

SUMMARY

- Titles: (a) Elimination of ^{14}C -Glyphosate in Rhesus Monkeys Following A Single Dose
- (b) Percutaneous Absorption of ^{14}C -Glyphosate in Roundup® Formulation in Rhesus Monkeys Following A Single Topical Dose

Conducted by: Howard I. Maibach, M.D., U. California School of Medicine

Study No.: MA-81-349

Date of Reports: (a) April 1, 1983
(b) April 1, 1983

Study Director: Howard I. Maibach, M.D.

The accompanying report contains the results of the referenced study conducted by Howard I. Maibach, M.D., Dermatologist, School of Medicine, University of California. A review of the data and an evaluation of the conclusions are summarized below.

PURPOSE

This study was conducted to quantify the dermal penetration of topically applied ^{14}C -labelled ROUNDUP formulation through monkey skin. A preliminary study was conducted to determine the rate of elimination of ^{14}C -glyphosate, the active ingredient in ROUNDUP herbicide formulation, following an intramuscular injection of labelled MON 0139 (the isopropylamine salt of glyphosate). The elimination data are required to quantify dermal penetration.

METHODS

For the elimination phase of this study, uniformly labelled N-(phosphono- ^{14}C -methyl)glycine or ^{14}C -glyphosate was mixed with isopropylamine and unlabeled isopropylamine salt of glyphosate (MON 0139) and diluted with water to produce a treating solution where 1 milliliter of solution = 4 milligrams glyphosate = 2 microcuries (specific activity 84.6 microcurie/mole). One milliliter (ml) of this solution was injected into the thigh muscle of each of four male Rhesus monkeys. Scintillation counting indicated that a 1.0 ml dose of this solution contained 1.49 microcuries of C- 14 labeled glyphosate. Urine samples were collected at 4-, 8- and



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24-hours post-injection and every 24 hours thereafter for 7 days. Urine samples were then processed and analyzed by liquid-scintillation spectrophotometry for radiocarbon content. Results were expressed as mean percent of the applied dose excreted during each collection interval.

For the dermal penetration phase of this study, 0.74 mg ^{14}C -glyphosate was diluted with ROUNDUP formulation containing 357 mg unlabeled glyphosate to produce a treating solution where 25 microliters of solution = 8.9 milligrams glyphosate = 1.0 microcurie (specific activity 19.4 microcurie/mmmole). Scintillation counting indicated that there was 0.80 microcuries of C-^{14} labeled glyphosate in the applied dose.

Twenty-five microliters of the labeled ROUNDUP formulation were spread over 7.9 square centimeters of the shaved abdomen of each of six male Rhesus monkeys. After 24 hours each abdomen was swabbed twice with water, twice with acetone and again twice with water to remove the residual ROUNDUP. Urine samples were collected at 4-, 8-, 12-, 24-, 36- and 48-hours post-application and then processed and analyzed by liquid scintillation spectrophotometry. Results were expressed as mean percent of the applied dose. Urine sample values were corrected for incomplete urinary excretion determined in the first phase of this study.

RESULTS

During the seven-day collection period following intramuscular injection of ^{14}C -glyphosate in MON 0139, an average of 89.9% of the applied radiocarbon was excreted in the urine. A total of 85.6%, 1.8%, 0.8%, 0.5%, 0.4% and 0.4% of the applied dose were excreted in the urine during days 1 through 7, respectively. The calculated time required for elimination of one-half of the total dose ($t_{1/2}$) was 19.7 hours overall. However, there were two distinct phases to the excretion kinetics for glyphosate. The $t_{1/2}$ for the more rapid, early excretion phase from 0-24 hours was 6.9 hours, whereas the second phase of excretion had a $t_{1/2}$ of 35.1 hours. The remaining radiocarbon which was not excreted in the urine may have been excreted partially in the feces or eliminated as $^{14}\text{CO}_2$ via the lungs or skin or it may have remained at the site of injection or elsewhere in the monkey's body. Since the majority of the radiocarbon was rapidly eliminated in the urine, urinary excretion of radiocarbon following topical application was deemed acceptable for monitoring dermal penetration using a correction factor of 89.9%.

