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METABOLIC EVALUATION OF SKIN ABSORPTION OF TRITIATED FORMALDEHYDE IN HAIRLESS RATS

ÉVALUATION DU MÉTABOLISME DU FORMALDÉHYDE TRITIÉ ABSORBÉ PAR LA PEAU DE RATS SANS POILS

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RÉSUMÉ

Il y a des composés organiques tritiés présents sous forme de traces d'impuretés dans les installations de manutention de tritium. On a détecté le formaldéhyde tritié dans l'atmosphère et les effluents gazeux de sites de manutention et stockage de tritium. La capacité de diffusion du formaldéhyde tritié à travers la peau est une voie d'absorption possible. Étant donné qu'on n'a pas établi actuellement cette forme de contamination, on a exécuté des essais dans lesquels on a appliqué de façon topique (localement) du formaldéhyde tritié sur la peau dorsale de rats sans poils. Ces essais ont démontré que le tritium a été assimilé et retenu par la peau exposée sous forme de tritium lié organiquement (TLO) (OBT). Le TLO régit la vitesse de transformation du tritium dans le corps. On a observé la rétention du TLO par la peau, le foie, le coeur et les reins non exposés. La perte de tritium par les animaux a montré qu'environ 10% du tritium appliqué a été évacué par excrétion dans l'urine. On suppose qu'il a pu y avoir perte du reste du tritium appliqué par d'autres voies excréteuses ou par non pénétration de celui-ci dans le corps. On décrit mieux la vitesse biologique de l'excrétion du tritium par la somme de trois fonctions exponentielles. La plus grande partie du tritium évacué par excrétion a été sous la forme de TLO (90%) et l'évacuation par excrétion à été rapide dans les 1 à 2 jours qui ont suivi l'exposition. Ainsi, l'exposition de la peau au formaldéhyde tritié a entraîné le passage rapide du TLO de la peau dans l'urine et la rétention dans d'autres tissus. L'évaluation des valeurs de débit de dose a montré que le débit de dose, dans le cas de la peau exposée, a été supérieur d'un facteur 10 au débit de dose, dans le cas des autres organes.

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Abstract

Tritiated organics are present as trace impurities in tritium handling facilities. Tritiated formaldehyde has been detected in the atmosphere and gaseous effluents of tritium handling and storage sites. The ability of tritiated formaldehyde to diffuse through the skin is a possible route of intake. Since the metabolism of tritium through this mode of contamination is not currently established, experiments were performed in which tritiated formaldehyde was applied topically on the dorsal skin of hairless rats. These experiments demonstrated that tritium was assimilated and retained in the exposed skin as organically bound tritium (OBT). This retained OBT dominates tritium turnover in the body. OBT retention in the unexposed skin, liver, heart and kidneys was also observed. The loss of tritium from the animals showed that about 10% of the applied tritium was excreted in urine. It is assumed that the rest of the applied activity may have been lost through other excretory pathways, or may not have entered into the body. The biokinetics of tritium excretion is best described by a sum of three exponential functions. Most of the excreted tritium was in the form of OBT (90%) and excreted rapidly within 1-2 days post-exposure. Thus, skin exposure to tritiated formaldehyde results in the rapid clearing of OBT in the skin to urine and retention in other tissues. The evaluation of the dose-rate data showed that the dose-rate to exposed skin was almost a magnitude greater than the dose-rate to other organs.

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1. INTRODUCTION

Evidence indicates that tritiated volatile compounds occur within tritium process systems at tritium handling facilities (Krasznai et al., 1992; Belot et al., 1993). Tritiated formaldehyde has been detected in the atmosphere and gaseous effluents of a tritium removing facility, and in gaseous effluents released from solid miscellaneous wastes (Belot et al., 1992). Tritiated formaldehyde is an intermediate in the oxidation of tritiated impurities (e.g., tritiated methane) at the air-surface of contaminated materials (Krasznai and Mowat, 1993). Krasznai et al. (1992) have also measured tritiated methane, ethane and ethylene in the air above pump oil that has been contaminated with tritium gas. Tritiated formaldehyde is not the only tritiated by-product formed in the oxidation of tritiated methane or other tritiated hydrocarbons in tritium handling facilities: other tritiated by-products (e.g., tritiated methanol and formic acid) have been identified in gaseous effluents (Girard et al., 1989).

Since the metabolism of tritiated organics is not currently established, the metabolic assimilation and radiological consequences of these tritiated organics need to be addressed. Skin-contact is one of the possible routes of tritium intake. Our earlier experiments on skin-contact exposure to metal surfaces and oil contaminated with tritium demonstrated that tritium is retained in exposed skin (Trivedi et al., 1989; Trivedi, 1991; Trivedi, 1992). Metabolic evaluation showed that tritium retention in exposed skin is different from tritium retention following skin-contact exposure to tritiated water (HTO)[1] and tritiated gas (HT) (Johnson et al., 1988), and is probably governed by the ability of tritium (or tritiated species) to traverse the skin to the systemic fluids (Horvath et al., 1992). Most of the tritium excreted from the body after skin contamination from contaminated surfaces or oils was in the form of organically bound tritium (OBT)[2] (Trivedi, 1992).

Skin-contact exposure to tritiated formaldehyde can lead to tritium fixation at the point of contact (by replacing hydrogen moieties in the exposed skin). Tritium retention in exposed skin can affect tritium turnover in the body. To gain insight into the metabolic behaviour of topically applied tritiated formaldehyde, the present study examines tritium excretion in urine and tritium retention in parts of the body. A comparison is made between the biokinetics of tritium in urine from tritiated-formaldehyde- and tritiated-oil-contaminated animals, to determine whether the involvement of tritiated organics (such as tritiated formaldehyde), as the source of contamination, influences the uptake and retention characteristics of tritium.

2. MATERIALS AND METHODS

2.1 Tritiated Formaldehyde

Tritiated formaldehyde was purchased from NEN® Research Products, Wilmington, USA. The activity concentration of tritiated formaldehyde was 988 $MBq \cdot mL^{-1}$. The substance had a radiochemical purity of at least 99%. The stock solution of tritiated formaldehyde was diluted

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[1]Tritiated water (HTO) in this report means the sum of tritium as water and the exchangeable tritium. The exchangeable tritium may be organically or inorganically bound, but is in equilibrium with the tritium in body fluids.

[2]Organically bound tritium (OBT) means the tritium bound to biological molecules that is not exchangeable with hydrogen in body fluids.

with tritium-free water. The concentration, purity of tritiated formaldehyde and ratio of the organic form of tritium to total tritium activity water was verified using established laboratory procedures (Trivedi and Duong, 1993). Formaldehyde of 2% concentration is known to cause irritant skin reactions and allergy in humans (Gibson, 1985). In our experiment, the formaldehyde concentration applied on the skin of hairless rats was less than 2%. The activity concentration of tritiated formaldehyde was 48 ± 2 MBq in 0.05 mL.

2.2 Animals' Exposure

Four-month-old male hairless rats (Sprague-Dawley:hy.hy), weighing 420 ± 30 g, were used, to prevent abrasion to the skin and limit the stress to the animals by avoiding the necessity of shaving them before the exposure. Each animal was caged separately and kept in a fully controlled environment. Temperature was maintained at 24.5 ± 1.5°C, and the relative humidity was 40 ± 10%. The animals' room was adequately ventilated. Animals had free access to unlabelled drinking water and food throughout the experiment. Water bottles and cages were cleaned daily.

