

EVALUATION OF THE INTERNAL EXPOSURE DUE TO VARIOUS ADMINISTERED DOSAGES OF URETHANE TO MICE

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1. Introduction

This is a companion paper to that of Margaret R. White [1]. Using Miss White's data, the purpose is to develop the methodology needed to evaluate internal exposure to urethane following the injection of this chemical into mice, administered in varying doses measured in milligrams of urethane per gram of body weight (mg/g). Experimental details, including the use of urethane labeled in two ways, ethyl ($1\text{-}^{14}\text{C}$) carbamate and ethyl carbamate (carbonyl- ^{14}C), denoted E and C labeled, respectively, will be found in Miss White's paper. Here a brief description illustrated by Figure 1 must suffice.

2. Experimental setup

Each of the 70 separate experiments (or runs) performed by Miss White consisted of: (1) injecting a randomly selected group of four mice with the same dose D of ^{14}C labeled urethane (D measured in mg/g); (2) placing the mice in the metabolism cage I (see Figure 1); (3) establishing a flow of fresh air, at a constant rate F , through chamber I, then through chambers II and III; and (4) measuring the radioactivity in the ionization chamber III. These measurements, made every 20 seconds, were automatically recorded giving the values that will be called $Y_3(t)$. This quantity is supposed to be proportional to the number of atoms of the radioactive carbon ^{14}C present in chamber III at time t . Chamber II in Figure 1 was filled with water absorber.

The arrangement of the experiments was based on the premise that, after being injected into mice, the urethane molecules are catabolized into at least two daughter molecules. Further catabolism results in practically all the labeled ^{14}C atoms being incorporated into CO_2 molecules which are gradually exhaled. Calculations performed at the Donner Laboratory (University of California,

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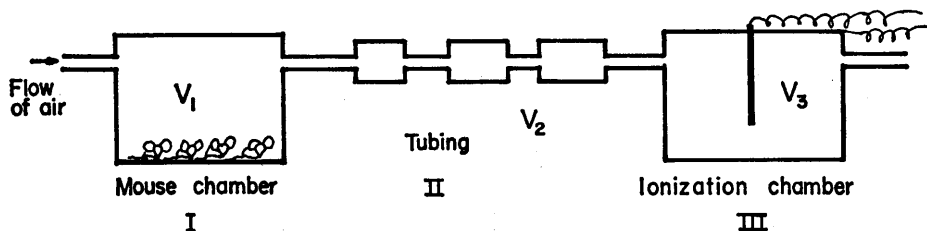


FIGURE 1

Experimental setup.

Berkeley) determined the quantities $X(0, D)$ proportional to the numbers of radioactive ^{14}C atoms injected in dose D of urethane. After the lapse of time t , a certain part of the original $X(0, D)$ is exhaled leaving the quantity $X(t, D)$ still unexhaled, possibly remaining in the bodies of the mice, but possibly partly eliminated in urine and feces. For simplicity of notation, we shall write $X(t)$ for $X(t, D)$.

Let V_1, V_2, V_3 denote the volume of air in chambers I, II, and III. Also, let F stand for the known constant rate of flow of fresh air through the whole apparatus. Finally, let $Y_1(t), Y_2(t)$, and $Y_3(t)$ be the measures of the numbers of molecules of ^{14}C present at time t in chambers I, II, and III.

The purposes of the calculations performed are: (1) to estimate $X(t)$ for $t > 0$; and (2) to calculate what is termed the internal exposure over a period T , due to the injection of D mg/g, that is,

$$(1) \quad E(T, D) = \int_0^T X(t) dt.$$

3. Method of estimating $X(t)$

The calculations were performed on a deterministic model, involving the following differential equations:

$$(2) \quad \begin{aligned} Y_1' &= -X' - c_1 Y_1, \\ Y_2' &= c_1 Y_1 - c_2 Y_2, \\ Y_3' &= c_2 Y_2 - c_3 Y_3, \end{aligned}$$

where the primes indicate derivatives with respect to t and where $c_1 = F/V_1$.

Easy manipulations yield

$$(3) \quad X' = -c_3 Y_3 - \frac{c_1 c_2 + c_2 c_3 + c_3 c_1}{c_1 c_2} Y_3' - \frac{c_1 + c_2 + c_3}{c_1 c_2} Y_3'' - \frac{1}{c_1 c_2} Y_3''',$$

which implies

$$(4) \quad \begin{aligned} X(t) &= X(0) - c_3 \int_0^t Y_3(x) dx - \frac{c_1 c_2 + c_2 c_3 + c_3 c_1}{c_1 c_2} [Y_3(t) - Y_3(0)] \\ &\quad - \frac{c_1 + c_2 + c_3}{c_1 c_2} [Y_3'(t) - Y_3'(0)] - \frac{1}{c_1 c_2} [Y_3''(t) - Y_3''(0)]. \end{aligned}$$

Since the actual measurements refer to radioactivity of the dose injected and of the air in chamber III, it is convenient to express all the variables as percentages of the dose injected $X(0)$. At time $t = 0$, the value of Y_3 must be zero. Whether the values of the first and the higher derivations of Y_3 must vanish at $t = 0$, is not clear *a priori*, but they were assumed to be zero.

