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The care and use of animals for scientific purposes

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Table of changes

Change No.	Date	Scope

Abstract

The code encompasses all aspects of the care and use of, or interaction with, animals for scientific purposes in medicine, biology, agriculture, veterinary and other animal sciences, industry and teaching. It includes their use in research, teaching, field trials, product testing, diagnosis, the production of biological products and environmental studies.

Keywords

animal husbandry, animals, care, experimental animals, laboratory animals, laboratory testing, veterinary medicine, veterinary science

Acknowledgement

This South African standard is based on the Australian Code of Practice for the care and use of animals for scientific purposes, September 1997, drawn up by the National Health and Medical Research Council of Australia (copyright Commonwealth of Australia, reproduced by permission), and on the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes

Foreword

This South African standard was approved by National Committee STANSA SC 5140.38D, *The care and use of animals for scientific purposes*, in accordance with procedures of Standards South Africa, in compliance with annex 3 of the WTO/TBT agreement.

Annex N
(Normative)
Care and management of laboratory animals - Fish

N.1 Introduction

Fish and aquaculture research focuses mainly on the areas of environmental or ecological pollution, conservation, protection of marine and estuarine habitats, health and husbandry of food fishes, molecular, genetic and toxicology studies.

Fish need to be maintained in controlled environments and emphasis should be placed on limitation of stress, humane handling and animal welfare aspects.

Researchers and all persons involved with the advancement of scientific knowledge through the use of fish, need to understand and appreciate these animals, their ecosystems and their requirements for essential life processes.

N.2 Availability of fish species

Choice of species depends on the research demands, and selection of the correct or suitable model. Ease of maintenance and suitable housing facilities and trained staff are important

considerations. Certain fish species are aggressive, require more space, specialised diets, social compatibility, specialised housing, or life support systems.

N.3 Capture and acquisition

Irrespective of the purpose for which live fish are being collected, a strict ethic of habitat conservation and humane treatment of the animals shall be observed.

Collection of large series of animals from breeding populations should be avoided, as well as unacceptable collection techniques and habitat destruction.

Sampling equipment and strategies should be designed to minimize “bycatch” and non-target species.

The choice of collection method shall take into account the welfare of the animals, worker safety, research objectives, seasonal conditions, and habitat.

N.3.1 Representative samples

The study design usually dictates the number of animals required, but the principle of only taking the smallest number of animals required shall be observed.
Poor handling of large numbers of captured fish can result in high and unnecessary mortalities.

N.3.2 Collection of imperilled species

“Imperilled species” applies to those animals listed officially as threatened or endangered. It also applies to those animals identified as candidates for listing. It is important to know if an area supports imperilled species and how to identify them.

Collection of imperilled species should be avoided, unless the research being conducted is to the benefit of that species, and necessary permits can be obtained.

Collection techniques such as injurious or lethal ichthyocides, are not recommended.

Translocation of imperilled species may require specialized equipment and conditions. This will include their return to the wild transport. Biosafety and biosecurity issues shall be considered.

N.3.3 Wild stock and captive bred stock

Wild caught fish - may be captured by the research team (with necessary Conservation or CITES permits) or bought from suppliers. Collection techniques will need to be declared. Where dead fish, fish products and eggs are collected, it is wise to ascertain the disease status and disease transmission risks before transport to the laboratory.

Captive bred fish are available from hatcheries and other laboratory supply houses, aquaria, and hobbyists.

Applicable animal welfare laws and other relevant legislation shall be considered.

N.3.4 Killed and museum specimens

The collection of fish from natural populations, for preservation, is necessary for:

- Understanding of basic biology, evolution and life history;
- Documenting and recording biodiversity;
- Establishing reference collections;
- Environmental impact assessments, ecological surveys (voucher specimens); and
- Geographic variation and delineation of new species

Each animal collected should serve as many types of study as possible to reduce the total numbers collected to a minimum.

The use of piscicidal (ichthyocides) agents for capture shall take into account the effects on other species in the environment. Conservation authority approval will need to be obtained, as well as justification for their use to the AEC.

Fish should be euthanazed prior to immersion in formalin.

N.3.5 Acquisition of hatchery fish

Fish should come from hatcheries with defined and acceptable health status, and preferably known genetic history.

