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## Biocontrol, new questions for Ecotoxicology?

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## Introduction

Biocontrol is viewed as an environment friendly alternative to the use of conventional (synthetic) pesticides to control pests or weeds in agrosystems. It relies on the use of biopesticides (Table 1) gathering either macro/micro-organisms (also called biocontrol agents), natural substances purified from living organisms (biopesticides *sensus stricto*) or mineral preparations as defined in the Article L253-6 from the "Code rural et de la pêche maritime" (Code rural et de la pêche maritime 2014). The organisms can be insects, nematodes, bacteria, fungi or viruses which could act as predators, parasitoids or pathogens of the pests or weeds (Flint and Dreistadt 1998). The natural substances can be plant extracts at various purification degrees or toxins from animals, plants or bacteria. Depending on their nature and composition, they can induce toxic effects or manipulate the behaviour of targeted organisms due to an attractive (as lures in traps) or repulsive effects. As for synthetic pesticides, the natural substances can be sprayed or associated to inert material or artificial food in order to optimize their efficiency in the field.

For many reasons, the biocontrol solutions which were until a recent time rarely used make their coming out. The politics, responding to the public pressure asking for safer agricultural practices, launched several new policies to reduce the use of pesticides such as the European Union (EU) regulation on the sustainable use of pesticides (Directive 2009/128/EC 2009) or the French law for the Agroecology transition (Loi n° 2014-1170 2014) and the Ecophyto II program (Ecophyto II 2015). This new paradigm paves the way to an important development of biocontrol solutions including biopesticides in the coming decades. However, their regulation is still following the same path than that of synthetic pesticides ((EC) N° 1107/2009/EC 2009) which seriously slow down their release on the market, except for the macro-organisms. The International Biocontrol Manufacturers' Association (IBMA) asked EU for considering an adaptation of the regulation to facilitate the homologation of biocontrol agents, notably by considering the concepts of 'substantial equivalence' and of 'low risk substances'. This trend is pushed in front by the French ministry of agriculture to release on the market biocontrol solutions and facilitate the adoption of the Agroecology transition (Chomienne 2017).

The "bio-" prefix in the word "biopesticides" implies the organic origin of such compounds/organisms (the updated list of the authorized biopesticides in France is edited by the "Direction Générale de l'Alimentation (DGAL 2018) but does it necessarily guarantee their environment safe profile? The advantages of their biodegradation and thus

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their expected low persistence in the environment are often evoked to elude the key questions on the potential side effects of biopesticides. Indeed, they comprised one or several active ingredients which can be harmful for non-target organisms and/or ecosystem processes. Do we have to assess the ecotoxicological risk of biopesticides in the same way as it is currently done for synthetic pesticides? Unfortunately, there are few available pertinent data on the assessment of the ecotoxicological impact on non-target organisms of biopesticides to feed this debate. Many reasons can be evoked to explain this lack of data among which: (i) biocontrol solutions are not enough present on the market despite their evident interest because of different reasons listed above, (ii) the industrial sector of the biocontrol is principally made of small to medium size companies with low R&D budget and facilities, (iii) very few academic laboratories are active in this domain.

Within this context, we identified four questions, listed thereafter, that were discussed with scientists in a roundtable during the conference of the French ECOTOX network (Dec. 2017, Valence, France), gathering researchers working on ecotoxicology:

I. Which biopesticides are in need to be studied today?

II. To what extent their side effects should be studied and on which organisms?

III. What role for ecotoxicology in regulation and homologation processes for the release of biopesticides on the market?

### **I. Which biopesticides are in need to be studied today?**

We did not identify a biopesticide to address more urgently than another in the framework of the Ecotoxicological Risk Assessment (ERA; Fig.1). Actually, such a selection should occur as what exists for conventional pesticides: the most used, the most toxic or the most present in the environment have to be the most studied. Furthermore, a compound authorized as biopesticide in one country could be forbidden in another one, depending on national legislation, like for synthetic pesticides. There is a clear need for a global homogenization, at the widest level, of the regulations for bio- and synthetic pesticides. Academic researchers also study biopesticides that are not yet on this market, such as Essential Oils (See Pavela and Benelli 2016 for a review) to test their toxicity on pests and their ecotoxicity towards non-target organisms. Actually, they precede the ERA requirements.

