B.29 SUB-CHRONIC INHALATION TOXICITY STUDY

90-DA Y REPEATED INHALATION DOSE STUDY USING RODENT SPECIES

1. METHOD

1.1. Introduction

See General Introduction Part B.

1.2. Definitions

See General Introduction Part B.

1.3. Reference substances

None.

1.4. Principle of the test method

Several groups of experimental animals are exponed daily for a defined period to the test substance in graduated concentrations, one concentration being used per group, for a period of 90 days. Where a vehicle is used to help generate an appropriate concentration of the test substance in the atmosphere, a vehicle control group should be used. During the period of administration the animals are observed daily to detect signs of toxicity. Animals which die during the test are necropsied and at the conclusion of the test surviving animals are necropsied.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

The animals are kept under the experimental housing and feeding conditions for at least five days prior to the experiment. Before the test, healthy young aimals are randomized and assigned to the treatment and control groups. Where necessary, a suitable vehicle may be added to the test substance to help generate an appropriate concentration of the substance in the atmosphere. If a vehicle or other additives are used to facilitate dosing, they should be known not to produce toxic effects. Historical data can be used if appropriate.

Test conditions

Experimental animals

Unless there are contra-indications, the rat is the preferred species. Commonly used laboratory strains of young healthy animals should be employed. At the commencement of the study the range of weight variation of animals used should not exceed ± 20% of the appropriate mean value. Where a subchronic inhalation study is conducted as a preliminary to a long-term study, the same species and strain should be used in both studies.

Number and sex

At least 20 animals (10 female and 10 male) should be used for each exposure concentration. The females should be nulliparous and non-pregnant. If interim sacrifices are planned the number should be increased by the number of animals scheduled to be sacrified before the completion of the study. In addition, a satellite group of 20 animals (10 animals per sex) may be treated with the high concentration level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for 28 days post treatment.

Exposure concentrations

At least three concentrations are required, with a control or a vehicle control (corresponding to the concentration of vehicle at the highest level) if a vehicle is used. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to the test group subjects. The highest concentration should result in toxic effects but no, or few, fatalities. Where there is a usable estimation of human exposure the lowest level should exceed this. Ideally, the intermediate concentration should produce minimal observable toxic effects. If more than one intermediate concentration is used the concentrations should be spaced to produce a gradation of toxic effects. In the low and intermediate groups, and in the controls, the incidence of fatalities should be low to permit a meaningful evaluation of the results.

Exposure time

The duration of daily exposure should be six hours after equilibration of the chamber concentrations. Other durations may be used to meet specific requirements.

Equipment

The animals should be tested in inhalation equipment designed to sustain a dynamic air flow of at least 12 air changes per hour to ensure an adequate oxygen content and an evenly distributed exposure atmosphere. Where a chamber is used its design should minimize crowding of the test animals and maximize their exposure by inhalation to the test substance. As a general rule, to ensure stability of a chamber atmosphere the total volume of the test animals should not exceed 5% of the volume of the test chamber. Oro-nasal, head only, or whole body individual chamber exposure may be used; the first two will minimize uptake by other routes.

Observation period

The experimental animals should be observed daily for signs of toxicity during the entire treatment and recovery period. The time of death and the time at which signs of toxicity appear and disappear should be recorded.

Procedure

The animals are exposed to the test substance daily, five to seven days per week, for a period of 90 days. Animals in any satellite groups scheduled for follow-up observations should be kept for a further 28 days without treatment to detect recovery from, or persistence of, toxic effects. The temperature at which the test is performed should be maintained at 22 ± 3 °C. Ideally, the relative humidity should be maintained between 30% and 70%, but in certain instances (e.g. tests of aerosols) this may not be practicable. Food and water should be withheld during exposure.

A dynamic inhalation system with a suitable analytical concentration control system should be used. To establish suitable exposure concentrations a trial test is recommended. The air flow should be adjusted to ensure that conditions throughout the exposure chamber are homogeneous. The system should ensure that stable exposure conditions are achieved as rapidly as possible.

Measurements or monitoring should be made of:

(a) the rare of air flow (continuously);

(b) the actual concentration of the test substance measured in the breathing zone. During the daily exposure period the concentration should not vary by more than ± 15% of the mean value. However, in the case of dusts and aerosols, this level of control may not be achievable and a wider range would then be acceptable. During the total duration of the study, the day-to-day concentrations should be held as constant as practicable. During the development of the generating system, particle-size analysis should be performed to establish the stability of aerosol concentrations. During exposure, analysis should be conducted as often as necessary to determine the consistency of particle-size distribution;

(c) temperature and humidity;

(d) during and following exposure, observations are made and recorded systematically; individual records should be maintained for each animal. All the animals should be observed daily and signs of toxicity recorded including the time of onset, their degree and duration. Cageside observations should include: changes in the skin and fur, eyes, mucous membranes, respiratory, circulatory,

autonomic and central nervous systems; somatomotor activity and behaviour pattern. Measurements should be made of food consumption weekly and the animals weighed weekly. Regular observation of the animals is necessary to ensure that animals are not lost from the study due to causes such as cannibalism, autolysis of tissues or misplacement. At the end of the exposure period all surviving animals are necropsied. Moribund animals should be removed and necropsied when noticed.

