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Focus

Implementation of the new French regulations on microorganisms and toxins: ANSES's experience

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The new French regulatory measures for operations involving microorganisms and toxins (MTs) are making lasting changes to the sector of microbiology laboratories. This new regulatory framework reinforces the control measures in this area to improve biological safety and security. In practice, it results in increased administrative and operating requirements that call for greater vigilance on the part of operators. To fulfil these new requirements, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) has implemented an in-house methodology for risk assessment, taking into account the specificities of its reference and research laboratories.

Introduction

The French Public Health Code in Chapter IX, Article L.5139-1 defines microorganisms and toxins (MTs) as biological agents that are pathogenic to humans and toxins whose use may involve a risk for public health, as well as products that contain these agents. In practical terms, these MTs are likely to pose a real public health risk in the event of accidental exposure (*biosafety*) or intentional exposure (*biosecurity*) outside their containment area. The list of microorganisms and toxins is determined by the Minister of Health. The amendment of Article L.5139-2 of the Public Health Code resulted in the publication of Decree No. 2010-736 of 30 June 2010 concerning MTs, which came into effect on 1 July 2012. The new regulatory framework, which includes seven implementation orders and one decision, is applicable to all French laboratories involved in any operation using MTs for diagnostic, research, development or teaching purposes.

The regulations primarily aim to protect workers, the environment and the population from accidental or intentional dissemination of a hazardous biological agent by establishing appropriate rules for safety and security to effectively reduce risks for public health. Control of the appropriate implementation of these rules is the responsibility of the French National Agency for Medicines and Health Products Safety (ANSM), which issues authorisations, and administers and monitors all operations involving MTs, including production, manufacture, transport, import, export, retention, supply, sale, purchase and use.

An exemption regime has been established for:

- certain proprietary medicinal products and investigational medicinal products containing MTs that have been inactivated or attenuated, ensuring a satisfactory safety level for public health;
- reagents intended for analyses in the veterinary and plant protection fields;
- operations carried out by establishments that receive biological samples purely for analysis (with a storage duration of less than 30 days);
- operations carried out by the establishments within the Ministry of Defence (except operations for import and export).

Another exception to these regulations is worth mentioning, even though the general notion of all or part of an MT remains valid. It involves fragments of genetic material (DNA or RNA) that are no longer considered part of MTs if they are less than 500 nucleotides in length.

Concerning toxins, protein toxin fragments containing fewer than 167 amino acids are also excluded from the regulations.

To demonstrate compliance with the requirements of the Decree of 30 June 2010, the authorisation application dossier is now made up of two separate parts:

- a technical dossier intended to describe in detail the facilities, procedures, and safety and security systems implemented by the laboratory to ensure protection of its personnel, the population, and the environment. In this dossier, the applicant must justify the utility of using MTs;
- a risk assessment concerning safety and security taking into account existing protection measures.

The ANSES reference and research laboratories are particularly affected by these new measures since they are called on to work with all types of MTs, including bacteria, viruses, proteins, DNA, and toxins. As a result, to respond to the new requirements, ANSES quickly set up an action plan to avoid some of its activities being called into question. Faced with the relative complexity of the new regulations and the wide range of MTs it studied and used, the Agency needed to set up an in-house working group with the task of analysing the regulatory requirements, proposing a joint method to harmonise the applications and to help and support the ANSES laboratories through the procedure. The Committee for control of biological risks in the laboratory (CMRBL) was therefore established and included the expertise needed to fulfil the requirements of the new regulations. In particular, the committee worked on the basis of the methodology for risk assessment provided by the ANSM, and proposed to ANSES laboratories a methodological guide for risk assessment adapted to the issues specific to research and reference activities. We felt that it could be useful to make this methodology accessible to laboratories that have the same specificities as those within ANSES. In addition, the CMRBL was to play the role of sole contact for forwarding questions to ANSM from the various laboratories within ANSES, which enabled constructive exchanges to be set up with ANSM and helped to find answers to most of the questions posed.

