Mr. Saeger

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

#3794

SUBJECT: Request Temporary Tolerances for combined residues of N'-(2,4-dimethylphenyl)-N-[[(2,4-dimethylphenyl) imino]methyl]-N-methylmethanimidamide and its metabolites N-(2,4-dimethylphenyl)-N'-methylformamidine and 2,4-dimethylformamilide in or on apples and pears at 1.0 ppm.

DATE: 19 FEB 1975

FROM:

TO:

Product Manager
Special Registrations Section

Pesticide Petition No: 5G1558

Chemical Identity: U-36, 059EC; BTS 27419

Use: Miticide

Petitioner: The Upjohn Company
Kalamazoo, MI 49001

Toxicity Data:

(1) Chronic, 2 yr. Feeding Study (DOG)---

Group of 8 beagle dogs (4M & 4F) were given 0.1, 0.25 and 1.0 mg/kg/day for 2 years. Dosing was in the morning and afterwards the dogs were provided w/ Spratts Dry Diet; water available ad libitum.

METHOD

Dogs were examined clinically before dosing began and immediately after dosing on the first two days and again during weeks 4, 13, 26, 39, 52, 78 and 102. Heart rate and rectal temperature were monitored serially for up to 24 hr. after dosing on day 1, and during wks. 39, 52, 79, and 103. Signs of toxicity and faecal appearance were recorded daily and body weights weekly.

Laboratory investigations were carried out before dosing and during weeks 4, 13, 26, 52, 78 and 102 as follows:

1/14

(a) Haematology: erythrocyte, leukocyte and differential leukocyte, and thrombocyte counts; Hb concentration; haematocrit; erythrocyte sedimentation rate; thrombotest activity.

(b) Blood chemistry: bilirubin; sugar; urea-N; GPT; GOT; alkaline phosphatase; Na; K.

(c) Urine analysis: pH; protein; total reducing substances; glucose; bile pigments; blood.

Dogs were killed and examined macroscopically on the day after the final dose and the following organs weighed:

adrenals	pituitary
brain	prostate
heart	spinal cord
kidneys	spleen
liver	testes
lungs	thyroid
ovaries	uterus
pancreas	

These organs and the following tissues and organs were prepared for histological examination:

aorta	gall bladder	sciatic nerve
bladder	ileum	skeletal muscle
bone	jejunum	skin
bone marrow	lymph nodes	stomach
caecum	mammary glands	tongue
colon	oesophagus	trachea
duodenum	optic nerve	thymus
eyes	salivary gland	vagina

Results:

All eight dogs given 1.0 mg/kg exhibited signs of slight CNS depression 3 hours after dosing on days 1 and 2, and all appeared normal again by the following morning. One male receiving 1.0 mg/kg had a slight subnormal temperature at 3 hours which returned to normal within 24 hours. All dogs in this group appeared clinically normal, except for one female that was slightly hypothermic 3 hours after dosing during weeks 52 and 79 (decrease 1°-2°F) No clinical reaction to treatment seen in dogs on lower dosage.

Blood samples collected during weeks 40 and 53 showed significant increase in mean blood sugar concentration 3 hours after dosing in the 1.0 mg/kg group.

2

NEL = 0.25 mg/kg
(Thomas J. Fakuk, D.V.M., Ph.D; The Upjohn Co.)

(2) Chronic, 2 yr. Feeding Study (RAT)--

Groups of 40 M and 40 F Ash-Wistar pathogen free rats were fed doses of 15, 50 or 200 ppm daily for 2 years. Food and water available ad libitum.

Methods

Haematological and biochemical investigations obtained from tail vein of 8 males and 8 females at 26, 52, 78, and 103 weeks. Methaemoglobin estimations made during week 90.

(a) Haematology: erythrocyte count; reticulocyte count; haemoglobin; methaemoglobin; hematocrit, leukocyte count (total & differential); thrombocyte count; thrombotest activity (Thromiotest Owren).

(b) Blood chemistry: sugar; urea-N.

(c) Plasma biochemical analyses from blood obtained by heart puncture immediately after death were: GPT; GOT; bilirubin; alkaline phosphatase; Na and K.

