PP# 9G0821

Petitions Control Branch and
Division of Pharmacology and Toxicology

June 9,1969

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Petitions Evaluation Branch
Division of Pesticides

PP #9G0821: RH-315[3,5-dichloro-N-(1,1-dimethyl-2-propynyl)benzamide] on various commodities. Evaluation of analytical method and residue data.

The Rohm and Haas Company proposes temporary tolerances for residues of the herbicide 3,5-dichloro-N-(1,1-dimethyl-2-propynyl)benzamide (trade name RH-315) and its metabolites (calculated as the parent compound) at 3 ppm in or on alfalfa, clover, lespedeza, trefoil, and vetch, 1 ppm in or on lettuce, 0.2 ppm in liver and kidney, and 0.01 ppm in milk.

This is the first request for either a temporary or permanent tolerance for this chemical.

Conclusions

1. The metabolism of RH-315 in plants is satisfactorily delineated for purposes of these temporary tolerances.

2. The analytical method is adequate for enforcement of the proposed temporary tolerances.

3. The proposed 3 ppm tolerance is adequate to cover residues of RH-315 and its metabolites in or on the subject forage legumes (including hay) resulting from the proposed uses, provided the 45 day harvest and grazing restriction interval for applications of <1.5 lbs. act/A is increased to 60 days.

4. The proposed 1 ppm tolerance is adequate to cover residues of RH-315 and its metabolites inlettuce resulting from the proposed use.

5. The proposed milk and liver and kidney tolerances are adequate to cover residues of RH-315 and its metabolites transferring to these items as a result of the ingestion by livestock of feed items bearing residues of RH-315 and its metabolites at 3 ppm [Section 120.6 (a)(1)]. With regard to other edible tissues, this is a Section 120.6(a) (3) situation.

6. Since no poultry feeding study was submitted, we are unable to determine whether or not finite residues would be present in poultry, or in eggs, as a result of feeding alfalfa and alfalfa hay or

its derivatives (chiefly alfalfa meal) However, since the latter usually constitutes only 5 percent of the poultry ration, and since the milk and tissue studies show that RH-315 does not store in the usual sense the amount of residue in poultry tissue and eggs if any, would be very low.

7. Residues in follow-up crops resulting from the proposed uses of this temporary tolerance will not be a problem.

Recommendations

Pharmacological considerations permitting, we recommend that the proposed temporary tolerances for residues of the herbicide 3,5-dichloro-N-(1,1-dimethyl-2-propynyl)benzamide (trade name RH-315) and its metabolites (calculated as the parent compound) of 3 ppm in or on alfalfa, clover, lespedeza, trefoil, and vetch, 1 ppm in or on lettuce, 0.2 ppm in kidney and liver, and 0.01 ppm in milk be established. This favorable recommendation is contingent upon the imposition of a 60 day harvest and grazing restriction (rather than the proposed 45 day interval) for the subject forage legumes in connection with applications of <1.5 lbs. act/A.

The petitioner should be informed that for consideration of a permanent tolerance request, we will need the following:

1. More positive identification of Metabolites 6,7, and 8 as determined in alfalfa would be desirable. However, we defer to DPT on this point since the metabolism of RH-315 in plants, soil and rats is quite similar and the residue method would be expected to determine these metabolites.

2. Additional residue data for clover reflecting broader geographical representation. (The data reflect one location only.)

3. Residue data for lespedeza, trefoil, and vetch. (No data were presented for these feeds.)

4. Additional residue data for lettuce from other geographical areas.

5. Additional residue data for Calmar variety lettuce reflecting treatment-harvest intervals <40 days.

6. A poultry feeding study would be in order particularly if future RH-315 tolerance proposals include poultry feed items.

