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QUALITY ASSURANCE PROCEDURES AND GOOD LABORATORY PRACTICES (GLP) GUIDELINES,

Quality Assurance Unit RIVM

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Fifth revised draft
For internal use only
Compliance to quality assurance and GLP and their spirits is not strictly limited to these guidelines

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QUALITY ASSURANCE AND GLP STUDY AND INSPECTION GUIDELINES

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Background

The importance of laboratory studies to governmental decisions demands that all studies be conducted according to scientifically sound study plans and with detailed attentention to quality control.
Compliance with quality assurance and GLP regulations meets these requirements.

Good Laboratory Practice guidelines are rules for laboratory research on chemicals, divices, biological products and electronic products to assure the quality and integrity of the safety data. GLP describes how a laboratory should work, how it should be organized and how it can produce valid data.
It does not prescribe the methods that have to be used. GLP is a policy and strategy for all the aspects in the laboratory which influence the quality of the experimental work, for laboratory spaces in which the work is done, for staff, analysts and technicians, for safety of equipment, handling of chemicals, for reporting and filing the results
Guidelines are implemented through a quality assurance program. This program is delineated in a quality assurance manual or in quality assurance operating procedures.
Some activities in research, e.g. methods development, validation do not come under the strict requirements for GLP or quality assurance compliance, however these activities will be carried out in the "spirit of GLP's". This is interpreted to mean that study plans and SOP's for general procedures will be written and followed, instrumentation will be calibrated and maintained, reagents etc. will meet the high standards required under GLP's and the data will be complete and provide an adequate data trail to allow reconstruction of the conduct of the study.
The reports will be received by quality control and quality assurance procedures to assure that the data support the conclusions.

Definitions

Good Laboratory Practice

GLP is concerned with the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported.

Quality Assurance

Quality Assurence is a planned system of activities whose purpose is to provide assurance that the quality control program is actually effective.

Quality Assurance Program

Quality Assurance Program means an internal control system designed to ascertain that the study is in compliance with the principles of Good Laboratory Practice.

PRINCIPLES AND PURPOSES OF GLP

I. Study design

A study is generated, designed and approved before the experiments are initiated.

II. Study plan

A written study plan is generated, approved, changed or revised and describes overall test system monitoring, specimen labeling, and data collection procedures.

III. Test and reference substances

The laboratory has procedures designed to ensure that the identity, potency, quantity and composition of test and reference substances are in accordance with their specifications, and to properly receive and store test reference substances.

Materials, reagents and specimens are properly labeled, used and stored.

IV. Study conduct

Studies are being conducted in conformance with written study plans and the conduct of the studies are in accordance with GLP Principles.

V. Animal care and biological test systems

The test facility has adequate support facilities and conditions for the care, housing and containment of animals and other biological test systems, to prevent stress and other problems which could affect the test system and hence the quality of data.

VI. Organization and Personnel

The test facility has sufficient qualified personnel, staff resources and support services, for the variety and number of studies undertaken; the organisational structure is appropriate; and management has established a policy regarding training and staff health surveillance appropriate to the studies undertaken in the facility.

VII. Quality Assurance Programme

The quality assurance programme is the mechanism by which the facility management is assured that laboratory studies are conducted in a manner that will guarantee the quality and integrity of date generated therein.

VIII. Facilities

The test facility is of suitable size, construction, design and location to meet the demands of the studies being undertaken. The design and the complexicity of the facility are governed by the need to ensure adequate control of cleanliness and sterility, safety and accuracy and reproducibility of experimental results.

IX. Equipment

The laboratory has suitably located, operational apparatus in sufficient quantity and adequate capacity to meet the requirements of the studies being conducted.

X. Standard operating procedures

The laboratory has written standard operating procedures (SOPs) relating to all the important aspects of the laboratory's operations, considering that one of the most important management techniques for controlling laboratory operations is the use of written SOPs. These relate directly to the routine methods and administrative procedures of studies, not specified in study plans or test guidelines.

XI. Report

Final (or interim) report has to reflect accurately the methods and the conditions and the raw data, to be in accordance with GLP principles and has to be completed in accord with the proper time schedule. The report is a complete presentation of the objectives, the results of the observations made, and the conclusions drawn.

XII. Archives

Study data, reports, specimens etc. are stored safe and adequate and retrieved in a manner which maximizes their integrity and utility in order to facilitate a necessary reconstruction of part or all of the study for full evaluation.

XIII. Critical in process phases

The critical in process phases of a laboratory study that will be inspected by the quality assurance unit are defined.
Inspection of phases not represented in this definition will be determined at study plan submission.

XIV Computers

GLP requirements are also applicable to computerized data acquisition and processing, hardware, software and validation.

XV Health and safety

All necessary precautions will be taken to protect personnel and the environment against possible exposure to the compounds under test.

XVI Laboratory inspections and study audits

Laboratory inspections and study audits are conducted to determine the degree of conformity of a laboratory and laboratory studies with GLP principles and to determine the integrity of data to assure that resulting data are of adequate quality for assessment and decision making.

SECTION I STUDY DESIGN*

A study is generated, designed and approved before the experiments are initiated.

1. Each study shall have an approved written study design before the study is initiated.
2. A descriptive title.
3. Statement of purpose of the study.
4. Name/address of the sponsor (e.g. VHI, HIL, IARC, WHO, etc).
5. Name and address of the testing facility.
6. Proposed completion date of the study (this means the date the final report is signed by the studydirector).
7. Estimated date of the final report.
8. A short description of the experimental design, including the method used for randomization or reference to OECD Test Guidelines or other test guidelines to be used.
9. The date of approval of the study design by the sponsor.
10. Signature of the study director and his deputy (this means the study initiation date).
11. Signature and date of approval of the study design by the testing facility management.

* = projectbeschrijving

SECTION II STUDY PLAN

A written study plan is generated, approved, changed or revised and describes overall test system monitoring, specimen labeling, and data collection procedures.

