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DB-ALM Report, 2016

EURL ECVAM DataBase service on ALternative Methods to animal experimentation

To promote the development and uptake of alternative and advanced methods in toxicology and biomedical sciences

Purpose of the document: Evidence of Deliverable 8 - JRC F3 Project/ WPK 1 816 Disseminate-1

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Executive Summary

This **deliverable report** outlines the activities performed during January 2014-June 2016 for the **DB-ALM** (EURL ECVAM DataBase service on advanced and Alternative Methods) enhancements regarding: information content, system evolution and innovation, as well as enlarging its outreach.

In October 2014, the first version of the **entirely innovated and revised DB-ALM** was publicly launched that provoked several positive reactions at International conferences. Completion is foreseen in 2017, progressively phasing out the previous version data sector by data sector. Several initiatives have been taken to ensure the state-of-the-art for both the scientific content and technological development. The **online information content** is continuously updated with current emphasis on the methods originating from EU funded research projects and/or undergoing validation studies towards international acceptance. The online information content covers today: 325 method descriptions (168 Summaries and 157 Protocols) linked to 82 method evaluations with 3017 test compounds, 9231 results, and experts` contact details. Further development refers particularly to the **innovation** of the content management system in addition to the public DB-ALM internet version. The Method Summary is the main data sector of the DB-ALM, providing the key-features of the comprehensively described methods, with regularly updated conent and revised nomenclature. This ensures a harmonised framework for adequately describing alternative methods in an OECD accepted format, **robust and flexible for various types of alternative approaches to testing of chemicals, pharmaceuticals, medical devices or biologicals.**

A further consolidation and growing interest in the DB-ALM was observed with a **steady increasing user community and usage**, amounting to a total of nearly 5,000 individual registrations from 82 countries, as well as an enhanced usage with over 40,000 accesses to the DB-ALM through the internet during the last calendar year. DB-ALM is, for example, referenced in *OECD Test Guidelines* and *Guidance documents*; *scientific books and cited in scientific article,* in addition to *website* references of relevant international organisations in and outside of Europe. The European Chemicals Agency (ECHA) suggests the DB-ALM as useful information source on in vitro methods to be considered for REACH registration purposes and the OECD recommended further the use of the DB-ALM for the storage and dissemination of method descriptions compliant with the new Guidance Document for describing non-guideline in vitro test methods GD 211.

1. Introduction

1.1 Project Deliverable within the WP 2014-2016

The goal of the project **Deliverable 8 (D.8)** is to provide *ready-to-use* standardised and *evaluated* descriptions of both *in vitro* and *in silico* methods for regulatory and research purposes in toxicology and biomedical sciences *via* **web-accessible dissemination platforms to facilitate knowledge sharing and their uptake by end-users**.

This **report** refers to activities performed for the **DB-ALM between January 2014 and June 2016** (EURL ECVAM DataBase service on advanced and ALternative methods to animal experimentation in biomedical sciences and toxicology) **with emphasis on *in vitro* methods,** but now also includes **non-experimental approaches**.

An equivalent report has been defined for the QSAR Model database (JRC102362) and both will constitute the **final deliverable**, deposited in the JRC central repository that can be made available on demand.

Related main deliverables of JRC 816 BioALM/Disseminate1 project: **D.2-4** and **7**.

D.2-4 refer to efforts undertaken to enhance and *update the information content* and are summarised in respective reports: JRC98655, JRC100695, JRC100696 available from the JRC central repository that can be made available on demand.

D.7 refers to the *development of a harmonised classification/indexing system* on key-features of experimental and non-experimental advanced and alternative methods with emphasis on the provision of mechanistic information by EURL ECVAM's information systems. The resulting reports will serve as the basis for their future interoperability, opening the doors for further applications, such as for Integrated Approaches for Testing and Assessment, based on the Integrated Testing Strategies or Adverse Outcome Pathways. First report (2016): JRC98655: "Provision of Mechanistic Information by EURL ECVAM's Information Systems on Advanced and Alternative methods for Toxicological Investigations" is available from the JRC central repository that can be made available on demand.

The project implements a **mandate of the Directive on the protection of animals used for scientific purposes [(2010/63/EU art.48 annex VII(2)(d)]** with an impact also on:

- Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (EC No. 1907/2006)
- Cosmetic Products Regulation (EC No.1223/2009)

1.2 Rationale

The ready access to suitable, ***ready-to-use*** and ***adequately described methods is a prerequisite for their use within decision making processes*** by regulators and scientists or any end-user in the field of ***biomedical sciences*** in general, and for safety assessment of chemicals or medical devices; or efficacy testing of pharmaceuticals, biologicals or vaccines. It supports international efforts to facilitate the acceptance of scientific approaches of integrated testing or adverse outcome pathways. In particular, regarding the use of novel technologies as advanced tools for screening and analysing modes of action, it has a great potential to translate the extensive R&D knowledge into a standardised, understandable and reliable source of information supporting decision makers.

Based on a legal requirement, the JRC has established and is managing dedicated dissemination platforms for different policy areas and purposes to enhance the knowledge about and the uptake of alternative and advanced methods ***at all stages of development and regulatory acceptance.***

The DB-ALM supports in this way the knowledge management and dissemination activities of the JRC. This is achieved by making ***high quality, expert written*** and ***reviewed information***, originating directly from research projects, thematic literature reviews on *in vitro* and other alternative approaches publicly available in a ready-to-use format *via* the internet to all stakeholders, but mainly to: national and international authorities, industry, the scientific community and animal welfare movements, the Commission services.

The work on the DB-ALM is primarily focused around two main pillars:

I. The revision and updates of the online information content including:

- **A. New and updated data sheets for the following sectors (page 7):**
  - Method Summaries and Protocols (at all stages of development, validation and/or regulatory acceptance)
  - Evaluations, EU integrated projects and formal validation studies
  - Test results (generated by documented methods as available)
  - Persons and institutions active in the field of alternative methods
- **B. Information standardisation and content criteria** (page 12)
- **C. Harmonisation of the scientific vocabularies** (page 14)

II. System evolution:

These activities comprise two main branches: *ordinary activities* (technological development of the informatics infrastructure) as well as the *extraordinary activities* (further evolution of the databases, content management enhancements and their respective websites, page 19).

2. Status at the end of June 2016

2.1 Public launch of the revised DB-ALM version

In October 2014 the **revised DB-ALM Internet version** was formally launched by the JRC. It was previously presented on occasion of the *9th World Congress on alternatives to animal use in life sciences* in August and received throughout positive comments during online demonstrations.

The DB-ALM search interfaces have been redesigned entirely to offer more flexibility during data retrieval procedures. The new interface provides the users with visible options for clarifying and refining queries *based on the controlled scientific vocabularies and/or classifications maintained at the JRC and covering the key features of advanced and alternative methods developed.* The next year will see the completion of the remaining sectors in support to phase out completely the current version also considering the feed-back resulting from the first release.

Figure 1: Screen capture of the revised DB-ALM Internet version

2.2 Users & Usage of the DB-ALM

Preamble

The revised DB-ALM version provides more information, such as methods abstract, status as well as all data retrieval procedures without the need of registration. Even if the core application is public now, the entire transition from the previous to the revised database service is made gradually, as more data sectors become available. Thus, currently both websites coexist. The login is no longer necessary to perform searches on the revised version and therefore less is known, for the time being, about the type of the user community in its entirety. Thus, the statistics are continuing to being redesigned to informatively and comprehensively reflect the usage of this service. Meanwhile, for the two transition years combined statistics are maintained to better follow user's interests in the service and are captured and reported at the end of each calendar year.

2.2.1 Status, December 2015

A further consolidation of the DB-ALM was observed with a steady increasing interest in the service amounting to a total of close to 5000 registrations over the entire time period since first online access was provided. The response of the user community to the public launch of the revised DB-ALM in 2014 was immediate and showed a rapidly growth of accesses (number of pages visited) that was confirmed in **2015** with over 42000 accesses, which corresponds to an **increase of about the 29% compared to the previous year.** In particular, the number of data retrieval procedures in the Topic Summaries and Methods data sectors **had more than doubled in 2015**, counting over 16500 searches with an **increase of about 130% compared to the 2014**.

Even though data requests could be performed without registration as well as access to a subset of additional information is provided, the service still can refer to a solid number of new users **with 499 new registrations in 2015,** being **the fourth highest during 9 years of public access** (see ANNEX II).

In 2015 the DB-ALM could refer to a total of **2867 unique active users** (a person who performs search requests in advanced and free text search fields, data sorting or navigating the website) **performing 10 accesses on average,** with a total of **2661 documents viewed** in addition to the abstracts view which does not require to login. Furthermore, Newsletters have been sent to over 2000 subscribers in 2014-2015.

The major customers of the DB-ALM originate from Italy and the USA (c.a.10% each), Germany and France (c.a. 9% each) and Spain and United Kingdom (c.a. 7% each), followed by Belgium (c.a. 5%), India, the Netherlands and Brazil (c.a. 3% each), and Japan, China and Poland with c.a. 2% each.

2.2.2 Information content display/Citations

Top Ten of the most popular **Methods downloaded**, **Biological Endpoints** and **Topics** searched in 2015 are included in the ANNEX II along with the **Total Accesses per Year** (number of pages visited) and the **Downloaded documents** per year. Method descriptions are cited in regulatory documents as well as in the scientific literature. More information is available in section 2.6 "Outreach" (page 22).

NOTE: The annexes contain the *Registration Tendency* showing the evolution of new registrations, thus demonstrations of interest in the service, since the first online access of the database service. The *users' geographical distribution* and *profile* are also accessible on the DB-ALM website from the top menu bar at:

https://ecvam-dbalm.jrc.ec.europa.eu/ (select *Use of the DB-ALM* from the top menu)

2.3 The Update of the Online Information Contents

All information content-related activities have been performed according to the following priorities:

1. Regulatory accepted methods
2. EURL ECVAM recommended and validated methods
3. Methods from EU Integrated Projects (e.g. SEURAT 1, ACuteTox)
4. Other emerging methods in development and related updates of DB-ALM information contents and its public website

2.3.1 Method Descriptions

Method descriptions are provided on two levels of detail by the DB-ALM:

- **Method Summaries** are usually defined as an outcome of systematic bibliographic reviews on priority topics or more recently refer to original work providing adequately documented method description as a stand-alone review according to the criteria for data content in place.
- **Protocols** always represent original work referring to individual technical descriptions of a method to such a detail that allows its transfer to another laboratory with no need of additional information. This is ensured by following EURL ECVAM standards for information content in a harmonised way. The original information is provided by the method experts or owners and reviewed by experts from DB-ALM for compliance before its publication.

At the time of writing the DB-ALM provided **325 Method descriptions** online: **168 Method Summary** and **157 Protocol** data sheets, covering 9 main topic areas (fields of application), further differentiated into more specific categories. **29 Method descriptions (comprehensively described ready to-use and stand-alone descriptions, together with related information) have been completed which were either newly defined, or thoroughly updated in the period January 2014-June 2016.**

The overview is provided in the Table 1, and discussed in detail in the text. Further 5 data sheets are prioritised and are close to finalisation, followed by another 10 data sheets with a lower level of prioritisation.

The various collaborations with colleagues for defining validated methods confirmed a best practice of having a Protocol and Method Summary available for every method with EURL ECVAM Recommendation (discussed in the section "*Support for EURL ECVAM Recommendation*", page 13).

In addition, efforts were made to further develop contacts with the SEURAT-1 consortium and as an outcome **the DB-ALM was chosen to become the repository for disseminating particular promising methods developed by their projects** and to be published as the "Methods Catalogue" by using the DB-ALM format for comprehensively describing advanced and alternative methods. Material and guidance were provided to the Consortium. In the course of 2015-2016 the information on the methods developed within various consortium projects was made available to the DB-ALM, leading to 8 publications so far and 5 compiled method descriptions that are approaching finalisation.

In the course of 2013-2015 OECD released a number of new or updates of existing Test Guidelines on genotoxicity and local toxicity testing. The information necessary to perform an update on the methods involved was collected. First updates were completed; others are in various stages of progression, prioritised for finalisation in 2016-2017.

Table 1 New and updated methods published in 2014-June 2016, listed by topic

Priority	Topic	Data sector	Title	Year
1	Haematotoxicity	Protocol	No. 101 Colony Forming Unit-Granulocyte/ Macrophage (CFU-GM) Assay (Update)	2014
2	Carcinogenicity *Cell transformation*	Method Summary	In vitro BALB/c 3T3 Cell Transformation Assay (CTA)- Summary	2014
			Bhas 42 Cell Transformation Assay in 6- and 96-well plates - Summary	
		Protocol	No. 156 Bhas 42 Cell Transformation Assay in 6- and 96-well plates	
			No. 137 In vitro BALB/c 3T3 Cell Transformation Assay (CTA)	
1 and 3	Local Toxicity Eye Irritation	Protocol	No. 157 Ocular Irritection	2014
		Method summary	EpiOcularTM Assay - Summary	2015
		Protocol	No. 164 EpiOcularTM Assay	
1	Skin sensitisation and allergic contact dermatitis	Method Summary	Direct Peptide Reactivity Assay*	2014
			KeratinoSens*	
		Protocol	No. 158: Human Cell Line Activation Test (h-CLAT)	

			No.155: KeratinoSens	
3	Systemic Toxicity	Protocol	No. 145: Aggregating brain cell cultures for neurotoxicity testing, a high content approach: measurements of RNA synthesis and gene expression	2014
			No. 167: HepaRG, repeated and single dose exposure for Mitochondrial Health and Lipid Toxicity	2016
		Method summary	No. 152:Neural network models for the prediction of blood-brain-barrier passage and human intestinal absorption	2014
			No. 161 Physiologically-Based Kinetic models	2015
			No. 162 Virtual Cell Assay model	
			No. 163: In vitro to in vivo extrapolation	
1	Genotoxicity/ Mutagenicity	Method summary	No. 30 Bacterial reverse mutation test (Ames test)	2015
		Protocol	No. 30: The Ames Test (with S. typhimurium) (update)	
			No. 160: Bacterial Mutation Assay (with S.typhimurium and E. coli)	
3	Stem cell culture	Method summary	No. 165: Differentiation of induced-pluripotent stem cells into post-mitotic neurons and glial cells (mixed culture)	2016
		Protocol	No. 166: Standard operating procedure for extension and propagation of pre-differentiated neural stem cells	
			No. 165: Standard operating procedure for differentiation of human induced pluripotent stem cells into post-mitotic neurons and glial cells (mixed culture)	
1	Effects on endocrine system	Method summary	No. 455: Transactivation Assays to Detect Estrogen Receptor Agonists In Vitro with Stably Transfected Human Cell Lines	2016
			No. 456: H295R steroidogenesis assay	
3	Mechanistic Studies	Method summary	No 174 : DNA methylation analysis using HumanMethylation450k microarray	2016
		Protocol	No 174: DNA methylation analysis using HumanMethylation450 microarray	
			No 171: Analysis of histone modifications via chromatin immunoprecipitation and sequencing (ChIP-seq)	

Priorities: 1 regulatory accepted · 2 recommended and validated · 3 originating from EU integrated projects

*Method summaries first published in 2014, migrated to the new OECD format and updated in 2015

The primary focus was put on the definition/updates of Methods with regulatory acceptance, undergoing validation, and/or participating in the EU funded research projects. The following topics were covered:

- **Skin sensitisation**

  New Method summaries and corresponding protocols were written and published online for the recently validated "*DPRA*", "*KeratinoSens*" and "*hCLAT*" methods. In 2015 the method summaries for "*DPRA*", and "*KeratinoSens*" were transferred to the new GD211 compliant reporting format, revised and republished. Unfortunately due to unforeseeable circumstances, the method summary for "*hCLAT*" could not be finalised yet.

- **Carcinogenicity**

  New Method summaries and corresponding protocols were written and published online for the recently completed validation studies of two cell transformation assays: "*BHAS 42"* and "*BALBc 3T3".*

- **Local toxicity – Eye irritation**

  Together with the test submission of the Ocular Irritection® Assay System, which supersedes the EYETEX method, a new protocol was published and the data sheet on the previous version of the method updated accordingly. The EpiOcular™ Eye irritation Assay reached regulatory acceptance as OECD TG 492 on 28th July 2015. Accordingly, the existing Methods Summary was updated and migrated to the new data format and accompanied by a new validation study protocol.

- **Genotoxicity/Mutagenicity**

  A review of the bacterial reverse mutation test (Ames test) was finalised which included a compilation of a new method summary and a new protocol representing the current SOP used by a contract research organization. A protocol for the regulatory accepted (OECD TG 487) *In Vitro* Micronucleus method was compiled and sent to the author for approval. Unfortunately, so far without response. DB-ALM is searching for another source of an SOP compliant with the OECD TG.