7. Additional data pertaining to soil persistence in a variety of soil types.

Detailed Considerations

Proposed Use

The requested experimental permit would allow the use of 8650 lbs. of the 75% wettable powder (WP) formulation (equivalent to 6488 lbs. active ingredient) in various geographical areas. The proposed experimental acreages and amount of active ingredient to be applied to the experimental crops are as follows: alfalfa, 2750 acres (4800 lbs. active); lettuce, 1050 acres (1575 lbs. active); Southern turf (Bermuda grass), 140 acres (140 lbs. active).

For control of certain broadleaf weeds and annual grasses in alfalfa, clover, lespedeza, trefoil, and vetch, RH-315 is to be applied in a 75% WP formulation at rates of 0.75-2.0 lbs. act/A. Application to established alfalfa is to be made during the dormant season (September to February), while application to newly-planted alfalfa, clover and related small-seeded legumes is to be made postemergent to the crop and either pre-emergent or early postemergent to the weeds.

Treated fields are not to be harvested or grazed for 90 days after application of 1.5-2.0 lbs. act/A (45 days if dosage is <1.5 lbs act/A).

For control of certain annual grasses and broadleaf weeds in lettuce, RH-315 is to be applied at rates of 1-2 lbs act/A. Application (soil incorporation) is to be made just prior to planting in areas where furrow irrigation is employed, and just after planting (surface treatment) in areas where natural rainfall or overhead irrigation is the source of water.

There is also a non-food use involving application of 0.75-1.5 lbs act/A to Bermuda grass (Southern turf) lawns and golf courses for annual bluegrass control.

Nature of the Residue

Three adjuvants are utilized in the 75% WP formulation. All except [redacted] However, an exemption from the requirements of a tolerance for this surfactant has been proposed.

Plant Metabolism

RH-315 is readily absorbed from the soil by the roots of the subject crops and translocated throughout the entire plant. Absorption from the leaf also occurs, but to a lesser extent.

The metabolism of RH-315 in alfalfa was studied under both field and

Inert ingredient information may be entitled to confidential treatment

greenhouse conditions (See Figure 1). Established alfalfa was treated with C^{14}-carbonyl labeled RH-315 at the maximum recommended rate of 2 lbs act/A. Samples were obtained at intervals of 17, 50, and 112 days after treatment. Total radioactive residues (fresh weight basis) were 168 ppm at 0 days, and 25.5, 1.76, and 0.50 ppm at the intervals discussed above. At intervals up to 50 days, the majority of the C^{14} residue (>95%) was methanol-extractable; for the 112 day samples, only 54% of the C^{14} residue was methanol--extractable (76% was acetone-extractable). Evidently an insoluble complex is formed as the interval increases.

Alfalfa samples were analyzed for RH-315 and metabolites at the above-mentioned intervals. Plant extracts were subjected to various TLC systems; the various metabolites were separated, identified, and quantitated.

At the 17 day interval, about 99% of the residue was identified as the parent RH-315 (about 25 ppm on fresh weight basis); traces of each of 8 metabolites were also present (<0.01 ppm). The results for the 50 and 112 day alfalfa samples, together with the identification of the metabolites, are given in Table 1.

Since the proposed harvest and grazing restrictions are 45 and 90 days, depending on the application rate (see Proposed Use) the nature of the residue at theearliest possible PHI's will be somewhat comparable to the 50 and 112 day results tabulated above. Therefore, with regard to the forage legumes, we estimate that at the shortest possible proposed interval (45 days), the residue will consist of the parent compound and total combined metabolites in the ratio of approximetely 2:1. At the 90 day interval, residues of the parent compound and Metabolite 1 will be present in slightly greater amounts than indicated in the preceeding table, while residues of Metabolites 2-6 and 8 will be slightly lower than shown. The level of Metabolite 7 remains almost constant over this 45-90 day interval. The analytical method will determine residues of all of these compounds.

The metabolism of RH-315 in plants is satisfactorily delineated for purposes of these temporary tolerances. For a future permanent tolerance, more positive identification of Metabolites 6,7 and 8 as determined in alfalfa would be desirable.

