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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: ID# 010352-00021. Glutaraldehyde, Technical. Evaluation of an *in vitro* dermal penetration study (not submitted to fulfill data requirements for registration of glutaraldehyde).

Tox. Chem. No.: 468
PC No.: 043901
Project No.: 1-1524

FROM: Linnea J. Hansen, Ph.D. 12/4/91
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Health Effects Division (H7509C)

TO: John Lee, PM Team 31 Manager
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THRU: Marion P. Copley, D.V.M., D.A.B.T. 12/4/91
Section Head, Section IV, Tox. Branch I
Health Effects Division (H7509C)

CONCLUSIONS:

TB-I has reviewed the *in vitro* dermal penetration study submitted by Union Carbide Corporation (MRID No. 418902-01).

Under the conditions of this study, ^{14}C-glutaraldehyde at concentrations of 0.75% and 7.5% penetrated excised skin preparations from Fischer 344 rat, CD-1® mouse, New Zealand White rabbit, Hartley guinea pig or human female only slightly during a 6 hr exposure. Dose absorbed/surface area ranged from 1.0 - 160 ug/cm^2 and percent dose recoveries in media effluents ranged from 0.05% in female rats to 1.73% in male mouse at 0.75%. Dose absorbed/cm^2 was higher at the higher dose but % recovered was similar between the two doses. Rat and human female skin showed the least amount of dermal penetration; mouse and rabbit the highest. All species (except humans, no male skin sample) showed sex-related differential penetration: at 0.75% glutaraldehyde, males tended to absorb more compound than females, while females absorbed more than males at 7.5%.

In vitro dermal penetration studies cannot be used to satisfy

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guideline requirements and this study therefore is not acceptable for regulatory purposes. However, it was submitted voluntarily and was not requested by EPA as part of guideline requirements for registration of glutaraldehyde. It is considered as additional information on glutaraldehyde. At this time, the toxicological data on glutaraldehyde does not suggest the need to conduct dermal penetration studies; however, this is subject to change should future studies indicate a significant hazard.

Core-classification - Supplementary

ACTION REQUESTED:

On May 7, 1991, Union Carbide Chemicals and Plastics Company Inc., Specialty Chemicals Division submitted for review an *in vitro* skin penetration study of glutaraldehyde in 5 species including human. The study was not requested by EPA and was submitted as additional data. It is not a required study for registration of glutaraldehyde.

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DATA EVALUATION REPORT

STUDY TYPE: Dermal Penetration, *in vitro* (85-2) TOX. CHEM NO: 468

MRID NO.: 418902-01 PC NO.: 043901

TEST MATERIAL: Glutaraldehyde

SYNONYMS: 1, 5-pentanedial, Ucarcide 250®. CAS# 111-30-8

STUDY NUMBER: 53-15

SPONSOR: Union Carbide Chemicals and Plastics Company, Specialty Chemicals Division, Danbury, CT 06817

TESTING FACILITY: Bushy Run Research Center, Export, PA 15632

TITLE OF REPORT: Glutaraldehyde: Species Comparisons of *In Vitro* Skin Penetration Following a Single Application to the Excised Skin of Humans, Fischer 344 Rats, CD-1® Mice, Hartley Guinea Pigs, and New Zealand White Rabbits

AUTHORS: M.J. Tallant, J.L. Beskitt, S.W. Frantz

REPORT ISSUED: April 2, 1991

CONCLUSION:

Doses administered: 0.75% and 7.5% ^{14}C-glutaraldehyde, aqueous; 250 ul (5-10 uCi) applied to 1.77 cm^2 skin preparations *in vitro* for 6 hr. Skin taken from male and female Fischer 344 rat, CD-1® mouse, New Zealand White rabbit, Hartley guinea pig and human (breast, female only).

Glutaraldehyde showed very slight penetration of skin in all species tested under the conditions of this study: percent of total radioactivity recovered in media effluent ranged from 0.05% (female rat, 0.75% glutaraldehyde) to 1.73% (male mouse, 0.75% glutaraldehyde) and dose absorbed/cm^2 ranged from 1 ug/cm^2 (male and female rat, 0.75% glutaraldehyde) to 160 ug/cm^2 (female rabbit, 7.5% glutaraldehyde). Human skin preparations absorbed 2 and 20 ug/cm^2 at 0.75% and 7.5%, respectively. Human and rat skin showed the least amount of penetration and rabbit and mouse skin the greatest. Males of all species tended to absorb more compound than females at

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0.75% but this was reversed at high dose. Dose absorbed/cm^2 was higher at 7.5% than 0.75% but the difference was not necessarily proportional (range 2x to 50x increase at 7.5%).

