Reviewed for OREB.
For Fol. Les. Dissipation
Sent to Jeff
Evans on 11/30/93

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

NOV 23 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Chlorothalonil: Metabolism/dermal penetration study in monkeys

EPA IDENTIFICATION NUMBERS: P.C. Code: 081901
Caswell No.: 215B
DP Barcode: D194404
Submission No.: S446678

FROM: Robert F. Fricke, Ph.D. Robert F Fricke 19 Nov 93
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Health Effects Division (H7509C)

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THRU: Jess Rowland, M.S. Jess Rowland 11/22/93
Toxicology Branch II, Head, Section IV
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 11/22/93
Chief, Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Fermenta ASC Corp, Mentor, OH

Chemical: Chlorothalonil, ASC-2787, Daconil 2787
Tetrachloroisophthalonitrile

Action Requested: Review metabolism/dermal penetration study in monkeys submitted in support of the painter exposure study currently in method development in OREB.

1. The registrant submitted a study entitled "Study to evaluate the urinary metabolites of chlorothanlonil following dermal application to male rhesus monkeys". This special study was submitted is support of a painter exposure study which is in method development by the Occupational and Residential Exposure

Branch (OREB). The data from this study will be used by OREB in their evaluation of exposure to chlorothalonil.

2. Study Conclusions: Rhesus monkeys were exposed dermally (non-occlusive patch, 48 hours) to test material at a dose of 5 mg/kg (≈ 130 $\mu g/cm^2$). One group of animals was terminated after removal of the dressing, while a second group was terminated at 120 hours. Results of the study reveal most (> 86%) of the applied dose remained on the skin surface. Less than 0.5% of the radioactivity was extractable from the excised skin, while approximately 2% remained bound to the skin following homogenization. Approximately 2.3% of the radioactivity was actually absorbed and, over the course of the study, was eliminated in both the urine (≈ 1%) and feces (≈ 1%). Very little (< 0.64%) tissue accumulation occurred. Analysis of selected, pooled urine samples did not reveal any detectable (detection limit 0.5ng/μl or 0.0041%) di- or tri-thiol metabolites.

CORE CLASSIFICATION: Acceptable. This study provides scientific data, but does not fulfil a specific guideline requirement. It is considered to be a "special study".

3. The risk of developing nephrotoxicity following dermal application of test compound is small. Washing of contaminated skin would significantly reduce the risk.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 19 Nov 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/22/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism, Dermal Absorption
Guideline: none (Special Study)

DP BARCODES: D194404 **SUBMISSION NO.:** S446678

P.C. CODE: 081901 **CASWELL NO.:** 215B

MRID NO.: 428759-01

TEST MATERIAL: Chlorothalonil

SYNONYMS: ASC-2787, Daconil 2787
Tetrachloroisophthalonitrile

STUDY NUMBER: 3382-89-0214-AM-001

SPONSOR: Fermenta ASC Corp., Mentor, OH

TESTING FACILITY: Ricerca, Inc., Painesville, OH

TITLE OF REPORT: Study to evaluate the urinary metabolites of chlorothanlonil following dermal application to male rhesus monkeys

AUTHOR: T.A. Magee, J.P. Marciniszyn and J.C. Killeen

REPORT ISSUED: 2 November 1990

CONCLUSIONS: Rhesus monkeys were exposed dermally (non-occlusive patch, 48 hours) to test material at a dose of 5 mg/kg (≈ 130 $\mu g/cm^2$). One group of animals was terminated after removal of the dressing, while a second group was terminated at 120 hours. Results of the study reveal most (> 86%) of the applied dose remained on the skin surface. Less than 0.5% of the radioactivity was extractable from the excised skin, while approximately 2% remained bound to the skin following homogenization. Approximately 2.3% of the radioactivity was actually absorbed and, over the course of the study, was eliminated in both the urine (≈ 1%) and feces (≈ 1%). Very little (< 0.64%) tissue accumulation occurred. Analysis of selected, pooled urine samples did not reveal any detectable (detection limit 0.5ng/μl or 0.0041%) di- or tri-thiol metabolites.

CORE CLASSIFICATION: Acceptable. This study provides scientific data, but does not fulfil a specific guideline requirement. It is considered to be a "special study".

I. OBJECTIVE: This special study was submitted is support of a painter exposure study which is in method development by the Occupational and Residential Exposure Branch (OREB). The data from this study will be used by OREB in their evaluation of exposure to chlorothalonil. This study design was not intended to meet either regular metabolism (85-1) or dermal absorption (85-2) guideline protocol/requirements.

II. MATERIALS

A. Test Compound and Dosing Solutions

1. Radiolabeled material: ^{14}C-Chlorothalonil (ICN Chemical and Radioisotope Division) was uniformly labeled on the benzene ring. Radiochemical purity was 96.3% and had a specific activity of 124.5 mCi/mmole. The lot number was not given in the study.

