2011 International Nuclear Atlantic Conference - INAC 2011
Belo Horizonte,MG, Brazil, October 24-28, 2011
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-04-5**

INTERNAL DOSIMETRY FOR [4-^{14}C]-CHOLESTEROL IN HUMANS

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ABSTRACT

This study proposes a biokinetic model for use in the assessment of the internal dose received by human subjects administered orally with [4-^{14}C]-cholesterol. The proposed model includes three systemic pools representing the short-term (T½ = 1 d), intermediate-term (T½ = 16 d) and long-term (T½ = 78 d) physiological exchanges and two excretion pathways: urine and feces. This model used the ANACOMP software to estimate radiometric doses with MIRD techniques (Medical Internal Radiation Dose). To validate the model, the profile curve of excretion prediction by the model in the range of seven days was compared with those curves described in literature. No statistical difference was detected (P = 0.416). The estimated effective dose coefficient calculated for the reference man described on ICRP publication 23 was 3.39×10^{-10} SvBq^{-1}. The organs that received the highest equivalent dose were the lower large intestine (2.459×10^{-9} GyBq^{-1}), upper large intestine (9.023×10^{-10} GyBq^{-1}) and small intestine (3.717×10^{-10} GyBq^{-1}).

1. INTRODUCTION

[4-^{14}C]-cholesterol is one of the most widely used radiotracer for biomedical research due to the importance of this compound in life maintenance and to understand the etiology of coronary heart diseases. The labeled molecule provides information regarding cholesterol physiology and its substrates (biliary acids, hormones and vitamins) in the body pools. Radiotracers constitute a simple and accurate tool for metabolic studies; however, the scientific community has shown reservations concerning the use of radioisotopes in young and pregnant women, children and normal patients [1]. This apprehension is probably the result of the deleterious effects of radiation. Although studies that utilize radioisotopes are approved by ethic committees, most do not mention the radiometric doses at which the human subjects are exposed to during the study period. The dose parameter should be the main concern taken into account for ethic committee approval. The radiometric dose

calculation is performed in order to assess the risks to health associated with the administration of the radioisotopes in human subjects. The calculation of the internal radiation energy delivered by radionuclides following injection into the body requires a biokinetic model that describes the biodistribution and retention of the radionuclide within body tissues [2]. The distribution of the radiotracer and the pathway of excretion are dictated by chemical reactions and tissue affinity of the labeled compound. Radionuclides distributed in the body emit radiation isotropically, i.e., without preferential directions. The flow of radiation (per unit of area) in regions close to the radionuclide emission is greater than in regions farther from the emission. In the case of ^{14}C (T½ = 5700 years, β^-_{max} = 156 keV) the deposited energy for the β-particles will be completely absorbed by those tissues closest to the β-emission region [3].

In 1968, the Society of Nuclear Medicine created the MIRD Committee (Medical Internal Radiation Dose) to develop and provide a standardized framework and methodology for the calculation of internal dose quantities for nuclear medicine. This committee has published many useful reports and other aids for calculating absorbed dose estimates for nuclear medicine applications [4]. The publications describe mathematical representations of the human body, which provide the absorbed fractions and radionuclide masses in organs. According to MIRD formalism, in any real internal dose problem, there will be more than one organ which concentrates the activity, and many targets for which the absorbed dose is required. In the case of the emissions of β-radiation, the target and source organs can be the same tissue [4].

The International Commission on Radiological Protection (ICRP) provides specific biokinetic models for a limited number of ^{14}C-labeled compounds only. For those compounds that do not have a described model, the generic radiocarbon model (GCM) is commonly used, as described in ICRP Publications [5, 6]. Despite the importance of cholesterol in human physiology, the ICRP does not provide a specific biokinetic model for [4-^{14}C]-cholesterol, and in such cases, the GCM would be advisable. The GCM is based upon the average rate of carbon intake for Reference Man[7] and it is assumed that ^{14}C-labeled compound is instantaneously and uniformly distributed in the body tissue and excreted with a half-life of 40 days [5,6].

