide stunning in pigs. Vet. Rec. 1987; 121(22): 517-518.

GRIER, R.L. and COLVIN, T.L., eds. Euthanasia guide for animal shelters. 3rd Ed. Ames, IA: Moss Creek Pubs., 1990: 41-42. (R.R. No. 1, Arrowsmith Trail Publication, Ames, IA.)

HANSEN, N.E, CREUTZBERG, A. and SIMMONSON, H.B. Euthanasia of mink (*Mustela vison*) by means of Carbon dioxide (CO_2), Carbon monoxide (CO), and Nitrogen (N_2). Brit. Vet. J. 1991; 147: 140-146.

HELLEBREKERS, L.J., BAUMANS, V., BERTENS, A.P.M.G. and HARTMAN, W. On the use of T-61 for euthanasia of domestic and laboratory animals: An ethical evaluation. Lab. Anim. 1990; 24: 200-220.

HERIN, R.A., HALL, P. and FITCH, J.W. Nitrogen inhalation as a method of euthanasia in dogs. Amer. J. Vet. Res. 1978; 39: 989-991.

HEVNER, J.E. and DEJOHNG, R.H. Magnesium electroencephalographic and behavioural effects in cats. Can. J. Physiol. Pharmacol. 1973; 31: 308.

HOCHACHKA, P.W. Living without oxygen. Closed and open systems in hypoxia tolerance. Cambridge, MA: Harvard University Press, 1980.

IKARASHI, Y., MARUYAMA, Y. and STAVINOHA, W.B. Study of the use of the microwave magnetic field for the rapid inactivation of brain enzymes. Japan J. Pharmacol. 1984: 371-387.

KIM, S.U. Brain hypoxia studied in mouse central nervous system cultures I. Sequential cellular changes. Lab. Invest. 1975; 33: 658.

KNOWLES, T.G. and WARRISS, P.D. (letters) The use of blood cortisol levels as a measure of short-term stress. Animal Welfare 1992; 1(1): 229.

LONGAIR, J.A., FINLEY, G.G., LANIEL, M.A., MACKAY, C., MOULD, K., OLFERT, E.D., ROWSELL, H. *et al.* Guidelines for euthanasia of domestic animals by firearms. Can. Vet. J. 1991; 32: 724-726.

LUMB, W.V. Euthanasia by noninhalant pharmacologic agents. J. Amer. Vet. Med. Assoc. 1974; 165: 851-855.

McARTHUR, J.A. Carbon dioxide euthanasia of small animals (including cats). In: Humane destruction of unwanted animals. Herts, U.K.: Universities Federation for Animal Welfare, 1976: 9-17. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

McKIE, D. The Animals (Scientific Procedures) Act 1986. The Lancet 1986 March 1: 513.

MIKESKA, J.A. and KLEMM, R.R. EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. Lab. Anim. Sci. 1975; 25: 175-179.

NATIONAL RESEARCH COUNCIL. Euthanasia. In: Education and training in the care and use of laboratory animals. A guide for developing institutional programs. Washington, DC: National Academy Press, 1991: 67-74.

ORLANS, F.B., ed. Impact on animal care and use committees. Field research guidelines. Bethesda, MD: SCAW (Scientists Center for Animal Welfare), 1988: 1-2. (4805 St. Elmo Ave., Bethesda, MD 20814.)

OWENS, C.E., DAVIS, R. and SMITH, B.H. The psychology of euthanizing animals: The emotional components. J. Inst. Stud. Anim. Prob. 1981(2); 1: 19.

QUINE, J.P., BUCKINGHAM, W. and STRUNIN, L. Euthanasia of small animals with nitrogen; comparison with intravenous pentobarbital. Can. Vet. J. 1988; 29(9): 724-726.

ROLLIN, B.E. Euthanasia and moral stress. In: Suffering. Psychological and social aspects in loss, grief and cure. DeBellis, R. *et al*., eds. New York, NY: Howorth Press, 1986.

ROLLIN, B.E. and KESEL, M.L., eds. The experimental animal in biomedical research. Volume 1. Boca Raton, Ann Arbour, Boston, MA: CRC Press, 1990.

ROWSELL, H.C. Euthanasia: The final chapter. In: Proc. second symposium on the pet and society, Vancouver, B.C. Toronto: Standard Brands Food Company, 1979: 125-139. (Dr. Ballard's Pet Food Division, Standard Brands Food Company, 1 Dundas St. W., Toronto, Ont. M5G 2A9.)

ROWSELL, H.C. Euthanasia: acceptable and unacceptable methods of killing. In: Rollin, B.E. and Kesel, M. L., eds. The experimental animal in biomedical research, Vol. 1., Boca Raton, Ann Arbor, Boston, MA: CRC Press, 1990: 381-391.

ROWSELL, H.C. The future of control of pain in animals used in teaching and research. In: Short, C.E. and Van Poznak, A., eds. New York, Edinburgh, London, Melbourne, Tokyo: Churchill Livingstone, 1992: 525-537.

RUSSELL, W.M.S. and BURCH, R.L. The principles of humane experimental technique. Springfield, Charles C. Thomas, 1959.

SCHATZMANN, U., LEUENBERGER, T., FUCHS, P. *et al*. Jet injection: The possibility of using a high pressure water jet for stunning of slaughter pigs. Fleischwirtschaft 1991; 71: 899-901.

SHORT, C.E. and VAN POZNAK, A., eds. Animal pain. New York, Edinburgh, London, Melbourne, Tokyo: Churchill Livingstone, 1992.

SMITH, R.A. and LEWIS, D. Suicide by ingestion of T-61. Vet. Hum. Toxicol. 1989; 31(4): 319-320.

STAVINOHA, W.B., FRAZER, J. and MODAK, A.T. Microwave fixation for the study of acetylcholine metabolism. In: Jenden, D.J., ed. Cholinergic mechanisms and psychopharmacology. New York, NY: Plenum Publishing Corporation, 1977: 169-179.

STAVINOHA, W.B. Study of brain neurochemistry utilizing rapid inactivation of brain enzyme activity by heating and microwave radiation. In: Black, C.L., Stavinoha, W.B. and Maruyama, Y., eds. Microwave radiation as a tool to study labile metabolites in tissue. Elmsford, NY: Pergamon Press, 1983: 1-12.

UNIVERSITIES FEDERATION FOR ANIMAL WELFARE. Physical methods. In: Humane killing of animals. 2nd Ed. Herts, U.K.: UFAW, 1968: 4-14. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

UNIVERSITIES FEDERATION FOR ANIMAL WELFARE. Report on euthanasia of unwanted, injured or diseased animals for education or scientific purposes. Herts, U.K.: UFAW, 1986. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

UNIVERSITIES FEDERATION FOR ANIMAL WELFARE. Humane slaughter of animals for food. Herts, U.K.: UFAW, 1987. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

UNIVERSITIES FEDERATION FOR ANIMAL WELFARE. Humane killing of animals. 4th Ed. Herts, U.K.: UFAW, 1988. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

VANDERWOLF, C.H., BUZSAKI, G., CAIN, D.P., COOLEY, R.K. and ROBERTSON, B. Neocortical and hippocampal electrical activity following decapitation in the rat. Brain Res. 1988; 451: 340-344.

WALL, P. Neglected benefits of animal research. New Scientist, April 18, 1992.

WATTS, R.Z. The development of a captive bolt instrument for killing horses. In: Universities Federation for Animal Welfare. Humane destruction of unwanted animals. Herts, U.K.: UFAW, 1976: 25-27. (8 Hamilton Close, South Mimms, Potters Bar, Herts, England EN6 3QD.)

WHELAN, G. and FLECKNELL, P.A. The assessment of depth of anesthesia in animals and man. Lab. Anim. 1992; 26: 153-162.

WHITE, D.J. High altitude euthanasia is not recommended. American Humane Shop Talk 1984; 2(2): 1.

ZWART, P., DEVRIES, H.R. and COOPER, J.E. The humane killing of fishes, amphibia, reptiles and birds. Tijdschr Dirgenteskd 1989; 114: 557-565.

ZWEIGHAFT, H.M. (letters) Euphemisms for "euthanasia". Can. Vet. J. 1990; 31: 611.

XIII. THE USE OF ANIMALS IN PSYCHOLOGY

The focus of psychology is the organization of behaviour. It is concerned with processes that control and direct adaptive and maladaptive activity, and so the range of phenomena studied is broad, and the paradigms and research settings employed are varied. The study of behaviour may incorporate physiology, pharmacology, ethology, or even sociology, and for that reason the distinction between psychology and closely related disciplines is often blurred. Through the history of the discipline, extending back, for example, to nineteenth century work on reflex organization, through Pavlov's fundamental discoveries about conditioning, and more recently to the identification of motivational and reward systems through electrical stimulation of the brain, animal behaviour has played a central role in research and conceptualization. Although there are clearly recognized limitations to the use of animals in research (e.g., no access to verbal report), it does permit a control of hereditary and experiential variables that could seldom be achieved in other ways. Furthermore, animal research places humans in an evolutionary context and makes possible a comparative and biological perspective on human behaviour.

Basic animal research in psychology has played a significant role in advancing our understanding of processes of learning, memory, perception, motivation, and emotion, and of behavioural adaptations of individuals and species to their environments. While much of this work has addressed theoretical issues, it can have direct implications for contemporary applied problems. Examples of such problems that affect animals directly include captive and domestic animal care, non-lethal means of predator control, and the reintroduction of endangered species to their former habitats. Examples of problems that have a direct effect on human welfare include the control of depression, phobias, pain, addiction, and the pathological effects of stress and anxiety.

Although it may be convenient to dichotomize research activities as basic and applied, it is important to recognize that there is a continuum and that it is difficult to know *a priori* where along that continuum the implications of some research program will fall. Applications sometimes follow quite directly from basic research, and fundamental discoveries often arise in applied research. It is also important to appreciate, as Hebb (1966) has pointed out: "Before we can have applied science, we must have a science to apply." This is a position shared by many researchers who, while cognizant of applied implications, orient their work toward the elucidation of issues that are basically scientific and theoretical in nature.

Since psychological research approaches a wide range of topics with a diversity of methodologies, it is not surprising that animal research in psychology may not be well understood outside the discipline. The following points are directed at research strategies and general experimental procedures that have apparently been sources of misunderstanding:

1. Many problems studied by psychologists deal with the understanding and control of psychopathology, such as depression, phobias, psychosomatic disorders, psychoses, hyperactivity and learning disabilities, obesity and addiction. Many aspects of these problems cannot be studied satisfactorily in human patients because of the difficulties associated with non-experimental paradigms in determining the causal relationship among variables. In other words, when one studies patients with a form of psychopathology, all one can establish is that some variable, X, is correlated with the pathology, P. Yet an understanding and control of the problem requires knowing more than correlations. It is necessary to establish whether that variable X in some sense causes or is an antecedent of the pathology P, whether the pathology P is an antecedent for the change in variable X, or whether the two variables are related only indirectly and non-causally through their relationship to some common underlying variables yet to be determined. Seldom is it practicable to study these kinds of important issues in human patients using the necessary experimental (as opposed to correlational) research designs. One alternative and productive research approach has been to use animal models. In the present context, such models refer to "the production, under controlled conditions, of phenomena analogous to naturally occurring disorders."

A more extensive discussion of the concept of animal models in psychology may be found in the literature

(Abramson and Seligman, 1977).

Using such models, it becomes possible to conduct experimental studies involving the active manipulation of variables, and this permits clarification of the relationships among variables. It is important to recognize, however, that a model is simply that, and requires validation through a detailed study of its essential features and an analysis of its similarities to the psychopathology in question.

2. In addition to their use in applied problems, animal models play an important role in the development of fundamental behaviour theory. Research may study, for example, the behaviour of animals pressing a lever in an isolated chamber, in order to examine the way in which the frequency or patterning of lever presses is controlled by the schedule of food reward or reinforcement. No one is terribly interested in lever pressing as a behaviour in itself; however, this simple and easily quantified behaviour can be viewed as a model or analogue of more complex forms of behaviour. The assumption is that if one can understand the basic principles that control a simple behaviour, one will have at least a place to start in developing principles that govern more complexly organized behaviour systems.

Similarly, when researchers use electric shock as a means of producing stress or of motivating animals to escape or avoid, they are fully recognizant that electric shock does not normally occur in nature. They do assume, however, that this particular easily controlled aversive event can serve as a model or analogue of other unpleasant events that do occur naturally and affect behaviour. This use of models in psychology is often misunderstood by those outside the discipline. It is important that they note that questions of the validity of the assumptions and the adequacy and generalizability of a model cannot be answered *a priori* and are more productively approached through empirical* research than logical argument.

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* It should be noted that the word empirical has two quite different, but equally correct meanings of which the reader should be aware. The first refers to work that is based on a systematic observation and the application of scientific principles in methods. It is in this sense that the word is normally used in psychology (although there also occurs the word **empiricism**, which refers to a philosophy of knowledge). The second meaning of empirical is found in the medical sphere, and refers to work that "relies on or is based on practical experience without reference to scientific principles as, an empirical remedy". It is related to the noun, **empiric**, which refers to one "ignorant of scientific principles" and who "lacks regular training and proper qualifications" (*Webster's New World Dictionary*, Second College Edition, 1970). The reader should be aware of these two quite opposite meanings and of what is implied by the psychologist when characterizing research as "empirical".

3. A basic assumption of contemporary psychology is that the brain is the organ of the mind, a term which simply refers to the internal processes that determine the organization of complex behaviour. Accordingly, one approach to the study of the properties of mind is to study the functioning of the brain. Such research is sometimes correlational in that it relates indices of brain function (e.g., electroencephalograms [EEG], evoked potentials) in humans and animals to behavioural processes. Often, however, and for reasons related to the control and sorting out of relationships among variables discussed under point 1), the research involves an experimental study of the effects of manipulation of the brain on behaviour. No one assumes that the brains of experimental animals are miniature human brains; however, it is assumed that the basic principles of brain organization are common across mammalian species and that the brain of the particular animal species **may** serve as a model for certain aspects of human brain function.

4. Psychologists incorporate and manipulate motivational variables in their research for three somewhat dissimilar reasons. The first is when the subject of study is the motivational system in question, e.g., the control of feeding or drinking behaviour. In such a context, it is obvious to all why an animal might be on a water or food- restricted regimen. The second is when the motivational system in question is being used as a model for other appetitive or aversive motivational systems, as discussed under point 2). A third reason, and one that is often not well appreciated by those outside the discipline, is that such manipulation represents an effective means of facilitating the controlled study of phenomena

only indirectly related to the motivational manipulation.

Behaviour that is oriented towards obtaining access to food and water, or escaping or avoiding an aversive event, can form the basis of sound inferences about non-motivational processes associated with, for example, learning, memory or perception. The origin of the misunderstanding is that, although motivation is manipulated, the dependent variables of the study relate to learning. The misunderstanding is compounded by the fact that although sometimes the interest is in learning as a process itself, other times it is in learning as a process that is affected by sensory, perceptual, motor or other processes that are in fact the principal foci of the study. As a simple example, consider the case of studying the capacity of an animal for pattern discrimination. One approach might be to place the animal repeatedly into a chamber with two doors. Behind the door with pattern A, food is always to be found. Behind the door with pattern B, there is no food. The animal is then taught to enter one of the doors each time it is placed in the chamber. To learn to enter the door behind which the food is located requires the animal to be motivated and interested in finding and consuming the food, and that is why motivation is manipulated. When the animal can consistently perform the correct response, i.e., when it has learned to go to the door with pattern A, one is in a position to make some statement about the perceptual capability of the animal, even though what was measured was learning, and what was manipulated was motivation. The analysis of learning is central to experimental psychology, and motivation is necessary for that analysis. The rationale for the choice of motivation manipulated is not always obvious, but the later discussion of Guideline 7 attempts to identify some of the considerations that are made. Often these considerations relate to practicability and minimization of behavioural variability, which promotes the economical and efficient use of animal resources.

Over the years, members of the general public, the discipline of psychology itself, and other disciplines, have expressed concerns about aspects of psychological research involving animals. In part, this may be due to psychology including as subjects of study phenomena to which one can easily relate on a highly personal level (e.g., stress, pain, anxiety, motivation, etc.). In part, this may be due to a poor understanding of the nature of the model systems as a research tool, and we have tried to address that in these introductory remarks. However, in large measure, this is undoubtedly due to a language, vocabulary, or jargon used to characterize phenomena, events, or hypothetical processes that can sometimes conjure unrealistic, distressing images. Those with concerns are to be encouraged to become fully informed about the nature of psychological research, and those engaged in research are to be encouraged to be sensitive to these concerns and to discuss openly their work and its implications with concerned individuals outside their discipline.

