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PROBLEMS AND POTENTIALITIES OF STABLE ISOTOPES AS TRACERS FOR STUDYING POLLUTANT BEHAVIOR UNDER FIELD CONDITIONS

by

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ABSTRACT

The separated stable isotopes of carbon (^{12}C, ^{13}C), oxygen (^{16}O, ^{17}O, ^{18}O), and nitrogen (^{14}N, ^{15}N) (the ICONs) have been available in limited quantities for many years. These non-radioactive, naturally occurring forms of the elements are the basic building blocks of natural compounds and of pollutants, and their great potential utility for tracer studies has long been recognized. However, in practice, the use of these isotopes has been limited primarily for two reasons. First, the separated isotopes in the required chemical forms have been relatively expensive. Second, the analytical instrumentation necessary for their detection and assay has been expensive to acquire and difficult to maintain. Recently this situation has begun to change rapidly and, in this paper, we review the problems and potentialities of stable isotopes as tracers for studying pollutant behavior under field conditions.

In this review, the radically changed situation with respect to increased supplies of the ICONs at low cost will be discussed against the backdrop of the continued high cost of certain isotopically labeled compounds and of analyses. Prospects for improvements in the latter areas will be explored in the context of the experience accumulated at the Los Alamos Scientific Laboratory in ICONs research and development activities directed toward ICONs production, synthesis of labeled compounds, and analytical techniques for ICONs. The potential and actual applications of ICONs for studying pollutant behavior under field conditions will be surveyed with emphasis on: (1) The use of ^{15}N and ^{14}N (depleted in ^{15}N) for tracing N tagged fertilizer; (2) the use of mass spectrometry and

nuclear magnetic resonance spectroscopy for studying the distribution and fate of ^{13}C and ^{15}N tagged pesticides in the biosphere; and (3) the use of tagged methane as a tracer for transport and dispersion studies in the atmosphere.

INTRODUCTION

In recent years there has been a wide-spread resurgence of interest in the use of the stable isotopes of carbon (^{12}C, ^{13}C), oxygen (^{16}O, ^{17}O, ^{18}O), and nitrogen (^{14}N, ^{15}N) (the ICONs, Table 1) in science and technology that is attested to by the appearance of a large number of review articles (1-12) and the convening of at least two international symposia on the subject (13,14). Of course, the ICONs have been available in limited quantities for more than forty years. Indeed, by 1940 some impetus, fueled by the availability of the enriched isotopes from H. C. Urey's laboratory and the development of the Nier mass spectrometer (1, 15-19), was building to develop ^{13}C and ^{15}N tracers in the biosciences and medicine. Particularly with respect to ^{13}C, this impetus was blocked by three events: the onset of World War II and its diversion of key scientists; the production of radioactive-^{14}C in nuclear reactors; and the development of sensitive and reliable liquid scintillation counters for the detection of radioactive isotopes.

During the war the tools for radiotracer studies were developed to a high level of sophistication. Following it, the technology was quickly applied in fundamental research by scientific investigators, particularly in the biosciences for it filled an urgent need -- e.g., the concept of metabolic pathways was well accepted but experimental techniques for elucidating them were crude, cumbersome, and, as in massive feeding experiments designed to build up intermediates, frequently perturbed the subject system. In contrast, studies of the metabolic fates of compounds labeled with the radiotracers ^{32}P, ^{14}C, and ^{3}H could be accomplished with low administered doses and, of more importance, since separation procedures for the metabolites could be developed empirically by monitoring the radioactivity of fractionated samples, no detailed knowledge of the chemical nature of the contained metabolites was required. In this context, it is of some historical interest to note that, until recently, research with the stable isotopes, ^{18}O and ^{15}N which have no long lived radioactive counterparts, continued at a much higher, albeit subdued, level than studies with ^{13}C (4, 6, 9, 20).

The reasons for the increased interest in and use of ICONs in science and technology can be traced to recent significant progress in reducing two significant barriers to their earlier use: the limited availability of the isotopes in the required chemical form at low cost; and the expense and sophistication of analytical instrumentation necessary for their detection and assay. In addition, public concern in many countries about the spread of radioactivity, in any form and at any level, has induced a serious search by concerned scientists for an alternate to radiotracer techniques. That search has uncovered two somewhat surprising facts, discussed at greater length in later sections of this article: stable isotopes frequently can be detected with more sensitivity than can radioisotopes with existing instrumentation; and structural information about the location of the stable isotope label in a molecule can be obtained at a lower cost than is incurred with radiotracer techniques. In the latter case one trades off an initial high capital cost of a sophisticated instrument capable of many measurements per unit period of time for a reduction in continued

high cost of personnel devoted to chemical processing.

Despite the markedly improved picture with respect to the cost and availability of compounds labeled with stable isotopes and isotopic analyses in recent years, the development of ICONs tracers in the biosciences is limited still by synthetic and analytical difficulties. In this paper we review the problems and potentialities of the enriched ICONs as tracers for studying pollutant behavior under field conditions with special emphasis upon the latter problems.

Production of Stable Isotopes

A number of methods have been used for the separation of stable isotopes including thermal diffusion, distillation, electrolysis, chemical exchange, electromagnetic separation, gas centrifugation, and solvent extraction (21). In practice, two methods have been found most appropriate for enriching the ICONs on a large scale at low capital cost, and these are: chemical exchange especially for the ^{15}N isotope (the so-called nitrox process) and cryogenic distillation of carbon monoxide (for the ^{12}C and ^{13}C isotopes) and of nitric oxide (for the ^{14}N, ^{15}N, ^{16}O, ^{17}O, and ^{18}O isotopes). In Table 2 are listed the production capacities for the ICONs achieved recently at the Los Alamos Scientific Laboratory. A number of other facilities have been able to achieve production of significant quantities of the enriched ICONs: Prochem/BOC Ltd., 1-2 kg ^{13}C (90+%) per year and ^{15}N (99+%), ca. 1 kg/year; and Institut für Neutronphysik and Reactortechnik, Karlsruhe, 3 - 4 kg. ^{18}O (99+%) per year; Weizman Institute of Science, Israel. The increased production of the separated ICONs has resulted, in the United States experience, in a substantial reduction in the cost of the raw isotope. For example, in 1967 the costs (per gram contained isotope at the 90% enrichment level) of $^{13}CO_2$, $(^{15}NH_4)_2SO_4$, and $^{18}OH_2$ were quoted at approximately \$900, \$400, and \$700 (22); today the costs are \$56, \$95, and \$110, respectively (23). Of course the prices of the enriched isotopes usually are a sensitive function of the level of enrichment, some representative costs for $^{15}NH_3$ and $^{18}OH_2$ as a function of enrichment level (obtained from the catalogues of commercial suppliers) are, respectively: \$400 (99+%), \$70 (5%); and \$513 (97%) \$200 (3%).

