REGULATORY SERVICES

Consultancy
for Environment
and Human
Toxicology
and Risk
Assessment

ECHA

Report on Survey of Worldwide CROs:

Costs and Practicalities of Two New OECD Guidelines for Testing Chemical Substances

OECD 443, Extended One-Generation Reproductive Toxicity Study, and

OECD 488, Transgenic Rodent Somatic and Germ Cell Mutation Assay

CEHTRA UK Project No.: ECA/RAR/1101

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Attention of: Mr Jukka Malm

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This report was prepared under the terms and conditions as specified in the technical annex to the request for offer ECHA/2011/217 (Annex I), the Contractor's offer dated 11 November 2011 and the clarification sent by e-mail on 25/11/2011.

For **CEHTRA UK Ltd**

Peter Jenkinson, PhD

Genetic Toxicologist

Date: 02 JUL 2012

SIGNATURE

1. Summary

A total of 50 CROs were identified as being potentially able to perform the OECD 443 extended one generation reproductive toxicity study and/or the OECD 488 transgenic rodent somatic and germ cell mutation assay. A preliminary survey was sent to these CROs in order to confirm their capability to do one or both of these tests and to determine which of them may be prepared to complete a follow-up survey answering more detailed questions about their capability, capacity, experience and prices. In the event many CROs were not able to perform these studies or they did not complete the surveys. In addition, many CROs are multinational and in these cases the European division was contacted to complete the survey on behalf of the entire company, thereby capturing the capability and capacity information for all their sites.

For the OECD 443, the survey data are compared and contrasted with the information submitted by COM to CARACAL in March 2012. This COM document is currently not publicly available.

The results of the survey are presented in the relevant sections below and a short discussion follows each set of responses. The overall results and conclusions are summarized below and in addition a series of 'author comments' are included. These comments are given in italics to distinguish them from the objective results and conclusions. The author comments are generally subjective in nature and express the opinion of the author based on his many years of experience in the CRO industry. They are included to help the reader to interpret the results and give a guide as to the level of confidence that may be appropriately applied to the conclusions.

1.1 OECD 443

Capability - a total of 16 CROs indicated that they were currently able to offer the OECD 443 study and a further 5 CROs intend to offer this study in the future. Five CROs answered that they have no plans to offer this service in the future. For these five CROs the reasons for not offering this study were the high cost compared to standard methods and that they believe that there is insufficient market demand. From a technical point of view most of the CROs indicated that they saw some difference between this assay and the OECD 416 2-generation test but primarily in terms of project management and scheduling complications rather than technical issues. However, some indicated that they would have to sub-contract the immunotoxicity cohort if that was required. The survey data indicate that a minimum of approximately 21 CROs worldwide have, or will have at some time in the future, the capability to do the OECD 443 test guideline.

Author comment: the technical and organizational complications of the OECD 443 test method are such that in my opinion some of the CROs that have indicated that they offer, or will offer this test in the near future, are being overly optimistic. It is clear that for most CROs that already perform reproductive toxicology studies then the basic protocol and the extension to two generations should present few problems.

However, the DNT and DIT cohorts are a different matter because they demand previous experience in the relevant end-points and methods that may not be available in-house. The reality is that perhaps only the European and American CROs and a handful of Asian CROs will actually be able to adequately perform this test. It should be noted that only one CRO indicated in the survey that it has actually performed this test (a single study), although another CRO, that did not respond to the main survey, is known to have completed three studies. Thus the number of CROs known to have actual experience of doing the test is only 2.

Capacity – not all the CROs that had claimed in the preliminary survey to have the capability to perform the OECD 443 assay went on to complete the follow-up questionnaire, consequently it has been necessary in some cases to extrapolate from the information provided by those that did respond to the main survey. It is clear that for most CROs the total capacity for OECD 416 and OECD 443 studies is quite limited and finite, and it is a matter of utilizing this capacity to do one test method or the other. On the basis of an average of 3 studies per year for the 12 CROs that responded to the main survey, it is estimated that the worldwide capacity for the OECD 443 test may be in the region of 63 studies per year (21 CRO offering or planning to offer the study multiplied by 3 studies per CRO). This number is calculated as being 50% of the total number of OECD 416 and OECD 443 combined, i.e. 126 per year. Some CROs indicated that their total capacity is reduced by doing OECD 443 studies because of the need for greater flexibility to accommodate the various choices of additional cohorts that may be triggered as the study proceeds, so this could be an over-estimate. In addition, it should be noted that some or most of this capacity may be utilized for pharmaceuticals and plant protection products.

Author comment: The annual capacity for the OECD 443 is closely linked to the capability of CROs to perform the test. In the section above I made comment about the probable over-estimation of the number of CROs that in practice are likely to be able to perform the test reliably, particularly in respect of the developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) cohorts. It is logical therefore to conclude that if the capability is over-estimated then so also is the capacity, perhaps by as much as 50% for the basic and 2-generation protocols and more for the DNT and DIT cohorts. Of course the problem that many CROs face is that they do not know for sure which cohorts will be needed until the results of the first generation are available.

Cost – Nine CROs provided details of their prices for the OECD 443 and a further two prices were obtained from another source; some CROs stated that they have not yet costed the study because they have not had any firm requests for a quotation. Others suggested that they anticipated that even the basic study without additional cohorts would be approximately double the price of the OECD 416. The data from the CROs that provided pricing information supports this assumption. The price range for the OECD 416 test guideline (without formulation analysis) is €141,000 to €408,000 and the worldwide average price is €285,842 (European average is €318,295). For

the OECD 443 the average price for the basic study (without second generation and extra cohorts) is €414,273, and if the second generation is included then the price is €469,778, which is approximately 1.6 times average cost of the OECD 416 protocol. If the extra cohorts are included, then the price increases to €507,444 for the neurotoxicity cohort or €440,414 for the immunotoxicity cohort. If both cohorts and the second generation component of the study are performed then the average cost may be as much as €655,195 (minimum €429,950, maximum €895,000. For the basic OECD 443 study protocol there is a three-fold difference in price between the maximum and minimum prices, this is explained because the eleven values include two outliers at the high end of the range; one was a US CRO and the other European, although the latter is arguably the CRO with the most experience of performing the OECD 443. If the two outliers are excluded then the European average for the basic one-generation protocol is €350,000, which is 10% more than the European average for the OECD 416.

Author comment: the prices provided by the respondents for the OECD 416 study may be considered to be very reliable because they are similar to those that I am familiar with when I used to work at a CRO. Furthermore, this is an established test and the CRO industry generally has a lot of experience of running these studies and therefore they can quote their prices based on many years of experience. For the OECD 443 study we must accept a lower level of confidence because very few of the CROs have actually performed this study (only two) and therefore they have no direct experience. However, most CROs have reliable tools with which to estimate study prices based on animal numbers, duration, technical procedures, experience of similar tests, etc. Therefore, I believe that even for the OECD 443 that we can consider the prices provided by the respondents to be relatively accurate. The price for the basic module and the basic module with the second generation may be taken with the same level of confidence as the OECD 416 prices because in this case there is little technical difference that would introduce a significant element of uncertainty. The prices for the additional cohorts should be taken as being less reliable than the basic test because most CROs have no experience of doing them so their estimates will be based on predictions or extrapolations from their standard prices. However, it is my opinion that they are sufficiently reliable because there was a high degree of consistency in the data between CROs in terms of the relative price for each variant of the test. For example, all the CROs estimated that the DNT cohort added significantly more to the basic price than did the DIT cohort.

Conclusion - For the OECD 443 test guideline there is the capability and capacity within the CRO industry worldwide to conduct a significant number of these studies. Furthermore, there is the potential for many more CROs to extend their current expertise in reproductive toxicology to include the OECD 443 in their service portfolio. This may be acceptable for the basic protocol, and for the basic protocol with the second generation. However, for the developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) cohorts there are concerns within the industry

about the lack of expertise. This includes the performance and interpretation of neurotoxicity tests, the pathological examination for neurotoxicity and the performance and interpretation of immunoassays. Furthermore, the lack of availability of positive control substances for the validation of the DNT and DIT cohorts is seen as a significant technical barrier. In terms of cost, the OECD 443 test guideline is more expensive (~50% greater) than the OECD 416 it is designed to replace, even when used in its most basic format.

