I

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

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: May 11, 2011

SUBJECT: Chlorfenapyr; Review and generation of a Data Evaluation Records

PC Code: 129093
Decision No.: 426130
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0055519
MRID No.: 46654701, 46654702

DP Barcode: D383302
Registration No.: NA
Regulatory Action: NA
Case No.: 7419
CAS No.: 122453-73-0
40 CFR: §180.513

Ver.Apr. 2010

FROM: Anwar Y Dunbar, Ph.D.
Pharmacologist, Risk Assessment Branch 1
Health Effects Division (HED) (7509P)

THROUGH: Dana Vogel,
Chief, Risk Assessment Branch 1
Health Effects Division (HED) (7509P)

TO: Susan Bartow , Risk Review Manager
Registration Division (7505P)

I. CONCLUSIONS

RAB1 has reviewed the 90-day inhalation exposure toxicity and 28-day dermal toxicity studies and they are both acceptable/guideline studies.

II. ACTION REQUESTED

Please review this 90-day inhalation exposure toxicity study and this 28-day dermal toxicity study in rodents.

Received 6/28/2011

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R192871 - Page 2 of 33

EPA Reviewer: Anwar Y. Dunbar, Ph.D. **Signature:** Anwar Y. Dunbar
Risk Assessment Branch 1, Health Effects Division (7509C) **Date:** 05-11-11
EPA Secondary Reviewer: Sheila Healy, Ph.D. **Signature:** Sheila Healy
Risk Assessment Branch 3, Health Effects Division (7509C) **Date:** 5/11/11

Template version 02/06

TXR# 0055519

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity -Rats; OPPTS 870.3465 [§82-4]; OECD 413.

PC CODE: 129093 **DP BARCODE:** D383302

TEST MATERIAL (PURITY): BAS 306 1 (Chlorfenapyr 97.8%)

SYNONYMS: (4-bromo-2-(-4-chlorphenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile)

CITATION: Ma-Hock, L, Deckardt, D, Kauffman, DW, Thoolen, RJMM and Ravenzwaay, BV (2005) BAS 306 1- Subchronic 90-Day inhalation study in Wistar rats dust aerosol exposure. Experimental Toxicology and Ecology, BASF Akiengeselischaft, 67056 Ludwigshafen/Rhein, Germany. MRID [46654701]. Unpublished.

SPONSOR: BASF Aktiengeselischaft, 67056 Ludwigshafen/Rhein, Germany

EXECUTIVE SUMMARY:

In a subchronic inhalation toxicity study (MRID 46654701) Chlofenapyr, (% 97.8, batch/lot # 2181H88HV) was administered to 15 HanRCC Crl:WI (Han) rats /sex/concentration by dynamic inhalation (nose only) exposure at concentrations of 0, 5, 20, 40 or 80 mg/m^3 (0, 0.005, 0.02, 0.04 or 0.08 mg/L) for 6 hours per working day, 5 days/week for a total of 90 days. Two groups were designated for each concentration; a "main group" where parameters were examined one day following the end of the exposure period, and a "recovery group" where the animals were observed for four weeks following the end of exposure and then sacrificed following the end of the recovery period.

At the 80 mg/m^3 concentration, four males from the main group and three males from the recovery group died prior to termination of the study. All animals in this concentration test group were subsequently sacrificed prior to completion of the study. The 80 mg/m^3 concentration was replaced with a lesser concentration of 40 mg/m^3. For the 40 mg/m^3 group, one male animal died from the main group and one male animal died from the recovery group, prior to the completion of the exposure period.

Most of the animals exposed to 80 mg/m^3 and all animals exposed to the 40 mg/m^3

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R192871 - Page 3 of 33

concentrations showed slight visually increased respiration on study days 0, 1 and 2 during and after exposure.

There were no treatment- related effects on body weight, body weight gains, food consumption, or organ weights at any of the concentrations tested.

With regard to hematological parameters, treatment-related statistically significant increases in white blood cell counts, prolonged prothrombin time, and a slight increase in lymphocyte counts were observed in both sexes at the concentration of 40 mg/m^3 immediately following exposure. For the 40 mg/m^3 recovery group, decreased white blood cell and lymphocyte counts were observed in both sexes.

In the recovery group there were treatment related decreases in inorganic phosphates, calcium, globulins and cholesterol in both sexes, as well as increased creatinine and urea levels, and decreased total protein in females at the 40 mg/m^3 concentration.

With regard to histopathological observations, the females had an increased incidence of dilation of the submucosal gland in the larynx at the 40 mg/m^3 concentration.

The LOAEC is 40 mg/m^3 (10.9 mg/kg/day), based on mortality (males only), visually slightly accelerated respiration, increased white blood cell and lymphocyte counts, increased clotting times, changes in clinical chemical parameters in both sexes following immediately following exposure and after recovery, and injury to the larynx in females. The increase in white blood cell counts was reversible in the recovery groups. An increased incidence of mortality occurred in males in the 80 mg/m^3 dose. The NOAEC is 20 mg/m^3 (5.43 mg/kg/day).

This subchronic inhalation toxicity study in the rats is **acceptable/ guideline** and satisfies the guideline requirement for a subchronic inhalation study OPPTS 870.3465; OECD 413 in the rats.

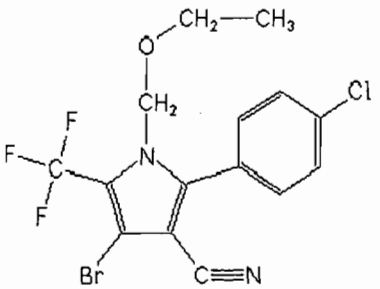
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: BAS 306 1

Description:	Technical grade active ingredient, solid/ powder white to light brown, stable at 5 to 30 ° C
Lot/batch #:	2181H88HV
Purity:	97.8 % a.i.
Compound stability:	Expiry date; April 08, 2008 stored at room temperature.
CAS # of TGAI:	122453-73-0
Structure:	



2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat *(or Rattus norvegicus)*
Strain:	Crl:Wl (Han)
Age/weight at study initiation:	9 weeks old
Source:	Charles River Deutschland GmbH, Sandhofer Weg 7, 97633 Sulzfeld
Housing:	During the period when the rats were not exposed, they were housed individually in Makrolon/wire cages (type MD III, Becker & Co., Castrop-Rauxel, Germany) with type ¾ dust free bedding, (SSNIFF, Soest, Germany).
Diet:	The animals were maintained on milled mouse/rat laboratory diet "GLP" (Provimi Kliba SA, Kaiseraugst, Basel Switzerland), *ad libitum,* except during exposure.
Water:	Tap water, *ad libitum* except during exposure
Environmental conditions:	**Temperature:** 20 – 24 ° C **Humidity:** 30-70% **Air changes:** Not specified **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	1-3 Weeks (See page 31 of the Study Report)

B. STUDY DESIGN:

1. In life dates: Start: August 25, 2004; End: January 18, 2005

2. Animal assignment: Animals were assigned randomly, stratified by body weight, to the test groups noted in Table 1. Two groups were designated for each concentration; "main groups" (Groups 0 through 4) in which animals were sacrificed at the end of the exposure period, and "recovery groups" (Groups 01, 11, 21, 31 and 41) in which the animals were observed for four weeks following the end of exposure period and then sacrificed.

TABLE 1. Analytical Measurements and Study Design[a]					
Test groups and Designations	**Nominal conc. (mg/m^3)**	**Analytical conc. (mg/m^3)**	**MMAD (µm)**	**Rats/sex (Main Group)**	**Rats/sex (Recovery Group)**
Control (0 and 01)	0	0	N.D.	15	5
Low (1 and 11)	5	5.1 ± 0.6	1.9	15	5
Mid (2 and 21)	20	20 ± 3	2.8	15	5
High (4 and 41)	40	41 ± 5	2.1	15	5
High (3 and 31)	80	79 ± 7	N.D.	15	5

[a] Data pertaining to the analytical concentration were obtained from pages 20,52-53 and 911-920 in the study report.
For MMAD measurements, values of 1-4 µm is considered acceptable according to OECD document 39.
GSD values were reported as being in the range of 2.1-2.7 µm on page 53 of the study report.

