

TRIP 003527689

AAEC/E.589



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**AUSTRALIAN ATOMIC ENERGY COMMISSION  
RESEARCH ESTABLISHMENT**

**LUCAS HEIGHTS RESEARCH LABORATORIES**

**STUDIES ON  $^{99m}\text{Tc}$ -PERTECHNETATE FROM THE  
MEK SOLVENT EXTRACTION GENERATOR**

by

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**DECEMBER 1984**

ISBN 0 642 59799 5

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ABSTRACT

Analysis by gas chromatography-mass spectrometry and high performance liquid chromatography has revealed organic residues in  $^{99m}\text{Tc}$ -pertechnetate obtained from  $^{99}\text{Mo}$ -molybdate by extraction, using the organic solvent methylethylketone (MEK). The organic residues have been identified as either

- (i) low molecular weight carbonyl compounds such as formaldehyde, acetaldehyde and acetone, presumably caused by the effects of  $\gamma$ -radiation on MEK, or
- (ii) condensation products resulting from the action of strong alkali on MEK during the extraction process.

The quantities of organic residues varied from batch to batch of extracted pertechnetate; up to  $40 \mu\text{g mL}^{-1}$  was found. When these compounds were tested, in rats, by addition to a pyrophosphate bone-seeking radiopharmaceutical, the tissue distribution was not significantly different from that in the control, which contained no added compound.

Assay for  $^{99}\text{Tc}$  in MEK-derived pertechnetate indicated up to  $10 \mu\text{g mL}^{-1}$  of  $^{99}\text{Tc}$  carrier. An assessment of the biological effect of  $^{99}\text{Tc}$  carrier was obtained by (i) red blood cell labelling, where  $6 \text{ ng mL}^{-1}$  of  $^{99}\text{Tc}$  was sufficient to reduce labelling efficiency; and (ii) pyrophosphate tissue distribution, where a significant effect was obtained in the presence of  $10 \mu\text{g mL}^{-1}$  of  $^{99}\text{Tc}$  carrier.

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National Library of Australia card number and ISBN 0 642 59799 5

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ERYTHROCYTES; IMPURITIES; KETONES; LABELLING; PERTECHNETATES; RADIONUCLIDE KINETICS; SOLVENT EXTRACTION; TECHNETIUM 99

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## 1. INTRODUCTION

Solvent extraction generators offer many advantages for the efficient production of  $^{99m}\text{Tc}$ , an isotope used for a wide range of radiopharmaceutical applications [Boyd 1982]. These include low cost per unit activity and the ability to use low specific activity  $^{99}\text{Mo}$ , yielding a high radioactive concentration of  $^{99m}\text{Tc}$  in the extract. Of major concern, however, is a number of reports that  $^{99m}\text{Tc}$  extracted with methylethylketone (MEK) has produced poor imaging quality in various  $^{99m}\text{Tc}$  labelled radiopharmaceuticals compared with those obtained with  $^{99m}\text{Tc}$  extracted by adsorption chromatography with alumina. For example, Wieland *et al.* [1974] and Yeates *et al.* [1974] claimed poor labelling efficiency of albumin microspheres with  $^{99m}\text{Tc}$  obtained from an MEK extraction generator.

Two reasons have been suggested as the cause of poor labelling characteristics:

- (a) the presence of organic residues in the MEK derived  $^{99m}\text{TcO}_4^-$  [Boyd 1982]; and/or
- (b) the presence of excessive quantities of  $^{99}\text{Tc}$  carrier [Richards 1975].

An investigation has been made of these possibilities.

## 2. EXPERIMENTAL

### 2.1 Materials

The following materials were supplied by the AAEC's Radioactive Products Research Section:

- (i) **Pertechnetate-M.** Samples of MEK-extracted sodium pertechnetate (which conforms with BP specifications) in isotonic saline solution were allowed to decay until the total activity was less than 2 MBq.
- (ii) **Pertechnetate-Q.** Samples of pertechnetate from the alumina chromatographic generator (which conforms to BP specifications) with an average activity of 1 GBq.
- (iii) **MEK-generator washing.** Samples of MEK flushed through the solvent generator.

Carrier Tc was obtained from Amersham International Ltd as  $^{99}\text{Tc}$ -ammonium pertechnetate in 0.1 M  $\text{NH}_4\text{OH}$ .

Methylethylketone (MEK - Ajax Chemical Co, LR grade) was redistilled before use, after drying with anhydrous potassium carbonate, and collecting the fraction boiling in the range 79 to 81°C at atmospheric pressure.

Alkali-treated MEK was prepared by thoroughly mixing equal volumes of distilled MEK and 5 M KOH for one hour. The two phases were separated and the MEK layer was evaporated to dryness under  $\text{N}_2$ . One hundred microlitres of redistilled MEK were added to redissolve the residue for further analysis. Samples were irradiated in the AAEC's gamma irradiation pond.

Phenylhydrazine and 2,4-dinitrophenylhydrazine (BDH Chemicals) were used as derivatising agents according to the method of Vogel [1979]. All other chemicals and solvents were AR grade (Ajax Chemical Co).

### 2.2 Instrumentation

A Hewlett Packard model 5720A gas chromatograph (GC), equipped with a hydrogen flame ionisation detector, was used with a 5 mm  $\times$  1 m glass column packed with 3 per cent OV-17 on Gas-Chrom Q (Applied Science Lab.) and nitrogen as carrier gas (60 mL  $\text{min}^{-1}$ ).

An Altex model 330 isocratic high performance liquid chromatograph (HPLC), consisting of a model 110A pump and fixed wavelength (254 nm) u.v. detector, was used with a reverse phase RP-8, 10  $\mu\text{m}$  column (Brownlee Laboratories) and methanol-water (70:30) as the mobile phase.

Gas chromatography-mass spectrometry (GC-MS) was performed on a Finnigan 6110-9500 system, with a 1 m column of 3 per cent OV-17 on Gas-Chrom Q using methane as carrier gas or, alternatively, on a 50 m capillary column coated with SE-30.

Chemical ionisation mass spectra of freeze-dried fractions from HPLC were obtained using the solid probe inlet of the Finnigan system.

Polarograms were obtained using a Princeton Applied Research Corporation model 364 polarographic analyser in the differential pulse mode. A three-electrode system, consisting of a dropping mercury electrode with a controlled drop time of 1 s, a platinum counter electrode and a reference saturated calomel electrode (SCE), was used. The polarographic scan was made from -0.2 V to -0.7 V (v. SCE) at a rate of 2 mV s<sup>-1</sup> and recorded on an Omnigraphic 2000 X-Y plotter.

Liquid scintillation counting was performed with a Packard Tri-Carb model 3255 liquid scintillation spectrometer using Instagel (Packard) as the scintillation cocktail. Gamma-counting was carried out on either a Nuclear Chicago gamma-scintillation counter or a Centronic ionisation chamber type 1G12-A20.

## 2.3 Biological Studies

### 2.3.1 Red blood cell labelling

The labelling method is a modification of one developed at Brookhaven National Laboratory [Smith and Richards 1975]. About 6 mL of whole blood was collected from male Australian Albino Wistar (AAW) rats in a 10 mL syringe containing 0.05 mL of 250 IU heparin (Commonwealth Serum Laboratory, Melbourne) as an anti-coagulant. Two millilitres of isotonic saline were added to freeze-dried renal imaging agent (AAEC) which had been allowed to warm to room temperature. After five minutes of incubation at room temperature, 0.2 mL of the solution was added to the blood sample contained in the evacuated tube. This was again incubated at room temperature for five minutes, with intermittent gentle mixing. The tube was centrifuged in the inverted position (so that the red blood cells settled on the stopper and were readily removed by syringe) for five minutes at 1300 g.