The ANSES methodology

Of the eleven laboratories within ANSES, six work with microorganisms and toxins. These laboratories are located in various regions in France and, depending on the laboratory, have level 2 or 3 containment facilities and/or animal housing. Since

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June 2012, 13 renewal applications and three authorisation applications have been submitted to ANSM.
The CMRBL is made up of 14 members of staff with complementary expertise: laboratory head, scientist, engineer, technician, quality manager, biosafety manager, head of animal housing, health and safety officer, and security-defence representative. Two committee members participated in a three-day training course on risk assessment, based on the Failure Mode Effects and Criticality Analysis (FMECA) method.
Given the significant delay in the schedule for publication of implementation orders related to the MT regulation, the CMRBL was only able to start its work at the beginning of 2012, leaving the laboratories with very little time to finalise their applications, with the deadline stipulated in the 2010 Decree of 30 June 2012.
To begin with, the CMRBL analysed the technical dossier and asked ANSM for clarification on points that seemed unclear. ANSM always replied clearly to each question, by email or by telephone, and indicated as a general rule that the applicant can give a wide range of responses provided that they are well substantiated. The main answers provided to ANSES's questions are shown in **Table 1**. Further to this analysis, the CMRBL issued a template of the technical dossier for ANSES laboratories, along with an explanatory text and suggested responses.
The second phase involved the development of a "Methodological guide for risk assessments concerning biological safety and security" [http://www.ansespro.fr/euroreference/], drawing on the model proposed by ANSM ("Risk management method in biological safety and security", version dated 3 May 2011), available on request. However, this model proved to be relatively unsuitable for the issues faced by ANSES laboratories, both in terms of description and semantics. As a result, the hazard identification questionnaires were adapted to ANSES specificities (reference and research). The rating scales for risks related to biological safety and security initially proposed were amended qualitatively and quantitatively. The limits defining "low", "average" and "unacceptable" risk levels were also changed. Concerning the biological safety aspect, the methodology for calculating risk was completely revised, with introduction of the concept of extrinsic severity and a change in calculation of the criticality index. These calculation methods were tested in several ANSES laboratories and then adjusted, before being adopted by the CMRBL.
Furthermore, ANSES chose to integrate the biological risk management system into its overall risk management policy, and then to apply the policy depending on the specificities of each entity.

Implementation of the regulation: impact on the laboratories

Personnel training

The Ministerial Order of 17 March 2011 defines a minimum level of competence and qualifications required for the authorisation holder, and for the persons whom he/she duly authorises. In addition, the requirements of the Ministerial Order of 23 January 2013 are very clear concerning authorisations, and initial and continuing training of personnel before they can be granted access to facilities and MTs. Clearly, each laboratory will need to implement an individual training plan, suitable for each activity. Certain universities or private organisations already offer specific training programmes on biological risks, which can be adapted to the area of MTs. It is interesting to note that a working group, sponsored by the French Society for Microbiology, is working on the development of a national reference standard on training concerning biological risks to harmonise knowledge and practices, and to provide a formal framework so that personnel do not need to start training again, if they change laboratories. In effect, these training and authorisation requirements for personnel working on MTs exclude short-duration interns from working on projects involving all or part of a microorganism or toxin. This could have significant consequences for some research laboratories.

Facilities, equipment and materials

The design and use of facilities and equipment are based on the process of risk management, which involves a number of requirements in terms of resources that have to be provided for in the budget, before working on MTs. The operating capacity of the facilities must be documented in normal and limit conditions, depending on the volume of activity of the laboratory, in order to avoid any overuse. Moreover, operations intended for the validation, qualification, maintenance and monitoring of safety and security equipment will account for a large proportion of the running costs of a laboratory. "Older" laboratories should expect to incur significant costs to upgrade their facilities.

Subcontracting

Faced with such constraints, some laboratories may be tempted to outsource certain tasks. Here again, the regulations define very clearly the roles and responsibilities of each party, and require contracts to be established for all operations related to study or use of MTs. In this way, the responsibility of the client is clearly emphasised.

Document management

As in any quality system, document management should enable tracking of all operations carried out and secure storage of documentation certifying implementation of biological safety and security measures. All of these documents must be made available, requiring implementation of a specific document management system.