Metabolism in Soil and Mammals

The metabolism of RH-315 in soil and mammals (rats and cow) has also been investigated. It is apparent that the metabolic pathway is similar to that for plants, i. e., cyclization of the parent compound to M-1, followed by hydrolysis to M-2 or hydroxylation to M-3 (See Figure 2). The hydroxylated metabolites are further oxidized to carboxylic acids.

Analytical Methods

The residue method is a gas chromatographic procedure capable of determining the parent compound and its metabolites. Two versions of the method are presented; the earlier version (RAR Memorandum No. 560) was utilized only for determination of residues in alfalfa, while an improved, simplified method (RAR Memorandum No. 561) was employed for residue determinations for all subject crops, and meat and milk. The two versions of the method differ only in minor procedural matters;we will therefore limit our discussion to the improved, simplified version.

According to this method, the ground sample (or representative sample of milk) is mixed with sulfuric acid and methanol. The resulting slurry is refluxed, and the parent compound and its metabolites are all hydrolyzed to 3,5-dichlorobenzoic acid, which in turn is methylated to form the methyl ester (methyl-3,5-dichlorobenzoate). The methyl ester is then co-distilled from the reaction mixture with the methanol. The ester is extracted from the distillate with petroleum ether. Additional clean-up consists of a Florisil column deactivated with 4-5% water; the methyl ester is eluted with 20% benzene in hexane (v/v). The determinative step involves injection of an aliquot of the eluate into a gas chromatograph fitted with an electron capture dector (ECGC). The gas chromatograph may be fitted with either of two columns; the alternate column may be utilized for confirmatory purposes.

Representative chromatograms for the subject crops indicate that control values are negligible (<0.2 ppm for alfalfa and clover and <0.05 ppm for lettuce) with respect to the proposed tolerances. Control values for milk and tissues (liver, kidney, and muscle) are <0.005 ppm. Recovery data are summarized in the following table.

Substrate	Fortification (ppm)	Recovery (%)
Alfalfa hay	1.07-10.7	59-85(71 av)
Clover	1.1-2.2	65,71
Lettuce	0.008-0.2	53-108(82 av)
Milk	*0.005-0.04	61-89(76 av)
Muscle	0.02-1.1	55-83(70 av)
Liver	0.04-0.08	57,74
Kidney	0.08-0.56	65-80(71 av)
Fat	0.08-0.25	42-73(59 av)

* The 0.005 ppm fortification level results in a peak height of <0.5 in.

On the basis of representative chromatograms and control values, our estimate of the method's practical sensitivity for the substrates listed above are as follows: forage legumes, 0.2 ppm; lettuce, 0.05 ppm; milk,

0.01 ppm; tissues, 0.02 ppm (0.04 ppm for fat). (Residues in the 0.005-0.01 ppm range can be detected in milk, but cannot be quantitated accurately.)

No data with regard to method specificity have been presented. However, with the use of the alternate GLC column and other potentially available techniques (e. g., p-values) the method's specificity is adequate for the purposes of this temporary tolerance. There are no compounds with established tolerances on the subject legumes and lettuce which would interfere because of hydrolysis to 3,5-dichlorobenzoic acid.

We conclude that the method is of adequate specificity and sensitivity for enforcement of the proposed temporary tolerances.

Residue Data

Alfalfa

The majority of the residue data for alfalfa reflect residues found in alfalfa hay. However, one study involving application of C^{14}-labeled RH-315 (2 lb act/A) indicates that total residues found in fresh alfalfa are about one-fourth those found in the air-dried hay at PHI's of 52 and 112 days; this is in line with the customary dry-down factor.

Residue data and decline curves for alfalfa hay indicate that there is a rapid initial decrease in residue levels up to about 100 days after application, followed by a very slow decrease thereafter.