Five millilitres of saline were added to the sample with shaking to dissolve all the residue. Of this solution, 2 mL was taken into a separate tube, 300 mBq (0.05 mL) of pertechnetate-Q was added and the solution thoroughly mixed.

The tube was removed, in the inverted position, from the centrifuge and a 5 mL syringe (with all air expelled) was inserted into the tube, keeping the needle point below the packed red blood cells. Two millilitres of the red blood cells were gently drawn into the syringe and added to the tube containing the pertechnetate/sample mixture. The solution was mixed and incubated at room temperature for another five minutes with occasional stirring. After incubation, the tube was again centrifuged at 1500 g for five minutes. The supernatant was then carefully withdrawn into a separate tube. The activity was measured in an ion chamber by first measuring the total activity and then the individual activity of the red blood cells and the supernatant. The percentage of labelled red blood cells was calculated. The control determination used 2 mL of saline instead of the sample solution. Each determination was performed in duplicate.

### 2.3.2 Tissue distribution studies

A bone-seeking radiopharmaceutical, Technescan PYP, containing 12.0 mg of sodium pyrophosphate and 3.4 mg of anhydrous stannous chloride, was obtained from Mallinckrodt as a freeze-dried preparation. One millilitre of pertechnetate-Q (approximate activity 100 MBq mL<sup>-1</sup>) was added to either

- (i) 1 mL of isotonic saline containing the test compounds at appropriate concentrations; or
- (ii) 2 mL of saline solution containing <sup>99</sup>Tc carrier at the required concentration. This solution was added to the PYP reagent, shaken and allowed to equilibrate for 10 min. A control was prepared with no added test compound. A standard was made by diluting 90 µL of the solution to 10 mL with water; two 1 mL samples of the diluted solution were then counted.

The radiochemical purity of the radiopharmaceutical was checked by thin-layer chromatography (TLC) using Gelman ITLC strips developed in butanol-ethanol (1:1) mixture [Farrington 1983]. Less than 5 per cent free pertechnetate was found in all samples.

Five female AAW rats were used for each set of samples. The rats were anaesthetised by placing them in an ether box. Each rat was held in a rat restrainer and 90 µL of the test solution injected into the tail vein. The treated rats were then placed in the metabolic cage. After 90 minutes, the rats were sacrificed, ensuring that they had urinated fully before removal from the metabolic cage. The body weight of each animal was recorded to 0.1 g. The rat was dissected and tissue samples of approximately 1 to 2 g were collected. The following tissues were sampled:

Blood (approximately 1 mL)	One kidney
Whole gut including caecum and rectum (subsamped later)	Skin (~ 10 cm <sup>2</sup> )
Bladder	Muscle from hind leg
Whole liver (subsamped later)	Both femurs (scraping off all clinging muscle)
Lungs	Tail (about two thirds, including the site of injection)
Spleen	Urine
Heart	

The weight of the whole liver was recorded and about 1 to 2 g sampled for counting. The sides of the metabolic cage were washed with water and the washings collected together with the urine. These were then weighed and a 1 mL aliquot put into the sample tube. The weights of all samples were recorded, and the sample tubes and standards counted in the scintillation counter. Activities were corrected to the initial counting time of the standard.

The final results were calculated, using the TISCON program [Maddalena 1983], both as a percentage of the initial dose and as a percentage injected dose (% ID) per gram of tissue, where:

$$\% ID = \frac{\text{Total organ counts}}{\text{Standard counts}} \times 100$$

$$\% ID \text{ per gram of tissue} = \frac{\% ID}{\text{Total organ weight}}$$

For muscle, skin and blood the following factors were used to estimate the total organ weights [Maddalena 1983]:

Muscle	=	0.45	×	body weight
Skin	=	0.20	×	body weight
Blood	=	0.065	×	body weight

Statistical analysis of the tissue distribution studies was performed using the Statistical Package for the Social Sciences (SPSS version 8.0) on the University of Sydney's Cyber 70 computer. The tests included (i) one-way analysis of variance (F-ratio), and (ii) the unpaired two-tailed t-test of significance [Nie *et al.* 1975].

### 3. RESULTS AND DISCUSSION

#### 3.1 Identification and Quantification of Organic Residues from MEK

##### 3.1.1 Pertechnetate samples

Pertechnetate-M samples in which the <sup>99m</sup>Tc had decayed to a total activity of less than 2 MBq were analysed. Several approaches were tried.

Direct GC-MS analysis was performed, after concentrating any organic residues, on extracts obtained by passing 10 mL of pertechnetate-M through a Sep-pak cartridge (filled with ODS HPLC stationary phase packing) then washing through with methanol. The methanol extract was reduced to ~ 25  $\mu$ L; 1  $\mu$ L was then injected into the GC. Small amounts of seven components were detected, five of which had a molecular ion with an m/e value of 142, corresponding to a molecular formula of C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>, which could be a dimer of MEK (mass 72) with the loss of two protons. The other components had m/e values of 114 and 140. The quantities detected were extremely small and varied from sample to sample.

On the basis of this evidence, it is likely that the residues contained at least one carbonyl function. If this were the case, carbonyl derivatisation would improve the sensitivity of detection to the nanogram level [Fung and Grosjean 1981].

Phenylhydrazone derivatives were prepared and chromatographed on OV-17 (initial temperature 150°C for 6 min, then programmed to 230°C at 8°C per min). Although several peaks were observed, together with batch-to-batch variation, GC-MS analysis indicated that some thermal decomposition had occurred and that the mass spectra were difficult to interpret.

Derivatisation with 2,4-dinitrophenylhydrazone (DNPH) yielded more stable compounds, but the higher molecular weight led to long retention times at high operating temperatures ( $> 210^{\circ}\text{C}$ ) for derivatives of ketones having molecular weights less than 100. Separation was therefore effected on reverse phase HPLC; fractions which corresponded to peaks in the chromatogram were freeze-dried and analysed by mass spectrometry.

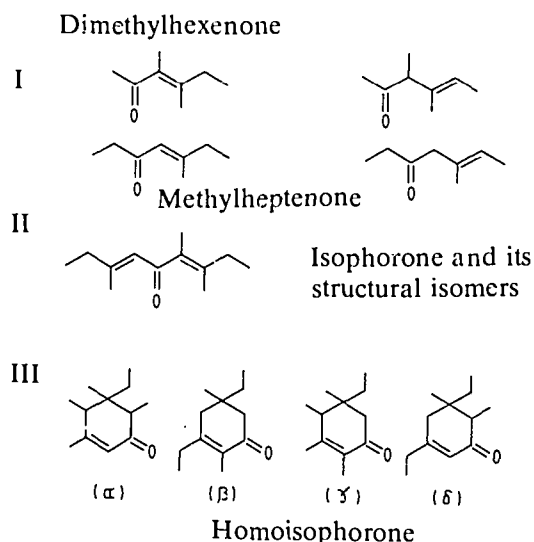
By these means, pertechnetate-M samples were found to contain low molecular weight carbonyl compounds (formaldehyde, acetaldehyde, acetone and MEK) following derivatisation by DNPH and HPLC analysis. Batch-to-batch variation of the amounts of these carbonyls in pertechnetate-M samples was of an order of magnitude, as shown in Table 1. Significant quantities of other carbonyl-DNPH derivatives were not detected. These low molecular weight carbonyl compounds are present as contaminants in the solvents used but at levels an order of magnitude less than the average found in pertechnetate-M.

