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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Triphenyltin Hydroxide, Review of Additional Data from Dermal Absorption Study

TO: Betty Shackelford, PM-71SR
Registration Division (TS-767)

FROM: Robert P. Zendzian PhD 3/3/86
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THROUGH: Reto Engler PhD, Head
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Theodore M. Farber PhD, Chief
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Compound Triphenyltin hydroxide — Tox Chem #896E

Registration #083601 — Registrant Hoechst

Accession #N/A — Tox Project #1248

Action Requested

Review the additional data submitted on the following dermal absorption study.

A Dermal Absorption Study in Rats with ^{14}C-Triphenyltin Hydroxide, J. Laveglia, Will Research Laboratories, WIL-39020, June 5, 1985, Add analysis of carcass, skin & muscle under application site. add Ltr WIL Feb. 24 1986.

Conclusion

Dermal absorption of triphenyltin hydroxide appears to be very small under the experimental conditions, ranging from <0.01 to 0.82 percent of applied dose depending upon dose and duration of exposure. However, additional information is needed for more accurate quantitation. (Data supplied see below.)

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Additional analytical data indicate significant residue remaining on and/or in the skin and potentially available for absorption. Additional studies are necessary to quantitate the ability of this residue to enter the body.

The study is scientifically acceptable.

Recommendation

It is recommended that the following additional study be performed to determine the ability of the residue on and/or in the skin to be absorbed.

Three groups of 8 rats each are to be dosed dermally with 0.1, 1.0 and 10 mg of radiolabeled TPTH. After 10 hours exposure in a metabolism cage for collection of feces and urine, the application site in all animals is to be washed with a soap/detergent solution in water and rinsed several times with water. The wash/rinse is to analysed. Four animals in each dose group are then sacrificed and the samples taken in the initial study are collected for analysis. The remaining four animals are placed in fresh metabolism cages and exposed with collection of feces and urine for at least 24 hours. After this second exposure the application site is again washed as above, the animals sacrificed and the tissue samples collected for analysis.

A copy of the latest Procedure for Dermal Absorption is attached for the Registrant's information. It is strongly recommended that the Registrant discuss the protocol for the additional study before performing it.

Attachments

DER

Procedure for Dermal Absorption, 3rd Edition w/California modifications

Data Evaluation Report
(revised)

Compound Triphenyltin Hydroxide

Citation
A Dermal Absorption Study in Rats with ^{14}C-Triphenyltin Hydroxide, J. Laveglia, Will Research Laboratories, WIL-39020, June 5, 1985, Add analysis of carcass, skin & muscle under application site. add Ltr WIL Feb. 24 1986.

Reviewed by Robert P. Zendzian PhD 3/3/86
Pharmacologist

Core Classification Acceptable

Conclusion
Dermal absorption of triphenyltin hydroxide appears to be very small under the experimental conditions, ranging from <0.01 to 0.82 percent of applied dose depending upon dose and duration of exposure. However, additional information is needed for more accurate quantitation. (Data supplied see page 5.)

Additional analytical data indicate significant residue remaining on and/or in the skin and potentially available for absorption. Additonal studies are necessary to quantitate the ability of this residue to enter the body.

Materials

^{14}C-labeled tiphenyltin hydroxide, from Hoerst Aktiengesellschaft
Batch 11009 I, Specific activity 32.4 mCi/gram radiopurity 98%
Batch 11009 II, Specific activity 3.88 mCi/gram radio purity 98%

Sexually mature Sprague Dawley COBS® CD® male rats (Crl:CD(SD)BR), charles River Breeding Laboratories

Methods

Twenty rats per group were assigned to the following test groups.

