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.

RESUMO - No Brasil, a Lei de Pesticidas (Lei no. 7,802/89) introduziu novos critérios ambientais, de saúde pública e de desempenho agronômico na análise das atividades que envolvem pesticidas. Assim, radioisótopos são utilizados nos estudos de comportamento ambiental e in planta porque apresentam algumas vantagens em relação às medidas químicas, incluindo maior sensibilidade; descrição dos passos de um elemento em particular em um sistema metabólico; e localização e detecção de pesticidas através de filmes de raio-X e/ou radioimagem (em plantas) e cintilação líquida (em plantas e solo), respectivamente. Esta revisão descreve as metodologias relativas à utilização de radioisótopos em estudos de absorção, translocação e metabolismo de herbicidas em plantas, bem como em estudos de biodegradação, mineralização, lixiviação e sorção-dessorção de herbicidas no solo. O passo a passo das metodologias descritas é baseado nas diretrizes estabelecidas, na sua maioria, pela Organização para Economic Co-operation and Development (OECD) e pela Environmental Protection Agency (EPA). Nesta revisão, informações metodológicas dos estudos de solo e plantas, utilizando radioisótopos, são disponibilizadas aos pesquisadores brasileiros. Portanto, tal revisão se propõe a incentivar a condução de mais estudos que empregam as metodologias aqui descritas.

Palavras-chave: espectrometria de cintilação líquida, comportamento ambiental, molécula radiomarcada com 14C.
INTRODUCTION

In Brazil, Act no. 7,802/89, known as the Pesticide Act, was shared by the Ministries of Agriculture, Health, and the Environment, aiming at recording new herbicides. The role of the Ministry of Agriculture (MAPA) was to evaluate the agronomical performance of the product; the Health Ministry (ANVISA), the toxicological evaluation; and the role of the Ministry of the Environment (IBAMA) is the environmental evaluation (Brasil, 1989). Therefore, before receiving the approval regulated by the Brazilian authorities, all pesticides must go through rigorous tests related to their toxicology, residual effect and determination of the physical-chemical and biological properties.

The use of isotopes as tracers may offer additional information, since they allow and may be differentiated with great accuracy from the ions of the compound, which are already in the environment, even when both show a similar chemical behavior. According to Zagatto (2015), under certain conditions, it is possible to measure amount of up to $10^{-15}$ g of several elements, such as carbon. Isotopes may be used as tracers, and their advantages are: greater sensitivity in relation to the chemical measures; they allow a stepwise description of a particular element through a metabolic system; and they may be detected and located by X-ray films and liquid scintillation.

The laboratories that work with radiolabeled molecules must be registered by the National Commission of Nuclear Energy (CNEN). CNEN is a federal authority connected to the Ministry of Sciences, Technology and Innovation (MCTI), created in 1956 and structured by Act no. 4,118, from August 27, 1962, to develop the national policy on nuclear energy. As a high authority for planning, orientation, supervision and monitoring, CNEN establishes rules and regulations on radioprotection and it is responsible for regulating, licensing and monitoring the production and use of nuclear energy in Brazil (CNEN, 2015).

Currently, CNEN has 229 installations that are authorized to manipulate radiolabeled molecules for research purposes applied to several areas of knowledge, such as electric power generation, nuclear medicine, industry-related, environmental and agricultural applications, among others. For researches on the area of agrarian sciences, several laboratories in Brazil are authorized by CNEN (private multinational companies from the phytosanitary sector and several Universities and research Institutes) to develop researches using radiolabeled molecules on the areas of soil and genetic fertility and plant improvement, nematology, phytopathology, animal nutrition, environmental biogeochemistry, ecotoxicology, among others. Within that context, radiolabeled herbicides have been used for over half a century to study their behavior in the environment (Nandula and Vencill, 2015).

Considering the above, the objective of this review was to conduct a description of the research procedures and the methodology related to the use of radioisotopes to study the absorption, translocation and metabolism of herbicides on plants and the biodegradation, mineralization, leaching and sorption-desorption of herbicides on the soil.

USE OF RADIOLABELED MOLECULES WITHIN THE CONTEXT OF PLANT STUDIES

Among the biochemical and physiological mechanisms, the change in the absorption, translocation or metabolism of resistant weed biotypes has been reported on several species for different herbicides. These resistance mechanisms have been studied over the last years, allowing the development and improvement of analytical techniques to diagnose this type of resistance (Heap, 2015).

Radioisotopes are used on several research areas, such as for the metabolism of drugs and pesticides, environmental studies to determine biological routes and mass balance studies for organic compounds, and the ones that are most frequently used are tritium and $^{14}$C. More specifically, the use of labeled herbicides with $^{14}$C to study the absorption, translocation and metabolic degradation in planta has been the most used method to conduct these studies (Beffa et al., 2012). The method for using radiolabeled herbicides may be quantitative or qualitative, allowing associating the resistance to the reduced absorption and/or translocation, and/or to the accelerated metabolism in several weed species. Therefore, it is important to understand
concepts and measurement units of the main analytical techniques that use labeled molecules with $^{14}$C to study the biochemical and physiological resistance mechanisms to herbicides, as well as for studies that evaluate the destination of these molecules on the environment.

MEASUREMENT UNITS

Radiolabeled organic molecules are simply molecules constituted by an atom with unstable nucleus. The number of neutrons on the atom of an element determines which is the isotope at hand. There are three carbon isotopes with natural formation: $^{12}$C (98.89%) and $^{13}$C (1.11%), which are stable, and $^{14}$C, which is the radioactive isotope and, therefore, it is unstable, and found on the atmosphere at a proportion of one atom at every $10^{12}$ atoms of $^{12}$C (Zagatto, 2015).

$^{14}$C is a radionuclide continuously produced through nuclear reactions from interactions between the energetic particles (cosmic rays) and atmospheric nuclides. In order to obtain $^{14}$C, several reactions may occur, such as: $^{16}$O + n → T + $^{14}$C; $^{17}$O + n → α (alpha radiation) + $^{14}$C; $^{13}$C + n → γ (gamma radiation) + $^{14}$C; $^{16}$O + n → d (deuteron) + $^{14}$C; and $^{14}$N + n → $^{1}$H + $^{14}$C (Zagatto, 2015).