Specific requirements

The Ministerial Order of 23 January 2013 related to good practice rules to ensure biological safety and security defines "specific requirements" in Chapter 7 concerning the use of vertebrates and invertebrates (arthropods) exposed to MTs and genetically modified MTs. These requirements are additional and without prejudice to the regulations concerning animal experimentation (Decree of 1 February 2013 and corresponding orders) and genetically modified microorganisms (GMMs) (Directive 2009/41/EC of the European Parliament).
For animal testing facilities, these requirements now include new constraints that were previously not mandatory. For example, vertebrates must have individual and lasting marking in order to ensure their traceability. This requirement is not difficult or expensive to fulfil for medium or large sized animals such as lagomorphs, dogs, cats, primates, and production livestock, etc. which are already identified individually before they enter animal testing facilities (Articles L.212 and R.214 of the Rural Code).
However, for small laboratory rodents such as mice and rats, it is more complex to identify animals individually and this involves significant additional costs depending on the

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technique used (tattooing, banding, or electronic chips). The most simple and above all safest identification method for small animals is subcutaneous implant of electronic transponders. This technology does however have some disadvantages: 1) it cannot be used systematically because of changes to the immune system related to a local inflammatory response at the transponder's implant site that could interfere with experimental findings; 2) its cost can reach €3 to €4 before tax per animal, depending on the size and quality of the transponder, for an animal that has a commercial value of €2 to €3 before tax (for instance in the case of OFI or Swiss mice). A biological safety risk assessment, depending on the type of MT and the specific animal model, will help in selecting the most suitable technique for individual identification.

Concerning arthropods, the regulations require that a biological safety risk assessment be performed before MTs are used in invertebrates to avoid dissemination of arthropods outside the chosen containment systems. This risk assessment should take account of whether the arthropods can fly, e.g. mosquitoes, or not, e.g. fleas, lice or ticks. Additional precautions must also be taken to avoid manipulation of free arthropods or those attached to vertebrates in class I or II biosafety cabinets. There are two main types of precautions: 1) protection of personnel with personal protective equipment that must cover the skin entirely to avoid a risk of bites by arthropods; 2) installation of a cold airlock or sticky mats in front of exit doors in facilities housing arthropods, to prevent the risk of insects escaping to the outside. Finally, the regulations require systematic careful counting of all individuals before and after manipulation, with all the constraints in terms of working time that this implies.

It should be noted that during development of the risk assessment methodology prepared by the CMRBL within ANSES, these specific points concerning animal testing were integrated both in terms of biological safety and biological security.

Emergency plans and restricted access areas

Importantly, laboratories will be required to implement an internal emergency plan to address any situations that may endanger its personnel, the public, or the environment. This emergency plan includes a clear description of the internal alert circuits and the information exchanges with external emergency services and administrative authorities. It must also include periodic simulation exercises. To develop this plan, the laboratory will necessarily need to work with external services (local authorities, fire-fighters, paramedics, police, etc.). Finally, in addition to these safety measures, laboratories will also have to implement security measures aimed at limiting the risk of malicious use of microorganisms and toxins. To avoid weighing down the system, these measures will need to comply with the requirements of the Decree of 2 November 2011 regarding the protection of the scientific and technical potential of the nation, which requires the creation of restricted access areas (ZRRs) for material and immaterial assets with dual use, that could be misappropriated or diverted.

Furthermore, a specific intervention plan must be implemented for the microorganisms and toxins included in Annex I of the Ministerial Order of 30 April 2012. This plan defines the assistance measures implemented and the way in which they are managed in the event of an accident with consequences that extend beyond the installation at risk. This includes the arming, alert and intervention phases, but also the emergency services exercises carried out periodically to ensure adoption of the system. The specific intervention plan is part of the system for the organisation of emergency services (ORSEC) in each *Département*.

Conclusion

Although these regulations are clearly part of the movement to protect public health that is gradually being implemented at the European level, it is also true that the administrative burden of this regulatory framework, and the significant time constraints imposed by the public authorities, have led to difficulties in implementation for certain laboratories. Moreover, implementation of the new regulations leads to a disparity between the laboratories that work with MTs and level 3 containment laboratories that do not work with MTs, since the latter are not subject to systematic control or inspections to verify the implementation of the Ministerial Order of 16 July 2007 stipulating the preventive measures required for workers who may be exposed to pathogenic biological agents. As a reminder, this order concerns the recommendations that are to be implemented in a laboratory to ensure compliance with biological safety requirements, and to a lesser extent biological security measures. It is therefore surprising that laboratories handling class 3 agents, though they are not MTs, are not subject to controls. On the contrary, laboratories working with MTs, whether in class 2 or 3, are subject to very strict regulatory constraints. For some laboratories, MT regulations will overlap with ZRR regulations, or even with those concerning sectors of vital importance, and those indicated in the Defence Code concerning toxins which are considered chemical products included in **Table 1** of the Chemical Weapons Convention (CWC), and those on dual-use items (Regulation (EU) No 388/2012 of 19 April 2012). Finally, even though the set of constraints imposed by MTs enabled some clarification for the actors involved in the MT area, the withdrawal of certain laboratories from such activities could lead to gaps in the health network in France for microorganisms that are highly regulated in the laboratory, but present in the natural environment in the country (ultra-resistant *Mycobacterium tuberculosis* in hospitals, *Francisella tularensis* regularly isolated in wildlife, etc.).

