# radiosynthesis

## *a vital role supporting drug development?*

The key *in vivo* drug metabolism and pharmacokinetic studies continue to be undertaken using radiolabelled versions of drug molecules. Traditionally, the preparation of these isotopically labelled compounds was largely the domain of specialist internal radiochemistry groups within large pharmaceutical companies (Big Pharma). However, the growth of mid-size biotech and pharmaceutical companies has resulted in the emergence of specialist custom radiolabelled synthesis companies operating as contract research organisations. This article explores the status and evolution of this market, examines the impact of new technologies on the discipline and considers the possible consequences of application of cGMP principles to the preparation and use of radiotracers in clinical studies.

**By Dave Roberts and Professor Bill Lockley**

Today, radioactivity is often associated, in the mind of the general pubic at least, with hazard and danger. However, artificial radioactive compounds have made possible a revolution in biomedical science, and have underpinned our understanding of the behaviour of modern drugs. Now, as part of the FDA's safety assessment process, more than 80% of all drugs have used radioactive materials in their testing programme.

Currently, these radiolabelled compounds are used as tracers to facilitate the research and regulatory process, primarily for studies of pharmaceuticals, agrochemicals, veterinary compounds (animal health) and related xenobiotics. Moves to increase the regulation of other chemicals (for example through REACH in the European Union[1]) may also widen the need for studies requiring such tracer compounds in the future. Radiolabelled compounds are frequently used to understand the distribution, flux, metabolic fate and tissue localisation of a drug *in vivo*, in pre-clinical and environmental (plants, soil etc) studies. Clinical studies may also involve administration of radiolabelled tracers to healthy human volunteers. It should be noted that this type of study is quite different from radiopharmaceutical applications in which drugs labelled with short-lived isotopes are used to treat sick individuals or as medical imaging agents.

### Why use a radiolabelled compound?

The radioisotopically labelled parent drug or its metabolite acts like, and therefore can trace, its non-radioactive counterpart providing one of the fundamental tools for biomedical science. These radiolabelled compounds have allowed scientists to study virtually every aspect of a drug's behaviour *in vivo*. One key objective is to evaluate the mass balance of the drug to understand how much of the applied dose is recovered with respect to time. Another, and no less important objective, is to understand and explain the metabolism of the drug. The metabolic profile will determine if any metabolites might represent a potential toxicological hazard and hence require additional testing.

The key advantages of using radiolabelled tracers can be summarised thus:

● **Determination of the total dose administered and excreted (mass balance)**
Radioactivity is easily quantified, using scintillation counters or other radio-detectors. Monitoring of excreta (urine, faeces, exhaled air) is therefore facile and over time provides information on the drugs pharmacokinetic profile.

● **Drug metabolism studies**
Allows study of metabolism in different species and individuals providing quantitative information on parent and any metabolites produced even those of unknown structure.

● **Whole body autoradiography**
Sensitive detectors, such as phosphorimagers, have replaced the use of x-ray film and are able to produce images of the distribution of radioactivity in multiple, part or whole body slices. This technique can also be used quantitatively to provide information on tissue distribution (QWBA).

All the above drug metabolism and pharmacokinetic (DMPK) or adsorption, distribution, metabolism and excretion (ADME) studies can be undertaken without resorting to sophisticated (and expensive) analytical techniques to follow unlabelled parent and metabolites.

Information from studies with a radiolabel can therefore provide a competitive advantage by providing key safety information, particularly in the area of reactive metabolites and adverse drug reactions (ADR), thereby raising potential valuations of the candidate drug.

## Radiosynthesis

Typically the objective of radiosynthesis is to synthesise an analogue (isotopomer) of the drug candidate in which one or more atoms have been replaced by a radioisotope. This requires the element to have an isotope which is both radioactive and has a suitably long half-life. Examples of such isotopes are carbon ($^{14}C$), hydrogen ($^{3}H$, tritium), sulphur ($^{35}S$) and iodine ($^{125}I$, for protein labelling).