To reach stability, the $^{14}$C atom releases energy as beta-type ($\beta$) ionizing radiation. This energy release is known as “radioactive decay”, characterized by the disintegration (whether spontaneously or not) of the nucleus through the emission of $\beta$ particles. The magnitude of the activity of a radioactive substance is characterized by the rate between the number of nuclear disintegrations and one unit of time. In order to measure the activity of a radioactive substance, the use of the Curie (Ci) unit has been gradually replaced by the use of the Becquerel unit (Bq), adopted by the International System of Units (SI). Becquerel (Bq), however, is the most adequate unit to be used on researches with radiolabeled herbicides (Nandula and Vencill, 2015).

\[
1 \text{ Bq} = 1 \text{ disintegration per second (1 dps)} \\
1 \text{ Ci} = 3.7 \times 10^{10} \text{ dps} = 3.7 \times 10^{10} \text{ Bq}
\]

The half-life of a radioisotope is the time that is necessary for its radioactive activity to be reduced to half of its initial activity. The half-life of $^{14}$C is 5.7 x 10^5 years (Godwin, 1962); this is the reason why it is important to know the concepts and measurement units of the analytical techniques that use labeled molecules with $^{14}$C. Knowing this helps in preventing contaminations by labeled substances with $^{14}$C, and it is fundamental for the radiological protection plan of any licensed laboratory in order to work with radioactive substances. This review focuses specifically on herbicide molecules labeled with $^{14}$C.

MEASUREMENT EQUIPMENT

Observing the presence and activity of radioisotopes on different biological compartments or the compartments of a physical installation is important for a research laboratory working with radioisotopes. This is called monitoring. The objective of monitoring may be controlling the contamination or the evaluation of the radiation exposure, including the interpretation of the results. The equipment used on research laboratories for this purpose is the Geiger-Müller monitor (Figure 1A), which must be adequately calibrated by accredited authorities.

The liquid scintillation spectrometry technique (LSS) is knowingly the most adequate one in order to determine the radiation with low penetration power, such as $\beta$ radiation, emitted by $^{14}$C, since it shows adequate sensitivity and reproducibility. The use of a certified pattern for the studied herbicide is indicated for the adequate calibration of the liquid scintillation equipment (scintillation counter) (Figure 1B).

For liquid scintillation, the radioactive sample is incorporated to a scintillating solution, which emits light photons as the result of the interaction between $\beta$ particles and the molecules of the solvent of the scintillating solution. The amount of light produced is directly proportional to the amount of energy lost. Since the $\beta$ particles emitted by $^{14}$C have a short reach on liquid media and they lose all of their energy on the scintillating solution, the amount of light produced is proportional to the energy of these particles (L’Annunziata and Kessler, 2012). The light photons
produced are captured by photomultiplier valves and they are converted into electric pulses. The counting of these electric pulses corresponds to the counting of the radiations emitted by the sample. The measurements of a scintillation counter are, therefore, expressed as a counting per minute (cpm). The scintillation counter also registers the rate of atomic disintegrations per unit of time, in dpm (disintegrations per minute). Therefore, the dpm unit is equivalent to the number of atoms that disintegrate in 1 min (Knoll, 2000).

\[ 1 \text{ Bq} = 60 \text{ dpm} \]

For the types of studies considered in this review, the scintillating solution is kept inside small containers, known as scintillation vials. There are scintillation cocktails based on solvents, such as diisopropyl naphthalene (DIN), toluene and xylene (Oliveira, 2014). However, the choice for the solvent fundamentally depends on the chemical characteristics of the sample to be measured. Scintillating solutions that contain non-biodegradable solvents, such as toluene, must be treated as both a radioactive and a chemical waste, and have an adequate disposal according to the waste management plant of each laboratory.

In order to quantify the radiolabeled herbicide, the main purpose of preparing samples is obtaining a homogeneous and stable solution that is adequate for the LSS analysis. Soil samples and parts of plants are not readily soluble on scintillation cocktails. Due to this reason, such samples go through biological combustion on equipment called oxidizers (Figure 2A). The

![Figure 1](image1.png)  
*Figure 1 - Geiger-Müller monitor (A) and liquid scintillation equipment, Tri-Carb 2910 TR LSA counter (PerkinElmer) (B) from the Laboratory of Ecotoxicology of CENA/USP.*

![Figure 2](image2.png)  
*Figure 2 - Oxidizer OX500 (R.J. Harvey Instrument Corporation) (A) and Radio Scanner (Packard-Cyclone) (B) from the Laboratory of Ecotoxicology of CENA/USP.*
combustion of the sample creates an atmosphere that is rich in hydrogen, which is oxidized by the water, while the entire carbon content is oxidized by the carbon dioxide containing $^{14}$C ($^{14}$CO$_2$). An adequate scintillating cocktail is mixed with the product resulting from the combustion for reading on a scintillation counter (L’Annunziata, 2012).

Several chromatography techniques are available in order to determine and separate a series of compounds in mixtures, and the choice for each technique depends on several factors, including the objective of the study, characteristics of the sample and physical-chemical properties of the studied molecule (Vuckovic, 2012). Currently, on the metabolic degradation studies with herbicides on plants using radiolabeled herbicides, the High Performance Liquid Chromatography technique (HPLC) has been the one that is mostly used and mentioned in the scientific literature. The purpose of the HPLC technique is to separate and analyze non-volatile compounds or sensitive terms, and it is ideal for the separation of these chemical and biological compounds. If a compound is volatile, the most indicated separation technique is gas chromatography (GC). For example, Farag et al. (2007) used the chromatography with an ultraviolet detector (HPLC-UV), mass spectrometry (MS) and GC to identify and quantify polyphenols on *Medicago truncatula* roots. The HPLC-UV and MS techniques did not detect all the compounds, and only the GC technique identified sugars and functional groups.

The identification of individual compounds through HPLC offers both quantitative and qualitative information. The quantitative analysis is conducted by the determination of the peak height, since its base, on the chromatogram or by determining the area of such peak. On the qualitative analysis, the identification of compounds is given by the retention time, or, depending on the detector used, this information is based on other parameters, including molecular structure and weight. Another important use of HPLC on environmental studies is the trace analysis, in which the studied compound occurs at very low concentrations. For such, high resolution separations and very sensitive detectors are necessary. The purification of compounds may also be reached with the use of HPLC by collecting elutes from chromatography peaks on the output of the detector and subsequent concentration of the compound, through the removal/evaporation of the solvent and subsequent resuspension on an appropriate solvent.

Radioactivity detection technologies on HPLC were developed throughout time, including flow monitors, fraction collections for liquid scintillation counting and counting of $^{14}$C atoms using laser. For samples with low radioactivity concentrations, as is the case of absorption, translocation and metabolism studies on plants, online monitors of radioisotopes have been mainly used to detect the radioactivity by HPLC, since they offer greater cost-efficiency in comparison to the offline methods. The adequate choice for the column, the mobile phase and the type of detector is the most important factor for the HPLC technique to be successful on studies with radiolabeled herbicides.