Following topical application of ^{14}C -glyphosate in the ROUNDUP formulation, a mean total of 1.8% of the applied dose was recovered in the urine during the seven-day collection period. This value includes a correction for incomplete urinary excretion mentioned above. Swabbing the application site with water and acetone after 24 hours removed 14.2% of the applied dose. Mean dermal penetration values for the six monkeys based on corrected urine values were 0.4%, 0.3%, 0.25%, 0.2%, 0.2%, 0.1% and 0.2% during days 1 through 7 respectively. The $t_{1/2}$ for elimination of the topically applied dose was calculated to be 59 hours.

The total percent recovery (percent label removed by washing plus total percent label contained in urine) was low, i.e., 16.0%. A definitive explanation for the low recovery is not provided in the report, but the author does state that previous experience would suggest that much of the test material may in some way bind to or in the skin and cannot be removed by washing. In support of this, it has been reported (Vickers, 1963) that a "chemical reservoir" is formed in the skin after drug application which is eventually shed without penetration. Thus it is concluded that "the bound material is not apparently available for systemic absorption."

CONCLUSION

Systemic doses of glyphosate in MON 0139 are rapidly eliminated in monkeys, predominantly via the urine. The half-time for elimination in the urine following a single intramuscular dose was 19.7 hours. A total of 1.8% of the glyphosate in a single topically applied dose of ROUNDUP formulation penetrated the skin of monkeys during a seven-day period as evidenced by its appearance in urine. Glyphosate penetrated the monkey skin slowly as only 0.4% of the topically applied dose appeared in the urine after 24 hours. The half-time for penetration and elimination via the kidneys of topically applied glyphosate was 59 hours.

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Vickers, C.F.H.: Arch. Dermatol., 88:20, 1963

Elimination of ¹⁴C - Glyphosate In Rhesus Monkeys
Following A Single Parenteral Dose.

STUDY NO. MA-81-349

Conducted For: Monsanto Company
REDACTED REDACTED
St. Louis, Missouri REDACTED

Conducted By: Howard I. Maibach, M.D.
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Study Initiated: 3/30/82
Date

Study Completed: 4/20/82
Date

Study Director: Howard I. Maibach 4-1-83
Howard I. Maibach, M.D. Date

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Elimination of C-14 Labeled Glyphosate in Rhesus Monkeys Following a Single Parenteral Dose

Introduction:

This study was performed to determine the excretion parameters of the C-14 label on glyphosate into the urine from a single parenteral dose utilizing the rhesus monkey experimental animal model.

Procedures:

Four adult male rhesus monkeys each received a single one ml dose of C-14 labeled glyphosate (specific activity of 84.6 uCi/mM, molecular weight of 169.1) by intramuscular injection into the thigh. Scintillation counting determinations showed that a 1.0 ml dose contained 1.49 microcuries of C-14 labeled glyphosate. Urine samples were collected at 4, 8 and 12 hours the first day, then every 24 hours for seven days. A five ml aliquot of each urine sample was assayed in 14 ml PCS (Amersham Corp.) with a liquid scintillation spectrophotometer. A C-14 internal standard was added to triplicate vial of each sample to determine the extent of quenching.

