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Combined retrospective analysis of 498 rat multi-generation reproductive toxicity studies: On the impact of parameters related to F1 mating and F2 offspring☆

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ABSTRACT

The multi-generation reproductive toxicity study (OECD TG 416 and USEPA 870.3800) has been extensively used internationally to assess the adverse effects of substances on reproduction. Recently the necessity of producing a second generation to assess the potential for human health risks has been questioned. The present standardized retrospective analysis of the impact of the second generation on overall study outcome combines earlier analyses and includes 498 rat multi-generation studies representing 438 different tested substances. Detailed assessment of study reports revealed no critical differences in sensitivities between the generations on the basis of a consideration of all endpoints evaluated. This analysis indicates that the second generation mating and offspring will very rarely provide critical information. These findings are consistent with the conclusions of previous retrospective analyses conducted by RIVM, USEPA and PMRA and support adoption of the proposed OECD extended one-generation reproductive toxicity study protocol in regulatory risk assessment testing strategies.

1. Introduction

In the early 1980s, the two-generation reproduction toxicity study (OECD Test Guideline 416) [1] was introduced as the globally agreed standard test protocol for assessing potential adverse effects of industrial substances and pesticides on fertility, reproduction, and postnatal development. This study protocol has been employed extensively for more than 25 years, and hundreds of such studies have been performed worldwide. The guideline was officially updated in 2001 in OECD TG 416 and similarly in 1998 by USEPA 870.3800 to include additional endpoints capable of detecting endocrine-mediated and other effects on reproductive development. The protocol requires continued exposure beginning prior to mating of mature male and female animals, usually rats (P0), through mating and pregnancy to generate F1 offspring (Fig. 1). The F1 offspring are exposed lactationally through weaning and then directly through to adulthood (P1). The P1 animals are

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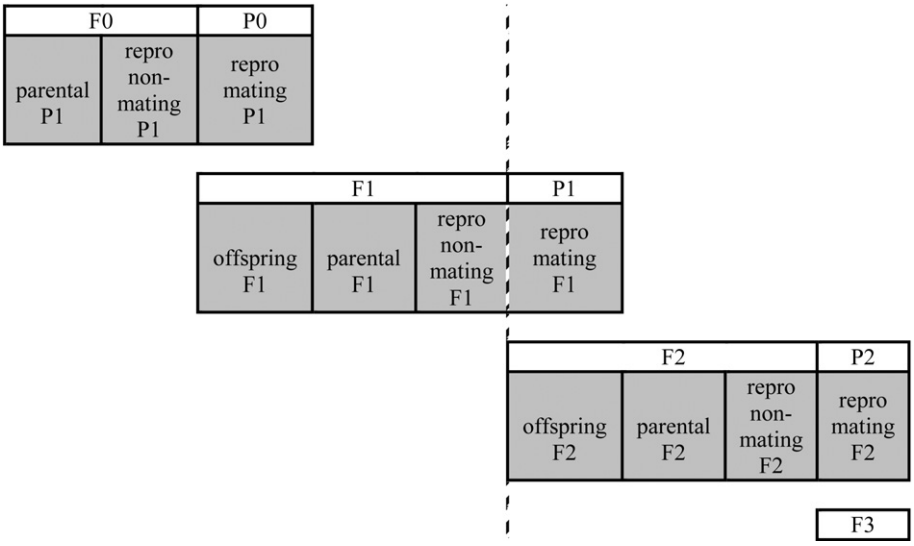

Fig. 1. Schematic representation of the different stages in multigeneration reproduction toxicity study protocols. Within generations, four groups of parameters are distinguished in the database (see shaded boxes): "offspring" parameters observed until weaning at postnatal day 21, "parental" general toxicity parameters observed from weaning onward and including adulthood, "repro"ductive "nonmating" parameters observed in adult animals, and "repro"ductive parameters related to "mating". In the text, life phases before mating are indicated as (F) (white boxes), and after mating as (P), whereas the index (0,1,2,3) indicates the generations such that the first offspring generation emanating from the study is designated as the F1. The generation indications in the grey boxes are the original EPA ToxRefDB nomenclature. The analysis of the impact of the second generation is primarily done by comparing parameters occurring to the left of the dashed line (F0/P0/F1 effects, white boxes) with those observed to the right of that line (P1/F2 effects, and if present also P2/F3 effects). In the manuscript text, we refer to the codes in the white boxes.

