Use of Isotopic Tracers in Pesticide and Environmental Contamination Research

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The insecticide DDT was used extensively for a quarter of a century with remarkable benefits in agricultural production and public health. As a consequence, most parts of the world are now contaminated with trace amounts of DDT, a legacy we leave for future generations.

In order to enjoy the benefits of an expanding chemical science and technology, it is necessary to thoroughly evaluate the risks and long-term consequences of our actions on an international basis. We must know the chemical and physical fate of the contaminant in the multiple components of the environment, an area of research admirably suited for isosopic tracers. These points will be illustrated with emphasis on pesticide research and utilization.

Pesticide chemicals are designed to control or kill harmful pests, for example insects, fungi and weeds. There are 200 to 400 pesticides in widespread usage, totalling in amount about one billion or 10^9 kilograms per year. Some are highly toxic to man while others are relatively safe. They are economic poisons that, at the time of application, are intentional environmental contaminants.

The organic pesticides are composed of carbon and hydrogen and sometimes chlorine, phosphorus or sulphur, for which radioisotopes are readily available, and of oxygen or nitrogen, where stable isotopes can be used. The labelled pesticide can be traced in the presence of an existing and chemically identical contaminant with great sensitivity and specificity, and usually by the use of non-destructive analytical or remote detection techniques. Radiocarbon is the tracer of choice for most environmental contamination research.

The era of synthetic organic pesticides, starting with DDT and the herbicide 2,4-D about 1940, coincides with that of rapid advances in radiotracer applications. This is indeed fortunate since isotopic experiments are an essential step in evaluating each new pesticide and in continually reassessing older compounds for safety and most efficient utilization. This research is carried out in all developed nations with important supplementation on local problems or use conditions from investigations in the developing countries. Several slides will help illustrate the sequence of studies for establishing the disposition and fate of pesticides and other environmental contaminants.

Slide 1. This picture shows the application of a herbicide to sugar cane in Brazil. Some of the chemical contacts the sugar cane but most of it contaminates the soil and the agro-ecosystem involved. Prior to using the herbicide on a commercial basis, we need to know what happens to the chemical in the sugar cane and the soil.

Slide 2. This time we see the helicopter application of an insecticide to a forested area. Very little of the chemical ever contacts the small insect larvae in the tops of the trees. The rest contaminates the trees, any streams or lakes in the area, and the forest soil. We must define the environmental fate of such a chemical or it cannot be safely used.

It is clear that very little of the pesticide ever contacts the pest. Pesticide chemicals are generally applied at dosages of 0.2 to 2 kilogram per hectare from one to five or more times per crop season. Less than 0.01% of an insecticide is absorbed or ingested by the pest insect. The remaining amount, more than 99.99%, is an environmental contaminant, a portion of which is a potential residue in food, feed and fibre. Isotopic research is critical in understanding or solving several aspects of the problem.

The isotopic label is introduced into the chemical by synthesis in a commercial or university laboratory or in a national or regional atomic research centre. The most common radioisotopes used are tritium, ^{14}carbon, ^{32}phosphorus, ^{35}sulphur and ^{36}chlorine. Stable isotopes are becoming increasingly important in pesticide research, particularly ^{13}carbon, ^{15}nitrogen and ^{18}oxygen.

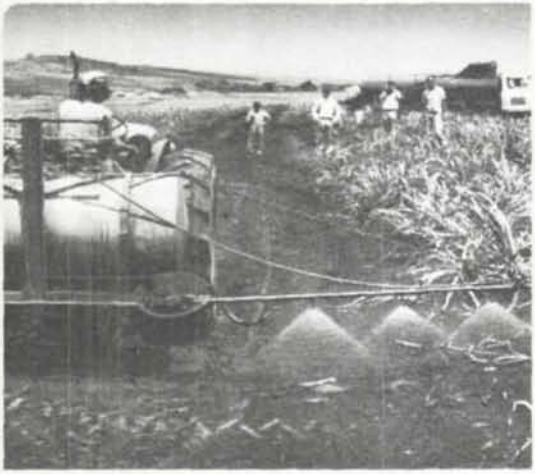
The initial studies usually involve administration of the ^{14}carbon-labelled pesticide to rats, which are then held in metabolism cages that allow separate collection of expired gases, urine and faeces. The products in the excreta are identified by various chromatographic and spectroscopic techniques (**Slide 3**). The persistence of the chemical and its metabolites in various tissues is also determined to make sure that the material is not stored in fat, like DDT, or any other place in the body.

Slide 4. This figure shows an autoradiogram of a thin-layer chromatogram involving separation of the metabolites of a radiolabelled pesticide, carbaryl or Sevin. This compound is radiolabelled at three different positions in the molecule to detect which products retain all three labels and which ones are formed by cleavage of the pesticide. Each labelled product is mixed with various unlabelled standard compounds to see if they chromatograph in an identical manner, that is they cochromatograph, thus providing an important criterion for metabolite identification. This cochromatographic procedure with labelled compounds is used for almost all types of metabolism and photodegradation studies.

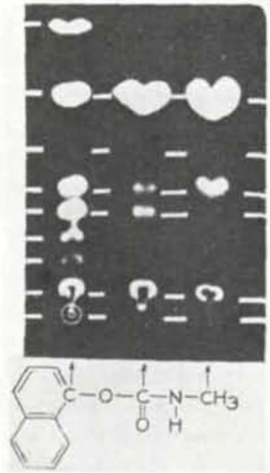
Slide 5. It is important to understand the extent of uptake of a chemical by plants and its distribution within the plants. The remote detection technique is illustrated in this autoradiogram of a herbicide after plant uptake. The autoradiograms on the top can be compared with the soybeans on the bottom to see the localization in the growing tips.

