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The Use of Radiolabelled Pesticides in R & D

by Dr John B Unsworth

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1.0 Introduction

Pesticides are a significant tool in the production of sufficient food to feed the growing global population. Defending crops against pests and diseases has always been important and probably dates from the beginnings of agriculture about 10,000 years ago in the Fertile Crescent of Mesopotamia (part of present day Iraq, Turkey, Syria and Jordan). Even today it has been estimated that losses due to pests and diseases range from 10-90%, with an average of 35 to 40%, for all potential food and fibre crops.

Today intensive agriculture still requires the use of pesticides to control a wide range of pests and it is essential to understand the fate of these pesticides when applied to crops or soil. Similarly, the degradation of these chemicals can result in the formation of degradation products which may also have properties similar to the parent compound and themselves pose a risk to human health or the environment.

2.0 Regulation

Registration is now a legally binding prerequisite for marketing a pesticide in most countries and over the years has become more stringent and data requirements for registration have become more formalised.

The US EPA state that *"The purpose for conducting metabolism studies is to determine the qualitative metabolic fate of the active ingredient..... To obtain this information, the pesticide is labelled with a radioactive atom"*.

For the successful registration of a pesticide, data are required to demonstrate that there are no significant adverse effects to the environment or human health when a pesticide is used according to the label instructions. Although the first radiolabelled pesticides were synthesised in the 1950s their use in regulatory studies did not become routine until the 1970s. Today, guidelines for obtaining the required data are published by government agencies and in many cases the use of radiolabelled material is considered to be mandatory, for example:

Similarly, for the EU it is stated that for aerobic soil studies *"Results obtained must be presented in the form of schematic drawings showing the pathways involved, and in the form of balance sheets which show the distribution of radio-label as a function of time"*.

3.0 Choosing the Radiolabel

Normally the radiolabel of choice is carbon-14. Tritium has been used, but its use should be avoided as hydrogen-tritium exchange can readily occur between the radiolabelled pesticide and endogenous compounds, thus rendering interpretation of the data difficult. The radiolabel should be positioned in the molecule so that potentially significant toxicological moieties can be tracked, usually this means labelling in a ring system. Many pesticides contain two ring systems

and in this case either both rings should be labelled or each ring should be labelled and used in separate experiments. In this way if the molecule is cleaved to give two separate fragments the fate of each fragment can be ascertained. If more than two ring systems are present then the molecule should be labelled accordingly. The purity of the radiolabelled compound should be at least 95%, preferably greater than 98%, both chemically and radiochemically.

4.0 Detection

To determine the total radioactivity in a given solution liquid scintillation counting is the method of choice and for solid matrices, e.g. soil, combustion techniques converting ^{14}C -labelled material to $^{14}\text{CO}_2$ are used. Chromatographic methods such as gas-chromatography (GLC),

high performance liquid chromatography (HPLC) (Figure 1) and thin layer chromatography (TLC) (Figure 2) can all be used, with appropriate detectors, to determine the number and quantity of radiolabelled compounds in a solution.

GC methods are less used than HPLC or TLC since many radiolabelled compounds found in pesticide studies have high molecular weights and relatively low volatility. This can result in poor chromatography and condensation between the gas-chromatograph and radioactivity detector with a subsequent loss of efficiency and resolution. One way of overcoming these problems is to use a catalytic reactor which is placed at the column exit inside the GC oven, ^{14}C containing compounds are converted to $^{14}\text{CO}_2$ which is quantified with a radioactivity detector. If a mass detector is also used a splitting device is required. HPLC methods are more versatile and can be used for both polar and non-polar compounds. Using flow cells the monitoring of radiolabelled compounds separated by chromatographic techniques is a powerful tool for the quantitation of metabolites and degradation products found in pesticide studies. TLC techniques have the advantage that there is no loss of radiolabelled components due to irreversible absorption to columns, as can occur with HPLC, although in rare cases where volatile components