The thin layer chromatography technique (TLC) may also be used on environmental studies and studies on the metabolic degradation of herbicides in plants. The TLC technique complements the GC and HPLC techniques for the separation, detection, identification and quantification of pesticides, since it has unique advantages when compared to the column chromatography, including: simple preparation of samples, simplicity due to the positioning of the TLC plate on a closed vat containing the mobile phase, and its low operation cost (Sharma, 2005).

On the TLC technique, the supernatants resulting from the preparation of treated and untreated plants are not injected into columns, such as on the HPLC technique. The supernatants are evaporated and dissolved again on an adequate solvent. This solution, containing the radiolabeled herbicide or eventually its metabolites, is applied on TLC plates with the use of a micro-syringe. The TLC plate is positioned inside a glass vat, containing a relatively large volume of the mobile phase, and it is then closed in order to favor the movement of the samples on the plate. The ascending unidirectional movement on a capillary flow on the plate is the most used technique on TLC for pesticides in general (Fried and Sharma, 1999). The methodology was established according to the Environmental Protection Agency – EPA – on the Soil thin layer chromatography guideline (EPA, 1998).

The zones containing radioactivity are detected by reading the TLC plate on phosphorus plate scanners (radio scanners) or with radiographic film (Figure 2B), and the chemical nature
of the components is identified by comparing the $R_f$ values and the analytical standards. The $R_f$ acronym comes from “retention factor”, and its value is defined as the rate between the distance travelled by the herbicide and the distance traveled by the solvent. For example, if an herbicide travels 2.4 cm on a TLC plate and the solvent travels 3.2 cm, $R_f$ is 0.75 ($R_f$ is dimensionless). Since maintaining the experimental conditions on a constant level is a challenge, relative $R_f$ values are usually considered on the studies that use TLC. Relative $R_f$ values are referred to in that manner because the $R_f$ value of the studied herbicide is based on the $R_f$ value of its analytical standard (the analytical standard of the herbicide and the sample to be studied are applied on the same TLC plate in order to compare the $R_f$ values) (EPA, 1998).

In addition to comparing the $R_f$ values, the herbicide zones on the TLC plate may be identified at visible light, as colored zones, or at ultraviolet light with wavelength of 366 nm, as fluorescent zones on their natural states, or after post-chromatographic derivation with a chromogenic or fluorogenic reactant applied on the TLC plate (Bawer et al., 1991).

In the case of simple molecules, the identity of the compounds on the sample may be confirmed through the production and interpretation of the emission spectrums or absorption of electromagnetic radiations by the compounds on the plate. This technique is called spectroscopy and it may be conducted by several methods, such as infrared, ultraviolet, fluorescence, X-rays, visible light, among others (Sharma, 2005). In order to identify more complex molecules on a sample, the use of mass spectrometry is the most indicated technique, because it is based on the determination of atomic masses, providing information on the structure of these molecules. The purification of compounds is also conducted by TLC, by scraping the region represented by the compound on the TLC plate and the dissolution of the scraped material on an adequate solvent.

**ABSORPTION AND TRANSLOCATION IN PLANTS**

Studies on the absorption and translocation of herbicides on plants are usually conducted to evaluate the behavior of a new herbicide on a certain plant species, comparing two or more herbicides, specific formulations, additives, or the effect of environmental standards. The growing problem regarding the resistance of weeds to herbicides promoted the studies on the absorption, translocation and metabolism of herbicides as the methodology to elucidate the resistance mechanisms (Nandula and Vencil, 2015).

The studies on the absorption of herbicides use a destructive sampling of treated plants on several post-treatment periods, which allows the characterization of the absorption standard on the plant, considering the planning and adequate statistical analyses (Kniss et al., 2011). The steps to conduct the study on the absorption and translocation of herbicides in plants aiming at resistance studies are described as follows, based on Ahmad-Hamdani et al. (2013).

On the adequate phenological stage for each species, susceptible and resistant plants must be adequately identified by treatment. The leaves that have been predetermined to receive the radiolabeled herbicide must be covered with plastic film, aluminum paper or small paper envelopes. Then, the “cold” herbicide is applied to the plats (without the radioisotope) at the dose recommended by the manufacturer, as a solution with adjuvant (when indicated) and water, followed by the immediate removal of the protective plastic film of the applied leaf.

The radiolabeled herbicide solution must be prepared on a solution containing its commercial formulation at the recommended dose for the considered phonologic stage. After applying the “cold” product, its radiolabeled version is applied. It is important for the radiolabeled herbicide to be applied with at least 170 Bq of specific activity, in the case of studies with most of the annual weeds (Nandula and Vencil, 2015).

The radiolabeled product is applied using a micro-syringe, by applying a 1 μL droplet (the total applied depends on the molecule and the radioactivity of the radiolabeled molecule) on the leaf blade of the upper part of the expanded leaf of each plant (Figure 3). The choice for the leaf on which the application will occur depends on the studied species. Each plant (or part of the plant) must be collected according to the pre-established times for each situation. However, it is suggested that at least six collection times are used, in addition to time zero (immediately after
Differently from the absorption, which occurs within hours after the treatment, the translocation of herbicides may take up to days after the treatment. Due to this reason, in order to evaluate the translocation, the previous knowledge must be considered in order to determine the times after the treatment in which this variable should be evaluated.

The biological combustion is the most used procedure to quantify the translocation of herbicides on plants. However, care must be taken when stating that the detection of the radioactivity on other parts of the plant, outside the treated leaf, means that the herbicide is on its parental form. It might have been converted into a non-phytotoxic metabolite. In order to state this, one must investigate the potential for the herbicide to have been metabolized by the studied weed, through the information available in the literature.

To study the movement of herbicides on plants, the qualitative techniques involving autoradiography or phosphorus blade images have been used for over 50 years (Nandula and Vencil, 2015). While the biological combustion offers a quantitative estimation of the herbicide on the treated plant, autoradiography (Figure 4) or the phosphorus blade image provide a qualitative measurement of the movement of the herbicide on the plant, in addition to the location where it occurs.

For the exposition of the treated and untreated plants, the use of phosphorus blade images is safer in comparison to the use of autoradiography, since it does not require handling chemical compounds that are harmful to the health. Despite more expensive, the technique is also quicker. A single day of exposition of a plant on a phosphorus blade resulted on images with superior quality than the exposition for three weeks with the X-ray film (Wehtje et al., 2007).