The concepts, approaches, methodologies and skills applied for ERA of pesticides are readily available (Fig. 1) and can be immediately remobilized and adapted for developing

1 appropriate ERA of biocontrol solutions. Yet, a problem often encountered when  
2 performing ERA of biopesticides lies in the difficulty to identify and have available the  
3 active ingredient. In line with this, the development of standardized biopesticides  
4 formulations is as crucial as for synthetic pesticides. Indeed, for both bio- and synthetic-  
5 pesticides one can have questions relative to the role, the effect and the origin of a  
6 potential co-formulant? Attention should be paid to develop suitable formulations to ensure  
7 the efficacy of both bio- and synthetic-pesticides and limit as far as possible  
8 ecotoxicological risk for the environment.  
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13 During the roundtable discussion, it appears that only a few numbers of laboratories of  
14 the French Network of Ecotoxicology are dealing with ecotoxicology in biocontrol. The  
15 same remark can be done at the EU level and worldwide.  
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## 19 **II. To what extent, side effects should be studied and on which organisms**

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22 In France the agro-ecology transition is part of the 'law for the future of agriculture (Loi n°  
23 2014-1170 2014). This law was adopted in year 2014 with the objectives to diminish the  
24 dependency of agriculture to agrochemicals and to decrease the negative effects of  
25 agriculture on environmental and human health. Within this context, the use of biocontrol  
26 solutions is promoted by the French Ministry of Agriculture. Despite this political pressure,  
27 the healthiness of biopesticides remains a matter of debate as recently underlined in a  
28 report from the French General Council for Food, Agriculture (Chomienne 2017). Indeed,  
29 there is still the need for a careful toxicological, ecotoxicological and ecological evaluation  
30 of the biocontrol products and solutions. Biopesticides as synthetic pesticides both contain  
31 one or several active ingredients designed to control a given pest which can harm non-  
32 targeted organisms.  
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42 Several ecotoxicological studies of various biopesticides on diverse non-targeted  
43 organisms have already been published such as arthropods (Biondi et al. 2012a; Biondi et  
44 al. 2012b; Nakasu et al. 2014; Renzi et al. 2016; Amichot et al. 2016), soil organisms  
45 (Ipsilantis et al. 2012; Romdhane et al. 2016; Chelinho et al. 2017), aquatic organisms  
46 (Duchet et al. 2010; Manachini et al. 2013) or mammals (Rahioui et al. 2014). The choice  
47 of such non-target organisms reflects on one hand the ecosystem service they provide: the  
48 beneficial arthropods are involved in biocontrol and pollination services when the beneficial  
49 soil organisms are involved in decomposition or nutrient transfer (e.g. mycorrhizas)  
50 services. Looking for the side effect of biopesticides on such organisms implies the  
51 potential side effect of biopesticides on ecosystem services. On the other hand, this choice  
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1 reflects the different components of the ecosystem (e.g. hydrosphere representing by  
2 aquatic organisms, lithosphere by soil organisms and biosphere by all of them) in order to  
3 evaluate the healthiness of biopesticides on all the environmental components.