(d) The following organs were dissected free of fat and weighed:

adrenals
brain
heart
kidneys
liver
lungs
ovaries
prostate w/ seminal vesicles
submaxillary salivary gland
spleen
testes
thymus
uterus

(e) These organs and the following tissues and organs were prepared for histological examination:

aorta
cervix & vagina
epididymis
lacrimal gland
larynx
lymph nodes
mammary gland
oesophagus
ureters
eyes
femur
pituitary
spinal column
bone marrow
ileum

3

muscle	bladder
pancreas	colon
sciatic nerve	duodenum
skin	stomach
trachea	mesenteric lymph node

All tissues and organs were examined for any abnormal growth, tissues or tumors using the naked eye, dissecting less and microscope.

Results:

There was a temporary reduction in food intake and depressed growth rate in rats fed 200 ppm of BTS 27419. They also tended to be nervous, aggressive and excitable. No haematological or biochemical change was detected; terminal plasma biochemistry and organ weights were no different from control values. No dose related histopathological changes.

Signs of ill-health, such as red tears (chromodacryorrhea), fur loss, skin abscesses, swelling and cornified skin on the feet, glomerulonephrosis, etc., were seen in a similar proportion of rats in control and treated groups. While these results are considered unrelated to treatment they do shade the effectiveness of the results obtained as well as the accuracy of an evaluation, and are therefore somewhat less than optimal for purposeful experimental technique.

Pathological examination of the cardiovascular system, respiratory tract, gastrointestinal system, urinary system, genital system, endocrine glands, lymph-reticular system, skeletal muscle, lacrymal gland, eye, integumentary system, and brain and nervous system demonstrated no dose-related responses. There were also no significant changes in the incidence, type or time of appearance of tumors in the treated groups as compared to the control group.

NEL 50 ppm (2.5 mg/kg) (Huntington, Research Centre, Dr. J. Offer, pathologist, Oct. 7, 1974)

(3) Teratogenicity Study in the RAT-

Groups of primiparous rats of the Boots-Wistar strain were given BTS 27419 in doses of 1, 3, or 12 mg/kg daily from day 8 to 20 of pregnancy inclusive, day 1 being the day on which sperm were detected in the vaginal smear. A 16 hr light and 8 hr dark cycle and TA of 70-74°F were maintained. The rats were killed on day 21 of pregnancy and the uterine contents examined.

4

The live fetuses were examined externally, weighed and after killing, were dissected. The heads removed and free-hand transverse sections in three regions were prepared, through the nose and palate, the eyes and the cerebral region of the brain, for microscopic examination. The carcases were processed for skeletal examination.

Results:

There were no deaths or clinical signs of toxicity. The average litter size, fetal viability and implantation index were similar for both treated and control groups. An unusually high proportion of male fetuses was found in the 12 mg/kg group (male : female was 1.65 compared with 0.81 in the control). The fetuses in the 12 mg/kg group weighed slightly less than control and calcification of their skeletal system was less advanced as judged by the number of calcified centers in the sternum.

Conclusion:

BTS 27419 is not embryotoxic or teratogenic at the doses administered.

(In another separate study which investigated the effect on pregnancy, parturition and care of the young, the same dosages were given and administered at day 1 thru day 21 of pregnancy. There were no effects on implantation, course of pregnancy, parturition and lactation in adult rats, or on the viability, care and development of the young).

(4) Teratogenicity Study in the Rabbit--
First Experiment-

BTS 27419 given by oral intubation to pregnant New Zealand white rabbits in doses of 1, 5, 25, 50 or 100 mg/kg daily from day 6 to 18 of pregnancy inclusive, the day on which mating occurred being counted as day 0. The rabbits were weighed regularly and changes in behavior and condition were noted. They were killed on day 30 of pregnancy and examined macroscopically. The uterine contents were examined, and the number of live, dead and resorbed fetuses and the number of corpora lutea were recorded. Live fetuses were weighed, examined externally, killed and dissected.

5

Results-

Three out of four rabbits on the high doses (100 and 50 mg/kg) died, and the fourth aborted on day 21 of pregnancy.

The average litter size and weight of the fetuses obtained from rabbits with viable young were similar in the 25, 5 and 1 mg/kg and control groups. The implantation index did not differ from that for the control group.

The maximally tolerated dose in pregnant rabbits was 25 mg/kg daily.

Second Experiment-

Procedure was the same as Exp. #1 except the dose levels were 1, 5, or 25 mg/kg daily from day 6 to 18 of pregnancy inclusive, and samples of liver, kidneys, spleen, lungs and any abnormal tissues were prepared for microscopic examination. Also, samples of brain, eyes, genitals, kidneys, liver and lungs were taken from fetuses in the highest dose and control groups for microscopic examination,, and the carcases of all fetuses prepared for skeletal examination.