Animals were mildly anaesthetized prior to skin-contact exposure. The exposure area was approximately 6 cm^2. The exposure site was just behind the neck (to minimize licking). The applied tritiated formaldehyde was rubbed mildly against the skin in the exposed area. Controlled experiments have determined that approximately 0.05 mL of normal formaldehyde readily absorbed through the exposed area. The animals were watched for 30 min post-exposure to prevent licking and ingestion of applied activity.

Following exposure, the animals were returned to their cages. To investigate the urinary excretion of tritium, the exposed animals were kept in metabolic cages. Urine samples were collected at 0.2, 0.3, 1, 1.1, 1.3, 2, 2.1, 2.3, 3, 3.1, 7, 8, 10, 15, 17, 22, 24, 29, 36, 43, 50, 57, 64, 71 and 85 days post-exposure, to determine the HTO and OBT rate of excretion. Urine samples from the four contaminated animals were collected for 29 days post-exposure, and at that time two animals were euthanized for tissue collection (exposed skin, unexposed skin, liver, kidneys and heart). The remaining two animals were used for the rest of the period for urine samples at specified periods, and then were sacrificed for tissue analysis 93 days post-exposure.

Urine samples were collected over 24 h before exposure to provide background specimens, and then subsequently at regular periods, to determine the HTO and OBT rate of excretion. The experimental animals in this study excreted 20 ± 5 mL of urine daily.

Tissue samples were collected from two unexposed animals, to determine average background tritium burden in the organs. The results reported here for tissue samples were corrected for the background activities.

2.3 Sample Preparation

All tissues and organs were chopped, weighed into plastic containers, and stored frozen (-20°C). The urine samples were stored at 4°C. All samples were analysed within two weeks of their sampling date.

The separation of exchangeable tritium and HTO was effected by alternating equilibration of the sample with distilled water until tritium activity in the tissue-soaked water fell to

background levels. Tritium activities in all tissue-soaked water of the organs were determined for HTO and exchangeable tritium activity.

2.4 Low-Temperature Distillation

The low-temperature distillation (LTD) method was used to determine HTO content in urine (Trivedi and Duong, 1993). The total tritium concentration of the initial urine sample was measured by liquid scintillation counting. The OBT activity in urine was the difference between total activity of tritium and HTO in urine.

2.5 Tissue Solubilisation

Tritium in the organic fractions of tissues was estimated by solubilising the tissue samples (obtained after removing HTO and exchangeable tritium) in Soluene-350 (Canberra-Packard, Mississauga, Ontario, Canada). One mL of Soluene-350 was added to 100 mg of tissues in a glass vial. The vial was heated for about 10 h at 40°C. After the complete tissue solubilisation, the sample was cooled and 0.2 mL of isopropanol and 0.2 mL of 30% of hydrogen peroxide were added, to bleach the color of the sample (if any); the sample was then kept in the dark, to avoid chemiluminescence. The solubilised portion of the tissue was used to determine the organic fraction of tissue samples.

2.6 Activity Measurement

The concentration of tritium in urine, in water (extracted by low-temperature distillation or by tissue-soaking) and in the solubilised tissue samples was measured by liquid scintillation counting (LKB Wallac 1219). Aliquots (1.0 mL) of urine and water (HTO and exchangeable tritium) were mixed with 9.0 mL of a pre-mixed liquid scintillation cocktail (Ecoscint, National Diagnostic, USA). Similarly, 10 mL of Hinoic-Fluor (Canberra-Packard, Mississauga, Canada) was mixed with the solubilised tissue samples. The scintillation vial was allowed to sit in the dark for at least 2 h, to decrease chemiluminescence. To determine the total tritium activity of a sample, it was normally counted for 30 min. The low-activity samples were counted for 60 min, to improve the counting statistics. The error on individual concentration measurements was about 5% for the high-activity samples (1-10 days post-exposure), and about 10% for the low-activity samples (>15 d post-exposure, 1 $kBq.L^{-1}$). These errors are mainly due to counting statistics, since the error on volume measurements is negligible compared to the counting error (Trivedi and Duong, 1993). The results of the tritium activity measurements were corrected for background and quench. Background activities were consistently $\leq 1\%$ of total activity.

2.7 Regression and Statistical Analysis

The time variation in activity concentrations of tritium in urine was measured for individual experimental rats. The excretion rate was calculated for each animal (n = 4). The changes in tritium activity concentration with time in urine were analysed using an iterative, non-linear regression analysis. The KaleidaGraph™[3] software program for Macintosh™ personal computers was used. Double- or triple-exponential models were selected, based on an examination of residual values and plots of the respective models. The biological half-lives

[3]Synergy Software, Reading, PA, USA.

(a_i), zero time concentrations ($U_{0,i}$), and standard deviation for each component were calculated.

3. RESULTS

Skin-contact exposure to tritiated formaldehyde results in the binding of some of the tritium in an exchangeable form and some in a non-exchangeable form (OBT). While labile tritiated biomolecules (exchangeable form) are transmitted quickly to the systemic fluids, tritium fixed as non-exchangeable tritium is released solely by metabolic processes. Thus, the elimination of tritium from the exposed skin to urine could be a function of faster and slower turnover rates of tritium in the body.

3.1 Tritium in Urine

The urinary excretion of tritium for four male hairless rats is listed in Table 1. Large variabilites in the amount of tritium excreted in urine were observed (particularly for periods of less than 24 h post-exposure). However, the overall tritium elimination for these animals (e.g., ratio of OBT to HTO) did not differ significantly among animals. The urinary concentrations of tritium in four rats were monitored for 29 days post-exposure. At that time, two rats were sacrificed for tissue collection (as discussed in section 2.2). After 29 days post-exposure, the daily activity variation of urinary tritium in rats was minimal. The results from two animals were therefore considered to evaluate the long-term excretion. Our previous studies showed that most of the tritium is excreted in urine within 28 days post-exposure following skin contamination with tritiated metal surfaces or tritiated pump oil (Trivedi et al., 1989; Trivedi, 1992).

Figure 1 shows the average tritium urinary excretion values for four animals 29 days post-exposure, and for two animals for the rest of the post-exposure period. Following the skin-contact exposure to tritiated formaldehyde, urinary concentrations of tritium rose rapidly, plateaued within 16-24 h, and then quickly declined (Figure 1). The initial rise in urinary excretion of tritium indicates that tritiated formaldehyde at the point of contact is absorbed rapidly into and through the skin to the systemic circulation. Tritium in urine between 8-24 h post-exposure represents a fast rate of tritium uptake and diffusion across the skin. While variabilities in OBT concentration in urine (orders of magnitude difference) were observed for early post-exposure periods, no significant variability in HTO concentration in urine was seen (Table 1). Declining tritium concentrations then exhibited a prolonged terminal phase, which contributed less than 5% of the total tritium in urine.