1. Before any trial starts, it shall be covered by an approved experimental plan in writing.
2. Identification of the test and reference substances by chemical name, chemical abstract number or code number and/or certificate of analysis.
3. Where applicable, the number, body weight, and/or age range, sex, source of supply, species, strain, substrain of the test system or other pertinent information (e.g. P, F_1, F_2).

   The preexperimental isolationperiod in which the animals are acclimatized and conditioned.
4. Procedure for identification of the test system (animals).
5. The method of assignment of animals to control and test groups.
6. A description and/or identification of the diet used, as well as solvents, carriers or other materials used to solubilize or suspend the test substances before mixing with the carrier.
   A specification for acceptable levels of contaminants is given.
7. Each dosage level expressed in the appropriate units (mg/kg), the method (route) and frequency (duration) of preparation and administration of the test or reference substance to the test animals.
8. Detailed information on the experimental design, including a description of the chronological procedure of the study, sampling methods and frequency, all methods, materials and conditions, instruments type and frequency of analysis, parameters measurements, observations and examinations to be performed.
   Citation of readily available documents such as SOP's is in order.
9. A list of the records to be maintained.
10. A statement of the proposed statistical methods to be used.
11. Nonroutine health and safety requirements.
12. CAS Number on cover sheet if applicable.
13. Procedure for animals dying prior to scheduled termination.

14. Procedure for replacement of animals on test.
15. All changes, modifications, or revisions of the study plan as agreed to by the Study Director, including justification(s), should be documented, signed and dated by the Study Director, and maintained with the study plan.
16. The names and addresses of contract laboratories if analytical or other experimental work is contracted out.

SECTION III TEST AND REFERENCE SUBSTANCES

The laboratory has procedures designed to ensure that the identity, potency, quantity and composition of test and reference substances are in accordance with their specifications, and to properly receive and store test reference substances.
Materials, reagents and specimens are properly labeled, used and stored.

1. The identity , strength, purity and composition, solubility, storage conditions or other characterization defining the test or reference substance is determined and documented, c.q. certificate of analysis.
2. Methods of synthesis, manufacturing, or derivation of the test and reference substances are documented.
3. The stability of the test and reference substances is determined before the initiation of the study. If stability cannot be determined, provisions for periodic re-analysis are documented.
4. Each storage container for a test or reference substance is labeled by name, code or batch number, date of manufacture, manufacturer, quantity, expiration date if any, and any nonroutine storage conditions.
5. For studies of more than 4 weeks' duration, substance mixtures are analyzed at regular intervals. These intervals may vary from 6 - 13 weeks.
6. Distribution of test and reference substances is made in a manner to preclude contamination, deteriorations or damage.
7. Proper identification of test and reference substances is maintained throughout the distribution process.
8. The receipt and disposition of each batch is documented by at least the date, quantity of, and person receiving each batch.
9. As appropriate, test or reference substance mixture are analyzed to:
   a. Determine the uniformity (homogeneity) of the mixture.
   b. Periodically determine the concentration of the mixture (concentration check). Weekly at least the first month of the study.
   c. Determine the stability of the mixture under the conditions of administration.

10. Where any component(s) of the test or reference substance mixture has an expiration date, the earliest date is shown on the container.
11. There are separate areas for:
    a. Receipt and storage of test and reference substances to preserve the concentration, purity and stability.
    b. Mixing of test and reference substances with a carrier.
    c. Appropriate storage of test and reference substance mixtures.
    d. The above areas are separate from areas housing animals.
12. All materials, reagents and solutions are properly labeled to indicate source, identity, titer or concentration and stability, and also specific storage requirements, data of formulation and expiration date. These are reviewed and documented annually.
13. Quality of reagents at the time of receipt and subsequent use are verified.
14. Records are kept of the composition, characterisation, concentration, and stability of test and reference substances.
15. Containers holding mixtures (or dilution) of the test and reference substances are labelled and records are kept of homogeneity and stability of their contents.
16. Test chemicals and solvents will not be stored in unvented areas.
17. A use log of the test chemical will be kept.
18. Standard procedures are established to prepare standard solutions.
19. New standard solutions are controlled on their actual concentration.

SECTION IV STUDY CONDUCT

Studies are being conducted in conformance with written study plans and the plans and the conduct of the studies are in accordance with GLP principles.

1. There is an approved final study plan before the initiation of the study.
2. Measurements, observations, and examinations are in accordance with the study plan and relevant SOPs. All deviations from the study plan are properly documented and authorized by the study director.
   If during a test or experiment the analyst deviates from a SOP the reason why the deviation took place and the way the test was conducted must be documented. Such deviations or other observations are recorded in a notebook. The pages of this book are numbered. The book will be archived.
3. All deviations from standard operating procedures are documented and authorized by the study director.
4. Specimens are identified by test system, study, nature, and date of collection or by a code number that precludes error in the recording and storage of data.
   In drug metabolism/kinetic studies the number of capsules prepared for each animal or drug administration have to be recorded.
   Records of time of administration and time of withdrawal of blood have to be available.
5. Records of gross findings for a specimen from postmortem observations are available to a pathologist when examining that specimen histopathologically.
6. All data generated are recorded directly, accurately, promptly and legibly in ink, except those generated by automated data collection systems.
7. In automated data collection systems, the individual responsible for direct data input is identified at the time of data input and the record is dated. The data are protected against unauthorized amendments or loss.
8. All study documents are identified by the study file number.

9. All data entries are signed or initialed and dated by the person entering the data.
   There should be a verification procedure of input in any automated data collection system.
10. Any change in data entries are made so as not to obscure the original entry, are dated and initialed at the time of the change, and are explained.
11. Correspondence relating to the interpretation of the study results should be retained.
12. A copy of the study plan, with all addenda and pertinent SOPs are kept by study personnel or are located in or adjacent to the study room.
    A computer program before use should be verified to show that it actually works and produces the type of data required.
    Any changes in the program have to be documented. The program has to be reverified.
    Statistical packages should be checked and validated.
    A historical file should be kept which documents the program in use at the time the study was conducted.
13. Raw data:
    Raw data are defined as laboratory worksheets, records, notebooks, memoranda, notes, or exact and accurate reproductions or true copies thereof, that are the result of original observations and activities and are necessary for the reconstruction and evaluation of the final report. Such copies are photocopies, microfilm, microfiche, signed computer printouts.
    In automated systems, data generated as graphs, recorder traces or cumputer print-outs are documents of raw data.
    - Histopathology raw data refer only to the signed and dated final report of the pathologist (interim reports and notes of the pathologist do not need to be retained).

