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Normative Update on Preclinical Studies: Can Animal Testing Be Avoided?

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Abstract
In previous years, much attention has been given to animal welfare issues regarding their use in preclinical analysis. As a result, Directives, Regulations, and International Standard Organizations have been updated to recognize the need for change in current preclinical study methods.

According to ISO 10993 standards for biocompatibility of medical devices (MD), skin irritation is one of the three required toxicological endpoints in a biological risk assessment. Different models of reconstructed human epidermis (RhE) have been investigated in order to understand if they could represent a suitable alternative to assess skin irritation of medical device extracts *in vitro* and ultimately replace the Draize rabbit test. Currently, a new part of ISO for *in vitro* irritation testing of medical devices is under development as a replacement for the animal irritation studies now indicated in ISO 10993-10. The following paper is intended to provide an overview of current normative situations regarding medical device preclinical analysis and perspectives of alternative methods that could be used in the future at Eurofins Medical Device Testing.

Normative background information
Directive 2010/63/EU on the protection of animals used for scientific purposes is firmly based on the principle of the "3 Rs": to replace, reduce, and refine the use of animals used for scientific purposes.[1] This Directive impacts all regulations pertaining to the marketing of products that are safe for humans, animals, and the environment.[2]

The 20106 EMA Guideline entitled Guideline on the Principles of Regulatory Acceptance of 3Rs Testing Approaches asks not to perform animal testing when alternatives that provide the same type of information without using animals are available, as in accordance with Directive 2010/63/EU. Among these alternative methods, computer modeling methods can be used in combination with many *in vitro* models.

The European Union (EU) is committed to promoting the development and validation of alternative techniques which provide the same level of information as current animal tests. Such methods must be considered whenever possible for hazard characterization, consequent classification, labeling for intrinsic hazards, and chemical safety assessment.

Within the EU Directive 2010/63/EU, the principles of the 3Rs are invoked whenever toxicological test methods are necessary. All pre-clinical studies are required to obtain marketing authorization, check product quality, and should adopt these alternative methods following this guideline. The EMA is available for advice during new 3Rs method development by encouraging companies and authorities to support and accept the 3Rs approach to development and use. This applies to regulatory studies on medicinal products for humans and animals, as well as quality control studies and medical devices biological evaluation.

Medical devices essential requirements
Old Directives related to the medical device sector have been replaced with two new Regulations to ensure safety, innovation, and competitiveness, including EU Regulation 2017/745 on medical devices and EU Regulation 2017/746 on *in vitro* diagnostic medical devices. The new Regulation 2017/745, in line with recent regulations on different product sectors, promotes alternative approaches to the use of animals, in particular, avoiding unnecessary duplication of tests.[3]

Medical device regulation requires that devices be designed and manufactured in such a way that they will not compromise the clinical condition, safety of patients, and/or the safety and health of users or other persons when used under the conditions for the intended.[3, 4]

Particular attention is focused on the choice of materials used (such as their potential toxicity) and the biocompatibility of the materials used during the intended purpose of the device (EU Regulation 2017/745, Annex 1). For this reason, pre-clinical analysis must be performed on medical devices to evaluate their safety and quality. Indeed, pre-clinical tests are essential for the evaluation of biocompatibility and biological safety according to ISO 10993-1. The new ISO 10993-1:2018 promotes the use of alternative approaches to *in vitro* test methods as long as they are validated, reasonably and practically available, reliable and reproducible, and considered for use in preference to *in vivo* tests.[5]

Skin irritation test overview

ISO 10993-10, published in 2010, describes only *in vivo* assays[6]; however, it recognizes the need to follow scientific progress that already utilizes recognized methods as validated alternatives to *in vivo* tests[7]. Since then, various studies have been published on the evaluation and validation of *in vitro* assays for the determination of chemical irritation as an alternative for *in vivo* irritation tests.[8-10]

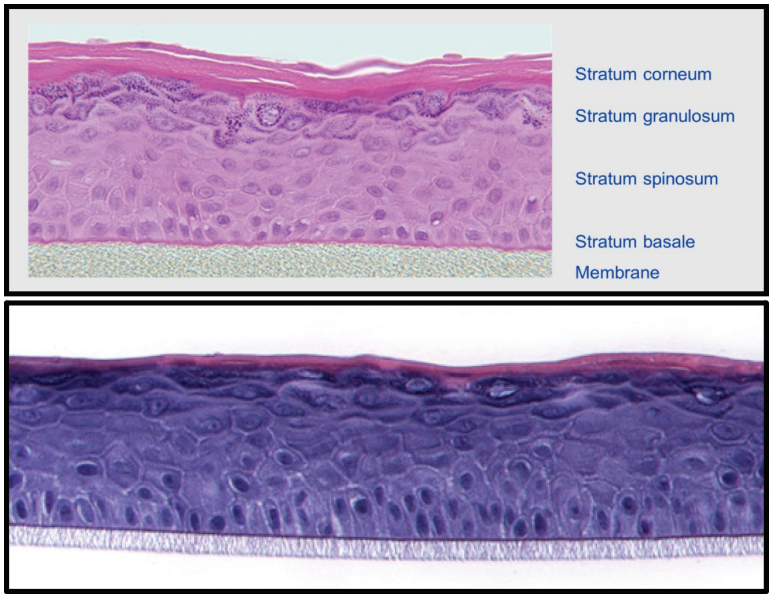
Indeed, besides the ethical issue, the major limits of *in vitro* based experimental models is that they are expensive, time-consuming, and sometimes not allowed due to the requirements of Directive 2010/63/EU on the protection of animals used for scientific purposes. Furthermore, an animal model does not provide information about the mechanism of action nor on barrier action. It is not useful to demonstrate the non-pharmacological mechanism of action. It is not relevant for mucosa and it has a poor predictive ability because of species-species extrapolation.

