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Apparatus for Monitoring the Mineralization of Volatile ^{14}C-Labeled Compounds†

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Quantitative mineralization studies on radiolabeled compounds having high vapor pressures need to cope with several technical difficulties. An incubation and trapping system is described that was successfully used in mineralization studies on highly volatile trichlorobenzenes and other xenobiotic pollutants.

The measurement of $^{14}CO_2$ evolution from radiolabeled compounds incubated in aqueous or soil systems is a sensitive and popular technique for the prediction of the environmental fate of xenobiotic pollutants. Since alkali drastically lowers the efficiency and accuracy of scintillation counting, $^{14}CO_2$ is usually trapped using quaternary ammonium bases. Goswami and Koch (1) have described a system in which $^{14}CO_2$ in the headspace gas of incubation vessels is trapped by flushing it through a phenethylamine-containing scintillation cocktail, thus allowing the direct counting of the trapped radioactivity. Difficulties are encountered, however, if the parent compound is volatile. In such cases, radioactivity in the phenethylamine cocktail may represent undegraded parent compound instead of $^{14}CO_2$. In addition to this error, radiolabeled parent compound may be lost from the system, leading to poor recovery budgets and ambiguous test results. Such problems are compounded by slow biodegradation rates or by an affinity of the test compound for rubber and certain plastic components of the incubation-trapping assembly, or by both. The use of a gas chromatograph connected to a proportional flow radioactivity counter (4) is an alternative approach for separating $^{14}CO_2$ from other labeled volatiles, but this technique requires an expensive apparatus and a relatively high specific activity of the compounds in question.

A quite extreme case of the described experimental difficulties was encountered in our attempts to measure the mineralization of 1,2,3- and 1,2,4-trichlorobenzenes in soil. The apparatus to be described was developed to cope with these difficulties. In addition to its use in the trichlorobenzene study (3), it was used in mineralization studies on organophosphates (2) and Kepone (U. George and R. Bartha, unpublished data) and should facilitate the testing of other similar compounds.

Because of the high affinity of the trichlorobenzenes for rubber and tygon, the contact parts of the incubation and trapping system were constructed of glass, stainless steel, and Teflon only. The incubation flask shown in Fig. 1 consisted of a 125-ml micro-Fernbach flask (Bellco, Vineland, N.J.) closed with a Teflon-lined screw cap. The screw cap was drilled, and two 16-gauge syringe needles were inserted through the Teflon lining. The syringe needles were secured to the screw cap with epoxy cement and, when not in use for flushing, were closed with 0-size polyvinylchloride stoppers (Caplugs, Protective Closures Inc., Buffalo, N.Y.). The incubation vessel was connected to the trapping unit with Teflon tubing. All flushing was done with negative pressure, using a small aquarium vacuum pump to prevent loss of unscrubbed flask atmosphere in case of a small undetected leak.

Leakage studies from soil-free flasks showed that the Teflon-lined caps retained 90 to 95% of the added trichlorobenzene after 1 week. An aluminum screw-cap liner was also tested and yielded results similar to those with the Teflon liner, but was not routinely used because of its lack of durability. A rubber-lined cap was included in this study and showed only a 1% trichlorobenzene retention after 1 week.

After the flask was charged with the appropriate soil mixture and other additives, the radiolabeled volatile test compound was introduced by a microliter syringe inserted through the shorter 16-gauge needle. After an appropriate incubation period, which should take into account not only the prospective mineralization rate of the test compound but also the background respiration rate of the soil sample (5), each flask was flushed with air or another ap-

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propriate gas mixture for 5 min. This time period allowed 7 to 10 flask volumes to pass through the incubation vessel. Assuming complete mixing, approximately 99% of the original gas in the flask is replaced.

