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CHINA NUCLEAR SCIENCE AND TECHNOLOGY REPORT

放射性同位素示踪技术及其在中国农药科学中的应用研究

STUDIES OF RADIOISOTOPE TRACER TECHNIQUE AND ITS APPLICATIONS TO PESTICIDE SCIENCES IN CHINA

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放射性同位素示踪技术 及其在中国农药科学中的应用研究*

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摘　要

化学农药的不合理使用造成了严重的环境问题和食品污染，影响生态平衡，危害人类健康。在中国，越来越多的科学家正在利用核技术研究农药学问题，仅中国农业科学院原子能利用研究所同位素应用研究室就已合成了80余种农用标记化合物，包括杀虫剂、杀菌剂、杀螨剂、除草剂、化肥和生物试剂等。在过去的几年里，放射性同位素示踪技术及其在中国农药科学中的应用研究已取得了巨大的成就，特别是在放射性同位素化学标记合成技术、利用示踪手段研究在动植物中的农药残留、降解和代谢、在环境中的行为和归宿以及农药安全使用技术等方面均有较大进展。文中简要介绍了在过去几年中，同位素示踪技术及其在中国农药科学中的应用研究进展，提出了存在的问题，并对其未来的发展予以展望。

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Studies of Radioisotope Tracer Technique and Its Applications to Pesticide Sciences in China*

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ABSTRACT

The improper use of chemical pesticides has resulted in serious environmental problems and food pollutions, affecting the ecosystem balance and human being health. There are more and more scientists and research institutions being engaged in the area of radioisotope tracer techniques for pesticide sciences in China. So far, more than 80 labeled compounds, including insecticides, fungicides, acaricides, herbicides, metabolic intermediates, fertilizer and biological agents, etc. have been synthesized at the laboratory for application of isotopes in Institute for Application of Atomic Energy, Chinese Academy of Agricultural Sciences. Over past several years, the great achievements have been made in the researches of radioisotope tracer techniques and their applications to pesticide sciences in China, especially in the researches for isotopic labeling, residues, degradation and metabolism of pesticides in plant and animal, behavior and fate of pesticides in environment, and techniques for safe application of pesticide, and so on. The researches of radioisotope tracer techniques and their applications to pesticide sciences in China in the past years are briefly introduced. Some problems are put forward and the development in future is predicted also.

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The use of large quantity of chemical pesticide in China played an important role in the control of insect, disease and weed in agriculture and led to growth of food output. Meanwhile, this also resulted in serious environmental problems and food pollutions, affecting the ecosystem balance and human being health. For this reason, the researches related to environmental behavior, especially to metabolism and residue of pesticide have been encouraged and supported by government and become topical subjects recently. Many concerning projects are in progress by the various advanced approaches, of which radioisotope tracer techniques are known to be one of the most effective means and employed by more and more scientists. Over past several years the great achievements have been made in the researches of radioisotope tracer techniques and their applications to pesticide sciences in China, especially in the researches for isotopic labeling, residues, degradation and metabolism of pesticides in plant and animal, and behavior and fate of pesticides in environment. The techniques for safe application of pesticide have been adopted.

1 HISTORY IN BRIEF

Radioisotope tracer techniques in pesticide sciences were initiated in the 1960s in China with Professor Xu Guanren, former Director General of the Institute For Application of Atomic Energy (IAAE), Chinese Academy of Agricultural Sciences (CAAS) in the lead. The main scopes of research field involved the techniques of radioisotopic labeling and their practices in the studies for pesticide residue, degradation and metabolism at the beginning, and then for pesticide toxicology and pharmacology and so on. In the early 1980s, when reform and open policies were established in China, the first group of Chinese scientists were sent to International Atomic Energy Agency (IAEA) and other developed countries. The new technology of isotopic labeling and the radioisotope tracer application to pesticide sciences was studied and introduced to china. In recent years, a research network of radioisotope tracer techniques for pesticide sciences, involving institutes, universities and experimental stations or centers, have been established in China. An enormous quantity of articles for pesticide researches by use of nuclear techniques were published in Acta Agricultural Nucleatae Sinica, Acta Environmental Sciences, J. Environmental Chemistry and J. Pesticide Science and Administration and so on. 60 ~70 per cent of them are relative to radioisotope tracer techniques for pesticide residue and metabolic researches. For the past three decades, radioisotope tracer techniques and their applications to pesticide sciences were developed as important research area in China [1,2]