A number of points need to be considered when deciding which isotope to select for radiolabelling purposes. These factors will include the level of specific activity required to gain information from the study (eg, ligand binding studies may require high specific activities necessitating labelling with $^{125}I$ or $^{35}S$ or with more than one $^{3}H$ atom per molecule, highly potent drugs may require $^{3}H$ rather than $^{14}C$, etc). The ideal position of the label (from the point of metabolism or to track a specific breakdown product or fragment) will need to be determined. This latter point also has implications for the synthetic route, the economics of synthesis, and the stability of the final radiolabelled molecule.

While tritium may be used for initial research studies (because it can be introduced non-specifically, greatly simplifying the synthesis and shortening the preparation time) the isotope of choice for definitive DMPK or ADME studies is usually carbon-14.

## Carbon-14

Although this isotope of carbon is present naturally in the environment and is assimilated by living organisms, its abundance is less than one atom in a million. It was first prepared artificially in 1940 and its significance was immediately recognised. With a half life of 5,730 years, this low energy ß-emitter could be used as a marker without affecting the biological properties of a drug. Today the raw material for radiosynthesis is produced by high-flux neutron bombardment of a suitable nitrogen compound in a nuclear reactor. Isolation and purification ultimately provides carbon-14 as barium [$^{14}$C] carbonate with a high specific activity of ca 2GBq/mmol.

Radiosynthesis often has to start at a much earlier stage than the non-labelled drug since very few advanced carbon-14 intermediates are available. A route may have to be developed from [$^{14}$C]cyanide or from [$^{14}$C]carbon dioxide. If a substituted aromatic is required, uniformly labelled [$^{14}$C]benzene may serve as a precursor while even the total synthesis of a labelled heterocyclic from simple labelled acyclic precursors may be needed. Thus synthetic options and route development are key challenges for all specialist, highly skilled radiosynthesis groups.

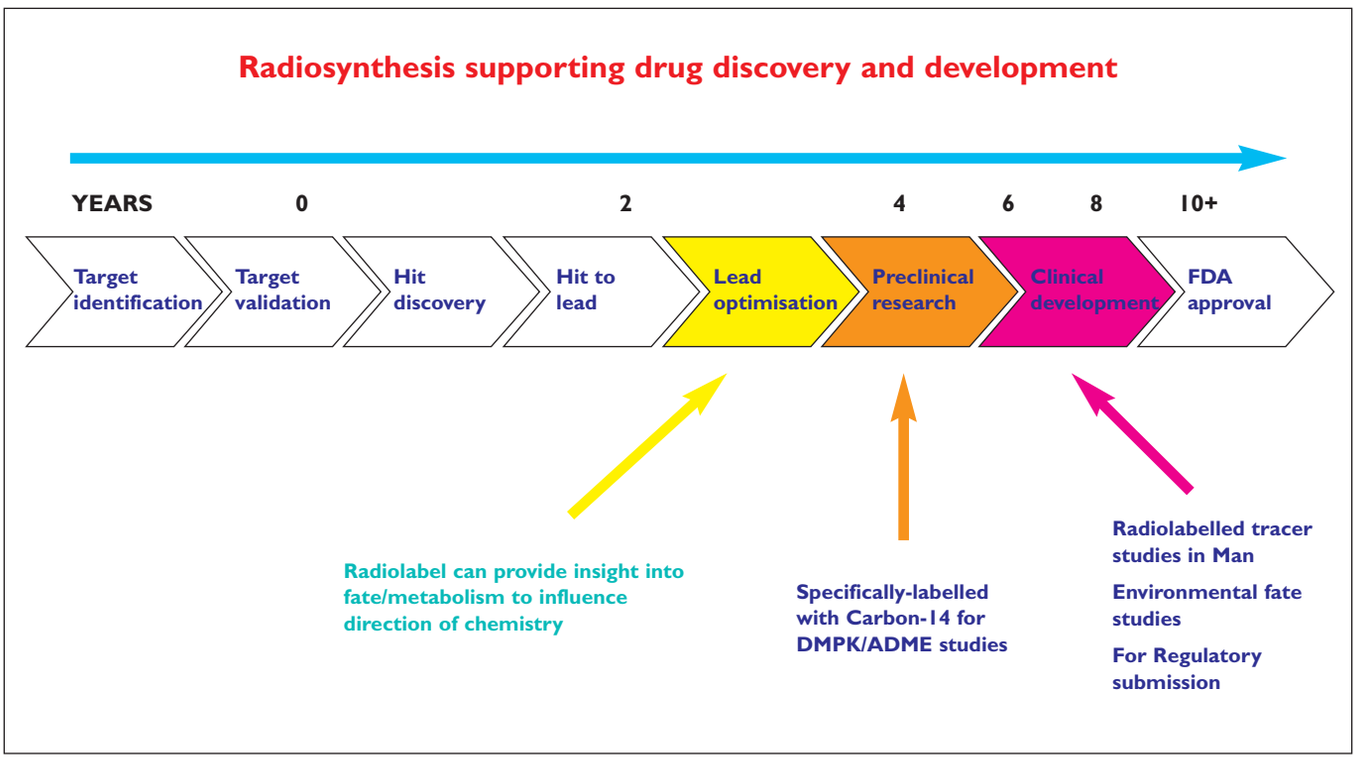
Because $^{14}$C is a low energy beta emitter, standard laboratory glassware can be used to store radioactive $^{14}$C compounds and is more than adequate to completely contain the beta radiation emitted. An accidental spill of a solid phase $^{14}$C compound on healthy, unbroken skin would have no detrimental effect so long as it was washed away.