Hatcheries regularly supplying fish to laboratories should be encouraged to develop husbandry and management practices consistent with those of the laboratories.

N.4 Transportation

Contingency plans should be drawn up for any vehicle breakdowns during transport.

Important considerations are water quality, oxygen, temperature, and ammonia levels. Cooling the water will reduce the metabolic rate and thus decrease the amount of ammonia excreted into the water, and reduce the oxygen requirement. Fish excreta lowers pH levels.

Fish may be taken off food for 2 - 3 days prior to transport. They will then have voided their digestive tract contents and will not excessively foul transport water.

Transport boxes are usually of cardboard, and lined with polystyrene (styrofoam) panels for insulation and protection. Ideally fish should be packed into square bottomed plastic bags that provide better protection. Bags should be filled to half with original aquaria housing water. Inflate the bag to balloon capacity with oxygen and seal off with an elastic band. Use newspapers to isolate bags from each other and absorb any excess water. Spiny fish have the capacity to puncture a plastic bag.

This packing method can sustain fish comfortably for 12 - 24 h. Express shipping should always be used to limit transport time to less than 24 h.

N.5 Quarantine and acclimation

The primary purpose of quarantine is the containment and isolation of newly introduced fish and associated biota, for a period of observation, testing, and acclimation.

This will also ensure acceptable health status (freedom from unwanted disease and parasites) and suitability for reliable research studies. Any structural and management changes should be approved to ensure continued biosafety standards.

It is recommended that a Quarantine Manual be developed with accompanying Standard Operating Procedures for dealing with all quarantine requirements and contingencies. The responsibilities of the manager and personnel should be defined.

Personnel working in the quarantine facility should be adequately trained in quarantine procedures and disease recognition.

A quarantine period of 30 days is recommended for new introduction shipments.
Access to the quarantine area(s) should be controlled and limited to designated staff only, and suitable signage displayed. Protective clothing (caps, gloves, goggles, gumboots, gowns or coats)

should be provided. Suitable wash facilities should be provided, i.e. elbow operated hand basins, paper towel dispenser and approved detergent or surgiscrub dispensers.

Each quarantine tank shall have its own independent filtration system and each tank specifically designated to a particular shipment or introduction. Separation of shipments of fish, of unknown health status, is vital. All tanks should be clearly marked and comprehensive tank records kept. Separation distance between tanks shall be such as to prevent splashing of water from one tank to another.

Fish arriving in transport bags should be acclimated by placing the bags in the tank water to equilibrate temperatures between bags and tank water (normally 30 minutes). Bags may be clamped to the side of the tanks so that they may be opened for aeration. Handle as little as possible and keep lighting levels low, to reduce stress. Transfer of fish should be done gently using appropriate fine mesh nets.

On entering the quarantine facility fish should be inspected for any abnormalities, external lesions or sampling. They may be routinely treated with approved broad-spectrum antibiotics, anti parasiticides, and anti fungal agents.

Progeny of fish which breed in quarantine may be moved to another tank but should remain in the quarantine facility for the duration of the quarantine period.

Dead fish shall be removed immediately and post mortem or laboratory testing or both carried out.

All designated cleaning and other equipment should remain in the quarantine area.

Lighting provision shall be adequate for inspection purposes. Dimmer systems may be incorporated.

Aquaria should have at least one side made of clear material for inspection of fish, and fitted with lids to prevent fish jumping out, and to minimise splash or spillage.

It is recommended that the holding room be floor to wall coving be 150 mm high to contain any accidental water spills (tank ruptures).

All wastewater, when discharged from the facility, shall enter directly to an approved municipal sewer system. It may be necessary to treat wastewater. This water should be chlorinated for a period of 20 hours with an available chlorine level for this period of not less than 200 mg/L.

Solid waste shall be disposed of by approved method (incineration, or approved waste company collection).

N.6 Quality assurance and standard operating procedures

Quality Assurance (QA) plans and Standard Operating Procedures (SOPs) are recommended as essential tools in the management and operation of animal research facilities. They provide guarantees that systems are operating and functioning efficiently, thereby promoting valid research data obtained via consistency in repeated procedures, limiting unnecessary replications, reducing the overuse of animals, and the protection of animals and staff.

QA plans and SOPs form the basis of essential and effective training programs.