Comparison of the Survey Data to those of COM Paper for CARACAL, March 2012 – in March 2012 the COM submitted a paper to the 10th meeting of competent authorities for REACH and CLP (CARACAL) that included some information about the cost of the OECD 416 and OECD 443 test methods and also some technical commentary about the OECD 443 (this document is not currently publically available). The information of this paper is compared below with the information obtained of the survey in order to gauge the validity of both data sets and to create a weight of evidence perspective.

The COM paper included some price/cost estimates for the OECD 416 and OECD 443 test methods; these are summarized, together with the survey prices in the table below.

	OECD 416		**OECD 443 Including 2nd Generation, no DNT or DIT**		
	€	% vs ^	€	% vs ^	% vs ^^
Fleischer[a]	375,000^	100	NA	NA	NA
Service provider	350,000	93	630,000*	168	198
Chemical Co.	400,000	107	415,000‡	111	130
Survey All	285,842	76	469,778	125	148
Survey Europe	318,295^^	85	449,000	120	141

* There was insufficient data to calculate this figure; this is the cost for the basic protocol only

‡ Calculated by subtracting the DNT and DIT costs from the total cost – maybe an underestimate

a Research Paper – Testing Costs and Testing Capacity According to the REACH Requirements – Results of a Survey of Independent and Corporate GLP Laboratories in the EU and Switzerland (by Manfred Fleischer, Journal of Business Chemistry, vol 4, issue 3, September 2007)

The survey for the OECD 416, which includes all the major European CROs, resulted in a European average price of €318,295, which was 85% of the Fleischer price

(adjusted for inflation) used by the COM in their paper. This suggests that prices may have come down since Fleischer did his survey, or at least prices have not kept pace with inflation. This is probably the result of a downward pressure on CRO prices since the worldwide financial crisis that began in late 2007, which has significantly reduced the spending of the pharmaceutical industry on pre-clinical development.

The price of the OECD 416 given in the COM paper for the service provider is at the upper end of the range of the survey (second most expensive), and the Chemical company cost was almost identical to the most expensive European CRO. This comparison of the data gives a high degree of confidence in the accuracy of the survey data for the OECD 416 price. The European average for a full OECD 443 (including all cohorts) is €607,119, which is 191% of the survey European average for the OECD 416. The figure for the same comparison in the COM paper is 223%.

For the OECD 443 the COM service provider price is extremely high compared to the survey European average (40% greater) and only one other CRO in the survey had a price that was higher and that was a US-based CRO. Overall we can see the same trend in the price comparisons between the COM paper and the survey data, however, the larger sample size of the survey and the fact that the service provider used by COM is an outlier in terms of price, suggests that the survey may provide a more accurate basis for price comparisons. On this basis it is clear that when comparing like for like (OECD 416 vs OECD 443 with 2nd generation) then the extended one-generation study is 41% more expensive than the OECD 416.

Practicalities – the COM paper included some commentary about the practicalities of the OECD 443, which can be compared to the results of the survey. In the COM paper it was reported that only two commercial laboratories have hands on experience of the OECD 443, this was confirmed by the survey. In the COM paper it was estimated that only 8 – 10 laboratories are capable of performing the OECD 443; in the main survey 12 CROs claimed to be able to do the test and in the preliminary survey 21 CROs made this claim. It may be prudent to conclude that 8 -12 may be the most accurate figure although it is possible that the source of this estimate for the COM paper may not have a complete worldwide appreciation of the CRO industry. The COM paper suggests that approximately 50 OECD 416 tests is the capacity of European and US CROs, with some non-defined capacity outside the EU/US. The survey estimates the equivalent of 126 studies worldwide, which is a similar figure after accounting for the number of CROs in the calculation (8 - 10? vs 21). The other comments made in the COM paper about the technical issues surrounding the OECD 443 are reflected in the responses of the survey participants (organizational issues, lack of DNT and DIT experience, etc.).

1.2 OECD 488

Capability – very few CROs currently have the capability to perform the OECD 488 test guideline. The preliminary survey revealed three laboratories that claim to have the capability and one other CRO was identified via other sources. Two of the four CROs are located in the USA and one of them does not work to OECD GLP standards and restricts its operations to servicing the pharmaceutical industry. Of the two European CROs, one has a proven track record of conducting this assay over many years, whereas for the other CRO has only completed a small number of

studies (less than six). The other major European CROs have a mixed view on the OECD 488; some have no interest whilst others are keeping a 'watching brief' and may be prepared to establish the assay if there is sufficient demand. However, the time required to establish and validate the assay is considerable, perhaps 9 to 18 months, and the cost will be significant (individual animals may cost approximately €200). Furthermore, there are few scientists and technicians with practical experience of the test.

Authors comment: In reality there is only one CRO with significant experience of performing transgenic mutation assays, primarily for the pharmaceutical industry. Two other CROs have limited experience, perhaps only a single study each. A fourth CRO in the USA has an unknown level of experience but does not work for the chemical industry and so has been excluded from the survey. Although the technical aspects to the test are not especially demanding it does involve a mix of techniques (toxicology, genetic toxicology, microbiology) that requires a breadth of experience that is not commonly found in the workforce at a CRO. The recruitment of staff with the relevant experience from academia is possible but, judging from recent publications in the scientific press, most work in this field seems to be done predominantly at research institutes in Japan and the USA, which may make recruitment by European CROs difficult. The barriers to any CRO wishing to establish this assay also include the difficulty of obtaining supplies of the animal models and the high cost of validating the assay.

The issue of species is important and, although out of the scope of the survey, it is worthy of comment. The original models that were developed using transgenic methodologies were murine and only later applied to the rat. The European and US CROs that responded to this survey have used exclusively murine TGRA models. Therefore, although rat models do exist, there is no experience in the CRO industry of their use and consequently no historical data.

Capacity – from the previous section it is obvious that the worldwide capacity for transgenic assays is extremely limited. In Europe there are two CROs that offer the OECD 488 transgenic assay and one of them has completed around 15 studies in total, and 1 to 5 have been performed by the other CRO. The US CRO offering these studies to OECD GLP has completed 1 to 5 non-GLP studies and will start their first GLP OECD 488 in the near future. Therefore, the survey has shown that a total of 16 to 20 studies have been performed by CROs in Europe over the previous 20 years, i.e. a maximum of 1 to 2 per year. In the USA the number is less than six if we exclude the CRO that only works to FDA GLP standards. Furthermore, none of these studies are formally compliant with the OECD 488 test guideline, either because they were performed before the guideline was published, or because they were custom-designed studies for specific pharmaceutical purposes. It is concluded that the current total capacity for OECD 488 studies in Europe is a maximum of six studies per year, and those available to REACH registrants may be considered to be half of that number, i.e. three studies per year; the others being taken up by the

pharmaceutical and plant protection industries. The US CRO offering OECD GLP studies has a capacity of up to five studies per year, which may contribute a further two or three studies to the available annual capacity. It is clear that the annual capacity for these studies is restricted, perhaps to only three to five per year, and that the available capacity is unlikely to increase in the short-term. Thus there will be a limited 'supply' of this test in the short-term and no significant number of follow-up genotoxicity tests can be made with this method.

Author comment: the capacity for these studies is dictated by the market demand. Until now the market for such studies has been dominated by academic research and the pharmaceutical industry. However, the number of studies performed by CROs for the pharmaceutical industry is clearly very small, perhaps only 16 to 25 in the last 20 years, excluding the FDA CRO in the USA. In addition there are a number of studies that have been done in-house by pharmaceutical companies but this is not possible to estimate. Since the publication of the OECD 488 test guideline one may anticipate an increase in demand from those areas where adherence to a published test method is of greater importance, e.g. chemicals and plant protection products. In the latter case there is some discussion in the agrochemical industry on the implications of the draft SANCO documents (SANCO 11802-2010 for AIs and PPP), where it states:

5.4.2. In vivo studies in somatic cells

Circumstances in which required

If all the results of the in vitro studies are negative, at least one in vivo study shall be done with demonstration of exposure to the test tissue (e.g. cell toxicity and/or toxicokinetic data).

A negative result in the first in vivo test in somatic cells will provide sufficient reassurance for active substances that are negative in the three in vitro tests.

For active substances for which an equivocal or a positive test result is obtained in any in vitro test, the nature of additional testing needed should be considered on a case-by-case basis taking into account all relevant information using the same endpoint as in the in vitro test.

