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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

014126

OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM April 3, 2000

SUBJECT CGA 329351 (Metalaxyl) Dermal absorption studies *in vivo* and in vitro

TO Thomas Ellwanger PM21
Fungacide Br
Registration Div (7505C)

4/13/2000

FROM Robert P Zendzian Ph.D.
Senior Pharmacologist
Science Evaluation Br
Health Effects Division (7509C)

THROUGH William Burnam
Chief
Science Analysis Br
Health Effects Division (7509C)

3/31/2000

DP Barcode # D264340 Case # 290692 Submission # S577268 Chemical # 113502
ID # 9F05044 Registrant Navartis MRID # none

Action Requested

Review the following studies;

Citation Dermal absorption of [phenyl-u-^{14}C] CGA 329351 formulated as Ridomil Gold EC (A-9408 B) in the rat. K.E. Mewes. Novartis, Study 034AM04. May 14, 1998. MRID 45085901

Core Classification Acceptable (not guideline)

Summary

12 male rats per dose were dosed at 0.094 or 4.7 mg/cm^2 CGA 329351 in the formulation (high dose) or a water dilution thereof. The application site on all rats was washed at 8 hours and 4 rats per dose were sacrificed at 8, 24 or 48 hours after start of dosing. Percent absorbed was 25.48, 35.33 and 34.77 for the low dose and 3.00, 9.28 and 16.49 for the high dose. The value of 35.33% absorbed should be used for converting oral to dermal doses for risk assessment. The

study is considered of minimal value and use of the data should not be unduly extended.

Citation The *in vitro* percutaneous absorption of [Phenyl-(U)-^{14}C] CGA 329351 formulated as Ridomil Gold 480 EC (A-9408B) through rat and human epidermis. K.E. Mewes. Novartis. Study 034AM05. May 28, 1998. MRID 450 85 902

Core Classification Unacceptable (invalid procedure)

Summary The Agency possesses sufficient information to conclude that this experimental *in vitro* procedure utilizing the 'isolated epidermal membrane' does not accurately determine *in vivo* dermal penetration of test chemicals. Results using this procedure have been shown to over or under estimate in vitro penetration in an inconsistent and unpredictable manner in relation to dose and duration of exposure. Further the data generated cannot be used to convert rat dermal penetration values to human values. This is clearly show by the data generated in this study. The relation between percent absorbed in rat and human varies with dose and duration of exposure in an inconsistent and unpredictable manner.

Attachment

DERs

Data Evaluation Report 014126

Chemical Metalaxyl, CGA 329351

Citation The *in vitro* percutaneous absorption of [Phenyl-(U)-^{14}C] CGA 329351 formulated as Ridomil Gold 480 EC (A-9408B) through rat and human epidermis. K.E. Mewes. Novartis. Study 034AM05. May 28, 1998. MRID 45085902

4/3/2002

Reviewed by Robert P. Zendzian PhD
Senior Pharmacologist

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Discussion

The study is summarized as follows from the report:

"The percutaneous penetration of the fungicide CGA 329351, I-e (R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester, formulated as 480 EC (A9408 B), was determined in vitro using epidermal membranes of rat and human origin.

The epidermal membranes were set up in flow through diffusion cells and the perfusates collected at defined time intervals. Three dose levels were used. The low dose Al reflects the highest concentration recommended for foliar application, a 0.2% dilution of formulation. The middle dose A2 reflects the highest concentration recommended for soil application, while the high dose A3 represents the exposure of skin to the undiluted formulation.

Dose Level	Species	Concentration [mg/cm^2]	Applied Dose [mg/cm^2]	Number of Replicates	Collection period[h]
low dose	rat	1.06	0.083	7	0-48
Al	human	1.06	0.083	6	0-48

middle dose	rat	9.7	0.76	7	0-48
A2	human	9.8	0.77	4	0-48
high dose	rat	512.8	40.1	6	0-48
A3	human	514.3	40.2	6	0-48"

The results are summarized in Table 13 from the report and are plotted in the graph 'Metalaxyl in vitro comparison'.