Certain procedures used in the study of psychological problems undoubtedly do produce some distress in animal and human subjects, and this places directly on the investigator a responsibility to question seriously what is being done, why it is being done, and where the work will lead. The Canadian Council on Animal Care (CCAC) and the scientific community have had a continuing concern for these and other issues related to the care and use of experimental animals. In the recognition that there are certain aspects of behavioural research that are misunderstood and can be problematic with respect to research ethics, the Canadian Psychological Association (CPA), in consultation with the CCAC, has prepared the following set of guidelines to assist psychologists as researchers and instructors in making the ethical decisions viewed to be an integral part of behavioural research with animals. It is hoped that these guidelines will complement those of the CCAC and facilitate the conduct of ethically responsible research that will promote our understanding of basic processes underlying behaviour.

GUIDELINES FOR THE USE OF ANIMALS IN RESEARCH AND INSTRUCTION IN PSYCHOLOGY: COMMENTARY AND ELABORATION

The discipline and profession of psychology in Canada shares with contemporary society a deep concern for the welfare and humane treatment of animals, especially in scientific research and instruction. While it is recognized that animal research is essential to the further development of scientific knowledge, it is also recognized that there are limits to what should be done to animals in the conduct of that research. In

response to this concern, the Canadian Psychological Association (CPA), in consultation with the Canadian Council on Animal Care (CCAC), has formulated these guidelines to assist psychologists as researchers and instructors in making the ethical decisions that are an integral aspect of working with animals. These have been published in a form suitable for posting in laboratories. The purpose of this commentary is to provide some elaboration of considerations that psychologists should give with respect to implementation of the preamble and each guideline.

Psychologists have an obligation to advance knowledge and promote welfare through the competent conduct of research, the accurate communication of findings, and the effective instruction of students. However, their values and goals as scientists sometimes come into conflict with their values related to the treatment of living organisms. Dilemmas posed by the conflict cannot be resolved by rigid rules and regulations, but require a careful weighing of values and alternatives. In many cases, the decisions reflect a relative judgement of the value of the research and the effects of the procedures on the animals. Psychologists using animals for research or instruction should be prepared to make such decisions and to explain the bases of their decisions to an informed audience. The following guidelines are intended to assist the scientist in making these ethical decisions.

The CPA's *Guidelines for the Use of Animals in Research and Instruction in Psychology* were formulated from the ethical perspective advocated by Diener and Crandall (1978a) in their book *Ethics in Social and Behavioural Research*. They consider research ethics to be a process of decision-making rather than one of devising explicit rules and regulations intended to govern the conduct of all research under all circumstances. Their approach is well represented in the following passage:

"The ethical or moral scientist makes individual judgements about research practices in light of his/her own values. According to this approach to ethics, the moral person is not one who blindly follows ethical codes, no matter how enlightened. The ethical decision-maker is one who realizes that his/her choices are related to values, and weighs these values carefully when making important decisions. For the moral person there may be a few moral absolutes (Szasz, 1967); however, he or she realizes that most moral decisions must be made individually in each case (Smith, 1969). This meaning of ethics emphasizes the process by which the decisions are made as well as the final choice. The decision is made by a person **who is educated about ethical guidelines, carefully examines moral alternatives, exercises judgement in each situation and accepts responsibility for his/her choices** (Diener and Crandall, 1978b) (emphasis added)."

As reflected in the Preamble to these Guidelines, this ethical framework clearly places the responsibility for the careful consideration of personal, social, and professional values on the individual scientist. A corollary of this is that the individual researcher or instructor must accept responsibility for choices through accountability to an informed public. Specifically, the investigator must be able to explain the rationale for both the research problem and its methodology to informed colleagues, peers, and institutional committees. If necessary, the investigator must be able to defend the research against the criticisms that suffering inflicted on animal subjects was unnecessary in view of the objectives of the research, or unconscionable in view of the balance between the suffering inflicted and expectation of gain in scientific knowledge or education. The present commentary is intended to provide the scientist with an indication of problematic issues and concerns that require special attention and consideration.

A. THE SCIENTIST

1. Prior to undertaking a research or instructional project with animals, the scientist has a responsibility to be sufficiently knowledgeable to ensure compliance with these guidelines. When in doubt about compliance, the scientist should consult with informed colleagues and the institutional Animal Care Committee (ACC) and give due regard to their advice. Investigators are reminded that it is the policy of the major granting agencies, e.g., Medical Research Council (MRC) and the Natural Sciences and Engineering Research Council (NSERC), of government departments and of most universities that ACC approval must be received prior to commencing any project with animals.

The position that the ethical scientist is one who can make informed decisions and who is prepared to give a reasoned judgment for the values and appropriateness of the objectives and procedures of the research or teaching assumes a considerable depth and breadth of knowledge by the scientist. The decisions involved are usually complex and multifaceted. If they are to be informed, scientists must be familiar with the recent literature relevant to the problem, aware of the current status of the problem, familiar with procedures involved, either through study of the literature or direct experience with the techniques, and aware of potential risks. When undertaking research in a new area, or when the research involves severe stress, pain, or privation, the investigator may have doubts about the breadth and depth of his/her knowledge and experience. Under such circumstances, there is an obligation to consult with informed colleagues and/or the ACC and to give due regard to their advice. Such consultation does not absolve the investigator from responsibility for the decisions; however, it is evidence of a responsible attitude towards becoming sufficiently knowledgeable to undertake research in an ethical manner.

2. A scientist trained in research methods and experienced in the care of laboratory animals should ensure that the comfort, health and humane treatment of experimental animals are given appropriate consideration.

Psychological research frequently requires that an investigator interact with animal subjects over an extended period of time. Accordingly, the psychologist has a vested interest, quite apart from humane considerations, in ensuring that experimental animals are well treated and healthy; otherwise, it is likely that behavioural data will be unreliable and the research objectives not achieved. Although the day-to-day maintenance and/or behavioural testing of animals may be done by trained technical staff, it is the responsibility of the individual scientist to be able to recognize good and poor practices by staff and students. This ability requires training in research methods and experience in animal care procedures. While our attention is naturally drawn to practices that involve pain or physical illness, the scientist should be sensitive to problems that can arise from the capacity of some animals to form human attachments (e.g., the distress that might be experienced by the animal when returned to isolated quarters at the end of a prolonged study).

3. The scientist should ensure that all individuals under his/her supervision have the training and competence needed to carry out their responsibilities for experimental procedures, care, maintenance, and handling of the species being used.

A variety of technical skills is required in any laboratory to ensure proper care and handling of animals, and it is the responsibility of the scientist to ensure that all supervised individuals have the skills and attitudes required to carry out competently their assigned duties. Since every species has unique biological and social needs, the design of experimental and maintenance protocols should take into consideration the species' normal ecology, evolutionary history, and behavioural adaptations to the natural environment. It is the responsibility of the scientist to acquire sufficient expertise in these regards, and to ensure that the training of technical staff, students, and research associates is adequate to meet their respective responsibilities. This is necessary, not only from the point of view of the humane treatment of animals, but also from the scientific perspective of generating reliable data.

4. The scientist should be fully cognizant of the CCAC's guidelines and of current federal, provincial, and local laws and regulations concerning the acquisition, care, use, and disposal of animals.

This guideline is self-explanatory. As professionals and as members of society, psychologists have a responsibility to be aware of and to follow federal, provincial, and local laws and regulations concerning animals. In cases of doubt, the scientists should consult with the chairman of the ACC or with the CCAC. Compliance with this *Guide* is a requirement of Canada's major granting agencies, many journals in Canada, and ACCs.

B. RESEARCH

5. There must be a reasonable expectation that studies involving animals will: a) increase understanding of structures and processes underlying behaviour; b) increase understanding of the particular animal species used in the experiment; or c) result eventually in benefits to the health and welfare of humans or other animals.

This guideline outlines the general spheres to which psychological research should contribute if animals are legitimately to be involved. It specifically recognizes the value of research, the implications of which are largely theoretical or philosophical, and is consistent with the CCAC guidelines in that there is a reasonable expectation that the development of new scientific knowledge and conceptualization may result in eventual benefits to the health and welfare of humans or other animals.

This guideline refers more to research programs than to individual studies. It is intended to recognize that there is no such thing as the "definitive study" and that the significance of any individual experiment, especially when viewed after the fact, is not always immediately apparent and can be easily trivialized. Accordingly, a particular experiment must be judged within the context of a research program as to whether it will contribute in a meaningful way to a systematic empirical or theoretical base.

An integral part of any research program is the use of small pilot studies, the results of which are at best suggestive, but which are important for decisions about directions to proceed, research design, parameters, etc. The value of such studies is often indirect, and therefore must be evaluated in the broader context of a research.

This guideline bears also on the problem of replication or reproducibility of results in psychological research. Replication is a necessary and desirable aspect of science when it is seen as a manipulation of a special class of factors (e.g., different laboratories, different experimenters, different times of the year, etc.), which can provide new scientific knowledge of value in understanding a phenomenon. As with the case of pilot studies, replications must be seen within the broader context of a research program. To the extent that there is a reasonable expectation that a replication will contribute to new scientific knowledge, it is consistent with this guideline.

6. Procedures subjecting animals to pain, stress, privation, or death should be used only when an acceptable alternative procedure is unavailable.

In psychological research, the subjection of animals to procedures involving pain, stress, privation, or death occurs in two broad contexts. The first is when the subject of study is pain, stress, motivational systems, or aspects of death, all of which are legitimate and important areas of the discipline. In such a context, there are seldom realistic alternatives, and the attention of the scientist must focus on ways of minimizing discomfort. For example, the study of stress must necessarily involve manipulations that will produce stress; nevertheless, the investigator must consider ways to minimize the trauma of these manipulations. In the second context, procedures are employed to induce motivational states that facilitate the controlled study of phenomena only indirectly related to the motivational manipulations (for example, learning discrimination, memory, sensory thresholds, etc.). Under such circumstances, the scientist has an ethical obligation to consider whether the research objectives could be met using broad alternatives not involving pain or discomfort.

7. Scientists should examine methodological and procedural techniques for the purpose of minimizing discomfort, illness, and pain to animals.

When acceptable alternative procedures to ones that involve pain or privation are not available, there remains a responsibility to examine methodologies and procedures that will minimize discomfort, illness, or pain, and that are consistent with the objectives of the research. The judgement involved clearly requires considerable knowledge of the species and its behavioural repertoire, as well as the research

problem. Issues that may arise in considering this guideline include: Is the species appropriate for the study? Have the motivational systems and the biological/social needs of the species been assessed so that a reasonable judgment can be made about the relative discomfort and stress produced by various potentially painful procedures or different kinds of levels of privation? Can motivational states in the given species be better controlled through appetitive motivation (e.g., water or food deprivation) or through aversive motivation (e.g., mild electric shock)? Have parameters of the aversive stimuli or the deprivation been selected judiciously so as to be optimal in light of the behavioural requirements of the research and the principle of minimizing discomfort? Could lower levels of shock or privation be used and still produce reliable behaviour? Could a lower level of aversive stimulation or privation be used, even though more animals might be required because of less reliable or stable behaviour? In studies involving food motivation, is privation to a fixed percentage (e.g., 80%) of *ad lib* body weight required, or would controlled daily access to food (e.g., every 23 hours), perhaps in combination with a preferred incentive, be sufficient?

Manipulations involving surgery can be problematic with respect to discomfort, and issues that may arise in this regard include: Is the method of an anesthetization optimal? Are the surgical procedures sufficiently aseptic to minimize post-operative infection and other stress? Is an analgesic required during post-operative recovery? Does the manipulation, such as implantation of a chronic cannula or electrode assembly, cause irritation, and if so are there ways to minimize this? Does a physiological or pharmacological manipulation (including administration of toxins) cause a generalized deterioration of the well-being of the animal, and if so, how can this be minimized in a manner consistent with the research objectives?

It is sometimes suggested that one approach to minimizing discomfort is to minimize the number of animals used in the research. However, since the detection of treatment effects is against a background of uncontrollable behavioural variance, one must be careful to ensure that there is sufficient power to detect those effects when they are there. Otherwise, the animals that were used would have suffered needlessly. However, investigators should consider single-subject designs, repeated measures designs, and other techniques to minimize variance, all of which could lead to fewer total subjects being required for the research.

8. An experiment should be terminated whenever it becomes apparent to the scientist or the institutional ACC, that its continuation will result in injury or suffering that is incompatible with these guidelines.

With the best of planning, some experiments simply do not work out as expected. This can be due to various reasons, such as equipment failure, procedures having unforeseen effects, experimenter error, or behavioural variance so large as to obliterate any possible treatment effects. Also, on occasion, new research results become known and may make ongoing research redundant. In such situations, the experimenters must give careful consideration to whether it is worthwhile to continue the work, and do so only if they are satisfied that it is justified in light of these guidelines.

9. The killing or other disposition of experimental animals at the termination of the experiment must be accomplished in a humane manner.

At the end of most experiments in psychology, experimental animals are killed. This must be done in a humane manner, as described in this *Guide*, and in Volume 2 (CCAC, 1984). While this is obvious and is the accepted practice, problems can arise when research animals are not killed. For example, releasing trapped animals back into the wild may or may not be humane depending on the species, its territorial behaviour, feeding habits, time of year, etc. As noted earlier, some animals can form human attachments and problems can arise after experiments involving long-term interaction with them if they are returned to isolated laboratory housing. The responsibility of the scientist to his/her animals extends beyond the actual termination of the experiment, and careful consideration must be given to whether the means of disposing of the experimental animal is actually humane.

C. INSTRUCTION

10. The decision to use animals for instructional purposes must be based on a consideration of educational objectives rather than contributions to new scientific knowledge. In other respects, ethical practices in the care and treatment of animals are the same as those that apply to the use of animals in research.

A committee of the British Psychological Society (BPS) has commented that, "...No psychology undergraduate can for long remain unaware of the extent to which the empirical basis of much psychological theory is derived from experimental work with animals. Accordingly, it is appropriate that, as a matter of course, all undergraduate students of psychology should receive specific instruction on the issues which arise from animal experimentation, issues scientific, intellectual, methodological, practical, and ethical" (BPS, 1979).

The CPA shares the view that instruction on the use of animals in psychology is desirable and necessary. On occasion, the actual use of animals in instruction is required to achieve educational objectives. In such cases, the same general considerations must apply to animal use in instruction as in research, that is, a balancing of the expected benefits against the costs, with the benefits seen in terms of advancing education rather than incrementing scientific knowledge. Clearly, classroom demonstrations of animal behaviour by their very nature involve phenomena which may not intrinsically advance scientific knowledge. However, to the extent that they assist the student's understanding of existing knowledge, they make a substantive contribution. As contained in the BPS position, there is an obligation to incorporate material on ethical issues into discussions of animal use. In this way, instructors can, by example, promote the ethical use of animals by future scientists and instill in students an appropriate sensitivity to associated issues.

11. Classroom demonstrations involving animals should only be used when instructional objectives cannot be achieved through the use of videotapes, films, or other alternative methods. Careful consideration should be given to whether the type of demonstration is warranted by the anticipated instructional gain.

Videotapes and films represent effective means of demonstrating principles of animal behaviour and experimentation. However, there are often advantages of using real animals, not the least of which is to convey the realism of the phenomenon. In deciding on the medium of instruction, instructors have an obligation to evaluate carefully their instructional objectives and

In making the evaluation, the instructor should be sensitive to the possible trauma that the animals may experience in being brought into a classroom, and to the possibility of disease transmission to or from the animal. The instructor must also consider whether the animal will be used solely for the demonstration, or whether it has already been used in experimentation, or is breeding stock, or is maintained for the purpose of demonstrations.

The guideline makes reference to "type of demonstration" to alert the instructor to the possibly adverse reactions that a demonstration, live or filmed, may produce in unprepared students. Procedures which to the naive viewer may appear to involve pain or stress (e.g., showing animals with chronic cannulae or electrode assemblies, animals having epileptic seizures, animals being operated on, injected with drugs or social animals raised in isolation, etc.) are especially problematic.

12. Student projects involving pain or distress to animals should be undertaken judiciously and only when the training objectives cannot be achieved in any other way.