Today, more scientists and research institutions than ever before are engaged in the scope of radioisotope tracer techniques for pesticide sciences in most of provinces in China. Under the supervision of Professor Wen Xianfang, IAAE has become the center of the researches on Nuclear-Agricultural Sciences in the whole country. A laboratory for studies of isotopic labeling and radio-tracer applications with advanced facilities and skilled technicians were built up in the IAAE. The basis for the chemical synthesis of labeled compounds and applications of radio-tracers to pesticide sciences have been founded. The first international technology cooperation program for pesticide research by use of nuclear techniques all over the country supported by IAEA is in progress at IAAE. Up to now, many problems in both theory and practice have been verified and resolved with remarkable economic benefit. Before industrial manufacture and registration of a new pesticide, it's necessary to assess its environmental effect by radioisotope techniques so as to estimate the potential harm of the pesticide to eco-environment and human being health. According to the tracing experimental findings resulted from investigating the movement and fate of pesticides, and tracing the pathway and residue of the poisonous agro-chemicals, the rules on proper and safe use of pesticides have been formulated with significant scientific and social impacts. The further studies are ongoing.

2 ESSENTIAL APPROACHES AND FEATURES

2.1 Exact and Quick Measurement of Distributions and Original Residues of Pesticide

Tracing function of radioisotopes labeled into pesticide molecules make it exact and quick that the distributions and original residues of pesticides in biological samples can be detected and measured under the undisturbed condition.

2.2 More Direct Study Through Vision

The studies of pesticide, especially the studies in pharmacology and toxicology of pesticide, can be conducted more directly through vision by radioautographic techniques.

2.3 Pathway and Fate of Pesticide Residues in Environment and Food-Chain

By using radiotracers, the pathway and fate of pesticide and its contamination to environment and accumulation of pesticide residue in food chain among different living things can be detected conveniently. The loss originated from pesticide residues during food processing can also be determined systematically.

2.4 More Simple and Accurate Analysis

The recoveries of pesticide residues from the sample by extraction can be detected conventionally and verified by isotopic tracer techniques without interference of co-extracted materials in sample matrix through a simple sample preparation. Combined with chromatographic analysis, radioisotope tracer techniques can not only detect the parent pesticides, but also detect accurately their degradate and metabolite in samples, especially bondresidues existing in soils and plants that can't be detected by other approaches.

3 STUDIES ON PREPARATION OF RADIOISOTOPIC LABELED PESTICIDES

Table 1 Catalogue of labeled pesticides

Labeled pesticides	Specific activity mCi/mmol	Labeled pesticides	Specific activity mCi/mmol
(^{14}C) Benzene hexachloride	1~5	(^{14}C) 2, 2, 2-Trichloroethyl styrene	1~5
(^{14}C) Methylene chlordimeform	1~5	(^{14}C) Amino-1, 3, 4-thiadiazole	10~30
(^{14}C) Dicyandiaonide	1~10	(^{14}C) N, N-Methylene bis (2-amino-	
(^{14}C) 2.4-D	5~10	1.3.4-thiadiazole	10~30
(^{14}C) X-naphthaleneacetic aid	10~20	(^{14}C) Indolyl acetous acid	10~20
(^{14}C) Amidino thiourea	10~20	(^{14}C) Thiophanate methyl	1~10
(^{14}C) S-triazine	1~10	(^{14}C) Trichlorfon	5~10
(^{14}C) Cydophosphamide	1~10	(^{14}C) 2, 3-Bis (p-chlorophenyl)	
(^{14}C) N- (3, 5-dichlorophenyl) -succimide	1~5	1, 1, 1-trichloroethane DDT	1~5
(^{14}C) Ethoxy-phozim	10~30	(^{14}C) N- (phosphonomethyl) glycume	10~30
(^{14}C) 1, 2-dimethyl-3, 5-diphonyl		(^{14}C) Triazol-Friadimefon	1~10
pyrazolium methyl sulfate	5~10	(^{14}C) Primicarb	1~10
(^{14}C) Paclobutrazol	1~5	(^{14}C) Carbofuran	1~5
(^{14}C) Amitraz	1~10	(^{14}C) trifluormethyl-Trifluralin	1~10
(^{14}C) Carbonyl-Chlorturon	1~10	(^{14}C) carboxyl-propoxur	1~10
(^{14}C) methyl-ISP	1~10	(^{14}C) Methoxy-Tetrachlorvinpho	1~10
(^{14}C) Chlorsulfuron	1~10	(^{14}C) Bensulfuron	1~10
(^{35}S) Rogor	1~10	(^{35}S) Thiram	1~10
(^{35}S) Parathion	1~5	(^{35}S) Zineo	1~5
(^{35}S) Thimet	1~5	(^{35}S) Phenthoate	0.2~2
(^{35}S) Nereistoxin	1~5	(^{35}S) Mencoxeb	1~10
(^{35}S) Thiocyclam	1~10	(^{35}S) Cyolane	1~10
(^{35}S) 0, 0-Diethy-S-1, 2-dicardoeth		(^{35}S) 2-Dimethylamino-1, 3-bis (car-	
oxyethyl dithiophosphae	1~10	bsmoylthio) propane hydrochloride	1~5