The urinary excretion data for each animal was fitted with two, or alternatively, three decaying exponentials. The data with two decaying exponentials gave residual plots with non-random scatter (data not shown). Table 2 summarizes the non-linear regressional fit to the urinary excretion data. The result shows the average $\pm$ standard deviation of each kinetic parameter obtained from the urinary excretion of tritium. All data points were considered in estimating the kinetic values. The urinary excretion data from each animal was analysed independently and then averaged (i.e., the urinary data for four independent animal experiments were considered 29 days post-exposure, and beyond that period the urinary data from two animals were evaluated).

The biological half-life for the rapidly eliminating component has an average of 0.25 ± 0.09 days. The excretion of medium- and long-term components exhibited biological half-lives of 1.55 ± 0.16 days and 17.3 ± 4.2 days, respectively. Further analysis of the data showed that the short-term components, with biological half-lives of 0.2 and 2.8 days, accounted for more than 90% of the tritium excreted in urine. The majority of the total tritium in urine was measured in OBT form. The cumulative activity of tritium eliminated via urine was estimated to be 10% of the applied activity on the skin. The percentage activity excreted in urine was determined by dividing the integrated activity of tritium excreted in urine by the applied activity of tritiated formaldehyde.

The excretion of OBT and HTO in urine was also examined (Figures 2 and 3). A non-linear regression analysis, similar to total tritium in urine, was performed for OBT and HTO in urine (Table 2). The statistical evaluation of parameters obtained from the non-linear regression of the data concluded that both OBT and HTO in urine are best represented by three exponential functions.

The level of OBT in urine elevated very sharply and reached a plateau within 8-12 h post-exposure (the first sampling period after exposure). The maximum level of OBT in urine, however, varied among the rats, and was higher than the level of HTO. Table 2 shows the kinetics of OBT excretion in urine; it reveals a faster OBT clearance with an effective half-life of 0.24 ± 0.07 days. The slower components are represented with 1.3 ± 0.4 days and 19.1 ± 4.4 days. Around 90% of OBT in urine was eliminated a short time after exposure, with components having biological half-lives of 0.2 and 1.3 days. Table 1 shows that the higher ratio of OBT to HTO in urine declined 48 hours post-exposure. Less than 10% of the excreted tritium was excreted in the terminal phase of OBT excretion (with a biological half-life of 19 days).

The HTO in urine initially rose gradually from the commencement of exposure up to 24-36 h post-exposure. An HTO excretion component (the second-component) with an average half-life of about 3.7 ± 0.4 days was observed (Table 2). Note that the normal turnover of body water in rats is around 3.5 days (Yasumura et al., 1990). A much faster clearance with a biological half-life of 0.66 ± 0.26 days was also observed in our animals, indicating that the nonexchangeable forms of tritium were also excreted in urine. The low-temperature distillation method for tritium in urine measurement cannot discriminate between HTO and exchangeable tritium. The results suggest that a fraction of the applied tritiated formaldehyde might have been absorbed quickly through the skin or oxidized as HTO in the skin. The biological half-life of long-term HTO excretion is 17.6 ± 5.9 days and is comparable with the half-life of the delayed OBT in urine of 19.1 ± 4.4 days. Thus, it is probable that the delayed excretion of HTO in urine arose from the metabolic degradation of the stored OBT in the body.

3.2 Tritium in Skin

The level of tritium accumulation in exposed skin and unexposed skin was examined 29 days and 93 days post-exposure. Table 3 shows the retained concentration of tritium in the exposed and unexposed skin of the contaminated animals. The OBT concentration in the exposed skin was higher than the HTO concentration. The OBT contribution was more than 85% of the total tritium found in skin at all post-exposure examinations.

The concentration of HTO in the exposed skin remained at approximately 10-15% of the total tritium concentration. The ratio of OBT to HTO increased with time after exposure. The retained activity in the exposed skin at 29 days post-exposure was estimated to be less than 1% of the total applied activity of tritiated formaldehyde. The tritium concentration in unexposed skin was ten times lower than in exposed skin at 29 days post-exposure. At 93 days post-exposure, the tritium concentration in unexposed skin was about that of exposed skin. The ratios of OBT to HTO are greater for exposed skin than for unexposed skin (Table 3). For example, at 29 days post-exposure, the ratios of OBT to HTO were 7 and 2 for exposed and unexposed skin, respectively.

3.3 Tritium in Tissues

Since our previous animal studies have shown that, after skin, the liver, heart and kidneys contain high concentrations of tritium from contamination by tritiated oil and tritiated surfaces (Trivedi et al., 1989; Trivedi, 1992), these organs were analysed for retained tritium activity. The liver, heart and kidneys, analysed at 29 and 93 days post-exposure, were found to have tritium concentrations considerably lower than the exposed skin (Table 3). About 50% of tritium in the liver was retained in OBT form. The accumulation of tritium in the liver is attributed to the metabolic degradation of tritiated formaldehyde intake from the exposed skin and the liver's assimilation of OBT products. Feinman (1987) has shown that, within hours of skin-contamination, formaldehyde is usually carried in body fluids to the liver, for clearance.

Tritium retention in the kidneys was mainly retained, up to 70%, in the form of OBT. This is probably due to the kidneys filtering the tritiated contaminants in the systemic fluids, as the clearance of OBT in systemic fluids to urine occurs through the kidneys. After the exposed skin, the OBT accumulation was highest in the heart, with around 85% of the retained tritium as OBT. Except for the exposed skin, the ratio of OBT to HTO in each organ was relatively constant in both post-exposure examinations. The level of HTO in all these organs was independent of OBT enrichment.

The tritium retention in the exposed skin and other tissues (unexposed skin, liver, heart and kidneys) of tritiated-oil-contaminated animals at 29 days and 93 days post-exposure, as determined earlier (Trivedi, 1992), is given in Table 3 for comparison with tritiated formaldehyde exposure. Similar to the tritiated formaldehyde data, the exposed skin retained the highest concentrations of tritium in OBT form (up to 95%) relative to other tissues. Following the exposed skin, the liver had the second-highest concentrations of tritium, as opposed to the heart, from the tritiated formaldehyde exposure. However, HTO levels in all analysed organs were comparable in both forms of exposure. The ratios of OBT to HTO in the exposed skin were similar for both types of exposure.

The dose-rates to organs were calculated from their accumulated activity concentrations (Table 3). The maximum dose-rate was received by the exposed skin for both tritiated formaldehyde and oil. The data showed that the exposed skin had approximately a ten-times larger dose-rate than the four other organs analysed. The dose-rates from retained OBT were high compared to HTO in those organs (from 47% to 95% of the total dose-rate).

4. DISCUSSION

Formaldehyde fixes rapidly with the cellular constituents of the exposed tissue, because of its high affinity for certain chemical groups in biomolecules. Exposure to formaldehyde causes reversible and largely irreversible binding in exposed tissues (i.e., the formation of stable cross-links and unstable macromolecule adducts). Several studies have shown that the topical application of formaldehyde results in the retention of activity in exposed skin (Usdin and Arnold, 1979; Jeffcoat et al., 1983; Gottschling et al., 1984). A recent review of laboratory animals and some studies in humans have suggested that there is rapid percutaneous absorption of formaldehyde (Feinman, 1987).

