International Journal of Engineering, Mathematical and Physical Sciences
ISSN: 2517-9934
Vol:9, No:10, 2015

Human Absorbed Dose Estimation of a New IN-111 Imaging Agent Based on Rat Data

H. Yousefnia, S. Zolghadri

Abstract**—**The measurement of organ radiation exposure dose is one of the most important steps to be taken initially, for developing a new radiopharmaceutical. In this study, the dosimetric studies of a novel agent for SPECT-imaging of the bone metastasis, ^{111}In-1,4,7,10-tetraazacyclododecane-1,4,7,10 tetraethylene phosphonic acid (^{111}In-DOTMP) complex, have been carried out to estimate the dose in human organs based on the data derived from rats. The radiolabeled complex was prepared with high radiochemical purity in the optimal conditions. Biodistribution studies of the complex was investigated in the male Syrian rats at selected times after injection (2, 4, 24 and 48 h). The human absorbed dose estimation of the complex was made based on data derived from the rats by the radiation absorbed dose assessment resource (RADAR) method. ^{111}In-DOTMP complex was prepared with high radiochemical purity of >99% (ITLC). Total body effective absorbed dose for ^{111}In-DOTMP was 0.061 mSv/MBq. This value is comparable to the other ^{111}In clinically used complexes. The results show that the dose with respect to the critical organs is satisfactory within the acceptable range for diagnostic nuclear medicine procedures. Generally, ^{111}In-DOTMP has interesting characteristics and can be considered as a viable agent for SPECT-imaging of the bone metastasis in the near future.

Keywords**—**In-111, DOTMP, Internal Dosimetry, RADAR.

I. Introduction

NOWADAYS, many of patients with the prostate, breast, lung, bladder, and thyroid cancers suffer from the skeletal metastasis [1], [2]. The radioisotope bone scan has been known as the standard method for the detection of the bone metastasis since the introduction of technetium-based scan agents [3]. Also, imaging is useful for the skeleton screening of the patients with non-skeletal tumors to assess probable metastatic disease and to determine its extent [4].

Although the ^{99m}Tc-methylene diphosphonate (MDP) is a well-established tracer for the diagnosis of the bone metastasis in nuclear medicine domain using SPECT imaging [5], utilization of labeled compounds with radionuclides of greater half-life providing the authorization of the long term skeletal studies in SPECT procedures and dosimetry of therapeutic analogs [6]. Recently, ^{111}In-1,4,7,10-tetraazacyclododecane-1,4,7,10 tetraethylene phosphonic acid (^{111}In-DOTMP) has been prepared and showed promising results such as very high target-to-soft-tissue ratios and ultrafast clearance [6].

The interesting physical properties of ^{111}In (a cyclotron produced radionuclide with half-life of 2.8 days, decaying by electron capture (EC) with subsequent emission of gamma photons of 173 and 247 keV; 89% and 94% intensity, respectively) as well as its easy production and availability, make it an interesting nuclide for radiopharmaceutical researches [7]-[9] using single photon emission computed tomography (SPECT).

The amount of energy that is deposited in any organs by ionizing radiation termed absorbed dose, plays an important role in evaluating the risks associated with the administration of radiopharmaceuticals and thus the maximum amount of activity that should be undertaken [10]. Many resources for facilitating dose calculations are available, once appropriate biokinetic data are gathered in animal or human experiments [11]. In nuclear medicine, the most commonly used method these days for calculation of the internal absorbed dose estimates is the radiation dose assessment resource (RADAR) method [12].

In this research, ^{111}In-DOTMP was prepared and injected to the Syrian rats. The biodistriburion of the organs was evaluated using authentic procedures. The human absorbed dose estimation for this complex was done based on the data taken from the Syrian rats by RADAR.

II. Materials and Methods

The enriched cadmium-112 with purity of >99% was obtained from Merck (Darmstadt, Germany). DOTMP was prepared from Fluka Co., respectively. All other chemical reagents were purchased from Sigma-Aldrich (Heidelberg, Germany). Whatman No. 2 paper was provided from Whatman (Buckinghamshire, U.K.). 30 MeV cyclotron (Cyclone 30, IBA, Belgium) was used for the production of ^{111}In via ^{112}Cd(p, 2n)^{111}In reaction. Radio-chromatography and imaging studies were performed using a thin layer chromatography scanner (Bioscan AR2000, Paris, France) and a Dual Head SPECT system (DST-XL, SMV, Buc, France). The activity of the samples was measured by a p-type coaxial high-purity germanium (HPGe) detector (EGPC 80-200R) coupled with a multichannel analyzer card system (GC1020-7500SL, Canberra, U. S. A.). Calculations were based on the 172 keV peak for ^{111}In. All values were expressed as mean ± standard deviation (Mean ± SD) and the data were compared using student T-test. Statistical significance was defined as $P<0.05$. Animal studies were conducted in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, second edition.