Therefore, in order to study the translocation, the plants treated as on the absorption study must be exposed on phosphorus blade for 72 hours, in order to scan the image for qualitative analysis. The usual procedure to quantify the translocation of herbicides on plants is the biological combustion, in which dry samples of each part of the plant (both the treated leaf and the part above and below it, as well as the roots) are oxidized by the presence of O₂, and the resulting CO₂ is captured on a special solvent. Then, the radioactivity must be measured on the scintillation counter.

The quantitative analysis of the translocation may also be conducted through the volume analysis, offered by the software provided together with the image scanner, as of its purchase. The volume is the total signal intensity of the radioactivity within defined limits of the image. The translocation is then expressed as the rate between the percentage of signal intensity on the applied zone, as well as above and below it, and the total signal intensity on a defined image containing ¹⁴C (Ahmad-Hamdani et al., 2013).
Figure 4 - Autoradiography of *Tecoma stans* with application of the leaf of $^{14}$C-aminocyclopyrachlor at 2, 4, 8, 12, 24 and 72 HAT (horas after the application).

(a) pressed plant to the right and (b) autoradiography of the plant translocation to the left.
Source: Reis et al. (2015).
METABOLISM IN PLANTS

The use of radiolabeled herbicides to investigate whether the herbicide is being metabolized on the plant is an efficient method, and it is the most indicated method to diagnose the resistance related to other phenomena that are not related to the change on the action site of the herbicide (Tranel, 2015). The analytical method aiming at studying the metabolism of herbicides in plants comprehends three fundamental steps: preparation of the plants and application of treatments; extraction and separation; and identification of the herbicide and its metabolites, if any.

The steps to conduct the study on the metabolism of herbicides in plants are described as follows. The preparation of plants and application of the treatments must be conducted as described for the absorption and translocation study. In case the fresh samples of plants are not adequate for processing after the collection, the ideal is to store them at -20 °C to assure the stability of the active substances and metabolites. The techniques employed on studies on the metabolism of herbicides in plants are TLC, HPLC and GC, depending on the herbicide molecule.

For the extraction, the adequate system of solvents for the studies herbicide must be known. The treated leaf must be rinsed with non-polar solvent (usually ethanol or methanol). Then, the plant must be dried with an absorbing paper, immediately frozen in liquid nitrogen and stored at -80 °C up to its use. The plant tissue must be macerated in crucibles that must be previously cooled with N₂, and homogenized with the specific cold solvent at a concentration of 80% (v v⁻¹). A stainless steel homogenizer may also be used. The solution must be centrifuged; the supernatant, decanted; and the residue must go through re-extraction with the chosen cold solvent at 80%, followed by extraction with the same cold solvent at 50% (v v⁻¹). The supernatants must be mixed, and the radioactivity must be determined by LSS, in order to know the mass balance, which is expressed as the rate between the radioactivity applied at the beginning of the experiment and the total radioactivity measured (originated from rinsing all parts of the plant). The mass balance may be also referred to as the radioactivity recovery percentage. Approximately 7 mL of the supernatant must be evaporated, resuspended in 300 mL of the solvent at 50% and centrifuged. The final sample may be analyzed by any previously described technique, usually TLC or HPLC, with the respective solvent system (Monquero et al., 2004).

BIODEGRADATION AND MINERALIZATION ON THE SOIL USING BIOMETRIC FLASKS

The study of biodegradation with the use of radiometric techniques is based on measuring the mineralization and degradation rates of a test-substance according to the microorganisms of the soil. The method consists in treating soil samples with the test-substance (pesticide) and incubating them in biometric flasks, under controlled temperature and soil humidity conditions. The molecule biodegradation process is quantified at previously established intervals, in which soil samples are extracted and analyzed in order to determine the concentrations of the original substance or metabolites. The mineralization is evaluated by the evolution of the ¹⁴CO₂ captured in the solution, as well as by the formation of bound residue through the oxidation of the soil samples (Dias, 2012).

The methodology described as follows was established according to the guidelines of OECD - 307 Aerobic and Anaerobic Transformation in Soil (OECD, 2002a).

Collection and preparation of soil samples

The soil samples must be collected from a layer with at most 20 cm in depth, having previously cleaned the vegetable layer that covers the soil. This is important because it is on the superficial layers that most of the microorganisms on the soil are. Whenever necessary, after the collection, the soil samples must be stored at 4 ± 2 °C for a maximum period of three months.

Before applying the test-substance, the soil samples must be sifted on a sieve with a ± 2 mm mesh. After sifting, the water content of the soil and the field capacity of the soils that will be used must be determined. This determination may be conducted as described below.
Determining the water content of the soil (U)

The amount of 10 g of soil must be placed on Petri dishes, previously weighted. These dishes, together with the soil, must be taken to the oven, at 105 ± 1 °C, for 24 hours. After this period, the dishes must be removed from the oven and placed on a desiccator until cooled. Then, they must be weighted again. The water content of the soil is calculated by the difference between the masses of the humid and dry samples, using the following equation: \( U \) (kg kg\(^{-1}\)) = \( \frac{(m_p + m) - (m_p + m_s)}{(m_p + m_s) - (m_s)} \), where \( m_p \) corresponds to the mass of the Petri dish, \( m \) is the mass of the humid soil, and \( m_s \) is the mass of the dry soil.

Determining the field capacity (CC)

The determination of the field capacity may be conducted according to the TSFM technique (Separated Clod by the Wetting Front), described by Costa (1983). Under that technique, the air-dried soil, sifted on a sieve with a 2 mm mesh, must be placed on a 500 mL beaker until it is filled. Then, an amount of 2 mL of distilled water must be dripped for a period of 10 seconds; thus, a moist clod will be created, which must be separated from the rest of the soil. This clod must be placed on a container, whose weight must be known, and weighted together with the container, and then taken to the oven at 60 ± 1 °C for 24 hours.

After the drying period, the clod is weighted, and the field capacity (CC %) is calculated by the difference between the masses (g) of the humid and dry samples, using the following equation: 
\[
CC (\%) = \frac{[(m_p + m) - (m_p + m_s)]}{(m_p + m_s) - (m_s)} \times 100,
\]
where \( m_p \) corresponds to the mass of the Petri dish, \( m \) is the mass of the humid soil, and \( m_s \) is the mass of the dry soil.

It is noteworthy that other techniques may be used in order to determine the field capacity, such as by calculating the density of the water flow, for its determination in the field, and by the water content corresponding to a certain tension on the retention curve, determined in the field and at the laboratory, as described by Brito et al. (2011).

Amount of soil to be weighted

The amount of soil to be weighted and placed inside each Bartha flask (biometric flask) must be determined by using the following equation: 
\[
m = (1 + U) \times m_s,
\]
where \( m \) is the mass of the soil to be weighted, at the current humidity (g), \( m_s \) is the desired mass of the dry soil (g), and \( U \) is the current humidity on the soil (kg kg\(^{-1}\)).

