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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: EPA ID # 007969-00053. Vinclozolin; Review of a Report of Penetration Through Human and Rat Skin *In Vitro* (MRID# 424831-03).

Tox. Chem. No.: 323C.
Shaughnessy No.: 113201.
Submission No.: S419316.
Barcode: 183806.
Case: 037677.
Company No.: 007969.
Action: 320.

From: David G Anderson, PhD. David G Anderson 3/31/92
Toxicologist, Section 3,
Toxicology Branch-1
Health Effects Division (H7509C)

To: S Lewis/R Rose, PM 21
Fungicide and Herbicide Section
Registration Division (H7505C)

KB 4/20/93

Thru: Karen Hamernik, PhD.
Acting Section 3 Head, K. Hamernik 4/5/93
Toxicology Branch I
Health Effects Division (H7509C).

CONCLUSIONS:

In vitro dermal penetration of [C^{14}phenyl]vinclozolin was studied. The study used post-mortem human male and female (mean = 65 years of age) abdominal skin (17 and 16 samples at low and high dose levels, respectively) and Sprague Dawley 26 day old female rat skin (17 and 16 rats at low and high dose levels, respectively). The concentrations studied were about 2.0 $\mu g/cm^2$ for the low dose level and about 200 $\mu g/cm^2$ for the high dose level. The study adds support to the concept that rat skin is more permeable to vinclozolin than human skin; rat skin was 2.3 to 16.7 times more permeable than human skin by this *in vitro* method.

Core classification: Acceptable for a non-guideline study on the comparison of skin penetration *in vitro*.

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REQESTED ACTION:

RD requested that the Toxicology Branch-1 review the following submitted report: *In vitro* Percutaneous Absorption of [C^{14}]vinclozolin, A Comparison Using Rat and Human Epidermis Plus Summary (MRID# 424831-03). The report was initially submitted to the OREB of HED who suggested that the report should be reviewed by toxicology.

Primary reviewer: David G Anderson, PhD. David G Anderson 4/5/93
Section 3, Tox. Branch 1 (H7509C).
Secondary reviewer: Karen Hamernik, PhD. K. Hamernik 4/5/93
Section 3, Tox. Branch-1 (H7509C).

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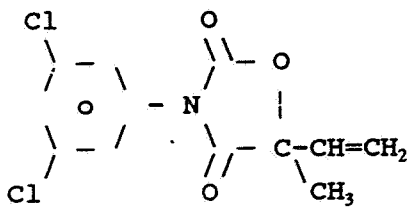
DATA EVALUATION REPORT

STUDY TYPE: Special Study: *In Vitro* Percutaneous Absorption of Vinclozolin/Human and Rat Skin.

Shaughnessy No.: 113201.
TOX. CHEM. No.: 323C
DP Barcode No.: D183806.
Submission No.: S419316.
MRID No.: 424831-03.
Action: 320.

TEST MATERIAL: 83 258, Vinclozolin, technical; A.I. is [3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedi-2,4-one] and ^{14}C-vinclozolin.

STRUCTURE:



SYNONYMS: Ronilan™.

SPONSOR: BASF Aktiengesellschaft, Department of Toxicology ZST/E-Z470 D W6700 Ludwigshafen, Germany.

TESTING FACILITY: Inveresk Research International Tranent, EH33 2NE, Scotland.

STUDY NO.: IRI 150582; BASF 92/10221 and 92/10222.

REPORT TITLE: *In Vitro* Percutaneous Absorption of C^{14}-Reg No. 83258 (Vinclozolin). A Comparison Using Rat and Human Epidermis Plus Summary.

AUTHOR(S): B.D. Cameron, L. Jack and Dr. Ben van Ravenzwaay.

REPORT ISSUED: February 7, 1992.

