OECD GUIDELINE FOR TESTING OF CHEMICALS

Adopted by the Council on 27th July 1995

Delayed Neurotoxicity of Organophosphorus Substances
Following Acute Exposure

INTRODUCTION

1. OECD Guidelines are periodically reviewed in light of scientific progress and in consideration of animal welfare. This updated guideline uses revised methods including measurement of neuropathy target esterase inhibition (NTE; formerly neurotoxic esterase) for determination of the effects of an adequate high dose and no longer requires a repeated dose on Day 21 (1)(2)(3). Inhibition of NTE in brain and spinal cord within 24-48 hours after dosing correlates well with the clinical and morphological effects of delayed neurotoxicity seen 10-20 days later. The NTE test model was found to be valid for all organophosphorus esters known to cause delayed neuropathy in man (4). Therefore, quantitative data on NTE inhibition will significantly improve the ability to determine whether ambiguous results sometimes seen in behavioral or histopathological data should be considered to indicate a potential delayed neurotoxicant (1)(2)(3)(4).

2. This updated version of Guideline 418 resulted from a Consultation Meeting of the ad hoc Working Group of Experts on Systemic Short-term and (Delayed) Neurotoxicity, held in Paris in February, 1992 (5). It is further based on an earlier proposal, discussed at the OECD ad hoc Meeting on Neurotoxicity Testing, held outside Washington, in March 1990 (6), and the comments received on that proposal from Member countries.

INITIAL CONSIDERATIONS

3. In the assessment and evaluation of the toxic effects of substances, it is important to consider the potential of certain classes of substances to cause specific types of neurotoxicity that might not be detected in other toxicity studies. Certain organophosphorus substances have been observed to cause delayed neurotoxicity and should be considered as candidates for evaluation by this guideline (7)(8) (see Annex). In addition, in vitro screening tests could be employed to identify those chemicals which may cause delayed polyneuropathy (9)(10)(11). However, negative findings from in vitro studies do not provide evidence that the test substance is not a neurotoxicant.

4. Negative results on the endpoints selected in this Guideline (biochemistry, histopathology and behavioural observation) would not normally require further testing for delayed neurotoxicity. Equivocal or inconclusive results for these endpoints may require further evaluation.

5. Definitions used are given in the Annex.
PRINCIPLE OF THE TEST

6. The test substance is administered orally in a single dose to domestic hens which have been protected from acute cholinergic effects, when appropriate. The animals are observed for 21 days for behavioral abnormalities, ataxia, and paralysis. Biochemical measurements, in particular NTE, are undertaken on hens randomly selected from each group (normally 24 and 48 hours after dosing). Twenty-one days after exposure, the remainder of the hens are killed and histopathological examination of selected neural tissues is undertaken (see para. 23).

DESCRIPTION OF THE METHOD

Selection of animal species

7. The young adult domestic laying hen (Gallus gallus domesticus), aged 8 to 12 months, is recommended. Standard size breeds and strains should be employed and the hens normally should have been reared under conditions which permitted free mobility.

Housing and feeding conditions

8. 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 provided along with an unlimited supply of drinking water.

Preparation of the animals

9. Healthy young adult hens free from interfering viral diseases, and medication and without abnormalities of gait should be randomized and assigned to treatment and control groups and acclimatized to the laboratory conditions for at least 5 days prior to the start of the study.

Route of administration and preparation of doses

10. Dosing with the test substance should normally be by the oral route using gavage, gelatine capsules, or a comparable method. Liquids may be given undiluted or dissolved in an appropriate vehicle such as corn oil; solids should be dissolved if at all possible since large doses of solids in gelatin capsules may not be absorbed efficiently. For non-aqueous vehicles the toxic characteristics of the vehicle should be known, and if not known should be determined before the test.

PROCEDURE

Number of animals and treatment groups

11. In addition to the treatment group, both a vehicle control group and a positive control group should be used.

12. The treatment group and the vehicle control group should contain a sufficient number of hens so that six can be killed for biochemical determinations (three at each of two time points) and six survive the 21 day observation period and can be used for pathology.

13. The vehicle control group should be treated in a manner identical to the treatment group, except that administration of the test substance is omitted.
14. The positive control group may be run concurrently or be a recent historical control group. It should contain at least six hens (three for biochemistry and three for pathology) treated with a known delayed neurotoxicant. An example of a widely used neurotoxicant is tri-o-cresylphosphate (TOCP). Periodic updating of historical data is recommended. New positive control data should be developed when some essential element (e.g. strain, feed, housing conditions) of the conduct of the test has been changed by the performing laboratory.

**Preliminary dose selection study**

15. A preliminary study using an appropriate number of hens and dose levels should be performed to establish the level to be used in the main study. The objective is to maximize the dose to be used in the main study, since the results of this acute study will be used to determine whether a 28-day study is necessary. Some lethality is typically necessary in this preliminary study to define an adequate main study dose. However, to prevent death due to acute cholinergic effects, atropine or another protective agent, known to not interfere with delayed neurotoxic responses, may be used. A variety of test methods may be used to estimate the maximum non-lethal dose of test substances (See Guideline 420). Historical data in the hen or other toxicological information may also be helpful in dose selection.