Amount of the water volume to be added to the flasks

In order to reach 75% of the field capacity, water must be added to the soil. In order to determine the volume to be added, the following formula may be used: 
\[
Va = \frac{[(CC \times 0.75)/100 - U] \times m_s}{m_s},
\]
where \( Va \) is the water volume to be added to the soil, mL; \( CC \), the field capacity (%); \( U \), the current humidity on the soil (kg kg\(^{-1}\)); and \( m_s \), the mass of the dry soil (g).

Calculating the dose and preparing the work solution

According to the standards published by OECD (2002a), for biodegradation studies, the highest recommended dose for the culture must be tested for the herbicide that will be studied. However, depending on the objective of the study, other doses may be used. In order to calculate the equivalent of the dose to be used for the preparation of the work solution, the soil density equal to 1.2 g cm\(^{-3}\) must be considered, as well as the homogeneous distribution of the test-substance (herbicide) on the first 10 cm of the soil profile (Dias, 2012).

The volume of the work solution to be applied on each flask depends on the soil mass that will be added to the soil. In general, for 50 g of soil (dry base) 200 μL of the work solution may be applied.


**Study installation**

Before applying the test-substance, weight the equivalent to 50 g of dry soil on Bartha flasks, add the necessary water volume and incubate the soil for a period of 2 to 28 days at 20 ± 2 °C (pre-incubation). It is important to point out that, during the study, periodic verifications of the water content of the soils on the Bartha flasks must be conducted. If the difference between the initial water content and the determined content was superior than 5%, the adjustment must be made by adding water.

The application of the work solution on the soil must be made with a micropipette, taking care for it to be distributed across the entire soil surface. After the application, the soil must be homogenized, and the flask, closed. On the side arm of the Bartha flasks (Figure 5A, B) a share of 10 mL of sodium hydroxide solution must be added (NaOH 0.2 mol L⁻¹) and, then, the flasks must be incubated at 20 ± 2 °C.

For the mineralization study, collections of the (NaOH 0.2 mol L⁻¹) solution must be conducted 7, 14, 21, 28, 35, 42, 56, 63 and 70 days after the application of the test-substance. On each evaluation period, samples of the solution must be collected and transferred into liquid scintillation flasks, containing the scintillating solution, in order for the ¹⁴C on the solution to be determined using the Liquid Scintillation Spectrometer (LSS). The rest of the solution must be removed, and a new solution must be added to the side arm.

The determination of the radioactivity on the soil using the extraction method must be conducted with the incubated soil collections, on the same incubation periods. The extraction is conducted to determine the extractable radioactivity from the soil and, then, with other available techniques, the concentration of the original product and its possible metabolites. In some cases, this extraction period may be extended, in order to reach a higher evolved ¹⁴CO₂ percentage. The extraction must be conducted according to the methodology available in the literature; in some cases, pre-tests are recommended in order to verify whether the solvents and extraction methods are adequate.

The study of the degradation of herbicides with the use of thin layer chromatography is conducted after the reduction of the extracts on a rotaevaporator. Shares of the reduced extract and the standard solution of the radiolabeled herbicide must be applied on silica plates. The

![Figure 5](Image)

**Figure 5** - Study conducted with herbicides on an acclimatized room (A) and scheme for a Bartha flask with 50 g of soil and NaOH 0.2 mol L⁻¹ solution added on the side arm to study the biodegradation of herbicides (B).

Source: Adapted from Dias (2012).
elution must be conducted with adequate solvents for each test-substance. The products and possible metabolites may be observed with the help of ultraviolet light, X-ray film or radioscanner.

After the extraction, the remaining $^{14}$CO$_2$ (non-extractable residue) from the soil samples must be determined. For such, soil samples must be oxidized on a biological oxidizer, and the resulting $^{14}$CO$_2$ from the combustion must be collected on a scintillating solution and quantified on a LSS.

At the end of the study, the sum of percentages of the test-substance found on the NaOH solution (0.2 mol L$^{-1}$), on the soil extract and on the oxidized soil offers the recovery of the study (also referred to as mass balance), which must vary between 90 and 110% for radiolabeled substances.

### Biodegradation model and herbicide mineralization

The accumulated $^{14}$CO$_2$ mineralization data are adjusted to a first-order kinetics model: ln$C/C_0$ = -$k$ x $t$, where $C$ is the concentration of the remaining herbicide on the soil according to time $t$ (%); $C_0$ the concentration of the herbicide at time zero; $k$, the mineralization speed constant; and $t$, the incubation time in days.

From the $k$ values, it is possible to calculate the mineralization half-life ($t_{1/2}$ min.) of the herbicides, which is defined as the time, in days, needed for 50% of the herbicide molecules to become an end product of the reaction: $t_{1/2}$ min. = ln2/$k$ (Dias, 2012).

There are other models that may be adjusted, such as the second order exponential decay model: $C_t = C_1 \times \exp(-K_1 \times t) + C_2 \times \exp(-K_2 \times t)$, where $t$ represents time; $C_i$ is the herbicide concentration on the soil, at time $t$; $C_1$ and $C_2$ are the initial concentrations of the herbicide on the soil; and $K_1$ and $K_2$ are the dissipation speed constants of each phase ($K_1 > K_2$) (Oliveira Jr. et al., 2013). On a biphasic model, degradation rates are usually much slower during the second phase; therefore, it is relevant in order to determine the necessary time for the dissipation of 50 and 90% of the initial concentration, DT50 and DT90, respectively (Oliveira Jr. et al., 2013).

### LEACHING IN SOIL COLUMNS

The methodologies used on leaching studies in soil columns with radiolabeled herbicides with $^{14}$C are established according to the guidelines from OECD - 312 “Leaching in soil columns” (OECD, 2002b).

### Fractioning and characterization of soils

The soil samples are collected on the superficial layer (at most 0 to 20 cm in depth), with the previous cleaning of the vegetable layer, on locations with cultivated areas with agricultural crops of an agronomic interest. After drying, the samples go through a sieve with a 2.0 mm mesh and stores at room temperature.

The physical-chemical properties must be analyzed (at least, the cation exchange capacity, pH, organic carbon or organic matter, clay content, mineralogy, density and porosity) of the samples in reference laboratories, and the classification of the studied soils must be described according to the Brazilian Soil Classification System (Embrapa, 2013).

### Preparation of the soil columns

Glass columns with glass wool with 0.50 m in height are used for the study, with soil samples (Figure 6A, B). At least two columns per soil must be prepared (two repetitions) for each herbicide treatment.

