RTC

RESEARCH TOXICOLOGY CENTRE - ROMA

THE PROCESS OF VALIDATION THE POINT OF VIEW OF THE CRO

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ecopa

Rema

The role of contract organization in toxicological development

General trend of industry → contract out non-clinical development

Reasons:

- small and mid-size companies (limited experience)
- preference to rely on established techniques
- importance of trained personnel
- assign valuable resources on internal projects
- rare occasions to carry out the methods

Features of the CROs

Industry is not homogeneous

CROs have more knowledge than average industrial companies for their:

- involvement in the safety assessment of a wide range of products
- tendency to deal with different industry segments (different needs, approaches, flexibility, drivers...)

Main functions of CROs

- comply with regulatory requirements
- ensure an updated knowledge of the new method status
- follow both scientific and regulatory points of view

The importance of the CRO experience

Important roles of the CRO in the alternative method validation process:

- ensure laboratory personnel training
- guarantee high quality level and proficiency in a wide variety of techniques

This generally occurs only in laboratories that regularly conduct a wide range of assays

Reasons for an early involvement of the CRO in the validation process

Validation and acceptance process should be as efficient as possible

Possible causes of attrition are:

- lack of rigorous controls
- lack of technical experience
- lack of information on regulatory requirements and industry needs
- design of inappropriate tests or unreliable results

An early involvement of the CRO in the validation process may facilitate its progression

Time of CRO involvement in validation process

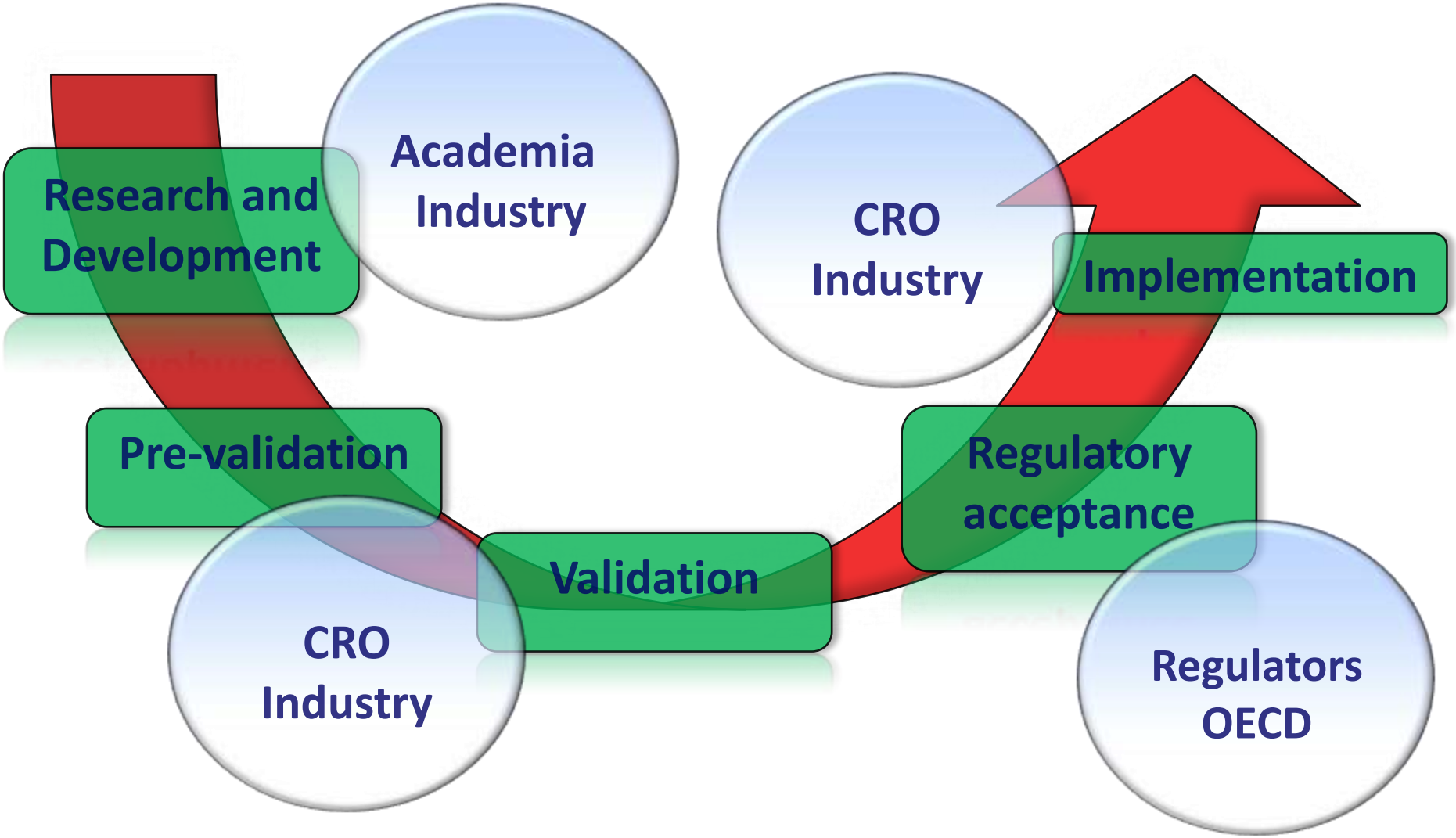
- New alternative methods find common ethical driver both in industry and CRO environment
- CROs are rarely involved in the preliminary phase → no real economical incentive (until the method is accepted and requested)
- Industries are often the developers of new alternative methods for economical motivation and law pressure

