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REPUBLIC OF KOREA

Technical report: Genetic and reproductive toxicology *

Prepared for the Government of the Republic of Korea
by the United Nations Industrial Development Organization,
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Explanatory Notes

Abbrevations

KRICT - Korea Research Institute of Chemical Technology

GLP - Good Laboratory Practice

SOPS - Standard Operating Procedures

UNDP - United Nations Development Programme

Abstract

This mission forms part of the expert assistance provided for the KRICT Toxicology Research centre in Genetic and Reproductive Toxicology. It was undertaken between 17 February and 3 March 1987.

The main objective was to guide the staff of the Centre towards a fuller understanding of genetic and reproductive toxicology principles and stategies to improve the quality and range of experimental techniques available to them and to help them to move towards conforming to GLP standards acceptable to International Regulatory Agencies.

The staff were given guidance in the form of lectures, seminars, informal discussions, written material and samples. Recommendations were made on equipment and further training.

Contents

Introduction

This report covers a mission of 3 weeks, commencing 17th February 1987, during which 14 days were spent at the KRICT Toxicology Research Centre. This centre established with UNDP aid and guidance is intented to develop as the main facility for Contract Toxicology in the Republic of Korea. To achieve this goal it is necessary to develop expertise in genetic and reproductive toxicology to a standard acceptable to Regulatory Authorities worldwide. This mission is aimed to provide advice, guidance and training which would further the devlopment of the Research Centre and its staff.

The objectives of the mission were to work closely with the Director of the centre(Dr. Roh) and his staff(Dr. Kim and others) to provide assistance in the planning, operation and administration of genetic short-term tests and longer term reproductive experiments and specifically to :

1. Advise on the design of protocols for short-term and reproductive tests.
2. Help train the genetic toxicology and reproductive toxicology staff for the assessment of various genetic and segment II reproductive end points.
3. Instruct and train staff of the genetic and reproductive toxicology groups in
   a) Observations during test methods
   b) Procedures and evaluation of end-points

4. Advise on experimental data processing and safety limits during such studies.

Following briefings with United nations staff and Dr.Roh and his senior staff these were redefined as :

1. Advise on the design and conduct of animal genetic and reproductive toxicology studies.
2. Train staff in a variety of practical techniques and review current methodology.
3. Advise on further training requirements.
4. Review the existing and planned equipment and advise on its suitability.
5. Advise on GLP compliance and assist in the preparation of SOPS.

These objectives were achieved through individual discussions, practicial seminars and formal lectures as is described more fully later in this report.

Recommendations

1. The centre should broaden its experience to include animal genetic toxicology and reproductive toxicology segment II studies to comply with international regulatory guidelines.
2. Compliance with GLP and safety procedures should be a high priority objective for the centre.
3. The training programme in methods should continue with existing staff.

1. Objectives

The intention of the mission was to provide assistance and advice which would develop the understnding and practical expertise required to enable the research centre to conduct genetic and reproductive toxicological studies to internationally acceptable standards.

The Research Centre has progressed rapidly since its foundation but some areas of genetic and reproductive toxicology have yet to be developed. Currently evaluation of segment II teratology using serial dissection of foetuses has not yet been performed, nor have studies involving bone marrow metaphase or micronucleus analysis or assay of dominant lethals. It was felt that practical advice was needed on the methodology.

At present the Research Centre does not reach GLP or safety standards required by international Regulatory Authorities and some SOP's were lacking or inadequate.
Instruction was necessary for their construction as well as advice on safety procedures to be used.

II. Actions

The general and specific objectives which were established were addressed by various actions which fell into a number of categories.

These are discussed under the following headings:

A. Informal discussions

B. Lectures

C. Seminars

D. Documentation

E. Equipment

F. Training Requipments

A. Informal Discussions

These contributions took place both on an ad hoc basis whenever staff had any specific questions to discuss, and on a pre-arranged time basis.Some time was spent in the microbial and tissue culture and animal areas in the laboratories looking at procedures and making suggestions where appropriate.