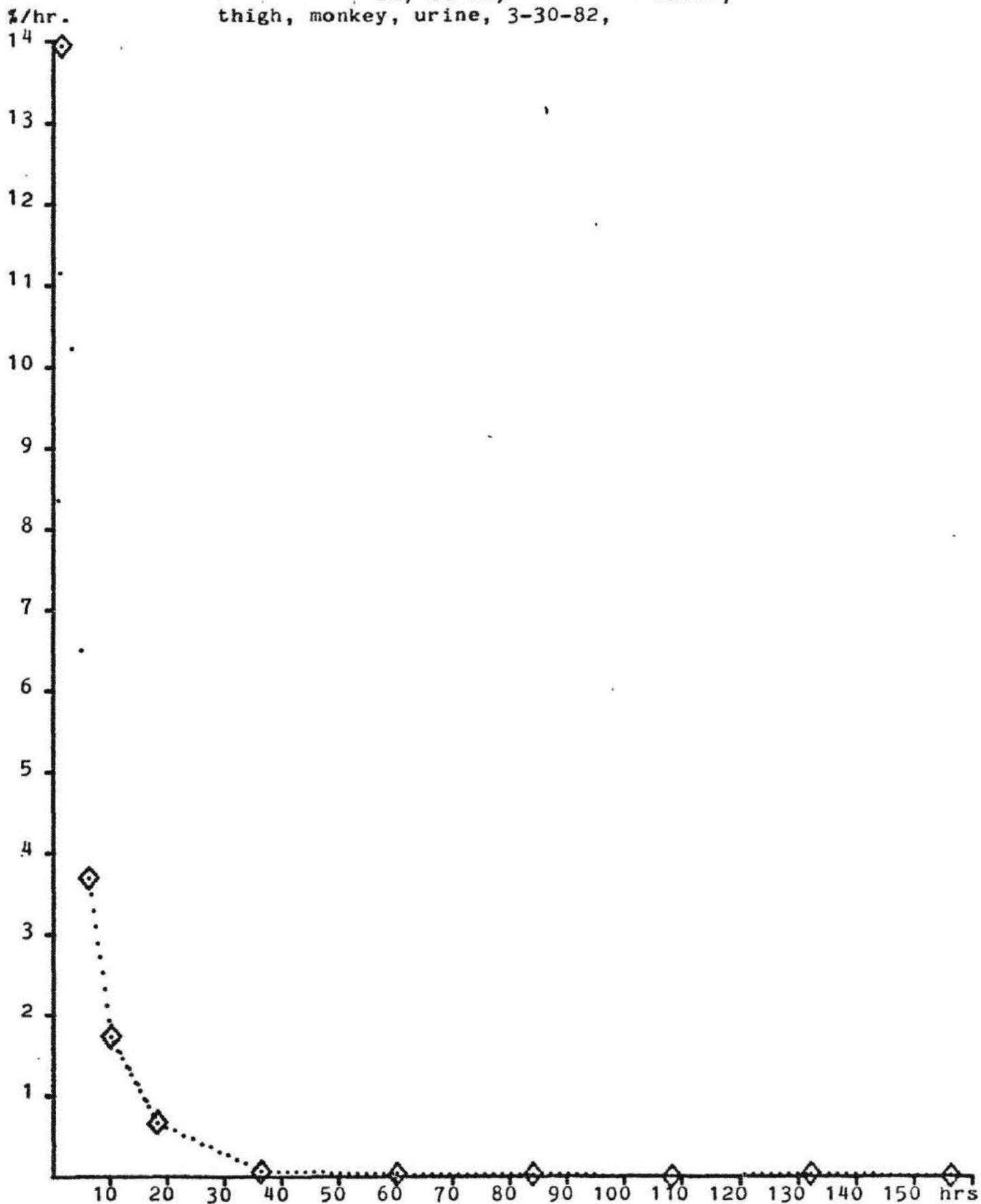
A sigma-minus analysis was performed to determine the C-14 elimination half-life. The differences between the total % dose excreted over all the collection intervals and the total % dose excreted up to the end of each collection interval are equivalent to the amount of the compound not yet excreted. A semilog plot of these differences versus time yields a straight line with a slope proportional to the elimination half-life. The rate constant, k_e , is equivalent to the slope times 2.303 and the elimination half-life equal to 0.693 divided by k_e (Fundamentals of Clinical Pharmacokinetics, first edition; Wagner, J., pg 77).

Results:

The enclosed table lists the dpm values, the total volume of urine collected and the calculated % of the applied dose excreted during each collection time interval for each subject. For example, under 0-4 hours for subject 1, 13522.4 and 13489.2 are the calculated dpm values, 245 is the volume of urine collected in milliliters, and 22.071 is the calculated % of the applied dose excreted. On the bottom of each column the average % dose excreted, the number of hours in the collection interval, and the rate of excretion in % dose per hour are reported. The urine contained an average total value of 89.9% (standard deviation of 12.5%) of the C-14 label on the glyphosate. Peak excretion occurred between 0-4 hours.

