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CFFTP

METABOLISM OF TRITIUM UPTAKE DUE TO HANDLING OF METAL SURFACES EXPOSED TO TRITIATED HYDROGEN GAS

CFFTP-G-87026 / (AECL-9518).
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by

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1. INTRODUCTION

Tritiated hydrogen gas (HT)* has been observed to adsorb onto metal surfaces following the Langmuir adsorption process (1). After adsorption tritium desorbs from metal in the form of HT gas and tritiated water (HTO) (1). This desorption process takes months to complete (1).
Future tritium extraction and fusion reactor facilities pose a potentially significant radiological hazard due to the handling of high concentrations of HT gas, as workers may come into contact with metals adsorbed with HT.

Earlier studies with both rats (2) and humans (3) have shown that when contact is made between skin and metals adsorbed with HT, tritium can be transferred from the metal to the skin and subsequently to body fluids. Eakins et al. (3) have shown that besides HTO, organically bound tritium (OBT) is excreted in urine. Although the OBT was not identified, there is some evidence that the OBT contains carboxylic acid and/or ketonic functional groups (3).

Hutchin and Vaughan (2) also observed the presence of OBT in skin of rats after they were exposed to HT contaminated planchets.

Johnson and Dunford (4) proposed a model for tritium uptake by skin contact. An experimental program was initiated to obtain data to improve and refine the proposed models, which could then be used to calculate doses to workers exposed to HT contaminated surfaces.

*HT is used throughout this report to denote tritiated hydrogen gas. No significant differences in the adsorption onto surfaces, or the uptake through skin and subsequent metabolism of HT and T_2 are expected.

In particular, in order to evaluate the radiological hazard from HT sorbed on metal surfaces, the following questions need to be answered:

(a) What are the excretion patterns of OBT and tritiated water after exposing the skin to HT adsorbed on metal surfaces?

(b) What are the retention patterns in exposed skin and some other organs such as unexposed skin, liver, muscle and blood?

(c) What are the accumulation patterns of tritium activity in skin (i.e. what are the activity profiles across the skin)?

(d) What is the chemical nature of OBT? and

(e) What are the implications for the dosimetry and radiation protection?

This report discusses the work carried out to date to answer the above questions.

2. EXPERIMENTAL

2.1 Methods of Exposure

2.1.1 Exposure of Metal Planchets

Stainless steel planchets of approximately 2.5 cm in diameter and 0.5 mm in thickness were exposed to 369 torr of HT gas for 30 minutes in the CRNL tritium facility. The HT was flushed out, and the container was taken to the Dosimetric Research Branch's laboratory, where the container was opened and after 15-30 minutes of waiting period (to allow HT to escape from the container) the planchets were taken out and used for the contact exposures. All procedures were carried out in a fume hood and the concentration of HT in air was monitored with a tritium monitor AEP-5321 (Atomic Energy of Canada Limited).

2.1.2 Animal Exposure

Hairless rats (Spraque-Dawley/CRNL:hy) used for the exposures were obtained from the CRNL Animal Facility. The rats were exposed by rubbing the rear flanks with ten strokes of 1 second duration with HT contaminated stainless steel planchets. Following the exposure the animals were placed in metabolic cages purchased from Canadian Laboratory Supply Company, Ottawa, Ontario.

2.1.3 Fractionation of Skin

To find which macromolecular fraction (liquid, protein or DNA) contained OBT, a patch (~ 5 g) of exposed skin was cut into small pieces and dried under vacuum for 24 hours to remove HTO. The dried skin was then rehydrated and homogenized in a blender. Chloroform extraction was employed to separate liquid from the rest of the homogenate (5). Chloroform was removed from the solution by heating the lipid solution. The activity of lipid fraction was then measured by means of a scintillation counter (2.2.1). The remaining homogenate contained proteins and DNA. Proteins were removed by phenol extraction. Phenol was removed from the protein solution by ether extraction. DNA was separated from the remaining homogenate by water. Both protein and DNA solutions were counted for tritium activity. The remainder containing insoluble proteins (collagen) was dried and combusted for the determination of tritium (see Section 2.2.3).

2.2 Measurement of Tritium Activity

2.2.1 Measurement of Tritium Activity in Urine

Aliquots of urine (0.2 mL to 1.0 mL) were mixed with scintillation liquid and counted in a liquid scintillation counter (Beckman LS100 or LS7000). The samples were spiked with NBS traceable HTO standard solution to determine the tritium concentration.

2.2.2 Measurement of OBT Activity in Urine

In order to determine the OBT content of the urine, low temperature distillation was used. This is the procedure that was used by Eakins et al(3) and was previously tested and used in our laboratory as reported previously (6). Briefly, a volume of approximately 2.0 mL of urine was put in a glass petrie dish, and a beaker of ice was placed on the cover. The sample was heated to about 40°C and the water that condensed on the cover was collected. The tritium activity of condensed water was measured using the same procedure described earlier (Section 2.2.1). The difference between the initial urine activity and condensate activity was attributed to OBT.