Taylor [2] calculated the effective dose coefficients for 27 ^{14}C-labeled compounds (including [4-^{14}C]-cholesterol) for which dose information had not yet been published. The kinetic models proposed by Taylor are very similar to the GCM structure and uses kinetic literature data. The [4-^{14}C]-cholesterol model proposed by Taylor is based on kinetic data from reference [9]. In Taylor's [2] model the [4-^{14}C]-cholesterol uptake is exclusively intravenous. This can be considered a significant limitation, considering that the common uptake of cholesterol is by ingestion. Moreover, (a) the excretion profile from Taylor's model was not validated in terms of excretion (feces and urine) comparing the model prediction with experimental data and (b) the model description is highly simplified and is unable to predict an overall view of the dose in different organs as can be previewed by the MIRD protocol. Taylor's model predicts an effective dose coefficient for injection of 3×10^{-10} $SvBq^{-1}$. Assuming that Taylor model is more realistic than GCM, despite its limitations described above, the GCM [5, 6] (5.8×10^{-10} $SvBq^{-1}$) overestimates the dose in 93.3 % in this case. An improvement of the model by taking into account the uptake by ingestion, the most usual intake of cholesterol, still remains open.

A new generic model for systemic radiocarbon that is less conservative than the current ICRP model [6] but maintains sufficient conservatism to avoid under estimating the effective dose by most radiocarbon–compound-specific models was proposed by Manger [10]. The Manger model is based upon common characteristics of current biokinetic models and excretion data derived from biokinetic studies in human subjects and rats. This model accounts for the short, moderate and long half-times that are present in many radiocarbon compounds that have been studied. Upon ingestion the radiocarbon proceeds according to the Human Alimentary Tract Model (HATM) described in ICRP Publication 30 [5]. The radiocarbon reaches the small intestine (SI) and it is rapidly absorbed and distributed to tissues by body fluids. In the Manger model, the excretion profile is based on the average of fourteen ^{14}C-labeled compounds. The profile of 60% in urine, 25% in breath and 15% in feces is used as a benchmark for the fine-tuning of the transfers coefficients.

The excretion profile of [4-^{14}C]-cholesterol is very different from that quoted by both generic models (ICRP GCM and Manger). Therefore, a new model or a deep modification in these models is required to fit the cholesterol biokinetical parameters. In contrast to the average of 15% for breath excretion used in the generic model proposed by Manger [10], Hellman [9] and co-workers reported that no radioactivity was found in the expired air after the administration of [4-^{14}C]-cholesterol. The percentage of cholesterol intestinal absorption investigated with radioactive tracers varies from 15% to 75% in humans [11]. Experimental results show that the plasma peak specific activity is not reached until the second or third day after ingestion [1, 9-15]. Most of the unabsorbed cholesterol appears in fecal samples on the second or third day after the meal [14, 15]. Only 0.35 to 1.76 % of the ingested [4-^{14}C]-cholesterol appeared in the urine, against 60% used as benchmark by the Manger generic model. Therefore, the kidney is a minor route for the excretion of [4-^{14}C]-cholesterol [9].

The purpose of the present study was to improve the current radiocarbon generic model proposed by Manger [10] for the assessment of the radiometric doses of orally administered ^{14}C-cholesterol. To validate the proposed kinetic model, the excretion and absorption profiles for [4-^{14}C]-cholesterol ingestion were compared with the profile curves described in the literature [15] and [16]. The radiometric doses were calculated for the Reference Man [7] using the MIRD protocol [16] and the ANACOMP software [17].

2. DEVELOPMENT OF THE PROPOSED MODEL

2.1. [4-^{14}C]-Cholesterol Excretion Data

Hellman et al. [9] reported that no radioactivity was found in expired air after the [4-^{14}C]-cholesterol administration; indicating that as little as 0.1 per cent of the administered dose per day could have been detected by the method used. The fact that no labeled carbon dioxide was detected after the administration of [4-^{14}C]-cholesterol shows that ^{14}C remained attached to the rest of the cholesterol nucleus [9]. Others references [18] exclude the possibility of [4-^{14}C]-cholesterol excretion in $^{14}CO_2$ form.