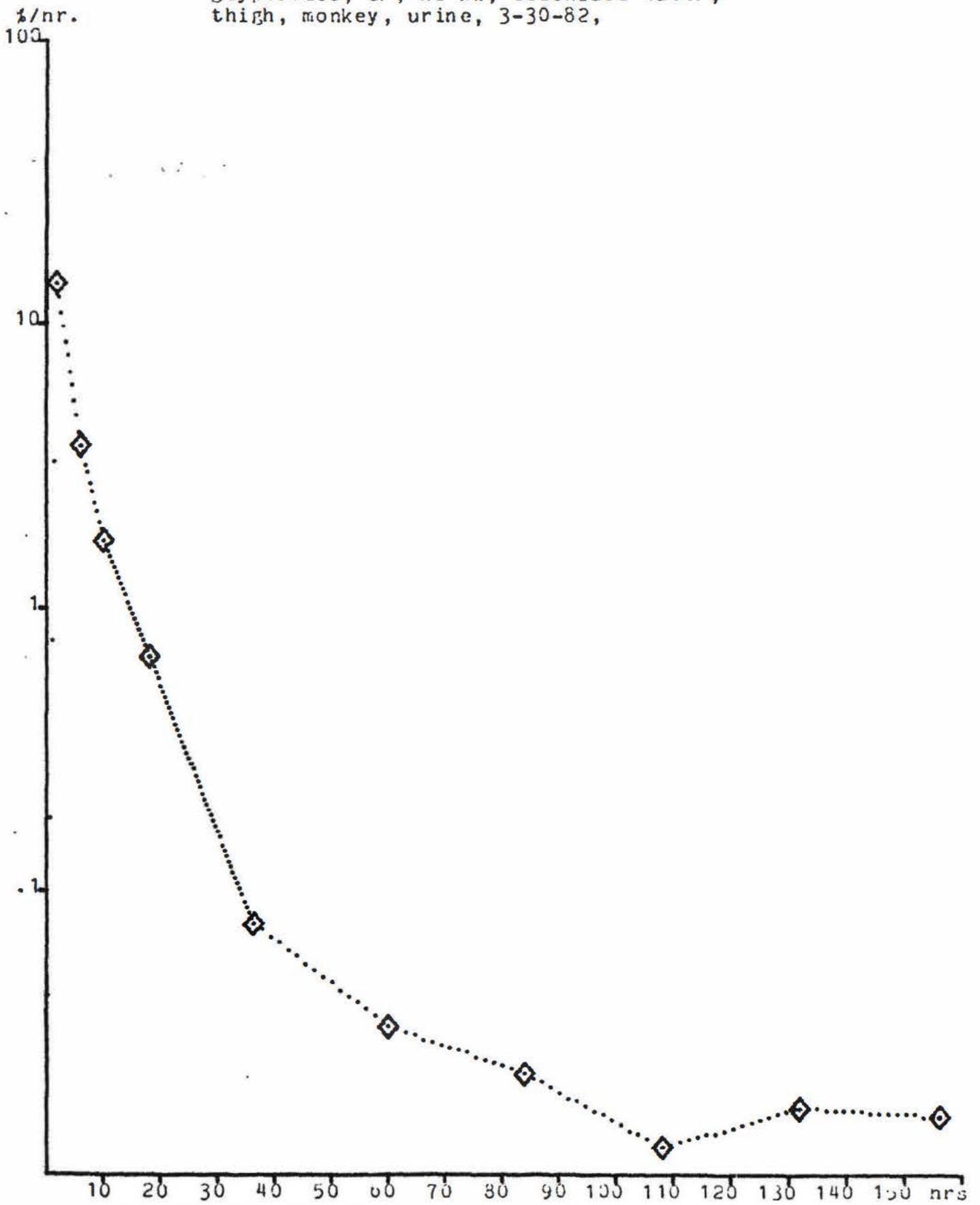
Three graphs are enclosed. The first is a linear plot of the % dose excreted per hour versus time. The second is a semilog plot of the % dose excreted per hour versus time. The last is a sigma-minus plot. The C-14 label on the glyphosate had an average elimination half-life of 19.7 hours; however, two phases of excretion were noted. The first phase from 0-24 hours had an elimination half-life of 6.9 hours, whereas the second phase of excretion had an elimination half-life of 35.1 hours.