Four discussions took place relating to GLP and the construction of SOPS. There was one disscussion relating to microbial assays, another on mammalian cell assays, another on animal assays and a general GLP talk with the QAU officer. During these discussions a need for stringent safety requirements in handling of carcinogens and mutagens in laboratory areas was identified. Many points were raised :

1) The Toxicology laboratories should develop a Health and Safety Policy and Codes of Practice based on available guidelines.
2) The Genetic and Reproductive Toxicology and other departments within Toxicology should have their own departmental safety manual.
3) There should be a room for weighing out carcinogens and mutagens known as the Carcinogen Room or the Hazard RoomThe weighing machine should be enclosed in a relatively 'still air' box with an extract to a fume cupboard. There should be a side entrance for the hands but the face should be protected by a glass shield at the front of the box. Carcinogens should be stored in a refrigerator-preferably a locked one. New carcinogens should be logged in and amounts taken logged out. The date of arrival and removal should be recorded and the person using them should sign for them.

Only diluted solutions should leave the room, dilution taking place in the fume cupboard. Personnel in the Carcinogen room should be suitably gowned with mask, gloves, cuffs, disposable aprons or boiler suits. After the procedures, these should be removed into a plastic bag which should be sealed, inserted into another plastic bag, sealed and then disposed of. The plastic bag should be labelled "Potential Carcinogenic waste." In the fume cupboard the bench should be covered with disposable "benchkote"(material porous on one side) to collect spillages during dilution procedures. This should be removed after dilution procedures and disposed of in the bag system. Any excess volumes of solvent for carcinogens should be poured into sawdust contained in the bag system and disposed of.

Radioactive material could be handled in such a room on a separate bench but preferably there should be another room for this purpose. Radioactive waste should also be monitored. Amounts disposed of should be logged.

4) There should be lockers for clothes.

5) There should be a separate room for tea and coffee. No smoking, eating or drinking should be allowed in laboratories.

6) According to GLP, there should be operating manuals for all equipment in plastic folders attached to each item of equipment. A service record and maintenance checks for equipment should also be available.

7) All bottles containing chemicals(liquids or solids) should be labelled and marked with expiry dates. All shelves and drawers should be labelled.

8) An incinerator, preferably on site, to operate at very high temperatures should be available to dispose of carcinogenic waste (One such on item may be purchased by several of the Research Institutes around the area of KRICT)

9) Solvents need to be stored in solvent cabinets.

10) Animals dosed with hazardous chemicals need to be dosed in a special ventilated area. Litter of urine and faeces can be collected on disposable paper which can be rolled off each day and disposed of in in bag system and then incinerated. This should also be the case for the carcasses of animals exposed to carcinogens. Very hazardous studies should be conducted in isolators. Personnel handling animals should be suitably protectively clothed(as above). Respirators may also be worn. All post mortems or dissections of animals should be carried out in a ventilated area.

11) In microbial and tissue culture laboratories, incubators should be contained in a vented box which extracts to a fume cupboard. Vertical laminar flow cabinets should have about a ten percent extract to the outside. Outside extracts should be about ten feet above the tops of buildings.

12) Disposable petri-dishes and pipettes should be used for experiments involving carcinogens and mutagens. After use, any contaminated dishes should be incinerated using the bag system. A part-time washing up person would be very useful to help clean routine culture glassware. In the first instance he/she may only be needed two mornings a week. Eventually, when the work volume builds up, a part-time slide reader might also be necessary.

13) A general safety committee should be formed for the toxicology laboratories. It should consist of a Chairman and one safety representative from each Department. Before major experiments with hazardous chemicals are performed, safety aspects relating to experimental conduct should be identified and any decisions adhered to. The safety representative in each department should check on any safety features which need attending to and a six-monthly audit performed eg check that centrifuges have locks, check for loose floor tiles, wobbly benches, electrical overloading, etc.

14) For general GLP purposes a line management chart is required so that personnel can identify immediate supervisors in case of absence or crisis.

B. Lectures

Ten lectures were given to senior staff. Most of these used slides but two used overhead projection material, one prepared on site and the other was pre-prepared. A tape recording was made of the lectures. Comprehension appeared to be high. Since the material was presented informally, discussion was active.

Titles and summaries of lectures were as follows :

Wed. February 18th Introductory Discussion

This was to explain the objectives of the visit and to describe the regulatory requirements for genetic toxicology worldwide e.g OECD, EPA, UK, EEC, etc., and the current role of short-term tests in the prediction of human mutagenicity and carcinogenicity ; how the animal tests were used for risk evaluation and the bacterial and mammalian cell in vitro assays

were used for prediction ; also to explain the difference between the main-stream and supplementary assays. Currently the EPA are re-evaluating the premise that a single short-term test can trigger a cancer bioassay. A meeting was held in January 1987 under the auspices of the EPA to determine the relationship between short-term test information and carcinogenicity. The main findings of the meeting were described.