The soil columns are prepared closing their end with quartz wool, filling the conic part with rinsed quartz sand dried on an oven at 100 °C and adding soil samples in the column up to 0.30 m in height, placing small air-dried soil portions on the bottom, and vibrating the set in
order to accommodate the soil, avoiding the creation of air bubbles. Remove 100 g of soil from the upper part and set apart for application of the herbicide, if applied on dry soil; if not, do not remove the 100 g portion from the surface. Avoid excessive vibrations in order not to separate the soil particles. The soil samples stored on the columns must be weighted, in order to control the reproducibility of the packaging process of the columns.

The soil columns are placed inside a 2 L test tube, and they are slowly moistened with the ascending flow of a CaCl$_2$ 0.01 mol L$^{-1}$ solution, so that the level of the solution is not 0.10 m higher than the wetting front of the soil sample. The soil sample must be flooded for approximately 30 min. After the flooding process of the columns, when the CaCl$_2$ solution reached the top of the columns due to the ascending flow, they are removed from the test tube and installed on the holder for one or two hours in order for the CaCl$_2$ solution to be drained. The amount of 100 g initially removed where the herbicide was applied (if that is the case) must be placed inside the column, or the herbicide must be directly applied on the humid soil.

**Amount and application of the herbicide**

The herbicide must be applied at the highest recommended dose for the studied culture in the field, and radioactivity around 18.7 kBq must be applied per soil column.

Approximately 200 to 250 $\mu$L of a solution containing the radiolabeled herbicide plus a technical product or analytical standard per soil column are applied directly on the humid soil on the surface of each column (Figure 7A) or on the dry sample of 100 g set apart during the packaging of the column, with posterior homogenization of the soil, and after putting it back on the column. The radioactivity (Bq) applied per soil column must be reported, with its radiochemical purity (%) and specific activity (MBq mg).

After the application, the surface of the soil sample must be covered by a quartz wool disk (Figure 7B), with an inverted funnel fitted in, where a tube will be connected, through which the CaCl$_2$ 0.01 mol L$^{-1}$ solution will go through (Figure 7C). A flow of approximately 8 mL h$^{-1}$ must be simulated for 48 hours, using the CaCl$_2$ 0.01 mol L$^{-1}$ solution, resulting on a rain simulation of approximately 200 mm for 48 hours.

The study must be conducted on an acclimatized environment, with little light and controlled temperature between 18 and 25°C.

**Leachate collection and removal of the soil column**

At every 12 hours (preferably), three 10 mL portions of the leachate are collected, having 10 mL of insta-gel solution added for measurement on the Liquid Scintillation Spectrometer.
(LSS). After 48 hours of the application of radiolabeled herbicides, corresponding to the end of the drainage of the CaCl₂ 0.01 mol L⁻¹ solution, the glass columns are removed from the holder, and the soil samples are removed from the columns, injecting air at the end of the column in order to force the soil out, which is cut into six sections of equal sized (at least five sections are used) (Figure 8A, B and C). Whenever this is not possible, the glass column with the soil must be frozen right after the end of the leaching and draining of the soil (since the excess of water on the soil may break the glass column), in order for the soil to be removed from the glass column afterwards. The soil samples are air-dried, weighted, macerated and homogenized (Figure 8D). Three sub-samples (0.2 g) from each layer (~ 5 cm) of dried soil are burnt on a biological oxidizer, in order to quantify the total radioactivity.

For the sections that show high radioactivity, the soil must go through an extraction process, aiming at determine the herbicide and its possible metabolites with radioactivity higher than 10%, in relation to the applied radioactivity.

**Recovery of the total leachate**

The leaching study on soil columns does not quantify the leaching potential of the herbicide in the field. The results are expressed as a percentage of the radioactivity found on the leachate and on each segment of the column, in relation to the initially applied radioactivity. These results help in the decision-making in order to request in-field tests for herbicides that show a high leaching potential in the laboratory. The recovery of the studies (sum of the percentages of radiolabeled herbicides found on the soil segments and on the leachate) must vary between 90 and 110% for radiolabeled herbicides, according to OECD (2002b). In order to verify the repeatability and analytical sensitivity of the method, the oxidized soil samples and leachate samples are in duplicates.

**SORPTION-DESORPTION IN SOILS USING THE BATCH EQUILIBRIUM METHOD**

The methodologies used on the sorption and desorption studies with radiolabeled herbicides with ¹⁴C are established according to the guidelines from OECD - 106 “Adsorption - Desorption Using a Batch Equilibrium Method” (OECD, 2000).
Fractioning and characterization of the soils

The soil samples are collected on the superficial layer (at most 0 to 20 cm of depth), with the previous cleaning of the vegetable layer, on locations with cultivated areas with agricultural crops of an agronomic interest. After drying, the samples go through a sieve with a 2.0 mm mesh and are stored at room temperature.
The analyses of the physical-chemical properties must be conducted (at least, the cation Exchange capacity, pH, organic carbon or organic matter, clay content, mineralogy, density and porosity) of the samples at a reference laboratory, and the classification of the studied soils must be conducted according to the Brazilian Soil Classification System (Embrapa, 2013). These procedures reduce the biomass and/or microbial activity of the soil, preventing the biodegradation of the herbicide. If necessary, the soil samples may be stored in a laboratory, on adequately identified plastic bags, with the location, date and name of the collector.

Experimental design

The experimental design is completely randomized, with at least two replications. Each experimental unit is constituted by a Teflon flask with capacity for 50 mL and screw cap. Portions of each soil are weighted in duplicates on the flasks, and the volume of the calcium chloride solution (CaCl₂) 0.01 mol L⁻¹ used depends on the soil:solution (m/v) relationship, described on the preliminary study.

Preliminary study

The pre-test must be conducted in order to determine the adequate soil:solution relationship, as well as the equilibrium time and the amount of the herbicide sorbed on the soil and on the walls of the Teflon flasks.

At least 45 mL of CaCl₂ 0.01 mol L⁻¹ solution are added on the dry soil samples, 12 hours before the beginning of the study, and the amount of stock-solution must not exceed 10% of the final volume (50 mL).

In order to conduct this study, samples in duplicates from two soils with contrasting texture must be used. It is suggested that the soil:solution relationship is 1:25 (2 g of soil and 50 mL of solution), 1:10 (5 g of soil and 50 mL of solution) or 1:2 (25 g of soil and 50 mL of solution), with at least 50 mL of volume used in all relationships.