- **Systemic Toxicity**

  Two new protocols and one update were finalised and published originating from the EU integrated project **ACuteTox**. The remaining seven are close to finalisation, awaiting authors final approval.

  In March 2015 the work has started on the **Seurat-1 Methods' catalogue** and led so far to 8 method descriptions published (JRC100696):

  - Three new method summaries were compiled and published in collaboration with EURL ECVAM colleagues directly involved in the project. The methods represent three computational approaches developed within the COSMOS project.
  - A method summary and pair of protocols were compiled in collaboration with the EURL ECVAM colleagues directly involved in the Scr&Tox project. "*The differentiation of induced-pluripotent stem cells*

into post-mitotic neurons and glial cells (mixed culture)" was developed at the JRC. The method has also been classified as the first one to be registered under the topic *Stem cell culture method.*

- The recently finalised report on the *"HepaRG, repeated and single dose exposure for Mitochondrial Health and Lipid Toxicity"* was made available to DB-ALM project team in December 2015 and the work has started on preparing the respective method summary and a protocol for online publication.
- NOTOX project provided DB-ALM with a method summary and protocol for two Methods: *DNA methylation analysis using HumanMethylation450 microarray* and *Analysis of histone modifications via chromatin immunoprecipitation and sequencing (ChIP-seq).* The topic Mechanistic Studies was judged as the most appropriate for both methods. The respective data sheets were published, with the exception of the method summary *Analysis of histone modifications via chromatin immunoprecipitation and sequencing (ChIP-seq).* The draft provided too little information to justify a creation of a new datasheet and more background information is needed before its completion.

- **Effects on endocrine systems**

  A new topic *Effects on endocrine systems* was set up in the DB-ALM to extend the information coverage to a new policy area, namely the area of endocrine disruption. In collaboration with the EURL ECVAM colleagues working in this area five method summaries were compiled. They are representative examples of the assays for testing of Androgen, Estrogen and PPARγ –specific effects and steroidogenensis. Two were published (JRC100695) and remaining three are expected to be published in the course of 2016. In addition, background materials were collected and contact persons identified for additional method summaries for studying effects on thyroid signalling.

Summary of the work progress January 2014-June 2016:

Method Summaries/Protocols processed (44)			**Work on hold/pending (53)**	
Published online	**Near finalisation**	**Work in progress**	**Contact lost**	**Insufficient information or lower priority**
29	**5**	**10**	**21**	**32**

NOTE: The list of methods included in the yearly project planning is based on the ready available information on the on-going validation studies, test submissions, activities on the OECD level and contacts with individual experts from the research communities. In addition DB-ALM staff is aware of several relevant EU research projects where alternative methods are being developed/evaluated, however as long as no detailed information is available, no planning can be made in advance. Such items are not included in the planning table, but will be taken up on case by case basis when made available.

2.3.2 Who's Who in the field of alternative methods

The **Who's Who** data sector of the DB-ALM was originally established from the results of a survey launched by EURL ECVAM on major players in the field of alternatives in the various fields of science and regulatory environment to promote the exchange of information on advanced and alternative approaches at different levels. Today this data sector is mainly composed by the information of **those actors involved in the preparation, submission and revision of methods included in the DB-ALM sharing their respective expertise**. Therefore, the data sector became an integral part of the Method descriptions.

In preparation for the periodical update of the whole sector, which is performed about every 2 years in line with the Personal Data Protection Regulation of the EU (Regulation (EC) N° 45/2001), a full status assessment of the entire sector was made before proceeding with the review which started in December 2014 and was concluded in May 2016. Information on the method data sheets will finally be adapted, where contact has been lost.

Periodical review 2014-2016:

- In the first stage of the periodical review, a total of 131 persons have been contacted in October 2014 leading to *53 revised and published records*. Those who did not reply to the first call and the following reminder, which mainly refer to very early registrations without involvement in current method developments, were removed from the system.
- In the second stage a total of 164 persons have been contacted in October 2015 leading to *14 revised and published records* within the same month.
  A reminder was sent to contacts who did not reply to the call.

NOTE: Those who do not reply twice are removed from the service according to the policy in place for data protection.

2.3.3 Standardisation of information content and criteria

The purpose of the **DB-ALM Method Summary** is to provide adequately documented method description as a stand-alone review according to the criteria for data content in place. It provides the context for standard operating procedures, which may be available from DB-ALM as Protocols, but equally so from other sources. The Method Summary includes the intended purpose or actual applications in research or for regulatory purpose, its scientific principles, the status of development and acceptance, the eventual applicability domain and overall performance as appropriate and available, of the method. In contrast, the **DB-ALM Protocol** addresses the practical aspects of the method's implementation in a laboratory and routine use, provided in standardised and controlled manner.

2.3.3.1 Compliance with OECD reporting standard for non-guideline methods

In 2014 the **DB-ALM Method Summary** was positioned by the OECD as the reference format for the description of non-standard experimental methods augmented by the expertise of an internationally composed drafting group and their

respective organisations. This resulted in the "**OECD Guidance Document for Describing Non-guideline in vitro Test Methods (N◦211),** an initiative of the OECD Advisory Group on Molecular Screening and Toxicogenomics and its programme on the development of Adverse Outcome Pathways. In the progress of drafting this document the key elements of the method description were defined and finally recommended for reporting formats. The final Guidance preserved the information elements of the original DB-ALM format for adequately describing alternative methods.

The **Method Summary content criteria** and format were subsequently refined by the BioALM project team (see *ANNEX III,* page 36) to ensure that all new Method Descriptions provided by DB-ALM are visually similar and thus compliant with the new OECD reporting standard. At the same time, care has been taken **to preserve the flexibility of the format to representing all relevant alternative approaches for various test materials in addition to chemicals, such as medical devices or biologicals, or even for non experimental techniques.**

Practical implementation into the DB-ALM & challenges:

Once the refined content criteria were implemented in the Content Management System of the DB-ALM (see section "*Method Summary alignment with the OECD Guidance for in vitro methods*", page 20), the project team evaluated possible follow up steps. This was done in such a way as to minimise the impact on the database structure, in other words to allow for very easy switch between the old and the revised format, as illustrated in the Figure 4 (*ANNEX IV*, page 51). *However, prior to re-publication each data sheet has to be inspected and adjusted where necessary to ensure that the quality after the change to the new format is maintained.*

At present it is not possible to ensure the quality control and migration for all 168 method summaries in the DB-ALM. Therefore, it was agreed to apply the revised format for all new/updated methods published from 2015 onwards.

In addition, the revised format (intended by the OECD for in vitro methods only) was immediately challenged by using it on three *in silico* methods (from Seurat-1) and one *in chemico* assay (DPRA). **The DB-ALM format has proven to be robust and flexible for various types of alternative approaches.** Some needs for further fine tuning were noted, regarding additional free text fields, display of contact information and need for support of mathematical formulas. These observations will be considered in the further work planning for 2016-2017.

The revised format has been distributed among the **SEURAT-1 consortium** members as a recommended format for the **Methods Catalogue of the Consortium**. It was also used in a small feasibility study for the endocrine disruptor methods.

2.3.3.2 Support for EURL ECVAM Recommendation

Starting from 2013 both documents **(Method Summary and Protocol) are to be issued together with the EURL ECVAM Recommendation**, where the Protocol is supplied and revised by the method developer/contact, and the Method Summary is compiled by the validation study responsible (or a nominated person) in EURL ECVAM. **6 such pairs were published so far** *(see* Table 1*,* page 8*).*

2.4 Harmonised Terminologies & Classifications

Data compilation and retrieval procedures in the DB-ALM are based on the use of **classification lists** of **controlled scientific vocabularies** maintained in-house by the data dissemination team covering:

1. **Biological endpoints:** terms that describe the processes, responses or effects assessed completed by the techniques to measure them
2. **Endpoint values:** precise values investigated in a given assay
3. **Experimental systems:** are the (bio)systems used to study the investigated effect
4. **Topic:** field of application
5. **Area of concern:** chemicals (generic), pharmaceuticals, biologicals, medical devices etc.
6. **Models and strategies:** type of method used with their sub-categories

These lists contain hundreds of terms used for **describing, classifying and indexing or retrieving database contents** which have to be maintained, reviewed and harmonised, as appropriate each time a new method is added that would require a new term and/or is done in the light of new scientific findings.

Controlled scientific vocabularies are key elements of the revised data retrieval approach of the new DB-ALM user interface. The DB-ALM free text search has been enhanced with the autocomplete function. When the user starts to enter a search term, the autocomplete function uses the **classification lists** and **scientific vocabularies** to provide suggestions for a successful search. Based on the analysis on the website use, since its introduction in 2014, this feature was used in approximately 80% of search procedures conducted on the DB-ALM Internet version.

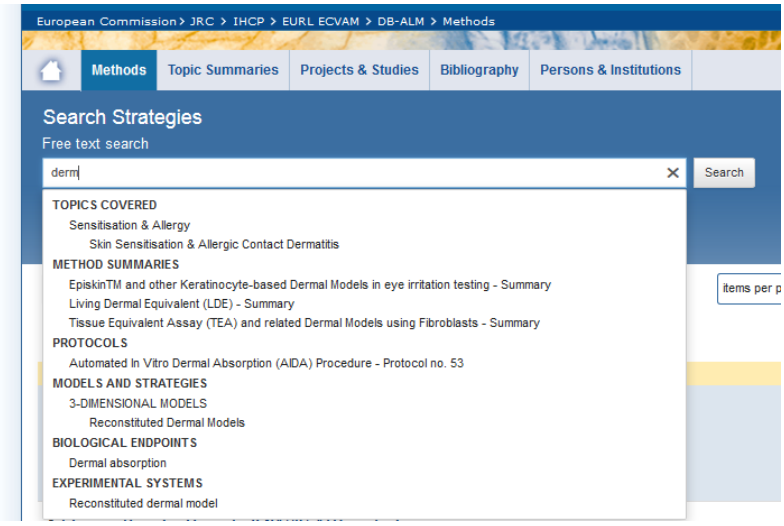


Figure 2: Example of Autocomplete function activated by typing *derm* in the free text search box

The activities to standardise the scientific vocabularies are carried out with a clear viewpoint of the **further development of the Unit's information systems and their interoperability** to serve future applications, such Integrated Approach to Testing and Assessment or Adverse Outcome Pathways [see also D.2, 7 Provision of Mechanistic Information by EURL ECVAM's Information Systems on Advanced and Alternative methods for Toxicological Investigations (JRC98655)].

2.4.1 Biological endpoints

The **Biological endpoints** are key elements of the new data retrieval approach of the revised DB-ALM launched in 2014. The terms refer to the **biological processes, responses or effects assessed by a given test method and can be categorised by the level of molecular organisation** as shown in the Table 2.

Table 2: DB-ALM Biological Endpoint 'Clusters'

Biological endpoint clusters categorised by the level of molecular organisation		Number of terms
Molecular level	Physicochemical or biochemical properties of test substance	19
	Physicochemical properties of test system	2
Total = 21		
Cellular level	Specific features to cultured cells	5
	properties and structures common to all cells	47
	Regulation of cell adhesion	5
	Mutagenesis	16
	Oxidative stress and damage	5
Total = 78		
Tissue and organ level	Blood and vascular system function and morphology	12
	Central and peripheral nervous system	28
	Connective tissue	1
	Corneal function and corneal inflammation markers	12
	Embryo development: histology and molecular markers	18
	Embryo implantation	2
	Eye and eye lens characteristics	5
	Germ cell maturation	12
	Hepatic functions	21
	Immune responses	9
	Inflammatory responses	3
	Muscle tissue function and morphology	4
	Non-hepatic metabolism (seminal vesicles)	1
	Placental tissue function and morphology	11
	Renal tissue function and morphology	1
	Skin function and morphology	1
	Thyroid tissue function and morphology	2
Total = 143		
Organism level	Conception	1
	Characteristic features of model organisms	26
Total = 27		
Grand Total = 269		

The first part of the *new DB-ALM content management system* was released already in 2013 with a dedicated data sector for each of the controlled vocabularies that enabled more user-friendly comprehensive revisions by the data specialist which started in due course and the biological endpoints (in total over 250 terms) were thoroughly revised and updated *(see Table 2)*, followed by with the remaining segment "gene expression". For example, this term was listed as an endpoint in app. 50 documents and reviewed/updated accordingly.

2.4.2 Experimental Systems

The list of experimental systems used by DB-ALM until 2015 was heterogeneous and could benefit from further harmonisation. Accordingly, the list of controlled terms underwent a thorough review for consistency and opportunities for improvement.

In the **first step**, the different types of experimental systems were evaluated, with a particular focus on the characteristic features (identifiers) used to adequately describe each type of a system. The experimental systems recorded by the DB-ALM methods fall into several categories:

- *Mathematical models and computerised systems*
- *Physico-chemical measurements*
- *Cell-free extracts and (bio)polymers*
- *Tissue and cell culture including 3 D Models*
- *Organs or organ parts used in the ex-vivo studies*
- *Complete organisms*

In the **second step**, the *existing scientific terminology resources available in the public domain were evaluated*, relevant for each type of (Experimental) system, **to define solid reference sources and basic rules on nomenclature.**

When proposing a name for the experimental system, the terminology already in use must be taken into account from:

- Scientific publications
- Literature databases (e.g. Scopus, Medline, National Agricultural Library, Google Scholar)
- Specialised databases (e.g. cell banks, Tree of Life project, product manufacturers and or distributors)
- Controlled vocabularies/thesauruses (e.g. Medical Subject Headings, US National Cancer Institute – Thesaurus, Cellosaurus)
- Ontologies (e.g. NCI Thesaurus, Ontology for Biomedical Investigations, Experimental Factor Ontology, Cell Line Ontology, Gene Ontology, The comprehensive enzyme information system tissue ontology BRENDA).

Proposed approach to main categories of experimental systems (selected examples):

- *Whole organisms / Species*
  Both common name and Latin name can be used, unless one of the two is very uncommon. For example: when spelling out the names of human cell lines, adding *Homo sapiens* would be unusual, whereas for other animal species Latin names are frequently used. Proposed syntax for mouse:
  Mouse (Mus musculus)

- *Organs and tissues*
  Usually the type of tissue is followed by the name of the species, the life stage and/or further specifications:
  Conjunctiva (mouse)
  Chorioallantoic Membrane (CAM) (chicken egg)
  Whole blood (human), cryopreserved

- *Cell lines*
  In order to harmonise different ways of naming the cell lines, the following convention is proposed:

  name of the cell line **|** species **|** cell/tissue type:
  V79 | Chinese hamster | lung fibroblast cell line

In total approximately 1000 names of experimental systems were evaluated by 2016. 501 terms, linked to method summaries or protocols, were updated to a new syntax. Duplicates were merged and unused terms deleted, resulting in 375 final terms being updated and republished. A detailed overview of the work was provided in the JRC99991 report.

2.4.3 Models and Strategies and Area of Concern

The other two important classification headings are the "**Models and Strategies**" and "**Area of Concern"**. The new content management system enables to quickly detect data sheets where this information is missing. **It was added or updated in 72 documents, where appropriate.**

The Area of Concern classification list was revised in 2015, to better reflect the regulatory distinction between the types of substances. Although from the scientific point of view all test substances are chemicals, the applicability domain of a given method is usually defined during the validation process and with a specific regulatory purpose in mind.

The users performing their queries often look for methods applicable within a specific regulated industry sector. To facilitate this type of search **the categories of the test materials were re-assigned into 9 main groups:**

1. *(Industrial)* Chemicals
2. Pharmaceuticals
3. Vitamins
4. Cosmetics
5. Consumer Products
6. Food Additives
7. Environmental pollutants
8. Nanoparticles
9. Biologicals
10. Medical devices.

2.4.4 Objective & Applications

For the purpose of the *online application for creating, updating and editing Method Summaries*, the free text fields Objective & Applications (purpose and type of testing, level of assessment) have been replaced by a pick-list, created using the content of all public documents (data sheets). **The appropriate terms were selected and the new improvement to the content management tested**.

2.4.5 Bibliography

Since 19.09.2014, the references can be managed and published entirely from the new content management system. This enabled a **more efficient management and quality control of bibliographic references** by the data specialist. After the implementation was made available, it was tested and it was started to clean up accordingly bibliographic references to be continued in the forthcoming years.