Thurs. February 19th Microbial assays

The use of the microbial assays with and without metabolic activation was discussed : how a record should be kept of positive results using a polaroid camera ; how important it is to determin a true from a pseudo-positive result(pseudo revertants develop on histidine released from dead cells); how the tests used should have equally high sensitivity and specificity for predicting carcinogens. The Ames test is the most well-validated of the assays and is required by all regulatory authorities ; Escherichia coli is also required by the Japanese ; Bacillus subtilis can be used as a pre-screen, so can Salmonella and E. coli if repair proficient and repair-deficient strains are used. The use of the Yeast system was also outlined.(Salmonella and Yeast systems are currently in use at the centre).

Fri. February 20th. Lecture at a mammalian Cell culture workshop at Yonsei University

Mon. February 23rd a.m. Mammalian Cell Culture and In Vitro Cytogenetics

The different cell systems used for regulatory purposes were outlined as well as how some could be used for research purposes.

The use of the immortal cell lines such as Chinese hamster ovary cells and the use of primary cells was highligthed e.g Sertoli germ cells could be used for detecting germ cell toxins such as pthalates, hepatic cells for detecting peroxisome proliferators such as clofibrate and pthalates, and muscle cells for detecting the Bhopal disaster toxin, methyl isocyanate.

Tues. February 24th, . . Reproductive Toxicology

The use of multigeneration versus segment I, II and III studies was described as well as details of the regulatory requirements by different regulatry authori ties. The segment II, Wilson sectioning technique was described in detail, with appropriate illustration of sliced animal tissue sections and identification of the organs. This was compared to the single incision dissection method. The two generation as opposed to the three generation study accepted by the EPA was also discussed.

Tues. February 24th p.m. In Vivo Cytogenetics

The use of the bone manrow assay was described, both for metaphase analysis and micronucleus determination. The question of gaps versus other categories of chromosome danage was discussed as well as the value of single versus multiple dosing and different sampling times. The concept of unbalanced DNA precursor pools giving rise to genetic danage was highlighted, since some chemicals may not attack DNA directly but upset pool balance.

Wed. February 25th Animal assays

The dominant lethal and Heritable translocation assays were described including both the one-week treatment, 8 or 10 week mating regimes for mice and rats respectively versus the 8 week-treament, one week mating technique. The value of both assays was outlined, the former being the better method. Males are generally tested for risk evaluation purposes. Although the females can be treated, confounding factors arise from systemic involvement. It was illustrated with CAPTAN, the pesticide, how thedominant lethal assay can be used for risk assessment using such concepts as Radiation Equivalent Doses and Doubling Doses.

Thurs. February 26th A general seminar for

personnel From KRICT was given on "The Mutagenicity, Teratogenicity and Carcinogenicity of Chemicals".

Fri. February 27th Human Monitoring

It was explained how the blood from a workforce exposed for example to vinyl chloride can be used as an indicator of exposure. Chromosome damage both in terms of metaphase analysis and sister chromatid exchange can be measured in small blood samples from men working at different areas of a vinyl chloride plant. Men such as autoclave workers(highest exposure group), maintenance men(medium exposure), packers and baggers(low exposure) were compared with controls and a dose response relationship was found.

When the exposure levels on the plant were lowered, chromosome damage levels returned to control levels. Also a normal healthy population at a sports club was investigated and females were shown to have a higher incidence of sister chromatid exchange. This was not related to contraceptive pill intake but probably to DNA synthesis and cell turnover rates, as was suggested when these parameters were measured.

Mon. March 2. The Predictibility of Bioassays by Comparison with Short-term tests

It was discussed how information from animal studies and short-term tests can be used to predict potential carcinogenicity or mutagenicity for man using a parallelogram approach. It was illstrated how smoking was nearly a proven human mutagen. Then it was described how rats and mice are only about 80% predicitive for each other with established genotoxic animal carcinogens and about 60% predicitve for target organ specificity. Rats and mice together are only about 75% predictive for human carcinogens and short-term tests with their predictions of greater than 80% on established genotoxic carcinogens perform as well as the rodents. However the use of short-term tests for the ultimate prediction of carcinogenicity for man is not yet emotionally acceptable ; consequently, animal studies still have to be used. When non-genotoxic carcinogens are included as carcinogens the short-term tests obviously do not predict as well as the animal tests.