N.7 Animal welfare considerations

Capture techniques (seines and traps, gill nets, ichthyocides (piscicides), electrofishing, hooks and spears) shall be justified by the AEC as to their necessity, and the possibility of capture distress and pain.

All statutory laws and regulations shall be observed. There should be as little as possible or no disturbance of natural habitats. The use of experienced personnel is essential.

Appropriate attention shall be given to experimental design and procedures whilst ensuring the humane treatment of experimental subjects.

There are essential differences between fish and other vertebrates that are critically important to the conduct of scientifically valid research.

- Mortality patterns differ in fish, especially in egg survival.
- Fish field research, or early life stage research, requires much larger numbers.
- Handling, housing, care and maintenance requirements.

The AEC has the responsibility to carry out scientific reviews that guarantee the effective, efficient and valid design of protocols, experiments, animal welfare considerations, and veterinary treatments. It is recommended that the AEC carries out regular inspections and audits of research facilities.

N.7.1 Pain and distress

Researchers should take great care to avoid inducing stress and pain in animal research subjects, especially on a prolonged basis.

In fish, any deviations from normal homeostasis, will result in stress.

Appropriate use of anaesthetics and analgesics in procedures that can cause pain is essential.

N.8 Aquatic facilities and housing

N.8.1 Security and access

Access to aquatic facilities should be designed, and controlled, to minimize traffic through the area(s). Access should be restricted to authorized personnel only.

N.8.2 Types of systems – Flow through, recirculation, static

Correct water management is critical to the well-being and survival of aquaria held fish. All water should be analysed prior to setting up an aquarium to establish the current levels of pH, ammonia, nitrates, calcium, etc, in that water.

N.8.3 Environmental monitoring and control

Aquatic environments should be designed to meet the established physical and behavioural requirements of species of fishes, and life stages, in terms of social grouping and housing criteria.

All architectural and engineering specifications and drawings should be available on site, for staff and those persons responsible for the running and maintenance of the facility. Records should be kept of maintenance programs and schedules.

The staff responsible for the facility management and animal care should be available 24 hours per day for routine and emergency needs.

N.8.4 Water quality and management

A sound environmental monitoring system is essential, and the complexity should be designed to adequately monitor and control the water management system(s).

All monitoring equipment should be regularly serviced and calibrated.

Detailed records should be kept of all maintenance and repairs, for retrospective analysis.

N.8.5 Temperature

The health, nutrient requirements, performance, reproduction and survival are dependant on water temperature and optimum temperature criteria vary for different species.

Very gradual equilibration of water temperature is crucial when transferring, shipping, breeding, acclimating, and changing tank water. An optimal temperature variation is 1 °C/hour.

N.8.6 Oxygen and supersaturation

Temperature variation affects the saturation of gases especially oxygen. There is less dissolved oxygen at higher temperatures. In closed aquaria, sudden large increases in temperature are very hazardous.

N.8.7 pH value

PH values of between 6.5 - 9.0 are desirable. pH value has multiple effects on dissolved gases and metals in the water, as well as oxygen uptake by fish. It will also affect organic acids, phosphates, and the ratio of non-ionized to ionized ammonia in the water.

Fish vary in their tolerance to pH values at various stages of their life cycle. PH values of 6.5 and above are required for normal breeding and reproduction.

N.8.8 Salinity, alkalinity and hardness

The total amounts of solid materials dissolved in the water is important as fish need specific elements to carry out vital biochemical processes, and they depend on their surrounding medium for these requirements.

Salinity – the amount of dissolved salts in the water that affect the density of the water and temperature requirements of certain species. When transferring fish salinity changes should be monitored and gradual.

Alkalinity – the measure of the acid neutralising capacity of the water. Bicarbonates, carbonates, borates, phosphates, and other anions contribute to alkalinity (milli-equivalents/L). Adequate alkalinity ensures buffering of acid metals and proper functioning of biofilters.

Hardness – is the measure of mineral content (primarily calcium, magnesium, and other divalent cations). Appropriate hardness may decrease stress toxicity due to dissolved metals and ammonia.

N.8.9 Nitrogenous compounds and toxic agents

Nitrogen is present in water as gas, nitrates, nitrites and ammonia.