4 In addition, the simultaneous use of several biopesticides as part of the biocontrol  
5 strategy applied on a given crop, such as the combination of parasitoids and micro-  
6 organisms or even the combination of biopesticide with conventional pesticides, asks the  
7 question of mixtures. Finally, studies performed with laboratory model organisms such as  
8 *Drosophila* or Mouse might also be extremely helpful to identify the mechanisms  
9 responsible for the putative side effects of biopesticides.  
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11 As mentioned above, insects, plants, bacteria, plant or microbial extracts can be used  
12 as biopesticides (Table 1). Being diverse by nature, they are diverging by their mode of  
13 action with a wide diversity of mechanisms involved in their toxicity, and thus they may  
14 have various side effects not only on target-organisms but also on ecosystem functions.  
15 ERA for pesticides cannot be readily transferred to other biocontrol solutions involving  
16 macro- and microscopic organisms. Regarding biocontrol agents, the risk assessment  
17 focus more on invasive species or ecosystem functions malfunctioning. It is noteworthy  
18 that ecosystem functions have been proposed by the European Food Safety Agency as  
19 specific protection goals for ERA of pesticides. Indeed, release of biocontrol agents in the  
20 field can be compared to invasive species thus questioning which is relevant to the  
21 ecology field, and more specifically to the community ecology (See De Castro et al 2010  
22 and Sundh & Goettel 2013 for a comparison between macro- and micro-organisms).  
23 Furthermore, it was underlined that for micro-organisms releasing toxins, it will be  
24 necessary to trace the fate of micro-organisms and of their toxins as they may have  
25 different persistence trajectories in the environment (See Sundh & Goettel 2013 for a  
26 review).  
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28 From this point of view, the discussion points out the need for the academics to clarify  
29 the contribution of both ecology and ecotoxicology concepts to environmental risk  
30 assessment of biopesticides. As a conclusion we agreed that ecotoxicologists and  
31 ecologists should overcome disciplinary boundaries and share their concepts, approaches  
32 and tools to remobilize them for a more global environmental risk assessment of  
33 biopesticides.  
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### 35 **III. What role for ecotoxicology in regulation and homologation processes for the** 36 **release of biopesticides on the market?** 37

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The complexity of the biopesticide regulation has been underlined in the previous sections. Indeed, two difficulties were highlighted: the first one is the nature of the biocontrol solution which can either be a substance or a living organism. On one hand, the biopesticides *stricto sensu* and the microorganisms are until now submitted to the same EU-Regulation (i.e. (EC) N° 1107/2009) like the synthetic pesticides, but with different data requirements for microorganisms ((EC) N° 283/2013). On the other hand, macro-organism use is submitted to a peculiar regulation (Loi “Grenelle II” 2010, Décret n° 2012-140 2012), based on their fundamental biology and their ecological interactions.

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The second difficulty comes from the frequent bypass of current regulations by proposing biostimulants doped with biopesticides to improve their intrinsic quality and functions (See (Pavela and Benelli 2016) for the essential oils case and (Sundh and Goettel 2012) for the micro-organism case). In fact, the regulation for biostimulants is much less binding than the one for pesticides and biopesticides ((EC) N° 1069/2009 2009, (EC) N° 1107/2009 2009). Consequently, we concluded that there is an urgent need for the overall reconsideration of the regulations for the use of organisms, biopesticides and biostimulants doped with biopesticides under the general umbrella of biocontrol solutions.

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The process leading to the approval of biopesticides should also question the ecotoxicity of their co-formulants entering in the recipe of their formulation which currently is under the REACH regulation ((EC) N° 1907/2006 2006): the potential side effect of the mixture of co-formulants entering in the composition of the formulation have to be addressed.

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We recognized the importance of ecotoxicology for (i) *a priori* ecotoxicological risk assessment of biopesticides as a part of the homologation process and for (ii) *a posteriori* ecotoxicological risk assessment of formulated biopesticides which may lead to their restriction of use when danger was obvious. Thus, ecotoxicology can play a pivotal role to secure the development of biocontrol solutions.

## 47 48 49 **Conclusion**

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To conclude, we recognized that among the French ECOTOX network and likely more widely, there are today only few academic laboratories working in the field of ecotoxicology involved in the ERA for biocontrol solutions. Thus, it appeared that the *a priori* ERA of biopesticides should be done in a similar manner as carried out for conventional pesticides. The richness of the discussions among the participants to the roundtable confirmed the relevance of the questions asked. We agreed that further discussions have to be planned



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to define a common ground between ecotoxicological and ecological concepts that Ecotoxicology should definitely help to reach the crucial challenges for the biocontrol.