Student research projects fall along a continuum from casual classroom projects at one end, to doctoral dissertation research (which should contribute to new scientific knowledge) at the other. The value of such projects is seldom to be found in their making substantive new contributions to knowledge, but is

found in their advancing knowledge through communication. If students are to acquire the knowledge and expertise required by these guidelines, it is necessary that they gain experience in working with animals. Especially in the case of students who show every indication of embarking on a research-oriented career, that experience may involve using procedures that involve minimal pain or distress. If so, the instructor must consider very carefully the appropriateness of the project and its procedures against its training objectives for the individual student and the development of student sensibilities towards animals. Students and instructors are reminded that ACC approval must be received prior to commencing any project with animals.

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These guidelines shall be conspicuously posted in every laboratory teaching facility and applied setting where animals are being used.

The CPA recommends that a copy of the guidelines and this commentary be included in laboratory manuals as well as that selected sections be posted in all laboratory facilities. It is hoped this will not be seen as an empty formality, but rather as an invitation for all laboratory personnel to become acquainted with the ethical process. Like science itself, ethical procedures are advanced by communication and discussion.

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In any situation involving decisions and judgment, there will, on occasion, be disagreements and misunderstandings. When these arise in the context of the use of animals in research or instruction in psychology, they should be resolved as quickly as possible so as not to impede legitimate research nor to prolong unacceptable procedures. The well-being of the animal must be a paramount concern. In general, there are two classes of problems. In the first, there may be allegations by students, colleagues, or the public that some on-going or completed research or instruction is inappropriate in light of these guidelines. In this case, the concerned individual should attempt to work through the ACC of the institution in which the research is carried out. In the second, individual psychologists may find their ACC or a granting agency unwilling to approve some research which they feel is scientifically and ethically warranted. The CCAC has developed a formal appeal mechanism of which researchers may take advantage. Part of this mechanism will involve consultation with the CPA.

D. REFERENCES

ABRAMSON, L.Y. and SELIGMAN, M.E.P. Modelling psychopathology in the laboratory: History and rationale. In: Maser, J.D. and Seligman, M.E.P., eds. Psychopathology: experimental models. San Francisco, CA: W.H. Freeman & Co., 1977: 1-26.

BRITISH PSYCHOLOGICAL SOCIETY. Policy statement. Brit. Psych. Soc. Bull. 1979; 32: 50.

CANADIAN COUNCIL ON ANIMAL CARE. Guide to the Care and Use of Experimental Animals, Vol. 2,

Ottawa, Ont.: CCAC, 1984.

DIENER, E. and CRANDALL, R. Ethics in social and behavioural research. Chicago, IL: University of Chicago Press, 1978a.

DIENER, E. and CRANDALL, R. Ethics in social and behavioural research. Chicago, IL: University of Chicago Press, 1978b: 4-5.

HEBB, D.O. Textbook on psychology. 2nd Ed. Philadelphia, PA: W.B. Saunders, 1966: 19.

SMITH, M.B. Social psychology and human values. Chicago, IL: Aldine, 1969.

SZASZ, T.S. Moral man: A model of man for humanistic psychology. In: Bugenthal, J.T., ed. Challenges of humanistic psychology. New York, NY: McGraw-Hill, 1967.

XIV. GUIDELINES ON THE USE OF ANIMALS IN NEUROBIOLOGICAL RESEARCH

A. INTRODUCTION

Research in the neurosciences contributes to the quality of life by expanding knowledge about living organisms. This improvement in quality of life stems in part from progress toward ameliorating human disease and disability, in part from advances in animal welfare and veterinary medicine, and in part from the steady increase in knowledge of the abilities and potentialities of human and animal life. Continued progress in many areas of biomedical research requires the use of living animals in order to investigate complex systems and functions because, in such cases, no adequate alternatives exist. Progress in both basic and clinical research in such areas cannot continue without the use of living animals as experimental subjects. The use of living animals in properly designed scientific research is therefore both ethical and appropriate. Nevertheless, our concern for the humane treatment of animals dictates that we weigh carefully the benefits to human knowledge and welfare whenever animal research is undertaken. The investigator using research animals assumes responsibility for proper experimental design, including ethical as well as scientific aspects.

The scientific community shares the concern of society at large that the use of animals in research should conform to standards that are consonant with those applied to other uses of animals by humans. While it is unlikely that any particular set of standards will satisfy everyone, it is appropriate for scientific societies to formulate guidelines that apply to the humane use of laboratory animals in particular areas of research. Ideally, such guidelines should also be acceptable to society at large as reasonable and prudent.

Most of the more specific sections of this document were formulated with respect to research using warm-blooded vertebrates. As a general principle, however, ethical issues involved in the use of any species, whether vertebrate or invertebrate, are best considered in relation to the complexity of that species's nervous system and its apparent awareness of the environment, rather than physical appearance or evolutionary proximity to humans.

B. FACTORS THAT RELATE TO THE DESIGN OF EXPERIMENTS

The primary factor used to evaluate humane treatment in animal research is degree of distress or discomfort, assessed by anthropomorphic judgements made by reasonable and prudent human observers. *The fundamental principle of ethical animal research is that experimental animals must not be subjected to avoidable distress or discomfort*. This principle must be observed when designing any experiment that uses live animals.

Although most animal research involves minimal distress or discomfort, certain valid scientific questions may require experimental designs that inevitably produce these effects. Such situations, while uncommon, are extremely diverse and must be evaluated individually. It is critical that distress and discomfort be minimized by careful experimental design. It is also important to recognize that there is no difference between distress and discomfort that may be inherent in a valid experimental design and that which may occur as an unintended side effect. It is therefore incumbent on the investigator to recognize and to eliminate all *avoidable* sources of distress and discomfort in animal subjects. This goal often requires attention to specifics of animal husbandry as well as to experimental design.

Invasive procedures and paralytic drugs should never be employed without benefit of anesthetic agents unless there is a very strong scientific justification and careful consideration is given to possible alternatives. Advances in experimental techniques, such as the use of devices chronically implanted under anesthesia, can offer alternative approaches. If these are not feasible, it is essential to monitor

nociceptive responses (for example, recordings of EEG, blood pressure and pupillary responses) that may indicate stress in the animal subject, and to use these as signals of the need to alleviate pain, to modify the experimental design, or to terminate the experiment.

When designing research projects, investigators should carefully consider the species and numbers of animals necessary to provide valid information, as well as the question of whether living subjects are required to answer the scientific question. As a general rule, experiments should be designed so as to minimize the number of animals used and to avoid the depletion of endangered species. Advances in experimental methods, more efficient use of animals within-subject designs, and modern statistical techniques all provide possible ways to minimize the numbers of animals used in research. This goal is completely consistent with the critical importance of replication and validation of results to true progress in science.

C. FACTORS THAT RELATE TO THE CONDUCT OF EXPERIMENTS

Research animals must be acquired and cared for in accordance with the guidelines published in the *NIH Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Publication No. 85-23, Revised 1985). The use of an animal scheduled for euthanasia by a pound or shelter saves the life of another; therefore, the use of pound or shelter animals is endorsed for research projects in which they are suitable subjects. In using animals acquired from a pound or shelter, as with all other aspects of research, investigators must adhere to the relevant local, state and federal law. (The reference to pound or shelter animals was added to the *Guidelines* following the recommendation by the Committee on Animals in Research and approval by Council.) The quality of research data depends in no small measure on the health and general condition of the animals used, as well as on the specifics of the experimental design. Thus, proper animal husbandry is integral to the success of any research effort using living animal subjects. General standards for animal husbandry (housing, food quality, ventilation, etc.) are detailed in the *NIH Guide*. The experienced investigator can contribute additional specifics for optimum care for particular experimental situations, or for species not commonly encountered in laboratory settings.

Surgery performed with the intent that the animal will survive, (for example, on animals intended for chronic study), should be carried out, or directly supervised, by persons with appropriate levels of experience and training, and with attention to asepsis and prevention of infection. Major surgical procedures should be done using an appropriate method of anesthesia to render the animal insensitive to pain. Muscle relaxants and paralytics have no anesthetic action and should not be used alone for surgical restraint. Post-operative care must include attention to minimize discomfort and the risk of infection.

Many experimental designs call for surgical preparation under anesthetic agents with no intent that the animal should survive. In such cases, the animals ordinarily should be maintained unconscious for the duration of the experiment. At the conclusion of the experiment, the animal should be killed without regaining consciousness and death ensured before final disposition.

Certain experiments may require physical restraint, and/or withholding of food or water, as methodological procedures rather than experimental paradigms. In such cases, careful attention must be paid to minimize discomfort or distress and to ensure that general health is maintained. Immobilization or restraint to which the animals cannot be readily adapted should not be imposed when alternative procedures are practical. Reasonable periods of rest and readjustment should be included in the experimental schedule unless these would be absolutely inconsistent with valid scientific objectives.

When distress and discomfort are unavoidable attributes of a valid experimental design, it is mandatory to conduct such experiments so as to minimize these effects, to minimize the duration of the procedure and to minimize the numbers of animals used, consistent with the scientific objectives of the study.

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* Reproduced with permission of the Society for Neuroscience from *Handbook for the Use of Animals in*

Neuroscience Research (1992). References to American publications and to American states are not relevant to Canadian researchers.

APPENDIX I

HOUSING AND ENVIRONMENT

The following charts are based on successful experiences and professional judgement ever cognizant of the performance evaluation of the animals being maintained. As there are few objective data available on the ideal space requirements for individual laboratory species, veterinary judgement must be considered in evaluating housing requirements. The Social and Behavioural Requirements of Experimental Animals chapter provides additional guidelines for specific species.

SPECIES (weight)	SPACE PER ANIMAL			TEMPERATURE ºC		R. H. %	Ventilation Changes/ Hour	B.T.U. Animal/ Hour
	Single-Floor Area	Minimal Height	Group or Loose Housing	Room/ Cage ºC	Pen/Free Ranging			
CAT >4 kg	0.28 m^2 0.37 m^2	0.76 m Perch	0.56 m^2 Perches	20-22	15-25	45-60	10-18	25-30
CATTLE Calf Cow	1.5 m^2 3.0 m^2		2.4 m^2 7-10 m^2	10-25	2-27	40-70	5-20	300-800
CHICKEN	Chapter IV Table 3			18-22	12-27	45-70	5-15	30
DOG <12 kg 12-30 kg >30 kg	0.75 m^2 1.20 m^2 2.23 m^2	0.8 m 0.9 m pen-2.0 m	1.5 m^2 2.0 m^2 3.0 m^2	18-21	5-25	45-55	8-12	80-150
GERBIL	116 cm^2	15 cm	pair + litter 900 cm^2	15-24		40-50	8-10	4.0
GOAT	1.4 m^2	2.0 m	1.0 m^2	15-24	7-30	55-65	6-12	350-500
GUINEA PIG <350 g >350 g	300cm^2 650cm^2	18 cm 22 cm	500 cm^2 800 cm^2	18-22		50-60	4-8	5-6
HAMSTER >100 g	100cm^2 120cm^2	18 cm 18 cm	fem. + litter 900 cm^2	21-24		45-65	6-10	2.5
HORSE	4-5 cm^2	3 m	13-17 m^2	10-24	2-27	25-75	4-8	
MOUSE <20 g >20 g	65 cm^2 100cm^2	13 cm 15 cm	fem. + litter 160 cm^2	22-25		50-70	8-12	0.6
NON-HUMAN PRIMATE Baboon (*Papio* sp) 5-12 kg >12 kg	0.74 m^2 1.39 m^2	0.91 m 1.22 m	2.8 m^2	21-26	15-30	45-60	12-16	60-140

(*Macaca* sp) <7 kg 7-15 kg >15 kg	0.4 m^2 0.6 m^2 0.75 m^2	0.81 m 0.91 m 1.2 m	2-3 m^2 perches	22-25	18-29	45-60	10-15	60-200
OPOSSUM	0.56 m^2	0.75 m		21-25	10-27	45-65	10-12	
PIGEON	0.18 m^2	0.38 m		16-20	5-27	45-70	12-15	1.2
QUAIL	400cm^2	15 cm max. 30 cm	200 cm^2	21-22	20-30	45-70	10-15	
RABBIT <4 kg >4 kg	0.37 m^2 0.46 m^2	0.40 m 0.45 m	fem. + litter 0.93 m^2	16-22	10-28 shade	40-50	10-20	30-40
RAT <150 g >150 g	150cm^2 250cm^2	18 cm 18 cm	fem. + litter 800 cm^2	20-25		50-55	10-20	4.0
SHEEP	Chapter IV Table 2			5-21	0-24	50-75	15-25	500-800
SWINE	2.0 to 4.0 m^2 of pen space per animal		fem. + litter 5-8.5 m^2	17-24	10-25	55-75	15-20	250-500

APPENDIX II

BREEDING AND REPRODUCTION DATA

SPECIES (age and weight)	Breeding Age range female-male	Cycle Type* Length (days)	Duration of Sexual Receptivity	Breeding Behaviour** and Season**	Gestation mean (d) range	Litter Size and Range	Optimal*** Reproductive Span (yrs/mos)	Light Hours
CAT	7-10 mos	14-24 S.P.	3-8 d irregular	Ph (f. to m.) Jan. to Sept.	62 57-65	2-6	6-7 yrs	12-14
CATTLE	16-24 mos	18-24 P	10-24 hrs	Ph (A1) all year	283 279-290	1	8-10 yrs	8-12
CHICKEN	4-6 mos			Poly all year	21 incubation		9-12 mos	13-14
DOG	10-14 mos	21 M	4-8 d	Ph (f. to m.) Biannual	63 58-68	breed 4-10 dependent	6-7 yrs	10-12
GERBIL	9-12 wks	4-6 P	14-18 hrs	MP all year	25 24-26	4-5	15 mos	12-14
GOAT	15-18 mos	14-21 S.P.	48-72 hrs	Poly Sept. to Feb.	151 149-153	1-2	4-5 yrs	8-12
GUINEA PIG	3 mos	15-19 P	6-14 d	H (1 to 6) all year	65 59-72	2-6	2 yrs	12-15
HAMSTER	6-8 wks	4 P	6-20 hrs	M.P. or H (1 to 5) all year	16 15-18	5-8	15 mos	12
HORSE	36-60 mos	19-24 S.P.	3 d 2-6	Ph (f. to m.) Feb. to Aug.	335 320-360	1	12-15 yrs	8-12
MOUSE	6 wks	4-5 P	10-20 hrs	H (1 to 4) all year	20 19-21	6-12	7-8 mos	14
NON-HUMAN PRIMATE Baboon (*Papio* sp)	48-66 mos	31-32	3-4 day menses day 15-17 optimal breeding period	H (1 to 6) all year	175 154-185	1	5-20 yrs	12-14
(*Macaca* sp)	36-48 mos	28	3-4 day menses day 10-12 optimal breeding time	H (1 to 8) or Ph (f. to m.) all year	165 150-180	1	5-20 yrs	10-14
OPOSSUM	8-12 mos	22-27 P	7-14 d post-partum	M.P.	marsupial 12-13	1-12	5 yrs	
PIGEON	6 mos			M.P. all year	13 incubation		2 yrs	12-14
QUAIL	6 wks			H (1 to 3) or poly all year	16 incubation		5-6 mos	14
RABBIT	6-9 mos	induced P	ovulation variable	Ph (f. to m.) all year	31 28-34	6-10	3 yrs	12-14

RAT	10-12 wks	4-5 P	10-20 hr	H (1 to 6) all year	21 20-22	7-14	9-10 mos	12-14
SHEEP	18-24 m	16-17 S.P.	1-1 1/2 d	Poly mid-Sept. to mid-Jan.	145 144-148	1-3	4-5 yrs	12
SWINE	9-11 mos	21 P	2-3 d	Poly all year	114 112-116	6-16	3-4 yrs	10-12

* P = Polyestrus; S.P. = Seasonal Polyestrus; M = Monoestrus

** H = Harem mating (1 male (m) to # females (f)); Poly = Polygamous; Ph = Polygamous but usually hand mated, take (male to female) or (female to male); A.I. = Artificial Insemination; M.P. = Monogamous Pair, set up at weaning for life.

*** Optimal Reproductive Span - refers to that period of the female's life during which fertility and litter size are maximal and reproductive complications minimal. Breeding stock will usually be replaced at the end of this span of time for economic reasons.