Typically for DMPK/ADME studies some 10-20mCi (370-740MBq) of final radiolabelled product are produced at a cost in the region of $20-50,000. In isolation, the 'raw' cost of a radiolabelled tracer may appear expensive to the customer. However, when viewed within the context of the information the tracer can provide, the true cost is far less prohibitive.

In any multi-step synthesis most of the feedstock will end up as radioactive waste. Great care is therefore needed to optimise the synthetic route. Discharge to the environment and radioactive disposal are strictly regulated, require licences, are monitored, reported and inspected. Radiochemists must also be aware of the radiolytic instability of the radiolabelled tracer, typically carbon-14 compounds degrade at a rate of several per cent per year. However, this radiolysis rate is neither predicable nor constant and means that compounds which have been stored for any length of time require purity checks and often need repurification before use.

## Radiosynthesis in Big Pharma

Clearly, radiolabelled compounds need to be provided in good time to meet Big Pharma's study deadlines, but not so early that radioactive degradation compromises the purity of the compound. Confidentially is also important as many of these labelled final products are key proprietary drug development candidates. These are some of the reasons why Big Pharma has internal radiosynthesis




groups. However, since isotopes are utilised within the entire R&D process, such groups are often organisational orphans neither operating fully in drug discovery (eg, in Medicinal Chemistry), nor fully within a relevant development function.

Stricter regulation, a diminishing pool of trained radiochemists, highly focused and limited company objectives and high costs associated with decommissioning radioactive facilities, have led new mid-size and biotech companies to explore radiochemistry outsourcing options. Big Pharma has also found that these new CROs can provide buffer capacity and can function as an extension of their own internal laboratories.

There are some 25 Big Pharma companies with significant internal radiosynthesis capability. These vary from large groups on single sites, such as Merck (US) and GSK (UK); to companies having two groups, for example Sanofi-Synthelabo (UK and France) and Novartis (US and Switzerland). AstraZeneca is unique in having groups in UK, Sweden and the US, each supporting different therapeutic areas.