Akhtar *et al.* [1975] reported the presence of acetaldehyde and acetone among the  $\gamma$ -radiolysis products of MEK, which also included  $\text{H}_2$ ,  $\text{CO}$ , and  $\text{CH}_4$ . They attributed the formation of acetaldehyde and ethylene to an intramolecular rearrangement involving the transfer of a  $\beta$ -hydrogen from an excited MEK molecule. Ausloos and Paulson [1953] also found acetaldehyde and ethylene in the vapour and liquid phase radiolysis of MEK. It is conceivable that some of the low molecular weight carbonyl compounds are due to the effect of  $^{99}\text{Mo}$  or  $^{99\text{m}}\text{Tc}$   $\gamma$ -radiation on the solvent MEK during the extraction process.

### 3.1.2 Alkali-treated MEK

In the production of  $^{99\text{m}}\text{Tc}$  by the solvent generator method, the MEK must come into contact with concentrated alkali to allow the formation of condensation products from the MEK. Narasimhan and Mani [1975] studied the reaction and found traces of condensation products in the extract; however, these were neither identified nor quantified.

The processes taking place in the solvent extraction generator were simulated by reacting alkali with MEK at room temperature. The object of this simulation was to prepare larger quantities of all possible contaminants of pertechnetate-M to enable identification of the minute amounts actually detected. The conditions of simulation were varied, with KOH concentrations of 1 to 10 *M* and equilibrium times of one hour to seven days. Longer equilibration time and stronger KOH solution led to altered product distribution.



Analysis of the resulting MEK by GC-MS revealed that the major product had an  $m/e$  of 144, suggesting keto-alcohol, the condensation product of MEK. Four other products were formed in increasing quantities as the KOH concentration, temperature and equilibration time increased. Two had molecular weights of 126, and others had molecular weights of 140 and 180. The 126 products correspond to a molecular formula of  $\text{C}_8\text{H}_{14}\text{O}$  and can be derived from the intermolecular condensation of MEK followed by dehydration to the various structural isomers of I [Franke and Kohler 1923; Abbott *et al.* 1928]. The 180 compound ( $\text{C}_{12}\text{H}_{20}\text{O}$ ) is attributed to isophorone II, homioisophorone III or their structural isomers [Ekeley and Howe 1923]. It is not understood how a compound with a molecular weight of 140 ( $\text{C}_8\text{H}_{12}\text{O}_2$ )



could be formed from the condensation of MEK.

High performance liquid chromatography of alkali-treated MEK reacted with DNPH confirmed the existence of molecules containing one carbonyl function each and having molecular weights of 144 and 140. However, derivatives corresponding to carbonyls of molecular weight 126 or 180 were not detected.

### 3.1.3 Generator washings

During the production of  $^{99m}\text{TcO}_4^-$  by the solvent extraction process, the MEK containing the extracted pertechnetate is evaporated to dryness, before dissolution of the residue in saline. The apparatus is washed at regular intervals to avoid the accumulation of contaminants in the system. Potentially, the generator washings provide a good source for investigating the presence of organic residues. The washings contain significant amounts of phthalate plasticisers, apparently as a result of minimal contact with plastic tubing during washing.

The washings were concentrated 100-fold and analysed by conventional (OV-17) and capillary (SE-30) column GC-MS. Apart from traces of common laboratory solvents, the principal species detected had molecular ions of 126, 128 and 72 (the last indicating a breakdown in the GC-MS) and very small amounts of 124, 140 and 142. The origin of the 128 peak is not clearly understood, but the molecular formulae  $\text{C}_7\text{H}_{12}\text{O}_2$  (diketone) or  $\text{C}_8\text{H}_{16}\text{O}$  (monoketone) are possible candidates. The other compounds were encountered in samples of alkali-treated MEK.

### 3.1.4 Irradiated MEK samples

Studies were made of the effect of external  $\gamma$ -irradiation on MEK, MEK- $\text{H}_2\text{O}$  (1:1) and MEK-5 *M* KOH (1:1). Each sample received a dose of 0.5 or  $1 \times 10^4$  Gy at the  $\gamma$ -irradiation pond and was then treated in the same manner as the alkali-MEK samples (see Section 3.1.2). Chromatograms of the irradiated MEK and MEK- $\text{H}_2\text{O}$  samples showed only the solvent (MEK) peak. The irradiated MEK-KOH sample gave a number of peaks which corresponded to those obtained from the unirradiated MEK-KOH, together with small amounts of a substance with molecular ions of 124 ( $\text{C}_8\text{H}_{12}\text{O}$ ) and 138 ( $\text{C}_8\text{H}_{10}\text{O}_2$ ). It appears that the effect of  $\gamma$ -irradiation on MEK is less significant than the effect of alkali.

## 3.2 Effect of MEK Residues on Sn(II)

The effect of residues from MEK and alkali-treated MEK on Sn(II) ions was analysed by polarography to determine whether oxidation of Sn(II) or complexation of Sn(II) were possible mechanisms for the production of contaminating organic residues. The half-wave potentials for Sn(II) and Sn(IV) are -0.40 V and -0.47 V (*v.* SCE) respectively. To obtain the Sn(II) wave, a supporting electrolyte of 1 *M* perchloric acid was required. If chloride ion ( $\text{Cl}^-$ ) is added, a proportion of the Sn(II) is oxidised to Sn(IV). Total tin content can be obtained (as Sn(IV)) using a supporting electrolyte containing 1 *M* HCl and 3 *M* NaCl.

Methylethylketone has an influence on the polarographic behaviour of Sn(II); the peak current is reduced but the half-wave potential remains the same. The reason for this effect is not understood, but it may be due to adsorption phenomena at the mercury drop surface [Heyrovsky and Kuta 1966] and not to a chemical reaction of the MEK.

Because of these potential interferences from MEK and the saline in which the pertechnetate was supplied, the standard addition technique was used. Two series of solutions were prepared: (a) 1 mL of pertechnetate-M was added to 25 mL of 1 *M* perchloric acid followed by from 6 to 36  $\mu\text{g L}^{-1}$  of Sn(II); and (b) 2 mL of pertechnetate-M was added to 25 mL 1 *M* perchloric acid followed by a lower range of Sn(II) concentration (4 to 12  $\mu\text{g mL}^{-1}$ ).

The peak current versus added Sn(II) concentration for the two assays is plotted in Figure 1. The intercept on the Sn(II) axis indicates the extent of the reaction of  $\text{TcO}_4^-$  with Sn(II). The Sn(II) equivalents per millilitre of added  $\text{TcO}_4^-$  were  $1.0 \pm 0.3 \mu\text{g mL}^{-1}$  for (a) and  $1.3 \pm 0.3 \mu\text{g mL}^{-1}$  for (b), and agree within experimental error. When the dilution (25-fold) and the stoichiometric equivalence (2 moles  $\text{TcO}_4^-$  to 3 moles Sn(II)) is taken into account, this corresponds to  $14 \pm 5 \mu\text{g }^{99}\text{Tc}$  per millilitre of pertechnetate-M sample, which is the upper limit of  $^{99}\text{Tc}$  found in the pertechnetate-M sample by liquid scintillation counting (Section 3.3). Because of the dilution, this determination was much less accurate than required for the identification of an effect which might be related to organic impurities. Consequently, it was necessary to use the simulated alkali-treated MEK samples.

Methylethylketone was equilibrated with 5 *M* KOH for 1 hour in sufficient quantities to ensure at least five 10 mL samples when the MEK layer was separated. Each of the 10 mL MEK layers was evaporated under nitrogen and 25 mL of 1 *M* perchloric acid was added to the residue. Amounts of Sn(II) in the range 6 to 36  $\mu\text{g mL}^{-1}$  were added and the peak current was plotted versus Sn(II) concentration (Figure 2). It can be seen that a straight line passes through the origin.