In the development of a field experiment, the cost of the isotope at a given level of enrichment can be as important a design parameter as the sensitivity of the analytical method and the apparent natural variation in the isotope ratio being measured (vide infra). For example, in the field studies of the plant uptake of fertilizers tagged with stable nitrogen isotopes discussed later, one has the choice of using enriched ^{15}N or material depleted of ^{15}N. For ^{15}N depleted (<100 ppm ^{15}N) materials, the minimum level of detectability of the tagged material is at a ratio of dilution with untagged material of approximately 1 part in one hundred. In contrast, if material tagged with ^{15}N (99+%) is used, the tracer can be detected at a dilution of approximately four parts in one hundred thousand. Considering only the minimum detectable levels of the ^{14}N and ^{15}N tagged materials, one might choose the ^{15}N tracer. However, since the relative prices (per gram contained isotope) of ^{14}N and ^{15}N tagged fertilizers currently stand in the ratio of approximately 1 to 600, cost considerations would dictate the use of ^{14}N tagged material. Stated in another way, for every 250 g of ^{14}N (<100 ppm ^{15}N), applied in a field study one could apply 1 g of ^{15}N (99+%) and detect the same isotope dilution. However, 250 g of ^{14}N costs only \$25 but 1 g of ^{15}N costs \$65. We emphasize, however, that the choice of isotope and its

enrichment level frequently will be dictated by the special character of the experiment. In the example just given, attention was focused on the determination of the plant $^{14}N/^{15}N$ isotope ratio as an indicator of the efficiency of fertilizer uptake. If, on the other hand, the question of interest is the efficiency of denitrification of fertilizer derived NO_3^- ion to N_2 gas (a problem of ecological significance) then the tracer of choice would probably be ^{15}N given the low natural background of $^{15}N_2$ $(3.65 \times 10^{-3})^2$ and the high backgrounds of $^{14}N_2$ and $^{14}N^{15}N$.

Some special notes are in order with respect to the level of isotope enrichment and experiment design for tracing organic pollutants under field conditions. Although significant quantities of ^{15}N and ^{18}O are available at the 99+% enrichment level, most of the ^{13}C currently available is enriched only to ~90 atom %. Consequently, if a particular study required the use of a uniformly labeled molecule containing eight carbon atoms (e.g. 2, 4, 5-T), the use of the 90% ^{13}C for the synthesis would result in mixture of isotope isomers in which only a small fraction, 0.43 $[(0.9)^8]$ would contain molecules with all eight carbon atoms labeled with ^{13}C. In some of the tracer and isotope dilution studies to be discussed later, the mixture would be unacceptable from the standpoint of analysis and the use of 99+ atom % ^{13}C in the synthesis would be required.

Carbon-13 enriched to the 97-99% level is available in small quantities but at more than twice the cost of 90% ^{13}C (23). The factor that limits the enrichment of ^{13}C to ~93% in the distillation of CO is the lack of chemical exchange among the carbon and oxygen atoms. Consequently, $^{13}C^{18}O$ is enriched as a contaminant in the $^{13}C^{16}O$ product. At the Los Alamos Scientific Laboratory, chemical exchange methods are being developed to induce isotope scrambling and within a year 99+% ^{13}C should be available at low cost.

Isotopically Labeled Compounds

As described above, the costs of the ICONs in the simple chemical forms, $^{13}CO_2$, $^{15}NH_3$, and $^{18}OH_2$, has dropped drastically to ~$100 per gram of contained isotope by means of the economies achieved through large scale production methods. Nonetheless, this price is still high enough to serve as a significant deterrent to their large scale use, primarily because most studies require that the isotope be incorporated into complex organic molecules by synthetic procedures which can involve significant losses and risks. For example, a field study with a ^{13}C labeled pesticide (eight carbon atoms, F.W. = 264), might require 100 g of labeled product synthesized in five steps of efficiency, 60% each, the cumulative yield being 7.8%. Depending on the reaction pathway and assuming that no catastrophe occurred in any step, the potential loss of ^{13}C in unrecoverable or useless by-products is approximately 450 g at an approximate cost of $26,000.

Now there are a number of ways that this problem might be solved. The most obvious potential solution, and the one least likely to be successful in the general case, is to improve the yields. However, even if 80% yields could be achieved in each step, the cumulative yield would still be only 33%, equivalent to losses of 80 g of ^{13}C at a cost of $5000. Of course in this analysis we have neglected the costs of the research and development devoted to improving product yields. In our experience the latter frequently exceeds the cost of the isotope conserved.

Another potential solution to the problem is to reduce the costs of the ICONs in their simple chemical forms. This can be achieved by increasing production capacities. In our ICONs program, few difficulties have been experienced in scaling distillation columns from a yearly production rate for nitrogen and oxygen heavy isotopes from the hundred gram to the one kilogram to the ten kilogram level. There is no technical reason that production levels could not be increased another order of magnitude for the ICONs with an attendant drop in their costs by a corresponding amount, putting the inorganic forms of the enriched ICONs in the cost category of many common synthetic intermediates. However, this fortunate turn of events would require a high initial cost for expanded production facilities which may not be incurred until it is demonstrated to industries or government agencies that a large scale requirement for these stable isotopes does exist. It is likely that such requirements will develop over the long term (> five years) but for the short term, barring an accelerated development of stable isotopes in biomedical research (1), supplies and costs of stable isotopes are likely to remain near the present levels.