The initial solution must be prepared with the herbicide at the highest dose to be used on the sorption study on CaCl₂ 0.01 mol L⁻¹. Then, this solution is applied on the Teflon flasks, and it is mechanically stirred on a horizontal table for 48 hours. Sub-samples of 50 to 100 μL are removed from the supernatant (Figure 9A, B) on the periods equivalent to 2, 4, 8, 24 and 48 hours of stirring. The final volume of the portions must not exceed 10% of the volume, in order to determine the equilibrium time and the amount of herbicide absorbed on the soil.

At least 20% of the applied herbicide must have been sorbed on the soil, preferably between 50 and 80%. This allows the selection of the most adequate soil:solution relationship in order to conduct the sorption study.

Chemical products

The stock-solutions must be prepared using five different concentrations of the technical products or analytical standards of the non-radiolabeled herbicides, with purity close to 100%, acquired from specialized laboratories. For the radiolabeled herbicides, the radiochemical purity (%) and the specific activity (MBq mg⁻¹) must be described, and acquired from international laboratories, due to the fact that there are no suppliers in Brazil. The solutions are prepared containing the herbicide (radiolabeled + non-radiolabeled) at different concentrations (mg L⁻¹), with 0.01 mol of L⁻¹ of CaCl₂ (1.11 g of CaCl₂ per liter of deionized water). The analytical standards (non-radiolabeled) and amounts of the radiolabeled herbicide are added in order to obtain a final concentration corresponding to the recommended dose of the active ingredient (g ha⁻¹) for the culture in which the herbicide is recorded.

Whenever possible, one of the concentrations of the herbicide used must correspond to the maximal in-field dose recommended and contain at least 80 Bq mL⁻¹ of radioactivity. The calculations of the dilutions to prepare the stock-solutions depend on the solubility, polarity, hydrophobicity (Kow) and the recommended dose of the herbicide.
The scintillating solutions are prepared by mixing 0.2 g of POPOP ([1.4-di-2.5-pheniloxazol) benzene) and 300 mL of toluene, stirred up to its dissolution. Then, add 4 g of PPO (2.5-diphenyloxazole) and stir until it is dissolved. Add 333 mL of RENEX 95% (nonylphenol ethoxylate) and stir. Complete with toluene up to 1 L.

**Herbicide sorption-desorption studies**

On sorption studies, shares of the radiolabeled solutions are transferred in duplicates to independent flasks containing 10 mL of scintillating solution insta-gel plus, and the amount of the initial concentration of $^{14}$C-herbicide is determined within five minutes according to the counting on a Liquid Scintillating Spectrometer (ECL).

In duplicate, 10 mL of the radiolabeled solutions of all concentrations are added to the Teflon flasks containing the soils with the exact amount of the soil:solution concentration found on the preliminary studies, which is usually 1:10. The flasks are stirred on an horizontal shaker table, in a dark room (20 to 25 °C), at approximately 180 rpm (4 g) to reach the equilibrium concentration during the time found on the preliminary studies, which is usually 24 hours. Although the equilibrium period of 24 hours is common, other periods may be determined upon previous assays with this purpose, in order to assure that 20 to 80% of the substance is sorbed on the soil.

At the end of the equilibrium period, the flasks are centrifuged between 3,000 and 3,500 rpm for 15 minutes, and shares of 1 mL of the supernatant from each flask are transferred in duplicate to scintillation flasks containing 10 mL insta-gel plus® scintillating solution (Perkin-Elmer) and analyzed in LSS, in order to determine the concentration of $^{14}$C-herbicide on the solution, by counting the radioactive activity. The sorbed amount of the herbicide is calculated by the difference between the initial concentration and the concentration on the supernatant after the equilibrium.

The desorption studies are conducted immediately after the sorption one, under the same conditions. On the Teflon flasks containing soil and the sorbed radiolabeled herbicide from the sorption study, 10 mL of the CaCl$_2$ 0.01 mol L$^{-1}$ solution are added. The flasks are stirred on an horizontal shaker table, aiming at the resuspension of the soil+solution, on a dark room (20 to 25 °C), at approximately 180 rpm, in order to reach the equilibrium concentration during the time found on the preliminary studies, which is usually 24 hours. After the stirring period, the flasks are centrifuged, and 1 mL shares of the supernatant are pipetted in duplicate to a scintillation flask containing 10 mL of scintillating solution and analyzed by LSS. The desorped amount is calculated by the difference between the sorbed radioactivity on the soil and the one remaining on the supernatant.

The studies must contain two controls: flasks with the CaCl$_2$ 0.01 ol$^{-1}$ solution and soil; and flasks with the radiolabeled solution added to the flask with no soil; they are usually conducted with the maximum recommended dose, in order to determine the stability of the herbicide and evaluate the possible adherence of the radiolabeled material to the walls of the flasks.

The soils from the Teflon flasks are dried on an oven at 40 °C for 48 hours. After drying, they are grinded and stored on plastic packages. Sub-samples from each soil are weighted in triplicate (0.2 g) and oxidized on a biological oxidizer, in order to determine the amount of radioactive herbicide bound to the soil (non-extractable) and to the residues of the herbicide that may be extracted by solvents.

From that, the mass balance is conducted by the total amount of herbicide applied, represented by the sum of the sorption, desorption and soil combustion sum. The adopted quality control admits an acceptable recovery interval between 90 and 110% for those that use studies with radiolabeled substances (OECD, 2000).

**Sorption-desorption model of the herbicide**

The $K_f$ and $1/n$ sorption coefficients are determined by the isotherm model by Freundlich: $C_s = K_f \times C_e^{1/n}$, where $C_s$ is the amount of the herbicide sorbed on the soil (μmol kg$^{-1}$); $K_f$ is
Freundlich’s equilibrium constant ($\mu$mol ($^{1-1/n}$) L$^{1/n}$ kg$^{-1}$); $C_s$ the concentration of the herbicide on the equilibrium solution ($\mu$mol L$^{-1}$); and $I/n$, the linearity degree of the isotherm. The sorption linear coefficient is also calculated ($K_s$, L kg$^{-1}$), where $K_s = C_s/C_r$, and the normalized sorption coefficient for the organic carbon content of the soil ($K_{oc}$, L kg$^{-1}$), where $K_{oc} = (K_r/\%CO) \times 100$.

The equilibrium constant $K_{foc}$ of the normalized sorption for the organic carbon content of the soil is adjusted by the formula: $K_{foc} = (K_f/\%CO) \times 100$. The $K_f$ and $I/n$ values of the desorption are determined in a similar manner than the sorption coefficients, from the remaining amount of the sorbed herbicide on the soil at every desorption versus the equilibrium concentration. The $K_d$ value of the desorption is also calculated in a similar manner than the $K_d$ values of the sorption. The hysteresis coefficient ($H$) is calculated using the parameters originated from the Freundlich isotherms for sorption ($I/n_{sorption}$) and desorption ($I/n_{desorption}$), with the formula: $H = (1/n_{desorption})/(1/n_{sorption})$ (Barriuso et al., 1994).