H. Yousefnia and S. Zolghadri are with the Nuclear Science and Technology Research Institute (NSTRI), Tehran, Iran (e-mail: hyousefnia@aeoi.org.ir, szolghadri@aeoi.org.ir).

A. Production and Quality Control of $^{111}InCl_3$ Solution

In order to prepare Cd targets for the production, cadmium electroplating was performed on a copper surface. Indium-111 chloride was prepared by 22 MeV proton bombardment of the cadmium target at a 30 MeV cyclotron, with a current of 100 μA for 48 min (80 μAh). Indium-111 was eluted with 1 N HCl (25 mL) as $^{111}InCl_3$ for labeling use. Radionuclidic purity of the final solution was carried out by counting in an HPGe detector for 1000 seconds. The concentrations of cadmium (from target material) and copper (from target support) were determined using polarography. The radiochemical purity of the $^{111}InCl_3$ solution was checked by instant thin layer chromatography method (ITLC) in two solvent systems, 1 mM DTPA and 10% ammonium acetate:methanol mixture.

B. Preparation and Quality Control of ^{111}In-DOTMP

Preparation and quality control of the ^{111}In-DOTMP complex was done according to the previously reported method [6]. Briefly, DOTMP with the concentration of 50 mg/mL was prepared by means of 2N NaOH solution and the pH was adjusted to 7.5–8. 185 MBq of ^{111}In activity was added to the 0.75 mL of the resulting solution following the addition of 0.5 mL phosphate buffer (pH 8). The pH of the final solution was adjusted to 7–8. The complex solution was incubated at room temperature for 1 h. The radiolabeling yield and radiochemical purity were determined by paper chromatography using NH_4OH:MeOH:H_2O (1:10:20) as the eluting solvent and both the Whatman No. 1 Paper and SG sheet as the stationary phase.

C. Biodistribution of Radiolabeled Complex in Syrian Rats

100 μL of final ^{111}In-DOTMP solution with approximately 3.7 MBq radioactivities (pH 7) were injected intravenously into the male Syrian rats through their tail veins. The total amount of radioactivity injected into each animal was measured by counting the 1-mL syringe before and after injection in a dose calibrator with fixed geometry. The biodistribution of the solutions among tissues were determined by scarification of 5 rats with around 18 weeks old for each selected interval time (2, 4, 24 and 48 h) after injection under the animal care protocols.

Blood samples were rapidly taken after scarification. The tissues (the skin, heart, kidneys, spleen, stomach, intestine, bone, muscle, lung and liver) were weighed and rinsed with normal saline and their activities were determined with a p-type coaxial HPGe detector coupled with a multichannel analyser according to (1) [13]:

$$A = \frac{N}{\epsilon\, \gamma\, t_s\, m\, k_1\, k_2\, k_3\, k_4\, k_5} \quad (1)$$

where, ε is the efficiency at photopeak energy, γ is the emission probability of the gamma line corresponding to the peak energy, t_s is the live time of the sample spectrum collection in seconds, m is the mass (kg) of the measured sample, k_1, k_2, k_3, k_4 and k_5 are the correction factors for the nuclide decay from the time. The sample is collected to start the measurement, the nuclide decay during counting period, self-attenuation in the measured sample, pulses loss due to random summing and the coincidence, respectively. N is the corrected net peak area of the corresponding photopeak given as:

$$N = N_s \frac{t_s}{t_b} N_b \quad (2)$$

where N_s is the net peak area in the sample spectrum, N_b is the corresponding net peak area in the background spectrum and t_b is the live time of the background spectrum collection in seconds.

The percentage of injected dose per gram (%ID/g) for different organs was calculated by dividing the activity amount of each tissue (A) to the decay-corrected injected activity and the mass of each organ. Five rats were sacrificed for each time interval. All values were expressed as mean ± standard deviation and the data were compared using Student's T-test.

