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

OFFICE OF PREVENTION, PESTICIDE AND TOXIC SUBSTANCES

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

MEMORANDUM

Date: May 19, 2011

SUBJECT: **Ethiprole** [AE 0316423 or RPA107382] **Title:** Bilateral Joint Review Project. Toxicology and Metabolism: Evaluation and Assessment of the Data Submitted

PC Code: 005550
Decision No.: D407907
Petition No.: 9E7750
Risk Assessment Type: NA
TXR No.: 0055806
MRID No.: See list below for multiple listing

DP Barcode: D385959
Registration No.: NA
Regulatory Action: Registration
Case No.: NA
CAS No.: 181587-01-9
40 CFR: 180.652

FROM: Judy Facey, Ph.D. Judy Facey 5/19/2011
Risk Assessment Branch 6
Health Effects Division (7509P)

THROUGH: Felecia Fort, Branch Chief Felecia Fort 5/19/2011
Risk Assessment Branch 6
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TO: Carmen Rodia
Insecticide Branch
Registration Division (7504P)

Sheila Piper
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I. CONCLUSIONS

The toxicology data base for the new chemical global joint project (bilateral review with Canada) **Ethiprole** [AE 0316423 or RPA107382] is attached as eleven separate files:
File 1: Ethiprole Acute Studies
File 2: Ethiprole Genotoxicity Studies
File 3: Ethiprole Developmental Studies

Rec'd in RRC 6/8/2011

File 4: Ethiprole Long-term Studies
File 5: Ethiprole Mouse Carcinogencity Studies
File 6: Ethiprole Neurotoxicity Studies
File 7: Ethiprole Reproduction Studies
File 8: Ethiprole Short-Term Repeat dose Studies
File 9: Ethiprole 90daydog&1yr dog Studies
File 10: Ethiprole Toxicokinetics Studies
File 11: 48016801.der

This document contains the toxicity study summaries prepared by the submitter. The responsibility for the primary review of these studies was with the Pest Management Regulary Agency (PMRA) of the Canadian government and secondary reviews of these studies were conducted by the Health Effects Division (HED) of the USEPA. Comments from USEPA were considered by PMRA and incorporated into the final version of the study summaries.

HED has prepared its own reports on cancer classification (CARC report dated, October 28, 2010, HED Document TXR # 0055516) and selection of the reference doses.

This submitted data was considered in support of the Human Health Risk Assessment for proposed insecticide uses on rice and tea.

RAB VI made minor adjustments to the PMRA evaluations as follows:

- The MRID number was added to each study adjacent to the Annex points (study name).
- The TXR number and DP Barcode were added to the header so that it occurs on each page of the monograph.
- The pagination in the header was changed to page x of y format.
- USEPA study classification was added to each study conclusion.

II. ACTION REQUESTED

Risk Assessment Branch VI (RAB VI) was requested to conduct a secondary review the toxicity data base for ethiprole submitted in support of proposed tolerances for food uses and to work in conjunction with the global partner Canada. PMRA of Canada conducted the primary reviews of the toxicity studies.

III. MRID Summary Table and location of study in the monograph.

The following tables list the study type, MRID and year of the study, the NOAEL and LOAEL or other result, study classification according to HED criteria and the page within the monograph where the study is located. Acute, subchronic, chronic, developmental, mutagenicity, general metabolism and neurotoxicity studies are listed. Studies with metabolites are also included. Studies in the reports are linked to the Table of Contents.

Study Type	MRID (year)	Results	Pages	Toxicity Category
870.1100/ Acute oral -rat	47622801 (1997)	LD_{50} > 5000 mg/kg (both sexes) LD_{50} > 7080 mg/kg (both sexes)	1-3	V
870.1100/ Acute oral-rat	476222802 (2001)	LD_{50} > 5000 mg/kg (both sexes)	4-6	V
870.1100/ Acute oral -rat	47622803 (1999)	LD_{50} > 2000 mg/kg (both sexes)	7-9	V

Study Type	MRID (year)	Classification (Y/N/O)[a]	Pages	Comments
870.5100/ *In vitro* genotoxicity - Bacterial assay for gene mutation	47622816 (1999)	Acceptable/Guideline (Y)	1-13	negative
870.5100/ Genotoxicity- *In vitro* genotoxicity - Bacterial assay for gene mutation	47622815 (2001)	Acceptable/Guideline (Y)	14-27	negative
870.5100/ *In vitro* genotoxicity - Bacterial assay for gene mutation.	47622814 (1998)	Acceptable/Guideline (Y)	28-39	negative
870.5375/ Genotoxicity/*In vitro* genotoxicity - Test for clastogenicity in mammalian cells.	47622818 (1998)	Acceptable/Guideline (Y)	40-50	negative
870.5300/ Genotoxicity/*In vitro* genotoxicity - Test for gene mutation in mammalian cells.	47622817 (1999)	Acceptable/Guideline (Y)	51-60	negative
870.5395/ Genotoxicity/*In vivo* genotoxicity (somatic cells) - Bone marrow or micronucleus.	47622819 (1999)	Acceptable/Guideline (Y)	60-67	negative
870.5550/ Genotoxicity/*In vivo* genotoxicity (somatic cells) - DNA repair.	47622820 (2001)	Acceptable/Guideline (Y)	61-74	negative

Study Type	MRID (year)	Classification (Y/N/O)[a]	Pages	Comments
870. 3700a/ Developmental Toxicity in the Rat by Gavage- Range-Finding Study	Not available (1999)	Acceptable/ Non-guidance (Y)	1-10	Adequate for providing data for use in dose selection for the definitive developmental study in rats.
870.3700/ Developmental toxicity study in rats	47622808 (2000)	Acceptable/ Guidance (Y)	11- 23	**NOAEL** for maternal and developmental toxicity = 3 mg/kg/day. **Maternal LOAEL** = 10 mg/kg/day based on increased mean liver weight. **Dev. LOAEL** = 10 mg/kg/day based on increased incidences of ossification delay for a few bones.
870.3700/ Developmental toxicity study n rabbit by gavage-range finding	Not available (1999)	Acceptable/ Non-guidance (Y)	24-33	Adequate for providing data for use in dose selection for the definitive developmental study in rabbits.
870.3700/ Developmental toxicity study in the rabbit	47622809 (2000)	Acceptable/ Guidance (Y)	34-47	**NOAEL** for maternal and developmental toxicity = 0.05 mg/kg/day. **Maternal LOAEL** = 2 mg/kg/day based on increased number of abortions, reduced body weight gains and food consumption. **Dev. LOAEL** = 2 mg/kg/day based on skeletal variations (incomplete ossification of pubis, metacarpal and /or middle phalanges).

Study Type	MRID (year)	Classification (Y/N/O)[a]	Pages	Comments
870.4300/ Combined chronic and carcinogenicity in rats.	47622813 (2001)	Acceptable/ Guidance (Y)	1- 25	**NOAEL**= (20 ppm) 0.85/1.117 (M/F) mg/kg/day. **LOAEL** = (75 ppm) 3.2/ 4.4 (M/F) mg/kg/day based on increased liver weight (↑13%, F)) and thyroid weight (↑27%/22%; M/F) associated with hepatocellular hypertrophy and thyroid follicular hypertrophy. Higher TSH plasma levels and reduced T4 plasma levels

Table 4. Chronic toxicity and carcinogenicity/ Long-term - Rats (2 years)				
870/4300 Combined chronic and carcinogenicity in rats.- Mechanism of action	47622827 (2001)	Acceptable/ Non-Guidance (Y)	26-37	Adequate for providing supporting data to inform the combined chronic toxicity/ carcinogenicity study in the rat on the potential effects on thyroid function after two weeks of treatment in the males rats using the Perchlorate discharge test.
870/4300 Combined chronic and carcinogenicity in rats.- Mechanism of action	47622826 (2001)	Acceptable/ Non-guidance (Y)	38- 47	Adequate for providing supporting data to inform the combined chronic toxicity/ carcinogenicity study in the rat on the effect of repeated oral doses of ethiprole on the biliary excretion of intravenously administered ^{125}I-Thyroxine (T4) in the male rats.
870/4300 Combined chronic and carcinogenicity in rats.- Mechanism of action	47622825 (2001)	Acceptable/ Non-guidance (Y)	48-55	Adequate for providing supporting data to inform the combined chronic toxicity/ carcinogenicity study in the rat on the effect of repeated oral doses of ethiprole on the pharmacokinetics of intravenously administered ^{125}I-Thyroxine (T4) in the male rats.

Table 5. Chronic toxicity and carcinogenicity/ Long-term - Mouse (2 years)				
Study Type	**MRID (year)**	**Classification (Y/N/O)[a]**	**Pages**	**Comments**
870.4200b/ Carcinogenicity study in C57BL/6 mice.	47622812 (2001)	Acceptable/ Guidance (Y)	1-12	**NOAEL** = (300 ppm) 50.8 mg/kg/day in Males; (150 ppm) 36.3 mg/kg/day in Females. **LOAEL** = (300 ppm) 73.5 mg/kg/day in Females based on Reduced survival rate. Slight increased in the incidence of hepatocellular adenomas (↑12%). **LOAEL** not established in Males.
870.4200b/ 28 day explanatory toxicity study in the C57BL/6 mouse- Mechanism of action and supporting data.	47622829 (2002)	Acceptable/ Non-guidance (Y)	13-23	**NOAEL** = (100 ppm) 23.2/ 23.4 mg/kg/day); this study was conducted in provide supporting data to inform the Carcinogenicity study.

Table 6. Neurotoxicity Studies				
Study Type	**MRID (year)**	**Classification (Y/N/O)[a]**	**Pages**	**Comments**
870.6200a/ Acute neurotoxicity screen in rats	47622821 (2001)	Acceptable/ Guidance (Y)	1- 12	**NOAEL** = Not established. **LOAEL** = 100 mg/kg/day based on Reduced landing foot splay(↓28%/25%; M/F) at FOB and reduced motor activity.

870.6200a/ Acute neurotoxicity screen in rats	47622822 (2001)	Acceptable/ Guidance (Y)	13- 24	**NOAEL** = 35 mg/kg/day. **LOAEL** = 250 mg/kg/day based on increase tremors (females), decrease grooming (both sexes), decreased arousal alert (females), increased number of animals for which no assessment of gait was possible (females), increase eye closure (females), increased standing/sitting hunched (females), deceased activity and rearing counts (females), increased hindlimb and forelimb grip strength (males), and decreased forelimb grip; strength (day 8) (females), decreased splay (females, day 1), increase splay (males, Day 8).
870.6200b/ Subchronic neurotoxicity screen in rats	47622823 (2001)	Acceptable/ Guidance (Y)	25-35	**Systemic NOAEL**= 1.4/8.4 mg/kg/day (M/F). **Systemic LOAEL**= 7.2/33 mg/kg/day (M/F) based on increased thyroid weight (↑24%) in males and higher thyroid weight (↑35%) and liver weights (↑65%) in females. **Neurotoxicity NOAEL** = 28.7/33 mg/kg/day (M/F). **Neurotoxicity LOAEL** not established. No FOB changes, no neuropathology observed.

Study Type	**MRID (year)**	**Classification (Y/N/O)[a]**	**Pages**	**Comments**
870.3800/ Two generation reproduction in Rats- Range finding study.	Not available (2000)	Acceptable/ Non-guidance (Y)	1- 18	**Parental toxicity** NOAEL = 50 ppm (3.7 mg/kg/day M/F) LOAEL = 250 ppm, (19.3 mg/kg/day) based on increased body weight gains, organ weight changes in thyroid and liver. **Reproductive toxicity** NOAEL = 1000 ppm (70 mg/kg/day) (HDT) **Offspring toxicity** NOAEL = 250 ppm (19.3 mg/kg/day) LOAEL = 1000 ppm (70 mg/kg/day) based on ↓ F1pup body weights with associated delays in acquisition of puberty.
870.3800/ Two generation reproduction in Rats	47622810 (2001)	Acceptable/ Guidance (Y)	19- 47	**Parental toxicity** NOAEL = 75 ppm (4.77-6.03 mg/kg/day M/F) LOAEL = 500 ppm, (32.33-39.63 mg/kg/day) based on increase liver

				weight in both P and F1 adults (14% and 16%, respectively). **Reproductive toxicity** NOAEL = 500 ppm (32.33-39.63 mg/kg/day males; 37.36-45.20 mg/kg/day females) (HDT) **Offspring toxicity** NOAEL = 75 ppm (4.77-6.03 mg/kg/day) males LOAEL = 500 ppm (32.33-39.63 mg/kg/day) males based on Reduced F1 and F2 pup body weights associated with delays in acquisition of puberty.

Study Type	MRID (year)	Classification (Y/N/O)[a]	Pages	Comments
870.3050/ 28-day feeding study in rats	47622804 (2001)	Acceptable/ Guidance (Y)	1- 17	**NOAEL** = (20ppm) 9.2/9.6 mg/kg/day (M/F). **LOAEL** = (100 ppm) 46.1/46.3 mg/kg/day (M/F) based on increased prothrombin time, ALAT activity, cholesterol, triglyceride and total protein concentration, imbalance of thyroid hormones, increased liver weights (35%/96%; M/F), thyroid weight (41%/40%; M/F) and adrenals weights (16%/15%; M/F)
870.3050/ 28-day feeding study in rats- Metabolite of ethiprole RPA112916	47622805 (2000)	Acceptable/ Non-Guidance (Y)	18- 34	**NOAEL** = (50 ppm) 5.2 mg/kg/day. **LOAEL** = (500 ppm) 53.5 mg/kg/day based on increase TSH levels, increased liver weights associated with a dark liver and/or a diffuse centrilobular to panlobular hepatocellular hypertrophy, and an increased incidence of microvacuolation of the adrenal zona fasciculate.
870.3050/ 28-day feeding study in mice	476222828 (1999)	Acceptable/ Guidance (Y)	35- 47	**NOAEL** = (50 ppm) 9.3/11.8 mg/kg/day (M/F). **LOAEL** = (250 ppm) 47.4/57.9 mg/kg/day (M/F) based on Lower total birirubin (↓64-69%), increased liver weights (↑10-16%) and histopathologic changes in the liver.
870.3100/ 90-day feeding study in rats	47622806 (2000)	Acceptable/ Guidance (Y)	48-66	**NOAEL** = (20 ppm) 1.2/1.5 mg/kg/day (M/F). **LOAEL** = (500 ppm) 30.5/37.6 mg/kg/day (M/F) based mortality, higher prothrombin time, Cholesterol, Triglyceride, Total protein, Calcium concentrations and lower chloride concentration, imbalance of thyroid hormones, increased

				liver weight (57%/96%; M/F) and thyroid weight (48%/44%; M/F) and histopathologic changes in the liver and thyroid.

Study Type	MRID (year)	Classification (Y/N/O)[a]	Pages	Comments
870.3150/ 90-day feeding in the dog	47622807 (2001)	Acceptable/ Guidance (Y)	1- 16	**NOAEL** = (30/60 ppm) 1.0/3.6 mg/kg/day (M/F). **LOAEL** = (30/200 ppm) 3.2/8.5 mg/kg/day (M/F) based on In Males: decrease prostate weight (↓59%), testis weight (↓31%) and epididymis weight (↓34%) and increase thymus weight (29%). In Females: Mortality, increased alkaline phosphatase activity and thymic atrophy
870.4100b/ Chronic feeding in dogs	47622811 (2001)	Acceptable/ Guidance (Y)	17- 25	**NOAEL** = (30 ppm) 0.70/0.76 mg/kg/day (M/F). **LOAEL** = (90 ppm) 2.73/2.51mg/kg/day (M/F) based on overall reduced body weight gain.

Study Type	MRID (year)	Classification (Y/N/O)[a]	Pages	Comments
870.7485/ Metabolism in rats	47622824 (1999)	Acceptable/ Guidance (Y)	1-34	Metabolism, absorption, pharmacokinetics, excretion – metabolic path

Study Type	MRID (year)	Classification (Y/N/O)[a]	Pages	Comments
870.7800/ Immunotoxicity- rats	48016801 (2010)	Unacceptable/ Guidance (Y)	1-10	Not immunotoxic at 500 ppm (HDT)

[a]Y= The study satisfies the guideline requirement. N= Does not satisfy the guideline requirement. O= satisfies the guideline requirement when combined with other studies.

EPA Reviewer: Judy A. Facey, Ph.D. **Signature:** Judy Facey
Risk Assessment Branch VI, Health Effects Division (7509P) Date: 5/18/2011
EPA Secondary Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G. Yang
Risk Assessment Branch VI, Health Effects Division (7509P) Date: 5/18/2011

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DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity Study in Rats [diet]; OPPTS 870.7800

PC CODE: 005550 **DP BARCODE:** D385959
TXR #: 0055806

TEST MATERIAL (PURITY): Ethiprole (95.9% a.i.)

SYNONYMS: 5-amino-1-[2, 6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1*H*-pyrazole-3-carbonitrile

CITATION: Odin-Feurtet, M. (2010). 28-Day immunotoxicoty study in the female rat by dietary administration. Bayer CropScience, Sophia Antipolis Cedex, France. Sponsor Identification No.: Lynx-PSI N°TXEHX015, March 02, 2010. MRID 48016801. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience. Alfred Nobel Str.50. 40789 Monheim, Germany.

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 48016801), Ethiprole (95.9% a.i.; batch # ETP090704) was administered to female Wistrar Rj:WI (IOPS HAN) rats (10/dose) in the diet at dose levels of 0, 20, 100, or 500 ppm (0, 1.6, 8.4, or 41.2 mg/kg/day, respectively) for 28 days. An additional group of 10 female rats were dosed cyclophosphamide daily by gavage (3.5 mg/kg/day) for 28 days at a dose volume of 5 mL/kg to serve as the positive control group. On Study Day 25, all animals were immunized with 0.5 mL Sheep Red Blood Cell (SRBC) (5 x 10^8 SRBC/mL) by intravenous injection via the tail vein. On Study Day 29, blood samples were collected from the retro-orbital venous plexus of each animal for T-dependent antibody response (TDAR), ant-SRBC IgM concentrations with an Enzyme-Linked Immunosorbent Assay (ELISA). All animals were necropsied, gross pathology observations were performed and selected organs (spleen and thymus) weighed.

There were no treatment related effects on mortality, clinical observations, food consumption, and thymus and spleen weights in all treatment groups compared to the control group. Higher mean body weight and body weight gains were observed at the 500 ppm compared to the control. For ant-SRBC IgM response, decreased anti-SRBC IgM concentration was observed at 500 ppm with the mean IgM concentration lower by 63% (not statistically significant) relative to the control animals. The positive control (cyclophosamide) induced no treatment-related mortality, no clinical signs or change in body weight parameters, food consumption. Mean terminal body weight and mean absolute and relative spleen and thymus weights were statistically lower compared to the vehicle control group. Mean anti-SRBC IgM concentration of the positive control group was markedly lower than the vehicle control group. However, a high inter-

individual variability was noted in all the groups exposed to ethiprole as well as in the control group. There were several animals (20-40%) in each group were considered either non-responders or severely immunosuppressive (compared with positive control). For example, 40% (4/9) of animals in the vehicle control group resulted in the same ranges of positive control and were considered non-responders. The validity of the positive data is questionable. The individual animal data suggested that the performing laboratory had difficulty conducting the assay. The quality and accuracy of the study is unacceptable. It is also noted that the anti-SRBC Ig M antibody level was measured at day 4 after immunization which may not have reached the optimum antibody response. The laboratory should establish an antibody response curve and identify the optimum post-immunization day for maximum antibody production before conducting the assay.

The Natural Killer (NK) cell activity assay was not performed and no justification was provided. The HED Guidance stated that if the test substance produces dose-related suppression of the TDAR (anti-SRBC response) without evidence of overt toxicity to non-immune system, then the test substance is considered as immunotoxic and no further study is required. If the TDAR is negative and observational immune system endpoints (e.g., changes in lymphoid organ weights, hematology, histopathology) suggest any evidence of immunotoxicity, then NK cell activity must be evaluated as a measure of nonspecific (innate) immune function. In contrast, if the TDAR assay is negative and evaluation of observational endpoints provide no evidence of immunotoxicity, the test article is considered negative for immunotoxicity and evaluation of NK activity is not necessary. Finally, if the TDAR is negative and limited or no observational immune system endpoints are available, a NK assay must be performed. Justification must be provided to support classifying modulation of the TDAR as secondary to overt toxicity, or to conclude that an NK cell assay is not required and this justification should be submitted to the Agency with the immunotoxicity study.

This immunotoxicity study is classified **Unacceptable/guideline** and does not satisfy the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

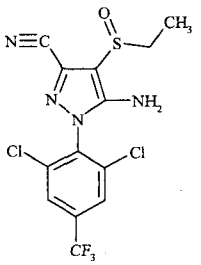
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Ethiprole

Description:	Beige powder
Batch #:	ETP090704
Purity:	95.9% a.i.
Compound Stability:	The test substance was stored in an air-tight, light resistant container at room temperature.
CAS # of TGAI:	181587-01-9



2. Vehicle: Diet

Positive control: Cyclophosphamide (Batch # 097K1311; purity 100.2%) in sterilized water.

3 Test animals

Species:	Rat	
Strain:	Wistar Rj:WI (IOP HAN)	
Age/weight at study initiation:	Approximately 7 weeks /176-209 g females	
Source:	R. Janvier, Le Genest St Isle, France.	
Housing:	Individually in stainless steel, wire-mesh cages suspended over cage board	
Diet:	Certified rodent powdered and irradiated diet A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France)	
Water:	Reverse osmosis-treated tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	20- 24°C
	Humidity:	40- 70%
	Air changes:	10 to 15 /hr
	Photoperiod:	12h light/12 h dark
Acclimation period:	12 days	

B. STUDY DESIGN:

1. In life dates: Start: 10/14/2009 End: 11/23/2009

2. Selection of test species/gender and animal assignment: The female rat was determined to be the appropriate species and sex for testing in an immunotoxicity study. There was no significant difference between rats and mice in general toxicity parameters. However, decreased spleen weights were observed in female rats while male rats and both sexes of female mice were similar to control in the 28-day studies.

Animals were randomly assigned, stratified by body weight in a block design, to the test groups noted in Table 1.

TABLE 1: Study design			
Test group	**Conc. in diet (ppm)**	**Dose to animal (mg/kg/day; F)**	**# Female**
Control	0	0	10
Low	20	1.6	10
Mid	100	8.4	10
High	500	41.2	10
Positive control Cyclophosphamide	N/A	3.5	10

Data were obtained from page 18 of the study report.
N/A Not applicable

3. **Dose selection:** The dose was selected based on previous toxicity studies. In a 28-day toxicity study in rats, the dose of 2500 ppm induced a treatment-related increase of mortality in males and an increase in cholesterol, triglycerides and total protein concentration in both sexes. A significant increase in thyroid stimulating hormone in the plasma was also observed at 2500 and 500 ppm in both sexes. Significant increases in liver and thyroid weights in conjunction with hepatocellular hypertrophy and follicular cell hypertrophy were observed in both males and females at 500 and 2500 ppm. In a 90-day toxicity study, treatment-related mortalities, increases in cholesterol, protein and calcium concentrations, marked thyroid hormone changes and increases in liver and thyroid weights associated with microscopic observations in these two organs were observed at 500 and/or 2500 ppm in both sexes.

4. **Diet preparation, administration, and analysis:** The test substance was incorporated into the diet to provide the required dietary concentrations. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. There was one preparation for each concentration. When not in use, the diet formulations were stored at room temperature. The stability of the test substance in the diet has been determined in a previous study (9) at concentrations of 5 and 2500 ppm for a time period which covers the period of storage and usage for the current study. The homogeneity of the test substance in the diet was verified for the lowest and highest concentrations to demonstrate adequate formulation procedures. The mean value obtained from the homogeneity check was taken as measured concentration. Dietary levels of the test substance were verified for each concentration.

Results

Homogeneity (% RSD): 1.7- 4.1%

Concentration (mean % nominal): 92-102%

The analytical data indicate that the test substance was homogeneous in the suspensions and that the nominal concentrations were achieved. Provided that the cited stability data indicate that the test material is stable in the vehicle or at room temperature, the slight variation between the nominal and actual dosages to the animal was acceptable.

5. **Positive controls:** Cyclophosphamide, a known immunosuppressive drug, (batch number 097K1311: a white powder, 100.2% purity) was used throughout the study. Information on the chemical characterization of cyclophosphamide was documented by the supplier Sigma-Aldrich,

USA. Cyclophosphamide was stored in an air-tight, light-resistant container at approximately 4°C.

6. **Statistics:** The following statistical procedures (two-tailed) were used:

Parameter	Statistical procedure
Body weight, body weight gain, food consumption, hematology parameters, and organ weights	Bartlett test followed by either the ANOVA or Kruskal-Wallis Test. If the ANOVA test is use it is followed by the Dunnett Test; If the Kruskal-Wallis Test is used it is followed by the DUNN Test.
Antibody-forming cell (AFC) assay data (absolute and relative to body spleen weights, mean number of spleen cells IgM AFC/10^6 spleen cells, and IgM AFC/spleen)	MANN-WHITNEY (2-sided test) followed by Kruskal-Wallis test and/or DUNN Test (2- sided test)
Positive control data	MANN- WHITNEY for comparison with vehicle controls

C. METHODS

1. **Observations:** All animals were checked for moribundity and mortality twice daily (once daily on weekends or public holidays).

2. **Body weight:** Each animal was weighed at least weekly during the acclimatization period, on the start of treatment (Study Day 1), then at weekly intervals throughout the treatment period and before necropsy.

3. **Food consumption and compound intake:** The weight of food supplied and of that remaining at the end of the food consumption period was recorded weekly for all animals during the treatment period. The weekly mean achieved dosage intake in mg/kg body weight/day for each week and for Weeks 1 to 4 was calculated (except for the group exposed to the immunosuppressive agent cyclophosphamide).

4. **Sacrifice and pathology:** At study termination on Day 29, all animals (non-fasted) were sacrificed by exsanguination while under deep anesthesia (Isoflurane inhalation). Blood samples were collected via the inferior vena cava of all dosed group animals at the time of necropsy. Blood (0.5 ml) was placed into tubes with clot activator (for serum). After centrifugation, serum aliquots were frozen (approximately -80°C) until analysis. The spleen and thymus was collected from all animals and weighed.

5. **Immunotoxicity**

   a. **Immunization:** On Day 25, all animals were immunized with an intravenous injection (0.5 mL/animal) of Sheep Red Blood Cells (SRBC) (5 x 10^8 cells/mL) via the tail vein.

   b. **Anti-SRBC IgM concentration, ELISA:** On Day 29, the T-dependent antibody immune response was evaluated using an Enzyme-Linked Immunosorbent Assay (ELISA) to determine the level of anti-SRBC IgM. Rat Anti-Sheep Red Blood Cell IgM ELISA kits from Life Diagnostics (West Chester, PA 19380 - USA) were used. Detailed procedures were not provided.

   c. **Natural Killer (NK) cell activity assay**: Not performed

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All rats survived to scheduled termination.

2. **Clinical signs of toxicity:** There were no treatment-related clinical observations. Few clinical findings (hair loss) were observed in the positive control (cyclophosphamide) group; these were considered not to be treatment related.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Higher mean body weight gain/day was observed at 500 ppm over the entire study period but was statistically significant only during the first and third weeks of the study ($p<0.05$ and $p<0.01$, respectively); the overall mean body weight gain was 32% higher ($p<0.01$) than the control group. At 100 and 20 ppm, body weight and body weight gain were unaffected by treatment with the test substance ethiprole. For positive control at 3.5 mg/kg body weight/day, body weight and body weight gain in females were unaffected by treatment with cyclophosphamide.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** There was no test-substance related effect on food consumption. Food consumption was unaffected in females by treatment with cyclophosphamide.

2. **Compound consumption:** Test substance intake is presented in Table 1.

D. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Up to the highest dose level tested of 500 ppm, spleen and thymus weights were not affected by the treatment. In the positive control group, mean absolute spleen and thymus weight and mean spleen and thymus to body weight ratio were statistically significantly lower compared to the controls.

TABLE 2. Terminal body, spleen, and thymus weights[a]

Treatment group (ppm)	Mean weight				
	Terminal body weight (grams ±SD)	Spleen		Thymus	
		Absolute weight (grams ±SD)	Relative weight (%)	Absolute weight (grams ±SD)	Relative weight (%)
Females (n=10/group)					
0 (Control)	252.1±20.2	0.733±0.146	0.289±0.05	0.601±0.147	0.238±0.058
20	257.5±14.6	0.768±0.103	0.299±0.05	0.587±0.076	0.229±0.034
100	260.0±18.4	0.803±0.160	0.306±0.04	0.660±0.114	0.254±0.038
500	270.8±15.5	0.723±0.106	0.267±0.04	0.653±0.157	0.240±0.051
Positive control – cyclophosphamide	255.2±16.7	0.483±0.068**	0.189±0.03**	0.433±0.113**	0.169±0.04**

[a]Data were obtained from pages77-79 of the study report.

**Statistically significant from the control group ($p<0.01$)

2. **Gross Pathology:** In the animals treated with cyclophosphamide (positive control), atrophic/small spleen was found in 5/10 animals and atrophic/small thymus was found in 4/10 animals.

3. **Microscopic pathology:** Microscopic examinations were not performed but are not a guideline requirement for immunotoxicity studies.

E. Immunotoxicity

Anti-SRBC response (ELISA): Decreased anti-SRBC IgM concentrations were observed at 500 ppm with the mean IgM concentration lower by 63% (not statistically significant) relative to the control animals. In the positive control, anti-SRBC IgM concentrations were markedly lower ($p<0.01$) with a mean anti-SRBC IgM concentration lower by 95% when compared to the vehicle control. A high inter-individual variability was noted in all the groups exposed to ethiprole as well as in the control group. There were several animals (20-40%) in each group were considered either non-responders or severely immunosuppressive (compared with positive control) (Table 4). For example, 40% (4/9) of animals in the vehicle control group resulted in the same ranges of positive control and were considered non-responders. The validity of the positive control data was questionable. The individual animal data suggested that the performing laboratory had difficulty conducting the assay. The quality and accuracy of the study is questionable.

TABLE 3 Results of the ELISA[a]

Treatment group (ppm)	Mean anti-SRBC IgM titer (u/mL±SD)
0 (Control)	6789 ± 6527
20	5625 ± 5230
100	9131 ± 13045
500	2536 ± 1706
Positive control - cyclophosphamide	343 ± 187**

**Statistically different from the control group ($p<0.01$)
[a]Data were obtained from pages 72-73 of the study report.
Note: The study report mistakenly using mg/kg/day as the dose unit for treatment groups.

a. **NK cell activity assay**: Not performed.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Exposure of female rats to ethiprole at 500 ppm (corresponding to 41.2 mg/kg body weight/day) showed lower concentration of SRBC-specific IgM antibody after the intravenous injection of the antigen SRBC as compared to the control group. This difference was not associated with any difference in organ weights or macroscopic changes of the main immune system target organs thymus and spleen. Considering the absence of gross pathology findings in target organs and the significant toxicological findings observed in previous toxicological studies conducted with this test compound at similar dose levels, the

lower IgM response after injection of the antigen SRBC is considered to be secondary to systemic toxicity and not evidence of a primary immunotoxic effect.

B. REVIEWERS COMMENTS: There were no treatment-related effects on mortality, clinical observations, feed consumption, or thymus and spleen weights. No gross pathology findings were observed on spleen and thymus. At 500 ppm, the mean body weight was higher (↑8.5%) than in the control group throughout the entire study period; the overall mean body weight gain was higher by 32%.

For anti-SRBC IgM response, decreased anti-SRBC IgM concentrations was observed at 500 ppm with the mean IgM concentration lower by 63% (not statistically significant) relative to the control animals. The positive control (cyclophosamide) induced no treatment-related mortality, no clinical signs or change in body weight parameters, food consumption. Mean terminal body weight and mean absolute and relative spleen and thymus weights were statistically lower compared to the vehicle control group. Mean anti-SRBC IgM concentration of the positive control group was markedly lower than the vehicle control group. However, a high inter-individual variability was noted in all groups exposed to ethiprole as well as in the control group. There were several animals (20-40%) in each group were considered either non-responders or severely immunosuppressive (compared with positive control). For example, 40% (4/9) of animals in the vehicle control group resulted in the same ranges of positive control and were considered non-responders. The individual animal data suggested that the performing laboratory had difficulty conducting the assay. The quality and accuracy of the study is questionable. It is also noted that the antibody level was measured at day 4 after immunization which may not have reached the optimum antibody response. The laboratory should establish an antibody response curve and identify the optimum post-immunization day for maximum antibody production before conducting the assay.

The Natural Killer (NK) cell activity assay was not performed and no justification was provided. The HED Guidance stated that if the test substance produces dose-related suppression of the TDAR (anti-SRBC response) without evidence of overt toxicity to non-immune system, then the test substance is considered as immunotoxic and no further study is required. If the TDAR is negative and observational immune system endpoints (e.g., changes in lymphoid organ weights, hematology, histopathology) suggest any evidence of immunotoxicity, then NK cell activity must be evaluated as a measure of nonspecific (innate) immune function. In contrast, if the TDAR assay is negative and evaluation of observational endpoints provide no evidence of immunotoxicity, the test article is considered negative for immunotoxicity and evaluation of NK activity is not necessary. Finally, if the TDAR is negative and limited or no observational immune system endpoints are available, a NK assay must be performed. Justification must be provided to support classifying modulation of the TDAR as secondary to overt toxicity, or to conclude that an NK cell assay is not required and this justification should be submitted to the Agency with the immunotoxicity study.

This immunotoxicity study is classified **Unacceptable/guideline** and does not satisfy the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

C. STUDY DEFICIENCIES:

- High inter-individual variability was noted in all groups exposed to ethiprole as well as in the control group. There were several animals (20-40%) in each group were considered either non-responders or severely immunosuppressive (compared with positive control). The data suggested that the performing laboratory had difficulty conducting the assay. The quality and accuracy of the study is questionable.

- It is also noted that the antibody level was measured at day 4 after immunization which may not be the optimum antibody response. The laboratory should establish an antibody response curve and identify the optimum post-immunization day for maximum antibody production.

- Mislabeled dietary levels were observed throughout the study including many tables with mg/kg as dietary levels, instead at ppm levels. These errors suggested poor QA/QC of the study.

- No NK cell activity assay was conducted.

Table 4. Individual anti-SRBC IgM response

ANIMAL NUMBER	SEX	GROUP/ SUBGROUP	DAY OF PHASE	IgM [illegible]
[illegible]	F	1/1	29	[illegible]
[illegible]	F	1/1	29	[illegible]
[illegible]	F	1/1	29	[illegible]
[illegible]	F	1/1	29	900
[illegible]	F	1/1	29	[illegible]
[illegible]	F	1/1	29	[illegible]
[illegible]	F	1/1	29	[illegible]
[illegible]	F	1/1	29	5170
[illegible]	F	1/1	29	[illegible]
[illegible]	F	2/1	29	4441
[illegible]	F	2/1	29	[illegible]
[illegible]	F	2/1	29	[illegible]
[illegible]	F	2/1	29	[illegible]
[illegible]	F	2/1	29	[illegible]
[illegible]	F	2/1	29	[illegible]
[illegible]	F	2/1	29	[illegible]
[illegible]	F	2/1	29	8150
[illegible]	F	2/1	29	[illegible]
[illegible]	F	3/1	29	[illegible]
[illegible]	F	3/1	29	[illegible]
[illegible]	F	3/1	29	[illegible]
[illegible]	F	3/1	29	[illegible]
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[illegible]	F	4/1	29	[illegible]
[illegible]	F	4/1	29	[illegible]
[illegible]	F	4/1	29	[illegible]
[illegible]	F	4/1	29	4515
[illegible]	F	4/1	29	[illegible]
[illegible]	F	4/1	29	[illegible]
[illegible]	F	4/1	29	[illegible]
[illegible]	F	4/1	29	[illegible]

ANIMAL NUMBER	SEX	GROUP/ SUBGROUP	DAY OF PHASE	IgM [illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]
[illegible]	F	5/1	29	[illegible]

STUDY PROFILE

MRID 47622824

Name of Chemical/ Technical

Ethiprole

Study Type: Metabolism Study - Rat

OPPTS Guideline Number: 870.7485

Title of the Study:

[^{14}C]-RPA 107382:
Absorption, Distribution, Metabolism and Excretion in the Rat

Study Identification:

Study Number: 160889
Report Number: 16729

Study Report Date:
December 3, 1999
Amended Report Date:
June 17, 2002

Executive Summary:
The biokinetic behaviour and metabolism of [phenyl-UL-^{14}C]-Ethiprole was investigated in the rat following a low and a high dose. Groups of five male and five female animals received orally a single dose at a level of 5 or 1000 mg kg^{-1} bw. Additional male and female rats received fourteen non-labelled doses followed by a single radiolabelled dose at the low dose level. Furthermore, the absorption of Ethiprole was examined with bile duct cannulated animals of both sexes. The distribution of radioactivity originating from [^{14}C]-Ethiprole in organs and tissues was studied after sacrifice at different intervals after administration. The metabolism could be derived from the observed metabolites in urine, feces and bile. The kinetics of excretion were determined from the radioactivity in urine and feces collected at different intervals after dosing.

The gastro-intestinal absorption was rapid and nearly complete at the low dose in both genders (≥ 89% of the oral dose), with the bulk of the dose being absorbed within 24 h after dosing (bile cannulation experiment). The absorption was more prolonged and significantly lower at the high dose level (14 and 20% of the dose in male and female animals, respectively) indicating a certain saturation of the absorption process at the higher dose levels. The excretion was rapid and high with 86% of the low dose (both sexes) and 77% (males) and 64% (females) of the high dose recovered within the first 48 hours after administration. The predominant route of elimination for both sexes was *via* the feces with 55% (females) and 67% (males) of the low dose and 88% of the high dose excreted over the 168 hour collection interval. Urinary excretion resulted in the recovery of a further 24% (males) and 36% (females) of the administered low dose. Urinary excretion played a minor role in elimination at the high dose (3-5%). After multiple dosing (14 non-labelled followed by 1 radiolabelled dose) at the low dose level the excretion pattern was similar to that following a single low dose. Exhalation of radioactivity was negligible (< 0.1% of the dose).

The pattern of tissue distribution of the [^{14}C]-Ethiprole radioactivity was similar for both sexes, and did not differ substantially following a single low dose, single high dose, or repeated low dosing. At the end of the study (168 hours after dosing) the radioactivity levels in all organs and tissues were generally low. The highest levels were observed in the organs associated with metabolism and excretion (kidney and liver) and in glandular tissues (adrenal, pancreas and thyroid). Slightly higher concentrations of radioactivity were also observed in the renal fat. The tissue levels at different times of sacrifice (tissue kinetics experiment) indicated that there is no retention of the radioactivity in a particular tissue type and the distribution and elimination was similar in male and female animals. At the end of the study, 168 h after dosing, the radioactive residues in tissues represented only < 1% of the low dose and < 0.05% of the high dose.

Analysis of the excreted radioactivity indicated that the orally administered [^{14}C]-Ethiprole was extensively metabolized. The pattern of metabolites in urine was similar in male and female rats following administration of the low dose. The major components were the polar glucuronide conjugate of hydroxy-MB 45897 (U1), the sulfinic acid of RPA 104615 (U2), as well as the less polar (non-conjugated) MB 45897 and in female urine the carboxylic acid RPA 112705. A similar pattern of metabolites was observed after repeated low doses or a single high dose. The metabolic profile in the feces at the high dose level was dominated by unabsorbed ethiprole. The excreted radioactive components could be identified to a proportion of ca. 65% (low dose level) or 80-85% (high dose level) of the administered radioactivity.

The primary metabolism of ethiprole was observed to proceed via three different routes:

1. Hydrolysis of nitrile group of ethiprole to form the amide RPA 112916 (no further metabolites detected, therefore this is considered to be a terminal metabolite).
2. Reduction of the sulfoxide group of ethiprole to form the sulfide RPA 107556. This metabolite is further metabolized by an alkyl oxidation reaction to generate the carboxylic acid RPA 112716 (terminal metabolite).
3. Oxidation of the sulfoxide group of ethiprole to form the major metabolite, the sulfone RPA 097973. RPA 097973 is subsequently further metabolized via three different possible routes:

   - RPA 097973 can be alkyl hydroxylated to generate RPA 114345, which is subsequently metabolized by alkyl oxidation to form RPA 112705 and by conjugation with sulfuric acid to form the sulphate conjugate of RPA 114345. Further metabolism of RPA 112705 can follow.
   - An oxidative dealkylation of RPA 097973 forms the sulfuric acid RPA 104615. Replacement of the sulfuric acid group by a hydroxy group generates the intermediate hydroxy-MB 45897. This intermediate is subsequently further metabolized by sulphation (to generate the sulphate of the hydroxyl-MB45897), by reduction (to generate MB 45897) and by conjugation (to produce the glucuronide conjugate of hydroxyl-MB 45897).
   - Oxidation of the nitrile group of RPA 097973 produces RPA 112917 (another terminal metabolite).

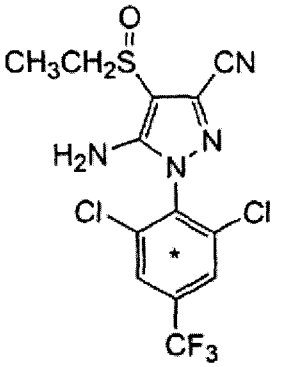
This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Compound:

Radiolabelled Test Material:	[Phenyl-UL-^{14}C]-Ethiprole
Radiochemical purity	≥99.3%, The identity and purity of the parent compound was determined by TLC and HPLC analysis prior to dose administration.
Specific Activity	796.6 MBq/mmol (54.1 µCi/mg), confirmed by u.v. spectroscopy
Lot/Batch #:	Batch No. GRX 485A
Non-Radiolabelled Test Material:	Ethiprole
Description:	White, crystalline powder, stored in the dark at about +4 °C
Lot/Batch #:	Batch No. 2CFS94
Purity:	99.7%
Contaminants:	Not specified
CAS # of TGAI:	181587-01-9



* = Position of [^{14}C]-radiolabel

2. **Vehicle and/or positive control:** The vehicle was identified as 0.05% or 0.5% w/w aqueous methyl cellulose solution containing 0.01% w/w Tween 80.

3. Test animals:

Species:	Rat
Strain:	Sprague Dawley rats of body weight ca 151g to 355 g
Age/weight at study initiation:	Age unspecified / body weight ca 151g to 355 g
Source:	Charles River (UK) Limited
Housing:	The animals used in excretion collections were housed singly in all glass metabolism cages designed for the separate collection of urine and feces. All other animals were housed in individual propylene and stainless steel cages with raised wire mesh floors to inhibit coprophagy.
Diet:	SDS Rat and Mouse, Maintenance Diet No. 1, (Special Diet Services, Witham, Essex), *ad libitum*
Water:	Tap water, *ad libitum*

Environmental conditions:	**Temperature:**	Recorded but not specified (room temperature)
	Humidity:	Recorded but not specified
	Air changes:	Not specified
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	≥7 days	

4. Preparation of dosing solutions: For all formulations, [^{14}C]ethiprole and non-radiolabelled ethiprole were dissolved in acetone in a volumetric flask and the flask made up to volume with acetone. Six replicate aliquots were taken from the flask for LSC to determine the specific activity of the radiodiluted material. The radiodiluted solution was added drop-wise, with stirring, to a pre-weighed 0.05% or 0.5% w/w aqueous methyl cellulose solution containing 0.01% w/w Tween 80. The resulting suspension was placed under a stream of nitrogen until all traces of acetone had evaporated. The suspension was re-weighed and adjusted for water lost during the evaporation. Non-radiolabelled ethiprole dose suspension was formed as described for the [^{14}C]ethiprole dose suspensions, by addition of a solution of the test material in acetone to dose vehicle, followed by evaporation of the acetone. The resulting solution was re-adjusted for any water lost.

B. STUDY DESIGN AND METHODS:

1. Group Arrangements Animals were assigned to the test groups noted in Table 1. Target dose levels were 5 or 1000 mg/kg body weight. Rats were weighed accurately immediately prior to dose administration.

TABLE 1: Dosing groups

Test Group (Dose Regime)	Dose of labeled material (mg/kg)	Number/sex	Remarks (eg. time of sacrifice)
Single Oral	5	2	Pilot experiment
Single Oral Single Oral Repeat Oral*	5 1000 5	5 5 5	Main absorption, distribution, metabolism and excretion experiment
Single Oral Single Oral	5 1000	5 5	Pharmacokinetic experiment
Single Oral Single Oral	5 1000	12 12	Tissue distribution experiment
Single Oral Single Oral	5 1000	5 5	Bile duct cannulation experiment

*[Phenyl-UL-14C]ethiprole administered as a single oral dose targeting 5 mg/kg following multiple oral administration (14 days) of non-radiolabelled ethiprole targeting 5 mg/kg/day.

2. Dosing and sample collection: The target dose levels were 5 and 1000 mg/kg and the rats were weighed accurately immediately prior to dose administration. The radioactive content of the dose suspension was determined by measuring the radioactivity associated with weighed samples (ca 15 mg) taken after dose formulation and prior to dosing and from mock doses taken at the time of dosing. The concentration of [^{14}C]ethiprole in the dose suspension was calculated from the radioactive content of the dose formulation and the specific activity of the radiodiluted [^{14}C]ethiprole. Individual doses from the dosing suspension were weighed into a syringe and administered via oral gavage. The syringe was re-weighed after dosing to determine the exact

weight of dose administered. The exact dose administered to each animal was calculated from the weight of dose administered and the concentration of [^{14}C]ethiprole in the dose suspension.

a. Pharmacokinetic studies

Preliminary experiment: A pilot study in which 2 male and 2 female rats each received a single oral administration of [phenyl-UL-^{14}C]ethiprole at the low dose level of 5 mg/kg was performed to establish methodology for the main phases of the study. Following administration, the animals were transferred to individual all-glass metabolism cages, specially designed for the separate, quantitative collection of urine, feces and expired air. Urine and feces were collected daily for 7 days. Expired air was collected daily into 2 serial traps containing 2-ethoxyethanol:ethanolamine (7:3, v/v) for 2 days. The cages were rinsed with water at the time of each feces collection and the rinsing retained. The animals were humanely killed by CO_2 narcosis at 7 days post dose and the carcasses retained. The total radioactive residue was determined in all samples collected.

Main Absorption, Distribution, Metabolism and Excretion (ADME) Study: Two groups of 5 male and 5 female rats received a single oral administration of [phenyl-UL-^{14}C]ethiprole at a target dose level of either 5 mg/kg or 1000 mg/kg. A third group of 5 male and 5 female rats received an oral administration of non-radiolabelled ethiprole (target dose level 5 mg/kg) once daily for 14 days. On day 15, [phenyl-UL-^{14}C]ethiprole was administered as a single oral dose at a target dose level of 5 mg/kg. Following administration of [^{14}C]ethiprole, the animals were transferred to individual all-glass metabolism cages. Samples of urine were collected into containers cooled by solid CO_2 (up to 96 h), at 6 h, 12 h and 24 h post dose and then at 24 h intervals until the end of the collection period (168 h post dose). Feces was collected into containers cooled by solid CO_2 (up to 96 h), at 12 h and 24 h post dose and then at 24 h intervals until the end of the collection period (168 h post dose). Cages were washed at the time of each feces collection, and the rinsings retained.

The animals were humanely killed by CO_2 narcosis at 7 days post dose and the following organs, tissues and biological samples retained from each animal: adrenals, pancreas, bone and marrow, perirenal fat, brain, plasma, gastrointestinal tract and contents, residual carcass, heart, skin and fur, kidneys, spleen, liver, stomach and contents, lung, testes/ovaries, muscle (leg), thyroid, and whole blood.

Pharmacokinetic Study: Two groups of 5 male and 5 female rats received a single oral administration of [phenyl-UL-^{14}C]ethiprole at target dose levels of either 5 mg/kg or 1000 mg/kg. Following dose administration, the animals were placed in individual polypropylene and stainless steel cages with raised wire mesh grids to inhibit coprophagy. Samples of blood (*ca* 0.2 ml) were collected into heparinized tubes by venesection of a tail vein at the following target times: 0.5, 1, 2, 4, 6, 8, 24, 48, 72, 96, 120, and 144 h post dose. At 168 h post dose, the animals were humanely killed by CO_2 narcosis and a terminal blood sample taken. The levels of total radioactivity were determined in each sample collected.

Tissue Kinetics Study: Two groups of 12 male and 12 female rats each received a single oral administration of [phenyl-UL-^{14}C]ethiprole at target dose levels of 5 mg/kg and 1000 mg/kg. Following dose administration, the animals were placed in individual polypropylene and stainless steel cages with raised wire mesh grids to inhibit coprophagy. Four male and 4 female rats were sacrificed at 8, 24 and 48 h after administration at the low dose level and 48, 72 and 96 h after administration of [^{14}C]ethiprole at the high dose level. These timepoints corresponded approximately to Cmax, Cmax/2 and Cmax/4 as determined during the pharmacokinetic experiment. Samples of organs, tissues and biological fluids were collected from each animal as detailed for the main ADME experiment.

Bile Duct Cannulation Study: Sixteen male and 16 female rats were surgically prepared with indwelling bile duct and duodenal cannulae under general anaesthesia (mixture of isofluorane in oxygen/nitrous oxide). The cannulae were passed through the abdominal wall, truncated subcutaneously and externalized through the ventral surface of the tail. The cannulae were protected by a metal tail cuff, overlying the exit site, and a spring assembly. The animals were returned singly to glass metabolism cages suitable for the separate collection of urine and feces and the free end of each cannula attached to a swivel joint fixed above the cage. Bile was collected into a clean container and stored at *ca* 4°C. Physiological saline was infused through the duodenal cannulae for one day at a rate of 0.5 mL/hr. After this one day period, the rat was infused with artificial bile salts into the duodenum, via the duodenal cannula. The animals were treated with Carprofen (Zenecarp™,C-Vet VP, 50 mg/mL) at a dosage of 5 mg/kg by the subcutaneous route as a premedication prior to surgery and approximately 24 h after surgery. Following satisfactory clinical examination, bile flow rate and body weight gain profile, [^{14}C]ethiprole was administered as a single oral dose to 2 groups of 6 male and 6 female rats at a target dose level of either 5 mg/kg or 1000 mg/kg (only 5 male rats were dosed at 1000 mg/kg). Animals were housed in all-glass metabolism cages suitable for the quantitative collection of urine, feces and bile during the surgical procedure and following dosing. Urine was collected into containers cooled by solid CO_2 at 6, 12 and 24 h post dose and then at 24 h intervals until 96 h post dose. Bile was collected at the same time intervals as urine. Feces was collected into containers cooled by solid CO_2 at 12 and 24 h post dose and then at 24 h intervals until 96 h post dose. Cages were rinsed with water at the time of feces collection and the rinsings retained. The total radioactive residues were determined in all samples collected. Where 6 animals survived to 96 h, only the results from 5 animals have been reported.

Biological samples were analysed on the day of collection wherever possible, prior to storage at *ca* -20°C. Where immediate analysis was not possible, biological samples were stored at *ca* -20°C prior to and following analysis. Cage wash was stored at ambient temperature.

To determine radioactivity levels, duplicate samples of urine, bile, plasma and cage wash were analyzed directly by liquid scintillation counting (LSC). Duplicate samples of whole blood were taken for combustion/LSC. Feces samples were homogenized with water or acetone (high dose level bile duct cannulated rats) using a blender and tissues (except thyroid, ovaries and adrenals which were not homogenized because of their small size) were homogenized by chopping and/or blending and were taken for combustion/LSC analysis. Carcasses were minced using a Newscan

Mincer followed by homogenization with water using a Waring Blender. Two *ca* 0 .3 g samples of homogenate were taken for combustion.

Representative blank sample values were subtracted from sample count rates to give net dpm per sample. A limit of quantification (LOQ) of 30 dpm above background was reported. The combustion efficiency was shown to be greater than 96% throughout the experimental period.

b. Metabolite characterization studies

Preparation of samples for Chromatography:
Based on the levels of total radioactivity, the following proportionately pooled samples were formed prior to chromatographic analysis. The percentage of each total sample weight used to form the pool ranged from 5 to 20%. The pooled samples were prepared for chromatographic analysis by procedures which were determined by the amounts of total radioactivity in each sample. During the extraction and processing procedures, aliquots were removed for LSC to assess the efficiency of the extraction process. The extraction efficiencies of each sample were provided in an Appendix in the report.

Main ADME Study

Urine. For the low dose urine, samples from the 0-12 h and 12-24 h timepoints were centrifuged prior to analysis. Samples from the 24- 48 h and 48-72 h pools were centrifuged, evaporated to dryness under nitrogen and reconstituted in de-mineralized water prior to analysis. For the high dose urine, samples from the 0-24 h pools were applied to C_{18} Solid Phase Extraction (SPE) cartridges and eluted with de-mineralized water and methanol. The organic eluents were evaporated under N_2 and reconstituted in mobile phase prior to analysis. Urine samples from the 24-48 h and 48-72h pools were reduced in volume under N_2 prior to analysis. All samples of repeat dose urine pools were concentrated under N_2 prior to HPLC analysis.

Feces. Each pooled feces sample was extracted (3x) with acetonitrile, centrifuged, and the supernatant decanted. Duplicate samples were taken from the combined extracts for LSC and samples of the post extracted solid (PES) were taken for combustion, to determine the efficiency of extraction. The combined extracts were evaporated to dryness under N2, and the dried extracts were transferred to vials with washes of water:acetonitrile (50:50, v/v) and evaporated to dryness, or a small volume, under nitrogen. The extracts were reconstituted in acetonitrile:water (50:50, v/v), partitioned against hexane, and analyzed by HPLC.

Several additional procedures were used to produce extracts suitable for HPLC analysis, determined by the nature of the extract. Certain samples formed a bi-phasic solution on concentration and were further processed using C18 SPE cartridges. Extracts were frozen and the organic layer decanted. The aqueous layer was thawed and applied to a pre-conditioned C18 SPE cartridge. The cartridges were washed with de-mineralized water and eluted with methanol:acetonitrile (50:50, v/v). The organic eluate was collected and combined with the decanted organic layer. The combined organic extracts were concentrated under N2 and

reconstituted in acetonitrile or acetonitrile:water (80:20, v/v) prior to analysis.

Bile Duct Cannulation Study

An aliquot of each pooled urine and bile sample was evaporated under N_2 and reconstituted in de-mineralized water prior to analysis. Each pooled feces sample was extracted (3x) with methanol, centrifuged, decanted, and the combined extracts were concentrated under N_2 prior to analysis.

Preparation of Samples for HPLC Chromatography:
Analysis of selected pooled samples of urine, feces and bile was carried out by radio-HPLC and LC-MS in an attempt to identify the metabolites of ethiprole. The following non-radiolabelled reference standards were used RPA 112716, MB 45897, RPA 94569, RPA 112916, RPA 112705, RPA 114345, RPA 112917, RPA 107566, RPA 97973, RPA 103343, and RPA 104615. The identities of those metabolites which co-chromatographed with supplied reference standards were confirmed by LC-MS. Structures for several other metabolites were postulated following MS analysis.

Samples were analyzed admixed with a solution containing the supplied metabolite reference standards during initial profiling. This helped to identify any potential co-chromatography between metabolite components and reference standards. The standard solution was prepared by dissolving the metabolite standards in a mixture of acetonitrile and water (50/50 v/v). The order of elution of the reference standards was determined by analyzing samples of the individual reference standards.

3. Statistics: Common descriptive statistics were used. Radioactivity in urine, feces, blood, and tissues (in % dose and/or concentration (µg equivalents of [^{14}C]-ethiprole/g)) was reported for the individual animals and as the mean (± standard deviation) for the number of animals/sex.

II. RESULTS

A. PHARMACOKINETIC STUDIES:

1. Preliminary experiment: In the pilot experiment, following a single oral administration of [phenyl-UL-^{14}C]ethiprole to male and female rats at a target dose level of 5 mg/kg, the mean recovery of total radioactivity over the 168 h collection period was 90.2% and 89.3% for male and female rats, respectively. The predominant route of excretion was via feces, with means of 68.9% (males) and 49.2% (females) of the dose excreted by this route. Excretion of total radioactivity via urine accounted for a mean of 20.7% of the dose for males and 38.3% for females. Less than 0.1% of the dose was eliminated in expired air over the collection period (0-48 h). The bulk of the administered dose (78-85%) was excreted by 48 h post dose. At the end of the collection period (168 h), <1.1% of the administered dose was retained in the carcass. From the results of the pilot experiment it was concluded that the methodology of excreta

collection and analysis proposed for the main study were appropriate. The low amount of the dose recovered in expired air (0.1%) did not warrant the collection of expired air in the main study. Results for the cumulative excretion, residual carcass levels and overall recovery of total radioactivity were provided in summary tables in the study report.

2. Absorption: The radioactivity in whole blood following oral administration of [^{14}C]ethiprole at 5 mg/kg or 1000 mg/kg are presented in Table 2. The calculated pharmacokinetic parameters are presented in Table 3.

a) Single Low-Dose: Following single oral administration of [phenyl-UL-^{14}C]ethiprole at 5 mg/kg, concentrations of total radioactivity observed in whole blood increased rapidly from mean concentrations of 0.48 and 0.62 µg/g in males and females respectively at 0.5 h to peak mean concentrations of 2.11 (males) and 1.63 (females) µg/g at 8 h post dose. Mean concentrations declined in a biphasic manner with concentrations decreasing rapidly to 1.47 µg/g (males) and 1.03 µg/g (females) at 24 h post dose. Thereafter, the rate of decline slowed, with low concentrations of radioactivity in the whole blood from males and females (0.09 and 0.17 µg/g, respectively) observed at 168 h post dose.

b) Single High-Dose: Following single oral administration of [phenyl-UL-^{14}C]ethiprole at 1000 mg/kg, mean concentrations of radioactivity in whole blood at 0.5 h post dose were 4.3 and 3.3 µg/g in male and female rats, respectively. Concentrations increased gradually to mean values of 14.7µg/g (males) and 9.9 µg/g (females) at 4 h and further to 20.1 µg/g (males) and 12.0 µg/g (females) at 8 h post dose. The peak mean concentrations of 38.7 µg/g in males and 29.8 µg/g in females were observed at 24 and 48 h post dose, respectively. Following each peak, concentrations decreased slowly to means of 12.2 and 20.9µg/g in male and female rats, respectively at 72 h post dose. Thereafter, the rate of decline remained at a steady level, with mean concentrations of 2.9 and 3.0 µg/g observed in the whole blood of males and females, respectively, at 168 h post dose.

TABLE 2. Radioactivity in whole blood of rats after administration of [phenyl-UL-[^{14}C]-labeled ethiprole

Time Post-Dosing (hours)	Low-Dose (5 mg/kg) (µg [^{14}C]-Ethiprole equivalents per g blood)		High-Dose (1000 mg/kg) (µg [^{14}C]- Ethiprole equivalents per g blood)	
	Males	Females	Males	Females
0.5	0.475	0.615	4.3	3.3
1	0.796	0.979	6.8	5.8
2	1.191	1.290	10.5	7.8
4	1.587	1.383	14.7	9.9
6	1.837	1.482	18.0	10.5
8	2.114	1.633	20.1	12.0
24	1.473	1.030	38.7	24.4
48	0.693	0.451	38.4	29.8
72	0.359	0.311	12.2	20.9
96	0.215	0.248	6.1	7.0
120	0.143	0.199	4.9	4.2
144	0.085	0.178	2.6	2.6
168	0.088	0.173	2.9	3.0

Data obtained from pages 151-154 in the study report.
Values are a mean for 5 rats/sex/dose.

Pharmacokinetic Analysis. Following oral dosing with [phenyl-UL-^{14}C]ethiprole, the mean peak whole blood concentrations of compound, Cmax(obs), were observed at 8 and 48 hours post-dose (= Tmax(obs)) at the low dose level and high dose levels, respectively. Within dose groups, Tmax(obs) estimates demonstrated minimal variability. At both dose levels, whole blood concentrations of [^{14}C]ethiprole had mean elimination phase half-lives ($T_{½}$el) of 44.33 to 49.18 hours, except in low dose females (113.98 hours), where inter-animal variation was more pronounced. This parameter was not, however, considered to change significantly as a function of dose. Estimates for Cmax(obs) and both AUC parameters increased with dose of [phenyl-UL-^{14}C]ethiprole. The mean $AUC_{(0-t)}$ was 94.88 and 79.38 (µg equiv.h/mL) for males and females respectively at the low dose level and 2620 and 2240 (µg equiv.h/mL) for males and females respectively at the high dose level. $AUC_{(0-\infty)}$ and Cmax(obs) demonstrated similar trends. This increase was not dose proportional, as a 200-fold increase in dose resulted in fold increases (male and female respectively) of 28 and 22 for $AUC_{(0-\infty)}$, 28 and 28 for $AUC_{(0-t)}$ and 20 and 18 for Cmax(obs). There was no consistent effect of dose upon $T_{½}$el in this study.

TABLE 3. Pharmacokinetic parameters for rats after administration of [phenyl-UL-[^{14}C]-labeled ethiprole

Pharmacokinetic Parameters	Low Dose (5 mg/kg) Whole Blood		High Dose (1000 mg/kg) Whole Blood	
	Males	Females	Males	Females
$Tmax_{(obs)}$	8.00	8.00	33.60	48.00
$Cmax_{(obs)}$	2.114	1.633	41.66	29.78
$AUC_{(0-\infty)}$	101.24	110.46	2849	2436
$AUC_{(0-t)}$	94.88	79.38	2620	2240
$T_{½}$ el	48.45	113.98	49.18	44.33
Rsq	NA	NA	NA	NA

Data obtained from pages 155-158 in the study report.
NA = Not applicable.
Values are means calculated from individual pharmacokinetic parameters.

3. Tissue distribution The distribution of radioactivity in tissues and organs collected from the low- and high-dose rats is summarized in Table 4.

a) Single Low-Dose: At 168 h after a single oral dose of [phenyl-UL-^{14}C]ethiprole at 5 mg/kg, the distribution of radioactivity among the tissues of both male and female rats was widespread, with low concentrations of radioactivity observed in all tissues collected. In males, the highest concentrations of radioactivity were in kidney, liver and the skin and fur (0.062, 0.058 and 0.040 µg/g, respectively). All other tissues contained concentrations of radioactivity which were similar to, or below that observed in plasma (0.036 µg/g). The distribution of radioactivity in female rats was equally extensive, with concentrations of radioactivity in most tissues, with the exception of whole blood, observed at similar levels to male animals. Concentrations of radioactivity present in whole blood were higher in female rats (0.266 µg/g) compared to males (0.041 µg/g). Within the remaining tissues, the highest concentrations were found in the kidney, lung, thyroid gland, spleen, liver, heart, skin and fur and adrenal gland (means of 0.087, 0.068, 0.063, 0.062, 0.061, 0.049, 0.049 and 0.043µg/g, respectively). All other tissue concentrations were similar to, or below that observed in plasma (0.027µg/g). Low amounts of radioactivity were found in the contents of gastrointestinal tract, with a mean value of 0.04% of the administered dose in both male and female rats. Levels of radioactivity within the contents of the stomach were close to the limit of reliable determination for all animals. Levels of radioactivity remaining in the carcass accounted for less than 1.3% in all animals.

b) Single High-Dose: At 168 h after a single oral dose of [phenyl-UL-^{14}C]ethiprole at 1000 mg/kg, low concentrations of radioactivity, in comparison with the dose administered, were observed in all tissues collected from male and female rats. In males, the highest concentrations of radioactivity were located in the skin and fur, liver and thyroid gland (9.9, 1.8 and 1.8 µg/g, respectively). All other tissues contained concentrations of radioactivity which were similar to, or below that observed in plasma (1.2 µg/g), with concentrations in individual animals close to the limit of reliable determination for many tissues. The distribution of radioactivity in female rats followed a similar pattern to the males, with concentrations in many of the tissues close to the limit of reliable determination for individual animals. The highest concentrations were found in the thyroid gland, skin and fur, adrenals, liver, ovaries, kidney and whole blood (3.4, 2.3, 1.7, 1.7, 1.5, 1.3 and 1.3 µg/g, respectively). The remaining tissues contained concentrations which were similar to or below that of plasma (0.5µg/g). Radioactivity was not observed in the contents of the gastrointestinal tract or stomach suggesting that absorption of the radiolabelled dose was complete by 168 h post dose. No significant radioactivity was detected in the carcasses from male and female rats.

c) Multiple Dose: Following sacrifice of the animals at 168 h post dose, concentrations of radioactivity present in tissues were similar to those observed following the single administration of [phenyl-UL-^{14}C]ethiprole at the low dose level for female animals, but slightly higher in males. Low concentrations of radioactivity were measured in all tissues, with the highest concentration for both males and females observed in the skin and fur (0.29µg/g (males) and 0.32 µg/g (females). Concentrations in all other tissues collected were similar to those present in plasma (0.09 and 0.03 µg/g for males and females, respectively), with the exception of whole

blood in female animals (0.195 µg/g). Low amounts of radioactivity were found in the contents of gastrointestinal tract, with means of 0.07% and 0.03% of the dose for male and female rats respectively, while levels of radioactivity within the contents of the stomach were close to the limit of reliable determination. Levels of radioactivity remaining in the carcass accounted for less than 0.8% in all animals, suggesting that no accumulation of the test material had occurred.

TABLE 4: Distribution of radioactivity in rat tissues/organs after administration of [phenyl-UL-^{14}C]-labeled ethiprole

	Mean Radioactivity in µg equivalents of [^{14}C]-ethiprole/g [a]					
	5 mg/kg		1000 mg/kg		5 mg/kg [b]	
Tissue/organ	Male	Female	Male	Female	Male	Female
Adrenals	*0.026	*0.043	*1.2	*1.7	0.103	*0.048
Bone Marrow	0.011	0.032	*0.3	*0.7	0.025	0.032
Brain	*0.006	0.011	*0.1	*0.2	0.010	0.010
Fat-Renal	0.021	*0.027	*0.3	*0.4	0.040	0.024
Heart	0.020	0.049	*0.4	*0.5	0.034	0.038
Kidneys	0.062	0.087	1.6	1.3	0.112	0.062
Liver	0.058	0.061	1.8	1.7	0.113	0.055
Lungs	0.024	0.068	*0.6	*0.5	0.049	0.058
Muscle	0.009	0.014	*0.2	*0.2	0.015	0.011
Pancreas	0.018	0.026	*0.3	*0.4	0.034	0.022
Ovaries	NA[c]	*0.039	NA	*1.5	NA	0.039
Skin and Fur	0.040	0.049	9.9	*2.3	0.294	0.322
Spleen	0.016	0.062	*0.3	*0.6	0.032	0.047
Testes	0.011	NA	*0.3	NA	0.023	NA
Thyroid	*0.036	*0.063	*1.8	*3.4	*0.122	*0.065
Whole Blood	0.041	0.266	*1.3	*1.3	0.076	0.195
Plasma	0.036	0.027	*1.2	*0.5	0.087	0.025

Data obtained from pages 129-130, 137-138, and 145-146 in the study report.

a An asterisk (*) indicates that the mean includes results calculated from data less than 30 d.p.m. above background.

b [^{14}C]ethiprole administered as a single oral dose (target dose level: 5 mg/kg) following multiple oral administration (14 days) of non-radiolabelled ethiprole (target dose level: 5 mg/kg.day).

c NA = Not Applicable

4. <u>Excretion</u> Average recoveries of radioactivity in urine, feces, and cage washings from the low- and high-dose rats are presented in Table 5.

a) <u>Single Low-Dose</u>: Following administration of [phenyl-UL-^{14}C]-ethiprole to male and female rats at a target dose level of 5 mg/kg, results for the cumulative recovery of total radioactivity were consistent with the findings of the pilot experiment. The predominant route of elimination for both males and females was via feces with means of 67.3% (males) and 54.9% (females) of the administered dose excreted by this route by 168 h post dose. Urinary excretion resulted in the recovery of a further 23.5% and 36.4% of the administered dose in males and females, respectively. Excretion of the dose was rapid with mean values of 85.6% (males) and 86.4% (females) of the administered radioactivity recovered within 48 h post dose. By the end of the 168 h collection period, the overall mean recovery in males and females was 92.3% and 94.1%, respectively.

b) <u>Single High-Dose:</u> Following administration of [phenyl-UL-^{14}C]-ethiprole to male and female rats at a target dose level of 1000 mg/kg, excretion of total radioactivity was slower than that

observed following administration at the low dose level. By 48 h post dose, 77.3% (males) and 64.0% (females) of the administered dose had been recovered, which increased by 72 h post dose to mean values of 87.5% and 90.2% in male and female rats, respectively. The major route of elimination was again via feces, with means of 88.4% (males) and 87.5% (females) of the dose excreted by this route. Urinary excretion played a minor role in elimination with only 3.0% and 5.1% of the dose recovered from males and females, respectively. By the end of the 168 h collection period, the overall mean recovery in male and female rats was 91.7% and 93.0%, respectively.

c) Multiple Dose: Results for the recovery of total radioactivity following administration of [phenyl-UL-^{14}C]-ethiprole to male and female rats at a target dose level of 5 mg/kg after the multiple oral administration of non-radiolabelled ethiprole were similar to those obtained following the single administration of [^{14}C]ethiprole at the low dose level. The predominant route of elimination for all animals was via feces, with means of 70.7% (males) and 55.5% (females) of the dose excreted by this route. Urinary excretion resulted in the recovery of a further 22.5% and 35.1% of the administered dose in males and females, respectively. Excretion of the dose was rapid with means of 81.6% (males) and 85.9% (females) of the radioactivity recovered within the first 48 h post dose. By the end of the 168 h collection period, the overall mean recovery in male and female rats was 94.1% and 93.7%, respectively.

TABLE 5: Recovery of radioactivity in excreta of rats after administration of [phenyl-UL-^{14}C]-labeled ethiprole

Component	Mean Recovery of Radioactivity (% Dose Administered)					
	5 mg/kg		1000 mg/kg		5 mg/kg [a]	
	Male	Female	Male	Female	Male	Female
Urine	23.5	36.4	2.96	5.13	22.5	35.1
Feces	67.3	54.9	88.4	87.5	70.7	55.5
Cage wash	1.53	2.67	0.37	0.37	0.85	3.12
Total	**92.3**	**94.1**	**91.7**	**93.0**	**94.1**	**93.7**

Data obtained from pages 127-128, 135-136, and 143-144 in the study report.
a [^{14}C]ethiprole administered as a single oral dose (target dose level: 5 mg/kg) following multiple oral administration (14 days) of non-radiolabelled ethiprole (target dose level: 5 mg/kg.day).

5. Tissue Kinetics Experiment Average concentration of radioactivity in tissues and organs collected from the low- and high-dose rats is summarized in Table 6 and Table 7.

a) Single Low-Dose: Following a single oral administration of [phenyl-UL-^{14}C]-ethiprole at a target dose level of 5 mg/kg, groups of 4 male and 4 female rats were humanely killed by CO_2 narcosis at each of 8, 24 and 48 h post dose respectively, approximately TCmax, TCmax/2 and TCmax/4 as determined from the pharmacokinetic experiment.

At 8 h post dose, the distribution of total radioactivity was widespread and followed a similar pattern in the tissues from male and female rats. The highest concentrations of total radioactivity were observed in the liver and renal fat with mean concentrations of 14.485 and 11.713 µg in males and 13.291 and 11.418 µg in females. High concentrations were also observed in the adrenal gland, pancreas, kidney, thyroid gland and lungs, with mean concentrations of 7.919,

6.429, 5.364, 5.316 and 4.249 µg in males and 9.805, 7.599, 5.867, 5.854 and 4.454 µg in females. High concentrations of radioactivity were observed in the ovaries from all female rats (mean 5.228 µg). However, concentrations of radioactivity within the testes were lower (mean 2.376 µg). Of the remaining tissues, concentrations were similar to or below that observed in plasma with mean values of 4.099 and 2.454 µg in male and female rats, respectively. High levels of radioactivity were observed in the contents of the gastrointestinal tract accounting for means of 30.2% (males) and 24.2% (females) of the administered radioactivity). Levels of radioactivity within the contents of the stomach were lower, accounting for means of 2.97% and 5.54% of the radioactivity recovered. High amounts of radioactivity were present in the carcass, with means of 34.2% and 35.2% of the dose observed in males and females, respectively.

By 24 h post dose, levels of radioactivity had decreased rapidly in all tissues. The highest concentration was in the liver, where mean concentrations of 5.304 µg (males) and 3.172 µg (females) were observed. Concentrations within the kidney, renal fat, lungs, adrenal gland, pancreas, and thyroid gland had decreased to means of 1.633, 1.472, 1.035, 0.986, 0.949 and 0.884 µg in males and 1.151, 1.623, 0.689, 1.123, 0.897 and 0.709 µg in females. Concentrations in all other tissues were below that observed in plasma (means of 2.311 and 0.806 µg in males and females, respectively). Lower levels of radioactivity were also observed in the gastrointestinal tract and contents, with mean levels accounting for 16.7% and 13.5% of the radioactivity administered to males and females, respectively. The amounts of radioactivity present in the stomach and contents were similar to those observed at 8 h post dose (means of 3.05% (males) and 2.00% (females)). Within the carcass, levels of radioactivity had decreased to means of 6.70% (males) and 5.53% (females).

By 48 h post dose, concentrations of total radioactivity had decreased considerably in all tissues in both the male and female rats. Within the liver, concentrations had decreased to means of 1.608 and 0.773 µg in males and females, respectively. All other tissues contained concentrations which were similar to, or below that observed in plasma (means of 0.805 µg (males) and 0.296 µg (females)). Levels of radioactivity within the gastrointestinal tract and stomach had decreased to means of 4.60% and 1.12% of the administered dose in males and 3.24% and 0.34% in females. Levels of radioactivity present in the carcass accounted for means of 1.84% and 2.28% of the dose in male and female animals, respectively.

TABLE 6: Mean concentration of radioactivity in rat tissues/organs 8, 24 and 48 hours after administration of [phenyl-UL-^{14}C]-labeled ethiprole at 5 mg/kg

	Mean Radioactivity in µg equivalents of [^{14}C]-ethiprole/g [a]					
	8 h post-dose		24 h post-dose		48 h post-dose	
Tissue/organ	Male	Female	Male	Female	Male	Female
Adrenals	7.919	9.805	0.986	*1.123	*0.138	0.312
Bone Marrow	1.509	1.704	0.364	0.314	0.086	0.078
Brain	1.612	2.012	0.194	0.186	0.032	0.056
Fat-Renal	11.713	11.418	1.472	1.623	0.153	0.371
Heart	3.809	3.918	0.839	0.665	0.220	0.230
Kidneys	5.364	5.867	1.633	1.151	0.496	0.328
Liver	14.485	13.291	5.304	3.172	1.608	0.773
Lungs	4.249	4.454	1.035	0.689	0.298	0.255
Muscle	2.600	3.165	0.472	0.403	0.079	0.117
Pancreas	6.429	7.559	0.949	0.897	0.150	0.265
Ovaries	NA[b]	5.228	NA	0.707	NA	0.196
Skin and Fur	4.412	3.520	0.714	0.722	0.242	0.261
Spleen	2.384	*2.418	0.423	0.401	0.097	0.156
Testes	2.376	NA	0.563	NA	0.132	NA
Thyroid	5.316	5.854	0.884	0.709	0.250	0.223
Whole Blood	2.211	1.525	1.379	0.723	0.564	0.428
Plasma	4.099	2.454	2.311	0.806	0.805	0.296

Data obtained from pages 159-160, 163-164, and 167-168 in the study report.
a An asterisk (*) indicates that the mean includes results calculated from data less than 30 d.p.m. above background.
b NA = Not Applicable

b) Single High-Dose: Following a single oral administration of [phenyl-UL-^{14}C]-ethiprole at a target dose level of 1000 mg/kg, groups of 4 male and 4 female rats were humanely killed by CO_2 narcosis at each of 48, 72 and 96 h post dose respectively, approximately TCmax, TCmax/2 and TCmax/4 as determined from the pharmacokinetic experiment.

High concentrations of total radioactivity were observed throughout the tissues of the male and female rats sacrificed at 48 h post dose. The highest concentrations of total radioactivity in male rats were observed in the renal fat, liver and adrenal gland, with mean concentrations of 208.1, 160.5 and 119.7 µg, respectively. A high mean concentration was also observed in the thyroid gland (192.0 µg). However, this value may be artificially high, resulting from one outlying value (568.0 µg for Animal 77, male). Concentrations of radioactivity in the remaining animals were within the range of 61.0-73.8 µg (mean 66.6 µg). Lower concentrations of radioactivity were observed in the pancreas and kidneys (92.9 and 65.6 µg, respectively). The remaining tissues all contained concentrations which were similar to or below the level observed in plasma (63.3 µg). High levels of radioactivity were present in both the gastrointestinal tract and stomach, where mean concentrations of 14.2% and 10.4% of the dose were observed. Only a small proportion of radioactivity (5.74%) remained in the carcass at this time, although this was slightly higher in the case of one animal (77, male), where approximately 12% of the administered radioactivity remained associated with the residual carcass. The tissue distribution pattern was similar in female rats, with the highest concentrations of radioactivity located within the renal fat, liver and adrenal gland (means of 138.0, 137.9 and 122.7 µg, respectively). Of the remaining tissues, only the bone marrow, muscle, spleen and whole blood contained concentrations which were below that in plasma (39.9 µg). In the gastrointestinal tract and contents, 7.91% of the dose was recovered, compared to 24.5% in the stomach and contents. Levels of radioactivity present

within the carcass were low, accounting for a mean of 2.48% of the administered radioactivity.

By 72 h post dose, the concentrations of radioactivity had declined rapidly in all tissues in male rats. The highest concentrations of radioactivity were present in the liver and renal fat where mean concentrations of 45.3 and 30.4 µg, respectively were observed. Within all other tissues, the concentrations of radioactivity had deceased to levels which were below concentrations in plasma (mean 22.6 µg). The amounts of radioactivity contained in the contents of both the gastrointestinal tract and stomach had decreased to means of only 1.76% and 0.93% of the dose, respectively. Less than 1.1% of the dose remained associated with the residual carcass. The decrease in levels of radioactivity was more gradual in the tissues collected from female rats at 72 h post dose. High mean concentrations were observed for a number of tissues such as renal fat (115.4 µg), liver (111.7 µg) and adrenal gland (87.7 µg). Lower concentrations of radioactivity were present in the ovaries, pancreas, kidneys, thyroid gland, lungs and heart (means of 56.7, 56.5, 47.4, 43.8, 37.7 and 33.9 µg). The remaining tissues contained concentrations which were similar to, or below, the mean concentration observed in plasma (30.1 µg). Moderate amounts of radioactivity were present in the gastrointestinal tract and stomach with means of 6.39% and 4.71% of the administered dose, respectively. Radioactivity remaining in the carcass accounted for less than 2.3% of the administered dose for all animals.

By 96 h post dose, concentrations of radioactivity had declined to levels which were close to the limit of reliable determination for many tissues from male rats. In male rats, the highest concentrations were observed in the liver and skin and fur (means of 14.5 and 11.8 µg). In females, the concentrations for individual tissues remained higher than in males, with the highest concentrations observed in the liver, renal fat, adrenal gland and ovaries (means of 56.3, 30.7, 27.6 and 27.5 µg, respectively). Lower concentrations were present in the pancreas, thyroid gland, kidneys and lungs (23.7, 20.0, 19.7 and 16.2 µg, respectively). Concentrations of radioactivity in all other tissues were similar to, or below plasma (mean 14.1 µg). Only small amounts of radioactivity were present in the gastrointestinal tract (means of 0.68% (males) and 1.80% (females) of the dose administered) and stomach (means of 0.04% (males) and 1.06% (females) of the dose administered). The levels of radioactivity remaining in the carcass were low, accounting for a mean of 0.23% of the dose in males and 0.60% of the dose in females.

TABLE 7: Mean concentration of radioactivity in rat tissues/organs 48, 72 and 96 hours after administration of [phenyl-UL-^{14}C]-labeled ethiprole at 1000 mg/kg

	Mean Radioactivity in µg equivalents of [^{14}C]-ethiprole/g [a]					
	48 h post-dose		72 h post-dose		96 h post-dose	
Tissue/organ	Male	Female	Male	Female	Male	Female
Adrenals	119.7	122.7	*17.5	87.7	*4.5	27.6
Bone Marrow	23.6	18.5	4.0	22.0	*1.6	7.1
Brain	24.8	68.4	*4.2	20.7	*1.0	6.8
Fat-Renal	208.1	138.0	30.4	115.4	6.9	30.7
Heart	49.8	46.4	10.6	33.9	3.0	14.1
Kidneys	65.6	63.4	20.1	47.4	7.3	19.7
Liver	160.5	137.9	45.3	111.7	14.5	56.3
Lungs	55.7	49.0	15.0	37.7	4.3	16.2
Muscle	31.7	33.6	6.4	22.7	*1.6	6.9
Pancreas	92.9	86.1	15.6	56.5	*3.8	23.7
Ovaries	NA[b]	63.8	NA	56.7	NA	27.5
Skin and Fur	45.2	37.5	12.5	30.3	11.8	13.1
Spleen	32.1	31.4	*5.0	25.3	1.8	9.3
Testes	36.7	NA	8.3	NA	2.1	NA
Thyroid	192.0	64.6	16.7	43.8	*5.2	20.0
Whole Blood	50.7	28.9	13.3	21.8	5.1	9.6
Plasma	63.3	39.9	22.6	30.1	7.9	14.1

Data obtained from pages 171-172, 175-176, and 179-180 in the study report.
a An asterisk (*) indicates that the mean includes results calculated from data less than 30 d.p.m. above background.
b NA = Not Applicable

6. <u>Bile Duct Cannulation Experiment</u> Average recoveries of radioactivity in urine, feces, cage washings, and carcass from the low- and high-dose rats are presented in Table 8.

a) <u>Single Low-Dose</u>: Five male and 5 female rats fitted with indwelling bile duct cannulae, each received an oral dose of 5 mg/kg of [phenyl-UL-^{14}C]-ethiprole. Following administration, the major route of excretion was *via* bile, with a mean value of 67.2% (males) and 51.6% (females) of the dose recovered by 96 h post dose. Excretion *via* urine and feces over the same period accounted for ca 11.0% and 10.5% (males) and ca 30.4% and 10.5% (females) of the administered dose. Absorption of the administered dose was extensive and rapid. The low excretion *via* feces (10.5%, (males and females) of the dose) indicated that at least ca 89% of an oral dose of [phenyl-UL-^{14}C]-ethiprole at the low dose level was absorbed, with the bulk of this absorbed within 24 h post dose. Excretion was rapid, with mean recoveries (in bile, urine, feces and cage wash) of 35.2%, 68.5% and 88.3% of the administered dose recovered by 12, 24 and 48 h post dose, respectively, in males. The rate of recovery in female rats was similar to males with mean recoveries of 27.1%, 51.3% and 80.2% of the administered dose recovered by 12, 24 and 48 h post dose, respectively. By the end of the study period at 96 h post dose the mean recovery of total radioactivity from bile, urine, feces and cage wash accounted for ca 92.4% and ca 95.6% of the dose in males and females, respectively. Recovery of total radioactivity from the remaining carcasses was low, accounting for mean values of 1.49% and 3.49% of the dose in males and females, respectively, suggesting low residues remained in the tissues. Recovery in all samples by the end of the study period represented mean values of 93.9% and 99.1% of the administered dose in males and females, respectively.

a) Single High-Dose: Five male and 5 female rats fitted with indwelling bile duct cannulae, each received an oral dose of 1000 mg/kg of [phenyl-UL-^{14}C]-ethiprole. Following administration, the major route of excretion was *via* feces with a mean value of 86.0% (males) and 79.3% (females) of the dose recovered by 96 h post dose. Excretion *via* urine and bile over the same period accounted for ca 1.1% and 8.9% (males) and ca 1.5% and 7.0% (females) of the administered dose. Absorption of the administered dose was low. Excretion *via* feces (86.0% (males) and 79.3% (females) of the dose) indicated that it was likely that only ca 14% (males) and 20% (females) of an oral dose of [phenyl-UL-^{14}C]-ethiprole at the high dose level was absorbed. Excretion was rapid with mean recoveries (in bile, urine, feces and cage wash) of 60.3%, 82.2% and 92.3% of the administered dose by 24, 48 and 72 h post dose respectively in males. The rate of recovery in female rats was similar to males with mean recoveries of 40.6%, 64.0% and 77.5% of the administered dose recovered by 24, 48 and 72 h post dose respectively. By the end of the study period at 96 h post-dose the mean recovery of total radioactivity from bile, urine, feces and cage wash accounted for 96.5% and ca 87.8% of the dose in males and females, respectively. Recovery of total radioactivity from the remaining carcasses was low, accounting for mean values of 0.36% and 5.78% of the dose in males and females, respectively suggesting low residues remained in the tissues. Recovery in all samples by the end of the study period represented mean values of 96.9% and 93.6% of the administered dose in males and females, respectively.

TABLE 8: Recovery of radioactivity in excreta of bile-duct-cannulated rats after administration of [phenyl-UL-^{14}C]-labeled ethiprole

Component	Mean Recovery of Radioactivity (% Dose Administered)			
	5 mg/kg		1000 mg/kg	
	Male	Female	Male	Female
Urine	Mean	Mean	Mean	Mean
6	1.69	1.38	0.09	0.10
12	3.37	5.81	0.19	0.11
24	7.30	14.7	0.35	0.20
48	9.92	24.8	0.69	0.56
72	10.6	28.8	0.98	0.96
96	11.0	30.4	1.14	1.53
Feces	Mean	Mean	Mean	Mean
12	3.18	1.24	19.7	11.3
24	8.03	6.04	57.9	39.6
48	10.0	9.24	76.0	60.3
72	10.4	10.1	82.9	71.0
96	10.5	10.5	86.0	79.3
Bile	Mean	Mean	Mean	Mean
6	6.99	7.05	0.40	0.07
12	26.1	19.2	0.95	0.23
24	49.9	28.6	1.88	0.75
48	64.8	43.6	5.13	2.90
72	66.7	49.9	7.92	4.95
96	67.2	51.6	8.92	7.03
Cage Wash	Mean	Mean	Mean	Mean
12	2.49	0.83	0.03	0.01
24	3.26	2.01	0.21	0.10
48	3.56	2.63	0.36	0.25
72	3.71	2.95	0.46	0.57
96	3.79	3.10	0.49	0.98
Recovery	Mean	Mean	Mean	Mean
6	8.68	8.43	0.49	0.13
12	35.2	27.1	20.9	11.6
24	68.5	51.3	60.3	40.6
48	88.3	80.2	82.2	64.0
72	91.5	91.8	92.3	77.5
96	92.4	95.6	96.5	87.8
Carcass	1.49	3.49	0.36	5.78
Recovery, all samples	93.9	99.1	96.9	93.6

Data obtained from pages 183-194 in the study report.

B. METABOLITE CHARACTERIZATION STUDIES: Summaries of the metabolites identified and characterized in excreta of rats dosed with ethiprole are presented in Tables 10-13.

Analysis of selected pooled samples of urine, feces and bile was carried out by radio-HPLC and LC-MS in an attempt to identify the metabolites of ethiprole. The identities of those metabolites which co-chromatographed with supplied reference standards were confirmed by LC-MS. Structures for several other metabolites were postulated following MS analysis and are listed below.

Metabolite Name	Metabolite Identity
U1	Glucuronide conjugate of hydroxy-MB45897
U2	Sulphinic acid of RPA 104615
U3	RPA 104615
U4	Sulphate conjugate of hydroxy-MB 45897
U5	Unknown, related to U4
U6	Cyclic structure, related to U7
U7	Cyclic amide of RPA 112705
U8	Sulphate conjugate of RPA 114345
B3	RPA 104615

It was not possible to identify several metabolites in bile, due to the occurrence of coeluting endogenous material.

Radio-HPLC profiling of urine indicated that [14C]-ethiprole was extensively metabolized, with the metabolites covering a wide range of polarities. The major metabolites observed were the polar components U1, U2 (ca 5-7% dose at the low dose level), RPA 112705 (ca 7% dose in low dose female animals) and the less polar MB 45897 (ca 2-3% at the low dose level). Other components, identified as U3, U4, U5, U6, U7, U8 (female animals only), RPA 97973 (female low dose animals only) and RPA 114345, were also observed. The metabolite profiles in male and female animals at both low and high dose levels were qualitatively quite similar, indicating that the routes of metabolism are very similar at both the low and high dose levels for male and female animals. Radio-HPLC profiles of urine from male and female rats following repeat administration showed no significant differences to the profiles obtained from animals receiving a single dose. This indicated that the metabolic pathway was unchanged following repeat administration of ethiprole.

Radio-HPLC profiling of feces indicated the presence of RPA 112705, RPA 114345, MB 45897, ethiprole parent (RPA 107382), RPA 97973 and RPA 107566, as well as minor unidentified metabolites, in male and female animals at the low dose level. Ethiprole parent represented only ca 0.2-0.3% of the dose, suggesting that administered material was almost totally absorbed. The major component was RPA 112705 representing ca 22% and 10% of the dose in male and female animals, respectively. The profile obtained from high dose level animals was dominated by unabsorbed ethiprole (72-77%). However, small quantities of RPA 112705, RPA 114345, MB 45897 and RPA 107566 were also observed, indicating that similar metabolic routes were involved at both the low and high dose levels. The fecal metabolite profile in male and female rats following repeat administration of ethiprole was similar to that observed following the single low dose.

Radio-HPLC profiling of urine from male and female bile duct cannulated rats gave profiles which were qualitatively similar to non-cannulated rats at the low dose level. The major component was U2 (4.8% dose male, 10.6% dose female), with RPA 112705 representing 5.23% dose in female animals. Components RPA 114345, MB 45897 and RPA 97973, along with U1, U3, U4, U5, U6 and U7 (male animals only), were also observed. The major difference between

the cannulated and non-cannulated profiles were the proportions of U1, U4 and MB 45897, which were all greater in non-cannulated animals compared to cannulated animals.

The metabolite profile in urine of bile duct cannulated animals at the high dose level showed some differences from the profile obtained for intact animals. In male animals the parent compound RPA 107382 (0.05%) and RPA 97973 (0.04%) were detected, however they were not observed in non-cannulated males at this dose. The metabolites U4, U6, and U7, which were observed in non-cannulated males, were not detected in cannulated males, and U1 was present in significantly less abundance than in non-cannulated rats. In females, there were significant quantitative differences in the metabolic profile of cannulated rats compared to intact female rats at the high dose level. The component RPA 114325 was not detected in non-cannulated female rats. Metabolites U1, U2, U3, U6, and U8 were observed at considerably lower levels in cannulated females than in non-cannulated females at this dose.

Analysis of feces from male and female bile duct cannulated animals at the low dose level revealed extensive metabolism, with the components RPA 114345, MB 45897, ethiprole parent, RPA 97973 and RPA 107566 observed in male and female animals. The main difference between the cannulated and non-cannulated animals was the absence of RPA 112705 in cannulated rats (which was the major metabolite in the feces of intact animals of both sexes), indicating that this was a systemic metabolite eliminated partially through biliary excretion.

The extensive metabolism in feces from cannulated animals suggests that gut microflora may be involved in the metabolism of ethiprole. Alternatively, systemic metabolites may be entering the gut by a mechanism other than biliary excretion.

Profiling of high dose level feces was dominated by unchanged ethiprole (76% in males, 63 % in females), but the presence of RPA 97973 and RPA 107566 indicate mechanisms of metabolism similar to that observed at the low dose level.

Radio-HPLC profiling of bile from male and female rats dosed at the low dose level revealed similar patterns of extensive metabolism. Several polar components, B4, B5, B6, B1 and B2 were observed, but not identified by mass spectrometry. It was thought possible that components B1 and B2 may be the same metabolites as U1 and U2, however it was not possible to confirm this. A further component, B3, was identified as RPA 104615. The major metabolites in bile were B1 and B3 (10.66% and 15.7% dose in males, 9.16 and 11.44% dose females). The metabolic profile in the bile from male and female rats dosed at the high dose level was very similar, qualitatively, to that observed at the low dose.

TABLE 10. Metabolite profile in urine and feces from rats dosed with [phenyl-UL-^{14}C]-labeled ethiprole at 5 mg/kg (expressed as % administered dose)

Identified / or Characterized[a]	Sampling Time point (hours)				Sampling Time point (hours)			
	0-12	12-24	24-48	48-72	0-12	12-24	24-48	48-72
	Urine (male, animal no. 1-5)				Urine (female, animal no. 6-10)			
U1	2.00	2.96	2.09	0.14	0.61	1.63	1.78	0.16
U2	3.21	1.51	0.22		3.72	1.79	0.66	0.03
U3	0.52	0.37	0.12		0.83	0.68	0.38	0.03
RPA 112705	0.33	0.53	0.13		1.08	3.25	2.28	0.16
U4	0.82	1.27	1.05	0.09	0.43	0.42	0.47	0.06
U5	0.26	0.21	0.05			0.35	0.29	0.02
U8					1.12	1.50	0.70	0.09
U6	0.20	0.21	0.12	0.01	0.29	1.16	0.84	0.07
U7		0.10	0.12	0.03		0.14	0.09	
RPA 114345	0.41	0.48	0.07	0.01	0.49	0.86	0.35	0.04
MB 45897	1.03	0.50	0.15		2.09	0.88	0.36	0.04
RPA 97973					0.58	0.63		0.02
Unknown			0.03	0.01			0.19	
Unknown			0.01	0.01				
Unknown			0.06	0.01				
Unknown			0.06	0.01				
Unknown			0.12	0.01				
Unknown			0.10					
%TRR in pooled sample	100	100	87.9	35.3	100	100	91.2	36.6
Mean %Dose in pooled sample	8.78	8.13	5.06	1.06	11.24	13.3	9.2	2.00
Identified in Urine	--	--	--	20.80	--	--	--	32.79
Characterized in Urine	--	--	--	0.95	--	--	--	0.85
Total Urine	--	--	--	21.75	--	--	--	33.64
	Feces (male, animal no. 1-5)				Feces (female, animal no. 6-10)			
RPA 112705		10.06	11.8	0.38		2.67	6.51	0.94
RPA 112917 (?)							0.77	
RPA114345	0.53	4.64	0.97	0.31	0.56	3.88	2.67	0.43
MB 45897	1.01	6.38	1.58	0.23	1.01	4.58	1.95	0.46
RPA 107382	0.29				0.19			
RPA 97973	0.92	3.38	0.76		0.58	3.16	1.59	
RPA 107566	0.35				0.13	0.39		
Unknown	0.13	1.02	0.94	0.35	0.10	0.99	0.73	0.47
Unknown	0.13	1.77	1.15	0.26	0.51	1.23	0.46	
Unknown		1.00	0.59	0.25	0.23	0.83	0.78	
Unknown					0.09	0.62		
%TRR in pooled sample	82.3	81.4	75.4	50.8	68.4	79.1	70.3	64.0
Mean %Dose in pooled sample	4.07	34.7	23.6	3.50	4.97	23.2	22.0	3.60
Identified in Feces	--	--	--	43.59	--	--	--	32.47
Characterized in Feces	--	--	--	7.59	--	--	--	7.04
Total Feces	--	--	--	51.18	--	--	--	39.51
Total Identified	--	--	--	**64.39**	--	--	--	**65.26**
Total Characterized	--	--	--	**8.54**	--	--	--	**7.89**
Total Accounted For	--	--	--	**72.93**	--	--	--	**73.15**

Data obtained from pages 195-196 (urine), 201-202 (feces), and 229 (sum identified/characterized) in the study report.

[a] Identified is where a structure has been proposed from MS data and/or from co-chromatography with an authentic reference standard. For some timepoints, extrapolation was made from MS data produced for metabolites from other samples. Characterized is where no structure has been proposed for a metabolite and applies to unknowns, the urinary metabolite U5 and the biliary metabolites B1, 2 and 4-8. Total accountability is the sum of identified and characterized metabolites.

TABLE 11. Metabolite profile in urine and feces from rats dosed with [phenyl-UL-^{14}C]-labeled ethiprole at 1000 mg/kg (expressed as % administered dose)

Identified / or Characterized[a]	Sampling Time point (hours)				Sampling Time point (hours)			
		0-24	24-48	48-72		0-24	24-48	48-72
	Urine (male, animal no. 11-15)				Urine (female, animal no. 16-20)			
U1		0.12	0.39	0.57		0.07	0.43	0.81
U2		0.03	0.03	0.06			0.04	0.42
U3		0.05	0.06	0.03		0.04	0.08	0.12
RPA112705			0.02	0.02		0.09	0.31	0.42
U4		0.06	0.07	0.04		0.05	0.05	0.07
U8						0.04	0.03	
U6		0.02				0.05	0.12	0.12
U7			0.03	0.02			0.06	0.06
RPA114345		0.04	0.02					
MB 45897		0.17	0.22	0.10		0.25	0.50	0.09
RPA 97973								
Unknown			0.02	0.03		0.03		
Unknown				0.02				
Unknown				0.02				
Unknown				0.01				
%TRR in pooled sample		95.7	96.7	100		100	100	94.3
Mean %Dose in pooled sample		0.52	0.88	0.92		0.61	1.63	2.25
Identified in Urine		--	--	2.17		--	--	4.32
Characterized in Urine		--	--	0.10		--	--	0.03
Total Urine		--	--	2.27		--	--	4.35
	Feces (male, animal no. 11-15)				Feces (female, animal no. 16-20)			
	0-12	12-24	24-48	48-72	0-12	12-24	24-48	48-72
RPA 112705			1.24	0.74				
RPA 114345			0.20	0.07				
MB 45897		0.14	1.14	0.33			0.26	0.79
RPA 107382	25.55	13.88	27.8	5.01	10.38	21.93	23.54	21.12
RPA 97973		0.08	0.28	0.10				
RPA 107566		0.34	0.67	0.21		0.34	1.02	1.09
Unknown				0.18				
%TRR in pooled sample	93.6	87.5	98.2	72.1	99.8	86.0	98.1	96.6
Mean %Dose in pooled sample	27.3	16.5	31.9	9.2	10.4	25.9	25.3	23.8
Identified in Feces	--	--	--	77.78	--	--	--	80.47
Characterized in Feces	--	--	--	0.18	--	--	--	0.00
Total Feces	--	--	--	77.96	--	--	--	80.47
Total Identified	--	--	--	**79.95**	--	--	--	**84.79**
Total Characterized	--	--	--	**0.28**	--	--	--	**0.03**
Total Accounted For	--	--	--	**80.23**	--	--	--	**84.82**

Data obtained from pages 197-198 (urine), 203-204 (feces), and 229 (sum identified/characterized) in the study report.

[a] Identified is where a structure has been proposed from MS data and/or from co-chromatography with an authentic reference standard. For some timepoints, extrapolation was made from MS data produced for metabolites from other samples. Characterized is where no structure has been proposed for a metabolite and applies to the unknowns. Total accountability is the sum of identified and characterized metabolites.

TABLE 12. Metabolite profile in urine and feces from rats dosed with [phenyl-UL-^{14}C]-labeled ethiprole at 5 mg/kg following 14 days of dosing with non-radiolabelled ethiprole at 5 mg/kg (expressed as % administered dose)

Identified / or Characterized[a]	Sampling Time point (hours)				Sampling Time point (hours)			
	0-12	12-24	24-48	48-72	0-12	12-24	24-48	48-72
	Urine (male, animal no. 21-25)				Urine (female, animal no. 26-30)			
U1	1.25	2.75	2.96	0.77	0.99	1.43	1.90	0.51
U2	2.49	0.82	0.14	0.03	2.40	1.36	1.29	0.18
U3	0.54	0.26	0.14	0.03	0.85	0.57	0.16	0.07
RPA112705	0.14	0.21	0.12	0.04	2.56	3.66	2.19	0.29
U4	0.53	1.24	1.97	0.46	0.36	0.33	0.53	0.11
U5	0.16	0.14			0.53	0.54	0.46	0.05
U8					0.63	0.52	0.09	
U6	0.09	0.15	0.17	0.07	0.50	1.00	0.82	0.16
U7		0.16	0.23	0.13	0.38	0.19	0.13	
RPA114345	0.35	0.33			3.29	0.42	0.20	0.06
MB 45897	1.09	0.45	0.11		0.42	1.79	0.20	
RPA 97973	0.07					0.30	0.11	0.03
Unknown		0.15	0.30	0.07			0.10	
Unknown				0.17				
Unknown				0.07				
Unknown				0.03				
%TRR in pooled sample	97.0	100	100	100	100	100	100	98
Mean %Dose in pooled sample	6.92	6.74	6.15	1.87	12.9	12.1	8.18	1.5
Identified in Urine	--	--	--	20.29	--	--	--	32.98
Characterized in Urine	--	--	--	1.09	--	--	--	1.68
Total Urine	--	--	--	21.38	--	--	--	34.66
	Feces (male, animal no. 21-25)				Feces (female, animal no. 26-30)			
	0-12	12-24	24-48	48-72	0-12	12-24	24-48	48-72
RPA 112705		10.76	10.70	0.94		1.20	9.92	0.38
RPA 114345	0.26	5.50	2.18	0.27	0.60	3.89	1.47	0.62
MB 45897	0.20	2.05	1.89	0.29	0.94	4.61	0.57	0.33
RPA 107382	1.21				0.38	3.59		
RPA 97973	0.23		1.52		0.78	1.41		
RPA 107566	0.31				1.43	2.13		
Unknown	0.25	1.77	0.99	1.76	0.24	0.67	1.89	0.27
Unknown		1.39	1.42	0.29			1.08	
Unknown		0.65		0.22			1.39	
Unknown							1.15	
Unknown								
%TRR in pooled sample	66.8	77.6	64.7	60.0	82.4	82.6	74.0	38.0
Mean %Dose in pooled sample	3.78	28.5	28.9	6.3	5.31	21.2	23.6	4.2
Identified in Feces	--	--	--	38.31	--	--	--	34.25
Characterized in Feces	--	--	--	8.74	--	--	--	6.69
Total Feces	--	--	--	47.05	--	--	--	40.94
Total Identified	--	--	--	**58.60**	--	--	--	**67.23**
Total Characterized	--	--	--	**9.83**	--	--	--	**8.37**
Total Accounted For	--	--	--	**68.43**	--	--	--	**75.60**

Data obtained from pages 199-200 (urine), 205-206 (feces), and 229 (sum identified/characterized) in the study report.

[a] Identified is where a structure has been proposed from MS data and/or from co-chromatography with an authentic reference standard. For some timepoints, extrapolation was made from MS data produced for metabolites from other samples. Characterized is where no structure has been proposed for a metabolite and applies to the unknowns. Total accountability is the sum of identified and characterized metabolites.

TABLE 13. Metabolite profile in excreta of bile-duct-cannulated rats dosed with [phenyl-UL-^{14}C]-labeled ethiprole at 5 or 1000 mg/kg (expressed as % administered dose)

Identified / or Characterized[a]	Sampling Time point (0-72 hours) 5 mg/kg		Sampling Time point (0-72 hours) 1000 mg/kg	
	Urine (male, animal no. 105-110)	Urine (female, animal no. 112-116)	Urine (male, animal no. 117-121)	Urine (female, animal no. 123-127)
U1	0.72	1.93	0.07	0.10
U2	4.81	10.60	0.27	0.03
U3	0.81	2.18	0.12	0.10
RPA112705	0.48	5.23	0.05	0.18
RPA 112716		0.31		
U4	0.79	0.89		
U5	0.29	0.57		
RPA 112917		0.33		
U8				0.04
U6	0.46	1.25		0.04
U7	0.23			
RPA114345	0.71	1.84	0.06	0.04
MB 45897	0.35	2.84	0.29	0.34
RPA 107382			0.05	
RPA 97973	0.12	0.57	0.04	
RPA 107566	2.25			
Unknown	0.12			0.03
Unknown	0.26			0.02
Unknown	0.24			
Unknown	0.21			
%TRR in pooled sample	100	99.1	97.2	97.6
Mean %Dose in pooled sample	10.6	28.8	0.98	0.96
Identified in Urine	9.48	27.97	0.95	0.87
Characterized in Urine	1.12	0.57	0.00	0.05
Total Urine	10.60	28.54	0.95	0.92
	Feces (male, animal no. 105-110)	Feces (female, animal no. 112-116)	Feces (male, animal no. 117-121)	Feces (female, animal no. 123-127)
RPA114345	2.94	3.29		
MB 45897	0.51	0.37		
RPA 107382	1.53	0.44	76.35	62.94
RPA 97973	2.35	4.54	0.63	
RPA 107566	2.25	2.05	4.73	2.54
%TRR in pooled sample	92.2	94.0	97.6	92.1
Mean %Dose in pooled sample	10.39	11.38	82.9	71.1
Identified in Feces	9.58	10.69	81.71	65.48
Characterized in Feces	0.00	0.00	0.00	0.00
Total Feces	9.58	10.69	81.71	65.48

(Table continued, footnotes follow)

TABLE 14. Metabolite profile in excreta of bile-duct-cannulated rats dosed with [phenyl-UL-^{14}C]-labeled ethiprole at 5 or 1000 mg/kg (expressed as % administered dose) – cont.

Identified / or Characterized[a]	Sampling Time point (0-72 hours) 5 mg/kg		Sampling Time point (0-72 hours) 1000 mg/kg	
	Bile (male, animal no. 105-110)	Bile (female, animal no. 112-116)	Bile (male, animal no. 117-121)	Bile (female, animal no. 123-127)
B4	1.29	1.70	0.90	0.40
B5	6.68	4.73	0.58	0.42
B6	3.94	2.38	0.29	0.17
B1	10.66	9.16	1.75	0.94
B2	8.41	8.21	1.68	1.00
B3 (RPA 104615)	15.70	11.44	0.58	0.45
RPA112705	6.63	2.55	0.67	0.30
RPA112716	0.91	1.34	0.15	0.47
RPA112916	1.93	1.03		
B7	2.06	0.96	0.34	0.10
RPA114345	6.38	3.37		
MB 45897	0.89	0.62	0.15	0.08
B8	1.21	0.98	0.05	
RPA 97973				0.06
Unknown		1.43	0.13	0.17
Unknown			0.16	0.05
Unknown			0.05	0.09
Unknown			0.23	0.17
Unknown			0.06	0.08
Unknown			0.04	
%TRR in pooled sample	100	100	98.6	100
Mean %Dose in pooled sample	66.7	49.9	7.75	4.95
Identified in Bile	32.44	20.35	1.55	1.36
Characterized in Bile	34.25	29.55	6.26	3.59
Total Bile	66.29	49.90	7.81	4.95
Total Identified	**51.50**	**59.01**	**84.21**	**67.71**
Total Characterized	**35.37**	**30.12**	**6.26**	**3.64**
Total Accounted For	**86.47**	**89.13**	**90.47**	**71.35**

Data obtained from page 207-218 and 229 in the study report

[a] Identified is where a structure has been proposed from MS data and/or from co-chromatography with an authentic reference standard. For some timepoints, extrapolation was made from MS data produced for metabolites from other samples. Characterized is where no structure has been proposed for a metabolite and applies to unknowns, the urinary metabolite U5 and the biliary metabolites B1, 2 and 4-8. Total accountability is the sum of identified and characterized metabolites.

III. INVESTIGATORS' DISCUSSION AND CONCLUSIONS:

Oral administration of [14C]-ethiprole to male and female rats, at a target dose level of 5 mg/kg, indicated rapid absorption and excretion of the test material. The bulk of the administered radioactivity was excreted by 48 h, mainly *via* feces (62.4% dose male, 50.2% dose female). The higher level of excretion in male feces compared to female was reflected in a consistent decrease in male urinary excretion (23.5% of the dose at 168 h) compared with females (36.4%). This may indicate a possible sex dependent difference in the routes of excretion.

Low levels of total radioactivity were measured in tissues from male and female rats at 168 h following administration at 5 mg/kg. The highest residue levels were measured in liver and kidney, the organs associated with metabolism. In general, concentrations in tissues were slightly higher in female animals, with the exception of whole blood, where residues in female animals were ca 5-6 fold higher than male animals.

Investigation of the pharmacokinetics in whole blood following single oral administration at 5 mg/kg demonstrated rapid absorption and excretion of test material. The maximum concentration of total radioactivity observed occurred 8 h following administration in both male and female animals. Levels of radioactivity in blood had dropped significantly by 48 h, consistent with rapid excretion. The terminal elimination phase half-lives ($t_{½}$el) of female animals was considerably greater (114 h) than male animals (48 h). This was reflected in the levels of radioactivity in female whole blood at 168 h, which were ca 2 fold higher than male animals.

Repeat dosing with unlabelled ethiprole followed by a single administration of [^{14}C]-ethiprole gave patterns of absorption and excretion very similar to those observed following a single low dose. The dose was quickly absorbed and excreted, with the bulk of the dose excreted by 48 h post dose. The major route of excretion was again feces, with a higher amount excreted by male rats (70.7% dose) compared to female (55.5% dose). The higher excretion *via* feces in male rats was consistent with observations made following single administration at the low dose level. Similarly, the increased urinary excretion in female rats (35.1% dose) compared to male rats (22.5%) was also noted following a single administration.

The similarity in the routes and rates of excretion following single and multiple administration indicated that there were no changes in the metabolic route following repeat administration.

Tissue levels in male rats following multiple administration were comparable with, or slightly higher than, those of female rats, with the exception of whole blood, where concentrations in female rats were 2-3 times higher than males. This result was also observed following single administration. Comparison of tissue concentrations following single and multiple administration revealed very similar concentrations in tissues from female rats and concentrations which were slightly higher in repeat dose male animals. With the exception of skin and fur, no significant differences were observed between single and repeat dose administration. The high concentrations in skin and fur may have been due to contamination, or absorption of material

from urine.

The similarity of residue levels in tissues following single and multiple oral administration indicated that there was no accumulation in the tissues or changes in the pattern of distribution and elimination from tissues following repeated administration of the test material.

Administration of [^{14}C]-ethiprole at the high dose level of 1000 mg/kg indicated slower absorption than that observed at the low dose level, with the bulk of the dose being excreted by 72 h post dose. The large amounts of unabsorbed material excreted *via* feces dominated the excretion profile and it was not possible to determine whether there were any significant differences in the pattern of excretion between male and female animals.

Tissue concentrations of total radioactivity following high dose administration were similar in both male and female animals. Considerably higher residues were noted in male skin and fur compared to females but this may have been due to urinary contamination. As with the low dose animals the highest residues were found in the organs of metabolism (liver, kidneys). High levels in the adrenals and thyroid may have been exaggerated by the low weight of these tissues.

Investigation of whole blood pharmacokinetics at the high dose level revealed much slower absorption and excretion than that seen at the low dose level. The maximum concentration of total radioactivity was observed at 48 h post dose, with concentrations after this declining rapidly at first, then more slowly after 96 h. The terminal elimination half-lives for male and female animals were similar and consistent with low dose male animals, indicating that there was no effect of dose upon $t_{½}$el. Both AUC values measured for male and female animals were 22-28 times greater than at the low dose level. This was in comparison to an increase in dose level of 200 fold, indicating non-dose linearity and a saturation of the absorption pathways at the high dose level.

The levels of total radioactivity in tissues from male and female animals declined rapidly throughout the course of the low dose level tissue kinetics experiment. Tissue concentrations in male and female animals were very similar at 8 h post dose and remained comparable in most tissues at 48 h post dose (although residues in liver and plasma were ca 2 fold higher in male animals at 48 h post dose). In general these results indicate that there is no retention of total radioactivity in a particular tissue type and that the tissue distribution and elimination in male and female animals is similar.

At the high dose level, tissue residues in female animals declined more slowly than in males. Concentrations were comparable at 48 h post dose, but by 96 h, levels in most female tissues were ca 3-4 fold higher than males. This was not noted with whole blood where concentrations were only ca 1.5-2 fold greater. The results again demonstrate similar distribution of total radioactivity to male and female rats and show no retention of residues in a particular tissue.

Analysis of low dose bile duct cannulated male and female rat feces indicated identical amounts (10.5% dose) of unabsorbed material. This indicated that absorption in male and female animals

was identical and represented ca 90% of the administered dose. The difference in excretion *via* urine (11.0% male, 30.4% female) is consistent with that observed in non-cannulated animals. Similarly, the increased excretion in male bile (67.2%) compared to female (51.6%) is consistent with the increased fecal excretion in non-cannulated male rats compared to female. This suggests a sex specific difference in excretion between male and female animals, due to increased biliary excretion in male rats. The lower amounts of dose excreted in the urine of both male and female cannulated animals (and the increase in bile/feces) compared to intact animals, may indicate that a proportion of the dose excreted in bile in intact animals undergoes enterohepatic re-circulation and is subsequently excreted *via* urine.

Analysis of feces from male and female bile duct cannulated rats at the high dose level indicated that ca 86% and 79%, respectively, of the administered dose was unabsorbed. The excretion profile is dominated by this unabsorbed material and it was not possible to determine if there were any significant differences in the pattern of excretion between male and female animals. Of the material absorbed, the bulk of this was excreted *via* bile (ca 9% dose (male) and ca 7% dose (female)).

Radio-HPLC profiling of feces, urine and bile indicated that [14C]-ethiprole was extensively metabolized following oral administration. Several of the metabolites observed co-chromatographed with supplied reference standards and the identity of these were confirmed by LC-MS experiments. The structures of significant metabolites which did not co-chromatograph with reference standards were also investigated by LC-MS and the suggested identities of these metabolites are shown in the tables below.

Metabolite	Metabolite Identity (in Urine)
U1	Glucuronide conjugate of hydroxy-MB45897
U2	Sulphinic acid of RPA 104615
U3	RPA 104615
U4	Sulphate conjugate of hydroxy-MB 45897
U5	Unknown, related to U4
U6	Cyclic structure, related to U7
U7	Cyclic amide of RPA 112705
U8	Sulphate conjugate of RPA 114345

Metabolite	Metabolite Identity (in Bile)
B1	Unknown
B2	Unknown
B3	RPA 104615
B4	Unknown
B5	Unknown
B6	Unknown
B7	Unknown
B8	Unknown

Postulated structures for these metabolites are shown in the suggested metabolite pathway (Figure 1).

Analysis of urine from male and female rats following administration at 5 mg/kg revealed similar, qualitative, metabolite profiles. The major components were the polar, conjugated,

metabolites U1 and U2, the less polar MB 45897 and in female urine RPA 112705.

In general the amounts of each metabolite detected were greater in female urine, reflecting the higher amount of urinary excretion in female rats. However, the level of RPA 112705 detected in female rats was far greater than detected in males. In addition the sulphate conjugated RPA 114345 (U8) and RPA 97973 were observed in female urine but not male. A similar pattern emerged in the high dose urine. The major metabolites were again the polar components U1 and U2, the less polar MB 45897 and in female rats, RPA 112705 which was present in far greater quantities than in male urine. Again the component U8 was detected in female urine but not male. The only major qualitative difference between low and high dose urine was the absence of the component U5 at the high dose levels. Otherwise the pattern of metabolism at the high dose level was consistent with that observed at the low dose level.

Analysis of urine from males and females following repeat dosing showed very similar qualitative and quantitative metabolite profiles to those observed in male and female rats following a single dose. This indicates that the metabolism of ethiprole is not changed by repeated exposure. It was again noted that the levels of RPA 112705 were considerably higher in female urine and the sulphate conjugate of RPA 114345 was not seen in male rats.

Radio-HPLC profiling of male and female feces at the low dose level indicated a very similar metabolite profile. The proportion of each metabolite was consistent between the sexes, with the exception of RPA 112705. This metabolite occurred in far greater abundance in male feces compared to female. This is in comparison to urine where RPA 112705 was more predominant in female rats. It is also significant that very small quantities of unchanged ethiprole were observed in both male and female rats, suggesting that nearly all of the administered dose was absorbed.

Analysis of high dose feces is dominated by unabsorbed ethiprole (72-77% of the administered dose). However small quantities of RPA 112705, RPA 114345, MB 45897 and RPA 107566 were observed. These components were also observed at the low dose level, suggesting a similar metabolic pathway at both dose levels.

Investigation of the metabolite pattern in feces following repeat administration showed similar quantitative and qualitative profiles to those observed following a single low dose administration in both male and female rats. This indicated that the pattern of metabolism is unchanged by repeated administration of ethiprole.

Analysis of urine from bile duct cannulated male rats revealed the major metabolite to be U2. This was observed in similar proportions to those in non-cannulated animals. However amounts of U4 (the proposed sulphate conjugate of hydroxy MB 45897), MB 45897 and the metabolite U1, were observed in far greater amounts in noncannulated rats. This may be due to enterohepatic re-circulation which is not observed in cannulated animals. The pattern of the remaining metabolites is similar to that observed in intact animals.

Analysis of urine from bile duct cannulated female rats also indicated lower quantities of several

metabolites, most obviously U1, U5 and U6. The metabolite proposed as a sulphate conjugate of RPA 114345 (U8) was not observed in cannulated rats.

The reduced amounts of these metabolites in urine from cannulated animals suggests that metabolites may be excreted via bile and re-absorbed, undergoing further metabolism before being excreted in the urine.

Comparison between male and female rats again shows a far higher proportion of RPA 112705 in the urine of female animals compared to males. The component U2, a proposed sulphate conjugate of RPA 104615, was also seen in greater proportion in female animals.

Analysis of bile from male and female rats at the low dose level revealed similar qualitative metabolite profiles. Several metabolites B1, B2 and B4-8 were observed, but it was not possible to obtain any structural information on these from mass spectroscopy. It is highly likely that these are conjugated metabolites with B1 and B2 possibly being the same metabolites as U1 and U2 observed in urine. The component B3 was identified by mass spectroscopy as RPA 104615. All metabolites were observed in greater quantities in male bile compared to female. This was consistent with the greater biliary excretion in male animals, compared to females. The greater abundance of RPA 112705 in male bile compared to female was also noted. This increase in RPA 112705 was also seen in male feces.

Profiling of feces from bile duct cannulated animals indicated extensive metabolism, with nearly all the metabolites observed in the feces of intact animals also present in cannulated animals. The major difference was the absence of RPA 112705 in cannulated animals, indicating this metabolite was excreted in feces *via* bile. The presence of the remaining metabolites may be due to metabolism by gut microflora or may be due to systemic metabolites entering the gut by processes other than biliary excretion.

Analysis of urine from male and female bile duct cannulated rats at the high dose level indicated a similar metabolite profile to that seen at the low dose level, indicating that qualitatively, the pattern of metabolism was similar at low and high doses. Several minor metabolites present at the low dose (U4, U5, U6 and U7) were not seen at the high dose level in male animals while U4 and U5 were not observed in female animals.

The same metabolites were observed in high dose bile as were detected in the low dose with the exception of RPA 114345 which was absent at the high dose level. This again indicates the same metabolite pathways are involved at the low and high dose levels.

Analysis of feces from male and female bile cannulated rats at the high dose level is dominated by the presence of unabsorbed ethiprole. However the presence of components such as RPA 97973 and RPA 107566 indicate that potentially, metabolism by gut microflora is taking place or that systemic metabolites are entering the gut *via* an alternative mechanism to biliary excretion.

Metabolism of ethiprole in the rat. The proposed metabolic pathway is presented in Figure 1. The primary metabolism of ethiprole was observed to proceed *via* three different routes:

1. Oxidation of the nitrile group to produce RPA 112916, although this metabolite was only tentatively identified by co-chromatography during HPLC analysis. No further metabolites that were thought to be derived from the further metabolism of RPA112916 were detected so it is suggested that this may be a terminal metabolite.