Importance of independence

Desired attributes of a Reference Laboratory:

- independence from assay developers and manufacturers
- unbiased position in the successful application
- neutral toward scientific and economical interests

Validation process



CRO as the ideal partner for a successful validation strategy

Understanding the needs of industry and regulatory framework is a key factor

CRO in pre-validation phase

CRO as the ideal partner in the **pre-validation** phase:

- established attitude to work in GLP compliance
- aptitude to facilitate assay transfer
- habit to assess test reproducibility (intra- and inter-laboratories)
- properly trained personnel

CRO in validation phase

CRO as the ideal partner in the **validation** phase:

- ability to analyse a large group of chemicals → test the predictivity of the proposed method
- habit to conduct a significant number of GLP-compliant studies in a timely manner

The point of view of CRO in the validation process

CRO involvement in the validation process represents an excellent occasion to:

- gain experience regarding alternative method opportunities
- get used to the procedural difficulties
- become skilled with the interpretation of results of the new method

CRO must consider balance between immediate investments and delayed payback (rate and time)

Status of validated alternative methods

Test Method	Regulatory acceptance	
	OECD	EU
Eye irritation		
BCOP Test Method	OECD TG 437 (2009)	EU Test Method B.47
Isolated Chicken Eye Test Method	OECD TG 438 (2009)	EU Test Method B.48
Skin Corrosion		
CORROSITEX Skin Corrosivity test	OECD TG 435 (2006)	
3D skin model Skin Corrosivity Test	OECD TG 431 (2004)	EU Test Method B.40 Bis
Rat Skin TER Corrosivity Test	OECD TG 430 (2004)	EU Test Method B.40
EST-1000 (Cell Systems)	OECD TG 431 (2004)	EU Test Method B.40 Bis
Skin Irritation		
3D skin model Skin Irritation Test	OECD TG 439 (2010)	EU Test Method B.46
In vitro skin absorption test	OECD TG 428 (2004)	EU Test Method B.45
Skin Sensitization		
LLNA	OECD TG 429 (2002)	EU Test Method B.42
LLNA:BrdU-ELISA	OECD TG 442B (2010)	
LLNA:DA	OECD TG 442A (2010)	

Test Method	Regulatory acceptance	
	OECD	EU
Acute phototoxicity		
3T3 NRU Phototoxicity Test	OECD TG 432 (2004)	EU Test Method B.41
Acute toxicity		
Up and Down Procedure (UDP)	OECD TG 425 (2001)	
Fixed Dose Procedure (FDP)	OECD TG 420 (2001)	EU Test Method B.1 Bis
Acute Toxic Class Method (ATC)	OECD TG 423 (2001)	EU Test Method B.1 Tris
3T3 NRU to estimate starting doses for oral acute toxicity	Guidance document adopted in 2010	
Genotoxicity		
In vitro micronucleus test	OECD TG 487 (2010)	Mentioned in Annex VIII of Reg.1907/2006
In vitro COMET and MN test 3D skin models		

