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

MAY 11 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 279-3052, -3053. Command Herbicide. Review of Metabolism Studies and Field Trials Conducted as Follow-Up to Off-Target Incidents.

MRID Numbers	RCB Nos.
400700-00	2110
-01	2086
401236-01	2164
-02	
-03	
401474-01	
-02	
-03	
-04	

FROM: Lynn M. Bradley, Chemist
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

THRU: Andrew R. Rathman, Section Head
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

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Registration Division (TS-767C)

and

Toxicology Branch
Hazard Evaluation Division (TS-769C)

FMC Agricultural Chemicals Group submits studies on Command® which have been presented in a series of meetings with the Agency, held as follow-up to the off-target contamination incidents which occurred in 1986. A number of restrictive changes have been made to the Command label, and FMC has requested that the Agency review these studies for the purpose of making a more definitive assessment of the significance of any off-target incidents which might occur in the future.

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We will discuss the various metabolism studies and simulated spray drift studies, and estimate residue levels for alfalfa and for ruminants consuming treated alfalfa. TOX Branch should be consulted for evaluation of the possible toxicological significance of residue levels.

Some determination should be made concerning the legal status of crops which show bleaching due to off-target contamination. These aspects are beyond RCB's purview, in that action levels have not previously been established for spray drift but only for truly unavoidable contaminants. RCB suggests that the question of legitimizing residues from Command spray drift be referred to the FDA/EPA Liaison Group or the Policy Group.

Simulated Drift/Volatility (MRID #401474-02, -03)

In the summer of 1985, FMC conducted a field study which simulated off-site drift. This study was based on greenhouse tests, and in general confirmed the greenhouse results. Lima beans were more sensitive and onions more tolerant in the field than in the greenhouse. Part of this study was designed to evaluate direct application at rates ranging from 1 g/ha to 250 g/ha, and another part to study off-target effects.

In the simulated drift trial, crops were examined at 7, 15, and 30 days after treatment with 1, 4, 16, 63, or 250 g/ha. The growth stage at treatment is specified and is young seedling for all (<10 leaves). Radish was the most sensitive, showing >5% injury at the 1 g/ha rate at all 3 times. Lima beans and sunflowers showed initial (day 7) injury at 4 g/ha, with sunflowers recovering by day 15 and limas continuing to show injury throughout the test. Wheat, lettuce, tomatoes, cucumber, and broccoli (and lambsquarter, a weed) showed initial injury at 16 g/ha; tomato and cucumber were recovering by day 15, and the others by day 30.

Peanuts and corn showed injury on days 7 and 15, but had recovered by day 30. Rice, cotton, and onions showed initial injury at 250 g/ha; rice recovered by day 15 and cotton by day 30, while onions continued to show injury at day 30. Soybeans and peppers showed no injury at 250 g/ha, the maximum rate. None of the species was killed and all had begun recovering by the end of the test. Affected leaves were lost, however.

The other part of the study showed off-target injury in terms of relative distance from portions of the plot treated at 250 g/ha. Peanuts, rice, and cotton showed no injury while corn, wheat, and sunflowers showed injury at respectively increasing distance from the treatment zone, in the same direction as wind at application time. Several "weeds" were also tested, and showed injury at approximately the same distance as did sunflowers. Velvetleaf, by far the most sensitive "weed," showed injury even several feet into the wind.

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A follow-up report (June 9, 1986) on this same study reports analyses for Command of the mature crops harvested at appropriate time intervals (reported). Control and treated samples of all crops were analyzed; PHIs were 38-112 days. Only broccoli (PHI 60) showed detectable residues (0.02 ppm, limit of detection 0.11 ppm, method sensitivity 0.05 ppm). This demonstrates that injury levels do not correlate with command levels. Radish had a persistent interference, but FMC states that additional work-up "gave a good indication that the treated sample had very little or no FMC 57020 [Command] residue." The analytical method is referenced and acceptable recovery data are presented.

Both of these studies are submitted in the form of FMC internal memos (at RCB's request in our most recent meeting, February 12, 1987) in order to document the varying sensitivity of different crops.

Translocation Potential (MRID #401474-01)

This internal memorandum discusses additional evidence that Command is not translocated. This evidence is summarized below.