3. **Dose selection rationale:** Exposure concentrations were initially selected to produce a dose-response curve. The high dose was reduced to 40 mg/m^3 after fatality was observed at 80 mg/m^3. Preliminary range-finding data were not provided or referenced.

4. **Generation of the test atmosphere / chamber description:**

Time to equilibrium: The time to reach equilibrium was not described.

Analytical chemistry: The concentrations of the inhalation atmospheres were analyzed by gravimetrical measurements in low and high test groups (main group/recovery group) 1/11-4/41.

Daily mean values were calculated based on two measured samples per concentration and exposure. From the daily mean values of each concentration, mean concentrations and standard deviations for the entire study were derived.

In these groups, the constancy of concentrations in each inhalation system was continuously monitored using scattered light photometers.

The control atmosphere (test group 0 and 01) was not sampled.

Test atmosphere concentration: The test substance was used as provided by the Sponsor (no vehicle) and a primary dust aerosol was generated by means of a solid particle feeder (BASF). This aerosol was fed to a stainless steel mixing tube (BASF), diluted by conditioned supply air and passed into the inhalation systems. The concentration of the test atmosphere was adjusted by varying the piston feed and by varying the brush rotation indicated in the column "substance feed rate (g/h)" in the table below from page 33 of the study report:

Test atmosphere generation conditions

Test group	Substance feed rate [g/h]	Supply air 1 conditioned [m³/h]	Supply air 2 compressed [m³/h]	Exhaust air [m³/h]
0+ 01	–	6.0 ± 0.3	–	5.4 ± 0.3
1 +11	0.03 – 0.20	4.5 ± 0.3	1.5 ± 0.3	5.4 ± 0.3
2 + 21	0.12 – 0.80	4.5 ± 0.3	1.5 ± 0.3	5.4 ± 0.3
3 +31	0.50 – 3.00	4.5 ± 0.3	1.5 ± 0.3	5.4 ± 0.3
4 +41	0.25 – 1.50	4.5 ± 0.3	1.5 ± 0.3	5.4 ± 0.3

Particle size determination: The particle size analysis was carried out with a cascade impactor equipped with pre-weighed fiber glass filter discs and a backup particle filter (3 L/min limiting orifice). The impactor was connected to a vacuum pump. Three samples from the breathing zones of the animals were taken for each concentration group (5, 20 and 40 mg/m^3; Mark III Stack sampler with 7 mm internal probe diameter [Andersen]). The sampling velocity was 1.25 m/sec. Dust concentration was calculated from difference between weight of preweighed filter and weight of the filter after sampling. Results are in table 1 above.

5. Statistics:

Statistic analyses of Neurofunctional and Clinical Examinations

Parameter	Statistical test	Markers in the tables	References
Food consumption, body weight and body weight change, food efficiency	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means A comparison of group 4 (41) with group 0 (01) was performed using the welch t-test (two sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 – 1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 – 491 Welch B.L. (1947): The generalization of Student's problem when several differentpopulation valiances are involved. Biometrika, 34, 28-35
Feces, rearing, grip strength forelimbs, grip strength hind-limbs, landing foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of the dose groups with the control group was performed using the WILCOXON-test (two-sided) for the hypothesis of equal medians A pairwise comparison of group 4 (41) with group 0 (01) was performed using Wilcoxon-test (two sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York

Statistical analyses of Clinical Pathology

Groups 0 – 2 or 01 - 21

Parameter	Statistical test	Markers in the tables	References
Clinical pathology parameters, except reticulocytes and differential blood count	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided).If the resulting p-value was equal or less than 0.05, a pair-wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York

Groups 0 + 4 or 01 + 41

Parameter	Statistical test	Markers in the tables	References
Clinical pathology parameters, except reticulocytes and differential blood count	Comparison of the dose group with the control group using Wilcoxon-test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York

Groups 0-2 (0, 1 and 2), 01-21 (01, 11 and 21)
Statistical procedures were considered appropriate.

C. METHODS:

1. Observations:

1a. Cage side observations: The animals were examined for evident signs of toxicity or mortality twice a day, Mondays through Fridays and once a day on Saturdays, Sundays and public holidays.

1b. Clinical examinations: Detailed clinical observationss were performed weekly for the duration of the study. The clinical condition of study animals were recorded at least thrice daily during treatment (before, during and after exposure) and otherwise, once daily.

1c. Neurological evaluations: From each main group, neuro-functional tests were performed in five male and five female animals for each concentration group once before the beginning of the exposure period and once prior to gross necroscopy. The FOB for the recovery groups was carried out at the first day of observation period and one day prior to gross necroscopy. The tests used were as follows:

X	Home Cage Observations	X	Sensorimotor Tests/Reflexes
X	Posture	X	Approach response
X	Tremor	X	Touch response
X	Convulsions	X	Vision ("visual placing response")

X	Abnormal Movements	X	Pupillary reflex
X	Impairment of Gait	X	Pinna reflex
X	Other Findings	X	Audition ("startle response")
X	**Open Field Observations**	X	Coordination of movements ("righting response")
X	Behavior when removed from cage	X	Behavior during "handling"
X	Fur	X	Vocalization
X	Skin	X	Pain perception ("tail pinch")
X	Salivation	X	Grip strength of forelimbs
X	Nasal discharge	X	Grip strength of hindlimbs
X	Lacrimation	X	Landing foot-splay test
X	Eyes/pupil size	X	Other findings
X	Posture		
X	Palpebral closure		
X	Respiration		
X	Tremors		
X	Convulsions		
X	Abnormal movements		
X	Impairment of gait		
X	Activity/arousal level		
X	Feces (number of fecal pellets/appearance/consistency) within two minutes		
	Urine (appearance/quantity) within two minutes		
X	Number of rearings within two minutes		
X	Other findings		

2. **Body weight:** The body weight data of the animals was determined before and at the start of the pre-flow (pre-exposure period, with animals in restraint tubes receiving clean off-exposure air), at the start of the exposure period, once a week thereafter, and prior to gross necropsy. The animals, which underwent neurofunctional testing, were additionally weighed on the days of neurobehavioral examination. .

3. **Food consumption:** Food consumption was determined before (except groups 4 and 41) and at the start of the pre-flow, at the start of the exposure period and then, as a rule, once a week thereafter. Food consumption was calculated as mean food consumption (g) per animal per day.

   Food efficiency: Food efficiency (group mean) was calculated based upon individual values for body weight and calculated values for food consumption:

$$\frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100 = \text{Food efficiency for day x}$$

BW_x = body weight on day x (g)

BW_y = body weight on day y (last weighing date before day x) (g)

$FC_{y\ to\ x}$ = mean food consumption from day y to day x; calculated as mean daily food consumption on day x, multiplied with the number of days from day y to day x (g)

4. **Ophthalmoscopic examination:** Ophthalmoscopic exams were performed on all animals in main treatment groups prior to study initiation (Days -12 and -5) and at termination. The

eyes of recovery group animals were examined only on Day 92 and again on Day 118, 119 or 120. They were not examined prior to exposure.

5. **Hematology and clinical chemistry:** Blood was collected from the retroorbital venous plexus from fasted animals following anesthesia with isoflurane 24 hours following the end of exposure for main groups and 4 weeks following the end of exposure for the recovery groups. The CHECKED (X) parameters were examined.

   a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

   b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
X	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase*	X	Total serum protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
X	Serum-γ-glutamyltransferase		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

6. **Urinalysis**: Urinalysis was performed.

