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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378. Thiodicarb on Cotton and Soybeans. Amendment of August 30, 1984 (Accession No. 129074, 129075, 129077, and 129080).

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THRU: Charles L. Trichilo, Ph.D., Chemist
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and

Toxicology Branch
Hazard Evaluation Division (TS-769)

Note: This review was expedited per the request of Mr. Douglas D. Campt, Director of Registration Division (see memo of 9/12/84).

Introduction

Union Carbide Agricultural Products Company, Inc. has submitted this amendment, consisting of a cover letter from J. S. Lovell of Union Carbide to J. S. Ellenberger of EPA, information regarding the oncogenic potency of thiodicarb and the acetonitrile/acetamide ratio in milk, and methods of analyses for acetamide in beef and poultry liver, and acetonitrile in milk and eggs.

Background information relating to the proposed use of thiodcarb on cotton and soybeans can be found in RCB's review of the 6/12/84 amendment to PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378 (see A. Smith memo of 6/12/84). In that memorandum, RCB made a favorable recommendation contingent upon resolution of questions concerning the metabolites acetonitrile and acetamide, i.e., RCB indicated that if residues of these two metabolites were found to be of toxicological concern and should be regulated, then validated analytical methodology would be required.

RCB waits for an answer to a deference to TOX concerning whether or not acetamide and/or acetonitrile are toxicologically significant and should be regulated.

Detailed Considerations

Sensitivity of Methods (SOM) Policy

In the cover letter (EPA Correspondence No. 318-84), the petitioner states the following:

"Using FDA's procedures, the acetamide level in the diet, So, which corresponds to a theoretical maximum lifetime oncogenic risk of 1 X 10^{-6} is 40 ppb. That is, the entire human diet could contain 40 ppb, or 60 ug in a 1500 gm/day diet as used by FDA in its calculations. Following FDA's procedures for calculation of allowable levels in target tissues, Sm, we find that Sm = So/T, where T is the fraction of the total daily diet represented by an individual edible tissue. These fractions and the resulting Sm for edible tissues are as follows:

Edible Tissue	T	Sm
Milk	1	So, or 40 ppb
Meat	1/3	3 So, or 120 ppb
Eggs	1/3	3 So, or 120 ppb . ...

Allowable levels (Sm) in each edible tissue group are as follows:

Food	Food Intake	Allowed Acetamide Intake	Sm in ppb
Milk	900 gm	20 ug	22.2
Meat	300 gm	20 ug	66.7
Eggs	300 gm	20 ug	66.7
Total	1500 gm	60 ug	

As can be seen in this table, total daily allowed intake of acetamide at these levels does not exceed the So of 60 ug/day. For milk, Union Carbide has developed a method for acetonitrile as a marker residue for acetamide based on a lowest observed ratio of acetonitrile:acetamide of 800:1. In the July 27, 1984 meeting, EPA expressed some reservation with this choice based on minimal substantiating data to definitively demonstrate the quantitative relationship of these two metabolites of thiodcarb. Based upon the considerations discussed in the attached memorandum from R. W. Heintzelman to me dated

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August 10, 1984, we feel it will be impractical, if not impossible, to provide more definitive data, and that such data is unnecessary based on the extremely low levels of acetamide that might be present in milk, if present at all. Thus, we feel the 800:1 ratio is conservative and, if in doubt, is more likely to overestimate the presence of acetamide than to underestimate it. For these reasons, an analytical method is submitted for acetonitrile at 17 ppm as a marker residue capable of indicating presence of acetamide in milk at a level of 22.2 ppb or greater. (Recent information reveals that endogenous acetamide is present in milk at ~ 150 ppb. Since experimental data indicates [sic] that maximium theoretical acetamide residues in milk resulting from the use of thiodicarb on cotton and soybeans is 0.006 ppb, it would be entirely appropriate and in accordance with FDA policy for EPA to waive the requirement for an analytical method for acetamide in milk).

For eggs, Union Carbide has developed a method for acetonitrile as a marker residue for acetamide based on the observed quantitative relationship of acetonitrile to acetamide residues in eggs of 4.5:1. Thus, a method is submitted for acetonitrile in eggs at 0.3 ppm (4.5 X 66.7 ppb).