APPENDIX III

PHYSIOLOGICAL AND NUTRITIONAL PARAMETERS*

SPECIES	Rectal Temp. $^{o}C \pm 0.5$	Resp. Rate/ Mean and (range)	Heart Rate/ Mean and (range)	Average Daily Water Consumption	Urine Excreted Daily	Daily Feed Recommendations	Digestible Protein** %
CAT	38.5	31 (20-40)	150 (110-226)	150 ml 100-200	50-120 ml	110-225 g	30
CATTLE	38.5	29 (26-35)	58 (46-55)	45-65 L	14-23 L.	7.5-12.5 kg	8.5-10
CHICKEN	39.5	(12-36)	300 (150-400)	ad lib		85-115 g	13-17
DOG	39.0	24 (20-34)	110 (77-138)	25-35 ml/kg body wt	65-400 ml breed dependent	250-1200 g breed dependent	20
FERRET	38.5	34 (33-36)	240 (200-400)	75-100 ml	26-28 ml	140-190 g	9.5
GERBIL	38.5	90 (70-120)	360 (260-600)	3-4 ml or green feed	few drops	10-15 g	15
GOAT	39.0	19 (12-35)	90 (70-135)	1.5-4 L	1-2 L	1-4 kg	15
GUINEA PIG	39.0	86 (42-104)	280 (230-380)	12-15 ml/ 100 g body wt	15-75 ml	20-35 g + Vit. C supp.	25-30
HAMSTER	39.0	77 (35-135)	332 (250-500)	8-12 ml	6-12 ml	7-15 g	16
HORSE	38.0	12 (10-14)	44 (23-70)	25-55 L	3-15 L	8-16 kg	5.5-14
MOUSE	37.5	138 (94-163)	470 (325-780)	3-7 ml	1-3 ml	3-6 g	12
NON-HUMAN PRIMATE Baboon (*Papio* sp)	39.0	25 (22-35)	115 (105-150)	400-600 ml	150-400 ml	1-1.5 kg + Vit. C supp.	17
Cynomolgus (*M. fascicularis*)	39.0	40 (30-54)	220 (165-243)	350-950 ml	150-550 ml	350-550 g + Vit. C supp.	17
OPOSSUM	34.5	36-65	(140-220)	100-200 ml		85-150 g	20-25
PIGEON	41.0	25-30	(140-244)	40-50 ml		25-75 g	10-15

RABBIT	39.0	40 (32-60)	260 (130-325)	80-100 ml/ kg body wt	50-90 ml/ kg body wt	75-100 g	14
RAT	37.0	92 (70-115)	350 (250-450)	20-45 ml	10-15 ml	10-20 g	12
SHEEP	39.5	25 (20-34)	76 (70-80)	600-1800 ml	400-1200 ml	1-2 kg	5
SWINE	39.0	40 (32-58)	70 (60-75)	4.5-6.5 L	2.5-4.5 L	1.5-3 kg	14

* Averages and ranges derived from literature mean values for **young adult animals** under various conditions (from various sources).

** Refers to (ideal or digestible protein required; crude protein (CP)) levels in most prepared laboratory animal diets may be considerably higher.

References

FOX, J. Biology and diseases of the ferret. Lea and Febiger, 1988.
FOX, J., COHEN, B. and LOEW, F. Laboratory animal medicine. Academic Press, 1984.
HARKNESS, J. and WAGNER, J. The biology and medicine of rabbits and rodents. Lea and Febiger, 1983.
HECKER, J.F. The sheep as an experimental animal. Academic Press, 1983.
MERCK VETERINARY MANUAL. 6th Ed. Merck and Co., 1986.
SWENSON, M. Dukes' Physiology of domestic animals. 10th Ed. Cornell Un. Press, 1984.

APPENDIX IV

HEMATOLOGY*

SPECIES	RBC X 10^{12}/L	Hb g/L	PCV L/L	Platelets X 10^9/L	WBC X 10^9/L	Neutrophils 10^9/L	Lymphocytes 10^9/L	Blood Vol. (ml/kg)
CAT	7.5 5.0-10.0	120 80-150	0.37 0.24-0.45	190-400	12.5 5.5-19.5	7.5 2.5-12.5	4.0 1.5-7.0	66.7 45-75
CATTLE	7.0 5.0-10.0	110 80-150	0.35 0.24-0.46	220-640	8.0 4.0-12.0	2.0 0.6-4.0	4.5 2.5-7.5	57 55-60
CHICKEN	3.0 2.5-3.5	90 70-130	0.3 0.22-0.35	14-60	12.0 12.0-30.0	3.0-6.0	14.0 7.0-17.5	83 60-90
DOG	6.8 5.5-8.5	150 132-193	0.45 0.38-0.57	145-440	11.5 6.0-17.0	7.0 3.9-12.0	2.8 1.0-4.8	83-101
GERBIL	8.5 7.0-10.0	150 121-169	0.48 0.41-0.52	638	4.3-21.6	0.3-4.1	3.2-9.7	60-85
GOAT	13.0 8.0-18.0	100 80-120	0.35 0.24-0.48	250-750	9.0 4.0-13.0	3.2 1.2-7.2	5.0 2.0-9.0	70 55-80
GUINEA PIG	5.2 4.8-5.9	110-140	0.43 0.37-0.46	450-630	3.8-13.5	2.6 2.0-3.1	6.4-7.5	65-90
HAMSTER	7.5 5.0-9.2	168 146-200	0.5 0.46-0.52	300-570	7.6 5.0-10.0	1.5-3.5	6.1-7.0	65-80
HORSE	9.0 6.8-12.9	144 111-190	0.41 0.32-0.53	80-397	9.0 5.4-14.3	4.7 2.3-8.6	3.9 1.7-6.8	72 75-100
MOUSE	9.1 7.9-10.1	110-145	0.37-0.46	600-1200	5.0-13.7	0.4-2.7	7.1-9.5	70-80
NON-HUMAN PRIMATES Baboon (*Papio* sp)	5.0 4.0-6.0	120 90-150	0.42 0.36-0.49	135-400	3.0-11.4	2.7-7.3	2.6-5.9	50-70
CYNOMOLGUS (*M. fascicularis*)	5.0 3.9-7.1	116-145	0.38-0.50	90-140	8.1-21.3	1.3-8.1	3.5-8.3	55-75
OPOSSUM	5.0 3.4-7.1	121-198	0.42 0.30-0.58	235-1235	3.0-27.0	1.5-6.5	1.9-9.2	45-65
QUAIL	4.7 4.0-5.5	110-150	0.42 0.3-0.45		12.5-25.0	2.5-5.0	5.0-7.0	55-75
RABBIT	6.5 4.5-8.5	94-175	0.40 0.31-0.50	468 180-750	4.0-13.0	3.0-5.2	2.8-9.0	57-65

RAT	5.4-8.5	115-160	0.37-0.49	450-885	4.0-10.2	1.3-3.6	5.6-8.3	50-65
SHEEP	12.0 9.0-15.0	115 90-150	0.35 0.27-0.45	250-750	8.0 4.0-12.0	2.4 0.7-6.0	5.0 2.0-9.0	58-66.4
SWINE	6.5 5.0-8.0	130 100-160	0.42 0.32-0.50	300-700	16.0 11.0-22.0	4.0-7.5	6.0-10.0	52-69

* The normal values may vary according to age, sex, breed and function of animals.

References

ARCHER, R.K., JEFFCOTT, L.B. and LEHMANN, H. Comparative clinical haematology. Blackwell Scientific Publications, 1977.
FOX, J.G., COHEN, B.J. and LOEW, F.M. Laboratory animal medicine. Academic Press, 1984.
HAWKEY, C.M. Comparative mammalian haematology; cellular components and blood coagulation of captive wild animals. William Heinemann Medical Books Ltd., 1975.
JAIN, N.C. Schalm's Veterinary Haematology. 4th Ed. Lea and Febiger, 1986.
MITRUKA, B.M. and RAWNSLEY, H.M. Clinical biochemical and hematological reference values in normal experimental animals. New York, Paris, Barcelona, Milan: Masson Publishing USA Inc., 1977.
SWENSON, M.J. Dukes' physiology of domestic animals. 10th Ed. Cornwell Un. Press, 1984.
UNIVERSITY OF GUELPH. Veterinary teaching hospital (Manual), 1988.

APPENDIX V

CLINICAL BIOCHEMISTRY REFERENCE VALUES[a]

SPECIES	Glucose mmol/L	Urea mmol/L	Cholesterol Total mmol/L	Protein			Aspartate Amino-transferase (AST, SGOT) U/L	Alanine Amino-transferase (ALT, SGPT) U/L	Alkaline Phosphatase U/L
				Total g/L	Albumin g/L	Globulin g/L			
CAT[b]	3.89-6.11 (5.05 ±0.42)	14.28-21.42	2.46-3.37	54-78 (66 ±7)	21-33 (27±2)	26-51 (39±7)	26-43 (35±9)	6-83 (26±16)	25-93 (50±35)
CHICKEN[b]		(9.30)		(56)	(25)	(31)	(175)		
COW[b]	2.5-4.16 (3.19 ±0.38)	14.28-21.42	2.07-3.11	67-75 (71 ±2)	30-35 (33±1)	30-35 (32±2)	78-132 (105±27)	14-38 (27±14)	0-488 (194±126)
DOG[b]	3.61-6.55 (5.05 ±0.67)	7.14-19.99 (12.14 ±2.86)	3.50-6.99 (4.61±0.98)	54-71 (61 ±5)	26-33 (29±2)	27-44 (34±5)	23-66 (33±12)	21-102 (47±26)	20-156 (66±36)
GOAT[b]	2.78-4.16 (3.49 ±0.39)	7.14-14.28 (10.71 ±1.43)	2.07-3.37	64-70 (69 ±5)	27-39 (33±3)	27-41 (36±5)	167-513	24-83	93-387 (219±76)
GUINEA PIG[c] Hartley (500-800g)	4.94-5.29 (5.12)	15.35-17.99 (16.67)		48-56 (52)	24-27 (25)		46-48 (47)	38-45 (41)	66-74 (70)
HAMSTER[c] Syrian (100g)	3.61-4.07 (3.84)	14.85-21.49 (18.333.08)	4.71-6.13 (5.42)	64-73 (675)	32-37 (352)		53-124 (7932)	21-50 (3511)	8-18 (135)
HORSE[b]	4.16-6.39 (5.30 ±0.47)	7.14-17.14	1.94-3.89 (2.88±0.04)	52-79 (63 ±6)	26-37 (31±3)	26-40 (33±7)	226-366 (296±70)	3-23 (14±11)	143-395 (244±101)
MOUSE[d] CD-1 [Crl:CD-1 (ICR)BR][e]	9.71-18.60 (15.00)	12.14-20.59 (16.07)	1.27-2.48 (1.89)	42-60 (51)	21-34 (28)	18-82 (22)	55-251 (139)	28-184 (95)	28-94 (67)
CF-1 [Crl: CF-1BR][e]	9.10-20.48 (14.46)	8.57-19.99 (14.99)	2.72-4.16 (3.49)	54-65 (60)	30-40 (35)	18-31 (24)	30-314 (177)	76-208 (143)	67-303 (167)
B6C3F1 [B6C3F1/ CrlBR][f]	7.6-26.0 (17.3)	4.3-13.5 (7.85)	1.53-3.63 (2.29)	47-60 (52)	26-34 (30)	17-29 (22)	0-111 (43)		46-289 (207)
NON-HUMAN PRIMATE Baboon (*Papio* sp)[c]	(6.72 ±1.16)			(63 ±6)	(37±4)		(25±3)	(16±4)	
Cynomolgus (*M. fascicularis*)[g]	2.20-4.70	3.80-10.00	1.91-4.52	68-86	34-45	27-47	9-68	0-138	102-1163

Rhesus (*M. mulatta*)[c]	(3.89 ±0.57)	12.07-14.85 (13.46)	3.31-4.43 (3.87)	66-80 (70 ±8)	43-44		27-79 (55±27)	27-42 (35)	(149)
PIG[b]	4.72-8.33 (6.61 ±0.96)	7.41-21.42	0.93-1.40	79-89 (84 ±7)	(26±7)	53-64 (59±6)	32-84 (61±26)	31-58 (45±14)	118-395 (194±84)
RABBIT[b]	2.78-5.18 (4.08 ±0.53)	(10.212.14)	0.14-1.86 (0.69±0.41)	(64 ±3)	(27±3)		(47)	(79)	(120±14)
RAT[d] Wistar[Crl:(W)BR][h]	4.71-7.33 (6.22)	11.42-19.28 (14.64)	1.20-2.38[f] (1.79)	63-86 (73)	33-49 (47)	24-39 (31)	39-92 (64)	17-50 (32)	39-216 (123)
F-344[i] [CDF(F-344) CrlBR]	4.24-20.04 (10.85)	7.85-19.99 (10.00)	0.54-2.22 (1.29)	60-78 (66)	34-43 (39)	24-35 (29)	56-436 (233)	108-375 (232)	147-399 (248)
CD[Crl:CD (SD)BR][j]	5.55-16.71 (11.69)	9.28-22.13 (14.64)	1.18 (0.52-1.914)	59-79 (70)	28-44 (38)	26-39 (32)	39-262 (129)	110-274 (216)	46-264 (161)
SHEEP[b]	2.78-4.44 (3.80 ±0.33)	5.71-14.28	1.34-1.97 (1.66±0.31)	60-79 (72 ±5)	24-30 (27±2)	35-57 (44±5)	(307±43)	(30±4)	68-387 (178±102)

[a] Ranges with the means and standard deviations in parenthesis. Reported in S.I. units.

[b] KANEKO, J.J., ed. Clinical chemistry of domestic animals. Academic Press, 1989: 886-891.

[c] LOEB, W.F. and QUIMBY, F.W., eds. The Clinical Chemistry of Laboratory Animals. Pergamon Press, 1989: 417-476.

[d] Sexes combined, 19-21 weeks.

[e] Baseline haematology and clinical chemistry values for Charles River outbred mice: Crl:CD-1(ICR)BR. Crl:CF-1BR. Charles River Laboratories Techn. Bull., 1986.

[f] Values from Parke Davis Research Institute, Mississauga, Ontario.

[g] CLARKE, D., TUPASI, G., WALKER, R. and SMITH, G. Stability of serum biochemical parameters in Beagle Dogs and Cynomolgus monkeys. Clin. Chem. Newsl. (In press).

[h] Baseline haematology and clinical chemistry values for Charles River Wistar rats (CRL:(W)BR) as a function of sex and age. Charles River Techn. Bull., Vol. 1, No. 2, 1982.

[i] Baseline haematology and clinical chemistry calues for Charles River Fischer-344 rats - CDF(F-344)CrlBR as a function of sex and age. Charles River Techn. Bull., Vol. 3, No. 1, 1984.

[j] Baseline haematology and clinical chemistry values for Charles River CD[Crl:CD(SD)BR] rats as a function of sex and age. Charles River Techn. Bull., Vol. 3, No. 2, 1984.