2.2.3 Measurement of Organically Bound Tritium

To estimate the amount of OBT in tissue, each tissue sample was cut into small pieces and dried. The samples were vacuum dried at room temperature for about 10-15 hours until the constant weight (see Fig. 1) was achieved (except for blood which was freeze dried). The dried weights were in average 32%, 27%, 30% and 19% for skin, muscle, liver and blood, respectively, of the wet weights. Free water removed from the samples was trapped by a cold trap at liquid nitrogen temperature placed between the sample and the pump. The dried tissue samples were then pelletized by means of a pellet press (Parr Instruments). Since dried tissue is brittle the samples were wrapped in tissue paper before the pellet was pressed. The pellets were then combusted in a Parr Instrument Co. (Moline, Illinois, USA) oxygen combustion bomb. The oxygen pressure before the combustion was 25 psi. After combustion, which occurs in a fraction of a second, the bomb was placed into an icewater bath (about $0^{o}C$) for about 15 minutes to condense the water vapours onto the walls of the bomb. The gases from the bomb were then released through a cold trap to trap uncondensed water vapors. Using a known amount of distilled water, the inside of the bomb, the lid of the combustion bomb, and the trap were rinsed, and the tritium activity in the rinse was counted in a liquid scintillation counter (Beckman LS7000, overnight count).

The percentage recovery of the combustion procedure was determined by combusting known amounts of tritiated thymidine. Eleven experiments indicate that the recovery of the procedure was 89 $\pm$ 7% and this number was used to calculate the activities of combusted samples.

It should be noted that the procedure given above measures total organically bound tritium, whereas OBT refers to the non-exchangable tritium. Hence the concentration given in subsequent sections for OBT will be an overestimation.

3. RESULTS

3.1 Pattern of Tritium Excretion in Urine

Four animals were followed over a 17 day period with urine samples being analyzed each day for total tritium activity and for the activity in the inorganic phase (i.e. tritium activity in the condensate). The difference of the two activities is attributed to the activity of OBT (3).

Figure 2 shows an example of the retention of tritium activity as a function of time. As can be seen, the OBT component represents a significant fraction of the initial activity excreted. After about 5 days the activity of OBT excreted was negligible in all four animals. The retention curve for OBT could be approximated with a sum of two exponential, one with a half-life of about 0.4 days and another with a half-time of about 1.4 days. The retention of HTO was well fitted by a single exponential curve with a half-life of 3.1 days, which agrees well with the values for rats observed previously (6-10). Table 1 shows the half-lives for the excretion of OBT and HTO for four animals with the respective amplitudes. In the case of the second animal the second component could not be identified. Included in Table 1 are the halflifes estimated by Johnson and Dunford(4) from the work of Eakins et al(3) for humans. The reasonably good agreement between the OBT retention in rats and humans supports our contention that the organic constituents formed in this mode of tritium uptake, and their subsequent metabolism and excretion are the same in rats and humans. The difference in HTO retention is expected from other experiments on HTO retentions in rats and humans.

3.2 Distribution of Free and Organically Bound Tritium in Some Tissues

Exposed skin, unexposed skin, liver, muscle and blood of six animals (two sacrificed at 4 hours after exposure, two at 24 hours after exposure and two at 48 hours after exposure) were removed and assayed for free HTO and for organically bound tritium (Section 2.2.3). The activities expressed in nCi/g of wet tissue for HTO and OBT are given in Table 2. Each value represents an average of at least two measurements. As expected, the highest activity was found in the exposed skin. The other organs which also show particularly high activity are the unexposed skin and liver. It should be noted that there were

differences observed between animals which are probably due to the difficulties of reproducing the exposure conditions but could also result from differences in the metabolism of the animals. Two general trends with time can be observed from Table 2.

i) the activity in the exposed and unexposed skin decreases, and

ii) the activity of HTO increases after exposure especially in liver, muscle and blood. It is assumed that this results from oxidization of the OBT, either at the site of exposure, or elsewhere.

Table 2(c) is useful as it gives an indication of the difference in our ability to estimate the doses from this type of exposure compared to an HTO exposure. In an HTO exposure, the ratios in Table 2(c) would all be essentially one as very little HTO would have been incorporated into OBT up to 48 hours past exposure. The average tritium concentration in tissue would be about 70 - 80% of that in urine (11). The factor by which the ratios in table 2(c) exceed one is the factor by which urinary concentrations would underestimate the dose rate if the exposure was assumed to be an HTO exposure, rather than an HT exposure.

3.3 Examination of Exposed Skin

The exposed skin samples were longitudinally cut into thin slices using a microtome (Spencer-Lens, Model 810) in order to study the distribution of tritium activity as a function depth in skin. Skin patches of rats sacrificed 4 and 24 hours after exposure were used for the experiments. The skin patches (approximately 1 cm x 1 cm) were immobilized in agar before cutting. Wet skin samples were cut into 40 m thick layers and the dried skin samples (after drying of skin under vacuum for 24 hours) were cut into 25 m thick layers. Ten slices were combined and combusted in an oxygen combustion bomb and the product of combustion counted for tritium activity. The activity profile measured in the dried skin which was removed 4 and 24 hours after the exposure can be seen in Figure 4. It can be seen that the activity retained after drying, due to the presence of bound tritium, is heavily concentrated in the uppermost layers of the skin. The distribution of organically bound tritium 4 hours after eposure seemed to be similar, but slightly steeper, than the 24

hour OBT profile. This could be due to the combined effect of tritium leaving the skin surface (returning to air) and penetrating further into the skin.