For meat, Union Carbide has chosen the liver as a target tissue because earlier data showed residue levels, if present, would be higher in liver than any other tissue. Based on food intake, a method for acetamide in liver could have a sensitivity 50X that of the allowable residue in meat, since liver consumption. However, in beef the liver has been shown to contain residues as low as 17X that found in meat, and in poultry the minimum concentration is 6X. Thus, the appropriate acceptable level for acetamide in beef liver is 17X that in meat, or 1.1 ppm. In poultry, the Sm for acetamide in liver is 6X that allowed in poultry muscle tissue, or 0.4 ppm.

Confirmatory method: We are currently developing a GC/MS confirmatory method which we expect to submit to EPA within 2 months. When validating the above methods, the laboratory may wish to save the final extracts of samples for use in validation of the confirmatory method.

In summary, Union Carbide has developed and submits herewith the following method which comply with the requirement of FDA in its SOM policy.

Target Tissue	Marker Residue	Detection Limit
Milk	Acetonitrile	17 ppm
Beef Liver	Acetamide	1.1 ppm
Poultry Liver	Acetamide	0.4 ppm
Eggs	Acetonitrile	0.3 ppm ."

RCB's Comments/Conclusions re: SOM Policy

a. Milk and Eggs

In RCB's review of an 8/7/84 letter from J.S. Lovell of Union Carbide regarding, PP#0F2413/FAP#0H5275 (see R. Schmitt/ A. Smith memo of 9/7/84), it was indicated that the use of acetonitrile as an alternative marker residue is contingent upon the submission of data by Union Carbide which establishes a quantitative relationship between acetonitrile and acetamide levels.

^{14}C-Thiodicarb metabolism data previously submitted in PP#0F2413/FAP#0H5275 is summarized below.

Commodity	Feeding Level (ppm)	Residue Level (ppm)		Acetamide: Acetonitrile Ratio
		Acetamide	Acetonitrile	
milk	(7.02 mg/kg)[a]	--------	---------	1:4 - 1:9
milk	10[b]	ND[c]	0.051	----------
"	30[b]	ND[c]	0.263	----------
"	100[b]	0.001 - 0.010[d]	0.814	1:80 - 1:800
egg white	15.4[e]	0.009	0.037	1:4.1
"	28.6[e]	0.024	0.142	1:5.9
"	102[e]	0.065	0.269	1:4.1
"	15.4[b]	0.007	0.024	1:3.4
"	28.6[b]	0.026	0.082	1:3.2
"	102[b]	0.304	0.210	1:6.2

a) single oral dose feeding study, ratio based on reported percentages (4%:17-37%)
b) 21-day continuous feeding study
c) ND = non-detectable
d) value originally reported as <0.01 ppm. J.V. Rodricks (8/14/84) report "Evaluation of Thiodicarb Under FDA's Sensitivity of Methods Procedure" uses a value of 0.001 ppm
e) 14-day continuous feeding study

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The above results for egg white indicate a minimum ratio of approximately 1:3 (not 1:4.5). Assuming this ratio, the required detection limit for acetonitrile in egg white would be 0.32 ppm (0.108 ppm X 3), rather than the reported 0.3 ppm. (Note: TOX has indicated - see C.Chaisson memo of 9/21/84 re: PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378 - that the value of Sm for acetamide in eggs is 108 ppb, versus 66.7 ppb as calculated by the petitioner.)

The submitted analytical method - see following discussion - has a reported lowest limit of reliable measurement for acetonitrile in egg whites of 0.285 ppm, which translates to an acetamide level of 95 ppb in eggs.