2. Reduction of the sulphoxide group to produce RPA 107566 was observed. This metabolite was further metabolized by an alkyl oxidation reaction to generate RPA112716. Again no further metabolism of this metabolite was detected therefore it maybe considered to be terminal metabolite.

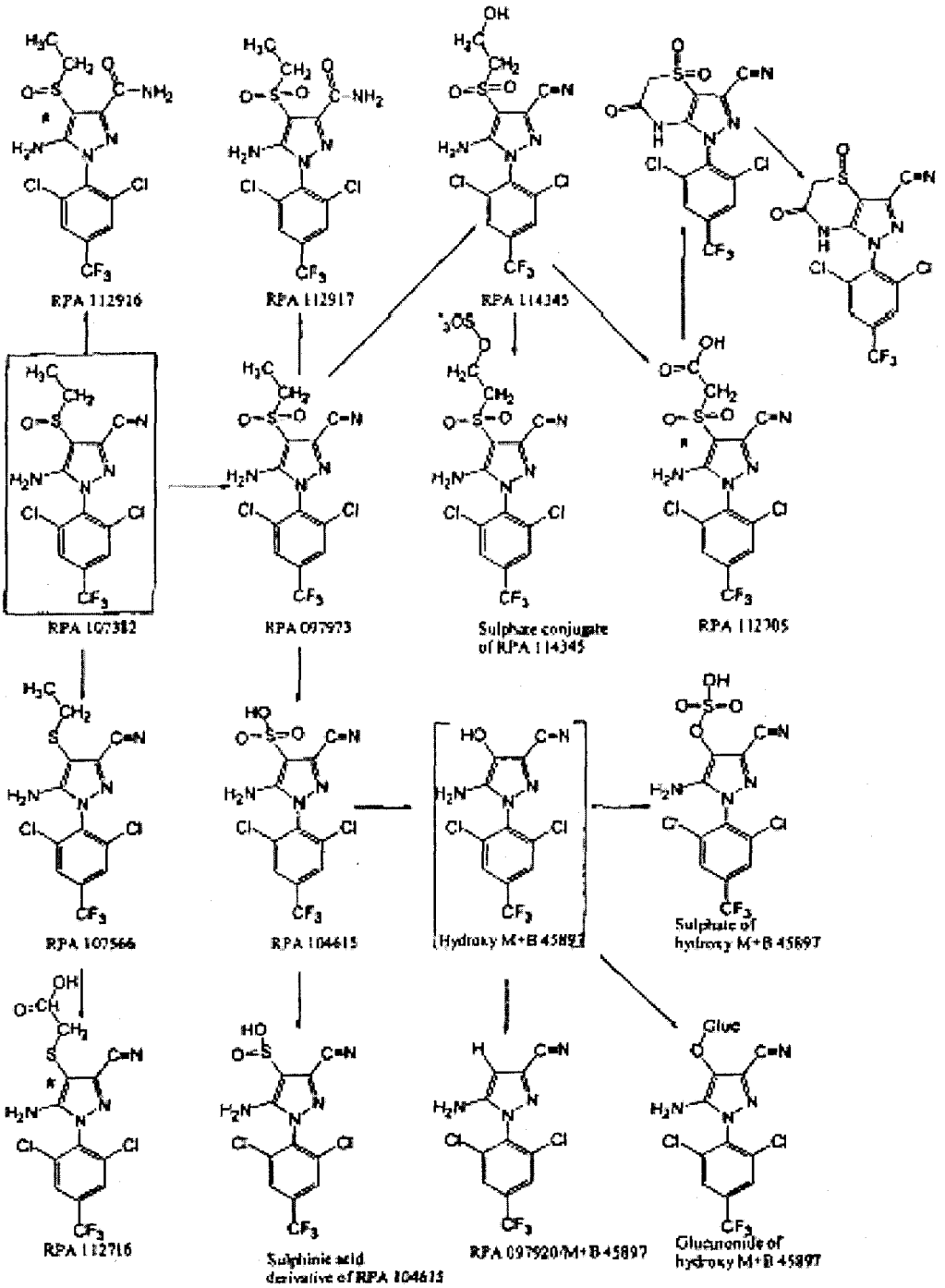
3. Oxidation of the sulphoxide group to the sulphone produced RPA 097973, the major metabolite. RPA 097973 was subsequently further metabolized via three different possible routes.

a. An alkyl hydroxylation reaction produced RPA 114345, which was subsequently further metabolized by an oxidative route to RPA 112705 and by sulphation to the sulphate conjugate of RPA 114345. The metabolite RPA 112705 underwent acyclisation reaction to generate the cyclic amide of RPA 112705, which was further metabolized by reduction of the sulphone function.

b. An oxidative dealkylation reaction produced the metabolite RPA 104615, which was further metabolized by substitution of the sulphate function with a hydroxyl group to produce hydroxy-M&B 45897. This intermediate was subsequently further metabolized by sulphation to generate the sulphate of hydroxy-M&B 45897, reduction to generate M&B 45897 and by conjugation to produce the glucuronide conjugate of hydroxy-M&B 45897. RPA 104615 was also metabolized by reduction of the sulphone function to produce RPA 104615 sulphinic acid.

c. Oxidation of the nitrile function produced RPA 112917, a possible terminal metabolite.

Figure 1. Proposed Metabolic Pathway



* Tentatively identified by co-chromatography with a reference standard
[ ] Postulated intermediate

Acute oral toxicity in rats with ethiprole (RPA 107382)

1.	Annex point(s)	KIIA 5.2.1; Acute toxicity/Acute oral toxicity: **MRID 47622801**
2.	Location in dossier	KIIA 5.2.1 /01
3.	Authors (year)	Steiblen, G. (1997)
	Title	Acute oral toxicity in the rat RPA107382
	Company, report No.	Bayer CropScience AG, Report-No.: R016801 (M-192389-01-1)
	Date	10.12.1997
4.	Testing facility	Rhone-Poulenc Agro, Sophia Antipolis, France
5.	Dates of work	Exp. Start date: September 30th 1997; Exp. Term. Date: October 21st 1997
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC (Batch-No.: CDR9706)
7.	Test method	EU (=EEC): 92/69/EEC, Annex V, Method B1; JMAF: 59 NohSan No. 4200, (1985); OECD: 401, (1987); USEPA (=EPA): FIFRA 81-1, (1984)
	Deviations	
8.	GLP	yes

Executive Summary:

The acute oral toxicity of RPA 107382 (ethiprole; batch number CRD 9706, 93% purity) was assessed after a single oral administration of the test substance at 5000 or 7080 mg/kg body weight to groups of fasted, 6-7 week old Wistar rats (5/sex/dose). RPA 107382 was suspended in 0.5% methylcellulose. All animals were observed for clinical signs daily for fifteen days.

Death occurred in 3 rats (1 male and 2 females) on Day 2 dosed at 5000 mg/kg/bw day and in 1 female rat on Day 3 dosed at 7080 mg/kg/bw day.

Clinical signs of toxicity were observed in the 3 rats at 5000 mg/kg/bw/day that died on Day 2 and included reduced motor activity, palpebral ptosis, hunched posture, and/or soiled fur and snout. No signs were observed for the one female that died in the 7080 mg/kg/bw day dose group. No gross lesions were noted at necropsy for surviving animals.

The three decedents dosed at 5000 mg/kg/bw day exhibited a marked dilation of the stomach (one male), moderate general autolysis (one female) or black spots on the granular surface of the stomach (one female). The female at 7080 mg/kg/bw day that died on Day 3 revealed a white area on the liver and black spots on non-glandular surface of the stomach.

According to OECD guideline 401, the LD_{50} of RPA 107382 (ethiprole; 93% purity) was greater than 7080 mg /kg/bw day in males and females. RPA 107382 is of **Low Toxicity** by the oral route based on the LD_{50} in rats. No signal words are required on the label.

MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 107382

Description: White powder
Lot/Batch: CRD 9706
Purity: 93.0%
CAS 181587-01-9
Stability of test compound: Stable at room temperature

2. Vehicle and /or positive control: 0.5% methylcellulose in distilled water

3. Test animals:

Species: Rat
Strain: Wistar (AF) RJ: WI (IOPS AF)
Age: 6 to 7 weeks approximately
Weight at dosing: 162-224 g ♂; 147-185 g ♀
Source: R. Janvier, Le Genest St Isle, France
Acclimation period: 6 days (rats dosed at 5000 mg/kg/bw) or 13 days (rats dosed at 7080 mg/kg bw)
Diet: Certified Rodent Pellet Diet "M20 contrôlé" (Pietrement, Provins, France), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Animals were housed individually in suspended stainless steel wire mesh cages

Environmental conditions –
Temperature: 20 to 24 °C
Humidity: 40 – 70 %
Air changes: Approximately 10 – 15 air changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: From September 30th to October 21st 1997

2. Animal assignment and treatment

Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of RPA 107382 (93% a.i.) by gavage (volume of 10 mL/kg) then observed approximately one hour after dosing, at least once on the day of dosing (Day 1) and once daily thereafter. Body weights were recorded on Days 1, 8 and 15, and when found dead. Survivors were sacrificed and a necropsy was performed.

Table 1. Doses, mortality/animals treated

Test Group	Dose (mg/kg bw)	Males	Females	Combined
Group 1	5000	1/5	2/5	3/10
Group 2	7080	0/5	1/5	1/10

II. RESULTS

A. MORTALITY

Mortality is given in Table 1. Mortalities occurred at both dose levels. At 5000 mg/kg bw, 1/5 males and 2/5 females died on Day 2 whereas; at 7080 mg/kg bw, 1/5 females died on Day 3. The oral LD_{50} for males is > 7080 mg/kg/bw
females is > 7080 mg/kg/bw
combined is > 7080 mg/kg/bw

B. CLINICAL OBSERVATIONS

Clinical signs noted prior to death in the 3 decedents (1 male and 2 females) dosed at 5000 mg/kg bw included reduced motor activity, palpebral ptosis, hunched posture, and/or soiled fur and snout. No other clinical signs were detected in this study.

C. BODY WEIGHT

All surviving rats gained weight throughout the study.

D. NECROPSY

No gross lesions were noted at necropsy for surviving animals. The three decedents dosed at 5000 mg/kg bw day revealed a marked dilation of the stomach (one male), a moderate general autolysis (one female) or black spots on the granular surface of the stomach (one female). White area on the liver and black spots on non-glandular surface of the stomach was observed in the female at 7080 mg/kg that died on Day 3.

III. CONCLUSIONS

The oral LD_{50} of RPA 107382 (ethiprole) was higher than 7080 mg/kg bw day. Ethiprole does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity. No signal words are required on the label.

(Steiblen, G. 1997)

Acute oral toxicity in rats with RPA 112916

1.	Annex point(s)	KIIA 5.8; Toxicity studies on metabolites: **MRID 47622802**
2.	Location in dossier	KIIA 5.8 /01
3.	Authors (year)	Dange, M. (2001)
	Title	Acute oral toxicity in the rat RPA 112916
	Company, report No.	Bayer CropScience AG, Report-No.: C012671 (M-203164-01-1)
	Date	05.04.2001
4.	Testing facility	Aventis CropScience S.A., Sophia Antipolis, FRA;
5.	Dates of work	Exp. Start date: February 20th 2001; Exp. Term. Date: March 7th 2001
6.	Test substance(s)	Molecule(s): (14C)-RPA112916, AE 0616301, ethiprole Substance(s): ethiprole TC AE 0616301 TC (Batch-No.: PAN01/99) (14C)-RPA112916 TC
7.	Test method	EU (=EEC): 92/69 B1 (1992); MAFF: Nohsan 4200 (1985); OECD: 401 (1987); USEPA (=EPA): OPPTS No 870.1100 (1998)
	Deviations	
8.	GLP	yes

Executive Summary:

The acute oral toxicity of RPA 112916 (batch number PAN01/99; 945 g/kg purity) was assessed after a single oral administration of the test substance at 2000 or 5000 mg/kg body weight to groups of fasted, 8 week old Wistar rats (5/sex/dose). RPA 112916 was suspended in 0.5% methylcellulose. All animals were observed for clinical signs daily for fifteen days.

No mortalities, clinical signs of toxicity or body weight changes were observed at any dose level in either sex.

A renal capsular cyst was detected in one low-dose (2000 mg/kg/bw) male, a moderate renal pelvic dilation was noted in one low-dose male and one high-dose (5000 mg/kg bw) female, and moderate bilateral dilated uterine horns were observed in two females (one at low-dose and one at high-dose). A diffuse mottled white spleen was observed in 2 males dosed at 5000 mg/kg/bw and a brown color in the cranial lung lobes was observed in one male at this dose level.

The acute oral toxicity (LD_{50}) of RPA 112916 in male and female Wistar rats was found to be > 5000 mg/kg/body weight. RPA 112916 (945 g/kg purity) is of **Low Toxicity** by the oral route based on the LD_{50} in rats. No signal words are required on the label.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 112916, a metabolite of Ethiprole (RPA 107382)
   Description: White powder
   Lot/Batch #: PAN01/99
   Purity: 945 g/kg
   Compound Stability: The test substance was stored in an air-tight, light-resistant container at room temperature.
   CAS #: Not known

2. **Vehicle and/or Positive Control:** The dosing formulations were prepared by suspending the test substance in 0.5% methylcellulose in distilled water to produce the required dosing concentrations (w/v). The formulations were placed in glass air-tight bottles at room temperature and were used as quickly as practicable after preparation.

3. **Test Animals:**
   Species: Rat
   Strain: Wistar (AF) rats RJ: WI (IOPS HAN)
   Age/weight at dosing: 8 weeks / 272-287 g males; 175-185 g females
   Source: R. Janvier, Le Genest St Isle, France
   Housing: Individually in suspended stainless steel, wire mesh cages.
   Diet: Certified and irradiated Rodent Pelleted diet A04C (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France), *ad libitum*
   Water: Filtered and softened water from the municipal water supply, *ad libitum*
   Environmental conditions:
   Temperature: 20°C - 24°C (target)
   Humidity: 40% - 70% (target)
   Air changes: 10 to 15 air changes per hour (average, not monitored)
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: 6 or 7 days

B. STUDY DESIGN:

1. **In-Life Dates:** February 20, 2001 to March 07, 2001

2. **Animal Assignment and treatment:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution by sex to the test groups noted in Table 1.

Table 1. Doses, mortality/animals treated

Test Group	Dose (mg/kg bw)	Males	Females	Combined
Group 1	2000	0/5	0/5	0/10
Group 2	5000	0/5	0/5	0/10

Following an overnight fast, rats were given a single oral administration of RPA 112916 at dose levels of either 2000 mg/kg/bw or 5000 mg/kg bw (volume of 10 mL/kg). Animals were observed approximately 1 hour after dosing, at least once on the day of dosing (Day 1), and once daily thereafter until Day 15. In addition, animals were checked twice daily for mortality, except on weekends and public holidays when they were checked once daily. Body weights were recorded on Days 1, 8, and 15. All animals were necropsied.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** There were no clinical signs at any dose level in either sex.

2. **Mortality:** There were no mortalities at any dose level in either sex.
   The oral LD_{50} for males is > 5000 mg/kg bw
   females is > 5000 mg/kg bw
   combined is > 5000 mg/kg bw

B. BODY WEIGHT: All animals gained weight throughout the study.

C. NECROPSY: One male dosed at 2000 mg/kg bw exhibited a renal capsular cyst, one male dosed at 2000 mg/kg bw and one female dosed at 5000 mg/kg bw showed a moderate renal pelvic dilation, and one low-dose and one high-dose females revealed moderate bilateral dilated uterine horns. A diffuse mottled white spleen was observed in 2 males dosed at 5000 mg/kg bw and a brown color in the left and right cranial lung lobes was noted in one high-dose male. No other gross lesions were found upon necropsy.

III. CONCLUSIONS

The acute oral toxicity (LD_{50}) of RPA 112916 in male and female Wistar rats was found to be > 5000 mg/kg bw. Based on the LD_{50}, RPA 112916 is of **Low Toxicity** by the oral route in rats. No signal words are required on the label.

Acute oral toxicity in rats with RPA 097973

1.	Annex point(s)	KIIA 5.8; Toxicity studies on metabolites: **MRID 47622803**
2.	Location in dossier	KIIA 5.8 /07
3.	Authors (year)	Bigot, D. (1999)
	Title	Acute oral toxicity in the rat RPA097973
	Company, report No.	Bayer CropScience AG, Report-No.: R016844 (M-192471-01-1)
	Date	11.01.1999
4.	Testing facility	Rhone-Poulenc Agro, Sophia Antipolis, France
5.	Dates of work	Exp. Start date: November 3rd 1998; Exp. Term. Date: November 17th 1998
6.	Test substance(s)	Molecule(s): MB 46118 Substance(s):MB 46118 TC (Batch-No.: 64EAR37)
7.	Test method	EU (=EEC): 92/69/EEC, Annex V, B1; OECD: 401, (1987); USEPA (=EPA): OPPTS 870.1100, (1998)
	Deviations	
8.	GLP	no

Executive Summary:

The acute oral toxicity of RPA 097973 (batch number 64EAR37, 99.1% purity) was assessed after a single oral administration of the test substance at 2000 mg/kg body weight to groups of fasted, 8 week old Wistar rats (5/sex). RPA 097973 was suspended in 0.5% methylcellulose. All animals were observed for clinical signs daily for fifteen days.

No mortalities, clinical signs of toxicity or body weight changes were observed at 2000 mg/kg bw in either sex.

The acute oral toxicity (LD_{50}) of RPA 097973 in male and female Wistar rats was found to be > 2000 mg/kg body weight. RPA 097973 (99.1% a.i.) is of **Low Toxicity** by the oral route based on the LD_{50} in rats. No signal words are required on the label.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 097973, a metabolite of the insecticide ethiprole (RPA 107382)
   - Description: a white crystalline powder
   - Lot/Batch #: 64EAR37
   - Purity: 99.1%
   - Compound Stability: The test substance was stored in an air-tight, light-resistant container at approx. 4°C.
   - CAS #: 120068-68-0

2. **Vehicle and/or Positive Control:** The dosing formulations were prepared by suspending the test substance in 0.5% methylcellulose in sterile water to produce the required dosing concentrations (w/v). The formulations were placed in glass air-tight bottles at room temperature and were used as quickly as practicable after preparation.

3. **Test Animals:**
   - **Species:** Rat
   - **Strain:** Wistar (AF) rats RJ: WI (IOPS AF)
   - **Age/weight at dosing:** 8 weeks / 264.83- 306.38 g males; 187.20-213.83 g females
   - **Source:** R. Janvier, Le Genest St Isle, France
   - **Housing:** Individually in suspended stainless steel, wire mesh cages.
   - **Diet:** Certified Rodent Pellet diet "M 20 contrôlé" (Pietrement, Provins, France), *ad libitum*
   - **Water:** Filtered and softened water from the municipal water supply, *ad libitum*
   - **Environmental conditions:**
     - **Temperature:** 20°C - 24°C (target)
     - **Humidity:** 40% - 70% (target)
     - **Air changes:** 10 to 15 air changes per hour (average, not monitored)
     - **Photoperiod:** 12 hrs dark/12 hrs light
   - **Acclimation period:** 6 days

B. STUDY DESIGN:

1. **In-Life Dates:** November 03, 1998 to November 17, 1998

2. **Animal Assignment and treatment:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution by sex to the test groups noted in Table 1.

Table 1. Doses, mortality/animals treated

Test Group	Dose (mg/kg bw)	Males	Females	Combined
Group 1	2000	0/5	0/5	0/10

Following an overnight fast, rats were given a single oral administration of RPA 097973 at a dose level of 2000 mg/kg bw (volume of 10 mL/kg). Animals were observed approximately 1 hour after dosing, at least once on the day of dosing (Day 1), and once daily thereafter until Day 15. In addition, animals were checked twice daily for mortality, except on weekends and public holidays when they were checked once daily. Body weights were recorded on Days 1, 8, and 15. All animals were necropsied.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** There were no clinical signs at 2000 mg/kg bw in either sex.

2. **Mortality:** There were no mortalities at 2000 mg/kg bw in either sex.

The oral LD_{50} for males is > 2000 mg/kg bw
females is > 2000 mg/kg bw
combined is > 2000 mg/kg bw

B. BODY WEIGHT: All animals gained weight throughout the study.

C. NECROPSY: There were no macroscopic changes at 2000 mg/kg bw in either sex.

III. CONCLUSIONS

The acute oral toxicity (LD_{50}) of RPA 097973 in male and female Wistar rats was found to be > 2000 mg/kg body weight. Based on the LD_{50}, RPA 097973 is of **Low Toxicity** by the oral route in rats. No signal words are required on the label.

KIIA 5.4.1 - In vitro genotoxicity - Bacterial assay for gene mutation

1.	Annex point(s)	KIIA 5.4.1; Genotoxicity/In vitro genotoxicity - Bacterial assay for gene mutation: **MRID 47622816**
2.	Location in dossier	KIIA 5.4.1 /01
3.	Authors (year)	Dawkes, N. (1999)
	Title	RPA 097973 (metabolite of ethiprole): Reverse Mutation in four Histidine-requiring strains of *Salmonella typhimurium* and one Tryptophan-requiring strain of *Escherichia coli*.
	Company, report No.	Bayer CropScience AG, Report-No.: R016832 (M-192448-01-1)
	Date	06.1999
4.	Testing facility	Covance Laboratories Limited, Harrogate;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): RPA097973 Substance(s):Batch-No.: 64EAR37
7.	Test method	USEPA (=EPA): 84-2
	Deviations	
8.	GLP	yes

Executive summary:

In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and WP2*uvr*A of *E.coli* were exposed to RPA 097973 (a metabolite of ethiprole) (99.1%) in DMSO at concentrations of 8, 40, 200, 1000, 5000 µg/plate (Experiment 1) and 20.48, 51.2, 128, 320, 800, 2000, 5000 µg/plate (Experiment 2) in the presence and absence of mammalian metabolic activation, where treatments in the presence of S9 included a pre-incubation step (Experiment 2).

RPA 097973 was tested up to the limit concentration of 5000 µg/plate, this being the best estimate of the limit of precipitation. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **acceptable/guideline** and it satisfies the requirement for Test Guideline OPPTS 870.5100 and OECD 471 for *in vitro* mutagenicity (bacterial reverse mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

Description: White, crystalline powder

Lot/Batch #: 64EAR37

Purity: 99.1% a.i.

CAS # of TGAI: 120068-68-0

Solvent Used: Sodium azide was dissolved in water. RPA097973 and the other positive controls were dissolved in sterile anhydrous analytical grade DMSO.

2. Control Materials:

Negative:

Solvent (final conc'n): DMSO at 0.1 mL/plate (0.05 mL for preincubation treatments)

Positive:

Nonactivation: 2-Nitrofluorene(2NF) 5 µg/plate, TA98
Sodium azide (NaN_3) 2 µg/plate, TA100 and TA1535
9-Aminoacridine (AAC) 50 µg/plate, TA1537
4-nitroquinoline 1-oxide (NQO) 2 µg/plate, WP2 uvrA

Activation: 2-Aminoanthracene (AAN) 5 µg/plate, at least one TA strain: 10 and 20 µg/plate for WP2 uvrA for Tests 1 and 2 , respectively
S9 derived from

3. Activation:

X	induced	X	Aroclor 1254	X	Rat	X	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Describe S9 mix composition: S9 fraction (10% v/v), sodium phosphate buffer pH 7.4 (500 mM), glucose-6-phosphate (180 mg/mL), NADP (25 mg/mL), $MgCl_2$ (250 mM), KCl (150 mM). L-histidine HCl in 250 mM $MgCl_2$ and d-biotin (1 mg/mL each) were added to solutions for treatments with *S. typhimurium* strains and DL-tryptophan (1 mg/mL) and casamino acids (20% w/v) were added to solutions for treatments with *E. coli* strain only. Batches of MolTox™ S9 were stored frozen at -80°C, and thawed just prior to incorporation into the top agar (8). Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens, and cytochrome P-450-catalysed enzyme activities (alkoxyresorufin-O-dealkylase activities).

4. Test Organisms:										
S. typhimurium strains										
		TA97	X	TA98	X	TA100		TA102		TA104
	X	TA1535	X	TA1537		TA1538		list any others		
E. coli strains										
		WP2	X	WP2 *uvr*A				list any others		

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?	X	Yes		No

5. Test compound concentrations used:

Concentration of the Test Substance Used	Number of Replicates Per Dose	Strains Assayed	Conditions
First Test (Range Finding and Mutation Experiment 1): Activated and Non-Activated Conditions			
8, 40, 200, 1000 and 5000 µg/plate	3	*S. typhimurium,* (strains TA98, TA100, TA1535, and TA1537) *E. coli,* (strain WP2 *uvr*A)	Plated on agar plates and incubated at 37 °C for 3 days.
Second Test (Mutation Experiment 2): Activated and Non-Activated Conditions			
20.48, 51.2, 128, 320, 800, 2000, and 5000 µg/plate	3	*S. typhimurium,* (strains TA98, TA1535, and TA1537) *E. coli,* (strain WP2 *uvr*A)	Plated on agar plates and incubated at 37°C for 3 days. Treatments in the presence of S9 included a 1 hour pre-incubation step at 37 °C prior to plating and normal incubation.
4.096, 10.24, 25.6, 64, 160, 400, and 1000 µg/plate	3	*S. typhimurium,* (strain TA100)	

B. TEST PERFORMANCE

1. Type of *Salmonella* and *E. Coli* assay:

X standard plate test
X pre-incubation (60 minutes) (Mutation Experiment 2)

2. Protocol: For all assays, bacteria were cultured for 10 hours at 37°C in nutrient broth (containing ampicillin for strains TA98 and TA100). Incubation was carried out in a shaking incubator. Bacteria were taken from vials of frozen cultures, which had been checked for strain characteristics of histidine or tryptophan dependence, *rfa* character (Salmonella strains) and presence (strains TA98 and TA100) or absence (strains TA1535, TA1537 and E. coli) of the pKM101 ampicillin resistance factor. Checks were carried out according to Maron and Ames (1983) and De Serres and Shelby (1979). All experimentation commenced within 2 hours of the end of the period of incubation.

Range-finder experiment: RPA097973 was tested in strain TA100 in triplicate plates at 8-5000 µg/plate without and with S9 mix (±S9 activation). Negative (solvent) and positive controls were included in quintuplicate and triplicate respectively (±S9 activation). These platings were achieved by the following sequence of additions to 2.5 mL molten agar at 46°C: 0.1 mL bacterial culture; 0.1 mL test article solution or control solution (0.5 mL 10% S9 mix) or buffer solution followed by rapid mixing and pouring on to Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible revertant colonies were counted electronically using a Seescan® Colony

Counter. Colonies were counted electronically using a Seescan Colony Counter (Seescan pic) or manually, where confounding factors such as split agar or the presence of test article precipitation prevented accurate automated counts from being obtained. In each case the background bacterial lawn of every plate was inspected for signs of toxicity.

Mutation Experiments: In two separate experiments, RPA097973 was tested in four strains of S. *typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of E *coli* (WP2 uvrA) using triplicate plates at 8-5000 µg/plate (±S9 activation). Experiment 1 mutagenicity data for strain TA100 was provided by the range-finding experiment treatments. Negative (solvent) and positive controls were included in quintuplicate and triplicate respectively (±S9 activation). The activity of the S9 mix used in each experiment was confirmed by AAN treatments (again in triplicate) of at least one strain in the presence of S9. The platings were achieved as described above for the range-finding experiment.

As the results of the first experiment were negative, treatments in the presence of S9 in Experiment 2 included a pre-incubation step, where the quantities of test article or control solution (reduced to 0.05 mL), bacteria and S9 mix, as described above for the range-finding experiment, were mixed together incubated for 1 hour at 37°C, with shaking, before the addition of 2.5 mL molten agar at 46°C. Plating of these treatments then proceeded as for the normal plate-incorporation procedure. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Volume additions for the Experiment 2 pre-incubation treatments were reduced to 0.05 mL due to the solvent (DMSO) employed in this study. This and some other organic solvents are known to be near to toxic levels when added at volumes of 0.1 mL in this assay system when employing the pre-incubation methodology. By reducing the addition volume to 0.05 mL per plate, it was hoped to minimise or eliminate any toxic effects of the solvent that may have otherwise occurred.

3. **Statistical Analysis:** The m-statistic was calculated to check that the data were Poisson-distributed (Mahon et. al., 1989), and Dunnett's test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis.

4. **Evaluation Criteria:** The assay was considered valid if (1) mean negative control counts fell within the accepted normal ranges for mean numbers of spontaneous revertants on solvent control plates for the laboratory; (2) the positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S9 preparation; and (3) no more than 5% of the plates were lost through contamination or some other unforeseen event. The test article was considered to be mutagenic if (1) the assay was considered valid, as described above; (2) the Dunnett's test gave a significant response ($p < 0.01$) and the data set(s) showed a significant dose correlation; and (3) the positive responses described in (2) were reproducible.

II. RESULTS

The stability of RPA097973 and the stability and homogeneity of RPA097973 in the solvent were not determined as part of this study. In addition, analysis of achieved concentration was not performed as part of this study.

The mean negative control counts fell within the accepted normal ranges for mean numbers of spontaneous revertants on solvent control plates for the laboratory. Historical control data were provided in the study report.

Appropriate positive control chemicals (with S9 mix where required) induced substantial increases in revertant colony numbers with all strains, confirming sensitivity of the cultures and activity of the S9 mix. Results from the first and second tests are detailed below.

A. RANGE-FINDING:

An initial toxicity range-finding experiment was carried out in strain TA100 only, using final concentrations of RPA097973 at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Following these treatments, evidence of toxicity (manifest as thinning of the background bacterial lawn) was observed at the two highest treatment concentrations both in the absence and in the presence of S9. These data were considered acceptable for mutagenicity assessment, and were presented in the report as the data for strain TA100, for Mutagenicity Experiment 1. Precipitation of test agent was observed on all plates treated at 5000 µg/plate.

B. FIRST TEST (MUTAGENICITY DATA):

Experiment 1 treatments of the remaining test strains were performed using the same dose-range as employed in the range-finding experiment. Following these Experiment 1 treatments, evidence of toxicity was apparent at the maximum treatment concentration with strain TA1537, both in the absence and in the presence of S9, and strain TA98 solely in the absence of S9. The results are summarized in Table 1.

TABLE 1. Summary of Mean Revertant Colonies - Experiment 1.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA98**	**-S9**					
Solvent (DMSO)	28.40	5	---	8.91	---	---	---
8	30.67	3	1.08	5.03	0.16	0.28	0.58
40	35.33	3	1.24^	0.58	0.44	0.17	1.57
200	33.00	3	1.16	5.57	0.21	0.02	1.06
1000	24.67 (PPN+M)	3	0.87	6.11	0.35	-0.01	-0.80
5000	16.00 (PPN+M+S)	2	0.56	0.00	0.63	-0.00	-2.63
Pos. Con. 2NF (5)	943.67	3	33.23	84.36	---	---	---
	M Statistic = 1.365						
Test strain:	**TA98**	**+S9**					
Solvent (DMSO)	35.40	5	---	9.45	---	---	---
8	33.67	3	0.95	9.71	0.10	-0.22	-0.32
40	36.00	3	1.02^	2.65	0.06	0.02	0.18
200	26.00	3	0.73	5.29	0.47	-0.05	-1.76
1000	30.00 (PPN+M)	3	0.85	6.24	0.24	-0.00	-0.97
5000	24.00 (PPN+M)	3	0.68	5.57	0.44	-0.00	-2.20
Pos. Con. AAN (5)	1087.33	3	30.72	71.07	---	---	---
	M Statistic = 1.673						
Test strain:	**TA100**	**-S9**					
Solvent (DMSO)	95.20	5	---	11.21	---	---	---
8	105.67	3	1.11	8.39	0.49	1.31	1.53
40	98.33	3	1.03	2.52	0.06	0.03	0.50
200	102.67	3	1.08	12.10	0.17	0.02	1.09
1000	114.33 (PPN+M+S)	3	1.20^	10.69	0.54*	0.02	2.72*
5000	107.00 (PPN+M+S)	3	1.12	8.19	0.26	0.00	1.72
Pos. Con. NaN_3 (2)	560.00	3	5.88	47.44	---	---	---
	M Statistic = 0.917						
Test strain:	**TA100**	**+S9**					

Solvent (DMSO)	116.60	5	---	7.23	---	---	---
8	112.33	3	0.96	9.07	0.29	-0.53	-0.48
40	114.67	3	0.98	15.50	0.05	-0.03	-0.24
200	107.33	3	0.92	13.43	0.32	-0.04	-1.07
1000	125.33 (PPN+M+S)	3	1.07^	11.06	0.37	0.01	0.94
5000	120.67 (PPN+M+S)	3	1.03	18.01	0.22	0.00	0.41
Pos. Con. AAN (5)	1353.67	3	11.61	167.43	---	---	---
	M Statistic = 1.296						

(Table continued; footnotes follow)

TABLE 1. Summary of Mean Revertant Colonies - Experiment 1.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA1535**	**-S9**					
Solvent (DMSO)	19.40	5	---	6.23	---	---	---
8	23.00	3	1.19^	7.21	0.29	0.45	0.91
40	19.67	3	1.01	1.53	0.04	-0.01	0.17
200	14.00	3	0.72	4.58	0.47	-0.03	-1.52
1000	12.33 (PPN+M)	3	0.64	2.08	0.52	-0.01	-1.99
5000	12.00 (PPN+M)	3	0.62	3.61	0.45	-0.00	-2.15
Pos. Con. NaN_3 (2)	500.33	3	25.79	15.31	---	---	---
	M Statistic = 1.331						
Test strain:	**TA1535**	**+S9**					
Solvent (DMSO)	14.60	5	---	4.51	---	---	---
8	16.67	3	1.14	2.52	0.28	0.26	0.77
40	17.00	3	1.16^	4.00	0.25	0.05	0.85
200	14.67	3	1.00	4.51	0.09	-0.00	0.03
1000	12.67 (PPN+M)	3	0.87	2.52	0.32	-0.00	-0.65
5000	11.33 (PPN+M)	3	0.78	3.79	0.40	-0.00	-1.23
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 1.036						
Test strain:	**TA1537**	**-S9**					
Solvent (DMSO)	8.60	5	---	1.67	---	---	---
8	8.00	3	0.93	2.00	0.18	-0.08	-0.23
40	11.67	3	1.36^	6.11	0.42	0.08	0.88
200	6.67	3	0.78	1.53	0.26	-0.01	-0.77
1000	5.67 (PPN+M)	3	0.66	0.58	0.41	-0.00	-1.19
5000	2.67 (PPN+M+A)#	3	0.31	2.52	0.61	-0.00	-3.50
Pos. Con. AAC (50)	503.00	---	58.49	42.93	---	---	---
	M Statistic = 1.019						
Test strain:	**TA1537**	**+S9**					
Solvent (DMSO)	8.80	5		3.35			
8	8.33	3	0.95	1.53	0.09	-0.06	-0.11
40	9.00	3	1.02^	3.61	0.05	0.01	0.11
200	6.67	3	0.76	2.52	0.30	-0.01	-1.00
1000	7.33 (PPN+M)	3	0.83	2.52	0.19	-0.00	-0.64
5000	3.67 (PPN+M+S)	3	0.42	2.08	0.56	-0.00	-2.83
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 1.038						

For one of the three plates, "toxic, no revertent colonies"

(Table continued; footnotes follow)

TABLE 1. Summary of Mean Revertant Colonies - Experiment 1.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**WP2 uvrA**	**-S9**					
Solvent (DMSO)	14.80	5	---	3.56	---	---	---
8	15.00 (M)	3	1.01^	5.57	0.03	0.03	0.01
40	14.67	3	0.99	5.69	0.02	-0.00	-0.10
200	13.33	3	0.90	3.21	0.16	-0.01	-0.48
1000	7.67 (PPN+M)	3	0.52	1.53	0.61	-0.01	-2.72
5000	10.33 (PPN+M)	3	0.70	2.52	0.36	-0.00	-1.60
Pos. Con. NQO (2)	783.67	3	52.95	114.29	---	---	---
	M Statistic = 1.097						
Test strain:	**WP2 uvrA**	**+S9**					
Solvent (DMSO)	21.20	5	---	3.49	---	---	---
8	22.00	3	1.04	6.93	0.09	0.10	0.18
40	24.00	3	1.13^	2.65	0.29	0.07	1.02
200	17.67	3	0.83	0.58	0.41	-0.02	-1.31
1000	21.67	3	1.02	3.06	0.03	-0.00	0.19
5000	14.33	3	0.68	2.08	0.57	-0.00	-2.73
Pos. Con. AAN (10)	315.67	3	14,89	66.29	---	---	---
	M Statistic = 0.625						

Data extracted from the study report, Appendix 1, Tables 1-10. pages 29-40.

Key to significance:
* = $p \leq 0.05$
** = $p \leq 0.01$
*** $p \leq 0.005$

Key to postfixes:
^ = maximum increase above control

Positive Control:

2NF: 2-Nitrofluorene
NaN_3: Sodium azide
AAC: 9-Aminoacridine
NQO: 4-Nitroquinoline 1-oxide
AAN: 2-Aminoanthracene

A: Absence of background lawn
S: Slight thinning of background bacterial lawn
PPN: Precipitation of Test article observed
M: Plate(s) counted manually

C. SECOND TEST (MUTAGENICITY DATA, PRE-INCUBATION UNDER +S9):

Experiment 2 treatments were performed with the maximum treatment concentration of 5000 µg/plate retained for all test strains except TA100. For strain TA100 treatments, both in the absence and in the presence of S9, the maximum treatment concentration was reduced to 1000 µg/plate (an estimate of the lower limit of the toxic range). Narrowed dose intervals were employed in order to more closely investigate those doses of RPA097973 considered most likely to induce any mutagenic response. In addition, all treatments in the presence of S9 employed a pre-incubation step. Following these Experiment 2 treatments, evidence of toxicity was apparent both in the absence and in the presence of S9 with strains TA98, TA100 and TA1537. Toxicity was observed at the two highest test doses with strain TA1537 but solely at the maximum treatment concentration with strains TA98 and TA100.

Precipitation of the test article was observed following all treatments at test concentrations of 800 µg/plate and above. Results are summarized in Table 2.

Overall, it was shown that mean solvent control counts were all within the normal historical ranges, and that the positive control chemicals all induced characteristic increases in revertant numbers in the appropriate strains. Less than 5% of plates were lost, leaving adequate numbers of plates at all treatments. The study was accepted as valid according to the specified validity criteria. No RPA097973 treatment of any of the test strains, either in the absence or in the presence of S9, resulted in a statistically significant increase in revertant numbers (when the data were analysed at the 1% level using Dunnett's test).

TABLE 2. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA98**	**-S9**					
Solvent (DMSO)	27.60 (M)	5	---	6.31	---	---	---
20.48	21.67 (M)	3	0.79	4.04	0.51	-0.29	-1.54
51.2	27.00	3	0.98	2.00	0.06	-0.01	-0.09
128	18.67	3	0.68	4.51	0.51	-0.06	-2.44
320	23.67 (M)	3	0.86	3.06	0.23	-0.01	-0.97
800	28.33 (PPN+M)	3	1.03^	5.13	0.18	0.00	0.21
2000	27.33 (PPN+M)	3	0.99	9.61	0.20	0.00	-0.15
5000	20.00 (PPN+M+S)	3	0.72	2.00	0.19	-0.00	-1.99
Pos. Con. 2NF (5)	972.00	3	35.22	25.87	---	---	---
	M Statistic = 1.086						
Test strain:	**TA98**	**+S9**					
Solvent (DMSO)	36.60	5	---	4.56	---	---	---
20.48	36.67	3	1.00	3.51	0.01	0.00	0.03
51.2	40.00	3	1.09^	8.19	0.28	0.07	0.76
128	32.67	3	0.89	3.06	0.28	-0.03	-0.95
320	37.67	3	1.03	10.02	0.01	-0.00	0.17
800	31.00 (PPN+M)	3	0.85	4.00	0.33	-0.01	-1.39
2000	26.33 (PPN+M)	3	0.72	5.77	0.56	-0.01	-2.68
5000	33.00 (PPN+M+S)	3	0.90	3.61	0.29	-0.00	-0.87
Pos. Con. AAN	1746.33	3	47.71	241.61	---	---	---
	M Statistic = 0.919						
Test strain:	**TA100**	**-S9**					
Solvent (DMSO)	117.20	5	---	14.55	---	---	---
4.096	124.67	3	1.06	23.97	0.22	1.82	0.68
10.24	133.33	3	1.14^	9.29	0.43	1.58	1.57
25.6	128.33		1.09	9.29	0.28	0.42	1.10
64	125.67		1.07	8.96	0.13	0.08	0.85
160	131.33	3	1.12	8.33	0.22	0.05	1.39
400	123.00	3	1.05	21.63	0.01	0.00	0.54
1000	126.00 (PPN+S)	3	1.08	2.65	0.02	0.00	0.89
Pos. Con. NaN_3 (2)	758.33	3	6.47	84.91	---	---	---
	M Statistic = 1.619						
Test strain:	**TA100**	**+S9**					
Solvent (DMSO)	147.20	5	---	9.09	---	---	---
4.096	170.33	3	1.16^	5.69	0.85***	5.65	2.63*
10.24	159.00	3	1.08	12.29	0.41	1.20	1.36
25.6	143.67	3	0.98	8.02	0.25	-0.33	-0.41
64	153.67	3	1.04	15.28	0.09	-0.05	0.74
160	139.33	3	0.95	12.34	0.39	-0.10	-0.95
400	140.67	3	0.96	15.82	0.38	-0.04	-0.80
1000	166.67 (PPN+S)	3	1.13	14.01	0.20	0.01	2.21
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 0.920						

(Table continued; footnotes follow)

TABLE 2. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA1535**	**-S9**					
Solvent (DMSO)	13.00	5	---	5.39	---	---	---
20.48	21.00	3	1.62^	2.00	0.70*	0.39	2.45
51.2	17.00	3	1.31	5.29	0.33	0.08	1.27
128	12.00	3	0.92	4.58	0.19	-0.02	-0.32
320	16.00	3	1.23	3.61	0.03	-0.00	1.03
800	13.33 (PPN+M)	3	1.03	3.51	0.16	-0.00	0.19
2000	18.33 (PPN+M)	3	1.41	3.21	0.15	0.00	1.71
5000	19.00 (PPN+M)	3	1.46	6.08	0.26	0.00	1.84
Pos. Con. NaN_3 (2)	572.67	3	44.05	43.62	---	---	---
	M Statistic = 1.366						
Test strain:	**TA1535**	**+S9**					
Solvent (DMSO)	19.20	5	---	5.81	---	---	---
20.48	18.67	3	0.97	3.06	0.06	-0.03	-0.10
51.2	22.67	3	1.18	4.04	0.32	0.07	1.21
128	23.67	3	1.23	4.51	0.41	0.04	1.51
320	21.33	3	1.11	3.21	0.20	0.01	0.79
800	27.33 (PPN)	3	1.42^	4.04	0.51*	0.01	2.60*
2000	18.33 (PPN+M)	3	0.95	3.51	0.05	-0.00	-0.22
5000	14.67 (PPN+M)	---	0.76	2.52	0.42	-0.00	-1.56
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 0.864						
Test strain:	**TA1537**	**-S9**					
Solvent (DMSO)	8.40	5	---	2.70	---	---	---
20.48	8.67	3	1.03	4.51	0.04	0.01	0.00
51.2	9.00	3	1.07	6.56	0.07	0.01	-0.03
128	9.67	3	1.15^	7.02	0.11	0.01	0.17
320	6.00	3	0.71	1.00	0.21	-0.01	-0.84
800	9.33 (PPN)	3	1.11	1.53	0.01	0.00	0.36
2000	5.67 (PPN+M+S)	3	0.67	0.58	0.24	-0.00	-0.97
5000	3.33 (PPN+M+S)	3	0.40	2.31	0.45	-0.00	-2.20
Pos. Con. AAC (50)	740.67	3	88.17	208.20	---	---	---
	M Statistic = 1.782						
Test strain:	**TA1537**	**+S9**					
Solvent (DMSO)	8.00 (M)	5	---	3.74	---	---	---
20.48	12.67	3	1.58	2.89	0.60	0.23	1.99
51.2	9.00	3	1.13	2.65	0.14	0.02	0.54
128	9.33	3	1.17	4.73	0.02	0.00	0.53
320	7.33	3	0.92	3.51	0.21	-0.01	-0.30
800	13.00 (PPN)	3	1.63^	2.65	0.28	0.00	2.12
2000	6.33 (PPN+M+S)	3	0.79	0.58	0.19	-0.00	-0.65
5000	4.33 (PPN+M+S)	---	0.54	1.15	0.46	-0.00	-1.80
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 1.101						

(Table continued; footnotes follow)

TABLE 2. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**WP2 uvrA**	**-S9**					
Solvent (DMSO)	9.00 (M)	4	---	2.71	---	---	---
20.48	10.00 (M)	3	1.11	2.65	0.21	0.05	0.34
51.2	8.67	3	0.96	3.51	0.06	-0.01	-0.16
128	9.00	3	1.00	4.36	0.05	-0.00	-0.10
320	10.33	3	1.15	7.09	0.11	0.00	0.21
800	7.67 (PPN+M)	3	0.85	3.21	0.13	-0.00	-0.52
2000	12.00 (PPN+M)	3	1.33^	2.65	0.22	0.00	0.96
5000	9.00 (PPN+M)	3	1.00	2.65	0.04	0.00	-0.00
Pos. Con. NQO (2)	976.33	3	108.48	12.22	---	---	---
	M Statistic = 1.534						
Test strain:	**WP2 uvrA**	**+S9**					
Solvent (DMSO)	15.60	5	---	5.50	---	---	---
20.48	17.00	3	1.09	5.20	0.14	0.07	0.54
51.2	10.00	3	0.64	1.00	0.46	-0.11	-1.95
128	17.33	3	1.11	2.08	0.07	0.01	0.71
320	14.67	3	0.94	2.89	0.01	-0.00	-0.19
800	18.00	3	1.15^	2.00	0.24	0.00	0.93
2000	13.00	3	0.83	3.61	0.10	-0.00	-0.83
5000	12.00	3	0.77	3.61	0.26	-0.00	-1.21
Pos. Con. AAN (20)	97.67	3	6.26	5.03	---	---	---
	M Statistic = 0.966						

Data extracted from the study report, Appendix 2, Tables 11-20. pages 42-53.

Key to significance:
* = $p \leq 0.05$
** = $p \leq 0.01$
*** $p \leq 0.001$

Key to postfixes:
^ = maximum increase above control

Positive Control:

2NF: 2-Nitrofluorene
NaN_3: Sodium azide
AAC: 9-Aminoacridine
NQO: 4-Nitroquinoline 1-oxide
AAN: 2-Aminoanthracene

S: Slight thinning of background bacterial lawn
PPN: Precipitation of Test article observed
M: Plate(s) counted manually

III. CONCLUSIONS:

An initial toxicity range-finding experiment was carried out with strain TA100 only, using final concentrations of RPA097973 at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Evidence of toxicity (manifest as thinning of the background bacterial lawn) was observed at the two highest treatment concentrations both in the absence and in the presence of S9. Experiment 1 treatments of the remaining test strains were performed using the same dose-range as employed in the range-finding experiment. Evidence of toxicity was apparent at

the highest treatment concentration with strain TA1537 both in the absence and in the presence of S9 and strain TA98 solely in the absence of S9.

Experiment 2 treatments were performed with the maximum treatment concentration of 5000 µg/plate retained for all test strains except TA100. For strain TA100 treatments, both in the absence and in the presence of S9, the maximum treatment concentration was reduced to 1000 µg/plate (an estimate of the lower limit of the toxic range). Narrowed dose intervals were employed in order to more closely investigate those doses of RPA097973 considered most likely to induce any mutagenic response. In addition, all treatments in the presence of S9 employed a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Evidence of toxicity was apparent with strains TA98, TA100 and TA1537 both in the absence and in the presence of S9 at the higher test doses. Precipitation of the test article was observed following all treatments at test concentrations of 800 µg/plate and above.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments. No RPA097973 treatment of any of the test strains produced an increase in revertant numbers sufficient to be considered as indicative of mutagenic activity.

It was concluded that RPA097973, a metabolite of an insecticide RPA 107382 (also known as ethiprole), did not induce mutation in four strains of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and one strain of Escherichia coli (WP2 uvrA), when tested under the conditions employed for this study, which included treatment at up to 5000 µg/plate, or concentrations extending into the toxic range, both in the absence and in the presence of a rat liver metabolic activation system (S9).

The stability of RPA097973 and the stability and homogeneity of RPA097973 in the solvent were not determined; in addition, analysis of achieved concentration was not performed as part of this study. These omissions are considered minor and do not impact on the review of this study.

KIIA 5.4.1 - In vitro genotoxicity - Bacterial assay for gene mutation

1.	Annex point(s)	KIIA 5.4.1; Genotoxicity/In vitro genotoxicity - Bacterial assay for gene mutation: **MRID 47622815**
2.	Location in dossier	KIIA 5.4.1 /01
3.	Authors (year)	Ballantyne, M. (2001)
	Title	RPA 112916 (metabolite of ethiprole): Reverse Mutation in four Histidine-requiring strains of *Salmonella typhimurium* and two Tryptophan-requiring strain of *Escherichia coli*.
	Company, report No.	Bayer CropScience AG, Report-No.: R016832 (M-192448-01-1)
	Date	11.05.2001
4.	Testing facility	Covance Laboratories Limited, Harrogate;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): RPA112916 Substance(s):Batch-No.: PAN01/99
7.	Test method	USEPA (=EPA): 84-2
	Deviations	
8.	GLP	yes

Executive summary:

In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and WP2*uvr*A and WP2*uvr*A pKM101 of *E.coli* were exposed to RPA 112916 (a metabolite of ethiprole) (94.5%) in DMSO at concentrations of 1.6, 8, 40, 200, 1000, 5000 µg/plate (Experiment 1) and 156.25, 312.5, 625, 1250, 2500, 5000 µg/plate (Experiment 2) in the presence and absence of mammalian metabolic activation. Treatments in the presence of S9 included a pre-incubation step (Experiment 2).

RPA 112916 was tested up to the limit concentration of 5000 µg/plate, this being the best estimate of the limit of precipitation. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **acceptable/guideline** and it satisfies the requirement for Test Guideline OPPTS 870.5100 and OECD 471 for *in vitro* mutagenicity (bacterial reverse mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA112916

Description: White powder

Lot/Batch #: PAN01/99

Purity: 945g/kg

Description: White powder

Solvent Used: Sodium azide was dissolved in water. RPA112916 and the other positive controls were dissolved in DMSO.

2. Control Materials:

Negative: DMSO was used as a negative control

Solvent (final conc'n): DMSO at 0.1 mL/plate (0.05 mL for preincubation treatments)

Positive:

Nonactivation: 2-Nitrofluorene(2NF) 5 µg/plate, TA98
Sodium azide (NaN_3) 2 µg/plate, TA100 and TA1535
9-Aminoacridine (AAC) 50 µg/plate, TA1537
4-nitroquinoline 1-oxide (NQO) 2 µg/plate, WP2 uvrA pKM101; 10 µg/plate, WP2 pKM101

Activation: Benzo[a]pyrene (B[a]P) 10 µg/plate, TA98
2-Aminoanthracene (AAN) 5 µg/plate, TA100, TA1535, TA1537, and WP2 uvrA pKM101*

*WP2 pKM101 fails to provide consistent increases when tested with AAN in the presence of S9. The metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data.

3. Activation: S9 derived from

X	induced	X	Aroclor 1254	X	Rat	X	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Describe S9 mix composition: S9 fraction (10% v/v), sodium phosphate buffer pH 7.4 (500 mM), glucose-6-phosphate (180 mg/mL), NADP (25 mg/mL), $MgCl_2$ (250 mM), KCl (150 mM). L-histidine HCl in 250 mM $MgCl_2$ and d-biotin (1 mg/mL each) were added to solutions for treatments with *S. typhimurium* strains and DL-tryptophan (1 mg/mL) and casamino acids (20% w/v) were added to solutions for treatments with *E. coli* strains only. Batches of MolTox™ S9 were stored frozen at -80°C, and thawed just prior to incorporation into the top agar. Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens, and cytochrome P-450-catalysed enzyme activities (alkoxyresorufm-0-dealkylase activities).

4. Test Organisms:

***S. typhimurium* strains**										
		TA97	X	TA98	X	TA100		TA102		TA104
	X	TA1535	X	TA1537		TA1538		list any others		
***E. coli* strains**										
		WP2	X	WP2 pKM101	X	WP2 *uvr*A pKM101		list any others		

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?	X	Yes		No

5. Test compound concentrations used:

Concentration of the Test Substance Used	Number of Replicates Per Dose	Strains Assayed	Conditions
First Test (Range Finding and Mutation Experiment 1): Activated and Non-Activated Conditions			
1.6, 8, 40, 200, 1000 and 5000 µg/plate	3	*S. typhimurium,* strains TA98, TA100, TA1535, and TA1537; and *E. coli,* strains WP2 pKM101 and WP2 *uvr*A pKM101)	Plated on agar plates and incubated at 37 °C for 3 days.
Second Test (Mutation Experiment 2): Activated and Non-Activated Conditions			
156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate	3	*S. typhimurium,* strains TA98, TA100, TA1535, and TA1537, and *E. coli,* strains WP2 pKM101 and WP2 *uvr*A pKM101	Plated on agar plates and incubated at 37°C for 3 days. Treatments in the presence of S9 included a 1 hour pre-incubation step at 37 °C prior to plating and normal incubation.

B. TEST PERFORMANCE

1. Type of *Salmonella* and *E. Coli* assay:

_X_ standard plate test
_X_ pre-incubation (60 minutes) (Mutation Experiment 2)

2. Protocol: For all assays, bacteria were cultured for 10 hours at 37°C in nutrient broth (containing ampicillin for strains TA98 and TA100 and *E. coli*). Incubation was carried out in a shaking incubator. Bacteria were taken from vials of frozen cultures, which had been checked for strain characteristics of histidine or tryptophan dependence, *rfa* character (*Salmonella* strains), uvrB character *Salmonella* strains), uvrA character (Escherichia coli) and presence (strains TA98, TA100 and *Escherichia coli*) or absence (strains TA1535 and TA1537) of the pKM101 ampicillin resistance factor. Checks were carried out according to Maron and Ames (1983) and De Serres and Shelby (1979). All experimentation commenced within 2 hours of the end of the period of incubation.

Range-finder experiment: RPA112916 was tested in strain TA100 in triplicate plates at 1.6-5000 µg/plate without and with S9 mix (±S9 activation). Negative (solvent) and positive controls were included in quintuplicate and triplicate respectively (±S9 activation). These platings were achieved by the following sequence of additions to 2.5 mL molten agar at 46±1°C: 0.1 mL bacterial culture; 0.1 mL test article solution or control solution (0.5 mL 10% S9 mix) or buffer solution followed by rapid mixing and pouring on to Vogel-Bonner E agar plates. When set, the plates were inverted and incubated at 37±1°C in the dark for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible revertant colonies were counted electronically using a Seescan® Colony Counter. Where confounding factors such as split agar or precipitation of test agent

prevented an accurate automated count from being obtained, plates were counted manually. Experiment 2 treatments of strains TA98 and TA1535 (-S9) had low levels of bacterial contamination on the test plates. These prevented an accurate automated count, and so all of these solvent and treated plates were counted manually. The bacterial contamination was sufficiently slight, and distinguishable from the revertant colonies, that this was considered to have had no impact upon assay functioning or the accuracy of revertant colony counting.

Mutation Experiments: In two separate experiments, RPA112916 was tested in four strains of S. *typhimurium* (TA98, TA100, TA1535 and TA1537) and two strains of E *coli* (WP2 pKM101 and WP2 uvrA pKM101) using triplicate plates at 1.6-5000 µg/plate (±S9 activation). Negative (solvent) and positive controls were included in quintuplicate and triplicate respectively (±S9 activation). The activity of the S9 mix used in each experiment was confirmed by AAN or B[a]P treatments (again in triplicate) of the majority of strains in the presence of S9. The platings were achieved as described above for the range-finding experiment.

As the results of the first experiment were negative, treatments in the presence of S9 in Experiment 2 included a pre-incubation step, where the quantities of test article or control solution (reduced to 0.05 mL), bacteria and S9 mix, as described above for the range-finding experiment, were mixed together and incubated for 1 hour at 37±1°C, with shaking, before the addition of 2.5 mL molten agar at 46±1°C. Plating of these treatments then proceeded as for the normal plate-incorporation procedure. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Volume additions for the Experiment 2 pre-incubation treatments were reduced to 0.05 mL due to the solvent (DMSO) employed in this study. This and some other organic solvents are known to be near to toxic levels when added at volumes of 0.1 mL in this assay system when employing the pre-incubation methodology. By reducing the addition volume to 0.05 mL per plate, it was hoped to minimize or eliminate any toxic effects of the solvent that may have otherwise occurred.

3. **Statistical Analysis:** The m-statistic was calculated to check that the data were Poisson distributed (Mahon et. al., 1989), and Dunnett's test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis.

4. **Evaluation Criteria:** The assay was considered valid if (1) mean negative control counts fell within the accepted normal ranges for mean numbers of spontaneous revertants on solvent control plates for the laboratory; (2) the positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S9 preparation; and (3) no more than 5% of the plates were lost through contamination or some other unforeseen event. The test article was considered to be mutagenic if (1) the assay was considered valid, as described above; (2) the Dunnett's test gave a significant response ($p < 0.01$) and the data set(s) showed a significant dose correlation; and (3) the positive responses described in (2) were reproducible.

II. RESULTS

The stability of RPA112916 and the stability and homogeneity of RPA112916 in the solvent were not determined as part of this study. In addition, analysis of achieved concentration was not performed as part of this study.

The mean negative control counts fell within the accepted normal ranges for mean numbers of spontaneous revertants on solvent control plates for the laboratory. Historical control data were

provided in the study report. Less than 5% of plates were lost, leaving adequate numbers of plates at all treatments. The study therefore demonstrated correct strain and assay functioning and was accepted as valid.

Appropriate positive control chemicals (with S9 mix where required) induced substantial increases in revertant colony numbers with all strains, confirming sensitivity of the cultures and activity of the S9 mix. Experiment 2 treatments of strain TA98 (-S9) resulted in solvent control counts that were just below the lower limit of the historical control range. These values were considered sufficiently comparable to the historical control range to have confirmed the correct strain and assay functioning, and the data were accepted as valid. Results from the first and second tests are detailed below.

A. RANGE-FINDING:

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final concentrations of RPA112916 at 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. No evidence of toxicity was observed following these treatments. The results are summarized in Table 1.

TABLE 1. Summary of Mean Revertant Colonies - Range Finder Experiment.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA100**	**-S9**					
Solvent (DMSO)	86.80	5	---	13.07	---	---	---
1.6	105.00	3	1.21	7.21	0.66	11.38	2.55
8	114.33	3	1.32	5.13	0.71	3.13	3.76
40	98.33	3	1.13	5.51	0.11	0.09	1.66
200	97.00	3	1.12	13.23	0.04	-0.01	1.45
1000	98.67	3	1.14	7.09	0.01	-0.00	1.70
5000	125.33	3	1.44^	11.15	0.59	0.01	5.09
Pos. Con. NaN_3 (2)	796.67	3	9.18	17.50			
	M Statistic = 1.034						
Test strain:	**TA100**	**+S9**					
Solvent (DMSO)	98.80	5	---	14.86	---	---	---
1.6	111.67	3	1.13	6.03	0.50	8.04	1.45
8	113.67	3	1.15	17.01	0.40	1.62	1.62
40	108.67	3	1.10	4.51	0.15	0.12	1.13
200	119.67	3	1.21	8.33	0.41	0.07	2.27
1000	102.33	3	1.04	18.72	0.11	-0.00	0.37
5000	122.67	3	1.24^	8.08	0.34	0.00	2.58
Pos. Con. AAN (5)	2299.00	3	23.27	147.65	---	---	---
	M Statistic = 1.508						

Data extracted from the study report, Appendix 1, Tables 1 and 2, pages 29-30.

Key to significance:
* = $p \leq 0.05$
** = $p \leq 0.01$
*** $p \leq 0.005$

Key to postfixes:
^ = maximum increase above control

Positive Control:

NaN_3: Sodium azide
AAC: 9-Aminoacridine

B. FIRST TEST (MUTAGENICITY DATA):

Experiment 1 treatments of all the test strains were performed using the same test doses employed for the range-finder experiment. Strain TA100 data from the range-finder data were considered valid for mutation assessment, but treatments of this strain were included in Experiment 1 to determine the reproducibility of the results obtained. Following these treatments there was again no evidence of any test article toxicity. The results are summarized in Table 2.

TABLE 2. Summary of Mean Revertant Colonies - Experiment 1.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA98**	**-S9**					
Solvent (DMSO)	30.60	5	---	8.56	---	---	---
1.6	33.00	3	1.08	2.00	0.19	1.50	0.62
8	34.33	3	1.12^	3.06	0.25	0.43	0.90
40	33.00	3	1.08	1.73	0.11	0.03	0.62
200	29.33	3	0.96	8.96	0.19	-0.01	-0.30
1000	21.33	3	0.70	4.62	0.59	-0.01	-2.20
5000	27.33	3	0.89	4.51	0.27	-0.00	-0.68
Pos. Con. 2NF (5)	935.00	3	30.56	86.88	---	---	---
	M Statistic = 1.220						
Test strain:	**TA98**	**+S9**					
Solvent (DMSO)	30.00	5	---	1.87	---	---	---
1.6	32.33	3	1.08	2.08	0.56	1.46	0.75
8	39.67	3	1.32^	7.51	0.75***	1.20	2.88*
40	34.33	3	1.14	2.52	0.23	0.07	1.37
200	27.33	3	0.91	5.69	0.39	-0.03	-0.96
1000	34.33	3	1.14	4.73	0.06	0.00	1.34
5000	30.33	3	1.01	4.93	0.14	-0.00	0.07
Pos. Con. B[a]P (10)	358.67	3	11.96	29.14	---	---	---
	M Statistic = 0.576						
Test strain:	**TA100**	**-S9**					
Solvent (DMSO)	93.00	5	---	12.02	---	---	---
1.6	94.67	3	1.02	6.11	0.09	1.04	0.29
8	99.33	3	1.07	8.74	0.29	0.78	0.97
40	100.33	3	1.08^	8.62	0.29	0.16	1.12
200	89.00	3	0.96	7.00	0.26	-0.03	-0.58
1000	95.33	3	1.03	8.62	0.03	-0.00	0.38
5000	91.00	3	0.98	7.81	0.16	-0.00	-0.28
Pos. Con. NaN_3 (2)	847.00	3	9.11	47.15	---	---	---
	M Statistic = 0.876						
Test strain:	**TA100**	**+S9**					
Solvent (DMSO)	100.80	5	---	12.93	---	---	---
1.6	114.00	3	1.13	13.08	0.49	8.25	1.20
8	113.67	3	1.13	20.82	0.31	1.35	1.14
40	117.00	3	1.16	10.58	0.32	0.28	1.47
200	108.67 (M)	3	1.08	21.83	0.02	0.00	0.68
1000	118.33	3	1.17	11.06	0.22	0.01	1.58
5000	126.00	3	1.25^	15.87	0.37*	0.00	2.22
Pos. Con. AAN (5)	3056.33	3	30.32	186.57	---	---	---
	M Statistic = 2.126						

(Table continued; footnotes follow)

TABLE 2. Summary of Mean Revertant Colonies - Experiment 1.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA1535**	**-S9**					
Solvent (DMSO)	19.80	5	---	5.17	---	---	---
1.6	20.33	3	1.03	3.79	0.06	0.33	0.19
8	24.33	3	1.23^	5.86	0.41	0.58	1.24
40	20.33	3	1.03	7.23	0.00	-0.00	0.09
200	19.33	3	0.98	3.06	0.13	-0.01	-0.08
1000	20.00	3	1.01	3.61	0.08	-0.00	0.11
5000	21.33	3	1.08	3.06	0.04	0.00	0.49
Pos. Con. NaN_3 (2)	714.33	3	36.08	41.55	---	---	---
	M Statistic = 1.120						
Test strain:	**TA1535**	**+S9**					
Solvent (DMSO)	19.60	5	---	3.05	---	---	---
1.6	18.00	3	0.92	1.00	0.33	-1.00	-0.48
8	17.33	3	0.88	7.57	0.22	-0.26	-0.86
40	24.33	3	1.24	1.53	0.52*	0.14	1.41
200	17.67 (M)	3	0.90	6.51	0.09	-0.01	-0.72
1000	27.33	3	1.39^	5.77	0.51*	0.01	2.18
5000	19.67	3	1.00	1.53	0.04	0.00	0.04
Pos. Con. AAN (5)	262.33	3	13.38	9.02	---	---	---
	M Statistic = 1.018						
Test strain:	**TA1537**	**-S9**					
Solvent (DMSO)	15.20	5	---	2.05	---	---	---
1.6	20.00	3	1.32^	1.73	0.81**	3.00	1.78
8	12.33	3	0.81	3.51	0.47	-0.49	-1.24
40	17.67	3	1.16	3.51	0.14	0.03	0.92
200	8.00	3	0.53	3.00	0.65	-0.04	-3.38
1000	10.67	3	0.70	2.89	0.43	-0.01	-1.98
5000	11.33 (M)	3	0.75	4.93	0.28	-0.00	-1.77
Pos. Con. AAC (50)	152.00	3	10.00	31.19	---	---	---
	M Statistic = 0.807						
Test strain:	**TA1537**	**+S9**					
Solvent (DMSO)	14.60	5	---	5.94	---	---	---
1.6	13.33 (M)	3	0.91	5.51	0.12	-0.79	-0.31
8	16.67	3	1.14	5.69	0.20	0.30	0.53
40	17.33	3	1.19	7.77	0.22	0.08	0.63
200	16.33	3	1.12	2.52	0.11	0.01	0.51
1000	18.00	3	1.23^	7.21	0.18	0.00	0.78
5000	15.67	3	1.07	4.73	0.02	0.00	0.32
0=Pos. Con. AAN (5)	490.33	3	33.58	8.08	---	---	---
	M Statistic = 2.154						

(Table continued; footnotes follow)

TABLE 2. Summary of Mean Revertant Colonies - Experiment 1.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**WP2 pKM101**	**-S9**					
Solvent (DMSO)	62.20	5	---	5.89	---	---	---
1.6	61.00	3	0.98	8.72	0.10	-0.75	-0.21
8	58.33	3	0.94	6.35	0.26	-0.47	-0.63
40	59.00	3	0.95	8.54	0.16	-0.06	-0.54
200	48.00	3	0.77	11.27	0.58	-0.07	-2.49
1000	57.67	3	0.93	9.50	0.13	-0.00	-0.77
5000	65.33	3	1.05^	7.77	0.26	0.00	0.49
Pos. Con. NQO (10)	615.67	3	9.90	74.00	---	---	---
	M Statistic = 1.178						
Test strain:	**WP2 pKM101**	**+S9**					
Solvent (DMSO)	67.20	5	---	7.69	---	---	---
1.6	77.00	3	1.15	3.61	0.64	6.13	1.52
8	79.67	3	1.19^	12.66	0.50	1.38	1.87
40	76.67	3	1.14	14.36	0.22	0.14	1.41
200	77.33	3	1.15	5.13	0.18	0.02	1.57
1000	78.67	3	1.17	3.79	0.20	0.00	1.77
5000	79.33	3	1.18	11.85	0.19	0.00	1.83
	M Statistic = 1.116						
Test strain:	**WP2 uvrA**	**-S9**					
Solvent (DMSO)	166.20	5	---	11.54	---	---	---
1.6	173.67	3	1.04^	13.32	0.32	4.67	0.59
8	167.00	3	1.00	19.16	0.02	-0.08	0.05
40	165.67	3	1.00	30.17	0.07	-0.07	-0.11
200	151.00	3	0.91	10.39	0.40	-0.09	-1.25
1000	145.00	3	0.87	8.00	0.48	-0.02	-1.75
5000	158.00	3	0.95	19.00	0.17	-0.00	-0.68
Pos. Con. NQO (2)	2063.00	3	12.41	93.66	---	---	---
	M Statistic = 1.720						
Test strain:	**WP2 uvrA**	**+S9**					
Solvent (DMSO)	289.00	5	---	30.65	---	---	---
1.6	322.00	3	1.11^	21.66	0.55	20.63	2.11
8	317.00	3	1.10	7.21	0.37	2.83	1.81
40	292.67	3	1.01	14.05	0.15	-0.23	0.27
200	289.67	3	1.00	24.01	0.23	-0.08	0.07
1000	285.67	3	0.99	8.02	0.27	-0.02	-0.17
5000	263.33	3	0.91	15.89	0.51	-0.01	-1.66
Pos. Con. AAN (5)	1017.00	3	3.52	17.44	---	---	---
	M Statistic = 1 496						

Data extracted from the study report, Appendix 2, Tables 3-14, pages 33-44.

Key to significance:
* = $p \leq 0.05$ ** = $p \leq 0.01$ *** = $p \leq 0.005$

Key to postfixes:
^ = maximum increase above control

Positive Control: 2NF: 2-Nitrofluorene; NaN_3: Sodium azide; AAC: 9-Aminoacridine; NQO: 4-Nitroquinoline 1-oxide; AAN: 2-Aminoanthracene; B[a]P: Benzo[a]pyrene

M = plates counted manually

C. SECOND TEST (MUTAGENICITY DATA, PRE-INCUBATION UNDER +S9):

The results for Experiment 2 are summarized in Table 3. Experiment 2 treatments of all the tester strains retained 5000 µg/plate as the maximum test dose, but employed a narrowed dose range (156.25-5000 µg/plate), this way, those concentrations of RPA112916 approaching the limit dose, and therefore considered most likely to provide evidence of any mutagenic activity, were more closely investigated. In addition, all Experiment 2 treatments in the presence of S9 were further modified by the inclusion of a pre-incubation step.

Following the treatments in Experiment 2 there was again no evidence of toxicity in the absence of S9. Pre-incubation treatments in the presence of S9 did however demonstrate evidence of toxicity in most of the tester strains at doses of 2500 µg/plate and above (the only exception being strain TA1535, where no clear evidence of toxicity was observed). This evidence of toxicity was in the form of a diminution of the background bacterial lawn, and/or a marked reduction in revertant numbers. In addition, precipitation was also observed over these same dose levels in the presence of S9 only.

The observation of both toxic effects and precipitation only following pre-incubation treatments was attributed to the enhanced exposure of the bacterial cells during this preincubation period, compared to that of the plate-incorporation treatments.

Overall, the mutation data were evaluated as follows:

Following RPA112916 treatments of all the tester strains, both in the absence and in the presence of metabolic activation (S9), only strain TA100 treatments resulted in any increases in revertant numbers that were statistically significant when the data were analysed at the 1% level using Dunnett's test. Statistically significant increases were observed in this strain in the absence of S9 in the range-finder experiment, and in the presence of S9 in Experiment 2. In both cases, the magnitude of the increases was no more than 1.5 times the mean solvent control revertant colony counts, which is a level that is considered to be within the variability range of data in this strain. In neither of these cases was there any clear evidence of a dose relationship, nor were the data reproduced in independent experiments (although for the increase in the presence of S9 which occurred when a pre-incubation methodology was employed, no directly comparable experimental treatments were available, as the Experiment 1 treatments employed a plate incorporation methodology). It was therefore considered that the observed increases were due to chance (biological variability), rather than to any RPA112916 mutagenic activity. This study was therefore considered to have provided no clear evidence of an ability of RPA112916 to induce mutation in this assay system.

TABLE 3. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA98**	**-S9**					
Solvent (DMSO)	20.75 (M)	4	---	2.99	---	---	---
156.25	19.33 (M)	3	0.93	3.21	0.26	-0.01	-0.54
312.5	19.00 (M)	3	0.92	1.73	0.31	-0.01	-0.64
625	23.33 (M)	3	1.12^	5.51	0.29	0.00	0.88
1250	20.00 (M)	3	0.96	2.00	0.07	0.00	-0.26
2500	23.00 (M)	3	1.11	6.00	0.25	0.00	0.74
5000	23.00 (M)	3	1.11	2.00	0.30	0.00	0.83
Pos. Con. 2NF (5)	918.67	3	44.27	115.74	---	---	---
	M Statistic = 0.610						
Test strain:	**TA98**	**+S9**					
Solvent (DMSO)	34.80	5	---	4.66	---	---	---
156.25	38.00	3	1.09^	3.46	0.38	0.02	0.47
312.5	36.67	3	1.05	10.69	0.16	0.01	0.22
625	36.33	3	1.04	15.50	0.06	0.00	0.09
1250	32.00	3	0.92	5.20	0.16	-0.00	-0.43
2500	18.67 (M+S+PPN)	3	0.54	2.89	0.63	-0.01	-2.76
5000	12.00 (M+V+PPN)	3	0.34	8.54	0.77	-0.01	-4.58
Pos. Con. B[a]P (10)	242.33	3	6.96	20.98	---	---	---
	M Statistic = 2.334						
Test strain:	**TA100**	**-S9**					
Solvent (DMSO)	112.40	5	---	13.32	---	---	---
156.25	116.00	3	1.03	19.92	0.13	0.02	0.27
312.5	103.00	3	0.92	19.29	0.23	-0.03	-0.80
625	117.67	3	1.05^	16.80	0.06	0.00	0.41
1250	110.67	3	0.98	22.81	0.01	-0.00	-0.17
2500	105.67	3	0.94	3.06	0.14	-0.00	-0.52
5000	115.00	3	1.02	20.07	0.02	0.00	0.18
Pos. Con. NaN_3 (2)	693.67	3	6.17	30.01	---	---	---
	M Statistic = 2.610						
Test strain:	**TA100**	**+S9**					
Solvent (DMSO)	127.80	5	---	8.84	---	---	---
156.25	116.67	3	0.91	2.52	0.65	-0.07	-0.95
312.5	118.00	3	0.92	8.72	0.53	-0.03	-0.84
625	117.00	3	0.92	11.27	0.43	-0.02	-0.93
1250	191.33	3	1.50^	40.43	0.70***	0.05	4.73***
2500	71.67 (M+S+PPN)	3	0.56	16.56	0.26	-0.01	-5.47
5000	87.00 (M+S+PPN)	3	0.68	11.79	0.42	-0.01	-3.78
Pos. Con. AAN (5)	2198.67	3	17.20	261.97	---	---	---
	M Statistic = 2.122						

(Table continued; footnotes follow)

TABLE 3. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA1535**	**-S9**					
Solvent (DMSO)	17.80 (M)	5	---	2.49	---	---	---
156.25	19.33 (M)	3	1.09^	7.77	0.17	0.01	0.33
312.5	15.67 (M)	3	0.88	1.53	0.19	-0.01	-0.65
625	16.67 (M)	3	0.94	2.08	0.19	-0.00	-0.34
1250	19.33 (M)	3	1.09	4.73	0.09	0.00	0.41
2500	16.00 (M)	3	0.90	1.00	0.11	-0.00	-0.54
5000	14.00 (M)	3	0.79	7.81	0.29	-0.00	-1.41
Pos. Con. NaN_3 (2)	640.33	3	35.97	66.12	---	---	---
	M Statistic = 1.225						
Test strain:	**TA1535**	**+S9**					
Solvent (DMSO)	15.40	5	---	6.43	---	---	---
156.25	20.33	3	1.32^	2.52	0.45	0.03	1.58
312.5	16.00	3	1.04	1.00	0.12	0.00	0.35
625	16.33	3	1.06	5.69	0.01	0.00	0.34
1250	17.00	3	1.10	3.61	0.02	0.00	0.61
2500	15.33 (M+PPN)	3	1.00	1.53	0.11	-0.00	0.14
5000	17.33 (M+PPN)	3	1.13	4.62	0.01	0.00	0.68
Pos. Con. AAN (5)	148.33	3	9.63	22.81	---	---	---
	M Statistic = 1.233						
Test strain:	**TA1537**	**-S9**					
Solvent (DMSO)	14.60	5	---	3.51	---	---	---
156.25	14.67	3	1.00	5.69	0.01	0.00	-0.03
312.5	19.67	3	1.35^	4.62	0.45	0.02	1.55
625	13.00	3	0.89	3.46	0.05	-0.00	-0.54
1250	12.33	3	0.84	3.21	0.26	-0.00	-0.76
2500	12.00	3	0.82	2.00	0.34	-0.00	-0.85
5000	13.67	3	0.94	5.51	0.20	-0.00	-0.38
Pos. Con. AAC (50)	165.33	3	11.32	25.15	---	---	---
	M Statistic = 1.161						
Test strain:	**TA1537**	**+S9**					
Solvent (DMSO)	24.20	5		4.21	---	---	---
156.25	21.33	3	0.88	7.77	0.27	-0.02	-0.77
312.5	18.33	3	0.76	2.31	0.49	-0.02	-1.45
625	19.00	3	0.79	5.29	0.42	-0.01	-1.33
1250	18.33	3	0.76	6.81	0.36	-0.00	-1.54
2500	(T+PPN)	3	---	---	---	---	---
5000	(T+PPN)	3	---	---	---	---	---
Pos. Con. AAN (5)	354.33	3	14.64	50.34	---	---	---
	M Statistic = 1.430						

(Table continued; footnotes follow)

TABLE 3. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**WP2 pKM101**	**-S9**					
Solvent (DMSO)	40.40	5	---	6.66	---	---	---
156.25	44.67	3	1.11	4.51	0.37	0.03	1.28
312.5	47.00	3	1.16^	2.00	0.52*	0.02	1.95
625	43.00	3	1.06	5.57	0.21	0.00	0.79
1250	43.33	3	1.07	3.21	0.10	0.00	0.91
2500	47.00	3	1.16	1.73	0.29	0.00	1.95
5000	44.33	3	1.10	4.51	0.17	0.00	1.19
Pos. Con. NQO (10)	799.00	3	19.78	34.70	---	---	---
	M Statistic = 0.527						
Test strain:	**WP2 pKM101**	**+S9**					
Solvent (DMSO)	79.60	5		11.19	---	---	---
156.25	90.33	3	1.13^	7.09	0.51	0.07	1.68
312.5	74.67	3	0.94	1.53	0.13	-0.01	0.74
625	82.33	3	1.03	7.23	0.02	-0.00	0.46
1250	73.33	3	0.92	4.16	0.32	-0.01	-0.97
2500	30.33 (M+PPN)	3	0.38	9.24	0.85	-0.02	-9.68
5000	25.00 (M+PPN)	3	0.31	3.00	0.88	-0.01	-11.01
	M Statistic = 0.973						
Test strain:	**WP2 uvrA**	**-S9**					
Solvent (DMSO)	208.60	5	---	30.55	---	---	---
156.25	213.67	3	1.02^	23.71	0.10	0.03	0.33
312.5	177.33	3	0.85	14.19	0.45	-0.09	-1.93
625	172.33	3	0.83	6.51	0.59	-0.07	-2.25
1250	194.67	3	0.93	14.19	0.28	-0.02	-0.81
2500	193.33	3	0.93	27.21	0.15	-0.00	-0.93
5000	207.33	3	0.99	20.03	0.09	0.00	-0.04
Pos. Con. NQO (2)	2075.00	3	9.95	58.59	---	---	---
	M Statistic = 2.470						
Test strain:	**WP2 uvrA**	**+S9**					
Solvent (DMSO)	258.40	5	---	25.32	---	---	---
156.25	246.33	3	0.95	15.53	0.29	-0.08	-0.41
312.5	258.67	3	1.00	18.50	0.03	-0.00	0.02
625	261.67	3	1.01^	15.04	0.11	0.01	0.12
1250	242.67	3	0.94	30.04	0.19	-0.01	-0.55
2500	128.33 (M+PPN)	3	0.50	46.31	0.81	-0.05	-5.35
5000	102.33 (M+PPN)	3	0.40	45.54	0.87	-0.04	-6.70
Pos. Con. AAN (5)	726.00	3	2.81	78.17	---	---	---
	M Statistic = 6.104						

Data extracted from the study report, Appendix 3, Tables 15-26. pages 47-58.

Key to significance:

* = $p \leq 0.05$
** = $p \leq 0.01$
*** = $p \leq 0.005$

Key to postfixes:

^ = maximum increase above control

Positive Control:

2NF: 2-Nitrofluorene
NaN_3: Sodium azide
AAC: 9-Aminoacridine
NQO: 4-Nitroquinoline 1-oxide
AAN: 2-Aminoanthracene
B[a]P: Benzo[a]pyrene

S: Slight thinning of background bacterial lawn

PPN: Precipitation of Test article observed

T = Toxic, no revertant colonies

V = Very thin background lawn

M: Plate counted manually

III. CONCLUSIONS:

RPA112916, a metabolite of ethiprole, was assayed for mutation in four histidine-requiring strains (TA98, TA100, TA1535 and TA1537) of *Salmonella typhimurium*, and two tryptophan-requiring strains (WP2 pKM101 and WP2*uvr*A pKM101) of *Escherichia coli*, both in the absence and presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S9), in two separate experiments.

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final RPA112916 concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Following these treatments, and those of all the test strains in Experiment 1 which employed the same test doses, no evidence of toxicity was observed.

Experiment 2 treatments of all the tester strains were performed at concentrations extending up to 5000 µg/plate, but employed a narrowed dose range (156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate), in order to more closely examine those concentrations of RPA112916 considered most likely to provide evidence of any mutagenic activity. Treatments in this experiment in the presence of S9 were modified by the inclusion of a pre-incubation step, in order to increase the range of mutagenic chemicals that could be detected using this assay system. Following these treatments, no evidence of toxicity was observed in the absence of S9, but at concentrations of 2500 µg/plate in most tester strains in the presence of S9 clear evidence of toxicity was observed. In addition, precipitation of test agent was also observed over these test doses, again only in the presence of S9.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.

No increases in revertant numbers were observed following any of the strain treatments that were sufficient in terms of dose-relationship or reproducibility to be considered as indicative of any RPA112916 mutagenic activity.

It was concluded that RPA112916 did not induce mutation in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and two strains of *Escherichia coli* (WP2 pKM10l and WP2 uvrA pKM101), when tested under the conditions employed for this

study. These conditions included treatments at concentrations up to 5000 ug/plate, both in the absence and in the presence of a rat liver metabolic activation system.

The CAS number of RPA112916 was not provided. The stability of RPA112916 and the stability and homogeneity of technical ethiprole in the solvent were not determined; in addition, analysis of achieved concentration was not performed as part of this study. These omissions are considered minor and do not impact on the review of this study.

KIIA 5.4.1 - In vitro genotoxicity - Bacterial assay for gene mutation

1.	Annex point(s)	KIIA 5.4.1; Genotoxicity/In vitro genotoxicity - Bacterial assay for gene mutation: **MRID 47622814**
2.	Location in dossier	KIIA 5.4.1 /01
3.	Authors (year)	Ballantyne, M. (1998)
	Title	RPA107382: Reverse mutation in four histidine-requiring strains of Salmonella typhimurium and one tryptophan-requiring strain of Escherichia coli.
	Company, report No.	Bayer CropScience AG, Report-No.: R016832 (M-192448-01-1)
	Date	21.09.1998
4.	Testing facility	Covance Laboratories Limited, Harrogate;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s): ethiprole TC (Batch-No.: CDR9706)
7.	Test method	USEPA (=EPA): 84-2
	Deviations	
8.	GLP	yes

Executive summary:

In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2*uvr*A of *E.coli* were exposed to ethiprole (92.7% a.i.) in DMSO at concentrations of 8, 40, 200, 100 and 5000 µg/plate in the presence and absence of mammalian metabolic activation (plate incorporation method). As the results of this experiment were negative a second experiment was conducted at concentrations of 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate in the presence and absence of mammalian metabolic activation, where treatments in the presence of S9 included a pre-incubation step (Experiment 2).

Ethiprole was tested up to the limit concentration of 5000 µg/plate, this being the best estimate of the limit of precipitation. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **acceptable/guideline** and it satisfies the requirement for Test Guideline OPPTS 870.5100 and OECD 471 for *in vitro* mutagenicity (bacterial reverse mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Ethiprole

Description: White powder

Lot/Batch #: CDR9706

Purity: 92.7% a.i.

CAS # of TGAI: 181587-01-9

Solvent Used: Sodium azide was dissolved in water. Ethiprole and the other positive controls were dissolved in DMSO.

2. Control Materials:

Negative: DMSO was used as a negative control

Solvent (final conc'n): DMSO at 0.1 mL/plate (0.05 mL for preincubation treatments)

Positive:

Nonactivation: 2-Nitrofluorene(2NF) 5 µg/plate, TA98
Sodium azide (NaN_3) 2 µg/plate, TA100 and TA1535
9-Aminoacridine (AAC) 50 µg/plate, TA1537
4-nitroquinoline 1-oxide (NQO) 2 µg/plate, WP2 uvrA

Activation: 2-Aminoanthracene (AAN) 5 µg/plate, at least one TA strain; 10* or 20 µg/plate for WP2 uvrA

*When using the pre-incubation methodology, this AAN concentration was often too low to provide clear increases in revertant numbers in the laboratory, so treatments at 20 µg/plate were also included.

3. Activation: S9 derived from

X	induced	X	Aroclor 1254	X	Rat	X	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Describe S9 mix composition: S9 fraction (10% v/v), sodium phosphate buffer pH 7.4 (500 mM), glucose-6-phosphate (180 mg/mL), NADP (25 mg/mL), $MgCl_2$ (250 mM), KCl (150 mM). L-histidine HCl in 250 mM $MgCl_2$ and d-biotin (1 mg/mL each) were added to solutions for treatments with the *S. typhimurium* strains and DL-tryptophan (1 mg/mL) and casamino acids (20% w/v) were added to solutions for treatments with the *E. coli* strain only. Batches of MolTox™ S9 were stored frozen at -80°C, and thawed just prior to incorporation into the top agar. Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens, and cytochrome P-450-catalysed enzyme activities (alkoxyresorufm-0-dealkylase activities).

4. Test Organisms:										
***S. typhimurium* strains**										
		TA97	X	TA98	X	TA100		TA102		TA104
	X	TA1535	X	TA1537		TA1538		list any others		
***E. coli* strains**										
		WP2	X	WP2 *uvr*A				list any others		
Properly maintained?							X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?							X	Yes		No

5. Test compound concentrations used:

Concentration of the Test Substance Used	Number of Replicates Per Dose	Strains Assayed	Conditions
First Test (Range Finding and Mutation Experiment 1): Activated and Non-Activated Conditions			
8, 40, 200, 1000 and 5000 µg/plate	3	*S. typhimurium,* (strains TA98, TA100, TA1535, and TA1537) *E. coli,* (strain WP2 *uvr*A)	Plated on agar plates and incubated at 37 °C for 3 days.
Second Test (Mutation Experiment 2): Activated and Non-Activated Conditions			
156.25 (with S9 only), 312.5, 625, 1250, 2500 and 5000 µg/plate	3	*S. typhimurium,* (strains TA98, TA100, TA1535, and TA1537) *E. coli,* (strain WP2 *uvr*A)	Plated on agar plates and incubated at 37°C for 3 days. Treatments in the presence of S9 included 1 hour pre-incubation step at 37 °C prior to plating and normal incubation.

B. TEST PERFORMANCE

1. Type of *Salmonella* and *E. Coli* assay:

_X_ standard plate test
_X_ pre-incubation (60 minutes) (Mutation Experiment 2)

2. Protocol: For all assays, bacteria were cultured for 10 hours at 37°C in nutrient broth (containing ampicillin for strains TA98 and TA100). Incubation was carried out in a shaking incubator. Bacteria were taken from vials of frozen cultures, which had been checked for strain characteristics of histidine or tryptophan dependence, *rfa* character (Salmonella strains) and presence (strains TA98 and TA100) or absence (strains TA1535, TA1537 and E. coli) of the pKM101 ampicillin resistance factor. Checks were carried out according to Maron and Ames (1983) and De Serres and Shelby (1979). All experimentation commenced within 2 hours of the end of the period of incubation.

Range-finder experiment: Ethiprole was tested in strain TA100 in triplicate plates at 8-5000 µg/plate without and with S9 mix (±S9 activation). Negative (solvent) and positive controls were included in quintuplicate and triplicate respectively (±S9 activation). These platings

were achieved by the following sequence of additions to 2.5 mL molten agar at 46°C: 0.1 mL bacterial culture; 0.1 mL test article solution or control 0.5 mL 10% S9 mix or buffer solution followed by rapid mixing and pouring on to Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible revertant colonies were counted electronically using a Seescan® Colony Counter. Where confounding factors such as precipitation of test agent prevented an accurate automated count from being obtained, plates were counted manually. For certain treatments in Experiment 2, as three or more test doses required manual counting due to the presence of precipitate, the remaining test doses and solvent control plates were also counted manually for consistency of counting methodology.

Mutation Experiments: In two separate experiments, ethiprole was tested in four strains of S. *typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of E *coli* (WP2 uvrA) using triplicate plates at 8-5000 µg/plate (±S9 activation). Experiment 1 mutagenicity data for strain TA100 was provided by the range-finding experiment treatments. Negative (solvent) and positive controls were included in quintuplicate and triplicate respectively (±S9 activation). The activity of the S9 mix used in each experiment was confirmed by AAN treatments (again in triplicate) of at least one strain in the presence of S9. The platings were achieved as described above for the range-finding experiment.

As the results of the first experiment were negative, treatments in the presence of S9 in Experiment 2 included a pre-incubation step, where the quantities of test article or control solution (reduced to 0.05 mL), bacteria and S9 mix, as described above for the range-finding experiment, were mixed together incubated for 1 hour at 37°C, with shaking, before the addition of 2.5 mL molten agar at 46°C. Plating of these treatments then proceeded as for the normal plate-incorporation procedure. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Volume additions for the Experiment 2 pre-incubation treatments were reduced to 0.05 mL due to the solvent (DMSO) employed in this study. This and some other organic solvents are known to be near to toxic levels when added at volumes of 0.1 mL in this assay system when employing the pre-incubation methodology. By reducing the addition volume to 0.05 mL per plate, it was hoped to minimize or eliminate any toxic effects of the solvent that may have otherwise occurred.

3. **Statistical Analysis:** The m-statistic was calculated to check that the data were Poisson-distributed (Mahon et. al., 1989), and Dunnett's test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis.

4. **Evaluation Criteria:** The assay was considered valid if (1) mean negative control counts fell within the accepted normal ranges for mean numbers of spontaneous revertants on solvent control plates for the laboratory; (2) the positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S9 preparation; and (3) no more than 5% of the plates were lost through contamination or some other unforeseen event. The test article was considered to be mutagenic if (1) the assay was considered valid, as described above; (2) the Dunnett's test gave a significant response ($p < 0.01$) and the data set(s) showed a significant dose correlation; and (3) the positive responses described in (2) were reproducible.

II. RESULTS

The stability of technical ethiprole and the stability and homogeneity of technical ethiprole in the solvent were not determined as part of this study. In addition, analysis of achieved concentration was not performed as part of this study. The mean negative control counts fell within the accepted normal ranges for mean numbers of spontaneous revertants on solvent control plates for the laboratory. Historical control data were provided in the study report. Appropriate positive control chemicals (with S9 mix where required) induced substantial increases in revertant colony numbers with all strains, confirming sensitivity of the cultures and activity of the S9 mix. Results from the first and second tests are detailed below.

A. RANGE-FINDING:

An initial toxicity range-finding experiment was carried out in strain TA100 only, using final concentrations of ethiprole at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Following these treatments, evidence of toxicity in the form of a thinning of the background bacterial lawn was observed at the maximum test dose only in the presence of S9. These data were considered acceptable for mutagenicity assessment, and were presented in the report as the data for strain TA100, for Mutagenicity Experiment 1. Precipitation of test agent was observed on all plates treated at 5000 µg/plate.

B. FIRST TEST (MUTAGENICITY DATA):

Experiment 1 treatments of the remaining test strains were performed with 5000 µg/plate retained as the maximum test dose, since this was the best estimate of the limit of precipitation, and in the absence of S9, toxicity. The same test doses as employed in the range-finding experiment were used for the remaining Experiment 1 treatments. Evidence of toxicity was only observed in strain TA98 without S9 and in strain TA1537 with S9, in each case at the highest test dose of 5000/µg/plate only. Precipitation of test agent was observed on all plates treated at 5000 µg plate. The results are summarized in Table 1.

Table 1. Summary of Mean Revertant Colonies - Experiment 1 (standard plate test).

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA98**	**-S9**					
Solvent (DMSO)	29.00	5	---	9.30	---	---	---
8	30.33	3	1.05	3.06	0.10	0.17	0.42
40	32.33	3	1.11^	4.04	0.22	0.08	0.86
200	23.67	3	0.82	5.69	0.38	-0.03	-1.21
1000	26.33	3	0.91	2.52	0.23	-0.00	-0.50
5000	25.67 (PPN+M+S)	3	0.89	3.21	0.21	-0.00	-0.67
Pos. Con. 2NF (5)	460.33	3	15.87	58.62	---	---	---
	M Statistic = 1.255						
Test strain:	**TA98**	**+S9**					
Solvent (DMSO)	43.80	5	---	3.90	---	---	---
8	41.67	3	0.95	5.51	0.26	-0.27	-0.47
40	43.00	3	0.98	10.54	0.03	-0.01	-0.25
200	60.67	3	1.39^	7.51	0.77***	0.09	3.28*
1000	47.00	3	1.07	3.46	0.16	-0.00	0.67
5000	49.33 (PPN+M)	3	1.13	8.50	0.13	-0.00	1.11
Pos. Con. AAN (5)	1170.00	3	26.71	186.94	---	---	---
	M Statistic = 0.951						
Test strain:	**TA100**	**-S9**					
Solvent (DMSO)	107.60	5	---	10.50	---	---	---
8	99.67	3	0.93	5.13	0.44	-0.99	-0.91
40	111.67	3	1.04	14.19	0.24	0.14	0.45
200	115.67	3	1.07	12.66	0.38	0.05	0.90

1000	122.33	3	1.14^	20.55	0.45*	0.02	1.59
5000	115.00 (PPN+M)	3	1.07	6.08	0.20	0.00	0.85
Pos. Con. NaN_3 (2)	661.00	3	6.14	37.27	---	---	---
	M Statistic = 1.325						
Test strain:	**TA100**	**+S9**					
Solvent (DMSO)	133.00	5	---	13.08	---	---	---
8	137.00	3	1.03^	10.58	0.18	0.50	0.44
40	110.00	3	0.83	13.45	0.67	-0.62	-2.58
200	126.33	3	0.95	9.71	0.17	-0.03	-0.71
1000	130.00	3	0.98	9.17	0.04	0.00	-0.31
5000	121.00 (PPN+M+S)	3	0.91	16.46	0.17	-0.00	-1.32
Pos. Con. AAN (5)	1518.00	3	11.41	92.26	---	---	---
	M Statistic = 1.238						

(Table continued; footnotes follow)

TABLE 1. Summary of Mean Revertant Colonies - Experiment 1 (standard plate test).

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA1535**	**-S9**					
Solvent (DMSO)	14.60	5	---	3.05	---	---	---
8	13.00	3	0.89	3.61	0.27	-0.20	-0.72
40	11.67	3	0.80	3.21	0.38	-0.07	-1.35
200	10.67 (M)	3	0.73	1.53	0.43	-0.02	-1.78
1000	12.33	3	0.84	1.53	0.14	-0.00	-0.97
5000	13.33 (PPN+M)	3	0.91	4.04	0.05	0.00	-0.60
Pos. Con. NaN_3 (2)	433.33	3	29.68	66.06	---	---	---
	M Statistic = 0.685						
Test strain:	**TA1535**	**+S9**					
Solvent (DMSO)	20.80	5	---	4.21	---	---	---
8	15.67	3	0.75	2.31	0.61	-0.64	-1.61
40	17.33	3	0.83	2.08	0.30	-0.06	-1.04
200	18.33	3	0.88	7.51	0.05	-0.00	-0.85
1000	19.33	3	0.93	5.77	0.07	0.00	-0.47
5000	16.67 (PPN+M)	3	0.80	1.53	0.15	-0.00	-1.25
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 1.033						
Test strain:	**TA1537**	**-S9**					
Solvent (DMSO)	7.00	5	---	3.39	---	---	---
8	8.00	3	1.14	2.00	0.18	0.13	0.62
40	10.67	3	1.52^	1.53	0.55*	0.09	1.81
200	8.67	3	1.24	4.51	0.14	0.01	0.77
1000	8.67	3	1.24	2.08	0.07	0.00	0.93
5000	6.00 (PPN+M)	3	0.86	1.00	0.30	-0.00	-0.37
Pos. Con. AAC (50)	496.50	3	70.93	12.02	---	---	---
	M Statistic = 1.002						
Test strain:	**TA1537**	**+S9**					
Solvent (DMSO)	12.20	5	---	1.30	---	---	---
8	13.00	3	1.07^	5.57	0.13	0.10	0.15
40	5.33	3	0.44	1.53	0.74	-0.18	-3.35
200	10.33	3	0.85	3.79	0.15	-0.01	-0.89
1000	9.67	3	0.79	4.04	0.11	-0.00	-1.19
5000	8.33 (PPN+M+S)	3	0.68	0.58	0.22	-0.00	-1.69
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 0.888						

(Table continued; footnotes follow)

TABLE 1. Summary of Mean Revertant Colonies - Experiment 1 (standard plate test).

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**WP2 uvrA**	**-S9**					
Solvent (DMSO)	15.40	5	---	1.82	---	---	---
8	15.67	3	1.02	6.66	0.04	0.03	-0.09
40	15.33	3	1.00	2.52	0.02	-0.00	-0.03
200	18.33	3	1.19	4.04	0.33	0.01	0.92
1000	19.00	3	1.23^	5.29	0.34	0.00	1.08
5000	15.00 (PPN+M)	3	0.97	3.61	0.09	-0.00	-0.17
Pos. Con. NQO (2)	1019.67	3	66.21	59.88	---	---	---
	M Statistic = 0.986						
Test strain:	**WP2 uvrA**	**+S9**					
Solvent (DMSO)	23.00	5	---	1.87	---	---	---
8	26.67	3	1.16	6.66	0.44	0.46	1.45
40	30.67	3	1.33^	3.06	0.66*	0.18	3.11*
200	19.33	3	0.84	1.53	0.44	-0.03	-1.67
1000	18.67	3	0.81	0.58	0.52	-0.01	-1.99
5000	21.00	3	0.91	3.61	0.28	-0.00	-0.93
Pos. Con. AAN (5)	164.67	3	7.16	33.65	---	---	---
	M Statistic = 0.433						

Data extracted from the study report, Appendix 1, Tables 1-10. pages 28-39.

Key to significance:
* = $p \leq 0.05$
** = $p \leq 0.01$
*** $p \leq 0.005$

Key to postfixes:
^ = maximum increase above control

Positive Control:

2NF: 2-Nitrofluorene
NaN_3: Sodium azide
AAC: 9-Aminoacridine
NQO: 4-Nitroquinoline 1-oxide
AAN: 2-Aminoanthracene

S: Slight thinning of background bacterial lawn
PPN: Precipitation of Test article observed
M: Plate counted manually

C. SECOND TEST (MUTAGENICITY DATA, PRE-INCUBATION UNDER +S9):

For Experiment 2 treatments of all strains, 5000 µg/plate was retained as the maximum test dose. This dose was selected as the best estimate of the lower limit of precipitation, and where toxic effects were observed, also as the best estimate of the lower limit of toxicity. A narrowed dose range was observed for Experiment 2 treatments, in order to more closely investigate those concentrations of ethiprole considered most likely to induce any mutation. In addition, all Experiment 2 treatments in the presence of S9 were performed with a pre-incubation step included. Evidence of toxicity was observed following treatments of strains TA98, TA100 and TA1537 treatments (±S9), and following treatment of strain TA1535 treatments, -S9 only. In each case evidence of toxicity was observed at 2500 µg/plate and above where it occurred in the

absence of S9, and 625 or 1250 µg/plate (the latter being applicable for strain TA1537 only) and above where it occurred in the presence of S9. Precipitation of test agent was again observed, occurring on all plates treated at 625 µg/plate and above in the presence of S9, and all plates treated at 1250 µg/plate and above in the absence of S9. Although relatively few test doses in Experiment 2 were in the soluble or non-toxic range, these data were still considered satisfactory for mutagenicity assessment, as lower test dose treatments in Experiment 1 confirmed the absence of any increases in revertant numbers at those dose levels. Results are summarized in Table 2.

TABLE 2. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA98**	**-S9**					
Solvent (DMSO)	25.80 (M)	5	---	5.22	---	---	---
312.5	25.67 (M)	3	0.99	9.45	0.01	-0.00	-0.15
625	26.00 (M)	3	1.01	5.29	0.01	0.00	0.06
1250	33.33 (PPN+M)	3	1.29^	1.53	0.46*	0.01	1.83
2500	29.33 (PPN+M+S)	3	1.14	3.06	0.33	0.00	0.90
5000	28.33 (PPN+M+S)	3	1.10	4.04	0.19	0.00	0.65
Pos. Con. 2NF (5)	850.00	3	32.95	83.35	---	---	---
	M Statistic = 1.090						
Test strain:	**TA98**	**+S9**					
Solvent (DMSO)	49.00 (M)	5	---	8.43	---	---	---
156.25	39.33 (M)	3	0.80	1.15	0.62	-0.06	-2.28
312.5	47.33 (M)	3	0.97	1.53	0.19	-0.01	-0.32
625	40.67 (PPN+M+S)	3	0.83	3.14	0.35	-0.01	-2.00
1250	32.00 (PPN+M+S)	3	0.65	5.57	0.65	-0.01	-4.30
2500	38.00 (PPN+M+S)	3	0.78	1.00	0.49	-0.00	-2.62
5000	36.33 (PPN+M+S)	3	0.74	3.21	0.44	-0.00	-3.07
Pos. Con. AAN	1651.33	3	33.70	213.04	---	---	---
	M Statistic = 0.736						
Test strain:	**TA100**	**-S9**					
Solvent (DMSO)	121.40	5	---	12.62	---	---	---
312.5	118.33	3	0.97	15.01	0.13	-0.01	-0.46
625	119.33	3	0.98	1.15	0.10	-0.00	-0.26
1250	117.33 (PPN)	3	0.97	4.16	0.15	-0.00	-0.56
2500	106.67 (PPN+M+S)	3	0.88	6.03	0.50	-0.01	-2.17
5000	121.67 (PPN+M+S)	3	1.00^	3.79	0.08	-0.00	0.07
Pos. Con. NaN_3 (2)	636.67	3	5.24	75.48	---	---	---
	M Statistic = 0.735						
Test strain:	**TA100**	**+S9**					
Solvent (DMSO)	150.60 (M)	5	---	16.10	---	---	---
156.25	142.00 (M)	3	0.94	10.44	0.32	-0.06	-1.12
312.5	141.00 (M)	3	0.94	8.72	0.35	-0.03	-1.25
625	158.33 (PPN+M+S)	3	1.05^	7.64	0.22	0.01	1.04
1250	137.67 (PPN+M+S)	3	0.91	6.03	0.20	-0.01	-1.70
2500	145.00 (PPN+M+S)	3	0.96	8.00	0.13	-0.00	-0.71
5000	145.33 (PPN+M+S)	3	0.97	2.31	0.09	-0.00	-0.65
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 0.733						

(Table continued; footnotes follow)

TABLE 2. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**TA1535**	**-S9**					
Solvent (DMSO)	20.20	5	---	4.60	---	---	---
312.5	22.00 (M)	3	1.09^	5.20	0.20	0.01	0.45
625	18.00	3	0.89	5.20	0.17	-0.00	-0.61
1250	16.67 (PPN)	3	0.83	6.66	0.32	-0.00	-1.05
2500	19.67 (PPN+M+S)	3	0.97	6.43	0.12	-0.00	-0.17
5000	16.00 (PPN+M+S)	3	0.79	1.00	0.28	-0.00	-1.09
Pos. Con. NaN_3 (2)	461.67	3	22.85	36.56	---	---	---
	M Statistic = 1.379						
Test strain:	**TA1535**	**+S9**					
Solvent (DMSO)	23.40 (M)	5	---	2.61	---	---	---
156.25	19.00 (M)	3	0.81	4.00	0.62	-0.03	-1.08
312.5	20.33 (M)	3	0.87	3.51	0.43	-0.01	-0.73
625	20.00 (PPN+M)	3	0.85	2.65	0.35	-0.00	-0.81
1250	28.00 (PPN+M)	3	1.20^	8.72	0.37	0.00	0.91
2500	22.33 (PPN+M)	3	0.95	10.60	0.15	0.00	-0.46
5000	17.00 (PPN+M)	---	0.73	4.36	0.21	-0.00	-1.62
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 1.405						
Test strain:	**TA1537**	**-S9**					
Solvent (DMSO)	10.40 (M)	5	---	3.44	---	---	---
312.5	6.33 (M)	3	0.61	3.79	0.54	-0.01	-1.95
625	9.00	3	0.87	2.00	0.24	-0.00	-0.51
1250	8.00 (PPN)	3	0.77	1.73	0.23	-0.00	-0.94
2500	5.00 (PPN+M+S)	3	0.48	3.00	0.48	-0.00	-2.59
5000	6.33 (PPN+M+S)	3	0.61	1.15	0.40	-0.00	-1.71
Pos. Con. AAC (50)	620.67	3	59.68	127.26	---	---	---
	M Statistic = 1.052						
Test strain:	**TA1537**	**+S9**					
Solvent (DMSO)	15.40 (M)	5	---	4.10	---	---	---
156.25	14.67 (M)	3	0.95	4.16	0.10	-0.00	-0.31
312.5	16.00 (M)	3	1.04^	2.00	0.06	-0.00	0.33
625	13.33 (PPN+M)	3	0.87	3.06	0.20	-0.00	-0.86
1250	8.67 (PPN+M+S)	3	0.56	1.15	0.63	-0.01	-3.16
2500	9.67 (PPN+M+S)	3	0.63	2.52	0.61	-0.00	-2.66
5000	10.67 (PPN+M+S)	---	0.69	1.15	0.49	-0.00	-2.09
Pos. Con. N/A	---	---	---	---	---	---	---
	M Statistic = 0.656						

(Table continued; footnotes follow)

TABLE 2. Summary of Mean Revertant Colonies - Experiment 2.

Treatment/Dose Level (µg/plate)	Mean	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Test strain:	**WP2 uvrA**	**-S9**					
Solvent (DMSO)	24.00	5	---	3.16	---	---	---
312.5	26.00	3	1.08	2.65	0.35	0.01	0.67
625	23.67	3	0.99	3.51	0.00	-0.00	-0.11
1250	26.00 (PPN)	3	1.08	4.00	0.18	0.00	0.65
2500	27.00 (PPN+M)	3	1.13^	7.94	0.25	0.00	0.87
5000	20.33 (PPN+M)	3	0.85	1.15	0.27	-0.00	-1.25
Pos. Con. NQO (2)	817.00	3	34.04	51.88	---	---	---
	M Statistic = 0.663						
Test strain:	**WP2 uvrA**	**+S9**					
Solvent (DMSO)	24.20 (M)	5	---	3.83	---	---	---
156.25	22.67 (M)	3	0.94	4.73	0.20	-0.01	-0.57
312.5	29.67 (M)	3	1.23^	5.13	0.43	0.02	1.84
625	25.00 (PPN+M)	3	1.03	2.65	0.18	0.00	0.31
1250	23.33 (PPN+M)	3	0.96	3.21	0.07	-0.00	-0.29
2500	26.00 (PPN+M)	3	1.07	3.00	0.06	0.00	0.65
5000	23.33 (PPN+M)	3	0.96	4.16	0.10	-0.00	-0.31
Pos. Con. AAN (10)	29.67	3	1.23	10.02	---	---	---
Positive (20)	39.7	3	1. 6	5.9	---	---	---
	M Statistic = 0.612						

Data extracted from the study report, Appendix 2, Tables 11-20. pages 41-52.

Key to significance:
* = $p \leq 0.05$
** = $p \leq 0.01$
*** $p \leq 0.005$

Key to postfixes:
^ = maximum increase above control

Positive Control:

2NF: 2-Nitrofluorene
NaN_3: Sodium azide
AAC: 9-Aminoacridine
NQO: 4-Nitroquinoline 1-oxide
AAN: 2-Aminoanthracene

S: Slight thinning of background bacterial lawn
PPN: Precipitation of Test article observed

M: Plate counted manually

Note (-S9): All strain TA98 solvent control and treatment plates counted manually for consistency of counting method throughout, as the highest three treatment doses in each case required counting manually due to the presence of precipitation.

Note (+S9): All solvent control and treatment plates counted manually for consistency of counting method throughout, as at least the highest three treatment doses in each case required counting manually due to the presence of precipitation.

III. CONCLUSIONS:

The mutagenic potential of technical ethiprole was assessed in histidine dependent auxotrophic mutants of *S. typhimurium,* strains TA1535, TA1537, TA98 and TA100, and a tryptophan dependent mutant of *E. coli,* strain WP2*uvr*A.

Two independent mutation tests were performed in the presence and absence of liver preparations from Aroclor 1254-induced rats (S9 mix). The first test was a standard plate incorporation assay using final concentrations of ethiprole at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Precipitation of test agent was observed on all plates treated at 5000 µg/plate without affecting the viability of colonies.

In the second test, the maximum test dose of 5000 µg/plate was retained, this being the best estimate of the precipitation limit and in some cases the toxicity limit. Narrowed dose ranges were employed in order to more closely investigate those concentrations of ethiprole considered most likely to induce any mutagenic response. The final concentrations of ethiprole tested were 156.25 (with S9 only), 312.5, 625, 1250, 2500 and 5000 µg/plate, plus negative (solvent) and positive controls. In addition, all treatments in the presence of S9 employed a pre-incubation step. Precipitation of test agent was again observed on all plates treated at the higher test doses, again both in the absence and in the presence of S9. This did not affect the viability of the colonies and is not considered as evidence of toxicity.

The mean solvent control counts were all within the normal historical ranges, the positive control chemicals all induced characteristic increases in revertant numbers in the appropriate strains, and less than 5% of plates were lost, leaving adequate numbers of plates at all treatments. Therefore, the study was accepted as valid.

No ethiprole treatment of any of the test strains produced an increase in revertant numbers sufficient to be considered as indicative of mutagenic activity. Ethiprole treatments of all the test strains, either in the absence or in the presence of metabolic activation (±S9), resulted in no increases in revertant numbers that were statistically significant when the data were analysed at the 1% level using Dunnett's test. Therefore, this study was considered to have provided no evidence of an ability of ethiprole to induce mutation, with treatments being performed up to precipitating, and in some cases, toxic doses.

It was concluded that ethiprole did not induce mutation in four histidine-requiring strains (TA98, TA100, TA1535 and TA1537) of *S. typhimurium*, and one tryptophan-requiring strain (WP2 uvrA) of Escherichia coli, when tested under the conditions employed for this study, which included treatments up to 5000 µg/plate (a precipitating dose), both in the absence and in the presence of a rat liver metabolic activation system (S9).

The stability of technical ethiprole and the stability and homogeneity of technical ethiprole in the solvent were not determined. In addition, analysis of achieved concentration was not performed as part of this study.

KIIA 5.4.2 - In vitro genotoxicity - Test for clastogenicity in mammalian cells

1.	Annex point(s)	KIIA 5.4.2; Genotoxicity/In vitro genotoxicity - Test for clastogenicity in mammalian cells: **MRID 47622818**
2.	Location in dossier	KIIA 5.4.2 /01
3.	Authors (year)	Marshall, R. (1998)
	Title	RPA 107382: Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes
	Company, report No.	Bayer CropScience AG, Report-No.: R016593 (M-191907-01-1)
	Date	14.10.1998
4.	Testing facility	Covance Laboratories Ltd., England;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC (Batch-No.: OP9750149)
7.	Test method	USEPA (=EPA): 84-2
	Deviations	
8.	GLP	yes

Executive summary:

In a mammalian cell cytogenetics/clastogenicity assay, human lymphocyte cultures were exposed to ethiprole (93% a.i.) in DMSO at concentrations of 19.01, 25.34, 33.79, 45.05, 60.07, 80.09, 106.8, 142.4, 189.8, 253.1, 337.5, 450.0, 600.0, and 800.0 µg/mL (first test) and 106.8, 142.4, 189.8, 253.1, 337.5, 450.0, 600.0, 800.0 µg/mL (second test), both with and without S9 metabolic activation.

Ethiprole was tested up to a limit concentration (800 µg/mL) however, incidences of mitotic inhibition was higher at lower dose levels. The absence of a clear dose-relationship for mitotic inhibition is a reflection of an accumulation of cells in mitosis at the higher concentrations. Positive controls induced the appropriate response. **There was no evidence of chromosome aberrations induced over background.**

This study is classified as **acceptable/guideline** and satisfies the requirement for Test Guideline OPPTS 870.5375 and OECD 473.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 107382 (Ethiprole)

Description: White powder
Lot/Batch #: OP9750149
Purity: 93%
CAS #: 181587-01-9

2. Control Materials:

Culture medium: Hepes-buffered RPMI medium containing 20% (v/v) fetal calf serum and 50 µg/mL gentamycin. Phytohaemagglutinin (PHA, reagent grade) was included at a concentration of approximately 10 µg/mL of culture to stimulate the lymphocytes to divide.

Negative/Solvent control: DMSO in culture

Positive control:
Nonactivation: 4-nitroquinoline 1-oxide (NQO) in DMSO at 1.25, 2.50 and 5.00 µg/mL (final concentration in the medium).
Activation: Cyclophosphamide (CPA) in DMSO at 12.5 or 25.0 µg/mL (final concentration in the medium).

3. Activation: S9 derived from

X	induced	X	Aroclor 1254	X	Rat	X	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Describe S9 mix composition: The mammalian liver post-mitochondrial fraction (S9) used for metabolic activation was prepared from male Sprague Dawley rats induced with Aroclor 1254 and obtained from Molecular Toxicology Incorporated, USA. The batches of MolTox™ S9 were stored frozen in aliquots at -80°C and thawed just prior to use. Each batch was checked by the manufacturer for sterility, protein content (minimum 32 mg/mL), ability to convert known promutagens to bacterial mutagens and cytochrome P-450-catalyzed enzyme activities (alkoxyresorufin-O-dealkylase activities). Glucose-6-phosphate (180 mg/mL), NADP (25 mg/mL), 150 mM KC1 and rat liver S9 were mixed in the ratio 1:1:1:2. An aliquot of the resulting S9 mix was added to each cell culture designated for treatment in the presence of S9 to achieve the required final concentration in a total of 10 mL. The final concentration of liver homogenate in the test system was 2%. Cultures treated in the absence of S9 received an equal volume of 150 mM KC1.

4. **Test Cells:** Two healthy, non-smoking volunteers (male in Experiment 1 and female in Experiment 2) were used in this study. Neither donor was suspected of any virus infection nor had been exposed to high levels of radiation or hazardous chemicals. For each experiment an appropriate volume of whole blood was drawn from the peripheral circulation either one day prior to culture (Experiment 1) or on the day of culture (Experiment 2). Blood was stored refrigerated until use. Whole blood cultures were established in sterile disposable centrifuge tubes by placing 0.4 mL heparinized blood into 9.0 mL Hepes-buffered RPMI medium containing 20% (v/v) fetal calf serum and 50 µg/mL gentamycin. Phytohaemagglutinin (PHA, reagent grade) was included at a concentration of ~10 µg/mL of culture to stimulate the lymphocytes to divide. Blood cultures were incubated for approximately 48 hours at 37°C and rocked continuously.

	V79 cells (Chinese hamster lung fibroblasts)
X	Human lymphocytes
	Chinese hamster ovary (CHO) cells

Media: Hepes-buffered RPMI tissue culture medium supplemented with 20% fetal calf serum and 50 µg/mL gentamycin. The blood was heparinized.

Properly maintained?	X	Yes		No
Periodically checked for *Mycoplasma* contamination?		Yes		No
Periodically checked for karyotype stability?		Yes		No

5. **Test Compound Concentrations Used:**

First Test:

Nonactivated conditions: 20 hour treatment + 0 hour recovery
19.01, 25.34, 33.79, 45.05, 60.07, 80.09, 106.8, 142.4, 189.8, 253.1*, 337.5*, 450.0*, 600.0*, 800.0*

Activated conditions: 3 hour treatment + 17 hour recovery
19.01, 25.34, 33.79, 45.05, 60.07, 80.09, 106.8, 142.4, 189.8, 253.1*, 337.5*, 450.0*, 600.0*, 800.0*

Second Test:

Nonactivated conditions:
20 hour treatment + 0 hour recovery
253.1, 337.5*, 450.0*, 600.0*, 800.0*
44 hour treatment + 0 hour recovery
106.8, 142.4, 189.8, 253.1, 337.5*, 450.0*, 600.0*, 800.0*

Activated conditions:
3 hour treatment + 17 hour recovery
253.1*, 337.5*, 450.0*, 600.0*, 800.0*
3 hour treatment + 41 hour recovery
253.1*, 337.5*, 450.0*, 600.0*, 800.0*

*Precipitation observed

Solubility data indicated that the test article was soluble (with warming to 37 °C) in dimethyl sulphoxide (DMSO) and that limited precipitation was observed at a concentration of approximately 518 µg/mL following a 100-fold dilution into culture medium. A maximum concentration of 800 µg/mL was chosen as an appropriate top dose for the assay as being close to, but in excess of, the solubility limit in culture medium. Osmolality measurements on post-treatment media were not performed because the highest concentration tested was less than 10 mM (3972 µg/mL, data provided by Sponsor).

B. TEST PERFORMANCE

1. **Preliminary Cytotoxicity Assay** A preliminary cytotoxicity assay was not performed. However, in order to assess the toxicity of Technical ethiprole to cultured human lymphocytes, the mitotic index was calculated for all treated cultures as described below.

2. **Cytogenetic Assay:** S9 mix or KC1 (0.5 mL) were added appropriately, as described above. One set of quadruplicate cultures (A, B, C and D) for each of the treatment regimes was then treated with the solvent and one set of duplicate cultures with the test article (0.1 mL per culture). Additional duplicate cultures for 20+0 hour treatments in the absence of S9 and 3+17 hour treatments in its presence were treated with 0.1 mL of the positive control chemicals as needed. This scheme is illustrated below:

Treatment	S9	Number of cultures (Hours treatment + Hours recovery)			
		20+0	3+ 17	44+0	3+41
Experiment 1					
Negative control	-	4	---	---	---
	+	---	4	---	---
Test article (all doses)	-	2		---	---
	+	---	2	---	---
Positive controls (all doses)	-	2	---	---	---
	+	---	2	---	---
Experiment 2					
Negative control	-	4	---	4	---
	+	---	4	---	4
Test article (all doses)	-	2	---	2	---
	+	---	2	---	2
Positive controls (all doses)	-	2	---	---	---
	+	---	2	---	---

Treatment media remained on cultures receiving continuous treatments in the absence of S9 until sampling, that is, 20 hours and 44 hours after the beginning of treatment. Cultures were treated in the presence of S9 for 3 hours only. They were then pelleted (300 x 'g', 10 minutes), washed twice with sterile saline, and resuspended in fresh medium containing fetal calf serum and gentamycin. Cultures were incubated for a further 17 or 41 hours before harvesting. The delayed sample was adopted for test and negative control cultures only, not for positive controls.