Results-

At the high dose tested of 25 mg/kg daily there were 4/10 litters aborted, and 1/10 fetuses were dead (5 dead, 44 alive, 1 resorbed). The average fetal weight was somewhat less than control and low test animals.

There were 4 fetuses with gross malformations. Two in a litter of 3 underweight fetuses in the 25 mg/kg group had hydramnios and in one case, gastroschisis. One in the 5 mg/kg group had cleft palate, meningocoele associated with small ears, and a displaced toe, and one fetus in the 1 mg/kg group was without lower incisors.

Skeletal development did not differ from dosed groups and control.

BTS 27419 is therefore, not considered teratogenic at 5 mg/kg/day or less.

(5) Multigeneration Feeding Study in the Rat-

Newly weaned Boots-Wistar strain rats were randomly allocated to groups of 12 M and 24 F. When rats were 14 wks old,

10 M and 20 F from each group (200, 50, and 15 ppm and control) were selected at random and bred. Each male was housed w/ 2 females from the same group for 1 wk. and then crossed over to a second pair of females from the same group for another week, then each female was exposed to 2 males. After the F1 generation was weaned on day 21 post partum, 12 M and 24 F from each group were retained for breeding and maintained continuously on the test diet. Procedure repeated until the F3 generation was weaned. Investigation of the 200 ppm group was terminated when F1 generation was weaned because of low survival.

Observations-

Parent: bodyweights, food consumption, estrus cycles (by vaginal smear), gestation period, autopsied after young weaned.

Young: number born vs number alive on days 4 and 21, bodyweight, examined internal and external (except those bred); F3 generation weaned, killed examined histologically (liver, kidneys, brain, eyes, and gonads, as well as skeleton).

Results: 200 ppm (20 mg/kg/day) caused a depression of growth and food consumption for the first 2 wks in the P1 generation, and a marked mortality among the young during the suckling period 48% viability index).

50 ppm (5 mg/kg) caused slightly increased mortality above the control to the offspring during suckling period through all generation, but the difference was only significantly different in the F3 generation. Litter size was affected significantly in the F3 generation.

No such effects observed at the low dose level of 15 ppm (1.6 mg/kg).

NEL 15 ppm.

(6) 90-DAY Subacute Oral (RAT) (April 1971)-

BTS 27419 was given to Ash-Wistar rats according to the following schedule:

Group 1	200 mg/kg/day for 7 days
Group 2	50 mg/kg/day for 7 days followed by 11 weeks untreated, then again for 7 days
Group 3	12 mg/kg/day for 90 days
Group 4	3 mg/kg/day for 90 days
Group 5	control

At autopsy, blood was taken from the heart for estimation of: alkaline phosphatase, bilirubin, GPT, GOT, Na and K. Microscopic examination of the following: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate w/ seminal vesicles, spleen, testes, thyroid, uterus, bladder, bone marrow, eyes, duodenum, colon, ileum, mesenteric lymph node, pancreas, pituitary, salivary gland, stomach and thymus.

Results-

200 mg/kg/day - rats became irritable immediatly after dosing; lethargic, emaciated, and such poor physical condition they were killed after receiving 7 doses.

50 mg/kg/day - developed abnormal behavior pattern, aggressive, and squealing; reduced weight gain; organ/body weight ratios became significant in adrenals, liver, seminal vessicles w/ prostate; spleen congestion; thymic involution; 2/42 died.

12 mg/kg/day - appeared irritable and excitable; no deaths, slightly depressed growth, normal organ weights (except males w/ slightly underweight livers); no histological changes attributable to treatment.

3 mg/kg/day - NEL

(7) 90-DAY Subacute Feeding (RAT) - (August 1971)-

BTS 27419 given to Wistar rats in doses of 50, 12 or 3 mg/kg/day for 90 days.

Weight gain and food consumption were measured along with the following:

hematology: leukocyte count, erythrocyte count, hematocrit, Hb, total serum protein, GOT, GPT, alkaline phosphatase, cholesterol, urea-N, blood sugar, Na, K, Cl and creatinine.

urine: sugar, protein, bilirubin, pH, Na, K, ketone bodies.

After death the main organs were weighed and examined histologically: brain, heart, lungs, liver, kidneys, spleen, testes, prostate, adrenals, thyroid, pituitary, thymus.