    The final printout version of the computer summary, signed by the pathologist, are the raw data too.
14. An intralaboratory testing program for periodic checks of performance to determine precision and accuracy is in operation. This program includes analysis of duplicate and check samples, peer check of chart readings and calculations, and validation of methodology.
15. An interlaboratory testing program for determining the ability of the laboratory to achieve comparable results with participating laboratories, when same samples are analyzed.

16. The analysis of appropriate reference materials (e.g. some matrixes) is performed to investigate bias during analysis or to test the reliability of the results of an analytical method.
17. The following information regarding purchased reference material must be recorded:
    - the data
    - the amount purchased
    - the stability
    - the distribution
    - the amount used for a test and the kind of test
    - the amount left after using
    - the goal
    - the preparation (if necessary)
    - the results of the tests (see control charts).
18. Results of several quality parameters like reference materials, blanks, recordings of signals of instruments (e.g. sensitivity), standard solutions etc. should be recorded on a control chart.
19. Sampling strategy.
    Before sampling starts it is necessary to have a sampling model and a sampling plan.
    In the sampling plan all steps of sampling and all protocols used during the sampling are specified and documented.
    Several questions should be adressed, for example:
    - What is the goal of the study?
    - What should samples be analyzed for?
    - In which environmental compartment should samples be collected?
    - Where should samples be collected?
    - When should samples be taken?
    - What type of samples should be collected?
      (the single discrete or the composite)
    - What is the size of the sample?
    - What is the number of samples that should be taken?
    - What is the frequency of sampling?
    - What is the storage and holding time?
20. <u>Way of sampling</u>
    A written protocol for the sampling itself and procedures before and after sampling is established. It specifies also conditions during transport.

21. Storage and preservation

For each type of container, the parameter analysed and the compartment involved, the cleaning procedure of the containers per parameter is written down in a protocol. Also a protocol of the storage procedure and preservation is be present and known by the technicians.

22. Sample identification

For each sample or group of samples a document is present on which several data are recorded. These are items such as:

- conditions during sampling
- time
- sampling point description
- lot number etc.

23. Laboratory analysis

The analytical method used is the set of written instructions completely defining the procedure to be adopted by an analyst in order to obtain the required analytical result.
The analyst must be alert to recognise problems during the analysis of the sample, such as:

- Contamination
  To check on contamination procedural blanks are processed in the same way as the samples.
- Matrix effects
  To overcome matrix effects for certain analyses it is necessary to analyse the sample and spiked samples.
- Poor recovery
  For certain analyses it is necessary to check the recovery by analysing a sample with a known concentration of the analyte.
- Bad instrument settings
  For each instrumental analysis, the basic settings per parameter per instrument are written down in a manual.

24. Test sample

Every test sample is uniquely identified. The sample is stored in an adequate way before and after the tests.

25. Contract laboratories

Contract laboratories have a program in which the qualifications and the control of their quality assurance program is specified.
Audits of the quality assurance programs of contract laboratories are made during the study.

SECTION V ANIMAL CARE AND BIOLOGICAL TEST SYSTEMS

The test facility has adequate support facilities and conditions for the care, housing and containment of animals and other bilogical test systems, to prevent stress and other problems which could affect the test system and hence the quality of data.

1. All newly received animals from outside sources are isolated, e.g. not specific placed in quarantine and their health status is evaluated.
   Document the receipt and assignment, health examination, veterinary release and examination during the acclimation period of all the animals placed on study.
   Criteria used to determine when animals should be isolated and/or quarantined are documented.
   Newly received animals are identified.
2. Diseased animals are isolated if appropriate.
3. Treatment of diseased animals is documented and retained, including diagnosis, authorization of treatment, description of treatment and each date of treatment.
4. All warm-blooded animals on test, excluding suckling rodents, are uniquely identified by a permanently attached code, such as a tattoo, eartag, toe clip, earpunch or neck chain.
5. All information needed to specifically identify each animal on tests within an animal housing unit appears on the outside of that unit.
6. Animals of different species are housed separately when appropriate.
7. Animals of the same species but different studies housed in the same room are differentiated by space and identification.
8. Animal cages, racks and accessory equipment are cleaned and sanitized at appropriate intervals.
9. Animal rooms are adequately cleaned and sanitized before, during and after housing animals on study.
10. All feed, water and bedding used for animals is analyzed periodically for contaminants that may reasonably be expected to interfere with the study. Analyses have to be provided before use.
11. Bedding used in animal cages and pens does not interfere with the purpose or conduct of the study, is changed as often as necessary to

keep the animals dry and clean, and is stored separately from areas housing animals.

12. The use of any pest control materials is documented and does not interfere with the study.
13. Animal care facilities provide adequate space; temperature humidity, lighting, ventilation and drainage. Although it is ideal to record the information of temperature and humidity with sensitive equipment on a continuous 24-hour basis, a minimum of two observations a day should be made at approximately the same times each day, air changes of the ventilation system can be monitored (in about a 15 minute period monthly).

    Document particle size, passage and functioning of filters, and drainage.
14. Food supplies are stored to prevent contamination, mix-up and infestation, are stored separately from areas housing animals, and have a documented batch production or expiration date. Storage areas for feed and bedding use a first in first out system.
15. Equipment and supplies are of design and composition to maintain adequate sanitation practices and to minimize interference with the conduct of studies.

    A scheduled list is provided for preventive maintenance and the procedure to be followed in any case of equipment malfunction. A description of all equipment malfunctions, the corrective action taken and the length of time the equipment was not operative are entered in the daily log book.

