

Dinâmica dos pesticidas no ambiente, panorama geral dos estudos e *guidelines* para uso de ^{14}C -pesticidas

Ph.D. candidate Vanessa Takeshita

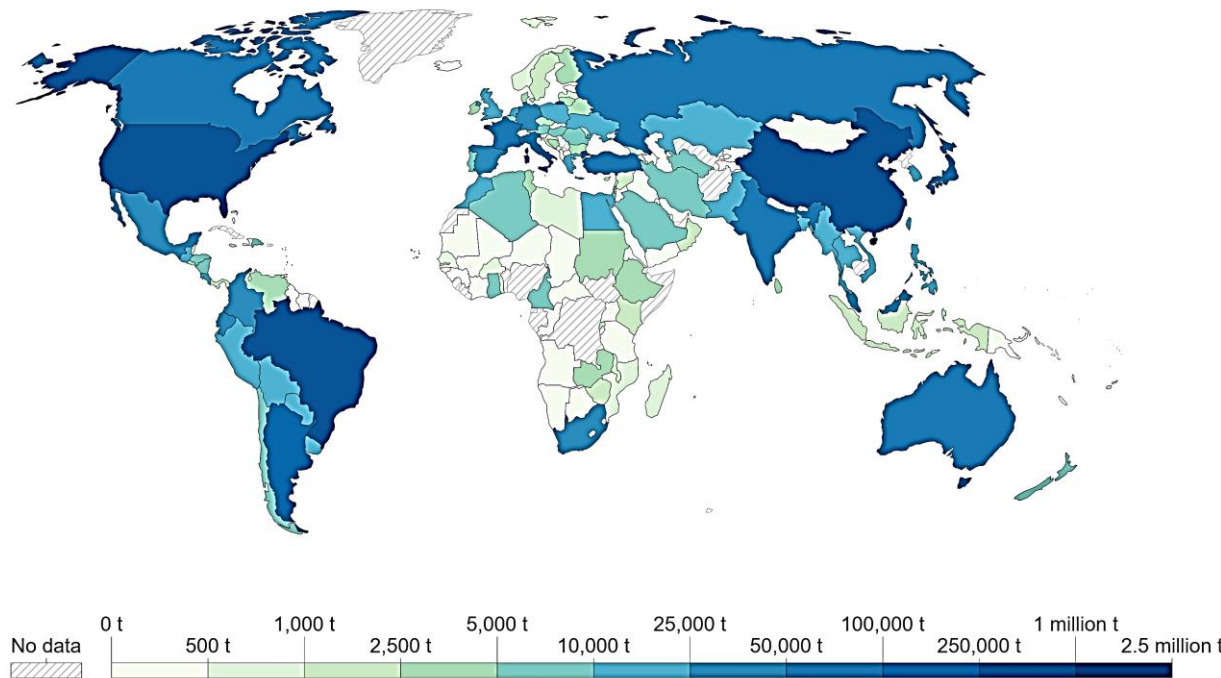
Cenário global: Uso dos pesticidas

Panorama global do uso de pesticidas

Pesticide use, 2020

Total pesticide use measured in tonnes of pesticide consumption per year.

Our World
in Data



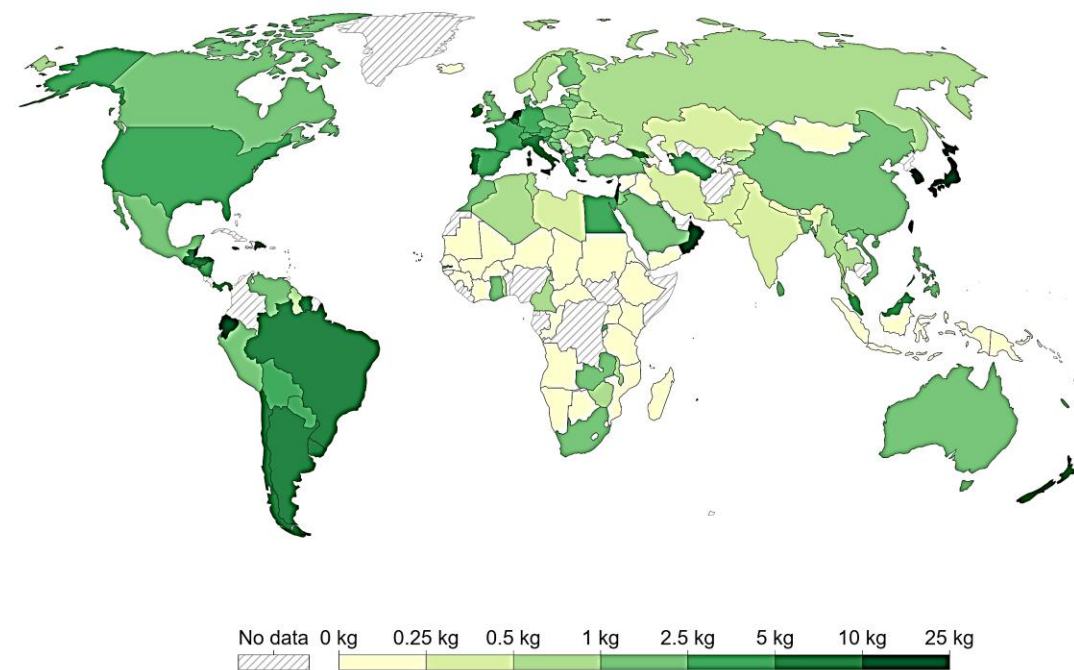
Source: Food and Agriculture Organization of the United Nations

OurWorldInData.org/pesticides • CC BY

Pesticide use per hectare of cropland, 2020

Average pesticide application per unit of cropland, measured in kilograms per hectare.

Our World
in Data



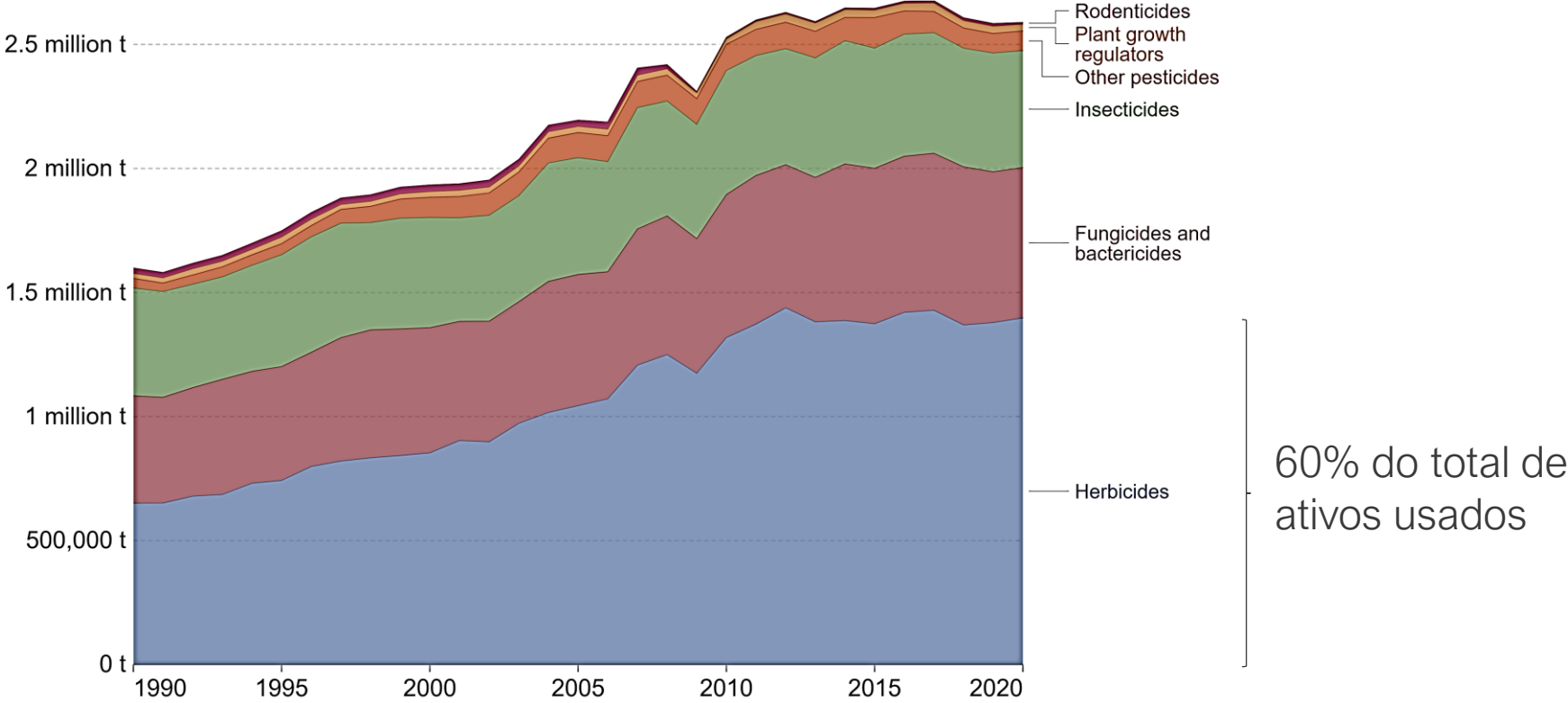
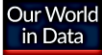
Source: Food and Agriculture Organization of the United Nations

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Panorama global do uso de pesticidas

Pesticide breakdown by type, World, 1990 to 2020

Pesticide use, broken down by product type, measured in tonnes of active ingredient.