The activity in the uppermost layers of the skin (0 - 150 m) was examined in more detail. Figure 4 shows the activity profiles for both wet and dried skin samples. The basal layer, which is probably located somewhere between 50 μm and 100 μm, is exposed to a tritium concentration of between 70-90% of that at the surface.

3.4 Identification of Macromolecular Fraction Containing OBT

The objective of this experiment was to determine which fraction (lipid, protein or DNA) contains OBT. The skin (removed 24 hours after the exposure) was dried, rehydrated in water, homogenized and fractionated as discussed in Section 2.1.3. After separation, the activity in each fraction was counted (see Table 3). As can be seen in Table 3 the two fractions containing the highest amount of radioactivity were lipid and insoluble protein (mainly collagen). The total activity per gram of starting material of 555 nCi/g agrees quite well with the value reported in Table 2(a). The dry weight of the lipid in the skin sample was estimated to be 3.8% of the wet tissue weight. The dry weight of protein and DNA was estimated at 29% of the wet weight. The activity per unit weight of lipid is about 4.6 times higher than that for protein and DNA combined. Furthermore, from Table 3 it can be seen that all macromolecular components are labelled with tritium. Such non-specific labelling of HT in the presence of metals was observed previously (12).

4. DISCUSSION AND CONCLUSIONS

This experiment confirmed the results reported previously (2,3) for skin contact with HT contaminated surfaces. That is, there is a long term, elevated bound tritium concentration in skin at the point of contact; and OBT is excreted in urine following these exposures. In addition, liver and unexposed skin were found to have higher concentrations of OBT than other soft tissue.

Pri-Bar and Buchman (12) observed HT exchange between the HT and hydrogen atoms of organic molecules in the presence of metal. It is believed that such exchange (labelling) can also occur on skin and that metals are not required to catalize the reaction (3). Some of the labelled molecules will diffuse across the skin and will enter the body fluids. Some of the labelled compounds entering the body fluids are probably of small molecular size and will be excreted from the body via urine. Other perhaps larger molecules will be concentrated in liver, and distributed throughout other soft tissue.

Collagen located at or near the skin surface will also be tritiated. These molecules constitute the support structure of the skin and will remain in the skin for longer times. The activity in these molecules can be removed by shedding and replacing of the horny layer of the skin, a process with a half life of a few days (13). Thus, any model should include the possibility of the removal of OBT from the skin via the shedding of contaminated skin as well as by diffusion into the body fluids.

Our results indicate that the concentration of OBT in skin can be about 200-400 times higher than that in soft tissue such as muscle and the activity in liver about 5 - 8 times that in muscle. The latter results will necessitate a model with a compartment for liver separate from that for other soft tissues.

Johnson and Dunford (4) proposed two dosimetric models for HT skin exposure (see Figures 5 and 6). Both models were adjusted so that the model results for the concentration of HTO and OBT in urine was consistent with the retention patterns observed by Eakins et al(3). The first model gives the maximum retention of OBT in skin and the second the minimum retention of OBT in skin. Both models consist of six compartments. In model 1 the skin consists of four compartments from which HTO and OBT enter the body fluids to form two independent components. In model 2 the skin consists of two compartments from which HTO and OBT enter the body fluid compartment to form four interconnected compartments.

The results obtained in this experiment tend to support a model somewhat between the two models described previously (4), with perhaps three compartments in the skin and with two to describe body fluids and one for the liver. However, measurements of retention at longer times are required before an improved model can be developed.

TABLE 1

HALF LIFE OF EXCRETION OF OBT AND HTO

ANIMAL #	OBT (DAYS)		HTO (DAYS)
	COMPONENT 1	COMPONENT 2	
1	0.40	-	3.25
2	0.34	1.40	3.10
3	0.64	1.91	3.44
4	0.47	1.04	3.10
MEAN	0.46	1.45	3.22
HUMANS (Johnson and Dunford)	0.2	1.6	10

TABLE 2(a)

ACTIVITY OF BOUND TRITIUM IN nCi PER GRAM OF WET TISSUE

TISSUE	TIME POST EXPOSURE					
	4 HOURS		24 HOURS		48 HOURS	
	RAT # 1	RAT # 2	RAT # 3	RAT # 4	RAT # 5	RAT # 6
Exposed Skin	2522	4925	580	569	518	763
Unexposed Skin	177	318	104	122	48	74
Liver	37	22	44	35	78	63
Muscle	6.3	6.2	6.2	6.0	11.3	13.5
Blood	4.7	3.0	4.3	3.0	7.8	2.4

TABLE 2(b)