In vivo one can expect two relationships between dose and between species to occur. For any species as the dose increases from very small the flux will increase but not in proportion to the increase in dose. Thus the percent absorbed will decrease with increasing dose. This is because as dose increases the flux increases asymptotically to saturation at which point it no longer increases with increasing dose. Between species one can expect the flux at a common dose to be greater in the rat than in the human. Thus at the same dose the percent absorbed in the rat will be greater than the percent absorbed in the human. For the same chemical human dermal absorption is saturated at a lower dose than rat dermal absorption

This leads to a third conclusion, the difference in flux of a chemical between rat and human will vary with dose becoming a constant only at doses which saturate both species. As a percentage the difference will be largest at the smallest dose and decrease, with increasing doses, asymptotic with zero as saturation is exceeded.

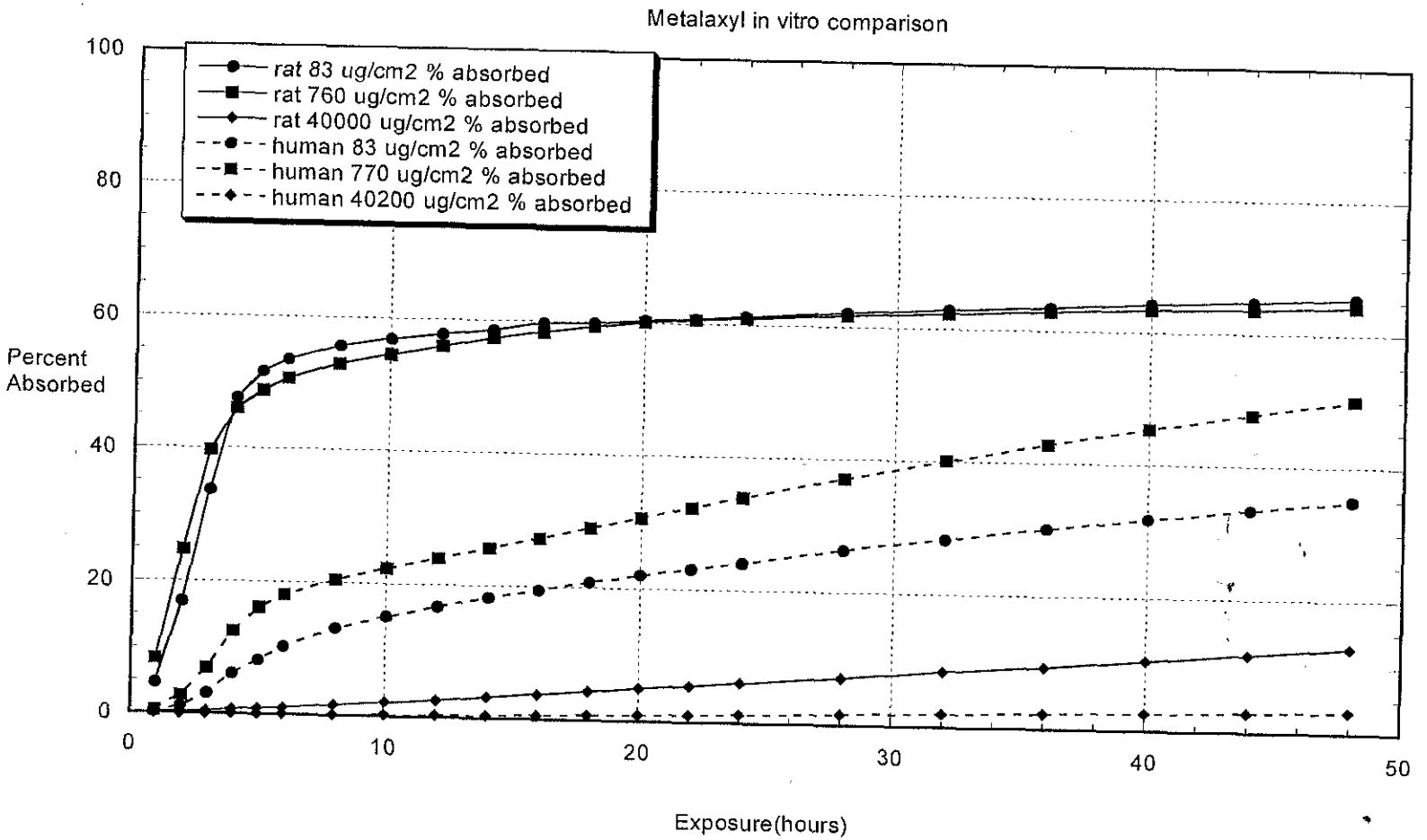
Now let us see what these data show. In this study the doses go up by factors of 10 and 50. In the rat the percent absorbed at 48 hours is essentially equal for doses of 83 and 760 ug/cm^2, a 10 fold increase in flux with a 10 fold increase in dose. The dose of 40000 ug/cm^2 is 50 fold higher, the percent absorbed at 48 hours decreases by a factor of 5 and the flux increases by a factor of 10. The first step is unexpected but possible the second step is expected.