Tues. March 3rd. Research Programmes using Genetic Toxicology and Reproductive toxicology methods

The research programme at BIBRA using such methods was outlined. Within the Genetic and Reproductive Toxicology and Cell Biology Department at BIBRA, there are six research students, three of whom are concerned with reproductive methods. One examines the effect of manipulating the DNA precursor pools on sperm morphology and sperm production and function. Another examines the effect of manipulating basic, food components such as carbohydrates, proteins and fats. Another investigates the effect of treating the male to produce teratogen-like effects both in vivo and in vitro. Of the other three, one is investigating a benzene-exposed population in a human monitoring study, another is measuring hypoxanthine guanine phosphoribosyl transferase mutants in a population from a sports club local to BIBRA as well as investigating at the molecular biological level of the DNA for base sequence changes in oxygen-mediated mutants at the same locus. The last is investigating the effects of in vitro digestive techniques on di- and polypeptidesin the Ames test as well as effects of novel nitrosopetides in animals.

C. Seminars/Demonstrations

Practical seminars and demonstrations were held in the laboratories to present various methodologies. These included bone marrow metaphase analysis and micronucleus assays.

The femur of the animal was removed and cells aspirated and prepared for the assays. Dominant lethal dissections with instruction on how to dose animals, how to score early deaths, late deaths and live implants were also shown. Segment II methods i.e. teratogenic sectioning using Wilson's method and a single dissection method were performeed. Fixed embryos were brought from England to explain this last technique. The staff were also encouraged to try the methods.

D. Documentation

Standard operating procedures were discussed as well as guidelines for the different countries. Overhead projection material was copied and distributed.

E. Equipment

A review of existing equipment was undertaken. Some recommendations for safety were made and additional apparatus suggested(see earlier-under Informal Discussion)

F. Training

Various aspects of training were discussed. It was felt that existing staff were already well-placed to cope with the extra methodologies required. Two staff of the Genetic Toxicology group at KRICT had recently visited laboratories overseas. One had visited my own laboratory at BIBRA and another had been to Dr. Casciano's laboratory at NCTR in America. The third member of the term is already a publishing scientist.

All three appeared to be enthusiastic and well-qualified for the tasks at hand. In combination with staff effort fromthe animal toxicology group,the genetic toxicology animal studies should easily be carried out. In terms of reproductive toxicology, once the segment II techniques have been mastered, the teratology programme should run quite smoothly. Already, teratology studies are being undertaken and alizarin-red/alcian blue skeletal preparations are well-conducted. The staff in these areas are already quire well-trained and practice should be able to cope easily with the extra tequirements for the guidelines

FUTURE RESEARH

The staff of KRICT and BIBRA are keen to collaborate in a joint research project. During the course of the lectures, shikimic acid was mentioned as having been investigated in the dominant lethal and heritable translocation assays. Shikimic acid was thought to be the active ingredient in bracken fern. However, this was not found to be the case in these two assays ; some other ingredient probably is. Bracken fern is eaten widely in Korea and Japan. One of the genetic toxicology staff at KRICT currently has wide experience in extracting mutagenic as opposed to growth inhibiting factors. If this could be done for the widely consumed bracken fern, this would be a useful extract to examine in these assays, as well as provideing useful practice in the methodology of the assays not already carried out at KRICT.

The research already under taken at KRICT with extracts of medicinal herbs appears to be scientifically sound and the idea of separating the mutagenic from the growth inhibiting factor in the microbial assays a novel approach.

Another suitable research area would be human monitoring studies. Here in the first instance at least 20 males and 20 females from the local community e.g. a sports club could be investigated for their incidence of chromosome damage both in terms of metaphase analysis and sister chromatid exchange. This would provide useful background information on a normal Korean population with which to compare an exposed population in future years. Volunteers should be offered a small financial imbursement. The work should be carried out in conjunction with a medical officer and a medical questionnaire should be answered at the time of taking the blood sample. GOOD CLINICAL PRACTICE guidlines are available for such procedures. Haematology profiles should be prepared at the same time. Tenml of blood is sufficient per person for such a study.

CONCLUSIONS

Within the Toxicology centre much has already been achieved by the Genetic and Reproductive Toxicology groups with little effort a complete package of tests could soon be available to meet the battery of tests acceptable to international Regulatory Agencies. However, compliance with GLP is a prereguisite for the use of the Centre as a contract Facility by client companies who wish to market their products outside Korea.

Before handling chemicals of unknown hazard together with known positive control chemicals to test system efficacy, various safety procedures will have to be adapted. Compliance with both safety procedures and GLP has already begun.