Moreover, since protocols currently described in ISO 10993 suffer from limited biological relevance and predictive value with respect to MD product complexity and heterogeneity, it could be worthy to take into consideration other approaches that are scientifically more comprehensive and meaningful as the assessment of dermal irritation is an essential component of the biological safety evaluation of medical devices.

For example, the use of an approach based on 3D human tissue models for medical device evaluation could support medical device biocompatibility evaluation. Reconstructed human epidermis (RhE) models have already replaced rabbit skin irritation testing for neat chemicals and their mixtures (OECD Test Guideline 439). However, this guideline cannot be directly applied to medical devices since for these products, the non-toxicity assessment is largely based on the testing of medical device extracts that may have very low irritation potential. Therefore, the RhE-methods previously validated with neat chemicals had to be modified to reflect the needs for detection of low levels of potential irritants and the general move in the industry towards the use of a RhE model for the assessment of skin irritation.[11, 12] New scientific methods must be evaluated before being included and described; meaning their reliability and relevance of the new procedures need to be established.

Following an international round robin for the detection of irritant activity of medical device extracts, an *in vitro* skin irritation test based on two different RhE models (EpiDerm and SkinEthic RHE reported in Figure 1) has been proposed as a replacement method for the rabbit skin irritation test. Nineteen different laboratories, including Eurofins Medical Device Testing, independently performed the test several times, employing

Figure 1



these two *in vitro* models. The objective was to verify the new alternative test method efficiency and to determine its reproducibility among different laboratories. All laboratories were able to discriminate between irritants and non-irritants with an accuracy of more than 92%.[13, 14] Indeed, these results demonstrated that RhE tissue models can detect the presence of strong skin irritants at low levels in dilute medical device polymer extracts. Therefore, these models may be suitable replacements for the rabbit skin irritation test to support the biological evaluation of medical devices.[15]

For these reasons, a new guideline for preclinical studies suggests that the characterization and biological evaluation of medical devices should be done according to ISO 10993, using new experimental approaches based on the use of reconstructed human tissues (such as RhE) for the evaluation of medical device biocompatibility.

Moreover, a new international standard, ISO 10993-23, for *in vitro* irritation testing of medical devices, has been drafted as a replacement for the animal irritation studies indicated in ISO 10993-10.[15, 16]

This new standard "Tests for Irritation" is currently under the characterization and biological evaluation of medical devices should be done according to ISO 10993, using new experimental approaches based on the use of reconstructed human tissues (such as RhE) for the evaluation of medical device biocompatibility. Moreover, a new international standard, ISO 10993-23, for in vitro irritation testing of medical devices, development and will reflect the requirements described in ISO 10993-1 and 10993-10 that refer to the application of the "3R" principles that have been previously reported, and it will take into account ISO 10993-2 that is focused on animal welfare.[17] Although this new alternative method is not part of the current ISO 10993-10 nor has the new upcoming ISO 10993-23 been released yet, a paper on this validation has been recently published.[15] Eurofins Medical Device Testing played an active part in the validation of this alternative method and is prepared to provide all possible support to medical device manufacturers.

Future possible approaches

The Organization for Economic Co-operation and Development (OECD) proposes an Integrated Approach on Testing and Assessment (IATA) for hazard identification of skin corrosion or irritation potential of chemicals that provides adequate information for classification and labeling (CLP) with the purpose of minimizing the use of animals, while ensuring human safety.

IATA provides consistent information on strengths and limitations as well as the potential role and contribution of each of the individual information sources, how to integrate the information for decision making within the approach (including decisions on the need for further testing), and how to integrate all existing and generated information on the corrosive and irritant hazard potential of test chemicals for final decisions for classification and labeling.[18, 19]

Indeed, coupling CLP Regulation with *in vitro* irritation tests could represent a possible alternative method for testing medical devices and formulations.

Moreover, the FDA is engaged in considering additional test methods for qualification through the Medical Device Development Tools (MDDT) program regarding MD potential irritation. The FDA's MDDT program is a way for the FDA to qualify tools that medical device sponsors can use in the development and evaluation of medical devices. Qualification means that the FDA has evaluated the tool and concurs with available supporting evidence that the tool produces scientifically plausible measurements and works as intended within the specified context of use.

Conclusions

There is great potential to apply scientific and technological advances to reduce reliance on animal tests and to establish testing paradigms that hold more human and ethical relevance.

While *in vivo* animal testing remains one of the major tools to evaluate potential toxicities, more and more importance is given to mechanism-based approaches. Many different components, such as different mechanistic information

Many different components, such as different mechanistic information and existing data, can be brought together into Integrated Approaches for Testing and Assessment (IATA) where two or more non-animal methods are combined to provide a sufficient level of information to make regulatory safety decisions.[3]

Unfortunately current *in vivo* options for medical devices are not always up-to-date compared to drug and chemical industry testing. There is a discrepancy between the language in the ISO 10993- 1 standard, which recognizes a potential tiered approach, giving more weight to *in vitro* data, and the reality that this is not translated into regulatory decision-making. An evolving regulatory, scientific, and legislative landscape is driving a fundamental change in how chemical safety decisions are made. However, suitable *in vitro* alternatives are now being accepted.[20]

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