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WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Triphenyltin Hydroxide (TPTH). Evaluation of the Relative Absorption by Oral and Dermal Routes. Route Conversion Factors.

TO: Jack Housinger, Head
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Special Review Branch, SRRD (H7508C)

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Attached is a copy of the Case Study, Triphenyltin Hydroxide, Determination of a Systemic Dose Following Oral or Dermal Dosing, Comparison Factors for an Oral NOEL and a Dermal Exposure (10/11/91). This document provides the factors necessary for a risk assesment from a dermal dose of TPTH based on oral toxicity studies. It is written rather simply for ultimate use as a training document. This is the document requested in the Letter from Jellinek et al. re TPTH Technical (EPA Reg. No. 8340-17) Response to EPA's Risk Assessment Dated May 30,1991, Aug 16. 1991. I received a copy of the subject letter in late September 1991.

By the oral route, a single dose of TPTH is only partially absorbed to a maximum systemic load of 9%. Because of its slow excretion ($T^{1/2}$ = 60 hrs), repeated daily doses will bioaccumulate. Following repeated, equal, daily oral doses for a minimum of 20 days (8 half times of excretion) the maximum systemic load would be 37% of the daily dose. Other dose regimens would require individual calculation.

By the dermal route, up to 50.3% of the dermal dose remains on the washed skin[1] and is slowly absorbed ($T^{1/2}$ = 72 hrs). Considering its slow excretion, a single dermal dose will produce a maximum systemic load of 11% of the applied dose. Because of bioaccumulation, repeated, equal, daily dermal doses, will

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1. This is only true for the very small dermal doses that occur in human exposure. Order of magnitude higher doses will leave a significantly smaller portion on the washed skin.

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produce a maximum systemic load of 30% of the daily dose. Other dermal dose regimens would require individual calculation.

Factors for determination of Margins of Safety

In determining a specific MOS one divides the dose of the NOEL by the dose of the exposure. In the case of TPTH we have oral NOEL doses and dermal exposure doses. The oral doses are to rats and the dermal exposures are to people. People don't absorb TPTH the same way rats do. It would be much easier if we had NOELs from dermal rat studies and could calculate MOSs directly as we do for oral human exposures and oral NOELs. However, we can compare the oral and dermal dose by pretending that both are to rats and converting both into a rat systemic dose utilizing the appropriate absorption and excretion data. We then determine the MOSs for these systemic doses. That is;

$$\text{MOS} = \frac{\text{\% Oral Dose Absorbed as Maximum Systemic Dose}}{\text{\% Dermal Dose Absorbed as Maximum Systemic Dose}}$$

There are four posible comparisons of single and repeated oral and dermal doses as presented below. For simplification of calculation, one divides the percent for oral by the percent for dermal for each case to obtain a single factor. In each case the repeated doses are equal daily doses, for a minimum of 20 days (8 halftimes of excretion). Any other condition(s) of the repeated doses must be calculated individually.

$$\frac{\text{Single Oral Dose}}{\text{Single Dermal Dose}} = \frac{0.09 \times \text{Oral Dose}}{0.11 \times \text{Dermal Dose}} = 0.8 \times \frac{\text{Oral Dose}}{\text{Dermal Dose}}$$

$$\frac{\text{Single Oral Dose}}{\text{Repeated Dermal Dose}} = \frac{0.09 \times \text{Oral Dose}}{0.30 \times \text{Dermal Dose}} = 0.3 \times \frac{\text{Oral Dose}}{\text{Dermal Dose}}$$

$$\frac{\text{Repeated Oral Dose}}{\text{Single Dermal Dose}} = \frac{0.37 \times \text{Oral Dose}}{0.11 \times \text{Dermal Dose}} = 3.4 \times \frac{\text{Oral Dose}}{\text{Dermal Dose}}$$

$$\frac{\text{Repeated Oral Dose}}{\text{Repeated Dermal Dose}} = \frac{0.37 \times \text{Oral Dose}}{0.30 \times \text{Dermal Dose}} = 1.3 \times \frac{\text{Oral Dose}}{\text{Dermal Dose}}$$

Attachment
Case Study

cc
Dorsey
Chow

008724

CASE STUDY

Triphenyltin Hydroxide

Determination of a Systemic Dose Following Oral or Dermal dosing

Comparison Factors for an Oral NOEL and a Dermal Exposure

10/11/91

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9/11/91

TRIPHENYLTIN HYDROXIDE 008724

A kinetic comparison of the systemic dose(s) following oral and dermal doses in the rat

The NOELs and LOELs for triphenyltin hydroxide (TPTH) toxicity were obtained from oral dosing studies but the major route of human exposure is dermal. Dermal absorption studies have been performed to provide a bridge between these two exposure routes and to allow calcualtion of Margins of Safety (MOSs) for various exposure scenarios. This involves determining the systemic dose, more correctly the maximum systemic concentration following an oral dose and a dermal dose and comparing these two values. This paper shows how the systemic dose may be calculated following oral or dermal dosing and how a 'conversion' factor for the standard MOS calculation can be obtained.

In order to perform these calculations the following parameters of TPTH kinetics must be determined;

1. The portion of an oral dose that is absorbed.
2. The rate at which the oral dose is absorbed.
3. The rate at which a systemic dose in excreted.
4. The portion of a dermal dose that is absorbed.
5. The rate at which the dermal dose is absorbed.

The first three parameters are determined from oral kinetic studies and the last two from dermal kinetic studies.

Estimate of absorption by comparing LD_{50}s

A very crude estimate of the relative absorption of TPTH by inhalation, oral and dermal routes can be made by comparing its acute LD_{50}s, in the same species, by inhalation, oral and dermal routes. This method includes rate of absorption, distribution, metabolism and mechanism (target) of toxicity which may differ by each route. It can be used to provide some indication that the kinetic analysis is in the right direction and may indicate the existance of other significant route related differences. TPTH is most toxic by the inhalation route and we will consider absorption as 100% by this route. LD_{50}s and relative absorptions by the three routes are as follows;

Route	LD_{50} (mg/kg)	Dermal Absorption as Ratio to Inhalation
inhalation	16.3	100%
oral	156	10%
dermal	1600	1%

The oral kinetic studies

There are four oral kinetic studies available, two of which used ^{14}C labeled material and two used ^{113}Sn labeled material. From these oral studies we will use only data from males dosed at 2 mg/kg (no sex related differences in metabolism were observed). This is the lowest dose tested in the oral kinetic study and therefore closest to the NOELs and LOELs determined in the oral toxicity studies. All of these studies clearly establish that TPTH is absorbed orally and excreted in the bile. Three of the studies are in quantitative agreement on percent absorbed and halftime for biliary excretion. However, the fourth study, a ^{113}Sn bile collection study, differs significantly both from the three and internally. These differences will be considered under the discussion of the study.

Oral study 1

The first oral study, using ^{14}C labeled material, was reported on 10/29/86[1]. It was a standard guideline metabolism study, both sexes were given single oral doses of 2 or 10 mg/kg and 14 daily doses of unlabeled material followed by one of labeled material all at 2 mg/kg/dose. The male single dose of 2 mg/kg was excreted as presented in Table 1.

Table 1 (^{14}C)

	percent of dose		
day(24hr)	urine	Feces	
1	6.2	56.4	
2	2.8	15.1	
3	0.7	1.5	
4	0.4	0.6	
5	0.2	0.7	
6	0.2	0.2	
7	0.1	0.3	
Totals	10.6	74.8	85.4% excreted

Metabolite identification showed that phenol and other hydroxylated ring metabolites were excreted in the urine while parent compound and di- and monophenyl tin were excreted in the feces. Evidence was presented that intestinal bacteria can produce benzene from the parent compound. Based on this study from zero to 10.6 percent of the tin containing compound is absorbed. That is, the urinary excretion may represent solely phenolic metabolites absorbed from the intestine (zero percent absorbed) or, at the other extreme, the urinary

1. HOE 029664-14C, TPTH, Metabolism in rats after single and repeated oral administration at two dose levels 2 and 10 mg/kg body weight, W.L. Burkle, Hoechst Aktiengesellschaft, Study No. CM011/85, 10/29/86, MRID 400294-06

excretion may represent phenolic metabolites produced systemically after TPTH was absorbed (10.6 percent absorption).

