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On the impact of second generation mating and offspring in multi-generation reproductive toxicity studies on classification and labelling of substances in Europe

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ABSTRACT

The possible impact on classification and labelling decisions of effects observed in second generation parental (P1) and offspring (F2) parameters in multi-generation studies was investigated. This was done for 50 substances classified as reproductive toxicants in Europe, for which a multi-generation study was available. The P1 and F2 effects were compared to parental (P0) and first generation offspring (F1) effects with regard to type of effect as well as incidence, magnitude and severity (IMS), at any dose level. For every study with unique P1/F2 effects, or differences in IMS, the influence of the P1/F2 findings on the classification decision was investigated. Unique P1/F2 generation findings did not play a crucial role in the classification decision of any of the 50 classified substances, except for fenarimol. This substance however provided abundant alerts on the basis of its endocrine activity and developmental neurotoxicity and would therefore also be expected to be identified as a developmental neurotoxicant in an Extended One Generation Reproductive Toxicity Study (EOGRTS). These findings, in addition to the increased number of parameters analysed, increased statistical power and reduced animal use, provide strong further support for replacement of the classical two-generation reproductive toxicity study by the EOGRTS in regulatory reproductive toxicity assessment.

1. Introduction

Reproductive toxicity hazard assessment of substances in Europe is based on all relevant toxicological information retrieved from studies ranging from repeat dose tests in adult animals to the two-generation reproductive toxicity study (OECD Test Guideline 416, (OECD, 2001)). The latter study design includes exposure of adult males and females before mating (P0), and continued exposure of dams throughout pregnancy and weaning, exposure of the first generation offspring (F1) throughout life, including their mating (P1) and reproduction into a second generation offspring (F2), which is terminated at weaning. This study is time-consuming, requires no less than 2600 animals, and is limited as to the number of parameters included and the number of animals assessed for each parameter.

The Extended One Generation Reproductive Toxicity Study (EOGRTS) (Cooper et al., 2006; OECD, 2010) has an innovated study design that includes extensive additional end point determinations. Novel end points include reproductive and endocrine parameters as well as developmental immunotoxicity and developmental neurotoxicity parameters. In addition, end points are assessed in more offspring than in the classical multi-generation study (e.g. the OECD TG 416 two-generation reproductive toxicity study (OECD, 2001)) whilst the mating of the second generation (P1) and the second generation offspring (F2) are omitted from the protocol, unless triggered in specific cases. This new EOGRTS protocol is expected to provide a higher level of scientific information and at the same time substantially reduces animal use when no second generation offspring is produced.

The EOGRTS has been suggested as a possible replacement of the OECD TG 416 study. Discussion has focused on the necessity

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of producing a second generation offspring. These studies are applied in risk assessment as well as in classification and labelling of substances, for both of which European legislation is in place (EU, 2006, 2008b). For risk assessment, the impact of the second generation offspring was addressed in our previous publication (Piersma et al., 2011). We produced a unique database containing 498 rat multi-generation studies with 438 substances, mainly pesticides and industrial chemicals. A retrospective analysis of risk assessment reports considering these studies showed that the impact of the second generation had been negligible. This implied that the production of a second generation offspring might be omitted without impacting risk assessment outcome, saving significant time, and reducing animal numbers from 2600 to 1400 animals per study. These advantages are even more significant in the light of the EU REACH legislation (EU, 2006), which requires extensive animal toxicity testing in the coming 5 years. The contribution of reproductive toxicity generation studies has been estimated to amount to around 35% of all animal testing in REACH (van der Jagt et al., 2004), and omission of the second generation as indicated would therefore reduce animal use in REACH by around 15%.

We have concluded that also for classification and labelling in Europe (ECHA, 2011; EU, 2008b) it is highly unlikely that the second generation offspring would contribute significantly (Piersma et al., 2011). This analysis was based on relative parameter sensitivity in terms of lowest observed adverse effect levels (LOAELs) as compared between generations. However, it has been argued that the nature and the incidence, magnitude, and severity of effects might play a significant role specifically in view of classification and labelling. Thus, in this view, although the same LOAEL might have been derived for the first and the second generation, if the nature of the effect, or its incidence, magnitude or severity would be judged as more serious in the second generation, it might lead to a higher classification level.