Source: Food and Agriculture Organization of the United Nations

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ABOUT PESTICIDES IN AGRICULTURE

1 The global consumption of pesticides is increasing, even though the health and ecological consequences have long been known. International goals of **BIODIVERSITY CONSERVATION** can only be achieved if the use of pesticides is significantly reduced.



2 Herbicides are applied against unwanted plants and are the **MOST USED GROUP OF ACTIVE SUBSTANCES**. Insecticides are effective against insects. Often even in smallest amounts and even against other insects that were not targeted.



3 About 385 million cases of **PESTICIDE POISONING** occur worldwide every year. People in the Global South working in rural areas are particularly affected.

4 Pesticides that are **NOT PERMITTED IN EUROPE FOR ECOLOGICAL OR HEALTH REASONS** are still produced here and exported to other countries. European companies are also involved in this business.



5 The EU has strict criteria for the authorisation of pesticides. But the harmful **EFFECTS OF PESTICIDES ON WHOLE ECOSYSTEMS** are not taken into account.



6 Pesticide active ingredients usually do not stay in the place they were applied. They can seep into the soil and **GROUNDWATER**, become airborne, or blow away – some can be found over 1,000 kilometres away.



7 Pesticides **CONTAMINATE** water via infiltration, surface runoff and drift. They also accumulate in the soil and exert adverse effects on soil life – sometimes for decades.



8 Pesticide residues in food can be **HARMFUL TO PEOPLE'S HEALTH**. Despite attempts to reach globally harmonized standards, maximum residue levels vary widely from country to country.



9 Four corporations from the Global North control 70 percent of the global pesticide market. They are **EXPANDING THEIR BUSINESS** to the Global South where pesticides are less strictly regulated.



10 Beneficial insects are the **NATURAL ENEMIES OF PESTS** and creating beneficial environments for them can help reduce the use of pesticides.



11 The EU has so far failed to reduce the use of pesticides. Its **FARM TO FORK STRATEGY** aims to change that by introducing a new regulation to half the use of pesticides by 2030. The EU's Common Agricultural Policy is not yet aligned.



12 Unlike industrial monocultures, agroecological cultivation practices, including more crop rotations and combinations, empower farmers to use less or no pesticides. Some regions of the world are going ahead. But a binding international **TREATY ON THE REDUCTION OF PESTICIDES** does not yet exist.

11 The EU has so far failed to reduce the use of pesticides. Its **FARM TO FORK STRATEGY** aims to change that by introducing a new regulation to half the use of pesticides by 2030. The EU's Common Agricultural Policy is not yet aligned.



https://food.ec.europa.eu/horizontal-topics/farm-to-fork-strategy_en

European Green Deal

<p>The first climate-neutral continent by 2050</p>	<p>At least 55% less net greenhouse gas emissions by 2030, compared to 1990 levels</p>	<p>3 billion additional trees to be planted in the EU by 2030</p>
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 **OBJETIVOS**  **DE DESENVOLVIMENTO SUSTENTÁVEL**