IM, no Rx,
 thigh, monkey, urine, 3-30-82,

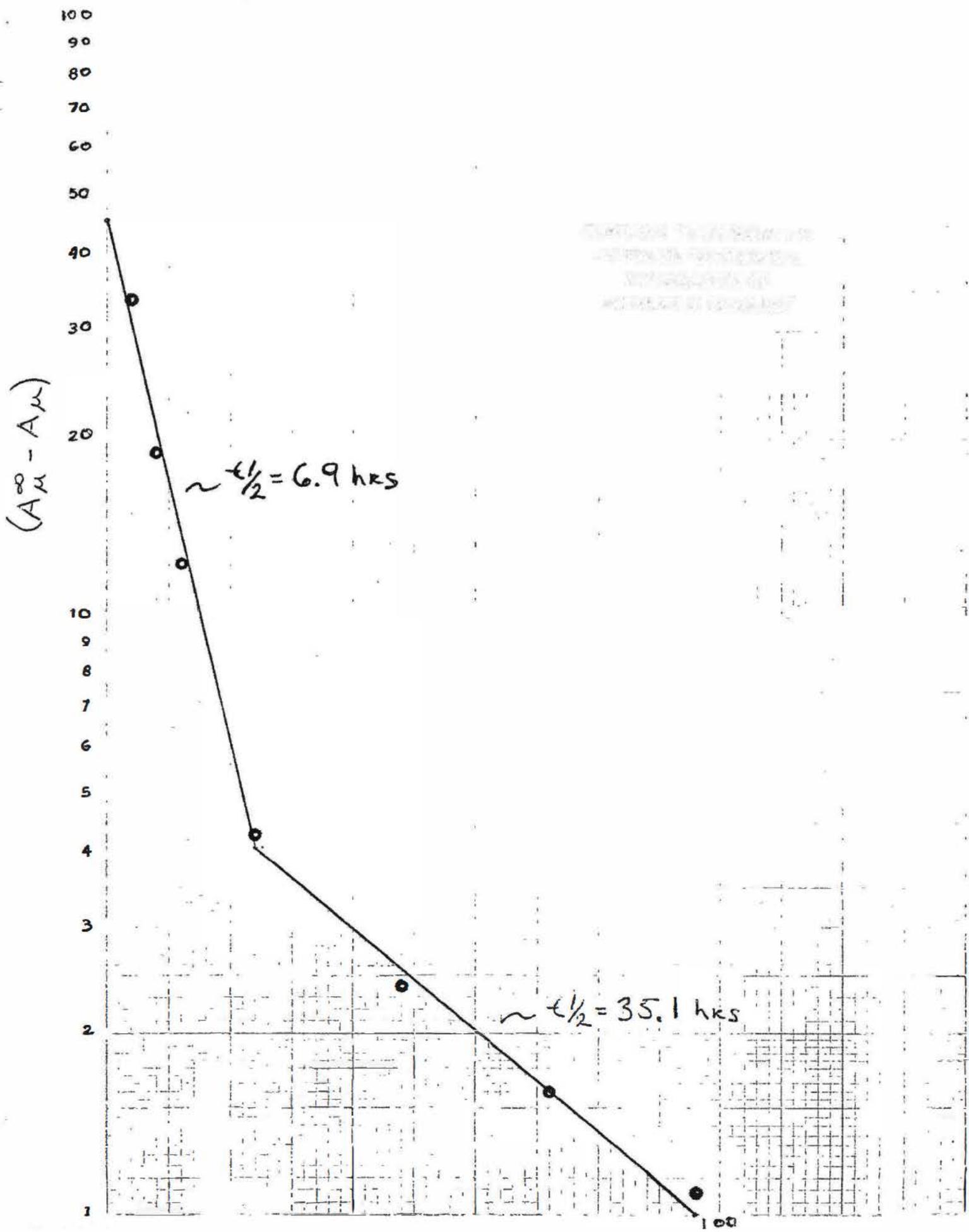


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glyphosate, IM, no Rx, deionized water,
thigh, monkey, urine, 3-30-82,



(3)



¹⁴C-Glyphosate: Urine Samples for Intramuscular Injection Into Thigh of Rhesus Monkeys;
Solvent Vehicle - Propylene Glycol (3-30-82)

