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Chantra Eskes
Maurice Whelan *Editors*

Validation of Alternative Methods for Toxicity Testing

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Validation of Alternative Methods for Toxicity Testing

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Foreword

Why do we need to validate alternative test methods?

The validation of alternative methods ultimately serves the decision-making process towards the safe use of chemicals. Whether they are based on *in vitro* tests, computer models or combinations of both, validated methods can be used to determine the properties of chemicals used in all sorts of products and processes, including pharmaceuticals, cosmetics, household products, food and industrial manufacturing.

Hazard property information influences risk management decisions at numerous stages of the life cycle of a chemical. For example, during the research and development stage of a new chemical, industry uses non-test methods such as (quantitative) structure–activity relationships to predict its hazards and estimate the risks involved with its use to decide whether the chemical should move towards production. Industry and authorities use results from laboratory tests and non-test methods to classify and label chemicals, which in turn, can trigger specific risk management measures, such as the use of personal protective equipment by workers handling those chemicals or even marketing restrictions to protect consumers and the environment.

These kinds of risk management decisions have to be taken for all the many thousands of chemicals on the market in so many different sectors, even if only one result is available for each relevant hazard endpoint. It is therefore important that authorities, industry and the public at large, have the assurance that the results of the methods used are reliable and relevant. Furthermore, only on these grounds can the data generated be exchanged and accepted across countries for regulatory purposes. This is why demonstration of relevance and reliability are the requirements for the validation and regulatory use of OECD Test Guidelines. Also, both the Test Guidelines (developed following validation studies) and their accompanying guidance documents, generally provide sufficient details to allow all studies to be replicated in any state-of-the-art laboratory.

Research laboratories are continuously developing new methods that better characterise the hazardous properties of chemicals (e.g., for new effects such as

endocrine disruption) or alternative methods that do not use laboratory animals (e.g., *in vitro* methods or toxicogenomics). But decision-makers often do not feel confident to use the results from these methods for risk-reduction decisions before they have been demonstrated to be scientifically valid. Furthermore, many non-animal testing-based methods do not sufficiently establish the link with the predicted adverse outcome in humans or wildlife.

But regulatory toxicology is changing. Toxicologists are now seeking to understand the mode of action of chemicals or the adverse outcome pathway that they trigger, i.e., how they interact at a molecular level resulting in effects at the organ or organism level. With increasing knowledge about the modes of action or the adverse outcome pathways that chemicals can trigger, decision-makers are more comfortable using results from alternative methods if it can be shown that the results are linked to key events along the chain of events that constitute the adverse outcome pathway.

This also means that, ultimately, individual animal test methods will be replaced by a number of *in chemico*, *in vitro* and/or *in silico* methods that collectively allow the gathering of information needed to characterise the hazardous property of a chemical. In parallel, as alternative methods become more sophisticated, they will better predict adverse effects in a specific species of interest—e.g., humans.

While this new approach to safety testing will challenge the current approach taken to standardise and validate test methods for regulatory purposes, the objectives of validation will remain the same. The novel test methods used to identify the modes of action will need to be validated in the sense that their reliability and relevance will need to be demonstrated when used to make regulatory decisions. Validation of alternative test methods will therefore remain one of the cornerstones of a successful toxicological (r)evolution.

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Preface

This book provides a comprehensive overview of the best practices and new perspectives regarding the validation of alternative methods for animal procedures used in toxicity testing. Alternative methods cover a wide range of non-animal techniques and technologies, including: *in vitro* assays based on various biological tests and measurement systems; chemoinformatics approaches; computational modelling; and different ways of weighting and integrating information to make predictions of a toxicological effect or endpoint. Validation of an alternative method or approach aims not only to establish the reproducibility and robustness of an alternative method but also to determine its capacity to correctly predict effects of concern in a species of interest. This latter aspect is one of the most critical considerations when striving to replace or reduce animal testing and promoting new approaches in toxicology that are more relevant for human hazard assessment. This book covers the validation of experimental and computational methods and integrated approaches to testing and assessment. Furthermore, validation strategies are discussed for methods employing the latest technologies such as tissue-on-a-chip systems, induced human pluripotent stem cells, bioreactors, transcriptomics and methods derived from pathway-based concepts in toxicology.

