DATA EVALUATION RECORD

PYRACLOSTROBIN (BAS 500F)

Study Type: §85-3a; Dermal Penetration Study - Rats

Work Assignment No. 3-01-113 GG (MRID 45118402)

Prepared for 5/24/2001

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PYRACLOSTROBIN (BAS 500F) **Dermal Penetration Study - Rats (§85-3a)**

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DATA EVALUATION RECORD

STUDY TYPE: Dermal Penetration - Rat
OPPTS Number: 870.7600 OPP Guideline Number: §85-3a

DP BARCODE: D269669
P.C. CODE: 099100 SUBMISSION CODE: S583112
TOX. CHEM. NO.: None

TEST MATERIAL (RADIOCHEMICAL PURITY): Pyraclostrobin (>95%)

SYNONYMS: BAS 500 F; methyl-N-[[[1-(4-chlorophenyl)pyrazol-3-yl]oxy]-*o*-tolyl]-N-methoxycarbamate

CITATION: Leibold, E. (1999) BAS 500 F - Study of the Dermal Absorption in Rats. BASF Aktiengesellschaft, D-67056 Ludwigshafen/Rhein, FRG. Laboratory Study Id.: 01B0363/966044, June 18, 1999. MRID 45118402. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 45118402), male Wistar rats (4/dose group/interval) received a single dose of [^{14}C-tolyl]-pyraclostrobin (≥95% radiochemical purity) at 0.015, 0.075 or 0.375 mg/cm^2 in a commercial formulation (BAS 501 00 F) with unspecified components. Rats were exposed to the [^{14}C]-test material for up to 8 hours, after which the application site was washed. Rats were terminated 4, 8, 24 or 72 hours post-dose. The mean recovery of the applied doses ranged from 99.1-110.1%.

In all three doses the largest portion of the dose was found in the Dressings (72.38-84.36%, 78.51-81.58% and 76.38-80.24% for the respective doses) The dressings consisted of silicone ring, nylon mesh cover and porous bandge. This material must be considered **unavailable** for absorption.

Dermal penetration of [^{14}C-tolyl]-pyraclostrobin was low for all dose groups and time intervals (0.44-2.60%). After an 8-hour exposure period, absorbed radioactivity accounted for 0.65%, 0.86% and 0.50% of the applied dose at nominal doses of 0.015, 0.075 and 0.375 mg/cm^2, respectively. After removal of the test substance and washing the site, absorption continued - an additional 0.93-1.74% of the dose in each dose group over the next 64 hours; however, there was

no concomitant decrease in radioactivity in the skin.

The percent of the dose absorbed was not dose dependent; however, the actual amount of the test material absorbed did increase with increasing dose level. Immediately following the 8-hour exposure period, absorbed radioactivity accounted for 0.105, 0.757 and 2.27 $\mu g/cm^2$ at mean achieved doses of 0.017, 0.087 and 0.428 mg/cm^2. Comparing the increases from the low to high-dose groups, the amount of actual absorption increased by 21.6x compared to the 25.2x increase in the dose level. This pattern of absorption is commonly seen with a chemical which directly damages the stratum corneum of the skin.

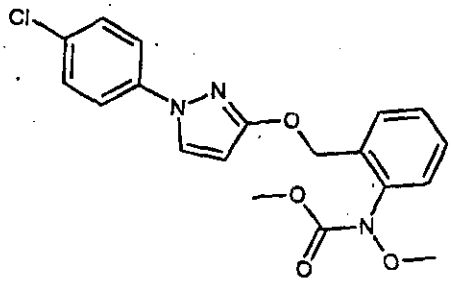
The submitted study is classified as **unacceptable/guideline [§85-3]** and **cannot** be upgraded because the material retained on the dressings is unavailble for absorption and the actual dose cannot be determined.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Pyraclostrobin
   Chemical Name: methyl-N-[[[1-(4-chlorophenyl)pyrazol-3-yl]oxy]-o-tolyl]-N-methoxycarbamate
   Description: Solid
   Batch Number: H2037
   Purity: 98.5% (w/w)
   CAS #: 175013-18-0
   Structure:



   [^{14}C-U-tolyl]-pyraclostrobin

   Batch Number: 566-2101
   Radiochemical purity: >95% (determined by HPLC)
   Specific activities: 4.62 MBq/mg (277,000 dpm/μg)

