# INTEGRATING BIOAVAILABILITY MEASUREMENTS IN PERSISTENCE ASSESSMENTS OF PARTIALLY-BIODEGRADABLE ORGANIC CHEMICALS IN SOIL

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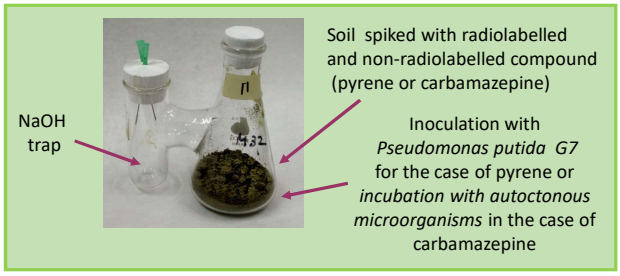
## OBJETIVES

The integration of bioavailability assessments in standardized procedures for monitoring the biological transformation of organic chemicals in soil would help to explain biodegradation rates, leading to more realistic assessments of persistence and risk, especially with partial transformation reactions. We prospected this integration through a two-steps procedure: the first one was to follow the biodegradation (by the OECD 307 guideline) of 14C-labelled chemicals (pyrene –a PAH- and carbamazepine-a pharmaceutical compound) under solid-phase conditions leading to partial transformation products. The second step was to assess bioavailability of the parent chemicals and the metabolites at different stages of this incubation, using a standardized method (ISO 16751:2020) or a adaptation of it, with soils samples at the start and the end of the incubation. For the case or pyrene we have previous studies about the cometabolism, but for the carbamazepine we needed to start with the adaptation and optimization of the methodology that we often use.

## MATERIALS AND METHODS

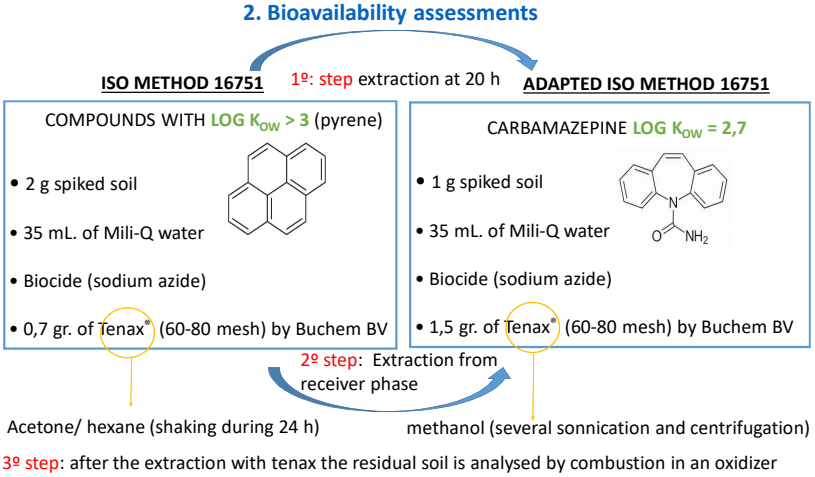
### 1. Approach to study mineralization and biodegradation

This experiment of mineralization and biodegradation was carried out following the OECD 307 method. The soil moisture was adjusted with sterilized water to 40 % WHC before inoculation.




The soil used in this study, for both cases (pyrene and carbamazepine) is an agricultural soil with these physicochemical properties: pH 8.44; 0.44% total organic carbon (TOC); 0.75% organic matter; 0.046% organic nitrogen (Kjeldahl); 8.0 mg $kg^{-1}$ Olsen phosphorus; and 122 mg $kg^{-1}$ available potassium; the particle size distribution was 71.6% coarse-grained sand, 6.9% fine-grained sand, 10.6% silt, and 10.8% clay.