Approximately 1½ hours prior to harvest, colchicine was added to give a final concentration of approximately 1 µg/mL to arrest dividing cells in metaphase. Cultures were then centrifuged at 300 x 'g' for 10 minutes, the supernatant was carefully removed and cells were resuspended in 4 mL hypotonic (0.075 M) KCI and incubated at 37 °C for 15 minutes to allow cell swelling to occur. Cells were then fixed by dropping the KCl suspension into an equal volume of fresh, ice-cold methanol/glacial acetic acid (3:1, v/v). The fixative was changed by centrifugation (300 x 'g', 10 minutes) and resuspension. This procedure was repeated several times (centrifuging at 1250 x 'g', 2-3 minutes) until the cell pellets were clean.

a. Cell exposure time

Treatment Description	(Hours exposure + Hours recovery)	S9	Duration of treatment (hours)
Continuous:	20+0	-	20*
	44+0	-	44
Pulse:	3+17	+	3*
	3+41	+	3

* Included positive control treatments

b. Spindle inhibition

Inhibition used/concentration: Colchicine, at a final concentration of 1 µg/mL
Administration time: 1.5 hours (before cell harvest)

c. Cell harvest time after termination of treatment:

Treatment Description	(Hours exposure + Hours recovery)	S9	Harvest time (hours after start of treatment)
Continuous:	20+0	-	20*
	44+0	-	44
Pulse:	3+17	+	20*
	3+41	+	44

* Included positive control treatments

d. Details of slide preparation: Lymphocytes were kept in fixative in the refrigerator before slides were prepared but slides were not made on the day of harvest to ensure cells were adequately fixed. Cells were pelleted and resuspended in a minimal amount of fresh fixative (if required) so as to give a milky suspension. Several drops of 45 % (v/v) aqueous acetic acid were added to each suspension to enhance chromosome spreading, and several drops of suspension were transferred to clean microscope slides. Slides were flamed, as required, to improve metaphase spreading. After the slides had dried the cells were stained for 5 minutes in 4% (v/v) filtered Giemsa stain in pH 6.8 buffer. The slides were rinsed, dried and mounted with coverslips.

e. Metaphase analysis: Slides were examined, uncoded, for mitotic index (MI) or percentage of cells in mitosis. Slides from enough dose levels from each treatment regime were scored to determine if chemically induced mitotic inhibition had occurred. This is defined as a clear decrease in mitotic index compared with negative controls (based on at least 1000 cells counted), preferably dose-related.

Rationale for dose selection. The top dose for chromosome analysis from 20+0 hour, -S9 and 3+17 hour, +S9 treatments, in Experiments 1 and 2, was to be the one at which a 50-80% reduction in MI occurred or was to be the highest dose treated. Slides from this dose and the next two lower doses were to be taken for microscopic analysis. For 20 hour treatments in the absence of S9, slides from an additional concentration were also analysed for reasons discussed in the results section ("toxicity data").

If a negative or equivocal result was obtained in Experiment 1, a single dose from the delayed harvest (44+0, -S9 and 3+41, +S9) was to be scored in Experiment 2. The dose level selected was to correspond to the top dose scored following the 20+0 hour treatment -S9 or the 3+17 hour treatment +S9, unless mitotic inhibition was too severe in which case the highest scorable

dose was to be selected. For each treatment regime, two solvent control cultures were initially to be analysed for chromosome aberrations. Slides from the remaining solvent control cultures were only to be analysed if considered necessary, for example, to help resolve an equivocal result. A single positive control dose level, which gave satisfactory responses in terms of quality and quantity of mitoses and extent of chromosomal damage, was to be analysed.

Scoring of aberrations Slides from the selected treatments and from solvent and positive controls were coded using randomly generated letters by a person not connected with the scoring of the slides. Labels bearing only the study reference number, the sex of the donor, replicate and the code were used to cover treatment details on the slides. Where possible, 100 metaphases from each code were analysed for chromosome aberrations. Only cells with 44-46 chromosomes were considered acceptable for analysis of structural aberrations. Any cell with more than 46 chromosomes that is polyploid, endoreduplicated and hyperdiploid cells, observed during this search was noted and recorded separately. Classification of structural aberrations was based on the scheme described by ISCN and were detailed in an appendix in the report.

No. of cells examined/dose: 100 cells in metaphase

Scored for structural	X	Yes		No
Scored for numerical	X	Yes, polyploid, endoreduplicated cells		No
Coded prior to analysis	X	Yes		No

f. **Evaluation criteria:** The human lymphocyte assay was to be considered valid if the following criteria were met: (1) the binomial dispersion test demonstrated acceptable heterogeneity between replicate cultures, and (2) the proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the normal range, and (3) at least 160 cells out of an intended 200 were analyzable at each dose level, and (4) the positive control chemicals induced statistically significant increases in the number of cells with structural aberrations.

The test article was to be considered as positive in this assay if: (1) a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurred at one or more concentrations, and (2) the proportion of cells with structural aberrations at such doses exceeded the normal range, and (3) the results were confirmed in the second experiment.

A positive result only at the delayed harvest in Experiment 2 was to be taken as evidence of clastogenicity provided criteria 1 and 2 were met. Increases in numbers of cells with gaps or increases in the proportions of cells with structural aberrations, not exceeding the normal range or occurring only at very high or very toxic concentrations, were likely to be concluded as "equivocal".

Full assessment of the biological importance of such increases is likely only to be possible with reference to data from other test systems. Cells with exchange aberrations or cells with greater than one aberration were to be considered of particular biological significance.

g. **Statistical analysis:** After completion of microscopic analysis, data were decoded. The aberrant cells in each culture were categorized as follows: 1) cells with structural aberrations including gaps 2) cells with structural aberrations excluding gaps 3) polyploid, endoreduplicated or hyperdiploid cells. The totals for category 2 in negative control cultures were used to determine whether the assay was acceptable or not. The proportions of aberrant

cells in each replicate were used to establish acceptable heterogeneity between replicates by means of a binomial dispersion test. The proportion of cells in category 2 for each test treatment condition was compared with the proportion in concurrent negative controls using Fisher's exact test. Probability values of $p \leq 0.05$ were accepted as significant. The proportions of cells in categories 1, 2 and 3 were examined in relation to historical negative control (normal) ranges.

II. RESULTS

Samples of dose formulations were not retained for analysis of achieved concentration.

A. TOXICITY DATA

In order to assess the toxicity of technical ethiprole to cultured human lymphocytes, the mitotic index was calculated for all cultures treated with the test substance and the solvent control. Mitotic indices of cultured human lymphocytes treated with technical ethiprole are summarized in Table 1 for the first and Table 2 for the second test.

First Test:

The results of mitotic index determinations for the treatments in Experiment 1 (±S9) sampled at 20 hours are shown in Table 1. Treatment in the absence of S9 resulted in a decrease in mitotic index at concentrations up to 337.5 µg/mL at which 73% mitotic inhibition was seen. At concentrations higher than this, the mitotic index increased such that at 800 µg/mL only 42% mitotic inhibition (reduction in mitotic index) was apparent. The absence of a clear dose-relationship for mitotic inhibition is a reflection of an accumulation of cells in mitosis at the higher concentrations. In order to include an examination of the effects of the test article at concentrations where greater than 50% mitotic inhibition was seen, cells were analysed for chromosome aberrations at 253.1, 450, 600 and 800 µg/mL. Approximately 61% mitotic inhibition was observed at 253.1 µg/mL. Chromosome aberrations were analysed at three consecutive dose levels following treatment in the presence of S9 (450, 600 and 800 µg/mL). The highest concentration chosen for analysis, 800 µg/mL, induced approximately 31% mitotic inhibition. The dose levels 253.1, 450, 600, 800 µg/mL (20+0 hours, without S9) and 450, 600, 800 µg/mL (3+17 hours, with S9) were selected for chromosome analysis according to the "Rationale for dose selection" criteria discussed above.

TABLE 1. Mitotic Index Data, First Test

Treatment µg/mL)	Mitotic Index		Mitotic Inhibition	Mitotic Index		Mitotic inhibition
	Without S9 Mix (20+0)			With S9 Mix (3+17)		
	A/C	B/D		A/C	B/D	
Solvent	2.8/3.2	3.0/3.4	---	5.6/4.2	4.9/3.9	
19.01	3.6	3.4	0%	NS	NS	-
25.34	2.6	3.2	6%	NM	NM	-
33.79	3.1	3.0	2%	NM	NM	-
45.05	3.0	2.7	8%	NM	NM	-
60.07	2.4	3.6	3%	NM	NM	-
80.09	2.3	2.1	29%	NM	NM	-
106.8	3.0	1.8	23%	NM	NM	-
142.4	1.9	2.7	26%	NM	NM	-
189.8	2.1	1.3	45%	NM	NM	-
253.1	1.1	1.3	61%	NS	NS	-
337.5	0.9	0.8	73%	NS	NS	-
450	1.8	2.4	32%	2.6	3.2	38%
600	1.8	1.9	40%	3.5	3.8	22%
800	1.9	1.7	42%	2.7	3.7	31%

NS = not scored; NM = slides not made

Second Test:

The results of mitotic index determinations for the treatments in Experiment 2 (±S9) sampled at 20 and 44 hours are shown in Table 2. A pattern of mitotic inhibition, similar to that seen in Experiment 1 was seen. Slides from cells receiving 253.1 µg/mL where approximately 66% mitotic inhibition was observed,were included. The dose levels 253.1, 450, 600, 800 µg/mL (20+0 hours, without S9); 450, 600, 800 µg/mL (3+17 hours, with S9); 800 µg/mL (44+0 hours, without S9); and 800 µg/mL (3+41 hours, with S9) were selected for chromosome analysis according to the "Rationale for dose selection" criteria discussed above.

TABLE 2. Mitotic Index Data, Second Test

Treatment µg/mL)	Mitotic Index		Mitotic Inhibition	Mitotic Index		Mitotic inhibition
	Without S9 Mix (20+0)			Without S9 Mix (44+0)		
	A/C	B/D		A/C	B/D	
Solvent	3.0/3.3	2.2/3.4		1.9/2.2	2.1/2.2	
106.8	NT	NT	-	0.9	0.9	57%
142.4	NT	NT	-	1.2	1.1	45%
189.8	NT	NT	-	1.1	0.4	64%
253.1	0.9	1.1	66%	0.6	0.4	76%
337.5	1.4	1.5	51%	1.7	1.0	36%
450	1.3	2.7	33%	2.1	2.1	0%
600	2.2	1.9	31%	2.6	2.3	0%
800	2.1	2.9	16%	2.6	1.8	0%
	With S9 Mix (3+17)			With S9 Mix (3+41)		
	A/C	B/D		A/C	B/D	
Solvent	2.7/2.8	4.6/2.1	-	2.7/2.8	2.0/2.0	-
253.1	4.0	3.1	0%	NM	NM	-
337.5	2.4	2.1	26%	NM	NM	-
450	2.2	1.6	38%	NM	NM	-
600	2.8	2.4	15%	2.5	1.5	16%
800	3. 9	2. 5	0%	3.4	1.7	0%

NS = not scored; NM = slides not made

B. CYTOGENETIC ASSAY:

The effects of technical ethiprole on the chromosomes of cultured human lymphocytes are summarized in Table 3 for the first and second tests. Treatment of cultures with ethiprole (±S9) resulted in frequencies of cells with aberrations which were similar to those in concurrent negative controls. A small but statistically significant ($p<0.05$) increase in cells with aberrations at the highest concentration following treatment in the absence of S9 for 20 hours in Experiment 2 was considered to be of no biological significance because (i) no statistically significant increase in numbers of cells with aberrations were observed under the same conditions in Experiment 1 (ii) no dose-response relationship was observed and (iii) the numbers of aberrant cells observed fell within the historical negative control range. An appendix was included in the report which described the numbers and types of structural aberrations seen per cell. Normal frequencies of cells with numerical aberrations were seen under all treatment/sampling conditions (detailed supporting raw data were given in the report appendices).

The tests were considered valid, as 1) no evidence of significant heterogeneity between replicate cultures was obtained in the binomial dispersion test, and 2) the proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the normal range, and 3) at least 160 cells out of an intended 200 were analysed at each dose level, and 4) the positive control chemicals NQO and CPA induced statistically significant increases in the number of cells with structural aberrations.

TABLE 3. Summary of Results, Cells with Structural Aberrations

Treatment (ng/mL)	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Statistical Significance	Mitotic index (mean)
20 hour treatment without S9, 0 hour recovery (20+0), Experiment 1, Donor sex: male					
Solvent	200	1	0	---	2.9
253.1	200	3	2	NS	1.2
450	200	4	2	NS	2.1
600	200	3	3	NS	1.9
800	200	6	3	NS	1.8
NQO, 2.5	200	17	14	$p \leq 0.001$	---
3 hour treatment with S9, 17 hour recovery (3+ 17), Experiment 1, Donor sex: male					
Solvent	200	1	0	---	5.3
450	200	3	1	NS	2.9
600	200	2	1	NS	3.7
800	200	2	1	NS	3.2
CPA, 12.5	200	52	48	$p \leq 0.001$	---
20 hour treatment without S9, 0 hour recovery (20+0), Experiment 2, Donor sex: female					
Solvent	200	1	0	---	2.6
253.1	182	2	1	NS	1.0
450	200	3	0	NS	2.0
600	200	4	1	NS	2.1
800	200	7	5	$p \leq 0.05$	2.5
NQO, 2.5	200	25	19	$p \leq 0.001$	---
3 hour treatment with S9, 17 hour recovery (3+ 17), Experiment 2 Donor sex: female					
Solvent	200	2	0	---	3.7
450	200	3	1	NS	1.9
600	200	3	1	NS	2.6
800	200	2	1	NS	3.2
CPA, 12.5	200	37	26	$p \leq 0.001$	---
44 hour treatment without S9, 0 hour recovery (44+0), Experiment 2 Donor sex: female					
Solvent	200	1	1	---	2.0
800	199	4	3	NS	2.2
3 hour treatment with S9, 41 hour recovery (3+41), Experiment 2 Donor sex: female					
Solvent	200	3	2	---	2.4
800	200	3	3	NS	2.6

Data are from two replicates (A and B), NS = Not significant (Fisher's exact test)

III. CONCLUSIONS:

In Experiment 1, treatment in the absence of S9 was continuous for 20 hours (20+0). Treatment in the presence of S9 was for 3 hours only followed by a 17 hour recovery period prior to harvest (3+17). The test article dose levels for chromosome analysis were selected by evaluating the effect of ethiprole on mitotic index. Treatment in the absence of S9 resulted in a decrease in mitotic index at concentrations up to 337.5 µg/mL at which 73% mitotic inhibition was seen. At concentrations higher than this, the mitotic index increased such that at 800 µg/mL only 42% reduction in mitotic index (mitotic inhibition) was apparent. The absence of a clear dose-relationship for mitotic inhibition is a reflection of an accumulation of cells in mitosis at the

higher concentrations. In order to include an examination of the effects of the test article at concentrations where greater than 50% mitotic inhibition was seen, cells were analysed for chromosome aberrations at 253.1, 450, 600 and 800µg/mL. Approximately 61% mitotic inhibition was observed at 253.1 µg/mL.

Chromosome aberrations were analysed at three consecutive dose levels following treatment in the presence of S9 (450, 600 and 800 µg/mL). The highest concentration chosen for analysis, 800 /ug/mL, induced approximately 31% mitotic inhibition.

Experiment 2 included a delayed sampling time following treatment in the absence and presence of S9 (44+0, 3+41 respectively). Chromosome aberrations were analyzed in cells receiving 20+0 hour treatments in the absence of S9 at 253.1, 450, 600 and 800 µg/mL. Approximately 66% and 16% mitotic inhibition was observed at 253.1 and 800 µg/mL, respectively. Chromosome aberrations were again analyzed at three consecutive dose levels following treatment in the presence of S9. The highest concentration chosen for analysis, 800 µg/mL, induced no mitotic inhibition. The effects of a single concentration only, 800 µg/mL (±S9) were investigated at the delayed sampling time.

Appropriate negative (solvent) control cultures were included in the test system in both experiments under each treatment condition. The proportion of cells with structural aberrations in these cultures fell within historical solvent control ranges. NQO and CPA were employed as positive control chemicals in the absence and presence of liver S9 respectively. Cells receiving these were sampled in each experiment, 20 hours after the start of treatment; both compounds induced statistically significant increases in the proportion of cells with structural aberrations.

Treatment of cultures with ethiprole in the absence and presence of S9 resulted in frequencies of cells with aberrations which were similar to those in concurrent negative controls. A small but statistically significant ($p \leq 0.05$) increase in cells with aberrations at the highest concentration following treatment in the absence of S9 for 20 hours in Experiment 2 was considered to be of no biological significance because (i) no statistically significant increase in numbers of cells with aberrations were observed under the same conditions in Experiment 1 (ii) no dose-response relationship was observed and (iii) the numbers of aberrant cells observed fell within the historical negative control range.

It was concluded that ethiprole did not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested to its limit of solubility in both the absence and presence of S9.

The study author did not indicate if mycoplasm and karyotype checks were conducted for the test cells. In response to a clarification sent to address this issue, the study authors explained that mycoplasma checks and karyotyping are only rountinely conducted on immortalised cell line stocks, as these may have undergone multiple passages or long term storage. The human peripheral blood lymphocytes are primary cell cultures, and therefore, such checks are not strictly appropriate. Standard human karyotype is relevant, but no deviations were noted during metaphase scoring. Samples of dose formulations were not retained for analysis of achieved concentration; however, this is considered to be a minor omission and does not impact on the review of this study.

KIIA 5.4.3 - In vitro genotoxicity - Test for gene mutation in mammalian cells

1.	Annex point(s)	KIIA 5.4.3; Genotoxicity/In vitro genotoxicity - Test for gene mutation in mammalian cells: **MRID 47622817**
2.	Location in dossier	KIIA 5.4.3 /01
3.	Authors (year)	Fellows, M. (1999)
	Title	RPA 107382: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the microtitre fluctuation technique
	Company, report No.	Bayer CropScience AG, Report-No.: R000204 (M-158105-01-1)
	Date	15.04.1999
4.	Testing facility	Covance Laboratories, Harrogate, North Yorks, GBR;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC
7.	Test method	USEPA (=EPA): Guideline 84-2
	Deviations	
8.	GLP	yes

Executive summary:
In a mammalian cell gene mutation assay the thymidine kinase locus in mouse lymphoma cells cultured *in vitro* were exposed to ethiprole (93% a.i.) in DMSO, at concentrations of 15.625, 31.25, 62.5, 125, 250, 375 and 500 µg/mL in the presence and absence of mammalian S9 metabolic activation.

Ethiprole was tested up to cytotoxic concentrations of 500 µg/mL, where precipitate and toxicity were observed. The positive controls induced the appropriate response. **There was no evidence of induced mutant colonies over background or evidence of a concentrated-related positive response.**

This study is classified as **acceptable/guideline** and satisfies the requirement for Test Guideline OPPTS 870.5300 and OECD 476.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 107382 (Ethiprole)
Description: White powder
Lot/Batch #: OP9750149
Purity: 93%
CAS # of TGAI: 181587-01-9

2. Control Materials:

Culture medium: Media: RPMI 1640 medium supplemented with the following:
For "RPMI 10" gentamycin (100 µg/mL), amphotericin B (2.5 µg/mL), pluronic (0.5 mg/mL) and heat-inactivated horse serum (10% v/v)
For "RPMI 20" gentamycin (100 µg/mL), amphotericin B (2.5 µg/mL) and heat-inactivated horse serum (20% v/v)
For "RPMI A" gentamycin (100 µg/mL), amphotericin B (2.5 µg/mL) and pluronic (0.5 mg/mL)

Negative/Solvent control: 1% DMSO (v/v) in culture medium (200 µL in 20 mL of medium)

Positive control:
Nonactivation: 4-nitroquinoline 1-oxide (NQO) in DMSO at 0.05 and 0.10 µg/ (final concentration in the medium).
Activation: Benzo(a)pyrene (BP) in DMSO at 2 and 3 µg/mL (final concentration in the medium).

3. Activation: S9 derived from

X	induced	X	Aroclor 1254	X	Rat	X	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Describe S9 mix composition: The S9 was obtained from Molecular Toxicology Incorporated, USA. The batches of MolTox™ S9 were stored frozen at - 80°C prior to use. Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens and cytochrome P-450-catalysed enzyme activities (alkoxyresorufin-O-dealkylase activities). Glucose-6-phosphate (180 mg/mL), NADP (25 mg/mL), 150 mM KC1 and rat liver S9 were mixed in the ratio 1:1:1:2. For all cultures treated in the presence of S9, a 1 mL aliquot of the mix was added to each cell culture (19 mL) to give a total of 20 mL. Cultures treated in the absence of S9 received 1 mL 150 rnM KC1.

4. Test Cells: Mammalian cells in culture.

X mouse lymphoma L5178Y cells
V79 cells (Chinese hamster lung fibroblasts)
Chinese hamster ovary (CHO) cells
list any others

Media: RPMI 1640 medium supplemented with the following:
For "RPMI 10" gentamycin (100 µg/mL), amphotericin B (2.5 µg/mL), pluronic (0.5 mg/mL) and heat-inactivated horse serum (10% v/v)
For "RPMI 20" gentamycin (100 µg/mL), amphotericin B (2.5 µg/mL) and heat-inactivated horse serum (20% v/v)
For "RPMI A" gentamycin (100 µg/mL), amphotericin B (2.5 µg/mL) and pluronic (0.5 mg/mL)

Properly maintained?	X	Yes	No
Periodically checked for *Mycoplasma* contamination?	X	Yes	No
Periodically checked for karyotype stability?	X	Yes	No
Periodically "cleansed" against high spontaneous background?	X	Yes	No

5. Locus Examined:

	X	Thymidine kinase (TK)	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)	Na^+/K^+ ATPase
Selection agent:		bromodeoxyuridine (BrdU)	8-azaguanine (8-AG)	ouabain
		fluorodeoxyuridine (FdU)	6-thioguanine (6-TG) concentration, 6 µg/mL	
	X	trifluorothymidine (TFT)		

6. Test Compound Concentrations Used:

Preliminary Cytotoxicity Assay:

Nonactivated conditions:	31.25, 62.5, 125, 250, 500, 1000 µg/mL (3 hr treatment period)
Activated conditions:	31.25, 62.5, 125, 250, 500, 1000 µg/mL (3 hr treatment period)

Mutagenicity Assay (First Test):

Nonactivated conditions:	15.625, 31.25, 62.5, 125, 250, 500 µg/mL (3 hr treatment period)
Activated conditions:	15.625, 31.25, 62.5, 125, 250, 500 µg/mL (3 hr treatment period)

Mutagenicity Assay (Second Test):

Nonactivated conditions:	15.625, 31.25, 62.5, 125, 250, 500 µg/mL (3 hr treatment period)
Activated conditions:	15.625, 31.25, 62.5, 125, 250, 375, 500 µg/mL (3 hr treatment period)

Preliminary solubility data indicated that ethiprole was soluble in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO) up to at least 450µg/mL and in tissue culture medium (RPMI 1640) in the range of 250 to 500 µg/mL. A top dose of 1000 (µg/mL was selected for the cytotoxicity range-finder to ensure the limit of solubility was achieved. Osmolality measurements on post-treatment medium were not required because the top dose of ethiprole tested (1000 µg/mL) was less than 10 mM (molecular weight of 397.21 provided by Sponsor).

B. TEST PERFORMANCE

1. **Preliminary Cytotoxicity Assay:** Treatment of cell cultures for the cytotoxicity range-finding experiment was as described below for the main mutation experiments; however, single cultures only were used and positive controls were not included. Following treatment, cells were washed with tissue culture medium and then resuspended in 20 mL RPMI 10. The cell concentrations were adjusted to 8 cells/mL and, for each dose, 0.2 mL was plated into each well of a 96- well microtitre plate. The plates were incubated at 37 °C in a humidified incubator gassed with 5% v/v CO_2 in air for 4 days. Wells containing viable clones were identified by microscope and counted.

2. **Cytogenetic Assay:** At least 10^7 cells in a volume of 18.8 mL of RPMI 5 (cells in RPMI 10 diluted with RPMI A [no serum] to give a final concentration of 5 % serum) were placed in each of a series of sterile disposable 50 mL centrifuge tubes. Solvent, test article or positive control solution (0.2 mL) was added, and S9 mix or 150 mM KC1 added, as required, such that each treatment, in the absence or presence of S9, was in duplicate (single cultures only used for positive control treatments). After 3 hours rocking at 37°C, the tubes were centrifuged at 200 x 'g' for 5 minutes, the cells washed with tissue culture medium and resuspended further in 20 mL RPMI 10/tube. Cell densities were determined using a Coulter counter (or haemocytometer) due to the presence of precipitate at the top three doses tested in the cytotoxicity range-finding experiment [±S9], the top two doses tested [-S9] and the top dose tested [+S9] in Experiment 1, and the top two doses tested in Experiment 2 [±S9]) and, where possible, the concentrations adjusted to 2 x 10^5/mL. Cells were transferred to flasks for growth through the expression period.

For expression, cultures were maintained in flasks for a period of 2 days during which the TK mutation would be expressed. Sub-culturing was performed as required with the aim of not exceeding 1 x 10^6 cells per mL and, where possible, retaining at least 1 x 10^7 cells/flask. From observations on recovery and growth of the cultures during the expression period, all the cultures were selected to be plated for viability and 5-trifluorothymidine (TFT) resistance (mutation assessment):

Plating for viability. At the end of the expression period, cell concentrations in the selected cultures were determined using a Coulter counter and adjusted to give 1 x 10^4/mL in readiness for plating for TFT resistance. Samples from these were diluted to 8 cells/mL as follows: Using an 8-channel pipette, 0.2 mL of final concentration of each culture was placed into each well of two 96-well microtitre plates (192 wells at an average of 1.6 cells per well). The plates were incubated at 37°C in a humidified incubator gassed with 5% v/v CO2 in air until scoreable (8 to 10 days). Wells containing viable clones were identified by eye using background illumination and counted.

Plating for TFT resistance. At the end of the expression period, the cell densities in the selected cultures were adjusted to 1 x 10^4/mL. TFT (300 µg/mL) was diluted 100-fold into these suspensions to give a final concentration of 3 µg/mL. Using an eight-channel pipette, 0.2 mL of each suspension was placed into each well of four 96-well microtitre plates (384 wells at 2 x 10^3 cells per well). Plates were incubated at 37°C in a humidified incubator gassed with 5% v/v CO2 in air until scoreable (12 days) and wells containing clones were identified as above and counted. In addition, the number of wells containing large colonies and the number containing small colonies were scored for the negative and positive controls.

a. Cell treatment:

- Cells were exposed to test compound, negative/solvent or positive controls for 3 hours (both activated and non-activated conditions).
- After washing, cells were cultured for 2 days (expression period) before cell selection.
- After expression, 2000 cells/microtitre well (384 wells/replicate/group) were cultured for 12 days in selection medium to determine numbers of mutants and 1.6 cells/ microtitre well (192 wells/replicate/group) were cultured for 8 to 10 days without selective agent to determine cloning efficiency.

b. Statistical Methods:

Statistical significance of mutant frequencies (total wells with clones) was carried out according to the UKEMS guidelines. Thus the control log mutant frequency (LMF) was compared with the LMF from each treatment concentration based on Dunnett's test for multiple comparisons, and secondly the data was checked for a linear trend in mutant frequency with treatment concentration using weighted regression. The test for linear trend is one-tailed, therefore negative trend was not considered significant. These tests required the calculation of the heterogeneity factor to obtain a modified estimate of variance.

c. Evaluation Criteria:

The assay was considered valid if all the following criteria were met: (1) the mutant frequencies in the negative (solvent) control cultures fell within the normal range (above 60 mutants per 10^6 viable cells but not more than three times the historical mean value); (2) at least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value); and (3) the plating efficiencies of the negative controls from the mutation experiments were between the range of 60% to 140% on Day 0 and 70% to 130% on Day 2.

The test article was considered to be mutagenic if all the following criteria were met: (1) the assay was valid (see acceptance criteria, described above); (2) the mutant frequency at one or more doses was significantly greater than that of the negative control ($p<0.05$); (3) there was a significant dose-relationship as indicated by the linear trend analysis ($p<0.05$); and (4) the effects described above were reproducible.

Determination of survival or viability

From the zero term of the Poisson distribution the probable number of clones/well (P) on microtitre plates in which there are EW empty wells (without clones) out of a total of TW wells is given by:

$$P = -\ln (EW/TW)$$

The plating efficiency (PE) in any given culture is therefore:

$$PE = P/N° \text{ of cells plated per well}$$

and as an average of 1.6 cells per well are plated on all survival and viability plates,

$$PE = P/1.6$$

The percentage relative survival (%RS) in each test culture was therefore determined by comparing plating efficiencies in test and control cultures thus:

$$\% RS = [PE (test)/PE (control)] \times 100$$

To take into account any loss of cells during the 3 hour treatment period, percentage relative survival values for each concentration of test article were adjusted as follows:

$$\text{Adjusted } \%RS = \%RS \times \frac{\text{Post-treatment cell concentration for dose}}{\text{Post-treatment cell concentration for solvent control}}$$

All percentage relative survival (%RS) values were adjusted as described above.

Relative Total Growth (RTG) was also calculated and this is the product of the cumulative relative suspension growth (compared with the control), RSG, and the relative plating efficiency for each culture:

$$RTG = RSG \times \text{Day 2 relative } PE/100$$

Where,

Suspension growth (SG) = [Day 1 cell count/2 x 10^5*] x [Day 2 cell count/2 x 10^3*]
* Or appropriate cell concentration if lower

Relative Suspension Growth (RSG) = SG (test) / SG (control)

Day 2 relative PE = Day 2 PE(test) / Day 2 PE (control)

Determination of mutant frequency

It is usual to express mutant frequency (MF) as "mutants per 10^6 viable cells". In order to calculate this, the plating efficiencies of both mutant and viable cells in the same culture were calculated,

$$MF = [PE (mutant)/PE (viable)] \times 10^6$$

From the formulae given above and with the knowledge that 2 x 10^3 cells were plated/well for mutation to 5-trifluorothymidine resistance,

$$PE\ (mutant) = P\ (mutant)/2 \times 10^3$$

$$PE\ (viable) = P\ (viable)/1.6$$

where, in each case, P = -ln (EW/TW)

Therefore,

$$MF = [P\ (mutant)/2 \times 10^3] \times [1.6/P\ (viable)] \times 10^6$$

$$= \{-\ln [EW/TW\ (mutant)]/-\ln [EW/TW\ (viable)]\} \times 800$$

Small and large colony mutant frequencies were calculated in an identical manner, using the relevant number of empty wells for small and large colonies as appropriate.

II. RESULTS

Samples of dose formulations were not retained for analysis of achieved concentration.

A. Preliminary Cytotoxicity Assay:

In the cytotoxicity range-finding experiment, six doses of ethiprole were tested, separated by two fold intervals and ranging from 31.25 to 1000 µg/mL. Upon addition of the test article to the cultures, precipitate was observed at the top two doses tested 500 and 1000 µg/mL, ±S9). Furthermore after the 3 hour treatment incubation period, precipitate was observed at these doses and also at 250 µg/mL (±S9). Marked toxicity was observed at the top three doses tested in the absence of S9 (250 to 1000 µg/mL), and the top two doses tested in the presence of S9 (500 and 1000 µg/mL). The top dose tested (1000 µg/mL) yielded 14.88% relative survival in the absence of S9 and 30.44% relative survival in the presence of S9. It may be noted that the precipitate observed at the top dose tested was believed to have affected post treatment cell counting. Hence a top dose of 500 µg/mL was considered to be appropriate for Experiments 1 and 2. The range-finder raw plate counts and relative survival values are shown in Table 1.

Table 1. Raw plate counts and adjusted relative survival in the cytotoxicity range-finder

Treatment (µg/mL)	Without S9 Mix					With S9 Mix				
	Day 0 Cells x 10^5	Survival § (Day 0)	%CE (Day 0)	%S	%RS	Day 0 Cells x 10^5	Survival § (Day 0)	%CE (Day 0)	%S	%RS
0	5.22	69	79.28	79.28	100.00	4.79	72	86.64	86.64	100.00
31.25	5.21	67	74.82	74.67	94.19	4.97	67	74.82	77.63	89.59
62.5	4.57	60	61.30	53.67	67.69	4.91	71	84.09	86.20	99.49
125	3.72	62	64.87	46.23	58.31	4.56	69	79.28	75.48	87.11
250	0.98	68	77.01	14.46	18.24	2.75	73	89.30	51.27	59.17
500	1.05	69	79.28	15.95	20.11	2.08	49	44.64	19.38	22.37
1000	1.30	51	47.36	11.79	14.88	1.05	82	120.33	26.38	30.44

§ Positive wells per plate. 96 wells scored unless otherwise stated, *e.g.* 52/95
1.6 cells/well plated for survival.

B. Mutagenicity Assay:

A summary of the results for Experiments 1 and 2 is shown in Table 2. The details of the raw plate counts and statistical analyses needed to calculate the results were provided as appendices in the report and included individual plate counts observed (on two plates for viability and on four plates for TFT resistance for each culture); summary of individual replicate results; heterogeneity factors for the survival, viability and mutant plates; comparison of negative and positive controls with historical means; % relative survival and relative total growth values, the mutant frequencies and the statistical analyses; and the results of small and large colony analysis. The acceptance criteria were met and the study was accepted as valid.

No statistically significant increases in mutant frequency were observed following treatment with ethiprole at any dose level tested, in the absence or presence of S9, in Experiment 1 or 2. It may be noted that in Experiments 1 and 2 in the presence of S9 a weak linear trend was observed ($P<0.05$). This linear trend was solely due to a very small, and not statistically significant, increase in mutant frequency at the top dose tested. Reanalysis of the data with the top dose excluded confirmed that without this dose there was no evidence of a linear trend in Experiment 1 or 2. Furthermore, no statistically significant increases in mutant frequency were observed at any dose level tested. Accordingly the weak linear trend was not considered to be biologically significant.

In addition, for the negative and positive controls, the number of wells containing small colonies and the number containing large colonies were scored. Thus the small and large colony mutant frequencies could be estimated and the proportion of small mutant colonies could be calculated. For the negative controls, the proportion of small colony mutants in the absence and presence of S9 ranged from 52% to 65% in Experiments 1 and 2. Marked increases in the number of both small and large colony mutants were observed following treatment with the positive control chemicals NQO and BP.

A summary of the results for Experiments 1 and 2 is shown in Table 2. The details of the raw plate counts and statistical analyses needed to calculate the results were provided as appendices in the report and included individual plate counts observed (on two plates for viability and on four plates for TFT resistance for each culture); summary of individual replicate results; heterogeneity factors for the survival, viability and mutant plates; comparison of negative and positive controls with historical means; % relative survival and relative total growth values, the mutant frequencies and the statistical analyses; and the results of small and large colony analysis. The acceptance criteria were met and the study was accepted as valid.

No statistically significant increases in mutant frequency were observed following treatment with ethiprole at any dose level tested, in the absence or presence of S9, in Experiment 1 or 2. It may be noted that in Experiments 1 and 2 in the presence of S9 a weak linear trend was observed ($P<0.05$). This linear trend was solely due to a very small, and not statistically significant, increase in mutant frequency at the top dose tested. Reanalysis of the data with the top dose excluded confirmed that without this dose there was no evidence of a linear trend in Experiment 1 or 2. Furthermore, no statistically significant increases in mutant frequency were observed at any dose level tested. Accordingly the weak linear trend was not considered to be biologically significant.

In addition, for the negative and positive controls, the number of wells containing small colonies and the number containing large colonies were scored. Thus the small and large colony mutant frequencies could be estimated and the proportion of small mutant colonies could be calculated. For the negative controls, the proportion of small colony mutants in the absence and presence of

S9 ranged from 52% to 65% in Experiments 1 and 2. Marked increases in the number of both small and large colony mutants were observed following treatment with the positive control chemicals NQO and BP.

Table 2. Main Mutagenicity Assay for Ethiprole - Summary of Results

Treatment (µg/mL)	Without S9 Mix				Treatment (µg/mL)	With S9 Mix			
	%RS	RTG	MF§			%RS	RTG	MF§	
Experiment 1									
0	100.00	1.00	95.90	---	0	100.00	1.00	116.24	---
15.625	89.04	0.96	79.92	NS	15.625	97.35	1.13	99.02	NS
31.25	94.50	0.87	101.71	NS	31.25	99.84	1.04	79.30	NS
62.5	71.74	0.65	100.52	NS	62.5	88.38	0.95	93.16	NS
125	69.95	0.42	107.49	NS	125	75.37	0.75	86.31	NS
250	28.38	0.42	127.22	NS	250	53.92	0.48	92.37	NS
500	17.98	0.12	102.30	NS	500	10.44	0.22	146.04	NS
Linear trend			NS		Linear trend			*	
NQO					BP				
0.05	88.32	0.74	401.31		2	41.54	0.39	1555.90	
0.1	67.15	0.60	736.77		3	46.37	0.33	1130.71	
Experiment 2									
0	100.00	1.00	104.94	---	0	100.00	1.00	81.74	
15.625	94.27	0.99	80.30	NS	15.625	100.19	1.13	68.60	NS
31.25	90.68	1.00	94.99	NS	31.25	91.56	1.08	80.01	NS
62.5	68.10	0.79	115.05	NS	62.5	78.38	0.80	82.82	NS
125	48.67	0.46	100.68	NS	125	60.71	0.44	75.21	NS
250	33.97	0.41	124.57	NS	250	48.17	0.25	82.11	NS
500	19.13	0.28	100.05	NS	375	16.89	0.57	81.03	NS
					500	17.33	0.24	112.74	NS
Linear trend			NS		Linear trend			*	
NQO					BP				
0.05	84.16	0.68	353.15		2	61.33	0.48	788.43	
0.1	66.20	0.55	576.43		3	55.26	0.50	725.36	

§ 5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS Percent relative survival adjusted by post treatment cell counts,
NS Not significant,
* $P<0.05$. As no statistically significant increases in mutant frequency were observed at any dose level tested, this was not considered to be biologically significant

III. CONCLUSIONS:

Ethiprole was tested up to cytotoxic concentrations. In the cytotoxicity range-finding experiment, six doses were tested, separated by two-fold intervals and ranging from 31.25 to 1000 µg/mL (limited by solubility). Marked toxicity was observed at the top three doses tested in the absence of S9 and the top two doses tested in the presence of S9. Furthermore, at the top dose tested (1000 µg/mL) heavy post-treatment precipitate was observed in both the absence and presence of S9. This precipitate was believed to have affected post-treatment cell counting. Hence, a top dose of 500 µg/mL was considered to be appropriate for the main mutagenicity experiments 1 and 2.

Accordingly, six doses were chosen for the first experiment, separated by two-fold intervals and ranging from 15.625 to 500 µg/mL. Two days after treatment all doses were selected to determine viability and TFT resistance. The top dose tested (500 µg/mL) yielded 17.98% and 10.44% relative survival in the absence and presence of S9, respectively. In Experiment 2, the same dose range was tested in the absence of S9; however, at the request of the Sponsor, an extra dose (375 µg/mL) was tested in the presence of S9. The top dose tested in this experiment was again 500 µg/mL, which yielded 19.13% and 17.33% relative survival in the absence and presence of S9, respectively.

Negative (solvent) and positive control treatments were included in each mutation experiment in the absence and presence of S9. Mutant frequencies in negative control cultures fell within normal ranges, and clear increases in mutation were induced by the positive control chemicals NQO (without S9) and BP (with S9). Therefore, the study was accepted as valid.

No statistically significant increases in mutant frequency were observed following treatment with ethiprole at any dose level tested, in the absence or presence of S9, in Experiment 1 or 2.

When tested up to its solubility limit and toxic doses, ethiprole did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells in two independent experiments, in the absence or presence of S9.

It was concluded that, under the conditions employed in the study, ethiprole was not mutagenic in the test system.

The study author stated that preliminary solubility data indicated ethiprole was soluble in DMSO up to at least 450 mg/mL and in the range of 250 to 500µg/mL in tissue culture medium. Therefore a top dose of 1000 µg/mL was selected for the cytotoxicity range-finder to ensure the limit of solubility was achieved. Based on this top dose of 1000 µg/mL, the reviewer will assume that the study author intended to write 450 µg/mL instead of 450 mg/mL for solubility in DMSO.

The stability of technical ethiprole and the stability and homogeneity of technical ethiprole in the solvent were not determined; in addition, analysis of achieved concentration was not performed as part of this study. These omissions are considered minor and do not impact on the review of this study.

KIIA 5.4.4 - In vivo genotoxicity (somatic cells) - Bone marrow or micronucleus

1.	Annex point(s)	KIIA 5.4.4; Genotoxicity/In vivo genotoxicity (somatic cells) - Bone marrow or micronucleus: **MRID 47622819**
2.	Location in dossier	KIIA 5.4.4 /01
3.	Authors (year)	Burman, M. (1999)
	Title	RPA 107382: Induction of micronuclei in the bone marrow of treated mice.
	Company, report No.	Bayer CropScience AG, Report-No.: R000181 (M-158055-01-1)
	Date	01.02.1999
4.	Testing facility	Covance Laboratories, Harrogate, North Yorkshire, United Kingdom
5.	Dates of work	1998-07-23 - 1998-10-20
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC
7.	Test method	USEPA (=EPA): 84 - 2
	Deviations	
8.	GLP	yes

Executive summary:

In a bone marrow micronucleus assay, CD-1 mouse (15/sex/dose) were treated by oral gavage with ethiprole (93% a.i.) at doses of 500, 1000 or 2000 mg/kg bw. Bone marrow cells were harvested at 24, 48 and 72 hours post-treatment. The vehicle was 0.5% w/v methylcellulose.

There were no signs of toxicity during the study. In the preliminary toxicity assay, abnormal gait was exhibited by both sexes between 2 and 4 hours after administration of a single dose at 2000 mg/kg/day. Ethiprole was tested at an adequate dose based on the limit dose of 2000 mg/kg/bw. The positive control induced the appropriate response. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time at any dose level.**

This study is classified as **acceptable/guideline** and satisfies the requirement for Test Guideline OPPTS 870.5395 and OECD 473.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CDR9706
Purity:	93%
CAS # of TGAI:	181587-01-9

2. Control Materials:

Vehicle:	0.5% w/v methylcellulose	**Final Volume:** 20 mL/kg body wt	**Route:** oral by gavage
Positive control:	Cyclophosphamide	**Final Dose(s):** 20 mg/kg body wt	**Route:** oral by gavage

3. Test animals:

Species:	Mouse
Strain:	CD-1
Age/weight at study initiation:	35-38 days / males weighed 25 - 34 g and females weighed 21 - 27 g
Source:	Charles River UK Limited, Margate, Kent, England.
No. animals used per dose	Preliminary Toxicity Test: One dose group at 2000 mg/kg, 3 males; 3 females Micronucleus Test: Vehicle control, 18 males, 15 females Low-dose, 15 males, 15 females Mid-dose, 15 males, 15 females High-dose, 15 males, 15 females Positive control, 5 males, 5 females
Properly Maintained?	Yes

4. Test Compound Administration:

	Dose Levels	Final Volume	Route
Preliminary:	2000 mg/kg body wt	20 mL/kg body wt	Oral by gavage
Main Study:	500, 1000, or 2000 mg/kg body wt	20 mL/kg body wt	Oral by gavage

B. TEST PERFORMANCE

1. Treatment and Sampling Times: From the results obtained in the preliminary toxicity study, dose levels of 500, 1000, and 2000 mg/kg bodyweight were used for the micronucleus test. The experimental design is shown below:

Treatment	Conc. (mg/mL)	Dosage (mg/kg)	Number of Treated Animals					
			24 Hr Sample		48 Hr Sample		72 Hr Sample	
			M	F	M	F	M	F
Negative/ (Vehicle Control)	---	---	5	5	8	5	5	5
Ethiprole	25	500	5	5	5	5	5	5
Ethiprole	50	1000	5	5	5	5	5	5
Ethiprole	100	2000	5	5	5	5	5	5
Positive Control (Cyclophosphamide)	2	40	5	5	---	---	---	---

Negative/Vehicle Control: 0.5% w/v Methylcellulose
Positive Control: Cyclophosphamide

For each treatment group, a single dose of the test substance, vehicle control, or positive control was administered by oral gavage in a volume of 20 mL/kg body weight. Clinical signs of toxicity were recorded over the period of the main study.

Test article and vehicle treated mice were killed in groups, 24, 48 or 72 hours after administration; CPA-treated mice were killed after 24 hours.

a. Test compound and vehicle control:

Dosing: X once twice (24 hrs apart) Other
Sampling (after last dose): 6 hr 12 hr X 24 hr X 48 hr X 72 hr

b. Positive control:

Dosing: X once twice (24 hrs apart) Other
Sampling (after last dose): 6 hr 12 hr X 24 hr 48 hr 72 hr

2. Tissues and Cells Examined:

Bone marrow: X
No. of polychromatic erythrocytes (PCE) examined per animal: At least 2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: <1000
Other (if other cell types examined, describe):

3. Details of Slide Preparation: Mice were killed by asphyxiation with carbon dioxide (subsequently ensured by cervical dislocation) in the same order as they were dosed. Both femurs from each animal were removed and the ends removed from the shanks. Using a syringe and needle, bone marrows were flushed from the marrow cavity with 1 mL fetal bovine serum into appropriately labelled centrifuge tubes (one per animal) labelled with study number, sex, animal number and sampling time.

The tubes were centrifuged (1250 x 'g', 2-3 minutes) and the serum was aspirated to leave one or two drops and the cell pellet. The pellet was mixed into this small volume of serum in each tube and from each tube a small volume of suspension was placed on the end of each of two slides labelled with the appropriate study number, sampling time, sex, date of preparation and tag number. The latter served as a code so analysis could be conducted "blind". A smear was made from the drop by drawing the end of a clean slide along the labelled slide.

Slides were allowed to air-dry and were fixed for 5 minutes in absolute methanol before being stained according to the modification of Gollapudi and Kamra (1979). One slide from each set of two was then taken, the other was kept in reserve. After rinsing several times in water, slides were stained for 10 minutes in filtered Giemsa stain diluted 1:6 (v/v) in distilled water. Stained slides were rinsed, and allowed to dry thoroughly before clearing in xylene for 3 minutes. When dry, the slides were mounted with coverslips.

Scoring. The slides from all control and dose groups sacrificed after 24, 48 and 72 hours were arranged in numerical order by sampling time and sex and analyzed by a person not connected with the dosing phase of the study. Initially the relative proportions of polychromatic erythrocytes (PCE), seen as pale blue or blue/grey enucleate cells, and normochromatic erythrocytes (NCE), seen as smaller yellow/orange stained enucleate cells, were determined until a total of at least 1000 cells (PCE plus NCE) had been analysed. Counting continued (but of PCE only) until at least 2000 PCE had been observed. All PCE containing micronuclei observed during these two phases of counting were recorded. The vernier coordinates of all cells containing micronuclei were recorded to a maximum of six per 2000 cells scored.

4. Evaluation Criteria: The criteria for a valid test was as follows: 1) the heterogeneity chi-square test provided evidence of acceptable variability between animals within the same group, and 2) the incidence of micronucleated PCE in vehicle control groups fell within or close to the historical vehicle control range, and 3) at least eight animals (males plus females) out of each group at each kill time were available for analysis, and 4) the positive control chemical (CPA) induced a statistically significant increase in the frequency of micronucleated PCE.

The following criteria were considered when interpreting the data: The test article was to be considered as positive in this assay if (1) a statistically significant increase in the frequency of micronucleated PCE occurred at least at one dose, at one sampling time, and (2) the frequency of micronucleated PCE at such a point exceeded the historical vehicle control range. It should be noted that increased, but statistically insignificant increases in frequencies of micronucleated PCE at one dose, at one sampling time in conjunction with either 1 or 2 of the above at the other sampling time may be cause for additional testing or analysis.

5. **Statistical Methods:** The group mean frequency of micronuceated PCE (male and female) and standard deviation were calculated. In addition, for each group, interindividual variation in the numbers of micronucleated PCE was estimated by means of a heterogeneity X^2 test (Lovell, et. al., 1989). The numbers of micronucleated PCE in each treated group (males and females, separately and combined) were then compared with the numbers in vehicle control groups by using a 2 x 2 contingency table to determine X^2. Probability values of $p < 0.05$ were to be accepted as significant. A further statistical test (for linear trend) was used to evaluate possible dose-response relationships.

II. RESULTS

Samples of 25, 50 and 100 mg/mL formulations of ethiprole prepared during the study were analysed in duplicate. Ethiprole recoveries ranged from 103 to 110% of the nominal concentrations, verifying the accuracy of preparations.

A. Preliminary toxicity assay:

The choice of dose levels was based on an initial toxicity range-finding study in which ethiprole, made up in 0.5% methyl cellulose (0.5% MC), was administered to mice orally by gavage. The test article was administered as a single dose to groups of three male and three female mice at 2000 mg/kg/day. Observations were made over a 4 day period following administration and signs of toxicity and body weight recorded. Abnormal gait was exhibited by both sexes between 2 and 4 hours after administration. No other clinical signs or loss of body weight were observed. Based on these data, a dose of 2000 mg/kg was chosen as the top dose for the main study.

B. Micronucleus assay:

In most cases, the heterogeneity X^2 test provided evidence of acceptable variability in the number of micronucleated PCE between animals within each group. Heterogeneity of marginal significance ($p \leq 0.05$) was seen among animals treated with the vehicle and sampled at 48 hours and was attributable to one mouse with four micronucleated PCE/2000. Insofar as such a frequency fell within the historical negative control range and has previously been seen among control animals, and other animals in the group had low frequencies of cells with micronuclei, it was considered to be of no biological significance. The incidence of micronucleated PCE in vehicle control groups fell within the historical vehicle control range. At least eight animals (males plus females) out of each group at each kill time were available for analysis. The positive control chemical (CPA) induced a statistically significant increase in the frequency of micronucleated PCE (X^2, $p \leq 0.001$).

No animals receiving the test article exhibited clinical signs of toxicity. The following mortalities were observed:

Treatment	Conc. (mg/mL)	Dosage (mg/kg)	Mortality (# of dead animals)/Number of Treated Animals					
			24 Hr Sample		48 Hr Sample		72 Hr Sample	
			M	F	M	F	M	F
Ethiprole	25	500	0/5	0/5	0/5	0/5	0/5	0/5
Ethiprole	50	1000	0/5	1/5	1/5	0/5	0/5	0/5
Ethiprole	100	2000	1/5	0/5	0/5	2/5	0/5	0/5

Groups of mice treated with ethiprole exhibited PCE/NCE ratios which were similar to vehicle controls at all sampling times. Group mean frequencies of micronucleated PCE were also similar to those seen in vehicle control and were not significantly different by X^2 analysis. These

frequencies fell within the historical negative control range. The results of the micronucleus test are summarized in Table 1.

TABLE 1. Summary of Micronucleus Test Result, Group Mean Data

Treatment group (mg/kg)	Kill time (hours)	Sex	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000)	
				per sex	per treatment group (± sd)
Vehicle control	24	F	1.21	0.30	0.50 ±0.6
		M	0.99	0.70	
	48	F	0.79	0.00	0.44 ±0.7
		M	0.81	0.79	
	72	F	1.16	0.25	0.39 ±0.3
		M	0.89	0.50	
500	24	F	0.96	0.69	0.64 ±0.6
		M	1.14	0.60	
	48	F	0.65	0.30	0.50 ±0.5
		M	0.98	0.70	
	72	F	0.96	0.20	0.15 ±0.2
		M	0.90	0.10	
1000	24	F	1.15	0.30	0.22 ±0.4
		M	1.14	0.12	
	48	F	0.61	0.25	0.22 ±0.3
		M	0.80	0.20	
	72	F	0.80	0.30	0.25 ±0.4
		M	1.04	0.20	
2000	24	F	1.38	0.10	0.17 ±0.3
		M	0.98	0.25	
	48	F	0.71	0.20	0.17 ±0.3
		M	0.76	0.12	
	72	F	0.97	0.10	0.17 ±0.4
		M	0.82	0.25	
CPA, 40	24	F	0.98	12.50	12.34 ±3.7
		M	0.80	12.18	

III. CONCLUSIONS:

The negative (vehicle) control in the study was 0.5% methylcellulose, also administered orally as a single dose. Groups of five male and five female mice treated with this were killed and sampled 24, 48 or 72 hours after administration. Cyclophosphamide, the positive control, was dissolved in saline and administered orally as a single dose at 40 mg/kg to groups of five male and five female mice which were killed after 24 hours. All positive control animals exhibited increased numbers of micronucleated polychromatic erythrocytes such that the micronucleus frequency in the positive control group was significantly greater than in concurrent controls (2 x 2 contingency X^2 test). Negative (vehicle) control mice exhibited normal group mean ratios of PCE to NCE (normochromatic erythrocytes) and normal frequencies of micronucleated PCE within historical negative control (normal) ranges.

Mice treated with ethiprole at all doses exhibited group mean ratios of PCE to NCE and frequencies of micronucleated PCE which were similar to the values for vehicle control groups at all sampling times and also fell within normal ranges. There were no instances of statistically significant increases in micronucleus frequency for any of the groups receiving the test article at either sampling time.

It was concluded that ethiprole did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice treated up to 2000 mg/kg/day.

KIIA 5.4.5 - In vivo genotoxicity (somatic cells) - DNA repair

1.	Annex point(s)	KIIA 5.4.5; Genotoxicity/In vivo genotoxicity (somatic cells) - DNA repair: **MRID 47622820**
2.	Location in dossier	KIIA 5.4.5 /01
3.	Authors (year)	Howe, J. (2001)
	Title	Ethiprole (RPA 107382): Measurement of unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure
	Company, report No.	Bayer CropScience AG, Report-No.: C021030 (M-210455-01-1)
	Date	29.11.2001
4.	Testing facility	Covance Laboratories Ltd., North Yorkshire, GBR;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):AE 0316423 00 1C (Batch-No.: AE 0316423 00 1C92 0001)
7.	Test method	EU (=EEC): 1999/11/EC (1999); JMAF: 12-NohSan no 8628 (2000); OECD: env/mc/chem(98)17 (1998); USEPA (=EPA): OPPTS 870.5550
	Deviations	
8.	GLP	yes

Executive summary MRID 47622820:
In an unscheduled DNA synthesis assay, male Han Wista rats were orally dosed once via oral gavage with ethiprole (91.5% a.i.) in 0.5% w/v methylcellulose (0.5% MC) at concentrations of 800 and 2000µg/mL. Primary hepatocytes were cultured from the dosed animals approximately 12-14 hours (Experiment 1) and 2-4 hours (Experiment 2) after dosing.

Ethiprole was tested up to a limit concentration of 2000 µg/mL. The positive controls induced the appropriate response. **There was no evidence of a dose related positive response that unscheduled DNA synthesis, as determined by radioactive tracer procedures (nuclear silver grain counts), was induced.**

This study is classified as **acceptable/guideline** and satisfies the requirement for Test Guideline OPPTS 870.5550 and OECD 482.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:

Description:	White powder
Lot/Batch #:	CDR9706
Purity:	91.5% a.i.
Compound Stability:	Determinations of the stability and characteristics of the test article were stated as the responsibility of the Sponsor.
CAS #:	181587-01-9

2. Control Materials:

Negative control:	0.5% w/v methylcellulose (0.5%MC)	
Solvent:	0.5% w/v methylcellulose	**Concentration:** Test substance was dissolved in 0.5% MC at 80 or 200 mg/mL and administered at 800 or 2000 mg/kg in a volume of 10 mL/kg for each dose level
Positive control /solvent:	1. 2-Acetamidofluorene (2-AAF) freshly suspended in corn oil at 7.5 mg/mL for the 12-14 hour experiment.	1. **Concentration:** 75 mg/kg in administered in a volume of 10 mL/kg
	2. Dimethylnitrosamine dissolved in purified water at 1.0 mg/mL for the 2-4 hour experiment.	2. **Concentration:** 10 mg/kg in a volume of 10 mL/kg

3. Test animals:

Species:	Rat, male
Strain:	Han Wistar
Age/weight at study initiation:	7 - 8 weeks / weighed 205 – 240 g
Source:	Charles River UK Limited, Margate, Kent, England.
No. animals used per dose	Preliminary Toxicity Test: One dose group at 2000 mg/kg, 3 males Main Test: Vehicle control, 4 males Low-dose, 4 males Mid-dose, 4 males High-dose, 4 males Positive control, 4 males
Properly Maintained?	Yes

4. Test Compound Administration:

	Dose Levels	Final Volume	Route
Preliminary:	2000 mg/kg body wt	10 mL/kg body wt	Oral by gavage
Main Study:	800 or 2000 mg/kg body wt	10 mL/kg body wt	Oral by gavage

The LD_{50} value for ethiprole, as determined previously by the Sponsor, was >2000 mg/kg. confirmatory toxicity range-finding experiment was performed in which three male rats were dosed once with 2000 mg/kg ethiprole. During a 2 day post-dose observation period only weight loss was observed; therefore, 2000 mg/kg was selected as the maximum dose for the main study

experiments. In the main experiments, four rats each were treated once orally with 800 or 2000 mg/kg of ethiprole suspended in 0.5% w/v methylcellulose (vehicle) with the aid of a Silverson homogenizer and stirring. The test article preparations were protected from light, stored at 1-10 °C, and used within 89 hours of initial formulation. The vehicle (0.5% MC), was tested in an homogeneity trial and found to produce an acceptable homogeneous formulation. In addition, samples of the dosing preparations were taken from the main study and analyzed for determination of achieved concentration.

5. Media: The isolated liver cells were cultured in culture media (Williams E medium-Complete) as described in Section 6, Cell Preparation, below.

6. Test Cells: Liver cells were isolated from male rats treated with the negative/vehicle control (0.5 %MC), the test substance (at 800 or 2000 mg/kg), or two positive controls, (2-AAF in corn oil at 75 mg/kg or DMN in water at 10 mg/kg). All doses were administered once orally in a volume of 10 mL/kg. The negative control and test substance groups were sacrificed 12-14 or 2-4 hours after treatment for Experiment 1 and 2, respectively. Four male rats were randomly assigned to each test group as shown in Table 2.

TABLE 1 Animal Assignments

Test Group	Compound Administered	Dose Level (mg/kg)	Volume (mL/kg)	No. of Animals Dosed	
				Experiment 1 (12-14 hours)	Experiment 1 (2-4 hours)
Negative Control	Vehicle	---	10	4	4
Test Substance	Ethiprole	800	10	4	4
		2000	10	4	4
Positive Control	2-AAF	75	10	4	---
	DMN	10	10	---	4

2-AAF = 2-Acetylaminofluorene and DMN = Dimethylnitrosamine, Vehicle = 0.5% w/v methylcellulose
Hepatocytes were prepared from three of the four animals in each dose group. Animals not required were humanely killed.

A sufficient number of male Han Wistar rats were obtained from Charles River (UK) Ltd, Margate, UK. They were housed in groups of no more than four animals in solid-floored cages, cleaned and dried before use with wood shavings for bedding. Bottled water (public supply) and diet, (Special Diets Services Ltd,) were provided *ad libitum.* Animal holding rooms were designed to be illuminated continuously by fluorescent light for 12 hours out of each 24 hour cycle and to receive at least 15 fresh air changes per hour. Group weights were checked within 24 hours prior to treatment to ensure individual group weights differed from the mean by no more than 5%. A description of the animals used, husbandry and environmental conditions are summarized below in Table 2.

TABLE 2. Animals and Husbandry

	Range-finder	Main study
Number of animals used in study	3	32
Weight range on first day of assay (g)	170-185	205-240
Approximate age on first day of assay (weeks)	7	7-8
Acclimatization period (days)	2	6-8
Temperature range during assay†	18*-22°C	19-22°C
Humidity range during assay†	52-66%	47-60%

† From receipt to last day of sampling
* Temperature slightly out of protocol stated range (19-24°C); however, this minor deviation did not effect the validity of the study in any way.

7. **Cell Preparation:** Livers were perfused and hepatocytes were harvested as described below for all control (negative and positive) and treated animals.

a. Perfusion Technique: Livers were perfused *in situ* as follows. Individual animals were anesthetized with halothane and maintained under deep anesthesia to prevent any likelihood of recovery. The liver was surgically exposed, the hepatic portal vein and superior vena cava cannulated with suitable cannulars and the liver perfused with suitable buffers. Approximately 400 mL of calcium free Buffer 1 was pumped at a flow rate of approximately 40 mL/min to wash the liver free of blood. The liver was then perfused with Buffer 2 also at a flow rate of 40 mL/min for approximately 5 minutes. Both buffers were gassed with 5% CO_2 in air (v/v) prior to use and Buffer 2 throughout perfusion. Calcium and collagenase (approximately 50 mg of collagenase dissolved in 1 mL of 769 mM $CaCl_2$ and 10 mL of Buffer 2) were added to the reservoir and after 1-2 minutes the waste line was placed in the Buffer 2 reservoir so that the perfusate recirculated and the flow rate was reduced to 20 mL/minute. When the reticular pattern of the liver had begun to break up and the liver became 'spongy', the perfusion was stopped. The liver was cut free into a suitable container with Buffer 2.

b. Hepatocyte Harvest/Culture Preparation: The liver was transferred to a sterile dish, cut open and the hepatocytes carefully teased out. The hepatocyte suspension was gently washed through 150 µm nylon mesh with Williams E medium-Complete (WE-C) to a volume of approximately 100 mL. Of this suspension, about 50 mL was taken and centrifuged at approximately 40 x 'g' for 2-3 minutes. The resultant pellet was resuspended in WE-C. The centrifugation and resuspension procedure was repeated at least twice and the pellet resuspended finally in approximately 20 mL of WE-C. A sample (0.5 mL) of this suspension was taken, diluted with an equal volume of 0.4% (w/v) trypan blue in phosphate buffered saline (PBS) and the proportion of viable cells (those with unstained nuclei) determined using an hemocytometer. The culture was diluted where possible to provide approximately 1.5×10^5 viable cells/mL. The isolated liver cells were subsequently cultured as described below in B. Test Performance.

B. TEST PERFORMANCE

Isolated hepatocytes from all control (negative and positive) and treated animals were cultured and processed for cytotoxicity and UDS evaluations as described below.

1. **Cytotoxicity Assay:** A sample (0.5 mL) of the hepatocyte suspension was taken, diluted with an equal volume of 0.4% (w/v) trypan blue in PBS and the proportion of viable cells (those with unstained nuclei) determined using an hemocytometer.

2. **UDS Assay:** Three mL of hepatocyte suspension was added to each well of a six-well multiplate containing 25 mm round plastic coverslips and incubated at 37°C± 1°C in a 5% CO_2 in air (v/v) atmosphere for at least 90 minutes to allow cells to attach. Medium was removed from the cells and the monolayers washed with 2 mL Williams E medium-Incomplete (WE-I) which was then replaced with 2 mL WE-I containing 10 µCi/mL [^{3}H]thymidine. After 4 hours incubation at 37°C in a 5% CO_2 in air (v/v) atmosphere, the medium was removed and the cells washed with three changes of WE-I containing 0.25 mM thymidine. Cultures were then incubated overnight with 3 mL of the same medium.

a. **Preparation of Autoradiographs/Grain Development:** To prepare for autoradiography, coverslips were washed with 2 mL PBS and the cells fixed with three changes of 2 mL glacial acetic acid:ethanol (1:3 v/v). The coverslips were then washed four times with purified water, allowed to dry and mounted onto previously labelled microscope slides, cells side up, with DPX. Three of the six slides from each animal were coated in Ilford K2 liquid emulsion using a dipping technique. Each slide was dipped individually into the molten emulsion, ensuring that no air-bubbles were generated. After gelling over ice, for 10 minutes face upwards, the slides were incubated in a light-tight box at room temperature for approximately 90 minutes to let the emulsion dry. The slides were then packed in light-tight boxes containing desiccant, sealed with tape and refrigerated for 14 days. At the end of this time, the emulsion was developed in Kodak D19 developer and fixed using Ilford Hypam fixer.The cell nuclei and cytoplasm were then stained with Meyers haemalum/eosinY. Slides were then dehydrated in ethanol, cleared in xylene and mounted with coverslips for microscopic examination. The spare, duplicate set of slides were not required.

b. **Grain Counting:** Grain counting was performed using a microscope with a video camera connected to an image analysis system (Perceptive Instruments) and a computer programmed for automatic data capture. Each slide was examined to ensure that the culture was viable. A patch of cells was selected as a starting point and cells were scored in a regular fashion by bringing new cells into the field of view, moving only in one axis. If the desired number of cells had not been scored before coming to the edge of the slide, the stage was moved one or two fields on the other axis and counting resumed. The circular field was centered over the nucleus of a suitable cell and the grains counted. The field of view was moved and counts obtained for three separate adjacent areas of cytoplasm. Nuclear and mean cytoplasmic grain counts were then recorded, and the net grains/nucleus (NNG) determined. One hundred cells were analysed per animal, where possible using two of the three slides in each case. The following criteria were used for cell analysis:

1. only cells with normal morphology were scored
2. isolated nuclei with no surrounding cytoplasm were not scored
3. cells without nuclear and/or cytoplasmic graining were not scored
4. cells with unusual staining artifacts were not scored
5. heavily labelled cells in S-phase were not scored
6. all other normal cells, 100 per animal were scored
7. all slides were analysed blind (coded).

The following were calculated for each slide, animal and dose point:

1. the population average NNG and standard deviation (SD)
2. the percent of cells responding or in repair (i.e., >5 NNG)
3. the population average cytoplasmic and nuclear grain count.

c. **Evaluation Criteria:** The study would be considered valid if the negative control animals had a group mean NNG value that did not exceed the upper limit of the historical range. The positive control treatments should have group mean values of five or more NNG with 50% or more cells having NNG counts of five or greater.

The test article would be considered as positive in the assay if, at any dose and at either time point, (1) the test article yielded group mean NNG values greater than 0 NNG and 20% or more of cells responding (mean NNG values >5), and (2) an increase above solvent control levels was seen in both NNG and the percentage of cells in repair. Cytoplasmic and nuclear grain count values as well as the concurrent negative control data would be considered in relation to the overall NNG values of cultures from treated animals. If the test article failed to induce UDS at any dose tested after both 2-4 and 12-14 hours exposure, it would be considered clearly negative in this system.

d. **Statistical Analysis:** Descriptive statistical methods were used. Mean NNG, cytoplasmic and nuclear grain count and standard deviations were calculated for each group.

II. RESULTS

The vehicle (5% MC) was tested for homogeneity and found to produce an acceptable homogeneous formulation. Ethiprole recoveries from triplicate aliquots taken from the top and the bottom of 80 and 200 mg/mL formulations were 110-112% and 122-126%, respectively. In addition, samples of the dosing preparations saved from the main study were analysed for achieved concentration. Samples prepared 10 and 13 September 2001 were analysed in duplicate. Ethiprole recoveries were 88-114% and 96-117% from the 80 and 200 mg/mL formulations, respectively.

A. Preliminary cytoxicity assay:

The percentage of viable cells from the individual animals as determined by trypan blue exclusion were 70-82% in Experiment 1 (12-14 hour experiment) and 59-78% in Experiment 2 (2-4 hour experiment).

B. UDS Assay: The results of the UDS assay are summarized in Table 3.

In the main study weight loss was observed in the 2000 mg/kg dose group (12-14 hour experiment). No other clinical sign was observed in any other main study animal.

The group mean net grain count for vehicle-treated animals was less than the upper limit of the historical control range (-1.4 and -3.2 for Experiments 1 and 2, respectively), with only 0 and 1% cells in repair. In addition, the positive control chemicals 2-AAF and DMN induced increases in group mean net grain count of five or more (19.5 and 28.0 respectively), and 50% or more of cells (96.3% and 96% respectively) had net grain counts of five or more. This result showed that the test system was sensitive to two known DNA damaging agents requiring metabolism for their action and that the experiment was valid.

Treatment with ethiprole at doses up to 2000 mg/kg yielded NNG values less than zero, producing group mean NNG values over the two experiments in the range -2.0 to -7.0, well below the threshold value of 0 NNG required for a positive response. No more than 1% cells were seen in repair at any dose of ethiprole.

TABLE 3. UDS Assay, Mean Grain Count and Cell Viability per Dose Group

Dose Level (mg/kg)	Test Group	Compound Administered	Net grain count (NNG)		Percent of cells in repair (NNG≥5)		Net grain count of cells in repair		Cell viability %
			mean	SD	mean	SD	mean	SD	
Sacrifice 12-14 hours post-treatment									
0	Negative Control	0.5% MC	-1.4	0.6	-	-	-	-	72-82
800	Test Substance	Ethiprole	-2.0	0.4	1.0	1.7	6.3	-	73-82
2000	Test Substance	Ethiprole	-2.2	0.8	0.7	1.2	6.7	-	71-81
75	Positive Control	2-AAF	19.5	5.2	96.3	4.0	20.0	5.8	70-81
Sacrifice 2-4 hours post-treatment									
0	Negative Control	0.5% MC	-3.2	0.5	1.0	1.0	6.3	1.4	64-78
800	Test Substance	Ethiprole	-4.4	0.5	0.3	0.6	5.0	-	59-74
2000	Test Substance	Ethiprole	-7.0	1.2	1.0	1.0	8.5	3.5	62-77
10	Positive Control	DMN	28.0	3.3	96.0	0.0	29.1	3.5	69-75

There were four rats for each test group and dose level.
Cell viability % = percentage of viable cells as determined by trypan blue exclusion (range of individual values from three animals in each dose group is shown).

III. CONCLUSIONS:

Ethiprole was tested for its ability to induce unscheduled DNA synthesis in the livers of orally dosed male rats using an *in vivo/in vitro* procedure.

A toxicity range-finding experiment was performed in which three male rats were dosed once with 2000 mg/kg ethiprole. During a 2 day post-dose observation period only weight loss was observed; therefore, 2000 mg/kg was selected as the maximum dose for the main study experiments.

Groups of four male rats were treated once by oral gavage with the solvent (0.5% w/v methylcellulose), ethiprole at 800 mg/kg or 2000 mg/kg, or the required positive control, all at a dose volume of 10 mL/kg. The positive controls used were 75 mg/kg 2-acetamidofluorene suspended in corn oil (12-14 hour experiment) and 10 mg/kg dimethylnitrosamine dissolved in purified water (2-4 hour experiment). In the main study weight loss was observed in the 2000 mg/kg dose group (12-14 hour experiment). No other clinical signs were observed in any other main study animal.

The vehicle control net grain count value was consistent with both published and historical control data, and the system was shown to be sensitive to two known DNA damaging agents requiring metabolism for their action. The assay was therefore accepted as valid.

Male Han Wistar rats, when treated once via oral gavage with ethiprole at doses up to 2000 mg/kg, showed no induction of UDS in hepatocytes isolated ex vivo approximately 12-14 or 2-4 hours after dosing. Treatment with 800 or 2000 mg/kg ethiprole did not produce a group mean net grain count value greater than -2.0, nor were any more than 1% cells found in repair at either dose. It was concluded that ethiprole had no genotoxic activity detectable in this test system under the experimental conditions employed.

KIIA 5.6.2.1 - Teratogenicity test by the oral route in the rat – Range-Finding

1.	Annex point(s)	KIIA 5.6.2.1; Teratogenicity test by the oral route in the rat
2.	Location in dossier	KIIA 5.6.10 /01
3.	Authors (year)	Foulon, O. (1999)
	Title	Ethiprole (RPA 107382) Range-Finding Study for Developmental Toxicity in the Rat by Gavage.
	Company, report No.	Bayer CropScience AG, Report-No.: SA99036
	Date	24.09.1999
4.	Testing facility	Aventis CropScience, Sophia Antipolis;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC (Batch-No.: CDR9706)
7.	Test method	EU (=EEC): 92/69/EEC, V, B31; JMAF: 59 NohSan No. 4200, (1985); OECD: Draft 1998, for updating guideline 414; USEPA (=EPA): OPPTS 870.3700, (1998)
	Deviations	
8.	GLP	yes

Executive summary:

In a range-finding developmental toxicity study, ethiprole (92.7 % a.i.) was administered to 8 female Sprague Dawley Crl: CD (SD) BR rats/dose by gavage at dose levels of 0, 7.5, 25, 50, or 150 mg/kg bw/day from gestation days 6 through 20.

No deaths occurred in the study. At 150 mg/kg bw/day, adverse clinical signs were noted as evidenced by the decreases in maternal body weight gain and food consumption. At 25 and 50 mg/kg bw/day, the decreases in the mean body weight gain and food consumption were significantly reduced at the beginning of the dosing period. A body weight loss was observed in animals that received ≥ 25 mg/kg bw/day during GD 6-8. At 7.5 mg/kg bw/day, slight decrease in food consumption was observed at the early stage of treatment. A significant increase was noted in liver weights associated with accentuated lobular pattern of liver lobes from 25 mg/kg bw/day.

No gestational parameters were adversely affected. At 150 mg/kg bw/day, the mean fetal body weight was lower than the control group value. No treatment-related external findings were noted at any dose.

This study is classified **acceptable/non-guideline** providing data for use in dose selection for the definitive developmental toxicity study in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Ethiprole (RPA 107382)

Description:	White powder
Lot/Batch #:	CDR9706
Purity:	92.7% a.i.
Compound Stability:	The test substance was stored in an air-tight, light-resistant container at room temp.
CAS #of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** Control animals received 10 mL/kg body weight of vehicle alone (0.5% aqueous solution of methylcellulose 400).

3. **Test Animals*:**

Species:	Rat	
Strain:	Sprague Dawley Crl: CD (SD) BR	
Age/weight at study initiation:	Adult virgins / 237 to 301 g at mating	
Source:	Charles River France (St Aubin-les-Elbeuf)	
Housing:	Individually in suspended, stainless steel, wire mesh cages	
Diet:	U.A.R. Certified Rodent meal A04C 10 (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France), *ad libitum*	
Water:	Filtered water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	20°C - 24°C
	Humidity:	40% - 70%
	Air changes:	approximately 15 changes /hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	12 days	

*NOTE: Age and weight of males was not provided in the study report.

B. PROCEDURES AND STUDY DESIGN:

1. **In-Life Dates:** Start: February 16, 1999; End: March 11, 1999

2. **Mating:** Females were mated on a one-to-one basis with stock males of the same strain and same supplier. Each morning following pairing, rats showing spermatozoa in a vaginal smear or sperm plug in situ were considered as pregnant animals. The day when evidence of mating was noted, was designated as gestation day 0 (GD 0).

3. **Animal Assignment:** The females were assigned to control and treated groups each day of pairing using a body weight dependent procedure. Permanent identification numbers were assigned to animals within each group.

Table 1. Study design

Dose (mg/kg bw/day)	0	7.5	25	50	150
# Females	8	8	8	8	8

4. **Dose selection rationale** - Doses were selected in agreement with the sponsor representative based on results obtained in a previous range-finding study (M. Repetto-Larsay, 1998) where pregnant females received doses of ethiprole at 0, 25, 100, 200, 400 and 800 mg/kg bw/day from GD 6 to 15.

5. **Dosage Preparation and Analysis:** "The appropriate amount of test substance was suspended (w/v) in an aqueous solution of methylcellulose 400 at 0.5% and stored at approximately 5°C (± 3°C). The test substance suspensions will be prepared periodically based on stability data from a previous study (Repetto-Larsay, 1995) in which suspensions of RPA 107382 in aqueous methylcellulose was found to be stable during a 14-day period at concentrations of 0.125 and 50 mg/ml.

   Before the start of the dosing period, the homogeneity of the suspensions of the first formulation was checked at 0.75 and 15.0 g/l. All concentrations of the first and the second formulation were checked. Stability of the test substance in suspension in the vehicle was determined during the study at 0.075 and 15.0 g/l during a 21-day period."

   No further information was given regarding formulation details and amount of formulations made

   Results **Homogeneity Analysis:** Results for homogeneity of the first formulations of ethiprole in the vehicle at 0.75 and 15.0 g/L were within the range of 92-98% of the nominal concentrations. (Data obtained from the study report, page 101).

   Stability Analysis: The suspensions were stable between 0.075 g/L and 15 g/L over a 21-day period at 5°C±3°C. (Data obtained from the study report, page 103).

   Concentration Analysis: Results for the concentration analyses were within 93-101% of the nominal concentrations. (Data obtained from the study report, page 102).

6. **Dosage Administration:** All doses were administered once daily by gavage, on gestation days 6 through 20, in a volume of 10 mL/kg of body weight/day. Dosing was based on the most recent body weight determination.

C. OBSERVATIONS

1. **Maternal Observations and Evaluations**: The animals were checked for mortality or clinical signs daily. Body weight data and food consumption data were recorded on gestation days 0, 6, 8, 10, 12, 14, 16, 18 and 21.

   Animals killed *in extremis* by inhalation of carbon dioxide or found dead were autopsied. The number of implantations and corpora lutea were noted when present. Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulfide (2) to visualize any sites which were not apparent. Then tissues and carcass were discarded.

   On Gestational Day 21, surviving females were killed by inhalation of carbon dioxide, for examination of uterine content. Each female was first subjected to macroscopic examination of the visceral organs and the liver of each pregnant female was collected and weighed. The reproductive tract was weighed (gravid uterine weight), dissected out and the following parameters recorded: number of corpora lutea, number of implantations, number of resorptions, number of live and dead fetuses, sex of live fetuses, and the individual weights of live fetuses. Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulfide to visualize any sites which were not apparent.

2. **Fetal Evaluations**: Live fetuses were killed by intrathoracic injection (0.02 ml/fetus) of Dolethal (Sanofi, Libourne, France) and subjected to an external examination. Dead fetuses were also subjected to an external examination. Tissue and carcass were discarded. Resorptions were classified as early (macroscopic discrimination between fetal residues and placental material not possible) and late (distinct macroscopic discrimination between fetal and placental remains possible). Dead fetuses were defined as fetuses showing distinct digits on fore-and hind-paws.

D. DATA ANALYSIS

1. **Statistical Analyses:** Results of maternal body weight changes, corrected body weight change, liver weight, food consumption and foetal weight per litter were intercompared between the treated groups and the control group by use of:
   - Bartlett's test for homogeneity of variances
   - Analysis of variance (ANOVA) when Bartlett's test indicated homogeneous variances
   - Dunnett's test if Anova was significant

   When Bartlett's test indicated heterogeneous variances, non parametric tests were performed using the Kruskal-Wallis non parametric one-way analysis of variance followed by the Mann-Whitney test when the Kruskal-Wallis test was significant.

   Litter data were statistically treated using the Kruskal-Wallis test followed by the Mann-Whitney test when the Kruskal-Wallis test was significant.

   - When the mean and variance of the control group were equal to zero, means of treated groups only (different from zero) were compared with the control group using the Mann-Whitney test.
   - When the means and the variances of the control group and the treated groups were equal to zero, no statistical analysis of the variable was performed.

- Statistical analysis was performed on all pregnant females and on pregnant females with viable foetuses. As all pregnant females had viable foetuses, results of analysis performed on all pregnant females only was included in the report.

The alpha levels for each statistical comparison were 0.05 and 0.01.

Statistical analyses were performed using SAS programs.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

$$\textbf{Pre-implantation loss index} = \frac{\textbf{(Number of corpora lutea - Number of implantations)}}{\textbf{Number of corpora lutea}} \times 100$$

$$\textbf{Post-implantation loss index} = \frac{\textbf{(Number of implantations - Number of live fetuses)}}{\textbf{Number of implantations}} \times 100$$

$$\textbf{Percentage of live fetuses (per litter)} = \frac{\textbf{Number of live fetuses}}{\textbf{(Number of live fetuses + Number of dead fetuses)}} \times 100$$

$$\textbf{Percentage of dead fetuses (per litter)} = \textbf{100-percentage of live fetuses}$$

$$\textbf{Percentage of male fetuses} = \frac{\textbf{(Number of male fetuses)}}{\textbf{Total number of live fetuses}} \times 100$$

3. **Historical Control Data:** Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and Clinical Observations:** No treatment-related deaths occurred in the study. At 50 mg/kg bw/day, one pregnant female was killed on GD 11. At necropsy, this female presented white liquid between thorax and muscles and the esophagus was perforated, demonstrating a gavage error. At 150 mg/kg bw/day, five pregnant females exhibiting curling up at handling for 2, 3 or 4 days between GD 8 and 11. In the same treatment group, one female had clonic convulsions on GD 9; one other female had reduced motor activity on GD 8 and 9 and staggering step from GD 8 to 11. The other clinical signs (hairloss, piloerection or red vaginal discharge) were considered spontaneous in origin or not toxicologically significant as these signs were noted for a few females with no apparent dose-related effect.

2. **Body Weight:** Mean body weight changes showed negative values representing a loss in body weight, hence they were statistically different from control values for the period GD 6-8 in animals treated from 25 mg/kg bw/day and above. In addition, at 150 mg/kg bw/day, the mean body weight gain was lower than control values from GD 8 to 10 and GD 18 to 21 (64% and 25% respectively) but did not show any statistically significant difference. Corrected overall body weight change was lower than the control values at 50 and 150 mg/kg bw/day and the difference was statistically significant at 150 mg/kg bw/day.

Table 2. Maternal body weight (g) and body weight gain (g)[a]

mg/kg bw/d	0 n = 8	7.5 n = 8	25 n = 8	50 n = 8	150 n = 8
Initial BW (pretreatment)	258.5 ± 8.93	256.6 ± 9.71	255.9 ± 10.74	256.2 ± 9.39	263.4 ± 8.16
BW Day 6 (pretreatment)	291.1 ± 9.82	287.3 ± 10.00	291.3 ± 10.63	287.2 ± 13.85	292.0 ± 16.49
BW day 14 (treatment)	327.3 ± 12.81	319.6 ± 14.34	317.4 ± 12.51	309.0 ± 15.82	300.9 ± 13.64
BW day 21 (treatment)	431.8 ± 20.07	417.8 ± 23.53	416.0 ± 10.66	409.0 ± 18.31	387.5 ± 21.90
BWG Day 0-6	32.6 ± 6.89	30.6 ± 5.15	35.4 ± 3.74	31.0 ± 5.87	28.6 ± 10.06
BWG Days 6-8	5.5 ± 2.73	4.5 ± 1.85	-2.6** ±6.784	-6.8 **± 5.98	-24.6**± 6.78
BWG Days 8-10	11.1 ± 2.17	8.8 ± 3.96	9.4 ± 4.96	10.8 ± 2.99	4.0 ± 12.09
BWG Days 10-14	19.5 ± 2.88	19.1 ± 2.85	19.4 ± 5.01	17.8 ± 3.43	26.5 ± 12.25
BWG Days 14-18	45.5 ± 4.07	44.3 ± 8.17	43.9 ± 7.28	46.0 ± 10.41	42.4 ± 9.52
BWG Days 18-21	59.0 ± 9.53	53.9 ± 13.31	54.8 ± 7.65	54.0 ± 8.94	44.3 ± 12.90
Corrected BW Change[b]	69.5 ± 13.36	59.4 ± 17.47	59.5 ± 19.47	49.5 ± 10.73	28.5** ± 17.68

[a] Data obtained from pages 29-33 in the study report.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

[b] Corrected body weight change = (Body weight on Gestational Day 21 - Body weight on Gestational Day 0) - (gravid uterine weight)
BW body weight, BWG body weight gain

3. **Food Consumption:** Food consumption data are summarized in Table 3. At 150 mg/kg bw/day, food consumption was reduced during the dosing period, showing statistical differences during each interval except from GD 16 to 18. At 25 and 50 mg/kg bw/day, the mean food consumption was significantly lower than the control values from GD 6 to 8 and GD 8 to 10 and from GD 12 to 14 at 50 mg/kg bw/day only. At 7.5 mg/kg bw/day, the mean food consumption was slightly reduced at the beginning of the dosing period, showing a statistically significant difference from GD 8 to 10.

Table 3. Mean (±SD) Maternal Food Consumption in (g/day)[a]

mg/kg bw/d	0 n = 8	7.5 n = 8	25 n = 8	50 n = 8	150 n = 8
			Female		
FC Days 1 – 6	26.4 ± 1.77	25.3 ± 2.12	27.1 ± 1.81	26.8 ± 2.79	25.5 ± 2.67
FC Days 6 – 8	27.6 ± 2.77	25.4 ± 1.60	22.66**± 3.42	19.2**± 4.31	12.9**± 2.59
FC Days 8 – 10	26.9 ± 2.10	24.4*±1.69	22.9**±1.73	21.3**±2.80	13.5**± 4.69
FC Days 10 – 12	27.1 ± 2.75	25.9 ± 2.53	25.6 ± 2.07	24.2 ± 2.71	21.1**±2.75
FC Days 12 - 14	27.9 ± 1.96	25.1 ± 1.89	26.8 ± 2.31	24.0* ± 1.67	22.4**± 3.58
FC Days 14 - 16	28.8 ± 1.91	26.6 ± 1.77	27.1 ± 2.64	25.5 ± 2.51	24.6*± 3.66
FC Days 16-18	30.8 ± 2.66	29.5 ± 8.17	31.1 ± 2.30	29.5 ± 2.51	28.6 ± 3.29
FC Days 18 - 21	29.4 ± 2.50	28.0 ± 1.93	30.1 ± 3.04	26.8 ± 2.23	25.9*± 2.42

a Data extracted from pages 35-36 of the study report
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01
FC food consumption

4. **Gross Pathology:** Gross pathology data are summarized as follows: A significant dose-related increase in absolute liver weight, associated with accentuated lobular pattern, was observed at doses of 25 mg/kg bw/day and above.

TABLE 4. Mean (±SD) Maternal Liver Weights (g)[a]

Organ Weight (g)	Dose in mg/kg bw/day				
	0 (n=8)	**7.5 (n=8)**	**25 (n=8)**	**50 (n=8)**	**150 (n=8)**
Liver Weight (g)	14.6±0.69	15.7±1.58	18.8**±1.86	20.3**±1.55	21.6**±2.69

a Data obtained from the study report page 34.
*** Statistically different (p <0.001) from the control

5. **Cesarean Section Data:** Data are as follows: The mean number of corpora lutea, uterine implantation sites and the mean pre-implantation loss percentage in the treated groups did not show any significant differences from the control group. There were no adverse effects on gestational parameters, including number of early and late resorptions, dead and live fetuses and post-implantation loss percentages. At 150 mg/kg bw/day, the mean fetal body weights were slightly lower (not statistically significant) than the control group value. At 7.5, 25 and

50 mg/kg bw/day, the mean fetal body weights were not affected by treatment; as summarized in Table 5.

Table 5. Cesarean section observations[a]

Observation	Dose (mg/kg bw/day)				
	0	7.5	25	50	150
# Animals Assigned (Mated)	8	8	8	8	8
# Animals Pregnant	8	8	8	6	8
Pregnancy Rate (%)	100	100	100	75	100
# Nonpregnant	0	0	0	1	0
Maternal Wastage					
# Died	0	0	0	2	0
# Died Pregnant	0	0	0	1	0
# Died Nonpregnant	0	0	0	1	0
# Aborted	0	0	0	0	0
# Premature Delivery	0	0	0	0	0
Total # Corpora Lutea Corpora Lutea/Dam	18.0 ± 4.31	16.6 ± 1.60	16.5 ± 0.93	16.5 ± 1.38	17.0 ± 1.77
Total # Implantations (Implantations/Dam)	15.6 ± 1.06	14.8 ± 2.92	15.3 ± 3.01	15.3 ± 2.42	14.9 ± 2.80
Total # Litters	8	8	8	6	8
Total # Live Fetuses (Live Fetuses/Dam)	14.3 ± 1.49	14.3 ± 3.20	13.9 ± 3.04	14.5 ± 2.43	13.9 ± 3.23
Total # Dead Fetuses (Dead Fetuses/Dam)	0	0.3 ± 0.46	0	0	0
Total # Resorptions					
Early	1.4 ± 1.30	0.3 ± 0.46	1.4 ± 1.60	0.8 ± 0.41	0.9 ± 0.83
Late	0	0	0	0	0.1 ± 0.35
Mean Fetal Weight (g)	5.37 ± 0.453	5.32 ± 0.273	5.42 ± 0.189	5.36 ± 0.184	4.94 ± 0.625
Preimplantation Loss (%)	10.4 ± 13.78	11.4 ± 14.67	7.7 ± 17.29	7.5 ± 7.85	12.6 ± 13.69
Postimplantation Loss (%)	8.7 ± 7.83	4.0 ± 4.52	9.0 ± 9.44	5.6 ± 2.94	7.3 ± 8.06

[a] Data obtained from pages 38-41 in the study report.

B. <u>DEVELOPMENTAL TOXICITY</u> Developmental toxicity was observed at the high dose level as increased incidences of a skeletal anomaly, skeletal variations, and a visceral variation (enlarged thymus). No treatment-related malformations were noted at any dose level.

1. **<u>External Examination:</u>** A summary of the external examinations is presented in Table 6. The number of runt fetuses increased in the 150 mg/kg bw/day group. No other dose-related variations or malformations were observed.

TABLE 6. External Examinations [a]

	Dose (mg/kg bw/day)					Dose (mg/kg bw/day)				
	0	7.5	25	50	150	0	7.5	25	50	150
	Number of fetuses examined					Number of litters examined				
	114	114	111	87	111	8	8	8	6	8
Findings	Number (%) fetuses affected					Number (%) litters affected				
Variation:										
Runt (body weight <4.0g).	0 (0.0)	1 (0.8)	1 (0.8)	1 (1.5)	10 (9.9)	0 (0.0)	1 (12.5)	1 (12.5)	1 (16.7)	3 (37.5)
Placentae: fused: two implantations	0 (0.0)	0 (0.0)	2 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Malformation:										
Eye buldges (bilateral):absent. Mandibular: micrognathia	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)

a Data obtained from the study report, page 43.

III. CONCLUSIONS:

1. **Maternal toxicity:** No treatment-related deaths occurred in the study. At 150 mg/kg bw/day, five pregnant females exhibited curling up at handling for 2, 3 or 4 days between GD 8 and 11. In the same treatment group, one female had clonic convulsions on GD 9 and one other female had reduced motor activity on GD 8 and 9 and staggering step from GD 8 to 11.

   Animals that received ≥ 25 mg/kg bw/day lost body weight between GD 6-8. Overall corrected body weight gain was lower than the control values, at 50 and 150 mg/kg bw/day and showing a statistically significant difference at 150 mg/kg bw/day.

   Food consumption was significantly lower during the dosing period at 25, 50 and 150 mg/kg bw/day. At 7.5 mg/kg bw/day, the mean food consumption was slightly reduced at the beginning of the dosing period, showing a statistically significant difference from GD 8 to 10.

2. **Developmental toxicity:** The number of runt fetuses (body weight < 0.4g) was increased in 150 mg/kg bw/day and concerned three litters. At this dose level, the mean fetal body weights were slightly lower (not statistically significant) than the control group value. One fetus at 25 mg/kg bw/day was externally malformed: this observation was considered spontaneous in origin.

   The mean number of corpora lutea, uterine implantation sites and the mean pre-implantation loss percentage in the treated groups did not show any significant differences from the control group. There were no adverse effects on gestational parameters.

   a. Deaths/Resorptions: There were no significant effects of treatment on gestational parameters including early and late resorptions and fetal death status.

   b. Altered Growth: The number of runt fetuses was increased at 150 mg/kg bw/day on both fetal and litter basis.

   c. Developmental Variations: No treatment-related variations were noted in any of the dose groups.

   d. Malformations: One fetus at 25 mg/kg bw/day was externally malformed with absent eye buldges and micrognathia mandibular: this observation was considered spontaneous in origin.

Historical data were not included. Since this was only a range-finding study external fetal examination was performed (not visceral or skeletal).

KIIA 5.6.10 - Teratogenicity test by the oral route in the rat

1.	Annex point(s)	KIIA 5.6.2.1; Teratogenicity test by the oral route in the rat: **MRID 47622808**
2.	Location in dossier	KIIA 5.6.10 /01
3.	Authors (year)	Foulon, O. (2000)
	Title	Developmental toxicity study in the rat by gavage Ethiprole (RPA107382)
	Company, report No.	Bayer CropScience AG, Report-No.: R018797 (M-196111-01-1)
	Date	19.12.2000
4.	Testing facility	Aventis CropScience, Sophia Antipolis;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC (Batch-No.: CDR9706)
7.	Test method	EU (=EEC): 92/69/EEC, V, B31; JMAF: 59 NohSan No. 4200, (1985); OECD: Draft 1998, for updating guideline 414; USEPA (=EPA): OPPTS 870.3700, (1998)
	Deviations	
8.	GLP	yes

Executive summary:

In a developmental toxicity study, ethiprole (92.7 % a.i.) was administered to 25 female Sprague Dawley Crl: CD (SD) BR rats/dose by gavage at dose levels of 0, 3, 10, or 30 mg/kg bw/day from gestation days 6 through 20.

No deaths occurred in the study. Adverse maternal effects were noted at 30 mg/kg bw/day as evidenced by body weight loss from GD 6 to 8 and reduced food consumption from GD 6 to 12. The general condition of females at 3 and 10 mg/kg bw/day were similar to those of controls. Macroscopic examination of females revealed increased incidence of accentuated lobular pattern in the liver at 30 mg/kg and increased mean liver weight at 10 and 30 mg/kg bw/day. **The maternal LOAEL is 10 mg/kg bw/day, based on increased mean liver weight. The maternal NOAEL is 3 mg/kg bw/day**.

Gestational parameters and mean fetal body weights were not affected by treatment. There was no indication of abnormal embryo-fetal development. At 10 mg/kg/day, a slight increase in ossification delay was observed for a few bones and at 30 mg/kg/day there was increased incidences of enlarged thymus. **The developmental LOAEL is 10 mg/kg bw/day, based on incidences of ossification delay for a few bones. The developmental NOAEL is 3 mg/kg bw/day.**

This study is classified **acceptable/ guideline** and statisfies the guideline requirement (OPPTS 870.3700; OECD 414) for a developmental toxicity study in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Ethiprole (RPA 107382)

Description:	White powder
Lot/Batch #:	CDR9706
Purity:	92.7% a.i.
Compound Stability:	The test substance was stored in an air-tight, light-resistant container at room temp.
CAS #of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** Control animals received 10 mL/kg body weight of vehicle alone (0.5% aqueous solution of methylcellulose 400 (Fluka, Mulhouse, France)).

3. **Test Animals*:**

Species:	Rat	
Strain:	Sprague Dawley Crl: CD (SD) BR	
Age/weight at study initiation:	Adult virgins / 237 to 301 g at mating*	
Source:	Charles River France (St Aubin-les-Elbeuf)	
Housing:	Individually in suspended, stainless steel, wire mesh cages	
Diet:	U.A.R. Certified Rodent meal A04C 10 (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France), *ad libitum*	
Water:	Filtered water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	20°C - 24°C
	Humidity:	40% - 70%
	Air changes:	approximately 15 changes /hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	13 days	

*NOTE: Age and weight of males was not provided in the study report.

B. PROCEDURES AND STUDY DESIGN:

1. **In-Life Dates:** May 25, 1999 to June 29, 1999

2. **Mating:** Females were mated on a one-to-one basis with stock males of the same strain and same supplier. Each morning following pairing, rats showing spermatozoa in a vaginal smear or sperm plug *in situ* were considered as pregnant animals. The day where evidence of mating was noted was designated as gestation day 0 (GD 0).

3. **Animal Assignment:** The females were assigned to control and treated groups as indicated in Table 1 using a body weight procedure for each day of pairing. Body weight means were checked after the mating period to ensure similar means among all groups.

Table 1. Study design

Dose (mg/kg bw/day)	0	3	10	30
Dose formulation concentration (g/L)*	0	0.3	1.0	3.0
Dosing volume (mL/kg)	10	10	10	10
# Females	25	25	25	25

4. **Dose selection rationale** - The dose levels were selected based on the results obtained in a previous range finding study in the rat (Study Number: SA 99036, PMRA 1730916) in which pregnant rats received ethiprole at doses of 0, 7.5, 25, 50 or 150 mg/kg bw/day. Dose-related reduction in body weight changes and food consumption at the beginning of the dosing period were observed at 25, 50 or 150 mg/kg bw/day. Based on these results, a dose of 30 mg/kg bw/day was expected to cause maternal toxicity in the present definitive developmental toxicity study.

5. **Dosage Preparation and Analysis:** Test material-vehicle mixture was prepared three times (F1, F2, and F3) by mixing appropriate amounts of test substance with an aqueous solution of 0.5% methylcellulose 400. The formulations were stored at approximately 5°C (± 3°C) while not in use. The suspensions were mixed continuously before and during dosing with an electromagnetic stirrer.

   Prior to study initiation, the stability of ethiprole in aqueous methylcellulose at concentrations of 0.075 and 15 g/L was demonstrated over 21 days under conditions similar to those used in the current study (Study Number: SA 99036). Results were not summarized in the current study report.

   The homogeneity (top, middle, and bottom) of the suspensions were checked on the first formulation (Fl) of each concentration (0.3, 1.0 and 3.0 g/L).

   Mean values obtained from the homogeneity checks were used as measured concentrations. In addition, all three concentrations of the second (F2) and third (F3) formulations were evaluated (single sample each of each dose level for each formulation).

   Results

   Homogeneity Analysis: Results for homogeneity of the first formulations of ethiprole in the vehicle at 0.3, 1.0, and 3.0 g/L were within the range of 87-98% of the nominal concentrations. (Data obtained from the study report, page 162).

   Stability Analysis: The suspensions were stable between 0.075 g/L and 15 g/L over a 21-day period at 5°C±3°C. (Data obtained from the study report, page 161).

   Concentration Analysis: Results for the concentration analyses were within 94-107% of the nominal concentrations. (Data obtained from the study report, page 163).

6. **Dosage Administration:** All doses were administered once daily by gavage, on gestation days 6 through 20 in a volume of 10 mL/kg of body weight/day. Dosing was based on the body weight on the most recent body weight determination.

C. OBSERVATIONS

1. **Maternal Observations and Evaluations**: The animals were checked for clinical signs daily. Mortality was checked twice daily, except for weekends and public holidays when mortality was checked once daily.

   Body weights were recorded on Gestational Days 0, 6, 8, 10, 12, 14, 16, 18 and 21. Body weight changes for the following intervals were determined: Gestational Days 0-6, 6-8, 8-10, 10-14, 14-18, 18-21, and 0-21 (corrected for gravid uterine weight). Full feeders were weighed on Gestational Days 1, 6, 8, 10, 12, 14, 16 and 18. Empty feeder weights were measured on Gestational Days 6, 8, 10, 12, 14, 16, 18 and 21. Food consumption was determined for the following intervals: Gestational Days 1-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18, and 18-21.

   On Gestational Day 21, surviving females were killed by inhalation of carbon dioxide, for examination of uterine content. Each female was first subjected to macroscopic examination of the visceral organs and the liver of each pregnant female was sampled, weighed and fixed in formol. The reproductive tract was weighed (gravid uterine weight), dissected out and the following parameters recorded: number of corpora lutea, number of implantations, number of resorptions, number of live and dead fetuses, sex of live fetuses, and the individual weights of live fetuses. Resorptions were classified as early (macroscopic discrimination between fetal residues and placental material not possible) and late (distinct macroscopic discrimination between fetal and placental remains possible). Dead fetuses were defined as fetuses showing distinct digits on fore-and hind-paws. Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulfide in order to visualize any sites which were not apparent.

2. **Fetal Evaluations**: Live fetuses were killed and subjected to an external examination. Approximately half of the live fetuses from each litter were immersed in Bouin fluid for subsequent internal examination following free-hand sectioning. The remaining half was skinned, eviscerated and then placed in absolute ethanol before staining of bone and cartilage. A modified staining technique (Tyl R. W. and Marr M. C., 1997) was used and a subsequent examination of bones and cartilages was performed. Structural deviations were classified as follows: malformations (very rare or obviously lethal changes), minor anomalies (slight, relatively rare structural changes not obviously detrimental), or common variants (structural changes occurring in more than approximately 5% of the control population).

D. DATA ANALYSIS

1. **Statistical Analyses:** Means and standard deviations for all maternal, litter and fetal parameters were calculated for each group. A detailed description of each statistical test was provided in the study report. A brief summary follows herein.

   Statistical analyses were performed on the following maternal endpoints: body weight change (Gestational Days 0-6, 6-8, 8-10, 10-14, 14-18, 18-21, and 0-21 (corrected for gravid uterine weight)), gravid uterine weight, liver weight, and food consumption in g/day (Gestational Days 1-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18, and 18-21) and food consumption in g/kg/day (Gestational Days 6-8, 8-10, 10-12, 12-14, 14-16, 16-18, 18-21 g/kg/day).

Statistical analyses were performed on the following litter based endpoints: number corpora lutea per dam, number implantation sites per litter, percent pre- and post-implantation loss per litter, and number early and late resorptions per litter.

The following fetal endpoints were analyzed statistically: fetal death status (% dead fetuses), fetal body weight of each fetus (combined, male, and female), and fetal sex (% male fetuses).

All maternal and litter parameters were analyzed using a combination of parametric ANOVA and nonparametric techniques, followed by adjustment for multiple comparisons and multiple endpoints via bootstrap resampling methods (Westfall and Young, 1993). Fetal parameters were analyzed using Generalized Estimating Equations (GEE) linear and logistic regression models, which assumed exchangeable intralitter correlations and used a robust variance estimator for regression parameters (Zeger and Liang, 1986). The linear regression model was used for analyzing fetal body weight and fetal death status. The logistic model was used for analyzing fetal sex. Although fetal death status is a binary outcome (dead vs. live for each fetus), this parameter was still analyzed via a linear regression model, because the distribution of the variable was too sparse for computations required for logistic regression. The GEE methods are robust to misspecifications of the response variance as well as assumptions about the intralitter correlation structure, as long as robust variance estimators are used. Individual /-tests for pairwise comparisons to control were evaluated when the overall treatment effect was statistically significant ($p<0.05$ via a Wald chi-square test), and a Wald chi-square test for linear trend was also conducted regardless of the significance of the overall treatment effect.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

$$\textbf{Pre-implantation loss index} = \frac{\textbf{(Number of corpora lutea - Number of implantations)}}{\textbf{Number of corpora lutea}} \times 100$$

$$\textbf{Post-implantation loss index} = \frac{\textbf{(Number of implantations - Number of live fetuses)}}{\textbf{Number of implantations}} \times 100$$

$$\textbf{Percentage of live fetuses (per litter)} = \frac{\textbf{Number of live fetuses}}{\textbf{(Number of live fetuses + Number of dead fetuses)}} \times 100$$

$$\textbf{Percentage of dead fetuses (per litter)} = \frac{\textbf{Number of dead fetuses}}{\textbf{(Number of live fetuses + Number of dead fetuses)}} \times 100$$

$$\textbf{Percentage of male fetuses} = \frac{\textbf{(Number of male fetuses)}}{\textbf{Total number of live fetuses}} \times 100$$

3. **Historical Control Data:** Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and Clinical Observations:** No deaths occurred during the study and all females were euthanized on Gestational Day 21. There were no treatment-related clinical signs. In the treated groups, the general conditions of females were similar to those of controls. A few observations concerned fur (hair loss, soiled), incisors (broken) and eyes (ocular discharge) and were unrelated to treatment

2. **Body Weight:** Body weight data are summarized in Table 2 and as follows: No treatment-related effects on maternal body weight or body weight gains were noted at the 3 mg/kg bw/day dose level.

   In the 10 mg/kg bw/day group, the body weight gain from Gestational Days 6 to 8 was reduced (-43%) when compared to the control group, but was not statistically significant. Nevertheless, from Gestational Days 8 to 21, no changes in body weight gain were recorded at 10 mg/kg bw/day when compared to control.

   At 30 mg/kg bw/day, a statistically significant body weight loss (-0.6 g) was noted during Gestational Days 6 to 8, while the control group gained weight (6.0 g). Further, the mean body weights at 30 mg/kg bw/day were lower than controls from Gestational Days 8 to 21.

TABLE 2. Mean (±SD) Maternal Body Weight (g) and Maternal Body Weight Change (g)[a]

Interval	Dose in mg/kg bw/day			
	0 (n=25)	3 (n=23)	10 (n=23)	30 (n=25)
Maternal Body Weight (g)				
Pretreatment: Day 0	267.0± 2.6	267.6± 3.1	267.1± 2.7	267.7± 3.6
Pretreatment: Day 6	301.0± 3.2	299.4± 3.3	301.1± 3.7	300.6± 4.1
Treatment Day 8	307.0+ 3.5	305.0+ 3.8	304.5± 4.5	300.0± 4.4
Treatment Day 10	317.7± 3.5	315.1± 4.1	315.9± 4.3	309.6± 4.4
Treatment Day 12	328.8± 3.8	324.2± 4.1	328.0± 4.4	320.6± 4.8
Treatment Day 14	337.7± 3.9	334.0± 4.3	338.8± 4.9	329.3± 5.0
Treatment Day 16	355.2± 4.5	353.5± 4.5	357.2± 5.3	347.2± 5.5
Treatment Day 18	383.4± 5.0	382.7± 4.4	387.2± 5.7	377.9± 6.0
Treatment Day 21	438.8± 6.6	439.9± 5.1	443.7±7.0	430.5± 7.3
Maternal Body Weight Change (g)				
Pretreatment: Day 0 to 6	34.1± 1.6	31.8± 1.6	34.0±2.1	32.9± 1.1
Treatment Day 6 to 8	6.0± 1.0	5.6± 0.9	3.4±1.7	-0.6±0.7***
Treatment Day 8 to 10	10.7±0.6	10.1± 0.7	11.3±1.3	9.7± 0.8
Treatment Day 10 to 14	20.04± 0.8	18.9± 0.9	23.0±1.1	19.6± 1.1
Treatment Day 14 to 18	45.7± 2.0	48.7± 1.1	48.4±1.6	48.6± 1.6
Treatment Day 18 to 21	55.4± 2.4	57.1± 1.5	56.5±1.9	52.6± 1.9
Pretreatment: Day 6 to 21[b]	137.8	140.5	142.6	129.9
Corrected Body Weight Change[c]	65.8± 3.6	64.0± 3.6	66.8±4.8	56.8± 3.2
Gravid Uterine Weight (g)	106. 1±4.4	108.3±2.3	109. 8±2.6	106. 0±3.0

a Data obtained from the study report pages 32-34.
b Calculated by reviewer
c Corrected body weight change = (Body weight on Gestational Day 21 - Body weight on Gestational Day 0) - (gravid uterine weight)
*** Statistically different ($p < 0.001$) from the control.

3. **Food Consumption:** Food consumption data are summarized in Table 3. At 3 and 10 mg/kg bw/day, food consumption was considered unaffected by treatment. At 30 mg/kg bw/day, food consumption was statistically significantly reduced during intervals Gestational Days 6-8, 8-10 and 10-12 when compared to controls.

TABLE 3. Mean (±SD) Maternal Food Consumption in (g/kg/day)[a]

Interval	Dose in mg/kg bw/day			
	0 (n=25)	3 (n=23)	10 (n=23)	30 (n=25)
Maternal Food Consumption (g/kg/day)				
Treatment: Days 6 to 8	90.5±1.9	91.2±1.8	88.5±2.8	81.1***±1.5
Treatment: Days 8 to 10	91.1±1.7	90.7± 2.7	90.1± 1-7	81.8**±2.1
Treatment: Days 10 to 12	87.8±1.5	87.8± 1.6	88.4± 1.8	81.0*±1.8
Treatment: Days 12 to 14)	85. 9± 1.0	86.1±1. 8	86.8±2.3	84.1±1.7
Treatment: Days 14 to 16	80.1± 1.8	82.3±1.6	84.2± 1.5	80.3±1.6
Treatment: Days 16 to 18	82.4±1.3	82.4±1. 9	83.6±1.5	83.5±1.3
Treatment: Days 18 to 21	71.2± 0.9	72.3± 1.2	72.6± 1.6	72.2±1.3

a Data obtained from the study report pages 35-37.
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.
*** Statistically different (p <0.001) from the control.

4. **Gross Pathology:** Gross pathological findings are summarized as follows. Maternal liver weight was statistically significantly increased at 10 and 30 mg/kg bw/day when compared to control value (see Table 4). Accentuated lobular pattern of the liver lobes was observed in five pregnant females at 30 mg/kg bw/day but not seen in the control group. Other findings were not considered to be treatment-related as no apparent dose-related effect was noted.

TABLE 4. Absolute and Relative Maternal Liver Weights[a]

Organ	Dose in mg/kg bw/day			
	0 (n=25)	3 (n=23)	10 (n=23)	30 (n=25)
Abs. Liver Weight (g)	14.2±0.3	14.8±0.4	16.4***±0.4	18.8***±0.4
Rel. Liver Weight (%)[b]	3.2	3.4	3.7	4.3

a Data obtained from the study report page 34.
b Calculated by reviewer: (Abs. Liver Weight/Body weight on Gestational Day 21) x 100%
*** Statistically different (p <0.001) from the control.

5. **Cesarean Section Data:** Data are summarized in Table 5 and as follows. There were no significant effects of treatment on gestational parameters, including early and late resorptions, fetal death status and percent of male fetuses. Analysis of mean fetal body weights (calculated by sex and sex combined) revealed no significant differences between treated and control groups.

TABLE 5. Cesarean Section Observations [a]

Observation	Dose in mg/kg bw/day			
	0	3	10	30
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant **Pregnancy Rate (%)**	25 (100)	23 (92)	23 (92)	25 (100)
# Nonpregnant	0	2	2	0
Maternal Wastage:				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
No. Corpora Lutea per Dam	16.9± 0.3	17.2±0.5	17.0± 0.4	17.0± 0.4
No. Implantation Sites per Litter	15.6±0.7	16.1±0.3	16.1±0.4	15.5± 0.4
Percent Pre-implantation Loss per Litter	7.6±3.6	5.4± 1.6	4.9± 1.3	8.4±1.8
No. Early Resorptions per Litter	0.7± 0.2	1.0± 0.3	1.0± 0.2	0.8±0.2
No. Late Resorptions per Litter	0.1±0.1	0.0±0.0	0.0± 0.0	0.1± 0.1
Percent Post-implantation Loss per Litter	6.6± 2.1	6.0±1.6	6.5± 1.4	5.2±1.0
No. Live Fetuses per Litter	14.8± 0.7	15.1±0.3	15.0±0.4	14.7± 0.4
No. Dead Fetuses per Litter	0	0	0	0
No. Female Fetuses per Litter	7.4±0.5	7.6±0.5	7.3±0.4	7.9±0.5
No. Male Fetuses per Litter	7.4± 0.4	7.5±0.4	7.7±0.5	6.8±0.5
Fetal Death Status (% dead fetuses)	0 (N=369)	0 (N=348)	0 (N=346)	0 (N=367)
Percent Male Fetuses	50.1±2.2 (N=369)	49.7±2.8 (N=348)	51.2±2.6 (N=346)	46.3±3.0 (N=367)
Fetal Body Weight (g)	5.38± 0.05 (N=369)	5.33±0.06 (N=348)	5.40±0.05 (N=346)	5.35± 0.06 (N=367)
Male Fetal Body Weight (g)	5.52±0.05 (N=185)	5.48±0.06 (N=173)	5.47±0.06 (N=177)	5.51± 0.06 (N=170)
Female Fetal Body Weight (g)	5.24±0.05 (N=184)	5.19±0.06 (N=175)	5.33±0.04 (N=169)	5.22±0.07 (N=197)

a Data obtained from pages 32, 39, 40, and 41 in the study report.

B. DEVELOPMENTAL TOXICITY: Developmental toxicity was observed at the high dose level as increased incidences of a skeletal anomaly (short, discontinuous or absent costal cartilage on the 13th rib), skeletal variations, and a visceral variation (enlarged thymus). No treatment-related malformations were noted at any dose level.

1. **External Examination:** A summary of the external examinations is presented in Table 6a. No abnormal external fetal observations were noted in any groups and the number of runt fetuses (BW <4.0 g) was not increased in a dose-related manner.

2. **Visceral Examination:** A summary of selected findings from the internal examinations is presented in Table 6b. No observed malformations or anomalies were considered to be treatment-related. The incidence of enlarged thymus appeared increased at 30 mg/kg bw/day. All variations were distributed among all groups, although none of the other observed variations were considered to be related to treatment as a relationship to dose level was not noted.

3. **Skeletal Examination:** A summary of the skeletal examinations is presented in Table 6c. No dose-related increase in skeletal malformations was observed. A few anomalies were observed in different groups but no dose-related increase was noted except at 30 mg/kg bw/day, where a higher incidence of short 13th ribs (with no, a short or a discontinuous costal cartilage) and a bipartite thoracic centrum were observed. At 30 mg/kg/day, a slight ossification delay was noted for the 7th cervical centrum, lst metatarsal and for the dumbbell thoracic centrum. Increased incidences of unossified 1st metatarsal noted at 3 and 10 mg/kg bw/day, and of dumbell thoracic centrum noted at 10 mg/kg bw/day were either within, or barely exceeding, historical control ranges (table 7), and therefore, not considered to be adverse. In all cases, the associated cartilages were present and normal.

TABLE 6a. External Examinations [a]

	Dose (mg/kg bw/day)				Dose (mg/kg bw/day)			
	0	3	10	30	0	3	10	30
	Number of fetuses examined				Number of litters examined			
	369	348	346	367	25	23	23	25
Findings	Number (%) fetuses affected				Number (%) litters affected			
Variation:								
Runt (body weight <4.0g).	2 (0.6)	5 (1.5)	1 (0.3)	1 (0.2)	2 (8.0)	3 (13.0)	1 (4.3)	1 (4.0)

a Data obtained from the study report, page 43.

TABLE 6b. Visceral Examinations [a]

Observation	Dose (mg/kg bw/day)				Dose (mg/kg bw/day)			
	0	3	10	30	0	3	10	30
	Number of fetuses examined				Number of litters examined			
	180	168	169	176	24	23	23	25
	Number (%) fetuses affected				Number (%) litters affected			
Variations:								
Thymus (unilateral/bilateral): enlarged	7 (3.8)	2 (1.2)	6 (3.6)	16 (9.3)	5 (20.8)	2 (8.7)	4 (17.4)	11 (44.0)
Ureter(s) (unilateral/bilateral): convoluted	66 (36.8)	46 (26.8)	47 (27.5)	59 (33.3)	23 (95.8)	18 (78.3)	19 (82.6)	21 (84.0)
Ureter(s) (unilateral/bilateral): dilated	54 (30.6)	33 (18.9)	39 (23.2)	48 (26.5)	20 (83.3)	15 (65.2)	19 (82.6)	17 (68.0)
Renal pelvis(es) (unilateral/bilateral): dilated	24 (13.6)	8 (4.2)	18 (10.8)	15 (8.1)	12 (50.0)	6 (26.1)	9 (39.1)	9 (36.0)
Anomalies:								
Esophagus: dilated	4 (2.1)	3 (1.8)	3 (1.4)	2 (1.1)	4 (16.7)	2 (8.7)	1 (4.3)	2 (8.0)
Left ventricle: enlarged.	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
Renal papilla (unilateral/bilateral): enlarged	1 (0.5)	2 (1.3)	1 (0.6)	2 (1.1)	1 (4.2)	2 (8.7)	1 (4.3)	2 (8.0)
Renal papilla (unilateral): absent	0 (0.0)	2 (1.1)	1 (0.5)	0 (0.0)	0 (0.0)	2 (8.7)	1 (4.3)	0 (0.0)
Malformations:								
Esophagus:malpositioned (laterally); aortic arch and ascending aorta: dilated; aorta:abnormal origin from right ventricle, ventricular septum defect in cranial region; all lung lobes and kidney (unilateral): misshapen; adrenal glands (bilateral): enlarged	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)	0 (0.0)
Right ventricle: enlarged and wall partially thin with muscular defect; ventricle septum: thin; ascending aorta and aortic arch: dilated; aortic valves: absent.	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)

a Data obtained from page 44 in the study report.

TABLE 6c. Skeletal Examinations [a]

Observation	Dose (mg/kg bw/day)				Dose (mg/kg bw/day)			
	0	3	10	30	0	3	10	30
	Number of fetuses examined				Number of litters examined			
	189	180	177	191	25	23	23	25
	Number (%) fetuses affected				Number (%) litters affected			
Variations:								
Interparietal/parietal suture (unilateral) or posterior fontanelle: extra ossification site	1 (0.5)	3 (1.5)	0 (0.0)	1 (0.4)	1 (4.0)	3 (13.0)	0 (0.0)	1 (4.0)
Parietal (unilateral) or interparietal: incompletely ossified; or interparietal: split; or anterior fontanelle: enlarged	2 (0.9)	4 (2.3)	2 (1.1)	3 (1.5)	2 (8.0)	3 (13.0)	2 (8.7)	2 (8.0)
Hyoid centrum: incomplete ossification;/ normal cartilage.	2 (1.1)	0 (0.0)	3 (1.7)	3 (1.5)	1 (4.0)	0 (0.0)	3 (13.0)	3 (12.0)
7th cervical centrum: unossified / normal cartilage.	6 (3.2)	4 (2.2)	14 (7.9)	13 (6.8)	5 (20.0)	3 (13.0)	7 (30.4)	9 (36.0)
2nd and/or 3rd and/or 4th and/or 5th sternebrae: misaligned / normal cartilage and/or 2nd stenebra: incompletely ossified / normal cartilage.	6 (3.4)	8 (4.6)	7 (4.1)	6 (2.9)	5 (20.0)	7 (30.4)	6 (26.1)	3 (12.0)
5th and/or 6th sternebra: unossified / normal cartilage.	1 (0.6)	2 (1.2)	1 (0.5)	3 (1.4)	1 (4.0)	2 (8.7)	1 (4.3)	2 (8.0)
14th thoracic rib (unilateral).	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
14th thoracic rib (unilateral/bilateral): short.	5 (2.5)	0 (0.0)	0 (0.0)	7 (3.9)	4 (16.0)	0 (0.0)	0 (0.0)	5 (20.0)
Extra ossification point(s) (unilateral/bilateral) on 14th thoracic vertebra	11 (5.7)	10 (5.6)	5 (2.6)	10 (5.4)	7 (28.0)	7 (30.4)	5 (21.7)	8 (32.0)
Presence of 27 presacral vertebrae.	1 (0.5)	2 (1.0)	0 (0.0)	1 (0.4)	1 (4.0)	2 (8.7)	0 (0.0)	1 (4.0)
Thoracic centrum: dumbbell / normal cartilage.	6 (3.2)	6 (3.5)	12 (7.6)	18 (9.6)	4 (16.0)	6 (26.1)	7 (30.4)	14 (56.0)
1st metatarsal (bilateral): unossified / normal cartilage.	11 (5.6)	25 (14.0)	24 (13.8)	55 (29.1)	6 (24.0)	11 (47.8)	11 (47.8)	19 (76.0)
3rd and 4th proximal phalanges of forepaws (bilateral): unossified / normal cartilage	1 (0.6)	2 (1.3)	3 (1.6)	4 (2.4)	1 (4.0)	2 (8.7)	2 (8.7)	3 (12.0)
Anomalies:								
13th rib(s) (unilateral/bilateral): short / discontinuous costal cartilage or costal cartilage absent.	4 (2.1)	1 (0.6)	3 (1.7)	9 (5.0)	3 (12.0)	1 (4.3)	2 (8.7)	6 (24.0)
7th costal cartilages (bilateral) not attached to the sternum.	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.0)
4th costal cartilage (unilateral): misshapen.	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
Presence of 25 presacral vertebrae.	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.5)	0 (0.0)	0 (0.0)	1 (4.3)	1 (4.0)
Thoracic centrum: bipartite and/or dumbbell or bipartite cartilage	2 (1.1)	2 (0.6)	2 (1.3)	5 (2.5)	2 (8.0)	1 (4.3)	2 (8.7)	5 (20.0)
Malformations:								
Cervical rib (unilateral) on 7th cervical vertebra: short, 3rd and 4th cervical centrum: cartilage bipartite and fused (bilateral)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
1st and 2nd costal cartilage (unilateral): fused and/or 2nd costal cartilage(s) (unilateral/bilateral): not attached to the sternum and/or 1st and 2nd sternebrae : fused	0 (0.0)	0 (0.0)	4 (1.9)	1 (0.5)	0 (0.0)	0 (0.0)	1 (4.3)	1 (4.0)

a Data obtained from page 45-47 in the study report.