7. **Sacrifice and pathology:** All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC

10

	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+	**X**	**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*	**X**	**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	XX	Thyroid*
XX	Liver*+	XX	Testes*+	**X**	**OTHER**
	Gall bladder* (not rat)	XX	Epididymides*+		Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin
X	**RESPIRATORY**	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Head with Nasal Cavities
XX	Lung*	X	Oviducts, Vagina		
X	Nose*	X	Mammary gland*		
X	Pharynx*				
X	Larynx*				

* Recommended for subchronic rodent studies based on Guideline 870.3465
+ Organ weights required

II. RESULTS:

A. OBSERVATIONS :

1. **Mortality:** 4/10 males in group 3 and 3/5 male animals in group 31 exposed to the 80 mg/m^3 concentration died during the first three exposure days. Due to the lethality that occurred in the high concentration male animals, the surviving male and female animals of the 80 mg/m^3 group were sacrificed after the 4th/3rd exposures. The 80 mg/m^3 concentration group was not included in analyses, and was subsequently replaced by the 40 mg/m^3 group. For the 40 mg/m^3 group, one male animal died from the main group and one male animal died in the recovery group as well, prior to the completion of the exposure period on Day 80 and 35 respectively.

2. **Clinical signs of toxicity:** Most (8/10) of the animals exposed to 80 mg/m^3 and all animals exposed to the 40 mg/m^3 concentrations showed slight visually increased respiration on study days 0, 1 and 2 during and after exposure. No other treatment-related clinical findings were observed.

3. **Neurological evaluations:**

Detailed Clinical Observations: The detailed clinical observations did not reveal any additional abnormalities in all groups.

Functional observational battery: No treatment-related effects were observed.

Motor Activity: All changes observed were not different than the values generated in the

control groups.

B. BODY WEIGHT AND WEIGHT GAIN:

1. **Body Weight Gain:** There were no significant, treatment-related effects on animal body weights or body weight gains. Body weights of the 40 mg/m^3 recovery group males were statistically significantly lower than the untreated group, however this difference in weight was present on day zero of exposure and even prior to exposure (see page 119 of the study report). See tables 2 and 2a.

TABLE 2. Average body weights and body weight gains during 90 days of treatment [a]

Test Group (mg/m^3)	Body weights (g±SD)				Overall body weight gain	
	Day 0	Day 7	Day 49	Day 92	g	% of control
Male (Main Group)						
0	242.9 ± 20.8	253.0 ± 23.9	302.7 ± 33.0	320.8 ± 40.9	77.9	-
5	248.3 ± 21.7	254.3 ± 24.3	306.9 ± 27.0	334.5 ± 30.1	86.2	111
20	247.0 ± 11.7	253.2 ± 12.0	301.4 ± 19.6	323.2 ± 27.4	76.2	98
40	223.2± 8.0	235.2 ± 8.8*	280.2 ± 17.2	307.7 ± 18.9	84.5	108
Female (Main Group)						
0	164.8 ± 9.7	167.1 ± 9.4	189.5. ± 10.0	201.5 ± 12.4	36.9	-
5	164.6 ± 10.7	164.5 ± 10.4	186.4. ± 13.2	199.4 ± 14.0	34.8	94
20	163.9 ± 11.0	168.6 ± 11.4	187.3 ± 14.8	198.0 ± 12.3	34.1	92
40	166.8± 4.5	174.7 ± 6.7	195.4 ± 10.2	207.3 ± 11.0	40.5	110

[a] Data obtained from pages (95-117) in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

TABLE 2a. Average body weights and body weight gains during 90 days of treatment [a]

Test Group (mg/m^3)	Body weights (g±SD)					Overall body weight gain	
	Day 0	Day 7	Day 49	Day 92	Day 119	g	% of control
Male (Recovery Group)							
0	261.2 ± 5.4	271.7 ± 10.0	325.1 ± 22.6	342.4 ± 36.6	401.4 ± 22.3	77.9	-
5	253.2 ± 15.7	260.5 ± 19.4	304.6 ± 29.1	321.2 ± 34.9	374.8 ± 38.3	86.2	111
20	254.1 ± 23.1	261.4 ± 26.0	311.0 ± 40.5	331.7 ± 41.4	380.5 ± 55.1	76.2	98
40	229.5**± 8.9	236.8** ± 11.9	272.9* ± 22.4	291.4* ± 24.5	340.9** ± 22.5	84.5	108
Female (Recovery Group)							
0	170.1 ±	177.1 ±	200.4 ±	214.6 ±	234.9 ±	64.9	-

	11.0	19.6	21.7	27.2	25.6		
5	167.9 ± 6.6	171.6 ± 4.4	197.1 ± 8.1	212.3 ± 11.6	233.7 ± 10.8	65.8	101
20	163.9 ± 4.3	168.0 ± 3.3	189.3 ± 8.0	20.9 ± 10.3	219.3 ± 6.4	55.4	85
40	168.6 ± 7.1	178.0 ± 5.6	198.8 ± 6.5	205.9 ± 13.5	221.9± 12	53.3	82

[a] Data obtained from pages (95-117) in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. FOOD CONSUMPTION:

1. **Food consumption:** There was no difference in food consumption between the control and the other exposure groups (5, 20 and 40 mg/m^3).

2. **Food efficiency**: There were no treatment-related differences in food efficiency for any of the exposure groups in comparison to the control.

D. OPHTHALMOSCOPIC EXAMINATION: The ophthalmologic examinations did not show any treatment-related impairment of the refracting media. Spontaneous findings such as remainders of the papillary membrane or corneal stippling were observed in several animals of all test groups and the control group without any dose-response relationship.

E. BLOOD ANALYSES:

1. **Hematology:** At the end of the exposure period, slight but statistically significantly treatment-related increased white blood cell counts were observed in males (↑28%) and females (↑43%) exposed to 40 mg/m^3 of the test compound. After a recovery period of 4 weeks, decreased white blood cells (↓46% for males and ↓47% for females) and lymphocytes (↓22% for males and ↓4% for females) were observed in the 40 mg/m^3 groups suggesting a rebounding effect. Clotting analysis revealed prolonged prothrombin times in the blood of animals of the 40 mg/m^3 groups at the end of the 3-month exposure period (↑11% for males and ↑6% for females). After cessation of treatment, prothrombin times were comparable to control values. No test substance-related changes were seen in any of the other hematological parameters of either sexes. See Table 3.

TABLE 3. Summary of Hematological Parameters (± SD) following 90 days of treatment and 30 days of recovery. [a]

Parameters	Analytical concentration (mg/m^3)			
	0	5	20	40
Males				
WBC[b] (10^9 cells/l), Day 93	4.62± 1.04	4.69± 0.87	5.21± 1.41	5.92* ± 1.35 (↑28%)
WBC[b] (10^9 cells/l), Day 120	6.11± 1.44	4.56*± 0.77	6.25± 1.31	3.97* ± 0.54 (↓46%)

LYMPH (%), Day 93	76.7± 5.5	73.3± 7.1	75.1± 9.9	77.5± 5.2
LYMPH (%), Day 120	75.3± 2.7	76.9± 3.9	76.8± 2.7	59.9 ± 9.3(↓22%)
LEUK (%), Day 93	0.8± 0.6	0.6± 0.3	0.7± 0.3	0.6± 0.3
LEUK (%), Day 120	0.4± 0.1	0.5± 0.2	0.5± 0.2	0.6± 0.2
PT (seconds), Day 93	29.5± 1.3	30.4± 1.8	30.6± 1.5	32.9** ± 1.6 (↑11%)
PT (seconds), Day 120	28.7± 1.0	30.2± 0.9	29.5± 1.1	29.7± 1.3
Females				
WBC[b] (10^9 cells/l), Day 93	2.76± 0.59	3.52± 1.26	3.22± 0.75	3.96* ± 1.14 (↑43%)
WBC[b] (10^9 cells/l), Day 120	4.88± 1.19	3.86± 0.52	3.70± 0.35	2.59** ± 0.51 (↓47%)
LYMPH (%), Day 93	78.0± 5.7	78.8± 7.1	79.9± 3.8	80.0± 3.3
LYMPH (%), Day 120	81.4± 5.8	82.7± 6.1	80.4± 2.5	78.9 ± 5.9 (↓4%)
LEUK (%), Day 93	0.6± 0.3	0.8± 0.7	0.6± 0.2	0.7± 0.1
LEUK (%), Day 120	0.5± 0.2	0.5± 0.2	0.5± 0.3	0.7± 0.1
PT (seconds), Day 93	26.9± 0.8	26.7± 2.2	27.3± 1.4	28.7*± 1.9 (↑6%)
PT (seconds), Day 120	26.6± 1.5	26.4± 0.8	26.5± 0.3	27.1± 1.2

a Data obtained from pages 287-318 in the study report.
b Units expressed as giga/l in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

2. **Clinical chemistry:** No treatment-related changes were seen in the clinical chemistry parameters of the male and female rats at the end of the exposure period. At the end of the recovery period, decreased concentrations of inorganic phosphate (↓22% for males and ↓19% for females), calcium (↓6% for both sexes), globulins (↓11% for both sexes) and cholesterol (↓19% for males and ↓32% for females) were found in the serum of the male and female rats exposed to 40 mg/m^3 of the test article. The decrease in inorganic phosphate in females, however, was not statistically significantly different to the respective control, but appeared only as a trend towards a reduced concentration. In females exposed to 40 mg/m^3 of the test compound, non-statistically significant increases in urea (↑8%) and creatinine (↑2%) immediately following exposure were observed, and statistically significant decreased total protein values were also observed at the end of the recovery period (↓5%). In females exposed to 40 mg/m^3 of the test compound, the amount urea was further increased (↑26%) as well as creatinine (↑14%) following the recovery period. Both of these changes were statistically significant. See Table 4.