CONCLUSIONS: *In vitro* penetration of [C^{14}phenyl]vinclozolin was studied through human males and female (mean = 65 ± 12 years of age) abdominal skin (17 and 16 individuals at low and high dose levels, respectively) and female (26 days old) Sprague Dawley rat skin (17 and 16 individuals at low and high dose levels, respectively) at low dose levels of about 2.0 $\mu g/cm^2$ and at high dose levels of about 200 $\mu g/cm^2$. The study adds support to the concept that rat skin is more permeable to vinclozolin than human skin. Rat skin was 2.3 to 16.7 times more permeable than human

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skin by this *in vitro* method.

Core classification: Acceptable for a non-guideline study on the comparison of skin penetration *in vitro*.

A. MATERIALS:

1. Test compound: Vinclozolin, purity was unspecified (other studies have indicated > 99%) and [C^{14}phenyl]-radio-labeled vinclozolin purity was > 97%; specific activity was 7.56 MBq./mg. Solubility in water was approximately 10 micromoles/liter (2.9 mg/liter). The following data were extracted from the 1989 Merck Index: Melting point 108°C. Slowly hydrolyzed in dilute alkaline solution (solubility in water stated to be 1 g/liter; may be in error). Mole weight is 289 g.

2. Test Skins: Species: Abdominal post-mortem full thickness human males and females (mean = 65 ± 12 years of age)(17 samples were tested for the low dose and 16 samples were tested for the high dose). Skin samples were obtained from 3 men for the low dose (ages were 61 to 70 years of age and from 3 men for the high dose with ages from 49 to 62 years. Skin samples were obtained from 4 females for the low dose with ages from 35 to 77 years and 5 for the high dose with ages 62 to 77 years of age) and 26 day old female Sprague Dawley rat dorsal skin obtained from Charles River (17 for the low dose and 16 for the high dose).

3. Environmental: Laboratory conditions.

4. Food and Water: NA.

B. STUDY METHOD:

Skin Samples - Full thickness abdominal human male and female (mean = 65 ± 12 years of age) and dorsal female Sprague Dawley rat skin (26 days old) were removed and trimmed of fat, examined for damage and stored in aluminum foil at -20°C prior to use. To remove the epidermis, human skin was soaked for 1 minute at 60°C and floated on water and rat skin was soaked in 2M sodium bromide for approximately 24 hours, blotted dry and floated on water. The skin was used immediately or stored at 4°C for a maximum of 7 days. The integrity of the skin sample was determined by determining the tritiated water penetration through the skin into 0.9% NaCl for 6 hours, Kp < 1.5×10^{-3} cm/hour for human skin and Kp < 2.5×10^{-3} cm/hour for rat skin. The skin samples were left in the cell overnight between the tritiated water test and the test with [C^{14}phenyl]-vinclozolin.

The effect of the 2M sodium bromide soaking for 24 hours on human skin was compared with untreated human skin.

Skin samples were solubilized in methanolic sodium

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hydroxide.

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Dose preparation - Two dose levels were used, approximately 2 $\mu g/cm^2$ and 200 $\mu g/cm^2$. The concentrations used were reported to 4 significant figures. The concentrations of the low dose ranged from 1.944 to 2.419 or a mean of 2.234 $\mu g/cm^2$ for the tests with the human skin and 1.944 to 2.556 or a mean of 2.239 $\mu g/cm^2$ for the tests with the rat skin; at the high dose concentrations ranged from 191.9 to 203.0 or a mean of 196.3 $\mu g/cm^2$ for the tests with human skin and 191.9 to 203.0 or a mean of 195.4 $\mu g/cm^2$ for tests with rat skin. Each preparation was counted and amount of radiolabel calculated from the specific activity. The amount of label and cold vinclozolin mixture used was 0.64 and 64 μg in 5 μl of ethanol, which was added to 5 μl of water on the 0.32 cm^2 of exposed sample skin. The added solution was allowed to evaporate from the skin sample.