ACTIVITY OF FREE WATER TRITIUM IN nCi PER GRAM OF WET TISSUE

TISSUE	TIME POST EXPOSURE					
	4 HOURS		24 HOURS		48 HOURS	
	RAT # 1	RAT # 2	RAT # 3	RAT # 4	RAT # 5	RAT # 6
Exposed Skin	13.1	19.3	15.5	13.0	34.5	13.3
Unexposed Skin	4.6	7.4	15.7	11.5	44.1	12
Liver	3.0	8.8	17.8	13	56.0	12.7
Muscle	1.8	6.0	16.1	12.9	60.4	13.5
Blood	2.0	7.6	23.2	15.2	78.3	15

TABLE 2(c)

RATIO OF TOTAL TRITIUM TO FREE WATER TRITIUM

TISSUE	TIME POST EXPOSURE					
	4 HOURS		24 HOURS		48 HOURS	
	RAT # 1	RAT # 2	RAT # 3	RAT # 4	RAT # 5	RAT # 6
Exposed Skin	196	356	38	44	16	58
Unexposed Skin	39	44	7.6	12	2.1	7.2
Liver	13	3.5	3.5	3.7	2.4	6.0
Muscle	4.5	2.0	1.4	1.5	1.2	2.0
Blood	3.4	1.4	1.2	1.2	1.1	1.2

TABLE 3

ACTIVITY IN SEPARATED FRACTIONS IN SKIN

FRACTION	ACTIVITY (nCi)	WEIGHT* %
Lipid	691	3.8
DNA	16	
Protein	19	29
Insoluble Protein (collagen)	1099	
TOTAL	2480	

* Weight percent of wet tissue weight

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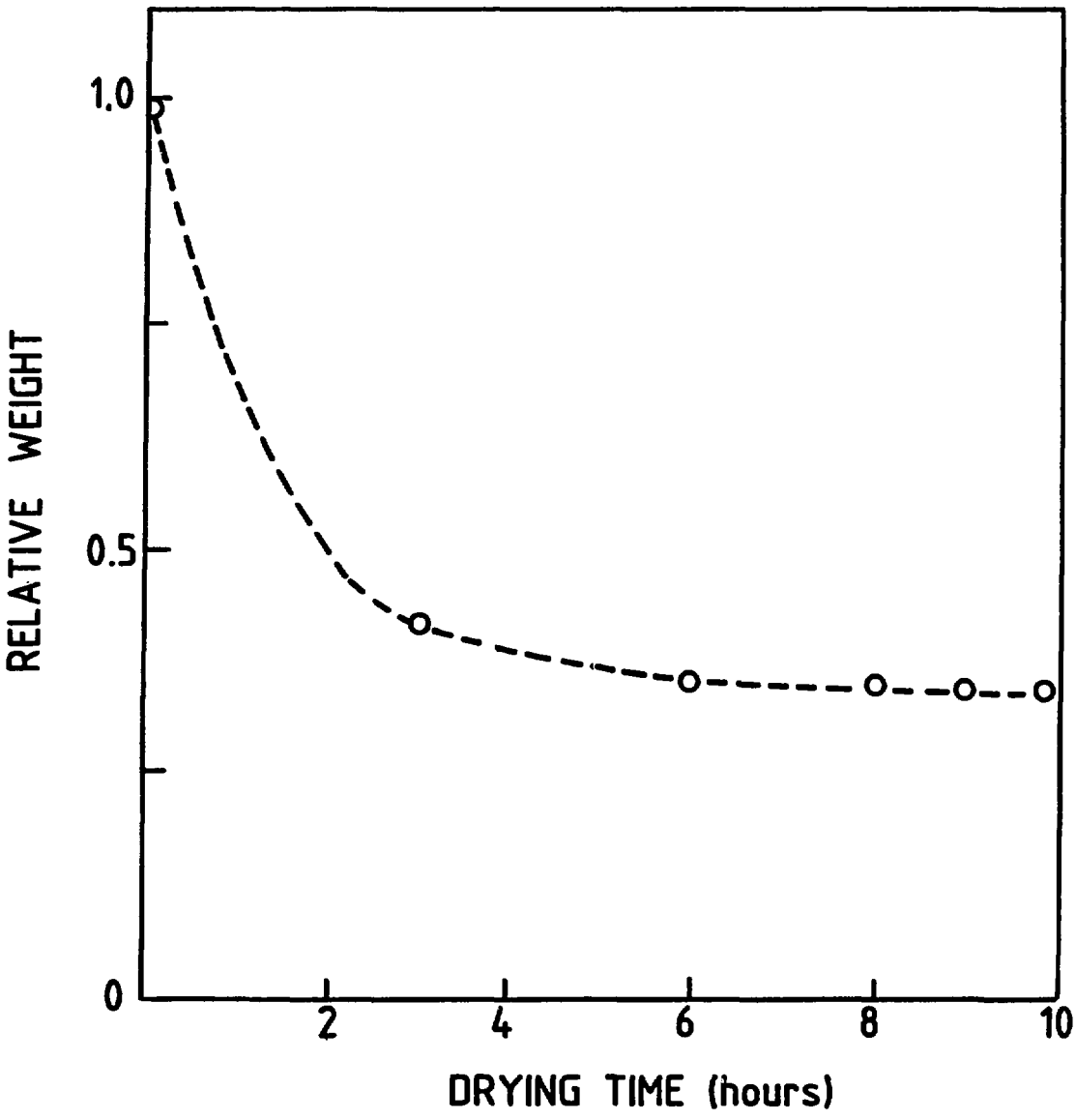

FIGURE 1: Weight of skin samples as a function of drying time.