In a second series, the MEK was equilibrated overnight (18 hours) with 5 *M* KOH with a lower Sn(II) concentration range of 4 to 12  $\mu\text{g mL}^{-1}$ . Again, the line passes through the origin (Figure 2). From both series of experiments, it is concluded that the organic residue from alkali-treated MEK has no detectable effect on Sn(II).

In a third series, the equilibration was carried out in the presence of molybdate (300 mg per mL of MEK). After equilibration for one hour, the MEK layer was treated as described above. The presence of molybdate affected the shape and height of the Sn(II) peak. However, when the peak current at -0.4 V (*v.* SCE) was plotted against Sn(II) concentration (Figure 2), a straight line was obtained with an intercept on the [Sn(II)] axis at 1  $\mu\text{g mL}^{-1}$ . This value is presumed to be the Sn(II) equivalent of molybdate dissolved in MEK.

### 3.3 Assay of Carrier $^{99}\text{Tc}$ in Pertechnetate-M

The  $\beta$ -emitting  $^{99}\text{Tc}$  in pertechnetate-M samples was determined by liquid scintillation counting. The counting system was calibrated with known amounts of standard  $^{99}\text{Tc}$  ammonium pertechnetate solution. The theoretical count rate calculated from the concentration and decay constant of  $^{99}\text{Tc}$  was correlated to the measured count rate. Over the range 5 to 12000  $\text{ng mL}^{-1}$ , the agreement averaged  $98 \pm 4$  per cent. Table 2 shows the amounts of  $^{99}\text{Tc}$  measured in different batches of pertechnetate-M; the overall range was 0.5 to 10  $\mu\text{g mL}^{-1}$ .

### 3.4 Biological Studies

#### 3.4.1 Red blood cell (rbc) labelling studies

The site and mechanism of  $^{99m}\text{Tc}$  binding to rbc components have been reported by Dewanje [1974] and Rehani and Sharma [1980]. Apparently, the process of pertechnetate binding to the rbc involves passive diffusion of pertechnetate into the cell. There appears to be no mechanism inside the cell to reduce pertechnetate since, in the absence of a reducing agent, intracellular  $^{99m}\text{TcO}_4^-$  ion readily diffuses when the rbc is suspended in normal saline. Stannous ion (Sn(II)) then reduces  $^{99m}\text{TcO}_4^-$  ion to a lower valence state, probably Tc(V), and is then believed to bind to the beta chain of the globin.

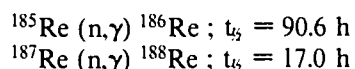
Since the binding of  $^{99m}\text{Tc}$  depends on the ability of the  $^{99m}\text{TcO}_4^-$  ion to be reduced by Sn(II) ions, any interference with this process will manifest itself by a lowering in labelling efficiency. Consequently, the rbc labelling method was used to investigate the effects of organic residues, molybdate, perrhenate and  $^{99m}\text{Tc}$  carrier.

Alkali-treated MEK was used to determine the effect of organic residues on rbc labelling. Methylethylketone which had been equilibrated with 5 *M* KOH for one hour was divided into 5 and 40 mL portions. These were then evaporated under nitrogen and used for the labelling experiment. The rbc labelling efficiencies, expressed as percentages, are given in Table 3.

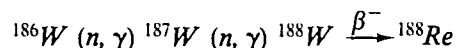
The rbc labelling efficiency for the 40 mL sample was lower than those obtained for the 5 mL sample and the control. However, using the t-test, there is no significant difference between the two sets of results and the control. This means that the organic residue from 40 mL of alkali-treated MEK has no significant effect on the rbc labelling efficiency.

The effect of molybdate was tested because of the possibility of molybdate contamination due to a slight variation in the extraction process. The amount of molybdate, measured by detecting 740 keV photons of  $^{99}\text{Mo}$  in a dose calibrator or in an NaI (TI) detector coupled to a pulse height analyser [USP 1975], was usually less than 5 ppm. As can be observed in Table 3, the percentage rbc labelled in the presence of 10 ppm molybdate was very high (99.4%), indicating that at this level molybdate does not affect the efficiency.

The presence of perrhenate in pertechnetate samples is due to trace impurities of rhenium and tungsten in the molybdenum trioxide target material [Boyd 1982]. Rhenium impurities undergo neutron activation as follows:



When neutron-activated, tungsten undergoes the following reaction:



The amount of rhenium in the final pertechnetate solution depends on the initial amounts of rhenium and tungsten impurities in the target material.

When 100 ppm of perrhenate was used in the rbc labelling study the labelling efficiency was high (99%). The result indicates that at this level perrhenate does not affect the rbc labelling efficiency.

The  ${}^{99}\text{Tc}$  carrier effect on the rbc labelling efficiency was investigated by diluting the  ${}^{99}\text{Tc}$  serially and spiking it with freshly eluted  ${}^{99m}\text{Tc}$  pertechnetate-Q. From Table 4 it can be seen that the percentage of rbc labelled decreases with increasing concentration of  ${}^{99}\text{Tc}$  carrier. There is a relatively sharp drop in the percentage of rbc labelled for  ${}^{99}\text{Tc}$  concentrations in the range 5.6 - 56 ng mL<sup>-1</sup>, resulting in about 70 per cent labelling efficiency. A further increase in the amount of  ${}^{99}\text{Tc}$  did not produce a corresponding reduction in the percentage rbc labelled.

### 3.4.2 Effect of carbonyl compounds on tissue distribution

Chemical analysis of pertechnetate-M revealed the presence of several low molecular weight carbonyl compounds, including formaldehyde, acetaldehyde, acetone and MEK. These carbonyl compounds were studied to determine whether they have an effect on the tissue distribution of radiopharmaceuticals.

A bone-seeking radiopharmaceutical containing pyrophosphate was used since most of the poor quality scans reported were associated with the pertechnetate-M when used in bone studies. Throughout the experiment, the same batch of lyophilised pyrophosphate sample was used to avoid batch variation which might occur in the manufacture of the radiopharmaceutical kit. Other controlled parameters were the age and sex of the animals, both of which affect the uptake and tissue distribution of bone agent [Thrall *et al.* 1974; Wilson 1981]. Thus only female rats between the ages of 7 and 8 weeks were used.

The quantity of test compound added to the radiopharmaceutical was approximately ten times the average value found in the assayed pertechnetate-M samples (except for MEK, which was used at twice the average amount). With this elevated value, any change that might occur in the tissue distribution would be enhanced and thus more readily detected. Subsequent experiments at lower concentrations would then be used to ascertain the minimum detectable level of the effect.

Acetylacetone was used as a representative of a dicarbonyl compound to determine if such a structural group affects the distribution of the bone-seeking agent. Table 5 shows the tissue distribution (presented as %ID) of  ${}^{99m}\text{Tc}$ -pyrophosphate in the absence and presence of formaldehyde, MEK, acetaldehyde and acetylacetone. Table 6 shows the same results as %ID per gram of tissue. The latter representation enables a more accurate comparison of uptake by different tissues since the results are compared for the same amount of each tissue. Also included in the latter are the ratios of femur to blood and femur to muscle (calculated for individual animals and then averaged). This value indicates the contrast between the activity in the bone and surrounding tissues.

