ASSESSMENT OF MALE REPRODUCTIVE TOXICITY IN RATS UNDER ICH GUIDELINES

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A major goal of the tripartite international conference on harmonization (ICH) guidelines for detection of reproductive toxicity was to maintain flexibility of study designs and to avoid strict adherence to rigid, and often unscientific, rules. The general guiding principles inherent in the design of the guidelines were that 1) they should describe screening studies to identify agents that specifically disrupt reproduction, 2) screening studies should not be designed to identify the critical stage, mechanism of action or full expression of the effect, and 3) results of screening studies should be used to determine if further investigation is warranted. It is implicit in the guidelines that interpretation of reproductive effects would be made in context with general systemic toxicity studies at similar doses when possible. As was stated in the guidelines, an area where more basic research would be useful in optimization of study designs was in male fertility assessment, especially the optimal treatment period for males prior to mating. Recent revision in the guidelines agreed on at the ICH meeting in Yokohama in 1995 have further justified the use of a 4 week pretreatment period of males prior to cohabitation. This was based on data from a variety of test agents in a prospective collaborative study in Japan (Takayama et al.) and a retrospective literature analysis (Ulbirch and Palmer). One specific issue which has not been adequately addressed in the guidelines is the utility of quantitative staging of spermatogenesis, which was implied but not specifically stated as a required endpoint. At this time, sufficient data does not exist for a consensus. Staging has been employed in our laboratory to clarify findings in specific cases, but we do not feel that this technique is warranted or useful as a general screen. There is also insufficient information to put findings from staging analysis into perspective for human risk assessment.

It has been evident for some time that mating trials of males, even after prolonged exposure to test compounds, is an insensitive indicator of male fertility. The focus of male reproductive assessment has now shifted to include study designs with several overlapping endpoints, as well as reliance on previous subchronic toxicity studies which include organ weights and histopathology of the reproductive tract. The current design for male fertility studies in our laboratory is diagrammed below, assuming no significant effects have been observed in the reproductive organs in subchronic studies, usually 5 to 14 week duration.

Figure; Fertility Studies in Male Rats

<table>
<thead>
<tr>
<th>Week</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Cohabitation Laparotomy Necropsy (max. 10 days) (GD 15 or 21) Organ weight Routine histology Sperm count/motility</td>
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4 weeks

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This paper was presented at the 5th Satellite Symposium of the Japanese Society of Toxicological Sciences on July 24, 1996 in Fukuoka.
The major study endpoints are:

Function (mating behavior/pregnancies produced)
Sperm count - epididymis
Sperm motility - vas deferens
Organ weights
Histology

This design, in conjunction with results of the routine subchronic toxicity studies, is adequate to detect possible effects on male reproduction and provide preliminary data for more detailed studies should positive findings occur.

In the past several years, we have examined a number of compounds that have shown effects on male reproduction. In several cases we have had to use lengthy exposures and/or unusual study designs to demonstrate effects on male fertility. In one case, it was found that the age of the male at the start of treatment had significant effects. Younger males (6-week old) required 12 weeks of treatment to show a moderate decrease in fertility whereas 15-week old males required 24 weeks to show similar effects (Wise et al.). In examining the mechanism of these effects observed matings, intrauterine sperm counts and artificial insemination were utilized to resolve the mechanism of decreased fertility (Cukierski et al.). In another case, treatment required 11 to 15 weeks to show significant effects on fertility parameters. However, in all cases we have experienced in our laboratory the above findings would not have been missed by use of the routine study design; as changes observed in organ weight or histopathology of the reproductive tract were evident within two days to two weeks even though it required up to six months to see effects on fertility. Data from these studies will be presented to illustrate the need for adequate toxicity data prior to initiation of male fertility studies and the need for flexibility in design and selection of endpoints.

References