    Any changes that are observed in the test animals and can be attributed to environmental change resulting from equipment malfunction are also noted.
16. There is adequate personnel to assure treatment, care and handling of animals.
17. Animal care facilities are periodically inspected by the veterinarian in charge of animal health and/or designee, and records of such inspections are maintained.
18. Animal facilities are designed, constructed, and located so as to minimize disturbances that interfere with the study.
19. Facilities exist for the collection and disposal of all animal waste and refuse and such facilities are operated as to minimize vermin infestation, odors, disease hazards, and environmental contamination.
20. Health, behaviour or other aspects, as appropriate to the test system is monitored and recorderd.

21. A record will be kept of any incident resulting in minor or major personal injury (including animal bites) or probable personnel exposure to test chemicals.

22. All dose preparation, gavage, filling of dosed feed containers, skin painting, intraperitoneal injection, and inhalation administration will be performed in hoods or other vented enclosures.

23. Engineering controls:

    1. Laboratory hoods and all other local exhaust ventilation enclosures (e.g. mixer enclosures, vented necroscopy and histology work stations, dumping stations) will have proper operation verified by measurement of air flow at least quarterly during long-term studies. For studies of ninety days or less duration, each hood or vented enclosure will be verified within forty-five days prior to the beginning of the study. Face velocities of laboratory hoods and enclosures for gavage, skin painting, intraperitoneal injection, and dosed feed will be 30 $\pm$ 7 m/min.
    2. Relative pressures of laboratory areas will be checked monthly with smoke tubes to verify that air flows from relatively clean to relatively dirty areas.
    3. Confermation of at least 10 air changes per hour in animal rooms will be verified at least twice yearly.
       Records will be maintained by the engineering control department.
    4. Engineering controls will be employed to ensure that exposure to formaldehyde are kept below 1 ppm at histology, necropsy tissue trimming operations, and storage of wet tissues.
       Formaldehyde exposure in tissue trimming areas shall be measured annually, or monthly if TWA is 1 ppm or above.
    5. Emergency power generation systems will be provide (or on lease contract available in operation within short time) to assure continued operation in critical areas and will include routine maintenance and testing programs to assure continued operation.
       Critical areas are animal room, inhalation chamber, storage freezers, refrigerators, autotechnicons etc.

SECTION VI ORGANIZATION AND PERSONNEL

The test facility has sufficient qualified personnel, staff resources and support services, for the variety and number of studies undertaken; the organisational structure is appropriate; and manegement has established a policy regarding training and staff health surveillance appropriate to the studies undertaken in the facility.

1. There is an adequate number of personnel for timely and proper conduct of each study.
2. Personnel have adequate education, training, practice and experience, or combination thereof, to enable them to perform satisfactorily their assigned functions.

   For each study or project an individual is designated with the appropriate qualifications, training and experience as the Study Director before the study is initiated. If it necessary to replace a Study Director during a study, this should be documented.

   Study Director's responsibilities

   1) The Study Director has the responsibility for the overall conduct of the study and for its report.
   2) These responsibilities should include, but not be limited to, the following functions:
      a. Should agree to the study plan;
      b. Ensure that the procedures specified in the study plan are followed, and that authorization for any modification is obtained and documented together with the reasons for them;
      c. Ensure that all data generated are fully documented and recorded;
      d. Sign and date the final report to indicate acceptance of responsibility for the validity of the data and to confirm compliance with these Principles of Good Laboratory Practice;
      e. Ensure that after termination of the study, the study plan, the final report, raw data and supporting material are transferred to the archives.

3. There are curriculum vitae covering qualifications, training and experience for all professional and technical personnel.
4. There are job descriptions for all personnel.
5. There are on-job-training records for all personnel.
6. There is an organizational chart that delineates line management responsibility and scientific organization.
   It shows how the quality assurance unit fits into the overall organizational structure.
7. There is updating of the records from 3., 4., 5., and 6. above at least bi-annually.
8. Personnel take necessary personal sanitation and health precautions to minimize risk to themselves and to avoid interference with studies.
   Safety precautions are applied according to national regulations.
9. Personnel are instructed to report to their immediate supervisors any health or medical conditions that may reasonably be considered to have an adverse effect on a study. They should be excluded from operations that may affect the study.
10. The institute uses a program to increase training and qualifications of personnel.
    Additional experience and expertise in gained by participation in workshops, visits to expert laboratories, courses for handling the equipment in use.
11. The facility has procedures to identify, recognize and deal with health problems of employees that may affect the quality and integrity of studies.
12. The workload of personnel participating in a study has to be adequate in order to accomplish tasks specified by the study design and study plan in time.

SECTION VII QUALITY ASSURANCE PROGRAMME

The quality assurance programme is the mechanism by which the facility management is assured that laboratory studies are conducted in a manner that will guarantee the quality and integrity of date generated therein.

1. There is a quality assurance unit (QAU) in order to guarantee that the experiments adhere to Good Laboratory Practice.
2. The QAU maintains a list of on-going and completed studies at the testing facility.
3. The list contains information on the test system, nature of study, date study was initiated/completed, current status of each study, name of the sponsor, and the study director.
4. The QAU maintains copies of study plans of all on-going studies.
   The QAU ensures that appropiate Standard Operating Procedures are established and available to those conducting an experiment.
   The QAU maintains a historical file of all Standard Operating Procedures.
   The QAU ensures that the study plan and the SOPs are followed by periodic inspections and/or by auditing the study in progress.
   The QAU establishes that no deviations from the experimental plan or SOPs have been made without authorization.
   The QAU promptly reports to management and the Study Director unauthorized deviations from the study plan and from SOPs, likely to affect the quality or integrity of a study.
5. The QAU inspects phases of a study at intervals adequate to assure the integrity of the study and maintains written and signed records of each periodic inspection.
6. The inspection reports show the date of the inspection, the study inspected, the phase or segment of the study inspected, the person performing the inspection, findings and problems, action recommended and taken to resolve existing problems, and any scheduled date for re-inspection.
7. For studies lasting more than 6 months, the QAU inspects the study every 3 months.