Absorption Cells - The temperature of the adsorption cells were controled at ≈ 30°C. The receptor fluid (ethanol:water = 50:50 v/v) was delivered by peristaltic pump at a rate of 1.5 ml/hour from the cells into scintillation vials over 24 hours as follows:

0.5 hour fractions from 0 to 8 hours post dose
2.0 hours fractions from 8 to 24 hours post dose.

At 24 hours, the skin surface was washed twice with the 50% ethanol to remove unabsorbed material and counted.

C. RESULTS:

The percentage of the dose absorbed and rate of penetration are summarized in Table A below. The ratio of rat absorption to human absorption at 8 and 24 hours and the retained label in the skin at 24 hours at the low and high dose levels were deterimined.

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Table A.
Summary of percent absorption of the low and high dose applied and maximum absorption rates found in human and rat skin. Coefficient of variation (CV) was calculated from the standard deviations of data presented.

	Low dose ≈2.0 μg/cm²			High dose ≈200 μg/cm²		
	Human	Rat	Ratio*	Human	Rat	Ratio*
8 hr absorp., % dose CV (%)	16.4 95	69.4 21	4.2	1.18 70	19.7 56	16.7
24 hr absorp., % dose CV (%)	28.0 77	74.0 13	2.6	2.25 62	35.4 54	15.7
Washed solubilized skin after 24 hours CV (%)	30.9 59	15.1 73	0.49	56.4 18.6	22.2 63	0.39
Max. absorption[a] rate (ng or μg/cm²/hr) CV (%)	162 107	689 38	4.3	0.98 92	10.9 54	11.1
Rate absorption[b] rate (ng or μg/cm²/hr) CV (%)	154.8 32	360.2 3.8	2.3	2.30 NA	15.2 28	6.6

*Ratio = Rat/Human.
[a] = Absorption rates comparing human skin soaked 1 minute in water at 60°C and rat skin soaked in 2M NaBr for 24 hours: ng/cm²/hr at the low dose, μg/cm²/hr at the high dose.
[b] = Absorption rates comparing human and rat skin soaked in 2M NaBr for 24 hours: ng/cm²/hr at the low dose; μg/cm²/hr at the high dose.
NA = data not available because insufficient data points were available.

Rat skin was completely permeable to 69.4/16.4 = 4.2 times the amount of vinclozolin to that of human skin at the low dose of ≈2.0 μg/cm² and 19.7/1.18 = 16.7 times the amount at the high dose of ≈200 μg/cm² in 8 hours. Rat skin was completely permeable to 74/28.0 = 2.6 times to amount of vinclozolin to that of human skin at the low dose of ≈2.0 μg/cm² and 35.4/2.25 = 15.7 times the amount at the high dose of ≈200 μg/cm² in 24 hours.

Similarly the amount remaining in the skin at 24 hours for rat skin was 15.1/30.9 = 0.49 times more vinclozolin at ≈2.0 μg/cm² and 22.2/56.4 = 0.39 times more than human skin at ≈200 μg/cm². The washed human skin after 24 hours contained 30.9% of 2.23 μg/cm² and 56.4% of 196 μg/cm² of the applied dose. The washed rat skin after 24 hours contained 15.1% of 2.24 μg/cm² and 22.2% of the 195 μg/cm² dose level.

Accumulated absorption of the labeled dose was 16.4% of 2.23 μg/cm² and 1.18% of 196 μg/cm² at 8 hours through human skin and 69.4% of 2.24 μg/cm² and 19.6% of 195 μg/cm² at 8 hours through rat skin. Accumulated absorption of the labeled dose was 28.0% of 2.23 μg/cm² and 2.25% of 196 μg/cm² at 24 hours through human skin and 74% of 2.24 μg/cm² and 35.4% of 195 μg/cm² at 24 hours through rat skin. The data in the previous paragraphs were extracted from Table 1 and 2, reproduced in the Appendix from the submitted report.

The standard deviation of the results in the various *in vitro* experiments was large, shown by coefficients of variation

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(CVs) of around 100% for the human and around 50% for the rat experiments (See Table A).

