



# A “good practice” approach to the quality and consistency of morphological examination of the internal head structures of the term rabbit fetus

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## ABSTRACT

Variation in the interpretation of the regulatory guidelines has resulted in a diversity of techniques employed to examine the internal structures of the foetal rabbit head.

Examination of the foetal rabbit brain, using a single transverse section as the sole technique, is considered not to be sufficiently thorough to be regarded as an adequate examination method. It is not compliant with published EPA and OECD guidelines covering required examination of the internal head structures, nor is it considered to conform to the spirit of the safety assessment required by the ICH guideline.

Fixation of approximately half of the heads in each litter to allow the examination of multiple transverse sections enables the major structures within the head to be assessed effectively. This method is compliant with current guidelines, represents “good practice” and should be consistently adopted for the examination of the internal head structures of the term rabbit foetus.

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## 1. Introduction

The conduct of regulatory embryo–foetal development and prenatal developmental toxicity studies is covered in three guidelines, ICH (1993) – *Harmonised Tripartite Guideline. Detection Of Toxicity To Reproduction for Medicinal Products & Toxicity To Male Fertility S5(R2)* [1], EPA (1998) – *Health effects test guidelines OPPTS 870.3700. Prenatal developmental toxicity study* [2] and the OECD (2001) – *TG414: OECD guideline for the testing of chemicals. Prenatal developmental toxicity study, for industrial chemicals* [3].

Studies addressed in each of these guidelines are designed to provide specific information on non-clinical safety assessment, including the ability of the test compound to produce structural abnormalities or alterations in growth in the developing organism following exposure of the pregnant animal to a test compound. The information acquired from these studies, using both a rodent (typically rat) and non-rodent species (typically rabbit), forms part of the prenatal developmental hazard assessment of the test compound, and the risk of causing foetal harm in pregnant women should there be human exposure, either intentional or accidental.

All three guidelines specify the proportion of foetuses from each litter that are assigned for either soft tissue and/or skeletal

evaluation. For rodents, the EPA and OECD guidelines identify the methods that should be used for the examination of soft tissues as “dissection techniques” together with “serial sectioning methods”. The guidance given for non-rodents (typically rabbits) is more detailed and states which head structures should be examined, in the case of the OECD guideline specifically mentioning the use of “standard serial sectioning methods or an equally sensitive method” to achieve this. The ICH guideline makes no mention of specific head structures which should be examined and refers only to using fresh microdissection techniques for soft tissue alterations.

The relevant section from each of the guidelines is reproduced below:

ICH Harmonised Tripartite Guideline. Detection of toxicity to reproduction for medicinal products and toxicity to male fertility S5 (R2):

“When using techniques requiring allocation to separate examination for soft tissue or skeletal changes, it is preferable that 50% of foetuses from each litter be allocated for skeletal examination. A minimum of 50% rat foetuses should be examined for visceral alterations, regardless of the technique used. When using fresh microdissection techniques for soft tissue alterations – which is the strongly preferred method for rabbits – 100% of rabbit foetuses should be examined for soft tissue and skeletal abnormalities.”

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## EPA Health Effects Test Guidelines OPPTS 870.3700 Prenatal Developmental Toxicity Study:

“For rodents, approximately one-half of each litter should be prepared by standard techniques and examined for skeletal alterations, preferably bone and cartilage. The remainder should be prepared and examined for soft tissue anomalies, using appropriate serial sectioning or gross dissection techniques. It is also acceptable to examine all foetuses by careful dissection for soft tissue anomalies followed by an examination for skeletal anomalies. For rabbits, all foetuses should be examined for both soft tissue and skeletal alterations. The bodies of these foetuses should be evaluated by careful dissection for soft-tissue anomalies, followed by preparation and examination for skeletal anomalies. An adequate evaluation of the internal structures of the head, including the eyes, brain, nasal passages, and tongue, should be conducted for at least half of the foetuses.”

## TG414: OECD guideline for the testing of chemicals. Prenatal developmental toxicity study:

“For rodents, approximately one-half of each litter should be prepared and examined for skeletal alterations. The remainder should be prepared and examined for soft tissue alterations, using accepted or appropriate serial sectioning methods or careful gross dissection techniques. For non-rodents, e.g. rabbits, all foetuses should be examined for both soft tissue and skeletal alterations. The bodies of these foetuses are evaluated by careful dissection for soft tissue alterations, which may include procedures to further evaluate internal cardiac structure. The heads of one-half of the foetuses examined in this manner should be removed and processed for evaluation of soft tissue alterations (including eyes, brain, nasal passages and tongue), using standard serial sectioning methods or an equally sensitive method. The bodies of these foetuses and the remaining intact foetuses should be processed and examined for skeletal alterations, utilising the same methods as described for rodents.”

It is possible that the complexity of the foetal processing requirements (multiple species being described, multiple examinations, litters divided for processing and examination), resulted in ambiguity in the guideline wording, which has caused the guidelines to be interpreted differently in different laboratories. This confusion has resulted in different perceptions of the level of detail expected for the head examination, and so the implementation of different techniques being used for the examination of the internal structures of the foetal rabbit head. This, in turn, has led to inconsistency in the areas examined, and considerable variation in the level of detail and quality of these examinations.

The purpose of this document is to propose the rationale for a technique which could be considered as an industry standard for the morphological examination of the internal head structures of the foetal rabbit. It is intended that this will provide clarity to foetal morphologists, and ensure that examination data are of sufficient detail and rigour. Consistency across the industry should provide confidence to the regulatory reviewers.

## 2. Routinely used methods for internal head examination of rabbit foetuses

The methodology of the techniques in common use is reviewed, together with their benefits and disadvantages.



**Fig. 1.** Single transverse section of alcohol preserved specimen – normal appearance.

### 2.1. Single transverse section of fresh/alcohol preserved specimens

Stuckhardt [4] described the use of a single transverse section exclusively to “observe the brain for hydrocephaly” and the removal of the eyes to facilitate their examination. This paper is included in the bibliography of both the EPA and OECD guidelines and allows both lateral and 3rd ventricles, left and right cerebral hemispheres to be examined at a single level (Figs. 1 and 2).

Advantages:

- Simple, quick and typically performed either at the time of fresh visceral examination or following a short time in ethanol. The results of this examination can be available with the fresh external/visceral examination data.



**Fig. 2.** Single transverse section of alcohol preserved specimen – hydrocephaly (internal).

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