With regard to the acetamide:acetonitrile ratio in milk, the value reported from a continuous feeding study can be as high as 1:800. RCB considered the data on which this ratio is based to be very scanty. This ratio is apparently based on the analysis of one sample of milk from the 21 day cow feeding study, thus, the petitioner was informed at a 7/27/84 meeting that additional data on the ratio may be needed. The petitioner subsequently informed RCB (memorandum of Telecon 8/6/84, R. D. Schmitt) that a new goat feeding study was bring initiated to better define this ratio. As part of this current submission, the petitioner now indicates that the new goat study will not be carried out. Since no new data on the ratio of acetamide to acetonitrile in milk is forthcoming as anticipated, RCB has reexamined the available data on the acetamide:acetonitrile ratio in milk. In a report submitted with PP#0F2413/FAP#0H5275 concerning the metabolism of ^{14}C-thiodicarb in a lactating cow fed a single oral dose of 7.02 mg/kg, (approximate 350 ppm in the diet for one day), the following narrative was presented:

"^{14}C-residues in the milk appeared in the first milking 6 hours after treatment and peaked at 18 hours. Subsequently they declined with an estimated half-life of one day. Most of the milk radioactivity was incorporated into natural products (lactose, lipids, lactalbumin and casein). ^{14}C-lactose (specific activity 160,000 dpm/g) was isolated by standard biochemical techniques and its identity confirmed by melting point and NMR analysis. ^{14}C-acetonitrile (17-37% of milk radioactivity) was isolated by distillation and characterized by isotope dilution and glc analysis. The organic residue contained less than 4% of the milk radioactivity and the analysis showed mainly ^{14}C-acetamide."

The above data indicate that the acetamide:acetonitrile ratio can be as low as about 1:4 (4%:17%) following a high level, single oral dose of thiodicarb. Given the wide variability in this ratio from 800:1 to 4:1, RCB concludes that the 4:1 ratio is appropriate. Additional data are needed before RCB could conclude that the acetonitrile:acetamide ratio would always be 800:1 or greater.

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Assuming a ratio of 1:4, the required detection limit for acetonitrile in milk would be 0.14 ppm (0.036 ppm X 4), versus 17 ppm as calculated by the petitioner using a ratio of 1:800. (Note: TOX has indicated - see C. Chaisson memo of 9/21/84 re: subject petitions - that the Sm value of acetamide in milk is 36 ppb versus a value of 22.2 ppb as calculated by the petitioner.) However, the submitted analytical method - see following discussion - has a stated lowest limit of reliable measurement for acetonitrile in milk of 1.3 ppm, which would translate to an acetamide level in milk of 325 ppb.

With regard to the petitioner's claim that endogenous acetamide is present in milk approximately 150 ppb, RCB reiterates that when these data are submitted, an evaluation will be made, and appropriate conclusions, relating to endog enous acetamide reached (see R. Schmitt/A. Smith memo of 9/7/84 re: PP#0F2413/FAP#0H5275).

b. Meat

The following ^{14}C-thiodicarb metabolism data were presented in PP#0F0413/FAP#0H5275:

Acetamide Levels in Tissue of Lactating Cows After 21-Day Feeding of ^{14}C-Thiodicarb

Feeding Level (ppm)	Acetamide Residue Level (ppm)		Liver:Muscle Ratio
	muscle	liver	
30	0.007	0.166	1:24
100	0.040	0.677	1:17

Acetamide Level in Hen Tissue Six Hours Following Termination of ^{14}C-Thiodicarb Feeding at 102 ppm in Diet

Acetamide Residue Level (ppm)		Liver:Muscle Ratio
muscle	liver	
0.10	0.60	1:6

The petitioner has used liver:muscle ratios of 17:1 and 6:1 for the calculation of detection limits of acetmide in poultry and cattle liver, respectively. These values are suspect due to the extremely limited amount of data upon which they were based.

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Analytical Methodology

a. Acetamide in Beef Liver

The petitioner has submitted a method with the designation "Acetamide-NPD-Beef Liver." Briefly, acetamide is extracted from liver with acetone, cleaned up by silica gel column chromatography, and quantified by gas-liquid chromatography (fused silica capillary column coated with 0.25 um thickness of bonded Carbowax PEG 20M) using a nitrogen-phosphorus specific detector. The lowest limit of reliable measurement is reported at 0.77 ppm, below the Sm calculated by TOX (C. Chaisson memo of 9/21/84 re: PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378) for acetamide in beef liver (i.e., 1.8 ppm).