8. For studies lasting less than 6 months, the QAU inspects the study at intervals adequate to assure the integrity of the study.
   Where studies are of such short duration that monitoring of each study is impracticable experiments are monitored on a sample basis.
9. The QAU reviews the final study report to assure that the report accurately describes the methods, SOPs, procedures and observations and the reported results accurately reflect the raw data.
10. The QAU prepares and signs a statement to be included with the final study report which specifies the dates inspections were made findings reported to management and to the study director.
11. The responsibilities and procedures of the QAU and the records maintained by the QAU are in writing and are maintained.
12. The QAU maintains a record of inspections which specify the date of inspection, the study inspected, the phase or segment of the study inspected and the name(s) of the individual(s) performing the inspection.
13. External GLP inspection will be conducted in presence of the internal QAU.

SECTION VIII FACILITIES

The test facility is of suitable size, construction, design and location to meet the demands of the studies being undertaken.

1. The testing facilities are of suitable size, construction, and location to facilitate the proper conduct of studies.
The animal facility should be designed to prevent access by unauthorized persons.
The facilities appear clean and well maintained.
Facility maintenance is periodically performed and documented.
Sanitation/cleaning/housekeeping is periodically performed and documented.
A biological monitoring or measurement technique is used to check cleanliness.
Chemicals used by the housekeeping personnel are approved by management.
There is a system to assure the quality of:
- compressed air
- sanitation hot water systems; water temperature at least 82°C.
- steam sanitation systems
- distilled water systems

Critical areas, i.e. animal rooms, biological test system rooms, test substance storage areas, laboratory areas, handling of bio-hazardous material, etc. are environmentally controlled and monitored.
There is a written pest control program which:
- describes responsibility
- approved pesticides/rodenticides
- approved methods
- documentation
- schedule
- area maps application

In rooms where two or more functions requiring separation are performed a separation is maintained.

2. There are separate areas as appropriate provided for the diagnosis, treatment and control of animal diseases.

3. There is separate laboratory space as needed for the performance of routine and specialized procedures required by a study.
4. There are specialized areas for performing such activities such as surgery, necropsy, histology, radiography and the handling of biohazardous materials.
5. There is separate space for cleaning, sterilizing and maintaining equipment and supplies.
6. There is separate space for the administration, supervision, and direction of the testing facility.
7. There are personnel facilities provided including lockers, showers and toilets, with adequate cleanliness items such as soap and towels.
8. There is adequate safety equipment; it is periodically inspected, and records of such inspection are maintained.
9. Separate areas should be provided for e.g. microbiology, tissue culture, cytogenetics, Drosophila testing.
10. An area is specifically designated for weighing mutagens and carcinogens and for the preparation of stock solutions.
11. Working surfaces, storage areas, ventilation systems etc. are aimed at making clean and sterile working practices easily.
12. Incubators used for testing procedures are in an area where the ventilation system removes any hazardous vapours from volatile test chemicals, when incubator doors are opened.
13. Areas where the test and control substances are handled at high concentrations are separate from areas where these materials are encountered at only low concentrations.

SECTION IX EQUIPMENT

The laboratory has suitably located, operational apparatus in sufficient quantity and adequate capacity to meet the requirements of the studies being conducted.

1. Equipment is of appropriate design, adequate capacity and suitably located for operation, inspection, cleaning, maintenance and function.
2. Equipment is inspected, cleaned, maintained and used as necessary in accordance with established SOPs.
3. Equipment used for the generation, measurement, or assessment of data is adequately tested, calibrated and/or standardized in accordance with established SOPs.
4. Written records (logbooks) are maintained of apparatus operation, all inspections, maintenance, testing, calibration and/or standardizing operations.
5. Records of maintenance operations contain the date of the operation and describe the operation if not routine and if in accordance with SOPs.
6. Records of nonroutine repairs document the nature of the defect, how and when the defect was discovered, and any remedial action taken in response to the defect.
7. Where necessary, equipment is located in a controlled environment.
8. A manual is present nearby every instrument.
9. A short manual version in the local language consisting of a description of the instrument and its operation and a part about trouble shooting is described in a SOP.
10. For control of graduated glassware, syringes, micropipettes, balances etc., when applicable, procedures are developed and documented.
11. The preventive maintenance of the instruments by outside contracts or internal technical services is done with a check list. This list is filed.
12. Someone is appointed who is responsible for the instruments and in- and external contacts in case of difficulties that cannot be solved by the operator itself.
    This person sees that the instrument SOP's and the including schedules are followed and carried out.

13. Cleaning procedures for glassware and non-glassware are established depending on the type of determinations.
14. Refrigerators, if appropriate are capable of safe storage of flamable solvents.
15. Suitable non-residual detergent is used to provide clean glassware.
16. In biological safety cabinets a curtain of filter-sterilized air protects the cultures from contamination.

SECTION X STANDARD OPERATING PROCEDURES

The laboratory has written standard operating procedures (SOPs) relating to all the important aspects of the laboratory's operations, considering that one of the most important management techniques for controlling laboratory operations is the use of written SOPs. These relate directly to the routine methods and administrative procedures of studies, not specified in study plans or test guidelines.

1. There are SOPs in writing for each laboratory area setting forth methods adequate to insure the quality and integrity of the data generated in the course of a study.
2. SOPs are appropriately authorized by management and dated.
   Any amendments or changes in established SOPs are authorized in writing and dated by management.
3. There is maintained a historical file of SOPs and all revisions including the dates of such revisions.
4. Each laboratory has manuals and authorized copies of SOPs relative to the procedures being performed immediately and freely available to personnel in the area where the procedure is carried out.
5. SOPs in written form should be worked out for but not be limited to the following laboratory activities. The details below can be seen as examples.
   1) Test, reference and control substances
      a. Recording the receipt, identification, marking, handling, sampling, usage, formulation, specimen-taking, stocking or storage and transportation.
      b. Properly labeling of test and reference substances.
      c. The determination of identity, purity, composition, stability, and for prevention of contamination of test and reference substances, when applicable.
      d. Analysis of the homogeneity and stability of tested substances in mixture with carriers (e.g. feed).
      e. Mixing of substances designed to prevent errors in identification or cross contamination.
      f. Administration of the tested substances.
      g. Preparation of reagents, media and formulations.
      h. Waste disposal.

