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# Comprehensive framework for overcoming scientific challenges related to assessing radioactive ultra-fine (nano/micro) particles transfer at the atmosphere-leaf interface

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## Essential title page information

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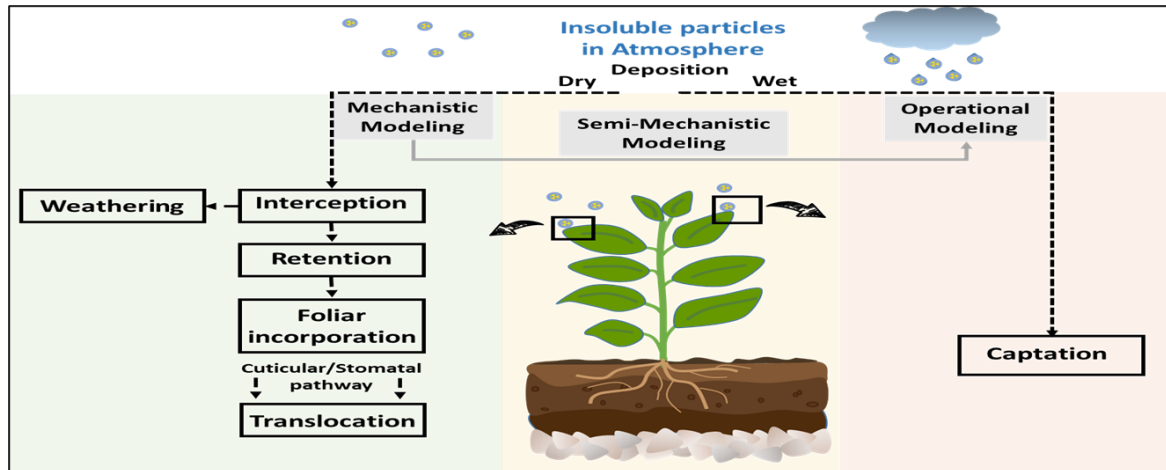
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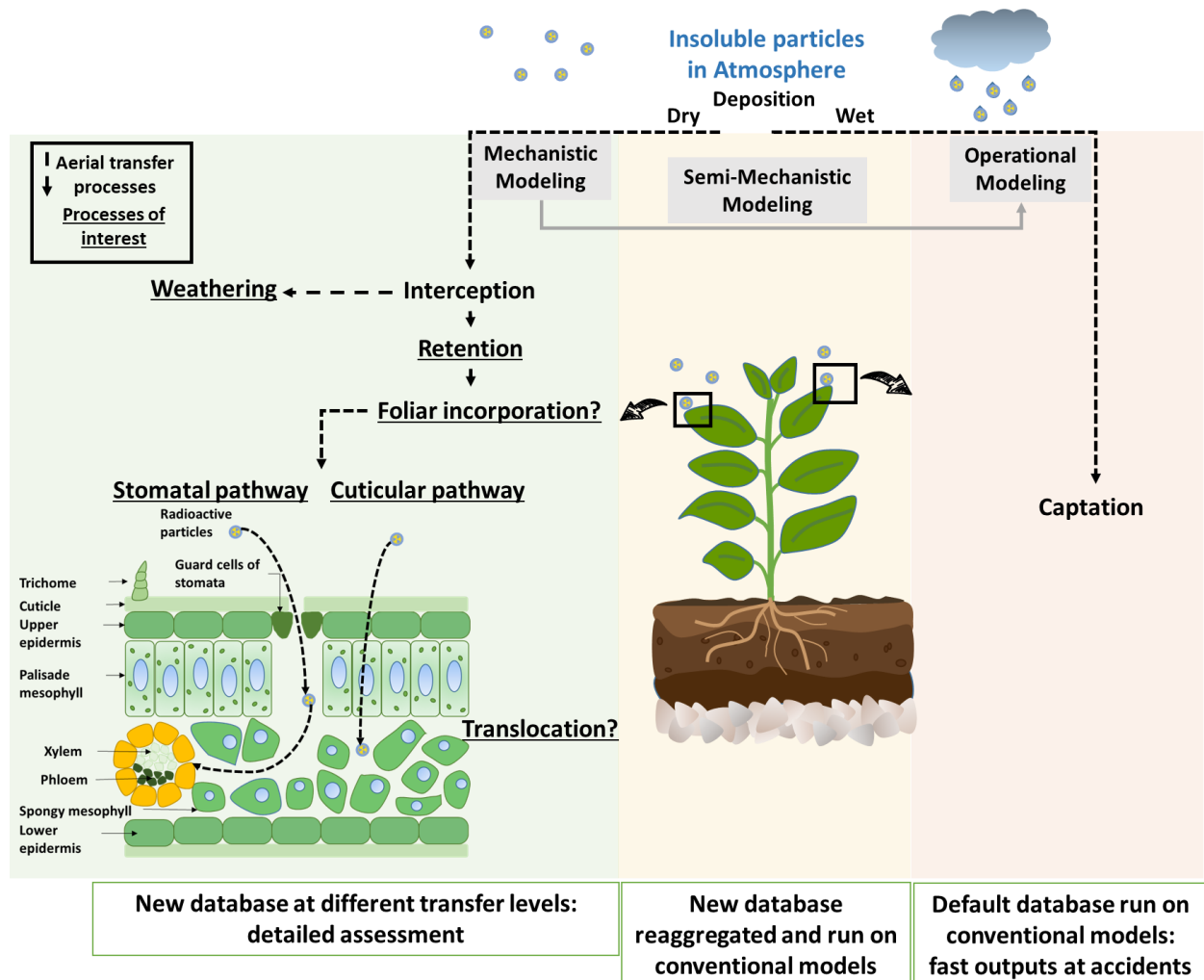
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# Graphical abstract