First, the low levels of radioactivity in mature soybeans from pre-plant soil application is taken to indicate that neither parent nor metabolites are translocated. This study was discussed in detail in our review of PP 4G2987 (L. Propst, 4/17/84), and again for PP 4F3128 (J. Worthington, 9/24/84). The tolerance for soybeans was accordingly set at method sensitivity. However, the forage data do indicate uptake from soil.

Second, the fact that perennials "often put out new growth . . . which is normal" after exposure to Command is taken to demonstrate absence of translocation. No studies are given to support this statement; apparently it comes from field observations.

Third, use as postemergent herbicide is generally unsuccessful, since new growth does not show symptoms. Again, we assume this is supported by field observations.

Fourth, in perennials having underground stems, new plants emerging along the rhizome show no symptoms. Again, we presume this is supported by field observations.

If we accept the evidence as presented, we could conclude that, as demonstrated in the simulated drift study, residues are not likely to translocate after foliar application to plant parts not directly contacted. We could extend this conclusion to state that subsequent alfalfa cuttings are not expected to contain Command residues (see discussion on alfalfa, below).

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Soybean Forage Metabolism (MRID #401236-01)

This study was conducted in 1982-3 and was not submitted or needed for the soybean tolerance request due to the grazing and feeding restriction imposed. It was presented in a meeting during the past year, and has been written up and submitted at our request.

Methylene-labeled Command [2-(2'-chlorophenyl)-^{14}C-methyl-4,4-dimethyl-3-isoxazolidinone] was spray applied at a rate equivalent to 2 lb ai/A at planting to soybeans grown in pots in a greenhouse. Plants were thinned at 2 weeks and harvested at 30 and 60 days after planting. A similar, larger scale study was conducted (PHI 30 only) to provide additional material for identification of metabolites.

The plant material was extracted with methanol which was concentrated, diluted with water, and reconcentrated. A dark precipitate which formed was returned to the non-extractable fraction. The supernatant was partitioned with methylene chloride and the methylene chloride extracts dried and concentrated. This fraction was expected to contain the non-conjugated metabolites. The aqueous methanol fraction was subjected to cellulase hydrolysis, then chromatographed on a C-18 reverse phase Sep Pak to separate the polar metabolites (water-soluble). Organosoluble aglycones were then eluted with methylene chloride, which was dried and concentrated. A final elution with methanol was combined with the initial polar effluent.

Organic extracts and aglycones were anaylzed by TLC and autoradiography, with a percentage distribution calculated by scraping, eluting, and counting all bands of radioactivity. Eluted bands were also subjected to HPLC, and certain metabolites also chromatographed on GC, with mass spec analyses for most using derivatives of several polar metabolites. Specific location of functional groups was deduced from mass spec fragment patterns and confirmed by NMR.

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Command on Soybean Plants, 2 lb a.i./A
Metabolite Distribution, Methylene Label

	30 day		60 day		Difference
non conjugated	0.8 ppm	8%	0.14 ppm	3%	-5%
conjugates	(8.14 ppm)	(78%)	(4 ppm)	(81%)	
aglycones	6.3 ppm	60%	3.2 ppm	66%	+6%
polar	1.9 ppm	18%	0.8 ppm	16%	-2%
non-extractable	1.5 ppm	14%	0.7 ppm	16%	+2%
	approx 10 ppm		approx 5 ppm		

further breakdown of aglycone metabolites

A 5-Keto Command	0.3 ppm	5%	0.15 ppm	4.5%
B (aglycone not identified)	0.5 ppm	8%	0.11 ppm	3%
C o-chlorobenzyl alcohol	3 ppm	48%	1.8 ppm	54%
D (aglycone/non-conjugate not identified)	0.5 ppm	9%	0.1 ppm	3%
E 5-OH Command, aglycone	0.2 ppm	3%	0.06 ppm	2%
F,G F = 5'-OH Command, G = hydroxymethyl Command	1.2 ppm	19%	0.6 ppm	20%
origin	0.5 ppm	8%	0.4 ppm	14%