are present some material may be lost. Originally, in order to achieve good resolution, quantitation of TLC plates was achieved by photographic film autoradiography followed by zonal analysis which was a time consuming process. However, with the current instant imagers and phosphorimagers, separated components in mixtures can be detected quickly by autoradiography without significant loss of resolution enabling *in situ* quantitation of the thin layer chromatograms. Indeed, current technology for TLC can provide higher sensitivity than HPLC detectors. TLC provides a useful technique for determining the radiochemical purity of ^{14}C containing pesticides. In each of the above techniques dedicated software allows sophisticated analysis of the data obtained. After detection and quantitation by chromatographic techniques identification is achieved by co-chromatography with known compounds and subsequent mass-spectroscopy. Film autoradiography is still used in some circumstances, for example, to show the uptake of a radiolabelled pesticide in plants.

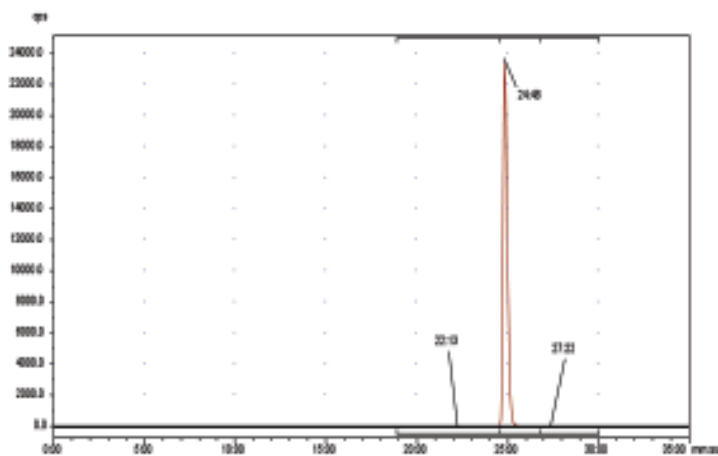


Figure 1. HPLC- ^{14}C Chromatogram of Test Item

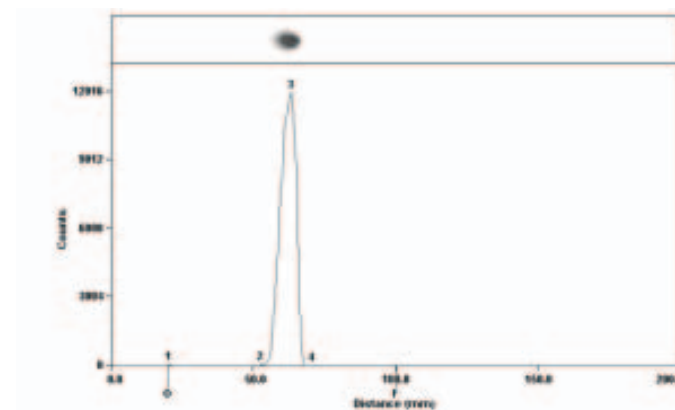


Figure 2. TLC- ^{14}C Chromatogram of Test Item

5.0 Studies with Radiolabelled Pesticides

Studies with radiolabelled pesticides are mainly used in the development phase, specifically for registration purposes, however, these studies can be adapted for the research phase in order to choose between active compounds in a given family, e.g. to determine differences in soil persistence. This latter use, however, is often precluded because of the expense in synthesising the molecules in question.

When pesticides are applied either to crops or directly to soil they are subject to degradation and transport processes which lead to their dissipation in the environment. Thus defining the fate and behaviour of pesticides in the soil environment is a key aspect in the registration procedure. Initial studies include aqueous photolysis and hydrolysis studies which are usefully carried out using radiolabelled pesticides, particularly with lipophilic compounds that are relatively insoluble in water. With low concentrations, often in the sub-ppm range, the high sensitivity for the detection of radiolabelled compounds allows for 100% of the initial concentration (material balance) to be accounted for. Additionally, it allows for individual degradation products to be isolated and subsequently identified to define the degradation pathway. Laboratory or wind tunnel volatilisation studies have also been carried out with radiolabelled pesticides. The data obtained from these studies can be used to give a first indication of the fate of a given pesticide in the environment.