Experimental results for [4-^{14}C]-cholesterol in humans show that the plasma peak specific activity is not reached until approximately the second or third day after ingestion [1, 9-15]. The percentage of cholesterol intestinal absorption investigated with radioactive tracers varies from 15% to 75% in humans [11], a broad range suggestive of metabolic or genetic regulation [1]. The efficiency of absorption also may be related to the large difference in the amount of

dietary cholesterol intake, Borgstrom [15] reported the averages of 45.7 ± 6.1 % for 150 mg, 48.5 ± 15.0 % for 550 mg), 33.3 ± 10.0 % for 950 mg and 22.8 ± 2.6 % for 1910 mg of cholesterol. All cited values for cholesterol absorption are from normal subjects.

In the present study, data from 43 normal subjects from references [14] and [15] were used to calculate the cholesterol absorption in function of the amount of ingested cholesterol. The amount of percent cholesterol absorbed was linearly related to dose fed over the range from 110 to 1910 mg (Figure 1). This linear fit is shown in equation 1,

$$C_{absorbed} = 50 - 0.014 \cdot C_{ingested} \quad (1)$$

where $C_{absorbed}$ is the amount of cholesterol absorbed (%) and $C_{ingested}$ (mg) is the amount of cholesterol ingested in a single test meal.

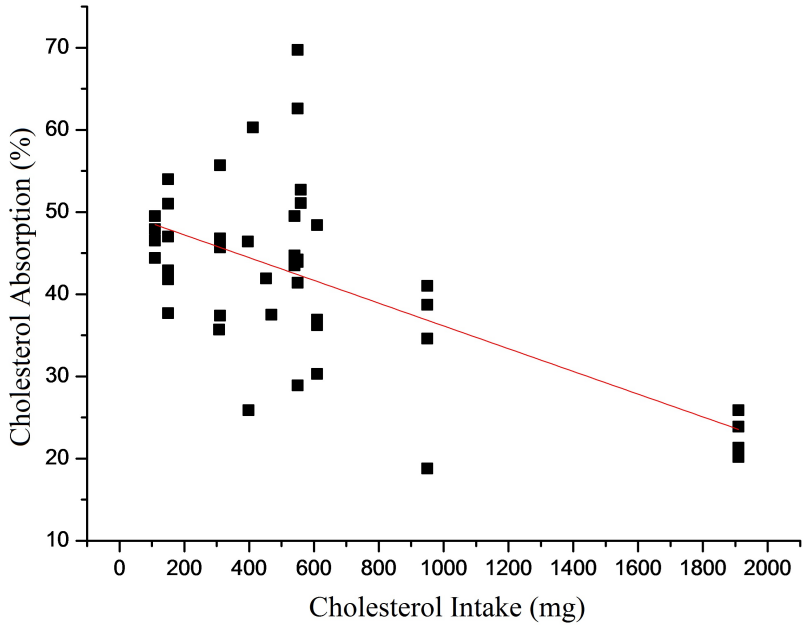

Figure 1. The cholesterol absorption (%) of 43 normal subjects after the ingestion of a single test meal containing different amounts of cholesterol (mg). The data are derived from references [14] and [15].

In published studies [14, 15], the radioactivity of the unabsorbed dietary cholesterol was determined in daily fecal samples over six days to provide an estimate of the absorbed cholesterol. Most of the unabsorbed cholesterol appeared in fecal samples on the second or third day after meal ingestion and the total of the unabsorbed cholesterol was recovered over 6 days [14, 15].

In this study, data from 43 normal subjects derived from references [14] and [15] were used to calculate the average cholesterol excretion in function of time after ingestion. The amount of [4-^{14}C]-cholesterol ingested by the subjects of the select data varied from 110 to 1910 mg. The cholesterol excretion average for the range of six days is listed in Table 1.

Table 1. Cumulative [4-^{14}C]-cholesterol radioactivity recovered in feces in function of time after cholesterol uptake (days). The data are from 43 normal subjects that ingested a single test meal containing different amounts of cholesterol (110 to 1950 mg), the data are derived from references [14] and [15].

Time (days)	Cumulative Radioactivities (%)
1	7.22 ± 10.15
2	34.21 ± 21.30
3	47.44 ± 20.15
4	53.65 ± 16.69
5	57.19 ± 12.50
6	58.01 ± 12.90

Only 0.35 to 1.76 % of the ingested [4-14C]-cholesterol appeared in the urine since the kidney is a minor route for the excretion of the cholesterol nucleus. A considerable fraction of the urinary radioactivity is in the form of steroid hormone metabolites [9].