* 1 Ci=3.7×10^{10} Bq.

To study pesticide by using radioisotope tracer techniques, it's necessary to prepare labeled compounds at first. So far, more than 80 labeled compounds, including insecticide, fungicide, acaricide, herbicide, metabolic intermediate, and biological agent, etc. have been synthesized at the laboratory for applications of isotopes in IAAE to meet the needs of various researches in pesticide sciences. A series of labeled pesticides with organic phosphorus, organic sulfur, organic nitrogen, organic chloride, organic arsenic, and organic mercury, and so on are listed in Table 1.

The methodology for preparations of labeled pesticides is actually the development of techniques for the synthesis organic compound. When synthesize a pesticide, one/several element (s) in special location (s) of the pesticide molecules can be taken the place by it/their radioisotope (s) through some chemical approaches. Attentions to radioisotopic labeling are focused as follows:

3.1 Choice of Radioisotopes for Labeling Pesticide Molecule

At first, the choice of radioisotopes depends on the chemical structure and element composition of labeled pesticide molecule and second on the synthesis routes, testing aim, means and safety. There are many radioisotopes including ^{14}C, ^{32}P, ^{35}S, ^{35}Cl, ^{74}As, ^{75}As, ^{208}Hg and ^{3}H being used for labeling pesticide molecules.

^{14}C is used as the most popular isotope for labeling pesticide on the following accounts: (1) the proportion of C atom is the largest in whole pesticide molecule and ^{14}C intermediate of pesticide is relative easy to prepare; (2) ^{14}C is β-ray radioisotope, so that shielding from radiation, cleaning of the contaminitions and treatment of sample are more simple; (3) the half time of ^{14}C is long enough without decay calibrations and the efficiency of detection for ^{14}C is more than 80%; (4) detection equipments have been popularized.

Compared with ^{14}C, ^{3}H has the same advantages except for its lower energy and character easier to exchange, and thus resulting in indefinite detection. ^{32}P and ^{35}S, with a short half time and need for decay calibrations, are only used to label such pesticides as those containing P and S elements in molecules, for example, the organic phosphorus pesticides, etc.

3.2 The Labeling Location of Pesticide Molecule

It's very important to choose the labeling site in studies of pesticides. If the labeling location had been chosen improperly, the labeling isotopic atom might escaped from the body of pesticide molecule through decomposition by degradaton, metabolisms and exchange reactions, and the experimental results would deviate from real value, even got a reverse conclusion. There were contradictious results in earlier studies siuce improper choices of the labeling site. In the studies for the metabolism of ^{14}C-carbonyl carbofuran in the body of cow, for example, high rate of radioactivity was found in the milk. The further researches showed that ^{14}C-carbonyl carbofuran was easily decomposed to $^{14}CO_2$ in the body of cow, and then was removed out of the cow body in the form of $^{14}CO_3^{2-}$ to milk. This case indicated that the labeling location should be chosen at a proper part of pesticide molecule so that the labelling isotope can trace the whole process of metabolism of pesticide molecule in the living body and environment in each step of experiments. On the other hand, the choice of labeling location also depends upon the research purpose. In the case of study of the total amount of pesticide residues, the labeling site is proper at the most stable group of the pesticide molecule, in the study of the fate for a certain metabolite of pesticide, the group of metabolite in the pesticide molecule should be labeled. For example, the methoxy group was labeled in the studies of demethylation of malathion, the labeling site was chosen at sulfur atom in its oxidization and hydrolysis studies and the total amount of malathion residue was determined through labeling phosphorus atom of the pesticide. Moreover, some complex pesticides in molecular structure such as pyrethroid insecticide were labeled simultaneously at two or more sites in the molecule with same or different isotopes because only one labeled site was not enough for the tracing researches. However, the difficulty and expense for labeling process should not be neglected.