Given the fact that biopesticides *sensus stricto* follow the same regulation than synthetic pesticides, their ecotoxicological risk assessment is not really a matter of discussion except that the overall process is time consuming and costly like it is for synthetic pesticides. This may result in slowing down the release of biocontrol solutions on the market in particular for small- or medium-size companies who have less budget than pesticide industries to carry out all the requirements needed to claim an authorization to put on the market a new active ingredient. On the contrary, for living organisms used as biocontrol agents, ecotoxicological risk assessment passes beyond the concepts of ecotoxicology and have recourse to ecology concepts and researches on invasive species. Therefore, their mode of action and their putative side effects on non-target organisms are expected to be extremely different. We believe that it is an interesting path to follow. Furthermore, the use of living organisms which produce toxins (as *Bacillus thuringiensis* for instance) should be the object of specific attention since the toxins component can be submitted to ERA whereas the bacteria component can be considered as a putative invasive organism (See Sundh & Goettel 2013 for a review).

We feared a major difficulty in the ecotoxicological risk assessment scheme of biocontrol agents because of the diversity of their nature which can either be living organisms or substances (plant extracts, toxins...). Nevertheless, we believe that well mastered concepts, approaches and methodologies of ecotoxicology and also of ecology can be adapted and transferred for a better ecotoxicological risk assessment of biocontrol agents to truly ensure their safe development and to consolidate them as a credible alternative to conventional plant protection products.

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## Legends

### Figure 1

Flowchart of the Ecotoxicological Risk Assessment for pesticides.

This flowchart is also conceptually suitable to address the putative risks associated with the use of biopesticides or biostimulants. After the problem formulation, laboratory experiments are conducted to address the putative toxicity of the compound on pertinent organisms, for instance organisms present in the ecological niche in which the compound is planned to be used. If toxicity is detected, then field experiments will determine the environmental distribution of the compound and the modes of exposition of the organisms. If a new use is intended for a known compound, it is possible to directly plan exposure measures as shown by the dotted arrow because the toxicity of the compound is already known. We boxed the lower area to emphasize the close relationships that exist between Ecotoxicological Risk Assessment and the Environmental Risks Assessment (the boxed area), although separated in the regulations. As for synthetic pesticides, the choice of the non-targeted organisms to be tested with biopesticides in laboratory experiments is crucial for the relevancy of the tests. Furthermore, there is a gap to fill for the exposition measures relatively to biopesticides as the data are very scarce in the literature.

### Table 1: Biopesticides *sensus lato* authorised for use in France

Synthesis of biocontrol solutions authorized in France. Many products are natural extracts from plant or mineral origins. Most of them have a broad range of targets but some are very specific such as the pheromones. The reader may note that not all products are used to kill pests, a few impact the behaviour of the pests (mainly semiochemicals category) or stimulate the ability of plants to defend against pests (included in the plant extract) \*.

Based on the list of authorized biopesticides in France for biopesticides *stricto sensu* (DGAL 2018), the mineral solution and the microorganisms and for the macro-organisms (Arrêté AGRG1502673A 2015).