APPENDIX VI

SERUM ELECTROLYTE REFERENCE VALUES[a]

SPECIES	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Bicarbonate mmol/L	Phosphorus mmol/L	Calcium mmol/L	Magnesium mmol/L
CAT[b]	147-156 (152)	4.0-4.5 (4.3)	117-123	17-21	1.45-2.62 (2.00)	1.55-2.55 (2.06 ±0.24)	(0.90)
CHICKEN[b]					(2.52)	(7.10)	
COW[b]	132-152 (142)	3.9-5.8 (4.8)	97-111 (104)	17-29	1.81-2.10	2.43-3.10 (2.78 ±0.15)	0.74-0.95 (0.84±0.10)
DOG[b]	141-152	4.37-5.35	105-115	18-24	0.84-2.00 (1.39±0.29)	2.25-2.83 (2.55 ±0.15)	0.74-0.99 (0.86±0.12)
GOAT[b]	142-155 (150 ±3.1)	3.5-5.4	99-110 (105 ±2.9)		(4.62±0.25)	2.88-3.20 (2.58 ±0.15)	0.90-0.31 (1.03±0.12)
GUINEA PIG[c] Hartley (500-800g)	122-125	4.7-5.3	92-97	22-24	1.71-1.72	2.40-2.67	0.97-1.01
HAMSTER[c] Syrian (100g)	128-145	4.9-5.1	94-99	(30±2.9)	1.71-2.13	2.60-3.09	0.91-1.03
HORSE[b]	132-146 (139 ±3.5)	2.4-4.7 (3.5±0.6)	99-109 (104 ±2.6)	20-28	1.00-1.81	2.80-3.40 (3.10 ±0.14)	0.19-1.15 (1.03±0.13)
MOUSE[d] CD-1 [Crl:CD-1(ICR) BR][e]	143-150 (148)	3.8-10.0 (6.3)	96-111 (105)		2.68-3.62 (3.08)	2.77-3.02 (2.90)	
CF-1 [Crl:CF-1BR][e]	139-157 (148)	4.8-8.9 (6.9)	104-119 (111)		2.91-4.65 (3.76)	2.25-2.89 (2.57)	
NON-HUMAN PRIMATE Baboon (*Papio* sp)[c]	(142 ±3.5)	(3.8±0.5)	(107 ±3.7)		(2.26±0.48)	(2.10 ±0.02)	
Cynomolgus (*M. fascicularis*)	142-153[f] (149)	3.0-4.8[f] (3.9)	101-112[f] (107)		1.18-2.30[g]	2.17-2.55[g] (2.36)	
Rhesus (*M. mulatta*)[c]	154-158	3.6-4.6	110-114		1.41-1.62	2.42-2.70	0.68-0.75

PIG[b]	135-152	4.4-6.7	94-106	18-27	1.71-3.10	1.78-2.90 (2.41 ±0.25)	1.11-1.52 (1.31±0.20)
RABBIT[b]	(141 ±0.93)	(5.30.5)	85-105.3 (96.5 ±6.7)	(47)	(1.34±0.15)	1.46-3.60	(0.92±0.07)
RAT[d] Wistar[Crl:(W) BR]	141-150[h] (145)	5.2-7.8[h] (6.2)	99-114[h] (106)		1.99-3.77[f] (2.95)	2.67-3.43[f] (3.05)	1.07-1.28[c]
F-344[i] [CDF(F-344) CrlBR]	139-150 (145)	3.9-7.5 (5.7)	82-99 (93)		2.42-5.62 (4.13)	2.47-3.32 (2.82)	
CD[Crl:CD(SD) BR][j]	139-150 (145)	3.6-8.4 (5.7)	84-99 (93)		2.42-5.62 (4.13)	2.47-3.22 (2.82)	0.66-1.79[c]
SHEEP[b]	139-152	3.9-5.8	95-103	20-25	1.62-2.36	2.88-3.20 (2.78 ±0.07)	0.90-0.31 (1.03±0.12)

[a] Ranges with the means and standard deviations in parenthesis. Reported in S.I. units.
[b] KANEKO, J.J., ed. Clinical chemistry of domestic animals. Academic Press, 1989: 886-891.
[c] LOEB, W.F. and QUIMBY, F.W., eds. The clinical chemistry of laboratory animals. Pergamon Press, 1989: 417-476.
[d] Sexes combined, 19-21 weeks.
[e] Baseline haematology and clinical chemistry values for Charles River outbred mice: Crl:CD-1(ICR)BR. Crl:CF-1BR. Charles River Laboratories Techn. Bull., 1986.
[f] Values from Parke Davis Research Institute, Mississauga, Ontario.
[g] CLARKE, D., TUPASI, G., WALKER, R. and SMITH, G. Stability of serum biochemical parameters in beagle dogs and cynomolgus monkeys. Clin. Chem. Newsl. (In press).
[h] Baseline haematology and clinical chemistry values for Charles River Wistar rats (CRL:(W)BR) as a function of sex and age. Charles River Techn. Bull., Vol. 1, No. 2, 1982.
[i] Baseline haematology and clinical chemistry values for Charles River Fischer-344 rats - CDF(F-344)CrlBR as a function of sex and age. Charles River Techn. Bull., Vol. 3, No. 1, 1984.
[j] Baseline haematology and clinical chemistry values for Charles River CD[Crl:CD(SD)BR] rats as a function of sex and age. Charles River Techn. Bull., Vol. 3, No. 2, 1984.

APPENDIX VII

ZOONOSES--EXPERIMENTAL ANIMALS TO MAN

A. BACTERIAL DISEASES:

Disease in Man	Causative Agent	Vertebrate Hosts[1]	Means of Spread	Vectors and Notes on Cycle
Anthrax Woolsorters disease	*Bacillus anthracis*	Farm animals wild and zoo animals	contact, inhalation, ingestion	Spores: long lived in soil
Brucellosis[2] Undulant Fever Malta Fever Zang's disease	*B.suis*	swine	contact and ingestion of milk, milk products, raw meat	
	B.abortus	cattle, sheep, buffalo		
	B.melitensis	sheep, goats	direct contact primarily with semen	
	B.ovis	sheep		
	B.canis	dogs	contact with infected semen, fetuses, fetal membranes and vaginal secretions	
Campylobacteriosis	*C.fetus* *C.jejuni*	cattle, sheep, pigs, dogs, non-human primates, poultry	ingestion	may survive inadequate heating
Chlamydiosis[3] Psittacosis	*Chlamydia* spp.	Psittacine birds, poultry, pigeons	inhalation	recovered nestlings
Colibacillosis[4]	*E.coli*	cattle, swine, poultry, misc. animals	ingestion	
Leptospirosis Weil's disease	*Leptospira* spp.	rodents, dogs, farm and wild animals	contact, urine contaminated soil and water	
Pasteurellosis	*P.multocida* *P.hemolytica* *P.pneumotropica*	cats, dogs, rabbits, misc. mammals, birds	contact, bite wounds, inhalation	
Plague	*Yersinia pestis*	rodents	contact, flea bites, inhalation	fleas
Pseudotuberculosis	*Yersinia pseudotuberculosis*	rodents, lagomorphs, pigeons, turkeys, canaries, wild birds	contact, contaminated food and water ingestion	
Rat Bite Fever	*S.moniliformis* *Spirillum minus*	rodents	rodent bites, ingestion	infected saliva
Salmonellosis	*Salmonella* spp.	farm animals, rodents, reptiles, amphibians, zoo and wild animals	ingestion, inhalation, contact	

Shigellosis Bacillary dysentery	*Shigella* spp.	non-human primates	contact, fecal contamination, ingestion	direct or by fomites
Tetanus[5]	*Cl.tetani*	dog, cat, equine spp.	bite wounds, contaminated puncture wounds	soil
Tuberculosis	*M.tuberculosis* *M.bovis* *M.avium*	non-human primates, cattle, dogs cattle, dogs poultry, swine, sheep	contact, ingestion, inhalation	Anthropozoonotic[6]
Tularemia Rabbit fever	*F.tularensis*	lagomorphs, wild rodents, birds, dogs	inhalation contact, tick and insect bites, ingestion of contaminated food and water	biting insects and ticks

B: RICKETTSIAL DISEASES:

Causative Agent	Diseases in Man	Common Vertebrate Hosts[1]	Means of Spread, Vectors, Cycle Notes
Coxiella[8]	Q fever	cattle, sheep, goats	inhalation, ingestion of contaminated raw milk, blood sucking anthropods, contact with amniotic fluid or placenta
R.akari	Rickettsial pox	wild mice, rats	mite bites: *A. sanguineus*
R.rickettsia	Rocky mountain spotted fever	wild rodents, rabbits, dogs	tick bites: *Dermacentor* spp., American dog tick
R.siberica	Asian tick fever	various wild rodents	tick bites: ticks themselves may act as reservoirs with tick to tick passage
R.typhi	Murine typhus	wild mice, rats	flea bites from rat fleas, rat to rat spread by lice, ingestion of contaminated food

C. ARBOVIRUS DISEASES:

Causative Agent	Diseases in Man	Common Vertebrate Hosts[1]	Means of Spread, Vectors, Cycle Notes
Asian arboviruses	various tickborne hemorrhagic fevers	wild rodents, hares, wild-caught monkeys	tick bites, sub-tropical climate conditions favour cycle
California encephalitis	California encephalitis	wild rabbits, rodents	natural cycle wild rabbits and rodents/ mosquito
Colorado tickborne virus	Colorado tick fever	ground squirrels, *Deromyscus* spp.	tick bite, tick/small rodent natural cycle
E.E.E.	Eastern equine encephalitis	horses, birds	mosquito bites: bird/mosquito/horse natural cycle

Powassan virus	Powassan encephalitis	wild rabbits, rodents	tick bites
S.L.E.	St. Louis encephalitis	birds	natural cycle bird/mosquito only
V.E.E.	Venezuelan equine encephalitis	horses	natural cycle horse/mosquito only
W.E.E.	Western equine encephalitis	horses, birds	mosquito bites: bird/mosquito/horse natural cycle

D. OTHER VIRUS DISEASES:

Causative Agent	Diseases in Man	Common Vertebrate Hosts[1]	Means of Spread, Vectors, Cycle Notes
Filovirus	Marburg disease Ebola hemorrhagic fever	African green monkey *Macaca* sp.	direct contact with monkey tissues
Hemorrhagic fever virus	S. American and Korean hemorrhagic fever	wild rodents *Mastomys ratalensis*	contact, contamination of food, etc., with rodent excreta; direct contact
Hepatitis virus	Hepatitis A	chimpanzees	contact, anthropozoonotic diseases[9]
Herpes simiae	Herpes B. encephalitis	rhesus; other Macaca	contact, bite wounds, Old World monkeys
L.C.M. virus	Lymphocytic Chorio--Meningitis	rodents; numerous other mammals	contact, inhalation; congenital transmission, tissue culture transmission
Rabies virus	Rabies	dogs, cats, bats and many others	bites; saliva contact, virus concentrate in saliva

E. FUNGAL AND PROTOZOAN DISEASES:

Causative Agent	Diseases in Man	Common Vertebrate Hosts[1]	Means of Spread, Vectors, Cycle Notes
Balantidium coli	Balantidiasis	non-human primates	ingestion by contamination of food or fomites
Coccidioides immitis	Coccidioidomycosis	cattle, dogs and occasionally other spp.	inhalation of air-borne spores; fungus present in desert soil
Entamoeba histolytica	Amebiasis Amebic dysentery	non-human primates, dogs	contamination of food, usually by man (natural host) to dogs
Giardia intestinalis	Giardiasis	non-human primates, dogs, beaver	man is main reservoir, ingestion of cysts in contaminated water or food
Histoplasma capsulatum	Histoplasmosis	dogs, other domestic and wild species	inhalation of fungi; may also grow in soil

Toxoplasma gondii	Toxoplasmosis	cats; occasionally other domestic and lab spp.	ingestion of oocysts from cats; inhalation infected meat; fetal transmission may occur
Trichophyton spp. *Microsporum* spp. Other dermatophytes	Ringworm, dermatomycosis	dog, cat, guinea pig, other rodents and farm animals, rabbits	direct contact, ringworm of man can be transmitted to animals and visa-versa; soil may be reservoir
Trypanasoma spp. *Plasmodium* spp. *Leishmania* spp.	Blood protozoan diseases	non-human primates, rodents, domestic and wild spp.	insect vectors--saliva transmission; some few species direct transmission

[1] Only more common host species are listed.

[2] *Brucella abortus* has also been reported in bactrian and dromedary camels, alpacas, and caribou. *B.suis* has been reported in African rodents, European hares (it is the reservoir). Brucellosis has also been reported in desert rats in the U.S. and in foxes and mustelids in S. America.

[3] One case of cat to human transmission causing conjunctivitis.

[4] *E.coli* has many serotypes; those with capsular K antigen are especially pathogenic to man and animals. Some serotypes are species specific. Man is the main reservoir of colibacillosis for humans with the route of infection the handling of human feces or not washing hands after using the bathroom.

[5] Tetanus is not considered a true zoonoses.

[6] Man is the primary vertebrate host.

[7] In addition to the G.I. signs, this organism is associated with abortion in women.

[8] Organism concentrated in placenta and fetal membranes and fluids.

[9] Man is primary host. Measles (Rubeola) is another anthropozoonotic virus to non-human primates.

Reference

ACHA, P.N. and SZYFRES, B. Zoonoses and communicable diseases common to man and animals. Washington, DC: Scientific Publishers No. 503, World Health Organization, 1989.

APPENDIX VIII

COMMON BLEEDING SITES

SPECIES	ANT, VENA CAVA	CEPHALIC VEIN	EAR VEIN	FEMORAL VEIN	HEART	JUGULAR VEIN	ORBITAL SINUS	TAIL V/A	TOE/ TAIL NICK	WING VEIN
CAT		Xc		Xc,r,t		Xc,r,t				
COW						Xj,t		Xt		
BIRD					Xc,f	Xc				Xc,f
DOG		Xc,r,t,u		Xc		Xc,r,t,u				
FERRET					Xl	Xl		Xl	Xl	
FISH					Xe				Xe	
FROG					Xg					
GERBIL					Xv		Xv	Xv		
GUINEA PIG	Xaa		Xn,o		Xo		Xo		Xc,o	
HAMSTER	Xx		Xp		Xp,x		Xx		Xx	
NHP				Xc,w		Xc				
MOUSE					Xc,h,i, n,s		Xc,h,n,s	Xh,s	Xc,i,n, s	
OPOSSUM						Xk		Xk		
RABBIT			Xa,c, b,n		Xa,c,b					
RAT					Xc,n,z, y	Xy	Xc,n,z,y	Xd,y	Xc,n,z, y	
SMALL RUMINANT		Xt				Xt				
SNAKE					Xm					
PIG	Xq,t		Xq,t							
TURTLE					Xm				Xm	

X Recognized bleeding site, followed by reference.

References

[a] ADAMS, C.E. The laboratory rabbit. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 415-435.

[b] BIVIN, W.S. et TIMMONS, E.H. Basic biomethodology. In: Weisbroth, S.H., Flatt, R.E. and Kraus, A.L., eds. The biology of the laboratory rabbit. New York, NY: Academic Press, 1974: 74-89.

[c] BIVIN, W.S. et SMITH, G.D. Techniques of experimentation. In: Fox, J.G., Cohen, B.J. and Loew, F.L., eds. Laboratory animal medicine. Orlando, FL: Academic Press, 1984: 564-588.

[d] BOBER, R. Technical review: Drawing blood from the tail artery of a rat. Lab. Anim. July/August, 1988: 33-34.

[e] CAMPBELL, T.W. Fish cytology and hematology. In: Stoskopf, M.K., ed. The Veterinary Clinics of North America. Small Animal Medicine. Tropical Fish Medicine. Philadelphia, PA: W.B. Saunders, 1988: 349-364.

[f] COOPER, D.M. et HARRY, E.G. The domestic fowl and turkey. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 640-662.

[g] CRAWSHAW, G.J. Amphibian medicine. In: Kirk, R.W. and Bonagura, J.D., eds. Current veterinary therapy XI small animal practice. Philadelphia, PA: W.B. Saunders, 1992: 1219-1230.

[h] CUNLIFFE-BEAMER, T.L. Biomethodology and surgical techniques. In: Foster, H.L., Small, J.D. and Fox, J.G., eds. The mouse in biomedical research. Vol. III. Normative biology, immunology, and husbandry. New York, NY: Academic Press, 1983: 402-439.

[i] CUNLIFFE-BEAMER, T.L. et LES, E.P. The laboratory mouse. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 275-308.

[j] EWBANK, T. Cattle. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 525-534.

[k] FINNIE, E.P. Monotremes and marsupials. Restraint. In: Fowler, M.E., ed. Zoo and wild animal medicine. Philadelphia, PA: W.B. Saunders, 1986: 181-184.

[l] FOX, J.G. Anesthesia and surgery. In: Fox, J.G., ed. Biology and diseases of the ferret. Philadelphia, PA: Lea and Febiger, 1988: 289-302.

[m] FRYE, F.L. Hematology of captive reptiles. In: Fowler, M.E., ed. Zoo and wild animal medicine. Philadelphia, PA: W.B. Saunders, 1986: 181-184.

[n] HARKNESS, J.E. et WAGNER, J.E. Clinical procedures. In: The biology and medicine of rabbits and rodents. Philadelphia, PA: Lea and Febiger, 1989: 55-76.

[o] HOAR, R.M. Biomethodology. In: Wagner, J.E. and Manning, P.J., eds. The biology of the guinea pig. New York, NY: Academic Press, 1976: 13-17.

[p] HOBBS, K.R. Hamsters. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 377-410.

[q] HOLTZ, W. Pigs and minipigs. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 496-515.

[r] KIRK, R.W., BISTNER, S.I. et FORD, R.B., eds. Routine diagnostic procedures. In: Handbook of veterinary procedures and emergency treatment. 5th Ed. Philadelphia, PA: W.B. Saunders, 1990: 447-455.