Group Number	Dose mg/kg	Batch used	Amount of test material to be Administered to each rat uCi	ug
I	0.1	11009 I	1	25
II	1.0	11009 I	10	250
III	10.0	11009 II	10	2500

On the day prior to dosing the back of each rat was clipped and 30 minutes prior to dosing the clipped area was washed with acetone. A 2" by 2" application zone was marked with felt tipped pen. Dose was applied as a suspension and the application site was wrapped with a non-occlusive cover. Animals were placed in individual metabolism cages and urine and feces collected. Four animals per dose group were sacrificed at 0.5, 1, 2, 4 and 10 hours after dose application. The wrap, blood sample and skin and muscle at the application site were collected for ^{14}C-analysis. The skin was extracted with ethanol for analysis. The remaining carcass was retained for possible analysis.

Results

Table 1, Mean actual dose applied. From tables 3, 4, 5, 6, 7, & 8 of the report.

Duration of exposure(hr)		0.5	1.0	2.0	4.0	10.0
Group #						
I	uCi	0.614	0.553	0.720	0.523	0.863
	mg/kg	0.09	0.07	0.10	0.07	0.11
II	uCi	7.045	7.150	7.281	7.820	8.295
	mg/kg	0.90	0.91	0.95	1.01	1.05
III	uCi	12.384	11.538	12.237	11.975	12.534
	mg/kg	13.02	11.99	12.72	12.37	14.01

Table 2. Mean percent of applied dose in excreta. From tables 9, 10 & 11 of the report.

Duration of exposure(hr)	0.5	1.0	2.0	4.0	10.0
Group #					
I	<0.04	<0.07	0.32	0.24	0.82
II	<0.01	<0.01	0.13	0.08	0.27
III	<0.01	<0.01	<0.01	0.01	0.20

Table 3. Mean percent of applied dose recovered from the skin after extracton with ethanol. From Table 21 of the report.

Duration of exposure(hr)	0.5	1.0	2.0	4.0	10.0
Group #					
I	ND	ND	ND	ND	16.1
II	ND	ND	ND	ND	20.5
III	ND	ND	ND	ND	26.0

ND = not determined

Table 4, Mean concentraion of material in the blood and in muscle under the application site. From tables 13, 14 & 15 of the report.

Duration of exposure(hr)	0.5	1.0	2.0	4.0	10.0
Group #	equivilants of test material (ppb)				
I blood	<1.2*	<1.2	<1.2	<1.2	<1.2
muscle	<1.2	<1.2	<1.2	<1.2	<1.2
II blood	<1.2	<1.2	1.4	<1.2	<1.2
muscle	3.0	2.8	1.5	<1.2	7.6
III blood	<10*	<10	<10	<10	<10
muscle	67	39	36	88	14

*limit of detection

Table 5. Mean percent of applied dose recovered from application site by ethanol extraction and from the wrap. From Tables 18, 19 & 20 of the report.

Duration of exposure(hr)	0.5	1.0	2.0	4.0	10.0
Group #					
I	62	71	24	116	33
II	44	76	67	81	70
III	55	53	54	51	55

Discussion

The data in Table 2, mean percent of applied dose in excreta, show that absorption of the compound follows the most common pattern observed in this type of study. Percent absorbed increases with time of exposure and decreases with increasing dose. The percent absorbed is small by this measure and the quantities found in the blood and muscle below the application site support this conclusion. The highest concentration of compound found, in the muscle, represents approximately 0.05% of the particular applied dose.

Confounding the conclusion that the precent absorbed is small is the relatively low recovery of compound from the application site. Under the protocol used in this study the absorption can be quantitated in two ways, 1) determining the amount of compound found in the animal and excreta and 2) determining the amount 'lost' from the application site. The latter determination is relatively insensitive at low absorption rates because of the problems of obtaining quantitative recovery from the application site. In this study a large portion of each total dose is missing. Table 6 shows the apparent absorption obtained by this approach for the ten hour exposures. These values are considerably larger than those obtained from the direct absorption data and the report indicates that some of this material may be bound to the wrap. Since the carcasses were not analyzed the possibility also exists that a 'significant' portion of the missing material was absorbed and is present in the carcasses. On the other hand of the analysis shows little or no compound one may conclude that dermal absorption of small even without finding the 'missing' material from the application site.