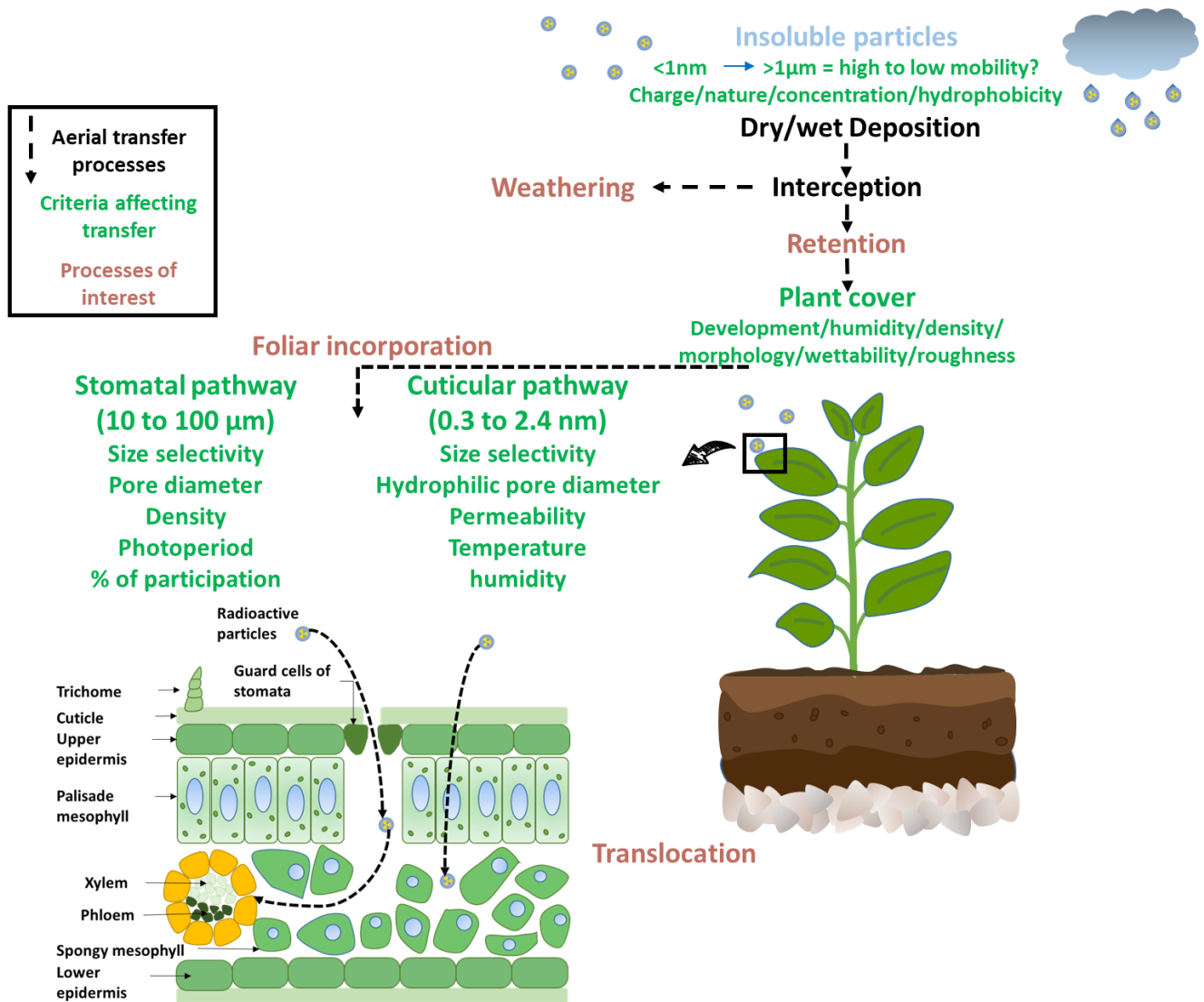


## Highlights

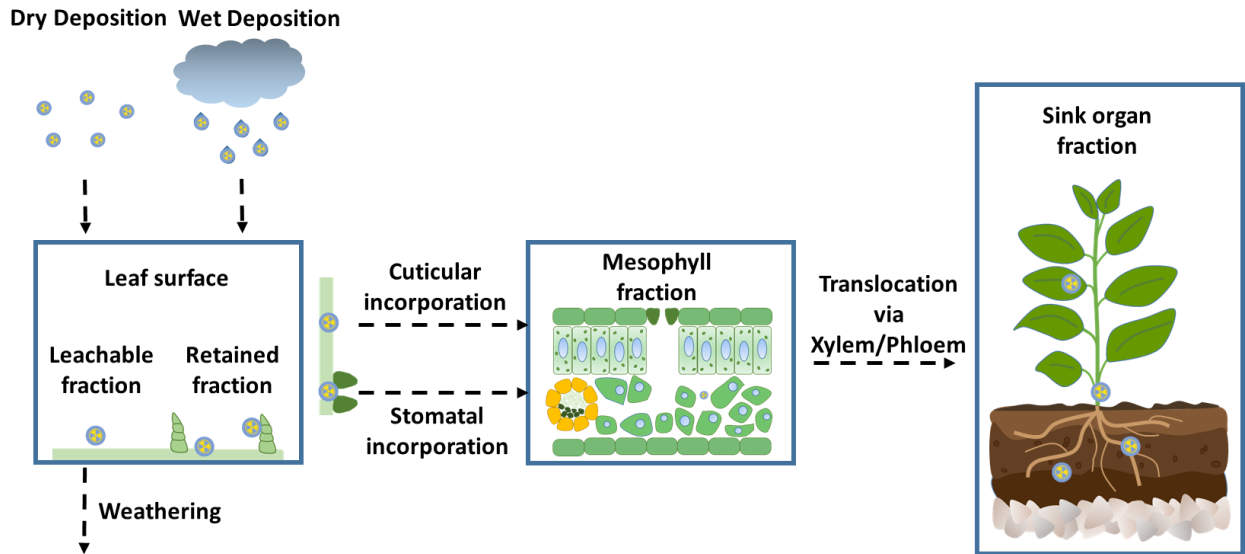
- Modeling tools estimating plants radioactive exposure via particles are prone to several configurational modifications.
- Foliar transfer Processes of particulate form of radionuclides are not well characterized.
- Internalization and translocation of radioactive particles is determined by plants and particles interactions.
- Fluxes of particles distributed on and within the plant needs to be quantified and added to the database of risk assessment modules.
- Mechanistic modelling discriminating different processes can be a promising approach for more accurate predictions for all sorts of radionuclides.