The following examinations are customarily made on all animals including the controls:

(a) ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the exposure to the test substance and at the termination of the study, preferably in all animals but at least in the high-dose and control groups. If changes in the eyes are detected all animals should be examined;

(b) haematology, including haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, and a measure of clotting potential, such as clotting time, prothrombin time, thromboplastin time, or platelet count, should be investigated at the end of the test period;

(c) clinical biochemistry determination on blood should be carried out at the end of the test period. Test areas which are considered appropriate to all studies are electrolyte balance, carbohydrate metabolism, liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations are calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species), serum glutamic pyruvic transaminase ([1]), serum glutamic oxaloacetic transaminase ([2]), ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methaemoglobin and cholinesterase activity. Additional clinical biochemistry may be employed where necessary to extend the investigation of observed effects;

(d) urinalysis is not required on a routine basis but only when there is an indication based on expected or observed toxicity.

If historical baseline data are inadequate, consideration should be given to determination of haematological and clinical biochemistry parameters before dosing commences.

Gross necropsy

All animals should be subjected to a full gross necropsy which includes examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals and testes should be weighed wet as soon as possible after dissection to avoid drying. The following organs and tissues should be preserved in a suitable medium for possible future histopathological examination: all gross lesions, lungs - which should be removed intact, weighed and treated with a suitable fixative to ensure that lung structure is maintained (perfusion with the fixative is considered to be an effective procedure), nasopharyngeal tissues, brain - including sections of medulla/pons, cerebellar cortex and cerebral cortex, pituitary, thyroid/parathyroid, any thymic tissue, trachea, lungs, heart, aorta, salivary glands, liver, spleen, kidneys, adrenals, pancreas, gonads, uterus (accessory genital organs), (skin), gall bladder (if present), oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, urinary bladder, representative lymph node, (female mammary gland), (thigh musculature), peripheral nerve, (eyes), sternum with bone marrow, (femur, including articular surface), and (spinal cord at three levels - cervical, mid-thoracic and lumbar). The tissues mentioned between brackets need only be examined if indicated by signs of toxicity, or target organ involvement.

Histopathological examination

(a) Full histopathology should be carried out on the respiratory tract and other organs and tissues of all animals in the control and high-dose groups.

(b) All gross lesions should be examined.

(c) Target organs in other dose groups should be examined.

([1]) Now known as serum alanine aminotransferase.

([2]) Now known as serum aspartate aminotransferase.

(d) Lungs of animals in the low - and intermediate-dose group should also be subjected to histopathological examination; since this can provide a convenient assessment of the state of health of the animals. Further histopathological examination may not be required routinely on the animals in these groups but must always be carried out on organs which show evidence of lesions in the high-dose group.

(e) When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in other treated groups.

2. DATA

Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion. Results should be evaluated by an appropriate statistical method. Any recognized statistical method may be used.

3. REPORTING

3.1. Test report

The test report shall, if possible, contain the following information:

-species, strain, source, environmental conditions, diet,
-test conditions:

Description of exposure apparatus: including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air and the method of housing animals in a test chamber then this is used. The equipment for measuring temperature, humidity and, where appropriate, stability of aerosol concentrations or particle size, should be described.

Exposure data: these should be tabulated and presented with mean values and a measure of variability (e.g. standard deviation) and should include:

(a) air flow rates through the inhalation equipment;
(b) temperature and humidity of air;
(c) nominal concentrations (total amount of test substance fed into the inhalation equipment divided by the volume of air);
(d) nature of vehicle, if used;
(e) actual concentrations in test breathing zone;
(f) median particle sizes (where appropriate),

-toxic response data by sex and concentration,
-no-effect level when possible,
-time of death during the study or whether animals surived to termination,
-description of toxic or other effects,
-the time of observation of each abnormal sign and its subsequent course,
-food and bodyweight data,
-ophthalmological findings,
-haematological tests employed and results,
-clinical biochemistry tests employed and results (including results of any urinalysis),
-necropsy findings,
-a detailed description of all histopathological findings,
-statistical treatment of results where appropriate,
-discussion of the results,
-interpretation of the results.

3.2. Evaluation and interpretation

See General Introduction Part B.

4. REFERENCES

See General Introduction Part B.