Ammonia is the most toxic inorganic nitrogen produced by fish and by heterotrophic bacteria of biological filters. 0.02 mg/L is considered a safe level for ammonia. Nitrite toxicity can occur in recirculating water systems and causes methaemoglobinaemia and ultimately hypoxia. Combined excess levels of ammonia and nitrites are responsible for “new tank syndrome“.

All chemical products should be stored well away from aquatic housing and the water supply. Chemical storage facilities should be lockable and secure.

Where there is reason to believe hazardous materials have entered the water system(s), such system(s) should be immediately isolated and tested.

N.8.10 Water supply

Four main processes are necessary to maintain optimum water quality in closed systems.
- Biological filtration - removal of bacteria and nitrification processes.
- Mechanical filtration – particulate removal.
- Chemical filtration – granulated activated carbon, foam fractionation, ion exchangers.
- Disinfection – ozonization, UV light treatment

N.8.11 Engineering, design and materials

The correct materials need to be chosen (concrete, plastic, fibre, glass, glues, plumbing). These should not contribute toxic products to the tank or holding container water. Construction materials should not contain copper, nickel, cadmium, or brass.

N.8.12 Mechanical and electrical requirements

All electrical systems should be professionally installed to compliance standards.

Extension cords should be avoided as well as system(s) overloading.

Electrical components and equipment should be located outside the splash zone, and in moisture proof enclosures. Seawater is corrosive and has a high electrical conductivity.

Machinery that produces noise and vibration should be isolated from the areas housing fish.

Critical systems, including pumps, should be duplicated to ensure failures cause only minimal disruption. Emergency power supply shall be available at all times. These should be tested regularly to ensure proper and efficient functioning.

N.8.13 Lighting

Both photoperiod and light intensity are important, as well as species variations.

Most species do well at a 12/12 hours light/dark cycle, although some tropical fish prefer 10/14 hours light/dark.

Fluorescent lighting is most commonly used. Full spectrum lighting can be used over tanks.

N.9 Husbandry and breeding

N.9.1 Record keeping

Detailed SOPs and checklists should be developed for the maintenance and care of all fish species, sanitation and cleaning procedures of tanks and rooms, equipment maintenance, and daily records.

N.9.2 Density and carrying capacity

Each species should be housed at a density that optimises the well being of the fish while meeting experimental parameters. Where necessary the ideal environment will have to be developed using performance based criteria, such as growth rate.

N.9.3 Food, feeding and nutrition

Fish are one of the most efficient animals in converting food nutrients into body tissues. They are poikilotherms and excrete waste products efficiently and require little energy for support and transportation.

Essential amino acids (proteins make up (60 – 70) % of fish tissue on a dry weight basis. Vitamins and minerals shall be given in proper ratios to ensure a well balanced diet. The research being undertaken, may determine these requirements. Other important factors are the stability of the food in the water and the levels of resultant pollution. Overfeeding and pollution of the tank water should be avoided.

Fish food should only be purchased from approved sources, and according to the standards required.

Feed bags should be labelled, including manufacture date, and provide detailed analysis information. Bags should be stored at optimal or recommended temperatures, in a designated feed storage area. All bins shall be lidded and sealed.

N.9.4 Broodstock and breeding

Holding systems and environmental conditions shall be appropriate for the species being held.

Attention shall be given to environmental cues for the maintenance, stimulation, or manipulation of endogenous reproductive rhythms.

N.10 Health and disease control

N.10.1 Fish health program

All facilities should have a fish health-monitoring program, and fish should be observed daily for signs of illness and abnormal behaviour.

A health management program should focus on early diagnosis and identification of causal agents and rapid initiation of control measures. It may be necessary to remove sick fish from the aquarium and collect specimens for laboratory examination. Each tank should have a mortality record.

Dead fish should be incinerated.

Drug and chemical administration to fish and tanks should be subject to the approval of the facility manager or veterinarian. Records shall be kept of all treatments.

N.10.2 Injuries and handling

Fishes should be fasted prior to handling or manipulations.

Personnel handling fish shall be trained and experienced to reduce handling injuries.

Handling shall be reduced to the minimum essential episodes. Protect fish from bright direct lighting or rapid changes in lighting whilst being restrained.

Fish should not be kept in the open air for more than 30 seconds.