2.5 Innovation and System Evolution

The DB-ALM has been further innovated and evolved from January 2014-June 2016 regarding:

2.5.1 Software Developments

2.5.1.1 The public launch of the first release of the revised DB-ALM in 2014

The revised DB-ALM follows the Commission's development standards and industry best practices. Based on the experience gained during more than 6 years of operation of the DB-ALM on the Internet offered to over 4000 registered users, the DB-ALM search interfaces have been entirely redesigned in due course to offer more flexibility during data retrieval procedure ***based on key features*** of the methods.

The new interface provides the users with visible options for clarifying and refining queries. The user can combine text searches beginning with a classic keyword search narrowing the search results with a number of offered choices. *Using this progressive incremental query construction, users will be enabled to formulate the equivalent of a sophisticated query by taking a series of small, simple steps.*

In 2014, following a preliminary (internal) launch and thus broader use, a number of functionalities were further refined and completed, the Homepage slightly reviewed and functionalities of the content management system adapted. In 2015 further developments and refinements where implemented (*ANNEX IV,* page *49*)

The year 2016-2017 will see the upgrade and/or completion of the revised search interfaces and content management system also for the ***methods'-supporting data sectors.***

2.5.1.2 Restructuring formats for Method Summaries and Protocols

After EURL ECVAM's internal request, it was decided that the protocol should not include any descriptive sections that can be accomplished by the Method Summary. *The protocol will maintain those values necessary only for classifying and retrieving the document*. Thus, the following descriptive sections were deleted from the introduction section of the Protocol format:

- Objective & Applications, Data Analysis/Prediction Model, Test Compounds and Results Summary, Modifications of the Method, Acceptance Criteria and Proficiency Testing.

Current Protocols that have content in these fields can still be edited but new Protocols do not show these fields anymore.

2.5.1.3 Adaptations in the light of evolving science and requirements for data management

Much of the work made in 2014-2016 on the database was related to the development of the new DB-ALM website and realization of the new requirements for the support of the data dissemination of the STU, which include:

- Changed the Method Summary fields 'Type of Testing', 'Purpose of testing', 'Level of Assessment' from free text fields to controlled lists. This will allow searches to be performed on these fields in the future

- Added the 'Exposure regime' section to the Method Summary
- Added the ability to create and edit publishers and acronyms
- Add method number for Method Summaries
- Rèsumès were re-developed with enhanced functionality. They can now exist independently of any other item. A Rèsumè can be linked to many Method Summaries, Protocols and Validation Studies and a Method Summary or Protocol or Validation Study can be linked to many Resumes
- The 'Evaluated Test Method' list was updated to include the new functionality of Rèsumès related to projects
- New content management system further developed with the ability to Publish and Delete individual datasheets from the database: Topics, Method summaries, Protocols, Validation studies, Resumes, Evaluation studies, Contacts, Laboratories, Institutes, Bibliographies, Sources, Acronyms, Publishers, Experimental systems, Endpoints, Compounds, Test results, Study participants, Study labs, Study compounds, Study test results

2.5.1.4 Method Summary alignment with the OECD Guidance for in vitro methods

Adopting the Method summary format to the OECD Guidance required the following changes to be made:

- Added the ability to display a different layout for a Method Summary based on the content criteria version
- Added a new layout of the Method Summary that matches the format required by the OECD
- Added the ability to assign Contacts to a Method Summary that are linked to the who's who data sector to ensure maintenance according legislative rules
- Added the ability to assign a function for a Contact e.g. Corresponding author, Method developer etc.
- Added the ability to assign a type-value for a Bibliography e.g. Method development, General references

2.5.1.5 User Registration

The user registration process, forgotten password process and user account page were re-written to meet modern development standards and security best practices for applications storing user information.

2.5.1.6 Online applications for providing and updating information contents

The development of version 2 of the *Online Submission application for Method Summaries* and *Protocols* has commenced.

2.5.1.7 Planned activities

A complete working list is available in the project folder. In particular during the forthcoming years the following work has to be carried out and/or completed on the content management system, in addition to the finalisation of the public DB-ALM 2016 version basis for phasing out the previous one (see before):

- Protocol and Method-Summary submission application and creation of the same functionality for the remaining data sectors
- Migration and updating of Test Results

- Management of Who's who
- Content Management for Navigation pages – the text displayed on web pages etc.
- Publication process of website contents

A document management functionality to interconnect with external documents has to be developed and preparation/feasibility evaluations to be done in view of revisiting the classification systems in line with the currently ongoing project on *harmonising classification schemes (addressed by Deliverable 7)*. Consequently, at a longer term, the integration with the TSAR and related system has to be ensured.

The website usage statistics needs to be readapted for the DB-ALM 2016 version.

2.5.2 Upgraded server environment

A new development environment has been upgraded to always match the production environment. This allows us to test all website updates and operating system upgrades before applying the changes to the production environment.

2.5.3 Documentation

The work done on the DB-ALM and related activities are documented stating the date of entry, priority and completion date of requests in internal project file repository at JRC.

3. Conclusions and Outreach

In addition to the steady increasing user community (close to 5000 registered users from 82 countries covering the entire time period since first online access was provided; see Section 2.2: " Users & Usage of the DB-ALM". page 6), the DB-ALM has been presented at the following events and/or using the following communication channels:

1. At the end of 2014 the **OECD has approved the "Guidance for describing non-guideline in vitro test methods"** (N° 211) for methods at all stages of development, including at early stage. It was based on the format in use by the DB-ALM augmented with the expertise of an internationally composed drafting group and their respective organisations. The OECD recommended the use of the DB-ALM for the storage and dissemination of methods described according to it.

2. **Public awareness** was raised by sending a series of newsletters to more than 2000 DB-ALM newsletter subscribers.

3. Growing interest and use of the DB-ALM information content has been observed. For example:

    - DB-ALM is referenced in 6 OECD Test Guidelines and 2 OECD Guidance documents (for more details refer to "*ANNEX V: Bibliographic Citations*" *page 53*)

    - more than 160 scientific books and journal articles included numerous references to the DB-ALM documents [Google Scholar and SCOPUS search performed on 22-06-2016 (for more details on the most recent publications refer to *ANNEX V: Bibliographic Citations* " *page 53*)

    - the European Chemicals Agency (ECHA) indicates the DB-ALM as useful information source on *in vitro* methods to be considered for REACH registration purposes

    - the National Brazilian network for Alternative Methods (RENAMA) features DB-ALM on their homepage, and a substantial number visits to the DB-ALM are redirected from this page. A complete list of the websites referring most of the visitors to the DB-ALM are listed in the *ANNEX V: Bibliographic Citations*" (page 70) and include not only search engines but also the Swedish 3R' centre, e-Learning platform for laboratory animal science LAS interactive and the Shiseido cosmetic company.

    - the US Health and Environmental Sciences Institute (HESI) emphasises the usefulness of the JRC database.

4. **SEURAT-1 consortium** has chosen the DB-ALM to be the repository for descriptions of particularly promising methods developed by the participating projects. The revised DB-ALM format has been adopted as the recommended format for the Methods Catalogue of the consortium.

5. At the **9th World Conference for alternatives to animal** use in August 2014 (close to 1000 attendees) the new search interface of the DB-ALM was presented and tested by the individual visitors at the stand of the JRC in the

exhibition area and can refer to only positive feedback documented in a questionnaire.
A poster and oral presentation were given as well on the EURL ECVAM dissemination activities and the session on dissemination *via* databases co-chaired.

6. The poster and leaflet of EURL ECVAM database services were included in the online Poster and Flyer collection *"Animals used for scientific purposes"* on the **DG Environment** website and received positive responses through social media:
   http://ec.europa.eu/environment/chemicals/lab_animals/pubs_posters_en.htm

7. At the 51st Congress of The European Societies of Toxicology in September 2015 (EUROTOX 2015, app. 1500 attendees) the EURL ECVAM information systems were presented to individual visitors at the stand of the JRC in the exhibition area and received a very positive feedback.

8. At Workshop: **"Mechanisms, markers and models – the 3M strategy in risk assessment"** in October 2015 (organised jointly by SSCT and Swetox, 61 attendees) the poster on EURL ECVAM dissemination activities was presented and the Content Criteria for Method Summaries were distributed to the participants.

9. In order to provide a better visibility of the BioALM/STU-Disseminate WPK1 project (JRC 816) within the Commission, a dedicated project space was created on Connected. It offers background information, documents, the links to all information systems and the possibility to interact directly with the project team (accessible to JRC personnel only):

   https://connected.cnect.cec.eu.int/groups/816-bioalm-dissemination

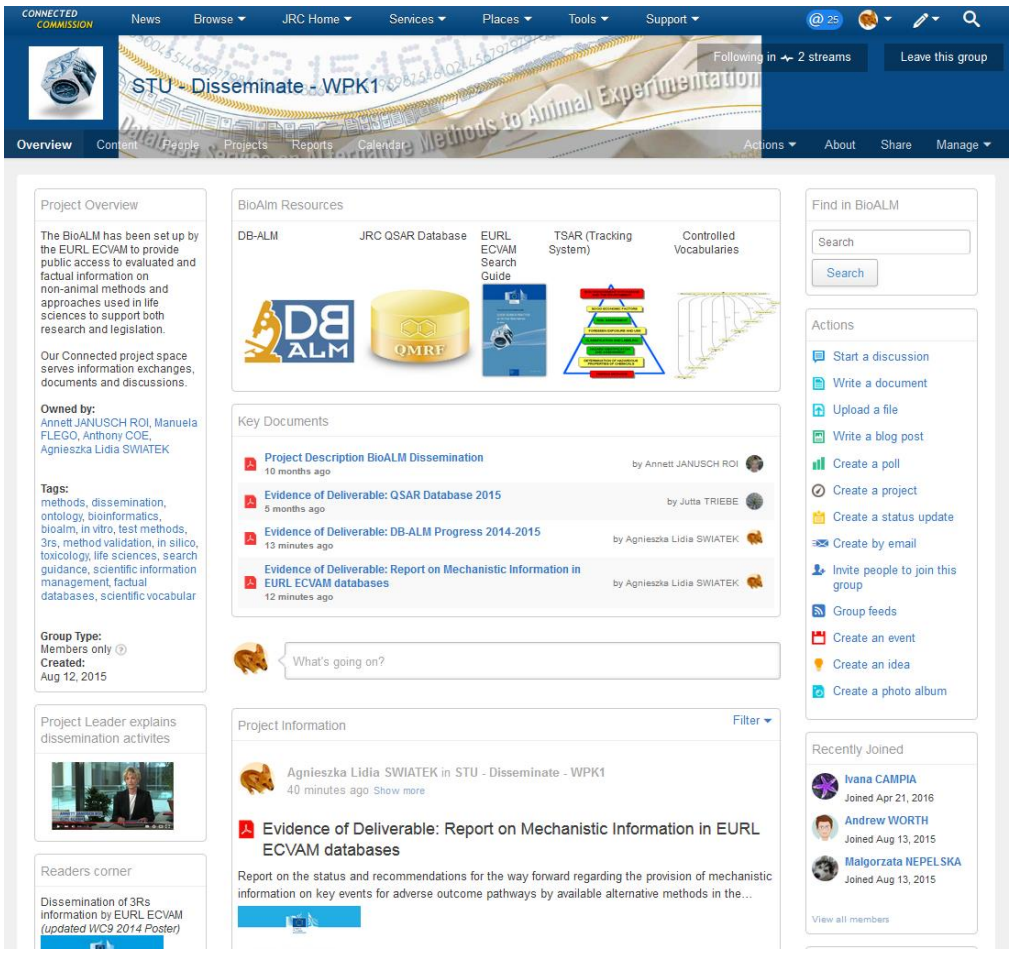


Figure 3: Screen capture of the BioALM Dissemination project space on Connected (last access 10 June 2016).

4. Access to the deliverable

Public access to the DB-ALM:

https://ecvam-dbalm.jrc.ec.europa.eu

Public access to the revised DB-ALM version:

https://ecvam-dbalm.jrc.ec.europa.eu/

DB-ALM staging environment providing access to all applications and to the content management systems (accessible to JRC personnel only):

https://staging-ecvam.jrc.ec.europa.eu/dbalm-staging/

5. Contacts

Helpdesk: dbalm-contact@jrc.ec.europa.eu

DB-ALM Responsible: annett.janusch-roi@ec.europa.eu

6. Abbreviations

- 3D – three dimensional
- DB-ALM – EURL ECVAM DataBase service on ALternative Methods to animal experimentation
- EU – European Union
- EURL ECVAM - European Union Reference Laboratory for alternatives to animal testing. Formerly a part of the Systems Toxicology Unit at JRC. As of 01.07.2016 its duties are transferred to the Chemical Safety and Alternative Methods unit (F.3)
- JRC – Joint Research Centre
- OECD – The Organisation for Economic Co-operation and Development
- R&D – Research and Development
- SEURAT-1 – Safety Evaluation Ultimately Replacing Animal Testing
- WP – Work Program
- WPK - Work Package

7. Annexes

I. DB-ALM Newsletters

II. DB-ALM User Community

III. Method Summary in OECD Compliant Format

IV. Software developments (extract)

V. Bibliographic Citations

7.1 ANNEX I: DB-ALM Newsletters

October 2014

JOINT RESEARCH CENTRE

EURL ECVAM DataBase service on ALternative Methods to animal experimentation (DB-ALM)

1. DB-ALM Updates, October 2014

DB-ALM 2014 Version – now online

In line with the commitment of the Joint Research Centre to enhance the dissemination of knowledge on advanced and alternative methods, the EURL ECVAM is aiming at constantly improving its services. We now have the pleasure to inform you that the entirely revised DB-ALM 2014 Version is available online. It provides a completely redesigned data retrieval approach where more flexibility and support is offered. This version was presented during the 9th World Conference on Alternatives and Animal Use in the Life Sciences in Prague this August where first positive feedback has been collected. We wish you happy navigating!

2. Updated DB-ALM Information Content

Together with the revised DB-ALM 2014 version and since our last communication, the following method descriptions have been published for in vitro methods compliant with regulatory requirements and/or undergoing validation by the EURL ECVAM in the areas of Carcinogenicity, Eye Irritation and Skin Sensitisation:

- In vitro BALB/c 3T3 Cell Transformation Assay (BALB/c 3T3 CTA) (Method Summary and Protocol No. 137)
- Bhas 42 cell transformation assay in 6- and 96-well plates (Method Summary and Protocol No. 156)
- Ocular Irritection® Assay System (Protocol No. 157)
- Human Cell Line Activation Test (h-CLAT) (Protocol No. 158)

Access: DB-ALM 2014 Version

European Commission - Joint Research Centre
Institute for Health & Consumer Protection
European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)
EURL ECVAM DataBase service on ALternative Methods to animal experimentation (DB-ALM)

April 2015

JOINT RESEARCH CENTRE

EURL ECVAM DataBase service on ALternative Methods to animal experimentation (DB-ALM)

Updated information content on QSAR methods, April 2015

We have the pleasure to inform you that, in addition to the updated information on ***in vitro*** methods, computational methods which are publicly available from EURL ECVAM's Information Systems, will be also covered in our future communications.

Ten new reports have been published in the JRC QSAR Model Database that uses an internationally recognised format to provide key information on models which are all peer-reviewed before publication. The new reports refer to the following topic areas:

- six reports for the main endpoint ***Human health effects*** with the following QSAR inventory numbers: Q35-50-46-429; Q32-48-43-426; Q32-48-43-425; Q31-47-42-424; Q29-44-39-423; Q28-43-38-420
- four reports for the main endpoint ***Ecotoxic effects*** with the following QSAR inventory numbers: Q33-49-44-427; Q34-49-44-428; Q19-46-41-422; Q30-45-40-421

The complete list of published QSAR models (80 in total) can be freely downloaded from the JRC QSAR Model Database list of published reports.

More information on QSAR models and free download of all published QSAR reports can be obtained directly from the JRC QSAR Model Database .

European Commission - Joint Research Centre
Institute for Health & Consumer Protection
European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)
EURL ECVAM DataBase service on ALternative Methods to animal experimentation (DB-ALM)

August 2015

JOINT RESEARCH CENTRE

EURL ECVAM DataBase service on ALternative Methods to animal experimentation (DB-ALM)

DB-ALM now provides non-guideline in vitro test methods in OECD compliant format, August 2015

The JRC's European Union Reference Laboratory for Alternative Methods to Animal Testing (EURL ECVAM) has adapted the format of DB-ALM, its unique public database on alternative methods, to fully comply with the OECD guidance for describing non-guideline *in vitro* test methods.