1 ERRADICAÇÃO DA POBREZA



2 FOME ZERO



3 BOA SAÚDE E BEM-ESTAR



4 EDUCAÇÃO DE QUALIDADE



5 IGUALDADE DE GÊNERO



6 ÁGUA LIMPA E SANEAMENTO



7 ENERGIA ACESSÍVEL E LIMPA



8 EMPREGO DIGNO E CRESCIMENTO ECONÔMICO



9 INDÚSTRIA, INOVAÇÃO E INFRAESTRUTURA



10 REDUÇÃO DAS DESIGUALDADES



11 CIDADES E COMUNIDADES SUSTENTÁVEIS



12 CONSUMO E PRODUÇÃO RESPONSÁVEIS



13 COMBATE ÀS ALTERAÇÕES CLIMÁTICAS



14 VIDA DEBAIXO D'ÁGUA



15 VIDA SOBRE A TERRA



16 PAZ, JUSTIÇA E INSTITUIÇÕES FORTES



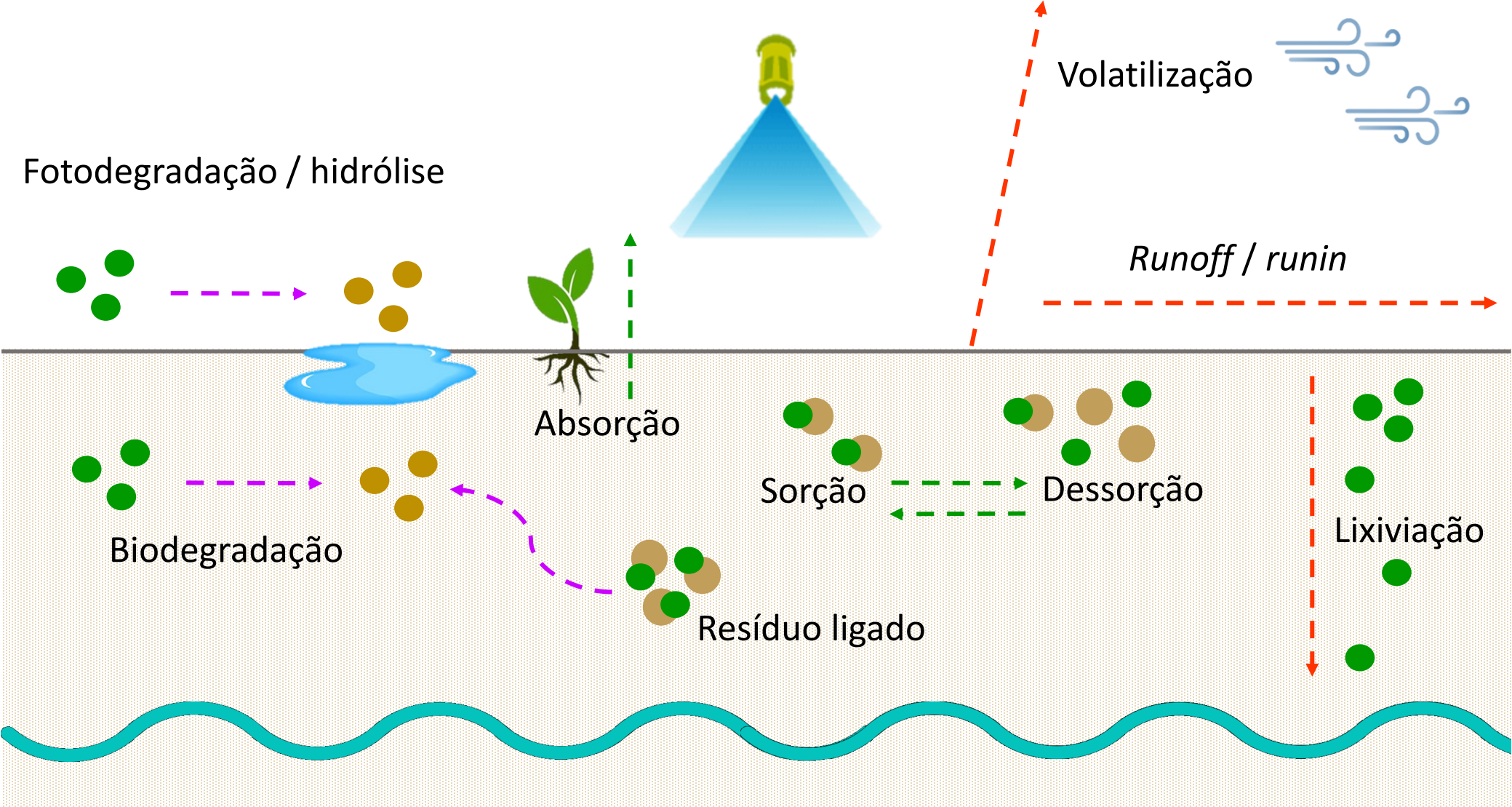
17 PARCERIAS EM PROL DAS METAS



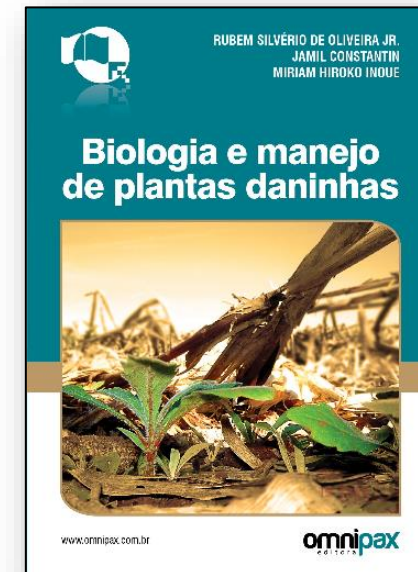
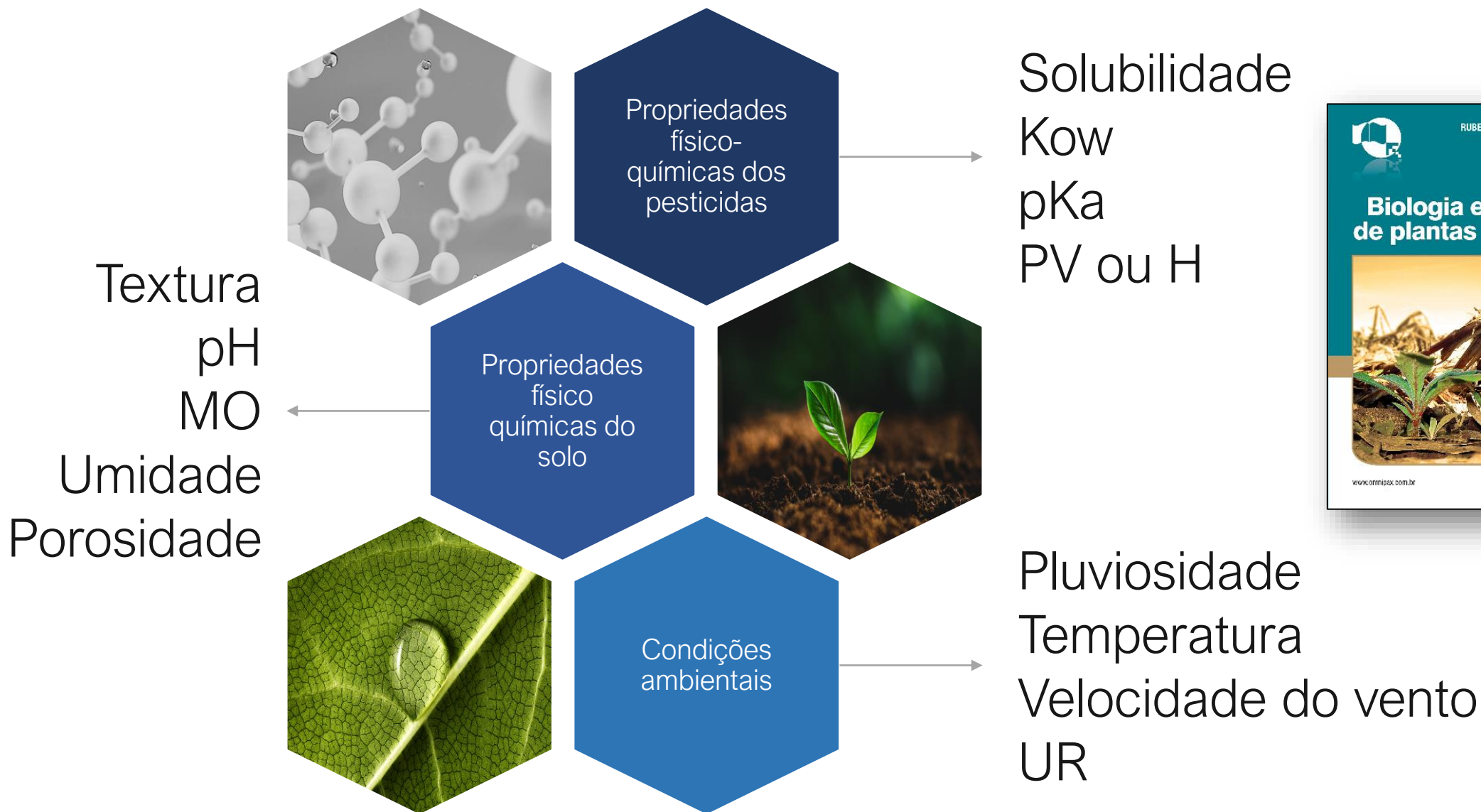

OBJETIVOS 
DE DESENVOLVIMENTO
SUSTENTÁVEL

Cenário ambiental: Dinâmica dos pesticidas

Dinâmica dos pesticidas no ambiente



Dinâmica dos pesticidas no ambiente



Dinâmica dos pesticidas no ambiente

Processos gerais	Processos específicos	Propriedades físico-químicas e outros fatores	Coeficientes e dados importantes gerados
Retenção	Sorção-dessorção Resíduo ligado		Kd, Koc, Kf, % sorvida, % biodisponível, % dessorvida, H
Transporte	Volatilização / deriva Lixiviação <i>Runoff / runin</i>	pKa, Kow, Sw, PV, textura, MO, pH, Umidade do solo, UR, pluviosidade, vento, T°C	% de transporte, Rf
Degradação	Hidrólise Fotodegradação Biodegradação		T _{1/2} ou DT ₅₀ , % de degradação, % de mineralização, número e quantidade de metabólitos formados