t (hrs)	<u>0-4</u>	<u>4-8</u>	<u>8-12</u>	<u>12-24</u>	<u>24-48</u>	<u>48-72</u>	<u>72-96</u>	<u>96-120</u>	<u>120-144</u>	<u>144-168</u>	<u>Total %</u>
<u>Subject 1</u>											
DPM/5ml	13522.4	9216.0	7857.1	11432.1	433.2	249.1	82.2	71.4	67.4	58.0	
	13489.2	9316.2	7877.2	11514.5	438.5	242.6	80.6	68.3	66.4	55.5	
Total Vol.	245	218	277	224	797	738	939	653	860	790	
% Applied Dose	22.071	13.474	14.536	17.143	2.317	1.210	0.510	0.304	0.384	0.299	72.247
<u>Subject 2</u>											
DPM/5ml	59446.5	8412.8	3282.2	1109.2	264.6	104.2	109.0	57.6	52.7	46.6	
	59043.8	8390.5	3277.2	1119.5	244.6	107.1	106.0	58.5	54.1	46.0	
Total Vol.	161	287	263	507	831	826	628	809	1042	814	
% Applied Dose	63.624	16.084	5.753	3.769	1.411	0.582	0.450	0.313	0.371	0.251	92.608
<u>Subject 3</u>											
DPM/5ml	63604.72	3720.8	3524.2	1621.8	389.7	167.8	161.3	67.6	130.3	105.8	
	63441.92	3573.5	3502.6	1627.4	390.7	163.4	161.1	58.5	128.9	103.1	
Total Vol.	158	119	229	495	731	800	775	823	702	814	
% Applied Dose	66.947	18.770	5.367	5.364	1.903	0.884	0.833	0.346	0.607	0.567	101.587
<u>Subject 4</u>											
DPM/5ml	33194.3	7921.1	1228.9	2039.3	286.1	112.1	65.5	45.4	53.4	71.8	
	33079.1	7844.5	1259.5	2064.8	292.0	113.1	71.2	48.6	52.0	60.4	
Total Vol.	320	205	251	429	902	719	854	719	722	906	
% Applied Dose	70.729	10.779	2.075	5.872	1.739	0.540	0.389	0.225	0.254	0.339	93.002
<hr/>											
Av-(%)	55.843	14.777	6.933	8.037	1.842	0.804	0.546	0.297	0.404	0.379	89.861
Collect. Period	4	4	4	12	24	24	24	24	24	24	SD=
% /hour	13.961	3.694	1.733	0.670	0.077	0.033	0.023	0.012	0.017	0.016	12.5

(5)

Percutaneous Absorption of ¹⁴C - Glyphosate in ROUNDUP® Formulation
In Rhesus Monkeys Following A Single Topical Dose

STUDY NO. MA-81-349

Conducted For: Monsanto Company
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Date

Study Completed: 5/4/82
Date

Study Director: *Howard I. Maibach* 4-1-83
Howard I. Maibach, M.D. Date

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Percutaneous Absorption of Glyphosate in the Roundup Formulation

Introduction:

Carbon-14 labeled glyphosate in the Roundup formulation was applied to the abdomen of rhesus monkeys. Percutaneous absorption of the glyphosate was determined by measurement of the total amount of the C-14 label excreted into the urine.

Procedures:

Six male rhesus monkeys each received a single dose of 0.80 microcuries (as determined by scintillation counting) C-14 labeled glyphosate (specific activity of 19.4 uCi/mM, molecular weight of 169.1). Twenty-five microliters of the glyphosate preparation was applied over 7.9 square centimeters of abdomen lightly clipped of hair using an Oster clipper. After 24 hours, the site of application was washed two times with distilled water, two times with acetone, then two times with distilled water. The wash solvent was applied to a cotton ball attached to a pair of curved blunt forceps. The site of application was wiped with the solvent laden cotton ball. The amount of C-14 label from the wash was then determined by scintillation spectroscopy of the cotton ball. A C-14 internal standard was subsequently added to each sample to determine the extent of quenching.

Urine samples were collected at 4, 8 and 12 hours the first day, every 12 hours the second day, then every 24 hours for 7 days. A five ml aliquot of each urine sample was assayed in 14 ml PCS (Amersham Corp.) with a liquid scintillation spectrophotometer. A C-14 internal standard was added to a triplicate vial of each sample to determine the extent of quenching.

A sigma-minus analysis was performed to determine the C-14 elimination half-life. The differences between the total % dose excreted over all the collection intervals and the total % dose excreted up to the end of each collection interval are equivalent to the amount of the compound not yet excreted. A semilog plot of these values versus time yields a straight line with a slope proportional to the elimination half-life. The rate constant, k_e , is equivalent to the slope times 2.303 and the elimination half-life equal to 0.693 divided by k_e (Fundamentals of Clinical Pharmacokinetics, first edition; Wagner, J., pg 77).

(1)

Results:

The washing procedure removed 14.2% (standard deviation of 3.5%) of the applied C-14 label on the glyphosate.

The enclosed table lists the dpm values, the total volume of urine collected, and the calculated % of the applied dose excreted during each collection time interval for each subject. For example, under 0-4 hours for subject 1, 18.5 and 14.6 are the calculated dpm values, 340 is the volume of urine collected in milliliters and 0.071 is the calculated % of the applied dose excreted. On the bottom of each column the average % dose excreted, the number of hours in the collection interval, and the rate of excretion in % dose per hour are reported. The urine contained an average corrected total value of 1.8% (standard deviation of 0.6%) of the applied C-14 label on the glyphosate. Peak excretion occurred between 8-36 hours.