2.2. Biokinetic Model

The [4-^{14}C]-cholesterol model proposed in this paper is an improvement of the current generic biokinetic model proposed by Manger [10] based on kinetic data derived from experimental studies with [4-^{14}C]-cholesterol in human subjects [9, 14, 15]. It is important to observe that the proposed model is limited to tracers capable of following the cholesterol molecule through its entire metabolic pathway, as is in the case of [4-^{14}C]-cholesterol. Some tracers like the ^{14}C-cholesteryl oleate, which is labeled in the fatty acid portion of molecule, can be hydrolyzed and deviate from the cholesterol pathway core.

The structure of the kinetic model contains nine compartments (Figure 3). The pathway of [4-^{14}C]-cholesterol uptake is through ingestion and the input of the labeled particle is represented by an arrow with asterisk (C1). The pathways of excretion considered are renal (C9) and fecal (C4). No measurable ^{14}C activity was reported in expired air after [4-^{14}C]-cholesterol administration [9, 18], thus this excretion pathway was suppressed of Manger's model. Furthermore, the proposed model includes one more systemic pool of cholesterol exchange (moderate-term, T½ = 16 d) and the colon is divided in two compartments (U.L.I. and L.L.I) in agreement with ICRP 30 [5] and Eve model [19, 20].

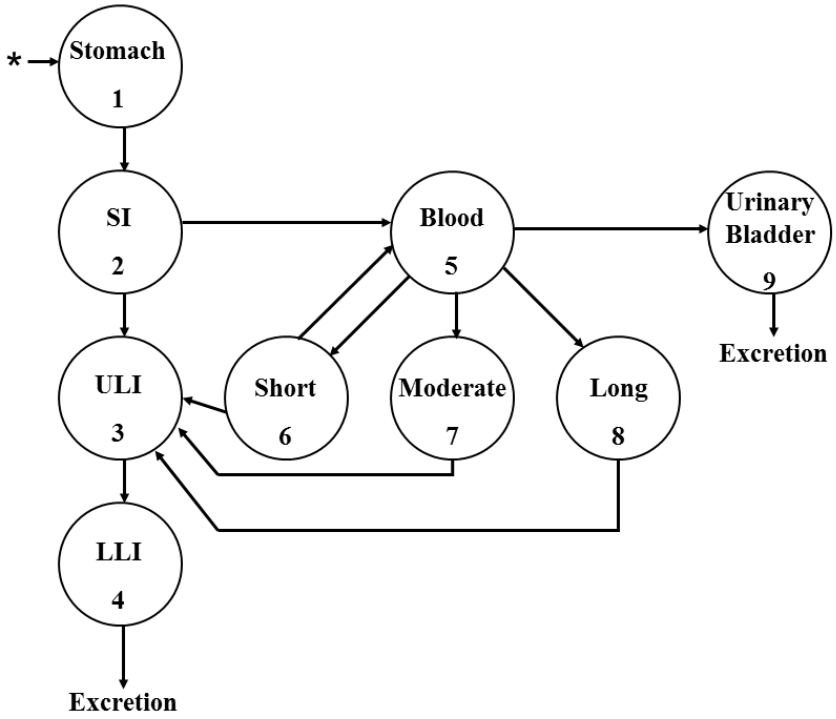

Figure 3. Proposed biokinetic model for [4-^{14}C]-cholesterol. C1: stomach; C2: small intestine (SI); C3: upper large intestine (ULI); C4: lower large intestine (LLI); C5: blood, C6: systemic short (T½ = 1 d), C7: systemic moderate (T½ = 16 d); C8: systemic long (T½ = 78 d) and C9: urinary bladder. The input of the [4-^{14}C]-cholesterol is represented by an asterisk.