**Figure 1** Different modeling levels of insoluble radioactive particles transfer on plant. Mechanistic approach accounts for fluxes quantification at different processes: retention or loss, incorporation, and translocation, while operational modeling relies on default aggregated parameters to produce fast estimations of exposure. The semi-mechanistic approach ensures using the new particle-related data while aggregated to run on operational models to assess validity of default data.



**Figure 2** Transfer processes of insoluble radioactive particles deposited on plant foliage. Particles of specific properties can interact with plants macro and micro properties (green), to tune underlying mechanisms of interest (red): retention or loss, incorporation, and translocation.



*Figure 3 Disaggregated conceptual model of particles transfer processes in plants. The boxes denote different plant compartments that the particle might reach upon transfer depending on particles and plants properties interaction.*



## Abstract

Food products are prone into contamination after a nuclear emission of radionuclides. While the mechanisms of emission and deposition of ultrafine radioactive particles are well documented, the transfer of these species from the atmosphere into plants is poorly assessed. This is evident in the lack of quantification of particles distributed within plants, especially regarding particles physical-chemical criteria to plant of different properties. Such knowledge gaps raise the concern about the representativeness of risk assessment tools designed for the transfer evaluation of ionic/soluble species to be qualified for simulating insoluble species exposure and proposes a possible underestimation. This highlights the possible need for special particle codes development to be implemented in models for future emissions. In addition, the later tools utilize transfer factors aggregating relevant sub-processes, suggesting another weak point in their overall reliability. As researchers specialized in the nuclear safety and protection, we intend in this perspective, to develop a compressive analysis of the interaction of ultrafine particles with plants of different specificities at different level processes starting from particles retention and gradual translocation to sink organs. This analysis is leveraged in providing insights for possible improvements in the current modeling tools for better real-life scenarios representation.

## Keywords

radioecological models, hot particles, risk assessment, plants exclusion limits.

# Comprehensive framework for overcoming scientific challenges related to assessing radioactive ultra-fine (nano/micro) particles transfer at the atmosphere-leaf interface

## Abstract

Food products are prone into contamination after a nuclear emission of radionuclides. While the mechanisms of emission and deposition of ultrafine radioactive particles are well documented, the transfer of these species from the atmosphere into plants is poorly assessed. This is evident in the lack of quantification of particles distributed within plants, especially regarding particles physical-chemical criteria to plant of different properties. Such knowledge gaps raise the concern about the representativeness of risk assessment tools designed for the transfer evaluation of ionic/soluble species to be qualified for simulating insoluble species exposure and proposes a possible underestimation. This highlights the possible need for special particle codes development to be implemented in models for future emissions. In addition, the later tools utilize transfer factors aggregating relevant sub-processes, suggesting another weak point in their overall reliability. As researchers specialized in the nuclear safety and protection, we intend in this perspective, to develop a compressive analysis of the interaction of ultrafine particles with plants of different specificities at different level processes starting from particles retention and gradual translocation to sink organs. This analysis is leveraged in providing insights for possible improvements in the current modeling tools for better real-life scenarios representation.

## 1. Introduction

Nuclear accidents can lead to worldwide emissions (Mathieu et al., 2018). Radionuclides can be among the constituents forming the particle released, in association to materials of the nuclear installation (P. G. Martin et al., 2020), or be incorporated to aerosols present in the atmosphere like soil particles, feldspars, sea salts, sulfates aerosols and carbonaceous compounds (Higaki & Hirota, 2013; Kaneyasu et al., 2012; Masson et al., 2013; Miyamoto et al., 2014). These radionuclide-bearing-particle are defined by the International Atomic Energy Agency (IAEA) as “localized aggregation of radioactive atoms that give rise to an inhomogeneous distribution of radionuclides significantly different from that of the matrix background” (IAEA, 2011). The composition of radioactive particles, its activity concentration ( $\text{Bq kg}^{-1}$  or  $\text{Bq L}^{-1}$ ), and atom/isotopic ratios of the matrix depend on the source. The particle properties like particle size distribution, crystallographic structure or oxidation state will depend on release scenarios and the environmental conditions like temperature, pressure and presence of oxygen (Salbu & Lind, 2020). Release conditions can lead to the appearance of particles ranging from 100 nm (Acsente et al., 2015) reaching hundred micrometers (Salbu, 2000). Populations of tungsten dust particles having the size of 2  $\mu\text{m}$  and 20 nm have been recently characterized within the WEST tokamak (Arnas et al., 2023), which allows to get an insight into plausible release scenario that will be assessed for the future ITER facility. Within the whole process of risk assessment, there is a great uncertainty linked to the assessment of this source term as even for past and real releases, we have only partial characterization. In addition, the possibility of particles presence in the atmosphere to be secondary carrier of emitted radionuclides is not addressed. The particles in accumulation mode (0.1 - 1  $\mu\text{m}$ ) are capable of long-range transport and their deposition have been studied extensively due to the fact that they account for significant specific activity compared to other particle classes (Kaneyasu et al., 2012). This was evident after Fukushima Daiichi