The total percent recovery (percent label removed by washing plus percent label contained in urine) was low, i.e., 16.0%. Although a definitive explanation can not be offered for the low recovery, previous experience suggests that much of the test material may in some way bind to or in the skin and can not be removed by washing. This bound material is not apparently available for systemic absorption.

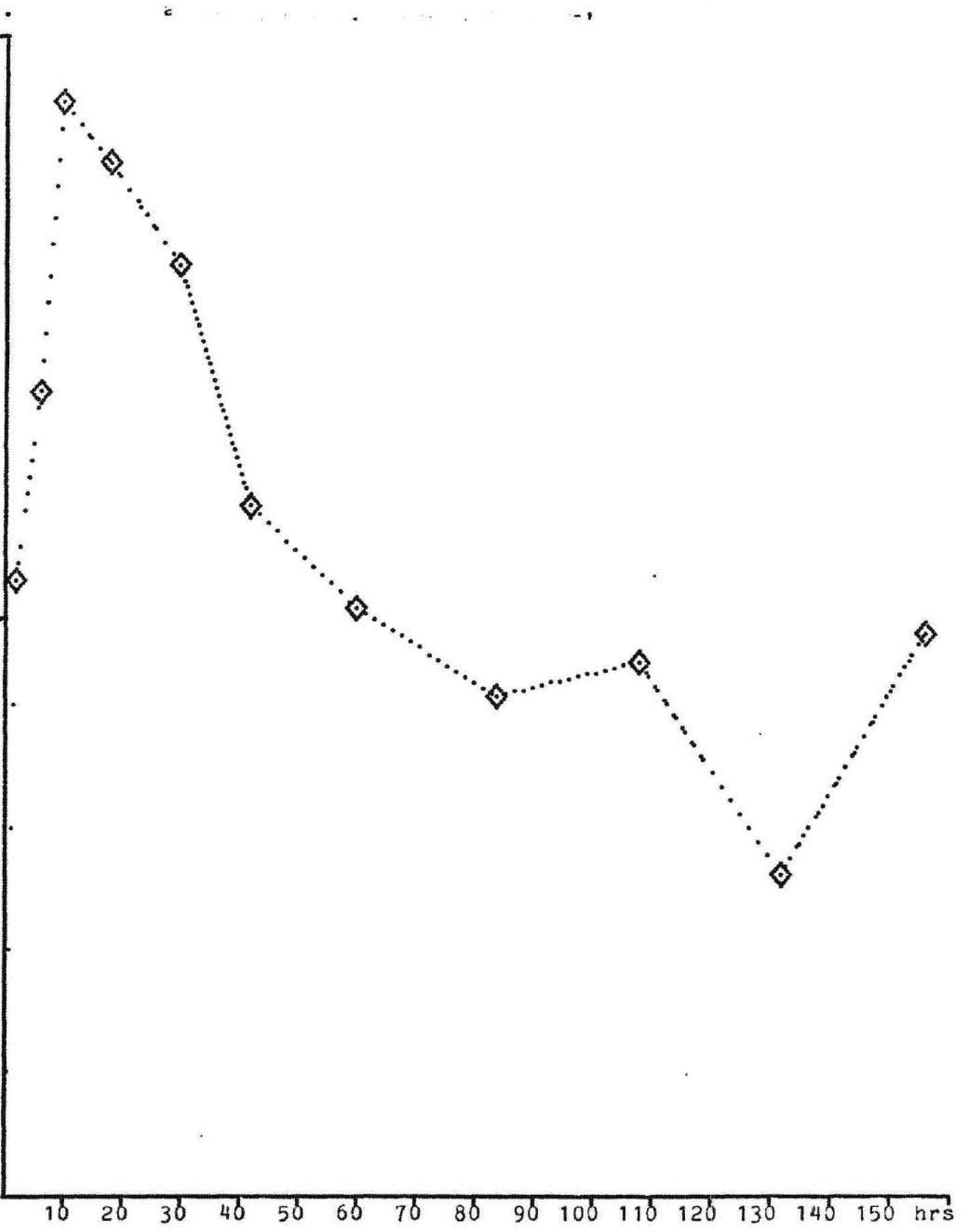
Three graphs are enclosed. The first is a plot of the % dose excreted per hour versus time. The second is a semilog plot of the % dose excreted per hour versus time. The third is a sigma-minus plot. The C-14 label on the glyphosate had an average elimination half-life of 59 hours.