### 2. Bioavailability assessments




A standardized method (ISO 16751:2020), based on Tenax extraction at 20 hours was used to measure bioavailability at initial and final times of these mineralization and biodegradation experiments**.** In this method the potential bioavailable fraction is the amount of contaminant present in the matrix that can be released from the solid phase to the aqueous phase in a well-mixed water soil mixture and in presence of a receiving phase (Tenax). This method is described for compounds with LOG KOW > 3 (pyrene) for this reason this method need to be adapted for compounds like the carbamazepine with LOG KOW = 2,7

## RESULTS

### PYRENE

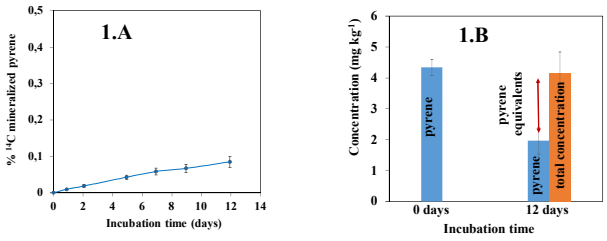



**Figure 1:** (**A**) Mineralization by *P. putida* G7 of $^{14}$C-pyrene added to a sterelized soil. Mineralization was less than 0,2 %, indicating a cometabolic transformation. (**B**) Initial and final concentrations of pyrene and the pyrene equivalents (transformation products). The biodegradation in this experiment was 47,3 %. With the analysis of soil at final time by combustión in a oxidazer, we can asume that pyrene has been transformed to metabolites in a 52,65 %.

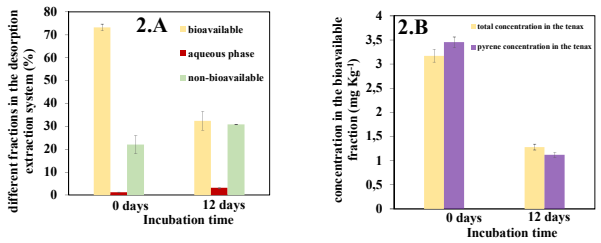



**Figure 2**: **(A)** Determination of the phase distribution in our system of the 14C-labelled parent compound and metabolites among soil, water and Tenax. In the case of pyrene, the cometabolic transformation carried out by the microbial inoculum in the soil accounted for 52,65 % of the initial concentration (4 mg $kg^{-1}$), from which only 3 % were present as hydrophilic transformation products that were not trapped by Tenax but partitioned into the water. The rest remained as non-bioavailable residues. However, the bioavailable pyrene fraction in soil passed well to the Tenax and decreased as long as cometabolism proceeded. **(B)** It can be also corroborated with the combined use of liquid scintillation and HPLC fractionation and we can assumed that only the pyrene is present in the tenax and not the metabolites formed in the process of cometabolism. **These results indicate that cometabolism decreased efficiently the risks from pyrene in the soil.**

### CARBAMAZEPINE

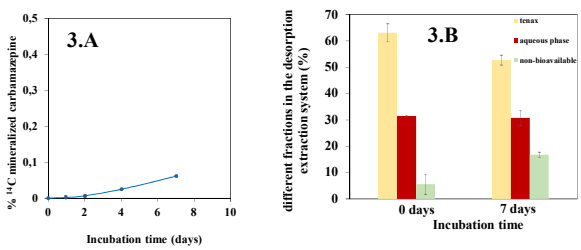



**Figure 3:** (**A**) Mineralization of $^{14}$C-carbamazepine added to a non-sterelized and non inoculated soil. (**B**) Determination of the phase distribution in this system of the 14C-labelled compound among soil, water and Tenax. In the case of carbamazepine the bioavailable fraction will be the sum of water fraction and tenax fraction. The mass balance in this system is almost 100 %. These are preliminary date because to observed the possible production of metabolites in this system, more incubation time is necessary. The finally results will presented in Copenhagen.

## CONCLUSIONS

*** The results obtained with pyrene in this integration of the bioavailability assessment in the OECD 307 simulation test, showed very good effectiveness of Tenax-based ISO method and possible ways of optimization in the presence of polar metabolites are prospected.**

*** The metabolites formed by cometabolism are hydrophilic compounds that remain in the aqueous phase and not in the Tenax, what facilitates their discrimination during the assessment.**

*** The results could be applicable to other non-polar compounds, such as pharmaceuticals, which are usually transformed partially as a result of their biological processing in soil.**

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