Table 7 shows a statistical analysis of the results. There were significant differences for kidney, skin, lung, stomach and femur tissues at P = 0.05. A t-test was then performed comparing the carbonyl compounds to the control. Significant differences were observed for acetylacetone in liver, skin and femur tissues and in the femur-to-blood ratio; for acetaldehyde, significant differences were found for kidney, skin and lung tissues. In the case of MEK, skin and blood were significantly different and for formaldehyde only the skin showed any significant difference.

Since the radiopharmaceutical was a bone-seeking agent, of most interest is the difference that affects distribution in the bone. A change in bone distribution was observed only in the presence of acetylacetone, *i.e.* a dicarbonyl compound.

### 3.4.3 Effect of $^{99}\text{Tc}$ carrier on tissue distribution

The assay of  $^{99}\text{Tc}$  carrier in pertechnetate-M samples showed the presence of  $^{99}\text{Tc}$  in the range 0.5 to 9.9  $\mu\text{g mL}^{-1}$ . Red blood cell labelling studies showed that even at nanogram quantities the presence of  $^{99}\text{Tc}$  affects the labelling efficiency. At microgram levels, the rbc labelling efficiency is only about 60 to 70 per cent. Because the amounts of  $^{99}\text{Tc}$  in the pertechnetate-M samples are in the microgram range, tissue distribution studies were carried out to investigate the effect of  $^{99}\text{Tc}$  at that level.

After the addition of  $^{99}\text{Tc}$ , the quality of the radiopharmaceutical was monitored by ITLC, which showed that it had insufficient free pertechnetate to interfere with the distribution of  $^{99\text{m}}\text{Tc}$ -pyrophosphate.

The tissue distribution studies were performed at three different  $^{99}\text{Tc}$  concentrations corresponding to two, three and five times the maximum amount of  $^{99}\text{Tc}$  found in 20 batches of pertechnetate-M sample. The results are presented in Tables 8 and 9 as %ID and %ID per gram of tissue respectively.

An increase in liver uptake and a reduction in femur uptake is to be expected if  $^{99}\text{Tc}$  carrier affects tissue distribution. This can be observed by comparing the values of tissue uptake for different  $^{99}\text{Tc}$  concentrations to that of the control. There is a considerable increase in liver uptake at 56  $\mu\text{g mL}^{-1}$  of  $^{99}\text{Tc}$  and a subsequent decrease in the femur uptake. The same trend was observed at the other  $^{99}\text{Tc}$  concentrations although they were not so pronounced. The femur-to-blood ratio decreased as the  $^{99}\text{Tc}$  concentration increased; this indicated a larger blood uptake causing background activity to be high, thus resulting in poor bone imaging. This trend was also observed for femur-to-muscle ratio, although in this case there were large variations in the values within a group owing to variations in muscle sampling.

A statistical evaluation of the results is given in Table 10. There are significant differences for liver, spleen, lungs, blood, urine, bladder and femur tissues and for femur-to-blood ratio. The t-test indicates highly significant differences for liver, blood, and femur tissues and for femur-to-blood ratio at 56  $\mu\text{g mL}^{-1}$  of  $^{99}\text{Tc}$ ; at 33.6  $\mu\text{g mL}^{-1}$ , the differences for femur tissue and femur-to-blood ratio are also highly significant. Significant differences are observed at  $P = 0.05$  for liver, spleen and lung tissue and for blood. This study indicates that pyrophosphate distribution is affected by the amount of  $^{99}\text{Tc}$  in the radiopharmaceutical.

The radiopharmaceutical systems used in these biological studies were chosen because of adverse reports about MEK-derived pertechnetate. This has not been the case for other bone-seeking agents such as methylene diphosphonate (MDP) or pertechnetate from a chromatographic generator. In the case of MDP, it is possible that the equilibrium constant for the formation of the Tc-MDP complex is larger than that for pyrophosphate, so all  $^{99}\text{Tc}$  and  $^{99\text{m}}\text{Tc}$  is complexed, and good bone imaging results. Of equal importance is the fact that most contemporary MDP formulations consist of a large excess of free ligand, whereas the brand of pyrophosphate used in this study (Mallinckrodt Technescan PYP) is formulated to contain equivalent amounts of pyrophosphate and tin, and hence could provide only a low concentration of free pyrophosphate ligand. Consequently, the effect of Tc carrier might be more significant in Mallinckrodt pyrophosphate than in most MDP formulations containing large excesses of free ligand.

Usually, chromatographic generators are first eluted four days after loading, by which time  $^{99}\text{Tc}$  levels have risen to about 50 per cent of the loaded  $^{99}\text{Mo}$ . For example, a typical generator loaded with 2 Ci (74 GBq) of  $^{99}\text{Mo}$  and first eluted 67 hours later will yield about 2  $\mu\text{g}$  of  $^{99}\text{Tc}$  in 5 mL of eluant. At this concentration (0.4  $\mu\text{g mL}^{-1}$ ), the  $^{99}\text{Tc}$  carrier is expected to interfere with rbc labelling only. (The instructions accompanying chromatographic generators carry such a warning.)

The presence of non-uniform amounts of  $^{99}\text{Tc}$  in pertechnetate-M samples can be attributed to two variations in the extraction and dispensing procedures:

- (i) The time between successive extractions of the  $^{99}\text{Mo}$ - $^{99\text{m}}\text{Tc}$  mixture with MEK varies. When a generator is loaded, there is 2  $\mu\text{g}$  of  $^{99}\text{Mo}$  (calculated as Mo atoms) per curie (37 GBq) of  $^{99}\text{Mo}$  activity. The amount of technetium (as  $^{99}\text{Tc}$  and  $^{99\text{m}}\text{Tc}$ ) at the time of extraction is governed by the half-life of  $^{99}\text{Mo}$  (67 h) and the time elapsed since the previous extraction. The amount of  $^{99}\text{Tc}$  extracted will usually be small (0.3 mg  $\text{Ci}^{-1}$  at 24 h) unless the sample of  $^{99}\text{Mo}$  is very old (*i.e.* many half-lives since the previous extraction).
- (ii) Freshly extracted  $^{99\text{m}}\text{Tc}$  decays with a much shorter half-life ( $t_{1/2} = 6$  h) thereby accumulating  $^{99}\text{Tc}$  at a rate greater than that inside the generator. For example, if 15 mCi ( $\sim 0.5$  GBq) of  $^{99\text{m}}\text{Tc}$  is required for injection and the time for dispensing the pertechnetate is 24 hours earlier,

240 mCi (~ 8 GBq) will be extracted. The technetium content of a freshly extracted 240 mCi sample is about 45 ng. Thus the total technetium in the 15 mCi used 24 hours later will be 45 ng. On the basis of these results, if 15 mCi of pertechnetate was then used for rbc labelling studies, it would reduce the labelling efficiency significantly, but would not affect the tissue distribution of Tc-pyrophosphate complex.

When a sample of  $^{99}\text{Mo}$ - $^{99m}\text{Tc}$  of particularly high activity is extracted with MEK, then taken up in a fixed volume of saline, the  $^{99m}\text{Tc}$  activity could be greater than desired for dispensing. If the sample is allowed to decay to the desired level of activity, the accumulation of  $^{99}\text{Tc}$  per Ci of activity will increase by a factor of 16 for every 24 hours of decay. This is the only means by which  $^{99}\text{Tc}$  levels in pertechnetate could rise high enough to interfere with the pyrophosphate bone imaging.

#### 4. CONCLUSIONS

Analysis by HPLC and GC-MS of sodium pertechnetate derived by extraction of alkaline pertechnetate-molybdate mixtures has revealed the presence of organic residues in quantities which vary widely from batch to batch. The principal components were identified as the low molecular weight carbonyl compounds formaldehyde (up to  $3.2 \mu\text{g mL}^{-1}$ ), acetaldehyde (up to  $34.2 \mu\text{g mL}^{-1}$ ), acetone (up to  $6.1 \mu\text{g mL}^{-1}$ ) and MEK (up to  $3.7 \text{mg mL}^{-1}$ ).