If the in vitro mammalian chromosome aberration test or the in vitro micronucleus test are positive for clastogenicity, an in vivo test for clastogenicity using somatic cells such as metaphase analysis in rodent bone marrow or micronucleus test in rodents or in vivo Comet assay, when validated, shall be conducted.

If the in vitro micronucleus test for numerical chromosome aberrations on mammalian cells is positive (or the in vitro mammalian chromosome test is positive for numerical chromosome changes), an in vivo micronucleus test should be conducted. In case of positive result in the in vivo micronucleus assay, appropriate staining procedure such as FISH (fluorescence in-situ hybridization) should be used to identify an aneugenic and/or clastogenic response.

If either of the in vitro gene mutation tests are positive, an in vivo test to investigate the induction of gene mutation shall be conducted.

The implication of the final sentence is that a positive Ames (or L5178Y tk large colony inducer or HPRT if done) should be followed up by a TGRA. If this is correct, then once this document is finalized, it may be expected that there will be an increased need for such studies by the agrochemical industry. In the short-term this may absorb a significant amount of the available capacity but in the mid- to long-term it may stimulate the existing CROs to increase capacity and other CROs to introduce

the method to their portfolio. In the preliminary survey seven CROs indicated that they may offer the OECD 488 in the future. If half of these actually do implement the assay then it could double the capacity of the CRO industry. In my opinion four of the seven CROS have, or could develop, the capability within a reasonable period of time.

Cost – the cost of an OECD 488 study is at least €40,000 for a single tissue and at least €100,000 for three tissues. However, the range of the prices collected for the survey was extremely wide and the average price for a single tissue was €97,000 and >€125,000 for three tissues. This is greater than any of the standard in vivo genetic toxicology studies such as the micronucleus test or UDS assay in the liver, which in Europe may cost in the region of €12,000 and €21,000 respectively. The price of the COMET assay, where there is no OECD guideline, is approximately €28,000 for up to three tissues. [The prices for standard studies given above are based on the experience of the author and are for guidance only]. It can be seen that the average price of the OECD 488 for a single tissue is approximately 4.5 times the price of a UDS assay, which is the standard method used to follow up on in vitro positive substances with a gene mutation mechanism of action.

Author comment: The prices provided by the respondents are considered to be reliable, in particular the CRO with experience of ~15 studies. It should be noted that if demand increases in the future because of changes in the regulatory requirements for agrochemicals (see above) and for follow-up testing requirements under REACH then the price may be expected to escalate quite quickly because of the limited number of CROs offering the test and the high barrier to entry by other CROs.

Conclusion - For the OECD 488 test guideline the capability and capacity is limited and there is little prospect of the situation changing in the short to medium term. The technical resources required to establish and validate this assay are considerable, and the transgenic animal models are currently in short supply (small colonies and/or maintained as frozen embryos) and perhaps difficult to import. The cost needed to establish, validate, and generate historical data for this assay may act as a further deterrent to CROs to add this test to their portfolio. Therefore, any REACH registrant that is required to do this study will be limited to a choice of two or three CROs and may be competing with pharmaceutical and plant protection product customers for the limited capacity of these laboratories. The cost of an OECD 488 study is greater than any of the current standard in vivo tests, perhaps five times the cost depending on the scope of the protocol. Furthermore, no CRO currently offers this test using the inhalation route so, at the moment, gases, volatiles and respirable dusts cannot be tested. It is not known why the inhalation route is not currently on offer but it may be assumed to be because of several factors, including the cost of validation or the absence or non-availability of a suitable positive control. In the principle there should be no technical barrier to performing this assay using the inhalation route but the cost may be expected to be significant.

2. Introduction and Objectives

As required by the ECHA, CEHTRA UK Ltd performed the following actions, in reference to:

> **Contract No.: ECHA/2011/217,** Costs and Practicalities of Two New OECD Guidelines for Testing Chemical Substances (OECD Test Guidelines 443, extended one generation reproductive toxicity test and OECD 488, transgenic rodent somatic and germ cell gene mutation assay).

CEHTRA UK investigated the prices and capacity, including practicalities and availability, of contract research laboratories worldwide to conduct GLP-compliant studies of chemical substances using two new OECD test guidelines.

- OECD test guideline 443, including an assessment of specific testing options permitted within the guideline, and including a price comparison with studies conducted to OECD guideline 416.
- OECD test guideline 488.

The specific tasks to be performed, as identified by ECHA, may be summarized in the form of a list as follows:

- Identify major GLP-compliant Contract Research Organizations (CRO) companies worldwide (Europe, North America, Japan, India and Brazil) that can perform one, or both, of OECD 443 and 488.
- Confirm the capacity (both annual throughput and number of concurrent studies), lead-time, resource limitations impacting on lead-times, overall capacity, and the cross-interference on capacity by similar studies utilizing the same resources.
- Collate detailed information on prices for the studies (both local currency and euro equivalent), and comparative prices for the OECD 443 and OECD 416 2-generation reproductive toxicology study.
- Gather comments and information from the CROs on perceived technical and practical challenges in the conduction of these studies.

3. Methodology used to Achieve the Objectives

3.1 Identification of study-competent CROs

a. Network contacts – CEHTRA staff have worked with many of the major CRO companies and have good contacts with their toxicology and business development staff. We have contacts at CROs in Europe, North America, Japan, India and Brazil. These sources were contacted to identify CRO companies that may be invited to take part in the survey

b. European and International government institutions provide lists of GLP compliant laboratories in their area of jurisdiction, for example the EPA in the USA. These lists were examined to identify CROs that may wish to take part in the survey.

c. CRO Directories on the internet were accessed and used to provide information on CROs worldwide.

d. Agilent Technologies, suppliers of animal models for the Big Blue system, were contacted and asked if they could supply the contact names of their users.

e. Perform an internet search on publications on both study types to identify practitioners.

3.2 Survey methodology

CROs identified as potentially having the capability to do either of the study types were contacted to establish whether or not they offer one or both of the tests. Once identified, those CROs with the capability to perform one or both study types were invited to participate in the survey.

A preliminary survey questionnaire and two 'follow up' surveys were prepared using the commercial web-based service 'Freeonlinesurveys' because this was considered to be the most effective way to optimize survey responses as the completion of the survey is simplified. The basic service offered by 'Freeonlinesurveys' was not adequate so a subscription was taken out for the upgrade that allows more functionality in the survey.

The preliminary survey was designed to identify those laboratories that were eligible to take part in the full survey for one or both of the study types. Following receipt of responses to the preliminary survey, the main survey was issued by email as two links to the online website of 'Freeonlinesurveys', the first link was for the survey on OECD 443 and the second link was for the survey on OECD 488.

The main survey asked questions about capability including extent of experience and historical data, capacity, price and technical issues. The questions were structured to constrain the answers in order to ensure comparability between CROs. Free text answers were allowed for the questions on the investigation of experimental and technical challenges, and limitations of the assays as perceived by the CROs. The three surveys are included in Appendix 1.

3.3 Follow-up

Once the surveys were returned they were examined for completeness and clarity. Any ambiguous answers were followed up by direct contact (telephone or email) with the CRO in order to clarify the response to ensure comparability between CROs and to check the veracity of the answers.

4. Results

4.1 Identification of CROs

Using the resources mentioned in section 2.1 above, CROs that were considered to have the capability to perform GLP studies using either the OECD 443 or OECD 488 guidelines were identified. The key resources used to identify CROs were:

Network contacts – identified all European and majority of the North American CROs, the Brazilian CRO and also all the Japanese and some of the Indian and Chinese CROs, perhaps 80% of the total.

European and International government lists of GLP compliant laboratories – identified one additional CRO in Canada.

CRO Directories on the internet – identified the majority of Indian CROs and the Taiwanese CRO, approximately 20% of the total.

Agilent Technologies, suppliers of animal models for the Big Blue system – no names were provided by Agilent.

Internet search on publications on both study types to identify practitioners – no additional CROs were identified using this method; the majority of publications are by academic institutions.

The websites of these CROs were accessed and a judgment made as to whether they offered the OECD 443 or OECD 448 studies, or if they offered similar study types such as general toxicology, reproductive toxicology or *in vivo* genetic toxicology studies. For some of the CROs it was not always obvious whether or not they operate under OECD GLP criteria. However, all of the countries, except China and Taiwan, presented in Table 1 are known to be members of the OECD scheme for the mutual acceptance of studies. At least one CRO in China claims to operate to OECD standards and has been inspected by a European GLP monitoring team. The contact details for each of the CROs were taken either from known sources or from the website.