Table 6. Mean percent of applied dose absorbed at 10 hours by subtraction from dose applied of total dose recovered from application site and wrap and recovered from the skin after extraction with ethanol.

Group #	application site and wrap	skin after extraction	total	percent absorbed
I	33	16.1	49.1	50.9
II	70	20.5	90.5	9.5
III	55	26.0	81.0	19.0

Recommendations (noted these recommendations have been completed. See below)

It is recommended that;

1. Data from the full analysis of wrap and skin be obtained in order to better quantitate the 'missing' material.

2. The remaining carcasses be analyzed, starting with the 10 hour exposures, in order to complete quantitation of the absorbed material.

Additional Data (ltr WIL Feb 24, 1986)

As a result of the recommendations above, a complete analysis was performed on the carcass, the skin residue and the muscle samples from under the application site. The quantity of radioactivity found in the carcass and the muscle samples was insignificant. However a major portion of the total dose was found in the skin samples when they were subjected to an ethanol extract and an alkaline digestion.

Table 7 presents a material ballance determination which shows that essentially all the radioactivity (TPTH) is now accounted for. The values for 2, 4 and 10 hours in the 0.1 mg dose group are rather far off but this can be expected when dealing with such small quantities.

The information on the carcass, the muscle samples and the material ballance allows one to say that the excreta data in Table 2 represent essentially all the material that can be shown directly as having been absorbed.

Table 8 presents the material that was detected as remaining in and/or on the skin. An ethanol extract of the skin was first performed and analysed. The remaining material was subjected to an alkaline digest and analysed. Taken together these two analysis reveal that a significantly high percentage of the applied dose remained in and/or on the skin. This material must be considered as potentially available for absorption and requires additional work to clarify its availability.

Additional studies required

Is is necessary to determine if the material detected in this study as remaining on and/or in the skin may be 1) washed off with soap and water and 2) if any material remaining after washing is available for absorption.

Table 7. Material ballance of radioactivity (nCi) administered to the skin and radioactivity recovered from the skin. Values presented are means of four animals except as noted.

Group #		Duration of exposure (hours)				
		0.5	1.0	2.0	4.0	10.0
I	ADA[a]	614	553	720	523	863
	recovered[b]	384	396	169	641	288
	alk digest[e]	265	259	124	294	139*
	total	649	655	293	935	427
	% recovered	106	118	41	179	49
II	ADA	7045	7150	7281	7820	8295
	recovered[c]	3104	5439	4913	6356	5830
	alk digest[f]	3303	2476	3059	2416	1725*
	total	6407	7915	7972	8772	7555
	% recovered	91	111	109	112	91
III	ADA	12384	11538	12237	11975	12534
	recovered[d]	6758†	6065	6646	6102	6904
	alk digest[g]	4549†	4643	4667	5115	3263*
	total	11307	10708	11313	11214	10167
	% recovered	91	93	92	94	81

a. Actual Dose Applied from Table 1.
b. Total from Table 15 of report.
c. Total from Table 16 of report.
d. Total from table 17 of report.
e. Alkaline digest of skin from Table 4 addendum.
f. Alkaline digest of skin from Table 5 addendum.
g. Alkaline digest of skin from Table 6 addendum.
*. From 'residual skin' column of addendum.
†. One sample lost.

Table 8. Material remaining in or on the skin as mean radioactivity (nCi) and mean percent of applied dose. Values presented are means of four animals except as noted.

Group #		Duration of exposure (hours)				
		0.5	1.0	2.0	4.0	10.0
I	ADA[a]	614	553	720	523	863
	EtOH Extract[a]	35	31	22	37	21
	alk digest[e]	265	259	124	294	139*
	Total	301	290	146	331	160
	% Available for absorption	49	52	20	62	19
II	ADA	7045	7150	7281	7820	8295
	EtOH Extract[b]	862	672	766	690	510
	alk digest[f]	3303	2476	3059	2416	1725*
	Total	4165	3148	3861	3106	2235
	% Available for absorption	59	44	53	40	27
III	ADA	12384	11538	12237	11975	12534
	EtOH Extract[c]	1237†	1381	1471	1441	941
	alk digest[g]	4549†	4643	4667	5115	3263*
	Total	5786	6024	6138	6556	4202
	% Available for absorption	47	52	50	55	34