RTC experience - Validation of BCOP

Preamble:
Mainly pushed by the ethical driver, RTC participated in COLIPA validation trial and promoted this test especially as screening method

Gautheron P; Giroux J; Cottin M; Audegond L; Morilla A; Mayordomo-Blanco L; Tortajada A; ***Haynes G; Vericat JA***
"Interlaboratory assessment of the bovine corneal opacity and permeability (BCOP) assay"
Toxicology in vitro; 8(3); 381-392; 1994

RTC experience - Validation of BCOP

Outcome:

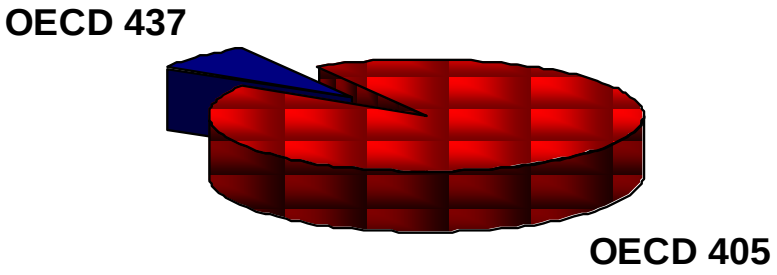
- Few requests at the beginning (lack of regulatory requirements)
- Sponsor requests increased after issue of OECD 437
- RTC method adjusted to comply with the new guideline

RTC Sponsor's requests for ocular irritation

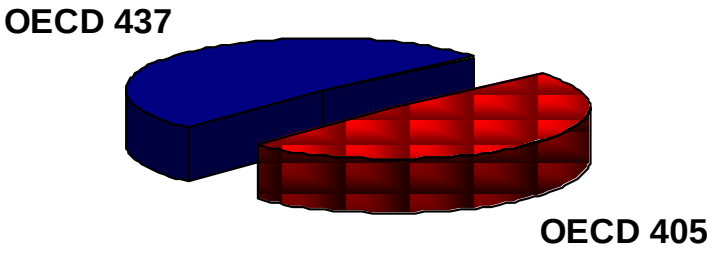
OECD 405 - Acute Eye Irritation/Corrosion

OECD 437 - BCOP Test

2005



2010 -2011



RTC experience - Validation of *in vitro* MNT

Preamble:

RTC participated in the French validation trial and promoted this test as screening method.

Clare MG;Lorenzon G; Akhurst LC; Marzin D; van Delft J; Montenero R; Botta A; Bertens A; ***Cinelli S****; Thybaud V; Lorge E.*

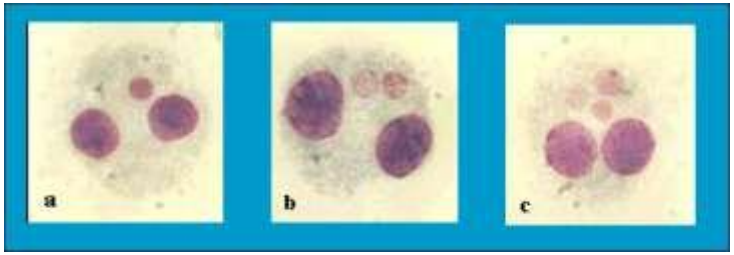
"SFTG international collaborative study on in vitro micronucleus test II. Using human lymphocytes"

Mutat. Res. 2006 Aug 4;607(1):37-60

RTC experience - Validation of *in vitro* MNT

Outcome:

- Immediate success of this kind of study as genotoxicity screening
- Sponsor requests increased following EU regulatory acceptance and rapid integration into REACH legislation
- RTC method adjusted to comply with the new versions of OECD guideline



RTC experience - Validation of *in vitro* MNT

- Economical driver
- Improvement of in vitro test battery predictivity →limitation of animal testing
- Ethical consequences: Intelligent Testing Strategy