Oral study 2

The second oral study, using ^{14}C labeled material, was reported on 12/7/87[1]. An oral dose of 2 mg/kg was administered to three male rats having a cannulated bile duct. Bile was collected for a total of 30 hours. Mean excretion was bile 2.8%, urine 11.0% and cage wash 0.4%, total 14.2% of administered dose. Parent compound was not identified in the urine but the radiolabeled material could not be identified. The radiolabeled material in the bile was not identifiecd Based on this study from zero to 14.2 percent of the dose was absorbed. The study has the same problems as study 1, it did not identify tin absorption.

Oral study 3

The third oral study, using ^{113}Sn labeled material, was reported on 7/30/89. An oral dose of 2 mg/kg was administered as a single dose or as seven consecutive daily doses. Urine and feces were collected for 7 days after dosing. Recovery from the single dose is presented in Table 2.

Table 2 (^{113}Sn)

day(24hr)	percent of dose urine	Feces	
1	0.11	62.20	
2	0.16	24.80	
3	0.12	5.77	
4	0.10	1.56	
5	0.07	0.73	
6	0.05	0.61	
7	0.04	0.43	
Totals	0.66	96.17	96.82 excreted

Based on this study up to 0.66% of the dose, the material found in the urine, was absorbed. This does not agree with our crude, LD_{50} based, estimate of absorption but it does introduce data which indicates strongly that the bile data from study 2 represents absorbed compound. Excretion half lives were determined as urine 55.7 hours and feces 9.6 and 57.0 hours. The biphasic excretion in the feces is indicative of two processes, the relatively fast passage of label directly through the digestive tract and the delayed excretion of label in the bile.

1. HOE 029664 (TPTH) - 14 - C, Excretion study in rats with bile fistula following oral administration of 2 mg a.i./kg body weight, W.L. Burkle & H.M. Kellner, Hoechst Aktiengesellschaft, A 36660, (B) 97/87, 12/1987, MRID

Half-life data

From study 2 we have information that ^{14}C radio labeled ring was excreted in the bile. From study 3 we have a biphasic fecal excretion of ^{113}Sn labeled compound. The first phase ($t^{1/2}$ 9.6 hrs) represents passage of unabsorbed compound through the intestine and the second phase ($t^{1/2}$ 57.0 hrs) represents the compound excreted in the bile. Together these two studies clearly show absorption of tin containing compound and its enterohepatic excretion in the bile. We can now use the fecal excretion data from study 3 to quantitate the compound that was absorbed and excreted through the bile.

Figures 1 and 2 present the excretion data via urine and feces respectively from study 3. Urinary excretion is clearly monophasic and fecal excretion biphasic. Using the last three data points of fecal excretion as indicative of biliary excretion (phase 2), we extrapolate a straight line backwards to separate early bilary excretion from total excretion. We then subtract the excretion values of this line for 1, 2, 3 and 4 days from the total excretion for these days to determine excretion of unabsorbed compound (phase 1). Percent of dose absorbed and unabsorbed taken from Figure 2 are presented in Table 3.

Table 3 (^{113}Sn) percent of administered dose in feces

day(24hr)	Unabsorbed (phase 1)	Absorbed (phase 2)	
1	59.9	2.3	
2	23.1	1.7	
3	4.47	1.3	
4	0.56	1.0	
5	0.00	0.8	
6	0.00	0.6	
7	0.00	0.4	
Totals	88.03	8.1	95.12 % + 0.66% urine

Because of the extended $t^{1/2}$ for biliary excretion it is necessary to correct the phase 2 total for the portion remaining in the animal. Using a $t^{1/2}$ of 60 hours[1], the fecal collection period represents 2.8 halftimes. During this period 90% of the absorbed dose will have been excreted. We correct for this as follows;

Total % absorbed = 8.1% / .9 = 9%

As a check on the accuracy of this analysis we can compare this quantitative data with the bile excretion data, from the cannulated rats, of study 2. In study 2, a mean of 2.8% of the ^{14}C label was excreted in the bile in 30 hours.

1. We have $t^{1/2}$s of 57 and 60 hours for bile excretion and will use 60 hours rather than their mean for simplicity of calculations.

Figure 5 presents the phase 2 data from Table 3 plotted as cumulative excretion. At 30 hours this graph gives the cumulative excretion of ^{113}Sn label as 2.7% of the dose. Considering experimental variation and the relative crudeness of the extrapolation this is almost to good to be true.

As a further check, we apply the biphasic approach to the data from study 1. Figures 3 and 4 present the excretion data via urine and feces respectively from study 1. Here too urinary excretion is clearly monophasic and fecal excretion biphasic. Using the last four data points of fecal excretion as indicative of biliary excretion (phase 2), we extrapolate a straight line backwards to separate biliary excretion from total excretion. We then subtract the excretion values of this line for 1, 2, and 3 days from the total excretion for these days to determine excretion of unabsorbed compound (phase 1). Percent of dose absorbed and unabsorbed taken from Figure 4 are presented in Table 4. We can also determine the $t^{1/2}$ of the two phases of fecal excretion from the graph. The first phase (intestinal passage of unabsorbed compound) has a $t^{1/2}$ of 10.8 hours and the second phase (biliary excretion of absorbed compound) has a $t^{1/2}$ of 60 hours. This is in good agreement with the $t^{1/2}$ figures from the ^{113}Sn study (9.6 hrs & 57 hrs).

Table 4 (^{14}C)

	percent of administered dose in feces		
day(24hr)	Unabsorbed (phase 1)	Absorbed (phase 2)	
1	56.4	2.9	
2	13.2	1.9	
3	0.3	1.2	
4	0.0	0.6	
5	0.0	0.7	
6	0.0	0.2	
7	0.0	0.3	
totals	69.9	7.8	77.7% + 10.6% urine*

*Metabolite identification showed that this material did not contain tin and thus may not represent absorbed parent compound.

Here too it is necessary to correct the phase 2 total for the portion remaining in the animal. We correct as follows;

Total % absorbed = 7.8% / .9 = 8.7%

As with the study 3 data, we can compare the bile excretion of the cannulated rats in study 2 with the phase 2 biliary excretion by graphing the cumulative excretion (Figure

6). This graph gives a phase two excretion of 3.2% at 30 hours, in good agreement with the 2.7% in 30 hours of cannulated biliary excretion from study 2. All three studies agree on an entrohepatic excretion of between 8.0 and 9.0 % of the administered dose of 2 mg/kg which also agrees with the crude estimate from the LD_{50} comparisons. We are also reasonably certain that the urinary excretion of ^{14}C labeled material does not represent absorption of tin containing compound. Also, within the limits of the data, we can say that essentially all of the metabolism occurs in the intestine.

Study 4

This study, dated 5/29/89, is the outlier which agrees that absorbed tin containing compound and metabolites are excreted in the bile but does not agree quantitatively with the other studies[1]. The study used ^{113}SN labeled TPTH administered in a single oral dose of 2 mg/kg to rats with a cannulated bile duct. Bile was collected for 30 hours and bile and carcass without the digestive tract, were analyzed for ^{113}SN. Results are presented in Table 5.

Table 5. Dose distribution in bile duct cannulated male rats.

Rat Number	Dose mg/kg	% Dose Absorbed by Direct Calculation			Corrected % dose Absorbed ($t^{1/1}$ = 60 hrs)		Calculated $t^{1/2}$ (hrs)
		Bile	Carcass	Total	Bile 30% excreted	Carcass 70% remaining	
227	2.00	3.26	7.6	10.88	10.9	10.9	67
228	2.05	3.64	15.80	19.51	12.1	22.6	100
229	1.93	13.29	29.66	43.02	44.3	43.4	57
233	2.33	4.70	19.31	24.02	15.7	27.6	92
Mean	——	6.21	21.59	27.81	——	——	——
SD	——	4.76	7.21	12.48	——	——	——

In this study only rat # 227 has an oral absorption, determined directly, which is similar to that determined from the first three studies. We can run two mathematical checks on the data. First we calculate total absorption from biliary excretion and carcass residue using the previously determined $t^{1/2}$ of 60 hours for excretion. For rats 227 and 229 the calculated total absorbed is similar to the total determined directly indicating a $t^{1/2}$ similar to that determined from the three other studies.

1. HOE 029664 (TPTH)-^{113}Sn, Absorption studies in rats with bile fistula after a single oral dose of 2 mg/kg body weight, W.L. Burkle, H.G. Eckert & H.-M. Kellner, Hoechst AG, CM079/87, A 41409, 5/29/89

As a second check we calculate $t^{1/2}$ for each rat from the % carcass, % total absorbed and the collection time. As expected rats 227 and 229 have $T^{1/2}$s that are similar yet they have a four fold difference in total absorption. Rats 228 and 233 have $T^{1/2}$s that are similar to each other but the $T^{1/2}$ is significantantly longer than that of the other two rats and the values obtained from the first three studies. In general the individual absorption and $T^{1/2}$ values are much more variable then one would expect among four male rats of the same strain, sex, age and source. There is something wrong with this study both internally and in relation to the other studies and there is no way of determining the error(s). Therefore I will not use this data as part of the evaluation.