The expense of preparing complex isotopically labeled compounds can also be reduced if there is a plentiful supply of key labeled intermediates. For the interim period, this appears to be the most likely area where economies can be achieved. Within recent years, the number of commercial suppliers of isotopically labeled compounds has increased and the variety of selections in their catalogues has expanded significantly. In Tables 3 & 4 are listed a selection of some of the labeled compounds available and their suppliers. Accompanying the increased commercial supplies of labeled compounds has been a significant decrease in their price. For example, sodium [1-^{13}C] acetate was offered for sale in 1967 at prices ranging from \$136 - \$246 (50-60% enriched) per gram whereas today the price is approximately \$70 per gram (90% enriched).

Nonetheless, the cost of the isotope itself accounts only for a minor proportion (15% in the example above) of the cost, the majority of it being the labor involved. The problem apparently is that the syntheses are usually performed on a small scale. For example, we estimate that sodium [1-^{13}C] acetate could be obtained for approximately \$11 per gram if synthesized on a five mole scale, which one anticipates will be approached as the demand increases for labeled compounds.

The use of efficient large scale production methods for the simple organic compounds is also important in lowering the costs of more complex materials. The many illustrative synthetic pathways used in our laboratories and summarized in Fig. 1 illustrate this dependence. The synthetic network suggests also that selection of a particular procedure depends, to an important extent, on previously established capabilities and libraries of labeled starting materials. Significant progress has been made in this area; the reader is referred to the literature for the details (13, 14). We would emphasize here only that a considerable amount of success has been achieved in many laboratories (13) in labeling with stable isotopes molecules as complex as those likely to be encountered in tracing the behavior of organic pollutants. A selection of those compounds is summarized in Table 5.

Assay Techniques for Stable Isotopes

The analytical methods for assaying stable isotopes, the most common and convenient of which are listed in Table 6, can be divided into two general categories: (1) Those that provide precise measurements of isotope ratios in simple molecules like CO, CO_2, NO, and N_2; and (2) Those in which, for complex molecules and even mixtures, not only can the isotope ratios be measured but frequently the precise location of the label can be determined also.

The isotope ratio methods generally allow rapid and convenient measurements to be made, and require only modest training for operation of the equipment and data analysis. In addition the capital equipment cost is low, frequently less than $20,000. A disadvantage associated with the use of the simple isotope ratio methods is that the high natural background of the ICONs limits the sensitivity in tracer studies and requires the administration of high levels of the isotopically labeled compound. For example, because the probability of the natural occurrence of 2,4,5-T labeled with 100% ^{13}C at all eight carbon atom positions is approximately 2 parts in 10^{-16}, that material would provide an elegantly sensitive field tracer, permitting detectable dilutions with natural 2,4,5-T of more than one part in 10^{8} (as a practical limit) provided that the analytical method is specific for the intact ^{13}C labeled compound. In the simple isotope ratio analysis, however, the material analyzed would first be converted to CO_2. Since most routine isotope ratio methods have an accuracy limit of ~1% on the isotope ratio [the natural variation of isotope ratios usually being limiting in this respect (4) (12)], these methods could be used to detect a maximum dilution of one part of the ^{13}C labeled compound with 10^{4} parts of the natural abundance material (1.1% ^{13}C). Of course the trade-off in the loss of sensitivity is for convenience and rapidity of analysis. In addition, however, one may destroy information about the existence of degradation products developed under field conditions (vide infra). Nonetheless, abundant supplies of certain of the ICONs make feasible some experiments in which the "tracer" undergoes only a hundred to a thousand fold dilution.

The analytical techniques in the second category are characterized by high initial capital cost of the equipment ($50,000 - $250,000), and a great deal of skill is required for operation and data analysis. However the techniques offer the advantages of determination of label location, direct analysis of complex mixtures and, in the case of mass spectrometry, sensitivities approaching that for radiotracers in some cases. Significant progress has been made recently in reducing the complexity and cost of nmr and mass spectrometers and in improving their sensitivities. Notable among these are the development of the quadrupole mass spectrometer with multiple ion detection for mass fragmentography (24, 25) and the recent introduction of field desorption techniques which should simplify sample preparation and reduce the complexity of mass spectra (26). Similarly in the field of nmr spectroscopy, the development of pulse Fourier techniques has markedly improved the sensitivity and reduced the complexity of spectrometers. Also important in improving the nmr sensitivity have been the introduction of large (20 mm) sample tubes (27) and the development of special techniques for the cross polarization of ^{1}H and ^{13}C and ^{15}N nuclear spins, the so-called proton-enhanced nuclear induction spectroscopy (28).

In the following discussion of selected potential applications of stable isotopes in tracing pollutants under field conditions certain of these analytical techniques will be considered in more detail.

POTENTIAL APPLICATIONS OF MULTIPLY LABELED COMPOUNDS IN FIELD STUDIES OF THE FATE OF PESTICIDES

In field studies of the fate of pesticides, there are two important problem areas (29-31) in which multiply labeled compounds may find wide use: (1) Quantitation of low levels of residues in tissues, foods, crops, soil, and water; and (2) Identification of chemical and metabolic products of pesticides. A number of elegant and sensitive analytical techniques already have been developed for quantitation of residues and their metabolites. These include enzyme inhibition (32), gas chromatography with electron capture detection (33), radiotracers (29, 30, 34) and gas chromatography - mass spectrometry (35-37). Each of these techniques has limitations associated with specifity, interfering substances, use of "external" standards for quantitation, or losses during sample work-up.

In a sense, the analytical problems faced by the environmental scientist are similar to those of the clinical pharmacologist: drugs and drug metabolites are present at low levels and the compounds to be anlayzed must be isolated from very complex mixtures (38, 39). Significant advances in solving these problems are being made by using drugs labeled with stable isotopes and then, in the analysis, monitoring several peaks in the isotope cluster in the mass fragmentogram of the drug or its metabolites (Table 7). In these studies the "artificial" isotope cluster allows the positive identification of the drug and its metabolites and, in some cases, provides an internal standard for quantitative studies.