2. Vehicle: The neat solvent of commercial formulation, BAS 501 00 F.

3. Test animals: Species: Rat
   Strain: Wistar Chbb:THOM (SPF)
   Sex: Male
   Age: 7 weeks
   Weight upon receipt: 200-250 g
   Source: Boehringer Ingelheim Pharma KG, Biberach a.d. Riss (FRG)
   Diet: Kliba lab diet for rat-mouse-hamster (Klingentalmühle AG, CH-4303 Kaiseraugst, Switzerland), *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: Prior to the experiment, type III Macrolon cages; after treatment, individually in all-glass metabolism cages equipped for the separate collection of urine and feces
   Environmental conditions:
   Temperature: 20-24°C
   Humidity: 30-70%
   Air changes: Not specified
   Photoperiod: Not specified
Acclimatization period: Not specified

B. STUDY DESIGN and METHODS

1. Dose selection

Nominal doses of [^{14}C-tolyl]-pyraclostrobin were selected to simulate expected exposure in the field. Assuming exposure predominantly through hands and forearms, the Sponsor indicated that model calculations suggest a dose of 0.015-0.110 mg/cm^2. Thus, nominal doses of 0.015, 0.075, and 0.375 mg/cm^2 were selected for a single application each to male rat skin with a dermal exposure of 4 or 8 hours or an exposure of 8 hours with a post-dose period of 16 or 64 hours. The study was conducted from June 22, 1998 to October 9,1998.

2. Dose preparation

[^{14}C-tolyl]-pyraclostrobin was dissolved in toluene, isotopically diluted with unlabelled pyraclostrobin for the high- and mid-doses, and then reduced to dryness at 30°C under vacuum. Radiochemical purity was >95%. Nominal concentrations were achieved following the addition of the vehicle, a commercial formulation (BAS 500 01 F). The Sponsor stated that the formulation stability, homogeneity and concentration accuracy was tested and found acceptable; however, the data were not presented.

3. Animal preparation and dosing

Sixteen animals/dose group were assigned in subgroups of 4 males to 4 sampling intervals/dose. An area of the skin on the back shoulders (about 10 cm^2) was clipped and the skin washed with acetone 24 hours prior to dosing. A silicone ring was glued to the skin. The dose formulation (about 10 μl/cm^2) was applied with a syringe. A nylon mesh gauze was glued to the rings and secured with a porous bandage. Rats received mean achieved doses of 0.0167, 0.0868 and 0.428 mg/cm^2. The specific activity of the high-, mid- and low-dose groups was 17, 686, 75, 076, and 277,332 dpm/μg respectively. For application the test substance was diluted with a blank formulation of unspecified components. The rats were then placed into metabolism cages equipped for separate collection of urine and feces.

4. Animal observations and sample collection

Following either a 4- or 8-hour exposure, the protective cover was removed and the skin at the treatment site was washed with a mild soap solution. A total of 4 male rats/dose/interval were sacrificed under anesthesia by cardiac puncture immediately following a 4- or 8-hour dermal exposure or following an 8-hour exposure with either a 16- or 64-hour post-dose interval. Urine, feces, cage washings, blood, plasma, kidney, liver, carcass, application site skin, adjacent skin, and protective cover were collected at the time of sacrifice.

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5. Radioassay of samples

Liquid samples were mixed with scintillation fluid and analyzed by liquid scintillation counting (LSC). Feces were suspended in water. Carcasses were homogenized in water. Aliquots of these suspensions were lyophilized. Aliqouts of the lyophilized samples, tissue homogenates and skin were solubilized, bleached and radioassayed with LSC.

6. Data analysis

Means and standard deviations were calculated using computer software.

II. RESULTS

Analytical values of Blood cells, Plasma, Kidney and Liver were at or below the limit of detection (0.01 percent of dose) and are not included in the Tables.

Recovery of the applied dosage was 99.1-110.1% for each group at each sampling interval (Table 1). Following either a 4- or 8-hour exposure period, the majority of the applied dose for each group was not absorbed (83.8-95.8% dose; Table 2), with the majority of the non-absorbed material being recovered from the protective covering (72.4-84.4% dose). This latter material must be considered **unavailable** for absorption. Absorbed radioactivity accounted for 0.44-2.60% of the dose for all groups. Recovery of radioactivity in samples of blood, plasma, kidneys and liver was ≤0.02% at each dose.

For the 0.015 mg/cm^2 dose group, absorption was low (<1.6% dose) at all intervals. Increasing the duration of exposure increased absorption only slightly (4-hours, 0.56% dose; 8-hours 0.65% dose). However, low levels of absorption continued after removal of the test substance, with 1.46 and 1.58% of the dose being absorbed by 16 and 64 hours, respectively, after removal of the test substance. Radioactivity remaining in/on the treated skin after an 8-hour exposure did not decline at longer post-treatment intervals (13.5-15.5% dose).