Conclusions from the oral studies

We now have answers to the three questions asked of the oral studies.

1. The portion of an oral dose that is absorbed. Correcting for incomplete excretion collection, 8.7-9% of the administered dose is excreted in the bile and this represents the portion of the dose absorbed by the oral route. This is in good agreement with the absorption estimated by comparing LD_{50}s.

2. The rate at which the oral dose is absorbed. Although the data do not allow quantitation of the rate of absorption, we can say that is is relatively rapid for the following reasons;

a. Most of the unabsorbed dose is excreted in the feces in 24 hours and is therefore no longer available for absorption (68 to 81 percent).

b. A significant portion of the absorbed dose appears im the bile within 24 hours (28 to 37 percent).

3. The rate at which a systemic dose in excreted. Studies 1 and 3 give $t^{1/2}$s for billary excretion of 60 and 57 hours respectively. Since this is the sole route of excretion, the values represent the $t^{1/2}$ for excretion. They also indicate that bioaccumulation will occur following repeated daily dosing of TPTH.

The dermal absorption studies

There are three dermal absorption studies of TPTH all of which used a ^{14}C-ring label. The first study, a standard dermal absorption study, showed that only a very small portion of the dose was absorbed during the exposure period but the major portion of the dose remained on/in the skin following the soap and water wash. The second and third studies were designed to determine the fate of the retained material.

Dermal study 1

This study was reported in 1985 and, because it had some significant experimental deficiencies, was classified as incomplete[1]. Additional analytical data was supplied and we were able to conclude that only a very small portion (<1.0%) of the dose was absorbed at exposure periods of up to 10 hours. In the initial analysis the skin was extracted with ethanol indicating approximately 10-20% of the dose had remained on/in the skin but at the same time the majority of the dose was missing. Combustion of the skin residue found the missing material. Thus, although quantitation was poor, in the order of 50% of the dose was found on/in the washed skin. The dermal absorption data from this study cannot be used for risk assesment because of assay problems and the significant portion of the dose remaining on/in the washed skin. This material is available for absorption over time.

Dermal study 2

This study was designed to determine the fate of the material remaining on the skin[2]. Three doses were used, the application site on all of the rats was washed after 10 hours and groups of 4 rats from each dose were carried for 10 and 24 hours and 7, 14 and 21 days after dosing. The data are summarized in Table 6. Several items stand out in the table. The skin wash for the 10 hour sacrifice removes only a fraction of the material removed from the other animals in the respective dose groups. Subsiquemtly we determined that this was a function of how the wash was performed. The 10 hour rats were sacrificed, the skin was removed and then it was washed. The other rats were washed in situ. Removing the skin exposed the cut edge and underside of the skin to the wash solution and a significant amount of TPTH became bound to the skin. Thus, we do not know how much TPTH would have been washed of the 10 hours rats in situ but we may be assured that it should have been considerably more, in the order of that removed from the remaining rats.

Dispite the 10 hour wash problem, the data clearly show that the material remaining on the skin is absorbed with time. Taking the one day data as a base, one sees that the majority of the material on the washed skin disappears within 14 days and about half of this material appears in the absorbed column. During the same time period total recovery decreases leaving us with the question as to whether or not the missing material from the skim was absorbed. This material ballance problem will be discussed under study 3.

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1. A dermal absorptiom study in rats with ^{14}C-Triphenyltin Hydroxide, J. Laveglia, WIL Research Laboratories, WIL-39020, June 5, 1985

2. An extended duration dermal absorption study in rats with ^{14}C-triphenyltin hydroxide, E.M. Caine, WIL Research Laboratories Inc. WIL-39033, Feb 6, 1987 MRID 400730-01.

Table 6. Distribution of applied ^{14}C labeled triphenyltin hydroxide. Dosing material prepared from an emulsifiable concentrate. The application site was washed with soap and water after 10 hours. Values are means of four animals per dose duration group. Dosing area 10.8 cm^2

Rat Group	Time of Sacrifice (days)	Mean dose applied (ug)	Mean Skin Wash (% dose)	Mean TPTH equivalents (% of Dose) in urine TOT	urine /Day	feces TOT	feces /Day	carcass	washed skin	Mean TPTH equivalents absorbed[b] (% dose) Tot	/Day	Recovery (% dose)	Missing 100% - Recovery (% dose)
I	10hrs	18.8	14.3[a]	0.1	0.3	<0.1	<0.1	<1.45	91.8	<1.61	<1.61	106.1	----
	1	18.8	56.7	0.1	0.1	<0.1	<0.1	<1.51	41.8	<1.61	<1.6	100.2	----
1.73	7	19.0	55.8	3.4	0.5	2.7	0.4	1.63	22.6	7.73	1.1	86.1	13.9
ug.cm^2	14	18.5	51.7	7.1	0.5	8.7	0.6	1.49	2.2	17.29	1.2	71.2	28.8
	21	18.3	52.6	7.4	0.4	8.9	0.4	<2.9	2.0	18.79	0.9	73.8	26.2
II	10hrs	211.2	21.5[a]	<0.1	<0.1	<0.1	<0.1	<0.2	77.2	<0.4	<0.4	98.7	1.3
	1	211.0	61.0	0.1	0.1	<0.1	<0.1	0.4	24.5	0.6	0.6	86.1	13.1
19.53	7	212.2	51.9	3.5	0.5	5.6	0.8	2.1	10.3	11.2	1.6	73.4	26.6
ug/cm^2	14	209.2	53.4	4.7	0.3	7.8	0.6	0.6	0.4	13.1	0.1	66.9	33.1
	21	211.5	56.1	4.0	0.2	7.2	0.3	0.3	<0.2	11.5	0.5	67.8	32.2
III	10hrs	1955.3	39.3[a]	<0.1	<0.1	<0.1	<0.1	<0.1	57.4	<0.3	<0.3	96.7	3.3
	1	1953.8	81.0	<0.1	<0.1	<0.1	<0.1	0.2	7.8	<0.4	<0.4	89.2	10.8
181.1	7	1954.8	80.3	0.6	0.1	0.8	0.1	1.3	3.8	2.7	0.4	86.8	13.2
ug/cm^2	14	1954.8	76.5	1.3	0.1	3.2	0.2	0.2	0.1	4.7	0.1	81.3	18.7
	21	1960.5	78.6	1.4	0.1	2.8	0.1	.1	0.03	4.3	0.2	82.9	17.1

a. application site was washed only after sacrifice.
b. Totals urine, feces and carcass.

The label was excreted in both the urine and feces. From the oral studies we can reasonably assume that the label in the feces represents tin containing material and the label in the urine represents only the ring metabolite. From the oral studies we know that ^{14}C-ring label in the urine does not represent orally absorbed tin since the ring is released in the intestine, absorbed and then excreted in the urine. In the case of dermal dosing the ring may have been released in the skin and then may or may not represent absorbed tin. Or the ring metabolite may represent bile excreted compound from which was then released in the intestine, absorbed and excreted in the urine. Since we cannot distinguish these posibilities we will make the worst case assumption that urinary excretion of label represents dermally absorbed tin. This problem would not have occured if ^{113}Sn had been used for the label in the dermal absorption studies.

The data from study 2 will not be used for risk assesment because of the questionable 10 hour sacrifice skin wash data and because the vehicle used was not appropriate to the risk exposures.

Dermal Study 3

This study was performed with the same goals and experimental design as study 2 except that the dosing material was a suspension and the ten hour rats were washed before sacrifice[1]. The data are summarized in Table 7. The effect of the change in washing is clear, all time groups within each dose group had a similar amount of material washed off the skin. This study has the same label excretion questions as was noted in study 2 and we will assume that urinary excretion of label also represents dermally absorbed tin. In utilizing this data to determine the fate of the material remaining on the skin after washing one must note that the low dose most nearly approximates the dermal exposure in the field and its absorption data will be used in the risk assesment.

The skin wash is the hardest procedure to quantitate as can be seen in the variation of the mean values for the exposure durations within each dose group. This also means that there will be a similar variation in the quantity remaining on the skin for the extended absorption periods. This variation must be accepted as unavoidable in this type of study.