2. Nonradioactive material: Analytical grade Chlorothalonil (Lot No. not given) had a purity of 98.9%.

3. Dosing solutions: An appropriate amount of labeled and unlabeled test material was dissolved in 20 ml of dichloromethane. After the solution was mixed to homogeneity, the solvent was evaporated under a stream of nitrogen and the resulting solid material pulverized with a small amount of vehicle (Bravo 720 formulation blank) to a particle size of < 5 microns. The test material suspension was brought to a final volume of 70 ml.

B. Test animals: Species: Monkey (Macaca mulatta) Strain: Rhesus Sex: Male Age: Not given Weight (kg): 3.5 - 5.0 Source: Buckshire Corp., Perkasie, PA Housing: Individually Feed: Purina Monkey Chow, *ad libitum* Water: Tap water, *ad libitum* Environment: Temperature, 23 ± 5 °C; Humidity, ambient; Light cycle, 12 hr light/12 hr dark

III. METHODS

A. Study Design: Five male rhesus monkeys were assigned to two study groups. Group 1 consisted of two treated and one control animal, while Group 2 consisted of two treated animals. The backs of all the animals were prepared by clipping a 300 cm^2 area free of hair. Four animals received sufficient amount of test material suspension, applied to a 180 cm^2 application site, to yield a dose of 5 mg/kg; the fifth animal served as a control and received vehicle only. The application site was covered with a non-occlusive dressing for 48 hours. During this time, the animals were restrained in metabolism chairs to facilitate collection of blood, urine and feces. After 48 hours, the non-occlusive dressing was removed and the application site scrubbed with separate cotton balls moistened first with soapy water and then twice with distilled; the skin was dried with a fourth

cotton ball. After skin washing, two treated animals and the one control animal were euthanized immediately (Group 1 animals). The animals in Group 2 were placed in metabolism cages and euthanized 72 hours later (120 hours post dosing). Following euthanasia, skin, consisting of the application site and surrounding untreated skin, was removed. The liver, kidneys, small and large intestines were collected, weighed and stored frozen.

B. Sample Collection and Analysis

1. Blood: Blood (2 ml) was collected at 0.5, 1, 2, 3, 5, 7, 9, 12, 15, 18, 24, 36 hours during the study and at terminal sacrifice (48 or 120 hours post dosing). Duplicate blood samples were oxidized and the amount of trapped $^{14}CO_2$ determined by liquid scintillation counting (LSC).

2. Urine: For the first 48 hours of the study, urine was collected using a catheter-type device attached to the penis. After 48 hours, urine was collected using the metabolism cages. In all cases, urine was freeze-trapped over dry ice. For treated animals, samples were collected at 2-hour intervals during the first 12 hours of the study and at 12-hour intervals through terminal sacrifice. At terminal sacrifice, the bladder contents were collected. For control animals, samples were collected at 24 and 48 hours only. Urine samples were thawed and, after measuring the total volume, duplicate, 1 ml aliquots were taken for determination of radioactivity using LSC. If necessary, samples were diluted prior to determination of radioactivity.

3. Feces: For treated animals, fecal samples were collected at 4-hour intervals during the first 12 hours of the study and at 12-hour intervals through terminal sacrifice. For control animals fecal samples were collected at 24 and 48 hours. Samples were collected at room temperature. For analysis, fecal samples were weighed and homogenized frozen in dry ice. Duplicate, weighed samples were combusted and the amount of $^{14}CO_2$ determined by LSC.

4. Non-occlusive patch and skin washes: Non-absorbed test material was determined by measuring the amount of radioactive residues remaining in the non-occlusive dressing and removed by the skin washes. The patch was extracted five-times with 6 ml of acetone; an aliquot of each extract was assayed in duplicate. Each set of cotton balls were extracted three times with 20 ml acetone; pooled extracts were assayed in duplicate.

5. Skin: Surface residues were determined by extracting the excised skin twice with 100 ml of acetone for one hour with intermittent agitation. After each extraction, the acetone was decanted off and radioactivity determined in 1 ml aliquot. For

determination of extractable residues, the skin was cut into small pieces an sequentially homogenized in acetone, twice in methanol, and finally in acetone; 200 to 240 ml of solvent was used for each homogenization. After each homogenization, the solvent was decanted, the volume determined and the radioactivity determined in a 1 ml aliquot. Bound residues were determined on combusted skin samples.

6. Tissues: Tissues were homogenized frozen with dry ice and duplicate, weighed samples were combusted for determination of radioactivity.