3.3 Specific Activity and Radiochemical Purity

The requirement for specific activity depends on the purpose and method of the research. Over-low specific activity affects the detection sensitivity and the over-high one leads to leave over. Therefore, it is important to choose the optimum specific activity in accordance with precision, accuracy and detection limit of the method so as to decide the input amount of radioactivity in synthesis of labeling. In addition, the content of radioactive impurities affects the experimental results. For this reason, it's necessary to purify the labeled compounds by TLC technique and other available methods and to determine their purity and to assure the radioactive impurity less than 5%.

4 APPLICATION OF LABELED PESTICIDE

4.1 Studies of Residue, Metabolism and Safe Use of Pesticide in Plants and Animals

4.1.1 Original Residue in Crops after Pesticide Application

In the study of original residue in crops after pesticide application, the use of labeled pesticide greatly simplified the complex procedures of extraction and purification in the analysis: through sampling in different stages after application of pesticide and determining the radioactivity in the samples, the amount of original residue, the dynamic curve and decomposing rate of the pesticide in crops are calculated according to the specific activity and application rate of the labeled pesticide. The pesticide residual criteria are formulated on the basis of experimental results.

4.1.2 Absorption, Transfer and Distrbution of Pesticide in Plants and Animals

Experiment of radioautography showed that the physical absorption was dominative in absorption of pesticide on crop surface and that some of pesticides in internal tissues of crops were transferred to other parts of the crops. In the studies of absorption, transfer and residual dynamics for ^{14}C-labeled isofenphos-methyl in peanut and soybean and for a ^{32}S-labeled insecticide of nereistoxin in rice, Chinese scientist [3,4] have found that isofenphos-methyl could be transfered two-dimensionally in phloem and xylem of peanut and soybean and the nereistoxin insecticide was absorbed strongly by the internal tissues of rice and transfered quickly to other parts of plant through roots and leaves of the crop. The results of many researches showed that there were great differences in the characteristics of absorption and transfer of pesticide in various formulations. In addition, the circulation and accumulation of pesticide residue in food chain for different animals were also studied by using labeled pesticide as an efficient means. These studies indicated that, it is significant to improve the formulations and processing forms of pesticide to get better efficiency of pesticide applications and the pollution control.

4.1.3 Metabolisms and Degradations of Pesticides in Plants and Animals [5]

Some of phosphate ester analogues of organic phosphorus insecticides, dechloride products of organic chloride insecticides, hydrolysis products of carbamate pesticides and oxidation products of sulfonylurea herbicide, and so on in plants and/or animals were studied by means of labeled compounds. The results [4] from the studies on metabolism and degradation of ^{14}C-triazol triadimefon indicated that triadimefon in cucumber plant was changed into triadimenol and two uncertain products by radioisotope tracer techniques and thin layer chromatography (TLC).

4.2 Studies of Behavior and Fate of Pesticides in Environment [6,7]

Pesticide residue retained in the environment affects ecosystem by transferring, leaching, dissipating and volatilizing or in other ways. Absorption and desorption of ^{14}C-labeled chlorsulfuron in 8 sorts of soils in China had been studied by using radio-tracer techniques [6]. The certain influence of the soil pH value on absorption was observed, but there was not obvious influence of organic substance content in soils. The experiments also showed that the most absorption rate of

19%~23% was in allic soil of Jilin and yellow fluvo-aquic soil. Chlorsulfuron absorbed in the soils could be desorbed through leaching water. Moreover, the fate of some pesticides such as primicarb and so on in a plant-soil microcosm was also studied by using radio-tracer techniques combined with HPLC and TLC scanner.

Over past several years, pesticide residues bound with some components of soil, plant and seed, i. e. bound residues, not being extracted with solvents, has been concerned by Chinese scientists. The bound residues would not only affect non-target animals and plants, but also be detrimental to human health through food chain. Therefore, it is important to study the dynamics of bound residues in eco-environment. The use of radioisotope-labeled compounds is only available method for quantitative analysis of bound residues [7]. Dynamics and bioavailability for bound residue of ^{14}C-trifluralin and ^{14}C-chlorsulfuron in paddy and black soil were investigated respectively [8,9]. The bound residues of ^{14}C-trifluralin in yellow-brown earth with soybean were 0.24 mg/kg (about 1.18% of applied dose) and could be uptaken by step-plants, e. g. wheat and rye-grass. The bound residues of ^{14}C-chlorsulfuron in soils would increase with the incubation time and were affected remarkably by the organic substances and humidity in soil. The bound residues could also be absorbed by step-crop (rice) and transfered to the shoots of the crop.