This study shows that a small portion of each dose is absorbed in 10 hours (1.9, 0.8 & <0.1% respectively) and a significant portion of each dose remains on the washed skin (47.0, 31.9 & 8.4% respectively). Most of this latter material leaves the skin over a period of three, two or one weeks respectively. This prolonged absorption leads to a maximum

1. An extended duration dermal absorption study in rats with ^{14}C-triphenyltin hydroxide, E.M. Caine, WIL Research Laboratories, Inc. WIL-39037, May 11, 1987. MRID 401983-01.

Table 7. Distribution of dermally applied ^{14}C labeled triphenyltin hydroxide. Dosing material prepared as a suspension. The application site was washed with soap and water after 10 hours. Values are means of four animals per dose duration. Dosing area 10.8 cm^2.

Rat Group mean dose	Time of Sacrifice (days)	Mean dose applied (ug)	Mean Skin Wash (% dose)	Mean TPTH equivalents (% of Dose) in urine Tot	urine /day	feces Tot	feces /day	carcass	washed skin	Mean TPTH equivalents absorbed[b] (% dose) Tot	(% dose) /day	Recovery (% dose)	Missing 100% - Recovery (% dose)
I	10hrs	25.66	49.8	0.2	0.5	<0.1	<0.1	1.7	47.0	1.9	4.6	98.7	1.3
	1	26.00	51.5	0.6	0.6	0.3	0.3	2.5	40.8	3.4	3.4	95.7	4.7
1.83	7	25.48	43.6	4.4	0.6	18.9	2.7	3.2	7.3	26.5	3.8	77.4	22.6
ug/cm^2	14	25.35	46.6	6.3	0.6	24.5	1.8	3.2	3.1	34.0	2.4	83.7	16.3
	21	25.20	40.8	6.0	0.3	20.5	1.0	0.2	0.4	26.7	1.3	67.9	32.1
II	10hrs	278	55.1	0.4	1.0	<0.1	<0.1	0.4	31.9	0.8	1.9	87.8	12.2
	1	278	56.4	0.3	0.3	<0.1	<0.1	1.0	28.5	1.3	1.3	83.3	16.7
25.97	7	281	61.6	2.4	0.3	10.2	1.5	3.2	1.0	15.7	2.2	78.4	21.6
ug/cm^2	14	282	67.1	3.2	0.2	10.1	0.8	0.4	0.4	13.7	1.0	81.2	18.8
	21	281	69.2	2.6	0.1	10.0	0.5	0.3	<0.2	12.9	0.6	82.3	17.7
III	10hrs	2598	85.4	<0.1	<0.1	<0.1	<0.1	<0.1	8.4	<0.1	<0.1	93.5	6.5
	1	2589	81.6	<0.1	<0.1	<0.1	<0.1	0.2	12.9	0.3	0.3	94.9	5.1
239.6	7	2587	77.9	1.2	0.2	5.0	0.7	2.6	2.6	8.8	1.3	89.3	10.7
ug/cm^2	14	2584	71.3	2.9	0.2	12.2	0.9	0.4	<0.1	15.5	1.1	86.9	13.1
	21	2592	75.9	1.8	0.1	7.9	0.4	0.1	<0.1	9.8	0.5	85.8	14.2

a. Totals urine, feces and carcass.

percent of dose absorbed, by direct measurement, of 34, 15.7 and 15.5 percert respectively for doses of 1.83, 25.97 and 239.6 ug/cm^2. These values are far larger than the one percent dermal absorption estimated from comparing LD_{50}s as well as larger than the 8-9% calculated directly for the oral route.

These data are neither mysterious nor necessarily contradictory. The biological activity, including the toxicity, of a compound depends not on the total amount of compound absorbed but on the maximum systemic concentration obtained from the dose administered. A single oral dose is absorbed relatively rapidly, within hours, leading to the rapid attainment of a maximum systemic concentration which is only slightly less than the total amount absorbed. On the other hand the portion of the dermal dose that is in the skin does not contribute directly to the toxicity of TPTH in the organism until it passes from the skin into the organism. This portion of the dermal dose is absorbed over a period of 2-3 weeks leading to the slow attainment of a much lower maximum systemic concentration expressed as portion of the dose. Thus, determining the percent of dose absorbed from the skin is not sufficient for comparing oral and dermal dosing. One must determine the rate at which TPTH is absorbed from the washed skin.

In determining a rate for dermal absorption of TPTH one must take into account the data showing that most of the applied dose remains on the washed skin and is absorbed slowly from the skin. Under such conditions one must consider the washed skin a compartment and determine the kinetics between that compartment and the systemic compartment. In the case of TPTH one can assume that the rate of passage of TPTH from skin compartment to systemic compartment is first order[1]. Using the data from group I, (1.83 ug/cm^2) one may determine the $t^{1/2}$ of this rate in two ways, material remaining in the skin with time or material leaving the skin (absorbed) with time. There is one thing wrong with this approach, at 14 days the skin residue data says that 43.9% of the dose has gone some place (absorbed?), the absorption data says that only 34% of the dose was absorbed and 16.3% of the dose is missing. The missing material has been attributed, at least partially, to evaporation of ring labeled metabolite from excreta. This does not tell us how much of the missing material can be considered absorbed. This can make considerable difference in $t^{1/2}$ values.

Taking a worst case approach we will assume that all the material disappearing from the skin is absorbed systemically and determine our systemic $t^{1/2}$ for absorption from the rate of disappearance from the skin data. Fitting a straight line

1. See appendix I for a detailed analysis of skin to systemic kinetics.

to a plot of log percent dose in skin against time gives a $t^{1/2}$ of 74.4 hours. Direct mathmatical determination utilizing the means from each duration-concentration gives a mean $t^{1/2}$ of 72.5 hours. The numerical agreement of the values determined by the two methods is further evidence of a first order process. Since the smallest $t^{1/2}$ will give the maximum systemic dose we will consevatively use 72 hours.

Conclusions from the dermal absorption studies

We now have answers to questions 4 and 5 for the low dose animals.

4. The portion of a dermal dose that is absorbed. From direct data a maximum of 34% of the applied dose is absorbed (but 16.3% of the dose is missing). Using a conservative estimate, this gives a total absorption of 50.3%. From the residue on the skin at ten hours (47%) and the direct absorption (1.9%) we obtain a value of 48.9% absorbed. Adding the missing 1.3% gives a conservative estmate of absorption of 50.2%[1].

5. The rate at which the dermal dose is absorbed. The first order kinetics of disappearance from the skin gives $T^{1/2}$s of 74.4 and 72.5 hours and we will conserativelyuse a $T^{1/2}$ of 72 hours.

Determining the systemic dose with time

The systemic dose of TPTH, from a dermal dose, is a function of the rate at which TPTH leaves the skin entering the organism and the rate at which TPHT is excreted from the organism. We determined the first process as first order with a $T^{1/2}$ of 72 hours. The second process is also first order and a $T^{1/2}$ of 60 hours was determined from the oral kinetic study.

Using the $t^{1/2}$ skin to systemic and $t^{1/2}$ excretion we can calculate the portion (percent) of the material remaining on the washed skin that is present in the body at any time after dosing (Fig. 7). Following a single low dermal dose of TPTH, the portion of the dose in the body increases to a maximum at three days after dosing. At that time 22% of the material remaining on the skin after washing will be found in the organism where it can produce toxicity. If 47% of the dose remains on the skin after washing, 10% of the applied dose will be present in the organism at 3 days after dosing. Using the conservative value of 50% absorbed we have a maximum systemic dose of 11% of the applied dose.

1. There is a third method of estimating absorption by back calculation of the portion of dose on/in the skin at zero time using 47% at 10 hours and the T1/2 of 72 hours. This gives a value of 51.6% available for absorption.

Unlike oral absorption, this figure of 10-11% dermal absorption does not agree with our LD_{50} derived estimate of 1% absorbed from a dermal dose. This is because the percent absorption of a dermal dose increases with decreasing dose per unit area. The dose we are using for the dermal absorption determination is much smaller than the doses uses for the determination of a dermal LD_{50}. The surface area of a 250 gram rat is approximately 400 cm^2(1) and the guideline calls for dosing 10% of that area (40 cm^2). At the dose we are using from the dermal absorption study (1.83 ug/cm^2) this would give a dose of 2.95 mg/kg. The rat demal LD_{50} of TPTH is 1600 mg/kg or a dermal dose of 10,000 ug/cm^2. This dermal dose is approximately three orders of magnitude larger than the dose we are using and can be expected to reduce the percent absorption significantly. We do not have the dermal absorption data necessary to make a determination of absorption at the dose per unit area used in the LD_{50} study.