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D. DISCUSSION:

The *in vitro* penetration of radio-labeled vinclozolin through human abdominal epidermis and rat dorsal epidermis was studied at a low dose of ≈2.0 $\mu g/cm^2$ and a high dose of ≈200 $\mu g/cm^2$ every 0.5 hour for 8 hours and every 2 hours from 8 to 24 hours. After applying the radio-labeled vinclozolin samples in 50% ethanol, the solvent was allowed to evaporate. Accumulated absorption of the labeled dose through human skin was 16.4% of 2.23 $\mu g/cm^2$ and 1.18% of 196 $\mu g/cm^2$ at 8 hours and through rat skin 69.4% of 2.24 $\mu g/cm^2$ and 19.6% of 195 $\mu g/cm^2$ at 8 hours. After 24 hours, the washed solubilized human skin contained 30.9% of the low dose and 56.4% of the high dose; the washed and solubilized rat skin contained 15.1% of the low dose and 22.2% of the high dose.

The ≈2.0 and ≈200 $\mu g/cm^2$ concentrations covered the range of concentrations used in the *in vivo* dermal penetration study conducted previously in rats (MRID# 418243-09). In this latter study, 13.3% of 0.002 mg/cm^2 was absorbed in 10 hours and 11.9% was retained by the skin at 10 hours. At 0.200 mg/cm^2, 0.51% was absorbed and 5.65% was retained in the washed skin at 10 hours. Since the rat skin in the in vivo study was washed after the 10 hour absorption period, the retained radioactivity in the rat skin at 24 hours can not be compared with the rat skin washed after 24 hours in the *in vitro* experiment.

There are several variables that may reduce the applicability of the *in vitro* data to the relative *in vivo* dermal penetration of vinclozolin in test rats and humans. The vehicle used in the *in vivo* rat dermal developmental toxicity study (MRID# 414130-01) and the rat dermal penetration study (MRID# 418243-09) was 1% carboxymethylcellulose whereas 50% ethanol was used in the *in vitro* studies and human workers are exposed to vinclozolin in [redacted] agents. The young female rat skin (26 days) may have been less than optimally comparable with the human skin (the male and female age had a mean of 65 ± 12 years with a range of 35 to 77 years). The rat skin gave less variable results than the human skin, probably because of smaller variation in the skin samples. The experimental data on the skin from the 35 year old female demonstrated more permeability than most of the specimens from older humans, however, dermal penetration was not as high in the specimen from the 35 year old human as it was in several specimens from 60-70 year old humans and the 49 year old male was similar to the specimens from the 60-70 year old humans. Thus, it is not clear from these data that age of the source of the skin affected the results.

Marzulli and Maibach (1983) indicate that female rat skin is more permeable than male rat skin. The permeability of very young skin relative to the skin from old subjects may be comparable in some cases, but may differ in others. Also there is a large difference in the permeability of skin from various

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areas of the human body.

The *in vitro* rat dermal penetration can not be compared with the *in vivo* rat dermal penetration because of many of the above considerations. Chemicals tested by these experiments are frequently not comparable. However, the *in vitro* study on vinclozolin supports the generally held view that rat skin is 2 to 5 times more permeable to substances than the human skin. The study supports the concept that vinclozolin could be expected to penetrate rat skin more readily than human skin. The difference in human skin permeability could be a factor 1/2.3 times less permeable or as much as a factor of 1/16.7 times less permeable. This was a well conducted study where the details and precision reported and the comparison of the quality of the skin preparations added credibility to the results.

E. REFERENCES:

1. Marzulli, F.N. and Maibach, H.I. Dermatotoxicology, 2nd ed., Hemispher Publishing Corp., N.Y., 1983.

F. APPENDIX: Tables 1 and 2, copied from the submitted report.

VINCLOZALIN Sh # 113201

Page_____ is not included in this copy.

Pages 9 through 10 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

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