Agència de Gestió d'Ajuts Universitaris i de Recerca

Memòria justificativa de recerca de les convocatòries BE, PIV, BCC, NANOS i BP

La memòria justificativa consta de les dues parts que venen a continuació:
1.- Dades bàsiques i resums
2.- Memòria del treball (informe científic)

Tots els camps són obligatoris

1.- Dades bàsiques i resums

Nom de la convocatòria

BP

Llegenda per a les convocatòries:

BCC	Convocatòria de beques per a joves membres de comunitats catalanes a l'exterior (BCC)
BE	Beques per a estades per a la recerca fora de Catalunya (BE)
BP	Convocatòria d'ajuts postdoctorals dins del programa Beatriu de Pinós (BP)
NANOS	Beques de recerca per a la formació en el camp de les nanotecnologies (NANOS)
PIV	Beques de recerca per a professors i investigadors visitants a Catalunya (PIV)

Títol del projecte: ha de sintetitzar la temàtica científica del vostre document.
Establiment de bases científiques per al desenvolupament d'una estratègia integrada per avaluar toxicitat en reproducció

Dades de l'investigador

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Número d'expedient

2005 BP-A10062

Paraules clau: cal que esmenteu cinc conceptes que defineixin el contingut de la vostra memòria.
Toxicitat reproductiva, desenvolupament, fertilitat, estratègies integrades, extrapolació in vitro-in vivo

Data de presentació de la justificació

20-02-2008

Resum del projecte: cal adjuntar dos resums del document, l'un en anglès i l'altre en la llengua del document, on s'esmenti la durada de l'acció

Resum en la llengua del projecte (màxim 300 paraules)

Resum en anglès(màxim 300 paraules)

Summary of the work done in the period June 2006 to February 2008.

This project aimed at creating scientific elements that could help deriving an integrated testing strategy for reproductive toxicity. Part of the project focused on the use of alternative tests for regulatory purposes. An in vitro-in vivo extrapolation method for embryotoxicity was proposed and evaluated. In vitro and in vivo dose descriptors were correlated; however, the scatter in the correlation was too large to allow an accurate estimation of an in vivo dose from an in vitro dose. The in vitro-in vivo extrapolation method together with toxicokinetic data was also applied to a category of substances (phthalates). Although based on a limited number of substances, the results suggested that in vitro-in vivo extrapolation for embryotoxicity is possible within a category of compounds if adequate toxicokinetic data is available.
Because of the limitations that still remain in the use of alternative tests for reproductive toxicicity, other approaches to reduce animal testing were explored. Thus, a database of reproductive toxicity studies was created to retrospectively evaluate the comparative value of some studies or elements in a particular study. When compared to the subchronic toxicity study, the rat two-generation reproductive toxicity study had a considerable impact on the identification of hazard for reproductive toxicity, but not on the overall NOAEL. Among the two-generation studies included in our database, the second generation affected neither the overall NOAEL nor the critical effect. The rat and the rabbit developmental toxicity studies were, on average, similarly sensitive. However, for certain substances the developmental study in either one of the two species appeared to be more sensitive than in the other species.

2.- Memòria del treball (informe científic sense limitació de paraules). Pot incloure altres fitxers de qualsevol mena, no més grans de 10 MB cadascun d'ells.

Preface

The work performed during this postdoctoral fellowship has resulted in four articles published in international peer reviewed journals. These publications provide detailed scientific insight on the project; therefore this memory will only provide an extended summary of the project. Potential interested readers can find further information in the following manuscripts:

Janer G; Hakkert BC; Slob W; Vermeire T; Piersma AH. 2007. A retrospective analysis of the two-generation study: What is the added value of the second generation? Reproductive Toxicology. 24: 97-102.

Janer G; Hakkert BC; Piersma AH; Vermeire T; Slob W. 2007. A retrospective analysis of the rat two-generation study versus the rat subchronic study. Reproductive Toxicology. 24: 103-113.

