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

DEC 6 1984

9001AA

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378. Thiodicarb on Cotton and Soybeans. Evaluation of Amendment dated November 19, 1984 (Accession Numbers 134927, 134928, 134929, and 134930 - Recorded on RD Data Review Record, only).

FROM: Michael P. Firestone, Ph.D., Chemist
Tolerance Petition Section II
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

THRU: Charles L. Trichilo, Ph.D., Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

TO: Jay S. Ellenberger, Product Manager, No. 12
Insecticide-Rodenticide Branch
Registration Division (TS-767)

and

Toxicology Branch
Hazard Evaluation Division (TS-769)

Note: This review was expedited per the request of the Director of Registration Division, Mr. Douglas D. Campt (see memo of 11/27/84).

Introduction

Union Carbide Agricultural Products Company, Inc. has submitted this amendment, consisting of a cover letter dated 11/19/84 from J. S. Lovell of Union Carbide to J. S. Ellenberger of EPA, a GC-MS confirmatory method of analysis for acetamide in beef and poultry liver, and a request for a waiver of regulatory analytical method requirements for acetamide in milk and eggs (including a method of analysis for acetamide in milk and eggs; and a report concerning the determination and GC-MS confirmation of endogenous acetamide in milk and eggs).

Background information relating to the proposed use of thiodicarb on cotton and soybeans can be found in RCB's review of 6/12/84 (A. Smith memo) and the OPP Director's (S. Schatzow) memo of 10/9/84 re: the subject petitions.

In RCB's 9/24/84 review of the 8/30/84 amendment to PP#0F2413/FAP#0H5275/PP#3F2793/FAP#3H5378, the following conclusions were reached:

RCB again defers to TOX on the toxicological significance of acetamide. If TOX concludes that residues of acetamide are not toxicologically significant in relationship to the proposed uses on cotton and soybeans and, thus, would not need to be regulated, RCB recommends for establishment of the proposed tolerances on cotton and soybeans.

If TOX concludes that residues of acetamide should be regulated in conjunction with the proposed thiodicarb use on cotton and soybeans, the following conclusions will hold true:

1. RCB will request method trials of all analytical procedures submitted with this amendment.

2a. Previously submitted metabolism data indicate that the acetamide:acetonitrile ratio in milk may be as low as about 1:4.

RCB concludes that the 1:4 conservative ratio is appropriate. Additional data are needed before RCB could conclude that the acetamide:acetonitrile ratio would always be 1:800 or greater.

Assuming a ratio of 1:4, the required detection limit for acetonitrile in milk would be 0.14 ppm, not 17 ppm as calculated by the petitioner using a ratio of 1:800. However, the submitted method which analyzes for acetonitrile in milk has a stated lowest limit of reliable measurement of 1.3 ppm. Thus, this method may require revisions to increase its sensitivity based upon the outcome of the above requested method trial.

2b. Previously submitted metabolism data indicate that the minimum acetamide:acetonitrile ratio in egg white is approximately 1:3.

Assuming this ratio, the required detection limit for acetonitrile in egg white would be 0.32 ppm (0.108 ppm X 3). The submitted analytical method has a reported lowest limit of reliable measurement for acetonitrile in egg white of 0.285 ppm. Thus, this method should have adequate sensitivity.

3. The submitted methods for detection of acetamide in beef and poultry liver have reported lowest limits of reliable measurement (0.77 ppm and 0.40 ppm, respectively) at or below the Sm values calculated by the petitioner (1.1 ppm - beef liver, 0.40 ppm - poultry liver), or TOX (1.84 ppm - beef liver, 0.65 ppm - poultry liver).

4. With regard to the petitioner's claim that endogenous acetamide is present in animal commodities, RCB reiterates its previous remarks that when these data are submitted, an evaluation will be made, and appropriate conclusions relating to endogenous acetamide can then be reached (see R. Schmitt/A. Smith memo of 9/7/84 re: PP#0F2413/FAP#0H5275). The confirmatory GC/MS method(s) discussed below should be useful in determining whether the apparent acetamide residue in liver control samples, and possibly milk, is actually acetamide or interfering GLC peaks. The confirmatory method(s) will not resolve whether any acetamide which may be found in control samples is a result of normal metabolic processes, or results from other environmental sources or laboratory contamination.