Nuclear Power Plant (FDNPP) accident where up to  $0.71 \mu\text{m}^{137}\text{Cs}$  particles were able to reach Europe (Masson et al., 2013). The particulate source term may display distinct behavior from their ionic counterparts, causing different biological uptake in living organisms (Salbu et al., 2018). Difference in uptake among nanometric and micrometric size classes is also evident where the nanoparticles are more mobile as we explain in the next sections, thus presenting an important vector for food chain radiocontamination. Such contamination calls for reliable estimation of environmental contamination using risk assessment tools. These tools run on aggregated transfer factors which assess the ratio of activity between two related compartments, like atmosphere and foliage for example, to describe the transfer processes. However, questions of assessment tools uncertainties arise because of input variabilities, knowledge gaps and too aggregated structures of conceptual models compared to reality. Models are prone to continuous developments needed for accurate estimations of radioactivity exposure simulating real case scenarios. At present, these tools do not consider the particulate speciation of radionuclides in plant transfer although generation of ultrafine radioactive particles is foreseen in assessment scenarios associated to future nuclear projects (Bernard et al., 2019; Sanles Sobrido et al., 2021). Previous work have been dedicated to soluble radioactive particles, in which the radionuclides transferred to the plant as ionic form are due to solubilization prior to incorporation (Henner et al., 2013; Madoz-Escande & Santucci, 2005). Previous studies showed that non-soluble radioactive particles were retained on plant leaves after the Fukushima nuclear accident (Itoh et al., 2014), but no further fluxes of quantification were done. In fact, the nearest quantification of particles fluxes transfer into plants dates back to the early 1980's (Cataldo et al., 1981). Therefore, since the behavior of radioactive particles is highly dependent on its particulate nature and is expected to be similar whether they bear radionuclides or not, we will focus on the literature assessing the fate of non-soluble, non-radioactive particles (Joshi, 1982), since the literature regarding these objects is less scarce. We relied on these studies to describe the physical-chemical parameters that will affect foliar uptake and transfer so that we can improve the reliability of our models. In addition, aggregated TFs do not distinguish between various contamination pathways, or mechanisms like leaching and translocation. Thus in this perspective article, we (1) highlighting the incomplete safety of risk assessment tools in particles transfer prognosis, (2) discuss the underlying transfer processes of hot ultrafine (micro/nano) particles into plants, (3) and develop a comprehensive analysis on the variable properties of plants and particles affecting the behavior of particles in plants, (4) and eventually, recommendations are provided to improve model representativeness for future risk assessment.

## 2. An incomplete safety of risk assessment tools in estimating particles fluxes transferred to plants: SYMBIOSE case

Risk assessment models are developed to estimate the exposure to radionuclides. Modelling levels can vary from operational to mechanistic. Operational models are used in crisis situations where rapid outputs are needed, and they run on simple aggregated parameters.

Various models/dedicated softwares whose output is radiological dose assessment exist, including in their structure parts dedicated to the calculation of transfer to plants by foliar and/or root uptake pathway, treated in close to similar manner: ECOSYS (Müller and Pröhl, 1993), FARMLAND (ref), JRODOS (ref), SYMBIOSE (Gonze et al., 2011; Simon-Cornu et al., 2015). In addition, values of radioecological transfer parameters such as the one from International Atomic Energy Agency (IAEA) had been widely incorporated into the databases used to run these models, with variation in the manner to fill the gaps.

Some of these models, including SYMBIOSE, the platform developed by the French institute of radioprotection and nuclear safety (IRSN), aggregates transfer processes under the term “captation” (figure 1). Captation includes well identified processes in radioecology like interception, retention or weathering, foliar incorporation, and possible translocation. While the interception fraction accounts for physical adherence of deposited particles on the leaf surface, the retention would represent the fraction that is not immediately blown or washed off from the foliage. The extent of aerial contamination of vegetation was shown to be greatly dependent on the process of foliage interception and retention (Anspaugh et al., 2002). Percentage of interception and retention is dependent on many factors like plant species and morphology (H. Wang & Shi, 2021), particles size (Joshi, 1982) and rainfall duration (Xu et al., 2017). Particles have the possibility to translocate from plants exterior surface into the mesophyll (Figure 1). Many pathways have been discussed in the literature, with the most dominant pathways being the stomatal and cuticular pathways (Lv et al., 2019). Though cuticle is the first natural barrier against biotic and abiotic stresses, several studies have documented the uptake of particles of few nanometers via this waxy layer (Avellan et al., 2019; Schreck et al., 2012). The stomata forming another pathway for particles entry, serve as an aperture for gas and water exchange between plant interior and atmosphere. These pores theoretically have a relatively higher size exclusion limit for particles. Once the particle is in the plant’s interior, it has the possibility to translocate to other organs like fruits or roots via the conducting vessels (xylem and phloem). Sections 3.2 and 3.3 provide a detailed overview of the critical elements influencing particle transport at each level.