JRC/EURL ECVAM scientists were important contributors to the formulation of Guidance Document No.211 which was released by the OECD last December 2014. The guidance was motivated by a growing awareness within the scientific and regulatory communities that data derived from *in vitro* methods (e.g. mechanistic information) can have considerable value in supporting chemical safety assessment, even in cases where the *in vitro* method is not included within an OECD Test Guideline or has not been formally validated, provided the method is properly described in terms of its scientific/technical basis and its reliability and relevance.

Making such method descriptions readily available via the widely accessed DB-ALM (over 4400 registered users from 82 countries) will encourage their uptake and use in a variety of sectors for multiple applications, such as chemical toxicity screening or within integrated approaches to chemical safety assessment. The data generated by end-users may also aid the retrospective validation of methods intended for more routine regulatory use which may eventually be incorporated into future OECD Test Guidelines. DB-ALM already contains over 300 entries for alternative methods, at all stages of development and with various levels of detail (summary descriptions or detailed protocols). It covers mainly *in vitro* methods, but also includes non-experimental approaches.
Access:

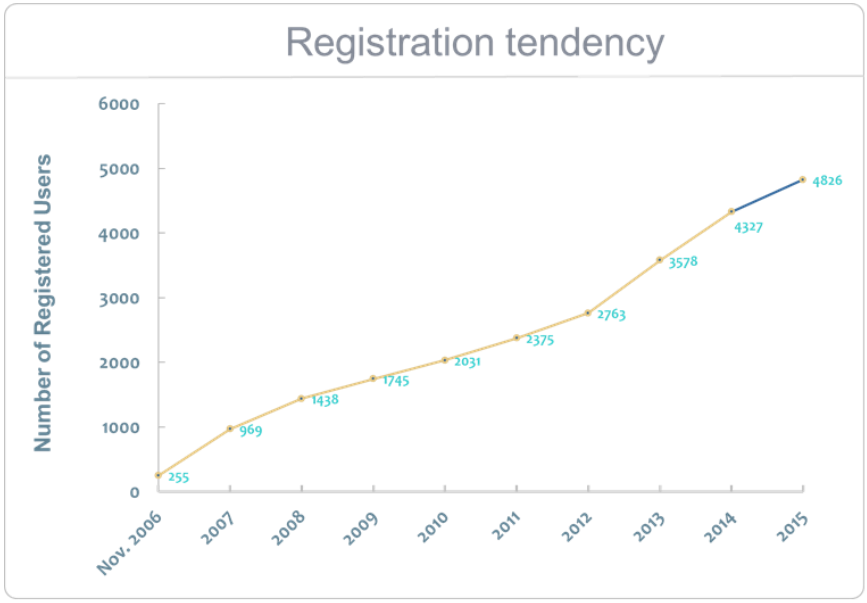
- DB-ALM: http://ecvam-dbalm.jrc.ec.europa.eu
- OECD Guidance document for describing non guideline in vitro test methods no 211

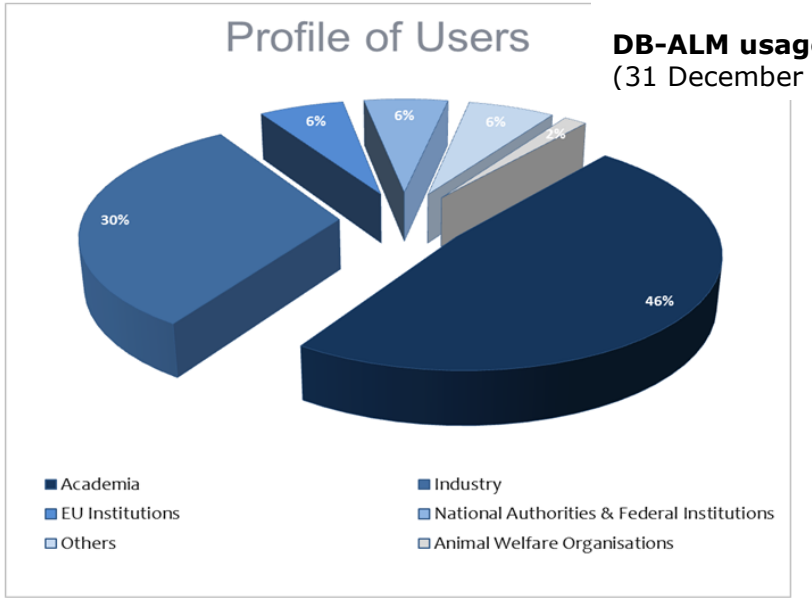
European Commission - Joint Research Centre
Institute for Health & Consumer Protection
European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)
EURL ECVAM DataBase service on ALternative Methods to animal experimentation (DB-ALM)

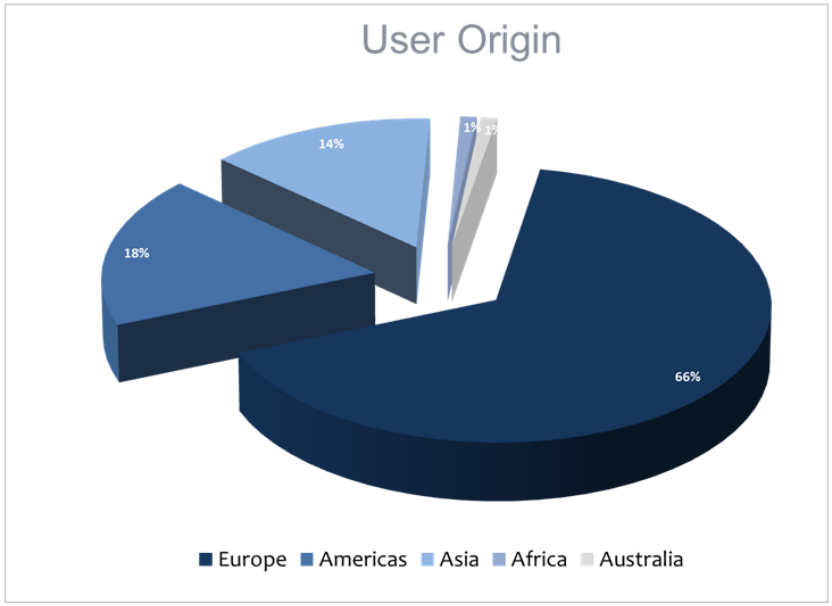
7.2 ANNEX II : DB-ALM User Community/Usage (31 December 2015)[1]

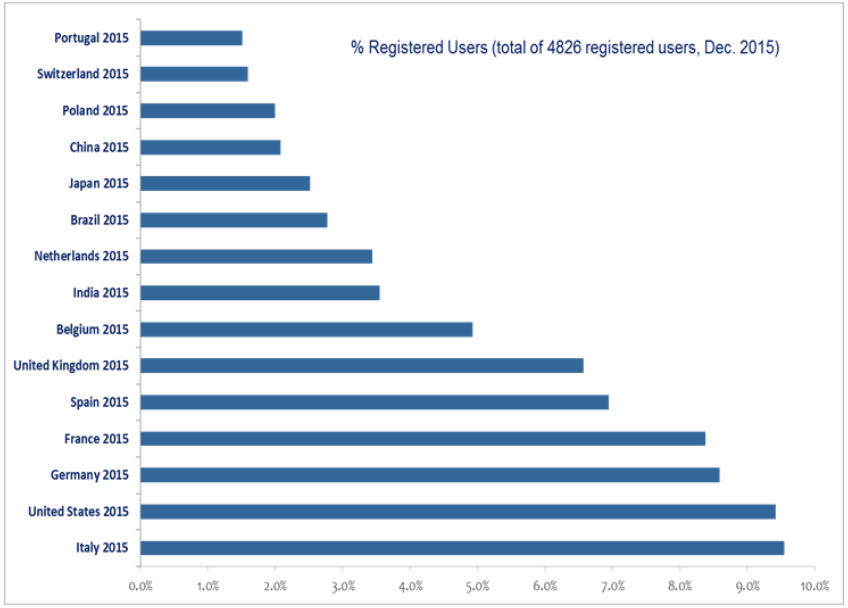
[1] **NOTE:** In September 2014 the revised DB-ALM version was released that allows data retrieval and access to method features also without registration.

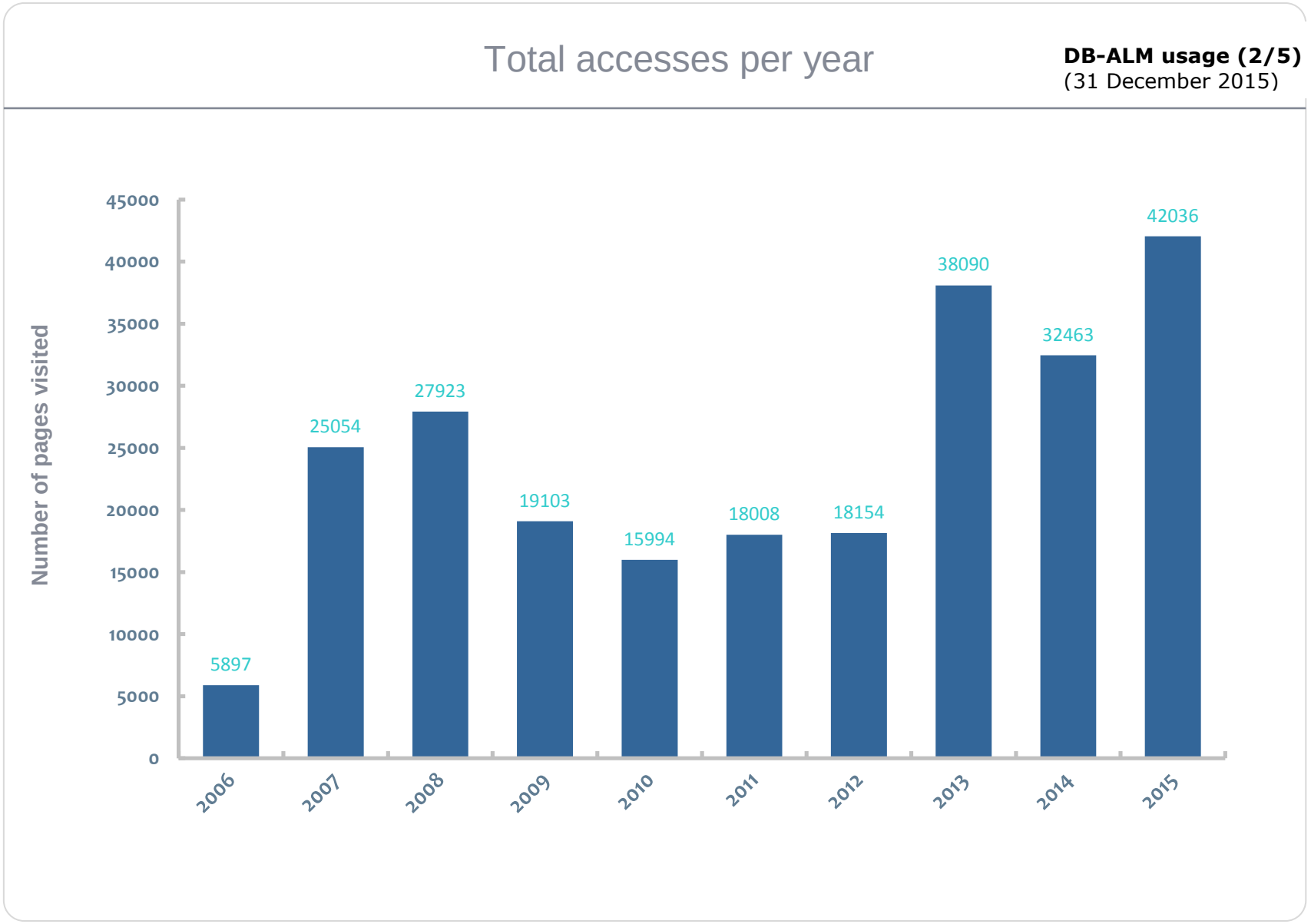
DB-ALM usage (1/5)
(31 December 2015)





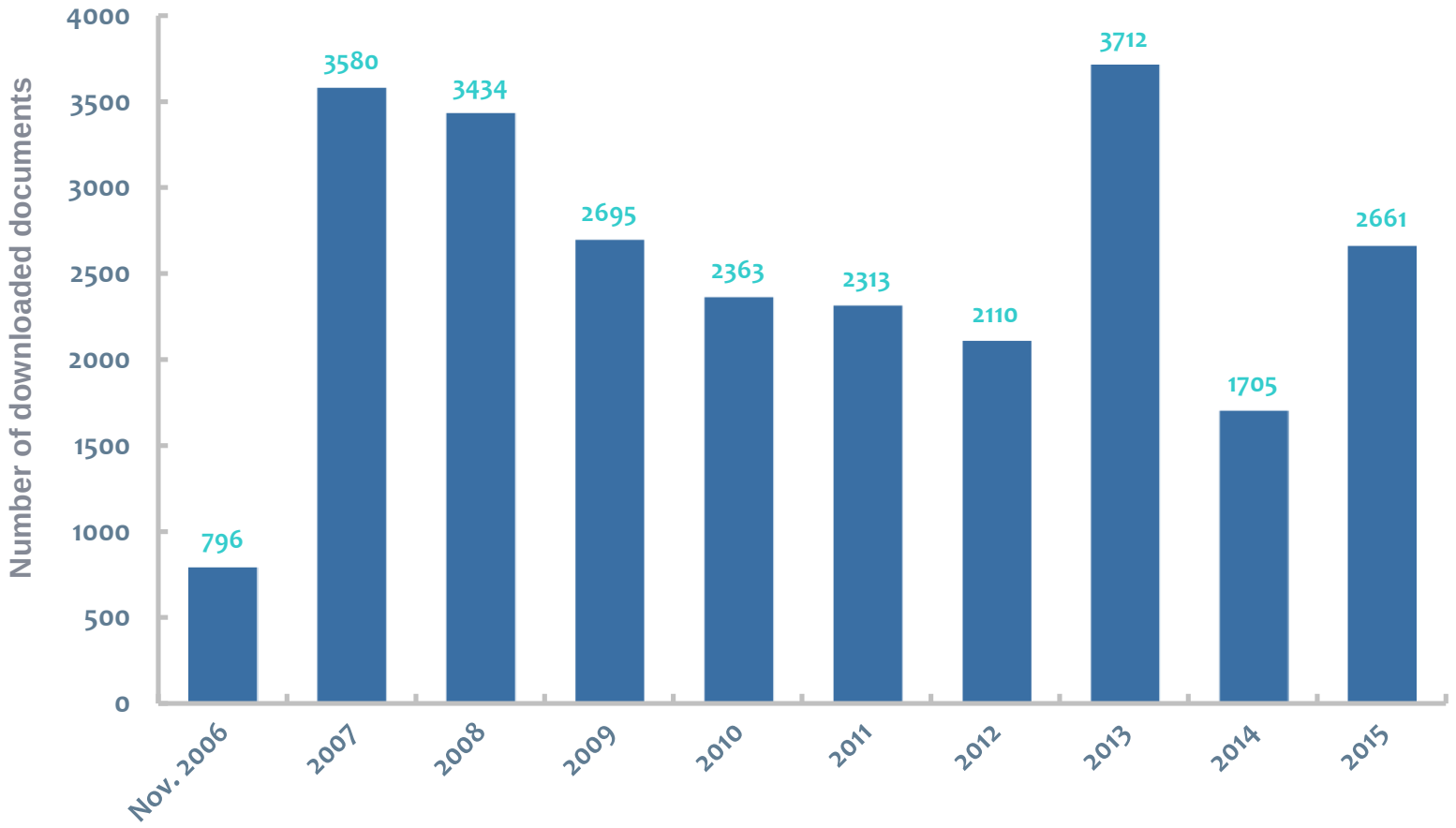




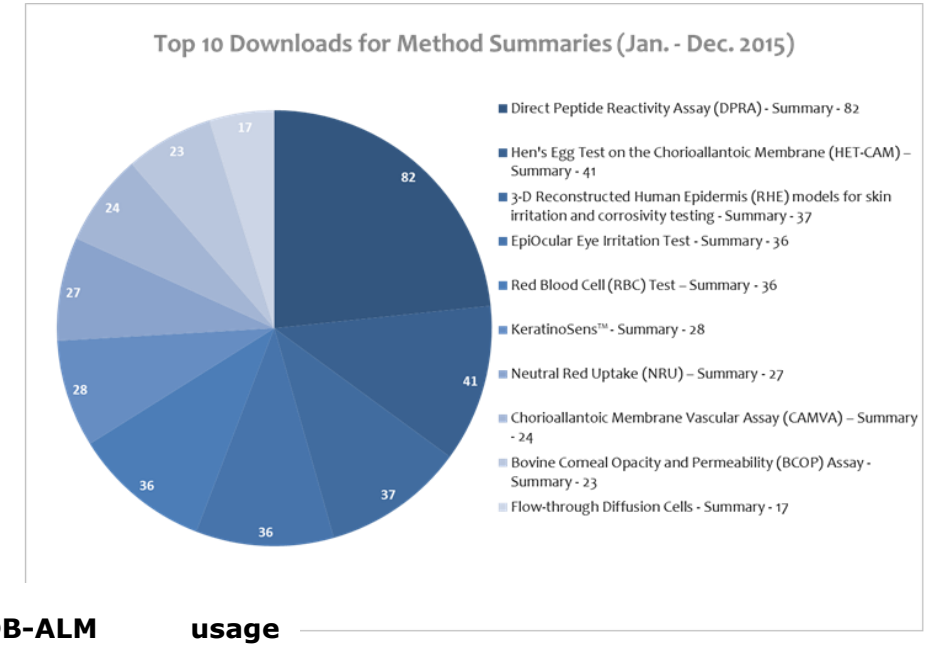

Total accesses per year
DB-ALM usage (2/5)
(31 December 2015)
Number of pages visited
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
5897
25054
27923
19103
15994
18008
18154
38090
32463
42036
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015