Validation of Alternative Methods for Toxicity Testing provides practical insights into state-of-the-art approaches that have resulted in successfully validated and accepted alternative methods. In addition, it explores the evolution of validation principles and practices that will ensure that validation continues to be fit for purpose and has the greatest international impact and reach. Indeed, validation needs to keep pace with the considerable scientific advancements being made in biology and toxicology, the availability of increasingly sophisticated tools and techniques, and the growing societal and regulatory demands for better protection of human health and the environment.

This book is a unique resource for scientists and practitioners working in the field of applied toxicology and safety assessment who are interested in the

development and application of new relevant and reliable non-animal approaches for toxicity testing and in understanding the principles and practicalities of validation as critical steps in promoting their regulatory acceptance and use.

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Acknowledgments

The quest for the development and implementation of alternative methods to animal testing really took hold in the 1980s, driven by both heightened ethical concerns surrounding animal testing and the scientific advances being made in the *in vitro* field. Since then, additional motivation has emerged including an increasing emphasis on the need for more human-based and scientifically relevant models for use in basic biomedical research and safety assessment. However, only through the development and implementation of validation principles, establishing the relevance and reliability of new methods for specific applications, have the regulatory acceptance and use of alternative methods been possible. The editors of this book would like to acknowledge the huge contribution and sustained commitment of so many pioneers, too numerous to mention here, who have progressed the field to the point where we can now truly believe in better safety assessment without the use of animals.

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Chapter 1
Introduction

Chantra Eskes and Maurice Whelan

Abstract Alternative approaches to animal testing are gaining momentum with an increasing number of test methods obtaining international acceptance, thanks in large part to the validation efforts conducted on these assays. The principles and process of validation were first established in the 1990s in Europe and USA, and further gained international recognition ensuring the broader acceptance of alternative test methods at a regulatory level. If these principles were successful in pioneering the regulatory acceptance of alternative methods for less complex endpoints, an evolution of concepts is needed to embrace emerging technologies and the increased complexity of endpoints. Innovative concepts and approaches of scientific validation can help to ensure the continued regulatory and international acceptance of novel alternative methods and technologies for toxicity testing such as human-based *in vitro* models derived from induced pluripotent stem cells and significant advances in bioengineering. This chapter provides a historical overview of the establishment and evolution of the principles of the scientific validation of alternative methods for toxicity testing as well as the challenges and opportunities for adapting those principles to keep pace with scientific progress whilst ensuring human safety and best serve the needs of society.

1 The Need for Validation

Alternative methods refer to procedures that can replace the need for animal experiments, reduce the number of animals required, or diminish the amount of distress or pain experienced by animals (Smyth 1978). This definition embodies the "Three Rs" concept proposed by Russell and Burch in The Principles of Humane Experimental Technique (Russell and Burch 1959), which was considered by many

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countries in defining regulatory requirements concerning the protection of animals used for scientific purposes (Council Directive 86/609/EEC 1986; Directive 2010/63/EU 2010; Brazil 2008).

During the last quarter of the twentieth century, public concern over ethical aspects regarding the use of animals for scientific purposes has steadily increased, especially in the USA and in Europe. Humane societies have questioned in particular the need for animals in product-safety testing, medical research and science education (Wilhelmus 2001). For example, eye irritation testing procedures on rabbits has often been used as a symbol for cruelty by animal welfare activists, since at times such procedures can be very painful and result in visible suffering, trauma and reactions in the rabbit eyes. In April 1980, a group of animal welfare activists specifically targeted the rabbit eye test by publishing a full-page advertisement in the New York Times stating "*How many rabbits does Revlon blind for beauty's sake?"*, followed by a second advertisement published in October 1980. Such campaigns resulted in grant investments to support the development of alternatives to the rabbit eye test (Wilhelmus 2001).