The identity of the CROs is kept confidential to CEHTRA, in order to encourage them to participate; hence CROs are listed by number and/or location by country/region in the tables.

Table 1. CROs Identified for Potential Inclusion in the Survey

Region	*CROs Identified as Known or Assumed to be Offering Studies:*		*Number of CROs Contacted for Preliminary Survey*
	OECD 443	OECD 488	
Europe	10	1	10
USA	11	2	11*

Canada	2	0	2
Japan	3	0	2‡
India	19	0	18≠
Brazil	1	0	1
Taiwan	1	0	1
China	5	0	5
Total	**52**	**3**	**50**

* One of these CROs later discovered to have closed
‡ One Japanese CRO represented by US CRO
≠ One 'CRO' shown to offer brokering service only

4.2 Preliminary Survey

The preliminary survey was sent on the 12 March 2012 to the email contacts identified for the 50 CROs and was re-sent on 23 March 2012. In five cases the original email address was found to be 'undeliverable'. One of these was because the laboratory had closed, two were because the contact people had left the company and for the remaining two (one in China and one in India) for reasons that could not be determined. The responses of the 26 CROs that responded to the preliminary survey are summarized in Tables 2 to 9. Question 1 was related to the identity of the CRO and the contact details of the respondent and is therefore excluded from the results.

Table 2. Answers to Q2

2) Does your laboratory currently offer the OECD 443 Extended one-generation reproductive/developmental toxicity test?	
Yes	16
No	10
Total	26

A total of 16 CROs claim to currently offer the OECD 443 test. Eight of these are based in the EU, two in India, one in the USA and the other five were not identified (completely the questionnaire anonymously). It is not clear whether the anonymous responses should be taken seriously or included in the analysis, however, I have included them for the sake of completeness. Note that the US CRO claimed only to perform the assay for drug evaluation (US FDA/ICH) but not for chemical evaluation (EPA/OECD).

Table 3. Answers to Q3

3) If you answered NO to question 1 then do you plan to offer the OECD 443 Extended one-generation reproductive/developmental toxicity test in the future?	
Yes	21
No	5
Total	26

Of the 10 CROs that do not currently offer the OECD 443 test, six claimed to be prepared to offer the test in the future, three of these were Indian, two from the USA and one from Canada.

Table 4. Answers to Q4

4) If you answered No to both questions 2 and 3 then please provide the reasons why, tick all that apply. If you answered yes to either question 2 or 3 then please skip this question.	
We do not have the necessary equipment	1
We do not have the necessary experience	3
We do not believe there is sufficient market demand	5
The test is too expensive compared to standard methods	2
Other	

This question seemed to confuse some respondents; for example two respondents that answered yes to Q3 also commented that they do not believe there is sufficient market demand. However, it is clear that the majority opinion of those CROs that do not intend to offer this study is that there is insufficient market demand.

Table 5. Answers to Q5

5) If you answered Yes to either question 2 or 3 then will you be prepared to complete a second survey designed to collate more detailed information. Note that the identity of respondents will not be revealed to ECHA or any third party.	
Yes	20
No	6

Of the 21 CROs that offer or plan to offer the OECD 443 study all but one agreed to participate in the main survey. The one CRO that declined was Canadian and one of the USA laboratories agreed but stipulated that a confidentiality agreement was required before they would release any information. Another USA CRO indicated that they only do work according to US FDA GLP and not to EPA GLP, which means that they do not do work on chemicals but only on pharmaceuticals. However, they agreed to participate in the survey.

Table 6. Answers to Q6

6) Does your laboratory currently offer the OECD 488 transgenic rodent assay (TGRT)?	
Yes	3 (4)*
No	15

* A further CRO was identified from information on their website

Eight of the respondents that completed questions 1 to 5 failed to complete questions 6 to 9, it is assumed for these CROs that they do not, and do not intend to offer the OECD 488 test guideline. Two of the three CROs offering the study are based in the EU and the one is based in the USA. However, this CRO is also the one that only provides services to the pharmaceutical industry, so unless their policy changes, their experience and capacity cannot be accessed by the chemical industry. One further US CRO was identified to offer this study on the basis of their website content and they were contacted directly to encourage them to participate.

Table 7. Answers to Q7

7) If you answered NO to question 6 then do you plan to offer the OECD 488 TGRT test in the future?	
Yes	7
No	8

Seven CROs that currently do not offer the OECD 488 test indicated that they plan to offer this in the future. Three of these are based in the EU, three in India and one in the USA.

Table 8. Answers to Q8

8) If you answered No to both questions 6 and 7 then please provide the reasons why, tick all that apply. If you answered yes to question 6 or 7 then please skip this question.	
We do not have the necessary equipment	2
We do not have the necessary experience	4

We do not believe there is sufficient market demand	5
The test is too expensive compared to standard methods	1
Other: “Never had an enquiry”, “We intend to gear up for it once a stable supply of test animals is available”, “Alternative tests such as the Pig A gene may provide a more realistic alternative which takes account of the need to minimise animal usage”, “Difficult to import genetically modified animals due to lengthy, time consuming and tedious procedures.”	

The predominant reason for not offering this study is the perceived lack of market demand, but this was closely followed by the lack of experience. However, other comments point to problems of availability of the animal models and importation problems with genetically modified animals, which appears to be a problem in some countries.

Table 9. Answers to Q9.

9) If you answered Yes to either question 6 or 7 then will you be prepared to complete a second survey designed to collate more detailed information. Note that the identity of all respondents will not be revealed to ECHA or any third party.	
Yes	12
No	6

A total of 12 CROs agreed to participate in the follow-up survey on the OECD 488 test method, including one CRO that answered no to questions 6 and 7. However, as there are only 3 or 4 CROs that claim to offer this test method at the present time then it is clear that there is a dearth of experience in the CRO industry and few scientists with the relevant training.

4.3 Main Survey – OECD 443

The main survey on the OECD 443 test guideline was sent as a link included in an email on the 2 April 2012 to the email contacts of the CROs that had indicated in the preliminary survey that they were willing to participate in the main survey. Several of the CROs were re-contacted directly by telephone or email in subsequent weeks to encourage them to complete the questionnaire. However, thirteen CROs eventually completed the survey and one of those only completed question 1 (their contact details) and so was excluded from the results but was contacted to request that the survey be completed (a completed questionnaire was never received from this CRO). The responses of the CROs that responded to the main survey are summarized in Tables 10 to 25. Question 1 was related to the identity of the CRO and the contact details of the respondent and is therefore excluded from the results.

Table 10. Answers to Q2

2) Does your laboratory currently offer the OECD 443 extended one generation reproductive toxicity test	
Yes	8
No	4

All but 1 of the CROs currently offering the OECD 443 are based in Europe and the other one is based in the USA. Of the four CROs that plan to offer the test, two are in India, one in the USA and one in Brazil. However, it is known from website information that a small number of laboratories in the USA also offer this test but in some cases only to the pharmaceutical industry. In addition, two of the European laboratories also have facilities in the USA and it is known that they have capacity for toxicology studies on both sides of the Atlantic.

Table 11. Answers to Q3

3) If you answered no to the previous question then when does your laboratory plan to begin offering the OECD 443 study?	
2012	3
2013	1
>2013	0

Those CROs that have identified a potential market for the OECD 443 study have indicated that they will begin to offer the study within the next 6 to 18 months; note that these CROs are based in India and Brazil, so it is not clear whether they will be able to contribute to the overall capacity available to REACH registrants because this

will also depend on the willingness of registrants to place their studies at CROs based in countries where they may have no experience of placing studies before. Furthermore, even though Brazil and India are members of the OECD scheme for the mutual acceptance of GLP, this does not guarantee that studies performed in these countries are equivalent to studies done in Europe, for example the animal models may be produced in-house because of the difficulties and cost associated with the import of live animals. One of the CROs that proposes to begin offering the test in 2012 is based in the USA, but does offer OECD GLP so may be available for use by REACH registrants.

Table 12. Answers to Q4

4) Whether or not your laboratory currently offers the OECD 443 guideline study please estimate the annual capacity for OECD 443 studies in your laboratory (all available facilities). If there is an exact number please enter this in the 'other' box.	
1 - 5 per year	8
6 - 10 per year	3
> 10 per year	1

Eight of the CROs have a capacity they estimate to be in the range of 1 to 5, three CROs have a capacity in the range of 6 to 10 and 1 CRO claims a capacity in excess of 10 studies per year. This means that the total capacity for these twelve CROs is in the range of 36 to 80+ with a mean capacity of 45 studies per year.