In this manuscript, we have addressed the impact of the second generation parental and offspring parameters on classification and labelling in Europe. We have used the multi-generation study database to select those substances which had both a multi-generation study and in addition had been classified and labelled for effects on fertility, development or lactation. We identified 50 substances in the database satisfying these criteria, relevant for this analysis. For these substances the public records of the EU Specialized Expert (SE) and Technical Committee (TC) meetings were studied to assess the impact that the second generation had on classification and labelling. For those compounds for which such records could not be retrieved, we did our own assessment of the likelihood that the second generation in the study would have specifically impacted on the classification and labelling. This analysis shows that, except for a single case, effects observed in second generation mating and offspring did not impact the decision on classification and labelling for reproductive toxicity. Moreover, the single case where second generation mating and offspring effects appeared to be instrumental for classification would be identified without any doubt as a reproductive toxicant in an EOGRTS without second generation mating and offspring. Therefore, this analysis supports the replacement of the OECD Test Guideline 416 two-generation reproductive toxicity study (OECD, 2001) with the EOGRTS (OECD, 2010). This replacement is expected to allow at least the same level of scrutiny for both risk assessment and classification and labelling, and moreover, in view of increased parameter number and enhanced power of the EOGRTS, it is anticipated to increase the likelihood for reproductive toxicants to be detected. The significant reduction in time and animal use provides further advantages that are more than relevant in view of implementation of the REACH legislation in Europe.

2. Methods

The multi-generation reproduction toxicity study database was developed as described in detail before (Piersma et al., 2011). Briefly, the USEPA ToxRefDB format (Martin et al., 2009) was used and its content was extended with the database generated by Janer et al. (2007) and additional studies. The final database contained 498 multi-generation studies covering 438 substances. The substance list of the database was matched with the EU Classification and Labelling compound list, Annex VI to Regulation (EC) No. 1272/2008 (EU, 2008a). In the database, 50 substances were found to carry a classification for reproductive toxicity on Annex VI. For these substances the multi-generation studies in the database were analysed in detail as to the nature, magnitude and severity of adverse effects found in the different generations within multi-generation studies, irrespective of the dose level at which they occurred. Study reports were consulted where necessary and whenever possible, and the reports of the EU Specialized Experts (SE) and Technical Committee (TC) on Classification and Labelling were taken into account to address the possibility of a unique contribution of the second generation mating and offspring (P1/F2). Summaries of the SE and TC meetings were available until 2010 on the website of the former European Chemicals Bureau and most of them are still available through the H-class database from the Nordic Council of Ministers (http://apps.kemi.se/hclass/).

Throughout this manuscript, reference is made to the EU C&L system as used during the period of time addressed. This entails classification as Cat.1 for proven human reproductive toxicants, as Cat.2 for substances that should be considered as reproductive toxicants for humans based on animal studies, and as Cat.3 where there is some evidence for reproductive toxicity from animal data, but where the evidence is insufficient for Cat.2. Cat.1 and 2 reproductive toxicants are labelled with risk phrase R60 for fertility effects, and R61 for developmental effects, and Cat.3 reproductive toxicants are labelled with R62 for fertility effects and R63 for developmental effects. In the GHS system (UN, 2007) which is currently being introduced in Europe, Cat.1, 2 and 3 are generally replaced by the new Cat.1a, 1b and 2, with some (minor) changes as to the criteria for these categories. As mentioned, this manuscript refers to the old EU C&L system as it is based on references in which the old EU classification scheme has been used throughout.

3. Results

The multi-generation study database (Piersma et al., 2011) of 438 substances contained 50 substances that had an EU classification for fertility, development, and/or lactation. These substances are given in Table 1. The multi-generation study summaries for these substances were analysed in order to assess whether the P1/F2 generation showed different types of effects, or the same effects but at lower doses as compared to the P0/F1 generation. It appeared that for 24 substances at least one multi-generation study showed effects that had been scored uniquely in the P1/F2 generation (Table 1). For the remaining 26 substances, the effects found in the first and second generation mating and offspring was not different in nature or toxicological relevance as indicated by the study summaries. Therefore, we conclude that for these 26 classified substances, the second generation mating and offspring was not crucial for the classification given.

Of the 24 substances with specific effects noted in the P1/F2 generation, five had a Cat.2, R60 classification and eight had a Cat.3, R62 classification for fertility. Most of these substances had an additional classification for development. Of the remaining 11 substances without a classification for fertility, six had a Cat.2,