TABLE 4. Summary of Clinical Chemical parameters (± SD) following 90 days of treatment and 30 days of recovery.[a]

Parameters	Analytical Concentration (mg/m^3)			
	0	5	20	40
	Male			
Inorganic Phosphate (mmol/l), 93 days	1.73± 0.14	1.69± 0.1	1.78± 0.15	1.69± 0.17
Inorganic Phosphate (mmol/l), 120 days	1.82± 0.22	ND	ND	1.42 ± 0.18 (↓22%)

Calcium (mmol/l), 93 days	2.60± 0.03	2.58± 0.05	2.61± 0.05	2.60± 0.06
Calcium (mmol/l), 120 days	2.71± 0.05	ND	ND	2.55* ± 0.06 (↓6%)
Globulins (g/l), 93 days	27.22± 1.02	27.15± 1.02	26.77± 1.38	28.06± 1.33
Globulins (g/l), 120 days	30.29± 0.92	ND	ND	27.24* ± 1.82 (↓11%)
Cholesterol (mmol/l), 93 days	2.1± 0.3	2.06± 0.3	2.1± 0.5	2.13± 0.34
Cholesterol (mmol/l), 120 days	2.17± 0.28	ND	ND	1.76* ± 0.21 (↓19%)
	Female			
Inorganic Phosphate (mmol/l), 93 days	1.42± 0.18	1.56± 0.2	1.69**± 0.12	1.41± 0.33
Inorganic Phosphate (mmol/l), 120 days	1.63± 0.13	1.42± 0.18	1.59± 0.18	1.33 ± 0.31 (↓19%)
Calcium (mmol/l), 93 days	2.64± 0.07	2.67± 0.04	2.68± 0.04	2.67± 0.04
Calcium (mmol/l), 120 days	2.70± 0.07	2.67± 0.06	2.72± 0.10	2.56** ± 0.04 (↓6%)
Globulins (g/l), 93 days	28.21± 2.17	28.74± 1.5	29.23± 1.23	29.51± 1.22
Globulins (g/l), 120 days	30.29± 0.92	29.50± 2.83	28.9± 1.61	27.24* ± 1.82 (↓11%)
Cholesterol (mmol/l), 93 days	1.63± 0.37	1.59± 0.18	1.75± 0.42	1.83± 0.26
Cholesterol (mmol/l), 120 days	2.06± 0.52	1.53± 0.21	1.72± 0.44	1.41* ± 0.35 (↓32%)
Urea (mmol/l), 93 days	7.05± 0.58	7.29± 0.49	7.85± 0.87	7.66± 0.87 (↑8%)
Urea (mmol/l), 120 days	5.59± 0.3	5.83± 0.25	5.99± 0.51	7.06** ± 0.35 (↑26%)
Creatinine (µmol/l), 93 days	58.0± 3.4	56.6± 3.5	57.1± 3.8	59.1± 3.7 (↑2%)
Creatinine (µmol/l), 120 days	52.1± 1.8	53.3± 2.0	52.4± 3.0	59.4** ± 2.7 (↑14%)
Total Protein (g/l), 93 days	66.47± 2.61	66.51± 1.67	67.74± 1.85	68.68± 1.89
Total Protein (µmol/l), 120 days	67.77± 1.74	68.77± 3.96	67.59± 1.88	65.0* ± 1.74 (↓5%)

a Data obtained from pages 319-342 in the study report 46110601.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.
ND-Not determined

F. URINALYSIS: Urinalysis was not carried out.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight: There were no significant treatment related changes in the absolute organ weights at any of the concentrations tested. Relative weights were unaffected in the treated groups compared to the control animals. See table 5.

TABLE 5. Summary of Absolute Organ Weights (± SD) immediately following 90 days of treatment and 30 days of recovery. [a]	
Parameters	**Analytical concentration (mg/m³)**

	0	5	20	40
Males				
Lung, Day 93	0.95± 0.137	1.098± 0.112	1.026± 0.112	0.886± 0.101
Lung, Day 120	0.97± 0.069	1.028± 0.042	0.98± 0.069	0.878± 0.121
Brain, Day 93	1.948± 0.082	1.954± 0.111	1.935± 0.072	1.876± 0.059
Brain, Day 120	2.068± 0.051	1.994± 0.037	1.988± 0.055	1.873± 0.107
Spleen, Day 93	0.493± 0.056	0.569± 0.074	0.561± 0.063	0.506± 0.054
Spleen, Day 120	0.706± 0.056	0.654± 0.087	0.658± 0.095	0.638± 0.046
Liver, Day 93	6.898± 0.858	7.777± 0.688	7.581± 0.862	7.138± 0.847
Liver, Day 120	10.662± 0.861	9.612± 2.015	9.03± 1.94	8.22± 1.07
Adrenal Glands (mg), Day 93	64.3± 7.6	69.3± 10.995	70.6± 6.7	58.556± 6.444
Adrenal Glands (mg), Day 120	64.8± 5.805	63.2± 7.463	62.0± 6.595	55.5± 3.416
Thymus (mg), Day 93	193.2± 36.7	187.9± 33.4	189.7± 41.2	193.2± 45.7
Thymus (mg), Day 120	199.6± 34.4	199.4± 16.3	227.8± 32.8	175.3± 22.9
Females				
Lung, Day 93	0.642± 0.091	0.631± 0.095	0.659± 0.059	0.783± 0.094
Lung, Day 120	0.866± 0.16	0.908± 0.054	0.776± 0.075	0.772± 0.07
Brain, Day 93	1.768± 0.02	1.751± 0.084	1.773± 0.084	1.76± 0.046
Brain, Day 120	1.768± 0.087	1.812± 0.036	1.762± 0.047	1.834.9± 0.105
Spleen, Day 93	0.518± 0.451	0.382± 0.06	0.356± 0.045	0.415± 0.049
Spleen, Day 120	0.464± 0.048	0.53± 0.085	0.438± 0.058	0.46± 0.085
Liver, Day 93	4.385± 0.591	4.323± 0.374	4.439± 0.222	4.98± 0.465
Liver, Day 120	5.818± 1.102	5.62± 0.284	4.998± 0.093	5.486± 0.4
Ovaries, Day 93 (mg)	84.3± 15.833	81.5± 8.746	84.3± 8.718	99.7± 16.269
Ovaries, Day 120 (mg)	100.4± 3.362	103.0± 5.523	92.6± 8.649	98.0± 11.091
Thymus (mg), Day 93	199.6± 46.9	185.2± 30.8	174.1± 38.7	182.3± 25.4
Thymus (mg), Day 120	248± 47.2	243.6± 49.7	196.2± 17.1	216.6± 48.4

a Data obtained from pages 672-735 in the study report.

2. **Gross pathology:** All test animals from the 80 mg/m^3 group died or were sacrificed prior to the scheduled termination of the study. Animals exposed to 80 mg/m^3 that died prior to scheduled termination showed a diffuse red discoloration and edema in all lobes of the lung.