5. The petitioner has stated that a GC/MS confirmatory method, currently under development, is expected to be submitted to EPA within 2 months. RCB awaits the submission of the GC/MS confirmatory method. It is unclear whether the confirmatory method(s) are for acetamide and/or acetonitrile in liver, milk, and eggs.

Present Considerations

RCB continues to defer to TOX as the toxicological significance of acetamide in animal commodities resulting from the proposed use of thiodicarb on cotton and soybeans.

Although TOX has not yet presented its conclusions re: acetamide, RCB has requested method trials be performed on the analytical procedures submitted in Union Carbide's 8/30/84 amendment to the subject petitions (see M. Firestone memo of 10/26/84).

RCB can not comment further on conclusions 2a, 2b, and 3 re: the adequacy of the submitted methods for analysis of acetonitrile in milk and egg whites and acetamide in beef and poultry liver, until results of the method trial have been received from EPA's method trial unit (BUD).

In the present amendment, the petitioner has responded to conclusions 4 and 5 of RCB's 9/24/84 memo (see above). The petitioner's latest submission will be evaluated below.

GC-MS Confirmatory Methods for Analysis of Acetamide

a. Beef and Poultry Liver

The petitioner has submitted a procedure entitled "Confirmation Method of Analysis for Acetamide in Beef and Poultry Liver" dated 11/14/84.

Acetamide residues are quantitatively determined according to the techniques discussed in RCB's last review of the subject petition (see M. Firestone memo of 9/24/84). In brief, acetamide is extracted from liver with acetone, cleaned up by silica gel column chromatography, and analyzed by capillary gas-liquid chromatography using a nitrogen-phosphorus specific detector.

The same extracts are then qualitatively analyzed by GC-MS using selected ion monitoring (SIM) of the chromatographic peak with the retention time of the acetamide standard. In the SIM mode, the mass spectrometer does not scan over the entire mass range since only one compound is of interest. In the case of acetamide, the mass spectrum consists of two concerned peaks, mass 44 and 59. Since carbon dioxide also has a mass of 44, the ion of choice is mass 59, the ion representing the molecular weight of acetamide.

Fortification-recovery data using the nitrogen-phosphorus detector (GC method) and GC-MS confirmatory results are presented below:

Sample	Fortification (ppm)	Found (ppm)	GC-MS Confirmation	%Recovery*
Beef Liver	0	0.06	No	----
"	1	0.92	Yes	86
"	2	2.23	Yes	109
"	4	4.45	Yes	110
"	0	0.04	No	---
"	1	1.16	No	112
"	2	1.91	No	93
"	4	3.24	Yes	80
Poultry Liver	0.0	0.08	Yes	---
"	0.4	0.51	Yes	108
"	0.8	0.94	Yes	108
"	1.2	1.31	Yes	103
"	0.0	0.11	Yes	---
"	0.4	0.54	Yes	108
"	0.8	0.83	Yes	90
"	1.2	1.19	Yes	90

average = 100%

*% Recoveries corrected for blank values

The GC-MS data for poultry liver adequately demonstrate the validity of this method for confirmatory purposes. The two blank values (0.08 ppm, 0.11 ppm) indicate that acetamide might be endogenously present in poultry liver at a level of approximately 100 ppb. However, since the poultry liver samples were purchased at local grocery stores, RCB does not know the history of the samples, i.e., RCB can not conclude as to what the source(s) of acetamide are.

GC-MS was able to confirm the presence of acetamide in only half the beef liver samples. According to the petitioner:

"This was probably due to concentration of acetamide in the final extracts. Careful concentration of the extracts should allow for results comparable to those of the poultry liver to be obtained."