RTC experience in promoting alternative methods

RTC position with other alternative methods

- EpiOcular assay
- Inflammatory cytokine measurement in 3D skin models
- Genotoxicity tests in 3D skin models

CRO plays a central role to encourage the use of promising methods as preliminary screening

CRO strategy in the selection of alternative tests

- business area (Cosmetic, Pharmaceutical, Chemical)
- CRO technical and scientific vocation
- white or position papers which foresee regulatory requirements
- number of Sponsor enquiries
- possibility to apply the new test as a stand-alone substitute of conventional method
- expectations of economical return after investments for internal implementation and demonstration of proficiency

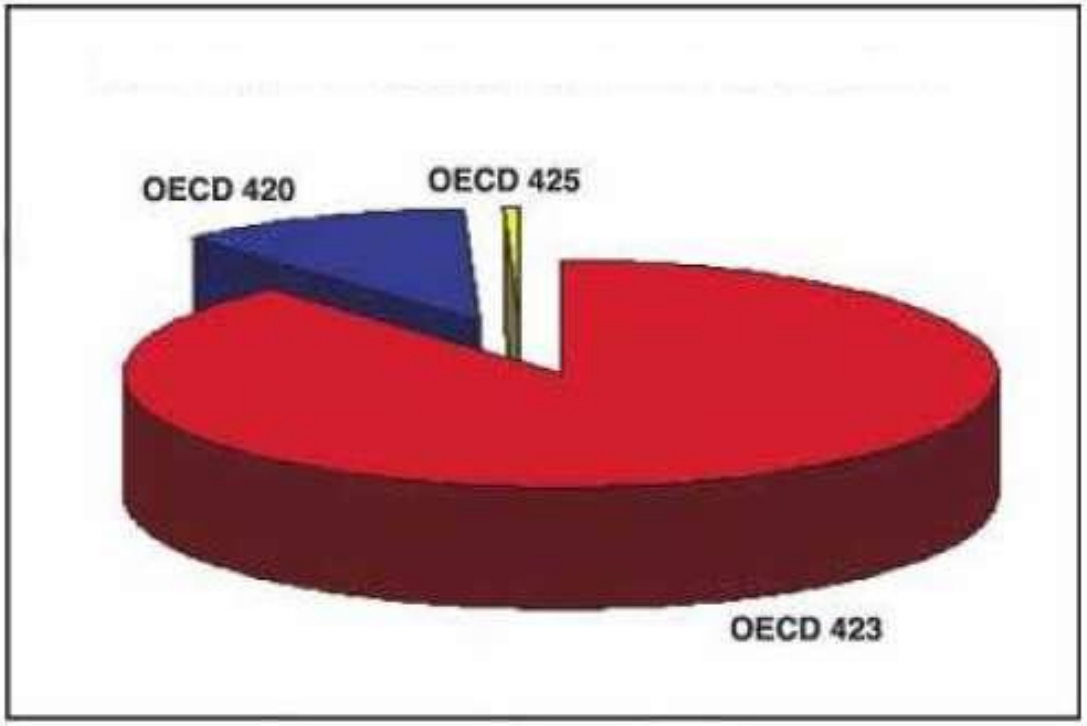
RTC experience in the alternative test selection – OECD 425

Preamble:

- OECD 401 (LD50) guideline deleted in December 2001
- Alternative test methods introduced in the mid 1990's
- RTC implemented the 3 OECD guidelines
  - OECD 423 (Toxic class)
  - OECD 420 (Fixed dose)
  - OECD 425 (Up and Down)
- OECD 425 is the most complex, difficult to manage and requires more time to carry out the test

RTC experience in the alternative test selection – OECD 425

Distribution of RTC Sponsor's requests for acute toxicity studies



RTC experience in the alternative test selection – LLNA

Radioactive LLNA

- included in RTC services following issue of OECD 429 guideline
- offered as sensitization test alternative to M&K and Buehler tests
- high costs of disposal for radioactive waste → test poorly profitable

RTC experience in the alternative test selection – LLNA

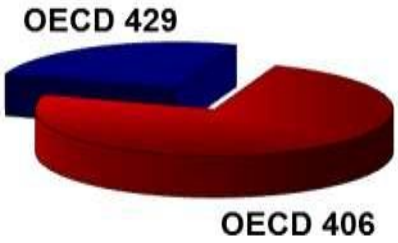
BrdU LLNA

- OECD guideline did not present radioactive method as the only acceptable one
- Sponsors did not trust “cold” alternatives until the specific guideline was issued in 2010 (OECD 442B).