2) Apparatus and reagents

a. Use, care and maintenance, cleaning, calibration and/or standardization of measuring instruments and environmental control equipment.

b. Production of reagents. All reagents and solutions at the laboratory that are used in a trial should be labeled with identity, titer or concentration and, where appropriate, with date of storage and date of manufacture.

3) Facilities

Environmental control and monitoring in critical areas, e.g. animal and other biological test system rooms, test substance storage areas, laboratory areas.

4) Test systems

a. Routines for receipt, removal, characterization, placement and care of animals and other test systems.

b. Room maintenance and control of environmental conditions for test systems.

c. Production of test systems.

d. Experimental work with microorganisms.

e. Observations and investigations of test systems.

f. Laboratory tests.

g. Handling of dead or dying animals in the course of a trial.

h. Routines for necropsy or other post mortem examinations of animals.

i. Histological investigations and other post mortem examinations of tissues.

j. Collection, identification and handling of specimens, including necroscopic and histopathological specimens and preparations.

5) Safety

The protective clothing and respirator program.

Entry and exit from the testing area (including traffic patterns of dose preparation facility and animal handling and testing room).

Employee training

Respirator protection and fit.

Safety glasses.

Ventilation system maintenance.

General housekeeping practices.

Chemical exposure monitoring (requirements, sampling).

6) Data: collection, reporting, storage and retrieval, coding of trials, data collection, production of reports, indexing systems, handling of data, including use of computerized systems.

7) Quality assurance programme operations.

6. SOPs are written by the laboratory expert.

7. The Quality Assurance Unit edits all SOPs.

8. After editing a SOP may be used.

9. In general a SOP is subdivided into the following sections:

1) Aim/principle

2) Reagents

3) Equipment

4) Method/procedure

5) Calculations

6) References

10. Further information on SOPs is given in note 113/86 BKG Str/lg, "SOP-Guidelines".

11. SOPs are revised and updated on a regular basis by existing procedures.

SECTION XI REPORT

Final (or interim) report has to reflect accurately the raw data, to be in accordance with GLP principles and has to be completed in accord with the proper time schedule.
The authors of the report have to follow the RIVM instructions for authors ("Indeling en vorm van het RIVM-onderzoeksrapport; juli 1987.)

1. Name and address of the testing facility.
2. Dates on which the study was started, completed and reported. An animal toxicity study has to be reported in less than six months after necropsy.
3. Objectives and procedures stated in the approved study plan or study plan addenda.
4. Statistical methods (including transformations and calculations) employed for analyzing the data. The use of International System (SI) Units.
5. The test and reference substances identified by name, code, strength, purity, and composition or other appropriate characteristics (IUPAC, CAS number etc.).
6. Stability and homogeneity of the test and reference substances under the conditions of administration.
7. All relevant information about the samples (sample strategy, way of sampling, storage and preservation, sample identification)
8. A description and/or identification of the methods (biological tests, laboratory determinations, statistical methods) pathologic diagnosis procedures (e.g. group review, blinding) used.
   Reference to OECD Test Guidelines, other test guidelines,literature or a methods appendix.
   Any deviation from the proposed method in the study is noted.
   Results of reference samples are reported.

9. A description of the test system used. Where applicable, including number, sex, weight and/or age range, source of supply, species, strain, substrain, and procedure used for identification.
10. A description of the dosage, dosage regimen, route of administration and duration.
11. A description of significant circumstances that may have affected the quality or integrity of the data.
12. The report accurately presents the results of the study consistently and completely and reflects the raw data.

    The Quality Assurance Unit reviews (audits) the final report to assure that the report accurately describes the methods, SOPs, procedures, and observations and the reported results accurately reflect the raw data.

    The entire report should receive scientific review.
13. A signature sheet as part of the last page in the report, including:
    a. The name, date and signature of the study director.
    b. The names, date and signatures of other (principal) scientists or professionals coauthoring the report.
    c. The name, data and signature of the reviewer of the final report.
    d. The names of all supervisory personnel involved in the study.
14. A description and/or identification of the nonroutine transformations, calculations, or operations performed on the data.
15. A summary and analysis of the data.
16. A statement of the conclusions drawn from the analysis of the data.
17. When appropriate, the signed and dated reports of each of the individual scientists or other professionals involved in the study.
18. The locations where all samples, specimens, raw data, and the final report are to be stored.
19. The study director signs a statement indicating acceptance of responsibility for the validity of the study and confirming that the study was conducted in accordance with GLP principles.
20. A Quality Assurance statement certifying the dates inspections were made and the dates any findings were reported to management (i.e. Laboratory Head c.q. Division Director) and to the Study Director.

21. All pages of the report consecutively numbered, including graphs, tables, figures, etc.
22. Corrections and additions to a final report should be in the form of an amendment. The amendment should clearly specify the reason for the corrections or additions and should be signed and dated by the Study Director and by the principal scientist from each discipline involved. The amendment is audited by the Quality Assurance Unit.
23. The reporting of (pre)chronic animal studies according to U.S. N.T.P. (National Toxicology Program) prechronic and chronic report format may improve relevant description of the study conduct.
24. The reporting of nonclinical pharmacology/toxicology studies may improve by applying the "Guideline for the format and content of the nonclinical pharmacology section of an application", issued under 21 CFR 10.90.

SECTION XII ARCHIVES

Study data, reports, specimens etc. are stored safe and adequate and retrieved in a manner which maximizes their integrity and utility.