In the human the picture is different. As the dose increases from 83 to 770 ug/cm^2 the percent absorbed at 48 hours increases 1.4 times and the flux increases 13 times. This pattern is unexpected and most likely in error. As the dose increases from 770 to 40200 ug/cm^2 the percent absorbed decreases and the flux increases as expected.

These data show a variable and inconsistent difference between rat and human flux at common doses and thus cannot be used to determine species related differences for risk assessment.

Table 13 Mean cumulative penetration of [Phenyl-(U)-^{14}C] CGA 329351 through rat and human epidermis (% of dose)

Cumulative penetration [% of dose]						
Species	rat			human		
Group	Q1			Q2		
Dose level	A1	A2	A3	A1	A2	A3
Applied Dose [mg·cm^{-2}]	0.083	0.76	40.1	0.083	0.77	40.2
Number of replicates	7	7	6	6	4	6
Time period						
0 - 1 h	4.66	8.29	0.16	0.10	0.44	< 0.01
0 - 2 h	17.03	24.87	0.30	1.06	2.72	0.02
0 - 3 h	33.92	39.91	0.47	3.16	7.01	0.04
0 - 4 h	47.78	46.23	0.69	6.15	12.60	0.06
0 - 5 h	51.88	49.03	0.88	8.28	16.33	0.08
0 - 6 h	53.84	50.88	1.10	10.41	18.22	0.11
0 - 8 h	55.90	53.22	1.58	13.30	20.66	0.17
0 - 10 h	57.10	54.73	2.10	15.19	22.46	0.24
0 - 12 h	58.02	56.15	2.64	16.83	24.14	0.34
0 - 14 h	58.82	57.52	3.24	18.31	25.79	0.46
0 - 16 h	59.51	58.65	3.80	19.66	27.44	0.61
0 - 18 h	60.07	59.57	4.41	20.94	29.11	0.77
0 - 20 h	60.61	60.38	5.00	22.15	30.80	0.95
0 - 22 h	61.09	60.88	5.51	23.29	32.54	1.13
0 - 24 h	61.53	61.27	6.04	24.26	34.19	1.30
0 - 28 h	62.42	62.04	7.20	26.55	37.36	1.68
0 - 32 h	63.17	62.61	8.38	28.50	40.32	2.01
0 - 36 h	63.87	63.22	9.38	30.25	43.11	2.35
0 - 40 h	64.56	63.78	10.56	31.93	45.75	2.67
0 - 44 h	65.24	64.23	11.73	33.51	48.13	2.97
0 - 48 h	65.79	64.51	12.84	35.16	50.33	3.26


Metalaxyl in vitro comparison
rat 83 ug/cm2 % absorbed
rat 760 ug/cm2 % absorbed
rat 40000 ug/cm2 % absorbed
human 83 ug/cm2 % absorbed
human 770 ug/cm2 % absorbed
human 40200 ug/cm2 % absorbed
Percent Absorbed
100
80
60
40
20
0
0
10
20
30
40
50
Exposure(hours)

014126

Data Evaluation Report

Chemical Metalaxyl, CGA 329351,

Citation Dermal absorption of [phenyl-u-^{14}C] CGA 329351 formulated as Ridomil Gold EC (A-9408 B) in the rat. K.E. Mewes. Novartis, Study 034AM04. May 14, 1998. MRID 45085901