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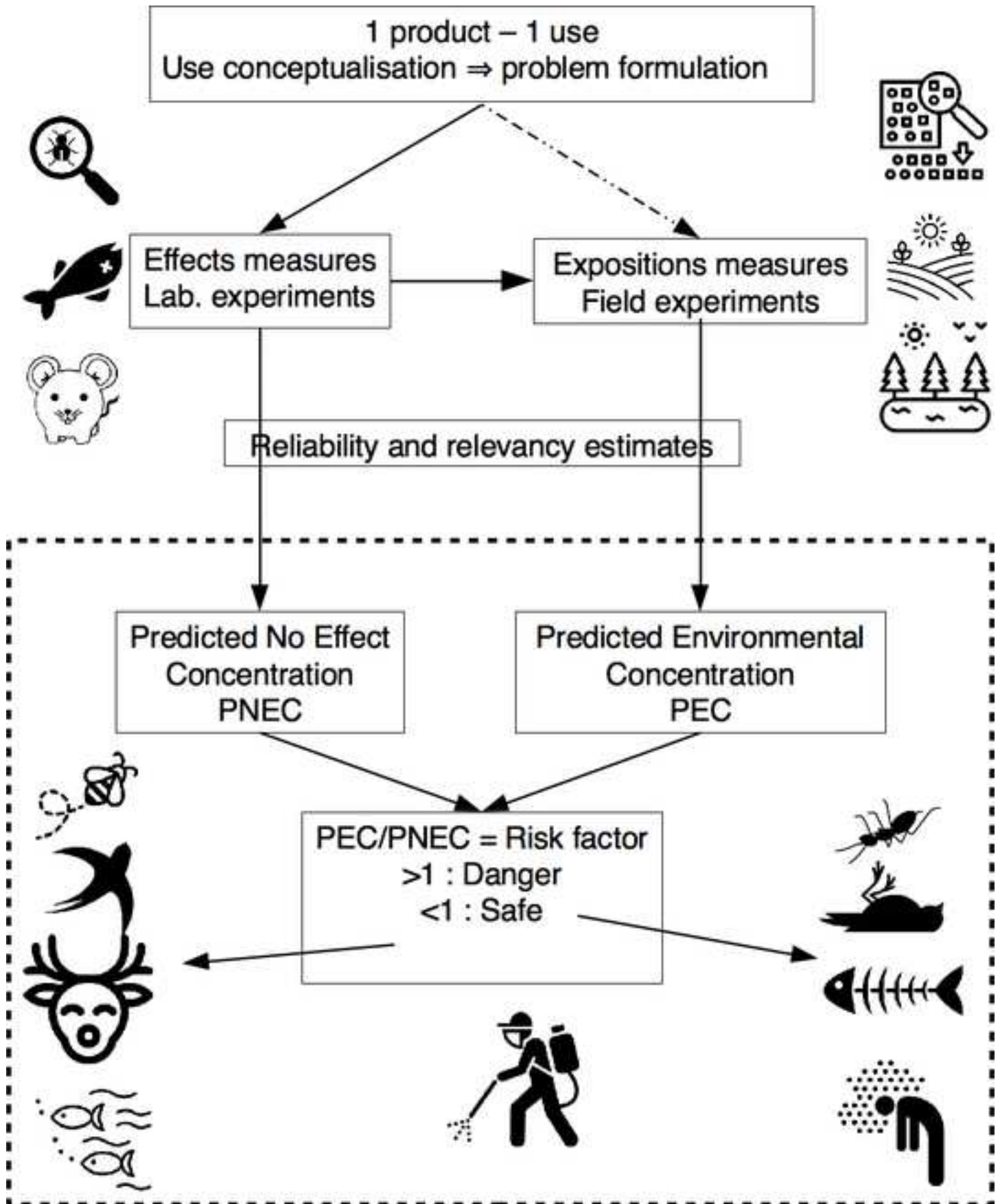


Table 1

	<i>Living Organisms = Biocontrol agents</i>			<i>Natural substances = Biopesticides sensus stricto</i>					<i>Mineral substances</i>		
	Macro-organisms		Microorganisms	Natural extract			Semiochemicals				
	Pathogens	Predators	Parasitoids	Pathogens	Plant origin*	Animal origin	Microorganism origin	Complex		Pheromones	Allelochemicals
	Nematodes	Insects		Bacteria, Fungi, Virus							
Mode of Action	Insecticide			Insecticide, Fungicide, Bactericide, Herbicide						Behavioural effect on insects	
Regulation	<b>Loi “Grenelle II”</b> Décret n° 2012-140			Regulation (EC) No 1107/2009 Commission Regulation (EU) No 283/2013 For biostimulants*: (CE) No 1069/2009 (CE) No 1107/2009							
List of official biopesticides in France	Décret 26 Feb 2015 <a href="https://www.legifrance.gouv.fr/eli/arrete/2015/2/26/AGRG1502673A/jo/texte">https://www.legifrance.gouv.fr/eli/arrete/2015/2/26/AGRG1502673A/jo/texte</a>			Bimonthly edited list at: <a href="https://info.agriculture.gouv.fr/gedei/site/bo-agri/instruction-2018-528">https://info.agriculture.gouv.fr/gedei/site/bo-agri/instruction-2018-528</a>							