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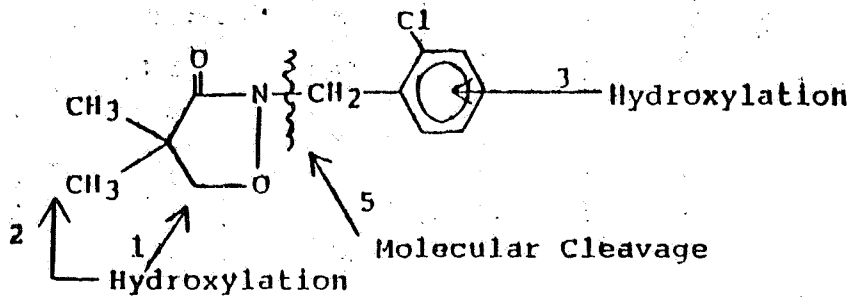
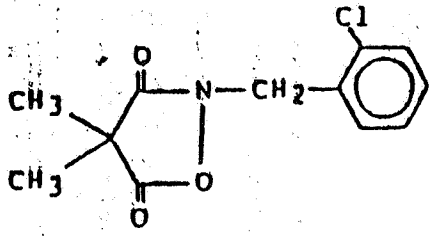
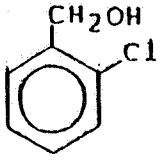
FMC 57020

SOYBEAN FORAGE METABOLITES

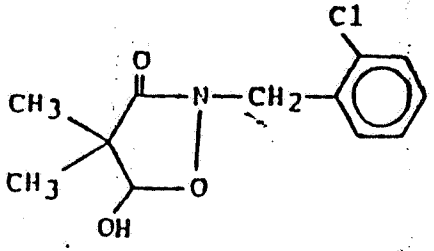




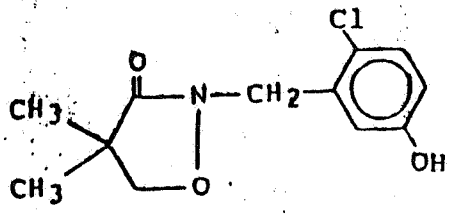
A (5-Keto FMC 57020)



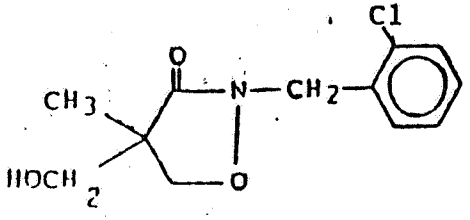
C (OCBA)



E (5-Hydroxy FMC 57020)



F (5'-Hydroxy FMC 57020)



G (Hydroxymethyl FMC 57020)

Alfalfa Foliar Metabolism (MRID #401236-02)

This study was conducted to aid in assessing the off-target incidents of 1986. Alfalfa was one of most frequent and perhaps the most economically important of the crops which were contaminated, and certainly the biggest feed item.

Based on spray drift/volatility studies, a dose rate of 0.25 lb ai/A was selected for alfalfa. This dose causes extensive bleaching, but does not halt plant growth. Higher rates were feared to kill alfalfa. Both methylene-labeled and aromatic ring-labeled Command were used.

Field grown alfalfa plants were brought into a greenhouse in pots and acclimated for 8 weeks before being sprayed with Command. Three separate studies were conducted.

Ring-labeled Command was used to study dissipation of (total ^{14}C) residues as a function of time (harvest at 1 hr, 3 days and 7 days, Study A) and to characterize by solubility and enzyme hydrolysis the residue distribution over time (days 0, 3, 7, 15 and 33, Study B). This characterization procedure was identical to that described for soybeans, above. The methylene-labeled study provided sufficient quantities for identification of components of the aglycone fraction (Study C).

The aglycone fraction from alfalfa was compared to that from soybeans. TLC shows that the metabolites are qualitatively alike, but that metabolites B and D (which were minor and unidentified in soybeans) constitute a larger portion of the aglycone fraction in alfalfa. Study C was thus conducted (5 day PHI) using methylene label to provide quantities sufficient for identification. The preparative work-up was the same as for soybeans. Mass spectra indicate that both B and D are monohydroxylated parent. Exact locations of the -OH group on the benzene ring were not confirmed, but the 3' and 6' position (on benzene ring) are postulated from mass spec of the trimethylsilyl derivatives.

The fraction designated "polar" is the water-soluble remnant after cellulase digestion. FMC's report suggests that these "polar" components likely have multiple hydroxy groups. Although not demonstrated, this is a reasonable explanation. FMC presents a discussion of the extreme possibilities for this polar fraction including the unextractables. Either it consists primarily of parent-related metabolites, in which case the majority of residue (>90% 2-2.5 ppm) would be parent-related, or it consists of molecular cleavage products, of which the radiolabeled portion would be ortho-chlorobenzyl alcohol (OCBA). An equal quantity of heterocyclic ring metabolites would necessarily be present, also. If cleavage were the major route, about 40-50% of the residue (1-1.5 ppm) would be cleavage products.