   Animal number 128 (40 mg/m^3, male) who died 80 days after the start of exposure did not show any macroscopic abnormalities. Animal number 144 (40 mg/m^3, male) who died 35 days after start of exposure showed diffuse discolored red lung lobes. All other animals survived to scheduled termination.

   Two main and two recovery group males showed a white deposition in the nasal cavity. All other macroscopic changes were all within the normal background alterations, which may be seen in untreated rats of this age and strain and are not considered to be related to administration of test substance BAS 306 1 (4-bromo-2-(-4-chlorphenyl)-1-(ethoxymethyl)-5-

(trifluoromethyl)-1H-pyrrole-3-carbonitrile).

3. **Microscopic pathology:** The 5 and 20 mg/m^3 groups were not analyzed for microscopic changes. All microscopic observations in both surviving male and female animals were judged to be normal background findings.

   The lungs of animals (nos. 128 and 144), dosed with 40 mg/m^3 that did not survive to termination, showed autolytic changes (not treatment-related) to a different degree, and marked pulmonary congestion in both cases.

   With regard to the respiratory tract, both the control and 40 mg/m^3 groups showed similar potential markers of injury. The only treatment related effect was dilation of the submucosal gland in the larynx of females at the 40 mg/m^3 concentration.

   Unlike in the oral studies, there were no signs of injury in the central or peripheral nervous systems. See table 6.

TABLE 6. Selected histopathological observations (total occurrence/# of rats) in rats exposed following inhalation exposure to BAS 306 I[a]

Clinical observation	Concentration (mg/m^3)	
	0	40
Males		
Nasal Lesions	4/10	4/10
Lung Congestion	3/10	2/10
Brain	0	0
Autolyzed; sultifocal; marginal		1
Spinal Cord	0	0
Liver		
Foci of mononuclear cells	1	1
Absent		
Subacute inflammation		1
Autolyzed; slight		1
Lymphnodes		
Mesenteric;sinus hitocytosis;slight	1	1
Mediastinal; pigment deposition; focal; slight	3	1
Sinuses; pigment deposition; areas; moderate	1	1
Sinuses; pigment deposition; areas; marginal	2	
Sinuses; erythrophagocytosis; slight	1	
Sinuses; erythrophagocytosis; slight	3	1
Sinuses; erythrophagocytosis; areas; marginal	1	
Sinuses; histocytosis; marginal	1	
Sinuses; blood filled	1	
Iliac; plasmacytosis; marked	1	
Fibrosis; focal	1	
Plasmacytosis; moderate		
Autolyzed; moderates.		1
Testes		
Seminiferous tubules;unilateral;loss of germinal epithelium		
Seminiferous tubules; multinucleate giant cell (s)	3	2
One semineferous tubule with dystrophic calcification	1	
Atrophy; bilateral; severe		
Seminiferous tubules; bilateral; loss of germinal epithelium; many (more than 3)	1	
Autolyzed; slight	1	
	1	
		1
Epididymides		
Granulomatous inflammation; area; marked	1	
Granulomatous inflammation adjacent to spermatocele; unilateral		
Autoyzed; Slight	1	
		1
Prostate		
Focal; acute inflammation	1	
Acini; subacute inflammation	1	1
Reactive hyperplasia/squamous metaplasia of acinar epithelium		
Area of inflammation with extensive necrosis	1	
Autolyzed; slight		1
		1
Kidneys; cortical tubules; regeneration; a few	1	2
Autolyzed; slight		1
Stomach	0	
Glandular mucosa; near limiting ridge; cyst		2
Adrenal Glands	0	
Autolyzed; moderate		1
Hyperaemia		1
Bone Marrow, Caecum, Colon, Duodenum, Heart, Ileum, Jejunum, Rectum, Spleen, Seminal vesicle, Thyroids, Thymus, Urinary bladder,	0	

coagulating gland, Stome (fore and glandular Autolyzed; slight and marked		 1
Nasal Lesions	7/10	5/10
Lung Congestion	3/10	2/10
Larynx Injury (dilated submucosal glands)	0	4/10
Brain	0	0
Spinal Cord	0	0
Lymphnodes Sinus histocytosis; slight Sinus histocytosis; marginal Sinuses; erythrophagocytosis; areas; marginal Sinuses; pigment deposition; areas; slight Sinuses; pigment deposition; areas; marginal Sinuses; pigment deposition; areas; moderate Pigment deposition; focal; marginal Mediastinal; absent Sinuses; erythrophagocytosis; slight Sinuses; erythrophagocytosis; moderate	 2 2 3 1	 2 1 1 1 2 2 2
Mammary Glands Absent Development; slight Development; marginal	 1 8	 6 1
Stomach and Forestomach Forestomach; non-glandular; squamous cyst Glandular mucosa; cysts (1)	 1	 1 1
Adrenal Glands Cortex (zona fasciculate); aggregations of chromaffin cells; a few Dystrophic Calcification	 1 1	0
Uterus Uterus horn; endometrial polyp (s); unilateral; two		 1

a Data were obtained from Table 1D on pages 736-898 of the study report.

III. DISCUSSION AND CONCLUSIONS:

A. <u>INVESTIGATORS CONCLUSIONS</u>: A stable attainment of the desired atmosphere concentrations was obtained throughout the study. Cascade impactor measurement resulted in particle size (MMAD) ranging from 1.7 to 2.8, which were well within the respirable range. The study fulfilled the technical requirement of the guidelines.

The high concentration (80 mg/m^3) clearly exceeded the maximum tolerated dose (MTD) in males. This concentration produced lethality in 7/15 male animals (4/10 main group, 3/5 recovery group) within the first three exposure days. Because of the high lethality, the surviving animals of this group were sacrificed prematurely. Substitute groups of animals were exposed to 40 mg/m^3. However, two animals were found dead after study day 35 and 80.

Besides the lethality that occurred, slightly increased respiration rates were observed in animals exposed to 40 mg/m^3. This finding is considered to be substance related, because it was neither observed in controls nor in other animals exposed to lower concentrations of test substances.

Regarding clinical pathology findings, inhalation of 40 mg/m^3 of the test compound caused slight increases in white blood cells and lymphocytes at the end of the exposure period. These findings were assessed as being treatment-related. At the end of the recovery period leukocytes and lymphocytes were decreased when compared with the respective control. The prolonged prothrombin times in the animals of both sexes exposed to 40 mg/m^3 are also assessed as being treatment-related and are indicative of slight perturbation of coagulation.

Regarding pathology, the two cases (nos. 128 and 144, both dosed 40 mg/m^3) that did not survive to termination of the study were examined by histopathology and showed autolytic changes to a different degree, marked pulmonary congestion in both cases, with animal no. 144 showing additional macroscopic lung changes (all lung lobes diffuse discoloration, dark red). This is considered to be related to the mode of death due to asphyxiation and/or agonal respiratory distress. Considering the lethality that occurred at 80 mg/m^3, the two deaths at 40 mg/m^3 are considered to be substance related.

There were no major organ weight changes for both sexes in the treated groups, and those observed were non-dose related.

All further macroscopic or histopathologic findings were as well regarded to be incidental in origin and not related to treatment.

Inhalation of a dust aerosol of BAS 306 I led to premature death of male animals at a concentration of 80 mg/m^3 within the first few exposure days. At 40 mg/m^3, two male animals did not survive the whole exposure period. Moreover, slight increases of white blood cells and lymphocytes and the mild disturbance of the blood coagulation were noted in both sexes. At the end of the recovery period the white blood cells and the lymphocytes were slightly decreased. No treatment-related effects were observed in the animals exposed to 5 mg/m^3 and 20 mg/m^3 of the test compound. Thus, the No Observed Adverse Effect Concentration (NOEAC) was 20 mg/m^3 under the current condition of the study.