When these compounds were tested by addition to a pyrophosphate bone-seeking radiopharmaceutical and injection into rats, the tissue distribution was not significantly different from that in the control, even when the added amounts were up to ten times the maximum levels specified above. These low molecular weight carbonyl compounds seem to result from the radiation-induced decomposition of MEK.

In addition, compounds were detected at very low levels ( $\leq 1 \mu\text{g mL}^{-1}$ ) and suspected of being derived from alkaline condensation reactions of MEK followed by dehydration. Sample mixtures of such compounds were prepared and tested with the sensitive rbc labelling technique. Again, with added amounts of condensation products of the order of  $1 \text{mg mL}^{-1}$ , there was no significant difference from the control.

Other possible contaminants (molybdate and perrhenate) did not affect rbc labelling studies. Polarographic studies of the effect of various organic contaminants on the radiopharmaceutical reducing agent Sn(II) gave similar results. It is concluded that organic contaminants derived from MEK do not affect the performance of radiopharmaceuticals.

However,  $^{99}\text{Tc}$  carrier at nanogram levels produced a reduction in rbc labelling efficiency. This reduction was observed at  $^{99}\text{Tc}$  concentrations as low as  $6 \text{ng mL}^{-1}$ , at which level the percentage rbc labelled dropped to about 95 per cent compared to 99 per cent for the control. Further reduction was observed at  $11 \text{ng mL}^{-1}$  (corresponding to about 84 per cent labelled). Subsequent increases in  $^{99}\text{Tc}$  did not produce corresponding reductions in labelling efficiency. Values of about 60 per cent were obtained for microgram amounts of  $^{99}\text{Tc}$ .

An assay of pertechnetate-M samples revealed microgram quantities of  $^{99}\text{Tc}$ , *i.e.* levels far in excess of those needed to reduce the rbc labelling efficiency. Animal tissue distribution studies with two, three and five times the maximum amount detected showed that tissue distribution of pyrophosphate is susceptible to the amount of  $^{99}\text{Tc}$  at these levels. This was observed as a highly significant reduction in bone uptake and an equally significant increase in liver uptake. The effect apparently increases with an increase in added  $^{99}\text{Tc}$ . Thus, it would seem that  $^{99}\text{Tc}$  affects the characteristics of pyrophosphate radiopharmaceuticals.

On the basis of these experiments, maximum levels of  $^{99}\text{Tc}$  in  $^{99m}\text{Tc}$  can be defined for particular applications. For example, in rbc labelling,  $6 \text{ng mL}^{-1}$  of  $^{99}\text{Tc}$  carrier appears to be sufficient to influence the labelling, whereas a significant effect on pyrophosphate distribution in bone is found only above  $10 \mu\text{g mL}^{-1}$ .

#### 5. ACKNOWLEDGEMENTS

This report is based upon work described in a Master of Science thesis submitted to the University of Sydney by Rohani Mohammad, who was sponsored by the Australian Development Assistance Bureau and PUSPATI (Malaysia). The Australian Institute of Nuclear Science and Engineering provided assistance with Project Grants in 1981 and 1982.

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**TABLE 1**  
**AMOUNTS OF CARBONYL COMPOUNDS FOUND IN PERTECHNETATE-M SAMPLES**

AAEC Code No.	Batch No.	Concentration ( $\mu\text{g mL}^{-1}$ )			
		Formaldehyde	Acetaldehyde	Acetone	MEK ( $\times 10^3$ )
33151-14	1	0.45	16.72	0.00	1.380
-15	2	1.50	4.05	0.72	0.001
-16	3	1.20	2.19	0.77	0.001
-17	4	1.16	19.39	0.00	1.270
-18	5	2.67	4.75	3.30	0.010
-19	6	1.66	3.38	3.47	0.006
-20	7	2.10	5.91	4.48	0.034
-22	8	1.08	5.80	3.67	0.061
-23	9	2.16	26.86	1.51	3.670
-24	10	2.14	34.15	2.58	2.060
-25	11	2.82	3.36	4.38	0.013
-26	12	1.82	2.32	3.03	0.005
-27	13	3.03	11.30	3.98	0.060
-28	14	2.61	11.80	3.05	0.179
-29	15	3.19	14.96	3.86	0.057
-30	16	1.53	0.69	6.07	0.007
-31	17	3.18	14.09	4.66	0.242
-33	18	2.14	0.00	0.27	0.020

**TABLE 2**  
**AMOUNT OF  $^{99}\text{Tc}$  IN DIFFERENT BATCHES OF PERTECHNETATE-M SAMPLES**

AAEC Batch No.	Batch No.	Amount of $^{99}\text{Tc}$ ( $\mu\text{g mL}^{-1}$ )	AAEC Batch No.	Batch No.	Amount of $^{99}\text{Tc}$ ( $\mu\text{g mL}^{-1}$ )
33151-24	1	4.4	33151-57	11	2.0
-43	2	1.4	-58	12	0.5
-46	3	2.6	-60	13	9.9
-49	4	6.2	-61	14	5.3
-50	5	1.8	-62	15	8.3
-51	6	3.9	-64	16	5.0
-54	7	2.6	-65	17	2.8
-55	8	3.4	-66	18	7.5
-59	9	3.3	-67	19	1.4
-56	10	0.8	-69	20	8.2

**TABLE 3A**  
**THE EFFECT OF ORGANIC RESIDUES ON**  
**RED BLOOD CELL LABELLING EFFICIENCY**

	rbc Labelling Efficiency (%)	
Control	99.0	97.9
5 mL sample	99.1	95.5
40 mL sample	96.7	95.0

Each value is an average of two determinations. The two sets of readings were obtained on separate days.

**TABLE 3B**  
**THE EFFECT OF MOLYBDATE AND PERRHENATE ON THE**  
**RED BLOOD CELL LABELLING EFFICIENCY**

	Conc. ( $\mu\text{g g}^{-1}$ )	rbc Labelling Efficiency (%)
Control		98.8
Molybdate	10	99.4
Perrhenate	100	99.1

Value is an average of two determinations.

**TABLE 4**  
**THE EFFECT OF  $^{99}\text{Tc}$  CARRIER ON THE**  
**RED BLOOD CELL LABELLING EFFICIENCY**

Conc. of $^{99}\text{Tc}$ ( $\text{ng mL}^{-1}$ )	rbc Labelling Efficiency (%)
Control	98.9 $\pm$ 0.6
5.6	95.0 $\pm$ 1.0
11.2	83.0 $\pm$ 1.0
56.0	68.0 $\pm$ 0.7
112.0	65.6 $\pm$ 1.0
8440.0	60.8 $\pm$ 3.0



**TABLE 5**  
**TISSUE DISTRIBUTION OF <sup>99m</sup>Tc ACTIVITY IN RATS**  
**FOR PYROPHOSPHATE WITH ADDED CARBONYL COMPOUNDS**