Of course natural isotope clusters have been used for many years in mass spectrometry to assist in molecular weight and structure determinations (40). An example of natural isotope clusters appearing in the mass spectrum of DDT is presented in Fig. 2. The compound contains the stable isotopes ^{35}Cl and ^{37}Cl having the ratio of abundances .745/.246 ($\sim$3:1), and isotopes clusters for the $C_{14}H_9Cl_2^+$ (R) and $C_{14}H_9Cl_3^+$ (Q) ions can be detected readily in the spectrum. Using the relative abundances of the isotopes, it can be shown that the fragments R and Q should give rise to ions at the following m/e values and relative intensities (in parentheses): R(1), R+2(0.65), and R+4(0.11); and Q(1), Q+2 (0.98), Q+4(0.32), and Q+6(0.04). As shown in the figure there is a close correspondence between the observed and predicted patterns. The schematic mass spectra of the parent molecular ions of "natural" and ^{13}C labeled DDT, depicted in Fig. 3, illustrate a point discussed earlier regarding the desirability of working with high levels of ^{13}C in these applications. There are peaks at odd mass values (M+1, +3,...) in the natural DDT spectrum but absent from that for the labeled material. They arise from those molecules containing natural abundance ^{13}C and exhibit appreciable intensity because of the large number of carbon atoms in the molecule. When working at the <90% level of ^{13}C, these odd mass peaks not only lower the sensitivity but also complicate structure determinations. For example, even in the spectrum of a molecule tagged with three ^{13}C atoms at the 90% level (41) the intensity of the M+2 peaks relative to M+3 peaks if $\sim$1:3.

The artificial isotope clusters of multiply labeled drugs provide excellent internal standards for mass fragmentography in which a narrow region of the mass spectrum is repetitively scanned. In clinical pharmacology the use of these internal standards, which are added to tissue, urine, or plasma samples prior to chemical work up, has extended the range of sensitivity of the mass spectrometer to the level of 10^{-15} gram (41). Another technique used by clinical pharmacologists which should find wide application in tracing pollutants is the administration of a 1:1 mixture of the labeled (M+n) and unlabeled (M) drugs to a subject followed by specimen analysis by mass chromatography and mass fragmentography, the appearance of 1:1 doublet at interval n in the mass spectrum providing positive identification of the drug and its metabolites (42). The relatively high background of pesticides that has accumulated over the years will probably necessitate using a variant of this technique in field studies, viz. the application of two isotopically labeled pesticides, M+n and M+n', and the monitoring of the mass spectra for doublets with an interval n-n'. The chemical synthesis of such materials will present a challenge to the organic chemist but his task could be simplified by prior knowledge of potential degradation routes which would result in loss of label under field conditions and the use of mass spectrometry techniques which minimize the fragmentation of parent molecular ions. Significant progress has been made in both these subjects in recent years.

Insofar as analytical techniques are concerned, the emphasis in this review has been placed on the quantitative detection of labeled molecules present at submicrogram levels and, up to this point, the structures of the labeled molecules have not been considered. With regard to pesticides, structural questions have become more important with the realization that transformation in the ecosystem may be rapid enough that adverse biological effects and/or the efficacy of the agent may be due to chemical or metabolic residues rather than to the pesticide itself. Although mass spectrometry provides the ultimate in sensitivity for the detection of complex molecules labeled with stable isotopes, at present the method has limited utility in structural studies despite recent advances (43). In contrast, ^{13}C and ^{15}N nmr spectroscopy provides powerful method for structural investigations. Particularly for ^{13}C, the use of nmr spectroscopy in structural investigations has been documented well (44, 45), and, in this context, we note especially the elegant tour de force by Dalling and Grant (46) in their recent study of the structure and stereochemistry of the perhydro-anthracenes and phenanthrenes. We shall not consider ^{13}C and ^{15}N nmr as a structural tool here. Instead, we should like to emphasize two significant, but not generally appreciated aspects of nmr investigations with these isotopes.

First, it has been emphasized frequently that nmr spectroscopy is not a sensitive analytical technique (35, 47). However, the refinements in instrument design discussed previously, together with recent advances in detection methods (48) should allow the detection of pesticides at the 100-500 nanogram level. This sensitivity approaches that for many mass spectrometric studies, the data acquisition time in the nmr study exceeding that for mass spectrometry (at equivalent sensitivity levels) by a factor of 2000-5000:1. However, the additional time spent in the nmr data acquisition (spectrometer stability is only a minor consideration) may well be worth the effort given the second aspect of the methods considered here -- the potential for studying complex mixtures while conserving the high structural content of the nmr spectrum. Indeed it has been shown recently (49-52) that the molecular structures of cellular con-

stituents can be deduced from the ^{13}C nmr spectra of intact cells and tissues. In addition, the nmr spectra of molecules multiply labeled with ^{13}C and ^{15}N contain characteristic nuclear spin multiplets which, like the appearance of isotope clusters in mass spectra, can be used for the positive identification of pesticide degradation products (2, 47, 53, 54).

Nitrogen Tracers for Quantitating the Nitrogen Cycle under Field Conditions

For many years ^{15}N labeled fertilizers have been used in greenhouse experiments to obtain quantitative descriptions of nitrogen transformations in the soil, especially with respect to the efficiency of the incorporation of fertilizer nitrogen by plants (55-58). The use of nitrogen tracers in field studies has been more recent and, of course, much more limited (59-67). The uncertainties inherent in extrapolating the parameters obtained in greenhouse experiments to models of the nitrogen cycle under field conditions, the rising costs of fertilizer, the pressures to expand food and fiber production through more efficient use of fertilizer, and the increasing concern about the potential effects on the environment from increased input of fertilizer nitrogen into the biosphere combine to suggest that a marked increase in the number of field experiments with nitrogen tracers will occur in the near future. Certainly the cost of the isotopes can no longer be considered a serious impediment. In the United States three such field studies are currently in progress under the sponsorship of the National Science Foundation (at the University of California) (66, 67), the Tennessee Valley Authority, and the United States Department of Agriculture. A novel feature in these studies is the use of metric ton quantities of the "reverse" tracer -- the abundant ^{14}N isotope depleted to <100 ppm of the "rare" ^{15}N isotope. In the following we high light some of the problems and areas of promise in field studies with nitrogen isotopes. We will not consider here the use of variations in the "natural" $^{14}N/^{15}N$ ratios (68) which, for all their qualitative utility, present a number of difficulties in quantitative studies (69).