[s] KRAUS, A.L. Research methodology. In: Baker, H.J., Lindsey, J.R. and Weisbroth, S.H., eds. The laboratory rat. Vol. II. Research applications. New York, NY: Academic Press, 1980: 5-10.

[t] LUCAS, M.J. et LUCAS, S.E. Diagnostic sampling and treatment techniques. In: McCurrin, D.M., ed. Clinical Textbook for Veterinary Technicians. 2nd Ed. Philadelphia, PA: W.B. Saunders, 1990: 191-209.

[u] MACARTHUR, J.A. The dog. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 456-473.

[v] NORRIS, M.L. Gerbils. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 360-376.

[w] OLSON, M.E., MORCK, D.W. et NABROTZKY, V.C.A. Manual of standard operating procedures for animal facilities. Calgary, Alberta: University of Calgary, 1992.

[x] SILVERMAN, J. Biomethodology. In: Van Hoosier, G.L. and McPherson, C.W., eds. Laboratory hamsters. Orlando, FL: Academic Press, 1987: 7-91.

[y] WAYNFORTH, H.B. et FLECKNELL, P.A. Methods of obtaining body fluids. In: Experimental and surgical techniques in the rat. 2nd Ed. London, U.K.: Academic Press, 1992: 68-87.

[z] WEIHE, W.B. The laboratory rat. In: Poole, T.B., ed. UFAW (Universities Federation for Animal Welfare) handbook on the care and management of laboratory animals. 6th Ed. Harlow, Essex: Longman Scientific and Technical, 1987: 309-330.

[aa] WHORTON, J.A. Collecting blood via the anterior vena cava in the guinea pig. Lab Animal 1982; 11(6): 66-68.

APPENDIX IX

TRANQUILLIZER, SEDATIVE AND ANTICHOLINERGIC DRUG DOSAGES

SPECIES	Acepromazine Maleate (Atravet)		Xylazine (Rompum)[d]		Midazolam[a] (Versed)		Diazepam[a] (Valium)		Atropine Sulfate[c]		Glycopyrrolate[c]	
	Mg/Kg	Route[b]	Mg/ Kg	Route	Mg/ Kg	Route	Mg/ Kg	Route	Mg/Kg	Route	Mg/Kg	Route
CAT	0.2-0.5 1-3	IM IV PO	1-3	IM SC	0.2-0.5	IM IV	1.0-max 5 mg		0.02-0.05	SC IM IV	0.011 0.005	IM IV
CATTLE	0.1	IV	0.1	IM					not effective			
DOG	0.1-0.5	IM IV SC	1-2	IM	0.2-0.5	IM IV	1.0 max 20.0	IM IV	0.02-0.05	SC IM IV	0.011 0.005	IM IV
GUINEA PIG					5.0	IP	2.5	IM IP	0.02-0.05	SC IM IV		
HAMSTER/ GERBIL					5.0 5.0	IP IP	5.0	IP	0.02-0.05	SC IM IV		
MOUSE					5.0	IP	1.0	IM IV	0.1-0.2	SC IM IV		
NON-HUMAN PRIMATE	0.5-1	SC IM	1-2	IM			1.0	IM IV	0.05	SC IM IV		
RABBIT	1.0	SC IM	1-3.0	IM	2.0	IP	1.0	IM IV	0.1-0.2	SC IM IV	0.1	SC
RAT					2.5	IP	2.5	IP	0.02-0.05	SC IM IV		
SHEEP/ GOAT	0.1-0.2	IM IV	1.0 0.05	IM IM					0.05	SC IM		
SWINE	0.2	IM IV					1-2	IM IV	0.05-0.1	SC IM IV		

[a] The tranquillizers listed are not marketed in Canada under veterinary labels. These are human Schedule F, Part II (H&W Canada)
drugs requiring prescription.

[b] PO = oral; SC = subcutaneous; IM = intramuscular; IP = intraperitoneal; IV = intravenous.

[c] Atropine and Glycopyrrolate should be administered 35-50 minutes prior to surgery, SC or IM.

[d] Xylazine--is an analgesic as well as a sedative.

APPENDIX X

ANALGESIC DRUG DOSAGES

SPECIES	Acetylsalicylic Acid (Aspirin)		Meperidine HCl (Pethidine) (Demerol)		Fentanyl + Droperidol** (Innovar-vet)*		Morphine		Butorphanol		Buprenorphine	
	Mg/ Kg Route	Duration	Mg/ Kg Route	Duration	ml/kg Route	Duration	Mg/ Kg Route	Duration	Mg/ Kg Route	Duration	Mg/ Kg Route	Duration
CAT			2-6 IM SC	2-3 hr			0.05-0.1 SC	4 hr	0.4 SC	3-4 hr	0.005-0.01 SC IV	8-12 hr
CATTLE												
DOG	10 PO	8-12 hr	2-6 SC IM	1-2 hr	0.2-0.5 IM		0.3-2.0 SC IM	2-4 hr	0.2-0.4 SC IM	3-4 hr	0.01-0.02 IM SC IV	8-12 hr
GOAT			up to 200 total dose IM	4 hr			up to 10 total dose IM	4 hr			0.005 SC IM	8-12 hr
GUINEA PIG	85 PO	4 hr	10-20 SC IM	2-3 hr			2-5 SC	2-4 hr			0.05 SC	6-12 hr
HAMSTER					0.02-0.05 ml/ 100 g IM 0.1 of 1:10 dilution IP						0.5 SC	6-8 hr
MOUSE	120-300 PO	4 hr	10-20 SC IM	2-3 hr	0.02-0.05 ml/ 100 g IM		2-5 SC	2-4 hr	1-5 SC	4 hr	0.05-0.1 SC	6-8 hr
NON-HUMAN PRIMATE	10-20 PO	6 hr	2-4 IM	3-4 hr	0.05-0.2 IM	required dosages vary greatly with different NHP species	1-2 SC IM	4 hr	0.025 IM	4 hr	0.01-0.05 IM IV	8-12 hr
RABBIT	10 PO	4 hr	10-20 SC IM 5 IV	2 hr 2-4 hr	0.15-0.3 IM		2-5 SC IM	2-4 hr	0.1-0.5 IV	4 hr	0.02-0.05 SC IV IM	8-12 hr
RAT	100 PO	4 hr	10-20 SC IM	2-3 hr	0.10-0.25 IM 0.2-0.5 IM	Sedation/ Anesthesia. Dilute to 10% solution prior to administra-tion	2-5 SC	2-4 hr	2 SC	4 hr	0.01-0.5 SC	8-12 hr

SHEEP			up to 200 total dose IM	4 hr			up to 10 total dose IM	4 hr			0.005 IM	4-6 hr
SWINE	10 PO	4 hr	2 IM	4 hr	0.5 IM 0.03 IV		up to 10 total dose IM	4 hr	0.1-0.3 IM	4 hr	0.1 IV IM	8-12 hr

* Innovar-vet = Fentanyl 0.4 mg/ml + Droperidol 20 mg/ml. ** Neurolepranalgesic.

References

Anesthesia and analgesia in laboratory animals. In: Proc. American College of Laboratory Animal Medicine (ACLAM) Forum, 1990.
FLECKNELL, P. Laboratory animal anesthesia. Orlando, FL: Academic Press, 1987.
FLECKNELL, P. Anesthesia of laboratory animals. Short course. University of Guelph, Guelph, Ontario 1992.

APPENDIX XI

INJECTABLE ANESTHETIC AGENTS--DOSAGE

SPECIES	Pentobarbital		Thiopental		Ketamine HCl[a]		Urethane[b]		Ketamine/ Xylazine		Alphaxalone/ Alphadolone (Saffan)[c]	
	Mg/Kg	Route	Mg/ Kg	Route	Mg/ Kg	Route	Mg/ Kg	Route	Mg/ Kg	Route	Mg/Kg	Route
CAT	25	IV	10-15	IV	20	IM	1250	IV	15/ 1	IM IM SC	9-12 12-18	IV IM
DOG	20-30	IV	25	IV			1000	IV			contraindicated	
GOAT	30	IV	15	IV	20	IM						
GUINEA PIG	37	IP	20	IV	100-200	IM	1500	IP IV	40-100/ 4-5	IM IM SQ	40	IP
HAMSTER	50-90	IP	20-40	IV IP							150	IP
MOUSE	30-40	IP	30-40	IV IP	100-200	IM			200/ 10	IM IP	10-15	IV
NON-HUMAN PRIMATE	5-15	IV	15-20	IV	5-25	IM			7/ 0.6	IM IM	6-9 12-18	IV IM
RABBIT	45	IV	20	IV	50	IM	1000	IV IP	35-50/ 5-10	IM IM	6-9	IV
RAT	40	IP	20-40	IV IP	60-100	IM	1000	IP	90/ 5-10	IP IM IP IM	10-12	IV
SHEEP	30	IV	15	IV	20	IM						
SWINE	30	IV	6-8	IV	10	IM			20/ 2	IM IM	2 5	IV IM

[a] Ketamine useful for birds at 15-20 mg/kg for immobilization and from 40-100 mg/kg for anesthesia in healthy birds, alone or in combination with a suitable tranquillizer.

[b] May only be used for non-survival surgery--gives prolonged anesthesia. CAUTION: Urethane is carcinogenic.

[c] Saffan useful as anesthetic for birds given IV rapidly at 12-14 mg/kg body weight.

APPENDIX XII

ANESTHETIC AND SEDATIVE DRUG DOSAGE--AMPHIBIANS AND REPTILES*

Species	Agent	Dosage and Administration
Chemical Restraint of Amphibians		
Frog	MS-222 (Tricaine methane-sulfonate)	1:1000 adult by immersion 1:5000 juvenile by immersion - prepare 1:1000 stock solution by adding 1 gm MS-222 to 1 liter of water - buffer with 2 grams Na bicarbonate; use of unbuffered acidic MS-222 has caused acidosis, increased BUN,ACTH and cholesterol and is believed to be a stressor in other aquatic species - for recovery use administer at holding or ambient temperature to avoid shock and use an airstone to oxygenate solution, induction 15 minutes - for rapid induction, use warmed solution; however, may induce shock - for maintenance, dilute induction concentration 50 percent, cover animal with paper towel soaked in solution - recovery 30 to 90 minutes
Newt/ Salamander	MS-222 (Tricaine methane-sulfonate)	1:2000-1:7500 by immersion - prepare and administer solution as for frog - induction more rapid; 3-5 minutes
	Benzocaine	1:10,000 by immersion - dissolve 100 mg crystalline solid in 5 ml ethanol, add to1 liter of water - 5 minute induction at room temperature
Chemical Restraint of Reptiles		
Crocodilian	Ketamine	40-60 mg/kg IM - administer in forelimb muscles - induction 15-30 minutes - require adjunct anesthesia for surgery
	Pentobarbital	7.5-15 mg/kg IP; recovery up to 5 days, unpredictable effects - not recommended for recovery use
	Halothane/ Isoflurane	- use after premedication with ketamine; otherwise, apnea results in prolonged induction in diving species - use nasal mask, preoxygenate for 3 minutes with 100%

		oxygen, then give 4% halothane or isoflurane until jaw tone slackens - block mouth open, intubate and maintain on positive pressure ventilation, use 100% oxygen and 0.5-2% anesthetic gas - do not exceed 10 cm water pressure during PPV; ventilate 4-6 times per minute 10-20 ml/kg tidal volume
Turtle	Ketamine	40-80 mg/kg IM in forelimb - results highly variable, prolonged induction common, resp. arrest and death at doses over 110 mg/kg common, prolonged recovery 6 hours - 3 days - use at low dose to abolish apnea during gas anesthesia
	Halothane/ Isoflurane	- use after ketamine premedication - mask induction using N_2O as with crocodilian or direct intubation and maintain on gas anesthesia - can spontaneously ventilate, if in dorsal recumbency use PPV at 6 breaths per minute, maintain at 0.5-1.0 percent gas
	Pentobarbital	60 mg/kg IP - dilute stock solution to 25 mg/kg to lessen irritation - prolonged induction (1-3 hours) and recovery (3 days) - may have no effect in 10 percent of turtles
Lizard	Ketamine	20-30 mg/kg IM in forelimb - apnea common after induction, intubate and ventilate as for turtles with oxygen during apnea
	Halothane/ Isoflurane	- mask induction with oxygen or nitrous oxide/oxygen and 4 percent gas, apnea common; use ketamine premedication - maintain at 0.5-2.0 percent gas - recovery rapid (30 minutes or less)
Snake	Ketamine	40-80 mg/kg IM in dorsal epaxial muscles - 3-5 minute induction, muscle rigidity common - recovery dose-dependent, 30-90 minutes
	Halothane/ Isoflurane	4 percent by mask/chamber - apnea uncommon, use of N_2O will hasten induction which is rapid (5-10 minutes) - maintain at 0.5-2 percent, intubate and allow to spontaneously ventilate - exercise caution if using PPV due to fragility of snake air sac

* BENNETT, R.A. A review of anesthesia and chemical restraint in reptiles. J. Zoo. Wildlife Med. 1991; 22 (3): 282-303.

APPENDIX XIII

ANESTHETIC AND SEDATIVE DRUG DOSAGE--FISHES

Anesthetic	Species	Dose	Induction and Recovery	Comments
MS-222 (Tricaine methanesulfonate)	Salmonids Carp/ Minnows Cod	25 mg/l 75-100 mg/l 40 mg/l 75 mg/l	Induction: <3 min Recovery: <10 min	Use the higher concentration for deep anesthesia. Slower at low temperatures
Benzocaine hydrochloride	Salmonids Striped Bass Carp Cod	25-45 mg/l 55-80 mg/l 50-100 mg/l 40 mg/l	Induction: 2-4 min Recovery: <10 min	Very small safety margin between effective and lethal doses
Lidocaine plus 1 g/l $NaHCO_3$	Carp Tilapia Catfish	350 mg/l 250 mg/l 350 mg/l	Induction: 1-1.5 min Recovery: 10-13 min	Can also be used successfully in conjunction with CO_2
Metomidate	Cod Rainbow Trout	5-20 mg/l 5 mg/l	Induction: <3 min Recovery: 8-20 min	Very large safety margin between effective and lethal doses
Etomidate	Salmonids Channel catfish Striped Bass Golden Shiners	1.0 mg/l 0.6-2 mg/l 1.0 mg/l 0.5-1.5 mg/l	Induction: 3-5 min Recovery: 5-20 min	More effective in alkaline waters
Propoxate	Various	1-4 mg/l	Induction: 0.5-1 min	Safe for 16 hours at 0.25 mg/l

Ketamine hydrochloride	Salmonids Shark	30 mg/kg IM 10-30 mg/kg IM	Induction: 10-300 secs Recovery: 1-2 hours	Does not block ventilatory rhythm
Quinaldine sulfate	Salmonids Channel Catfish Bluegill Largemouth Bass	15-40 mg/l 30-70 mg/l 10-30 mg/l 15-70 mg/l	Induction: 2-5 min Recovery: 2-60 min	Lower toxicity in soft water
2-Phenoxyethanol	Cod Salmonids	0.1-0.5 ml/l 0.25-0.5 ml/l	Induction: 2-4 min Recovery: 3-6 min	Narrow margin of safety between lethal and effective doses
Methylpentynol	Trout	1.5-8 ppt	Induction: 2-30 min Recovery: 4-60 min	Toxic to small fish
Chlorobutanol	Salmonids	600-750 mg/l	Induction: ~3 min Recovery: 6-20 min	Toxic to small fish. Causes death at all concentrations
Hypothermia	Salmonids	Immersion in crushed ice and water	Induction: 10-15 min Recovery upon return to normal temperature range	Fish which live at 10ºC will still show activity at 1ºC
Halothane	Rainbow trout	200 u/l		Lethal if duration is >10 minutes
Urethan	Various	5-40 mg/l	Induction: 2-3 min Recovery: 10-15 min	Known to be carcinogenic
Diethyl ether	Various	10-15 ml/l	Induction: 2-3 min Recovery: 5-30 min	Highly irritating to skin
Chloral hydrate	Various	0.8-0.9 g/l	Induction: 8-10 min Recovery: 20-30 min	Anesthesia is not deep, more appropriate as a sedative
Propanidid	Salmonids	1.5-3 ml/l	Induction: 1-4 min Recovery: 4-10 min	Does not cause blood chemistry alteration

Carbon Dioxide	Salmonids Carp	50% CO_2:50% O_2 250-350 mg/l 290-460 ml/ min 1-2/min @ 50% CO_2	Induction: 3-4 min Recovery: 10-15 min Induction: 20-30 min Recovery: 20-30 min	Lethal if held in solution for more than two minutes Solution should be buffered to reduce the water acidity Violent induction
Carbonic acid		150-600 mg/l H_2CO_3		
Sodium bicarbonate	Trout/Carp Adult salmon	pH 6.5 + 642 mg/l 900 mg/l	Induction: 5 min Recovery: 10-12 min	
Electroanesthesia	Salmonids	AC 100V for 5-7 sec DC 0.6v/cm or 400V @ 5 amps pulsed for 13 sec RC 100Hz, 8ms @ 2ms intervals RC 0.64-0.82V/ cm	Induction: rapid Recovery: 20-30 min Induction: rapid Recovery: immediate Induction: rapid Recovery: 80 secs	Less impact on blood chemistry, but may be more physically damaging

See references on following page.

