PEPPER: a tool to increase the availability of standardised methods for the identification of endocrine disrupters

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INTRODUCTION

Currently, the identification of endocrine disrupters (EDs) for regulatory purposes is hindered by a lack of test methods. Few test methods have been validated and they mainly focus on the estrogenic, androgenic and, to a lesser extent, thyroid pathways, as well a s on steroidogenesis. Thus, the regulatory identification of endocrine disrupters acting through other pathways is complex.

Even though the use of data from non-validated methods published in the scientific literature is required, the practice is often difficult due to a description of the method which does not allow the method and its limitations to be properly assessed.

These observations have been the basis for the creation of the Public-Private platform for the pre-validation of endocrine disrupter characterisation methods (PEPPER). The platform aims to foster translational research with the aim of developing tools to monitor or limit human/environmental exposure to endocrine disrupters and constructing the evidence required for them to be validated by bodies such as OECD, ECVAM and ISO.

METHODOLOGY

PEPPER is a French initiative supported by the National Strategy on Endocrine disrupters, ministries and international institutions (such as BfR, RI.SE, EURL-ECVAM and OECD). It organises and funds the pre-validation of endocrine disrupter characterisation methods.

The methods to be pre-validated are selected by a Relevance Committee with representatives from regulatory authorities, NGOs, civil society, European institutions, industry and academia. The methods are subject to a Committee vote and therefore need to be sufficiently developed and to fill a regulatory gap. Methods relevant to the identification of a mode of action, an adverse outcome, or supporting the establishment of weight-of-evidence for the identification of endocrine disrupters are considered.

The pre-validation work involves at least three laboratories. The transferability of the method is assessed, as well as its relevance and reliability. The success of the pre-validation is evaluated by a Scientific Council, composed of independent scientists.

RESULTS

Three methods are undergoing pre-validation.

The first one assesses the effects of xenobiotics on placental cells (JEG-3), measuring the activation of the P2X7 receptor, as well as the production of E2, P4, hPl and βhCG. These endpoints are implicated pathologies such as pre-eclampsia, miscarriages and premature births.

The second pre-validation study evaluates the enhancement of the current OECD Test Guideline on the H295R steroidogenesis assay, extending it to 19 hormones and precursors. This enhancement has been awaited by several stakeholders.

The third method is a glucocorticoid receptor transactivation assay. This method is based on a stably transfected human cell line, and is relevant to adverse outcomes linked to metabolic dysfunction and male fertility/reproduction.

The design of the pre-validation is based on two steps. The first one is a transfer phase during which the method is implemented in a naïve lab, testing a few chemicals. During the second phase, more chemicals are tested, allowing for a further assessment of several aspects of the method such as inter-laboratory variability, predictive capacity and applicability domain.

DISCUSSION

The current regulatory identification of EDs is limited by the number of available methods, as well as by the lack of specific information requirements for endocrine disruption. Additional and more appropriate methods are required in order to facilitate the identification of EDs. Translational research should therefore be supported in order to fill the gap between research and regulatory needs.

The PEPPER initiative helps to translate academic research into regulatory science through the organisation and funding of pre-validations. It is recommended that this effort be amplified at European level.

Another lesson learnt from initial PEPPER studies is that awareness on this topic must also evolve as must the scientific culture. Method developers need to have a better understanding of what is needed for a method to reach regulatory use. Lack of knowledge has been one of the main obstacles explaining the difficulty in identifying methods to be pre-validated.

Training may be offered to method developers by those involved in regulation in order to explain what criteria a method should meet, in addition to training on validation so that this can be taken into account from the first steps of development.