For the 0.075 mg/cm^2 dose group, absorption was also low (<2.7% dose) at all intervals. As in the low-dose group, increasing the duration of exposure increased absorption only slightly (4-hours, 0.44% dose; 8-hours 0.86% dose). However, low levels of absorption continued after removal of the test substance, with 2.57 and 2.60% of the dose being absorbed by 16 and 64 hours, respectively, after removal of the test substance. Radioactivity remaining in/on the treated skin after an 8-hour exposure did not decline at longer post-treatment intervals (13.0-14.4% dose).

For the 0.375 mg/cm^2 dose group, absorption was also low (<1.6% dose) at all intervals. As in the other groups, increasing the duration of exposure increased absorption only slightly (4-hours, 0.51% dose; 8-hours 0.50% dose). However, low levels of absorption continued after removal of the test substance, with 1.19 and 1.59% of the dose being absorbed by 16 and 64 hours, respectively, after removal of the test substance.

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Radioactivity remaining in/on the treated skin after an 8-hour exposure did not decline at longer post-treatment intervals (10.7-12.5% dose).

Excretion occurred predominantly in the feces. By 64 hours after the 8-hour exposure, urinary excretion in all three dose groups accounted for 0.22-0.38% of the dose and fecal excretion in all the groups accounted for 0.91-1.76% of the dose.

III. DISCUSSION

F. Investigator's Conclusion - Absorption of [^{14}C-tolyl]-pyraclostrobin through rat skin is low, amounting to ≤2.6% of the dose. Concentrations of radioactivity in organs and tissues analyzed were very low with the remaining carcass showing the highest values.

B. Reviewer's Discussion - Following a 4- or 8-hour dermal exposure of male rats to [^{14}C-tolyl]-pyraclostrobin at 0.015, 0.075 or 0.375 mg/cm^2, 99.1-110.1% of the administered dose was recovered.

In all three doses the largest portion of the dose was found in the Dressings (72.38-84.36%, 78.51-81.58% and 76.38-80.24% for the respective doses) The dressings consisted of silicone ring, nylon mesh cover and porous bandge. This material must be considered **unavailable** for absorption.

Regardless of the dose level, absorption was low (0.44-2.60% of the dose) at all intervals. After either a 4- or 8-hour exposure, the absorbed radioactivity (0.44-0.86% dose) was recovered mostly in the carcass (0.42-0.77% dose). However, by 64 hours after the 8-hour exposure, the absorbed radioactivity (1.58-2.60% dose) had been mostly excreted (1.17-2.24% dose), with the level of fecal excretion (0.91-1.76% dose) being approximately five times higher than urinary excretion (0.22-0.38% dose). Recovery of radioactivity in each sample of blood, plasma, kidneys and liver was ≤0.02% for all doses at each interval.

For each dose group, 12.0.-13.5% of the dose remained in/on the washed skin following the 8-hour exposure. By 16 and 64 hours after the 8-hour exposure period, absorption increased slightly (0.69-1.74% dose) in each group; however, there was no concomitant decline in radioactivity remaining on the skin (12.5-14.4% dose at 64 hours post-treatment).

The percent of the dose absorbed was not dose dependent; however, the actual amount of the test material absorbed did increase with increasing dose level. Immediately following the 8-hour exposure period, absorbed radioactivity accounted for 0.105, 0.757 and 2.27 μg/cm^2 at mean achieved doses of 0.017, 0.087 and 0.428 mg/cm^2. Comparing the increases from the low to high-dose groups, the amount of actual absorption increased by 21.6x compared to the 25.2x increase in the dose level. This pattern of absorption is commonly seen with a chemical which directly damages the stratum corneum of the skin.

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IV. STUDY DEFICIENCIES

The following deficiencies were noted in this dermal penetration study:

1.In all three doses the largest portion of the dose was found in the Dressings (72.38-84.36%, 78.51-81.58 and 76.38-80.24 for the respective doses) The dressings consisted of silicone ring, nylon mesh cover and porous bandge. This material must be considered **unavailable** for absorption.

2. The Sponsor stated that the formulation stability, homogeneity and concentration accuracy was tested and found acceptable; however, the data were not presented.

The submitted study is classified as **unacceptable/guideline [§85-3]** and **cannot** be upgraded because the material retained on the dressings is unavailable for absorption and the actual dose cannot be determined.

