S7,

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

SEP 12 1988

SEP 12 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#8F3633. Cyromazine Surface Application in Swine Tissues. Evaluation of Residue Data and Analytical Method (MIRD#'s 405932-00,-01,-02,-03 and 0.4). RCB#3733

FROM: Freshteh Toghrol, Chemist F. Toghrol
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

THRU: Philip Errico, Section Head
Tolerance Petition Section Head III
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

TO: Philip Hutton, PM-17
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

The Ciba-Geigy Corporation proposes the establishment of tolerances for combined residues of the insecticide cyromazine (N-cyclo-propyl-1,3,5-triazine-2,4,6-triamine) and its metabolite, melamine (1,3,5-triazine-2,4,6-triamine) in or on swine meat, fat and meat by-products (including kidney) of 0.2 ppm and swine liver of 0.4 ppm.

Technical cyromazine is registered by the agency (EPA REG. NO. 100-632).

Tolerances are established [40 CFR 180.414 (a) and (d)] for combined cyromazine and its metabolite, melamine in/on eggs at 0.25 ppm, head lettuce at 5 ppm, and celery at 10 ppm.

Tolerances are established (40 CFR 180.414b) for residues of the insecticide cyromazine in or on fat, meat, and meat by-products of chicken layer hens at 0.05 ppm.

Tolerances are established (40 CFR 180.414c) for residue of cyromazine metabolite, melamine in meat, fat, and meat by-products in chicken layer hens at 0.05 ppm.

Conclusion

1a. The nature of the residue in plants is adequately understood. The significant components of the residues are the parent compound cyromazine and its metabolite melamine.

1b. The nature of the residue in nonruminants (hogs, horses, poultry) is adequately understood. Cyromazine is excreted mainly unmetabolized, with some deposition in eggs and meat. The significant components of the residue are the parent compound cyromazine and its metabolite melamine.

2. The analytical methods are available for enforcement purposes in PAM II(method I,) for residues of cyromazine and its metabolite melamine in animal tissues.

3. From information discussed under "Other considerations" below, we believe cyromazine would be used more than the three times described in the residue data submitted in this petition. In order to assess the cumulative effects of season long use, the petitioner should submit residue data for swine tissue reflecting sampling after repeated applications. These samples should include both raw and cooked (pork rinds) skin. Alternatively, the petitioner can submit a revised section B which allows the use of cyromazine only in the absence of swine and their food and water.

4. With a revised section B removing swine and their food and water from livestock holding areas during treatment, and an adequate answer to conclusion 5, we can conclude the proposed tolerances will be adequate.

5. The petitioner should submit complete information on how the tissue samples were handled after slaughter and through storage and analysis.

6. An International Residue Limit Status sheet is attached. There are no internationally established tolerances for this chemical.

Recommendation

RCB recommends against the proposed tolerances because of the deficiencies identified in storage stability, data gap on swine skin[raw and cooked], and or a revised section B (see residue data in this memo for more information).

Detailed Consideration

Manufacture and Formulation

The manufacturing process and composition of technical cyromazine has been adequately discussed (A. Rathman, PP#9G2230, 11/14/79). The impurities are not likely to produce a problem.

This product will be formulated as Larvadex 2SL Containing 2% active ingredient cyromazine (0.17 lb. cyromazine per gallon) and 98% inert ingredient.

The petitioner has requested (PP#7F3477) the registration of Larvadex 2SL (EPA Reg. No. 100-AAE)for use in or around swine operations and the approval of an alternate brand name "Cycle 2SL" (cover letter in this petition).

Proposed use

To control house and stable flies in and around swine operations. Applied as a 0.05% or 0.1% spray at a rate of one gallon of finished spray per 100 sq. ft. of the surface of the manure or other areas where flies breed. Applications are repeated at 14-day intervals or as needed based upon larval (Maggot) activity in manure and other areas. The proposed label includes a note, not to apply cyromazine spray directly to swine or swine feed. No pre-slaughter interval is required, Larvadex 2SL is not intended for use in animal feeds.

There is no maximum number of applications indicated. Swine do not have to be removed from area during application to premises.

Nature of the Residue

Plant Metabolism

Plant metabolism studies were not submitted with this petition. However based on the plant metabolism data submitted earlier in support of other petitions (PP# 5G3176 and PP# 5F3180, celery metabolism, PP# 5G3176, lettuce metabolism and PP# 5F3180,tomatoes metabolism) it was concluded that the nature of the residue in plants, is adequately understood. The significant components of the residue are the parent compound cyromazine and its metabolite, melamine.