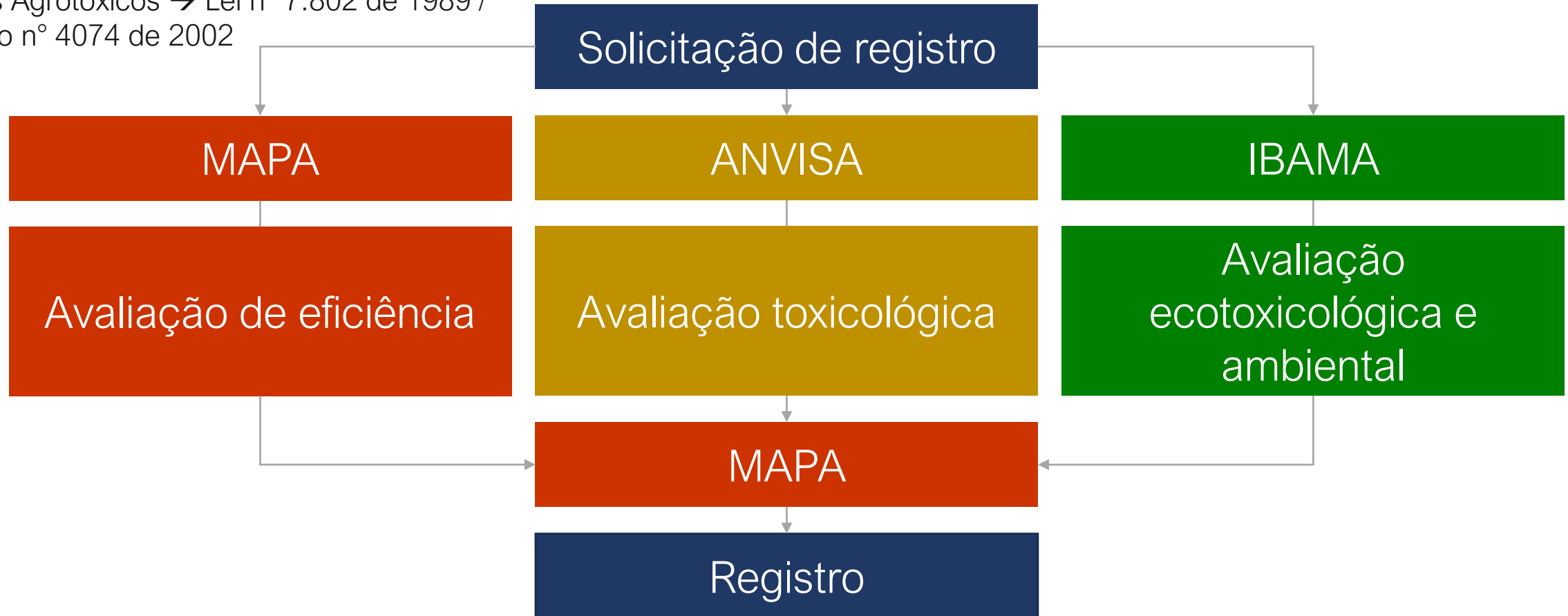
Dinâmica dos pesticidas no ambiente

Outros estudos	Processos específicos	Propriedades físico-químicas e outros fatores	Coeficientes e dados importantes gerados
Absorção e translocação em plantas	Absorção Translocação Metabolismo	pKa, Kow, Sw, PV, textura, MO, pH, Umidade do solo, UR, pluviosidade, vento, T°C	% absorvida, % translocada, Fator de absorção, número e quantidade de metabólitos formados
Ecotoxicologia	Bioacumulação		BAF (fator de bioacúmulo), % bioacumulada

Cenário regulatório: Registro e regulação

Registro de Agrotóxicos no Brasil

Lei dos Agrotóxicos → Lei nº 7.802 de 1989 /
Decreto nº 4074 de 2002



Registro de Agrotóxicos no Brasil

Lei dos Agrotóxicos → Lei nº 7.802 de 1989 /
Decreto nº 4074 de 2002

- 8 parâmetros são avaliados:
Transporte (mobilidade e retenção)
Persistência ($T_{1/2}$)
Bioacumulação
Toxicidade organismos do solo
Toxicidade organismos aquáticos
Toxicidade aves e abelhas
Toxicidade sistêmica e tópica para mamíferos



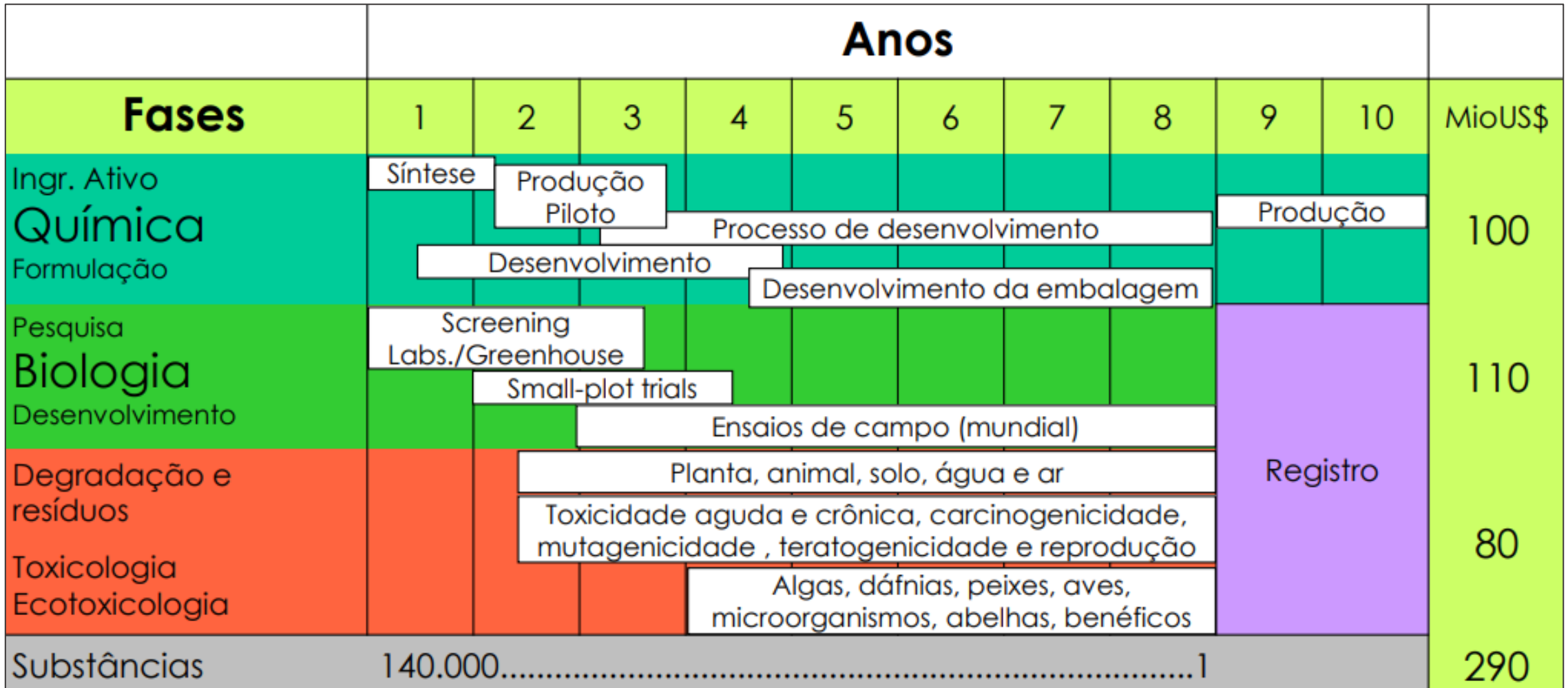
O que é importante considerar para a aprovação de um pesticida?

Visão global (EFSA):

- Não apresentar **nenhum perigo imediato e a longo prazo aos humanos e animais**, através da água potável, alimentos e ar, ou na exposição do trabalhador, bem como efeitos cumulativos ou sinérgicos de perigo
- **Não apresentar efeitos inaceitáveis ao ambiente** (contaminação do solo e água), principalmente relacionados a organismos não-alvo e biodiversidade geral

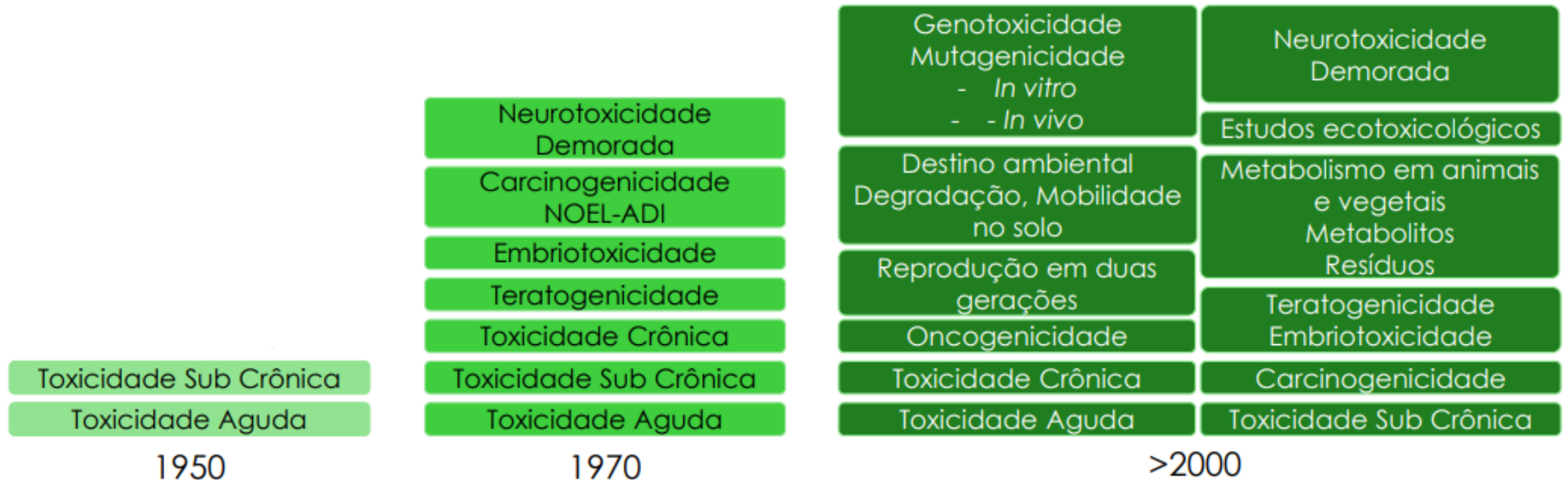
Submissão → Avaliação → Aprovação → Autorização → Monitoramento / Reavaliação

O que é importante considerar para a aprovação de um pesticida?