Results-

Significant suppression of body weight increase in the 12 and 50 mg/kg groups. Male rats in the 50 mg/kg group showed organ to body weight increase in the weight of brain, heart, lungs,

liver, kidney, spleen, seminal vessicles, and thymus gland, and suppression of the weight of adrenals. Those of the 12 mg/kg group showed increased organ to body weight of brain only. Female rats show similar results though there was no suppression in adrenal weights. There was a decrease in alkaline phosphatase, and GOT in the 50 mg/kg group.

NEL = 3 mg/kg
(Japan Experimental Medical Research Co.)

(8) 90-DAY Subacute Feeding (Mouse) - (August 1972)-

BTS 27419 was given to ICR-SLC mice in doses of 50, 12 and 3 mg/kg/day for 90 days.

Same parameters as the foregoing 90-Day rat study were used in this study also.

Results-

There was suppression in body weight increase in both M & F mice in groups 50 and 12 mg/kg, but not 3 mg/kg.

M mice of 50 mg/kg group showed significant increase in weight of brain, and heart, and the F mice showed decrease kidney weight.

The 12 mg/kg group did not show any symptom except increase heart weight of M mice.

No remarkable symptom observed in any dosage group both microscopically and histologically.

NEL = 3 mg/kg.
(Japan Experimetnal Medical Research Co.)

(9) 90-DAY Subacute Feeding Study (Dog) - (April 1973)-

BTS 27419 was given to beagle dogs in doses of 0, 0.25, 1, and 4 mg/kg/day for 90 days. Food consumption and body weights were recorded daily and weekly, respectively.

The following laboratory analyses were carried out before and at internals during the dosing period:

9

Haematology - erythrocyte, leukocyte, differential leukocyte and thrombocyte count, Hb concentration, packed cell volume, erythrocyte sedimentation rate and thrombotest activity.

Blood chemistry - bilirubin, sugar, urea-N, GPT, GOT, alkaline phosphatase, Na and K.

Urine analysis - bilirubin, protein glucose, total reducing substance, blood.

The dogs were killed by an IV injection of sodium pentobarbitone and exsanguinated from the carotid artery. Organ weights were recorded of the adrenals, brain, gonads, heart, kidneys, liver, lungs, pancreas, pituitary, secondary sex organs, spinal cord, spleen and thyroids, and samples of these organs and of the aorta, bladder, bone marrow, colon, duodenum, eyes, gall bladder, ileum, jejunum, lymph nodes, esophagus, optic nerve, mammary gland, salivary gland, sciatic nerve, skeletal muscle, skin, stomach, tongue, trachea, thymus and vagina were taken for histological examination.

Results-

4 mg/kg - CNS depression/ataxia 3 hrs following dosing (persisted for 6 hrs); vomiting; subnormal rectal temperatures and pulse rates 3 hrs. after dosing (returned to normal within 24 hrs); acute catarrhal conjunctivitis (started at 8 wks); increased blood sugar (maximal at 6 hrs. post dosing, normal at 24 hr); small amount of glucose in urine;

PATHOLOGY - small uteri; increased liver weight (hyperplasia of the small periportal hepatocytes and increase in binucleate cells); hyperplasia of zona glomerulosa (subsequent w/ decrease in zona fasciculata and reticularis); neutrophilia in bone marrow; pigment noted in bone marrow and spleen.

1 mg/kg - CNS depression; decrease in rectal temperature and pulse rate at 3 hr. after dosing; similar but less pronounced changes in blood sugar concentration from the 4 mg/kg group; small amount of glucose in urine in one dog;

PATHOLOGY - slight enlargement of central and midzonal hepatocytes in liver; hyperplasia of zona glomerulosa w/ decrease in zona fasciculata and reticularis; neutrophilia in bone marrow

10

0.25 mg/kg - little or no overt reaction to treatment except for one occasion of slight CNS depression in a male 3 hr. after dosing during week 8; increase in blood sugar on day 38 in one dog (samples taken on day 1, 38 and 78).

PATHOLOGY - slight neutrophilia in bone marrow; slight increase in extent of larger central and midzonal hepatocytes.

NEL = 0.25 mg/kg

(10) 90-DAY Subacute Feeding Study in Dogs using BTS 27271

BTS 27271 was given orally (gelatin capsule) to beagle dogs in doses of 0.1, 0.25, and 1.0 mg/kg/day for 90 days.

