1. INTRODUCTORY INFORMATION

- Prerequisites
  - Solid or liquid test substance
  - Chemical identification of test substance
  - Purity (impurities) of test substance
  - Stability of test substance
  - Solubility characteristics
  - Melting point/boiling point
  - pH (where appropriate)

- Standard documents

  There are no relevant international standards.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

Organophosphorus substances should be considered as candidates for delayed neurotoxicity studies using the adult hen as the test animal. This test has certain limitations, e.g. in predicting effects from repeated exposures. These limitations may be minimised by conducting an adjunct test in which the inhibition and ageing of neurotoxic esterase of hen neural tissue are measured.

- Definitions

  Acute delayed neurotoxicity is a prolonged, delayed-onset locomotor ataxia resulting from single administration of the test substance, repeated once if necessary.
"Acute Delayed Neurotoxicity of Organophosphorus Substances"

- **Reference substances**

  A substance which is known to produce acute delayed neurotoxicity should be used as a positive control. Examples of such substances are triorthocresyl phosphate (TOCP) and leptophos.

- **Principle of the test method**

  The test substance is administered orally in a single dose to domestic hens (*Gallus gallus domesticus*) which have been protected from acute cholinergic effects, when appropriate. The animals are observed for at least 21 days for delayed neurotoxicity, with redosing and observation for another 21 days if no effects or equivocal responses are seen. The animals are observed daily for behavioural abnormalities, locomotor ataxia and paralysis. Histopathological examination of selected neural tissues is undertaken on all animals surviving the initial cholinergic phases.

B. **DESCRIPTION OF THE TEST PROCEDURE**

- **Preparations**

  A preliminary LD50 test using an appropriate number of animals, dosages and dose groups, as recommended in Test Guideline 401, should be performed in unprotected hens to establish the dose level to be used in this test. Healthy young adult hens free from interfering viral diseases and medication and without abnormalities of gait should be acclimatised to the laboratory conditions for at least five days prior to randomisation and assignment to treatment and control groups.

- **Experimental animals**

  **Selection of species**

  The adult domestic laying hen, aged between 8-14 months, is recommended. Standard size breeds and strains should be employed.
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Number

A sufficient number of hens should be utilised so that at least six survive the observation period.

Controls

Appropriate control groups should be used. These should include a positive control group of at least two hens treated with a known delayed neurotoxicant and a concurrent control group of at least six hens treated in a manner identical to the treated group, except that administration of the test substance and any protective agents are omitted.

Housing and feeding conditions

Cages or enclosures which are large enough to permit free mobility of the hens and easy observation of gait should be used. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. Appropriate diets should be administered as well as an unlimited supply of drinking water.

• Test conditions

Dose level

The selected dose level of the test substance should not be less than the unprotected LD50 dose. Atropine or another non-interfering protective agent may be used to prevent death due to acute cholinergic effects. Doses of test substance higher than 5000 mg/kg of body weight need not be tested.

Route of administration

Dosing with the test substance should normally be by the oral route using gavage, gelatine capsules, or a comparable method.

• Procedure

The test or control substance should be administered and observations begun. All hens should be carefully observed at least once daily for a period of at least 21 days and signs of toxicity recorded, including the time of onset, degree and duration. Observations should include,
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but not be limited to, behavioural abnormality, locomotor ataxia and paralysis. At least twice a week the hens should be taken outside the cages and subjected to a period of forced motor activity, such as ladder climbing, in order to enhance the observation of minimal responses.

If neurotoxic responses are not observed or if equivocal responses are seen, then the dose should be administered again and the animals observed for an additional 21 days. The hens should be weighed weekly. Any moribund hens should be removed and sacrificed.

- Pathology

Gross necropsy

In the presence of clinical signs of delayed neurotoxicity, useful information may be provided by gross necropsy.

Histopathology

All animals should be subjected to microscopic examination. Tissues should be fixed in situ, preferably using perfusion techniques. Sections should include medulla oblongata, spinal cord and peripheral nerves. The spinal cord sections should be taken from the upper cervical bulb, the mid-thoracic and the lumbo-sacral regions. Sections of the proximal region of the tibial nerve and its branches should be taken. Sections should be stained with appropriate myelin and axon-specific stains.

3. DATA AND REPORTING

- Treatment of results

Data may be summarised in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions or effects, the types of lesions or effects and the percentage of animals displaying each type of lesion or effect.
Evaluation of results

The findings of an acute delayed neurotoxicity study should be evaluated in terms of the incidence and severity of neurotoxic effects and of any other observed effects and histopathological findings in the treated and control groups.

Test report

The test report should also include the following information:

- toxic response data by group with a description of clinical manifestations of nervous system damage; where a grading system is used the criteria should be defined;
- for each animal, time of death during the study or whether it survived to termination;
- the day of observation of each abnormal sign and its subsequent course;
- body weight data;
- necropsy findings for each animal, when performed;
- a detailed description of all histopathological findings; and
- statistical treatment of results, where appropriate.

Interpretation of results

This study provides information on the acute delayed neurotoxic effects of exposure to organophosphorus substances. Extrapolation from the results of the study to man is valid only to a limited degree, although it can provide useful information on the degree of neurotoxic activity of a substance.

4. Literature