1. Study data, reports speciments etc. in support of an application for a research, marketing or experimental use permit or tolerance level, waiting period, or emergency exemption have to be stored.
2. There are archives for the orderly storage and expedient retrieval of all raw data, correspondance and other documents relating to the interpretation and evaluation of data, study plans, samples, specimens exept those specimens obtained from mutagenicity tests and wet specimens of blood, urine, feces and biological fluids and final reports.
   The Study Director is obligated to assure that, after termination of a study, the study plan, the final report, raw data and supporting materials are transferred to the archives.
   Personnel responsibilities, training and experience records are to be preserved.
   Equipment maintenance and calibration records, superseded editions of SOP's, masterschedules of studies and quality assurance reports are to be archived too.
3. The archives storing raw data and specimens from completed studies is limited to access by authorized personnel only.
4. Conditions of storage minimize deterioration of the documents or specimens.
5. An individual is identified as responsible for control and care of the archives.
6. Material retained or referred to in the archives is indexed to permit expedient retrieval.
7. Material retained or referred to in the archives is retained at least 5 years after the date on which the results of the study were submitted to a governmental authority or for a longer time period specified by government regulation or RIVM records retention policy.
8. There is a system (sign-out sheets) for the documentation of removal and replacement of material in the archives under the supervision of person(s) responsible.

9. The records and materials are protected from loss or damage by fire, adverse environmental conditions, other prevailing risks and unauthorized access.

10. Retaining of records and materials as required by different authorities:

OECD

For the required period of time - as appropriate.

USA

FFDCA-FDA

At least 5 years after submitting to the FDA in support of an application for a research or marketing permit.
In other situations at least 2 years.

TSCA-EPA

At least 10 years after the effective date of a test rule or EPA acceptance of a negotiated test agreement.

FIFRA-EPA

The period during which the sponsor holds any permit to which the study is pertinent.
At least 5 years after submitting to the FDA/EPA in support of an application for a research or marketing permit.
In other situations at least 2 years.

Japan

Pharm. and Agric.: until 5 years after approval of application for approval to manufacture (or import) the drug concerned is approved.

New drugs : for the purpose of application of new drugs after approval for reevaluation. 5 years after that reevaluation.

Trade/industry 10 years after the receipt of notice that the substance comes under the Chemical Substances Control Law.

England

For a reasonably period consistent with the objectives of the study; in normal circumstances 10 years would be appropriate.

France

The period required will be indicated by the competent authorities.

The Netherlands

The period required will fixed by the competent authorities.

Sweden

Upon conclusion of a trial all primary data, documentation, specimens, preparations and final reports should be filed in archives for at least 10 years after the results have been submitted to the National Board of Health and Welfare, Departement of Drugs, as a basis for clinical trial or registration of pharmaceutical speciality, or for at least 5 years after the drug has been registered.

SECTION XIII CRITICAL IN PROCESS PHASES

The critical in process phases of a laboratory study that will be inspected by the quality assurance unit are defined.
Inspection of phases not represented in this definition will be determined at study plan submission.

Basic repeated feeding, water, gavage

1. Test and control article (carrier mixture)
   a. Test article characterization and stability determination
   b. Test article - carrier mixture preparation
   c. Test article - carrier mixture sampling
   d. Test article - carrier mixture homogeneity determination
2. Test systems weigh and randomize to housing
3. Test and control article (carrier mixture) distributed to test systems
   3a. Dose calculations
   3b. Test and control article dosing of test system
   Periodical measurements (where applicable)
   - Test system weights
   - Feeder weight
   - Water bottle weights
   - Clinical signs
5. Bleeding, necropsy, clincal chemistry, and hematology
6. Histopathology

Inhalation-Acute, Basic Repeated and Subchronic

1. Test systems weigh and randomize to housing
2. Test and control article (carrier mixture) exposure to test systems
3. Chamber concentration analysis
4. Instrument calibration
5. Periodic measurements (where applicable)
   - Test system weights
   - Clinical signs
6. Bleeding, necropsy, hematology, clinical chemistry (where applicable)
7. Histopathology

Acute Oral LD50

1. Test system weights and randomize to housing
2. Test article (carrier mixture) preparation
3. Dose calculations
4. Test article (carrier mixture) dosing of test systems
5. Clinical signs
6. Test system body weights at 1 week
7. Test system body weights at 2 weeks

Acute Aquatic LC50

1. Test article weigh/measure and mix with carrier
2. Test system addition to test article carrier mixture and control article
3. Periodic measurements at 0-96 hours
   - Dissolved oxygen
   - pH
   - Temperature
   - Clinical signs

Ames Bacterial Mutagenesis

1. Cytotoxicity
2. Test and control article (carrier mixture) preparation
3. Test article (carrier mixture) dilutions
4. Test system addition to test and control article (carrier mixture)/pour plates
5. Scoring of revertant colonies

Cell transformation

1. Cytotoxicity
2. Test system preparation (seeding to flaks)
3. Test and control article (carrier mixture) preparation
4. Test article (carrier mixture) dilutions
5. Test and control article dosing of test system
6. Remove dose, wash cells and replace with fresh medium
7. Stain and count colonies to determine relative cloning efficiency
8. Biweekly refeeding of flasks
9. Staining
10. Count foci

Micronucleus

1. LD50
2. Test and control article (carrier mixture) preparations
3. Test system weight
4. Dose calculations
5. Test and control article (carrier mixture) dosing
6. Specimen collection (harvest femur) at 24, 48 and 72 hrs.
7. Specimen preparation (harvest bone marrow)
8. Slide preparation
9. Staining
10. Count slides 1000 PCE/mouse

Chinese Hamster Ovarian Test

1. Cytotoxicity
2. Seed flasks
3. Test and control article (carrier mixture) preparation and dilutions
4. Test and control article (carrier mixture) dosing of test systems
5. Remove dose, wash cells and replace with fresh medium
6. Subculture for expression of mutants (day 1)
7. Plate treatment survival flasks
8. Count colonies and determine relative cloning efficiency
9. Subculture for expression of mutants (every 2-3 days)
10. Plate cells in selection medium and absolute cloning efficiency (day 7 at the earliest)
11. Staining and fixing of cells
12. Count colonies

Teratology

1. Vaginal smears and/or check for plugs
2. Test system randomization
3. Test and control article (carrier mixture) distribution to test systems
4. Bleeding, necropsy, hematology and clinical chemistry
5. Caesarian section
   - count corpora lutea
   - count implantation sites
   - sex determination of fetuses
6. Soft tissue sectioning and examination of fetuses
7. Processing and skeletal examination of fetuses

Inhalation toxicology

1. Test system weigh, randomize to housing
2. Pre-exposure testing (where applicable)
   - flow volume
   - pressure volume
   - ventilatory efficiency
3. Test and control article exposure to test systems
4. Post exposure testing (where applicable)
   - flow volume
   - pressure volume
   - ventilatory efficiency
   - necropsy - clinical chemistry
6. Histopathology

SECTION XIV COMPUTERS

GLP requirements applicable to computerized data acquisition and processing, hardware, software and validation.