References

IWAMA, G.K. and ACKERMAN, P.A. Anesthetics. In: Hochachka, P. and Mommsen, T., eds. Biochemistry and molecular biology of fishes. Analytical techniques. Elsevier, 1993; Vol. 3. In press.

STOSSKOPF, M.K. Fish medicine. New York: W.B. Saunder Co. 1993: 79-91.

APPENDIX XIV

METHODS FOR EUTHANASIA BY SPECIES
(Methods in order of Acceptability)

Species	Most Acceptable	Acceptable
AMPHIBIANS	Barbiturates Inhalant anesthetics Tricaine methanesulfonate MS-222 Benzocaine	Double pithing Decapitation and pithing Stunning and pithing CO_2 + O_2 mixture
AVIAN SPECIES (birds)	Barbiturates Inhalant anesthetics	Electrocution stunning followed by exsanguination CO_2 + O_2 mixture Physical stunning followed by exsanguination or decapitation
BOVINE SPECIES (calves, cows, goats, sheep and other ruminants)	Barbiturates Penetrating captive bolt followed by exsanguination	Shooting followed by exsanguination
CATS	Inhalant anesthetics Barbiturates	CO_2 + O_2 mixture
DOGS	Inhalant anesthetics Barbiturates	CO_2 + O_2 mixture
EQUINES (horses)	Barbiturates	Shooting Penetrating captive bolt
FISH	Tricaine methanesulfonate MS-222 Benzocaine	Stunning followed by cervical dislocation or decapitation
FUR BEARING ANIMALS (mink, fox, others raised for fur)	Barbiturates Electrical stunning using special equipment followed by cervical dislocation Inhalant anesthetics in specially designed chamber	CO CO_2 + O_2
INVERTEBRATES (cephalopods, crustacea)	Tricaine methanesulfonate MS-222 Benzocaine	CO_2 bubbling through water
MARINE MAMMALS (seals, porpoises, cetacea)	Barbiturates Etorphine hydrochloride	Stunning followed by exsanguination
NON-HUMAN PRIMATES	Barbiturates Inhalant anesthetics	Tranquillization and CO_2 + O_2 mixture
RABBITS	Barbiturates Inhalant anesthetics	CO_2 + O_2 mixture
REPTILES	Inhalant anesthetics in special chamber Barbiturates	CO_2 + O_2 mixture
RODENTS (and similar small species)	Inhalant anesthetics in special ' chambers Barbiturates Microwave irradiation in specially designed units	CO_2 + O_2 mixture CO
SWINE	Barbiturates Inhalant anesthetics	Electrocution using special equipment

WILD AMIMALS (200 species, etc.)	Shooting by expert marksman Immobilization followed by barbiturates	Sedation followed by penetrating captive bolt

Barbiturates includes an overdose of all pharmacological agents which depress the Central Nervous System (CNS) producing irreversible unconsciousness and death (see also Euthanasia and CCAC policy statements).

Competence and knowledge of agents and procedures are mandatory requirements for personnel conducting euthanasia.

Any other means (not listed here) of killing an experimental animal, must not be undertaken without prior review and approval by an Animal Care Committee (ACC), and should only be done in expert hands.

APPENDIX XV-A

ETHICS OF ANIMAL INVESTIGATION

The use of animals in research, teaching, and testing is acceptable only if it promises to contribute to understanding of fundamental biological principles, or to the development of knowledge that can reasonably be expected to benefit humans or animals.

Animals should be used only if the researcher's best efforts to find an alternative have failed. A continuing sharing of knowledge, review of the literature, and adherence to the Russell-Burch '3R' tenet of 'Replacement, Reduction and Refinement' are also requisites. Those using animals should employ the most humane methods on the smallest number of appropriate animals required to obtain valid information.

The following principles incorporate suggestions from members of both the scientific and animal welfare communities, as well as the organizations represented on Council. They should be applied in conjunction with the Canadian Council on Animal Care's (CCAC) 'Guide to the Care and Use of Experimental Animals.'

1. If animals must be used, they should be maintained in a manner that provides for their physical comfort and psychological well-being, according to CCAC's 'Policy Statement on Social and Behavioural Requirements of Experimental Animals.'

2. Animals must not be subjected to unnecessary pain or distress. The experimental design must offer them every practicable safeguard, whether in research, in teaching or in testing procedures; cost and convenience must not take precedence over the animal's physical and mental well-being.

3. Expert opinion must attest to the potential value of studies with animals. The following procedures, which are restricted, require independent, external evaluation to justify their use:

i) burns, freezing injuries, fractures, and other types of trauma investigation in anesthetized animals, concomitant to which must be acceptable veterinary practices for the relief of pain, including adequate analgesia during the recovery period;

ii) staged encounters between predator and prey or between conspecifics where prolonged fighting and injury
are probable.

4. If pain or distress is a necessary concomitant to the study, it must be minimized both in intensity and duration. Investigators, Animal Care Committees (ACC), grant review committees and referees must be especially cautious in evaluating the proposed use of the following procedures:

a) experiments involving withholding pre- and post-operative pain-relieving medication;

b) paralyzing and immobilizing experiments where there is no reduction in the sensation of pain;

c) electric shock as negative reinforcement;

d) extreme environmental conditions such as low or high temperatures, high humidity, modified atmospheres,

etc., or sudden changes therein;

e) experiments studying stress and pain;

f) experiments requiring withholding of food and water for periods incompatible with the species specific physiological needs; such experiments should have no detrimental effect on the health of the animal;

g) injection of Freund's Complete Adjuvant (FCA). This must be carried out in accordance with *'CCAC Guidelines on Acceptable Immunological Procedures*.'

5. An animal observed to be experiencing severe, unrelievable pain or discomfort, should immediately be humanely killed, using a method providing initial rapid unconsciousness.

6. While non-recovery procedures involving anesthetized animals, and studies involving no pain or distress are considered acceptable, the following experimental procedures inflict excessive pain and are thus unacceptable:

a) utilization of muscle relaxants or paralytics (curare and curare-like) alone, without anesthetics, during surgical
procedures;

b) traumatizing procedures involving crushing, burning, striking or beating in unanesthetized animals.

7. Studies such as toxicological and biological testing, cancer research and infectious disease investigation may, in the past, have required continuation until the death of the animal. However, in the face of distinct signs that such processes are causing irreversible pain or distress, alternative endpoints should be sought to satisfy both the requirements of the study and the needs of the animal.

8. Physical restraint should only be used after alternative procedures have been fully considered and found inadequate. Animals so restrained, must receive exceptional care and attention, in compliance with species specific and general requirements as set forth in the *'Guide.'*

9. Painful experiments or multiple invasive procedures on an individual animal, conducted solely for the instruction of students in the classroom, or for the demonstration of established scientific knowledge, cannot be justified. Audiovisual or other alternative techniques should be employed to convey such information.

Revised October 1989

APPENDIX XV-B

CATEGORIES OF INVASIVENESS IN ANIMAL EXPERIMENTS

Investigators and teachers who consider it essential to use vertebrates or invertebrates in their research, teaching or testing in the laboratory or in the field, must adhere to humane principles, and take cognizance of the Canadian Council on Animal Care's (CCAC) Ethics of Animal Investigation *and other CCAC documentation in assigning a category. Protocols must be submitted to an appropriate review committee for all studies and courses which involve the use of vertebrates and some invertebrates in*

Categories B through E. Cephalopods and some other higher invertebrates have nervous systems as well developed as in some vertebrates, and may therefore warrant inclusion in Category B, C, D, or E.

*The following list of categories provides **possible examples** of experimental procedures which are considered to be representative of each category.*

A. Experiments on most invertebrates or on live isolates

Possible examples: the use of tissue culture and tissues obtained at necropsy or from the slaughterhouse; the use of eggs, protozoa or other single-celled organisms; experiments involving containment, incision or other invasive procedures on metazoa.

B. Experiments which cause little or no discomfort or stress

Possible examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral, but not intrathoracic or intracardiac (Category C); acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose, or decapitation preceded by sedation or light anesthesia; short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

C. Experiments which cause minor stress or pain of short duration

Possible examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies, laparoscopy; short periods of restraint beyond that for simple observation or examination, but consistent with minimal distress; short periods of food and/or water deprivation which exceed periods of abstinence in nature; behavioural experiments on conscious animals that involve short-term, stressful restraint; exposure to non-lethal levels of drugs or chemicals. Such procedures should not cause significant changes in the animal's appearance, in physiological parameters such as respiratory or cardiac rate, or fecal or urinary output, or in social responses.

Note: During or after Category C studies, animals must not show self-mutilation, anorexia, dehydration, hyperactivity, increased recumbency or dormancy, increased vocalization, aggressive-defensive behaviour or demonstrate social withdrawal and self-isolation.

D. Experiments which cause moderate to severe distress or discomfort

Possible examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioural stresses such as maternal deprivation, aggression, predator-prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organization; the use of Freund's Complete Adjuvant (FCA) (see *CCAC Guidelines on Acceptable Immunological Procedures*).

Other examples include induction of anatomical and physiological abnormalities that will result in pain or distress; the exposure of an animal to noxious stimuli from which escape is impossible; the production of radiation sickness; exposure to drugs or chemicals at levels that impair physiological systems.

Note: Procedures used in Category D studies should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioural patterns or attitudes, the absence of grooming, dehydration, abnormal vocalization, prolonged anorexia,

circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.

E. Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals

This Category of Invasiveness is not necessarily confined to surgical procedures, but may include exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress; completely new biomedical experiments which have a high degree of invasiveness; behavioural studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; a euthanasia method not approved by the CCAC; any procedures (e.g., the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain tolerance threshold and cannot be relieved by analgesia (e.g., when toxicity testing and experimentally-induced infectious disease studies have death as the endpoint).

Revised February 1991

APPENDIX XV-C

CCAC GUIDELINES ON ACCEPTABLE IMMUNOLOGICAL PROCEDURES

When beginning an immunization, choosing the correct adjuvant may be difficult. As a general suggestion, Freund's Complete Adjuvant (FCA) may be used when only small amounts of soluble immunogens are available. FCA is considered to be an emulsion consisting of equal volumes of FCA to antigen (1 part FCA or less to 1 part antigen). If large amounts of particulate, or highly immunogenic immunogens are available, other adjuvants should be considered.

An important aspect in immunization procedures is the utilization of skilled, competent, technical staff experienced in the handling of the species being used and in performing the technique. They must be knowledgeable and capable of recognizing signs of distress in all injected animals, and be responsible for taking action when necessary.

FCA should be used only for the most problematic immunization situations. It must never be given either intravenously or in repeated doses. FCA must not be used in horses.

Intradermal Route

Sound scientific evidence and justification must be available if the intradermal route of injection of FCA is to be used, because of the frequent ulceration and infections that occur at the site of such injections. The use of the intradermal route may be justified only when the purpose is to induce cell-mediated response.

In rabbits, volumes of inoculum in excess of 0.05 mls (50 microliters) per site should not be used. The location of the site(s) should be carefully selected so as to prevent mutilation. A minimal number of sites should be selected, and the distance between each site be maximized.

The intradermal route is inappropriate in the mouse. Nor is it recommended in other rodents.

Subcutaneous Route

In guinea pigs, up to a total volume of 0.4 ml (400 microliters) of inoculum may be injected subcutaneously dorsally in the neck, in one or divided into several sites. In rabbits, the site of choice is the interscapular region (between the shoulder blades) on the dorsum (back), administering up to 0.25 ml of inoculum (250 microliters) per site, to a maximum of four sites. The distance between sites should be maximized. In the mouse, up to 0.1 ml (100 microliters) may be administered in the neck region.

Intramuscular Route

In rabbits, intramuscular injections of FCA may be administered in the thigh muscle; up to 0.5 ml (500 microliters), preferably in one site. Intramuscular injection of FCA is not recommended for small laboratory animals such as rats, mice, hamsters, gerbils, etc.. For larger animals such as cats, dogs and poultry, up to 1 ml of FCA injected into the thigh muscles is acceptable. In livestock such as pigs, cattle, sheep and goats, the intramuscular route is acceptable.

Intraperitoneal Route

The intraperitoneal route for injection of FCA is permitted in small rodents only. FCA should be administered only once, and be limited to minimal volumes of up to 0.1 ml (100 microliters).

Intravenous Route

FCA is not to be injected intravenously.

Footpad Injection

FCA should not be injected in the feet of rabbits. Footpad injection of FCA in rodents is not permissible unless there is scientific evidence indicating this route is essential as a specific requirement for the production of immune response. In rats and mice, only one footpad may be used. Animals should be maintained on soft bedding and not on wire-bottomed cages.

Induction of Ascites Fluid in Animals

Pristane or other recognized priming agent(s) (excluding FCA) may be used.

Ascites may be collected only for as long as the animal is not experiencing pain or distress, is in good body condition, and does not show signs of debilitation, dehydration or other complications from the procedure. Upon recognition of loss of condition, pain, or distress the animal must be euthanized according to a method approved by the Canadian Council on Animal Care (CCAC).

Observation of Injection Sites

The injection site(s) must be observed by the investigator or his/her designate, a minimum of three times per week, for four weeks after each injection.

If a lesion(s) develops at any injection site, it must be reported through established channels, e.g., the animal resources supervisor or veterinarian, and must receive appropriate veterinary treatment. Such lesions should be inspected at least three times per week by the investigator or his/her designate, until

all lesions are healed.

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APPENDIX XVI

JOURNALS HELD BY CCAC

Alternatives To Laboratory Animals
American Journal of Veterinary Research
Animal Behaviour
Animal Technology
Animal Welfare
Applied Animal Behaviour Science
Canadian Journal of Veterinary Research
Canadian Veterinary Journal
Compendium on Continuing Education for Practising Veterinarians
Federation of American Societies for Experimental Biology Journal
Journal of Agricultural & Environmental Ethics
Journal of the American Veterinary Medical Association
Journal of Wildlife Diseases
Lab Animal
Laboratory Animals
Laboratory Animal Science
Laboratory Investigation
Nature
Veterinarian Magazine
Veterinary Record
Veterinary Technician

NEWSLETTERS

CCAC holds a number of newsletters, among them:

Animal Welfare Institute Quarterly
Canadian Association for Laboratory Animal Science Newsl.
Canadian Association for Laboratory Animal Medicine Newsl.
Resource (CCAC)
Caring for Animals (CFHS)
Council for Laboratory Animals Newsl.
Eubios Ethics Institute Newsl.
Future Health (Canadians for Health Research)
Institute of Laboratory Animal Resources Newsl.
Laboratory Primate Newsl.
Scientists Center for Animal Welfare Newsl.

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APPENDIX XVII

GLOSSARY

***ABNORMAL BEHAVIOUR**: Behaviour that deviates from a defined, comparable standard. Such a standard may be a behavioural inventory typical for a given genotype, age group, sex, nutritional level, housing condition or management system, etc.

AD LIBITUM: Free choice.

ADJUVANT: A substance which non-specifically enhances the immune response to an antigen.

AMBIENT TEMPERATURE: The temperature surrounding the animal: under caging conditions may refer to the temperature in the microenvironment inside the cage as opposed to temperature outside the cage in the room or enclosure.

ANALGESIC: Substance which reduces or ameliorates the sensation of pain.

ANESTHESIA: The loss of sensation in a part or portion (local) or all (general) of the body, usually produced by the administration of a chemical or a drug.

ANTIBODY: A molecule produced by animals in response to antigen, and which has the particular property of combining specifically with the antigen which induced its formation.

ANTICOAGULANT: A substance added to whole blood to prevent clotting.