a. Actual Dose Applied from Table 1.
b. Ethanol extract from Table 15 of report.
c. Ethanol extract from Table 16 of report.
d. Ethanol extract from table 17 of report.
e. Alkaline digest of skin from Table 4 addendum.
f. Alkaline digest of skin from Table 5 addendum.
g. Alkaline digest of skin from Table 6 addendum.
*. From 'residual skin' column of addendum.
†. One sample lost.

10/9/85

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Procedure for Studying Dermal Absorption

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Introduction

This paper presents a general procedure for dermal absorption studies on pesticides which is applicable to any compound or formulation of a compound. The study requires application of various doses of radiolabeled compound to the shaven skin of male rats followed, at specific intervals after dosing, by total urine and fecal collection, determination of blood concentration, determination of the quantity in the body and determination of the quantity remaining on the skin. It is assumed that a metabolism study of the test compound has been performed in the rat before the dermal absorption study is undertaken.

The rat is used for purely practical reasons, it is not intended as a model of absorption through the human skin but rather as a test system for dermal absorption. The domestic rat is a conveniently sized animal, which is readily available and used for most of the toxicology studies on pesticides including metabolism. Because of its small size, several animals can be used per dose and several dose levels per compound within the constraints of time and resorces. Foreign compounds in general pass more rapidly through rat skin than through human skin and thus determination of dermal penetration in the rat offers a built-in safety factor for projection to human exposure.

The study described here combines two different types of dermal absorption studies in a manner which can compensate for their individual deficencies and simultaniously cover the full range of possible dermal absorption patterns. The first type of study involves placing a measured quantity of compound on the skin for a specific period of time. The animal is then killed and the treated skin is removed. The quantity remaining on the skin is determined and the quantity of compound absorbed is calculated by subtraction. This method works very well for small quantities of a compound which does not fall or vaporize off of the skin. Large quantities, volatile compounds or strange solvents, cannot be used in this procedure.

The second type of study measures what goes into the animal. The compound is applied to the skin in a measured dose and the quantity in the body and the quantity excreted for a specific time period is measured. The procedure has greator possibilities for error in very low doses, for compounds which are not rapidly excreted and for compounds which are completely metabolized to CO_2, water and urea.

Materials

Twenty-four young adult male rats, 225-250 grams in weight, are used at each dose point. It is prefered that the rats be of the same strain used for metabolism studies on the test compound.

The compound should be chemically pure and radiolabeled, usually with carbon-14, in a position which is part of the "core" of the compound. The label should follow the compound and its major metabolites until excreted. The label should not be exchangable nor should it be metabolically removed to CO_2 or become part of the one-carbon pool of the organism.

Methods

Twenty-four hours prior to dosing the back and shoulders of the rats are clipped free of hair and the area washed with acetone. Do not damage the skin.

Twenty-four animals are used per dose. A minimum of three but preferably four doses, at log intervals should be used. The doses should span the range of dose per unit area of skin which can be expected to occur in human exposure. Experiance has shown that the highest useful dose is in the order of 10mg/rat with descending doses of 1, 0.1, and 0.10mg/rat. If less then four doses are used it is prefered that the lower dose range be used.

The compound is applied to a measured area of the rat's skin, at least 10 cm^2, in the form applied in the field utilizing the field solvent. When no solvent is specified, as for the technical material or a dust, the compound is dissolved or suspended in water. Organic solvents should not be used. The material is spread evenly until dry. The spreader should be checked for loss of material. The treated area is covered with a nonocclusive cover to prevent loss by falling or being rubbed off.

Experiance has shown that the application area must be covered. A combination cover consisting of a rubber ring glued to the skin and a filter paper or gauze glued to the ring appears to be most effective.