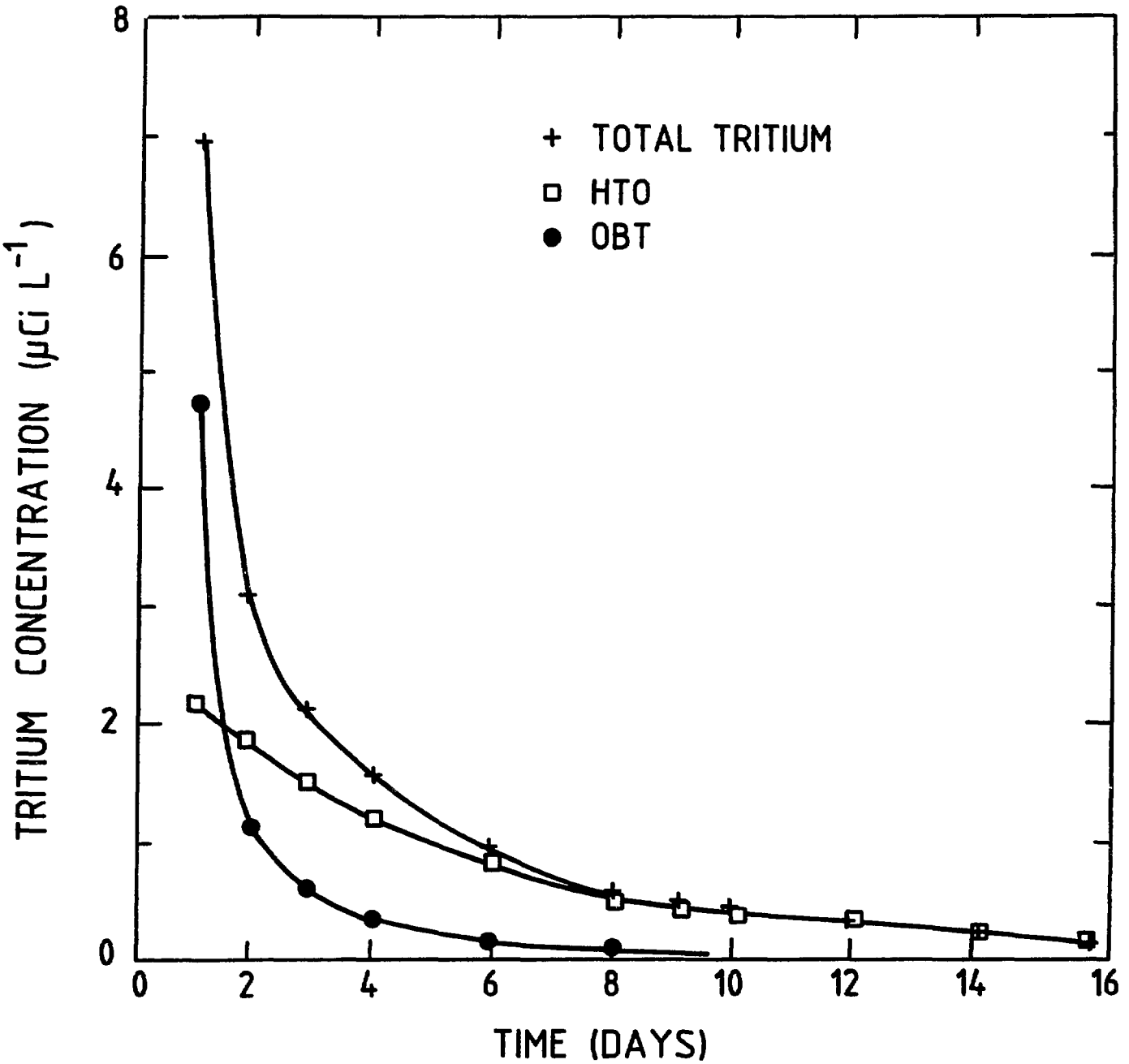

FIGURE 2: Tritium concentration in urine of rats following exposure to T_2 contaminated planchets.

