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Commentary

Mind the gap: Concerns using endpoints from endocrine screening assays in risk assessment

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ABSTRACT

Endocrine screening assays not only provide mechanistic information on the potential of a substance to interact with the endocrine system, but also data potentially relevant for risk assessment. However, these screening assays have a number of limitations that should be considered before the direct use of such data for risk assessment purposes. This paper discusses the limitations that should be considered for both human and environmental risk assessment. A proposal is made to provide an objective and transparent process in order to consider which endpoint(s) might be incorporated into a risk assessment, and when more definitive studies may be of value. The proposal is complemented with an easy-to-follow flowchart to aid industry scientists and regulators when evaluating the relevance of these data. Such an approach is necessary to ensure the appropriate use of screening data to further our understanding of the ecotoxicological profile of substances undergoing screening.

1. Introduction

The Organization for Economic Co-operation and Development (OECD) and United States Environmental Protection Agency (US-EPA) have both developed a suite of screening assays for use in endocrine screening programmes of chemicals. Examples of such programmes are US-EPA's Endocrine Disruptor Screening Programme[1] and Japan's ExTEND programme.[2] These assays may also be required under different European regulations (COM, 1999) following concerns raised by the evaluation of a substance. The primary purpose of these screening assays is to establish if a substance has the potential to interact with the endocrine system. Currently this covers interactions with oestrogen and androgen pathways and the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) axes. An evaluation of the screening assays outcomes alongside other relevant information is then used to trigger appropriate higher tier or definitive tests to confirm whether the identified endocrine activity leads to an adverse effect in an intact organism (Bars et al., 2011, 2012). These higher tier tests are used for regulatory action according to region and substance specific legislation. This may be a scientific risk assessment (e.g. in the US and Japan) or a hazard based restriction, without risk assessment, in the European Union (Wheeler et al., 2012).

Despite the original purpose of the *in vivo* mammalian, fish and amphibian screening assays in current regulatory programmes as described above, there has also been interest in using certain data from these assays for risk assessment (Dang et al., 2011; US-EPA, 2013a). In principle, the use of relevant data from screening assays is an interesting proposition, making use of all the available information on a specific substance for human and environmental risk assessment. This is particularly important in the ecotoxicology area, where the endocrine screens provide information on endpoints (e.g. fish reproduction) and a taxon (amphibia) not routinely required for the evaluation of substances. Nevertheless, the limitations of the assays concomitant with their primary purpose, screening for endocrine activity, should be carefully considered when interpreting whether the data are relevant for risk assessment. This paper highlights some key limitations of using these screening assays for risk assessment purposes for both the toxicological and ecotoxicological areas. It also makes a proposal on how these data can be effectively integrated into an assessment.

2. Toxicology

The Hershberger (OCSPP, 2009c; OECD, 2009a), uterotrophic (OECD, 2007; OCSPP, 2009f) and male and female pubertal assays

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[1] http://www.epa.gov/endo/.
[2] http://www.env.go.jp/en/chemi/ed.html.

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Table 1
Summary of the mammalian endocrine screening assays.

Mammalian screens				
Study type	Hershberger assay	Uterotrophic assay	Male pubertal assay	Female pubertal assay
Relevant regulatory guidelines	OECD 441, OCSPP 890.1400	OECD 440, OCSPP 890.1600	OCSPP 890.1500	OCSPP 890.1450
No. treatments	2 for androgenicity 3 for anti-androgenicity	2	2	2
Recommended spacing factor	~3 (0.5 log)	~3 (0.5 log)	2	2
Animals/ replicate	6 orchidoepididymectomised male rats	6 ovariectomised/ immature female rats	15 prepubertal male rats	15 prepubertal female rats
Mandatory endpoints	Mortality, clinical signs, body weight, food consumption, weights of 5 androgen-dependent organs	Mortality, clinical signs, body weight, food consumption, wet and blotted uterus weights	Mortality, clinical signs, body weight, food consumption, age and body weight at preputial separation, seminal vesicle + coagulating glands weight, ventral and dorsolateral prostate weights, levitator ani/bulbocavernosus muscle complex weight, epididymides weights and histology, testes weights and histology, thyroid weight and histology, liver weight, kidney (paired) weight and histology, pituitary weight, adrenal (paired) weight), serum testosterone, T_4 and TSH, clinical chemistry panel including creatinine and blood urea nitrogen	Mortality, clinical signs, body weight, food consumption, age and body weight at vaginal opening, uterus weight and histology, ovary (paired) weight and histology, thyroid weight and histology, liver weight, kidney (paired) weight and histology, serum T_4 and TSH, assessment of oestrus cyclicity, clinical chemistry panel including creatinine and blood urea nitrogen
Optional endpoints	Liver weight, kidney (paired) weight, adrenal (paired) weight, serum testosterone, serum luteinizing hormone	Histopathological evaluation of uterus and vagina	None specified	None specified

(OCSPP, 2009e,d) are the primary assays with regulatory guidelines that use mammalian species explicitly for endocrine screening. These assays are described briefly in Table 1.

2.1. Intact status of the test animals

The Hershberger and uterotrophic assays commonly use gonadectomised rodents in order to produce exquisitely sensitive model organisms for evaluation of (anti)androgenicity and oestrogenicity, respectively. These animal models cannot be considered physiologically-relevant owing to the lack of a functional hypothalamic-pituitary-gonadal (HPG) axis. The lack of physiological relevance of these models is recognised in the most widely accepted definition of an endocrine disruptor, which states that an adverse effect must be observed in an intact organism in order to be considered truly relevant (IPCS, 2002). Hence, results of the Hershberger and uterotrophic assays using surgically modified animals can only be used to help investigate potential mechanisms as part of a broader investigation and should not be used in risk assessment.

2.2. Single time point assessments of hormones

The male and female pubertal assays both require that thyroid stimulating hormone (TSH) and thyroxine (T_4) be measured at termination. In addition, testosterone measurements are also required in the male pubertal assay. Although the relevant guidelines make some effort to control for the high level of intrinsic variability of these hormones, a single time point assessment is insufficient to reliably assess effects on these parameters, making them unsuitable for use in risk assessment, as highlighted in Box 1.

Box. 1. Pubertal assays summarising the issues around hormone measurements.

Test Guideline Requirements

The male and female pubertal assays both require that thyroid stimulating hormone (TSH) and thyroxine (T_4) be measured at termination. In addition, testosterone measurements are also required in the male pubertal assay.

Significant Issues

Hormone levels demonstrate a high level of variability, both between individuals but also the same individual at different times of the day, as well as being sensitive to other factors, such as when the animal last ate. Such factors mean that when measured in toxicology studies, these endpoints can show extremely high variability. Examples of the extreme variability in these endpoints are exemplified in the guideline for the male pubertal assay, which specifies control ranges for Sprague Dawley rats of 4.212–24.112 ng/mL and 0.260–3.960 ng/mL for TSH and testosterone, respectively, with coefficients of variation of 34.04% and 58.82%, respectively.

Conclusions

Owing to their extreme variability, apparent effects on hormone levels should not be used as endpoints for risk assessment, particularly in the absence of any correlating functional effect. Although the reliability of hormonal measurements would be increased greatly by sampling multiple times over the course of the study, the relatively high volumes of blood required for hormonal analyses and the additional stress involved in taking these this samples preclude this approach from being taken in these studies, which use potentially sensitive juvenile animals, as well as assessing a number of parameters which are known to be significantly affected by stress, including the hormone levels themselves.

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