Tritiated formaldehyde is capable of fixing in the exposed skin and therefore is of radiological concern. Entry of the tritium atom of tritiated formaldehyde into the body, either as non-exchangeable tritium (OBT) or as exchangeable tritium (e.g., oxidized tritium, HTO), can account for tritium turnover in the body. If the rate of oxidation of tritium is low in the exposed skin, it is expected that most of the tritiated formaldehyde is retained and excreted as OBT. However, the urine data have demonstrated that, following skin-contact exposure to tritiated formaldehyde, tritium excretes in the form of HTO and OBT, and is represented by the sum of three exponential functions. OBT was identified as the major component in urine (90%) 24-36 h post-exposure. The delayed elimination of tritium in urine was also observed.

The kinetics of tritium in urine showed that more than 95% of the activity in urine was eliminated within 48-72 h post-exposure, indicating that a significant fraction of tritium in systemic circulation was cleared quickly through the urine. However, our results have shown that only a small fraction (5-18%) of the applied activity was eliminated via urine. An examination of the kinetic parameters of tritium in urine has shown that the biological half-lives for delayed elimination of HTO and OBT are closely coupled. This may suggest that HTO in urine with a biological half-life of 17 days is a metabolic by-product of stored OBT in the body. Thus, the long-term storage (if any) of tritium in the body can be related to delayed excretion of both HTO and OBT.

Like most low molecular weight polar compounds, formaldehyde is known to diffuse rapidly through the skin to enter the body (Usdin and Arnold, 1979). As such, some free formaldehyde could be present in the systemic fluids (e.g., blood) immediately after exposure. Possibly, the early excretion of tritium is a result of quick labelling of macromolecules in systemic fluids (e.g., plasma protein) with tritiated formaldehyde. Most of these labelled tritiated metabolites are organic, and tritium labelling is mostly exchangeable. Neely (1964) has demonstrated that labelled urinary artifacts are formed spontaneously by mixing normal urine and labelled formaldehyde. Also, previous studies of the metabolism of formaldehyde by rats have demonstrated that formate, protein adducts and urinary artifacts of urea are the main components excreted in the early phase of urinary excretion (Mashford and Jones, 1982).

An evaluation of the applied activity of tritiated formaldehyde and total excreted amount of tritium in urine showed an accountability of about 10-15% of tritium that was transferred onto the skin. This implies that a significant fraction of tritium is either retained somewhere in the body or has never entered into the body. Previous animal studies on the percutaneous absorption of non-labelled formaldehyde have demonstrated that a large proportion of applied activities remains in skin, in contrast with a small amount that is absorbed and excreted (Usdin and Arnold, 1979; Gottschling et al., 1984). Jeffcoat et al. (1983) applied ^{14}C-labelled

formaldehyde solution in three monkeys epicutaneously and studied the radioactivity retention in the body. Activity was retained in all monkeys 72 h after dosage at the application site (on the posterior, to prevent scratching), but this retention varied from 4 to 17% in individual primates. For each monkey, the sum of radioisotope excreted in expired air, urine, and feces accounted for less than 1% of the applied dose. On the other hand, Bartnik et al. (1985), using rodents as a model system, reported that 70% of the topically applied ^{14}C-formaldehyde is retained in the exposed skin, and only a small fraction is lost through the urine ($\leq$10%). Similar to the results of Bartnik et al. (1985), about 5-18% of the applied activity was eliminated in urine. This means that most of the applied tritiated formaldehyde has been retained in the exposed skin, and that only a small fraction has diffused across the skin rapidly.

The present study has demonstrated that, following skin uptake of tritiated-formaldehyde, the exposed skin had the highest concentration of the retained tritium, compared to other organs at 29 days and 93 days post-exposure. A preferential incorporation and retention of tritium in the form of OBT was observed. The total retained tritium activity in the exposed skin is estimated to be less than 1% of the applied tritiated formaldehyde (48 $\pm$ 2 MBq) at 29 days post-exposure. Therefore, even though the exposed skin remains the major storage tissue for the applied tritiated formaldehyde, a significant amount of long-term storage of tritium was not seen in the exposed skin, suggesting that probably most of the applied tritiated formaldehyde never entered into the systemic circulation.

Autoradiography of the treated skin (from ^{14}C-formaldehyde) has shown a very sharp localization of the retained radioactivity in the different layers of the skin over a long period (Bartnik et al., 1985). Most of the applied activity (80%) was shown to be localized in the dead uppermost layers of the skin at 48 h post-exposure, probably by reaction of formaldehyde with macromolecules (e.g., keratin and collagen). If this is the case with tritiated formaldehyde contamination, then the heavy localization of tritium in dead epidermis would be unable to enter the body. Tritium fixed in the labelled dead layer of the skin is likely to be shed off, as the dead epidermis has a fast turnover rate of a few days (Bartnik et al., 1985).

This study also examined the biokinetics and metabolism of tritium from tritiated formaldehyde and tritiated-oil exposures. The results show a strong match between the experimental findings of skin-contact exposure to tritiated formaldehyde and oil. Table 4 summarizes the biokinetics of the urinary excretion of tritium between tritiated-oil-contaminated animals (Trivedi, 1991; Trivedi, 1992) and tritiated formaldehyde-exposed animals (this study). Estimation of the HTO and OBT peaks in activity concentration of urine showed that the peaks occur about 24 h and 8 h post-exposure in both types of contamination. A short equilibrium period of 24-36 h in the tritium excretion curve was observed in both cases. The comparison can be extended to the kinetic parameters, where the clearance of HTO in urine follows similar values. The difference mainly occurs in the kinetic values for OBT in urine, which are usually influenced by tritiated impurities in the source of contamination. Skin contamination with tritium oil has also indicated that a very small fraction (less than 5%) of the total applied activity is excreted in urine (Trivedi, 1992). Thus, it is possible that under both types of skin contamination (tritiated oil and formaldehyde), a significant portion of the applied tritium activity never entered into the body.

So far, the results from this study have shown that, similar to our earlier studies (Johnson et al., 1988; Trivedi et al., 1989; Trivedi, 1991; Horvath et al., 1992), the appropriate dosimetric

understanding can be derived through the collection of urinary data, and the use of a modified model of Horvath et al. (1992) (Figure 4). Here, the present study has discussed mainly the metabolism and excretion of tritium after skin-contact exposure to tritiated formaldehyde, and compared them with tritiated oil exposure. The dose-rate to the skin and other tissues was estimated only to quantify the radiological concern from this type of exposure. The appropriate dosimetric understanding for tritiated formaldehyde can be derived, however, by adjusting the parameters in the proposed dosimetric model (McElroy et al., 1992; Horvath et al., 1992) to obtain a good agreement between the experimental observations and model predictions.

Our metabolic evaluation of skin-contact exposure to tritiated formaldehyde, and previously to tritiated oil, has demonstrated that the exposed skin had approximately a ten-times larger dose rate than most of the other organs. The dose-rate contributions from retained OBT in the exposed skin are high (up to 90%). Thus, it is possible that the skin dose will be limiting for skin-contact exposure to tritiated formaldehyde, since the magnitude of doses to other organs will be small in comparison.

5. CONCLUSIONS

(i) Skin-contact exposure to tritiated formaldehyde results in HTO uptake and OBT accumulation in the body. A significant amount ($\geq$85%) of retained tritium in the exposed skin is in the form of OBT. It can be assumed that most tritium remains attached to biomolecules in the skin, which metabolized or released gradually into body fluids for metabolism.