4. Validation of the theory behind formula (4)

In order to obtain some idea of the relationship between formula (4) and the actual phenomena, Miss White performed ten experiments, or runs, conducted so that both the quantities $X(0)$ and $X(t)$ could be measured by a particular, say direct, method, while the radioactivity $Y_3(t)$, in chamber III, was measured by exactly the same procedure as was done with the runs with injected mice. Then the use of formula (4), and also the result of integration to obtain the quantity defined by (1), provided "theoretical" counterparts to be compared with independently obtained "direct" measurements of the same quantities.

These validating experiments consisted of replacing the radioactivity exhaled by experimental mice by a steady flow of radioactive CO_2 through the whole apparatus, at an approximately known rate K per unit of time. Such flow was maintained over a known period of time T . Then (and here was an experimental difficulty) the flow of radioactive CO_2 was interrupted and replaced by the flow of fresh air, intended to be at the same constant rate K . In some of such runs noninjected mice were placed in chamber I, but not in all of the runs. The measurements of Y_3 in the ionization chamber III continued up to such time as the readings were essentially zero.

It will be realized, that in the validating experiment, the presumed known rate K of flow of labeled CO_2 corresponds to the derivative

$$(5) \quad X'(t) = -K \quad \text{for } t < T.$$

For values of $t > T$, we have $X'(t) = 0$. Thus,

$$(6) \quad X(0) - X(t) = \begin{cases} Kt & \text{for } t < T, \\ KT & \text{for } t \geq T, \end{cases}$$

and the integration yields

$$(7) \quad \int_0^t [X(0) - X(x)] dx = \begin{cases} Kt^2/2 & \text{for } t < T, \\ KT(t - T/2) & \text{for } t > T. \end{cases}$$

The difference $X(0) - X(T)$ will be called *activity loss*. For values of t at which the $Y_3(t)$ was essentially zero, the integral in (7) will be called the *exposure loss*. The values of these quantities obtained from the presumed known T , K , and t will be described as *direct measurements*. The values of the same quantities obtained through measurements of $Y_3(x)$ and the use of the formula (4) will be called *theoretical values*.

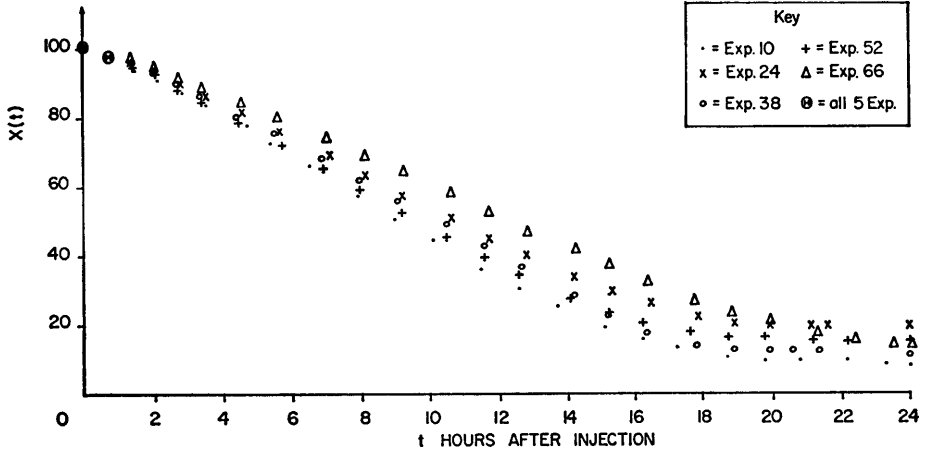


FIGURE 2

C labeled urethane, dose 1.00 mg/g.