During the course of a field experiment with nitrogen tracers, thousands of measurements of the $^{14}N/^{15}N$ isotope ratio may be required and there is a need for a simple, rapid, and sensitive analytical method. The two methods used most often in greenhouse and field experiments have been isotope ratio mass spectrometry and optical emission spectroscopy. Of the two, the preferred method of analysis at present appears to be mass spectrometry -- the expense of the equipment and its maintenance, and the time required for analysis notwithstanding. The development of less versatile, perhaps single purpose mass spectrometers (e.g. devoted to ^{14}N-^{15}N, $^{14}N^{14}N$ analyses), is highly desirable and it is certain that the product would find wide use if the loss of versatility were traded for a decrease in cost and a greater simplicity and rapidity of analysis. Similarly, the optical emission ^{15}N analyzer, which has been used more in Europe than in America, deserves more attention (70-73). The memory effects and chemical problems in emission spectroscopy appear to be no more severe than those in mass spectrometery, and the cost of the instrumentation is less. Although the sensitivity and precision of the optical methods are less, particularly at low isotope ratios, this should not be a problem in some applications, a relative error of 5% being acceptable for some nitrogen tracer studies from which macro-samples are usually available.

As emphasized recently by Hauck (55) and Norman (58) there is a need to define more closely the biological significance of nitrogen in soil organic fractions particularly with respect to a determination of the structures and turn-over rates of the labile and passive fractions. In those field studies where ^{15}N is used as a tracer, structural studies appear possible with ^{15}N nmr techniques on the soils themselves and on crude soil fractions. In this context, greenhouse experiments with both ^{15}N and ^{13}C tracers, in conjunction with ^{13}C and ^{15}N nmr spectroscopy, should also prove useful. The beneficial results of a strong research program in this interesting area would likely be a determination of the conditions for minimum reversion of labile nitrogen into the passive pool and optimum release of nitrogen from the passive organic constituents.

Closely related to the problem of increasing food production with minimal environmental impact is the question of more precisely defining human nutrition requirements in normal and diseased states (74). The enormous quantities of nitrogen labeled amino acids which could be available as a result of field experiments with nitrogen tracers provide the opportunity to perform elegant and sensitive tracer experiments in nutrition on statistically significant numbers of human subjects. A few tracer experiments of this type, but limited in scope, have already been accomplished (75,76). In view of the enormous potential value of such nutritional tracer experiments, we believe some thought should be given to their design now and, in particular, to the difficult task of separating and purifying the labeled amino acids on the hundred gram to the kilogram scale.

Labeled Methane as an Atmospheric Tracer

In collaboration with Dr. Lester Machta of the U. S. National Oceanic and Atmospheric Administration (NOAA) and Dr. Norman Daly of the Aldermaston Weapons Research Establishment in the U. K., we have recently begun some preliminary field experiments to establish the limits of detection for mass-21 methane ($^{13}CD_4$) as an atmospheric tracer. This isotope of methane is presumably almost non-existent in the atmosphere although mass-16 methane is present, to the extent of 1-2 parts per million. It is possible to detect less than one part in 10^{10} of mass-21 methane in the presence of mass-16. Thus, one can measure mass-21 methane in 10^{21}-fold dilution in the atmosphere. The use of mass-20 methane is almost as promising.

The lifetime of methane in the atmosphere is several years, its chief sink being photolytic decomposition in the stratosphere. It is hoped that it will be possible to use tagged methane repeatedly in quantities of the order of 100 g to follow the transport and dispersion of air masses for distances of several hundred miles. A ground sampling network for collection of suitable samples presently exists under the direction of NOAA.

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TABLE 1.

SOME CHARACTERISTICS OF THE STABLE ISOTOPES OF CARBON, OXYGEN AND NITROGEN

Isotope	"Natural" abundance (%)	Nuclear spin
^{12}C	98.9	-
^{13}C	1.1	1/2
^{14}N	99.6	1
^{15}N	0.4	1/2
^{16}O	99.8	-
^{17}O	0.04	5/2
^{18}O	0.2	-

TABLE 2.

CURRENT PRODUCTION CAPACITIES (kg.) FOR THE ICONS AT THE LOS ALAMOS SCIENTIFIC LABORATORY

^{12}C (<10 ppm ^{13}C)	^{13}C (90+%)	^{14}N (<100 ppm ^{15}N)	^{15}N (99+%)
12	5.5 kg	240	1.3

^{15}N (40+%)	^{16}O (<100 ppm ^{17}O, ^{18}O)	^{17}O (20+%)	^{18}O (95+%)
5.3	2700	0.3	1.5

TABLE 3.

COMMON LABELED COMPOUNDS AND INTERMEDIATES AVAILABLE

Carbon-13 Labeled	Nitrogen-15 Labeled	Oxygen-18 Labeled
Acetic Acid and Acetyl Derivatives	Acetamide	Acetone
Algal Amino Acid Mixtures	Alanine	Acetyl Chloride
Algal Sugar Mixtures	Ammonia & Ammonium Halides	Barium Carbonate
Algal Hydrolysate	Ammonium Nitrate & Sulfate	Ethanol
Benzene & Derivatives	Aspartic & Glutamic Acids, Lysine	Methanol
Benzyl Derivatives	Glycine	Phenol
Butyl Halides		
Ethyl Halides	Methylamines	Sodium Acetate
Fatty Acids		
Formaldehyde	Nitric Acid	Sulfuric Acid
Glucose	Nitrogen Gas	Water
Glycine	Potassium Azide	
Methyl Halides	Phthalinide	
	Tyrosine	
Phenyl Alanine	Urea	
	Uracil	
Pyruvic Acid	Uric Acid	
Serine	Valine	
Succinic Acid		
Tetramethyl Silane		
Tyrosine		
Urea		

TABLE 4.

