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# Satellite rats are redundant in embryo-fetal development studies

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ABSTRACT

Routinely in many laboratories, satellite rats are added to embryo-fetal development (EFD) studies for pharmaceuticals to assess toxicokinetic (TK) properties, because it is assumed that collection of multiple blood samples with relatively large volumes might affect the study outcome. With recent refinement of blood sampling techniques, this belief requires reevaluation. The current work showed successful implementation of jugular vein blood sampling in an EFD rat study without satellite animals, thereby reducing the number of rats in standard EFD studies for pharmaceuticals by 20%. Although not evaluated in this study, microsampling has shown to be very successful and eliminates the need of satellite animals. However, currently not all laboratories have implemented this method and regularly the bioanalytical method is already developed with a limit of quantification that is insufficiently sensitive. Therefore in those cases, a quick win to omit satellite animals can be established by using jugular vein blood sampling.


## 1. Introduction

Guidance document ICH M3 (R2) [1] describes the type of nonclinical toxicity studies required for safety assessment of pharmaceuticals and its timing in respect of the clinical phases. Developmental toxicity is generally assessed in two species: a rodent and non-rodent (usually the rat and rabbit). In the EU and Japan definitive developmental toxicity studies in two species are required, in general, before inclusion of women of childbearing potential (WOCBP) in any clinical study. However, there are some exemptions to this. One circumstance could be a short-duration (e.g., 2 weeks) clinical study with precautions taken to prevent pregnancy. Another circumstance is where the clinical study must include WOCBP since the disease is prominent in women, but sufficient precautions are used to prevent pregnancy. In the relevant regions, the guidance also allows for the inclusion of WOCBP (up to 150) using adequate birth control methods for a relatively short duration (up to 3 months) if preliminary developmental toxicity data are available from two species. In the United States, assessments of embryo-fetal development (EFD) can be deferred up to but not including phase 3 clinical studies for WOCBP as long as precautions are taken to prevent pregnancy.

Details on the study design are described in ICH guideline S5 (R2) Section 4.1.3. Study for effects on embryo-fetal development [2]. In short, three groups of pregnant animals are treated with the test item during organogenesis to evaluate the effect of in utero exposure on embryo-fetal development. A fourth group serves as vehicle control. The ICH guideline describes "*For all but the rarest events (such as malformations, abortions, total litter loss), evaluation of between 16 to 20 litters for rodents and rabbits tends to provide a degree of consistency between studies. Below 16 litters per evaluation, between study results become inconsistent, above 20–24 litters per group consistency and precision is not greatly enhanced*". It is common practice to use a group size of 22 mated rats to assure 16–20 litters per group. For rats, this would result in an animal usage of 88 females for the $F_0$-generation and, according to European animal welfare rules, approximately 1056 fetuses for the $F_1$-generation (assuming a litter size of 12 fetuses/litter) per EFD study. It should be realized that these numbers are without the addition of satellite animals for toxicokinetic (TK) assessment.

The ICH guideline S5 (R2) also provides some details on why TK information is needed in developmental and reproductive toxicity (DART) testing [2]. In the development of a new drug, it is important to know if the No Observed Adverse Effect Level (NOAEL) in an EFD study provides an adequate safety margin over the anticipated human exposure. As a rule, blood samples are collected for TK evaluation on the first and last day of treatment with up to six time points per 24 hours, and in EFD rat studies this has always been done by using satellite animals [3]. For each EFD study in the rat,

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this results in an additional 21 $F_0$-females and, according to European animal welfare rules, approximately 252 $F_1$-fetuses (assuming a litter size of 12 fetuses/litter). These numbers are based on 6 $F_0$-females per treatment group (as each female would be bled at 3 time points) and 3 $F_0$-females for the control group (as these will only be bled around the anticipated $T_{max}$ for the test item groups; 2 time points).

Historically, satellite animals for TK have been used to circumvent possible adverse effects on the study outcome as it was thought that multiple occasions of blood sampling (usually under anesthesia) of relatively large volumes (~300 μl per time point) would impact maternal health and/or embryo-fetal development.

Microsampling is a relatively new technique for obtaining small amounts of blood (~30 μl) using an EDTA coated capillary. It was developed in 2012 at AstraZeneca [4]. Since then, it has been validated by several organizations, pharmaceutical companies and contract research organizations [5,6]. It has already been applied to obtain repeated blood samples from adult rats, mice and dogs in general toxicity studies [4,7–13], in juvenile rat studies [14,15] and even on mouse fetuses and pregnant mice [16]. These data have paved the way for adopting microsampling in study animals, thereby reducing or even preventing the use of satellite animals for TK blood collection, and allowing a direct link of exposure data to treatment related findings for individual animals [5].