C. Identification of Urinary Metabolites: Aliquots (250 µl) of each urine sample were applied to a reversed phase HPLC column and eluted with a pH 7.0 KH_2PO_4-methanol gradient; UV and radiation detectors were used to monitor the eluate. Radioactive peaks were isolated, acidified to pH 2 with 2N HCl and extracted three times with ethyl acetate (previously saturated with 1N HCl). The residue remaining following rotary evaporation of the pooled ethyl acetate fractions was dissolved in 10 ml of methanol. A 250 µl aliquot was rechromatographed using HPLC, while the remainder of the sample was methylated using diazomethane. The residue, following removal of the solvents, was dissolved in 2 ml of methanol. A 250 µl aliquot of the methylated residue was chromatographed using HPLC; a 1 ml aliquot was subjected to preparative HPLC (four runs, with pooling of fractions based on retention times and radioprofile). Methylated standards of mono-, di- and tri-thiol derivatives of test material were used to identify HPLC fractions based on retention times. Fractions containing the thiol derivatives were pooled, brought to dryness using a rotary evaporator, and dissolved in methanol. The methanol solution was applied to a C-18 column and eluted with methanol. The eluate was reduced to dryness under a stream of nitrogen and the residue dissolved in toluene (100 µl). Samples were subjected to GC/MS analysis.

D. Statistics: Sample means and standard deviations were determined.

IV. REGULATORY COMPLIANCE

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

V. RESULTS

A. Material Balance: The excretion and distribution of radiolabel is presented in Table 1. Most of the test material was not absorbed and remained on the non-occlusive patch (≈ 81%) or was removed during the skin washing

procedure (≈ 8%). Of the test material that was absorbed, approximately 2 to 4% remained in the skin. Labeled residues were approximately equally distributed between the urine (0.91 to 1.01%) and feces (0.82 to 1.26%). Tissue accumulation accounted for 0.44% after 48 hours and 0.08% after 120 hours. Essentially all (91.26 to 98.37%) of the applied radioactivity was accounted for.

Table 1: Material Balance (Data summarized from study Table 2)

Fraction	Percent of Administered Dose[a] Group 1	Group 2
Patch	78.07	84.00
Skin Washes	7.15	9.73
Urine	1.01	0.93
Feces	0.82	1.26
Tissues	0.44	0.08
Skin	3.78	2.39
Total	91.26	98.37

[a] Animals dosed at 5 mg/kg

B. Urinary Elimination: The urinary elimination data for individual animals, expressed as percent of administered dose, μg equivalents/ml and total μg equivalents, are summarized in Table 2, below. Most (59%) of the labeled residues were eliminated during the first 24 hours.

C. Fecal Elimination: The fecal elimination data for individual animals are presented in Table 3, below. For animals in Group 1 (terminated at 48 hours), 0.592 to 1.040% of the administered dose appeared in the feces; for Group 2 animals, 1.089 to 1.421% was eliminated over 120 hours.

D. Tissue Distribution of Radioactivity: Total tissue accumulation of labeled residues ranged from 0.239 to 0.642% at 48 hours to approximately 0.08% at 120 hours (Table 4). Most of the radioactivity was associated with the intestine.

E. Urinary Metabolites: Selected urine samples were pooled and thiol derivatives isolated and quantified. No di- and tri-thiols was present at concentrations greater than or equal to the detection limit of 0.5 ng/μl (equivalent to 0.0041 % of the administered dose).

F. Data Summary: Table 5 summarizes the absorption data, expressed as μg equivalents and μg equivalents/cm^2 of skin, for the dermal application of test material.

Table 2: Urinary elimination of labeled residues by individual animals dosed at 5 mg/kg (Data summarized from Tables 3, 4, and 5 of the study)

Time (hrs)	Monkey No.[a] 1	2	3	4	Monkey No. 1	2	3	4	Monkey No. 1	2	3	4
	% of Administered Dose[b]				µg Equivalents/ml				Total µg Equivalents			
0-2	0.039	0.025	0.088	0.025	0.180	0.749	0.321	0.118	9.73	5.97	17.66	5.92
2-4	0.125	0.063	NS[c]	NS	0.508	0.844	NS	NS	31.00	15.04	NS	NS
4-6	0.065	0.073	NS	0.044	4.220	0.725	NS	2.060	16.01	17.40	NS	10.30
6-8	0.075	NS	0.063	NS	4.133	NS	1.491	NS	18.59	NS	12.67	NS
8-10	NS	0.093	0.115	0.036	NS	2.011	1.303	1.127	NS	22.14	23.17	8.45
10-12	0.104	0.049	NS	0.047	3.670	0.223	NS	1.294	25.70	11.59	NS	11.02
12-24	0.346	0.264	0.378	0.187	0.340	0.580	0.794	0.319	85.74	62.62	76.21	44.03
24-36	0.217	0.170	0.198	0.118	0.572	0.603	0.506	0.356	53.71	40.37	39.96	27.75
36-48	0.173	0.128	0.178	0.109	0.516	0.360	0.765	0.777	42.84	30.28	35.94	25.64
48-60			0.082	0.020			0.159	0.287			16.48	4.74
60-72			0.028	0.024			0.045	0.106			5.66	5.77
72-84			0.018	0.017			0.021	0.083			3.71	3.97
84-96			0.020	0.013			0.059	0.083			4.02	3.09
96-108			0.015	0.009			0.019	0.064			2.99	2.21
108-120			0.011	0.010			0.015	0.028			2.11	2.42
0-24	0.753	0.568	0.644	0.338	13.051	5.132	3.909	4.918	186.8	134.8	129.7	79.71
0-48	1.144	0.865	1.021	0.565	14.139	6.095	5.180	6.051	283.3	205.4	205.6	133.1
0-72			1.131	0.609			5.384	6.445			227.8	143.6
0-96			1.169	0.639			5.463	6.610			235.5	150.7
0-108			1.183	0.649			5.483	6.674			238.5	152.9
0-120			1.194	0.659			5.497	6.702			240.6	155.3