SOME SUPPLIERS OF COMMON LABELED COMPOUNDS

Bio-Rad Laboratories
32nd & Griffin Avenue
Richmond, California

Isocommerz - GMBH
1115 Berlin-Buch, den
Lindenberger Weg 70

Merck Chemical USA
Rahway, New Jersey 07065

Merck, Sharp & Dohme, Canada,
Montreal
Quebec, Canada

Miles-Yeda, Ltd.
Rehovot, Israel

Junta de Enerjia Nuclear
Madrid, Spain

Norsk Hydro
Oslo 2, Norway

Prochem
Deer Park Road
London SW19 3 UF, England

Office National Industriel
de L'Azote
40 Avenue Hoche
Paris (VIII^{e}) France

Stohler Isotope Chemical
49 Jones Road
Waltham, Massachusetts

Techsnabexport
Moscow, USSR

TABLE 5.

SOME COMPLEX COMPOUNDS PREPARED IN QUANTITY

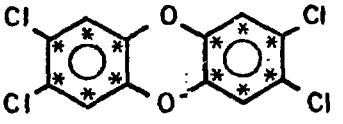
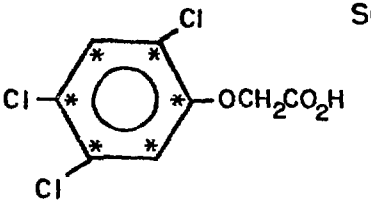
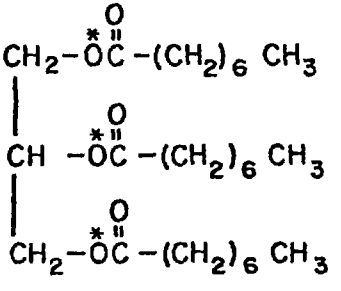
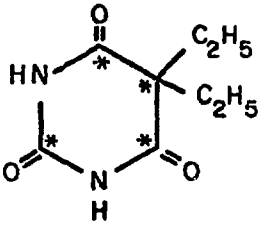
NAME	STRUCTURE	INVESTIGATORS
TETRACHLORODIBENZODIOXIN (TCDD)		D.G.OTT *etal*, LOS ALAMOS SCIENTIFIC LABORATORY
2,4,5-TRICHLOROPHENOXY ACETIC ACID (2,4,5T)		D.G.OTT *etal*, LOS ALAMOS SCIENTIFIC LABORATORY
TRIOCTANOIN		P.D. KLEIN *etal*, ARGONNE NATIONAL LABORATORY
PHENOBARBITAL		M.E. WALL *etal*, RESEARCH TRIANGLE INSTITUTE

TABLE 5. (contd.)

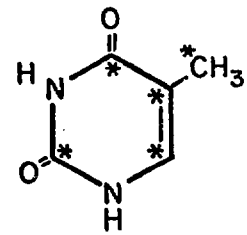
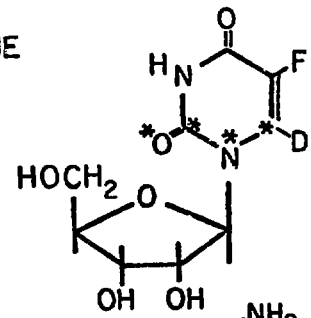
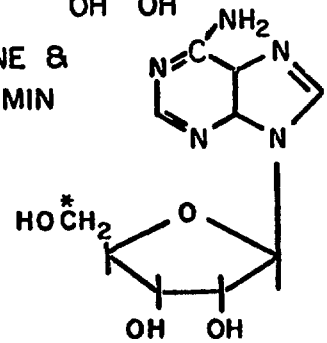
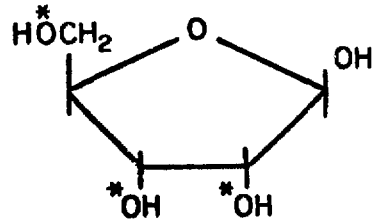
NAME	STRUCTURE	INVESTIGATORS
THYMINE		M. E. WALL *etal*, RESEARCH TRIANGLE INSTITUTE
5-FLUOROURIDINE		M. ANBAR *etal*, STANFORD RESEARCH INSTITUTE
$[5^{1}-^{13}C]$ ADENOSINE & ADENOSYLCABALAMIN		H.P.C. HOGENKAMP *etal*, UNIV. OF IOWA
$[2,3,\text{or } 5-^{18}O]$ RIBOSE, ADENOSINE & ATP		H.P.C. HOGENKAMP *etal*, UNIV. OF IOWA

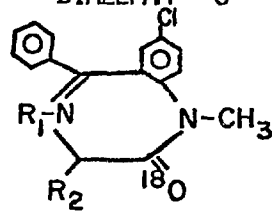
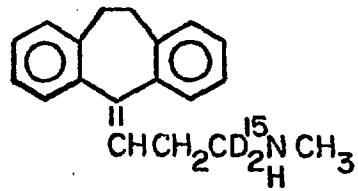
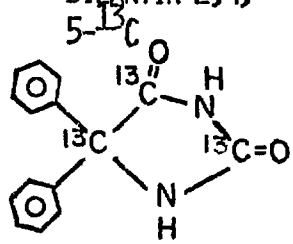
TABLE 6.

SOME COMMON TECHNIQUES FOR ISOTOPE ANALYSIS

Technique	Minimum sample size	Sample form	Information obtained
Infrared spectroscopy	Milligram-microgram	Simple gases (CO_2,NO)	Isotope ratio($^{13}C/^{12}C$; $^{15}N/^{14}N$)
Optical emission spectroscopy	Microgram	Simple gases(N_2)	Isotope ratio
Mass spectrometry	Milligrams-fentograms	Simple gases to complex mixtures (gas chromatographic separation)	Isotope ratio,frequently label location and molecular structure
NMR spectroscopy	Milligrams-micrograms	Simple molecules complex mixtures	^{13}C, ^{15}N content, label location,and direct analysis of mixtures

TABLE 7.