O que é importante considerar para a aprovação de um pesticida?

Quem participa da regulação a nível global?



Adaptado de: Palestra ao Senado Federal sobre a modernização e regulamentação dos agrotóxicos - Prof. Dr. José Otávio Mentem

Organizações responsáveis pela regulação dos testes com pesticidas no mundo

- **U.S. Environmental Protection Agency**
 - Agência federal americana que trabalha para **desenvolver e aplicar regulamentos** para a proteção ambiental e à saúde humana no país, criada em 1970
 - Estrutura organizacional: Chefiada por um administrador com status de ministro (apesar de não ser um ministério do governo)
 - Responsável pela avaliação ambiental e toxicológica, bem como pela regulamentação dos pesticidas nos EUA, previsto por lei (FIFRA e FFDCA)



Organizações responsáveis pela regulação dos testes com pesticidas no mundo

- **European Food Safety Authority**
 - Agência europeia de **aconselhamento científico** doa gestores para a comunicação de risco associados a cadeia alimentar, como fornecedores da **base científica para a legislação e regulamentação**
 - Estrutura organizacional: Comissão Europeia + Parlamento Europeu + Estados-Membros (conselho de administração) > agência
 - Auxilia a Comissão Europeia nas decisões sobre o registro de pesticidas



Intervalo?

Organizações responsáveis pela regulação dos testes com pesticidas no mundo

- Organisation for Economic Co-operation and Development - OECD
 - Organização internacional que trabalha para **formular políticas**, informar e aconselhar países, e **definir padrões** e fornecer suporte a políticas no mundo a mais de 60 anos
 - Estrutura organizacional: Conselho geral (decisões gerais) > Comitês (grupos especialistas de trabalho) > Secretariado (formulação das políticas internacionais)
 - 38 países membros (Brasil é candidato atualmente, mas já participa e colabora com a organização a alguns anos)



OECD e os *guidelines* para estudos com pesticidas

- 5 seções para testes em químicos:

Seção 1: Propriedades físico-químicas (27)

Seção 2: Efeitos em sistemas bióticos (52)

Seção 3: Destino ambiental e comportamento (24)

Seção 4: Efeitos na saúde (86)

Seção 5: outros *Test Guidelines* (10)

Elaborados com a participação de:

Agências regulatórias, academia,
indústria e organizações ambientais e de
bem-estar animal



OECD – guidelines com aplicação de ^{14}C

Test Guideline No. 316: Phototransformation of Chemicals in Water – Direct Photolysis

Test Guideline No. 320: Determining Anaerobic Transformation of Chemicals in Liquid Manure

Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure

Test No. 314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater



OECD – guidelines com aplicação de ^{14}C

Test No. 315: Bioaccumulation in Sediment-dwelling Benthic Oligochaetes

Test No. 317: Bioaccumulation in Terrestrial Oligochaetes

Test No. 312: Leaching in Soil Columns

Test No. 309: Aerobic Mineralisation in Surface Water – Simulation
Biodegradation Test

Test No. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment
Systems



OECD – guidelines com aplicação de ^{14}C

Test No. 307: Aerobic and Anaerobic Transformation in Soil

Test No. 306: Biodegradability in Seawater

Test No. 301: Ready Biodegradability

Test No. 304A: Inherent Biodegradability in Soil

Test No. 123: Partition Coefficient (1-Octanol/Water): Slow-Stirring Method

Test No. 111: Hydrolysis as a Function of pH



OECD – guidelines com aplicação de ^{14}C

Test No. 106: Adsorption - Desorption Using a Batch Equilibrium Method

Test No. 502: Metabolism in Rotational Crops

Test No. 501: Metabolism in Crops


Test No 506: Stability of Pesticide Residues in Stored Commodities

Test No. 503: Metabolism in Livestock



Cenário científico: Publicações e protocolos

Porque são usadas moléculas radiomarcadas nos testes?



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Article

METHODOLOGIES TO STUDY THE BEHAVIOR OF HERBICIDES ON PLANTS AND THE SOIL USING RADIOISOTOPES


Metodologias para Estudos de Comportamento de Herbicidas na Planta e no Solo Utilizando Radioisótopos

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MARTINS, B.A.B.¹
REIS, F.C.¹
DIAS, A.C.R.²
TORNISIELO, V.L.¹

ABSTRACT - In Brazil, the "Pesticide Act" (Act no. 7,802/89) has introduced new criteria related to the environment, public health and agronomic performance in the analyses of pesticide-related activities. Likewise, radioisotopes are used for environmental behavior and *in planta* studies, since they provide some advantages in comparison to chemical measures, including greater sensitivity, stepwise description of a particular element in a metabolic system, and pesticide position and detection through X-ray films and/or radio image (in plants) and liquid scintillation (in plants and soil), respectively. This review describes methodologies related to radioisotope utilization in studies on herbicide absorption, translocation and metabolism in plants, as well as in studies on herbicide biodegradation, mineralization, leaching and sorption-desorption on the soil. The step-by-step of the described methodologies is based on the guidelines that were established, mostly by the Organization for Economic Co-operation and Development (OECD) and the Environmental Protection Agency (EPA). On this review, methodological information on soil and plant studies, using radioisotopes, is available to Brazilian researchers. Thus, the objective of this review is to stimulate the conduction of further studies that use the methodologies described herein.

Keywords: liquid scintillation spectrometry, environmental behavior, ¹⁴C-labeled molecule.


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Radiometric strategy to track nanopesticides: An important approach to understand the fate, mechanisms of action and toxicity