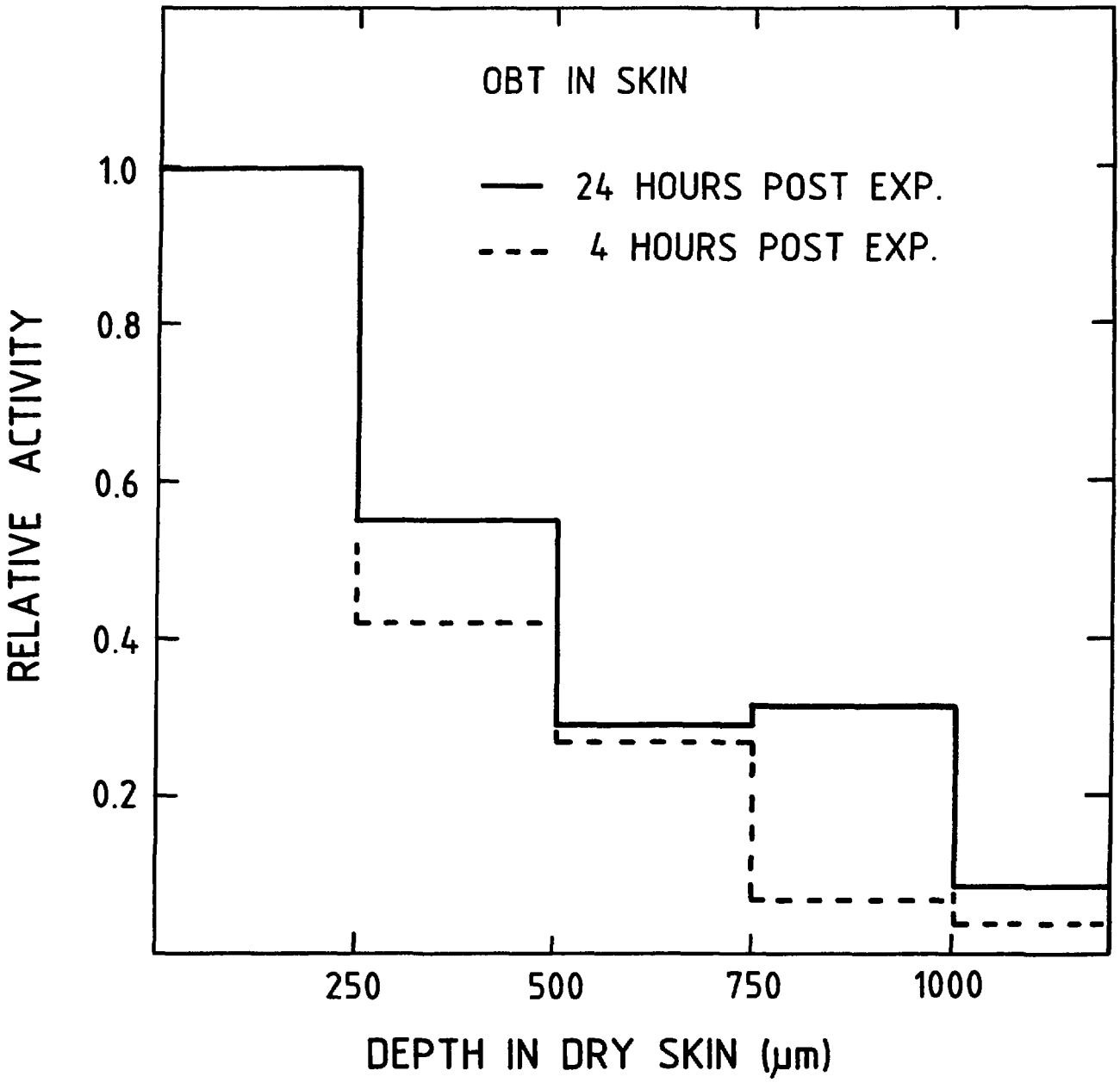

FIGURE 3: Bound tritium activity in exposed skin as a function of depth for two times post exposure.