Laboratory soil studies play an important role in the understanding of what will happen to a pesticide under use conditions. Pesticides will reach the soil either by direct application, indirectly during application to the crop or by wash-off after application. Studies that are required include soil surface photodegradation, leaching, aerobic and anaerobic soil metabolism and dissipation in soil/sediment systems. For these studies the conditions used should be as close to those pertaining in the environment as is possible in the laboratory. It is also important that the fate of any significant metabolites or degradation products is also determined. This involves using sophisticated methods for extracting, quantifying and subsequently identifying these products, together with, as far as possible, a material balance. In these studies the use of GLC, HPLC and TLC using radio-detector technique involving ^{14}C can give a good estimate of the concentration of the parent pesticide, its metabolites and its degradation products in soil extracts. Any radiolabelled material remaining in soil, e.g. as bound residues, can be quantified by a combustion technique. These laboratory studies can be supplemented by lysimeter studies using a radiolabelled pesticide. Lysimeters consist of isolated, undisturbed soil cores which are set up in the open so that any leachates can be collected and by using radiolabelled pesticide a mass balance can be attempted. Lysimeters are treated with the pesticide at the normal use rate and, as they are in the open, experience the same environmental



conditions as a field study, hence providing a more realistic means of assessment than laboratory techniques, without the complications and uncertainties of field studies. In this way the decline of the parent pesticide, the formation of metabolites and degradation products and their mobility can be determined, together with uptake by a crop if this has been planted in the lysimeter. Similarly, microcosm and mesocosm studies can be used to mimic the fate of a pesticide in water/sediment systems under more natural conditions. These studies provide data which can be used in environmental risk assessments for regulatory purposes. As part of these assessments pesticides can be characterised as persistent and/or bioaccumulative depending, of course, on their properties and the results of fish bioaccumulation studies.

Plant metabolism studies, which are required on food or feed crops, are conveniently carried out with radiolabelled pesticides. Crops are treated with the pesticide and samples of foliage, fruits and roots are taken and analysed for the parent compound and any metabolites. Plant metabolism studies are usually required for a minimum of three diverse crops. If the metabolism in three diverse crops is similar, then the metabolism in other crops is assumed to be similar. Based on the results, the compounds arising from the pesticide treatment are characterised and this information is used to determine which should be included in the residue definition and analysed in treated crops. By using soil previously treated with a radiolabelled pesticide the uptake of more persistent pesticides or metabolites by rotational crops can be determined. These data are then used to establish realistic crop rotation restrictions and to provide information for determining if tolerances are needed in rotational crops.