Table 7. Historical Control Data for Select Skeletal Variations [a]

Finding	% Incidence (fetal range)	% Incidence (litter range)
7th cervical centrum unossified	1.7 - 11.8	8.3 - 41.7
Thoracic centrum-dumbbell ossification	0 - 7.2	0 - 39.1
1st metarasal (bilateral) unossified	0 - 13.2	0 - 40

[a] Results from 8 studies conducted between March 1998 and January 2006.

III. CONCLUSIONS:

1. Maternal toxicity: No deaths occurred in the study. Adverse maternal effects were noted at 30 mg/kg bw/day through body weight loss from GD 6 to 8 and reduced food consumption from GD 6 to 12. The general conditions of females at 3 and 10 mg/kg bw/day were similar to those of controls. Macroscopic examination of females revealed increased incidence of accentuated lobular pattern at 30 mg/kg and increased mean liver weight at 10 and 30 mg/kg bw/day. The liver is a known target organ for toxicity in the rat following ethiprole administration, and a histopathological examination of the liver was not conducted in the study (nor is it required in a developmental toxicity study). Therefore, the possibility of microscopic changes in the liver at the 10 mg/kg bw/day dose level cannot be dismissed. **Therefore, the maternal LOAEL is set at 10 mg/kg bw/day, based on increased mean liver weight. The maternal NOAEL is 3 mg/kg bw/day.**

2. Developmental toxicity:

a. Deaths/Resorptions: There were no significant effects of treatment on gestational parameters, including early and late resorptions, fetal death status and percent of male fetuses.

b. Altered Growth: No altered growth found at any dose level.

c. Developmental Variations:
The study author considered there to be an increase in ossification delay for the 7th cervical centrum, 1st metatarsal, and for the dumbell thoracic centrum at 30 mg/kg bw/day; however did not consider any increase in variations to be present at the lower dose levels. Although there were apparent increases in the incidences of unossified 1st metatarsal and dumbell thoracic centrum at the lower dose levels of 3 and 10 mg/kg bw/day, after receipt of the requested historical control data the reviewer concedes that the incidences were within or just barely surpassing the historical control range for these variations. Therefore, these variations were not considered to be adverse under the condition of the present study, and the reviewer agrees with the conclusions reached by the study author.

d. Malformations: No malformations or anomalies were considered to be treatment-related.

The developmental LOAEL is 10 mg/kg bw/day, based on incidences of ossification delay for a few bones. The developmental NOAEL is 3 mg/kg bw/day.

KIIA 5.6.2.2- Teratogenicity test by the oral route in the rabbit – Range-finding

1.	Annex point(s)	KIIA 5.6.2.2 Teratogenicity test by the oral route in the rabbit
2.	Location in dossier	KIIA 5.6.11 /01
3.	Authors (year)	Foulon, O. (1999)
	Title	Ethiprole (RPA 107382) Range-Finding Study for Developmental Toxicity in the Rabbit by Gavage
	Company, report No.	Bayer CropScience AG, Report-No.: SA99078
	Date	15.10.1999
4.	Testing facility	Aventis CropScience, Sophia Antipolis;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC (Batch-No.: CDR9706)
7.	Test method	EU (=EEC): 92/69/EEC, V, B31; JMAF: 59 NohSan 4200, (1985); OECD: Draft 1998, for updating 414, (1981); USEPA (=EPA): OPPTS 870.3700
	Deviations	
8.	GLP	yes

Executive summary

In a range-finding developmental toxicity study, ethiprole (92.7 % a.i.) was administered to 8 female New Zealand White rabbits /dose by gavage at dose levels of 0, 0.25, 0.75, 2 or 5 mg/kg bw/day from gestation days 6 through 28.

At 5 mg/kg bw/day, pregnant females presented a body weight loss from GD 6 to 18 in correlation with reduced food intakes. All females in this group produced low amounts of feces for several days during the study period. At 2 mg/kg bw/day, although no statistically significant observations were noted, the mean body weight gain and food consumption were lower than the control values during the first half of the dosing period. The general conditions of females at 0.25 and 0.75 mg/kg bw/day were considered similar to those of the controls. At necropsy, no maternal gross findings were considered treatment-related.

When examining the litter parameters for all pregnant females, an increase in post-implantation loss at 2 mg/kg bw/day was seen. This includes the two females found with implantations only and one with total resorptions following impregnation. However, none of the litter parameters were statistically significantly different when compared to control values. The external foetal malformations observed in the one litter at 5 mg/kg bw/day were probably spontaneous and not treatment-related.

This study is classified **acceptable/non-guideline** providing data for use in dose selection for the definitive developmental toxicity study in rabbits.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Ethiprole (RPA 107382)

Description:	White powder
Lot/Batch #:	CDR9706
Purity:	92.7% a.i.
Compound Stability:	The test substance was stored in an air-tight, light-resistant container at room temp.
CAS #of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** Control animals received 4 ml/kg body weight of vehicle alone (0.5% aqueous solution of methylcellulose 400 (Fluka, Mulhouse, France), based on the most recent body weight recorded.

3. **Test Animals:**

Species:	Rabbits	
Strain:	New Zealand White rabbits Crl: Kbl/BR (SPF)	
Age/weight at study initiation:	Sexually mature and nulliparus females/ 2.96-4.01 kg at Gestational Day 0	
Source:	(Charles River, Saint-Aubin-les-Elbeuf, France)	
Housing:	Rabbits housed individually in plastic cages on a perforated cage floor.	
Diet:	Certified Rabbit Pellet diet UAR 110C-10 (Usine d'Alimentation Rationnelle, 91360 Villemoisson-sur-Orge, France), *ad libitum*	
Water:	Filtered water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	17°C - 21°C
	Humidity:	40% - 70%
	Air changes:	approximately 15 changes per hour (not monitored)
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	19 days	

B. PROCEDURES AND STUDY DESIGN

1. **In-Life Dates:** March 29, 1999 to April 30, 1999

2. **Mating:** Female rabbits were artificially inseminated with semen of fertile males of the same strain and the same supplier. The day of insemination was designated as Gestation Day 0.

3. **Animal Assignment:** females were allocated to the different groups (using a body weight dependent procedure) to ensure similar body weight means among all groups on GD 0. Permanent identification numbers were assigned to animals within each group. Each animal was identified by a cage card and an ear tag bearing a unique sequential number.

Table 1. Study design

Dose (mg/kg /day)	0	0.25	0.75	2	5
# Females	8	8	8	8	8

4. **Dose selection rationale:** Doses were selected in agreement with the sponsor representative based on results obtained in a previous range-finding study (M. Repetto-Larsay, 1996) where pregnant females received 0, 0.3, 1, 3 and 10 mg/kg bw/day from GD 6 to 19.

5. **Dosage Preparation and Analysis:** The appropriate amount of test substance was suspended (w/v) for the two formulations F1 and F2 in an aqueous solution of methylcellulose 400 (Fluka, Mulhouse, France) at 0.5% and stored at approximately 5°C (± 3°C). Before the start of the dosing, homogeneity of the suspensions was first checked on the first formulation (F1) for the lowest and highest concentrations. Concentrations of 187.5 and 500 mg/l for F1 and all concentrations for the second formulation (F2) were checked. Stability of the test substance in suspension in the vehicle at the lowest concentration (62.5 mg/l) was determined during the study. Stability of ethiprole at higher concentrations (75 and 15000 mg/l) were demonstrated in a previous study (O. Foulon, 1999). The suspensions were mixed continuously before and during dosing with an electromagnetic stirrer.

Results

Homogeneity Analysis: Homogeneity results for preparations at 62.5 and 1250 mg/L of the first formulation sequence were within the range of 95-103% of the nominal concentrations. (Data obtained from the study report, page 85).

Stability Analysis: After 21 days of storage at 5±3°C, the ethiprole formulations in aqueous methylcellulose at 62.5 mg/L were 98-99% of the nominal concentrations, demonstrating stability for the intervals and conditions used in this study. (Data obtained from the study report, page 89)

Concentration Analysis: Results for the concentration analyses were within 95-103% of the nominal concentrations. (Data obtained from the study report, page 88).

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage Administration:** All doses were administered once daily by gavage, on gestation days 6 through 28, in a volume of 4 mL/kg of body weight/day. Dosing was based on the body weight on the most recent body weight determination

C. OBSERVATIONS

1. **Maternal Observations and Evaluations:** The animals were checked for mortality or clinical signs daily. Body weight was recorded on GD 0, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 29. Food consumption data were recorded on gestation days 1 and every day through 28. Dams were sacrificed on day 29 of gestation. Examinations at sacrifice consisted of: macroscopic examination of the visceral organs and the number of ribs was recorded. The liver of each pregnant female was collected and weighed. The reproductive tract was weighed (gravid uterine weight), dissected out and the following parameters recorded: number of

corpora lutea, number of implantations, number of resorptions, number of live and dead fetuses, sex of live fetuses, and the individual weights of live fetuses.

2. **Fetal Evaluations:** Live fetuses were killed by intrathoracic injection (0.02 ml/fetus) of Dolethal (Sanofi, Libourne, France) and subjected to an external examination. Dead fetuses were also subjected to an external examination. Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulfide to visualize any sites which were not apparent. Tissue and carcass were discarded. Resorptions were classified as early (macroscopic discrimination between fetal residues and placental material not possible) and late (distinct macroscopic discrimination between fetal and placental remains possible). Dead fetuses were defined as fetuses showing distinct digits on fore-and hind-paws.

D. DATA ANALYSIS:

1. Statistical Analyses: Maternal and Litter-Based Endpoints

For endpoints measured on the *dam* or the *litter* as a unit, we used a combination of standard ANOVA and nonparametric data analysis techniques. Examples of endpoints measured on the dam included *maternal body weight gain throughout gestation, corrected body weight gain, liver weights,* and *feed consumption throughout gestation.* Since these parameters are all measured on acontinuous scale, the treatment groups were compared using either parametric ANOVA or nonparametric techniques. The homogeneity of variance assumption was examined via Bartlett's test (Bartlett, 1937), implemented through a user-written SAS® macro. If Bartlett's test indicated lack of homogeneity of variance (p <0.05), nonparametric tests were used. Nonparametric testsincluded the Kruskal-Wallis test (Hollander and Wolfe, 1973) for overall treatment group differences, followed by the Mann-Whitney U test for individual exposed vs. control groupcomparisons, if the Kruskal-Wallis test was significant (p <0.05). Jonckheere's test (Jonckheere, 1954) was used to examine dose-response trends. All nonparametric tests (except Jonckheere's test) were performed using the NPAR1WAY procedure in SAS® 6.12 (SAS Institute, 1996). Jonckheere's test was implemented through a user-written SAS® macro.

If Bartlett's test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 (SAS Institute, 1996) was used to evaluate the overall effect of treatment, test the significance of the linear dose-response relationship, and, when a significant treatment effect was present (p<0.05), to compare each exposed group to control via Dunnett's test (Dunnett, 1955, 1964). Examples of *litter-based* endpoints include number of *corpora lutea, implantations, early and late resorptions,* and the *pre- and post-implantation loss percentage.* These parameters were analyzed using the same methods as for body weights in SAS® 6.12. Namely, the treatment groups were compared using either parametric ANOVA or nonparametric techniques. The homogeneity of variance assumption was examined via Bartlett's test. If Bartlett's test indicated lack of homogeneity of variance, nonparametric tests were used. Nonparametric tests included the Kruskal-Wallis test for overall treatment group differences, followed by the Mann-Whitney U test for individual exposed vs. control group comparisons, if the Kruskal-Wallis test was significant. Jonckheere's test was used to examine dose-response trends. If Bartlett's test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 was used to evaluate the overall effect of treatment, test the significance of the linear dose-response relationship, and, when a significant treatment effect was present, to compare each exposed group to control via Dunnett's test.

Fetal Endpoints

Fetal endpoints are those which are measured on each pup or fetus in the litter. In the range-finding study, such endpoints included fetal death status (live vs dead) and fetal body weight (continuous). When multiple pups or fetuses from the same litter are used in the analysis, care must be taken to adjust for the resulting correlation of responses within litters. Intracluster correlation, or the tendency for littermates to respond similarly, tends to inflate the true variance of parameter estimates, including percentages and regression coefficients used in these analyses. In other words, fetuses within litters are not statistically independent, and failure to account for this in the statistical analysis will result in underestimated standard errors and false-positive tests for treatment effects (Haseman and Kupper, 1979). Hence, the chance of finding false-positive results is increased when the clustering is ignored.

To incorporate the effects of intracluster correlation and reduce the chances of finding spurious results, a model-fitting method, designed specifically for clustered data in which the outcomes may or may not be normally distributed, is recommended. Recent advances in analyzing longitudinal and cluster-correlated data for generalized linear models (Zeger and Liang, 1986; Zeger, Liang, and Albert, 1988) have led to new methods for handling binary, categorical, and continuous outcomes on fetuses clustered within litters. The techniques (otherwise referred to as Generalized Estimating Equations or GEEs) make no strict distributional assumptions about the endpoint of interest (e.g., normality) or the correlations among clustered observations, thereby providing flexibility for a variety of analytical settings.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

Corrected body weight change = (Body weight on GD 21 –Body weight on GD 0) – (gravid unterine weight)

$$\textbf{Pre-implantation loss index} = \frac{\textbf{(Number of corpora lutea - Number of implantations)}}{\textbf{Number of corpora lutea}} \times 100$$

$$\textbf{Post-implantation loss index} = \frac{\textbf{(Number of implantations - Number of live fetuses)}}{\textbf{Number of implantations}} \times 100$$

$$\textbf{Percentage of live fetuses (per litter)} = \frac{\textbf{Number of live fetuses}}{\textbf{(Number of live fetuses + Number of dead fetuses)}} \times 100$$

$$\textbf{Post-implantation loss index} = \textbf{100 – percentage of live fetuses}$$

2. **Historical Control Data:** Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and Clinical Observations:** One female at 5 mg/kg bw/day and one female at 0.75 mg/kg bw/day aborted on GD 26 and 20, respectively. On GD 24, one control female (JR1F0319) died after clonic convulsions, dyspnea and nasal discharge; the autopsy confirmed a gavage error. In the 5 mg/kg bw/day group, all females had few feces during several days. No other clinical signs were considered treatment-related. The pregnancy rate was between 50 and 100% and at least 4 females per group had live fetuses at caesarean section. The variability in pregnancy rate was due to the low fertility of a few males used for insemination.

2. **Body Weight:** Body weight data are summarized in Table 2 and as follows: At 5 mg/kg bw/day, a body weight loss was noted from GD 6 to 18 (-0.30kg) while the control group maintained or gained weight. In the high dose group, the body weight change values during gestation was significantly different (weight loss or decreased weight gain) compared to control values during every interval starting from GD 6. At 2 mg/kg bw/day, the mean body weight change was slightly lower than in the control group during the first half of the dosing period but was not significantly different. At 0.25 and 0.75 mg/kg bw/day, the mean body weight changes were considered unaffected by treatment. Other body weight analysis indicated a significant dose-related decrease in corrected body weight gain from GD 0-29 although no individual dose groups were significantly different from controls.

Table 2. Maternal body weight (kg) and body weight gain (kg)[a]

mg/kg bw/d	0 n = 8	0.25 n = 8	0.75 n = 8	2 n = 8	5 n = 8
Initial BW (pretreatment)	3.21 ± 0.03	3.21 ± 0.02	3.25 ± 0.03	3.27 ± 0.02	3.24 ± 0.02
BW Day 6 (pretreatment)	3.48 ± 0.06	3.50 ± 0.06	3.48 ± 0.04	3.48 ± 0.04	3.47 ± 0.02
BW day 14 (treatment)	3.58 ± 0.09	3.63 ± 0.12	3.61 ± 0.07	3.53 ± 0.05	3.20 ± 0.06
BW day 22 (treatment)	3.74 ± 0.09	3.77 ± 0.09	3.76 ± 0.09	3.69 ± 0.04	3.25 ± 0.09
BW day 29 (treatment)	3.86 ± 0.10	3.86 ± 0.11	3.92 ± 0.11	3.84 ± 0.04	3.59 ± 0.03
Gravid uterine weight	0.40 ± 0.06	0.28 ± 0.07	0.42 ± 0.08	0.49 ± 0.03	0.26 ± 0.05
Corrected BW Change	0.25 ± 0.05	0.38 ± 0.15	0.25 ± 0.12	0.12 ± 0.04	0.09 ± 0.06
BWG Day 0-6	0.28 ± 0.05	0.29 ± 0.05	0.24 ± 0.03	0.21 ± 0.03	0.23 ± 0.02
BWG Days 6-18	0.19 ± 0.03	0.21 ± 0.05	0.18 ± 0.03	0.12 ± 0.04	-0.30**± 0.09
BWG Days 6-29	0.37 ± 0.05	0.37 ± 0.05	0.44 ± 0.07	0.36 ± 0.04	0.13**± 0.04

[a] Data obtained from pages 31-33 in the study report.
** Significantly different from control, p<0.01
BW body weight, BWG body weight gain

3. **Food Consumption:** Food consumption data are summarized as follows: Maternal food consumption was significantly (p<0.05) reduced in a dose-related fashion during every time interval from GD 6 through 22 and significantly increased in a dose-related fashion during GD 22-26 and GD 26-29. Food consumption was significantly reduced in the high dose group vs controls during the interval GD 6-8, GD 8-10, GD 10-14 and GD 14-18. At 2 mg/kg bw/day, slight reductions of food intakes were noted at some intervals during the dosing period without showing any statistically significant differences. At 0.25 and 0.75 mg/kg bw/day, the mean food consumption were considered unaffected by treatment.

Table 3. Mean (±SD) Maternal Food Consumption in (g/day)[a]

mg/kg bw/d	0 n = 8	0.25 n = 8	0.75 n = 8	2.00 n = 8	5.00 n = 8
			Female		
FC Days 1 – 6	201.5 ± 10.4	212.5 ± 8.5	197.2 ± 11.3	203.9 ± 7.7	213.0 ± 3.4
FC Days 6 – 8	188.3 ± 11.8	193.0 ± 16.8	178.4 ± 13.9	181.4 ± 11.2	123.5**± 12.5
FC Days 8 – 10	188.8 ± 12.1	197.3 ±12.8	170.5 ± 6.2	164.1 ± 13.1	52.8**± 20.1
FC Days 10 – 14	173.3 ± 18.9	203.9 ± 14.1	180.5 ± 11.8	162.0 ± 13.3	35.0**± 13.7
FC Days 14 - 18	184.1 ± 14.3	178.8 ± 29.2	174.0 ± 7.5	157.9 ± 12.3	57.0**± 23.7
FC Days 26 - 29	134.9 ± 4.9	132.4 ± 28.0	149.1 ± 18.0	147.4 ± 13.0	180.2 ± 11.3

a Data extracted from pages 35-36 of the study report
* Statistically different from control, p<0.05

** Statistically different from control, p<0.01
FC food consumption

4. **Gross Pathology:** No findings were considered treatment-related.

5. **Cesarean Section Data:** Data are as follows: For females with live foetuses, a high variability was noted in many litter parameters but no trend or adverse effects were noted. When the litter parameters were calculated for all pregnant females, the post-implantation loss at 2 mg/kg bw/day was increased. This value of post-implantation loss at 2 mg/kg bw/day was due to two females with implantations only and one female with total resorptions. However in all cases, none of the litter parameters were statistically significantly increased when compared to control values.

Analysis of litter and fetal parameters (fetal death status and foetal body weight) revealed no significant treatment effects.

TABLE 4. Cesarean Section Observations [a]

Observation	Dose in mg/kg bw/day				
	0	0.25	0.75	2	5
# Animals Assigned (Mated)	8	8	8	8	8
# Animals Pregnant	6	4	4	8	6
with live fetuses	6	4	4	5	6
with resorptions only	0	0	0	1	0
with implantations only	0	0	0	2	0
Pregnancy Rate (%)	88	50	63	100	88
# Nonpregnant	1	4	3	0	1
Maternal Wastage:					
# Died	1	0	0	0	0
# Died Pregnant	0	0	0	0	0
# Died Nonpregnant	1	0	0	0	0
# Aborted	0	0	1	0	1
# Premature Delivery	0	0	0	0	0
No. Corpora Lutea per Dam	9.5 ± 1.0	9.8 ± 0.6	10.0 ± 1.8	9.5 ± 1.1	9.2± 1.0
No. Implantation Sites per Litter	7.2 ±1.1	4.5 ±1.6	7.3± 1.9	8.1 ± 0.9	5.8 ± 1.7
Percent Pre-implantation Loss per Litter	22.1±10.9	54.9 ± 13.8	26.1± 15.2	12.1± 4.9	36.1 ± 16.0
No. Early Resorptions per Litter	0.3± 0.2	0.0± 0.0	0.3 ± 0.3	1.4± 1.4	0.8±0.4
No. Late Resorptions per Litter	0.0±0.0	0.0±0.0	0.3 ± 0.3	0.0± 0.0	0.2± 0.2
Percent Post-implantation Loss per Litter	16.4± 7.8	0.0±0.0	9.4 ± 6.0	38.9± 17.9	19.5±6.8
No. Live Fetuses per Litter	6.2 ± 1.2	4.5 ± 1.6	6.3 ± 1.3	8.8 ±0.6	4.2± 1.0
No. Dead Fetuses per Litter	0.7 ± 0.5	0.0 ± 0.0	0.3± 0.3	0.2± 0.2	0.7 ± 0.4
Fetal Death Status (% dead fetuses)	9.8 ± 6.8 (N=41)	0.0± 0.0 (N=18)	0.3 ± 0.3 (N=4)	0.2 ± 0.2 (N=5)	0.7 ± 0.4 (N=6)
Fetal Body Weight (g)	40.5 ± 1.5 (N=37)	39.6± 4.5 (N=18)	43.2 ± 1.0 (N=25)	38.1± 1.9 (N=44)	39.6± 2.9 (N=25)

a Data obtained from pages 30, 38- 40 in the study report.

B. **DEVELOPMENTAL TOXICITY:** A high variability was noted in many litter parameters but no trend or adverse effects were noted. Analysis of litter and fetal parameters (fetal death and fetal body weight) revealed no significant treatment effects.

External Examination: No external observations were noted at 0, 0.25, 0.75 and 2 mg/kg bw/day. At 5 mg/kg bw/day, two fetuses in one litter (JR5F0353) had multiple external malformations. At 2 and 5 mg/kg bw/day, two and one litter respectively had runt fetuses (BW < 28.0g).

TABLE 5. External Examinations [a]

	Dose (mg/kg bw/day)					Dose (mg/kg bw/day)				
	0	0.25	0.75	2	5	0	0.25	0.75	2	5
	Number of fetuses examined					Number of litters examined				
	37	18	25	44	25	6	4	4	5	6
Findings	Number (%) fetuses affected					Number (%) litters affected				
Variation:										
Runt (body weight <28.0g).	0 (0.0)	1 (3.1)	0 (0.0)	4 (9.4)	2 (4.2)	0 (0.0)	1 (25.0)	0 (0.0)	2 (40.0)	1 (16.7)
Malformation:										
Domed head, cleft palate (total), fore/hindpaw (s) (unilateral/bilateral): malrotated: outward/inward. Fore/hindlimbs (bilateral): hyperflexion	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)
Eye buldges (bilateral):absent. Mandibular: micrognathia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)

a Data obtained from the study report, page 42.

III. CONCLUSIONS:

1. Maternal toxicity: One female at 5 mg/kg bw/day and one female at 0.75 mg/kg bw/day aborted on GD 26 and 20, respectively. In the 5 mg/kg bw/day group, all females had few feces during several days. No other clinical signs were considered treatment-related.

At 5 mg/kg bw/day, a body weight loss was noted from GD 6 to 18 (-0.30kg) while the control group maintained or gained weight. In the high dose group, the body weight change values during gestation were significantly different (weight loss or decreased weight gain) compared to control values during every interval starting from GD 6. At 2 mg/kg bw/day, the mean body weight change was slightly lower than in the control group during the first half of the dosing period but was not significantly different. At 0.25 and 0.75 mg/kg bw/day, the mean body weight changes were considered unaffected by treatment. Other body weight analysis indicated a significant dose-related decrease in corrected body weight gain from GD 0-29, although no individual dose groups were significantly different from controls.

Maternal food consumption was significantly ($p<0.05$) reduced in a dose-related fashion during every time interval from GD 6 through 22 and significantly increased in a dose-related fashion during GD 22-26 and GD 26-29. Food consumption was significantly reduced in the high dose group vs controls during the interval GD 6-8, GD 8-10, GD 10-14 and GD 14-18. At 2 mg/kg bw/day, slight reductions of food intakes were noted at some intervals during the dosing period without showing any statistically significant differences.

2. Developmental toxicity: A high variability was noted in many litter parameters but no trend or adverse effects were noted. Analysis of litter and fetal parameters (fetal death and fetal body weight) revealed no significant treatment effects.

a. Deaths/Resorptions: The number of post-implantation loss was increased at 2 mg/kg bw/day. This value of post-implantation was due to two females with implantations only and one female with total resportions.

b. Altered Growth: Fetal body weights were considered unaffected by treatment.

c. Developmental Variations: At 2 and 5 mg/kg bw/day, two and one litter respectively had runt fetuses (BW < 28.0g).

d. Malformations: At 5 mg/kg bw/day, two fetuses in one litter (JR5F0353) had multiple external malformations.

Historical control data were not provided to allow comparison with concurrent controls. Since this was only a range-finding study external fetal examination was performed (not visceral or skeletal).

KIIA 5.6.2.2 - Teratogenicity test by the oral route in the rabbit

1.	Annex point(s)	KIIA 5.6.2.2; Teratogenicity test by the oral route in the rabbit: **MRID 47622809**
2.	Location in dossier	KIIA 5.6.11 /01
3.	Authors (year)	Foulon, O. (2000)
	Title	Developmental toxicity study in the rabbit by gavage Ethiprole (RPA107382)
	Company, report No.	Bayer CropScience AG, Report-No.: R016684 (M-192124-02-1)
	Date	09.11.2000, Amended: 13.05.2002
4.	Testing facility	Aventis CropScience, Sophia Antipolis;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC (Batch-No.: CDR9706)
7.	Test method	EU (=EEC): 92/69/EEC, V, B31; JMAF: 59 NohSan 4200, (1985); OECD: Draft 1998, for updating 414, (1981); USEPA (=EPA): OPPTS 870.3700
	Deviations	
8.	GLP	yes

Executive summary:

In a developmental toxicity study, ethiprole (92.7 % a.i.) was administered to 30 female New Zealand White rabbits/dose by gavage at dose levels of 0, 0.25, 0.5, 2.0 and 4.0 mg/kg bw/day from days 6 through 28 of gestation. The 0.25 mg/kg bw/day group, along with a concurrent control group were added as supplemental groups to the initial study following abortions in the high dose group, in order to provide a study with three treated groups with at least 20 pregnant females at scheduled sacrifice.

No deaths were considered to be treatment-related. Abortions occurred at 2.0 and 4.0 mg/kg bw/day. All abortions occurred between GD 21 to 28 and in all cases, following a reduction in food intake for several days and a body weight loss. Further body weight changes and food consumption at 2.0 and 4.0 mg/kg bw/day were significantly reduced for many intervals during the dosing period. The mean number of late resorptions was slightly increased at 4.0 mg/kg bw/day. Pale and/or accentuated lobular pattern of liver was observed in 10 of the 11 high-dose females (4.0 mg/kg bw/day) which were sacrificed early due to abortions. No other treatment-related gross pathologic findings were noted at any dose level. **The maternal LOAEL is 2.0 mg/kg bw/day, based on increased number of abortions, reduced body weight gains and food consumption. The maternal NOAEL is 0.50 mg/kg bw/day.**

The mean number of late resorptions was slightly increased at 4.0 mg/kg bw/day. All the other litter parameters did not show any significant treatment-related effect. Fetal body weights were considered unaffected by treatment. No malformations at external, visceral or skeletal examination were considered to reflect any abnormal development linked to treatment except two fetuses at 4.0 mg/kg with a diaphragmatic hernia. Incomplete ossification was noted for a very few bones; it was limited to 2.0 and 4.0 mg/kg bw/day and

did not reflect any general ossification delay. **The developmental LOAEL is 2.0 mg/kg bw/day, based on skeletal variations (incomplete ossification of pubis, metacarpal and/or middle phalanges). The developmental NOAEL is 0.50 mg/kg bw/day.**

This study is classified **acceptable/guideline** and statisfies the guideline requirement (OPPTS 870.3700; OECD 414) for a developmental toxicity study in rabbits.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Ethiprole (RPA 107382)

Description:	White powder
Lot/Batch #:	CDR9706
Purity:	92.7% a.i.
Compound Stability:	The test substance was stored in an air-tight, light-resistant container at room temp.
CAS #of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** Control animals received 4 ml/kg body weight of vehicle alone (0.5% aqueous solution of methylcellulose 400 (Fluka, Mulhouse, France)), based on the most recent body weight recorded.

3. **Test Animals:**

Species:	Rabbits	
Strain:	New Zealand White rabbits Crl: Kbl/BR (SPF)	
Age/weight at study initiation:	Sexually mature and nulliparus females/ 2.96-4.01 kg at Gestational Day 0	
Source:	(Charles River, Saint-Aubin-les-Elbeuf, France)	
Housing:	Rabbits housed individually in plastic cages on a perforated cage floor.	
Diet:	Certified Rabbit Pellet diet UAR 110C-10 (Usine d'Alimentation Rationnelle, 91360 Villemoisson-sur-Orge, France), *ad libitum*	
Water:	Filtered water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	17°C - 21°C
	Humidity:	40% - 70%
	Air changes:	approximately 15 changes per hour (not monitored)
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	12 days	

B. PROCEDURES AND STUDY DESIGN

1. **In-Life Dates:** August 23, 1999 to December 09, 1999

2. **Mating:** Female rabbits were artificially inseminated with semen from New Zealand White bucks from the same supplier and the same strain. The day of insemination was designated as Gestation Day 0.

3. **Animal Assignment:** The females were allocated to the control and treated groups as indicated in Table 1 (using a body weight dependent procedure) to ensure that the females inseminated with the same male were distributed among the different groups. Two groups, a second control group and a low-dose (0.25 mg/kg bw/day), were added to the study after a high abortion rate was observed at 4.0 mg/kg bw/day. The lower dose group was added to ensure that at sacrifice, there would be at least 20 pregnant females in each of three treated groups. The additional control group was added to assure that the newly added dose group (0.25 mg/kg bw/day) would have concurrent controls for statistical comparisons. For statistical purposes, the study report refers to "Block 1" as encompassing the initial study

design with dose levels of 0 (vehicle control), 0.5, 2.0, and 4.0 mg/kg bw/day ethiprole, and "Block 2" using dose levels of 0 (vehicle control) and 0.25 mg/kg bw/day ethiprole.

TABLE 1: Animal Assignment [a]

	Control		LDT	MDT-1	MDT-2	HDT
Group #	1	5 [b]	6 [b]	2	3	4
Dose (mg/kg bw/day)	**0**	**0**	**0.25**	**0.50**	**2.0**	**4.0**
Dose formulation concentration (mg/L) [c]	0	0	62.5	125	500	1000
Dosing volume (mL/kg)	4 [d]	4 [d]	4	4	4	4
# Females	30	30	30	30	30	30

a Data obtained from the amended study report on page 15.
b Groups 5 and 6 (a second control group, and the LDT group) were added to the initial study following the abortion rate observed in group 4 (HDT) in order to provide a study with three treated groups with at least 20 pregnant females at the scheduled sacrifice.
c In terms of material as supplied.
d Volume of vehicle administered.

4. **Dose selection rationale:** The dose levels were selected based on the results obtained in a previous range finding study for developmental toxicity in the rabbit by gavage (Study Number: SA 99078). In the previous study, the highest dose level (5 mg/kg bw/day) induced body weight loss from Gestational Day 6 to 28. In the present study, dose levels originally selected were 0.5, 2.0, and 4.0 mg/kg bw/day. The high dose level (4.0 mg/kg bw/day) was chosen to induce some maternal toxicity, and the low dose level was selected to obtain a maternal/developmental No Observable Adverse Effect Level (NOAEL). As indicated above, an additional treated group (0.25 mg/kg bw/day) and a concurrent control group were added because of a high percentage of abortions observed in the 4.0 mg/kg bw/day dose group.

5. **Dosage preparation and analysis:** Test material-vehicle mixture was prepared seven or eight times (F1, F2, F3, etc.) by mixing appropriate amounts of test substance with an aqueous solution of 0.5% methylcellulose 400 (Fluka, Mulhouse, France) to provide the 62.5, 125, 500, and 1000 mg/L concentration levels for dosing. The formulations were stored at approximately 5±3°C while not in use. The suspensions were mixed continuously before and during dosing with an electromagnetic stirrer.

   The homogeneity (top, middle, and bottom) of the suspensions was checked on the first formulation (Fl) of each concentration (62.5, 125, 500, and 1000 mg/L).

   Mean values obtained from the homogeneity checks (Fl) were used as measured concentrations. The concentrations of all other preparations (F2 to F7 or F8) were also checked.

   Ethiprole (RPA 107382) formulations in aqueous methylcellulose were previously demonstrated to be stable over a 21-day period at 5±3°C during two earlier studies using concentrations from 62.5 to 15,000 mg/L (Study Numbers: SA 99036yand SA 99078). The results of these stability checks were summarized in the current study.

   Results **Homogeneity Analysis:** Homogeneity results for the first formulations of ethiprole in the vehicle at each concentration (62.5, 125, 500, and 1000 mg/L) were within the range of 92-101% of the nominal concentrations.

Stability Analysis: After 21 days of storage at 5±3°C, the ethiprole formulations in aqueous methylcellulose ranging in concentration from 62.5 to 15,000 mg/L were 97-100% of the nominal concentrations, demonstrating stability for the intervals and conditions used in this study.

Concentration Analysis: Results for the concentration analyses were within 90-106% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

5. **Dosage Administration:** All doses were administered once daily by gavage, on gestation days 6 through 28, in a volume of 4 mL/kg of body weight/day. Dosing was based on the body weight on the most recent body weight determination.

C. OBSERVATIONS

1. **Maternal Observations and Evaluations:** The animals were checked for clinical signs daily. Mortality was checked twice daily, except for weekends and public holidays when mortality was checked once daily. Any animal showing signs of abortion or morbidity, particularly if death appeared imminent, were killed by intravenous injection of Dolethal® to prevent loss of tissues through autolysis.

   Body weights were recorded on Gestational Days 0, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 29. Body weight changes for the following intervals were determined: Gestational Days 0-6, 6-8, 6-10, 6-14, 6-18, 6-22, 6-26, 6-29, and 0-29 (corrected for gravid uterine weight). Full feeders were weighed on Gestational Day 1 and every day through Gestational Day 28. Empty feeder weights were measured on Gestational Day 2 and every day through Gestational Day 29. Food consumption was determined for the following intervals: Gestational Days 1-6 (g/day only), 6-8, 8-10, 10-14, 14-18, 18-22, 22-26, and 26-29.

   Animals killed in extremis (including aborted females) and those found dead, were necropsied. The number and type of implantations and corpora lutea were noted when present. Uterine horn(s) without visible implantations were immersed in a 20% solution of ammonium sulfide. Tissues and carcass were discarded.

   On Gestational Day 29, surviving females were killed by intravenous injection of Dolethal® for examination of their uterine content. Each female was first subjected to macroscopic examination of the visceral organs and the number of ribs was recorded. Uterine horn(s) without visible implantations were immersed in a 20% solution of ammonium sulfide. Tissues and carcass were discarded. The reproductive tract was weighed (gravid uterine weight), dissected out and the following parameters recorded: number of corpora lutea, number of implantations, number resorptions, number of live and dead fetuses, and the individual weight of live fetuses. Resorptions were classified as early (macroscopic discrimination between fetal residues and placental material not possible) and late (distinct macroscopic discrimination between fetal and placental remains possible). Dead fetuses were defined as fetuses showing distinct digits on fore-and hind-paws.

2. **Fetal Evaluations:** Live fetuses were killed by subcutaneous injection of Dolethal® and subjected to an examination to detect external abnormalities. After internal examination of the neck, the head of fetuses from approximately half of each litter was immersed in Bouin

fluid and the internal structures examined after fixation. The bodies of all fetuses were dissected to detect soft tissue abnormalities and sexed. Then the fetuses were fixed in absolute ethanol before staining. A modification of the Staples and Schnell staining technique was used and a subsequent skeletal examination was performed. The heads after examination and the skeletons were archived. Structural deviations were classified (Palmer, 1977) as follows: Malformations (very rare or obviously lethal changes), minor anomalies (slight, relatively rare structural changes not obviously detrimental), and common variants (structural changes occurring in more than approximately 5% of the control population).

D. DATA ANALYSIS:

1. **Statistical Analyses:** Means and standard deviations for all maternal, litter and fetal parameters were calculated for each group. Data from non-pregnant, dead or killed *"in extremis"* animals were not included in group mean calculations of all maternal parameters. Animals which aborted were included in body weight and food consumption calculations up to the day of abortion. A detailed description of each statistical test was provided in the study report. A brief summary follows herein.

   Statistical analyses were performed on the following maternal endpoints: body weight change of pregnant females (Gestational Days 0-6, 6-8, 6-10, 6-14, 6-18, 6-22, 6-26, 6-29 and 0-29 (corrected for gravid uterine weight); gravid uterine weight; and food consumption in g/day and g/kg of body weight/day (Gestational Days 1-6 (g/day only), 6-8, 8-10, 10-14, 14-18, 18-22, 22-26, and 26-29.

   Statistical analyses were performed on the following litter based endpoints: number corpora lutea per doe, number implantation sites per litter, percent pre- and post-implantation loss per litter, and number early and late resorptions per litter.

   The following fetal endpoints were analyzed statistically: fetal death status (% dead fetuses), fetal body weight of each fetus (combined, male, and female), and fetal sex (% male fetuses).

   Because the study was conducted in two blocks (Block 1: 0, 0.5, 2.0 and 4.0 mg/kg bw/day; Block 2: 0 and 0.25 mg/kg bw/day), treatment groups were compared using linear regression techniques for maternal and litter parameters, and GEE (Generalized Estimating Equations) linear and logistic regression methods for analysis of fetal parameters. For each analysis, the categorical effects of dose and study block were included in the model. All treatment effects were adjusted for study blocks. However, since the study design was not fully replicated in each block, an interaction effect between dose and study block was not be evaluated.

   For maternal and litter parameters, Dunnett's test was used to compare exposed groups to controls when the overall treatment effect was statistically significant ($p<0.05$ via the ANOVA F-test). The GEE linear regression model was used for analyzing fetal body weight and fetal death status, and the GEE logistic model was used for analyzing fetal sex. Although fetal death status is a binary outcome (dead vs. live for each fetus), this parameter was still analyzed via a linear regression model because the distribution of the variable was too sparse for computations required for logistic regression. All GEE regression models assumed exchangeable intralitter correlations and used a robust variance estimator for regression parameters (Zeger and Liang, 1986). GEE methods were considered robust to misspecifications of the response variance as well as assumptions about the intralitter correlation structure, as long as robust variance estimators were used. Individual t-tests for

pairwise comparisons to control were evaluated when the overall treatment effect was statistically significant ($p<0.05$ via a Wald chi-square test).

2. **Indices:** The pre- and post-implantation loss indices as well as the percent dead and percent male fetuses were calculated from cesarean section records of animals in the study. The pre- and post-implantation indicies were calculated separately for all pregnant does, and for does with live fetuses. Formulas for the pre- and post-implantation loss indices as provided in the study report are follows:

$$\textbf{Pre-implantation loss index} = \frac{\textbf{(Number of corpora lutea - Number of implantations)}}{\textbf{Number of corpora lutea}} \times 100$$

$$\textbf{Post-implantation loss index} = \frac{\textbf{(Number of implantations - Number of live fetuses)}}{\textbf{Number of implantations}} \times 100$$

3. **Historical Control Data:** Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and Clinical Observations:** Three control rabbits (two from Group 1, one from Group 5 see Table 4) and two rabbits dosed at 0.25 mg/kg bw/day died due to gavage errors during the study. None of the deaths were considered to be related to treatment with ethiprole. No other unscheduled deaths occurred during the study.

   In the 2 mg/kg bw/day dose group, two females aborted (on Gestational Days 25 and 28) after a body weight loss and a reduced food intake. Eleven females in the 4.0 mg/kg bw/day dose group aborted between Gestational Day 21 and 27. In all cases it was after a severe reduction of food intake since the start of dosing (Gestational Day 6) and a body weight loss. At necropsy, 10 out of these 11 females had pale and/or accentuated lobular pattern of liver.

   All clinical signs (except for dead or aborted females) were those commonly observed, recorded once only or distributed between different groups with no dose related increase and thus were not considered to be related to treatment.

2. **Body Weight:** Body weight data are summarized in Table 2 and as follows: No statistically significant differences were noted in the mean maternal body weights or in the corrected body weight change (for gravid uterine weight) between the treated and control groups.

   At 0.25 and 0.50 mg/kg bw/day, body weight changes were considered to be unaffected by treatment when compared to controls. At 2.0 mg/kg bw/day, the body weight changes were reduced for many intervals, showing statistical differences for Gestational Days 6-8, 6-14, 6-18, 6-22 and 6-26. At 4.0 mg/kg bw/day, the mean body weight changes for all intervals during the dosing period were significantly lower than the corresponding control values. From Gestational Days 6 through 18, a body weight loss of -0.27 kg was observed compared to a gain of 0.21 kg in the control group.

TABLE 2. Mean (±SD) Maternal Body Weight (g) and Maternal Body Weight Change (g)[a]

Interval	Dose in mg/kg bw/day				
	0	0.25	0.50	2.0	4.0
Maternal Body Weight (kg)					
Pretreatment Day 0	3.49 ±0.03 (N=50)	3.57 ± 0.04 (N=27)	3.43 ± 0.02 (N=26)	3.44 ± 0.02 (N=24)	3.43 ± 0.02 (N=28)
Pretreatment Day 6	3.65 ±0.03 (N=50)	3.71 ± 0.04 (N=27)	3.57 ± 0.03 (N=26)	3.60 ± 0.02 (N=24)	3.56 ± 0.02 (N=28)
Treatment Day 8	3.67 ± 0.03 (N=50))	3.72 ± 0.04 (N=27)	3.57 ± 0.03 (N=26)	3.58 ± 0.03 (N=24)	3.49 ± 0.03 (N=28)
Treatment Day 10	3.70 ± 0.03 (N=50)	3.76 ± 0.05 (N=27)	3.60 ± 0.03 (N=26)	3.60 ± 0.03 (N=24)	3.41 ± 0.03 (N=28)
Treatment Day 14	3.79 ± 0.03 (N=50)	3.84 ± 0.05 (N=27)	3.69 ± 0.03 (N=26)	3.63 ± 0.04 (N=24)	3.33 ± 0.05 (N=28)
Treatment Day 18	3.86 ± 0.03 (N=50)	3.92 ± 0.05 (N=27)	3.76 ± 0.03 (N=26)	3.67 ± 0.05 (N=24)	3.29 ± 0.06 (N=28)
Treatment Day 22	3.94 ± 0.03 (N=50)	4.01 ± 0.05 (N=27)	3.81 ± 0.03 (N=26)	3.72 ± 0.06 (N=24)	3.33 ± 0.08 (N=26)
Treatment Day 26	4.01 ± 0.03 (N=50)	4.03 ± 0.05 (N=27)	3.89 ± 0.03 (N=26)	3.83 + 0.04 (N=23)	3.60 ± 0.09 (N=18)
Treatment Day 29	4.06 ± 0.04 (N=50)	4.06 ± 0.05 (N=27)	3.94 ± 0.03 (N=26)	3.90 ± 0.04 (N=22)	3.73 ± 0.08 (N=17)
Maternal Body Weight Change (kg)					
Pretreatment Days 0 to 6	0.17 ± 0.01 (N=50)	0.14 ± 0.01 (N=27)	0.14 ± 0.01 (N=26)	0.16 ± 0.01 (N=24)	0.14 ± 0.01 (N=28)
Treatment Days 6 to 8	0.02 ± 0.01 (N=50)	0.01 ± 0.01 (N=27)	0.00 ± 0.02 (N=26)	-0.02 * ± 0.01 (N=24)	-0.08*** ± 0.01 (N=28)
Treatment Days 6 to 10	0.05 ± 0.01 (N=50)	0.05 ± 0.02 (N=27)	0.03 ± 0.02 (N=26)	0.00 ± 0.01 (N=24)	-0.15*** ± 0.03 (N=28)
Treatment Days 6 to 14	0.13 ± 0.01 (N=50)	0.13 ± 0.02 (N=27)	0.13 ± 0.02 (N=26)	0.03* ± 0.03 (N=24)	-0.24*** ± 0.05 (N=28)
Treatment Days 6 to 18	0.21 ± 0.01 (N=50)	0.21 ± 0.02 (N=27)	0.19 ± 0.02 (N=26)	0.07* ± 0.04 (N=24)	-0.27*** ± 0.0 6 (N=28)
Treatment Days 6 to 22	0.28 ± 0.02 (N=50)	0.29 ± 0.03 (N=27)	0.25 ± 0.03 (N=26)	0.12* ± 0.05 (N=24)	-0.23*** ± 0.08 (N=26)
Treatment Days 6 to 26	0.36 ± 0.02 (N=50)	0.31 ± 0.03 (N=27)	0.33 ± 0.03 (N=26)	0.22* ± 0.03 (N=23)	0.05*** ± 0.08 (N=18)
Treatment Days 6 to 29	0.40 ± 0.02 (N=50)	0.34 ± 0.04 (N=27)	0.38 ± 0.03 (N=26)	0.29 ± 0.03 (N=22)	0.17*** ± 0.07 (N=17)
Corrected Body Weight Change [b]	0.12 ± 0.03 (N=46)	0.05 ± 0.04 (N=26)	0.09 ± 0.04 (N=24)	0.07 ± 0.05 (N=20)	0.02 ± 0.07 (N=13)
Gravid Uterine Weight (kg)	0.456 ± 0.020 (N=46)	0.443 ± 0.033 (N=26)	0.433 ± 0.020 (N=24)	0.390 ± 0.025 (N=20)	0.370 ± 0.041 (N=13)

a Data obtained from the study report, pages 31, 32, and 33 (original pagination). Note that data from non-pregnant, dead or killed *"in extremis"* animals were not included in group mean calculations of all maternal parameters. Statistical comparisons were made according to study block, (see Section D.1 of this DER).

b Corrected body weight change = (Body weight on GD 21 - Body weight on GD 0) - (gravid uterine weight)

* Statistically different (p <0.05) from the control.

*** Statistically different (p <0.001) from the control.

3. **Food Consumption:** Food consumption data are summarized in Table 3. At 0.25 and 0.5 mg/kg bw/day, mean food consumption was unaffected by treatment. At 2.0 mg/kg bw/day, food consumption was statistically significantly reduced for Gestational Days 10-14 and for

Gestational Days 14-18. At 4.0 mg/kg bw/day, mean food consumption was reduced during each interval measured from Gestational Days 6-22 and increased for Gestational Days 26-29.

TABLE 3. Mean (±SD) Maternal Food Consumption in (g/kg/day)[a]

Interval	Dose in mg/kg bw/day				
	0	0.25	0.50	2.0	4.0
Maternal Food Consumption (g/kg/day)					
Treatment Days 6 to 8	53.4 ± 0.9 (N=50)	50.4± 1.5 (N=27)	51.6± 1.7 (N=26)	49.1± 2.0 (N=24)	34.0 ***± 2.6 (N=28)
Treatment Days 8 to 10	52.6±1.0 (N=50)	50.7±1.5 (N=26)	51.2± 1.3 (N=26)	47.9± 2.4 (N=24)	19.7 ***± 4.0 (N=28)
Treatment Days 10 to 14	51.1± 0.9 (N=49)	47.7± 1.7 (N=27)	50.4± 1.1 (N=26)	41.7**± 3.2 (N=24)	17.7***± 4.1 (N=28)
Treatment Days 14 to 18	46.9±1.2 (N=49)	46.4±1.6 (N=27)	48.3± 1.1 (N=26)	39.0*± 3.5 (N=24)	16.9***± 4.2 (N=28)
Treatment Days 18 to 22	47.2± 0.9 (N=50)	46.9± 1.4 (N=27)	45.0± 1.7 (N=25)	41.3± 3.2 (N=24)	25.4***± 4.6 (N=26)
Treatment Days 22 to 26	39.5± 1.1 (N=49)	35.3±2.1 (N=26)	37.8± 2.0 (N=25)	38.2± 2.4 (N=23)	44.9± 3.0 (N=18)
Treatment Days 26 to 29	31.4 ± 1.4 (N=50)	26.5± 2.0 (N=27)	32.9± 1.9 (N=26)	34.2± 2.7 (N=22)	45.6***± 3.1 (N=17)

a Data obtained from the study report, pages 34 and 35 (original pagination). Note that data from non-pregnant, dead or killed *"in extremis"* animals were not included in group mean calculations of all maternal parameters. Statistical comparisons were made according to study block, (see Section D.1 of this DER).

* Statistically different (p <0.05) from the control. *** Statistically different (p <0.001) from the control.

4. **Gross Pathology:** At the scheduled sacrifice, no treatment-related findings were observed at necropsy at any dose level. Pale and/or accentuated lobular pattern of liver was observed in 10 of the 11 females dosed at 4.0 mg/kg bw/day which were sacrificed early due to abortions. No other treatment-related gross pathologic findings were noted.

5. **Cesarean Section Data:** Data are summarized in Table 4 and as follows. Treatment-related findings were observed only at the 4 mg/kg bw/day dose level. At 4.0 mg/kg bw/day, the mean post-implantation loss percentage was increased (not statistically significant) when compared to the control group value. It was linked to an increased number of late resorptions (statistically significant for females with live fetuses) and an increased percentage of dead fetuses (not statistically significant). Subsequently, at 4.0 mg/kg bw/day, the number of live fetuses was slightly reduced when compared to control group value. None of the other litter parameters in any treated groups were considered to be affected by the treatment. The mean fetal body weights (per sex or combined) did not show any significant differences between treated and control groups.

TABLE 4. Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)					
	0	0	0.25	0.50	2.0	4.0
	Dose Group					
	1	5	6	2	3	4
# Animals Assigned (Mated)	30	30	30	30	30	30
# Animals Pregnant	26	27	29	26	24	28
with live fetuses	23	23	26	24	20	13
with resorptions only	0	0	0	0	0	2
with implantations only	2	2	1	2	2	2
Pregnancy Rate (%)	87	90	97	87	80	93
# Nonpregnant	4	3	1	4	6	2
Maternal Wastage						
# Died (pregnant)	1	2	2	0	0	0
# Aborted	0	0	0	0	2	11
All Pregnant Does [b]						
No. Corpora Lutea per Doe	10.0 ± 0.3 (N=50)	10.3 ± 0.4 (N=27)	9.3 ± 0.5 (N=26)	9.5 ± 0.6 (N=22)	9.4 ± 0.7 (N=17)	
No. Implantation Sites per Litter	8.0 ± 0.4 (N=50)	7.6 ± 0.7 (N=27)	7.4 ± 0.5 (N=26)	7.0 ± 0.7 (N=22)	6.3 ± 0.9 (N=17)	
% Pre-implantation Loss per Litter	22.2 ± 3.2 (N=50)	26.2 ± 5.7 (N=27)	19.8 ± 4.2 (N=26)	27.8 ± 4.9 (N=22)	35.1 ± 7.5 (N=17)	
No. Early Resorptions per Litter	0.340 ± 0.133 (N=50)	0.519 ± 0.188 (N=27)	0.423 ± 0.168 (N=26)	0.182 ± 0.084 (N=22)	0.294 ± 0.143 (N=17)	
No. Late Resorptions per Litter	0.020 ± 0.020 (N=50)	0.000 ± 0.000 (N=27)	0.017 ± 0.053 (N=26)	0.000 ± 0.000 (N=22)	0.176 ± 0.128 (N=17)	
% Post-implantation Loss per Litter	17.1 ± 3.9 (N=50)	9.8 ± 4.2 (N=27)	17.9 ± 5.8 (N=26)	16.9 ± 6.3 (N=22)	38.7 ± 10.0 (N=17)	
Does with Live Fetuses: [c]						
No. Corpora Lutea per Doe	10.4 ± 0.2 (N=46)	10.3 ± 0.5 (N=26)	9.8 ± 0.3 (N=24)	10.1 ± 0.5 (N=20)	10.5 ± 0.6 (N=13)	
No. Implantation Sites per Litter	8.3 ± 0.4 (N=46)	7.9 ± 0.7 (N=26)	7.9 ± 0.4 (N=24)	7.5 ± 0.6 (N=20)	7.6 ± 0.9 (N=13)	
% Pre-implantation Loss per Litter	21.0 ± 3.3 (N=46)	23.7 ± 5.3 (N=26)	18.5 ± 3.9 (N=24)	26.9 ± 5.3 (N=20)	28.6 ± 7.3 (N=13)	
No. Early Resorptions per Litter	0.370 ± 0.144 (N=46)	0.538 ± 0.194 (N=26)	0.458 ± 0.180 (N=24)	0.200 ± 0.092 (N=20)	0.231 ± 0.166 (N=13)	
No. Late Resorptions per Litter	0.022 ± 0.022 (N=46)	0.000 ± 0.000 (N=26)	0.083 ± 0.058 (N=24)	0.000 ± 0.000 (N=20)	0.231* ±0.166 (N=13)	
% Post-implantation Loss per Litter	9.9 ± 1.9 (N=46)	6.3 ± 2.4 (N=26)	11.0 ± 3.7 (N=24)	8.6 ± 3.0 (N=20)	19.8 ± 6.8 (N=13)	
No. Live Fetuses per Litter	7.5 ± 0.4 (N=46)	7.3 ± 0.6 (N=26)	7.1 ± 0.5 (N=24)	6.8 ± 0.6 (N=20)	6.2 ± 0.9 (N=13)	
No. Dead Fetuses per Litter	0.3 ± 0.1 (N=46)	0.0 ± 0.0 (N=26)	0.1 ± 0.1 (N=24)	0.4 ± 0.2 (N=20)	0.8 ± 0.4 (N=13)	
No. Female Fetuses per Litter	4.1 ± 0.3 (N=46)	4.0 ± 0.4 (N=26)	3.8 ± 0.5 (N=24)	3.2 ± 0.3 (N=20)	3.0 ± 0.5 (N=13)	
No. Male Fetuses per Litter	3.5 ± 0.3 (N=46)	3.4 ± 0.3 (N=26)	3.3 ± 0.3 (N=24)	3.6 ± 0.5 (N=20)	3.2 ± 0.6 (N=13)	
% Dead Fetuses	3.3 ± 0.9 (N=359)	0.0 ±0.0 (N=191)	1.7 ± 0.9 (N=174)	4.9 ± 2.7 (N=143)	11.1 ± 5.3 (N=90)	
% Male Fetuses	45.8 ± 2.8 (N=347)	46.1 ± 2.8 (N=191)	46.2 ± 4.7 (N=171)	52.9 ± 4.1 (N=136)	51.3 ± 4.3 (N=80)	

Fetal Body Weight (g)	39.7 ± 0.5 (N=347)	39.8 ± 0.7 (N=191)	40.5 ± 0.6 (N=171)	37.4 ± 1.0 (N=136)	38.3 ± 1.4 (N=80)
Male Fetal Body Weight (g)	40.5 ± 0.6 (N=159)	40.7 ± 0.7 (N=88)	41.6 ± 0.7 (N=79)	36.8 ± 1.2 (N=72)	38.7 ± 1.1 (N=41)
Female Fetal Body Weight (g)	39.1 ± 0.6 (N=188)	39.0 ± 0.8 (N=103)	39.5 ± 0.6 (N=92)	38.0 ± 1.0 (N=64)	37.9 ± 2.1 (N=39)

a Data obtained from the study report, pages 30 and 36-38 (original pagination). Statistical comparisons were made according to study block, (see Section D.1 of this DER).
b Includes does that had live fetuses, that had all resorptions, and that had implant sites only at scheduled sacrifice.
c Includes only does that had live fetuses.
* Statistically different (p <0.05) from the control.

B. DEVELOPMENTAL TOXICITY: No malformations at external, visceral or skeletal examination were treatment-related, with the exception of two fetuses (from different litters) at 4.0 mg/kg bw/day with a diaphragmatic hernia. Incomplete ossification, noted for a very few bones, was limited to the 2.0 and 4.0 mg/kg bw/day dose groups and was not considered to reflect a general ossification delay. No other treatment-related developmental findings were observed at any dose level.

1. **External Examination:** A summary of the external examinations is presented in Table 5a. The number of runt fetuses (body weight <28.0 g) was not affected by treatment. No fetal external findings were considered to be related to treatment as all findings concerned only one fetus per group.

2. **Visceral Examination:** A summary of the internal examinations is presented in Table 5b. The examination of heads of half of the number of fetuses did not reveal any treatment-related findings. At visceral examination, a few malformations were observed but none of them was considered treatment-related with the exception of the two diaphragmatic hernia recorded at 4 mg/kg bw/day. All variations and anomalies were considered to be randomly distributed between treated and control groups without any treatment-related increase.

3. **Skeletal Examination:** A summary of the skeletal examinations is presented in Table 5c. No dose-related increase of malformations was observed. Incomplete ossification was noted in a few bones at 2.0 and 4.0 mg/kg bw/day (incomplete ossification of pubis, metacarpal and/or middle phalanges); however, no marked ossification delay was noted at any dose level. All other variations and anomalies observed were randomly distributed between all groups and were not attributed to treatment.

TABLE 5a. External Examinations [a]

	Dose (mg/kg bw/day)						Dose (mg/kg bw/day)					
	0	0	0.25	0.50	2.0	4.0	0	0	0.25	0.50	2.0	4.0
	Dose Group						Dose Group					
	1	5	6	2	3	4	1	5	6	2	3	4
	Number of fetuses examined						Number of litters examined					
	178	169	191	171	136	80	23	23	26	24	20	13
Findings [b]	Number (%) fetuses affected						Number (%) litters affected					
Variations:												
Runt fetus (BW<28.0g).	7 (3.2)	3 (1.2)	4 (1.5)	1 (0.4)	7 (3.8)	4 (3.1)	5 (21.7)	2 (8.7)	3 (11.5)	1 (4.2)	3 (15.0)	2 (15.4)
Malformations:												
Snout: short. Tongue: protruding.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)
Omphalocele.	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.6)	0 (0.0)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)

Dorsal skin: folded.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)

a Data obtained from page 40 in the study report (original pagination).
b Some observations may be grouped together.

TABLE 5b. Visceral Examinations [a]

	Dose (mg/kg bw/day)						Dose (mg/kg bw/day)					
	0	**0**	**0.25**	**0.50**	**2.0**	**4.0**	**0**	**0**	**0.25**	**0.50**	**2.0**	**4.0**
	Dose Group						**Dose Group**					
	1	**5**	**6**	**2**	**3**	**4**	**1**	**5**	**6**	**2**	**3**	**4**
	Number of fetuses examined						**Number of litters examined**					
	178	**169**	**191**	**171**	**136**	**80**	**23**	**23**	**26**	**24**	**20**	**13**
	Number of heads examined						**Number of litters examined (heads)**					
	83	**80**	**90**	**78**	**63**	**38**	**22**	**23**	**26**	**23**	**20**	**12**
Findings [b]	**Number (%) fetuses affected**						**Number (%) litters affected**					
Variations:												
Thymus (unilateral/bilateral): enlarged.	17 (9.8)	15 (7.6)	17 (9.2)	25 (13.2)	2 (1.6)	2 (2.1)	8 (34.8)	6 (26.1)	11 (42.3)	12 (50.0)	2 (10.0)	2 (15.4)
Innominate artery: short	10 (3.9)	4 (3.8)	3 (1.7)	4 (2.3)	4 (3.3)	2 (1.5)	3 (13.0)	4 (17.4)	2 (7.7)	4 (16.7)	3 (15.0)	1 (7.7)
Caudate lung lobe: absent	15 (12.2)	9 (6.0)	4 (3.6)	7 (4.4)	7 (6.6)	3 (2.9)	7 (30.4)	4 (17.4)	4 (15.4)	5 (20.8)	4 (20.0)	3 (23.1)
Malformations:												
Ventricular septum defect in cranial/median/caudal region (one case: communication between ventricle and auricle: absent (unilateral)).	1 (0.4)	2 (1.5)	1 (0.4)	0 (0.0)	3 (1.8)	0 (0.0)	1 (4.3)	2 (8.7)	1 (3.8)	0 (0.0)	2 (10.0)	0 (0.0)
Diaphragmatic hernia.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (15.4)

a Data obtained from pages 41 and 42 in the study report (original pagination).
b Some observations may be grouped together.

TABLE 5c. Skeletal Examinations [a]

	Dose (mg/kg bw/day)						Dose (mg/kg bw/day)					
	0	**0**	**0.25**	**0.50**	**2.0**	**4.0**	**0**	**0**	**0.25**	**0.50**	**2.0**	**4.0**
	Dose Group						**Dose Group**					
	1	**5**	**6**	**2**	**3**	**4**	**1**	**5**	**6**	**2**	**3**	**4**
	Number of fetuses examined						**Number of litters examined**					
	178	**169**	**191**	**171**	**136**	**80**	**23**	**23**	**26**	**24**	**20**	**13**
	Number of heads examined						**Number of litters examined (heads)**					
	95	**89**	**101**	**93**	**73**	**42**	**23**	**23**	**26**	**24**	**20**	**13**
Findings [b]	**Number (%) fetuses affected**						**Number (%) litters affected**					
Variations:												
Anterior and/or posterior fontanelle(s): enlarged.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (4.3)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)	1 (7.7)
Nasal and/or frontal and/or parietal (unilateral/bilateral): split	1 (0.7)	4 (5.2)	1 (0.8)	3 (3.1)	1 (1.0)	1 (1.5)	1 (4.3)	3 (13.0)	1 (3.8)	3 (12.5)	1 (5.0)	1 (7.7)
Hyoid centrum:	16	6	14	17	19	11	8	6	9	8	8	7

incomplete ossification.	(13.9)	(6.0)	(14.1)	(17.0)	(22. 0)	(20. 8)	(34.8)	(26.1)	(34.6)	(33. 3)	(40.0)	(53.8)
Hyoid centrum: unossified.	1 (0. 7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	4 (6.2)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	2 (15.4)
13th thoracic rib(s) (unilateral/bilateral) (bilateral, short unilateral).	107 (59.6)	95 (53.4)	115 (57.2)	105 (63.8)	77 (52.6)	57 (71.3)	21 (91.3)	20 (87.0)	25 (96.2)	24 (100.0)	19 (95.0)	12 (92.3)
13th thoracic rib(s) (unilateral/bilateral): short.	31 (16.8)	31 (17.8)	33 (18.6)	33 (18.0)	24 (21.0)	11 (17.3)	15 (65.2)	16 (69.6)	19 (73.1)	16 (66.7)	11 (55.0)	8 (61.5)
13th thoracic rib(s) (unilateral/bilateral): detached (including short) .	19 (9.3)	13 (7.1)	12 (7.2)	18 (9.4)	10 (10.0)	11 (13.3)	13 (56.5)	7 (30.4)	10 (38.5)	9 (37.5)	8 (40.0)	7 (53.8)
Presence of 27 pre-sacral vertebrae.	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	5 (2.6)	3 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)	2 (10.0)	2 (15.4)
1st metacarpal: incomplete ossification or unossified. Forepaws: 4th or/and 5th middle phalanges: unossified.	18 (9.8)	19 (11.4)	20 (12.1)	17 (10.6)	39 (27.6)	31 (35.4)	6 (26.1)	12 (52.2)	13 (50.0)	11 (45.8)	13 (65.0)	8 (61.5)
Insertion point (unilateral/bilateral) of pelvic girdle on 2nd sacral vertebra.	85 (46.4)	75 (41.3)	78 (39.4)	85 (51.5)	65 (45.9)	40 (50.7)	20 (87.0)	20 (87.0)	23 (88.5)	24 (100.0)	17 (85.0)	12 (92.3)
Pubis (unilateral/bilateral): incomplete ossification.	4 (3.1)	3 (1.2)	4 (2.5)	2 (1.0)	14 (8.2)	11 (11.0)	3 (13.0)	2 (8.7)	4 (15.4)	1 (4.2)	6 (30.0)	4 (30.8)
Anomalies												
Cervical ribs or cervical rib(s) short (unilateral/bilateral) on 7th cervical vertebra.	3 (1.4)	1 (0.7)	5 (2.4)	8 (4.0)	5 (2.5)	0 (0.0)	3 (13.0)	1 (4.3)	5 (19.2)	5 (20. 8)	3 (15.0)	0 (0.0)
Sternebrae: misshapen and/or misaligned or malpositioned and/or fused	0 (0.0)	6 (3.1)	3 (2.8)	8 (5.3)	8 (7.5)	5 (7.2)	0 (0.0)	5 (21.7)	3 (11.5)	6 (25.0)	7 (35.0)	3 (23.1)
5th sternebra: absent. Cartilage of 7th rib (bilateral) not attached to the sternum.	0 (0.0)	1 (0.6)	4 (3.5)	3 (1.7)	6 (4.0)	1 (0.8)	0 (0.0)	1 (4.3)	4 (15.4)	3 (12.5)	3 (15.0)	1 (7.7)
Malformations												
Axis centrum and axis arch (unilateral): fused. 6th cervical centrum and 6th cervical arch (unilateral): fused.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)
Thoracic region: (two or three) ribs: fused / one hemivertebra / (one or two or three or four) centrum or hemi-centrum: fused, unossified, malpositioned, misshapen, bipartite or incomplete ossification / (two or three) arches: fused.	1 (0.5)	1 (0.4)	3 (3.2)	0 (0.0)	3 (1.8)	0 (0.0)	1 (4.3)	1 (4.3)	2 (7.7)	0 (0.0)	3 (15.0)	0 (0.0)

a Data obtained from pages 43-45 in the study report (original pagination).
b Some observations may be grouped together.

III. CONCLUSIONS:

The NOAELs for both maternal and developmental toxicity are 0.5 mg/kg bw/day with the LOAELs for maternal and developmental toxicity at 2 mg/kg bw/day. The maternal LOAEL is based on increased number of abortions, reduced body weight gains and food consumption while the developmental LOAEL is based on skeletal variations.

1. Maternal toxicity: Eleven females in group 4 (4.0 mg/kg bw/day) aborted between GD 21 and 27. In all cases it was after a severe reduction of food intake since the start of dosing (GD 6) and a body weight loss. At autopsy, ten out of eleven females had pale and/or accentuated lobular patter of liver. In group 3, (2 mg/kg bw/day), two females aborted (on GD 25 and 28) after a body weight loss and a reduced food intake. All clinical signs, except for the dead or aborted females, were those commonly observed and not considered to be related to treatment.

2. Developmental toxicity:

a. Deaths/Resorptions: The mean number of late resorptions was slightly increased at 4.0 mg/kg bw/day. The percent of pre-implantational and post-implantational loss per litter was higher in all pregnant does and ones with live fetuses.

b. Altered Growth: Foetal body weights were considered unaffected by treatment.

c. Developmental Variations: Incomplete ossification was noted in a few bones at 2.0 and 4.0 mg/kg bw/day (incomplete ossification of pubis, metacarpal and/or middle phalanges); however, no marked ossification delay was noted at any dose level. All other variations and anomalies observed were randomly distributed between all groups and were not attributed to treatment.

d. Malformations: No malformations at external, visceral or skeletal examinations were considered to reflect any abnormal development linked to treatment except two foetuses at 4.0 mg/kg with a diaphragmatic hernia.

Historical data was not provided.

KIIA 5.5.1 - Long-term (2 years) oral toxicity in the rat

The long term (2 years) oral toxicity of ethiprole in rats was investigated in a combined chronic/carcinogenicity study reported under point IIA 5.5.2.

KIIA 5.5.2 - Carcinogenicity study in the rat

1.	Annex point(s)	KIIA 5.5.1; Long-term toxicity and carcinogenicity/Long-term (2 years) oral toxicity in the rat: **MRID 47622813**
2.	Location in dossier	KIIA 5.5.1 /01 also filed: KIIA 5.5.2 /01
3.	Authors (year)	Dange, M.; Foulon, O. (2001)
	Title	Chronic toxicity and carcinogenicity study of Ethiprole (RPA 107382) in wistar rat by dietary administration
	Company, report No.	Bayer CropScience AG, Report-No.: C012607 (M-203024-02-1)
	Date	05.04.2001, Amended: 05.03.2002
4.	Testing facility	Aventis CropScience S.A., Lyon, France
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole (radioactive), ethiprole Substance(s): ethiprole TC ethiprole (radioactive) TC
7.	Test method	
	Deviations	
8.	GLP	yes

Executive Summary:

Ethiprole was administered continuously via the diet at 0, 5, 20, 75 or 250 ppm to separate groups of rats (85 rats/sex were used in the control and high dose groups and 70 rats/sex were used in the intermediate groups). Mortality and clinical signs were checked daily. Body weight and food consumption were measured weekly for the first 13 weeks, every 4 weeks thereafter. Ophthalmic examinations were performed on all animals during the acclimatization period and at one and two years. Clinical chemistry, hematology determinations and urinalysis were performed during Months 6, 12, 15, 18 and 24 on selected animals. In addition, a blood sample was collected during Weeks 2, 9, 24 and 52 and during Weeks 2, 6 and 13 of the recovery period for T3, T4 and TSH analysis. All remaining animals of the chronic, recovery and carcinogenicity phases were subjected to necropsy, selected organs were weighed and designated tissues sampled and examined microscopically.

Long term administration of ethiprole produced no treatment-related changes in the mortality rate or clinical signs. The body weight changes in males and females at 75 and 250 ppm were slightly higher when compared to controls especially during the first year of treatment. The overall food consumption was generally higher in males and lower in females. At 250 ppm and to a lesser extend at 75 ppm, increases in prothrombin time, cholesterol, and total protein and a decrease in total bilirubin were observed at several intervals during the study. All of these changes were found to be completely reversible during the 3-month recovery period. Ethiprole induced an imbalance of thyroid hormones resulting in higher levels of TSH and lower levels of T4 in females and in males at 75 and 250 ppm. During the recovery period, TSH and T4 returned to control levels within 2 weeks of the withdrawal of treatment at 250 ppm. The imbalance of TSH

and T4 was most likely secondary to a treatment-induced increase in the metabolic activity of the liver causing an increased biliary clearance of T4 and consequently, an overstimulation of the thyroid due to increased levels of TSH. Consistent with this mechanism, an increase in liver and thyroid weights were observed at the sacrifice intervals for the chronic (after 52 weeks) and carcinogenicity (after 104 weeks) phases in both sexes at 250 ppm. In addition, liver weight was increased in females at 75 ppm for both of these sacrifice intervals while thyroid weight was increased at 75 ppm in males only after 52 weeks and in both sexes at 75 ppm after 104 weeks.
After 1 year of treatment, these organ weight changes were associated with transient centrilobular hepatocyte hypertrophy and thyroid follicular cell hypertrophy in males and/or females at 250 and 75 ppm.
After two years of treatment, slight increased incidences of thyroid follicular cell hyperplasia and follicular cell adenomas and hepatocellular adenomas were observed in males and/or females at 250 ppm.
The thyroid follicular cell hypertrophy noted for both sexes at 75 and 250 ppm after 52 weeks of treatment and in females at 250 ppm after 104 weeks of treatment and the follicular cell hyperplasia observed in both sexes at 250 ppm after 104 weeks of treatment are consistent with a chronic overstimulation of the thyroid due to increased circulating levels of TSH in conjunction with decreased levels of T4. Non proliferative lesions associated after long term exposure to ethiprole were also observed as follows:

- thyroid gland colloid mineralization in both sexes at 250 ppm and in females at 75 ppm ;
- thyroid diffuse follicular cell hypertrophy in females at 250 ppm;
- liver bile duct hyperplasia and sclerosis and focal sinusoidal dilatation in 250 ppm females;
- liver eosinophilic and basophilic tigroid foci of cellular alteration in males at 250 ppm;
- an increase in the severity, but not the incidence, of renal chronic nephropathy in both sexes at 250 ppm associated with an increased incidence of arteritis/periarteritis in the kidney of females at 250 ppm;
- foci of alveolar macrophages in lungs of females at 250 ppm.

There was no treatment-related change at 20 ppm. Therefore, the No Observed Adverse Effect Level (NOAEL) was established at 20 ppm corresponding to 0.85 mg/kg/day in males and 1.17 mg/kg/day in females.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4300; OECD 453) for a chronic study in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	93% (930 g/kg)
Compound Stability:	The test substance was stored in an air-tight, light resistant container at room temperature until May 18, 1998 then, at approximately +4°C thereafter. A note in the deviations section says that the test diet formulations were stored below -15°C rather than at room temperature. The timing of this deviation is unknown.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. Control animals received untreated diet.

3. **Test Animals:**

Species:	Rats	
Strain:	Wistar rats R.J: WHOPS AF	
Age/weight at study initiation:	6 weeks/ 246.0 - 249.4 g males; 178.0 - 182.7g females [a]	
Source:	R. Janvier (Le Genest St Isle, France).	
Housing:	Rats were housed 5/sex/cage at the start of the study. Cages were suspended over trays.	
Diet:	Ground diet, "M20 contrôlé" (Pietremont BP 59, 77482 Provins Cedex, France), *ad libitum*	
Water:	Filtered and softened water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	20°C - 24°C (target)
	Humidity:	40% - 70% (target)
	Air changes:	10 to 15 changes per hour (average, not monitored)
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	10 days	

a Body weight values are the range at Week 0 of the mean body weights for each treated and control group in the three designated phases, or groupings, in this study (chronic, recovery and carcinogenicity phases)

B. STUDY DESIGN:

1. **In-Life Dates:** Start: January 15, 1998; End: January 31, 2000

2. **Animal Assignment/Dose Levels:** All animals were subjected to an ophthalmological examination and a detailed physical examination during the acclimation phase. Any animal deemed unsuitable for selection based on weight, health status or ophthalmic abnormalities was not used for the study. Animals were assigned randomly, stratified by body weight, to the test groups noted in Table 1. Control animals received untreated diet. All other groups received the appropriate dietary concentrations at a constant (ppm) level. Diets were

available *ad libitum* throughout the study period, except during specific fasting periods. There were three designated phases in this study as follows: the chronic phase (sacrificed after 52-weeks of treatment, 10/sex/dose group), the recovery/reversibility phase (sacrificed after 52 weeks of treatment followed by 13 weeks of recovery with untreated diet, 15/sex/dose group – control and high dose only), and the carcinogenicity phase (sacrificed after 104 weeks of treatment, 60/sex/dose group). As a result of these groupings, 85 rats/sex were used in the control and high dose groups and 70 rats/sex were used in the intermediate dose groups as shown below.

TABLE IIA 5.5.2-1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg bw/day) Weeks 1-48 / 1-101 [a]		Main Study 104 weeks		Interim Sac. 52 weeks	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	60	60	10+15 [b]	10+15 [b]
Low (LDT)	5	0.25 / 0.22	0.34 / 0.29	60	60	10	10
Mid-1 (MDT-1)	20	0.98 / 0.85	1.34 / 1.17	60	60	10	10
Mid-2 (MDT-2)	75	3.73 / 3.21	5.01 / 4.40	60	60	10	10
High (HDT)	250	12.39 / 10.79	16.51 / 14.68	60	60	10+15 [b]	10+15 [b]

a Mean achieved dietary intake of ethiprole (RPA 107382) for Weeks 1-48 and Weeks 1-101
b Only the control and high-dose group were included in the 13 week recovery/reversibility phase of the study.

The dose levels were selected based on the results of previously conducted 90-day rat toxicity study (Study Number SA 97233, see KIIA 5.3.2/01).

3. **Diet Preparation and Analysis:** Test diets were prepared approximately every 4 weeks by mixing appropriate amounts of test substance with Ground diet, "M20 contrôlé" (Pietremont BP 59, 77482 Provins Cedex, France), and were stored at approximately -18°C temperature when not in use. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing.

The stability of the test substance in the diet was demonstrated prior to the start of the study in the previous subchronic rat study (Study Number SA 97233, see KIIA 5.3.2/01). The following storage intervals and conditions were investigated: 10, 17, 24, 31, or 45 days frozen, each followed by 7 days at room temperature; and 17, 24, 31, 38 and 52 days at room temperature (no preceding frozen storage interval).

During the current study, the homogeneity of the test substance in diet was verified on the first formulation to demonstrate adequate formulation procedures at 5, 20, 75 and 250 ppm. In addition, the concentration of each diet preparation was verified prior to administration to the animals for the first three formulations, and then every 3 months thereafter.

Results: **Homogeneity Analysis:** All the results for homogeneity checks were within the target range of 85-115% of the nominal concentration, except for one result out of nine at 5 ppm which was slightly below the target ranges (80%).
Stability Analysis: In the previously conducted study (SA 97233), samples of 5 and 2500 ppm diets were found to be stable after storage at room temperature for up to 7 weeks, or after frozen storage for up to 45 days followed by 7 days at room temperature.

Concentration Analysis: All results for concentration checks were within 85-114% of the nominal concentration. One result for the ninth formulation at 5 ppm was slightly below the target ranges (84%).

4. **Statistics**: The following parameters were subjected to statistical analyses: mortality, body weights, body weight changes, food consumption, hematology parameters (except eosinophils, basophils, monocytes and large unstained cells), clinical chemistry parameters, urinary parameters (only pH, volume and refractive index), organ weights, organ/body weight ratios, organ/brain weight ratios, hormone data, and tumor incidence.

A combination of standard ANOVA and non parametric data analysis techniques were used. The treatment groups were compared using either parametric ANOVA or non-parametric techniques. The homogeneity of variance assumption was examined via Bartlett's test in SAS® PROC GLM (SAS Institute, 1989, 1996). If Bartlett's test indicated lack of homogeneity of variance ($p<0.05$), non-parametric tests were used. Non-parametric tests included the Kruskal-Wallis test for overall treatment group differences, followed by the Mann-Whitney U test for individual exposed vs. control group comparisons when the Kruskal-Wallis test was statistically significant ($p<0.05$). All non-parametric tests were performed using the NPAR1WAY procedure in SAS® 6.12 (SAS Institute, 1989, 1996).

If Bartlett's test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 was used to evaluate the overall effect of treatment, and, when a significant treatment effect was present ($p<0.05$), to compare each exposed group to control via Dunnett's test.

Adjusted mortality rates were estimated using Kaplan-Meier estimation procedures (Kaplan E.L. and Meier P., 1958). Kaplan-Meier estimates were calculated separately for each sex and treatment group. Mortalities which were the result of animals killed following accidents or at scheduled sacrifices (interim or terminal sacrifices) were considered to be censored observations. Statistical significance of differences in survival rates between treated and control groups and dose-related trends in survival were assessed using Cox's test and Tarone's test. Trend test were conducted based on actual doses. They were conducted firstly including all the groups. When the trend test including all the doses was significant, a second trend test excluding the high dose group was also done. Probability values presented are two-sided for pairwise comparisons and one-sided for the trend test.

Statistical analysis of tumors was conducted using the Fisher Exact test which forms part of the statistical package from Roelee 84.

C. METHODS:

1. Observations:

1a. **Cageside Observations and Clinical Examinations:** Animals were checked for clinical signs, moribundity and mortality twice daily (once daily on week-ends or public holidays). Observed clinical signs were recorded at least once daily for all animals. The nature, onset, severity, duration and recovery of clinical signs were recorded. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose feces. Detailed physical examinations including palpation for masses were performed twice monthly during the first 13 weeks of the study and weekly thereafter. The onset, location and dimension of the masses were recorded. Debilitated animals were observed carefully and were eventually isolated.

1b. **Neurological Evaluations:** Neurological evaluations were not performed. Separate neurotoxicity studies are available (see section IIA 5.7).

2. **Body Weight:** Each animal was weighed twice during the acclimatization period then weekly for the first 13 weeks of study, and every 4 weeks thereafter.

3. **Food Consumption and Compound Intake:** The weight of food supplied and of that remaining at the end of the food consumption period was recorded for each cage. Food consumption was recorded weekly during the first 13 weeks of treatment, and once every 4 weeks thereafter.

   The mean achieved dosage intakes in mg/kg/day for each week and for Weeks 1 to 48 and for Weeks 1 to 101 were calculated. Ethiprole intake was calculated using the average of the body weight at the beginning of the interval and the body weight at the end of the interval for the measurement period for weeks 1 through 13, and was calculated using the single body weight available for the interval for the measurement period for weeks 17 through 101.

4. **Ophthalmoscopic Examination:** Funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were performed on all animals during the acclimatization phase and on all surviving animals at one and two years. Each eye was firstly examined by direct ophthalmoscopy, and then after instillation of an atropinic agent (Mydriaticum, Merck Sharp and Dohme), each eye was re-examined by means of a slit lamp and an indirect ophthalmoscope.

5. **Hematology & Clinical Chemistry (including thyroid hormone analysis:** Hematology and clinical chemistry were performed during Months 6 and 12 on the surviving animals of the interim sacrifice groups (chronic phase) and on the first ten surviving animals of the recovery groups and of the terminal sacrifice groups (carcinogenicity phase). These investigations were also performed during Month 15 on the first ten surviving animals of the recovery groups and during Months 18 and 24 on the first ten surviving animals of the terminal sacrifice groups (carcinogenicity phase). Blood was sampled from isoflurane anesthetized animals by puncture of the retro-orbital venous plexus after overnight diet fasting. Blood was collected in tubes containing EDTA for hematology (0.5 ml), lithium heparin (for plasma) for clinical chemistry (2.5 ml) and sodium citrate for coagulation (0.9 ml). Any significant change in the general appearance of the plasma was recorded. Additionally, a blood sample was collected in tubes containing lithium heparin for T3, T4 and TSH analysis from animals of the chronic and recovery groups at Weeks 2, 9, 24, 52 and at Weeks 2, 6 and 13 of the recovery phase. T3, T4 and TSH were measured by radioimmunoassay.

The checked (X) hematology and clinical chemistry parameters were examined. In addition, when possible, a blood smear was prepared for the moribund animals, just before necropsy. However, based on the study results these smears were not 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		Reticulocyte count
X	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
	Magnesium	X	Urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose (fasting)
	ENZYMES (more than 2 hepatic enzymes)	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Thyroid hormones (T3, T4, TSH)
X	Alanine aminotransferase (ALT/ SGPT)		
X	Aspartate aminotransferase (AST/ SGOT)		
	Gamma glutamyl transferase (GGT)		
	Sorbitol		
	Glutamate dehydrogenase		

NOTE: T3, T4 and TSH were monitored at Weeks 2, 9, 24, 52 and at Weeks 2, 6 and 13 of the recovery phase. T3, T4 and TSH were measured by radioimmunoassay.

6. **Urinalysis:** Urinalyses were performed at the same time points and for the same animals that blood was collected for hematology and clinical chemistry. Urine samples were collected overnight. Animals had diet and water withdrawn during the overnight (approximately 16 hours) collection period. . The checked (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
	Specific gravity / osmolality	X	Bilirubin
X	Refractive index		
X	pH	X	Blood/ red blood cells
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

7. **Sacrifice and Pathology**: On approximately Day 365 for the chronic phase, Day 458 for the recovery phase and Day 738 for the carcinogenicity phase, all surviving animals from all groups were sacrificed by exsanguinations under deep anesthesia (pentobarbital, intraperitoneal injection of 50 or 60 mg/kg bw). All animals that died and those sacrificed on schedule were subjected to gross pathological examination. The checked (X) tissues were collected for histological examination and the (XX) organs, in addition, were weighed. Paired organs were weighed together. Histopathology was performed on the collected tissues as follows:

   Chronic Phase: Histopathological examinations were performed for all animals in the control and high dose groups for all collected tissues. In addition, liver, lung, kidney, thyroid glands (with parathyroids), and tumors were examined for all the animals from the intermediate dose groups.

   Reversibility Phase: Histopathological examinations were performed on target organs (liver and thyroid glands) as identified in the chronic phase of the study. All tissues were examined in animals which died prematurely (before scheduled sacrifice). In addition, all suspected tumors and gross findings detected at necropsy were also examined in all animals.

   Carcinogenicity Phase: Histopathological examinations were performed in all animals from all groups.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic	XX	Brain (multiple sections)
X	Salivary glands	XX	Heart	X	Periph.nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	XX	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (retina, optic nerve)
X	Jejunum	XX	Thymus		**GLANDULAR**
X	Ileum			XX	Adrenal gland
X	Cecum		**UROGENITAL**	X	Lacrimal gland
X	Colon	XX	Kidneys	XX	Parathyroids
X	Rectum	X	Urinary bladder	XX	Thyroids
XX	Liver+	XX	Testes		**OTHER**
	Gall bladder (not rat)	XX	Epididymides	X	Bone (sternum and/or femur)
	Bile duct (rat)	XX	Prostate	X	Skeletal muscle
X	Pancreas	X	Seminal vesicle	X	Skin
	RESPIRATORY	XX	Ovaries	X	All gross lesions and masses
X	Trachea	XX	Uterus	X	Articular surface (femoro-tibial joint)
X	Lung	X	Mammary gland		
	Nose	X	Vagina		
	Pharynx				
X	Larynx				

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** There were no treatment-related clinical signs noted at any dose level during any phase of the study.

2. **Mortality:** There was no treatment-related effect on mortality at any dose level for either sex.

3. **Neurological Evaluations**: Neurological evaluations were not performed. Separate neurotoxicity studies are available (see section KIIA 5.7).

B. **BODY WEIGHT**: In males treated at 20, 75 and 250 ppm and in females treated at 75 and 250 ppm, body weight gain was statistically significantly increased from Week 0 to Week 48, when compared to controls. In females treated at 20 ppm, body weight gain was statistically significantly increased for the first 4 weeks of the study. From Week 48 to Week 101, no significant differences in body weight gain were recorded in both males and females, when compared to controls. During the recovery period, body weight gain in animals of both sexes treated at the high dose level was not significantly different compared to controls.

TABLE IIA 5.5.2-2. Mean Body Weights (BW) and Body Weight Gains (BWG) During Chronic and Carcinogenicity Phases of the Study [a]

Study Week (Study Day)	Dose level (ppm in diet)				
	0	5	20	75	250
Chronic Phase - Male Body Weights (g)					
Initial BW - Week 0	248.2 (N=85)	249.3 (N=70)	247.2 (N=70)	246.8 (N=70)	247.6 (N=85)
Final BW - Week 48	665.3 (N=83)	679.5 (N=68)	689.3 (N=70)	697.3* (N=70) [105%]	705.1*** (N=83) [106%]
Chronic Phase - Male Body Weight Gain (g)					
BWG - Weeks 0-48	417.1 (N=83)	430.0 (N=68)	442.1* (N=70) [106%]	450.5** (N=70) [108%]	457.3 *** (N=83) [110%]
Chronic Phase - Female Body Weights (g)					
Initial BW - Week 0	181.5 (N=85)	182.3 (N=70)	180.8 (N=70)	179.9 (N=70)	178.8 (N=85)
Final BW - Week 48	338.5 (N=83)	341.1 (N=70)	342.6 (N=70)	350.9 (N=69)	357.9** (N=83) [106%]
Chronic Phase - Female Body Weight Gain (g)					
BWG - Weeks 0-48	157.2 (N=83)	158.9 (N=70)	161.8 (N=70)	171.0* (N=69) [109%]	179.0*** (N=83) [114%]
Carcinogenicity Phase - Male Body Weights (g)					
Initial BW- Week 0	249.0 (N=60)	249.3 (N=60)	247.1 (N=60)	247.4 (N=60)	247.8 (N=60)
Final BW - Week 101	638.6 (N=32)	645.5 (N=32)	670.2 (N=30)	665.6 (N=35)	668.1 (N=36)
Carcinogenicity Phase - Male Body Weight Gain (g)					
BWG - Weeks 0-101	392.2 (N=32)	397.3 (N=32)	422.7 (N=30)	420.3 (N=35)	421.3 (N=36)
Carcinogenicity Phase - Female Body Weight (g)					
Initial BW - Week 0	182.3 (N=60)	182.7 (N=60)	181.3 (N=60)	180.5 (N=60)	178.0* (N=60) [98%]
Final BW - Week 101	431.8 (N=40)	430.9 (N=48)	424.0 (N=42)	408.9 (N=41)	432.7 (N=44)
Carcinogenicity Phase - Female Body Weight Gain (g)					
BWG - Weeks 0-101	249.6 (N=40)	248.4 (N=48)	243.7 (N=42)	228.1 (N=41)	254.5 (N=44)

a The values shown in brackets are the % of control and are given for the body weights and weight gains that are statistically different from the controls.

* Statistically different ($p<0.05$) from the control.

** Statistically different ($p<0.01$) from the control.

*** Statistically different ($p<0.001$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food Consumption:** The overall mean food consumption was higher in males at all dose levels, when compared to controls. Generally, the overall mean food consumption was lower in females at 5, 75 and 250 ppm. The differences achieved statistical significance on several occasions but no dose-related effect was noted.

2. **Compound Consumption (time-weighted average):** The mean achieved dose levels expressed in mg/kg body weight/day received by the females during the study are presented in Table 4.

TABLE IIA 5.5.2-3. Mean Achieved Dietary Intake of Ethiprole (mg/kg/day)

Test Group	Dose Level (ppm in diet)	Test Compound Intake (mg/kg/day) for Indicated Study Intervals			
		Chronic Phase (Weeks 1 - 48)		Carcinogenicity Phase (Weeks 1-101)	
		Males	Females	Males	Females
Low (LDT)	5	0.25	0.34	0.22	0.29
Mid-1 (MDT)	20	0.98	1.34	0.85	1.17
Mid-2 (MDT)	75	3.73	5.01	3.21	4.40
High (HDT)	250	12.39	16.51	10.79	14.68

D. OPHTHALMOSCOPIC EXAMINATION:

There were no treatment-related changes at the ophthalmological examination at one and two years.

E. BLOOD ANALYSES:

1. **Hematology:** Prothrombin times were higher (in males) and lower (in females) throughout the study at 75 and 250 ppm (Table IIA 5.5.2-4). In addition at 250 ppm in females, the mean platelet counts were statistically significantly higher on Months 12 (+25%, α=0.001) and 24 (+35%, α=0.01).
   After the recovery period no relevant differences were noted between control and high dose animals indicating that these effects were reversible.

   Some other slight variations in erythrocyte parameters noted at 250 ppm. However, as these changes were of low magnitude and high inter-individual variability was noted, they were not considered toxicologically significant. Other statistically significant changes found in the hematological parameters were considered to be incidental and unrelated to treatment as they were not dose-related and/or not biologically relevant.

   No treatment-related hematological changes were observed at 5 or 20 ppm.

TABLE IIA 5.5.2-4. Mean Prothrombin Time (seconds) for Animals from the Chronic, Recovery and Carcinogenicity Phases

Interval	Dose Level (ppm in diet)				
	0	5	20	75	250
Males - Chronic Phase[a]					
6 month	12.64 (N=26)	12.93 (N=20)	13.09 (N=18)	13.48** (N=16)	15.87 *** (N=28)
12 month	13.06 (N=27)	13.16 (N=19)	13.05 (N=17)	12.89 (N=19)	14.27** (N=28)
Females - Chronic Phase[a]					
6 month	12.32 (N=22)	12.17 (N=14)	11.99 (N=17)	11 .34*** (N=18)	11.00*** (N=27)
12 month	12.70 (N=24)	12.88 (N=16)	12.88 (N=17)	11.82** (N=18)	11.04*** (N=28)
Males - Recovery Phase					
15 month	12.56 (N=9)	---	---	---	12.83 (N=9)
Females - Recovery Phase					
15 month	12.98 (N=9)	---	---	---	11.84 (N= 7)
Males - Carcinogenicity Phase					
18 month	13.24 (N=8)	12.86 (N=9)	12.86 (N=10)	12.97 (N=9)	14.31* (N=10)
24 month	13.51 (N=10)	13.56 (N=9)	13.81 (N=9)	13.06 (N=10)	13.50 (N=10)
Females - Carcinogenicity Phase					
18 month	12.62 (N=9)	12.36 (N=10)	12.47 (N=10)	11.14*** (N=9)	11.02*** (N=10)
24 month	13.56 (N=10)	12.71 (N=7)	13.41 (N=9)	12.20** (N=9)	11.79*** (N=11)

a: N = number of animals examined and includes all animals from chronic, recovery and carcinogenicity phases
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

2. Clinical Chemistry: In females at 250 ppm, mean total cholesterol concentration was higher at Months 6 (+44%, α=0.001), 12 (+55%, α=0.001), 18 (+84%, α=0.01) and 24 (+33%, not statistically significant). Higher mean total protein concentrations were observed at 250 ppm in males and females in Months 6 (approx. +10%, α=0.001), 12 (approx. +7%, α=0.001), and in females only on Month 18 (+7%, α=0.05) (Table IIA 5.5.2-5). Mean total bilirubin concentrations were lower at 75 and 250 ppm in both sexes on Months 6 (approximately -34% and -48%), 12 (approx.-30% and -51%), 18 (approx. -50% and -49%) and 24 (approx. -36% and -48%), and at 20 ppm in males on Month 18 (-31%). After the 3-month recovery period, no relevant changes were noted between controls and the high dose group indicating that the effects were reversible.

Also at 75 and 250 ppm, a few statistically significant changes were noted in enzyme activities (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) in males and/or females at different sampling times. These changes were of low magnitude and not consistent over time and thus were not considered to be toxicologically significant. Other statistically

significant variations noted in clinical chemistry were of low magnitude or not dose-related, thus they were not considered to be toxicologically significant.

No treatment-related biochemical changes were observed at 5 or 20 ppm.

TABLE IIA 5.5.2-5. Mean Total Cholesterol, Total Protein and Total Bilirubin Concentrations for Animals from the Chronic, and Carcinogenicity Phases [a]

Interval	Parameter	Dose Level (ppm in diet)				
		0	5	20	75	250
Males - Chronic Phase [b]						
Number of animals examined		30	20	20	20	29
Month 6	TBIL (µmol/l)	2.45	2.48	2.10	1.87**	1.49***
	CHOL (mmol/l)	2.098	2.185	1.996	1.975	2.229
	TPROT (g/l)	65.1	66.3	66.4	65.9	71.3***
Month 12	TBIL (µmol/l)	2.67	2.68	2.38	2.00*	1.55***
	CHOL (mmol/l)	2.485	2.518	2.527	2.527	2.699
	TPROT (g/l)	67.7	67.9	69.1	68.9	71.8***
Females - Chronic Phase [b]						
Number of animals examined		30	20	20	20	30
Month 6	TBIL (µmol/l)	2.97	2.36**	2.59	1.67***	1.27***
	CHOL (mmol/l)	2.108	1.901	2.119	1.944	3.040***
	TPROT (g/l)	68.3	66.3	66.3	68.9	74.3***
Month 12	TBIL (µmol/l)	2.92	3.13	2.58	1.91***	1.17***
	CHOL (mmol/l)	2.283	2.223	2.335	2.471	3.540***
	TPROT (g/l)	70.7	68.9	71.3	73.6*	76.7***

(Table continued; footnotes to follow)

TABLE IIA 5.5.2-5. Mean Total Cholesterol, Total Protein and Total Bilirubin Concentrations for Animals from the Chronic, and Carcinogenicity Phases [a] *(cont.)*

Interval	Parameter	Dose Level (ppm in diet)				
		0	5	20	75	250
Males- Carcinogenicity Phase						
Number of animals examined		10	10	10	10	10
Month 18	TBIL (µmol/l)	3.77	2.85	2.59*	2.42**	1.88***
	CHOL (mmol/l)	3.196	3.153	2.794	3.059	2.854
	TPROT (g/l)	73.0	70.3	71.7	70.1	72.9
Month 24	TBIL (µmol/l)	3.07	2.80	2.60	2.14**	1.66***
	CHOL (mmol/l)	3.177	3.230	3.021	3.025	3.397
	TPROT (g/l)	67.8	71.2	66.9	70.3	72.1

Interval	Parameter	Dose Level (ppm in diet)				
		0	5	20	75	250
Females- Carcinogenicity Phase						
Number of animals examined		10	10	10	10	11
Month 18	TBIL (µmol/l)	3.25	3.16	2.71	2.19**	1.72***
	CHOL (mmol/l)	2.393	2.354	2.182	2.633	4.405***
	TPROT (g/l)	74.6	74.1	74.4	77.7	79.9*
Month 24	TBIL (µmol/l)	3.17	2.79	2.73	1.88***	1.59***
	CHOL (mmol/l)	2.394	2.620	2.519	2.826	3.185
	TPROT (g/l)	73.6	71.9	74.0	77.0	74.5

a TBIL = Total Bilirubin, CHOL = Total cholesterol, TPROT= Total protein., b: N=number of animals examined and includes all animals from chronic, recovery and carcinogenicity phases

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

3. Hormone analysis: Statistically and biologically significant effects were observed on TSH and T4 levels at 250 ppm (table IIA 5.5.2-6). No treatment-related hormonal changes were observed at 5 and 20 ppm.

At Week 2, increases in TSH levels (approx.+52%) were associated with decreases in T4 levels (approx.-11%), both in males and in females. The decrease observed in females was not statistically significant, but in view of the effect on TSH, it was considered biologically significant. At 75 ppm, a trend showing an increase in TSH in association with a T4 decrease was observed in females. These effects were not statistically significant.

At Week 9, a statistically significant increase in TSH levels at 250 ppm and 75 ppm was observed in females (+49% and +20%, respectively). This increase was associated with a decrease in T4 levels (-14% and -9%, respectively). A trend toward an increase in TSH associated with a decrease of T4 level was also noted at 20 ppm, but was not statistically significant. The other changes observed in females were not considered to be related to treatment. In males, an increase in TSH levels at 250 and 75 ppm (+13% and +9%, respectively) associated with a decrease in T4 levels (-13% and -2%, respectively) was also observed. In view of this association between TSH and T4 level variations, these effects were considered related to treatment.

At Week 24, a statistically significant increase in TSH levels at 250 ppm was observed in females (+55%). This increase was associated with a decrease in T4 (-14%). A trend toward an increase in TSH associated with a decrease of T4 levels was also noted at 75 and 20 ppm, but these changes were not statistically significant or dose-related. In males, a statistically significant increase in TSH at 250 ppm (+36%) was observed without a corresponding decrease in T4 (+8%). A statistically significant increase in T3 levels were observed at 20 and 250 ppm. Toxicological significance of these T3 variations was considered doubtful due to the absence of a dose response.

At Week 52, statistically significant effects were observed at 250 ppm in females for TSH (+43%) and T4 plasma levels (-14%). A trend toward an increase in TSH (+16%) associated with a decrease in T4 (-23%) was noted at 75 ppm, but these effects were only statistically significant for T4.

At Week 2, 6 or 13 of the recovery period, no biologically significant effects were observed on TSH, T4 or T3 plasma levels at 250 ppm in either males or females.

TABLE IIA 5.5.2-6. Mean T3, T4, and TSH Plasma Levels During Chronic Phase

Interval	Parameter	Dose Level (ppm in diet)				
		0	5	20	75	250
Males						
Number of animals examined		25	10	10	10	25
WEEK 2	TSH (ng/ml)	6.278	6.300	6.584	6.062	9.011
	T4 (ng/ml)	35.226	34.066	36.451	34.136	32.465*
	T3 (ng/ml)	0.675	0.661	0.770	0.691	0.699
WEEK 9	TSH (ng/ml)	6.479	6.983	6.794	7.071	7.320
	T4 (ng/ml)	27.653	27.613	28.917	27.153	24.063**
	T3 (ng/ml)	0.352	0.412	0.421	0.391	0.372
WEEK 24	TSH (ng/ml)	6.509	6.495	7.153	5.724	8.872***
	T4 (ng/ml)	23.733	24.465	26.693	25.640	25.643 (N=24)
	T3 (ng/ml)	0.260	0.287	0.372**	0.339	0.402*** (N=24)
WEEK 52	TSH (ng/ml)	6.658±1.760 (N=24)	5.957	6.759	5.554	7.106±2.165 (N=24)
	T4 (ng/ml)	23.963 (N=24)	22.502	22.953	22.966	22.276 (N=24)
	T3 (ng/ml)	0.420 (N=23)	0.389	0.397	0.376	0.469 (N=24)
Females						
Number of animals examined		25	10	10	10	25
WEEK 2	TSH (ng/ml)	5.054	5.445	5.085	5.485	8.072***
	T4 (ng/ml)	29.099	28.889	26.646	27.309	25.456
	T3 (ng/ml)	0.656 (N=24)	0.759	0.660	0.692	0.646
WEEK 9	TSH (ng/ml)	4.749	4.911	5.216	5.693*	7.086***
	T4 (ng/ml)	24.562	25.604	22.995	22.308	21.092*
	T3 (ng/ml)	0.503	0.531	0.478	0.473	0.483
WEEK 24	TSH (ng/ml)	4.641 (N=24)	5.502	5.205	4.949	7.213***
	T4 (ng/ml)	22.700 (N=24)	23.133	19.269	19.138	19.453
	T3 (ng/ml)	0.410 (N=24)	0.415	0.397	0.392	0.472 (N=24)
WEEK 52	TSH (ng/ml)	5.087	4.695	5.486 (N=9)	5.906	7.295** (N=23)
	T4 (ng/ml)	21.942 (N=25)	19.915	23.424 (N=9)	16.804***	18.946* (N=23)

	T3 (ng/ml)	0.548	0.527	0.600 (N=9)	0.472	0.538 (N=23)

* $p < 0.05$; ** $p < 0.01$; *** $p<0.001$

F. **URINALYSIS:** Urinary pH values were higher in females at 250 ppm at Months 6 and 12 (+9% and +7%, respectively, α=0.001) and at 75 ppm at Month 12 (+7%, α=0.05). In addition, for females at 250 ppm the mean refractive index was slightly lower at Month 6. No treatment-related changes were noted at Months 18 and 24 in either males or females.

After a 3-month recovery period, no relevant changes were noted between controls and the high dose group indicating that these findings were reversible.

No treatment-related urinalysis changes were observed at 5 or 20 ppm.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ Weight:** Liver and/or thyroid weights were increased at 75 and/or 250 ppm as described below (table IIA 5.5.2-7 and table IIA 5.5.2-8). Treatment-related histopathology was noted at 75 ppm in the thyroid (females only) and at 250 ppm in the liver and thyroid (both sexes).

Chronic Phase: Treatment-related organ weight changes were noted in the liver and thyroid gland. There was a statistically significant, dose-related, higher mean liver weight at 75 ppm in females only (approx.+15%) and at 250 ppm in both sexes (approx 26% in males and 46% in females). The mean thyroid weight was increased (statistically significant) at 75 ppm in males only (approx.+30%) and at 250 ppm in both sexes (approx. 63% in males and 24% in females).

Reversibility Phase: No treatment-related changes in organ weights were noted compared to controls.

Carcinogenicity Phase: At the end of the carcinogenicity phase, treatment-related organ weight changes were noted in the liver and thyroid gland. Mean liver weights were increased (statistically significant) at 75 ppm in females (approx.+14%) and 250 ppm in both sexes (approx. 24% in males and 38% in females). There was a statistically significant higher mean thyroid weight at 75 and 250 ppm in both males (approx.+38% and +21%, respectively) and females (approx.+30% and +35%, respectively). In males, this change was only statistically significant when expressed as the ratio to brain weight.

A few statistically significant minimal differences were observed as compared to control group, namely a higher mean kidney weight at 250 ppm in both sexes, at 75 ppm in females, and at 5 ppm in males; and a higher mean heart weight at 250 ppm in females. These differences, only noted at the end of the 104-week phase of the study, were minimal (8-13%), not observed for all the modes of expression of the organ weights, not dose-related, and not correlated to gross or microscopic changes. Therefore, they were not considered to be biologically or toxicologically significant.

No treatment-related organ weights changes were observed at 5 or 20 ppm.

TABLE IIA 5.5.2-7. Mean Terminal Body Weight, Absolute and Relative Liver Weights

Organ	Parameter	Dose level (ppm in diet)				
		0	5	20	75	250
Chronic Phase- males						
		10	10	10	10	9
Terminal body weight (g)		612.5	641.9	650.6	648.0	666.0 (N=10)
Liver	Absolute weight (g)	11.42	12.11	12.58	12.39	14.80***
	% of terminal body weight	1.87	1.90	1.93	1.92	2.22***
	% of brain weight	516.33	543.23	558.70	555.28	678.71**
Carcinogenicity Phase- males						
Number ofanimals examined		27	30	29	34	29
Terminal body weight (g)		603.8	597.3	591.3	613.8	598.2
Liver	Absolute weight (g)	12.37	12.40	12.33	13.51	15.20***
	% of terminal body weight	2.06	2.11	2.12	2.23	2.57***
	% of brain weight	533.33	528.78	530.58	578.94	666.80***
Chronic Phase- females						
Number of animals examined		10	10	10	10	10
Terminal body weight (g)		314.2	314.9	311.8	310.2	308.4
Liver	Liver (g)a	6.90	6.74	7.12	7.75	10.00*
	% of terminal body weight	2.21	2.15	2.28	2.51**	3.23***
	% of brain weight	331.12	325.33	353.66	390.77**	491.12***
Carcinogenicity Phase- females						
Number of animals examined		38	44	37	34	41
Terminal body weight (g)		314.2	314.9	311.8	310.2	308.4
Liver	Absolute weight (g)	9.82 (N=36)	9.87	9.75	11.14*	13.55***
	% of terminal body weight	2.46	2.48	2.50	2.81***	3.37***
	% of brain weight	465.74	470.08	469.30	532.19*	644.07***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

TABLE IIA 5.5.2-8. Mean Terminal Body Weight, Absolute and Relative Thyroid Weights

Organ	Parameter	Dose level (ppm in diet)				
		0	5	20	75	250
Chronic Phase- males						
Number of animals examined		10	10	10	10	9
Terminal body weight (g)		612.5	641.9	650.6	648.0	666.0 (N=10)
Thyroid	Absolute weight (g)	0.022	0.029	0.027	0.030*	0.037***
	% of terminal body weight	0.0038	0.0044	0.0042	0.0046	0.0057***
	% of brain weight	1.006	1.278	1.194	1.333*	1.713**
Carcinogenicity Phase- males						
Number of animals examined		27	30	29	34	29
Terminal body weight (g)		603.8	597.3	591.3	613.8	598.2
Thyroid	Absolute weight (g)	0.028	0.030	0.032	0.039	0.034
	% of terminal body weight	0.0047	0.0052	0.0056	0.0065	0.0057
	% of brain weight	1.2241	1.284	1.379	1.666*	1.478**
Chronic Phase- females						
Number of animals examined		10	10	10	10	10
Terminal body weight (g)		314.2	314.9	311.8	310.2	308.4
Thyroid	Absolute weight (g)	0.020	0.016	0.017	0.021	0.024*
	% of terminal body weight	0.0063	0.0052	0.0055	0.0068	0.0078*
	% of brain weight	0.947	0.778	0.856	1.056	1.199*
Carcinogenicity Phase- females						
Number of animals examined		38	44	37	34	41
Terminal body weight (g)		314.2	314.9	311.8	310.2	308.4
Thyroid	Absolute weight (g)	0.025	0.026	0.026	0.032*	0.034***
	% of terminal body weight	0.0063	0.0064	0.0066	0.0084*	0.0085***
	% of brain weight	1.193	1.226	1.232	1.549	1.601***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

2. **Gross Pathology**: No treatment-related gross pathologic changes were noted at any dose level during any phase of the study.

3. Microscopic Pathology:

a) Non-neoplastic:

Chronic Phase: Selected non-neoplastic microscopic findings from the carcinogenicity phase of the study are presented in Table IIA 5.5.2-9.
Treatment-related changes were noted in the liver and thyroid gland as follows. In the liver, centrilobular hepatocyte hypertrophy attributable to treatment was noted in all females at 250 ppm. This change characterized by an appreciable increase in the size of hepatocytes in the area of the central vein was generally graded slight to mild and is considered to represent a minor adaptive change (table IIA 5.5.2-9).

In the thyroid, follicular cell hypertrophy was noted in animals of both sexes dosed at 250, 75 or 20 ppm. This change which is characterized by an increase in height of the follicular epithelial cells accompanied by a decrease in the follicular size and in colloid content was graded slight to moderate. There was evidence of a dose-effect relationship in both sexes, more prominently in females than in males. In addition, colloid mineralization was observed in a small proportion of males (2/9) and females (4/10) at 250 ppm.

Reversibility Phase: In the liver, there was a markedly lower occurrence of periportal hepatocyte microvacuolation in females at 250 ppm (2/11) compared to the control (9/15). Centrilobular hepatocyte hypertrophy, which was considered to be treatment-related at the end of the 52-week chronic phase of the study in females at 250 ppm, was fully reversible.

In the thyroid gland, the occurrence of colloid mineralization, characterized by the presence of basophilic material within the colloid of the majority of the follicles, was higher in males (7/14) and females (4/11) from the 250 ppm group as compared to controls (0/12 and 0/15 for males and females, respectively). Colloid mineralization in the thyroid gland was also observed in 1 of the 4 high-dose females which died prior to the scheduled sacrifice. This was the only treatment-related finding noted in the animals which died early for this phase of the study.

In addition in the thyroid, the occurrence of slight follicular cell hypertrophy was still higher in males from the 250 ppm group (5/14) as compared to the control group (1/13), indicating that reversibility was in progress but incomplete in males. However, in females which were more affected than males at the end of the 52-week chronic phase, this change was fully reversible.

Carcinogenicity Phase: In the liver, bile duct hyperplasia and sclerosis and focal sinusoidal dilatation were noted in many animals with the incidence and severity being slightly higher in females at 250 ppm (table IIA 5.5.2-10). Also in the liver, eosinophilic and basophilic tigroid foci of cellular alteration were noted in many animals from all groups. The incidence of both types (and the severity of tigroid foci) was slightly higher in males dosed at 250 ppm in comparison with controls. In females, the incidence of tigroid altered hepatocyte foci was lower in animals dosed at 250 ppm with no effect on the incidence of eosinophilic foci of cellular alteration.

In the thyroid gland, colloid mineralization was the predominant finding in rats of both sexes at 250 ppm and females at 75 ppm. Females at 250 ppm also showed significant diffuse follicular hypertrophy. These changes are consistent with minor, chronic stimulation.

In the kidneys, chronic progressive nephropathy was noted in most animals from all groups. The severity, but not the incidence, was slightly higher in rats of both sexes at 250 ppm. In addition, the incidence of arteritis/periarteritis in the kidney was marginally higher than controls in females at 250 ppm. This change may be related to the increased severity of chronic progressive nephropathy.
In the lungs, foci of alveolar macrophages were noted in many animals from all groups. The incidence was marginally higher than controls in females dosed at 250 ppm.
There were no treatment-related non neoplastic changes observed at 5 or 20 ppm.

TABLE IIA 5.5.2-9. Non-Neoplastic Microscopic Findings in Rats at Wk 52 (Chronic Phase)

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	5	20	75	250	0	5	20	75	250
	Males					Females				
Liver number examined	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**10**	**10**	**10**
Hepatocyte hypertrophy - centrilobular	0	1	0	0	0	0	0	1	0	10
Thyroid number examined	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**10**	**10**	**10**
Follicular cell hypertrophy	0	0	1	3	5	0	0	2	3	9

TABLE IIA 5.5.2-10. Non-Neoplastic Microscopic Findings in Rats at Wk 104 (Carcinogenicity Phase)

Observation		Males					Females				
		Dose Level (ppm in diet)					Dose Level (ppm in diet)				
		0	5	20	75	250	0	5	20	75	250
LIVER	**Number examined**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
	Hyperplasia, Bile Ducts:										
	Slight	23	33	29	26	30	16	22	15	24	18
	Mild	14	1	4	14	8	3	1	2	5	14
	Moderate	3	0	1	0	1	0	0	0	0	1
	Total	**40**	**34**	**34**	**40**	**39**	**19**	**23**	**17**	**29**	**33**
	Sclerosis, Bile Ducts:										
	Slight	18	29	26	28	33	9	13	6	17	22
	Mild	11	9	10	11	9	1	0	0	0	7
	Moderate	0	0	0	0	0	0	0	1	0	0
	Total	**29**	**38**	**36**	**39**	**42**	**10**	**13**	**7**	**17**	**29**
	Dilatation, Sinusoidal, Focal:										
	Slight	4	2	9	8	5	14	13	14	17	28
	Mild	0	0	0	0	0	1	0	0	0	2
	Total	**4**	**2**	**9**	**8**	**5**	**15**	**13**	**14**	**17**	**30**
	Cellular Alteration, Basophilic, Tigroid, Focal:										
	Slight	30	33	38	33	37	30	30	27	32	17
	Mild	2	2	3	6	10	10	13	8	4	4
	Moderate	0	0	0	0	3	2	0	0	2	0
	Marked	0	0	0	0	1	0	0	0	1	0
	Total	**32**	**35**	**41**	**39**	**51**	**42**	**43**	**35**	**39**	**21**
	Cellular Alteration, Eosinophilic Type, Focal:										
	Slight	23	20	16	16	32	22	32	24	17	29
	Mild	3	3	3	4	8	5	3	6	6	2
	Moderate	0	0	1	0	0	0	0	2	0	0
	Total	**26**	**23**	**20**	**20**	**40**	**27**	**35**	**32**	**23**	**31**

(Table continued; footnotes to follow)

TABLE IIA 5.5.2-10. Non-Neoplastic Microscopic Findings in Rats at Wk 104 (Carcinogenicity Phase) ***(cont.)***

Observation		Males					Females				
		Dose Level (ppm in diet)					Dose Level (ppm in diet)				
		0	5	20	75	250	0	5	20	75	250
THYROID	**Number examined**	**60**	**60**	**59**	**60**	**59**	**59**	**59**	**60**	**60**	**60**
	Mineralization, Colloid:										
	Slight	4	4	4	9	12	0	1	3	5	16
	Mild	3	2	0	2	6	0	0	0	2	16
	Moderate	1	1	1	3	11	0	0	0	2	10
	Marked	1	0	0	0	0	0	0	0	0	0
	Total	**9**	**7**	**5**	**14**	**29**	**0**	**1**	**3**	**9**	**42**
	Hypertrophy, Follicular, Diffuse:										
	Slight	1	0	2	3	3	0	0	0	3	14
	Mild	1	1	2	0	0	0	0	0	1	6
	Total	**2**	**1**	**4**	**3**	**3**	**0**	**0**	**0**	**4**	**20**
KIDNEYS	**Number examined**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
	Nephropathy, Chronic, Progressive:										
	Slight	33	32	33	36	28	37	36	30	28	26
	Mild	10	8	9	6	16	1	2	2	9	11
	Moderate	6	3	5	7	10	3	1	2	1	5
	Marked	0	0	1	1	3	0	0	0	3	2
	Severe	0	1	0	0	0	0	0	0	1	3
	Total	**49**	**44**	**48**	**50**	**57**	**41**	**39**	**34**	**42**	**47**
	Arteritis/Periarteritis:										
	Slight	0	0	1	2	0	0	1	0	2	2
	Mild	0	0	0	0	1	0	0	0	1	2
	Moderate	0	0	0	0	0	0	1	0	0	2
	Total	**0**	**0**	**1**	**2**	**1**	**0**	**2**	**0**	**3**	**6**
LUNGS	**Number examined**	**60**	**60**	**60**	**60**	**60**	**60**	**59**	**60**	**60**	**59**
	Alveolar Macrophages, Focal:	21	26								
	Slight	22	22	24	18	21	17	18	19	24	29
	Mild	3	4	1	1	3	6	3	4	3	6
	Moderate	2	0	0	0	0	0	0	0	0	1
	Total	**21**	**26**	**25**	**19**	**30**	**23**	**21**	**23**	**27**	**36**

b) Neoplastic

Reversibility and Chronic Phases: There was no evidence of treatment-related increased incidence of proliferative changes at the end of either the chronic or reversibility/recovery phases of the study.

Carcinogenicity Phase: For the unscheduled deaths, there was no indication of a significantly higher incidence of tumors overall in either sex. Findings in the target organs (liver and thyroid) in the animals that died prematurely are as follows. Thyroid follicular cell adenoma was seen in four males dosed at 250 ppm with no similar lesion in controls (table IIA 5.5.2-11). There was no evidence of an effect of treatment in females. Focal follicular cell hyperplasia was seen in one control male, one 5 ppm male, four 250 ppm males and one 250 ppm female. In the liver, hepatocellular adenoma was seen in one male dosed at 250 ppm.

At the scheduled sacrifice, there was no evidence of an effect of treatment in the thyroid. Follicular cell adenoma was noted in two females in the 250 ppm group. In the liver, hepatocellular adenoma was noted in two males at 250 ppm and in one female at 75 ppm.

For the overall incidence (scheduled + unscheduled deaths), when focal follicular cell hyperplasia is included in the analysis, a higher incidence of focal proliferative lesions in comparison with controls becomes apparent, especially in males. In the context of the stimulatory effect of treatment on the thyroid gland, these results provide evidence of a treatment-related effect on focal proliferative lesions at 250 ppm especially in males. In the liver, hepatocellular adenoma was found in three males at 250 ppm and in one female at 75 ppm with no similar lesion in controls.

There was no indication of a significantly higher overall tumor incidence in either sex. Although a slight increase was observed at 250 ppm for follicular hyperplasia and adenomas in the thyroids (both sexes) and for adenomas in the liver (males only), no statistical differences were noted. A variety of spontaneous tumors was noted in animals from all groups with no evidence of an effect of treatment. Tumors were commonly encountered (10 or more in a group) in the pituitary gland, thyroid gland (C-cells), subcutaneous tissue (males) and mammary gland (females). The spectrum was consistent with the range of spontaneous lesions encountered in aging rats of this strain.

There were no treatment-related neoplastic changes at 5, 20 or 75 ppm.