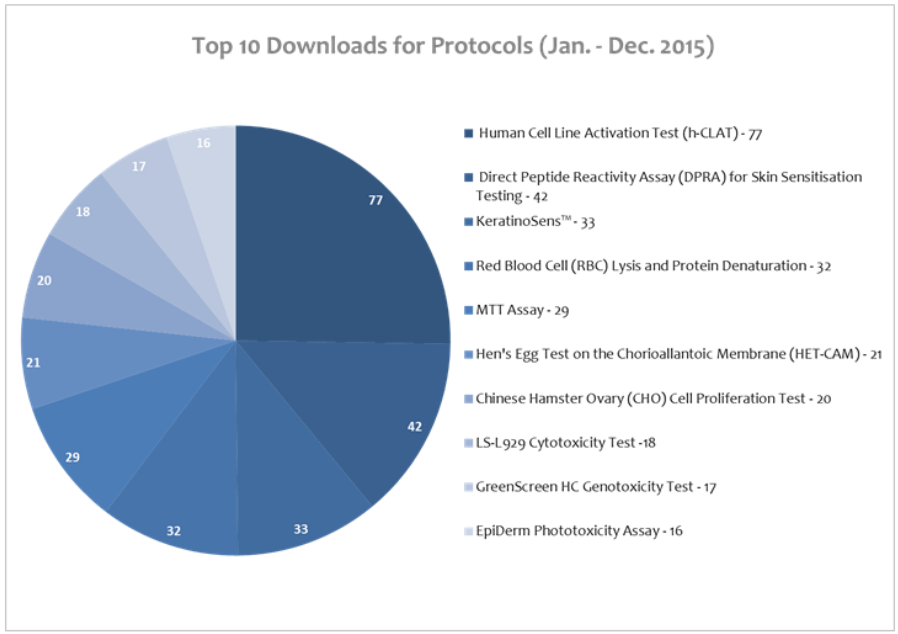
Downloaded documents

DB-ALM usage (3/5)
(31 December 2015)

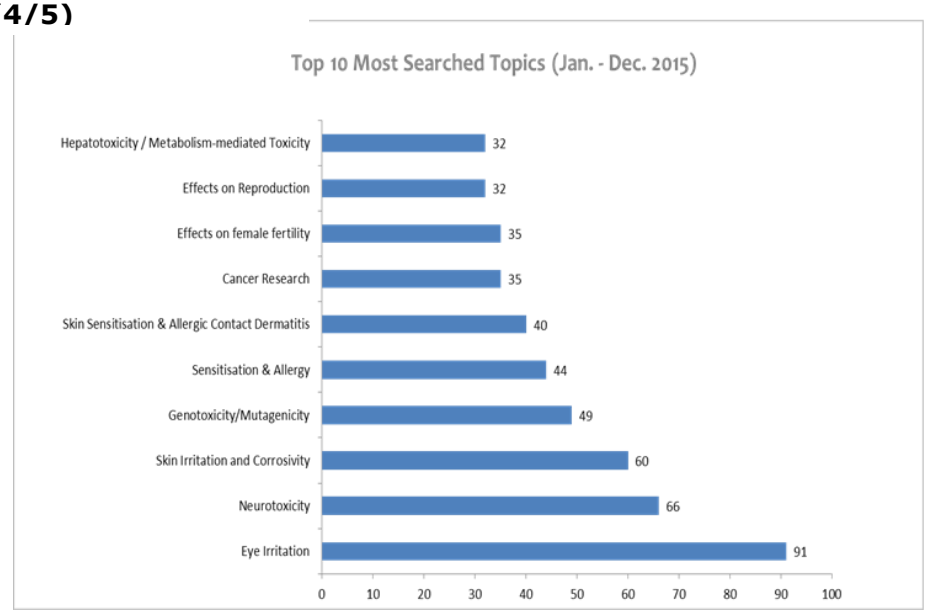


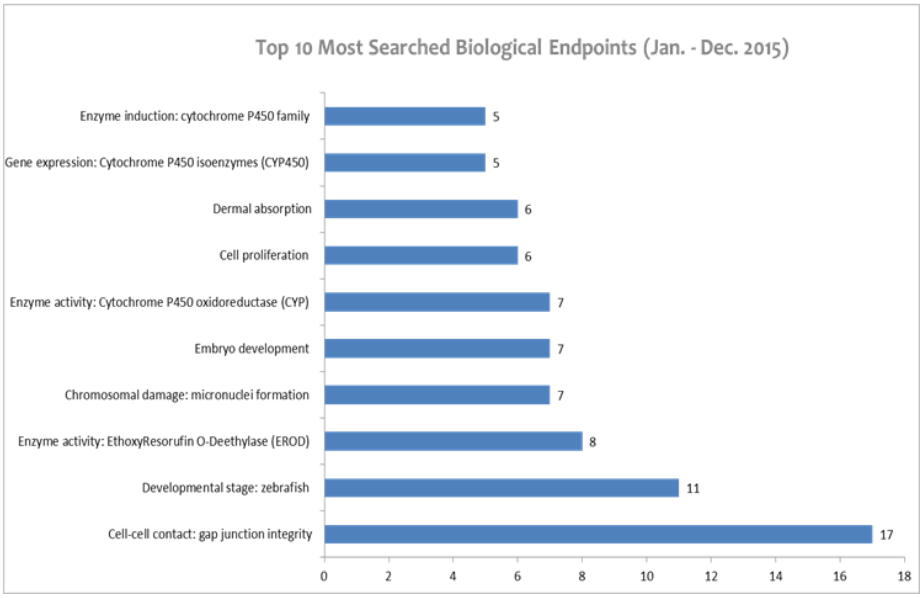
NB: Since 2014 information on key features of methods is provided without the need for registration/download





DB-ALM usage (4/5)





7.3 ANNEX III:

Method Summary in OECD Compliant Format: Criteria for Data Content

Last Update: January 2015 (v.10)

EURL ECVAM DATABASE SERVICE ON ALTERNATIVE METHODS TO ANIMAL EXPERIMENTATION

The **DB-ALM Criteria for Data Content** are based on the analysis of common descriptors from hundreds of different non-animal experimental methods and techniques collected for the purpose of providing public access *via* the EURL ECVAM DataBase service on Alternative Methods (DB-ALM).

These criteria shall ensure the comprehensive descriptions of advanced and alternative methods at *all* stages of development, validation and/or acceptance in the field of **biomedical sciences** and **toxicology** used for various compounds and materials in **research** and for **regulatory purposes**.

The present revised criteria (v10) are compliant with the "**OECD Guidance for describing non-guideline in vitro test methods"** developed to facilitate their consideration in regulatory applications.

However, the **DB-ALM format is designed to be flexible** and broad enough to allow for summary descriptions of both experimental and non-experimental methods, as appropriate, also for non-regulatory applications (see NOTE on page 3).

The DB-ALM is operated by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of the European Commission's Joint Research Centre.

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6. Abbreviations & Definitions 45
7. Bibliographic References: 46

NOTE: The content criteria are periodically reviewed to respond to emerging needs. Not all fields or sections are applicable to all methods equally. Options for free text additions are always offered by the system. It is the responsibility of the author to provide content where relevant and as appropriate related to a precise method. A review for consistency and compliance of the content provided with the criteria in place is always performed before any method will be published *via* the DB-ALM. Any comment or suggestions for amendments should be sent to dbalm-contact@jrc.ec.europa.eu .

Method Name: name of the method

Summary: One-two sentence summary on the method features

1. General information

Method developer (s)[2]

- Name, Institution, Country
  Date of data sheet creation

  Last version number

  Last update

  Notes:

Proprietary and/or confidentiality issues

Short paragraph containing information on intellectual property rights (if applicable), including the information whether the method details or any equipment, materials, software or mathematical models needed to carry out the procedure is copyright protected, trademarked, access- restricted or undisclosed.

If a distribution of the method or any of its components is limited by a copyright protection or confidentiality agreement, this should be indicated with a reference to the holder of the intellectual property right.

Assay throughput

If applicable, indication of the throughput of the assay and/or likely resource intensity should be given, e.g. low (manual assay, one chemical tested at a time), low-moderate, moderate, moderate-high, high throughput/footprint (e.g. in 96 well-plate and higher), and qualify with e.g. approximate number of chemicals/concentrations per run. If appropriate indicate whether a manual assay could be run in a higher throughput mode

Status

Should be compiled with the appropriate sections, as applicable:

In development:

Indication if the method is under development and the estimated timeline for completion as far as possible

Known laboratory use:

Summary of the current and/or past use of the method by different research groups

Participation in evaluation studies:

List of the main studies conducted using the method

Participation in validation studies: Indication whether the method has undergone any formal validation (as defined in OECD Guidance Document No. 34) or similar activities

Regulatory acceptance/compliance/standards:

Indication of any existing regulatory application and of the toxicological hazard endpoint being addressed by the method, as appropriate. If applicable, the relevant guidelines and/or on-going guideline developments with indication of the step(s) within the regulatory acceptance process (Draft Test Guideline or accepted) or any other compliance with

[2] Affiliation details are listed at the end of this document.

(inter)national standards should be listed. The details and rationale should be provided in the next section: Purpose of the Method.

2. Method Definition

Purpose of the Method

Type of Testing	:	e.g. partial (full) replacement, adjunct (3Rs relevance)
Level of Toxicity Assessment	:	e.g. toxic potential, dose response, toxic potency, hazard
Purpose of Testing	:	e.g. LOEL, mechanistic study, screening, priority setting, classification and labelling, biocompatibility, quality control or efficacy testing

NOTE: These terms are part of a controlled vocabulary used for data retrieval purposes during dissemination of the DB-ALM based on the information provided below.

Context of use & potential applications

Short paragraph on the intended use referring to the particular investigation with a proposal for the practical applications of the method:

- **Regulatory applications** (e.g. safety testing of chemicals REACH, classifications under GHS, cosmetics and pharmaceuticals regulation such as international guidelines and requirements for pharmaceutical (ICH) and veterinary (VICH) product registration, biocompatibility testing of medical devices, safety evaluations of nanomaterials, quality control of vaccines)
  and/or
- **Non-regulatory** purposes (e.g. cell culture studies, exploratory research)

The following supporting information should be provided to describe the level of scientific confidence for the previously suggested uses, such as:

- An alternative to an existing method/approach, provision of novel information for regulatory decision making or non-regulatory uses
- Positioning in the context of any existing regulatory hazard endpoint (i.e. adverse outcome), such as support to grouping of substances to support read-across and weight of evidence evaluations, priority setting, screening, mechanistic activities
- Indication of all suggested applications, e.g. either based on experimental evidence or reliable prediction model; or if it is wider, providing a justification for this (and referring to the *'Applicability'* section as appropriate)
- Its possible use in test batteries, integrated approaches to testing and assessment (IATA)/integrated testing strategies (ITS), or adverse outcome pathways (AOPs)
- In case of non-regulatory applications exhaustive information on the methods purpose and eventual evidence should be provided

NOTE: If the information specified above is not available to such a detail, the objectives and proposed application/s of the method should be summarised in one paragraph laying down the intended purpose/context for its use. The linkage between the biological endpoint investigated and the suggested use(s) should always be given.

Scientific Principle of the Method

Short paragraph containing information on the origin and the basic scientific principles of the method including the:

- *scientific rationale* and the history of the method development
- *biological basis* (e.g. modelling a specific organ, e.g., reconstituted human skin models)
- *mechanistic basis* of the method (i.e. modelling a particular mechanism by biochemical parameters e.g., the Haemoglobin Denaturation Test for Eye Irritation)
- *toxicological/biological relevance* (i.e. mode of action addressed, toxicological endpoints); in case the measured biological endpoint refers to a molecular initiating event (MIE) or any key event (e.g. mechanism of action, AOP), together with the context, also at which level of biological organisation the method can be attributed (e.g. sub-cellular, cellular, tissue and organ or individual) including the anchor point within an AOP (as applicable)

NOTE: Any biological endpoint(s) investigated and indicated should always be put in the context concerning biology/physiology leading to the *in vivo* response or effects.

Procedure Description

Biological endpoint & Endpoint measurement

Indication of the *biological processes, responses or effects* assessed and techniques used for their measurement with a short description, such as e.g. "*corneal opacity measured using an opacitometer*".

NOTE: Biological endpoint terms will be reviewed against DB-ALM controlled vocabulary.

Biological endpoint value

Precise endpoint assay investigated (e.g. IC50, NOEL) if applicable

Experimental system

Name, category and species source of the material for the endpoint being measured, such as *"freshly isolated bovine cornea" (organotypic)*
NOTE: Terms will be reviewed against DB-ALM controlled vocabulary

NOTE: Provide information if the material is readily available commercially or whether it is developed in the laboratory on site (e.g. cell suspension from tissue). Indicate source/manufacturer of the biological material used.

Metabolic competence

If applicable, indicate to which extend the system can be considered metabolically competent, either by itself or enzymatic fractions added. Indicate the name of the system used

Exposure regime

Application volume	:	Volume of the test substance applied and volume unit
Concentration	:	Concentration of test substance applied (dose and/or min and max used)
Dosage	:	Amount of substance administrated (dosing and/or min and max dosage used)
Application type	:	Single/repeated dose or continuous dosing
Exposure Time	:	Period in time unit of contact between the tested

		substance and the experimental system
Controls	:	Negative and positive controls and other references or Standards used
Other Details	:	Other useful parameters not listed above, e.g.: name of the method used for detecting the test substance

Summary

A short description of the basic procedure; e.g. the exposure regime, including observation frequency, replicate number, and key steps, together with the references to the appropriate section of the DB-ALM protocol (as applicable). A DB-ALM Method Summary may apply to multiple protocols with different exposure regimes where the structured *Exposure regime* section above cannot be filled in detail, but an overall outline should be given here.

Quality/Acceptance Criteria

Whenever appropriate, information should be provided on the availability of acceptance criteria and quality assurance in place in the form of a short and easily overseeable list, as it pertains to, such as:

- Criteria for experimental system acceptance
- Criteria for acceptance or rejection of experimental data
- Experimental data storage/archiving done
- Internal reference standards/chemicals/performance benchmarks
- Equipment used/calibration programmes
- Quality systems in place, such as Good Laboratory Practice (GLP) or Good Cell Culture Practice (GCCP), ISO 9001 or similar.

Data Interpretation and/or Prediction Models

Data analysis

Summary of the data analysis procedures applied to the raw data, specialised software (if applicable), statistical approach used and rationale for its choice. If applicable, information on data interpretations and/or evaluations should be given.

- Specification of the software name and version used for data analysis

Prediction model (PM)

If appropriate, the rules for the prediction of the *in vivo* toxicity potential from *in vitro* test data or any other PM used should be provided, including any eventual criteria for the classifications. Detailed descriptions of large and complex models can be attached as supporting document(s) and references to publications that describes it into detail be made.

- Specification of the software name and version used for algorithm/prediction model generation.

The prediction model could part of a more complex PM that relies on different methods (e.g. IATA, test battery). In such case, it is recommended to provide this information separately with individual method descriptions or by compiling a project/study summary in the DB-ALM where it can be described entirely or make reference to relevant publications.

In some cases it may be helpful to consider the intended purpose of the prediction model in the context of an AOP: e.g. a desire to predict a given outcome.