Consolidation within the pharmaceutical industry has led to site closures and rationalisation of activities. As a result many radiosynthesis groups have disappeared altogether. This consolidation however has provided opportunities for spin outs of radiosynthesis groups.

## Custom radiosynthesis companies

Mid-size pharma and biotech companies, focused on expanding and progressing compounds in their pipelines, have a clear requirement for information on the DMPK profile of their compounds. When a candidate has been identified to enter Phase 1, *in vivo* studies using a radiolabelled compound can provide key information on its metabolic fate. The knowledge gleaned at this stage will help progress or kill a compound, thus reducing costly late attrition. These studies are often performed by contract laboratories (eg, Quintiles, Inveresk, Covance, etc) that may outsource synthesis of the radiolabelled compound.

These are some of the key factors that are driving market evolution, creating a requirement for the synthesis of final products, as opposed to early stage intermediates and off-the-shelf catalogue items such as radiolabelled ligands.

This has provided an opportunity for new CRO companies to synthesise these final radiolabelled compounds.

Amersham (now GE) has been in this market since the 1960s. In the 1980s the company was part of the UK government's first privatisation wave. The carbon-14 business operates from its Cardiff research centre and represents only a small percentage of the UK operation. Among joint ventures announced by Amersham are those with

radiosynthesis groups in Russia (Revis) and Hungary (Izotop).

Another long established player is PerkinElmer which began life as New England Nuclear (NEN) before being taken over and then later sold by DuPont. It operates from a facility in Boston (Massachusetts) where most of its radiosynthesis activity is focused on radiopharmaceuticals.

ChemSyn has a 20-year pedigree as part of the Mid West Research Institute. The company is now owned by EaglePicher and continues to be based in Lenexa (Kansas). Other companies in the US include Moravek (Los Angeles, CA) and American Radiolabelled Chemicals (ARC), the latter having an extensive catalogue of intermediates. Recent newcomers include Vitrax (California) and Girindus (Ohio). Some companies provide synthesised radiolabelled compounds as part of a wider DMPK service and environmental fate studies regulatory packages, for example ABC Labs (Kansas).

Biodynamics Research has recently moved to a new facility in Rushden (UK) where a radiosynthesis group supports internal drug metabolism and environmental fate study group. The company has disclosed links with ACE for environmental fate work with pesticides and LCE in the area of clinical trials. Biodynamics had its origins in the former Hoechst research site in Milton Keynes while their radiosynthesis group had its origins with ICI at Billingham, both in the UK.

SCYNEXIS, headquartered in Research Triangle Park (North Carolina), span out of Aventis in 2000 and is a chemistry-focused company supporting pharmaceutical research. Its radiosynthesis group is based at its European subsidiary, SCYNEXIS Europe which originated from within Aventis when the company consolidated its crop science research in Germany and France at the end of 2001. Rather than establish a new radiochemistry group, Aventis CropScience sub-contacted the work previously undertaken by its internal group to SCYNEXIS. This radiosynthesis group includes experienced industrial radiochemists from Rhone Poulenc and AgrEvo and now numbers 10 radiochemists. One of the key features is the close coupling of analytical services to radiochemistry to ensure the chemical and radiochemical purity of the products.

Tocris is based in Bristol (UK) and has a small radiosynthesis group. It offers radiolabelled ligands as part of its extensive catalogue of ligands and patent markers.

Daiichi Pure Chemical has a large share of the Japanese market for custom radiosynthesis just behind Amersham. It has published collaborations with PerkinElmer, EaglePicher and Moravek all from the US. The company operates from the former Japanese government radioactive facility at Ibaraki.

## Future trends

### Microdosing

Microdosing is an emerging technique receiving some attention as a tool for 'first in human' studies. It relies on parent detection using a new technology known as accelerator mass spectrometry

## Reference

1 European Commission Proposal from 29 October 2003COM (2003) 644 final: Proposal for the Registration, Evaluation and Authorization and Restriction of Chemicals (REACH), establishing a European Chemicals Agency and amending Directive 1999/45/EC and Regulation (EC) on Persistent Organic Pollutants.