N.10.3 Vermin control

Surveillance should be maintained for the presence of unwanted vermin, and a control program undertaken if required.

N.11 Laboratory activities with fishes

Fishes should not be held indefinitely without an AEC approved protocol.

The manager of the facility has the responsibility to maintain comprehensive and up to date records of all fish and activities in the facility, and to ensure compliance with all quality assurance programs. Routine auditing of facilities is recommended.

Key personnel should be listed.

N.12 Experimental procedures

N.12.1 Statistical design

The number of animal subjects required for an investigation will depend on the research questions being asked. Field and laboratory studies require very different experimental statistical designs. Field and early life stage studies require very large numbers.

The use of adequate and valid numbers to establish variance and assure reliability of results is essential to prevent needless repetition and animal over use. A statistician should be consulted to develop study designs that have the appropriate statistical power to accomplish sound objectives.

N.12.2 Restricted movements

Every effort should be made to provide fish held in restricted environments with as non-stressful environment as possible. Restraints, as required by research design, shall be justified and approved by the AEC.

N.12.3 Surgery

Surgery should be performed by personnel with appropriate training and expertise.

Surgical sites should be prepared in a sterile manner and which minimizes tissue damage and contamination.

During prolonged surgery, water quality should be maintained at a high level. Water for anaesthesia should be from the same tank source to minimize shock.
Appropriate anaesthetics shall be used to provide adequate safety margins, predictable results and rapid recovery. Under field conditions, anaesthetic effects vary with temperature, water quality, species, size of fish, and life stage.

Any incisions should avoid the lateral line and follow a longitudinal axis.

Suture materials should be strong, inert, non hygroscopic and with atraumatic needles.

N.12.4 Administration of compounds and devices

If a treatment compound is to be administered orally, the dose rate should not exceed 1% of body weight (1mL/100gm).

Intramuscular injections may be made into the large dorsal epaxial and abdominal muscles, avoiding the lateral line and ventral blood vessels.

Intraperitoneal injections should avoid penetrating the abdominal viscera.

Implanted materials should be biocompatible and aseptic and implanted using sterile techniques.

Care should be taken to introduce the suture needle in spaces between scales.

N.12.5 Marking and tagging

Marking methods are used mainly for movement assessments, management and population dynamics. It is important to consider the effects of marking and tagging on fish health, physiology, behaviour and survival.

Methods used include DNA markers, fin clipping, electrocauterization or freeze branding (under general or local anaesthesia), tattooing, radio telemetry, radioisotope injections (13C, 15N, or 34S), and tagging. Any proposed method shall be justified and approved by the AEC.

Release of fish back to the wild shall comply with Nature Conservation regulations. Fish shall be in good health, able to function normally in the new environment, be released back to the natural home range, and not introduce any pathogenic agents into the surroundings.

N.12.6 Collection of body fluids and tissues

Results obtained from careful collection and examination of blood and tissues samples are often critically important to research results. Sterility under field conditions is not always possible.

Sedation or anaesthesia should be used for restraint when collecting or cannulation purposes. Restraint will affect serum glucose and hormonal levels.

Blood is collected via 3 main routes – cardiac puncture, venous puncture and caudal vein bleeding. Tissues are collected after fish have been appropriately anaesthetised, or euthanased.

N.12.7 Endpoints and monitoring

Experimental endpoints, other than death of the experimental subjects, should be developed, clearly outlined, and understood, unless death is required, and justified by an AEC approved protocol.

Researchers should eliminate, mitigate or minimize potential pain and distress whenever possible. The use of a Pilot Study should be considered where appropriate monitoring parameters have not yet been defined.

In any study, where there is expected morbidity and mortality, the criteria for endpoints and early euthanasia should be defined. A list of parameters should be established to permit objective assessment of health, pain and distress status.

The frequency of monitoring should allow for the timely removal of fish, before severe morbidity occurs.

N.12.8 Negative reinforcement

Pilot studies and literature searches should be used to establish the least invasive method of obtaining a consistent response when using negative reinforcement modalities in fish.

N.12.9 Exercise to exhaustion

Studies involving swimming to the point of exhaustion, often in conjunction with negative reinforcement, should be justified and approved by the AEC. Strict attention shall be given to continuous monitoring and the elimination of undue distress.