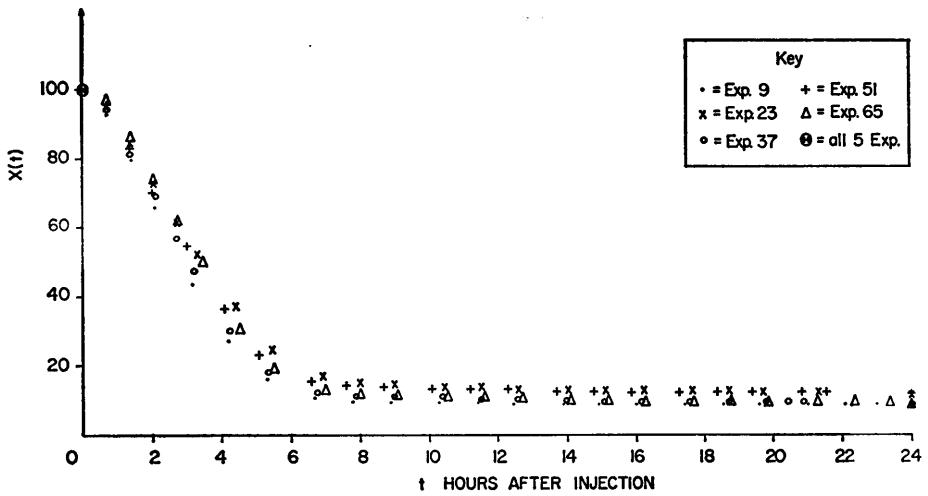


FIGURE 3

C labeled urethane, dose 0.125 mg/g.

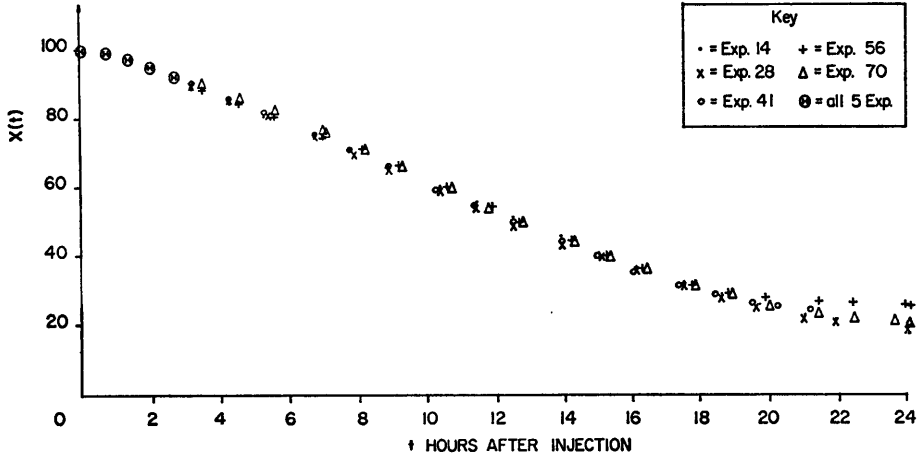


FIGURE 4

E labeled urethane, dose 1.00 mg/g.

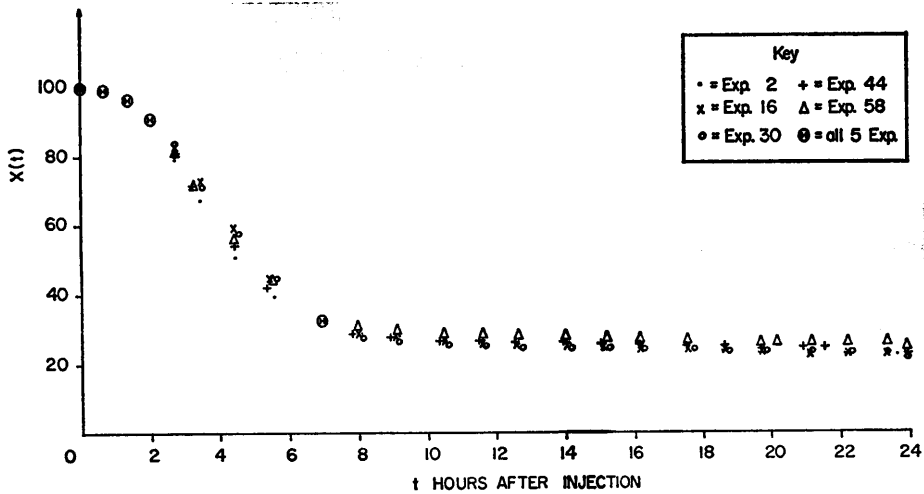


FIGURE 5

E labeled urethane, dose 0.125 mg/g.