Recently, model ecosystem box has been developed and applied successfully in China, which provided more convenient means for exploiting radioisotope tracer techniques to study the behavior of pesticide in eco-environments [10]. The behaviors of sulfonylurea herbicides such as metsulfuron etc. at specific agricultural eco-environments in China is being studied by a research group using nuclear techniques and immunochemical methods combined with an advanced model ecosystem box. This project was supported by the National Natural Science Foundation of China. Some interesting researches are well under way.

4.3 Application of Labeled Compounds to Formulation of Pesticides

The formulations and processing of some pesticides were studied by using radioisotopic tracing techniques. In the process of pesticide formulation, labeled pesticide was mixed with certain polymer as matrix material which could bind pesticide to its molecules, and the radio-tracing atoms could show the release rate of pesticide from the matrix after application of pesticide. In this way, release-controlled formulations of pesticides, which can control the release rates of pesticides in the formulations, have been developed successfully through special physical or chemical process. The relative slow release rate of pesticide was enough for pest control and the pesticide residue, however, was lower than that of the routine treatments. As a result, the use of pesticides was more proper and safevfor control of pests and with a great decrease of environmental pollution.

5 PROBLEMS AND DEVELOPMENT IN FUTURE

5.1 Problems

In the research area of radioisotope tracer technique and its applications to pesticide sciences, the great progress has been made in China. However, there are still some problems in it. For example, the techniques are not well understood and exploited by some researchers being engaged in pesticide research because worry too much about the harm of radioactivity and the extreme expense of radioisotopes. Some of them would rather use improper methods in their researches than try to use radioisotope tracer technique. The studies of some urgent projects in pesticide science are limited due to the shortage in funds. The export of radio-isotopic labeled compounds is very difficult because of less of international exchange. In spite of the above problems, the research area is optimistic in future.

5.2 Development in Future

In the future, radioisotope tracer technique and its applications to pesticide sciences will be developed further. The joint division of FAO/IAEA has listed the programs in the plan of research projects in 1995～2000 [11], including studies by using nuclear techniques on decomposition, fate and transfer of pesticide in local environment; new formulation of pesticide; quality control of pesticide in commercial manufactures and enhancement of monitoring level of pesticide residue in environment. Chinese government has focus its attention on the development of new pesticides and regarded this research area as an important direction in the Ninth Five-Year Plan. To realize the aim, it's essential to exploit radioisotope tracing technique in basic studies of the new pesticide. This will facilitate the development and application of nuclear techniques in the pesticide studies.

Some development directions in future will be emphasized on as follows:

5.2.1 Development of Theoretical Research and Extension of Applicaton Scope

Many problems in the application of the present pesticides need to be solved urgently. At the same time, however, new pesticides will be bound to lead to the new problems in both theory and practice. In the further researches for these problems, rodioisotope tracing technique can provide an essential means, especially in some basic theoretical research. All of such studies as the resistance to pesticide dealt with molecular ecotoxicology; the residue, degradation and metabolism involving envronmental toxicology, the mechanism of plant pharmacology of a new pesticide, and so on, are the fields of radioisotope tracer technique application. Therefore, the developement of new pesticides with high efficiency and low residue will more greatly rely on the technique as a efficient means utilized in basic theoretical research in future.

5.2.2 To Renew the Technique in Combination With Other Advanced Techniques

21st Century Agenda has attached great importance to the protection of eco-environment being regarded as a strategy of the whole global economic development. For sustainable agriculture, the control of environmental pollution resulted from pesticide and reduction of the pollution to a minimum is a long-term research program. New techniques in large numbers have been found as efficient means in the research area. In future, combination and cross of isotope tracing technique with some advanced means such as chromatographic, molecular biological, biosenser, immunochemical and computer techniques, will not only promote the development of pesticide sciences, but also make the isotope tracing technique itself get greater vitality.

5.2.3 To Develop New Techniques and System for Labeling Pesticide

It is essential for using radioisotope tracer technique to label pesticides with isotopes for the studies of pesticide science. Therefore, more and more related compounds will be labeled by new synthesis techniques. In future, these studies will open up to more area. With greater achievement in China, a new assembly metwork for radioisotopic labeling pesticide including research, development, manufacture, commerce and application, will be formed in the future and the products of labeling pesticide will go into international market. Meanwhile, this is also bound to further promote pesticide science.

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