then mated to produce an F2 generation. The F2 animals are terminated at weaning (postnatal day 21). One unique component of the study design is in the exposure of the F1 animals during all life stages, starting with exposure of the adult gametes in the P0 which will give rise to the F1, continuing through fertilization, embryofetogenesis and postnatal development of the F1 and reproduction of the P1 generating the F2. This comprehensive exposure design encompassing the entire reproductive cycle in the F1/P1 generation allows adverse effects on reproductive function at any time in the reproductive cycle to be detected.

This comprehensive 2-generation study design which includes exposure of the F1 from conception through adulthood and mating has been important in regulatory assessments for over three decades. However, extensive experience with the protocol suggests that P1 mating to produce the F2 generation may actually have little added value for assessing reproductive toxicity. This may in part be caused by the fact that fertility in rats is generally very insensitive, and F2 litter data are extremely apical, being mainly limited to pup growth and survival. In cases where the endpoints measured during P1 mating and in the F2 offspring occur at doses equal to or greater than in P0 and F1 animals, the P1 mating (and subsequent generation of the F2) would not impact on the overall lowest observed adverse effect level (LOAEL) of the study. Removal of the P1 mating and beyond would reduce animal use by around 1200 animals (approx. 40%) as well as cost (approx. 25%), and time in the study. This is in keeping with the goals of the international community to reduce animal use and refine testing paradigms as described by US National Academy of Science in its Toxicity Testing for the 21st Century report [2]. In 2006, Cooper et al. [3] proposed a novel protocol, the Extended One-Generation Reproduction Toxicity Study (EOGRTS), in which the F1 generation would be followed until adulthood, while the subsequent mating of the P1 to generate an F2 would be triggered on the basis of existing information or data collected within the study. Furthermore, additional parameters and increases in the number of observations were suggested to enhance the sensitivity and statistical power of the study. Subsequent to the publication of the Cooper et al. [3] proposal, several retrospective analyses assessing the impact of the second generation on the overall conclusions of the two-generation study in existing risk assessment were conducted. Janer et al. [4] evaluated 176 multi-generation study risk assessment reports and observed that in all cases the second generation affected neither the overall NOAEL nor the nature of the critical effect. Therefore, Janer et al. concluded that the second generation had no impact on the risk assessment or on classification and labelling. The Janer et al. [4] paper specifically included all available two-generation studies for substances classified and labelled as reproductive toxicants under European and Californian law. Two smaller studies followed which suggested that the second generation parameters might affect overall study outcome [5,6]. In addition, Martin et al. [7] using a USEPA ToxRefDB dataset of 329 multigenerational studies supported the hypothesis that the F2 generation would rarely impact either the qualitative or quantitative evaluations of these studies. The USEPA followed up with a retrospective analysis of 350 multigenerational studies, generally reaching the same conclusions about the limited impact of the F2 generation [8].

Meanwhile, the EOGRTS protocol was formally forwarded by the USA, Germany and the Netherlands for adoption as a globally agreed OECD test guideline. In October 2008 and 2009 the OECD convened Expert Panel meetings in Paris. The discussions on the relevance of the F2 generation focused on the concept of the need to assess effects of exposure during the entire reproductive cycle within one generation, versus the practical argument that in retrospect the P1 mating and F2 generation parameters hardly ever if at all impacted on the study interpretation. The OECD Expert Panel concluded that a systematic combined retrospective analysis of all available two-generation studies would be necessary before adoption and implementation of the OECD EOGRTS protocol. This manuscript describes this combined retrospective analysis performed at RIVM with input from the OECD expert group. It combines the ToxRefDB and RIVM databases with smaller datasets from Beekhuijzen (NOTOX) and EU New Substance data as provided by the German authority (BfR). The analysis focuses on the impact of