We can also calculate the maximum systemic dose following repeated dermal dosing. Since the $T^{1/2}$ for excretion is 60 hours we will expect bioaccumulation following dosing at 24 hour intervals. To perform this analysis we will make an assumption for which their is absolutely no supporting data. We will assume that each day when we dose the animal we 'fill up' the skin compartment to the same 'level' so that 50% of the dose is available for absorption. This is based on the assumption that applying the 'same' dose (in volume, concentration and vehicle) will present TPTH with the same free energy so that it will equilibrate between washable and nonwashable in the same manner. This is also the only assumption one can make to get a usable number.

Figure 8 presents the systemic dose following a repeated equal dermal dose at 24 hour intervals. The maximum systemic concentration, 59% of the residue on the washed skin, is reached after 14 days dosing. Using the conservative value of 50% absorbed we have a maximum systemic dose of 27% of the applied dose.

Factors for determination of Margins of Safety

In determining a specific MOS one divides the dose of the NOEL by the dose of the exposure. In the case of TPTH we have oral NOEL doses and dermal exposure doses. The oral doses are to rats and the dermal exposures are to people. People don't absorb TPTH the same way rats do. It would be much easier if we had NOELs from dermal rat studies and could calculate MOSs directly as we do for oral human exposures and oral NOELs. However, we can compare the oral and dermal dose by pretending that both are to rats and converting both into

1.Freireich, E. et al. 1966, Quantative comparison of toxicity of anticancer agents in mouse, rat, dog, monkey and man. Cancer Chemother. Repts. 50(4):219-244

a rat systemic dose utilizing the appropriate absorption and excretion data. We then determine the MOSs for these systemic doses. That is;

$$MOS = \frac{\%\ \text{Oral Dose Absorbed as Maximum Systemic Dose}}{\%\ \text{Dermal Dose Absorbed as Maximum Systemic Dose}}$$

There are four posible comparisons of single and repeated oral and dermal doses as presented below. For simplification of calculation, one divides the percent for oral by the percent for dermal for each case to obtain a single factor. In each case the repeated doses are equal daily doses, for a minimum of 20 days (8 halftimes of excretion). Any other condition of the repeated doses must be calculated individually.

$$\frac{\text{Single Oral Dose}}{\text{Single Dermal Dose}} = \frac{0.09 \times \text{Oral Dose}}{0.11 \times \text{Dermal Dose}} = 0.8 \times \frac{\text{Oral Dose}}{\text{Dermal Dose}}$$

$$\frac{\text{Single Oral Dose}}{\text{Repeated Dermal Dose}} = \frac{0.09 \times \text{Oral Dose}}{0.30 \times \text{Dermal Dose}} = 0.3 \times \frac{\text{Oral Dose}}{\text{Dermal Dose}}$$

$$\frac{\text{Repeated Oral Dose}}{\text{Single Dermal Dose}} = \frac{0.37 \times \text{Oral Dose}}{0.11 \times \text{Dermal Dose}} = 3.4 \times \frac{\text{Oral Dose}}{\text{Dermal Dose}}$$

$$\frac{\text{Repeated Oral Dose}}{\text{Repeated Dermal Dose}} = \frac{0.37 \times \text{Oral Dose}}{0.30 \times \text{Dermal Dose}} = 1.3 \times \frac{\text{Oral Dose}}{\text{Dermal Dose}}$$