Control values reportedly ranged from 0.13 to 0.26 ppm (average of 7 samples = 0.21 ppm). Recoveries from cattle liver samples fortified at from 0.4 to 3.6 ppm acetamide reportedly ranged from 63 to 178% (average of 14 trials = 102 ± 30%) when corrected for a control value of 0.21 ppm.

RCB can not determine whether control GLC peaks represent endogenous acetamide or background interference.

b. Acetamide in Poultry Liver

The method of analysis submitted for acetamide in poultry liver is designated "Acetamide-NPD-Poultry Liver." This method is the same as that previously described for analysis of acetamide in beef liver. For poultry liver, the lowest limit of reliable measurement is reported at 0.4 ppm, which is less than the Sm calculated by TOX (C. Chaisson memo of 9/21/84 re: PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378) for acetamide in poultry liver (i.e., 1.1 ppm).

Control values reportedly ranged from 0.09 to 0.16 ppm (average of 5 trials = 0.11 ppm). When corrected for a blank value of 0.11 ppm, recoveries of acetamide from poultry liver reportedly ranged from 57 to 106% (average of 14 trials = 83 ± 14%) for fortifications ranging from 0.28 to 1.28 ppm.

RCB can not determine whether control GLC peaks represent endogenous acetamide or background interference.

c. Acetonitrile in Milk

The analytical method submitted for the detemination of acetonitrile in milk is designated "Acetonitrile-HECD-Milk." Briefly, this method involves acetonitrile extraction from milk with methanol, and quantification by gas-liquid chromatography (Porapak Q, 80/100 mesh) using a Hall electrolyitc conductivity detector operated in the nitrogen mode. The lowest limit of reliable measurement for acetonitrile in milk is reported at 1.3 ppm. This value is considerably above the required SOM policy detection limit of 0.14 ppm which is based on an acetamide:acetonitrile ratio in milk of 1:4 (see RCB's Comments/Conclusions re: SOM Policy) and a Sm value of 36 ppb as calculated by TOX (C. Chaisson memo of 9/21/84 re: PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378).

All (4) control values were reported as 0 ppm. Recoveries of acetonitrile from milk samples fortified at from 2.01 to 12.0 ppm reportedly ranged from 97 to 118% (average of 11 trials = 103 $\pm$ 6%).

d. Acetonitrile in Egg White

A method designated "Acetonitrile-HECD-Egg White" has been submitted for the determination of acetonitrile in egg white (albumen). This method is very similar to that described previously for analysis of acetonitrile in milk. In brief, acetonitrile is extracted with methanol and quantified by GLC using a Hall electrolytic conductivity detector. The lowest limit of reliable measurement for acetonitrile in albumen is reported at 0.285 ppm. This value is less than the required SOM policy detection limit of 0.32 ppm which is based on an acetamide:acetonitrile ratio in albumen of 1:3 (see RCB's Comments/Conclusions re: SOM Policy) and a Sm value of 108 ppb as calculated by TOX (C. Chaisson memo of 9/21/84 re: PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378.)

Control values reportedly ranged from 0 to 0.058 ppm (average of 4 trials = 0.0145 ppm). When corrected for a blank value of 0.014 ppm, acetonitrile recoveries from milk fortified at 0.235-1.01 ppm reportedly ranged from 51 to 83% (average of 15 trials = 70 $\pm$ 11%).

e. Confirmatory Method(s)

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.

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The confirmatory GC/MS method(s) 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.

Residue in Meat, Milk, Poultry and Eggs

Cottonseed, cottonseed hulls, soybeans, and soybean hulls may be fed to cattle and/or poultry.