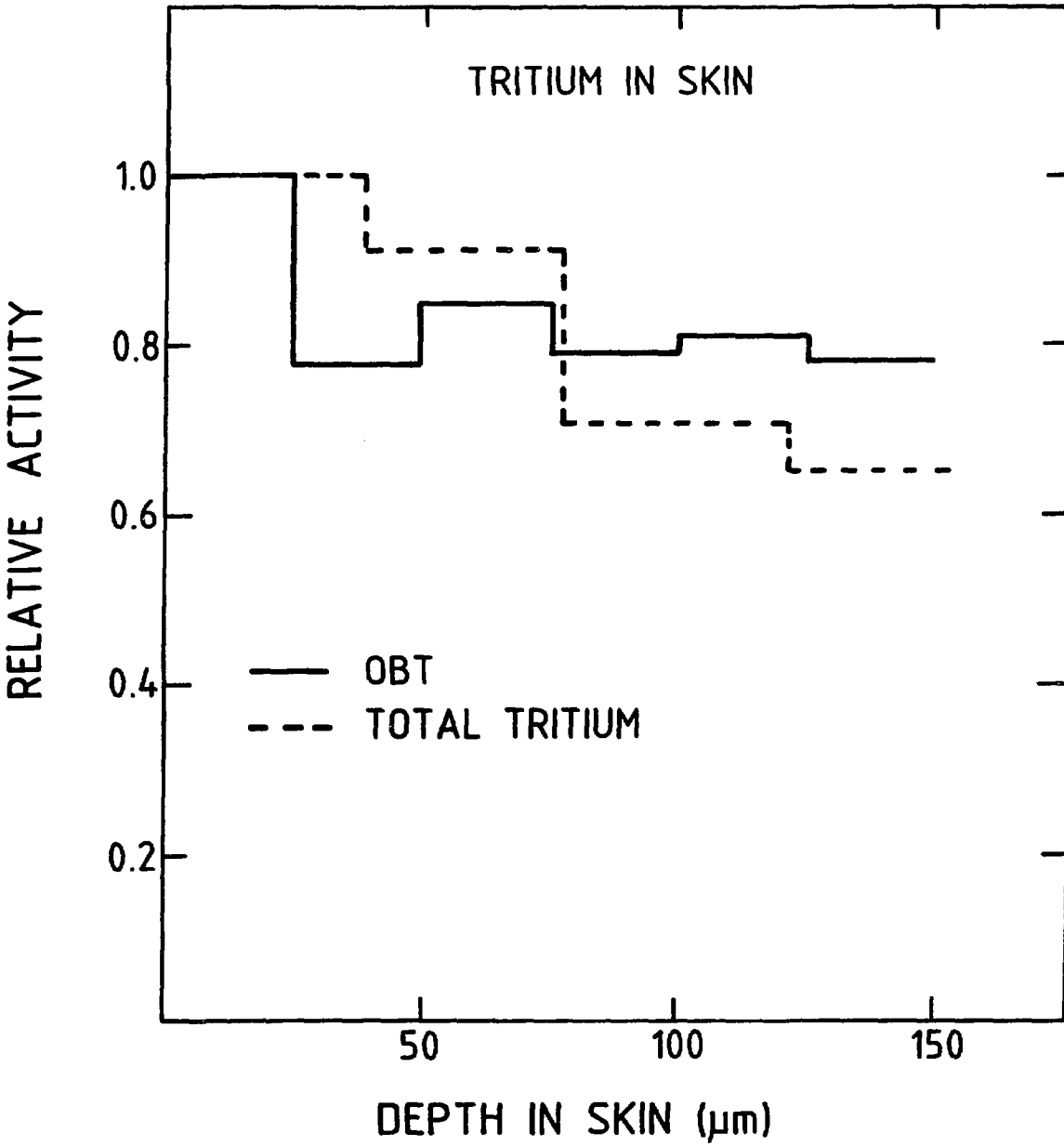

FIGURE 4: Total tritium and OBT in the top layer of exposed skin at 24 hours post exposure.