Though the approach of aggregation simplifies the complexity of transfer pathways in natural ecosystems and provides an easy way for assessing impact, some drawbacks are still present and may require improvements. Although particulate radionuclides have been identified at many contamination sites (Byrnes et al., 2020; Igarashi et al., 2019), data related to the transfer of particles of different properties to plants and the rest of the food chain is still lacking. This alarming fact is evident in the real case assessment reported by Maro et al. (2014) measuring the fluxes of tritium deposited from the Canadian CANDU (CANada Deutrium Uranium) reactors. The study included the gas forms, tritium in soil and in grass as tissue-free water tritium (TFWT) and organically-bound tritium (OBT), besides the particulate tritium collected from the air by filters of aerosols sampling system. The measurements indicated a mean activity value of 0.09 Bq/m<sup>3</sup> varying from 0.007 Bq/m<sup>3</sup> to 0.187 Bq/m<sup>3</sup>. The calculation of an average particle content in the atmosphere of 10 µg/m<sup>3</sup> (mass concentration), of activity 0.1 Bq/m<sup>3</sup>, yields an activity of 10 x 10<sup>6</sup> Bq/Kg, in comparison to 1 – 10 Bq/Kg in soil or grass. Thus, a small contribution of radioactive particles can result in a significant contribution to dose concentration. Authors warned that neglecting radioactive particles would lead to discrepancies between field measures and modelling outputs. So far, transfer factors data have been collected mainly for non-particulate species (Henner et al., 2013; Larionova et al., 2018; Vandecasteele et al., 2001). If the plant transfer of such source term is significant, then its exclusion upon nuclear emissions can contribute to underestimations in real exposure (IAEA, 2011). On the other side, using the same transfer factors to evaluate the dose transferred to plants via particulate and non-particulate radionuclides might lead to biased risk estimation. Thus, the first need is to test the existing data and depending on the test outputs, two possible improvements are envisioned. First represented by adopting a semi-mechanistic approach in modelling, through proposing new specific transfer factors values for particles, or a new model structure with more parameters to discriminate the underlying processes (figure 1). The later will result in disaggregating the “captation” mentioned earlier by detailed process like particles retention, incorporation into the foliage and distribution within plant as

presented in the mechanistic model (figure 1). In the following sections, we will explain how each parameter of the plant and particle will tune the transfer of particle at each level.

### 3. Process of transfer of radioactive particles

Having any sort of nuclear release near an agricultural crop would lead to possible human exposure to radioactivity through food-chain transport (Anspaugh et al., 2002). Anthropogenic releases of radioactive particles have been studied from the moment of release, passing through the different process that would end up by settling of the radioactive fallouts on various surfaces including aerial parts of vegetation. Among the differentiated processes, we can mention deposition, interception that have been extensively studied before (Connan et al., 2018; Laguionie et al., 2014; Madoz-Escande & Santucci, 2005; Pellerin et al., 2017; Roupsard et al., 2013). Therefore, it is in our interest in this perspective to focus on the interaction of ultra-fine particles, that are in the micro and nano size range, with the atmosphere-leaf interface and the processes after. These processes include particles retention on the foliage surface or their loss, incorporation into the internal foliage and translocation to other parts. Figure 2 summarizes the specific properties of plants and particles that would affect the particles retention and incorporation, thus the fluxes distribution. The transfer would be tuned by the plant's macro (humidity at leaf surface, orientation of leaves) and micro (trichomes, stomatal sizes and density, cuticular pores) features interacting with radioactive particles of specific properties (size, hydrophobicity) that will either enhance or hinder further movement. Further related details are discussed in the following sections. But because of the scarcity of studies dealing with the interaction of radioactive particles with plants, most of studies referred to in the following sections rely on literature using conventional nanomaterial to express the effect of particulate species of radionuclides in the processes of transfer. This approach has been common in the domain of radioecology and risk assessment. Stable particles are commonly used to generate transfer parameters like interception fraction for radionuclide dynamics and are used in international compilations for human food chain assessment models (IAEA, 2010). Previous studies have validated the use of stable elements as a proxy too radioactive isotope. The transfer factor of stable cesium showed a strong correlation with that of the radioactive cesium proving to be a useful index for  $^{137}\text{Cs}$  (Ogasawara et al., 2019). Recommendations for model improvements suggested studying the dynamics of stable chlorine who have similar behavior to that of chloride-36 (Le Dizès & Gonze, 2019).

#### 3.1.Retention

##### 3.1.1. Plant properties affecting particles retention

Prior to retention, particles physical encounter with foliage is termed interception. The increase in the vegetative cover increases the interception of radionuclides by the plant, and this is the case when reaching advanced growth stages assigned by increased leaf area index (LAI), and limited with the leaves super imposition (Madoz-Escande et al., 2004; Madoz-Escande & Santucci, 2005). Interception fraction calculated for plants in homogenous growth or at their vegetative growing period like cereals where the biomass and the leaf area are in good correlation, differs from the stage of generative developments. At the later stage, the biomass continues to increase while the leaf area remains the same (Vandecasteele et al., 2001). Once intercepted by the plant cover, a fraction of radioactive nano/micro particles can be retained or recycled from plant by process like weathering. Retention can be defined as the ratio of total particles-bound activity retained by the plant at harvest to the initial quantity deposited on it. Retention is determined as a function of interaction of both plant and radionuclide properties (figure 2). Particles retention can vary among different plant species (H. Wang & Shi, 2021). Higher retention levels are expected with horizontal leaf orientation due to higher moistening capacity in comparison to erect foliage