References

Regulatory texts concerning MTs

Code de la santé publique : article L.5139-2 modifié par la Loi n°2009-879 Art 111. [http://www.legifrance.gouv.fr/affichCodeArticle.do?cidTexte=LEGITEXT000006072665&idArticle=LEGIARTI000020889763&dateTexte=]

Décret n°2010-736 du 30 juin 2010 relatif aux micro-organismes et toxines. [http://legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000022415024&categorieLien=id]

Arrêté du 30 juin 2010 fixant les mentions qui figurent sur les états annuels des stocks prévus à l'article R. 5139-14 du code de la santé publique. [http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000022415125&dateTexte=&categorieLien=id]

Arrêté du 30 juin 2010 fixant les renseignements qui figurent dans le registre ou les enregistrements mentionnés à l'article R. 5139-17 du code de la santé publique. [http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000022415149&dateTexte=&categorieLien=id]

Arrêté du 30 juin 2010 fixant les renseignements qui figurent sur l'autorisation mentionnée à l'article R. 5139-1 du code de la santé publique. [http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000022415141&dateTexte=&categorieLien=id]

Arrêté du 17 mars 2011 relatif aux compétences et qualifications dont le titulaire de l'autorisation mentionnée à l'article R. 5139-1 du code de

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la santé publique justifie pour lui-même ainsi que pour les personnes qu'il habilite pour contribuer sous sa responsabilité aux opérations faisant l'objet de cette autorisation. [http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000023776935&dateTexte=&categorieLien=id]

Arrêté du 30 mai 2011 fixant la liste des médicaments mentionnée à l'article R. 5139-26 du code de la santé publique. [http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000024185577]

Arrêté du 30 avril 2012 fixant la liste des micro-organismes et toxines prévue à l'article R. 5139-1 du code de la santé publique. [http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000025837146&dateTexte=&categorieLien=id]

Arrêté du 11 juin 2013 modifiant l'arrêté du 23 janvier 2013 relatif aux règles de bonnes pratiques tendant à garantir la sécurité et la sûreté biologiques mentionnés à l'article R. 5139-18 du code de la santé publique. [http://legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000027047902&categorieLien=id]

Décision du 20 octobre 2010 fixant le contenu du dossier technique mentionné à l'article R. 5139-3 du code de la santé publique et accompagnant la demande d'autorisation prévue à à l'article R. 5139-1 du code de la santé publique. http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000022993011

Regulatory texts concerning containment measures

Arrêté du 16 juillet 2007 fixant les mesures techniques de prévention, notamment de confinement, à mettre en œuvre dans les laboratoires de recherche, d'enseignement, d'analyse, d'anatomie et cytologie pathologiques, les salles d'autopsie et les établissement industriels et agricoles où les travailleurs sont susceptibles d'être exposés à des agents biologiques pathogènes. http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000465273&dateTexte=&categorieLien=id

Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32009L0041:EN:NOT

Regulatory texts concerning the protection of laboratory animals

Décret n° 2013-118 du 1er février 2013 relatif à la protection des animaux utilisés à des fins scientifiques. http://legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000027037840&categorieLien=id

Arrêté du 1er février 2013 fixant les conditions d'agrément, d'aménagement et de fonctionnement des établissements utilisateurs, éleveurs ou fournisseurs d'animaux utilisés à des fins scientifiques et leurs contrôles. http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000027037983&dateTexte=&categorieLien=id

Arrêté du 1er février 2013 relatif à l'acquisition des compétences des personnels des établissements utilisateurs, éleveurs et fournisseurs d'animaux utilisés à des fins scientifiques. http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000027037960&dateTexte=&categorieLien=id