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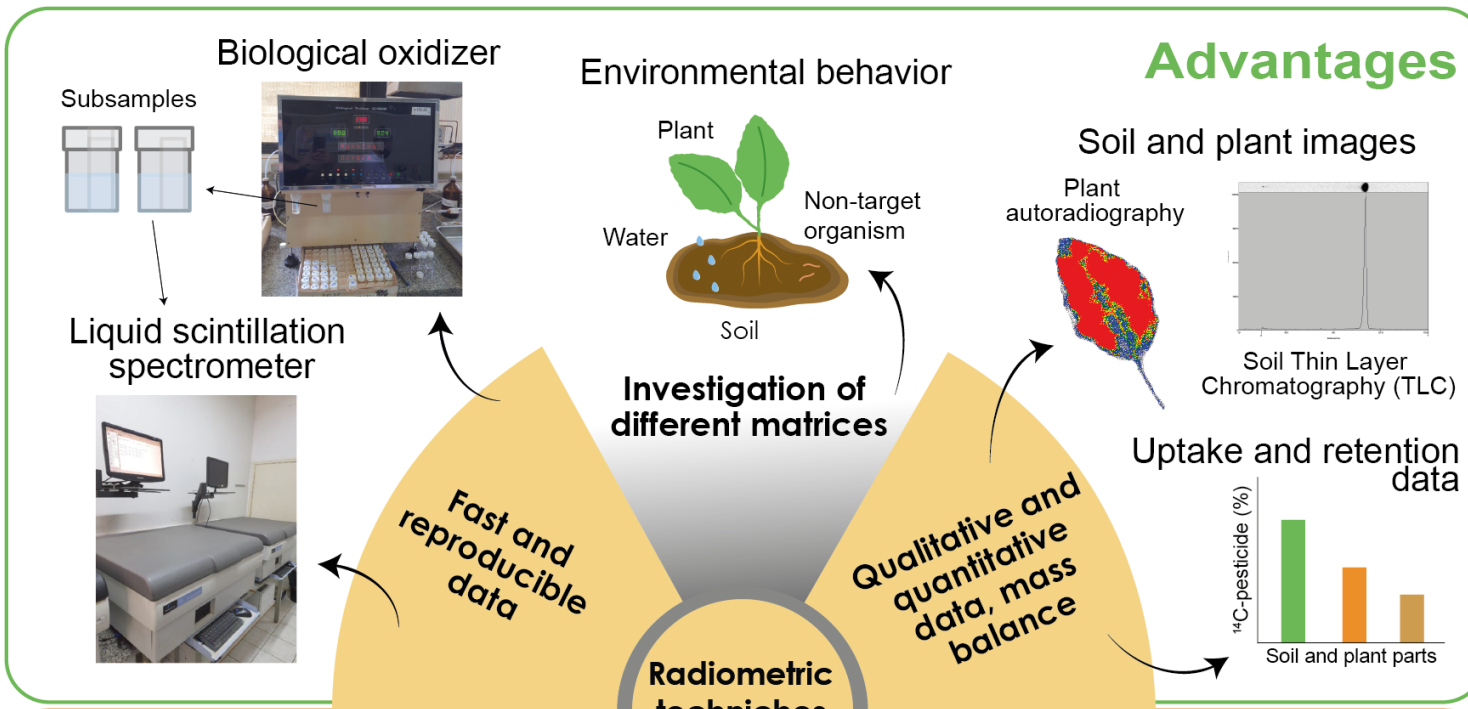
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Nanoformulation
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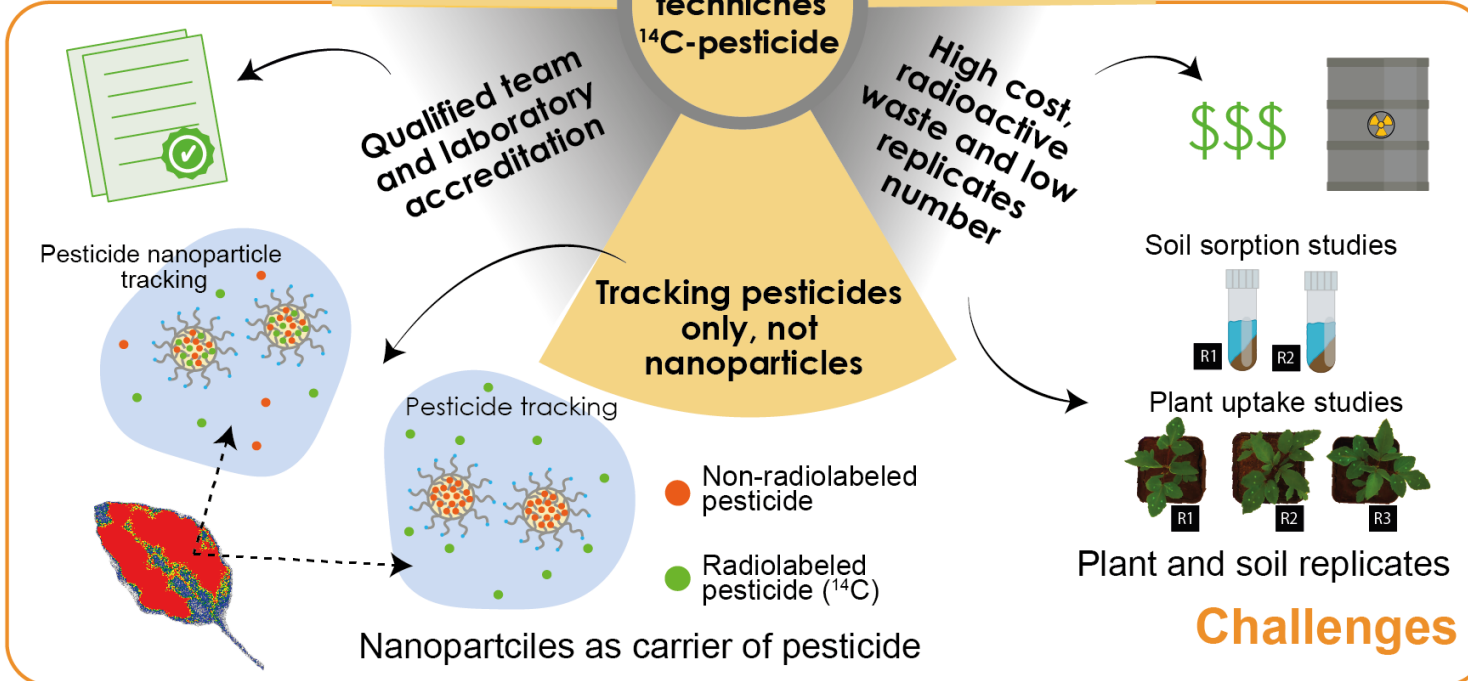
ABSTRACT

Nanopesticides are an innovative tool for crop protection, demonstrating the potential to replace conventional pesticides used in agriculture worldwide. Before their commercialization and use, careful evaluation of nanopesticides (environmental fate, toxicity, efficacy, modes of action, and agroecosystem interactions) is required. To ensure the environmental safety of nanopesticides, the standardization of protocols in the evaluation of nanoformulation regulatory aspects is needed. In this sense, we consider that radiometric techniques may be applied in the same way as conventional pesticide regulatory tests. This method can be used to track active ingredients in a biological and environmental system, together with other techniques for characterizing nanomaterials. In this review (1) we share our point of view about the use of radiolabeled pesticides to track nanoformulations in biological and environmental scenarios; and (2) we offer a critical discussion about the advantages and challenges of using radiometric techniques to track these active ingredients associated with nanoformulations.

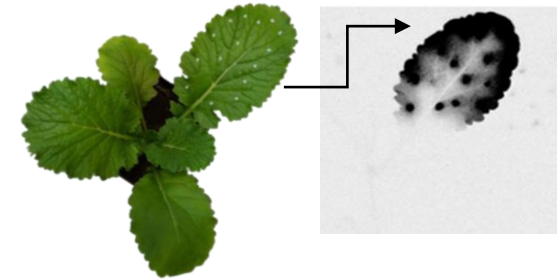
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Porque são usadas moléculas radiomarcadas nos testes?



Outras aplicabilidades do ^{14}C em estudos com pesticidas



Absorção e translocação de pesticidas em plantas:

- Usado para entender e elucidar o **mecanismo de resistência** de absorção e translocação diferencial
- Associado ao estudo de **metabolismo** serve para elucidar mais um mecanismo de resistência
- Pode ter diversas outras aplicabilidades, como distribuição a curta e longa distância, entendimento do modo de ação, padrões de absorção entre espécies e muitas outras



Porque são usadas moléculas radiomarcadas nos testes?

Weed Science 2015 Special Issue:140–151

IF: 2.58



Herbicide Absorption and Translocation in Plants using Radioisotopes

Vijay K. Nandula and William K. Vencill*

2,4-D, discovered independently in the United States and Europe in the mid-1940s, was one of the first synthetic herbicides to be used selectively for weed control (Cobb and Reade 2010). Since then, several herbicides belonging to different chemical classes and possessing diverse mechanisms of action have been synthesized and marketed globally. Herbicides have vastly contributed to increasing world food, fiber, fuel, and feed production in an efficient, economic, and environmentally sustainable manner. Before receiving regulatory approval, all herbicides (pesticides) undergo rigorous testing for their toxicological, residual, physicochemical, and biological properties. Additionally, herbicides are suitably formulated to reach their target site and maximize their efficacy on target weeds while being safe on crops. One of the main principles behind the design of herbicide formulations is getting the active ingredient across the leaf surface barrier in the case of

Terminology (Mostly Pertains to Radioactive Isotopes)

Mass Balance. This item is described as the sum of the radioactivity measured in all plant parts, leaf (or root) washes, and root exudates and calculated as a proportion of applied radioactivity at the start of the experiment (Kniss et al. 2011). It is expressed as percent recovery. Mass balance will help account for losses through volatilization, radioactive dust, root exudation, and experimental techniques.

Uptake or Absorption. It is the amount or quantity or percentage of applied active ingredient that has been absorbed by the leaves or roots of the treated plant. This is also calculated as the sum of the radioactivity measured in all parts and is generally expressed as percentage of applied radioactivity.