The purchased beef liver samples had background (possible endogenous) levels of 40 ppb and 60 ppb. Based on the submitted data, it is not possible for RCB to declare that acetamide is endogenous to cattle meat (liver) since the cattle liver samples were purchased at a local grocery store, i.e., there is no known history of the samples.

However, the results indicate (see the above table) that the GC-MS method submitted in this amendment is adequate for confirmatory purposes with regard to analysis of acetamide residues in animal tissues (i.e., liver), pending BUD's assessment of the extraction procedures.

b. Milk and Egg Whites

The petitioner has submitted a method for the quantitative determination of acetamide in milk and eggs entitled "Method of Analysis for Acetamide in Milk and Eggs" dated 11/15/84, as well as a confirmatory method entitled "Determination and GC/MS Confirmation of Endogenous Acetamide in Milk and Eggs" dated 11/14/84.

In brief, acetamide is extracted from milk or egg whites with acetone. Interfering co-extractives are removed by using activated charcoal and a cation-exchange column. Detection and quantification are performed by capillary gas-liquid chromatography using a nitrogen specific detector. The same extracts are then qualitatively analyzed by GC-MS using the selected ion monitoring technique.

The petitioner cites several problems including:

1. Difficulty in concentrating acetamide residues due to its affinity for water.

2. The eluting solvent used for the cation-exchange chromatography step presented potential problems with the capillary gas-liquid chromatography.

3. The GLC capillary column quickly loses efficiency.

The following fortification-recovery data are presented in the 11/15/84 and 11/14/84 reports, respectively:

> "The average recovery of acetamide from eight milk samples fortified at 96 to 800 parts per billion was 77 percent with a standard deviation of 13 percent. The average recovery of acetamide fortified at 400 and 800 ppb was 79 percent with a standard deviation of 11 percent."
>
> "The average percents recovered of the acetamide added after correcting for the untreated controls (0 ppm) was (sic) 66% for milk and 71% for eggs."

So that RCB's files are complete, the petitioner should submit all raw data and calculations used to generate the fortification-recovery data cited above, as well as the residue data re: endogenous acetamide in milk and eggs which are cited in the following section of this review.

Data relating to the GC-MS confirmation of "endogenous" residues in milk and eggs are discussed in the next section of this review.

Residue Data re: "Endogenous" Acetamide in Milk and Eggs

Using the methods described in the last section of this review, the petitioner assayed milk and egg samples purchased from grocery stores in twelve states (FL, NC, RI, PA, IN, MD, TX, MN, CO, WA, CA, and AZ) for the presence of acetamide.

The reported results (corected for acetamide recoveries of 66% and 71% from milk and eggs, respectively) are presented below:

State	Milk Acetamide (ppb)	GC-MS Confirmation	Eggs Acetamide	GC-MS Confirmation
FL	441	Yes	71	N/A*
NC	507	Yes	194	Yes
RI	410	Yes	154	N/A
PA	433	Yes	143	Yes
IN	413	Yes	180	Yes
MO	328	Yes	114	N/A
TX	274	Yes	128	Yes
MN	350	Yes	126	N/A
CO	433	Yes	119	N/A
CA	503	Yes	248	Yes
AZ	388	Yes	358	N/A
WA	---	---	178	Yes
Average =	407 ± 70		Average =	168 ± 75

* N/A - not analyzed

The petitioner's results do support the assertion that acetamide is endogenous in milk and eggs. However, the history for these samples is unknown, i.e., since these samples were purchased at local grocery stores, RCB cannot conclude as to what the source(s) of acetamide are. Perhaps a carefully controlled study could provide this information.