Core Classification: Supplementary

A signed Quality Assurance Statement was present.

EXPERIMENTAL DESIGN:

Discs of skin were prepared from the experimental animals and from human breast skin removed during surgery and placed into skin penetration chambers. Skin samples were equilibrated with MEM culture medium (dermal side), then treated with 0.75% and 7.5% (w/w) glutaraldehyde ~~containing~~ on the epidermal surface. Amount of test substance that penetrated skin was determined from the cumulative percent absorbed radioactivity (^{14}C-glutaraldehyde recovered in media effluent). ^{14}C-ethanol was used as a positive control for dermal penetration.

MATERIALS:

1. Test compound: Unlabelled: Glutaraldehyde (Union Carbide Corp.). Colorless, non-viscous liquid, strong odor, Batch # IS-455-245, Purity - 50% (w/w) aqueous solution, <1% contaminants. Labelled: [1,5-^{14}C]-glutaraldehyde (Dupont NEN, Boston, MA). Lot # 2534-069, assay # 88-19720, Purity - >99%, Specific activity - 10.50 mCi/mmol.

2. Control compound: Ethanol-1-^{14}C (Sigma Chemical, St. Louis), Lot no. 058F9218, Specific activity - 9.5 mCi/mmol, Purity- >99%.

3. Test animals:

a) Species: Rat, Strain: Fischer 344, Age: 10-12 wks, Weight: not specified, Source: Harlan Sprague Dawley, Indianapolis, IN.

b) Species: Mouse, Strain: CD-1®, Age: 5 - 7 weeks, Weight: not specified, Source: Charles River, Kingston, NY

c) Species: Guinea Pig, Strain albino Hartley, Age: 5-7 weeks, Weight: not specified, Source: Hazleton Dutchland, Inc., Denver, PA.

d) Species: Rabbit, Strain: New Zealand White, Age: 10-12 weeks, Weight: not specified, Source: Hazleton Dutchland, Denver, PA.

4. Human skin samples

Skin samples from women undergoing reconstructive

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mammoplasty were obtained from the University of Pittsburgh Hospitals and placed in minimum essential medium (MEM/d-valine) until preparation for the skin chamber. Although fresh samples were obtained for each experiment, the actual time between sample removal and the experiment was not specified.

METHODS:

1. Preparation of skin samples

Preparation and treatment of skin was based on methods described by Kao *et al.*, Toxicol. Appl. Pharmacol. 58:206, 1983 and Holland *et al.*, Toxicol. Appl. Pharmacol. 72:64, 1984.

a) Animal - Rats, mice and guinea pigs were anesthetized with Metofane® and rabbits injected with T-61 euthanasia solution. Fur was clipped on the thoracic region of the dorsal trunk and a 6 x 6 cm piece of skin was removed and placed in MEM. Fat and connective tissue were scraped from the skin with a spatula and one-inch discs of skin were cut from the pieces. Samples were kept moist with MEM prior to placing in chamber.

b) Human - Skin was scraped of fat and connective tissue as above and one-inch discs were cut (total of 6, number of individual skin specimens not specified). Samples were kept moist with MEM prior to placing in chamber.

2. Test substance exposure: Skin discs (exposed surface area 1.77 cm^2) were placed in perfusion chambers. The dermal surface of the skin discs were perfused with MEM/d-valine for 30 min. at a flow rate of 2.5 ml/min prior to treatment. 250 ul aqueous solutions of 0.75% and 7.5% glutaraldehyde containing 5-10 uCi ^{14}C were placed on the epidermal side of each skin preparation. Skin preparations were exposed for 6 hr to glutaraldehyde.

3. Positive control substance exposure: Skin discs prepared as above were exposed to 250 ul ethanol containing 2-4 uCi ^{14}C-ethanol.

3. Determination of skin penetration/material balance: Skin preparations were removed from the chambers, placed in petri dishes and swabbed with water-wetted cotton applicators to remove any remaining adhering test chemical. Skin discs were stored frozen in vials until analysis using a Biological Materials Oxidizer to determine radioactivity. Media effluent collected during treatment and rinsing solution containing applicators

were radioassayed by liquid scintillation spectrometry.