Radiometric strategy to track nanopesticides: An important approach to understand the fate, mechanisms of action and toxicity



Vanessa Takeshita ^{a,*,1}, Gustavo Vinícios Munhoz-Garcia ^{a,1}, Anderson Espírito Santo Pereira ^{b,c}, Valdemar Luiz Tornisielo ^a, Leonardo Fernandes Fraceto ^{c,*}

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Outras aplicabilidades do ^{14}C em estudos com pesticidas além dos guidelines

SunTest para degradação de pesticidas:

- Usado para entender e elucidar a fotodegradação de pesticidas (água, solo, sedimentos, plantas)

Volatilização de pesticidas em ambiente controlado:

- Usado para capturar moléculas voláteis ou o CO_2 desprendido no processo de degradação pelas plantas

Fitorremediação

- Usado na mesma lógica de um estudo de absorção e metabolismo, para encontrar o valor de pesticida extraído ou transformado pelas plantas



Exemplos de estudos com aplicação da técnica

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ORIGINAL ARTICLE



WILEY

Aminocyclopyrachlor sorption–desorption and leaching in soil amended with organic materials from sugar cane cultivation

Vanessa Takeshita¹ | Kassio Ferreira Mendes² |
 Thiago Francisco Ventoso Bompadre¹ | Felipe Gimenes Alonso¹ |
 Rodrigo Floriano Pimpinato¹ | Valdemar Luiz Tornisielo¹

IF: 2.11

TABLE3 Sorption–desorption parameters for the aminocyclopyrachlor in the soils without addition and with addition of sugarcane straw (12 t/ha), sugarcane filter cake (90 t/ha) and sugarcane vinasse (200 m³/ha)

Soil conditions	Ageing ^a	K_d (sorption)	Sorption
		(L/kg)	(%)
Soil unamended	-	0.41 (0.40–0.42) ^{b*}	28.97 (28.44–29.50)*
Soil + sugarcane straw	T0	0.35 (0.35–0.35)Aa ^d	25.89 (25.67–26.11)Ab
	T15	0.33 (0.30–0.36)Aa	24.70 (23.23–26.17)Ab
	T30	0.33 (0.32–0.35)Aa	24.90 (24.31–25.49)Ab
	T60	0.27 (0.27–0.27)Ba	21.42 (21.37–21.48)Bb
Soil + sugarcane filter cake	T0	0.35 (0.35–0.35)Aa	27.30 (26.57–28.04)BCab
	T15	0.33 (0.30–0.36)Aa	28.85 (28.66–29.05)Aba
	T30	0.33 (0.32–0.35)Aa	30.03 (29.40–30.66)Aa
	T60	0.27 (0.27–0.27)Ba	26.06 (25.96–26–15)Ca
Soil + sugarcane vinasse	T0	0.38 (0.37–0.39)Aa	27.60 (27.09–28.11)Aa
	T15	0.23 (0.23–0.23)Bb	18.58 (18.47–18.69)Bc
	T30	0.18 (0.18–0.18)Cb	15.49 (15.44–15.55)Cc
	T60	0.17(0.17–0.17)Cb	14.59 (14.09–15.08)Cc
SEM		0.0139	1.0276
p value _{treatment*control}		<.0001	<.0001
p value _{ageing}		<.0001	<.0001
p value _{soil}		<.0001	<.0001
p value _{ageing*soil}		<.0001	<.0001

Exemplos de estudos com aplicação da técnica

Geoderma 316 (2018) 11–18

IF: 7.42

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journal homepage: www.elsevier.com/locate/geoderma



Animal bonechar increases sorption and decreases leaching potential of aminocyclopyrachlor and mesotrione in a tropical soil

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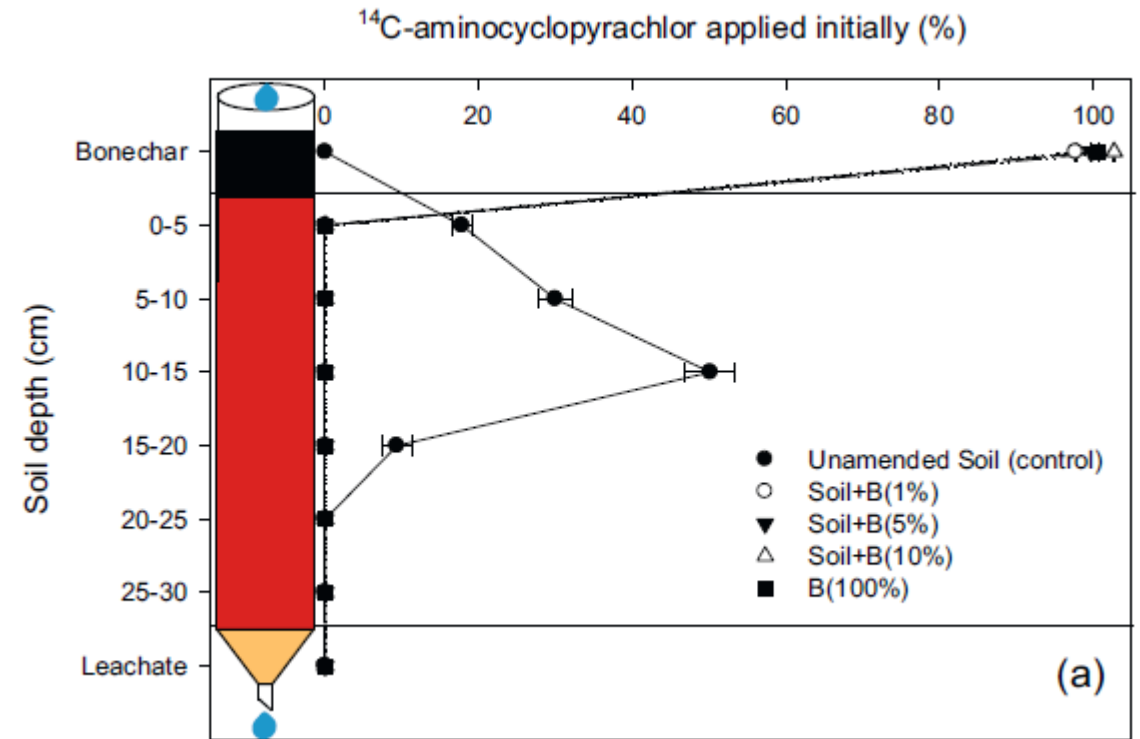


Fig. 6. Aminocyclopyrachlor (a) and mesotrione (b) distribution in soil columns and leachate after 200 mm rainfall simulation over 48 h. Herbicide distributions in unamended soil, bonechar (B)-amended soil (1, 5, 10% w w⁻¹), and pure bonechar (100%) are compared. Data shown are the average of the two particle sizes (0.3–0.6 and 0.15–0.3 mm). Error bars represent the standard deviation of the mean ($n = 4$). Symbols may overlap.

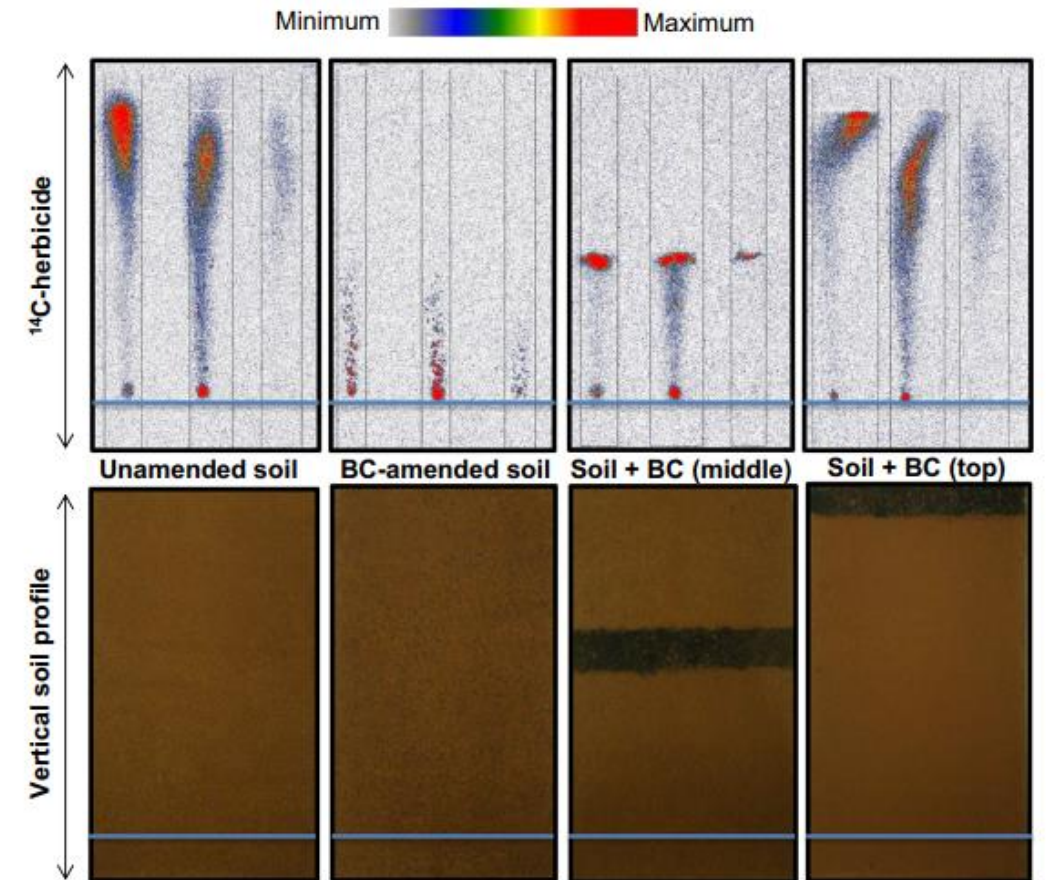
Exemplos de estudos publicados com aplicação da técnica