N.12.10 Environmental extremes

Studies involving the exposure of fish to environmental extremes should be justified, and approved by the AEC. Endpoints shall be clearly defined.

N.12.11 Transgenic fish – genetically modified fish

Genetically modified fish shall not be permitted to enter the food chain.

N.13 Holding and disposition of experimental fish

N.13.1 Statutory requirements

It is the responsibility of the researcher, and institution, to ensure the all regulations and permits pertaining to the animals being captured, transported, held in captivity and under study, are complied with.

Work with many species is regulated by the provisions of CITES.
The Organization for Economic Cooperation and Development (OECD) is concerned with toxicological testing methods for human health and eco toxicological test methods, including testing on fish.

N.14 Dangerous aquatic animals and safety considerations

It is important to note the human safety aspects when working with fish of unknown origin and health status. The risk of zoönoses shall be assessed

Even the smallest fish can have defence mechanisms that can be dangerous to humans. Diseases can be transmitted to and from the animals.

Risk activities are feeding and handling procedures. Emergency procedures shall be outlined and understood by trained staff. It is advisable that 2 persons are present at all times when working with dangerous animals.

Traumatogenic animals are those that cause injury mainly via bites, stings, electric shock, and punctures. In many cases secondary bacterial infection can be serious. The stings of certain venomous fish can cause serious cardiovascular effects and irreversible cardiac arrest.

All staff working in designated laboratories should follow safety protocols and guidelines set out in Safety Manuals with regard to biohazards, chemicals, radioisotopes, and dangerous animals.

N.15 Anaesthetics and analgesics

Anaesthetics and analgesics shall be used in a regulated, judicious and appropriate manner to effect pain relief sedation, immobility, loss of equilibrium and controlled loss of consciousness, for surgery, handling, transport and capture.

Most commonly used are the substances that mix easily with water, and allow minimal physical restraint once fish have been placed in the solution.

For recovery, fish are placed in a well-oxygenated anaesthetic free environment. Jaw tone returns before opercular activity. It may be necessary to propel fish through the water to force water through the mouth and gills.

MS–222 (Tricaine methanesulfonate)- is absorbed rapidly via gill diffusion. The anaesthetic dose range varies for species and is between 50-200mg/liter. Aeration should be provided in the anaesthetic solution, as hypoxia is a potential side effect.

Benzocaine and Benzocaine Hydrochloride – benzocaine is a highly insoluble powder that shall first be dissolved in ethanol or acetone. A stock solution of 100gm/liter is generally made up and concentrations of 25-200mg/liter used.

Metomidate – often used for transport sedation at a dose rate of 0.06-0.20 mg/L. For most fish anaesthesia is achieved at a dose rate of 2.5-5.0 mg/L. Induction is rapid, but recovery can be prolonged according to the time animals are exposed to the drug.

Ketamine Hydrochloride – provides excellent anaesthesia in teleosts (bony fish) when injected intramuscularly at a dose of 60-80 mg/kg. In most cases induction takes 10 - 20 min and provides 10 - 20 min of surgical time. Ketamine (12 mg/kg) and Xylazine (6mg/kg) mixture is used for sharks.

N.15.1 Stages of anaesthesia in fish

Stage 1 – erratic swimming, excitement, some loss of equilibrium, disorientation, increased respiration, some loss of tactile response and reduced activity.

Stage 2 – loss of equilibrium, slow swimming movements with loss of direction, decreased respiration.

Stage 3 – complete loss of equilibrium, slow swimming and respiration, reduced responses to stimuli. Surgical plane is reached when fish are unable to swim, respiration is shallow, and no response to stimuli. Cessation of opercular movements.

Stage 4 – spasmodic over distension of opercules and cardiac failure.

N.16 Euthanasia

Wherever possible, a two-step process shall be used – anaesthesia to loss of equilibrium followed by physical or chemical method to cause brain death.

MS-222 is recommended at 500mg/liter followed by an acceptable method to ensure brain death.

N.17 References

ILAR – Guidelines for the Care and Use of Fish in Research

NZ MAF – Transitional Facilities for Ornamental Fish and Marine Invertebrates.

ASF – Guidelines for the Use of Fishes in Research.

CCAC – Guidelines on the Care and Use of Fish in Research, Teaching and Testing.

UFAW Handbook