In order to ensure the acceptance of the developed alternatives to animal testing, regulatory action was also taken. In Europe for example, the original Directive on the protection of laboratory animals for experimental and other scientific purposes stated that *"An* (animal) *experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available*" (Directive 86/609/EEC).

The final acceptance of an alternative test method may depend on various factors such as national regulatory requirements, the test method purposes, uses and applicability. However, demonstrating the scientific validity of an *in vitro* method is usually required for its use within the regulatory framework especially for detecting both hazardous and non-hazardous effects as a replacement, reduction or refinement of animal testing (OECD Guidance Document No. 34 2005; Regulation (EC) No 1907/2006). As such, for an alternative method to gain regulatory acceptance, it is current practice to demonstrate that the method is scientifically satisfactory, i.e., valid, for the purpose sought. This is generally carried out through a validation process through which the scientific validity of a test method can be demonstrated.

2 Historical Developments

The criteria and processes for the validation of a test method were first developed in the 1990s. In Europe, the European Centre for the Validation of Alternative Methods (ECVAM) was created in 1991 as part of the European Commission's Joint Research Centre (JRC), to respond to the requirement from the original EU Directive on the protection of animals for scientific purposes, namely that "*The Commission and Member States should encourage research into the development and validation of alternative techniques (…) and shall take such other steps as they consider appropriate to encourage research in this field"* (Directive 86/609/EEC). This was followed in the United States by the creation in 1997 of the Interagency Coordinating

Committee on the Validation of Alternative Methods (ICCVAM), and subsequently in Japan in 2005 with the establishment of the Japanese Center for the Validation of Alternative Methods (JaCVAM). Reflecting the growing awareness of the importance of validation worldwide, internationally agreed principles of validation were adopted by the Organization for Economic Co-operation and Development (OECD) in 2005 (OECD Guidance Document No. 34 2005). More recently, the implementation of the EU Directive 2010/63 on the protection of animals used for scientific purposes (Directive 2010/63/EU 2010), which came into full force in 2013, has reinforced Europe's commitment to place the 3Rs at the heart of EU policy and to strengthen legislative provision to minimize the reliance on animal procedures in different contexts whenever possible. Moreover, outreaching countries have since also established national centers for the validation of alternative methods such as the South Korean Center for the Validation of Alternative Methods (KoCVAM) established in 2010 and the Brazilian Centre for the Validation of Alternative Methods (BraCVAM) established in 2011 (see Chap. 14).

Based upon the experiences gained during earlier multi-laboratory evaluation studies on e.g. eye irritation, and in consultation with various international experts, ECVAM published under the enriching leadership of Michael Balls, recommendations on the principles, practical and logistical aspects of validating alternative test methods (Balls et al. 1990, 1995; Curren et al. 1995). These documents represent the first basic principles for the validation of alternative methods including the management and design of a validation study that were later integrated at an international level (OECD Guidance Document No. 34 2005).

An alternative method for the replacement (or partial replacement) of an animal test is defined as the combination of a "test system", which provides a means of generating physicochemical or *in vitro* data for the chemicals of interest, and a "prediction model (PM)" or "data interpretation procedure" (Archer et al. 1997). The prediction model or data interpretation procedure plays an important role in the acceptance process, as it allows converting the obtained data (e.g., *in vitro* or physicochemical) into predictions of toxicological endpoints in the species of interest e.g., animals or humans (OECD Guidance Document No. 34 2005).