D. Calculation of Accumulated Activity in Human Organs

The accumulated source activity for each animal organ was calculated according to (3), where A (t) is the activity of each organ at time t.

$$\tilde{A} = \int_{t_1}^{\infty} A(t)\, dt \quad (3)$$

For this purpose, the data points, which represent the non-decay corrected percentage-injected dose, were created. A linear approximation was used between the two experimental points of times up to 48 hours after injection. After 48 h, the curves were extrapolated to infinity by fitting each curve to a monoexponential curve. The exponential coefficient of the curves was equal to the physical decay constant of ^{111}In. Because the activity of each organ had been measured from 2 hours after injection, the activity of blood at t=0 was assumed to be the total amount of injected activity and the activity of the all other organs was supposed zero at t=0.he accumulated activity was calculated by computing the area under the curves.

The accumulated activity in the animals was extrapolated to the accumulated activity in humans by the proposed method of Sparks et al. (4) [14].

$$\tilde{A}_{human\ organ} = \tilde{A}_{human\ organ} \times \frac{\text{Organ Mass}_{human} / \text{Body Mass}_{human}}{\text{Organ Mass}_{animal} / \text{Body Mass}_{animal}} \quad (4)$$

In order to extrapolate this accumulated activity to human, the standard mean weights of each organ for human were used [15].

E. Equivalent Absorbed Dose Calculation

The absorbed dose in the human organs was calculated by RADAR formalism based on the biodistribution data in the rats [12]:

$$D = \tilde{A} \times DF \quad (5)$$

where $\tilde{A}$ is the accumulated activity for each human organ, and DF is:

$$DF = \frac{k \sum_i n_i E_i \phi_i}{m} \quad (6)$$

In (6), n_i is the number of radiations with the energy E emitted per nuclear transition, E_i is the energy per radiation (MeV), ϕ_i is the fraction of energy emitted that is absorbed in the target, m is the mass of the target region (kg) and k is some proportionality constant ($\frac{\text{mGy.kg}}{\text{MBq.s.MeV}}$). DF represents the physical decay characteristics of the radionuclide, the range of the emitted radiations, and the organ size and configuration [16] expressed in mGy/MBq.s. In this research, DFs have been taken from the amount presented in OLINDA/EXM software [12].

Since D in (5) is the absorbed dose in the target organ directly from a source organ, the total absorbed dose for each target organ was computed by the summation of the absorbed dose delivered from each source organ.

F. Effective Absorbed Dose Calculation

The effective absorbed dose for each organ was computed by:

$$E = \sum_T W_T H_T \quad (7)$$

where H_T is the equivalent absorbed dose for each organ and W_T is the tissue-weighting factor which represents a subjective balance between the different stochastic health risks [17]. W_T was obtained from the reported value in ICRP 103 [18].

III. Results

A. Radionuclide Production

^{111}In, in the form of $InCl_3$, was prepared by 22 MeV proton bombardment of the enriched ^{112}Cd target at Cyclone-30. Radionuclidic control showed the presence of 173 and 247 keV gamma energies, all originating from ^{111}In that indicated the radionuclidic purity of higher than 99%. The concentrations of cadmium (from target material) and copper (from target support) determined by polarography, showed to be below the internationally accepted levels, i.e. 0.1 ppm for Cd and Cu [19], [20].

The radiochemical purity of the ^{111}In solution was checked using Whatman No.2 paper in two solvent systems. In 1 mM DTPA solution, free In^{3+} cation is converted to the In-DTPA form (the more lipophilic form than free In^{3+} cation) and migrates to the higher R_f (0.8). Any other in ionic species in the solution such as $InCl_4^-$ or colloids, do not form a complex with DTPA and therefore in the presence of these ionic species, small radioactive fraction would be observed at the origin (not seen in this experiment). In the case of 10% ammonium acetate:methanol mixture, In^{3+} and/or colloids remain at R_f=0.1, whereas, the other ionic ^{111}In species would elute faster and migrate to higher R_f (not seen in this experiment).