B. <u>REVIEWER COMMENTS</u>: At concentrations of 40 mg/m^3 or greater, the technical form of chlorfenapyr administered as an inhalable dust aerosol is clearly a respiratory irritant and is toxic to the respiratory tract. Numerous treatment-related, adverse effects were observed including deaths at both the 40 and 80 mg/m^3 concentrations, injury to the larynx in females, and visually increased respiration. The statistically significant increase in white blood cell counts and prothrombin times, as well as a marked increase in lymphocytes, indicate that the test article caused systemic inflammation most likely due to irritation in the respiratory tract. These treatment-related effects were also considered adverse. The changes in the clinical chemical parameters with and without recovery further demonstrate that the compound had systemic effects (changes in calcium, cholesterol, creatinine, urea, and globulins).
Gross pathological examination further demonstrated respiratory irritation due inhalation exposure of chlorfenapyr. All of the animals in the 80 mg/m^3 group that died prior to termination of the study show diffuse red discoloration and edema in all lobes of the lung. Both of the animals who died in the 40 mg/m^3 group prior to termination of the study showed marked pulmonary congestion. One of these animals showed diffuse red discoloration similar to the 80 mg/m^3 group. It would have been interesting to see whether or not a more extensive

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R192871 - Page 21 of 33

histopathological analysis would have revealed lung fibrosis. Also it would have would have been interesting to see what the rate of survival of the animals would have been had the recovery gone for a longer duration.

Through the oral route of exposure, Chlorfenapyr's critical effects were injury to the nervous system (vacuolar myelinopathy in the white matter of the brain, spinal cord, and/or spinal nerve roots). No neurotoxic effects were observed in this study behaviorally or histologically. There were histological markers of injury in the control rats which made interpretation of this section difficult. The authors did not discuss why there were markers of injury in the control and the high concentration groups.

Upon request of clarification from the authors, the low- and mid-dose groups were not observed for microscopic tissue alterations. Furthermore the histopathological effects observed in the control and 40 mg/m^3 animals were considered to be background effects and not treatment-related according to the study authors. This reviewer is in agreement with this interpretation. Even though these examinations would have eliminated uncertainty regarding these results, the weight of evidence suggests that based upon the available data, the treatment-related, adverse effects described above first occur at the 40 mg/m^3 concentration, and this study is thus adequate for risk assessment purposes.

The LOAEC is 40 mg/m^3 (10.9 mg/kg/day), based on mortality (males only), visually slightly accelerated respiration, increased white blood cell and lymphocyte counts, increased clotting times, changes in clinical chemical parameters in both sexes following immediately following exposure and after recovery, and injury to the larynx in females. The increase in white blood cell counts was reversible in the recovery groups. An increased incidence of mortality occurred in males in the 80 mg/m^3 dose. The NOAEC is 20 mg/m^3 (5.43 mg/kg/day).

C. STUDY DEFICIENCIES:

- For the clinical chemical measurements, inorganic phosphate, calcium, cholesterol and globulins were not determined in the low- and mid-concentration recovery males, similar to the histopathological examinations.
- Opthamoscopic analysis was not performed on the recovery groups prior to exposure to the test article.

EPA Reviewer: Chester Rodriguez, Ph.D. **Signature:** Chester Rodriguez
Risk Assessment Branch I, Health Effects Division **Date:** 05/11/11
EPA Secondary Reviewer: Anwar Y. Dunbar, Ph.D. **Signature:** Anwar Y. Dunbar
Risk Assessment Branch I, Health Effects Division **Date:** 05-11-11

TXR#: 0055519

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity - Rat;
OPPTS 870.3200 [§82-2] (rodent); OECD 410.

PC CODE: 129093 **DP BARCODE:** D383302

TEST MATERIAL (PURITY): BAS 306 I (97.8%)

SYNONYMS: Chlorfenapyr

CITATION: Kaspers, U., Deckardt, K., Kaufmann, W. (2005). BAS 306 I: Repeated Dose 28-d Dermal Toxicity Study in Wistar Rats. Experimental Toxicology and Ecology, BASF Aktlengeselischaft, 67056 Ludwigshafen/Rhein, Germany. Project No: 33SO346/03061. MRID 46654702. Unpublished.

SPONSOR: BASF Aktiengesellschalft

EXECUTIVE SUMMARY:

In a 28-day dermal toxicity study (MRID# 46654702), BAS 306 I (97.8%, batch #2181H88HV) was applied to the shaved skin of 10 Wistar rats/sex/dose at nominal dose levels of 0, 100, 300, or 1000 mg/kg bw/day, 6 hours/day for 5 days/week during a 4-week period. Based on recovery analyses, the effective doses applied were actually lower at 72.1, 205.5, and 835 mg/kg bw/day, respectively. The discrepancy was presumably due to the test substance adhering to the glass container. The validity of the study was not affected, nonetheless, since treatment-related effects were identified and a clear NOAEL was established.

At the high dose of 835 mg/kg bw/day, effects included clinical signs including anogenital region smearing with urine in both male and female animals and piloerection exhibited by 2 females on day 14. Blood analyses indicate mild decreases in serum albumin levels, mild increases in globulin levels, and more moderate increases in triglyceride levels. These effects were noted along with absolute and relative liver weight increases in both sexes and absolute and relative spleen weight increases in males and females, respectively.

At the mid-dose of 205.5 mg/kg bw/day, 3 females showed slight to moderate smearing of the anogenital region with urine for several days. Male rats exhibited a statistically significant increases in relative liver weight. Females showed a similar liver increase, though not statistically-significant as compared to the respective controls.

28

EPA's Records Disposition Schedule PEST 361 Scientific Data Reviews HED Records Center - File R192871 - Page 23 of 33

At the low dose of 72.1 mg/kg bw/day, liver weight increases (absolute and relative) similar to the mid-dose group were observed in male rats, but in the absence of any other findings, its adverse nature is questionable.

No treatment-related changes in bodyweight, food consumption, FOB, motor activity, brain weight, or neuropathology were identified at any dose level.

The LOAEL is 205.5 mg/kg bw/day based on clinical signs consisting of slight to moderate urine smearing of the anogenital region for several days in female rats and relative liver weight increase in both sexes. All of these effects increased in incidence and severity at the higher dose of 835 mg/kg bw/day in both sexes. The NOAEL is 72.1 mg/kg bw/day.

This 28-day dermal toxicity study in the rat is **acceptable/guideline** and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200; OECD 410) in rats.

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

23

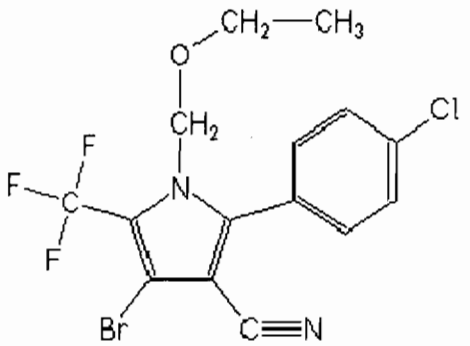
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: BAS 306 I

Description:	White to light brown solid
Lot/batch #:	2181H88HV
Purity:	97.8 %
Compound stability:	Stable for at least 7 days before initiation of study
CAS #:	122453-73-0

Structure:



2. Vehicle and/or positive control: aqua bidest

3. Test animals:

Species:	Rat	
Strain:	Wistar CrlGlxBrlHan:WI	
Age/weight at study initiation:	55 ± 1 days (males) and 54 ± 1 days (females)	
Source:	Charles River, Sulzfeld, Germany	
Housing:	Housed singly in stainless steel wire mesh cages	
Diet:	Kilba maintenance diet mouse/rat GLP, ad libitum	
Water:	Water bottles, ad libitum	
Environmental conditions:	**Temperature:**	20 - 24 °C
	Humidity:	30 - 70 %
	Air changes:	Not provided – air conditioned room
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	3 days	

B. STUDY DESIGN:

1. In life dates: Start : July 27th 2004 ; End: September 1st, and 2nd 2004

2. Animal assignment: Animals were assigned according to a list of randomization instructions compiled with a computer to the test groups listed in Table 1.

24

TABLE 1: Study design			
Test group	**Dose (mg/kg bw/day)**	**# Male**	**# Female**
Control	0	10	10
Low	100	10	10
Mid	300	10	10
High	1000	10	10

3. **Dose selection rationale:** The dose levels were selected at the request of the sponsor. The high dose was expected to cause toxicity while no information was provided for the other two doses, except that were expected to serve as mid- and low- dose, respectively.