TABLE IIA 5.5.2-11. Neoplastic Microscopic Findings in Thyroids of Rats from Carcinogenicity Phase (Week 104)

Males at both unscheduled and terminal sacrifices (Week 104)						
	Dose level in diet (ppm)	**0**	**5**	**20**	**75**	**250**
THYROID	**Number examined**	**60**	**60**	**59**	**60**	**59**
	Hyperplasia, follicular cell, focal	2	1	0	1	5
	Adenoma, follicular cell	0	0	0	0	4
	Carcinoma, follicular cell	0	0	0	0	0
	Total with focal proliferative lesions	**2**	**1**	**0**	**1**	**8**
Females at both unscheduled and terminal sacrifices (Week 104)						
	Dose level in diet (ppm)	**0**	**5**	**20**	**75**	**250**
THYROID	**Number examined**	**59**	**59**	**60**	**60**	**60**
	Hyperplasia, follicular cell, focal	0	1	0	1	2
	Adenoma, follicular cell	0	0	0	0	2
	Carcinoma, follicular cell	0	0	1	1	0
	Total with focal proliferative lesions	**0**	**1**	**1**	**2**	**4**

III. CONCLUSIONS:

In conclusion, the dietary administration of ethiprole in rats for 104 weeks produced systemic toxicity at 250 ppm and to a lesser extent at 75 ppm. The NOAEL is 0.85 mg/kg/day in males and 1.17 mg/kg/day in females (20 ppm in the diet) based on observed effects in the thyroid and/or liver (histopathologic changes, increased organ weights, and/or altered thyroid hormone or bilirubin levels) at75 ppm.

1.	Annex point(s)	KIIA 5.5.4; Long-term toxicity and carcinogenicity/Mechanism of action and supporting data: **MRID 47622827**
2.	Location in dossier	KIIA 5.5.4 /02
3.	Authors (year)	Webber, C. (2001)
	Title	Ethiprole Investigation into potential effects on thyroid function after two weeks of treatment in male rats using the Perchlorate discharge test
	Company, report No.	Bayer CropScience AG, Report-No.: C017842 (M-205038-01-1)
	Date	12.11.2001
4.	Testing facility	Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole (radioactive), ethiprole Substance(s): ethiprole TC ethiprole (radioactive) TC
7.	Test method	
	Deviations	
8.	GLP	yes

Executive Summary:

The objective of the study was to obtain an insight into the mechanism of action of ethiprole on thyroid function in the rat. Three groups of male Wistar strain rats (initially 24 animals/group) received oral (gavage) administration of 0 and 20 mg/kg/d of ethiprole (batch N°CRD9706, purity 92.7% (927 g/kg). An additional group received oral (gavage) administration of 200 mg/kg/d of propylthiouracil (positive control). For each group, 14 consecutive daily doses were given at intervals of about 24 hours.

Body weight and food consumption were recorded twice weekly and clinical signs monitored daily for each individual animal throughout the study. After the completion of the 14-day dose administration period, and 24 hours after the final dose administration, each rat received an intraperitoneal injection of sodium ^{125}I-iodide solution. Six hours after this, further intraperitoneal injections were administered as follows: 0.9% saline to six rats from each treatment group; potassium perchlorate (10 mg/kg) to six rats from each treatment group; potassium perchlorate (20 mg/kg) to six rats from each treatment group. Two and half minutes following these second intraperitoneal injections, samples of whole-blood were taken from each animal prior to sacrifice and the thyroid gland then removed.

There were no unscheduled deaths during the course of the study. Group mean food intake and body weight gain for rats receiving ethiprole was unaffected by treatment. However, for rats receiving propylthiouracil, both food consumption and body weight gain were significantly reduced.

Animals receiving 200 mg/kg/day propylthiouracil (positive control for direct effect on thyroid) showed clinical signs characteristic of this treatment after dosing throughout the treatment period. No clinical signs or a reaction to treatment were noted for rats treated with ethiprole.
Statistical analysis of the data indicated that pre-treatment of male rats for 14 days with propylthiouracil resulted in a large increase in thyroid weight ($p<0.001$), consistent with hypertrophy. In addition, significant decreases in thyroid radioactivity concentration and total thyroid radioactivity ($p<0.001$ in each case) were observed. In contrast, the only statistically

significant change seen in male rats pre-treated with the test substance, ethiprole, was an increase in thyroid activity concentration ($p<0.001$) and total thyroid radioactivity ($p=0.002$).
These data indicate that the effects of ethiprole on thyroid function are not mediated by a direct toxic effect.

Comparison of the data obtained from a group of rats to which ethiprole was administered orally during 14 days with data from rats which had received 14 daily doses of propylthiouracil (positive control), suggests that ethiprole at 20 mg/kg/day does not affect thyroid function by of a direct toxic effect.

This study is classified as **acceptable/non-guideline** and provides supporting data to the combined chronic toxicity/ carcinogenicity study in the rat.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	92.7% (927 g/kg)
Compound Stability:	The test substance was stored at room temperature in the dark
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** Propylthiouracil (5-propyl-2-thiouracil) was used as a positive control. Propylthiouracil is a thyroid peroxidase inhibitor. The vehicle, used for both the test substance and positive control, was 0.5% aqueous (w/v) methylcellulose.

3. **Test Animals:**

Species:	Rat	
Strain:	Wistar (AF) rats RJ: WI (IOPS AF)	
Age/weight at study initiation:	6 weeks old/ 173- 245 g (males only used in this study)	
Source:	Elevage Janvier, Le Genest St Isle, France	
Housing:	Housed 4 rats per cage in suspended cages with wire mesh floors.	
Diet:	Pelleted SDS Rat and Mouse No. 1 modified maintenance diet, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19 - 22°C
	Humidity:	38 - 57%t
	Air changes:	not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	15 days	

B. STUDY DESIGN:

1. **In-Life Dates:** November 04, 1999 to November 18, 1999

2. **Animal Assignment:** Animals were assigned randomly to the test groups noted in Table IIA5.5.4.2-1. Only male animals were used because of the greater sensitivity of this sex to thyroid perturbation by goitrogens.

   Oral Dosing Phase: Fourteen consecutive daily doses were given by oral gavage to 24 rats/treatment group. The test substance group received ethiprole administered at 20 mg/kg/day as a 4 mg/mL suspension in 0.5% aqueous methylcellulose. The positive control group received propylthiouracil at 200 mg/kg/day as a 40 mg/mL suspension in 0.5% aqueous methylcellulose. The control group received the vehicle alone (0.5% aqueous methylcellulose). The dosage volume used for all groups was 5 mL/kg, according to the most recent recorded bodyweight.

   Terminal Studies Phase: After the completion of the 14-day oral dose administration period, all animals were transferred to the test facility's Department of Drug Metabolism for the

Terminal Studies phase. At 24 hours after the final oral dose administration, each rat received an intravenous injection of sodium ^{125}I-iodide solution. Six hours after this, intraperitoneal injections were administered as follows: 0.9% saline to 6 rats from each treatment group; potassium perchlorate ($KClO_4$) at 10 mg/kg to 6 rats from each treatment group; or $KClO_4$ at 20 mg/kg to 6 rats from each treatment group. Two and half minutes following the intraperitoneal injections, samples of whole-blood were taken from each animal prior to sacrifice and the thyroid gland then removed.

TABLE IIA 5.5.4.2-1. Study Design

Oral Dosing Phase by Gavage				
Test Group (#)	**Treatment**	**Dosage (mg/kg/day)**	**Dose volume (mL/kg)**	**No. of Male Animals [b]**
Control (1)	Control [a]	0	5	24
Test Substance (2)	Ethiprole	20	5	24
Positive Control (3)	Propylthiouracil	200	5	24

Terminal Studies Phase (24 hours following the last oral dose) [c]		
Test Group (#) / Treatment	**Intraperitoneal Injection**	**No. of Male Animals [b]**
Control (1)	0.9% Saline	6
	$KClO_4$ at 10 mg/kg	6
	$KClO_4$ at 20 mg/kg	6
Test Substance (2) / Ethiprole	0.9% Saline	6
	$KClO_4$ at 10 mg/kg	6
	$KClO_4$ at 20 mg/kg	6
Positive Control (3) / Propylthiouracil	0.9% Saline	6
	$KClO_4$ at 10 mg/kg	6
	$KClO_4$ at 20 mg/kg	6

a The control group was administered the vehicle alone (0.5% aqueous methylcellulose) at 5 mL/kg body weight.

b NOTE: Spare rats (6/treatment group) were included in the study to replace any animals dying during the study and to ensure that 6 rats per group were available for metabolism studies. As there were no unscheduled deaths during the study, these animals were killed and discarded without further investigations.

c Six hours prior to the intraperitoneal injections, each rat received an intravenous injection of sodium ^{125}I-iodide solution.

The dose level and route of administration of ethiprole was selected by the Sponsor to approximate an intermediate dose level which had produced thyroid hormone changes in a 90-day toxicity rat study (Reference IIA 5.3.2, Study No. M-192116-02-1). The dose level of the positive control is one which was reported to be routinely used in studies of this type.

3. **Dose Preparation and Analysis:** Formulations of ethiprole were prepared weekly and stored at 4°C. Ethiprole was suspended in the vehicle of 0.5% aqueous (w/v) methylcellulose at a concentration of 4 mg/mL. Formulations of propylthiouracil were prepared on the day of use by suspension of the propylthiouracil in 0.5% aqueous (w/v) methylcellulose at a concentration of 40 mg/mL.

The stability and homogeneity of the test substance (ethiprole) in 0.5% aqueous (w/v) methylcellulose were established previously (References IIA 5.7.1 and 5.7.2, Study Nos .M-201848-01-1 and M-202573-01-1, respectively). These data were not summarized in the current study.

During the study, samples of the ethiprole formulations, prepared for use in Week 1, were analyzed to check the accuracy of preparation.

Results: **Concentration Analysis:** The mean concentration of ethiprole in the test formulation analyzed for Week 1 of the study was -2.3% of the nominal concentration, confirming the accuracy of formulation.

5. **Statistics:** Statistical analyses were performed on food consumption and body weight data as well as radioactivity measurements in the blood and thyroid as follows.

Food Consumption and Body Weight Data: Food consumption data were analyzed using cumulative cage totals. Bodyweight data were analyzed using weight gains. For these parameters, analysis of variance was applied, followed by Student's *t* test for comparison of treated groups with the control group. Additional or alternative statistical methods were used when considered appropriate

Radioactivity Measurements: The following radioactivity measurements in blood and thyroid subjected to statistical analyses: thyroid radioactivity concentration; thyroid radioactivity (% dose); blood radioactivity concentration; blood radioactivity (% dose); ratio of thyroid radioactivity concentration / blood radioactivity concentration; ratio of blood radioactivity concentration / thyroid weight; and thyroid weight.

There were three groups of male rats, an untreated control and two groups pre-treated for 2 weeks with ethiprole or the positive control (propylthiouracil). Following an intraperitoneal dose of sodium ^{125}I-iodide, the animals were split into three subgroups receiving saline, potassium perchlorate at 10 mg/kg, or potassium perchlorate at 20 mg/kg.

Analyses of variance were carried out on the data with treatment group (control, ethiprole and propylthiouracil), subgroup (saline, $KClO_4$ at 10 mg/kg, or $KClO_4$ at 20 mg/kg) and their interaction as factors.

To confirm the effect of perchlorate on the thyroid, comparisons were made between the subgroups for each treatment group separately. Comparisons were made between the saline and perchlorate groups using Student's *t* test.

Given there was little evidence of an interaction between treatment group and subgroup (F-test $p \geq 0.039$) and the differences from the control were generally similar for the subgroups, comparisons were made using the overall treatment means. Comparisons were made between the control and treated groups using Student's *t* test.

Final bodyweight was considered as a possible covariate in the analysis of thyroid weights; however, there was no evidence of a relationship between organ weight and bodyweight (F-test $p>0.05$) and the results from the unadjusted analysis have been presented.

C. METHODS:

1. **Observations:** Individual animals were observed at least twice daily (except after transfer to Drug Metabolism) for any signs of behavioral changes, reaction to treatment or ill health. These checks were made early in each working day and again in the afternoon to look for

dead or moribund animals. A detailed physical examination was performed weekly for the duration of the study.

In addition, animals were observed in association with dosing on a daily basis during Week 1 and on two occasions during Week 2. These observations were made just prior to dosing, at the end of dosing for each group, between 1 and 2 hours after completion of dosing of all groups, and as late as possible in the working day.

2. **Body Weight:** The weight of each rat was recorded the week before treatment commenced and then on Day 1, 5, 8, 12 and 14 of treatment. Bodyweight was also recorded during the Terminal Studies phase on Day 15.

3. **Food Consumption and Food Conversion Efficiency:** Food consumption was recorded at intervals from one week prior to start of treatment, but not after transfer to The Department of Drug Metabolism for the Terminal Studies phase of the study. Food conversion efficiency was assessed by calculation of food conversion ratios using weight of food consumed per unit bodyweight.

4. **Terminal Studies:** On completion of 2 weeks of treatment, all rats were transferred to the Department of Drug Metabolism at the testing facility (Huntingdon Life Sciences, Huntingdon Research Centre, Cambridgeshire, England). At 24 hours after the last dose of the test compound the rats were dosed intravenously (in tail vein) with sodium ^{125}I-iodide (~1 µCi) in 0.5 mL 0.9% (w/v) saline solution. Six hours later, 6 animals in each treatment group received an intraperitoneal injection of one of the following: potassium perchlorate in 0.9% saline at a dosage of 10 or 20 mg/kg bodyweight, or 0.9% saline alone to serve as the control (see Table 1). All intraperitoneal injections were made in a volume of 2 mL/kg body weight. All solutions (sodium^{125}I-iodide and KCl_4) were made fresh on the day of dosing.

Two and a half minutes following the intraperitoneal injection of the potassium perchlorate or saline solutions, the animals were anaesthetized using isofluorane. Approximately 2 ml whole blood was obtained by cardiac puncture, weighed into pre-weighed heparinised blood tubes and analyzed for radioactivity.

Three replicate aliquots (0.5 ml) of sodium I-iodide solution were taken for measurement of its radioactivity concentration. The mean value (2,334,999 dpm) of these three checks was used as the radioactive dose to each animal.

Thyroid removal/analysis: Immediately after the blood sampling, the animals were killed by cervical dislocation and the thyroid gland from each rat dissected from the trachea following ventral midline incision and skin reflection. Adhering tissue was removed, and the thyroids rinsed in ice cold saline solution, blotted dry and weighed into pre-weighed storage tubes prior to measurement of radioactivity by gamma scintillation counting.

The carcasses were discarded without further investigation.

Measurement of radioactivity: Radioactivity in duplicate weighed aliquots (0.5 mL) of blood and in complete thyroid glands was measured using a gamma scintillation counter with automatic correction for counting efficiency (about 85%) and reference date (set to 15 November 1999, consistent with that given on the Certificate of Analysis for the sodium ^{125}I-iodide). Radioactivity measurements in the sample analyzed of less than twice background at the time of measurement were considered to be below the limit of detection (ND).

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** No clinical signs of a reaction to treatment or signs of ill health were observed among those animals receiving 20 mg/kg/day ethiprole.

   Post-dosing clinical signs for animals receiving 200 mg/kg/day propylthiouracil included; salivation and abnormal gait on most occasions, with partially closed eyes and reduced body tone also noted on two occasions. These signs were evident on completion of dosing from Day 2. All animals appeared to have recovered fully before dosing the next day.

2. **Mortality:** There were no unscheduled deaths.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

Body weight gain for animals receiving 20 mg/kg/day ethiprole was essentially similar to that of controls after 14 days of treatment (table IIA 5.5.4.2-2). Overall bodyweight gain for those animals receiving 200 mg/kg/day propylthiouracil was significantly lower than that of controls.

TABLE IIA 5.5.4.2-2. Body Weight and Body Weight Gain During Dosing Phase

Interval	Test Group		
	Control	Ethiprole	Propylthiouracil
	Dose Level (mg/kg/day)		
	0	20	200
Body Weight (g)			
1	218	213	216
14	311	311	255
Body Weight Gain (g)			
Overall Days 1-14 (% Control)	**93.9**	**98.1 (104)**	**38.5** (41)**

N=24 for each test group, ** Statistically different from control (p<0.01).

C. FOOD CONSUMPTION:

Food intake for animals receiving 20 mg/kg/day ethiprole was unaffected by treatment (table IIA5.5.4.2-3). Food intake for animals receiving 200 mg/kg/day propylthiouracil was altered consistent with the reduced bodyweight gain.

TABLE IIA5.5.4.2-3. Overall (Mean) Food Consumption

Interval	Test Group		
	Control	Ethiprole	Propylthiouracil
	Dose Level (mg/kg/day)		
	0	20	200
Food Consumption (g/day)			
Overall Days 1-13 (% Control)	32.1 (-)	32.7 (102)	23.9** (74)

N=24 for each test group.
** Statistically different from control (p<0.01).

C. TERMINAL STUDIES:

1. **Thyroid Weights:** The mean weight of thyroid glands taken from animals of the control group was 0.0138 g. The corresponding mean for animals pre-treated with ethiprole was 0.0148 g.

   For the positive control group (those pre-treated with propylthiouracil), the mean thyroid weight was 0.0349 g. This was about 2.5 times greater than the control group and, when expressed as % mean bodyweight, was over 3 times greater than the control group. This increase in thyroid weight (compared to the thyroid weights for the control group) was statistically highly significant ($p<0.001$).

2. **Radioactivity Measurements:**
   Controls: The increase in whole-blood radioactivity concentration following administration of perchlorate at the lower dose level was statistically significant ($p=0.006$) compared to the saline treatment (table IIA5.5.4.2-4). As a consequence of this, statistically significant changes in the thyroid/blood radioactivity concentration ratio (decrease, $p=0.020$) and blood radioactivity concentration/thyroid weight ratio (increase, $p=0.002$) were also seen.

   Thyroid radioactivity concentrations in these animals were 393.8 ±84.1% dose/g (saline sub-group), 375.2 ± 79.0% dose/g and 357.5 ± 77.4% dose/g (10 mg/kg and 20 mg/kg perchlorate sub-groups, respectively). These results correspond to about 5.9, 4.5 and 4.9 total % dose in the thyroid for the three sub-groups, respectively.

   Ethiprole treated rats: Whole-blood radioactivity concentrations in ethiprole-treated animals were similar across the subgroups. Thyroid radioactivity concentrations in these animals were 494.2 ± 140.6% dose/g (saline sub-group), 473.8 ± 74.7% dose/g and 428.9 ± 63.9% dose/g (10 mg/kg and 20 mg/kg perchlorate sub-groups respectively). These results correspond to about 7.4, 7.3 and 6.1 total % dose in the thyroid for the three sub-groups respectively and were statistically greater ($p<0.001$ for the concentration and 0.002 for the total radioactivity content) than those in the control rats.

   The ratio of radioactivity concentration in the thyroid to that in the blood was between 1600 and 1800 for each sub-group and was in each case significantly ($p<0.001$) higher than in the corresponding sub-group from the control animals. The ratio of the blood radioactivity concentration (dpm/g) to the thyroid weight (g) was about 450,000 for each sub-group.

   Propylthiouracil treated rats (positive controls): Whole-blood radioactivity concentrations in propylthiouracil-treated animals were increased in the perchlorate sub-groups. At the 20 mg/kg dose level for perchlorate, the increase in whole-blood radioactivity concentration (compared to the saline treated rats) was statistically significant ($p=0.011$).

   Thyroid radioactivity concentrations in these animals were 112.8 ± 17.5% dose/g (saline sub-group), 54.05 ± 22.57% dose/g and 34.92 ±11.21% dose/g (10 mg/kg and 20 mg/kg perchlorate sub-groups respectively). These results correspond to about 3.4, 1.9 and 1.3 total % dose in the thyroid for the three sub-groups respectively and were significantly ($p<0.001$) decreased compared to those in control rats.

   Within the propylthiouracil treated group, the decrease in thyroid radioactivity content in the rats treated with perchlorate (20 mg/kg) was also statistically significant ($p=0.035$) compared to the saline treated rats.

The ratio of radioactivity concentration in the thyroid to that in the blood was about 400, 160 and 100 for the saline, 10 mg/kg perchlorate and 20 mg/kg perchlorate sub-groups respectively. These ratios were all much lower than those for the corresponding sub-groups for both the control and ethiprole-treated animals. The ratio of the blood radioactivity concentration (dpm/g) to the thyroid weight (g) for each of the sub-groups was about 220,000. In each case, the ratio was about 50% or less than those for the corresponding sub-groups in the control and ethiprole-treated groups and was statistically different ($p<0.001$).

TABLE IIA 5.5.4.2-4. Whole-Blood and Thyroid Radioactivity Measurements [a]

Treatment	Radioactivity				Thyroid : blood radioactivity ratio [c, e]	Blood : thyroid weight ratio [d, e]
	Whole-blood (% dose/g)	Whole-blood[b] (total % dose)	Thyroid (% dose/g)	Thyroid (total % dose)		
Control: Saline treated sub-group	0.258	5.594	393.8	5.885	1531	428700
Perchlorate sub-group 1 (10 mg/kg)	0.339	7.191	375.2	4.544	1145	659700
Perchlorate sub-group 2 (20 mg/kg)	0.303	6.814	357.5	4.870	1196	532500
Ethiprole: Saline treated sub-group	0.282	6.251	494.2	7.352	1767	487000
Perchlorate sub-group 1 (10 mg/kg)	0.286	6.427	473.8	7.345	1688	433400
Perchlorate sub-group 2 (20 mg/kg)	0.270	6.100	428.9	6.094	1600	474800
Propylthiouracil Saline treated sub-group	0.287	5.057	112.8	3.394	395.4	225300
Perchlorate sub-group 1 (10 mg/kg)	0.342	5.861	54.05	1.948	162.5	215600
Perchlorate sub-group 2 (20 mg/kg)	0.362	6.264	34.92	1.323	97.09	228500

a Radioactivity concentrations in % dose/g and content in total % dose.
b Assumes that whole-blood accounts for 7% of bodyweight.
c Ratio of the radioactivity concentrations, thyroid : whole-blood.
d Ratio of the whole-blood radioactivity concentration : weight of the thyroid
e Calculated from the concentrations of radioactivity in dpm/g

TABLE IIA5.5.4.2-5. Statistical Comparisons for Overall Treatment Means and for Saline- and Perchlorate-Treated Sub-Group Means (Treatment 1 = Control; Treatment 2 = Ethiprole; Treatment 3 = Propylthiouracil) [a]

Parameter	Effect in analysis of variance							
	Treatment Group		Sub-group (10 mg/kg perchlorate *v* saline) within treatments			Sub-group (20 mg/kg perchlorate *v* saline) within treatments		
	2	3	1	2	3	1	2	3
Thyroid weight (g)	---	↑(p<0.001)	---	---	↑(p<0.005)	---	---	↑(p<0.004)
Thyroid radioactivity content (% dose)	↑(p<0.002)	↓(p<0.001)	---	---	---	---	---	↓(p=0.035)
Thyroid radioactivity concentration (% dose/g)	↑(p<0.001)	↓(p<0.001)	---	---	---	---	---	---
Blood radioactivity concentration (% dose/g)	---	---	↑(p<0.006)	---	---	---	---	↑(p<0.011)
Thyroid : blood radioactivity concentration ratio	↑(p<0.001)	↓(p<0.001)	↓(p=0.020)	---	---	↓(p=0.043)	---	---
Blood radioactivity concentration: thyroid weight ratio	---	↓(p<0.001)	↑(p=0.002)	---	---	---	---	---

a Data obtained from the study report, page 28. The table presents the direction of change, and p-values for statistical comparisons with, the means for animals in the control group, or in the saline sub-group: ↑ increase, ↓- decrease, - no (significant) change. Because there was little evidence of an interaction between treatment group and subgroup (F-test p≥0.039) and the differences from the control were generally similar for the subgroups, statistical comparisons were also made using the overall treatment means.

III. CONCLUSIONS:

Oral Dosing Phase: There were no unscheduled deaths during the course of the study. Group mean food intake and bodyweight gain for rats receiving ethiprole was unaffected by treatment. However, for rats receiving propylthiouracil, both food consumption and bodyweight gain were significantly reduced.

Animals receiving 200 mg/kg/day propylthiouracil showed clinical signs characteristic of this treatment after dosing throughout the treatment period. These included; salivation and abnormal gait on most occasions, with partially closed eyes and reduced body tone also noted on two occasions. These signs were evident on completion of dosing from Day 2. All animals appeared to have recovered fully before dosing the next day. No clinical signs or a reaction to treatment were noted for rats treated with ethiprole.

Terminal Studies:

Propylthiouracil treated rats (positive controls): Statistical analysis of the data from the Terminal Studies (perchlorate discharge test), indicated that pre-treatment of male rats for 14 days with propylthiouracil (PTU) resulted in a large increase in thyroid weight, consistent with hypertrophy. Significant decreases in thyroid radioactivity concentration and total

thyroid radioactivity were also observed. Specifically, comparison of the amounts of thyroid radioactivity in PTU/saline treated rats with control/saline treated animals shows a reduction (to about 30% of the concentration and about 60% of the total in the thyroid) in the ability of the thyroid to take up and retain ^{125}Iodide. In addition, a comparison of the thyroid radioactivity in PTU/saline treated rats with PTU/perchlorate treated animals shows that about 40 or 60% of thyroid radioactivity was displaced by perchlorate at the dose levels of 10 mg/kg or 20 mg/kg respectively and can, therefore, be considered to have been present as ^{125}Iodide. This is a consequence of the inhibition of the thyroid peroxidases that would normally convert the ^{125}Iodide to organic compounds and is in contrast to the control rats. Finally, a comparison of the whole-blood radioactivity concentrations in PTU treated rats with those measured in control rats shows slightly higher concentrations in the PTU treated groups. This may reflect the reduced ability of the thyroid to take up and metabolize ^{125}Iodide.

Ethiprole treated rats: In contrast, the only statistically significant change seen in male rats pre-treated with the test substance, ethiprole, was an increase in thyroid radioactivity concentration and total thyroid radioactivity content. An increase in the thyroid radioactivity in this test is often seen where the test compound is an inducer of microsomal drug metabolizing enzymes (for example, phenobarbital).

In conclusion, a comparison of the data obtained from a group of rats to which ethiprole was administered orally during 14 days with data from rats which had received 14 daily doses of propylthiouracil (a goitrogen acting directly on the thyroid), suggests that ethiprole at 20 mg/kg/day does not affect thyroid function by means of a direct toxic effect.

1.	Annex point(s)	KIIA 5.5.4; Long-term toxicity and carcinogenicity/Mechanism of action and supporting data: **MRID 47622826**
2.	Location in dossier	KIIA 5.5.4 /04
3.	Authors (year)	Webber, C. (2001)
	Title	The effect of repeated oral doses of ethiprole on the biliary excretion of intravenously administered ^{125}I-Thyroxine (T4) in male rats
	Company, report No.	Bayer CropScience AG, Report-No.: C017804 (M-204974-01-1)
	Date	12.11.2001
4.	Testing facility	Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole (radioactive), ethiprole Substance(s): ethiprole TC ethiprole (radioactive) TC
7.	Test method	
	Deviations	
8.	GLP	yes

Executive Summary:

The objectives of the study were to determine the effect of ethiprole on the biliary excretion of ^{125}I-thyroxine and, from these data, to obtain an insight into the effect of ethiprole on thyroid function in the rat.

Three groups of male Wistar strain rats (initially 7 animals/group) were treated in the following way: group 1 - 0.5% aqueous methylcellulose (5 ml/kg/day) as the control group (oral, by syringe and gavage), group 2 - ethiprole (20 mg/kg/day as a 4 mg/ml suspension in 0.5% aqueous methylcellulose, oral, by syringe and gavage), and group 3 - phenobarbital (80 mg/kg/day, intraperitoneal injection).

For each group, 14 consecutive daily doses were given at intervals of about 24 hours. Body weight and food consumption were recorded twice weekly and clinical signs monitored daily for each individual animal throughout the study. Immediately after administration of the final of the 14 doses, all animals were transferred to the Department of Drug Metabolism where 5 from each group were anaesthetised prior to cannulation of the common bile duct. After recovery from anaesthesia, a single intravenous dose of sodium ^{125}I-thyroxine was administered 4 hours after the final dose of test substance or control. Following administration, complete collections of bile were made at selected intervals during 24 hours after dosing. Upon completion of bile collection at 24 hours, rats were sacrificed and the livers removed. There were no unscheduled deaths during the course of the study. Group mean food intake and bodyweight gain for rats receiving phenobarbital or ethiprole were not considered to have been significantly affected by treatment.
Animals receiving 80 mg/kg/day phenobarbital showed clinical signs characteristic of phenobarbital treatment after dosing throughout the treatment period. No clinical signs of a reaction to treatment were noted for rats treated with ethiprole.

Pre-treatment of male rats for 14 days with either ethiprole or phenobarbital resulted in a significant increase in the rate and extent of biliary excretion of radioactivity during 24 hours after intravenous administration of ^{125}I-thyroxine. There were no statistically significant differences between these parameters for the groups pre-treated with ethiprole and phenobarbital.

The mean amount of radioactivity excreted in the bile during this time was 26% dose (control rats), 43% dose (ethiprole treated rats) and 47% dose (phenobarbital treated rats).
Metabolite profiling confirmed that, for each group of rats, about 60% of radioactivity in bile during the 24 hours after administration of ^{125}I-thyroxine was present as conjugated ^{125}I-thyroxine.

These data indicate an indirect effect, presumably via induction of hepatic drug metabolizing enzymes including β-D-glucuronyl transferases, of ethiprole on thyroid function.

This study is classified as **acceptable/non-guideline** and provides supporting data to the combined chronic toxicity/ carcinogenicity study in the rat.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	92.7% (927 g/kg)
Compound Stability:	The test substance was stored at room temperature in the dark
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** The vehicle used for the test substance was 0.5% aqueous (w/v) methylcellulose. Phenobarbital was used as a positive control. Phenobarbital is a known inducer of hepatic drug metabolizing enzymes including the β-D-glucuronyl transferases responsible for the metabolism of thyroxine (T4). The positive control was dissolved in sterile water prior to administration.

3. **Test Animals:**

Species:	Rat	
Strain:	Wistar (AF) rats RJ: WI (IOPS AF)	
Age/weight at study initiation:	6 weeks old/ 158-264 g (males only used in this study)	
Source:	Elevage Janvier, Le Genest St Isle, France	
Housing:	Housed 3 or 4 rats per cage in suspended cages with wire mesh floors.	
Diet:	Pelleted SDS Rat and Mouse No. 1 modified maintenance diet, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-22°C
	Humidity:	38-57%
	Air changes:	not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	13-17 days	

B. STUDY DESIGN:

1. **In-Life Dates:** November 16, 1999 to April 20, 2000

2. **Animal Assignment:** Animals were assigned randomly to the test groups noted in Table IIA5.5.4.4-1. Only male animals were used.

Dosing Phase: Male rats (7/group) were administered 14 consecutive daily doses of the vehicle, the test substance, or the positive control. The test substance group received ethiprole administered at 20 mg/kg/day as a 4 mg/mL suspension in 0.5% aqueous methylcellulose (5 mL/mg body weight dose volume) by oral gavage. The vehicle control group received 5 mL/kg body weight of the vehicle alone (0.5% aqueous methylcellulose) by oral gavage each day. The positive control group received phenobarbital at 80 mg/kg/day by intraperitoneal injection (2.5 mL/kg body weight dose volume).

Terminal Biliary Excretion Studies: Following the last administration of the test substance, vehicle, or positive control, all animals were transferred to the Department of Drug Metabolism for completion of the terminal biliary extraction studies as follows: Five rats from each group were selected for surgery where a flexible plastic cannula was inserted into the common bile duct to allow complete collection of bile. After recovery from anesthesia and the collection (during about 30 minutes) of control bile, sodium iodide solution (1 mL of an aqueous solution containing 4 mg/ml) was administered by intravenous injection into a caudal vein. Subsequently, a fixed volume of ^{125}I-thyroxine (T4) solution (0.4 mL, equivalent to *ca* 12-15 µCi) was administered intravenously by syringe into a lateral tail vein 4 hours after the final dose of the vehicle, ethiprole, or phenobarbital. Following administration of ^{125}I-thyroxine, complete collections of bile were made into pre-weighed tubes cooled by solid CO_2 and were stored frozen at *ca* -20°C until taken for measurement of radioactivity and profiling. Samples were collected during 0-1, 1-2, 2-3, 3-4, 4-5, 5-6 and 6-24 hours after dosing and weights of bile collected were recorded. Upon completion of bile collection at 24 hours, rats were sacrificed and the livers removed. The remaining carcasses were discarded without analysis.

TABLE IIA 5.5.4.4-1. Study Design [a]

Test Group / Treatment	Route of Administration	Dosage (mg/kg/day)	Dose volume (mL/kg)	No. of Male Animals [b]
Control / Vehicle [b]	Oral Gavage	0	5	7
Test Substance / Ethiprole	Oral Gavage	20	5	7
Positive Control / Phenobarbital	Intraperitoneal	80	2.5	7

a For all test groups, NaI (1 mg NaI in 0.5 ml 0.9% saline) followed by ^{125}I-thyroxine (T4) were administered intravenously within 4 hours after the last of 14 daily doses of the test substance, positive control, or vehicle alone (control).

b The control group was administered the vehicle alone (0.5% aqueous methylcellulose) at 5 mL/kg body weight.

3. **Dose Selection Rationale:** The dose level and route of administration of ethiprole was selected to approximate an intermediate dose level which had produced thyroid hormone changes in a 90-day toxicity rat study (KII A 5.3.2, Study No. M-204794-01-1). The dose level of the positive control is one which was reported to be routinely used in studies of this type.

4. **Dose Preparation and Analysis:** Formulations of ethiprole were prepared weekly and stored at 4°C. Ethiprole was suspended in the vehicle of 0.5% aqueous (w/v) methylcellulose at a concentration of 4 mg/mL. Phenobarbital was dissolved in sterile water at a concentration of 32 mg/mL, and was prepared up to 7 days in advance and stored at 4°C until used. The ^{125}I-thyroxine (T4) solution was prepared in 0.9% sterile saline on the day of use and stored in the dark at about 4°C when not in use. The ^{125}I-thyroxine (T4) solutions were prepared to provide a concentration of 38.1, 38.7, and 29.1 µCi/mL for the vehicle control, test substance, and positive control groups, respectively.

   The stability and homogeneity of the test substance (ethiprole) in 0.5% aqueous (w/v) methylcellulose has been previously established (Study N° M-201848-01-1 and M-202573-01-1). These data were not summarized in the current study.

During the study, samples of the ethiprole formulations, prepared for use in Week 1, were analyzed to check the accuracy of preparation.

Results: **Concentration Analysis:** The mean concentration (n=2) of ethiprole in the test formulation analyzed for Week 1 of the study was -2.0% of the nominal concentration (3.92 vs. 4.0 mg/mL actual and nominal, respectively), confirming the accuracy of formulation. (Data obtained from the study report, page 74).

5. **Statistics:** Analyses of variance were carried out on the biliary excretion data with treatment group (control, ethiprole and phenobarbital) as the factor of interest. Comparisons were made between the control and treated groups using Student's *t* test.

   Food consumption data were analyzed using cumulative cage totals. Bodyweight data were analyzed using weight gains. The following sequence of statistical tests was carried out: Levene's test was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was applied to see if a more stable variance structure could be obtained. If significant heterogeneity of variance could not be removed by transformation, an analysis of ranks was used. Analysis of variance was applied, followed by Student's *t* test for comparison of treated groups with the control group. Where analysis of ranks was indicated, comparisons were made by Wilcoxon rank sums test.

C. METHODS:

1. **Observations:** Individual animals were observed at least twice daily (except after transfer to Drug Metabolism) for any signs of behavioral changes, reaction to treatment or ill health. These checks were made early in each working day and again in the afternoon to look for dead or moribund animals. A detailed physical examination was performed weekly for the duration of the study.

   In addition, animals were observed in association with dosing on a daily basis during Week 1 and on two occasions during Week 2. These observations were made just prior to dosing, at the end of dosing for each group, between 1 and 2 hours after completion of dosing of all groups, and as late as possible in the working day.

2. **Body Weight:** The weight of each rat was recorded approximately one week before treatment commenced and then twice weekly during the dosing period.

3. **Food Consumption and Food Conversion Efficiency:** Animals were allowed access to food *ad libitum.* Food consumption was recorded twice weekly at intervals from one week prior to start of treatment.

4. **Terminal Biliary Excretion Studies:** On completion of 2 weeks of treatment, 5 rats/group were selected for the terminal biliary extraction studies as follows: Surgery was performed to insert a flexible plastic cannula into the common bile duct to allow complete collection of bile. After recovery from anesthesia and the collection (during about 30 minutes) of control bile, sodium iodide solution (1 mL of an aqueous solution containing 4 mg/ml) was administered by intravenous injection into a caudal vein. Subsequently, a fixed volume of ^{125}I-thyroxine (T4) solution (0.4 mL, equivalent to *ca* 12-15 µCi) was administered

intravenously by syringe into a lateral tail vein 4 hours after the final dose of the vehicle, ethiprole, or phenobarbital.

Following administration of ^{125}I-thyroxine, complete collections of bile were made into pre-weighed tubes cooled by solid CO_2 and were stored frozen at *ca* -20°C until taken for measurement of radioactivity and profiling. Samples were collected during 0-1, 1-2, 2-3, 3-4, 4-5, 5-6 and 6-24 hours after dosing and weights of bile collected were recorded. Upon completion of bile collection at 24 hours, rats were killed by cervical dislocation, the livers removed at post-mortem and stored at about -20°C pending analysis. The remaining carcasses were discarded without analysis.

Sample Preparation for Radioactivity Measurement: Aliquots of bile were taken directly for measurement of radioactivity concentration and chromatography by a high performance liquid method. Livers were weighed, and then homogenized in the minimum volume of distilled water. The homogenate weights were recorded and two sub-samples (known masses of about 0.5 g) taken for radioactivity measurement.

Measurement of Radioactivity: Radioactivity (^{125}I) was measured using an automatic gamma scintillation counter with automatic correction for counting efficiency and reference date (set to 15 November 1999, consistent with that given on the Certificate of Analysis for the ^{125}I-thyroxine).

Chromatography of Bile Samples: A total of 18 bile samples were prepared for high performance liquid chromatography by pooling equal proportions of individual samples to give one 0-1, 1-2, 2-3, 3-4, 4-6 and 6-24 hour sample for each experimental treatment.

Aliquots from each bile pool were diluted with an equal volume of 0.2M acetate buffer containing about 330000 Units/g of a mixed p-glucuronidase/arylsulphatase preparation (Type HI from *Helix Pomatia).* These mixtures were incubated in a water bath at about 37°C during 4 hours. HPLC analysis of bile samples was carried out in duplicate (prior to enzyme incubation) or singlicate (after enzyme incubation). The eluent was collected at 30 second intervals for the duration of the run, and analyzed by liquid scintillation spectroscopy.

Data Processing: Biliary excretion data in the form of percentage of the administered dose excreted during a collection interval up to 24 hours post-dose were analyzed to determine the rate of biliary excretion. The method used was the cumulative excretion or ARE (amount remaining to be excreted) method. The cumulative amount excreted at the end of each collection period was subtracted from the 24-hour total excretion and the resulting data analyzed using the software WinNonlin Pro version 3, to determine the rate constant λ. Log-linear regression analysis was performed on the amount remaining to be excreted versus time data and the slope of the regression line was taken as λ. The half-life of biliary excretion of radioactivity was calculated as $\ln 2/\lambda$.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** No clinical signs of a reaction to treatment or signs of ill health were observed among those animals receiving 20 mg ethiprole/kg/day.

Animals receiving 80 mg/kg/day phenobarbital showed clinical signs, characteristic of phenobarbital treatment. These signs included underactive behavior, abnormal gait and prostrate in cage from approximately 1 hour after dosing. These animals remained underactive and displayed abnormal gait for the rest of the working day. All animals appeared to have recovered fully before dosing the next day.

2. **Mortality:** There were no unscheduled deaths.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** The group mean bodyweights for animals receiving ethiprole or phenobarbital were marginally higher than controls at the start of treatment. This difference from controls was largely on account of these groups of animals being older (2 days and 4 days respectively) due to the staggered start between treatment groups which was incorporated into the study design to allow for the terminal metabolic procedures.

Therefore, taking into account these initial intergroup differences in age and weight, no importance is attached to the apparent reduction in overall bodyweight gain, which attained statistical significance, for those animals treated with phenobarbital.

Bodyweight gain for those animals receiving ethiprole was considered to be essentially similar to controls.

C. **FOOD CONSUMPTION:** Food consumption was unaffected by treatment with either ethiprole or phenobarbital.

D. **TERMINAL BILIARY EXCRETION STUDIES:**

Biliary Radioactivity: Pre-treatment with either ethiprole or phenobarbital resulted in significantly increased biliary excretion of radioactivity compared to the control group (Table IIA5.5.4.4-2). The cumulative excretion over 24 hours was 26.1, 42.9 and 47.4% total dose in the control, ethiprole and phenobarbital groups, respectively.

Rate constants and half-lives for the biliary excretion of radioactivity in each group of rats are presented in Table 5.5.4.4-3. Both ethiprole and phenobarbital increased the rate of biliary excretion of radioactivity.

TABLE IIA 5.5.4.4-2. Mean ± SD Cumulative Biliary Excretion of Radioactivity (total % dose) [a]

Time (hours)	Test Group		
	Control	Ethiprole	Phenobarbital
1	2.23	4.05	3.05
2	4.26	7.92	7.05
3	5.97	10.88	10.75
4	7.25	13.33	13.06
5	8.32	15.45	15.91
6	9.33	17.42	18.54
24	26.05	42.94 ($p<0.001$)	47.38 ($p<0.001$)

a n = 5 rats/group

TABLE IIA5.5.4.4-3. Rate Constants and Half-Lives of Biliary Excretion of Radioactivity [a]

Parameter	Test Group		
	Control	Ethiprole	Phenobarbital
Rate Constant (λ, hours^{-1})	0.0703	0.0832 (p=0.014)	0.0841 (p=0.010)
$t_{1/2}$ (hours)	9.9	8.3	8.2

a n= 5 rats/group

Subsequent analysis of pooled bile samples by HPLC showed that there were no significant differences in metabolite profiles between each of three treatment groups or between sample collection times within each group. Prior to enzyme incubation, three major regions of radioactivity were observed in profiles of bile samples. One of these eluted about 4 minutes after sample injection, close to the solvent front, and represented about 20% of sample radioactivity. This was considered likely to be free ^{125}I. The major region, representing about 60% of sample radioactivity, eluted after about 8 minutes and it appeared from some of the profiles that this may comprise more than one component. The other region, typically representing about 5% sample radioactivity, eluted at about 16 minutes after sample injection. Remaining radioactivity was associated with minor peaks, including one eluting after about 11 minutes that contained up to 3% sample radioactivity.

Following enzyme incubation, a significant change was seen in the profile for each of the bile samples analyzed. The proportion of radioactivity eluting at about 8 minutes was significantly reduced (from about 60% to about 5% total) and in some cases was absent altogether. There was a corresponding increase in the proportion of radioactivity eluting at about 11 minutes (to about 10% total) and 16 minutes (to about 50% total). The proportion of sample radioactivity eluting at about 4 minutes was generally similar to the pre-incubated samples.

The major radioactive peak observed after enzyme incubation eluted about 3 minutes later than the reference solution of non-radiolabelled thyroxine. However, upon isolation and re-injection of this peak, it was seen to co-chromatograph with the reference. This indicates that the major radioactive peak present in the bile samples represents conjugated ^{125}I-thyroxine, either as the glucuronide or sulphate conjugate. Upon deconjugation, the elution time of the ^{125}I-thyroxine was probably affected by the presence of bile salts and consequently differed from that of the thyroxine reference.

Radioactivity in the Liver: Data for liver weights and radioactivity in the liver are presented in Table IIA5.5.4.4-5. Liver weights of both ethiprole and phenobarbital treated rats were higher than control. However, only the phenobarbital-treated rats showed a significant increase in the concentration and total amount of radioactivity in the liver (about 50% in each case) compared to the control rats.

TABLE IIA 5.5.4.4-4. Radiolabelled Metabolites in Bile (Results are Expressed as % Sample Radioactivity) [a]

Test Group	Enzyme Treatment	Time (hours)	Typical retention time(minutes)			
			4	8	11	16
Control	Prior to enzyme incubation	0-1	22	50	1	11
		1-2	20	59	3	7
		2-3	21	60	0	6
		3-4	20	62	0	4
		4-6	22	59	2	3
		6- 24	23	58	3	4
	After enzyme incubation	0-1	25	2	9	50
		1-2	23	0	9	55
		2-3	22	2	8	52
		3-4	20	3	10	50
		4-6	22	3	11	49
		6- 24	24	2	15	47
Ethiprole	Prior to enzyme incubation	0-1	21	62	0	5
		1-2	18	66	0	4
		2 -3	19	65	0	3
		3 -4	20	63	0	3
		4 -6	21	63	0	2
		6 - 24	24	63	0	1
	After enzyme incubation	0-1	25	0	4	55
		1-2	22	4	6	58
		2-3	22	4	6	55
		3-4	27	2	9	54
		4-6	27	2	8	49
		6- 24	30	2	8	46
Phenobarbital	Prior to enzyme incubation	0-1	23	55	0	7
		1-2	18	62	0	5
		2-3	18	67	0	3
		3-4	19	59	0	2
		4-6	19	60	2	3
		6- 24	23	59	3	2
	After enzyme incubation	0-1	26	2	9	49
		1-2	23	2	9	54
		2-3	21	5	8	55
		3-4	28	3	7	49
		4-6	25	3	10	50
		6- 24	30	3	11	46

a For samples collected prior to enzyme incubation, results are the mean of two analyses. For samples collected after enzyme incubation, results are for a sample analysis.

TABLE IIA 5.5.4.4-5. Mean Liver Weights (g), Radioactivity Concentrations (% dose/g) and Total Amounts of Radioactivity (total %dose) [a]

Parameter	Control	Ethiprole	Phenobarbital
Liver Weight (g)	10.6	12.1	12.0
Radioactivity Concentration (% dose/g)	0.72	0.59	0.93
Total Radioactivity (total % dose)	7.6	7.2	11.1

a n= 5 rats/group

III. CONCLUSIONS:

Dosing Phase: No unscheduled deaths occurred during the study. Group mean food intake and bodyweight gain for rats receiving phenobarbital or ethiprole were not considered to have been significantly affected by treatment. Animals receiving phenobarbital showed clinical signs characteristic of phenobarbital treatment after dosing throughout the treatment period. No clinical signs of a reaction to treatment were noted for rats treated with ethiprole.

Terminal Biliary Excretion Studies: Both ethiprole and phenobarbital resulted in a significant increase in the rate and extent of biliary excretion of radioactivity during 24 hours after intravenous administration of ^{125}I-thyroxine. There were no statistically significant differences between these parameters for the groups pre-treated with ethiprole and phenobarbital. The mean amount of radioactivity excreted in the bile during this time was 26% dose (control rats), 43% dose (ethiprole-treated rats) and 47% dose (phenobarbital-treated rats).

Metabolite profiling confirmed that, for each group of rats, about 60% of radioactivity in bile during the 24 hours after administration of ^{125}I-thyroxine was present as conjugated ^{125}I-thyroxine.

These data indicate an indirect effect, presumably via induction of hepatic drug metabolizing enzymes including (3-D-glucuronyl transferases, of ethiprole on thyroid function.

1.	Annex point(s)	KIIA 5.5.4; Long-term toxicity and carcinogenicity/Mechanism of action and supporting data: **MRID 47622825**
2.	Location in dossier	KIIA 5.5.4 /03
3.	Authors (year)	Webber, C. (2001)
	Title	The effect of repeated oral doses of Ethiprole on the pharmacokinetics of intravenously administered ^{125}I-thyroxine (T4) in male rats
	Company, report No.	Bayer CropScience AG, Report-No.: C016605 (M-202728-01-1)
	Date	22.02.2001
4.	Testing facility	Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole (radioactive), ethiprole Substance(s): ethiprole TC ethiprole (radioactive) TC
7.	Test method	
	Deviations	
8.	GLP	yes

Executive Summary:

The objective of this study was to assess the effect of ethiprole on the pharmacokinetics of intravenously administered ^{125}I-thyroxine (^{125}I-T4) in male rats treated for 14 days with separate daily oral doses of ethiprole (20 mg/kg/day) prior to the administration of ^{125}I-thyroxine. Comparison was made with untreated rats (controls administered dose vehicle alone) and with phenobarbital treated rats (80 mg/kg/day administered by intraperitoneal injection) which was included as a "positive control" since phenobarbital is a known inducer of hepatic drug metabolising enzymes including the β-D-glucuronyl transferases responsible for the metabolism of thyroxine (T4). This comparison provides an insight into whether the effect on thyroid function of ethiprole was an indirect effect, following from the induction of hepatic drug metabolizing enzymes.

The dose level of ethiprole (20 mg/kg/day) was selected to approximate an intermediate dose level which had produced thyroid hormone changes in a Regulatory 90-day toxicology study (see KII A 5.3) conducted in the same strain of rat. Administration of the test substance was by the oral route since this is a likely route of human exposure.

Bodyweight and food consumption were recorded twice weekly and weekly respectively, and clinical signs monitored daily for each animal throughout the study. At 24 hours after the completion of the 14-day dose administration period *(ie* Day 15), all animals were transferred to the Department of Drug Metabolism, where 6 from each Group were administered a single intravenous dose of sodium ^{125}I-thyroxine. Following administration, blood samples were taken at selected times during 48 hours after dosing.

There were no unscheduled deaths during the course of the study. Group mean food intake and bodyweight gain for rats receiving phenobarbital or ethiprole were unaffected by treatment.
Animals receiving 80 mg/kg/day phenobarbital showed clinical signs characteristic of phenobarbital treatment after dosing throughout the treatment period. No clinical signs of a reaction to treatment were noted for rats treated with ethiprole.

Statistical analysis of the data indicated that pre-treatment of male rats for 14 days with either ethiprole or phenobarbital resulted in a statistically significant decrease in AUC and C0.
In addition, λ_z, CL and V_{ss} were significantly higher in ethiprole and phenobarbital pre-treated animals. The data therefore indicated that ethiprole and phenobarbital pre-treatment resulted in increased whole body clearance and steady-state volume of distribution of radioactivity.

These data would indicate an indirect effect, presumably via induction of hepatic drug metabolizing enzymes, of ethiprole on thyroid function.

This study is classified as **acceptable/non-guideline** and provides supporting data to the combined chronic toxicity/ carcinogenicity study in the rat.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	92.7% (927 g/kg)
Compound Stability:	The test substance was stored at room temperature in the dark
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** The vehicle used for the test substance was 0.5% aqueous (w/v) methylcellulose. Phenobarbital was used as a positive control. Phenobarbital is a known inducer of hepatic drug metabolizing enzymes including the β-D-glucuronyl transferases responsible for the metabolism of thyroxine (T4). The positive control was dissolved in sterile water prior to administration.

3. **Test Animals:**

Species:	Rat	
Strain:	Wistar (AF) rats RJ: WI (IOPS AF)	
Age/weight at study initiation:	6 weeks old/ 177- 221 g (males only used in this study)	
Source:	Elevage Janvier, Le Genest St Isle, France	
Housing:	Housed 4 rats per cage in suspended cages with wire mesh floors.	
Diet:	Pelleted SDS Rat and Mouse No. 1 modified maintenance diet, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	21 ± 2°C
	Humidity:	55 ± 15%
	Air changes:	not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	15 days	

B. STUDY DESIGN:

1. **In-Life Dates:** October 28, 1999 to December 15, 1999

2. **Animal Assignment:** Animals were assigned randomly to the test groups noted in Table IIA5.5.1.3-1. Only male animals were used.

Dosing Phase: Male rats (8/group) were administered 14 consecutive daily doses of the vehicle, the test substance, or the positive control. The test substance group received ethiprole administered at 20 mg/kg/day as a 4 mg/mL suspension in 0.5% aqueous methylcellulose (5 mL/mg body weight dose volume) by oral gavage. The vehicle control group received 5 mL/kg body weight of the vehicle alone (0.5% aqueous methylcellulose) by oral gavage each day. The positive control group received phenobarbital at 80 mg/kg/day by intraperitoneal injection (2.5 mL/kg body weight dose volume).

Terminal Metabolic Studies: Six rats from each of the treatment groups were subjected to the following metabolic studies (the remaining 2 rats/group were sacrificed and discarded). A fixed volume of ^{125}I-thyroxine (T4) solution (0.4 ml, equivalent to *ca* 17 µCi) was administered intravenously by syringe into a lateral tail vein 4 hours after the final dose of the vehicle, ethiprole, or phenobarbital. In addition, each of these rats received a further intraperitoneal injection of 1 mg NaI in 0.5 ml 0.9% saline at about 10 hours after the final dose of the vehicle, ethiprole, or phenobarbital. Whole-blood samples were taken from each rat at 1, 2, 4, 6, 8, 12, 24, 36 and 48 hours after the NaI dosing for radioactivity measurements. After the final blood sample was collected, animals were sacrificed and the carcasses discarded.

TABLE IIA 5.5.4.3-1. Study Design [a]

Test Group / Treatment	Route of Administration	Dosage (mg/kg/day)	Dose volume (mL/kg)	No. of Male Animals [b]
Control / Vehicle [b]	Oral Gavage	0	5	8
Test Substance / Ethiprole	Oral Gavage	20	5	8
Positive Control / Phenobarbital	Intraperitoneal	80	2.5	8

a For all test groups, ^{125}I-thyroxine (T4) was administered intravenously (0.4 ml, equivalent to *ca* 17 µCi) followed by intraperitoneal injection of NaI (1 mg NaI in 0.5 ml 0.9% saline) at 4 and 10 hours, respectively, after the last of 14 daily doses of the test substance, positive control, or vehicle alone (control).

b The control group was administered the vehicle alone (0.5% aqueous methylcellulose) at 5 mL/kg body weight.

3. **Dose Selection Rationale:** The dose level and route of administration of ethiprole were selected to approximate an intermediate dose level which had produced thyroid hormone changes in a 90-day toxicity rat study (KII A 5.3.2 study report N° M-202728-01-1; see study profile). The dose level of the positive control is one which was reported to be routinely used in studies of this type.

4. **Dose Preparation and Analysis:** Formulations of ethiprole were prepared weekly and stored at 4°C. Ethiprole was suspended in the vehicle of 0.5% aqueous (w/v) methylcellulose at a concentration of 4 mg/mL. Phenobarbital was dissolved in sterile water at a concentration of 32 mg/mL, and was prepared up to 7 days in advance and stored at 4°C until used. The NaI and ^{125}I-thyroxine (T4) solutions were prepared in 0.9% sterile saline on the day of use and stored in the dark at about 4°C when not in use. The ^{125}I-thyroxine (T4) solutions were prepared to provide a nominal concentration of 50 µCi/mL, (measured at 43.2 µCi/mL), and the NaI solutions were prepared at a concentration of 1 mg NaI in 0.5 ml 0.9% saline.

The stability and homogeneity of the test substance (ethiprole) in 0.5% aqueous (w/v) methylcellulose were established previously (Study N° M-201848-01-1 and M-202573-01-1). These data were not summarized in the current study.

During the study, samples of the ethiprole formulations, prepared for use in Week 1, were analyzed to check the accuracy of preparation.

Results: **Concentration Analysis:** The mean concentration (n=2) of ethiprole in the test formulation analyzed for Week 1 of the study was -1.0% of the nominal concentration (3.96 vs. 4.0 mg/mL actual and nominal, respectively), confirming the accuracy of formulation.

5. **Statistics:** Statistical analysis of the pharmacokinetic parameters was performed using analysis of variance with treatment group (control, ethiprole, phenobarbital) as the factor of interest. The data were logarithmically transformed prior to analysis in order to stabilize the variances. Comparisons were made between the control and treated groups using Student's *t* test. Further comparisons were made between the ethiprole and phenobarbital treated groups.

   Food consumption data were analyzed using cumulative cage totals. Bodyweight data were analyzed using weight gains. The following sequence of statistical tests was carried out: Levene's test was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was applied to see if a more stable variance structure could be obtained. If significant heterogeneity of variance could not be removed by transformation, an analysis of ranks was used. Analysis of variance was applied, followed by Student's *t* test for comparison of treated groups with the control group. Where analysis of ranks was indicated, comparisons were made by Wilcoxon rank sums test.

C. METHODS:

1. **Observations:** Individual animals were observed at least twice daily (except after transfer to Drug Metabolism) for any signs of behavioral changes, reaction to treatment or ill health. These checks were made early in each working day and again in the afternoon to look for dead or moribund animals. A detailed physical examination was performed weekly for the duration of the study.

   In addition, animals were observed in association with dosing on a daily basis during Week 1 and on two occasions during Week 2. These observations were made just prior to dosing, at the end of dosing for each group, between 1 and 2 hours after completion of dosing of all groups, and as late as possible in the working day.

2. **Body Weight:** The weight of each rat was recorded the week before treatment commenced and then on Day 1, 5, 8, 12 and 14 of treatment.

3. **Food Consumption and Food Conversion Efficiency:** Animals were allowed access to food *ad libitum.* Food consumption was recorded at weekly intervals from one week prior to start of treatment.

4. **Terminal Studies:** On completion of 2 weeks of treatment, 6 rats/group were selected for administration of ^{125}I-thyroxine. Rats not receiving the radiolabelled dose were sacrificed and discarded. Remaining rats were housed 3 per cage according to treatment group and were subjected to daily observation for clinical signs prior to sacrifice.

   A fixed volume of ^{125}I-thyroxine solution (0.4 ml, equivalent to *ca* 17 µCi) was administered intravenously by syringe into a lateral tail vein 4 hours after the final dose of vehicle/ethiprole/phenobarbital. In addition, each of these rats received a further intraperitoneal injection of 1 mg NaI in 0.5 ml 0.9% saline at about 10 hours after the final dose of vehicle/ethiprole/phenobarbital. Aliquots of ^{125}I-thyroxine solution were taken for

measurement of radioactivity concentration (dpm/mL of dose solution). Doses administered to each animal (38343000 dpm, equivalent to *ca* 115 ng thyroxine) were calculated by multiplying the volume of dose administered (0.4 mL) by this measured concentration.

Samples of whole-blood *(ca* 0.2 mL) were taken from a tail vein (not that used for administration) into heparinised tubes and stored frozen at *ca* -20°C until taken for measurement of radioactivity. Samples were taken from each rat at 1, 2, 4, 6, 8, 12, 24, 36, and 48 hours after dosing, after which these animals were sacrificed and the carcasses disposed of.

Measurements of radioactivity: Two aliquots of 50 µL of each whole-blood sample were dispensed directly into tubes for radioactivity measurement. A further 50 µL aliquot of each sample was dispensed into a tube containing 1 mL of 0.9% saline. To these samples, 10% w/v aqueous trichloroacetic acid (1 mL) was added and, after standing on ice for at least 15 minutes, the samples were centrifuged at about 3000 'g' during 15 minutes and the supernatant and pellet taken separately for radioactivity measurement.

Radioactivity (^{125}I) was measured using an automatic gamma scintillation counter with automatic correction for counting efficiency and reference date (set to 18 October 1999, consistent with that given on the Certificate of Analysis for the ^{125}I-thyroxine).

Data processing: Whole-blood radioactivity concentration-time data were analyzed using the software WinNonlin Pro version 3 (Pharsight Corp., USA). Concentrations at time zero (C_0) were calculated by back extrapolation following log-linear regression analysis of the first two data points. Areas under concentration-time curves to the last measurable sample time (AUC_t) were calculated using the linear trapezoidal rule, and areas under the concentration-time curves to infinite time (AUC) were calculated by means of the expression;

$$AUC = AUC_t + C_{last} / \lambda_z$$

where, C_{last} is the predicted concentration at the last measurable sample point and λ_z is the terminal rate constant determined by log-linear regression analysis of those points which constitute the terminal phase of the concentration-time curve. The following criteria were applied to the regression analysis for it to be acceptable: (i) the points used were randomly distributed about a single straight line on visual inspection; (ii) the regression coefficient was ≥0.95 and the variance accounted for by the regression was ≥0.90; (iii) the number of data points used in the regression was ≥3; and (iv) the ratio of the measurement period of the regression to the estimated half-life was ≥2.

Terminal half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda_z$. Total body clearance (CL) was calculated as Dose/AUC and volume of distribution at steady-state (V_{ss}) was calculated as CL.MRT where MRT is the mean residence time.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity**: No clinical signs of a reaction to treatment or signs of ill health were observed among those animals receiving 20 mg ethiprole/kg/day.

Animals receiving 80 mg phenobarbital/kg/day showed clinical signs, characteristic of phenobarbital treatment. These signs included under active behavior, partially closed eyes and prostrate in cage for approximately 1 hour after dosing. These animals remained under active and displayed abnormal gait for the rest of the working day. All animals appeared to have recovered fully before dosing the next day.

2. **Mortality:** There were no unscheduled deaths.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** Body weight and body weight gains were unaffected by treatment with either ethiprole or phenobarbital.

C. **FOOD CONSUMPTION:** Food consumption was unaffected by treatment with either ethiprole or phenobarbital.

D. **TERMINAL METABOLIC STUDIES:**
Whole-blood radioactivity concentrations are presented in Table IIA5.5.4.3-2. Examination of the mean data indicated that whole-blood radioactivity concentrations were ranked in the order control > ethiprole > phenobarbital with maximum mean measured radioactivity concentrations of 1.822, 1.478 and 1.181 %dose/mL, respectively at 1 hour after dosing (the first sampling time). The decline in whole-blood radioactivity concentrations appeared to be parallel in all three treatment groups.

TABLE IIA 5.5.4.3-2. Mean Whole-Blood Radioactivity Concentrations (%dose/mL)

Time(hours)	Test Group		
	Control	Ethiprole	Phenobarbital
1	1.822	1.478	1.181
2	1.397	1.185	0.997
4	1.173	0.983	0.815
6	1.051	0.907	0.660
8	0.971	0.823	0.695
12	0.809	0.666	0.530
24	0.524	0.370	0.273
36	0.304	0.222	0.173
48	0.182	0.127	0.105

Pharmacokinetic analysis of the whole-blood radioactivity concentration-time data resulted in the pharmacokinetic parameters described in Table IIA5.5.4.3-3. Analysis of the mean pharmacokinetic parameters indicated that pre-treatment of male rats with either ethiprole or phenobarbital resulted in a decrease in the area under the whole-blood radioactivity concentration-time curve to infinite time, an increase in total body clearance of radioactivity, and an increase in the steady-state volume of distribution of radioactivity. The terminal half-life of whole-blood radioactivity was slightly shorter in rats pre-treated with either ethiprole or phenobarbital when compared to the vehicle control group. Therefore, both ethiprole and phenobarbital pre-treatment resulted in increased whole body clearance and steady-state volume of distribution of radioactivity.

TABLE IIA 5.5.4.3-3. Mean Pharmacokinetic Parameters Derived from Whole-Blood Radioactivity Concentrations[a]

Test Group	C_0 (%dose/mL)	AUC_t (%dose.h/mL)	λ_z (hours^{-1})	AUC (%dose.h/mL)	$t_{1/2}$ (hours)	CL (mL/h)	V_{ss} (mL)
Control	2.379	30.0	0.0412	34.5	16.8[b]	2.916	67.2
Ethiprole	1.862**†	23.6**†	0.0467*	26.4**†	14.9[b]	3.798**†	77.6*†
Phenobarbital	1.414**	18.7**	0.0465*	20.8**	14.9[b]	4.835**	97.4**

a (n=6/test group).
b Calculated as ln2/mean λ_z
* p=<0.05 compared to the control group
** p=<0.01 compared to the control group
† p=<0.01 in comparison of the ethiprole and phenobarbital groups

The mean proportions of radioactivity precipitable by TCA in whole-blood samples from the control rats declined from 94% at 1 hour after administration of ^{125}I-thyroxine to 86% at 48 hours after administration (Tables IIA5.5.4.3-4). Similar declines were also observed over the same period in rats pretreated with ethiprole or phenobarbital (from 87% to 80% in each case).

TABLE IIA 5.5.4.3-4. Proportion (%) of Whole-Blood Radioactivity Precipitable by TCA [a]

Time (hours)	Test Group		
	Control	Ethiprole	Phenobarbital
1	93.6	87.4	86.9
2	92.3	86.2	85.5
4	90.6	85.0	85.3
6	89.1	83.0	83.5
8	89.1	82.7	81.3
12	90.3	83.9	85.1
24	87.9	81.7	81.3
36	86.6	81.4	81.0
48	85.5	79.6	79.8

a (n=6/test group).

III. CONCLUSIONS:

Positive control: In the phenobarbital group, statistically significant decreases were noted in C_0 and AUC. There were also statistically significant increases in clearance and the volume of distribution. These changes are consistent with the known effect of phenobarbital as an inducer of the microsomal β-D-glucuronyl transferases which catalyze the metabolism of thyroxine (T4) to its glucuronide conjugate.

Ethiprole: In the ethiprole group, statistically significant decreases in C_0 and AUC were observed. There was also a statistically significant increase in clearance.

The results of this study support that ethiprole is an inducer of β-glucuronyl transferase enzymes.

KIIA 5.5.3 - Carcinogenicity study in the mouse

1.	Annex point(s)	KIIA 5.5.3; Long-term toxicity and carcinogenicity/Carcinogenicity study in the mouse: **MRID 47622812**
2.	Location in dossier	KIIA 5.5.3 /01
3.	Authors (year)	Richard, J. (2001)
	Title	Ethiprole (RPA 107382) Carcinogenicity study by oral route (dietary admixture) in C57BL/6 mice
	Company, report No.	Bayer CropScience AG, Report-No.: C014046 (M-205769-02-1)
	Date	30.05.2001, Amended: 21.05.2002
4.	Testing facility	CIT Centre Internationale de Toxicologie, Evreux, France
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole (radioactive), ethiprole Substance(s):ethiprole TC ethiprole (radioactive) TC
7.	Test method	EU (=EEC): 96/54; JMAF: 59 NohSan 4200; OECD: 451; USEPA (=EPA): 870.4200
	Deviations	
8.	GLP	yes

Executive summary:

Groups of 50 male and 50 female C57BL/6J mice were fed diet containing 0, 10, 50, 150 or 300 ppm of ethiprole (batch number CDR 9706, purity 93%) for at least 78 weeks. The mean intake of ethiprole over the 18 months was calculated to be 0, 1.7, 8.6, 25.6 or 50.8 mg/kg/day in males and 0, 1.7, 12.5, 36.3 or 73.5 mg/kg/day in females, at 0, 10, 50, 150 and 300 ppm, respectively. For evaluation of toxic effects, 10 satellite males and 10 satellite females per group were sacrificed after a one-year treatment. Throughout the study, clinical signs and mortality were checked daily, and careful examination was carried out weekly to assess possible neurotoxic effects. Palpation of possible masses was carried out every 4 weeks from weeks 4 to 52 and every 2 weeks thereafter. Body weight and food consumption were measured at weekly intervals during the first 13 weeks of the study and every 4 weeks thereafter. Hematological investigations (Differential White Cell count) were carried out in week 52 and at the end of the study (principal animals). Before the sacrifice of satellite animals in week 52, blood was taken for the determination of liver enzyme activities (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase). At the end of the appropriate scheduled treatment period (weeks 52 or 78), all animals were sacrificed and submitted to a macroscopic *post-mortem* examination. Designated organs were weighed. A microscopic examination was performed on all samples of tissues from satellite animals, from principal animals of the control and high-dose groups, and from principal animals which died or were killed prematurely during the study.

A reduced survival rate was observed in females at 300 ppm. The incidence, nature and onset of the clinical signs were similar in controls and treated animals. No treatment-related changes were observed on body weights and food consumption. There were no treatment-related changes in hematological parameters. In week 52, an increase in alanine amino transferase activity was observed in males at 300 ppm. However, in the absence of macroscopic or microscopic changes in the liver, the relationship to treatment with the test substance was considered to be doubtful.

No treatment-related necropsy findings were noted in any group. Increases in absolute and relative liver weight were observed in males and females at the highest dose (300 ppm), however they were not correlated with any histopathological changes.

After 52 weeks of treatment, no non-neoplastic or neoplastic findings were observed that could be related to treatment with the test substance. After 78 weeks of treatment, there were no non-neoplastic findings which were related to treatment with the test substance. After 78 weeks of treatment, the only neoplastic change was a slight increase in the incidence of hepatocellular adenomas in females at 300 ppm.

In conclusion, a dietary administration of ethiprole over a chronic, 18-month period to C57bl/6J mice resulted in a reduced survival rate and a slight increase in the incidence of hepatocellular adenomas in females at 300 ppm.

The NOAEL is 50.8 mg/kg/day (300 ppm) in males and 36.3 mg/kg/day in females (150 ppm).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD 9706
Purity:	92-93%
Compound Stability:	The test substance was stored at +4°C at receipt, and after at room temperature from 21 July 1999, protected from light.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** The vehicle was "Pietrement M20" irradiated, controlled diet, batch Nos. 980622, 981103, 990310, 990630,991103 and 000309, supplied by Extralabo Pietrement (Provins, France). Control animals received untreated diet.

3 **Test Animals:**

Species:	Mouse	
Strain:	C57BL/6 N Crl: BR mice	
Age/weight at study initiation:	~ 7 - 8 weeks / 18.2 - 24.9 g males, 13.3 - 20.2 g females	
Source:	Charles River, Saint-Aubin-les-Elbeuf, France.	
Housing:	The mice were housed individually in polycarbonate cages (24.0 x 13.5 x 13.0 cm) and the floor of each cage was covered with autoclaved sawdust (SICSA, Alfortville, France). Cages and sawdust were changed once a week.	
Diet:	"Pietrement M20" irradiated, controlled diet (Extralabo Pietrement, Provins, France), *ad libitum*	
Water:	Filtered tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	21 ± 2°C (target)
	Humidity:	50 ± 20% (target)
	Air changes:	approximately 12 cycles/hour
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	16 days	

B. STUDY DESIGN:

1. **In-Life Dates:** November 5, 1998 to May 19, 2000

2. **Animal Assignment/Dose Levels:** Animals were assigned to the test groups noted in Table IIA 5.5.3-1 according to body weight and clinical condition and allocated to groups (by sex), using a computerized stratification procedure, so that the average body weight of each group was similar.

TABLE IIA5.5.3-1. Study Design

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg/day) [a]		Main Study (78-80 weeks)		Interim Sac. (53 weeks)	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	50	50	10	10
Low (LDT)	10	1.7	1.7	50	50	10	10
Mid-1 (MDT-1)	50	8.6	12.5	50	50	10	10
Mid-2 (MDT-2)	150	25.6	36.3	50	50	10	10
High (HDT)	300	50.8	73.5	50	50	10	10

a Values are the mean achieved dose levels over the course of the entire study (Weeks 1-78). The study duration was 78 to 80 complete weeks, depending on necropsy dates.

The dose-levels were chosen by the Sponsor according to a preliminary 4-week toxicity study carried out by the Sponsor in C57BL/6 mice. Results from this preliminary study were not included in the study report.

3. **Diet Preparation and Analysis:** The test substance was given by dietary admixture in Pietrement M20 diet. The test substance was blended with a small quantity of diet using a mortar and pestle; this premix was then transferred into a mixer with the required total quantity of diet in order to achieve the concentrations of 10, 50, 150 and 300 ppm. The diet formulations were prepared on a weekly basis from day 1 to week 38, and on a 4-weekly basis from week 39 to the end of the study (except in week 63: prepared for one week), and were stored at room temperature, protected from light, in closed bags, prior to use.

The homogeneity of the mixing procedures used was verified prior to the start of the study using 5 and 500 ppm dietary preparations. During the treatment period, homogeneity of the 10 and 300 ppm diets were checked in weeks 1, 13, 39 and 65. From each dietary mixture, two samples were taken at three different levels (top, middle, bottom) of the mixer and analyzed for homogeneity.

The stability of the test substance was also verified prior to the start of the study. Samples of the 5 and 500 ppm diets (also used for the homogeneity analysis) were stored either in closed bags or in open feeders at room temperature. Samples were collected in duplicate from each the closed bags (after 0, 5, 10, 16, 23 and 35 days of storage) and open feeders (after 0, 5, 10 and 15 days of storage). Samples taken on days 10 and 35 (closed bags) or on days 10 and 15 (open feeders) were analyzed as soon as possible after sampling. All other samples were placed in a freezer (-20°C) and not analyzed, with the exception of selected samples from the 5 ppm closed bag. These samples (5 ppm closed bag, collected at days 0, 16, 23 and 35 and stored in the freezer) were re-analyzed to verify the initial results. The mean result of the homogeneity test was taken as the initial value for the stability test (except for re-analyses of the 5 ppm concentration).

During the study, each dietary mixture (including the control) prepared for use in weeks 1, 2, 3, 4,13, 26, 39, 52, 65 and 78 was sampled in duplicate (2 x 50 g) and one sample was analyzed to determine the concentration of the test material.

Results: **Homogeneity Analysis:** Results for homogeneity of the 5 and 500 ppm diets were within 97 and 102% of the nominal concentrations. Results for the homogeneity of the 10 and 300 ppm diets used during the study were within 96-

108% of nominal. Values are for the means of top, middle, and bottom analyses. For samples analyzed during the study, coefficients of variation ranged between 1.5 to 8.1%. Data obtained from the study report, pages 195 and 199-202 original pagination).

Stability Analysis: After 35 days of storage in closed bags at room temperatures, the 500 ppm diet was 94% of nominal and the 5 ppm diet was 86% (initial analysis) or 88% (confirmation analysis) of nominal. After 15 days of storage at room temperatures in open feeders, the 5 and 500 ppm diets were 99% and 98% of nominal, respectively. (Data obtained from the study report, pages 197 and 198, original pagination).

Concentration Analysis: Results for the concentration analyses were within 91-110% of the nominal concentrations. (Data obtained from the study report, page 203, original pagination).

4. **Statistics:** The main study group and interim sacrifice group animals were evaluated separately. Body weight, food consumption, blood biochemistry, hematology and organ weight data were subjected to the following statistical analyses. If the number of animals in the group were less than 5, the data were analyzed according to Dunn's test (3 or more groups) or Mann-Whitney's test (2 groups). If the number of animals in the group were greater than or equal to 5, a test for normality of the distribution was performed using the Kolmogorov-Lilliefors test.

   If a normal distribution was found using the Kolmogorov-Lilliefors test, an assessment of homogeneity of variance between groups was determined using the Bartlett (3 or more groups) or the Fisher (2 groups) test. If the groups were homogeneous (using either test), the data were analyzed using a Dunnett (3 or more groups) or Student (2 groups) test. If the groups were heterogeneous, the data were analyzed using a Dunn (3 or more groups) or Mann-Whitney test (2 groups).

   If the result using the Kolmogorov-Lilliefors test indicated that the data were not normally distributed, a logarithmic transformation of the values was performed (except for organ weights). The data were then re-assessed for normality using the Kolmogorov-Lilliefors test. If the transformed data were now normally distributed, they were assessed as above for the non-logarithmically transformed data. If the logarithmic transformation failed to normalize the distribution, a comparison of the treated and control groups was performed using the original (untransformed) values (including organ weights) using Dunn's test.

   Survival analysis was performed by Chi-square and log-rank tests and using the Kaplan-Meier procedure. For the Kaplan-Meier analysis, the software used was not validated according to GLP principles. A pair-wise comparison of the number of neoplasm (per group and per organ) was performed by Fisher's exact test. Where appropriate, a comparison for non-neoplastic lesions was also performed by Fisher's exact test.

C. METHODS:

1. **Observations:** Each animal was checked at least twice a day (except on weekends and public holidays: at least once a day), for mortality or signs of morbidity. Any animal showing signs of poor clinical condition was humanely killed.

Each animal was observed at least once a day, at approximately the same time, for the recording of clinical signs. In addition to this routine check, a careful examination was carried out at least once a week. This observation included (but was not limited to) evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects (such as salivation), central nervous system (including tremors and convulsions), changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength and stereotypies or bizarre behavior (e.g., self-mutilation, walking backwards).

From week 4, all animals were palpated every 4 weeks, and then, from week 52, every 2 weeks for the remainder of the study in order to record the date of detection, number, location, size, appearance and progression of palpable masses.

2. **Body Weight:** The body weight of each animal was recorded once before allocation of the animals to groups, on the first day of treatment, once a week during the first 13 weeks of treatment, then once every 4 weeks until the end of the study.

3. **Food Consumption and Compound Intake:** The quantity of food consumed by each animal was recorded, over a 7-day period, once a week during the first 13 weeks of treatment then once every 4 weeks until the end of the study. Food intake per animal and per day was calculated by subtracting the quantity of food left in each food hopper from that given. Achieved intake of test substance was calculated on a weekly basis for each sex and each treated group as follows:

   Achieved dosage (mg/kg/day) = nominal diet level (ppm) x (mean food cons. (g/animal/day) / mean bwt (g))

4. **Ophthalmoscopic Examination:** Not performed.

5. **Hematology & Clinical Chemistry:** In week 52 and before the terminal sacrifice (week 78), blood smears were prepared from blood samples obtained without anesthesia from a tail vein of each surviving animal and when practicable, from any animal killed prematurely. The animals were not fasted before sampling on any occasion. The leukocyte count (WBC) and leukocyte differential count were determined for all animals of control and high dose-level groups and when possible in all animals of any group killed prematurely.

b. **Clinical Chemistry:** In week 52, before sacrifice, and after an overnight fasting period, blood samples were collected under light isoflurane anesthesia from the orbital sinus of each surviving male and female mouse of all satellite groups. The alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) plasma levels were determined:

6. **Sacrifice and Pathology:** On completion of the chronic (week 52) or carcinogenicity (week 78) treatment periods, after at least 14 hours fasting, all surviving animals were killed by carbon dioxide asphyxiation and exsanguination. During the study, any moribund animals were killed in the same way (except for fasting). A complete macroscopic *post-mortem* examination was performed on all animals, including any that died during the study or were killed prematurely. This included examination of the external surfaces, all orifices, the

cranial cavity, the external surface of the brain and spinal cord, the thoracic, abdominal and pelvic cavities with their associated organs and tissues and the neck with its associated organs and tissues. All macroscopic observations were recorded individually. For animals in the chronic and carcinogenicity phases of the study, the checked (X) tissues were collected; histological examinations were performed on selected groups as described below. The (XX) organs, in addition, were weighed.

All listed tissues were examined microscopically for all dose levels in the interim sacrifice animals, and for any animal in the study sacrificed prematurely. At the final sacrifice, all tissues listed were examined for the control and high dose groups. In addition at the final sacrifice, all macroscopic lesions, lungs, liver (with gall bladder) and kidneys were examined for all the animals, including those from the low- and intermediate-level dose groups.

	DIGESTIVE SYSTEM		**CARDIOVASC./HEMAT.**		**NEUROLOGIC**
X	Tongue	X	Aorta, thoracic	X, XX	Brain (multiple sections)
X	Salivary glands	X, XX	Heart	X	Periph.nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X, XX	Spleen	X	Eyes (retina, optic nerve)
X	Jejunum	X	Thymus		**GLANDULAR**
X	Ileum			X, XX	Adrenal gland
X	Cecum		**UROGENITAL**		Lacrimal gland
X	Colon	X, XX	Kidneys	X	Parathyroids
X	Rectum	X	Urinary bladder	X	Thyroids
X, XX	Liver	X, XX	Testes		**OTHER**
X	Gall bladder (not rat)	X, XX	Epididymides	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate	X	Skeletal muscle
X	Pancreas	X	Seminal vesicle	X	Skin*
	RESPIRATORY	X, XX	Ovaries	X	All gross lesions and masses
X	Trachea	X, XX	Uterus (with cervix)	X	Articular surface (femoro-tibial joint)
X	Lung	X	Mammary gland	X	Harderian gland
X	Nose	X	Vagina		
X	Pharynx				
X	Larynx				

II. RESULTS AND DISCUSSION:

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** There were no treatment-related clinical signs noted at any dose level during either phase of the study. No signs of neurotoxic effects were observed during the careful examination performed at weekly intervals. There was no evidence that treatment produced an increase in the incidence or reduction in the onset time of palpable tumors.

2. **Mortality:** The survival rates (%), at study termination (beginning of week 79), are presented in Table IIA5.5.3-2. The number and occurrence of unscheduled deaths was low and similar in all the control and treated groups, except in the females of the 300 ppm group

where mortality was slightly higher (11/50) than in the control group (4/50). This was due to a higher number of animals which needed to be euthanized during the last weeks of the study, due to poor clinical condition. Although not statistically significant, a relationship to treatment with the test substance was considered to be probable.

The factors contributing to death or morbidity were similar in nature and incidence in all groups, including the 300 ppm female group. The most frequent causes of death were malignant lymphomas and general amyloidosis; these are common findings in geriatric mice.

TABLE IIA 5.5.3-2. Survival Rates (%) at Study Termination (Beginning of Week 79)

Test Group	Dose Level (ppm in diet)				
	0	10	50	150	300
Males	94	88	92	92	92
Females	92	86	84	88	80

B. **BODY WEIGHT**: The body weight gain was similar in the control and the treated groups, for the males, but was slightly lower for the females given 50, 150 or 300 ppm during the first 4 weeks of the treatment period, without correlation with variations in food consumption (table IIA5.5.3-3). However, since the lower gain was slight, transient, of low magnitude, without any dose-relationship and only recorded in females, it was not considered to be of toxicological significance. Thereafter, body weight changes were similar to controls.

TABLE IIA 5.5.3-3. Mean Body Weights and Body Weight Gains (g)

Study Week	Dose Level (ppm in diet)				
	0	10	50	150	300
Males					
Body Weight (g)					
Week 4	24.2	24.2	24.3	23.8	24.2
Week 78	34.9	35.5	34.8	34.4	35.2
Body Weight Gain (g)					
Weeks 1-78	13.2	14.3	13.6	13.3	13.8
Females					
Body Weight (g)					
Week 4	21.1	20.8	20.4**	20.6	20.4**
Week 78	30.2	29.2	29.9	29.9	29.3
Body Weight Gain (g)					
Weeks 1-4	3.3	3.5	3.5	3.1	3.1
Weeks 1-78	12.4	11.9	13.0	12.4	12.0

* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food Consumption:** The food consumption was similar in the control and the treated groups during the treatment period. Statistically significant increases in food consumption noted for female treated groups during Week 2 were considered to be attributable to spillage.

2. **Compound Consumption (time-weighted average):** The mean achieved dose levels expressed in mg/kg body weight/day received by the animals during the study are presented in Table IIA5.5.3-4.

TABLE IIA 5.5.3-4. Mean Achieved Dietary Intake of Ethiprole (mg/kg/day) for Weeks 1-78

Test Group	Dose Level (ppm in diet)	Test Compound Intake (mg/kg/day)	
		Males	Females
Low (LDT)	10	1.7	1.7
Mid-1 (MDT)	50	8.6	12.5
Mid-2 (MDT)	150	25.6	36.3
High (HDT)	300	50.8	73.5

D. **OPHTHALMOSCOPIC EXAMINATION:** Not performed.

E. **BLOOD ANALYSES:**

1. **Hematology:** There were no treatment-related qualitative or quantitative differences between the control and the treated groups in the hematology parameters examined. Among females given 300 ppm, when compared to controls, there was a slight reduction in the proportion of neutrophils and an associated slight increase in lymphocytes after 52 weeks only. However, this was not confirmed after 78 weeks of treatment. Consequently, the fluctuations of these quite variable parameters were considered to be of spontaneous occurrence.

2. **Clinical Chemistry:** No treatment-related changes were noted in the clinical chemistry parameters examined. At week 52, an increase in alanine aminotransferase activity (90 ± 89 vs. 21 ± 5 IU/L in controls) was observed in males given 300 ppm. However, since this parameter is quite variable (as shown by the standard deviations), since two values were particularly high at 300 ppm (241 and 258 IU/L) whereas the other values were within the normal range (historical control values ranged from 8 – 202 IU/L), and since the other liver enzyme activities were not increased, a relationship to treatment was considered to be doubtful. Also, these changes were not associated with any macroscopic or microscopic changes in the liver. In addition, a high individual variability was observed in this group of males at 300 ppm as well as in the historical control groups.

F. **SACRIFICE AND PATHOLOGY:**

1. **Organ Weights:** Minor differences were noted in the liver after 52 or 78 weeks (Table IIA5.5.3-5). After 52 weeks, statistically significant increases in absolute and relative liver weight were observed at the high dose (300 ppm) in males (absolute + 20%, relative +19%) as well as females (absolute +15%, relative +14%). As the differences between treated and control animals in the absolute and relative liver weights after 52 weeks were not of a large magnitude and were without correlative histopathological changes, they were considered to be of doubtful toxicological importance. At 78 weeks, similar increases in absolute and/or relative liver weight were observed at 300 ppm in both sexes and at 150 ppm in females. The increases noted in females were not dose-related, and the increases noted at 78 weeks were of a lesser magnitude than those observed at 52 weeks. In the males of the top dose group (300 ppm), the increased liver weights were mainly the contribution of three individuals whose

absolute liver weight was moderately to markedly higher than the highest liver weight in the controls. Consequently, the differences in organ weights were considered to be of doubtful toxicological importance.

An increase in absolute (25%) and relative (23%) spleen weight was observed in males from the 300 ppm dose group at 52 weeks, however a similar increase was not noted at the 78 week terminal sacrifice and no corresponding histopathological changes were noted.

Other differences noted in the organ weights for the treated animals relative to the controls were slight, not dose-related, without similar trend in both sexes, and without correlative histopathological changes. These differences, therefore, were considered to be of no toxicological importance.

TABLE IIA 5.5.3-5. Mean terminal body weights (g) and Absolute and Relative (to Body or Brain) Liver Weights (g)

	Dose Level (ppm in diet)				
	0	**10**	**50**	**150**	**300**
Males					
52 Weeks					
Number of Animals	10	10	9	9	10
Terminal body weight	32.30	32.73	32.37	32.17	32.69
Absolute	1.58	1.54	1.66	1.69	1.89** (↑20%)
Relative to body (%)	4.87	4.73	5.12	5.26	5.80** (↑19%)
Relative to brain (%)	348.7	347.2	370.8	378.0	424.1** (↑22%)
78 Weeks					
Number of Animals	47	43	44	44	45
Terminal body weight	32.52	33.08	32.61	32.50	32.93
Absolute	1.60	1.72	1.70	1.68	1.88** (↑18%)
Relative to body (%)	4.95	5.26	5.25	5.24	5.81** (↑17%)
Relative to brain (%)	357.4	388.6	383.2	373.6	417.2** (↑17%)
Females					
52 Weeks					
Number of Animals	10	10	9	8	10
Terminal body weight	28.48	30.62	30.31	28.33	28.64
Absolute	1.43	1.52	1.57**(↑10%)	1.52 (↑6%)	1.64** (↑15%)
Relative to body (%)	5.06	5.00	5.24	5.42	5.75* (↑14%)
Relative to brain (%)	315.8	331.1	340.8	340.6	359.3**(↑14%)
78 Weeks					
Number of Animals	46	42	42	41	39
Terminal body weight	28.54	27.39	27.55	27.82	27.66
Absolute	1.55	1.70	1.56	1.75*(↑13%)	1.66
Relative to body (%)	5.50	6.25	5.71	6.34**(↑15%)	6.06**(↑10%)
Relative to brain (%)	332.7	368.5	335.5	376.0*(↑13%)	362.6*(↑9%)

* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

2. **Gross Pathology:** No treatment-related necropsy findings were noted in any group.

3. **Microscopic Pathology:**

a) **Non-neoplastic:** There were no treatment-related non-neoplastic findings at any dose level after treatment for either 52 or 78 weeks. The non-neoplastic findings observed in all organs and tissues were recognized as those which are commonly recorded in aging mice of this strain and age and were regarded as being of no toxicological importance.

b) **Neoplastic:** After 78 weeks of treatment, the only neoplastic change was a slight increase (p=0.0133) in the incidence of hepatocellular adenomas in females at the 300 ppm (6/50 vs. 0/50 in controls, table IIA5.5.3-6). The incidence and time of onset of the liver neoplasms was similar in all groups. All neoplasms were recorded in animals at terminal sacrifice, suggesting that treatment with the test substance did not shorten the time of onset of this lesion. In addition, the incidence and onset time of the hepatocellular carcinoma were similar in all groups.

Other (without any dose relationship) observed neoplastic lesions found in the hemolymphoreticular system (as assessed from the microscopic examination of the liver and lymphoid organs) were diagnosed as malignant lymphoma (lymphocytic, pleiomorphic or heterogenous (mixed cell) types) and histiocytic sarcoma.
The incidence and morphological types of the remarkably few neoplastic lesions found in the testes, ovaries and uterus, were similar in both control and treated animals and showed no treatment-related changes.

All the other neoplastic lesions were recognized as those which are commonly occurring in mice of this strain and age and showed no indication of treatment-relationship.

TABLE IIA 5.5.3-6. Incidence of Hepatic Neoplastic Lesions.

Finding	Dose Level (ppm in diet)					Contemporary Control Study	
	0	10	50	150	300		
	Males						
Hepatocellular adenoma	5/49	5/50	4/50	1/50	1/50	3/50	3/49
Hepatocellular carcinoma	0/49	3/50	1/50	0/50	1/50	1/50	0/49
Total number of animals bearing neoplastic lesions	5/49	8/50	5/50	1/50	2/50	4/50	3/49
	Females						
Hepatocellular adenoma	0/50	2/50	1/50	2/50	6/50	1/50	1/50
Hepatocellular carcinoma	0/50	0/50	0/50	0/50	0/50	0/50	1/50
Total number of animals bearing neoplastic lesions	0/50	2/50	1/50	2/50	6/50	1/50	2/50

III. **CONCLUSIONS:** The test substance, ethiprole (RPA 107382), when administered daily for 78 weeks by the oral route (dietary admixture) to C57BL/6 mice was clinically well tolerated at all dose-levels, producing a slightly lower survival rate in females at 300 ppm. Increased liver weights were also observed in both males and females at 300 ppm. There were no treatment-related non-neoplastic changes in any organ at any of the dose-levels tested, but

treatment with the test substance produced a slight increase (p=0.0133) in the incidence of hepatocellular adenomas in female mice at 300 ppm.

An increase in these benign tumours was only observed in high dose females reaching the Maximum Tolerated Dose (MTD) where a reduced survival rate was observed. Since no increase in hepatocellular adenomas was observed at the lower dose levels, this effect was thus devoid of any dose-relationship. In addition, no hepatocellular carcinomas were noted in females, and only a few were noted in males, with no dose-response relationship. Given the lack of genotoxicity potential, the absence of carcinoma following a prolonged exposure to ethiprole, and the absence of any dose-relationship, this increased incidence of hepatocellular adenomas in high dose female mice was therefore considered to be subsequent to a threshold mechanism with a probable Phenobarbital-like mechanism of action: hepatocellular hypertrophy associated with transient liver cell proliferation followed by a steady state (Grasso, P. *et al.*, 1991, Hildebrand, B. *et al.*, 1991, Jirtle, R.I., *et al.*, 1991). To address this mechanism of action, the liver enzyme induction potential as well as the liver cell proliferation potential (BrdU assessment) were investigated for ethiprole and Phenobarbital in 28-day explanatory toxicity studies.

The NOAEL is therefore considered to be 50.8 mg/kg/day (300 ppm) for males and 36.3 mg/kg/day for the females (150 ppm) based on a decrease in survival rate and a slight increase in the incidence of hepatocellular adenomas at 300 ppm.

KIIA 5.5.4 - Mechanism of action and supporting data

1.	Annex point(s)	KIIA 5.5.4; Long-term toxicity and carcinogenicity/Mechanism of action and supporting data: **MRID 47622829**
2.	Location in dossier	KIIA 5.5.4 /01
3.	Authors (year)	Langrand-Lerche, C. (2002)
	Title	28-Day explanatory toxicity study in the C57BL/6 mouse Ethiprole
	Company, report No.	Bayer CropScience AG, Report-No.: C026077 (M-210729-01-1)
	Date	09.09.2002
4.	Testing facility	Bayer CropScience S.A., Sophia Antipolis, France
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC
7.	Test method	EU (=EEC): 1999/11/EC; MAFF: 12 NohSan 8628; OECD: 1997; USEPA (=EPA): 40 Cfr part 160
	Deviations	
8.	GLP	yes

Executive Summary:

Ethiprole was administered in the diet to 15 female mice at dose levels of 0, 100, 300 or 1000 ppm for 28 days (equivalent to 23.2/23.4, 64.5/67.5, or 201.1/234.5 mg/kg bw/day for 1 or 4 weeks of treatment, respectively). Satellite subgroups of 15 female mice were added to each group for interim sacrifice after 7 days of treatment. Bromodeoxyuridine (BrdU) was administered in drinking water for 7 days before scheduled sacrifices (interim and final) for liver cell proliferation assessment. In addition, at interim sacrifice after 7 days, hepatic cytochrome P-450 isoenzymes were assessed. Bodyweights, food and water consumption were recorded throughout the study. At both interim and final sacrifices, liver were removed, weighed and processed for further microscopic examination and cell proliferation assessment.

At 1000 ppm, one female was found dead on Day 22. There were no treatment-related clinical signs. At interim and final sacrifice, mean absolute and relative liver weights were significantly higher, associated with macroscopically enlarged and dark livers. This change was correlated with a slight to mild diffuse panlobular hepatocellular hypertrophy and with the appearance of a diffuse midzonal hepatocellular macrovacuolation. Hepatocellular proliferation index was 5-fold increased after 7 days of treatment whereas no difference was found with the controls after 28 days of treatment. After 7 days of treatment, total cytochrome P-450 content was 1.7 fold increased, and EROD, BROD and PROD activities were 2.7, 3.6 and 1.6 times higher than controls, respectively.

At 300 ppm, clinical signs, body weight parameters, food and water consumption, and liver weight and microscopic findings were comparable to control. Hepatocellular proliferation was unchanged after 7 or 28 days of treatment. At interim sacrifice, total cytochrome P-450 content was 1.4 fold increased. BROD and PROD activities were 2.7 and 1.6 fold increased, respectively.

At 100 ppm, there were no changes in any parameters measured.

The NOAEL is 100 ppm in the diet (23.18 or 23.37 mg/kg/day for 1 or 4 weeks of treatment, respectively).

This study is classified as **acceptable/non-guideline** and provides supporting data to the carcinogenicity study in the mice.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White crystals
Lot/Batch #:	CRD9706
Purity:	91.5%
Compound Stability:	The test substance was stored in an air-tight, light-resistant container at room temperature.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing.

3. **Test Animals:**

Species:	Mouse	
Strain:	C57BL/6 J@Ico mice	
Age/weight at study initiation:	Approx. 9 weeks old / 17.4 to 20.8 g, only females used in this study	
Source:	Iffa-Credo, L'Arbresle, France	
Housing:	Individually in suspended stainless steel, wire mesh cages.	
Diet:	Certified rodent diet A04C-10 PI from U.A.R. (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France), *ad libitum*	
Water:	Filtered and softened water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	22 ± 2°C (target)
		55 ± 15% (target)
	Humidity:	15 changes per hour (average, not monitored)
	Air changes:	12 hrs dark/ 12 hrs light
	Photoperiod:	
Acclimation period:	8 days	

B. STUDY DESIGN:

1. **In-Life Dates:** February 14, 2002 to March 15, 2002

2. **Animal Assignment:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution among the test groups noted in Table 5.5.4-1. Ethiprole was administered at dose levels of 0, 100, 300, and 1000 ppm in the diet to female C57BL/6 mice for either 7 or 28 days.

   In addition bromodeoxyuridine (BrdU) was administered to all treatment groups at 0.8 g/l in drinking water for 7 days before sacrifice (starting at Day 0 or Week 3 for the interim and final sacrifice groups, respectively) to assess hepatocytic cell cycling

TABLE IIA 5.5.4.1-1. Study Design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day) at Week 1/ Weeks 1-4[a]	No. of Female Mice	
			Interim Sacrifice after 1 Week of Treatment	Final Sacrifice after 4 Weeks of Treatment
Control	0	0	15	15
Low	100	23.18 / 23.37	15	15
Mid	300	64.46 / 67.48	15	15
High	1000	201.12 / 234.54	15	15

The test substance dose levels based on the results of a subacute toxicity study (Reference KIIA 5.3.1/01, Study Number M-171883-01-1) and a chronic toxicity study (Reference KIIA 5.5.3, Study Number M-205769-02-1). Effects on liver were observed at 1000 ppm in the subacute toxicity study. Hepatocellular adenomas were observed in females at 300 ppm in the chronic/carcinogenicity study and the No Effect Level was 150 ppm. Groups of 30 female mice (15 females for each sacrifice time) were given control diet or the appropriate diet mixture.

3. **Diet Preparation and Analysis:** The test substance was ground to a fine powder before being incorporated into the diet (Certified rodent diet A04C-10 PI from U.A.R.) by dry mixing to provide the required concentrations. Diet formulations were prepared once for the study. When not in use, the diet formulations were stored at approximately -18° C.

The stability of ethiprole in diet was demonstrated in a previous study at 5 and 2500 ppm (Reference KIIA 5.3.2, Study Number M-192116-02-1) under storage and usage conditions similar to those of the current study.

During the current study, the homogeneity of the 100 and 1000 ppm preparations was checked. The sampling was done at three levels, the surface (H), middle (M) and bottom (B). At each level, 2 samples (1 & 3) were taken and analyzed. The mean value obtained from the homogeneity checks (ethiprole) was used as measured concentration. For the intermediate preparation at 300 ppm, the concentration was checked on a single sample, the sampling being done at three levels, the surface (H), middle (M) and bottom (B) and pooled.

Results: **Homogeneity Analysis:** Results for homogeneity of ethiprole in the 100 and 1000 ppm dose level diet formulations were within the range of 92-103% of the nominal concentrations.

Stability Analysis: Stability results for up to 38 days at room temperature and 31 days of frozen storage followed by 7 days at ambient temperature ranged from 88-99% of the nominal concentrations and were thus within the target range of 85-115% of nominal concentration (see Study Profile for Study Number M-192116-02-1).

Concentration Analysis: Results for the concentration analyses were within 95-98% of the nominal concentrations.

4. **Drinking Water Treatment, Preparation and Analysis:** Solutions of BrdU in drinking water were prepared twice during the study. When not in use, the solutions were stored at ambient temperature. Homogeneity, concentration and stability of BrdU in drinking water were checked on a pre-study solution at concentration of 0.8 g/l after 4, 7 and 14 days at ambient temperature. Concentration was checked for each of the preparations used on the study.

   Results for homogeneity and concentration on the pre-study solution at 0.8 g/l were within the range of 101-103% and BrdU was found to be stable in drinking water over a 14-day period at ambient temperature. The results for the BrdU solutions used in the study were 100% of the nominal concentrations.

5. **Statistics:** Statistical analyses were performed on the following endpoints: body weight parameters, food consumption, water consumption, total cytochrome P-450 content, cytochrome P-450 isoenzyme activities, BrdU labeling indexes, organ weight parameters, organ/body weight ratios, and organ/brain weight ratios.

   Means and standard deviations were calculated separately for each group at each time period.

   Water consumption: Group variances were compared using the Bartlett test. When Bartlett's test indicated heterogeneous variances, any significant differences were identified by Dunnett's test. When Bartlett's test indicated heterogeneous variances, any significant differences were identified by using a modified t-test. All tests were performed at 5% and 1% using Path/Tox System version 4.2.2.

   Body Weight, Food Consumption, Organ Weight parameters: Two analyses were conducted; the first one on the interim sacrifice phase of the study, the second one on the final sacrifice phase of the study. Group variances were compared using the Bartlett test. When the Bartlett test was not significant (α=0.05), means were compared using the analysis of variance (ANOVA). When the ANOVA was significant (α=0.05), the means of the exposed groups were compared to the control mean using the Dunnett test (2-sided). When the Bartlett test was significant (α=0.05), means were compared using the non-parametric Kruskal-Wallis analysis of variance. When the Kruskal-Wallis test was significant (α=0.05), the mean of each exposed group was compared to the mean of the control group using the exact Mann-Whitney test (2-sided).

   P450 Content: Group variances were compared using the Bartlett test. When the Bartlett test was not significant (α=0.05), means were compared using the analysis of variance (ANOVA). When the ANOVA was significant (α=0.05), the means of the exposed groups were compared to the control mean using the Dunnett test (2-sided). When the Bartlett test was significant (α=0.05), means were compared using the non-parametric Kruskal-Wallis analysis of variance. When the Kruskal-Wallis test was significant (α=0.05), the mean of each exposed group was compared to the mean of the control group using the exact Mann-Whitney test (2-sided).

   Cytochrome P450 isoenzyme activities BROD, EROD, PROD and lauric acid hydroxylation: Group means were compared using the non-parametric Kruskal-Wallis analysis of variance. When the Kruskal-Wallis test was significant (α=0.05), the mean of each exposed group was compared to the mean of the control group using the exact Mann-Whitney test (2-sided).

Centrilobular and Perilobular BrdU labeling indexes: Two analyses were conducted; the first one on the interim sacrifice phase of the study, the second one on the final sacrifice phase of the study. Group means were compared using the non-parametric Kruskal-Wallis analysis of variance. When the Kruskal-Wallis test was significant (α=0.05), the mean of each exposed group was compared to the mean of the control group using the exact Mann-Whitney test (1-sided). Because several independent null hypotheses were tested and consequently the probability of a false positive was high, significance levels were adjusted by using Bonferronni corrections. The threshold to keep overall risk of type I error equal to 0.05 was 0.017; the threshold to keep overall risk of type I error equal to 0.01 was 0.003.

Statistical analyses were carried out using SAS version 8.2, except results from water consumption.

C. METHODS:

1. **Observations:** All animals were checked for moribundity and mortality twice daily (once daily on week-ends or public holidays). Observed clinical signs were recorded at least once daily for all animals. Detailed physical examinations were performed at least weekly during the treatment period. The nature, onset, severity, reversibility and duration of clinical signs were recorded. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose feces.

2. **Body Weight:** All animals were weighed twice during the acclimatization phase. Each animal was weighed on the first day of test substance administration, then weekly throughout the treatment period and before scheduled necropsy. Animals for interim sacrifice were weighed on Days 1 and 7 and before sacrifice only.

3. **Food Consumption and Compound Intake:** The weight of food supplied and of that remaining at the end of the food consumption period was recorded weekly for all animals during the treatment period, except for animals for interim sacrifice which were recorded for a 6-day interval only. Weekly group mean achieved test material intake was calculated using the formula:

   Test material intake (mg/kg/day): $\frac{\text{Dose level (ppm) x group mean food consumption (g/mouse/day)}}{\text{Group mean end-period body weight (g)}}$

4. **Treated Drinking Water Consumption:** Drinking water bottles containing BrdU were weighed on the first day of BrdU administration. Empty water bottles were weighed on the day of scheduled sacrifice, 7 days later. The mean water consumption in g/day was calculated at each scheduled sacrifice.

5. **Sacrifice and Pathology:** On study Days 8 and 29, all surviving designated animals from all groups were sacrificed. All sacrifices were performed by exsanguination under deep anesthesia (pentobarbital, intraperitoneal injection of approximately 60 mg/kg body weight). Animals were fasted overnight prior to sacrifice, except one animal from the low-dose group at final sacrifice. All animals were necropsied.

   The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded but not sampled, because they were considered to be incidental for this species at this age. Duodenum and liver were sampled and fixed in 10% neutral buffered formalin for histological and immunohistochemical assessment. Histological

sections of the liver (left and median lobes), including a sample of duodenum, were prepared for all animals in all groups for all scheduled sacrifice and stained with hematoxylin and eosin. Histopathological examination was performed on the liver of all surviving animals from all groups. Liver and duodenum for the animal which was not fasted were not processed for cell proliferation.

6. **Cell Proliferation Assessment:** Immunohistochemical staining to visualize the incorporation of an analog of a nucleic acid (BrdU) was used to assess hepatocytic cell cycling. A section from a formalin-fixed paraffin-embedded block containing 2 liver samples and one duodenum sample was prepared (the duodenum has a high proliferation rate and serves as a positive staining control). The immunohistochemical reaction involved incubation with a monoclonal antibody raised against BrdU, amplification with a secondary biotinylated antibody and a streptavidin-horseradish peroxidase complex, and detection of the complex with the chromogen diamino-benzidine (DAB) and a Feulgen nuclear counterstaining. The immunohistochemical staining for BrdU and determination of the labeling index were performed on all surviving and fasted study animals.

   The labeling index, expressed as the number of BrdU-positive hepatocytes per thousand hepatocytes, was measured separately on random fields comprising at least 1000 centrilobular and 1000 periportal cells using an automatic image analysis system. The mean labeling index with standard deviation was calculated for each zone, each liver and each group.

7. **Hepatotoxicity Testing:** At interim sacrifice, the remaining portions of the liver pooled by three within each group were homogenized for microsomal preparations in order to determine total cytochrome P-450 content and specific cytochrome P-450 isoenzyme profile to check the hepatotoxic potential of the test substance. Microsomal preparations were not performed from animals sacrificed at the final sacrifice date. Each microsomal sample was identified by the animal number of the first animal from the pool of three.

   Total cytochrome P-450 content in microsomal preparations was determined by spectrophotometry using a reduced CO differential spectrum. Specific cytochrome P-450 enzymatic activities were evaluated by spectrofluorimetry using the following substrates: benzoxyresorufin (BROD), ethoxyresorufin (EROD), pentoxyresorufin (PROD) and by HPLC with fluorimetric detection following derivatization by 4-(bromomethyl)-7-methoxycoumarin of 12-hydroxy-lauric acid (lauric acid used as substrate).

   Rat microsomes induced by well known reference compounds β-naphtoflavone, phenobarbital and clofibric acid were measured at the same time as the study samples to have positive controls for each assay. Results are not reported in the present report.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** There were no treatment-related clinical signs at any dose group during the study.

2. **Mortality:** At 1000 ppm, one female was found dead on Day 22. Necropsy revealed mottled and brown liver, but no clear cause of death could be determined. No other unscheduled deaths occurred during the study.

B. BODY WEIGHT AND WEIGHT GAIN:

Mean body weight and body weight gain were unaffected by treatment at all dose levels.

C. FOOD, COMPOUND, AND TREATED DRINKING WATER CONSUMPTION:

1. **Food Consumption:** Mean food consumption was unaffected by treatment.

2. **Compound Consumption:** The mean achieved dosage intake per group is presented in Table IIA5.5.4.1-2.

TABLE IIA 5.5.4.1-2. Mean Achieved Dietary Intake of Ethiprole

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	
		Week 1 (Interim Sacrifice)	Weeks 1-4 (Final Sacrifice)
Control	0	0	0
Low	100	23.18	23.37
Mid	300	64.46	67.48
High	1000	201.12	234.54

3. **Treated Drinking Water Consumption:** At 1000 ppm, mean water consumption was statistically significantly reduced by 13% in animals sacrificed on Day 8, compared to controls (mean = 3.4 g/day vs. 3.9 g/day for controls, $p<0.05$). However, this result did not impair BrdU incorporation through drinking water as demonstrated by BrdU labeling index assessment. No other differences from the control animals were noted in any other treatment group (including the high-dose group sacrificed at Day 29).

D. SACRIFICE AND PATHOLOGY:

1. **Organ Weight:** At the interim sacrifice, the mean absolute and relative liver weights were statistically significantly higher in animals at 1000 ppm, when compared to controls (↑40-41%) (table IIA5.5.4.1-3). At 300 ppm, mean relative but not absolute liver weights were slightly statistically significantly higher in animals. This change was considered as not toxicologically relevant. No gross or microscopic changes in the liver were noted at 300 ppm. No changes were observed at 100 ppm.

   At the final sacrifice, the mean absolute and relative liver weights were statistically significantly higher in animals at 1000 ppm (↑34-39%). Mean relative to body weight ratio was statistically significantly higher at 300 ppm. This was considered as not toxicologically relevant. No gross or microscopic changes in the liver were noted at 300 ppm. No change was observed on organ weights at 100 ppm.

TABLE IIA 5.5.4.1-3. Mean Terminal Body Weights, Absolute and Relative (to brain and to terminal body weight) Liver Weights

Parameter	Dose Level (ppm in diet)			
	0	100	300	1000
Interim sacrifice				
Terminal body weight (g)	15.96	15.67	15.95	16.03
Absolute Liver (g)	0.71	0.71	0.77	1.00**
Liver to Body Weight Ratio	4.45	4.52	4.85*	6.23**
Liver to Brain Weight Ratio	164.53	162.09	180.75*	230.51**
Final sacrifice				
Terminal body weight (g)	16.76	17.40	16.87	17.41 (N=14)
Absolute Liver (g)	0.77	0.82	0.83	1.07** (N=14)
Liver to Body Weight Ratio	4.58	4.72	4.95*	6.14** (N=14)
Liver to Brain Weight Ratio	176.50	189.87	190.79	244.51** (N=13)

N=15 unless otherwise noted.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

2. **Gross Pathology:** Only one unscheduled death occurred during this study; on Day 22 one high-dose animal was found dead. Necropsy of this animal revealed mottled and brown liver but no cause of death was found.

   At the interim sacrifice, no treatment-related gross pathologic changes were seen at any dose level.

   At the final sacrifice, treatment-related findings were noted at the high-dose only. At 1000 ppm, a liver enlargement was observed in 13 of 14 animals, and a dark color of the liver was found in 5 of 14 females. No other treatment-related gross pathologic findings were noted at any dose level.

3. **Microscopic Pathology:** A treatment-related histological change was found in liver on Day 8 and Day 29 at 1000 ppm. At interim and final sacrifice, a slight to mild diffuse panlobular hepatocellular hypertrophy was observed in all animals at 1000 ppm. This change was associated with the appearance of a diffuse midzonal hepatocellular macrovacuolation (12/15 animals at interim sacrifice and 5/14 animals at final sacrifice). In addition, a lower incidence of diffuse centri- to pan-lobular hepatocellular microvacuolation was noted at 1000 ppm as compared to the controls.

   No other treatment-related microscopic findings were noted at any dose level at either the interim or final sacrifice.

4. **Cell Proliferation Assessment:** At the interim sacrifice, the mean BrdU labeling index was approximately 5-fold increased at 1000 ppm, when compared to the controls (table

IIA5.5.4.1-4). No change was observed at 300 and 100 ppm. After 28 days of treatment, the mean BrdU labeling index was not significantly different for any treatment group.

TABLE IIA5.5.4.1-4. Mean ± SDev BrdU Labeling Index (Total for Centrilobular and Perilobular Hepatocytes) at both interim and final sacrifices

Parameter	Dose Level (ppm in diet)			
	0	100	300	1000
Interim sacrifice				
Labeling index	15.0±15.05	40.6±38.15	29.2±32.17	73.4*±49.22
Final sacrifice				
Labeling index	32.5±27.17	28.6±31.23 (N=14)	33.2±23.25	29.4±19.63 (N=14)

N=15 unless otherwise noted.
*p<0.01

5. **Hepatotoxicity Testing:** Hepatotoxicity testing was only performed on the animals at the interim sacrifice (table IIA5.5.4.1-5).
Total cytochrome P-450 content: Ethiprole administration via the diet to C57B1/6 mice induced a dose-related increase in total cytochrome P-450 content. At 1000 and 300 ppm, there was an increase of 1.7 and 1.4 times, respectively, compared to control mean. No effect was observed at 100 ppm.

Enzymatic activities: Statistically significantly increases in BROD (2.7 and 3.6-fold increases) and PROD (1.6-fold increases) activities were observed at 300 and 1000 ppm, whereas increase in EROD (2.7-fold increases) was obtained at the high dose level only. The statistically significant decrease in lauric acid hydroxylation observed at 1000 ppm was not considered toxicologically relevant.

TABLE IIA 5.5.4.1-5. Total Cytochrome P-450 and Related Activities

Parameter	Dose Level (ppm in diet)			
	0	100	300	1000
Total Cytochrome P450 Content (nmol/mg prot.)	1.141±0.208	1.203±0.075	1.618**±0.128	1.891**±0.133
BROD (pmol/min./mg prot.)	19.23±3.11	43.78±6.00	51.97*±11.71	69.89*±28.90
EROD (pmol/min./mg prot.)	37.28±7.08	35.56±5.57	37.71±15.72	102.44**±10.67
PROD (pmol/min./mg prot.)	12.46±2.46	14.24±2.40	20.51**±2.32	20.25**±4.35
LAURIC ACID (nmol/min./mg prot.)	10.76±1.51	12.58±2.86	10.49±2.95	4.96**±0.79

*p<0.05
**p<0.01

III. CONCLUSIONS

At interim (7 days) and final sacrifice (28 days), mean absolute and relative liver weights were significantly higher in animals that received 1000 ppm. This increase was associated with macroscopically enlarged and dark livers. This change was correlated with a slight to mild diffuse panlobular hepatocellular hypertrophy and with the appearance of a diffuse midzonal hepatocellular macrovacuolation. Cell proliferation assessment indicated that the dietary administration of ethiprole at 1000 ppm induced a transient hepatocellular proliferation in C57BL/6 mice after 7 days of treatment which returned to control levels after 28 days of treatment. No effect on hepatocellular proliferation was noted at 300 ppm after 7 or 28 days of treatment. At interim sacrifice, total cytochrome P-450 content and related enzymatic activities were increased at 300 ppm and 1000 ppm compared to controls.

At 100 ppm, there were no changes in any parameters measured.

The NOAEL under the conditions of this study is therefore 100 ppm in the diet (23.18 or 23.37 mg/kg/day for 1 or 4 weeks of treatment, respectively).

KIIA 5.7.1 - Acute neurotoxicity - rat

1.	Annex point(s)	KIIA 5.7.1; Neurotoxicity/Acute neurotoxicity – rat: **MRID 47622821**
2.	Location in dossier	KIIA 5.7.1 /03
3.	Authors (year)	Palmer, H. A. (2001)
	Title	RPA 107382 - Neurotoxicity to Rats by acute oral administration
	Company, report No.	Bayer CropScience AG, Report-No.: C016157 (M-201848-01-1)
	Date	24.05.2001
4.	Testing facility	Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC
7.	Test method	USEPA (=EPA): OPPTS 870.6200
	Deviations	
8.	GLP	yes

Executive summary MRID 47622821:

A single acute dose of ethiprole (RPA 107382) at 0, 100, 500, or 2000 mg/kg was administered to rats by oral gavage. Observations were made at 4 hours, 7 days, and 14 days after dosing. There were no treatment-related clinical signs noted at any dose level. Decreased food consumption was noted among females treated at 2000 mg/kg, whereas males exhibited slightly increased food consumption and body weight gain. Behavioral changes were confined to the day of treatment. Behavioral observations at 2000 mg/kg included licking and chewing behaviors, decreased arousal, activity, and rearing counts in the arena, increased forelimb grip strength, decreased landing foot splay, decreased rectal temperature, and a lower level of locomotor activity when assessed in the Coulbourn system. At 500 mg/kg, behavioral observations in males and females were increased forelimb grip strength, decreased landing foot splay, decreased activity counts, decreased rectal temperature (in females only) and a lower level of locomotor activity when assessed in the Coulbourn system. Females were also observed in the arena as chewing and with lower rearing counts. At 100 mg/kg, behavioral observations were limited to decreased landing foot splay in both males and females. Females also had lower level of locomotor activity when assessed in the Coulbourn system. Females were also observed in the arena as chewing. All changes appeared to be fully reversible since behavioral parameters, rectal temperature and activity counts were normal at later evaluation times. There was no evidence of neuropathology in this study.

Based on the effects seen in this study, the LOAEL was 100 mg/kg bw based on decreased landing foot splay (both sexes) and a lower level of activity compared with controls (females only). This was the lowest dose tested in this study. The NOAEL was not established.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (870.6200; OECD 424) for an acute neurotoxicity study in rats.

MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	93%
Compound Stability:	Stored at room temperature in the dark.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** The test substance, ethiprole, was administered as a suspension in aqueous 0.5% w/v methylcellulose.

3. **Test Animals:**

Species:	Rats	
Strain:	Crl:CD BR	
Age/weight at dosing:	42-49 days/ 176 - 197 g males, 143 - 173 g for females	
Source:	Charles River Breeding Laboratories, Mansion Road, Margate, Kent, England	
Housing:	Individually in suspended cages with wire mesh floors	
Diet:	Pelleted SDS Rat and Mouse No. 1 maintenance diet, *ad libitum* (except when animals were fasted overnight before dosing)	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	20 ± 0.5°C
	Humidity:	60 ± 10%
	Air changes:	Filtered, not recirculated.
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	5 days for males and 12 days for females (for first group of rats dosed, see Study Design for details).	

B. STUDY DESIGN:

1. **In-Life Dates:** March 23, 1998 to April 17, 1998

2. **Animal Assignment and Treatment:** Animals were randomly assigned to the test groups noted in Table 1, stratified by body weight, in such a way that the initial group means were approximately equal. Four groups of 10 male and 10 female Crl:CD BR rats were given ethiprole (RPA 107382) as a single oral dose (by gavage) at levels of 100, 500, or 2000 mg/kg body weight. A similar sized group (10/sex) was given the vehicle alone by oral dose to serve as the controls. The dosage volume used for all test groups was 10 mL/kg body weight. Animals were fasted overnight before dosing.

   In order to facilitate the neurobehavioral screening not all rats were dosed in one day. Animals were assigned in pairs to the control and dose groups (total of 8 batches, 2 rats/sex/dose level in each batch) for treatment. An additional acclimation period (at least 7 days) was allowed between allocations of animals to these batches. On each treatment day, dosing was balanced across the groups.

   All animals in the study were subjected to a functional observational battery and motor activity assessments prior to treatment, at the estimated time of peak effect at 4 hours, and at 7 days, and 14 days after dosing. Throughout the study, clinical signs were monitored daily, and body weights and food consumption were monitored weekly. At the end of the observation period, all animals were killed and the tissues fixed by whole body perfusion. Examination was confined to

designated tissues of the nervous system which were subsequently examined microscopically for the control and high-dose animals only (5/sex/dose level).

The dosages and time to peak effect used in this study were based the results from a dose-ranging study conducted in the same laboratory.

TABLE IIA 5.7.1.2-1. Study Design

Experimental Parameter	Dose Group (mg/kg bw)			
	0	100	500	2000
Total number of Animals/sex/group	10/sex	10/sex	10/sex	10/sex
Behavioral Testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	---	---	5/sex

3. **Test Substance Preparation and Analysis:** The test substance was administered by gavage as a suspension in 0.5% methylcellulose. For the highest required concentration, the test substance was weighed then ground in a mortar with a small amount of the vehicle until a smooth paste was formed. Further amounts of the vehicle were gradually added and mixed in to form a suspension. Once the formulation had been made up to volume, it was mixed using a high shear homogenizer. The required lower concentrations were prepared by serial dilution. Formulations were made up on one occasion and dispensed as daily aliquots on 8 occasions. During dosing, suspensions were stirred using a magnetic stirrer.

   Prior to the start of treatment, the homogeneity and stability of ethiprole in 0.5% methylcellulose was confirmed. Samples of a 2 and 200 mg/mL formulation were stored up to 2 days at ambient temperature or up to 15 days in a refrigerator (4 °C). Triplicate aliquots (from the top, middle, and bottom) were taken for analysis at each storage interval. Samples were mixed by inversion and magnetic stirring prior to analysis.

   Samples of the formulations prepared for dosing were analyzed once during the study to check the accuracy of preparation.

   Results **Homogeneity Analysis:** Results from the homogeneity analyses indicated that the coefficients of variation ranged from 0.50 to 4.25%.

   Stability Analysis: The mean measured concentrations (triplicate analysis, top, middle, bottom) of the 2 and 200 mg/mL formulation stored for up to 2 days at ambient temperatures were 1.85 and 195 mg/mL, respectively. After 15 days of storage at 4°C, the mean measured concentrations of the 2 and 200 mg/mL formulations were 1.95 and 200 mg/mL, respectively.