B. Preparation and Quality Control of ^{111}In-DOTMP

In the case of ^{111}In-DOTMP, the paper chromatography using NH_4OH:MeOH:H_2O (1:10:20) as the eluting solvent and both the Whatman No. 1 Paper and Silica-Gel sheet as the stationary phase, ^{111}In-DOTMP complex moved toward the solvent front (R_f = 0.8) while uncomplexed $^{111}InCl_3$ migrated to the lower part (R_f = 0.1) under identical condition according to previously reported diagrams [6]. The radiochemical purity reached > 99% (Fig. 1).

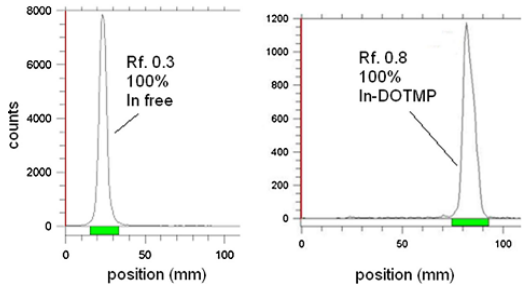

Fig. 1 ITLC of $^{111}InCl_3$ (left) and ^{111}In-DOTMP (right) in NH_4OH: MeOH: H_2O (0.2:2:4) solution on Whatman No.2 papers

C. Biodistribution of Radiolabeled Complex in Syrian Rats

The percentage of the injected dose per gram in rats' organs up to 48 h after injection of the radiolabeled complexes was determined. The non-decay corrected clearance curves from the main organ sources of the rats for ^{111}In-DOTMP are shown in Fig. 2. As expected, the major portion of the injected radioactivity, remaining in the body is transferred from the blood circulation into the bones for both complexes. The radioactivity uptake in the bone is enhanced with time up to 4 h after injection. In addition, the significant excretion of the radioactivity is observed in the kidneys showing the major route of excretion for the labeled compound is through the urinary tract.

D. Dosimetric Studies

Dosimetric evaluation in human organs was carried out by RADAR method based on biodistribution data in the rats' organs. The equivalent and the effective absorbed dose in human organs after intravenously injection of ^{111}In-DOTMP are presented in Table I. The total body effective absorbed dose for ^{111}In-DOTMP is 0.061 mSv/MBq.

IV. Discussion

According to the importance of the target and non-target organs uptakes in the quality of images, and furthermore, in the unnecessary radiation exposure to the patients, biodistribution studies after intravenous injection of ^{111}In-DOTMP to the male Syrian rats were surveyed.

A prerequisite for the clinically application of a new diagnostic radiopharmaceutical is the measurement of organ radiation exposure dose from the biodistribution data in animals [21]. These results can be used to estimate the maximum permissible administered activity, which maintains the organ doses within an acceptable range. Minimizing the

radiation exposure to the patients while providing scintigraphic images with good quality is the key point, this should be considered.

In this study, the radiation-absorbed dose of a new ^{111}In bone-seeking agent in human organ was calculated based on the biodistribution data as regards the Syrian rats. Calculation of the radiopharmaceuticals absorbed dose in human organs from biodistribution in small animals can be useful for the determining the injected activity and accelerating the development of radioactive compounds to be used in clinical settings, and is a common initial step, consistent with the recommendations of ICRP 62 [22]. The equivalent and effective absorbed dose in human organs after intravenous injection of ^{111}In-DOTMP are presented in Table I. The dose received to the organs is well within the acceptable considered range for diagnostic nuclear medicine procedures [23], [24].

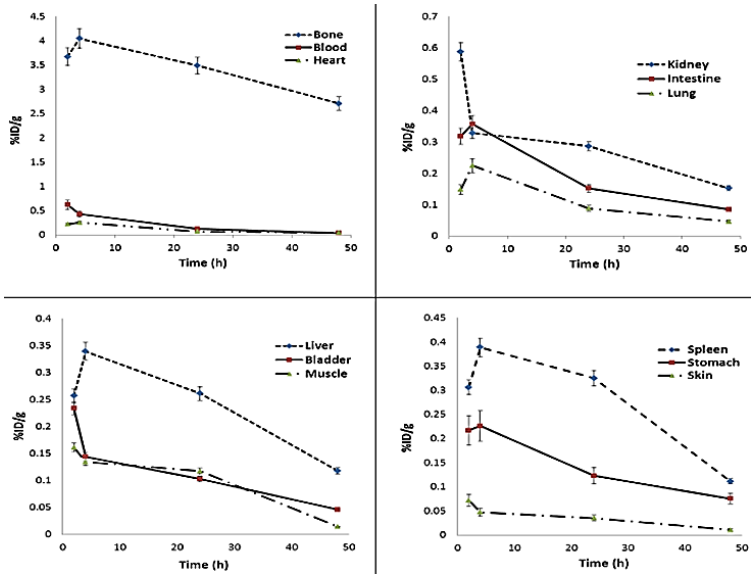

Fig. 2 Non-decay corrected clearance curves for each organ of Syrian mice after intravenously injection of ^{111}In-DOTMP