Test method validation is defined as the process whereby the relevance and reliability of the method are characterized for a particular purpose (OECD Guidance Document No. 34 2005; Balls et al. 1990). In the context of a replacement test method, relevance refers to the scientific basis of the test system and to the predictive capacity of the test method as compared to a reference method. Reliability refers to the reproducibility of test results, both within and between laboratories, and over time. The "purpose" of an alternative method refers to its intended application, such as the regulatory testing of chemicals for a specific toxicological endpoint (e.g., eye irritation). Adequate validation (i.e., to establish scientific validity) of an alternative test requires demonstration that, for its stated purpose:

- the test system has a sound scientific basis;
- the predictions made are sufficiently accurate; and
- the results generated by the test system are sufficiently reproducible within and between laboratories, and over time.

Furthermore, some of the key principles of the validation process encompass (Balls et al. 1990):

- An alternative method can only be judged valid if the method is reliable and relevant;
- The prediction model should be defined in advance by the test developer;
- The aspired performance criteria should be set in advance by the management team (for a prospective validation study);
- Performance is assessed by using coded chemicals;
- There should be independence in:
  - the management of the study,
  - the selection, coding and distribution of test chemicals,
  - the data collection and statistical analysis;
- Laboratory procedures should comply with GLP criteria.

In addition, a prevalidation scheme has been recommended to ensure that a method included in a formal validation study adequately fulfils the criteria defined for inclusion in such a study, so that financial and human resources are used most efficiently with a greater likelihood that the expectations will be met. The prevalidation process includes three main phases: protocol refinement, protocol transfer and protocol performance (Curren et al. 1995).

In 2004, a "Modular Approach to the ECVAM Principles on Test Validity" was proposed with the objective to make the validation process more flexible by breaking down the various steps of validation into seven independent modules, and defining for each module the information needed for assessing the scientific validity of a test method (Hartung et al. 2004). One of the main advantages of the Modular Approach to Validation is the possibility to complete the different modules in any sequence, allowing the use of data both gathered retrospectively and generated prospectively as required. This approach has the potential to increase the evidence gathered on a specific test method whilst decreasing the time necessary if only prospective data were to be considered. The seven modules are:

1. Test definition;
2. Within-laboratory reproducibility;
3. Transferability;
4. Between-laboratory reproducibility;
5. Predictive capacity;
6. Applicability domain; and
7. Definition of performance standards.

A consequence of the replacement in 2010 of Directive 86/609/EEC with Directive 2010/63/EU was the formalization and broadening of the role of ECVAM, reflected in its name being changed by the JRC to the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM, see also http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam). EURL ECVAM's duties and tasks (Article 48/Annex VII of Directive 2010/63) now encompass the coordination and promotion of

the development, validation and use of alternative methods; acting as a focal point for the exchange of information; setting up, maintaining and managing public databases and information systems on alternative methods; and promoting dialogue between legislators, regulators, and all relevant stakeholders with a view to the development, validation, regulatory acceptance, international recognition, and application of alternative approaches.

Regarding the USA, the NIH Revitalization Act of 1993 (Public Law 103-43) required the National Institute of Environmental Health Sciences (NIEHS) to establish criteria for the validation and regulatory acceptance of alternative toxicological testing methods, and that NIEHS recommend a process to achieve the regulatory acceptance of scientifically valid alternative test methods. To respond to requirements of this Act, NIHS created ICCVAM initially as an *ad hoc* committee in 1994, and subsequently as a standing committee in 1997 (see also http://www.iccvam.niehs.nih.gov) with the aim to (i) implement a process by which new test methods of interest could be evaluated and (ii) coordinate interactions among US agencies related to the development, validation, acceptance, and national and international harmonization of toxicological test methods. ICCVAM was then formally established as a permanent interagency committee of the NIEHS under the National Toxicology program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) in 2000 by the ICCVAM Authorization Act Public Law 106-545.

Criteria for validation and regulatory acceptance of alternative test methods were published in 1997 by ICCVAM-NIEHS (Validation and Regulatory Acceptance of Toxicological Test Methods 1997). The definition and principles of scientific validity are similar to those adopted in the European Union, although a specific format of data compilation is required including for example: test method protocol components, intra- and inter- laboratory reproducibility, test method accuracy, protocol transferability, information on the selection of reference substances, information on the reference species, supporting data and quality, animal welfare considerations and practical considerations.