4/3/2001

Reviewed by Robert P. Zendzian PhD
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Summary
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The following is abstracted from the report

3 Materials

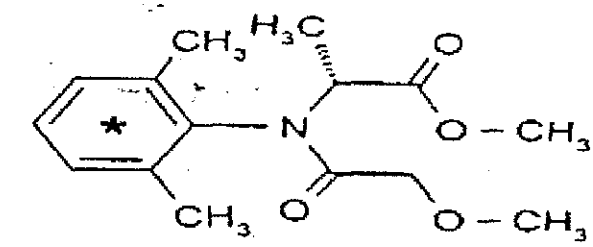
3.1 Test Substance

Company Code	CGA 329351	
Chemical *Name (APACE)*	(R)-2-[(2,6-dimethyl-phenyl)-methoxyacetylamino]-propionic acid methyl ester	
Chemical Name (CA)	N-(2,6-dimethylphenyl)-N- (methoxyacetyl)-D-alanine methyl ester	
CAVES Registry Number	70630-17-0	
Empirical Formula	$C_{15}H_{21}NO_4$	
Molecular Weight	279.3	
Appearance	pale yellow, clear, viscous liquid	
Vapor Pressure at 25°C	3.3Pa (extrapolated)	
Density at 20°C	1. 125 g/cm	
Solubility in water at 25 ° c	26 g/l	
Solubility in organic solvents at 25°C	methanol	completely miscible
	acetone	completely miscible
	toluene	completely miscible
	n-hexane	59 g/l

ethyl acetate	completely miscible
dichloromethane	completely miscible

Partition Coefficient log Pow = 1.71
*(n-octanol /*water) *at 20°C*

Structure / Label



* = [^{14}C]

3.1.1 Radiolabeled Test Substance

Name for the Label	[Phenyl-U-^{14}C]
Batch Number	ILS- 114.1
Specific Activity	1440 kBq/mg
Purity	97.3 %
Expiration date	April 15, 1998
Radiodilution	For the high dose administration (Group P2) this material was diluted with the non-radiolabeled test substance to a specific radioactivity of 21 kBq/mg,

3.1.2 Non-radiolabeled Test Substance

Batch Number	AMS 758/101
Purity	99.40%
Expiration Date	August 2000

3.1.3 Formulation

Dose Formulation	The test substance was formulated according to protocol A. 9408 B, The composition of A-9408 B is as follows:
active ingredient	CGA 329351 (Metalaxyl-M) 46.2 % w/w
formulation ingredients	33.8 %

For the low dose P1 the composition was: CGA 329351 31.4%, formulation ingredients 68.6%

3.2 Test System

3.2.1 Animals

Species	Rats
Strain	Tif RAI f (SPF)
Number, Sex	24, males
Source	Biological Research Laboratories (BRL), Fullinsdorf, Switzerland
Age and Body Weight	Male rats about 8 weeks of age were used in the experiment.

4 Methods

4.1 Animal Groups

Twelve rats each were dosed at a low dose (group P1) and a high dose level (group P2). The groups were further divided into subgroups, e.g. P1t1, P1t2 and P1t3 consisting of 4 animals each. Exposure time to the formulated test substance was 8 hours for all animals. Animals of subgroup tl were sacrificed at 8 hours directly after washing-off the test substance, animals of subgroup t2 were sacrificed at 24 hours and animals of subgroup t3 at 48 hours after treatment.

4.2 Formulation of [Phenyl-U-^{14}C] CGA 329351

[Phenyl-U-^{14}C] CGA 329351 and unlabeled CGA 329351 (only group P2) were mixed with the formulation ingredients (blank formulation). The formulation was prepared separately for dose level P1 and P2.