(Madoz-Escande et al., 2004). Leaf surface structures play a pivotal role in the variation of retention among different plant type, this includes epidermal hairs, stomata, and cuticular waxes (Chen et al., 2022). For example, the presence of small hairs on the surface of squash and soybean lead to a more homogenous distribution of  $^{86}\text{Rb}$  labeled quartz particles, in comparison to the V-shaped leaves of sorghum and peanuts which rolled the particles toward the midvein (Witherspoon & Taylor, 1970). Cuticle is a multifunctional layer that is composed of cutin layer made out of fatty acids, and integrated waxes that gives it its characteristic hydrophobic property and that are composed of esters of long chain acids and long chain alcohols (Barthlott et al., 1998). The presence of waxy layer showed a positive correlation with the entrapment of particles from the atmosphere (Sæbø et al., 2012). Dappe et al. (2019) tested the high level of epicuticular wax crystals in cabbage plants (*Brassica oleracea*) in which they subjected it to inorganic particles like  $\text{PbSO}_4$ ,  $\text{CuO}$ , and  $\text{CdO}$  and noticed their immobilization and trapping by encapsulation in the wax layers up to 15 days post application. Also cuticular thickness can vary from few nm up to 500nm according to plant species thus affecting the efficiency of particle entrapment (Stevens & Baker, 1987). The development stage can affect cuticular absorption as the older leaves tend to have more resistance than younger leaves because of the optimum level of maturity and fully developed epidermal barriers (Hull et al., 2000; Segado et al., 2016). Another factor is surface wettability that's determined by the collective measure of polar and dispersive surface forces or the foliar surface free energy (SFE). The variation of the microstructure of leaves in terms of roughness at the level of cuticle or epicuticular waxes and hairs will strongly influence foliar wettability and particulate retention and thus the subsequent possibility of particle contamination by wet deposition (Koch et al., 2009). For instance, plants with lower SFE have reported higher retention levels for particles applied through wet deposition (Kranjc et al., 2018). Hydrophilic surface of plants like lettuce can allow particles to be easily washed out in comparison to hydrophobic surfaces of plants like kale and collard (Keller et al., 2018). Trichomes proved to serve in physical immobilization of particles (Avellan et al., 2019; Sæbø et al., 2012; T Vu et al., 2013) whether glandular (Spielman-Sun et al., 2020) and non-glandular (Li et al., 2018). Yet in the later study no difference was recognized with  $\text{ZnO}$  nanoparticles absorption upon application on soybean leaves having 10 fold more trichomes density (Li et al., 2018). The role of trichomes needs further investigations to clarify whether its presence can affect the process of retention or incorporation of particles as function of their properties, especially that it can be cuticle free in some species (Schreel et al., 2020).

### 3.1.2. Particle's properties affecting particles retention

The fact that the deposited material is in the form of particles, makes its retention vary from the ionic form. Opposite retention behavior had been documented for copper nanoparticles vs ionic forms by Kah et al (2019) and Shen et al (2022). Further work is required to identify the conditions and mechanisms favoring the retention of a certain species of radionuclides. At physiological pH, the negative charge of plant cuticle favors retention of cations in comparison to anions (Hoffman et al., 1995; Schönherr & Bukovac, 1973). The same concept of retention applies for particles of positive and negative charge (Spielman-Sun et al., 2017). Functionalized surface charge of amino-modified polystyrene nanoplastics (PS-NH<sub>2</sub>) lead to increased retention to maize foliage (*Zea mays L.*) compared to carboxyl-modified polystyrene nanoplastics (PS-COOH) (Sun et al., 2021). Such kind of functional groups can enhance retention with the aid interactions like hydrogen bonds, electrostatic attractions, and covalent bonds (Yu et al., 2017). Another property that could modify the particles retention is hydrophobic-hydrophilic interactions. Unlike the hydrophilic citrate coated gold nanoparticles, the hydrophobic PVP coat caused the nanoparticles to have more interaction with the waxy cuticle after rinsing regardless of its negative

zeta potential value (Avellan et al., 2019). Same study has shown that particles of smaller sizes like 3 nm are capable of being retained more than the larger ones of 50 nm that were almost entirely washed off. Previous study revealed that most radioactivity in plant leaves was derived from  $^{89}\text{Sr}$  and  $^{131}\text{I}$  particles of  $<5\ \mu\text{m}$  diameter while undetectable contribution was from larger particles of  $>44\ \mu\text{m}$  (W. E. Martin, 1965). This observation have been redundant in other studies where the smaller the size of the particles the higher retention would be compared to larger ones (Creek, 2017; Kah et al., 2019). Shape of the particle can be an important factor, where articular shapes favored retention of micron-size particles compared to nanos (Kah et al., 2019). The submicronic particles, whether radioactive or non-radioactive of equivalent composition, will result in similar behavior based on their surface properties. This will aid in better anticipation of the possible fate of radioactive particles in environment based on non-radioactive proxies.

### 3.1.3. Environmental conditions affecting particles retention

External environmental conditions can tune particles retention. Capacity of retention can vary with seasonality (Lu et al., 2019), wind speed (Xie et al., 2022) and different rain scenarios (Xu et al., 2017). Timing elapsed between the contamination and the first weathering event would interfere with particles retention level. After 2 days of exposure to  $^{86}\text{Rb}$ -quartz particles, the loss by wind from the plants leaves reached 63% but decreased after 3 weeks to reach 3.3% (Witherspoon & Taylor, 1970). This was also evident in the case of wet deposition, where the rain after 2 days caused more wash off of radionuclides than the one occurring after 6 days (Madoz-Escande et al., 2004). Likewise, other properties like rain frequency, intensity and duration contribute to determining quantity of washed off ultra-fine particles (Madoz-Escande et al., 2005; Xu et al., 2017). The acidity of rainwater was proved to alleviate the attractive forces that adhere  $^{234}\text{Pu}$  and  $^{241}\text{Am}$  particles to the leaf surface, thus inducing more leaching (Cataldo et al., 1981).