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PYRACLOSTROBIN

Dermal Penetration Study (§85-3[a])

Table 1. Distribution of radioactivity following a single dermal exposure of male rats to [^{14}C-tolyl]-pyraclostrobin. [a]

Nominal dose (mg/cm^2)	Actual dose		Sacrifice time (hours) [b]	Recovered radioactivity expressed as % of applied dose							
	(mg/cm^2)	mg/rat		Dressing(s)	Skin wash	Skin [c]	Urine	Feces	Carcass	Cage wash	Total Recovered
0.015	0.0179	0.179	4	78.86	10.28	13.68	0.01	0.00	0.54	0.01	103.36
	0.0161	0.161	8	84.36	9.16	14.91	0.03	0.01	0.59	0.02	109.06
	0.0158	0.158	24	81.86	9.82	12.04	0.17	0.49	0.80	0.03	105.20
	0.0170	0.170	72	72.39	11.40	14.60	0.27	1.04	0.27	0.00	99.97
0.075	0.080	0.80	4	80.35	11.43	9.87	0.01	0.01	0.42	0.00	102.07
	0.088	0.88	8	78.51	12.63	12.96	0.05	0.02	0.77	0.02	104.96
	0.084	0.84	24	81.58	12.16	13.80	0.22	0.56	1.74	0.05	110.10
	0.095	0.95	72	79.79	7.71	14.39	0.38	1.76	0.36	0.10	104.47
0.375	0.400	4.00	4	80.33	15.51	10.96	0.01	0.01	0.47	0.02	107.30
	0.454	4.54	8	76.38	10.22	11.97	0.04	0.01	0.44	0.01	99.08
	0.425	4.25	24	77.07	11.13	10.72	0.16	0.42	0.59	0.02	100.11
	0.432	4.32	72	80.24	10.84	12.48	0.22	0.91	0.42	0.04	105.13

a Data are the mean of 4 rats/dose/interval. Data were calculated by reviewer from or found in Tables 1-3 (pages 26-28) of the study report.
b Dose exposure was for 4 or 8 hours. Groups sacrificed after 8 hours had the application site washed at 8 hours post-dose.
c Includes treated skin and skin adjacent to the application site.
d Includes carcass, blood cells, plasma, kidney, and liver.

Table 2. Summary of dose distribution values for [^{14}C-tolyl]-pyraclostrobin following a single dermal exposure of male rats. [a]

Nominal dose (mg/cm²)	Actual dose		Sampling interval (hrs) [b]	Amount of dose							
				Not Absorbed [c]	Skin at test site [d]		In Carcass [e]	Excreted [f]	Absorbed [g]		Total of skin and absorbed
	mg/cm²	mg/rat		(% dose)	(% dose)	µg/cm²	(% dose)	(% dose)	% dose	µg/cm²	(% dose)
0.015	0.0179	0.179	4	89.14	16.18	2.90	0.54	0.02	0.56	0.100	16.74
	0.0161	0.161	8	93.52	13.47	2.17	0.59	0.06	0.65	0.105	14.12
	0.0158	0.158	24	91.68	15.46	2.44	0.80	0.66	1.46	0.231	16.92
	0.0170	0.170	72	83.79	13.90	2.36	0.27	1.31	1.58	0.269	15.48
0.075	0.080	0.80	4	91.78	9.87	7.90	0.42	0.02	0.44	0.352	10.31
	0.088	0.88	8	91.14	12.96	11.40	0.77	0.09	0.86	0.757	13.82
	0.084	0.84	24	93.74	13.80	11.59	1.74	0.83	2.57	2.16	16.37
	0.095	0.95	72	87.5	14.39	13.67	0.36	2.24	2.60	2.47	16.99
0.375	0.400	4.00	4	95.84	10.96	43.84	0.47	0.04	0.51	2.04	11.47
	0.454	4.54	8	86.60	11.97	54.34	0.44	0.06	0.50	2.27	12.47
	0.425	4.25	24	88.20	10.72	45.56	0.59	0.60	1.19	5.06	11.91
	0.432	4.32	72	91.08	12.48	53.91	0.42	1.17	1.59	6.87	14.07

a Data are the mean of 4 rats/dose/interval. Data were calculated by reviewers from or found in Tables 1-15 (pages 26-40) of the study report.
b Dose exposure was 4 or 8 hours. Groups sacrificed after 8 hours had the application site washed 8 hours post-dose.
c Includes radioactivity in protective cover (dressings) and skin wash. The material in the dressing is considered unavailable for absorption.
d Includes skin at application site and surrounding skin.
e Includes carcass, blood cells, plasma, kidney, and liver.
f Includes radioactivity in urine, feces and cage wash.
g Includes excreted radioactivity and radioactivity remaining in the carcass.