Residues in alfalfa hay resulting from the proposed use ranged from 0.9 ppm (0.75 lb act/A, 50 day PHI) to 2.7 ppm (2 lbs act/A, 173 day PHI). The residue data and residue decline curves for alfalfa indicate that the proposed 90 day harvest and grazing restriction is adequate for applications of 1.5-2.0 lbs act/A, but that a 60 day harvest and grazing restriction (rather than the proposed 45 day interval) is necessary to insure that residues resulting from applications <1.5 lbs act/A would not exceed the proposed 3 ppm temporary tolerance. In the absence of adequate data for the other subject legumes, we recommend that the 60 day interval be extended to cover all the subject forage legumes for applications of ≥1.5 lbs act/A.

We consider the proposed 3 ppm tolerance adequate to cover residues of RH-315 and its metabolites (calculated as the parent compound) in or on alfalfa (green) and alfalfa hay resulting from the proposed use, provided the 45 day harvest and grazing interval for applications of <1.5 lb act/A is increased to 60 days.

Clover and other legumes

The residue data for clover reflects residues found in dried red clover from one Pennsylvania location involving applications of 0.75-3.0 lbs act/A (86 and 210 day PHI's). Residues ranged from 0.13 to 4.4 ppm; values which exceeded 3 ppm resulted from the exaggerated 3 lb act/A rate.

There are no residue data for lespedeza, trefoil and vetch. However, for purposes of this temporary tolerance we can extend the data for clover and alfalfa to include these legumes. For purposes of a permanent tolerance request, additional data for clover from other geographical areas and some data for lespedeza, trefoil and vetch will be necessary.

We conclude that the proposed 3 ppm tolerance is adequate to cover residues of RH-315 and its metabolites (calculated as the parent compound) in or on clover, lespedeza, trefoil and vetch resulting from the proposed use, provided the 45 day harvest and grazing interval for applications of ≤1.5 lbs act/A is increased to 60 days.

Lettuce

Residue data from several California locations and one Texas location were submitted. Residues resulting from the proposed use ranged from <0.05 ppm to 0.27 ppm at PHI's of 35-113 days, with the exception of results obtained in one Soledad, Calif. study. In this study (test 68-28), Calmar variety lettuce treated according to the proposed use had residues ranging from 0.48 to 2.6 ppm; five of a total of 12 samples had residues exceeding the 1 ppm proposed temporary tolerance.

The petitioner states that this series of Calmar lettuce samples did not appear normal, i. e., the leaves were smaller than normal and roots appeared abnormal. Also, the samples contained dirt; no information is given with regard to washing of samples prior to analysis. In light of the above, we consider the excessive values reported in this study atypical.

We consider the proposed 1 ppm tolerance adequate to cover residues resulting from the proposed use in lettuce. For purposes of a permanent tolerance request, we will need additional residue data from other geographical areas and for the Calmar variety reflecting PHI's ≤40 days.

Meat, Milk, Poultry and Eggs

Feeding studies involving dairy cows and rats have been conducted to

determine the propensity for transfer of RH-315 residues (and metabolites) to meat and milk. One study utilized a single lactating Guernsey cow which was fed C^{14}-carbonyl labeled RH-315 at different levels for several time intervals. After a control period of 7 days, the labeled material was fed at the 0.2 ppm level (based on daily feed ration of 45 lbs) for 14 days, 1.0 ppm for 14 days and 5.0 ppm for 14 days. Samples of milk were collected daily throughout all dosage periods, representative samples of urine and feces were also collected during each test period.

No C^{14} residues were detected in the milk samples from the 0.2 ppm feeding level by a method with claimed sensitivity of 0.009 ppm. C^{14} residues in milk were detected in two of the 14 samples from the 1 ppm feeding level (0.009 and 0.013 ppm). In the final feeding period (5 ppm), C^{14} residues were detected in all daily milk samples, and ranged from 0.015 to 0.044 ppm (0.028 ppm av).

Analyses of urine and feces from each test period indicated that the majority of the radioactivity was eliminated in the urine (about 80%); about 10-15 % appeared in the feces.