Importance of regulatory position on reliability of alternative methods

Industry may have a conservative approach driven by the risk avoidance

- non-chemical industry (skin sensitization tests) → M&K or Buehler still requested in place of LLNA



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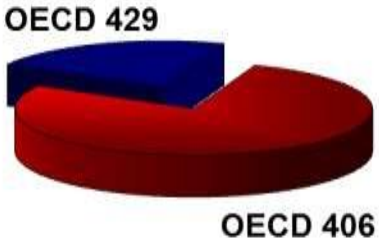


2010 - 2011

Importance of regulatory position on reliability of alternative methods

- non-chemical industry (skin irritation tests) → *in vivo* study requested instead of Intelligent Testing Strategies (QSAR, pH, alternative *in vitro* methods)
- pharmaceutical industry (photosafety assessment) → requests of complete battery in presence of negative results in *in vitro* phototoxicity assay

Toxicology is a conservative science

Often toxicologists rely on previous experience of dossier acceptance

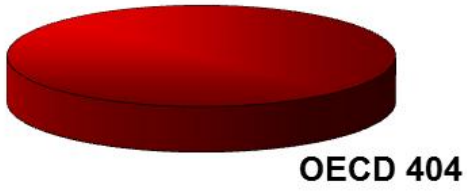
- fear that results from alternative studies might be not easily accepted
- rare requests for methods abolished for ethical reasons

Role of CRO in updating sponsors on validation progress

- promote acceptance of methods
- facilitate quick adoption in routine use

RTC experience in the alternative test selection – Episkin

RTC in the recent years witnessed an increasing number of requests for *in vitro* skin studies, in particular for skin irritation tests



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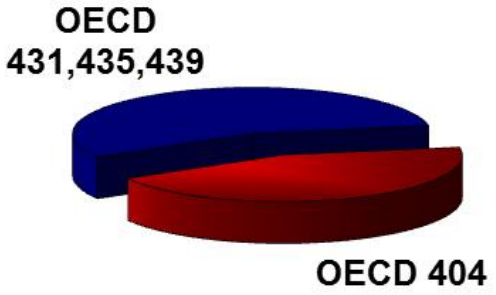


2010 - 2011

RTC experience in the alternative test selection – Episkin

Reasons:

- predictivity comparable to *in vivo* conventional studies
- good reliability as screening assay (e.g. pharmaceutical formulations)
- regulatory driver (Cosmetic and REACH regulation)
- costs

Conclusion

CRO an ideal reference laboratory during the validation process

- independence from assay developers and manufacturers
- GLP-compliance
- experience in routine use of *in vitro* assays
- understanding of scientific and regulatory needs of industry
- knowledge of regulatory agencies requirements
- aptitude to meet programmed deadlines

Conclusion

- Promotion of alternative tests may be challenging
- CRO may find economic benefit if involved in the validation process and subsequent application of alternative methods

CRO plays a central role to encourage the use of new tests and overcome the natural cautious aptitude of industrial sponsors

Responsive

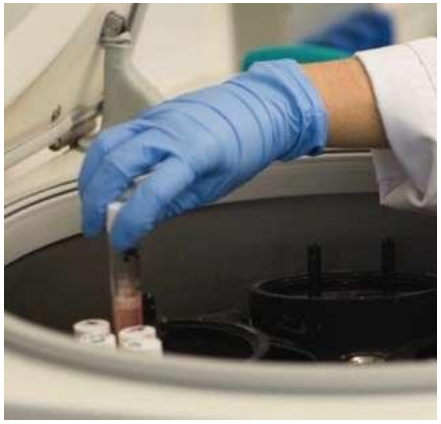
Thorough

Competent

Reliable

Team focused

Committed



Reactive

Tailored

Cost effective

Thank you

Receptive

Timely

Capable

Respected

Trustworthy

Collaborative

Right size

Toxicology

CRO

RTC

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