(ii) Less than 10% of the applied tritium was excreted in urine. The kinetics of tritium in urine showed fast, medium and slow components. Most of the excreted tritium ($\geq$90%) was removed from the skin to the body within two days of the exposure.

(iii) The long-term storage of tritium in the exposed skin and other organs was in the form of OBT. The retention of OBT in organs was independent of the concentration of HTO.

(iv) The uptake and retention characteristics of tritium resulting from exposure to tritiated formaldehyde and tritiated oil was observed to have similar characteristics.

(v) Metabolic evaluation of skin-contact exposure to tritiated formaldehyde (and also tritiated oil) has demonstrated that the exposed skin had approximately a ten-times larger dose-rate than most of the other organs, and that the dose-rate from retained OBT in the exposed skin is high: up to 90% of the total dose-rate.

6. ACKNOWLEDGMENTS

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Table 1 Excretion of tritium in the urine following percutaneous absorption of tritiated formaldehyde of four rats (i-iv).

Rat #i

Days Post-Exposure	3H-Total # (Bq.L-1)	OBT $ (Bq.L-1)	HTO (Bq.L-1)	OBT / HTO
0.2	1.12E+07	9.81E+06	1.43E+06	6.86
0.3	1.43E+07	1.05E+07	3.82E+06	2.75
1.0	3.49E+07	2.38E+07	1.11E+07	2.15
1.1	3.42E+07	2.66E+07	7.59E+06	3.50
1.3	3.63E+07	2.57E+07	1.06E+07	2.43
2.0	3.79E+07	1.99E+07	1.80E+07	1.10
2.1	4.04E+07	2.34E+07	1.70E+07	1.38
2.3	3.21E+07	1.93E+07	1.29E+07	1.50
3.0	2.06E+07	8.51E+06	1.20E+07	0.71
3.1	1.95E+07	8.43E+06	1.11E+07	0.76
7.0	5.44E+06	5.49E+05	4.90E+06	0.11
8.0	5.28E+06	6.49E+05	4.63E+06	0.14
10.0	3.38E+06	2.21E+05	3.16E+06	0.07
15.0	9.51E+05	8.57E+04	8.66E+05	0.10
17.0	8.65E+05	1.52E+05	7.13E+05	0.21
22.0	3.31E+05	5.44E+04	2.77E+05	0.20
24.0	2.53E+05	4.70E+04	2.06E+05	0.23
29.0	1.36E+05	2.25E+04	1.13E+05	0.20

Rat #ii

Days Post-Exposure	3H-Total # (Bq.L-1)	OBT $ (Bq.L-1)	HTO (Bq.L-1)	OBT / HTO
0.2	4.45E+05	3.82E+05	6.37E+04	5.99
0.3	9.61E+06	8.17E+06	1.44E+06	5.69
1.0	3.21E+07	2.32E+07	8.93E+06	2.59
1.1	2.79E+07	2.27E+07	5.20E+06	4.37
1.3	2.75E+07	1.97E+07	7.85E+06	2.51
2.0	2.39E+07	1.27E+07	1.12E+07	1.13
2.1	2.42E+07	1.14E+07	1.28E+07	0.89
2.3	2.05E+07	1.05E+07	9.99E+06	1.05
3.0	1.53E+07	5.75E+06	9.60E+06	0.60
3.1	1.49E+07	5.35E+06	9.50E+06	0.56
7.0	4.76E+06	4.65E+05	4.29E+06	0.11
8.0	4.73E+06	4.11E+05	4.32E+06	0.10
10.0	3.04E+06	3.54E+05	2.68E+06	0.13
15.0	9.80E+05	1.23E+05	8.56E+05	0.14
17.0	9.34E+05	1.06E+05	8.28E+05	0.13
22.0	4.11E+05	8.17E+04	3.29E+05	0.25
24.0	3.20E+05	5.97E+04	2.60E+05	0.23
29.0	1.81E+05	2.83E+04	1.53E+05	0.19

Measured concentration of tritium in urine.
$ Difference of measured concentration of total tritium and HTO in urine.

Table 1 (Concluded)

Rat #iii

Days Post-Exposure	3H-Total # (Bq.L-1)	OBT $ (Bq.L-1)	HTO (Bq.L-1)	OBT HTO
0.2	1.39E+07	1.23E+07	1.59E+06	7.72
0.3	1.27E+07	1.06E+07	2.11E+06	5.03
1.0	2.73E+07	1.99E+07	7.39E+06	2.69
1.1	2.81E+07	2.00E+07	8.11E+06	2.46
1.3	2.85E+07	1.90E+07	9.53E+06	1.99
2.0	2.18E+07	1.12E+07	1.06E+07	1.06
2.1	2.25E+07	1.06E+07	1.18E+07	0.90
2.3	1.94E+07	9.50E+06	9.87E+06	0.96
3.0	1.92E+07	8.64E+06	1.05E+07	0.82
3.1	1.75E+07	7.17E+06	1.04E+07	0.69
7.0	5.30E+06	4.98E+05	4.80E+06	0.10
8.0	5.24E+06	5.70E+05	4.67E+06	0.12
10.0	3.75E+06	3.79E+05	3.37E+06	0.11
15.0	1.17E+06	6.91E+04	1.10E+06	0.06
17.0	1.11E+06	8.05E+04	1.03E+06	0.08
22.0	4.91E+05	6.75E+04	4.23E+05	0.16
24.0	3.83E+05	5.54E+04	3.28E+05	0.17
29.0	2.05E+05	1.89E+04	1.86E+05	0.10
36.0	9.43E+04	1.41E+04	8.03E+04	0.18
43.0	5.52E+04	1.06E+04	4.46E+04	0.24
50.0	4.24E+04	1.05E+04	3.19E+04	0.33
57.0	3.79E+04	9.76E+03	2.82E+04	0.35
64.0	3.22E+04	8.35E+03	2.39E+04	0.35
71.0	2.38E+04	5.47E+03	1.83E+04	0.30
85.0	1.58E+04	3.47E+03	1.23E+04	0.28

Rat #iv

Days Post-Exposure	3H-Total # (Bq.L-1)	OBT $ (Bq.L-1)	HTO (Bq.L-1)	OBT HTO
0.2	9.15E+05	8.77E+05	3.88E+04	22.60
0.3	2.02E+07	1.51E+07	5.08E+06	2.97
1.0	4.52E+07	3.71E+07	8.10E+06	4.57
1.1	4.76E+07	3.67E+07	1.09E+07	3.37
1.3	4.54E+07	3.67E+07	8.77E+06	4.18
2.0	4.37E+07	2.78E+07	1.60E+07	1.74
2.1	4.19E+07	2.40E+07	1.78E+07	1.35
2.3	3.51E+07	2.20E+07	1.31E+07	1.68
3.0	2.72E+07	1.24E+07	1.48E+07	0.83
3.1	2.40E+07	1.07E+07	1.33E+07	0.80
7.0	6.76E+06	7.18E+05	6.04E+06	0.12
8.0	7.94E+06	5.48E+05	7.39E+06	0.07
10.0	4.14E+06	2.93E+05	3.85E+06	0.08
15.0	1.22E+06	1.52E+05	1.07E+06	0.14
17.0	1.18E+06	1.52E+05	1.03E+06	0.15
22.0	4.65E+05	8.80E+04	3.77E+05	0.23
24.0	3.71E+05	8.39E+04	2.87E+05	0.29
29.0	1.69E+05	2.79E+04	1.41E+05	0.20
36.0	6.83E+04	1.61E+04	5.22E+04	0.31
43.0	5.80E+04	1.76E+04	4.04E+04	0.44
50.0	4.63E+04	1.61E+04	3.02E+04	0.53
57.0	3.48E+04	1.15E+04	2.33E+04	0.49
64.0	3.49E+04	1.26E+04	2.24E+04	0.56
71.0	2.70E+04	8.03E+03	1.90E+04	0.42
78.0	1.89E+04	4.17E+03	1.48E+04	0.28
85.0	1.29E+04	3.99E+03	8.90E+03	0.45