1. Computer hardware

Equipment design

- appropriate design
- adequate capacity
- functioning acoording to study plan
- suitable located

Maintenance/calibration of equipment

- adequately maintained and inspected
- adequately calibrated and/or standardized
- written SOPs and records for above

2. Computer software

- written SOPs approved by management for data handling, storage and retrieval
- space required for archives
- archival control - access limited to authorized personnel
- individual responsible for direct driven data collection system of data input shall be identified at time of data input
- changes in computer entry shall be made so as not to obscure original entry, shall indicate reason for change and shall be dated
- responsible individual identified
- raw data shall be archived

3. Computer validation

SOPs necessary for

- hardware
  description (includes schematics)
  operations
  maintenance
  environmental controls
  logs for routine and non-routine
  security measures
  testing and use of diagnostics

- software
  development and testing
  documentation of testing
- review and approval
  responsible parties including user and quality assurance
- software security
  limited access
    password control
    user I.D. log
  data security
    shutdown procedures
    alarm systems
  physical security
- user and operator training
- conditions revalidation
  hardware changes
  program chances
  periodic re-check

4. Auditing

Auditing of computer system by Quality Assurance Unit.

SECTION XV HEALTH AND SAFETY

All necessary precautions will be taken to protect personnel and the environment against possible exposure to the compounds under test.

Health and safety aspects shall include:

- acquisition, storage and ultimate disposal of test material,
- general housekeeping practices,
- eating and smoking areas,
- precoutionary signs and labels,
- emergency procedures,
- chemical storage,
- personal protective equipment,
- respirators,
- engineering controls,
- waste disposal,
- employee training,
- fire protection and prevention,
- personal and environmental monitoring,
- laboratory safety inspection,
- formaldehyde control,
- plumbed eyewash stations,
- emergency showers.

All the safety rules are compiled in a safety manual and accessible to everybody in the laboratory.
In appropriate biological safety cabinets a curtain of filter-sterilized air protects the worker from chemical exposure.

SECTION XVI LABORATORY INSPECTIONS AND STUDY AUDITS

Laboratory inspections and study audits are conducted to determine the degree of conformity of a laboratory and laboratory studies with GLP principles and to determine the integrity of data to assure that resulting data are of adequate quality for assessment and decision making.

Laboratory inspections are conducted on a regular, routine basis to establish and maintain a record of the compliance status of a laboratory. The inspection results in a report which describes the degree of adherence of a laboratory to the GLP principles.

1. Laboratory inspection

   The purpose of the internal RIVM laboratory inspection carried out by the Quality Assurance Unit (Bureau Kwaliteits Garantie) is to determine and to check wether the principles and purposes of GLP are adequately and sufficiently followed as described on page 4 and 5 of this report.

2. Study audits

   Study audits are brief reviews of on-going or completed studies and carried out by QAU (BKG). The objective is to reconstruct the study from the study plan using relevant SOPs, raw data, and other archived material. In some cases, inspectors may need assistance from other experts in order to conduct an effective study audit - e.g., where there is a need to examine tissue sections under the micoscope.

   When conducting a study audit, the inspector should:

   - obtain names, job descriptions and summaries of training and experience for selected personnel engaged in the studu(ies) such as the study director and principal scientists,
   - check that there is sufficient staff trained in relevant areas for the study(ies) undertaken,
   - identify individual items of apparatus or special equipment used in the study and examine the calibration, maintenance and service records for the equipment,
   - review the records relalting to the stability of the test substances, analyses of feed etc.,

- attempt to determine, through the interview process if possible, the work assignments of selected individuals participating in the study at the time of the study to ascertain if these individuals had the time to accomplish the tasks specified in the study plan or report,
- obtain copies of all documentation concerning control procedures or forming integral parts of the study, including:
  - i) the study plan,
  - ii) SOPs in use at that time the study was done,
  - iii) log books, laboratory notebooks, files, worksheets, print-outs of computer-stored data, etc, and
  - iv) the final report.

In studies in which animals (rodents and other mamifers) are used, the inspectors should follow a certain percentage of individual animals from their arrival at the laboratory to autopsy. They should pay particular attention to the records relating to:

- animal body weights, food/water intake, dose formulation and administration, etc.,
- clinical observation and autopsy findings,
- clinical chemistry,
- pathology.

3. Critical in process phase inspection.

The critical in process phases of a study are periodically inspected by the Quality Assurance Unit. The phases are defined as in Section XIII or determined at study plan submission.

The QAU ensures the study director and management in this manner that study plan and SOPs are followed.

REFERENCES

- Guide to Short-term Tests for Detecting Mutagenic and Carcinogenic Chemicals.
  Environmental Health Criteria 51, UNEP, ILO, WHO, Geneva 1985.
  (Part: Laboratory facilities and Good Laboratory Practices, p. 125-139, describes the reflection of a widely-used aproach that may be regarded as good contemporary practice. The basic principles of laboratory design for conducting mutagenicity and genetic toxicology studies are described as well as the application of GLP in genetic toxicology research laboratories).

- GLP regels FDA.

- GLP regels OECD.

- NEN norm 2653

- Quality Assurance boeken.