

ECETOC Document

No 29

**Proposal for the Updating of OECD
Test Guideline No 407**

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ECETOC COMMENTS FOR THE UPDATING OF OECD TEST GUIDELINE 407
REPEATED DOSE ORAL TOXICITY - RODENT: 28-DAY OR 14-DAY STUDY

ECETOC has combined the practical experience of toxicologists from its member companies and special expertise from members of its Task Forces "Neurotoxicity" and "Immunotoxicity" to specifically address three issues in respect to the updating of OECD Test Guideline 407:

1. CONCEPTUAL BACKGROUND

The OECD Test Guideline 407 for 'Repeated Dose Oral Toxicity in the Rodent: 28-day or 14-day Study' is seen as outlining a study which is part of the initial toxicological evaluation of a chemical. EEC regulations require it for all new chemicals which enter the market at the level of 1 ton per annum. The results of such a study therefore become part of a Base Set Dossier. In other cases such studies, often 14-day studies, are conducted as dose-range-finding studies as precursors to longer term studies. In all cases the aim is definition of a chemical's toxic profile from repeated oral dosing.

Objectives of such a 14 or 28 day study are determination of toxic effects, the shape of the dose response curve and the establishment of a NOEL. To achieve these objectives it is often necessary to administer relatively high but non-lethal doses in order to ensure that all toxic effects have been detected. Since these objectives are essential for hazard identification, ECETOC supports certain modifications, specifications and extensions of the methods and parameters investigated in these studies. However, in carrying out such repeated dose studies it is important that methods used in the execution of the study are validated, and yield interpretable results.

There is a need to maintain flexibility of study design so that further parameters can be measured, either as a result of anticipated toxic properties or as a consequence of the study results. For instance, additional histopathology examinations may need to be added if indicated by the clinical observations or other parameters.

While the inclusion of a recovery group may be indicated by a chemical's predicted toxicity, its inclusion should not be a mandatory part of the 407 Test Guideline. Recovery studies are better incorporated into specific, in-depth studies, the need for which will become evident from results of tests following Test Guideline 407.

2. SCREENING FOR NEUROTOXIC POTENTIAL

ECETOC supports the integration of methods to improve the evaluation of the nervous system as a component of the updated Test Guideline 407. Additional clinical observations, organ weight determinations and pathologic examinations related to the nervous system are appropriate for the initial detection of neurotoxicity and could be included (1).

The repeated exposures and appropriate dose levels make repeated dose 14- or 28-day studies suitable for the initial evaluation of the nervous system and of any other systemic toxicity. The guidelines should not, however, be rigid but scientific flexibility should be allowed. In some cases preliminary results from repeated dose studies or data from previous studies will provide information which triggers more detailed evaluation. In other cases integration of some additional clinical and pathologic evaluations similar to those in the proposed OECD Guideline "Neurotoxicity Screening Battery" would allow Test Guideline 407 to fulfil completely the role of a neurotoxicity screen. For many substances, however, the proposed "Neurotoxicity Screening Battery" will not be necessary.

The specific neurotoxicity testing guideline should be restricted to methods for characterisation of neurotoxicity (Tier 2) if the need is indicated by initial studies.

¹ ECETOC considers evaluations of the nervous system in the context of standard toxicological studies to be true "screening" tests (Tier 1) for the detection of potential neurotoxicity. These screening studies should not include methods that are designed to characterise neurotoxic effects (Tier 2).

ECETOC supports the inclusion of appropriate clinical observations in Test Guideline 407. Carefully conducted and well documented clinical observations are important components of any toxicologic evaluation and are essential for the assessment of potential neurotoxicity. The clinical examinations should not routinely include any test requirement which may confuse interpretation of the results. For example changes in motor activity and grip strength do not necessarily indicate nervous system dysfunction because they are affected by many other factors. Although grip strength does indicate neuromuscular function, it can also be detected readily by sickness, weight loss, toe-nail injuries and habituation. Motor activity tests can be automated and a large quantity of data can be generated but a dose-related change in activity counts may well reflect effects on the nervous system which are secondary to systemic toxicity. Therefore, ECETOC cannot support a quantified measurement of grip strength or motor activity as a component of Test Guideline 407; such investigations may become useful in more detailed studies on potential neurotoxicity indicated by preliminary findings.

3. ENHANCED PATHOLOGY including SCREENING FOR IMMUNOTOXICITY

Enhanced pathologic evaluations of the nervous system in Test Guideline 407 are supported by ECETOC. The inclusion of the brain in the list of tissues to be weighed is appropriate. Preservation of the brain, spinal cord, peripheral nerves and skeletal muscle in fixative should also be considered. Histopathologic examination of the spinal cord and skeletal muscle, however, should not be required on a routine basis; the spinal cord and skeletal muscle could be included in the microscopic examination if other findings suggest a possible treatment-related effect. Appropriate sections of the brain and a single peripheral nerve are adequate for routine microscopic examination unless clinical signs or other data suggest a potential neuropathy.

ECETOC also supports the introduction into the Test Guideline 407 of additional pathological evaluation of the immune system to give a more comprehensive screen for potential immunotoxicity.