The animal was sacrificed after the feeding was terminated (42 days total feeding), and various tissues analyzed for C^{14} residues. No detectable residues were found in samples of fat and bone (method sensitivity, 0.13 ppm), or in muscle, heart, brain, tripe, tongue, lung and blood samples (method sensitivity, 0.06 ppm). Total C^{14} residues found in liver averaged 0.58 ppm; kidney samples averaged 0.11 ppm. Residues in these organs would be expected since they play a major role in metabolism and excretion, and since there was no withdrawal period.

Balance studies in rats also indicated rapid elimination of carbonyl-labeled C^{14} RH-315. Two female rats were fed at the 1000 ppm level (based on total diet) for 6 days. Over 95% of the material administered was recovered; of this, more than 99% was eliminated in the urine and feces within 192 hours (end of study). This essentially complete elimination indicates that RH-315 and its metabolites have very little propenisty for storage in animal tissues.

Another study was conducted in which dairy cows were fed alfalfa hay bearing field-aged residues arising from RH-315. Four groups of three cows each were involved; each group was fed alfalfa hay bearing total residues determined as RH-315 of 0, 0.9, 2.3, 4.6 and 9.9 ppm (0,0.7, 1.8,3.5, and 7.5 ppm based on total ration) for periods of 16-30 days after a 7 day control period. The group fed at the 0.7 ppm level for 21 days was subsequently fed at the 3.5 ppm level for 16-30 days. Milk was sampled daily throughout the test periods; urine and feces samples were taken on the 15th day. The animals were sacrificed upon termination of feeding; liver, kidney, muscle, and fat samples were taken. The method discussed under Analytical Method was used for all milk, tissue, urine and feces analyses.

No residues were detected (method sensitivity, 0.005 ppm) in the milk samples from the 0.7 ppm feeding level. Of the 37 milk samples reflecting the 1.8 ppm feeding level, 34 had no detectable residues, while 3 samples from one cow had residues ranging from 0.005-0.007 ppm. The milk of cows fed at the 3.5 ppm level (after being fed at the 0.7 ppm level) had residues ranging from <0.005-0.01 ppm (2 of 38 samples had residues reported as 0.01 ppm). Milk from the group fed at the highest level (7.5 ppm) had residues ranging from 0.005-0.015 ppm; the majority of the samples had residues of 0.01 ppm or more. Residues in milk reached a plateau 2-3 days after feeding began. Residues are not concentrated in milk fat; analyses of skim milk from several test periods showed residue levels comparable to those in the whole milk.

Results of analyses of urine and feces samples were similar to those of the C^{14} study discussed above.

Analyses of selected tissues representing all feeding levels showed no detectable residues in various muscles (method sensitivity, 0.02 ppm) or fat from several locations (method sensitivity, 0.04 ppm). Residues of RH-315 and metabolites in liver ranged from 0.02-0.05 ppm at the 1.8 ppm feeding level, from 0.06-0.08 ppm at the 3.5 ppm feeding level, and from 0.12-0.17 ppm at the 7.5 ppm feeding level. Residues in kidney samples were $\leq$ 0.02 ppm at the 1.8 ppm feeding level, <0.02-0.04 ppm at the 3.5 ppm feeding level, and 0.03-0.04 ppm at the 7.5 feeding level.

If we assume that the entire diet of livestock consists of alfalfa hay bearing residues at the 3 ppm proposed tolerance level, residues approaching 0.01 ppm will transfer to milk. Residues in liver and kidney generally would not exceed 0.1 ppm, but due to the metabolic and excretory functions of these organs and variations between animals, residues could approach 0.2 ppm. There is no reasonable expectation of finite residues in other edible tissues. Therefore, we conclude that this is a Section 120.6(a)(1) situation with respect to milk, liver, and kidney. Other edible tissues fall into Section 120.6(a)(3).

We consider the proposed milk and liver and kidney tolerances adequate to cover residues transferring to these items as a result of the ingestion by livestock of feed items bearing residues of RH-315 and its metabolites at 3 ppm.