ANTIGEN: A foreign material or substance that stimulates the formation of antibodies when introduced into the tissues and blood stream.

ANTISEPTIC: 1. Preventing decay or putrefaction; 2. A substance which will inhibit the growth and development of microorganisms.

ANXIETY: An aroused state in which there is involuntary and voluntary nervous activation.

ASEPTIC: The absence of living germs, free from septic or poisonous putrefactive products.

AXENIC: Free of foreign organisms; germ-free.

BACK CROSS: The cross of an F1 hybrid to either of its parents (see F1 below).

BARRIER HOUSING: Housing for research animals that protects them from outside contamination through both procedure and facility design. In contrast, containment housing protects the outside environment from contaminants within the animal housing facility.

BIOSAFETY CABINET: A special exhaust hood with an enclosed work surface used for biological testing and experiments. Biosafety cabinets protect the surrounding room, and they protect workers from hazardous materials being used in the cabinet.

BIOTECHNOLOGY: The use or development of techniques using organisms or parts of organisms to provide or improve goods or services.

BIOPSY: The surgical removal of a cell or sample of tissue for diagnostic purposes.

BREED: A population of animals within a species, which differs from those in other populations within the same species in respect to definite genetically determined traits.

CANNULA: A tube (may be plastic or glass) which is inserted into the intravascular compartment or into the body to facilitate administration or withdrawal of gases or liquids.

***CIRCADIAN:** Referring to cyclic rhythmicity corresponding closely to a 24 hour interval.

CLOSED COLONY: A colony not recruiting breeding animals from outside itself.

***COGNITION:** A process of perception, reasoning and development of expectations.

COLONY (HERD, FLOCK): An animal population maintained under some degree of control for the purpose of reproduction. A group of animals representing a single genetic pool produced at a single site under identical conditions of management.

CONDITIONING: Term applied to examination and preparation of animals for research.

CONGENIC: Animals which genetically differ at one particular locus.

CONJUNCTIVITIS: Inflammation of the conjunctiva (the membrane that lines the eyelids and covers the exposed surfaces of the eyeball).

CONTAGIOUS: A disease or disorder easily transmitted from individual to individual.

CONTAINMENT HOUSING: Housing for research animals that protects the environment from contaminants within; accomplished through procedure and facility design.

***DEPRIVATION:** Removal of needed substances (feed deprivation, water deprivation), perceptual isolation from things desired (social isolation) or prevention of the performance of necessary behaviours (sleep deprivation, exercise deprivation). Deprivation frequently is used experimentally to induce a detectable drive.

DIFFERENTIAL PRESSURE: The difference between pressures measured at two points or levels in a system.

DOMINANT: Controlling. Usually applied to controlling trait or gene governing genetic patterns.

DRIVE: An internal state causing increased activity, e.g., hunger drive.

EDEMA: The presence of abnormally large amounts of fluid in the intercellular tissue spaces of the body; usually applied to accumulation of fluid in subcutaneous tissues.

EMBRYO: The early or developing stage of any organism, especially the developing product of

fertilization of an egg.

***ENVIRONMENTAL COMPLEXITY:** The diversity and intensity of environmental stimuli relevant to a given organism, age group, species, etc. Environmental complexity may range from very low to very high, and thus be characterized as insufficient, adequate, or excessive.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA): Rapid, sensitive and cost-effective test for screening large numbers of serum samples. ELISA kits are commercially available.

ESTRUS: The period when mating may occur.

ETHICS: A system of moral principles or standards governing conduct.

ETHOLOGY: The scientific study of animal behaviour.

F1 HYBRID: The first generation cross between two strains, between two inbred strains, between two lines, etc.

FARROW: The act of giving birth by sow (guinea pigs or swine).

FERTILIZATION: The union of the sperm of the male with the ovum (egg) of the female leading to reproduction.

FETUS: A developing embryo in utero.

FOMITE: Non-living objects that can carry disease organisms (e.g., restrainers, feeders, mops, etc.).

FREUND'S COMPLETE ADJUVANT (FCA): An emulsion of aqueous antigen in oil. Contains killed *Mycobacterium tuberculosis* while Incomplete Freund's Adjuvant does not.

FULL SPECTRUM LIGHTING: Fluorescent lighting that very closely matches the spectral energy distribution of sunlight.

FUME HOOD: A negative airflow cabinet designed to prevent exposure of personnel to hazardous materials being handled in it, chemical or microbiological.

GENE: The hereditary unit that occupies a fixed chromosomal locus, which through transcription has a specific effect upon phenotype.

GENOME: The total genetic material contained within the cell.

GENOTYPE: The genetic constitution of an animal, as distinguished from its phenotype.

GESTATION: The period between conception and birth which includes embryonic and fetal life.

GNOTOBIOTES: Animals which are completely germ-free or may have one or more clearly-identified microorganisms existing in the animal.

GOOD LABORATORY PRACTICES (GLP): Standards for conducting non-clinical research studies as

published by the U.S. Federal Food and Drug Administration (USFDA).

GROSS SQUARE METERS: All of the floor space inside building measured from the outside surface of exterior walls.

HAREM MATING: Mating of one male with more than two females.

***HEALTH:** A relative state of physical, psychological and social well-being.

HEAT: The period during which the mating desire is prominent in the female.

HEMATOCRIT: The volume percentage of erythrocytes (red blood cells) in whole blood. Also Packed Cell Volume (PCV).

HEMOGLOBIN: The oxygen carrying pigment of erythrocytes (red blood cells) composed of iron complex and protein.

HIGH EFFICIENCY PARTICULATE AIR (HEPA) FILTER: Used in cleanrooms, biological safety cabinets, laminar flow units, etc., to filter out contaminating particles as small as 0.5 microns in diameter.

HERITABILITY: A measure of the degree to which a phenotype is genetically determined.

HUMIDITY (RELATIVE): The ratio of the quantity of water vapour actually present in the air to the amount of water vapour that air is capable of holding at the given temperature.

IMPRINTING: The learning process involved in developing, during an early sensitive period, the tendency to follow or otherwise approach an object.

INFECTION: Disease process caused by the invasion of microorganisms into the body tissue.

INBRED: Inbreeding - resulting from mating between closely related animals.

INFLAMMATION: The condition into which tissues enter as a reaction to injury or an infectious agent.

INTRADERMAL: Delivered into the dermis or skin.

INTRAPERITONEAL (IP): Delivered into the peritoneal or abdominal cavity.

INTRAVENOUS (IV): Delivered into a vein.

LAMINAR AIRFLOW: Uniform direction movement of air. Laminar airflow is generally associated with fume hoods or biological safety enclosures that utilize this characteristic to capture and carry away airborne particles.

LATENT OR MASKED INFECTION: An infection or condition which is not clinically expressed in the animal but may, under stress or certain conditions, develop into an overt, recognizable diseased state.

LITTER: a) numerous young born at one time of a single female; b) in reference to bedding may mean

straw, hay or other material used for the purpose of bedding.

MAJOR SURGERY: A surgical procedure in which there is direct visual access to a major body cavity (cranium, spinal canal, thorax, abdomen, pelvis) and/or exposure of major vascular, muscular, skeletal, neural, lymphatic or glandular structures and/or removal of, or alteration to, a functionally significant amount of tissue. There is no clear boundary between Major and Minor Surgery; thus Animal Care Committees (ACC) should use definitions of these terms only as adjuncts to the "Categories of Invasiveness", and should seek additional professional judgment when the level of invasiveness and injury is unclear.

MALIGNANT: Tending to become progressively worse and to result in death.

MASS AIR DISPLACEMENT CLEANROOM (MADC): A cleanroom used in conjunction with animal housing in research facilities to keep the environment free of hair, dandruff and other airborne contaminants. The level of cleanliness is determined by the number of air changes per hour and the numbers and types of animals held in the cleanroom.

MATERIAL SAFETY DATA SHEETS (MSDS): Technical documents that provide detailed and comprehensive information on controlled products related to health effects of overexposure to the products; hazard evaluation related to the products handling, storage or use; measures to protect employees at risk of over-exposure; and emergency procedures.

MICROENVIRONMENT: A small, isolated habitat, usually within a cage.

MICROINJECTION: A technique used for the insertion of genes from one cell into another cell.

MICROISOLATION CAGING: A caging system that protects animals from becoming contaminated via other lab animals or personnel by placing the barrier at cage level and never allowing that barrier to be opened except in a protected class 100 environment by personnel whose pertinent body surfaces are covered and decontaminated with a sterilant.

MICROORGANISM: A microscopic living agent, often a producer of disease.

MINIPUMP: A small device, implanted in the body (usually subcutaneously or intraperitoneally), which through osmotic pressure on a drug-containing chamber, provides continuous controlled delivery of drugs to the body.

MINOR SURGERY: A surgical procedure that does not result in removal of, or alteration to, a functionally significant amount of tissue. There is no clear boundary between Minor and Major Surgery; thus Animal Care Committees (ACC) should use definitions of these terms only as adjuncts to the "Categories of Invasiveness", and should seek additional professional judgment when the level of invasiveness and injury is unclear.

MORBIDITY: The occurrence of sickness.

MORIBUND: Close to death.

MUTANT: An organism bearing a mutant gene that expresses itself in the phenotype of the organism.

MYCOTIC INFECTION: Disease caused by a fungus.

NECROPSY: Systematic dissection of an animal after death to elucidate the cause of death. Same as postmortem examination. Necropsy preferred term for animal postmortem examinations as opposed to autopsy for human-beings.

NECROSIS: The death of a portion of tissue or organ.

NET ASSIGNABLE SQUARE METERS: The net floor space in a building measured from the inside surfaces of exterior walls and excluding interior walls and partitions, mechanical equipment rooms, lavatories, janitorial closets, elevators, stairways, major circulation corridors, aisles, and elevator lobbies.

NON-HUMAN PRIMATES: Any non-human member of the order *primates* of mammals including prosimians, monkey, apes. Synonyms: infrahuman primate, sub-human primate.

NON-SENTIENT MATERIAL: Material that fails to visually demonstrate pain, without or almost devoid of nervous and sensory systems.

NUDE MOUSE: A genetically athymic mouse, it also carries a closely-linked gene producing a defect in hair production.

ORAL OR *PER OS* (PO): The act of administering a substance through the mouth.

OVUM: Egg or germ cell produced by the female reproductive organ, the ovary.

PATHOGEN: An organism which causes disease.

PARTURITION: The act or process of giving birth.

PHENOTYPE: The outward visible expression of the hereditary constitution of an organism.

***PICA:** Abnormal appetite for unusual and often inappropriate feed, e.g., dirt, hair, feces, etc.

PLASMA: The fluid portion of blood, without cells, in which anticoagulants have prevented clotting.

***POLYDIPSIA:** The consumption of large amounts of liquids (frequently used interchangeably with the term, excessive thirst).

***POLYPHAGIA:** Consumption of an unusually broad variety of foods. Compare: Hyperphagia.

***POLYURIA:** Excessive excretion of urine.

POST PARTUM: The immediate period following parturition or birth of young.

PROGENY: The young of a species.

PROGNOSIS: The prospect as to recovery from a disease as indicated by the nature and symptoms of the case.

PROPHYLAXIS: Prevention.

PUBERTY: The onset of sexual maturity.

QUARANTINE: The segregation or isolation of animals from all others to prevent the spread of disease.

RESTRAINT: Holding or securing to reduce activity in order to prevent the animal from causing harm to itself or harm to the handler.

***REWARD TRAINING:** A type of operant conditioning in which a reward (positive reinforcer) is directly contingent on the performance of the subject. According to the training objectives, the performance resulting in reward may be either a produced response or a withheld response.

RISK: The probability of adverse effects, their nature and their severity over a range of exposures.

ROUGHAGE: Food that is high in fibre and low in digestible nutrients.

RUMINANT: A cud-chewing polygastric animal having usually four digestive compartments; includes such animals as cows, goats, sheep.

SANITIZE: To reduce the level of microorganisms to an acceptable health level.

SEMEN: The ejaculate of the male reproductive organs containing spermatozoa and including material from accessory glands and the testes.

SERUM: Non-cellular components of blood which remain after clotting.

SERVICE: In reference to animal breeding, refers to the act of copulation by the male animal. The male animal serves (breeds) the female.

SEVERE COMBINED IMMUNE DEFICIENCY (SCID) MOUSE: Mice that possess a genetic autosomal recessive mutation. SCID mice lack functional lymphocytes, a defect that is manifested in a number of ways including lymphopenia, agammaglobulinemia and a high susceptibility to infection. SCID mice are desirable research models for implantation of foreign tissues and tumours**.**

SEXUAL MATURITY: The age at which the animal is first able to reproduce.

***SOCIAL DOMINANCE:** Ascendency of an individual over another individual(s).

SPECIFIC PATHOGEN FREE (SPF): Defines the health status of animals raised free of specific disease organisms.

STANDARD OPERATING PROCEDURES (SOP): Written documents specifying the procedures that must be followed to ensure the quality and integrity of the study.

***STEREOTYPED BEHAVIOUR:** Behaviour repeated in a very constant way. The term generally is used to refer to behaviour that develops as a consequence of a problem situation such as extended social isolation, low level of environmental complexity, etc.

STERILIZATION: The complete destruction of microorganisms by heat, chemical compounds, mechanical or physical means. In animal breeding, refers to any procedure which renders the animal

incapable of reproduction.

STOCK: A collection of outbred animals being grown or maintained for breeding or for experimental use.

STRAIN: A group of animals of known ancestry maintained by a planned inbreeding mating system; generally with some distinguishing characteristics.

STRESS: A strain upon the normal physiological or psychological processes or functions of the body, organ or tissue. Some stresses may cause pathology or diseased states or weaken the normal body defences.

SUBCUTANEOUS (SC): Occurring beneath the skin.

SUSCEPTIBLE: Lacking in resistance to infection or injury or permitting a weak defense.

SYNDROME: A group of signs (animals) or symptoms (humans) occurring together designating a state or a disease.

SYSTEMIC: A condition occurring throughout the entire system of the entire animal body.

THRESHOLD LIMIT VALUE (TLV): An airborne concentration of a substance to which indoor workers may be exposed repeatedly without adverse effects.

TISSUE CULTURE: The propagation of tissue removed from organisms in a laboratory environment that has strict sterility, temperature and nutrient requirements.

TOXIN: A product poisonous to the animal, arising from a plant or animal cell. It may be produced by the cell itself and excreted from the cell or it may be contained within the cell, such as the bacterial cell, and released only on the death of the cell.

TRANQUILLIZER: An agent, usually a drug, capable of making the animal quiet and docile.

TRANSGENIC ANIMALS: Animals whose hereditary DNA has been augmented by the addition of DNA from a source other than parental germplasm, usually from another animal or a human, using recombinant DNA techniques.

TRAUMA: An injury.

VACCINE: A substance used to stimulate the production of antibodies against a specific disease-producing agent, usually as a preventive measure.

VASCULAR ACCESS PORT: Catheters terminating subcutaneously in "ports" which allow transcutaneous access with needles.

VECTOR: A living thing that is capable of carrying and transmitting infectious agents.

VERMIN: Any undesirable or disturbing offender such as flies, lice, fleas, cockroaches, ticks, mice, rats, weasels.

VIABILITY: Usually refers to the ability of the young to live after birth.

VIRUS: Any of a large group of organisms containing genetic material, but unable to reproduce outside a host cell.

VITAL CENTER: Any one of a various group of nerve cells located in the *medulla oblongata* of the central nervous system (CNS) which co-ordinates functions essential to life, e.g., respiration, heart beat.

***WELL-BEING:** A state or condition of physical and psychological harmony between the organism and its surroundings. Good health and manifestation of a normal behavioural repertoire are the most commonly used indicators of (an) animal's well-being.

WHELP: The act of parturition in the bitch, the birth of puppies.

WORKPLACE HAZARDOUS MATERIALS INFORMATION SYSTEM (WHMIS): A federal system to provide information on hazardous materials used in the workplace, it concentrates on three key elements; labels, material safety data sheets, legislation and employee education.

ZOONOSIS: A disease of animals that may under natural conditions be secondarily transmitted to humans.

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* Excerpted from: 1) Dictionary of farm animal behaviour. Hurnik, J.F., Webster, A.B. and Siegel, P.B., eds. University of Guelph 1985; 2) Glossary of terms relevant to farm animal behaviour and welfare. In: Farm animal behaviour and welfare. Fraser, A.F. and Broom, D.M., eds., Ballière Tindall, London 1990: 385-391.