Piersma A, Janer G, Bessems J, Woltering G, Hakker B, Slob W. 2008. Quantitative extrapolation of in vitro whole embryo culture embryotoxicity data to developmental toxicity in vivo using the Benchmark approach. Toxicological Sciences. 101: 91-100.

Janer G, Hakkert B, Slob W, Vermeire T, Piersma A. 2007. A retrospective analysis of developmental toxicity studies: What is the added value of the rabbit as an additional species? Regulatory Toxicology and Pharmacology, 50: 206-217.

An additional manuscript has been submitted:

Janer G, Verhoef A, Gilsing HD, Piersma AH. Use of the WEC test to assess the embryotoxic potency within a chemical category and to identify toxic metabolites.Toxicology in vitro, submitted.

This work has as well been presented in three international congresses:

06/2007—47th Teratology Society Meeting, Pittsburgh (US). Janer G, Slob W, Hakkert B, Vermeire T, Piersma A. A retrospective analysis of the two-generation study: What is the added value of the second generation?

09/2007—35th Annual Conference of the European Teratology Society, Bratislava (Slovakia). Janer G, Slob W, Hakkert B, Vermeire T, Piersma A. A retrospective analysis of developmental toxicity studies: What is the added value of the rabbit as an additional species?

10/2007--44th EUROTOX Meeting, Amsterdam (The Netherlands). Janer G, Slob W, Hakkert B, Vermeire T, Piersma A. A retroespective assessment of the added value of the two-generation reproductive toxicity study.

Introduction

Increasing pressure is exerted by some stakeholders to reduce the number of animals used for experimentation, and particularly, toxicity testing. Reproductive toxicity testing uses most of the animals in regulatory toxicology. Therefore, there is a great interest in refining the testing strategy for this endpoint. Several *in vitro* tests for reproductive toxicity have been proposed. However, their use as stand-alone tests for regulatory purposes is precluded by the difficulties in extrapolating *in vitro* dose descriptors to *in vivo* dose descriptors. We proposed and evaluated an *in vitro-in vivo* extrapolation method both in a broad group of chemicals and in a chemical category. In addition, another approach to reduce animal use in reproductive toxicity

testing was explored. We created a database of reproductive toxicity studies to retrospectively evaluate the comparative value of some studies or elements in a particular study.

Objective

The overall objective of the project was to create scientific elements that would help deriving an integrated testing strategy for reproductive toxicity. This objective was divided in the following sub-objectives:

A. Explore the potential and limitations of the use of *in vitro* data to extrapolate *in vivo* results. Particularly,

- To propose and evaluate an *in vitro-in vivo* extrapolation method.
- To evaluate the potential of alternative tests in chemical category approaches.

B. Explore possible changes in current *in vivo* tests to refine the current testing strategy for reproductive toxicity. Particularly,

- To retrospectively evaluate both in terms of the types of effects observed and in terms of the effective doses and NOAELs (not-observed-adverse-effect-levels):
    - The differences between the first and the second generation in a two-generation reproductive toxicity study.
    - The added value of the two-generation reproductive toxicity study when a subchronic study (90-day repeated dose toxicity study) is available.
    - The added value of the rabbit developmental toxicity study when a rat developmental toxicity study is available.

Methods

A. *In vitro-in vivo* extrapolation

We used the data generated in an European Center for the Validation of Alternative Methods validation study for the *in vitro* Whole Embryo Culture (WEC) test to examine whether a correlation existed between *in vitro* embryotoxicity data and *in vivo* developmental toxicity tests. We applied the Benchmark Dose (BMD) approach to estimate equipotent *in vitro* concentrations (Benchmark Concentrations [BMCs]) for the three endpoints analyzed in the *in vitro* test (head length, crown-rump length, and total morphological score) and equipotent *in vivo* doses (BMDs) for malformations or fetal weight.

We applied a similar approach to a category of chemicals (the phthalates). We generated *in vitro* embryotoxicity data with the WEC test and collected *in vivo* developmental toxicity and toxicokinetic data from literature. The *in vitro* data together with the toxicokinetic information were compared to the *in vivo* data to evaluate the concordance between the *in vitro* and *in vivo* outcomes.