Clinical signs were recorded daily; bodyweight once/wk; food consumption periodically.

The following laboratory analyses were performed:

Haematology - ESR; PCV, Hb conc., red cell count, reticulocyte count, MCB, mean corpuscular Hb conc. (MCHC), total white cell count, differential leukocyte count, platelet count, and prothrombin index

Blood chemistry - plasma urea, glucose, total serum proteins, alkaline phosphatase (SAP), GPT, bilirubin (serum), Na, and K.

Urinalysis - specific gravity, pH, protein, glucose, ketones, bile pigments, urobilinogen, and Hb; also examined microscopically for epithelial cells, erythrocytes, polymorphonuclear leukocytes, mononuclear leukocytes.

The dogs were killed by IV injection of sodium pentobarbitone and exsanguinated from the carotid artery. The following organs were removed and weighed:

brain	liver	prostate	adrenals
pituitary	spleen	uterus	gonads
heart	pancreas	kidneys	
lungs	thymus	thyroid	

Small portions of these tissue together with the following were prepared for microscopic examination:

aorta	duodenum
trachea	jejunum
lymph nodes	ileum
gall bladder	colon
urinary bladder	skin
salivary gland	mammary gland
tongue	skeletal muscle
oesophagus	bone marrow
stomach	peripheral nerve
eye	optic nerve

Results -

No adverse effects were noted as pertains to bodyweight, food consumption, water consumption, ophthalmoscopy.

Decreased rectal temperature were noted in the 0.25 and 1.0 mg/kg groups for 1 to 2 hrs. following dosing.

There was a significant reduction in heart rate for the 1.0 mg/kg group one to two hrs. after dosing on days 41 and 83 (only checked 3 times).

Haematology, biochemistry and urinalysis were within normal limits and there was no dose relationship for the exceptional cases that occurred.

No morphological change or variation from normal was seen in any of the tissues or organs examined that is considered to be associated with administration of BTS 27271.

NEL = 0.25 mg/kg

NOTE: Dr. Kent J. Davis was consulted concerning the aforementioned 90-DAY feeding studies in dogs and confirms no significant dose related pathology.

(11) 21-DAY Subacute Dermal Toxicity in the Rabbit (BTS 27419)-

BTS 27419 in acetone was appled to the intact skin of New Zealand white rabbits in doses of 50 or 200 mg/kg for a total of 15 doses over a 21 day period.

Condition of rabbits observed daily and local reactions to treated area of skin (10 cm square) were noted. Blood samples obtained before treatment and on the final day of treatment. Bodyweights recorded weekly and food consumption daily. Ophthalmoscopy, rectal temperature and heart rate also observed.

12

The rabbits were killed with sodium pentobarbitone given IV on the day after the last treatment. The following organ weights taken:

adrenals	lungs	thyroid
brain	ovaries	uterus
heart	pituitary	
kidneys	spleen	
liver	testes	

Tissues of these organs plus the following tissues were examined microscopically:

bladder	stomach
bone marrow	thymus
duodenum	
eyes	
ileum	
mesenteric lymph node	
pancreas	
treated skin	
untreated skin	

Results-

Both dosed groups showed moderate erythematous reactions with desquamation of skin and subcutaneous hemorrhage. All dosed animals displayed inappetence and became sedated after dosing.

No changes in rectal temperature, heart rate or ophthalmoscopy.

Most of the dosed animals (all males) lost weight during the experiment.

Deaths -	200 mg/kg	1/4 M
		3/4 F
	50 mg/kg	1/4 M
		0/4 F

Underweight testes with variable tubular degeneration were noted in males on 200 mg/kg.

Blood sugar concentration was slightly elevated in both dosed groups.

PATHOLOGY (50 & 200 mg/kg) - nodular hyperplasia in lymph nodes; marked neutrophilia and eosinophilia in spleen; leukocytoses in the liver; pigment noted in lymph nodes; generalized neutrophilia (lung, skin, pituitary, bladder).

Findings/Recommendations-

TB finds that the data in the petition supports the safety of the proposed tolerance for BIS 27419 and its metabolites BIS 27271 and BIS 27919 in or on apples and pears at 1.0 ppm.

Robert B. Jaeger, Physiologist
Toxicology Branch
Registration Division (WH-567)

cc: CB, EEEB, Branch File, PP No. 5G1558

R/D Init: G.E. Whitmore 2/10/75

Init: G.E. Whitmore G.E.W.

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