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GUIDANCE DOCUMENT 117 ON THE CURRENT IMPLEMENTATION OF INTERNAL TRIGGERS IN TEST GUIDELINE 443 FOR AN EXTENDED ONE GENERATION REPRODUCTIVE TOXICITY STUDY, IN THE UNITED STATES AND CANADA

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The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. UNDP is an observer. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

This Guidance Document provides guidance on the current implementation of internal triggers in TG 443: Extended One Generation Reproductive Toxicity Study, in the United States and Canada.

A combined meeting of the National Coordinators of the Test Guidelines Programme and of an expert group on reproductive toxicity studies, held in Arlington on 19-21 October 2010, agreed to remove the text concerning the procedure for the decision on the internal triggers from TG 443. The Arlington meeting and the Joint Meeting of the Chemicals Committee and the working Party on Chemicals, Pesticides and Biotechnology (Joint Meeting), however, agreed to include internal triggers in a Guidance Document that would be published at the same time as TG 443.

The Guidance Document was prepared by the United States and Canada. It was revised and approved by the WNT at its meeting in April 2011. The Joint Meeting agreed to the declassification of this document on 12 July 2011.

This document is published under the responsibility of the Joint Meeting.

Guidance Document 117 on the current implementation of internal triggers in Test Guideline 443 for an Extended One Generation Reproductive toxicity Study, in the Unites States and Canada

1. This Guidance Document has been developed to support the use of Test Guideline 443 for an Extended One-Generation Reproduction Toxicity Study (EOGRTS) under Canadian (Pest Management Regulatory Agency) and US regulatory authorities (United States Environmental Protection Agency, Office of Pesticide Programs). Internal triggers for the assessment of the second generation required by these authorities are discussed in Annex 1. This document is intended to provide guidance to users of TG 443 under these authorities. Other regions or member states can have different requirements or approaches for implementing TG 443. A more general OECD Guidance Document on the EOGRTS is in preparation.

2. Under the Canadian Pest Management Regulatory Agency and the United States Environmental Protection Agency, Office of Pesticide Programs, the decision to assess a second generation will take into consideration all available information including data gathered during the conduct of the EOGRTS. In making this determination, consideration should be given to issues such as the sensitivity of the young noted in the database and seriousness of pre- or post-natal endpoints, although other factors can influence the assessment (i.e.., dose-response, toxicokinetics and/or metabolism, mode of action, confidence in study and/or endpoints, and human exposure information). It is expected that, with the additional parameters evaluated in the F_1 generation in the EOGRTS, the F_2 with their limited parameter assessments would seldom affect the hazard characterization for risk assessment (Piersma et al., 2010). However, until sufficient empirical data can be provided to support this hypothesis, the decision by the Canadian Pest Management Regulatory Agency and the United States Environmental Protection Agency, Office of Pesticide Programs to assess the second generation will be determined through the implementation of triggers, as defined in this Guidance Document.

3. As noted above, these triggers are intended to be used for the hazard characterization in risk assessments rather than for Classification and Labelling within the mentioned jurisdictions. Companies wishing to register chemicals in other regions are expected to consult regulatory authorities to determine the appropriate testing protocol.

4. When a maximum of 20 EOGRTS have been conducted with an assessment of the second generation, the available results and appropriate scientific evidence will be analysed. Subsequently, this Guidance Document will be reviewed and, if necessary, revised in light of experience gained. In this regard, OECD member countries and regions are encouraged to share available results.

Annex

Current implementation of TG 443 in the US and Canadian regulatory frameworks for pesticides

Text provided by Canada and USA[1]:

Introduction

5. The OECD expert group for the EOGRTS held several meetings to discuss the interpretation of the data derived from Test Guideline 443. The meetings recognized the need for a Guidance Document on triggers for assessing the second generation and on methodological aspects and interpretation of data for all three cohorts within the Test Guideline (reproductive, developmental immunotoxicity, and developmental neurotoxicity).

6. This document constitutes an essential supplement to OECD Test Guideline 443 (EOGRTS) to describe the use of data gathered within the study to determine if a second generation should be produced. However, as a starting point, available mode of action information and data from other toxicity studies (e.g., repeat dose toxicity studies for systemic toxicity, and ADME) should be considered in the assessment as they may already indicate the potential for reproductive toxicity.