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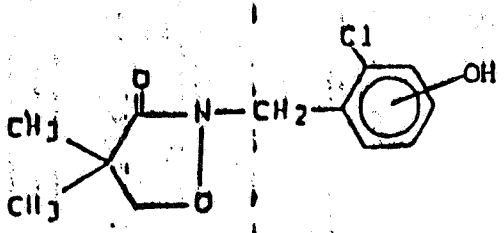
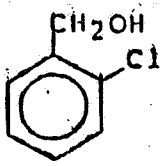
FMC 57020

ALFALFA FORAGE METABOLITES



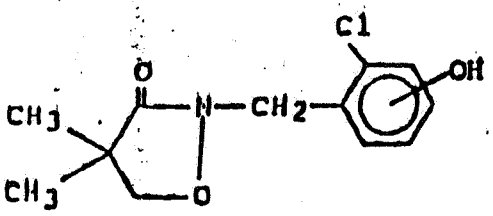
B (3'-Hydroxy FMC 57020)

(10.2%)



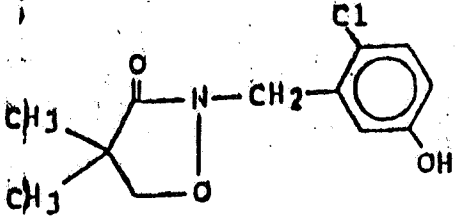
C (OCBA)

(5.0%)



D (6'-Hydroxy FMC 57020)

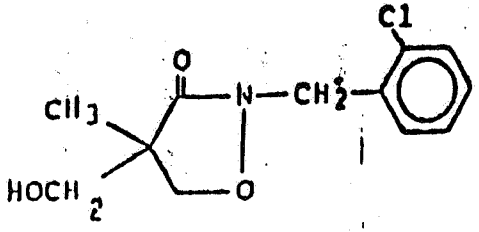
(9.6%)



F (5'-Hydroxy FMC 57020)

FMC 77039

(<1%)



G (Hydroxymethyl FMC 57020)

(<1%)

-6a-

2C

The rapid drop in residue level after day 0 is attributed to volatilization of residue prior to penetration of the leaf surface. This is consistent with the vapor pressure and the reasons for performing the study.

Distribution of Command Metabolites in Alfalfa

	day 0	day 3	day 7	day 15	day 33
STUDY A					
non-conjugates	96.9%	0.6%	0.6%		
conjugates					
aglycone	0.3%	32%	28%		
polar	1.1%	46%	52%		
non-extractable	2.2%	22%	19%		
total residue ^{14}C as parent	10.2 ppm	2.3 ppm	3.6 ppm		
STUDY B					
non-conjugates	96%	<1%	<1%	<1%	<1%
conjugates					
aglycone	1%	46%	48%	47%	29%
polar	2.4%	27%	41%	38%	42%
non-extractable	0.5%	28%	10%	15%	29%
total residue ^{14}C as parent	3.7 ppm	2.4 ppm	1.9 ppm	1.3 ppm	0.6 ppm

AGLYCONE FRACTION, STUDY A

	day 3	day 7
Metabolite B	13%	10%
Metabolite C (OCBA)	7%	5%
Metabolite D	9%	10%
Metabolite F/G	1.2%	2.5%
origin	1.1%	0.7%
total	32%	28%

To summarize, alfalfa is expected to have about 3 ppm total residue; at most, about 1.5 ppm (half of total) would be OCBA (probably less). If all the material identified as non-conjugate were parent, by day 3 (and maybe earlier) after exposure, <0.1 ppm parent would be present. In effect, after 3 days, only metabolites should exist.

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Incident reports from summer of 1986 found 30 ppb-32 ppm Command in 25 samples of bleached alfalfa; only 3 of these were >3 ppm, and another 52 samples had no detectable parent. Almost all "positives" were resampled later, and showed lower or no detectable parent. (All crops other than alfalfa showed no detectable to <0.3 ppm, except one garlic sample having 0.6 ppm). The time from contamination or visible injury to sampling is unknown (see L. Bradley memo of 9/16/86).