SEMI+LOGARITHMIC 2 CYCLES X 175 DIVISIONS

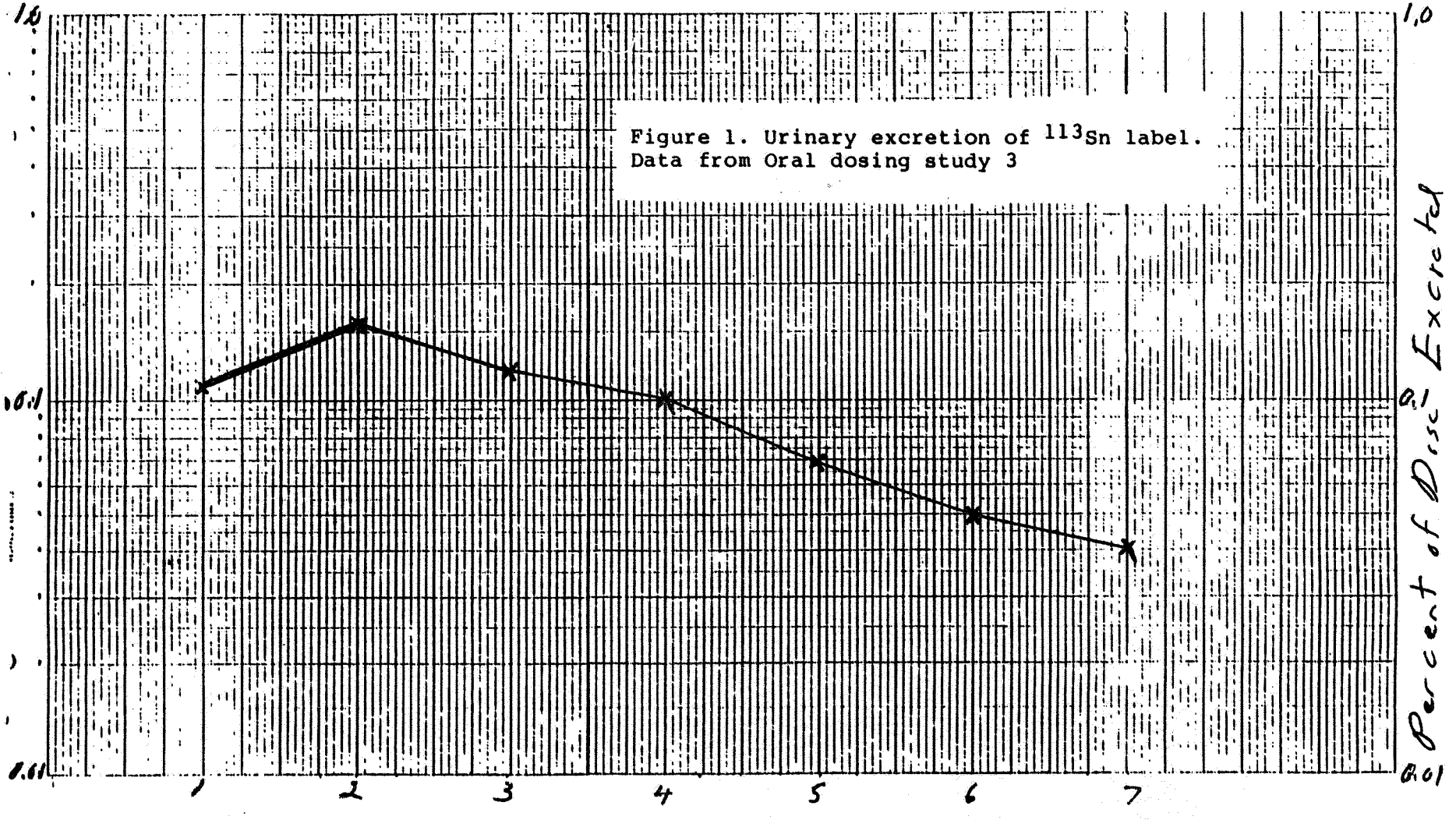

Figure 1. Urinary excretion of ^{113}Sn label.
Data from Oral dosing study 3

008724

Percent of Dose Excreted

Figure 2. Fecal excretion of 113Sn label.
Data from Oral dosing study 3

SEMI-LOGARITHMIC 3 CYCLES X 175 DIVISIONS

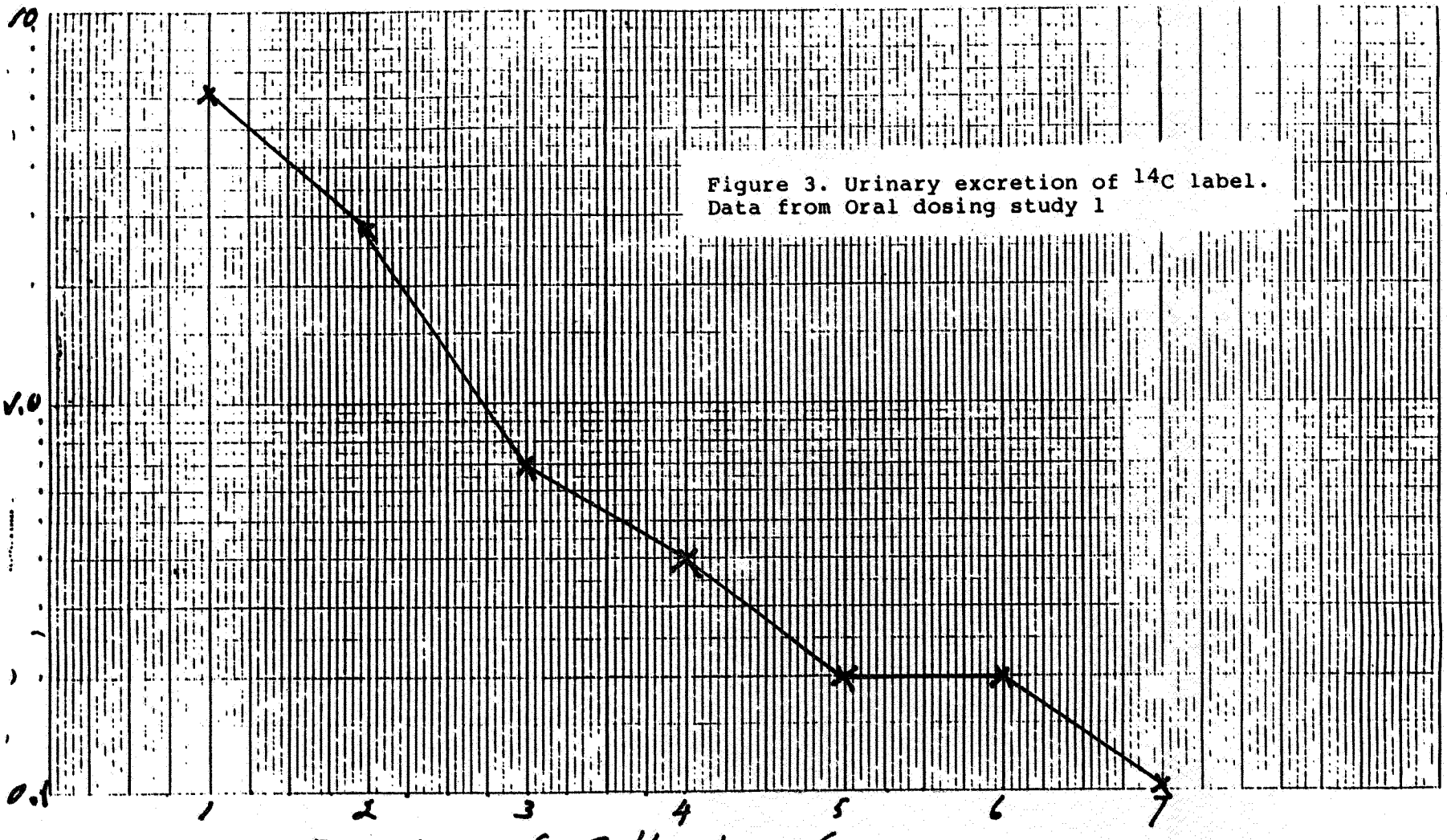

Figure 3. Urinary excretion of ^{14}C label. Data from Oral dosing study 1

008724

Percent of dose excreted

Figure 4. Fecal excretion of ^{14}C label. Data from Oral dosing study 1

SEMI-LOGARITHMIC 2 CYCLES X 175 DIVISIONS

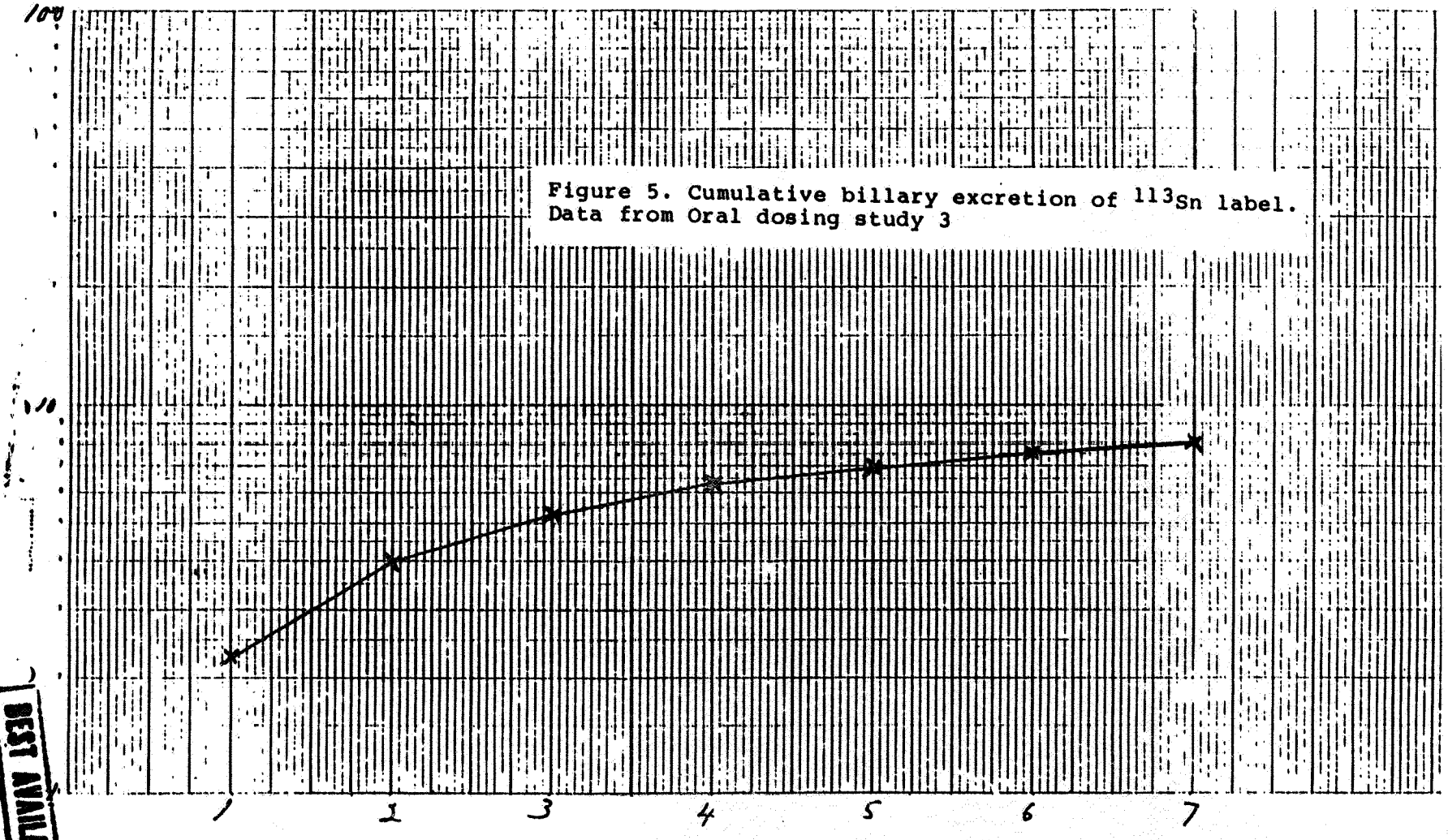

Figure 5. Cumulative billary excretion of ^{113}Sn label. Data from Oral dosing study 3

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Figure 6. Cumulative billary excretion of ^{14}C label. Data from Oral dosing study 1