7. The EOGRTS design includes evaluations of numerous sensitive structural, functional, and endocrine-mediated components. Thus, it is unlikely that a critical effect on development and reproduction would be missed. Using a science and risk based approach (as described in Cooper et al., 2006) to determine the need for an F_2 evaluation allows for a tailored approach to testing, reduces the numbers of animals used (1200 animals are used to generate an F_2), and the resources needed to manage, review, and document the study. If required, the production of an F_2 (i.e., breeding of the Cohort 1 F_{1b} animals) does represent a critical decision point integral to the study design. This decision will need to be made rapidly with a clear understanding of supporting data. When determining whether production of an F_2 is needed, one should consider how the additional information gained by breeding a second generation will be used in the hazard evaluation or overall risk assessment.

8. Typically, the F_2 generation allows for a replicate assessment of reproductive performance, litter size, offspring survival and development (including anogenital distance and nipple retention), and weanling necropsy endpoints (organ weights and histopathology) after a lifetime of exposure. A weight of evidence will be essential to determine if the relative sensitivity of the young and seriousness of pre- and post-natal endpoints have been sufficiently evaluated for the hazard characterization for risk assessment.

9. Lastly, when predicted human exposures are considered adequately characterized, margin of exposure (MOE) considerations may be factored into the decision to require the assessment of a second generation. For example, if toxicity triggers are limited to the high dose level alone (with no apparent dose-related trend), MOE of this dose relative to either estimated human exposures or those directly measured through human biomonitoring studies could help guide the triggering decision.

[1] This annex is provided for transparency reasons. It represents the views from the US and Canada. It has not been approved by other OECD Member States.

Determining the level of concern

10. Hazard identification involves understanding the inherent properties of a substance that may lead to adverse responses. It involves defining the relationship between the dose of a substance administered to or received by the test species and the qualitative and quantitative response to the substance.

11. When results of animal testing reveal unique effects in the young (e.g. a different pattern of effects of concern) relative to adults, this is referred to as susceptibility or qualitative sensitivity. Evidence of quantitative sensitivity arises when the effects in the young occur at doses lower than those causing effects in adults, occur more quickly, or occur with greater severity or duration than those observed in adults.

12. If the critical endpoint is based on a serious toxicological effect, a high degree of concern would be identified. The temporal nature of the effect (e.g. time of onset, persistence, recovery, etc.) will influence the determination of the degree of concern, with irreversible findings eliciting greater concern. Examples of serious endpoints of concern include, but are not limited to, reduced viability of offspring, and the occurrence of malformations. Less serious endpoints of concern could include transient effects such as organ weight changes with no or minimal associated histopathological changes.

13. As described above, an evaluation of pre- and post-natal toxicity, primarily determined by an analysis of the sensitivity of the young and seriousness of pre- or post-natal endpoints, is critical to the hazard characterization for risk assessment under certain regulatory authorities. A recent retrospective analysis of 498 multi-generation reproductive toxicity studies (OECD TG 416 and USEPA 870.3800), representing 438 different tested substances, determined that the second generation mating and offspring will rarely provide critical information for hazard identification (Piersma et al., 2010). However, until sufficient empirical data can be provided to support this hypothesis in the context of the EOGRTS, under certain regulatory authorities, the assessment of the second generation will be determined through the implementation of triggers, as defined below.

Determination of Triggers for the Assessment of the Second Generation in the EOGRTS

14. Table 1 below provides a list of endpoints (or "triggers") which can be used to determine whether or not an assessment of the second generation is required. Criteria for establishing triggers were based on the original proposal by Cooper et al. (2006) as well as discussions within the expert group for the EOGRTS.

15. Several general categories of triggers for the production of a second-generation are proposed in order to better characterize equivocal results and/or the relative sensitivity of the young and/or seriousness of pre- and post-natal endpoints:

    - an adverse effect on fertility or fecundity of the parental generation,
    - indications of abnormal sexual development of the F_1 pups,
    - adverse effects on F_1 litter parameters and developmental landmarks,
    - death or evidence of toxicity to the F_1 pups pre-weaning, and
    - equivocal effects on F_1 parameters or unusual control data compared to historical background may also trigger a second generation

Adverse effects on fertility or fecundity of the parental generation

16. There are some endpoints that are only examined in the P animals and/or F_1 offspring (Table 1). These include reproductive performance (number of implantations, pregnancy rate, and gestation interval) and estrous cyclicity. Dose-dependant and biologically relevant alterations in these endpoints justify the production of a second generation, particularly if these findings occur in the absence of severe maternal toxicity. In this situation, evaluating a second generation would provide an assessment of the consequences of these types of effects, after a lifetime of exposure.

