exposures as necessary to determine adequately the consistency of the particle distributions to which the animals had been exposed.

- Duration of study

The duration of the exposure period should be at least 12 months.

3. DATA AND REPORTING

- Observations

A careful clinical examination should be made at least once each day. Additional observations should be made daily with appropriate actions taken to minimise loss of animals to the study, e.g. necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals. Careful observations should be made to detect onset and progression of toxic effects as well as to minimise loss due to diseases, autolysis or cannibalism.

Clinical signs, including neurological and ocular changes as well as mortality, should be recorded for all animals. Time of onset and progression of toxic conditions, including suspected tumours, should be recorded.

Body weight should be recorded individually for all animals once a week during the first 13 weeks of the test period and at least once every 4 weeks thereafter. Food intake should be determined weekly during the first 13 weeks of the study and then at approximately three-month intervals unless health status or body weight changes dictate otherwise.

Haematological examination

Haematological examination (e.g. haemoglobin content, packed cell volume, total red blood cells, total white blood cells, platelets, or other measures of clotting potential) should be performed at 3 months, 6 months, and at approximately 6-month intervals thereafter and at termination on blood samples collected from all non-rodents and from 10 rats/sex of all groups. If possible, these collections should be from the same rats at each interval. In addition, a pre-test sample should be collected from non-rodents.

If clinical observations suggest a deterioration in health of the animals during the study, a differential blood count of the affected animals should be performed.

A differential blood count is performed on samples from those animals in the highest dosage group and the controls. Differential blood counts are performed for the next lower group(s) only if there is a major discrepancy between the highest group and the controls, or if indicated from the pathological examination.

Urinalysis

Urine samples from all non-rodents and from 10 rats/sex of all groups, if possible from the same rats at the same intervals as haematological examination, above, should be collected for analysis. The following determinations should be made from either individual animals or on a pooled sample/sex/group for rodents:

- appearance: volume and density for individual animals;
- protein, glucose, ketones, occult blood (semi-quantitatively); and
- microscopy of sediment (semi-quantitatively).

Clinical chemistry

At approximately 6-month intervals, and at termination, blood samples are drawn for clinical chemistry measurements from all non-rodents and 10 rats/sex of all groups, if possible, from the same rats at each interval. In addition, a pre-test sample should be collected from non-rodents. Plasma is prepared from these samples and the following determinations are made:

- total protein concentration;
- albumin concentration;
- liver function tests (such as alkaline phosphatase activity, glutamic-pyruvic transaminase* activity and glutamic oxalacetic transaminase** activity), gamma glutamyl transpeptidase, ornithine decarboxylase;
- carbohydrate metabolism such as fasting blood glucose;
- kidney function tests such as blood urea nitrogen.

• Pathology

The pathological examination, macroscopy as well as microscopy, if often the cornerstone of the chronic toxicity study. These aspects should therefore get all necessary attention and should be described and reported in detail including diagnosis.

Necropsy procedures

A well-performed gross necropsy may provide optimal information for microscopic examination and may in certain cases facilitate more restrictive microscopic examination. An inadequate gross necropsy cannot be replaced by microscopic examination no matter how well-performed. Gross necropsy should be carried out under the guidance of a trained laboratory animal pathologist.

Complete gross examination should be done in all animals, including those which died during the experiment or were killed in moribund conditions. Prior to sacrifice of all animals, samples of blood should be collected from all animals for differential blood counts. All grossly visible lesions, tumours, or lesions suspect of being tumours should be preserved. An attempt should be made to correlate gross observations with the microscopic findings.

* Now known as serum alanine aminotransferase.

** Now known as serum aspartate aminotransferase.

All organs and tissues should be preserved for microscopic examination. This usually concerns the following organs and tissues: brain* (medulla/pons, cerebellar cortex, cerebral cortex), pituitary, thyroid (including parathyroid), thymus, lungs (including trachea), heart, aorta, salivary glands, liver*, spleen, kidneys*, adrenals*, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, uterus, urinary bladder, lymph nodes, pancreas, gonads*, accessory genital organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord (cervical, thoracic, lumbar), sternum with bone marrow and femur (including joint) and eyes. Although inflation of lungs and urinary bladder with a fixative is the optimal way to preserve these tissues, the inflation of the lungs in inhalation studies is essential for appropriate histopathological examination. In special studies such as inhalation studies, the entire respiratory tract should be studied, including nose, pharynx, and larynx.