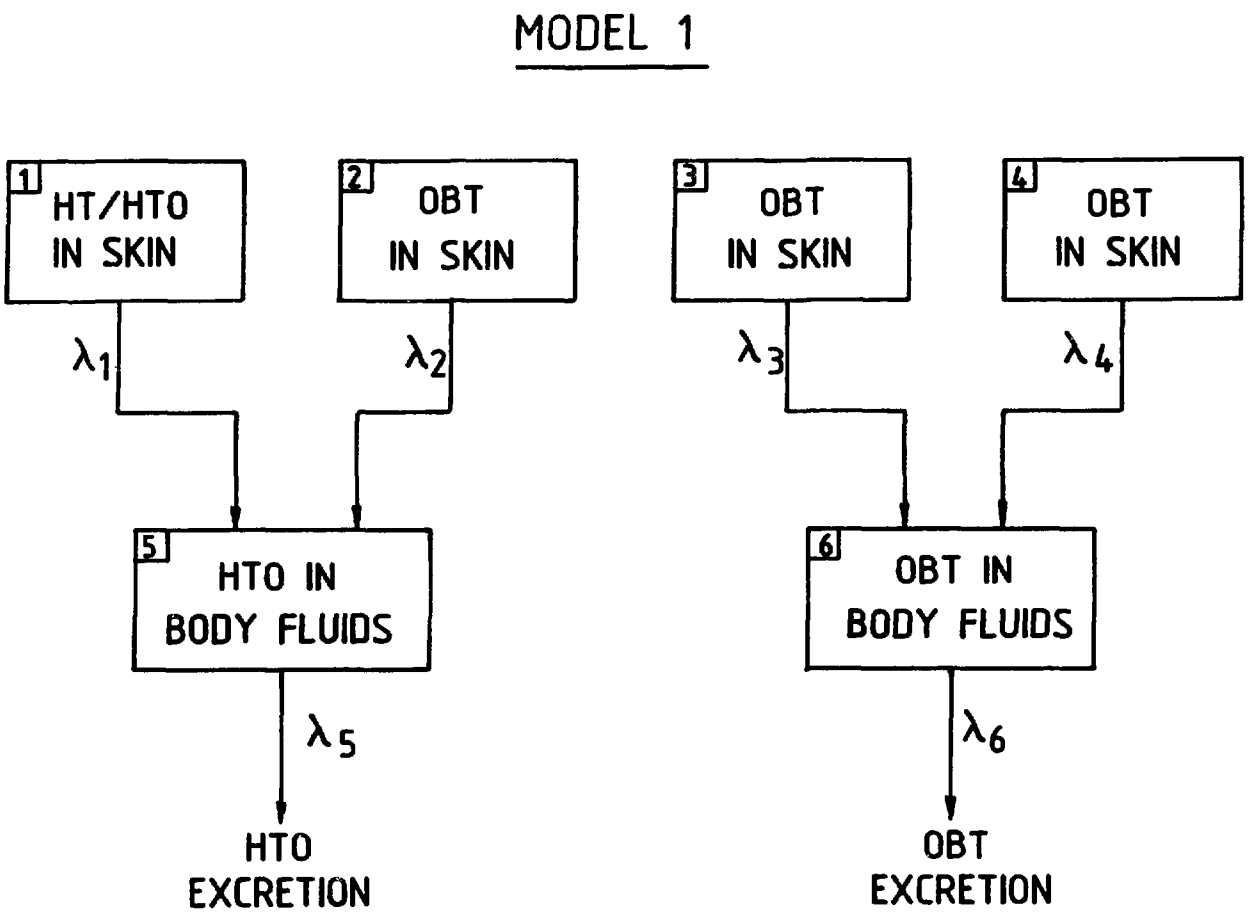

FIGURE 5: This model maximizes the dose to exposed skin.

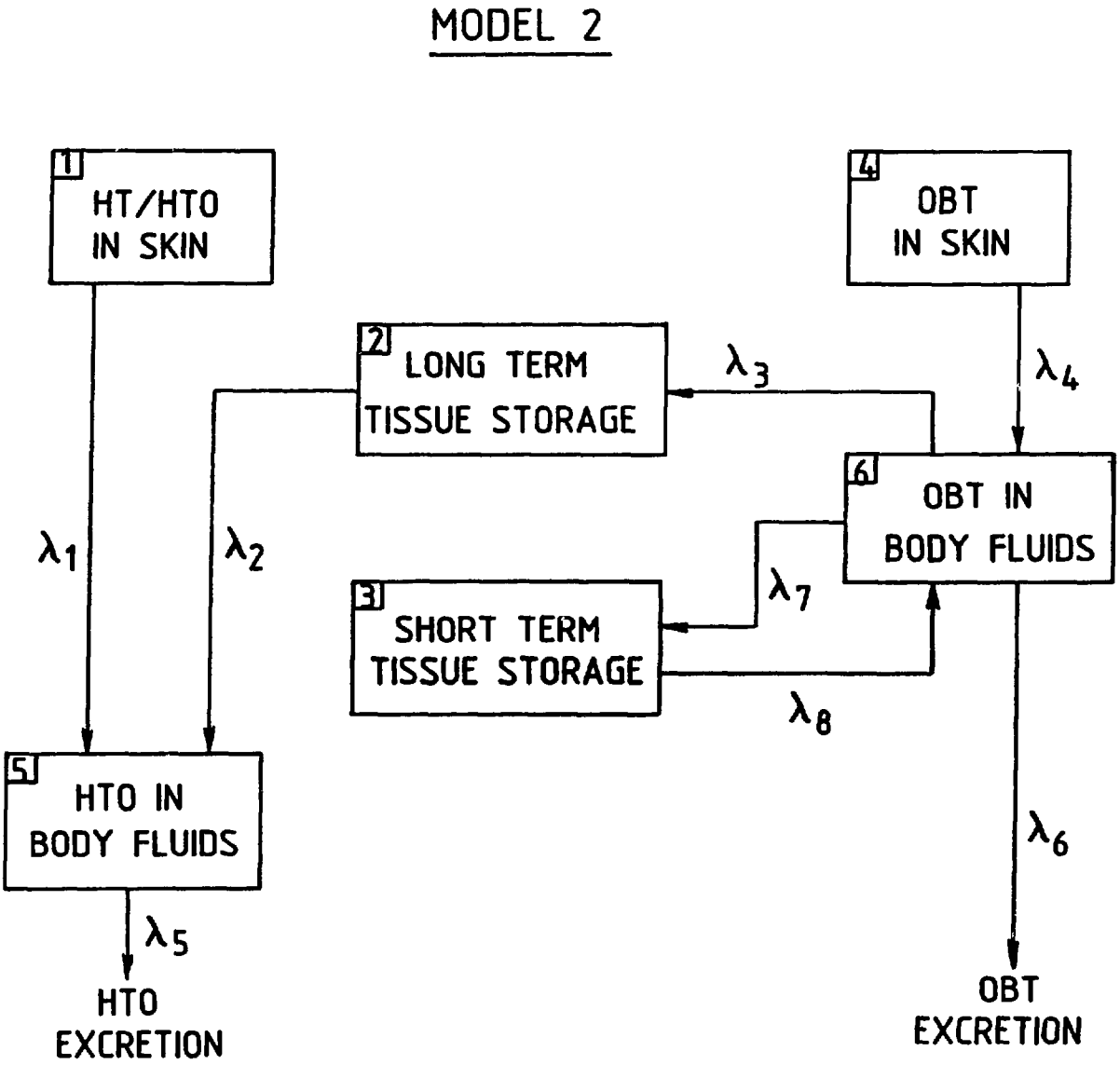

FIGURE 6: This model minimizes the dose to exposed skin.