17. It is generally accepted that male reproductive organ histopathology is the most sensitive endpoint for detecting minor changes in spermatogenesis in rats (Mangelsdorf *et al.,* 2003; Ulbrich and Palmer, 1995). Functional evaluations of fertility are less sensitive due to the excess sperm reserve in rodents. Mangelsdorf *et al.* (2003), in an assessment for the German Federal Institute on Occupational Safety and Health, reported that reproductive/accessory sex gland organ weights and sperm parameters (motility and counts) were more sensitive endpoints for detecting toxicant effects on reproduction than fertility parameters (number of implantations and pregnancies). These results are consistent with a limited analysis by Gray *et al.* (1989) who reported effects on sperm and gonadal toxicity occurred at lower doses than effects on fertility. These data consistently support the premise that alterations in sperm parameters will be more sensitive at detecting potential adverse effects than the assessment of a second generation.

18. Given the greater sensitivity of histopathology and sperm evaluations to detect changes in male reproductive toxicity compared to the functional observation by the assessment of a second generation, neither effects on reproductive histopathology nor effects on sperm parameters warrant breeding a second generation. These endpoints are assessed twice in the EOGRTS, including an assessment of F_1 offspring that have been exposed *in utero*, during lactation and maturation. F_1 offspring data on reproductive organ weights, histopathology and andrology will not be available when a decision to mate the F_1 adults is needed (Table 1); however, the greater sensitivity of these endpoints fulfills risk assessment needs better than the assessment of a second generation. Furthermore, neither mature testicular histopathology nor sperm assessment is assessed in F_2 animals, which are euthanized at weaning.

19. With respect to the female reproductive endpoints (estrous cycle evaluation, reproductive organ weights and histopathology, and ovarian follicle counts), there are fewer data available for the comparison of endpoint sensitivity. Generally, it is recognized that alterations in ovarian follicle development in female rats may not affect fertility (Hirshfield, 1987). Female rats have robust reproductive performance and hormone production even in the presence of reproductive system alterations (*e.g.*, within 24 hours of removing an ovary, the remaining ovary can ovulate a full complement of 10-12 follicles; only 2-3 corpora lutea are needed to maintain pregnancy – Hirshfield, 1987). Thus, ovarian histopathology is believed to be a sensitive indicator of female reproductive toxicity (Regan *et al.*, 2005). Examination of adult ovarian histopathology is favoured as decreases in primordial follicle numbers will be exacerbated in adults due to continuous recruitment of the remaining follicles (Regan *et al.*, 2005). Therefore, examination of adult P and F_1 offspring is preferred over an examination of prepubescent F_2 weanlings. While the Society of Toxicologic Pathology favours qualitative ovarian histopathology (conducted in the Ps) as an initial assessment of ovarian effects (Regan *et al.*, 2005), Bolon *et al.* (1997) has suggested that ovarian follicle counts (conducted in the F_1) also provide a more sensitive indicator of female reproductive toxicity than fertility, again favouring a histopathology endpoint over fertility.

20. With respect to reproductive organ weights, uterine weight is highly variable, depending on the stage of the estrous cycle at necropsy. The stage of the estrous cycle at the time of necropsy is also not a predictive endpoint, being limited to a single time-point. Effects on reproductive organ weights and histopathology do not warrant the assessment of a second generation as these endpoints are either more, or as, sensitive to toxicant alterations than fertility and because additional information on these endpoints would not be obtained in F_2 pups. A second assessment of reproductive organ weights and histopathology will be available from the F_1 adults, including ovarian follicle counts. These data should fulfil risk assessment needs.

Abnormal sexual development of the F_1 pups, adverse effects on F_1 litter parameters and developmental landmarks, and death or evidence of toxicity to the F_1 pups pre-weaning:

21. Effects on 1) F_1 litter size in the absence of P reproductive organ histopathology changes or 2) pup survival in the absence of severe maternal toxicity or 3) pup developmental landmarks (discussed below) would require the assessment of a second generation in order to determine the relative sensitivity of the young and seriousness of pre- and post-natal endpoints.