Preparation of the Stock Solution	[Phenyl-U-14C] CGA 329351 was dissolved in toluene/methanol 9:1 (v:v). 50 ul aliquots were transferred into three 10 ml volumetric flasks and filled to volume with toluene, 100 ul aliquots were taken in triplicate and assayed for the radiocarbon content by LSC. Based on these results the concentration of the stock solution was determined.
Formulation Group P1 (Low) dose)	A volume of the stock solution corresponding to 24.0 mg [Phenyl-U-14C] CGA 329351 was transferred into a conical vial (approx. volume 5 ml). The solvent was removed using a gentle stream of nitrogen gas. 52.4 mg of the blank formulation A-9408 B was added to the dried material and the flask was sonicated to yield a homogenous solution.
Formulation Group P2 (high dose)	1185.9 mg CGA 329351 was transferred into a conical vial (approx, volume 5 ml), A volume of the stock solution corresponding to 17.4 mg [Phenyl-U-14C] CGA 329351 was added to the unlabeled CGA 329351. The solvent was removed using a

gentle stream of nitrogen gas.
1397.4 mg of the blank formulation A-9408 B was added to the vial and the flask was sonicated to yield a homogenous solution.

4.3 Preparation of the Application Solution

Dilution of the Application solution: On the day of application the formulated test substance for Group P1 was diluted with 2450 ul water to yield the application solution for the low dose.
The undiluted formulation served as the high dose.

4.4 Stability of the Application Solution

The purity of the formulated test substance mixed with water at the time of application was checked by TLC using solvent system SS2 and SS3.

4.5 Application of the Test Substance

Shaving: The day prior to application a dorsal area of about 15 cm^2 was shaved with an electric clipper taking care not to abrade the skin,

Anesthesia: Anesthesia was induced with isofluranel (5% v/v) and maintained at an isoflurane concentration of (1.5% v/v) during dermal application and removal of the unabsorbed dose.

Dose Level: Rats were dosed at two dose levels:
Group P1 0.094 mg/cm^2
Group P2 4.66 mg/ cm^2

Application: Prior to dosing, a non-absorbing 'O'-ring (Normatec, diameter: 36 mm) with an inside area of approximately 10 $cm^2$2 was glued to the shaved skin using cyanoacrylate adhesive (Patex Supergel). The application solution (100 ul) was applied to the skin inside the 'O'-ring using a Hamilton syringe and spread evenly. In order to prevent uncontrolled loss of the test substance the 'O'-ring was covered with a permeable tape (Flawa fix; non-occlusive conditions). Ingestion of the test substance was prevented by a collar around the rat's neck.

Removal of the unabsorbed Dose: After an exposure time of 8 h the cover was removed and retained for analysis, The unabsorbed test substance was removed from the application site by washing (at least 3 times) with a mild soap solution (Lux Duschgel, pH 5.3) using cotton swabs. The moist skin area was dried with cotton swabs and a fresh cover tape was applied to the 'O'- ring.

4.6 Specimen Collection

The specimens were collected at the following time points. All volumes or weights were recorded:

Excreta	
Urine	0 - 8 (tl, t2, t3), 8 - 24 (t2, t3), 24 - 48 h (t3) after application (individually and separately collected)
Feces	0 - 8 (tl, t2, t3), 8 - 24 (t2, t3), 24 - 48 h (t3) after application (individually and separately collected)
Application site	
Skin wash	8 hours after application (tl, t2, t3),
'O'-ring + cover	after sacrifice, at 8 h (tl), 24 h (t2) and 48 h (t3)
Skin treated area	after sacrifice, at 8 h (t1), 24 h (t2) and 48 h (t3)
Blood and Tissues	At the defined time points (i.e. 8h, 24h, and 48 hours after application) the animals were sacrificed by exsanguination after anesthesia with carbon dioxide in a desiccator. The following specimens were retained for analysis:
Blood	was taken from four animals from subgroup t3 at time points as follows: 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h after application. Serial blood specimens were taken from the tail vein (vena sacralls media) by cutting the tip of the tail.
Whole blood and plasma	after sacrifice, at 8 h (t1), 24 h (t2) and 48 h (t3) At sacrifice terminal blood from each animal was collected into heparinized tubes. After taking aliquots of whole blood, plasma was separated by centrifugation.
Skin (non-treated area)	after sacrifice, at 8 h (tl), 24 h (t2) and 48 h (t3) A small piece of non-treated skin was excised from the shaved area in some distance from the application site.
Carcass	The residual carcasses after sacrifice, at 8 h (tl), 24 h (t2) and 48 h (t3) were retained.
Cage Wash	At the end of the collection period the cages were rinsed thoroughly with water/ethanol (1:1 v/v).