The maximum likely ingestion levels, based on the proposed tolerance levels of 0.4 ppm (cottonseed), 0.8 ppm (cottonseed hulls and soybean hulls), and 0.2 ppm (soybeans) are as follows:

dairy cattle - 0.16 ppm
beef cattle - 0.25 ppm
poultry - 0.10 ppm

The following 21 day cattle feeding study submitted in PP#0F2413/FAP#0H5275 will be used to estimate secondary acetamide levels in cattle meat, meat by-products, and milk as a result of the proposed use on cotton and soybeans:

Acetamide Residue (ppm) in Cow Tissues and Milk

	Thiodicarb Feeding Level							
	10 ppm		30 ppm		100 ppm		30 ppm equivalent	
Tissue	Residue (ppm)	Adj.[a] (ppb)	Residue (ppm)	Adj. (ppb)	Residue (ppm)	Adj. (ppb)	Residue (ppm)	Adj. (ppb)
Liver	0.143	3.7	0.166	1.4	0.677	1.8	-----	------
Muscle	-----	---	0.007	0.1	0.040	0.1	-----	------
Milk	-----	---	-----	---	<0.01	<0.02	00.053[b]	0.3

a) Adjusted acetamide values (ppb) are based on a thiodicarb intake of 0.16 ppm for dairy cattle (milk) and 0.26 ppm for beef cattle (meat and mbyp)

b) Based on 30 ppm 21-day feeding level of acetonitrile (0.263) and the assumption that acetamide levels may account for up to 20% of the total acetamide plus aetonitrile residue in milk (i.e., acetamide:acetonitrile ratio of 1:4)

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Thus, the likely levels of secondary acetamide residues resulting from the proposed use in cattle would be 3.7 ppb in meat by-products, 0.1 ppb in meat, and 0.3 ppb in milk.

The following two chicken feeding studies submitted in PP#0F2413/FAP#0H5275 will be used to estimate levels of secondary levels in poultry meat, meat by-products, and eggs:

Acetamide Residue (ppm) Present 6 Hours After Termination of 21 Day Administration of 102 ppm Thiodicarb in the Diet of Poultry

Tissue	Acetamide Level (ppm)	Acetamide Level (ppb) Adjusted to 0.1 ppm Thiodicarb Intake
Liver	0.6	0.6
Breast	0.1	0.1

Acetamide Residue (ppm) Present in Egg White of Hens Fed Thiodicarb for 14 Days at 15.4, 28.6, and 102 ppm in the Diet

	Thiodicarb Feeding Level 15.4 ppm	28.6 ppm	102 ppm
Acetamide Level (ppm)	0.009	0.024	0.065
Acetamide Level (ppb) Adjusted to a Thiodicarb Intake of 0.1 ppm	0.06	0.07	0.07

Thus, the likely level of secondary acetamide residues resulting from the proposed use on cotton and soybeans would be 0.1 ppb in poultry meat, 0.6 ppb in poultry meat by-products, and 0.1 ppb in eggs.

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Conclusions

The following chart tabulates a comparison between the maximum expected level of secondary acetamide residues in meat (muscle), meat by-products (liver), milk and eggs, versus the Sm values calculated by the petitioner (Union Carbide) and TOX (C. Chaisson memo of 9/21/84 re: PP#0F2413/FAP#0H5275 and PP#3F2793/FAP#3H5378), and the petitioner's reported lowest limit of reliable measurement (LLRM):

Acetamide Residue Level (ppb)

Target Tissue	Expected	Sm(UC)	Sm(TOX)	LLRM
milk	0.3	22.2	36	325[a]
cattle liver	3.7	1134[b]	1836[b]	770
chicken liver	0.6	400[c]	648[c]	400
eggs	0.1	66.7	108	95[d]

a) based on analysis of acetonitrile and an acetamide: acetonitrile ratio of 1:4 in milk

b) based on a cattle liver:muscle abundance ratio of 17:1

c) based on a poultry liver:muscle abundance ratio of 6:1

d) based on a analysis of acetonitrile and an acetamide: acetonitrile ratio of 1:3 in albumen

It should be noted that although the method for determination of acetamide levels in milk has a reported LLRM greater than the Sm value calculated by the petitioner or by TOX Branch, all reported Sm values are much higher than expected levels of secondary acetamide residues.

Recommendations

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 soybean.

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 (0.65 ppm - beef liver, 1.1 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.

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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.

cc:R.F., Circu, Reviewer:TOX, EAB, EEB, PP#OF2413/FAP#0H5275
RDI:J. Onley:9/19/84:R. D. Schmitt:9/19/84
TS-769:RCB:CM#810:CM#2:X7484:M. Firestone:wh:9/21/84