A strategy for assessing immunotoxicity in experimental models was proposed by ECETOC (ECETOC, 1987. Monograph No 11. Identification of Immunotoxic

Effects of Chemicals and Assessment of their Relevance to Man). It was concluded that "sub-acute and chronic studies, provided they include a full gross and histopathological assessment of the lymphoid organs are capable of providing considerable insight into the integrity and activity of the immune system of a treated animal". ECETOC further concluded that if such studies indicate that a substance has affected the immune system, an assessment of immune function, using specialised function tests, could be considered.

Since the publication of ECETOC's recommendations, a number of initiatives have been taken to implement them. In particular, an international collaborative study, under the auspices of the IPCS and the EEC (ICICIS) is currently validating the most appropriate pathological, histopathological and functional assessments of the immune system required to detect potential immunotoxicity. Although the results of this and other inter-laboratory studies will not be available for some time, it is considered prudent to extend the gross and histopathological assessment of lymphoid organs beyond that currently required by Test Guideline 407.

It is recommended that the spleen and thymus should be removed and weighed at necropsy and that these two organs, a local (draining) and a distant lymph node and bone marrow should be preserved and may be examined histopathologically.

The recommended changes in the text of OECD Test Guideline 407 resulting specifically from the above considerations are added in annex.

ANNEX:
RECOMMENDED CHANGES IN
OECD TG 407

"Repeated Dose Oral Toxicity - Rodent:
28-day or 14-day Study"

407

1. I N T R O D U C T O R Y I N F O R M A T I O N

° P r e r e q u i s i t e s

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° S t a n d a r d d o c u m e n t s

....

2. M E T H O D

A. I N T R O D U C T I O N , P U R P O S E , S C O P E , R E L E V A N C E ,
A P P L I C A T I O N A N D L I M I T S O F T E S T

In the initial assessment and evaluation of the toxic characteristics of a chemical the determination of oral toxicity using repeated doses may be carried out after information on toxicity has been obtained by acute testing. It provides information on possible health hazards likely to arise from repeated exposures over a limited period of time.

The use of a range of doses, including high doses, allows an evaluation of the toxicological profile of a chemical and identification of target organs, the nature of the dose response curve, and the No Observed Effect Level.

There is sufficient similarity between the considerations involved in the conduct of a 28-day or 14-day repeated dose oral study to allow one Guideline to cover both test durations. The main differences lie in the time over which dosing takes place (indicated in the text) and in the extent of the clinical and pathological investigations which might be considered appropriate for the shorter test duration.

As the primary purpose of these studies is to define the toxicological profile, it is not necessary to use a recovery group routinely. However, it may be incorporated into any subsequent studies used for more comprehensive toxicological evaluation if the results of initial studies indicate a specific need to examine the question of recoverability.

° D e f i n i t i o n s

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° Principle of the test method

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B. DESCRIPTION OF THE TEST PROCEDURE

° Preparations

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° Experimental animals

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° Test conditions

Dose levels

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Limit test

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Observations

The observation period should be for 14 or 28 days. The selection of duration is not fixed rigidly, but should be determined based on the expected toxic reactions and characteristics of the chemical. **If animals in a satellite group are scheduled for follow-up observations they should be kept for at least a further 14 days without treatment to detect recovery from, or persistence of, toxic effects. A careful clinical examination of all animals should be made at least once each day, with additional examinations to detect and if possible to quantify, specific abnormalities. Moribund animals or animals showing obvious signs of suffering should be removed, humanely killed and necropsied.**

° Procedure

The animals are dosed with the test substance ideally on 7 days per week for a period of 28 days or 14 days. Signs of toxicity should be recorded as they are observed, including the time of onset, degree and duration. **Cage-side observations provide valuable information on the occurrence of toxic effects and may specifically demonstrate neurotoxic actions. Observations should be detailed and carefully recorded; animals should be handled and observed both inside and outside the cage. Parameters noted should include,**

but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions or excretions and also clinical observations which indicate effects on the respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern. Measurement should be made of food consumption and water consumption at least once per week and the animals weighed at least once per week. 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. Moribund animals or animals showing obvious signs of suffering should be removed, humanely killed and necropsied. At the end of the study period all survivors are sacrificed.

° C l i n i c a l e x a m i n a t i o n s

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° P a t h o l o g y

Gross necropsy

All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals, testes and epididymides, thymus, spleen, brain and heart should be trimmed of any adherent tissue and weighed wet as soon as possible after dissection to avoid drying. The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examinations:

All gross lesions, brain - including sections of medulla/pons, cerebellar cortex and cerebral cortex, (thymus), (trachea and lungs), heart, liver, spleen, kidneys, adrenals, gonads, (uterus), (accessory genital organs), (urinary bladder), (representative lymph nodes from the gut and from a distant site), (skeletal muscle), peripheral nerve, (spinal cord), (bone marrow). (The tissues mentioned between brackets need only be examined if indicated by signs of toxicity or target organ involvement.)

Histopathology

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

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- (b) All gross lesions shall be examined.
- (c) To aid in the elucidation of NELs, target organs in other dose groups should be examined, particularly in groups claimed to show a NEL.
- (d) When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in the treated groups.

3. D A T A A N D R E P O R T I N G

° T r e a t m e n t o f r e s u l t s

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° E v a l u a t i o n o f r e s u l t s

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Note: The last para ("In any study ... should be considered") should be deleted.

° T e s t r e p o r t

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° I n t e r p r e t a t i o n o f t h e
r e s u l t s

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