Results are summarized in the following tables from the report.

7.3.1 Blood residue levels

The time course of radioactivity concentration in blood was determined at both dose levels P1 and P2 in the four animals of subgroup t3 and is presented in the summary table below and in Figure 2 and Figure 3. Individual data are presented in Table 3 and Table 4.

Blood kinetics (ppm CGA 329351 equivalents) - subgroup t3 (48 h)		
time (h)	P1 Low dose (0.094 mg/cm^{-2})	P2 High dose (4.7 mg/cm^{-2})
0.5	0.0303	< LQ
1	0.0567	< LQ
2	0.0346	0.414
4	0.0237	< LQ
6	0.0294	0.414
8	0.0356	0.444
12	0.0443	0.938
24	0.0261	1.501
48	0.0099	0.419

7.3.2 Absorption and Excretion

A summary of the absorption data is presented in the following table. All individual data are shown in Table 5 to Table 10.

SUMMARY TABLE : Values in % of applied dose						
Group	P1			P2		
Dose	Low dose (0.094 mg/cm^2)			High dose (4.7 mg/cm^2)		
Subgroup	t1 (8 h)	t2 (24h)	t3 (48 h)	t1 (8 h)	t2 (24h)	t3 (48 h)
Urine	2.31	11.85	13.56	0.33	2.82	6.45
Feces	0.07	9.13	16.00	< 0.01	2.32	6.35
Cage wash	0.32	0.69	0.47	0.04	0.18	0.66
Control skin and blood	0.04	0.04	0.01	< 0.01	0.01	0.07
Residual carcass	23.10	13.62	4.72	2.62	3.94	2.96
Systemic Absorption	**25.84**	**35.33**	**34.77**	**3.00**	**9.28**	**16.49**
Treated Skin	**20.49**	**8.52**	**5.53**	**15.14**	**10.18**	**10.72**
Dislodged dose	**60.24**	**55.81**	**54.58**	**82.10**	**81.14**	**70.93**
Recovery	**106.56**	**99.66**	**94.88**	**100.23**	**100.59**	**98.13**

7.3.3 Terminal Blood Residues

The terminal blood and plasma residues are summarized in the following table. Individual data are presented in Table 11 to Table 16.

SUMMARY TABLE : Terminal blood and plasma residues expressed as ppm CGA 329351 equivalents						
Group	P1			P2		
Dose	Low dose (0.094 mg/cm^2)			High dose (4.7 mg/cm^2)		
Subgroup	t1 (8 h)	t2 (24h)	t3 (48 h)	t1 (8 h)	t2 (24h)	t3 (48 h)
Blood	0.0314	0.0302	0.0117	0.300	0.425	0.323
Plasma	0.0423	0.0330	0.0075	0.386	0.481	0.257

Discussion

This study, although rated acceptable, is not considered guideline and is of minimal value for assessing the dermal penetration of the test chemical. The information provided may serve as a crude upper limit for risk assessment. The rational for dose selection appears to be acceptable but that for exposure duration is faulty. Unlike laboratory technicians farm workers do not work 8 hour days. Experience has shown that the maximum work day for farm labor is in the order of 10 hours from putting on ones dirty overalls to taking then off and washing off the residual pesticide.

In general the data conform to the expected pattern, percent absorbed decreases with increasing dose and increases with increasing duration following skin wash. This latter shows that the residue material in the washed skin continues to be absorbed for up to 48 hours. The apparent failure of this pattern at P1t3 is most likely to be due to the missing 7% of the dose.

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003142

Chemical:	**(R)-2{(2,6-dimethylphenyl)-methoxyacetyl**
PC Code:	**113502**
HED File Code	**13000 Tox Reviews**
Memo Date:	**04/03/2000**
File ID:	**TX014126**
Accession Number:	**412-01-0121**

HED Records Reference Center
02/12/2001