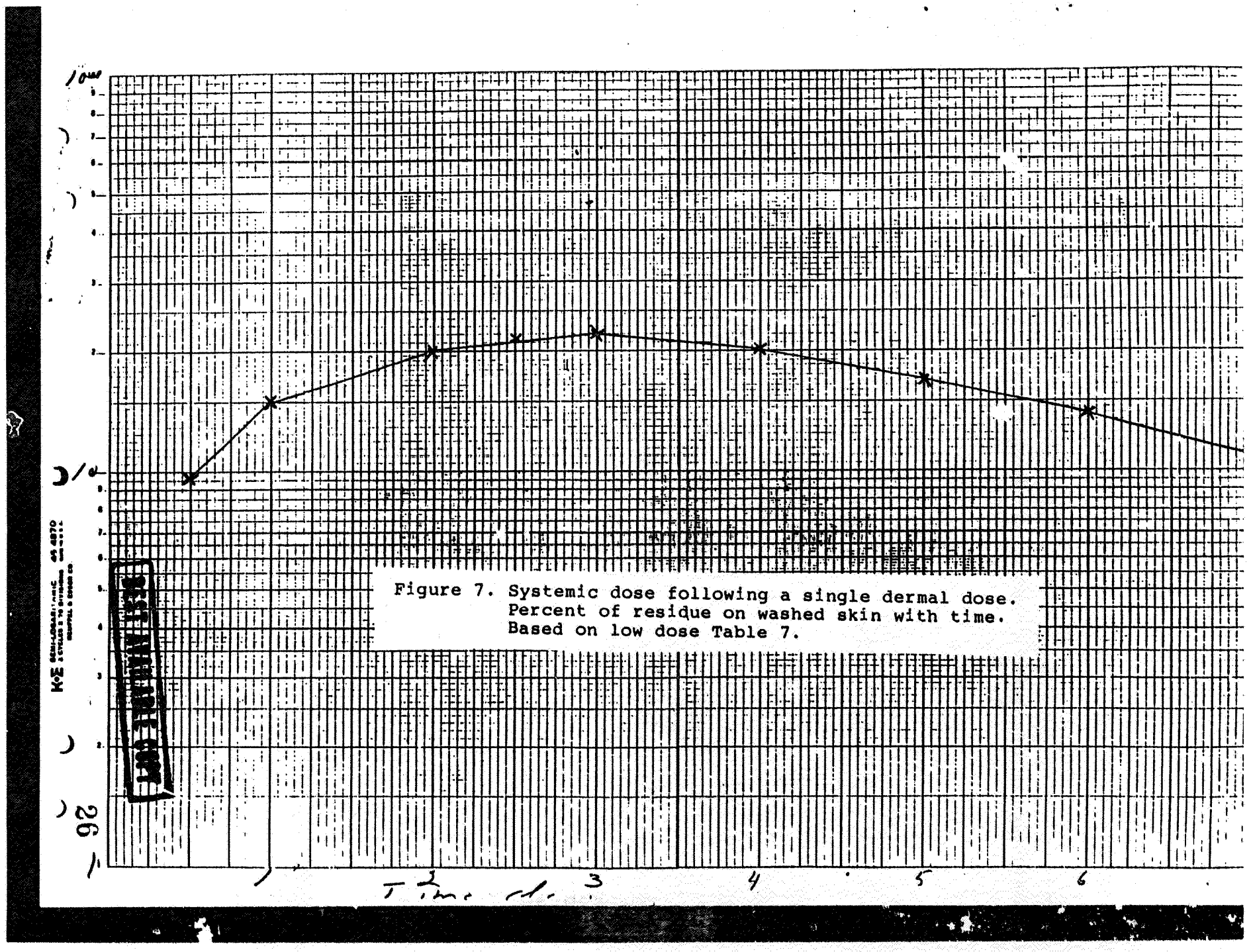

Figure 7. Systemic dose following a single dermal dose. Percent of residue on washed skin with time. Based on low dose Table 7.

SEMI-LOGARITHMIC 2 CYCLES X 175 DIVISIONS

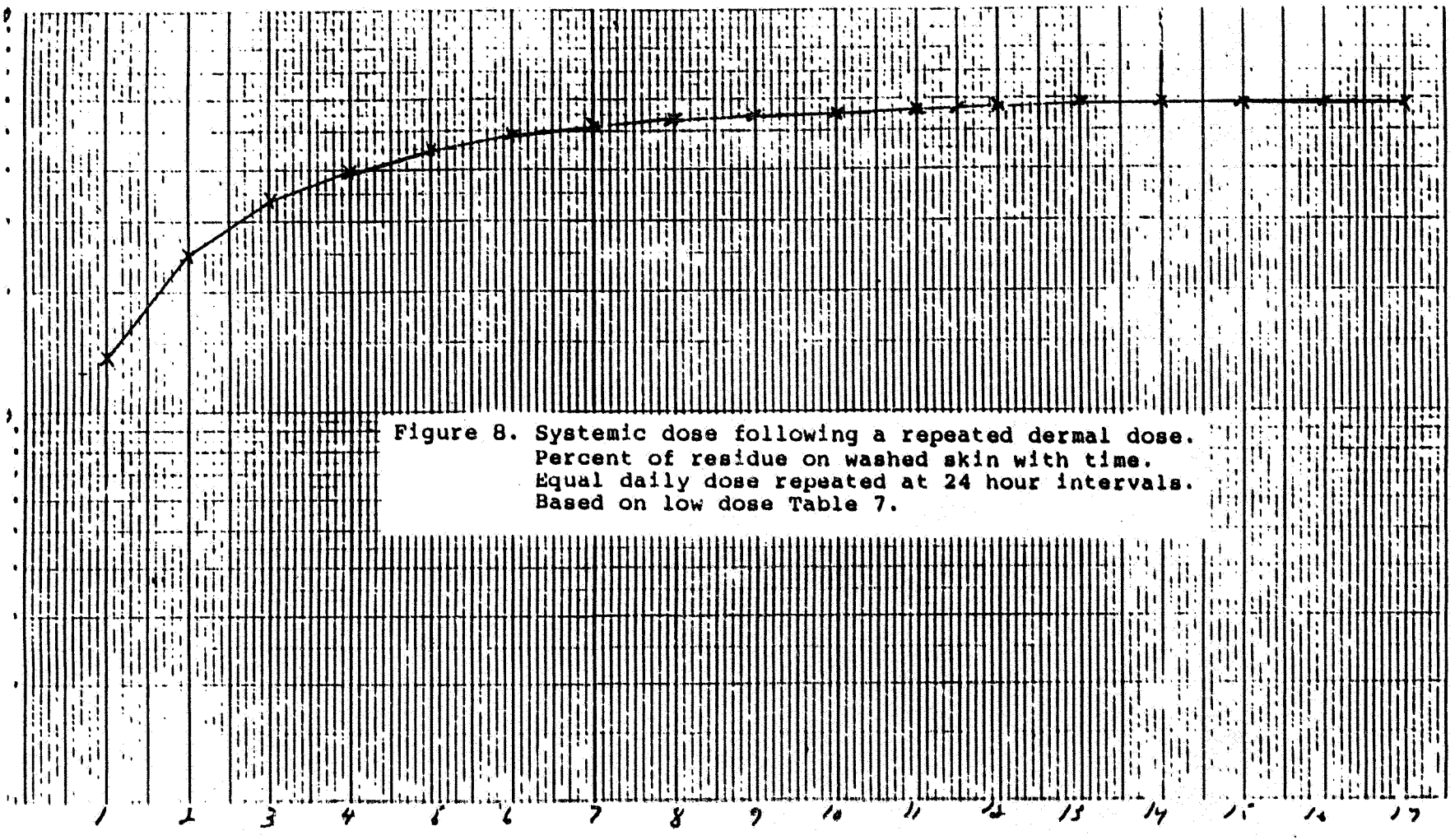

Figure 8. Systemic dose following a repeated dermal dose. Percent of residue on washed skin with time. Equal daily dose repeated at 24 hour intervals. Based on low dose Table 7.

APPENDIX I

The kinetics of distribution from the dermal compartment to the systemic compartment.

In the rat dermal absorption study the skin is washed with soap and water at the end of the exposure period and the application site skin is collected and analyzed for test material. This skin residue varies with the compound tested and ranges from a tiny portion of the dose to the largest single portion of the dose. For some compounds it is many times the amount absorbed systemically during the exposure period. Considering this skin material as part of the total material absorbed systemically can make significant differences in a dermal risk assesment for the compound. This residue has been noted in published studies and it has been postulated that the material binds to the protein in the statium cornium of the epidermis and is lost during exfoliation of this layer. Thus, the bound material will not contribute to the systemic toxicity of the compound.

A few studies have been performed with pestiides in a variation of the rat dermal study to determine the fate of the wash resistant material. Rats were dosed, washed after 10 hours and carried for up to three weeks during which period this material was found to leave the skin compartment and enter the systemic compartment. No clear evidence of loss by exfoliation has been found[1]. An example of this data from a triphenyltin hydroxide (TPTH) study is given in Table A.

Table A[2]. Distribution of dermally applied ^{14}C labeled triphenyltin hydroxide. Dosing material prepared as a suspension. The application site was washed with soap and water after 10 hours. Values are means of four animals per dose duration. Dosing area 10.8 cm^2. Mean dose 1.83 ug/cm^2.