Dermal penetration by the test substance was calculated as cumulative percent absorbed radioactivity determined by the total sum of counts in the media effluent divided by mean dosing solution counts.

RESULTS:

1. Positive control: Although dermal penetration by ^{14}C-ethanol to assess viability of skin preparations was discussed in the methods section, the results were not included in the study report.

2. Dermal penetration by glutaraldehyde: Results of the dermal penetration study are presented in Tables 1 - 3 taken from the study. Tables 1 and 2 below show *in vitro* material balance recoveries for 0.75% and 7.5% ^{14}C-glutaraldehyde, respectively, in each species (guinea pig skin not included in 7.5% glutaraldehyde exposure).

TABLE 1: IN VITRO MATERIAL BALANCE RECOVERIES FOR 0.75% GLUTARALDEHYDE: % APPLIED DOSE RECOVERED[1]

Fraction Recovered	Rat[2]	Mouse[3]	Guinea Pig[3]	Rabbit[2]	Human[4]
Males:					
Effluents	0.06±0.05	1.73±1.65	0.53±0.69	0.77±0.20	
Unabsorbed Dose	66.14±7.26	74.10±5.36	64.91±6.29	61.55±3.25	
Apparatus Rinse	1.16±0.54	2.27±0.94	1.38±0.58	1.31±0.17	
Skin	17.24±6.98	7.65±2.49	19.37±6.72	28.02±2.53	
Total Recovery	84.78±1.70	85.76±2.72	86.19±1.14	91.66±3.15	
Females:					
Effluents	0.05±0.01	0.26±0.04	0.17±0.16	0.34±0.11	0.16±0.14
Unabsorbed Dose	76.42±0.56	79.18±1.34	64.89±6.19	53.51±8.37	66.71±3.44[5]
Apparatus Rinse	1.64±0.47	1.54±0.20	1.38±0.13	0.53±0.29	1.95±1.56
Skin	6.82±1.73	5.85±0.94	19.52±5.14	20.87±4.29	6.36±2.79
Total Recovery	84.92±1.87	86.83±0.51	85.97±1.16	75.26±8.22	75.17±4.20

1 Table is same as Table 1 of study
2 Mean of 3 animals
3 Mean of 6 males and 3 females
4 Mean of 3 females
5 Unabsorbed dose excluding skin rinse

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TABLE 2: IN VITRO MATERIAL BALANCE RECOVERIES FOR 7.5% ^{14}C-GLUTARALDEHYDE: % APPLIED DOSE RECOVERED[1]

Fraction Recovered	Rat[2]	Mouse[3]	Rabbit[4]	Human[5]
Males:				
Effluents	0.08±0.04	0.39±0.14	0.85±0.71	
Unabsorbed	79.11±0.66	75.36±3.59	70.06±11.01	
Apparatus Rinse	4.32±2.37	3.28±1.69	1.95±0.63	
Skin	4.75±1.03	2.15±1.02	11.54±11.36	
Total Recovery	88.25±2.37	81.38±0.90	84.40±0.71	
Females:				
Effluents	0.33±0.10	1.43±1.10	1.55±2.09	0.20±0.08
Unabsorbed Dose	70.90±2.62	77.30±4.31	70.75±8.81	69.18±0.08[6]
Apparatus Rinse	5.36±0.52	2.48±0.65	1.87±0.83	2.07±1.74
Skin	8.49+2.13	4.98+3.02	8.89+6.03	4.56+1.67
Total Recovery	85.08+1.37	86.19+2.81	83.07+2.27	76.01+3.64

1 Table is same as Table 2 from study
2 Mean of 3 animals
3 Mean of 3 males and 3 females
4 Mean of 3 males and 5 females
5 Mean of 3 females
6 Unabsorbed dose excluding skin rinse

Total recovery of radioactivity varied between 75% and 92%: most of this was recovered from the unabsorbed dose. Radioactivity recovered from combusted skin discs varied from 2.15% (male mouse, 7.5% dose) to 28.02% (male rabbit, 0.75% dose) and was in general lower at 7.5%. Percent recoveries in effluent were all very low (less than 2% of total).

The fraction of applied dose recovered in the effluent and dose absorbed/surface area following application of doses are shown below in Table 3 for both doses.