Table 13. Answers to Q5

5) What is the combined annual capacity for OECD 443 and OECD 416 (2-gen) studies in your laboratory (all available facilities)? If there is an exact number please enter in the 'other' box.	
1 - 5 per year	6
6 - 10 per year	4
> 10 per year	2

The combined capacity for OECD 416 and OECD 443 studies for the 12 CROs was estimated by them to be in the range of 50 to >90 per year, with a mean of approximately 55 studies. This figure is very similar to the figure of 45 studies for OECD 443 alone, which suggests that in actuality the capacity of one study type is directly dependent on the number of studies of the other type, and of course this is not so surprising. However, it is also clear that overall; the OECD 443 study absorbs a greater proportion of the total capacity for reproductive studies than does the

OECD 416. This may be because familiarity with the OECD 416 means that CROs are more confident of the time taken to complete a study, whereas lack of experience with the OECD 443 means that there are many unknowns about the time and resources needed to complete the study. However, it is also indicated that the OECD 443 does actually consume more capacity that the OECD 416 because of the flexibility required to meet the demands of the protocol design.

Table 14. Answers to Q6

6) What is the impact of the OECD 443 on the overall capacity of your laboratory to perform reproductive toxicology studies? Do you anticipate an increase or a decrease in capacity following an increased throughput of OECD 443 studies? Free text response required.
The free text answers to this question are reproduced below: 1. Increase in the capacity. 2. Each study would absorb about 15% of room capacity for about 20 weeks and perhaps 20% of technical resources. At a rate of 2 studies per year we could probably maintain normal repro throughput but precise scheduling would be disturbed to fit the smaller studies around the larger study 3. It will reduce current capacity to perform all reproduction studies due to increased study duration. If OECD443 replaces a two generation study then it is replacing one study with another. If a second generation is not required for OECD443 then the impact will not be significant. This presumes that the study design is known before the start and extra modules are not added during the course of the study. 4. There will be a huge decrease in overall capacity, as the OECD 433 study needs a lot of flexibility and has many different endpoints 5. We anticipate a decrease in capacity with an increase of OECD 443 studies. However, the Management will enhance the capacity based on the requirement 6. There is no impact on our overall capacity, once we keep the limit of 1 to 5 studies in a year. 7. A decrease of capacities for reproductive toxicity studies can be expected, as the full OECD 443 panel requires high numbers of animals and extended downstream processing. 8. Whilst we can offer OECD 443 and have experience of all the components that make up the study we have not yet been awarded and conducted an OECD 443. We will maintain a watching brief as we do across all the industry sectors we work in and staff according to market demand. 9. Our facilities in Europe are well equipped to perform and schedule OECD 443 studies alongside other reproductive toxicology studies. 10. The OECD 443 is a large and complex study that requires considerable coordination of staff and other scientific resources. As those resources would also be used for other Reproductive Toxicology studies, it will reduce our overall capacity until we can respond to a real increase in OECD 443 studies with increased our resources. 11. No impact 12. We will have an increase in capacity

This question has provoked a variety of answers that include all scenarios, i.e. increase, no change or decrease. However, it is perhaps important to note that the CROs that claim to be already offering the OECD 443 all agree that capacity will be reduced, whereas those CROs that are not yet offering this study anticipate little change in overall capacity. On this basis it is considered prudent to take the opinion of the experienced CROs as more accurate than the inexperienced CROs and to

conclude that for a fixed level of resource fewer OECD 443 studies can be performed than OECD 416, regardless of the extent of the OECD 443 study in terms of cohorts.

Table 15. Answers to Q7

7) What is the lead-time for the OECD 443 at your laboratory (assuming availability of test substance, etc)?
The free text answers to this question are reproduced below: 1. 8 months 2. Four months - to ensure availability of 3 rooms at the required time points to accommodate the F1 cohorts. 3. The current lead time is affected by REACH 2013 deadline and volume of OECD 422/421 studies being conducted hence approximately 4-6 months 4. 5 months in-life + 4 months reporting = 9 months (assuming 2 weeks premating and no triggered mating) 5. Four to Six weeks 6. I don´t have that time available at that moment, once we never perform this study on our facility before. But, assuming this study would lead-time 70% of a 2-generation study, I could risk to say that would take 750 days, approximately. 7. Turnaround time: ~10 months without F2 generation and ~13 months including F2 generation 8. 2 months assuming test item availability and study paperwork is in place. 9. 2 months 10. 2 months 11. Approximately 4 to 6 weeks 12. 3 months

See the text below Table 16 for the analysis of Q7.

Table 16. Answers to Q8

8) What is the lead-time for the OECD 416 (2-gen) at your laboratory (assuming availability of test substance, etc)?
The free text answers to this question are reproduced below: 1. 18 months 2. Two months - to plan rooms and ensure that litter mate animals available for start of P generation 3. Similar to question 7 4. 9 months in-life + 3 months reporting = 1 year 5. Four to Six weeks 6. The lead-time for 2-generation studies is 1000 days. 7. Turnaround time: ~13 months 8. 1-2 months assuming test item availability and study paperwork is in place. 9. 2 months 10. Two months 11. Approximately the same as for OECD443 ie 4-6 months 12. Approx. 4 to 6 weeks 13. 3 months

These two questions seem to have been confusing in that some have understood that 'lead-time' is the time required before a study can begin (the correct interpretation), whereas others have taken it to mean the time needed to complete a study. However, comparing the answers of Q7 with those of Q8, it can be seen that the lead-time (time before the study is started) is longer for the OECD 443 than the OECD 416 but that the overall time taken to complete the OECD 443 may be shorter than the OECD 416, although this is highly dependent on which cohorts may be triggered. The answer by respondent 6 suggests that it may take 2 years to complete an OECD 443 study and 3 years to complete an OECD 416, which seems to be much longer than one may expect.

Table 17. Answers to Q9

	9) What is the standard price in Euros and/or local currency for the OECD 443 at your laboratory? Please exclude analysis of formulations costs.			
	Number	Min	Max	Average
Basic study	11	€250,000	€764,000	€414,273
Basic study with optional second generation	9	€310,000	€670,000	€469,778
Basic study with neurotox (DNT) module	9	€352,000	€809,000	€507,444
Basic study with immunotox (DIT) module	9	€269,000	€675,725	€440,414
Basic study with both modules	9	€369,950	€854,725	€567,964
Basic study with two generations and with both modules	10	€429,950	€895,000	€655,195

Nine of the respondents provided prices for at least the basic OECD 443 study and two further prices were obtained from another source. Nine values were obtained for the basic protocol with a second generation, the average of these were €414,273 and €469,778 respectively. The average price for the OECD 416 study in 10 European CROs is €318,295 and the worldwide average is €285,842. Therefore in this direct comparison with the average European price for the OECD 416 the OECD 443 is 1.3 times more expensive for the basic protocol and 1.5 times more expensive if a second generation is included. The OECD 443 with all modules included is 2 times more expensive than the OECD 416. For the basic OECD 443 study protocol there is a three-fold difference in price between the maximum and minimum prices, this is explained because the eleven values include two outliers at the high end of the range; one was a US CRO and the other European, although the latter is arguably the CRO with the most experience of performing the OECD 443. If the two outliers are excluded then the European average for the basic one-generation protocol is €350,000, which is 10% more than the European average for the OECD 416.

Two of the non-European CROs both responded to say that they have not estimated the prices because they have not started to perform the study yet. This suggests that they may be at a very early stage in the implementation of the test

Table 18. Answers to Q10

10) What is the price of a standard OECD 416 2-generation study in Euros and/or local currency at your laboratory?			
1. € 295,000 2. € 350,000 3. € 400,000 4. € 317,000 5. € 408,000 6. € 294,000 7. € 185,000 8. € 321,000 9. € 141,000 10. € 207,000 11. € 219,950 12. € 300,000 13. € 278,000	Minimum € 141,000	Maximum €408,000	Average € 285,842 European Average € 318,295 Non-European Average € 177,667

Eleven CROs provided their current price for the OECD 416 and a further two prices were obtained via another source; three of the thirteen were non-European (the one US CRO that provided prices for the OECD 443 did not provide a price for the OECD 416). The average price for European CROs was € 318,295.