This type of study defines where the chemical is located but not the chemical identity of the labelled products. Supplementation by thin-layer cochromatography studies and other forms of chemical analysis solves this problem.

Slide 6. Detailed studies are necessary on the fate of a new labelled chemical in plants. This can be accomplished by applying the labelled compound to the soil, placing the rice or



Slide 1



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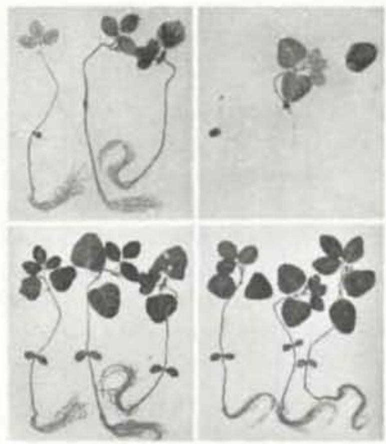

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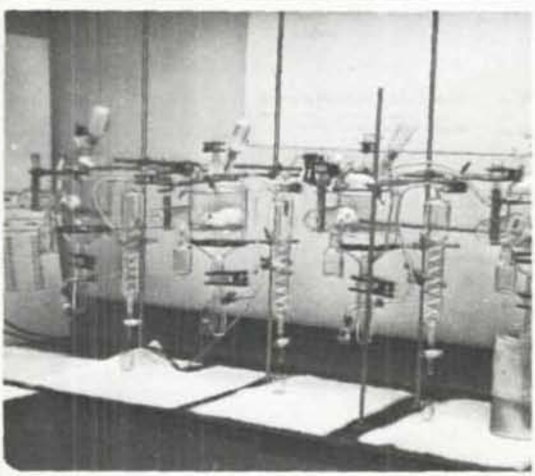

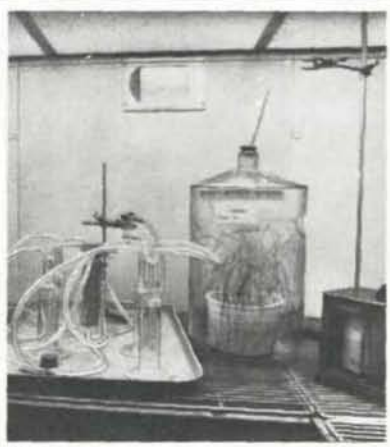

Slide 3

Slide 6

other plants in a suitable metabolism chamber such as the one illustrated, and analysis of the gases as well as the products in the plant and soil. This type of study confirms the autoradiogram approach and extends it to the chemical identification of the products.

Studies of the type discussed define the physical fate of the contaminant under simulated environmental conditions. With suitable design, they establish how the chemical moves within and between air, water and soil. They can be applied to model ecosystems to determine possible accumulation through food chains. They also define the chemical changes that occur within each organism and in each stage of breakdown.

Isotopic tracers provide the most important approach in determining the chemical fate of an environmental contaminant. These studies involve photodecomposition and metabolism and a precise definition of the sequence of products or the degradation pathway for the pesticide. Each pesticide or environmental contaminant usually generates dozens of new products as it decomposes. They vary greatly in toxicity. Where possible all products from metabolism and photodecomposition are identified including both the initial "activation" products which are sometimes formed and the terminal residues. These terminal residues are often chemically identical with natural endogenous materials from which they are easily differentiated because of the isotopic label.

For most efficient utilization of a pesticide, it is necessary to understand its fate in the pest and the response of the pest to the pesticide. The amount of pesticide absorbed and translocated to the site of action, such as a specific region of the insect nerve, is less than 0.1% of the dose taken up by the pest. Much of the rest is detoxified and excreted, sometimes so efficiently that the pest survives. Repeated pesticide use selects the pest population for the resistant individuals, eventually yielding resistant populations of insects and fungi. With herbicides, the susceptibel weed species may practically disappear only to be replaced by other, more tolerant weeds. Pest resistance requires increased treatment levels and eventually leads to collapse of control programmes. DDT resistance involving the detoxifying removal of hydrogen chloride from the molecule is only one of many possible examples. Thus, pesticide applications are often only temporary solutions to pest problems, lasting for only a few seasons or years unless great care is taken in the amount and timing of application.

Isotopic techniques are important in almost all areas of pesticide research. They help to optimize for selective toxicity, suitable persistence and optimal delivery. They aid in understanding the fate and action in the pest. They also help define the physical and chemical fate of the environmental contaminants. These studies must be made to meet national and international requirements on pesticide registration and use. Thus, isotopic tracer research is a critical feature in establishing the use-safety of pesticide chemicals.

In the final stages of pesticide research, the studies are extended from laboratory plants to field crops, from model ecosystems to environmental conditions, and form laboratory animals to larger mammals or even man. In these studies the isotopic techniques are generally replaced by conventional chemical methods of analysis. Never again will the DDT case of intolerable persistence, environmental pollution, and biomagnification be repeated, thanks in part to isotopic tracer research.

Important information is also obtained by the use of labelled enzyme substrates and other biochemicals rather than a labelled pesticide. These studies on disruptions in normal metabolic pathways help define the mode of action of the toxicant, knowledge which often leads to improved pesticides. They also assist in understanding deleterious biochemical lesions in mammals in order to define scientifically, and in appropriate policies or laws, the conditions for safe pesticide use.

In summary, the discovery of radioactivity by Becquerel in 1895 and the first use of radioactive tracers by Hevesy in 1923 laid the background for many developments important in expanding food production and improving public health. Radiotracer experiments were an important aspect in developing the current pesticide chemicals and in defining conditions for their safe and efficient use. Further isotopic research may lead to even safer and more effective pesticides for the future.