(AMS) to provide quantification at several orders of magnitude below conventional limits. With doses 100-fold below those encountered for therapeutic purposes, there is no biological effect, thus reducing any potential risk to human volunteers yet providing initial PK and ADME information.

By the application of AMS as the analytical procedure the dose of carbon-14 administered in studies involving human volunteers can be reduced from microCuries to nanoCuries. The increased detection efficiency relies upon detection of all the carbon-14 atoms via a specialised accelerator-induced ionisation (coupled with MS quantification), not just the small percentage of atoms that undergo radioactive decay during ß-counting. A consortium is currently working on this project lead by Xceleron of York (UK). Time will tell how this technique will evolve. It is not clear yet how this will impact on the numbers of projects for human trials work with carbon–14. While this technique may allow 'first in human studies' to be done with early stage candidates there is a possible contradiction with the move to apply cGMP synthesis for all materials administered to humans.

**cGMP 14C-labelled APIs for use in human mass balance studies**

While basic quality principles have been applied by many CROs to the synthesis of radiolabelled compounds for non-clinical studies, the implementation of the clinical trials directive, 2001/20/EC, in May 2004 has caused prolonged debate throughout the industry. Radiolabelled compounds, by their nature, are unstable creating analytical challenges. Moreover, the material is frequently diluted with 'cold' material before administration so the radiolabel component may even be present at lower levels than impurities in the parent compound. However, the directive is clear in that the drug substance used in the formulation of investigational medicinal products destined for clinical trials must be manufactured in accordance with appropriate principles of cGMP.

Previously, material for human use was prepared under the FDA guidelines 21 CFR pt 361. Now, CROs work with individual clients to develop auditable procedures that ensure the risk assessment demonstrates that the labelled material is safe for administration to humans. This may involve use of segregated and dedicated facilities, use of new glassware, full audit trial of all materials, solvents and procedures all carried out and witnessed 'Quality Assured' by trained and competent staff. It is clear that many of the leading players are now able to offer synthesis of radiolabelled compounds to GMP principles similar to the above.

## Conclusion

In the last decade poor control of physico-chemical properties and a lack of understanding of the importance of ADME were among the major reasons why new drug candidates failed during development. With the emergence of computational tools and new *in vitro* HTS screening, these issues have been largely overcome.

However, the attrition rate stubbornly fails to improve and one of the major issues today is how to predict poor drug safety including adverse drug reactions.

The quality of risk assessment more than ever relies upon the availability of radiolabelled compounds for *in vivo* studies in man. Indeed, a sound safety file backed up with information on intrinsic clearance, the metabolic pattern and incidence of reactive intermediates will provide a competitive advantage which adds to any drug candidate's valuation.

Testing using radiolabelled compounds in preclinical studies and clinical trials has the potential to minimise errors of extrapolation between other species and humans. Microdosing also now offers the prospect of the possibility of going directly into human studies using lower doses of radiolabelled compounds with reduced risk to volunteers.

Radiolabelled compounds will continue to play a key role is ensuring new drugs meet ever challenging safety standards. With improved quality and radiosynthesis to GMP principles, custom radiosynthesis companies will continue the trend of increased outsourcing of radiosynthesis from Big Pharma companies. DDW

***Dave Roberts*** *is Head of Radiochemistry with SCYNEXIS Europe Ltd. He has more than 20 years' industrial experience with Aventis and Rhone-Poulenc. He is one on the co-inventers of the animal health insecticide Fipronil and the maize herbicide Isoxazflutole.*

***Bill Lockley*** *is currently a Professorial Research Fellow at Surrey University. He was previously Head of Radiochemistry at AstraZeneca, Charnwood and has more than 25 years of experience in industrial radiochemistry, DMPK and bioanalysis.*