Tissue	% Injected Dose (Average of 5 Rats)				
	Control	Formaldehyde	MEK	Acetaldehyde	Acetylacetone
Liver	10.9 ± 2.8	11.2 ± 1.7	10.4 ± 3.1	10.5 ± 2.4	8.7 ± 1.6
Spleen	0.2 ± 0.06	0.27 ± 0.08	0.24 ± 0.10	0.43 ± 0.15	0.28 ± 0.16
Kidney	1.3 ± 0.2	1.31 ± 0.18	1.13 ± 0.08	1.65 ± 0.12	1.32 ± 0.09
Muscle	2.7 ± 1.1	*2.31 ± 0.49	2.4 ± 1.0	2.17 ± 0.15	2.09 ± 0.23
Skin	2.1 ± 0.4	2.92 ± 0.35	3.00 ± 0.29	3.06 ± 0.51	3.31 ± 0.19
Lungs	0.09 ± 0.03	0.08 ± 0.00	0.09 ± 0.02	0.16 ± 0.05	0.10 ± 0.008
Heart	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.001	0.03 ± 0.00
Blood	1.12 ± 0.14	1.08 ± 0.08	1.09 ± 0.10	1.15 ± 0.32	1.52 ± 0.14
Urine and Bladder	33.3 ± 3.2	33.7 ± 3.3	31.6 ± 5.1	37.1 ± 1.5	31.7 ± 7.5
Stomach	0.27 ± 0.24	1.1 ± 1.1	0.34 ± 0.20	0.14 ± 0.07	0.82 ± 0.11
Gut	2.6 ± 1.7	3.3 ± 1.4	2.9 ± 1.1	2.6 ± 1.6	2.78 ± 0.93
Tail	0.87 ± 0.43	1.1 ± 0.1	1.6 ± 0.6	1.94 ± 0.33	1.54 ± 0.52
Femur	1.27 ± 0.03	1.30 ± 0.06	1.38 ± 0.08	1.35 ± 0.25	1.27 ± 0.12
Body Weight (g)	144.0 - 168.0	153.8 - 179.1	150.7 - 202.3	144.9 - 180.3	185.3 - 207.9

Results shown are the average ± standard deviation of determinations on 5 rats except those indicated by \* where outlying values (>2 s.d.) have been eliminated.

**TABLE 6**  
**DATA OF TABLE 5 EXPRESSED AS % INJECTED DOSE PER GRAM OF TISSUE**

Tissue	% Injected Dose/g Tissue (Average of 5 Rats)				
	Control	Formaldehyde	MEK	Acetaldehyde	Acetylacetone
Liver	1.44 ± 0.44	1.47 ± 0.21	1.26 ± 0.53	1.07 ± 0.31	0.94 ± 0.27
Spleen	0.41 ± 0.11	0.44 ± 0.14	0.38 ± 0.18	0.67 ± 0.32	0.36 ± 0.17
Kidney	0.95 ± 0.18	0.98 ± 0.12	0.87 ± 0.16	1.21 ± 0.17	0.86 ± 0.04
Muscle	0.049 ± 0.025	0.032 ± 0.006	0.031 ± 0.014	0.029 ± 0.004	0.023 ± 0.002
Skin	0.069 ± 0.011	0.088 ± 0.008	0.085 ± 0.010	0.092 ± 0.018	0.085 ± 0.004
Lungs	0.092 ± 0.019	0.087 ± 0.006	0.085 ± 0.010	0.148 ± 0.039	0.089 ± 0.007
Heart	0.051 ± 0.008	0.044 ± 0.002	0.048 ± 0.010	0.054 ± 0.011	0.043 ± 0.004
Blood	0.112 ± 0.011	0.101 ± 0.008	0.095 ± 0.008	0.105 ± 0.025	0.119 ± 0.010
Urine and Bladder	1.21 ± 0.20	1.12 ± 0.17	1.43 ± 0.36	1.43 ± 0.32	1.38 ± 0.38
Stomach	0.079 ± 0.063	0.47 ± 0.39	0.135 ± 0.076	0.047 ± 0.031	0.204 ± 0.058
Gut	0.20 ± 0.13	0.25 ± 0.10	0.23 ± 0.13	0.17 ± 0.11	0.171 ± 0.055
Tail	0.45 ± 0.23	0.58 ± 0.05	0.56 ± 0.18	0.69 ± 0.09	0.50 ± 0.12
Femur	3.49 ± 0.30	3.40 ± 0.25	3.13 ± 0.46	3.39 ± 0.26	2.74 ± 0.32
<b>Ratio</b>					
Femur/ Blood	31.3 ± 4.3	33.7 ± 3.2	32.9 ± 5.1	35 ± 13 *28.8 ± 2.4	23.0 ± 2.2
Femur/ Muscle	86 ± 36 *97 ± 29	*114 ± 26	113 ± 37 *127 ± 25	117 ± 9	117 ± 10

Results shown are the average ± standard deviation of determinations on 5 rats except those indicated by \* where outlying values (>2 s.d.) have been eliminated.

TABLE 7  
STATISTICAL EVALUATION OF DATA IN TABLE 6

Tissue	Analysis of Variance		t-test
	F Ratio	Significance	Significance
Liver	1.845	NS	Significant for acetylacetone
Spleen	1.871	NS	NS
Kidney	4.699	S	Significant for acetaldehyde
Muscle	2.019	NS	NS
Skin	3.117	S	All significant
Lung	8.438	S	Significant for acetaldehyde
Heart	1.735	NS	NS
Blood	2.205	NS	Significant for MEK
Stomach	4.234	S	NS
Tail	1.744	NS	NS
Femur	4.215	S	Significant for acetylacetone
<b>Ratio</b>			
Femur/Blood	2.213	NS	Significant for acetylacetone

S = significant      NS = not significant at P = 0.05

TABLE 8  
DISTRIBUTION OF  $^{99m}\text{Tc}$  PYP IN RATS  
EFFECT OF ADDED  $^{99}\text{Tc}$

Tissue	% Injected Dose			
	Control	22.4 $\mu\text{g mL}^{-1}$	33.6 $\mu\text{g mL}^{-1}$	56 $\mu\text{g mL}^{-1}$
Liver	9.24 $\pm$ 0.93	13.21 $\pm$ 1.7	13.0 $\pm$ 1.3	22.0 $\pm$ 3.4
Spleen	0.30 $\pm$ 0.06	0.66 $\pm$ 0.05	0.48 $\pm$ 0.10	0.59 $\pm$ 0.10
Kidney	1.25 $\pm$ 0.26	1.08 $\pm$ 0.10	1.28 $\pm$ 0.12	1.13 $\pm$ 0.12
Muscle	1.92 $\pm$ 0.28	2.5 $\pm$ 1.1	2.42 $\pm$ 0.52	2.32 $\pm$ 0.47
Skin	2.29 $\pm$ 0.34	2.33 $\pm$ 0.25	2.70 $\pm$ 0.27	3.26 $\pm$ 0.61
Lungs	0.08 $\pm$ 0.02	0.10 $\pm$ 0.03	0.13 $\pm$ 0.05	0.14 $\pm$ 0.02
Heart	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.04 $\pm$ 0.02	0.04 $\pm$ 0.08
Blood	1.05 $\pm$ 0.16	1.30 $\pm$ 0.21	1.486 $\pm$ 0.034	1.81 $\pm$ 0.24
Urine and Bladder	30.6 $\pm$ 4.0	31.4 $\pm$ 3.0	28.7 $\pm$ 3.3	26.8 $\pm$ 4.4
Stomach	0.34 $\pm$ 0.19	0.53 $\pm$ 0.63	0.164 $\pm$ 0.061	0.214 $\pm$ 0.085
GI Tract	2.9 $\pm$ 1.4	1.73 $\pm$ 0.64	1.21 $\pm$ 0.21	*1.60 $\pm$ 0.49
Tail	1.04 $\pm$ 0.54	1.67 $\pm$ 0.26	1.63 $\pm$ 0.48	0.98 $\pm$ 0.13
Femur	1.28 $\pm$ 0.03	1.18 $\pm$ 0.06	1.16 $\pm$ 0.06	0.90 $\pm$ 0.05
Body Weight (g)	163.3 - 189.6	137.1 - 172.6	162.7 - 187.5	182.8 - 213.1

Results shown are the average  $\pm$  standard deviation of determinations on 5 rats except those indicated by \* where outlying values ( $>2$  s.d.) have been eliminated.

