INTERNATIONAL UNION OF
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ANALYTICAL CHEMISTRY DIVISION

COMMISSION ON ANALYTICAL RADIOCHEMISTRY
AND NUCLEAR MATERIALS

# AN ENQUIRY INTO THE PURITY OF COMMERCIAL RADIOCHEMICALS

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# AN ENQUIRY INTO THE PURITY OF COMMERCIAL RADIOCHEMICALS

## INTRODUCTION

The purity of reagents has always been and will always be a prime concern of all chemists. Manufacturers have responded well to the many demands that the laboratory worker has made in this connection and a relatively wide variety of reagents for general and specific purposes is available, relieving the chemist of the necessity of purifying inadequate commercial products. In general, reagents are available nowadays for almost all purposes in a state of high purity and stability. As a recent example, one may cite the production of especially pure reagents for use in the manufacture and analysis of transistor material in the electronics industry.

One class of modern reagent, however, is by its very nature unstable and that is the radioactively labelled reagent, the "labelled" or "tagged" molecule. Within a very few years of their introduction, hundreds of radiochemical reagents, the majority labelled with carbon-14 or tritium, but also including sulphur-35, phosphorus-32, iodine-125 and -131 and many others have been put on the market. The volume of business today amounts to several millions of dollars per year. It is a common experience that at the beginning of most developments there is dissatisfaction as difficulties are recognised and first attempts to solve them are often unsuccessful. At the present time, the radiochemical manufacturers are paying serious attention to the problems of stability and an excellent survey has been published by Bayley and Evans [*J. Labelled Molecules*, II(1)1, (1966)] which gives much practical information about the decomposition of labelled molecules. However, it cannot be claimed that the problem of self decomposition is well understood. Empirical experiments have shown certain general rules which minimise decomposition on storage but much remains to be clarified. This is well illustrated by the reported result that sulphur 35–DL methionine is more stable on storage in the solid state than similarly labelled L-methionine (see ref. above p. 179).

The reason labelled compounds decompose is easy to understand. In the first instance, a radioactive atom decaying by beta- or positron emission (the

† *Titular Members:* G. B. Cook, Chairman (Austria); W. W. Meinke, Secretary (U.S.A.); M. B. A. Crespi (Argentina); J. Minczewski (Poland); A. A. Smales (U.K.); *Associate Members:* F. Hecht (Austria); L. Kosta (Yugoslavia); J. Platzer (France); R. Wainerdi (U.S.A.).

commonest form of radionuclide employed) produces an atom of different atomic number to that originally incorporated into the molecule. In addition to this, the act of radioactive decay imparts considerable excitation to the molecules or fragments produced. Thus in the case of the decay of the tritium-labelled toluene more than 25 ionised fragments have been recognised. While none of these will be radioactive (since double labelling of a molecule is relatively rare), the fragments can react with the environment to produce other chemical species. However, this effect is not very important, as the numbers of radioactive atoms decaying in the usable lifetime of a preparation is relatively small. Much more important is the effect of beta particles and gamma rays of the preparation on itself by producing ions and free radicles from the compound and any solvent or added material present. Such species can react both with unlabelled and labelled molecules to produce new chemical species, in some cases in appreciable amount. In addition to this source of impurity, one should consider also the normal chemical stability of the preparation and the possibility of impurities present due to incomplete purification of preparations often made in relatively small quantity.

As a result of several bad experiences reported by some laboratories to the Joint Commission on Applied Radioactivity of the International Council of Scientific Unions, this Commission interested Commission V-7 of the Analytical Division of IUPAC in this problem. The most direct method of assessing the situation seemed to be that of a questionnaire circulated to workers actively engaged in the use of labelled molecules. This was done. during 1967–1968 with the results reported here.

## THE QUESTIONNAIRE

The questionnaire was a relatively simple one and five questions were put. The first two asked whether the laboratory concerned was a regular or only an occasional user of labelled molecules, and whether each shipment received was always, occasionally or never analysed. The third question was "Have you ever found that the purity was insufficient for the intended uses without further purification". The fourth and fifth questions required answers to the analytical methods used and whether any particular class of compound (e.g. small molecule intermediates; carbohydrates; steroids and lipids; proteins, peptides and amino acids; nucleic acids, nucleotides, nucleosides, purines, pyrimidines, etc.) was in the experience of the laboratory worse than others.

## THE REPLIES

There was about a 60% response to the questionnaire with answers received from 66 laboratories in 18 countries, 63 answers being of use to the full scope of the enquiry. The majority of laboratories were concerned with biochemical and organic research and some with clinical investigations. This is not surprising as it is in biochemistry that the widest use of labelled molecules is made. The major part of the respondents were regular users, only 18 of them classed themselves as occasional.