Animal Metabolism

The animal metabolism studies (goat, rat, poultry and sheep) were submitted in support of PP#9G2230 (A. Rathman, 11/14/79), and PP#2F2707/#2H5355(P.V. Errico, 2/23/83) and are summarized below.

Summary:

Rat-Report No. ABR-78072:

Radiolabelled ^{14}C-cyromazine (A. Rathman, 11/14/79, PP#9G2230) equivalent to 5 ppm in the diet was fed to the rat in a single oral dose and 95% (83% of it was un metabolitzed cyromazine) of the activity was found in the urine (within 72 hours). In the feces 3% of the activity was found and the tissues had less than 0.003-0.007 ppm cyromazine, which was too low to identify.

Chicken-Report Nos. ABR-79043 and ABR-81035:

Radiolabelled ^{14}C-cyromazine at a level of 5 ppm was fed (for 7 days) to egg-laying chickens. 99.1% of the activity was found in the excreta(77.6% unmetabolized cyromazine). 0.4% of the activity was found in the egg white (60% unmetabolized cyromazine), 0.2% in the egg yolk(70% cyromazine and 7% melamine), and 0.1% in the tissues. The egg white and the yolk each had separate unidentified minor components, less than 25% of the radioactivity.

Sheep-Report No. ARB-79056:

Radiolabelled ^{14}C-cyromazine was fed daily to sheep at a level of 5 ppm for 9 days. 83% of the activity was recovered in the urine (84% of it was unchanged cyromazine), 3.6% in the feces (44% Cyromazine), 0.174 ppm in the liver (12% cyromazine, 44% melamine), and 0.048 ppm in the kidney.

Goat(PP# 2F2707 Report Nos. ABR-80051 and Biol-80006):

5 ppm ^{14}C-cyromazine in feed was fed to a lactating goat for 10 days. 89% of the total activity was recovered and the major portion of it was in the urine(83%), and feces(4.4%). The rest of the activity was in tissues (0.26%), the blood (0.03%) and the milk (0.1%). No expired carbon dioxide was recovered, indicating that cyromazine's triazine ring was not ruptured. In the urine, cyromazine was about 65% of the total activity, an unidentified metabolite 26% and at least two more unidentified compounds about 6%. In the feces 44% was unmetabolized cyromazine, while two unidentified metabolites each constituted 12 and 13% of the total activity. In the milk 15% was unmetabolized cyromazine, 34% was an unidentified compound (at zone 2), and 38% unidentified compound (zone 4). Zone 2 and 4 refer to the location of the compounds on TLC(thin layer chromatography) plates (PP#2F2707). Since the unidentified component of the milk makes up a significant portion of the residues and the zone 4 has been reported to be 1-methylcyromazine (PP#6F3332) but no study submitted for review, it was requested that (A. Smith, PP#6F3329, 1/28/87) the petitioner identify and characterize these compounds.

The metabolism of cyromazine in animals was established to proceed through dealkylation to form melamine and it was mainly excreted as unchanged cyromazine. There was some deposition of residues in eggs, milk and tisses. As a result, the nature of the residue in nonruminants(hogs, poultry) is adequately understood for this proposal use. The significant component of the residues are the parent compound cyromazine and its metabolite melamine (see attached sheet for the cyromazine and melamine formula). In ruminants (cows, goats, sheep) the nature of residues is not adequately understood.

Analytical Methods

Residue methods were submitted for cyromazine and its metabolite melamine in swine tissues. The samples were homogenized and extracted with acetonitrile:water, the supernatant was decanted. An aliquate was cleaned up by C-18 sep^{pak} filtration followed by cation exchange (Dowex 50w-X4) column and was eluted with ammonium hydroxide:methanol and evaporated to dryness. The residues were dissolved in water and cleaned up in anion exchange column (AG-417:Borex 9 and AG-417A: Dowex 1-X8). The column was eluted with water, the eluate was submitted to a (Dowex 50w-X4) cation exchange column and was reeluted with ammonium hydroxide:methanol, which was evaporated to dryness. Then the residues were dissolved in methanol and were analyzed (cyromazine and melamine) by HPLC.