All calculated excretion values reported in this study were corrected for incomplete urinary excretion with a parenteral excretion factor of 89.9%.

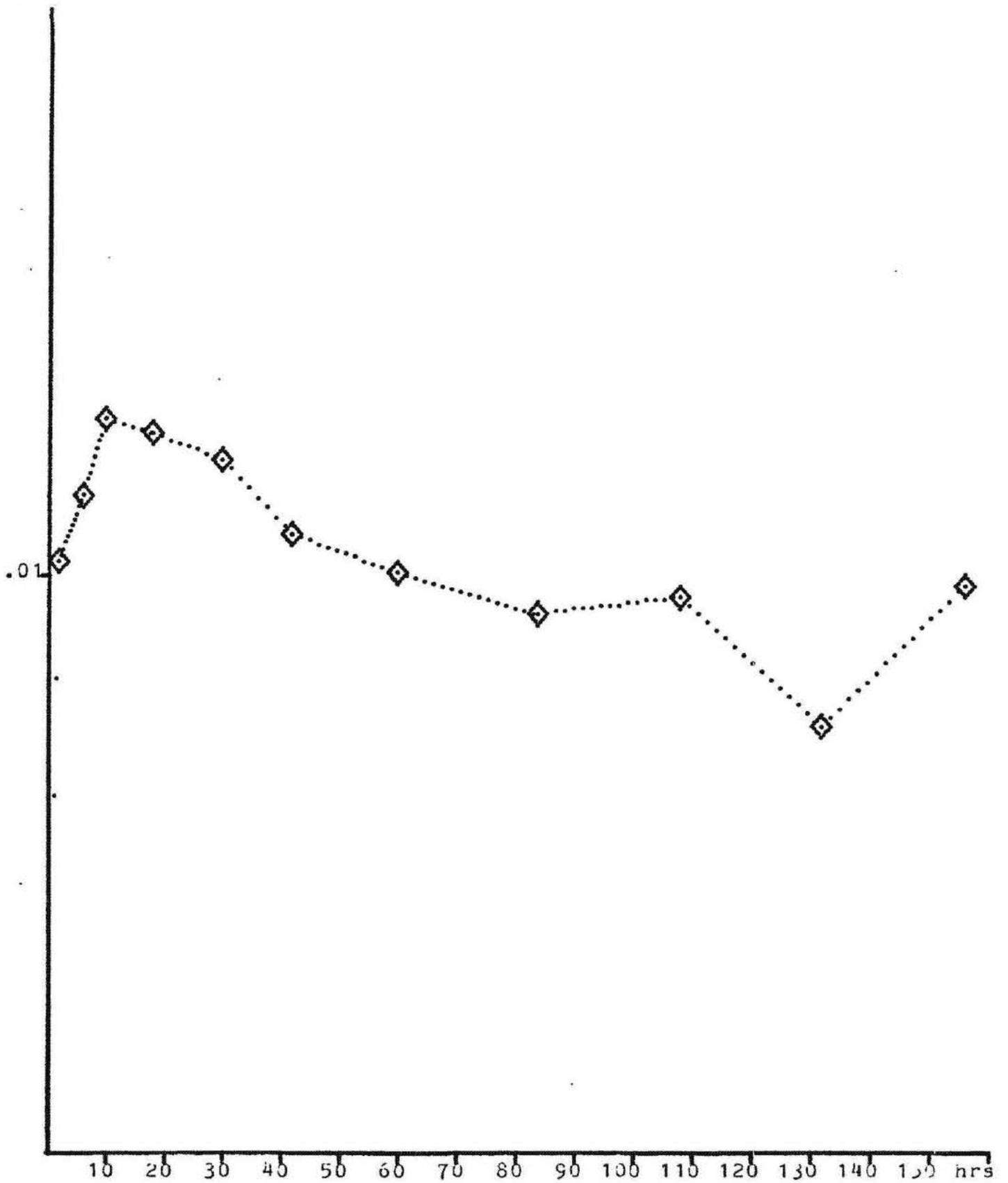
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(3)



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A - ΔH