Arrêté du 1er février 2013 fixant les conditions de fourniture de certaines espèces animales utilisées à des fins scientifiques aux établissements utilisateurs agréés. http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000027037949

Arrêté du 1er février 2013 relatif à l'évaluation éthique et à l'autorisation des projets impliquant l'utilisation d'animaux dans des procédures expérimentales.

http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000027038013&dateTexte=&categorieLien=id

Regulatory texts concerning safety and security plans

Regulation (EU) No 388/2012 of the European Parliament and of the Council of 19 April 2012 amending Council Regulation (EC) No 428/2009 setting up a Community regime for the control of exports, transfer, brokering and transit of dual-use items.http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:129:0012:0280:EN:PDF

Décret n°2005-1158 du 13 septembre 2005 relatif aux plans particuliers d'intervention concernant certains ouvrages ou installations fixes et pris en application de l'article 15 de la loi n° 2004-811 du 13 août 2004 relative à la modernisation de la sécurité civile. http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000786335&dateTexte=&categorieLien=id

Décret du 2 novembre 2011 relatif à la protection du potentiel scientifique et technique de la nation. http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000024749915&dateTexte=&categorieLien=id

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Table 1. Overview of the questions posed and responses from ANSM.

Question	ANSM response
What does the term "fate" of MTs refer to, used in Part 2.2 of the technical dossier?	The authorisation applicant must indicate the "fate" of the MT: destruction after handling, possible storage by freezing, destruction of the batch at the end of the project, etc.
What is meant by the term "operation" in Part 3.4 "Description of operations" in the technical dossier?	"Operation" is a general term that can be defined by the applicant depending on the specific activities of the laboratory.
In Chapter 3.4.4 "Description of implementation", it is difficult to respond from the outset, before the protocols are effectively implemented: maximum number of animals used for the experiment; maximum inoculated infectious dose per animal; duration and frequency of animal experiment; maximum culture volume and surface area; duration and frequency of cultures, etc.	The description of implementation must be drafted using average figures in the case of research laboratories that often change their protocols.
Risk management system: must a risk assessment be performed for each protocol or can risk operations be organised into groups?	The aim is to evaluate the risks concerning "general" hazards (risk of bites, risk of theft, escape of an animal, etc.) encountered when implementing the protocols.
In the particular area of these specificity validations, can an NRL keep DNA extracted from strains of organisms classed as MTs and use the DNA for a period not exceeding 30 days, thereby enabling an exemption from authorisation?	- either the DNA contains fewer than 500 base pairs, making it exempt from the MT regulation (Ministerial Order of 30 April 2012); - or you consider the DNA fragment to be a veterinary reagent, also rendering it exempt.
Most of the available methods are PCR protocols which require a positive control. How can Departmental veterinary laboratories have a positive reference control that they keep for less than 30 days? What criteria are used to determine whether a DNA fragment is a veterinary reagent?	Article R.5139-2 of the Public Health Code provides for an exemption from authorisation specifically for reagents containing MTs, when they are reagents intended for analyses carried out in the veterinary and plant protection fields, as defined in Article L.202-6 and in paragraph 1 of Article R.203-1 of the Rural and Maritime Fishing Code (CRPM). The only exempt veterinary reagents are those validated by the NRL.
For avian flu viruses, how should we interpret the term "causing human infection"? Should we only consider the availability of effectively reported cases in humans, or the suspected zoonotic potential given certain documented viral characteristics, or in the absence of this data, a default classification in this category in line with the principle of precaution?	The regulation is based on the availability of effectively reported human cases. The Ministerial Order of 30 April 2012 stipulates for Orthomyxoviridae: - Type A avian influenza virus and H5N1 subtype, causing human infection; - Type A avian influenza virus and H7N7 and H7N3 subtypes, causing human infection This list may change if other cases are reported.
Some of the data requested in the dossier are security-defence related: must this information be submitted?	The documents can be classified security-defence confidential if necessary before submission to ANSM, which has authorised personnel to handle this type of document.
How can we evaluate the physical and psychological capacity of persons who work with MTs?	During the occupational medical assessment, the physician attempts to identify fears related to handling MT agents or working in a confined space (claustrophobia). The decision "able" is sufficient if these questions were asked.
What are the training requirements for MT auditors?	No specific requirements, only a need to validate their competence in auditing and knowledge of MTs.