The headspace gas from incubation vessels, containing radiolabeled parent compound vapors and $^{14}CO_2$, was scrubbed in the unit illustrated in Fig. 2. Connection between the incubation flask and scrubbing unit was made with Teflon spaghetti tubing. The volatile parent compound and its degradation products other than CO_2 were trapped in units A_1 and A_2, containing toluene- or xylene-based universal scintillation cocktail. In the case of trichlorobenzenes, trapping efficiency in each unit was over 99%, and only the contents of A_1 were routinely counted, A_2 serving only as a backup trap. $^{14}CO_2$ was then trapped in units O_1 and O_2 containing phenethylamine. Trapping efficiency was checked by evolving $^{14}CO_2$ in incubation flasks from acidified $Na_2{}^{14}CO_2$ before flushing. The results showed that 90 to 95% of the $^{14}CO_2$ passing through the phenethylamine scrubbers (O_1 or O_2) was removed by each vial. Both $^{14}CO_2$ trapping units were routinely counted, achieving an overall 99% recovery of $^{14}CO_2$. Units T_1 and T_2 were kept empty as an insurance against loss or mixing of trap contents by back pressure. All trapping units consisted of 24-mm-diameter glass scintillation vials. Vial caps and stainless-steel tubing connections were permanently mounted on a common bracket by epoxy cement. Counting vials filled with the appropriate counting fluid were attached or detached by screwing them into the cap threads, thus eliminating any need for fluid transfer between trapping and counting. The dual trapping system permitted an acceptable recovery budget of the originally applied radioactivity, and eliminated the error of measuring volatile parent compound as $^{14}CO_2$.

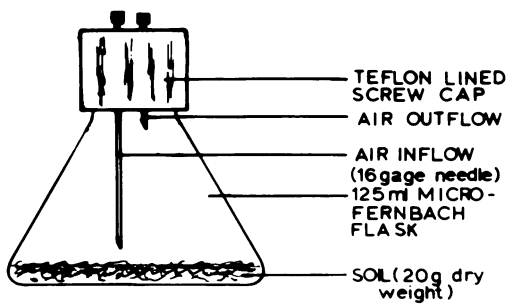

FIG. 1. *Soil incubation vessel.*

Some backflow problems were encountered with this sytem, but these could be eliminated by connecting the system starting at the source of vacuum (trap O_2) and proceeding upstream to the incubation flask. Disconnection should proceed in reverse order, starting at the incubation flask and proceeding through to the vacuum source.

With weekly flushings, the described system was successfully used for 2- to 3-month incubation periods while studying the fate of 1,2,3- and 1,2,4-trichlorobenzenes in soil, allowing the detection of mineralization rates as low as 0.05 nmol/day per 1 g of soil (3).

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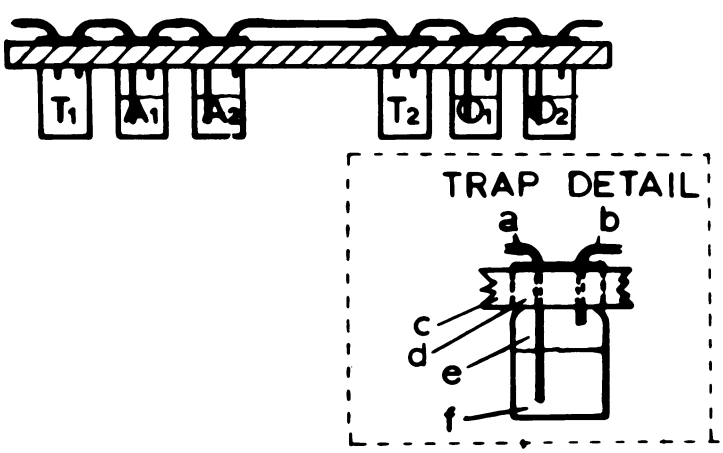

FIG. 2. *Radioactive trichlorobenzene and CO_2 trapping system. T_1 and T_2 are backflow traps. A_1 and A_2 contain a toluene- or xylene-based fluor for trichlorobenzene trapping. O_1 and O_2 contain a phenethylamine fluor for CO_2 trapping. Trap detail: (a) inflow (18-gauge stainless steel); (b) outflow (18-gauge stainless steel); (c) 0.625-in. (ca. 1.6-cm) wood mounting board; (d) epoxy-cemented scintillation vial cap (24 mm); (e) glass scintillation vial; (f) trapping fluor.*

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