**STATISTICAL ANALYSIS OF DATA FROM RADIOACTIVITY STUDIES**

The data must follow the normal distribution, and the variances must be homogeneous in order to provide an appropriate interpretation of tests and, also, the additivity of the effects of variation factors.

According to the information adapted from Nandula and Vencill (2015) and Kniss et al. (2011), the non-linear regression analysis may be applied to the absorption and translocation data, if the number of collection intervals used throughout time is three or more, considering that it is recommended to use at least six times (points), in addition to the initial time (zero). In case of comparison between behavioral studies on herbicides between two or more soils or plants, within the same collection time, the data may be analyzed using separation procedures for common means. Autoradiography is purely a qualitative measurement and, therefore, it does not involve any statistical application.

For the metabolism, also with a qualitative measurement, its quantification on the extracts of plants may be expressed by a percentage, in relation to the extracted and the applied radioactivity, found as the herbicide and/or on metabolite(s). The peaks obtained by chromatography may be separated and identified according to the ($R_f$) values available in the scientific literature or by the use of the metabolite standards.

Regarding the data related to biodegradation, in case of significant effects, tests must be applied for multiple comparisons of the means of these significant effects. Multiple linear regression models may be developed based on the “stepwise” method for the selection of variables in order to evaluate the association between mineralization and the physical-chemical properties of the soil (Dias, 2012).

In order to interpret the results, the leaching data are compared to the ones from the treatment without the herbicide (dose zero); however, when there is no interaction between the variation sources, the means of the treatments on the curves are used. The data are subjected to analysis of variance according to the F test, using a statistical program and, then, regression equations and the curves are plotted, when the data are quantitative, and a comparison test for the means is conducted, when the data are qualitative. Multiple linear regression models may be developed based on the “stepwise” method for the selection of variables in order to evaluate the association of the leaching and the physical-chemical properties of the soils. The calculations may be made through the GLIMMIX and REG procedures when the SAS software is used (Dias, 2012).

On the sorption-desorption data, the sorption coefficients may be transformed into log ($x + 1$) when necessary, and the analysis of variance may be conducted, in order to determine the differences among the herbicides on each soil, and among the soils for each herbicide. Pearson’s correlation between the sorption coefficients and the physical-chemical properties of the soil may be estimated for each herbicide, and linear regressions may be obtained with statistical packages (Oliveira Jr. et al., 2001).
MANAGEMENT OF RADIOACTIVE WASTE

Waste storage location

Every radioactive installation or radioactive waste deposit must have a radioactive waste management plan, within the context of the licensing and control processes (CNEN, 2014). All and every material that gets directly in contact with the radioactive solution are considered as radioactive waste. All radioactive waste from the laboratory shall be stored in packages identified with the international symbol for the presence of radiation, fixated on a clear and visible manner, and stored on an adequate deposit room for such purposes, which shall be sufficiently far away from the work area. This location shall be rigorously controlled by a responsible technician, designated to receive, store and control all the radioactive material, as well as for the several protection measurements; the access must be limited to the staff that is authorized to handle the radioactive material. After stored on the radioactive waste deposit, the waste is sent to IPEN (Institute for Energetic and Nuclear Researches), in order to be stored or eliminated.

Segregation of the radioactive waste

The segregation of waste must be conducted on the same location where the waste was generated or on an adequate environment, taking into consideration the characteristics of the radioactive material, such as: physical state; half-life; compactable or non-compactable; organic or inorganic; biological (putrescible and pathogenic); in addition to hazardous characteristics regarding explosivity, combustibility, inflammability, corrosivity and chemical toxicity (CNEN, 2014).

Solid waste

The solid waste is placed on plastic containers with a screw cap and adequately labeled. The materials (soils and plants), after conducting the studies on radiolabeled herbicides, must be dehydrated at room temperature, on stainless steel trays. Then, the materials must be oxidized with a biological oxidizer in order to determine the activity per kilogram of soil or plant; if this value is higher than $1 \times 10^4$ kBq kg$^{-1}$ for a quantity $\leq 1,000$ kg of material, and 1 kBq kg$^{-1}$ for a quantity $> 1,000$ kg (CNEN, 2014), they must be disposed as radiolabeled waste. In case their value is below the value specified as an activity concentration limit for disposal, the materials must be disposed as regular waste.

Liquid waste

The liquid waste (aqueous solution) with low activity (lower than $5.6 \times 10^5$ Bq m$^{-3}$) (CNEN, 2014) must be stored on adequately identified flasks disposed on the regular sewage (sink). This solution is only disposed if the activity value found is equal to the control (“background” - BG). In the case of scintillating and organic solutions, they must be carefully transferred from the scintillating flasks to the polypropylene tanks, with a sealing screw cap, adequately identified.

All scintillating solutions with radioactive activity above the allowed limit for disposal on the sanitary swage network must be stored on polypropylene tanks with a sealing screw cap, adequately identified. When the volume of the solution on the flask reaches the limit, the responsible technician must be immediately warned in order to measure the final specific activity (Bq m$^{-3}$). The technician must adequately fill the waste record book of the laboratory and send the flask to the deposit of radiolabeled materials.

Any leftover of the radiolabeled work solution must be transferred to the glass flasks, with a spigot, screw cap and capacity for 10 mL or more. After identification (product, radioisotope, total activity, specific activity, responsible person and date), these flasks must be stored on the freezer designated for such purposes.

Radioactive waste

All disposed material, such as absorbing paper, tips, gloves, broken glass and TLC plates, must be radioactively monitored with the Geiger-Müller counter after the use and, if
contaminated, it must be stored on a reinforced plastic bag, adequately closed and labeled. Use the radiolabeled waste collector from the laboratory. Otherwise, this material might be disposed as regular waste. At the end of each month, the responsible technician must send the radioactive waste to the deposit, filling out the waste records.

**FINAL CONSIDERATIONS**

There is the need of further disclosure within the scientific community connected to the study of weeds regarding the use of radiolabeled herbicides on absorption, translocation and metabolism studies on plants and the biodegradation, mineralization, leaching and sorption-desorption on the soil according to the Brazilian conditions. On this review, a step-by-step was suggested for the methodologies in order to meet this need, including the management of resulting radioactive waste from the studies conducted in the laboratory. Techniques that use radioisotopes such as tracers are extremely useful to study the dynamics of herbicides on the environment, since the radiometric techniques offer the possibility of accurately determining very small amounts in a relatively short time.

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