**TABLE 9**  
**DATA OF TABLE 8 EXPRESSED AS % INJECTED DOSE PER GRAM OF TISSUE**

Tissue	% Injected Dose/g Tissue			
	Control	22.4 $\mu\text{g mL}^{-1}$	33.6 $\mu\text{g mL}^{-1}$	56 $\mu\text{g mL}^{-1}$
Liver	1.16 $\pm$ 0.11	1.95 $\pm$ 0.37	1.84 $\pm$ 0.29	2.71 $\pm$ 0.48
Spleen	0.41 $\pm$ 0.10	0.91 $\pm$ 0.17	0.71 $\pm$ 0.16	0.80 $\pm$ 0.20
Kidney	0.84 $\pm$ 0.20	0.843 $\pm$ 0.096	0.941 $\pm$ 0.058	0.76 $\pm$ 0.13
Muscle	0.027 $\pm$ 0.006	0.037 $\pm$ 0.018	0.031 $\pm$ 0.008	0.027 $\pm$ 0.005
Skin	0.069 $\pm$ 0.009	0.076 $\pm$ 0.010	0.078 $\pm$ 0.005	0.084 $\pm$ 0.017
Lungs	0.076 $\pm$ 0.015	0.111 $\pm$ 0.026	0.122 $\pm$ 0.002	0.140 $\pm$ 0.014
Heart	0.044 $\pm$ 0.012	0.054 $\pm$ 0.009	0.066 $\pm$ 0.017	0.059 $\pm$ 0.006
Blood	0.103 $\pm$ 0.020	0.140 $\pm$ 0.021	0.143 $\pm$ 0.009	0.155 $\pm$ 0.019
Urine and Bladder	1.20 $\pm$ 0.19	1.36 $\pm$ 0.12	0.86 $\pm$ 0.13	1.18 $\pm$ 0.19
Stomach	0.096 $\pm$ 0.053	0.23 $\pm$ 0.25	0.080 $\pm$ 0.036	0.058 $\pm$ 0.028
GI Tract	0.21 $\pm$ 0.11	0.131 $\pm$ 0.058	0.086 $\pm$ 0.020	*0.100 $\pm$ 0.031
Tail	0.38 $\pm$ 0.16	0.724 $\pm$ 0.074	0.58 $\pm$ 0.12	0.262 $\pm$ 0.027
Femur	3.19 $\pm$ 0.23	3.48 $\pm$ 0.42	2.93 $\pm$ 0.23	1.992 $\pm$ 0.078
<b>Ratio</b>				
Femur/Blood	31.9 $\pm$ 5.1	25.6 $\pm$ 4.9	20.50 $\pm$ 0.60	12.9 $\pm$ 1.1
Femur/Muscle	*133 $\pm$ 19	*124 $\pm$ 32	*107 $\pm$ 12	*81.2 $\pm$ 5.8

Results shown are the average  $\pm$  standard deviation of determinations on 5 rats except those indicated by \* where outlying values ( $>2$  s.d.) have been eliminated.

**TABLE 10**  
**STATISTICAL EVALUATION OF DATA IN TABLE 9**

Tissue	Analysis of Variance		t-test
	F Ratio	Significance	Significance
Liver	13.33	S	All significant. Highly significant for (3)
Spleen	6.94	S	All significant
Kidney	1.06	NS	NS
Muscle	0.87	NS	NS
Skin	1.15	NS	NS
Lungs	7.29	S	All significant
Heart	2.62	NS	NS
Blood	6.26	S	(1) and (3) highly significant (2) significant at P = 0.05
Urine and Bladder	6.31	S	Significant for (2)
Stomach	2.31	NS	NS
Gut	1.50	NS	NS
Tail	1.30	NS	NS
Femur	23.59	S	(1) significant at P = 0.05 (2) and (3) highly significant
<b>Ratio</b>			
Femur/Blood	19.89	S	(1) Not significant (2) and (3) highly significant

S = significant      NS = not significant

(1), (2) and (3) denotes the level of  $^{99}\text{Tc}$  carrier corresponding to 22.4, 33.6 and 56.0  $\mu\text{g mL}^{-1}$  respectively.

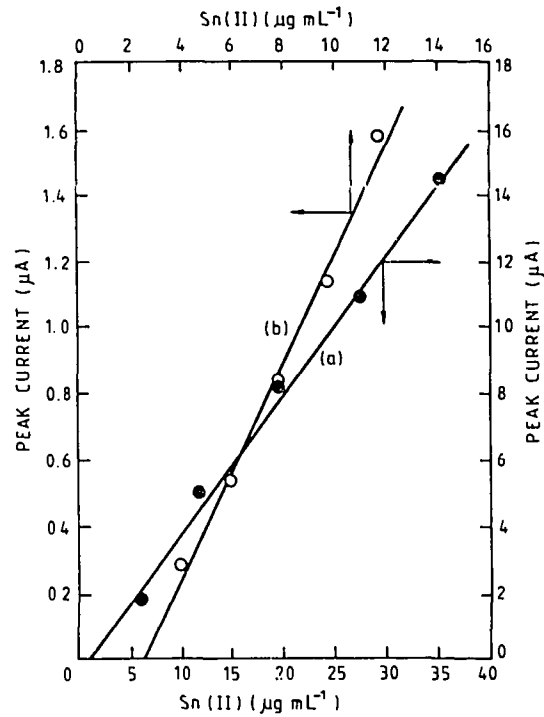


FIGURE 1 Effect of pertechnetate on Sn(II) (a) 1 mL pertechnetate, and (b) 2 mL pertechnetate

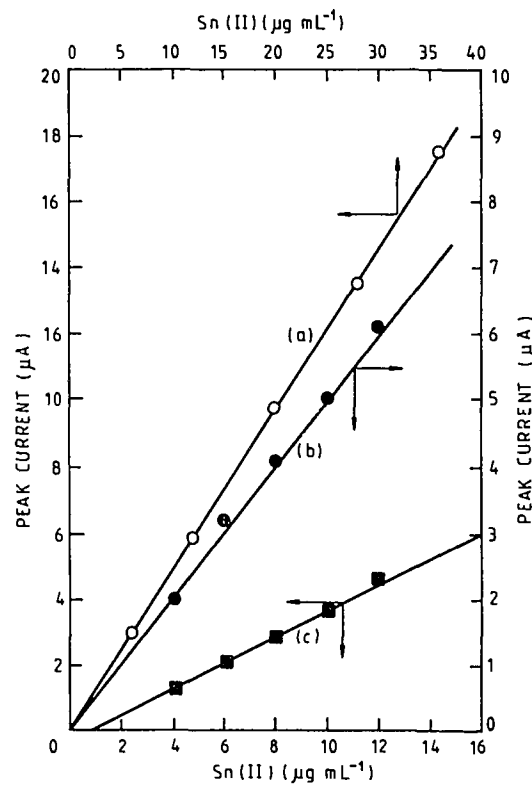


FIGURE 2 Effect of alkali treated MEK on Sn(II) (a) MEK equilibrated with KOH for 1 hour, (b) MEK equilibrated with KOH overnight, and (c) MEK equilibrated with KOH and molybdate