Upon ingestion the [4-^{14}C]-cholesterol proceeds according to the Human Alimentary Tract Model (HATM) described in ICRP 30 [5]. The ingested [4-^{14}C]-cholesterol reaches the the small intestine (C2) and is absorbed into the bloodstream (C5). The [4-14C]-cholesterol that reaches the blood is transferred to urinary bladder and to three systemic pools representing the short-term (T½ = 1 d), moderate-term (T½ = 16 d) and long-term (T½ = 78 d) physiological exchanges of cholesterol between the body tissues [8, 9]. The $k_{i,j}$ parameters (Table 2) represent the transfer of material from compartment i (C_i) to compartment j (C_j) and are associated with the biological half-life by the equation $k_{i,j} = \log_e(2)/T½$. The parameters $k_{2,5}$ (S.I. to Blood) and $k_{2,3}$ (SI to ULI) can be calculated in function of the amount of cholesterol ingestion by equations 2 and 3 respectively:

$$k_{2,5} = \frac{(50 - 0.014 \cdot C_{ingested})}{100} \cdot 0.115 \quad (2)$$

$$k_{2,3} = \left[1 - \left(\frac{50 - 0.014 \cdot C_{ingested}}{100}\right)\right] \cdot 0.115 \quad (3)$$

Table 2. Transfer coefficients for the proposed [4-^{14}C]-cholesterol biokinetic model.

Pathway	Transfer coefficient (d^{-1})
$k_{1,2}$ (Stomach to SI)	24
$k_{3,4}$ (ULI to LLI)	1.8
$k_{4,0}$ (LLI to excreta)	1
$k_{5,6}$ (Blood to short)	1.5
$k_{5,7}$ (Blood to moderate)	0.03
$k_{5,8}$ (Blood to long)	0.002
$k_{5,9}$ (Blood to bladder)	0.002
$k_{9,0}$ (Bladder to excreta)	0.693
$k_{6,5}$ (Short to blood)	13.44
$k_{6,3}$ (Short to ULI)	0.693
$k_{7,3}$ (Moderate to ULI)	0.0433
$k_{8,3}$ (Long to ULI)	0.009

3. RESULTS AND DISCUSSION

3.1. Excretion Profile after [4-^{14}C]-Cholesterol Ingestion

In order to validate the proposed model, the predicted values were compared with the experimental data (Figure 4) [14, 15].

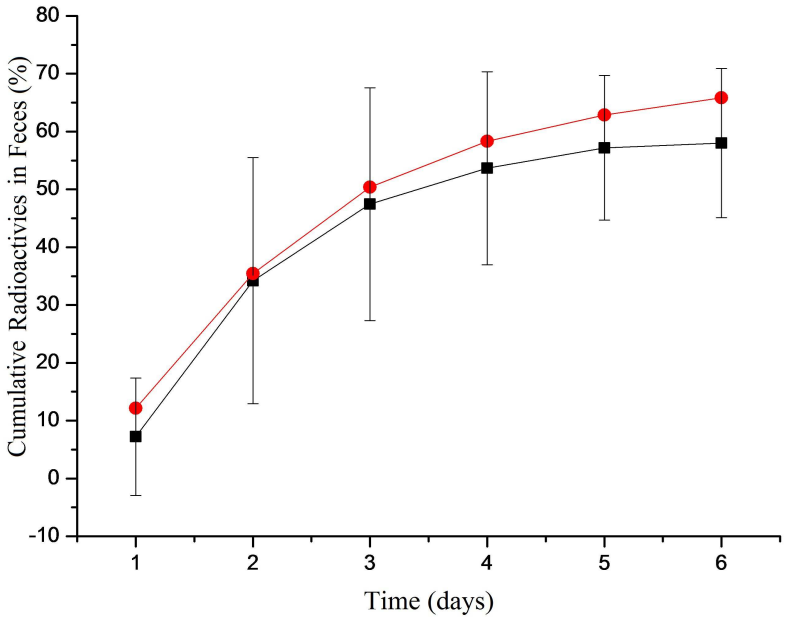

Figure 4. Average of the cumulative fecal radioactivity in function of time after [4-^{14}C]-Cholesterol ingestion. ■: Experimental data from 43 normal subjects derived from [14] and [15]. The bars represent the standard deviation. ●: Predicted by the proposed kinetic model.

The [4-^{14}C]-cholesterol excretion profile predicted by the proposed model was compared with the excretion profile listed in Table 1. The ingestion for the same amounts of cholesterol (110 to 1910 mg) was simulated for the proposed model and the weighted average calculated. There was no statistically significant difference between the excretion profiles according to the Kruskal-Wallis test (P = 0.416).