For three non-European CROs (India and Brazil) the average price is €177,667, which is approximately 48% of the average of European CROs.

Table 19. Answers to Q11

11) Are additional costs (e.g. analytical charges) significantly different for the OECD 443 and OECD 416 in your laboratory? If so please give brief description of the differences.

Two of the non-European CROs both responded to say that they have not estimated the prices because they have not yet started to perform the study. None of the European CROs made any comment to this question, so we may assume that ancillary costs are no different for the OECD 443 as compared to the OECD 416.

Table 20. Answers to Q12

12) What technical challenges have you identified for the OECD 443 test guideline?

1. Logistics: numbers of subsets and animals in F1 generation. Decision point planning for F1B mating, 2. No technical challenges; just a lot of flexibility 3. In-house standardisation of some techniques and procurement of additional instruments 4. No challenges were found. The only part of the study that we might subcontract is the immunologic part, once we don´t perform it routinely. 5. Setup and organisation (logistics) of the study is a challenge esp. with the different modules. Quick evaluation of F1 data for the decision if a 2nd generation is required is another challenge. We have not run a study yet however we have run all the individual components eg neutotoxicity and immunotoxicity as standard. 6. We have the technical expertise to conduct all aspects of the OECD 443 guideline "Challenges have been as follows: Complex coordination, Positive control studies required before FOB; motor activity, Functional immunotoxicity assessment may not be optimized, Complex Neurohistopathology and interpretation, Trigger decision for additional generation" 7. Identification of developmental immunotoxicants in particular that would make relevant positive controls I question whether such chemicals exist for both DIT and DNT that would allow the test systems to be challenged; where true effects would be seen in the absence of effects such as reduction in litter size or litter weights 8. None

The technical challenges were clearly identified as being predominantly related to scheduling and the flexibility required for the resources available to the CRO. The main problem being that at the outset, because of the various triggers for the use of the additional cohorts, it is not clear how long it may last or how large the study may eventually become. The DIT cohort was also identified as being a problem for the CRO that has little or no experience with this endpoint. In addition, there is a perceived absence of positive control data for DIT and DNT effects.

Table 21. Answers to Q13

13) How many OECD 443 studies have been performed at your laboratory (include studies currently in progress)? If possible state the actual number in the 'other' box.	
0	10
1 - 5	1
6 - 10	0
10 - 20	0
> 20	0

Table 22. Answers to Q14

14) How many OECD 443 studies that have been performed at your laboratory have included the neurotox module (include studies currently in progress). If possible state the exact number in the 'other' box.	
0	10
1 -5	1
6 - 10	0
10 - 20	0
>20	0

Table 23. Answers to Q15

15) How many OECD 443 studies that have been performed at your laboratory have included the immunotoxicity module (include studies currently in progress). If possible state the exact number in the 'other' box.	
0	10
1 -5	1
6 - 10	0
10 - 20	0
>20	0

Table 24. Answers to Q16

16) How many OECD 443 studies that have been performed at your laboratory have included a second generation (include studies currently in progress)? If possible state the exact number in the 'other' box.	
0	11
1 -5	0
6 - 10	0
10 - 20	0
>20	0

Two of the CROs did not reply to questions 13 to 16 and all but one of the other CROs indicated that they have not yet performed an OECD 443 study in any of its permutations. Only one CRO indicated that it has performed an OECD 433 study but

another European CRO that did not respond to the survey is believed to have completed three studies, making a total of only four studies completed by European CROs.

Table 25. Answers to Q17

17) This question is for any additional comments that you wish to make regarding the OECD 443 test guideline and or your laboratories capability and capacity to perform the test.
1. We have world class infrastructure, state of the art laboratories, highly qualified and experienced personnel. With this background and the willingness of the management to allocate required resources , we are very confident that we will be able to carry out OECD 443 studies
2. We considered this guideline very similar with 2-generation study (OECD 416), but much more focused on animal care, avoiding the unnecessary usage of animals.
3. We are monitoring the requirement for this test through our enquiry reporting metrics and will maintain a watching brief
4. Our safety assessment facility has an excellent reputation in the field of reproduction toxicology based on our team's expert scientific and technical knowhow gained over many years with studies that assess fertility, developmental toxicity or littering. Study plans can be developed for all types of studies required based on, or adapted from, the corresponding recognized guidelines. Our team has unrivalled experience in the design and conduct of studies in our state-of-the-art, GLP-compliant and AAALAC accredited facilities using the routes of administration and animal models needed to support the reproductive components of safety assessment. Applicable studies are performed according to ICH, EPA, FDA and OECD guidelines.
5. Technically the study can be performed but I do not believe it to be a practical option to determine study design whilst the study is in progress. I also have concerns that all modules will be included where there is no significant reason to include them

Only five of the CROs (two non-European) chose to make additional comments, and clearly these CROs believe that they have the necessary resources and skills to perform the OECD 443 test guideline. This can be taken to support the view that the estimate of the worldwide capacity of the CRO industry for the OECD 443 may be considered to include the capacity of the majority of GLP-compliant laboratories outside of the EU and USA.

4.4 Main Survey – OECD 488

The main survey on the OECD 488 test guideline was sent as a link included in an email on the 2 April 2012 to the email contacts of the CROs that had indicated in the preliminary survey that they were willing to participate in the main survey. Several of the CROs were re-contacted directly by telephone or email in subsequent weeks to encourage them to complete the questionnaire. Nine CROs eventually completed the survey and three of those have experience with the test method, although not necessarily with the OECD 488 test guideline. The responses of the CROs that responded to the main survey are summarized in Tables 26 to 33. Question 1 was related to the identity of the CRO and the contact details of the respondent and is therefore excluded from the results.

Table 26. Answers to Q2

2) Does your laboratory currently offer the OECD 488 transgenic rodent assay?	
Yes	3
No	6

Of the nine laboratories that answered this question three currently offer the OECD 488 test guideline; two of the CROs are European and one is based in the USA. From the initial phase of the survey, where CROs were identified using internet searches, websites, etc., only four CROs worldwide were identified as offering transgenic assays on a contract basis. Two of these are in Europe and two in the USA. Direct contact with one of the USA laboratories has confirmed that that facility has FDA GLP compliance but not with the US EPA. Consequently, they do not officially comply with OECD GLP, which means that a REACH registrant would be unlikely to place a study with them. Therefore, there are only two CROs in Europe and one in the USA that currently offer this study type to the chemical industry.

Table 27. Answers to Q3

3) Whether or not your laboratory currently offers the OECD 488 study, please estimate the annual capacity for OECD 488 studies in your laboratory (all available facilities)? If there is an exact number please enter this in the 'other' box.	
1 - 5 per year	5
6 - 10 per year	0
> 10 per year	2

Of the three laboratories that already offer the OECD 488 two indicated that they have a capacity for 1 to 5 studies per year and one has a capacity greater than ten

studies per year. One of the CROs not currently offering the test answered that they will have the capacity to do greater than ten per year from approximately 2013/14. The remainder of the CROs estimated one to five studies per year. If we combine the capacities of the three CROs with current capability then it indicates a total capacity of approximately 12 to >20 studies per year.

If we project the current capacity into a forecast of potential future capacity based on the seven CROs that completed the survey and also anticipate offering the study in the future, then we have a range of 25 to 45 studies. However, two of these laboratories are non-European/non-USA and it is known that some countries have difficulties to import standard laboratory animals and will probably have great difficulty to import genetically modified animals. Even the UK has problems to import laboratory animals and there are only one or two airlines that continue to transport such animals into the UK. It is reasonable to conclude that the current situation is that the worldwide maximum capacity for OECD 488 studies, accessible to REACH registrants, is in the range of 12 to 20 per year, with an average of 15 studies per year. However, it should be remembered that much of this potential capacity may be taken up by the pharmaceutical and agrochemical industries.

It may further be proposed that if the market demand was to increase for this test that other European CROs may be able to implement the assay in their laboratory. However, it is also reasonable to assume that the period required to setup and validate this assay will not be short. This is because there are few people in the CRO industry with the relevant technical experience of the assay and the availability of the animal models is also restricted (see discussion after Table 32).