TABLE I
EQUIVALENT AND EFFECTIVE ABSORBED DOSE DELIVERED INTO HUMAN ORGANS AFTER INJECTION OF ^{111}IN-DOTMP

Target organs	Equivalent absorbed dose (mSv/MBq)	Wt[a]	Equivalent absorbed dose (mSv/MBq
Adrenals	0.037	0.12	0.004
Brain	0.039	0.01	0.000
Breasts	0.011	0.12	0.001
GB Wall	0.017	0.12	0.002
LLI Wall	0.036	0.12	0.004
Small Intestine	0.020	0.12	0.002
Stomach Wall	0.015	0.12	0.002
ULI Wall	0.017	0.12	0.002
Heart Wall	0.022	0.12	0.003
Kidneys	0.027	0.12	0.003
Liver	0.024	0.04	0.001
Lungs	0.024	0.12	0.003
Muscle	0.026	0.12	0.003
Ovaries	0.023	0.08	0.002
Pancreas	0.023	0.12	0.003
Red Marrow	0.135	0.12	0.001
Bone Surf	0.349	0.01	0.003
Spleen	0.022	0.12	0.003
Testes	0.014	0.12	0.003
Thymus	0.017	0.12	0.002
Thyroid	0.025	0.04	0.001
UB Wall	0.015	0.04	0.001
Uterus	0.017	0.12	0.002
Total Body	0.052		0.061

A comparison between the absorbed doses of the new radiolabeled complex with the other ^{111}In clinically used radiopharmaceuticals indicates that the novel product is advantageous. The total body effective dose of the adult after injection of ^{111}In labeled platelets, ^{111}In labeled red blood cells and ^{111}In labeled WBC's are 0.52, 0.20 and 0.67 mSv/MBq, respectively [24]. This value is much less in ^{111}In-DOTMP. The difference can be related to the fast clearance of the new bone-seeking agent and the low amounts of the non-target organs uptake and can be considered as a remarkable benefit.

V. CONCLUSION

Biodistribution studies of the complex performed in the Syrian rats, showed major accumulation of the labeled compounds in the bone tissue, while the other tissues uptakes were almost negligible. The total body effective absorbed dose for ^{111}In-DOTMP was 0.061 mSv/MBq, which is comparable to the other ^{111}In clinically used complexes. The dose received to the critical organs for the complex is well within the acceptable considered range for diagnostic nuclear medicine procedures. The results show ^{111}In-DOTMP has interesting characteristics and they can be considered as a viable agent for SPECT-imaging of the bone metastasis.

ACKNOWLEDGMENT

The authors wish to thank Nuclear Sciences and Technology Research Institute (NSTRI) for financial supports.