Cow bone char as a sorbent to increase sorption and decrease mobility of hexazinone, metribuzin, and quinclorac in soil

Kassio Ferreira Mendes*, Rodrigo Nogueira de Sousa, Vanessa Takeshita, Felipe Gimenes Alonso, Ana Paula Justiniano Régo, Valdemar Luiz Tornisielo

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Exemplos de estudos publicados com aplicação da técnica

Development of a Preemergent Nanoherbicide: From Efficiency Evaluation to the Assessment of Environmental Fate and Risks to Soil Microorganisms

Vanessa Takeshita,* Lucas Bragança Carvalho, Juliana Aparecida Galhardi, Gustavo Vinícios Munhoz-Garcia, Rodrigo Floriano Pimpinato, Halley Caixeta Oliveira, Valdemar Luiz Tornisielo, and Leonardo Fernandes Fraceto*

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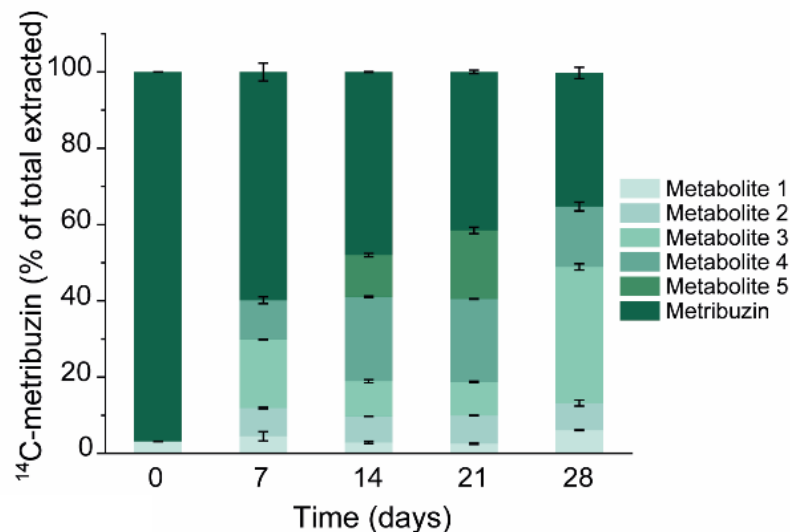


Table 1. Biodegradation Parameters of the Nanoformulation and Commercial Formulation of ¹⁴C-Metribuzin, 28 after Incubation, in Different Soils

parameters	soil	formulation	
		nanoMTZ	MTZ
$C_0 \pm se^a$ (%)	clay	89.14 ± 8.94	91.14 ± 6.56
	SL-1	84.10 ± 14.76	90.63 ± 12.89
	SL-2	84.20 ± 14.93	84.29 ± 15.75
$k \pm se$ (day ⁻¹)	clay	0.0417 ± 0.0095	0.0406 ± 0.0067
	SL-1	0.0395 ± 0.0161	0.0610 ± 0.0173
	SL-2	0.0483 ± 0.0182	0.0485 ± 0.0193
R^2	clay	0.88	0.93
	SL-1	0.67	0.83
	SL-2	0.71	0.68
p -value	clay	<0.1	<0.1
	SL-1	<0.1	<0.1
	SL-2	<0.1	<0.1
DT ₅₀ (days)	clay	16.62	17.07
	SL-1	14.35	14.29
	SL-2	17.55	11.36

^aStandard error of mean ($n = 2$).

Exemplos de estudos publicados com aplicação da técnica

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Research Paper

Foliar absorption and field herbicidal studies of atrazine-loaded polymeric nanoparticles

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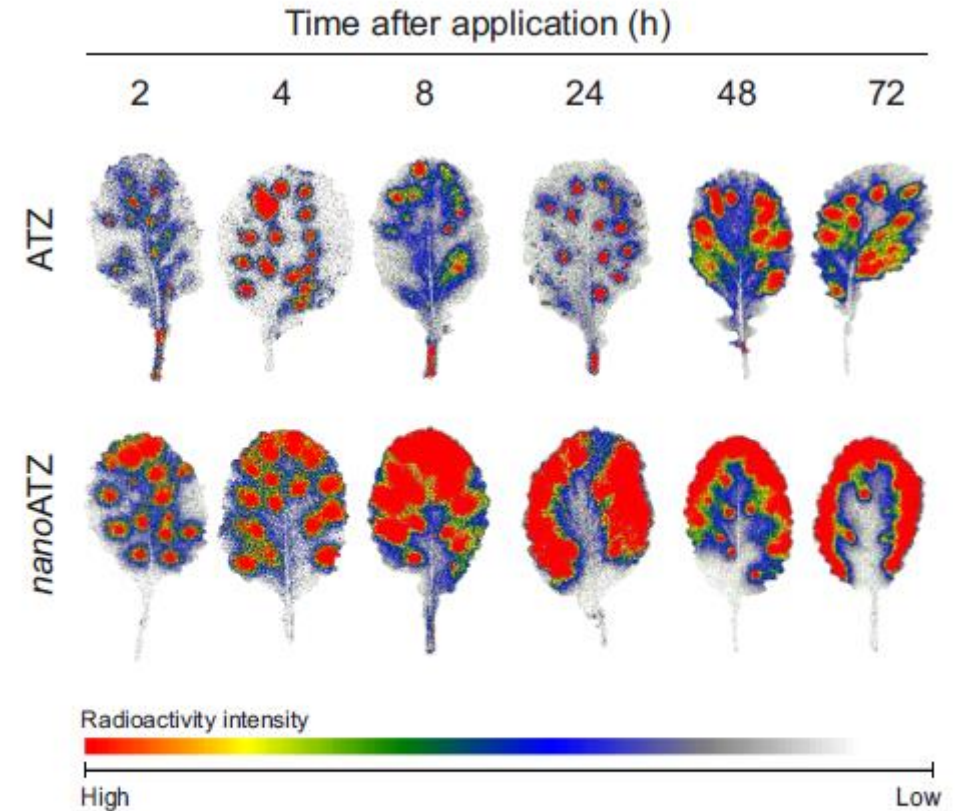


Fig. 3. | Autoradiography of ^{14}C -atrazine ($2,000 \text{ Bq plant}^{-1}$) absorbed by mustard leaves over 72 h after the application of nanoatrazine (*nanoATZ*, $30 \mu\text{g a.i.}$) or commercial atrazine (*ATZ*, $30 \mu\text{g a.i.}$).

Dúvidas?

Proposta de projeto:

- Aplicação das guidelines e/ou papers, bem como os conhecimentos da disciplina para propor um projeto de pesquisa
- **Proposta:** Vincular estudos ao projeto original de cada um (com testes diferentes dos propostos inicialmente), que contribuam com a elucidação dos problemas e complemento dos objetivos iniciais
- **Entrega e apresentação ao final da disciplina:** Introdução ao problema, Hipóteses, Objetivos, Justificativa, Material e métodos, Resultados esperados, Cronograma, Considerações finais

Processo avaliativo:

- Projeto final = 6 pontos (4 escrita + 2 apresentação)
- Atividades propostas ao longo das aulas = 4 pontos ($A1 + A2 + A_n \dots \div n$)
- Nota final = Projeto + Atividades = 10 pontos

Responder até dia 31 de agosto
Apresentar dia 14 de setembro

Estudo dirigido

1

Lixiviação

2

Biodegradação

3

Mobilidade (solo)

4

Sorção-dessorção

5

Biodegradação

6

Sorção-dessorção

7

Bioacumulação (solo)

Dúvidas?

Muito obrigado!

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