Table 28. Answers to Q4

4) What is the lead-time for the OECD 488 at your laboratory (assuming availability of test substance, etc.)?
1. Six to Eight weeks 2. I´m not able to estimate a lead-time for OECD 448, once we never performed such study before. 3. Approximately 6-8 weeks 4. We assume that we will be available to offer the study in 2013/2014 5. Not applicable 6. Whilst we don't currently offer OECD 488 we continually review assays with a view to validating and offering tests in house. For this, we have a Technology Transfer group. Where clients have specific requirements, we are always pleased to discuss them and collaborate in validations. 7. approx. 4 to 6 weeks 8. NA 9. 3 months

The lead time for the European CRO that has the most experience of this assay is 6 to 8 weeks, so it is assumed that this is an accurate estimate. But this will be dependent on the availability of animals, which may differ from one CRO to another depending on whether they use the Muta™Mouse model, one of the Big Blue® models or one of the other available models.

Table 29. Answers to Q5

5) What is the standard price in Euros and/or local currency for the OECD 488 at your laboratory? Please exclude analysis of formulation costs.

	Average price	Average European Price
Oral study with 1 tissue	€97,000*	€60,000
Oral study with 3 tissues	€125,000*	€125,000
Dermal study with 1 tissue	€100,000*	€65,000
Dermal study with 3 tissues	€140,000*	€140,000

* The prices for studies with 1 tissue are the average of three CROs but the most expensive, USA-based, CRO did not provide prices for studies with 3 tissues, so the true average for 3 tissues is much higher. The range for an oral study with one tissue is €40,000 to €172,000!

As far as is known, no CRO currently offers this study using the inhalation route of exposure and no prices were submitted for this dose route. This means that currently, gases and volatile substances cannot be routinely tested using the OECD 488 test guideline or substances where the most relevant route of exposure is the inhalation route, e.g. substances where workers are exposed to respirable powders. There is no technical reason why the inhalation route cannot be used but a relevant positive control and validation study would be required before a GLP study could be done.

The European average price of this study for a single tissue, at €60,000, is greater than any of the standard in vivo genetic toxicology studies such as the micronucleus test or UDS assay in the liver, which in Europe may cost in the region of €12,000 and €21,000 respectively. The price of the COMET assay, where there is no OECD guideline so far, is approximately €28,000 for up to three tissues. The prices of the standard tests given above are based on the experience of the author and are for guidance only. However, it can be seen that the price of the OECD 488 for a single tissue is approximately 3 to 4 times the price of a UDS assay, which is the standard method used to follow up on in vitro positive substances with a gene mutation mechanism of action.

Table 30. Answers to Q6

6) Are additional costs (e.g. analytical charges) significantly different for the OECD 488 and other in vivo mutation assays in your laboratory? If so please give a brief description of the differences.
1. We have not yet estimated the cost since the OECD 488 study has not started 2. We are not able to estimate a cost for that study right know, once we never performed that study before. Maybe in the near future. 3. Due to 28 day administration, dosing/treatment charges may be higher than for other in vivo studies, but costs for dose formulation analysis, tk analysis etc. would only be elevated if the number of samples and/or time-points correspondingly increases from other in vivo assays.

The sub-acute dosing regimen of this study necessarily adds extra cost to this study type; however, this is not additional to the figures given in Table 29. Therefore, in principle there are no additional costs above and beyond those quoted for the standard test method.

Table 31. Answers to Q7

7) How many OECD 488 studies have been performed at your laboratory (include studies currently in progress)? If there is an actual number available please enter it in the 'other' box.	
0	6
1 - 5	2
6 - 10	0
10 - 20	1*
> 20	0
Other: *Please note, we have conducted many (~15) transgenic rodent mutation assays, but as OECD 488 is a relatively recently issued guideline, only a few recent/current studies actually claim OECD 488 compliance	

In Europe there are two CROs that offer the OECD 488 transgenic assay and one of them has completed around 15 studies in total, and 1 to 5 have been performed by the other CRO. The US CRO offering these studies to OECD GLP has completed 1 to 5 non-GLP studies and will start their first GLP OECD 488 in the near future. Therefore we can conclude that a total of 16 to 20 studies have been performed in Europe over the previous 20 years, i.e. a maximum of one to two per year. In the USA the number is less than six if we exclude the CRO that works to FDA GLP standards only. The CRO that has completed the most studies has indicated that the

most they have done in one year is three studies and there are often years when no studies are performed.

Table 32. Answers to Q8

8) What technical challenges have you identified for the OECD 488 test guideline?
1. In-house standardisation of some techniques and procurement of additional instruments.
2. We have found some challenges, starting from a specific animal purchasing to molecular assays.
3. DNA extraction for certain cell types, Identification of appropriate positive controls for certain tissue types, possible technical constraints with vehicles for dermal studies

As mentioned previously, the procurement of animals for use in these transgenic assays in not straightforward. The Muta™Mouse system, as developed and used by one of the EU CROs, is available commercially but the stocks of animals are maintained at low levels, so the lead time following an order for animals can be extensive (information provided directly by the CRO). In addition, studies for that CRO take precedence for the available animals. This means that any new entrants into the market may choose to use the Agilent Big Blue® mouse or rat. Information provided by Agilent indicated that these animals are maintained in the USA and the lead time for delivery to Europe may be expected to be about one month plus the age of the animals as required. However, the UK and most other countries require an import licence to be obtained before the animals can be shipped and it is not clear how long this process may take. CROs in the UK may have a particular problem with the import of animals from the USA because currently there are only one or two airlines that continue to offer this service to the UK.

Alternative models include the gpt Delta rat and mouse, and the lac Z plasmid mouse. From internet searches it seems that these are maintained only as frozen embryos, (although it may be possible to obtain live animals from an academic institution that has an active colony). In this case, the suppliers of frozen embryos provide a small number of breeding animals once they have been recovered from frozen, it is then up to the purchaser to breed the requisite number of animals for the study. However, most CROs do not keep their own colonies of animal models and prefer to purchase them from specialist breeders (Charles River, Harlan, etc.). The necessary knowledge and skills needed to maintain a colony of animals, particularly a strain that has been transgenically modified, may not be available and may deter CROs from utilizing such models. I was informed by one CRO that they have purchased young adult lacZ plasmid mice from Charles River in the past but it has not been able to confirm whether Charles River continue to offer this model (it is the sort of information that such companies do not disclose easily).

5. Conclusions

5.1 OECD 443, Extended one-generation reproductive toxicity test

For the OECD 443 test guideline there is the capability and capacity within the CRO industry worldwide to conduct a significant number of these studies. Furthermore, there is the potential for many more CROs to extend their current expertise in reproductive toxicology to include the OECD 443 in their service portfolio. This may be acceptable for the basic protocol, and for the basic protocol with the second generation. However, for the developmental neurotoxicity (DNT) and developmental immunotoxicity (DIT) cohorts there are concerns within the industry about the lack of expertise. This includes the performance and interpretation of neurotoxicity tests, the pathological examination for neurotoxicity and the performance and interpretation of immunoassays. Furthermore, the lack of availability of positive control substances for the validation of the DNT and DIT cohorts is seen as a significant technical barrier. In terms of cost, the OECD 443 test guideline is more expensive (~50% greater) than the OECD 416 it is designed to replace, even when used in its most basic format.

5.2 OECD 488, Transgenic rodent somatic and germ cell mutation assay

For the OECD 488 test guideline the capability and capacity is limited and there is little prospect of the situation changing in the short to medium term. The technical resources required to establish and validate this assay are considerable, and the transgenic animal models are currently in short supply (small colonies and/or maintained as frozen embryos) and perhaps difficult to import. The cost needed to establish, validate, and generate historical data for this assay may act as a further deterrent to CROs to add this test to their portfolio. Therefore, any REACH registrant that is required to do this study will be limited to a choice of two or three CROs and may be competing with pharmaceutical and plant protection product customers for the limited capacity of these laboratories. The cost of an OECD 488 study is greater than any of the current standard in vivo tests, perhaps five times the cost depending on the scope of the protocol. Furthermore, no CRO currently offers this test using the inhalation route so, at the moment, gases, volatiles and respirable dusts cannot be tested. It is not known why the inhalation route is not currently on offer but it may be assumed to be because of several factors, including the cost of validation or the absence or non-availability of a suitable positive control. In the principle there should be no technical barrier to performing this assay using the inhalation route but the cost may be expected to be significant.

Appendix 1: Questionnaires Used in the Survey

ECHA Preliminary Survey on OECD 443 and 488

This survey is being performed by CEHTRA UK Ltd on behalf of the European Chemicals Agency (ECHA). The purpose of the survey is to provide information to ECHA on the capability and capacity of the CRO industry worldwide to perform the OECD 443 extended one-generation reproductive and developmental toxicity study and also the OECD 488 transgenic rodent assay.