When the results were examined, the presence of impurities in all classes

of compounds is recognised as an important factor. It should perhaps be pointed out here that in the preamble to the questionnaire, respondents were asked to try to distinguish between the products of self radiolysis and the impurities resulting from a poor purification of the initial preparation. In view of the incomplete knowledge about autoradiolytic products, however, this aspect of the question is probably meaningless and answers reflect impurities from any cause. 36 laboratories reported having found significant impurities and this represents 84 reports on (often multiple) examinations of the various general classes of compounds listed because many laboratories reported observations on more than one class. Sixty of these noted the presence of an unacceptable level of impurities corresponding to 70% of the total number of results.

On the question of the frequency of analysis, 17 laboratories analysed all shipments, 36 occasionally and 10 never. When these results are combined with those just indicated on satisfaction concerning purity, some interesting observations can be made. As mentioned above, 36 laboratories have found the purity of preparations at some time or other insufficient. Of the rest, three did not provide enough data and 26 had always found purity sufficient. However, of the latter class only one always analysed shipments (out of a total of 17 who always analyse), 16 occasionally (out of 36 who occasionally analysed) and 9 never (out of 10). At least two interpretations can be placed on these results. Either the purity was sufficient for the purpose and impurities had no disturbing effects, or the effect of impurities, if present, did not obviously manifest themselves in the results. However, such effect may have been present and not recognised and could have effected the interpretation of the results if taken into account.

Regarding the methods of analysis of preparations, these were in practically all cases thin-layer or paper chromatography combined with autoradiography or radio scanning, gas chromatography with radioactive detectors and sometimes electrophoresis. It was also noticed that no laboratory which was satisfied with purity and which analysed occasionally gave their analytical methods, so it is difficult to judge the adequacy of the techniques used.

On the final question of whether any class of compound was generally less satisfactory than others, no definite answer can be given. Impurity was found in almost every class and few laboratories singled out a particular one as worse than others, although it should be pointed out that most laboratories did not use all or even a majority of the classes of compounds available so that their direct comparative experience is limited. Of the classes mentioned more than once, steroids were classed as worst by 4 laboratories; but as 3 worked only with steroids, there is no real comparative basis. Carbohydrates were mentioned by two groups, one of which has experience with all classes of compounds and the other only with 3. Several cases of aminoacids were also singled out but only in terms of individual compounds and not as a class. The only other class mentioned specifically was lipids but this by only one laboratory.

Another aspect of the situation is presented by remarks from some workers. Two commented that, although they had found significant impurity, the trouble was rare or seldom. Another, with many years of

experience with nucleotides and nucleic acids, commented that during recent years, the situation has improved considerably, which is supported by that of another who found trouble relatively infrequent with nucleosides and nucleotides (and also aminoacids).

One other general aspect arises out of the remarks from three laboratories that in general tritium-labelled compounds were worse than the carbon-14 analogues. Two of these laboratories specifically referred to steroids. This may be due to two reasons. Firstly the specific activity of tritium-labelled compounds is in general much higher than those of $^{14}C$ to such an extent that, after allowing for the difference in beta-particle energy of the two radionuclides, the energy dissipated in the sample is greater for tritium than for carbon 14 and correspondingly the amount of self-radiolysis is expected to be greater for the tritium compound. A second consideration may be that some methods of production of tritiated compounds, e.g. the Wilzbach and related processes, produce a wide variety of products, many of high specific activity which inevitably complicate purification procedures. However, it is not known to what extent these methods are used for commercial production.

## CONCLUSION

The occurrence of significant levels of impurity in commercial radiochemicals is undoubtably wide spread, although what constitutes a significant amount is not a constant quantity as the requirements for one experiment may be more stringent than for another. No one class of compound is clearly worse than another. Although several were mentioned by more than one laboratory, statistically they cannot be regarded as very significant and could perhaps reflect a larger variety of compounds available in that class or more stringent requirements for the experiments undertaken. What is not revealed is the extent to which impurities are due to production methods, in which case a rapid improvement is possible, and to what extent they are due purely to autoradiolysis. In the latter case, undesirable compounds will always be present as nothing can stop the dissipation of beta-particle and gamma-ray energy in the sample environment, and the manufacturer can only improve the situation by research into storage methods, but he cannot eliminate it.

What can clearly be recommended is that all users of radioactive compounds analyse their shipments, as the majority of experienced laboratories already do, by suitably sensitive methods. It should be remembered, however, that the system is not static, and analysis may be necessary before each use to check that significant changes have not occurred in the time between experiments.

It is hopeful that the situation seems to be improving and the problem is, we believe, receiving serious and continuous attention by the manufacturers and we can hope for further improvement with time.