22. Puberty is only examined in the F_1 offspring (3/sex/litter), but effects seen in the absence of body weight effects may indicate that the underlying development of the reproductive system has been affected. Some regulatory authorities may therefore require an assessment of the second generation.

23. There are some endpoints that, without producing a second generation, are only examined in the F_1 offspring (Table 1). These include litter size and weight, offspring survival, offspring development (including anogenital distance and nipple retention) and endpoints assessed at weanling necropsy. Treatment- and dose-related alterations in these endpoints in the absence of severe maternal toxicity, justifies the production of a second generation. As with other toxicological studies, weight of evidence will be applied when interpreting the results of the EOGRTS. The weight of evidence concept becomes especially important given the number of endpoints examined and the opportunity for Type I error. The laboratory's historical control data (HCD) can assist in the interpretation of data on reproductive toxicity endpoints.

Equivocal effects on F_1 parameters or unusual control data compared to historical background may also trigger a second generation:

24. As discussed in the Test Guideline 443, the assessment of a second generation may help clarify equivocal findings or provide further characterization of effects on fertility observed in the first generation mating.

25. Statistical significance does not need to be present to validate the biological significance of treatment-related effects. This is particularly true of findings with low incidence (i.e., rare malformations) or high variability, or in situations where the concurrent control data have an unusual incidence profile. In the same way, statistical significance does not necessarily signify biological significance, and scientific judgement and relevant historical control data should be used to distinguish between spurious and real findings (OECD GD 43).

26. Comparison of concurrent study control data with the data from treated animals should always take precedence over comparison with historical control data. The primary goal of evaluating the historical control data is to determine if concurrent control animals are behaving within the margins of normal variability for the species and strain. If historical control data are demonstrably different from concurrent control data, it may be an indication that the study contains some flaw. In the most egregious case, it may not be appropriate to utilise the historical control data in the interpretation of data from treated groups (OECD GD43).

27. If historical control data are used, the most appropriate of these are from studies conducted in the same laboratory, within a reasonable amount of time prior to the study being interpreted (*e.g.*, ± 2 years) in order to avoid genetic drift in the laboratory animal population, and under the same study conditions (*e.g.*, identical species, strain, source, age, vehicle, route and duration of administration, technical personnel, etc.). It is important that the data include sufficient information to render it meaningful in the context of the concurrent study. For example, definitions of terminology should be provided; incidental and continuous data should be fully characterized and summarised with appropriate data ranges, maximum, minimum, median, and mean values; data variance should be addressed. Historical control information that is compiled by animal suppliers or through surveys (or including genetic drift) across multiple laboratories (Clemens *et al.*, 1994, MARTA and MTA, 1995, 1996) can also be useful in some situations and under the appropriate caveats. Overall, the interpretation and use of historical control data requires careful consideration, and the application of scientific judgement and expertise (OECD GD43).

Table 1. Criteria for assessing the second generation in the EOGRTS

Trigger Endpoints[a]	Recommendations
Adult Endpoints	
P Fertility (# implantations, pregnancy rate, gestational interval)	Mate F_1 in the absence of corresponding biologically relevant and dose-related changes in reproductive histopathology
F_1 Estrous Cycle Evaluation	Mate F_1 if biologically relevant and dose-related changes in estrous cycle length without severe toxicity in the dams[b]
Offspring Endpoints	
F_1 Litter parameters (litter size)	Mate F_1 if biologically relevant and dose-related decreases in litter size are seen in the absence of severe maternal toxicity or lethality[b]
F_1 Developmental landmarks (AGD, nipple retention, puberty onset, PPS, VO)	Mate F_1 if biologically relevant and dose-related effects in the absence of body weight-mediated changes in these parameters
↓ F_1 pup survival post-natally	Mate F_1 in the absence of severe maternal toxicity[b]
F_1 pup malformations	Mate F_1 in the absence of severe maternal toxicity[b]
↓ F_1 live birth index	Mate F_1 in the absence of severe maternal toxicity[b]
↓ F_1 pup body weight	Mate F_1, if pup body weight decrease is biologically relevant and in the absence of maternal body weight decrements

[a] Each endpoint will be available in sufficient time to determine whether or not the F1 should be mated.

[b] Type, incidence, magnitude and severity of effect(s) should be considered in relation to maternal toxicity.

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