Mash poultry rations commonly include 5% dehydrated alfalfa meal. No poultry feeding study was submitted. Therefore, we are unable to determine whether or not finite residues would be present in the

meat of poultry, or in eggs, as a result of feeding alfalfa and alfalfa hay or its derivatives (chiefly alfalfa meal). Since alfalfa meal usually comprises only 5 percent of the diet and since the studies with the rat, milk and meat show that RH-315 does not store in the usual sense, residues in poultry tissue and eggs, if any, would be very low. A poultry feeding study would be in order, particularly if future RH-315 tolerance proposals involve additional poultry feed items.

Soil Persistence

The metabolism of RH-315 in soil has been discussed (see Nature of the Residue). Laboratory, greenhouse, and field studies with regard to the residual life of RH-315 in soil have been conducted using the C^{14}-carbonyl labeled compound. The half-life of RH-315 in laboratory soil is about 35 days at moderate temperatures; under greenhouse conditions (warm soil) it was reduced to about 20 days. Under high temperature field conditions, the half-life was about 15 days. The half-life of RH-315 is also affected by soil moisture content; higher moisture contents increase the half-life. Leaching does not contribute significantly to loss of soil residues.

Total C^{14} residues in Pennsylvania soil treated with 2 lbs act/A ranged from 0.10-0.17 ppm 115 days from treatment after rototilling (1-6 in. sample depths). After 223 days, total C^{14} residues ranged from 0.05-0.16 ppm. Total soil residues from application of 1.5 lbs. act/A (Pennsylvania) ranged from 0.13 to 0.22 ppm at intervals of 206 and 360 days as determined by the GLC method.

Carrots and cabbage were grown from seed in the soil treated with C^{14}-labeled RH-315 (2 lb act/A). Planting was at the 115 day interval discussed above. At maturity, total C^{14} residues in the cabbage and carrots were 0.005 and 0.003 ppm, respectively.

We conclude that residues in follow-up crops resulting from the proposed uses of this temporary tolerance will not be a problem.

For a permanent tolerance request, we will need additional data pertaining to soil persistence in a variety of soil types.

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CC:
SC-13 SC-12 SC-1 SC-330 SC-300
SC-970 CS-40 PP #9G0821

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TABLE 1

COMPOUND	% of residue		**equiv. ppm	
	50 days	112 days	50 days	112 days
RH-315[3,5-dichloro-N-(1,1-dimethyl-2-propynyl)benzamide]	63.1	11.3	1.11	0.06
*M-1[2-(3,5-dichlorophenyl)-4,4-dimethyl-5-methyleneoxazolene]	0.7	0.3	0.01	<0.01
M-2[N-(1,1-dimethylacetonyl)-3,5-dichlorobenzamide]	0.7	3.1	0.01	0.02
M-3[2-(3,5-dichlorophenyl)-4,4-dimethyl-5-hydroxymethyloxazoline]	1.5	5.4	0.03	0.03
M-4[N-(1,1-dimethyl-3-hydroxyacetonyl)-3,5-dichlorobenzamide]	4.3	7.1	0.08	0.04
M-5[N-(1,1-dimethyl-2,3-dihydroxypropyl)3,5-dichlorobenzamide]	2.5	8.0	0.04	0.04
M-6 presently unidentified (a 3,5-dichlorobenzoic acid derivative)	4.1	6.9	0.07	0.03
M-7 presently unidentified (a 3,5-dichlorobenzoic acid derivative)	9.9	8.8	0.17	0.04
M-8[N-(1,1-dimethylacetic acid)-3,5-dichlorobenzamide]	8.1	25.1	0.14	0.12
or				
[N-(1,1-dimethyl-2-keto-2-carboxyethyl)-3,5-dichlorobenzamide] (TAM 35:42)				
TOTAL	95	76***		

* M=metabolite
** ppm calculated on fresh weight basis
*** 24% of radioactivity remained as acetone-insoluble residue