TABLE 2. Initial urine concentrations (Uo,i) and biological half-lives (ai) of tritium determined from excretion data following percutaneous absorption of tritiated formaldehyde.

The kinetic data are derived from non-linear regression analysis and curve-fitting of the experimental data from each animal. Values reported are the mean of four independent animal experiments. The standard deviation for each value is included in parentheses.

Tritium	Uo,1. (Bq.L-1)	a1 (days)	Uo,2 (Bq.L-1)	a2 (days)	Uo,3 (Bq.L-1)	a3 (days)
Total Tritium	6.08E+07 (1.10E+07)	0.25 (0.09)	3.82E+07 (1.63E+07)	1.55 (0.16)	3.90E+05 (1.94E+05)	17.33 (4.21)
HTO	1.86E+07 (6.62E+06)	0.66 (0.26)	1.87E+07 (5.51E+06)	3.68 (0.43)	1.34E+05 (1.24E+05)	17.60 (5.85)
OBT	5.26E+07 (1.06E+07)	0.24 (0.07)	1.94E+07 (1.03E+07)	1.33 (0.37)	3.80E+05 (1.93E+05)	19.08 (4.43)

Table 3. Average tritium concentrations in the organs of two animals and the contributing radiation dose-rate.

Tissues	Days Post-Exposure	Tritium Concentration (Bq per g wet tissue)			OBT	Dose-rate ¢ (nGy per s)	
		HTO#	OBT	Total *	HTO	Total (OBT) ∞	
TRITIATED FORMALDEHYDE							
Exposed Skin	29	528	3490	4018	7	3.66	(87%)
	93	17	288	305	17	0.28	(94%)
Unexposed Skin	29	158	276	434	2	0.40	(64%)
	93	60	145	205	2	0.19	(71%)
Liver	29	184	163	347	1	0.32	(47%)
	93	31	29	60	1	0.06	(49%)
Kidneys	29	192	240	432	1	0.39	(56%)
	93	16	36	52	2	0.05	(69%)
Heart	29	86	641	727	7	0.66	(88%)
	93	12	66	78	6	0.07	(85%)
TRITIATED OIL							
Exposed Skin	29	129	1090	1219	8	1.11	(89%)
	93	8	141	149	18	0.14	(95%)
Unexposed Skin	29	84	107	190	1	0.17	(56%)
	93	22	35	57	2	0.05	(62%)
Liver	29	64	373	436	6	0.40	(85%)
	93	33	109	142	3	0.13	(77%)
Kidneys	29	21	72	93	3	0.08	(77%)
	93	8	16	24	2	0.02	(66%)
Heart	29	23	95	118	4	0.11	(81%)
	93	7	18	25	2	0.02	(71%)

The sum of HTO and exchangeable tritium in organs.

* The sum of HTO and OBT in organs.

¢ The dose-rate is computed by the multiplication of activity concentration of tritium in tissues with 9.12E-4 nGy per Bq per g per s. The factor, 9.12E-4 nGy per Bq per g per s is for the absorbed dose-rate per unit activity concentration of tritium in tissues. A is the average measured tritium concentration in Bq per g of the tissue. The effective average energy of tritium is 5.7E-3 MeV per decay.

∞ OBT contribution is expressed as a percentage of total dose-rate, which is shown in parentheses.

Table 4. Summary of tritium biokinetics and metabolism following skin-contact exposure to tritiated oil and tritiated formaldehyde.

Category	Tritium Contamination	
	Oil*	Formaldehyde
General Information		
Sample area for exposure (cm)	6.0	6.0
Activity applied (MBq in 0.05 mL)	50	48
Tritium in Urine		
HTO Peak (h)	22 ± 5	24 ± 5
OBT Peak (h)	8 ± 3	8 ± 3
Ratio OBT/HTO at the Peak	12 ± 2	4 ± 1
Approximate Plateau Duration (h)	18-24	24-36
HTO Excretion Half-Life (d) (Medium-term component)	3.4 ± 0.4	3.7 ± 0.4
HTO Excretion Half-Life (d) (Long-term component)	14.6 ± 4.2	17.6 ± 5.9
OBT Excretion Half-Life (d) (Short-term component)	1.0 ± 0.1	0.2 ± 0.1
OBT Excretion Half-Life (d) (Medium-term component)	4.2 ± 0.6	1.3 ± 0.4
OBT Excretion Half-Life (d) (Long-term component)	26.6 ± 5.9	19.1 ± 4.4

*Trivedi (1992)