The identity of all respondees will remain confidential and **not** be provided to ECHA or released in any future publications. All data generated in the survey will be coded according to the responding CRO and the decode will **not** be revealed by CEHTRA UK to any other party. All respondees will be provided with a summary of the data obtained together with their own laboratory decode information.

This is a **preliminary survey** designed to identify those CROs that currently offer, or that plan to offer in the near future, either the OECD 443 or OECD 488. Those laboratories that indicate that they currently offer, or plan to offer one or both of these test guidelines will be invited to participate in second more detailed survey.

1) Please provide the contact details of the respondent so that I can contact you again for further information.	
Name	
Company	
Email address	
Contact telephone number	

2) Does your laboratory currently offer the OECD 443 Extended one-generation reproductive/developmental toxicity test?	
Yes	
No	

3) If you answered NO to question 1 then do you plan to offer the OECD 443 Extended one-generation reproductive/developmental toxicity test in the future?	
Yes	
No	

4) If you answered No to both questions 2 and 3 then please provide the reasons why, tick all that apply. If you answered yes to either question 2 or 3 then please skip this question.	
We do not have the necessary equipment	
We do not have the necessary experience	
We do not believe there is sufficient market demand	
The test is too expensive compared to standard methods	
Other	

5) If you answered Yes to either question 2 or 3 then will you be prepared to complete a second survey designed to collate more detailed information. Note that the identity of respondents will not be revealed to ECHA or any third party.	
Yes	
No	

6) Does your laboratory currently offer the OECD 488 transgenic rodent assay (TGRT)?	
Yes	
No	

7) If you answered NO to question 6 then do you plan to offer the OECD 488 TGRT test in the

future?	
Yes	
No	

8) If you answered No to both questions 6 and 7 then please provide the reasons why, tick all that apply. If you answered yes to question 6 or 7 then please skip this question.	
We do not have the necessary equipment	
We do not have the necessary experience	
We do not believe there is sufficient market demand	
The test is too expensive compared to standard methods	
Other:	

9) If you answered Yes to either question 6 or 7 then will you be prepared to complete a second survey designed to collate more detailed information. Note that the identity of all respondents will not be revealed to ECHA or any third party.	
Yes	
No	

ECHA Follow-up Survey on OECD 443

This survey is being performed by CEHTRA UK Ltd on behalf of the European Chemicals Agency (ECHA). The purpose of the survey is to provide information to ECHA on the capability and capacity of the CRO industry worldwide to perform the OECD 443 extended one-generation reproductive and developmental toxicity study and also the OECD 443 transgenic rodent assay.

The identity of all respondees (both contact persons and CRO names) will remain confidential and not be provided to ECHA or released in any future publications without permission. All data generated in the survey will be coded according to the responding CRO and the decode will not be revealed by CEHTRA UK to any other party. All respondees will be provided with a summary of the data obtained together with their own laboratory decode information.

This survey has been sent to you because you completed a preliminary survey and indicated that you may be prepared to complete a second survey designed to collate more detailed information on your capability to perform the OECD 443 test guideline.

1) Please provide the contact details of the respondent so that I can contact you again for further information.	
Contact name	
Contact email	
Name of laboratory	
Address of laboratory	
Contact telephone number	

2) Does your laboratory currently offer the OECD 443 extended one generation reproductive toxicity test	
Yes	
No	

3) If you answered no to the previous question then when does your laboratory plan to begin offering the OECD 443 study?	
2012	
2013	
>2013	

4) Whether or not your laboratory currently offers the OECD 443 guideline study please estimate the annual capacity for OECD 443 studies in your laboratory (all available facilities). If there is an exact number please enter this in the 'other' box.	
1 - 5 per year	
6 - 10 per year	
> 10 per year	
Other (Please Specify):	

5) What is the combined annual capacity for OECD 443 and OECD 416 (2-gen) studies in your laboratory (all available facilities)? If there is an exact number please enter in the 'other' box.	
1 - 5 per year	
6 - 10 per year	
> 10 per year	
Other (Please Specify):	

6) What is the impact of the OECD 443 on the overall capacity of your laboratory to perform reproductive toxicology studies? Do you anticipate an increase or a decrease in capacity following an increased throughput of OECD 443 studies? Free text response required.

7) What is the lead-time for the OECD 443 at your laboratory (assuming availability of test substance, etc)?

8) What is the lead-time for the OECD 416 (2-gen) at your laboratory (assuming availability of test substance, etc)?

9) What is the standard price in Euros and/or local currency for the OECD 443 at your laboratory? Please exclude analysis of formulations costs.	
Basic study	
Basic study with optional second generation	
Basic study with neurotox (DNT) module	
Basic study with immunotox (DIT) module	
Basic study with both modules	
Basic study with two generations and with both modules	

10) What is the price of a standard OECD 416 2-generation study in Euros and/or local currency at your laboratory?

11) Are additional costs (e.g. analytical charges) significantly different for the OECD 443 and OECD 416 in your laboratory? If so please give brief description of the differences.

12) What technical challenges have you identified for the OECD 443 test guideline?

13) How many OECD 443 studies have been performed at your laboratory (include studies currently in progress)? If possible state the actual number in the 'other' box.	
0	
1 - 5	
6 - 10	
10 - 20	
> 20	
Other (Please Specify):	

14) How many OECD 443 studies that have been performed at your laboratory have included the neurotox module (include studies currently in progress). If possible state the exact number in the 'other' box.	
0	
1 -5	
6 - 10	
10 - 20	
>20	
Other (Please Specify):	

15) How many OECD 443 studies that have been performed at your laboratory have included

the immunotoxicity module (include studies currently in progress). If possible state the exact number in the 'other' box.	
0	
1 -5	
6 - 10	
10 - 20	
>20	
Other (Please Specify):	

16) How many OECD 443 studies that have been performed at your laboratory have included a second generation (include studies currently in progress)? If possible state the exact number in the 'other' box.	
0	
1 -5	
6 - 10	
10 - 20	
>20	
Other (Please Specify):	

17) This question is for any additional comments that you wish to make regarding the OECD 443 test guideline and or your laboratories capability and capacity to perform the test.

ECHA Follow-up Survey on OECD 488

This survey is being conducted by CEHTRA UK Ltd on behalf of the European Chemicals Agency (ECHA). The purpose of the survey is to provide information to ECHA on the capability and capacity of the CRO industry worldwide to perform the OECD 488 transgenic rodent assay.

The identity of all respondees will remain confidential and not be provided to ECHA or released in any future publications without their permission. All data generated in the survey will be coded according to the responding CRO and the decode will not be revealed by CEHTRA UK to any other party. All respondees will be provided with a summary of the data obtained together with their own laboratory decode information.

This survey has been sent to you because you completed a preliminary survey and indicated that you may be prepared to complete a second survey designed to collate more detailed information on your laboratories capability to preform the OECD 488 guideline.

1)	
Name of contact	
Email of contact	
Name of Laboratory	
Address of Laboratory	
Telephone number of contact	

2) Does your laboratory currently offer the OECD 488 transgenic rodent assay?	
Yes	
No	

3) Whether or not your laboratory currently offers the OECD 488 study, please estimate the annual capacity for OECD 488 studies in your laboratory (all available facilities)? If there is an

exact number please enter this in the 'other' box.

1 - 5 per year	
6 - 10 per year	
> 10 per year	
Other:	

4) What is the lead-time for the OECD 488 at your laboratory (assuming availability of test substance, etc)?

5) What is the standard price in Euros and/or local currency for the OECD 488 at your laboratory? Please exclude analysis of formulation costs.	
Oral study with 1 tissue	
Oral study with 3 tissues	
Dermal study with 1 tissue	
Dermal study with 3 tissues	
Inhalation studywith 1 tissue	
Inhalation study with 3 tissues	

6) Are additional costs (e.g. analytical charges) significantly different for the OECD 488 and other in vivo mutation assays in your laboratory? If so please give a brief description of the differences.

7) How many OECD 488 studies have been performed at your laboratory (include studies currently in progress)? If there is an actual number available please enter it in the 'other' box.	
0	
1 - 5	
6 - 10	
10 - 20	
> 20	
Other:	

8) What technical challenges have you identified for the OECD 488 test guideline?