TABLE 3: FRACTION OF APPLIED DOSE RECOVERED IN EFFLUENTS AFTER APPLICATION OF A 0.75% AND 7.5% GLUTARALDEHYDE SOLUTION[1]

		0.75% Dose		7.5% Dose	
Species	Sex	% Recovered	Dose Absorbed/Surface Area (mg/cm^2)	% Recovered	Dose Absorbed/Surface Area (mg/cm^2)
Rat	M	0.06±0.05	0.001±0.001	0.08±0.04	0.01±0.004
	F	0.05±0.01	0.001±0.00001	0.33±0.10	0.04±0.01
Mouse	M	1.73±1.65	0.02±0.02	0.39±0.14	0.04±0.01
	F	0.26±0.04	0.003±0.001	1.43±1.10	0.15±0.12
Guinea Pig	M	0.53±0.69	0.01±0.01		
	F	0.17±0.16	0.002±0.002		
Rabbit	M	0.77±0.20	0.01±0.002	0.85±0.71	0.09±0.08
	F	0.34±0.11	0.004±0.001	1.55±2.09	0.16±0.22
Human	F	0.16+0.14	0.002+0.002	0.20+0.08	0.02+0.01

1 Table is same as Table 3 from study

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The amount of ^{14}C-glutaraldehyde penetrating the skin preparations was very small for all tissues after 6 hr of exposure (average 0.5% recovery for 0.75% solution and 0.8% recovery for 7.5% solution). The individual percent recoveries of total radioactivity in effluent ranged from 0.05 for female rat skin to 1.8% in mouse skin, both at 0.75% glutaraldehyde. Dose absorbed/surface area ranged from 1 - 160 ug/cm^2 (average 6 ug/cm^2, 0.75% and 73 ug/cm^2, 7.5%) and tended to be higher at 7.5% than 0.75% glutaraldehyde for all species (human male skin not tested and guinea pigs not tested at the high dose). Human skin preparations absorbed 2 and 20 ug/cm^2 at 0.75% and 7.5%, respectively. Rates of penetration and permeability coefficients were not presented for this study.

DISCUSSION/CONCLUSIONS:

Under the conditions of this *in vitro* study, ^{14}C-glutaraldehyde penetrated isolated skin preparations from male and female rat, mouse, guinea pig, rabbit and female human only slightly when applied to the epidermal surface for 6 hr. Percent of applied dose recovered ranged from 0.05% - 1.73% (average 0.5% recovery at 0.75% and 0.8% recovery at 7.5%) and dose absorbed ranged from 1 - 160 ug/cm^2 (average 6.3 ug/cm^2 at 0.75% and 73 ug/cm^2 at 7.5%). Most species showed differential absorption between skin derived from males and females: at the low dose, male skin absorbed more compound, whereas at the high dose, female skin had higher absorption than male. Doses absorbed/cm^2 were higher at 7.5% than 0.75% glutaraldehyde but percents of dose absorbed were comparable. Rat and human skin preparations absorbed the least amount of applied compound; mouse and rabbit the most. Results were only given as means and standard deviations: no individual skin preparation data was provided in this study.

Results of this study were compared with a previous *in vivo* dermal penetration study (McKelvey *et al.*, BRRC Report 47:91, 1985) in rats and rabbits. Dose absorbed in rabbit skin preparations at 0.75% glutaraldehyde but not 7.5% was comparable for *in vivo* and *in vitro* studies when adjusted for area treated. However, the dose was administered for 24 hr in the *in vivo* study compared to 6 hr in the *in vitro* study, and penetration rates and penetration coefficients were not calculated in the *in vitro* study for comparison to the *in vivo* study. Because *in vitro* dermal penetration studies cannot duplicate the absorption process as it occurs *in vivo*, the results obtained from them are not necessarily relevant to dermal penetration in the live animal and are of limited use for hazard assessment.

Study deficiencies: 1) No positive control for assessment of integrity of sample preparation - ethanol-1-^{14}C positive control data was not presented, although the study authors discuss it in the Introduction and Methods sections, 2) data for individual skin preparations not included in study: only calculated means and

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standard deviations were given, 3) Unabsorbed dose should have also included the skin rinse, 4) penetration rate and permeability coefficient calculations were not included, although calculation formulas were described in the Methods section.

Since dermal penetration studies are currently required to be conducted *in vivo*, this study is considered supplementary information and cannot be upgraded. It is not acceptable for regulatory purposes, although a dermal penetration study is not required as part of the data requirements for registration of glutaraldehyde (this study was submitted voluntarily as additional information).

Core-Classification - Supplementary