Research conducted namely by the French Institute for Radiological Protection and Nuclear Safety have highlighted the importance of evaluating radioactive particles retention for reliable assessment of plant contamination level. Thus, the acquisition of data for particulate retention is indispensable for evaluating risk posed by cultures that are consumed for their foliage such as leafy vegetables. Quantification should be done under different conditions that will simulate real scenarios, thus with varying properties of particles besides those of the plants.

## 3.2. Incorporation routes and *in-planta* behavior

Following the retention of ultrafine particles to the leaf's surface, it's possible for them to be incorporated into the plant's foliage. Foliar uptake of particles has been documented in agrochemistry and phytotoxicity literature. Particles incorporation can result from deliberate application like pesticides and fertilizers, or nonintentional, as byproducts contaminating ecosystems (Kah et al., 2019; Larue et al., 2014; Nair et al., 2010; Shen et al., 2022; W. N. Wang et al., 2013). Uptake can occur through openings like hydrophilic stomata, hydathodes, trichomes and possible leaf injuries (Etxeberria et al., 2016; Hong et al., 2014; Spielman-Sun et al., 2020), while hydrophobic pathway comprise the cuticular pores (Larue et al., 2014). Stomata can be mainly present on the leaves especially the abaxial side (Burkhardt et al., 2012), but the cuticular pathway can be of great importance as it covers the whole plant. After incorporation, particles can translocate to other organs or reach a dead end. Distribution of fluxes can be tuned as a function of properties of the particles and the plant (figure 2). Radioecology literature mainly studied the incorporation and translocation of dissolved products of particles (Henner et al., 2005, 2013; Hurtevent

et al., 2013). Limited information is present on the uptake of radioactive particles in plants, and the latest quantification of fluxes dates back to the 1980s by Cataldo et al. (Cataldo et al., 1981). The two main entry pathways for radioactive ultrafine particles are discussed in the following sub-sections while presenting the possible interactions in case of different properties.

### 3.2.1. Cuticular pathway

Though cuticle is of hydrophobic nature, cuticular pathway represents a combination of hydrophilic and lipophilic pores (Popp et al., 2005). The lipophilic diffusion has no size selectivity while the hydrophilic pathway have been proved to have size selectivity ranging between 0.3 nm and 2.4 nm (Buchholz et al., 1998; Eichert & Goldbach, 2008; Popp et al., 2005). However, some studies show that pore size might not prevent some particles from overcoming the cuticular barrier into plant interior. One study showed that 12nm NPs accumulated in the mesophyll of stomata devoid areas, which were believed to enter through the cuticle layer by disruption or diffusion (Avellan et al., 2019). Some particle might have photocatalytic effect that can degrade cuticular surface and get trapped in the cuticular wax (Larue et al., 2014). These studies have highlighted the importance of the hydrophilicity/hydrophobicity of particles in possible uptake through the lipophilic cuticle (Avellan et al., 2019; Popp et al., 2005). This makes the penetration dependent on particle's properties interacting with cuticle. Better understanding is required regarding the interaction of particles physical-chemical characteristics with cuticles of different species enabling their entry.

### 3.2.2. Stomatal pathway

Stomatal pores have larger pores than cuticular ones, reaching up to 100  $\mu\text{m}$  proximately (Edwards et al., 1998). Stomatal pathway was proved as an important route for particles entrance. It has been justified by the overexpression of some genes like *BoIC.CHLI1* which is responsible for the stomatal opening and  $\text{CO}_2$  assimilation, after increased accumulation of CuO nanoparticles (Dappe et al., 2019). Abscisic acid is known to induce stomatal closure, so ABA-sensitive *Arabidopsis thaliana* plants had lower levels of Ag NPs after ABA-induced stomatal closure. The ABA-insensitive plants showed no difference of particles content upon ABA application (He et al., 2022). Stomata have higher size exclusion limit with Au nanoparticles ranging from 5-50nm were believed to cross stomatal pores (Luo & Cao, 2018). Another study validated the stomatal entry of 38.6nm and 38.2 nm  $\times$  57.8 nm Ag nanoparticles (Larue et al., 2014). Up to 200 nm  $\text{TiO}_2$  MPs were also able to penetrate inside the leaf tissue providing a higher size exclusion limits of lettuce plants (Larue et al., 2014). However, uptake is not driven by mass flow of particles smaller than the stomatal pore, as the 1.1  $\mu\text{m}$  particles size didn't show a possibility to be incorporated in the *Vicia faba* (L.) stomata although its pore length 25 $\mu\text{m}$  and widths between approximately 3 $\mu\text{m}$  and 10  $\mu\text{m}$  (Eichert et al., 2008). This could be explained by stomata's conical geometry and the magnitude of drop contact angle with wall angel formed by the cuticular ledges (Schönherr & Bukovac, 1972). Also, particles with hydrophilic coats can aid in traversing stomatal pores (Avellan et al., 2019). Stomatal penetration can vary as function of particle's concentration (Hong et al., 2014) delivered as aerosols or by droplets (W. N. Wang et al., 2013). In addition, deposits that reduce surface tension to  $\sim 22$  mN/m, by improving wettability and contact with plant, induce rapid uptake of larger particles (Hu et al., 2020). Incorporation can be enhanced by light induced stomatal opening, increased humidity and density of stomata participating in the uptake (Driesen et al., 2020; Eichert & Burkhardt, 2001). Humidity fluctuations by wetting cycles can decrease the hydrophobic character of leaf surface and promote penetration through water films connecting leaf's surface to the apoplast (Burkhardt et al., 2012).