   Concentration Analysis: The actual concentrations of the 10, 50, and 200 mg/mL formulations used in the study were 10.8, 49.8, and 214 mg/mL, respectively. The relative mean error, representing the deviation from nominal for these formulations were -0.4 to +8.0%. A mean of two replicates were determined for each dose concentration.

   The results of these analyses demonstrate that the formulations were homogeneously prepared and stable for the duration of use. Further, the achieved concentrations were in good agreement with the intended nominal concentrations (see Formulation Analysis).

4. **Statistics:** All statistical analyses were carried out separately for males and females.

   For all parameters, the analyses were carried out using the individual animal as the basic experimental unit. Food consumption data was analyzed on weekly totals, and bodyweight data was analyzed using weight gains. The following sequence of statistical tests was used for food

consumption and bodyweight: Bartlett's test was applied to test for heterogeneity of variance between treatments. As no significant heterogeneity was detected, a one-way analysis of variance was carried out. Except for pre-dose data, analyses of variance were followed by Student's t-test and Williams' test for a dose-related response, although only the one thought most appropriate for the response pattern observed was reported.

For the behavioral data, the following were subjected to statistical analysis: rearing and activity counts, grip strength, hindlimb splay, bodyweight, temperature and Coulboum activity data. One-way analyses of variance were applied to the data, followed by Williams' test for a dose-related response. The pre-dose data were considered as a possible covariate for grip strength, hindlimb splay, bodyweight and temperature. The results from the adjusted analyses have been presented where the relationship between the pre-dose and post-dose values was statistically significant at ($p<0.10$) and where the adjustment changed the statistical significance of the initial analysis of variance. Pre-dose data were analyzed by analysis of variance followed by Student's t-test.

The categorical data were handled in the following manner: Where the recorded observations suggest a possible difference between controls and treated groups, the data were analyzed using the Jonckheere-Terpstra test.

C. METHODS / OBSERVATIONS:

1. **Mortality and Clinical Observations:** Individual animals were observed at least once daily for any signs of behavioral changes, reaction to treatment or ill health. Further checks were made early in each working day and again in the afternoon to look for dead or moribund animals.

2. **Body Weight:** The weight of each rat was recorded at the time of allocation of animals to groups, on Week -1, on the day of treatment and once a week thereafter (on each occasion that the functional observational battery was performed).

3. **Food Consumption:** For a given batch of animals, the quantity of food consumed by each rat was recorded on a weekly basis. As animals were fasted overnight prior to dosing, the initial recording interval, Week -1, was 6 days. Food intake per rat was calculated using the amount of food given to and left by each rat during each week using the following formula:

$$\text{Food consumption (g/ rat/ week)} = \frac{\text{Total food given - Total food left}}{\text{Number of animal days*}} \times 7$$

* The term "animal day' counts one animal day for each animal alive for a whole day

Efficiency of food utilization (food conversion ratios) was calculated, where possible, on a weekly basis from the body weight and food consumption data as weight of food consumed per unit gain in body weight. The following formula was used:

$$\text{Food conversion ratio} = \frac{\text{Food consumed}}{\text{Body weight gain}}$$

4. **Neurobehavioral Assessment:** The functional observational battery and motor activity were performed at approximately the same time of day, before initiation of treatment, at the estimated time of peak effect (at about 4 hours post dosing), and at 7 and 14 days after dosing. Estimation of time of peak effect was based on a dose range study conducted in the same laboratory. Because dosing did not occur on one day, not all rats were tested in one day. However, the time of testing was balanced across the groups.

a. **Functional Observational Battery (FOB):** This battery comprised 4 sets of observations. The first set of observations was performed while the animal was in its home cage. The second set of observations was performed when initially handling the animal. The third set of observations was

performed in a test arena and the fourth set comprised handling/specific testing of the animal. All these observations were made with the observer blind to the treatment condition of the animal. The CHECKED (X) parameters were examined.

X	**HOME CAGE OBSERVATIONS:**	X	**OBSERVATIONS IN THE ARENA:**
X	Posture in the cage	X	Occurrence of convulsions, tremors, twitches
X	Presence of convulsions, tremors, twitches	X	Level of activity in arena
X	Presence of spontaneous vocalizations	X	Level of arousal
X	Palpebral closure.	X	Rearing count
		X	Grooming
X	**OBSERVATIONS IN THE HAND:**	X	Assessment of gait
		X	Record presence of fecal boluses, urine
X	Ease of removing rat from cage		
X	Ease of handling rat	X	**MANIPULATIONS:**
X	Salivation/lacrimation		
X	Palpebral closure	X	Approach response
X	Exophthalmus	X	Touch response
X	Piloerection	X	Startle response
X	Vocalization on handling	X	Righting reflex
		X	Tail pinch response
		X	Pupil response
		X	Grip strength: fore and hindlimb
		X	Landing foot splay
		X	Body temperature
		X	Bodyweight

b. **Locomotor Activity:** Motor activity was evaluated at the same intervals and at the same time of day that the FOB observations were made. Motor activity was monitored using a Coulboum Infra-Red Activity Monitoring System.

This system uses an infra-red detector to monitor activity. The following categories of activity were recorded: the time spent in no movement, locomotor, and non-locomotor activity. The number of occurrences (events) of each category was also recorded. For reporting this data, only the locomotor activity was presented.

For testing, designated animals were placed singly into observation cages. The placement of an animal into a given cage was balanced as much as possible across groups. Once all animals had been placed into the cages, the test session program was started. The test session for each animal was 1 hour. Data was collected every 2 minutes and written onto a floppy disk.

5. **Sacrifice and Pathology:** All animals were killed Day 15, following the completion of the 14-day observation period. Neuropathology was performed as follows.

All animals were perfused and tissue samples were taken from them, but neuropathological examination was restricted to the control and high dose groups (5 rats/sex/dose group).

The animals were given an anesthetic overdose of sodium pentobarbital (i.p.) and perfused *in situ* with a heparinized flushing agent followed by a 1.5% glutaraldehyde: 4% paraformaldehyde solution. After perfusion, the cranial vault was removed and the brain removed, weighed and measured as detailed below. The eyes and optic nerves were removed. The skin was removed from the dorsal region and hindlimbs, and the sciatic and tibial nerves and their branches were exposed. The spinal column and cord were fixed *in toto* integral with the carcass.

The brain was transected from the spinal cord above the first cervical spinal nerve, and the olfactory lobes were removed. The length was measured between the rostral part of the cerebral

hemispheres to the most caudal part of the cerebellum, and the width between the widest parts of the cerebral hemispheres.

Following immersion fixation, peripheral nerve samples were taken and processed for epon/toluidine blue sections and tissues for paraffin wax/H&E sections were also taken. Both cross and longitudinal sections were evaluated for spinal cord, sciatic and tibial nerve tissues.

Brains, spinal cords, ganglia, and dorsal and ventral root fibers of the perfused animals were processed for paraffin embedding, sectioned at approximately 4-5 microns and stained with haematoxylin and eosin. Peripheral nerves from the right side were embedded in epon, sectioned at approximately 2 microns, and stained with toluidine blue.

The checked (X) tissues were evaluated. Lesions were graded as to severity, where possible, into 4 categories (trace, minimal, moderate or marked)

X	**CENTRAL NERVOUS SYSTEM**	X	**PERIPHERAL NERVOUS SYSTEM**
	BRAIN		**SCIATIC NERVE**
X	Forebrain	X	Mid-thigh
	Center of cerebrum	X	Sciatic Notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons		Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
X	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
	Gasserian Ganglion	X	Cervical dorsal root fibers
	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve	X	Skeletal muscle, gastrocnemius
X	Eyes		

6. **Positive Controls:** A positive control study was submitted to the PMRA to validate the ability of the laboratory to conduct FOB measurements.

Study Title: Further Validation of Neurotoxicity Procedures Following Oral Gavage Administration of D-Amphetamine or Diisopropyl Fluorophosphateto CD Rats to Meet EPA FIFRA Requirements. Huntingdon Life Sciences Ltd. Eye, Suffolk, England. Study conducted: December 1997.

Summary:
Two groups of female CD rats, (35 days old, 142-180 g) each comprising 15 animals, received diisopropyl fluorophosphate (DFP) by gavage, at a single dosage of 2 or 3 mg/kg. A third group of 15 females received D-amphetamine by gavage, at a single dosage of 2 mg/kg and a similarly constituted Control group received the vehicle (purified water).
Functional observational battery (FOB) assessments were performed on all animals before treatment, approximately one hour after treatment (time of peak effect) and one day after treatment. Grip strength recordings were repeated approximately 12 days after treatment. Five members of staff were either trained or re-assessed during the course of this study - each person testing the same 12 animals (three from each of the four groups) on each occasion, although the same animals were not necessarily examined by the same person for each FOB test. All FOB testing was performed "blind" to treatment.

One hour after treatment, animals which received DFP at 3 mg/kg generally showed gait abnormalities, chewing mouth movements, decreased reactivity, reduced rearing in the standard arena, reduced body temperature, and reduced activity during the one hour of automated activity recording. Effects in animals which received DFP at 2 mg/kg were limited to decreased motor activity scores and

the observation of chewing mouth movements in two individuals. In contrast, animals which received D-amphetamine showed increased levels of reactivity and arousal, increased activity and rearing counts in the standard arena, and markedly increased motor activity scores during the automated activity recording period. At 24 hours after treatment, animals which had received D-amphetamine still showed some evidence of increased reactivity to handling and motor activity scores were still slightly higher than those of control animals. Animals which had received DFP at 3 mg/kg showed slightly decreased reactivity and, in contrast to the finding on the day of treatment, motor activity scores were increased when compared with controls. Grip strength recordings showed no treatment-related effects, however some inter-observer variation was noted.

Conclusions:
The treatment related findings noted above would be anticipated following treatment with D-amphetamine or DFP. It should be noted that this study was conducted in a different laboratory than that utilized for assessing the neurotoxicity of ethiprole, although it is a branch of the original laboratory. Also, no neuropathology control data were provided. All FOB measurements were consistent with the parameters examined in both the acute and subchronic neurotoxicity studies with ethiprole. Individual animal data was provided and the study illustrated inter-observer reliability. The above described study illustrates the laboratory's successful ability to conduct FOB measurements in a neurotoxicity study.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs**: In respect of clinical signs, there were no obvious signs of reaction to treatment immediately following dosing.

2. **Mortality:** There were no mortalities during this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Bodyweight gains during Week 1 were comparable with controls. During Week 2, group mean weight gain for males treated at 2000 mg/kg was statistically significantly increased relative to controls.

TABLE IIA 5.7.1.2-2. Body Weight and Body Weight Gain

Interval	Males				Females			
	Dose Level (mg/kg bw)				Dose Level (mg/kg bw)			
	0	100	500	2000	0	100	500	2000
Body Weights (g)								
Pre-dose	197	197	197	197	163	164	163	164
Week 0	206	210	210	212	160	161	162	162
Week 1	274	288	288	282	205	209	207	205
Week 2	313	331	332	332	213	221	217	217
Body Weight Gains (g)								
Week 0 to 1	68	78	77	70	46	48	44	43
Week 1 to 2	39	43	45	50*	8	12	10	12

* Statistically different ($p<0.05$) from control.

C. **FOOD CONSUMPTION**: During Week 1, the group mean food consumption for females at 2000 mg/kg was statistically significantly lower than controls. There were no similar effects at lower dosages. During Week 2, food consumption compared favorably with controls in females, but was slightly significantly increased in high dose males. There were no marked differences in food conversion ratios.

TABLE IIA 5.7.1.2-3. Mean Food Consumption (g/rat/week)

Interval	Males				Females			
	Dose Level (mg/kg bw)				Dose Level (mg/kg bw)			
	0	100	500	2000	0	100	500	2000
Pre-dose	188	188	190	195	141	138	144	143
Week 1	224	236	225	213	185	184	177	162**
Week 2	231	238	250	252*	173	176	179	184

* Statistically different ($p<0.05$) from control. ** Statistically different ($p<0.01$) from control.

D. NEUROBEHAVIORAL RESULTS

1. **FOB Findings:** Changes associated with treatment were confined to the day of treatment. At 2000 mg/kg, both sexes exhibited the following: Licking behavior was observed in the arena (table IIA 5.7.1.2-2). Temperature was statistically significantly reduced. Forelimb grip strength was statistically significantly increased. Landing foot splay was statistically significantly lower. Statistically significantly lower activity and rearing counts were noted in females while the animals were in the arena. Certain non-statistically significant findings that were decreased compared to pre-dose values were noted in male (activity and rearing counts) and male and female animals (arousal levels). These findings were considered to be treatment related due to the number of control animals which exhibited atypically lowered counts for these parameters, thus, pre-dose measurements of treated animals serve as superior baseline values for the above mentioned parameters. In addition for females only, chewing, and an absence of grooming behavior, were noted while the animals were in the arena (table IIA 5.7.1.2-3).

   At 500 mg/kg, both sexes exhibited the following: Forelimb grip strength was statistically significantly increased. Landing foot splay was statistically lower. Activity counts were lower compared to pre-dose values in both sexes, and decreased arousal was noted in male animals. Rectal temperature was also statistically significantly lower compared with controls for females only. In addition for females only, chewing and licking behaviors and decreased rearing counts were observed while the animals were in the arena.

   At 100 mg/kg, both sexes exhibited a statistically significantly lower landing foot splay compared with controls. Females also showed decreased locomotor activity and increased forelimb grip strength and chewing behavior in the arena.

   For the most part, all results were similar to control on Days 8 and 15. The number of control males which exhibited a low level of arousal was considered atypical. For that reason, statistically significant differences from the controls in related findings (increased activity counts and alert arousal level, and rearing counts) in the high-dose males at Day 8 and/or 15 were not considered treatment-related.

TABLE IIA 5.7.1.2-4. Functional Observation Battery (FOB) Results [a]

Parameter / Interval	Males				Females			
	Dose Level (mg/kg bw)				Dose Level (mg/kg bw)			
	0	100	500	2000	0	100	500	2000
OPEN FIELD OBSERVATIONS								
Arousal, alert								
Pre-dose	10	10	10	10	9	9	10	9
4-Hours (Day 1)	3	8	3	5	8	7	8	5
Day 8	5	8	7	10*	9	9	10	10
Day 15	6	8	8	10*	8	9	10	10
Grooming								
Pre-dose	4	1	4	5	5	3	1	1*
4-Hours (Day 1)	3	7	3	2	6	5	3	0*
Day 8	2	3	2	4	6	4	2	3
Day 15	1	3	0	4	1	5	4	1
Piloerection								
Pre-dose	0	0	0	0	0	0	0	0
4-Hours (Day 1)	0	0	0	1	0	1	1	1
Day 8	0	0	0	0	0	0	0	0
Day 15	0	0	0	0	0	0	0	0
Gait, unable to assess								
Pre-dose	1	1	0	1	1	1	0	1
4-Hours (Day 1)	9	2	8	7	2	4	3	6
Day 8	5	2	4	2	0	1	0	0
Day 15	4	2	2	0	1	1	0	0
ADDITIONAL COMMENTS								
Chewing								
Pre-dose	0	0	0	0	0	0	0	0
4-Hours (Day 1)	0	0	0	0	0	2	3	5**
Day 8	0	0	0	0	0	0	0	0
Day 15	0	0	0	0	0	0	0	0
Eye closure								
Pre-dose	0	0	0	0	0	0	0	0
4-Hours (Day 1)	4	3	5	6	2	2	3	5
Day 8	3	2	1	1	0	0	0	0
Day 15	3	2	1	0	1	0	0	0
Licking of the arena								
Pre-dose	0	0	0	1	0	0	0	2
4-Hours (Day 1)	0	0	1	3*	0	1	2	4*
Day 8	0	0	0	0	0	0	0	0
Day 15	0	0	0	0	0	0	0	0

a N=10 for all test groups.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

TABLE IIA 5.7.1.2-5. Additional FOB Results: Activity and Rearing Counts (events/hour), Forelimb and Hindlimb Grip Strength (kg), Splay Values (cm), Temperature (°C) [a]

Parameter	Dose Level (mg/kg bw)			
	0	100	500	2000
Males				
Activity counts (events/h)				
Pre-dose	16±6.6	17±7.3	16±8.6	15±7.0
4-Hours (Day 1)	3±3.2	11±7.2	5±7.2	5±4.8
Day 8	5±4.4	13±8.4	12±10.0	15*±8.1
Day 15	12±11.2	21±11.4	17±12.2	21±8.8
Rearing counts (events/h)				
Pre-dose	7±3.9	9±3.3	6±4.3	8±5.2
4-Hours (Day 1)	2±2.6	5±4.4	2±2.8	2±2.0
Day 8	2±2.2	4±3.5	5±5.1	7*±4.8
Day 15	6±7.2	8±5.5	8±6.0	10±5.8
Forelimb grip strength (kg)				
Pre-dose	0.82±0.105	0.77±0.102	0.82±0.157	0.76±0.065
4-Hours (Day 1)	0.85±0.087	0.89±0.098	0.96*±0.089	0.94*±0.133
Day 8	0.97±0.245	0.87±0.137	1.03±0.123	0.88±0.136
Day 15	1.15±0.213	1.04±0.217	1.09±0.107	1.07±0.201
grip strength (kg)				
Pre-dose	0.48±0.112	0.44±0.043	0.45±0.108	0.46±0.076
4-Hours (Day 1)	0.51±0.091	0.45±0.099	0.47±0.072	0.49±0.076
Day 8	0.62±0.124	0.56±0.110	0.62±0.203	0.63±0.110
Day 15	0.61±0.073	0.66±0.086	0.66±0.122	0.69±0.125
Splay values (cm)				
Pre-dose	10.2±2.06	9.1±1.64	9.7±1.72	9.7±2.36
4-Hours (Day 1)	9.8±1.85	7.1**±1.47	7.2**±1.13	7.6**±2.45
Day 8	9.7±1.67	9.2±1.80	10.9±1.33	11.0±2.32
Day 15	10.2±2.08	9.2±1.53	10.0±1.49	10.3±1.86
Temperature (°C)				
Pre-dose	38.3±0.31	38.4±0.35	38.3±0.31	38.3±0.25
4-Hours (Day 1)	37.4±0.58	37.3±0.45	36.9±0.34	36.8*±0.56
Day 8	38.4±0.32	38.4±0.24	38.5±0.48	38.4±0.28
Day 15	38.4±0.32	38.1±0.49	38.2±0.44	38.3±0.24
Females				
Activity counts (events/h)				
Pre-dose	19±12.0	20±10.8	21±9.3	17±8.1
4-Hours (Day 1)	18±13.3	11±9.0	9*±6.6	5**±3.2
Day 8	23±13.7	25±11.8	32±11.1	24±12.7
Day 15	27±14.7	25±11.3	27±10.2	30±8.0
Rearing counts (events/h)				
Pre-dose	9±6.8	10±5.5	11±4.5	7±3.4
4-Hours (Day 1)	10±11.4	5±4.5	4*±4.1	1**±1.3
Day 8	12±8.8	12±5.7	14±5.1	10±5.9
Day 15	13±9.6	11±6.3	14±5.7	15±4.2
Forelimb grip strength (kg)				
Pre-dose	0.78±0.112	0.87±0.114	0.78±0.176	0.76±0.094
4-Hours (Day 1)	0.78±0.072 (0.78)	0.92*±0.116 (0.88)	0.87**±0.145 (0.88*)	0.91**±0.135 (0.93**
Day 8	0.99±0.202	0.97±0.207	0.94±0.176	0.88±0.132
Day 15	1.06±0.162	0.98±0.155	0.98±0.118	0.93±0.269

(Table continued; footnotes to follow)

TABLE IIA 5.7.1.2-5. Additional FOB Results: Activity and Rearing Counts (events/hour), Forelimb and Hindlimb Grip Strength (kg), Splay Values (cm), and Temperature (°C) (cont.) [a]

Parameter	Dose Level (mg/kg bw)			
	0	100	500	2000
		Females		
grip strength (kg)				
Pre-dose	0.50±0.093	0.44±0.088	0.46±0.080	0.48±0.089
4-Hours (Day 1)	0.47±0.101	0.49±0.122	0.48±0.084	0.48±0.076
Day 8	0.54±0.540	0.57±0.570	0.57±0.570	0.55±0.550
Day 15	0.57±0.136	0.56±0.138	0.67±0.112	0.57±0.097
Splay values (cm)				
Pre-dose	8.9±1.52	8.1±1.60	8.8±2.09	9.8±1.82
4-Hours (Day 1)	8.9±1.48	6.7**±1.55	6.9**±1.51	6.2**±1.21
Day 8	9.2±1.41	8.7±1.47	9.6±1.63	10.2±1.49
Day 15	9.3±1.46	8.0±2.23	9.1±1.81	9.3±1.62
Temperature (°C)				
Pre-dose	38.5±0.38	38.6±0.33	38.6±0.40	38.5±0.43
4-Hours (Day 1)	37.9±0.64	37.4±0.70	37.1**±0.54	36.8**±0.40
Day 8	38.5±0.47	38.7±0.20	38.7±0.33	38.6±0.28
Day 15	38.5±0.35	38.6±0.27	38.6±0.34	38.5±0.25

a Values are mean±SD for all parameters. N=10 for all test groups.
b Forelimb grip strength values in parentheses are adjusted for pre-dose values.
* Statistically different (p<0.05) from the control.
** Statistically different (p<0.01) from the control.

2. **Locomotor Activity:** Four hours after treatment, there was a statistically significant decrease in locomotor activity among both males and females treated at 500 and 2000 mg/kg and among females treated at 100 mg/kg (table IIA 5.7.1.2-4). At 8 and 15 days after treatment, there were no significant differences in locomotor activity.

TABLE IIA 5.7.1.2-6. Locomotor Activity [a]

Parameter	Dose Level (mg/kg bw)			
	0	100	500	2000
		Males		
Pre-dose	468±167.1	577±243.7	517±170.0	614±322.3
Day 1	231±96.9	280±106.2	110**±54.2	76**±44.1
Day 8	632±198.9	707±166.0	676±215.7	813±303.0
Day 15	619±244.7	721±291.6	715±233.2	656±282.2
		Females		
Pre-Dose	754±218.6	824±294.0	759±283.6	724±269.2
Day 1	354±199.6	176**±54.1	96**±54.6	39**±22.1
Day 8	551±217.3	575±237.5	478±125.8	597±183.5
Day 15	661±242.1	725±234.2	645±208.6	695±233.6

a Values are total (mean±SD) for large movements (in secs) during the 1 hour observation session at the indicated test day.
** Statistically different (p<0.01) from the control.

E. SACRIFICE AND PATHOLOGY:

1. **Brain weight:** There were no statistically significant differences in brain weight or in brain measurements.

Neuropathology: There were no microscopic findings in the sections of nervous tissue examined which were considered to be related to the administration of ethiprole. Incidental findings were noted as follows. One control animal and two high-dose animals showed minimal axonal degeneration in

tibial nerve, spinal nerve root(s), or spinal cord. Two control animals and one high-dose animal showed minimal or slight focal retinal rosettes in one eye. These minor changes were considered to be incidental and of no toxicological importance. Furthermore, all incidental findings of axonal degeneration were within historical control values. Historical control ranges were compiled from counts of minimal axonal degeneration from eight different acute neurotoxicity studies conducted between January 1998 and October 2005, at Huntingdon Life Sciences.

TABLE IIA 5.7.1.2-7. Microscopic Neuropathology [a]

Parameter	*Males*		*Females*	
	Dose Level (mg/kg bw)		Dose Level (mg/kg bw)	
	0	2000	0	2000
Spinal Cord (C3-6)	0	0	1	0
Spinal Cord (L1-4)	0	1	0	0
Tibial Nerve (at knee)	0	1	0	0
Eyes (retinal rosettes) [b]	0	1	2	0

a Values indicate the number of animals observed with minimal axonal degeneration in a particular neurological tissue type.
b Microscopic abnormalities observed in the eyes were in the form of retinal rosettes.

TABLE 5.7.1.2-8 Historical Control Range in percent for Microscopic Neuropathology

Parameter	Males	Females
Spinal Cord (C3-6)	0-40%	0-20%
Spinal Cord (L1-4)	0-40%	0-20%
Tibial Nerve (at knee)	0-20%	0-20%

III. CONCLUSIONS: The acute oral (gavage) administration of ethiprole to rats produced behavioral changes confined to the day of treatment at 100, 500 and 2000 mg/kg. Behavioral observations at 2000 mg/kg included licking and chewing behaviors, decreased arousal, activity and rearing counts in the arena, increased forelimb grip strength, decreased landing foot splay, decreased rectal temperature, and a lower level of locomotor activity when assessed in the Coulbourn system.

Based on the effects seen in this study, the LOAEL was 100 mg/kg bw based on decreased landing foot splay (both sexes) and a lower level of activity and decreased forelimb grip strength compared with controls (females only). This was the lowest dose tested in this study. A NOAEL was not established.

KIIA 5.7.1 - Acute neurotoxicity - rat

1.	Annex point(s)	KIIA 5.7.1; Neurotoxicity/Acute neurotoxicity – rat: **MRID 47622822**
2.	Location in dossier	KIIA 5.7.1 /01
3.	Authors (year)	Palmer, H. A. (2001)
	Title	RPA107382 Neurotoxicity study by a single oral gavage administration to CD rats followed by a 14-day observation period
	Company, report No.	Bayer CropScience AG, Report-No.: C016526 (M-202573-01-1)
	Date	12.07.2001
4.	Testing facility	Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC
7.	Test method	USEPA (=EPA): OPPTS 870.6200
	Deviations	
8.	GLP	yes

Executive Summary:

The objective of this study was to assess the potential neurotoxicity of ethiprole (batch CDR 9706, 93.0% purity) and to establish a NOAEL in rats, by oral administration. Four groups of 10 male and 10 female Crl:CD BR rats were given ethiprole as a single oral dose (by gavage) at levels of 10, 25, 35 or 250 mg/kg bw. A similar sized group was given the vehicle by oral dose and acted as controls. All of these animals were subjected to a functional observational battery (FOB) prior to treatment and at 4 hours after dosing and seven and fourteen days after treatment. Motor activity of each animal was also quantitatively assessed at the same intervals. Throughout the study, clinical signs, bodyweights and food consumption were monitored. At the end of the observation period, all animals were killed. There were no further investigations.

There were no clinical signs of reaction to treatment following dosing. There were no differences in bodyweights, food conversion ratios, or food consumption between treated and control groups. The functional observational battery changes associated with treatment were confined to the day of treatment at 250, and 35 mg/kg bw and included low arousal levels, increased eye closure, increased incidence of body tremors and decreased rearing counts in females at 35 mg/kg bw. At 250 mg/kg bw additional effects were noted including: males that were awkward to handle, decreased grooming behavior, reduced activity in females, and increased grip strength in males. Seven days after treatment, slight changes in behavioral indices (decreased grooming and increased walking on toes) were noted. There were no differences between groups fourteen days after treatment. In a quantitative assessment of locomotor activity, four hours after treatment, there was a statistically significant decrease in locomotor activity among females treated at 250 mg/kg bw, while males exhibited non-statistically significant decreases in locomotor activity. No effects were noted for the lower dose animals.

There were no behavioral differences at 25 mg/kg/bw. The NOAEL for acute neurotoxicity was set at 25 mg/kg/bw.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (870.6200; OECD 424) for an acute neurotoxicity study in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White crystals
Lot/Batch #:	CRD9706
Purity:	93%
Compound Stability:	Stored at 4°C in the dark.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** The test substance, RPA 107382, was administered as a suspension in aqueous 0.5% w/v methylcellulose.

3. **Test Animals:**

Species:	Rats	
Strain:	Crl:CD BR	
Age/weight at dosing:	42-49 days/ 203-228 g for males, 135-160 g for females	
Source:	Charles River Breeding Laboratories, Mansion Road, Margate, Kent, England	
Housing:	Individually in suspended cages with wire mesh floors	
Diet:	Pelleted SDS Rat and Mouse No. 1 maintenance diet, *ad libitum* (except when animals were fasted overnight before dosing)	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	21 ± 1°C
	Humidity:	57 ± 10%
	Air changes:	Filtered, not recirculated.
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	5 days for males and 12 days for females (for first group of rats dosed, see Study Design for details).	

B. STUDY DESIGN:

1. **In-Life Dates:** June 28, 1999 to July 24, 1999

2. **Animal Assignment and Treatment:** Animals were randomly assigned to the test groups noted in Table IIA 5.7.1-1, stratified by body weight, in such a way that the initial group means were approximately equal. Four groups of 10 male and 10 female Crl:CD BR rats were given ethiprole (RPA 107382) as a single oral dose (by gavage) at levels of 10, 25, 35, or 250 mg/kg bw. A similar sized group (10/sex) was given the vehicle alone by oral dose to serve as the controls. The dosage volume used for all test groups was 10 ml/kg body weight. Animals were fasted overnight before dosing.

   In order to facilitate the neurobehavioural screening not all rats were dosed in one day. Animals were assigned in pairs to the control and dose groups (total of 10 batches, 2 rats/sex/dose level in each batch) for treatment. An additional acclimation period (at least 7 days) was allowed between allocations of animals to these batches. On each treatment day, dosing was balanced across the groups.

   All animals in the study were subjected to a functional observational battery and motor activity assessments prior to treatment, at the estimated time of peak effect at 4 hours, and at 7 days, and

14 days after dosing. Throughout the study, clinical signs were monitored daily, and body weights and food consumption were monitored weekly. At the end of the observation period, all animals were killed and no further investigations were performed.

The dosages and time to peak effect used in this study were based the results from a dose-ranging study and an acute neurotoxicity study previously performed at the same laboratory.

TABLE IIA 5.7.1.1-1. Study Design

Experimental Parameter	Dose Group (mg/kg bw)				
	0	10	25	35	250
Total number of Animals/sex/group	10/sex	10/sex	10/sex	10/sex	10/sex
Behavioral Testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10/sex	10/sex

3. **Test Substance Preparation and Analysis**: The test substance was administered by gavage as a suspension in 0.5% methylcellulose. The required amount of test substance was weighed then ground in a mortar with a small amount of the vehicle until a smooth paste was formed. Further amounts of the vehicle were gradually added and mixed in to form a suspension. Once the formulation had been made up to volume, it was mixed using a high shear homogenizer. The formulations were stored at 4°C until use. The required concentrations were prepared by direct dilution. Formulations were made up on one occasion and divided into 10 aliquots. An aliquot was used on each occasion of dosing. During dosing, suspensions were stirred using a magnetic stirrer.

   Prior to the start of treatment, the homogeneity and stability of ethiprole in 0.5% methylcellulose was confirmed. Samples of a 0.2 mg/mL formulation were stored up to 2 days at ambient temperature or up to 15 days in a refrigerator (4 °C), representing the maximum storage intervals and conditions used in this study. Triplicate aliquots (from the top, middle, and bottom) were taken for analysis at each storage interval. Two trials were conducted. In the first trial, aliquots for analysis were taken from samples directly out of storage, without pre-mixing, or allowing the temperature to equilibrate. In the second trial, samples were mixed and allowed to equilibrate to room temperature prior to analysis.

   Samples of the formulations prepared for dosing were analyzed once during the study to check the accuracy of preparation.

   Results **Homogeneity Analysis:** For the samples that were not mixed prior to analysis, the coefficients of variation ranged from 2.18 to 6.04%. For the samples that were mixed and equilibrated to ambient temperatures before analysis, the coefficients of variation ranged from 0.38 to 2.87%. The homogeneity results confirmed that the test substance must be equilibrated to ambient temperature before use to ensure a free flowing solid.

   Stability Analysis: The mean measured concentration (triplicate analysis, top, middle, bottom) of the 0.2 mg/mL formulation stored for up to 2 days at ambient temperatures was 0.187 mg/mL, and after 15 days of storage at 4 °C, the mean measured concentration was 0.190 mg/mL. These data indicate that ethiprole is stable in a 0.5% methylcellulose formulation for the intervals and conditions used in this study.

   Concentration Analysis: The actual concentrations of the 1, 2.5, 3.5, and 25 mg/mL formulations used in the study were 0.999, 2.45, 3.54, and 25 mg/mL, respectively. The relative mean error, representing the deviation from nominal for these formulations were -2.0 to +1.1%. A mean of two replicates were determined for each dose concentration.

4. **Statistics:** All statistical analyses were carried out separately for males and females.

For all parameters, the analyses were carried out using the individual animal as the basic experimental unit. Food consumption data was analyzed on weekly totals, and body weight data was analyzed using weight gains.

The following sequence of statistical tests was used for food consumption, body weight, and brain parameters: Bartlett's test was applied to test for heterogeneity of variance between treatments. As no significant heterogeneity was detected, a one-way analysis of variance was carried out. Except for pre-dose data, analyses of variance were followed by Student's t-test and Williams' test for a dose-related response, although only the one considered most appropriate for the response pattern observed was reported.

The following data were subjected to statistical analysis: rearing and activity counts, grip strength, hindlimb splay, body weight, temperature, and Coulbourn locomotor activity data. This data was analyzed using a one-way analysis of variance followed by Williams' test for a dose-related response. Pre-dose data was analyzed by analysis of variance followed by a Student's t-test.

Categorical data, where the recorded observations suggested a possible difference between control and treated groups, were analyzed using the Jonckheere-Terpstra test.

C. METHODS / OBSERVATIONS:

1. **Mortality and Clinical Observations:** Individual animals were observed at least once daily for any signs of behavioral changes, reaction to treatment or ill health. Further checks were made early in each working day and again in the afternoon to look for dead or moribund animals.

2. **Body Weight:** The weight of each rat was recorded at the time of allocation of animals to groups, on Week -1, on the day of treatment and once a week thereafter.

3. **Food Consumption:** For a given batch of animals, the quantity of food consumed by each rat was recorded on a weekly basis. As animals were fasted overnight prior to dosing, the initial recording interval, Week -1, was 6 days. Food intake per rat was calculated using the amount of food given to and left by each rat during each week using the following formula:

$$\text{Food consumption (g/ rat/ week)} = \frac{\text{Total food given - Total food left}}{\text{Number of animal days*}} \times 7$$

* The term "animal day' counts one animal day for each animal alive for a whole day

Efficiency of food utilization (food conversion ratios) was calculated, where possible, on a weekly basis from the body weight and food consumption data as weight of food consumed per unit gain in body weight. The following formula was used:

$$\text{Food conversion ratio} = \frac{\text{Food consumed}}{\text{Body weight gain}}$$

4. **Neurobehavioral Assessment:** The functional observational battery and motor activity were performed at approximately the same time of day, before initiation of treatment, at the estimated time of peak effect (at about 4 hours post dosing), and at 7 and 14 days after dosing. Estimation of time of peak effect was based on a dose range study. Because dosing did not occur on one day, not all rats were tested in one day. However, the time of testing was balanced across the groups.

a. **Functional Observational Battery (FOB):** This battery comprised 4 sets of observations. The first set of observations was performed while the animal was in its home cage. The second set of observations was performed when initially handling the animal. The third set of observations was

performed in a test arena and the fourth set comprised handling/specific testing of the animal. All these observations were made with the observer blind to the treatment condition of the animal. The checked (X) parameters were examined.

X	**HOME CAGE OBSERVATIONS:**	X	**OBSERVATIONS IN THE ARENA:**
X	Posture in the cage	X	Occurrence of convulsions, tremors, twitches
X	Presence of convulsions, tremors, twitches	X	Level of activity in arena
X	Presence of spontaneous vocalizations	X	Level of arousal
X	Palpebral closure.	X	Rearing count
		X	Grooming
X	**OBSERVATIONS IN THE HAND:**	X	Assessment of gait
		X	Record presence of fecal boluses, urine
X	Ease of removing rat from cage		
X	Ease of handling rat	X	**MANIPULATIONS:**
X	Salivation/lacrimation		
X	Palpebral closure	X	Approach response
X	Exophthalmus	X	Touch response
X	Piloerection	X	Startle response
X	Vocalization on handling	X	Righting reflex
		X	Tail pinch response
		X	Pupil response
		X	Grip strength: fore and hindlimb
		X	Landing foot splay
		X	Body temperature
		X	Bodyweight

b. **Locomotor Activity:** Motor activity was evaluated at the same intervals and at the same time of day that the FOB observations were made. Motor activity was monitored using a Coulboum Infra-Red Activity Monitoring System.

This system uses an infra-red detector to monitor activity. The following categories of activity were recorded: the time spent in no movement, locomotor, and non-locomotor activity. The number of occurrences (events) of each category was also recorded. For reporting this data, only the locomotor activity was presented.

For testing, designated animals were placed singly into observation cages. The placement of an animal into a given cage was balanced as much as possible across groups. Once all animals had been placed into the cages, the test session program was started. The test session for each animal was 1 hour. Data was collected every 2 minutes and written onto a floppy disk.

5. **Sacrifice and Pathology:** All animals were killed Day 15, following the completion of the 14-day observation period. No macroscopic examinations were performed.

6. **Positive Controls:** A positive control study was submitted to the PMRA to validate the ability of the laboratory to conduct FOB measurements.

Study Title: Further Validation of Neurotoxicity Procedures Following Oral Gavage Administration of D-Amphetamine or Diisopropyl Fluorophosphateto CD Rats to Meet EPA FIFRA Requirements. Huntingdon Life Sciences Ltd. Eye, Suffolk, England. Study conducted: December 1997.

Summary:
Two groups of female CD rats, (35 days old, 142-180 g) each comprising 15 animals, received diisopropyl fluorophosphate (DFP) by gavage, at a single dosage of 2 or 3 mg/kg. A third group of 15 females received D-amphetamine by gavage, at a single dosage of 2 mg/kg bw and a similarly constituted Control group received the vehicle (purified water).

Functional observational battery (FOB) assessments were performed on all animals before treatment, approximately one hour after treatment (time of peak effect) and one day after treatment. Grip strength recordings were repeated approximately 12 days after treatment. Five members of staff were either trained or re-assessed during the course of this study - each person testing the same 12 animals (three from each of the four groups) on each occasion, although the same animals were not necessarily examined by the same person for each FOB test. All FOB testing was performed "blind" to treatment.

One hour after treatment, animals which received DFP at 3 mg/kg bw generally showed gait abnormalities, chewing mouth movements, decreased reactivity, reduced rearing in the standard arena, reduced body temperature, and reduced activity during the one hour of automated activity recording. Effects in animals which received DFP at 2 mg/kg bw were limited to decreased motor activity scores and the observation of chewing mouth movements in two individuals. In contrast, animals which received D-amphetamine showed increased levels of reactivity and arousal, increased activity and rearing counts in the standard arena, and markedly increased motor activity scores during the automated activity recording period. At 24 hours after treatment, animals which had received D-amphetamine still showed some evidence of increased reactivity to handling and motor activity scores were still slightly higher than those of control animals. Animals which had received DFP at 3 mg/kg bw showed slightly decreased reactivity and, in contrast to the finding on the day of treatment, motor activity scores were increased when compared with controls. Grip strength recordings showed no treatment-related effects, however some inter-observer variation was noted.

Conclusions:
The treatment related findings noted above would be anticipated following treatment with D-amphetamine or DFP. It should be noted that this study was conducted in a different laboratory than that utilized for assessing the neurotoxicity of ethiprole, although it is a branch of the original laboratory. Also, no neuropathology control data were provided. All FOB measurements were consistent with the parameters examined in both the acute and subchronic neurotoxicity studies with ethiprole. Individual animal data was provided and the study illustrated inter-observer reliability. The above described study illustrates the laboratory's ability to successfully conduct FOB measurements in a neurotoxicity study.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs**: There were no treatment-related clinical signs noted at any dose level.

2. **Mortality:** There were no mortalities during this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN: No treatment-related effects were observed on body weights or body weight gains at any dose level.

C. FOOD CONSUMPTION: No treatment-related changes in food consumption or food efficiency were noted at any dose level.

D. NEUROBEHAVIORAL RESULTS

1. **FOB Findings:** In summary, changes associated with treatment were mostly confined to the day of treatment (4 hours after dosing) and were seen at the 35 and 250 mg/kg bw dose levels (tables IIA 5.7.1.1-2 and IIA 5.7.1.1-3). The following effects were noted 4 hours post-dose. At 35 mg/kg bw a lower level of arousal, decreased rearing counts and increased incidence of body tremor, and a higher incidence of closure of the eyes were observed in females only. At 250 mg/kg, the observations included the following: a higher incidence of animals being slightly awkward to handle (males); a decrease in the number of animals grooming (both sexes); a lower level of arousal (females); a higher incidence of animals for which no assessment of gait was possible (females); a higher incidence of closure of the eyes (females); significantly reduced

activity and rearing counts (females); increased incidence of body tremor (females); increased forelimb and hindlimb grip strength in (males); increased incidence of hunched posture (females); and significantly reduced hindlimb splay in females.

Statistically significant increases in body tremors and statistically significant decreases in rearing counts were also noted in females at 25 mg/kg; however, due to a lack of corroborating neurobehavioral findings in females at 25 mg/kg, body tremors and decreased rearing counts were not considered biologically significant findings at this dose level.

For the most part, results were similar to control on Days 8 and 15. On Day 8 at 250 mg/kg, fewer females were observed to groom in the arena and more males were recorded as walking on toes. Decreased incidence of grooming behavior in females is consistent with treatment related effects noted in a previous acute neurotoxicity study conducted with ethiprole (Study Number RPD 558/982938). These effects are considered to be treatment related.

On Day 8, forelimb grip strength was slightly but statistically significantly lower for 250 mg/kg bw females compared with controls. There were no similar differences among males and no differences in hindlimb grip strength for either sex. Although the mean forelimb grip strength value for females was lower relative to controls on Day 8, the group mean of 0.83 kg was higher than the value recorded in the pre-dose phase (0.70 kg). The observation at Day 8, therefore, was not considered to be attributable to treatment.

Also on Day 8, landing foot splay for 250 mg/kg bw males was statistically significantly increased compared with controls. Examining the pattern of mean splay values over the course of the study, the mean values for controls on Day 8, (for both males and females), were the lowest that were seen in these groups following the commencement of treatment. Furthermore, comparing the values recorded on Day 15 with those recorded on Day 8, it can be seen that the treated groups are tending to show fairly stable mean values across this period. The control values, however, are changing between Days 8 and 15. Furthermore, significant decreases, as opposed to increases in landing foot splay, are more consistent with effects noted following acute treatment of ethiprole. Significant decreases in landing foot splay were observed in females in this study, 4 hours post-dose, and in both sexes during the previous acute neurotoxicity study conducted with ethiprole. Considering these points, the observation at Day 8 was not considered to be attributable to treatment.

At 10 and 25 mg/kg bw, no treatment-related effects were noted.

TABLE IIA 5.7.1.1-2. Functional Observation Battery (FOB) Results

Parameter / Interval	Males					Females				
	Dose Level (mg/kg bw)					Dose Level (mg/kg bw)				
	0	10	25	35	250	0	10	25	35	250
HANDLING OBSERVATIONS:										
Removal from cage, handling, easy										
Pre-test	8	8	9	9	9	9	10	10	9	10
4-Hours (Day 1)	9	10	10	10	6*	10	10	9	10	8
Day8	9	10	9	10	9	9	8	10	10	10
Day 15	10	10	10	10	10	10	10	10	10	10
OPEN FIELD OBSERVATIONS										
Tremors										
Pre-test	0	0	0	0	0	0	0	1	0	0
4-Hours (Day 1)	4	5	3	3	7	1	0	5*	4*	3*
Day 8	2	2	1	1	0	0	0	1	0	0
Day 15	3	2	3	2	3	0	0	0	1	0
Grooming										
Pre-test	3	3	4	2	0	2	1	2	1	5
4-Hours (Day 1)	7	4	4	3	2*	6	6	4	3	2*
Day 8	1	3	2	1	0	6	3	2	2	1*
Day 15	3	1	1	1	0	6	2	0	5	2
Arousal, alert										
Pre-test	10	9	9	9	9	9	10	10	10	10
4-Hours (Day 1)	5	2	4	4	4	9	9	8	5*	5*
Day 8	7	6	8	9	7	9	10	8	9	8
Day 15	9	7	7	10	8	10	10	10	9	10
Gait, walking on toes										
Pre-test	1	3	3	3	2	5	5	9	7	8
4-Hours (Day 1)	3	1	1	1	1	7	8	4	5	4
Day 8	2	1	5	4	5*	9	9	9	9	10
Day 15	6	4	2	9	4	9	9	9	8	8
Gait, unable to assess										
Pre-test	0	1	1	1	1	0	0	0	0	0
4-Hours (Day 1)	6	8	8	6	7	3	1	2	4	6*
Day 8	3	5	2	1	3	0	0	1	1	0
Day 15	1	3	5	0	2	0	0	0	1	0
Closure of eyes										
Pre-test	0	0	0	0	0	0	0	0	0	0
4-Hours (Day 1)	8	6	7	6	5	1	1	2	4*	7**
Day 8	0	0	0	0	0	0	0	0	0	0
Day 15	0	0	0	0	0	0	0	0	0	0
Standing/sitting hunched										
Pre-test	0	0	0	0	0	0	0	0	0	0
4-Hours (Day 1)	0	1	0	0	0	0	0	0	1	3**
Day 8	0	0	0	0	0	0	0	0	0	0
Day 15	0	0	0	0	0	0	0	0	0	0

N=10 for all test groups.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

TABLE IIA 5.7.1.1-3. Additional FOB Results: Activity and Rearing Counts, Forelimb and Hindlimb Grip Strength (kg), and Splay Values (cm)

Parameter	Dose Level (mg/kg bw)				
	0	10	25	35	250
			Males		
Activity counts					
Pre-dose	19±10.2	13±7.3	17±9.2	16±9.3	16±10.7
4-Hours (Day 1)	9±10.9	5±8.5	4±3.0	6±5.7	4±2.6
Day 8	14±11.3	10±9.2	13±9.0	15±9.2	15±10.5
Day 15	20±11.9	12±9.3	12±11.7	18±9.5	15±9.2
Rearing counts					
Pre-dose	7±5.3	5±4.8	6±4.2	7±4.6	7±4.3
4-Hours (Day 1)	4±6.2	2±3.7	2±1.7	3±4.1	2±1.5
Day 8	6±7.0	5±5.3	6±5.5	7±4.4	7±5.5
Day 15	9±5.6	7±5.9	7±7.5	9±5.9	6±5.5
Forelimb grip strength (kg)					
Pre-dose	0.72±0.151	0.75±0.133	0.74±0.100	0.70±0.177	0.77±0.145
4-Hours (Day 1)	0.87±0.156	0.90±0.135	0.87±0.113	0.91±0.150	1.02*±0.171
Day 8	0.98±0.197	1.06±0.159	1.01±0.195	1.02±0.197	1.07±0.162
Day 15	1.08±0.227	1.19±0.155	1.08±0.164	1.16±0.207	1.23±0.112
grip strength (kg)					
Pre-dose	0.60±0.147	0.62±0.106	0.66±0.112	0.61±0.095	0.62±0.083
4-Hours (Day 1)	0.61±0.131	0.62±0.116	0.69±0.118	0.69±0.107	0.71*±0.071
Day 8	0.75±0.283	0.78±0.104	0.79±0.113	0.68±0.181	0.76±0.129
Day 15	0.74±0.224	0.81±0.135	0.82±0.144	0.71±0.195	0.87±0.148
Splay values (cm)					
Pre-dose	10.1±2.75	10.6±1.89	9.8±1.96	10.1±2.07	10.4±2.16
4-Hours (Day 1)	10.2±2.17	9.5±2.34	8.9±1.24	8.6±1.73	8.7±1.94
Day8	9.6±2.11	9.6±2.36	10.8±1.58	10.7±1.80	11.8*±1.67
Day 15	11.5±2.51	10.6±2.22	10.2±1.77	10.9±2.11	11.8±1.82
Temperature (°C)					
Pre-dose	38.3±.023	38.2±.033	38.0*±0.33	38.0*±0.33	38.0±0.52
4-Hours (Day 1)	36.8±0.65	36.8±0.65	36.5±0.40	36.6±.0.33	36.6±0.32
Day 8	38.5±0.63	38.5±0.48	38.3±0.31	38.4±0.37	38.3±0.48
Day 15	38.0±0.89	38.3±0.41	38.1±0.40	38.4±0.33	38.2±0.46

(Table continued; footnotes to follow)

TABLE IIA 5.7.1.1-3. Additional FOB Results: Activity and Rearing Counts, Forelimb and Hindlimb Grip Strength (kg), and Splay Values (cm)(*cont.*)

Parameter	Dose Level (mg/kg bw)				
	0	10	25	35	250
Females					
Activity counts					
Pre-dose	19±10.3	28*±8.5	30**±6.4	26±7.9	27±8.7
4-Hours (Day 1)	16±11.0	16±8.4	10±7.4	12±10.6	6*±5.2
Day 8	32±10.8	31±9.5	31±14.7	28±10.1	34±9.4
Day 15	27±14.2	29±12.1	27±13.0	22±11.7	25±10.7
Rearing counts					
Pre-dose	10±5.2	14±5.8	12±2.3	12±4.2	12±6.1
4-Hours (Day 1)	7±3.8	7±4.9	2**±2.2	4±3.9	2**±2.5
Day 8	16±4.3	13±4.2	14±7.8	13±6.5	17±5.6
Day 15	12±7.9	12±5.4	10±5.7	12±6.3	13±8.5
Forelimb grip strength (kg)					
Pre-dose	0.73±0.136	0.64±0.133	0.74±0.090	0.73±0.094	0.70±0.120
4-Hours (Day 1)	0.84±0.125	0.79±0.113	0.84±0.096	0.89±0.089	0.91±0.085
Day 8	0.94±0.111	0.86±0.107	0.94±0.088	0.91±0.148	0.83*±0.105
Day 15	1.02±0.150	0.86±0.124	1.01±0.115	0.97±0.155	0.96±0.116
grip strength (kg)					
Pre-dose	0.53±0.087	0.48±0.096	0.52±0.073	0.54±0.078	0.52±0.101
4-Hours (Day 1)	0.58±0.133	0.48±0.094	0.52±0.064	0.55±0.113	0.57±0.087
Day 8	0.58±0.167	0.49±0.123	0.54±0.111	0.61±0.123	0.50±0.125
Day 15	0.63±0.181	0.51±0.114	0.57±0.112	0.66±0.105	0.55±0.168
Splay values (cm)					
Pre-dose	7.8±1.73	7.4±1.93	9.0±1.62	9.0±2.12	8.4±2.42
4-Hours (Day 1)	8.4±1.59	7.7±1.70	8.0±0.90	8.6±1.61	6.3**±1.22
Day8	8.0±1.08	8.5±1.62	9.5±1.46	9.8±1.09	9.6±1.73
Day 15	8.9±1.38	8.6±1.75	9.6±1.26	9.0±1.23	9.1±2.30
Temperature (°C)					
Pre-dose	37.9±0.45	38.2±0.21	38.0±0.18	38.2±0.33	38.0±0.45
4-Hours (Day 1)	37.0±0.58	37.2±0.52	37.0±0.63	37.2±0.74	36.8±0.51
Day 8	38.5±0.56	38.4±0.30	38.4±0.43	38.4±0.32	38.5±0.36
Day 15	38.3±0.40	38.6±0.43	38.4±0.50	38.3±0.37	38.4±0.67

N=10 for all test groups.
* Statistically different (p<.005) from the control.
** Statistically different (p<0.01) from the control.

2. **Locomotor activity**: Four hours after treatment there was a statistically significant reduction in the total amount of locomotor activity for females treated at 250 mg/kg bw (table IIA 5.7.1.1-4). There were no clear differences among males at this dosage. Examining activity over ten minute blocks, however, indicated that there was a clear reduction in activity during the first 10 minutes among males treated at 250 mg/kg bw compared with controls.

   On Day 8 and 15, there were no statistically significant differences in mean locomotor activity in any dose group.

   At 10 and 25 mg/kg, no treatment-related effects were noted at any time point.

TABLE IIA 5.7.1.1-4. Locomotor Activity[a]

Test Day	Dose Level (mg/kg bw)				
	0	10	25	35	250
Males					
Total at Pre-dose	607±252.1	658±254.8	506±159.9	600±130.3	660±278.4
4-Hours (Day 1) - 10 minutes	168	146	171	149	119*
4-Hours (Day 1) -20 minutes	22	34	48	42	19
4-Hours (Day 1) -30 minutes	0	8	14	16	16
4-Hours (Day 1) -40 minutes	2	19	14	5	4
4-Hours (Day 1) -50 minutes	7	3	1	11	5
4-Hours (Day 1) -60 minutes	6	2	4	15	11
Total at 4-Hours (Day 1)	205±81.2	211±116.5	251±95.1	238±88.2	173±128.7
Total at Day 8	495±159.0	477±172.5	490±170.0	519±295.0	668±243.4
Total at Day 15	554±132.1	599±218.8	493±211.2	567±230.8	685±281.9
Females					
Total at Pre-dose	695±289.2	641±287.8	736±259.1	721±288.8	740±301.5
4-Hours (Day 1) - 10 minutes	181	185	169	146	68**
4-Hours (Day 1) -20 minutes	99	76	77	74	21**
4-Hours (Day 1) -30 minutes	36	35	35	20	28
4-Hours (Day 1) -40 minutes	8	28	35	4	18
4-Hours (Day 1) -50 minutes	16	22	15	11	15
4-Hours (Day 1) -60 minutes	20	53	19	7	11
Total at 4-Hours (Day 1)	360±130.5	398±136.4	350±148.4	261±108.5	160**±104.1
Total at Day 8	429±118.8	510±167.0	449±158.5	484±181.0	497±178.9
Total at Day 15	397±159.2	566±257.5	442±180.9	403±115.6	517±220.8

a Totals are the mean ± SD for large movements (in secs) during the 1 hour observation session at the indicated test day. Values in *italics* are the activity for each 10 minute block during the 1 hour session conducted at the 4-hour (Day 1) post-dosing observation interval. Activity over the 10 minute block sessions are shown only for this Test Day to highlight effects on the high-dose males.

* Statistically different ($p<0.05$) from the control.

** Statistically different ($p<0.01$) from the control.

E. <u>SACRIFICE AND PATHOLOGY</u>: Not performed.

III. CONCLUSIONS: The oral (gavage) administration of ethiprole to rats at 35 and 250 mg/kg bw produced behavioral changes primarily on the day of dosing. At 35 mg/kg bw, changes were observed in females only, and included decreased rearing and increased incidence of body tremor, indications of a lower level of arousal and a higher incidence of closure of the eyes. At 250 mg/kg bw, 4 hours after dosing, observations included the following: a higher incidence of animals being slightly awkward to handle (males); a decrease in the number of animals grooming (both sexes); a lower level of arousal (females); a higher incidence of animals for which no assessment of gait was possible (females); a higher incidence of closure of the eyes (females); significantly reduced activity and rearing counts (females); increased incidence of body tremor (females); increased forelimb and hindlimb grip strength in (males); and significantly reduced hindlimb splay in females. Quantitative assessment indicated that locomotor activity was decreased as well at 250 mg/kg bw in both sexes.

On Day 8 at 250 mg/kg bw, fewer females were observed to groom in the arena and more males were recorded as walking on toes. No effects were noted in Day 15.

Based on the effects seen in this study, the NOAEL in males is 35 mg/kg bw. The LOAEL is 250 mg/kg bw in males based on decreased locomotor activity, increased incidence of being awkward to handle, and increased fore- and hindlimb grip strength. The NOAEL in females is 25 mg/kg bw. The LOAEL in females is 35 mg/kg bw based on decreased rearing counts, increased incidence of body tremors, indications of a lower level of arousal, and a higher incidence of closure of the eyes.

KIIA 5.7.4 - Subchronic neurotoxicity - rat - 90-day

1.	Annex point(s)	KIIA 5.7.4; Neurotoxicity/Subchronic neurotoxicity - rat - 90-day: **MRID 47622823**
2.	Location in dossier	KIIA 5.7.4 /01
3.	Authors (year)	Palmer, H. A. (2001)
	Title	RPA107382 - 13 week neurotoxicity study in rats by dietary administration
	Company, report No.	Bayer CropScience AG, Report-No.: C016595 (M-202708-01-1)
	Date	12.07.2001
4.	Testing facility	Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole (radioactive), ethiprole Substance(s): ethiprole TC ethiprole (radioactive) TC
7.	Test method	USEPA (=EPA): OPPTS 870.6200
	Deviations	
8.	GLP	yes

Executive Summary:

In this assessment of the potential neurotoxicity of ethiprole (batch CDR 9706, 93% purity), on male and female rats, 3 groups of 10 male and 10 female Crl:CD BR rats were given ethiprole by dietary administration at levels of 20, 100, and 400 ppm for 13 weeks. A similar sized group were given the vehicle and acted as controls. All of these animals were subjected to a functional observational battery prior to treatment and after 4, 8 and 13 weeks of treatment. Motor activity of each animal was also quantitatively assessed at the same intervals. Throughout the study, clinical signs, body weights and food consumption were monitored.

At the end of the treatment period, all animals were killed and the tissues fixed by whole body perfusion. Examination was confined to designated tissues of the nervous system which were subsequently examined microscopically in five males and five females / group in controls and high dose animals.

At 400 ppm, findings were limited to increased liver and thyroid weights among males and females. There were no other signs of toxicity or of neurobehavioural changes. At 100 ppm, findings were limited to increased thyroid weights among males. There were no signs of toxicity at 20 ppm. There were no effects of treatment on the behavioural screening tests nor was there any evidence of neuropathology.

In terms of systemic toxicity, the No Observed Effect Level was 20 ppm for males (1.4 mg/kg/day) based on increased thyroid weight at 100 ppm, and 100 ppm for females (8.4 mg/kg/day) based on increased thyroid and liver weight at 400 ppm.

The No Observed Effect Level for neurotoxicity (both behavioural changes and neuropathology) was considered to be 400 ppm for both sexes (28.7 and 33.0 mg/kg/day in males and females, respectively).

The study is classified as **acceptable/guideline** and satisfies the Guideline requirements (870.6200b) for a subchronic neurotoxicity study in the rat.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	93%
Compound Stability:	Stored at 4°C in the dark.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. Control animals received untreated diet.

3. **Test Animals:**

Species:	Rats	
Strain:	Crl:CD BR	
Age/weight at dosing:	42-49 days/ 182 - 197 g males, 133 - 152 g for females	
Source:	Charles River Breeding Laboratories, Manston Road, Margate, Kent, England	
Housing:	Individually in suspended cages with wire mesh floors	
Diet:	Ground SDS Rat and Mouse No. 1 maintenance diet, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	21 ± 0.5°C
	Humidity:	54 ± 10%
	Air changes:	Filtered, not recirculated.
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	5 days (for first group of rats dosed, see Study Design for details).	

B. STUDY DESIGN:

1. **In-Life Dates:** January 18, 1999 to April 23, 1999

2. **Animal Assignment and Treatment**: Animals were randomly assigned to the test groups noted in Table 5.7.4-1, stratified by body weight, in such a way that the initial group means were approximately equal. The test substance was administered in the diet at dose levels of 20, 100, or 400 ppm for 13 weeks. Control animals received untreated diet. All other groups received the appropriate dietary concentrations at a constant (ppm) level. Diets were available *ad libitum* throughout the study period. Dose levels were chosen based on information from the Sponsor. Further details were not included in the study report.

   In order to facilitate the neurobehavioral screening, the initiation of treatment was staggered. Animals were assigned in batches to the control and treatment groups (total of 4 batches, 5 rats/sex/dose level in each batch). An additional acclimation period of 7 days was allowed between allocations of animals to these batches. On the initiation of treatment for each batch, dosing was balanced across the groups.

   All animals were subjected to a functional observational battery prior to treatment and after 4, 8 and 13 weeks of treatment. Motor activity of each animal was also quantitatively assessed at the same intervals. Throughout the study, clinical signs, bodyweights and food consumption were monitored. At the end of the treatment period, all animals were killed and the tissues fixed by whole body perfusion. Examination was confined to designated tissues of the nervous system

which were subsequently examined microscopically in the control and high-dose groups (5/sex/dose level).

TABLE 5.7.4-1. Study Design

Experimental Parameter	Dose Group ppm (mg/kg bw/day)			
	0	20	100	400
Total number of Animals/sex/group				
Behavioral Testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	---	---	5/sex

3. **Test Substance Preparation and Analysis:** Test diets were prepared at Weeks 1 and 12 by mixing appropriate amounts of test substance with ground SDS Rat and Mouse No. 1. Animals were fed fresh diet on a weekly basis.

   Prior to the start of the study, the homogeneity and stability of the test substance in diet was verified using a 20 ppm diet. To assess homogeneity, aliquots taken from the top, middle, and bottom of the dietary admixture were analyzed. To assess stability, samples were analyzed after 0, 8, and 22 days of storage at ambient temperatures. During the study, samples of each freshly prepared diet were analyzed to check the accuracy of the preparations.

   Results: **Homogeneity Analysis:** Results for homogeneity checks were within 93-100% of the nominal concentration.
   Stability Analysis: After 22 days of storage at ambient temperatures, the 20 ppm diet was found to be 97% of nominal, indicating that the ethiprole in the diet was stable for the intervals and conditions used in this study
   Concentration Analysis: Results for the concentration checks were within 94-106% of the nominal concentrations.

4. **Statistics:** All statistical analyses were carried out separately for males and females. For all parameters, the analyses were carried out using the individual animal as the basic experimental unit. Food consumption data was analyzed on weekly basis, and body weight data was analyzed using weight gains.

   The following sequence of statistical tests was used for food consumption, bodyweight, organ weight and brain parameters: Bartlett's test was applied to test for heterogeneity of variance between treatments. As no significant heterogeneity was detected, a one-way analysis of variance was carried out. Except for pre-dose data, analyses of variance were followed by Student's t-test and Williams' test for a dose-related response, although only the one thought most appropriate for the response pattern observed was reported.

   The following behavioral data were subjected to statistical analysis: rearing and activity counts, grip strength, hindlimb splay, body weight, temperature, and Coulbourn activity data. This data was analyzed using a one-way analysis of variance followed by Williams' test for a dose-related response. Pre-dose data were analyzed by analysis of variance followed by Student's t-test.

   The categorical data were handled in the following manner: Where the recorded observations suggested a possible difference between controls and treated groups, the data were analyzed using the Jonckheere-Terpstra test.

C. METHODS / OBSERVATIONS:

1. **Mortality and Clinical Observations:** Individual animals were observed at least once daily for any signs of behavioral changes, reaction to treatment or ill health.

Further checks were made early in each working day and again in the afternoon to look for dead or moribund animals.

2. **Body Weight:** The weight of each rat was recorded at the time of allocation of animals to groups, on Week -1, on the day of commencement of treatment and once a week thereafter.

Bodyweights were also recorded on each occasion that the functional observational battery was performed.

3. **Food Consumption and Compound Intake:** The quantity of food consumed by each rat was recorded on a weekly basis. Food intake per rat (g/rat/week) was calculated using the amount of food given to and left by each rat each week.

Food intake per rat was calculated using the amount of food given to and left by each rat during each week using the following formula:

$$\text{Food consumption (g/ rat/ week)} = \frac{\text{Total food given - Total food left}}{\text{Number of animal days*}} \times 7$$

* The term "animal day' counts one animal day for each animal alive for a whole day

Efficiency of food utilization (food conversion ratios) was calculated, where possible, on a weekly basis from the bodyweight and food consumption data as weight of food consumed per unit gain in bodyweight. The following formula was used:

$$\text{Food conversion ratio} = \frac{\text{Food consumed}}{\text{Body weight gain}}$$

At weekly intervals the group mean achieved intake of test substance (mg/kg/day) was calculated from the group mean bodyweight and food consumption and the dietary inclusion levels of the test material. The following formula was used:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{Food consumed (g/rat/week) x ppm test substance}}{\text{Mid-week bodyweight x 7}}$$

4. **Neurobehavioral Assessment:** The functional observational battery and motor activity were performed at approximately the same time of day, before initiation of treatment, and during Weeks 4, 8, and 13. Not all rats were tested in one day, but time of testing was balanced across the groups.

a. **Functional Observational Battery (FOB):** This battery comprised 4 sets of observations. The first set of observations was performed while the animal was in its home cage. The second set of observations was performed when initially handling the animal. The third set of observations was performed in a test arena and the fourth set comprised handling/specific testing of the animal. All these observations were made with the observer blind to the treatment condition of the animal. The checked (X) parameters were examined.

X	**HOME CAGE OBSERVATIONS:**	X	**OBSERVATIONS IN THE ARENA:**
X	Posture in the cage	X	Occurrence of convulsions, tremors, twitches
X	Presence of convulsions, tremors, twitches	X	Level of activity in arena
X	Presence of spontaneous vocalizations	X	Level of arousal
X	Palpebral closure.	X	Rearing count
		X	Grooming
X	**OBSERVATIONS IN THE HAND:**	X	Assessment of gait
		X	Record presence of fecal boluses, urine
X	Ease of removing rat from cage	X	Piloerection
X	Ease of handling rat		
X	Salivation/lacrimation	X	**MANIPULATIONS:**
X	Palpebral closure		
X	Exophthalmus	X	Approach response
X	Piloerection	X	Touch response
X	Vocalization on handling	X	Startle response
X	Occurrence of convulsions, tremors, twitches	X	Righting reflex
		X	Tail pinch response
		X	Pupil response
		X	Grip strength: fore and hindlimb
		X	Landing foot splay
		X	Body temperature
		X	Bodyweight

b. **Locomotor Activity:** Motor activity was evaluated at the same intervals and at the same time of day that the FOB observations were made. Motor activity was monitored using a Coulboum Infra-Red Activity Monitoring System. This system uses an infra-red detector to monitor activity. The following categories of activity were recorded: the time spent in no movement, locomotor, and non-locomotor activity. The number of occurrences (events) of each category was also recorded. For reporting this data, only the locomotor activity was presented.

For testing, designated animals were placed singly into observation cages. The placement of an animal into a given cage was balanced as much as possible across groups. Once all animals had been placed into the cages, the test session program was started. The test session for each animal was 1 hour.

5. **Sacrifice and Pathology:** On completion of 13 weeks of treatment, all rats were killed. All animals were perfused and tissue samples were taken, but neuropathological examination was restricted to 5 males and 5 females from the control and high dose groups.

The animals were given an anesthetic overdose of sodium pentobarbital (i.p.) and perfused *in situ* with heparinized flushing agent followed by a 1.5% glutaraldehyde: 4% paraformaldehyde solution. After perfusion, the cranial vault was removed and the brain removed, weighed and measured as detailed below. The eyes and optic nerves were removed. The skin was removed from the dorsal region and hindlimbs, and the sciatic and tibial nerves were exposed. The spinal column and cord were fixed *in toto* integral with the carcass.

The brain was transected from the spinal cord above the first cervical spinal nerve, and the olfactory lobes were removed. The length was measured between the rostral part of the cerebral hemispheres to the most caudal part of the cerebellum, and the width between the widest parts of the cerebral hemispheres.

Following immersion fixation, peripheral nerve samples were taken and processed for epon/toluidine blue sections and tissues for paraffin wax/H & E sections were also taken. Both cross and longitudinal sections were evaluated for spinal cord, sciatic and tibial nerve tissues.

For light microscopic examination, brains, spinal cords, ganglia, and dorsal and ventral root fibers of the perfused animals were processed for paraffin wax embedding, sectioned at approximately 4-5 microns and stained with haematoxylin and eosin. Peripheral nerves from the right side were embedded in epon, sectioned at approximately 2 microns, and stained with toluidine blue.

The checked (X) tissues were evaluated. Lesions were graded as to severity, where possible, into 4 categories (trace, minimal, moderate or marked). For axonal degeneration, minimal was defined as one to four fibers affected.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain	X	Mid-thigh
	Center of cerebrum	X	Sciatic Notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons		Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian Ganglion	X	Cervical dorsal root fibers
	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve	X	Skeletal muscle
X	Eyes		
X	Gastrocnemius muscle		

6. **Positive Controls:** A positive control study was submitted to the PMRA to validate the ability of the laboratory to conduct FOB measurements.

Study Title: Further Validation of Neurotoxicity Procedures Following Oral Gavage Administration of D-Amphetamine or Diisopropyl Fluorophosphateto CD Rats to Meet EPA FIFRA Requirements. Huntingdon Life Sciences Ltd. Eye, Suffolk, England. Study conducted: December 1997.

Summary:
Two groups of female CD rats, (35 days old, 142-180 g) each comprising 15 animals, received diisopropyl fluorophosphate (DFP) by gavage, at a single dosage of 2 or 3 mg/kg. A third group of 15 females received D-amphetamine by gavage, at a single dosage of 2 mg/kg and a similarly constituted Control group received the vehicle (purified water).
Functional observational battery (FOB) assessments were performed on all animals before treatment, approximately one hour after treatment (time of peak effect) and one day after treatment. Grip strength recordings were repeated approximately 12 days after treatment. Five members of staff were either trained or re-assessed during the course of this study - each person testing the same 12 animals (three from each of the four groups) on each occasion, although the same animals were not necessarily examined by the same person for each FOB test. All FOB testing was performed "blind" to treatment.

One hour after treatment, animals which received DFP at 3 mg/kg generally showed gait abnormalities, chewing mouth movements, decreased reactivity, reduced rearing in the standard arena, reduced body temperature, and reduced activity during the one hour of automated activity recording.

Effects in animals which received DFP at 2 mg/kg were limited to decreased motor activity scores and the observation of chewing mouth movements in two individuals. In contrast, animals which received D-amphetamine showed increased levels of reactivity and arousal, increased activity and rearing counts in the standard arena, and markedly increased motor activity scores during the automated activity recording period. At 24 hours after treatment, animals which had received D-amphetamine still showed some evidence of increased reactivity to handling and motor activity scores were still slightly higher than those of control animals. Animals which had received DFP at 3 mg/kg showed slightly decreased reactivity and, in contrast to the finding on the day of treatment, motor activity scores were increased when compared with controls. Grip strength recordings showed no treatment-related effects, however some inter-observer variation was noted.

Conclusions:
The treatment related findings noted above would be anticipated following treatment with D-amphetamine or DFP. It should be noted that this study was conducted in a different laboratory than that utilized for assessing the neurotoxicity of ethiprole, although it is a branch of the original laboratory. Also, no neuropathology control data were provided. All FOB measurements were consistent with the parameters examined in both the acute and subchronic neurotoxicity studies with ethiprole. Individual animal data was provided and the study illustrated inter-observer reliability. The above described study illustrates the laboratory's successful ability to conduct FOB measurements in a neurotoxicity study.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs:** There were no clinical signs of reaction to treatment.

2. **Mortality:** There were no mortalities during this study.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** Body weight gains among males treated at 400 ppm were slightly lower than controls; differences failed to attain statistical significance (table 5.7.4-2). Gains for females were comparable across all groups.

TABLE IIA 5.7.4-2. Body Weight and Body Weight Gain [a]

Interval	Males				Females			
	Dose Level (ppm in diet)				Dose Level (ppm in diet)			
	0	20	100	400	0	20	100	400
Body Weights (g)								
Week 0	249	245	246	249	168	168	170	170
Week 1	306	298	301	301	184	190	191	187
Week 4	417	403	404	405	229	239	236	232
Week 8	514	489	505	502	260	276	271	266
Week 13	586	570	574	572	282	302	299	286
Body Weight Gains (g)								
Weeks 0-13 [b]	337	325 [96]	329 [98]	322 [96]	115	134 [117]	129 [110]	117 [102]

a N=10 for all dose groups.
b Values in brackets are the % of control for overall weight gains.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food Consumption:** During the first week of treatment, food intake of males treated at 400 ppm was slightly but statistically significantly lower than controls (table 5.7.4-3). Thereafter, the cumulative food intakes were generally comparable among the groups. There were no similar

effects among females. The efficiency of food utilization was comparable among treated and control groups.

TABLE IIA 5.7.4-3. Mean Food Consumption (g/rat/week)

Interval	Males				Females			
	Dose Level (ppm in diet)				Dose Level (ppm in diet)			
	0	20	100	400	0	20	100	400
Week 1	230	218	219	213*	137	147	142	134
Week 4	230	218	217	212	143	152	151	145
Week 8	219	211	220	220	134	148	144	139
Week 13	211	206	207	218	138	142	135	128
Cumulative Intake:								
Weeks 1-13	2932	2790	2854	2827	1836	1943	1894	1814
% of Control	-	95	97	96	-	106	103	99

TABLE IIA 5.7.4-4. Mean Achieved Dietary Intake of Ethiprole (Weeks 1-13)

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	
		Males	Females
Control	0	0	0
Low	20	1.4	1.7
Mid	100	7.2	8.4
High	400	28.7	33.0

D. **NEUROBEHAVIORAL RESULTS:** No treatment-related effects were noted at any dose level or in either sex at the FOB or during the locomotor assessments.

1. **FOB Findings:** During the course of the study, there were two instances when statistically significant differences in the incidences of observations occurred (table 5.7.4-5). At Week 8, there was a higher incidence of males at 400 ppm for which gait could not be assessed. At Week 13, there were more females at 400 ppm which were observed to groom in the arena compared with controls. There was no consistent pattern to these observations which were therefore considered to be coincidental.

   There were no statistically significant differences in activity counts, rearing counts, fore- or hindlimb grip strength, footsplay values, body temperature, or body weights between the treated and control groups at any time point during this study.

TABLE IIA 5.7.4-5. Functional Observation Battery (FOB) Results

Parameter	Interval	Males				Females			
		Dose Level (ppm in diet)				Dose Level (ppm in diet)			
		0	20	100	400	0	20	100	400
OPEN FIELD OBSERVATIONS									
Grooming									
	Pre-dose	2	1	1	2	1	2	2	0
	Week 4	0	1	1	1	2	4	2	3
	Week 8	1	1	1	1	1	1	3	3
	Week 13	0	2	0	1	0	2	3	5*
Gait, unable to assess									
	Pre-dose	2	0	2	1	1	0	0	0
	Week 4	1	2	2	4	0	0	0	0
	Week 8	0	2	1	4*	0	0	0	0
	Week 13	2	1	3	4	0	0	0	0

N=10 for all test groups.
* Statistically different ($p<0.05$) from the control.

2. **Motor Activity:** There were no statistically significant differences in Coulbourn activity measurements between treated and control groups (table 5.7.4-6).

TABLE IIA 5.7.4-6. Locomotor Activity [a]

Interval	Dose Level (ppm in diet)			
	0	20	100	400
		Males		
Pre-dose	637	656	557	610
Week 4	863	1015	905	914
Week 8	894	924	866	851
Week 13	759	686	692	811
		Females		
Pre-dose	534	580	550	682
Week 4	896	917	1058	1143
Week 8	817	767	890	934
Week 13	775	743	937	866

a N=10 for all test groups. Values are total mean for large movements (in secs) during the 1 hour observation session at the indicated test interval.

E. SACRIFICE AND PATHOLOGY:

1. **Brain and Organ Weights:** Brain weights and measurements were generally comparable among treated and control groups.

At 20 ppm, the absolute thyroid weights of males were increased but not statistically significantly (table 5.7.4-7). At 100 ppm, absolute thyroid weights in males were increased (↑24%). For the high dose group, absolute thyroid weights were increased in both sexes (↑44% in males and ↑35% in females). Absolute liver weights were also increased in both males and females at 400 ppm (↑22 and ↑65%, respectively).

TABLE IIA 5.7.4-7. Brain Parameters and Organ Weights [a]

Parameter	Males				Females			
	0	20	100	400	0	20	100	400
Terminal Body Weight (g)	585	568	574	569	286	305	299	285
Brain								
Absolute Weight (g)	1.62	1.60	1.62	1.63	1.47	1.48	1.47	1.50
Length (mm)	22.00	21.55	21.78	22.13	21.20	20.76	20.21	21.40
Width (mm)	15.39	15.19	15.30	15.21	14.61	14.64	14.52	14.56
Thyroid								
Absolute Weight (mg)	21.9	26.1	27.2* [+124]	31.6** [+144]	19.4	20.6	22.1	26.1** [+135]
Liver								
Absolute Weight (g)	17.9	17.3	18.6	21.9** [+122]	10.4	12.1	13.3	17.2** [+165]

a N=10 for all test groups. Percent of control values for absolute thyroid and liver weights are shown in brackets.
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.

2. **Neuropathology:** There were no microscopic findings in the sections of nervous tissue examined which were considered to be related to the administration of the test substance.

Incidental findings included the following. In the control group, two males and one female showed minimal axonal degeneration in one or two levels of peripheral nerve (sciatic or tibial), and one affected male also showed minimal axonal degeneration in spinal root fibers. Four males and two females in the high-dose group showed minimal axonal degeneration in one or two levels of peripheral nerve (sciatic and/or tibial), and one additional female showed minimal axonal degeneration in spinal root fibers. One affected high-dose male also showed minimal axonal degeneration in the cervical spinal cord.

All incidental findings were within the range of historical controls values, except minimal degeneration in the ventral root fibers in one high dose female and one control male, as well as degeneration in the tibial nerve at the calf branch of two high dose males. No other findings of axonal degeneration were noted in any other tissue location in these two high dose males. Furthermore, the isolated occurrence of axonal degeneration in individual animals in this study, including controls, as well as a lack of any pattern of axonal degeneration within the individual animals or the high dose group indicate that these are not treatment related findings. Historical control ranges were compiled from counts of minimal axonal degeneration from ten different 13-week neurotoxicity studies conducted between June 1998 and November 2003, at Huntingdon Life Sciences.

TABLE IIA 5.7.4-8. Microscopic Neuropathology [a]

Parameter	*Males*		*Females*	
	Dose Level (ppm in diet)		Dose Level (ppm in diet)	
	0	400	0	400
Spinal Cord (C3-6)	0	1	0	0
Dorsal Root Fibres (L)	0	0	0	1
Ventral Root Fibres (L)	1	0	0	1
Sciatic Nerve (Sciatic Notch)	1	2	0	1
Sciatic Nerve (Mid-Thigh)	1	0	1	0
Tibial Nerve (Calf Branch)	0	2	0	0
Tibial Nerve (at knee)	1	1	0	1

a Values indicate the number of animals observed with minimal axonal degeneration in a particular neurological tissue type.

TABLE IIA 5.7.4-9. Historical Control Range in percent for Microscopic Neuropathology

Parameter	Males	Females
Spinal Cord (C3-6)	0-40%	0-40%
Dorsal Root Fibres (L)	0-20%	0-20%
Ventral Root Fibres (L)	0%	0%
Sciatic Nerve (Sciatic Notch)	0-40%	0-20%
Sciatic Nerve (Mid-Thigh)	0-40%	0-60%
Tibial Nerve (Calf Branch)	0-20%	0-40%
Tibial Nerve (at knee)	0-40%	0-20%

III. CONCLUSIONS: The oral dietary administration of ethiprole RPA 107382 for 13 weeks at dosages of 0, 20, 100, or 400 ppm included changes in organ weights. Thyroid and liver weights of males and females treated at 400 ppm and thyroid weights of males treated at 100 ppm were significantly increased. Behavioral screening tests and neuropathological evaluations showed no findings attributable to treatment.

In terms of indices of systemic toxicity, the NOAEL was 20 ppm in males (1.4 mg/kg bw/day) based on increased thyroid weight and 100 ppm in females (8.4 mg/kg bw/day) based on increased liver and thyroid weights.

No neurotoxicological effects, based on behavioral changes and neuropathology, were observed at any dose level in this study. The NOAEL for neurotoxicity was 400 ppm (28.7 mg/kg/day for males and 33.0 mg/kg/day for females.

KIIA 5.6.1 – Range-finding study for the two generation reproductive toxicity study in the rat

1.	Annex point(s)	KIIA 5.6.1; Reproductive toxicity/Two generation reproductive toxicity in the rat
2.	Location in dossier	KIIA 5.6.1 /01
3.	Authors (year)	Tyl, R. W.; Myers, C.; Marr, M. (2000)
	Title	Range-Finding Study for the Two-Generation Reproductive Toxicity Evaluation of Ethiprole (RPA107382) Administered in the Feed to CD® (Sprague-Dawley) Rats
Company, report No.		Bayer CropScience AG, Report-No.: 65C-7407-100, M-210173-01 **Aventis CropScience was original sponsor **
Date		03.03.2000
4.	Testing facility	Research Triangle Institute;
5.	Dates of work	May 11 1999 (study initiation) / July 6, 1999 (animal arrival)– October 20-1999 (lab completion)
6.	Test substance(s)	Molecule(s): ethiprole Substance(s): AE 0316423 00 1C (Batch-No.: CDR9706)
7.	Test method	USEPA (=EPA): 83-4
	Deviations	
8.	GLP	Yes

Executive summary:

Male and female CD® (Sprague-Dawley) rats (10 animals/sex/dose) were administered ethiprole in the feed at 0, 10, 50, 250 and 1000 ppm, for a total of ten weeks. Ethiprole was provided during two-week prebreed, two-week mating, three-week gestation, and three-week lactation periods. Body weights and feed consumption were recorded weekly, and clinical signs were recorded at least once daily. At necropsy of P males (after mating) and P females (after weaning), weights of reproductive organs (testes, epididymides, seminal vesicles plus coagulating glands, prostate, ovaries, uterus), liver, thyroid and kidneys were recorded. At the weaning of the offspring litters on postnatal day (PND) 21, ten weanlings/sex/group, designated the F1 generation, were retained and maintained on their dosed diets for a maximum of approximately four weeks, until the females had acquired vaginal patency and the males had acquired preputal separation. On the date of acquisition, animals were weighed, examined grossly, euthanized and discarded.

There were no treatment-related parental deaths or clinical signs. Maternal body weights were reduced at 1000 ppm during lactation, while maternal body weight gains were increased at 250 and 1000 ppm. Parental food consumption was reduced at 1000 ppm during prebreed and lactation. Mating and gestational indices were equivalent across all groups, including no effects on mating index, precoital interval, gestatioinal length, fertility index, postimplantation loss, total, livebirth, and all lactational survival indices. Stillbirth index was slightly elevated at 250 and 1000 ppm, although not these changes were not statistically significant, they were slightly above the historical range, and of unknown toxicological significance. Pup bodyweights per litter during lactation were significantly reduced at 1000 ppm on PND 7, 14 and 21. At necropsy, significant increases in absolute and relative liver and thyroid weights were observed at 1000 ppm in males and females, with increased absolute and relative liver weights also observed at 250 ppm (females only). Treatment-related gross lesions were limited to increased liver size and mottled discoloured liver for both sexes at 1000 ppm, and increased liver size in P females at 250 ppm.

F1 offspring (10/sex/group) were retained for approximately four additional weeks of dosing, following weaning, until acquisition of puberty. F1 male and female body weights and weight changes were significantly reduced at 1000 ppm during this period. Food consumption was reduced in F1 males and females at 1000 ppm. Acquisition of vaginal patency and preputial separation was significantly delayed (1.2 days for females and 3.7 days for males) at 1000 ppm. There were no effects of treatment at 10 or 50 ppm for P rats and no effects in F1 offspring at 10, 50 or 250 ppm.

Ethiprole intake during the parental prebreed period was approximately 0.75, 3.7, 19.3 and 70.0 mg/kg/day for both sexes at 10, 50, 250 and 1000 ppm, respectively. For P females during gestation, ethiprole intake was approximately 0.7, 3.6, 18.4 and 72.3 mg/kg/day, and during lactation approximately 1.8, 9.5, 43.5 and 172.6 mg/kg/day (confounded by pups self-feeding) at 10, 50, 250 and 1000 ppm, respectively. Ethiprole intake for F1 postweanling retained males and females, during the two week period (PND 21-35) was approximately 1.6-1.4, 7.5-7.3, 36.2-35.6 and 150.0-147.2 mg/kg/day at 10, 50, 250 and 1000 ppm, respectively.

The purpose of this range-finder study was to evaluate the potential toxicity of ethiprole with respect to parental fertility, maternal pregnancy and lactation, growth and development until puberty, and to set target dietary concentrations for the definitive two-generation study. Given the group sizes, observations made in the current study may not be supported by the statistical power of a full definitive study.

Parental toxicity was observed at 250 ppm (increased body weight gains, organ weight changes in thyroid and liver) and 1000 ppm (decreased body weights, increased body weight gain, decreased food consumption, organ weight changes in liver and thyroid and gross necropsy observations in the liver). The parental systemic NOAEL for this study is 50 ppm.

The offspring LOAEL is 1000 ppm based on decreases in F1 pup body weights with associated delays in acquisition of puberty. Decreases in food consumption were also observed at this dose. The offspring NOAEL is 250 ppm.

No adverse effects on reproductive performance were noted at any dose level, therefore the NOAEL is 1000 ppm.

This study is classified **acceptable/non-guideline** provides data for use in dose selection for the definitive 2-generation reproductive toxicity study in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)
   - Description: White or white/beige powder
   - Lot/Batch #: CRD9706
   - Purity: 92.7%
   - Compound Stability: The test substance was stored at room temperature.
   - CAS # of TGAI: 181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive controls were used.

3. **Test Animals:**
   - **Species:** Rat
   - **Strain:** Outbred albino CD® (Sprague-Dawley) rats (Crl:CD®[SD] BR)
   - **Age at study initiation:** Exposure of P animals began at ~9 wks of age (~11 wks at mating)
   - **Wt. at study initiation:** Exposure began when P animals were:
     (P) Males: 292.28 – 328.26 g; Females: 204.88 – 239.96 g
   - **Source:** Charles River Breeding Laboratories, Raleigh, NC
   - **Housing:** Individually housed during the quarantine period and upon the initiation of the treatment period in solid-bottom polycarbonate cages with stainless steel wire lids, with Sani-Chips® cage litter. Two per cage (1 male:1 female, same dose level) during the mating period. Females were caged separately and individually once they were successfully mated (or at the end of the mating period). Females were housed with their litters throughout the lactation period. Selected F1 weanlings, males and females, were singly housed for the postwean exposure period.
   - **Diet:** Purina Certified Ground Rodent Diet® (No. 5002, PMI Feeds, Inc., St. Louis, MO), *ad libitum*
   - **Water:** Municipal tap water, *ad libitum*
   - **Environmental conditions:**
     - **Temperature:** 18° to 26° C (target)
     - **Humidity:** 30 to 70% (target)
     - **Air changes:** not recorded
     - **Photoperiod:** 12 hrs dark/ 12 hrs light
   - **Acclimation period:** 9 days

B. PROCEDURES AND STUDY DESIGN

1. **In-Life Dates:** From July 6, 1999 to October 19, 1999

2. **Mating Procedure:** Mating for the P generation was accomplished by co-housing one female with one male from the same dose group for up to 14 consecutive days. Females were examined daily during the cohabitation period for the presence of a copulation plug or vaginal sperm. The day vaginal sperm/plug was observed was designated Gestation Day 0, and the male and female from that mating pair were individually housed. At the end of the

cohabitation period, all remaining females were placed individually polycarbonate cages in case evidence of mating was undetected.

3. **Study Schedule:** The P parental animals (10 rats/sex/dose group) were approximately 9 weeks of age at initiation of treatment and were given test diets for 2 weeks before they were mated (~11 weeks of age at mating).

   Fl litters were culled to ten pups each on Lactation Day 4 and weaned on Lactation Day 21. At weaning, at least one female and one male (whenever possible) from each F1 litter were selected and continued dosing for approximately four more weeks. Weanlings not selected for continued dosing were examined externally, euthanized and discarded. The F1 rats selected for continued dosing were monitored until sacrifice.

   At necropsy of P and Fl animals, gross lesions were recorded, and selected organs were weighed and discarded.

   Feeding of test diets to both parents and offspring (after weaning) was continuous throughout the study, and all offspring were fed the same dietary level as their corresponding parents.

4. **Animal Assignment:** P animals were assigned to the different test groups as seen in Table 1 by means of randomization stratified by body weight, such that the body weights of all groups by sex were homogeneous at study initiation.

TABLE 1. Animal Assignment

Test Group	Dose in Diet [a] (ppm)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	10	10	10	10
Low (LDT)	10	10	10	10	10
LowMid (LMT)	50	10	10	10	10
Mid (MDT)	250	10	10	10	10
High (HDT)	1000	10	10	10	10

a Diets were administered from beginning of the study until sacrifice.

The purpose of the range-finder study was to evaluate the potential of ethiprole, administered in the feed to CD® rats, to produce alterations in parental fertility, maternal pregnancy and lactation, growth and development of the offspring until approximately four weeks after weaning, and to set target dietary concentrations of ethiprole for the subsequent two-generation study. The selected dietary concentrations for the range-finder study were 10, 50, 250 and 1000 ppm.

5. **Dosage Preparation and Analysis:** Formulations were prepared at least monthly by mixing appropriate amounts of test substance with Purina certified ground rodent diet® (No. 5002) and were then stored frozen. Stability and homogeneity of the test diets at concentrations used during the range-finding study (10 and 1000 ppm) were established. Stability analyses were performed on dosed feed samples at 10 and 1000 ppm at room temperature in open containers (to simulate cageside exposures) for 0, 3-4, and 9-11 days, and on dosed feed samples stored frozen (-11 to -21°C) for 0, 9, 28, and 48 days. In addition, homogeneity analyses were performed on the 10 and 1000 ppm concentration diets (three samples each from the left, right, and center of the V-shell blender for each dietary level). During the

study, the concentration of the test material in the diets (all dose levels) was verified for the all doses and control for the first and last formulations. These analyses were performed prior to administration of the test diets to the animals.

Results: **Homogeneity Analysis:** Results for homogeneity of the 10 and 1000 ppm diets were within 89.1-104% of the nominal concentrations. (Data obtained from the study report, pages 293 and 296).

Stability Analysis: After 48 days of frozen (-11 to -21°C) storage, results for the 10 and 1000 ppm diets were 91.8-106% of the nominal concentration. After 9 days of storage at room temperature, the 10 and 1000 ppm diets were within 97.2-102% of the nominal concentrations. (Data obtained from the study report, pages 298, 299, 301, and 302).

Concentration Analysis: Results for the concentration analyses were within 90.7-111% of the nominal concentrations. (Data obtained from the study report, pages 96, 100 and 103). These data indicate that the variance between nominal and actual dosage to the study animals was acceptable with exception of one occasion, upon which the 50 ppm dose formulation was 111% of nominal concentration, and was not expected to have adversely affected the outcome of the study.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. In addition, the stability of ethiprole in the diets was verified for the intervals and conditions used in this study. The ethiprole in 10 and 1000 ppm diets was demonstrated as stable for at least 48 days under freezer conditions and at least 9 days at ambient temperatures.

C. OBSERVATIONS

1. **Parental Animals:** The following observations and the schedule for those observations for the parental rats (P generation) are summarized from the report. Observations for mortality were made twice daily (a.m. and p.m.), and the general condition of all animals was checked daily. Clinical examinations were conducted and recorded daily throughout the course of the study. These cage-side observations included, but were not limited to, changes in skin and fur, eyes, mucous membranes, respiratory and circulatory system, autonomic and central nervous system, somatomotor activity, and behavior pattern.

The body weights of the P male rats were determined and recorded initially and weekly through mating. The body weights of P female rats were recorded in the same manner until confirmation of mating. During gestation, females were weighed on Gestation Day 0, 7, 14, and 20. Dams producing litters were weighed on Lactation Day 0, 4, 7, 14, and 21, and body weight gains were computed.

Food consumption measurements were recorded weekly for all P parental animals throughout the prebreed treatment periods. During pregnancy of P females, food consumption was recorded for Gestation Days 0-7, 7-14, and 14-20. During lactation of Fl litters, maternal food consumption was measured for Lactation Day 0-4, 4-7, 7-14, and 14-21, although maternal food consumption after Lactation Day 14 was confounded by the contribution from the pups since pups were self-feeding by this time. Food consumption was not measured during the cohabitation period. Food consumption collection periods corresponded to the collection of the animals' body weight data. These data were employed to calculate their

actual exposure to the test chemical as mg ethiprole/kg body weight/day. In addition, food efficiency was calculated per animal per interval as weight gain in grams for the interval divided by food consumption in grams for the interval (x 100 to allow reporting as a percentage). Feed was changed at least weekly throughout the study.

Females were examined daily during the cohabitation period for the presence of a copulation plug and/or vaginal sperm.

2. **Litter Observations:** According to the study report, the following litter observations (X) were made (see Table 2).

TABLE 2. F_1 Litter Observations

Observation	Time of observation (lactation day)					
	Day 0	Day 4 [a]	Day 4 [b]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
External alterations [c]	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X	X

a Before standardization (culling)
b After standardization (culling)
c All pups were examined for physical abnormalities at birth and throughout the preweaning period.

Beginning on Gestation Day 20, each female was observed twice daily (a.m. and p.m.) for evidence of littering. All Fl pups were sexed and examined as soon as possible after birth (date of birth designated Lactation Day 0) to determine the number of viable and stillborn members of each litter. Thereafter, litters were evaluated for survival on 4, 7, and 14 days after birth and at weaning (Lactation Day 21).

On Lactation Day 4, the size of each litter was adjusted by eliminating extra pups by random selection to yield, as nearly as possible, a maximum of 10 pups/litter (5 pups/sex/litter). Culled pups were sacrificed by decapitation and discarded. All live pups were counted, sexed, weighed individually, and examined grossly at birth (Lactation Day 0), at 4, 7, and 14 days after birth, and at weaning (Lactation Day 21). The body weights and sexes were recorded on an individual basis, but the pups were not uniquely identified at this stage. All pups were examined for physical abnormalities at birth and throughout the preweaning period. All pups dying during lactation were necropsied, when possible, to investigate the cause of death.

3. **Postmortem Observations:**

a) **Parental Animals:** All P parental animals in all groups were subjected to a complete gross necropsy. The gross necropsy included examination of the external surfaces; all orifices; cranial cavity; carcass; external and cut surfaces of the brain and spinal cord; the thoracic, abdominal, and pelvic cavities and their viscera; and cervical tissues and organs. The following organs were weighed and discarded: ovaries, uterus with oviducts and cervix, prostate, testes, seminal vesicles, epididymides, liver, kidneys, and thyroid.

b) **Offspring:** Unselected Fl offspring were examined for gross external abnormalities, euthanized, and discarded. A complete gross internal examination was made on any pup appearing moribund or dying on test.

D. DATA ANALYSIS

1. **Statistical Analyses:** The unit of comparison was the P and F1 male, the P and F1 female, the pregnant P female, or the litter, as appropriate. Treatment groups were compared to the concurrent control group using either parametric ANOVA under the standard assumptions or robust regression method which does not assume homogeneity of variance or normality. The homogeneity of variance assumption was examined via Levene's Test, which is much more robust to the underlying distribution of the data than the traditional Bartlett's Test. If Levene's Test indicated lack of homogeneity of variance ($p<0.05$), robust regression methods were used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They were used to test for overall treatment group differences, followed by individual tests for exposed vs. control group comparisons (via Wald chi-square tests), if the overall treatment effect was significant. The presence of linear trends was analyzed by GLM procedures for homogenous data or by robust regression methods for nonhomogenous data (SAS Institute Inc.). The standard ANOVA methods, as well as Levene's Test, used are available in the GLM procedure of SAS® Release 6.12 (SAS Institute Inc., 1997), and the robust regression methods used are available in the REGRESS procedure of SUDAAN® Release 7.5.3 (Shah et al., 1997).

   If Levene's Test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 was used to evaluate the overall effect of treatment and, when a significant treatment effect was present, to compare each exposed group to control via Dunnett's Test. For the litter-derived percentage data (e.g., periodic pup survival indices), the data were arcsine square root transformed prior to analysis, since percentage data derived from litters tend to have unequal variances. The transformed data were then analyzed using ANOVA weighted by litter size in order to further stabilize the variances. A test for linear trend was also performed on the transformed data weighted by litter size. A one-tailed test (i.e., Dunnett's Test) was used for all pairwise comparisons to the vehicle control group, except that a two-tailed test was used for parental and pup body weight and organ weight parameters, food consumption, percent males per litter, and anogenital distance.

   Frequency data such as reproductive indices (e.g., mating and fertility indices) were not transformed. All indices were analyzed by Chi-Square Test for Independence for differences among treatment groups and by the Cochran-Armitage Test for Linear Trend on Proportions. When Chi-Square revealed significant ($p<0.05$) differences among groups, then a Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each treatment group and the control group. Acquisition of developmental landmarks (e.g., vaginal patency and preputial separation), as well as F2 newborn anogenital distance, were analyzed by Analysis of Covariance (ANCOVA; in addition to ANOVA analysis) using body weight at acquisition or measurement as the covariate. For correlated data (e.g., body and organ weights at necropsy of weanlings, with more than one pup/sex/litter), SUDAAN® software was used for analysis of overall significance, presence of trend, and pairwise comparisons to the control group values.

   A SAS Institute, Inc. test for statistical outliers was performed on parental body weights and food consumption (in g/day) and on adult and weanling necropsy organ weights. If

examination of pertinent study data did not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If food consumption data for a given animal for a given observational interval (e.g., study day 0-7, 7-14, 14-28, 28-35, etc., during the prebreed exposure period) were designated outliers or "unrealistic" (e.g., negative value), then summarized data encompassing this period (e.g., study day 0-70 for the prebreed exposure period) was not included in this value.

For all statistical tests, the significance limit of 0.05 (one- or two-tailed) was used as the criterion for significance.

2. Indices:

Reproductive Indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Females:

$$\text{Mating index (\%)} = \frac{\text{No. females sperm-positive}}{\text{No. females paired}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{No. females pregnant}}{\text{No. females sperm-positive}} \times 100$$

$$\text{Gestational index (\%)} = \frac{\text{Number of females with live litters}}{\text{Number of females pregnant}} \times 100$$

Males:

$$\text{Mating index (\%)} = \frac{\text{No. males impregnating females}}{\text{No. males paired}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{No . males siring litters}}{\text{No. males impregnating females}} \times 100$$

$$\text{Pregnancy index (\%)} = \frac{\text{No. pregnant females}}{\text{No. males impregnating females}} \times 100$$

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

$$\text{Live birth index (\%)} = \frac{\text{Number of live pups at birth}}{\text{Total number of pups born}} \times 100$$

$$\text{4-Day survival index (\%)} = \frac{\text{Number of pups surviving 4 days (precull)}}{\text{Total number of live pups at birth}} \times 100$$

$$\text{7-Day survival index (\%)} = \frac{\text{Number of pups surviving 7 days}}{\text{Total number of live pups at 4 days (postcull)}} \times 100$$

$$\text{14-Day survival index (\%)} = \frac{\text{Number of pups surviving 14 days}}{\text{Total number of live pups at 7 days}} \times 100$$

$$\text{21-Day survival index (\%)} = \frac{\text{Number of pups surviving 21 days}}{\text{Total number of live pups at 14 days}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{Number of pups surviving 21 days}}{\text{Total number of live pups at 4 days (postcull)}} \times 100$$

$$\text{Stillbirth index (\%)} = \frac{\text{Number of dead pups delivered}}{\text{Total number of pups delivered}} \times 100$$

3. **Historical Control Data:** Historical control data were provided from studies conducted at the laboratory between 1990 and 1998. Means and standard deviation values from individual studies were provided. Historical control data included the following: parental body weights and food consumption, female vaginal cytology data; various reproductive and lactation indexes (mating, fertility, gestation, and pregnancy indices), pre-coital intervals, gestation lengths, numbers of implantation sites per female, various offspring viability indices (live and still birth indices, percent post implantation loss per litter, survival and lactation indices), litter sizes, pup weights, percent male pups, male nipple evaluations during lactation; anogenital distances (F2 pups only), time to perusal separation or vaginal patency, sperm evaluation data, ovarian follicle counts, and selected parental and pup organ weights.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and Clinical Signs:** All P males survived until scheduled sacrifice. One female at 10 ppm was euthanized following observation of signs of dystocia on SD 40. There were no treatment- or dose-related clinical observations in either sex in the parental generation.

2. **Body Weight and Food Consumption:**

Food consumption and body weight data during the premating phase are summarized as follows:

P Generation Body Weights (table 3): Male body weight gains were significantly increased at 50 ppm for sd 0-14, and unaffected for any other time interval. Based on the transient nature of this effect, and the lack of a dose-response, it was considered to be of no toxicological consequence.

Female body weights were equivalent across all groups during the two-week premating phase.

P Generation Food Consumption expressed in g/kg/day (table 4): During the premating phase, male food consumption was significantly reduced at 1000 ppm for days 0-7 in g/day (by approximately 10%, $p<0.01$) and g/kg/day (by approximately 9%, $p<0.01$) and days 0-14 in g/kg/day (by approximately 6%, $p<0.01$) of the premating phase, relative to the controls.

Female food consumption was decreased at 1000 ppm during the premating phase during days 0-7 in g/kday and g/kg/day (by approximately 12% and 10%, respectively, $p<0.01$).

No changes in parental food consumption were noted in either sex at 10, 50 or 250 ppm.

TABLE 3. Mean Body Weight (g) and Body Weight Gains (g) [a]

Interval	Dose Group (ppm in diet)				
	0	10	50	250	1000
P Generation Males - Pre-mating					
Body Weight (g)					
Day 0	317.6	317.9	318.2	320.0	317.7
Day 7	364.6	367.8	369.1	367.7	357.4
Day 14	397.6	404.3	408.9	406.8	392.3
Body Weight Change (g)					
Days 0 to 7	47.0	49.9	51.0	47.7	39.6
Days 0 to 14	80.0	86.4	90.7*	86.8	74.6
P Generation Females - Pre-mating					
Body Weight (g)					
Day 0	224.4	221.5	221.7	221.5	221.5
Day 7	242.1	243.8	242.2	243.3	236.5
Day 14	253.1	256.2	255.0	257.5	244.4
Body Weight Change (g)					
Day 0 to 7	17.8	22.3	20.5	21.8	15.0
Day 0 to 14	28.7	34.7	33.3	35.9	22.9

a N=10 unless otherwise noted, * Statistically different from control, p<0.05, ** Statistically different from control, p<0.01, *** Statistically different from control, p<0.001.

TABLE 4. Mean Food Consumption (g/kg/day) - Pre-mating [a]

Interval	Dose Group (ppm in diet)				
	0	10	50	250	1000
P Generation Males-Pre-mating					
Days 0 to 7	77.3	76.3	77.1	76.7	70.2**
Days 0 to 14	72.7	71.3	72.4	71.8	68.1**
P Generation Females-Pre-mating					
Days 0 to 7	78.8	80.7	78.7	81.7	70.6**
Days 0 to 14	76.8	78.5	76.3	82.2	71.9

a N=10 unless otherwise noted, * Statistically different from control, p<0.05, ** Statistically different from control, p<0.01, ***Statistically different from control, p<0.001.

Food consumption and body weight data during gestation and lactation are summarized as follows:

P-Generation (table 5): No statistically significant differences from the controls were noted in the maternal gestational body weights, body weight change or food consumption at any dose level.

During the lactation phase, maternal body weights were significantly reduced at 1000 ppm on PND 4 (approximately 6%, p<0.05) compared to controls. Also at 1000 ppm, maternal body weights were significantly higher on PND 14 (approximately 1%, p<0.05) however based on the magnitude of this change, was not considered to be toxicologically relevant. Maternal body weight change was significantly increased at 250 ppm (approximately 240%, p<0.05) and 1000 ppm (approximately 331%, p<0.05) between PND 0-21.

Maternal lactational food consumption, expressed g/day, was significantly reduced at 1000 ppm between PND 7-14 (by approximately 105, $p<0.05$).

TABLE 5. Mean Body Weights (g), Body Weight Gain (g) and food consumption (g/kg/day) in dams during gestation and lactation periods

Interval	Dose Group (ppm in diet)				
	0	10	50	250	1000
P Generation-gestation					
Number of animals[d]	8	9	9	7	8
Day 0	261.5	256.0	255.9	260.0	252.3
Day 7	300.4	289.3	301.3	300.7	286.6
Day 14	334.3	321.7	333.2	336.7	315.5
Day 20	396.8	379.2	401.0	395.7	371.5
BWG[a] Days 0 to 20	135.3	123.3	145.1	135.6	119.2
FC[a] Days 0 to 20	71.0 (N=8)	70.8 (N=9)	72.6 (N=9)	73.5 (N=7)	72.3 (N=8)
P Generation-lactation					
Number of animals	10	9	8[b]	8[c]	8
Day 0	318.8	303.7	316.4	324.7	301.7
Day 4	330.8	324.3	336.7	339.2	309.7*
Day 7	330.9	311.4	340.0	349.4	315.4
Day 14	336.2	344.0	348.4	366.7	340.0*
Day 21	327.2	322.3	326.5	351.2	337.9
BWG[a] Days 0 to 21	8.4	18.6	10.1	28.6*	36.2**
FC[a] Days 0 to 21	174.4	179.3	190.0	173.5	172.7

a BWG: body weight gain, FC: food consumption
b All pups in the litter for female 66 were found dead or missing and presumed dead on PND 1
c All pups in the litter for female 56 were found dead or missing and presumed dead on or before PND 4
d Number of pregnant females found sperm and/or plug positive
* Statistically different from control, $p<0.05$, ** Statistically different from control, $p<0.01$.
*** Statistically different from control, $p<0.001$.

3. **Test Substance Intake:** Based on food consumption and body weight data, the doses expressed as mean daily mg test substance/kg body weight/day during the 2 week pre-mating period, gestation, and lactation are presented in Tables 6a and 6b.

TABLE IIA 6a. Mean Test Substance Intake During Pre-mating (Days 0-14) (mg/kg/day)

	Male				Female			
	10	50	250	1000	10	50	250	1000
P	0.71	3.62	17.96	68.10	0.79	3.82	20.55	71.92

Note: The purity of bulk ethiprole used as the test material was assumed to be 100% for diet formulation purposes.

TABLE IIA 6b.Mean Test Substance Intake During Gestation and Lactation (mg/kg/day)

	P Parental Females			
	10	50	250	1000
Gestation	0.71	3.63	18.38	72.33
Lactation	1.79	9.50	43.38	172.66

Note: The purity of bulk ethiprole used as the test material was assumed to be 100% for diet formulation purposes.

4. **Reproductive Performance:** Reproductive performance data are presented in Table 7. There were no compound-related effects on any parameter (e.g., mating, fertility, gestation or pregnancy indices, days to insemination, or gestation length) in either generation at any dietary level tested.

TABLE IIA 7. Reproductive Performance [a]

Observation	Dose Group (ppm in diet)				
	0	10	50	250	1000
P Generation – F1 Litters					
No. Females Paired	10	10	10	10	10
No. Females that Mated	10	10	10	10	9
Mating Index (Females)	100.0	100.0	100.0	100.0	90.0
No. of Pregnant Females	10	10	9	10	8
Fertility Index (Females)	100.0	100.0	90.0	100.0	88.9
No. of Females with Live Litters	10	9[a]	9	9	8
Gestational Index	100.0	100.0	100.0	90.0	100.0
No. Males Paired	10	10	10	10	10
No. Males that Mated	10	10	10	10	9
Mating Index (Males)	100.0	100.0	100.0	100.0	90.0
No. Males Siring Litters	10	10	9	10	8
Fertility Index (Males)	100.0	100.0	90.0	100.0	88.9
Pregnancy Index	100.0	100.0	90.0	100.0	88.9
Days until Sperm Positive	2.5 (N=8)	2.7 (N=9)	3.0 (N=10)	2.4 (N=7)	3.2 (N=9)
Gestational Length (days)	21.9(N=8)	22.1(N=9)	22.1 (N=9)	22.3(N=7)	22.4(N=9)

a Female 98 was euthanized moribund while in the process of delivering. She had one resorbing implant site in the right uterine horn and the left uterine horn was twisted upon itself blocking delivery of three fetuses. She was included as a pregnant female and her number of implantation sites were included but no other reproductive index or survival data was included for this female.

* Statistically different from control, $p<0.05$, **Statistically different from control, $p<0.01$

6. **Parental Postmortem Results:**

a) **Organ Weights:**

P Generation (table 8): In males, the absolute and relative thyroid weights were significantly increased at 1000 ppm (approximately 60% and 61%, respectively, $p<0.01$). Absolute and relative thyroid weights were also increased at 250 ppm (approximately 25% and 22%) compared to controls, although these changes were not statistically significant, they were considered biologically relevant. Absolute and relative liver weights were significantly increased at 250 ppm (approximately 24% and 22%; $p<0.01$ and 0.001, respectively) and

1000 ppm (62% and 65%, respectively, $p<0.001$). All other organ weights (absolute and relative) were equivalent across groups in parental males.

In the P generation females, absolute and relative liver weights were significantly increased at 250 ppm (approximately 46% and 37%, respectively, $p<0.01$) and 1000 ppm (approximately 77% and 73%, respectively, $p<0.001$). Absolute and relative thyroid weights were increased at 1000 ppm (approximately 15% for both, $p<0.01$), although not statistically significant, these changes were considered treatment-related. All other absolute and relative organ weights were equivalent across groups in parental females.

TABLE IIA 8. Selected Absolute and Relative Organ Weights for P Adults

Organ	Dose Group (ppm in diet)				
	0	10	50	250	1000
P Generation Males					
N	10	10	10	10	10
TBW[b] (g)	428.11	440.64	447.21	434.45	421.22
Absolute Organ Weights (g)					
Liver	18.3607	20.1384	20.0812	22.7291**	29.7800***
Thyroid	0.0306	0.0408	0.0360	0.0382	0.0490**
Organ weight relative to terminal body weight (%)					
Liver	4.2947	4.5577	4.4907	5.2320***	7.0679***
Thyroid	0.0072	0.0093	0.0081	0.0088	0.0116**
P Generation Females					
N	10	9	10	10	10
TBW[a] (g)	319.45	316.48	320.40	337.48	325.73
Absolute Organ Weights (g)					
Liver	18.7926	19.5127	19.6362	27.4243**	33.2388***
Thyroid	0.0238	0.0204	0.0240	0.0239	0.0274
Organ weight relative to terminal body weight (%)					
Liver	5.8868	6.1700	6.1426	8.0930**	10.1738***
Thyroid	0.0075	0.0064	0.0075	0.0072	0.0086

a TBW = terminal body weight,
* Statistically different from control, $p<0.05$, **Statistically different from control, $p<0.01$
*** Statistically different from control, $p<0.001$

b) Pathology

1) Macroscopic Examination:

P Generation: Gross necropsy observations included no apparent treatment or dose-related findings in any P generation males.

In P females, enlarged livers were seen at 250 ppm (5/10) and 1000 ppm (8/10) with discoloured livers observed at 1000 ppm (2/10). Gross findings for the one female at unscheduled necropsy at 10 ppm included pale liver and kidneys and retained fetuses in the left uterine horn, and were not considered related to treatment.

B. OFFSPRING

Viability and Clinical Signs *(table 9)*: There were no treatment-related deaths in the F1 offspring. No treatment-related clinical signs were observed at any dose level in F1 pups.

In the F1 litters, there were no test article-related effects on the number of implantation sites per dam, the postimplantation loss per litter, number of live and dead pups per litter on

Lactation Day 0, or on the number of litters delivered (table IIA 5.6.1-12). In addition, the live birth index, day 4, 7, 14, and 21 survival indices, and lactation index were all statistically equivalent across all groups. The sex ratio (% males per litter) and average number of pups per litter on Lactation Days 0, 4, 7, 14, and 21 were also statistically equivalent across all groups. The stillbirth index appeared elevated at 250 ppm and 1000 ppm (approximately 250% and 225%, respectively) when compared to control values, but were not statistically significant. Values for this parameter in the historical database generally fell within a range of 0.2 to 2.0 (one value of 10.2 was considered to be an outlier). The toxicological significance of this finding could not be discounted.

TABLE 9. Litter Parameters for F_1 Generation

Observation	Dose Group (ppm in diet)				
	0	10	50	250	1000
F1 Litters					
No. Live Litters Postnatal Day 0	10	9	9	9	8
No. Live Litters Postnatal Day 21	10	9	8[a]	8[b]	8
No. Implantation Sites per Litter	14.60	14.20	13.44	11.10	13.63
Percent Postimplantation Loss per Litter	8.47	12.45	4.71	10.97	11.08
Mean Number of Pups/Litter:					
Live Pups on Day 0	13.5	13.8	12.9	11.7	12.0
Dead Pups on Day 0	0.1	0.1	0.0	0.4	0.4
Total Pups on Day 0	13.6	13.9	12.9	12.1	12.4
Live Pups on Day 4 [c]	13.0	13.8	14.3	12.8	11.5
Live Pups on Day 7	9.9	9.6	10.0	9.0	9.5
Live Pups on Day 21	9.8	9.6	10.0	8.9	9.3
Stillbirth Index	0.8	0.8	0.0	2.8	2.6
Live Birth Index (no. live on pnd 0/total no. on pnd 0)	99.2	99.2	100.0	97.2	97.4
Lactational Index (no. surviving 21 days/no. live on pnd 4)	99.0	100.0	100.0	98.8	96.8
Percent Male Pups per Litter (Day 0)	54.3	53.6	49.0	45.1	49.8
Percent Male Pups per Litter (Day 21)	49.3	51.1	47.5	50.7	51.3

a The entire litter for female 66 was missing and presumed dead by PND 1
b The entire litter for female 56 was missing and presumed dead by PND 4
c Before standardization (culling) on Day 4.
* Statistically different from control, $p<0.05$ **Statistically different from control, $p<0.01$

2. Body Weight and Food Consumption:

F1 Generation Body Weights during Lactation (table 10): There were no treatment-related effects on F1 pup body weights at 10, 50 or 250 ppm. Average F1 pup body weight per litter was significantly reduced at 1000 ppm for all pups and female (but not male) pups on PND 7, and for all pups and separately by sex on PND 14 and 21.

F1 Generation Body Weights Post-Weaning (table 11): F1 body weights (males and females) were significantly reduced at 1000 ppm for PND 21, 28 and 35 (males only). Body weight changes were significantly reduced in males and females at 1000 ppm for PND 21-28 and 28-35.

F1 Generation Food Consumption expressed in g/day (table 12): Food consumption (g/day) was significantly reduced in males and females at 1000 ppm for PND 21-28 and in males for PND 28-35. When data were expressed as g/kg/day, food consumption was unaffected.

TABLE 10. Mean Pup Weights during Lactation (g)

Lactation Day	Pup Gender	Dose Group (ppm in diet)				
		0	10	50	250	1000
F1 Pups						
Day 0	Both Sexes	6.29	6.68	6.38	6.98	6.91
	Males	6.48	6.84	6.62	6.85	7.13
	Females	6.08	6.50	6.21	6.84	6.71
Day 4	Both Sexes	10.41	10.42	10.36	10.70	9.96
	Males	10.69	10.55	10.75	11.00	10.21
	Females	10.14	10.21	10.10	10.43	9.72
Day 7	Both Sexes	16.52	16.14	16.64	16.77	13.98*
	Males	16.84	16.22	17.12	17.15	14.19
	Females	16.19	16.05	16.23	16.38	13.74*
Day 14	Both Sexes	33.55	33.47	34.01	33.54	26.59***
	Males	34.01	33.40	34.93	34.07	27.05***
	Females	33.15	33.49	33.23	32.94	26.07***
Day 21	Both Sexes	50.04	50.19	53.58	50.50	42.56**
	Males	51.25	50.46	55.26*	51.50	42.99**
	Females	48.68	49.78	52.06	49.38	42.17**

TABLE 11. Body Weights Post-weaning (g)

Interval	Dose Group (ppm in diet)				
	0	10	50	250	1000
F1 Generation Males –Post-weaning					
Body Weight (g)					
PND 21	53.9	54.1	57.6	53.4	45.3**
PND 28	99.8	992.	106.3	101.3	82.8**
PND 35	159.0	159.1	165.5	163.0	128.0**
PND 42	211.8	213.2	220.5	224.9	186.4
Body Weight Change (g)					
PND 21 to 28	45.9	45.2	48.7	48.0	37.5*
PND 28 to 35	59.2	59.9	59.1	61.7	45.2*
PND 35 to 42	61.0	60.6	64.1	66.0	59.7
F1 Generation Females - Post-weaning					
Body Weight (g)					
PND 21	50.6	50.8	54.2	49.8	44.3*
PND 28	89.7	90.5	90.9	87.4	74.5**
PND 35	128.3	126.4	121.7	127.7	107.2
Body Weight Change (g)					
PND 21 to 28	39.0	39.7	36.7	37.6	30.3**
PND 28 to 35	41.6	44.2	36.4	40.2	33.6

TABLE 12. Food consumption Post-weaning (g/day)

Interval	Dose Group (ppm in diet)				
	0	10	50	250	1000
F1 Generation Males-Post-weaning					
PND 21 to 28	12.5	12.5	13.2	11.9	10.5*
PND 28 to 35	17.0	17.1	17.8	17.9	14.3*
PND 35 to 42	18.5	21.4	19.3	22.4	19.7
F1 Generation Females- Post-weaning					
PND 21 to 28	11.4	11.4	12.2	10.4	9.6*
PND 28 to 35	15.6	16.2	13.7	14.3	12.0

3. **Sexual Maturation** **(F_1)** *(table 13)*: Selected Fl offspring (10/sex/group) were examined daily for vaginal patency or preputial separation until acquisition, starting at postnatal day 22 (for females) or 35 (for males). Each animal was weighed on the day of acquisition. Age of acquisition for females was significantly delayed at 1000 ppm (by approximately 1.2 days) when analyzed by Analysis of Covariance (ANCOVA), with bodyweight at acquisition as the covariate. It was not significantly delayed when the day of acquisition was analyzed by non parametric methods. Female F1 body weight on the day of acquisition was significantly equivalent across groups, but the mean weight at 1000 ppm (102.26 g) was 88.0% of the control value (116.9 g). F1 male acquisition of preputial separation was also significantly delayed at 1000 ppm (by approximately 3.7 days) when analyzed by ANCOVA or by nonparametric methods. F1 male body weight on day of acquisition was statistically equivalent across all groups; the mean weight at 1000 ppm (209.51 g) was only slightly lower (97.7%) than the control value of 214.49 g.

TABLE 13. F1 Female Vaginal Opening and Male Preputial Separation

Observation	Dose Group (ppm in diet)				
	0	10	50	250	1000
Female vaginal opening					
N° females evaluated	10	10	10	10	10
Day of vaginal opening	32.3	31.4	31.9	32.2	33.5**
Body weight (g) on day of acquisition	116.19	109.68	114.13	111.49	102.26
Adjusted day of vaginal opening	N/a	N/a	N/a	N/a	N/a
Average female anogenital distance (mm) per litter	N/a	N/a	N/a	N/a	N/a
Male preputial separation					
N° males evaluated	10	10	10	10	10
Day of preputial separation	41.1	41.2	41.9	41.5	44.8**
Body weight (g) on day of acquisition	214.49	212.11	229.31	223.84	209.51
Adjusted day of preputial separation	N/a	N/a	N/a	N/a	N/a
Average male anogenital distance (mm) per litter	N/a	N/a	N/a	N/a	N/a

* Statistically different from control, $p<0.05$, **Statistically different from control, $p<0.01$,
***Statistically different from control, $p<0.001$

III. <u>CONCLUSIONS</u>: Following dietary exposure to ethiprole for two generations, at 0, 10, 50, 250 and 1000 ppm, parental toxicity was observed at 250 ppm (increased body weight gains, organ weight changes in thyroid and liver) and 1000 ppm (decreased body weights, increased body weight gain, decreased food consumption, organ weight changes in liver and thyroid and gross necropsy observations in the liver). The parental systemic NOAEL for this study is 50 ppm.