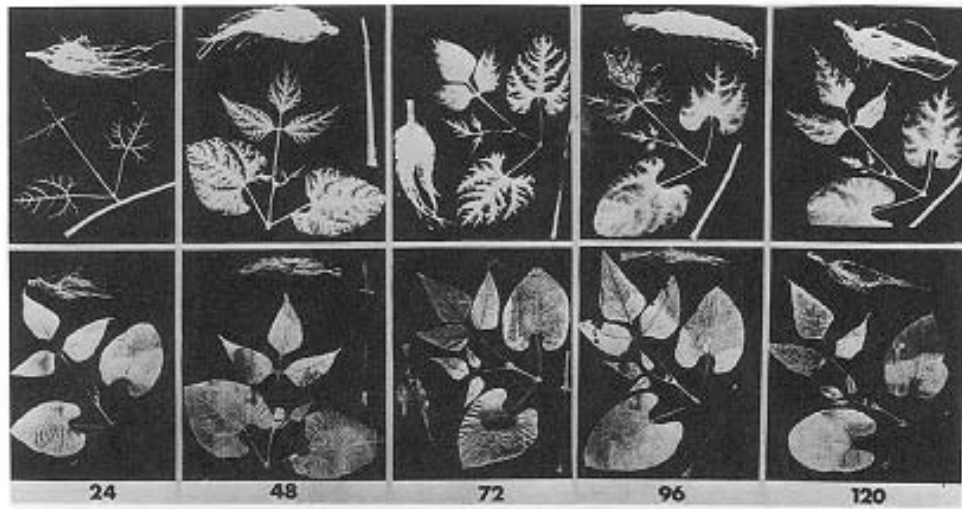


Figure 3. Time course (h) of translocation of ¹⁴C in snapbean plants treated with 4.15x10⁻⁶M in nutrient solution. Radioautograms above; mounted plants below. From Absorption and Translocation of Terbutyn and Fluometuron in Cotton (*Gossypium hirsutum*) and Snapbeans (*Phaseolus vulgaris*). B. Rubin and Y. Eshel, *Weed Science* 25(6) 499-505 (1977). Reproduced with permission of the Weed Science Society of America.

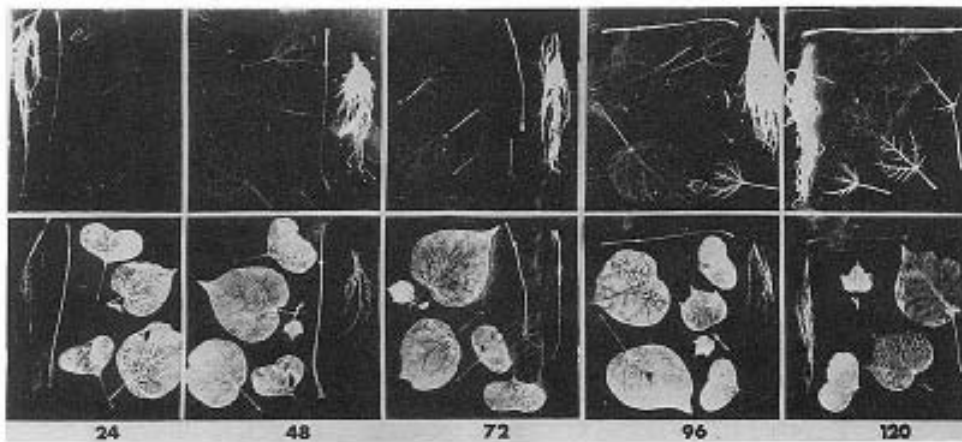


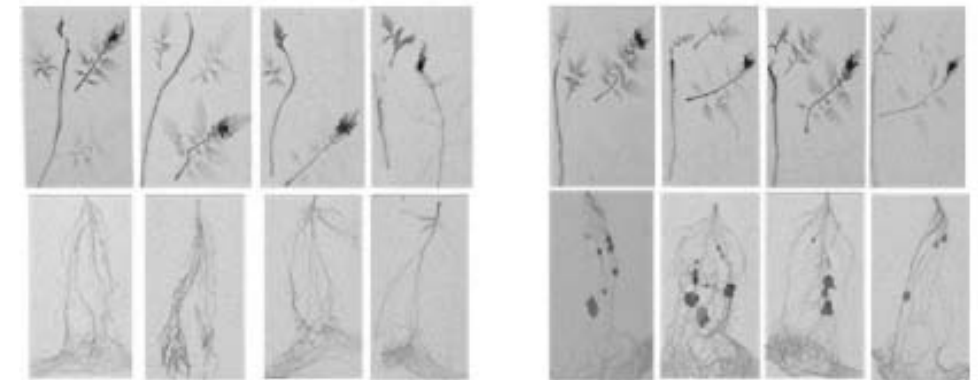
Figure 4. Time course (h) of translocation of ¹⁴C in cotton plants treated with 4.15x10⁻⁶M in nutrient solution. Radioautograms above; mounted plants below. From Absorption and Translocation of Terbutyn and Fluometuron in Cotton (*Gossypium hirsutum*) and Snapbeans (*Phaseolus vulgaris*). B. Rubin and Y. Eshel, *Weed Science* 25(6) 499-505 (1977). Reproduced with permission of the Weed Science Society of America.