In its latest presentation, FMC suggested that an "enforcement level" of 240 ppb parent would be appropriate. RCB has no objections to this value, except that we would prefer "0.25 ppm" for cosmetic reasons. Analytical methodology is available to enforce residue levels for the parent compound (Method A, PAM-II).

We would also suggest that alfalfa (or other crops) should not be harvested for 3 days after incidential exposure, since residue estimates and metabolite identifications are based on 3+ days after treatment.

Goat Metabolism Study (MRID #400700-01)

This is actually a material balance study using ^{14}C-ring labeled Command. Two lactating goats were given 0.5 ppm, and two 5 ppm. Dose was administered by capsule (1 and 10 mg/capsule per day) and nominal levels are based on projected feed intake of 2 kg/day. Milk samples as analyzed are composites of morning and evening milkings. Urine and fecal samples respresent 24 hour collections. The control goat and one from each dose level was slaughtered within 24 hrs of last dose, and the remaining animals within 72 hours. Tissue samples as listed were wrapped in aluminum foil, sealed in plastic bags or jars and frozen pending radioanalysis: blood, fat (omental & perirenal), kidneys, liver, gall bladder contents, heart and muscle (3 specific locations). Tissue levels are reported here only for the animals slaughtered within 1 day of last dose.

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From soybean consumption
where the OCBA is found.

^{14}C-Command Material Balance in Goats

	0.5 ppm dose		5 ppm dose	
	ppm Command equiv. found	% of total	ppm Command equiv. found	% of total
urine	0.15-0.6 ppm	73, 83	1-5 ppm	79, 68
feces	0.05-0.1 ppm	14, 11	0.4-2 ppm	11, 18
milk	<1-2 ppb	0.15, 0.24	2-9 ppb	0.16, 0.06
liver	5 ppb	0.07, 0.04	0.05 ppm	0.06, 0.04
kidney	0.8 ppb	0.002 (one only)	0.02 ppm	none given too low
muscles (various)	<6 ppb (ND)		<6 ppb (ND)	
fat (various)	<6-<7 ppb (ND)		<6-<7 ppb (ND)	
blood	<6 ppb (ND)		<6 ppb (ND)	
gall bladder contents	1 ppb	0.0001	.07 ppm	0.0003

FMC's chart illustrating rat metabolites is on the following page. RCB has not reviewed this study, but TOX undoubtedly has, prior to granting the tolerance for Command on soybeans. We have no information on the metabolites in the goat, and no basis for comparison. We would assume that metabolism occurs in ruminants and thus the levels based on radioactivity are higher than levels of parent, but the relative amount is unknown.

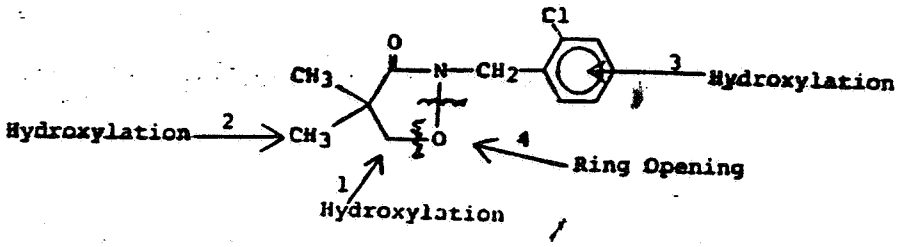
OCBA does not occur as a rat metabolite. TOX has been provided with references and data on 2-chlorotoluene, which metabolizes to OCBA (35-40% of oral dose) in rats. FMC has also submitted a rat pharmacokinetic study on OCBA, which RCB is not reviewing, but referring to TOX.

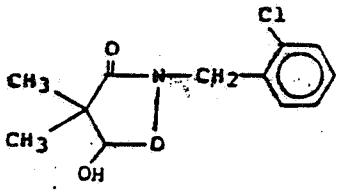
Based on the goat metabolism study and the "worst" residue estimates for alfalfa, no detectable residues are likely to occur in animals. Alfalfa may be up to 80% of the diet of dairy cattle, giving approximately 2.5 ppm total residue in the diet, virtually all of which is metabolites. Up to 1.2 ppm OCBA could possibly be present in the alfalfa (this is "worst case"): if the 25 ppm were all parent the total residue which could occur is <0.01 ppm in muscle, fat and milk, <0.1 ppm in kidney, and <0.03 ppm in liver. Since, in fact, <0.1 ppm parent is present in alfalfa, we do not anticipate that any detectable parent will occur in animals. Neither would we expect metabolites to accumulate but we have no chemical-specific data to support this expectation. We can make no conclusion about possible OCBA levels in animals.