A discussion is presented that acetamide could be formed by the reaction of acetyl-coenzyme A (an activated acetate involved in many biochemical reactions in animals including fatty acid metabolism) with ammonia (produced by the oxidative deamination of amino acids):

$$CH_3CO{\sim}SCoA + NH_3 \longrightarrow CH_3CONH_2 + CoASH$$

A discussion is also presented concerning the maximum amounts of secondary acetamide residues in animal commodities resulting from the ingestion of methomyl-treated feeds. The petitioner calculates these values to be 5.4 ppb in eggs and 25 ppb (based on single-dose cow feeding study) in milk (note: RCB predicts this value to be as great as 60 ppb based on the calculations presented on p.9 of M. Firestone's memo of 9/24/84 re: subject petitions). Thus, the maximum possible acetamide residue levels in milk and eggs resulting from established methomyl tolerances are well below the reported "endogenous" levels of 407 ppb and 168 ppb, respectively.

In conclusion, acetamide appears to be endogenous in milk and eggs. The source(s) for endogenous acetamide are unknown.

Other Considerations

An International Residue Limit Status sheet is attached. Since there are no Codex, Canadian or Mexican tolerance/limits established for thiodicarb on soybeans or cotton, there are no compatibility problems.

Conclusions

1. RCB continues to defer to TOX as to the toxicological significance of acetamide residues in meat, milk, and eggs resulting from the proposed uses of thiodicarb on cotton and soybeans.

2. Methods for the determination of acetonitrile in milk and eggs (acetonitrile is a marker compound for acetamide) and acetamide in beef and poultry liver have been submitted to EPA's method trial unit (BUD). RCB awaits the results of the method trials before commenting on the adequacy of these procedures for possible enforcement purposes.

8

3. The GC-MS method for determination of acetamide in beef and poultry liver submitted in this amendment is adequate for confirmatory purposes with regard to the analysis of animal tissues pending BUD's assessment of the extraction procedures.

4. Based on analysis of two unfortified (blank) poultry liver samples, there is an indication that acetamide is endogenous at a level of approximately 100 ppb. However, since the samples were purchased at local grocery stores, RCB can not conclude as to what the source(s) of the acetamide are. Perhaps a carefully controlled study could provide this information.

5. So that RCB's files are complete, the petitioner should submit all raw data and calculations used to generate the fortification-recovery data and residue data for analysis of acetamide in milk and eggs submitted in this amendment.

6. The petitioner assayed milk and egg samples purchased from grocery stores in twelve states. The results (corrected for acetamide recoveries of 66% and 71% in milk and eggs, respectively) reportedly show that "endogenous" acetamide levels range from 274 to 507 ppb (ave. = 407 ppb) in milk and from 71 to 358 ppb (ave. = 168 ppb) in eggs. The source(s) for endogenous acetamide in animal commodities have not been positively identified.

7. The petitioner presents a series of calculations which indicate that the established uses of methomyl could not account for more than a maximum of approximately 10% of the reported endogenous acetamide levels in milk and eggs.

8. An International Residue Limit Status sheet is attached. Since there are no Codex, Canadian, or Mexican tolerances/limits, there are no compatibility problems.

cc:R.F., Circu, Reviewer, TOX, EAB, EEB, PP#0F2413/FAP#0H5275, PP#3F2793/FAP#3H5378, FDA, Robert Thompson
RDI:J. Onley:12/03/84:R.D. Schmitt:12/03/84
TS-769:RCB:CM#2:RM810:X7484:M. Firestone:wh:12/05/84

INTERNATIONAL RESIDUE LIMIT STATUS

OF 2413/OH5275
3F 2793/3H5378

CHEMICAL: thiodicarb

PETITION NO.:

CCPR NO.:

REVIEWER: Michael P. Firestone

11/25/84

Codex Status

☑ No Codex Proposal Step 6 or above

Residue (if Step 9):

Proposed U.S. Tolerances

Residue: thiodicarb and its metabolite methomyl

Crop(s)	Limit (mg/kg)

Crop(s)	Tol. (ppm)
soybeans	0.2
soybean hulls	0.8
cottonseeds	0.4
cottonseed hulls	0.8

CANADIAN LIMIT

Residue:

Crop(s)	Limit (ppm)
none	

MEXICAN TOLERANCIA

Residue:

Crop(s)	Tolerancia (ppm)
none	

Notes:

10