3. Method Performance

The information on the robustness of the method i.e. the reliability of the (experimental) results and the prediction capability of the model used is expected in this section. Information is to be provided under the following paragraphs (as appropriate):

Robustness of the Method

The information may be available at various levels of detail according to the development status and the nature of the method. Information on repeatability, reproducibility and between laboratory transferability should be reported if known, or any other information relevant for the evaluation of the method depending on the method's purpose.

Within-laboratory reproducibility

Evidence of the agreement among results obtained from testing the same set of compounds over time using the same protocol in one laboratory, including an explanation on possible sources of variability (if applicable).

Between-laboratory transferability and reproducibility

The following information should be provided (if known):

- an estimation of the amount of training that is necessary to establish the test method in a naïve laboratory
- obstacles or difficulties that may impact on the transferability of the test method, e.g. level of complexity of some procedures in the protocol(s).
- the results of the assessment of the between-laboratory reproducibility of experimental data

General performance measures

For methods, where between-laboratory transferability and reproducibility studies might not be applicable, available and/or feasible, it may be helpful to include information on other performance measures or similar under this section. If applicable, the explanations of the curve fitting process together with the assumptions used to determine the goodness-of-fit and any limitations related to the data analysis, as well as any other means for evaluating the performance of a method can be provided here.

Test Compounds

As applicable, a summary record with information on the test compound categories, chemical classes and sets tested during the method's development, evaluations, PM development and /or validation (as applicable) is expected in this section. In particular the rationale for the choice of the given test compounds/reference chemicals/chemical libraries/test materials/nano-materials/ pharmaceuticals or other products.

The paragraph should include the references to the sources where the detailed lists of the tested compounds and results are published and/or made available and to which level of detail: e.g. names, MOL files, CAS umbers, IUPAC names, InCHI codes, availability from commercial sources, composition (for mixtures) characteristics/properties (for nano-materials) and the endpoint values obtained and unit of the raw data. If the access to data is restricted, the reason why should be stated.

Predictive capacity

Available information on the predictive capacity of the method should be provided here, with respect to its ability to measure or predict the effect/event/mechanism/response of interest with the test compounds for which the method has been intended. If available, the following accuracy values should be included: overall accuracy, sensitivity, specificity, positive and negative predictive values, false positive and negative proportions, comments on any unexpected outcome as appropriate and indication of preliminary evaluations performed.

Applicability

Brief description of the types of test material that are compatible with the method and/or for which the method has been intended and judged as appropriate, based on the available evidence and indicating possible limitations.

If possible, the applicability domain may be characterised and suggested by e.g.: use classes (chemicals (full spectrum, specific types and with specific uses), vaccines, hormones, medical devices) or physicochemical descriptors (e.g. in case of nanomaterials).

Issues encountered with any chemical classes during a study should be listed, whether it is not suitable or requires extra precautions (e.g. volatile, viscous, highly coloured or low solubility compounds).

In cases when only a limited number of substances (i.e.<10) would have been tested up to now, a list of the substances by names, grouped in logical chemical group/categories should be provided.

NOTE: Additional documents/data sets can be uploaded to this Method Description for further clarification. The section is also interlinked with the Test Results DB-ALM data sector where experimental data can eventually be entered/uploaded.

4. Discussion

Comments should be provided in brief on the following factors (as appropriate):

Ethical issues and considerations for 3R's impact

- e.g., use of animals to obtain primary cells, use of human stem cells

Known strength and/or limitations of the method

- Duration and complexity of the test
- Costs (approximate cost for testing e.g. 10 compounds)
- Comparison of the method to other similar assays (if known) that may characterise the same endpoint/key event as described in Procedure Description section
- Eventual advantages, issues/limitations of the experimental system (amenable to cryopreservation or only freshly or prepared, availability, reproducibility over time)
- Not suitable for certain classes of chemicals

Technical requirements

- Technical complexity of the method regarding special equipment, controlled environment, software and amount of training required

Modifications of the method

- Known differences in conducting this method, identified from different/same research group(s) and the rationale

Potential for future development

- Potential of the method for up-scaling and/or automation
- Other on-going developments

Comparisons to other methods

- As appropriate indication/comparison to other method developments or related

Additional Considerations

- Indication of information not covered by the previous headings

5. Contact Details

Contact details of persons and organisations familiar with the method (authors, developers or experienced users).

To be listed in the following format:

Contact Person(s):

Function:

- Method developer/user
- Data sheet provider

Full Address:

Tel:

E-mail:

Contact Person:

Function: (see above)

Full Address:

Tel:

E-mail:

Versioning:

Name	Type of changes	Version number	Date

6. Abbreviations & Definitions

To be listed in an alphabetical order for all key terms included in the method summary and using the following format:

NRR: Neutral Red Release

7. Bibliography

- **Method development**

List of all bibliographic references to original paper(s) explaining the method development (cited in the Section: **Scientific Principle of the Method**).

- **General**

Reports useful references other than those directly associated with the assay or prediction model development.

All cited articles and other sources should be listed in accordance with the rules of the data entry format of the content management system of the DB-ALM

Last Update: Last data sheet revision

NOTE: All indications or eventual statements shall be supported with references within the text and full details listed under "**Bibliographic References***"* data sector.

DB-ALM Content Criteria - Glossary

3Rs:

Reduction (of animal use); Refinement (to lessen pain or distress and to enhance animal well-being); and Replacement (of an animal test with one that uses non-animal systems or phylo-genetically lower species).

AOP: Adverse Outcome Pathway

For comprehensive information on the concept and realisation of the Adverse Outcome Pathway conceptual framework, access at:

- OECD Adverse Outcome Pathways: Molecular Screening and Toxicogenomics
- https://aopkb.org/

Biological Endpoint:

Comprises biological processes, responses or effects assessed by a given method. The techniques used to assess the biological endpoint are referred to as endpoint measurement.

CAS number: Chemical Abstract Service Registry is a unique numeric identifier that designates only one substance and has no chemical significance.

Category formation and read-across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across; see also

- OECD Guidance for Describing Non-guideline in vitro Test Methods

DB-ALM: EURL ECVAM DataBase service on ALternative Methods to animal experimentation; access at:

- http://ecvam-dbalm.jrc.ec.europa.eu

GHS: Globally Harmonized System of Classification and Labeling of Chemicals, an internationally agreed-upon system, created and implemented by the United Nations.

IATA: Integrated approaches to testing and assessment

The method described may form one component of an IATA; A detailed example and description of the concept and the realization of IATA can be found here:

- http://www.oecd.org/env/ehs/testing/

ICH: The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, a trilateral (EU-Japan-USA) programme bringing together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration

- www.ich.org

InCHI code: The IUPAC International Chemical Identifier, a non-proprietary identifier for chemical substances that can be used in printed and electronic data sources thus enabling easier linking of diverse data compilations.

ITS: Integrated Testing Strategies; the objective of an ITS is to give guidance on a stepwise approach to hazard identification with regard to a given endpoint; a key principle of the strategy is that the results of one study are evaluated before another is

initiated. The strategy should seek to ensure that the data requirements are met in the most efficient and humane manner so that animal usage and costs are minimised. (ECHA, Guidance on Information Requirements and Chemical Safety Assessment)

- Chapter R.7a: Endpoint specific guidance

IUPAC: The International Union of Pure and Applied Chemistry

LOEL: Lowest Observed Effect Level

MIE: Molecular Initiating Event

The first point of chemical- biological interaction within an organism which starts the adverse outcome pathway.

MOL: A file format for holding information about the atoms, bonds, connectivity and coordinates of a molecule

NOEL: No Observed Effect Level

The level of exposure of an organism, at which there is no biologically or statistically significant effects when compared to the appropriate control

Priority setting: The assay might help prioritise substances within an inventory for more detailed evaluation; see also

- OECD Guidance for Describing Non-guideline in vitro Test Methods

REACH: EU Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency

- http://echa.europa.eu/support/testing-methods-and-alternatives

Robust(ness): The insensitivity of test results to departures from the specified test conditions when conducted in different laboratories or over a range of conditions under which the test method might normally be used. If a test is not robust, it will be difficult to use in a reproducible manner within and between laboratories (see also OECD Series on Testing and Assessment Nr 34: Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment).

- http://www.oecd.org/env/ehs/testing/

Validation: The process by which the reliability and relevance of a particular approach, method, process or assessment is established for a defined purpose. A novel method can become a candidate for further validation studies and standardisation in order to achieve international acceptance (see also OECD Series on Testing and Assessment Nr 34: “Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment”)

- http://www.oecd.org/env/ehs/testing/

VICH: The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products, a trilateral (EU-Japan-USA) programme aimed at harmonising technical requirements for veterinary product registration

- www.vichsec.org

7.4 ANNEX IV:
Software Developments for System Evolution

DB-ALM version 2014/15/16

The following updates were made to the DB-ALM version 2014/15/16 and put live.

- Added major improvements to the free text search for Methods, especially those using logical operators e.g. AND, OR etc.

  Search finds results in

  - topic name
  - method name
  - method body
  - biological endpoint terms and measurements
  - experimental system terms and descriptions
  - compound names, trade names, synonyms, CAS and EC numbers
  - bibliography titles and authors
  - contact names
  - institution names
  - statuses
  - models
  - areas

- Resumes were completely re-developed. They are now a first class citizen with their own table and can exist independently of Protocols
- A Method number was added to Method Summaries with the ability to search for that number
- The Registration procedure was redeveloped. This including the forgotten password function and Account update page
- Added the ability to sort Method results by Models & Strategies, Experimental System and Regulatory Status

- Added the ability to create a Method summary in OECD compliant structure and view and convert existing Method Summaries to OECD format

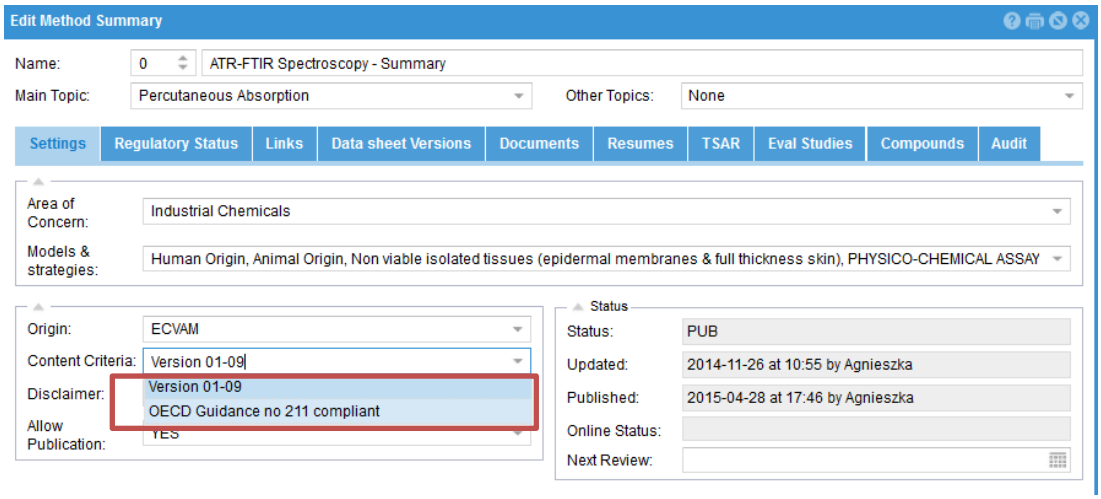


Figure 4: The screen capture of the DB-ALM Content Management System, showing the settings for applying new Content Criteria.

- Added printing for Topic Summary list, Methods list, Glossary list and Home pages
- Improved the usability of results page. When scrolling, the EU header scrolls off the page but the search options stay visible at the top of the page.
- Redesigned and implemented the layout the compound list displayed inline and in a popup
- Added a feedback questionnaire
- Based on user feedback modification to the layout of the menus and content on home page were made
- All items can be published from the new admin system, Topics, Method summaries, Protocols, Validation studies, Resumes, Evaluation studies, Contacts, Biblios, Sources, Acronyms, Publishers, Experimental systems, Endpoints, Compounds, Test results, Study participants, Study labs, Study compounds, Study test results
- All actions of the website visitors are logged from now on. This allows us to analyse exactly how users are using the website and perhaps make improvements in the future.
- Created a preview of a log analysis tool to report DB-ALM website usage
- Created a virtual machine with Windows 10 for testing with new MS Edge browser
- Moved all log tables to separate oracle user and table space for each environment e.g. staging, pre-public, public for better separation on environments and security
- Moved all security tables to separate oracle user and table space for each environment e.g. staging, pre-public, public for better separation on environments and security
- Replaced one poorly designed and very complicated database table that holds study information for Validation study/protocols with six separate tables. The new design also caters for the ability to have study information for Validation study/Method summaries

- Changed the website to run HTTPS using the JRC reverse proxy
- Made server configuration changes to improved security

The following updates were made to the DB-ALM version 2014/15/16 in staging and are ready to go live in the course of 2016:

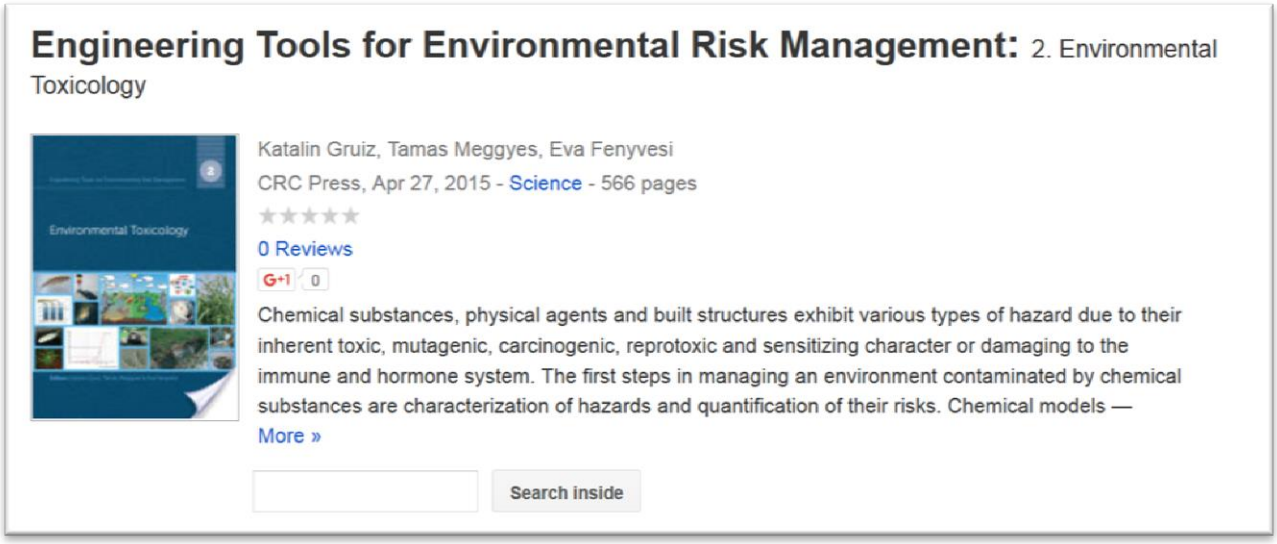
- The remaining sections of the old website were moved to /pre and made the /beta website the main site. This work included also additional changes and improvement to the service:
  1. Added pages Links, Faq, Privacy Statement
  2. Sub-pages on home page e.g. related projects, now have their own page/URL
  3. Added Projects and Studies section
  4. Added People and Institutions section
  5. Added Bibliography section
  6. Improved search engine optimisation by removing index.cfm from URL, giving each page a friendly human readable URL
  7. Improved search engine optimisation by giving all free text searches, filters, sorts etc. a friendly URL that can be linked to from other sites and indexed by search engines
  8. Converted the javascript to typescript/ES6 and rewrote to make code more generic, clean, and reusable
  9. Redesigned the CSS usage and page layout
  10. Added support for mobile devices
  11. Added favicons and configuration files for apple, android and microsoft devices
  12. Added facebook and twitter metadata so links to DB-ALM on their sites display our branding and content

7.5 ANNEX V: Bibliographic Citations

BOOKS published in 2014-2016 referring to the BD-ALM as information resource

(conducted on 22.06.2016; using Google Books search engine)

1. **Gruiz, K., Meggyes, T., and Fenyvesi, E. (2015). Engineering Tools for Environmental Risk Management: 2. Environmental Toxicology (CRC Press).**



Engineering Tools for Environmental Risk Management: 2. Environmental Toxicology

Katalin Gruiz, Tamas Meggyes, Eva Fenyvesi

CRC Press, Apr 27, 2015 - Science - 566 pages

0 Reviews

Chemical substances, physical agents and built structures exhibit various types of hazard due to their inherent toxic, mutagenic, carcinogenic, reprotoxic and sensitizing character or damaging to the immune and hormone system. The first steps in managing an environment contaminated by chemical substances are characterization of hazards and quantification of their risks. Chemical models — More »

Search inside

The book contains numerous references to the DB-ALM in the chapters on Carcinogenity, Genotoxicity, Eye Irritation and Corrosion and Toxicokinetics:

- Page 138 »

Draft OECD TG (NHK NRU, 2010)	Normal Human Keratinocyte (NHK) Neutral Red Uptake Assay	Adjunct to *in vivo* acute oral toxicity tests for determining starting doses	ICCVAM
ECVAM DB-ALM Protocol No.101 (CFU GM, 2006)	Colony Forming Unit-Granulocyte/Macrophage Assay	Hematotoxicity test for acute neutropenia	ECVAM DB-ALM

- Page 144 »

DB-ALM (2014) database include:

- *Cytotoxicity and Genotoxicity in Primary Cultures of Human Hepatocytes* of the test compound by measuring cell viability, DNA damage and unscheduled DNA synthesis (DB-ALM Protocol No.16);
- *Stable Cell Lines Expressing Cytochromes CYP cDNA* can be inserted into cell lines such as V79 Chinese hamster cells, which are used for *in vitro* toxicity testing. This means that the metabolites of xenobiotics are produced in the same cells in

- Page 145 »

chromatography (HPLC) (DB-ALM Protocol No.8);
- *Alkaline Unwinding Genotoxicity Test* applies to mouse lymphoma cells cultured in the presence of test chemicals, with or without a metabolic activating system, and resultant DNA-strand breaks detected by alkaline unwinding and hydroxyapatite elution (DB-ALM Protocol No.19);
- *Prostaglandin H Synthase (PHS) mediated Genotoxicity of Xenobiotics*. This protocol describes the use of SEMV cells (a cell line derived from ram seminal vesicles) for studying prostaglandin H synthase-mediated metabolism of xenobiotics in

- Page 148 »

guidelines and, additionally, the old *in vitro* chromosome aberration assay has been replaced with the *in vitro* micronucleus test for genotoxicity testing (see Table 3.6 in Section 3.3.2).