The treated animals are placed individually in metabolism cages. All urine and feces are collected, a single collection for the entire duration of exposure. At intervals of 1/2, 1, 2, 4, 10 and 24 hours, four animals per dose are anesthetized and a blood sample taken. The animals are killed and residual urine collected from the bladder and added to the collected urine. The exposed skin and residual compound are collected seperately by washing

the skin with a mild soap solution followed by several water rinses. Liquid Ivory or Dove for dishwashing is suggested. Any material on the protective appliance is measured. The remainder of the animal is prepared for determination of the quantity of compound in the carcass.

For each animals the following determinations are made. Results are expressed as quantity or concentration of the parent compound. Metabolites are not separately distinguished.

1) The quantity of the compound in/on the application device and the protective appliance.

2) The quantity of compound that can be washed from the skin

3) Quantity of compound remaining on or in the skin at the application site which cannot be removed by washing.

4) Concentration of compound in the blood and from this the quantity of compound in the blood.

5) Quantity of compound excreted in the urine and feces.

6) Quantity of material remaining in the carcass.

Results and Conclusions

From the quantity determined in parts 1 and 2 above one may calculate, by subtraction the quantity absorbed provided that other routes of loss are not significant. Excessive variation of results within groups at the same time and dose will indicate external loss of the dose.

From the quantity in the skin, the quantity excreted, the quantity in the blood and the quantity remaining in the carcass one may obtain directly the quantity absorbed.

The blood concentration of the compound can be used for a direct comparison with other studies on the compound.

Graphs relating dose, time and amount absorbed may be constructed and used to calculate absorption for doses which are not directly studied. Using proper assumptions one may extrapolate to estimate human absorption under conditions of normal exposure.

Additional procedures

1) Procedure to define compounds which are essentially not absorbed.

Results from a study of a compound expected to have little or no dermal absorption have suggested the necessity of treating an additional group of rats. In the study, analysis of the dermal residue indicated no absorption to a limit of 0.1 percent of the dose. This limit was defined by the variability of recovery of compound from the skin. The blood showed no radioactivity at any dose and duration of exposure. The urine showed radioactivity which did not appear to follow the dose and duration of exposure relationship expected. In only one of nine treatment groups were the results internally consistant with all four animals showing similar positive results. In the other eight groups the number of animals having radioactivity in the urine ranged from zero to three with a mean of 1.5. These results appear indicative of contamination of the urine rather than dermal absorption.

Under such circumstances an additional group of four rats should be treated with the high dose at the 10 and 24 hour durations of exposure. These animals should have their urinary bladders cannulated to avoid contamination of the urine collected during the exposure period. Samples of blood, urine and carcass should be counted for the longest practical time in order to produce the lowest possible limit of dermal absorption. In the case where no absorption occurs under the experimental conditions the limit of dermal absorption will be defined solely by the sensitivity of the method for detecting the radio tracer.

2) Procedure for examining compounds which show a major residue on/in the washed skin.

Several compounds have been tested which show a significant residue on/in the skin dispite vigorous washing. The concentration has appeared in short exposures and shows little or not increase with time and often does not appear to increase to any large extent with increase of dose. This suggests a binding process.

For regualtory purposes one must assume that this material is available for further absorption. However, this may not be true particularly in cases were little or no detectable compound appears in blood, excreta and/or carcass.

In such cases the following additional study is suggested.

1) Eight rats per dose are treated for the time period which shows the maximum skin concentration (or ten hours).

2) At the end of the exposure period 4 rats per dose are sacrificed and treated as in the basic protocol.

3) The skin of the remaining 4 rats per dose, is washed in the same fashion used in the origional study and the animals followed for at least an additional 24 hours.

4) The animals are then sacrificed and treated as in the basic protocol.

A balance comparison of the various residues should give some indication as to whether or not the quantity in the washed skin can be absorbed and some quantitation of any absorption. If absorption occurs it may be necessary to repeat this process with longer post washed periods to obtain a quantitation of absorption over time.

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