FMC 57020

RAT METABOLISM

BIODEGRADATION PATHWAY SUMMARY





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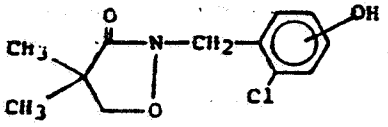

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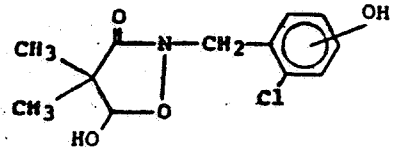
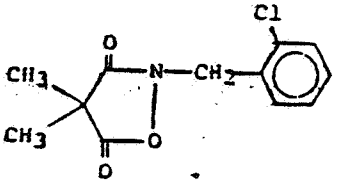


(1 & 3)



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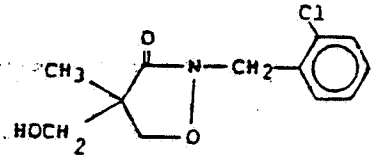

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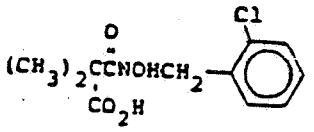
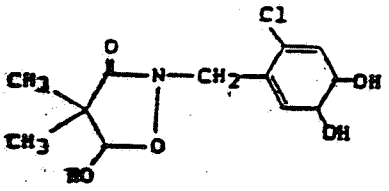
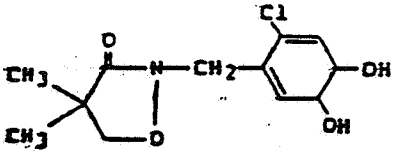


(1 & 4)



(1 + 3)



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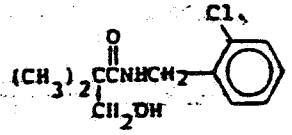

(4)

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Conclusions

1. Among different plant species, degree of visible injury is not expected to correlate with residue levels.

2. Translocation of Command or metabolites after foliar application is not likely to occur. Thus, fruits and fruiting vegetables are unlikely to have any residues from inadvertant exposure early in the growing season.

2a. RCB does not object to FMC's suggested enforcement level of 0.25 ppm Command. Based on the alfalfa metabolism study, we estimate that residues of parent Command will be <0.1 ppm, and that total residue levels will be 3 ppm or less (total activity calculated as parent). Of this, no more than 1.5 ppm (and perhaps far less) would be OCBA. These estimates are valid at 3 or more days after exposure.

2b. We do not expect any residues to occur in subsequent cuttings, since Command does not readily translocate from foliar application.

3. Based on the goat metabolism study, no detectable residues of parent Command are expected to occur in animals from consuming contaminated alfalfa. A "worst case" estimate for transfer of residues concludes that, if the total residue in alfalfa (3 ppm at 80% of diet) were parent, residue levels in animals (total activity calculated as parent) would be <0.01 ppm for meat, fat, and milk, <0.1 ppm for kidney and <0.3 ppm for liver.

4. We can reach no conclusion about possible OCBA levels in animals.

Recommendations

We recommend that crops, particularly alfalfa, which are inadvertantly contaminated with Command not be harvested for 3 days after spraying of adjacent fields if chlorosis has occurred, to allow volatilization and metabolism to reduce residue levels.

RCB does not recommend that an action level be established for Command residues resulting from unintentional contamination. This is not the purpose of action levels.

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We would refer the following questions to the FDA/EPA Liaison group and/or the Policy Group, after the results of TOX's review are available:

1) Is it necessary to set an enforcement level under these circumstances?

2) If so, what is an appropriate mechanism?

cc: Command s.f., PP 4F3128, PMSD/ISB, r.f., circ.

TS-769:LMB:5577378:CM2:804F
RDI:A.R.Rathman:4/29/87:R.D.Schmitt:4/29/87

92273:I:Bradley:C.Disk:KENCO:5/4/87:BJP:LF:SONJA

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