The residues were expressed as cyromazine residues. Control samples of tissues were fortified (spiked) with cyromazine and

its metablite melamine each at levels of 0.05 -0.2 ppm. Recoveries were 71-102 percent with an average recovery of 84 percent for cyromazine (AG-417 and AG-417A), and 77-109 percent with an average of 89 percent for melamine.

The method (No. AG-417 and addendum, AG-417A) has been validated with cyromazine at 0.3 ppm and melamine at 0.1 ppm on poultry tissues and eggs in a collaborative effort of four laboratories (see USDA report by R.L. Ellis, of 10/24/84, PP#2F2707). The limit of detection using these procedures are 0.05 ppm for cyromazine and 0.04 ppm for melamine,equivalent to 0.05 ppm of cyromazine.

These methods are available for enforcement purposes (PAM II, method I) for residues of cyromazine and its metabolite melamine.

Residue Data in Swine Tissue

Eight nine-week-old Landrace X Yorkshire littermate (similar size) pigs were divided into three separate groups. The first group consisted of two pigs which served as the controls, and two sets of three pigs each dosed at 1x and 10x levels, respectively. Each group had at least one representative of each sex, each group was housed in a 10' x 10' pen with concrete flooring. Each pen contained a separate self feeder and nipple water container. Each pig was sprayed three times at seven-day intervals with one gallon of solution per application per pen. The pigs were sprayed within the confines of their pen. The solution was applied to the back, sides and bottom of the pig's body with a hand sprayer until dripping occurred. The remaining solution was sprayed on the floor of each pen.

Body weight and feed consumption records were kept on a weekly bases. Blood profiles were taken prior to the test period and prior to slaughtering of the animals. The pigs were slaughtered at zero, three and seven days following the final application. The day-three sacrifice did not have a control.

The tissue samples taken from each pig included: round muscle, tenderloin, perirenal fat, omental fat, back fat, liver, kidney and blood. Samples were frozen immediately after collection and remained so until time of analysis.

No cyromazine and melamine (<0.1ppm) were detected in fat samples at 1X rate. No melamine residues (with the exception of a one seven day liver sample) were found in any of the tissues at the 1X rate. The maximum combined residues of cyromazine and melamine found at 1X rate were: liver (0.32), kidney (0.14), round (0.12), loin (0.12), and blood (0.06) ppm. At the 10X rate no melamine was detected in fat, back fat or loin . The maximum combined residues of cyromazine and melamine at the 10X rate were: liver (0.89), kidney (1.4) round (1.0), loin (0.61), omental fat (0.27), back fat (0.09), perirenal fat (0.26) and blood (0.56)ppm. The reported residue values 0, 3 and 7 days post treatment are as follows:

Combined Residues of Cyromazine and Melamine in Swine Tissues Resulting from Dermal Application:

Matrix	PTI days**	1X Total Residues-ppm*	10X
Round	0	0.08	0.71, 0.88
	3	0.05	0.83, 1.0
	7	0.12	0.44
Loin	0	0.12	0.61
	3	<0.10	0.55
	7	0.08	0.35
Liver	0	0.19	0.15
	3	0.09	0.89
	7	0.32	0.42
Kidney	0	0.12	1.20
	3	<0.10	1.40
	7	0.14	0.82
Omental Fat	0	<0.10	0.27
	3	<0.10	0.11
	7	<0.10	0.08
Perirental Fat	0	<0.10	0.26
	3	<0.10	0.16
	7	<0.10	0.11
Back Fat	0	<0.10	0.08
	3	<0.10	0.07
	7	<0.10	0.09
Blood	0	<0.10	0.21
	3	<0.10	0.56
	7	0.06	0.27

*One Gallon of 0.1% and 1% active ingredient of cyromazine represents 0.125 and 1.25 oz. a.i. of cyromazine respectively.

** PTI = Post Treatment Interval. Swine were sprayed three consecutive (with 7 day intervals) times and were sacrified at

0, 3 and 7 days following the last application.

The proposed use allows application, in the presence of swine and presumably their water and feed(every 14 days or as needed), around the premises and manure of swine operations. The residue data submitted was a worst case which reflected a direct application to the animals. In actual practice, cyromazine would not be applied directly to the animals; exposure would occur as a result of the swine, and their food and water, being present during the application to the walls, fence lines, under and around food troughs etc. of the holding facilities. We have received information (see "Other Considerations" below) that even if the interval between applications is restricted to every 14 days, cyromazine would be used more than the three times used in these submitted studies. No residue data was submitted which would help us to assess any cumulative residue levels from repeated use throughout the fly season.