SOME DEMONSTRATIONS OF THE FEASIBILITY OF QUANTITATIVE ANALYSIS OF LOW LEVELS OF LABELED COMPOUNDS IN CRUDE MIXTURES

STUDY	SAMPLE	COMPOUND DETECTED	TECHNIQUE
GLUCOSE AND ALANINE TURNOVER IN VIVO, BIER, KIPNIS, ET AL (1972)	BLOOD PLASMA	ALANINE-D_4 GLUCOSE-D_7	GC-MASS SPEC., 200 NG. SAMPLES
METABOLISM & PHARMACOKINETICS OF DIAZEPAM, SADEE, CASTAGNOLI, ET AL (1971)	PLASMA & URINE	DIAZEPAM-^{18}O 	GC-MASS SPEC., ~1 NG. SAMPLES
STUDIES OF THE DISPOSITION OF PROPANOLOL AND NORTRIPTYLENE IN MAN, GAFFNEY, KNAPP, ET AL (1972)	URINE	NORTRIPTYLENE-^{15}N, D_2 	GC-MASS SPEC., ~1 NG. SAMPLES
METABOLISM OF DILANTIN IN MAN, HORNING, HORNING, ET AL (1973)	BLOOD PLASMA	DILANTIN-2,4, 5-^{13}C 	GC-MASS SPEC., FENTOGRAM-PICOGRAM SAMPLES

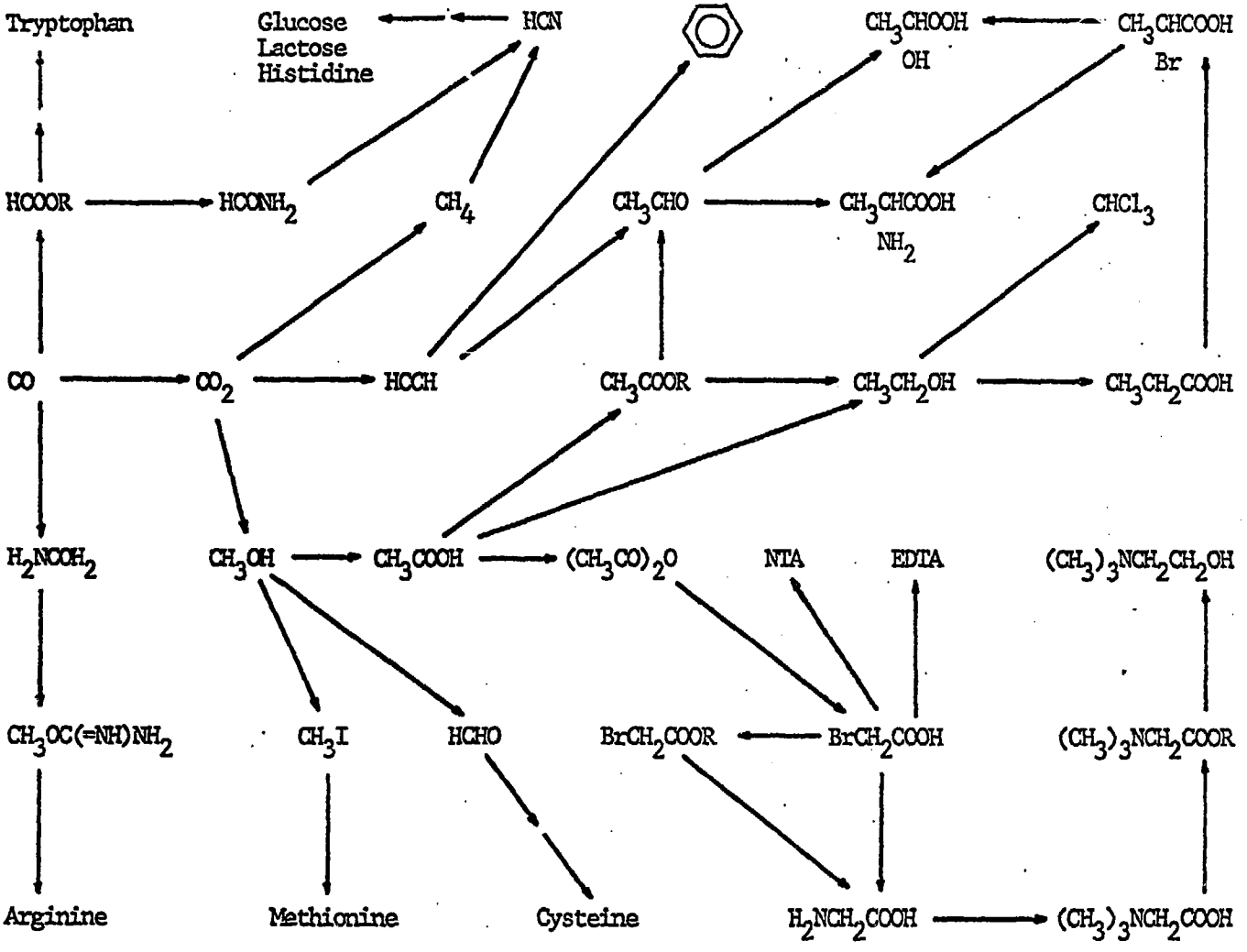
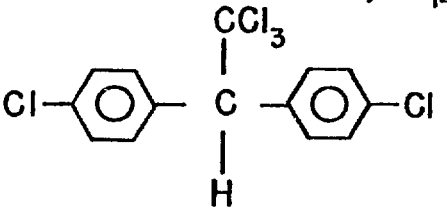
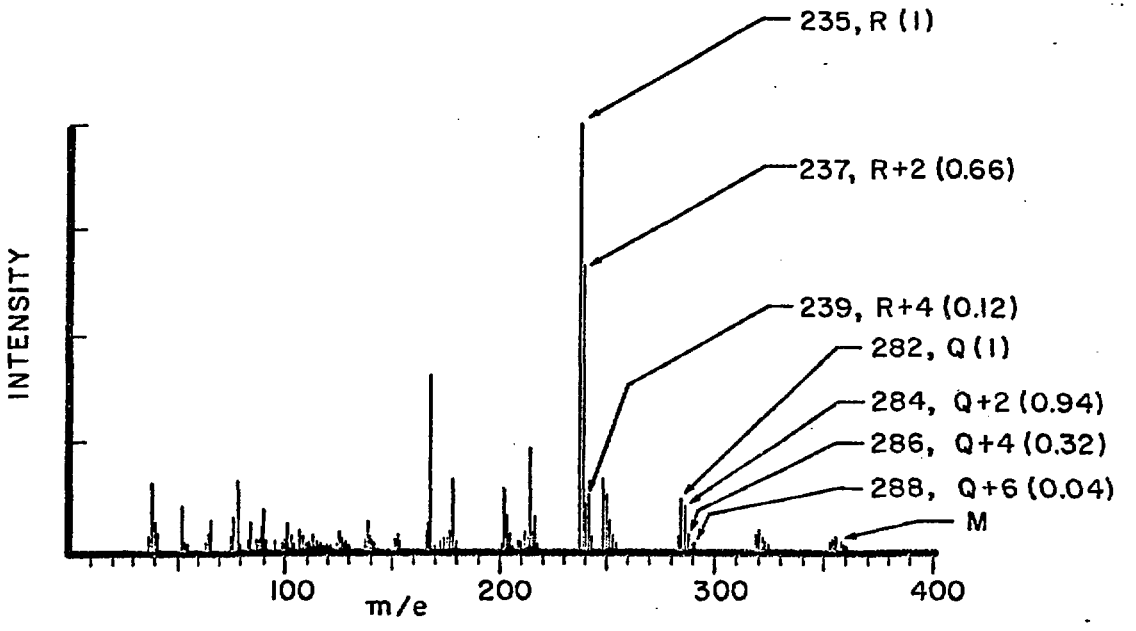