[a] Monkeys 1 and 2: Group 1; Monkeys 3 and 4: Group 2
[b] Animals dosed at 5 mg/kg
[c] NS = no sample

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Table 3: Percent of Administered Dose (5 mg/kg) Appearing in the Feces (Data summarized from Table 6 of the study)

Time (hrs)	Monkey No.[a] 1	2	3	4
0-4	NS[b]	<0.000	NS	NS
4-8	NS	<0.000	NS	NS
8-12	NS	NS	NS	NS
12-24	0.247	0.441	<0.000	NS
24-36	0.015	0.265	0.030	NS
36-48	0.330	0.334	NS	<0.000
48-60			0.227	NS
60-72			0.690	0.187
72-84			NS	NS
84-96			0.272	0.556
96-108			0.158	0.243
108-120	____	____	0.044	0.103
TOTAL	0.592	1.040	1.421	1.089

[a] Monkeys 1 and 2: Group 1
Monkeys 3 and 4: Group 2
[b] NS = no sample

Table 4: Tissue Distribution (Data summarized from Table 7 of the study)

Tissue	Monkey No.[a] 1	2	3	4
Liver	0.085	0.031	0.011	0.024
Kidney	0.014	0.016	0.009	0.003
Intestine	0.543	0.192	0.061	0.057
TOTAL	0.642	0.239	0.081	0.084

[a] Monkeys 1 and 2: Group 1; Monkeys 3 and 4: Group 2

Table 5: Summary of Data (Data summarized from Table 11 of study)

Group	Dose	Skin Residues: Surface	Extractable	Bound	Absorbed
			µg Equivalents		
1 only	24274	20914	97.5	581.5	549.3
1 & 2	23065	20742	83.9	479.0	518.4
			µg Equivalents/cm^2		
1 only	134.9	116.2	0.54	3.23	3.05
1 & 2	128.1	115.2	0.47	2.66	2.88

VI. DISCUSSION AND COMMENTS: The primary objective of this study was to determine if di- and tri-thiol derivatives appeared in the urine following dermal application of test material. Previous reports indicated that nephrotoxicity of the test material has been associated with these thiol compounds[1,2]. The scope of thisstudy was therefore limited to (1) the measurement of the absorption and elimination of following dermal application of radiolabeled test material and (2) the identification of di- and tri-thiol derivatives which may appear in the urine.

Test material was applied to the backs of rhesus monkey and the application site covered with a non-occlusive dressing. After 48 hours, the dressing was removed and the application site washed to remove any residual compound. One group of animals was terminated after removal of the dressing, while a second group was terminated at 120 hours. Results of the study reveal that greater than approximately 86% of the applied dose remained on the skin surface or non-occlusive patch. Less than 0.5% of the administered radioactivity was extractable from the skin, while approximately 2% remained bound. Approximately 2.3% of the radioactivity was actually absorbed and, over the course of the study, was eliminated in both the urine (≈ 1%) and feces (≈ 1%). Very little (< 0.64%) tissue accumulation occurred. Analysis of selected, pooled urine samples did not reveal any detectable (detection limit 0.5ng/μl or 0.0041%) di- or tri-thiol metabolites.

The study concludes the risk of developing nephrotoxicity following dermal application of test compound is small. Washing of contaminated skin would significantly reduce the risk.

CORE CLASSIFICATION: Acceptable. This study provides scientific data, but does not fulfil a specific guideline requirement. It is considered to be a "special study".

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[1] Ricerca, Inc. Document Number: 1504-87-0020-AM; "Summary of the available data on the animals metabolism studies conducted with chlorothalonil (Revised July, 1989), 1989.

[2] Ricerca, Inc. Document Number: 1117-87-0019-TX-003; "Summary of toxicology studies conducted with chlorothalonil (Revised July 1989)", 1989.