If other clinical examinations are carried out, the information obtained from these procedures should be available before microscopic examination, because it may give significant guidance to the pathologist.

Histopathology

All grossly visible tumours and other lesions should be examined microscopically. In addition, the following procedures are recommended:

(a) Microscopic examination of all preserved organs and tissues with complete description of all lesions found of

(1) all animals that died or were killed during the study, and

(2) all of the highest dose group(s) and controls.

(b) Organs or tissues showing abnormalities caused, or possibly caused, by the test substance are also examined in the lower dose groups.

(c) In case the result of the experiment gives evidence of substantial alteration of the animals' normal longevity or the induction of effects that might affect a toxic response, the next lower dose level should be examined as described above.

---

* These organs, from 10 animals per sex per group for rodents and all non-rodents, plus thyroid (with parathyroid) for all non-rodents, should be weighed.

(d) The incidence of lesions normally occurring in the strain of animals used (under the same laboratory conditions, i.e. historical control) is indispensible for correctly assessing the significance of changes observed in exposed animals.

• Test Report

Each test report must identify:

– the laboratory where the test was performed by name and address;

– the inclusive dates of the test; and

– the individual responsible for the conduct and report of the study.

The test report must include all information necessary to provide a complete and accurate description of the test procedures and an evaluation of the results. It should contain a summary of the data, an analysis of the data, and a statement of the conclusions drawn from the analysis. The summary must highlight data or observations and any deviations from control data which may be indicative of toxic effects.

4. LITERATURE

1. Chronic Oral Toxicity; Fitzhugh, O.G. In: Association of Food and Drug Officials: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, pp. 36-45, 1959.

2. Evaluation of Drugs; Goldenthal, E.I. and D'Aguanno, W. In: Association of Food and Drug Officials: Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, pp. 60-67, 1959.

3. *Principles for Pre-Clinical Testing of Drug Safety.* WHO Technical Report Series No. 341, Geneva, 1966.

4. Measurement of Chronic Toxicity. Benitz, K.F. In: *Methods of Toxicology* (ed. G.E. Paget), Blackwell Scientific Publications, Oxford, pp. 82-131, 1970.

5. Report of Chronic Studies Task Force Committee, National Center for Toxicological Research (Appendix B), April 13-21, 1972.

6. Toxicology of Neuroleptic Agents. Schwartz, E. In: *Industrial Pharmacology: Neuroleptics* (ed. S. Fielding and H. Lal), Vol. 1, Futura Publishing Co., Mt. Kisco, N.Y., pp. 203-221, 1974.

7. Drug Safety Evaluation - Pre-Clinical Considerations. D'Aguanno, W. In: *Industrial Pharmacology: Neuroleptics* (ed. S. Fielding and H. Lal), Vol. 1, Futura Publishing Co., Mt. Kisco, N.Y., pp. 317-332, 1974.

8. *Guidelines for Evaluation of Drugs for Use in Man.* WHO Technical Report Series No. 563, Geneva, 1975.

9. Chronic Toxicity and Carcinogenicity Guidelines. Page, N. *J. Env. Path. and Toxicol., 1*: 161-182, 1977.

10. Toxicity and Clinical Trial Subcommittee, Committee on Safety of Medicines, November, 1977.

11. United States Pharmaceutical Manufacturers Association, Guidelines for the Assessment of Drug and Medical Device Safety in Animals, February, 1977.

12. United States Environmental Protection Agency Pesticide Programs. Proposed Guidelines for Registering Pesticides in the U.S.; Hazard Evaluation: Humans and Domestic Animals. *Federal Register*, Vol. 43, No. 163, pp. 37336-37403, August 22, 1978.

13. *Principles and Procedures for Evaluating the Toxicity of Household Substances.* National Academy of Sciences, Washington, D.C., 1977.

14. WHO Publication: Environmental Health Criteria *6*, Principles and Methods for Evaluating the Toxicity of Chemicals, Part I. Geneva, 1978.

15. United States Environmental Protection Agency, Office of Testing and Evaluation. Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules, 40 CFR Part 772, Standards for Development of Test Data, Subpart D: Chronic Health Effects. *Federal Register*, Vol. 44, No. 91, pp. 27350-27362, 1979.

16. Proposed System for Food Safety Assessment. Prepared by the Scientific Committee, Food Safety Council. Food and Cosmetics Toxicology, Vol. 16, Supplement 2, December, 1978.