Film autoradiography can be used to provide qualitative information on the uptake of a radiolabelled pesticide (Figures 3 and 4), although it is not generally used to provide regulatory data. Similarly, it can also be used in research programmes to determine uptake and

movement of radiolabelled natural compounds. For example, indirect effects of pesticides can be investigated and an interesting application is in the determination of the effect of glyphosate on the translocation of ¹⁴C-sucrose from tomato to the parasitic plant *Orobanche* (Figure 5).

Effect of glyphosphate on ¹⁴C-sucrose movement from tomato treated leaf to Orobanche
1+24 Hours after Treatment

Tomato				Tomato and <i>Orobanche</i>			
+GLY		-GLY		+GLY		-GLY	
R	S	R	S	R	S	R	S



R = glyphosate resistant

S = glyphosate sensitive

Figure 5. Personal Communication, Baruch Rubin, J & R Liss Professor of Agronomy & Weed Science, RH Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Following on from the plant metabolism studies is the development of residue methods for the parent compound and any metabolites included in the residue definition. Whilst not widely practiced residue method development can be carried out more efficiently if radiolabelled compounds are used. At each step of the method, e.g. extraction and clean-up, any losses can be quickly identified by liquid scintillation counting of the various extracts. The nature of residues in a crop may be changed by food processing operations. Crops containing residues are processed and the fate of the residue is determined, if processing results in a change in the nature of the residue a study using radiolabelled material may be required. Livestock (cattle or goats and chickens) metabolism studies are carried out to determine the fate of residues in meat, milk and eggs. After feeding animals with radiolabelled pesticide meat, milk and eggs as appropriate are examined for the parent compound and any metabolites using radiochromatographic techniques. The results allow the development of analytical methods for residues which may be ingested when the various commodities are eaten.

As consumers may ingest residues present in foodstuffs and applicators etc. may be exposed to pesticides it is essential to determine the fate of a given pesticide in mammalian systems. One aspect of this is the absorption, distribution, metabolism and excretion (ADME) of the pesticide in mammalian systems. Studies are carried out with ^{14}C -labelled pesticides in rats, both orally and dermally, and a mass balance is achieved by measuring the radioactivity in expired air, urine, faeces and the various tissues. Using appropriate extraction techniques and the use of GLC, HPLC and TLC, with radio-detector techniques to measure the amount of metabolites, a good estimate of the concentration of the parent pesticide and its metabolites can be obtained. Radiolabelled material has also been used in *in vitro* dermal absorption studies of different formulations using human skin. Combining the information obtained with the data from toxicology studies allows human risk assessment studies to be carried out.

Conclusions

The use of radiolabelled compounds provides a highly sensitive means for the detection and quantitation of pesticides and their metabolites and degradation products in research and development studies. They can be used in

diverse systems and are now mandatory for many regulatory studies. With the use of modern radiodetectors, coupled with chromatographic techniques, low concentrations of compounds can be readily separated and quantified.

About the Author



Dr John Unsworth has been involved in the agrochemical industry for over 35 years covering all aspects of research and development activities. After graduating from London University with a B.Sc. and Ph.D in chemistry his industrial career started at the bench for May & Baker Ltd. in the UK. He subsequently held various technical management positions before becoming Director of Environmental Chemistry for Rhone-Poulenc Agro, based in Lyon, France. Following this came the management of R & D teams in the UK, US and France for Rhone-Poulenc Agro and finally with Aventis CropScience and Bayer CropScience. He has been involved with the International Union of Pure and Applied chemistry (IUPAC) for 15 years and is currently a member of the Subcommittee on Crop Protection Chemistry. He is also a member of the American Chemical Society (ACS). John now works as an independent consultant specialising in the registration of chemicals.



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