### 3.3. Translocation in plants

#### 3.3.1. Translocation in the mesophyll: Apoplastic and Symplastic pathways

Once internalized, particles can move through the tightly knitted cellulose cell wall matrix of the mesophyll cells named the apoplast, or through plasmodesmata-connected cytoplasm corresponding to the symplastic pathway. The pore size in cell wall allowed the transport of particles of diameter ranging from 5.4 nm (Etxeberria et al., 2016) reaching up to 30 nm (Zhu et al., 2020). Transport can be charge-specific favoring the negatively charged particles to cross cell wall channels (Avellan et al., 2017). This apoplast-mediated movement would lead to higher translocation of radioactive particles having negative charge to plant parts like shoots and roots. Authors have highlighted the effect of agglomeration of particles on limiting the transport to the apoplastic space rather than the smaller plasmodesmata pores. Transport into the symplast requires crossing the cell membrane (Corredor et al., 2009). This can happen mainly through endocytosis via endosomes (Kurepa et al., 2010; Valletta et al., 2014) with a possible mechanism of recognition, separation and redistribution of particle (Etxeberria et al., 2006). Various other mechanisms like ion channel transport, passive lipid membrane diffusion and aqua porins crossing have been reported and that would depend on particles size, charge and membranes fluidity and bounded molecular species (Ahmed et al., 2021; Karami Mehrian & De Lima, 2016). Once in cytoplasm, particle can move from one cell to another through plasmodesmata of diameter ranging 3-50 nm (He et al., 2022; P. Wang et al., 2016).

#### 3.3.2. Translocation to sink organs

Particle can reach out the phloem and transport to shoots and roots following a “source-sink pressure gradient” (W. N. Wang et al., 2013). Translocation can be a size selective mechanism where particle of <10 nm were found throughout the contaminated plant while >80 nm particles didn't reach the vascular system (Belhaj Abdallah et al., 2020). Roots can have sieving properties preventing exudation of particles larger than 50 nm (Avellan et al., 2019). In addition, Xylem-phloem-xylem transport has been previously documented (Z. Wang et al., 2012), making redistribution of particles throughout plant possible via xylem-phloem loading, though xylem have restrictive nanometric pores unlike micrometric phloem pores (Schwab et al., 2016). However, another work reported no translocation of 37 nm nanoparticles in maize (Birbaum et al., 2010). A better understanding of the factors that influence the pathway of radioactive particles starting from plants incorporation towards crossing or not each biological barriers reaching sink organs is required for fully estimating the fluxes distribution among plant parts.

### 4. An improved conceptual model of particles fluxes transferred in plants by foliar pathway

This disaggregation approach had formerly brought plus-value within studies dealing with soluble radionuclides, as it shows the impact of some parameters on different processes (Madoz-Escande et al., 2004). The conceptual model (Figure 3) shows the foliage of the plant separated into external and internal part. The deposited fraction on the leaf surface can either be lost by weathering or retained to be later incorporated by stomatal or cuticular pathways, and eventually translocated to sink organs. On the level of retention, it is necessary to acquire values for particles retention on different leaf morphology, as it can highly impact the quantity of leachable and retained fractions. For incorporation, it is essential to discriminate the incorporation via cuticular and/or stomatal pathway, which is mainly tuned by particles properties like size and charge. Plants cuticle that thickens with advanced developmental stage can have a barrier effect on particles incorporated. With the possibility of mesophyll fraction to translocate via

vasculature, distribution of nanometric particles within plant parts becomes critical and requires quantification of fluxes to different sink organs. This is of specific importance when the organ consumed in a plant is its roots or fruits rather than the leaves (Henner et al., 2013; Hurtevent et al., 2013). Contamination occurring at the stage of organ formation is to be highlighted as resources as well as radionuclides will allocate toward the newly grown part like new organs after flowering (Choi et al., 2002; Madoz-Escande et al., 2004). Therefore, experiments should be designed to acquire new data on the fluxes of particles transferred at each of the processes detailed. This will allow on one hand to analyze the contribution of each process under certain conditions to the overall fluxes' distribution. Data will also be used to generate the specific transfer factor values needed to use the actual tools in "adapted mode" whose output will be confronted to outputs of the default mode of the tool. Thus, adopting this improved approach of detailed mechanistic modelling of transferred particles will allow to detect any underestimation and to assess the representativeness of operational models in case of radioactive particle exposure prognosis.

## 5. Conclusion and recommendations

Particle emissions are anticipated in future occurrences as they were seen in prior nuclear releases. Models not differentiating between the transfer of radionuclides in the form of ions or particle can lead to biased risk estimations. The processes post interception of particles on plant are poorly understood and there is scarcity of quantification of particles fluxes throughout the plant based on properties of both. This knowledge gap highlights the need for new data sets related to the detailed transfer of particles in plants, to have more realistic approximations of the exposure to hazardous hot particles. This recommends the development of adapted mechanistic framework, while leveraging previous studies on the interaction of different submicronic particles with different plants species. First level of work includes the acquisition of new particles-specific transfer data at different processes like retention, incorporation, and translocation. Values at each process can then be aggregated to be run on current models and outputs can be compared to assess the adequacy of models' prognosis. This approach thus will expose possible underestimation, and thus verify the need to design a dedicated particulate model for future emissions.

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