Numerous other *in vitro* genotoxicity tests, including the *in vitro* Comet assay, are being developed but are not yet validated. ECVAM DB-ALM Database, (2014) comprises some protocols for carcinogenicity testing:

- *Lucifer Yellow Intercellular Exchange assay* for Tumor Promoters: the effect of the

- Page 156

... Table 3.15 In vitro ocular test methods considered valid for limited regulatory testing (OECD, 2014; DB-ALM, 2014). ... ECVAM **DB-ALM Protocol** No.85 Isolated Rabbit Eye (IRE) assay Eye corrosion Ongoing/CLP R41 **DB-ALM Protocol** ...

- Page 159

The permeability is expressed as apparent permeability coefficient (Papp) (**DB-ALM Protocol** No.142); – Metabolic Stability assay can be used to rank the test compounds with respect to their metabolic stability or biotransformation.

- Page 160

ECVAM **DB-ALM** established a **protocol** titled: "Whole Rat Brain Reaggregate Spheroid Culture''. This culture system (single cell Table 3.19 In vivo testing of endocrine disrupting effect of 160 Engineering Tools for Environmental Risk ...

- Page 161

... for Detection of Estrogenic Agonist-Activity of Chemicals suspension) allows the testing of neurotoxic compounds during development, differentiation and relative maturity of the brain reaggregate (**DB-ALM Protocol** No.11 (DB-ALM, 2014).

- Page 165

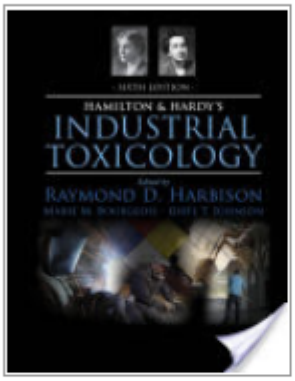
DB-ALM Protocol No 101. [Online] Available from: http://ecvam-dbalm.jrc.ec.europa.eu. [Accessed 20th October 2013]. Coecke, S., Blaauboer, B.J., Elaut, G. et al. (2005) Toxicokinetics and metabolism. Alternatives to Laboratory Animals, 33(1) ...

- Page 166

ECVAM-DB-AL INVITTOX **protocol**. [Online] Available from: http:// ecvam-**dbalm**. jrc.ec.europa.eu/public_view_doc.cfm?id=6E7E72104B2DEFD6BE979B3B13 9176C67180BB0BC12CB10496CDA74B54630A05A3291B895581F634.

2. **Harbison, R.D., Bourgeois, M.M., and Johnson, G.T. (2015). Hamilton and Hardy's Industrial Toxicology (John Wiley & Sons).**

Hamilton and Hardy's Industrial Toxicology



Raymond D. Harbison, Marie M. Bourgeois, Giffe T. Johnson

John Wiley & Sons, Mar 16, 2015 - Science - 1368 pages

★★★★★

0 Reviews

G+1 0

Providing a concise, yet comprehensive, reference on all aspects of industrial exposures and toxicants; this book aids toxicologists, industrial hygienists, and occupational physicians to investigate workplace health

More »

Search inside

Preview this book »

Makes references to a number of DB-ALM protocols:

ECVAM DB-ALM (1994a) *INVITTOX Protocol No. 100*. European Centre for the Validation of Alternative Methods, Database Service on Alternative Methods to Animal Experimentation, European Comission. Available at http://ecvam-dbalm.jrc.ec.europa.eu/ (last accessed April 10, 2014).

ECVAM DB-ALM (1994b) *INVITTOX Protocol No. 99*. European Centre for the Validation of Alternative Methods, Database Service on Alternative Methods to Animal Experimentation, European Commisson. Available at http://ecvam-dbalm.jrc.ec.europa.eu/ (last accessed April 10, 2014).

ECVAM DB-ALM (1995) *INVITTOX Protocol No. 108*. European Centre for the Validation of Alternative Methods, Database Service on Alternative Methods to Animal Experimentation, European Commisson. Available at http://ecvam-dbalm.jrc.ec.europa.eu/ (last accessed April 10, 2014).

3. **Baki, G., and Alexander, K.S. (2015). Introduction to Cosmetic Formulation and Technology (John Wiley & Sons).**

Introduction to Cosmetic Formulation and Technology

Gabriella Baki, Kenneth S. Alexander

John Wiley & Sons, Apr 27, 2015 - Science - 776 pages

0 Reviews

Designed as an educational and training text, this book provides a clear and easily understandable review of cosmetics and over the counter (OTC) drug-cosmetic products. The text features learning objectives, key concepts, and key terms at the beginning and review questions and glossary of terms at the end of each chapter More »

Search inside

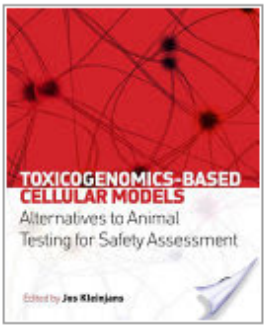
Preview this book »

Source of citations:

80. Fluorescein Leakage (FL) Test, DB-ALM Protocol n° 71, Accessed 3/15/2014 http://ecvam-dbalm.jrc.ec.europa.eu/public_view_doc2.cfm?id=C06709AA034D363CC6F6AB5BAFADFF7180BB0BC12CB10496CDA74B54630A05A3291B895581F634

4. **Kleinjans, J. (2014). Toxicogenomics-Based Cellular Models: Alternatives to Animal Testing for Safety Assessment (Academic Press).**

Toxicogenomics-Based Cellular Models: Alternatives to Animal Testing for Safety Assessment



Jos Kleinjans

Academic Press, Jan 2, 2014 - Medical - 362 pages

★★★★★

0 Reviews

G+1 0

Toxicogenomics-Based Cellular Models is a unique and valuable reference for all academic and professional researchers employing toxicogenomic methods with respect to animal testing for chemical safety. This resource offers cutting-edge information on the application More »

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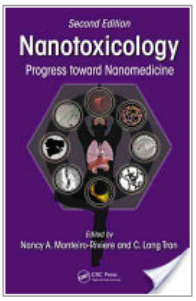
Source of citations:

[8] Piersma AH. Embryotoxicity Testing in Post-Implantation Whole Embryo Culture (WEC): Method of Piersma. ECVAM DB-ALM Protocol No. 123. 1999.

5. **Monteiro-Riviere, N.A., and Tran, C.L. (2014). Nanotoxicology: Progress toward Nanomedicine, Second Edition (CRC Press).**

Nanotoxicology: Progress toward Nanomedicine, Second Edition



Nancy A. Monteiro-Riviere, C. Lang Tran
CRC Press, Mar 3, 2014 - Medical - 514 pages
★★★★★
0 Reviews
G+1 0

Since the first publication of this book in 2007, the field of nanoscience and nanomedicine continues to grow substantially. This second edition, **Nanotoxicology: Progress toward Nanomedicine,** enlists internationally recognized experts to document the continuing development and rationale for the safe design of engineered nanomaterials (ENM). This includes new improved characterization endpoints, screening, and detection methods for in vitro and in vivo toxicity testing. These tools also contribute greatly to nanosafety research applied to nanomedicines.

Topics include

- The impacts of nanotechnology on biomedicine, including functionalization for tissue-specific targeting, the biointeractions of multifunctional nanoparticle-based therapy, and the ability to control specific physicochemical properties of nanoparticles
- The requirements for proper detection, measurement, and assessment both for workplace exposure and in consumer products—with a focus on potential health and safety implications
- Predictive modeling, using quantitative nanostructure activity relationships to predict the pharmacokinetics and biodistribution of nanomaterials in the body
- Specific methodologies, imaging, and techniques to assess nanomaterials from the manufacturing process to nanomedicine applications
- Tools for assessing nanoparticle toxicity and the limitations of detection methods for assessing toxicity in both in vivo and in vitro systems and at the single cell and tissue levels
- Toxicity of nanomaterials to specific organ systems, cell–based targeting to tumors, and other biomedical applications
- The difficulty of conducting risk assessments and the need for addressing knowledge gaps, especially with long-term studies
- A roadmap for future research

The development of nanotechnology-based products must be complemented with appropriate validated methods to assess, monitor, manage, and reduce the potential risks of ENM to human health and the environment. This volume provides a cogent survey of advances in this area by a well-respected and diverse group of international scientists.

Source of citations:

Page 362 »

Approach		Name/Description	Validation Stage and Protocol Reference (If Available)
Ex vivo	Whole embryo tests	Embryotoxicity testing in postimplantation WEC	ECVAM DB-ALM 123
		FETAX	Prevalidation
		DaRT	Prevalidation

Page 372 »

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14. Pamies D., Martinez C., Sogorb M. & Vilanova E. (2011). Mechanism-based models in reproductive and developmental toxicology. In: Gupta R. C. (ed.) *Reproductive and Developmental Toxicology*. Academic Press, London.
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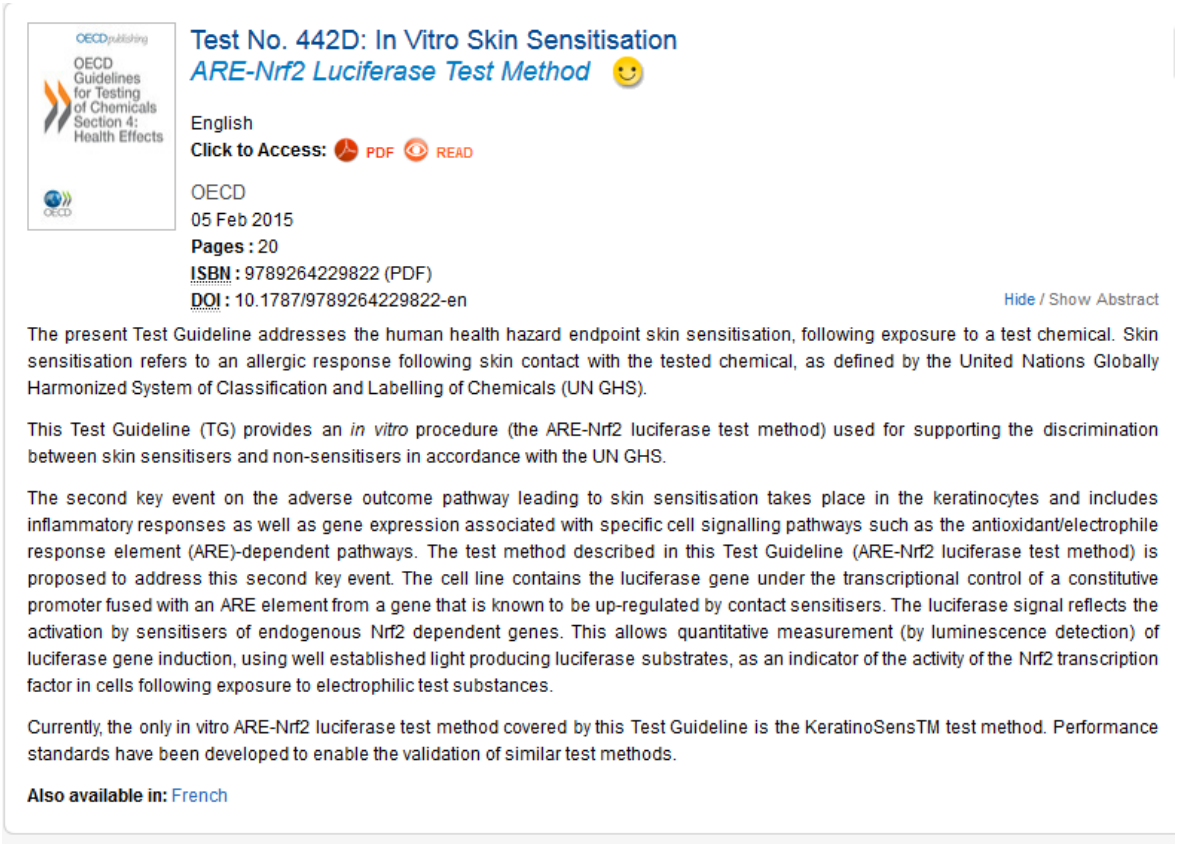
(search conducted on 22.06.2016 on the OECD website)

1. OECD TG 422D*:* Kreatinosens



Test No. 442D: In Vitro Skin Sensitisation
ARE-Nrf2 Luciferase Test Method

English
Click to Access: PDF READ

OECD
05 Feb 2015
Pages : 20
ISBN : 9789264229822 (PDF)
DOI : 10.1787/9789264229822-en

Hide / Show Abstract

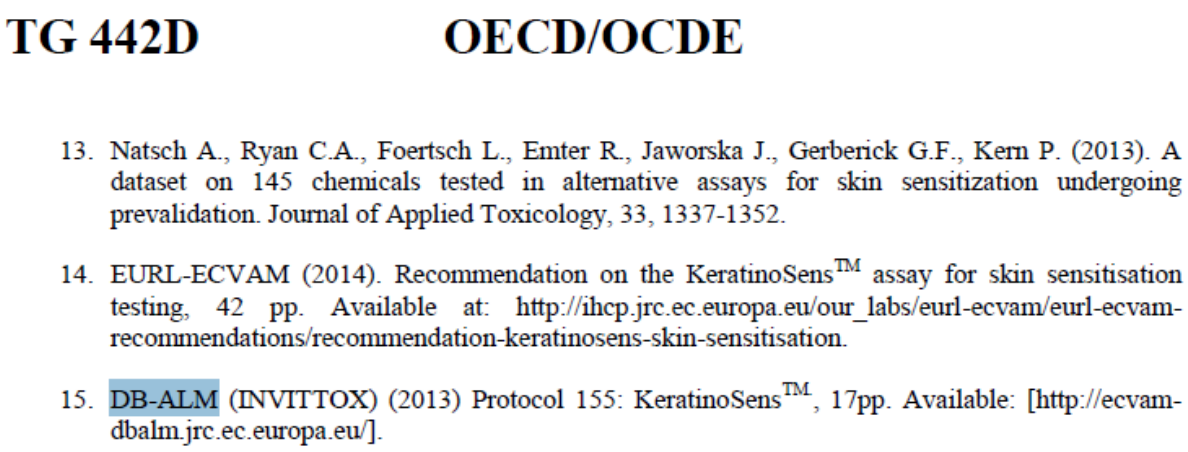
The present Test Guideline addresses the human health hazard endpoint skin sensitisation, following exposure to a test chemical. Skin sensitisation refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS).