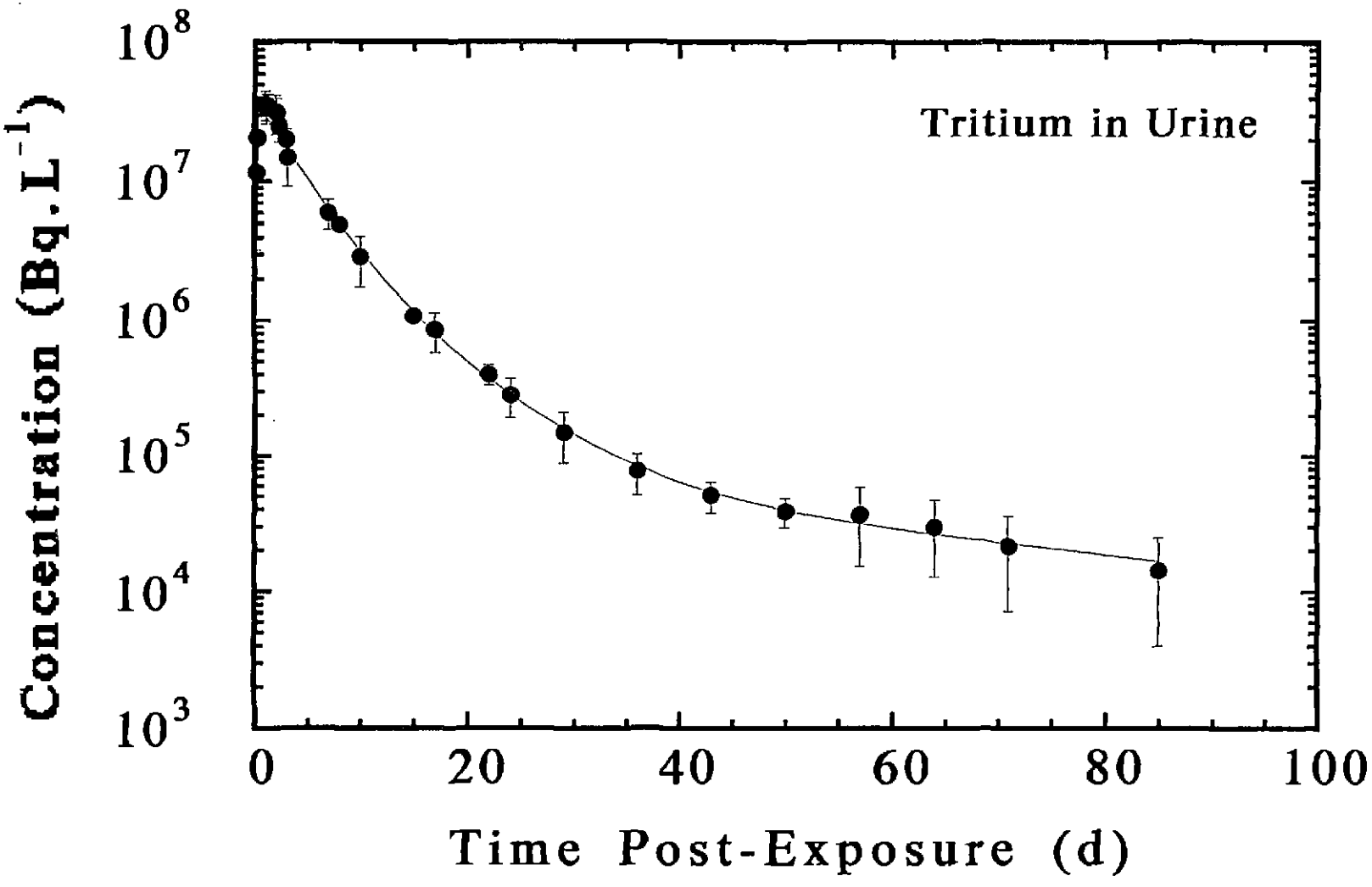

Figure 1 Excretion of total tritium in urine following percutaneous absorption of tritiated formaldehyde. Observed mean concentration-time points (± SE) and a non-linear fit to measured data using a sum of three exponential functions.

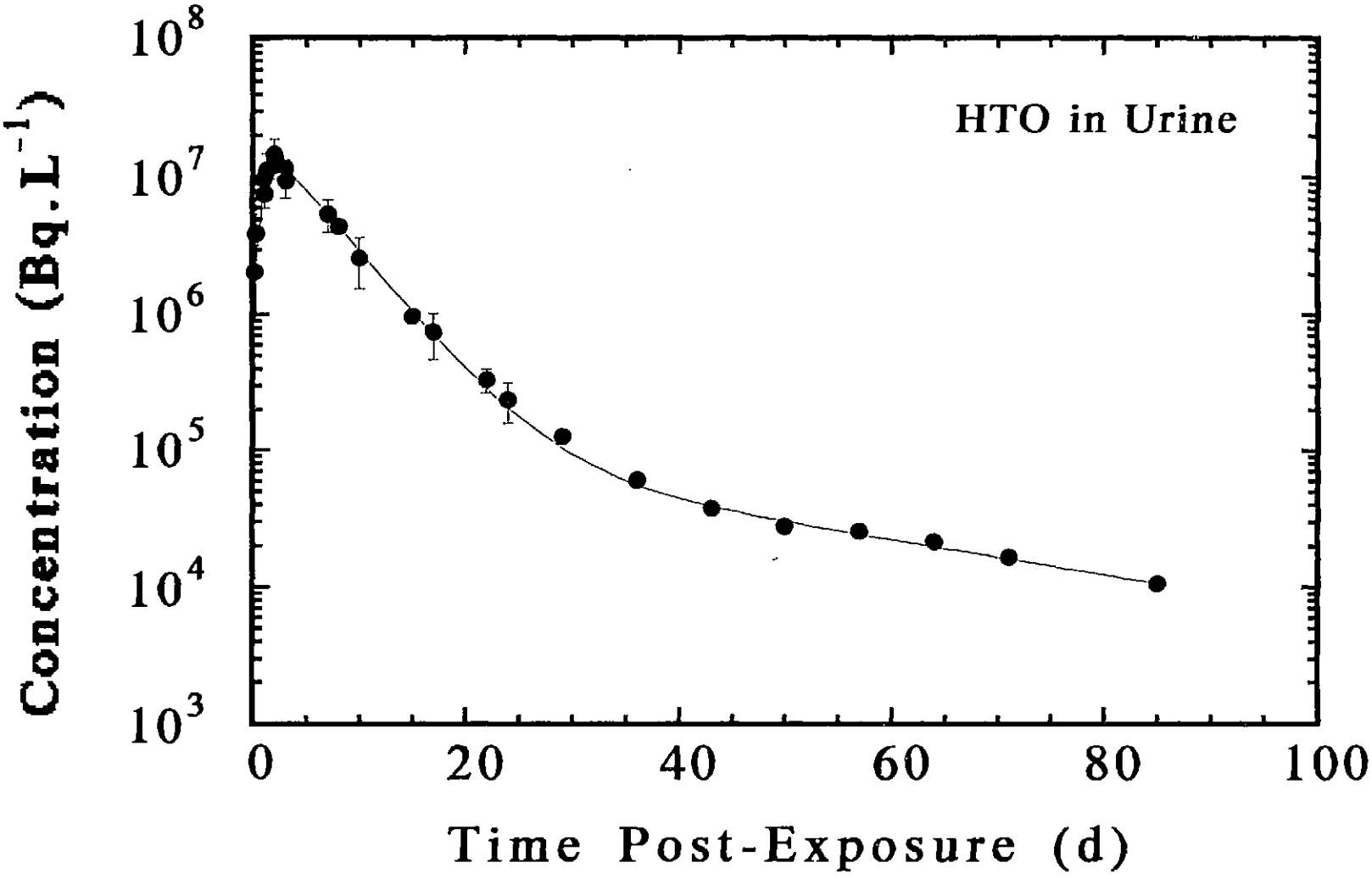

Figure 2 Excretion of OBT in urine following percutaneous absorption of tritiated formaldehyde. Observed mean concentration-time points (± SE) and a non-linear fit to measured data using a sum of three exponential functions.