4. **Preparation and treatment of animal skin:** The test substance was administered to the clipped dorsal skin (dorsal and dorsolateral parts of the trunk covering at least 10% of the body surface area) for about 4 weeks (5 days/week). The administration volume was 4 ml/kg body weight based upon the latest individual body weight determination. The skin was covered for 6 hrs after administration using a semiocclusive dressing, consisting of 4 layers of porous gauze dressing and an elastic dressing. After removal of the dressing, the treated skin was washed with lukewarm water.
Rats in the control group were exposed to the vehicle using the same procedure as described above.

5. **Statistics:** Means and standard deviations of each test group were calculated for several parameters including food consumption, body weight, body weight change, and food efficiency. Other parameters analyzed included feces, rearing, grip strength, forelimbs, length, hindlimbs, foot splay test, and motor activity.

C. METHODS:

1. Observations:

1a. Cageside observations:. The animals were observed in their closed home cages. Attention was paid to posture, tremor, convulsions, abnormal movements, and impairment of gait. Skin was examined for signs of local skin irritation after removing the gauze patches.

1b. Clinical examinations: Animals were observed daily (twice a day Monday to Friday and once during the weekend and public holidays) for signs of mortality and toxicity. Detailed examination of the skin was carried out daily just before treatment. Clinical examinations were conducted outside the home cage prior to the start of the administration period (day 0) and weekly thereafter (usually in the morning prior to test substance administration). The following parameters were examined: abnormal behavior during handling, fur, skin, posture, salivation, respiration, activity/arousal level, tremors, convulsions, abnormal movements, impairment of gait, lacrimation, palpebral closure, exophthalmus, feces (appearance/consistency), urine, and pupil size. The animals were also subjected to the

following sensorimotor or reflex tests: approach response, touch response, vision, papillary reflex, pinna reflex, audition (startle response).

1c. **Neurological evaluations:** Functional observational batteries (FOB) and motor activity measurements were carried out on day 25. FOB measurements started with passive observations without disturbing the animals, followed by removal from the home cage and open field observations in a standard arena. FOB measurements were performed in the morning while motor activity measurements were performed for each dose group starting at 12:45 pm for the first 5 animals and 2:00 pm for the remaining 5 animals.

2. **Body weight:** Animals were weighed prior to initiation of the study and at the beginning of each study week. The difference between the body weight on the respective day of weighing and day 0 (start of treatment) was calculated as body weight change.

3. **Food consumption:** Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. Food efficiency (group means) was calculated based upon individual values for body weight and food consumption.

4. **Ophthalmoscopic examination:** Ophthalmological examinations were carried out in all animals before treatment and in the control and high dose groups at the end of the administration period.

5. **Hematology and clinical chemistry:** Blood was collected from the retroorbital venous plexus in the morning from fasted animals that had been anaesthetized using isoflurane. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in randomized sequence. The examination was carried out in 10 animals/test group/sex. For urinalysis, individual animals were transferred to metabolism cages and urine was collected overnight. The CHECKED (X) parameters were examined.

a. **Hematology:**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
	(Thromboplastin time)		
X	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
X	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium* (K)	X	Globulins
X	Sodium* (NA)	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes, eg., *)	X	Total bilirubin
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		
X	Alanine aminotransferase		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

6. **Urinalysis:** Individual animals were transferred to metabolism cages (withdrawal of food and water) and urine was collected overnight. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	urobilinogen
X	Sediment (microscopic)	X	Blood / blood cells*
X	Protein*		Nitrate

* Optional for 28-day dermal toxicity studies

7. **Sacrifice and pathology:** All animals were sacrificed on schedule and subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+		Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Sciatic nerve
X	Jejunum*	XX	Thymus*+	X	Eyes (optic nerve)*
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*+
X	Colon*	XX	Kidneys*+	X	Lacrimal gland (extraorbital)
X	Rectum*	X	Urinary bladder*	X	Parathyroid*
XX	Liver*+	XX	Testes*+	X	Thyroid*
				X	Salivary glands (mandibular and sublingual)
				X	Female mammary gland
	Gall bladder* (not rat)	XX	Epididymides*+		OTHER
	Bile duct* (rat)	X	Prostate*	X	Bone (sternum and/or femur)
X	Pancreas*	X	Seminal vesicles*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin* (treated & untreated areas)
X	Trachea*	XX	Uterus*+	X	All gross lesions and masses*
X	Lung*	X	Mammary gland*		
X	Nose*	X	Bladder		
X	Pharynx*	X	Vagina		
X	Larynx*	X	Seminal vesicles		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200
+ Organ weights required.

II. RESULTS:

Concentration control analyses revealed a recovery of 68.5- 84.4 % of the nominal concentration. The discrepancy was attributed to the test substance adhering to the glass wall when suspended in water. The effectively applied dose levels were 72.1 (group 1), 205.5 (group 2), and 835 (group 3) mg/kg bw/day, respectively. The validity of the study was not, however, compromised since substance-related adverse findings were observed and a clear NOAEL was established.

A. OBSERVATION(s):

1. **Clinical signs of toxicity:** In the low dose group, skin lesions were observed in one female from day 22 to day 28. This single occurrence was considered to be spontaneous and not treatment-related. The mid-dose group (3 females) showed slight to moderate anogenital region smearing with urine on several days. The high dose group also showed slight (5 males) to moderate (6 females) anogenital region smeared with urine on several days. Piloerection was observed in 2 high dose females on day 14.

2. **Mortality:** No animal mortality was reported.

3. **Neurological evaluations**: FOB findings were considered incidental and not treatment related since observations were equally distributed between treated and untreated groups, not

based on a dose response relationship, occurred only before day zero, or occurred only in a single animal. Females of the high dose group exhibited reduced (66.2%) rearing, however, such finding was not considered treatment-related in the absence of other findings such as reduced motor activity. Additionally, no treatment-related changes in motor activity were identified.

4. **Dermal Irritation:** No dermal effects were observed

B. BODY WEIGHT AND WEIGHT GAIN:

TABLE 2. Average body weights and body weight gains during 28 days of treatment [a]

Dose rate (mg/kg bw /day)	Body weights (g±SD)					Total weight gain	
	Day 0	Day 7	Day 14	Day 21	Day 28	g	% of control
Male							
0	238.6 ± 7.5	254.8 ± 11.5	271.7 ± 14.2	282.1 ± 18.9	287.0 ±19.6	48.4 ± 12.8	-
72.1	243.1 ± 7.1	260.2 ± 8.0	277.8 ± 9.7	291.6 ± 11.4	297.7 ± 12.6	54.6 ± 10.9	112.8
205.5	239.4 ± 9.9	254.5 ± 16.5	272.1 ± 19.9	284.8 ± 22.7	288.6 ±25.3	49.2 ± 17.2	101.7
835	239.0 ± 5.8	255.6 ± 6.3	273.7 ± 11.2	288.0 ± 14.3	293.0 ± 18.9	54.0 ± 17.8	111.6
Female							
0	168.8 ± 5.0	175 ± 7.0	190.2 ± 9.9	196.0 ± 9.9	203.1 ±10.6	34.3 ± 8.1	-
72.1	167.6 ± 6.3	174.7 ± 5.8	185.3 ± 9.4	196.4 ± 15.4	207.4 ± 13.8	39.9 ± 11	116.3
205.5	167.6 ± 3.8	173.6 ± 6.4	184.4 ± 7.7	196.3 ± 11.1	201.4 ±12.3	33.8 ± 11.2	98.5
835	167.8 ± 7.4	176.8 ± 10.0	189.9 ± 13.2	204.0 ± 14.2	207.7 ± 15.6	39.9 ± 12.4	116.3

[a] Data obtained from pages 66-69 in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. FOOD CONSUMPTION AND EFFICIENCY:

1. **Food consumption:** No test substance-related findings were observed

D. OPHTHALMOSCOPIC EXAMINATION: No substance related findings were observed. Findings were spontaneous in nature and equally distributed between treated and untreated animals.

E. BLOOD ANALYSES:

1. **Hematology:** No test substance-related findings were reported.