The offspring LOAEL is 1000 ppm based on decreases in F1 pup body weights with associated delays in acquisition of puberty. Decreases in food consumption were also observed at this dose. The offspring NOAEL is 250 ppm.

No adverse effects on reproductive performance were noted at any dose level, therefore the NOAEL is 1000 ppm.

KIIA 5.6.1 - Two generation reproductive toxicity in the rat

1.	Annex point(s)	KIIA 5.6.1; Reproductive toxicity/Two generation reproductive toxicity in the rat: **MRID 47622810**
2.	Location in dossier	KIIA 5.6.1 /01
3.	Authors (year)	Tyl, R. W.; Myers, C.; Marr, M. (2001)
	Title	Ethiprole 2-generation Reproductive Toxicity Evaluation of Ethiprole (RP107382) Administered in the Feed to CD (Sprague-Dawley) Rats
	Company, report No.	Bayer CropScience AG, Report-No.: B003509 (M-240522-01-1)
	Date	19.09.2001
4.	Testing facility	Research Triangle Institute;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):AE 0316423 00 1C (Batch-No.: AE 0316423 00 1C92 0001)
7.	Test method	USEPA (=EPA): 83-4
	Deviations	
8.	GLP	yes

Executive summary:

Male and female CD® (Sprague-Dawley) rats (30 animals/sex/dose) were administered ethiprole in the feed at 0, 10, 75, and 500 ppm, for ten weeks. Body weights and feed consumption were recorded weekly, and clinical signs were recorded at least once daily. Vaginal cytology was evaluated for the last three weeks of the prebreed period. Animals were then randomly mated within treatment groups for a two-week mating period to produce the Fl generation, with exposure continuing. P males were necropsied after the delivery period, with histologic evaluation of reproductive and other organs and andrological assessments (reproductive organ weights, epididymal sperm number, motility and morphology, testicular homogenization-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production). Fl litters were culled to ten pups on postnatal day (pnd) 4 and weaned on pnd 21. At weaning, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as Fl parents of the F2 generation. P females were then necropsied with organ weights, stage of estrus at necropsy, enumeration of ovarian primordial follicles, and histopathology of reproductive and other selected organs. Selected Fl weanlings, were administered ethiprole in the diet for a ten-week prebreed exposure period, They were mated for a two-week period, as described above. At weaning of F2 litters, up to three weanlings/sex/litter were necropsied. Fl males were necropsied after the delivery period, with histopathology and andrological assessments (as described above). At weaning of the F2 litters, parental Fl females were then necropsied with histopathology, as described above.

There were no treatment-related deaths or clinical signs. P and/or F1 males and/or females had reduced body weight gains at 500 ppm for the prebreed period and during lactation. Feed consumption was significantly lower for variable time points in P males at 75 and 500 ppm and in females at 500 ppm. At necropsy, P and/or F1 parent absolute and relative liver, thyroid, and/or adrenal weights were significantly increased at 500 ppm, while pituitary weights were significantly decreased. Treatment-related histopathologic findings were present in the liver, thyroid and kidneys of P and/or F1 parents only at 500 ppm. There were no statistically significant effects of treatment in reproductive parameters in P and F1adult males and females.

Stillbirth index was slightly elevated at 1000 ppm in F1 and F2 litters, although these changes were not statistically significant, they were above the historical range.

F1 and F2 pup body weights per litter were significantly reduced at 500 ppm on pnd 4, 7, 14 and 21. During the postwean/prebreed exposure period of the selected F1 offspring, the age at acquisition of female vaginal opening was significantly delayed by approximately two days at 500 ppm. F1 male preputial separation was delayed by approximately three days when expressed as age at acquisition or adjusted age at acquisition. Delay in acquisition of puberty for F1 females was associated with significantly reduced body weights at acquisition at 500 ppm. These minimal effects on reproductive development in the F1 offspring triggered measurement of anogenital distance in F2 offspring at birth (PND 0), which showed no differences among groups (see below). At necropsy at weaning, F2 male and female pup body weights were significantly reduced at 500 ppm. Most likely due to reduced body weights, all absolute organ weights for F2 pups were also reduced at 500 ppm, with exception of liver weights, which were increased following treatment. Relative organ weights for both sexes were either increased or unaffected (again, due to body weight reductions) at 500 ppm. There were no treatment-related gross necropsy findings in either the F2 males or females at weaning.

In conclusion, the parental systemic LOAEL is 500 ppm (32.33 - 39.63 mg/kg bw/day in males, 37.36 – 45.20 mg/kg bw/day in females), based on decreased body weights and occasionally decreased weight gain, organ weight changes (liver, thyroid, adrenals, pituitary) and liver, thyroid and kidney histopathology (findings noted in one or both generations and sexes). The parental systemic NOAEL is 75 ppm (4.77 - 6.03 mg/kg bw/day in males, 5.82 – 6.76 mg/kg bw/day in females).

The offspring LOAEL is 500 ppm (32.33 - 39.63 mg/kg bw/day in males, 37.36 – 45.20 mg/kg bw/day in females), based on decreases in Fl and F2 pup body weights during lactation (beginning on Day 4 for Fl and on Day 14 for F2 offspring) and into the postwean/prebreed period, with associated delays in acquisition of puberty. Additional changes in organ weights (thymus, spleen, kidneys, brain, liver) were observed in weanlings, but largely attributed to body weight effects (with exception of the liver). The offspring NOAEL is 75 ppm (4.77 - 6.03 mg/kg bw/day in males, 5.82 – 6.76 mg/kg bw/day in females).

No adverse effects on reproductive performance were noted at any dose level in either generation. The reproductive NOAEL is 500 ppm (32.33 - 39.63 mg/kg bw/day in males, 37.36 – 45.20 mg/kg bw/day in females).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White/beige powder
Lot/Batch #:	CRD9706
Purity:	92.7%
Compound Stability:	The test substance was stored in an air-tight, light-resistant container at room temperature.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive controls were used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing.

3. **Test Animals:**

Species:	Rat	
Strain:	Outbred albino CD® (Sprague-Dawley) rats (Crl:CD®[SD] IGS BR)	
Age at study initiation:	At Day 0 of 10 week pre-mating period for both P and F1 parents: (P) ~7 wks (~17 wks at mating); (F1) ~3 wks (~13 to 15 wks at mating)	
Wt. at study initiation:	At Day 0 of 10 week pre-mating period for both P and F1 parents: (P) Males: 205.2 - 249.4 g; Females: 153.7 - 190.5 g (F1) Males: 62.6 - 71.8 g Females: 56.1 - 68.0 g (range of ave. over dose groups);	
Source:	Charles River Breeding Laboratories, Raleigh, NC	
Housing:	Individually housed during the quarantine period and upon the initiation of the treatment period in solid-bottom polycarbonate cages with stainless steel wire lids, with Sani-Chips® cage litter. Two per cage (1 male: 1 female, same dose level) during the mating period. Females were caged separately and individually once they were successfully mated (or at the end of the mating period).	
Diet:	Purina Certified Ground Rodent Chow® (No. 5002, PMI Feeds, Inc., St. Louis, MO), *ad libitum*	
Water:	Municipal tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18° to 26° C (target)
	Humidity:	30 to 70% (target)
	Air changes:	not recorded
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	1 week	

B. PROCEDURES AND STUDY DESIGN

1. **In-Life Dates:** From March 20, 2000 to November 28, 2000

2. **Mating Procedure:** The estrous cycle was determined by examining daily vaginal smears over a three week period prior to mating of the P and F1 generation females, immediately prior to the cohabitation period. Additionally, the estrous cycle stage was determined for all females just prior to termination.

Mating for both the P and F1 generation was accomplished by co-housing one female with one male from the same dose group for up to 14 consecutive days. Brother-sister matings were avoided. Vaginal smears were taken each morning and examined for the presence of sperm and/or an internal vaginal plug during the mating phase. Females found to be inseminated (designated Gestation Day 0) were placed in an individual polycarbonate cage. At the end of the cohabitation period, all remaining females were placed individually polycarbonate cages in case evidence of mating was undetected.

3. **Study Schedule:** The P parental animals (30 rats/sex/dose group) were approximately 7 weeks of age at initiation of treatment and were given test diets for 10 weeks before they were mated (~17 weeks of age at mating).

   Fl litters were culled to ten pups each on Lactation Day 4 and weaned on Lactation Day 21. At weaning, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as Fl parents of the F2 generation. At least one male and one female pup per litter, if possible, were randomly selected to produce the F2 generation. All Fl pups not selected as parents or for necropsy were examined externally, euthanized, and discarded. The F1 rats selected to become parents of the F2 generation were monitored for developmental landmarks (vaginal opening, preputial separation) prior to initiation of a 10 week pre-mating interval. The Fl animals were approximately 13 to 15 weeks of age at the initiation of the mating period.

   Three F2 weanlings per sex per litter were selected and subjected to a gross (external and visceral) necropsy examination. At necropsy of P and Fl parental animals and selected Fl and F2 weanlings, gross lesions were retained in fixative, and selected organs were weighed and/or retained in fixative for possible subsequent histopathologic evaluation.

   Feeding of test diets to both generations of parents and offspring (after weaning) was continuous throughout the study, and all offspring were fed the same dietary level as their corresponding parents.

4. **Animal Assignment:** P animals were assigned to the different test groups as seen in Table 1 by means of randomization stratified by body weight, such that the body weights of all groups by sex were homogeneous by statistical analysis at study initiation.

TABLE 1. Animal Assignment

Test Group	Dose in Diet [a] (ppm)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
Low (LDT)	10	30	30	30	30
Mid (MDT)	75	30	30	30	30
High (HDT)	500	30	30	30	30

a Diets were administered from beginning of the study until sacrifice.

The dose levels selected for this study were based on the results from the range-finding study (Study Number: 65C-7407-100, M-210173-01) where P generation CD® rats (10/sex/dose group) were exposed to ethiprole in the feed at 0, 10, 50, 250, and 1000 ppm *ad libitum* for the two-week prebreed, two-week mating, three-week gestation, and three-week lactation periods. At weaning of Fl offspring on Lactation Day 21, 10/sex/group were selected and exposed to the same dietary concentrations as their parents, for a maximum of approximately

four weeks until the females acquired vaginal patency and the males acquired preputial separation (i.e., puberty). P parental toxicity (reduced body weights and food consumption) was observed at 1000 ppm during lactation. At necropsy of the parental animals (P generation), male liver and thyroid weights (absolute and relative) and female liver weights (absolute and relative) were significantly increased at 1000 ppm. Fl offspring toxicity (reduced body weights per litter on Lactation Days 7, 14, and 21) was observed at 1000 ppm. Based on these results, the selected target dietary concentrations for the main study were 10, 75, and 500 ppm.

5. **Dosage Preparation and Analysis:** Formulations were prepared at least monthly by mixing appropriate amounts of test substance with Purina certified ground rodent diet® (No. 5002) and were then stored frozen. Prior to the start of the study, stability and homogeneity of the test diets at concentrations used during the range-finding study (10 and 1000 ppm) were established. Stability analyses were performed on dosed feed samples at 10 and 1000 ppm at room temperature in open containers (to simulate cageside exposures) for 0, 3-4, and 9-11 days, and on dosed feed samples stored frozen (-11 to -21°C) for 0, 9, 28, and 48 days. In addition, homogeneity analyses were performed on the 10 and 1000 ppm concentration diets (three samples each from the left, right, and bottom of the V-shell blender for each dietary level). During the study, the concentration of the test material in the diets (all dose levels) was verified for the first four formulation dates, and for every second formulation date thereafter. These analyses were performed prior to administration of the test diets to the animals.

Results: **Homogeneity Analysis:** Results for homogeneity of 10 and 1000 ppm diets were within 89.1-104% of the nominal concentrations. (Data obtained from the study report, pages 293 and 296).

Stability Analysis: After 48 days of frozen (-11 to -21°C) storage, results for the 10 and 1000 ppm diets were 91.8-106% of the nominal concentration. After 9 days of storage at room temperature, the 10 and 1000 ppm diets were within 97.2-102% of the nominal concentrations. (Data obtained from the study report, pages 298, 299, 301, and 302).

Concentration Analysis: Results for the concentration analyses were within 93.4-110% of the nominal concentrations. (Data obtained from the study report, page 80). These data indicate that the variance between nominal and actual dosage to the study animals was acceptable

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. In addition, the stability of ethiprole in the diets was verified for the intervals and conditions used in this study. The ethiprole in 10 and 1000 ppm diets was demonstrated as stable for at least 48 days under freezer conditions and at least 9 days at ambient temperatures.

C. OBSERVATIONS

1. **Parental Animals:** The following observations and the schedule for those observations for the parental rats of both generations (P and F1) are summarized from the report. Observations for mortality were made twice daily (a.m. and p.m.), and the general condition of all animals was checked daily. Clinical examinations were conducted and recorded daily throughout the course of the study. These cage-side observations included, but were not

limited to, changes in skin and fur, eyes, mucous membranes, respiratory and circulatory system, autonomic and central nervous system, somatomotor activity, and behavior pattern.

The body weights of the P and Fl male rats were determined and recorded initially and weekly through mating. The body weights of P and Fl female rats were recorded in the same manner until confirmation of mating. During gestation, P and Fl females were weighed on Gestation Day 0, 7, 14, and 20. Dams producing litters were weighed on Lactation Day 0, 4, 7, 14, and 21, and body weight gains were computed.

Food consumption measurements were recorded weekly for all P and Fl parental animals throughout the prebreed treatment periods. During pregnancy of P and Fl females, food consumption was recorded for Gestation Days 0-7, 7-14, and 14-20. During lactation of Fl and F2 litters, maternal food consumption was measured for Lactation Day 0-4, 4-7, 7-14, and 14-21, although maternal food consumption after Lactation Day 14 was confounded by the contribution from the pups since pups were self-feeding by this time. Food consumption was not measured during the cohabitation period. Food consumption collection periods corresponded to the collection of the animals' body weight data. These data were employed to calculate their actual exposure to the test chemical as mg ethiprole/kg body weight/day. In addition, food efficiency was calculated per animal per interval as weight gain in grams for the interval divided by food consumption in grams for the interval (x 100 to allow reporting as a percentage). Feed was changed at least weekly throughout the study.

For both the P and F1 generation females, vaginal smears for estrous cyclicity and normality were taken for the last three weeks of the prebreed exposure period. In addition, vaginal smears were taken daily during the 14-day mating period or until mating was confirmed. The slides taken during the mating period were retained but were not evaluated for estrous cyclicity or normality unless it was necessary for interpretation of mating results. Additionally, the estrous cycle stage was determined for all females just prior to termination.

The F1 rats selected to become parents of the F2 generation were monitored for developmental landmarks (vaginal opening, preputial separation).

2. **Litter Observations:** According to the report, the following litter observations (X) were made (see Table 2).

TABLE 2. F_1 / F_2 Litter Observations

Observation	Time of observation (lactation day)					
	Day 0	Day 4 [a]	Day 4 [b]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
External alterations [c]	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X	X
Anogenital distance (F2 pups only)	X					

a Before standardization (culling)
b After standardization (culling)
c All pups were examined for physical abnormalities at birth and throughout the preweaning period.

Beginning on Gestation Day 20, each female was observed twice daily (a.m. and p.m.) for evidence of littering. All Fl and F2 pups were sexed and examined as soon as possible after birth (date of birth designated Lactation Day 0) to determine the number of viable and stillborn members of each litter. Thereafter, litters were evaluated for survival on 4, 7, and 14 days after birth and at weaning (Lactation Day 21). Because there were delays in acquisition of puberty for Fl males and females, individual anogenital distance and body weight were recorded on Lactation Day 0 for all F2 offspring. Anogenital distance was measured using an ocular micrometer and microscope stage grid.

On Lactation Day 4, the size of each litter was adjusted by eliminating extra pups by random selection to yield, as nearly as possible, a maximum of 10 pups/litter (5 pups/sex/litter). Culled pups were sacrificed by decapitation and discarded. All live pups were counted, sexed, weighed individually, and examined grossly at birth (Lactation Day 0), at 4, 7, and 14 days after birth, and at weaning (Lactation Day 21). The body weights and sexes were recorded on an individual basis, but the pups were not uniquely identified at this stage. All pups were examined for physical abnormalities at birth and throughout the preweaning period. All pups dying during lactation were necropsied, when possible, to investigate the cause of death.

3. <u>Postmortem Observations</u>:

a) <u>Parental Animals</u>: All surviving parental males were sacrificed after the delivery period for each generation. Maternal animals were sacrificed after the last litter of each generation was weaned. These animals were subjected to postmortem examinations as follows.

On the day of necropsy, prior to euthanasia, all P and Fl parental females were subjected to vaginal lavage to determine the stage of estrus at termination. For each P and F1 parental male, the right cauda epididymis was removed immediately after sacrifice, weighed, and seminal fluid from the cauda was assessed for sperm number, motility, and morphology.

All P and Fl parental animals in all groups were subjected to a complete gross necropsy. The gross necropsy included examination of the external surfaces; all orifices; cranial cavity; carcass; external and cut surfaces of the brain and spinal cord; the thoracic, abdominal, and pelvic cavities and their viscera; and cervical tissues and organs. The following organs were weighed (and retained in fixative): ovaries, uterus with oviducts and cervix, prostate, pituitary, testes, seminal vesicles, epididymides, adrenal glands, spleen, brain, liver, kidneys, and thyroid.

Full histopathology of the tissues from the following organs was performed for the control and high dose parental animals (10/sex/dose level for each P and Fl generations): ovaries, vagina, uterus with oviducts and cervix, testis, seminal vesicles with coagulating glands and fluids, prostate, epididymides with contents, thyroid, liver, kidneys, and other tissues with gross lesions identified as being potentially treatment related. In addition, the liver, thyroid, and kidneys were examined histologically at the low- and mid-dose for either the P or F1 generations (10/sex/dose level), dependent on the findings at the high dose. The reproductive organs of any animal, regardless of dose group, were subjected to histopathological evaluation if it was an unsuccessful breeder or exhibited other evidence of possible reduced fertility. Pituitaries from all P and Fl parental animals were retained in fixative for possible subsequent histopathologic examination.

b) <u>Offspring</u>: Three Fl and F2 weanlings/sex/litter, if possible, were randomly selected for necropsy. Pups were 21 days of age at sacrifice. The following organs were weighed (and

retained in fixative): brain, liver, spleen, kidneys, and thymus. In addition, the thyroid with attached trachea was also retained in fixative from the Fl and F2 weanlings that were necropsied. The Fl offspring not selected as parents or for necropsy and the F2 offspring not selected for necropsy were examined for gross external abnormalities, euthanized, and discarded. A gross internal examination was made on any pup appearing moribund or dying on test.

D. DATA ANALYSIS

1. **Statistical Analyses:** The unit of comparison was the male, the female, the pregnant female, or the litter, as appropriate. Treatment groups were compared to the concurrent control group using either parametric ANOVA under the standard assumptions or robust regression method which does not assume homogeneity of variance or normality. The homogeneity of variance assumption was examined via Levene's Test, which is much more robust to the underlying distribution of the data than the traditional Bartlett's Test. If Levene's Test indicated lack of homogeneity of variance ($p<0.05$), robust regression methods were used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They were used to test for overall treatment group differences, followed by individual tests for exposed vs. control group comparisons (via Wald chi-square tests), if the overall treatment effect was significant. The presence of linear trends was analyzed by GLM procedures for homogenous data or by robust regression methods for nonhomogenous data (SAS Institute Inc.). The standard ANOVA methods, as well as Levene's Test, used are available in the GLM procedure of SAS® Release 6.12 (SAS Institute Inc., 1997), and the robust regression methods used are available in the REGRESS procedure of SUDAAN® Release 7.5.3 (Shah et al., 1997).

   If Levene's Test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 was used to evaluate the overall effect of treatment and, when a significant treatment effect was present, to compare each exposed group to control via Dunnett's Test. For the litter-derived percentage data (e.g., periodic pup survival indices), the data were arcsine square root transformed prior to analysis, since percentage data derived from litters tend to have unequal variances. The transformed data were then analyzed using ANOVA weighted by litter size in order to further stabilize the variances. A test for linear trend was also performed on the transformed data weighted by litter size. A one-tailed test (i.e., Dunnett's Test) was used for all pairwise comparisons to the vehicle control group, except that a two-tailed test was used for parental and pup body weight and organ weight parameters, food consumption, percent males per litter, and anogenital distance.

   Frequency data such as reproductive indices (e.g., mating and fertility indices) were not transformed. All indices were analyzed by Chi-Square Test for Independence for differences among treatment groups and by the Cochran-Armitage Test for Linear Trend on Proportions. When Chi-Square revealed significant ($p<0.05$) differences among groups, then a Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each treatment group and the control group. Acquisition of developmental landmarks (e.g., vaginal patency and preputial separation), as well as F2 newborn anogenital distance, were analyzed by Analysis of Covariance (ANCOVA; in addition to ANOVA analysis) using body weight at acquisition or measurement as the covariate. For correlated data (e.g., body and organ weights at necropsy of weanlings, with more than one pup/sex/litter), SUDAAN® software was used for analysis of overall significance, presence of trend, and pairwise comparisons to the control group values.

A SAS Institute, Inc. test for statistical outliers was performed on parental body weights and food consumption (in g/day) and on adult and weanling necropsy organ weights. If examination of pertinent study data did not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If food consumption data for a given animal for a given observational interval (e.g., study day 0-7, 7-14, 14-28, 28-35, etc., during the prebreed exposure period) were designated outliers or "unrealistic" (e.g., negative value), then summarized data encompassing this period (e.g., study day 0-70 for the prebreed exposure period) was not included in this value.

For all statistical tests, the significance limit of 0.05 (one- or two-tailed) was used as the criterion for significance.

2. **Indices:**

Reproductive Indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Females:

$$\text{Mating index (\%)} = \frac{\text{No. females sperm-positive}}{\text{No. females paired}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{No. females pregnant}}{\text{No. females sperm-positive}} \times 100$$

$$\text{Gestational index (\%)} = \frac{\text{Number of females with live litters}}{\text{Number of females pregnant}} \times 100$$

Males:

$$\text{Mating index (\%)} = \frac{\text{No. males impregnating females}}{\text{No. males paired}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{No . males siring litters}}{\text{No. males impregnating females}} \times 100$$

$$\text{Pregnancy index (\%)} = \frac{\text{No. pregnant females}}{\text{No. males impregnating females}} \times 100$$

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

$$\text{Live birth index (\%)} = \frac{\text{Number of live pups at birth}}{\text{Total number of pups born}} \times 100$$

$$\text{4-Day survival index (\%)} = \frac{\text{Number of pups surviving 4 days (precull)}}{\text{Total number of live pups at birth}} \times 100$$

$$\text{7-Day survival index (\%)} = \frac{\text{Number of pups surviving 7 days}}{\text{Total number of live pups at 4 days (postcull)}} \times 100$$

$$\text{14-Day survival index (\%)} = \text{Number of pups surviving 14 days} \times 100$$

$$\text{21-Day survival index (\%)} = \frac{\text{Total number of live pups at 7 days}}{\text{Number of pups surviving 21 days}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{Total number of live pups at 14 days}}{\text{Number of pups surviving 21 days}} \times 100$$

$$\text{Stillbrith index (\%)} = \frac{\text{Total number of live pups at 4 days (postcull)}}{\text{Number of dead pups delivered}} \times 100$$

$$= \frac{\text{Total number of pups delivered}}{\text{Number of dead pups delivered}} \times 100$$

3. **Historical Control Data:** Historical control data were provided from studies conducted at the laboratory between 1990 and 1998. Means and standard deviation values from individual studies were provided. Historical control data included the following: parental body weights and food consumption, female vaginal cytology data; various reproductive and lactation indexes (mating, fertility, gestation, and pregnancy indices), pre-coital intervals, gestation lengths, numbers of implantation sites per female, various offspring viability indices (live and still birth indices, percent post implantation loss per litter, survival and lactation indices), litter sizes, pup weights, percent male pups, male nipple evaluations during lactation; anogenital distances (F2 pups only), time to preputial separation or vaginal patency, sperm evaluation data, ovarian follicle counts, and selected parental and pup organ weights.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and Clinical Signs:** All P and Fl males and females (30/group) survived until scheduled sacrifice. There were no treatment- or dose-related clinical observations in either sex in any parental generation.

2. **Body Weight and Food Consumption:**

Food consumption and body weight data during the premating period are summarized as follows:

P Generation Body Weights (table 3): Male body weights and body weight gains were unaffected by treatment at all dose levels. Female body weights at week 10 (Day 70) were significantly ($p<0.01$) reduced at 500 ppm relative to the controls. In addition, the body weight gains for females were significantly reduced (by approximately 13%, $p<0.05$) at 500 ppm for the entire prebreed period (Days 0-70).

No changes in body weights were observed at 10 and 75 ppm.

P Generation Food Consumption expressed in g/kg/day (table 4): Male food consumption, was significantly reduced (by approximately 3-4%, $p<0.05$) at 75 and 500 ppm for week 2 (days 7-14) and at 75 ppm for week 3 (days 14-21) relative to the controls, however given the magnitude and transient nature of these changes, were considered to be of no toxicological consequence.

In females, at 75 ppm, food consumption (in g/kg/day) was significantly increased (by approximately 7.7%, $p<0.05$) for week 6, however returned to levels equivalent to controls for the remaining duration of the study, and therefore were not considered to be

toxicologically significant. At 500 ppm, food consumption was significantly reduced at weeks 1, 2, and 4 (by approximately 5-10%, $p<0.01$) as a result of treatment.

No changes in food consumption were noted in either sex at 10 ppm.

F1 Generation Body Weights (table 3): F1 male body weights were significantly reduced at 500 ppm through Day 28 of the premating phase, and body weight gain was also significantly reduced only at 500 ppm for Days 0 to 7 and 7 to 14. During the total ten-week prebreed period, body weight gains were equivalent across all groups in males.

F1 female weekly body weights were significantly reduced at 500 ppm for almost all the prebreed period. There was no effect on female F1 body weights for any other time period and dietary dose during the prebreed period. F1 female body weight gains were significantly reduced at 500 ppm for Days 0-7 and 42-49. Female body weight gains were equivalent across all other groups at all intervals and for the overall prebreed period at all dose levels.

F1 Generation Food Consumption expressed in g/kg/day (table 4): There were no statistically significant differences in food consumption at any time interval during the entire prebreed period in either sex.

TABLE 3. Mean Body Weight (g) and Body Weight Gains (g)- Pre-mating

Interval	Dose Group (ppm in diet)			
	0	10	75	500
P Generation Males - Pre-mating				
Body Weight (g)				
Day 0	230.0	230.2	229.2	227.5
Day 7	292.2	293.0	287.3	287.8
Day 70	558.1	557.9	530.0	544.5
Body Weight Gains (g)				
Days 0 to 7	62.2	62.8	58.1	60.3
Days 0 to 70	328.2	327.7	300.8	317.0
P Generation Females - Pre-mating				
Body Weight (g)				
Day 0	172.9	171.7	170.8	172.3
Day 7	200.4	196.2	196.2	197.1
Day 70	307.1	298.4	298.4	288.9 **
Body Weight Gains (g)				
Day 0 to 7	27.5	24.4	25.4	24.8
Day 0 to 70	134.2	126.7	127.6	116.6*
F1 Generation Males - Pre-mating				
Body Weight (g)				
Day 0	71.7	71.8	70.1	62.6 **
Day 7	122.8	121.7	120.6	109.1 **
Day 14	183.5	180.8	179.8	164.7**
Day 21	244.8	241.0	240.4	222.7 **
Day 28	306.0	299.6	299.8	280.8 **
Day 84	531.2	524.9	525.5	520.3
Body Weight Gains (g)				
Day 0 to 7	51.1	49.9	50.5	46.4*
Day 7 to 14	60.7	59.1	59.2	55.7*
Day 49 to 56	22.9	25.1 (N=29)	24.1	28.5 ***
Day 0 to 70	433.2	426.2	428.3	427.2
F1 Generation Females - Pre-mating				
Body Weight (g)				
Day 0	67.4	68.0	64.1 (N=29)	56.1 ***
Day 7	110.5	110.1	104.1	94.7 ***
Day 14	151.8	153.4	143.2	136.3***
Day 21	179.6	180.9	172.5	166.3**
Day 70	297.2	297.9	286.0	277.2 *
Body Weight Gains (g)				
Day 0 to 7	43.1	42.1	40.7 (N=29)	38.6 **
Day 7 to 14	41.3	43.2	39.0	41.6
Day 42 to 49	19.2	18.1	16.3	14.7**
Day 0 to 70	229.8	229.9	224.2 (N=29)	221.1

a N=30 unless otherwise noted, * Statistically different from control, $p<0.05$, ** Statistically different from control, $p<0.01$, *** Statistically different from control, $p<0.001$.

TABLE 4. Mean Food Consumption (g/kg/day) - Pre-mating [a]

Interval	Dose Group (ppm in diet)			
	0	10	75	500
P Generation Males-Pre-mating				
Days 0 to 7	95.7	97.4	92.6.	92.7
Days 7 to 14	85.6	87. (N=29)	82.2** (N=29)	82.2** (N=29)
Days 14 to 21	77.1	77.8	74.4*	75.0
Days 0 to 70	64.7 (N=28)	66.1 (N=28)	63.6 (N=29)	64.7 (N=29)
P Generation Females-Pre-mating				
Days 0 to 7	98.0	97.7	95.5	88.5***
Days 7 to 14	93.5 (N=29)	92.9	92.5	88.1**
Days 14 to 21	87.3	88.5	86.6 (N=29)	83.8
Days 21 to 28	80.9	83.4	82.7	77.0** (N=29)
Days 35 to 42	72.8	77.1 (N=29)	78.4*	74.4
Days 0 to 70	75.4 (N=27)	78.0 (N=27)	77.7 (N=29)	74.7 (N=29)
F1 Generation Males-Pre-mating				
Days 0 to 7	147.1	150.3	149.5	148.9 (N=29)
Days 7 to 14	125.0	127.0	128.9	128.1
Days 0 to 70	79.3 (N=29)	79.9 (N=28)	80.4	79.3 (N=28)
F1 Generation Females-Pre-mating				
Days 0 to 7	150.8	147.7	146.2 (N=28)	149.2
Days 7 to 14	125.7 (N=29)	127.0	124.1	128.7
Days 0 to 70	90.7 (N=28)	91.1	90.1 (N=28)	90.4

a N=30 unless otherwise noted, * Statistically different from control, p<0.05, ** Statistically different from control, p<0.01, *** Statistically different from control, p<0.001.

Food consumption and body weight data during gestation and lactation are summarized as follows:

P-Generation (table 5): No statistically significant differences from the controls were noted in the maternal gestational body weights at any dose level. Body weight gains during gestation was also not affected by treatment. Overall, maternal food consumption was considered to be similar across all groups during the gestation period.

Maternal lactational body weights were statistically significantly reduced at 500 ppm for Lactation Days 0, 4, and 7, which demonstrated recovery over the remainder of the lactation period such that lactational body weight gains were significantly increased at 500 ppm for the entire lactational period (Days 0-21), possibly indicating a compensatory response. Maternal lactational food consumption, expressed g/kg/day, was statistically equivalent across all groups for all time points evaluated.

F1 Generation (table 5): Fl maternal body weights were significantly reduced at 500 ppm for Gestation Day 0, 7, and 14. Fl maternal body weight gains were significantly reduced (by approximately 16-22%, p<0.001) at 10, 75 and 500 ppm for Gestation Day 0-7 with no dose-response relationship, and not considered toxicologically significant. Fl female body weight gain was significantly lower for the gestational period (Gestation Day 0-20) only at 500 ppm. Maternal gestational food consumption, expressed as g/kg/day, was not affected by the treatment at any dietary dose for any gestational interval.

Maternal lactational body weights were statistically significantly reduced at Lactation Days 0, 4, 7, and 14 at 500 ppm. Maternal lactation food consumption as g/kg/day, showed no differences at any time interval for any dose group.

TABLE 5. Mean Body Weights (g), Body Weight Gain (g) and food consumption (g/kg/day) in dams during gestation and lactation periods

Interval	Dose Group (ppm in diet)			
	0	10	75	500
P Generation-gestation				
Number of animals	25	27	25	25
Day 0	302.2	293.5	293.7	284.4
Day 7	334.3	321.7	328.2	316.0
Day 14	364.0	350.1	354.6	345.6
Day 20	435.7	425.2	430.2	425.0
BWG[a] Days 0 to 20	133.4	131.7	136.6	140.5
FC[a] Days 0 to 20	65.2	65.2	67.0	68.2
P Generation-lactation				
Number of animals	28	29	29	26
Day 0	341.9	330.7	334.0	320.2*
Day 4	356.5	343.4	348.6	330.9**
Day 7	358.2	347.5	347.7	337.1**
Day 14	368.6	366.0	368.2	356.0
Day 21	346.9	342.4	344.0	349.1
BWG[a] Days 0 to 21	2.5	11.7	9.9	28.9
FC[a] Days 0 to 20	151.7 (N= 27)	159.3 (N= 28)	162.4 (N=28)	158.1
F1 Generation-gestation				
Number of animals	23	26	25	27
Day 0	299.1	300.2	285.5	277.3*
Day 7	337.7	332.3	318.0	307.3*
Day 14	369.9	363.9	349.1	336.9**
Day 20	443.6 (N=15)[b]	445.1 (N=17)	426.1 (N=18)	414.0 (N=17)
BWG[a] Days 0 to 20	150.4 (N=15)[b]	140.5 (N=17)	139.4 (N=18)	134.2* (N=17)
FC[a] Days 0 to 20	71.0 (N=15)[b]	68.3 (N=17)	71.7 (N=18)	69.4 (N=17)
F1 Generation Females-lactation				
Number of animals	24	28	29	28
Day 0	344.1	339.0	327.2	313.6**
Day 4	359.4	351.7	343.3	320.6***
Day 7	364.6	358.6	350.1	331.5***
Day 14	365.9	363.2	354.0	343.8*
Day 21	360.1	351.0	346.8	340.1
BWG[a] Days 0 to 21	16.1	12.0	19.6	26.4
FC[a] Days 0 to 20	161.5	167.2	170.2	161.2

a BWG: body weight gain, FC: food consumption
b Decrease in N is due to one or more females inadvertently not being weighed on gestational day 20
* Statistically different from control, $p<0.05$, ** Statistically different from control, $p<0.01$.
*** Statistically different from control, $p<0.001$.

3. **Test Substance Intake:** Based on food consumption and body weight data, the doses expressed as mean daily mg test substance/kg body weight/day during the 10 week pre-mating period, gestation, and lactation are presented in Tables 6a and 6b.

TABLE 6a. Mean Test Substance Intake During Pre-mating (Days 0-70) (mg/kg/day)

	Male			Female		
	LDT	MDT	HDT	LDT	MDT	HDT
P	0.66	4.77	32.33	0.78	5.82	37.36
F1	0.80	6.03	39.63	0.91	6.76	45.20

Note: The purity of bulk ethiprole used as the test material was assumed to be 100% for diet formulation purposes.

TABLE 6b. Mean Test Substance Intake During Gestation and Lactation (mg/kg/day)

	P Parental Females			F1 Parental Females		
	LDT	MDT	HDT	LDT	MDT	HDT
Gestation	0.65	5.03	34.12	0.68	5.38	34.68
Lactation	1.59	12.18	79.05	1.67	12.77	80.58

Note: The purity of bulk ethiprole used as the test material was assumed to be 100% for diet formulation purposes.

4. **Reproductive Function:**

a.) **Estrous Cycle Length and Periodicity:** Vaginal cytology was evaluated for P and F1 generation females daily during the last three weeks of the ten-week prebreed exposure period. No treatment-related findings were noted.

P Generation (table 7): The number of P generation females exhibiting abnormal estrous cycles was 2 (6.7%), 5 (16.7%), 0 (0%), and 1 (3.3%) at 0, 10, 75, and 500 ppm, respectively. Cycle length was 4.15 days in the control group, 4.44 days at 10 ppm, 4.44 days at 75 ppm, and 4.56 days at 500 ppm. Cycle length was slightly longer (by less than one-half day) than the mean control value at 75 and 500 ppm (with the mean value at 10 ppm, 4.44 days, the same as the value at 75 ppm but not statistically significantly different from the control value due to larger variance). Values for all groups were within the performing laboratory's historical control values for this strain of rat.

In addition, at necropsy, the stage of estrus was equivalent across all groups. Ovarian primordial follicle counts for both ovaries from ten females each at 0 and 500 ppm were also statistically equivalent, although potential changes at lower doses could not be determined as females at 10 and 75 ppm were not evaluated.

F1 Generation (table 7): All females in all groups were cycling; 3, 5, 8, and 4 females in the 0, 10, 75, and 500 ppm groups, respectively, had abnormal cycles. Cycle length was equivalent across all groups, with a mean duration of 4.30, 4.58, 4.83, and 4.76 days at 0, 10, 75, and 500 ppm, respectively, and was comparable to historical control data provided (4.11 to 4.69 days).

In addition, the stage of the estrous cycle at demise was statistically equivalent across all groups, although the percentage of females in proestrus in controls (32.1%) was higher than in the treatment groups (11.5, 13.8, and 11.1% at 10, 75, and 500 ppm, respectively). The number of control females in metestrus (25.6%) was correspondingly lower than in the

treatment groups (46.2, 62.1, and 51.8% at 10, 75, and 500 ppm, respectively). Historical control data did not assist in the interpretation of these findings as the values provided from the two studies for these endpoints were highly variable. As there was no dose-related pattern to these findings, and there were no subsequent changes in fertility parameters, they were not considered toxicologically significant.

TABLE 7. Vaginal cytology in P and F1 females at the end of the prebreed period

Interval	Dose Group (ppm in diet)			
	0	10	75	500
P Generation				
Number of females started on study	30	30	30	30
Number of females with abnormal cycles	2	5	0	1
Cycle length (days)	4.15	4.44	4.44	4.56
F1 Generation				
Number of females started on study	30	30	30	30
Number of females with abnormal cycles	3	5	8	4
Cycle length (days)	4.30	4.58	4.83	4.76
Necropsy: N° in proestrus	9 (N=29)[a]	3	4	3 (N=28)[a]
Necropsy: N° in metestrus	8	12	18	14

a Decrease in N is due to one or more females inadvertently not being smeared at the time of necropsy

b.) <u>Sperm Measures</u>:

<u>P and F1 Generations:</u> No treatment-related findings were observed in sperm measures in either generation for any of the following parameters: percent motile sperm, percent progressively motile sperm, epididymal sperm concentration, testicular spermatid head concentration, daily sperm production per testis, efficiency of daily sperm production, and percent abnormal sperm. Aspermia was observed in one high dose P male, however this was not considered to be toxicologically significant.

5. **Reproductive Performance:** Reproductive performance data are presented in Table 8. There were no compound-related effects on any parameter (e.g., mating, fertility, gestation or pregnancy indices, days to insemination, or gestation length) in either generation at any dietary level tested.

TABLE 8. Reproductive Performance [a]

Observation	Dose Group (ppm in diet)			
	0	10	75	500
P Generation – F1 Litters				
No. Females Paired	30	30	30	30
No. Females that Mated	30	30	30	29
Mating Index (Females)	100.0	100.0	100.0	96.7
No. of Pregnant Females	29	29	29	27
Fertility Index (Females)	96.7	96.7	96.7	93.1
No. of Females with Live Litters	29	29	29	26 [a]
Gestational Index	100.0	100.0	100.0	96.3
No. Males Paired	30	30	30	30
No. Males that Mated	30	30	30	29
Mating Index (Males)	100.0	100.0	100.0	96.7
No. Males Siring Litters	29	29	29	27
Fertility Index (Males)	96.7	96.7	96.7	93.1
Pregnancy Index	96.7	96.7	96.7	93.1
Days until Sperm Positive	2.6	2.4	2.5	2.2
Gestational Length (days)	22.1	22.0	22.2	22.0
F1 Generation – F2 Litters				
No. Females Paired	30	30	30	30
No. Females that Mated	27	29	30	29
Mating Index (Females)	90.0	96.7	100.0	96.7
No. of Pregnant Females	25	28	29	28
Fertility Index	92.6	96.6	96.7	96.6
No. of Females with Live Litters	25	28	29	28
Gestational Index	100.0	100.0	100.0	100.0
No. Males Paired	30	30	30	30
No. Males that Mated	27	29	30	29
Mating Index (Males)	90.0	96.7	100.0	96.7
No. Males Siring Litters	25	2 8	2 9	28
Fertility Index	92.6	96.6	96.7	96.6
Pregnancy Index	92.6	96. 6	96. 7	96.6
Days until Sperm Positive	2.8	2.4	2.7	2.3
Gestational Length (days)	22.0	22.1	22.0	22.0

a One high-dose P female had implantation sites only.
* Statistically different from control, $p<0.05$, ** Statistically different from control, $p<0.01$

6. Parental Postmortem Results:

a) Organ Weights:

P Generation (table 9): In males, the absolute and relative liver and thyroid weights were significantly increased in the high dose group. Relative paired epididymides and testes weights were significantly increased at 75 ppm (but comparable to values in the historical control database), with no changes observed at 500 ppm and therefore not considered treatment related (most likely due to the reduced body weights in this group). No statistically significant differences among groups for any of the other organs were observed. There were no histological findings at any dose level in the P generation males.

In the P generation females, the absolute and relative paired adrenal and thyroid weights were significantly increased at 500 ppm, and absolute and relative liver weights were significantly increased at 75 and 500 ppm. Associated treatment-related histopathology was noted only in the liver and thyroid of the P generation females at 500 ppm. In the absence of any histopathologic correlate, the increased liver weight at 75 ppm was considered treatment-related, although not adverse. There were no statistically significant differences among groups for any of the other organs.

F1 Generation (table 10): At 75 ppm, in the F1 males, absolute and relative pituitary weights were statistically significantly reduced (approximately 9% and 7%, respectively, $p<0.01$), while at 500 ppm, the absolute and relative pituitary weights were also decreased (approximately 10% and 7%, respectively). Given the magnitude of these pituitary changes, and the body weight effects noted at these doses, the relation to treatment could not be determined. At 500 ppm, the absolute and relative weights of each thyroid and liver weights were increased. Absolute brain weights were significantly decreased at 10, 75, and 500 ppm; relative brain weights were not affected, and in light of the decreased body weights observed, the decreases in absolute brain weights were not considered to be toxicologically significant. There were no other statistically significant differences among groups for any of the other organs. Treatment-related histopathology was noted in the liver and thyroid of the F1 generation males at 500 ppm. Histopathologic changes were also seen in the kidneys of the F1 males at 500 ppm, with no associated changes in kidney organ weights.

For the F1 females, the following statistically significant differences were noted in the organ weights from treated animals relative to the controls. At 10 ppm, the absolute and relative pituitary weights were decreased (approximately 8% and 7%, respectively, $p<0.05$), while at 75 ppm pituitary weights (absolute only) were decreased (approximately 8%, $p<0.05$). Based on the magnitude of the pituitary changes noted at 10 and 75 ppm and the very small size of the organ in question, the decreases were not considered to be toxicologically relevant. At 75 ppm, absolute and relative liver weights were also slightly increased, with no histopathologic lesions in the liver at that dose and therefore were considered treatment-related, but not adverse. Absolute organ weight changes observed at 500 ppm in the spleen and brain were not considered toxicologically relevant based on body weight changes observed at this dose. At 500 ppm, increased liver (absolute and relative) and thyroid (relative only) weights were noted; and decreased pituitary (absolute and relative, approximately 21% and 17%, respectively, $p<0.001$) weights were observed. Associated histopathology was only found in the liver and thyroid of the F1 generation females at the high dose. Histopathologic changes were also seen in the kidneys of the 500 ppm F1 females, with no associated changes in kidney organ weights.

TABLE 9. Selected Absolute and Relative Organ Weights for P Adults [a]

Organ	Dose Group (ppm in diet)			
	0	10	75	500
P Generation Males				
N	30	30	30	30
TBW[b] (g)	619.19	619.68	583.70	608.13
Absolute Organ Weights (g)				
Liver	23.5888	23.9215	22.6508	31.4250***
Adrenal	0.0625	0.0640	0.0626	0.0659
Thyroid	0.0313	0.0285	0.0314	0.0402***
Brain	2.2155	2.1958	2.1672	2.1816
Organ weight relative to terminal body weight (%)				
Liver	3.8056	3.8539	3.8816	5.1797***
Adrenal	0.0101	0.0104	0.0108	0.0110
Thyroid	0.0051	0.0046	0.0054	0.0066***
Brain	0.3604	0.3569	0.3750	0.3630
P Generation Females				
N	30	30	30	30
TBW[b] (g)	340.90	338.85	340.87	338.78
Absolute Organ Weights (g)				
Liver	17.9372	18.5559 (N=29)	20.4816***	30.5256***
Adrenal	0.0864 (N=29)	0.0866	0.0853	0.0963*
Thyroid	0.0269 (N=28)	0.0255	0.0280.	0.0316*
Brain	1.9965	1.9606 (N=29)	1.9726	1.9372
Organ weight relative to terminal body weight (%)				
Liver	5.2493	5.4844 ± 0.0838 (N=29)	6.0137***	8.9914***
Adrenal	0.0253 (N=29)	0.0256	0.0251	0.0284**
Thyroid	0.0079 (N=28)	0.0075	0.0082	0.0093**
Brain	0.5876	0.5810 (N=29)	0.5806	0.5739

a Weights adrenal are paired organ weights, b TBW = terminal body weight,
* Statistically different from control, $p<0.05$, ** Statistically different from control, $p<0.01$
*** Statistically different from control, $p<0.001$

TABLE 10. Selected Absolute and Relative Organ Weights for F1 Adults [a]

Organ	Dose Group (ppm in diet)			
	0	10	75	500
F1 Generation Males				
N	30	30	30	30
TBW[b] (g)	588.31	580.38	579.92	571.64
Absolute Organ Weights (g)				
Liver	22.8712	23.1675	23.5611	28.9539***
Spleen	0.8957	0.9019	0.8805 (N=29)	0.9024
Thyroid	0.0316	0.0323	0.0335	0.0418***
Brain	2.2254	2.1585*	2.1777*	2.1267***
Pituitary	0.0171	0.0164	0.0156**	0.0154** (N=29)
Organ weight relative to terminal body weight (%)				
Liver	3.8900	3.9794	4.0514	5.0575***
Spleen	0.1524	0.1555	0.1523 (N=29)	0.1585
Thyroid	0.0054	0.0056	0.0058	0.0073***
Brain	0.3808	0.3747	0.3806	0.3747
Pituitary	0.0029	0.0028	0.0027*	0.0027* (N=29)
F1 Generation Females				
N	30	30	30	30
TBW[b] (g)	349.64	345.86	338.81	331.32
Absolute Organ Weights (g)				
Liver	18.2012	18.6734	21.1933***	30.7981***
Spleen	0.6054	0.6216	0.6321	0.5308 **
Thyroid	0.0225	0.0200	0.0236 (N=29)	0.0250
Brain	1.8896 (N=29)	1.8968	1.8764	1.7719***
Pituitary	0.0209 (N=29)	0.0192*	0.0192*	0.0164***

Organ weight relative to terminal body weight (%)				
Liver	5.2073	5.4157	6.2578***	9.2717***
Spleen	0.1735	0.1807	0.1868	0.1608
Thyroid	0.0064	0.0058	0.0070 (N=29)	0.0076**
Brain	0.5418 (N=29)	0.5502	0.5557	0.5375
Pituitary	0.0060 (N=29)	0.0056*	0.0057	0.0050***

a Weights adrenal are paired organ weights. b TBW = terminal body weight
* Statistically different from control, p<0.05, ** Statistically different from control, p<0.01
*** Statistically different from control, p<0.001

b) Pathology

1) Macroscopic Examination:

P Generation: Gross necropsy observations included no apparent treatment or dose-related findings in any P generation males.

In P females, the following findings were noted. Alopecia (various locations) was seen in two to six females at 0, 10, 75, and 500 ppm, bilateral red ears (one female each at 75 and 500 ppm), chromodacryorrhea in one female at 75 ppm, and rust-colored fur in one female at 0 and 500 ppm. These findings were similar to those noted at clinical exam, but were not considered treatment-related. In addition, brown-colored kidneys were seen in the high dose group in 50% of the females (15/30), and brown-colored liver was seen in 60% (18/30) of the females in the high dose group.

F1 Generation: Gross observations for Fl males, which were examined at scheduled sacrifice, included no apparent treatment- or dose-related findings, except for an apparent increase in the incidence of hydronephrosis of the right kidney at 500 ppm of 20% compared to 0% in controls.

Findings at necropsy for the F1 females included alopecia across all groups (three, three, six, and two at 0, 10, 75, and 500 ppm, respectively), chromodacryorrhea (one at 0 ppm), enlarged cervix (two at 10 ppm), rust-colored fur in all groups (two, two, three, and one at 0, 10, 75, and 500 ppm, respectively), and enlarged uterus or vagina with fluid present at 0 (one), 10 (three), and 75 ppm (one), respectively (at 10 ppm, for one additional female, the fluid was present at the posterior end of the vagina, indicating a possible blockage). Similar findings at clinical exam were reported, but not considered treatment-related. In addition, for the F1 females in the 500 ppm group, dark brown liver (in 25/30; 83%) and dark brown kidney bilateral (in 16/30; 53%) were noted, as well as left adrenal with two dark red areas in one female.

2) Microscopic Examination: Treatment-related histopathologic findings were noted at 500 ppm in the liver and thyroid of the P females, F1 males, and F1 females. In addition, histopathology was noted in the kidneys of the high-dose F1 males and females. The report noted the following observations upon microscopic examination.

No treatment-related microscopic changes were observed at 10 and 75 ppm in the P and F1 generations.

P Generation: There were no treatment- or dose-related histopathologic findings in any P generation male organs examined, including reproductive organs, kidneys, liver, and thyroid gland.

Treatment-related histopathologic findings in P generation females included hepatocyte hypertrophy and thyroid follicular cell hypertrophy at 500 ppm (table 11).

F1 Generation: Treatment-related, histopathologic findings in Fl males included follicular cell hypertrophy at 500 ppm. Hepatocyte cytoplasmic alterations were also observed in livers at 500 ppm. The histopathologic findings in the kidney (hydronephrosis, nephropathy) were slightly increased at the high dose. An apparent increase in the incidence of hydronephrosis of the right kidney was also observed at gross necropsy in the 500 ppm F1 males (20% compared to 0% in controls).

At 500 ppm, treatment-related histopathologic findings noted in the Fl females included hepatocyte hypertrophy and follicular cell hypertrophy. Hydronephrosis was also observed in one female each at 0 and 75 ppm, with none at 10 or 500 ppm, and nephropathy was observed in four of ten kidneys at 500 ppm, with one, two, and one of ten each at 0, 10, and 75 ppm.

TABLE 11. Incidence of Selected Microscopic Findings for P and F1 Adults

Observation	Dose Group (ppm in diet)			
	0	10	75	500
P Generation Females				
LIVER - Number Examined	10	10	10	10
Hypertrophy, Hepatocyte	0	0	0	8
THYROID – Number Examined	10	10	10	10
Hypertrophy, Follicular Cell	0	0	0	5
F1 Generation Males				
LIVER - Number Examined	10	10	10	10
Cytoplasmic Alteration, Hepatocyte	0	0	0	3
THYROID – Number Examined	10	10	10	10
Hypertrophy, Follicular Cell	0	0	0	9
KIDNEY – Number Examined	10	11	10	10
Hydronephrosis	1	1	1	3
Nephropathy	8	6	6	10
F1 Generation Females				
LIVER - Number Examined	10	10	10	10
Hypertrophy, Hepatocyte	0	0	0	8
THYROID – Number Examined	10	10	10	10
Hypertrophy, Follicular Cell	0	0	0	9
KIDNEY – Number Examined	10	10	10	10
Nephropathy	1	2	1	4

B. OFFSPRING

Viability and Clinical Signs: No treatment-related effects were seen at any dose level in F1 and F2 pups.

In the F1 litters, there were no test article-related effects on the number of implantation sites per dam, the postimplantation loss per litter, number of live and dead pups per litter on Lactation Day 0, or on the number of litters delivered (table IIA 5.6.1-12). In addition, the live birth index, day 4, 7, 14, and 21 survival indices, and lactation index were all statistically equivalent across all groups. The sex ratio (% males per litter) and average number of pups per litter on Lactation Days 0, 4, 7, 14, and 21 were also statistically equivalent across all groups. The stillbirth index in F1 litters appeared to be slightly elevated at 500 ppm; 5.0 vs 3.9 in controls, translating to approximately 28% increase, however these apparent differences were not statistically significant. This parameter is also slightly higher than the range provided with the historical database (0.2 - 2.0, with one study value of 10.2, considered to be an outlier). The toxicological significance of this observation could not be determined.

In the F2 litters, the percent prenatal (postimplantation) loss was significantly increased at 75 ppm (11.91%, $p<0.01$) compared to F2 controls (4.68%), however as this value was comparable to percent postimplantation loss observed in F1 controls (9.47%), and was also not subsequently observed at 500 ppm, this finding was not considered treatment related. The mean number of live pups per litter was significantly reduced on Lactation Day 0 at 75 ppm (13.9) and at 500 ppm (14.0); however, they were equivalent across all dose groups for the remainder of the lactational period and well within the historical control range (12.3-15.6). No treatment-related effects were noted on any other offspring viability parameter examined (table 12). The stillbirth index in F2 litters appeared to be elevated at 500 ppm; 5.3 vs 0.8 in controls, translating to approximately 563% increase, however these apparent differences were not statistically significant. This parameter is also slightly higher than the range provided with the historical database (0.2 - 2.0, with one study value of 10.2, considered to be an outlier). The toxicological significance of this observation could not be determined.

There were no significant treatment-related clinical signs noted during lactation at any dose level in the F1 or F2 pups. The anogenital distance of the F2 offspring at birth was measured and was found to be equivalent across all groups.

TABLE 12. Litter Parameters for F_1 and F_2 Generations

Observation	Dose Group (ppm in diet)			
	0	10	75	500
F1 Litters				
No. Live Litters Postnatal Day 0	29	29	29	26
No. Live Litters Postnatal Day 21	28 [a]	29	29	26
No. Implantation Sites per Litter	14.83 N=29	15.69 N=29	16.62 N=29	15.70 N=27
Percent Postimplantation Loss per Litter	9.47 N=29	10.02 N=29	7.58 N=29	13.49 N=27
Mean Number of Pups/Litter:				
Live Pups on Day 0	14.1	14.4	15.4	14.7
Dead Pups on Day 0	0.6	0.3	0.3	0.8
Total Pups on Day 0	14.7	14.8	15.8	15.5
Live Pups on Day 4 [b]	13.8	14.1	15.1	14.3
Live Pups on Day 7	9.5	9.6	9.9	9.7
Live Pups on Day 21	9.5	9.4	9.8	9.6
Stillbirth Index	3.9	2.2	2.0	5.0
Live Birth Index (no. live on pnd 0/total no. on pnd 0)	96.1	97.8	98.0	95.0
Lactational Index (no. surviving 21 days/no. live on pnd 4)	99.3	97. 6	97.6	96.9
Percent Male Pups per Litter (Day 0)	46.5	51.1	53.7	55.1
Percent Male Pups per Litter (Day 21)	46.3	50.1	51.4	50.1
F2 Litters				
No. Live Litters Postnatal Day 0	25 [c]	28	29	28
No. Live Litters Postnatal Day 21	24	28	29	28
No. Implantation Sites per Litter	15.08	16.57	15.59	15.32
Percent Postimplantation Loss per Litter	4.68	7.51	11.91 **	9.33
Mean Number of Pups/Litter:				
Live Pups on Day 0	15.6	15.4	13.9*	14.0*
Dead Pups on Day 0	0.1	0.3	0.4	0.7
Total Pups on Day 0	15.7	15.7	14.3	14.8
Live Pups on Day 4 [b]	14.7	15.3	13.6	13.6
Live Pups on Day 7	9.7	9.9	9.8	9.5
Live Pups on Day 21	9.5	9.5	9.8	9.5
Stillbirth Index	0.8	1.6	2.9	5.3
Live Birth Index (no. live on pnd 0/total no. on pnd 0)	99.2	98.4	97.1	94.7
Lactational Index (no. surviving 21 days/no. live on pnd 4)	98.3	95.4	99.7	99.3
Percent Male Pups per Litter (Day 0)	47.4	50.0	49.1	53.9
Percent Male Pups per Litter (Day 21)	49.6	50.4	48.2	52.4

a Entire litter for one P generation control dam was found dead or missing and presumed dead on/or before pnd 1.
b Before standardization (culling) on Day 4.
c The postnatal day 0 date for one F1 generation control dam was inadvertently missed, and therefore none of her litter data could be included since postnatal day 0 was not known. She was included as a sperm positive, pregnant female with a live litter, and her number of implantation sites was also included.

* Statistically different from control, $p<0.05$ ** Statistically different from control, $p<0.01$

2. **Body Weight:** There were no treatment-related effects on F1 and F2 pup body weights at 10 or 75 ppm.

   F1 Pups: Mean pup body weights per litter were statistically equivalent across all groups (for all pups or separately by sex) at birth (Day 0). Beginning on Lactation Day 4 and for Lactation Days 7, 14, and 21, pup body weights per litter (for all pups and separately by sex) were significantly reduced at 500 ppm (table 13). The second week of the lactational period corresponds to the time the pups generally begin to self feed, so they were likely to be directly exposed to the test material by ingestion, as well as potentially via the milk, during the second and third weeks of lactation.
   F1 Weanlings: F1 weanling body weight was significantly decreased in females at 75 ppm (approximately 6%). F1 weanling body weight was also decreased at 500 ppm in males and females (approximately 19% and 18%, respectively).

   F2 Pups: At 500 ppm, F2 pup body weights per litter were significantly reduced when analyzed as all pups or separately by sex for Lactation Days 14 and 21 (table 13).

   F2 Weanlings: F2 weanling body weight was significantly decreased at 500 ppm in males and females, each group by approximately 13% compared to control values.

TABLE 13. Mean Pup Weights (g)

Lactation Day	Pup Gender	Dose Group (ppm in diet)			
		0	10	75	500
F1 Pups					
Day 0	Both Sexes	6.43	6.37	6.36	6.31
	Males	6.62	6.58	6.57	6.48
	Females	6.26	6.17	6.13	6.10
Day 4	Both Sexes	10.20	9.97	9.75	8.98*
	Males	10.47	10.17	10.00	9.14*
	Females	9.91	9.76	9.45	8.76*
Day 7	Both Sexes	16.24	15.63	15.15	12.96***
	Males	16.68	15.92	15.68	13.21 ***
	Females	15.85	15.30	14.63	12.68 ***
Day 14	Both Sexes	32.76	31.75	31.15	26.66 ***
	Males	33.40	32.31	31.78	27.13***
	Females	32.24	31.18	30.49	26.18***
Day 21	Both Sexes	51.25	50.86	48.14	41.36***
	Males	52.78	52.12	49.64	42.34 ***
	Females	50.11	49.37	46.59*	40.36 ***
F2 Pups					
Day 0	Both Sexes	6.16	6.24 ± 0.08	6.47	6.30
	Males	6.34	6.42 ± 0.0 8	6.65	6.46
	Females	6.00	6.06 ± 0.0 8	6.30	6.12
Day 14	Both Sexes	32.62	33.13	33.42	29.49 ***
	Males	33.27	33.84	34.25	30.01 ***
	Females	31 .93	32.37	32.65	28.84 **
Day 21	Both Sexes	49.92	50.60	51.19	43.64 ***
	Males	51.21	51.80	52.90	44.32 ***
	Females	48.62	49.32	49.45	42.64 ***
	Females	48.62	49.32	49.45	42.64 ***

* Statistically different from control, $p<0.05$, ** Statistically different from control, $p<0.01$
*** Statistically different from control, $p<0.001$

3. **Sexual Maturation (F_1):** Fl offspring (30/sex/group) selected to become parents of the F2 litters were examined daily for vaginal patency or preputial separation until acquisition, starting at postnatal day 22 (for females) or 35 (for males). Each animal was weighed on the day of acquisition. Age of acquisition for females was not delayed; however, body weight at acquisition was significantly reduced for Fl females at 500 ppm (table 14). Age of acquisition of preputial separation was significantly delayed for males at 500 ppm, but body weight at acquisition was not affected. ANCOVA of age at acquisition, with body weight at acquisition as the covariate, indicated that puberty was significantly delayed for both sexes at 500 ppm; by 2.1 days for females and 2.9 days for males.

TABLE 14. F1 Female Vaginal Opening and Male Preputial Separation and F2 Anogenital Distance on Postnatal Day 0

Observation	Dose Group (ppm in diet)			
	0	10	75	500
Female vaginal opening				
N° females evaluated	30	30	30	30
Day of vaginal opening	31.3	31.6	31.5	32.0
Body weight (g) on day of acquisition	110.9	110.5	104.9	96.9**
Adjusted day of vaginal opening	30.8	31.1	31.6	32.9***
Average female anogenital distance (mm) per litter	2.05	2.09	2.08	2.06
Male preputial separation				
N° males evaluated	30	30	30	30
Day of preputial separation	40.9	41.4	41.4	43.6***
Body weight (g) on day of acquisition	206.9	206.0	206.1	205.4
Adjusted day of preputial separation	40.8	41.4	41.4	43.7***
Average male anogenital distance (mm) per litter	0.97	0.98	0.97	0.98

* Statistically different from control, $p<0.05$, ** Statistically different from control, $p<0.01$,
*** Statistically different from control, $p<0.001$

4. **Offspring Postmortem Results:**

a) **Organ Weights:** The following differences from the controls in organ weights were observed in the F1 and F2 weanlings at necropsy. The effects noted were attributed primarily to reduced pup body weights at the high dose.

F1 Offspring: Fl weanling male body weight was significantly reduced at 500 ppm (by approximately 19%. In male weanlings, the absolute mean thymus weight was significantly reduced at 500 ppm. However, relative male thymus weights were equivalent across all groups, indicating that the finding at 500 ppm was possibly related to the decreased body weight at that dose. Absolute spleen, paired kidneys, and brain weights were significantly reduced at 500 ppm. Absolute liver weights were unaffected. Relative spleen and paired kidney weights were significantly decreased at 500 ppm; however, relative liver and brain weight were significantly increased at 500 ppm (table 15).

Fl weanling female body weight was significantly reduced at 75 and 500 ppm (by approximately 6% and 18%, respectively). Absolute female thymus, brain, and spleen weights were also significantly reduced at 500 ppm, and absolute brain weight was significantly reduced at 10 ppm (table 15). Absolute paired kidney weights were significantly reduced at 75 and 500 ppm. Absolute liver weights were equivalent across all groups. Female relative paired kidney weight was decreased at 500 ppm. Relative brain and

liver weights were significantly increased at 500 ppm, indicating that these findings were related to the decreased body weight at that dose. Relative spleen weights were unaffected.

F2 Offspring: F2 male weanling body weight was significantly reduced at 500 ppm (87% of the control value). Absolute male thymus, spleen, paired kidney, and brain weights were also slightly but significantly reduced at 500 ppm, most likely due to the reduced body weight at this dietary dose (table 15). Relative liver and brain weight was significantly increased at 500 ppm; relative liver weight was also increased at 75 ppm. Relative paired kidney weight was significantly decreased at 500 ppm.

The F2 female weanling body weight was significantly reduced at 500 ppm (87% of the control value). Absolute thymus, spleen, paired kidney and brain weights were significantly decreased at 500 ppm (table 15). Relative paired kidney weight was significantly reduced at 500 ppm, and relative liver and brain weight was significantly increased at 500 ppm. The effects on absolute and relative organ weights at 500 ppm are considered most likely due to the reduced animal body weight at this dietary dose.

TABLE IIA 15. Relative Organ Weights to Terminal Body Weight (%) for F1 and F2 pups on Postnatal Day 21

Organ	Dose Group (ppm in diet)			
	0	10	75	500
F1 Males				
N	71	82	85	75
TBW[a] (g)	51.3	52.5	49.8	41.6***
Liver	4.6666	4.7439	4.6639	5.3575***
Spleen	0.4299	0.4300	0.4452	0.3867**
Kidney	1.3968	1.3751	1.3786	1.2896***
Brain	2.9969	2.9306	3.0645	3.5000***
Thymus	0.4245	0.4221	0.4220	0.4240
F1 Females				
N	80	79	84	74
TBW[a] (g)	48.53	46.99	45.67	39.80***
Liver	4.8079	4.9151	4.8041	5.6984***
Spleen	0.4202	0.4388	0.4343	0.4081
Kidney	1.4808	1.4529	1.4556	1.3608***
Brain	3.0716	3.0844	3.1982	3.5490***
Thymus	0.4465	0.4620	0.4536	0.4452
F2 Males				
N	70	84	83	82
TBW[a] (g)	50.45	51.33	52.73	43.98***
Liver	4.4587	4.5134	4.7183*	5.0813***
Spleen	0.4231	0.4284	0.4252	0.3954
Kidney	1.3461	1.3656	1.3291	1.2280***
Brain	3.0093	2.8852	2.8954	3.3014***
Thymus	0.4069	0.4188	0.4272	0.4155
F2 Females				
N	70	82	84	82
TBW[a] (g)	47.85	48.68	48.63	41.68***
Liver	4.7870	4.7971	4.9094	5.4452***
Spleen	0.4342	0.4438	0.4265	0.4010
Kidney	1.4491	1.4463	1.4275	1.3140***
Brain	3.0876	2.9699	3.0200	3.3832***

Thymus	0.4511	0.4597	0.4517	0.4502

a TBW = terminal body weight; * Statistically different from control, p<0.05; ** Statistically different from control, p<0.01; *** Statistically different from control, p<0.001

b) Pathology

1) **Macroscopic Examination:** There were no treatment-related findings at gross necropsy for the F1 or F2 pups available for examination at any dose level.

2) **Microscopic Examination:** Selected organ tissues from the F1 and F2 offspring were fixed and retained, but not examined microscopically.

III. CONCLUSIONS: Following dietary exposure to ethiprole for two generations, one litter per generation, at 0, 10, 75, and 500 ppm, systemic toxicity in the P and F1 parental generations was observed at 500 ppm. The parental systemic LOAEL is 500 ppm (32.33 - 39.63 mg/kg bw/day in males, 37.36 – 45.20 mg/kg bw/day in females), based on decreased body weights and occasionally decreased weight gain, organ weight changes (liver, thyroid, adrenals, pituitary) and liver, thyroid and kidney histopathology (findings noted in one or both generations and sexes). The parental systemic NOAEL is 75 ppm (4.77 - 6.03 mg/kg bw/day in males, 5.82 – 6.76 mg/kg bw/day in females).

The offspring LOAEL is 500 ppm (32.33 - 39.63 mg/kg bw/day in males, 37.36 – 45.20 mg/kg bw/day in females), based on decreases in F1 and F2 pup body weights during lactation (beginning on Day 4 for F1 and on Day 14 for F2 offspring) and into the postwean/prebreed period, with associated delays in acquisition of puberty. Additional changes in organ weights (thymus, spleen, kidneys, brain, liver) were observed in weanlings, but largely attributed to body weight effects (with exception of the liver). The offspring NOAEL is 75 ppm (4.77 - 6.03 mg/kg bw/day in males, 5.82 – 6.76 mg/kg bw/day in females).

No adverse effects on reproductive performance were noted at any dose level in either generation. Subtle changes in estrous cycle parameters noted in both the parental and F1 generations did not translate to changes in reproductive potential and therefore dismissed. The reproductive NOAEL is 500 ppm (32.33 - 39.63 mg/kg bw/day in males, 37.36 – 45.20 mg/kg bw/day in females).

KIIA 5.3.1 - Oral 28-day toxicity

1.	Annex point(s)	KIIA 5.3.1; Short-term toxicity/Oral 28-day toxicity: **MRID 47622804**
2.	Location in dossier	KIIA 5.3.1 /03
3.	Authors (year)	Dange, M. (2001)
	Title	28-day toxicity study in the rat by dietary administration Ethiprole (RPA107382)
	Company, report No.	Bayer CropScience AG, Report-No.: C011658 (M-201261-01-1)
	Date	17.01.2001
4.	Testing facility	Aventis CropScience S.A., Lyon, France
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole (radioactive), ethiprole Substance(s): ethiprole TC ethiprole (radioactive) TC
7.	Test method	EU (=EEC): 92/69 Annex V; OECD: 407
	Deviations	
8.	GLP	yes

Executive summary:

Ethiprole was administered continuously via the diet at 0, 5, 100, 500 or 2500 ppm to separate groups of 10 rats /sex/dose. Mortality and clinical signs were checked daily. Body weight and food consumption were measured weekly throughout the study. Ophthalmic examinations were performed on all animals during the acclimatization period and during Week 4 of dosing. Clinical chemistry, hematology and urinalysis were performed towards the end of the dosing phase. In addition, a blood sample was collected during Weeks 4 for T3, T4 and TSH analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues sampled and examined microscopically.

The short-term dietary administration of ethiprole (RPA 107382) to rats for at least 28 days produced signs of overt toxicity at 2500 ppm as evidenced by the premature death of two males.

Ethiprole induced no treatment-related clinical signs. The body weight evolution of animals at 2500 ppm was inferior to that of controls throughout the study in males and only during the first week of treatment in females. At 2500 ppm, statistically significant lower food consumption was noted in males during the first two weeks and in females during the first week.

Increases in prothrombin time were noted in males at 500 and 2500 ppm. A tendency towards higher platelet counts was noted in males and females at 2500 ppm. Increases in alanine amino-transferase (ALAT), cholesterol, triglycerides and total protein were observed in females at doses of 500 and 2500 ppm. In males, increased ALAT, cholesterol, and total protein were noted only at 2500 ppm, along with a decrease in albumin.

Ethiprole induced an imbalance of thyroid hormones in animals treated at 500 and 2500 ppm resulting in higher levels of TSH and T3 (2500 ppm only) and lower levels of T4 in both sexes.

Significant increases in absolute and relative thyroid and adrenal weights were observed in both males and females at 500 and 2500 ppm, Liver weights were also increased in males at ≥500 ppm, and in females at ≥100 ppm.

Liver enlargement and dark liver colour were noted in males and females at 500 and 2500 ppm, and dark kidney colour was seen in all males and females at 2500 ppm.

At the histopathological examination diffuse centrilobular to panlobular hepatocellular hypertrophy was observed in the livers of all males and almost all females at ≥500 ppm. Diffuse follicular hypertrophy in the thyroid was also noted in all animals at ≥500 ppm. An increased diffuse vacuolation of the adrenal cortex was noted in both sexes at ≥500 ppm. The kidney was also affected at 2500 ppm with both sexes showing tubular golden brown pigment.

In conclusion, the NOAEL is 9.2 mg/kg/day in males and 9.6 mg/kg/day in females (100 ppm in diet), based on slight changes in liver and adrenals weights without any untoward histological changes. The NOEL is 2 mg/kg/day in males and 1.8 mg/kg/day in (20 ppm in diet). The LOAEL is 46.1/46.3 mg/kg bw/day (500 ppm in diet), based on changes in hematology, clinical chemistry, and urinalysis parameters, and increased liver, thyroid and adrenal weights with associated histopathology.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3050; OECD 407) for a subchronic oral toxicity study in the rat.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)
   Description: White powder
   Lot/Batch #: CRD9706
   Purity: 93%
   Compound Stability: The test substance was stored in an air-tight, light resistant container at room temperature
   CAS # of TGAI: 181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. Control animals received untreated diet.

3. **Test Animals:**
   Species: Rats
   Strain: Wistar rats R.J: WI (IOPS AF)
   Age/weight at study initiation: 6 weeks old / 158 - 203 g males, 151 - 171 g females
   Source: R. Janvier (Le Genest St Isle, France).
   Housing: Individually in suspended stainless steel, wire mesh cages.
   Diet: Certified rodent powder diet "M 20 control" (Pietrement, Provins, France), *ad libitum*
   Water: Filtered and softened water from the municipal water supply, *ad libitum*
   Environmental conditions:
   Temperature: 20°C - 24°C (target)
   Humidity: 40% - 70% (target)
   Air changes: 10 to 15 changes per hour (average, not monitored)
   Photoperiod: 12 hrs dark/ 12 hrs light
   Acclimation period: 6 days

B. STUDY DESIGN:

1. **In-Life Dates:** Start: September 07, 1999; End October 08, 1999

2. **Animal Assignment/Dose Levels:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution by sex to the test groups noted in Table IIA 5.3.1.2-1.

TABLE IIA 5.3.1.2-1 Study Design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day) [a]		No. of Animals	
		Males	Females	Males	Females
Control	0	0	0	10	10
Low	20	1.8	2.0	10	10
Mid-1	100	9.2	9.6	10	10

Mid-2	500	46.1	46.3	10	10
High	2500	219.3	220.2	10	10

a Mean achieved dietary intake of ethiprole (RPA 107382), weeks 1 to 4

3. **Diet Preparation and Analysis:** The test substance was ground to a fine powder before being incorporated into the diet (Certified Rodent Pellet diet "M 20 control") by dry mixing to provide the required concentrations. Diet formulations were prepared one time for the study at four concentrations: 20, 100, 500 and 2500 ppm. When not in use, the diet formulations were stored at approximately -18° C.

   The stability of ethiprole in diet was demonstrated in a previous study at 5 and 2500 ppm (RPA 107382, 90-day toxicity in the rat by dietary administration, SA 97233) under storage and usage conditions similar to those of the current study.

   During the current study, the homogeneity of the 20 and 2500 ppm preparations were checked. Sampling was done on three levels, the surface (H), middle (M) and bottom (B). At each level, 2 samples (1 & 3) were taken and analyzed. Mean values obtained from the homogeneity checks were used as measured concentrations. The concentration of the other preparations was also checked. Sampling was done on 3 levels (the surface, middle and bottom) and pooled.

   Results: **Homogeneity Analysis:** Results for homogeneity of ethiprole in the 20 and 2500 ppm dose level diet formulations were within the range of 98-114% of the nominal concentrations. (Data obtained from the study report, page 350).

   Stability Analysis: Stability results for up to 38 days at room temperature and 31 days of frozen storage followed by 7 days at ambient temperature ranged from 88-99% of the nominal concentrations and were thus within the target range of 85-115% of nominal concentration (see Study Profile for Study Number SA 97233).

   Concentration Analysis: Results for the concentration analyses were within 103-110% of the nominal concentrations. (Data obtained from the study report, page 351).

4. **Statistics**: Statistical analyses were performed on the following endpoints: body weight parameters, food consumption, hematology parameters (except eosinophils, basophils, monocytes and large unstained cells), clinical chemistry parameters, urinary parameters (only pH, volume and refractive index), organ weight parameters, and hormone data.

   Since the above parameters were all measured on a continuous scale, the treatment groups were compared using a combination of parametric ANOVA and nonparametric data analysis techniques. The homogeneity of variance assumption was examined via Bartlett's test, implemented through a user-written SAS® macro. If Bartlett's test rejected the homogeneity of variance assumption ($p<0.05$), nonparametric tests was used. Nonparametric tests include the Kruskal-Wallis test for overall treatment group differences, followed by the Mann-Whitney U test for individual exposed vs. control group comparisons, if the Kruskal-Wallis test was significant ($p<0.05$). All nonparametric tests were performed using the NPAR1WAY procedure in SAS® 6.12 (SAS Institute Inc., 1989, 1996).

If Bartlett's test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 was used to evaluate the overall effect of treatment, and, when a significant treatment effect was present ($p<0.05$), to compare each exposed group to control via Dunnett's test.

C. METHODS:

1. Observations:

1a. Cageside Observations and Clinical Examinations: All animals were checked for moribundity and mortality twice daily (once daily on week-ends or public holidays). Observed clinical signs were recorded at least once daily for all animals. Detailed physical examinations were performed at least weekly during the treatment period. The nature, onset, severity, reversibility and duration of clinical signs were recorded. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose feces.

1b. Neurological Evaluations: Neurotoxicity assessments (grasping, righting, corneal, pupillary, auditory startle, and head shaking reflexes) were performed during the acclimatization phase and during Week 3 of the study.

2. Body weight: Each animal was weighed once during the acclimatization period, on the first day of test substance administration, then at weekly intervals throughout the treatment period, and before necropsy.

3. Food Consumption and Compound Intake: The weights of food supplied and of that remaining at the end of the food consumption period was recorded weekly for all animals during treatment. The mean achieved intake in mg/kg/day for each week and for weeks 1-4 was calculated.

4. Ophthalmoscopic Examination: During the acclimatization period all animals were subjected to an ophthalmic examination. After instillation of an atropinic agent (Mydriaticum, Merck Sharp and Dohme) each eye was examined by means of an indirect ophthalmoscope. During week 4, all surviving animals from control and high dose groups were re-examined.

5. Hematology & Clinical Chemistry: On Study Days 23, 24 or 25, blood samples were taken from all surviving animals in all groups by puncture of the retro-orbital venous plexus. An approximately equal number of animals randomly distributed amongst all groups were sampled on each day. Animals were fasted overnight prior to bleeding and anesthetized by inhalation of isoflurane. Blood was collected on EDTA for hematology (0.5 ml), on lithium heparin for plasma chemistry (3.0 ml) and on sodium citrate for coagulation parameters (0.9 ml). The Checked (X) parameters listed below were examined.

In addition, a blood sample was collected into tubes containing heparin during Week 4 from the retro-orbitalvenous plexus of each surviving animal following overnight fasting. After separation, plasma samples were frozen and kept for T3, T4 and TSH determinations.

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	Blood clotting measurements	X	Reticulocyte count (smear prepared but not examined)
X	(Platelet count)		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)	X	Thyroid Hormones (T3, T4, and TSH)
X	Serum alanine amino-transferase (ALAT/also SGPT)		
X	Serum aspartate amino-transferase (AST/also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Ornithine decarboxylase		

6. **Urinalysis:** On study Days 29, 30, 31 or 32, in the morning, prior to sacrifice, overnight urine samples were collected from all surviving animals in all groups. An approximately equal number of animals randomly distributed amongst all groups were sampled on each day. Feed and water were not accessible during urine collection. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen
X	Refractive index		

7. **Sacrifice and Pathology**: On study Days 29, 30, 31 or 32, all surviving animals from all groups were sacrificed by exsanguination under deep anesthesia (pentobarbital). An approximately equal number of animals randomly distributed amongst all groups were sacrificed on each day. Animals were fasted overnight prior to sacrifice. The Checked (X) tissues were collected for histological examination and the (XX) organs, in addition, were weighed. All listed tissues were examined microscopically for all the animals in the control and high dose groups and for any animal which died prior to the scheduled sacrifice, regardless of dose group. In addition, lung, liver, thyroid gland, kidney and adrenal gland

tissues were examined in the intermediate dose groups to identify the no-effect-level. Macroscopic findings were also examined in all intermediate dose groups.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta	X, XX	Brain
X	Salivary glands	X, XX	Heart	X	Periph.nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X, XX	Pituitary
X	Duodenum	X, XX	Spleen	X	Eyes (optic n.)
X	Jejunum	X, XX	Thymus		**GLANDULAR**
X	Ileum			X, XX	Adrenal gland
X	Cecum		**UROGENITAL**	X	Hardarian gland
X	Colon	X, XX	Kidneys	X	Mammary gland
X	Rectum	X	Urinary bladder	X, XX	Parathyroids
X, XX	Liver	X, XX	Testes +	X, XX	Thyroids
	Gall bladder	X, XX	Epididymides		**OTHER**
X	Pancreas	X, XX	Prostate	X	Bone
	RESPIRATORY	X	Seminal vesicle	X	Skeletal muscle
X	Trachea	X, XX	Ovaries	X	Skin
X	Lung	X, XX	Uterus	X	All gross lesions and masses
	Nose	X	Vagina	X	Target Organs
	Pharynx				
X	Larynx				

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** No treatment-related clinical signs were observed at any dose level in either sex, with the exception of the two males which were found dead or sacrificed moribund at 2500 ppm. These animals presented with a general pallor before death.

2. **Mortality:** Treatment-related mortalities were observed in males at 2500 ppm.

   One male at 2500 ppm was found dead on Day 25 and a second one was sacrificed moribund on Day 15.

No treatment-related mortalities were observed in males at 20, 100 or 500 ppm and in females at any dose level.

3. **Neurological Evaluations**: No treatment-related effects were observed in any of the reflexes assessed at any dose level in either sex.

B. **BODY WEIGHT**: Treatment-related decreases in body weight were observed at 2500 ppm throughout the study in males and only during the first week of treatment in females. Body weight gains were also reduced in both sexes during the first week of the dosing phase. Overall body weight gain was lower in males at 2500 ppm (↓18%); however terminal body weight in females treated at 2500 ppm was similar to controls (Table IIA 5.3.1.2-2).

No treatment-related changes in body weight and body weight gain at were observed at 20, 100 and 500 ppm in either sex.

TABLE IIA 5.3.1.2-2 Mean Body Weights (g) and Body Weight Gains (g/day)

Interval (Week)	Dose Level (ppm in diet)				
	0	20	100	500	2500
Males					
Body Weight (g)					
0	190.4	188.3	191.7	192.0	191.2
1	246.6	247.0	243.6	241.4	209.4 ** (↓15%)
2	299.3	300.1	295.7	296.3	261.7 ** (↓13%)
3	340.3	340.9	336.2	332.8	311.1 ** (↓9%)
4	358.0	359.3	353.0	350.9	328.0 ** (↓8%)
Mean Body Weight Gain/Day (g)					
1	56.2	58.7	51.9	49.4	18.2 **
2	52.7	53.1	52.1	54.9	52.3
3	41.0	40.8	40.5	36.5	48.7 **
4	17.7	17.9	16.8	18.1	17.9
Overall body weight gain (% of control	-	102	96	95	82
Females					
Body Weight (g)					
0	159.7	160.2	160.0	159.3	159.1
1	184.2	185.1	185.5	189.3	165.8 ** (↓10%)
2	203.9	207.6	205.8	209.6	194.8
3	214.4	220.8	220.2	227.3	214.4
4	225.6	226.9	229.5	237.8	226.1
Mean Body Weight Gain/Day (g)					
1	24.5	24.9	25.5	30.0	6.7 ***
2	19.7	22.5	20.3	20.3	29.0 **
3	10.5	13.2	14.4	17.7 *	19.6 *
4	11.2	6.1	9.3	10.5	11.7
Overall body weight gain % of control	-	101	105	119	102

Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control. *** Statistically different ($p<0.001$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food Consumption:** Treatment-related decreases in food consumption were observed at 2500 ppm during the first two weeks of dosing in males and only during the first week of treatment in females (Table IIA 5.3.1-11).

No treatment-related changes in food consumption were observed at 20, 100 and 500 ppm in either sex.

TABLE IIA 5.3.1-11 Mean Food Consumption (g/day)

Interval (Week)	Dose Level (ppm in diet)				
	0	20	100	500	2500
Males					
1	24.8	25.0	24.7	24.8	15.8 *** (↓36%)
2	27.3	27.1	26.8	26.9	23.8 *** (↓13%)
0-4	26.5	26.2	26.0	26.1	22.6 *** (↓15%)
Females					
1	18.7	19.4	19.1	18.4	12.8 *** (↓32%)
2	18.5	20.6 **	19.7	19.1	19.0
0-4	18.4	19.6	19.3	18.9	17.2

* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control. *** Statistically different ($p<0.01$) from the control.

2. **Compound Consumption (time-weighted average):** The mean achieved dose levels expressed in mg/kg body weight day are presented in Table IIA 5.3.1.2-3.

TABLE IIA 5.3.1.2-3 Mean Achieved Dietary Intake of Ethiprole (Weeks 1-4)

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	
		Males	Females
Control	0	0	0
Low	20	1.8	2.0
Mid-1	100	9.2	9.6
Mid-2	500	46.1	46.3
High	2500	219.3	220.2

D. **OPHTHALMOSCOPIC EXAMINATION:** No treatment-related changes were observed at the ophthalmological examination at any dose level in either sex.

E. **BLOOD ANALYSES:**

1. **Hematology:** At the end of the dosing phase, treatment-related effects were observed at 500 and 2500 ppm (Table IIA 5.3.1.2-4).

Prothrombin times were higher in males at 500 and 2500 ppm (+44% and +120%, respectively, $p<0.001$). In addition a tendency towards higher platelet counts was noted at 2500 ppm in males (+24%, $p<0.05$) and in females (+ 17% higher, $p<0.01$).

The decreased prothrombin times observed in females at 100 and 500 ppm were considered to be incidental and unrelated to treatment as they were not dose-related and/or not biologically relevant and the individual values remained in the normal range for rats this strain and age.

Some other slight variations in erythrocyte parameters were noted at 500 and 2500 ppm. However, as these changes were of low magnitude and high inter-individual variability was noted, they were not considered toxicologically significant. Other statistically significant changes found in the hematological parameters were considered to be incidental and unrelated to treatment as they were not dose-related and/or not biologically relevant.

No treatment-related hematological changes were observed at 20 and 100 ppm.

TABLE IIA 5.3.1.2-4 Mean Prothombin Time and platelet counts

Interval	Parameter	Dose Level (ppm in diet)				
		0	20	100	500	2500
Males						
Number of animals examined		10	9	8	10	9
Week 4	PLT (10^9/L)	1244.5	1233.9	1155.5	1359.0	1542.1*
	PT (s)	12.40	12.83	12.93	17.80***	27.32***
Females						
Number of animals examined		9	8	9	9	10
Week 4	PLT (10^9/L)	1259.8	1268.6	1353.2	1355.8	1475.4**
	PT (s)	13.45	12.74	11.98**	12.00**	14.67

* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control. *** Statistically different ($p<0.01$) from the control.

2. **Clinical chemistry:** At the end of the dosing phase, treatment-related effects were observed at 500 and 2500 ppm (Table IIA 5.3.1.2-5).

Higher mean alanine aminotransferase activity was observed in males at 2500 ppm (+364%, $p<0.001$) and in females at 500 (+52%, $p<0.01$) and 2500 ppm (+59%, $p<0.001$). Mean total cholesterol concentrations were higher in males at 2500 ppm (+112%, $p<0.001$) and in females at 500 and 2500 ppm (+69% and +147%, $p<0.001$). In females, these changes were associated with higher mean triglycerides concentrations at 500 and 2500 ppm (+84% and +249%, respectively, $p<0.001$). Mean total protein concentrations were higher in females at 500 and 2500 ppm (+ 13% and +16%, $p<0.001$). A slight non-toxicologically significant increase was observed in males at 2500 ppm (↑7%) and in females at 100 ppm (↑7%). Lower mean albumin concentration was noted in males at 2500 ppm (- 9%, $p<0.001$).

Other statistically significant variations noted in clinical chemistry parameters were of low magnitude and/or incidence and/or not dose-related, thus they were not considered to be toxicologically significant.

No treatment-related biochemical changes were observed at 20 and 100 ppm.

TABLE IIA 5.3.1.2-5 Selected clinical chemistry parameters in rats at Week 4

Interval	Parameter	Dose Level (ppm in diet)				
		0	20	100	500	2500
Males						
Number of animals examined		10	9	10	10	9
	ALAT (IU/l)	23.1	21.6	23.3	27.3	107.1***
	CHOL (mmol/l)	1.902	1.763	1.819	2.052	4.028***
	TRIG (mmol/l)	0.988	0.890	0.835	0.906	1.270
	TPROT (g/l)	63.5	61.6	62.8	65.6	68.1**
	ALB (g/l)	33.9	33.8	33.4	33.1	30.9***
Females						
Number of animals examined		10	10	10	10	10
	ALAT (IU/l)	20.6	20.4	21.3	31.4**	32.7***
	CHOL (mmol/l)	2.150	2.218	2.302	3.641***	5.311***
	TRIG (mmol/l)	0.427	0.444	0.382	0.785**	1.491**
	TPROT (g/l)	58.4	60.4	62.5*	67.9***	66.1***
	ALB (g/l)	33.0	33.6	34.3	33.8	32.0

* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control. *** Statistically different ($p<0.01$) from the control.

3. **Hormone analysis:** Treatment-related effects were observed at 500 and 2500 ppm (Table IIA 5.3.1.2-6).

Increases in TSH levels were observed at 500 ppm and 2500 ppm in males (+152% and +259%, respectively, $p<0.001$) and in females (+339% and +282%, respectively, $p<0.001$). These changes were associated with decreases in T4 levels at 500 and 2500 ppm in males (-29% and -52%, respectively, $p<0.001$) and in females (-15% and -11%, respectively) and increases in T3 levels at 2500 ppm in males (+38%, $p<0.05$) and females (+49%, $p<0.01$).

No treatment-related hormonal changes were observed at 20 and 100 ppm.

TABLE IIA 5.3.1.2-6 Mean T3, T4, and TSH Plasma Levels

Interval	Parameter	Dose Level (ppm in diet)				
		0	20	100	500	2500
Males						
Week 4	N° of animals examined	10	9	9	8	9
	T3 (mmol/l)	0.439	0.410	0.447	0.447	0.608*
	T4 (mmol/l)	31.708	29.173	26.308	22.452 ***	15.242 ***
	TSH (ng/ml)	2.748	2.139	3.639	6.929 **	9.878 ***
Females						
Week 4	N° of animals examined	10	9	10	9	9
	T3 (mmol/l)	0.407	0.430	0.438	0.355	0.605 **
	T4 (mmol/l)	17.888	21.941	21.620	15.070	15.935
	TSH (ng/ml)	1.180	1.229	2.716	5.181***	4.511**

* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control. *** Statistically different ($p<0.01$) from the control.

F. **URINALYSIS:** Treatment-related effects were observed in males at 500 and 2500 ppm and consisted in lower mean urinary volume (-41% and -57%, respectively). In addition, pH value was slightly reduced (12%) in the male high dose group.

No treatment-related urinalysis changes were observed at 20 and 100 ppm in males and at any dose level in females.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ Weight:** At terminal sacrifice, treatment-related organ weight changes were noted in the liver, thyroid and adrenals at 100, 500 and/or 2500 ppm (Table IIA 5.3.1.2-7).

Terminal body weight was slightly lower in males at 2500 ppm (-8%, $p<0.01$).

In the liver, a dose-related increase in absolute and relative weights were observed at 500 and 2500 ppm in males (+35% and + 98%, respectively, for absolute values, $p<0.001$) and in females at 100, 500 and 2500 ppm (approximately +20%, +96% and +162%, respectively for absolute values, $p<0.001$). In the thyroid, a dose-related increase in both absolute and relative weights were observed at 500 and 2500 ppm in males (+41% and +53% for absolute, $p<0.001$ and $p<0.01$, respectively) and in females (+40% and +60% for absolute, $p<0.01$ and $p<0.001$, respectively). In the adrenals, higher absolute and relative weights were observed in males at 500 and 2500 ppm (+16% and +23% for absolute, $p<0.01$ and $p<0.001$, respectively) and in females at 100, 500 and 2500 ppm (+23%, +15%, +35% for absolute, $p<0.01$, not statistically significant and $p<0.001$, respectively).

Statistically significantly lower absolute (-37%; p<0.001) and relative (-31%; p<0.01) prostate weights were noted in males at 2500 ppm. This change was considered to have a doubtful significance by the study authors, since no histological change was observed and in view of the lower terminal body weights. In females at 500 and 2500 ppm, lower absolute (-15%, -17%) and relative (-19%, -17%) spleen weights were observed but they were not considered to be biologically significant since the differences were minimal, they were not dose-related, and were not correlated to any gross or microscopic findings.

No treatment-related organ weights changes were observed at 20 ppm in either sex.

TABLE IIA 5.3.1.2-7 Mean Terminal Body Weight, Liver, Thyroid and Adrenal Weights

Organ	Parameter	Dose level (ppm in diet)				
		0	20	100	500	2500
Males						
Number ofanimals examined		10	9	10	10	8
Terminal body weight (g)		338.6	339.9	332.8	334.2	310.4 * (↓8%)
Liver	Absolute weight (g)	10.27	10.06	10.31	13.90 *** (↑35%)	20.38 *** (↑98%)
	% of terminal body weight	3.03	2.96	3.10	4.16*** (↑37%)	6.58*** (↑117%)
	% of brain weight	529.39	514.77	528.65	714.12***	1082.65***
Thyroid	Absolute weight (g)	0.017	0.018	0.019	0.024 *** (↑41%)	0.026 *** (↑53%)
	% of terminal body weight	0.0049	0.0053	0.0058	0.0073*** (↑49%)	0.0084** (↑71%)
	% of brain weight	0.861	0.914	0.985	1.254**	1.390**
Adrenals	Absolute weight (g)	0.057	0.052	0.054	0.066 ** (↑16%)	0.070 *** (↑23%)
	% of terminal body weight	0.017	0.015	0.016	0.020** (↑18%)	0.022*** (↑29%)
	% of brain weight	2.921	2.646	2.762	3.387*	3.691***
Females						
Number of animals examined		10	9	10	7	10
Terminal body weight (g)		201.4	212.3	215.2	221.1	210.3
Liver	Absolute weight (g)	5.98	6.27	7.20 *** (↑20%)	11.74 *** (↑96%)	15.64 *** (↑162%)
	% of terminal body weight	2.85	2.96	3.35*** (↑18%)	5.31*** (↑86%)	7.43*** (↑160%)
	% of brain weight	322.86	344.88	386.99**	630.56***	851.05***
Thyroid	Absolute weight (g)	0.015	0.016	0.014	0.021 ** (↑40%)	0.024 *** (↑60%)
	% of terminal body	0.0072	0.0076	0.0067	0.0095*	0.0113***

	weight				(↑32%)	(↑57%)
	% of brain weight	0.807	0.889	0.771	1.128**	1.301***
Adrenals	Absolute weight (g)	0.060	0.066	0.074 ** (↑23%)	0.069 (↑15%)	0.081 *** (↑35%)
	% of terminal body weight	0.029	0.031	0.035* (↑21%)	0.031 (↑7%)	0.039*** (↑34%)
	% of brain weight	3.263	3.641	3.964*	3.681	4.418***

* Statistically different (p<0.05) from the control. ** Statistically different (p<0.01) from the control. *** Statistically different (p<0.01) from the control.

2. **Gross Pathology**: At terminal sacrifice, treatment-related macroscopic findings were noted in the liver, kidney and thyroid at 500 and/or 2500 ppm (Table IIA 5.3.1.2-8).

   Liver enlargement and dark liver color were seen in males and females at 500 and 2500 ppm. Dark kidney color was seen in all males and females at 2500 ppm. Thyroid enlargement was noted in 2/8 males and 2/10 females at 2500 ppm.

   No treatment-related macroscopic changes were observed at 20 and 100 ppm in either sex.

TABLE IIA 5.3.1.2-8 Selected macroscopic findings in rats at Week 4

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	20	100	500	2500	0	20	100	500	2500
	Males					Females				
	N of rats examined					N of rats examined				
	10	9	10	10	8	10	10	10	10	10
Liver										
Enlarged	0	0	0	10	8	0	0	0	10	10
Dark	0	0	0	10	7	0	0	0	9	10
Kidney										
Dark	0	0	0	0	8	0	0	0	0	10
Thyroid										
Enlarged	0	0	1	0	2	0	0	0	0	2

3. **Microscopic Pathology**: At terminal sacrifice, treatment-related microscopic findings were noted in the liver, thyroid gland, kidneys and adrenals at 500 and/or 2500 ppm (Table IIA 5.3.1.2-9).

   In the liver, slight to moderate diffuse centrilobular to panlobular hepatocellular hypertrophy was noted in all males and in almost all females treated at 2500 and 500 ppm. The severity of this lesion was treatment-related and slightly more pronounced in females when compared to males. Slight amounts of golden brown intracytoplasmic pigment was also observed in 9/10 females at 2500 ppm.

   In the thyroid, diffuse slight to moderate follicular cell hypertrophy was noted in all males and females at 500 and 2500 ppm

   In the kidney, an increased degree of cortical tubular brown pigment was reported in all males and females treated at 2500 ppm.

In the adrenals, a diffuse vacuolation in the zona fasciculata and glomerulosa of the adrenal cortex was observed in all males and females treated at 2500 ppm. A similar change was found in the zona fasciculata for 3/10 females and 3/10 males at 500 ppm and in the zona glomerulosa of 3/10 females at 500 ppm

No treatment-related microscopic changes were observed at 20 and 100 ppm in either sex.

TABLE IIA 5.3.1.2-9 Selected microscopic findings in rats at Week 4 (terminal sacrifice)

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	20	100	500	2500	0	20	100	500	2500
	Males					Females				
Liver: number examined	**10**	**9**	**10**	**10**	**8**	**10**	**10**	**10**	**10**	**10**
Hepatocellular hypertrophy, centrilobular to panlobular, diffuse	0	0	0	10	8	0	0	0	9	10
Thyroid: number examined	**10**	**9**	**10**	**10**	**8**	**10**	**10**	**10**	**10**	**10**
Follicular cell hypertrophy, diffuse	0	0	0	10	8	0	0	0	10	10
Kidney: number examined	**10**	**9**	**10**	**10**	**8**	**10**	**10**	**10**	**10**	**10**
Golden brown pigment – cortical tubules	0	0	0	0	8	0	0	0	0	9
Adrenals: number examined	**10**	**9**	**10**	**10**	**8**	**10**	**10**	**10**	**10**	**10**
Increased vacuolation, zona fasciculata, adrenal cortex	0	0	0	3	8	0	0	1	3	10
Increased vacuolation, zona glomerulosa, adrenal cortex	0	0	0	0	8	0	0	0	3	10

III. CONCLUSIONS:

The short-term dietary administration of ethiprole (RPA 107382) to rats for at least 28 days produced signs of overt toxicity at 2500 ppm as evidenced by the premature death of two males. The body weight evolution of animals at 2500 ppm was inferior to that of controls throughout the study in males and only during the first week of treatment in females. At 2500 ppm, statistically significant lower food consumption was noted in males during the first two weeks and in females during the first week.

Increases in prothrombin time were noted in males at 500 and 2500 ppm. A tendency towards higher platelet counts was noted in males and females at 2500 ppm. Increases in ALAT, cholesterol, triglycerides and total protein and decreases in albumin were reported at 500 and/or 2500 ppm. Ethiprole induced an imbalance of thyroid hormones in animals treated at 500 and 2500 ppm resulting in higher levels of TSH (at ≥ 500 ppm) and T3 (at 2500 ppm) and lower levels of T4 (at ≥ 500 ppm) in both sexes.

Significant increases in thyroid and adrenal weights were observed in both males and females at 500 and 2500 ppm, Liver weights were also increased in males at ≥500 ppm, and in females at ≥100 ppm.

At the histopathological examination diffuse centrilobular to panlobular hepatocellular hypertrophy was observed in the livers of all males and almost all females at ≥500 ppm. Diffuse

follicular hypertrophy in the thyroid was also noted in all animals at ≥500 ppm. An increased diffuse vacuolation of the adrenal cortex was noted in both sexes at ≥500 ppm. The kidney was also affected at 2500 ppm with both sexes showing tubular golden brown pigment.

In conclusion, the NOAEL is 9.2 mg/kg/day in males and 9.6 mg/kg/day in females (100 ppm in diet), based on slight changes in liver and adrenal weights without any untoward histological changes. The LOAEL is 46.1/46.3 mg/kg bw/day (500 ppm in diet), based on changes in hematology, clinical chemistry, and urinalysis parameters, and increased liver, thyroid and adrenal weights with associated histopathology.

28-day toxicity study in rats with metabolite RPA112916

1.	Annex point(s)	KIIA 5.8; Toxicity studies on metabolites: **MRID 47622805**
2.	Location in dossier	KIIA 5.8 /03
3.	Authors (year)	Bigot, D. (2000)
	Title	28-day toxicity study in the rat by dietary administration RPA112916 Metabolite of ethiprole (RPA107382)
	Company, report No.	Bayer CropScience AG, Report-No.: R016688 (M-192137-01-1)
	Date	21.12.2000
4.	Testing facility	Aventis CropScience, Sophia Antipolis;
5.	Dates of work	Exp. Start date: November 3rd 1999; Exp. Term. Date: March 20th 2000
6.	Test substance(s)	Molecule(s): (14C)-RPA112916, AE 0616301, ethiprole Substance(s): AE 0616301 TC (Batch-No.: PAN01/99) (14C)-RPA112916 TC
7.	Test method	EU (=EEC): 92/69/EEC, V, B7; OECD: 407, (1995)
	Deviations	
8.	GLP	yes

Executive summary:

In a subchronic toxicity study, RPA 112916 (945 g/kg purity) was administered to 10 Wistar (AF) rats/sex/dose in the diet at dose levels of 0, 50, 500, 5000 or 10,000 ppm (equivalent to 0, 5.2, 51.4, 515.2 or 983.4 mg/kg bw/day in males and 0, 5.2, 53.5, 512.4 or 992.8 mg/kg bw/day in females) for 28 days.

RPA 112916 induced no treatment-related mortalities or clinical signs. Decreased body weight and body weight gains were noted in males at 10,000 ppm. Statistically significant decreases in mean food consumption were observed in males at 10,000 ppm for the first 3 weeks and in females for the first week.

Increases in prothrombin times were reported in males at 5000 and 10,000 ppm. Increases in mean total cholesterol and triglyceride concentrations were seen in females at 5000 and 10,000 ppm.

RPA 112916 induced an imbalance of thyroid hormones in animals treated with doses ≥ 5000 ppm in males and ≥ 500 ppm in females resulting in higher levels of TSH and/or lower levels of T4.

Increased crystals in the urine were observed in both sexes at ≥ 5000 ppm and were considered treatment-related.

Significant increases in liver, thyroid and/or adrenal weights were observed in males at 500, 5000 and/or 10,000 ppm and/or females at 5000 and 10,000 ppm. Decreased prostate weights were also noted in males at ≥ 5000 ppm.

At the histopathological examination, the liver with centrilobular to panlobular hepatocellular hypertrophy (≥ 500 ppm) and some with hepatocellular necrotic foci (≥ 5000 ppm), the thyroid with follicular cell hypertrophy (≥ 5000 ppm), and the adrenal gland with diffuse

microvacuolation of the zona fasciculata (≥ 500 ppm) were found to be the target organs. A reduced prostate size and an increased incidence of interstitial mononuclear cell infiltration were also observed in high-dose males.

In conclusion, the **LOAEL** is 51.4 mg/kg bw/day in males and 53.5 mg/kg bw/day in females (500 ppm in diet) based on increased TSH levels, increased liver weights associated with a dark liver and/or a diffuse centrilobular to panlobular hepatocellular hypertrophy, and an increased incidence of microvacuolation of the adrenal zona fasciculata. The **NOAEL** is 5.2 mg/kg bw/day in both sexes (50 ppm in diet).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3050; OECD 407) for a subchronic oral toxicity study in the rat.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 112916, a metabolite of Ethiprole (RPA 107382)
   Description: White powder
   Lot/Batch #: PAN01/99
   Purity: 945 g/kg
   Compound Stability: The test substance was stored in an air-tight, light-resistant container at room temperature.
   CAS #: Not known

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. Control animals received untreated diet.

3. **Test Animals:**

Species:	Rat	
Strain:	Wistar (AF) rats RJ: WI (IOPS AF)	
Age/weight at study initiation:	5 weeks old / 132 - 165 g males, 115 - 141 g females	
Source:	R. Janvier, Le Genest St Isle, France	
Housing:	Individually in suspended stainless steel, wire mesh cages.	
Diet:	Certified rodent powder diet "M 20 contrôlé" (Pietrement, Provins, France), *ad libitum*	
Water:	Filtered and softened water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	20°C - 24°C (target)
	Humidity:	40% - 70% (target)
	Air changes:	10 to 15 air changes per hour (average, not monitored)
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	7 days	

B. STUDY DESIGN:

1. **In-Life Dates:** November 03, 1999 to December 03, 1999

2. **Animal Assignment/Dose Levels:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution by sex to the test groups noted in Table IIA 5.8.2-1.

TABLE IIA 5.8.2-1 Study Design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day) [a]		No. of Animals	
		Males	Females	Males	Females
Control	0	0	0	10	10
Low	50	5.2	5.2	10	10
Mid-1	500	51.4	53.5	10	10
Mid-2	5,000	515.2	512.4	10	10
High	10,000	983.4	992.8	10	10

a Mean achieved dietary intake of RPA 112916, weeks 1 to 4.

3. **Diet Preparation and Analysis:** The test substance was ground to a fine powder before being incorporated into the diet (Certified rodent powder diet "M 20 contrôlé ") by dry mixing to provide the required concentrations. There was one preparation for each concentration. When not in use, the diet formulations were stored at a temperature below -15°C.

   Prior to study initiation, the homogeneity of test substance in diet was verified for the concentrations of 25 and 15,000 ppm to demonstrate adequate formulation procedures. Sampling was done at three levels, the top (T), middle (M) and bottom (B). At each level, a single sample was taken and analyzed. The stability for dietary concentrations of 25 and 15,000 ppm was also tested over 18, 25 and 36-day periods (~ 11, 18 and 28-day frozen storage followed by 7 or 8 days at ambient temperature).

   During the study, the homogeneity of the 50 and 10,000 ppm preparations was checked. Sampling was done at three levels, the top (T), middle (M) and bottom (B). At each level, two samples (1 & 3) were taken and analyzed. The mean value obtained from the homogeneity analysis was used as the measured concentration. The concentration of the 500 and 5,000 ppm preparations was checked on a single sample (pooled from the 3 levels).

   Results: **Homogeneity Analysis:** Results for homogeneity of RPA 112916 in the 25 and 15,000 ppm dose level diet formulations were within the range of 86-94% of the nominal concentrations. For the 50 and 10,000 ppm diets prepared during the study, results were within 86-94% of the nominal concentrations. (Data obtained from the study report, pages 334 and 337).

   Stability Analysis: After 36 days of storage (28 days frozen + 8 days at ambient temperature), the 25 and 15,000 ppm diets were within 102% and 97% of the nominal concentrations, respectively. (Data obtained from the study report, pages 336).

   Concentration Analysis: Results for the concentration analyses were within 87-102% of the nominal concentrations. (Data obtained from the study report, page 338).

4. **Statistics**: Statistical analyses were performed on the following endpoints: body weight parameters, food consumption, hematology parameters (except eosinophils, basophils, monocytes and large unstained cells), clinical chemistry parameters, urinary parameters (only pH, volume and refractive index), organ weight parameters, and hormone data.

When appropriate, means and standard errors were calculated separately for each sex and dose group at each time period.

"Since the above parameters were all measured on a continuous scale, the treatment groups were compared using a combination of parametric ANOVA and non-parametric data analysis techniques. The homogeneity of variance assumption was examined via Bartlett's test, implemented through a user-written SAS® macro. If Bartlett's test rejected the homogeneity of variance assumption ($p<0.05$), non-parametric tests was used. Non-parametric tests include the Kruskal-Wallis test for overall treatment group differences, followed by the Mann-Whitney U test for individual exposed vs. control group comparisons, if the Kruskal-Wallis test was significant ($p<0.05$). All non-parametric tests were performed using the NPAR1WAY procedure in SAS® 6.12 (SAS Institute Inc., 1989, 1996).

If Bartlett's test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 was used to evaluate the overall effect of treatment, and, when a significant treatment effect was present ($p<0.05$), to compare each exposed group to control via Dunnett's test."

The reviewer agrees with the statistical methods used.

C. METHODS:

1. **Observations:** Animals were inspected twice daily (once daily on weekends or public holidays) for moribundity and mortality. Clinical signs were recorded at least once daily for all animals. Detailed physical examinations were performed at least weekly during the treatment period. The nature, onset, severity, reversibility and duration of the clinical signs were recorded. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose feces.

   In addition, neurotoxicity assessments were performed. During the acclimatization phase and during Week 4 of the study, grasping, righting, corneal, pupillary, auditory startle, and head shaking reflexes were tested.

2. **Body weight:** Each animal was weighed once during the acclimatization period, on the first day of test substance administration and once weekly thereafter. Animals were also weighed before scheduled necropsies.

3. **Food Consumption and Compound Intake:** The weight of food supplied and of that remaining at the end of the food consumption period was recorded weekly for all animals during the treatment period. The mean achieved intake was calculated as mg/kg bw/day for each week and for weeks 1-4.

4. **Ophthalmoscopic Examination:** During the acclimatization period, all animals were subjected to an ophthalmic examination. After instillation of an atropinic agent (mydriaticum), the eyes of all animals were examined using an indirect ophthalmoscope. At Week 4, the eyes of surviving animals from the control and high dose groups were re-examined.

5. **Hematology & Clinical Chemistry (including Thyroid Hormone Analysis):** On study Days 22, 23, or 24, blood samples were collected from all surviving animals by puncture of the retro-orbital venous plexus. Animals were randomly distributed prior to each sampling. Animals were fasted overnight prior to bleeding and anesthetized by inhalation of isoflurane. Blood was

collected on EDTA for hematology (0.5 ml), on lithium heparin for plasma chemistry and on sodium citrate for coagulation parameters (0.9 ml). The CHECKED (X) parameters listed below were examined. In addition, on study Days 22, 23 or 24, blood was collected from all animals on lithium heparin for T3, T4 and TSH analysis (3.0 ml). TSH, T3 and T4 levels were measured on each plasma sample by radioimmunoassay.

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	Blood clotting measurements	X	Reticulocyte count (smear prepared but not examined)
X	(Platelet count)		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)	X	Thyroid Hormones (T3, T4, and TSH)
X	Serum alanine amino-transferase (ALT/also SGPT)		
X	Serum aspartate amino-transferase (AST/also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Ornithine decarboxylase		

6. **Urinalysis**: On study Days 29, 30, or 31, overnight urine samples were collected from all surviving animals in the morning, prior to sacrifice. Animals were randomly distributed among groups prior to each sampling. Feed and water were not accessible during urine collection. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen
X	Refractive index		

8. **Sacrifice and Pathology:** On study Days 29, 30, or 31, all surviving animals were fasted overnight and sacrificed by exsanguination under deep anesthesia (pentobarbital). Animals were randomly distributed among groups prior to sacrifice on each day. The CHECKED (X) tissues were collected for histological examination and the (XX) organs, in addition, were weighed. The listed tissues were examined microscopically for all the animals in the control and high-dose groups and for those that died prior to the scheduled sacrifice, regardless of dose group. In addition, lung, liver, thyroid gland, kidney and adrenal gland tissues were examined macroscopically and histopathologically in the intermediate-dose groups to identify the no-effect-level.

	DIGESTIVE SYSTEM		**CARDIOVASC./HEMAT.**		**NEUROLOGIC**
X	Tongue	X	Aorta	X, XX	Brain
X	Salivary glands	X, XX	Heart	X	Periph.nerve (sciatic nerve)
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X, XX	Pituitary
X	Duodenum	X, XX	Spleen	X	Eyes (optic n.)
X	Jejunum	X, XX	Thymus		**GLANDULAR**
X	Ileum			X, XX	Adrenal gland
X	Cecum		**UROGENITAL**	X	Hardarian (lachrymal) gland
X	Colon	X, XX	Kidneys	X	Mammary gland
X	Rectum	X	Urinary bladder	X, XX	Parathyroids
X, XX	Liver	X, XX	Testes	X, XX	Thyroids
	Gall bladder	X, XX	Epididymides		**OTHER**
X	Pancreas	X, XX	Prostate	X	Bone (including articular surface)
	RESPIRATORY	X	Seminal vesicle	X	Skeletal muscle
X	Trachea	X, XX	Ovaries	X	Skin
X	Lung	X, XX	Uterus	X	All gross lesions and masses
	Nose	X	Vagina	X	Target Organs
	Pharynx				
X	Larynx				

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity and Neurotoxicity Assessments:** There were no treatment-related clinical signs at any dose level in either sex. In addition, no impairment of any of the assessed reflexes was observed at any dose level in either sex.

2. **Mortality:** There were no treatment-related mortalities at any dose level in either sex. One female from the 50 ppm group died on Day 23 following anesthesia for blood sampling.

B. **BODY WEIGHT**: A slight statistically significant decrease in mean body weight gain was observed in males at 10,000 ppm on Weeks 1 (↓21%) and 2 (↓11%), leading to a statistically significant decrease (by approximately 7%) in mean body weight on Week 2 (table IIA 5.8.2-2). A non-statistically significant decrease in mean body weight (of about 7%) was still observed on Weeks 3 and 4.

There were no treatment-related changes in body weights in males at 50, 500 or 5000 ppm and in females at any dose level. Increased body weight gains observed on Week 2 in females dosed at ≥ 500 ppm were not considered to be biologically relevant since these effects were not dose-related and not persistent throughout the study.

TABLE IIA 5.8.2-2. Mean Body Weights and Body Weight Gains During 4 Weeks of Treatment[a]

Interval	Dose Level (ppm in diet)				
	0	50	500	5000	10,000
Males					
Body Weight (g)					
Week 0	147.3	147.0	147.5	148.2	147.1
Week 1	210.4	210.5	210.3	204.2	197.2 (↓6%)
Week 2	266.1	266.3	263.5	255.0	246.8* (↓7%)
Week 3	311.2	314.8	310.1	299.9	289.9 (↓7%)
Week 4	341.5	345.7	342.4)	329.1	317.7 (↓7%)
Body Weight Change (g)					
Weeks 0-1	63.1	63.5	62.8	56.0	50.1***(↓21%)
Weeks1-2	55.7	55.8	53.2	50.8	49.6*(↓11%)
Weeks 2-3	45.1	48.5	46.6	44.9	43.1(↓4%)
Weeks 3-4	30.3	30.2	32.3	29.2	27.8(↓8%)
Overall (Weeks 0-4)	194.2	198.7	194.9	180.9	170.6*(↓12%)
Interval	**Dose Level (ppm in diet)**				
	0	**50**	**500**	**5000**	**10,000**
Females					
Body Weight (g)					
Week 0	125.9	126.0	124.5	125.4	124.8
Week 1	160.5	162.7	161.2	158.7	157.1
Week 2	177.3	183.7	186.4	181.2	180.8
Week 3	191.3	201.4	202.9	194.2	195.3
Week 4	203.0	212.9	213.6	203.9	205.3

Body Weight Change (g)					
Weeks 0-1	34.6)	36.7	36.7	33.3	32.3
Weeks1-2	16.8	21.0	25.2**(↑50%)	22.5(↑34%)	23.7*(↑41%)
Weeks 2-3	14.0	17.7	16.5	13.0	14.5
Weeks 3-4	11.7	11.7	10.7	9.7	10.0
Overall (Weeks 0-4)	77.1	87.3	89.1	78.5	80.5

[a] Data obtained from pages 41-44 in the study report.
* Significantly different (p < 0.05) from the control. ** Significantly different (p <0.01) from the control.
*** Significantly different (p < 0.001) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food Consumption:** Treatment-related decreases in food consumption were observed at 10,000 ppm in males (↓18% to 11%) on Week 1 to 3 and in females (↓14%) during the first week of dosing (table IIA 5.8.2-3).
2.

There were no treatment-related changes in food consumption at 50, 500 or 5000 ppm in either sex.

TABLE IIA 5.8.2-3. Mean Food Consumption (g/day) [a]

Interval (Week)	Dose Level (ppm in diet)				
	0	50	500	5000	10,000
Males					
Week 1	24.7	24.7	24.3	24.0	20.3***(↓18%)
Week 2	27.0	27.2	26.5	25.5	24.1**(↓11%)
Week 3	28.3	28.4	27.8	26.7	25.3**(↓11%)
Week 4	25.9	26.3	26.5	25.8	24.8
Weeks 0-4	26.4	26.6	26.2	25.7	23.6**(↓11%)
Females					
Week 1	19.1	18.7	18.6	17.2	16.4**(↓14%)
Week 2	18.7	18.7	19.7	18.0	17.6(↓6%)
Week 3	18.7	18.9	19.6	18.0	17.7(↓5%)
Week 4	17.1	17.8	18.3	17.4	16.9
Weeks 0-4	18.0	18.5	19.0	17.5	17.1(↓5%)

[a] Data obtained from pages 46-49 in the study report.
** Significantly different (p <0.01) from the control.
*** Significantly different (p <0.001) from the control.

2. **Compound Consumption (time-weighted average):** The mean achieved dose levels expressed in mg/kg body weight/day are presented in Table IIA 5.8.2-4.

TABLE IIA 5.8.2-4 Mean Achieved Dietary Intake (Weeks 1-4) [a]

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	50	5.2	5.2
Mid-1	500	51.4	53.5
Mid-2	5,000	515.2	512.4
High	10,000	983.4	992.8

[a] Data obtained from pages 46-49 in the study report.

D. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related changes at the ophthalmological examination at any dose level in either sex.

E. BLOOD ANALYSES:

1. **Hematology**: At the end of the dosing phase, treatment-related effects were observed in males at 5000 and 10,000 ppm (Table IIA 5.8.2-5).

RPA 112916 induced a slight increase in mean prothrombin time in males at 5000 ppm (+7%) and 10,000 ppm (+15%) compared to controls.

There were no treatment-related hematological changes in males at 50 or 500 ppm and in females at any dose level. Slightly lower mean hemoglobin concentration was noted in females at 5000 and 10,000 ppm compared to controls. However, as the individual values remained within normal range, this isolated variation was not considered to be related to treatment.

TABLE IIA 5.8.2-5 Mean Prothrombin Time (PT) [a]

Interval	Parameter	Dose Level (ppm in diet)				
		0	50	500	5000	10,000
Males						
Number of animals examined		9	9	10	10	10
Week 4	PT (s)	12.43	11.64	11.91	13.33*(↑7%)	14.29**(↑15%)
Females						
Number of animals examined		9	9	9	7	7
Week 4	PT (s)	12.16	12.29	12.37	11.31	11.33

[a] Data obtained from pages 52 and 54 in the study report.
* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control.

2. **Clinical Chemistry**: At the end of the dosing phase, treatment-related effects were observed in females at 5000 and 10,000 ppm (Table IIA 5.8.2-6).

RPA 112916 induced increases in mean total cholesterol and triglyceride concentrations in females at 5000 (+35% and +150%, respectively) and 10,000 ppm (+23% and +110%, respectively).

There were no treatment-related hematological changes in males at any dose level and in females at 50 or 500 ppm.

The study author claimed that the total bilirubin variations noted in males at ≥ 500 ppm were not considered relevant since no clear dose-response was observed and the individual values were within the normal range. In addition, decreased aspartate aminotransferase and/or alkaline phosphatase activities in males at ≥ 5000 ppm, decreased albumin concentrations in males at 50 and 5000 ppm, and in females at 10,000 ppm and increased total protein concentrations in females at 10,000 ppm were not considered to be treatment-related because of the low incidence of results out of the normal range or the low magnitude of change.

TABLE IIA 5.8.2-6 Mean Total Cholesterol (CHOL) and Triglyceride (TRIG) concentrations [a]

Interval	Parameter	Dose Level (ppm in diet)				
		0	50	500	5000	10,000
Males						
Number of animals examined		10	10	10	10	10
Week 4	CHOL (mmol/l)	1.971	1.807	1.910	1.900	2.016
	TRIG (mmol/l)	1.030	1.004	0.882	1.167	1.206
Females						
Number of animals examined		10	9	10	10	10
Week 4	CHOL (mmol/l)	2.048	1.993	2.268	2.757*** (↑35%)	2.518** (↑23%)
	TRIG (mmol/l)	0.616	0.472	0.527	1.537** (↑150%)	1.295** (↑110%)

[a] Data obtained from pages 56-60 in the study report.
** Statistically different (p< 0.01) from the control. *** Statistically different (p<0.001) from the control.