3.2. Calculation of the Radiation Dose

The radiometric doses were determined using the MIRD (Medical Internal Radiation Dose) protocol [16] by means of the ANACOMP software computer [17], which calculates the radiometric doses using the kinetic parameters $k_{i,j}$ (listed on Table 2) from the compartmental model shown in Figure 3. The parameters $k_{2,5}$ and $k_{2,3}$ are used for an uptake of 300 mg of cholesterol. The effective dose and the equivalent dose in organs (shown on Table 3) were calculated according to ICRP Publications 60[21] to the theoretical phantom of an adult described in ICRP Publication 23 [7].

Table 3. Effective dose and absorbed dose to organs after a [4-^{14}C]- cholesterol ingestion for the reference man [7].

Organs	Absorbed dose ($GyBq^{-1}$)
Adrenals	9.18×10^{-11}
Brain	9.18×10^{-11}
Breast	9.18×10^{-11}
Gallbladder	9.18×10^{-11}
L.L.I.	2.46×10^{-9}
S.I.	3.72×10^{-10}
Stomach	1.47×10^{-10}
U.L.I.	9.02×10^{-10}
Heart	9.18×10^{-11}
Kidneys	9.18×10^{-11}
Liver	9.18×10^{-11}
Lungs	9.18×10^{-11}
Muscle	9.18×10^{-11}
Ovaries	9.18×10^{-11}
Pancreas	9.18×10^{-11}
Bone Marrow	1.13×10^{-11}
Cortical Bone	8.80×10^{-11}
Skin	9.18×10^{-11}
Spleen	9.18×10^{-11}
Testicles	9.18×10^{-11}
Thymus	9.18×10^{-11}
Thyroid	9.18×10^{-11}
Urinary Bladder	1.14×10^{-10}
Womb	9.18×10^{-11}
Effective Dose ($SvBq^{-1}$)	3.39×10^{-10}

The ICRP 60 [21] recommends that any activities that require radioactive sources should not expose human study participants to an effective dose greater than 1 mSv/year. Adopting this dose level as acceptable to submit adult volunteers to metabolic studies with [4-^{14}C]-cholesterol, the amount of radioactive tracer for a single dose that can be administered orally should be ≤ 3MBq (81 μCi). Most studies that utilize [4-^{14}C]-cholesterol uses radioactivity in the range of 3.7 to 185 kBq (1 to 50 μCi) [9-15]. In such cases, the effective dose will be in the range of 0.0122 to 0.609 mSv which is lower than the 1 mSv allowed dose for an adult person per year. In other words, these dose levels are lower than the environmental dose from the sum of cosmic rays (gamma rays and X-rays from space) and radiation from ^{238}U, ^{236}Th, ^{40}K, ^{14}C found naturally in the earth. About half of the total annual average U.S. individual's radiation exposure comes from natural sources. The other half is mostly from diagnostic medical procedures. For the American population the average annual radiation exposure from natural sources is about 3.1 mSv/year. Radon and Thoron gases account for two-thirds of this exposure, while cosmic, terrestrial, and internal radiation account for the remainder. No adverse health effects have been related from doses arising from these levels of natural radiation exposure [22].

4. CONCLUSIONS

The proposed kinetic model achieves an adequate level of agreement between the calculated excretion and the experimental values reported in published studies [14, 15]. For [4-^{14}C]-cholesterol ingestion the estimated effective dose for an adult subject was 3.39 x 10^{-10} $SvBq^{-1}$. The organs that received the highest equivalent dose were the lower large intestine (LLI) 2.459×10^{-9} $GyBq^{-1}$, upper large intestine (ULI) 9.023×10^{-10} $GyBq^{-1}$ and the small intestine (SI) 3.717×10^{-10} $GyBq^{-1}$. For oral administration in the range of 3.7 to 185 kBq (1 to 50 μCi), the effective dose will be 0.0122 to 0.609 mSv which is lower than the 1 mSv which is commonly accepted as safe for an adult person.

ACKNOWLEDGMENTS

The authors wish to thank CAPES for financial support.

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