Fig. 1. Some synthetic pathways illustrating the role of key intermediates.

SOME $^{35}Cl(75.4\%) - {}^{37}Cl(24.6\%)$ ISOTOPE CLUSTERS IN THE MASS SPECTRUM OF DDT, $C_{14}H_9Cl_5$, FW=354





$R = M - CCl_3 = C_{13}H_9Cl_2^+$; R : R+2 : R+4 = 1.00 : 0.65 : 0.11

$Q = M - Cl_2 = C_{14}H_9Cl_3$; Q : Q+2 : Q+4 : Q+6 = 1.00 : 0.98 : 0.32 : 0.04

Fig. 2

SCHEMATIC MASS SPECTRA OF DDT PARENT MOLECULAR IONS

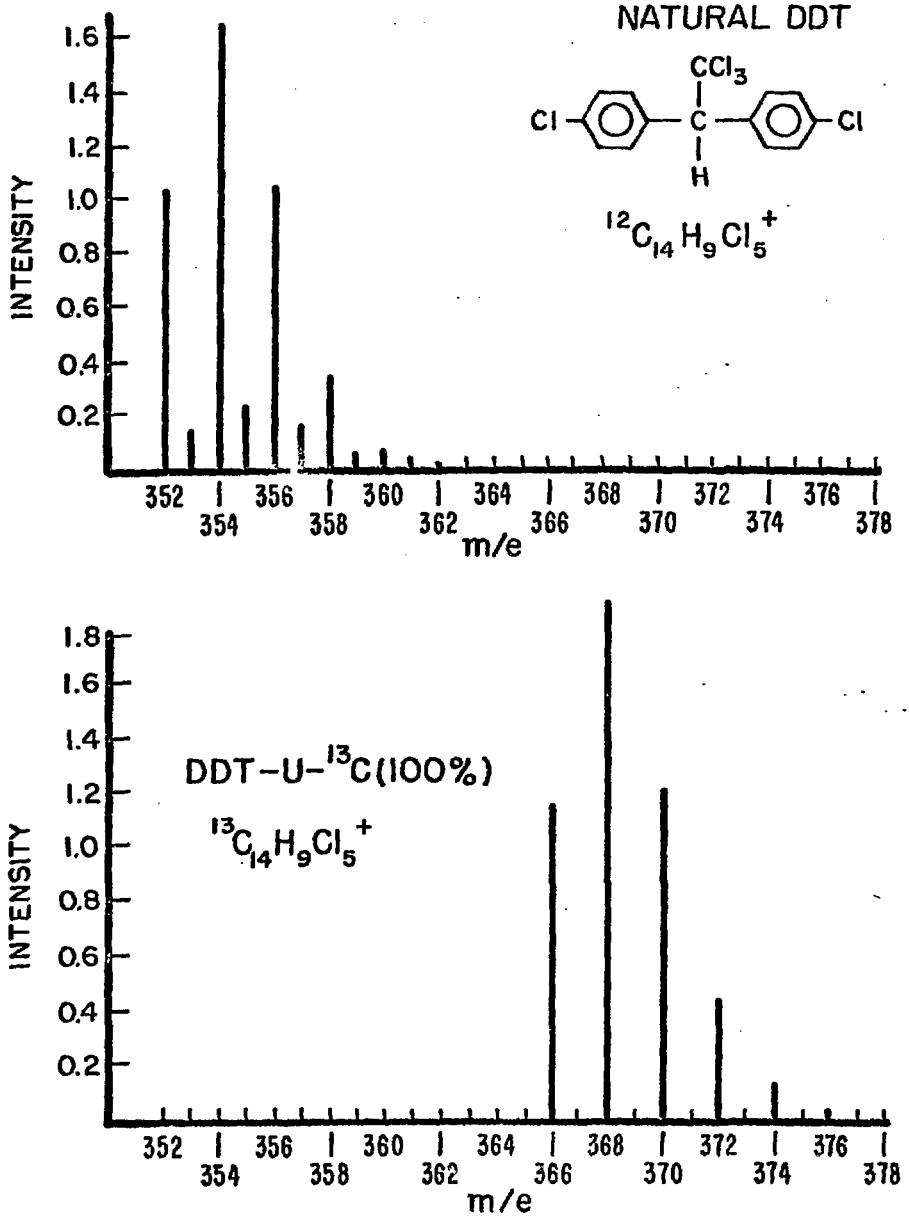


Fig. 3