The Japanese Center for the Validation of Alternative Methods (JaCVAM, see also http://jacvam.jp/en) was established in 2005 as part of the Biological Safety Research Center (BSRC) of the National Institute of Health Sciences (NIHS). Its key objectives are to ensure that new or revised test methods are validated, peer reviewed, and officially accepted by regulatory agencies (Kojima 2007). For this purpose, JaCVAM assesses the utility, limitations, and suitability for use of alternative test methods in regulatory studies for determining the safety of chemicals and other materials. JaCVAM also performs validation studies when necessary. Furthermore, JaCVAM establishes guidelines for new alternative experimental methods through international collaboration.

As validation is an important step within the regulatory acceptance of alternative methods, international efforts have been undertaken to favor the harmonization of its processes and principles with the ultimate goal of promoting harmonization of international acceptance and recognition of alternative methods. In particular, through a process of consultation with validation bodies and key

stakeholders, the OECD adopted internationally agreed validation principles and criteria for the regulatory acceptance of alternative test methods. Such internationally agreed principles are described in the OECD Guidance Document No. 34 on *"The Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment*" (OECD Guidance Document No. 34 2005). The OECD GD 34 details internationally agreed principles and criteria on how validation studies of new or updated test methods should be performed. It represents a document of key importance for promoting harmonized approaches and procedures for the validation and regulatory acceptance of alternative methods at the international level (see also Chap. 2).

3 Current Challenges and Opportunities

If the validation principles and processes established in the 1990s were successful in achieving international acceptance of a number of alternative test methods, the scientific advances made in the recent years in the area of *in vitro* toxicology call for an evolution of the traditional validation principles. Indeed, considerable progress was dictated by new technologies and discoveries, as well as by the increasing complexity of the endpoints assessed. For instance, the 2012 Nobel Prize Shinya Yamanaka opened the door for the reprogramming of mature cells to become pluripotent, the so-called induced pluripotent stem cells, which allow the use of human-based cells reprogrammed in any organ-type cell for the evaluation of toxicity. Furthermore, a number of scientific groups have developed new complex bioengineering technologies such as the human-on-a-chip models which allow combining various organ-specific cell types and obtaining a more holistic response to toxicants whilst providing a more complex model mimicking the *in vivo* toxicity. In the US, the use of high-throughput *in vitro* screening assays, systems biology and predictive *in silico* approaches have also been recently used within the twenty-first century NTP program to improve the hazard evaluation of environmental chemicals. Furthermore, the evaluation of more complex endpoints require not only complex models but also their integration into e.g., integrated approaches for testing and assessment as well as consideration of the mechanistic adverse-outcome pathways of toxicity, that call for new considerations regarding the approaches for the scientific validation of alternatives to toxicity testing. Finally, collaboration of the validation centers in the various geographical regions is critical to ensure the harmonized international acceptance of alternative methods, the removal of barriers and the promotion of harmonized human safety assessment across the globe.

This book provides two distinct yet complementary perspectives on the approaches used for the scientific validation of alternative methods. The first is more retrospective and describes the state-of-the-art in validation including the underlying principles and practical approaches that have been successful over the years in gaining international regulatory acceptance of alternative methods. The second, more forward-looking perspective addresses the need to foster innovation

and ensure progressive evolution of validation concepts and practices that are fit for the purpose of aiding the translation of emerging technologies and sophisticated methodologies in the field of alternative methods into internationally accepted solutions for regulatory toxicity testing.