This Test Guideline (TG) provides an *in vitro* procedure (the ARE-Nrf2 luciferase test method) used for supporting the discrimination between skin sensitisers and non-sensitisers in accordance with the UN GHS.

The second key event on the adverse outcome pathway leading to skin sensitisation takes place in the keratinocytes and includes inflammatory responses as well as gene expression associated with specific cell signalling pathways such as the antioxidant/electrophile response element (ARE)-dependent pathways. The test method described in this Test Guideline (ARE-Nrf2 luciferase test method) is proposed to address this second key event. The cell line contains the luciferase gene under the transcriptional control of a constitutive promoter fused with an ARE element from a gene that is known to be up-regulated by contact sensitisers. The luciferase signal reflects the activation by sensitisers of endogenous Nrf2 dependent genes. This allows quantitative measurement (by luminescence detection) of luciferase gene induction, using well established light producing luciferase substrates, as an indicator of the activity of the Nrf2 transcription factor in cells following exposure to electrophilic test substances.

Currently, the only in vitro ARE-Nrf2 luciferase test method covered by this Test Guideline is the KeratinoSensTM test method. Performance standards have been developed to enable the validation of similar test methods.

Also available in: French



TG 442D **OECD/OCDE**

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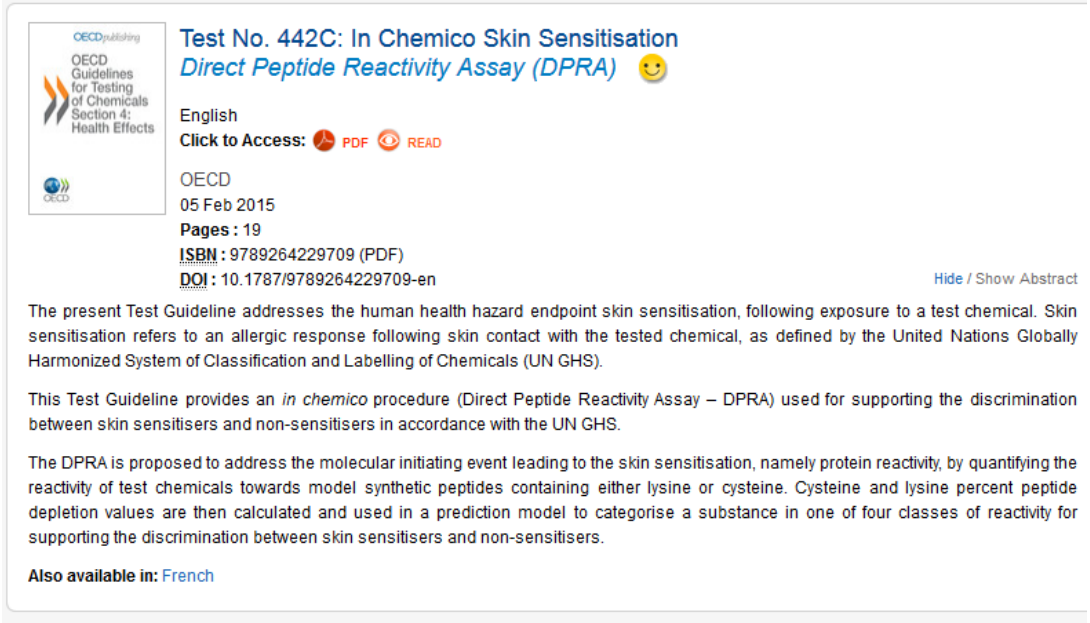
14. EURL-ECVAM (2014). Recommendation on the KeratinoSens™ assay for skin sensitisation testing, 42 pp. Available at: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/recommendation-keratinosens-skin-sensitisation.

15. DB-ALM (INVITTOX) (2013) Protocol 155: KeratinoSens™, 17pp. Available: [http://ecvam-dbalm.jrc.ec.europa.eu/].

2. OECD TG 442C –DPRA:



OECD Guidelines for Testing of Chemicals Section 4: Health Effects

Test No. 442C: In Chemico Skin Sensitisation
Direct Peptide Reactivity Assay (DPRA)

English
Click to Access: PDF READ

OECD
05 Feb 2015
Pages : 19
ISBN : 9789264229709 (PDF)
DOI : 10.1787/9789264229709-en

Hide / Show Abstract

The present Test Guideline addresses the human health hazard endpoint skin sensitisation, following exposure to a test chemical. Skin sensitisation refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS).

This Test Guideline provides an *in chemico* procedure (Direct Peptide Reactivity Assay – DPRA) used for supporting the discrimination between skin sensitisers and non-sensitisers in accordance with the UN GHS.

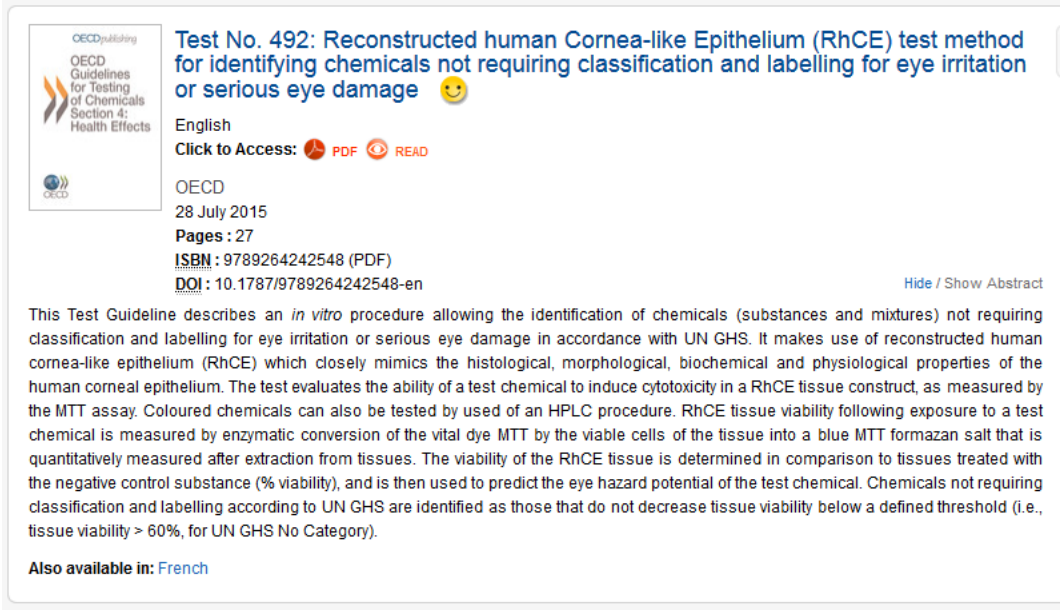
The DPRA is proposed to address the molecular initiating event leading to the skin sensitisation, namely protein reactivity, by quantifying the reactivity of test chemicals towards model synthetic peptides containing either lysine or cysteine. Cysteine and lysine percent peptide depletion values are then calculated and used in a prediction model to categorise a substance in one of four classes of reactivity for supporting the discrimination between skin sensitisers and non-sensitisers.

Also available in: French

PROCEDURE

16. This Test Guideline is based on the DPRA DB-ALM protocol n° 154 (20) which represents the protocol used for the EURL ECVAM-coordinated validation study. It is recommended that this protocol is

3. *OECD TG 492: EpiOcular:*



OECD Guidelines for Testing of Chemicals Section 4: Health Effects

Test No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage

English
Click to Access: PDF READ

OECD
28 July 2015
Pages : 27
ISBN : 9789264242548 (PDF)
DOI : 10.1787/9789264242548-en

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This Test Guideline describes an *in vitro* procedure allowing the identification of chemicals (substances and mixtures) not requiring classification and labelling for eye irritation or serious eye damage in accordance with UN GHS. It makes use of reconstructed human cornea-like epithelium (RhCE) which closely mimics the histological, morphological, biochemical and physiological properties of the human corneal epithelium. The test evaluates the ability of a test chemical to induce cytotoxicity in a RhCE tissue construct, as measured by the MTT assay. Coloured chemicals can also be tested by used of an HPLC procedure. RhCE tissue viability following exposure to a test chemical is measured by enzymatic conversion of the vital dye MTT by the viable cells of the tissue into a blue MTT formazan salt that is quantitatively measured after extraction from tissues. The viability of the RhCE tissue is determined in comparison to tissues treated with the negative control substance (% viability), and is then used to predict the eye hazard potential of the test chemical. Chemicals not requiring classification and labelling according to UN GHS are identified as those that do not decrease tissue viability below a defined threshold (i.e., tissue viability > 60%, for UN GHS No Category).

Also available in: French

28) EpiOcular™ EIT SOP, Version 8. (March 05, 2013). EpiOcular™ EIT for the Prediction of Acute Ocular Irritation of Chemicals. Available at: [http://www.ecvam-dbalm.jrc.ec.europa.eu/].

4. *Draft h-Clat test guideline –*

http://www.oecd.org/env/ehs/testing/151216-Draft-h-CLAT-TG-After-Expert-Meeting-(clean)-Final.pdf

PROCEDURE

16. This test guideline is based on the h-CLAT DB-ALM protocol no. 158 (24) which represents the protocol used for the EURL ECVAM-coordinated validation study. It is recommended that this protocol is used when implementing and using the h-CLAT method in the laboratory. The following is a description of the main components and procedures for the h-CLAT method, which comprises two steps: *dose finding assay* and *CD86/CD54 expression measurement*.

5. OECD TG 430-Ter. Updated in 2015:

OECD Guidelines for Testing of Chemicals Section 4: Health Effects

Test No. 430: In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)

Replaces Test No. 430: In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)

English

Click to Access: PDF READ

OECD

28 July 2015

Pages : 15

ISBN : 9789264242739 (PDF)

DOI : 10.1787/9789264242739-en

Hide / Show Abstract

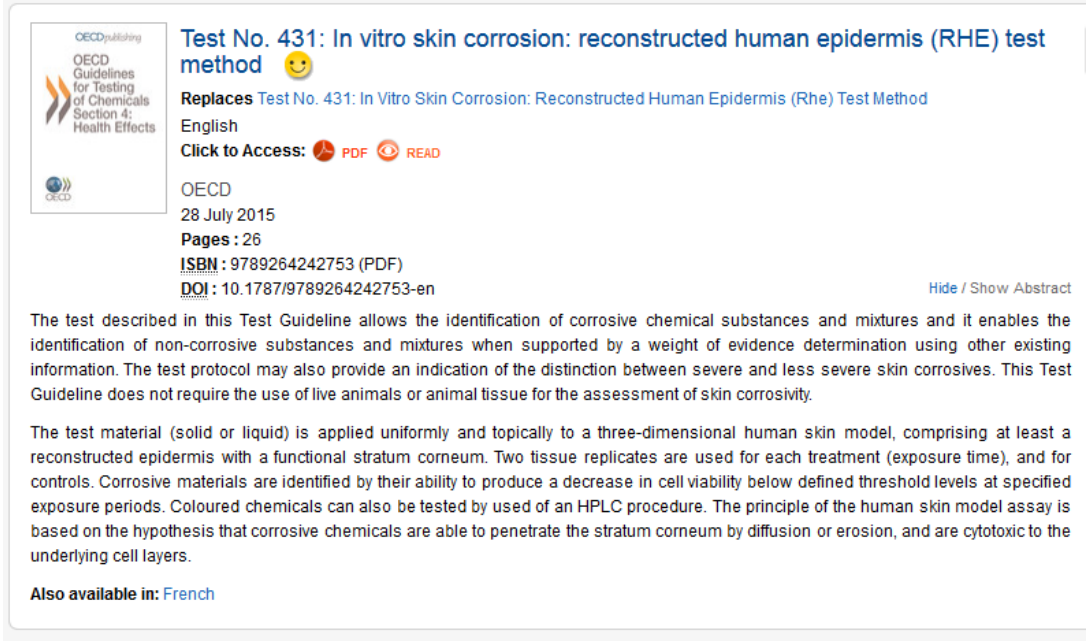
This Test Guideline addresses the human health endpoint skin corrosion. It is based on the rat skin transcutaneous electrical resistance (TER) test method, which utilizes skin discs to identify corrosives by their ability to produce a loss of normal *stratum corneum* integrity and barrier function. This Test Guideline was originally adopted in 2004 and updated in 2015 to refer to the IATA guidance document.

Also available in: French

The text refers to the SOP available from the DD-ALM:

(19) TER SOP. (December 2008). *INVITTOX* Protocol (No. 115.) Rat Skin Transcutaneous Electrical Resistance (TER) Test. Available at: [http://www.ecvam-dbalm.jrc.ec.europa.eu/].

6. OECD TG 431- Rhe Skin Corrosion updated in 2015:



OECD Guidelines for Testing of Chemicals Section 4: Health Effects

Test No. 431: In vitro skin corrosion: reconstructed human epidermis (RHE) test method

Replaces Test No. 431: In Vitro Skin Corrosion: Reconstructed Human Epidermis (Rhe) Test Method

English

Click to Access: PDF READ

OECD

28 July 2015

Pages : 26

ISBN : 9789264242753 (PDF)

DOI : 10.1787/9789264242753-en

Hide / Show Abstract

The test described in this Test Guideline allows the identification of corrosive chemical substances and mixtures and it enables the identification of non-corrosive substances and mixtures when supported by a weight of evidence determination using other existing information. The test protocol may also provide an indication of the distinction between severe and less severe skin corrosives. This Test Guideline does not require the use of live animals or animal tissue for the assessment of skin corrosivity.

The test material (solid or liquid) is applied uniformly and topically to a three-dimensional human skin model, comprising at least a reconstructed epidermis with a functional stratum corneum. Two tissue replicates are used for each treatment (exposure time), and for controls. Corrosive materials are identified by their ability to produce a decrease in cell viability below defined threshold levels at specified exposure periods. Coloured chemicals can also be tested by used of an HPLC procedure. The principle of the human skin model assay is based on the hypothesis that corrosive chemicals are able to penetrate the stratum corneum by diffusion or erosion, and are cytotoxic to the underlying cell layers.

Also available in: French

Refers to a number of protocols available from DB-ALM:

(33) EpiSkin™. (December 2011).SOP, *INVITTOX* Protocol (No. 118.). EpiSkin™ Skin Corrosivity Test. Available at: [http://www.ecvam.jrc.ec.europa.eu.hm;].

(34) EpiDerm™ SOP. (February 2012). Version MK-24-007-0024Protocol for: *In Vitro* EpiDerm™ Skin Corrosion Test (EPI-200-SCT), for Use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm. Available at: [http://www.ecvam.jrc.ec.europa.eu.html].

(35) SkinEthic™ RHE SOP, *INVITTOX* Protocol (January 2012). SkinEthic™ Skin Corrosivity Test. Available at: [http://www.ecvam.jrc.ec.europa.eu.html].

(36) EpiCS® SOP, Version 4.1 (January 2012). *In Vitro* Skin Corrosion: Human Skin Model Test Epidermal Skin Test 1000 (epiCS®) CellSystems. Available at: [http://www.ecvam.jrc.ec.europa.eu.html].

OECD Guidance documents (released in 2015)

1. ***DRAFT Guidance Document In Vitro Bhas 42 Cell Transformation Assay***
   available from
   https://www.google.com/url?q=http://www.oecd.org/env/ehs/testing/Bhas%252042%2520CTA%2520GD%2520after%25203rd%2520comments-F_CLEAN.pdf&sa=U&ved=0ahUKEwi92OfC5KnKAhUnnnIKHfAqDOc4ChAWCBEwBA&client=internal-uds-cse&usg=AFQjCNGD7KUQALT13faa07SlRjMsocYiNg)

   18. EURL ECVAM DataBase service on Alternative Methods to Animal Experimentation (DB-ALM) protocol No.156 on in vitro Bhas 42 cell transformation assay (http://ecvam-dbalm.jrc.ec.europa.eu/)

2. ***Guidance Document On The In Vitro Syrian Hamster Embryo (She) Cell Transformation Assay Series on Testing & Assessement No. 214 :***

   5. This Guidance Document (GD) provides an in vitro procedure of the SHE cell transformation assay, as specified in Maire et al. (13) or in the EURL ECVAM DB-ALM protocol on SHE CTA (14), conducted at pH 6.7 and 7.0. The assay can be performed at either pH 6.7 or 7.0 (see paragraphs 14) provided proficiency has been demonstrated at the chosen pH (see paragraph 54-55). The morphology of the normal colonies differs slightly at physiological pH compared to acidic pH, however, the conduct of the assay at either pH has been shown to give similar results. Other than the difference in the pH, the experimental protocol for both versions of the assay is the same.

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"DB-ALM" OR "INVITTOX" (Search performed on 22.06.2016)

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