B. Retroespective analysis

We collected data from subchronic and two-generation studies, focusing our efforts on substances classified as reproductive toxic. The database was later expanded with non-reproductive toxic substances, for which a two-generation study was available.

For each of these studies, we assessed differences between the first and the second generation in the two-generation study, both in terms of the types of effects observed and in terms of the effective doses. The outcomes of the subchronic and two-generation studies were also compared in view of the question what the impact would have been both for the derived NOAEL and for classification regarding toxicity to fertility.

In addition, developmental toxicity studies in rats and rabbits were collected for substances classified for developmental toxicity. These two studies were compared for each substance in order to evaluate the impact of the rabbit developmental toxicity study when a rat developmental study is available in terms of NOAEL setting and identification of developmental toxicity.

Results and Discussion

A) *In vitro- in vivo* extrapolation

A clear *in vitro-in vivo* correlation was found between BMCs and BMDs. However, a large uncertainty would remain if the BMDs were estimated from the BMC using this correlation. Differences in toxicokinetic properties among the compounds explained at least part of the scatter of the *in vitro-in vivo* correlation. But also heterogeneity in the design of the available *in vivo* studies underlied much of the scatter, and this puts a limit on validating *in vitro* data as predictors of *in vivo* data. Further analysis of the *in vitro-in vivo* correlation would therefore require high-quality *in vivo* data, generated by appropriate (and similar) study designs.

In a second part of the study, the approach was limited to a category of compounds, the phthalates, and to relatively homogeneous and high quality *in vivo* studies. The BMCs obtained in the WEC tests were considerably similar to the blood concentrations expected for the BMDs obtained in the *in vivo* studies. This example suggests that *in vitro* data together with toxicokinetic information can be used to estimate *in vivo* dose descriptors for a category of substances.

B) Retrospective analysis

Two-generation reproductive toxicity study versus subchronic study

On average, only a small difference (less than 2-fold) in overall NOAELs was found between the rat two-generation study and the rat subchronic study. For individual compounds the differences could be larger (up to around a factor of ten), but differences of this magnitude equally occur between NOAELs of subchronic studies (testing the same substance). The two generation study did have an impact on classification for reproductive toxicity: about one third of the substances shown to be toxic to fertility in the 2-generation study did not show any sign of that in the 90-day study. If the subchronic study did show toxicity to reproductive organs this often occurred at (much) higher doses than other toxic effects in the same study. Therefore, apart from including more fertility endpoints, a larger dose spacing (or more dose groups) in the subchronic study might increase its detection rate of fertility toxic substances.

Impact of the second generation in the two-generation reproductive toxicity study

The second generation in the two-generation studies considered affected neither the overall NOAEL nor the critical effect. Therefore, it had no impact on the ensuing risk assessment, nor on classification and labeling. However, several substances did show an increased sensitivity of the F1 adults in comparison to the P0. These results support the proposal of replacing the current two-generation study by a 1-generation study with a more extensive assessment of parameters at F1 adulthood.

The rabbit developmental toxicity study versus the rat developmental toxicity study

On average, the rat and the rabbit developmental toxicity studies were similarly sensitive: the average ratio of the NOAELs between the two species was about one, and for most compounds there were no differences between rat and rabbit studies in terms of classification for developmental toxicity. For certain substances the developmental study in either one of the two species appeared to be more sensitive than in the other species. However, these differences are partly due to differences between studies other than the test species used. Overall, our

analysis does not clearly indicate that the evaluation of developmental toxicity, as opposed to other types of toxicity, would specifically require the rabbit as an additional test species. The discrimination between direct and indirect (i.e., as a consequence of maternal toxicity) developmental effects was often doubtful, and is one of the factors that could explain the apparent differences between the two species. A more accurate assessment of maternal toxicity might improve the reliability of the results from a single developmental toxicity study. More knowledge about the interaction between maternal and developmental effects is required before decisions on omitting the requirement for the developmental toxicity testing in a second species can be considered.

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Barcelona, 20 de Febrer de 2008

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