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Chapter 2
Validation in Support of Internationally Harmonised OECD Test Guidelines for Assessing the Safety of Chemicals

Anne Gourmelon and Nathalie Delrue

Abstract Ten years elapsed since the OECD published the Guidance document on the validation and international regulatory acceptance of test methods for hazard assessment. Much experience has been gained since then in validation centres, in countries and at the OECD on a variety of test methods that were subjected to validation studies. This chapter reviews validation principles and highlights common features that appear to be important for further regulatory acceptance across studies. Existing OECD-agreed validation principles will most likely generally remain relevant and applicable to address challenges associated with the validation of future test methods. Some adaptations may be needed to take into account the level of technique introduced in test systems, but demonstration of relevance and reliability will continue to play a central role as pre-requisite for the regulatory acceptance. Demonstration of relevance will become more challenging for test methods that form part of a set of predictive tools and methods, and that do not stand alone. OECD is keen on ensuring that while these concepts evolve, countries can continue to rely on valid methods and harmonised approaches for an efficient testing and assessment of chemicals.

Keywords OECD validation principles • Test Guidelines • Integrated approaches • Mutual acceptance

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1 Introduction to the OECD Test Guidelines Programme

1.1 Context and Goal

Since 1981, OECD countries have tasked the Environment, Health and Safety Programme to develop harmonized methods for the testing of chemicals. The methods are intended to generate valid and high quality data to support chemical safety regulations in member countries. The OECD Guidelines for the testing of chemicals are a collection of the most relevant internationally agreed testing methods used by governments, industry and independent laboratories to assess the safety of chemical products. OECD Test Guidelines are covered by the OECD Council Decision on the Mutual Acceptance of Data (MAD) stating that test data generated in any member country—or partner country adhering to MAD—in accordance with OECD Test Guidelines and Principles of Good Laboratory Practice (GLP) shall be accepted in other member countries and adhering partner countries for assessment purposes and other uses relating to the protection of human health and the environment (OECD 1981). This Decision minimises the costs associated with testing chemicals by avoiding duplicative testing, and utilises more effectively scarce test facilities and specialist manpower in countries. Having harmonised Test Guidelines also avoids non-tariff barriers to international trade of chemicals through a level playing of environmental protection across countries.

Started in 1981, the collection of OECD Test Guidelines is augmented every year with new and updated Test Guidelines that have undergone a number of stages to demonstrate their validity in order to be accepted by regulatory authorities. The motivations for continuously improving testing standards at OECD level are keeping the pace with progress in science, responding to countries' regulatory needs, addressing animal welfare and improving cost-effectiveness of test methods. At various stages of Test Guidelines development, OECD-wide networks of scientists in government, academia, and industry provide input. The OECD Test Guidelines Programme is also fed by the work of validation centres established in certain countries or regions which establish and/or review the scientific validity of test methods proposed for the development of Test Guidelines. It is indeed essential that test methods undergo a critical appraisal of their relevance and reliability through experimental demonstration in laboratories who are potential future users, so that the utility of the method for a specific purpose, as well as its limitations, can be defined and understood by users and regulators. The use of Test Guidelines that are based on validated test methods promotes the generation of dependable data for human and animal health and environmental safety. In 2005, the OECD published a Guidance Document for test method validation outlining general principles, important considerations, illustrative examples, potential challenges and the results of experience gained (OECD 2005).

1.2 Participation (WNT, Nominated Experts, Industry Experts, Animal Welfare Organisations)

The development of OECD Test Guidelines is overseen by the Working Group of the National Coordinators of the Test Guidelines Programme (WNT). National Coordinators represent regulatory authorities in OECD member countries and countries adhering to MAD. Representatives from identified interest groups (industry and animal welfare non-governmental organisations, green NGOs) and from some additional countries having an economically important chemical industry also attend annual meetings of the WNT as invited experts, and can participate in technical expert groups. National Coordinators take decisions on Test Guidelines for approval (including updates of existing Test Guidelines) and decide on project proposals to include on the work plan. Experts in technical groups are nominated by their National Coordinators, Business and Industry Advisory Council to OECD (BIAC), the International Council on Animal Protection in OECD programmes (ICAPO) and the European Environmental Bureau (EEB). Expert groups are specialised by area of hazard assessment (e.g. reproductive toxicity, genotoxicity, toxicity to the aquatic environment, environmental fate), and thus can work on several projects of the work plan that fall under the same area.