TABLE I
RESULTS OF TEN VALIDATING RUNS

Run	Measurements of activity loss			Measurements of exposure loss		
	Direct = KT	Theory	Per cent difference	Direct $KT(t - T/2)$	Theory	Per cent difference
1	3.12	3.08	1.51	3.06	2.72	11.74
2	2.82	2.74	2.58	1.93	1.64	16.30
3	4.58	4.75	-3.61	5.18	4.83	7.01
4	3.46	3.42	1.21	2.37	2.54	-7.02
5	2.65	2.80	-5.52	3.01	2.93	2.67
6	5.38	5.31	1.19	5.78	5.24	9.85
7	2.84	2.91	-2.31	3.05	2.86	6.38
8	1.79	1.83	-1.94	1.20	1.33	-9.92
9	1.77	1.76	0.54	1.21	1.08	11.42
10	2.40	2.53	-5.28	3.00	2.93	2.37

Table I summarizes the results of ten validating runs performed by Miss White.

It is seen that the comparison of the direct and theoretical measurements of activity loss is satisfactory. For the exposure loss, however, the situation is not that good. The suspected sources of errors include the difficulty of maintaining a really constant rate of flow of labeled CO_2 and the sudden interruption of that flow, supposed to be immediately followed by an equal flow of fresh air. Since the real experiments with mice did not involve sudden changes in the procedure and used more precise flow regulators, it is hoped that the values of internal exposure obtained through the use of formula (4) and then of formula (1) will be more accurate than the data of Table I might suggest.

5. Estimates of $X(t)$ and of $E(T, D)$

As described by Miss White, her experiments covered a substantial range of urethane doses $D = 0.125, 0.25, 0.50, 0.75, 1.00, 1.20,$ and 1.40 , all measured in mg/g. Because quite a few mice did not survive the injections of urethane doses in excess of 1.00 mg/g, the numbers of experiments completed with the two largest doses 1.2 and 1.4 mg/g are small. It is plausible that the mice surviving the large doses of urethane are somehow more resistant than those that died. For this reason, the applicability to the whole population of mice of the results of the completed experiments with the two largest doses injected is subject to doubt.

For doses up to 1.00 mg/g, the results of calculation of $X(t)$ proved unexpectedly consistent. Figures 2 and 3 give graphs of calculated $X(t)$ for C labeled urethane and Figures 4 and 5 for E labeled urethane. The graphs in Figures 2 and 4 correspond to the highest dose of urethane, 1.00 mg/g, and those in Figures 3 and 5 to the smallest dose, 0.125 mg/g.

In each figure, there are five sequences of different symbols, each sequence corresponding to a particular experiment with four mice. The ordinate of each symbol gives the value of formula (4) calculated for the given value of t . Here

$X(0)$ was replaced by a conventional 100 and all amounts of radioactivity Y_3 , and so on, were expressed as percentages of the injected $X(0)$. For technical reasons, in performing the experiments, the values of t for which $X(t)$ was calculated for one experiment do not coincide with those for others.

With the possible exception of dose 1.00 mg/g of C labeled urethane (Figure 2), the $X(t)$ curves computed for five replicates of an experiment are remarkably consistent with each other.

6. Conclusions suggested by the graphs of $X(t)$

(i) The comparison of Figure 2 with 3 and Figure 4 with 5 leaves no room for doubt that the speed of elimination of ^{14}C atoms from the bodies of mice through exhaling depends very much on the dose injected, and this whether the urethane is C or E labeled. With the larger doses, twelve hours after the injection, the exhaled ^{14}C amounts to about 50 per cent of the injected quantity. With the minimal dose of 0.125 mg/g, the amount exhaled during the same period is something between 70 and 90 per cent.

(ii) All the curves $X(t)$ appear to approach a horizontal asymptote. For E labeled urethane this asymptote is higher than for that C labeled. In each case, the height of the asymptote indicates the percentage of the injected ^{14}C atoms that are "not exhalable" over the period studied. A part of these "unexhalable" atoms must be involved in some molecules eliminated through urine or feces. However, there may be a part of them remaining in the bodies of mice more or less indefinitely.

7. Values of internal exposure

The calculated values of the internal exposure E , over 24 and 48 hours are reported in Miss White's paper [1]. As the dose D in mg/g grows, the value of E increases somewhat faster than D . For $D \leq 1.00$ mg/g, the average number of lung tumors per mouse is very nearly proportional to E . This suggests that, for not too large doses of D injected, the number of initial events of carcinogenesis is proportional to E rather than to D . For higher values of D , the average number of tumors increases at a rate somewhat slower than that of E . This may be an indication of the anesthetic effect of urethane which slows down the functioning of the various organs, including the exhaling of the accumulated CO_2 .

Closer analysis of Miss White's experimental data is clearly indicated.



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REFERENCE

- [1] M. WHITE, "Studies of the mechanism of induction of pulmonary adenomas in mice," *Proceedings of the Sixth Berkeley Symposium on Mathematical Statistics and Probability*, University of California Press, Berkeley and Los Angeles, 1972, Vol. 4, pp. 287-307.