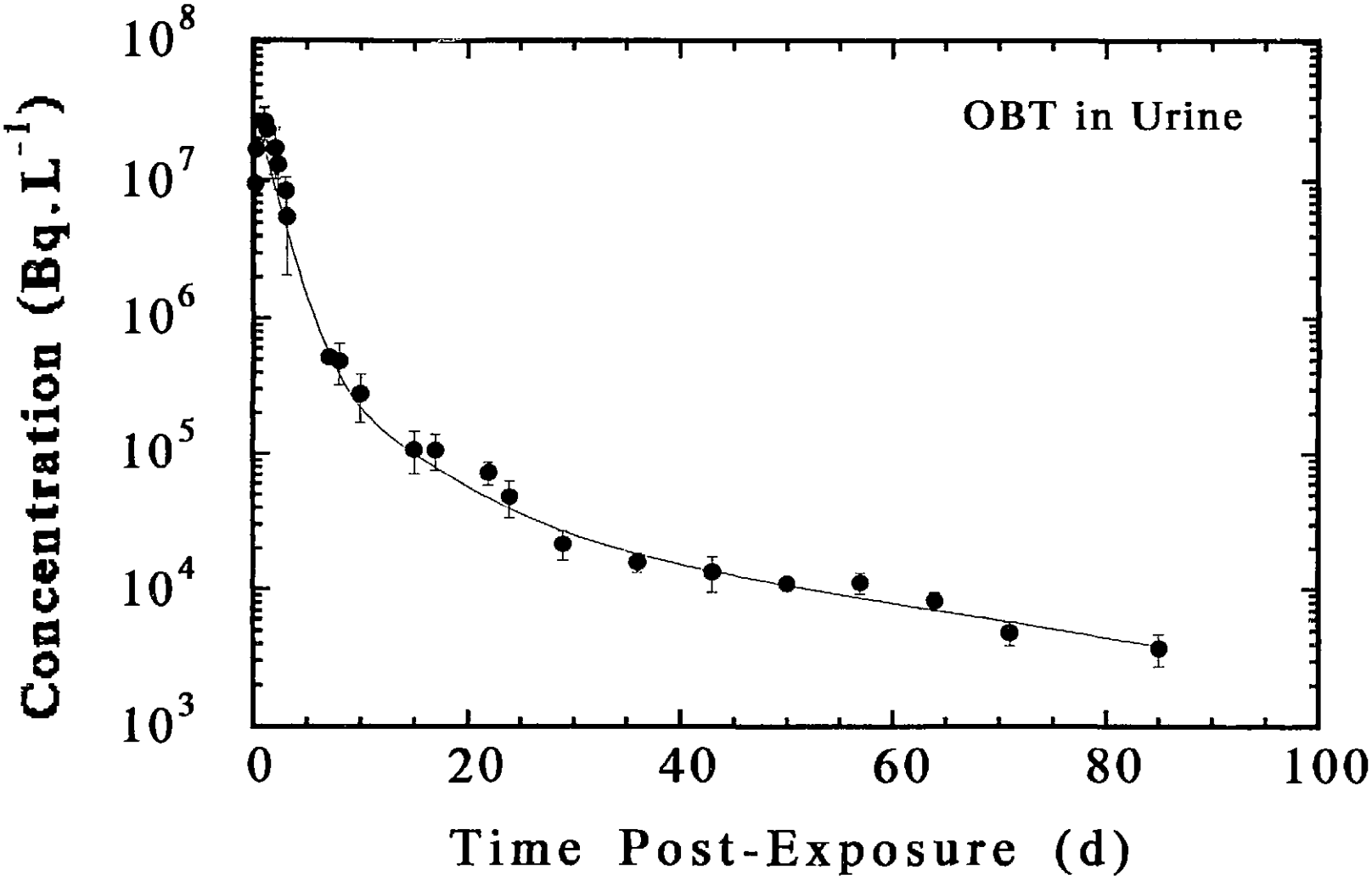

Figure 3 Excretion of HTO in urine following percutaneous absorption of tritiated formaldehyde. Observed mean concentration-time points (± SE) and a non-linear fit to measured data using a sum of three exponential functions.

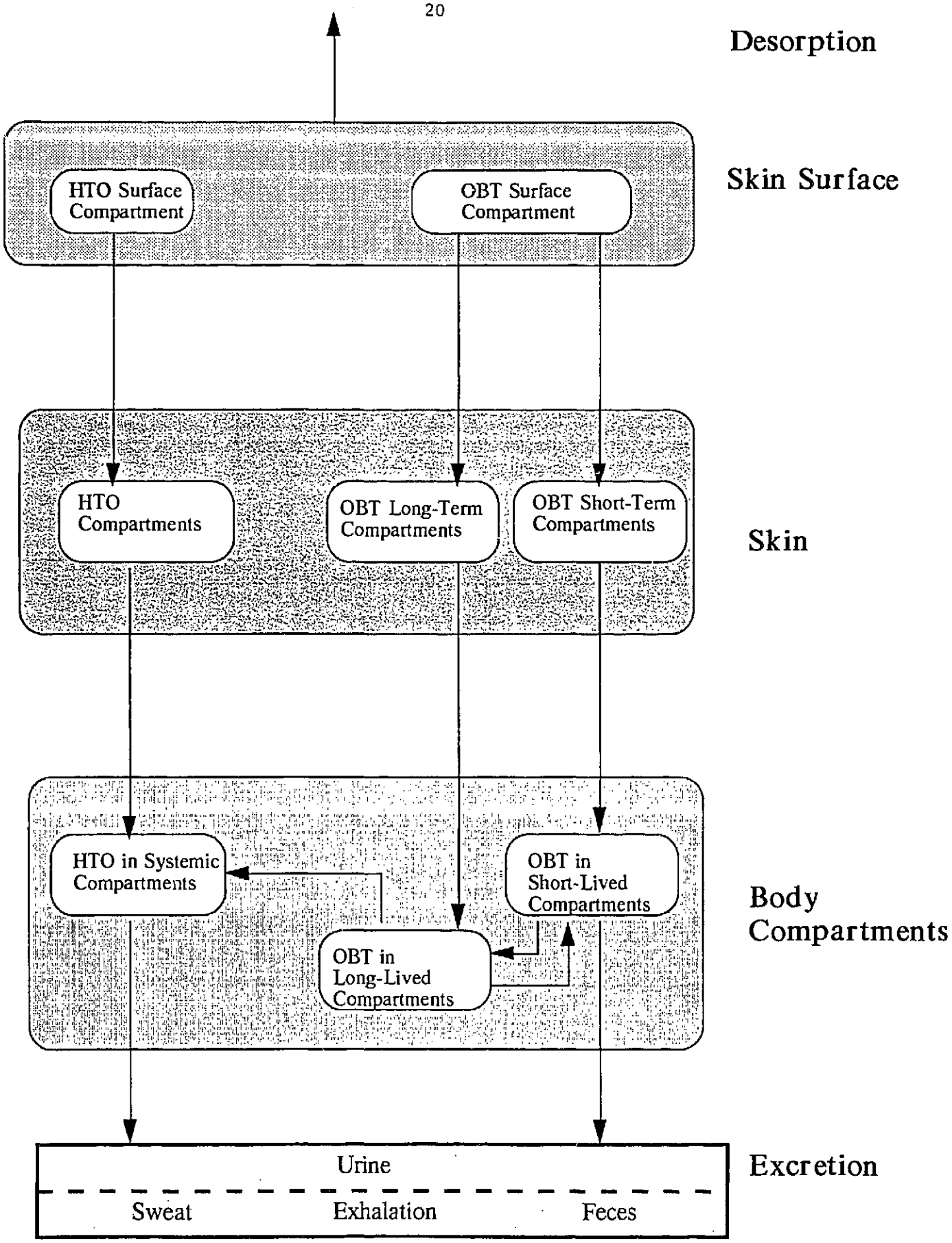

Figure 4. A physiologically based dosimetric model for percutaneous absorption of tritiated contaminants (modified from Horvath et al., 1992).

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