However, currently not all laboratories have implemented this method and regularly the bioanalytical method is already developed with a limit of quantification that is insufficiently sensitive. Therefore in those cases, a quick win to omit satellite animals could be established by using jugular vein blood sampling. This method does not require anesthesia and causes less discomfort to the animals in comparison to other methods (i.e. discomfort score changes from moderate to mild). In addition, multiple blood samples can be obtained from one animal making it a suitable method for sampling for TK assessment as it provides one TK profile per rat. However, a high degree of competence is required for this technique.

The present study was undertaken to investigate, if blood collection from the jugular vein would not affect health and/or embryo-fetal development, and therefore satellite animals would no longer be needed in EFD rat studies.

## 2. Material and methods

### 2.1. Guidelines

The study plan was reviewed and agreed by the Laboratory Animal Welfare Officer and the Ethical Committee as required by the Dutch Act on Animal Experimentation (February 1997). The study procedures were in compliance with the ICH S5 (R2): International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline on Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility, parent guideline dated 24 June 1993 (addendum dated 9 November 2000 incorporated in November 2005), Section 4.1.3. “Study for effects on embryo-fetal development”.

### 2.2. Test animals

Sixty-six time-mated female rats (Crl:WI(Han, outbred, SPF-Quality) from Charles River Laboratories Germany were assigned to the study. Untreated primiparous females were mated at the Supplier, and arrived at Day 0 or 1 post-coitum at the Test Facility (Day 0 post-coitum was the day of successful mating; confirmed by vaginal plug). The females were 10–14 weeks old on arrival and acclimatized for at least 5 days prior to start of treatment. A health inspection was performed upon receipt of the animals. The animals were randomized one day after receipt, by computer-generated random algorithm according to body weight, with all animals within ± 25% of the mean body weight per mating day. The animals were uniquely identified by indelible ink on the tail.

### 2.3. Animal husbandry

Environmental controls for the animal room were set to maintain 18 to 24 °C, a relative humidity of 40–70%, at least 10 air changes/hour, and a 12-hour light/12-hour dark cycle. Females were individually housed in Macrolon plastic cages (MIII type, height 18 cm). Sterilized sawdust as bedding material (Lignocel S 8–15, JRS – J.Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany) and paper as cage-enrichment/nesting material (Envirodri, Wm. Lillico & Son (Wonham Mill Ltd), Surrey, United Kingdom) were supplied. They had free access to pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany) and tap-water. Evaluation for contaminants was performed for diet, water, bedding and cage-enrichment/nesting material. There were no contaminants identified that could interfere with the study.

### 2.4. Experimental groups

There were 3 groups, each consisting of 22 time-mated female rats. The animals were dosed by oral gavage from Day 6 to 17 post-coitum, inclusive, with Elix water at 5 mL/kg body weight. Group 1 animals (nos. 1–22) were not used for TK blood sampling, Group 2 animals (nos. 23–44) were used for 3 time points per occasion, and Group 3 animals (nos. 45–66) were used for 6 time points per occasion. The number of animals per group was based on the standard group size as defined in ICH S5 (R2) [2].

### 2.5. In-life observations

At least twice daily, the animals were checked for mortality/viability. Clinical signs were observed at least once daily from Day 2 post-coitum onwards up to the day prior to necropsy. The time of onset, grade and duration of any observed signs were recorded. Body weights and food consumption were determined on Days 2, 6, 9, 12, 15, 18 and 21 post-coitum.

### 2.6. Toxicokinetic blood sampling

On Days 6 and 17 post-coitum, blood was collected from all animals of Groups 2 and 3 from the jugular vein by highly trained personnel. Animals were not fasted prior to sampling and warming of the animals was not required. The neck region of the animal was shaved and kept in hyperextended position. The jugular vein appears blue in color and is found 2–4 mm lateral to the sternoclavicular junction. A 23G needle was inserted in the caudocephalic direction (back to front) and blood was withdrawn slowly using a 1 mL syringe. Slight pressure with a finger was applied to stop bleeding. Approximately 0.3 mL blood samples were taken and collected into tubes containing Li-heparin as anticoagulant. The animals of Group 2 were sampled at 0.5, 2 and 8 hours post-dose, and the animals of Group 3 were sampled at 0.5, 1, 2, 4, 8 and 24 hours post-dose. Left and right sides were alternated. The samples were visually checked for quality and quantity, where after the samples were discarded.

### 2.7. Postmortem examination of the dams

On Day 21 post-coitum, all animals were sacrificed using an oxygen/carbon dioxide procedure and subsequently subjected to an external examination, followed by a thoracic and abdominal