3. **Hormone Analysis:** At the end of the dosing phase, treatment-related effects on hormone levels were observed at ≥ 500 ppm in females and at ≥ 5000 ppm in males (Table IIA 5.8.2-7).
Increases in TSH levels were observed at ≥ 500 ppm in females (↑34-38%; p<0.05. These changes were associated with a slight and non-statistically significant decrease in T4 levels at ≥ 5000 ppm (↓12-11%).

A non-statistically significant increase in TSH plasma levels was also observed in males at 5000 and 10,000 ppm (↑20-25%) This increase correlated with a statistically significant decrease in T4 plasma levels (↓28-30%, p<0.01) and a non-statistically significant decrease in T3 plasma levels (↓9-25%) at both 5000 and 10,000 ppm.

There were no treatment-related hormonal changes at 50 ppm in females and at ≤ 500 ppm in males.

TABLE IIA 5.8.2-7 Mean T3, T4, and TSH Plasma Levels [a]

Interval	Parameter	Dose Level (ppm in diet)				
		0	50	500	5000	10,000
Males						
Week 4	N° of animals examined	10	9	10	10	10
	T3 (ng/ml)	0.404	0.424	0.416	0.367 (↓9%)	0.301 (↓25%)
	T4 (ng/ml)	36.082	33.088	32.182	26.080** (↓28%)	25.360** (↓30%)

	TSH (ng/ml)	5.941	5.711	6.162	7.439 (↑20%)	7.148 (↑25%)
Females						
Week 4	N° of animals examined	10	7	8	9	9
	T3 (ng/ml)	0.382	0.370	0.350	0.374[c]	0.377[c]
	T4 (ng/ml)	20.231	22.794	20.067	17.864 (↓12%)	17.999 (↓11%)
	TSH (ng/ml)	3.134	3.401[b]	4.209* (↑34%)	4.337* (↑38%)	4.255 (↑36%)

[a] Data obtained from pages 72-73 in the study report.
[b] N = 6
[c] N = 8
* Statistically different (p<0.05) from the control. ** Statistically different (p<0.01) from the control.

F. URINALYSIS: A slight increase in the number of crystals observed after urine centrifugation was noted in both sexes at 5000 and 10,000 ppm (Table IIA 5.8.2-8). There were no treatment-related changes at 50 or 500 ppm in either sex. The variations observed in mean urinary volumes in both sexes were considered not to be treatment-related.

TABLE IIA 5.8.2-8 Incidence of crystal formation in urine following 4 weeks of treatment[a]

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	50	500	5000	10,000	0	50	500	5000	10,000
	Males					Females				
	N of rats examined					N of rats examined				
	10	10	9	10	10	10	8	10	9	10
Crystals										
Grade 2[b]	5	4	3	1	2	0	1	2	5	7
Grade 3[b]	2	1	1	6	5	0	0	0	0	2
Grade 4[b]	0	0	0	0	1	0	0	0	0	1

[a] Data obtained from pages 69-70 in the study report.
[b] Grading system from 0 to 4 (none to many) corresponding to the number of crystals observed per low power field.

G. SACRIFICE AND PATHOLOGY:

1. **Organ Weight:** Treatment-related organ weight changes were noted in the liver, adrenals, thyroid gland and prostate at 500, 5000 and/or 10,000 ppm (Table IIA 5.8.2-9).

In the liver, dose-related increases in absolute and relative liver weights were observed at ≥ 500 ppm in males (absolute weights: +14% [p<0.05], +46% [p<0.001] and +56% [p<0.001], respectively) and in females at 5000 and 10,000 ppm (absolute weights: +44% [p<0.001] and +56% [p<0.001], respectively).

In the adrenals, statistically significant increases in absolute and relative weights were noted in males at 10,000 ppm (absolute weights: +21% [p<0.05]) and in females at 5000 and 10,000 ppm (absolute weights: +23% [p<0.01] and +22% [p<0.01], respectively).

In the thyroid gland, statistically significant increases in absolute and relative weights were observed in males at 5000 and 10,000 ppm (absolute weights: +22% [p<0.05] and +33% [p<0.01], respectively).

In the prostate, statistically significant decreases in absolute and relative weights were observed in males at 5000 and 10,000 ppm (absolute weights: -30% [p<0.01] and [p<0.05], respectively).

There were no treatment-related organ weights changes at 50 ppm in males and at 50 or 500 ppm in females.

The study author claimed that decreased absolute and relative epididymis weights observed in males at 5000 and 10,000 ppm were considered of doubtful toxicological significance as no correlated microscopic change was seen. In addition, decreased absolute and relative uterus weights noted in females at ≥ 50 ppm were not considered to be toxicologically significant since no dose-response was observed and the individual values were within the normal range.

TABLE IIA 5.8.2-9 Mean Terminal Body Weight, Absolute and Relative Liver and Thyroid Weights [a]

Organ	Parameter	Dose level (ppm in diet)				
		0	50	500	5000	10,000
Males						
Number ofanimals examined		10	10	10	10	10
Liver	Absolute weight (g)	9.55	10.08	10.86* (↑14%)	13.98*** (↑46%)	14.88*** (↑56%)
	% of terminal body weight	2.99	3.12	3.37* (↑13%)	4.55*** (↑52%)	5.01*** (↑68%)
	% of brain weight	490.02	518.73	553.72* (↑13%)	733.08*** (↑50%)	759.98** (↑55 %)
Adrenals	Absolute weight (g)	0.058	0.057	0.057	0.064 (↑10%)	0.070* (↑21%)
	% of terminal body weight	0.018	0.018	0.018	0.021 (↑17%)	0.024*** (↑33%)
	% of brain weight	2.982	2.946	2.904	3.366 (↑13%)	3.574* (↑20%)
Thyroid	Absolute weight (g)	0.018	0.018	0.018	0.022* (↑22%)	0.024** (↑33%)
	% of terminal body weight	0.0056	0.0056	0.0058	0.0072** (↑29%)	0.0079*** (↑41%)
	% of brain weight	0.911	0.925	0.944	1.158* (↑27%)	1.206** (↑32%)
Prostate	Absolute weight (g)	0.361	0.363	0.335	0.252** (↓30%)	0.260* (↓30%)
	% of terminal body weight	0.112	0.112	0.104	0.083* (↓26%)	0.087 (↓22%)
	% of brain weight	18.540	18.700	17.068	13.350* (↓28%)	13.280* (↓28%)

Epididymis	Absolute weight (g)	0.792	0.719	0.779	0.710 (↓10%)	0.631*** (↓20%)
	% of terminal body weight	0.248	0.222	0.242	0.231 (↓7%)	0.213* (↓14%)
	% of brain weight	40.641	36.940	39.745	37.380 (↓8%)	32.214*** (↓21%)
Females						
Number of animals examined		10	9	10	10	10
Liver	Absolute weight (g)	5.78	6.23	6.57	8.35*** (↑44%)	9.00*** (↑56%)
	% of terminal body weight	3.05	3.12	3.32	4.42*** (↑45%)	4.75*** (↑56%)
	% of brain weight	317.10	349.36	357.06* (↑13%)	467.05*** (↑47%)	507.55*** (↑60%)
Adrenals	Absolute weight (g)	0.060	0.062	0.071* (↑18%)	0.074** (↑23%)	0.073** (↑22%)
	% of terminal body weight	0.032	0.031	0.036	0.039** (↑22%)	0.039** (↑22%)
	% of brain weight	3.264	3.452	3.835	4.145** (↑27%)	4.117** (↑26%)
Thyroid	Absolute weight (g)	0.014	0.016	0.016	0.016	0.015
	% of terminal body weight	0.0072	0.0079	0.0081	0.0083	0.0077
	% of brain weight	0.746	0.883	0.874	0.872	0.821
Uterus	Absolute weight (g)	0.608	0.419* (↓31%)	0.472 (↓22%)	0.360** (↓41%)	0.421* (↓31%)
	% of terminal body weight	0.322	0.209 (↓35%)	0.240 (↓25%)	0.190** (↓41%)	0.223* (↓31%)
	% of brain weight	33.565	23.568* (↓30%)	25.733 (↓23%)	20.120** (↓40%)	23.886* (↓29%)

[a] Data obtained from pages 75-88 in the study report.
* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control.
*** Statistically different ($p<0.001$) from the control.

2. **Gross Pathology**: At terminal sacrifice, treatment-related macroscopic findings were noted in the liver (enlargement and/or dark color) of males and females at 5000 and 10,000 ppm (Table IIA 5.8.2-10). One male at 500 ppm also exhibited a dark liver. Decreased prostate size was observed in three high-dose males. All other gross changes were considered to be incidental and not treatment-related.

There were no treatment-related macroscopic changes in males at 50 ppm and in females at ≤ 500 ppm.

TABLE IIA 5.8.2-10 Selected macroscopic findings in mice at Week 4 [a]

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	50	500	5000	10,000	0	50	500	5000	10,000
	Males					Females				
	N of rats examined					N of rats examined				
	10	10	10	10	10	10	9	10	10	10
Liver										
Enlarged	0	0	0	10	9	0	0	0	3	4
Dark	0	0	1	10	8	0	0	0	5	7
Prostate										
Obviously small	1	1	1	1	3	-	-	-	-	-

[a] Data obtained from pages 91-92 in the study report.

3. **Microscopic Pathology**: At terminal sacrifice, treatment-related microscopic findings were noted in the liver (≥ 500 ppm), thyroid gland (≥ 5000 ppm) and adrenals (≥ 500 ppm) in males and/or females, and in the prostate of high-dose males (Table IIA 5.8.2-11).

In the liver, a diffuse centrilobular to panlobular hepatocellular hypertrophy was noted in all animals (both sexes) treated at 5000 and 10,000 ppm, and in one male at 500 ppm. An increased incidence of hepatocellular necrotic foci was also observed in males at ≥ 5000 ppm (1-1-1-3-4). In the thyroid, follicular cell hypertrophy was noted in both sexes at 5000 and 10,000 ppm without any dose relationship. At 5000 ppm, 9/10 males and 1/10 females were affected whereas only 1/10 males and no females were affected at the higher dose level of 10,000 ppm.

In the adrenals, a diffuse microvacuolation of the zona fasciculata was observed in males at ≥ 500 ppm and in females at ≥ 5000 ppm.

An increased incidence of interstitial mononuclear cell infiltration in the prostate was observed in males at 10,000 ppm (3-0-0-0-7).

There were no treatment-related microscopic changes in males at 50 ppm and in females at ≤ 500 ppm.

TABLE IIA 5.8.2-11 Selected microscopic findings in rats at Week 4 (terminal sacrifice) [a]

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	50	500	5000	10,000	0	50	500	5000	10,000
	Males					Females				
Liver: number examined	**10**	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**10**
Hepatocellular hypertrophy, centrilobular to panlobular, diffuse	0	0	1	10	10	0	0	0	10	10
Hepatocellular necrotic foci	1	1	1	3	4	1	0	0	0	0
Thyroid: number examined	**10**	**10**	**10**	**10**	**9**	**10**	**9**	**10**	**10**	**9**
Follicular cell hypertrophy, diffuse	0	0	0	6	1	0	0	0	1	0
Adrenals: number examined	**10**	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**10**

Microvacuolation of the zona fasciculata, diffuse	0	0	2	2	4	0	0	0	1	3
Prostate: number examined	**9**	**1**	**1**	**1**	**10**	-	-	-	-	-
Interstitial mononuclear cell infiltration, multifocal	3	0	0	0	7	-	-	-	-	-

[a] Data obtained from pages 99 and 102 in the study report.

III. CONCLUSIONS:

There were no treatment-related effects on mortality, clinical signs, opthalmoloscopic or neurotoxic parameters.

Decreased body weights and body weight gains were observed in males at 10,000 ppm. Decreases in food consumption were noted in high-dose males during the first 3 weeks and in high-dose females during the first week.

Increased crystals in the urine were observed in both sexes at ≥ 5000 ppm and were considered to be treatment-related.

Increases in prothrombin times were noted in males at ≥ 5000 ppm, and increased cholesterol and triglyceride concentrations were observed in females at ≥ 5000 ppm. Significant increases in liver weights were observed in both sexes (males at ≥ 500 ppm and females at ≥ 5000 ppm) and were associated with an enlarged and/or dark liver, and with a diffuse centrilobular to panlobular hepatocellular hypertrophy. A slight increased incidence of hepatocellular necrotic foci was also noted in males at ≥ 5000 ppm. These findings are evidence of hepatotoxicity.

Significant increases in thyroid gland weights were observed in males at ≥ 5000 ppm and were associated with a follicular cell hypertrophy although not in a dose-related manner. One female at 5000 ppm also exhibited a similar lesion in the thyroid gland. These changes in the thyroid were consistent with increases in TSH levels and/or decreases in T4 and/or T3 levels observed in males at ≥ 5000 ppm and in females at ≥ 500 ppm and are suggestive of an imbalance of thyroid hormones resulting in the over-stimulation of the thyroid gland.

The reviewer disagrees with the study author pertaining to the effects on adrenal glands. Increases in adrenal gland weights were noted in males at 10,000 ppm and in females at ≥ 5000 ppm. In addition, six males and four females at ≥ 5000 ppm, and two males at 500 ppm exhibited a diffuse microvacuolation of the adrenal zona fasciculata. The study author claimed that: "As these changes showed a low incidence and severity, and were not associated with degenerative lesions, they were considered to be fortuitous." However, the reviewer considered the adrenal gland changes as treatment-related and toxicologically relevant since the increased adrenal weights were associated with histopathology changes in both sexes and that similar effects were observed in the 28-day dietary short-term study with the parent compound (ethiprole) (PMRA # 1730839).

The study author also claimed that: "The mean absolute and relative prostate weights were lower in males at 10,000 and 5000 ppm, when compared to controls. These differences were considered of doubtful toxicological significance as no correlated microscopic change was seen". However, in addition to the decrease in prostate weights observed in males at ≥ 5000 ppm, the reviewer noted a reduced prostate size and an increased incidence of interstitial mononuclear cell

infiltration in males at 10,000 ppm. These effects were considered to be toxicologically relevant by the reviewer.

The reviewer is in disagreement with the investigator's conclusions pertaining to the NOAEL. The study author stated that the NOAEL is 500 ppm in both sexes. The study author argued that the statistically significant increase in TSH levels observed in females at 500 ppm was not associated with a decrease in T4 levels, therefore its toxicological significance was considered doubtful at this dose level. The study author did not comment on the effects observed in the liver of males at 500 ppm, and as mentioned above, the study author also considered that changes in the adrenal gland at all doses were not treatment-related. However, the reviewer considered the NOAEL to be 50 ppm (5.2 mg/kg bw/day) and the LOAEL to be 500 ppm (51.4 mg/kg bw/day in males and 53.5 mg/kg bw/day in females) in both sexes based on increased TSH levels, increased liver weights associated with a dark liver and/or a diffuse centrilobular to panlobular hepatocellular hypertrophy, and an increased incidence of microvacuolation of the adrenal zona fasciculata.

KIIA 5.3.1 - Oral 28-day toxicity

1.	Annex point(s)	KIIA 5.3.1; Short-term toxicity/Oral 28-day toxicity: **MRID 47622828**
2.	Location in dossier	KIIA 5.3.1 /01
3.	Authors (year)	Dange, M. (1999)
	Title	RPA 107382 preliminary 28 day toxicity study in the C57 B1/6N mice by dietary administration
	Company, report No.	Bayer CropScience AG, Report-No.: R005957 (M-171883-01-1)
	Date	05.03.1999
4.	Testing facility	Rhone-Poulenc Agro, Sophia Antipolis, France
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC
7.	Test method	
	Deviations	
8.	GLP	yes

Executive Summary:

Ethiprole was administered continuously via the diet at 0, 50, 250, 1000 or 2500 ppm to separate groups of 15 mice /sex/dose. Mortality and clinical signs were checked daily. Body weight and food consumption were measured weekly throughout the study. Clinical chemistry was performed at the end of the dosing phase. All animals were subjected to necropsy, selected organs were weighed and designated tissues sampled and examined microscopically.

The short-term dietary administration of ethiprole (RPA 107382) to mice for at least 28 days produced signs of overt toxicity at 2500 ppm. This dose level, which induced the death or premature sacrifice of almost all animals, was considered to have exceeded the MTD.

Ethiprole occasionally induced increased motor activity in males and females at 2500 ppm. The body weight evolution of animals at 2500 ppm was comparable to controls. At 2500 ppm, statistically significant lower food consumption was noted in males and females during the first week of treatment.

Decreases in mean total bilirubin concentrations were seen in males from 50 ppm onwards and in females from 250 ppm onwards. Higher mean aminotransferase activities were seen in males at 1000 and 2500 and ppm. Decreases in alkaline phosphatase were noted in both sexes at 2500 ppm. Ethiprole also induced a dose-related increase in total liver cytochrome P-450 content in males at 1000 and 2500 ppm.

Significant increases in liver weights were observed in males and females at 250, 1000 and 2500 ppm.

At the histopathological examination, the liver; with centrilobular to panlobular hepatocellular hypertrophy, hepatocyte microvacuolation, and necrosis; was found to be the target organ in animals at 250 and 1000 ppm.

In conclusion, the **LOAEL** is 47.4 mg/kg/day in males and 57.9 mg/kg/day in females (250 ppm in diet), based on lower total bilirubin, increased liver weights and histopathologic changes in the liver. The **NOAEL** is 9.3 mg/kg/day in males and 11.8 mg/kg/day in females (50 ppm in diet).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3050; OECD 407) for a subchronic oral toxicity study in the mice.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	93%
Compound Stability:	The test substance was stored in an air-tight, light resistant container at 4°C
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. Control animals received untreated diet.

3. **Test Animals:**

Species:	Mouse	
Strain:	C57 B1/6N Crl BR mice	
Age/weight at study initiation:	7 weeks old / 19.9 to 25.1 g for the males and 12.6 to 18.8 g for the females	
Source:	Charles River France, St Aubin-les-Elbeuf, France	
Housing:	Individually in suspended stainless steel, wire mesh cages.	
Diet:	Certified rodent powder diet "M 20 controle" (Pietrement, Provins, France), *ad libitum*	
Water:	Filtered and softened water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	22 ± 2°C (target)
	Humidity:	55 ± 15% (target)
	Air changes:	10-15 changes per hour (average, not monitored)
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	6 days	

B. STUDY DESIGN:

1. **In-Life Dates:** Start: July 28, 1998; End August 30, 1998

2. **Animal Assignment/Dose Levels:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution by sex to the test groups noted in Table 5.3.1.-1.

TABLE IIA 5.3.1.-1 Study Design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day) [a]		No. of Animals	
		Males	Females	Males	Females
Control	0	0	0	15	15
Low	50	9.3	11.8	15	15
Mid-1	250	47.4	57.9	15	15
Mid-2	1000	186.2	234.4	15	15
High	2500	458.0	513.0	15	15

a Mean achieved dietary intake of ethiprole (RPA 107382), weeks 1 to 4

3. **Diet Preparation and Analysis:** The test substance was incorporated into the diet ("M 20 controle", Pietrement, Provins, France) by dry mixing to provide the required dietary concentrations. Each test diet concentration was prepared once for the entire study.

   The stability of ethiprole in diet was demonstrated in a previous study at 5 and 2500 ppm (Reference 1, Study Number SA 97233) under storage and usage conditions similar to those of the current study.

   Before the start of the study, the homogeneity of ethiprole in diet was verified for the lowest and highest concentrations to demonstrate adequate formulation procedures. The sampling was done at three levels, the surface, middle and bottom. At each level, 2 samples (1 & 3) were taken and analyzed. The mean value obtained from the homogeneity checks (ethiprole) was used as measured concentration. The concentration of the other preparations was also checked. Sampling was done on 3 levels (the surface, middle and bottom) and pooled.

   Results: **Homogeneity Analysis:** Results for homogeneity analyses conducted prior to the start of the study were within the range of 105-115% of the nominal concentrations.

   Stability Analysis: Stability results for up to 38 days at room temperature and 31 days of frozen storage followed by 7 days at ambient temperature ranged from 88-99% of the nominal concentrations and were thus within the target range of 85-115% of nominal concentration (see Study Profile for Study Number SA 97233).

   Concentration Analysis: Results for the concentration analyses conducted prior to the start of the study were within 110-115% of the nominal concentrations.

   On Day 11, following unexpected mortality at 1000 ppm, the diet of the animals found dead was analyzed. As the results clearly showed that these animals received diet containing 2500 ppm instead of 1000 ppm, diets from the feeders of all animals at 1000 and 2500 ppm were analyzed. The analytical results demonstrated an error in the delivery to the animals of the 1000 and 2500 ppm diets from Day 8 (August 04, 1998 in the morning) to Day 11 (August 07, 1998 in the afternoon). During that 4-day dosing period, animals in the 1000 ppm group received the 2500 ppm diet, and animals in the 2500 group received the 1000 ppm diet.

4. **Statistics**: Statistical analyses were performed on the following endpoints: body weight parameters, food consumption, water consumption, clinical chemistry parameters, organ weight parameters, organ/body weight ratios, organ/brain weight ratios, and total cytochrome P-450 content.

Means and standard deviations (STD) were calculated for each sex separately for each group at each time period. Results of body weight parameters, food consumption and of total cytochrome P-450 content were inter-compared for the treated groups and the control group by use of Bartlett's test (5) for homogeneity of variances and analysis of variance (ANOVA). If Bartlett's indicated homogeneous variances and the ANOVA was significant, the group means were inter-compared to the control mean using the Dunnett's test. If Bartlett's test indicated heterogeneous variances, a non-parametric statistical procedure was performed using the Kruskal-Wallis non-parametric one-way analysis of variance by ranks. If the Kruskal-Wallis test was significant, the Mann-Whitney test was used to compare each group to the control. Statistical analyses were performed using SAS programs.

Results of clinical chemistry and organ weight parameters were inter-compared for the treated groups and the control group with *PathTox* computer system by use of Dunnett's test if Bartlett's test indicated homogeneous variances. If Bartlett's test indicated non-homogeneous variances, variables were analyzed using SAS programs and a non-parametric statistical procedure was performed using the Kruskal-Wallis non-parametric one-way analysis of variance by ranks. If the Kruskal-Wallis test was significant, Mann-Whitney's test was used to compare each group to the control. The levels of significance for each statistical comparison were 0.05 and 0.01.

C. METHODS:

1. **Observations:** All animals were checked for moribundity and mortality twice daily (once daily on week-ends or public holidays). Observed clinical signs were recorded at least once daily for all animals. Detailed physical examinations were performed at least weekly during the treatment period. The nature, onset, severity, reversibility and duration of clinical signs were recorded. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose feces.

2. **Body weight:** Each animal was weighed once during the acclimatization period, on the first day of test substance administration, then at weekly intervals throughout the treatment period and before necropsy.

3. **Food Consumption and Compound Intake**: The weight of food supplied and of that remaining at the end of the food consumption period was recorded weekly for all animals during the treatment period.

4. **Clinical Chemistry:** On study Days 29, 30, 31, 32 or 35 blood samples were taken from all surviving animals in all groups by puncture of the retro-orbital venous plexus. An approximately equal number of animals randomly distributed amongst all groups were sampled on each day. Animals were fasted overnight prior to bleeding and anesthetized by inhalation of isoflurane. Blood (0.6 ml) was collected on lithium heparin for plasma chemistry determinations. Any significant change in the general appearance of the plasma was recorded. In addition, the following parameters were determined: total bilirubin, and aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities.

5. **Sacrifice and Pathology**: On study Days 29, 30, 31, 32 or 35, all surviving animals from all groups were sacrificed by exsanguination under deep anesthesia (intraperitoneal injection of pentobarbital, 60 mg/kg body weight). An approximately equal number of animals randomly

distributed amongst all groups were sacrificed on each day. Animals were fasted overnight prior to sacrifice. All animals, either found dead or killed by design, were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Significant macroscopic abnormalities were recorded and sampled.

The CHECKED (X) tissues were collected; and the (XX) organs were weighed. Only livers were examined microscopically. Livers from all animals in the control, 50, 250 and 1000 ppm groups were examined microscopically. Due to the high mortality at 2500 ppm, livers were not examined microscopically for this group.

	DIGESTIVE SYSTEM		**CARDIOVASC./HEMAT.**		**NEUROLOGIC**
	Tongue		Aorta, thoracic	XX	Brain (multiple sections)
	Salivary glands	XX	Heart		Periph.nerve
	Esophagus		Bone marrow		Spinal cord (3 levels)
	Stomach		Lymph nodes	X	Pituitary
	Duodenum	XX	Spleen		Eyes (retina, optic nerve)
	Jejunum	XX	Thymus		**GLANDULAR**
	Ileum			XX	Adrenal gland
	Cecum		**UROGENITAL**		Lacrimal gland
	Colon	XX	Kidneys	X	Parathyroids
	Rectum	X	Urinary bladder	X	Thyroids
					Harderian gland
XX	Liver	XX	Testes		**OTHER**
X	Gall bladder (not rat)	X	Epididymides		Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate		Skeletal muscle
	Pancreas	X	Seminal vesicle		Skin
	RESPIRATORY	X	Ovaries	X	All gross lesions and masses
X	Trachea*	X	Uterus (with cervix)		Articular surface (femoro-tibial joint)
X	Lung		Mammary gland		
	Nose	X	Vagina		
	Pharynx				
	Larynx				

6. **Hepatotoxicity Testing**: At terminal sacrifice, the remaining portions of the liver were pooled, when possible, into sub-groups of three animals for microsomal preparation in order to determine total cytochrome P-450 content. Total cytochrome P-450 content from hepatic microsomal preparation was determined by spectrophotometry using a reduced CO differential spectrum.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity**: Increased motor activity was occasionally observed in males and females at 2500 ppm.

No treatment-related clinical signs were observed at 50, 250 and 1000 ppm in either sex.

2. **Mortality:** Treatment-related mortalities were observed in 9/15 males and 12/15 females at 2500 ppm.

No treatment-related mortalities were observed at 50, 250 and 1000 ppm in either sex.

It should be noted, however, that there were a total of twenty seven mortalities during the study: One female in the control group, one male and four females at 1000 ppm as well as the nine males and twelve females at 2500 ppm. Among the animals which died in the 1000 ppm dose group the death of the male and three females (found dead on Days 9 and 10) were considered to be due to the erroneous administration of 2500 ppm from Day 8 to Day 11. The death of the fourth female in the 1000 ppm group was due to an accidental trauma. The control female animal was found dead on day 6, and histopathology revealed autolysis of all organs. All animals at 2500 ppm which died before terminal sacrifice were found dead between days 20-24 of the study.

B. **BODY WEIGHT**: No treatment-related changes in body weight and body weight gain were observed at any dose level in either sex.

C. **FOOD CONSUMPTION AND COMPOUND INTAKE**

1. **Food Consumption:** Treatment-related decreases in food consumption were observed at 2500 ppm during the first week of dosing in males and females (Table IIA 5.3.1.1-2).

No treatment-related changes in food consumption were observed at 50, 250 and 1000 ppm in either sex.

TABLE IIA 5.3.1.1-2 Mean Food Consumption (g/day)

Interval (Week)	Dose Level (ppm in diet)				
	0	50	250	1000	2500
Males					
1	4.44	4.68	4.85*	4.45	4.04*
4	4.73	4.55	4.74	4.64	5.15*
Females					
1	4.19	4.62	4.35	4.24	3.65**
4	4.95	4.79	4.89	5.06	4.60

* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control.

3. **Compound Consumption (time-weighted average):** The mean achieved dose levels expressed in mg/kg body weight day are presented in Table IIA 5.3.1-3. Food consumption and achieved dietary intake data for Week 2 was not used for these groups because of an error in the delivery of the treated diets. (Table IIA 5.3.1.1-3).

TABLE IIA 5.3.1.1.3 Mean Achieved Dietary Intake of Ethiprole (Weeks 1-4)

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	
		Males	Females
Control	0	0	0
Low	50	9.3	11.8
Mid-1	250	47.4	57.9
Mid-2	1000	186.2	234.4
High	2500	458.0	513.0

D. BLOOD ANALYSES:

1. **Clinical Chemistry:** At the end of the dosing phase, treatment-related effects were observed at all dose levels in males and at 250, 1000 and 2500 ppm in females (Table IIA 5.3.1.1-4).

   Ethiprole induced a dose-related decreases in mean total bilirubin concentrations in males from 50 ppm onwards (-31 to -74%) and in females from 250 ppm onwards (-49 to -69%). Higher mean aminotransferase activities were seen in males at 1000 and 2500 and ppm: the mean aspartate aminotransferase (ASAT) activities were increased by 87% and 100%, respectively; the mean alanine aminotransferase (ALAT) activities were increased by 368% and 432%, respectively. Alkaline phosphatase (ALP) was decreased in males and females at 2500 ppm (-14% and -34%, respectively).

   Other statistically significant variations noted in clinical chemistry were of low magnitude and/or incidence and/or not dose-related, thus they were not considered to be toxicologically significant.

   No treatment-related biochemical changes were observed at 50 ppm in females.

TABLE IIA 5.3.1.1-4 Selected clinical chemistry parameters in mice at Week 4

Interval	Parameter	Dose Level (ppm in diet)				
		0	50	250	1000	2500
Males						
Number of animals examined		15	15	15	14	6
Week 4	TBIL (µmol/1)	1.21	0.84*	0.59**	0.43**	0.32**
	ASAT (IU/l)	130.7	116.5	107.1	244.6**	261.2*
	ALAT (IU/l)	49.3	53.9	51.4	230.7**	262.2**
	ALP (IU/l)	84.8	83.9	86.4	98.9*	72.7*
Females						
Number of animals examined		14	15	15	11	3
Week 4	TBIL (µmol/1)	0.81	0.88	0.41*	0.25**	0.27*
	ASAT (IU/l)	141.7)	135.7	125.7	120.5	104.7
	ALAT (IU/l)	55.9	48.5	57.9	50.4	84.3
	ALP (IU/l)	151.7	153.1	153.1	154.7	100.0*

* Statistically different (p<0.05) from the control. ** Statistically different (p<0.01) from the control. *** Statistically different (p<0.01) from the control.

E. SACRIFICE AND PATHOLOGY:

1. **Organ Weight:** At terminal sacrifice, treatment-related organ weight changes were noted in the liver at 250, 1000 and 2500 ppm (Table IIA 5.3.1.1-5).

   Terminal body weight was slightly higher in females at 2500 ppm (+15%, p<0.01).

   In the liver, a dose-related increase in absolute and relative weights were observed at 250, 1000 and 2500 ppm in males (+15%, +40%, +70% for absolute, respectively, p<0.001) and in females (+14%, +43% and +110%, for absolute, respectively, p<0.001).

   A few statistically significant minimal differences were observed as compared to control group, namely a higher mean absolute brain and kidney weight at 2500 ppm in females; and a lower mean absolute and/or relative testicular weight in males at 50 (absolute), 250 and 2500 ppm (relative to brain). However, these differences were minimal, not observed for all the modes of expression of the organ weights, not dose-related, and not correlated to gross or microscopic changes. Furthermore, the 2500 ppm group of female animals remaining and available for organ weighing consisted only of three animals, thus organ weight means were calculated from a very small sample size and may not be representative of an actual trend in this group of animals. Therefore, none of the above mentioned organ weight changes were not considered to be biologically or toxicologically significant.

No significant treatment-related organ weights changes were observed at 50 ppm in either sex.

TABLE IIA 5.3.1.1.5 Mean Terminal Body Weight, Absolute and Relative Liver weights

Organ	Parameter	Dose level (ppm in diet)				
		0	50	250	1000	2500
Males						
Number of animals examined		15	15	15	14	6
Terminal body weight (g)		21.2	20.9	21.8	21.3	22.2
Liver	Absolute weight (g)	0.93	0.95	1.07**	1.30**	1.58**
	% of terminal body weight	4.38	4.52	4.93**	6.08**	7.09**
	% of brain weight	204.9	209.9	238.7**	289.0**	332.2**
Testes	Absolute weight (g)	0.189	0.168*	0.171	0.181	0.165
	% of terminal body weight	0.893	0.805	0.779*	0.850	0.739*
	% of brain weight	41.66	37.34	37.66	40.33	34.73*
Females						
Number of animals examined		14	15	15	11	3
Terminal body weight (g)		16.2	16.7	16.8	16.8	18.7 **
Liver	Absolute weight (g)	0.68	0.75	0.78**	0.97**	1.43**
	% of terminal body weight	4.24	4.47*	4.68**	5.74**	7.63**
	% of brain weight	158.4	169.0	180.2**	214.8**	302.8**

* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control. *** Statistically different ($p<0.01$) from the control.

2. **Gross Pathology**: Treatment-related macroscopic findings were noted in the livers of males and females at 1000 and 2500 ppm which survived to terminal sacrifice as well as in those animals which were found dead during the study (Table IIA 5.3.1.1-6)

   Liver enlargement and/or pale color were seen in most males at 1000 and 2500 ppm and females at 2500 ppm.

   Spleen enlargement was noted in the majority of 2500 ppm animals which were found dead before terminal sacrifice. It is expected that the splenomegaly observed in these animals is the product of blood accumulation in the spleen due to liver tissue damage (evidenced by liver macro- and microscopic findings) and subsequent hypertension of hepatic veins. Hypertension in the hepatic venous bed results in shunting of the blood to other bodily blood reservoirs, such as the spleen.

TABLE IIA 5.3.1.1-6 Selected macroscopic findings in mice

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	50	250	1000	2500	0	50	250	1000	2500
	Final Sacrafice									
	Males					Females				
	N of rats examined					N of rats examined				
	15	15	15	14	6	14	15	15	11	3
Liver										
Enlarged	0	0	2	9	3	0	1	1	2	3
Pale	1	0	1	7	1	0	2	2	1	2
Finding	Unscheduled Deaths									
	Males					Females				
	N of rats examined					N of rats examined				
	0	0	0	1	9	1	0	0	5	12
Liver										
Enlarged	0	0	0	0	8	0	0	0	1	12
Pale	0	0	0	1	1	0	0	0	2	5
Pominent Lobulation	0	0	0	0	4	0	0	0	1	3
Spleen										
Enlanged	0	0	0	1	6	0	0	0	1	9

3. **Microscopic Pathology**: At terminal sacrifice, treatment-related microscopic findings were noted in the liver at 250 and 1000 ppm (Table IIA 5.3.1.1-7). The 2500 ppm treated group was not examined due to the high mortality.

   In the liver, hepatocellular hypertrophy was noted in most males and females treated at 1000 ppm and at 250 ppm. Diffuse hypertrophy was noted in treated animals of both sexes, while centrilobular and periportal hepatocellular hypertrophy were noted only in either male or female animals, respectively. Hepatocyte microvacuolation, characterized by the cytoplasmic accumulation of small vacuoles in the hepatocytes, was noted in males at 250 and 1000 ppm. Hepatocyte necrosis was also observed in males at 250 and 1000 ppm.

   No treatment-related microscopic changes were observed at 50 ppm

TABLE IIA 5.3.1.1-7 Selected microscopic findings in mice at Week 4 (terminal sacrifice)

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	50	250	1000	2500	0	50	250	1000	2500
	Males					Females				
Liver number examined	15	15	15	14	-	14	15	15	11	-
Hepatocellular hypertrophy	1	0	1	8	-	0	0	2	12	-
Hepatocyte microvacuolation, diffuse	5	4	11	13	-	0	0	2	1	-
Focus(i) of hepatocyte necrosis	0	0	2	14	-	0	0	0	0	-

4. **Hepatotoxicity Testing**: At terminal sacrifice, treatment-related changes were noted in males at 1000 and 2500 ppm (Table IIA 5.3.1.1-8).

   Ethiprole induced a dose-related increase in total liver cytochrome P-450 content in males at 1000 and 2500 ppm (+23% and 42%, respectively).

   No treatment-related effects were observed at 50 and 250 ppm in males and at any dose level in females. The slight decrease at 250 ppm in females was not thought to be a significant effect, due to the absence of a dose-response relationship.

TABLE IIA 5.3.1.1.8 Cytochrome P-450 Content

Interval	Parameter	Dose Level (ppm in diet)				
		0	50	250	1000	2500
Males						
Number of animals examined		5	5	5	5	3
Total Cytochrome P450 Content (nmol/mg prot.)		1.306 (±0.155)	1.144 (±0.307)	1.347 (±0.044)	1.602* (±0.157)	1.855* (±0.089)
Females						
Number of animals examined		4	5	5	4	2
Total Cytochrome P450 Content (nmol/mg prot.)		1.775 (±0.459)	1.149 (±0.270)	1.118* (±0.098)	1.515 (±0.119)	1.521 (±0.050)

*Statistically different ($p<0.05$) from the control.

III. CONCLUSIONS:

The short-term dietary administration of ethiprole (RPA 107382) to mice for at least 28 days produced signs of overt toxicity at 2500 ppm. This dose level, which induced the death or premature sacrifice of almost all animals, was considered to have exceeded the MTD.

Ethiprole occasionally induced increased motor activity in males and females at 2500 ppm. Ethiprole also induced a dose-related increase in total liver cytochrome P-450 content in males at 1000 and 2500 ppm. Furthermore; decreases in mean total bilirubin concentrations were seen in

males from 50 ppm onwards and in females from 250 ppm onwards. Higher mean aminotransferase activities were seen in males at 1000 and 2500 and ppm, and decreases in alkaline phosphatase were noted in both sexes at 2500 ppm.

Significant increases in liver weights were observed in males and females at 250, 1000 and 2500 ppm. Macroscopic examination revealed an enlarged liver and spleen, as well as changes in liver colouration and lobulation at 2500 ppm in males and females, with some findings in 1000 ppm males. Therefore; following histopathological examination, the liver with centrilobular to panlobular hepatocellular hypertrophy, hepatocyte microvacuolation and necrosis was found to be the target organ in animals at 250 and 1000 ppm (the highest dose levels examined microscopically).

In conclusion, the NOAEL is 9.3 mg/kg/day in males and 11.8 mg/kg/day in females (50 ppm in diet). The LOAEL is 47.4 mg/kg/day in males and 57.9 mg/kg/day in females (250 ppm in diet), based on lower total bilirubin, increased liver weights and histopathologic changes in the liver.

KIIA 5.3.2 - Oral 90-day toxicity (rodents)

1.	Annex point(s)	KIIA 5.3.2; Short-term toxicity/Oral 90-day toxicity (rodents): **MRID 47622806**
2.	Location in dossier	KIIA 5.3.2 /02
3.	Authors (year)	Dange, M.; Foulon, O. (2000)
	Title	90-day toxicity study in the rat by dietary administration RPA107382
	Company, report No.	Bayer CropScience AG, Report-No.: R016682 (M-192116-02-1)
	Date	13.04.2000, Amended: 23.01.2002
4.	Testing facility	Aventis CropScience, Sophia Antipolis, France;
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC (Batch-No.: CDR9706)
7.	Test method	EU (=EEC): 92/69/EEC, Annex V, Method B26; JMAFF: 59 NohSan No. 4200, (1985); OECD: 408, (1981); USEPA (=EPA): FIFRA 82-1, (1984)
	Deviations	
8.	GLP	yes

Executive summary:
Ethiprole was administered continuously via the diet at 0, 5, 20, 500 or 2500 ppm (equivalent to 0, 0.3, 1.2, 30.5, and 154.7 mg/kg bw/day in males, respectively, and 0, 0.4, 1.5, 37.6, and 187.9 mg/kg bw/day in females, respectively) to separate groups of 10 rats /sex/dose. Mortality and clinical signs were checked daily. Body weight and food consumption were measured weekly throughout the study. Ophthalmic examinations were performed on all animals during the acclimatization period and during Week 12 of dosing. Clinical chemistry, hematology and urinalysis were performed towards the end of the dosing phase. In addition, a blood sample was collected during Weeks 2, 6, 13 for T3, T4 and TSH analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues sampled and examined microscopically.

The short-term dietary administration of ethiprole (RPA 107382) to rats for at least 90 days produced signs of overt toxicity at 2500 ppm and at 500 ppm to a lesser extent. These two dose levels, which induced death or premature sacrifice, were considered to have exceeded the MTD.

Clinical signs consisting of piloerection, increased and/or reduced motor activity and irritability to touch were noted mainly in animals at 2500 ppm on few occasions. The body weight evolution of animals at 2500 ppm was inferior to that of controls during the first five weeks for the males and only during the first week for the females. At 2500 ppm, statistically significant lower food consumption was noted in males during the first three weeks and in females during the first week.

Increases in prothrombin time were noted in males at 500 and 2500 ppm. Increases in cholesterol, total protein, triglyceride, calcium and potassium concentrations and a decrease in chloride concentration were reported at 500 and 2500 ppm.

Ethiprole induced an imbalance of thyroid hormones in animals treated at 500 and 2500 ppm resulting in higher levels of TSH and T3 and lower levels of T4.

Significant increases in liver and thyroid weights were observed at 500 and 2500 ppm. Increased adrenal weight was observed at 500 and 2500 ppm and increased prostate weight was observed at 2500 ppm.

At the histopathological examination, the liver with centrilobular hepatocyte hypertrophy, and the thyroid with follicular hypertrophy/hyperplasia were found to be the target organs in animals treated at 500 and 2500 ppm. The kidney was also affected at 2500 ppm with both sexes showing tubular golden brown pigment.

In conclusion, the **LOAEL** is 30.5 mg/kg/day in males and 37.6 mg/kg/day in females (500 ppm in diet), based on mortality, changes in thyroid hormone levels, clinical pathology parameters, and increased liver and thyroid weights associated with microscopic changes in these target organs. The **NOAEL** is 1.2 mg/kg/day in males and 1.5 mg/kg/day in (20 ppm in diet).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100; OECD 408) for a subchronic oral toxicity study in the rat.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CDR9706
Purity:	93%
Compound Stability:	The test substance was stored in an air-tight, light resistant container at room temperature.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. Control animals received untreated diet.

3. **Test Animals:**

Species:	Rats	
Strain:	Wistar rats R.J: WI (IOPS AF)	
Age/weight at study initiation:	6-7 weeks/ 243 - 280g males; 174 - 203g females	
Source:	R. Janvier (Le Genest St Isle, France).	
Housing:	Individually in suspended stainless steel, wire mesh cages.	
Diet:	Ground diet, "M20 contrôlé" (Pietremont BP 59, 77482 Provins Cedex, France), *ad libitum*	
Water:	Filtered and softened water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	20°C - 24°C (target)
	Humidity:	40% - 70% (target)
	Air changes:	10 to 15 changes per hour (average, not monitored)
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	14 days	

B. STUDY DESIGN:

1. **In-Life Dates:** Start: September 10, 1997; End: December 12, 1997

2. **Animal Assignment/Dose Levels:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution by sex to the test groups noted in Table 5.3.2-1. The dose levels were selected after evaluation of the results from a previous 90-day dietary study in Sprague Dawley rats performed with this test substance (Bigot, D. (SA 95219): 90-Day Toxicity Study in the Rat by Dietary Administration. Bayer Cropscience, Sophia Antipolis, France (August 26, 1996).

TABLE IIA 5.3.2-1 Study Design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)[a]		No. of Animals	
		Males	Females	Males	Females
Control	0	0	0	10	10
Low	5	0.296	0.373	10	10
Mid-1	20	1.174	1.503	10	10
Mid-2	500	30.484	37.567	10	10
High	2500	154.746	187.867	10	10

a Mean achieved dietary intake of ethiprole (RPA 107382), weeks 1 to 13.

3. **Diet Preparation and Analysis:** The test substance was ground to a fine powder before being incorporated into the diet (Certified Rodent Pellet diet "M 20 control") by dry mixing to provide the required concentrations. Diets were prepared approximately every three weeks with a total of four preparations for the study. When not in use the diet formulations were stored at approximately -18°C.

   The homogeneity of test substance in diets was verified prior to the initiation of the study on the first formulation at the lowest and highest concentrations to demonstrate adequate mixing procedures.

   Stability of the formulations was checked for the highest (2500 ppm) and lowest (5 ppm) concentrations in an analytical pre-study. The following storage intervals and conditions were investigated: 10, 17, 24, 31, or 45 days frozen, each followed by 7 days at room temperature; and 17, 24, 31, 38 and 52 days at room temperature (no preceding frozen storage interval).

   Dietary levels of the test substance were verified for each concentration following the first, the second and last dietary preparation. In addition, samples of each concentration from the third preparation were frozen for possible future analysis.

   Results: **Homogeneity Analysis:** Results for homogeneity of ethiprole in the 5 and 2500 ppm dose level diet formulations were within the range of 86-106% of the nominal concentrations. (Data obtained from the study report, pages 406 and 411, original pagination).

   Stability Analysis: Stability results were within the in-house target range of 85-115% of the nominal concentration with two exceptions. For the 5 ppm diet preparation held 52 days at ambient temperatures and for the 5 ppm diet held frozen for 45 days followed by 7 days at ambient temperatures, recoveries were slightly below the target (84% each, instead of 85%). However, animals only received diets which were frozen for less than 42 days and stability data within that time frame were acceptable. Results from all other storage stability analyses were within the range of 88-99% of the nominal concentrations. (Data obtained from the study report, pages 409 and 410, original pagination).

   Concentration Analysis: Results for the concentration analyses were within 86-99% of the nominal concentrations. (Data obtained from the study report, pages 407, 412, 413, original pagination).

4. **Statistics**: Statistical analyses were performed on the following endpoints: body weight parameters, body weight changes, food consumption, hematology parameters (except eosinophils, basophils, monocytes and large unstained cells), clinical chemistry parameters, urinary parameters (only pH, volume and refractive index), organ weight parameters, and hormone data.

Means and standard deviations (STD) were calculated for each sex separately for each group at each time period. The levels of significance for each statistical comparison were 0.05 and 0.01.

Results of body weight parameters, body weight gains, food consumption and hormone data were intercompared for the exposed groups and the control group by use of Bartlett's test for homogeneity of variances, analysis of variance (ANOVA). If Bartlett's test indicated homogeneous variances and the ANOVA was significant, the group means were intercompared to the control mean using the Dunnett's test. If Bartlett's test indicated heterogeneous variances, non-parametric statistical procedure was performed using the Kruskal-Wallis non-parametric one-way analysis of variance by ranks. If the Kruskal-Wallis test was significant, Mann-Whitney test was used to compare each group to the control. Statistical analyses were performed using SAS programs.

Results of hematology (except percentages and absolute values for eosinophils, basophils, monocytes and large unstained cells), clinical chemistry, urinalysis variables (pH, volume and refractive index) and organ weight parameters were intercompared for the exposed groups and the control group with PathTox computer system by use of Dunnett's test if Bartlett's test indicated homogeneous variances. If Bartlett's test indicated non-homogeneous variances, variables were analyzed using SAS programs and a non-parametric statistical procedure was performed using the Kruskal-Wallis non-parametric one-way analysis of variance by ranks. If the Kruskal-Wallis test was significant, Mann-Whitney's test was used to compare each group to the control.

C. METHODS:

1. Observations:

1a. Cageside Observations and Clinical Examinations: All animals were checked for moribundity and mortality twice daily (once daily on week-ends or public holidays). Observed clinical signs were recorded at least once daily for all animals. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose feces.
Detailed physical examinations were performed during the acclimatization phase and at least weekly during the treatment period.

1b. Neurological Evaluations: No neurological evaluations were performed in this study. These data are available in separate rat neurotoxicity studies using eithiprole.

2. **Body weight:** Each animal was weighed once during the acclimatization period, on the first day of test substance administration, then at weekly intervals throughout the treatment period, and before necropsy.

3. **Food Consumption and Compound Intake:** The weight of food supplied and of that remaining at the end of the food consumption period was recorded weekly for all animals during the treatment period. The mean achieved dosage intake in mg/kg/day for each week and for weeks 1 to 13 was calculated.

4. **Ophthalmoscopic Examination:** During the acclimatization period all animals were subjected to an ophthalmic examination. After instillation of an atropinic agent (Mydriaticum, Merck Sharp and Dohme) each eye was examined by means of an indirect ophthalmoscope. During week 12, all surviving animals from control and high dose group were re-examined.

5. **Hematology & Clinical Chemistry:** On study Days 85, 86 or 87, blood samples were taken from all surviving animals in all groups by puncture of the retro-orbital venous plexus. Animals randomly distributed amongst all groups were sampled on each day. Animals were fasted overnight prior to bleeding and anesthetized by inhalation of isoflurane. Blood was collected on EDTA for hematology (0.5 ml), on lithium heparin for plasma chemistry (2.5 ml) and on sodium citrate for coagulation parameters (0.9 ml). The checked (X) parameters listed below 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 (smear prepared, not examined)
X	Blood clotting measurements (Activated Partial Thromboplastin time) (Clotting time)	X	Thyroid hormones (T3, T4, TSH)
X	(Prothrombin time) (PT)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
	Magnesium	X	Urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium		Globulins
X	Sodium*	X	Glucose
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase	X	Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Thyroid hormones (T3, T4, TSH)
X	Alanine aminotransferase (ALT/also SGPT)		
X	Aspartate aminotransferase (AST/also SGOT)		
	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

NOTE: T3, T4 and TSH were monitored at Weeks 2,6 and 13. T3, T4 and TSH were measured by radioimmunoassay

6. **Urinalysis:** Overnight urine samples were collected from all surviving animals in all groups on study Days 91, 92, 93 or 94, in the morning, prior to sacrifice. An approximately equal number of animals randomly distributed amongst all groups were sampled on each day. Food and water were not accessible during urine collection. The checked (X) parameters were examined.

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

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 at scheduled sacrifice only. Paired organs were weighed together.

   Histopathological examinations were performed on all the animals, either found dead or killed by design, in the control and the 500 and 2500 ppm dose groups, all decedents in all groups, and on the liver, lung, testis, epididymis, adrenal, thyroid and kidney of all the animals in the study. Target organs were examined in the intermediate dose groups as necessary to identify the no effect level. Significant macroscopic findings were also examined in all intermediate dose groups.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta	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	XX	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (incl. optic nerve)
X	Jejunum	XX	Thymus		**GLANDULAR**
X	Ileum			XX	Adrenal gland
X	Cecum		**UROGENITAL**	X	Lacrimal gland
X	Colon	XX	Kidneys	XX	Parathyroid
X	Rectum	X	Urinary bladder	XX	Thyroid
XX	Liver	XX	Testes		**OTHER**
	Gall bladder (not rat)	XX	Epididymides	X	Bone (sternum and/or femur)
	Bile duct (rat)	XX	Prostate	X	Skeletal muscle
X	Pancreas	X	Seminal vesicles	X	Skin
	RESPIRATORY	XX	Ovaries		All gross lesions and masses
X	Trachea	XX	Uterus		
X	Lung	X	Mammary gland		
	Nose	X	Vagina		
	Pharynx				
X	Larynx				

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** Treatment-related clinical signs were observed at 2500 ppm and consisted of piloerection, increased and/or reduced motor activity and irritability to touch. These signs were observed on only very few occasions during the study. In addition, most of the males which died at 2500 ppm exhibited a general pallor before death.
   No treatment-related clinical signs were observed at 5, 20 and 500 ppm in either sex.

2. **Mortality:** Treatment-related mortalities were observed at 500 and 2500 ppm.

   One male and one female at 500 ppm and one male at 2500 ppm were sacrificed moribund,. One female at 500 ppm and seven males at 2500 ppm were found dead. All these mortalities occurred between Days 19 and 90.
   No treatment-related mortalities were observed at 5 and 20 ppm in either sex. One female in the 5 ppm group was sacrificed moribund, however in the absence of any mortality at 20 ppm the study author considered this death to be incidental.

3. **Neurological Evaluations**: Neurological evaluations were not performed. Separate neurotoxicity studies are available in section KIIA 5.7.

B. BODY WEIGHT: Treatment-related decreases in body weight were observed at 2500 ppm during the first five weeks of dosing in males and only during the first week of treatment in

females. Body weight gains were also reduced in both sexes during the first week of exposure (Table IIA 5.3.2-2).

There were no treatment-related changes in body weight and body weight gain at 5, 20 and 500 ppm in either sex.

TABLE IIA 5.3.2-2 Mean Body Weights (g) [%][a] and Body Weight Gains (g/day)

Interval (Week)	Dose Level (ppm in diet)				
	0	5	20	500	2500
Males					
Body Weight (g)					
0	263.3	260.0	261.5	261.4	258.9
1	309.5	303.7	309.1	310.5	259.6** [16]
2	333.2	326.2	334.7	332.5	287.8** [14]
3	377.1	367.1	378.5	373.9	322.6**(n=8) [14]
4	402.2	392.9	406.4	398.9	353.3**(n=7) [12]
5	419.5	406.4	424.6	419.9	378.7*(n=7) [10]
13 [b]	499.9	489.7	524.3	533.3 (n=9) [7]	482.0 (n=2)
Mean Body Weight Gain/Day (g)					
1	7.70	7.28	7.93	8.18	0.12**
13 [b]	0.22	0.80	0.50	1.61 (n=9)	-0.08 (n=2)
Females					
Body Weight (g)					
0	187.1	186.0	186.7	186.4	187.9
1	207.3	206.6	203.9	202.7	183.3** [12]
2	217.4	216.9	213.5	214.8	205.7 [5]
3	238.1	234.4	232.8	229.3	225.8 [5]
4	246.9	244.9	239.3	238.2	236.6
5	254.2	252.5	247.4	244.4	247.4
13	283.2	288.3 (n=9)	280.8	275.1 (n=7)	279.0
Mean Body Weight Gain/Day (g)					
1	3.37	3.43	2.87	2.72	-0.77**
13	-0.70	-0.61 (n=9)	-0.18	-0.55 (n=7)	0.03

a Percent different from control included if ≥ 5%.
b For the high dose group at Weeks 12 and 13, statistical analysis was not performed due to the small number of remaining animals.
* Statistically different (p<0.05) from the control. ** Statistically different (p<0.01) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food Consumption:** Treatment-related decreases in food consumption were observed at 2500 ppm during the first three weeks of dosing in males and only during the first week of treatment in females. (Table IIA 5.3.2-3).

   There were no treatment-related changes in food consumption at 5, 20 and 500 ppm in either sex.

TABLE IIA 5.3.2-3 Mean Food Consumption (g/day) [%][a]

Interval (Week)	Dose Level (ppm in diet)				
	0	5	20	500	2500
Males					
1	26.7	25.5	26.5	26.9 (n=9)	16.5**(n=4) [38]
2	25.1	23.1 [8]	24.1	23.9 [5]	21.3** [15]
3	25.9	24.2 [7]	24.5 (n=9) [5]	25.6	22.7* (n=8) [12]
13[b]	22.2	23.0	24.2 [9]	25.2 (n=9) [14]	23.4 (n=2)[b] [5]
Females					
1	19.7	19.2	19.0	17.6 (n=6) [11]	12.2** (n=8) [38]
2	18.7	18.6	18.2 (n=9)	17.5 [6]	17.4 [7]
3	20.1	18.9 [6]	18.9 [6]	18.8 [6]	19.1
13	16.2	17.5 (n=9) [8]	16.3	16.7 (n=7)	16.1 (n=9)

a Percent different from control included if ≥ 5%.
b For the high dose males at Weeks 12 and 13, statistical analysis was not performed due to the small number of remaining animals.
* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control.

2. **Compound Consumption (time-weighted average):** The mean achieved dose levels expressed in mg/kg body weight day are presented in Table IIA 5.3.2-4.

TABLE IIA 5.3.2-4 Mean Achieved Dietary Intake of Ethiprole (Weeks 1-13)

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	
		Males	Females
Control	0	0	0
Low	5	0.296	0.373
Mid-1	20	1.174	1.503
Mid-2	500	30.484	37.567
High	2500	154.746	187.867

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related changes were observed at the ophthalmological examination at any dose level in either sex.

E. BLOOD ANALYSES:

1. **Hematology:** At the end of the dosing phase, treatment-related effects were observed at 500 and 2500 ppm (Table IIA 5.3.2-5).

Prothrombin times were higher in males at 500 and 2500 ppm. In addition a tendency towards higher platelet counts was noted at 2500 ppm in males (+28%, not statistically analyzed) and in females (+ 22% higher, p<0.05).

Some other slight variations in erythrocyte parameters were noted at 500 and 2500 ppm. However, as these changes were of low magnitude and high inter-individual variability was noted, they were not considered toxicologically significant. Other statistically significant changes found in the hematological parameters were considered to be incidental and unrelated to treatment as they were not dose-related and/or not biologically relevant.

No treatment-related hematological changes were observed at 5 or 20 ppm.

TABLE IIA 5.3.2-5 Mean Prothombin Time and platelet counts

Interval	Parameter	Dose Level (ppm in diet)				
		0	5	20	500	2500
Males						
Number of animals examined		8	9	10	9	4
Week 13	PT (s)	15.79	15.19	15.15	22.68 **	41.78 [a]
	PLT (10^9/L)	1036.8 (n=9)	1038.6 (n=10)	1015.5	1161.8	1329.0 [a]
Females						
Number of animals examined		9	8	8	7	10
Week 13	PT (s)	16.14	15.60	15.58	14.16	17.54
	PLT (10^9/L)	1058.8	1050.5	1084.7	1228.1	1288.6 *

a Not statistically analyzed (n=4).
* Statistically different (p<0.05) from the control. ** Statistically different (p<0.01) from the control.

2. **Clinical chemistry:** At the end of the dosing phase, treatment-related effects were observed at 500 and 2500 ppm (Table IIA 5.3.2-6).
Higher mean total protein concentrations were noted in males at 500 and 2500 ppm (+ 8%, p<0.01 and +21%, not statistically analyzed) and in females at the same dose levels (+19% and +25%, p<0.01). Higher mean total cholesterol concentration observed in females at 500 and 2500 ppm (+114% and +233%, p<0.01).These changes were associated with higher mean triglycerides concentrations (+37%, not statistically significant and +41%, p<0.05).

In addition, a tendency towards slightly higher values in calcium and potassium concentrations and slightly lower values in chloride concentrations was noted at 500 and 2500 ppm.

Other statistically significant variations noted in clinical chemistry were of low magnitude or not dose-related, thus they were not considered to be toxicologically significant.

No treatment-related biochemical changes were observed at 5 or 20 ppm in either sex

TABLE IIA 5.3.2-6 Mean Total Cholesterol, Total Protein, calcium, potassium and chloride concentrations [%][a]

Interval	Parameter	Dose Level (ppm in diet)				
		0	5	20	500	2500
Males						
Number of animals examined		10	10	10	9	4
Week 13	TPROT (g/l)	69.6	69.2	68.4	75.0** [8]	84.0[b] [21]
	CHOL (mmol/l)	2.088	2.275 [9]	2.392 [15]	2.141	3.010[b] [44]
	TRIG (mmol/l)	0.898	0.812 [10]	0.763 [15]	0.967 [8]	0.998[b] [11]
	Ca (mmol/l)	2.696	2.693	2.692	2.801*	2.993[b] [11]
	K (mmol/l)	3.90	3.78	3.80	3.99	4.25[b] [9]
	Cl (mmol/l)	104.8	104.8	105.3	103.7	103.5[b]
Females						
Number of animals examined		10	9	10	7	10
Week 13	TPROT (g/l)	66.5	67.2	67.4	78.9 ** [19]	83.2 ** [25]
	CHOL (mmol/l)	2.152	2.132	2.463 [14]	4.596 ** [114]	7.158 ** [233]
	TRIG (mmol/l)	0.459	0.476	0.509 [11]	0.631 ≙ [37]	0.649 * [41]
	Ca (mmol/l)	2.624	2.630	2.631	2.776 ** [6]	2.868 ** [9]
	K (mmol/l)	3.50	3.38	3.40	3.40	3.79 * [8]
	Cl (mmol/l)	107.1	105.6	105.9	103.6 **	102.6 **

a Percent different from control included if ≥ 5%.
b Not statistically analyzed (n=4).
* Statistically different (p<0.05) from the control. ** Statistically different (p<0.01) from the control.

3. **Hormone analysis:** Treatment-related effects were observed at 500 and 2500 ppm (Table IIA 5.3.2-7).

At Week 2, increases in TSH levels were observed at 500 ppm and 2500 ppm in males (+98% and +105%, respectively, p<0.01) and in females (+58% and +102%, respectively, p<0.01). These changes were associated with decreases in T4 levels at 500 and 2500 ppm in males (-21% and -44%, respectively, p<0.01) and in females (-10% and -22%, respectively).

At Week 6, increases in TSH levels were observed at 500 ppm and 2500 ppm in males (+120 and +200%, respectively, p<0.01) and in females (+58% and +160%, respectively, p<0.01). These changes were associated with decreases in T4 levels at 500 and 2500 ppm in males (-22% and -46%, respectively, p<0.01) and females (-23% and -25%, respectively, p<0.01). In addition a statistically significant increase in T3 level was noted in females at 2500 ppm (+51%, p<0.01).

At the end of the dosing phase, increases in TSH levels were observed at 500 ppm and 2500 ppm in males (+81%, p<0.01, and +96%, p<0.05, respectively) and were associated with decreases in T4 levels (-19%, p<0.05, and -40%, p<0.01, respectively). In addition a statistically significant increase in T3 level was noted at 2500 ppm (+65%, p<0.05). In females, a statistically significant increase in TSH levels (+125%, p<0.01) was associated with a statistically significant increase in T3 level (+73%, p<0.01%) at 2500 ppm.

No treatment-related hormonal changes were observed at 5 or 20 ppm in either sex.

TABLE IIA 5.3.2-7 Mean T3, T4, and TSH Plasma Levels [%][a]

Interval	Parameter	Dose Level (ppm in diet)				
		0	5	20	500	2500
Males						
Week 2	N° of animals examined	10	10	10	10	10
	T3 (nmol/l)	0.78	0.72 [6]	0.80	0.77	0.89 [14]
	T4 (nmol/l)	36.17	36.53	37.72	28.58 ** [21]	20.27 ** [44]
	TSH (ng/ml)	0.57	0.65 [14]	0.62 [9]	1.13 ** [98]	1.17 ** [105]
Week 6	N° of animals examined	10	10	10	10	7
	T3 (nmol/l)	0.74	0.63 [15]	0.71	0.85 [15]	0.71
	T4 (nmol/l)	39.27	37.72	37.59	30.48 ** [22]	21.25 ** [46]
	TSH (ng/ml)	0.71	0.72	0.86 [21]	1.56 ** [120]	2.13 ** [200]
Week 13	N° of animals examined	8	7	8	8	4
	T3 (nmol/l)	0.66	0.75 [14]	0.66	0.83 [26]	1.09 * [65]
	T4 (nmol/l)	36.79	36.32	37.54	29.84 * [19]	22.17 ** [40]
	TSH (ng/ml)	0.83 (n=9)	0.80 (n=9)	0.74 (n=9) [11]	1.50 ** (n=9) [81]	1.63 * (n=3) [96]

Females						
Week 2	N° of animals examined	10	10	10	10	10
	T3 (nmol/l)	0.81	0.81	0.89 [10]	0.81	0.79
	T4 (nmol/l)	28.33	29.06	28.10	25.45 [10]	22.00 [22]
	TSH (ng/ml)	0.48	0.42 [13]	0.48	0.76 ** [58]	0.97 ** [102]
Week 6	N° of animals examined	10	10	10	9	10
	T3 (nmol/l)	0.77	0.72 [6]	0.85 [10]	0.96 [33]	1.16 ** [51]
	T4 (nmol/l)	31.41	34.60 [10]	32.87 [5]	24.13 ** [23]	23.57 ** [25]
	TSH (ng/ml)	0.52	0.52	0.48 [8]	0.82 ** [58]	1.35 ** [160]
Week 13	N° of animals examined	5	8	7	5	10
	T3 (nmol/l)	0.73	0.76	0.91 [25]	1.08 [48]	1.26 ** [73]
	T4 (nmol/l)	23.53 (n=6)	26.29 [12]	29.83 (n=8) [27]	22.13 (n=6) [6]	22.89 (n=9)
	TSH (ng/ml)	0.64 (n=8)	0.64	0.62 (n=9)	0.81 (n=6) [27]	1.44 ** [125]

a Percent different from control included if ≥ 5%.
* Statistically different (p<0.05) from the control. ** Statistically different (p<0.01) from the control.

F. URINALYSIS: No treatment-related urinalysis changes were observed at any dose level in either sex.

G. SACRIFICE AND PATHOLOGY:

1. **Organ Weight:** Treatment-related organ weight changes were noted in the liver and thyroid at 500 and 2500 ppm (Table IIA 5.3.2-8).

In the liver, a dose-related increase in absolute and relative weights was observed at 500 and 2500 ppm in males (+57% and +157% for absolute, p<0.01 and not analysed, respectively) and in females (+96% and +192% for absolute, respectively, p<0.01). In the thyroid, a dose-related increase in both absolute and relative weights was observed at 500 and 2500 ppm in males (+48% and +62% for absolute, p< 0.01 and not analysed, respectively) and females (+44% and +56%, for absolute, p<0.01, respectively).

Statistically significant increases, in comparison to control groups, were observed with absolute and/or relative heart weights of 500 and 2500 ppm males (+18% and +16% for absolute, p<0.05 and not analysed, respectively) and females (+7% and +12% for absolute, not statistically significant, respectively) and absolute and/or relative adrenal weights of 500 and 2500 ppm females (+15%, not statistically significant, and +27%, p<0.01, for absolute, respectively). There was also a non-statistically significant increase in absolute and/or relative adrenal weights in 500 and 2500 ppm males (+7% and +12% for absolute, respectively). The study author did not consider these weight changes biologically significant

since they were not observed for all modes of expression of the organ weights, they were not dose-related and they did not correlate to gross or microscopic changes. The increase in heart weight may not be treatment related, however increases in adrenal weights have been observed in other studies in the ethiprole toxicology database: 28-day rat toxicity study and 2-year rat chronic toxicity study. Although no corresponding changes in histopathology were observed, the increase in adrenal weight may be treatment related.

A non-statistically significant decrease in absolute and relative prostate weight was observed in 2500 ppm males (-21% for absolute). Since the finding did not reach statistical significance or correspond to any histopathological observations, it was not mentioned by the study author. This finding was found to be relevant since it has been observed in other studies in the ethiprole toxicology database: 28-day rat toxicity study, 90-day dog toxicity study and 2-year rat chronic toxicity study.

No treatment-related organ weights changes were observed at 5 or 20 ppm in either sex.

TABLE IIA 5.3.2-8 Mean Terminal Body Weight, Absolute and Relative Liver and Thyroid Weights [%][a]

Organ	Parameter	Dose level (ppm in diet)				
		0	5	20	500	2500
Males						
Number of animals examined		10	10	10	9	2
Terminal body weight (g)		475.1	461.7	495.7	503.7	458.4
Liver	Absolute weight (g)	10.38	10.39	11.01 [6]	16.30 ** [57]	26.65 [157]
	% of terminal body weight	2.18	2.25	2.23	3.23 ** [48]	5.81 [167]
	% of brain weight	503.9	514.0	522.6	785.8 ** [56]	1327.4 [163]
Thyroid	Absolute weight (g)	0.021	0.022 [5]	0.023 [10]	0.031 ** [48]	0.034 [62]
	% of terminal body weight	0.0044	0.0047	0.0046	0.0062 ** [41]	0.0075 [70]
	% of brain weight	1.013	1.063	1.083 [7]	1.499 ** [48]	1.681 [66]
Adrenal	Absolute weight (g)	0.481	0.415	0.481 [5]	0.440 [7]	0.555 [12]
	% of terminal body weight	0.012	0.012	0.012	0.012	0.014 [17]
	% of brain weight	2.698	2.790	2.808	2.865 [6]	3.148 [17]
Prostate	Absolute weight (g)	0.805	0.861 [7]	0.867 [8]	0.833	0.635 [21]
	% of terminal body weight	0.170	0.186 [9]	0.174	0.167	0.139 [18]
	% of brain weight	39.32	42.41 [8]	41.01	40.04	31.44 [20]

Females						
Number of animals examined		10	9	10	7	10
Terminal body weight (g)		267.6	269.8	263.9	260.6	262.5
Liver	Absolute weight (g)	6.16	6.22	6.13	12.06 ** [96]	18.00 ** [192]
	% of terminal body weight	2.31	2.31	2.33	4.63 ** [100]	6.85 ** [197]
	% of brain weight	314.9	316.1	318.0	613.7 ** [95]	954.6 ** [203]
Thyroid	Absolute weight (g)	0.016	0.015	0.015	0.023 * [44]	0.025 ** [56]
	% of terminal body weight	0.0060	0.0055 [8]	0.0058	0.0086 ** [43]	0.0094 ** [57]
	% of brain weight	0.810	0.751 [7]	0.789	1.150 ** [42]	1.300 ** [60]
Adrenal	Absolute weight (g)	0.067	0.073 [9]	0.071 [6]	0.077 [15]	0.085** [27]
	% of terminal body weight	0.025	0.027	0.027	0.030* [20]	0.033** [32]
	% of brain weight	3.424	3.718 [9]	3.697 [8]	3.926 [15]	4.514** [32]

a Percent different from control included if ≥ 5%.
* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control.

2. **Gross Pathology**: At terminal sacrifice, treatment-related macroscopic findings were noted in the liver, kidney and thyroid at 500 and 2500 ppm (Table IIA 5.3.2-9).
Liver enlargement and dark liver color were seen in all surviving males at 2500 ppm and the majority of females at 2500 ppm as well as in both sexes at 500 ppm. Dark kidney color was seen in all males and females at 2500 ppm and a proportion of males and females at 500 ppm. Thyroid enlargement was noted in single males at 500 and 2500 ppm and in a proportion of females.

No treatment-related macroscopic changes were observed at 5 or 20 ppm in either sex.

TABLE IIA 5.3.2-9 Selected macroscopic findings in rats at Week 13

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	5	20	500	2500	0	5	20	500	2500
	Males					Females				
	N of rats examined					N of rats examined				
	10	10	10	9	2	10	9	10	7	10
Liver										
Enlarged	0	0	0	5	2	0	0	0	4	9
Prominent lobulation	0	1	1	4	0	0	0	0	0	1
Green tinge	0	0	0	2	2	0	0	0	3	7
Dark	0	0	0	6	2	0	0	0	7	10
Pale foci	0	0	0	2	0	0	0	0	1	2
Red focus	0	1	0	0	1	0	0	0	1	0
White area	0	0	0	1	0	0	0	0	0	0
Kidney										
Dark	0	1	1	3	2	0	0	0	6	10
Green tinge	0	0	0	1	0	0	0	0	2	1
Thyroid										
Enlarged	0	0	0	1	1	0	0	0	2	3

3. **Microscopic Pathology**: At terminal sacrifice, treatment-related microscopic findings were noted in the liver, thyroid and kidney at 500 and/or 2500 ppm (Table IIA 5.3.2-10).

   In the liver, centrilobular hepatocyte hypertrophy attributable to treatment was noted in both sexes at 500 and 2500 ppm. Changes were generally more pronounced in females. Mild to moderate amounts of golden brown intracytoplasmic pigment was also observed in the majority of females at 2500 ppm.

   In the thyroid, follicular cell hypertrophy/hyperplasia was noted in almost all examined animals of both sexes dosed at 500 and 2500 ppm. In addition, slight follicular epithelial hypertrophy/hyperplasia was seen in a single male at 20 and 5 ppm, and mild follicular epithelial hypertrophy/hyperplasia in two males at 20 ppm. However these findings were not considered to be toxicologically significant in view of the low level of change and the small number of animals involved and the absence of corroborative changes in thyroid weights or thyroid hormone levels.

   In the kidney, an increased degree of cortical tubular brown pigment was reported in all surviving males and females at 2500 ppm.

   No treatment-related microscopic changes were observed at 5 or 20 ppm in either sex.

TABLE IIA 5.3.2-10 Selected microscopic findings in rats at Week 13 (terminal sacrifice)

Finding	Dose Level (ppm in diet)					Dose Level (ppm in diet)				
	0	5	20	500	2500	0	5	20	500	2500
	Males					Females				
Liver: number examined	**10**	**10**	**10**	**9**	**2**	**10**	**9**	**10**	**7**	**10**
Hepatocyte hypertrophy – centrilobular becoming generalized	0	0	0	2	0	0	0	0	5	6
Hepatocyte hypertrophy - centrilobular	0	0	0	6	2	0	0	0	0	0
Cytoplasmic golden brown pigment	0	0	0	0	2	0	0	0	2	8
Thyroid: number examined	**10**	**10**	**10**	**9**	**2**	**10**	**9**	**10**	**7**	**10**
Follicular epithelial hypertrophy/hyperplasia	0	1	3	9	2	0	0	0	6	10
Kidney: number examined	**10**	**10**	**10**	**9**	**2**	**10**	**9**	**10**	**7**	**10**
Golden brown pigment – cortical tubules	3	5	1	4	2	3	3	3	4	10

III. CONCLUSIONS:

The short-term dietary administration of ethiprole (RPA 107382–) to rats for at least 90 days produced signs of overt toxicity at 2500 ppm and at 500 ppm to a lesser extent. These two dose levels, which induced the death or premature sacrifice were considered to have exceeded the MTD. Clinical signs consisting of piloerection, increased and/or reduced motor activity and irritability to touch were noted mainly in animals at 2500 ppm on few occasions. The body weight evolution of animals at 2500 ppm was inferior to that of controls during the first five weeks for the males and only during the first week for the females. At 2500 ppm, statistically significant lower food consumption was noted in males during the first three weeks and in females during the first week.

Increases in prothrombin time were noted in males at 500 and 2500 ppm. Increases in cholesterol, total protein, triglyceride, calcium and potassium concentrations and decreases in chloride were reported at 500 and 2500 ppm.

Ethiprole induced an imbalance of thyroid hormones in animals treated at 500 and 2500 ppm resulting in higher levels of TSH and T3 and lower levels of T4. Significant increases in liver and thyroid weights were observed at 500 and 2500 ppm. The study author noted that increased adrenal weights were also observed at 500 and 2500 ppm, but were not biologically significant due to a lack of dose response and corresponding histopathological observations. However, since this finding has been observed elsewhere in the ethiprole toxicology database, it is believed to be treatment related by the study reviewer. Decreased prostate weight was observed in 2500 ppm males. This finding was not discussed by the study author. In isolation, this observation would not be biologically significant as there were no corresponding histopathological observations of prostate toxicity. However, it has been consistently observed in the ethiprole database in rats and dogs and is therefore considered treatment related by the study reviewer. At the histopathological examination, the liver with centrilobular hepatocyte hypertrophy, and the thyroid with follicular hypertrophy / hyperplasia were found to be the target organs in animals treated at 500 and 2500

ppm. The kidney was also affected at 2500 ppm with both sexes showing tubular golden brown pigment.

In conclusion, the **NOAEL** is 1.2 mg/kg bw/day in males and 1.5 mg/kg bw/day in (20 ppm in diet) based on mortality, changes in thyroid hormone levels, clinical pathology parameters, and increased liver and thyroid weights associated with microscopic changes in these target organs observed at 30.5 mg/kg bw/day in males and 37.6 mg/kg bw/day in females.

KIIA 5.3.3 - Oral 90-day toxicity (dog)

1.	Annex point(s)	KIIA 5.3.3; Short-term toxicity/Oral 90-day toxicity (dog): **MRID 47622807**
2.	Location in dossier	KIIA 5.3.3 /01
3.	Authors (year)	Bigot, D. (2001)
	Title	RPA 107382 90 day toxicity study in the dog by dietary administration
Company, report No.		Bayer CropScience AG, Report-No.: C014168 (M-206098-02-1)
	Date	04.05.2001, Amended: 02.05.2002
4.	Testing facility	Aventis CropScience S.A., Lyon, France
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole Substance(s):ethiprole TC (Batch-No.: CDR9706)
7.	Test method	EU (=EEC): 92/69, V, B27 (1992); MAFF: 4200 (1985); OECD: 409 (1981); USEPA (=EPA): F (1984)
	Deviations	
8.	GLP	yes

Executive summary:

Ethiprole (RPA 107382) was administered continuously via the diet at 0, 30, 90 or 200 ppm to separate groups of 4 dogs /sex/dose. Mortality and clinical signs were checked daily. Body weight was measured during the acclimatization period and weekly thereafter. Food consumption was measured weekly throughout the study. Ophthalmic examinations were performed on all animals during the acclimatization period and during Week 12 of dosing. Clinical chemistry, hematology and urinalysis were performed towards the end of the dosing phase. In addition, a blood sample was collected during Weeks 2 and 13 for T3, T4 and TSH analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues sampled and examined microscopically.

The short-term dietary administration of ethiprole to dogs for at least 90 days produced signs of systemic toxicity at 90 ppm and overt toxicity at 200 ppm as evidenced by the premature sacrifice of one female.

Clinical signs were limited to the female which was sacrificed moribund on Day 24 and consisted of bloody feces, diarrhea, soiled fur, prostration, tremors and increased salivation. Treatment-related decreases in body weight and/or body weight gain (often exhibited as body weight loss) were observed at 90 and 200 ppm in males and at 200 ppm in females. At 200 ppm, statistically significant lower food consumption was noted in males during the first five weeks.

Statistically significant increases were observed in alkaline phosphatase activities at 200 ppm in females in Weeks 7 and 13. The same tendency was noted at 200 ppm in males, in Week 13 only, but did not reach statistical significance.

Ethiprole did not induce any changes in thyroid hormone levels at any dose level in either sex.

Lower mean prostate, testis and epididymis weights were noted in males from all treated groups, and decreased thymus weights were observed in males at 90 and 200 ppm. These decreases may have been a result of the decreased body weights observed in these animals. Upon histopathological examination, centrilobular hepatocyte hypertrophy was noted in the livers of

males that received 90 or 200 ppm. Thymic atrophy was also observed in males at 90 and 200 ppm and in females at 200 ppm. In addition, immature male sex organs were reported in all males including controls, but the severity of this finding was more pronounced at 90 and 200 ppm. No treatment-related microscopic changes were observed at 30 ppm.

In conclusion, the **LOAEL** is 3.2 mg/kg/day in males (90 ppm in the diet) based on reduced body weight and body weight gain, decreased thymus and sexual organ weights, thymic atrophy and hepatocellular hypertrophy. The **LOAEL** is 8.5 mg/kg/day in females (200 ppm in diet), based on the premature sacrifice of one female, lower body weight gain, increased alkaline phosphatase activity, and thymic atrophy. The **NOAEL** is 1.0 mg/kg/day in males (30 ppm in the diet) and 3.6 mg/kg/day in females (90 ppm in diet).

This study is classified as **acceptable/guideline** and does satisfies the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in the dogs.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	93%
Compound Stability:	The test substance was stored in an air-tight, light resistant container at room temperature.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. Control animals received untreated diet.

3. **Test Animals:**

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	22 weeks 6.1 – 7.9 kg males; 4.7 – 6.6 kg females	
Source:	Marshall Farms, 5800 Lake Bluff Road, North Rose, NY	
Housing:	Individually housed in stainless steel kennels with 1.2 m^2 floor surface area.	
Diet:	Ground diet U.A.R. 125C (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France) (300 g of diet moistened with 500 ml of water), fed daily to each animal for about 2 hours in the morning.	
Water:	Filtered and softened water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	18°C - 21°C
	Humidity:	40-70%
	Air changes:	10-15 air changes/hr (average, not monitored)
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	21 days	

B. STUDY DESIGN:

1. **In-Life Dates:** Start: September 16, 1998; End: December 18, 1998

2. **Animal Assignment/Dose Levels:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution by sex to the test groups noted in Table IIA 5.3.3-1.

TABLE IIA 5.3.3-1 Study Design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day) [a]		No. of Animals	
		Males	Females	Males	Females
Control	0	0	0	4	4
Low	30	1.0	1.1	4	4
Mid	90	3.2	3.6	4	4
High	200	7.6	8.5	4	4

a Mean achieved dietary intake of ethiprole (RPA 107382), weeks 1 to 12.

3. **Diet Preparation and Analysis:** Diet was prepared four times during the study by mixing appropriate amounts of test substance with Ground diet U.A.R. 125C and was stored at -18°C.

Prior to the start of the study, homogeneity and stability of 20 and 200 ppm diet preparations were tested. To demonstrate stability, samples of the diets were analyzed after a 5 week storage interval (4 weeks of frozen storage (<-15°C) followed by 1 week at ambient temperatures). To check homogeneity, samples were collected from each diet at three levels, the surface, middle, and bottom. At each level, 2 samples were taken and analyzed.

During the study, the homogeneity (top, middle, bottom) of RPA 107382 in the diet was verified on the first preparation for the lowest (30 ppm) and highest (200 ppm) concentrations to demonstrate adequate formulation procedures. The dietary levels of the test substance were verified for each concentration of all preparations.

Results:

Homogeneity Analysis: Results were within 91-101% of the nominal concentrations. (Data obtained from the study report, pages 369 and 372, original pagination.)

Stability Analysis: Results were within 96 and 100% of the nominal concentration for the 20 and 200 ppm diets after 5 weeks of storage (4 weeks frozen + 1 week ambient). (Data obtained from the study report, page 371, original pagination.)

Concentration Analysis: Results were within 90-105% of the nominal concentrations. (Data obtained from the study report, pages 373-376, original pagination.)

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics**: For each hematology, clinical chemistry and urinalysis parameter, statistical analyses were performed on the differences calculated between Periods 1 and 2. Period 1was defined as the difference between the value observed before treatment and the value observed at Week 7. Period 2 was defined as the difference between the value observed before treatment and the value observed at Week 13.

Results of body weight, body weight change, food consumption, hormonal assays, hematology (except percentages and absolute values eosinophils, basophils, monocytes and large unstained cells), clinical chemistry and urinalysis parameters (pH and refractive index) were

intercompared for the exposed groups and the control group by use of Bartlett test for homogeneity of variances.

If the Bartlett test was not significant, the analysis of variance (ANOVA) was performed. When the analysis of variance was significant, the Dunnett test was performed to compare the group means to the control mean.

If Bartlett test indicated heterogeneous variances, the non-parametric statistical procedure was performed using the Kruskal-Wallis non-parametric one way analysis of variance by ranks. If Kruskal-Wallis test was significant, Mann-Whitney test was used to compare each group to the control group.

Statistical analyses were performed using SAS programs.

Results of organ weight and terminal body weight, organ to body weight ratio and organ to brain weight ratio intercompared for the exposed groups and the control group with PathTox computer system by use of Dunnett test when Bartlett test indicated homogeneous variances. If Bartlett test indicated nonhomogeneous variances, variables were analyzed using SAS programs and a non-parametric statistical procedure was performed using the Kruskal-Wallis non-parametric one way analysis of variance by ranks. If the Kruskal-Wallis test was significant, Mann-Whitney test was used to compare each group to the control.

Only the variables analyzed using the non-parametric statistical procedure figure in the statistical report. The levels of signification for each statistical comparison were 0.05 and 0.01.

C. METHODS:

1. Observations:

1a. Cageside Observations: Clinical signs were recorded once daily (3-6 h after offering the diet) and included but were not limited to changes in: general behavior and appearance, skin and fur, teeth and gum, eyes, ears, mucous, membranes. Any deviation from normal was recorded in respect to the nature and severity. Daily examination of the kennels was also recorded for vomitus, diarrhea or blood. Each animal was checked for ill-health, moribundity and mortality twice daily or once daily on week-ends and public holidays. Any animal suffering from severe distress, in moribund condition or considered unlikely to survive was sacrificed and necropsied.

1b. Clinical Examinations: Each animal was subjected to a physical examination once before initiation of treatment and weekly during the treatment period. Particular attention was paid to: fur and skin, eyes, ears, teeth, gum, mucous membranes, rectal temperature, gait, stance, general behavior, chest including heart and respiratory rate, abdomen including palpation, external genitalia, and mammary glands.
Additionally, a specific neurological examination was performed which included: cranial nerves reflexes: papillary light and consensual light, palpebral blink and corneal reflex; segmental reflexes: flexor (withdrawal) including crossed extensor; postural reactions: placing reactions, visual and tactile.

2. Body weight: Body weight was measured twice during the acclimatization phase, then at weekly intervals throughout the treatment period and before necropsy.

3. **Food Consumption and Compound Intake:** Food intake was measured for four days before treatment. The weights of food offered to each animal and that remaining were recorded daily throughout the treatment period. From these records, the mean weekly consumption was calculated for each dog. Food spillage was also recorded. The group mean achieved dosage for each sex, expressed as mg/kg/day was calculated weekly.

4. **Ophthalmoscopic Examination:** During the acclimatization phase all animals were subjected to an ophthalmological examination after instillation of an atropinic agent. Each eye was examined by means of an indirect ophthalmoscope. At the end of treatment all surviving animals were re-examined. In the case of treatment-related effects being identified, photographic records are made from the affected animals, and an equivalent number of controls.

5. **Hematology & Clinical Chemistry:** Once before initiation of treatment and on study Days 44 or 45 and 86 or 87, blood samples were taken from all surviving animals in all groups by puncture of the jugular vein. Blood was collected on EDTA for hematology (2.5 ml), lithium heparin (for plasma) and clot activator (for serum) for clinical chemistry (5 ml each) and sodium citrate for coagulation parameters (1.8ml). The CHECKED (X) parameters were examined. In addition, a blood sample was collected into tubes containing heparin before initiation of treatment and during Weeks 2 and 13 by puncture of the jugular vein from each animal; the plasma was separated and frozen for thyroid hormones (T3, T4, TSH) measurements.

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		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
	Magnesium	X	Urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)	X	Thyroid Hormones (T3, T4, and TSH)
X	Alanine amino-transferase (also SGPT)		
X	Aspartate amino-transferase (also SGOT)		
	Sorbitol dehydrogenase		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

NOTE: T3, T4, and TSH levels were monitored at Weeks 2 and 13.

6. **Urinalysis:** Once before initiation of treatment and on study Days 44 or 45, and 86 or 87 in the morning, overnight urine samples were collected from all surviving animals in all groups. Access to water was not restricted during urine collection. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
	Specific gravity / osmolality	X	Bilirubin
X	pH	X	Blood / blood cells
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen
		X	Urinary refractive index

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. Histopathological examinations were performed in all animals from all groups. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		**CARDIOVASC./HEMAT.**		**NEUROLOGIC**
X	Tongue	X	Aorta, thoracic	X, XX	Brain
X	Salivary glands	X, XX	Heart	X	Peripheral nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X, XX	Pituitary
X	Duodenum	X, XX	Spleen	X	Eyes (incl. optic nerve)
X	Jejunum	X, XX	Thymus		**GLANDULAR**
X	Ileum			X, XX	Adrenal gland
X	Cecum		**UROGENITAL**		Lacrimal gland
X	Colon	X, XX	Kidneys	X, XX	Parathyroid
X	Rectum	X	Urinary bladder	X, XX	Thyroid
X, XX	Liver	X, XX	Testes		**OTHER**
X	Gall bladder	X, XX	Epididymides	X	Bone (sternum and/or femur)
X	Pancreas	X, XX	Prostate	X	Skeletal muscle
	RESPIRATORY	X, XX	Ovaries	X	Skin
X	Trachea	X, XX	Uterus	X	All gross lesions and masses
X	Lung	X	Mammary gland		
	Nose	X	Vagina		
X	Pharynx				
X	Larynx				

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** Treatment-related clinical signs were observed in one female treated at 200 ppm which was sacrificed moribund on Day 24. These signs were limited to bloody feces, diarrhea, soiled fur, prostration, tremors and increased salivation before death. Emesis containing food was occasionally observed in males from control group and low and high dose treated groups.

   No treatment-related clinical signs were observed at 30 and 90 ppm in either sex.

2. **Mortality:** Treatment-related mortality was observed in one female treated at 200 ppm which was sacrificed moribund on Day 24.

   No treatment-related mortalities were observed at 30 and 90 ppm in either sex.

B. **BODY WEIGHT**: Treatment-related decreases in body weight and/or body weight gain were observed at 90 and 200 ppm in males and at 200 ppm in females (Table IIA 5.5.3-2).

In males the differences were statistically significant only at 200 ppm and included lower mean body weight from Day 36 onwards (reaching a maximum decrease of 26% when compared to controls on day 85). Decreases in body weight gain, often exhibited as body weight loss, were also observed throughout the study period in the high dose males, with the animals exhibiting an overall body weight loss for weeks 1-12. At 90 ppm, mean body weights were reduced 13% by Day 85 and overall body weight gain (Weeks 1-12) was reduced by 43%. In females, although no apparent effect was observed on mean body weight at 200 ppm, a lower body weight gain was observed frequently throughout the study and overall from weeks 1-12.

No treatment-related changes in body weight and body weight gain were observed at 30 ppm in males and 30 and 90 ppm in females.

TABLE IIA 5.3.3-2 Mean Body Weights (g) and Body Weight Gains (g/day)

Interval (day)	Dose Level (ppm in diet)			
	0	30	90	200
Males				
Body Weight (g)				
Day 1	7384.5	7226.5	7079.2	7015.0
Day 8	7380.2	7282.0	7069.2	6870.0
Day 36	8395.7	8208.7	7702.5	6902.7* (↓18%)
Day 43	8525.7	8362.0	7778.5	6895.0* (↓19%)
Day 50	8727.7	8566.5	7903.0 (↓9%)	6992.0* (↓20%)
Day 58	8813.0	8685.7	7902.7 (↓10%)	6952.0** (↓21%)
Day 64	8965.7	8822.7	7997.2 (↓11%)	6951.0** (↓22%)
Day 71	9200.0	9050.0	8175.0 (↓11%)	7050.0** (↓23%)
Day 78	9278.5	9094.7	8182.5 (↓12%)	6946.0** (↓25%)
Day 85	9463.5	9207.0	8278.5 (↓13%)	6970.7** (↓26%)
Body Weight Gain (g)				
Overall (Weeks 1-12)	2079.0	1980.5	1199.3	-44.3
Body Weight Gain (g/day)				
Overall (Weeks 1-12)	24.4	23.8	13.82	-0.6**
Females				
Body Weight (g)				
Day 1	5493.4	5613.8	5657.0	5708.2
Day 8	5548.2	5670.5	5631.5	5510.0
Day 43	6108.5	6275.7	6185.3	6161.6 (n=3)
Day 50	6189.5	6337.5	6275.0	6197.0 (n=3)
Day 58	6325.0	6494.2	6341.2	6146.0 (n=3)
Day 64	6431.0	6583.8	6484.9	6408.0 (n=3)
Day 71	6650.0	6800.0	6625.0	6400.0 (n=3)
Day 78	6680.7	6877.5	6709.5	6555.3 (n=3)
Day 85	6784.7	7099.7	6824.7	6716.0 (n=3)
Body Weight Gain (g)				
Overall (Weeks 1-12)	1291.3	1485.9	1167.7	1007.8 (↓22%)
Body Weight Gain (g/day)				
Overall (Weeks 1-12)	15.3	17.1	13.8	8.2 (n=3)

* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control. Statistics not calculated on overall body weight gain (weeks 1-12).

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food Consumption:** Treatment-related decreases in food consumption were observed at 200 ppm during the first five weeks of dosing in males without reaching statistical significance (Table IIA. 5.5.3-3).

   No treatment-related changes in food consumption were observed at 30 and 90 ppm in males and at any dose level in females.

TABLE IIA 5.3.3-3 Mean Food Consumption (g/day)

Interval (Week)	Dose Level (ppm in diet)			
	0	30	90	200
Males				
Week 1	633.7±97.7	635.1±91.8	582.4±86.9	545.4±29.2
Week 2	685.9±109.8	704.4±64.5	602.5±85.3	552.0±16.0
Week 3	701.7±76.8	741.8±55.1	679.5±107.1	591.0±100.2
Week 4	734.8±58.8	779.0±29.4	698 .6±72.1	675.9±94.8
Week 5	796.2±6.4	768.3±31.1	747.5±68.4	710.0±71.6
Week 13	799.1±0.3	782 .7±31.9	798.7±0.3	763.7±69.3
Females				
Week 1	524.4±53.1	468.9±131.6	507.4±15.9	488.1±49.6
Week 2	549.8±99.6	542.9±160.0	523.9±56.7	554.2±123.4
Week 3	593.8±68.7	583.9±160.1	554.0±71.4	606.6±129.7
Week 4	638.2±62.9	595.6±150.6	630.2±48.2	673.8±104.9
Week 5	668.7±112.8	618.5±137 .6	661.3±49.3	721.1±70.2
Week 13	794.4±7.9	679.6±134.5	755.5±85.6	798.8±0.4 (n=3)

2. **Compound Consumption:** The mean achieved dose levels expressed in mg/kg body weight day are presented in Table IIA 5.3.3-4.

TABLE IIA 5.3.3-4 Mean Achieved Dietary Intake of Ethiprole (Weeks 1-13)

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	
		Males	Females
Control	0	0	0
Low	30	1.0	1.1
Mid	90	3.2	3.6
High	200	7.6	8.5

Since one female in the 200 ppm dose group had to be sacrificed prematurely on Day 24, the individual achieved compound intake for all females from this dose group was calculated for Weeks 1 to 3. The results showed that the female that was sacrificed prematurely had the highest intake of the test compound in the group, which may have explained the clinical signs observed in this animal (10.8 mg/kg bw/day compared to 8.4, 5.9, and 5.7 mg/kg bw/day in the other 3 dogs).

D. **OPHTHALMOSCOPIC EXAMINATION:** No treatment-related changes were observed at the ophthalmological examination at any dose level in either sex.

E. **BLOOD ANALYSES:**

1. **Hematology:** At the end of the dosing phase, no treatment-related hematological changes were observed at any dose level in either sex.

2. **Clinical chemistry:** At the end of the dosing phase, treatment-related effects were observed at 200 ppm (Table IIA 5.3.3-5).

Statistically significant increases were observed in alkaline phosphatase activities at 200 ppm in females in Weeks 7 and 13. The same tendency was noted at 200 ppm in males, in Week 13 only, but did not reach statistical significance.

No treatment-related biochemical changes were observed at 30 and 90 ppm in either sex.

TABLE IIA 5.3.3-5 Mean Total Alkaline Phosphatase activity

Interval	Parameter	Dose Level (ppm in diet)			
		0	30	90	200
Males					
Number of animals examined		4	4	4	4
Pre-treatment	AP (UI/l)	143.7	154.0	111.5	171.0
Week 7	AP (UI/l)	131.5	117.0	106.7	161.2
Week 13	AP (UI/l)	100.5	101.2	104.2	168.7
Females					
Number of animals examined		4	4	4	4
Pre-treatment	AP (UI/l)	124.5	126.2	127.0	143.2
Week 7	AP (UI/l)	105.7	101.2	109.7	162.3 (n=3)
Week 13	AP (UI/l)	94.0	100.5	112.5	179.6 (n=3)
Mean Differences Between AP Levels Observed Before Treatment and at Weeks 7 and 13					
Males					
Number of animals examined		4	4	4	4
Week 7	AP (UI/l)	-12.2	-37.0	-4.7	-9.7
Week 13	AP (UI/l)	-43.2	-52.7	-7.2	-2.2
Females					
Number of animals examined		4	4	4	4
Week 7	AP (UI/l)	-18.7	-25.0	-17.2	9.6 * (n=3)
Week 13	AP (UI/l)	-30.5	-25.7	-14.5	27.0* (n=3)

3. **Hormone analysis:** No treatment-related changes were observed in T3 and T4 levels at any dose level in either sex.

   TSH concentrations were measured but results were not reported because the levels were below the acceptable range according to the immunometric assay procedure. The study author attributed the low levels to a loss of immunoreactivity due to prolonged frozen storage intervals (7-10.5 months).

F. URINALYSIS: No treatment-related urinalysis changes were observed at any dose level in either sex.

G. SACRIFICE AND PATHOLOGY:

1. Organ Weight:

At terminal sacrifice, mean body weights were lower in males from the 90 ppm (-13%) and 200 ppm (-26%, $p<0.01$) dose groups when compared to controls.

Decreases in the weights of the male sexual organs (prostate, testis and epididymis) and in thymus weights were noted in treated males, and associated with the lower terminal body weights in these animals (Table IIA 5.3.3-6). No organ weight changes were observed in any of the treated females when compared to control values.

Lower mean absolute and relative thymus weights were observed in males treated with 90 and 200 ppm of the test material. The differences were statistically significant for mean absolute thymus weight (decreased 68%) and mean thymus to brain weight ratio (decreased 69%) in males that received 200 ppm. These effects were considered to be related to the lower terminal body weights in these animals.

Mean absolute and relative (to body weight and brain weight) prostate, testis and epididymis weights were lower in males from all treated dose groups when compared with the control animals, reaching statistical significance in a number of cases. At the 90 and 200 ppm dose levels, these organ weight changes were considered to be related to the decreased body weights observed in the animals, and not a direct treatment-related effect. No significant decline in body weight was noted for males that received 30 ppm of the test material, however decreases in absolute and relative epididymis, testes and prostate weights were still observed in many animals at this dose.

As a result of the lower terminal body weights in the high dose males, the relative brain to body weight ratio was statistically higher in these animals (+30%, $p<0.05$).

Higher mean absolute and relative uterus weight were observed in females treated with 30 and 90 ppm when compared to control animals. However, these differences were associated with a large animal variability probably due to a different juvenile status, were not dose-related and not correlated to gross or microscopic changes. Therefore, they were not considered to be biologically significant.

TABLE IIA 5.3.3-6 Mean Terminal Body Weight, Absolute and Relative Brain, Thymus, Epididymis and Prostate Weights (percent decrease relative to controls)

Organ	Parameter	Dose level (ppm in diet)			
		0	30	90	200
Males					
Number of animals examined		4	4	4	4
Terminal body weight (kg)		9.5	9.2	8.3 (↓13%)	6.9 ** (↓27%)
Brain	Absolute weight (g)	76.2	78.2	77.5	75.2
	% of terminal body weight	0.825	0.850*	0.950	1.075*
	% of brain weight	-	-	-	-
Thymus	Absolute weight (g)	8.95	11.524	7.10 (↓21%)	2.90* (↓68%)
	% of terminal body weight	0.0950	0.1225	0.0800	0.0400
	% of brain weight	11.7500	14.7500	9.1500 (↓22%)	3.7000 * (↓69%)
Epididymis	Absolute weight (g)	2.87	1.90 (↓34%)	1.85 (↓36%)	1.45 (↓50%)
	% of terminal body weight	0.027	0.017 (↓37%)	0.020 (↓26%)	0.017(↓37%)
	% of brain weight	3.750	2.450 (↓35%)	2.325(↓38%)	1.925(↓49%)
Testes	Absolute weight (g)	13.32	9.22 (↓31%)	7.82* (↓41%)	5.85** (↓56%)
	% of terminal body weight	0.145	0.100 (↓31%)	0.092 (↓37%)	0.082 (↓43%)
	% of brain weight	17.375	11.800* (↓32%)	10.100* (↓42%)	7.725** (↓56%)
Prostate	Absolute weight (g)	6.10	2.52** (↓59%)	1.67** (↓73%)	1.05** (↓83%)
	% of terminal body weight	0.065	0.027** (↓58%)	0.022* (↓66%)	0.015** (↓77%)
	% of brain weight	7.975	3.275** (↓59%)	2.150** (↓73%)	1.400** (↓82%)

* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

2. **Gross Pathology**: At terminal sacrifice small testes and small prostate were seen in one male at 200 ppm. In addition, small thymus was noted in one male at 90 ppm and 2 males from the 200 ppm dose group.

   No treatment-related macroscopic changes were observed at 30 and 90 ppm in males and at any dose level in females.

3. **Microscopic Pathology**: The female from the 200 ppm dose group that was sacrificed moribund showed hemorrhages in the serosa of the stomach, gallbladder, and duodenum, and

in the cerebellum and spinal cord. There was a slight lymphoid depletion in the mesenteric lymph node and a moderate atrophy in the thymus also noted.

At terminal sacrifice, treatment-related microscopic findings were noted in the liver, thymus and sexual organs of males and/or females at 90 and/or 200 ppm (Table IIA 5.3. 7).

In the liver, slight to mild centrilobular hepatocyte hypertrophy attributable to treatment was noted in 2 males at 90 and one male treated at 200 ppm. Glycogen depletion was noted in one male and one female treated at 200 ppm.

In the thymus, atrophy was noted in 2 males at 90 ppm and 4 males and 2 females treated at 200 ppm.

In the sexual organs of males, immaturity was reported in all males including controls but the severity of this finding was more pronounced at 90 and 200 ppm. A complete absence of epididymal sperm was noted in one male at 90 ppm and 2 males at 200 ppm, compared with none in the control group.

No treatment-related microscopic changes were observed at 30 ppm.

TABLE IIA 5.3.3-7 Selected microscopic findings in dogs at Week 13 (terminal sacrifice)

Finding	Dose Level (ppm in diet)				Dose Level (ppm in diet)			
	0	30	90	200	0	30	90	200
	Males				Females			
Liver -number examined	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**3**
Hepatocyte hypertrophy – centrilobular becoming generalized	0	0	2	1	0	0	0	0
Glycogen depletion	0	0	0	1	0	0	0	1
Thymus - number examined	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**3**
Atrophy	1	0	2	4	0	0	0	2
Epididymis - number examined	**4**	**4**	**4**	**4**	-	-	-	-
No sperm	0	0	1	2	-	-	-	-
Few sperm	1	2	2	1	-	-	-	-
Testis - number examined	**4**	**4**	**4**	**4**	-	-	-	-
Immatirity	4	4	4	4	-	-	-	-
Prostate - number examined	**4**	**4**	**4**	**4**	-	-	-	-
Immaturity	0	1	3	4	-	-	-	-

III. CONCLUSIONS:

The short-term dietary administration of ethiprole (RPA 107382) to dogs for at least 90 days produced signs of systemic toxicity at 90 ppm and overt toxicity at 200 ppm as evidenced by the premature sacrifice of one female.

Clinical signs were limited to the female which was sacrificed moribund on Day 24 and consisted of bloody feces, diarrhea, soiled fur, prostration, tremors and increased salivation. The body weight evolution of males at 90 and 200 ppm and females at 200 ppm was inferior to that of

controls. At 200 ppm, statistically significant lower food consumption was noted in males during the first five weeks.

Statistically significant increases were observed in alkaline phosphatase activities at 200 ppm in females in Weeks 7 and 13. The same tendency was noted at 200 ppm in males, in Week 13 only, but did not reach statistical significance.

Lower mean prostate, testis and epididymis weights were noted in males from all treated groups, and decreased thymus weights were observed in males at 90 and 200 ppm. The study authors considered these changes to be related to the lower body weights observed in these animals, however the reviewer notes that the body weights of males at 30 ppm were not significantly reduced compared to controls. The decreases in the weight of the male sex organs were not associated with any histopathological changes in these organs, aside from immaturity which was noted at all dose levels, including controls. In addition, no effects on the male sex organs were observed in the subsequent 1-year dog study at doses up to and including 90 ppm, Therefore, these effects were considered not to be toxicologically relevant by the reviewer.

Upon histopathological examination, centrilobular hepatocyte hypertrophy was noted in the livers of males that received 90 or 200 ppm. Thymic atrophy was also observed in males at 90 and 200 ppm and in females at 200 ppm. In addition, immature male sex organs were reported in all males including controls, but the severity of this finding was more pronounced at 90 and 200 ppm. No treatment-related microscopic changes were observed at 30 ppm.

In conclusion, the LOAEL is 3.2 mg/kg/day in males (90 ppm in the diet) based on reduced body weight and body weight gain, decreased thymus and sexual organ weights, thymic atrophy, and hepatocellular hypertrophy. The NOAEL in males is 1.0 mg/kg bw/day (30 ppm in the diet). In females, the LOAEL is 8.5 mg/kg/day (200 ppm in diet) based on the premature sacrifice of one animal, lower body weight gain, increased alkaline phosphatase activity, and thymic atrophy.

KIIA 5.3.4 - Oral 1 year toxicity (dog)

1.	Annex point(s)	KIIA 5.3.4; Short-term toxicity/Oral 1 year toxicity (dog): **MRID 47622811**
2.	Location in dossier	KIIA 5.3.4 /01
3.	Authors (year)	Chevalier, G. (2001)
	Title	Ethiprole (RPA107382) 52-week dietary toxicity in beagle dogs
	Company, report No.	Bayer CropScience AG, Report-No.: C013286 (M-204326-01-1)
	Date	24.04.2001
4.	Testing facility	CIT Centre Internationale de Toxicologie, Evreux, France
5.	Dates of work	
6.	Test substance(s)	Molecule(s): ethiprole (radioactive), ethiprole Substance(s): ethiprole TC ethiprole (radioactive) TC
7.	Test method	EU (=EEC): 96/54, B32; MAFF: 59, Nohsan 4200; OECD: 409; USEPA (=EPA): OPPTS 870
	Deviations	
8.	GLP	yes

Executive Summary:

Ethiprole was administered continuously via the diet at 0, 9, 30 or 90 ppm to separate groups of 5 dogs /sex/dose. Mortality and clinical signs were checked daily. Body weight was measured during the acclimatization period and weekly thereafter. Food consumption was measured weekly throughout the study. Ophthalmic examinations were performed on all animals during the acclimatization period and during Week 12 of dosing. Clinical chemistry, hematology and urinalysis were performed towards the end of the dosing phase. In addition, a blood sample was collected during Weeks 2 and 12 for T3, T4 and TSH analysis. All animals were subjected to necropsy, selected organs were weighed and designated tissues sampled and examined microscopically.

The dietary administration of ethiprole (RPA 107382) to dogs for at least 52 weeks produced signs of systemic toxicity at 90 ppm as evidenced by reduction in the overall body weight gain in both sexes. No other treatment-related findings were noted in any of the parameters examined in this study.

In conclusion, the LOAEL is 2.73 and 2.51mg/kg/day in males and females, respectively (90 ppm in the diet) based on overall reduced body weight gain. The NOAEL is 0.70 and 0.76 mg/kg/day in males and females, respectively (30 ppm in the diet).

This study is classified as **acceptable/guideline** and does satisfies the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in the dogs.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** RPA 107382 (Ethiprole)

Description:	White powder
Lot/Batch #:	CRD9706
Purity:	93%
Compound Stability:	The test substance was stored at +4°C, then at room temperature (from July 21, 1999) protected from light.
CAS # of TGAI:	181587-01-9

2. **Vehicle and/or Positive Control:** No vehicle or positive control was used. The test substance was ground to a fine powder before being incorporated into the diet by dry mixing. Control animals received untreated diet.

3. **Test Animals:**

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	6 months 6.5-7.9 kg for the males, 5.8 - 7.7 kg for the females	
Source:	Marshall Farms, 5800 Lake Bluff Road, North Rose, NY	
Housing:	Individually housed in stainless steel kennels with wood shavings.	
Diet:	Powdered diet, 125C1 P 2.5 or 125C2 P 2.5, supplied by UAR, (Villemoisson, Epinaysur-Orge, France). (300 g of diet moistened with 500 ml of water), *ad libitum*	
Water:	Filtered and softened water from the municipal water supply, *ad libitum*	
Environmental conditions:	**Temperature:**	20 ± 5°C
	Humidity:	50 ± 20%
	Air changes:	12 air changes/hr (average, not monitored)
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	27 days	

B. STUDY DESIGN:

1. **In-Life Dates:** Start: June 21, 1999; End: July 7, 2000

2. **Animal Assignment/Dose Levels:** Animals were assigned using a randomization procedure that ensured a similar body weight distribution by sex to the test groups noted in Table IIA 5.3.4-1. The dose levels were selected after evaluation of the results from a previous 90-day dietary study in Beagle dogs performed with this test substance (Bigot, D. (SA98340), RPA 107382 90 day toxicity study in the dog by dietary administration. Bayer Cropscience (Formerly Aventis Cropscience and Rhone-Poulenc Agrochimie), Sophia Antipolis, France. (May 4, 2001), see section KIIA 5.3.3).

TABLE IIA 5.3.4-1 Study Design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)		No. of Animals	
		Males	Females	Males	Females
Control	0	0	0	5	5
Low	9	0.27	0.22	5	5
Mid	30	0.70	0.76	5	5
High	90	2.73	2.51	5	5

3. **Diet Preparation and Analysis:** Test diets were prepared as admixtures as follows. The test substance was blended with a small quantity of diet (125C1 P 2.5 or 125C2 P 2.5, supplied by UAR) using a mortar and pestle. This premix was then mixed for 10 minutes in a mixer with the appropriate amount of diet needed to achieve the dosing levels of 9, 30 and 90 ppm. The first dietary admixture was prepared for 2 weeks, and thereafter diets were prepared at 4 week intervals according to stability results. Diets were stored at room temperature, protected from light, in closed bags prior to use. On the day of administration, diets were moistened with water (300 g/500 mL water) before to feeding to the animals.

   Prior to the start of the study, two dietary admixtures were prepared without addition of water: a low concentration of 5 ppm (the lightest capacity of the mixer), and a high concentration of 100 ppm (the lowest capacity of the mixer). From each mixture two samples were taken at three different levels (top, middle, and bottom.) and analyzed to evaluate the homogeneity. In addition, during the study, the homogeneity (top, middle, and bottom) of the 9 and 90 ppm diets were tested at Weeks 1, 13, 39, and 52.

   The stability of the 5 and 100 ppm diets were confirmed at intervals up to 35 days at room temperature. In addition, diets were tested after 16 days of storage in a closed bag, and 24 hours of storage in an open feeder.

   During the study, the concentrations of the administered diets were verified at Weeks 1, 4, and 13.

   Results: **Homogeneity Analysis:** The homogeneity result obtained prior to the start of the study for the 5, 9, and 100 ppm diets were within 83-102% of nominal. During the study, the homogeneity results were for the 9 and 90 ppm diets were within 90-103% of the nominal values. (Data obtained from the study report, page 236-241).

   Stability Analysis: After 35 days of storage at room temperatures, results were within 92 or 93% of the Day 0 measured concentrations for the 5 and 100 ppm diets, respectively. For diets stored 16 days at room temperature in a closed bag, followed by 24 hours in an open feeder, diets were 92-104% of the nominal concentrations. (Data obtained from the study report, page 242).

   Concentration Analysis: Results of were within 90-100% of nominal. (Data obtained from the study report, page 243).

4. **Statistics**: Statistical analyses were performed on of body weight, food consumption, hematology, blood biochemistry, urinalysis and organ weight data:

If the number of animals/sex in each group was less than 5, then statistical analyses were performed using either the Dunn test (3 or more groups) or the Mann-Whitney/Wilcoxon test (2 groups).

If the number of animals/sex in each group was greater than or equal to 5, then the Kolmogorov-Lilliefors test for normality of the distribution was used. If the data were found to be of normal distribution, an assessment of homogeneity of variance between the groups was determined using Bartlett's test (3 or more groups) or Fisher's test (2 groups). Data found to be homogeneous (by either Bartlett's or Fisher's test) were assessed using the Student's test (2 groups) or Dunnett's test (3 or more groups). Data found to be heterogeneous using Bartlett's or Fisher's test were assess by Dunn's test or the Mann-Whitney/Wilcoxon test, respectively.

If the data were found by the Kolmogorov-Lilliefors test to not be normally distributed, then they were logarithmically transformed (except for organ weights) and then re-tested. If the data were found to be of normal distribution after the log transformation, then they were further assessed as described above. If, after the log transformation, the data were still not normally distributed, then the original values (untransformed data, including organ weights) were subjected to the Dunn's test.

C. METHODS:

1. Observations:

Each animal was checked at least twice a day (except on weekends and public holidays: at least once each day) for mortality or signs of morbidity.

Each animal was observed at least once a day, at approximately the same time each day, for the recording of clinical signs. A detailed clinical examination was carried out once before the beginning of the treatment period and once weekly during the study in all animals. These observations were made outside the kennels and included (but were not limited to) evaluation of skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. salivation, lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards) were recorded. As no abnormalities were recorded, individual tables are not reported in the study report, but are archived with the raw data of the study.

2. **Body weight:** Body weight was measured once during the acclimatization phase, then on the first day of treatment and weekly thereafter.

3. **Food Consumption and Compound Intake:** The amount of food consumed was recorded for each animal by weighing the difference between the quantity given and that remaining the next morning. Food intake per animal and per day was calculated for 7 days before the beginning of the treatment period and then throughout the study. Whenever fasting was required, the food was removed at the end of the day and the estimation was made at that time, food consumption being over a period of approximately 5 hours for that day.

   Achieved intake of test substance was calculated on a weekly basis for each sex and each treated group as follows:

$$\text{Achieved Dosage (mg/kg/day)} = \text{Nominal Conc. (ppm)} \times \frac{\text{Mean Food Consumption (g/animal/day)} \times 0.375^*}{\text{Mean Body Weight (g)}}$$

* represents the corrective factor, added water was not taken into consideration for the calculation of achieved doses.

4. **Ophthalmoscopic Examination:** Ophthalmological examinations were performed on all animals before the beginning of the treatment period and in weeks 13, 26 and 51. Pupillary light and blink reflexes were evaluated. The pupils of the animals were then dilated with tropicamide, and the appendages, optic media and fundus were examined by indirect ophthalmoscopy.

5. **Hematology & Clinical Chemistry:** Blood samples were taken without anesthesia and collected into tubes containing the appropriate anticoagulant. Prior to blood sampling, the animals were deprived of food for an overnight period of at least 14 hours. Blood was collected from all animals for the examination of the CHECKED (X) parameters once before the beginning of the treatment period and during weeks 12, 25 and 51.

   In addition, blood samples (~2.5 mL) were taken from all animals into tubes without anticoagulant before the beginning of the treatment period (at Weeks -2 and 1) and during the treatment period (at Weeks 1 and 12). At each sampling time, the blood samples were taken in the morning before feeding. The blood samples were immediately centrifuged and the serum was collected into individual tubes, frozen (at -20°C), and sent for thyroid hormones (T3, T4, TSH) measurements by radioimmunoassay.

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		
X	(Thromboplastin time)		
X	(Fibrinogen)		
X	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
	Magnesium	X	Urea nitrogen
X	Phosphorus	X	Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	ENZYMES (more than 2 hepatic enzymes)	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine kinase		Serum protein electrophoresis
X	Lactate dehydrogenase (LDH)	X	Amylase
X	Alanine aminotransferase (ALT/ SGPT)	X	Thyroid Hormones (T3, T4, and TSH)
X	Aspartate aminotransferase (AST/ SGOT)		
X	Gamma-glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

NOTE: T3, T4, and TSH levels were monitored before the beginning of the treatment period (at Weeks -2 and 1) and during the treatment period (at Weeks 1 and 12)

6. **Urinalysis:** Urine was collected from fasted animals once before the beginning of the treatment period and during weeks 12, 25 and 51. The urine was collected in the presence of thymol crystals. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity / osmolality	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Nitrate
X	Protein	X	Urobilinogen

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. Histopathological examinations were performed on all animals from all groups. The (XX) organs, in addition, were weighed.

Bone marrow smears were prepared from the sternum of all animals killed on completion of the treatment period. However, as there were no relevant abnormalities were observed in hematological investigations, the bone marrow differential cell count was not determined.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic	XX	Brain (multiple sections)
X	Salivary glands	XX	Heart	X	Periph.nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (retina, optic nerve)
X	Jejunum	XX	Thymus		GLANDULAR
X	Ileum			XX	Adrenal gland
X	Cecum		UROGENITAL		Lacrimal gland
X	Colon	XX	Kidneys	XX	Parathyroids
X	Rectum	X	Urinary bladder	XX	Thyroids
XX	Liver	XX	Testes		OTHER
X	Gall bladder	XX	Epididymides	X	Bone (sternum and/or femur)
X	Pancreas	XX	Prostate	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries	X	Skin
X	Trachea	XX	Uterus		All gross lesions and masses
X	Lung	X	Mammary gland		
X	Nose	X	Vagina		
X	Pharynx				
X	Larynx				

II. RESULTS

A. OBSERVATIONS:

1. **Clinical Signs of Toxicity:** There were no treatment-related clinical signs at any dose level in either sex.

2. **Mortality:** There were no treatment-related mortalities at any dose level in either sex.

B. BODY WEIGHT:

Slight decreases in the overall body weight gain were noted in both males and females at 90 ppm (-19% and -23% in males and females, respectively) (Table IIA 5.3.4-2).

There were no treatment-related changes in body weight and body weight gain at 9 and 30 ppm in either sex.

TABLE IIA 5.3.4-2 Mean Body Weights (g) and Body Weight Gains (g/day)

Interval (day)	Dose Level (ppm in diet)			
	0	9	30	90
Males				
Body Weight (g)				
Week 1	7.4±0.53	7.3±0.54	7.6±0.29	7.3±0.55
Week 13	8.7±1.16	8.7±0.89	10.0±0.39	9.0±0.57
Week 52	11.0±1.71	10.1±2.11	12.2±1.06	10.0±1.28
Body Weight Gain (g/day)				
Overall (Weeks 1-52)	3.7	2.9 [3.6][a]	4.8	3.0 (↓19%)
Females				
Body Weight (g)				
Week 1	6.7±0.60	6.6±0.51	6.6±0.61	6.6±0.66
Week 13	8.6±0.80	8.8±0.55	8.5±0.93	7.8±0.54
Week 52	9.6±1.11	10.3±1.84	9.9±1.26	8.9±1.00
Body Weight Gain (g/day)				
Overall (Weeks 1-53)	3.1	3.9	3.4	2.4 (↓23%)

* Statistically different ($p<0.05$) from the control. ** Statistically different ($p<0.01$) from the control.

[a] Value in brackets is the mean body weight gain excluding one low-dose male that lost weight for reasons that were unrelated to treatment with the test substance.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food Consumption:** There were no treatment-related changes in food consumption at any dose levels in either sex.

2. **Compound Consumption:** The mean achieved dose levels expressed in mg/kg body weight day are presented in Table IIA 5.3.4-3.

TABLE IIA 5.3.4-3 Mean Achieved Dietary Intake of Ethiprole (Weeks 1-52)

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day) [a]	
		Males	Females
Control	0	0	0
Low	9	0.27	0.22
Mid	30	0.70	0.76
High	90	2.73	2.51

D. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related changes at the ophthalmological examination at any dose level in either sex.

E. BLOOD ANALYSES:

1. **Hematology:** At the end of the dosing phase, no treatment-related hematological changes were observed at any dose level in either sex.

2. **Clinical chemistry:** At the end of the dosing phase, no treatment-related clinical chemistry changes were observed at any dose level in either sex.

3. **Hormone analysis:** No treatment-related changes in serum levels of the thyroid hormones were observed at any dose level in either sex.

F. **URINALYSIS:** No treatment-related urinalysis changes were observed at any dose level in either sex.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ Weight:** At terminal sacrifice, no treatment-related organ weights changes were observed at any dose levels in either sex.

2. **Gross Pathology**: At terminal sacrifice, no treatment-related macroscopic changes were observed at any dose level in either sex.

3. **Microscopic Pathology**: At terminal sacrifice, no treatment-related microscopic changes were observed at any dose level in either sex.

III. CONCLUSIONS:

The dietary administration of ethiprole (RPA 107382) to dogs for at least 52 weeks produced signs of systemic toxicity at 90 ppm as evidenced by reduction in the overall body weight gain in both sexes. No other treatment-related findings were noted in any of the parameters examined in this study.

In conclusion, the NOAEL is 0.70 and 0.76 mg/kg/day in males and females, respectively (30 ppm in the diet) based on overall reduced body weight gain at the next dose level (2.73/2.51 mg/kg bw/day in males and females, respectively).

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R192513

Chemical Name: Ethiprole

PC Code: 005550
HED File Code: 11000 Chemistry Reviews
Memo Date: 5/19/2011
File ID: 00000000
Accession #: 000-00-0137

HED Records Reference Center
6/9/2011