Experts participating in technical groups are nominated to provide their technical expertise in the area. Many experts participate over many years in the technical groups. This ensures consistency in the work done over time; however new expertise is always sought to ensure the best available science is taken into account and used in test method development. It is important that Test Guidelines development and regulatory science benefit from progress made in scientific research through networks and consortia of academic and industry laboratories. Gathering expertise and input from academia, industry, environmental and animal welfare organisations is essential for the OECD work on chemical safety to remain relevant for countries. Although industry and environmental organisations have been involved from the start in TG development, the participation of animal welfare NGOs is more recent, starting in the early 2000, and was encouraged by countries' uptake of ethical considerations in the use of laboratory animals for safety testing of chemicals. Occasionally, for specific areas of hazard assessment (e.g. endocrine disrupters), other interest groups are also involved. Furthermore, the European Commission, although not a member "country", participates in all the activities; indeed a large number of research activities in Europe relevant to the work of the Test Guidelines Programme are undertaken and coordinated by the European Union Reference Laboratory—European Centre for the Validation of Alternative Methods (EURL-ECVAM). Finally, countries like the People's Republic of China and the Russian Federation are invited to contribute to the work of the Test Guidelines Programme.

1.3 Workflow and Decision-Making Processes

National Coordinators can propose new projects. Such proposals have to be motivated by a regulatory need in more than one country or region (to benefit from international harmonisation), by a progress in science, by animal welfare considerations (e.g. making it possible to use fewer animals or to reduce duration of a test for example), or by an improvement in the cost-effectiveness of a test method. Proposals are reviewed and commented on by all members of the WNT a few months before the annual WNT meeting. At the meeting itself, the National Coordinators take a consensus decision on whether or not to include the project on the work plan following discussions. Project proposals can be submitted at different stages of test method development. In cases where the test method has already been validated, information and documents supporting the validation and the development of a Test Guideline are brought to the attention of the WNT upon submission of the project proposal. The WNT takes its decision to include the proposal in the work plan based on all available information.

If the project is accepted and the test method has already been validated, the lead country will take the first steps to make the first draft Test Guideline, while the Secretariat asks the WNT to nominate experts to a group, unless an existing group is competent and can take the new project on board. When the draft Test Guideline is sufficiently ready, it is circulated for a commenting round. The National Coordinators, industry, environmental organisations and ICAPO usually consult their expert networks when providing comments. In case of diverging views expressed by national experts, National Coordinators can take a national position. The Secretariat collects and compiles comments received and works with the lead country to address issues raised and revise the draft Test Guideline. Typically, following two rounds of WNT comments, the draft documents are mature enough for submission and eventually approval by the WNT, but there may be exceptions. The OECD Guidance Document 1 on the Development of Guidelines for the Testing of Chemicals, updated in 2009 (OECD 2009a), describes in more details the process and procedures for the development of OECD Test Guidelines and related documents (see Fig. 2.1). When Test Guidelines are approved by the WNT, they are subsequently endorsed by higher policy-level bodies of the Organisation until final adoption by the OECD Council and publication. Guidance documents approved by the WNT do not go to OECD Council for adoption (because they are not covered by the OECD Council Decision on the Mutual Acceptance of Data) and they are published under the responsibility of the policy body overseeing the work on chemical safety at OECD.

Projects may be included in the work plan at various stages of test method development, and the validity of the test method may not necessarily be fully established. In such cases, the project starts with experimental validation across laboratories, organised by the lead country(ies), with the assistance of the expert group or a Validation Management Group (VMG), with support from the OECD Secretariat as appropriate. When a project starts with a proposal for a test method that has not yet been validated, the whole process until approval of a Test Guideline takes more time, as the experimental validation is the most resource-intensive stage of the project.