**Limit test**

16. If a test at a dose level of at least 2,000 mg/kg body weight/day, using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a study using a higher dose may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used.

**Main Study Dose Selection**

17. The dose level of the test substance in the main study should be as high as possible taking into account the results of the preliminary dose selection study and the upper limit dose of 2,000 mg/kg body weight. Any mortality which might occur should not interfere with the survival of sufficient animals for biochemistry (six) and histology (six) at 21 days. Atropine or another protective agent, known to not interfere with delayed neurotoxic responses, should be used to prevent death due to acute cholinergic effects.

**Observations**

18. Observations should start immediately after exposure. All hens should be carefully observed several times during the first 2 days and thereafter at least once daily for a period of 21 days or until they are killed according to schedule. All signs of toxicity should be recorded, including the time of onset, type, severity and duration of behavioral abnormalities. Ataxia should be measured on an ordinal grading scale consisting of at least four levels, and paralysis should be noted (12). At least twice a week the hens selected for pathology should be taken outside the cages and subjected to a period of forced motor activity, such as ladder climbing, in order to facilitate the observation of minimal toxic effects. Any moribund hens should be removed and killed and subjected to gross necropsy.

**Body Weight**

19. All hens should be weighed just prior to administration of the test substance and at least once a week thereafter.
Biochemistry

20. Six hens randomly selected from each of the treatment and vehicle control groups, and three hens from the positive control group (when this group is run concurrently), should be killed within a few days after dosing, and the brain and lumbar spinal cord prepared and assayed for NTE activity (1)(13)(14)(15). In addition, it may also be useful to prepare and assay sciatic nerve tissue for NTE activity (16)(17)(18). Normally, three birds of the control and each treatment group are killed after 24 hours and three at 48 hours, whereas the three hens of the positive control group should be killed at 24 hours. If observation of clinical signs of intoxication [this can often be assessed by observation of the time of onset of cholinergic signs (2)] indicates that the toxic agent may be disposed of very slowly then it may be preferable to sample tissue from three birds at each of two times between 24 and as late as 72 hours after dosing.

21. Analyses of acetylcholinesterase (AChE) may also be performed on these samples, if deemed appropriate (19)(20). However, spontaneous reactivation of AChE may occur in vivo, and so lead to underestimation of the potency of the substance as an AChE inhibitor.

Pathology

Gross necropsy

22. Gross necropsy of all animals (scheduled killed and killed when moribund) should include observation of the appearance of the brain and spinal cord.

Histopathology

23. Neural tissue from animals surviving the observation period and not used for biochemical studies should be subjected to microscopic examination. Tissues should be fixed in situ, using perfusion techniques. Sections should include cerebellum (mid-longitudinal level), medulla oblongata, spinal cord, and peripheral nerves. The spinal cord sections should be taken from the upper cervical segment, the mid-thoracic and the lumbo-sacral regions. Sections of the distal region of the tibial nerve and its branches to the gastrocnemial muscle and of the sciatic nerve should be taken. Sections should be stained with appropriate myelin and axon-specific stains.

DATA AND REPORTING

Data

24. Individual data should be provided. Additionally, all 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, behavioral, or biochemical effects, the types and severity of these lesions or effects, and the percentage of animals displaying each type and severity of lesion or effect.

Evaluation of results

25. The findings of this study should be evaluated in terms of the incidence, severity, and correlation of behavioral, biochemical and histopathological effects and any other observed effects in the treated and control groups.

26. Numerical results should be evaluated by appropriate and generally acceptable statistical methods. The statistical methods used should be selected during the design of the study.
Test report

27. The test report must include the following information:

Test substance:

- physical nature (including isomerization, purity and physico-chemical properties);
- identification data.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- strain used;
- number and age of animals;
- source, housing conditions, etc.;
- individual weights of animals at the start of the test.

Test conditions:

- details of test substance preparation, stability and homogeneity, where appropriate;
- details of the administration of the test substance;
- details of food and water quality;
- rationale for dose selection;
- specification of doses administered including details of the vehicle, volume and physical form of the material administered;
- identity and details of the administration of any protective agent.

Results:

- body weight data;
- toxic response data by group, including mortality
- nature, severity and duration of clinical observations (whether reversible or not);
- a detailed description of biochemical methods and findings;
- necropsy findings;
- a detailed description of all histopathological findings;
- statistical treatment of results, where appropriate.

Discussion of results.

Conclusions.

LITERATURE


DEFINITIONS

Delayed neurotoxicity is a syndrome associated with prolonged delayed onset of ataxia, distal axonopathies in spinal cord and peripheral nerve, and inhibition and aging of neurotoxic esterase in neural tissue.

Organophosphorus substances include uncharged organophosphorus esters, thioesters, or anhydrides of organophosphoric, organophosphonic, or organophosphoramic acids or of related phosphorothioic, phosphonothioic, or phosphorothioamidic acids, or other substances that may cause the delayed neurotoxicity sometimes seen in this class of chemicals.