REFERENCES

[1] IAEA-TECDOC-1549, "Criteria for Palliation of Bone Metastases-Clinical Applications," Austria, Vienna: IAEA; 2007.
[2] A. Lipton, "Pathophysiology of Bone Metastases: How This Knowledge May Lead to Therapeutic Intervention," J. Support. Oncol. vol. 2, pp. 205-20, 2004.
[3] L. D. Rybak, D. I. Rosenthal, "Radiological imaging for the diagnosis of bone metastases," Q. J. Nucl. Med., vol. 45, pp. 53-64, 2001.
[4] Z.C. Traill, D. Talbot, S. Golding, F.V. Gleeson, "Magnetic resonance imaging versus radionuclide scintigraphy in screening for bone metastases," *Clin. Radiol*, vol. 54, pp. 448-51, 1999.
[5] G. Zettinig, T. Leitha, B. Niederle, K. Kaserer, A. Becherer, K. Kletter. et al. "FDG positron emission tomographic, radioiodine, and MIBI imaging in a patient with poorly differentiated insular thyroid carcinoma," Clin. Nucl. Med. Vol. 26, pp. 599–601, 2001.
[6] H. Yousefnia, A.R. Jalilian, S. Zolghadri, A. Mirzaei, A. Bahrami-Samani, M. Mirzaii, M. Ghannadi, "Development of ^{111}In DOTMP for dosimetry of bone pain palliation agents," J. Radioanal. Nucl. Chem. DOI 10.1007/s10967-014-3911-6.
[7] Zh. Zhou, N.K. Wagh, S.M. Ogbomo, W. Shi, Y. Jia, S.K. Brusnahan et al., "Synthesis and In Vitro and In Vivo Evaluation of Hypoxia-Enhanced ^{111}In-Bombesin Conjugates for Prostate Cancer Imaging., *J. Nucl. Med*., vol. 54, pp. 1605-12, 2013.
[8] J. Lai, S.M. Quadri, P.E. Borchardt, L. Harris , R. Wucher , E. Askew et al., "Pharmaco-kinetics of radiolabeled polyclonal antiferritin in patients with Hodgkin's disease," Clin. Cancer Res, vol. 5, pp. 3315−23, 1999.
[9] M.W. Nijhof, W.J. Oyen, A. Van Kampen, R.A. Claessens, J.W. Van der Meer, F.H. Corstens, "Evaluation of infections of the locomotor system with indium-111-labeled human IgG scintigraphy," J. Nucl. Med., vol. 38, pp. 1300−05, 1997.
[10] M. G. Stabin, M. Tagesson, S. R. Thomas, M. Ljungberg , S.E .Strand, "Radiation dosimetry in nuclear medicine," Appl. Radiat. Isot., vol. 50, pp. 73-87, 1996.
[11] M. G. Stabin, "Internal Dosimetry in Nuclear Medicine," Braz. J. Radiat. Sci., vol. 01, pp. 1-15, 2013.
[12] M.G. Stabin, J.A. Siegel, "Physical Models and Dose Factors for Use in Internal Dose Assessment," Health. Phys., vol. 85, pp. 294-310, 2003.
[13] IAEA-TECDOC-1401, "Quantifying uncertainty in nuclear analytical measurements," Austria, Vienna: IAEA; 2004.
[14] R. B. Sparks, B. Aydogan, "Comparison of the effectiveness of some common animal data scaling techniques in estimating human radiation dose," Sixth International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, TN: Oak Ridge Associated Universities, pp. 705–16, 1996.
[15] H. Yousefnia, S. Zolghadri, A. R. Jalilian, M. Tajik, M. Ghannadi-Maragheh, "Preliminary dosimetric evaluation of ^{166}Ho-TTHMP for human based on biodistribution data in rats," Appl. Radiat. Isot., vol. 94, pp. 260-5, 2014.
[16] J.J. Bevelacqua, "Internal dosimetry primer," Radiat. Prot. Manage., vol. 22, pp. 7-17, 2005.
[17] D. J. Brenner, "Effective dose: a flawed concept that could and should be replaced," British. J. Radiol., vol. 81, pp. 521–3, 2008.
[18] ICRP Publication 103, "The 2007 Recommendations of the International Commission on Radiological Protection," Ann. ICRP, vol. 37, pp. 2-4, 2007.
[19] United States Pharmacopoeia 28, NF 23, pp. 1009, 2005.
[20] United States Pharmacopoeia 28, NF 23, pp. 1895, 2005.
[21] A.L. Kesner, W.A. Hsueh, J. Czernin, H. Padgett, M.E. Phelps, D.H. Silverman, "Radiation dose estimates for (^{18}F)5-fluorouracil derived from PET-based and tissue-based methods in rats," Mol. Imaging Biol., vol. 10, pp. 341-8, 2008.
[22] ICRP Publication 62, "Radiological Protection in Biomedical Research," Ann. ICRP, vol. 22, pp. 3, 1993.
[23] ICRP Publication 53, "Radiation Dose to Patients from Radiopharmaceuticals," Ann. ICRP, vol. 18, pp. 1-4, 1988.
[24] Radiation Internal Dose Information Center, "Radiation dose estimates to adults and children from various radiopharmaceuticals," Oak Ridge Institute for Science and Education. Oak Ridge, TN 37831. Available at: orise.orau.gov/files/reacts/pedose.pdf. pp. 14, 1996.