2. **Clinical chemistry:** At the end of the treatment period, decreased albumin and increased globulin levels were observed in the serum of males and females of the high dose group. Increased inorganic phosphate and triglyceride levels were also found in the serum of high dose males. No other clinical chemistry changes were reported.

Table 3: Clinical Chemistry Changes [a]

	Dose (mg/kg bw/day)			
	0	**72.1**	**205.5**	**835**
		Males		
Albumin (g/L)	36.34 ± 0.91 (100%)	36.54 ± 1.06 (100%)	35.89 ± 0.71 (98.7%)	35.16 ± 0.77 ** (96%)
Globulin (g/L)	25.84 ± 1.09 (100%)	26.43 ± 1.52 (102%)	26.11 ± 1.49 (101%)	28.00 ± 1.54 ** (108%)
Triglycerides (mmol/L)	0.26 ± 0.10 (100%)	0.25 ± 0.05 (96%)	0.31 ± 0.09 (119%)	0.40 ± 0.13 ** (153%)
		Females		
Albumin (g/L)	38.39 ±1.71 (100%)	37.23 ± 1.61 (97%)	37.22 ± 1.39 (97%)	35.53 ± 1.53 ** (92%)
Globulin (g/L)	27.30 ± 1.32 (100%)	27.38 ± 1.19 (100%)	28.12 ± 1.05 (103%)	30.21 ± 1.71 ** (110%)
Triglycerides (mmol/L)	0.21 ± 0.04 (100%)	0.23 ± 0.04 (110%)	0.22 ± 0.04 (105%)	0.26 ± 0.04 (123%)

[a] Data obtained from pages 102-107 in the study report
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

F. URINALYSIS:

Males in the high dose group excreted increased amounts of urine with decreased specific gravity. No other changes were reported.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Tables 4 and 5 list statistically significant changes in absolute and relative organ weights, respectively. Liver weight increases (absolute and relative) were observed in both sexes particularly in males where mild increases were seen even at the low dose of 72.1 mg/kg bw/day. In the case of females, only the high dose group was considered to be treatment-related due to an equivocal relationship. Spleen weight increases (absolute weight in females and relative weight in males) were not considered to be of biological significance.

Table 4: Absolute Organ Weight (% relative to control group) [a]

	Dose (mg/kg bw/day)			
	0	**72.1**	**205.5**	**835**
		Males		
Liver (g)	6.505 ± 0.624 (100%)	7.217 ± 0.538 * (111%)	6.960 ± 0.817 * (107%)	7.557 ± 0.604 ** (116%)
Brain (g)	1.956 ± 0.043	1.98 ± 0.099	1.944 ± 0.085	2.024 ± 0.081

Spleen (g)	0.48 ± 0.065 (100%)	0.515 ± 0.091 (107%)	0.48 ± 0.052 (100%)	0.553 ± 0.047 (115%)
Females				
Liver (g)	4.827 ±0.462 (100%)	5.284 ± 0.631 (109%)	5.147 ± 0.503 (107%)	6.062 ± 0.599 ** (126%)
Brain (g)	1.816 ± 0.055	1.812 ± 0.059	1.775 ± 0.063	1.813 ± 0.054
Spleen (g)	0.406 ± 0.063 (100%)	0.466 ± 0.072 * (115%)	0.397 ± 0.049 (98%)	0.452 ± 0.072 (111%)

[a] Data obtained from pages 112-113 in the study report
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

Table 5: Relative Organ Weight (% relative to control group) [a]

	Dose (mg/kg bw/day)			
	0	**72.1**	**205.5**	**835**
Males				
Liver	2.545 ± 0.128 (100%)	2.752 ± 0.168 ** (108%)	2.706 ± 0.138 * (106%)	2.931 ± 0.101** (115%)
Brain	0.769 ± 0.045	0.756 ± 0.043	0.76 ± 0.047	0.787 ± 0.036
Spleen	0.188 ± 0.023 (100%)	0.197 ± 0.037 (105%)	0.187 ± 0.017 (99%)	0.215 ± 0.014* (114%)
Females				
Liver	2.714 ±0.215 (100%)	2.917 ± 0.223 (107%)	2.903 ± 0.188 (107%)	3.322 ± 0.182 ** (122%)
Brain	1.024 ± 0.065	1.006 ± 0.07	1.004 ± 0.048	0.998 ± 0.066
Spleen	0.229 ± 0.034 (100%)	0.258 ± 0.034 (113%)	0.224 ± 0.024 (98%)	0.248 ± 0.037 (108%)

[a] Data obtained from pages 114-115 in the study report
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

2. **Gross pathology:** No treatment-related findings were reported.

3. **Microscopic pathology**: No treatment-related findings were reported.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: BAS-306 I was applied via the dermal route to groups of 10 male and female Wistar rats (8 hours/day, 5 days/week) for 4 weeks at nominal doses of 0, 100, 300, and 1000 mg/kg body weight/day. Based on concentration control analyses, the actual dose levels effectively applied were lower at 72.1, 205.5, and 835 mg/kg body weight/day, respectively. The validity of the study was not affected by the lower effectively applied doses since treatment-related effects were identified and a clear NOAEL was obtained. The results of the study revealed no signs of local irritation or neurotoxicity based on clinical examinations and histopathology. There were, however, indications of general systemic toxicity including smeared urine of anogenital region and piloerection.
Clinical pathology findings included slightly decreased albumin and slightly increased globulin in the serum of both sexes. Inorganic phosphate and triglyceride levels were also increased in high dose males which also excreted increased amounts of urine with decreased specific gravity.

The high dose groups (males and females) also exhibited increased liver weights. The mild increase in liver weight in the low- and mid-dose groups and increase in spleen weight in the high dose group were regarded as incidental due to an equivocal dose response relationship. The LOAEL is 205.5 mg/kg/day based on general systemic toxicity. The NOAEL is 72.1 mg/kg body weight/day for both sexes.

B. REVIEWER COMMENTS:

The aim of this study was to investigate the toxicity of BAS 306 I (chlorfenapyr) including target organs following dermal application for 4 weeks. BAS-306 I was applied at nominal doses of 0, 100, 300, and 1000 mg/kg bw/day for 8 hours/day, 5 days/week. Based on recovery analyses, the doses that were effectively applied were lower at 72.1, 205.5, and 835 mg/kg bw/day. The validity of the study is not affected by the discrepancy between nominal and effectively applied doses since treatment-related effects were identified and a clear NOAEL was established.
The results of the study indicate that BAS 306 I induced the following effects via the dermal route of exposure:
At the high dose of 835 mg/kg bw/day, effects included clinical signs consisting of smearing of the anogenital region with urine in both male and female animals and piloerection in 2 females on day 14. In addition, blood analyses indicate mild decreases in serum albumin levels, mild increases in globulin levels, and more moderate increases in triglyceride levels. These effects were noted along with liver weight increases (absolute and relative in males and females) and spleen weight increases (absolute in females and relative in males).
At the mid-dose of 205.5 mg/kg bw/day, 3 females showed slight to moderate smearing of the anogenital region with urine for several days. Male rats exhibited a statistically significant increase in relative liver weight. A similar liver increase was also observed in female rats, although not statistically-significant as compared to the respective controls.
At the low dose of 72.1 mg/kg bw/day, liver weight increases (absolute and relative) similar to the mid-dose group were observed in male rats, but in the absence of any other findings, its adverse nature is questionable and may actually be considered an adaptive response.
No treatment-related changes in bodyweight, food consumption, FOB, motor activity, brain weight, or neuropathology were identified.

Based on the evidence reported, the LOAEL is 205.5 mg/kg bw/day based on slight to moderate anogenital region smearing with urine on several days in female rats and relative liver weight increase in both sexes. All of these effects increase in incidence and severity at the higher dose of 835 mg/kg bw/day. The NOAEL is 72.1 mg/kg bw/day.

C. STUDY DEFICIENCIES: None noted.

13544

R192871

Chemical Name: Chlorfenapyr

PC Code: 129093
HED File Code: 11000 Chemistry Reviews
Memo Date: 5/11/2011
File ID: 00000000
Accession #: 000-00-0137

HED Records Reference Center
6/30/2011