Time of Sacrifice (days)	Mean Skin Wash (% dose)	Mean in washed skin (% dose)	Mean TPTH equivalents absorbed (% dose) Tot	/day	Recovery (% dose)	Missing 100% - Recovery (% dose)
10hrs	49.8	47.0	1.9	4.6	98.7	1.3
1	51.5	40.8	3.4	3.4	95.7	4.7
7	43.6	7.3	26.5	3.8	77.4	22.6
14	46.6	3.1	34.0	2.4	83.7	16.3
21	40.8	0.4	26.7	1.3	67.9	32.1

1. Compounds tested to date have been organic compounds having no chemical or physical properties such that one would expect them to bind to protein.
2. An extended duration dermal absorption study in rats with ^{14}C-triphenyltin hydroxide, E.M. Caine, WIL Research Laboratories, Inc. WIL-39037, May 11, 1987. MRID 401983-01.

The toxicity of a compound is a direct function of the maximum systemic dose (or systemic concentration) obtained following administration to an animal. This is a functon of the magnitude of the dose administered, its rate of absorption and its rate of excretion (or clearance). Since the passage of a compound from the washed skin compartment to the systemic compartment is the equivilant of absorption, the rate of this process must be obtained in order to quantitate systemic dose with time. It is proposed that this rate is a first order process such that a halftime ($T^{1/2}$) may be determined. With this $T^{1/2}$, and knowledge of the portion of the dose present in the washed skin at a known time interval after dosing, one may calculate the passage of the compound from the skin compartment to the systemic compartment with time. With a $T^{1/2}$ of excretion (clearance) of the compound one may then calculate the systemic dose of the compound with time.

Evidence for a first order process.

a. Physical/chemical conditions.

Passage from the skin compartment to the systemic compartment is a two compartment problem in which we can physically define the compartments and their interface. The interface between these compartment lies in the skin itself and is the interface between the epidermal and dermal layers. The epidermis lacks blood vessels and material moves through it by difusion. The dermis is vascularized and a compound difusing into it from the epidermis is carried by the blood which distributes the compound throughout the systemic compartment. Thus the epidermis is the skin compartment and the dermis is continious with, part of, the systemic compartment. Passage across the interface between these compartments is by difusion.

The rate of diffusion of a compound across an interface between two compartments is a function of the relative solubilities and concentrations in the two compartments and the common area between the compartments. The solubility of a particular compound in each of the two compartments in this system is a constant and therefore the relative solubilities have a constant effect on its diffusion between the compartments. The concentration of a compound is a function of the mass of the compound (which is in the order of micrograms in this system) and the volume of the compartments. Since the epidermal compartment is relatively small and poorly stirred and the systemic compartment is relatively large and well stirred, the concentration difference between compartments will be defined by the concentration in the epidermal compartment. Concentration in the systemic compartment will

be essentially zero in relation to concentration in the epidermal compartment. Finally the common area between the two compartments is constant. Therefore, the instantanious rate at which the compound leaves the epidermal (skin) compartment will be directly proportional to the concentration in that compartment. This is a first order situation.

b. Analysis of the TPTH data.

The TPTH data can be used to test the hypothysis that diffusion from the skin compartment to the systemic compartment is a first order process.

Figure A presents the TPTH skin residue data plotted as the log of the percent dose in the skin with time[1]. This data is best fitted with a straight line which is indicative of a first order process. The process has a $T^{1/2}$ of 74.4 hours.

One may also calculate $T^{1/2}$ directly for the skin residue data by considering the 10 hour data as 100%, each subsiquent data point as percent of the 10 hour data, determining the number of half-lives for that decrease in residue from a half time graph and calculating the $T^{1/2}$ for each subsiquent point. The calculations are presented in Table B.

Table B. Determination of half-time for difusion of TPTH from the skin compartment.

Time of Sacrifice (hours)	TPTH in Washed Skin (% dose)	(% of 10hr)	Number of Half times	Elapsed Time (hours)	$T^{1/2}$
10	47.0	100	----	----	----
24	40.8	87	0.2	14	70
168	7.3	16	2.7	158	59
336	3.1	7	3.8	326	86
504	0.4	1	6.6	494	75
				mean	72.5

The individual variation of $T^{1/2}$s from the graphically derived value is indicative that these values to not lie on the fitted line as can be seen in the graph. The mean value is very close to the graphically derived value. Only a slight difference in line fitting would be necessary to make them identical.

The TPTH date agree with the hypothysis that the diffusion of TPTH from the washed skin compartment is a first order process.

1. In a dermal absorption study the dose is mass per unit area (ug/cm^2). Thus, the volume of the epidermal compartment is constant and one may use mass per unit area or percent of applied dose for $T^{1/2}$ determination.

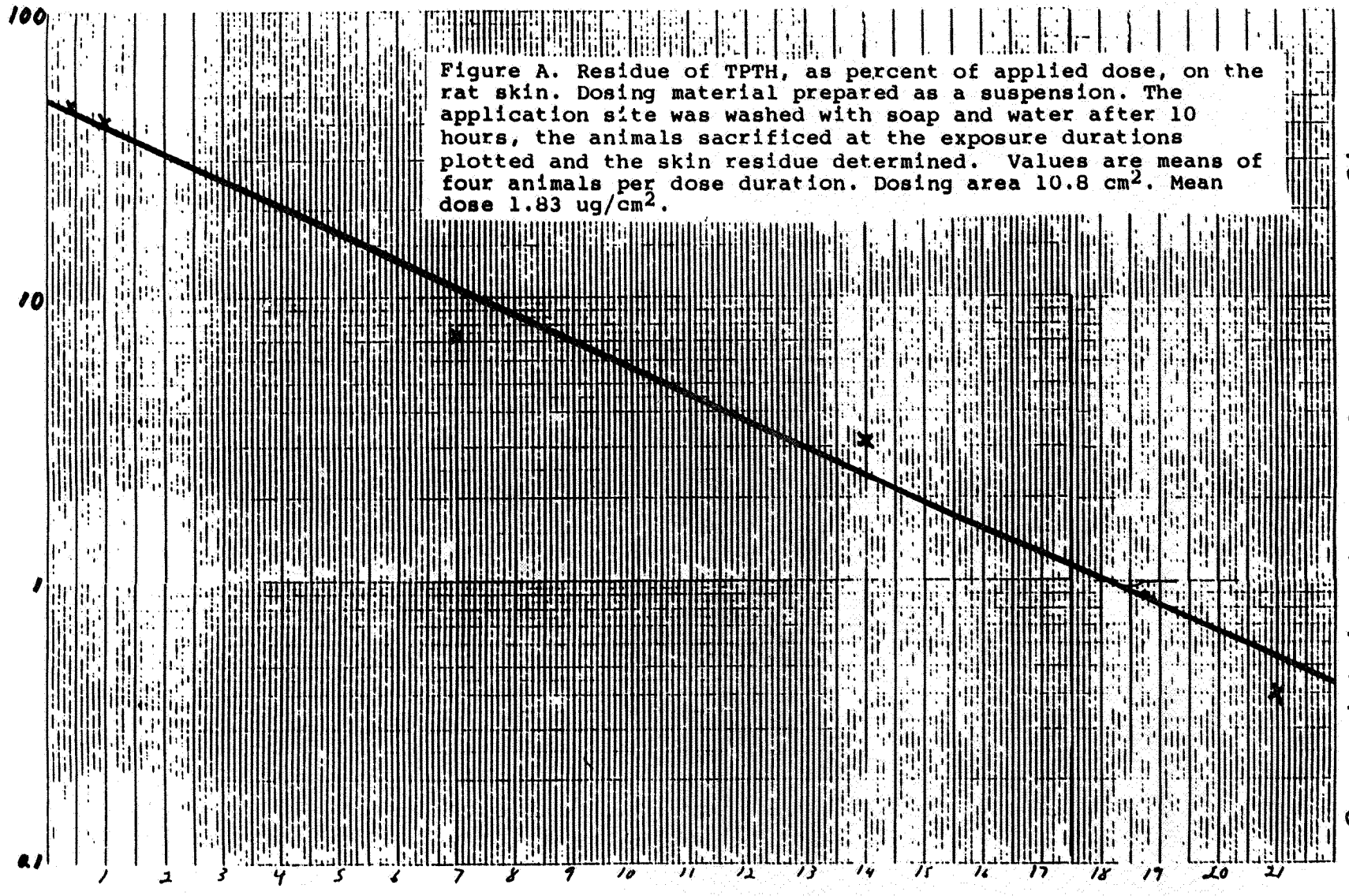

Figure A. Residue of TPTH, as percent of applied dose, on the rat skin. Dosing material prepared as a suspension. The application site was washed with soap and water after 10 hours, the animals sacrificed at the exposure durations plotted and the skin residue determined. Values are means of four animals per dose duration. Dosing area 10.8 cm^2. Mean dose 1.83 ug/cm^2.