The petitioner should submit residue data for swine tissue sampled after repeated applications to assess the cumulative effects of season long use. These samples should include both raw and cooked (pork rinds) skin. Alternatively, the petitioner can submit a revised Section B which allows the use of cyromazine only in the absence of swine and their food and water. With a revised Section B we can conclude the proposed tolerances for residues in swine meat, fat and meat by-products [except skin(raw and cooked)], and liver, will not be exceeded. The petitioner must submit residue data on skin, both raw and cooked (pork rinds), in order that we may assess the need for and level of the tolerances in these commodities.

The stability of cyromazine residues in storage samples has been done only on eggs(PP#6F3329); this data showed that the cyromazine residue in/on eggs were stable for 23 months. For any additional uses in or around livestock, storage stabilities on tissue should be submitted. The date of analysis was not mentioned for samples submitted in this hog tissue study, but from the date of the study's completion and the last date on which the animals were sprayed, the samples may have been stored in the freezer for up to 6 months. The petitioner should submit more complete information on how the tissue samples were handled after slaughter and through storage and analysis.

With a revised section B (removing swine and their food and water from livestock holding area during treatment) and adequate handling of samples(see questions on sample handling above paragraph), we can conclude the proposed tolerances will be adequate.

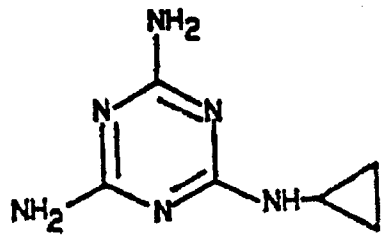
Other Considerations:

Iowa, the largest swine-producing state has a fly season of 7 months (April - October). This information is based on a telephone conversation with Dr. E. Steverman, USDA swine research facilities in Iowa, on 6/17/88. North Carolina also has the same fly season according to Dr. J. Arends 6/20/88, who said that three applications of larvicide at 14 day intervals (based on proposed tolerance data) would not be enough, to control flies during such a long fly season. Swines are kept in contained facilities for 4-7 months to reach market weight, and 2-3 years as breeders, sometimes breeding swine are kept in the facilities for up to 12 years.

The International Tolerance Status sheet is attached. There are no established tolerances for cyromazine in/on swine in other countries or Codex, therefore there are no compatibility problems.

cc: R.F.,S.F., Circ. Freshteh Toghrol, PMSD/IBS
RDI:PE:8/30/88 :RDS:9/7/88
TS-769C:RCB:FT: CM#2, RM:803, 557-7561, F.T,(8/8/88).

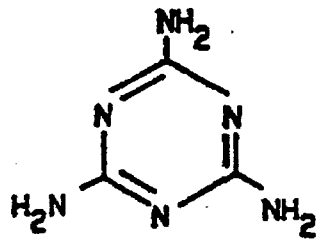
referenced from study with MRID #405932-03



Cyromazine (CGA-72662)

N-Cyclopropyl-1,3,5-triazine-2,4,6-triamine

$C_6H_{10}N_6$



Melamine

1,3,5-Triazine-2,4,6-triamine

$C_3H_6N_6$

FIGURE 1: CHEMICAL NAMES AND STRUCTURES

INTERNATIONAL RESIDUE LIMIT STATUS

Fred Lucas 6/8/88

CHEMICAL Cyromazine

CODEX NO. ____

CODEX STATUS:

☑ No Codex Proposal Step 6 or above

Residue(if Step 8): ____

Crop(s)	Limit (mg/kg)

PROPOSED U.S. TOLERANCES:

Petition No. BF 3633

RCB Reviewer F. Toghrol

Residue: N-cyclo-propyl-1,3,5-triazine-2,4,6-triamine

Crop(s)	Limit (mg/kg)
swine meat, fat and meat byproduct including kidney	0.2
swine liver	0.4

CANADIAN LIMITS:

☑ No Canadian limit

Residue: ____

Crop(s)	Limit (mg/kg)

MEXICAN LIMITS:

☑ No Mexican limit

Residue: ____

Crop(s)	Limit (mg/kg)

NOTES: