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Strengths and limitations of using repeat-dose toxicity studies to predict effects on fertility

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Abstract

The upcoming European chemicals legislation REACH (Registration, Evaluation, and Authorisation of Chemicals) will require the risk assessment of many thousands of chemicals. It is therefore necessary to develop intelligent testing strategies to ensure that chemicals of concern are identified whilst minimising the testing of chemicals using animals. Xenobiotics may perturb the reproductive cycle, and for this reason several reproductive studies are recommended under REACH. One of the endpoints assessed in this battery of tests is mating performance and fertility. Animal tests that address this endpoint use a relatively large number of animals and are also costly in terms of resource, time, and money. If it can be shown that data from non-reproductive studies such as in-vitro or repeat-dose toxicity tests are capable of generating reliable alerts for effects on fertility then some animal testing may be avoided. Available rat sub-chronic and fertility data for 44 chemicals that have been classified by the European Union as toxic to fertility were therefore analysed for concordance of effects. Because it was considered appropriate to read across data for some chemicals these data sets were considered relevant for 73 of the 102 chemicals currently classified as toxic to reproduction (fertility) under this system. For all but 5 of these chemicals it was considered that a well-performed sub-chronic toxicity study would have detected pathology in the male, and in some cases, the female reproductive tract. Three showed evidence of direct interaction with oestrogen or androgen receptors (linuron, nonylphenol, and fenarimol). The remaining chemicals (quinomethionate and azafenidin) act by modes of action that do not require direct interaction with steroid receptors. However, both these materials caused in-utero deaths in pre-natal developmental toxicity studies, and the relatively low NOAELs and the nature of the hazard identified in the sub-chronic tests provides an alert for possible effects on fertility (or early embryonic development), the biological significance of which can be ascertained in a littering (e.g. 2-generation) study. From the chemicals reviewed it would appear that where there are no alerts from a repeat-dose toxicity study, a pre-natal developmental toxicity study and sex steroid receptor binding assays, there exists a low priority for animal studies to address the fertility endpoint. The ability for these types of tests to provide alerts for effects on fertility is clearly dependent on the mode of action of the toxicant in question. Further work should therefore be performed to determine the 'failure rate' of this type of approach when applied to a larger group of chemicals with diverse modes of action.

Keywords: REACH; Fertility; Integrated testing strategy; Hazard identification; Repeat-dose; Two-generation; Screening tests; Mode of action

1. Introduction

The forthcoming European chemicals legislation REACH (Registration, Evaluation, and Authorisation of Chemicals) will necessitate the re-evaluation of any chemical that is produced in or imported into the European Union at levels of 1 tonne per annum (tpa) or more (EU, 2006). The safety studies required for this process will depend largely on the volume of the chemical produced or imported, with chemicals produced in large quantities requiring the most extensive data packages. The reproductive and developmental studies that are recommended under REACH are the OECD 421 screening test, the OECD 414 pre-natal development study, and the OECD 416 2-generation reproduction study. However, other

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pre-existing data will also need to be taken into account to prevent the unnecessary use of animals. For example, developmental toxicity studies performed to historical guidelines may be available, but many of these older tests involved the treatment of pregnant females for a shorter period during gestation than the current OECD 414 guideline. Furthermore, other good quality modern studies may be available which are not performed to an OECD guideline, such as the Continuous Breeding Protocol used by the US National Toxicology Programme. While these types of test do not match the current OECD requirements, they can provide valuable data to assess the potential for a chemical to affect the reproductive system or development.

The finalised REACH legislation requires reproductive toxicity testing to be performed once production/import reaches the 10 tpa threshold. At this level, if there is no evidence from available information on structurally related substances, structural activity relationship, or quantitative structural activity relationship ((Q)SAR) estimates, or in-vitro indicators that the substance may be a developmental toxicant, then an initial OECD 421 screening test is conducted. Alternatively an OECD 422 screening test (which combines the reproductive screen of the OECD 421 study with some extra assessments of general toxicity) could be used. The screening test does not need to be performed if either a prenatal developmental toxicity study (OECD 414) or a two-generation study (OECD 416) is available. Further, at this tonnage level one of the definitive tests should be performed instead of the screening test if there are indications of reproductive target organ toxicity from repeated-dose toxicity tests or a close structural relationship to a known developmental/reproductive toxicant.

The next tonnage trigger (100 tpa) requires a developmental toxicity study (OECD 414) unless already performed, while the two-generation study is required once production or import reaches the 1000 tpa level (unless alerts triggered this study at a lower tonnage level). The registrant therefore has a fundamental decision to make at 10 tpa; to perform the screening test at this tier then perform a prenatal developmental toxicity study if production/import exceeds 100 tpa in the future, or if it suspected that this will be the case go straight to the prenatal developmental toxicity study and bypass the screening test altogether. While it could be argued that both these studies are necessary because the OECD 421 screening test assesses endpoints not addressed in the OECD 414 developmental toxicity, the value of the screening test is diminished when data from a definitive developmental toxicity study are available. The endpoints not addressed in the pre-natal development study (OECD 414) that are covered by the screening test (OECD 421) are fertility, mating performance, parturition, early post-natal development and maternal care and lactation. The repeat-dose toxicity studies that are required at 100 tpa (28-day or 90-day repeat-dose toxicity tests OECD 407 or 408) can provide useful information that may point to the potential for effects on reproduction. This is because repeat-dose toxicity studies include organ weights and histological examination of the gonads and accessory sex organs, providing useful information on potential effects on fertility or endocrine effects. A brief summary of the types of effects that may point to a potential for effects in another reproductive endpoint is shown in Table 1.

The decision whether to perform a screening test when it is suspected that a pre-natal development study will eventually be needed therefore depends on the certainty with which effects on fertility and post-natal survival and development can be predicted from a combination of a pre-natal development study and the required repeat dose repeat-dose toxicity study. For most chemicals this will be a repeat dose study of 90-days' duration or less.

The aim of this investigation was to identify chemicals where significant amounts of published reproductive and repeat-dose toxicity data are available. The objective was to look for concordance between studies with a view to:

1. Identifying chemicals where data from pre-natal developmental toxicity and sub-chronic repeat-dose toxicity studies have not been predictive of effects on mating performance and fertility.

Table 1
Prediction of effects across studies

Study	Examples of findings that may give alerts for...		
	Fertility/mating performance	Pre-natal development	Postnatal survival/development
Pre-natal development study (OECD 414)	Early implantation loss; effects on reproductive organs (e.g. hypospadias, cryptorchidism)	Structural changes fully assessed in study	Certain abnormalities may indicate a possible effect on postnatal survival; in-utero mortality; significant growth retardation in the absence of maternal toxicity
Repeat-dose toxicity study (OECD 407 or 408)	Reproductive organ pathology; organ weight changes in reproductive or accessory organs	Neurotoxicity; pathology of the endocrine system	Pathology of the endocrine system
Developmental and reproductive toxicity screening test (OECD 421/422)[a]	Assessed in study	Smaller live litter size at birth; observance of abnormal offspring	Assessed in study (but only up to day 4 of age)

[a] The relative insensitivity of this investigation (low animal numbers, loss of information due to cannibalism, and short duration) mean that effects may need to be substantial in order to be detected.